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23° SILAE Congress

"Prof. Gugliemo Stagno d'Alcontres"

7th - 12th of September, 2014
Marsala, Italy

Abstracts Book

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Vincenzo Barbarulo, Giacomo Dugo, Luigi Russo,
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Abstracts Book

of

SILAE:

The Scientific And Cultural Network

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WELCOME TO SILAE EXPERIENCE

On behalf of the organizing committee, we are honored and delighted to welcome you to the 23° SILAE Congress, Marsala, Italy. We believe we have chosen a venue that guarantees a successful technical conference a mid the culture and scenery of Sicily.

The Congress is a multi-disciplinary event organized in different sessions: Anthropology and Ethnobotany, Phytochemistry and Pharmacognosy, Natural Products and Pharmacology, Analytical Chemistry, Chemistry of Food and Nutrition. For each of them the Congress will show the results of studies and research on traditional medicine and medicinal, food and aromatic plants, and will place the basis for new collaborations and future studies.

Our technical program is rich and varied with 8 plenary lectures and 2 invited talks, a round table, a documentary session and over 300 technical papers split between an oral session and 2 poster sessions each day. We also expect to provide technical demonstrations, and numerous opportunities for informal networking.

Furthermore, we have also organized many social programs for the participants to have a good time and relax after the intensity of the day sessions with live music, performances, folkloric dancers and by tasting special Sicily cuisine. We are most fortunate to have generous support from many national and international organizations and commercial enterprises.

We, today, dear SILAE members, gather here to work together, to promote the research of medicinal and food plants in different countries of the World and to consider strategies to start fruitful scientific collaborations and last but not least to consolidate our relations of friendship. Our website is a great interactive and scientific social networking with more than 4000 members and will constitute ever more a global platform open to all researchers of any nationality, who would like to present their studies as well as to build new collaborations and friendship among members.

As a conference chairs of 23° SILAE, we know that the success of the conference depends ultimately on the many people who have worked with us in planning and organizing both the technical program and supporting social arrangements. In particular, we thank the Organizer Committee for their wise advice and brilliant suggestion on organizing the technical program; the Scientific Committee for their thorough and timely reviewing of the papers, and our sponsors who have helped us to keep down the costs of 23° SILAE for all participants. Recognition should go to the Local Organizing Committee members who have all worked extremely hard for the details of important aspects of the conference programs and social activities.

Giacomo Dugo and Luca Rastrelli
Chairman and Co-chairman of 23° SILAE Congress

Congress Organization and Committees

Organizing Committee

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Prof. Giacomo Dugo

Prof. Luca Rastrelli, Dr. Nicola Cicero

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Vincenzo Ferrantelli (IZS)

marsala2014@silae.it
segreteria@silae.it

Graphic design
Rossella Bicco

Webmaster
Vincenzo Barbarulo

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Program

SUNDAY, SEPTEMBER 7 - Opening

San Pietro Monumental Center

17:00-19:00 **Registration**

19:30-20:30 **welcome to Marsala**
Prof. Giacomo Dugo, Chariman 23° SILAE Congress
Prof. Suzana Guimarães Leitão, President of SILAE
Prof. Paola Vita Finzi, Past President of SILAE
Dr. Antonino Salina, IZS Sicilia
Dr. Rossella Lelli, IZS Sicilia
Prof. Luca Rastrelli, Co-Chariman 23° SILAE Congress

Memorial award to
Prof. **Gugliemo Stagno D'Alcontres**

20:30 SILAE CAREER AWARD to
Prof. **Giovanni Dugo** and Prof. **Francesco De Simone**
For their contributions in the fields of food chemistry and food analysis

21:00 Welcome Cocktail Party - Traditional Sicilian Dancers

MONDAY, SEPTEMBER 8 - Anthropology and Ethnobotany

Villa Favorita Hotel (Congress Center)

9:00-9:45 Plenary Giovanni Dugo - University of Messina
Comprehensive two-dimensional chromatography in food analysis: an overview

I Session Chairman:
Alberto Nuñez Selles and Nunziatina De Tommasi

9:45 Nubilde Martinez - Instituto de Investigaciones Biomédicas "Dr. Francisco Javier Triana Alonso (BIOMED-UC)", Venezuela
The popular belief vs scientific knowledge about *Ficus carica* (fig) decoction

10:00 Cesar Augusto Martinez Cotacio - National University of Colombia, Colombia
Analysis of the neuroprotective activity of alcoholic extracts of Caquetá foothills and natural reserve Ucumarí - Risaralda Colombia

10:15 Yoleida Mendoza Vega - University of La Guajira, Colombia
The xerophytic forest as food pantry of the Wayuu, La Guajira Colombia

10:30 Gizem Bulut - Marmara University, Turkey
The folk medicinal plants of Istanbul (Turkey)

10:45 Rabia Afza - Hazara University Mansehra, Pakistan
Medicinal plants of hazarno (Malakand agency) and their conservation status

11:00 Coffee Break

II Session Chairman:
Luca Rastrelli and Silvia Quesada

11:30 Carolina Weber Kffuri - Universidade Estadual Paulista, Brazil
Antimalarial plants used by indigenous people of the Alto Rio Negro region - Brazilian Amazon

11:45 Katia Peralta - Instituto Peruano de Investigación Fitoterápica Andina, Perù
Peruvian amazon oleo resins

12:00 Reinaldo Correa Costa - National Institute of Amazonian Research, Brazil
Vegetal biodiversity and produtives chains in Amazonas (Brazil)

12:15 Sara M. Robledo - University of Antioquia, Colombia
In vitro and in vivo screening of colombian plants for potential antileishmanial, antimalarial and antitrypanosomal compounds

12:30 Qamar Abbas - Karakorum International University Gilgit-Baltistan, Pakistan
Ethnobotanical study of Bagrote valley of Gilgit district, Central Karakoram National Park, Gilgit-Baltistan, Pakistan

12:45 Luisa Schipilliti - Università degli Studi di Messina, Italy
Genotype differentiation of *Helichrysum italicum* (roth) G. don fil. subsp. italicum by means of essential oils carbon isotope ratio analysis

13:00 Free time and Research, development and collaboration agreements

13:15 **Lunch**

14:30-15:30 **FOCUS: Caribbean Herbal Pharmacopoeia**

Lionel Germosén-Robineau - Université des Antilles et de la Guyane, R. Dominicana
"Caribbean Herbal Pharmacopoeia"

Jairo Rosado - University of La Guajira, La Guajira, Colombia
"Guajiras plants: from medicinal to toxic"

III session Chairman:
Fredyc Diaz-Castillo and Rosangela Marchelli

15:30 Carlotta Crescenti - University of Messina, Italy
Sicilian rural cooking and therapeutic virtues of some mediterranean spontaneous plants

15:45 Patricio V. Noboa - Escuela Superior Politécnica de Chimborazo, Ecuador
Towards a de-colonial ethnobotany

16:00 Hassan Sher - University of Swat, Pakistan
Maximizing farm income and other livelihood opportunities through introduction of high value medicinal and aromatic plants in district Swat, Pakistan

16:15 Coffee Break

16:45-18:00 **Round Table: Recent advances in medicinal plants and biotecnhology**
Coordinator: Maria de Los Angeles Basiglio and Sandra Sharry (Argentina)

Participants:
Maria de Los Angeles Basiglio - Centro Experimental de Propagación Vegetativa
Argentina Tissue culture in species of the genus Erythrina

Marta Goleniowski - Ministry of Science and Technology, Córdoba, Argentina
Biotechnology applied to plant secondary metabolites production

Alicia Consolini - School of Exact Sciences, National University of La Plata
Argentina Ethnopharmacology of argentinian medicinal plants:
experimental models and results

Silvana Alvarenga Venutolo - Institute of Technology, Center for Biotechnology Research
Biotechnological tools for sustainable crop managementand marketing of
cat's claw (*Uncaria tomentosa*) in Costa Rica

Vilma Jimenez - Biotechnology Research Center (CIB) of the Technological Institute of Costa Rica
Antioxidants content and advances in micropropagation of
blueberry (*Vaccinium consanguineum*) native from Costa Rica

Ana Abdelnour-Esquivel - Instituto Tecnológico de Costa Rica, Cartago, Costa Rica
Cryopreservation of cat's claw (*Uncaria tomentosa*)

17:45 - 19:30 Poster Session I

18:00 Prof. Giacomo Dugo - Chairman of the Research Consortium Co.Ri.Bi.A. Palermo
Presentation of the project: "Traceability and food safety, add value to agricultural products of Sicily"

20:30 **Traditional Sicilian Dinner**

23:00 **Latin Disco Party**

TUESDAY, SEPTEMBER 9 - Pharmacognosy and Phytochemistry

Villa Favorita Hotel (Congress Center)

9:00-9:45 Plenary Tina De Tommasi - University of Salerno, Italy
"Probing plants chemical diversity for new leads"

I Session Chairman:
Paola Vita Finzi and Susana Abdo

9:45 Gianluca Gilardoni - Universidad Técnica Particular de Loja, Ecuador
Isolation of novel hydroquinolinic alkaloids from Ecuadorian plants of the genus Huperzia, employed in the traditional medicine of Saraguro people

10:00 Flavia Reis - Itajubá College of Medicine, Brazil
Effects of chronic treatment with aqueous extract of "*Passiflora edulis*" seeds in rats induced to obesity and dyslipidemia

10:15 Fernando Siller Lopez - Universidad de Guadalajara, Mexico
Increase in enzymatic antioxidant activities as a response to long term water arsenic exposure by phytoremediators *Zantedeschia aethiopica* and *Anemopsis californica*

10:30 Mario Alberto Sequeira Obando - Instituto Tecnológico de Costa Rica, Costa Rica
Cytotoxic effect of an ethanol extract of *Phyllanthus accuminatus* leaves on human epithelial cancer cells

10:45 Julio Alarcon - Universidad del Bío-Bío, Chile
Terpenoid from chilean rhamnaceae and biological activity

11:00 Coffee Break

II Session Chairman:
Ivana Bonaccorsi and Alicia Consolini

11:30 Gina M. Mendez - Universidad de Ciencias Aplicadas y Ambientales, Colombia
Antineoplastic activity of species of the genera ageratina and lourteigia

11:45 Alev Tosun - Ankara University, Turkey
Cytotoxic and anti-inflammatory potency of Seseli L. oils

12:00 Livia Marques Casanova - Universidade Federal do Rio de Janeiro, Brazil
Comparative study of the chemical composition and in vivo hypoglycemic activity of two Ocimum species (Lamiaceae)

12:15 Maria Silvana Alves - Federal University of Juiz de Fora, Brazil
Antibacterial activity assessment of *Bauhinia forficata* link (fabaceae)

12:30 Paola Andrea Cardenas - Universidad Nacional de Colombia Colombia
Pharmacokinetics profile of 6-Methylcoumarin in Wistar Rats after oral administration

12:45 Aman Khan - Hazara University, Pakistan
Evaluation of antimicrobial and antioxidant activities of Juniperus species used in traditional medicine in Pakistan

13:00 **Lunch**

14:30-15:15 Plenary: Prof. Omar Malagon - Universidad Técnica Particular de Loja, Ecuador
The research in the natural products field in a "hot spot" of biodiversity and culture and the impact of the scientiic collaboration: the UTPL and University of Pavia case

III session Chairman:
Alev Tosun and Juan Carlos Sepulveda

15:15 Ericsson David Coy-Barrera - Universidad Militar Nueva Granada, Colombia
Benzofuran-type neolignans from *Ocotea heterochroma* and their binding mode within paf-receptor

15:30 Laifa El-Adoui - University of Constantine, Algeria
Synthesis and study of physico-chemical property of the derivatives of dithiol-one from dithiol-thione

15:45 Sinem Aslan Erdem - Ankara University, Turkey
Contribution to the phytochemistry and bioactivity of *Eryngium kotschyi* Boiss

16:00 Cecilia Veronica Nunez - National Institute of Amazonian Research, Brazil
Antimycobacterial activity of monoterpene indole alkaloids of *Duroia macrophylla*

16:15 Domenico Rongai - CRA, Consiglio per la ricerca e la sperimentazione in agricoltura, Italy
Antifungal activity of pomegranate extract: effect of different extraction methods

16:30 Jaciara Lira - Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Brasil
Identification of monoterpene indolic alkaloid strictosidinic acid from *Palicourea guianensis* Aubl

16:45 Fejzo Selami - University of Tirana, Albania
An investigation about the antibiotic and dye resisdues in aquaculture product of Albania

17:00 Coffee Break

IV session Chairman
Susana Leitao and Vincenzo Ferrantelli

17:30 José Luis Martinez - University of Santiago of Chile
Effects of two bisbencylisoquinoline alkaloids, tetrandrine and antioquine in rat aortic rings: comparison to verapamil

17:45 Ericsson David Coy-Barrera - Universidad Militar Nueva Granada, Colombia
LC-based profiling combined with chemometrics of Lupinus species from Colombia

18:00 Marcela Hernández Ortega - Universidad Autónoma del Estado de México, Mexico
Antioxidant, antinociceptive and antiinflamatory effects of carotenoids extracted from dried pepper (*Capsicum annuum* L.)

18:15 Katherine Sánchez-Zúñiga - Instituto Tecnológico de Costa Rica, Costa Rica
Preliminary evaluation of the cytotoxic capacity of an ethanol extract from *Moringa oleifera* leaves

18:30 Yeraldine Velásquez Ladino - Universidad Militar Nueva Granada, Cajicá, Colombia
Antioxidant capacity of colored kernels of maize varieties (Zea mays) from Bogota plateu

18:45 SILAE Assembly

21:00 **Latin Disco Party**: Dinner & Drink at Discoteca "Chiedilaluna"

WEDNESDAY, SEPTEMBER 10

All day **SILAE SICILY TOUR** - HISTORY and ENOGASTRONOMY

THURSDAY, SEPTEMBER 11 - Natural Products and Analytical Chemistry

Villa Favorita Hotel (Congress Center)

9:00-9:45 Plenary Prof. Alberto Nuñez Selles - Universidad Nacional Evangelica, Dominican R.
"Oxidative stress in chronic degenerative diseases"

I Session Chairman:
José Luis Martinez Salinas and Luisa Mannina

9:45 Claudio Corradini - University of Parma, Italy
Analytical approaches for determination of molecular food markers and compounds of interest in food contact materials

10:00 Osorio Roa Coralia - Universidad Nacional de Colombia, Colombia
The role of volatile organic compounds and their glycosidic precursors on the curuba (*Passiflora mollissima* Kunth L. H. Bailey) aroma

10:15 Imma Pagano - University of Salerno, Italy
Valorization of artichoke by-products as source of bioactive compounds

10:30 Erica Wilson - University of Leiden, Netherlands
Extraction of galanthamine from narcissus bulbs using natural eutectic solvents (nades) and hot pressurized extraction (HPE)

10:45 Coffee Break

II Session Chairman:
Carolina Weber Kffuri and Nicola Cicero

11:15 Alongi Angelina - Istituto Zooprofilattico Sperimentale della Sicilia
Validation of a chromatographic analytical method for qualitative assessment of fatty acid methyl esters percentage in pistachio

11:30 Susana L. Abdo - Escuela Superior Politécnica de Chimborazo, Ecuador
Photoprotector activity of plants for elaboration of a sunscreen

11:45 Bonaccorsi, I.L. - Università di Messina, Italy
Fast and eco-friendly analysis of bioactive molecule

12:00 Andrea Macaluso - Istituto Zooprofilattico Sperimentale della Sicilia
Detection of lead in not processed vegetables commercialized in Sicily

12:15 Luisa Mannina - CNR, Italy
NMR metabolite profiling of blueberries

12:30 Rosendo Archbold - Universidad de Antioquia, Medellin, Colombia
Rheology studies implementation in developing solid dosage forms in natural products

12:45 Iftikhar A. Khan - Hazara University Mansehra, Pakistan
Conservation issues of tree flora in natural habitats of Totalai tract district buner

13:00 **Lunch**

14:30-15:15 Plenary Prof. Fredyc Diaz Castillo - Universidad de Cartagena, Colombia
"Phytochemical and biological studies of extracts from Colombian Caribbean plants as an alternative to the fight against dengue"

III session Chairman:
Giovanni Romussi and Giuseppa Di Bella

15:15 Crispin A. Celis Zambrano - Facultad de Ciencias, Bogotà, Colombia
Method comparison for establishment of antioxidant potential in natural products

15:30 Marcelo G.F. Araujo - Universidade Federal de São João Del-Rei, Brazil
Antidiarrhoeal activity of umbelliferone (7-hydroxycoumarin)

15:45 Frank Blanco - Instituto Venezolano de Investigaciones Científicas,
Venezuela Stevia.. more than a sweetener? Antiinflammatory activity of *Stevia lucida*

16:00 Maria D. Hernandez-Navarro - Universidad Autónoma del Estado de México, México
Effect of reduced-calorie avocado paste on the lipid profile in wistar rats feed with a hypercholesterolemic diet

16:15 Carmelo Corsaro - University of Messina, Italy
Characterization of Sicilian food by HR-MAS NMR

16:30 Rosalva Mora Escobedo - Instituto Politécnico Nacional, México
Characterization and evaluation of antioxidant capacity of aguamiel (*Agave atrovirens*)

16:45 Shujaul Mulk Khan - Hazara University Mansehra, Pakistan
Medicinal and edible plants; Phyto-therapeutic uses by Balti community of the Karakoram Range of mountains

17:00 Coffee Break

17:15-19:30 Poster session II

Video Documentaries:
"Malaria in indigenous communities in northeast Amazonia"
Carolina Weber Kffuri - Universidade Estadual Paulista

"The land of remorse: exploration of 'tarantism' in the rural communities of Souhern Italy"
SILAE Documentary Office - University of Salerno

18:30 Book Presentation:
"A tavola con gli elementi"
Author Chourmo (alias Michele Girlanda) Casa Editrice ETS Pisa
presented by Prof. Enrico Rotondo

20:30 **Social Dinner**

FRIDAY, SEPTEMBER 12 - Food Chemistry and Nutrition

Villa Favorita Hotel (Congress Center)

9:00-9:45 Plenary Rosangela Marchelli - University of Parma, Italy
Update on food allergy: evaluation of new epidemiological data, threshold values and methods of analysis

I Session Chairman:
Maurizio Bruno and Andreazza Nathalia Luiza

9:45 Gloria Pumilia - University of Messina, Italy
ß-cryptoxanthin bioaccessibility in milk-fruit beverages containing orange juice

10:00 Vella Antonio - Istituto Zooprofilattico Sperimentale della Sicilia
Determination of aflatoxin B1, B2, G1, G2 in almond milk

10:15 Marcela P. Carrillo - Instituto Amazónico de Investigaciones Científicas Sinchi, Colombia
Development of food products from amazon fruits of asaí (*Euterpe precatoria*), seje (*Oenocarpus bataua* mart.) and canangucha (*Mauritia flexuosa*) palms

10:30 Antonella Smeriglio - University of Messina, Italy
Extra virgin olive oil (evoo) as functional food: standardization process based on scientific evidences

10:45 Coffee break

11:00 Calvaruso Enza - Istituto Zooprofilattico Sperimentale della Sicilia
Validation and determination of Polycyclic Aromatic Hydrocarbons in Citrus by Gc-Ms coupled with modified quechers sample preparation procedure

11:15 Maria Daglia - University of Pavia
Antibacterial activity and chemical characterization of *Myrcianthes hallii* (Myrtaceae), used as traditional medicine in Ecuador

11:30-12:15 J. Siddiqui - University of Karachi, Pakistan
Bioactives from *Grewia asiatica* fruits: potential food-plant explored for nutraceutical development

12:30 Plenary Prof. Patricia Vit - Universidad Tecnica de Machala, Ecuador
"*Melipona favosa* honey from Venezuela: a multidisciplinary characterization of pot-honeys in the world, towards its inclusion in the Ecuadorian honey regulation"

Young Researcher Award

Closing

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SILAE SICILY TOUR - HISTORY and ENOGASTRONOMY

Wednesday, September 10

..Legend, history, beauty, culture, castles, churches, stunning views and the latest in science and technology: all this and more is at your disposal when you travel to and spend some time at the beautiful city of Erice in Sicily..

The "Stagnone" is the largest lagoon in Italy near Marsala. It is a designated marine nature reserve covering some 2,000 hectares. The "Stagnone" is home not only to the ancient tradition of sea-salt production but also to a flourishing variety of wildlife and a fascinating archipelago consisting of four mostly uninhabited islands.

Time duration: all day
Meeting place: your selected hotel in Marsala
Departure time: 8:30am from "Hotel President" and "Hotel Carmine"
9:00am from "Hotel Villa Favorita"

9:00 • Departure

10:15 • **Erice**. Visit to the *Borgo antico* (old town) and to the *Villa Comunale* (public garden)

12:15 • Departure for the equipped beach "*Elios Garden Club*" of **San Vito Lo Capo**
During the stay on the beach, a lunch will be served (Sicilian pizza, fruit, ice cream and soft drinks)

17:30 • Departure for the "*Riserva dello Stagnone*" of Marsala, visit to the salt pans, to the salt museum

20:30 • Food and drink at the "*L'incanto*"

Organization

Università Degli Studi Di Messina
Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute - S.A.S.T.A.S.

Università Degli Studi Di Salerno
Dipartimento di Farmacia

Co.Ri.Bi.A.
Consorzio di Ricerca sul Rischio Biologico in Agricoltura

Istituto Zooprofilattico Sperimentale della Sicilia

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PLENARY

Plenary Speakers Biographies

Prof. **Giovanni Dugo**
"*Comprehensive two-dimensional chromatography in food analysis: an overview*"
University of Messina, Italy
giodugo@unime.it

Full Professor of Food Chemistry at the University of Messina, Faculty of Pharmacy, Italy.
His research activity is directed towards the development of innovative methods and to the study of food matrices by using innovative methodologies such as: multidimensional liquid chromatography (comprehensive LC); multidimensional gas chromatography (MDGC and comprehensive GC); ultrafast-GC and ultrafast-GC/MS; on-line SPME-GC/MS; micro-HPLC and micro-HPLC/API/MS; multidimensional HPLC and micro-HPLC; superheated HPLC; LC x GC.
He is co-author of more than 300 national and international papers and several scientific book and

Prof. **Michael Heinrich**
"Value chains of herbal medicines - an emerging research challenge?"
UCL School of Pharmacy - Faculty of Life Sciences
University of London
michael.heinrich@pharmacy.ac.uk

Pharmaceutical biologist / pharmacognosist, and anthropologist, with a many years of research experience in a multitude of generally transdisciplinary aspects of medicinal and food plant research (esp. bioactive natural products), as well as at the interface of cultural and natural sciences especially on the traditional use of food and medicinal plants for example in Mexico and the Mediterranean.
Since 2012 Head and Professor at the Centre for Pharmacognosy and Phytotherapy, UCL School of Pharmacy, London (UK), 2011 - 2012 Founding

Prof. **Lionel Germosén-Robineau**
"Caribbean Herbal Pharmacopoeia"
Université des Antilles et de la Guyane,
R. Dominicana

MD, holds a M.Sc. in Public Health and is the scientific coordinator of the TRAMIL network that conducts applied research on Caribbean medicinal plants.
Editor of the Caribbean Herbal Pharmacopoeia. He is a member of UAG University, French West Indies.
www.tramil.net

Prof. **Tina De Tommasi**
"Probing plants chemical diversity for new leads"
University of Salerno, Italy
detommasi@unisa.it

Full Professor in Pharmaceutical Biology; her scientific field of research is biological and chemical plants used in traditional medicines and their bioactive metabolites.
Currently she studies the interaction between natural bioactive molecules and proteins that play key roles in

Prof. **Omar Malagon**
"The research in the natural products field in a "hot spot" of biodiversity and culture and the impact of the scientfic collaboration: the UTPL and University of Pavia case"
Universidad Técnica Particular de Loja, Ecuador
omalagon@utpl.edu.ec

Director of Biológical Area of University Tecnica Particular de Loja; his researches are based on the study of chemical composition of essential oil and

Prof. **Massoud Kaykhai**
"Application of microextraction techniques for Chemical Screening of Volatiles from plants"
University of Sistan & Baluchestan, Zahedan, Iran

Associate Professor, Department of Chemistry, Faculty of Sciences, University of Sistan & Baluchestan; his research is based on the study and development of new sample preparation techniques for trace analysis, including: Liquid Phase Microextraction (LPME), Solid Phase Microextraction (SPME), Membrane Extraction

Prof. **Alberto J. Nuñez Selles**
"Oxidative stress in chronic degenerative diseases"
National Evangelic University (UNEV),
Santo Domingo, Dominican Republic
nunez500412@hotmail.com

PhD in Analytical Chemistry (chromatography), Tchekoslovaquie, 1985. Doctor in Sciences (DrSc), Havana, 2007. Member of Academies of Sciences in Cuba, Spain and Puerto Rico and the International Council of Science (ICSU). Author of 16 books or book chapters and more than 130 articles in peer review scientific journals.
Member of the Scientific Editorial Board of scientific journals in Latin America and Europe. Invited lecturer of scientific meetings in 25 countries from Latin and North America, and Europe. Biography included in 2000 Outstanding Scientists of the XX Century and Profiles of Accomplished Leaders (American Biography Institute, USA). His present field of research are

Prof. **Rosangela Marchelli**

"Update on food allergy: evaluation of new epidemiological data, threshold values and methods of analysis"

University of Parma, Italy
rosangela.marchelli@unipr.it

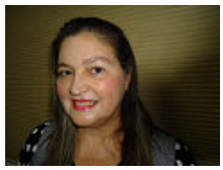
Full Professor of Organic Chemistry at the Università of Parma, Faculty of Agriculture. Member of the NDA Panel of EFSA (European Food Safety Authority). Past-President of the Interdivisional W.G. of Food Chemistry of the Italian Chemical Society. Delegate of the S.C.I. at the Division of Food Chemistry of EuCheMS (European Association for Chemical and Molecular Sciences).
Scientific interests: Molecular recognition of: a) DNA and RNA with peptide nucleic acids (PNAs) for food diagnostics (GMOs, food allergens, olive and tomato varieties, Norovirus) and biomedical applications (Cystic Fibrosis, Alzheimer Disease) and therapeutics; b) masked mycotoxins in food (identification and study of the masking mechanism), c) food allergens: methods for structural characterization and analysis; d) peptides as markers of authenticity and ageing in cheese and ham; e) enantiomers: chiral selectors for

Prof. **Fredyc Diaz Castillo**

"Phytochemical and biological studies of extracts from Colombian Caribbean plants as an alternative to the fight against dengue"

Universidad de Cartagena, Colombia
fredycdiazcastillo@yahoo.com

Full professor faculty of Pharmaceutical Science, University of Cartagena, Director of the laboratory of Phitochemical and pharmacological research.
His researches are based on the marine natural products, phytochemistry, infections diseases and



Prof. **Patricia Vit**

"Melipona favosa honey from Venezuela: A multidisciplinary characterization of pot-honeys in the world, towards its inclusion in the ecuadorian honey regulation"

Universidad Técnica de Machala,
Ecuador (Prometeo Senescyt)
Universidad de Los Andes, Venezuela
Honorary Associate,
The University of Sydney, Australia
vitolivier@gmail.com

Worked in the Food Science Departament, Faculty of Pharmacy and Bioanalysis at Universidad de Los Andes, Mérida, Venezuela since 1985. Honorary Associate at the Sydney Medical School – Medical Sciences, The University of Sydney, Australia since 2011. She was founder of a Museum of Apiculture "Ignacio Herrera" and sensory training of pot honey.
Her research interest on honey from *Apis mellifera* expanded to characterize and investigate bioactivity of tropical honey produced by Meliponini.
Joint efforts to communicate biological, medicinal and economical importance of honey processed by bees in cerumen pots instead of beeswax combs became a book of 40 chapters from all the continents: Vit P, Pedro SRM, Roubik DW, editors. 2013. Pot-honey. *A legacy of stingless bees. Springer, New York, USA. 654 pp.* Her last teaching award was received in 2014, First Class Mariano Picón Salas.
She holds a Prometeo-Senescyt scholarship from Ecuador for the project "Valorization of pot-honey produced by Meliponini of Ecuador". As a strategic National relationship, she was invited to the ongoing meetings to revise the Honey Norm INEN 1572, by the Technical Committee of the Ecuadorian Institute of Normalization.

PL1
COMPREHENSIVE TWO-DIMENSIONAL CHROMATOGRAPHY IN FOOD ANALYSIS: AN OVERVIEW

Giovanni Dugo

Introduction

In recent years, food samples have been widely analysed by using comprehensive two-dimensional chromatographic techniques (2D CC), in particular comprehensive 2D GC (GC×GC), 2D LC-GC (LC×GC) and 2D LC (LC×LC). Such approaches can be considered as innovative methods, and are gaining an excellent reputation as powerful analytical tools. The revolutionary aspect of comprehensive multidimensional (MD) techniques, with respect to classical MD chromatography, is that the entire sample is subjected to the 2D separation. The resulting unprecedented resolving power makes these approaches often an obliged choice when analysts are challenged with highly complex food mixtures.

The present lecture is focused on the description of the fundamental features of GC×GC, LC×GC and LC×LC. A variety of 2D CC food analysis experiments will be shown, highlighting the advantages of 2D techniques compared to the 1D counterparts, *viz.*, separation power, sensitivity, selectivity, speed (number of resolved compounds/unit of time) and structure (formation of 2D group-type patterns). The potential, and possible problems, of combining a third mass spectrometric dimension to a 2D CC system will also be discussed. Finally, a series of significant 3D CC-MS food applications will be illustrated.

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PL2
PROBING PLANTS CHEMICAL DIVERSITY FOR NEW LEADS

Nunziatina De Tommasi
Dipartimento di Farmacia, Università di Salerno, via Giovanni Paolo II, 84084 Fisciano, Italy
detommasi@unisa.it

Introduction

The use of plant molecules for the treatment of disease is an integral part of human culture. Plants provide an enormous number of biologically active secondary metabolites with a diversified pharmacological activity. The key idea of our research is to evaluate natural products (extracts, fractions, pure compounds) interacting with protein domain of different genetic origin, but structurally preserved, as starting points to develop libraries of compounds, biologically validated and selected from an evolutionist point of view.[1,3]. Database of natural products have a number of unused scaffolds, could represent new starting points for the discovery of new drugs[4]. The identification of target proteins and investigation of ligand-receptor interactions represents an essential step in the process of plant drug discovery and development. Recently a number of research projects are based on an approach aimed to the identification of biological activity and molecular target of extracts, and purified plant natural compounds[5]. In particular, chemical-biological and chemical proteomic studies are developed and optimized to identify proteins interacting with selected compounds[6,7]. This study allows the analysis of all potential macromolecular targets of a small bioactive molecules in a single experiment, leading to a complete and selective target mapping of a drug candidate. We have recently applied this approach to the study of cellular targets of some bioactive terpenes and polyphenols showing interesting results that will be described in this lecture[8-10]

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PL3
THE RESEARCH IN THE NATURAL PRODUCTS FIELD IN A "HOT SPOT" OF BIODIVERSITY AND CULTURE AND THE IMPACT OF THE SCIENTIFIC COLLABORATION: THE UTPL AND UNIVERSITY OF PAVIA CASE

Malagón, O. G.[1*]; Vita-Finzi, Paola[2]; Vidari, G.[2]; Armijos, C. P.[1]; Andrade, J. M.[1]; Morocho, S. V.[1]; Ramírez, J. Y.[1]; Gilardoni, G.[1,3]

[1]*Departamento de Química/ Universidad Técnica Particular de Loja, San Cayetano Alto s/n, Loja, Ecuador*
[2]*Dipartimento di Chimica, Università degli Studi di Pavia, Viale Taramelli 10, Pavia, PV, Italy*
[3]*Prometeo program, Secretaría Nacional de Educación Superior, Ciencia, Tecnología eInnovación (SENESCYT), Quito, Ecuador*
**omalagon@utpl.edu.ec*

Introduction

In 1988, Conservation International listed Ecuador as one of the "megadiverse" countries in the world (Mittermeier, 1988) due to its high biodiversity distributed in 4 natural regions: Coastal, Andean, Amazonian and the renowned Galapagos Islands. For example, Ecuador counts with more than 16,000 catalogued plant species, 4,200 endemic (Jorgensen *et. al*, 1999). Although Ecuador has a high potential for the biodiscovery of useful natural substances has been historically weak on research and development of products from its own "natural mine". Additionally, Ecuador counts with a large pool of non-renewable natural resources that are located below this source of natural wealth, and its exploitation could endanger the environment.

Southern Ecuador (Zamora Chinchipe, Loja and El Oro Provinces), has an area of 27113 km2 and it represents almost 10% of the national territory. This zone is one of the highest diversity and endemism of the country. This includes almost all national ecosystems: areas and marine-coastal islands, mangroves, dry forests, cloud forests of the Pacific, mountain rain forests, moors, forests of the Amazon forests, sandstone plateaus (Cordillera del Condor) and culture heritage semi-traditional and ancestral. Loja is part of one of the 25 hotspots of biodiversity mentioned by Myers *et. al*(2000): tropical Andes hotspot and the Amazonian region counts with high gold and copper reserves.

Method

From 1999, "Universidad Técnica Particular de Loja" (UTPL) created a research facility for developing research on natural products used in the southern region of Ecuador. In this context, in 2002 started a scientific collaboration with the Natural Organic Substances laboratory of the University of Pavia (UdP) with the challenge of training researchers, accomplishing collaborative scientific research and generating local facilities for high standard research. The collaboration has been continued and has resulted in a promissory research group with 4 Ph.D, 8 MSc., different successful projects, and publications, and at present 1 Italian researcher is collaborating *in situ* through a national program granted for SENESCYT.

Results / Discussion / Conclusion

The generation of local capacity ensures the development of research groups highly compromised with the development of its own region, where international efforts on cooperation are vital not only for the group *in se,* but for strengthening an international research group on sustainable biodiversity use. Our special gratitude and acknowledgment to Professors Paola Vita-Finzi and Giovanni Vidari for their unconditional assistance to the Chemistry Department - UTPL initiatives.

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PL4
APPLICATION OF MICROEXTRACTION TECHNIQUES FOR CHEMICAL SCREENING OF VOLATILES FROM PLANTS

Kaykhaii, M.
Associate Professor of Chemistry, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran.
Tel: (+98) 541 2446413; Fax: (+98) 541 2446888; Email: kaykhaii@chem.usb.ac.ir

Abstract

Compare with main constituents (carbohydrates, proteins and lipids), the concentrations of volatile compounds are very low in plants and to achieve trace analysis of such compounds, preconcentration techniques should be employed. Moreover, since plants have very complex matrices, a sample clean-up / extraction step is also necessary.

Microextraction techniques are non-exhaustive sample preparation methods which utilize microliter range of extracting phase to extract the analytes of interest from an aqueous phase of a few milliliters. In liquid-phase microextraction, this acceptor phase is less than 100 μL of an organic solvent while in solid-phase microextraction, it is a small volume of a solid or semi-solid (polymeric) material. Both techniques combine extraction and pre-concentration in a minimum number of steps, and thereafter, direct extract introduction into an analytical instrument. That's why in the past five years around 350 papers are published for the application of microextraction techniques (which have an overall *h*-index of 96 [1]) for the determination of volatile and semi-volatile chemicals in plants.

After a brief introduction of basic principles of microextraction techniques, this presentation will focus on applications of these techniques to plant matrices (including medicinal plants, fruits, and leaves) for the chemical screening of volatiles released from them (or their essence); such as smaller organic compounds, pesticides and secondary metabolites. Limitations of microextraction are also discussed. Finally, an outlook on the future of the techniques is given (e.g. the use of ionic liquids).

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PL5
OXIDATIVE STRESS IN CHRONIC DEGENERATIVE DISEASES

Alberto J. Núñez Sellés
National Evangelic University (UNEV), Santo Domingo, Dominican Republic

Abstract
A significant amount of data supports the hypothesis that oxidative stress is an important pathogenic factor in the development of chronic degenerative diseases and several attempts have been done to prove it. However, most of the clinical studies conducted to date have not demonstrated a clear beneficial effect of the antioxidant therapy on cancer, diabetes or neurological disorders, i.e. But it would be a mistake to reject the hypothesis on the basis of results from inadequated designed clinical interventions. The scientific evidence of the hypothesis, epidemiological and preclinical, must be re-examined in order to look for additional basic information needed for the appropiated design of clinical trials which will test the hypothesis. General and specific oxidative stress biomarkers in biological fluids must be selected in order to correlate disease progress to oxidative stress. Proper selection of therapeutical antioxidant products must consider all preventive, repairing and protective mechanisms, and not only the scavenging of free radicals excess in the organism. Determination of the Stress Oxidative Index (SOI) in diabetic patients is discussed as an example for the proper design of a clinical trial.

PL6
PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF EXTRACTS FROM COLOMBIAN CARIBBEAN PLANTS AS AN ALTERNATIVE TO THE FIGHT AGAINST DENGUE

Fredyc Díaz Castillo
Professor. University of Cartagena.
M.Sc. in Chemistry of Natural Products. Venezuelan Institute for Scientific Research, IVIC.
Ph.D. in Pharmacognosy, University of Illinois at Chicago, USA.
Director of the Laboratory of Phytochemical and Pharmacological Research of the University of Cartagena (LIFFUC)

Abstract

Since ancient times Nature has served as pantry to the humanity of essential products for life, including food, medicine and raw materials for various activities. Today the plants are still an invaluable source of compounds with medicinal properties for health care worldwide, and being in many cases, the main available resource in some health systems in developing countries. The mosquito Aedes aegypti L. is the main vector of the causative virus of dengue and yellow fever, two of the arbovirusis that more deaths produce, including all other causes of viral hemorrhagic fevers such as Ebola, Marburg, Lassa and Crimean -Congo. Furthermore, this vector is involved in the transmission of other major infections worldwide, such as chikungunya, western equine encephalitis and Rift Valley fever. Overall, the strategies for the control of arthropod-borne diseases include the use of methods aimed to control the vector and the development of vaccines and treatments aimed to the patient. Some yellow fever outbreaks continue to occur in the world, especially in Africa and in the jungle areas of South America, despite the existence of an efficient vaccine. In the case of dengue, the absence of a vaccine or specific antiviral treatment against all four serotypes of dengue virus, coupled with the great expansion in the geographical distribution of Aedes aegypti and the resistance that these mosquitoes are developing against synthetic insecticides, severely complicate the problem of dengue. It is estimated that worldwide there are about 50 to 100 million cases of dengue each year, including 250,000 to 500,000 cases of severe dengue. This indicates that massive spraying using adulticides based on organophosphates and pyrethroids of synthetic origin and the use of temephos as larvicide in domestic water bodies, is not producing the desired effect, mainly due to resistance developed by mosquitoes.

The mosquito that transmits the dengue virus has managed to evolve and fully adapt to man and his environment. Two clinical forms of the disease are presented, according to the World Health Organization; the first one is called dengue, which has no warning signs or warning signs and the second one is dengue grave, where plasma extravasation occurs, heavy bleeding and / or severe organ involvement, sometimes. This disease is caused by four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that in turn can be subdivided into different genotypes and viral strains.

Whereas Colombia is one of the tropical countries that has a large and varied number of promising plant species, many of which are located in the Colombian Caribbean region, it is imperative that our research groups profile themselves all their efforts and resources to exploration and search for natural alternatives against vectors and pathogens that cause tropical diseases such as Aedes aegypti and dengue viruses. In this lecture we summarize some of the results so far obtained by our research group, in the search for plant extracts, fractions and active molecules derived from them, as a contribution to the fight against dengue worldwide.

Keywords: Dengue, dengue virus, larvicides, temephos Bioinsecticides

Acknowledgments
To the University of Cartagena, University of Antioquia and Colciencias for financial support through the Research Projects with Codes 1115-493-26092 and 1107-519-28634. To Dr. Marlen Martinez, professors and students of the PECET Research Group of the University of Antioquia, for the bioassays on antiviral activity against dengue virus. To the professors Jorge Robles, John Diaz and Winston Quiñones, for their collaboration with the Nuclear Magnetic Resonance Spectroscopy. To my students of the LIFFUC Research Group.

PL7
UPDATE ON FOOD ALLERGY: EVALUATION OF NEW EPIDEMIOLOGICAL DATA, THRESHOLD VALUES AND METHODS OF ANALYSIS

Marchelli, R.
Prof, University of Parma, Parco Area delle Scienze 17A Parma,Italy
Member of the NDA Panel of EFSA, Chair of the WG on Food Allergy
rosangela.marchelli@unipr.it

Introduction
Food allergy is an important issue regarding the health of citizens, which involves clinical, immunological, chemical and regulatory aspects. On request of the European Commission, the Nutrition, Dietetics and Food Allergy (NDA) Panel of the European Food Safety Authority (EFSA) has produced a document on the update of the scientific knowledge concerning food allergy for labelling purposes. On the base of this document, at the moment launched for public consultation, methodology and problems encountered in establishing prevalence, threshold values as well as methods of detection of food allergens will be discussed.

Method
Literature searches and reviews related to the prevalence of food allergy in Europe have been conducted on two databases: Pub Med and Web of Science. Identification and characterisation of allergenic proteins have been carried out by proteomic (Mass Spectrometry), spectroscopic (NMR, CD) and immunological methods. Methods of detection and quantification of allergens in foods have been compared: immunological assays (ELISA), DNA analysis (PCR), and mass spectrometry. Methods to determine MOEDs (minimum observed eliciting doses) or MED (minimum eliciting doses) are provided. Methods of extrapolation to threshold probability distributions and eliciting doses (ED) are discussed.

Results / Discussion / Conclusion
Main attention is given to substances with known allergenic potential listed in Annex IIIa of Dir 2003/89/EC, as amended, as well as to emerging allergens.Prevalence of food allergy in the world is difficult to evaluate due to geographical variations, differences in genetic and environmental factors, and in food habits. However, the prevalence of food allergy in Europe has been estimated to be between 3% and 4%, both in children and adults. Most allergic reactions in children are due to egg, peanut, cows' milk, fish and nuts, in adults to fruits of the latex group of the *Rosaceae* family, vegetables of the *Apiaceae* family, and to nuts and peanuts. It is not possible to assess the allergenicity of a protein only on the base of its structural features and biological activity, but immunological and clinical data are required. The allergenic activity of a food may decrease, remain unchanged, or even increase by food processing depending on the nature of its proteins and on the conditions of the process. Immunological assays (ELISA) are mainly used for routine analyses although with limitations specially regarding processed foods, due to denaturation of the proteins, matrix effects, cross-reactions with other allergens, and variability among kits of different commercial brands. Mass spectrometry is very useful, but it is still presenting some challenges as far as quantification is concerned. DNA analysis by PCR does not detect the protein, but the allergenic food, it is robust and specific. At present it is very risky to establish safe allergen threshold levels that would not trigger adverse reactions in a sensitised individual. However, the approaches used to derive individual (MOED, MED) and population thresholds (ED) for allergenic foods will be discussed.

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PL8
Melipona favosa HONEY FROM VENEZUELA: A MULTIDISCIPLINARY CHARACTERIZATION OF POT-HONEYS IN THE WORLD, TOWARDS ITS INCLUSION IN THE ECUADORIAN HONEY REGULATION

Vit, P.

PhD/Prof Currently at [1]Centro de Investigaciones Agropecuarias (CIAP), Universidad Técnica de Machala/Av. Panamericana Km 5 ½ Via Pasaje, Machala, El Oro, Ecuador

[2]Departamento Ciencia de los Alimentos, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela

[3]Honorary Associate Biomedical Sciences, School of Medicalk Sciences, The University of Sydney, 75 East Street, Lidcombe, NSW 1825, Australia vitolivier@gmail.com

Introduction

Melipona favosa Fabricius –known as "erica" or "maba" in Venezuela, was the first stingless bee described in 1798. The state of the art of pot-honey received great benefits from interactions started during the first meetings of the International Honey Commission in Bern, organized by Stefan Bogdanov. Multidisciplinary collaborations for quality factors (Dr. Livia Persano Oddo from Rome, Italy), flavonoids (Dr. Tomás-Barberán and Federico Ferreres from Universidad de Murcia, Spain), sensory science (Dr. Rosires Deliza, EMBRAPA, Brazil), melissopalynology (Dr. Ortrud Monika Barth, Instituto FIOCRUZ, Brazil), antioxidant activity (Dr. Antonio Rodríguez-Malaver and Dr. Elizabeth Pérez from Universidad de Los Andes, Venezuela), anticancer activity (Dr. Fazlul Huq from The University of Sydney, Australia) along the years, conveyed into a comprehensive characterization of *M. favosa* pot-honey. The significance of contributions from pot-honeys studies in the world is discussed.

The *M. favosa* honey was extracted by syringe from cerumen pots in the nests. Quality factors were measured following the methods in the Venezuelan honey regulations (COVENIN 2136-84), extractive techniques, pollen analysis, spectrophotometric and sensory analysis.

The characterization of *M. favosa* pot-honey with compositional factors, pollen spectra, sensory and bioactive indicators generated a database useful for the proposal of standards for honey regulations (Vit, 2013a) of this thin sour sweet matrix with a distinctive floral nose. Classic quality factors from 40 honey samples varied as follows: ash (0.01-0.61g/100g), free acidity (12.7-97.1 meq/kg), diastase activity (2.64-3.50 DN), moisture (22.1-32.0 g/100g), nitrogen (10.5-102.0 mg/100g), HMF (5.0-24.7 mg/kg), reducing sugars (60.9-78.6 g/100g), apparent sucrose (0.5-5.1 g/100g) (Vit, 2013b). Anticancer activity of pot-honey collected in different seasons (Vit et al., 2013) was different possibly based on the flavonoid spectra (Tomás-Barberán et al., 2013). A constancy to collect pot-honey of one stingless bee along the years is advised in tropical countries to characterize the most abundant species available, for a better understanding of their similarities and differences with *Apis mellifera* honey. Pot-honeys of the world also deserve a place in the national and international regulations with systematic characterization to know their variability in composition and bioactivity. In Ecuador the target pot-honeys are produced by "catiana" *Scaptotrigona* spp., and "abeja de tierra" *Geotrigona* spp.; less frequent "abeja angel" *Tetragonisca angustula*, "cananambo" *Melipona indecisa* and "bermeja" *Melipona mimetica*. Currently, the *Melipona favosa* pot-honey model (Vit, 2013a) is used by the Technical Committee of the National Ecuadorian Institute of Norms (INEN) in the meetings to revise the Ecuadorian honey regulations INEN 1572. Preliminary data of Ecuadorian pot-honey composition is given. That may become the first honey regulation with standards for Meliponini.

Acknowledgements

To Prometeo, Senescyt, Ecuador for the grant to Patricia Vit at Universidad Técnica de Machala, Provincia El Oro. To Prof. JMF Camargo† from the Department of Biology, Faculty of Phylosophy, Sciences and Literature in Ribeirão Preto, Universidade de São Paulo, Brazil, for the entomological identification of M. favosa. To "erica" meliponicultors from Venezuela for providing pot-honey for the study. To the multidisciplinary experts who contributed with their facilities and knowledge to make possible a comprehensive approach to understand a honey made in cerumen pots by one of the most abundant bee species in the world. To Ecuadorian meliponicultors for their ancestral knowledge.

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FOCUS

Caribbean Herbal Pharmacopoeia

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FO1
CARIBBEAN HERBAL PHARMACOPOEIA

Lionel Germosén-Robineau
Université des Antilles et de la Guyane, R. Dominicana

Introduction
The 3rd edition of the Caribbean Herbal Pharmacopoeia (2014) contains 130 herbal monographs, with 393 significant uses (confirmed by >20% of families) and validated through 536 studies by the TRAMIL scientific group. These studies (90%) were performed by university laboratories in 30 participating territories of the Caribbean Basin and include the following categories: phytochemistry (49), biological activities (213), toxicology (267) and posology (7). In contrast, the 2nd edition (2005) presented 350 studies for 99 monographs which validated 315 uses.

In only 18 cases we were able to decide on a recommendation for a traditional use by means of research already published in the international scientific literature. In all other cases, we needed to conduct our own independent research.

Note that almost equal numbers of research studies already completed are not included in this 3rd edition, concerning traditional uses (of 230 other medicinal plants identified by our 11000 ethnopharmacological surveys) for which TRAMIL has not yet obtained sufficient evidence to validate. Hopefully they will be published in a 4th edition!

FO2
GUAJIRAS PLANTS: FROM MEDICINAL TO TOXIC

Rosado, J. R.[1]
[1]*M.Sc., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[1]*jairorosado@yahoo.com*

Introduction

Traditionally the tropical dry forest is used by the medicinal use of plants, however the community, especially children, of the suffer constant poisonings for its consumption. The Centers do not know for Medical Care its symptoms, treatments and contraindications. The objective of the research was to identify wild and ornamental plants of the department of La Guajira used for their medicinal properties but can they be potentially dangerous because of the content of toxic compounds affecting both humans and animals.

Method

A literature review was realized at the global, national and regional level, on plants that cause toxicity, then proceeding to their identification in the field and relating its medicinal uses, toxic principles, symptomatology and treatment. In order to evaluate its toxic impact a diagnosis was realized to departmental level in Hospitals, Health Centers, Technical Institutes Agricultural (ICA's), Colombian Corporation of Agricultural Investigation (CORPOICAs) and Units Agricultural Technical Assistance (UMATA's) Farmers and Ranchers.

Results / Discussion / Conclusion

There were identified 109 toxic species belonging to 37 families, presenting the major number the Euphorbiaceae (19), Apocynaceae (11), Poaceae and Solanaceae (8), Araceae (6), Caesalpiniaceae and Papilionaceae (5). The groups of toxic present beginning in major proportion in these species were the alkaloids (49,5 %), glycosides (36,7 %), terpenes (31,2 %), tannins (27,5 %), saponins (23,9 %), cyanogenic glycosides (18,3 %), nitrates and nitrites (14,7 %), and oxalates (13,8 %). It was concluded that La Guajira community does not identify the toxic plants and their compounds, the symptomatology that they cause and clinical treatment, making it necessary the implementation of programs or chats with the community on the toxic potential of the plants that are in their environment in order to take the pertinent measurements for prevention.

Keywords: toxic plants, toxic principles, symptoms, medicinal properties, La Guajira.

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ROUND TABLE

Recent advances in medicinal plants and biotecnhology

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RT1
TISSUE CULTURE IN SPECIES OF THE GENUS *Erythrina*

Basiglio, M. A.[1], Adema, M.[1]; Briones, M. V.[1]; Villarreal, B.[1]; Nikoloff, N.[1]; Sharry, S.[1]; Abedini, W.[1]; Valencia, T. G.[2], Garín A. M. E.[3]

[1]*Centro Experimental de Propagación Vegetativa, Facultad de Cs Agrarias y Forestales. Un. Nac. De La Plata, Buenos Aires, Argentina.*
[2]*PhD, Unidad Profesional Interdisciplinaria de Biotecnología-IPN, Acueducto S/N Col. Ticomán, México D. F.*
[3]*PhD, Facultad de Estudios Superiores Iztacala-UNAM, Av. De los Barrios 1, Tlal. Edo.México*
maribasiglio@hotmail.com, gvovaltor@yahoo.com.mx

Introduction

The genus *Erythrina* is represented by trees and shrubs with inflorescences and its seeds have red and chestnut color. In Mexico these plants receive the name of Colorín, pemoche, etc., and Seibo in Argentina. Species of the genus Erythrina have medicinal properties and have been used in different regions and ethnic groups in the world to cure various ailments, also, the alkaloids of *Erythrina* species have activity on Central Nervous System (CNS). Tissue culture in plants has been used as an alternative to obtaining secondary metabolites (Baladrin and Klocke, 1988). Therefore in this work the aim was the cultivation establishment in vitro of different species *Erythrina* as a biotechnological alternative for the development of production systems of secondary metabolites. The cultivation of plants or plant cells may have different degrees of differentiation, for example the entire plant or seeds can grow in a culture medium in aseptic conditions. Organ culture such as buds or roots can be propagated with the use of a medium supplemented with growth regulators (Petersen and Alfermann, 2008).

Method

In this work were used seeds of *Erythrina americana* and *Erythrina crista-galli* of species from Mexico and Argentina, respectively. To carry out the germination tests were conducted of scarification, for *E. americana* sulfuric acid was used and for *E. crista-galli* hydrogen peroxide, gibberellins and scraping of the tegument. The sections of nodal germinated seedlings were used to explant and were grown in Murashige and Skoog (MS) with different growth regulators: BAP 1 and 2 mg/L, ANA 0.5 mg/L, IBA 1 mg/L (for *E. crista-galli*) and 2,4 - D 2 to 8 mg/mL (for *E. americana*).

In *E. americana* once obtained the callus were inoculated into liquid MS medium and growth kinetics was obtained. On the other hand, were used as explants cotyledons and embryonic axes of immature seeds obtained from green vegetables of *E. crista-galli*, of trees of La Plata, Argentina. The cotyledons were separated from the embryo axes, were disinfected and cultured on MS, Broadleaved Tree Medium (BTM) and Woody Plant Medium (WPM) with 0.01 mg / mL of ANA and 1.5 mg / mL of BAP.

Results / Discussion / Conclusion

For E. crista-galli the optimal culture medium for shoot elongation was MS supplemented with BAP (1 mg/L) + ANA (0.5 mg/L), and the shoots obtained were subcultured with WPM medium complimented with IBA 0.1 mg/L to generate complete plants. The E. americana calluses were obtained 20 days of seeding, explants of hypocotyl and epicotyl presented more calluses formation, was established of cultivation of cells in suspension with a μ = 0.002 h-1 and t1/2 = 14.4 d. With regard to the formation of callus in E. crista-galli, 20 days after the seeding was observed callus formation of size and compact appearance and whitish located at the edges of the cotyledon, the best medium was WPM. Micropropagation of E. crista-galli will allow the production of native plants without degradation of the genetic basis of this resource, which turns out to be of vital importance for the conservation of the forest resources of the province of Buenos Aires. Obtaining callus in both species of Erythrina is an important step towards the use of these in the obtaining of secondary metabolites with interest in ethnomedicine

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RT2
BIOTECHNOLOGY APPLIED TO PLANT SECONDARY METABOLITES PRODUCTION

M. Goleniowski
CEPROCOR. Ministry of Science and Technology, Arenales 230, Córdoba, Argentina. CP X5004APP. goleniow@ceprocor.uncor.edu

Introduction

Traditional medicines are very important in primary health care, where they can be used instead of expensive western medicines. It is estimated that about 80 % of the world population depends on traditional medicinal plants for their primary health care (World Health Organization). Plants produce a broad spectrum of so called secondary metabolites and its represent an enormous value from economical point of view, used as drugs, insecticides, dyes, flavors and fragrances. In the past years different methods have been developed for obtaining these molecules, but the strategy of plant cell culture resulted to be an excellent alternative.

Plant biotechnology constitutes a potential model to elucidate the biosynthetic pathways and understand their regulation and to improve the production of many secondary metabolites of chemical and pharmaceutical interest. These techniques offer an advantage in comparison with the extraction of compounds on traditional whole plant growing in the field, inasmuch as the culture system occurs under controlled conditions and without the natural condition limitations, such as geographical location and seasonal variation factors.

This report gives a summary on the production of some secondary metabolites of *Larreadivaricata*, *Clinopodium odorum* among native species and other exotic plant as *Taxus* spp using biotechnology strategies.

The lignan nordihydroguaiaretic acid (NDGA) and its derivatives existing in *Larreadivaricata* species show a wide range of pharmacological activities which makes this genus an interesting target to consider the plant in vitro cultivation systems as a feasible alternative source for their production. These compounds are potentially useful in treating diseases related to heart condition, asthma, arteriosclerosis, viral and bacterial infections, inflammation and cancer. In order to improve the biomass formation and production of these phenolic compounds, four precursors (L-phenylalanine, cinnamic acid, ferulic acid, and sinapic acid) from the phenylpropanoid pathway were fed to *L. divaricata* cell cultures. The NDGA and phenylpropanoids production were affected by the precursors tested, presenting similar and different values to the standard medium.

The capacity of undifferentiated tissues to form phenolic compounds (NDGA and quercetin, p-coumaric acid, ferulic acid and sinapyl alcohol) was also studied. Undifferentiated tissues formed phenolic compounds in a very limited amount, but when the calli underwent organogenesis, developing mainly adventitious shoots, the phenolic compound production increased significantly. Plantlets regenerated from adventitious shoots of *L. divaricata* calli did not show the same phenolic pattern as wild plants.

To gain more insight into the mechanism by which elicitors can affect the biosynthesis of paclitaxel (Px) and related taxanes, the effect of coronatine (Cor) and methyl jasmonate (MeJA) on *Taxus media* cell cultures has been studied. Total taxane production in the cell suspension was significantly enhanced by both elicitors.

A micropropagation protocol was developed which may assist in the safeguarding and augmentation of dwindling natural populations of *Clinopodium odorum* (Griseb.) Harley. Best results for culture initiation with sustainable multiplication rates (100%) were obtained on WP (Lloyd and Mc Cown) medium without any growth regulator. WP with the addition of growth regulators promoted an optimum growth. The oils of *C. odorum* were submitted to qualitative and quantitative analysis by Gas Chromatograph and Mass Detector. A total of seventy-five components were identified in wild plants. Menthone, Pulegone and Limonene were found *in-vitro* plants as major components.

The efficiency of these bioprocesses showed that plant cell culture are a very useful model system for studying biosynthesis and mechanism of regulation of different plant secondary metabolites.

RT3
ETHNOPHARMACOLOGY OF ARGENTINIAN MEDICINAL PLANTS: EXPERIMENTAL MODELS AND RESULTS

Consolini, Alicia E.[1]
[1]*PhD/Prof, Pharmacology, School of Exact Sciences, National University of La Plata, 47 y 115, La Plata, Pcia de Buenos Aires, Argentina*
**dinamia@biol.unlp.edu.ar*

Introduction

The Ethnopharmacology has been defined as an interdisciplinary scientific study of the biologically active agents traditionally used or observed by the man (1). From the popular use given by a human community some hypothesis appear about the biological activity of the plant. But the evaluation of hypothesis needs an experimental model that resemble the medical effect. In South America there is a wide phytotherapy based on the traditional use, but only few of them have been pharmacologically validated. In our laboratory the Ethnopharmacology is applied with several models, some of them are revised here.

Results

Several plants are used as eupeptics and digestives, such as those of the genus *Aloysia* (two of them known as "cedrón" and "burrito", respectively). We have demonstrated their antispasmodic effects on isolated rat ileums, as non-competitive antagonists of muscarinic receptor and calcium (Ca^{2+}) influx, associated to activation of guanylate ciclase and potassium (K^{+}) channels (2, 3). Some medicinal plants have antispasmodic effect due to the essential oil, such as *Lippia alba* (known as "salvia") which has 4 chemotypes. The most effective as Ca^{2+} antagonist is that called "citral" due to the most abundant component (4). Another plant, *Stevia rebaudiana*, widely used as edulcorant and known as "yerba dulce", and its most active compound stevioside, have also demonstrated antispasmodic effect and inhibition of Ca^{2+} influx (5). In contrast, two plants of the genus *Mikania* (known as "guaco") exhibited a dual behavior as muscarinic agonist and non-competitive antagonist of cholinergic receptor, by which it could be a good regulator of the altered peristaltic movements, depending on the illness (6).

As antiinflammatory, we studied the effects of *Malva sylvestris* in topical application because it was the most frequently medicinal plant prescribed by the doctors in our region. We also studied the effects of an ecuatorian orchid *Catassetum macroglossum* via i.p. in the carrageenan-induced edema in rats. Both of them inhibited the edema between 30 and 90 minutes of administration of the carrageenan, almost as indometacin (7, 8).

At a cardiovascular level, we studied the effects of *Cecropia pachystachya* (known as "ambay") which grows in two regions of our country, a temperate and a subtropical region. It is widely used as antitussive, but it was suspected its cardiac toxicity. We evaluated the effect on the arterial pressure of normotensive rats, and found that the subtropical plant was more hypothensive than that from the temperate region (9), Also, it was a positive inotropic, acting as a cardiotonic (10).

Nowadays, we are evaluating extracts of plants with potential effect as cardioprotective in a model of angor, such as the "cardiac stunning" induced in isolated rat hearts by a period of no-flow ischemia followed by reperfusion (I/R). During this intervention, hearts decrease their contractility and increase the diastolic tone, in a characteristic dysfunction. We measured the contractile and energetical recovery when hearts are introduced in a flow calorimeter. Previous treatment with the compound stevioside at 0.3 mg/mL increased the contractile recovery and the muscle economy (as P/Ht, which is the ratio between pressure development in the contraction and total heat released). These results suggest that *Stevia rebaudiana* could prevent heart from dysfunction in short episodes of angor.

Conclusions

With Ethnopharmacology we have validated the tradicional use or the toxic effects of several medicinal plants from Argentina, and we could understand their mechanisms of action.

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RT4
BIOTECHNOLOGICAL TOOLS FOR SUSTAINABLE CROP MANAGEMENT AND MARKETING OF CAT'S CLAW (*Uncaria tomentosa*) IN COSTA RICA

Alvarenga-Venutolo, S.*[1], Aguilar, F.[2], Sánchez, L.[2], Rosales, C.[1], Salazar, T.[1]
[1] *(Biotechnology Research Center (CIB) of the Technological Institute of Costa Rica).* [2]*CENIBiot (National Center for Biotechnology Innovation) Research.*
**salvarenga@itcr.ac.cr*

Uncaria tomentosa (Willd.) DC, Rubiaceae (cat's claw, rangayo). It's a rainforest liana, is distributed from Peru to Belize. It is used in traditional medicine for its medicinal properties attributed to the activity of the oxindole alkaloids. Also, used as an anti-inflammatory (Obregon, 1997), immune stimulant (Keplinger et al 1999.) antitumor with cytotoxic effect on several types of breast cancer and sarcomas (García-Giménez et al.; 2010). Although U. tomentosa belongs to Costa Rica's flora diversity, there are not studies reported on phenology, domestication and management of the species in plantations or content of alkaloids in the germplasm of the country. In 2002, investigators from the CIB of the Technological Institute of Costa Rica began a research to establish in vitro cultivation, callogenesis from leaf segmentation and cell culture in suspension. The current state of the research is to obtain an aqueous standardized extract for medicinal uses, to do this the content of oxindole alkaloids (SILAE, 2013) was quantified and scaled to bioreactor cultivation. These studies were performed in order to generate biotechnological strategies to study and extract the active compounds of the plant, in order to increase production of secondary metabolites under controlled conditions.

U. tomentosa explants were collected in the province of Limón (Costa Rica). Establishment and micropropagation of in vitro material was made according to Alvarenga protocol (2010). Cell suspensions were obtained from callus induced from leaf segments of vitroplants (Sánchez and Alvarenga, 2014). Cell suspensions cultivation was optimized considering: initial cell volume, culture media, subculturing time and physical conditions of light and temperature suitable for growth (unpublished results-CENIBiot). Tests were conducted to determine the appropriate protocol for scaling bioreactor (7l), Applikon stirred tank type, dry weight was considered, substrate consumption, and bioreactor configuration (impeller type, agitation speed, ventilation (vvm), temperature and pH).

Protocols for in vitro establishment (Alvarenga, 2010), the callus formation and cultivation of suspension cells (Sánchez and Alvarenga, 2014) were obtained. Regarding optimization of culture medias at flask scale, a higher biomass production was observed by using Gamborg(1968) basal medium, in relation to M & S medium (1962), and the presence of 2,4-D and AIB, almost 60% PCV was obtained. About the Scale-7L, a maximum output of 18 g / L of dry biomass using fed-batch type strategies in open and closed circuit was reached. A correlation between the consumption of nutrients and dissolved oxygen consumption was observed, where a final conductivity value of 0.68 2 ìm/m2 appeared. This showed that sugars were not limiting subtracts and macroelement as the N, P or K (unpublished results-CENIBiot).

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RT5
ANTIOXIDANTS CONTENT AND ADVANCES IN MICROPROPAGATION OF BLUEBERRY (*Vaccinium consanguineum*) NATIVE FROM COSTA RICA

Jimenez, V.[1], Abdelnour, A.[2]
[1]*MSc. Prof Costa Rica Institute of Technology, Center for Biotechnology Research*
[2]*PhD.Prof. InstitutoTecnológico of Costa Rica, Biotechnology Research Center*
[1]*vijimenez@itcr.ac.crabdelnour@itcr.ac.cr*

Introduction

Blueberry is a shrub, part of the Ericaceae family, a Vaccinium gender.Its fruits constitute one of the most important sources of anthocyanins and carotenoids that give the fruits antioxidant properties. (Eroski, ND). Fruits and vegetables contain different compounds and antioxidant levels; determining and comparing their levels has become an area of interest.In Costa Rica there aresix species of wildblueberry(Montiel, 1991).This research was conducted to identify botanical aspects and determine the antioxidant levels of these species and develop a micropropagation protocol, in order to provide material for plantation and promote their crops and encourage diversification of agricultural activity in Costa Rica.

Methodology

Geographical distribution of these plants in Costa Rica was determined (INBio 2010). Sites were chosen to visit and samples were collected to identify botanical aspects.30 g of fruits were collected per sampled tree (five trees per site) and nutritional analysis was conducted, the method used was ORAC (total antioxidant capacity).Mini stakes were used for micropropagation, with 2 and 3 knots; they were washed with soap and water for 30 minutes, subsequently disinfected with a solution of 0.2% HgCl for 5 min.The media used was a WPM with 0,5g/l activated carbon.Aseptically set stakes were used to assess the effect of various growth regulators on bud sprouting. Zeatin (Z), BA and kinetin (K) will be use, among others, in concentrations of 0, 0.5, 1, 1.5 and 2 mg/l (Reed and Abdelnour-Esquivel, 1991; Ostroluca et al, 2007; Fira *et al.,* 2008).The shoots obtained in the previous stage will be separated from the initial explant and cultivated in the basic medium WPM with cytokinins in the concentration with better response from the preceding stage.

Results and discussion

The species was *vaccinium consanguineum*,the antioxidant content ranged from 676 545y, uM Trolox equivalent per g dry sample), higher than those shown by commercial blueberry (94 and 92 uM Trolox equivalent values/g fresh sample) (INKANATURAL 2008), and crops recognized for their antioxidant effect such as plum (73), blackberry (53), raspberry (48), apple (43) and orange (18).The only result reported as higher was the acai fruit *(Euterpe oleracea),* (INKANATURAL 2008). Disinfection was effective, cytokinins stimulated bud sprouting but not their development expected in dormant buds, for instance, evaluation of concentrations of 5, 10 and 15 mg/L of cytokinins and the effect of cold in order to stimulate the development of buds to start the process of multiplication will continue.

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RT6
CRYOPRESERVATION OF CAT'S CLAW (*Uncaria tomentosa*)

Abdelnour-Esquivel, Ana[1]*, Pérez, J.[2], Guillén, A.[2]; Araya, K.[2]
[1]*PhD, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica*
BS. Biotecnología, Instituto Tecnológico de Costa Rica, Cartago, Costa Rica.
**aaabdelnour@itcr.ac.cr*

Introduction

Uncaria tomentosa (Willd.) D.C. is known as cat's claw or uña de gato and it is native to Costa Rica. This species is attributed medicinal properties. Obregon (1995) mentioned positive effects for the treatment of a number of diseases and highly recommended to strengthen the immune and central nervous systems. As many tropical species of commercial value, U. tomentosa is threatened as a result of indiscriminate extraction from the forest and destruction of its habitat, therefore, there is an urgent need to develop strategies for conservation of this germplasm. Research have been focused on selecting phenotypes to initiate commercial plantations, the development of clonal propagation, by rooting stakes as well as using tissue culture techniques as micropropagation and the establishment of cell lines have been obtained (Alvarenga 2010; Alvarenga et al; 2008). For conservation of plant material generated in the laboratory, cryopreservation or storage at ultra-low temperature (Liquid nitrogen, LN, -196°C) is the only alternative. The major advantages of cryopreservation are the possibility to conserve not only seeds, but also embryos, meristems, shoots, callus, cell suspensions and materials produced by tissue culture, for indefinite time under genetic stability conditions. It is been utilized routinely for storage of cell lines and callus of species of pharmaceutical interest and embryogenic cultures and apex of species as a source of planting material of selected genotypes (Engelmann, 2011). This study showed the results obtained with the cryopreservation of shoots and cell suspensions of Cat's claw (*Uncaria tomentosa*).

Methodology

For cryopreservation of shoots the techniques of encapsulation-dehydration (Fabre, J.; Dereuddre, J. 1990) and vitrification (Sakai, 2000) were tested. For cryopreservation of suspension cultures a combination of preculture and cryoprotection with sucrose and DMSO were assayed (Aguilar et al., 1993).

Results and Discussion

Survival of shoot was observed with both techniques. When vitrification was used, survival rate reached up to 82% when shoots were preculture on 0.25 M sucrose during 24 h and incubated in loading solution (LS) during 20 min and in vitrification solution PVS2 during 30 min. On the other hand, when shoots were encapsulated, the highest survival rate after freezing in LN was observed when treatment consisted of preculture in increasing concentration of sucrose up to 0, 6M, dehydration between 22 and 20% water content under sterile air and rapid freezing (50% survival). When cell suspension were incubated during 24 h in 0.15M sucrose, followed by 1h in a solution of 0.15M sucrose + 5% DMSO, survival after immersion in LN reached 57.14%.

Conclusion

Cryopreservation showed to be a resourceful technique for the long term conservation of the valuable germplasm of U. tomentosa, and it is recommended for conservation of base collections.

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ORAL PRESENTATIONS

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OR1
MEDICINAL PLANTS OF HAZARNO (MALAKAND AGENCY) AND THEIR CONSERVATION STATUS

Rabia Afza[1], Akber Ali[1] and Jawad Khan[2]
[1]*Department of Botany, Hazara University Mansehra, Pakistan*
[2]*Govt. Degree collage Swabi, Pakistan*
ethnopk@gmail.com

Abstract
Pakistan is country of diverse ecological regions with a variety of flora and fauna of nearly 600 flowering species reported from Pakistan and Kashmir. Hazarnau representing the subtropical region of Pakistan is rich in natural resources. Local people use medicinal plants for curing different ailments since time immemorial. However increasing dependence of local people on herbal drugs along globalization trend slowly but surely have modified indigenous values and culture. In order to identify medicinal plants with high value, an ethnobotanical study was conducted. During the study, 57 plants of ethnobotanical importance belonging to 38 families have been recorded. The threats and conservation status of plant-resources has been identified according to IUCN red list 1996 criteria for species falling in to different categories. Unwise use, dual management, biotic pressure for fuel wood fodder and timber have been the threats that have made 3 species each as critically endangered, endangered, and vulnerable while 16 species as near threatened; the rest of 32 species are abundant.

Keywords: Medicinal plants, Hazarnau, Subtropical, deforestation and conservation

OR2
SYNTHESIS AND STUDY OF PHYSICO-CHEMICAL PROPERTY OF THE DERIVATIVES OF DITHIOL-ONE FROM DITHIOL-THIONE

Laifa E. A.[1], B. Khaled[2]
[1,2] *Département of Chemistry, Faculty of Science,Uuniversity of Constantine, Algeria*

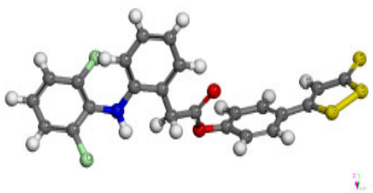
1,2- dithiole-3-ones and 1,2-D ithiole-3-thiones are an important class of oxygen and sulfur containing heterocycles with antioxidant, chemopreventive and radioprotective actvities, the biological action of which has been attributed to their redox reactions[1]. One of the main metabolites of the cancer chemopreventive agent, 4-methyl-5-(2-pyrazinyl)-3 H-1,2-dithiole-3-thione (Oltipraz)**1**, is a result of a reductive methylation process. This metabolite was synthesized also by chemical reduction of Oltipraz with sodium sulÿ'3fde followed by alkylation with methyl iodide[2].

Therefore, the reduction of 1,2-dithiole-3-thiones was intensively studied and has been shown that both biochemical[3], electrochemical[4] and reduction with sodium sulÿ'3fde affords a dianionic intermediate, which can be alkylated to give dithioester derivatives or undergoes an intramolecular cyclization as in case of Oltipraz **1** this product inhibits HIV-1(AIDS)[5]. An other group described the synthesis of 4-ÿ'3fuoro-5-polyÿ'3furoalkyl-1,2-dithiole-3-thiones (R 1=CF3, HCF 2 CF 2,R 2= F) and studied their cycloaddition properties as dienophiles and as 1,3-dipoles[6].

Also The natural product leinamycin **2**, an antitumor, antibiotic isolated from Streptomyces sp, displays potent antitumor and cytotoxic activities and an interesting activity against Gram-positive bacteria.

We therefore undertook a study in order to develop a method to synthesize, identification and study biological and pharmaceutical interest of various derivatives of 1,2-dithiolan-3-one 1-oxides.

The intramolecular cyclization of 2,2'dithiodibenzoic acid and they derivatives offered 3H-1,2-benzodithiol-3-thione **3** which, by oxidation gave 3H-1,2-benzodihiol-3-thione 1-oxide **4.** which we report in this communication.



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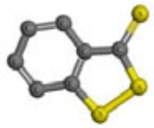

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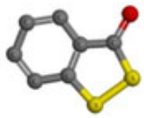

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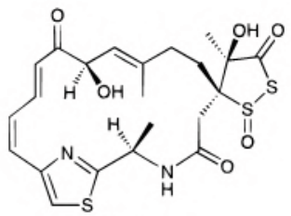

Leinamycin

4

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OR3
MAXIMIZING FARM INCOME AND OTHER LIVELIHOOD OPPORTUNITIES THROUGH INTRODUCTION OF HIGH VALUE MEDICINAL AND AROMATIC PLANTS IN DISTRICT SWAT, PAKISTAN

Dr. Hassan Sher
Center for Plant Sciences and Biodiversity, University of Swat

Executive Summary
The project is being implemented by University of Swat in collaboration with the International Food Policy Research Institute (IFPRI) under the Pakistan Strategy Support Program (PSSP), the office which is embedded in the Planning Commission of Pakistan.
This one year project (from 1st Jul 2012 to 30th Jun 2013) has evaluated opportunities to maximize farm income through introduction of high value medicinal and aromatic plants (MAPs) in the war-stricken district Swat of Pakistan, directly supporting new Framework for Economic Growth. The hypotheses is the establishment of ex-situ experimental production plots, leading to the development of skills in horticultural production and marketing among people in the valley and help rebuild commercial connections between this region and the rest of Pakistan.
District Swat host about 90% of its population depend directly or indirectly on agricultural and forestry products. This translate the importance of the present project for maximize farm income and other livelihood to uplift the socio-economic conditions of the farmers through introduction of high value MAPs and make them self sufficient to achieve the goal of poverty alleviation and to move the country towards economic stability.
This project has covered a range of interventions such as local awareness campaigns, capacity-building training, market survey, and MAPs production in the farms land of community. Local and indigenous communities have evolved traditional wisdom about the cultivation of conventional crops with very low economic return. This paper focuses on the cultivation of ten high value MAPswith farmers in four different locations/villages viz: Mingora (800meter), Khwazakhela (1000meters), Miandam(1500meters) and Behrain (2000meters).The cultivation of MAPs is totally new practice in the area and, therefore, the present IFPRIproject focus on to improve farm land production, specifically: 1) to establish agronomic techniques for cultivation and production of commercially important MAPs and to help farmers to produce them as cash crops, with compatible or higher farm income in comparison to present agriculture crops; 2) to identify commercially important MAP species whose cultivation and production techniques are known, assess the results with respect to attraction for the farmers, and to recommend agronomic techniques for production of the selected species; 3) to advise and transfer the agronomic techniques/cultivation technology of the selected species through capacity building training; and 4) to build capacity of the farmer communities regarding MAPs cultivation and pre-harvest and post-harvest treatments of economically important species. This study also focus on the economic analysis and profitability of selected MAPs production and to explore the prospects of MAPs cultivation as a potential economic venture in the region and a way of ensuring the long-term conservation of these plants in the wild.
Economic analysis/feasibility (in terms of cost comparisons/opportunity cost between cultivation of cereal/cash crops and the selected high value MAPs**)** and regular monitoring and evaluation of the adoption by farmers of improved agricultural practices were assessed after harvesting the crops. Results of the net farm income and economic analysis shows that three viz: Viola serpens, Sesamum indicum and Linum usitatissmum have higher net income than both the prevailing cash crop (Tomatoes) and cereal crops (maize and wheat) of the area. This study concluded that the farm land production of these three species were economically profitable than both the cereals and cash crops of the area. The study also found that labour, chemicals and land were over utilized for cereal and cash crops production compare to MAPs species. Economic analysis of four species Viz; Bistorta amplexicaule, Curcuma longa, Saussorea lappa, Crocus sativa and Valeriana jatamansii will be presented in Januray 2014 as these species are the long duration plants and reach to the level of commercial productions in two years.
The finding of the present MAPs productions IFPRI funded project are also analyzed through SWOT frame. Accordingly, the result was categories into: strengths, weaknesses, opportunities and threats with respect to MAPs production in the farm land of communities.
The study has also incorporate evaluation of its performance in introducing standardized production technology and appropriate post-harvest management, which represent the prime 'engines of growth' for the local economy. These strategic economic development areas are entirely based upon, and closely interlinked, with

the management and conservation practices of high value MAPs, and intact landscapes.
It was suggest that the cultivation of important and threatened MAPs is very effective means to satisfy market demand, to provide income to communities dependent on the wild natural resource, and to reduce pressure on the wild population of plants species. Moreover, the available marginal, sub-marginal and cultivable waste-land can be developed and planted with selected MAPs species. It was also recommended that the farmers should be further guided through extension visits in order to educate them regarding the adoption of new technologies related to MAPs production.

Key Words: medicinal plants production, economic analysis, people livelihood

OR4
THE XEROPHYTIC FOREST AS FOOD PANTRY OF THE WAYUU, LA GUAJIRA COLOMBIA

Rosado, J. R.[1]; Márquez, E. R.[2]; Vega, Y. M.[3]
[1]*M.Sc., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[2] *Prof., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[3]*M.Sc., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[1]*jairorosado@yahoo.com;* [2]*emarquez@uniguajira.edu.co;* [3]*yvega@uniguajira.edu.co*

Introduction

Traditionally Wayuu uses the tropical dry forest as an alternative in their diet. Despite this, the governmental entities has failed to provide the policies to generate agroindustrial enterprises for this products. These products could help improving the diet of the Wayuu people. The objective of this research was to determine what existing plant species in the dry forest are used by the Wayuu people for food, preparation methods and nutritional analyzes.

Method

Five fields were selected taking into account social and environmental criteria related to the concepts of territoriality assumed by the Wayuu ethnic group; they were: the Beach area, Rancheria River area, Carraipía area and the area traditional area of Savanna. Through pre-established guidelines and with the direct participation of the community, information on the species used in feeding and preparation methods was obtained. According to the part of the plant used in their diet, nutritional analysis was performed in a specialized laboratory.

Results / Discussion / Conclusion

There are 40 species used in their food, being Cactaceae, Capparidaceae and Mimosaceae families, the ones which are more consumed, towering species like Capparis pachaca, Stenocereus griseus, Prosopis juliflora, Malpighia punicifolia, Vitex compresa, Cassia occidentalis and Crescentia cujete. Roasting is the most common way to prepare food, also it is used to a lesser extent the squash, juices and cooking of food. Nutritional analysis showed that some species have chemical compounds with high contents of proteins, minerals and vitamins that correspond to high percentages at the daily nutritional requirement of a person. The results obtained allow to suggest elements for structuring nutritional programs and food security without altering their traditional customs.

Keywords: xerophytic forest, nutritional analysis, Wayuu people, La Guajira, plant species.

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Vega H, 2005. Guajira mi departamento. Ed. Guadalupe, Bogotá, Colombia, 113 p.

OR5
THE FOLK MEDICINAL PLANTS OF ISTANBUL (TURKEY)

Bulut, G.; Tuzlacı, E.; Doğan, A.; Şenkardeş, İ.
PhD, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
Prof. Dr., Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
PhD, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
PhD student, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
**gizem.bulut@marmara.com.tr*

Introduction

This study was made to represent to the plants used as traditional folk medicine in various localities of Istanbul based on our five ethnobotanical investigations (Bulut, 2011, Doğan and Tuzlacı, 2012).

Method

The specimens of the plants used as folk remedies have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration, and the duration of treatment has been recorded. The information was obtained from participants who were not only experienced adults but also patients in face to face interviews; furthermore, the specimens of the plants were collected. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University.

Results

As a result total 144 species, including also literature records in another ethnobotanical study, have been found. Among them, 118 species are wild or naturalized and 26 species are cultivated plants. According to the majority of the informants, the plants are mostly used for cold, gastrointestinal disorders, wound, diabetes, hemorrhoids and rheumatism.

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OR6
ANTIMALARIAL PLANTS USED BY INDIGENOUS PEOPLE OF THE ALTO RIO NEGRO REGION – BRAZILIAN AMAZON

Kffuri, C. W.[1]*; Ming, L. C.[1]; Kinnup, V. F.[2]; Chaves, F. C. M.[3]; Hidalgo, A. F.[4];
[1]PhD, Universidade Estadual Paulista/ Rua José Barbosa de Barros, n° 1780, Botucatu, SP, Brazil.
[3]PhD, IFAM, Campus Manaus-Zona Leste. Avenida Cosme Ferreira, 8.045, Herbário EAFM - Manaus, AM – Brazil.
[4]PhD/Emprabra, Amazônia Ocidental.Km 29 - AM 010-Zona Rural, Manaus, AM – Brazil.
[2]PhD, Universidade Federal do Amazonas/Av. General Rodrigo O.J. Ramos, 3000 Manaus, AM – Brazil.
**carolkffuri@gmail.com*

Introduction

More than 3.3 million people worldwide are exposed to malaria. The drugs used in combating the disease already show signs of resistance. In Brazil, 99% of malaria cases occur in Legal Amazonia. It is an endemic disease in the Upper Negro River region. This region considered to be a sui generis cultural region, as, in this area, more than 90% of the inhabitants are indigenous, 23 languages are spoken, and more than 22 ethnic groups exist. The forest is preserved and unknown to science. This is the first ethnobotanical research that has been conducted on antimalarial plants in the region.

Method

The fieldwork was carried out between September 2011 and July 2012 and between September and November 2013, in 5 indigenous communities. Semi-structured interviews were conducted with 89 indigenous people to record the plants used to treat malaria, the vegetal part used in the treatment, the method of preparation and the dosage. All interviews were recorded and filmed. The plants were collected, identified and deposited in the EAFM herbarium in Manaus. An extensive literature review was performed on data supplied by academic papers published in scientific journals until January 2014, with the goal of finding data on ethnopharmacological, ethnobotanical, phytochemical and/or antimalarial activity for the species cited as antimalarial in this research.

Results / Discussion / Conclusion

46 species used to treat malaria were recorded. These belong to 24 botanical families and are mostly native to the Amazonian phytogeographical area. Among the species collected, three families are distinguished by species richness, respectively: Fabaceae (17.39%), Arecaceae (13.04%) and Euphorbiaceae (6.52%). In the communities included in the study, there was only a consensus of opinion on the use of seven species. All except two of these species are widely used in the Amazon region for the treatment of malaria. The bark (33%) and root (29%) are the most used plant parts. Most of the preparations are made by decoction (65%); The most widely used form of administration is drinking tea (decoction and infusion), followed by taking a full-length bath. Most preparations are used in different doses (from a half-cup to three cups), but they are always administered three times per day (morning, noon and afternoon). Of the 46 species: 14 species have been the subject of studies that prove their antimalarial activity in the laboratory; 4 species have records of traditional use, but little or no research has been done on their antimalarial activity in the laboratory; 17 species have not been studied, but suggest activity based on their chemical compounds or tests of the same genre as antimalarial activity that have been positive; 8 species were not found in any studies of antimalarial activity in the literature; and 2 species showed inactivity in antimalarial tests. This work demonstrates the use of local flora by indigenous people in treating malaria and highlights the importance of cultural and environmental preservation in the search for and development of new medicines.

Acknowledgment: CNPq, CsF, indigenous communities which such knowledge belongs.

OR7
ISOLATION OF NOVEL HYDROQUINOLINIC ALKALOIDS FROM ECUADORIAN PLANTS OF THE GENUS *Huperzia*, EMPLOYED IN THE TRADITIONAL MEDICINE OF SARAGURO PEOPLE

Gilardoni, G.[1]*; Armijos Riofrio, C. P.[2]; Malagòn Avilés, O. G.[2]; Lozano Sarango, M. A.[3]; Fronza G.[4], Vidari, G.[5]; Vita Finzi, P. V.[5]

[1]*PhD Prometeo, Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
[2]*PhD, Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
[3]*Comunidad de Saraguro, Barrio Illincho, Saraguro, Ecuador*
[4]*Senior Associate of CNR, Institute of Chemistry for Molecular Identification at Politecnico di Milano, Via Mancinelli 7, Milano, Italy*
[5]*Professor of Organic Chemistry, Università degli Studi di Pavia and C.I.St.R.E., Dep. of Chemistry, Via Taramelli 10, Pavia, Italy*
**gianluca.gilardoni@gmail.com*

Introduction

The plants of the genus *Huperzia*, belonging to the family Lycopodiaceae, are well known as sources of biologically active alkaloids. More than 700 species and variety of *Huperzia* and more than 3000 genera, species and variety of Lycopodiaceae are recorded, from which more then 90 different alkaloids have been until now identified, with a molecular weight ranging from about 200 to about 500 amu. Many of the alkaloids show interesting biological activities, e.g. Huperzine A from *Huperzia* serrata is a promising lead for Alzheimer's disease drugs.

In this investigation we are studying some plants of the genus *Huperzia*, never studied before from the chemical viewpoint, collected in the region of Loja, South Ecuador, near the Saraguro town, at an altitude of about 3000 m MSL. These plants are employed by the Saraguro people as purgative, in mixture with other species to obtain psychotropic products with ritual purposes and in the treatment of supernatural diseases.

A preliminary study on the "light" fraction of alkaloids, extracted for the first time from some of these species was yet presented at this congress in 2012; now we are interested in a class of much heavier novel alkaloids, whose presence we confirmed in *Huperzia* crassa (Humb. & Bonpl. ex Willd.) Rothm. and *Huperzia* espinosana B. Øllg.

Method

The dried and grinded plant is extracted repeatedly by hydro-alcoholic maceration, until negative reaction to Dragendorff reagent. A solid extract, very rich in alkaloids, is than obtained by extraction of the macerate in a classical acid-base alkaloid extraction process.

These alkaloids are very difficult to purify and they can be obtained in a quite pure form through preparative liquid chromatography on SiO2, eluting with mixtures of THF/iPrOH/NH3(aq).

NMR spectroscopy, ESI-MS spectrometry and FT-IR spectrophotometry have been applied to the structural characterization of the samples.

Results / Discussion / Conclusion

H. crassa contains not less than two new "heavy" alkaloids (m.w. of about 600 amu). They are completely aliphatic and show a sequence of four hydroquinolinic and piperidinic nuclei. The exact structure can't be established only by spectroscopic methods and X-ray diffraction is needed. Analogous compounds are present in H. espinosana. Crystal derivatives of the alkaloids are in study and biological essays will also be performed.

This study is part of the PROMETEO PROJECT and is conducted with the financial support and patronage of the Secretaría de Educación Superior, Ciencia, Tecnología e Innovaciòn of Ecuador (http://prometeo.educacionsuperior.gob.ec).

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OR8
COMPARATIVE STUDY OF THE CHEMICAL COMPOSITION AND *IN VIVO* HYPOGLYCEMIC ACTIVITY OF TWO *Ocimum* SPECIES (LAMIACEAE)

Casanova, L. M.[1]*; Espíndola Neto, J. M.[2]; Da Silva, D.[2]; Sola-Penna, M.[2]; Costa, S. S.[1];
[1]*Núcleo de Pesquisa de Produtos Naturais, Universidade Federal do Rio de Janeiro, 21 941-902 Rio de Janeiro, RJ, Brazil*
[2]*Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, 21 941-902 Rio de Janeiro, RJ, Brazil*
**liviacasanova@ig.com.br*

Introduction

The genus *Ocimum* (Lamiaceae) comprises species ethnopharmacologically used to treat diabetes mellitus in Africa and Asia.[1,2] Some of them, such as *Ocimum basilicum* L. and *Ocimum gratissimum* L, have had their hypoglycemic activity confirmed by in vivo studies.[1] Recently, our group conducted a chemical investigation of a leaf decoction of *O. gratissimum* monitored by in vivo hypoglycemic activity assays, which allowed the identification of chicoric acid as a phenolic substance that contributes to its glucose lowering effect.[3] This phenolic can also be found in *O. basilicum*.[4] The aim of this study was to compare the chemical composition and hypoglycemic activity of leaf decoctions of O. *basilicum* (OB) and O. *gratissimum* (OG).

Method

Leafs from OB and OG collected at the flowering stage were triturated and extracted with boiling water for 10 minutes. The decoctions thus obtained had their chemical profile assessed by NMR spectroscopy (1D and 2D spectra) and HPLC-DAD. The acute hypoglycemic activities of both decoctions were evaluated in streptozotocin-induced diabetic mice.

Results and Discussion / Conclusion

^{1}H NMR spectra of OB and OG decoctions showed signals in three regions: ä 0.8- 3.0 ppm (aliphatic compounds and amino acids) ä 3.0-5.5 ppm corresponding to carbohydrates (signals most abundant and overlapped), and ä 6.0-8.0 ppm, which exhibited minor signals of phenolic substances. Signals compatible with organic acids could also be detected. 1D and 2D-NMR-based analyses allowed the tentative identification of 17 substances in OB and 12 substances in OG. The major metabolites in OB are malic and tartaric acids as well as asparagine. Tartaric, lactic and succinic acids predominate in OG.

HPLC-DAD analyses allowed the identification and quantification of four cinnamic acid derivatives in OB and OG decoctions: caftaric, chicoric, caffeic and rosmarinic acids. Chicoric acid is the major phenolic in both species, although its content is approximately three times higher in OG than in OB. On the other hand, OB has a higher content of caffeic and rosmarinic acids.

When administered to diabetic mice (n=5) by i.p. route at the dose of 200 mg/kg, both decoctions significantly reduced their glycemic levels ($p<0.05$, Student's t-test). OB and OG were able to reduce glycemia in 75% and 60%, respectively, 180 minutes after treatment. At this time, the glycemic levels of diabetic treated mice were not statistically different from normal untreated mice. Interestingly, OB, which has a lower content of chicoric acid is as active as OG, which clearly shows that other substances contribute to the hypoglycemic activity. These preliminary results corroborate the potential of both species to treat diabetes.

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OR9
PHARMACOKINETICS PROFILE OF 6-METHYLCOUMARIN IN WISTAR RATS AFTER ORAL ADMINISTRATION

Cárdenas P. A.[1]*, Baena Y.[1],Ospina L. F.[1,2], Aragón D. M.[1]*
[1]*Grupo Sistemas de Liberación Modificada de Moléculas Biológicamente Activas.* [2] *Grupo de Principios Bioactivos de Origen Natural. Departamento de Farmacia. Universidad Nacional de Colombia, Bogotá, Colombia.*
**Email contact: pacardenasc@unal.edu.co; dmaragonn@unal.edu.co*

Introduction

Some simple and complex coumarins have shown different biological activities, such as antibacterial, effects on the cardiovascular system, effects on the central nervous system, antioxidant activities,cytotoxicity, and anti-inflammatory properties (1-8). 6-methylcoumarin (6MC), a simple coumarin, previously has shown an important anti-inflammatory activity in *in vivo* and *in vitro* models(9-10). Although this drug has a remarkable pharmacological effect, it is necessary to evaluate other properties such as intestinal permeability, concentration-time profiles and biodistribution and metabolism, in order to identify drugs with optimal biopharmaceutical profile. The knowledge of the pharmacokinetics profiles, allows identify compounds with a good bioavailability and a desirable duration action. (11). Thus, the aim of this work was to evaluate 6MC pharmacokinetics after oral administration in Wistar rats.

Methodology

Male Wistar rats (12 weeks, 250± 10 g) were administrated oral administration (v.o) with 6MC at 200mg/Kg, suspended in saline solution (NaCl 0.9%) and 1% of Tween 80. The blood samples were collected at 2,6,10,15,20,25,30,45,60, and 120 minutes after administration. After each sampling, the blood was homogenized with trichloroacetic acid (20%) and was centrifuged at 13000rpm for 10 minutes and the supernatant was recovered for 6MC quantification by a validated HPLC-DAD method.
The peak plasma concentrations (Cmax) and the time for their occurrence (Tmax) were determined from the mean concentration in plasma (expressed as ìg/ml). The elimination rate constant (ke) and absorption constant were estimated by regression analysis from the slope of the line the log plasma concentration versus time curves, and the half-life (t1/2) was obtained by 0.693/ke. The area under the plasma concentration versus time curves from 0 to 2 h (AUC0ÿ'3f120) was calculated by the linear trapezoidal method. The kinetic parameters data were calculated using five individual plasma samples/time point.

Results, discussion and conclusion

The results show the pharmacokinetics parameters founded after oral administration of 200 mg/kg of 6MC. Finding a maximum concentration of 10,25 (μg/ml), and the maximum time is 39,3 minuts. The area under curve (AUC 0-120) finding was of 556,6 (μg/ml*min). For 6MC was found that its absorption and elimination is quickly Kabs = 0,041 min-1 and Kelim = 0,015 min-1. On the other hand, its half -life for 6MC (49,4 minuts) is smaller than Coumarin which it is about 1 to 2 hours in human and between 1 and 4 hours in other species as rat. The low 6MC absorbed fraction showed 0,28 %, (relation of AUC0-120 and the total administrated dose) could be explained for a low bioavailability of this drug. This behavior is agree with data reported for Coumarin, since this compound is completely absorbed from the gastrointestinal tract after oral administration but is extensively metabolized during the first pass through for the liver and only 2 to 6% of the intact compound reaches systemic circulation (12). These results suggested that 6MC is highly metabolized, which is confirmed by HPLC spectra since other peaks are found in samples of plasma after oral administration. These metabolites are studied for the identification.

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OR10
ETHNOBOTANY WAYUU: USES OF PLANTS PRESENT ON THE BEACH SAND AND DUNE, DEPARTMENT OF LA GUAJIRA, COLOMBIA

Moreno, M. I.[1]*; Rosado, J. R.[2];
[1]*Prof., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[2]*M.Sc., University of La Guajira, km 5 pathway to Maicao, Riohacha, La Guajira, Colombia*
[1]**mmoreno@uniguajira.edu.co; [2]jrosado@uniguajira.edu.co*

Introduction

In the beach sand and dunes there are a large number of plant species used by the Wayuu community in the cure and prevention of their diseases related with their traditional customs they are necessary to preserve. The objective of this research was to identify the plants settled on the beach sand and dunes, their use, preparations and plant parts used by the Wayuu ethnic.

Method

20 quadrants of 1000 m2 (50 m x 20 m) along the coast, ranging from Camarones to Cabo de La Vela (La Guajira), were established where significant Wayuu communities exist. Plants were identified and discussions were held with communities of different areas in order to determine their use, preparation, plant parts used and dose. A review of the existing literature on the active principles of plants was done.

Results / Discussion / Conclusion

37 species associated with 14 families were reported, of which 17 are used in the treatment and prevention of disease, 6 have medical and alimentary use, 8 the use as food and 6 report no use by the community. 23 species are related in the cure of 70 diseases, enhancing for its frequency: stomach pain, skin diseases, wound healing, organ inflammation, diarrhea and fevers. Families with the highest number of medicinal species are Euphorbiaceae, Caesalpiniaceae, Asclepiadaceae and Malvaceae. The most common forms of preparation are: cooking (56,5%), direct application (56,5%), infusion (37,7%) and beverages (30,4%), most often using the leaves (53,0%), roots (40,9%), whole plant (30,4%), fruits (34,7%) and cortex (17,4%). The active principles most reported in the species are: alkaloids, glycosides, flavonoids, tannins, essential oils, acids and polyphenols. Established communities along the coast have traditional values ÿ'3fÿ'3fregarding the use of medicinal plants. Therefore it is important to rescue the cultural heritage that has identified the Wayuu people.

Key words: Wayuu people, beach dune and sand, medicinal plants, active principles.

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OR11
ANTIDIARRHOEAL ACTIVITY OF UMBELLIFERONE (7-HYDROXYCOUMARIN)

Passos, T. F.[1]; Rodrigues, F. F.[1]; Brito, C. R.; Andrade, J. T.[1]; Araújo, M. G. F.[1]*
[1]*Universidade Federal de São João Del-Rei, Rua Sebastião Gonçalves Coelho, 400, Chanadour, Divinópolis, MG, Brazil.*
**mgfaraujo@ufsj.edu.br*

Introduction

Plant derived phenolic coumarins might play an important role in dietary due their consumption in the human diet in fruits and vegetables. Umbelliferone (Umb), a derivative of coumarin (7-hydroxycoumarin), is a benzo-pyrone in nature and it is present in the edible fruits like apple and orange. Some biological properties of Umb were described like antioxidant, antidiabetic, antihyperlipidemic (Kumar et al., 2013), anti-inflamatory (Islan et al., 2012), antimicrobial, among others. The aim of this study was to evaluate the effect of Umb on intestinal transit and exploit their antidiarrhoeal activity in castor oil induced diarrhoeal model.

Method

Swiss albino male mice (30-35 g) were used. The animals were randomly allocated to six groups of five animals each group: (1) Control non-treated group, received water; (2) Vehicle control group, received DMSO (0,2 mL orally); (3) Positive control group, received loperamide at the dose of 3 mg/kg orally (0,2 mL); and groupe 4, 5 and 6 groups, received the Umb at the doses of 50, 100 and 200 mg/kg (0,2 mL orally). Each animal was placed in an individual cage and the floor of which cage was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mice, 30 min after the above treatments. During an observation period of 4 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded, and the total faeces by group were weighted (Adeyemi et al., 2009). The effect of the treatments on normal intestinal transit was carried out as outlined above, without castor oil, and thirty minutes after, charcoal meal (0.2 ml orally) was administered to each animal. The animals were killed 30 min later and the small intestine was immediately isolated. The Peristaltic Index (PI), which is the distance traveled by the charcoal meal relative to the total length of small intestine (expressed in %), was determined for each mice (Sabai et al., 2014).The study protocol was approved by the Ethics Committee for Animal Care of the Federal University of Sao Joao Del-Rei (process 009/2014).

Results / Discussion / Conclusion

In the course of observation for 4 h after castor oil administration, all the mice in the control group (water) produced copious diarrhoea. Pretreatment of mice with 50, 100 and 200 mg/kg of Umb caused a significant decrease the frequency of purging (reduction of number of wet stools and total no of stools), and severity of diarrhoea. In positive control animals, the charcoal meal traversed 27,5% ± 13,4 % of the total length of the small intestine. The different doses of Umb produced significant ($P < 0.05$) reduction in normal intestinal transit when compared with negative control and show no significant differences when compared of positive control. The results obtained in this study suggest that the Umb possesses antidiarrhoeal activity due to inhibition of gastrointestinal propulsion and reduction of faeces elimination.

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OR12
ANTIBACTERIAL ACTIVITY ASSESSMENT OF *Bauhinia forficata* LINK (FABACEAE)

Gasparetto, C. M.[1,2]; Borges, M. L.[2]; Fernandes, L. S.[2]; Temponi, V. S.[2]; Rodrigues, K. C. M.[1]; Del-Vechio-Vieira, G.[2]; Mendonça, L. M.[3]; Sousa, O. V.[1,2]; Alves, M. S.[1,2*]
[1]Graduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, Brazil
[2]Faculty of Pharmacy, Federal University of Juiz de Fora, Juiz de Fora, Brazil
[3]Faculty of Pharmacy, Federal University of Juiz de Fora, Campus Avançado de Governador Valadares, Governador Valadares, Brazil
*Corresponding author E-mail: silvana.alves@ufjf.edu.br

Introduction

The genus *Bauhinia* (Fabaceae) contains about 300 species distributed in tropical areas with globally medicinal interest. *Bauhinia forficata* Link, known as "pata-de-vaca" in Brazil, is a native South America tree traditionally used as hypoglycemic, diuretic, hypocholesterolemic, and against cystitis, intestinal parasites and elephantiasis.

Method

The present study aimed to assess the antibacterial activity of ethanol extract (EE) and hexane (HF), dichloromethane (DF), ethyl acetate (AF) and butanol (BF) fractions obtained from *B. forficata* leaves. Minimal Inhibitory Concentration 100% (MIC_{100}), using microdilution method according to the Clinical Laboratory Standards Institute (CLSI) guidance, estimated the antibacterial activity. *Escherichia coli* ATCC 10536, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311, *Salmonella enterica* subs. *enterica* serovar Choleraesuis ATCC 10708, *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* ATCC 9027 were used as reference bacterial strains.

Results / Discussion / Conclusion

AF was active against *S. aureus* ATCC 25923 and *S. aureus*ATCC 6538, with MIC_{100} values of 2250 and 5000 µg/mL, respectively. The other fractions and EE had MIC_{100} values greater than 5000 µg/mL. Alves (2013) and Rocha (2012) also evaluated the antimicrobial activity of *B. forficata* against some species of fungi and bacteria. The present results agree with these two previous studies, providing additional scientific information about the antibacterial activity of *B. forficata*, and corroborating with the antimicrobial potential of the species of *Bauhinia* genus. The data indicates that *B. forficata* is a source of bioactive substances with antibacterial property and may be a promising alternative to therapeutic applications.

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OR13
TERPENOID FROM CHILEAN RHAMNACEAE AND BIOLOGICAL ACTIVITY

Alarcón, J.[1]*; Quiroz, S.[2]; Gonzalez, R.[2]; Ferrada, D.[2]; Cespedes, C. L.[1]
[1]PhD, Universidad del Bío-Bío, Avenida A. Bello s/n, Chillán, Chile
[2]M.Sc, Universidad del Bío-Bio, Avenida A. Bello s/n, Chillán, Chile
*jualarcon@ubiobio.cl

Introduction
The Rhamnaceae is a cosmopolitan family, in Chile this family is represented by 16 species distributed in seven genera. Some species are used in folk medicine. Our goal was correlate phytochemical composition and biological activity.

Method
Air-dried aerial portion of Talguenea quinquenervia, Trevoa trinervis, Colletia spinosissima, and C. spinosa were exhaustively extracted with MeOH in Soxhlet apparatus for 12 h. The resulting MeOH extract was filtered and concentrated under vacuum. The total extract was divided in two parts. One part was solvent partitioned using n-hexane, EtOAc, and water. The other part was used for alkaloid extraction. By successive chromatography and spectroscopic method were possible isolated, and identify several pentacyclic triterpenoid. Extracts, fractions and compounds were tested for their biological activity and inhibiting acetylcholinesterase and tyrosinase enzyme.

Results / Discussion / Conclusion
The methanol extract of aerial parts of the plants material were fractionated as described in the method. Pentacyclic triterpenes have been isolated by chromatography on silica columns and repeated preparative TLC. The compounds isolated: lupane, oleanane, ursane, and ceanothane skeleton type (fig.1). The insecticidal activity of the different fractions, and compounds obtained was evaluated using bioassay for insecticidal activity against larvae of D. melanogaster and Cydia pomonella. These results were correlated with enzymatic inhibitory activity showed by the compounds.

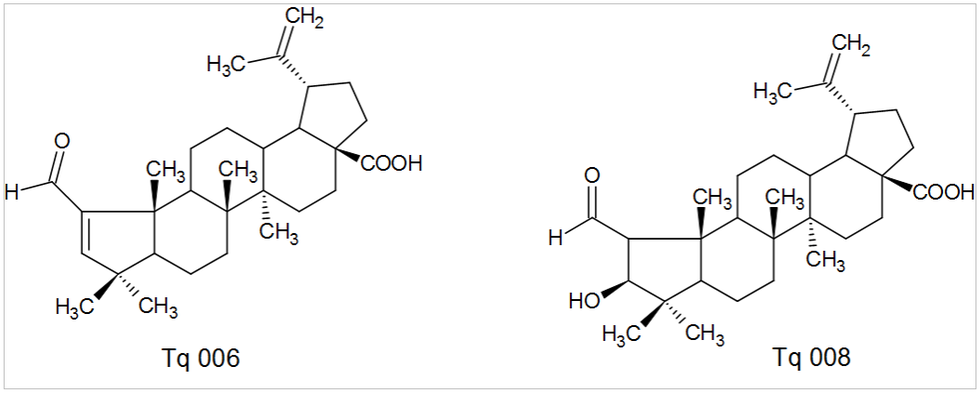

Fig.1

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OR14
ANALYSIS OF THE NEUROPROTECTIVE ACTIVITY OF ALCOHOLIC EXTRACTS OF CAQUETÁ FOOTHILLS AND NATURAL RESERVE UCUMARÍ-RISARALDA COLOMBIA

Martínez-Cotacio, C. A.[1]*, Mosquera-Martínez, O. M.[2], Trujillo-Calderón, W.[3], Arboleda-Bustos, G. H.[1+]

[1](c)Msc Neurosciences. National University of Colombia Bogotá D.C-Colombia.*
[1+]PhD. Professor Genetics Institute, building 426. National University of Colombia. Bogotá D.C-Colombia.
[2]Professor Biotechnology-Natural Products Technological University of Pereira (Risaralda-Colombia)
[3]Professor, Department of Biology. Amazon University Florencia (Caquetá- Colombia)
**ceamartinezco@unal.edu.co*

Introduction

Two cell lines, CAD and MO3.13, are being used to analyze the neuroprotective activity of four plant species of the Piper genus from the foothills of Caquetá department, to which secondary metabolites have been identified, as with two other plants of the Euphorbiaceae family extracted from Ucumarí-Risaralda Natural Reserve, that in previous studies have shown a high antioxidant activity.

Method

Plant Material

Plants were obtained from the departments of Caquetá and Risaralda, identified by a specialist and then botanized. Plants were dried, crushed and compounds were extracted with ethanol (Piper) and methanol (Euphorbiaceae).

Cell Material

Two brain-derived cell lines; CAD and MO3.13 cultured at 37C ° in 5% CO2 in DMEM F-12 medium (DMEM-MO3.13) supplemented with10% FBS. Differentiated in 96 well plates during 48 hours (7000 cells / CAD well) and 96 hours (50000 cells / MO3.13well)

Neuroprotection Assay

The extracts are applied pretreatment during one hour in differentiated cell lines adding C2-ceramide as neurotoxic agent, for a period of 6 and 24 hours.

C2-Ceramide dose of 25 and 12 μM at 6 and 24 hours respectively in CADÿ'3fÿ'3f, 50 and 20 μM at 6 and 24 hours respectively in MO3.13. MTT and LDH Essay used to analyze the results.

Results / Discussion / Conclusion

Four extracts on CAD cell line have been analyzed, two of which have demonstrated neuroprotective effect at 6 hours of treatment. In the MO3.13 cell line all the extracts have been analyzed and two have demonstrated significant neuroprotective activity at 6 hours of treatment. This activity may be attributed to the phenolic compounds such as flavonoids and amides present in the alcoholic extracts.

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OR15
MEDICINAL AND EDIBLE PLANTS; PHYTO-THERAPEUTIC USES BY BALTI COMMUNITY OF THE KARAKORAM RANGE OF MOUNTAINS

Zaheer Abbas[1], Shujaul Mulk Khan[1], Zahid Ullah[2], Muhammad Iqbal[1]
[1]*Department of Botany, Hazara University Mansehra, Pakistan*
[2]*Department of Plant Sciences, Quaid-i-Azam University Islamabad, Pakistan*
shuja60@gmail.com

Abstract
The Karakorum Range of mountains is one of the diverse habitats in the world. This study aimed to explore how the native people rely on indigenous plant resources to meet their needs in the form of medicinal plants, wild vegetables, wild fruits and other day today uses. Ethnobotanical data were collected in the study area for seven months in the summer of 2011. The trips were comprised of five phases viz. conduction of interviews, listing ethnobotanical species, specimens collection, group discussion and identification of plants species. Interviews were taken after seeking the consent from each respondent following show-and-tell/semi structured method. Interviews were chosen without distinction of gender after seeking the consent at each household. People from all age groups were interviewed on their knowledge about the uses of plants in this region. Families' data were also analyzed statistically for ANOVA and Residual Values using SPSS. Balti people consume 63 plant species in the region to fulfill their daily uses. Based upon their nature of usage these species were classified in different classes i.e. ethno medicinal, wild edible and cultural plants. A total of 26 species were used to treat 11 different human ailments. Findings of this paper advocate the need to ensure the conservation of therapeutic knowledge and phyto-diversity.

Keywords: Indigenous knowledge, Medicinal plants, Tormik Valley, Karakorum

OR16
INCREASE IN ENZYMATIC ANTIOXIDANT ACTIVITIES AS A RESPONSE TO LONG TERM WATER ARSENIC EXPOSURE BY PHYTOREMEDIATORS *Zantedeschia aethiopica* AND *Anemopsis californica*

del Toro-Sanchez, C. L.[1]; Zurita, F.[1]; Vaìzquez-Armenta, G.[2]; Gonzalez-Leal, N.[3]; Siller-Lopez, F.[4*]

[1] *PhD, Centro Universitario de la Ciénega, Universidad de Guadalajara, Av. Universidad 1115, Ocotlán, Jalisco, Mexico*
[2] *MD, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada 950, Guadalajara, Jalisco, Mexico;*
[3] *MD, Facultad de Medicina. Fundación Universitaria Autónoma de las Américas. Av. Sur 98-56. Pereira, Risaralda, Colombia.*
[4] *PhD, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada 950, Guadalajara, Jalisco, Mexico;*
Facultad de Medicina. Fundación Universitaria Autónoma de las Américas. Av. Sur 98-56. Pereira, Risaralda, Colombia.
**fsiller@cucs.udg.mx*

Introduction

In México, arsenic (As) contamination of groundwater is of increasing concern as 75 percent of drinking water comes from groundwater sources. Construction of artificial wetlands is being considered as a low-cost option for As remotion from drinking water. *Zantedeschia aethiopica* (calla lily) and Anemopsis californica (yerba mansa) are plant species commonly found in the occidental region of México capable of accumulating As and therefore proposed as phytoremediation for removal of As from drinking water. Plants have developed a number of mechanisms to control the homeostasis of essential elements and to cope with the oxidative stress induced by toxic elements. We aimed to assess the role of the antioxidant systems in leaves and stems of Z. aethiopica and *A. californica* in the ability to resist the stress induced by As.

Methods

The effects of a continuous six month As exposure (34 ± 11 µg/L) from local contaminated groundwater on the antioxidant response of *Z. aethiopica* and *A. californica* were evaluated in leaves and stems of the plants bimonthly in a subsurface flow constructed wetland. Oxidative stress and nonenzymatic activity were evaluated by analysis of thiobarbituric acid reactive substances (TBARS), total phenols and total antioxidant capacity determined with the oxygen radical absorptive capacity (ORAC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Activities of ascorbate peroxidase (APX), glutathione reductase (GSR) and catalase (CAT) were evaluated as part of the key antioxidant enzymatic plant response.

Results

No visible symptoms of inhibition of plant growth or death tissue sections were observed by As exposure on both plant species. As increased the activities of the antioxidant enzymes APX, GSR and CAT where higher levels were observed in *Z. aethiopica* than *A. californica*. No significant differences were detected on lipid peroxidation TBARS levels or antioxidant capacity evaluated by ORAC and DPPH assays or total phenol contents in any part of the plant, although in general the leaves of both plants showed the best antioxidant defense against the metal.

Discussion and Conclusion

The enzymatic and nonenzymatic antioxidant response mounted by plants in As detoxification is dependent on the concentration and exposure duration to the toxic. *Z. aethiopica* and *A. californica* were able to cope to As through induction of a more sensitive enzymatic antioxidant response mechanism than by a nonenzymatic antioxidant system. In the present study, the increased activities of the enzymatic antioxidants might be a consequence of a fine sensing redox mechanism of signal transduction that up-regulate the enzymes involved in antioxidant defense even when no effect on the oxidant status and antioxidant capacity were observed after As exposure.

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OR17
THE ROLE OF VOLATILE ORGANIC COMPOUNDS AND THEIR GLYCOSIDIC PRECURSORS ON THE CURUBA (*Passiflora mollissima* KUNTH L. H. BAILEY) AROMA

Conde-Martinez, N.; Sinuco, D.; Osorio, C.*
Departamento de Química, Universidad Nacional de Colombia-Sede Bogotá, AA 14490, Bogotá, Colombia
**cosorior@unal.edu.co*

Introduction

The genus *Passifloraceae* is mostly native of tropical America. Among these fruits, *Passiflora mollissima* (Kunth) L. H. Bailey, commonly known as "curuba de Castilla" or "banana passion fruit", is a native species of the southern Andes. It grows between 2000 and 3000 meters above sea level as a vigorous climber, in climates with average temperatures between 13 and 16 °C. The pulp is gelatinous and surrounds small black seeds. This fruit is a source of vitamins A, B, and C (93 mg/ 100 g fresh fruit), calcium, iron, phosphorus, and potassium. In Colombia, it has a year-round production and is exported to Europe, because it is versatile and not as perishable as are others. Usually, this fruit is eaten either fresh or in juices and sherbets, due to its flavour is smooth and pleasant, with an acid taste. The volatile composition of curuba had been reported [1], however no any sensory analysis was performed before.

Method

The odour-active volatiles of curuba fruit were isolated by Solvent Assisted Flavour Evaporation (SAFE) of n-pentane/ethyl ether (9:1, v/v) extract. GC-O and GCMS analyses allow identifying all volatile organic compounds relevant to the flavour of this fruit, by comparison with standards. From centrifugate curuba juice, a glycosidic-rich fraction was isolated by selective adsorption on Amberlite XAD-4 glass column (25 x 5 cm, 1.5 mL/min) and methanol elution [2]. Methanol fraction was concentrated to dryness under vacuum at 40 °C, extracted with diethyl ether to remove residual volatiles and lyophilized. Glycosidically bound volatiles were released by enzymatic hydrolysis with a â-glucosidase (emulsin, Sigma-Aldrich) and analysed also by GCMS. The identity of two glucosides were proposed from their analyses by HPLC-ESIMS, and confirmed by the analyses of their acetates.

Results / Discussion / Conclusion

GC-O and GC-MS analyses of curuba fruit SAFE extract allow identifying linalool, hexyl acetate, 1,8-cineole, and butyl acetate as key aroma compounds of this fruit. Other relevant odorants because their contribution to the overall aroma were: 2-methylpropyl acetate, (Z)-3-hexenol, and (Z)-3-hexenyl acetate. Sulphur compouds, 3-sulphanylhexyl acetate and methional, were reported here for first time as odour-active volatiles in curuba. The relevance of C6-compounds and monoterpenols as odour-active volatiles in curuba fruit was validated through the correlation found between fruit puree and the recombinated mixture during sensory evaluation.
Additionally, (Z)-3-hexenyl â-D-glucopyranoside and linalyl â-D-glucopyranoside were identified as aroma precursors after HPLC-ESIMS analyses of the glycosidic mixture and GCMS analyses of enzymatically released volatiles with a glucosidase. Thermal treatment of the glycosidic mixture at native pH of fruit (pH 3.3, 100 °C, 2 h) lead furanoid cis- and trans-linalool oxides, as well as, á-terpineol, compounds that exhibit flowery odour notes. Biogenic relationship among odour-active volatiles and their glycosidic precursors was proposed [3]. The fact that volatiles released by acid hydrolysis of glycosides exhibited pleasant odour notes, suggests that glycosides of P. mollissima are able to act as aroma enhancers in processed products from this fruit

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OR18
ANALYTICAL APPROACHES FOR DETERMINATION OF MOLECULAR FOOD MARKERS AND COMPOUNDS OF INTEREST IN FOOD CONTACT MATERIALS

Corradini, C.*, Bignardi, C., Cavazza, A., Laganà C., Lantano, C., Salvadeo, P.
University of Parma, Department of Chemistry, Parco Area delle Scienze 17/A, 43124 Parma, Italy
**Claudio.corradini@unipr.it*

Introduction

Food authentication and its traceability is becoming an issue of increasing relevance in food of Mediterranean area and is generally motivated by safety, quality and economic benefits.
Furthermore, there is a growing interest about chemicals entering food during farming, processing and packaging. The interest is from consumers, producers as well as the control authorities to guarantee that food products are safe.
More in generally, it can be concluded that in today's global marketplace, to assess food quality and its safety it is essential to characterize food by specific suitable molecular markes as well as to know all possible migration from food-contact materials into food. Consequently, it is essential to have suitable analytical methods to determine these substances in different foodstuffs at low concentrations. Our study aimed to develop and validate analytical methods to evaluate molecular markers associated with food quality, its origin and/or beneficial effects to human health. Results regarding innovative use of analytical techniques such as capillary electrophoresis (CE) coupled to mass spectrometry (MS), GC-MS and UHPLC-MS-MS will be presented and discussed.

Method

Detection and quantitative analyses of N-å- furoylmethyl-L-lysine (Furosine) and 5-hydroxymethyl-furfural (HMF) were carried out by capillary electrophoresis (CE) coupled with mass spectrometry according to previous published methods (1-2). Determination of maltose-maltulose and other oligosaccharides were performed by HPAEC-PAD, according to methods described in our previous papers (3-4). Targeted and non-targeted liquid chromatographic-mass spectrometry (LC-MS) methods developed for food contact material studies were performed on a Ultimate 3000 RSLC nano system operated in capillary-flow mode coupled to a Q Exactive Mass spectrometer (all from Thermo Scientific, Fremont, CA).

Results and Discussion

The first part of our presentation on innovative analytical techniques is focused on capillary electrophoresis coupled to tandem mass spectrometry, exploited for the first time for the determination in different foodstuffs of two of the Malillard reaction products (MRPs), furosine and HMF, which are most widely used as markers of food process as well as markers of the nutritional quality of food. The validated technique was also applied for the comparison of furosine behaviour against maltose:maltulose ratio, which was proposed as a marker of thermal treatment for flour-derived products and infant formulas. Development and validation of HPAEC-PAD methods for separation of disaccharides, as well as prebiotic oligosaccharides in food matrices were also proposed. Furthermore, UHPLC coupled with mass spectrometry has been exploited in the field of food-contact materials. In particular, a new line of hybrid mass spectrometer, namely Q-Exactive, has been used, for the first time, for characterization of plastic materials which intended to come in contact with food. As well known, the safety of materials in contact with food must be evaluated as molecules can migrate from materials into food. Results are present and discussed.

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OR19
ANTINEOPLASTIC ACTIVITY OF SPECIES OF THE GENERA *Ageratina* AND *Lourteigia*

Méndez, G. M.[1]*; Rodríguez, J.[1]; Torrenegra, R. D.[1]*; Diosa, S.[2]
[1]*Research Professors, Natural Products Group, Universidad de Ciencias Aplicadas y Ambientales U.D.C.A, 222 Street No.55-34 Bogotá, Colombia*
[2]*Farmaceutic Chemistry Student, Universidad de Ciencias Aplicadas y Ambientales U.D.C.A, 222 Street No.55-34 Bogotá, Colombia*
**gmendez@udca.edu.co, rtorrenegra@udca.edu.co*

Introduction

Cancer is a leading cause of death worldwide, and according to the World Health Organization, about 80% of people living in rural areas use medicinal plants as the first system of prevention and cure of diseases. In this regard, scientists around the world are directing their attention to research related to the use of medicinal plants and their active components (Manju et al, 2012).

Among genera used in traditional medicine in Colombia are Lourteigia and Ageratina, however, have been few studies related with these genera, especially with its antineoplastic effects. Our current study focuses on the analysis of antineoplastic potential of L. mycrophyllum and A. gracilis, two species of which it is known to contain methoxylated flavones in high concentrations previously identified in a phytochemical study (Torrenegra et al., 1987).

Method

The L. microphyllum and A. gracilis species were identified and collected around Guasca Municipality, Department of Cundinamarca and were taken to the National Herbarium of Colombia for classification.

Extracts of different polarity were obtained by using of conventional methods as soxhlet extraction. The comparative analysis of cytotoxic activities of extracts from dry leaves and inflorescences were tested on human cancer cells from breast (MCF-7) and healthy human embryonic kidney 293 cell line (HEK293), by MTT method to examine cell viability, (Thomas CM, et al., 2012) and by fluorescent microscopic methods for analyzing cellular morphology changes. IC50 of extracts was calculated by means of the percentage of cell viability with and without treatments. Vincristine was taken as cytotoxicity positive control for cancer cells.

Results / Discussion / Conclusion

There were noteworthy differences between the cytotoxic activities and morphological effects of extracts obtained from leaves and inflorescences of L. microphyllum and A. gracilis species on MCF-7 cancer cells, however no negative effects were found in healthy cells under same conditions. A potent cytotoxic activity was found in the extracts that containing flavones according to IC50. Furthermore, morphological changes, principally at nucleus and cytoskeleton were observed in the cancer cells treated with these extracts. These differential effects suggest an anticancer potential of some compounds in the species under study on breast cancer cells.

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OR20
METHOD COMPARISON FOR ESTABLISHMENT OF ANTIOXIDANT POTENTIAL IN NATURAL PRODUCTS

Ortiz-Ardila A. E.[1], Celis C. A.[12*], Sequeda L. G.[12], Correa-Cuadros J. P.[1]
[1]*Researcher Grupo Fitoquimica Pontificia Universidad Javeriana GIFUG, Faculty of Science, Carrera 7 N. 40-62, Bogotá D.C., Colombia*
[2]*Profesor Pontificia Universidad Javeriana, Faculty of Science, Carrera 7 N. 40-62, Bogotá D.C., Colombia*
**crispin.celis@javeriana.edu.co*

Introduction

After a lot of investigations around the world to understand the mechanisms of chronic diseases, was conclude that one of the main factors that interacts to produce or develop this disorders is the oxidative stress produced by Reactive Oxigen Species (ROS) and Reactive Nitrogen Species (RNS) which causes cellular damage in all of biological systems, especially in humans. ORAC method is one of the "newest" that determines the protection capacity of antioxidant against the peroxyl radicals attack produced by thermal decomposition of AAPH radical, the assay is based on the protection of the fluorescent probe by the antioxidant; the principal disadvantage is the assay only can determine antioxidant capacity on peroxyl or hydroxyl radicals. We seek to compare these antioxidant methods on experimental and statistical levels with analytical standard compounds and two vegetal samples to define if one is better or how many should use to describe the antioxidant capacity in natural products.

Method

We standardized the $ABTS^{+}$, DPPH and ORAC methods using as standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich) and Ascorbic Acid (Sigma-Aldrich) analytical grade in ranks of concentrations from 100 to 250ppm for Trolox; from 40 to 160ppm for Ascorbic Acid. To determine the antioxidant capacity in natural products was used two different plants: *Rosmarinus officinalis* Lin. Var. Govaerts as a comparison/control and *Cecropia mutisiana* Mildrb. as a sample. The measures was expressed on inhibition percentage, for the comparison between methods was standardized each treatment on IC_{50} concentrations, comparing by ANOVA method with post hoc test of orthogonal contrast, polynomial contrasts, Tukey´s test, Dunnet test and Scheffé, statistical comparisons was performed on Statistix Software and SPSS software.

Results / Discussion / Conclusion

Statistically was probe that IC_{50} Trolox values are not significant correlate with IC_{50}s values of Ascorbic acid (*p* Pearson: 0,183; *p* Spearman: 0,154) establishing the independence of the results at chemical and mathematical level. Significant differences between the used methods was found with Trolox (p=0,000) and Ascorbic acid (p=0,000) using one way ANOVA. The DHS-Tukey proves that any of the tested methods have similar responses between them. For Trolox standard, ORAC was the most sensitive method (p=0,001) due to the precision and specificity of fluorogenic probe and the propitious electrolytic condition of the buffer matrix, other reports shows that Trolox compound has a proton donor mechanism -Scavenger type which principally shows a protective effect (commonly detected by ORAC) but also acts as oxygen radical inhibitor. Also we proposed five response prediction models on base to the obtained data by the different used methods, that enable the theoretical obtaining of results without doing the specific test allowing a good approximation of the real result, using specific variables as "Compound" (Trolox= 1; Ascorbic acid= 2), "Concentration on ppm" (on range of 100-250 Trolox; 40-160 Ascorbic acid) and "Result of one or more methods" (inhibition percentage). With these data, it is possible to estimate the response in other method. The obtained model shows 97,5% of precision and data adjustment even a good reliability evidenced by the $\div^2$ statistical test between the observed data and expected (Real and obtained by prediction) proving that the results obtained are distributed same than real ones (p= 0,1154).

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OR21
EFFECT OF REDUCED-CALORIE AVOCADO PASTE ON THE LIPID PROFILE IN WISTAR RATS FEED WITH AHYPERCHOLESTEROLEMIC DIET

Pahua-Ramos, M. E.[1], Valadez-Carmona, L.[1], Plazola-Jacinto, C. P.[1], Hernández-Navarro, M. D.[2], Garduño-Siciliano, L.[1], Ortiz-Moreno, A.[1]*

[1]*Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Prolongación Carpio y Plan de Ayala s/n, Distrito Federal Ciudad de México, México*

[2]*Universidad Autónoma del Estado de México, Facultad de Química, Paseo Colón casi esq. Paseo Tollocán, Toluca de Lerdo, Estado de México, México*

**ortizalicia@hotmail.com*

Introduction

The consumption of high levels of fat and carbohydrates has an impact on the weight, and lipid profile of rats resulting in an increase in very low-density lipids (VLDL) and triglycerides (TG). The new technology of microwave-squeeze extraction of avocado oil generates a defatted pulp, which is called "reduced-calorie avocado paste". This paste contains phytochemicals that have protective effects on the cardiovascular system. Thus, the aim of this study was to evaluate the effect of reduced-calorie avocado paste alone and added with fiber on the lipid profile in Wistar rats feed with a hypercholesterolemic-high fructose diet (HHF).

Method

The hypercholesterolemia was achieved by administering of a hypercholesterolemic diet (cholesterol, sodium collate, butter, sucrose, casein, cellulose) supplemented with 10 ml/kg BW (body weight) of 60 % fructose solution. Rats were separated into five groups: a) standard commercial diet, b) hypercholesterolemic diet, c) hypercholesterolemic-avocado paste diet, d) hypercholesterolemic-reduced calorie avocado paste diet and e) hypercholesterolemic- reduced calorie avocado paste added with fiber. After seven weeks of treatment, the blood samples were taken from the retro-orbital sinus and centrifuged at 3,000 ×g for 15 min to isolate serum to determine total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG).Liver damage was evaluated by histological analysis.

Results / Discussion / Conclusion

Groups feed with diet high in fiber showed not only an increase in the fecal material and fecal lipids excretion, but also a decrease in their body weight gain, total cholesterol decreased around 40%, and glucose levels were reduced in a 8.5% compared with group feed with hypercholesterolemic diet (group b), while histological analysis of group b showed severe macro- and microvesicular stetatosis and chronic inflammation characterized by the presence of mononuclear cells. Rats supplemented with avocado paste clearly reduced liver damage (mild to moderate steatosis and panlobulillar microvesicles compared to group b. This reduction in hepatic steatosis might be due to the effect of phytochemicals and soluble dietary fiber present on avocado paste. In conclusion, this study has demonstrated that a diet supplementation with the reduced-calorie avocado paste prevents body weight gain and reduces serum cholesterol.

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OR22
ANTIOXIDANT, ANTINOCICEPTIVE, AND ANTI-INFLAMMATORY EFFECTS OF CAROTENOIDS EXTRACTED FROM DRIED PEPPER (*Capsicum annuum* L.)

Hernández-Ortega, M.[1], Hidalgo-Pérez Tejada, E. I.[2], Valadez-Carmona, L.[2], Plazola-Jacinto, C. P.[2], Hernández-Navarro, M. D.[1], Ortiz-Moreno, A.[1]*

[1]*Universidad Autónoma del Estado de México, Facultad de Química, Paseo Colón casi esq. Paseo Tollocán, Toluca de Lerdo, Estado de México, México*

[2]*Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Prolongación Carpio y Plan de Ayala s/n, Distrito Federal Ciudad de México, México*

**ortizalicia@hotmail.com*

Introduction

Peppers are important sources of carotenoids, are lipid-soluble pigments with antioxidant properties. Since pain stimulus and inflammation are correlated with free radical production, carotenoids may play an important role in pain reduction. The aim of this study was to determine the carotenoid content of three Mexican dried peppers, guajillo, pasilla, and ancho (*Capsicum annuum* L.), and to analyze their antioxidant, analgesic and anti-inflammatory activities.

Method

Carotenoid content of the pepper extracts was evaluated by the isochromic fractions method and identified by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). The antioxidant activity of each extract was evaluated using the ABTS+ and DPPH+ assays. Classic tests in mice were used to assay the analgesic (acetic acid-induced writhing test and hot plate test) and anti-inflammatory (carrageenan-induced paw oedema) properties of the extracts.

Results / Discussion / Conclusion

We found that all three peppers had a substantial carotenoid content: guajillo 3406.35 ± 4.13 ìg/g, pasilla 2932.98 ± 0.89 ìg/g, and ancho 1436.69 ± 5.79 ìg/g of sample in dry weight basis. A complex mixture of carotenoids was discovered in each pepper extract. The TLC analysis revealed the presence of chlorophylls in the pigment extract from pasilla and ancho peppers. Carotenoid extracts from peppers had good antioxidant activity, the best scavenging capacity for the ABTS+ cation was exhibited by pasilla pepper pigment extract (60%) and the best antioxidant activity against DPPH+ cation was exhibited by guajillo carotenoid extract (24%). The carotenoid extract obtained from guajillo peppers exhibited a safe margin of toxicity (LD50 > 2,000 mg/kg) and also generated significant peripheral analgesic activity at 5, 20, and 80 mg/kg and induced central analgesia at 80 mg/kg It was found that carotenoid extract from guajillo pepper exhibit anti-inflammatory activity at 5 mg/kg. The results suggest that the carotenoids in dried guajillo peppers have significant analgesic and anti-inflammatory benefits and could be useful for in pain and inflammation relief.

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OR23
CONTRIBUTION TO THE PHYTOCHEMISTRY AND BIOACTIVITY OF *Eryngium kotschyi* Boiss

Aslan Erdem, S.[1]*; Arıhan, O.[2]; Mitaine-Offer, A. C.[3]; Iskit, A. B.[2]; Kartal, M.[1]; Lacaille-Dubois, M. A.[3]

[1] *Ankara University/Faculty of Pharmacy/Department of Pharmacognosy, 06100, Tandoğan, Ankara, Turkey*
[2] *Hacettepe University/ Faculty of Medicine/Department of Physiology, 06100, Ankara,Turkey*
[3]*Laboratoire de Pharmacognosie, EA 4267(FDE/UFC), Faculté de Pharmacie, Université de Bourgogne, 7, Bd Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France*
**sinemaslanerdem@yahoo.com*

Introduction

The genus Eryngium L. is a cosmopolitan and rich genus of Apiaceae in respect to the number of species (Aslan Erdem, 2009; Yurdakök and Baydan, 2013). As a result of this widespread distribution, it is used traditionally for different therapeutic purposes such as antiinflammatory, antioedema, antitussive, diuretic, appetizer, stimulant, aphrodisiac and against pain, scorpion and snake bites (Kartal et al., 2005; Baydan et al., 2014). E. kotschyi Boiss. is an endemic taxa in Turkey; named as "Çakır dikeni" (Baydan et al., 2014). Eryngium species are known to contain triterpene saponins, acetylenes, flavonoids, coumarins and essential oils (Küpeli et al., 2006; Dunkic´ et al., 2013). The aim of this study is to present the phytochemical and bioactivity studies performed on E. kotschyi.

Method

Bioactivity Guided Fractionation: Acetic acid induced writhing test and hot plate tests, which are used to evaluate antinociceptive activity, were applied to the extracts and fractions obtained from underground parts of E. kotschyi in order to identify bioactive fractions and/or pure compounds. Column chromatography and medium-pressure liquid chromatography (MPLC) on silical gel and reversed-phase RP-18 were used for the fractionation and isolation studies. The structure elucidation of the pure compounds was achieved mainly by 1D and 2D NMR spectroscopic techniques (1H, 13C, 1H-1H COSY, TOCSY, NOESY, HSQC, HMBC) and by FAB-MS.

Results / Discussion / Conclusion

The total methanolic extract prepared from underground parts, was partitioned with hexane, dichloromethane and n-butanol (water saturated), respectively. Since the n-butanol fraction was found to possess the most significant antinociceptive activity, it was chosen for further isolation studies, yielding six pure compounds after multiple chromatographic steps. Their structures were elucidated as triterpene saponins, having mainly A1-barrigenol as aglycone, four of them being new. Since amount of the pure compounds was not sufficient, only one of them, a triglycoside of A1-barrigenol was examined for its antinociceptive activity by the hot plate test and found to be moderately active.

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OR24
THE POPULAR BELIEF VS SCIENTIFIC KNOWLEDGE ABOUT *FICUS CARICA* (FIG) DECOCTION

Martínez, N.[1]*; Greco, L.[2]; García, E.[2]; Castejón, O.[3]; Quiróz, J.[3]
[1]*Dr, Sección Fitofarmacología Instituto de Investigaciones Biomédicas "Dr. Francisco Javier Triana Alonso" (BIOMED-UC),final calle Cecilio Acosta Urb. Cantarrana Las Delicias Maracay, Aragua, Venezuela.*
[2]*M.D, Sección Fitofarmacología Instituto de Investigaciones Biomédicas "Dr. Francisco Javier Triana Alonso" (BIOMED-UC),final calle Cecilio Acosta Urb. Cantarrana Las Delicias Maracay, Aragua, Venezuela.*
[3]*Prof, Universidad de Carabobo sede Aragua, La Morita, Aragua, Venezuela.*
**nubildemartinez1@hotmail.com*

Introduction

The popular belief is that a decoction of the leaves of *F. carica* (fig) is like a magic potion that can speed up labor and they recommend drinking several cups of that decoction, this belief is opposed to the scientific knowledge which linking intake of decoction of *F. carica* to neonatal depression observed in several infants, so the aim of there search was viewed through optical and electron microscopy pathological findings that could produce a decoction of *F. carica* plant in the placentas of rabbits (*Oryctolagus cuniculus*).
The research was purely experimental, with unintentionally probability level, were sampled eleven (11) rabbits of which four (4) were found to be pregnant, three (3) of the New Zealand race and one (1) of the California race was randomly selected and two (2) control and two rabbits(2) experimental groups. For the latter, given a suspension of powdered *F. carica* leaves and water in a concentration of 5.22% m/v identified as number1, and of 38.09% m/v number 2 doses. The placenta of a rabbit from each group were collected, with the placenta in the experimental group that was given the suspension in higher concentration in dosed daily servings of 8.4 ml for five days, and proceeded to perform histological studies by optical microscopy (OM) and scanning electron microscopy (SEM).

As a result of studies significant histopathological changes were observed in relation to the Control placenta. In OM thrombi and foci of necrosis distribute din placental parenchyma were observed; SEM level changes in membrane decidual cells were observed. Therefore it was concluded that because of these major histopathological changes may explain the effects which lead to neonatal depression.

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OR25
ANTIMYCOBACTERIAL ACTIVITY OF MONOTERPENE INDOLE ALKALOIDS OF *Duroia macrophylla*

Martins, D.[1]; Carrion, L. L.[2]; Ramos, D. F.[2]; Da Silva, P. E. A.[2]; Nunez, C. V.[1]*
[1]*Bioprospection and Biotechnology Laboratory, National Institute of Amazonian Research - INPA, Manaus, AM, Brazil - INPA, Manaus, AM, Brazil.* [2]*Mycobacterial Laboratory, Federal University Foundation of Rio Grande - FURG, Rio Grande, RS, Brazil.*
**cecilia@inpa.gov.br*

Introduction
The species of Rubiaceae revealed great diversity of secondary metabolites, which are responsible for a range of biological activities. Among these species is Duroia macrophylla Huber, endemic to the Amazon Rainforest, popularly known as "cabeça-de-urubú". The aim of this work was to isolate the chemical constituents and evaluate the extracts and isolated compounds on antimycobacterial activity.

Method
The plant material was collected in the Natural Heritage Private Reserve, locally known as "Cachoeira da Onça", in "Presidente Figueiredo" County, AM, was carried out on May 18th, 2011. Leaves and branches were dried, grounded and extracted with dichloromethane and methanol. The isolated alkaloids were identified by spectroscopic methods (1H, 13C and two-dimensional NMR) and mass spectrometry. The antimycobacterial activity was carried using the Resazurin Microtiter Assay (Palomino et al., 2002). The extracts and alkaloids of D. macrophylla were evaluated against two Mycobacterium tuberculosis strains: pan-sensible (H37Rv) and isoniazid monoresistant (INHr).

Results / Discussion / Conclusion
There were identified from Methanol (MeOH) and dichloromethane (DCM) extracts of D. macrophylla, eight monoterpene indole alkaloids: 10-methoxy-ajmalicine, 11-methoxy-ajmalicine, 11-methoxy-3-iso-ajmalicine, 9-methoxy-3-isoajmalicine, 9-methoxy-19-epi-3-isoajmalicine, 10-methoxy-19-epi-3-isoajmalicine, 10-methoxy-3-isorauniticine and 10-methoxy-rauniticine. Only the alkaloids 10-methoxy-3-isorauniticine, 10-methoxy-3-isorauniticine, 10-methoxy-rauniticine and the mixture of 9-methoxy-3-isoajmalicine with 9-methoxy-19-epi-3-isoajmalicine, tested against M. tuberculosis (strain INHr) showed better results rather those obtained from crude extracts (Table 1). All the alkaloids isolated in this study were described for the first time in the genus Duroia.

Extracts/Alkaloids (μg/mL)	*M. tuberculosis* (μg/mL)	
	H37Rv	INHr
DCM extract of branches	25	50
10-methoxy-ajmalicine	50	25
11- methoxy-ajmalicine	100	50
11- methoxy-3-isoajmalicine	100	50
Mixture of 9-methoxy-3-isoajmalicine with 9-methoxy-19-epi-3-isoajmalicine	100	25
10-methoxy-19-epi-3-isoajmalicine	100	100
MeOH extract of leaves	100	100
10- methoxy-3-isorauniticine	100	50
10- methoxy-rauniticine	100	50

Table 1: MIC determination of extracts and alkaloids isolated from D. macrophylla

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OR26
ESTROGENIC ACTIVITY OF ETHANOLIC EXTRACTS FROM LEAVES OF *Ilex guayusa* Loes. AND *Medicago sativa* IN *Rattus norvegicus*

Contero, F.[1]*; Abdo, S.[1]; Vinueza, D.[1]*; Moreno, J.[1]; Tuquinga, M.; Paca, N.
[1]*Prof, Escuela Superior Politécnica de Chimborazo, Panamericana Sur Km 1 1/2, Riobamba, Chimborazo, Ecuador*
**bqffconterob@gmail.com*

Introduction

Anovulatory infertility is treated with ovulation-stimulating agents (OSA), as antiestrogens, gonadotrophins and aromatase inhibitors (Balen, 2012). The use of these substances, similarly to hormonal replacement therapy in postmenopausal women, increases the risk of development endometrial and breast cancer by genotoxic and cellular proliferative effect (Liehr, 2001). Several herbal supplements are used to female infertility, many of which have phytoestrogens. Ilex guayusa Loes. (Aquifoliaceae), an Andean tree, has numerous medicinal uses, as a stimulant beverage for its high caffeine content (Lewis et al, 1991). Also is used as treatment to gastritis, headache, body pain, and women infertility in southern and eastern Ecuadorian provinces. (Tene et al, 2007). Medicago sativa (Fabiaceae) has been cultivated as forage crop. This is a source of phytoestrogens coumestrol and genistein. Has been widely used in South America as diuretic, kidney and vesicular swelling, lung ailments, and reconstituent (Macía et al, 2005).

Method

Ilex guayusa and Medicago sativa were collected in Chimborazo and Pastaza, Ecuador. The dried and milled drug was macerated in 97% ethanol for 7 days. The extracts were concentrated to dryness under controlled pressure and reconstituted in 0,9 % sodium chloride solution.

Wistar female rats were used as experimental subjects, between 2.5 to 3.5 months old, and body weight 170±5 g, obtained from the animal facility center of the Faculty of Sciences, ESPOCH. 24 rats were divided into 8 experimental groups of 3 rats each. Based on the protocol of Telefo, P. B. et al (2002), after determination of basal estradiol in blood samples from facial vein by immunoassay Microplate, were treated daily for 15 days orally with 0.9% sodium chloride solution (negative control), standardized soy isoflavone extract (positive control) or various concentrations of plant extracts each corresponding to 9, 18 and 36 mg/kg/day. At the end of the experiment, it was obtained a sample of blood by cardiac puncture and determined the final concentration of estradiol. After euthanasia, ovaries and uteri were removed and weighed.

Results / Discussion / Conclusion

Administration of the medium and high doses of I. guayusa and M. sativa extracts caused a significant increase in serum estradiol concentration in comparison with the negative control. The administration of extracts of soy isoflavones did not cause this effect, since isoflavones are agonists of estrogen receptors. A significant increase in the weights of uteri and ovaries were observed in all experimental groups. The extracts tested could have effect on luteinizing hormone and follicle stimulating hormone, even on aromatase and the conversion of estrogen precursors in active estrogen.

The treatments with I. guayusa and M. sativa extracts at low dose (9 mg/kg), medium dose (18 mg/kg) and high dose (36mg/Kg) per day, have estrogenic effect manifested in the increase of the weight of ovaries and uterus, and serum estradiol concentration in immature Wistar rats. The use of these species in traditional Andean medicine for infertility is justified.

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OR27
ETHNOBOTANICAL STUDY OF BAGROTE VALLEY OF GILGIT DISTRICT, CENTRAL KARAKORAM NATIONAL PARK, GILGIT-BALTISTAN, PAKISTAN

Qamar Abbas[1]*, Sher Wali Khan[2], S Najamul Hassan[2], Shamsher Hussain[2], Rehmatullah Qureshi[3]
*Ýntegrated Mountain Area Reseach Center (IMARC), Karakorum International University Gilgit-Baltistan, Gilgit,Pakistan.**
[2]*Department of Biological Sciences, Karakorum International University Gilgit-Baltistan, Gilgit, Pakistan.**
[3]*Department of Botany, PMAS University of Arid Agriculture, Rawalpindi, Pakistan*
qamarabbaskiu@gmail.com

Abstract
The present study was carried out to explore natural flora, and record the ethnobotanical uses of plant wealth by the inhabitants of Bagrote valley, Central Karakoram National Park, Gilgit district, Gilgit-Baltistan of Pakistan during 2013-2014. Bagrote valley located near to core area CKNP, and is the model region of this park, due to its unique biodiversity and existing natural resources. The people of the area depended upon their agriculture, pastoral farming and other natural resources for their livelihoods.
The valley located between 2000m to 5000m altitude; consist of three major Glaciers, number of meadows and more than twenty forest patches. Bagrote valley consist of 10 major villages, and approximatly more than 15000 inhabitants. The valley is located at the northside of the river Indus, started from junction of three great mountainous ranges i.e. Karakoram, Himalaya, and Hindukush. The detailed information of the flora with respect to their ethnobotanical uses were collected through various field trips, specimen collection, using open ended questionnaires and detailed interviews from the native herbal healers (Hakeems), and elderly known people. For each species, botanical name, local name, habit, locality, parts used, medicinal and other multifarious uses have been recorded. During the survey, all collected plant specimens which were identified with the help flora of Pakistan are deposited in the herbarium Department of Biological Sciences Karakoram International University Gilgit. Global Positioning System (GPS) was used to record coordinates of the 23 different localities and their distribution map is designed using ArcGis 10.2. In total 61 medicinally plant species belonging to 56 genera and 41 families were collected and identified from the different areas of thevalley. The highest numbers of species (48) are used as medicinal purposes, followed by fodder and forage (39 species), fuel and timber (14 species), veterinary uses (6 species). Most of the species have reported multi-usages while three endemic species and five new species reported from this valley. Distribution of knowledge about the traditional use of medicinal plants was recorded that, the older people are much informative and habitual to use these medicinal plants for the treatment of different ailments as compare to younger people.The natural ecosystem is declining at rapid pace due to overgrazing, deforestation, anthtropoganic activities and un-sustainable management of flora, causing threat to natural biodiversity of the area. It is need of hour to aware the communities about natural resources, and governament and other stack holders should provide the alternative sources of fuel and timber for the inhabitants, than it is possible to protect the natural resources of the area.

Key words: Ethnobotany, Global Positioning System, Central Karakoram National Park, Gilgit- altistan

OR28
ANTIFUNGAL ACTIVITY OF POMEGRANATE EXTRACT: EFFECT OF DIFFERENT EXTRACTION METHODS

Rongai, D.[1]*, Milano, F.[1]; Pesce, B.[1]; Pulcini, P.[1];
[1]*Consiglio per la ricerca e la sperimentazione in agricoltura, Centro di ricerca per la patologia vegetale, via C.G Bertero, 22 - 00156 Roma, Italy*
domenico.rongai@entecra.it

Introduction

Punica granatum L., commonly known as pomegranate, is a small tree native of the Mediterranean region. A number of biological activities such as antibacterial and antifungal have been reported for P. granatum extracts (Orak et al., 2011; Quattrucci et al., 2013). In the present work several pomegranate extracts, obtained by different extraction methods, are being investigated as possible alternatives for control of Fusarium oxysporum f. sp. lycopersici (Sacc.). The presence of antifungal compounds in the extract was also determined through HPLC-MS analysis.

Method

Fruit peel of P. granatum var. "dente di cavallo" was cut in small pieces and put together at the solvent (water, ethanol, methanol and propanol). The heterogeneous mixture was stirred, sonicated, centrifuged and then the supernatant filtered through a 0.45 μm membrane filter. The solvent was vacuum evaporated in rotatory evaporator and, subsequently, the extract has been lyophilized. The powder of the extract obtained has been stored in a freezer until further use. To purify the extract has been used Solid Phase Extraction (SPE) procedure.
To determine the efficacy of the extracts, at 0.5, 1, 1.5 and 2% of concentration, 5 mm diameter of a colony of F. oxysporum was placed in the center of each Petri dish with the mycelium face down. The radial growth and the percentage of inhibition of each extract was calculated.
The content of the main antifungal compounds in the pomegranate extract (Lu et al., 2008), was determined through a simplified HPLC MS-MS analytical method (Fischer et al., 2011).

Results / Discussion / Conclusion

The findings show that increasing the concentration of the extract, the mycelia growth goes down. At the concentration of 0.5%, the fungal growth stood at 24 mm at fifth day and on the plates with 2% of pomegranate extract, the fungal growth seems to be completely inhibits. Regarding the solvent used, water extract seems to be more effectiveness than other solvents. It is possible to see that the chromatogram of water extract is a little bit different than ethanol and methanol extracts. The biological test response shows a most efficacy of water extract. At the 0.5% of concentration, the inhibition of water extract is in average 39%, statistically higher than 23 and 25% respectively for ethanol and methanol extract.
The use of SPE procedure, improve the efficacy of pomegranate extract. Comparing with chromatograms and biological tests, it can be seen that when the content of punicalagin is bigger (purified extract as showed by the relative peaks of the chromatogram), the extract has an higher level of inhibition (see Figure below). The superior efficacy of purified extract could be due at the most concentration of some phenolic compounds like the punicalagin which is held responsible for inhibiting fungal mycelial growth.

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OR29
IN VITRO AND *IN VIVO* SCREENING OF COLOMBIAN PLANTS FOR POTENTIAL ANTILEISHMANIAL, ANTIMALARIAL AND ANTITRYPANOSOMAL COMPOUNDS

Robledo, S. M.[1*]; Torres, O.[2]; Cardona, W.[3]; Upegui, Y.[4]; Muñoz, D.[4]; Ospina, V.[4]; Restrepo A. M.[4]; Arbelaez, N.[4], Murillo J.[4]; Torres, F.[2]; Quinones W.[2]; Escobar, G.[2]; Triana, O.[5]; Osorio E.[6]; Echeverri, F.[2]

[1]*Prof, PECET, Medical Research Institute, School of Medicine, University of Antioquia, Calle 70 No. 52 - 21, Medellin, Colombia*
[2]*Prof, QOPN, Institute of Chemistry, University of Antioquia, Calle 70 No. 52 - 21 Medellin, Colombia*
[3]*Prof, QPC, Institute of Chemistry, University of Antioquia, Calle 70 No. 52 - 21, Medellin, Colombia*
[4]*BSc, PECET, Medical Research Institute, School of Medicine, University of Antioquia, Calle 70 No. 52 - 21, Medellin, Colombia*
[5]*Prof, BCEI, Institute of Biology, University of Antioquia, Calle 70 No. 52 - 21, Medellin, Colombia*
[6]*Prof, Pharmaceutical Chemistry School, University of Antioquia, Calle 70 No. 52 - 21, Medellin, Colombia*
**sara.robledo@udea.edu.co*

Introduction

Parasite diseases such as malaria, leishmaniasis and Chagas disease cause high morbidity, mainly in poor countries. All of them kill million people annually while leishmaniasis, besides to health problems, is important cause of social stigma and psychological damage.

This landscape is more complex, due to lack of other therapeutic treatments, drug parasite resistance and secondary effects in humans of available drugs. Besides, the pharmaceutical industry is not interested in developing new drugs for this kind of diseases, due to high costs and low outcomes. Today, traditional medicine is a source of important bioactive substances, but its required to optimize this searching, especially, moving on from *in vitro* bioassays to an animal model of theses disease.

This point of view makes feasible to analyse not only the pharmacologic effect, but also pharmacokinetic parameters, preliminary toxicology and evaluate the drugability of the substance assayed under this methodology.

Method

More than 500 natural and synthetic related compounds from Colombian plants were analysed *in vitro* using intracellular amastigotes of Leishmania panamensis, asynchronic cultures of Plasmodium falciparum and intracellular amastigotes of Trypanosoma cruzi by flow cytometer, fluorometry and colorimetry test., respectively. Cytotoxicity of these compounds was also assessed using the MTT method. Compounds were identified as Hit compounds based on both *in vitro* antiparasitic and cytotoxic activities. Then, Hit compounds were tested for their therapeutic response against L. panamensis and P. falciparum using the corresponding *in vivo* bioassay: cutaneous leishmaniasis model on the dorsal skin of hamsters and the antimalarial Rane's test in mice experimentally infected with P. berghei.

Results / Discussion / Conclusion

Close to 40% of all the material assayed *in vitro* showed a moderate-high activity against L. panamensis, P. falciparum or T. cruzi. Ten percent of tested compounds showed good therapeutic response against L. panamensis or P. berghei and therefore they could be considered as good candidate as Lead compounds for drug development to combat these parasitic diseases.

Acknowledgments:
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OR30
EXTRA VIRGIN OLIVE OIL (EVOO) AS FUNCTIONAL FOOD: STANDARDIZATION PROCESS BASED ON SCIENTIFIC EVIDENCES

Smeriglio, A.[1]; Giovinazzo, C.[2]; Trombetta, D.[3]*;
[1] PhD, University of Messina,Viale Annunziata, Messina, Italy
[2] Prof, Freelance Consultant, P&P Farma Srl, Turin, Italy
[3] Prof, University of Messina,Viale Annunziata, Messina, Italy
* dtrombetta@unime.it

Introduction

The functional foods field has evolved recently thanks to the implementation of the research in the food area, the greater regulation which led to the coining of health claims and the increasing attention of consumers for health-promoting food products (De Boer A. et al., 2014; Van Buul VJ. and Brouns FJ., 2013).

Method

The aim of work is to enhance a typical functional food of the Mediterranean diet, the Extra Virgin Olive Oil (EVOO). According to scientific opinion on the substantiation of health claim related to polyphenols in olive oil and to á-tocopherol in foods published by European Food Safety Authority (EFSA) in the 2011 and 2010 respectively, the authors decided to focus their research on the standardization and validation of a production process, starting from cultivation and harvesting of the olives that guarantee product quality in terms of active ingredients as well as on the definition of a methodology/procedure to preserve them the best in time in order to guarantee to the consumer a product that retains its functional and organoleptic native characteristics.

Results and Discussion

The monitoring of cultivation process, in season 2013, of olive trees from a soil sample area according to the biological agriculture principles, the harvesting and milling of olives and oil extraction from these trees in accordance with a "coded" process as well as a careful quality control (shelf-life) of the analytical parameters taken into account, have allowed us to critically evaluate the nutritional values found (variation of poliphenols and á-tocopherol content < 15% and < 45 % respectively in 9 months) and identifying the particular features that will enable the achievement of an EVOO with functional and organoleptic characteristics suitable for the conferment of health claim.

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OR31
ANTIBACTERIAL ACTIVITY AND CHEMICAL CHARACTERIZATION OF *Myrcianthes hallii* (MYRTACEAE), USED AS TRADITIONAL MEDICINE IN ECUADOR

Daglia, M.[1]*; Chavez Carvajal, P.[1]; Di Lorenzo, A.[1]; Coppo, E.[2]; Gozzini, D.[3]; Bracco, F.[4]; Zanoni, G.[3]; Marchese, A.[2]

[1] *Department of Drug Sciences, University of Pavia, V.le Taramelli 12, 27100 Pavia, Italy*
[2] *Microbiology Unit, DISC, University of Genoa, Italy*
[3] *Department of Chemistry, University of Pavia, V.le Taramelli 10, 27100 Pavia, Italy*
[4] *Department of Territorial Ecology and Enviroment University of Pavia, Via S. Epifanio, 14-27100 Pavia, Italy***maria.daglia@unipv.it*

Introduction

Myrcianthes hallii (O. Berg) McVaugh is a medicinal and aromatic plant belonging to Myrtaceae family, commonly known in Ecuador as "arrayán". In traditional medicine, arrayán is used for its astringent, antiseptic, haemostatic, hypoglycemic and balsamic properties. Moreover, it is used to treat pulmonary disorders, diabetes, night sweats of tuberculosis and as an effective remedy for wounds. Despite the long history of use as traditional medicine, no literature data are available regarding its antimicrobial properties and chemical composition. Therefore, the aim of the present study was to evaluate arrayán antibacterial activity and the phytochemical composition.

Method

The investigation was performed on the hydro-methanolic extract obtained from dried leaves of Myrcianthes hallii.

First the extract was submitted to microbiological assays to determine the antibacterial activity. Ten clinical Staphylococcus aureus strains (five methicillin-resistant and five methicillin-susceptible) were studied. Among those resistant, three strains were multi-resistant (resistant to at least three classes of antibiotics). The antibiotype was determined using the disk diffusion test, according to the Clinical and Laboratory Standards Institute guidelines. Minimum inhibitory concentration (MIC) was determined by using the broth microdilution method, according to the Clinical and Laboratory Standards Institute guidelines.

To evaluate the phytochemical composition of the plant extract, it was submitted to a preliminary purification through dialysis (molecular weight cut-off of 3500 Da) and then analyzed by UHPLC-PDA-hESI-MSn.

Results

The extract shows antibacterial activity against both methicillin-resistant and methicillin-susceptible Staphylococcus aureus strains (MIC range 1.08 - 2.16 mg/mL).

The UHPLC-PDA-hESI-MSn analysis allowed the identification of more than thirty compounds, including organic acids, phenolic acids, and flavonoids. Since there are no literature data about the chemical composition of arrayán, this work represents the first available study about the chemical characterization of this plant.

Overall, the chemical composition and the antibacterial activity of Myrcianthes hallii determined in the present investigation can support the medicinal properties related to its traditional use in Ecuador.

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OR32
PHOTOPROTECTOR ACTIVITY OF PLANTS FOR ELABORATION OF A SUNSCREEN

Abdo S.[1], Contero F.[2], Ruiz M., Ortiz V., Vinueza D.[2]
[1]*MsC, Escuela Superior Politécnica de Chimborazo (ESPOCH) Facultad de Ciencias. Panamericana sur Km1, Riobamba- Ecuador*
[1]*MsC, (ESPOCH) Facultad de Ciencias*
[2]*Bioquímico Farmacéutico ESPOCH. Facultad de Ciencias*
abdosu@espoch.edu.ec

Introduction

By its geographical location and position in the Andes, Ecuador is the country that receives the most solar radiation in the world. UV radiation produces damage to DNA and oxidative stress in human skin, leading to photoaging and photocarcinogenesis. The adequate protection of the skin against solar radiation to which we are exposed is essential. Investigations show that cinamats, flavonoids and polifenoles have photoprotector characteristics for reason was leaded the verification of this activity (1), from Taxo (Passiflora tripartite),Maracuyá (Passiflora edulis), Basil (Ocimumbasilicum); and Ishpingo(Ocotea quixos) leaves extracts and a mixture of them.

Method

Flavonoids and total cinnamates, were quantified in fluids extracts of each plant by spectrophotometry and pharmacognosic analysis was performed (2).
The study was carried out through the application of a cream with 1.5% of fluid extracts of each plant and mixture of them: Passiflora tripartite-Ocimum basilicum (1:1) and Passiflora edulis-, Ocotea quixos (1:1) in volunteers with skin type III, with protective forearm divided into areas with six experimental surfaces subjected to solar radiation under specific conditions, according to the efficiency in solar products using the COLIPA method.

Results / Discussion / Conclusion

The results showed photoprotective levels of SPF of 3 for P. tripartite, SPF5 for O. basilicum, and the combination (1:1) SPF 7.
O. quixos presented an SPF of 8, P. edulis SPF 4, and the combination (1:1) of both an SPF 6.
Creams presented photoprotective levels and an interesting synergistic effect was observed in P. tripartite-Ocimun. basilicum combination. The combination of P edulis -(Ocotea quixos) has not synergistic effect. The plants with cinnamic compounds have better fotoprotector effect (Ocotea quixos and Ocimum basilicum) than plants with flavonoids (Passiflora ssp.)

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OR33
IDENTIFICATION OF MONOTERPENE INDOLIC ALKALOID STRICTOSIDINIC ACID FROM *Palicourea guianensis Aubl.*

Oliveira, J. L.[1]; Martins, D.[2]; Nunez, C.V.[3]*

[1]Graduation Student, Instituto Federal de Educação, Ciência e Tecnologia do Amazonas-CMC, Manaus,AM, Brasil; [2]PhD, Post-Doc at LABB-INPA [3]Prof., Laboratório de Bioprospecção e Biotecnologia-LABB, Coordenação de Tecnologia e Inovação, Instituto Nacional de Pesquisas da Amazônia-INPA, Av. André Araújo, 2936, Petrópolis, Manaus, AM, Brasil.

**cecilia@inpa.gov.br*

Introduction

Palicourea guianensis, belongs to Rubiaceae family and occurs in the Amazon region. *Palicourea* has some species that are toxic to cattle and also plants that are used in folk medicine to treat fungal infections, cough, stomach pains (El-Seedi, 1999) and as an antitumor agent (Hartwell, 1972). Chemically this genera characterized by biosynthesize indole alkaloids containing a secologaninic unit, extremely important class of substances from biological point of view (Dusman et. al., 2004).

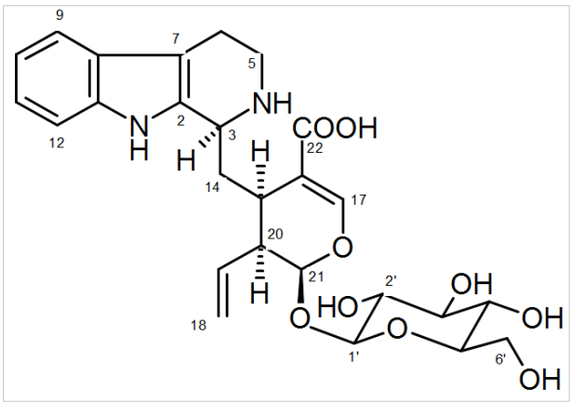
Method

Leaves were dried in a hot kiln of forced ventilation at 45 °C and grounded. Then, leaves were extracted with hexane using ultrasound for 20 min, filtered and repeating this procedure more two times. Then, the plant material was dried and extracted with methanol and finally with water, using the same procedure. The extracts were concentrated in rotary evaporator for hexane and methanol and liophilizator for water extract. The water extract was acid/base partitioned and used butanol to extract the alkaloids. This phase was chromatographed with Amberlite XAD4, and eluted with H2O/MeOH 9:1 to ethyl acetate 100%. 35 fractions were obtained and the fraction C2.12_24 was rechromatographed with Amberlite XAD2, and eluted with H2O/MeOH 8:2 to MeOH 100%, obtaining the fraction C6.1_5, analized by NMR of 300 MHz.

Results / Discussion / Conclusion

The chemical fractionation of the water extract of leaves of *P. guianensis* yielded the isolation of the glycosylated monoterpene indole alkaloid, named strictosidinic acid. The structural identification was made from the NMR data analyzes (1D and 2D) and compared to literature (Nascimento, 2006).

The biggest doubt in the chemical structure determination was the configuration of stereochemistry of the C-3. To clarify that, it was used the reference of Wenkert and collaborators (1961), were they determine the stereochemistry by the chemical displacement, were á position has the lower chemical displacement (around 3.4 ppm), while β is around 4.4 ppm. As our substance shows the signal in 3.74 ppm, we determine the relative stereochemistry as a.



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OR34
VALORIZATION OF ARTICHOKE BY-PRODUCTS AS SOURCE OF BIOACTIVE COMPOUNDS

Imma Pagano, Anna Lisa Piccinelli, Rita Celano, Luca Campone, Graziana Roscigno, Enrica De Falco and Luca Rastrelli
Department of Pharmacy, University of Salerno, via Giovanni Paolo II, 132, 84084, Fisciano, Salerno, Italy

Introduction

Artichoke (*Cynara scolymus L.*) is an ancient herbaceous perennial plant, originating from Mediterranean area (Lattanzio et al., 2009). Artichoke represents a rich source of bioactive compounds, like as phenolic compounds, inulin, fibers and minerals (Orlovskaya et al., 2007) and its extracts exhibit hepatoprotective, anticarcinogenic, antioxidative, antibacterial activity and inhibition of cholesterol biosynthesis and LDL oxidation (Gebhardt, 1997). The edible part of artichoke plants represents about the 30-40 % of its fresh weight, therefore huge amounts of by-products are produced, in particularly external bracts and leaves.

Method

The aim of this research is the valorization of the artichoke by-products as a potential source of bioactive compounds. Two variety of Campania region (Bianco di Pertosa and Romanesco C3 di Paestum) were studied and the content of secondary metabolites (caffeoylquinic acids and flavonoids) and inulin was evaluated in bracts and leaves.
Phenolic profiles of exhaustive extracts, obtained by ultrasound assisted extraction, were established by UHPLC-HRMS and tandem mass spectrometry (MS^n) analysis using a hybrid ion trap-orbitrap mass spectrometer.

Results / Discussion / Conclusion

Mono- and dicaffeoylquinic acids, luteolin and apigenin glycoside derivatives were identified as the main bioactive compounds of artichoke bracts and leaves. Their amounts were determined by quantitative UHPLC-UV analysis. Inulin content was established by AOAC method (McCleary et al., 1997).
According to our results, the artichoke by-products are a potential source of bioactive compounds for food and health applications. Further studies are in progress to obtain extracts rich in phenolic compounds and inulin.

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OR35
EXTRACTION OF GALANTHAMINE FROM NARCISSUS BULBS USING NATURAL EUTECTIC SOLVENTS (NADES) AND HOT PRESSURIZED EXTRACTION (HPE)

Rachmaniah, O.[1]; Wilson, E. G.[2*]; Choi, Y. H.[3]; Verpoorte, R.[4]; Vitkamp, G-J.[5]
[1]*PhD student, University of Leiden/Natural Products Lab.- IBL, Leiden,The Netherlands*
Delft University of Technology, Biotechnology Dept., Fac. Applied Sciences, Julianalaan 67, Delft, The Netherlands.
Inst. Technology of Sepuluh Nopember, Chemical Engineering Dept. Surabaya, Indonesia
[2]*PhD, University of Leiden/Natural Products Lab.- IBL, Sylviuweg 72, Leiden,Netherlands*
[3]*Assistant professor.University of Leiden/Natural Products Lab.- IBL, Leiden,The Netherlands*
[4] *Professor, University of Leiden/Natural Products Lab.- IBL, Sylviusweg 23, Leiden,The Netherlands*
[5] *Professor,Delft University of Technology, Biotechnology Dept., Fac. Applied Sciences, Julianalaan 67,Delft, The Netherlands.*
**{e.g.wilson@biology.leidenuniv.nl*

Introduction

Natural deep eutectic solvents (NADES) are combinations of two, or in a few cases three, common primary metabolites that form a liquid when mixed in a determined ratio in certain conditions (Dai *et al.,* 2013a). Their components are mono or disaccharides, amino acids, simple organic acids (malic, lactic or citric acid) and other compounds such as choline chloride. The NADES have several advantages over other eutectic solvents or ionic liquids, being non-toxic, cheap and having all features of a "green solvent". They have been proved to increase the solubility of a great variety of scarcely water-soluble compounds such as rutin, quercetin, cinnamic acid, taxol, carthamin, ginkgolide B as well as the macromolecules gluten, DNA, and starch (Dai *et al.*, 2013b, 2013c, 2014). There are different types of NADES according to the nature of their components: acid, basic, neutral or ionic liquids. In this case, *Narcissus pseudonarcissus* alkaloids were extracted from dried bulb material with hot pressurized extraction using diverse NADES to compare their efficiency and selectivity. The main alkaloid in *N.pseudonarcissus* is galanthamine, an acetyl cholinesterase inhibitor that is used for the treatment of Parkinson disease. This method is proposed as an alternative to its synthesis or acid extraction from diverse natural sources which are the current methods of industrial production.

Method

The extraction was carried out using a Speed Extractor E-916 (Buchi, Switzerland) (HPE) with 11 different NADES. The optimised HPE conditions were 50 °C, 50 bar, 2 cycles of approx. 7 minutes each. The qualitative and quantitative composition of extracts was analysed using GC-MS, GC-FID and HPLC/DAD. The correlation between the type of NADES and the extractability of *N. pseudonarcissus* alkaloids was performed applying a multivariate data analysis (PCA) from SIMCA-P software (version 13.0 Umetrics, Umeå, Sweden).

Results / Discussion / Conclusion

Galanthamine, lycoramine, norgalanthamine, oduline, and haemanthamine were identified in all NADES extracts. The maximum yield (0.19% (DW)) of galanthamine was observed with acid NADES-CHCA (choline chloride: citric acid: 1:1) and MAS (malic acid: sucrose:1:1). Different types of NADES resulted not only in different yields but in different alkaloid profiles of the extracts, showing that they are selective and can be tailored to obtain certain compounds. These results show the potential of NADES as alternative green solvents for extracting *N. pseudonarcissus* alkaloids.

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OR36
BENZOFURAN-TYPE NEOLIGNANS FROM *Ocotea heterochroma* AND THEIR BINDING MODE WITHIN PAF-RECEPTOR THROUGH MOLECULAR DOCKING

Rozo-Lugo, M. C.[1]; Cuca-Suárez, L. E.[2]*; Coy-Barrera, E.[3]
[1]*Master Student, Department of Chemistry, Universidad Nacional de Colombia, AA 14490, Bogotá, Colombia*
[2]*Prof. Dr., Department of Chemistry, Universidad Nacional de Colombia, AA 14490, Bogotá, Colombia*
3PhD, School of Science, Universidad Militar Nueva Granada, AA 49300, Cajicá, Colombia
**lecucas@unal.edu.co*

Introduction

The Lauraceae family is characterized as an excellent source of secondary metabolites, e.g., neolignan-type, which have exhibited a variety of biological activities [1]. One of the most important biological activities is the inhibition of the platelet-activating factor (PAF)-receptor. PAF is well-known to be involved in the body's inflammatory response as well as vascular diseases such as thrombosis. Currently, the use of computational techniques, such as molecular docking, has been widely used especially in the pharmacology field to establish the possible interaction mode of a molecule within the active site of an enzyme. Such approach becomes as useful tool for establishing the interaction/activity of naturally-occurring compounds within the active site of PAF-receptor. Thus, as part of our research on lauraceous compounds, two neolignans were isolated from the ethanol-soluble extract from leaves of Ocotea heterochroma by conventional and instrumental chromatographic methods. Their structures were elucidated by spectroscopic techniques. The structures of the isolated metabolites were docked within the active site of the PAF-receptor in order to establish their binding mode and determine its potentiality as PAF-antagonist.

Method

Starting from dried leaves of *O. heterochroma* (1220 g) an ethanol-soluble extract were obtained (120 g) by percolation. 80 g were fractionated through soxhlet using in turn several solvents (EdP, CHCl3, EtOAc, MeOH). CHCl3-soluble subfraction was fractionated by VLC on silica gel using EDP-AcOEt (95:5) by increased polarity to give 15 fractions. From fraction 7, by successively conventional chromatography, 20 mg of a yellow liquid (1) were isolated and 60 mg of a mixture that required instrumental separation (semiprep. HPLC). The separation of the second neolignan was carried out on a Phenomenex C18 semipreparative column (150 mm, 10 mm) with a mobile phase TFA (0.005% in Milliq water) and MeOH (HPLC-grade) in gradient elution. The two structures were elucidated by 1H and 13C NMR (1D and 2D) and they were found to have benzofuran moiety. The relative configuration of the C7, C8, and C1' carbons were defined through the corresponding coupling constants. In addition, in order to observe the binding mode, Autodock Vina was used to dock the most stable conformers of the two isolated neolignans within the active site of PAF-receptor, a rhodopsin-like G protein-coupled receptor (GPCR). Test structures were optimized at DFT level using B3LYP functional and 6-31G basis set. Stability of enzyme complexes between enzymes and neolignans were investigated through Vina scores and selected residues interactions.

Results / Discussion / Conclusion

Two benzofuran-type neolignans, 1 and 2, were isolated from leaves of *O. heterochroma*. These neolignans were found to have novel structures being similar to mirandin-A and –B, two neolignans reported for Nectandra miranda, a plant also belonging to Ocotea clade. Good Vina scores were obtained for enzyme-ligand complexes interactions at different levels. In addition, the results indicated that the best poses were found to be different for each test compound as well as the active site residues involved into the ligand-enzyme binding. Residue-ligand interaction profile was correlated with Vina scores, exhibiting important structure-interaction relationships useful in further studies. This is the first study to provide an explanation at atomic level of the binding of this kind of neolignans into the above-mentioned enzyme.

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OR37
LC-BASED PROFILING COMBINED WITH CHEMOMETRICS OF *Lupinus* SPECIES FROM COLOMBIA

Castañeda-González, L. Y. [1]; Angarita-Pabón, S. P.[1]; Guerrero-Perilla, C.[2]; Bernal, F. A.[3]; Coy-Barrera, E. D.[4]*

[1]*Undergraduate student, Universidad Distrital Francisco José de Caldas, Carrera 4 26B-54, Bogotá, Colombia*
[2]*Chemist, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, 2nd floor, Cajicá, Colombia*
[3]*MSc, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, 2nd floor, Cajicá, Colombia*
[4]*PhD/Professor, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, 2nd floor, Cajicá, Colombia*
**ericsson.coy@unimilitar.edu.co*

Introduction

The species belonging to genus *Lupinus* (Fabaceae family) are plants whose distribution along tropical regions is extensive. Lupinus plants are mainly characterized by their rapid growth allowing them to be predominating in some regions. Several studies have been carried out regarding to the proteic value of the seeds of Lupinus specimens (Pastor-Cavada et al., 2009). Moreover, alkaloidal and flavonoid compositions of different species of Lupinus have been also determined (Ranilla et al., 2009; Wojakowska et al., 2013). However, Colombian Lupinus species have not been studied respect their biological activity and/or chemical composition. Therefore, as part of our research on Colombian Fabaceous plants, a chemical characterization through chromatographic profiling for the ultrasound-assisted derived extracts of some Colombian Lupinus species are showed in the present study.

Method

Fifteen different specimens of Lupinus, including *L. bogotensis, L. mutabilis, L. guascensis,* and *L. pubescens*, were collected in different locations of Bogotá and Cundinamarca, Colombia. All specimens were corresponding to purple-blue colored flowers. Each sample was divided into leaves, seeds, flowers and stems and then dried and ground. Each sample was then submitted to ultrasound-assisted extraction protocol with ethanol as solvent. Resulting ethanol-soluble extracts were analyzed by UFLC-UV-DAD after separation conditions optimization. Finally, chromatographic profiles were compared by means of multivariate statistical tools including typical unsupervised two-way (PCA and HCA) and three-way (PARAFAC) methods.

Results / Discussion / Conclusion

Chromatographic profiling of the crude extracts of Colombian *Lupinus* species were developed by means of UFLC-UV-DAD. Some samples exhibited dissimilar profiles, including extracts derived from parts of the same plant. In addition, the location and taxonomy were variables that seemingly infer chemical variability to some samples. Multivariate-derived score plots demonstrated the above-mentioned remarks, indicated by the obtained clustering, which were examined according these variables. Furthermore, multivariate-derived loading plots allowing observe distinctive compounds for some samples, which are interesting for isolation purposes. The present work constitutes the first attempt to chemically describe, through LC-based profiling combined with chemometrics, some Lupinus plants growing in Colombia as an excellent tool conducted to further metabolomics studies on these plants.

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OR38
NMR METABOLITE PROFILING OF BLUEBERRIES

Mannina, L.[1,2]*; Capitani, S.[2]; Proietti, N.[2], Delfini, M.[3]; Carradori, S.[2]; Sobolev, A. P.[2]
[1]*Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", CNR, Monterotondo, Rome, Italy*
[2]*Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Rome, Italy*
3Dipartimento di Chimica, Sapienza Università di Roma, Rome, Italy
**luisa.mannina@uniroma1.it*

Introduction

The increasing ability of NMR spectroscopy to solve spectra of complex mixtures and to recognize and quantify each component without chemical separation, has found a constantly increasing application in metabolomics and food chemistry (Mannina et. Al). As a non-specific high-throughput analytical method, NMR spectroscopy is well suited to the requirements of metabolite profiling having the advantage to detect signals due to many different classes of compounds in the same experiment. This methodology has shown to be a valuable tool for the qualitative and quantitative analysis of metabolites and nutraceuticals presents in food-stuffs. In this presentation, untargeted NMR metabolite profiling of blueberries aqueous and organic extracts as well as targeted NMR analysis focused on anthocyanins and other phenols are reported. Bligh-Dyer and microwave-assisted extractions were carried out and compared.

Method

Classical extraction was performed according to a modified Bligh- Dyer methodology. MAE was performed using an automatic Biotage InitiatorTM 2.0. SPE was performed on C18 column to isolate phenolic compounds.
The NMR spectra of aqueous and organic blueberry extracts were recorded at 27°C on a Bruker AVANCE 600 NMR spectrometer operating at the proton frequency of 600.13 MHz.

Results

Water-soluble metabolites belonging to different classes such as sugars (glucose, sucrose, fructose and myo-inositol), amino acids (alanine, threonine, arginine, asparagine, aminobutyrate, glutamine, glutamate, valine and leucine), organic acids (malic, citric, quinic and shikimic acids), and phenolic compounds, as well as metabolites soluble in organic solvent such as triglycerides, sterols, and fatty acids, were identified. Five anthocyanins (malvidin-3-glucoside, malvidin-3-galactoside, delphinidin-3-glucoside, delphinidin-3-galactoside, and petunidin-3-glucoside) and 3-O-á-L-rhamnopyranosyl quercetin were identified in solid phase extract. Comparison of the two extraction techniques showed a better recovery of lipidic fraction in the case of microwave procedure.

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OR39
DEVELOPMENT OF FOOD PRODUCTS FROM AMAZON FRUITS OF ASAÍ (*Euterpe precatoria*), SEJE (*Oenocarpus bataua Mart.*) AND CANANGUCHA (*Mauritia flexuosa*) PALMS

Carrillo, M. P.[1]*; Peña, L. F. [1], Cardona, J. E. C.[1]; Díaz, R. O.[1]; Hernández, M. S.[1]
[1]*Instituto Amazónico de Investigaciones Científicas Sinchi, Calle 20#5-22, Bogotá D.C., Colombia*
**mcarrillo@sinchi.org.co*

Abstract
Biodiversity is one of the most important characteristics of the Amazonian region. Fruits of Amazonian palms are a significant source of bioactive compounds used for the development of food products with an economic and nutritional impact to the native communities of the Colombian Amazon. Bromatological and chemical composition of Asaí (Euterpe precatoria), Seje (Oenocarpus bataua Mart.) and Canangucha (Mauritia flexuosa) fruits were characterized to obtain food products for food sovereignty by local indigenous communities and/or their commercialization. Seje fruits presented high levels of ether extract (40.0% in mesocarp and 24.0% in seed) and high contents of oleic acid (86.4% d.w). Conversely, high levels of Anthocyanins (cyanidin-3-glucoside 1136.312 mg/kg of sample) and crude fiber (42.43% d.w), as well as low levels of carbohydrates (18.28% d.w) were found in Asai fruits. The main components of Canangucha fruits were carotenoids (beta-carotene 11.1 mg/100g of sample), oleic acid (61.9%) and ether extract (33.1 and 4.01% in mesocarp and seed, respectively). High amounts of carotenes and anthocyanins are associated with the antioxidant capacity of the latter species. The antioxidant properties and the ability to replace synthetic pigments (Del Pozo et al, 2004; Yahia, 2010), make these fruits suitable for their use in different applications, such as agro foods and nutraceutical products. From Asai fruit, pulp with high content of anthocyanins (cyanidin-3-glucoside 1026.6 mg/100g of sample) was obtained for the preparation of ice cream, yogurt and cookies in combination with native starch. Baked products and ice cream formulations were also obtained from Seje and Canangucha pulps. These baked products were mainly developed for nutritional purposes. From Amazonian palm fruits, it has been possible to obtain essential food products for local consumption within native communities of the Colombian Amazon, as well as the development of products for commercialization that can be included in the value chains of the region.

Keywords: Palm fruits, nutritional foods, food sovereignty, antioxidants

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OR40
PERUVIAN AMAZON OLEO RESINS

Katia Peralta Hinojosa, Elsa Rengifo Salgado
Instituto Peruano de Investigación Fitoterápica Andina (Ipifa). Instituto de Investigación de la Amazonia Peruana (IIAP)
erengifo@iiap.org.pekperaltah@terra.com

Introduction

The Peruvian Amazon, is a region world-renowned for its biodiversity in medicinal plants, and for being an important historical epicenter of traditional amazon medicine, for the benefit of health. These species of oleo resins are found in Peru in the departments of Ucayali, Madre de Dios and Loreto, and extend to Brazil in the area of the Acre River, to a lesser extent, Colombia, Guyana and the Antilles.

Method

The information was obtained by means of interviews with some ethnic groups of the amazon forest, and endorsed by their millennial experience, data survey and collection of samples, which were sent to the laboratory for their botanical identification and phytochemical routine analysis.

Results and discussions

The samples collected for the study are:
Ungurahui ------ Oenocarpus bataua
Copaiba---------Copaifera pauper
Sacha Inchi------Plukenetia volubilis

Sacha Inchi, is a vegetal oleaginous species found wild in the jungle of Peru, it is important for its high content of in saturated fatty acids, Omega 3,6,9 and proteins containing seeds, ideal for improving the human diet.

Ungurahui is a amazon palm, it is very important for the high content of omega oil 9 (82% to 85%) to bring antioxidants and nutrition's for the skin and to accelerate the reparation of the tissue.

Copaiba is a tree of the amazon jungle, from which en oil is extracted, it has cicatrizan, Detoxifying and anti-inflammatory properties, the main active substance are: Copaiba acid, Elacico acid, essentials oils, trementina and others.

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OR41
VEGETAL BIODIVERSITY AND PRODUTIVES CHAINS IN AMAZONAS (BRAZIL)

Costa, R. C.
Social Studies Laboratory, National Institute of Amazonian Research - INPA, Manaus, AM, Brazil - INPA, Manaus, AM, Brazil.
**rei@inpa.gov.br*

Introduction
Studies of the production chain (value and supply) are widely used to identify and analyze ways of market projection in society and nature in different scales of economy and market with differentiated uses of techniques and technologies that can add more value to final product. Thus, it is possible to define economic and social circuits of a given natural product, but also the social and ecological economic relevance of certain species. In that sense the presence of public policies for the improvement and strengthening of productive chains only highlight the importance of the issue as an social, economical and political reality connected to the use of plant biodiversity.

Method
For this study, a bibliographical research was done, there were done field work related to the spatiality of the production chain in different cities of the state of Amazonas, concerning about political, economic, technological, environmental and social matters.

Results / Discussion / Conclusion
Although the predominant idea of supply chain studies is only to identify bottlenecks in the production circuit and without the presence of social, economic relations only in scale from large companies, this is not our approach. Firstly, the idea of supply chain is to identify and map all the steps and subjects (social, economic, political, etc.) that are part of the production-distribution-circulation-consumption (P-D-C-C) involving its derivations such as: wholesale and retail, assistance technical and credit (including credit and funding policies), also involves the conflicts (social, landowners, environmental, competitions, training of value and price, exchange process, among others), due to the specificity of the productive chain, of the product, of the working conditions and the political and economic sphere in which it operates. Being carrier of circuits and spatial scales territorialized by division of labor and capital at different scales. Production chain is not restricted to a set of steps P-D-C-C, and processing and transfer of goods and technology being in the business or in the industrial sector. At each step of the P-D-C-C (internal and external) there are divisions of labor and access to capital and decision-making centers and market power, but in an interdependently way among subjects (economic and political). This occurs with pressures and demands of the market, the government and also derive specializations and more efficient interaction among individuals and corporations and institutions (public or private) involved. With greater aggregate capital, interest groups, technology, enter in the theme the communication between locations and the spatial distribution of goods and investments. Logistics as a gestion tool combined to stocks and flows that ensure the circulation of bioproducts, e.g., what makes the movement these bioproducts, in this context occurs the appreciation of the places at different scales of interaction, generating the expressions as glocal or glocalization. The understanding of the geo-economic and geoecological spaces identifies and analyzes the processes of different groups and / or societies, agricultural systems and production systems (social, economic, cultural and political), working with markets (commodities), property rights regime, division labor, income, training of the value / price, land use, among other process. As different forms of circuit and scales of economy and market.

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OR42
COMPARATIVE ANTIDIABETIC EFFECT OF *Tamarindus indica* Linn. SEDES AQUEOUS EXTRACTS WITH INSULIN AND GLIBENCLAMIDE

Naranjo-Rodríguez E. B.[1]*, Albores-Velasco M.[2], Toledo-Carrión C.[1a], Esquivel-Delgado, J. A.[1a.], Reyes-Valdés R.[1a]

[1]Prof/PhD/Prof, Facultad de Química, Departamento de Farmacia. Universidad Nacional Autónoma de México. Edificio A, Ciudad Universitaria, Delegación Coyoacán 04510, México D.F., México.

[1a] Student/Student. Facultad de Química, Departamento de Farmacia. Universidad Nacional Autónoma de México, Edificio A,Ciudad Universitaria, Delegación Coyoacán 04510, México D.F., México.

[2]ProfPhD/Prof, Facultad de Química, Departamento de Química Orgánica. División de Estudios de Posgrado. Universidad Nacional Autónoma de México. Edificio B. Ciudad Universitaria, Delegación Coyoacán 04510, México D.F., México.

**eliab@unam.mx*

Introduction

Diabetes Mellitus (DM) is a chronic disease discovered which effects carbohydrates, fats and proteins metabolism which produces hyperglycemia and different organs damage (Robbins and Cotran, 1991) as a result of the deficient secretion of insulin. The prevalence of DM in the World affects almost 6 % of the population (Norma Oficial Mexicana, 1994). Treatments to control the illness include a several activities and education. Some patients require the use of oral or intramuscular hypoglycemic. Sulfonylurea and metformin are valuable treatments for hyperglycemia in no-insulin-dependent DM, but they are often unable to lower glucose concentrations to normal levels, besides of producing side effects and high expenses for private and public health services [4] [5]. On the other hand more than 400 traditional plant treatments for DM have been recorded, but only a small number of them have received scientific and medical evaluation to assays their efficacy [6] [7]. An alternative low cost treatment, base don´t natural products is highly desirable [8] [9]. Tamarindus indica Linn (TiL) has been used as a traditional medicine for the treatment of diabetes its antidiabetic activity was studied by Maiti (Maiti et al., 2004). The objective was the evaluation of the antidiabetic activity of Til aqueous extracts on diabetic mice and its comparison with the activity of glibenclamide and insulin (Altshuler 2004).

Method

We used ICR Male mice (30 ± 2.5 g, UNAM-Harlan Laboratories), with diabetic (Mikio et al., 2001), with free access to food and water (12 light/12h periods). TiL (dose: 80 g/kg) was bought from a Mexico City market in September 2004. The pulp was eliminated and the seeds were cleaned and milled 150 mL distilled water were added to 1.5 g seeds. The suspension was boiled until evaporation of 75 mL water. After cooling, 75 mL were added methanol to the aqueous extract (AE) in order to eliminate proteins and complex carbohydrates. The methanol was fully evaporated and the initial volume (150 mL) was recovered with distilled water. This data were compared with administration of glibenclamide (2.5 mg/kg) and insulin (0.04 IU). We performed histopathological studies.

Results / Discussion / Conclusion

In animals treated with AE, the water consumption and body weight are similar to those observed when insulin was administered, whereas the treatment with glibenclamide, produced and increment in the food consumption and water demand. Animals body weight significantly diminished three symptoms for diabetics, polyphagy, polydipsy and poliuria. The hypoglycemic effect of Til on normal and diabetic mice is confirmed in this work. A toxicity evaluation of the AE and histological observations of internal organs of treated mice was carried out. No toxicity or lethality was found.

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OR43
EFFECTS OF CHRONIC TREATMENT WITH AQUEOUS EXTRACT OF "*PASSIFLORA EDULIS*" SEEDS IN RATS INDUCED TO OBESITY AND DYSLIPIDEMIA

Reis, F. O. V.[1*]; Tourino, J. G.[1]; Baracho, N. C. V[2]
[1]*Student, Medicine School of Itajubá, R: Tenente Lucrécio G Júnior, 368, Itajubá, Minas Gerais, Brazil*
[2]*PhD Prof, Medicine School of Itajubá, R: Tenente Lucrécio G Júnior, 368, Itajubá, Minas Gerais, Brazil*
**flavia9220@hotmail.com*

Introduction

A diet with poor nutrients, but with high lipid index adding a sedentary lifestyle is correlated with higher incidence of hyperlipidemia, hypertension and atherosclerotic disease. A study by the World Health Organization, predicts that by 2015 there will be 700 million obese worldwide and with obesity comes hyperlipidemia, hypertension or diabetes and these are risk for cardiovascular and atherosclerotic disease.
Due to the absence of a single drug for the treatment of these factors, the study *Passiflora edulis* seed is relevant due to its possible efficacy studies for the treatment of obesity and hyperlipidemia. Therefore, this study aimed to evaluate the effect of chronic treatment with aqueous extract of *Passiflora edulis* seeds on weight, lipid profile and blood glucose in rats induced to obesity and dyslipidemia.

Method

It used 20 male newborns Wistar rats. Obesity and hyperglycemia were induced by monosodium glutamate, 4mg/kg, subcutaneously, from the 2nd to the 11th day, on alternate day. From the 4th week until the end of the experiment, the animals were supplemented with cholesterol, to induce light dyslipidemia. On the 8th week, the animals were randomized into 4 groups (n =5): 1- Control- tap water; 2- Treatment 1- aqueous extract of *Passiflora edulis* seeds (500mg/Kg); 3- Treatment 2- aqueous extract of *Passiflora edulis* seeds (1,000mg/Kg); 4- Drug - simvastatin (10mg/kg/day). Finishing the experiment, under anesthesia, Ketamine (50mg/kg)/ Xylazine (25mg/Kg), intraperitaneously, intracardiac puncture was made to get blood samples to measure: total cholesterol; HDLc; LDLc; VLDLc; triglycerides; urea; serum creatinine and glucose.

Results / Discussion / Conclusion

Treatment 1 reduced significantly triglycerides and VLDL-C compared to control ($p<0.05$). Treatment 2 reduced significantly total cholesterol, triglycerides, LDL-c and VLDL-c ($p < 0.05$). As expected, simvastatin significantly reduced total cholesterol, LDL-c, VLDL-c and triglycerides ($p <0.05$). However, none treatment produced significant changes in glucose levels, HDL-c, creatinine and urea ($p >0.05$). In conclusion, Treatment with *Passiflora edulis* seeds extract was effective in reducing the levels of plasma lipids, since it reduced total cholesterol, triglycerides, LDLc and VLDLc, without producing significant changes in renal function. On the other hand, treatment with the extract produced no significant changes in glucose and HDL-c.

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OR44
FAST AND ECO-FRIENDLY ANALYSIS OF BIOACTIVE MOLECULE

Bonaccorsi, I. L.[1*]; Donato, P. [2]; Inferrera, V.[1]; Dugo, P.[1,2]; Mondello, L.[1,2];
[1] *Univerity of Messina, Dipartimento SCIFAR, V.le Annunziata, Messina, Italy*
[2]*University Campus Bio-Medico, Centro Integrato di Ricerca, Álvaro del Portillo 21, Rome, Italy*
**ivana.bonaccorsi@unime.it*

Introduction

This study is part of a wide project aimed to stimulate the reduction of the food waste and to recover biologically active ingredients (nutraceuticals) to be used in functional food. In this contribution, we describe the separation of carotenoids in foodstuffs without the need for time-consuming evaporation and reconstitution step after extraction, in a short time, and with organic solvent consumption reduced, noteworthy. The study was performed by Ultra Performance Convergence Chromatography (UPC^2) which brings in the advantage of a reduced system volume, resulting in a greatly reduced run time, improved resolution, and increased detection sensitivity The optimization of the carotenoid profile is deeply discussed and a quantitative analysis of beta-carotene is also demonstrated. An additional example of the advantage of UPC^2 is demonstrated in this presentation by the successful application on triacylglycerols (TAGs) determined in different vegetable oil fingerprint. The method here discussed allow to clearly differentiate different oils based on a short analysis (less that 10 minutes) compared with a similar analysis performed by conventional HPLC. Also in this case the method development and the results are deeply discussed.

Method

Carotenoids analysis:

System: ACQUITY UPC^2. Detector: ELS, nebulizer heating, 55°C, gas press. 40 psi, gain 100. Column: Acquity UPC^2 HSS C18 SB, 100×3.0 mm, 1.8 μm d.p. Column temperature: 20°C. ABPR: 2200 psi. Injection volume: 2 mL.. Sample solvent: 50:50 n-hexane/MTBE. Mobile phase A: compressed CO_2. Mobile phase B: EtOH.

For quantitative determination of beta-carotene:

Flow rate: 1.5 mL/min. Elution mode: gradient (0 min, 2% B, 3 min, 7% B, 6 min, 7% B, 8.50 min, 20% B, 8.60 min, 2% B, 11 min, 2% B).

For free xanthophylls separation:

Elution mode: gradient (0 min, 0% B x 5 min, from 5 to 20 min, 25% B, x 2 min).

Triacylglycerols analysis:

System: ACQUITY UPC^2. Detector: ELS, nebulizer heating, 55°C, gas press. 40 psi, gain 100. Column: Acquity UPC^2 HSS C18 SB, 100×3.0 mm, 1.8 μm d.p. ABPR: 3000 psi. Injection volume: 2 mL. Sample solvent: *n*-hexane, IPA, or 50:50 CH_2Cl_2/MeOH. Flow rate: 1.0 mL/min or 1.5 mL/min. Mobile phase A: compressed CO_2. Mobile phase B: ACN or EtOH. Elution mode: isocratic (2% B) or gradient (0-10% EtOH in 15 min; 0-40 ACN in 15 min).

Results / Discussion / Conclusion

The application of UPC^2 to carotenoid analysis has proved to be a reliable method for rapid quantitative determination of beta-carotene in vegetable extracts. In addition the chromatographic behavior of carotenoids separated by UPC^2 seams to be promising in order to separate and identify free xanthophylls, powerful antioxidant compounds in vegetable matrices.

Combining the use of supercritical CO_2 with sub-2-ÿ'3fm particle columns, good resolution can be obtained on a single column, in as little as 10 min analysis time, for the separation of TAGs in a variety of vegetable oils with excellent repeatability (average retention time CV 0.21%). Replacing the more commonly used ACN with EtOH as a co-solvent, leads to a series of advantages: four-times reduction of solvent consumption, one-third reduction of the flow rate, ten-fold increase in the sensitivity. Remarkably, such improvements are achieved by using a less toxic, and more "green" solvent.

ACKNOWLEDGMENTS
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OR45
BIOACTIVES FROM *Grewia asiatica* FRUITS: POTENTIAL FOOD-PLANT EXPLORED FOR NUTRACEUTICAL DEVELOPMENT

Siddiqui, J.[1]*, Abbaskhan, A.[1], Naheed, S.[2], Jalaluddin, A. J. A.[3] and Choudhary, M. I.[1, 3]
[1]*H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi; Karachi-75270, Pakistan*
[2]*Department of Biotechnology, Jinnah University for Women, 5-C, Nazimabad, Karachi-74600, Pakistan*
[3]*Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah-21412, Saudi Arabia*
**juverias@hotmail.com*

Introduction
Commonly consumed plant-foods available in local market were evaluated for their bioactive potential using antioxidant assays, which eventually explored for nutraceutical development in future.

Method
Globally consumed, commonly available plant foods in Pakistan were screened for their bioactive potential. Two radical scavenging assays namely, ABTS•+ and DPPH• radical scavenging assay and iron chelating capacity assay were used for primary screening of more than hundred plant food extract. Out of nineteen active extracts selected through the in-vitro assays, seven were selected for in-vivo anti-oxidative and hepato-protective evaluation. The extract of Grewia asiatica L. (Phalsa, a common fruit used in summer due to its cooling effects) exhibited good in-vitro and in-vivo antioxidant and hepatoprotective properties. Various fractions of G asiatica extract exhibit normalization of liver enzyme and bilirubin level as compared to standard (Trolox). Antioxidant activity-guided isolation on the fruits of G. asiatica L. has led to the isolation of a new compound, isorhamnetol 5-O-[6"-(3-hydroxy-3-methyl glutarate)] â-D-glucoside (1) in addition to ten known compounds, kaempferol 3-O-â-D-glucoside (2), kaempferol 3-O-á-D-rhamnoside (3), quercetin 3-O-â-D-glucoside (4), quercetin 3-O-â-D-rhamnoside (5), quercetin 3-O-(2-ñ-coumaroylglucoside) (6), myricetin 3-O-â-D-xyloside (7), 5-hydroxymethylfurfural (8), 3,4-dihydroxybenzoic acid (9), 1,5-dimethyl citrate (10), and trimethyl citrate (11). The structures of the isolated compounds were deduced by using mass, 1D- and 2D-NMR spectroscopic techniques. Except compounds 2 and 4, all others were obtained for the first time from this plant. A Trolox equivalent antioxidant capacity (TEAC) measurement on compounds 1-11 showed that some of them (3, 6, 8-9) possess potent antioxidant activity.

Results / Discussion / Conclusion
Plant-foods which are available for local population consumption and also commonly used internationally were first screened for their bioactive potential using antioxidant assays. Short listing and selection were done by analysis on more precise assays and animal model. Detailed phytochemical evaluation was achieved on selected Phalsa fruit. In addition to new compound, several active constituents as new source were reported from active fractions. Hence the underutilized fruit crop is explored as potential candidate for nutraceutical development.

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OR46
EFFECTS OF TWO BISBENCYLISOQUINOLINE ALKALOIDS, TETRANDRINE AND ANTIOQUINE IN RAT AORTIC RINGS: COMPARISON TO VERAPAMIL

José L. Martinez[1], Silvia Vargas-Rueda[2], Harold Gomez[3], Raúl Vinet[4] & Claudio Laurido[5]
[1]Vicerrectory of Investigation, Development and Innovation, Universisity of Santiago of Chile
[2]Facultad de Química Ambiental, Universidad Santo Tomás, Bucaramanga, Colombia
[3]Faculty of Pharmaceutical Science, University of Cartagena, Colombia
[4]Faculty of Pharmacy, University of Valparaíso, Valparaíso, Chile
[5]Faculty of Chemistry and Biology, University of Santiago of Chile
joseluis.martinez@usach.cl

Introduction

The search for new anti-hypertensive natural compounds is an interesting field of research. High blood pressure is a global problem causing thousands of deaths or permanent disabilities. Tetrandrine, a bisbenzylisoquinoline (BBQ) alkaloid isolated from the Chinese plant *Stephania tetrandra,* has been characterized as a calcium antagonist of natural origin and a Ca^{2+} voltage-dependent channel blocker. Also, Antioquine, BBQ isolated from the Colombian plant *Pseudoxandra esclerocarpa*, shows a similar effect to diltiazem. Numerous BBQ alkaloids are known (Schiff, 1991; Martinez, 2003), isolated from many species, with the genus Berberis the most studied chemically and pharmacologically in Chile (Martinez, 1986; Morales et al., 1989; Martinez et al., 1997). Such structures have been characterized as potential antagonists of naturally occurring calcium, being the most important studies developed with tetrandrine, and antioquine, among others. The BBQ alkaloids are constituted by two benciltetrahidroisoquinolinic structures, by one to three ether linkages.

Method

Adult white rats of the Wistar strain were used (weighing between and 200-300 g) of both sexes, with a balanced diet and water *ad libitum*. The aorta was isolated and placed in Krebs-Henseleit solution, bubbled with 95% O_2 and 5% CO_2 in a Petri dish. From each aorta, two rings of 4.58 ± 0.15 mm long and 1.47 ± 0.09 mg dry weight were obtained. They were mounted in a thermorregulated organ bath (37° C ± 1 °C) according to the technique described by Illanes et al. (1993). One hour stabilization later, the Krebs-Henseleit solution was changed to a depolarizing solution (70 mM KCl). Aortic rings developed maximum tension of 683 ± 59 mg/mg of dry (100% strain) tissue. After two KCl depolarization, the aorta was incubated with verapamil, tetrandrine and antioquine.

Results / Discussion / Conclusion

Tetrandrine, a BBQ alkaloid isolated from the Chinese plant *Stephania tetrandra*, antioquine, BBQ isolated from the Colombian plant *Pseudoxandra esclerocarpa*, After depolarized with KCl, a maximum contraction-calcium dependent occurs in two phases, assayed for tetrandrine and antioquine, using verapamil as control. The resulting effects on phases 1 and 2, antioquine showed a blockage of 57% compared with verapamil and tetrandrine, 37% for phase 1. For phase 2, antioquine reduces in 66% and tetrandrine in 9% compared to verapamil. We can conclude that these compounds block the movement of calcium from both intracellular and extracellular deposits, with the greatest effect when aortas are in the presence of endothelium. The development of new anti hypertensive drugs using these compounds could become a reality if pursued further investigation.

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OR47
β-CRYPTOXANTHIN BIOACCESSIBILITY IN MILK-FRUIT BEVERAGES CONTAINING ORANGE JUICE

Pumilia, G.[1,2] *; Stinco, C.[2]*; Giuffrida, D. [1]; Melende-Martinez, J-A.[2]; Dugo, G.mo[1]; Vicario, I-M. [2].

[1]. University of Messina Department of Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute (S.A.S.T.A.S), Viale F. Stagno d'Alcontres, 31, Messina, Italy

[2]. Seville University, Laboratory of Color y Calidad de Alimentos. Facoulty of Farmacy 41012,

*gpumilia@unime.it - * cstinco@us.es

Introduction

Fruits and juices are a healthy source of bioactive compounds such as carotenoids, tocopherols and vitamin C, which consumption is related to a reduction of the risk of suffering certain diseases (such as heart disease, cancer, macular degeneration)(Prior et. all., 2000 Kris-Etherton et. all., 2002). Interest in -cryptoxanthin, has increased recently in relation to its role in bone homeostasis (Yamaguchi., 2012). In this sense, fruit juice beverages containing also vitamins, milk and minerals, named Bio-functional fruit juice, are being widely consumed as an alternative to fruit juices.

One important aspect to consider in relation to bioactive compounds, such as carotenoids, is their bioavailability. A number of factor that affect the bioaccessibility/bioavailability are described in the literature, as the nature and contents of carotenoids, the presence of fat and fiber in the food matrix, the individual nutrient status, genetics aspects and interactions among these variables, as well as the effect of the industrial process (Rodriguez-Amaya., 2001). However, there is scarce information about the effect of the new matrix formulation (milk-fruit-juice), on the bioavailability of the bioactive compounds with respect to the equivalent in the fresh juice (Granado-Lorencio et. all., 2007). The aim of this study was to evaluate the relative bioaccessibility (BA) of -cryptoxanthin in commercial milk-fruit beverages containing orange juice actually present in the market.

Method

Samples: n=22 commercial milk-orange beverage were purchased in local hypermarkets in Seville Spain.

Bioaccesibility: An *in vitro* static digestion method proposed by the COST action FA 1005 INFOGEST (Dupont et al., 2011) was used. The samples were extracted with 3 ml of a mixture of hexane: acetone (1:1 v/v), dried, saponified with 300 ìl of dichloromethane and 300 ìl of 30% (w/v) of KOH in MEOH. The coloured dichloromethane extracts were concentrated to dryness in a rotary evaporator, dissolved in a known volume of ethyl acetate prior to their injection in the HPLC system. The analyses were performed in triplicated. The percentage of relative bioaccessibility (RBA) was calculated as follow: % bioaccessibility carotenoid = (ìg/ml carotenoid micellarized)/(ìg/ml carotenoid samples) x 100.

Results and discussion

The -cryptoxanthin (μg/100ml) content in the samples was in the range from 3 to 51 μg /100ml. The highest amount of -cryptoxanthin (51 ± 0,05 μg/100ml) was detected in the sample which contained a 15% of orange, carrot and peach from juice concentrated, 10% of skimmed milk, -carotene as colorant and pectin. However it showed only the 28 % RBA of -cryptoxanthin. On the contrary to this sample, the lowest content of -cryptoxanthin (3 ± 0,00μg/100ml), was detected in a sample, which contained 50% of orange, apple, pineapple and lemon mix from concentrated, 10% of skimmed milk, soluble fiber, ACE vitamins, and pectin. Also, it showed a higher RBA of the xanthophyll (82%). In addition to these results, two samples, which contained a mix of 25% of concentrated fruit juices that are rich in -cryptoxanthin, like papaya and orange, the xanthophyll, wasn't unexpectedly detected. According to the results obtained for RBA, a wide range of values for the -cryptoxanthin RBA was obtained (from 19% to 84%). Comparing the RBA of orange-milk-fruit juices beverages with fresh orange fruit juice (Stinco et al., 2012), the RBA of -cryptoxanthin in the formers were higher (50% vs 40%). On the other hand, in contrast with literature (Cilla et. all. 2012) the RBA of -cryptoxanthin in a sample containing a 17% of skin milk and sunflower-oil, was not higher (24%) than in those with no oil. Factors related to food processing (type and amount of fruit juice, thermal treatment etc.) could influence -cryptoxanthin RBA.

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OR48
TOWARDS A DE-COLONIAL ETHNOBOTANY

Noboa Viñán, Patricio
PhD, Escuela Superior Politécnica de Chimborazo (ESPOCH), Riobamba, Ecuador
gnoboa@espoch.edu.ec

Introduction

The traditional approach to ethno-botany is the ´study of plants used by primitive and aboriginal populations´ has evolved into a discipline which focuses on ´the relationship and influence of plants in the development of cultures´. Its application includes theoretical frameworks for reference and methodology that have covered areas such as: qualitative analysis of the usage and change (Gómez-Veloz, 2002; Martín, 2001); estimates of the ecological impact and its most effective use (Hall y Bawa, 1993); experiences of management and application in conservation (Prance et al., 1987); recognition of intellectual property rights (Zent, 2003); even, interdisciplinary studies of ethno-pharmacology, bio-prospecting, agro-ecosystems, sustainable development, bio-geography and biodiversity conservation (Khafagi y Dewedar, 2000; Frei et al., 2000). Despite this, the key pillars of ethno-botany remain the same as evidenced by limited change in its original functional theory, as well as epistemological and theoretical bases. As such, this work proposes alternatives to the traditional approach to ethno-botany from a de-colonial perspective.

Method

The methodology for a de-colonial approach to ethno-botany is based in the following:
Characterization of the political conditions of Ecuador as: a) Plurinational state; b) With the recognition of the "rights of nature"; c) With the constitutional mandate of "maintaining, protecting and developing collective knowledge; science, technology and ancestral knowledge; genetic resources that contain biological diversity and agro-biodiversity; traditional medicines and techniques, including the right to recover, promote and protect places of ritual and sacred significance, as well as plants, animals, minerals and ecosystems in the related territories; and the knowledge of resources and properties of fauna and flora" (Constitución de la República del Ecuador, 2008)
The de-colonial theory, which implies "the necessity to visualize, face up to and transform the structures and institutions that position groups, practices and thoughts in an order and logic […] based in a colonial approach […] and challenge and break down the socio-political and epistemological structures […] that support patrons with engrained powers […] based in a euro-centric perspective, and the categorization of some beings as sub-human and in the subordination or total exclusion of other theories, philosophies and ways of life" (Walsh, 2006).
The revitalization of culture as a key driver and promoter of strengthening the cultural identity of peoples
The mainstreaming of the participation of human groups as carriers of knowledge, technologies and management practices of biodiversity, in relation to their knowledge and experience

Conclusion

The socio-political context that Ecuador and Latin America currently exhibits, requires the elaboration and application of new ways of thinking and applying ethno-botany, based not only in methodological changes, but also epistemological ones. The de-colonial approach to ethno-botany overcomes the differentiation between primitive and civilized, and recognizing cultural differences, supports the recovery of the genetic heritage of the peoples and nationalities of Ecuador.

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OR49
CYTOTOXIC EFFECT OF AN ETHANOL EXTRACT OF *Phyllanthus accuminatus* LEAVES ON HUMAN EPITHELIAL CANCER CELLS

Sequeira-Obando, M. A.[1]*; Calvo-Castro, L. A.[1]; Moreira-González, I.[1]; Arnáez-Serrano, E.[1]; Navarro, M.[2]

[1] *Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica.*
[2] *Escuela de Química, Universidad de Costa Rica.*
**aldertos@hotmail.com*

Introduction

Cancer is responsible of one in four deaths in the United States (1). In 2012, 14.1 million new cases of cancer occurred worldwide; from which more than a half appeared in less developed regions. The worldwide most common cancers are lung, female breast, bowel and prostate. Lung cancer represents the 10% of all diagnosed cancers in men (2). In Costa Rica, lung cancer is the third most common type of cancer in men, and the fifth most common type of cancer in women (3). In this regard, it has been reported that plant extracts from the genus *Phyllanthus* sp. can suppress the growth of lung cancer cell lines, along with other types of cancer cells derived from cervix, liver, uterus, stomach, breast and colon (4). Particularly, Petit (5) has shown that the administration of secondary metabolites from an ethanolic extract of roots of *Phyllanthus accuminatus* could be useful for treating adenocarcinoma of lung, small cell carcinoma of lung, breast carcinoma, colon carcinoma, and others. Unpublished results from our group of research have shown that an ethanolic extract of leaves of *P. accuminatus* contains high concentrations of antioxidants, suggesting a possible anti-cancer effect. Thus, the objective of this study was to evaluate the cytotoxic effect of an ethanol extract of leaves of *P. accuminatus* (EPA) and to analyze the expression of molecular apoptosis biomarkers activated by this extract on diverse human cancer cell lines.

The half maximal inhibitory concentration (IC_{50}) of EPA was determined in lung (H460), colon (SW480), and breast (MCF7) cancer cell lines, in spontaneously transformed human skin keratinocytes (HaCaT), and in rat epithelial primary culture cells (QR3) from our laboratory. The cells were treated with six dilutions of EPA at 24, 48 and 72 hours, at which the number of metabolic active cells was measured with an MTT assay. The dose, at 24 hours of exposure to EPA, which reduced in 50% the viability of cells was lower in H460 cells (220 ìg.ml^{-1}) than in all other cell lines evaluated (228 ìg.ml^{-1} in SW480, 393 ìg.ml^{-1} in MCF7, 302 ìg.ml^{-1} in HaCaT, and QR3 wasn't affected at any of the evaluated doses). This indicates that EPA had a greater cytotoxic effect in lung cancer cell than in colon and breast cancer cells, and also that EPA was cytotoxic to transformed human epithelial cells, but did not affect rat epithelial primary culture cells.

Following this data, we studied several apoptosis biomarkers by western blot, immunofluorescence, and immunocytochemistry assays (6), in H460 cells treated with EPA for 24 hours. Caspase 3 fragmentation and PARP cleavage were detected, suggesting that EPA induced an apoptotic response on H460 cells. Also, we found evidence that EPA might activate the extrinsic apoptotic pathway by activation of the membrane receptor TNF-R1 and the death domain adaptor protein TRADD, coupled with the Fas-associated death domain (FADD). This was confirmed by caspase 8 cleavage. Caspase 9 cleavage was also detected, as well as the presence of apoptosis-inducing factor (AIF) at the nucleus, which suggests that EPA might also have involvement in the intrinsic apoptotic response pathway. Finally, the proliferating cell nuclear antigen (PCNA) wasn't affected by EPA. This study shows the potential of EPA to reduce the viability of lung cancer cells, and the induction of apoptosis through the extrinsic activation of the caspase signaling pathway. This study has been funded by Instituto Tecnológico de Costa Rica and Universidad de Costa Rica.

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OR50
STEVIA... MORE THAN A SWEETENER? ANTI-INFLAMMATORY ACTIVITY OF *Stevia lucida*

Blanco F., Salazar F., Fraile S., Michelangeli F., Ruiz M., Fernández A., Taylor P.
Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas, Venezuela
fblanco@ivic.gob.ve / ptaylor@ivic.gob.ve

Introduction

Venezuela shows great biodiversity with about 30.000 plant species. More than 1,500 are used medicinally by indigenous communities, but only a small number have been evaluated for their anti-inflammatory activity. The commercial use of Stevia rebaudiana as a sweetener has overshadowed the possible biomedical properties of the Stevia genus, such as for the treatment of diarrhea, stomach pain, dysmenorrhea, etc. Stevia lucida leaves are used for the treatment of rheumatism, inflammation and as a poultice to heal wounds in South and Central American communities1-4.

Methodology

We screened 165 plant extracts from 92 species for their capacity to inhibit nitric oxide (NO) production by macrophages stimulated with LPS for 48 h, using the Griess assay. Also, we assessed cytotoxicity on cells using the Sulforrodamine B assay. The anti-inflammatory activity *in vivo* was evaluated using carrageenan-induced edema in the hind paw of BALB/c mice. These assays were used to guide fractionation and identification of the bioactive compounds.

Results and discussion

The ethanolic extract of the leaf of S. lucida, selected from the 92 plant species evaluated, inhibited NO production *in vitro* with IC50=45 µg/ml. Of three fractions, separated by solubility in methanol/H2O and acetone, the two apolar fractions, E1 and E2, showed the greatest activity (IC50=26 and 20 ìg/ml respectively), but only E2 reduced inflammation in the carrageenan model by 30% compared to the control group. Further fractionation (solubility in CH3Cl) led to the separation of an active subfraction (E2-1) which showed a similar effect to E2, and which is presently being evaluated in terms of a) identification of the compound or compounds present, and b) their activity in other assays for inflammatory mediators such as tumour necrosis factor and interleukin-6.

We can conclude that the ethanolic extract of Stevia lucida leaves and the sub-fractions inhibit nitric oxide production by macrophages and diminish the inflammatory effect of carrageenan in BALB/c mice.

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OR51
CONSERVATION ISSUES OF TREE FLORA IN NATURAL HABITATS OF TOTALAI TRACT DISTRICT BUNER

Iftikhar Ahmad Khan and Habib Ahmad
Department of Genetics, Hazara University Mansehra, Pakistan
iftikharkhan2006@yahoo.com

Abstract
Generally trees are being combated by threats of different types. Totalai Tract, having an area of 29,853 hectares is no exception from the general rules. We communicate the result of an intensive scientific survey of 32 trees species belonging to 25 families recorded here. The data obtained from extensive field observation, interviews with the traditional experts, scientific professional and herbaria record, treated against Rabinowitz methods and categorized according to the IUCN Criteria revealed that out of the 32 recorded species 1, 1, 7, 12, 10 and 1 established under the Least Concerned, Near Threatened, Critically Endangered, Endangered, Vulnerable and Regionally Extinct categories respectively. It was concluded that almost all the trees found in the natural condition, are under severe extinction whose nature and extent varied from place to place. The conservation issues faced by the arboreal vegetation included; illegal cutting, habitat losses, agriculture extension and mineral mining, all these were due to the lack of the sense of ownership of natural conservation in the flora. The area needs complete protection, reintroduction of the suffered species, and recovery of the degraded habitat.

Key words
Trees; Conservation Issues; Rabinowitz Method; IUCN Criteria; Totalai Buner

OR52
RHEOLOGY STUDIES IMPLEMENTATION IN DEVELOPING SOLID DOSAGE FORMS IN NATURAL PRODUCTS

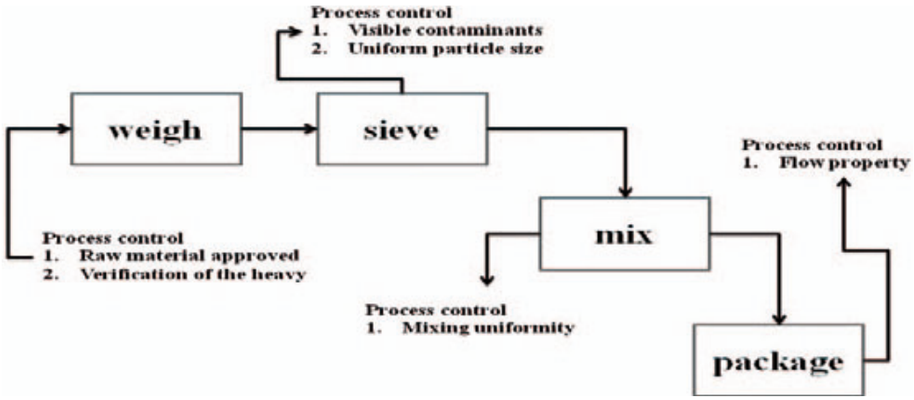
Archbold R.[1]; Caballero C. S.[2]; Quiñones W.[1]; Echeverri L. F.[1]; Escobar G.[1]; Torres L. F.[1]
1.Instituto de Química-Grupo de Química Orgánica de Productos Naturales Universidad de Antioquia, Calle 62 52-59, Medellín-Colombia
2.Facultad de Ciencias, Departamento de Física, Universidad de Burgos, Burgos, España

Introduction

Bingham (1929) defines "rheology" as the branch of physics that studies the deformation and flow of matter, when subjected to the action of external forces. From there, two behaviors that may benefit or otherwise impair the development of a product are released. In the first case, the state of deformation remains after the external force has ceased their action and in the second case, with elastic materials, the granular formulation recovers its original shape once the applied external force disappears, situation unwanted when we make any tablet or tablet.

For this reason and because it is widespread use of extracts to develop pharmaceuticals rheological studies are needed both to the active substance as to its components so that they can understand and control the features of the final formulation, allowing processes propose more efficient and thus achieving dosage forms of the highest quality possible.

The scheme 1, shows the different steps required involved in the production of the simplest dosage form and process controls required in accordance with Good Manufacturing Practices



Scheme 1, Steps and process controls required for the manufacture of solid dosage forms such powders

Methodology

Using the direct method, double compression method and wet method were developed three granules of a standardized extract of a natural product. To each of these granules were performed testing dynamic angle of repose, Carr index, median particle diameter, allowing to know the rheological properties of the different preparations and thus to analyze the behavior of the granules when a tablet is elaborate and their impact on the effectiveness of the product in terms of its dissolution.

Results

Comparing the results of the tests, we found significant differences between the granules, and when we make tablets, they showed variations in specifications (the testing of tensile strength, friability and dissolution).

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OR53
AN INVESTIGATION ABOUT THE ANTIBIOTIC AND DYE RESISDUES IN AQUACULTURE PRODUCT OF ALBANIA

SELAMI Fejzo[1]; KOLA Suzana[2]; VODICA Ani[2]
[1]PhD/Prof/, Agriculture University of Tirana, Faculty of Veterinary Medicine, Department of Veterinary Public Health, Tirana, Albania
[2]Msc. Food Safety and Veterinary Institute, Department of Toxicology and Veterinary Drugs residues, Tirane, Albania
*Prof.Associate Fejzo SELAMI *fselami@ubt.edu.al*

Abstract
The aquaculture in Albania is developed and a range of fish diseases have been encountered. The diseases in Aquaculture have a high impact due of the economical losses this lead to an increased use of veterinary drugs in production systems to combat diseases. Antibiotics are used to treat infections of farmed fish including infection from Aeromonas spp, Edwardsiella spp. Pasteurella spp. Vibrio spp. etc for all these pathogens the antibiotics are used as feed pellets.
The main hazards are antibiotic residues and the developed of the antimicrobial resistance in consumers. Chemicals dyes as Malachite green used as disinfectants for fungal and parasites infestation is a very potential factor for increase of the cancer rate. The monitoring of some antibiotics residues in aquaculture products will provide evidence of usage of those antibiotics that used in in aquaculture farms in random sampling of fish and other aquaculture products. Sample collection includes fish and shrimps and is performed by government officers and samples are analyzed in Department of Toxicology and Veterinary Drugs in Food Safety and Veterinary Institute.
We collected about 300 fish and shrimp's samples which means 100 samples per year. All samples were tested for the residues of different antibiotics and dyes. The investigation includes a 6 year period (2010-2013), the samples are randomly collected, the method used for the determination was ELISA test.

Key words: aquaculture, food safety, antibiotics, dye, samples, residues, Albania

OR54
SICILIAN RURAL COOKING AND THERAPEUTIC VIRTUES OF SOME MEDITERRANEAN SPONTANEOUS PLANTS

Crescenti, C., Cicero, N., Cardile, G., Alfa, M., Dugo, G.mo
Dipartimento S.A.S.T.A.S, Università degli studi di Messina, Viale F. Stagno D'Alcontres 31, Messina, Italy

Introduction

Poverty is the central issue of the history of Sicilian cooking. It's a well-known fact that a long series of dominations brings to Sicily heterogenous culinary traditions. Perhaps, throughout the ages, a set of recurrent and interconnected culinary *mores* comes to life. Certainly, it's a subject of intrinsic fascination but characterized by a lot of troublesome questions for all those who try to understand the influence of a certain typical dish. For them, there is the risk to get lost through plenty of subjects and disciplines, ranging across mythology, history, literature, medicine, religion, maybe astronomy too. The shepard of the Sicilian tradition – in Theocritus (III sec. B.C.) painted with some sophistication as a polished intellectual capable of speaking in metric about every subject – collected the spontaneous herbs destined to feed the beasts and cooked them with an arranged oven. These spontaneous plants, in the Sicilian language named *e.g, cavuliceddi, finucchieddi, cardiedda, pisciacani* represent an important way of expression of the traditional Sicilian cooking.

To put the question in an ancient medical way, it is to be reminded that in the history of knowledge about plants there is a significant quantity of ancient literary notations about therapeutic virtues of Mediterranean officinal herbs in human and veterinary medicine. So, if the wild fennel (*Foeniculum vulgare),* main ingredient of the typical Sicilian *Pasta con le sarde,* is considered by Plinius (I sec. A.D.) capable of heal eye diseases, the liquirice (*Glycyrrhiza glabra* L.) is considered by Theophrastus (IV sec. A.D.) useful against asthma or dry cough. Between poverty, foreign influences and popular medicine, the Sicilian farmer traditionally eats the above mentioned spontaneous plants or onion, bread, cheese, maybe a glass of wine. Breakfast at 10 a.m., after six long hours of hard work, generally consisting in a lemon salad, made with little peaces of lemon, water, oil and salt. Or, otherwise, *matarocco*, made with little pieces of tomatoes, water, salt, basilic and dry bread. It's interesting to notice how a typical Sicilian dish as *maccu di fave*, a fava bean soup, seems to get the name by a character of the Old Latin period, Maccus, an inextinguishable glutton of the ancient comedy. Alexis of Tarentum (IV sec. B.C.) affirms: "in Sicily I learnt to cook in a so delicious way that my dining companions would always eat even the plate". Sicilian cooking was famous in the ancient world and maybe the first real gourmet is Sicilian: Archestratus of Gela (IV sec. B.C.). In his *Life of Luxury* he canonizes the main gastronomy specialities of that time eulogizing the eolian crayfish, the eel of Messina, the smooth hound and the sturgeon of Siracusa. We believe that a sentence referred to *Pasta con le sarde* of Alberto Denti di Piraino, sophisticated scholar of Sicilian traditions, beautifully summarises the Sicilian soul: "Collect the wild fennel on the mountains where, even now, resound the echo of Pan's double chantered pipe (*zampogna*), catch the sardines in the sea where the Atenian triremes were defeated and cook them in Muslim way with pine nuts and grape, then put them in oven, gift of Norman seamen".

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OR55
CYTOTOXIC AND ANTI-INFLAMMATORY POTENCY OF *SESELI L.* OILS

Alev TOSUN, Jaemoo CHUN, Yeong Shik KIM
Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100, Ankara/TURKEY
Seoul National University, Natural Products Research Institute, College of Pharmacy, Seoul/REPUBLIC OF KOREA

Introduction

From ancient times, plants are huge sources for active constituents. Identification of pharmacologically active agents has an important side of natural product research. Essential oils are one of the active secondary metabolite of several medicinal and aromatic plants and have been demonstrated various important pharmacologic effects. Seseli is a genus of Apiaceae family and mostly grows in temperate locations of the World, known as aromatic and medicinal plant.

In the current presentation; two species called as Seseli gummiferum Pallas ex Smith (SG) and Seseli corymbosyum Boiss. & Heldr. ex Boiss. Pimenov and Kljuykov (SC) have been examined regarding their essential oils obtained by hydro-distillation from the fruits. Initially, the essential oils were analyzed by GC and GC/MS and the major compounds have been determined. Moreover, the cytotoxic and anti-inflammatory activity screening has also been performed on these oils. The cytotoxicity has been evaluated by MTT assay in three different human carcinoma cell lines HCT116, Caco-2, and AGS. The anti-inflammatory effects of essential oils have also been investigated in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. The oils of SC and SG inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expressions, as well as the downstream product, nitric oxide (NO). Investigation of the reporter gene assay on nuclear factor êB (NF-êB) showed that SC and SG inhibit the NF-êB transcriptional activity. Therefore, essential oils obtained from Seseli species have been investigated for their possible potency of cytotoxicity and anti-inflammatory effects in these assays.

KEY WORDS: Apiaceae, Seseli L., Cytotoxic, MTT, NF-êB

OR56
EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Juniperus* SPECIES USED IN TRADITIONAL MEDICINE IN PAKISTAN

Aman Khan[1], Gul Jan*[1] and Afsar Khan[2]
[1]*Department of Botany, Hazara University, Mansehra, Pakistan*
[2]*Department of Chemistry, COMSATS Institute of Information Technology, Abbottabad-22060, Pakistan*
drguljanhu@yahoo.com

Abstract

Present study intends to find out the antimicrobial activities and antioxidant potential of Juniperus communis L and Juniperus excelsa M.Beib of methanolic extracts and their fractions (n-hexane, chloroform, ethyl acetate, n-butanol and water). The antimicrobial activities of these extracts were tested against Gram negative, Gram positive strains of bacteria, and fungi. The crude methanolic extracts of J. communis L and J. excelsa M.Beib were fractionized in different solvents on the basis of polarity. The fractions (n-hexane, chloroform, ethyl acetate, n-butanol and water) were evaluated for their biological activities such as antioxidant, antibacterial, and antifungal. Antioxidant activities were determined by using stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and antibacterial activities were determined through Disc diffusion method against Gram negative strains of bacteria including Klebsiella pneumoniae, Pseudomonas aeruginosa and Gram positive strains of bacteria i.e., Bacillus atrophaeus, Bacillus subtilis, and fungi:Aspergillus niger, Aspergillus flavus. Extracts exhibited strong antioxidant activities and prominent zone of inhibition against both Gram positive and Gram negative strains of bacteria. Only n-butanol extracts inhibited the growth of fungal strain. Present work showed that J. communis L. and J. excelsa M.Beib possesses good antimicrobial activity against tested microorganisms. The results indicate that Juniperus species can be used as therapeutic agents for drug resistant bacteria.

Key words: Antimicrobial; Antioxidant; Juniperus communis L; Juniperus excels M.Beib

OR57
CHARACTERIZATION OF SICILIAN FOOD BY HR-MAS NMR

Corsaro, C.[1]*; Mallamace, D.[2]; Cicero, N.[2]; Salvo, A.[2]; Mallamace, F.[1]; Dugo, G.[2]
[1]Dipartimento di Fisica e Scienze della Terra, Università di Messina, Viale F. Stagno D'Alcontres 31, Messina, Italy
[2]Dipartimento SASTAS, Università di Messina, Viale F. Stagno D'Alcontres 31, Messina, Italy
*ccorsaro@unime.it

Introduction

We present our results on the metabolic profile of different Sicilian foodstuffs such as the PGI cherry tomato of Pachino, the PGI Interdonato Lemon of Messina, the red garlic of Nubia and several extra Virgin Olive Oils (VOOs) coming from many areas of Sicily. We use the experimental technique known as High Resolution Magic Angle Spinning Nuclear Magnetic Resonance on samples without any kind of treatment or extraction but only diluted in deuterated solvents.
Due to the relatively high price of this high quality products, commercial frauds originated in the Italian and international markets. Hence, there is a growing interest to develop analytical techniques able to reveal the origin of a particular foodstuff [1,2]. The most suitable approach in the field of biological systems seems to be that of Metabolomics, the scientific discipline able to identify and quantify the different metabolites that determine and characterize the studied bio-system in its particular state [3,4].
Our results indicate how by means of NMR spectroscopy it is possible to discriminate among different samples of different origin thanks to their different metabolic profile.
The experiments were performed by using a Bruker Avance spectrometer operating at 700 MHz equipped with the Magic Angle Spinning probe-head. By tilting samples of a precise angle (about 54.7°) with respect to the direction of the applied magnetic field, the Hamiltonian term corresponding to dipolar interactions vanishes and NMR peaks become narrower. Furthermore, by spinning the rotor at the magic angle by few thousands of Hertz, line broadening effects due to susceptibility differences within the sample are removed resulting in high resolution quality spectra. Few milligrams of sample were diluted in deuterated water or chloroform depending on the study, putted in 50 ÿ'3fl rotor and spun at 6000Hz. All spectra were processed (line broadening, Fourier transform, phase correction and baseline adjustment), by using the standard routines of the Bruker software Xwinnmr version 3.5. Peaks assignment was performed by means of bidimensional pulse sequences, literature data and a well-established software package: NMR Suite Professional version 7.1 (Chenomx, Alberta, Canada).

Results / Discussion / Conclusion

We were able to identify and quantify the main metabolic content of famous and protected Sicilian foodstuffs. Furthermore, we were able to find out which metabolites and the corresponding concentration values could be helpful to verify the origin of a chosen sample. In particular, the obtained metabolic concentrations may represent the fingerprint that would allow the discrimination between different species.

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OR58
CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT CAPACITY OF AGUAMIEL (*Agave atrovirens*)

Romero, L. M.[1]; Flores-Morales A.[2], Mora-Escobedo, R.[1]*
[1]*MSci/PhD/Escuela Nacional de Ciencias Biológicas. Instituto Politécnico Nacional.Plan de Ayala esq. Carpio s/n. Col. Sto. Tomás. C.P. 11320. México, D.F., México.*
[2] *PhD. Instituto Tecnológico del Altiplano de Tlaxcala*
**rosalmora@hotmail.com; shasen_33@hotmail.com*

Introduction

Aguamiel is used for elaboration of pulque by spontaneous fermentation. It has a higher proportion of water, followed of fructooligosaccharides (FOS), proteins, vitamins (B2, B6, and C) and minerals. Consumption of FOS has benefits against diseases such as hyperlipidemia, osteoporosis, compromised immune function, colon cancer, and ulcerative colitis. So that aguamiel may be a functional food (Jenkins et al., 1999).

Method

Aguamiel (A. atrovirens) was obtained from Contepec, Michoacán, Mexico. It is was sterilized (121 °C/15 min) in glass and stored at 4° C. The characterization was assessed following the AOAC, 2006 methods: moisture (986.21 method), ash (923.03 method), pH (981.12 method) and titratable acidity (939.05 method). Protein (Bradford, 1976), soluble solids (°Brix) by refractometer. Free, total and reducing sugars and sucrose (Ting, 1956), FOS (Ting, 1956), total saponins (Hiai et al., 1976); minerals (Perkin, 1996); hidrosoluble vitamins (Albdalá et al., 1997); ascorbic acid (USP, 1995); total phenols (Makkar et al., 1993) and antioxidant capacity by DPPH (Brand et al., 1995).

Results / Discussion / Conclusion

The moisture content was 89.61%, follow for the protein (0.35%), ash (03.%). The pH value was 6.29, titratable acidity (0.08%), soluble solids 11.10 °Brix. Total reducing sugars (2.9%), free fructose and glucose were (1.0% and 0.083%, respectively. The fructooligosaccharides content was 1.5.5% and total saponis 0.18%. It was found in major amount minerals such potassium (99.30 mg), calcium (10.49 mg) and sodium (7.45 mg). It was also determined iron (0.73mg), copper (0.64mg), magnesium (0.49mg), selenium (0.42mg) and zinc (0.16mg). The vitamins of the aguamiel were ascorbic acid (17.99mg), niacin (4.77mg), pyridoxine (0.57mg), riboflavin (0.38mg) and thiamine (0.09mg) per 100g sample. Total phenols reported as 302.49 μmol, gallic acid/100g dry weight and the antioxidant capacity was 872.0 μmol trolox/g dry weight. Aguamiel characterization shows a high content of FOS and similar values compared with other agave species as Agave mapisaga (Ortiz et al., 2008) and the antioxidant capacity was higher than the Tovar et al., 2011 (274 μmol trolox/g dry weight in Agave salmiana) found. Moreover the presences of phenols with a good antioxidant capacity are an additional characteristic of beverage. Although this activity may also be attributable to other components found. Due to the composition and prebiotics found in aguamiel, we are studying the effect of nutraceutical composition of aguamiel *in vivo* models, in order to propone healthier foods with aguamiel.

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OR59
GENOTYPE DIFFERENTIATION OF *HELICHRYSUM ITALICUM* (ROTH) G. DON FIL. SUBSP. *ITALICUM* BY MEANS OF ESSENTIAL OILS CARBON ISOTOPE RATIO ANALYSIS

Schipilliti L.[1]*, Bonaccorsi I.[1], Dugo G.[1,2], Dugo P.[1,2,3], Mondello L.[1,2,3]
[1] *University of Messina, Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute (SCIFAR), Viale Annunziata, Messina, Italy*
[2]*Chromaleont s.r.l. A start-up of the University of Messina, c/o Dipartimento SCIFAR, University of Messina, Viale Annunziata, 98168 Messina, Italy*
[3]*Università Campus Biomedico, Via Alvaro del Portillo, 21, 00128 Roma, Italy*
**lschipilliti@unime.it*

Introduction

Helicrysum italicum (Roth) G. Don fil. subsp. *italicum*, belongs to the *Asteraceae* family. This specie grows spontaneously on sandy and loamy soils, at altitudes ranging from sea level up to 1700 meters (Pignatti, 1984). Essential oils extracted from the green parts or from the flowers of thisplant are recognized to have several healing effects like, choleretic, diuretic and expectorant properties, as well as, anti-inflammatory, antioxidant, antimicrobial, anti-hypertension and anti-HIV activities (Leonardi et al., 2013). Active principles present in these medicinal plants can vary in base the genotypic and chemotypic of the same species. In order to differentiate and characterize the chemical composition of the samples, a preliminary study on the carbon isotope ratio of genuine *Helicrysum italicum* subsp. *italicum* essential oils, from Sicily and Corsica, was performed.

Method

In the present work, the essential oils, obtained from by steam distillation of the flavoring plants tops, were object of study. The samples were obtained from plants coming from Sicily and Corsica and harvested during the same flavoring period of June. The essential oils were preliminarily analyzed by GC-MS and the qualitative chemical profiles were established. Subsequently, the chosen common markers of the oils were analyzed by GC-C-IRMS.

Results / Discussion / Conclusion

GC-C-IRMS technique is able to establish the geographic origin of the matrices and it is employed in the metabolic studies of the living matter (Schipilliti et al. The secondary biogenetic pathways linked to the terpenes formation of *Helichrysum italicun* subsp. *italicum* oils, were investigated by GC-C-IRMS, highlighting the presence of two different samples genotypes. Moreover, the qualitative chemical profiles, performed by GC-MS analyses, suggested that the two genotypes show different chemotipyc, confirming that the environment aspects (geographic and climatic) contribute to influence strongly the chemical composition of these aromatic plants (Leonardi et al., 2013).

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OR60
PRELIMINARY EVALUATION OF THE CYTOTOXIC CAPACITY OF AN ETHANOL EXTRACT FROM *Moringa oleifera* LEAVES

Katherine Sánchez-Zúñiga[1*], Laura A. Calvo-Castro[1], Ileana Moreira-González[1], Mario Sequeira[1], Elizabeth Arnáez-Serrano[1], Mirtha Navarro[2]

[1]Centro de Investigación en Biotecnología, Instituto Tecnológico de Costa Rica, Costa Rica.[2]Escuela de Química, Universidad de Costa Rica, Costa Rica.

**kasazu021@gmail.com*

Introduction

Cancer is one of the leading causes of human death worldwide and on which there is not yet a definitive cure, or any medicines that can prevent it altogether. Most prevalent cancers include lung, liver, colon, stomach and breast (OMS, 2014). Current research is focused on searching fornew and betterways to prevent,detect andtreat this disease, including bioprospecting for relevant chemical and biologicalplant components. Many of the plant species thathave attracted the interest of these studiesis due totheir usein folk medicinethroughthe years. That is the case of *Moringa oleifera*, which has been used in traditional medicine for several therapeutic applications, including anti-inflammatory, hepatoprotective and anti-tumor treatments.(1). It has also been reported that moringa has lots of antioxidants, which have been associated with highly curative potential against cancer (2, 3).

In this project, we have studied the cytotoxic capacity of an ethanol extract from *M. oleifera* leaves, which was applied in two human cancer cell lines (H460 and SW480, lung and colon human cancer, respectively). The cell lineswere treatedfor 24 hours withvariousdoses of the extract (500 ìg/ml; 600 ìg/ml; 700 ìg/ml and 800 ìg/ml) and solvent controls (EtOH-500 ìg/ml; EtOH-600 ìg/ml; EtOH-700 ìg/ml and EtOH-800 ìg/ml). The cellswere washed oncewithPBSand cell viabilitywas determined byMTT assay. Then, a suitable half maximal inhibitory concentration (IC50) was determined. It was found thata dose of 500µg/mlofethanol extractfrommoringa's leaves was sufficient to reduce the viabilityofhumanlung cancercells by50.19%, whereas the samedosedecreasedby43.84% the viabilityof colon cancercells. This indicatesthat the extractofmoringahad a greatercytotoxic effect oncolon cancercells. In addition, we confirmed that theethanol solventdid not influence thecytotoxicityof the extract. Furthermore,the cytotoxicitywas confirmedby western blot, which indicated thatthis doseofextractinduced expression ofcaspase 3in both cell lines, indicating that the extractofmoringainduced celldeathbyapoptosis. This work has been funded by Instituto Tecnológico de Costa Rica and Universidad de Costa Rica.

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POSTERS

PO1
ANTI-AGES ALGERIAN VEGETAL EXTRACTS. PHYTOCHEMICAL STUDY OF *Daucus aureus* DESF. (APIACEAE)

E. Makhloufi,[1] S. Derbré,[2] K. Medjroubi,[1] A. El Omri,[3] H. Laouer,[4] E. Seguin[3] and S. Akkal[1*]

[1]Laboratoire de PAPB, Département de chimie, Faculté des Sciences exactes, Université Mentouri Constantine, Algérie. [2]Université d'Angers, SFR QUASAV 4207, EA 921 SONAS, UFR SPIS, Angers, France. [3] Université de Rouen, UMR CNRS 6014, COBRA, UFR Médecine-Pharmacie, Rouen, France. [4] Laboratoire de Valorisation des Ressources Naturelles Biologiques, Département de Biologie, Université Ferhat Abbas, Sétif, Algérie.

E-mail: salah4dz@yahoo.fr

Introduction

In a large project on Algerian plants, we are interested in their phytochemistry and biological properties (PHC franco-algérien Tassili, 2012-2016). Particularly, in diabetes, a chronic hyperglycaemia occurs which is responsible for complications of diabetes through advanced glycation end-products (AGEs) formation.[1] SONAS lab recently developed an automated HTS assay, suitable for compounds and extracts, to evaluate their anti-AGEs potential.[2] This assay was used to select potent anti-AGEs extract from a selection of plant species traditionally used for the treatment of diabetes in Algeria.[3] Among them, EtOAc and BuOH extracts from *Daucus aureus* (Apiaceae) were selected and their phytochemistry studied. Moreover, MeOH extract led to the isolation of flavonoid O-glucosides **1** and **2**, which structures were elucidated by spectroscopic methods, including UV, 1D and 2D NMR, MS.

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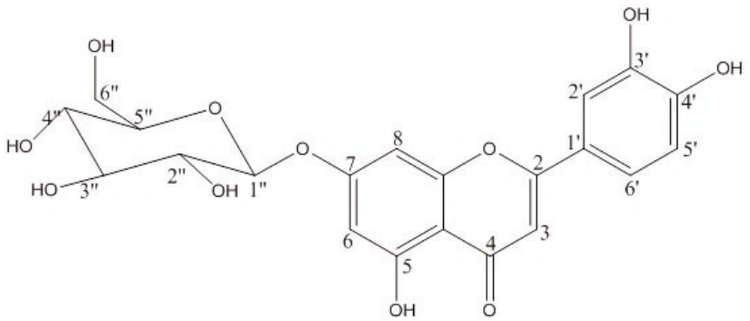
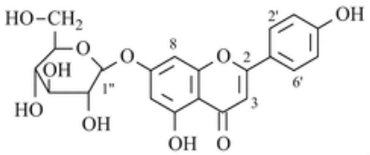


1



2

PO2
SQUARE WAVE VOLTAMMETRIC METHOD AS A TOOL FOR DETERMINATION OF OMEGA 3(Ω3) ESSENTIAL FATTY ACID QUANTITY IN OLIVE OILS AVAILABLE IN THE EASTERN ALGERIAN MARKET

Neghmouche Nacer Salah, Lanez Touhami*
University of El Oued, VTRS Laboratory, B.P.789, 39000, El Oued, Algeria
E-mail: neghmouchenacer-salah@univ-eloued.dz

Introduction

Fish oils, in general, are excellent sources for Omega-3 polyunsaturated fatty acids. One can find them in other plant sources like olive oil, wallnuts, almonds...etc [1]. Omega 3s have many beneficial effects on our health: they are considered as an important anti-inflammatory factor [2], show inhibitory effects on tumorigenesis, and reduce mortality from cardiovascular diseases [3]. Moreover, it has been shown that Omega-3 fatty acids may play a role in cognitive development [4], increase learning ability [5], and improve cognitive performance [6]. On the basis of these mechanisms, positive effects of Omega-3 fatty acids on dementia, schizophrenia, and other central nervous system diseases have been reported [7].

Omega 3s Fatty acids contained in foods and their implications on human health have been the subject of many scientific articles and reviews. Our study has for objective to determine the concentration of these fatty acids in olive oils extracted from olive fruits cultivated in different areas in East Algeria using SWV technique

Key words: olive oils, Omega-3, polyunsaturated fatty acids, Oils, square wave voltammetry.

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PO3
SYNTHESIS OF TETRACYCLIC TRAHYDRO-β-CARBOLINE

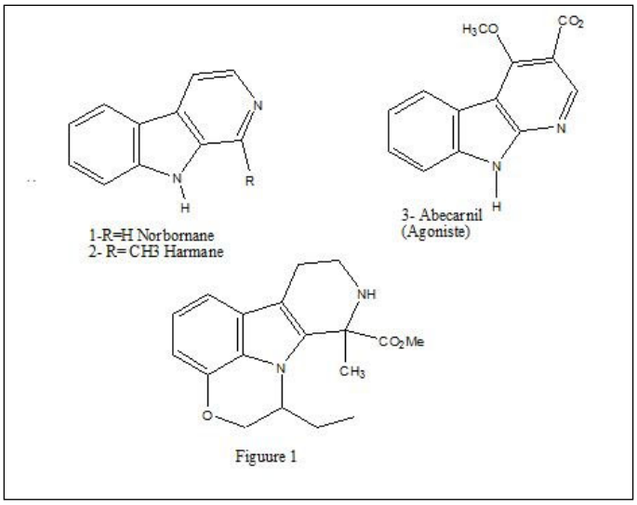
Abdelhamid Belattar*, Chafie Boukentoucha and Nedjda Belattar**
*Department of Chemistry, Faculty of Sciences, University of Constantine -I, 25000, Algeria**Hospital University Benbadis, Constantine - Algeria*

Abstract: There has been an enormous effort by various groups to achieve synthesis of β-carbolines, partly due the anxiolytic and hypnotic activity of a few of this class of compounds and the central place they occupy in the biosynthesis of monoterpene alkaloids**.**

Keywords: β-carboline, benzodiazepine receptor, muscarinic, alkaloid.

Introduction

The discovery of the biological activity and therapeutic effect of Norharman (1) and Harman (2) which contain a â-carboline skeleton as: antiparasitaire[1], antibiotic[2], antivirale[3], etc. has prompted intensive research activities for feasible syntheses of like compounds (3) (Figure1).



Figuure 1

Initially investigations were carried out towards the pentacyclic alkaloid which led to a synthesis of the important intermediate precursor compound (4). In view of the reported pharmacological properties of Abecarnil as anxiolytic, we hope that the new compound will have a significant therapeutically value.
During the last decade, the chemical and biological studies of â-carbolines have allowed a better comprehension bending affinities of the benzodiazepine receptor, muscarinic receptor and the development of the ZK 112119 (Abecarnil), first sedatif anxiolytic of â-carboline type.

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PO4
CHEMOTAXONOMY OF ERICALES

Rocha, M. E. N.[1*]; Figueiredo, M. R.[2]; Kaplan, M. A. C.[3]; Durst, T. [4]; Arnason, J. T. [5]
[1]PhD/Professor, Federal University of Rio de Janeiro – UFRJ, Carlos Chagas Filho, 373, office A1-050 – Health Sciences Centre, CEP: 21941-902 • Ilha do Fundão • Cidade Universitária - Rio de Janeiro, Brazil
[2]PhD/Researcher, Oswaldo Cruz Foundation,Rua Sizenando Nabuco, 100 – Manguinhos, Rio de Janeiro, Brazil
[3]PhD/Professor Emeritus, Federal University of Rio de Janeiro, Natural products research Institute, Ilha do Fundão, Rio de Janeiro, Brazil
[4]PhD/Professor Emeritus, University of Ottawa, Marie-Curie street, Ottawa, Ontario, Canada.
[5]PhD/Professor, University of Ottawa, Marie-Curie street, Ottawa, Ontario, Canada
*marcorocha04@ufrj.br

Introduction

The order Ericales, sensu APG III, belongs to the Asterids. It comprises around 25 families and 347 genera distributed in 4 clades: balsaminoids, polemonioids, primuloids and ericoids. These plants are widely distributed in arctic, temperate and tropical regions. Based on the recent modification related to the taxonomic systems of classification, was proposed for Ericales, the comparison between phylogenetic and chemical data. Most conclusions in the field of chemosystematics are still drawn from, and rely on non-phylogenetic botanical classifications such as those of Cronquist, Dahlgren and Takhtajan, whereas the new phylogenetic systems should yield a characterization of the chemosystematics profile consistent with the classification.

Method

Chemical data was obtained from surveys in different databases and from specific literature references, where 259 species had been the subject of some chemical investigation. The metabolites were organized with the occurrence number, ON = number of compounds of a chemical class produced by a taxon. The evolutionary advancement parameters related to hydroxyl protection mechanisms in flavonoids were: 1) protection by glycosylation (Evolutionary Advancement Parameter of Glycosylation, EAG), 2) protection by methylation (Evolutionary Advancement Parameter of Methylation, EAM), 3) double protection (Evolutionary Advancement Parameter of Glycosylation and Methylation, EAGM), 4) total protection (Evolutionary Advancement Parameter of Total Protection, EATP), 5) unprotected (Evolutionary Advancement Parameter of Unprotection, EAUN) and, 6) A-ring transformation (Evolutionary Advancement Parameter of A-ring transformation, EAATI), were evaluated by the weighted average of their flavonoid chemical indexes. These parameters were correlated to each other and to the following chemo-morphological and morphological indexes: the herbaceousness index (HI) and the Sporne index, (SI) which is based on the proportion of evolutionarily advanced characters displayed by a taxon.

Results / Discussion / Conclusion

Flavonoids and triterpenes were characterized as good taxonomic markers for the order, due to the great number of occurrences. Analyses of correlation among chemical parameters based on flavonoids and morphological and chemo-morphological indexes, showed a low phylogenetic affinity for certain groups, positioning Primuloids and Balsaminoids as the highest and the lowest evolutionary advanced taxa, respectively. Flavonoids produced are mainly flavonols rather than flavones. Hydroxyl protection occurs mainly by glycosylation and to a lower extent by methylation. These data confirmed the primitive status for the order.

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PO5
Chemical composition and evaluation of antibacterial activity of essential oil of *Espeletia schultzii* (Asteraceae) from state Trujillo – Venezuela

Judith Velasco[1*], Libia Alarcón[2], Alexis Peña[2], Deisy Ramírez[2], Alfredo Usubillaga[3], Billmary-Z Contreras-Moreno[3,4], Luis Rojas[3]

[1]Laboratorio de Síndromes Gastrointestinales y Urinarios (SGU) "Lcda. Luisa Vizcaya". Departamento de Microbiología y Parasitología. Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela

[2]Laboratorio de Productos Naturales Dr. Alfredo Nicolás Usubillaga del Hierro. Grupo de Investigación en Productos Naturales (GIPRONA). Departamento de Química y Biología. Universidad de Los Andes. Núcleo Universitario "Rafael Rangel". La concepción, Villa Universitaria. Trujillo-Venezuela

[3]Laboratorio ''C" de Productos Naturales del Instituto de Investigaciones de la Facultad de Farmacia y Bioanálisis. Mérida, Venezuela

[4]Laboratorio de Polímeros y Coloides (POLYCOL), Facultad de Ingeniería, Universidad de Los Andes, Mérida, Venezuela

[4]Laboratorio ''A" de Productos Naturales. Instituto de Investigaciones de la Facultad de Farmacia y Bioanálisis. Universidad de Los Andes. Mérida, Venezuela

* *judithvelasco2005@yahoo.es*

Introduction

The species *Espeletia schultzii*of the Asteraceae family, popularly known as frailejon is used in folk medicine of the venezuelan Andes for treatment of asthma. In the present study this plant was evaluated for its chemical composition and antimicrobial activity by two methods.

Method

Chemical composition of the essential oil from fresh leaves of *E. schultzii* (Asteraceae) obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS) is being described, in addition, antibacterial activity was also evaluated by two methods against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Salmonella* Typhi (CDC 57), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 23357) and *Pseudomonas aeruginosa* (ATCC 27853) and antifungal activity against *Candida albicans* (CDC- B385) and *Candida krusei* (ATCC 6258).

Results / Discussion / Conclusion

The results revealed this oil as rich in hydrocarbon monoterpenes according to the volatile compounds analysis performed by GC-MS that allowed the identification of 13 components, accounted for 100% of the essential oil, being the following major compounds: *α*-pinene (50.11%), *β*-pinene (16.28%) and *β*-myrcene (14.71%). This oil showed antibacterial activity with variation in the results depending on the method used. In this regard, by means of disc diffusion agar method, *E. schultzii* essential oil inhibited only Gram positive bacteria, *S. aureus* and *E. faecalis*, with inhibition zones of 7 and 9 mm of diameter and minimum inhibitory concentration values (MIC) of 280 and 580 µg/mL, respectively. Using broth microdilution method, the essential oil inhibited the growth of all tested bacteria with MIC values ranging from 10 to 100 µg/mL. These results proved that sensitivity on the method plays an important role in the evaluation of this oil as an antibacterial, being broth microdilution method more sensitive. Moreover, according to the literature reviewed this is the first report of antibacterial activity for the essential oil obtained from the leaves of this species.

Keywords:Espeletia schultzii, antibacterial activity, essential oil, á-pinene, â-pinene.

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PO6
BETULINIC ACID QUANTIFICATION IN LEAVES EXTRACTS OF *Eugeniaflorida* DC. (MYRTACEAE) OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

Nóbrega, A. B.[1,2*], Lessa, B. M.[2], Slana, G. B. C. A.[3,4*], Paiva, S. R.[1,5*]
[1]*Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, UFF, Rua Mário Viana N 523, Santa Rosa, Niterói, RJ, Brasil.*
[2]*Farmanguinhos, Fiocruz, Rio de Janeiro, Rua Sizenando Nabuco 100, Rio de Janeiro, RJ, Brasil*
[3]*Programa de Produtos Bioativos e Biociência, UFRJ, Macaé, Brasil*
[4]*Instituto Nacional de Propriedade Industrial, INPI, Praça Mauá N, Centro, Rio de Janeiro, RJ, Brasil*
[5]*Setor de Botânica, Departamento de Biologia Geral, Instituto de Biologia, UFF,* Outeiro de São João Batista s/n, *Niterói, RJ, Brasil.*
*abnobrega@gmail.com; glauciaslana@gmail.com; selmapaiva@id.uff.br

Introduction

Eugenia florida DC. belongs to the Myrtaceae family and is a commonly found throughout the Brazilian territory (Lorenzi, 2000). Betulinic acid is a triterpene found in the leaves of E. florida and presents several biological activities such as anti-inflamatory, antimalarial, antimicrobial and antiviral (Sami *et al.* 2006, Baltina *et al.* 2003; Hess *et al.* 1995; Pavlova *et al.* 2003). However, the most studied activity is the anticancer action (Pisha *et al.* 1995; Fulda *et al.* 1999; Ehrhardt *et al.* 2004). The aim of the present study was to compare the efficiency of five methods to extract betulinic acid from the leaves of Eugenia florida (static maceration, dynamics maceration, percolation, soxhlet and ultrasonic waves). The influence of some conditions such as different solvents and different particle sizes were also evaluated.

Method

The leaves of E. florida were collected at Fundação Oswaldo Cruz, Rio de Janeiro, Brazil during the month of August 2011 and a voucher was deposited in the Botanical Garden Herbarium of Rio de Janeiro, Brazil (RB 328.061). The leaves were dried in oven with air circulation at a temperature 25-30 °C and then were crushed in industrial blends. Five simultaneously experiments were carried out with different solvents: ethanol, methanol, ethyl acetate, chloroform and hexane applying different extraction techniques: static maceration, dynamics maceration, percolation, soxhlet and ultrasonic waves. The influence of particle sizes were also evaluated.

Results / Discussion / Conclusion

The results obtained on this study showed that the percolation and maceration extraction techniques presented the better content of betulinic acid. The solvents which best extracted betulinic acid were ethyl acetate and chloroform. It is known that the smaller of the particle size the greater will be the contact surface, this fact may favor the extraction of substances and reduce the process time. In the present study it was observed a significant increase in the extraction yield with smaller particle size of Eugenia florida, however for the betulinic acid content no significant differences were obtained for the different particle sizes of plant material.

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PO7
PROTECTIVEEFFECTSOF GREEN PROPOLIS EXTRACT AGAINST SYSTEMIC INFECTION RESPONSE: AN *IN VIVO* EXPERIMENTAL STUDY

Silva, M. V.[1]*; Paulino, N.[2]; Soares, A. C.[3]; Eudes-Filho, J.[4]; Assis, M. S.[5]; Ferreira, V. M.[6]
[1]PhD, Faculdade de Saúde, Universidade de Brasília, Campus Darcy Ribeiro, Brasília-DF, Brazil
[2] PhD, Universidade Bandeirante de São Paulo, Academia Paulista Anchieta, São Paulo-SP, Brazil;
[3]MSc Student, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas, Universidade de Brasília, Campus Darcy Ribeiro, Brasília-DF, Brazil
[4,5]Master, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas, Universidade de Brasília, Campus Darcy Ribeiro, Brasília-DF, Brazil
[6]PhD, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas, Universidade de Brasília, Campus Darcy Ribeiro, Brasília-DF, Brazil
* mvalero2@unb.br

Introduction
Sepsis is a systemic inflammatory response and it is on the major cause of morbidity and mortality in the intensive care unit. In recent years, even with many discoveries in the field of antibiotic therapy, only a few clinical benefits were observed in septic patients. The Brazilian flora is one of the biggest investments of pharmaceutical companies seeking alternative therapies. Propolis, for example, is one of these therapies because has antimicrobial properties.

Aim
Given that our country is rich in these natural resources, this study sought to evaluate the consumption of propolis in behavioral and cognitive changes resulting from systemic infection.

Method
It was used 20 Wistar male rats, 3 months old, anesthetized with ketamine + xylazine before the surgical procedure of cecal ligation and puncture (CLP). All animals were administered with the antibiotics clindamicine (25 mg/kg, i.p.) + gentamicin (3 mg/kg, i.p.) before surgery procedures, 24h and 48h latter. Propolis extract (100 mg/kg, i.p.) was administered in the same treatment regimen of the antibiotics. The behavioral and cognitive assessments were evaluated 72h after animals recovery. They were divided into 4 groups: 1) Sham+antibiotics, 2) Sham+propolis, 3) Sepsis+antibiotics, and 4) Sepsis+antibiotics, which were evaluated in the open field (locomotion), elevated plus maze-EPM (anxiety), forced swimming (depression) and step down inhibitory avoidance (memory) tests.

Results
The animals subjected to systemic infection decreased the percentage of frequency of entries and time spent in the open arms of the EPM. No changes were observed in locomotion, whereas in the forced swimming test animals that survived the infection showed a reduced immobility time, suggesting stress resulted from the reaction of flight and fight. Systemic infection survivor rats showed impairment in short and long term memories. Propolis extract maintained a response similar to the animals submitted to sham surgery, blocking the responses produced by systemic infection.

Conclusions
Propolis extract can prevent behavioral and cognitive changes resulting from systemic infection, leaving to investigate the cellular and molecular mechanisms involved in these therapeutic responses.

Keywords: Anxiety, Cognition, Depression, Propolis, Rats, Sepsis
Financial Support: PIBIC/CNPq/UnB.

PO8
ANTIOXIDANT ACTIVITY OF *Maytenus communis* REISSEK

Amaral, F. M. do[1]*; Anholeti, M. C.[1,2]; Valverde, A. L.[3]; Braga, J. M. A.[4]; Paiva, S. R.[1]; Joffily, A.[1]
[1]*Laboratório de Botânica Estrutural e Funcional,* UFF, Outeiro de São João Batista s/n, *Niterói, Brasil*
[2] *Pós-Graduação em Química de Produtos Naturais, IPPN, UFRJ, Bloco H, CCS, Ilha do Fundão, Rio de Janeiro, RJ, Brasil*
[3]Laboratório de Produtos Naturais, *Lapromar,* UFF, Outeiro de São João Batista s/n, *Niterói, Brasil*
[4] *Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, Rua Pacheco Leão 915, Jardim Botânico, Rio de Janeiro, RJ, Brasil*
**fernandamoreira@id.uff.br, anajoffily@id.uff.br*

Introduction

Maytenus communis Reissek is an endemic species of the Brazilian Atlantic Forest, occurring only in the state of Rio de Janeiro (Lombardi *et al.*, 2013). It belongs to the Celastraceae family, which contains several species said to be useful in folk medicine for treating a large number of diseases, such as gastritis and stomach ulcers (González *et al.*, 2000). This study aimed to evaluate the antioxidant activity of *Maytenus communis* crude extracts, since there is no previous work reported in literature involving its chemical aspects.

Method

For the present study, leaves and branches from an individual of *Maytenus communis* were collected at Mata do Entorno, Rio de Janeiro, Rio de Janeiro state, Brazil. The plant material was appropriately processed and subsequently subjected to static maceration extraction with ethanol, followed by the evaporation of the solvent under reduced pressure to obtain the crude extracts. The antioxidant activity of the ethanolic extracts of leaves and branches of *M. communis* was assessed by the scavenger of the free radical DPPH (1,1-diphenyl-2-picrylhydrazyl), using BHT (butylated hydroxytoluene) as positive control, according to the methodology proposed by Mensor *et al.* (2001) and Rufino *et al.* (2007), with modifications proposed by Silva and Paiva (2012). The experiments were performed in triplicate and results were expressed as mean ± standard deviation of three independent experiments. Statistical analysis was performed using ANOVA.

Results / Discussion / Conclusion

The extract concentration able to reduce to 50% the original concentration of DPPH is expressed by the EC50. The EC50 values of both extracts of *Maytenus communis* are statistically similar among each other, and lower than the value found for BHT (0,88 ± 0,09 g BHT/g DPPH), with 0,59 ± 0,08 g of extract/g DPPH for the leaves extract and 0,56 ± 0,10 g extract/g DPPH for the branches extract. Analyzing the reaction kinetics, it was observed that both extracts of *M. communis* showed an almost instantaneous response at each concentration evaluated. In 5 minutes of reaction, they exhibited an excellent antioxidant activity at the concentration of 50 µg/mL, with less than 10% of remaining DPPH, showing a better antioxidant activity than that observed for BHT. The results suggest that the extracts of *M. communis* are a source of antioxidant substances, and make this species an interesting target for further studies aiming at the chemical characterization of such substances.

The authors thank Universidade Federal Fluminense (UFF), CNPq and CAPES for financial support.

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PO9
CHEMICAL CONSTITUENTS OF THE RED SEA SPONGE *Biemna ehrenbergi*

Jihan M. Badr[1], Lamiaa A. Shaala[2], Gamal A. Mohamed[1], Faida H.Bamanie[3], Diaa T. A. Youssef[1]
[1]*Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia,*
[2] *Natural Products Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia,*
[3]*Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia*
jihanbadr2010@hotmail.com

Introduction

Marine sponges have gained a significant attention with respect to the diversity of their secondary metabolites. The biological activities of new metabolites from sponges have been reported in hundreds of scientific papers. Sponges have the potential to provide future drugs against important diseases such as inflammation (Festaa *et al.*, 2012), viral (Wellington *et al.*, 2000), plasmodial (Ang *et al.*, 2001) and bacterial (D_Ambrosio *et al.*, 1996). Moreover, as a result of the extensive efforts to explore bioactive metabolites offered by marine life, an interesting marine pipeline of significant anticancer agents was developed. Among the comprehensive examples are Psammaplins, Didemnin B and Dolastatin 10 (Quiñoà *et al.*, 1987; Rinehart *et al.*, 1987). In the present work, Biemna ehrenbergi sponge collected from Red Sea in Egypt was investigated for the active constituents that were isolated using different chromatographic techniques.

Method

Sponge was collected at Hurghada. Its characters comply with the specimen and subsequent descriptions of this common species. The Vouchers are registered in the collection of Netherlands Center for Biodiversity Naturalis under number ZMA Pro. 16622. The sponge was cut into very small pieces and macerated with methyl alcohol at room temperature. The total extract was concentrated under vacuum, fractionated using vacuum liquid chromatography, fractions were monitored by TLC and similar fractions were pooled together to obtain three main sub-fractions (A-C). Each of the three sub-fractions was concentrated under vacuum then fractionated several times using different chromatographic techniques including open column (packed with normal phase silica gel, reversed phase silica gel and Sephadex) and preparative thin layer chromatography. Finally, eight pure compounds were isolated.

Results / Discussion / Conclusion

Chemical investigation of the Red Sea sponge Biemna ehrenbergi extract resulted in isolation of eight compounds including an acetylinic acid, a hopanoid terpene, four steroids and two nucleosides. The isolated compounds were identified as (E)-tetracos-8-en-5-ynoic acid, 32,35-anhydrobacteriohopanetetrol, (24R)-ergosta-6,22-diene-5,8-epidioxy-3-ol, (22E)-ergosta-22-ene-5,6-epidioxy-6-methyl-3-one, (22E)-ergosta-5, 8, 22-trien-7-one-3â-ol, melithasterol B, thymidine and 2'-deoxyuridine. The structures of the isolated compounds were determined using different spectroscopic techniques including MS, 1D (1H NMR and 13C NMR) and 2D NMR (COSY, HSQC, and HMBC). On reviewing the literatures, three compounds were found to be new, isolated for the first time from a natural source. These compounds are (E)-tetracos-8-en-5-ynoic acid, (22E)-ergosta-22-ene-5,6-epidioxy-6-methyl-3-one, (22E)-ergosta-5, 8, 22-trien-7-one-3â-ol. The compounds were tested for their cytotoxic activities.

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PO10
SIMULTANEOUS DETERMINATION OF KHELLIN, VISNAGIN AND KHELLOL GLUCOSIDE USING VALIDATED HPLC METHOD

Jihan M Badr[1,2], Ghada M. Hadad[3], Khaled Nahriry[1] and Hashem A. Hassanean[1]
[1]*Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt*
[2]*Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia*
[3] *Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt.*
jihanbadr2010@hotmail.com

Introduction

Ammi visnaga crushed or powdered fruits have been used in folk medicine to relieve pain resulting from kidney stones after being prepared in the form of teas. Also its major components khellin and visnagin play a dynamic role in the prevention of stone formation hence protecting renal epithelial cells from damage (Haug et al, 2012; Vanachayangkul et al, 2010). Quantitative estimation of khellin or khellin and visnagin has been previously discussed via assorted methods of analysis but some of these procedures include several steps of purification by chromatographic methods before quantification (Orlov and Krupskaya, 1989; Abdel-Salam et al, 1985). More recently, the two furanochromones were estimated by a number of HPLC methods. (Zgorka, 1998). In Egyptian market, there are numbers of pharmaceutical formulations in which khellin, visnagin and khellol glucoside are included. To our knowledge, none of the most known pharmacopoeias or any journal includes the simultaneous determination of khellin, visnagin and khellol glucoside. Besides, the records concerned with follow-up studies of these drugs during their shelf life are not available.

Method

Ammi visnaga L. ripe and unripe fruits beside the selected pharmaceutical preparations (including tea bags and capsules) were extracted with methanol then directly injected without any purification and pre-separation processes. The isocratic reversed phase HPLC separation was performed on 5μm C18 column 25 cm length, 4.6 mm (internal diameter). Good resolution between khellin, visnagin and khellol glucoside was accomplished using a mixture of water, methanol and tetrahydrofuran (50: 45: 5, v/v/v) as a mobile phase at flow rate 1.3 mL/min. Quantitation was achieved with UV detection at 245 nm based on peak area.

Results / Discussion / Conclusion

The system suitability parameters such as retention time, retention factor, tailing factor, number of theoretical plates for khellin, visnagin and khellol glucoside peaks were calculated. All parameters comply with the acceptance criteria. When representative sample was tested, it was found that khellin, visnagin and khellol glucoside besides other components of Ammi visnaga extract were efficiently separated. The average retention time ± S.D. for khellol glucoside, khellin and visnagin were found to be 3.16 ± 0.03, 7.60 ± 0.04 and 8.90 ± 0.03 min respectively. Strict linearity was noticed between the peak area and the concentration over the range of 0.2-20, 1-30 and 0.5-20 ìg mL-1 for khellin, visnagin and khellol glucoside, respectively. Limits of detection were recorded as 2.26 x 10-3, 2.21 x 10-2 and 3.20 x 10-3 μg ml-1 while limits of quantitation were found to be 7.54 x 10-3, 7.13 x 10-2 and 10.68 x 10-3 μg ml-1 for khellin, visnagin and khellol glucoside respectively. The standard addition analysis method was used to validate accuracy. Additionally, intra-day precision, inter-day precision and method ruggedness were evaluated and all were satisfactory. The suggested method was successful in determination of the analytes of interest without any interference of the other compounds and matrix. All validation parameters were satisfactory and the procedure was relatively easy and fast as the extracts are evaluated without previous steps of purification. It is worthy to mention that, according to the available literature, this is the first study for the simultaneous evaluation of the khellin, visnagin and khellol glucoside and following up them during their shelf lives.

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PO11
PHYTOCHEMICAL INVESTIGATION OF *Phragmanthera austroarabica*

Jihan M. Badr[1]
[1]*Associate Professor, Department of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt*
jihanbadr2010@hotmail.com

Introduction

Family Loranthaceae comprises a number of the stem parasites; commonly known as mistletoes. Selected examples of them were investigated for chemical constituents and reported to accumulate flavonoids and phenolic compounds (Lin and Lin, 1999; Kim *et al.*, 2004; Al-Taweel *et al.*, 2012; Badr *et al.*, 2013). Phragmanthera austroarabica; a plant belonging to Family Loranthaceae was collected from Saudi Arabia. It is widely used in folk medicine among the kingdom in treatment of various diseases including diabetes mellitus (Din *et al.*, 2011). Upon reviewing the literature, it was found that no previous reports on the chemical constituents of P. austroarabica. In the present work, the plant was chemically investigated and the pure isolated compounds were evaluated for their free radical scavenging activities using DPPH reagent.

Method

The plant was collected during March 2011 from Abha – Khamis Mushat at the South of Saudi Arabia; identified at Faculty of Science, King Abdulaziz University. The plant was air dried, finely powdered and macerated with methyl alcohol at room temperature. The total extract was concentrated then successively fractionated with petroleum ether, chloroform and ethyl acetate. Each of the three extracts was concentrated under vacuum then fractionated several times using different chromatographic techniques including open column (packed with normal phase silica gel, reversed phase silica gel and Sephadex) and preparative thin layer chromatography. Finally, twelve pure compounds were isolated. The structures of the isolated compounds were determined using different spectroscopic techniques including MS, 1D (1H NMR and 13C NMR) and 2D NMR (COSY, HSQC, and HMBC). All the isolated phenolic compounds were tested for their free radical scavenging activities using DPPH reagent.

Results / Discussion / Conclusion

The total alcoholic extract of Phragmanthera austroarabica collected from Saudi Arabia was studied for the chemical constituents. Phytochemical investigation of the plant resulted in isolation of twelve pure compounds. The structures of the isolated compounds were established based on different spectroscopic data including MS, 1D (1H NMR and 13C NMR) and 2D NMR (COSY, HSQC, and HMBC). The isolated compounds were identified as chrysophanic acid, emodin, chrysophanic acid-8-O-glucoside, emodin-8- O-glucoside, pectolinarigenin, quercetin, dillenetin-3-O-glucoside, catechin, catechin-4'-O-gallate, methyl gallate, lupeol and ursolic acid. All the isolated phenolic compounds revealed significant free radical scavenging activities when tested using DPPH reagent where quercetin, methyl gallate and catechin-4'-O-gallate revealed the highest activities. Most of the isolated known compounds were previously proved to possess anti-inflammatory activities. These findings can justify the folk use of the plant in treatment of diabetes as it is well known the relation between both antioxidant and antiinflammatory activities from one side and the hypoglycemic activity (especially in type II diabetes) from the other side (Ceriello and Testa, 2009). The results of this study supported the traditional use of the plant as a hypoglycemic agent. Further pharmacological studies should be carried out to consider this plant in drug discovery.

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PO12
Capacity of inhibition of cholinesterase enzymes of plants used in traditional medicine in Ecuador

Jara, A.[1]; Rodríguez, Y. A.[1]; Cornejo, J.[1]; Gutiérrez, M.[1,2*]; Astudillo, L.[1,2]; Cazar, M.[3]
[1]Laboratory of Organic Synthesis, Institute of Chemistry of Natural Resources, Universidad de Talca, Chile.
[2]Interdisciplinary Excellence Research Program on Healthy Aging, Universidad de Talca, Talca, Chile
[3]Chemical SciencesFaculty, Universidad de Cuenca, Cuenca, Ecuador
* mgutierrez@utalca.cl

Introduction

Six plants used in traditional medicine in Amazonia of Ecuador were collected; the vegetable material was macerated in organic solvent obtained ethanolic (EtOH), ethyl acetate (EtOAc) and dichloromethane (DCM) extracts. Content of phenolic and flavonoids totals and the capacity of inhibition of cholinesterase enzymes were determinate. Thespeciescollectedwere: Curarina (*Potalia amara*), Zhute (*Salvia corrugata*Vahl,), Guayusa (*Ilex guayusa* Loes,), Tiatina (*Scopariadulcis)*, Iguila (*Monnina*sp,) and Moradilla (*Alternantheraporrigens*).

Method

The total phenolic content **(**TPC) of the extracts was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1965). The total flavonoids content (TFC) was determined spectrophotometrically based on the formation of a flavonoid-aluminum complex (Zhishen *et al.*, 1999). To assess acetyl cholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition, an adapted version of the Ellmann *et al.*, 1961, assaying 96 well plates was used. The extracts were assayed in the dilution interval of 500 - 15 µg/mL.Galantamine served as the positive control.

Results

All extracts obtained were evaluated as potential AChE/BChE inhibitors, IC_{50} values (concentration of sample required to inhibit 50% of cholinesterase enzyme) were calculated from the regression equations prepared from the concentrations of the samples. EtOAc Extracts from Curarina, Guayusa and Zhute showed activity and selectivity against AChE and Tiatina showed activity and selectivity against BChE, the extracts of Zhute DCM and Iguila were not selective over the enzymes evaluated.

Discussion

The results revealed that amazonic plants are good cholinesterasic inhibitors with potential application in neurodegenerative disease. The values of inhibition obtained are considered important, but not comparable to the reference inhibitor, considering that they are extracts. The most active extracts were Guayusa this activity can be related with a high content of caffeine and others methylxantines reported previously from this specie (McClatchey*et al.*, 2009).

Conclusion

The diversity of vegetables present in Ecuador can be an important source of bioactive compounds. This study allowed establishing the potential inhibitor of enzymes involved in neurodegenerative conditions of plant extracts from the Ecuadorian Amazon, showing them a good content of phenolic compounds which are directly related to the antioxidant activity, besides the potential against enzymes specific against Alzheimer disease. The phytochemical study of these species can provide important information in relation to their secondary metabolites responsible for the activity shown.

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PO13
CHEMICAL COMPOSITION, ANTIMICROBIAL, ANTIOXIDANT AND INSECTICIDAL ACTIVITIES OF ESSENTIAL OILS OF *Conyza linifolia* AND *Chenopodium ambrosioides*

Hala M. Hammoda[a], Fathallah M. Harraz[a], Maged G. Al Ghazouly [a,b], Mohamed A. Farag [c], Ahmed F. El-Aswad [d] and Samar M. Bassam [b]

[a] *Faculty of Pharmacy, Pharmacognosy Department, Alexandria University 21521, Alexandria, Egypt*
[b] *Faculty of Pharmacy, Pharmacognosy Department, Pharos University, Alexandria, Egypt*
[c] *Faculty of Pharmacy, Pharmacognosy Department, Cairo University, Cairo, Egypt*
[d] *Faculty of Agriculture (Elshatby), Pesticide Chemistry Department, Alexandria University, Alexandria, Egypt*
hala_hammoda@yahoo.com

Introduction

Genus Conyza is a native to North and South America. Different species of Conyza contain essential oils. Literature has proven that the essential oil of different Conyza species is rich in monoterpene hydrocarbons, sesquiterpene hydrocarbons and oxygenated compounds. The most common component among different Conyza oils was the monoterpene limonene. Few data are available concerning the essential oil of Conyza linifolia growing wildly in Egypt. Antimicrobial and insecticidal (Khalaf, 1999) activity was previously reported for some species of Conyza. Genus Conyza is also called fleabane. The name fleabane suggests the plant value as an insecticide or repellent. However, few studies proved this hypothesis (Mansour et al, 2011). Genus Chenopodium includes varieties of weedy herbs native to Europe, Asia and both North and South America. Chenopodium ambrosioides oil is rich in monoterpenes. Antimicrobial and insecticidal activities were proven for the essential oil of Chenopodium ambrosioides (Radwan et al, 2008; Owolabi et al, 2009).

Method

Conyza linifolia was collected in April 2011 from Nubaria, Alexandria, Egypt. Chenopodium ambrosioides was collected in October 2011 from Al Awayd, Alexandria, Egypt. The essential oils from both plants were prepared by hydrodistillation using a Clevenger-type apparatus, and analyzed by GC/MS using an Agilent Hewlett-Packard 6890 GC apparatus coupled to an Agilent Hewlett-Packard 5973 mass spectrometer. Antibacterial and antifungal screenings of essential oil of both plants were carried out using the agar-diffusion technique against S. aureus, B. subtilis, E. coli, P. aeruginosa, and C. albicans. MIC were calculated for both oils. Effect of essential oils on mosquitoe larvae and adults of Culex pipiens, and on rice weevil Sitophylus orizae were investigated and EC 50 values were calculated for both oils. Moreover, DPPH free radical scavenging activity assay was performed to evaluate the antioxidant activity of both oils.

Results/Discussion/Conclusion

According to literature, this is the first GC-MS analysis of the essential oil of Conyza linifolia growing in Egypt. Thirty one of 36 components were identified in the oil of Conyza linifolia and the major components were á-bergamotene and D- limonene. The oil was generally rich in sesquiterpenes. However, only 12 components were identified in the oil of Chenopodium ambrosioides, and the major components were o-cymene and á-terpinene. Chenopodium oil consisted mainly of monoterpenes. This study suggests an explanation for the low content of ascaridole found in the oil of chenopodium. Chenopodium ambrosioides essential oil had antibacterial activity against the B. subtilis and E. coli, while Conyza linifolia essential oil had antibacterial activity against B. subtilis only. Both oils showed no antifungal activity. The insecticidal activity screening showed the higher activity of Chenopodium ambrosioides oil against mosquito larvae of Culex pipiens, and on the contrary, the higher activity of Conyza linifolia against Culex pipiens mosquito adults. Both oils were found ineffective against the rice weevil Sitophylus orizae. Finally, the antioxidant activity of the essential oil of Conyza linifolia was higher than that of Chenopodium ambrosioides.

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PO14
BIOACTIVE COMPOUND FROM THE RED SEA MARINE CYANOBACTERIUM *Moorea producens*

Diaa Youssef[1,*], Lamiaa Shaala[2], Gamal Mohamed[1], Sabrin Ibrahim[3] Zainy Banjar[4], Jihan Badr[1]
[1]*Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia*
[2]*Natural Products Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia*
[3]*Department of Pharmacognosy and Medicinal Chemistry, Faculty of Pharmacy, Taibah University, Al Madinah Al Munawwarah 41477, Kingdom of Kingdom of Saudi Arabia*
[4]*Department of Clinical Buiochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, 21589, Kingdom of Saudi Arabia; dyoussef@kau.edu.sa; diaa22@yahoo.com*

Introduction

The genus of cyanobacteria Lyngbya (now Moorea) has been found to produce many interesting natural products. In addition, they are easily accessible from the tropic and subtropical regions. To date, the most important species of genus Lyngbya in terms of secondary metabolite production are L. majuscula, L. martensiana, L. aestuarii, and L. wollei (Liu and Rein 2010). Lyngbya majuscula yielded a variety of interesting secondary metabolites such as peptides (Marner *et al.* 1977; Tripathi *et al.* 2011), toxins such as lyngbyatoxins and aplysiatoxins (Ami *et al.* 1990;), malyngamides (Gross *et al.* 2010), alkaloids (Nogle and Gerwick 2003), and macrolides (Luesch *et al.* 2002). These compounds exhibit a variety of biological activities including cytotoxicity, antimalarial, antileishmanial, and antimicrobial (McPhail *et al.* 2007).

Method

The freeze-dried cyanobacterium M. producens (50 g) was extracted at room temperature with a mixture of MeOH/CH_2Cl_2 (4:1). The combined extracts were evaporated under reduced pressure to give a greenish organic extract. The extract was partitioned between 60% MeOH/H_2O (500 mL) and CH_2Cl_2 (3 150 mL). This CH_2Cl_2 extract (610 mg) was subjected to a flash silica gel column eluted with a solvent gradient of n-hexane/CH_2Cl_2-acetone to afford 10 fractions. Fraction 6 (eluted with 20% MeOH in CH2Cl2, 120 mg) was subjected to HPLC purification on a semi-preparative HPLC column (Cosmosil AR II, 5 mm, 250 10 mm) using 60% ACN-H_2O at a flow rate of 2 mL/min to give compounds 1 (3.1 mg), 2 and 3 (6.2 mg), 4 (6.9 mg), 5 (7.1 mg), and 6 (5.4 mg). Similarly, fractions 7 (eluted with 30% MeOH in CH_2Cl_2, 43 mg) was subjected to HPLC purification (Cosmosil AR II, 5 mm, 250 10 mm, 60% ACN-H_2O, 2 mL/min) to give 7 (5.5 mg).

Results / Discussion / Conclusion

Investigation of the organic extract of the Red Sea marine Moorea producens afforded a new compound, mooreatoxin, along with six known compounds including lyngbyatoxin A, majusculamides A and B, aplysiatoxin and debromoaplysiatoxin. Their structures were elucidated through extensive spectroscopic techniques including HRFABMS, 1D (1H and 13C) and 2D (COSY, HSQC, and HMBC) NMR spectra. Mooreatoxin is related to the lyngbyatoxin series but it lacks the indole moiety and possesses a formamide functionality. The isolated compounds showed different biological activities.

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PO15
CHEMICAL CONSTITUENTS OF *Penicillium* SP. ISOLATED FROM RED SEA ASCIDIAN *Didemnum* SPECIES

Samar S. A. Murshid, Jihan M. Badr, Diaa T. A. Youssef
Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
samurshid.pharma1@gmail.com

Introduction

Marine microorganisms have received a great attention lately, thus the fungi have began to be recognized as a liable source of potentially useful natural products. The growing interest in marine natural products gave rise to the discovery of a considerable number of metabolites that are worthy for clinical applications (Miller *et al.*, 1968). Certainly, one of the most well known natural product discoveries derived from a fungus (microorganism) is that of penicillin which was discovered by Fleming in 1929 from the fungus, Penicillium notatum (Abraham *et al.*, 1941). Each year numerous compounds with an array of biological activities are reported (Blunt *et al.*, 2007; Water *et al.*, 2010).The approved molecules include anti-cancer Cytarabine (Cytosar-U®) and anti-viral Vidarabine (Vira-A® ophthalmic ointment, 3%) which are two nucleosides based on sponge-derived nucleosides. Also, a cone snail peptide used in sever chronic pain; Ziconotide (Prialt®) and Trabectedin (Yondelis®) anti-cancer; a metabolite isolated from a tunicate (Mayer *et al.*, 2010) were reported. A good clarification for the high number of compounds reported from Penicillium species that they are salt tolerant, fast growing and are obtained easily from many substrates. Furthermore, many Penicillium species are known to yield derivatives with a wide range of activities.

Method

The fungus (Penicillium sp) isolated from Red Sea ascidian Didemnum species was cultivated using liquid cultures. The cultures were incubated at room temperature. After 30 days, 250 mL of EtOAc were added to each flask containing 500 mL culture medium and left overnight to stop cell growth. Culture media and mycelia were separated by vacuum filtration using Buchner funnel. The filtrates were collected and extracted with EtOAc till exhaustion. Fungal mycelia were separated from culture media and left in MeOH overnight for extraction. TLC examination of both extracts (broth & mycelia) showed different pattern. Each extract was studied separately. Different chromatographic techniques were used for isolation of pure compounds. This included column chromatography and preparative thin layer chromatography. The purity of the isolated compounds was checked using HPLC. Finally, the mycelia extract yielded four pure compounds while three pure compounds were offered by the broth extract.

Results / Discussion / Conclusion

The total alcoholic extract of each of the mycelia and the broth extracts of the fungus Penicillium sp. isolated from Red Sea ascidian Didemnum sp. were studied for the chemical constituents. Chemical investigation, resulted in isolation of seven pure compounds. The structures of the isolated compounds were established based on different spectroscopic data including MS, 1D (1H NMR and 13C NMR) and 2D NMR (COSY, HSQC, and HMBC). Compounds isolated from the mycelia extract were found to be new compounds and were identified as two fatty acid esters, in addition, two cerebrosides were identified. On the other hand, chemical investigation of the broth extract yielded three alkaloids, of which two were known assigned as tryptamine and indol-3-carbaldehyde. The third compound is a new one isolated for the first time from a natural source. All the isolated compounds were evaluated for their antimicrobial activities.

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PO16
BIOACTIVE COMPOUND FROM THE VERONGID SPONGE, *Pseudoceratina arabica*

Lamiaa Shaala[1,*], Diaa Youssef[2], Mansour Souliman[3], Gamal Hussein[1] and Alaa Khedr[4]
1Natural Products Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
2Department of Natural Products, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
3Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, 21589, Kingdom of Saudi Arabia;
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
Lamiaelnady@yahoo.com, Lshaala@gmail.com

Introduction

Sponges have been proven to be a prolific source of bioactive substances with antibiotic, anticancer, antiviral, antibacterial, anti-inflammatory and antihistaminic properties (Mayer *et al.* 2010). Marine sponges of the order Verongida are of much current biological and chemical interest. They are characterized by elaboration of typical brominated metabolites which are biogenetically related to tyrosine. These metabolites are considered as distinct markers for Verongid sponges. A diverse biological activities for these compounds were reported including antifungal (Kernan et al 1990), antibacterial (Tsuda *et al.* 2001), cytotoxic (Hirano *et al.* 2000) and enzyme inhibitory effects (Carroll *et al.* 2001). Secondary metabolites derived from members of the genus of Pseudoceratina displayed diverse bioactivities including antimicrobial, parasympatholytic HIV inhibition, enzyme inhibition, cytotoxic and antifouling activity.

Method

The fresh sponge material (600 g) was extracted exhaustively with MeOH and the resulting extract was partitioned between CHCl3 and water. The organic layer was subjected to VLC in SiO2 using CHCl3/MeOH gradients. The fraction eluted with 10% MeOH in CHCl3 was subjected to Sehadex LH-20 in CH2Cl2/MeOH (1:1). Final purification on HPLC using HPLC column (Cosmosil AR II, 5 mm, 250 10 mm) using 75% ACN-H2O at a flow rate of 2 mL/min afforded pseudocertin A-C (1-3). Fraction eluted with 30% MeOH in CHCl3 was subjected to Sephadex LH-20 using MeOH followed by HPLC purification HPLC purification (Cosmosil AR II, 5 mm, 250 10 mm, 50% ACN-H2O, 2 mL/min) yielded moloka'iamine (4), hydroxymolokiamine (5) and psammaplysin-A (6).

Results / Discussion / Conclusion

Investigation of the dichloromethane fraction of the methanolic extracts of the Red Sea sponge Pseudoceratina arabica afforded three new compounds, pseudocertin A-C, along with three known compounds psammaplysin-A, moloka'iamine and hydroxymolokiamine. Their structures of the compounds were determined by extensive interpretation of their spectral data including HRFABMS, 1D (1H and 13C) and 2D (COSY, HSQC, and HMBC) NMR spectra. The biological activities of the compounds will be presented and discussed.

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PO17
CHEMICAL STUDY OF *Limonium pruinosum* (L.) CHAZ.

Boudermine S.[1], Malafronte N.[2], Beghidja N.[1], De Tommasi N.[2,1], Benayech S.[1], Mencherini T.[2], Benayech F.[1], Aquino R.P.[2], Gualtieri M.J.[3]

[1]*Research Unit Development Of Natural Resources, Bioactive Molecules and Physicochemical and Biological Analysis. Department of Chemistry,University-Mentouri- Algeria*

[2]*Università degli di Studi di Salerno, Dipartimento di Farmacia, via Giovanni Paolo II, 132, 84084 Fisciano,Italy*

[3]*Departamento de Farmacognosia y Medicamentos Organicos. Universidad de Los Andes, Venezuela.*

detommasi@unisa.it

Introduction

Limonium is a genus of 120 flower species which belongs to the family of plumbaginaceae. This is a cosmopolitan family found in cold and tropical regions that include 775 species grouped in 24 genera, mostly herbs and small shrubby. The leaves are simple, glandular, without stipules, basal rosette, or alternate on the aerial stems. The inflorescences are racemes, in cymes or dense heads. The flowers are actinomorphic, bisexual and tetracyclic1. Different biological activities are attributed to the family of plumbaginaceae such as: antimicrobial, anti-ÿ'3finflammatory and antiviral activities2. Limonium priunosum was collected during the flowering period, in April 2012, in the region of Bechar, southwest of Algeria.

Materials and Method

The dried powdered aerial parts of Limonium priunosum (1.8 kg) were subjected to extraction with MeOH to 80%. Methanol extract was partitioned with CHCl3, ethyl acetate and n-butanol to afford the respectively residues, 0.8 g, 6 g and 12.0 g. The ethyl acetate and butanolic extracts were subjected to different chromatographic techniques as Silica gel, Sephadex LH-20, MPLC and rp-HPLC. The structures of isolated compounds were elucidated by 1D and 2D-NMR Spectroscopy (1H, 13C, 13C DEPT, DQF-COSY, HSQC, HMBC, ROESY) and confirmed by mass spectrometry studies. The extracts of L. pruinosum were also studied for the antioxidant activity.

Results and discussion

The phytochemical study of CHCl3 and Butanolic extracts led to the isolation of different known compounds and one new compound such as: kaempferol 3-O-rhamnopyranoside 3, myricétine-3-O-galactopyranoside, avicularine, quercitine-3-O-galactopyranoside, betmidin4 from ethyl acetate extract, kaempferol-3-O-(6''-O-Gallolyl)-â-O-Galactopyranoside, Typheramide, gallic acid, quercitine 3-O-rhamnopyranoside, while myricetin-ÿ'3f3-ÿ'3fO-ÿ'3f(6''-ÿ'3fgalloyl)ÿ'3fgalactoside, 1,ÿ'3f6-ÿ'3fDigalloyl-ÿ'3fâ-ÿ'3fD-ÿ'3fglucose5 and a new compound from butanolic extract.

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PO18
APPLICATION OF AN HPLC-PDA-MS METHODOLOGY TO THE CHARACTERIZATION OF CAROTENOIDS IN SOME EXOTIC FRUITS

Giuffrida, D.[*,1], Menchaca, D.[2], Dugo, P.[3,4,5], Donato, P.[3,4,5], Cacciola, F.[1,5] and Murillo, E.[2]
[1]*Università degli Studi di Messina, Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute, Messina, Italy;*
[2]*Departamento de Bioquimica, Facultad de Ciencias Naturales Exactas y Tecnologia, Universidad de Panama, Panama;*
[3]*Università degli Studi di Messina, Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute, Messina, Italy;*
[4]*Università Campus Bio-Medico di Roma, Centro Integrato di Ricerca, Roma, Italy;*
[5]*Chromaleont s.r.l. A start-up of the University of Messina, Italy.*
dgiuffrida@unime.it

Introduction

Our research group has recently started a research project aiming at the characterization of the native carotenoids composition in many tropical fruits [Murillo *et al.*, 2013], which can be considered a reservoir of bioactive substances with a special interest due to their possible health-promoting properties. Panama is a humid tropical country with a large native biodiversity, where there are many plants which have not been investigated for their native carotenoids composition. The interest in carotenoids from a nutritional standpoint has recently greatly increased, because of their important health benefits, which could make these compounds ideal for the always increasing functional food industry as well as promoting the consumption of the natural products in which are contained. Here we report the native carotenoids composition in four tropical fruits from Panama.

Method

About 2.5 kg of each fully mature fruits of Membrillo (Gustava superva), Guanabana toreta (Annona purpurea), Jobo (Spondias mombin), and Mamey (Mamea americana) from Panama, were collected directly from the tree, or purchased in local markets. The carotenoid pigments were extracted from the fresh fruits on the same day of collection, and analysed according to a similar methodology as reported in Murillo *et al.* (2013). The native carotenoid composition was directly investigated by an HPLC-DAD-APCI-MS methodology, for the first time.

Results and Discussion

Two fruits, Membrillo (Gustava superva) and Guanabana toreta (Annona purpurea), had never been investigated for their carotenoids composition before. In Membrillo, 5 different carotenoids were detected; the total carotenoids content was of 318.6 μg/g (fresh weight), with β-carotene showing a relative abundance of 75.3 %. In Guanabana toreta, 11 different carotenoids were detected; the total carotenoids content was of 48.3 μg/g (fresh weight), with β-carotene and zeaxanthin showing a relative abundance of 26.9 % and 27.5 % respectively. In Jobo (Spondias mombin), 9 different carotenoids were detected; the total carotenoids content was of 45.8 μg/g (fresh weight), with β-cryptoxanthin, α-cryptoxanthin and β-carotene showing a relative abundance of 25.4 %, 10.5 % and 8.5 %, respectively. In Mamey (Mamea americana), 15 different carotenoids were detected; the total carotenoids content was of 145.2 μg/g (fresh weight), in particular 10 different violaxanthin di-esters, of both cis and trans isomers, were determined.

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PO19
PHOTOTOXICITY TESTING OF NEW ALOE-EMODIN DERIVATIVES

Gámez, C.[1,2]; Colman, L.[2], Canudas, N.[1]; Villamizar, J. E.[2]; Taylor, P.[2]; Blanco, F.[2*]
[1]*Universidad Simón Bolívar, Apartado 89000, Caracas 1080-A, Venezuela.*
[2]*Instituto Venezolano de Investigaciones Científicas, Apartado 20632, Caracas 1020-A, Venezuela.*
fblanco@ivic.gob.ve

Introduction

Phototoxicity is a biological response to a substance which is either elicited or increased after exposure to light. In order to assess photodynamic activity, phototoxic compounds should ideally only become cytotoxic after photoactivation and the phototoxic activity is quantified as the Photo-Response-Factor (PRF). Aloe-Emodin, a hydroxyanthraquinone present in the leaves of *Aloe vera*, is reported to show a wide variety of biological activities in both ground and excited states, mainly related to diseases of the skin (Shelton, 1991) but also against tumours, virus and bacteria. The photoexcitation of Aloe-Emodin results in the formation of singlet oxygen which accounts for its excited state activity (Vath *et al.*, 2002). In this study, we tested 11 new Aloe-Emodin chalcone and stilbene derivatives for phototoxicity against tumour and normal cell lines.

Method

Four new chalcone and seven stilbene Aloe-Emodin derivatives were synthesized, purified by HPLC and characterized by infrared and UV-Vis spectroscopy, nuclear magnetic resonance (^{13}C and ^{1}H) and mass spectrometry. The compounds were tested for cytotoxicity on HT-29 and MDA-MB231 human tumour cell lines and a primary fibroblast cell line derived from foreskin. Cells were seeded on to plates, incubated with the compounds for 1 h (0-100 μg/ml), then irradiated for 10 min with an energy dosage of 21.8 J (84% Vis, 15% UVA and 1% UVB/UVC) using a 300W ceramic xenon lamp (SolSim: Luzchem Solar Simulator). After 48 h, cytostasis (GI_{50}- 50% growth inhibition, TGI - total growth inhibition) and cytotoxicity (LC_{50} - 50% lethal concentration) were assessed using the sulphorhodamine assay.

Results / Discussion / Conclusion

The majority of the compounds showed some degree of cytostatic activity in the low micromolar range, but only one, a stilbene derivative, was truly cytotoxic in its ground state with an LC_{50} of approximately 10 μM range for the three cell lines. Irradiation increased the cytotoxicity of the majority of the compounds to some degree, but the most notable results were those for three (trimethoxystilbene, p-nitrostilbene and p-methylchalcone) derivatives, which were not cytostatic or cytotoxic in their ground state, but became so, in the micromolar range, when irradiated. For example, the GI50, TGI and LC50 values for the trimethoxystilbene derivative tested against HT-29 cells decreased from >200, >200 and >200 μM to 1.2, 2.5 and 6.7 μM respectively, which translates into a PRF of over 200. Further studies are under way to determine the photostability of these compounds, as well as their mechanism of action.

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PO20
ENDOTHELIUM-INDEPENDENT VASORELAXANT EFFECT OF LQM-001, AN AMINOGUANIDINIC DERIVATIVE

Costa, C. D. F.[1*], Herculano, E. A.[3]; Ferreira-Rodrigues, A. K. B.[2]; Silva, J. C. G.[2]; Araújo-Júnior, J. X.[2,3]; Ribeiro, E. A. N.[1,2]
[1]*RENORBIO – Biotechnology's Northeast Network, Brazil*
[2]*Pharmacy and Nursing School, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
[3]*Chemistry and Biotechnology Institute, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
**cyntiadan@gmail.com*

Introduction

The high blood pressure has been identified as the most powerful one among the major risk factors for cardiovascular diseases and remains the leading cause of death worldwide and one of the world's greatest public health problems (Hobbs *et al.*, 2004). Although many new antihypertensive drugs with improved efficacy have been introduced to the market, they still possess serious side effects (Al-Salahi *et al.*, 2014). Hereby, attention has recently been focused on several medicinal chemistry strategies in the molecular design of new candidate therapeutic agents or new drugs prototypes (Wermuth *et al.*, 1993; Barreiro, 2009). The rational planning of new synthetic prototypes has been using a series of methods of structural modification that aim at the generation of new compounds presenting optimized pharmacodynamic and pharmacokinetic properties, exploring bioactive substances' fragments, active metabolites of drugs, bioisosterism, selective optimization of side effects of drugs (Barreiro and Fraga, 2001). The aminoguanidinics derivatives such as clonidine, guanabenz and guanfacine are important blood-pressure-lowering drugs. Therefore, the present study was designed to evaluate the cardiovascular effects of LQM-001, an aminoguanidinic derivative through *in vivo* and *in vitro* approach.

Method

Male spontaneously hypertensive rats (SHR) were used for all experiments. The approach the superior mesenteric arteries rings were maintained in organ baths. We studied the concentration-dependent relaxant effect of LQM-001 on endothelium-intact and endothelium-denuded mesenteric rings that were pre-contracted Phe (10-6M) or 80 mM KCl. All values were expressed as mean ± S.E.M. The results were analysed with student's t-test. Probability values < 0.05 were considered to be significant. The pD2 values were obtained by nonlinear regression. All analysis was performed using GraphPad™ Prism software, version 5.0®.Protocol approved by the ethics committee for animal experimentation: 009481/2011-21

Results / Discussion / Conclusion

In SHR the vascular effect was investigated. LQM-001 (3x10-8 - 10-4) induced vasorelaxation of a concentration-dependent manner in rings with intact endothelium, pre-contracted with Phe (10-6 M) (Emax = 114.19 ± 0.04% and pD2 = 6.00 ± 0.04), in endothelium-denuded rings, the relaxant effect induced was not changed (Emax = 107.97 ± 0.03 % and pD2 = 5.71 ± 0.03), suggesting that the presence of the endothelium not important for vasodilator effect. To check the participation of Ca+ channels in the mechanism of relaxation of this substance, the mesenteric rings were contracted with KCl 80 mM and cumulative relaxation responses were obtained by adding LQM-001 in denuded mesenteric rings (Emax = 39.31 ± 1.3%). These results confirms that the presences of endothelium is not essential for relaxant response expression and suggest the partial involvement of Ca+ channels in this effect.

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PO21
RED PROPOLIS PROMOVE HYPOTENSIVE EFFECT IN SHR

Herculano, E. A.[1]*; Costa, C. D. F.[2]; Oliveira, J. M. S.[2]; Nascimento, T. G.[2]; Ribeiro, E. A. N.[2]; Araújo-Júnior, J. X.[1]

[1]*Chemistry and Biotechnology Institute, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
[2]*Pharmacy and Nursing School, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
**edla_herculano@hotmail.com*

Introduction

Hypertension is one of the major risk factors associated with cardiovascular diseases (Hall *et al.*, 2012) and its control can significantly reduce complications such as heart attack, heart failure, stroke, kidney failure and premature death. Due to the complex nature of hypertension, your therapeutic is characterized only normalize blood pressure to lower the risk of complications from high blood pressure by use multiple antihypertensive agents (Kurtz, 2006). For that actually the research aims news strategies and drugs with fewer side effects and increase therapeutic efficacy (Destro *et al.*, 2010). How source of the biologically active compound are natural products (Camona *et al.*, 2013) such as red propolis. Studies have shown propolis' beneficial effect on human health (Daugsch *et al.*, 2008). Therefore Red Propolis from Alagoas' State (Northeastern Brazil) has a particular composition rich in flavonoids (no published data). And these cause a blood pressure lowering effect in normotensive and hypertensive subjects by improved endothelial function and increased nitric oxide bioavailability (Galeano *et al.*, 2012). In this sense, we evaluate the hypotensive effects from the crude extract and chloroformic fraction. Hence a formulation was produced and arise the patent (number register: 0120-INPI RE/AL) had its hypotensive and vasorelaxant effects available.

Method

Male spontaneously hypertensive rats were used for all experiments. For measurement hypotension and bradycardia catheters were inserted and after 24 hours the experiments were performed. For in vitro experiments the superior mesenteric arteries rings were maintained in organ baths.

Results / Discussion / Conclusion

In conscious SHR red propolis' etanolic extract (EE) (0.1–10 mg/kg, i.v, randomly), elicited immediate and dose-dependent decreases in mean arterial pressure (-6.3 ± 0.5%; -12.1 ± 1.6%; -12.2 ± 0%; -25.8 ± 0.4% and -16.5 ± 9.7%, respectively) the red propolis' chloroformic fraction (CF) (-5.5 ± 3%; -8.4 ± 1.8%; -16.3 ± 4.3%; -20.8 ± 5.5% and -22.7 ± 6.4%, respectively) and formulation's red propolis (FP) (-9.8 ± 2.9%; -14.4 ± 3.1%; -11.3 ± 3%; -19.4 ± 1.6% and -22.5 ± 3.8%, respectively) presented hypotensive effect without statistical difference and as well as heart rate effects. The same hypotensive effect was observed after administration of the FP in unconscious rats (-10.7 ± 2.2%; -14.2 ± 3%; -18.5 ± 4.6%; -18.7 ± 4% and -20.3 ± 3.5%, respectively). These data demonstrate that FP presented hypotensive effects without action in central nervous system, suggesting vascular effect. Therefore the vascular effect was investigated. FP (1–500 μg/mL) induced vasorelaxation of a concentration-dependent manner in intact endothelium rings pre-contracted with Phe (10-6 M) (Emax = 100% and pD2 = 1.06 ± 0.05), in endothelium-denuded rings, the relaxant effect induced was significantly different (Emax = 56.7 ± 4.01% and pD2 = 1.46 ± 0.1), suggesting that the presence of the endothelium is important for vasodilator effect.

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PO22
Cymbopogon winterianus LEAF ESSENTIAL OIL COMPLEXED IN β-CYCLODEXTRIN INCREASE PROTEIN FOS EXPRESSION IN CENTRAL NERVOUS SYSTEM AREAS ON RODENTS

Santos, P. L.[1]; Brito, R. G.[1]; Quintans, J. S. S.[1]; Blank, A. F.[2]; Menezes, P. P.[3]; Araújo, A. A. S.[3]; De Lucca Junior, W.[1]; Almeida, R. N.[5]; La Rocca, V.[5]; Araújo-Júnior, J. X.[4]; Quintans-Júnior, L. J.[1,*]

[1]*Department of Physiology,* [2]*Department of Agronomy. Department of Pharmacy, Federal University of Sergipe, São Cristóvão, SE, Brazil.*

[4]*Department of Pharmacy, Federal University of Alagoas, Maceió, AL, Brazil.*

[5]*Laboratory of Psychopharmacology. Federal University of Paraiba, João Pessoa, PB, Brazil.*

**lucindojr@gmail.com; lucindo@pq.cnpq.br*

Introduction

Cymbopogon winterianus is a medicinal plant belonging to the family Poaceae (Gramineae). Some pharmacological activities, as anticonvulsant and analgesic, are described in the literature for the C. winterianus leaf essential oil (CEO) (Leite *et al.*, 2010; Quintans-Júnior *et al.*, 2008). However, there are no data describing the involvement of the CNS in the CEO analgesic activity. So, we evaluated the central areas activated by CEO complexed in β-cyclodextrin (β-CEO) in mice subjected to the formalin-induced orofacial nociception protocol.

Method

CEO was extracted from C. winterianus leaves with hydrodistillation and analyzed using CG-MS/FID. CEO contains several bioactive compounds including geraniol (37.57%), citronellal (27.09%), geranial (9.63%) and citronellol (9.53%). The β-CEO was physicochemically characterized using the differential scanning calorimetry (DSC) and thermogravimetry derivative (TG) and showed that β-CEO was complexed as compared to reference standards. Male Swiss mice were pre-treated with vehicle (distilled water, p.o.) or β-CEO (50, 100 or 200 mg/kg; p.o.) and, 1 h after, it was performed the orofacial nociception induced by formalin (2%; 20 ul; s.c.) test. The nociceptive behavior was measured by the time (s) that the animal spent face-rubbing in the injected area with its fore or hindpaws at periods of 0 to 5 minutes (first phase) and 15 to 40 minutes (second phase) after the formalin injection. Then, the mice were perfused, the brains removed, frozen, cut (20 μm) and subjected to immunofluorescence for Fos protein. The protocols were approved by Animal Care and Ethics Committee at the UFS (CEPA/UFS: 42/12). The data were expressed in mean ± e.p.m. and analyzed by ANOVA (one-way) followed by Tukey's test ($p<0.05$).

Results / Discussion / Conclusion

The immunofluorescence essay demonstrated that the β-CEO treatment significantly ($p < 0.05$) increase the number of Fos positive cells in locus coeruleus (LC), trigemino-thalamic tract (TTT), trigeminal spinal nucleus (TSN) and rostroventromedial area (RVM) when compared with vehicle. The Fos protein has been used as a target for neuron activation. Its expression in specific areas can help elucidate the central mechanisms involved in the perception and response to pain (Burmeister *et al.*, 2008). The RVM and LC are involved in the descending pain modulatory system. The first one projects serotoninergic pathways to spinal cord and the second noradrenergic, controlling, both, the pain functions (Song *et al.*, 2013). The TSN has been viewed as an essential brainstem site for relaying facial pain information to higher levels of the CNS, being the TTT also involved in these pathways (Malnierca *et al.*, 2012). In this context, our results suggest that the activation of central areas is involved in the β-CEO analgesic activity.

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PO23
MODERN PHYTOCHEMICAL SCREENING OF RED PROPOLIS USING LC-ORBITRAP-FTMS AND MZ-MINE SOFTWARE: APPLICATION ON INVESTIGATION OF PHLOBATANNINS AND TERPENS

Souza, N. S.[1]*, Nascimento, T. G.[1] *; Araújo-Júnior, J.X.[1, 2]**; Oliveira, J. M. S.[1]; Silva, P. F.[1]; Azevedo, L. F.[1]

[1]School of Nursing and Pharmacy, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil
[2]Istitute of Chemistry and Biotechnology, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil
**nagari.bc@gmail.com, ticianogn@yahoo.com.br, **joaoxjr@yahoo.com.br*

Introduction

The Red Propolis (RP) is produced in mangrove area of the province of Alagoas, Brazil and its main plant source the "rabo de bugio" (Dalbergia ecastophyllum (L) Taud) and chemical compounds are being identified. Red propolis presents differences from the others propolis from Brazil due to the presence of isoflavones, chalcones, pterocarpans, terpens (Trusheva *et al.*, 2006) and other compounds which are presented in this work. Modern chromatographic methods combined with mass spectrometry have been used for detection and identification of new compounds in complex samples and has proven to be a suitable method for analysis of propolis because it allows performing a comprehensive analysis of bioproducts (Katajamaa & Orešiè, 2005). The aim of this study was to apply modern screening methods using LC-Orbitrap-FTMS and MZmine software for identification of new phlobatannins and terpens of red propolis from Alagoas, Brazil.

Method

Two samples of propolis (PVIZ and PVMR) was collected in Marechal Deodoro city, Alagoas-Brazil and a third sample (PVPE) was obtained from the Igarassu city, Pernambuco-Brazil in the form of crude extract, which was donated by LTF-UFPB during month of July/2012. Five grams of PVIZ and PVMR were subjected to extraction with ethanol (150 mL). The crude extracts (100mg) were solubilized in ethanol (10mL). The samples were submitted to classical screening or diluted for 1mg/mL and injected directly into the LC-Orbitrap-FTMS. The chromatograms acquired on Thermo system in flow rate of 300μL/min. in C18 columns from ACE® in gradient mode. LC-MS data acquired using LTQ Orbitrap ™ instrument (Thermo Fisher Scientific, Hemel Hempstead, UK) set at 30,000 resolutions. Sample analysis performed in negative mode. The mass scan range was m / z 50-1200 and capillary temperature was 250°C. The LC-Orbitrap-FTMS data were analyzed with MZmine software version 2.10. Multivariate statistical analysis, correlation analysis of variance (CV), histogram peaks m/z and identification of compounds were obtained by treating MZmine software data.

Results and Discussion and Conclusion

Classical screening method detected presence of phlobatannins, catequins, chalcones, aurones and pentacyclic triterpens after colorimetric reactions. The software MZmine 2.10 identified 2,010 ions considered valid, which 218 peaks were identified. A total of 51 ions classified as phlobatannins and 53 ions classified as terpens. Analysis of histogram (HT) and correlation of variance analysis (CVA) detected intense ions in molecular weight range between 300 to 800 m/z and allowed to view the elution time of each ion, both generated by MZmine analysis. Most new metabolities of isoflavonoids,chlacones, phlobatannins and biflavonoids as ruschalcona V, retusapurpurin B, 2-geranyl-2',4',3,4-tetrahydroxydihydrochalcone, 8-prenylluteone, 6,8-diprenylgenistein, makassaric acid, (+)-Syringaresinol, (+)-pinoresinol dimethyl ether, tanariflavanone A and pentacyclic terpens and its derivatives cycloartenol, α-amyrin, β-amyrin, Asprellic acid A, Nephelioside III were found between 300 and 780m/z, eluting with some substances between 4 and 50 minutes.

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PO24
LEISHMANICIDAL ACTIVITY OF AMINOGUANIDINE DERIVATIVES

Santos, M. S.[1*]; França, P. H. B.[2]; Queiroz, A. C.[1]; Araújo-Júnior, J. X.[2]; Alexandre-Moreira M. S.[1]
[1]*Biological and Health Sciences Institute, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
[2]*Chemistry and Biotechnology Institute, Federal University of Alagoas, Av. Lourival Melo Mota, Maceió, Alagoas, Brazil*
**nananiquel@yahoo.com.br*

Introduction

According to the World Health Organization (WHO), the leishmaniasis affects 12 million people around the world, with an average of more than 2 million new cases annually (Who, 2010). Despite epidemiological importance, the drugs available for treatment are limited and have many adverse effects (Singh; Sivakumar, 2004). Given the above, it became evident the need for new antileishmanial agents with fewer side effects and higher efficacy. The present study aimed the pharmacological evaluation of aminoguanidine derivatives as new prototypes of antileishmanial drugs.

Method

Initially, the cytotoxicity assay was executed using the MTT method. The evaluation of the conversion of MTT to formazan was performed after of the culture of cells (peritoneal macrophages from Swiss mice) with the substances for 48 hours. (Mosmann, 1983). Subsequently, the compounds were assayed for their activity against promastigote forms of Leishmania chagasi to evaluate the direct activity of substances against this evolutive forms of the parasite (Ávila *et al.*, 1997), and intracellular amastigotes of L. chagasi (Nunes *et al.*, 2005). Results were expressed as mean ± S.E.M. and analyzed by ANOVA with Dunnett post-test, considered significant when $p<0.05$. All analysis was performed using GraphPad™ Prism software, version 5.0®. Protocol approved by the ethics committee for animal experimentation of the Federal University of Alagoas (2013.02).

Results / Discussion / Conclusion

It was observed that the all compounds tested (WE 2, WE 3, WE 6, WE 8, WE 9, WE 10, WE 10(1), WE 12, WE 16, WE 17(1), WE 18, WE19 e WE 20) did not demonstrate cytotoxicity to host cell at a concentration of 10 μM. It was also observed that the derivatives WE 8, WE 10(1) e WE 17(1) showed a pronounced leishmanicidal activity against promastigotes forms, with inhibitory concentration 50% (IC50) of 6.20 ± 0.01, 1.00 ± 0.15 e 0.47 ± 0.09 μM, respectively, demonstrated a similar efficacy to the pentamidine, with IC50 value of 6.13 ± 0.20 μM. These compounds were selected and tested against amastigotes de L. chagasi. The substance WE 17(1) showed the best leishmanicidal activity in this assay. Thus aminoguanidine derivative WE 17(1) was identified as new leishmanicidal drug prototype candidate with low cytotoxicity and pronunciated leishmanicidal activity.

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PO25
THE EFFECT OF ETHANOLIC EXTRACT OF *Croton reflexifolius* H.B.K. ON TUMOR L5178Y AND ITS LETHAL DOSE (LD_{50}) ON MICE BALB/C, AND ANALYSIS QUALITATIVE FITOCHEMICAL

Hernandez Solis J. J.[1]*, Flores Mera J. F.[1]; Rezendiz Esparza E.[2]; Montejano Rodríguez J. R.[3], Almaguer Vargas Georgina[3]*;

[1]Pharmacy Students of Autonomous University of Hidalgo. Ex hacienda La Concepción road to Tilcuautla, Hidalgo, México, [2]Bachelor of Pharmacy Infant Hospital Teletón oncology. Querétaro México

[3]M. D. Autonomous University of Hidalgo. Ex hacienda La Concepción, road to Tilcuautla, Hidalgo, México

*1*jesushdz06@gmail.com; 3*georginaalmaguervargas@yahoo.com.mx.*

Introduction

Cancer represents one of the world's leading health problems due to its high mortality and frequency, especially in the countries with low monetary income. This could be the result of multiple factors such as late diagnostics and toxic treatments that include, high and unaffordable prices, because of this, the new antitumor treatment is truly important. In Mexico, in the area of La Huasteca is Crotón reflexifolius, found bibliographic data mentions that this specie has antitumor effect, thus the object of this study was to analyze the antitumor effect, the lethal dose (LD50), and also the qualitative fitochemical search for a new alternative of low cost, secure and effective.

Method

The branches of C. reflexifolius were collected in Huejutla, a state of Hidalgo, Mexico. An examplar of the plant was identified and preserved whithin a herbarium in UAEH, with the Boucher number GAV13.
The testing of the antitumor effect took place in lots with rising doses (a low dose of 2.92x10-4 mg/kg of mice, medium dose of 2.92x10-3 mg/kg of mice and a high dose of 2.92x10-2 mg/kg of mice) of the ethanolic solution of male mice Balb/C with murine lymphoma L5178Y, obtaining from ATCC. The growing of the tumor was valued with the weight gain of the animals and recorded the day of decease of each one. The lethal dose (LD50) was held by the Lorke method, and the qualitative fitochemical was carried out via colormetric testing. The statistic test of the data was made by Análisis de Varianza (ANOVA), continued by the Holm-Sidak test. They were considered statistically significant, those minor valors of 0.05. The management zootecnic and sacrifice of the animals used in this investigation, was performed according to NOM-062-ZOO-1999 and NOM-087-ECOL-SSA1-2002.

Results / Discussion / Conclusion

The results demonstrated the inhibition the growing tumor on medium and high doses, also more time of live on the last one. This results match with bibliografic reports in other species like C. lechleri, C. tiglium, C. cajucara, C. membranaceus where the antitumor effect was also identified. The extract did not show toxicity according to the following protocol, however observation of macroscopically hepatomegaly and nephritis on 5000 and 2900 mg/kg dose. In the bibliography of C. penduliflorus there was found damage to organs on lower doses, the fitochemical results showed the presence of cumarines, reducing sugars, tannins, flavanones, heterosides, leucoanthocyanidins, anthocyanidins, triterpenes and steroids which match when reported to other crotons where dditerpenes, alkaloids, flavonoids, lignans, protoanthocyanins and oils like eugenol, crotoflorin and β-sitostero were also found.

PO26
STUDY OF THE PHARMACOLOGICAL ACTION OF SPICES BASED ON MEDICINAL PLANTS ON LIPID PROFILE IN HYPERTENSIVE PATIENTS

Miranda, G. M.[1]; Rodrigues-das-Dores, R. G. C.[2]*

[1] *Medicine student, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy, Health Center, Campus UFOP, Ouro Preto, Minas Gerais, Brazil*

[2]. *PhD/Research/ Coordinator, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy, Health Center, Campus UFOP, Ouro Preto, Minas Gerais, Brazil*

**plantasmed@ufop.br ou rosanagrd@gmail.com*

Introduction

The use of condiments made from medicinal plants by feeding the world's population is singular habit and are being used for their cardioprotective effects. Cardiovascular diseases originate mainly from complications of atherosclerosis, related to the deposition of lipids and inflammatory cells in the inner walls of arteries and arterioles, aggravated by elevated plasma levels of cholesterol and/ or triglycerides.

Method

This study tested three spices preparations made from medicinal plants with proven cardioprotective activity. The experiment was conducted in a group of 20 hypertensive patients in drug therapy, aged 40 to 80 years, residents of district Bauxite in Ouro Preto (Brazil) assessing whether such preparations were effective on reduced of levels of cholesterol (total and fractions HDL and LDL), triglycerides, decreased of body weight and body mass index. The species used were cilantro, parsley, oregano, rosemary, basil, garlic and onion, grown organically in Viçosa (Brazil). The plants were dried, milled and tested in combinations of herbs, constituting three treatments: treatment 1 (T1 - garlic, onion, cilantro and oregano); treatment 2 (T2 - basil, parsley and garlic) and treatment 3 (T3 - garlic, onion, rosemary and coriander), which were added at 1 g of salt with low in sodium. The control, treatment 4 (T4) was consisting potassium salt (light) or hyposodic (66 % less sodium). All treatments were packed in aluminized and individual packages. Four groups of five hypertensive patients, randomly selected, were asked to use for 30 days, T1, T2, T3 and T4. Fourteen weekly-encoded aluminized sachets were distributed and patients were instructed to use them for two meals and replacing the conventional spices. The trial was a double blind, where neither the patient nor the researcher did not know what was tested seasoning. In outpatient care, anthropometric measurements (height (m), weight (kg), waist (m), body mass index (kg/m2) were made and we collected samples of 5 mL of blood in tubes dried (vacutainer) for biochemical determinations of total cholesterol and fractions (HDL and LDL) and triglycerides. Biochemical analyzes were performed by enzymatic colorimetric method in clinical analysis laboratory of the Federal University of Ouro Preto (LAPAC). Sampling and samples were taken every 15 days at 0, 15 and 30 days. The test results were presented to the participants who were told about the clinical procedures to be followed. The experimental design was randomized blocks with four treatments and five repetitions over time and compared with the control. The dependent variables were triglycerides (TRIGL), total cholesterol (Cholesterol) and fractions (LDL and HDL), body mass index (BMI), height (HGHT) and weight (WT), and the independent treatments (T1, T2, T3 and T4). Data were subjected to analysis of variance, average and regression, the 5% significance level, tailored to the studied model tests. Ethics committee on research approved the project (CAAE 0023.0.238.000-08).

Results / Discussion / Conclusion

The treatments did not differ in the variables analyzed, indicating that they are as effective as the use of salt hyposodic (T4). The values ÿ'3fÿ'3fof triglycerides and total cholesterol at 15 and 30 days of use of spices remained within normal limits. Levels of HDL cholesterol remained in the middle range of the reference values ÿ'3fÿ'3fin the same way that the values ÿ'3fÿ'3fof LDL cholesterol. Only T1 (garlic, onion, cilantro and oregano) was the most effective in reducing all biochemical parameters over time. This effect was attributed to the action of low lipid constituents of the preparation. The T1 and T2 treatments were effective in weight loss and BMI reduction, significant effect, according to the thermogenic properties of species used condiments.

Acknowledgment: FAPEMIG and UFOP

PO27
INDIGENOUS FOOD RESTRICTIONS DURING THE TREATMENT OF MALARIA IN THE BRAZILIAN AMAZON

Kffuri, C. W.[1]*; Ming, L.C.[1]; Hidalgo, A.[2]; Kinupp, V. F.[3]; Chaves, F. C. M.[4]
[1]*PhD, Universidade Estadual Paulista/ Rua José Barbosa de Barros, n° 1780, Botucatu, SP, Brazil.*
[2]*PhD, Universidade Federal do Amazonas/Av. General Rodrigo O.J. Ramos, 3000 Manaus, AM – Brazil.*
[3]*PhD, IFAM, Campus Manaus-Zona Leste. Avenida Cosme Ferreira, 8.045, Herbário EAFM - Manaus, AM – Brazil.*
[4]*PhD/Emprabra, Amazônia Ocidental.Km 29 - AM 010-Zona Rural, Manaus, AM – Brazil.*
**carolkffuri@gmail.com*

Introduction

The Negro river region, where malaria is endemic (IPA > 50), is considered to be a sui generis cultural region; in this area, more than 90% of the inhabitants are indigenous, and more than 22 ethnic groups exist. Over a hundred cultivars of cassava are grown, as this crop is part of the inhabitants' basic diet, supplemented by hunting animals and gathering fruit. The river water is very acidic, which restricts the supply of large fish in their diet. Processed products are rarely consumed. During previous research conducted in this region, it has been observed that patients with malaria maintained restricted diets during their illness until weeks later. This research aims to investigate what underpins this specialized diet.

Method

Between September and November 2013, semi-structured interviews were conducted with 89 indigenous peoples in 5 communities. Questions were asked about the meaning of the term reima and about the dietary restrictions followed by people who have malaria. The interviews were analyzed and the data was categorized in order to produce an analysis and ethical reflection by using the methodology of content analysis.

Results / Discussion / Conclusion

Food restrictions during the treatment of malaria relate to foods that, if eaten, "increase" malaria when you have malaria and/or "wake up" malaria that is still left in the body days, months or even years after the disappearance of symptoms. The ingestion of cold water is prohibited when the body is hot (fever). All kinds of very hot or very cold foods are forbidden because consuming them would "go against the heat inside the body." The foods that are avoided are: alcoholic beverages, sweets and foods considered reimosas. Reima is something that is bad for the blood and that damages the liver, reimosa foods increase body heat. These foods are classified into fruits, wild animals, fish, chicken farmed, pepper and cassava derivatives. The fruits considered reimosas are fatty, sour or very sweet. The umari (Poraqueiba sericea), the most cited reimosa fruit, is salty and rich in oil, has a strong odor and is known by the indigenous peoples as forest mayonnaise. Various palm fruits (Arecaceae) are also considered reimosos because they are very oily. Fish should not be eaten – especially fatty fish, fish without scales and fish that have a bad smell. Only the consumption of little fish is encouraged. Cassava is rich in starch, but contains cyanogenic compounds, which can produce hydrogen cyanide – a compound that is extremely toxic to the human body. The traditional processing of cassava (grating and pressing) eliminates most of these toxic compounds, but what is considered reimoso in regards to cassava is the smell that results from this process, as well as a fermented alcoholic beverage that is made ÿ'3fÿ'3ffrom cassava roots. Wild animals are considered reimosos because they eat reimosas fruits and because there are myths that associate their skin and meat with poisons. Sugar and sweets are avoided, as they would increase the temperature of the body and worsen a fever. Although there is a consensus of opinion regarding the foods considered to be most reimosos, there are specific reimosas foods for each type of person, suggesting that reima is not a quality inherent only to food, but also to the association of food with the consumer organism. The perception of the indigenous people on the need for a diet that restrictsmainly fat, sugar and alcohol seems to be important at the stage of malaria that involves liver weakness and for prevention of disease recurrence.

Acknowledgment: CNPq, CsF, Alto rio Negro indigenous communities.

PO28
BRAZILIAN LEGISLATION ON ACCESS TO TRADITIONAL KNOWLEDGE ASSOCIATED WITH GENETIC RESOURCES: THE CASE OF A MALARIA PROJECT IN AMAZONIAN INDIGENOUS COMMUNITIES

Kffuri, C. W.[1]*; Ming, L. C.[1]
[1]*PhD, Universidade Estadual Paulista/ Rua José Barbosa de Barros, n° 1780, Botucatu, SP, Brazil.*
**carolkffuri@gmail.com*

Introduction

In Brazil, the law governing access to Traditional Knowledge (TK) Associated with Genetic Resources (GR) is the Provisional Act 2.186-16 of 2001, that primarily provides: access to genetic resources, protection and access to associated traditional knowledge, benefit sharing and access and transfer of technology for conservation and its utilization. To conduct research involving TK and GR, it is necessary to receive authorization from the Board of Management of Genetic Patrimony (CGEN), a regulatory and deliberative agency of the Ministry of Environment that is responsible for establishing rules and regulating access to traditional knowledge associated with genetic resources. This abstract aims to briefly describe the process of obtaining permission to search for antimalarial plants in indigenous communities in the Brazilian Amazon by a Brazilian state university.

Method

The process of obtaining authorization for research began in 2010 and was partially completed in 2013. Results shown are based on official documents exchanged between the University and CGEN and on the experience of the researchers with the process of obtaining prior informed consent from indigenous communities in the Brazilian Amazon. The city of São Gabriel da Cachoeira is located in the extreme northeastern of Brazilian Amazon over 5,000 km from the university and the communities studied live several hours away from the city and can be reached only by boat or airplane.

Results / Discussion / Conclusion

The research on antimalarial plants used by indigenous communities in São Gabriel da Cachoeira is part of a large project entitled "Research network of plant chemicals for the control of malaria based on ethnopharmacology." The network is based on ethnopharmacological research in traditional communities, but also provides for the screening of chemical compounds found in plants and their effectiveness in combating the causative agent of malaria. This network involves nine federal institutes and universities. The first step in obtaining the CGEN authorization starts with acquiring the prior informed consent of indigenous communities. In the specific case of the municipality of São Gabriel da Cachoeira, other permits were necessary, such as permission from the Federal Indigenous Agency (FUNAI), as access to indigenous knowledge and indigenous lands was required, and the Brazilian Army, as the city is close to the ÿ'3fÿ'3fnational border. Onsite meetings with the local federation base and meetings with all of the indigenous communities studied were made. The indigenous representatives of local federations are very politicized. After obtaining the prior informed consent of the communities, the documents were sent to CGEN in July 2010. After much insistence from the investigators and some processing errors on the part of CGEN, in May 2012, CGEN sent a letter requesting additional documentation. To fulfill this request, it would have been necessary to travel back to São Gabriel da Cachoeira to meet with all of the indigenous communities again. This additional travel was not possible, so an extensive report was written by the investigators to explain the impossibility of acquiring the additional documents. In addition, the coordinator of the project attended meetings with CGEN to further explain this. On March 5, 2013, the authorization issued by CGEN was published in the Official Journal, 32 months after the beginning of the authorization process. CGEN only approved access to traditional knowledge and denied any chemical screening of plants. Due to the delay in the process, the indigenous communities felt misled about when the research would begin and repeatedly questioned the researchers about it. Some communities and their leaders were changed. Then, again, meetings were held with the communities to explain the entire project and its implications. After dealing with so much bureaucracy, the research on traditional knowledge was able to be conducted, and it was done, but now who will ensure the rights of the communities over their knowledge?

PO29
ANTI-INFLAMMATORY ACTIVITY OF THE ESSENTIAL OIL FROM *Xylopia sericea* FRUITS, ANNONACEAE

Mendes, R. F.[1]*; Santos, R. C.[2]; Scio, E.[3]
[1]MSc;[2] Pharmacy student [3]Prof/PhD, Laboratory of Bioactive Natural Products. Department of Biochemistry. Institute of Biological Science of Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil
**renatinhafmendes@gmail.com*

Introduction
The family Annonaceae is known for its medicinal properties. The species Xylopia sericea, popularly known as "embiriba", "banana-de-macaco", "pindaíba", "pimento-da-costa" or "pimenta-de-macaco", is popularly used as an analgesic, anti-inflammatory and for treating gastrointestinal disorders. Its seeds and fruits have carminative effect and are often used as a condiment in cuisine, replacing “black pepper”. So, the aim of this study was to evaluate the anti-inflammatory activity of the essential oil obtained from the fruits of X. sericea.

Method
Fruits of X. sericea were subjected to hydrodistillation using a Clevenger type apparatus. The test was performed using male Swiss mice, weighing 25 - 35 g. The inflammation was induced by topical application of croton oil 2.5% in acetone (20 μL/ear) on the right ear of each mouse. On the left ear (control) only the vehicle (acetone 20 μL) was applied. After 15 minutes, the animals (n = 8) received the following topical treatments: Group I (negative control), 20 μL of acetone; Group II (positive control), 20 μL of dexamethasone (0.1 mg/20 μL) diluted in acetone; and Group III: 20 μL of essential oil (0.5 mg/20 μL) diluted in acetone. Six hours after application of the phlogistic agent, the animals were sacrificed and identical disks of 6 mm diameter from both ears were obtained using a metallic punch. The disks were weighted on an analytical balance and the weight difference between the right and left ears indicated the intensity of edema. Data were subjected to analysis of variance (ANOVA) followed by Newman-Keuls test ($p < 0.05$).

Results / Discussion / Conclusion
The ear weights were: 6.2±0.7 mg for Group I; 0.7±0.6 mg for Group II; 2.8±1.6 mg for Group III. The essential oil showed significant anti-inflammatory activity ($p<0.001$) when compared to control (vehicle). It is possible that components of essential oil are involved on inhibition of inflammation chemical mediators, as well as dexamethasone. This work encourages further studies to identify the anti-inflammatory compounds of Xylopia sericea fruits and to elucidate their mechanism of action.

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PO30
ACTIVITY OF PHYTOL-RICH FRACTION FROM *Lacistema pubescens* AGAINST *Leishmania amazonensis*

Silva, J. M.[1]*; Antinarelli, L. M. R.[2]; Coimbra, E. S.[3]; Ribeiro, A.[4]; Scio, E.[4]
[1]MSc; [4]Prof/PhD, Laboratory of Bioactive Natural Products. Department of Biochemistry. Institute of Biological Science of Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil
[2]MSc;[3]Prof/PhD, Department of Parasitology, Microbiology and Immunology, Biological Sciences Institute, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil
** jmsjf@yahoo.com.br*

Introduction

Current chemotherapy for leishmaniasis is far from ideal due to a number of problems such as high cost and high toxicity (Sindermann *et al.*, 2004). Thus, there is an immediate need to obtain new drugs for the treatment of this disease.Estrogenic activity of friedelin rich fraction (IND-HE) separated from *Cissus quadrangularis* and its effect on female sexual function Estrogenic activity of friedelin rich fraction (IND-HE) separated from *Cissus quadrangularis* and its effect on female sexual function Originally from Brazil, Lacistema sp Mart. (Lacistemataceae) is widely distributed in other countries of South America. In Brazil, "sabãozinho" and "coffee" are some of the popular names found for this tree (Di Stasi and Hiruma-Lima, 2002). The present study was conducted to evaluate the in vitro effect of the phytol-rich fraction from L. pubescens against Leishmania amazonensis promastigotes and amastigotes forms and mouse peritoneal macrophages.

Method

The dried leaves (375 g) were powdered and macerated with methanol (5 x 300 mL) for five days at room temperature. The crude extract (65 g), after removal of solvent, was dissolved in MeOH-H2O (8:2) and partitioned with hexane. The hexane extract was then concentrated using a rotary evaporator under reduced pressure (yield 16 g). This extract was chromatographed on a 74 x 4 cm column of silica gel (70-230 mesh) with a gradient of increasing polarity (hexane, hexane-EtOAc, EtOAc, EtOAc-MeOH, MeOH) to obtain a total of 17 fractions. The fractions obtained were analyzed by GC-MS. The antileishmanial activity was determined by colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetra-zolium bromide (MTT) method (Mossman, 1983).

Results / Discussion / Conclusion

Results showed that, among the 17 fractions, the phytol-rich fraction was active against Leishmania amazonensis promastigote and amastigote forms and presented no toxicity for murine macrophages. This fraction was the most active for intracellular amastigotes (IC50 of 32.6 µg/mL) when compared to promastigote forms (IC50 of 44.0 µg/mL). For all bioassay, the IC50 values below 100 µg/mL were considered significant (Cos *et al.*, 2006). The results contributed to the research for new anti-leishmania drugs and suggested that phytol has promising antileishmanial potencial. In addition, this is the first report of the presence of diterpenes in the Lacistema genus, which may serve as a chemotaxonomical marker.

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PO31
CERVICAL CANCER STUDY OF *Opuntia joconostle*

Monroy, I. V.[1]; Figueroa, P. A.[2]; Silva, R. T.[1]
[1]*Student, Instituto Politecnico Nacional E.N.C.B. Av. Wilfrido Massieu s/n México D.F. C.P. 07738*
[2]*PhD/Prof Instituto Politécnico Nacional E.S.M. Calle Salvador Díaz Mirón y Plan de San Luis S/N, México, D.F. 11340*
[1]*PhD/Prof Instituto Politécnico Nacional. Av. Wilfrido Massieu s/n México D.F. C.P. 07738*
M. Salas No. 172 México D. F. C.P. 07070
rafaelsilvatorres@hotmail.com

Introduction

The cerviccal cancer is an important persists in problem in the public health in the world, mainly in the undeveloped countries. In Mexico, this cancer is the first reason of death of women between 25 and 64 years of malignant neoplacias. Women between 60 or more years the cervical cancer have a mortality of 41.88 per cent for 1000 woman. World health organization (WHO) contemplate the mortality of cancer in the world between 2007 and 2030, will increase 45% it is estimated that happen of 7.9 to 11.5 million of deaths for} year, result of population growth and aging, besides during the same period, the new cancer occurrences will grow from 11.3 to 15.5 million. On the other hand, it has been reported the uses of Opuntia joconostle (Xoconoxtle) in the Traditional Medicine as: laxative, cholesterol level control, reduce blood pressure, hypoglycemic and against cancer. All of the above it is necessary investigate our natural resources as Xoconoxtle in order to find new alternatives to treat the cancer.

Method

The mature fruit (7.6 Kg) was collected in Ozumba State of Mexico in April of 2013. It was obtained the ethanol crude extracted and the secondary metabolites were identified. A chromatography colon was made to separate different fractions from the extract. The biologicals tests lines HeLa (ATCC:CCL_23) were used to cervical cancer. Handling cell lines in terms sterility in laminar flow hood, using sterile equipment and solutions. Lines HeLa was grown in cell medium DMEM (Duldeco's ModififielEdgle Medium, Gibco) supplemented with fetal bovine serum (Fetal BovineSerum, Gibco) and antibiotic (Invitrogen penicillin streptomycin). The incubation was made in a incubator at 37 0C, CO 2 5%.

Results/Discussion/ Conclusion

It was identified in the crude extract, alkaloids, flavonoids, cumarins and quuinones. From the Colum chromatography were obtained 79 fractions, which were together the similar RF, 9 being obtained fractions and were identified, coumarins flavonoids saponins and quinones. Only 4 fraction of the extract showed cytotoxicity. The most cytotoxicity showed the fraction 7 a consentracion of 1000 μg/mL.

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PO32
THE FOLK MEDICINAL PLANTS OF ACIGÖL, DERINKUYU, GÜLŞEHIR, NEVŞEHIR-CENTRAL DISTRICT AND ÜRGÜP (NEVŞEHIR-TURKEY)

ªŞenkardeş, İ.*, Tuzlacı, E.
Ph. D. Student, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey.
Prof. Dr., Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey.
**isenkardes@marmara.edu.tr*

Introduction
This study was made to reveal the plants used as traditional folk medicine in Acigöl, Derinkuyu, Gülşehir, Nevşehir-Central district and Ürgüp. For this purpose, the field work was done between June 2012-March 2014. During this research 98 settlement centers (including 75 villages) were visited.

Method
The specimens of the plants used as folk remedies, have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration and the duration of treatment has been recorded. The information was obtained from 98 participants who were not only experienced adults but also patients in face to face interviews; furthermore, the specimens of the plants were collected. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University.

Results
As a result of identification of the plant specimens, 111 species used as a traditional folk medicine in Acigöl, Derinkuyu, Gülşehir, Nevşehir-Central district and Ürgüp, have been determined. Among them, 93 species are wild and 18 species are cultivated plants. According to the majority of the plants which have similar usage, the plants are mostly used for shortness of breath, diabetes, cough, gastric ulcer, abdominalgia and cardiovascular diseases.

PO33
ANTIVIRAL ACTIVITY AGAINST *Mayaro* VIRUS OF *Cassia australis*

Spindola, K. C. W.[1]*, Simas, N. K.[2], Sato, A.[3], Salles, T. S.[4], Meneses, M. D. F.[4], Ferreira, D. F.[4], Kuster, R. M.[1]

[1]*PhD Student, Núcleo de Pesquisas de Produtos Naturais, Centro de Ciências da Saúde, Bloco H, Universidade Federal do Rio de Janeiro, 21941-590. Rio de Janeiro, RJ, Brazil.*

[2]*Prof, Departamento de Produtos Naturais e Alimentos. Centro de Ciências da Saúde, Bloco A, Universidade Federal do Rio de Janeiro, 21941-590. Rio de Janeiro, RJ, Brazil.*

[3]*Prof, Departamento de Botβnica, CCBS, Universidade Federal do Estado do Rio de Janeiro-UNIRIO, Avenida Pasteur 458, 22290-040 Rio de Janeiro, Brazil*

[4]*Instituto de Microbiologia Prof. Paulo de Góes, Departamento de Virologia. Av. Brigadeiro Tropowski, s/n, CCS, Bloco I, Ilha do Fundão. 21941-590. Rio de Janeiro, RJ, Brazil.*

**kawaldhelm@gmail.com*

Introduction

The genus Cassia (Fabaceae, Leguminosae) comprises more than 600 species, including shrubs, trees and herbs. Due to the traditional use, several species, many already described in the literature, are medicinally used worldwide. Cassia australis is a medium sized shrub and may reach up to 2.5 meters of diameter. Air-dried leaves were extracted with Methanol/Water and the extract was partitioned with hexane. The remaining aqueous solution was partitioned successively with CH_2Cl_2, EtOAc, and n-BuOH. The EtOAc, n-BuOH and sub-partition EtOAc-Purp showed anti-viral activity against Mayaro virus, an important emerging arbovirus.

Method

Air-dried leaves (850 g) were extracted with MeOH:H2O (8:2) at room temperature by static maceration during 10 days. After concentration under reduced pressure, the methanol extract (25 g) was suspended in MeOH:H_2O (9:1), and partitioned with hexane. After removal of the methanol from the defatted extract, the remaining aqueous solution was partitioned successively with CH_2Cl_2, EtOAc, and n-BuOH. From EtOAc fraction was obtained EtOAc-Purp fraction, with XAD-2 column and 100% of water. The ESI(-)-FT-ICR MS, negative mode, was used to compare the samples and identify the main substances. The cytotoxicity of fractions was measured by the dye-uptake method while the antiviral activity was evaluated by virus yield inhibition assay and titrated by plaque assay.

Results / Discussion / Conclusion

EtOAc and n-BuOH fractions inhibited MAYV production, respectively, by more than 70% and 85% at 25μg/mL EtOAc-purp, fraction inhibited MAYV production by more than 90% at 10 μg/mL, displaying a stronger antiviral effect than the licensed antiviral ribavirin. The ESI spectra of EtOAc and n-BuOH fractions are very similar, containing mainly flavonoids glycosylated (m/z 513, 593, 447, 477). The main difference of EtOAc-purp spectrum is the presence of signs of condensed tannins (m/z 529). Catechins and proanthocyanidins have a documented antiviral activity, in the inhibition of virus adsorption (Ueda et al, 2013; Fukuchi et al, 1989). Therefore, we conclude that substances responsible for the remarkable activity of the AcOEt-purp fraction are the condensed tannins.

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- INBEB

PO34
PHENOLIC COMPOUNDS AND ANTIMICROBIAL ACTIVITIES OF *Serjania erecta* RADLK. (SAPINDACEAE)

Cardoso, C. A. L.[1], Coelho, R. G.[2], Honda, N. K.[2], Pott, A.[3], Pavan, F. R.[4], Fujimura Leite, C. Q.[4]*
[1]*Course of Chemistry, State University of Mato Grosso do Sul, Dourados, MS, Brazil,*[2]*Institute of Chemistry, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil*[3]*Biology Department, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil,*[4]*Faculty of Pharmaceutical Sciences, State University Paulista, Araraquara, SP, Brazil*

Introduction
Serjania erecta Radlk. is known in Brazil as “cinco-folhas” or “cipó-cinco-folhas”. Its leaves are used in folk medicine against inflammation, stomach ache, ulcerative diseases, and the roots to treat hypertension (Guarim Neto *et al.*, 2000; Pott *et al.*, 2004).

Method
For this work we used only the ethanol extract (12.0 g and 3.4 g) of leaves and roots, respectively. Both extracts were fractionated by XAD-2 (Supelco, Bellefonte, PA, USA) resin column chromatography (30 cm × 3 cm). The substances obtained were further purified by repeated column chromatography either on polyvinylpolypyrrolidone (Sigma, eluted with MeOH) eluted with methanol. NMR spectra were recorded on a Bruker DPX 300 spectrometer. IR spectrum were performed in a FT-IR-Nicolet Impact IMACT-400, KBr and UV spectrum were performed in a Hitachi 110 spectrophotometer.
The REMA (Resazurin Microtiter Assay) assay was determined using the leaves and roots ethanolic extract against M.tuberculosis H37Rv ATCC. The antimicrobial activity of the ethanolic extracts was assayed using the broth microdilution method, against four bacteria: Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC27853, Escherichia coli ATCC 11103 and Salmonella ATCC 19796, and two yeasts: Saccharomyces cerevisiae ATCC 2601 and Candida albicans ATCC 10231.

Results / Discussion/ Conclusion
From ethanolic extracts of leaves and roots were isolated (-)-Epicatechin, kaempferol aglycone and five glycoside derivates: kaempferol-3-O-a-l-rhamnopyranoside, kaempferol-3-O-a-l-rhamnopyranosyl-(1ÿ'3f6)-β-dglucopyranoside from the roots and kaempferol, kaempferol 3,7-di-O-a-L-rhamnopyranoside, vitexin, isovitexin and (-)-epicatechin in the leaves. The ethanolic leaf extract showed an MIC value of 128.0 µg mL-1 and root extract MIC value of 256.0 µg mL-1for the anti-M. tuberculosis activity. Studies of antimicrobial activity indicate that crude extracts of leaves and roots containing high content of flavonoids have showed significant activity against bacteria and fungi (5.0±0.1 µg mL-1, to 25.0±0.2 µg mL-1). This is the first chemical reported and antibacterial and antifungal activities study in the literature about this specie.

Uniterms: Serjania erecta Radlk/pharmacognosy. Serjania erecta Radlk/ethanolic extract/antioxidant activity. Serjania erecta Radlk/ethanolic extract/antimicrobial activity. Sapindaceae/pharmacognosy. Cipó-cinco-folhas/pharmacognosy. Flavonoids. Medicinal plants.

Acknowledgement
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PO35
STEROL AND TRITERPENES FROM *Qualea* SPECIES-BIOACTIVITY ON *Mycobacterium tuberculosis*

Nasser, A. L. M.[1], Calvo, T. R.[1], Campaner dos Santos, L.[1], Pavan, F. R.[2], Fujimura Leite, C. Q.[2]*, Hiruma-Lima, C. A.[3], Vilegas, W.[1]

[1]*Instituto de Química, Universidade Estadual Paulista, CP 355, 14801-970 Araraquara – SP*
[2]*Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, CP 502, 14801-902 Araraquara – SP*
[3]*Instituto de Biociências, Universidade Estadual Paulista, 18618-000 Botucatu – SP*

Introduction

Qualea species are known as "pau-terra" and are used in traditional medicine for many purposes. The Mycobacterium tuberculosis (TB), agent of tuberculosis, is responsible for high mortality rate in the world, annually killing about 1,7 million people. There are few effective drugs against tuberculosis. The plants through secondary metabolites pathways produce various compounds with biological activity against mycobacteria as terpenoids and physalins.

Method

The ethyl acetate extract of the barks of Qualea parviflora (Vochysiaceae) was fractionated by chromatographic columns on silica gel using silica gel 60H Merck (Darmstadt, Germany) and silica gel 60 PF254, Merck (Rio de Janeiro, Brazil). Some obtained compounds had been analyzed using gas chromatography-mass spectrometry. The anti- M. tuberculosis activity of ethyl acetate extract and those isolated compounds was analyzed by MABA determining the minimal inhibitory concentration (MIC) of the compounds necessary to kill 90% of the viable mycobacterial cells.

Resultados / Discussão / Conclusão

The fractionation process gives triterpenes (lupeol, lupenone, betulin, epi-betulinic acid, and friedelin) and sterol (b-sitosterol). The anti-M. tuberculosis activities were determined from Q. parviflora isolated compounds and the MIC value ranged from 250 to 31.2 μg/mL. This investigation is the first report of the chemical and antitubercular study of nonpolar compounds from Qualea species.

Keywords: Qualea species; triterpenes; anti- M. tuberculosis activity.

Aknowledgement: The authors thank to CNPq (306726/2012-2) and to PADC

PO36
EFFECT OF $CaCl_2$ E $MgCl_2$ ON THE PHOTODYNAMIC INACTIVATION OF GRAM NEGATIVE BACTERIA BY INDIGO ALKALOID

Andreazza N. L.[1], Caramano C. L.[1], Salvador M. J.[1]
[1]*Curso de Farmácia, FCF, PPG BTPB e BV, Depto de Biologia Vegetal, IB, UNICAMP, Campinas, SP, Brasil.*
nathilu@gmail.com; marcosjs@unicamp.br

Introduction

Photodynamic therapy (PDT) is a treatment based on the combination of light, oxygen and a photosensitizer that leads to reactive oxygen species (ERO's)production and consequently the host cell death. Indigo, a natural occurring pigment from Indigofera genus, has gained considerable interest due its mutagenic, cytotoxic and genotoxic activity (Calvo *et al.*, 2009). Studies regarding its photophysical (Gandra *et al.*, 2006) and spectroscopic characteristics rose relevant information about its application as a photosensitizer and photobiological studies conducted by our research group in previous work has shown photoactive activity of this substance in antimicrobial PDT. Gram negative bacteria are generally more resistant than gram positive due to differences in their cell wall composition that restrict the link and penetration of exterior substances. Certain additives, such as $CaCl_2$ and $MgCl_2$ may increase the permeability of gram negative bacteria outer membrane by selectively reacting with lipopolysaccharides, disrupting its integrity. Therefore, this study was undertaken to investigate the effect of $CaCl_2$ and $MgCl_2$ on gram negative bacteria photodynamic inactivation by indigo.

Method

Biological assay was performed employing the strain Escherichia coli ATCC 17099, according to the experimental procedure performed by Su *et al.* (2011), with modifications. Treatment mixtures were prepared in a 96-well microdilution plate containing microorganism suspension (107 CFU/ml, Mac Farland scale), additives (final concentration of 0,05M for both CaCl2 or MgCl2) and indigo (final concentration of 120 uM). The irradiation was performed with a 660 nm diode laser with 35 mW of output power, during 5 min, yielding an energy dosage of 28 J/cm2. Control treatments were also prepared. Treated and control microorganisms were serially diluted, plated and colonies were counted. The percentage of survivors were calculated according to the equation [(N1/N0)x100], where N0 represents the number of CFU/mL of each test sample (with or without additives) before irradiation and N1 represents CFU/mL after light exposure. Data were expressed as means of eight replicates ± standard error. Comparisons of microorganism grown within the same treatment (irradiated or not) were performed via t-tests ($P < 0.05$).

Results / Discussion / Conclusion

The presence of both additives with bacteria under or without irradiation or plus indigo without light did not affect microbial growth. However, the addition of $CaCl_2$ or $MgCl_2$ in the treatments contributed to a significant reduction in the number of CFU/ml, equals to 53% and 31,5%, respectively compared to treatment with indigo alone, after irradiation. It is believed that the increase in membrane permeability not only allowed greater alkaloid input as well as inner cytoplasmic production of ERO's (and not at the outer membrane, as usual in gram negative bacteria) responsible for the oxidative damage that leads to cell death. Despite the positive results, further investigations are necessary to characterize how this alkaloid is internalized by the bacteria at a cytoplasmic membrane level.

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PO37
TRADITIONAL USES OF MEDICINAL PLANTS AMONG FAMILY FARMERS OF REGION MURIAE, MG

Brasileiro, B. G.[1]; Calixto, J. S.[1]; Duarte, E. M. G.[1]; Paiva, M. S.[2]; Figueiredo, L.[2]
[1]*Prof./Federal Institute of Southest of Minas Gerais –Avenida Monteiro de Castro, 550- Muriaé-MG, Brazil.*
[1]*Student/Federal Institute of Southest of Minas Gerais –Avenida Monteiro de Castro, 550- Muriaé-MG, Brazil.*
beatriz.brasileiro@ifsudestemg.edu.br

Introduction

The use of medicinal plants and their derived forms has been the foundation of therapy through the centuries and that is often the only viable alternative for many communities in the treatment of diseases or health maintenance (Marchese *et al.*, 2009). Ethnobotanical studies in rural communities are very important, especially when we consider the great wealth of medicinal plants used in Brazil, which has been threatened because of human activities aimed at extraction plants. The objective of this study was to investigate the knowledge about medicinal plants among small farmers in rural communities in the region of Muriaé, MG.

Method

The research was conducted with farmers in the in the region of Muriaé, Zona da Mata, Minas Gerais, Brazil. Data were collected between August-November 2013, with the use of pre-established survey (Albuquerque & Lucena, 2004), with the purpose of retrieving information concerning the use of medicinal plants, which were part of the cultural tradition of these families. The identification of the plants mentioned was done with the group`s participation, according to the identification of morphological, vegetative and reproductive characteristics with the literature`s support (Lorenzi, 2006; Lorenzi & Matos, 2008; Lorenzi & Souza, 2008). The results were submitted to simple analyzes in order to place emphasis on the information obtained, classifying them into categories of responses that included the group`s featuring, the indications for use, the number of citations, plant part used and preparation methods.

Results and Conclusion

The characterization of the group in question showed that 53% of farmers were male and 47% female. For the preparation of various medicinal plant parts were cited, the sheet being the most widely used with 67% of 50% of quote and quote part pointed out the preparation in the form of tea as the primary means of utilization of medicinal plants. These species are distributed in 24 families and 50 gender, and the Lamiaceae and Asteraceae families were the most representative ones in this study, a result explained by the fact that these are cosmopolitan families that have adapted well in various environments and contains various essential oils. Mentha sp. was the most cited species and 18 other species are referred to in the resolution of the National Health Surveillance Agency (ANVISA), which regulates a list of medicinal plants traditionally used with scientifically proved effect beyond the correct ways of use and contraindications. Studies with medicinal plants should always aim to the medicinal uses optimization assigned by communities enhancing the prospects of future generations to take advantage of these resources. From these results can be drawn plans, proposing new management practices and/or replacements of plants with the same therapeutic values, as well as guidance on the storage and cultivation of these species, in addition to reinforcing the importance of preservation and dissemination of popular knowledge.

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PO38
Pipper aduncum EXTRACTS INDUCE CHANGES IN INTRACELLULAR CALCIUM CONCENTRATIONS IN CELLS EXPRESSING TRPV1 CHANNELS

Sierra C.[2], Sanchez R.[2], Casas Z.[1], Torres Y.[1]
[1] *Pontificia Universidad Javeriana, Bogotá, Colombia*
[2] *Universidad Distrital Francisco José de Caldas, Bogotá, Colombia*
yolima.torres@javeriana.edu.co

Introduction
Piper aduncum is a tropical shrub from *Piperaceae* family. The plant reports describe a broad range of activities such as antimicrobial (Torres Santos, *et al.* 1999), antifungal (Santos, *et al.* 2014), anti-inflammatory (Parise-Filho R, *et al.* 2011) and antinociceptive (Arroyo, *et al.* 2013). The antinociceptive mechanism used by *Piper aducum* extracts has not yet been elucidated. A well-known ion channel involved in nociceptive signaling is TRPV1 (reviewed in Adcock, *et al.* 2009; reviewed in Demir, et al 2013) which may be a good pharmacological molecular target. Herein, the hypothesis that *Piper aduncum* extracts can modulate TRPV1 channels activity is assessed.

Method
100g of dried leaves from *Piper aduncum* were used toobtainethanol extracts by percolation. The extract was concentrated under vacuum, lyophilized and solubilize in DMSO to prepare the following concentrations: 10, 50, 100 y 500 and 1000µg/ml. Cell viability was evaluated on HEK293 cells with MTT assay. Intracellular calcium concentration was measure in TRPV1 channels expressed on HEK293 cells using fluorometric assays. Capsaicin and Capsazepin were used as agonist and antagonist controls respectively.

Results
None of the evaluated concentrations of *Piper aduncum* ethanolic extracts caused cell death. An extract concentration of 100µg/ml induced changes in intracellular calcium concentration in HEK293 cells expressing TRPV1 channels similar to the effect caused by the agonist Capsaicin. In contrast, intracellular calcium concentrations did not change in cells void of TRPV1 channel. No changes in intracellular calcium concentration were observed in HEK293 cells expressing TRPV1 channels treated with the antagonist Capsazepin after extract treatment.

Discussion and Conclusions
Piper aduncum ethanolic extract induce an increase in intracellular calcium concentration because of TRPV1 channels activation. Our conclusion is based in the findings that the extract increases intracellular calcium concentrations in HEK293 cells expressing TRPV1 channels whereas in channel void cells no effect is seen. A similar effect is observed with the agonist compound while is block by the channel antagonist. Electrophysiological recordings are necessary to assure if the effects are by direct activation of the channel.

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PO39
DIRECT ANALYSIS OF DIFLUNISAL HYDRAZIDE-HYDRAZONE AND 4-THIAZOLIDINONES: DART-MS

Sevil Şenkardeş[1,a], Güniz Küçükgüzel[2]
[1]*PhD student, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Sciences, Istanbul, TURKEY*
[2]*Prof, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Sciences, Istanbul, TURKEY*
Corresponding Author: gkucukguzel@marmara.edu.tr (ª.Güniz Küçükgüzel)

Introduction
Mass spectrometry (MS) is one of the most powerful analytical method used for measuring the molecular mass of a sample. This application is sensitive, easier to use and available for structural identification of organic compounds. Mass spectrometers are used in industry, pharmaceuticals, biotechnology, environmental field and academia for research aims. Since the pioneering introduction on ambient ionization techniques for MS, a lot of research have been reported such as direct analysis in real time (DART) (1). DART is a new ionisation technique introduced by Cody *et al.* (2) and plays a major role in many applications.It is carried out in the open air, with no previous sampleand samplepreparation (3).

Method
In the present study, a series of new hydrazide-hydrazones and 4-thiazolidinones have been synthesized via diflunisal used as NSAID. The characterization of the synthesized compounds were identified by the help of DART-MS using ICR Apex-Qe DART.

Conclusion
Several hydrazide-hydrazone and 4-thiazolidinones were successfully detected by using DART-MS and it is showed that there were different types of high and low molecular weight compounds. DART-MS profiles of the compounds showed characteristic differences. Most of the identified compounds by DART-MS are basically biologically important. Thus, DART-MS is a promising tool for the rapid and correct analysis of importantmolecules in pharmaceuticals, functional foods, plants etc.

Acknowledgments
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PO40
HEPATOPROTECTIVE EFFECT OF *Leucophyllum frutescens* FOLLOWING THIOACETAMIDE-INDUCED NECROSIS IN RATS

Hernández Rodríguez Maricruz[1], Mirandeli Bautista[2]*; JA Gayosso-De Lucio[2], A. Chehue-Romero[2], C. Velázquez-González[2], M. De la O-Arciniega[2], E. Olvera-Hernández[2]

[1]*Student, Autonomous of Hidalgo State University /* Health Sciences Institute*/ Circuito Ex – Hacienda La Concepción, Km 1.5. C.P. 42160. San Agustín Tlaxiaca, Hidalgo, Mexico.*

[2]*PhD, Autonomous of Hidalgo State University /* Health Sciences Institute*/ Circuito Ex – Hacienda La Concepción, Km 1.5. C.P. 42160. San Agustín Tlaxiaca, Hidalgo, Mexico.*

**mirandeli@hotmail.com*

Introduction

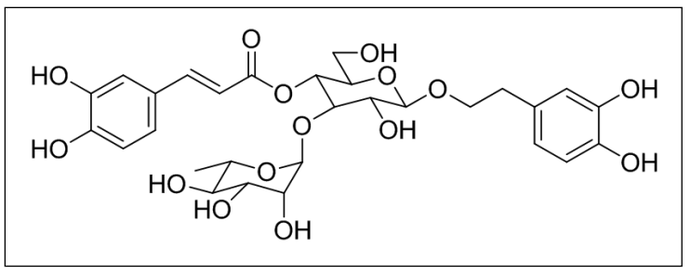
Leucophyllum frutescens (Lf) commonly known as "ashen", belongs to the family of Scrophulariaceaes which is used in airway diseases such as cough, tuberculosis and asthma; also used as healing, anti-oxidant, vasodilator and as analgesic. Some of the compounds isolated from this genus includes: diterpenes, phenolic compounds, polyalcohols and sterols. The methanolic extract of the aerial parts of Lf allowed the isolation and identification of verbascoside (1). In the present study the effect of this verbascoside were studied in reference to postnecrotic liver damage induced by thioacetamide (TA).

Method

The aerial parts of Lf were extracted by maceration, using hexane, ethyl acetate and methanol (5 L/500 g / 8 days), the solvent was removed with a rotary evaporator at 40 ° C under reduced pressure. Verbascoside isolated from methanol extact in about 30% of abundance. Wistar rats were intragastric pre-treated or not with a single dose of verbascoside (20 mg/kg) during 4 days, the fourth day of pretreatment were intraperitoneally injected with a single dose of TA (6.6 mmol/Kg). Samples of blood and liver were obtained from rats at 24 and 48 h following TA intoxication. Parameters related to liver damage like AST and ALT were carried out in blood using well established protocols and methods.

Results / Discussion / Conclusion

Verbascoside, an active phenylpropanoid glycoside found in many medicinal plants, displays various biological activities. Moreover previous studies had shown that attenuates carbon tetrachloride-induced hepatic injury. Our results show that the verbascoside significantly reduced the level of liver injury following thioacetamide-induced necrosis. The levels of AST and ALT were significantly lower in the rats pretreated with it. This result suggests that verbascoside may be used as an alternative for reduction of liver damage. However further investigation on the acute toxicity and on the mechanism of the hepatoprotective effect of it needs to be carried out.



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PO41
Metabolic profiling of Calabrain licorice (*Glycyrrhiza glabra*) by UHPLC-HRMS and tandem mass spectrometry (MSn)

Rita Celano[1*], Lidia Montero[2], Anna Lisa Piccinelli[1], Maria Anna Euterpio[1], Imma Pagano[1], Luca Campone[1], Alejandro Cifuentes[2], Mariateresa Russo[2], Luca Rastrelli[1]

[1]*Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II, 84084 Fisciano (SA), Italy*
[2]*Instituto de Investigaciün en Ciencias de la Alimentaciün (CIAL, CSIC). C/ Nicolás Cabrera, Universidad Autünoma de Madrid, 28049, Madrid, Spain.*
[3]*Dipartimento di Agraria, Università di Reggio Calabria, Feo di Vito, 89100 Reggio Calabria, Italy*
** rcelano@unisa.it*

Introduction

Licorice root is one of the oldest medicinal plants that have been used by human beings (Li *et al.,* 2000) Many biological studies have shown that licorice presents a wide variety of pharmacologic activities such as antioxidant, antiviral, antidepressant, anti-inflammatory, anti-carcinogenesis, and a bioactivity hepatoprotective (Finney *et al.,* 1958; Pompei *et al.,* 1979¸ Visavadiya *et al.,* 2006) These therapeutic activities are related to its metabolite composition, mainly flavonoids and tripterpene saponins (Montoro *et al.,* 2011).
The main constituent of licorice roots is glycyrrhizic acid and it is considered responsible for some adverse effects, it may interfere with the balance of mineral salts, causing the increase blood pressure (Heikens *et al.,* 1995). However, as shown in previous studies, the Calabrian licorice has the lowest content of glycyrrhizic acid. For these reason, in the present study, an analytical method for qualitative evaluation of the secondary metabolites present in Calabrian licorice has been developed, in order to identify geographical markers to trace the origins and history of the product.

Method

For metabolic profiling, appropriate aliquots of root material were mixed with ethanol:water (1:1, v/v; sample to solvent ratio 1:5, w/v) and subjected to ultrasonic agitation for 1 h and stored overnight at room temperature. The resulting extracts were filtered and diluted 1:10 (v/v) with ethanol:water (1:1, v/v) prior to qualitative or quantitative analysis (Montoro *et al.,* 2011).
The characterization of the chemical composition of licorice roots was obtained by coupling ultra high performance liquid chromatography (UHPLC) to two detectors in series, a Diode Array Detector, (DAD) and an high-resolution mass spectrometry, (HRMS).

Results / Discussion / Conclusion

In the extract of Calabrian licorice roots were detected 76 compounds, and most of them are phenolic compounds (flavones, flavanones and chalcones) and triterpenoid saponins. The detected peaks in the chromatographic profile were characterized by comparison with retention time, exact mass, UV spectra (range 200-600 nm), matching diagnostic ions, and fragmentation patterns.

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PO42
ANTIHIPERGLICEMIC ACTIVITY OF THE ETANOLIC EXTRACT OF *Annona cherimola* MILL. LEAVES (ANNONACEAE) IN RATS WITH DIABETES MELLITUS 2

Solares-Pascasio J. I.[1,2]; Calzada F.[1]

[1]*Unit of Medical Research in Pharmacology, Specialty Hospital, Centro Médico Nacional S. XXI, IMSS Av. Cuauhtémoc 330 Col. Doctores Del. Cuauhtémoc CP 06725, México D.F. México*

[2]*Escuela Superior de Medicina of the Instituto Politécnico Nacional, Post grade and Research Section, Plan de San Luis y Díaz Mirón s/n Col. Casco de Santo Tomás Del. Miguel Hidalgo CP 11340. México D. F. México.*

jeivsp@gmail.com

Introduction

In the world is recognized the use of traditional medicine and plants to treat of diabetes, in these sense México has around 383 plants used for the diabetes treatment. Annona cherimola is better known as "chirimoya", is used in Mexican traditional medicine to treat different diseases, like diarrhea, dysentery, abdominal pain, pneumonia, fever as well as diabetes. However, in the case of diabetes there are not enough studies that explain this activity.

Method

It was used healthy and diabetic male Sprague-Dawley rats, for the diabetes induction was used alloxan, it was administrated by an intraperitoneal injection. Animals with glucose levels above 200mg/kg were selected for the acute and chronic treatments. In the acute treatment it was administrated a single oral dose of the ethanolic extract (300mg/kg) or the vehicle (DMSO 2% in water), measuring the glucose levels at time of 0h, 2h and 4h. In the chronic treatment, the ethanolic extract was administrated every day for 4 weeks, the glucose levels were measured every 7 days.

Results / Discussion / Conclusion

The results show that the ethanolic extract causes glucose levels of 125mg/dL in diabetic rats in the acute assay after 4h. In the case of chronic assay, glucose levels were of 113 to 116mg/dL during second to fourth weeks of experiment. Further the compound responsible of the anti-diabetic activity was isolated.

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PO43
SYNTHESIS OF NEW STEROIDAL PYRANIC GLUCOSIDES, AS ANTICANCER AGENTS

Hilario-Martínez, J. C.*; Fernández-Herrera, M. A.[1]; Sandoval-Ramírez, J.[1]
[1] *Professor, Benemérita Universidad Autónoma de Puebla, Ciudad Universitaria, 72570 Puebla, Pue., México*
**ciciolil.hilario@correo.buap.mx*

Introduction

The steroidal saponins are secondary metabolites present in small amounts in plants; they have been widely employed through extracts in traditional medicine. Steroidal saponins have been employed as antidiabetic, antifungal, antiinflammatory, and anticancer, etc.1 The OSW-1 is a natural saponin with a potent antitumor activity; its anticancer activity is about 10-100 times more active than some other well-known anticancer agents, including taxol.2 For this reason the chemical synthesis of OSW-1 and analogues has become an attractive target. Our research group has reported the synthesis of analogues of OSW-1 and their in situ biological trials have shown a high anticancer selectivity.3 In the present report, the synthesis and biological evaluation of pyranic steroidal saponins is described.

Method

Starting from diosgenin (1) the (25R)-3β-hydroxy-22,26-epoxycholesta-5,22-dien-16β-yl acetate (2) was prepared in a single step, through a selective spirostanic E-ring opening. The E-ring fission process produces an acetate group at C-16 and an oxonium ion in the F-ring. A subsequent removal of proton from C-23, directs to a dihydropyranic compound with excellent yields. This methodology was also applied to sarsasapogenin and hecogenin obtaining the corresponding pyranic compounds, analogues of 2; which would be used as aglycons. The usefulness of compound 2 as a synthetic tool has been evidenced by its transformation in a variety of 22-oxocholestanic frameworks. In all cases the alcohol group at C-3 remained untouched which would allow creating glycosides. Sugar moieties play an important role in the biological activity and confer physiological solubility.4 The donor, a derivative of glucose, was prepared under standard procedures following a 3 step pathway. Glucose was first peracetylated and then selectively deprotected at the anomeric center by means of AcONH4. Finally, the formation of the corresponding 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl trichloroacetimidate 3 was performed by treatment with trichloroacetonitrile.4 Finally, for the linkage step the pyranic steroidal alcohol and 3 were submitted to the action of TMSOTf/DBU yielding the corresponding β-glycoside.

Results / Discussion / Conclusion

A route to synthesize new steroidal dihydropyranic 22,26-epoxycholestanes as 2 was developed. In this synthetic route the key step was the selective β-deprotonation of the oxonium ion. Under the reaction conditions the alcohol group at C-3 remained untouched which allows the formation of a glycosidic bond, so the (25R)-16β-acetoxy-22,26-epoxycholesta-5,22-dien-3β-yl glucopyranoside, was synthesized to evaluate its cytotoxic activity towards ViBo, HeLa and CaSki tumoral cells. Information of the anti-proliferative and apoptosis-inducing activity on cancer cells will be presented in detail at the Conference. All new products were characterized through their physical constants, and spectroscopic data (UV, IR, MS, and one and two-dimensional NMR).

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PO44
BASSIC ACID AND DERIVATIVES: SIMULATION OF INTERACTION WITH PEPTIDE DEFORMYLASE

Leite, S. R. A.[1*];
[1]Prof., Universidade Estadual Paulista/Instituto de Química, Rua Prof. Francisco Degni, 55, 14800-060 Araraquara, SP, Brazil
*leite@iq.unesp.br

Introduction

A few years ago bassic acid, a triterpene, was isolated from the plant Byrsonima fagifolia, which was shown to have activity against Mycobacterium tuberculosis1 (MTb). Later it was verified by computer simulation that this substance could interact with the active site of the enzyme peptide deformylase (PDF) of MTb and may therefore inhibit this enzyme2. In this work we use the same type of calculation to determine the effect of structural changes of the bassic acid molecule on its interaction with the active site of PDF, aiming to increase their inhibitory effect.

Method

The bassic acid was compared with two of its derivatives: the first obtained replacing the H by CH3 on carbon 3 (Deriv_Me), and the other exchanging CH3 by F at carbon 4 (Deriv_F)2. Such modifications aimed at intensifying the strength of the two hydrogen bonds formed between the bassic acid and the enzyme active site, involving the hydroxyl groups bonded to such carbon atoms, as foreseen by the previous simulation.
The simulation of bassic acid structure and of its derivatives were made by energy minimization with the ab initio quantum-mechanical DFT-B3LYP method (program Jaguar from Schrodinger, Inc.). The structure of peptide deformylase was obtained from the Protein Data Bank. The simulation of the interaction of PDF with the ligands was performed by docking calculation (program Glide from Schrodinger, Inc.). The binding energy of the ligand-enzyme pair was calculated by a mixed quantum-classical method: quantum ab initio for the ligand and enzyme interaction region, and classic for the rest of the protein molecule (program QSite from Schrodinger, Inc.).

Results / Discussion / Conclusion

It has been shown that the bassic acid can interact with the same binding site of the PDF with a known inhibitor3. The docking calculations suggest the formation of a hydrogen bond (HB) between the oxygen of the hydroxyl linked to the C3 and VAL 50 of the PDF, and a HB between the H of the hydroxyl group attached to C4 and GLY 105. These results suggested the modifications made in the structure of bassic acid, which resulted in the derivatives Deriv_Me and Deriv_F, aiming increasing such bond strenght and thereby intensifying the stability of enzyme-inhibitor pair and the inhibition constant. Below are the results of calculations:

Ligand	Docking GScore	Binding energy Ligand – Enzyme (Hartree)
Bassic acid	-5.46	-9.650422
Deriv_Me	-5.59	-9.933852
Deriv_F	-4.82	-9.744867

Deriv_Me reached this goal, since GScore (parameter that quantifies the quality of the ligand-macromolecule interactions in docking) increased compared to the bassic acid, and the binding energy with PDF increased too. On the other hand, Deriv_F had a small increase in binding energy, but a lower Gscore. This derivative interacted with the PDF with another geometry, and formed two HB involving different groups, which can justify a lower Gscore, although the calculated binding energy is slightly higher than that of the bassic acid, indicating a stable interaction. Concluding, it is expected that the derivative Deriv_Me is an inhibitor of PDF more potent than bassic acid, while for Deriv_F we cannot make a reliable prediction.

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PO45
OCCURRENCE OF *Mycobacterium bovis* AND NON-TUBERCULOUS MYCOBACTERIA (NTM) IN RAW AND PASTEURIZED MILK IN THE NORTHWESTERN REGION OF PARANÁ, BRAZIL

Sgarioni, S. A.[1]; Hirata, R. D. C.[2]; Hirata, M. H.[2]; Leite, C. Q. F.[3]; Prince, K. A.[3]; Leite, S. R. A.[4]*; Vedovello Fo, D.[5]; Siqueira, V. L. D.[1]; Ferracioli, K. R. C.[1]; Cardoso, R. F.[1]

[1]*Universidade Estadual de Maringá/Departamento de Análises Clínicas e Biomedicina, Maringá, PR, Brazil*
[2]*Universidade de São Paulo/Faculdade de Ciências Farmacêuticas, São Paulo, SP, Brazil*
[3]*Universidade Estadual Paulista/Faculdade de Ciências Farmacêuticas, Araraquara, SP, Brazil*
[4]*Universidade Estadual Paulista/Instituto de Química, Araraquara, SP, Brazil*
[5]*Secretaria de Estado da Saúde, Maringá, PR, Brazil*
**leite@iq.unesp.br*

Introduction

Milk is widely consumed in Brazil and can be the vehicle of agent transmission. Some species of mycobacteria, such as Mycobacterium bovis and non-tuberculosus mycobacteria (NTM), are zoonotic agents with a wide range of mammalian hosts(Konuk et al) 1. Although, in our country, bovine tuberculosis has been considered under control, the re-emergence of the disease has been reported. In this study, was evaluated the occurrence of Mycobacterium bovis and non-tuberculous mycobacteria (NTM) in raw and pasteurized milk consumed in the northwestern region of Paraná, Brazil.

Method

Fifty-two milk samples (20 pasteurized and 32 raw) from dairy farms near the municipality of Maringa, Parana State, Brazil were collected. Milk samples were decontaminated using 5% oxalic acid method and cultured on Löwenstein-Jensen and Stonebrink media at 35°C and 30°C, with and without 5-10% CO2 (Konuk et al) 1. Mycobacteria isolates were identified by morphological features, PCR-Restriction Fragment Length Polymorphism Analysis (PCR-PRA) and Mycolic acids analysis.

Results / Discussion / Conclusion

Thirteen (25 %) raw and 2 (4 %) pasteurized milk samples were positive for acid fast bacilli growth. Nine different species of NTM were isolated (M. nonchromogenicum, M. peregrinum, M. smegmatis, M. neoaurum, M. fortuitum, M. chelonae, M. flavescens, M. kansasii and M. scrofulaceum). Mycobacterium bovis was not detected. Raw and pasteurized milk may be considered one source for NTM human infection. The paper reinforces the need for intensification of measures in order to avoid the milk contamination and consequently prevent diseases in the south of Brazil.

Key words: Non-tuberculous mycobacteria; Milk; Mycobacterium; PCR-PRA; Mycolic acids analysis.

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PO46
ANTI-LYMPHOMA EFFECT AND LETHALITY AGAINST *Artemia salina* Leach OF *Maximowiczia sonorae* S. Wats

Valdés-Huerta M. A.[1,2*]; Trujillo-Ferrara J. G. [2], Barrientos-Salcedo C. [3]; García-Hernández N. [4]; Ordoñez-Razo R.[4]; Calzada F. [1]

[1]*QFB/Unit of Medical Research in Pharmacology, Specialty Hospital, National Medical Center S. XXI, IMSS Av. Cuauhtémoc 330 Col. Doctores Del. Cuauhtémoc CP 06725, México D.F. México*

[2]*Superior Medical School of the Instituto Politécnico Nacional, Post grade and Research Section, Plan de San Luis y Díaz Mirón s/n Col. Casco de Santo Tomás Del. Miguel Hidalgo CP 11340. México D.F. México.*

[3]*Faculty of Bioanalysis, Universidad Veracruzana, Veracruz, México*

[4]*Unit of Medical Research in Human Genetics, Pediatric Hospital, National Medical Center S. XXI, IMSS Av. Cuauhtémoc 330 Col. Doctores Del. Cuauhtémoc CP 06725, México D.F. México*

*aramivh4@gmail.com

Introduction

Cancer is a major public health burden in both developed and developing countries. Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Mexico, China and India from ancient time and an impressive number of modern drugs have been developed from them. In these sense several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world. In Mexican traditional medicine, many plant species had been cited as useful alternative in the treatment of cancer among these *Maximowiczia sonorae* S. Wats (Curcubitaceae). However, chemical and pharmacological studies had not been reported to support the use of this plant. The objectives of this project were to determine the anti-lymphoma effect of *M. sonorae* extracts in a murine model using U-937 cell line and the lethality against *Artemia salina.*

Methods

Evaluation of anti-lymphoma effect was tested in a murine model: The animals used were female and male Balb/c mice (20-30 g, n = 6) and the U-937 cell line. Five groups were inoculated with $1x10^6$ tumor cells (intraperitoneal administration), 24 hours after the inoculation, the groups were formed and were administered intragastrically daily for nine days. Experimental groups: DCM extract, acetone extract and MeOH extract (150 mg/kg) and negative control groups received vehicle (DMSO 2% in water), normal control group (animals healthy and untreated) and positive control (methotrexate 1.25 mg/kg). The animals were kept under observation for 30 days after the last administration, recording the daily survival and weight each week. At the end of the experiment it as realized a necropsy of the animals to remove the axillary and inguinal lymph nodes the evaluation of the antitumor activity was determined by comparing the total lymph nodes mass of the treated groups against the total nodal mass negative control group and normal control group. Evaluation of lethality against *A. salina*: Larvae groups of *A. salina* were exposed to four different concentrations of extract (500, 100, 10 and 1 ppm) for 24 hours. After these 24 hours of exposure it was realized the count of the dead bodies and the percentage mortality was calculated.

Results / Discussion / Conclusion

The acetone extract of *M. sonorae* showed the best reduction of lymph nodal growth in both genders mice. Also, it showed the best lethality in the *A. salina* test. These results make that *M. sonorae* as a candidate for the discovery of new products with anti-lymphoma potential.

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PO47
THE ENIGMA OF TARANTISM

Bicco R.[1]; Barbarulo V.[2]; Russo L.[3]; Rastrelli L.[3]
[1]Department of Architecture and Industrial Design Luigi VanvitelliAversa (CE), Italy, [2]Webmaster SILAE
[3]Departmentof Pharmacy, University of Salerno, Italy,
rossellabicco@live.it

Apulian Tarantism came in the Middle Ages and remained intensely popular until the end of the 1700 when beginning its decline in the next century. For the popular belief tarantism was a disease caused by the bite of the tarantula (Lycosatarentulathe most widespread species in the countryside of Salento),which manifested especially in the summer (harvest period) and that caused a state of general discomfort: abdominal pain, state of trance, sweats and palpitations; where music, dance and colors represent the basic elements of therapy that consisted precisely in a musical, choreographic and colorexorcism.Similar phenomena were found to tarantismo in history from all over the South, so much so that the tarantella is nothing other than the music of the little spider, and exist in Calabria, Sicily, Basilicata, Campania, reflecting the fact that the symbol of the tarantula was also operating in those territories; and also other peoples have known tarantism, such as Spanish, Sardinian and Tuscan.

The studies of Ernesto De Martino in 1959, it is clear that the "bite" becomes an excuse to resolve trauma, frustration, family conflict, and personal events: an unhappy love, the loss of a loved one, the crisis related to puberty and difficult socio-economic conditions. The music is the most important element of therapy, in fact the tarantata who lay on the floor or on the bed, listening began to move his head and legs, crawling on his back, he seemed unable to stand up and then remained adherent to the ground. Then knock his feet in time to music as to smash the spider, it did make several turns and acrobatic movements, until, exhausted by the effort, collapsed to the ground. The tarantata so graced by St. Paul, was conducted at the chapel of the Saint, in Galatina (LE), drank the sacred water of the well adjacent to it and repeated symbolically a short choreographic ritual.

The figure of St. Paul in the tarantismo is linked to the legend which tells how St. Paul one day, during his preaching in Judea, surrounded by snakes, vipers and grass snakes, captured by the Jews to scare and force him not to hear the voice of Jesus. But St. Paul, with a sign of the cross, did escape the ugly beasts that were killed by the people.

The phenomenon of tarantismo today has almost disappeared in its original form, or it's thought that has been changed in other aspects, being now radically changed the psychological, social, cultural, economic and religious componentsthat formed its base.

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PO48
ESSENCIAL OIL COMPOSITION OF TWO PLANT SPECIES OF *Myrsine* GENUS

Corrêa, A. L.[1]*; Tietbohl, L. A. C.[1]; França, H. S.[2]; Santos, M. G.[3]; Fuly, A. L.[4]; Rocha, L. M.[5]
[1] *PhD student, Fluminense University (UFF), Mário Viana Street, 523, Santa Rosa 24241-000, Niterói-RJ, Brazil*
[2] *Professor, Federal Institute of Education, Science and Technology (IFES) - Av. Ministro Salgado Filho, s/n°, Soteco 29106-010, Vila Velha-ES, Brazil*
[3] *Professor, Rio de Janeiro University State (UERJ), Francisco Portela Street, Patronato 24435-005, São Gonçalo-RJ, Brazil.*
[4] *Professor, Fluminense University (UFF), São João Batista Street, s/n°, Center, 24210-150, Niterói-RJ, Brazil*
[5] *Professor, Fluminense University (UFF), Mário Viana Street, 523, Santa Rosa 24241-000, Niterói-RJ, Brazil*
**arthur_farm@yahoo.com.br*

Introduction

The Primulaceae (Myrsinaceae) family is represented by 39 genera and about 1250 species with a cosmopolitan and pantropical distribution. The Myrsine genus is characterized by herbaceous plants which have internal secretory cavities which mainly produce compounds derived from hidroxibenzoquinones which can be used as chemotaxonomic characters. One of the main categories of metabolites produced by the this structures are essential oils, formed by terpenes. Myrsine rubra and Myrsine parvifolia are distributed in Atlantic Coast from Espirito Santo to Parana State, Brazil. Previews phytochemical studies performed with M. rubra by our research group showed the presence of myricetin 3-O-a-L-rhamnopyranoside, quercetin 3-O-a-L-rhamnopyranoside, Kaempferol 3-O-β-D-(6''-galloyl) glucopyranoside, luteolin 3'-O-a-L-rhamnopyranoside. The aim of the present study was to perform chemical analysis of the essential oil from leaves of Myrsine rubra and leaves and fruits of Myrsine parvifolia.

Method

Leaves of Myrsine rubra and Myrsine parvifolia were collected in Restinga de Jurubatiba National Park, Rio de Janeiro, Brazil. The essential oils were extracted by hydrodistillation of leaves of M. rubra and leaves and fruis of M. parvifolia and the chemical composition was defined by GC/MS.

Results / Discussion / Conclusion

The essential oil from leaves of M.rubra is mainly constituted by sesquiterpenes like β-Caryophyllene (17.2%), γ-Muurolene (11,1%) and Germacrene B (10,0%). The essential oil from leaves of M. parvifolia is also constituted only by sesquiterpenes like Caryophyllene-Oxide (14,4%) and β-Caryophyllene (12,6%). While in the essential oil from fruits of M. parvifolia was identified the monoterpenes a–pinene (0,4%) and β-pinene (0,3%) and others 37 sesquiterpenes like β-Caryophyllene (11,7%), a-Himachalene (6,1%) and a-copaene (6,0). Studies of the composition of essential oil of other species of the genus Myrcine revealed the presence of the β-caryophyllene (9.78%) in leaves of M. venosa, β-elemene (18.7%) in leaves of M. coriaceae and Naphthalene (7.7%) in the fruits of M. africana, as main constituents. These are the first reports regarding the comparative study of the chemical composition of essential oil of leaves and fruit of the same plant species of the Myrsine genus.

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PO49
(E)-N'-(2,4-DINITROBENZYLIDENE)-N,N-DIPHENYLHYDRAZINE

Cabrera-Vivas B. M.[1*], Meléndrez-Luévano R.[1*],Navarro O. M.[1], Ramírez Juan C.[1], Toledano M. Y.[2], García-Ramos J. C.[3]

[1]*PhD/Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

[2]*PhD/Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Inmunología. Av. Universidad 3000, Distrito Federal, C.P. 04510.*

[3]*PhD/Universidad Nacional Autónoma de México, Facultad de Química, Departamento de Química Inorgánica y Nuclear. Av. Universidad 3000, Distrito Federal, C.P. 04510.*

bmcabreravivas@yahoo.com

Introduction

Entamoeba histolytica is a microaerophilic protozoan parasite and the causative agent of amoebiasis, a disease that affects millions of people worldwide and claims up to 100,000 casualties per annum[1]. As is the case with other microaerophilic parasitic infections, such as giardiasis (caused by Giardia intestinalis) and trichomoniasis (caused by Trichomonas vaginalis), the 5-nitroimidazole drug metronidazole has established itself as the most effective treatment of amoebiasis. Due to the high prevalence of these infections[2] and due to its role as a second-line defense against Helicobacter pylori infections[3], metronidazole has been included in the ''essential medicines'' list by the World Health Organization[4].

Method

1.0 chemical equivalents of diphenilhydrazine were dissolved in ethanol; with continuous stirring was added 1.0 chemical equivalent of dinitrobenzaldehyde drop by drop previously dissolved in the same solvent. The reaction mixture was kept at room temperature and was monitored by TLC, then filtered at vacuum. The hydrazone was recrystallized several times until obtaining crystals with adequate size and purity for the x-Ray analysis. The characterization was performed by m. p., U.V, I.R. 1H NMR, 13C NMR and x-Ray.

Results / Discussion / Conclusion

Dark red crystals; yield: 78%; m. p. = 174-177 °C, UV λmax CHCl3= 443.36, nm. FT. IR (film): (cm-1): 3119 ν(N-H), 1683 ν(C=N), 1513 ν(C=N). MS-EI: m/z = 186 M+. $C_{11}H_{10}N_2O$.

RMN ^{1}H (400MHz, $(CD_3)_2CO$) δ (ppm)		J (Hz)	RMN ^{1}H (400MHz, $(CD_3)_2CO$) δ (ppm)		J (Hz)
8.73, d	1H,C3		7.68, s	2H, C=N	
8.62, d	1H,C5		7.53, td	4H, C3′	7.44
8.5, dd	1H,C6		7.33, t	4H, C2′	7.44
			7.28, m	2H, C4′	2.0

RMN ^{13}C (400 MHz, $(CD_3)_2CO$) δ (ppm)	
140.08 (C4)	128.79 (C1)
139.07 (C2)	128.21 (C6)
136.11 (C3)	126.89 (C4′)
135.03 (C1′)	126.06 (C5)
130.15 (C3′)	122.51 (C2′)
120.64 (C=N)	

The synthesized hydrazone was evaluated and has shown amoebicidal activity with IC50 = 8.6, which is an important growth inhibition potency comparable with metronidazole

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PO50
ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF THE ETHANOL EXTRACT FROM *Eryngium pristis* CHAM. & SCHLTDL (APIACEAE)

Del-Vechio-Vieira, G.[1*]; Fernandes, L. S.[2]; Santos, B. C. S.[2]; Minateli, M. M.[2]; Almeida, F. L. A.[2]; Pinheiro, R. P.[2]; Alves, M. S.[1]; Sousa, O. V.[1*];

[1]PhD/Professor, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, Rua José Lourenço Kelmer, s/n - Campus Universitário, Bairro São Pedro, Juiz de Fora, Minas Gerais, CEP 36036-900, Brazil

[2]PharmD, Faculty of Pharmacy, Federal University of Juiz de Fora, Rua José Lourenço Kelmer, s/n - Campus Universitário, Bairro São Pedro, Juiz de Fora, Minas Gerais, CEP 36036-900, Brazil

**glauciemar@gmail.com and orlando.sousa@ufjf.edu.br*

Introduction

Eryngium pristis Cham. & Schltdl (Apiaceae), known as gravatá or lingua-de-tucano, is a shrub that has been used in folk medicine as diuretic, anti-inflammatory and antidiabetic and for the treatment of canker sores, infections and throat and mouth ulcers. The present study evaluated the antinociceptive and anti-inflammatory activities of the ethanol extract from *E. pristis* leaves.

Method

E. pristis was collected in São João del Rei, Minas Gerais, Brazil, and a voucher specimen (R number 207576) has been deposited in the Herbarium of the Federal University of Rio de Janeiro. Dried and powdered leaves were subjected to extraction with ethanol by static maceration for the obtaining ethanol extract. The antinociceptive activity was assayed by acetic acid writhing (Collier *et al.*, 1968) and paw licking induced by formalin tests (Hunskaar; Hole, 1987), while the anti-inflammatory activity was evaluated by paw edema induced by carrageenan (Winter *et al.*, 1962). The data were expressed as mean ± S.E.M. Statistical significance was determined by the one-way analysis of variance followed by Student Newman-Keuls test ($p < 0.05$).

Results / Discussion / Conclusion

Doses of 100 (50.37±1.33; $p < 0.01$) and 200 mg/kg (44.00±1.49; $p < 0.001$) of the ethanol extract significantly reduced the abdominal contortions induced by acetic acid when compared to the control group (57.50±1.90). The first phase of the time paw licking was inhibited by extract at the dose of 200 mg/kg (55.00±1.49; $p < 0.001$). Doses of 100 (61.00±1.75; $p < 0.001$) and 200 mg/kg (47.87±1.19; $p < 0.001$) of the extract decreased the second phase. After 3 to 4 h of application of the carrageenan, doses of 100 (0.66±0.02 and 0.51±0.01, respectively) and 200 mg/kg (0.62±0.02 and 0.46±0.01, respectively) demonstrated significant reduction of the paw edema ($p < 0.01$ or $p < 0.001$). These results suggest that the ethanol extract from *E. pristis* can be a source of active compounds with antinociceptive and anti-inflammatory activities supporting the use in the Brazilian folk medicine.

Acknowledgement: CAPES, FAPEMIG, CNPq and UFJF.

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PO51
CHEMICAL COMPOSITION AND ANTINOCICEPTIVE EFFECT OF THE ESSENTIAL OIL FROM *Duguetia lanceolata* ST. HIL. (ANNONACEAE)

Del-Vechio-Vieira, G.[1*]; Santos, B. C. S.[2]; Minateli, M. M.[2]; Almeida, F. L. A.[2]; Pinheiro, R. P.[2]; Silvério, M. S.[1]; Alves, M. S.[1]; Sousa, O. V.[1*];

[1]PhD/Professor, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, Rua José Lourenço Kelmer, s/n - Campus Universitário, Bairro São Pedro, Juiz de Fora, Minas Gerais, CEP 36036-900, Brazil

[2]PharmD, Faculty of Pharmacy, Federal University of Juiz de Fora, Rua José Lourenço Kelmer, s/n - Campus Universitário, Bairro São Pedro, Juiz de Fora, Minas Gerais, CEP 36036-900, Brazil

**glauciemar@gmail.com and orlando.sousa@ufjf.edu.br*

Introduction

Duguetia lanceolata St. -Hil. (Annonaceae), known as pindaíba, is used in popular medicine for the treatment of diarrhea, rheumatism, inflammation, upset stomach, back and kidneys and as sedative (Sousa *et al.*, 2012). The present study investigated the antinociceptive effect of the essential oil from *D. lanceolata* branches.

Method

D. lanceolata was collected in Juiz de Fora, Minas Gerais, Brazil, and a voucher specimen (CESJ number 29750) has been deposited in the Herbarium of the Federal University of Juiz de Fora. The essential oil obtained from branches was identified by Gas Chromatography and Gas Chromatography/Mass Spectrometry. The antinociceptive effect was evaluated by acetic acid writhing (Collier *et al.*, 1968), paw licking induced by formalin (Hunskaar; Hole, 1987) and hot plate (Eddy; Leimbach, 1953) methods. The data were expressed as mean ± S.E.M. Statistical significance was determined by the one-way analysis of variance followed by Student Newman-Keuls test ($p < 0.05$).

Results / Discussion / Conclusion

β-pinene (1.08%), *trans*-pinocarveol (1.04%) and myrtenol (0.72%) were the most abundant monoterpenes, while β-elemene (8.32%), caryophyllene oxide (7.75%), β-eudesmol (7.22%) and β-selinene (7.15%) were the main sesquiterpenes. Doses of 100 (46.87±2.12; $p < 0.001$) and 200 mg/kg (36.50±2.34; $p < 0.001$) of the essential oil significantly reduced the abdominal contortions induced by acetic acid when compared to the control group (62.37±1.92). After 60 min of treatment, doses of 50 (53.751.41 and 56.751.78 s), 100 (43.122.17 and 43.402.08 s) and 200 mg/kg (34.872.12 and 35.502.36 s) inhibited both the phases (first and second) of paw licking induced by formalin ($p < 0.001$). The latency time on the hot plate increased significantly after 60 and 90 min of treatment at the dose of 50 (7.420.24 and 8.080.15 s, respectively), 100 (9.630.34 and 10.510.44 s, respectively) and 200 mg/kg (11.160.50 and 13.610.38 s, respectively). These results suggest that the essential oil from *D. lanceolata* branches has antinociceptive effect and this data could explain the popular application.

Acknowledgement: CAPES, FAPEMIG, CNPq and UFJF.

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PO52
ANTIOXIDANT ACTIVITY OF *Erythroxylum suberosum*

Morais, N. A.[1]; Fagg, C. W.[2]; Magalhães, P. O.[3]; Silveira, D. [4]; Fonseca, Y. M.*
[1]*post graduated student, Faculty of Health Sciences, University of Brasilia, Brasília, Distrito Federal District, Brazil.*
[2]*PhD, Faculty of Ceilβndia, University of Brasilia, Ceilβndia, Federal District, Brazil.*
[3], [4] *PhD, Faculty of Health Sciences, University of Brasilia, Brasília, Distrito Federal District, Brazil.*
**yrisfonseca@hotmail.com*

Introduction

E. suberosum, popularly known as "black hair", finds its use in folk medicine restricted to inflammatory processes and abortifacient plant (1). Although it is a little studied species there is evidence of some important the presence of compounds such as phenolic acids and polyphenols (2), which may be promising in preventing a of diseases, such as those related to oxidative stress. The aim of this study was to investigate the antioxidant activity from leaves and stems of E. suberosum in different extractive processes.

Method

E. suberosum leaves and stems were collected in Brazillian Cerrado, in Federal District-Brazil and were strored in the Herbarium of University of Brasilia (voucher number Fagg CW 2192). Extracts from dried leaves and stems were obtained by the percolation with ethanol and hexane solvents. Aqueous extracts were obtained by infusion with water at 70°C. The extracts were concentrated by reduced pressure or lyophilization. Antioxidant activity of different extracts was evaluated by DPPHÿ'3f method described by Blois (1958). Inhibition concentration of 50 percent (IC50) was calculated for leaf aqueous extract (LAE), leaf ethanolic extract (LEE), leaf hexanic extract (LHE) and stems ethanolic extract (SEE). Positive control compounds, rutin, BHT and ascorbic acid, were also evaluated.

Results / Discussion / Conclusion

It was observed that ethanolic and aqueous extracts showed antioxidant activity, however, hexanic extract did not show any activity by the used assay. The observerd IC50 values were: 150 μg/mL for LAE, 220 μg/mL for LEE, 160 μg/mL for SEE, 90 μg/mL for rutin, 30 μg/mL for ascorbic acid and 80 μg/mL for BHT. LAE extracts were 1.4 times more active with LEE and showed similar activity than SEE. In relation to positive controls, all extracts tested showed lower activity than positive controls. Therefore, these extracts from E. suberosum showed similar antioxidant activity with other plant extracts, as Apuleia leiocarpa and Brosimum guianense extracts (3). Thus, E. suberosum aqueous and hydroalcoholic extracts could contribute to the prevention of damage caused by oxidative stress.

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PO53
EVALUATION OF THIRTEEN NATURAL PRODUCTS ON CASTOR-OIL INDUCED DIARRHEA IN MICE

Abrin-Lopez Amanda[1]*; Valdés-Huerta M. A.[1,2]; Solares-Pascasio J. I.[1,2]; Calzada F.[1]
[1]*Unit of Medical Research in Pharmacology, Specialty Hospital, National Medical Center S. XXI, IMSS Av. Cuauhtémoc 330 Col. Doctores Del. Cuauhtémoc CP 06725, México D.F. México*
[2]*Superior Medical School of the National Polytechnic Institute, Post grade and Research Section, Plan de San Luis y Díaz Mirón s/n Col. Casco de Santo Tomás Del. Miguel Hidalgo CP 11340. México D.F. México.*

Introduction

Medicinal plants have an important role in our diseases and ailments history. One of these diseases with more incidents in children under 5 years old is the diarrhea. The diarrhea is an illness defined as a result of the increasing times of defecation, over 100-200g everyday as well as the change in the consistency and symptoms like weaknesses, fever, flatulencies and abdominal pain. The diarrhea is positioned as the third cause of death; it is considered as the principal cause of infants' mortality and malnutrition of under 5 year old population. The consequences of this illness are also reflected in different socio-economic areas of the Mexican population.

Method

Kaempferol, catechin, epicatechin, incomptine A, incomptine B, linearolactone, quercetin, rutin, tyramine, tiliroside, pinocembrin, pinostrobin and xanthomicrol, were previously obtained in bioguided studies from medicinal plants in the Unit of Medical Research in Pharmacology.
Natural products were given to mice at graded doses (0.1-5 mg/kg) to evaluate its antidiarrheic potential by using four experimentally induced diarrhea treated group consisted of six mice (Balb-c, 20-35g) and one control group. Castor oil was used to induce diarrhea in mice. Total number of stools and total weight of fecal output in 4 hours were measure.

Results / Discussion / Conclusion

The results of this study reveal that rutin was the best pharmacologically active substance with antidiarrheal properties. Activity demonstrated to rutin may explain the use of the several plants that contain it as antidiarrheal agent in the Mexican traditional medicine.

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PO54
SENSITIVITY OF *Meloidogyne* SPP. TO DIFFERENT ETHANOLIC EXTRACTS FRACTIONS OF *Ficus* SPP. LEAVES

Balbino, H. M.[2]; Oliveira, R. D. L.[1]; Leite, J. P. V.[1]; Almeida, A. A.[2]
[1]*PhD/Prof, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/n, 36570-000 Viçosa, MG, Brazil*
[2]*Undergraduate Student, Universidade Federal de Viçosa, Av. P.H. Rolfs, s/n, 36570-000 Viçosa, MG, Brazil*
rdlima@ufv.br

Introduction
Studies on the production of secondary metabolites in plants are continuously conducted to evaluate its biological action (Chitwood, 2002). Commonly, some species of Moraceae are used in the treatment of worms and extracts of Ficus spp. are investigated by acting on bacteria, fungi and insects, but there is no enough knowledge regarding their nematicidal action (Reschke *et al.*, 2007). Extracts of six species of Ficus were tested in order to assess the mortality of juveniles (J2) of Meloidogyne spp.

Method
Each hydroalcoholic extract (*Ficus obtusiuscula*, *F. maxima*, *F. adhatodifolia*, *F. piresiana*, *F. pulchella* e *F. nevesiae*) was obtained by exhaustive percolation of leaves powder using ethanol at 95% (v/v) and concentrated in a rotary evaporator. Each extract was fractionated in separating funnel using four solvents of increasing polarity in the following order: hexane, ethyl acetate, butyl alcohol and water. The phytochemical screening was performed using thin layer chromatography (TLC) with silica gel (Wagner *et al.* 1984). The biological assays were mounted in test tubes or Elisa plates. Each treatment consisted of 1.5 mL (tubes) or 100 μL (plates) of each extract prepared in 1% DMSO plus 300-500 J2 of M. javanica or M. incognita. After 24 or 48 hours, the immobile J2 was assessed. Confirmation of death J2 was made by using 1 M NaOH (Dickson & Chen, 2000).

Results / Discussion / Conclusion
The phytochemical screening of these extracts resulted in the presence of secondary metabolites belonging to the classes of coumarins, tannins, flavonoids and triterpenes, substances known to exhibit antibiotic and nematicidal activity (Chang et al, 2005; Chitwood, 2002). There was no significant difference between the hydroalcoholic extracts that were effective in causing 95-98% mortality (P ≤'3d 0.05), except F. pulchella that showed 62.7% mortality of juveniles of M. javanica. Related to M. incognita, only extracts of F. adhatodifolia and F. obtusiuscula caused mortality above 80% of J2. The treatment of J2 with the fractions of the extracts of these two species resulted in at least 80% of mortality of M. javanica juveniles, but lower mortality rate (60-80%) in J2 of M. incognita.
Nematicidal activity was detected in ethanolic extracts of Ficus spp. and in their fractions against M. javanica and M. incognita. The extracts have higher potential as a natural nematicide for M. javanica control. Further studies are needed to validate this biological activity *in vivo*.

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PO55
ANALYSIS AND QUANTIFICATION OF SUBSTANCES IN THE EXTRACT AND FRACTIONS OF *Annona squamosa* (ANNONACEA) BY ESI-MS AND UPLC-MS-MS

Lourenço, C. C.[2]*; Lopez, B. G. C.[1]; Salvador, M. J.[1]
[1]*PPGBTPB e PPGBV, DBV, Institute of Biology, Facult of Pharmaceutical Science, State University of Campinas (UNICAMP),Campinas (SP), Brazil*
[2] *PPGBV, DBV, Institute of Biology, State University of Campinas (UNICAMP), Campinas (SP), Brazil*
ca.caramano@gmail.com

Introduction
The Annonaceae family in Brazil is represented by 26 genera and approximately 260 species exist mainly in the Amazon and Atlantic forests. Reviews of the chemical constituents present in species of Annonaceae, show the presence of phenolic acids, flavonoids, essential oils and more than 130 alkaloids, mostly belonging to the class of isoquinoline, protoberberines and tetrahydroprotoberberines. Several alkaloids and acetogenins were isolated from Annona squamosa species (Patel et al, 2012.). The aim of this study was to identify and quantify the alkaloids present in the crude extract and fractions (alkaloid and neutral) of Annona squamosa.

Method
The chemical profile analysis, identification and quantification of alkaloids in the extracts was performed by UPLC-ESI(+)-MS and UPLC-ESI(+)-MS/MS. The fingerprint (direct injection) and chromatographic analysis were performed on a UPLC Acquity chromatographer coupled with a TQD Acquity mass spectrometer (Micromass-Waters Manchester, England), with an ESI source. In the chromatographic analysis, a C18 BEH Waters Acquity column (2.1mm x 50 mm x 1.7 μm particle size) was used. Solvent A was mili-Q purified water with 0.1% formic acid and solvent B was methanol with a linear gradient starting at 20% methanol and increasing to up 100% methanol in five minutes, held until 5.5 minutes and then returning to the initial conditions, followed by column re-equilibration. ESI ionization in the positive ion mode was used under the following conditions: Capillary - 3.00 kV, Cone - 30 V, Source Temperature 130°C, Dessolvation Temperature 250°C and Collision Energy 30V, acquiring data between 100 and 800 m/z. The identification of the alkaloids was performed by comparison of the fragmentation profile and chromatographic analysis with authentic stock standards available in the Laboratory of Pharmacognosy, bioassays and Plant Protection Technology Course of Pharmacy DBV - IB - UNICAMP. The standards used were anonaine, asimilobine, liriodenine, lysicamine, isomoschatoline, coreximine, isocoreximine, reticulin, subsicilin and palmatine. All data obtained were also compared to literature.

Results / Discussion / Conclusion
The alkaloids anonaine and palmatine were found in the neutral fraction, in the methanol extract was also detected liriodenine and in the alkaloid fraction were found the alkaloids already cited, plus lysicamine. The quantification was based on calibration curves of standards of these alkaloids in the concentration range between 1 mg/ml and 0.01 mg/ml. The assays were performed in duplicate. The methanol extract showed 0.16 mg/mL of liriodenine, 0.01 mg/mL of palmatine and 0.24 mg/mL of anonaine. In the alkaloid fraction were found 0.05 mg / mL of assimilobine, 0.01 mg / mL of liriodenine, and 0.04 mg/mL of anonaine. In the neutral fraction it was not possible to quantify the alkaloids, because it showed concentrations lower than the limit of quantification (0.01 mg/mL).

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PO56
OPTIMIZATION OF PRE-GERMINATION TREATMENTS OF FIVE NATIVE MEDICINAL SPECIES FROM PATAGONIA

Boeri, P.[1]; Cedrés Gazo M.[1]; Torres, M.[1]; Barrio, D.[1]; Sharry S.[1,2]
[1]*Departamento de Ciencias Exactas, Naturales y de Ingeniería. Sede Atlántica, Universidad Nacional de Río Negro. Don Bosco y Leloir (8500) Río Negro, Argentina. Mail: pboeri@unrn.edu.ar*
[2] *Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales. Argentina-CICPBA*

Introduction

In the last years the use of medicinal plants in the extra-Andean region of the Patagonia Argentina has gained a growing importance in the local communities. Some of those species are: Geoffroea decorticans (chañar), its decocted bark and fruit syrup are sedative, antitussive, expectorant, anti-catarral, balsamic, emollient, anti-asthmatic, anti-diarrheal and are also effective against infections of urinary and respiratory tracts. Larrea divaricata (jarilla), its dried leaves have numerous properties: antioxidants, antiulcer, antiviral, antimicrobial, anti-inflammatory and immunomodulatory. The alcoholic macerateds are usually used in the treatment of flues and coolings. Prosopis alpataco (alpataco) y P. caldenia (caldén) are both endemic species of Argentinean medicinal flora. P. alpataco has des-inflammatory and antioxidant properties and acts over nucleic acids too. P caldenia has influence on the intestinal flora and has laxative properties, coagulants, bactericides, anti-cancer, lowers cholesterol and protects the intestinal mucous membrane. Prosopis flexuosa (algarrobo dulce): a beverage with diuretic properties is made from its grinded and fermented seeds; the grinded paste is also astringent while the leaves infusion is antitussive.
The aim of this work was to make a selection of efficient sexual propagation techniques for these five native medicinal species from the Monte and Espinal phytogeographic provinces in Rio Negro. This will allow disposing a large amount of plants for the restoration of degraded ecosystems or initiating the domestication and cultivate production as well.

Method

In every case, a chemical scarification was made by submerging the seeds in concentrated sulfuric acid in different time lapses: for Prosopis sp y Larrea sp the times were 0 (control), 5, 10 and 30 minutes, and for Geoffroea decorticans, they were 0 (control), 8 and 12 hours. The seeds were surface disinfected and washed with filtered water thrice. The incubation was made at 30° C, for 7 days long, in Petri plates with filter paper moistened with filtered water. 10 seeds per treatment were used and three replicas by species were made. Germinative response was determined for each species, in accordance with the following parameters: mean germination time (MGT) and germination percentage. The data were subjected to the variation analysis (ANOVA) and Fisher Test.

Results / Discussion / Conclusion

100% of germination was obtained for L. divaricata sp y Prosopis spp seed at the 48 hours after the treatment and incubation. For the species G. decorticans, 51% of germination was obtained after 17 cultivation days. In the same period, non scarified seeds presented 6,67%, 23% y 0% of germination respectively.
The best treatment to the assessed species was the one with the largest exposition period to the sulfuric acid, with the exception of Geoffroea decorticans where the best answer was obtained at 8 hours of immersion. Highly significant differences were noticed in between treatments and control. In all cases, the MGT went in decrease at longer immersion periods.
In conclusion, simple sexual germination protocols were adjusted for five native species of medicinal use. The chemical pre-treatments accelerated the process without generating any damage in the emerging plants. These methodologies will allow a fast and successful massive propagation for these species.

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PO57
A VALIDATED SPECTROPHOTOMETRIC DETERMINATION OF BUTAMIRATE CITRATE IN BULK AND DOSAGE FORMS USING BROMOTHYMOL BLUE AS ION-PAIR REAGENT

Taşkın, D.[1,2*]; Çakır, G.[3,4]; Sungur, S.[5**]

1Marmara University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey
2Yeni Yüzyıl University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey
3Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul-Turkey
4Yeni Yüzyıl University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Istanbul-Turkey
5Prof, Yeni Yüzyıl University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey
**duygu.susuz@yeniyuzyil.edu.tr ** sidika.sungur@yeniyuzyil.edu.tr*

Introduction

An easy, sensitive, precise and valid spectrophotometric technique is described for the determination of butamirate citrate based on ion-pair formation with bromothymol blue. The technique involves the formation of yellow colored ion-pair in aqueous solution, extraction with dichloromethane and absorbance measurement at 410 nm against a blank solution.

The experimental parameters of the analysis were studied and optimized in terms of the pH of the aqueous solution, extraction solvent and the amount of the reagent. The effect of the pH was investigated in the interval of 2.0 to 8.0. Maximum absorbance was observed when the aqueous solution was buffered at pH 3.0. Among the various solvents tested for the extraction of the ion-pair such as chloroform, dichloromethane, benzene, carbon tetrachloride and toluene, dichloromethane was found to be the best solvent. The molar ratio of the ion-pair was determined using Job's Continuous Variation and Molar Ratio

Method

A linear relationship existed between absorbance and butamirate citrate concentration over the 4-20 μg/mL range. The proposed method was validated for specificity, linearity, LOD, LOQ, precision, accuracy and robustness. Using the present method, commercially available butamirate citrate syrup and tablet formulations were assayed. The results were statistically compared with those obtained by the reference method in terms of t- and F- tests.

Keywords: Butamirate citrate, ion-pair, spectrophotometry, validation, bromothymol blue.

PO58
EFFECT OF *Piper calceolarium* EXTRACTS ON TRPV CHANNEL ACTIVITY

Sierra C.[1], Sanchez R.[1], Bernal H.[1], Sequeda G.[1], Torres Y.[1], Casas Z.[1,2]
Grupo de Terapia Celular y Molecular, Pontificia Universidad Javeriana[1]. zcasas@javeriana.edu.co[2]

Abstract

Piper calceolarium is a plant traditionally used in different country such as Colombia for the treatment of pain (Bernal *et al.*, 2011). Despite its extensive use in traditional medicine, the mechanism by which Piper calceolarium exerts its anti-nociceptive action has not been fully characterized. One of the pathways involved in nociception, comprises the activation of the transient receptor potential cation channel (TRPV1) which is expressed by a subset of sensory neurons (Ramsey 2006; Levine JD, 2007; Ro, JY, 2009). In the present work, the potential of crude extracts of Piper calceolarium to regulate the activity of TRPV1 channels was evaluated using a heterologous expression system.

Method

Crude extract of Piper calceolarium were obtained using petroleum ether, chloroform, ethyl acetate, ethanol, and water. The obtained extracts were concentrated, lyophilized and solubilized in DMSO. To evaluate whether the Piper calceolarium extracts were toxic, a dose response curve (10, 50, 100 µg/mL) using the MTT assay was performed. Additionally, the effect of P. calceolariu extracts on the activation of TRPV1 channel was evaluated by measuring the changes in intracellular calcium using the Fluo-4 dye in the present of the agonist of TRPV1 channels Capsazepine or the antagonist Capsaicin.

Results

The MTT viability assays showed that extracts obtained by using petroleum ether, chloroform, ethyl acetate were toxic to all evaluated concentrations. In contrast the extracts obtained by using ethanol and water did not induce cell death. Interestingly, fluorometric assays using the Fluo 4 AM revealed that only a concentration of 100 µg/mL of the Piper calceolarium extract, obtained in ethanol, regulates the activity of the TRPV1 channel. It was found that this dose increased the concentration of intracellular calcium only in cells expressing the TRPV1 channels but not in non-expressing cells, suggesting that Piper calceolarium may have anti-nociceptive effects by inducing the activation of the TRPV1 channels.

Conclusions

The ethanol extract of Piper calceolarium increases intracellular calcium concentration in HEK cells stably expressing TRPV1 channels, suggesting that Piper calceolarium exerts its anti-nociceptive by activating these channels. Further trials are needed to determine whether electrophysiological effects observed directly affect channel activity or if additional pathways are involved in the increase in intracellular calcium concentration. Additionally, it is important to isolate secondary metabolites from the evaluated extract.

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PO59
CONSTITUENTS OF *Maytenus imbricata* AND OF ITS ENDOPHYTIC FUNGUS, IDENTIFIED BY GC-MS

Souza S. R.[1], Trindade I. C.[2], Okuma A. A.[3], Teixeira L. F. M.[1],Yamada-Oggatta S. F.[4], Duarte L. P.[5], Silva G. D. F.[5] and Vieira-Filho S. A[2].

[1]Faculdade de Ciências da Saúde, UNB. Campus Darcy Ribeiro. Brasilia, Distrito Federal, CEP 70910-900, Brazil; [2]Escola de Farmácia, UFOP. CEP: 34400-000. Ouro Preto. MG. Brazil; [3]CEFET. Belo Horizonte. CEP: 30421-169. MG, Brazil. [4] Centro de Ciências Biológicas, UEL, Campus Universitário, Londrina, Pr, CEP 86051-990, Brazil; [5]Instituto de Ciências Exatas, UFMG, 31270-901, Belo Horizonte, MG, Brazil.

Introduction

M. imbricata (Celastraceae) is a medicinal plant commonly found in the Cerrado regions of Brazil. Antiulcerogenic, antimicrobial, antispasmodic and other properties are popularly assigned to M. imbricate and other species of the genus1,2.

Method

Endophytic fungi (EF) (Fig.1) were isolated from this plant under Type A2 biological safety cabinet conditions. Triterpenes of the lupine series represent the main constituents isolated by phytochemical methods3.

Results / Discussion / Conclusion

Extracts from leaves and stems, and from oil of seeds and elaiosome, and from 44 EF were subjected to GC-MS. Thus, were identified 8 volatile constituents in polar extracts from leaves and stems, 18 in the oil from seeds and 15 from elaiosome. And, 22 compounds were identified in the extracts from in vitro cultures of EF. Glycerol, hexanedioic acid, palmitic acid, oleic acid and eicosanoic acid were identified has being constituents of both M. imbricata and EF inducing to suppose a symbioses process.

Acknowledgments: To FAPEMIG and CAPES

Fig.1: Photo of M. imbricata and some of its EF.

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PO60
DIASTEREOSELECTIVE SYNTHESIS OF BOTH CIS-4-METHYL/THIOL-PIPECOLIC ESTERS FROM NON-RACEMIC a,β - UNSATURATED PIPERIDIN-2- ONA

A. Zárate Ruiz, J. L. Terán Vázquez, M. L. Orea Flores, J. R. Juárez Posadas, D. Gnecco Medina
Centro de Química del Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla, Edif. 103H, Complejo de Ciencias, C.U., 72570 Puebla, Pue., México.
Email: aracelizarate81@hotmail.com

Introduction

Cyclic a-aminoacids are present in many biologically important compounds. Specifically, 4-substituted pipecolic acids and their derivatives are key fragments of compounds of pharmacological interest. For example, Palinavir, a highly potent inhibitor of HIV-1 (Ki= 31 pM) and HIV-2 (Ki = 134 pM) protease activities and of viral replication in vitro (EC50 = 3-30 nM). Argatroban, an important anticoagulant that is a small molecule direct thrombin inhibitor. Sulfur-containing aminoacids, pulcherrimine, a bitter principle from ovaries of the sea urchin Hemicentrotus pulcherrimus. Consequently, enantiopure 4-substituted-pipecolic esters, their esters and salts thereof are, in general, important synthetic intermediates.

Method

We present here a synthetic route for the access to a both enantiomers of cis-4-mehyl/thiol- pipecolic esters. It based on the ring-closing metathesis reaction to build the a,β-unsaturated piperidin-2-one derived from (S)-(-)-phenylethylamine, followed by a diastereospecific conjugate addition of methylorganocuprate to access at cis-4-methyl pipecolic ethyl esters or using the Lawesson's reagent to access at cis-4-thiol pipecolic ethyl ester.

Results/Discussion

We prepared the separable diastereomeric mixture of the a,β-unsaturated piperidin-2-ones 7a + 7b in six steps. With the a,β-unsaturated piperidin-2-one in hand, we started to explore the conjugate addition of methylcuprate to the a,β-unsaturated lactam 7a. Then, compound 7b was treated with methylcuprate following the same conditions. 4-Methyl piperidin-2-one 8b was obtained as a single diastereoisomer in 80%. The relative configuration at C-4 as (S) was confirmed from the X-ray analysis diffraction of compound 8b. Next, diastereoisomers 8a and 8b were treated with BH3.S(CH3)2 giving the corresponding piperidine 9a and 9b in 95% yield, which were subject to hydrogenolysis in the presence of di-tert-butyl dicarbonate to give the N-Boc-protected piperidines 10a and 10b. The comparison of its optical rotation, confirmed that 10a and 10b are enantiomers.We then oriented our attention to obtain the sulfur containing aminoesters. Compounds 7a and 7b were treated with Lawesson's reagent.In order to obtain the corresponding pipecolic ethyl ester, compound 11b was treated with BH3.S(CH3)2, affording the desired reduced compound 12b in 85 % yield. This compound was subject to hydrogenolysis in the presence of di-tert-butyl dicarbonate to give the N-Boc-protected piperidine 13b in 90 %.

Conclusion

In conclusion, an efficient method for the diastereoselective synthesis of cis-4-methyl/thiol-pipecolic ethyl esters from (S)-phenylethylamine has been developed.

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PO61
ANTIOXIDANT ACTIVITY TOTAL FLAVONOIDS CONTENT FROM METHANOLIC EXTRACT OF LEAVES OF *Dioscorea bulbifera* L.

Macedo, A. L.[1], Silva, L. R. G.[2], Martins, A. S. A.[1], da Silva, M. C. A.[3], Fagundes, T. S. F. [2], Paiva, S. R.[4], Boscolo, O. H.[4],Epifanio, R. A.[4], Chagas, E. F.[4], Couto, R. S.[5], Valverde, A. L.[4,*]

[1] *MSc/GraduateStudant, Universidade Federal Fluminense, Outeiro de São João Batista, s/n, Niterói, RJ, Brasil*
[2]*UndergraduateStudant, Universidade Federal Fluminense*
[3]*MSc/GraduateStudant, Universidade Federal do Rio de Janeiro, IPPN, Ilha do Fundão, CCS, RJ, Brasil*
[4]*PhD/Prof, Universidade Federal Fluminense*
[5]*MSc/GraduateStudant, Universidade Federal do Rio de Janeiro, Museu Nacional*
** alessandravalverde@id.uff.br*

Introduction

Many diseasesas cancer, neural disorder, Alzheimer, Parkinson, among others (Alam *et al.*, 2013)are attributed to oxidative stress. Natural compounds as flavonoids are widely studied as antioxidants aiming the reduction and prevention of these diseases (Chograni *et al.*, 2013). Several works describes the biological and nutritional value of *Dioscoreabulbifera*L. commonly known as yam or air potato. This specieis known by its antitumor, antioxidant, antibacterial, analgesic, and anti-inflammatory activities (Kuete *et al.*, 2012). Leaves of *D. bulbifera*are crop byproducts. The aims of this work are the evaluation of antioxidant activity, the flavonoid content and the preliminary chemical evaluation of themethanolic extract of leaves of *D.bulbifera.*

Method

Fresh mature leaves were collected in Niteroi/RJ and its extracts were prepared by maceration using methanol as solvent. After extracts were evaporated to dryness under reduced pressure at room temperature in rotary evaporatorfurnishing MED. Antioxidant activity was evaluated quantitatively by free radical DPPH scavenging assay as described by Silva and Paiva (2012). MED was evaluated by Thin Layer Chromatography (TLC) plate precoated with silica gel el 60 F254 to qualitative analysis utilizing BHT, rutin and quercetinas positive controls. NP/PEG (flavonoids), $FeCl_3$ (phenols) and BHT (antioxidants) was the chemical reagents. Solvent system was ethyl acetate:methanol:water (100:13,5:10; v/v). Flavonoid content was measured as described by Chang *et al.* (2002). Statistical analysis was performed by ANOVA with Tukey*post-hoc*.

Results / Discussion / Conclusion

MED reduced the reminiscent %DPPH immediately in all tested concentrations, been this reduction of 7 to 28% for 5 to 50 μg/mL. The concentration of 250 μg/mL stood out reducing 90% of DPPH. MED showed a dose dependent response ($p< 0,05$ between 5 and 10 μg/mL and $p< 0,01$ for the others). EC_{50} of MED was 5,96 ± 0,26 g/g of DPPH while for BHT was 0,88 ± 0,07 g/g of DPPH, approximately seven fold smaller. Furthermore, the treatment with 250 μg/mL of MED was statistically equal to the 125 μg/mL of BHT one ($p< 0,05$). These data are relevant, once BHT is a standard compound with market use and MED is a crude extract. TLC qualitative test showed many compounds with antioxidant activity against DPPH. Two phenols (Rf of 0,85 and 0,77), a flavonoid (Rf of 0,2, similar to rutin'sRf) and other aromatic compounds (Rf between 0,2 and 0,33) were active. Phenols are the major antioxidant class of phytochemicals (Prakash *et al.*, 2007), been flavonoids the most important ones (Vinson *et al.*, 1995). As MED is a crude extract with many compounds, that has approximately 5% of flavonoids, and the TLC showed other antioxidant compounds, the production of fractions rich in phenols from leaves of *D. bulbifera* will increase this activity. MED showed potential for future isolation and purification studies that may lead to use of leaves, crop's waste.

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PO62
GENOTOXIC EFFECT OF VENLAFAXINE IN A SHORT TERM ASSAY IN MOUSE

Alvarez-González, I.*; Madrigal-Bujaidar, E.; Gómez, P.
Dr, Laboratorio de Genética,
Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional,
Wilfrido Massieu s/n, Zacatenco, Gustavo A. Madero, CP 07738, México D.F., México
**isela.alvarez@gmail.com*

Introduction

During the last years the prevalence of depression has increased worldwide, and it is suggested that by 2020 it will be the second problem which cause discapacity. Venlafaxine is an antidepressive medicament from the group of inhibitors of serotonin and noradrenaline recapture, widely used in the treatment of the disease. In spite of this, no studies about its genotoxic capacity have been made, an evaluation which is relevant because such an effect may suggest the development of chronic-degenerative disease, including cancer. In this work, we determined the capacity of the medicament to induce micronuclei (MN) in an acute study in mouse. MN represents chromosomal fragments or whole chromosome delayed during mitosis.

Method

Five groups with six male mice each (Swiss Webster) were organized. Chemical were orally administered except daunorubicin. The negative control received distilled water and the positive control daunorubicin was intraperitoneally injected. Venlafaxine was administered to three groups (5, 50, and 250 mg/kg). Before the administration and at 24, 48, 72 and 96 h post-administration, we obtained blood samples from each individual, fixed in methanol and finally, they were stained with Giemsa to make the microscopic observations.

Results / Discussion / Conclusion

Venlafaxine in the three tested doses and in schedule examined showed MN values in the range of the negative control level, suggesting that the medicament did not have genotoxic effect; however, we observed that with the two high doses (50 and 250 mg/kg) the drug decreased the number of young erythrocytes respect to mature erythrocytes, a finding which suggest that venlafaxine affected the erythropoiesis in the mouse. These results suggest the pertinence of studying the genotoxic potential of venlafaxine in long term assays, as well as to carry out specific tests on its cytotoxicity. These studies will be useful to evaluate the use of the drug considering its risk/benefit proprieties.

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PO63
DIETARY FIBRE AS FUNCTIONAL INGREDIENT IN COOKED HAM

Salvadeo, P.[1], Cavazza, A.[1], Corradini, C.[1], Cardellini, C.[2], Padula, S.[2]
[1] *University of Parma, Department of Chemistry, Parco Area delle Scienze 17/A, 43124 Parma, Italy*
2 IFE I Fratelli Emiliani SpA, Via Don G. Corchia, 14 – 430013 Langhirano (PR)
paola.salvadeo@unipr.it

Introduction

The pronounced transformation of dietary habits has led to the manufacture of a vast variety of ready-to-eat (RTE) foods of animal origin. Many of these alternatives involve cooked meat products (CMP). At the same time, consumer's interest in the development of CMP, using different types of meat (pork, beef, poultry) and applying new technologies and formulations, mainly focused on reporting beneficial effects on health, is also growing.
Recently different aspects of the use of dietary fibre (DF) in the development of fibre-enriched meat products have been discussed. DF is one of the valuable components that can be incorporated in meat products from health point of view. Cooked ham is one of the most popular cooked meat products, and constitutes an important sector of the meat industry. The aim of the present study was to include DF ingredients in brine formulation employed in cooked ham production.

Methods

Fresh hind legs, after selection by weight, were manually de-boned. The average pH of the legs ranged from 5.8 to 5.9 for all pork which were of Italian origin. The injection of brine was done using multineedle syringes. The brine composition was obtained without adding phosphates, gluten source, OGM, caseinate and protein source. After brine injection, the samples were tumbled for 48h. Ham were moulded, pressed and cooked. During cooking the core temperature reached 69 C. Cooked hams were then cooled at 0 °C for about 24 h and, after de-moulding, were trimmed and packaged in vacuum plastic film-aluminium bags. Finally, products were pasteurized by autoclaving at 105 C for 15min, cooled and stored at 0 C. Analytical determination of DF used in brine formulations was carried out by HPAEC-PAD according to our previously published methods (1). Environmental scanning electron microscopy (ESEM) was used to make microstructure observations. Sensorial analysis was conducted by 6 pannellists, who were experienced in sensory evaluation of food, but received no specific training to this project.

Results and Discussion

As well known, The final quality of cooked ham products depend both on the raw material used and on processing which can be influenced by many factors. One of these factors is technology – the type of meat and quantity of brine injected, the rate and extent of tumbling or massaging, and the cooking time and temperature.
Injection of brine ensures a uniform distribution of all ingredients, whereas, brine injection level and ingredients used are characteristic of each product and determine the cooked ham quality. On other hand, the loss of water during cooling has a significant detrimental effect on the organoleptic quality of the cooked ham product.
Fibre is suitable for adding to meat products increasing the cooking yield due to its water-binding properties an to improve texture. Moreover, dietary fibre is a key ingredient widely used nowadays while developing nutritionally designed foods due to its significance in health promotion (2). In our study fructans (fructooligosaccharides and inulin at different degree of polymerization) having very distinct characteristics in terms of technological properties were characterized by Maldi-MS and HPAEC-PAD Results will be presented and discussed

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PO64
EFFECT OF RED CHILLI PEPPER ADDITION ON OLIVE OILS STABILITY DURING SHELF-LIFE

Cavazza, A.[1]*; Bignardi, C.[1]; Corradini, C.[1]; Corti, S. [2]; Mancinelli C.[2]
[1]University of Parma, Department of Chemistry, Parco Area delle Scienze 17/A, 43124 Parma, Italy
[2]Velp Scientifica srl, via Stazione 16, 20865 Usmate (MB), Italy
*antonella.cavazza@unipr.it

Introduction

Olive oil shows a limited shelf-life, and its progressive quality deterioration is mostly due to the occurrence of oxidation reactions leading to a reduction of total phenolic compounds. A relationship between the oxidative stability of an olive oil and its initial content of natural antioxidants has been reported[1].
Red chilli pepper is known to represent an excellent source of antioxidants, such as polyphenolic compounds and carotenoids. Therefore, its use as ingredient could be useful for protecting food products from spoilage. In this work the effect of the addition of red chilli pepper powder to vegetable oils has been evaluated, in order to determine its possible effect on oil stability during shelf-life. In particular, with the aim of investigating possible differences in the behavior of different oils, experiments have been carried out on samples of olive oil, extra-virgin olive oil and seed oil. The investigation was also extended to the evaluation of the effect of the addition of different amounts of chilli pepper.

Method

Three red chilli pepper powders characterized by different degree of piquancy were selected; the value of hotness was determined by the analysis of capsaicinoids content, and expressed as Scoville Units[2]. Antioxidant power was also measured by FRAP assay. Few grams of chilli powder were added to samples of three oils (olive, extra-virgin olive, and seed oil). Measures of the oxidative stability were carried out along 4 months of storage by OXITEST, based on accelerating oxidation process using high temperatures and pre-determined oxygen pressure.
Investigation of possible dose-effect relation was also carried out by adding three different percentage of powder to olive oil.

Results and Discussion

The addition of red chilli pepper powder to oils showed an extension of the stability of all samples. The effect was different depending on the type of oil considered: effect on olive oil was more evident than that on extra-virgin and seed oil.
Between the three pepper powders employed, the strongest effect was recorded with the less piquant one. Therefore, the antioxidant effect could not be attributed to the capsaicinoids, responsible for piquancy, but to the presence of other antioxidant phenolics[3]. FRAP values showed a good correlation with observed data confirming that antioxidant power is not linked to the pepper piquancy.
A dose-effect relation was found, and data collected on olive oil followed a linear dependence between amount of pepper powder and stability, suggesting interesting perspectives in the field of production of canned food in oil. Such relation was not evident in extra-virgin oil, probably for its higher stability to oxidation due to its high content of natural polyphenols[4].

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PO65
SHELF-LIFE ASSESSMENT DURING STORAGE OF TWO *Capsicum annuum* VARIETIES THROUGH COLORIMETRIC, NON-ENZYMATIC BROWNING INDICATOR AND CAPSAICINOIDS QUANTIFICATION

Bignardi, C.[1], Cavazza, A.[1]*, Corradini, C.[1], Rinaldi, M.[2]
[1]*University of Parma, Department of Chemistry, Parco Area delle Scienze 17/A, 43124 Parma, Italy*
[2]*University of Parma, Department of Food Science, Parco Area delle Scienze 47/A, 43124 Parma, Italy*
**antonella.cavazza@unipr.it*

Introduction

Chili peppers are well known all over the world as a delicious spice with characteristic colour, taste, pungency, and strong antioxidant activity. Pungency and red colour are considered the most important quality factors for commercial powders of dried peppers. Generally, colour quality of red pepper products deteriorate during processing and storage, resulting in decrease of market value1. In this study, changes in terms of colour, furosine and capsaicinoids during one year of storage were evaluated for the first time simultaneously on two different varieties, stored both at room temperature and at low temperature (-18°C), in order to determine if each variety shows a typical behaviour during storage.

Method

The powder of two chili pepper varieties belonging to Capsicum annuum sp. were obtained from red fresh berries subjected to drying process (60 °C for 48 hours) and grinded. Capsaicinoids were extracted and analysed by RP-HPLC-UV-DAD2. Colour determination was carried out using a Minolta Colorimeter. The spectral curves were determined over the 400–700 nm range. L* (lightness), a* (redness) and b* (yellowness) values were determined3. Analyses of furosine were performed by capillary electrophoresis coupled to mass spectrometry, following a method previously optimized and validated4 after the hydrolysis of the chili pepper powders.

Results and Discussion

The two chilli pepper varieties analysed were characterized by a very different degree of pungency corresponding to 15000 and 47000 Scoville Units for variety 1 and 2, respectively. After 12 months of storage at room temperature, capsaicinoids content, for both varieties, decreased significantly, reaching about 80% of the initial value, confirming data reported in a previous work5. Non-enzymatic browning, known to darken red colour of the powders, was evaluated by quantifying furosine content. This marker significantly increased during storage only at room temperature. Furthermore, variations of colour powders were recorded by colorimetric indexes a*, b* and L*. Redness (a*) showed the same behaviour in the two varieties with a significantly decrease at room temperature.

In general, as expected, at low temperature all the analytical indicators were more stable. In particular, capsaicinoids, furosine and redness (a*) did not significantly change, whereas lightness (L*), yellowness (b*) and Total Colour Change showed a significant variation.

Taking into account the high correlation between redness and furosine amounts (about -0.94, at both temperatures), we can conclude that capsaicinoids and redness variations appear to be two important quality parameters for the assessment of red chili pepper powder deterioration during shelf life.

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PO66
ANTIGENOTOXIC CAPACITY OF THE ROOT OF *Jatropha dioica* OVER CYCLOPHOSPHAMIDE AND METHYLMETANE SULFONATE EVALUATED BY THE COMET ASSAY

Hernández-Ceruelos A.[1]*, Martínez-García N.[1], Muñoz-Juárez S.[1], Almaguer Vargas G.[2], Vázquez-Alvarado P.[3]

[1]*Área Académica de Medicina,* [2]*Área Académica de Farmacia,* [3]*Área Académica de Odontología*
Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hidalgo, México
**alejandra.ceruelos@gmail.com*

Introduction

Leatherstem, known in Spanish as sangre de drago (dragon´s blood), is a small shrub of1 to 3 ft, with tiny pink or white flowers that appear in clusters during spring and early summer. The root of the plant spreads underground runners to form colonies as wide as 6 ft, and when the sab is exposed to air it turns blood red. The infusion of the root is used to treat alopecia, oral and skin lesions, infections and cancer. The aim of this work was to determine the potential chemoprotective capacity of the infusion of Jatropha dioica root over the genotoxic effect of cyclophosphamide (CCF) and methyl methanesulfonate (MMS) by the comet assay.

Method

Male mice with an average weight of 25 g, and kept under standard conditions were treated as follows: negative control with purified water, positive control one treated with CCF (50mg/kg, I.P. route), positive control 2 (MMS 40 mg/kg) and 3.5, 10.71and 21.42 ml/kg of Jatropha dioica decoction (JDD) (1g of root on 250 ml of purified boiling water for 15 minutes) administered by orally route in combination with the mutagens. After administration animals were sacrificed at 3, 9 15 and 21 h. Liver, kidney and bone marrow were dissected to prepare cellular suspensions. Viability of the cells was determined by Trypan blue dye exclusion, with 95% of cell survival. 10 µl of cell suspension were mixed with 75 µl of LMPA (0.5%). The mixture was expanded with a cover slide over a microgel of normal agarose (1%) and placed in-between microscope slides previously coded and left over ice for gelation. a second layer of LMPA was added and after immersed in solution of lysis then rinsed and placed immersed in buffer (pH > 13) in an electrophoresis chamber for 30 min, after electrophoresis was performed for 20 min (300 mA, 25 V). Slides were washed three times with 0.4M Tris pH = 7.5, and dehydrated with absolute methanol for 5 min for their preservation (Tice *et al.*, 2000). Slides were stained with 50 µl of ethidium bromide (0.02 mg/ml). 100 cells per sample were observed and classified in a visual scale according to Browne (2009). The criteria to determine the percentage of damaged nucleus and to calculate the damage index (DI) in order to obtain a numeric value for statistical analysis following this formula: DI = % of nucleus grade 0 (0) + % of nucleus grade 1 (1) + % of nucleus grade 2 (2) + % of nucleus grade 3 (3)+ % of nucleus grade 4 (4) (#): number of times the size of the head appeared to migrate on the tail, besides 100 nucleus were measured to determine tail moment (TM) and tail length (TL) using Metasystem Image Analyzer and the COMET 2.0 software, with fluorescence Microscope Carl Zeiss Axioimager using 20 X/0.065.

Results / Discussion / Conclusion

Chemoprotective effect was observed against the two alkylating agents at 3 h, in liver, kidney and bone marrow, but the protective capacity was observed only for CCF at 9 and 15 h. The difference between the effect over the two alkylating agents might be related with the antioxidant capacity of the compounds present in the decoction, that could inhibit the bioactivation of CCF, while apparently only blocked the initial alkylation provoked by MMS over DNA

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PO67
THE EFFECTS OF UNRIPE GRAPE EXTRACT ON SYSTEMIC BLOOD PRESSURE AND SERUM LEVELS OF SUPEROXIDE DISMUTASE, MALONDIALDEHYDE AND NITRIC OXIDE IN RATS

Behzad Zolfaghari[1], Mostafa Kazemi[1], Mehdi Nematbakhsh[2,3,4]
[1]*Department of Pharmacognosy, Isfahan University of Medical Sciences*
[2]*Water & Electrolytes Research Center, Isfahan University of Medical Sciences*
[3]*Department of Physiology, Isfahan University of Medical Sciences*
[4] *IsfahanMN Institute of Basic & Applied Sciences Research*

Introduction
The new lifestyle increases the incidence of hypertension. Potentially herbal are commonly uses by pupulation. In Iranian folk medicine, it is believed that Verjuice obtained by unripe grape (Vitis Vinifera) could control blood pressure. We tested the chronic effect of unripe grape extract (UGE) in blood pressure alteration, serum antioxidant level and aorta endothelial permeability in rats.

Methods
Four groups of rats treated daily by placebo and three different doses of UGE(50, 150 and 300 mg/kg/day). Four weeks later, the animals were anesthetized and catheterized. The direct mean arterial, systolic and diastolic pressures (MAP,SP,DP) were recoded. The endothelial permeability was determined and the serum levels of superoxide dismutase (SOD), *malondialdehyde* (MDA) and nitrite were measured.
Results: High dose of UGE increased MAP and SP significantly ($P<0.05$) when compared with control group. Decrease of MDA and increase of SOD and nitrite also were detected statistically in animals treated with high dose of UGE ($P<0.05$). No difference in aorta endothelial permeability was observed between the groups

Conclusion
The chronic effect of UGE on blood pressure was dose depended. High dose of UGE increased MAP and SP although its antioxidant activity was significantly high. Such observation mechanisms need to be defined.

PO68
SYNTHESIS OF COPPER PHTHALOCYANINE DOPED SILICA BY SOL-GEL METHOD

Ruth Meléndrez L.[1], Blanca M. Cabrera V.[1], Yanis Toledano M.[2*], Juan C. Garcia R.[2], Monica Cruz H.[1], Marcial Zamora T.[1], J. Aurelio Ortiz M., Estibaliz Sansinenea R.[1]

[1]*PhD/Facultad de Ciencias Químicas, Edificio 179, 18 Sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

[2*-2]*PhD/Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Inmunología e Inorgánica y Nuclear Av. Universidad 3000, Distrito Federal, C.P. 04510.*

ruthmelendrez@gmail.com

Introduction

Sol-gel is one of the soft chemistry routes for materials synthesis. Hydrid, nanostructured and mesoporous materials have been synthesized by sol-gel and used as sensors, membranes, catalysers and encapsulation materials for drug delivery among many other applications[1]. Phthalocyanines are been used in high-tech applications within the fields of energy conversion, eletrophotography, optical limiters, photosensitizers and gas sensors[2,3]. Phthalocyanines can be deposited on a porous polymeric matrix and used as photosensitizers in order to produce singlet oxygen from solar energy. This technology has led to new clean technologies based in renewable energy for water treatment and disinfection[4].

Supported photosensitizers offer great advantages, namely: sensitizer reuse, excellent stability, high yield fabrication, immobilization-based oligomerization resistance and superior performance in continuous operation compared to sensitizers in homogeneous medium[5]. In this work, synthesis of copper phthalocyanine (PcCu) doped silica was achieved using the sol-gel method by changing the concentration of phthalocyanine. The solids were characterised by X-Rays, UV-visible and nitrogen physisorption.

Method

Copper phthalocyanine (PcCu) doped silica was obtained using tetraethyl orthosilicate and ethanol as precursors under temperature reflux and agitation for 72 hrs. After hydrolysis and polycondensation, the gel was dried at 70 °C for 48 hrs. The thermal treatment was made at 400 °C for 5hrs. under air atmosphere with an increment of 2 °C/min. Textural characterization by nitrogen physisorption was performed using a Quantachrome Autosorb-1. X-ray diffraction analysis was made using a Siemens powder diffractometer model D-5005.

Results / Discussion / Conclusion

1.- The optimal conditions for copper phthalocyanine (PcCu) doped silica synthesis was found for different concentrations. 2.- After textural characterization, it was determined the BET areas and pore volume correspond to materials where doped silica contains PcCu impurities. 3.- Reordering of the crystalline lattice due to thermal treatment was confirmed by x-ray analysis. 4.- Lattice ordering is only observed at high PcCu concentration.

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PO69
CHECK THE ANTISEPTIC POWER OF A SEALER OF NIPPLES BASED ON AN INFUSION OF BRAD (*Erodium cicutarium*)

Sofía Milagros Leyton[1],Gonzalo Damián Monge[2], Dr Juan B. Beltramino[3]
[(1) (2) (3)] *Escuela Agropecuaria Provincial N° 1, Gobernador Gregores, Santa Cruz. Argentina*
beltra_154@yahoo.com.ar

Abstract
The disinfection of nipples is used in tambos routinely, existing a lot of methods available in the market. The antisepsis of nipples post milking seeks killing microorganisms living in the nipple and prevent colonization of these in the nipple's orifice.
This research work was performed to replace the antimicrobials that are usually used in the trading nipples' sealants, changing them by a local herbalist resource, the Brad (Erodium cicutarium), as an infusion in the active and this way detecting new antimicrobial without attacking the product effectiveness. To evaluate its efficiency, in-vitro analysis were made at school laboratory and in the laboratory of Universidad San Juan Bosco in Comodoro Rivadavia, Chubut, Argentina. The fieldwork was made over the bovines at Escuela Agropecuaria Provincial N°1 in Gobernador Gregores, Santa Cruz, Argentina, during April until September in 2013. The results obtained in this trial using sealant with brad as an infusion basis, showed a good antiseptic behavior as it reduced in more than 3 logarithms the units maker of colonies, coming from the nipple's skin, adapted estimate from Protocol A (National Mastitis Council). At fieldwork, it reduced the bacterial load from the nipple's skin in 71%, after 21 days of application. The cost of the elaborated sealant was up to 85% lower than the trading ones.

Keywords: Sealant of nipples. Brad. Antiseptics.

PO70
SURVEY OF EIMERIA SPP. IN SHEEP: FIRST NOTICE OF *Eimeria macusaniensis* IN THE REGION OF GOVERNOR GREGORES, SANTA CRUZ, ARGENTINA

Luciano Taibo[1], Gonzalo E. Maidana[2], Dr. Juan B. Beltramino[3]
[1] [2] [3]*Escuela Agropecuaria Provincial N°1, Gobernador Gregores, Provincia de Santa Cruz, Argentina.*
beltra_154@yahoo.com.ar

Abstract
The aim of this study was to evaluate the parasite level by Eimeria spp. in sheep in the region of Governor Gregores, Santa Cruz, Argentina. The herds belonging to the Agricultural School No.1, Governor Gregores and a rancher of the region were sampled. There were not helminthes observed eggs in any of the herds tested. At the Agricultural School No.1, Governor Gregores, there was observed a high number (29,430) of oocysts morphologically consistent with Eimeria macusaniensis, coccidia from camels. However, in the local livestock herd, the amount of this coccidia was 20 times less. This is the first report of sheep infestation with that species of protozoa in the studied area.

Key words: guanaco, sheep, Eimeria macusaniensis.

PO71
PRODUCTION OF CHITOSAN STARTING FROM SHELL OF PRAWNS

Aniba Sebastián Romero[1], Ayelen Alejandra Barra[2], Dr. Juan B. Beltramino[3]
[1] [2] [3] *Escuela Agropecuaria Provincial N° 1, Gobernador Gregores, Santa Cruz. Argentina*
beltra_154@yahoo.com.ar

ABSTRACT

Among the most abundant polymer molecules is chitin, component of the exoskeletons of invertebrates, fungi and algae, presents a very high replacement rate in the biosphere. The shores of Argentina are one of the main sources of crustaceans, but, at the same time, the shells of crustaceans, constitute a serious pollutant residue. Chitosan constitutes the most important derivative of chitin. Chitosan market lies mainly in nutraceuticals, protective food, medical applications, applications in agriculture and flocculation among others.

The aim of this trial was to obtain Chitosan from prawn shell. As raw material, were used exoskeletons of prawn Pleoticus muelleri, the exoskeletons of prawn were cleaned, dried and mashed. For the removal of the shell's calcium carbonate, 1.3 N hydrochloric acid was used at 25° C for one hour. In the second stage protein was separated with sodium hydroxide 0.8 N 80° c for 4 hours. Main chitin derivatization reaction is the hydrolysis of acetamide groups to generate the deacetylated polymer, Chitosan. This reaction was conducted under severe alkaline conditions, with hydroxide sodium 13 N to 100° C for 15 hours. Chitosan, with the following results, was obtained: solid waste: 15%, humidity: 9%, values of DA% (average obtained by FTIR): 65%, performance 20%. Under the experimental conditions used in this trial, Chitosan was obteined from the exoskeletons of prawn was with a degree of deacetylation acceptable.

Key words: chitin, Chitosan, prawns

PO72
IN VITRO ANTI-HSV-1 ACTIVITY AND CYTOTOXICITY OF CRUDE EXTRACTS OBTAINED FROM THREE SPECIES OF ASPIDOSPERMA

Pessini, G. L.[1]*; Ceole, L. F.[2]; Ueda-Nakamura, T.[3]; Oliveira, A. J. B.[3]; Mello, J. C. P.[3]; Santana, A. E. G.[4]; Nakamura, C. V.[3]; Araújo-Júnior, J. X.[4]*

[1]*Postgraduate student, Program of Postgraduate in Chemistry and Biotechnology, Federal of Alagoas University, Campus A. C. Simões. Tabuleiro dos Martins, 57072-970, Maceio, AL, Brazil*

[2]*Postgraduate student, Program of Postgraduate in Pharmaceutical Sciences, State of Maringá University, UEM, Maringá, PR, Brazil*

[3]*PHD /Prof, Program of Postgraduate in Pharmaceutical Sciences, State of Maringá University UEM, Program of Postgraduate in Pharmaceutical Sciences Av. Colombo 5790, 87020-900, Maringá-PR, Brazil*

[4]*PHD /Prof, Program of Postgraduate in Chemistry and Biotechnology, Federal of Alagoas University, UFAL, Campus A. C. Simões. Tabuleiro dos Martins, 57072-970, Maceio, AL, Brazil*

**joaoxjr@gmail.com or glpessini@hotmail.com*

Introduction

The species Aspidosperma tomentosum Mart., A. macrocarpon Mart. and A. pyrifolium Mart. belong to the Apocynaceae family and they are restrict in America being found between Mexico and Argentina. The barks of these plants are used in the form of infusions by the folk medicine of the Amazon (Barbosa *et al.*, 2010). Herpes simplex virus type 1 (HSV-1) has a high prevalence in adults and in immunocompromised patients it can be life threatening. HSV-1 strains have developed resistance to Acyclovir, the drug of choice for treatment of HSV-1 diseases, therefore the search for new, more potent and selective drugs are needed. The aim of this study was to evaluate the anti-HSV-1 activity and cytotoxicity of crude extracts of three species of Aspidosperma.

Method

Hydroethanolic crude extracts (HCE) obtained from root bark, stem barks, stems and roots of A. tomentosum and A. pyrifolium, from fruits and flowers of A. pyrifolium, and from leaves and stems of A. macrocarpon were prepared by dynamic maceration with 90% ethyl alcohol. In vitro antiviral activity against HSV-1 and cytotoxicity assay LLCMK2 cells were performed using the colorimetric sulforhodamine B method (570 nm) in 96-well plates, in three independent experiments. The EC50 and CC50 values were obtained by regression analysis of the dose response curve.

Results / Discussion / Conclusion

Among the hydroethanolic crude extracts evaluated for anti-HSV-1 activity A. tomentosum showed activity against the strain of HSV-1 presenting EC50 values of 47.0 μg/ mL for root, 49.5 μg/mL for the root bark and 54 μg mL for the stem. The HCE of the flowers and roots of A. pyrifolium showed EC50 of 33.5 μg/mL and 70.5 μg/mL, respectively. The HCE of the stem of A. macrocarpon showed an EC50 of 193.5 μg/mL. The crude extract of A. pyrifolium stem bark and stems had an EC50 of 128.5 μg/mL and 159.0 μg/mL, respectively. Studies with the aerial parts and roots of A. pachypterum showed antiviral activity in vitro against human immunodeficiency virus (HIV) (Suffredini *et al.*, 2002). The other extracts tested showed no activity against the HSV-1 strain at the highest concentration tested (1000 μg/mL). When the cytotoxicity was considered, the root of A. tomentosum showed the best selectivity index (SI): 4.3 (CC50 = 199.4 μg/mL). This study demonstrated the anti-HSV-1 activity for crude extracts of flowers of A. pyrifolium and the extract of the root, root bark and stem of A. tomentosum confirming their biological activity, which could contribute for the alternative treatment of herpes simplex.

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PO73
SYNHTESIS OF A NEW CHIRAL IMINE AND ITS Pd(II) and Cu(II) COMPLEXES WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

Hernández Téllez Ma. Guadalupe[1], Moreno Ceballos Andrea[1], Portillo Moreno Óscar[2],
Mendoza Martínez Angel G.[3], Rios Merino Francisco J.[3], Gutiérrez Pérez René[1].
[1] Lab. Síntesis de Complejos, Universidad Autónoma de Puebla, Facultad de Ciencias Químicas. Edificio 105 K, 14 Sur y Av. San Claudio, Col. San Manuel, C. P. 72570, Puebla, Puebla, México.
[2]Lab. de Materiales, [3]Unidad de Análisis Instrumental (Difracción de Rayos X)
guadalupe.hernandez@correo.buap.mx, jrgutie@correo.buap.mx

Introduction

Since the discovery of the tumor-inhibiting quality of the [cis-PtCl2(NH3)2] complex or cisplatin by Rosemberg, the complex has been in widespread use to date, being nowadays one of the most effective and successful drugs to treat a variety of human solid tumors. Nevertheless, cisplatin and other second-generation platinum drugs have such major drawbacks as severe tissue toxicity including nephrotoxicity, neurotoxicity and ototoxicity, the presence of or acquisition of resistance to the treatment and low water solubility, as salient limitations. Therefore, the search for safer platinum complexes is still continuing and also, in this regard, an alternative issue is replacing the metal atom by palladium on the basis of the structural and thermodynamic analogy between platinum(II) and palladium(II) complexes, and a vast array of platinum- and palladium-containing compounds have subsequently been synthesized and tested so far. We became interested in developing chiral imines as promising carrier ligands and by incorporation of a variety of selected functional groups into these carrier ligands, it is hoped that the clinical properties of the corresponding compounds can be modified systemically. We report herein our results concerning the preparation of the Pd(II) and Cu(II) complexes, derived from the a chiral imine and their crystal structures.

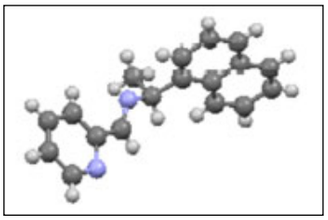
Experimental Method

Two new chiral Pd(II) and Cu(II) complexes are reported along with its ligand, derived from 2-piridilcarboxyaldehide and (S)-(+)-1-(1-naphthyl)-ethylamine. These results are interesting given that we employed a green method, the solvent-free approach affording high yields and faster reactions times, and for reasons of economy and pollution prevention, solvent-free methods are also used to modernize classical procedures by making them cleaner, safer, and easier to perform.

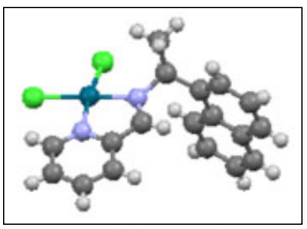
Results / Conclusion

The synthesis of the imine and its complexes was carried out and the structure of the compounds was established by spectroscopic means and fully confirmed by X-ray diffraction studies. The compounds are currently been tested in their pharmacological activity.

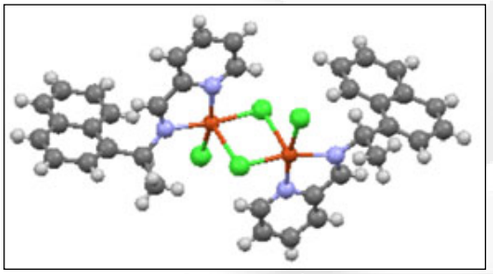
Crystal structures of the new compounds



Imine



Pd(II) complex



Cu(II) complex

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PO74
GREEN SYNTHESIS AND CHARACTERIZATION OF A NEW SULFUR-CONTAINING IMINE AND ITS Pd(II) COMPLEX

Hernández Téllez Ma. Guadalupe[1], Urbina García Laura[1], Mendoza Martínez Angel G.[2], Ríos Merino Francisco J.[2], Gutiérrez Pérez René[1]

[1] Lab. Síntesis de Complejos, Universidad Autónoma de Puebla, Facultad de Ciencias Químicas. Edificio 105 K, 14 Sur y Av. San Claudio, Col. San Manuel, C. P. 72570, Puebla, Puebla, México.
[2]Unidad de Análisis Instrumental (Difracción de Rayos X)
guadalupe.hernandez@correo.buap.mx, jrgutie@correo.buap.mx

Introduction

Green chemistry, also called sustainable chemistry, is a philosophy of chemical research that encourages the design of products and processes that minimize the use and generation of hazardous substances. Green chemistry seeks to reduce the impact of chemistry on the environment by preventing pollution at its source and using fewer natural resources.

Imines are widely studied and used, attracting much attention in both organic synthesis and metal ion complexation. Such versatile ligands are obtained by condensation of primary or secondary amines and carbonyl compounds. These compounds and their metallic complexes are important catalysts in many biological systems, colorants, polymers, etc.

Method

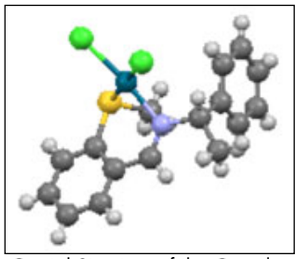
A new chiral Pd(II) complex is reported along with its ligand, derived from 2-(Methylthio)-benzaldehyde and (S)-(-)-1-phenylethylamine. These results are interesting given that we employed a green method, the solvent-free approach affording high yields and faster reactions times, and for reasons of economy and pollution prevention, solvent-free methods are also used to modernize classical procedures by making them cleaner, safer, and easier to perform.

The solvent-free approach is very attractive because the reactions occur under mild conditions and usually require easier workup procedures and simpler equipment. Other advantages of solvent-free reactions encompass cost savings, decreased reaction times along with reduced energy consumption, as well as safety is largely increased, working is considerably simplified, etc.

Results / Conclusion

The synthesis of the imine and its complex was carried out and the structure of the compounds was established by spectroscopic means and fully confirmed by X-ray diffraction studies. The crystal structure of the complex confirms that the Pd atom is linked to the S and N atoms of the imine.

YIELDS	IR (KBr) (cm^{-1}) IMINE	RMN- ^1H – IMINE ($CDCl_3$/TMS) (ppm)	RMN- ^1H - COMPLEX ($CDCl_3$/TMS) (ppm)	MS m/z IMINE
97%	1635 C=N	1.59, 1.62(d 3 H, $CHCH_3$), 2.45 (s 3 H, S-CH_3), 4.60 (q 1H, CH), 7.18-7.46 (m 8H, Ar), 7.91, 7.93 (d 1H, Ar), 8.83 (s 1H, HC=N)	1.92, 2.11(d 3 H, $CHCH_3$) 2.52 (s 3 H, S-CH_3),6.46 (q 1H, CH),7.26-7.65 (m 9H, Ar y 1H, HC=N)	255 (M^+) $C_{16}H_{17}NS$



Crystal Structure of the Complex

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PO75
PHOTOCHEMOPROTECTIVE ACTIVITY OF SOME MEXICAN PLANTS

Ana M. García-Bores[1], A. Monserrat Espinoza G.[1], Tzasna Hernández[2], Jorge Campos C.[3], José del Carmen Benítez[4] and José Guillermo Avila[1]

[1]Laboratorio de Fitoquímica, [2]Laboratorio de Farmacognosia, [3]Laboratorio de Bioquímica Molecular. UBIPRO, [4]Laboratorio 1, UMF, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios # 1, Los Reyes Iztacala, Tlalnepantla Estado de México. México.

tuncomaclovio2000@yahoo.com.mx

Introduction

Cutaneous overexposure to solar radiation, especially its ultraviolet (UVR) component can cause several skin related disorders. Some of these include sunburns, immunosuppression and skin cancer (both, melanoma and non-melanoma). In México, skin cancer ranks second in frequency among all malignancies, which constitutes 13.6%. Recently, considerable attention has been focused on identify natural products capable of inhibiting, retarding or reversing the multi-stage photocarcinogenesis. Our studies are aimed at evaluating the antioxidant and photoprotective (photochemoprotective) potential of the extracts and metabolites isolated from endemic plants of México. *Buddleja cordata*, *Lippia graveolens* and *Yucca periculosa* were collected in its natural growing areas. The preparation and isolation of extracts and compounds were made in accordance to conventional methods. Anitoxidant activities were made *in vitro* and *in situ* using scavenging measurements of DPPH, $O_2^{\bullet}$-, $^{\bullet}OH$ radicals. Acute and chronic photoprotective activities were evaluated using *Escherichia coli*, SKH-I mice and guinea pigs as models. In addition photoprotection was assessed at histological and genetic level (quantification of DNA adducts). Polar extracts of the three species have photochemoprotective activities in both, acute and chronic studies. The active components of the extracts were identified and belong to phenylpropanoids, stilbenes and flavonoids. The results of the acute experiments indicated that the extracts and compounds of the three plants substantially retracing the appearance of erythema, as well as, substances isolated of *B. cordata* and *Y. periculosa* delayed tumours formation in chronic experiments, and *L. graveolens* extract completely protected against the development of neoplasia.

PO76
ANTIBACTERIAL AND ALLELOPATHIC ACTIVITY OF ETHYL ACETATE EXTRACT FROM *Miconia caudata* (BONPL.) DC.

Ramírez Aristizabal, L. S.[1]*; Montes Ortiz, A. M.[2]; Martín Bedoya, J. G.[2]; Jiménez-González, F. J.[3]
[1]PhD, Grupo Polifenoles, Universidad Tecnológica de Pereira, Carrera 27 # 10 - 02, Pereira, Risaralda, Colombia
[2]Chemistry student, Grupo Polifenoles, Universidad Tecnológica de Pereira, Carrera 27 # 10- 02, Pereira, Risaralda, Colombia
[3]Q.I., Grupo Polifenoles, Universidad Tecnológica de Pereira, Carrera 27 # 10- 02, Pereira, Risaralda, Colombia
*luramire@utp.edu.co

Introduction

Miconia caudata (Bonpl.) DC. (Melastomataceae) is a small tree or shrub 3-7 m tall that is widely distributed in countries like Nicaragua and Panamá (Quiñones M, 2001). In Colombia known as "danto" and/or "panocho" and is used throughout the coffee belt region as ornamental. So far no have found ethnopharmacological studies for this species, and from the point of phytochemical view there are only investigations by the Polifenoles group (Isaza M. *et al.*, 2008).

Method

The antibacterial activity of the extract in ethyl acetate and its fractions were evaluated against strains Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923) and Pseudomonas aeruginosa (ATCC 27853) by the method of wells on agar (Valgas *et al.*, 2007). Allelopathic activity in extract in ethyl acetate and its fractions was determined by the inhibition/stimulation of the growth of germinated lettuce (Lactuca sativa L.) (Isaza et al., 2007), for seven days. The measurement of the seedling was performed on the hypocotyl and the epicotyl. The extract in ethyl acetate (8.0 g) obtained from the dried and ground leaves of Miconia caudata was fractionated by column chromatography (i.d.: 2.5 cm, h: 30 cm) packed with Diaion HP-20, eluting with a gradient system of stages 2-propanol-H_2O (95:5, 10:90) to give 20 fractions named as F1A to F1T. Chemical characterization tests were made to hydrolyzable tannins (Muller-Harvey, 2001) and condensate tannins, sesquiterpene lactones (Harborne, 1973), plus displacement reactions with sodium methoxide (MeONa), ferric chloride ($FeCl_3$) and sodium acetate (CH_3COONa) for identifying flavonoids cores (Harborne, 1973).

Results / Discussion / Conclusion

The extract in ethyl acetate, and the fractions evaluated for antibacterial activity, do not shown promising results in the inhibition against of *Escherichia coli*, *Staphyloccocus aureus* and *Pseudomonas aeruginosa*. To the ethyl acetate extract and fraction F1R was evident an inhibitory effect on hypocotyl and epicotyl of the lettuce seedlings, during the seven days of the trial. To the fraction F1M, the growth inhibitory effect on hypocotyl and epicotyl was evident during the first two days of the trial, after that, a promising effect shown steady growth until day seven.

According to the evidence of chemical characterization, in fractions F1R and F1M, were found sesquiterpene lactones, while only in the fraction F1M a higher concentration of condensed tannins was evident, and moderate for hydrolyzable tannins. Chemical shifts made to fractions showed bands characteristic for F1M phenolic compounds, which enables the presence of flavonoids and/or condensed or hydrolysable tannins.

The ethyl acetate extract and its fractions did not presented antibacterial effect against all strains tested. By contrast, the ethyl acetate extract and fraction F1R, presented inhibitory effect (herbicide), while for fraction F1M presented the inhibitory effect and at the third day presented a steady development of the seedling until the seventh day came. These results confirm the presence of tannins in plants of the genus Miconia, and its possible biological activity.

Acknowledgement: We thank Polifenoles group and microbiology laboratory (Escuela de Tecnología Química - Universidad Tecnológica de Pereira).

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PO77
IN VITRO PROPAGATION OF ANNATTO PLANTS (*Bixa orellana*) USING DIFFERENT HYPOCOTYL-DERIVED EXPLANTS

Faria, D. V.[2]; Gasparini, K.[2]; Matos, E. M. [3]; Batista, D. S.[2]; Otoni, W.C. [1]; Rogalski, M.[1]*
[1] *Prof of Federal University of Viçosa, Peter Henry Rolfs Avenue, –University Campus, 36570-000, Viçosa, MG, Brazil.*
[2] *Postgraduate student Federal University of Viçosa, Peter Henry Rolfs Avenue, –University Campus, 36570-000, Viçosa, MG, Brazil*
[3]*Postdoctoral student of Federal University of Viçosa, Peter Henry Rolfs Avenue, –University Campus, 36570-000, Viçosa, MG, Brazil*
** Rogalski@ufv.br*

Introduction

Bixa orellana L., commonly called annatto, has medicinal, nutritional and industrial properties, which make it an important and attractive species in the international market. The extract of seeds is a source of natural dye, the bixin, widely used in the cosmetics, pharmaceutical, and textile industries, and also as dye for various food products. In Brazil, the dye annatto extracted from seed is largely used as spice in traditional cooking. Due to its wide applications, annatto has a great economic value; it is solely responsible to support the global demand for bixin. There are few studies related to annatto, specially about biotechnological techniques such as tissue culture and plant regeneration. The optimization of plant in vitro regeneration protocols can facilitate the obtainment of transgenic plants, which can increase the production of carotenoids as well as assist studies involved to understand the bixin biosynthesis. Given the importance of annatto, this study aimed to investigate the influence of the length of hypocotyl segment explants on in vitro organogenesis of annatto.

Method

Etiolated annatto seedlings, established from in vitro germinated seeds, were used for obtaining hypocotyl segments with different lengths (1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mm). The explants were inoculated onto a medium supplemented with MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg *et al.*, 1968) and Zeatin (Zea) 0.0 and 4.56 μM. The frequency of organogenesis (%) and the length (mm) of shoots per explants were evaluated after 30 days of the beginning of the culture. Statistical analysis were performed by analysis of variance (ANOVA) and the difference between the means were calculated using Scott-Knott test.

Results / Discussion / Conclusion

As compared with control media, MS devoid of growth regulators, significant organogenic responses were observed only in MS medium supplemented with Zea (4.56 μM), which confirms previous results found by Paiva-Neto *et al.* (2003). The better condition for shoot induction (7.8 shoots) was observed when we used explants with 6 mm. Considering the average length of shoots and frequency of organogenesis (%) per explants, most significant results were observed in explants with 6 mm (2.61 mm and 96.67%) and 10 mm (2.66 mm and 93.33%), respectively. Studies by Paiva-Neto *et al.* (2003) using MS medium supplemented with 4.56 μM Zea, demonstrated a regeneration frequency of 78% and a number of shoots of 1.8 per explants, using explants with 10 mm. Carvalho *et al.* (2005) observed that 100% of hypocotyl explants with 10 mm, grown on MS medium supplemented with B5 vitamins, 2.28 μM Zea and 6 μM indole-3-acetic acid (IAA), induced an average of 3.35 shoots per explants. The data obtained in this study show that the reduction of the length of explants to 6 mm did not affect the efficiency of organogenesis. The size of explants is an important factor for efficient selection and growth of transformed shoots in presence of antibiotics during the genetic transformation. These results show that small explants can be used, with the same efficiency of organogenesis, for the development of transformation protocols aiming the increasing content of bixin and also to create mutant plants targeting the bixin biosynthesis pathway.

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PO78
HYPOGLYCEMIC ACTIVITY OF SERJANIC ACID OBTAINED FROM *Cecropia telenitida*: PRELIMINARY DATA

Balcazar, N.[1*]; Tabares-Guevara, Jorge H.[4]; Gonzalez, P.[5]; Ramirez-Pineda, Jose R.[2]; Montoya, G.[3*]

[1]*PhD/Grupo GENMOL. Sede de Investigación Universitaria, Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia.*
[2]*PhD/Grupo de Inmunomodulación. Sede de Investigación Universitaria, Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia.*
[3]*PhD/Grupo Natura. Facultad de Ciencias Naturales, Universidad ICESI., Calle 18 No. 122-135, Cali-Colombia.*
[4]*MSc/Grupo de Inmunomodulación. Sede de Investigación Universitaria, Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia*
[5]*MSc/Grupo GENMOL. Sede de Investigación Universitaria, Universidad de Antioquia, Calle 62 # 52-59, Medellín, Colombia*
**norman.balcazar@udea.edu.co, glmontoya@icesi.edu.co*

Introduction

Cecropia (cecropiaceae) is a neotropical genus frequently found in humid areas up to 2600 meters above sea level. *Cecropia telenitida* (Franco- Roselli P *et al.*, 1997) is restricted to the central and northern sections of the Andes. The principal ethno-pharmacological use comprises anti-inflammatory properties, and the treatment of diabetes type 2 (Schinella G *et al.*, 2008; Aragão DM., et al 2010). In our search for naturally occurring bioactive compounds from Colombian plant biodiversity, we initiated a phytochemical investigation of *C. telenitida* (vernacular names Guarumo, Guarumbo or Yarumo Plateado). Our preliminary studies indicated the presence of abundant pentacyclic triterpenes in the roots, such as Serjanic acid. In this study we evaluated the hypoglycemic potential of Serjanic acid by using an insulin resistance mouse model.

Methods

An ethyl acetate extract of air-dried roots of *C. telenitida* was partitioned successively with hexane and ethanol. The ethyl acetate was concentrated, diluted in methanol and purified by Sephadex LH-20. The fractions containing triterpenes were pooled and used for analytical and biological assays. Conventional isolations as preparative HPLC were used to purify Serjanic acid. We evaluated the effect of Serjanic acid on hyperglycemia, glucose intolerance and insulin blood levels in C57BL/6J mice fed with a high-fat (HF) diet. After 10 Serjanic acid oral doses, animals were sacrificed. Blood samples were taken to evaluate glucose and insulin plasma levels and the expression of pro-inflammatory cytokines genes of adipose tissue was evaluated by qRT-PCR.

Results / Discussion / Conclusion

We demonstrated that Serjanic acid was effective in ameliorating the hyperglycemia ($P < 0.05$) and glucose intolerance ($P < 0.001$) in mice fed with HF diet. Preliminary results have shown that the fraction containing triterpenes reduces the mRNA expression of pro-inflammatory cytokines MCP-1, IL-1β and IL-6 in adipose tissue of a mouse model of diet-induced obesity and insulin resistance. In addition, Serjanic acid increased insulin levels and this could contribute to reduce the hyperglycemia and improve glucose tolerance. *Conclusions:* This study demonstrates the beneficial effects of Serjanic acid, on glucose intolerance and hyperglycemia in an obese and insulin resistance mouse model. In addition, the reduction of pro-inflammatory cytokines expression levels in adipose tissue, allows us to suggest, that the systemic effects of Serjanic acid on carbohydrates metabolism could be regulated by their action on the inflammation process established in adipose tissue of this insulin resistance mouse model.

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PO79
HYPOGLYCEMIC/ANTIDIABETIC EFFECT OF TRITERPENES PRESENT IN *Eucalyptus tereticornis*

Balcazar, N.[1]*; Guillen, A.[3]; Granados, S.[4]; Echeverri, F.[2]
[1]PhD/Associate Professor, Universidad de Antioquia/GENMOL Group - School of Medicine, Calle 62 # 52-59, Medellín, Colombia.
[2]PhD/Full Professor, Universidad de Antioquia/QOPN Group - School of Natural and Exacts Sciences, Calle 62 # 52-59, Medellín, Colombia.
[3]MSc/ Assistant Researcher, Universidad de Antioquia/ GENMOL Group, Calle 62 # 52-59, Medellín, Colombia
[4]MSc/ Assistant Researcher, Universidad de Antioquia/QOPN group, Calle 62 # 52-59, Medellín, Colombia
*norman.balcazar@udea.edu.co

Introduction

Type 2 diabetes (T2D) is a progressive metabolic disorder with diverse pathological manifestation and is associated with abnormal carbohydrate and lipid metabolism due to underlying insulin resistance. T2D is a public health problem at worldwide level, as results of its high prevalence, high costs and rates of morbidity and mortality. Considering the limitations of current treatments, it is critical to identify new therapies with the least number of undesirable effects and more potent efficacies than conventional therapies. Natural products are valid alternative; our aim is to identify new pharmacological agents from plants used as hypoglycemic/anti-diabetic in the Colombian traditional medicine.

Method

Methanolic extract from leaves of Eucalyptus tereticornis (Eu) was partitioned with ethyl acetate, concentrated and purified by Sephadex LH-20. The fractions containing triterpenes were pooled and used for analytical and biological assays. Insulin resistance HepG2 and C2C12 (miotubes) cells models were established by adding palmitic acid (0.75 mmol/L) to the culture medium and then cells were treated by crude extracts or triterpene enriched fraction. Glucose consumption of C2C12 cells and glucose production of HepG2 cells were determined by glucose oxidase and HPLC method, respectively.
We evaluated the effect of fraction containing triterpenes on hyperglycemia, glucose intolerance and insulin resistance in C57BL/6J mice fed with a high-fat (HF) diet and treated with Streptozotocin (STZ). After 10 intraperitoneal doses, animals were sacrificed and the expression of pro-inflammatory cytokines and gluconeogenic genes of adipose and liver tissue was evaluated by qRT-PCR. Blood samples were taken, to evaluate glucose and insulin plasma levels.
In order to identify the active compound in triterpene fraction, we used different types of chromatography methods and evaluated its biological activity in C2C12 cells.

Results / Discussion / Conclusion

The triterpene-enriched fraction of Eu inhibits the glucose production in insulin resistant HepG2 cells and presents little effect in insulin resistant C2C12 cells. We demonstrated that this fraction was effective in ameliorating the hyperglycaemia ($P < 0.05$)insulin resistance ($P < 0.01$) and glucose intolerance ($P < 0.001$) in mice fed with HF diet and treated with STZ. Preliminary results have shown that the fraction containing triterpenes reduces the mRNA expression of pro-inflammatory cytokines MCP-1, IL-1β, IL-6 and TNF-a in adipose tissue and glucose-6-phosphatase in liver from diabetic mice. Finally, we isolated the main compound of triterpene fraction; 3b-hidroxi-urs-11-en-28, 13b-olide, which is the compound with major potential of hypoglycemic activity.
Conclusions: This study demonstrates the beneficial effects of triterpenes present in Eu, on insulin resistance, glucose intolerance and hyperglycemia in a diabetic mouse model. In addition, the reduction of pro-inflammatory cytokines expression levels in adipose tissue, allows us to suggest, that the systemic effects of the triterpenes of Eu on carbohydrates metabolism, could be regulated by their action on the inflammation process established in adipose tissue of mice with T2D

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PO80
Artemisia arborescens L. (ASTERACEAE): HPLC DETERMINATION OF FLAVONOIDS AND EVALUATION OF ANTI-ANGIOGENIC EFFECTS

Germanò, M. P.[1]; Russo, M.[1*]; Certo, G.[1,5]; Grasso, E.[1]; Costa, R.[1*]; Bonaccorsi, I.[1]; Ragusa, S.[4]; Dugo, P.[1,2,3]

[1] *University of Messina, Dipartimento di Scienze del Farmaco e dei Prodotti per la Salute (SCIFAR), Viale Annunziata, Messina, Italy*
[2] *Università Campus Biomedico, Via Alvaro del Portillo, 21, 00128 Roma, Italy*
[3] *Chromaleont s.r.l. A start-up of the University of Messina, c/o Dipartimento SCIFAR, University of Messina, Viale Annunziata, 98168 Messina, Italy*
[4] *Dipartimento di Scienze della Salute, Università degli Studi 'Magna Graecia' di Catanzaro, Viale Europa, 88100 Catanzaro, Italy*
[5] *Fondazione Prof. Antonio Imbesi, Piazza Pugliatti 1, 98100 Messina, Italy*
* *germanom@unime.it; costar@unime.it*

Introduction

Artemisia arborescens L. (Asteraceae), endemic of the southern Mediterranean area and commonly known as "arboreous absinth" or "arborent mugwort", is employed in traditional medicine as antispasmodic, antipyretic, anti-inflammatory and abortifacient. Previous reports on *A. arborescens* mainly focused on the volatile constituents of the essential oil and their biological activity (1-3). However, few data are present in literature about the polyphenolic composition of arboreous absinth (4,5). Considering that several plant derived flavonoids can prevent the development and progression of chronic diseases associated with extensive angiogenesis (6), the aim of this work was to analyze the flavonoid composition of a dichloromethane extract from *A. arborescens* leaves and to evaluate its potential use as a new source of antiangiogenic agents by assessing the inhibitory effects on vessel formation in zebrafish embryos.

Method

Plants of *Artemisia arborescens* L. were harvested at the end of January 2014 from the Natural Park of Nebrodi Mountains (Messina, Italy). Leaves (100g) were extracted by maceration with CH_2Cl_2 (1L x 72 h) and the extract was successively dried *in vacuo* (7). For HPLC analysis, 60 mg of extract were re-dissolved in 1 mL of acetonitrile, filtered through paper filters and injected into two HPLC systems (Shimadzu, Japan), equipped with UV and MS detectors, respectively. Quantification of flavonoids was carried out through the construction of a five-point calibration curve relative to the standard "casticin". To evualuate *in vivo* the inhibitory effect on angiogenesis, *A. arborescens* extract was tested on zebrafish (5-20 μg/embryo) by the quantitative endogenous alkaline phosphatase and staining assays (8).

Results / Discussion / Conclusion

Previous works about the polyphenolic composition of arboreous absinth have reported the presence of myricetin, kaempferol, two hydroxycinnamic acids and other flavonoids (santin, artemetin and casticin) (4-5). Literature data (4-5,7) along with ($APCI^+$-MS) information, allowed the identification of six flavonoids, namely artemetin, chrysosplenetin, casticin, eupatin, chrysosplenol-D and cirsilineol, in decreasing order of quantity. The total flavonoidic content accounted for 9.0 ÿ'3fg/Kg. Four compounds, even though not identified, could be ascribed to the flavonoid group from the observation of mass spectral data. Results of *in vivo* assays showed that *A.arborescens* extract produced a strong reduction on vessel formation in zebrafish (57% of inhibition at 10μg/embryo) inducing the appearance of an evident pericardial edema.
This study provided additional phytochemical investigations on *A.arborescens* suggesting its therapeutic efficacy as a new anti-angiogenic drug.

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PO81
CHEMICAL EVALUATION, CELL VIABILITY AND IMMUNOMODULATORY ACTIVITIES OF *Gossypium barbadense* L. (MALVACEAE)

Macedo, A. L.[1*], Riani, L. R.[2], Castro Junior, M., Correa, J. O. A.[3], Aguiar, J. A. K.[3], Medeiros,V. P.[3], Chedier, L. M.[3], Valverde, A. L.[4], Pimenta, D. S.[3]

[1] *MSc/Graduate Studant, Universidade Federal Fluminense,Outeiro de São João Batista, s/n, Niterói, Rio de Janeiro, Brasil*
[2] *Undergraduate Studant, Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, s/n, Juiz de Fora, Minas Gerais, Brasil*
[3] *PhD/Prof, Universidade Federal de Juiz de Fora*
[4] *PhD/Prof, Universidade Federal Fluminense*
* *arthur_macedo@id.uff.br*

Introduction

Gossypium barbadense L. (Malvaceae) leaves, popularly known as cotton, have been used for the treatment of bacterial vaginosis (BV) (Conde *et al.*, 2014), an infection caused by *Gardnerella vaginalis*. Some complications can occur during BV like lysis of vaginal epithelium cells and fetal membrane inflammation. The later can cause pregnancy complications (McCathie, 2006). Macedo *et al.* (2014) showed that *G. barbadense* extracts are active against *G. vaginalis*. The aims of present work are the evaluation of murin fibroblasts (3T3) cell viability and anti-inflammatory activity by the quantification of cytokines (IL-17, TNF-a and INF-γ) and NO.

Method

Extracts of leaves of *G. barbadense* were made by successively maceration with hexane (HCE) and methanol (MCE) and infusion with water (H_2OCE). MCE was successively submitted to liquid:liquid partition (v/v) with hexane, dichloromethane and ethyl acetate to produce Hexanic Fraction (HF), Dichloromethane Fraction (DMF), and Ethyl Acetate Fraction (EAF), respectively, as well as an Aqueous Residue (AR). The chromatographic profile was made by HPLC-DAD and GC-MS. One compound was isolated from EAF and characterized by ^{1}H RMN, COSY and HSQC. Cell viability test was made like described by Mosmann (1983), cytokines (IL-17, TNF-a and INF-γ) were dosed by ELISA and NO like described by Greiss (Ding *et al.*, 1988). Statistical analysis was made by ANOVA with tuckey *post-hoc*.

Results / Discussion / Conclusion

Polar extracts and fractions were rich in flavonoids and arylpropanoids, nonpolar ones were rich in terpenoids, saturated and unsaturated fatty acids. Cellular viability was increased in the bioassay against 3T3. The isolated compound of EAF was identified as quercetin substituted at the hydroxyl group of carbon 3. MCE, HF, DMF and EAF significantly increased cell viability, showing 130,4%, 134,8%, 180,7% and 147,0% respectively. Other extracts do not interfered significantly this parameter, showing 100,4% (HCE) and 97,4% (H_2OCE). HCE significantly reduced the production of IL-17, TNF-a, INF-γ and NO. This response can be related to methyl linolenate and ethyl palmitate, majoritarian compounds of HCE by GC-MS, once they are already known by their anti-inflammatory activity. H_2OCE and MCE significantly reduced IL-17, TNF-a and INF-γ production, this activity can be related to quercetin. Many flavonoids, as quercetin, are known by their anti-inflammatory activity. Extracts and fraction of *G. barbadense,* showed great potential for future *in vivo* studies for corroboration of these activities in complex biological systems, aiming the confirmation of popular use of this plant.

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PO82
PHENOLIC CONTENTS AND ANTIOXIDANT CAPACITY OF LEAVES EXTRACTS OF TEN ALGERIAN *Ficus carica* L. VARIETIES

Mahmoudi, S.[1,2,3]*; Ben Khaled, A. A.[2]; Baiti, I.[2]; Khali, M.[1]; Mahmoudi, N.[3]*
[1] *PhD student and teacher, University of Blida, Department of Food sciences, Algeria*
[2] *University of M'sila, Department of microbiology and biochemistry, Algeria*
[3] *University of M'sila, Department of agronomic sciences, Algeria*
**mahmoudisouhila@yahoo.fr*
**mahmoudinara@gmail.com*

Introduction

Ficus carica L., a deciduous tree belonging to the Moraceae family, is one of the earliest cultivated fruit trees. Seventy percent of the world's fig production is grown in the countries of the Mediterranean coast (Çaliskan and Aytekin Polat, 2011). Ficus carica leaves are an excellent source of phenolics and flavonoids and their several biological proprieties have been investigated.

In this work, phytochemical characters and antioxidant capacity of leaves extracts of ten Algerian Ficus carica L. varieties (Uniferous: Elbaidha, Elhamra, Onk Elhamam, Zarrouk, Elchatoui, Boughandjo and Elsafra; Biferous: Elbakkor and Elbither; caprifig tree: Eldhokkar) accessions were investigated.
Phenolics were extracted by Soxhlet method and analyzed by the colorimetric method of Folin-Ciocalteu (Boizot and Charpentier, 2006). Flavonoids were determined by aluminum trichloride assay (Bahourun, 1997) and the antioxidant capacity by the DPPH. radical scavenging assay (Koh and al., 2012).

Extract yields of fig leaves were ranged between 12.523 % for 'Elbakkor' and 19.805 % for 'Elsafra'. Leaves of biferous followed by uniferous varieties had the highest total phenolic contents (means 52.296 ± 5.232 and 48.973 ± 2.015 mg gallic acid equivalent/g of dry weight respectively), flavonoids (means 14.388 ± 0.333 and 14.136 ± 1.082 mg quercetin equivalent/g of dry weight) and antioxidant capacity (means IC50 798.754 ± 108.59 µg/ml and 825.004 ± 110.835 µg/ml). The caprifig tree had the lowest total phenolic and flavonoids values and antioxidant capacity (means 46.074 ± 0.134 mg gallic acid equivalent/g of dry weight, 11.667 ± 0.041 mg quercetin equivalent/g of dry weight and IC50 931.746 ± 5.158 µg/ml). Antioxidant capacity of fig leaves was significantly correlated with phenolic contents (r=0.748) but not with flavonoids values (r=0.007). Fig leaves appeared as a good source health-promoting polyphenols.

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PO83
SURVIVAL OF COLIFORM BACTERIA DURING PROCESSING OF FARMHOUSE OAXACA CHEESE

Colín-Cruz M. A.; Alva-Ortega P.; Reyes-García A.; Martínez-Hernández V.
Departamento de Alimentos, Facultad de Química, Universidad Autónoma del Estado de México
Paseo Colon y Paseo Tollocan S/N, Toluca 50 000. México
macolinc@uaemex.mx; ali_reyes@yahoo.com

Introduction
Farmhouse Oaxaca cheese is manufactured in small workshops in the whole country (Mexico). With the intention of reduce the content of coliform bacteria the producers add nitrate to raw milk. In addition, it has been proposed that kneading of curd in hot water (70-80°C) might replace the pasteurization (Sagarpa, 2009). Since presence of coliform bacteria in raw milk is common and milk is not pasteurized, the aim of this study was to evaluate the growth and survival of these bacteria during processing of Oaxaca cheese.

Method
Seven processes of manufacturing farmhouse Oaxaca cheese were evaluated. Samples were taken in representative stages of the process: raw milk, milk with nitrate, curd before and after kneading, salted cheese. Two control processes were carried out: with raw milk (and natural acidification), and with pasteurized milk (and starter added). In samples, total and MPN fecal coliform bacteria were estimated. All processes were evaluated in triplicate.

Results, Discussion, Conclusion
Results indicate that coliform bacteria in raw milk were high in all studied processes, even in control cheese made with raw milk: it ranged from 4 000 to 30 000 000 CFU/mL of total coliform and from 61 to 9 300 000 MPN/mL of fecal coliform. When nitrate was added the counts of coliform bacteria decreased 1 log10 in both total and fecal coliform bacteria, due certainly to the death of other present microorganisms in the milk, but not to the coliform bacteria (Vitozzi, 1992; Tudor *et al.*, 2007). During the 3-6 hours of milk acidification for reaching pH of 5.2-5.3, the population of coliform bacteria increased 1 to 3 log10. Addition of hot water in order to knead made increase the temperature in the curd to 45-68°C. As a result of kneading the content of coliform bacteria decreased 1 to 3 log10. In cheeses (salted and packed) the content of coliform bacteria ranged from 3 500 to 72 000 000 CFU/g and from 280 to 3 600 000 MPN/g of total and fecal coliform bacteria, respectively. In cheese made with pasteurized milk, no coliform bacteria were detected. It has been reported that coliform bacteria may growth in extreme acid conditions and high temperatures (Hengge-Aronis, 2002) and that only pasteurization may ensure that pathogens and coliform bacteria are destroyed (OMS, 2011). These results provide a better understanding of coliform bacteria behavior during processing of cheese made with raw milk, and warns on the danger to the consumers.

Key words: Oaxaca cheese, coliform bacteria, raw milk

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PO84
INVESTIGATION ON THE ANTICANCER ACTIVITIES OF *Origanum vulgare* SUBSP. *Vulgare* AND *Origanum bargyli*

Taşkın, T.[1]*; Abudayyak, M.[2]; Özhan, G.[2]; Sadıkoğlu, N.[3]; Biti°, L.[1]**
[1] *Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Turkey*
[2] *Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey*
[3] *Department of Pharmacognosy, Faculty of Pharmacy, Inönü University, Malatya, Turkey*
**turguttaskin@marmara.edu.tr, ** lbitis@yahoo.com*

Introduction

Twenty-three species and 31 taxa of Origanum (Labiatae) are recorded in the Flora of Turkey. Origanum are among the species recognized as "oregano" (= kekik) in Turkey (1). Aerial parts of Origanum species are aromatic and fragrant which are used as spice or herbal tea in addition to its medicinal uses since ancient times. The aromatic oregano water, rich in carvacrol and water soluble compounds, is consumed for gastrointestinal disorders, to reduce blood cholesterol and glucose levels, and also against cancer (2). The oils of Origanum species are widely used in the flavoring of food products and alcoholic beverages. Origanum vulgare L. is used to treat respiratory disorders, gastrointestinal (GI) diseases, painful menstruation, rheumatoid arthritis, scrofulosis and urinary tract disorders. Origanum bargyli Mouter is a rare plant of this genus in Turkey. The plant is a subshrub up to 40 cm, with subsessile cordate or ovate leaves, obovate or elliptic partlys, purple bracts and pink corolla. Native plants are found in the pine woods at 1150-1350 m in South Anatolia (3).

The purpose of the study is to investigate the anticancer activities of extracts, which were prepared by maceration in methanol from Origanum vulgare subsp. vulgare L. and Origanum bargyli Mouter aerial parts. For the extracts, anticancer activity was screened by using MTT viability test on cancer (mouse hippocampal cancer cell line, HT22) and normal (rat kidney epithelium cell line, NRK-52E) cells (4). It was revealed that two plants were more cytotoxic to HT22 cancer cells when compared with NRK-52E normal cells. For Origanum bargyli, the cell survival rates were 4.01 % and 74.04 % at 0.4 mg/mL concentration on HT22 and NRK-52E cells, respectively. However, cell death was seen less than 35% for both cell lines exposed with Origanum vulgare subsp. vulgare at 0.4 mg/mL concentration. It was concluded that Origanum bargyli might be potential source of anticancer agent. Indeed, further studies would be required to evaluate its true effectiveness on anticancer activities along with determination of chemical diversity and biological experiments.

Keywords: Origanum vulgare subsp. vulgare, Origanum bargyli, Anticancer activity

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PO85
A NEW ENDEMIC SUBSPECIES OF *ALLIUM PHANERANTHERUM* (AMARYLLIDACEAE) FROM HATAY, TURKEY AND ITS ANTIPROLIFERATIVE EFFECTS ON MCF-7 HUMAN BREAST CANCER

Ekşi, G.[1]; Bakar, F.[2]; Bona, M.[3]; Kılıç, C. S.[1]; Gençler Özkan, A. M.[1]
[1]*Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey*
[2]*Ankara University/Faculty of Pharmacy/Dept. Biochemistry, 06100, Tandoğan, Ankara, Turkey*
[3]*Istanbul University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 34116, Beyazıt, Istanbul, Turkey*
**erdurak@pharmacy.ankara.edu.tr*

Introduction

Allium phanerantherum subsp. *hatayensis* (Amaryllidaceae) is described and illustrated as a new subspecies. This taxon belongs to sect. *Allium* L. based on the tricuspidate inner filaments. It grows on the dry hillside in Hatay province (South Anatolia, Turkey). The new subspecies distinctly differs from related subspecies in the same area with its 1-valved, caducous spatha and involucrum like bracteoles. In this study we aimed to examine its potential to be used in breast cancer as an alternative to chemotherapeutic agents since extensive *in vivo* and *in vitro* studies has demonstrated that the garlic derivatives possess anti-cancer effects (Cao *et al.*, 2014) and we chose to work on breast cancer since it is the most frequently diagnosed cancer in women, and is the second most common cancer worldwide. According to GLOBOCAN (2012), 25% (1.67 million) of all new cancer cases and 15% (522,000) of all cancer deaths in women were due to breast cancer (Kaboli *et al.*, 2014).

Method

Time- and concentration-dependent anti-proliferative effects of *A. phanerantherum* subsp. *involucrata* on MCF-7 breast cancer cell line were determined by MTT method of Mossman (1983) in modification of Kuzma *et al.* (2012). Dried bulbs were submitted to extraction with ethanol and the extract was diluted in ethanol within the range between100 µg/ml to 0.05 µg/ml. The concentration of ethanolic extract required to reduce survival of cells by 50% (IC_{50}) was determined from the graph of the amount of visible cells against test compound concentration. Cell viability was determined by MTT assay. The results are expressed as percentage of live cells compared with untreated control.

Results / Discussion / Conclusion

The ethanolic extract has showed the highest anti-proliferalive effect at 24th hour with 5.33 µg/ml IC_{50} value, whereas the results were 12.11 µg/ml for 48 h and 16.32 µg/ml for 72 h, respectively. Therefore this species may represent a promising new agent and requires progressive studies for the identification of mechanism of action and also the responsible active substance(s).

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PO86
ACUTE DERMAL IRRITATION TEST OF A ROOT CANAL FILLING MATERIAL MADE WITH PROPOLIS

Cárdenas-Cruz, F. A.[1], Hernández-Ortega, N., Valadez-Carmona, L.[1], Plazola-Jacinto, C. P.[1], Hernández-Ortega, M.[3], Hernández-Navarro, M. D.[3], Garduño-Siciliano, L.[1], Ortiz-Moreno, A.[1,*]

[1]*Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Prolongación Carpio y Plan de Ayala s/n, Distrito Federal Ciudad de México, México*

[2] *Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Zaragoza, Av Guelatao 66, Distrito Federal Ciudad de México, México*

[3] *Universidad Autónoma del Estado de México, Facultad de Química, Paseo Colón casi esq. Paseo Tollocán, Toluca de Lerdo, Estado de México, México*

**ortizalicia@hotmail.com*

Introduction

Propolis is a byproduct of bees, also is an embalming substance responsible for the low incidence of bacteria inside the hive. The phenolic acids and aromatic fraction are the major part of propolis, to which is attributed the pharmacological action. Propolis has shown antimicrobial activity which has effectiveness in dental treatments. Thus the aim of this study was to evaluate the acute dermal irritation of a root canal filling material made with propolis.

Method

Propolis sample was obtained from bees farm placed in different states of Mexico and these samples were homogenized to get a representative sample of propolis. Root canal filling material was formulated with ethanolic extract of propolis, propylene glycol and calcium hydroxide. Due to the fact that the root canal filling material will be placed in a cutaneous zone, the primary irritation index was necessary to determine the possible irritation degree. According to OECD Test Guideline 404, the skin irritation was measured in male and female albino rabbits among 2-3.5 kg and determined as follows: 0.5g of root canal filling material was placed in two different regions a) zone intact and b) abraded area. These areas were monitored for three days in order to observe signs of erythema, edema and or eschar formation.

Results / Discussion / Conclusion

The primary irritation index is a test that shows local inflammatory reactions that occur on the skin intact and abraded skin. The root canal filling material showed, in intact skin, an index ranged between 1.7-1.93 meaning that the root canal filling material may be harmless to intact skin however, should be avoided in abraded skin, since it can generate mild irritation, thus may be caused by the slightly alkaline pH reached by the usage of calcium hydroxide. Other root canal filling materials has been tested for its biocompatibility and showed high toxicity, so it is very important to develop new safer alternatives for this dental treatment. These results found in the present study suggest that root canal filling material made with ethanolic extract of propolis can be used as an adjunct in the treatment of erosive and inflammatory skin conditions in oral health. More studies must be conducted in order to prove its toxicological safety as a root canal filling material.

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PO87
EFFECTS OF GENISTEIN ON A STUNNING MODEL OF RAT HEARTS EXPOSED TO ISCHEMIA/REPERFUSION

Colareda, G. A.[1]; Ragone, M. I.[1]; Consolini, A. E.[1]*
National University of La Plata, School of Exact Sciences, Pharmacology, 47 and 115 street, La Plata, Argentina
**dinamia@biol.unlp.edu.ar*

Introduction
Phytoestrogens were recommended to prevent cardiovascular diseases such as coronary failure. Genistein is a phytoestrogen isolated from soy, able to induce vasodilatation (1) and inhibition of tyrosine-kinases (2). However, there are no reports about its direct effects on the myocardium challenged by an ischemic insult. The heart is extremely dependent on aerobic metabolism, by which a period of ischemia and reperfusion (I/R) reduces its contractility and energetics (3). This work evaluated the effects of genistein (Gen) on the contractile and energetic recovery of rat hearts exposed to a model of stunned heart by I/R. The influence of gender was also evaluated.

Method
Isolated rat hearts were perfused with control Krebs (C) in the chamber of a flow-calorimeter. They were exposed to 20 μM Gen and other specific drugs before applying a period of no-flow ischemia (I) and reperfusion (R with Krebs-C). Left ventricular pressure (LVP, from which the maximal developed pressure, P, was calculated during the contraction) and total heat rate (Ht) were continuously measured (4). On the other hand, isolated cardiomyocytes loaded with Rhod-2 or Fluo-4 were used to evaluate the effects of Gen respectively on the free mitochondrial or cytosolic [Ca2+] by confocal microscopy (5).

Results / Discussion / Conclusion
Gen reduced the inotropism before I and the post-ischemic contractile recovery (PICR) in male rat hearts (MRH) from 66.9±6.9% of initial P in control hearts (n=12) to 25.5±8.6% in MRH (n=7) but maintained them in female rat hearts (FRH, with 65.6±13.5%, n=8). Muscle economy (Eco= P/Ht) showed similar behavior at low heart rate (HR). But at normal HR, Gen improved Eco in FRH (to 114±25%, n=7) during R. Addition of orthovanadate (OV, phosphatase inhibitor) reduced the negative effects of Gen in MRH, suggesting that they are due to inhibition of tyrosin-kinase. In FRH, OV did not induce changes. In both FRH and MRH, Gen reduced the relaxation rate of a contracture induced by reperfusing Krebs with 10 mM caffeine-36 mM Na. On this condition, the only transporter still able to extrude cytosolic Ca2+ was the mitochondrial uniporter (UCam) (exponential k: -0.025±0.012, -0.0055±0.0018 and -0.0135± 0.004 min-1 for control, Gen+FRH and Gen+MRH, respectively, ANOVA: p=0.058). These results suggest that Gen reduced the UCam flux with respect to control hearts.
In cardiomyocytes loaded with Fluo-4, perfusion of Krebs-10 mM caffeine-36 mM Na+ induced an increase in the relative fluorescence signal (F/Fo) followed by a fall before the reversion to control-Krebs perfusion. Gen did not modify these changes, suggesting that it did not affect the cytosolic free [Ca2+]. In the myocytes loaded with Rhod-2, the same perfusion also induced an increase in F/Fo signal during the fall in Fluo-4 signal, but Gen reduced it especially during the reversion. These results suggest that Gen reduced the free mitochondrial [Ca^{2+}].

Conclusion
Genistein was more cardioprotective in female than in male ischemic hearts. In male hearts, the negative effects of Gen were due to the inhibition of tyrosine kinases, which has been associated to inhibition of calcium channels. In both, female and male hearts, Gen also reduced the mitochondrial influx by the uniporter.

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PO88
EFFECTS OF ESSENTIAL OIL AND TINCTURE OF *Blepharocalyx salicifolius* ON A STUNNING MODEL OF RAT HEARTS BY ISCHEMIA/REPERFUSION

Ragone, M. I.[1]; Bonazzola, P.[2]; Bruno, F.[1]; Consolini, A. E.[1]
[1]National University of La Plata (UNLP), School of Exact Sciences, Pharmacology, 47 and 115 street, La Plata, Argentina.
[2] Cardiological Research Institute - School of Medicine-University of Buenos Aires(UBA)-CONICET
[1]dinamia@biol.unlp.edu.ar

Introduction

Blepharocalyx salicifolius Kunth O. Berg (Myrtaceae) is a tree known in Argentina as "anacahuita" and it is traditionally used as antitussive, in part due to its essential oil rich in 1,8-cineol (1, 2). Since in the intestinal smooth muscle it interferes with calcium influx and contractility, it was of interest to evaluate whether it could induce cardioprotection by reducing the calcium overload in hearts exposed to ischemia and reperfusion (I/R). Two preparations of ethnotherapeutic use were assessed, the ethanolic tincture and the distilled essential oil from the leaves, on the contractile and energetic recovery of isolated rat hearts exposed to a model of "cardiac stunning" by I/R.

Method

Leaves of Blepharocalyx salicifolius collected on last summer in the Botanical Garden of the School of Agronomy of La Plata, were dried and used to prepare the ethanolic tincture (T at 10% p/v) and to distillate the essential oil (EO). T was diluted to 0.002%, 0.02% and 0.2% in Krebs, while EO was diluted to 0.0001%. Isolated rat hearts were perfused with control Krebs (C) in the chamber of a flow-calorimeter up to stabilization of contractility and heat flow. They were exposed to diluted T or EO before applying a period of no-flow ischemia (I) and reperfusion (R with Krebs-C). Left ventricular pressure (LVP, from which the maximal developed pressure, P, was calculated during a contraction) and total heat rate (Ht) were continuously measured (3). Also, the muscle economy (P/Ht) was calculated. In some experiments, the drug clonazepam (Clzp) was perfused before the EO in order to block the mitochondrial Na/Ca exchanger by which Ca2+ is extruded to cytosol or to the sarcoplasmic reticulum (SR).

Results / Discussion

Both, T and EO reduced the inotropism before I. Then, only the EO slightly reduced the post-ischemic contractile recovery (PICR) which after 45 min R resulted 69.2±6.0% of initial P in the non-pretreated (C) hearts (n=7) and 47.3±3.5% in those treated with EO (n=5, $p<0.05$). 65.6±13.5%, n=8). T at 0.002% or at 0.2% did not significantly change PICR. Muscle economy (Eco= P/Ht) was strongly reduced by both T concentrations and by EO, before I and during R. At 45 minutes of R, Eco was 4.0±0.9 mmHg.g/mW in C-hearts and 2.5±0.2 mmHg.g/mW in hearts with EO, and 2.9±0.3 mmHg.g/mW and 2.2±0.4 mmHg.g/mW for hearts treated with 0.002% and 0.2% T, respectively. Also, T and EO induced an increase in the diastolic pressure (LVEDP) during I and R, which was higher in hearts pretreated with EO up to 66.5±7.8 mmHg at 45 minutes of R vs. 1.7±0.6 mmHg in C-hearts ($p<0.001$). To understand whether this contracture could be due to Ca2+ loss from mitochondria, hearts were pretreated with Clzp (to inhibit the mNCX) before perfusing with EO. After I/R hearts slightly improved the PICR and Eco but developed the same degree of diastolic contracture (ÄLVEDP of 70.0±5.4 mm Hg at 45 minutes R). These results suggest that mitochondria was not much altered by EO, since Clzp slightly increased [Ca2+]m (unpublished result) so that it improved the aerobic metabolism and ATP availability. Nevertheless, the effect on LVEDP was not improved by Clzp, suggesting that it is due to another organelle dysfunction. The strong diastolic contracture with reduced contractility and economy resemble the effects of caffeine in the heart. These caffeine-like effects may be associated to permeabilization of the SR by activation of Ca2+ release to cytosol, with the consequent energetic consumption to remove it by Ca-ATPases and Na/Ca exchanger.

Conclusion

The EO of Blepharocalyx salicifolius is not cardioprotective in this model of angor with stunning because it induces diastolic contracture by a caffeine-like effect, but Clzp could partially improved it.

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PO89
ANTISPASMODIC EFFECTS OF TWO ARGENTINIAN PLANTS: *Fuchsia magellanica* AND *Blepharocalyx salicifolius*

Bernal-Ochoa, A. M.[1,2]; Jimenez-Hernandez, J.[1,2]; Colareda, G. A.[1]; Consolini, A. E.[1]*
[1]*National University of La Plata (UNLP), School of Exact Sciences, Pharmacology, 47 and 115 street, La Plata, Argentina.*
[2]*Studenti della Maestria in Pianti Medicinali UNLP, La Plata, Argentina*
**dinamia@biol.unlp.edu.ar*

Introduction
These plants are used in Argentina to relieve certain spasms of smooth muscle. Fuchsia magellanica Lam. (Onagraceae) grows in the cold forest of Patagonic Andes, it is known as "chilco" and traditionally used by mapuche community to alleviate uterine spasms. It was also domesticated in the temperate Pampa region of Buenos Aires (1). Its pharmacological activities never have been studied. Blepharocalyx salicifolius Kunth O. Berg (Myrtaceae) grows near the costs of Rio de la Plata, it is known as "anacahuita" and is used to alleviate cough, broncospasm and diarrhea, in part due to its content in 1,8-cineol (2, 3). The aim of this work was to evaluate whether these plants have antispasmodic effect in the intestinal smooth muscle, and compare the effects of "chilco" from different regions and those of the tincture and essential oil of "anacahuita".

Method
Leaves of both plants were collected in the last summer and dried at air. Tinctures were prepared by maceration in ethanol 70° and dilluted in Tyrode the day of the experiment. Contractile concentration-response curves (CRC) of carbachol (Cbl, cholinergic agonist) were done in rat isolated ileon portions, in the absence and presence of: tincture of Fuchsia magellanica from Patagonia (T-F.m.P) or from Buenos Aires (T-F.m.BA), and tincture of Blepharocalyx salicifolius (T-B.s) or its essential oil (EO-B.s). Contractility was measured by a force transducer and acquisted on a computer. The T-F.m.P was also evaluated on CRC of calcium (Ca2+) in a high [K+] media (4).

Results
Both, T-F.m.P (yield 29.4%) and T-F.m.BA (yield 19.6%), induced a non-competitive inhibition of the Cbl-CRC, as well as verapamil, because they reduced the maximal effect of the agonist with IC50 of about 106 μg/mL T-F.m.P (n= 4) and 321 μg/mL T-F.m.BA (n= 4). Also, T-F.m.P non-competitively blocked the Ca2+ CRC with IC50 of 69 μg/mL (n=5), as well as verapamil (IC50: 240 40 μg/mL, pIC50: 6.28). The T-B.s. (yield 26.3%) also inhibited the Cbl-CRC in a non-competitive way, with IC50 of 17048 μg/mL T-B.s (n=6), while the EO-B.s (specific weight: 0.9019 g/mL) inhibited the Cbl-CRC with IC50 of 5.921.61 μg/mL (n=6).

Discussion / Conclusion
Both medicinal plants are good intestinal antispasmodics. The tincture of Fuchsia magellanica from Patagonia was a few more potent than that from Buenos Aires, and also it was demonstrated that its relaxant effect was due to inhibition of Ca2+ availability in the smooth muscle, a behavior similar to that of verapamil. The essential oil from the leaves of Blepharocalyx salicifolius was an antispasmodic more potent than the tincture, as it was expected from the purity of the EO, in which terpenes reach high concentration.

Acknowledgements: grants from National University of La Plata (UNLP 11X-642)

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PO90
(-)-DEOXIPODOFILOTOXINA, ISOLATED FROM STEMS OF *Bursera discolor, Bursera aptera* AND *Bursera fagaroides* VAR. *Purpusii*

PhD Juan D. Hernández-Hernández, QFB Angel Abad del Río-Chávez,
QFB Antonio Rodríguez-Luna and PhD Luisa Urania Román-Marín
Instituto de Investigaciones Químico-Biológicas de la UMSNH, Ed. B-1, Cd. Universitaria, 58030, Morelia, Michoacán, México;
**jdiego@umich.mx of the Author lroman@umich.mx*

Introduction
Species of Burseraceae Bursera section presents defoliation in its bark, are ecologically important in regions of Guerrero, Michoacan and Oaxaca, which recorded the highest rates endemism and biodiversity; exhudados bark and are used for respiratory conditions such as curing and healing gums. All three species have in common title defoliation of its bark and hexane extracts of the stems, isolated the (-)-deoxipodofilotoxina, lignan obtained from several species of yellow "cuajiotes", identification was made by comparison of their PMR spectroscopic data and RMC-13 in 1 and 2D.

Method
The collection of the three species was conducted during the months of March to June, fresh stems separately underwent hexánicas maceration for five days, an aliquot of the concentrate was subjected to chromatographic separation using silica gel-alumina as support and was eluted with mixtures of ascending polarity from hexane to AcOEt. Polarity in methylene chloride to separate the three isolated burseras the (-)-deoxipodofilotoxina, which was purified by successive.
Title of the paper (14 points, bolded) is followed by one blank line. The authors should be of 10 points plain font and presenting author's name should be underlined. Author's affiliations (9 points, italic, centered) and email address of the contact person are written in separate rows.

Results
Chromatographic separation of the hexane extracts separately fresh three Burseras stems, showed that the polarity of methylene chloride was isolated the (-)-Deoxipodofilotoxina. This metabolite was identified by its spectroscopic data of 1H and 13C in 1 and 2D and compared our data from other species besides those reported in the literature.

Conclusion
Genus our systematic study, showed the presence of metabolite lignánico -(-)-deoxipodofilotoxina, similar to those obtained from other species defoliating Burseras structure.

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PO91
COMPOSITION OF ESSENTIAL OIL OF IN THE AERIAL PARTS OF *E. Pubigerum* (DC.) MOREN & DECAISNE (BERBERIDACEAE) GROWING IN TURKEY

Cicek Polat, D.[1]*, Aslan Erdem, S.[2], Coskun, M.[3]
[1]*Research Assist., Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoğan, Ankara, TURKEY*
[2] *Assist. Prof. Dr., Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandoğan, Ankara, TURKEY*
[3]*Prof., Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoğan, Ankara, TURKEY*
**polatd@ankara.edu.tr*

Introduction

Epimedium L. (Berberidaceae) is a genus of approximately 52 taxa. A large part of this genus is endemic and found in the southwestern part of China. Epimedium spp. have been used to treat sexual dysfunction, prospermia, hyperdiuresis, osteoporosis, menopause syndrome, rheumatic arthritis, hypertension, and cardiovascular disease (Huiping *et al.*, 2011). Epimedium species are known as "Keşişkülahı" and "Tekeotu" in Turkey (Güner, 2012). Essential oils obtained from leaves of Epimedium have been studied before (Zhang *et al.*, 2013), however essential oil of the aerial parts of E. pubigerum (DC.) Moren & Decaisne growing in Turkey has not been studied.

Method

Aerial parts of E. pubigerum were collected in April 2013 from Uzungöl, Trabzon, were air-dried, essential oil of the aerial parts was obtained by hydrodistillation and analyzed by GC and GC-MS.

Results / Discussion / Conclusion

Main components of the essential oil were found to be rich in hydrocarbons; such as palmitic acid, myristic acid and lauric acid.

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PO92
PHOTOCATALYTIC DEGRADATION OF CIPROFLOXACIN

Ruth Meléndrez L.[1], Fernando Hernandez A.[1], Blanca M. Cabrera V.[1], Albino Moreno[1], Marcial Zamora T.[1], Lina Tlalmis L.[1], Juan C. Ramirez G.

[1]PhD/Facultad de Ciencias Químicas, Edificio 179, 18 Sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México. ruthmelendrez@gmail.com

Introduction

Drugs are one of the new contaminants responsible for severe enviromental damage and water contamination1. Heterogeneous photocatalysis is a key technology for mineralization of organic substances due to its strong oxidative properties. It uses photosensitizers like phthalocyanines, porphyrins and semiconductors2. Oxidation-reduction reactions take place at the semiconductor when a photon with high enough energy hits the surface. One of the most used semiconductors is TiO23. The properties and photocatalytic activity of TiO2 within the visible spectrum can be improved by metal doping. Thus, TiO2 metal doping has been subject of intensive research. In this work, TiO2 metal-doped materials were synthesised by sol-gel method4,5. The metals used for doping were Li, Na, K and Rb. The advantage of using the sol-gel method is that the metal ions are introduced before the formation of the material so they are better distributed compared to other techniques. The amount of dopant introduced was varied in order to study the impact on the photocatalytic activity. The catalytic activity was tested using low intensity UV light, H2O2 and ozone to degrade ciprofloxacino in water. H2O2 and ozone generate hydroxyl radicals, which accelerate the degradation of the antibiotic. The solids were characterized by nitrogen physisorption and x-ray.

Method

The materials TiO2 and TiO2-Mx (Mx=Li, Na, K, Rb at 1% and 3%) were synthesised by sol-gel using deionised water in n-butanol and changing the concentration of nitric acid to set the pH at 3. The salts were prepared with the corresponding concentration of metal and kept under reflux agitation. Then titanium n-butoxide is added drop by drop for 4 hrs. with a reflux time of 24h until the gel is formed. The gel is then dried at 90 °C for 24h. Sinterization is performed at 550 °C for 8 h at a speed of 2°C/min. The structure of the material was determined using x-ray. The porosity and specific superficial area was determined by adsorption and desorption of nitrogen. The catalytic activity was determined based on the degradation of ciprofloxacino inside a Batch photoreactor with radiation between 200 and 500nm. The final analysis was performed by UV-visible spectroscopy.

Results / Discussion / Conclusion

1. The photocatalysers TiO2 and TiO2-Mx (Mx=Li, Na, K, Rb at 1% and 3%) were obtained by sol-gel. 2. Textural analysis shows the solids are mesoporous. 3. The presence of metal ions Li, Na, K, Rb was confirmed by x-ray in all samples as well as the anatase structure. 4. In the photocatalytic degradation of ciprofloxacino, it was determined the solids doped at 1% were more efficient.

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PO93
ESSENTIAL OILCOMPOSITION OF THE FRUITS OF *Ferulago pauciradiata* BOISS. & HELDR. (APIACEAE) GROWING IN TURKEY

Delimustafaoğlu Bostanlık, F. G.[1]; Karakaya, S.[1]; Kılıç, C. S.[1]*; Demirci B.[2]; Coşkun, M.[1]
[1]*Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey*
[2]*Anadolu University/Faculty of Pharmacy/Dept. Pharmacognosy, 26470, Eski°ehir, Turkey*
**erdurak@pharmacy.ankara.edu.tr*

Introduction

Ferulago W. Koch. is a perennial genus of the Apiaceae family represented by nearly forty species, thirty-two of them growing naturally in Turkey, seventeen being endemic. This suggests that the gene centre for this genus is Anatolia (Kılıç, 2010). The genus is rich in essential oil and essential oils of several species have been studied. However, since only the composition of the fixed oil of the species was examined before (Bağcı, 2007), we decided to examine the composition of the essential oil of the fruits of F. pauciradiata in order to contribute to the literature on Ferulago spp.

Method

Air dried and crushed fruits (64 g) were subjected hydrodistillation for 3 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia to yield 2 ml essential oil (3.125%). GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. An Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1, injector temperature was set at 250°C. Mass spectra were recorded at 70 eV (mass range was from m/z 35 to 450). GC analysis: GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. To obtain the same elution order with GC/MS, simultaneous autoinjection was used on a duplicate column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Results / Discussion / Conclusion

Major components of the essential oil were found to be trans-chrysanthenyl acetate (25.2%), alpha-pinene (23.4%), 2,3,6-trimethyl benzaldehyde (21.1%), sabinene (9.5%) and myrcene (6.0%). The presence of trans-chrysanthenyl acetate might be an interesting finding since it is not generally found in Ferulago spp. One literature specifies that it was reported to be present in F. angulata growing in Iran; however the authors could not find this component in their study. Instead, cis- chrysanthenyl acetate is found in different Ferulago spp. in varying quantities (Sajjadi *et al.*, 2012; Cecchini *et al.*, 2010)

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PO94
IN VITRO ANTICANCER ACTIVITY OF *Heliotropium dolosum* DE NOT, *H. lasiocarpum* FISCH ET MEY AND *H. hirsutissimum* GRAUER (BORAGINACEAE) GROWING IN TURKEY AGAINST HUMAN PROSTATE CARCINOMA

Karakaya, S.[1]; Bakar, F.[2]; Kılıç, C.S.[1]*
[1]*Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey*
[2]*Ankara University/Faculty of Pharmacy/Dept. Biochemistry, 06100, Tandoğan, Ankara, Turkey*
**erdurak@pharmacy.ankara.edu.tr*

Introduction
Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues. Several clinically important anticancer drugs, such as paclitaxel, vinblastine, irinotecan, topotecan, etoposide and combretastatin, are derived from plants (Cragg and Newman, 1999; da Rocha *et al.*, 2001), and there are thousands of plant species that are yet to be examined in this respect. Heliotropium species are represented with 250-300 species in the world. Among these, 100 species are distributed in the Mediterranean and in temperate regions (Shah, 2012), and fourteen taxa grow naturally in Turkey. We aimed to examine the anti-cancer effects of Heliotropium species since they are traditionally being used against cancer in many countries (Koffluor et.al., 2012).

Method
In vitro cytotoxicity of ethanolic extracts of Heliotropium dolosum, H. hirsutissimum and H. lasiocarpum against human prostate-carcinoma (PC-3) cell line has been investigated using MTT assay. The cells were seeded at 5x104 cell/ml in DMEM:F12 medium and treated with different concentrations of ethanolic extracts for 48 hours. Results are expressed as percentage of live cells compared with untreated control. The data present the mean ± SD of four independent experiments.

Results / Discussion / Conclusion
H. dolosum decreased the proliferation of human prostate carcinoma (PC-3) cells. The percent of viable cells were 29.01±5.22% at 10mg/ml and 37.83±7.08% at 1mg/ml concentrations ($p<0.01$). H. lasiocarpum L. and H. hirsutissimum has also inhibited PC-3 cell proliferation, that the cell survival rate was 60.82±6.22% and 60.86±6.53%, respectively at 10 mg/ml concentration of ethanolic extracts.

Based on these results we can suggest that Heliotropium species may yield promising against some cancer line series. And we hope to identify its mechanism and the active substances responsible for this effect in our future studies.

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PO95
EFFECT OF LYOPHILIZED EXTRACTS OF *FERULAGO MUGHLAE* PEŞMEN (APIACEAE) ON ERECTILE DYSFUNCTION

Delimustafaoğlu Bostanlık, F. G.[1]; Karakaya, S.[1]; Başţaşkın, T.[2]; Önal, E.[2]; Kılıç, C. S.[1*]; Gür, S.[2]
[1]*Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey*
[2]*Ankara University/Faculty of Pharmacy/Dept. Pharmacology, 06100, Tandoğan, Ankara, Turkey*
**erdurak@pharmacy.ankara.edu.tr*

Introduction

Erectile dysfunction, the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance is an age-associated disease, with estimated prevalence rates of 39% among men 40 years of age and 67% among those 70 years old. Penile erection is a physiological event involving relaxation of smooth muscle of the corpus cavernosum. This relaxation process results in an increased flow of blood into the trabecular spaces of the corpora cavernosa and could further cause the erection of penis (Lue, 1983; Andersson and Wagner, 1995). *Ferulago* spp. are known with the name "Çakşır" in Turkey and are mostly well known for their aphrodisiac activities in Turkey like various plants in different countries (Ibrahim *et al.*, 2010). In our previous studies, we have found that water extract of *F. syriaca* roots produced relaxation in precontracted human corpus cavernosum (Ozturk, 2012), therefore we aimed to demonstrate the relaxant effect of the lyophilized water extract of *F. mughlae* Peşmen (Apiaceae) herba and roots, an endemic species for Turkey on erectile tissue.

Method

In order to measure isometric pressure, *in vitro* organ bath experiments were performed on male Sprague-Dawley rats. Corpus cavernosum was removed, put in a petri dish containing Krebs-bicarbonate solution and aerated continuously with 95% O_2 –5% CO_2. Cavernosal tissue strips (1x1x6 mm) were suspended into 20ml organ bath and mechanic activities were recorded with (COMMAT, Ankara, Turkey). Tissues were constricted with phenylephrine and relaxation responses related to acetylcholine (ACh, $10^{-'af8}$-$10^{-'af3}$M), SNP ($10^{-'af8}$-$10^{-'af3}$M) and electrical field stimulation (EFS, voltage 30 V, pulse duration 1ms, frequency 1-20 Hz) were obtained. Then dose-response curves were obtained for direct EFS (1-40 Hz) and phenylephrine ($10^{-'af8}$-$10^{-'af3}$M). All these concentration-response curves were repeated with lyophilized extract prepared from the herba and the roots.

Results / Discussion / Conclusion

It was found that lyophilized herba and root extracts yielded 59.375% and 97.8% relaxation, respectively. Therefore we can conclude that this species (especially the roots) can be used in erectile dysfunction and may represent a herbal alternative to synthetic drugs.

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PO96
PSILOCIN AND PSILOCIBIN CONTENT OF SOME HALLUCINOGENIC MUSHROOMS GROWING IN TURKEY

Kılıç, C. S.[1]*; Gümüştaş, M.[2]; Kaya, A.[3]; Solak, H.[4]; Işıloğlu, M.[4]; Gücin, F.[5]; Coşkun, M.[1]
[1]Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey
[2]Ankara University/Faculty of Pharmacy/Dept. Analytical Chemistry, 06100, Tandoğan, Ankara, Turkey
[3]Karamanoğlu Mehmetbey University / Kamil Özdağ Faculty of Science/Dept. Biology, 70100, Karaman, Turkey
[4]Muğla University/Faculty of Science and Arts / Dept. Biology, 48000 Kötekli, Muğla, Turkey
[5] Fatih University/Faculty of Science and Arts / Dept. Biology, 34500 Büyükçekmece, Istanbul, Turkey
*erdurak@pharmacy.ankara.edu.tr

Introduction

Psilocin and psilocybin are two hallucinogenic substances of tryptamine type, found naturally in some mushrooms. Turkey is known to be rich in mushroom species containing psilocin and psilocybin. In addition, they have been consumed as foodstuff in Anatolia. Therefore, mushrooms species containing these two hallucinogenic substances and their quantities should be determined. In recent years, usage of hallucinogenic mushrooms has increased in European countries and USA. But no case record has been found in Turkey. However, there are some criminal records in Gendarme and Police Narcotics archives. Therefore we aimed to examine some hallucinogenic mushroom species growing in the southern part of Turkey in respect to their psilocin and psilocybin contents.

Method

Lyophilized mushroom samples were grounded, 0,5-1 g were transferred into Erlenmeyer flasks and were subjected to maceration with 50-100 ml methanol for 6 hours; filtered through filter paper and the fluids were transferred into flasks. The filtrates were evaporated completely and then dissolved in HPLC grade methanol. Dissolved samples were filtered from filters with pore size of 0,45 μ, diluted in half with ultra pure water and readied for analysis with the addition of internal standard. HPLC conditions (Agilent 1100) were as follows: Mobile phase methanol: water (25:75 v/v) + 15 mM phosphoric acid, pH adjusted to 2.5 with NaOH; column temperature: 25°C; flow rate 0.7 ml/min; internal standard: 4-OH indole. Retention times of the compounds were determined to be 2.76, 4.30 and 6.36 min for psilocibin, psilocin and internal standard, respectively.

Results / Discussion / Conclusion

It was found that Turkey is actually rich in hallucinogenic mushrooms. Results of Psathyrella spp., Panaeolus spp. and Inocybe spp. were presented by our group previously (in ISOPS-10, ISE-13 and BIHAT 2012) and here, we are presenting the results of some species belonging to other genera along with the comparison to the literature (Koike *et al.*, 1981; Beug and Bigwood,1982; Ohenojaet al, 1987): Coprinellus, Coprinopsis, Marasmius, Conocybe and Stropharia species having psilocybin content within a range of 0-267.7mg/100g and psilocin content within a range of 7.9-54.63mg/100g).

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PO97
IN VITRO ANTIVIRAL ACTIVITY OF *Heliotropium dolosum* DE NOT, *H. lasiocarpum* FISCH ET MEY AND *H. hirsutissimum* GRAUER GROWING IN TURKEY

Karakaya, S.[1]; Yurdakök, B.[2]; Akkutay, Z.[3]; Aslan Erdem, S.[4]; Kılıç, C. S.[1]

[1]*Ankara University/Faculty of Pharmacy/Dept. Pharmaceutical Botany, 06100, Tandoğan, Ankara, Turkey*

[2]*Ankara University/Faculty of Veterinery Medicine, Dept. Pharmacology and Toxicology, Irfan Baştuğ Cad. 06110, Dışkapı, Ankara,Turkey*

[3]*Ankara University/Faculty of Veterinery Medicine, Dept. Virology, Irfan Bahtuğ Cad. 06110, Dışkapı, Ankara,Turke y 3Ankara University/Faculty of Pharmacy/Dept. Pharmacognosy, 06100, Tandoğan, Ankara, Turkey*

**ecz-songul@hotmail.com*

Introduction

Despite prophylactic treatment, high prevalence of viral diseases is still constant with no specific treatment and increasing resistant strains against antiviral agents in animal production. Alternative treatment methods including phytotherapy are recommended along with conventional treatment in production. Heliotropium species which are represented by fourteen taxa in the Turkish flora distributed in the arid zone parts contain pyrrolizidine alkaloids, and some are known to induce antiviral activity (Singh *et al.*, 2002)

Method

In the current study, we examined the cytotoxic and antiviral activities of the lyophilized extracts of Heliotropium dolosum, H. lasiocarpum and H. hirsutissimum against bovine herpes type 1 (BoHV-1) in Madin-Darby Bovine Kidney cell line (MDBK). Cytotoxic activity were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in MDBK cell lines at 15 doses varying 0.22-4000 μg/ml.

Results / Discussion / Conclusion

IC50 values for H. dolosum, H. lasiocarpum and H. hirsutissimum were found to be 430.35, 266.21, 534.97 μg/ml, respectively. Plant extracts at IC50/2, IC50 and 2XIC50 doses were administered against MDBK cell lines which were pre-incubated with BoHV-1. The preliminary findings showed good correlation with the increased dose of the plant extract and its potential antiviral activity; where the experiments are currently been repeated for lower multiplicity of infection (MOI) doses (< 0.1) of the virus. These results suggest that these Heliotropium species have a potential of being used as alternative antiviral agents and other species growing naturally in Turkey should also be examined.

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Read More: http://informahealthcare.com/doi/abs/10.1076/phbi.40.8.581.14659

PO98
MOLECULAR CHARACTERIZATION NATIVE *Pleurotus* STRAINS

Valencia, T. G.[1*]; Aguilar, D. L.[1]; Villanueva, A. R.[1]; Garín A. M. E.[2]
[1]*Profr, Unidad Profesional Interdisciplinaria de Biotecnología-IPN, Acueducto S/N Col. Ticomán, México D. F.*
[2]*PhD, Facultad de Estudios Superiores Iztacala-UNAM, Av. De los Barrios 1, Tlal. Edo.México*
**gvovaltor@yahoo.com.mx*

Introduction

The Cultivation of Edible mushrooms of genus *Pleurotus* has been widely developed in the world (Marquez *et al.*, 2012). These mushrooms are source of protein, minerals (Ca, P, Fe, K and Na) and vitamin C, B, thiamine, riboflavin, folic acid and niacin (Lopez *et al*, 2008; Patil *et al.*, 2010). Collection and characterization of native *Pleurotus* strains are important for commercial cultivation. Genetic analysis by specific conserved regions (ITS1 and ITS2) has enabled the molecular identification of *Pleurotus* strains.In this study were extracted genomic DNA from wild and commercial strains of *Pleurotus* from different states of the Mexican Republic, and fragments were amplified in ITS1 and ITS2 regions.

Method

Five*Pleurotus*strains collected in Oaxaca, San Luis Potosi, Hidalgo and Puebla, and three commercial strains were used. Genomic DNA extraction was performed according to protocol ChargeSwitch gDNA Plant Kit (Invitrogen). PCR amplification was performed using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The PCR products were purified using PCR Purification PureLink (Invitrogen). Sequencing was performed by Unit Synthesis and DNA Sequencing of Institute of Biotechnology UNAM. Sequences were aligned and analyzed by BioEdit Sequence Alignment Editor program and the Mega 6.6 software, determining consensus sequence by aligning two replicates per strain in directions 5'-3 'and 3'-5', and were compared with GenBank database of NCBI. Nucleotide differences between varieties and species within genus, and phylogenetic reconstructions were calculated based on the Neighbor-Joining method of MEGA 6.6 software.

Results / Discussion / Conclusion

The amplicons obtained from PCR using genomic DNA of strains RP, HTH, UTMB, UTMR, UAP9, IE201, TLA, TAM and molecular markers ITS1 and ITS4 are displayed as intense single bands between 700 to 800 bp in gel 1% agarose.Gardes and Bruns (1993), showed that among characteristics known in analysis of conserved regions in mushrooms, the complete sequences generated by amplification is in range of 600 to 800 bp, this is in accord with the markers through ITS1 and ITS4 regions of ITS1 and ITS2 of the DNA of native and commercial strains of *Pleurotus* amplified.The sequence analysis showed an homology of 98-99% of identity when was compared to Genbank being *Pleurotus djamur* species as identified for native strains. Phylogenetic analysis of Pleurotus strains was performed based on the sequence ITS1-5.8s - ITS2 giving a resulting dendrogram which showed 3 major clades, clade I represents to specie *Pleurotus ostreatus*, the second clade (II) represents to specie *Pleurotus djamur* and third clade as outgroup to *Fusarium phyllophium*.

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PO99
THE FOLK MEDICINAL PLANTS OF ALANYA (ANTALYA-TURKEY)

Tuzlacı, E.[1]; Bulut, G.[1]*; Çağlar, E.[2]
[1]*Prof. Dr., Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey*
[1]*PhD, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey*
[2]*Pharmacy student, Marmara University, Faculty of Pharmacy, Istanbul, Turkey*
**gizem.bulut@marmara.com.tr*

Introduction
This study was made to reveal the plants used as traditional folk medicine in Alanya (Antalya) situated in south of Turkey.

Method
The specimens of the plants used as folk remedies have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration, and the duration of treatment has been recorded. The ethnopharmacological information was obtained from the local people by personal interviews carried out face to face. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University.

Results
As a result of identification of the plant specimens, 53 species, used as a traditional folk medicine in Alanya, have been determined. According to the majority of the informants, the plants are mostly used for cold, stomach diseases, diabetes and rheumatism.

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PO100
PHENOLIC COMPOUNDS FROM *Clinopodium tomentosum* (KUNTH) GOVAERTS

Braca, A.[1]*; Vera Saltos, M. B.[2]; Naranjo Puente, B. F.[3]; Malafronte N.[4]; De Tommasi, N.[5]
[1]*Prof, Dipartimento di Farmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy*
[2]*PhD, Dipartimento di Farmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy*
[3]*Prof, Departamento de Ciencias de la Vida, Escuela Politecnica del Ejercito, Avenida El Progreso S/N, Sangolqui, Ecuador*
[4]*PhD, Dipartimento di Farmacia, Università di Salerno, via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy*
[5]*Prof, Dipartimento di Farmacia, Università di Salerno, via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy*
**alessandra.braca@farm.unipi.it*

Introduction

The genus Clinopodium (Lamiaceae) consists of flowering plants, wildly distributed in southern and southeastern Europe, in the United States of America and Mexico (Estrada-Reyes *et al.*, 2010). It is also found growing in Latin America between 3000 and 4000 m a.s.l. This wide distribution enables the use of these species as medicinal herbs. Clinopodium tomentosum (Kunth) Govaerts possesses small yellow-colored ÿ'3fowers, often reaches a height of 30–80 cm, and in Ecuador is commonly known by its vernacular name "Santa Maria". Local people use the aerial parts of the plant to prepare infusions for it relaxant effect and as antiinflammatory. Previous phytochemical studies on species of the Clinopodium genus have revealed the presence of ÿ'3favonoid glycosides, phenylpropanoids, caffeic acid oligomers, and saponins (Opalchenova and Obreshkova, 1999; Aoshima *et al.*, 2012; Murata *et al.*, 2009; Miyase and Matsushima, 1997). Despite its use in traditional medicine in Ecuador, to our knowledge, no data on the chemical composition or biological activity of the aerial parts of C. tomentosum are available.

Method

Aerial parts of C. tomentosum were collected in Tumbaco, Ecuador in September 2011. The dried and powdered plant material (560 g) was successively extracted for 48 h with n-hexane, CHCl3, CHCl3-MeOH (9:1) and MeOH, by exhaustive maceration (3 x 2 L), to give 7.6, 18.0, 8.5 and 13.1 g of the respective residues. The CHCl3-MeOH and MeOH extracts were separated through Sephadex LH-20 followed by RP-HPLC.

Results / Discussion / Conclusion

The phytochemical study of C. tomentosum aerial parts led to the isolation and structural characterization by spectroscopic and spectrometric methods of one new compound, named 2-O-benzoyl-3-O-cinnamoyl tartaric acid along with eleven known compounds. The new compound is an asymmetric tartaric acid; usually these derivatives are found rarely in nature, being isolated mostly from Echinacea genus (Soicke *et al.*, 1988; Lu *et al.*, 2012). The following known compounds were also identified: hesperitin, dihydrodehydroconiferyl alchol 9'-O-β-D-glucopyranoside, blumenol c glucoside, syringaresinol 4'-O-β-D-glucopiranoside, rosmarinic acid methyl ester, pinocembrin 7-rutinoside, clinopodic acid E, caffeic acid, caffeic acid methyl ester, caffeic acid ethyl ester, and p-coumaric acid.

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PO101
PHYTOCHEMICAL INVESTIGATION OF *Pseudolephantopus spiralis* (LESS.) CRONQUIST

Malafronte N.[1], Jimenez N.[2], Vassallo A.[3], Severino L.[4], Arango G.[2], De Tommasi N.[1]
[1]*Department of Pharmacia, University of Salerno, Fisciano, SA, Italy*
[2]*Universidad de Antioquia, Facultad de Química Farmacéutica, Grupo de Investigación en Sustancias Bioactivas, Cll 67 No 53-108 Medellin, Colombia*
[3]*Department of Science, University of Basilicata, Viale dell' Ateneo Lucano, 10- 85100 Potenza, Italia*
[4] *Department of Pathology and Animal Health, Division of Toxicology, School of Veterinary Medicine, University of Naples Federico II, Naples, NA, Italy.*
detommasi@unisa.it

Introduction

The genus Pseudoelephantopus (Asteraceae) consists of approximately three species native to temperate and tropical region of North and South America and distributed into the Lesser Antilles and moist uplands in the Galàpagos Islands1. Pseudolephantopus spiralis (Less.) Cronquist is erect perennial, stoloniferous herb, with leaves cauline, petioles indistinct blades oblanceolate to obovate, acute to obtuse at the apex, attenuate at the base. Some species belonging to this genus are used in folk medicine for the treatment of inflammatory condition such as edema, chest pain, nephritis, fever and coughing associated with pneumonia, scabies and anthralgia due to wounding2. In literature are reported the antinflammatory, antileishmanial2 and cytotoxic activities of different sesquiterpenes lactones isolated from the aerial parts of Pseudolephantopus spicatus3.

Materials and Methods

The dried aerial parts of Pseudolephantopus spiralis were subjected to extraction with methanol. Methanol extract was partitioned between n-butanol/water to afford a n-butanol soluble portion. The n-butanolic extract was carried to dry and then dissolved in chloroform. A portion of chloroform extract (5g) was subjected to different chromatographic techniques as Silica gel, MPLC and rp-HPLC. Methanol and aqueous extracts of Pseudolephantopus spiralis were also subjected to different biological tests: DPPH, Folin–Ciocalteu, TBARS, inhibition of nitric oxide production and citotoxicity through DOJINDO Cell Counting Kit-8, CCK-8.

Results and Discussion

The structures of isolated compounds were elucidated by 1D- and 2D-NMR Spectroscopy (1H, 13C, 13C DEPT, DQF-COSY, HSQC, HMBC, ROESY) and mass spectrometry studies The Pseudolephantopus spiralis chloroform extract phytochemical study allowed us to identify two new sequiterpenes lactones along with three known compounds 8a-acetoxy-10a-hydroxy-13-O-methylhirsutinolide4, spicatolide A4 and 8a,13-Diacetoxy-1a,10a,-1β,5β-diepoxygermacra-7(11)-en-12-olide5.

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PO102
POLYPHENOL PROFILE AS TRACEABILITY TOOL IN THE CHARACTERIZATION OF "LONG STORAGE" TOMATO GENOTYPES

Siracusa, L.[1]; Patanè, C.[2]; Ruberto, G.[1]
[1] *Istituto del CNR di Chimica Biomolecolare (ICB), Via Paolo Gaifami 18, 95126 Catania, Italy*
[2] *Istituto del CNR per la Valorizzazione del Legno e delle Specie Arboree (IVALSA), Via Paolo Gaifami 18, 95126 Catania,Italy*
laura.siracusa@icb.cnr.it

Introduction
The Mediterranean "long-storage" tomato, so-called for the textural properties of fruits that allow an extended shelf life after harvesting, provides a delightful product which combines a good taste with excellent nutritional properties. Due to the high drought tolerance of the plant, traditionally cultivated under no water supply, long-storage tomato may represent an interesting genetic source in breeding programs for water stress resistance in both fresh-market and processing tomatoes. Phenols have recently gained attention as effective chemotaxonomic markers, allowing to evidence even small differences within a wide number of wild and cultivated species; they are also used for classification purposes, including the determination of the origin area. We therefore decided to characterize a wide number of long storage tomato landrace through the analysis of their polyphenol profile, in search of evidences of peculiarity in this product.

Method
A total 28 long-storage tomatoes, belonging to the germplasm collection of the CNR-IVALSA of Catania (Italy), were investigated in this study. The landraces were recovered throughout Southern Italy. The commercial tomato 'Principe Borghese' (SAIS Sementi s.p.a., Cesena, Italy) was included in the study as control, being the sole long-storage tomato whose seeds are commercially available to farmers. The tomatoes were open-field cultivated in a flat site of eastern coast of Sicily, during the summer season of 2011. A fixed total volume of approximately 40 mm of water, split in two applications including transplanting, was applied. After that, irrigation was interrupted. High-performance liquid chromatography coupled with diode array detection and electron spray-mass spectrometry (HPLC/DAD/ESI-MS) was used to identify the phenolic profile in the landraces of long storage tomato. Sixteen different secondary metabolites, belonging to the classes of cinnamoylquinic acids and flavonoids, were identified. Quantitative analyses were also performed to monitor the changes in the phenolic content along the batch.

Results and Conclusion
The LC-MS analysis of the different long storage tomatoes showed the presence of chlorogenic acid (5-caffeolyquinic acid), rutin and quercetin in all samples; further studies on the data deriving from the MS allowed identifying 12 different molecules belonging to the class of cinnamoylquinic acids. Isomers of mono-, di- and tricaffeoylquinic acids, as well as coumaroylquinic and feruloylquinic acids, are already reported in literature to occur in tomato (1,2); nevertheless, the presence of some of these metabolites belonging to this class is a peculiarity of "long storage" tomato fruits. The results highlighted that landraces originating from the same area exhibit a different fruit morphology but own a similar biochemical profile, thus suggesting that these secondary metabolites in tomato fruits may be more genetics-dependent than environment-dependent (3).Given the analysis of phenols nowadays represents an useful tool to assess the genetic variability in tomato, these compounds could be adopted as chemotaxonomic markers in the traceability of this niche product.

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PO103
ANTI-INFLAMMATORY PROPERTIES OF DIHYDROSTILBENES FROM LIQUORICE LEAVES – *IN VITRO* ASSAYS AND DOCKING STUDIES OF SELECTIVE COX-2/COX-1 INHIBITION

Trombetta, D.[a]; Cristani, M.[a]; Tomaino, A.[a]; Saija, A.[a]; Giofrè, S.[a]; Romeo, R.[a]; Siracusa, L.[b]; Ruberto, G.[b]

[a]*Dipartimento Scienze del Farmaco e dei Prodotti per la Salute, Università di Messina, Contrada Annunziata, I-98168 Messina, Italy*
[b]*Istituto del C.N.R. di Chimica Biomolecolare, Via Paolo Gaifami, 18 I-95126 Catania, Italy*
laura.siracusa@icb.cnr.it

Introduction

Glycyrrhiza glabra L. (liquorice) roots are broadly known to posses a wide range of well documented therapeutic properties. Conversely, the aerial parts of this plant are scarcely used and considered an agrochemical waste. Nevertheless, the few phytochemical investigations on G. glabra leaves have shown the presence of some phenolic compounds which are scarcely or not present in the roots. Previous investigations of the lipid extract of Sicilian G. glabra leaves allowed the isolation of various known flavonoids and nine novel dihydrostilbenes, which have been considered responsible for the antioxidant, antigenotoxic and anti-inflammatory activities of the extract 1 In order to ameliorate our knowledge about the bio-properties of the dihydrostilbenes found in the Sicilian G. glabra leaves, three of them were in vitro tested to evaluate their capability to scavenge the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and to act as anti-inflammatory agents by inhibiting cyclo-oxigenase (COX) pathway. On the basis of the observed data, molecular docking study was carried out in order to understand in detail the ability of these compounds to bind COX-1 and COX-2.

Method

Besides to test the antioxidant properties of some of the liquorice dihydrostilbenes by evaluating their capability to scavenge the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), we characterized the anti-inflammatory properties of these compounds using a human whole blood assay to assess their capability to act as in vitro selective inhibitors of COX-1 or COX-2 pathways, by decreasing release of thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) respectively.

Results / Discussion / Conclusion

All the three stilbenoids under study appeared lacking of a significant capability to inhibit TxB2 release. Conversely they showed a good dose-dependent capability to inhibit PGE2 release, so suggesting a selective action of these molecules on the COX-2 pathway. Regarding the molecular docking study, in the complex formation with COX-1, the hydrogen bonding interaction with Ser530 is absent in two of the three studied compounds, which rationalizes the low activity of these molecules towards COX-1 inhibition observed in ex vivo experiments. On the other hand, the scoring function of the same compounds complexes with COX-2 confirms that both of them are preferred ligands for COX-2 rather than for COX-1. The absence of the isopentenyl group in the third molecule analysed leads to the loss of the hydrophobic interactions, essential for the anchoring of the compound in the enzyme active site.The results show that the liquorice dihydrostilbenes are preferred ligands for COX-2 rather than for COX-1, providing a good rational for the observed selectivity in ex vivo experiments. Therefore, they appear to be good candidates for employment in human therapy against inflammation-related pathological conditions.

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PO104
FATTY ACIDS PROFILE OF WILD BOAR FAT USED FOR TRADITIONAL HERBAL SALVES IN MORAVIAN SLOVAKIA

Svobodova, B.[1]*; Rak, J.[2]; Kuban, V.[3]; Kasparkova, V.[3]; Cubon, J.[4];
[1]*PhD, Faculty of Technology, Tomas Bata University in Zlin, T. G. Masaryka 275, 762 72, Zlin, Czech Republic*
[2]*PhD, Faculty of Logistics and Crisis Management, Tomas Bata University in Zlin, Studentske nam. 1532, 686 01 Uherske Hradiste, Czech Republic*
[3]*Prof, Faculty of Technology, Tomas Bata University in Zlin, T. G. Masaryka 275, 762 72, Zlin, Czech Republic*
[4]*Prof, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976, Nitra, Slovakia*
**bsvobodova@ft.utb.cz*

Introduction

Herbal salves made of wild boar fat are traditionally prepared in folk medicine among the region of Moravian Slovakia (Moravské Slovácko) in the southeastern part of the Czech Republic. The fat has unique properties and is widely used as salve base in combination of various herbs to treat dermal problems (such as atopic eczema, wounds, psoriasis) or arthritis.

Method

Salve bases were prepared from subcutaneous or visceral fat tissue of wild boar (*Sus scrofa*) using traditional slow melting on boiling water basin. Fatty acid composition in salve samples was studied with the emphasis on the proportional abundance of saturated and unsaturated fatty acids. Commercial visceral pork lard and shea butter were used for comparison. All samples undertook transesterification of fatty acids and then were measured by GC/FID method (Shantha, 1992).

Results / Discussion / Conclusion

The most abundant fatty acids in wild boar salve base were the palmitic, stearic, oleic and linoleic acids. Despite the content of monounsaturated fatty acids (MUFA) was comparable, around 40 % in all samples, the main difference lied in the content of polyunsaturated fatty acids (PUFA). Whilst the pork lard and shea butter had less than 10 % of PUFA, the visceral boar lard contained twice as much.

As expected, the saturated fatty acids (SFA) represented more than 50 % of total fatty acids in visceral pork lard, which proves it the most consistent fat from all the samples. On the other hand, the unsaturated fatty acids (MUFA + PUFA = 62 %) exceed the ratio of SFA (38 %) in visceral wild boar lard, which represents the most spreadable fat at room temperature. These results might explain the mild texture of wild boar salve and its easier transdermal absorption as a carrier of active compounds from the herbal ingredients.

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PO105
ANTIMICROBIAL ACTIVITY OF SEVEN MEDICINAL PLANTS FROM TEHUACÁN-CUICATLÁN VALLEY IN PUEBLA, MÉXICO

Claudia Tzasna Hernández Delgado[1], Samuel Meraz Martínez[1], Marisol Avila Romero[1], Rocío Serrano Parrales[1], Ana María García Bores[2], José Guillermo Avila Acevedo[2]*

[1]Laboratorio de Farmacognosia, [2]Laboratorio de Fitoquímica, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Autónoma de México, Av. de los Barrios # 1, Los Reyes Iztacala, Tlalnepantla Estado de México. México.

**E-mail: tuncomaclovio2000@yahoo.com.mx*

Introduction

The medicinal plants have been an alternative for health since prehispanic times. The valley of Tehuacán-Cuicatlán in Puebla, México is a worldwide center of mega diversity and endemism decreed by the International Union for Conservation of Nature. In the valley there is a long tradition of using the medicinal plants identified more than 90 species used in traditional medicine. Many of them have not been studied. Therefore, the aim of this investigation is to scientifically validate the traditional use of 7 species that are used for the treatment of illnesses of infectious origin and study the space-time variations in the chemical composition and biological activities. The species were Lippia graveolens, Lantana achiranthyfolia, L. camara, Cordia curassavica, C. globosa, Caesalpinia melanadenia, Gymnolaena oaxacana. The essential oils were obtained by steam distillation an analyzed by gas chromatography coupled to mass spectrometry. The extracts were obtained by maceration. The antimicrobial activity was evaluated qualitatively and quantitatively against 20 strains of microorganisms (bacteria, yeast and micelial fungi). The effect of oil and/ or extracts on the growth and viability of microorganisms were determined. All the studied plants exhibit antimicrobial activity and their chemical composition varies according to the season of collection. The use of medicinal plants in the treatment of infectious diseases by the habitants from Tehuacán-Cuicatlán in Puebla, México has a phytochemical basis.

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PO106
CHARACTERIZATION AND HYPERICINS CONTENT IN SOME *Hypericum* SPECIES FROM SICILY

Lazzara, S.[1]; Carrubba, A.[2]*; Napoli, E.[3]
[1]dr, CRA-SFM, Bagheria (PA), Italy
[2]Prof, D/SAF, Università di Palermo, Palermo, Italy
[3]dr, ICB-CNR, Catania, Italy
*alessandra.carrubba@unipa.it

Introduction

Different species belonging to the genus Hypericum are distributed into many environments of Sicily, where they represent an important component of wild Sicilian flora (Giardina *et al.*, 2007). Among these, H. perforatum (St John's Wort) is certainly the most common and famous; its floral parts are largely and traditionally used as a folk herbal remedy for treatment of wounds and burns, and considered an important raw matter for pharmaceutical industry due to their acknowledged antidepressant and sedative properties. Although it is not completely clear yet which compounds are responsible for the biological activity of Hypericum, the European Pharmacopoeia takes as a reference index for evaluating the quality of the drugs, the total content in naphthodianthrones, i.e., in hypericins (hypericin and pseudohypericin) on total dry extract. The concentration of hypericins in sprout and flowers may range from 0,06% and 0,75%, but for market quality a minimum hypericin amount of 0,04% is required (Wagner and Bladt,1994). Other Hypericum species besides H. perforatum contain appreciable hypericins amounts, and therefore could represent alternative raw matter sources for industry.

Method

In 2012 and 2013 a survey activity was performed in Sicily, collecting samples of Hypericum spp. in various areas and at different altitudes. The inflorescences were air-dried, and 5 g approx of the material was ground and treated with ethanol (50 ml) for extraction, being continuously shakered for 72 hh in the dark. The extract was filtered and the filter was washed thrice with 10 ml ethanol each, thereafter it was dried with a rotavapor. In this way, the yield in percent of each sample was calculated. Later on, the HPLC analyses were carried on, in triplicate, by injecting 20 μL of a 10 mg/mL solution in methanol for each extract.
The determination of the content in active substances (hypericin, hyperforin and pseudohypericin) was carried on by means of HPLC diode array analysis. The identification of hypericin was performed by comparison of the chromatographic behavior of the samples with that of solutions of standards (hypericin, pseudohypericin and iperforin) with a known concentration.

Results / Discussion / Conclusion

Species	Provenance	EXTR (%)	HYPC (mg kg^{-1})	HYPF (mg kg^{-1})	PSPC (mg kg^{-1})
H. hirsutum	Sicily	11,7	23,7	0,1	15,8
H. perfoliatum	Sicily	13,8	198,2	0,2	564,2
H. tetrapterum	N-C Italy	20,7	844,6	1,9	583,9
	Sicily	18,5	540,5	1,0	431,9
	Mean	19,6	692,6	1,4	507,9
H. perforatum	N-C Italy	16,6	301,6	1,2	227,1
	Sicily	17,6	345,2	1,1	265,2
	Mean	17,3	330,7	1,1	252,5
EXTR: extract yield (%); HYPC: hypericin d.m. (mg kg^{-1}); HYPF: hyperforin d.m. (mg kg^{-1}) ; PSPC: pseudohypericin d.m. (mg kg-1)					

Exception made for *H. hirsutum*, that expressed very low values, all the other examined species have shown interesting hypericins contents. *H. perforatum* from Sicily exhibited higher values than the other provenances.

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PO107
CYTOTOXIC ACTIVITY OF CRUDE EXTRACTS FROM *Annona muricata, Annona cherimola* Y *Physalis peruviana* ON BREAST CANCER CELL LINE MCF-7

Ardila, D.[1]; Vera, R.[2]; Mendez, G. M.[3*]; Robles, J. E.[2*];
[1]*Research Student,Faculty of Microbiology, Pontificia Universidad Javeriana, AV 7 No. 40-62, Bogotá, Colombia*
[3]*Research Professor, Faculty of Medicine, Universidad de Ciencias Aplicadas y Ambientales UDCA, 222 Street No.55-34 Bogotá, Colombia*

Introduction

The annonaceous acetogenins are promising antineoplastic agents that are found in the plant family Annonaceae (Hwan D, *et al.*, 2001). Some compounds have been isolated from the species *Annona muricata* and *Annona cherimola*, and according with taxonomic and chemical studies, these compounds could be have a high antineoplastic potential (Torres M, *et al.*, 2012).
*Physalis peruviana,*a member of the plant family Solanaceae have active compounds against inflammation, bacterial and fungal infections and tumor disease, among them withanolids (Fang, S *et al.* 2012)
The aim of this study was evaluated the cytotoxic activity of crude extracts from *Annona muricata, Annona cherimola* and *Physalis peruviana* on MCF-7 breast cáncer cells and to identify the secondary metabolites in the cytotoxic extracts.

Method

The plants *A. cherimola, A. muricata* and *P. peruviana* were collected at Urabá, Antioquia, were authenticated and were evaluated for cytotoxic activity using MTT assay, against MCF-7 and the healthy cells HEK293. Cells were treated at different concentrations of the crude extracts from seeds and leaves of*A. cherimola,* seedes of*A. muricata* and leaves and stems of *Physalis peruviana.* Qualitative assays were done to identify secondary metabolites in the cytotoxic extracts. Statistical analyses ANOVA test and the post-hoc Tukey's test were done by using of the Statistix 10 program

Results / Discussion / Conclusion

Ethanolic extracts from all species showed a high cytotoxic activity on MCF-7 but not on HEK 293 cell lines. However leaves and stems from Physalis peruviana had the most potent effect on viability of cancer cells MCF-7 with a IC50=8 µg/mL. Etanolic extract from seeds of Annona muricata was also powerful according with the IC50= 9 µg/mL, while IC50 obtained for extracts from seeds and leaves of Annona cherimola were higher than 11 µg/mL.

The secondary metabolites identified in the ethanolic extracts correspond to flavonoids, phenols, tannins, glycosides, alkaloids, saponins, steroids, sterols, terpenes and sesquiterpene lactones. The results of this study provide preliminary information about three plants of common use between Colombian populations, with a promising antineoplastic activity on breast cancer disease.

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PO108
ANTIOXIDANT, ANTIMICROBIAL AND CYTOTOXIC PROPERTIES OF *Naucleopsis glabra* (MORACEAE) FROM PERU

Svobodova, B.[1]*; Rak, J.[2]; Kuban, V.[3]; Kokoska, L.[4]; Leuner, O.[5];
[1]*PhD, Faculty of Technology, Tomas Bata University in Zlin, T. G. Masaryka 275, 762 72, Zlin, Czech Republic*
[2]*PhD, Faculty of Logistics and Crisis Management, Tomas Bata University in Zlin, Studentske nam. 1532, 686 01 Uherske Hradiste, Czech Republic*
[3]*Prof, Faculty of Technology, Tomas Bata University in Zlin, T. G. Masaryka 275, 762 72, Zlin, Czech Republic*
[4]*Prof, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6 – Suchdol, Czech Republic*
[5]*PhD, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6 – Suchdol, Czech Republic*
**bsvobodova@ft.utb.cz*

Introduction

Naucleopsis glabra Spruce ex. Pittier (Moraceae) is a medicinal plant used by the Shipibo-Conibo ethnic group in Peruvian Amazon to treat rheumatism, anemia, dyspepsia and gastric ulcers and wounds (Arevalo, 1994). Potential antimicrobial and antioxidant activity was previously described for 80% ethanolic extract of stem bark. Therefore, the toxicity of this extract and further fractionisation was performed.

Method

The dry 80% EtOH extract of stem bark was further fractionised by Soxhlet apparatus using solvents of various polarity (n-hexan, CH_2Cl_2, EtOAc, n-BuOH and H_2O). The antioxidant effect of the fractions was assessed in vitro using DPPH free radical scavenging assay in multiwell-plates (Fukumoto, 2000). Antimicrobial activity was tested on *Staphylococcus epidermidis* ATCC 12228 using broth microdilution method (Jorgensen, 1999). The extracts were screened for possible cytotoxic activity by brine shrimp (*Artemia salina* L.) lethality assay. All tests were done in triplicates and with standard negative and positive controls.

Results / Discussion / Conclusion

The antioxidant activity assay revealed that the EtOAc fraction has the most powerful effect on the DPPH radical scavenging with EC50 4.25 ± 0.31 μg/mL of dry matter (DM). The strongest antimicrobial activity was also performed by the EtOAc fraction with MIC = 62.5 ± 2.1 μg/mL DM. No toxicity on *Artemia salina* was exhibited in the tests up to concentration of 1000 μg/mL DM. The brine shrimp lethality assay correlates in most cases reasonably well with cytotoxic properties of plant extracts and therefore it could be considered as relatively non-toxic. These findings suggest that this plant remedy could serve as an important natural source of safe antioxidants and antimicrobials for use in the food and cosmetic industry.

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PO109
POTENTIAL ANTICANCER ACTIVITY OF LICHEN SECONDARY METABOLITE PHYSODIC ACID

Cardile, V.[1]; Graziano, A. C. E.[1]; Avola, R.[1]; Piovano, M.[2]; Russo, A.[3]*
[1]*Department of Bio-medical Sciences, University of Catania, V.le A. Doria 6, 95125 Catania, Italy*
[2]*Department of Chemistry, University T.F. Santa Maria, Casilla 110-V, Valparaiso, Chile.*
[3]*Department of Drug Sciences, Biochemistry Section, University of Catania, V.le A. Doria 6, 95125, Catania, Italy*
**alrusso@unict.it*

Introduction

Lichens and their metabolites have long been used by humans. Throughout the ages lichen extracts have been used for various purposes, in particular as dyes, perfumes and for various remedies in folk medicine since ancient Egyptian times. Chemical studies on the secondary metabolites present in lichens have led to the isolation of many new substances, which by today number over 800 [1]. These compounds, which comprise aliphatic, cycloaliphatic, aromatic, and terpenic compounds, are unique with respect to those of higher plants and show interesting biological and pharmacological activities [1]. Several well characterized depsidones and depsides exhibit anti-inflammatory, analgesic, antipyretic, antibacterial, antifungal and anticancer properties [1, 2]. However, only a few of these compounds have been assessed for their effectiveness against various in vitro cancer models. In the present study, we investigated the cytotoxicity of three lichen secondary metabolites (atranorin, gyrophoric acid and physodic acid) on A375 melanoma cancer cell line.

Method

The tested compounds arise from diverse lichen species collected in different localities of Continental and Antarctic Chile. The cells were treated with different concentrations (6.25–50 μM) of lichen compounds and incubated for 72 h. The cell viability was measured using MTT assay. LDH release, a marker of membrane breakdown, was also measured. For the detection of apoptosis, the evaluation of DNA fragmentation (COMET assay) and caspase-3 activity assay were employed. The expression of Bcl-2 and Bax proteins was also detected by western blot analysis. Generation of reactive oxygen species was measured by using a fluorescent probe [1, 2].

Results / Discussion / Conclusion

The results obtained confirm the major efficiency of depsidones [2]. In fact, it was observed that depsides atranorin and gyrophoric acid showed a lower activity inhibiting the melanoma cancer cells only at more high concentrations. Whereas the depsidone physodic acid showed a dose–response relationship in the range of 6.25–50 μM concentrations in A375 cells, activating an apoptotic process. The growth inhibition effect exhibited by this compound appears to be correlated with a modulation of redox-sensitive mechanisms. Although the molecular mechanism by which apoptosis is induced by physodic acid remains unclear, and of course further studies are needed, the results reported here confirm the promising biological properties of depsidone compounds, and may offer a further impulse to the development of analogues with more potent efficacy against melanoma cells.

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PO110
CYTOTOXIC EFFECT OF THREE *Centaurea* SPECIES COLLECTED FROM CENTRAL ANATOLIA REGION OF TURKEY ON HUMAN MELANOMA CELLS: PILOT STUDY

Russo, A.[1]*; Cardile, V.[2]; Graziano, A. C. E.[2]; Rigano, D.[3]; Formisano, C.[3]; Zengin, G.[4]; Senatore, F.[3]

[1] *Department of Drug Sciences, Biochemistry Section, University of Catania, V.le A. Doria 6, 95125, Catania, Italy*
[2]*Department of Bio-medical Sciences, University of Catania, V.le A. Doria 6, 95125 Catania, Italy*
[3]*Department of Pharmacy, University of Naples Federico II, Via D. Montesano, 49, I-80131 Naples, Italy*
4Selcuk University, Science Faculty, Department of Biology, Konya, Turkey
**alrusso@unict.it*

Introduction

Melanoma is one of the most invasive and deadly forms of skin cancer. Its incidence continues to increase at an alarming rate and contrary to other neoplasms, a relatively younger population is becoming affected by this tumor. Apoptosis represents an efficient and physiological strategy through which the organism eliminates neoplastic cells; however, melanoma cells, both *in vivo* and *in vitro*, are quite refractory to apoptosis [1]. Therefore, the agents that induce apoptotic death of melanoma cancer cells could be useful in controlling this malignancy. Centaurea is the largest genus within the Asteraceae family. Many members of this genus, such as Centaurea pulchella, are used in traditional folk medicine. Although biological activities of many Centaurea species have been investigated in different countries and Turkey [1], cytotoxic effect of C. patula, C. pulchella and C. tchihatcheffii has not been studied yet. Therefore, based on the above rationales and observations, in an ongoing effort to identify new natural anticancer products for the treatment and/or prevention of melanoma cancer, the present study was undertaken to investigate the effect of these Centaurea species, collected from Central Anatolia region of Turkey on cell growth and death in human melanoma cell line, A375.

Method

Centaurea species (Centaurea patula DC., Centaurea pulchella Ledeb., Centaurea tchihatcheffii Fisch. & Mey.) were collected in May and June 2009 from Konya and Golbası (Ankara), Turkey. The plants have been identified by Dr. Tuna UYSAL from Section of Botany, Department of Biology, Faculty of Science, Selcuk University. The voucher specimens have been deposited in KNYA herbarium at Department of Biology, Selcuk University. One of Centaurea species collected is endemic (C. tchihatcheffii) to Turkish flora. The biological activity of the methanolic extracts from aerial plant parts, containing phenolic compounds, was investigated against human melanoma cancer cells (A375), testing several biochemical parameters [3], such as cell vitality (MTT assay), cell membrane integrity (lactate dehydrogenase release) and caspase-3 activity. In addition, the expression of Bcl-2 and Bax proteins was evaluated.

Results / Discussion / Conclusion

The results revealed *that all* the *test extracts* were able to inhibit, after 48 h of treatment, the growth of cancer cells. Our data also demonstrate that these natural products induce apoptotic cell death that could be related to an overall action of the phenolic compounds present. In fact, C. pulchella, with the highest level of phenolics [2], showed a major activity followed by C. patula and C. tchihatcheffii. In conclusion, the study of plant extracts for their cytotoxic and apoptotic properties has shown that medicinal herbs from Centaurea species might have also importance in the prevention and treatment of melanoma.

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PO111
METABOLIC PROFILING OF *Baccharis latifolia* FROM BOGOTA PLATEAU

Prada, K. J.[1]; Guerrero-Perilla, C.[2]; Coy-Barrera, E. D.[3]*

[1]*Undergraduate student, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, Cajicá, Colombia*
[2]*Chemist, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, Cajicá, Colombia*
[3]*PhD/Professor, Universidad Militar Nueva Granada, km 2 vía Cajicá-Zipaquirá, Jose María Cabal Building, Cajicá, Colombia*
**ericsson.coy@unimilitar.edu.co*

Introduction

Baccharis latifolia is a shrub belonging to the Asteraceae family, distributed mainly in Brazil, Argentina, Colombia, Chile and Mexico (Gonzaga *et al.*, 2005). Several species of the genus Baccharis been shown to possess biological properties (Greco *et al.*, 2011). In the case of B. latifolia, a plant very abundant in the Bogota plateau, have not chemical studies, which adds importance to take it as target and model for metabolomics. Thus, as part of our research on Asteraceae plants, a UFLC-based metabolic profiling and fingerprinting was performed using several wild plant accessions of B. latifolia in order to analyze the chemical variability for this plant in the Bogota plateau.

Method

Plants of Baccharis latifolia (n=20) were collected in different areas of the Bogotá plateu through a field trips for collection purposes of different accessions of B. latifolia. Each sample was divided into leaves, fruits, flowers and stems. The samples were dried and ground, and they were separately extracted with 96% ethanol. The mixture was filtered to remove the solid residue of the ethanol solution. The solution was subjected to distillation under reduced pressure to remove solvent-soluble compounds in ethanol, obtaining thus the crude ethanolic extract. Each extract was analyzed by UFLC-DAD-MS after separation conditions optimization. All chromatographic data were compared by unsupervised multivariate statistical analysis (PCA, HCA).

Results / Discussion / Conclusion

UFLC-based profiling of the crude extracts of B. latifolia accessions exhibited diverse chromatographic profiles, including characteristic compounds for some samples, interesting for further studies. PCA-derived score plots clustered the samples depending of the location (e.g., natural and modified environments) and the plant part used for extraction. The variation was especially observed for those samples rich in flavonoids and sesquiterpene lactones. This study corresponds to an endeavor to characterize B. latifolia plants by metabolic profiling in the way for metabolomics studies of this plant.

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PO112
ESSENTIAL OILS CONSTITUENTS AND ANTIMICROBIAL ACTIVITY FROM HIGH ALTITUDE BRAZILIAN SPECIES: *Baccharis parvidentata, Hyptis monticola* AND *Lippia origanoides*

Perera, W. H.[1a]*, Bizzo, H.[2], Gama P.[2], Alviano C. S.[3], Salimena, F. R.[4], Alviano, D. S.[3] and Leitão, S.[1b]*

[1a] PhD, [1b] Prof. of Universidade Federal do Rio de Janeiro, Faculdade de Farmácia, CCS, Bl. A, Ilha do Fundão, 21.941-590 Rio de Janeiro, Brazil.Wilmer.Perera@gmail.com; sgleitao@superig.com.br

[2] PhD of Embrapa Food Technology, Avenida das Américas 29501, Guaratiba, 23020-470, Rio de Janeiro, Brazil.

[3] Prof. of Instituto de Microbiologia Paulo de Góes, Centro de Ciências da Saúde (CCS), Bloco I, Universidade Federal do Rio de Janeiro, Ilha do Fundão, 21941-590, Brazil.

[4] Prof. of Universidade Federal de Juiz de Fora, Inst. Ciências Biológicas, Depto. Botβnica, R. José Lourenço Kelmer s.n., Juiz de Fora, 36033-900, Minas Gerais, Brazil.

Introduction

Essential oils have been used in aromatherapy, pharmacy, perfumery, cosmetics, as well as food preservatives among other industrial uses (Lubbe & Verpoorte, 2011). Brazil is reputed by its floristic diversity, hence its flora should be considered as an important reservoir of active molecules with potential industrial applications. (Valli *et al.*, 2012). Herein, three wild species have been investigated: Baccharis parvidentata Malag (Asteraceae). Hyptis monticola Mart. ex Benth (Lamiaceae) and Lippia origanoides Kunth. (Verbenaceae).

Method

B. parvidentata (W: 43° 01.681'; S: 22° 27.594'; altitude: 2100m) was collected around Pedra dos Sinos, Teresópolis. H. monticola (W: 44° 41.166'; S: 22° 02.196'; 1229m) and L. origanoides (W: 43° 17.443'; S: 22° 25.341'; 1.239m) were harvested on the climb of Morro do Cuca, Vale das Videiras, Petrópolis. All species were collected in Rio de Janeiro, Brazil in October 2013.

200 g of fresh aerial parts of B. parvidentata and L. origanoides, and fresh leaves of H. monticola were submitted to hydrodistillation in a Clevenger-type apparatus for 3h (n=3). The oils were analyzed in an Agilent GC-FID and GC-MS gas chromatograph equipped with a HP5-MS (5%-phenyl-methylsilicone, 30 m x 0.25 mm x 0.25 μm) fused silica capillary column (n=3). The GC settings were as follows: the initial oven temperature was 60°C, the raised to 240°C at 3°C/min and hold for 10 min. Hydrogen was used as carrier gas at a flow rate of 1 mL/min. The injector was maintained at 250°C and detector (FID) was kept at 280°C. Quantification was performed by the normalization method from the electronic integration of the FID peak areas. Constituents were identified by comparison of both mass spectra and GC retention indices with those from NIST and Wiley libraries, as well as literature data (Adams, 2009). Antibacterial and antifungal activities for B. parvidentata and L. origanoides were assayed by broth micro dilution method according to procedures of the Clinical Laboratory Standard Institute against Escherichia coli ATTCC 11229, Staphylococcus aureus (MRSA BMB 9393), Candida albicans Serotype B ATCC 10231, Cryptococcus neoformans Serotype A, Aspergillus niger ATCC 16404, Fonsecaea pedrosoi 5VPL, Trycophyton rubrum T544. Results were obtained in triplicate and expressed as minimum inhibitory concentration (MIC).

Results / Discussion / Conclusion

Essential oil yields were 0.50 % for B. parvidentata, 0.05% for H. monticola and 0.56 % for L. origanoides. The most noteworthy compounds from B. parvidentata were sabinene (15.2%), himachalol (10.3%), β-pinene (9.2%) and ä-3-carene (5.7%). H. monticola was characterized predominantly by trans-caryophyllene (11.3%), trans-methyl-cinnamate (7.8%), germacrene-D (6.9%), limonene (6.6%), a-muurolene (6.4%) and β-pinene (5.6%) whereas the most prevalent components for L. origanoides were trans-methyl cinnamate (40.0%), hedycaryol (8.0%), a-eudesmol (8.0%) and β-eudesmol (7.0%). To the best of our knowledge, this is the first report about chemical composition of B. parvidentata and H. montila. C. neoformans yeast was the most sensitive strain against essential oil from L. origanoides with MIC of 78μg/mL whereas T. rubrum were the most sensitive filamentous fungus against essential oil from B. parvidentata with MIC of 156μg/mL.

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PO113
MAJOR SECONDARY METABOLITES FROM AERIAL PARTS OF BRAZILIAN *Amasonia lasiocaulis* (LAMIACEAE)

Perera, W. H.[1a]*, Julião, L.S.[1a], Barboso, S.[1a], Cabral, L. M.[1b], and Leitão, S. G.[1b]*
[1a]*PhD,* [1b]*Prof. of Universidade Federal do Rio de Janeiro, Faculdade de Farmácia, CCS, Bl. A, Ilha do Fundão, 21.941-590 Rio de Janeiro, Brazil.Wilmer.Perera@gmail.com; sgleitao@pharma.ufrj.br*

Introduction

The genus Amasonia L. nom. cons. described initially in Verbenaceae and reorganized recently in Lamiaceae, has neotropical distribution. Amasonia is composed by eight species and it might be easily confused in literature with other different genus, Amsonia Walt. (Apocynaceae). Taxa of the genus Amasonia thrive mainly in the North of South America, between Guyana and Brazil shield (Harley *et al.* 2004). A. lasiocaulis Mart & Schauer ex Schauer was described originally as A. lasiocaulos by Schauer and also quoted as A. lasiocaulus. The species is considered as a subshrub or shrub 0.4-2.0m high, distributed in Venezuela, Colombia, Perú, Guyanas and Brazil. In Brazil, A. lasiocaulis grows in Rondônia, Acre, Amazonas, Amapá, Pará, Tocantins, Mato Grosso, Maranhão and Ceará states (Silva Dos Santos, 2011). In folk medicine leaves and flowers of A. lasiocaulis have been used by Quilombola communities of Oriximiná, Pará state against ear pains, sexual impotence and as stimulant of the whole body (Ribeiro, 2009). However, to the best of our knowledge no biological activity and isolation of secondary metabolites have been reported in literature. Therefore, the aim of this research was to identify the major constituents from aerial part of A. lasiocaulis.

Method

Dried and powdered aerial parts (600 g) of A. lasiocaulis were extracted by successive macerations with solvents of increasing polarity to afford n-hexane extract (5.9 g), CH2Cl2 (1.8 g), EtOAc (1 g) and MeOH (23 g). n-Hexane extract was submitted to GC–MS analysis in a Shimadzu GC-2000 Plus instrument equipped with a DB-5 (30 m × 0.25 mm × 0.25 μm) capillary column. The GC settings were as follows: the initial oven temperature was held at 60°C for 5 min and ramped at 3 °C/min to 220 °C for 66 min and then increased at 4 °C min to 250 °C for 2 min. The injector temperature was maintained at 290 °C. One microlitter of the extract was injected, with a split ratio of 1:40. The carrier gas was helium at flow rate of 1.0 mL min-1. Spectra were scanned from 40 to 440 m/z at 1 scan s-1. Compounds were identified by interpretation of their GC–MS spectral data as well as by comparison with NIST 11 database and literature data.
EtOAc and MeOH crude extracts were fractioned in a Phenomenex Luna RP C-18 (250mm × 10mm, 5 m) and (250mm × 21.2mm, 10m) columns respectively with a Merck Hitachi, DAD-HPLC EliteLaChrom by using trifluoracetic acid (0.1%) and acetonitrile. 100 mg of EtOAc extract were fractionated by using TFA: AcN (85:15- 30:70) at 2.5 mL min-1. 300 mg of MeOH extract were separated with TFA: AcN (85:15- 30:70) at 8 mL min-1.

Results / Discussion / Conclusion

From n-hexane extract eight metabolites were identified: 3-methoxybutyl acetate (9.03 min), ethyl 3-ethoxypropionate (9.35 min), 2-hexadecene 3,7,11,15 tetramethylester (44.99 min), n-hexadecanoic acid (46.17 min), n-hexadecanoic ethyl ester (49.82 min), octadecadienoic acid (54.27 min), linoleic acid ethyl esther (54.86 min) and octadecanoic acid ethyl ester (55.82 min). From EtOAc three major metabolites were identified: p-hydroxybenzoic acid (10 min), kaempferol (12 min) and apigenin (15 min) whereas from MeOH extract two metabolites were isolated: one still unidentified (8.41 min) and apigenin-7-O-glucoside (17.49 min). Chemicals from EtOAc and MeOH were identified by NMR and/or MS. To the best of our knowledge our findings constitute the first report about chemical composition of the genus Amasonia.

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PO114
CHEMICAL COMPOSITION OF THE ESSENTIAL OILS FROM DIFFERENT SICILIAN POPULATIONS OF *Smyrnium* L. (APIACEAE)

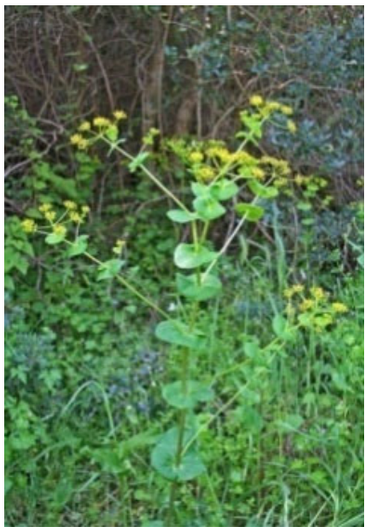
Maggio, A.[1]*; Spadaro, V.[2]; Bruno, M.[3]; Raimondo, F. M.[4]; Senatore, F.[5]; Campisi, P.[6]
[1]*Researcher, STEBICEF, University of Palermo, Parco d'Orleans II, 90128 Palermo, Italy*
[2]*Researcher, STEBICEF, University of Palermo, Parco d'Orleans II, 90128 Palermo, Italy*
[3]*Prof, STEBICEF, University of Palermo, Parco d'Orleans II, 90128 Palermo, Italy*
[4]*Prof, STEBICEF, University of Palermo, Parco d'Orleans II, 90128 Palermo, Italy*
[5]*Prof, Department of Pharmacy, University of Naples "Federico II", Via D. Montesano, 49 - 80131 Naples, Italy.*
[6]*Researcher, STEBICEF, University of Palermo, Parco d'Orleans II, 90128 Palermo, Italy*
**antonella.maggio@unipa.it*

Introduction

The genus Smyrnium, belonging to the Apiaceae family, is presented in Sicily with three species widely distributed (Smyrnium olusastrum L., S. perfoliatum L. and S. rutundifolium Mill.) [1].
A limited number of papers have been published on Smyrnium perfoliatum. Up to now no studies have been published on S. rutundifolium.

Material and method

Smyrnium rotundifolium: Monti Madonie (Sicily), Contrada Quacella, June 2012.
Smyrnium perfoliatum: Monti Nebrodi (Sicily), Portella Miraglia, June 2012.
Smyrnium sp.: Monti Madonie (Sicily), June 2012.



In this work we report and discuss the composition of the essential oils from aerial parts, flowers and roots of Smyrnium perfoliatum L., Smyrnium rutundifolium Mill. and of an indeterminate form of this genus from Sicily.
The essential oils from the epigeous (above ground) and hypogeous (below ground) parts of Smyrnium sp. pl. which were obtained by hydrodistillation, were subjected to analysis by GC and GC/MS.

Results

A large proportion of the oil was composed of sesquiterpene hydrocarbons and oxygenated compounds. The fraction of oxygenated compounds mainly contains furanosesquiterpenoids. It is noteworthy that the indeterminate form of Smyrnium (Smyrnium sp.: Monti Madonie) shows a peculiar profile with respect to the other two species.
The antioxidant and antibacterial activities is discussed.

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PO115
ASSESSMENT OF *Salvia officinalis* SUNSCREEN ACTIVITY

Michelin-Paganotte, D.[1,4]*; Santos, L. P.[2]; Chiari-Andréo, B. G.[3]; Martini-Marciano, P. C.[1]
[1]*Prof, Fund. Hermínio Ometto–UNIARARAS, Av. Maximiliano Baruto, 500, Jd. Universitário, Araras, SP, Brazil.*
[2]*Student, Fund. Hermínio Ometto–UNIARARAS, Av. Maximiliano Baruto, 500, Jd. Universitário, Araras, SP, Brazil.*
[3]*PhD student, Laboratório de Cosmetologia, LaCos, Dpto. Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista, UNESP, Rodovia Araraquara-Jaú, km1, Araraquara, SP, Brazil.*
[4] *Prof, Universidade Paulista – UNIP, Instituto de Ciências da Saúde, Campus de Limeira, Rua Miguel Guidotti, 405, Limeira – SP, Brazil*
**danielemichelin@yahoo.com.br*

Introduction

A cosmetic use of products extracted from plants that have been researched is its use as sunscreen substances. It is not easy to find plant derivatives with this property, since they have to present on its composition substances with structures related to the organic sunscreens actually used (Velasco de Paola, 2001; Ferrari *et al.*, 2008).

Salvia officinalis L. is a plant of the Lamiaceae family, popularly known as salvia. The major components of salvia are cineol, camphora, borneol, tuiona and other terpenes. Its medicinal properties are attributed to rosmarinic acid, flavonoids, and saponosides. Polyphenols, carnosic and ursolic acid, offer indicatives of antioxidant and sunscreen properties in salvia (Kosar; Goger, Baser, 2008).

Recent studies have demonstrated that polyphenols are able to protect the skin from the UV damage, since they exhibits sunscreen activity. Researches have proposed the association of the polyphenols with synthetic sunscreens, looking for an improvement of the Sun Protection Factor (SPF) and to a reduction of the amount of synthetic sunscreens used on the formulations (Ferrari *et al.*, 2008).

The SPF is a measure of UVB photoprotection. UVB is able to promote the erythema of the skin, and if in high intensities, also burn. Beyond that, the cumulative incidence of UVB could cause skin cancers (Sánchez, 2008). Considering the harmfull effects caused by the UV radiation, this study aimed to assess the sunscreen effect of a Salvia officinalis extract. It was assessed incorporated in an emulsion, added or not of synthetic sunscreens.

Method

The extract of the leaves of salviawere prepared by maceration in ethanol at 70%. This process happened during 10 days. This extract was filtered and concentrated in a rotaevaporator (45-55° C). This extract was added to a base emulsion added of not of Benzophenone-3, a syntethic sunscreen. Thus, the formulations assessed were: 1) base emulsion, 2) base emulsion with 15% of salvia extract, 3) base emulsion with 2% of Benzophenone-3 and 4) base emulsion with 15% of salvia extract and 2% of Benzophenone-3. The method described by Mansur *et al.* (1986) was used to determine the FPS of the formulation.

Results / Discussion / Conclusion

The concentrated extract obtained after the drying process give an FPS of 9,0, however, when added to the formulation at 15% were not observed sunscreen activity, neither when used in the formulation with synthetic sunscreen. Considering the high FPS obtained with the concentrated extract, our research group will continue this study assessing higher concentrations of salvia extract, which may offer better results.

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PO116
DEVELOPMENT AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF LIQUID SOAP INCREASED OF VOLATILE OIL FROM CINNAMON

Michelin-Paganotte, D.[1,3]*; Planche, N. C.[2]; Rodrigues, D. E.[2]; Navarro, F. F.[1] Martini-Marciano, P.[3]
[1]*Prof, Centro Universitário Herminio Ometto – UNIARARAS, Avenida Dr. Maximiliano Baruto, 500, Araras – SP, Brazil.*
[2]*Student, Centro Universitário Herminio Ometto – UNIARARAS, Avenida Dr. Maximiliano Baruto, 500, Araras – SP, Brazil.*
[3] *Prof, Universidade Paulista – UNIP, Instituto de Ciências da Saúde, Campus de Limeira, Rua Miguel Guidotti, 405, Limeira – SP, Brazil*
**danielemichelin@yahoo.com.br*

Introduction
The antimicrobial property is subject of numerous studies due to increased bacterial resistance to conventional antimicrobial drugs (Schelz & Hohmann, 2006).

The volatile oil of cinnamon (Cinnamomum zeylanicum Blume - Lauraceae) is used as fragrance, flavoring and natural food preservative. Studies have shown the ability to inhibit the growth of fungi (Lima *et al.*, 2006) and bacteria (Matan *et al.*, 2006).

The aim of this work was to evaluate the antimicrobial activity of the volatile oil of cinnamon and chlorhexidine gluconate against gram-positive bacterial strains (Staphylococcus aureus) and Gram-negative (Escherichia coli), as well as strains Candida albicans; and develop a formulation of liquid soap containing the volatile oil of cinnamon and evaluate the antimicrobial activity of the developed formulation.

Method
Four formulations of liquid soap, liquid soap base was used as a negative control, only increased the volatile cinnamon oil formulation, increased values of chlorhexidine formulation and increased the volatile oil of cinnamon and chlorhexidine gluconate formulation were developed.
The antimicrobial activity was evaluated by the agar diffusion method, using standard strains of Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922) and Candida albicans (ATCC10231)

Results / Discussion / Conclusion
Through testing, it can be concluded that the essential oil of cinnamon and chlorhexidine digluconate had antimicrobial activity under the conditions tested. It was possible to develop a formulation of three liquid soaps, all showed satisfactory antimicrobial activity, the soap containing cinnamon oil as an antimicrobial agent was less effective than soap containing chlorhexidine gluconate. The liquid soap plus volatile oil of cinnamon and chlorhexidine digluconate was more effective against all micro-organisms tested showing a possible synergism between the substances.

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PO117
DEVELOPMENT OF DENTRIFICE GEL PLUS CRUDE EXTRACT OF THE FRUIT OF *Morus nigra* L. AND EVALUTION OF *S. MUTANS* IN THE ORAL CAVITY IN HEALTHY INDIVIDUALS

Michelin-Paganotte, D.[1]; Guerrero, P. C.[2]; Martins, M. D. G.[2]; Navarro, F. F.[1]*
[1]*Professor, Herminio Ometto Fundation (UNIARARAS), 540 Maximiliamo Barutto Avenue, Araras, Sao Paulo, Brazil*
[2] *Student; Herminio Ometto Fundation (UNIARARAS), 540 Maximiliamo Barutto Avenue, Araras, Sao Paulo, Brazil*
**fernandaflores@uniararas.br*

Introduction

Currently 30% of available drugs as therapeutic agents derived from natural products (Calixto, 2005).
The removal of dental plaque has been emphasized in preventing the development of chronic inflammatory periodontal diseases. The role of toothpastes is supporting, with excellent vehicles for fluoride release, providing a polishing. The Morus nigra L., Moraceae, is a species belonging to the family Moraceae, commonly known as mulberry tree, having its origin in Asia (Remignton, 1995). The fruits of Morus contains phenolic compounds showed broad spectrum of biochemical activity as an antioxidant, hypoglycemic, anti-inflammatory and antimicrobial properties(Cruz, 1979; Lorenzi *et al.*, 2006).
The study aimed at the development of gel toothpaste plus crude extract of the fruit of Morus nigra L., obtained by turboextração, performing the evaluation of the reduction potential of S. mutans in oral cavity of healthy individuals.

Method

Getting extract:
Morus Nigra L. Fruit + Extractant solvent: 70% water-alcohol solution. Using the method of turboextração then rotoevaporation.
Formulation evolution:
After setting the base gel was incorporated 5% of the fruit extract of Morus nigra L.
In vivo evaluation:
15 volunteers were selected. After this selection, the volunteers were instructed to make a chewing a plate of paraffin to stimulate salivation. Yielded the salivary fluid and proceeded to the delivery of toothbrushes with 1.5 g of the dental gel developed, and then we collected salivary fluid again.

Results / Discussion / Conclusion

The obtained extract was performed by turboextração then was held at rotoevaporation alcohol, then was incorporated into the formulation.
The quantification of S. mutans in the saliva is an important method is used to count the microorganisms present in the oral cavity, it is associated with dental caries and plaque.
Data obtained during the evaluation *in vivo*:
The statistical analysis using the Student t test with $p < 0.001$, indicating a significant difference between the saliva sample collected before salivation (approximately 800 UFC/ML) and the sample collected after brushing with cranberry gel (aproximandamente 450 UFC/ML) ($n = 13$).
The Mitis salivarius agar, was used in this study, it is a selective medium for Streptococcus mutans, which causes dental caries and for being the most bacteria found in the oral cavity. Thus, the reduction in the number of colonies of streptococci is related to the prevention of oral diseases.We conclude that the results observed during the study render the formulation developed feasible and promising, but for marketing the dental gel is essential to carry out further tests.

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PO118
PRELIMINARY PHYTOCHEMICAL AND TOXICOLOGICAL *IN VIVO* STUDY OF *Morus nigra* L. (MORACEAE) LEAVES

Michelin-Paganotte, D.[1]; Breda, T. C. C.[4]; Guizzo, P. L.[2]; Pereira, N. R. C.[3]; Spatti, M. C.[3]; Navarro, F. F.[1]*

[1]*Professor, Herminio Ometto Fundation (UNIARARAS), 540 Maximiliamo Barutto Avenue, Araras, Sao Paulo, Brazil*
[2]*Drugs and Pharmaceuticals, University of Sao Paulo, 580 Professor Lineu Prestes Avenue, Sao Paulo, Sao Paulo, Brazil*
[3]*Toxicology and Toxicological Analysis, University of Sao Paulo, 580 Professor Lineu Prestes Avenue, Sao Paulo, Sao Paulo, Brazil*
[4]*Herminio Ometto Fundation (UNIARARAS), 540 Maximiliamo Barutto Avenue, Araras, Sao Paulo, Brazil*
**fernandaflores@uniararas.br*

Introduction

At the moment a plant is chosen as a form of therapy, studies are necessaries to make sure that they have the desired effect. In this aspect are necessaries requirements to ensure the authenticity this plant species as the correct specie, vegetal drug purite, planting, harvest, and evaluation of their active ingredients. Because of this a preliminary phytochemichal analysis is necessary to show what compounds the plant has. A toxicological study is also important to know if the plant can be used as a form of therapy. The present study had aimed the preliminary phytochemical analysis and a toxicological study of the Morus nigra L. study.

Method

For the leaf powder phytochemical characterization were realized classical tests for the main indentification the active ingredients (Simões *et al.*, 2007): 1) flavonoids (Shinoda, ferric chloride and aluminum chloride reactions); 2) saponins and Foam content; 3) tannins (ferric chloride and lead acetate reaction), 4) alkaloids (soluble in acidic and basic broth: Dragendorff, Bertrand, Valser-Mayer and Bouchardat reactions), 5) cardiotonic (Liebermann-Burchard, Balje, Keller-Kiliani and Kedde reactions) and 6) anthraquinones.
The toxicological study, preclinical acute toxicity study was performed in male Wistar mice with oral administration of the leaves aqueous extract of Morus nigra L., daily for 7 days, evaluating body weight and ingestion of water and food during treatment, at the end the animals were euthanized and was also evaluated macroscopic analysis and relative organ weights.

Results / Discussion / Conclusion

Different groups of chemical constituents have been investigated in the Morus genus, such as alkaloids, coumarins, flavonoids, triterpenes and steroids. The preliminary phytochemical showed the presence of flavonoids (reaction of aluminum chloride), alkaloids: Mayer reagents, Bertrand, and Bouchardout Fosfomolibidic acid and hydrolysable tannins: reaction with acid ethyl lead. According to literature data plants with these compounds can be used in the medicine to rheumatism treatment, bleeding, wounds, burns, stomach and kidney problems, inflammation, diarrhea antitumor actions, anti-bleeding, hormonal, anti-inflammatory, antimicrobial and antioxidant (Haslam, 1996; DeBrunye *et al.*, 1999; Dufrens&Farnworh, 2001; Toshio *et al.*, 2005 Simões *et al.*, 2007).
There was no significant difference in organ weights, however, we could observe a significant difference in body weight gain, but not in water intake and feed the animals treated with alcoholic extract of leaf, this change may be an indication of systemic toxicity (Rauber *et al.*, 2006), and so other toxicological tests are required.

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PO119
ACTIVITY OF AROYLMETHYL-4-PHENYLTHIOCHROMANS DERIVATIVES AGAINST *Leishmania panamensis*

Vargas E.*[1]; Quiñones W.[1]; Echeverri, F.[1]; Torres, F.[1]; Archbold, J.[1]; Upegui, Y.[2]; Robledo, S.[2]
[1]*Instituto de Química-Grupo de Química Orgánica de Productos Naturales Universidad de Antioquia, Calle 62 52-59, Medellín-Colombia*
[1]*Programa de Estudio y Control de Enfermedades Tropicales PECET, Universidad de Antioquia, Calle 62 52-59, Medellín- Colombia*
esteban.vargac@udea.edu.co

Introduction

Leishmaniasis is described as a series of anthropozoonotic diseases with clinical and epidemiological relevance (Desjeux, 2004). It is caused by parasites of the genus Leishmania and is transmitted through the bite of the female insect of the genus Phlebotomus and Lutzomyia (De Almeida, 2003). According to the World Health Organization (WHO, 2012), Colombia is one of the three countries with the highest incidence of leishmaniasis.

Previous work performed in our group found that compounds bearing the chroman and thiochroman moiety have important activity against leishmania parasites (Cardona, 2006). Moreover, with in vitro and in silico studies it has been shown that acyl hydrazones derivatives improve the activity of the compounds and the structural features allow it be improved in their druggability (Taha, 2013) (Coimbra, 2013). In the search for new antiparasitic substances in this work, some Aroylmethyl-4-phenylthiochromans derivatives were synthesized and their activity against Leishmania parasites were tested.

Method

Synthesis of cis-(aroylmethyl)-4-phenylthiochromans was carried out by iodine catalyzed reaction between cinnamylideneacetophenones and thiophenol (Mallik, 2012); acyl hydrazones derivatives are obtained by heating cis-2-(aroylmethyl)-4-phenylthiochromans with acyl hydrazides in the presence of an acid catalyst. Furthermore, by reaction with oxidizing agen
ts sulfone and sulfoxide derivatives are obtained.

The intermediates and final products were characterized by 1H NMR and 13C (NMR) and two-dimensional experiments.

Cytotoxicity test in the human promonocytic cell line U-937 was carried out using the enzymatic MTT micromethod. The intracellular leishmanicidal activity (L.panamensis strain pirGFP UA140) was tested using Flow cytometry.

Results

Some of the compounds showed a potential leishmanicidal activity at 20μg/ml, with percentages of inhibition higher than 50% of parasitic load. Some of the more active compounds (percentages of inhibition higher than 80%) also have a high cytotoxicity.

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PO120
PHYTOCHEMICAL COSNTITUENTS OF *Gunnera ticntoria* (NALCA) AND ITS ACTIVITY AGAINST *Helicobacter pylori*

Pastene, E.[1], Hebel S[1,3]., García A.[3], Vizcarra L.[2]*, Avello M.[1], Parada V.[1], Bustamante L.[1], Pinuer L., Aranda M.[1], Torres E.[1] and Urbina A.[2]

[1] Facultad de Farmacia, [2]Facultad de Agronomía, [3]Facultad de Ciencias Biológicas. Universidad de Concepción. Vicente Méndez 595, Chillán. CHILE

maurbina@udec.cl, lvizcarra@udec.cl, edgar.pastene@gmail.com

Introduction

Helicobacter pylori (Hp) is a pathogen whose global prevalence reach 50%, is involved in gastric diseases such as gastritis, duodenal ulcer and gastric cancer. To eradicate this bacterium, antibiotic triple therapy is used, which may show side effects associated with abandonment and failure. This is a critical factor because could promote selection of antibiotic resistance. Therefore, there is a constant search for new molecules with safer and selective profile against this pathogen. The aim of this work was to continue to identification of bioactive molecules from extracts of the edible petiole of Gunnera tinctoria (nalca), based on their ability to inhibit the growth of H. pylori.

Methods

Extracts were analyzed through HPLC-ESI-MS/MS HPTLC/MS/MS and GC-MS/MS allowing the assignation of the main polyphenol constituents identity. Antimicrobial effect was assessed using H. pylori strains 43504, SS1 and J99 and confirmed by transmission electron microscopy. Also, inhibition of urease and carbonic anhydrase enzymes were performed in order to evaluate the effect of extracts upon H. pylori virulence factors.

Results and conclusions

Tannin-rich exctracts from G. tinctoria promoted ultrastructure alterations characterized by the formation of "Blebs" in the bacterial membrane. Such modifications suggested that tannin fraction generate disruption of bacterial membrane leading to cell lyses and death. Additionally, extracts from this specie inhibit urease and carbonic anhydrase suggesting that Nalca activity not only affect bacterial morphology but also with two virulence factors that have a key role in the early steps of infection. In conjunction, our findings indicates that G. tinctoria could be a powerful resource for the preparation of nutraceuticals, phytotherapeutic and functional ingredients destined to prevent or complement the pharmacological therapy of H. pylori.

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PO121
THEORETICAL STUDIES OF HIDRAZONES

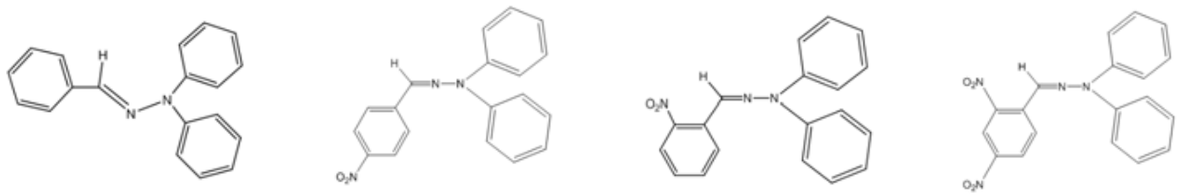
Ramírez Juan C.*, Cabrera-Vivas B. M., Meléndrez-Luévano R., Ortiz Márquez J. A., Sansinenea Royano E., Pineda Flor

[1]*PhD/Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.*

juan.ramirez@correo.buap.mx

Introduction

It is common to use Computational Methods to predict or compare numerous molecular properties of experimental data of synthesized compounds; they are reliable regarding the characterization of the molecule. These kind of theoretical studies are being consulted by researchers to support and predict experimental results. In this theoretical study are compared theoretical with experimental data of RMN, FTIR and MNR of 4 Hidrazones, to analyze and compare its capability of prediction of theoretical calculations. The molecules are: I (E)-1-(benzylidene)-2,2-diphenylhydrazine (C19H16N2) 1 II (E)-1-(4-Nitrobenzilidene)-2,2-diphenylhydrazine (C19H15N3O2) 2, III (E)-1-(2-Nitrobenzilidene)-2,2-diphenylhydrazine (C19H15N3O2) IV (E)-1-(2,4-diNitrobenzilidene)-2,2-diphenylhydrazine (C19H15N4O4).



I **II** **III** **IV**

Figure 1. StudiedHidrazones

Method

The molecules were synthesized and characterized by m. p., U.V, I.R. 1H NMR, 13C NMR and x-Ray. Also were modeled and theoretical calculations were carried out using a workstation with computational the suite of computational programs Gaussian 09, performing an optimization B3LYP/6-31(G), the calculation of IR with RB3LYP/6-31(G), and RMN 1H with RB3LYP/6-31G(d). UV data were calculated with B3LYP/6-31G(d,p).

Results / Discussion / Conclusion

MOLECULE	UV (λ max)		IR (C=N)		^{1}H RMN	
	Experimental	Theoretical	Experimental	Theoretical	Experimental	Theoretical
I	340.13	305.3	1586, 1490	1677, 1655	7.64	6.92
II	411.51	378.3	1592, 1556	1600, 1627	8.20	7.23
III	485.01	476.05	1577, 1513	1642.5, 1648	7.60	6.872
IV	443.36	415.6	1683, 1600	1710, 1650	8.73	7.57

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PO122
INHIBITORY CAPACITY OF EDIBLE FILMS ADDED WITH LACTIC ACID BACTERIA ON INDICATORS MICROORGANISMS (*S. aureus* AND *E. coli*)

Dublán-García, O.[1]*; García-Argueta, I. [3]; Peña-Santana, A.[1]; Gómez-Oliván, L. M.[1]; López-Martínez, L. X.[1]; Hernández-Navarro, M. D.[1], Quintero- Salazar, B.[2]

[1]*Universidad Autónoma del Estado de México /Facultad de Química, Cerrillo Piedras Blancas, Toluca, Edo. de México. CP 50090.*octavio_dublan@yahoo.com.mx; odublang@uaemex.mx*

[2]*Universidad Autónoma del Estado de México/ Facultad de Turismo y Gastronomía, Carretera Toluca-Atlacomulco Km. 14.5, Tlachaloya, Toluca, Edo de México. CP 50200.*

[3]*Universidad Autónoma del Estado de México/ Facultad de Medicina, Paseo Colón y Paseo Tollocan. Toluca, Edo de México. CP 50000.*

Introduction

The use of edible film (EF) in food applications and especially in highly perishable products, is based on certain characteristics such as availability, functional attributes, mechanical properties (tension and flexibility), optical properties, its barrier effect against the flow of gases, structural resistance to water and effect against microorganisms among others. Studies have shown, it can be used as vehicles for substances such as additives, antioxidants and microorganisms. Lactic acid bacteria (LAB) are a group of organisms represented by various genuses with morphological, physiological and metabolic characteristics in common. The conservative action of LAB, such as L. casei and L. acidophillus, is due to inhibition of a large number of pathogens and harmful microorganisms. Given features that have lactic acid inhibition of pathogenic microorganisms bacteria, joining an edible film based on whey, gelatin and inulin, could have intended the inhibition of microorganisms of importance in public health such as E. coli and S. aureus, in application in regular consumption food not dairy products, such as breads, fruits and vegetables and meat products. The objective of this study was to analyze the effect of an edible film with whey protein isolated, gelatin, glycerol and inulin added with lactic acid bacteria, on microorganisms of importance in public health (S. auereus and E. coli).

Method

Edible film (EF)was obtained employed a response surface Box Behnken type to determine the optimal formulation of EF from components: whey protein isolated (8%), glycerol (6%), inulin (0-4%), gelatin (2-5%) and Lactobacillus casei Shirota ® (Lc) as probiotics (0-2%) which was obtained from Yakult ® commercial product. 15 different formulations were tested which were measured in triplicate textural characteristics (strength, Young's Modulus, and elongation), as physicochemical characteristics pH, viscosity and particle size, and colorimetry (L * a * b *), the survival Lc was assessed on the agar plate technique and electron microscopy (SEM).

Results / Discussion / Conclusion

The different 15 combinations 5 were the most appropriate for its textural and physicochemical characteristics (hardness, viscosity, pH, light, elongation). Presence was observed in these 5 EF and survival of the BAL, however were the two EF that had the best growth of the BAL, observing a minimum 3x109 CFU/mL. The presence of BAL in the EF was observed by scanning electron microscopy (SEM). To carry out analyses of inhibition of indicator microorganisms (S. aureus and E. coli), it was found that two formulas (15 and BB1) were the most suitable for this purpose, observing halos of inhibition of 1 to 5 mm, when using 1.3 and 2% of BAL respectively. With the results, it is possible to say that the EF is a good inhibitor of indicator microorganisms as E. coli and S. aureus, also a good alternative as a vehicle for transport of probiotic bacteria.

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PO123
QUALITATIVE PHYTOCHEMICAL SCREENING, EFFECT IN AVIAN POX *IN VIVO* AND TOXICITY (DL_{50}) OF *Solanum cervantesii* LAG.

Reséndiz Esparza E.[1], Bautista Sánchez Urias[2], Bautista Sánchez Hiram[2], Baltazar Zavala Patricia Ivonne[2], Flores Hernández María Andrea[2], Hinojosa Espinoza Erik Alejandro[2], Montejano Rodríguez J.R.[3], Almaguer Vargas Georgina[3]*

[2]Pharmacy Students of Autonomous University of Hidalgo. Ex hacienda La Concepción road to Tilcuautla, Hidalgo, México
[2]Bachelor of Pharmacy. Infant Hospital Teletón oncology. Querétaro México
[3]M. D. Autonomous University of Hidalgo. Ex hacienda La Concepción, road to Tilcuautla, Hidalgo, México
*3*georginaalmaguervargas@yahoo.com.mx.*

Introduction

The impact of the fowl pox in Mexico is variable and it depends of the geographic area, the immunization schedule and the kind of work. At little productions it is more importance, because the morbidity is to high and mortality could be even 50% causing hard economic losses. Searching alternatives for economic treatment for this problem is very necessary. Solanum Cervatessii Lag it's from Mexico, it is known and used by its antiviral effect against the pox viruses it causes the smallpox.

In this work we did a plant qualitative phytochemical, we analyzed the effect on turkey's smallpox and we determinate the toxicological effect on mice.

Method

The plant was collected in San Agustin Tlaxiaca, Hidalgo. Mexico, and was identified at the University of Hidalgo's herbal. The qualitative phytochemical tests were on ethanolic extract. To prove the effect on turkey's we used infected 4 months turkeys. We administered them the extract over the injuries every 7 days for a month. We checked the survival and the recovery time. The toxicity was done according the method Lorke in male 25 g Balb/c mice. Past 15 days of observation they were sacrificed and we made the microscopic observation of liver, kidney, heart and spleen. The animal handling and sacrifice were made according to the NOM-062-ZOO-1999 and NOM-087-ECOL-SSA1-2002.

Results / Discussion / Conclusion

The plant was identified as Solanum cervantesii Lag. with voucher number GAV17. The qualitative phytochemical proved the existence of alkaloids, steroids, coumarins, saponins, sugars, phenols, tannins, amino acids, amines, carbohydrates and flavonoids.

The effect on the recovery of infected turkeys showed after 15 days of treatment without injuries. We get a 100% of survival of the animals with treatment. With the toxicity test any doses administered induced the dead of the animal, and the body without injuries.

The plant juice is uses etnopharmacologically for the treatment of fowl pox, this work proved the effect.

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PO124
BIOACTIVITY OF FRACTIONS OBTAINED FROM *Senna corymbosa*

Mattana, C. M.[1]*; Alcaráz, L. E.[1]; Satorres, S. E.[1]; Garro M.[2]; Petenatti, E.[2]; Saad, R.[3]; Laciar, A.[1]
*[1]PhD, Area Microbiología, [2] PhD, Area Farmacognosia y Herbario. [3]PhD, Area Química Orgánica. Facultad de Química, Bioquímica y Farmacia. Universidad Nacional de San Luis. Ejercito de los Andes 950, San Luis, Argentina.*cmmattan@unsl.edu.ar*

Introduction

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Ethnopharmacological studies on medicinally important plants are an area of interest for the investigators throughout the world. Plants belonging to Senna species are used extensively in various parts of the world against a wide range of ailments. Senna corymbosa Lam. (Fabaceae), commonly known as "sen del campo" or "black branch" is a shrub native to Brazil, Paraguay, Argentina and Uruguay. The infusion of the leaflets and decoction seed are used in folk medicine as laxative [1], the leaves are used as emollients and topical analgesic. The main components founded are anthraquinone, naphthoquinones and flavonoids glycosides. The objective of the present study was to test the antibacterial activity of fractions obtained from organic extract of S. corymbosa.

Method

The leaflets of S. corymbosa were extracted with different solvents of increasing polarity to obtain several fractions in which was determined the minimum inhibitory concentration (MIC) of the extracts against Gram positive (Staphylococcus aureus ATCC 43300, Staphylococcus aureus ATCC 35556, Listeria monocytogenes CLIP 74905) and Gram negative bacteria (Escherichia coli ATCC 35218 and Pseudomonas aeruginosa ATCC 27853). Broth microdilution test using 96-well microplates was employed. In each well, 95 µL triptone soy broth supplemented with 0.01% 2,3,5,-triphenyltetrazolium as visual indicator of bacterial growth, 5 µL of a suspension of 107 CFU/ mL strains and 100 µL serial dilution in base two of different fractions were added. Media, extract and strains controls were included. The test was performed in duplicate. The plates were incubated at 37°C 24 h, and read visually.

Results /discussion/conclusion

Tested the fractions corresponding to FI, FII, FVI and FVII no showed inhibitory effect on the growth of Gram-negative bacteria, whereas Gram-positive bacteria were inhibited with different values ÿ'3fÿ'3fof MICs showing the FVII best activity against S. aureus 35556 (MIC=5µg/mL). The FIV inhibited all the tested bacteria (MICs range between 36 and 290 µg/mL). The FVII, soluble in ethyl acetate, showed no activity inhibitory against Pseudomonas. The FVIII, soluble in hexane-methanol, showed no biological activity. The FIX showed MICs between 3 and 6 µg /mL for Staphylococcus, Listeria and Pseudomonas but not inhibited E. coli. The FX and XI were actives against Gram positive and negative bacteria.
In a previous study of S. corymbosa pure extracts showed good antibacterial activity [2], the fractions obtained from these extracts had higher biological activity indicating several active compounds presence. They are carrying out the separation and identification of bioactive compounds.

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PO125
ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OBTAINED FROM *Ligaria cuneifolia* AND *Tripodhantus flagellaris*

Alcaráz, L. E[1]*; Fusco, M. R[2]; Mattana, C. M[1]; Satorres, S. E[1]; Laciar, A. L.[1]
[1] *Área Microbiología.* [2]
Área Farmacognosia. Universidad Nacional de San Luis. Ejército de los Andes 950. San Luis. Argentina.
** lucesalca@gmail.com; cmmattan@unsl.edu.ar*

Introduction

In many places of Argentina, there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries, and other diseases. Ligaria cuneifolia, (Ruiz&Pav.) Tiegh, popularly known as "liga", "liguilla", "liga roja", or "muérdago criollo" and Tripodanthus flagellaris (Cham& Schlchdtl.) Tiegh, known as "liga blanca", "liguilla", "corpo", "liga", "pupusa", are two species belong to the Loranthaceae family widely distributed in the central and northern areas of the Argentina Republic. These species are shrubby plants hemiparasite, evergreen, living in epiphyte on trees and shrubs. In folk medicine, the infusion of these plants are used as hypotensive and to reduce excess cholesterol. Phytochemistry screening on Ligaria cuneifolia indicated the presence of tannins, flavonoids, saponins, anthraquinones, alkaloids and cardiac glycosides. Also, its anti-carcinogenic and anti-HIV activity has been reported. The objective of the present study was to test antimicrobial activity of aqueous and methanolic extracts obtained from Ligaria cuneifolia and Tripodhantus flagellaris against Staphylococcus aureus, Listeria monocytogenes, Escherichia coli and Pseudomonas aeruginosa strains.

Method

Antibacterial activity of the aqueous and methanolic extracts of both plants against Gram positive bacteria (Staphylococus aureus ATCC 43300, Staphylococcus aureus ATCC 25923, Listeria monocytogenes CLIP 74910) and Gram negative bacteria (Escherichia coli ATCC 35218 and Pseudomonas aeruginosa ATCC 27853) was determined using the agar diffusion method (variant holes).The minimum inhibitory concentration (MIC) values were determined by broth microdilution test using 96-well microplate. In each well, 95 µl triptone soy broth supplemented with 0.01% 2,3,5,-triphenyltetrazolium as visual indicator of bacterial growth, 5 µl of a suspension of 106 CFU/ml strains and 100 µl serial dilution in base two (8000 to 125 µg/ml) of the extracts were added. Media, extract and strains controls were included. The test was performed in duplicate. The plates were incubated at 37°C 24 h, and read visually.

Results /discussion/conclusions

All extracts were active against gram-positive bacteria and showed no activity against Gram-negative organisms at the concentrations tested. The high effect was observed for the aqueous and methanolic extracts of Tripodhantus flagellaris against S. aureus and L. monocytogenes for which the average diameters of the zones of inhibition were 15 to 18 mm respectively, and the MIC values were of 312.5 to 625 µg/ml, respectively. The MIC values of both Ligaria cuneifolia extracts against S. aureus and L. monocytogenes ranged 1250 to 2500 µg/ml. The results demonstrate the antibacterial activity of both plants which is interesting because, to our knowledge, there are no reports available of this activity in the literature. This antibacterial activity can be attributed to biologically active compounds whose presence was reported in previous studies. The separation and identification of bioactive principles could clarify the properties of these plants and to contribute to further understanding of these species from Argentina flora used in folk medicine.

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PO126
SPRAY-DRIED MICROPARTICLES CONTAINING *Passiflora quadrangularis* L. AQUEOUS LEAF EXTRACT: INFLUENCE OF EXPERIMENTAL PARAMETRES ON YIELD AND ENCAPSULATION EFFICIENCY

Hilbert I. Medina J.[1], Paola Cardenas, [1] Geison M. Costa [2], Diana M. Aragón N.[1]*
[1] *Grupo de Investigación Tecnología de Productos Naturales TECPRONA, Departamento de Farmacia, Facultad de Ciencias, Universidad Nacional De Colombia,* [2] *Grupo de Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia, Departamento de Química, Facultad de Ciencias, Universidad Nacional De Colombia,* *
e-mail: dmaragonn@unal.edu.co

Introduction
Passiflora quadrangularis L. is a plant widely distributed on South America, their fruits have been used traditionally in the preparation of foods and beverages while the tea of the leaves is employed in folk medicine as tranquilizer 1. Recently, pharmacological activities over Central Nervous System as sedative and anxiolytic, have been investigated and reported 2. The aim of this study was to evaluate the influence of experimental parameters on the manufacture of micro particles loaded with P. quadrangularis L. aqueous leaf extract prepared by Spray Drying method. Microparticles obtained could improve the P. quadrangularis extract stability, since they protect it from environmental conditions, additionally Eudragit ® E is a copolymer soluble in acid pH, thus would allow a gastric release.

Method
Micro particles were manufactured with Eudragit ® EPO (Poly(butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate) 1:2:1) by spray- drying an acid solution containing co-polymer and extract, experimental parameters such as Inlet Temperature, Air Flow, Pump Rate, Extract: Polymer Ratio and Solid Content were evaluated through Unbalanced Factorial Design 2 2-1 33-1. Yield was calculated by mass balance, and corrected taking into account the final micro particles water content. On the other hand, encapsulation efficiency was determinated by total flavonoid quantification after dissolve the micro particles in water. All the quantifications were carried out by a validated HPLC-DAD method.

Results and Discussion
Yield percentages ranging from 34,6% to 77,4%, and Encapsulation Efficiencies between 70,5% to 95,45%, were found. Experimental parameters modify responses evaluated, a Linear Model by Step Wise was made by R software, we found two different models, a different model for each response. For the Yield, the model show an influence by Inlet Temperature, Pump Rate, and Air Flow, and an interaction between Pump Rate and Air Flow was found. On the other hand, Encapsulation Efficiency was influenced by all evaluated parameters, and interactions between, Temperature and Pump Rate, Pump Rate and Air Flow, Pump Rate and Solid Content, Pump Rate and ratio, and finally Air flow and Proportion, were found. The microencapsulation of leaf extract led to good incorporation efficiencies with different yield. The better parameters to spray-dried Eudragit® EPO micro particles P. quadrangularis-loaded, in the Range of parameters evaluated are low Inlet Temperature and Pump Rate, a 3: 1 Extract: Polymer Ratio and a High Air Flow.

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PO127
DEVELOPMENT OF AN ANTIMICROBIAL EDIBLE FILM BASED ON WHEY PROTEIN ISOLATED AND INULIN WITH ONION AND GARLIC EXTRACT AND ITS APPLICATION TO FRESH COMMON CARP

Dublán-García, O.[1]*; Martínez del Prado, J. M.[1]; Gómez-Oliván, L. M.[1]; López-Martínez, L. X.[1]; Hernández-Navarro, M. D.[1], García-Argueta, I. [3]

[1]*Universidad Autónoma del Estado de México /Facultad de Química, Cerrillo Piedras Blancas, Toluca, Edo. de México. CP 50090.*octavio_dublan@yahoo.com.mx; odublang@uaemex.mx*

[2]*Universidad Autónoma del Estado de México/ Facultad de Turismo y Gastronomía, Carretera Toluca-Atlacomulco Km. 14.5, Tlachaloya, Toluca, Edo de México. CP 50200.*

[3]*Universidad Autónoma del Estado de México/ Facultad de Medicina, Paseo Colón y Paseo Tollocan. Toluca, Edo de México. CP 50000.*

Introduction

The current concern to bring adequate food, it is because the pace of life so have developed a number of options to meet the needs of the population. A problem with this situation is increasing mainly inorganic waste, comprising first bags and bottles. Alternatives have been developed in order to reduce the massive consumption of food packaging, such as edible coatings while maintaining the physicochemical and sensory characteristics of the product also has been observed that these coatings can be vehicles for compounds such as probiotics, prebiotics and / or antioxidants. The objective of this research was to develop an edible coating based on whey and inulin added to aqueous extracts of garlic (Allium sativum L.) and onion (Allium cepa L.) and evaluate their effect on microbiological, physicochemical and textural filet common carp (Cyprinus carpio).

Method

Quality of common carp was measurement by physicochemical parameters (pH, acidity, and color). Developed an edible film based on whey protein isolated, inulin, gelatin and glycerol, determining Yong modulus and strength. The aqueous extract of garlic and onion was obtained and was carried out mixing and total phenolics content (TPC) was quantified by the Folin-Ciocalteu method. The aqueous extract was added to the edible film and was evaluated Yong modulus and strength. The antimicrobial effect of edible film with added garlic and onion on common carp fillets and textural properties (hardness, cohesiveness, adhesiveness and gumminess) were evaluated during his shelf life. All tests were carried out by triplicate.

Results / Discussion / Conclusion

The quality parameters of carp were within reported by Huss (1999) and Arenas (1997), taking a fresh product with a pH = 6.7, and an acidity of 0.57%. The edible film whose composition was whey protein isolated, gelatin, inulin and glycerol (8, 3.5, 4, 6% respectively), had a light yellow color and a Yong module of 0.5 and a force of 3N, therefore, it could be considered as a functional edible film as a carrier for various compounds. The aqueous extract of garlic and onion had a content of 0.28 mg TPC/g, and when added to edible film it did not affect its color and texture. Had a significant antimicrobial effect by reducing up to 54% of CFU/mL of Salmonella; 62% of CFU/mL of S. aureus and 78% of mesophiles during storage; was observed a slightly decreased in texture parameters. It could say that this edible film can be used to extend the shelf life of meat and meat products, keeping their textural and physicochemical characteristics.

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PO128
SEASONAL MODULATION OF THE PHARMACOLOGICAL ALKALOID LIRIODENINE IN *Annona lutescens*

Gonzalez-Esquinca A. R[1]*., Castro-Moreno M.[1], De-la-Cruz-Chacón I.[1]
[1] *PhD/ Research Profesor Laboratorio de Fisiología y Química Vegetal, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas. Libramiento Norte-Poniente No. 1150. Colonia Lajas Maciel. CP. 29035. Tuxtla Gutiérrez, Chiapas, México.*
**aesquinca@unicach.mx*

Introduction

The liriodenine is a benzylisoquinolinic alkaloid (CAS 45-75-2) with a wide rank of pharmacological activities; including the antiproliferative on nearly twenty cancerous cellular lines (Wu *et al.* 1990; Chan et al 2014); which mechanism of action seems to be the block of the DNA synthesis while inhibiting the enzyme topoisomerase II (Wo et al 1997; Hsieh, *et al.* 2005). It is a powerful antimicrobial against more than fifty bacteria Gram (+) and Gram (-) like Staphyloccocus aureus, Streptococcus b-haemolyticus, Shigella dysenteriae, Salmonella typhi and over several fungi including Aspergillus flavus, A. niger, A. versicolor y Candida albicans (Mukhlesur *et al.*, 2005). Additionally, it has antiprotozoal activity against Leihsmania amazonensis, Plasmodium falciparum y Tripanosoma brucei (Wirasathien et al, 2006). The liriodenine is biosynthetized by several species, among them young plants of Annona lutescens, a native species of the deciduous low-altitude rainforest (Moreno *et al.*, 2013; González-Esquinca *et al.*, 2014). This plant, in consequence, is subject to seasonal changes which provoke contrasting morphological and physiological changes, among them, the modulation of its secondary metabolism. The goal of this research is to evaluate if the conditions of the dry season or rain season increase the accumulation of the alkaloid liriodenine.

Method

Young plants of A. lutescens at the age of one year were obtained and sown in a fragment of the deciduous low-altitude rainforest in Tuxtla Gutiérrez, Chiapas, Mexico. After a three months adaptation period, five young plants were recollected every month, during a year; each plant was taken as an experimental unity for the extraction of alkaloids. These were extracted from roots, stems and leaves by acid-base method. The liriodenine was quantified through HPLC (De-la-Cruz y González-Esquinca, 2012). In order to get a relative density in the seasonal effect, the hydrological potential of the seedlings was measured by a Scholander pressure pump. To compare the variation in the concentration of liriodenine between months and between the rainy season and dry season, it was used the repeated measures analysis of variance (ANOVA) and LSD mean separation technique.

Results/Discussion/Conclusion

Liriodenine is a molecule which is accumulated in the three organs of A. lutescens, its presence in the roots (0.24 μmol.g-1weave) is tenfold higher than in the rest of the seedlings. During the rainy season, the quantity of liriodenine remained stable all around the plant (P≥'3d0.05). When the dry season starts, the alkaloid proportion increases significantly in the roots, but the accumulation of the metabolic isn't apparently affected. The most important effect is observed during the driest month of the year, because the liriodenine reaches levels of 227 μmol.g-1 weave. The accompaniment of the hydrological potential let observe a modeling of the accumulation of liriodenine in respect to the hydrological stress, this metabolic has an inverse correlation with the water disposal for the roots (r= 0.8724 y r2 =0.761). Liriodenine is a molecule with pharmacological and therapeutic potential, in this study it is proved how its production might increase almost three thousand-fold, when young plants of A. lutescens are placed in hydrological stress conditions.

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PO129
ALKALOIDS FROM *Cissampelos tropaeolifolia* D.C.

Morales Escobar, L.[1], Milella L.[2], D'Ambola M.[3], Severino L.[3], Dal Piaz, F. [4], Malafronte N.[4]
[1]*Istituto de Investigaciones Quimicas, Universidad Mayor de San Andres, Calle 27, esq. A. Bello, Cota Cota Campus Universitario, IIQ, Casilla 303, La Paz, Bolivia*
[2]*Department of Science, University of Basilicata, Viale dell' Ateneo Lucano, 10- 85100 Potenza, Italy*
[3]*Department of Pathology and Animal Health, Division of Toxicology, School of Veterinary Medicine, University of Naples Federico II, Naples, NA, Italy.*
4Department of Pharmacia, University of Salerno, Fisciano, SA, Italy
nmalafronte@unisa.it

Introduction

Menispermaceae, a dioecious, largely pantropical family of vines and lianas, consists of approximately 70 genera. The Menispermaceae family is well known for the production of secondary metabolites.1 Cissampelos is a genus of flowering plants in the family Menispermaceae. Cissampelos tropaeolifolia is a plant used in the treatment of different diseases in South American regions. Local people use the aerial parts of the plant to prepare infusions for its relaxant effect and as anti-inflammatory.2 Previous phytochemical studies on species of the Cissampelos genus have revealed the presence of alkaloids.3 Biological study showed that extracts of C. tropaeolifolia were able to modulate the serotonine pathway.4 Despite its use in traditional medicine in South American region, to our knowledge, no data on the chemical composition of the aerial parts of Cissampelos tropaeolifolia are available.

Materials and Method

Aerial parts of Cissampelos tropaeolifolia were collected in Bolivia. The dried plant material was finely powdered and exhaustively extracted for 48 h with acetone by maceration at room temperature. The purification of each extract was accomplished by different chromatographic techniques such as allumina column, Sephadex LH-20, preparative TLC, MPLC, and HPLC.

Results / Discussion / Conclusion

The phytochemical study of Cissampelos tropaeolifolia aerial parts led to the isolation and structural characterization by spectroscopic and spectrometric methods of two morfinan-7-one alkaolid named 2 O-methyl-flavinantine and Fissistigine D. The following known compounds were also identified: caffeic acid, caffeic acid methyl ester, caffeic acid ethyl ester, and p-coumaric acid. The structures of isolated compounds were elucidated by 1D- and 2D-NMR Spectroscopy (1H, 13C, 13C DEPT, DQF-COSY, HSQC, HMBC, ROESY) and confirmed by mass spectrometry studies.

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PO130
LEAF ANATOMY OF TWO *Croton* (EUPHORBIACEAE) AND *Hyptis* (LAMIACEAE) SPECIES AND SECONDARY METABOLITES IDENTIFIED BY HISTOCHEMICAL ANALISIS

Arruda, R. C. O.[1]*; Rosa, A. C.[2], Paula L. P.[2], Victório, C. P. [3]
Universidade Federal de Mato Grosso do Sul, Mato Grosso do Sul, Brazil
[2]Student, Universidade Federal de Mato Grosso do Sul, Mato Grosso do Sul, Brazil
[3]Centro Universitário Estadual da Zona Oeste, Rio de Janeiro, Rio de Janeiro, Brazil
**rosaniarruda@gmail.com*

Introduction

Several studies have indicated nutrient-poor environments and with seasonal water availability tend to be occupied by plants whose leaves display secretory strucutres that could be related with resistance to environmental conditions. These traits include morphological features for physical defense and the production of compounds for chemical defense (Fustenberg-Haag *et al.* 2013). Simultaneously, ethnobotanical studies have indicated that many of these species exhibit properties that lead people to use them as a source of medicaments, especially when living in areas far from large towns and restricted access to manufactured drugs due to the high costs (Maciel et al 2002). The accreditation of a medicinal plant requires multidisciplinary studies, among which we mention the anatomical descriptions, chemical analysis and pharmacological tests. Aims to contribute to the knowledge of four native species (two Hyptis L. and two Croton L. species) considered promising from the point of medicinal view, the study of some species occurring was held in hot nutrient-poor tropical areas subjected to seasonal water provision and that exhibit different secretory structures related with the synthesis of alkaloids, lipophilic substances, phenols, and volatile.

Method

Mature leaves of two to three individuals were taken from the third or fourth nodes from the apex of the branch collected at Mato Grosso do Sul State, Brazil, from a Cerrado area (a Neotropical Savanna). The leaves were fixed in FAA, and preserved in 70% ethanol. The leaves were free-handed, sectioned in longitudinal and transverse planes. The sections were clarified in sodium hypochlorite and rinsed in 1% acetic acid and distilled water. Afterwards, the samples were stained with alcian blue and fucsin 0.1%. Histochemical tests were performed on fresh leaves, sectioned by the free-hand method, and submitted to Sudan III, Sudan IV and Red oil O staining to determine lipophilic compounds, followed by the Nile blue test for acid and neutral lipophilic compounds, and Draggendorff reagent for alkaloids. Histochemical tests were adapted and carried out following Victório *et al.* 2010. Control procedures for histochemical tests were carried out.

Results / Discussion / Conclusion

The analyzed species have external secretory structures: multicellular glandular trichomes containing lipophilic and phenolic substances indicated by histochemical tests. Species of Croton exhibit in mesophyll the laticiferous channels, a hallmark of the Euphorbiaceae family. Latex was demonstrated the presence of neutral and acidic lipids, phenols, and pectin. In Hyptis was recognized the presence of alkaloids. The species examined exhibit a wide range of chemical compounds possibly related with the properties indicated by the use of plants as medicinal. The peculiar leaf structure and the presence of secretory structures could be used for the identification of the species analyzed indicating the potential use of plant anatomy to pharmacognostic recognition.

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PO131
LEAF VOLATILES OF *Neomitranthes obscura* (MYRTACEAE) FROM MASSAMBABA RESTINGA, RIO DE JANEIRO

Victório, C. P.[1]*; Arruda, R. C. O.[2]; Silveira, E. G. P.[1], Azevedo, A. C.[1]; Gama, P. E.[3], Bizzo, H. R.[3];
[1] *Centro Universitario Estadual da Zona Oeste, Rio de Janeiro, RJ, Brazil*
[2] *Universidade Federal de Mato Grosso, Campo Grande, MT, Brazil*
[3]*Embrapa Agroindustria de Alimentos, Rio de Janeiro, RJ*
**cristianevictorio@uezo.rj.gov.br*

Introduction

The Atlantic coast sandbanks (restingas) in Brazil are one of the mapped biodiversity hotspots. This ecosystem presents a diverse flora, and Myrtaceae is one of the most important families in this plant community. The restinga of Massambaba is an environmental protection area created in 1986, with a wide area of sandy coastal plains, lagoons and low hills. Neomitranthes obscura is a large evergreen shrub or small tree, reaching from 1.5 to 5 m high. The genus Neomitranthes is restricted to Brazil and N. obscura is an endemic species of the Brazilian Atlantic Forest. It is popularly known by local population as cambuí-preto. In Brazil, N. obscura is commonly used for intestinal disorders and as food (Santos *et al.*, 2009; Sobral *et al.*, 2014). This study aimed to identify the composition of the essential oil from the leaves of N. obscura collected in the restinga of Massambaba.

Method

Leaves of Neomitranthes obscura N. (DC.) Silveira were collected from three individual plants growing in open shrub formation in the restinga of Massambaba surrounding the Jaconé Lagoon, City of Saquarema, Rio de Janeiro, Brazil. Marcelo da Costa Souza (Rio de Janeiro Botanical Garden, Brazil) The taxonomic identification of N. obscura was performed by Marcelo da Costa Souza (Rio de Janeiro Botanical Garden, Brazil). The oils from the fresh leaves were isolated separately by hydrodistillation for 3 h using a Clevenger-type apparatus. The oils were analyzed in Agilent 7890A GC-FID and 5973N GC-MS equipped with HP5-MS (5%-phenyl-95%-methylsilicone, 30 m x 0.25 mm x 0.25 µm) fused silica capillary columns (n=3). The initial oven temperature was 60°C, then raised to 240°C at 3°C/min and hold for 10 min. Hydrogen was used as carrier gas for FID and helium for MS, both at a flow rate of 1.0 mL/min. The injector was maintained at 250°C and detector (FID) was kept at 280°C. Mass spectra were obtained in electronic ionization mode at 70 eV. Quantification was performed by the normalization method from the electronic integration of the FID peak areas. Constituents were identified by comparison of both mass spectra and GC retention indices with those from NIST and Wiley libraries, as well as literature data (Adams, 2007).

Results / Discussion / Conclusion

The essential oils are composed manly by sesquiterpenes. A high content of β-caryophyllene (39%) was found in N. obscura from Massambaba (Jaconé Lagoon). Limonene and β-ocimene were the main monoterpenes representing 0.3%.

We especially acknowledge the FAPERJ by financial support and the taxonomist Marcelo da Costa Souza for species identification.

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PO132
NALCA (*Gunnera tinctoria*) PROPAGATION AND MORPHOLOGICAL DESCRIPTION OF GERMINATION PROCESS

Vizcarra L.[1]*, Urbina A.[1], Pastene E.[2] and Serri H.[1]
[1]*Facultad de Agronomía,* [2]*Facultad de Farmacia. Universidad de Concepción. Vicente Méndez 595, Chillán.CHILE.*
maurbina@udec.cl, lvizcarra@udec.cl

Introduction

The Gunneraceae family is represented in Chile by the species Gunnera tinctoria, commonly called nalca or pangue. It is a perennial herb that reaches large 2-3 m. tall, fast growing (Riedemann y Aldunate, 2003). Today, this species has gained interest in the pharmacological area, the inhibitory activity of the Gunnera tinctoria extracts on Helicobacter pylori (Hebel et al, 2013).

In the present study, two assays were performed. The first worked with chemical scarification for 0, 5 and 20 minutes and stratification at 4 ° C for 0, 30 and 60 days. In the second test the seeds were subjected to different concentrations of gibberellic acid for 24 hrs. Anatomically, it was described using a Scanning Electron Microscope.

The germination percentage of the assay I was 52% and the highest germination percentage was 89% in the assay II. The results suggest the presence of a primary structure following 21 days of germination process. The parasitic stomata are present on the abaxial surface of the cotyledon which are present in adult leaves (Urbina et al, 2012) The results are shown in photograph poster.

Based on the results it can be concluded that the most effective treatment for a high percentage of germination is GA3 (89%).

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PO133
MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION THE LIFE CYCLE *Lapageria rosea*

Suazo, C.[1], Urbina A.[1], Pastene E.[2], Figueroa, I.[1] and Serri, H.[1]
[1]*Facultad de Agronomía,* [2]*Facultad de Farmacia. Universidad de Concepción. Vicente Méndez 595, Chillán. CHILE.*
maurbina@udec.cl

Introduction

The copihue (Lapageria rosea R. et P.) is a climbing endemic bush from Chile, which belongs to the Philesiaceae family, it is distributed from the Valparaiso Region to Los Lagos Region. The leaves have heart-shaped base and apiculate apex with reticulate midrib. Its flowers are large and hanging, are isolated or grouped in bunches in the axils of the leaves. The flowering period of L. rosea goes from March to May. The fruit is a smooth, ovoid berry in green – yellow color, sweet mesocarp and inside it, there are many yellow seeds wrapped in a hyaline aryl.The copihue is used in a wide number of ways that goes from the ornamentation to medical purposes by the Mapuches. The objectives of this research were to study the biology of L. rosea through the morpho-anatomical characterization of the different phenological states, using photography techniques, light microscopy and electron microscopy. Visits were perfomed to the nursery "La Casa del Copihue" located in Quilacoya and then collected biological material. Morphologically, the samples were observed through magnifying glass and its weight, length and size were evaluated with digital scale and vernier caliper. Anatomically, it was described using a Scanning Electron Microscope. The lifecycle of L. rosea is determined by the three phenological states. It reach the reproductive status by the fifth year.

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PO134
DESCRIPTION OF DEVELOPMENT FROM CUTTINGS AND SEED GERMINATION IN *Eucryphia cordifolia* CAV.

Araya, N.[1], Urbina A.[1], Rodríguez, R.[2] Silva, G.[1] and Serri, H.[1]
[1]*Facultad de Agronomía,* [2]*Facultad de Ciencias Naturales y Oceanográficas.*
Universidad de Concepción. Vicente Méndez 595, Chillán. CHILE
maurbina@udec.cl

Introduction

The Chilean flora has a high level of endemism mainly due to geographic isolation generated by the presence of the Andes, the Pacific Ocean and on the north by the Atacama Desert. E. cordifolia is a characteristic tree of the south of Chile. Its distribution ranges from the Eighth Region to the province of Palena in the Tenth Region. This species has an ecological importance because it is associated with other species such as insects, which are attracted by its white flowers, which are also producers of Ulmo honey. This project aims sexually and asexually reproducing the species E. cordifolia by seed germination and rooting cuttings, as well as describing the morphological changes during the germination process. The seeds were treated with chemical and thermal scarification, stratification treatments well at 4 ° C for 30 and 60 days. To this end it is proposed that the scarification treatments allow softening of the cover increasing seed germination rate. Stratification treatments allow 60 days to break seed dormancy ensuring greater effectiveness of germination. The combined scarification and stratification treatments increase the percentage and rate of germination. In the case of asexual reproduction is stated that treatments indolebutyric acid rooting generate higher compared with treatments where it is not used. For E. cordifolia treatment stratification at 4°C for 60 days achieved a higher percentage and speed of germination, presenting significant differences from the control in the case of chemical scarification with 30 minutes exposure achieved a high germination percentage, however not presented significant differences from the control. For asexual reproduction no positive results were obtained since no rooting of cuttings, unless they appeared in a pair of stakes but were not representative of the test.

PO135
USE OF (S)-3-PHENYLBUTANOIC ACID IN THE SYNTHESIS OF (S)-4-METHYL-3,4-DIHYDRO-1 (2H)-ONE

Laura Orea Flores, Fermín Flores Manuel, Dino Gnecco Medina, Jorge Juárez Posadas, Joel Terán Vázquez, Ángel G. Mendoza Martínez, Araceli Zarate Ruiz

Lab. de Síntesis Orgánica, Centro de Química, Instituto de Ciencias.
Benemérita Universidad Autónoma de Puebla.
Edificio 103H Complejo de Ciencias. C.U. 72570 Puebla, Pue. México.
maria.orea@correo.buap.mx

Introduction

Substituted a-tetralones have played an important role in organic synthesis because they are highly reactive and suitable as starting materials for a wide range of compounds with biological activities, as well as being the precursors of several biologically active natural products.[1]

To date there are a number of interesting publications methodologies to synthesize differently substituted a-tetralones both the aromatic ring and the aliphatic cycle. Some are based on the aromatization of six-membered cyclic systems using conditions extreme and with yields generally moderate, or oxidation of tetralins. There are other reports describing the use of Friedel-Crafts reaction, both for the introduction of the hydrocarbon chain on the aromatic ring, as for the intramolecular cyclization.

The objective of this work is to establish a synthetic methodology for the chiral tetralone from (S)-3-phenylbutanoic acid.

Methodology

For the chiral a-tetralone, first we perform the reduction of (S)-3-phenylbutanoic acid generating the chiral alcohol. This alcohol was treated with HBr accessing brominated compound. The next step was to increase a carbon atom in the hydrocarbon chain of the bromo compound, for this KCN was used and DMSO as solvent, accessing the compound (S)-4-phenylpentanenitrile. The (S)-4-phenylpentanoic acid was obtained from acid hydrolysis of the compound (S)-4-phenylpentanenitrile. The next step was to obtain the (S)-4-methyl-3,4-dihydronaphthalen-1-(2H)-one via intramolecular cyclization of the (S)-4-phenylpentanoic acid. This cyclization is carried out with polyphosphoric acid (PPA), generating the desired compound.

Results / Discussion

Enter a stereogenic center at the benzylic position is very important in asymmetric synthesis. And hidronaftalenos exhibit such stereogenic centers so groups of researchers have shown great interest in synthesizing these compounds. Reduction of (S)-3-phenylbutanoic acid was carried out using LiAlH4 under nitrogen and THF solvent anh. The chiral alcohol was obtained in a yield of 72%. Subsequently, this alcohol was reacted with HBr at reflux for 2.5 h, we obtained brominated compound in 76% yield after being purified by column. Next step was to increase a carbon atom in the hydrocarbon chain of this compound, this is carried out using 1.1 eq. of KCN and DMSO as disolvente. The new compound was obtained as a colorless liquid in 91% yield after being purified by column chromatography. The compound (S)-4-phenylpentanenitrile was treated under acidic conditions and we obtained the corresponding acid in a yield of 70%. Finally we carry out the intramolecular cyclization of (S)-4-phenyl-pentanoic acid using polyphosphoric acid (PPA), so we obtained the (S)-4-methyl-3,4-dihydro-1-(2H)-one

Conclusion

Reaction conditions were established to access the intermediaries (S)-4-phenylpentanenitrile and (S)-4-phenylpentanoic acid. From the acid (S)-3-phenylbutanoic, it was possible to obtain the (S)-4-methyl-3,4-dihydro-1 (2H)-one in good yields. This tetralone will be used in the synthesis of the skeleton of 1,4-dialkyl-1,2,3,4-tetrahydronaphthalene

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PO136
ETNOMEDICINE OF A ZOQUE COMMUNITY: MEDICINAL AND FOOD PLANTS

Luna-Cazáres, L. M.[1]*; González-Esquinca, A. R.[1]; De la Cruz Chacón, I.[1]; Gutiérrez-Jiménez, J[2].; Schlie-Guzmán, M. A.[2]

[1] PhD/Profesor and Research Laboratorio de Fisiología y Química Vegetal, [2] PhD/Profesor and Research Laboratorio de Biología Molecular y Genética. Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas. Libramiento Norte-Poniente No. 1150. Colonia Lajas Maciel. CP. 29035. Tuxtla Gutiérrez, Chiapas, México.

**lorena.luna@unicach.mx; lucas58@hotmail.com*

Introduction

It is remarkable the rich knowledge that the different native human races which inhabit the state of Chiapas still keep. It is necessary to precise that a quantity of this traditional knowledge has been lost because of the environmental damage as well as by the process of acculturation that the group Zoque suffers. This is mainly due to the migration of the population to the cities or to foreign countries in search of better economic opportunities, due also to natural phenomena like the eruption of volcano Chichonal and to the modernization of the indigenous groups promoted by the State. The Zoque community from Rayón still saves and uses a great part of the wisdom of its ancestors in relation to the plants, knowledge that is the result of uncountable observations and empirical experiments of many generations of the native inhabitants.

Method

The field study was made from 1999 to 2004 with periodical visits to the community. Ninety-seven interviews were recorded and transcribed with traditional physicians, parteras, hueseros, yerbateros, and housewives. However, inspections were made in the farm, the mountain, the nearest fields, and the family farms; some pictures of the plants and of inhabitants' certain activities were also taken (Gispert *et al.*, 1979).

Results / Discussion / Conclusion

This study systematizes 62 medicinal species and 31 food species of more than 150 recollected species. 46% of them are wild, 33% are cultivated, 18% are partly wild and cultivated, and 3% has been forced to a wild condition; this reveals that the knowledge of the Zoques from Rayón incorporates in low degree elements of Mestizo's culture. Have been deposited at the Herbarium CHIP Tuxtla Gutierrez, Chiapas, Mexico; specimens corresponding to 45 families, 79 genera and 93 species, increasing the wealth of medicinal and food plants Zoque, enriching the stock of plant resources of Chiapas. The plants that were recollected, considered as medicinal, are used to alleviate different diseases, mainly gastrointestinal, respiratory, muscular and dermatological. Noteworthy forms of preparation and administration nothing simple and ingredients (plant parts) that are measured in semi-quantitative terms, and a deep knowledge of plants, all constitute a therapeutic, whose origins are long past. Of food plants collected 12 are wild, and many were also used as medicinal use of plants dual purpose, not only can cure ailments but also has managed the change and enrichment of the diet of the Zoque people.

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PO137
REVIEW OF MEDICINAL IMPORTANCE OF GENUS *Artemisia* BY RESEARCH PATENTS REQUESTS IN THE BRAZIL

Frickmann, F. d. S. e S.[1]*; Nóbrega, A. B.[2,3]*; Fragoso, T. P.[4]*; Frickmann, F. C.[5]*
[1]PhD/Prof/, Farmanguinhos/Fiocruz,Av. Comandante Guarany, 447, Rio de Janeiro, Rio de Janeiro,Brazil
[2]Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, UFF, Rua Mário Viana N□'3f 523, Santa Rosa, Niterói, RJ, Brazil.
[3]Farmanguinhos, Fiocruz, Rio de Janeiro, Rua Sizenando Nabuco 100, Rio de Janeiro, RJ, Brazil
[4]Farmanguinhos/Fiocruz,Av. Comandante Guarany, 447, Rio de Janeiro, Rio de Janeiro, Brazil
[5]Instituto Virtual Internacional de Mudanças Climáticas(IVIG)/COPPE/UFRJ, Rua Moniz Aragão- Ilha do Fundão, RJ, Brazil
fabianafrickmann@far.fiocruz,br, abnobrega@gmail.com, tainafragoso@gmail.com, fefrickmann@hotmail.com

Introduction

Genus Artemisia is in the family Compositae. These plants are recognized with medicinal in worldwide. In the Orient since V. Sung Dynasty (960-1279 d.C.) Artemisia spp. is used on moxibustion´s technique associated with the practice of acupuncture. In October of 1970, Zhenxing Wei obtained 30mg of a pure crystalline product that was not toxic, the artemisinin (Weina PJ, 2008). Dae-Kyun R. *et al.* (2006) described that Artemisinin, with a sesquiterpene lactone endoperoxide extracted from Artemisia annua L. is highly effective against multi-drug-resistant Plasmodium spp. a lethal malaria parasite. In Brazil, Artemisia absinthium L. is in the National List of Medicinal Plant of interest of the Single System of Health – RENISUS (Teixeira, 2013). The essential oils isolated from Artemisia absinthium L. have potent antifungal and antibacterial activity (Kordali et al., 2005). The objective of this study is analyzing the medicinal importance of the Artemisia genus for Brazil by raised patents applications.

Method

Papers were research in the sites: http://onlinelibrary.wiley.com/advanced/search/results, Google scholar (http://scholar.google.com). Thesis of Oswaldo Cruz Foundation was raised on the webpage (http://ppghcs.coc.fiocruz.br/images/dissertacoes). The Keywords surveyed: Artemisia, moxabustão, moxibustion, artemisinina, artemisinin, Artemisia annua, Artemisia absinthium. All the species names of Artemisia raised were analyzed about accepted for Latin name of species in website the Plant List: http://www.theplantlist.org (accessed on 05/06/2014). The search about patents applications was performed about National Institute of Industrial Property (INPI) database, which is referred to Artemisia compound or preparations. The time interval was the period from 1982 to 2014. The descriptive summaries of applications for patents from Brazil that have genus Artemisia were analyzed and identified the products.

Results / Discussion / Conclusion

Ten different species of the genus Artemisia were found with therapeutically properties in bibliographic review: (1) Artemisia argyi H. Lév & Vaniot, (2) A. vulgaris L., (3) A. siversiana Ehrh. ex Willd., (4) A. princeps Pamp., (5) A. montana (Nakai) Pamp., are used in moxibustion´s technique; (6) Artemisia dracunculus L., (7) A. absinthium L., (8) A. santonicum L.,(9) A. spicigera K.Koch were described with antifungal and antibacterial properties; (10) Artemisia annua L. was indicated with presence of artemisinin and sesquiterpenes used like promising and potent antimalarial. Fifteen patents applications were found with Artemisia in composition. The product patents were used to purpose therapeutically, cosmetics, phytotherapic drugs, immune modulator, herbal medicines, anti-obesity and others. The patents associating resources of the Brazilian genetic asset with traditional knowledge (CTA) have their processes analyzed by the Board of Management of Genetic Resources (CGen). The Resolution 23 of 200 of CGen establishes the mechanism of verification of accomplishment of MP N° 2186-16/2001, for purposes of granting of invention patents by INPI.

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PO138
CONTRIBUTION TO MICROENCAPSULATION OF CALYCES EXTRACT OF *Physalis peruviana*

Toro, R. M.,[1,4*] Aragón, D. M.,[1,2,4*] Ospina, L. F.,[2,4] Ramos, F. A.,[3,5] Castellanos, L.[3,5], Modesti, G.[3]

[1]*Grupo de Investigación en Tecnología de Productos Naturales-TECPRONA*
[2]*Grupo de Investigación Principios Bioactivos de Plantas Medicinales*
[3]*Grupo de Estudio y Aprovechamiento de Productos Naturales Marinos y Frutas de Colombia*
[4]*Departamento de Farmacia- Universidad Nacional de Colombia*
[5]*Departamento de Química-Universidad Nacional de Colombia*
**e-mail: rmtoroa@unal.edu.co, dmaragonn@unal.edu.co*

Introduction

Cape gooseberry (Physalis peruviana L.) is a widely used Andean species in folk medicine for treatment of diseases such as malaria, asthma, hepatitis, dermatitis, and rheumatism1,2. Moreover, according to previous studies this herbal material possesses an important antioxidant and anti-inflammatory activity by the presence of phenolic compounds3. Is important to note that the calyces that enclose the fruits are a waste material in the post-harvest operations of the cape gooseberry. The purpose of this work was to realize assays to microencapsulate an extract from calyces of Physalis peruviana. The benefits of encapsulating phenolic compounds in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release. Incorporation of antioxidant compounds in manufactured foods, nutraceuticals or cosmetic preparations is a growing area of research.

Method

The dried and powdered Physalis peruviana calyces were extracted by percolation with ethanol (EtOH). Emulsification-solvent evaporation was used as the microencapsulation method. In order to evaluate the influence of some variables in Eudragit® RS100 microspheres loaded with the extract, a statistic experimental design called Plackett-Burman was used, with seven variables at two levels. The following variables were studied: A) Eudragit® RS100 concentration (4 and 2%), B) PVA concentration (4 and 2%), C) Extract - Eudragit® RS100 ratio (1:1 and 1:2), D) temperature agitation (35 an 25°C), E) aqueous phase volume (50 and 100 mL), F) stirring speed (11000 and 24000 rpm), and G) stirring time (5 and 2 min). The response variables were: process yield, encapsulation yield, encapsulation efficiency, loading extract and particle size.

Results / Discussion / Conclusion

The microparticles of the extract were obtained by emulsification-evaporation of solvent. The following analysis was performed according to the top level of each variable, according to results of statistical analysis Plackett - Burman:

1. The yield of the process is positively affected by the variables F and G, and negatively by the variables C and D.
2. The particle size is positively affected by the variables E and G.
3. The extract loading is positively affected by the variable A, and negatively by the variables B, E and G.
4. The encapsulation efficiency is affected positively by the variable A, and negatively by the variable B and E.
5. The encapsulation efficiency is positively affected by the variables C and D, and negatively by the variable B.

According to the results the experimental conditions that produce an increased yield of microparticles and encapsulation, the extract loading, and the encapsulation efficiency, as well as a decrease of the particle size were selected. These variables are: Eudragit ® RS 100 4%, PVA 2%, Extract - Eudragit® RS100 ratio 2:1, stirring temperature 35 ° C, 100 mL of aqueous phase, stirring intensity of 11000 rpm and stirring time 2 minutes.

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PO139
THE *IN VITRO* BIOACTIVITY OF *Santolina corsica* JORD. & FOURR. (ASTERACEAE) EXTRACT

Tundis, R.[1]; Bonesi, M.[1]; Peruzzi, L.[3]; Bedini, G.[3]; Menichini, F.[1]; Bruno, M.[2]*; Loizzo M. R.[1]
[1]*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende (CS), Italy*
[2]*Department STEBICEF, University of Palermo, Viale delle Scienze, Parco d'Orleans II - 90128 Palermo, Italy*
[3]*Department of Biology, University of Pisa, Pisa, Italy*
**maurizio.bruno@unipa.it*

Introduction

Santolina species (Asteraceae) have been investigated for their ethnopharmacological uses which include anti-inflammatory, antispasmodic, antiseptic, antihelmintic, antimicrobial, antiproliferative, and antioxidant activities (Baig *et al.*, 1989; Sala *et al.*, 2000; Appendino *et al.*, 2005; Ruiz-Navajas *et al.*, 2013).
Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia and alterations in carbohydrate, lipid and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. The high prevalence of diabetes as well as its long-term complications has led to an ongoing search for hypoglycaemic agents from natural sources (Nicasio-Torres *et al.*, 2009). One therapeutic approach to treat the early stage of diabetes is to decrease postprandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, a-amylase and a-glucosidase, in the digestive tract. Consequently, inhibitors of these enzymes determine a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise (Tundis *et al.*, 2007). Interest in the search for new natural antioxidants has grown dramatically over the past years because reactive oxygen species (ROS) production and oxidative stress have been shown to be linked to a large number of human degenerative diseases, including diabetes. Taking into account these considerations, the objective of the present study was to determine the chemical composition, the antioxidant and hypoglycaemic properties of Santolina corsica Jord. & Fourr.

Method

S. corsica aerial parts were collected on ophiolithic substrate about 2 km north of Corte, Corsica (France) and were subjected to maceration using the n-hexane. The extract was analysed by gas chromatography (GC) and gas chromatography - mass spectrometry (GC-MS). In order to evaluate the hypoglycaemic potential S. corsica non polar extract, a-amylase and a-glucosidase inhibition assays were also performed (Menichini *et al.*, 2011). The radical scavenging activity was investigated through DPPH assay (Bonesi *et al.*, 2013).

Results / Discussion / Conclusion

This study reports for the first time the S. corsica antioxidant properties and hypoglycaemic activity via the inhibition of a-amylase and a-glucosidase enzymes. The n-hexane extract, characterized mainly by the monoterpenes β-myercene, β-phellandrene, 1,8-cineole, terpinen-4-ol, demonstrated a concentration-response relationship in all investigated tests. S. corsica exhibited a selective inhibitory activity against a-amylase with an IC50 value of 189.0 μg/ml. Moreover, antiradical scavenging effects in DPPH test were observed (IC50 value of 68.8 μg/ml). It is noteworthy that the antiradical and hypoglycaemic properties of the extract could be attributed to the presence of phenolic monoterpenes (Masteliæ *et al.*, 2008). However, the individual and synergistic bioactivities of other identified compounds could have contributed to the observed effects. Further studies could be done on the identification of effective active compounds in S. corsica. To confirm the obtained results, additional *in vivo* studies are needed.

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PO140
XANTHINE OXIDASE INHIBITORY EFFECT OF *Tamus communis* ROOTS EXTRACT

Fatima ZERARGUI[1], Abderrahmane BAGHIANI[1]*, Sabah BOUMERFEG[1], Meriem DJARMOUNI[1], Lekhmici ARRAR[1], Noureddine CHAREF[1], Seddik KHENNOUF[2], Musa H. Abu Zarga[3]*

[1] *Laboratory of Applied Biochemistry, Faculty of Nature and Life Sciences, University Setif1, Setif 19000, Algeria*

[2] *Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Setif1, Setif 19000, Algeria*

[3] *Chemistry Department, Faculty of Science, The University of Jordan, Amman 11942, Jordan.*

Abstract

In the course of our phytochemical studies of plant Tamus communis L., The methanol soluble extract (138 g) was chromatographied on a silica gel column. The column was eluted with chloroform and then with chloroform /methanol mixtures of increasing polarity. A total of 52 fractions (400 ml each) were collected and grouped according to their TLC behaviour into 11 main fractions (I-XI). Total phenolic and flavonoid contents in these extracts were determined by a colorimetric method. Values varied between 73,143±0.009 and 29,214±0.003equivalent gallic acid/g lyophilisate. All the extracts showed an inhibitory properties on xanthine oxidase, the IC50 ranges from 0,02948481±0,01703 mg/ml to 0,23685279±0,0256 mg/ml. The extracts exhibited an additional superoxide scavenging capacity by using both enzymatic methodsand IC50 values range from 0,03957896±0,02312 mg/ml to 0,14121141±0,08630 mg/ml. These results show that Tamus communis L. extracts have strong anti-oxidant effects and may have some clinical benefits.

Keywords: Xanthine oxidase, Antioxidant, Superoxide scavenger, Tamus communis L.

PO141
BIOACTIVITY OF *Salix martiana*

Guimarães, T. X.; Souza, M. C. S.; Fachin-Spinar, M. T.; Nunez, C. V.*
Laboratório de Bioprospecção e Biotecnologia, Instituto de Pesquisa da Amazônia, Av. André Araújo, 2936, 69067-375 Manaus- AM, Brasil
**cecilia@inpa.gov.br*

Introduction

The species Salix martiana is known as "oeirana" or "oeirana branca" and is used for diabetes treatment by folk medicine, by the people who live close to the Amazonas river (Fernandes, 2002). The Salix genera contains analgesic, antipyretic, and anti-inflammatory agents (Jeon, 2008). The present study aimed to investigate the bioactive potential of leaves and branches extracts of S. martiana.

Method

Branches and leaves were extracted with dichloromethane, methanol and water. The extracts were analyzed for their antioxidant capacity by using DPPH and Fe3+/Phenanthroline methods. The toxicity assay against Artemia salina was made at the concentration range from 1000 to 30 μg/mL (Sam, 1993). The determination of antibacterial activity was made by diffusion in agar method, were 20 μL solution of each extract (from a solution of 20 mg/mL) were placed in the agar surface, and 1 μL solution of oxytetracycline (from a solution of 0.2 μg/mL) was used as standard.

Results / Discussion / Conclusion

Both methanol extracts showed high antioxidant activity, by using DPPH method. The Artemia salina toxicity assay showed high activity of dichloromethane extract of branches as well as of leaves and branches methanol extracts. The results for antibacterial assay indicate activity against Bacillus cereus, Klebsiella pneumoniae, Nocardia brasiliensis, Pseudomonas fluorescens and Serratia marcescens.
The high antioxidant, antibacterial and toxic activity to A. salina activity of the extracts of S. martiana encourage the chemical fractionation to find the active substances.

Extract	Antioxidant (mg extract/mg ascorbic acid)	*Artemia salina* (30 μg/mL em %)	Antibacterial (cm)				
			Bacillus cereus	*Klebsiella pneumoniae*	*Nocardia brasiliensis*	*Pseudomonas fluorescens*	*Serratia marcescens*
Leaves DCM	23.8	67	1.1	0.9	In	1.2	0.7
Branches DCM	23.0	100	1.9	0.9	In	1.0	In
Leaves MEOH	1.6	70	1.7	0.9	1.1	1.2	In
Branches MEOH	1.7	90	1.1	0.9	In	0.9	0.8

Leg: In: Inactive

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PO142
INHIBITION OF HELA PROLIFERATION CELLS IN ATHYMIC MICE BY LAHERRADURIN, AN ANTITUMOR ANNONACEOUS ACETOGENIN

Schlie-Guzmán, M. A.[1*]; González-Esquinca, A. R.[1]; Luna-Cazáres, L. M.[1]; Gutiérrez-Jiménez, J.[1]
[1]*Doctora, Universidad de Ciencias y Artes de Chiapas, Instituto de Ciencias Biológicas. Libramiento Norte Pte. 1150 Colonia Lajas Maciel, Tuxtla Gutiérrez, Chiapas, 29039. México*
**adelina.schlie@unicach.mx*

Introduction

Plants of the annonaceae family have been used in diverse cultures for alimentary and medicinal purposes. Annonaceous acetogenins is a group of potent bioactive secondary metabolites that are exclusive to this family (Schlie-Guzmán *et al.*, 2009). laherradurin is one of these acetogenins, and it has been reported to have antiproliferative effects on several cancer cell lines, and strong inhibition of mitochondrial electron transport with a specific action at complex (Tormo *et al.*, 2003).

Method

6-week-old athymic mice were injected subcutaneously into its back with 1 x 106 human cervical carcinoma cells (HeLa cells). After 24 h, groups of five mice received subcutaneously at the base of the growing tumor different concentrations of the acetogenin o doxorrubicine as reference drug dissolved in 0.1mL of 0.9% NaCl solution, once daily for two consecutive weeks. Control animals were given 0.1mL vehicle solution.
Tumors were measured on alternate days using a vernier caliper from the beginning of treatment and its size in mm3 was calculated using the formula [(length x width2) / 2], where width represents the shortest dimension.

Results / Discussion / Conclusion

A 20-day *in vivo* evaluation of laherradurin against HeLa implanted cells in athymic mice showed interesting potential. Mice inoculated at the base of the tumor with laherradurin at a dose of 0.150 mg kg-1 day-1, showed a 44% inhibition of tumor growth (Treated/Control) at day 16th, and a 56% inhibition (Treated/Control) was observed at day 12th with a dose of 1.5 mg kg-1 day-1. Doxorrubicine treatments were as effective as laherradurin at the same time periods (T/C: 50.4 and 70%, respectively). These results suggest that laherradurin is potentially a promising anti-cancer compound.

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PO143
PHYTOCHEMICAL INVESTIGATION OF ALKALOIDS EXTRACTED FROM THE ECUADORIAN PLANT *Macrocarpaea lenae* J.R. GRANT

Gilardoni, G.[1]*; Armijos Riofrio, C. P.[2]; Iñiguez Iñiguez, N. J.[3]; Andrade Morocho, J. M.[4]; Malagón Avilés, O. G.[2]

[1]*PhD Prometeo, Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
[2]*PhD, Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
[3]*Student, Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
[4]*M.Sc., Universidad Técnica Particular de Loja, Dep. of Chemistry, San Cayetano Alto, Loja, Ecuador*
**gianluca.gilardoni@gmail.com*

Introduction

Macrocarpaea lenae J.R. Grant is a plant belonging to the family Gentianaceae, growing in Colombia, Ecuador and Peru between 1000-3500 m a.s.l., mainly on the eastern Andean slopes. The genus Macrocarpaea counts 75 recognized species, more than 30 of which are present in Ecuador. The species Macrocarpaea lenae J.R. Grant was classified for the first time in 2003 [1], after being collected in the Parque Nacional Podocarpus, in the Zamora-Chinchipe province; however the samples employed in this studies were recollected near the town of Saraguro, in the province of Loja. The collected samples were identified as Macrocarpaea lenae J.R. Grant at the herbarium of the Universidad Nacional de Loja (UNL), a specimen voucher is kept at the Universidad Técnica Particular de Loja (UTPL). In fact this plant is employed in the traditional medicine of the Saraguro people with the name of Tabaco de Cerro, were it is applied to the treatment of cold and of some supernatural diseases, such as mal aire (evil eye) and to purify the house. For treating cold, the dry leaves are burned and the patient's clothes passed through the smoke; for the treatment of mal aire the fresh branches with leaves are passed over the patient's body; for purifying the house the dry leaves are burned indoor, in order to flood the house with smoke. All these operations must be accomplished by a healer, called Yachak, from the Saraguro community. From the bibliographic viewpoint, this is the first chemical study on this species; moreover the entire genus Macrocarpaea counts only of one phytochemical publication [2], about the extraction of xanthones from Macrocarpaea glabra.

Method

The dried leaves were grinded and the alkaloids extracted through a classical acid-base aqueous extraction, producing an extract richer in alkaloids; these alkaloids are the object of the present study. At the date of this abstract, preliminary phytochemical analyses were performed; they will be followed by preparative liquid chromatography purification and characterization through NMR spectroscopy and MS spectrometry techniques.

Results / Discussion / Conclusion

Preliminary experimental evidences on the alkaloidal extract show the presence of at least two alkaloids, positively responding to Dragendorff's reagent. The chemical structure of these metabolites is at the moment under study; if possible some biological activities on the same compounds will be evaluated.

This study is part of the PROMETEO PROJECT and is conducted with the financial support and patronage of the Secretaría de Educación Superior, Ciencia, Tecnología e Innovaciòn of Ecuador (http://prometeo.educacionsuperior.gob.ec).

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PO144
PHYSICAL AND CHEMICAL CHARACTERISTICS OF CANOLA HYDROLYSATES PROTEIN ASSOCIATED WITH DIFFERENT PROFILES OF ANTIOXIDANT ACTIVITY

Anaid, Hernández Jabalera; Davila, Ortiz G.; Cristian, Jiménez Martínez
Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional. Prol. Carpio, Esq. Plan de Ayala S/N, Col. Casco de Santo Tomás, Del. Miguel Hidalgo, 11340 México, D.F., México.
Email: anaid.hdez.j@gmail.com; crisjm_99@yahoo.com

Introduction
The by product of oil canola production has been proposed as a suitable material for obtaining protein isolates, in these proteins there are bioactive peptides whose amino acid composition allows presenting antioxidant activity (AA); but there are also inactive peptides and phytochemicals associated with them; therefore the aim of this work was to define the most suitable criteria for the enrichment and purification of the responsible fragments for AA through identifying hydrolysates characteristics, associated to antioxidant profile criterion.

Method
Antioxidant activity (AA) was assessed in 36 hydrolysates obtained from a protein concentrate and a protein isolate (> 90 %) by the sequential action of Alcalase - FLavorzima (AF) or Pepsin, Pancreatin (PP) throught 7 in vitro methods and assay in line Caco-2 cells. An analysis of latent profiles (APL) for segmentation and grouping hydrolysates based on the existence of different profiles of AA was used, and to test the theoretical insights relating to the size of the peptides, the degree of hydrolysis and amino acid composition determined in mixtures with each of the profiles antioxidants multinomial analysis was used.

Results / Discussion / Conclusion
According to the segmentation we can infer that the shared characteristics of the hydrolyzed product of the specificity of the enzymes which were obtained (type of amino acid in the terminal position) are largely determining the hydrolysis time to preserve a pattern of features antioxidants determined. The same effect occurs for the presence of phenolic compounds. Membership in the group 3, characterized by having the largest number of antioxidant capacities belong to the group having a type 2 pattern all odds ratios are significant so that; for each additional unit in the degree of hydrolysis opportunities belong to the group having a type 3 with respect to type 2 pattern in 23 % increase. The odds of a sample with an additional unit of Hys+Lys+Arg 266 are sometimes opportunities present the type 3 pattern in relation to type 2 compared to a sample without that additional unit. In contrast with an increase in a unit of nonpolar amino acids the chances of having a type 3 pattern regarding the type 2 sample are 70 % lower compared to those without one additional unit. The concentration of Hys, Arg and Lys also resulted significant opportunities to influence belong to the group that shares a type 4 pattern characterized by exhibiting greater activity in Caco -2 cells as the chances of a sample becomes 2.62 times of a sample without an additional unit of these amino acids.
The increase in GH and the increased concentration of the Hys, amino acids Arg and Lys in peptide mixtures is associated with greater opportunities to provide patterns of properties characterized by enhanced cellular antioxidant activity.

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PO145
CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OILS FROM AERIAL PARTS OF *Sideritis syriaca* L. SSP. *nusairiensis* (POST) HUB.-MOR

Felice Senatore[1], Carmen Formisano[1], Filomena Oliviero[1], Daniela Rigano[1]
[1]*Department of Pharmacy, University of Naples "Federico II", Via D. Montesano 49, I-80131 Naples, Italy*

Introduction
The genus Sideritis (Lamiaceae) consists of more than 150 species, occurring mainly in the Eastern and Western Mediterranean regions The taxonomic classification of the genus is rather complex. The chemical investigations on Sideritis were concerned with flavonoids, essential oils, and especially diterpenoids. Diterpenoids occur in almost all the species and show a remarkable variability of carbon skeleta. Many species of the genus have been used for a long time in the traditional folk medicine of several countries, especially in the Middle East, as a herbal tea to treat different illnesses. In the continuation of our investigations on the essential oils of Sideritis species, in this paper we report the composition and the antioxidant activity of the essential oil obtained from aerial parts of S. syriaca L. ssp. nusairiensis (Post) Hub.-Mor.

Method
The oil from air-dried and ground aerial parts of the plant was isolated by hydrodistillation for 3 h, using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia. The GC and GC/MS analyses on a HP 5MS column evidenced eighty-six constituents, representing 91.4% of the total oil. Antioxidant activities were evaluated using both chemical DPPH and FRAP assays.

Conclusion
The most abundant compounds detected in the oil were β-pinene (8.9%), a-pinene (5.9%), pentacosane (3.7%), pinocarvone (3.6%), β-phellandrene (3.5%) and carvacrol (2.5%). The essential oil of S. syriaca showed a weak activity for FRAP test, meaning that it has an average ferric reducing/antioxidant power.

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PO146
OCHRATOXIN A OCCURRENCE IN SICILIAN BIOLOGICAL WINES

Gentile, F.; La Torre, G. L.*; Potortì, A. G.; Saitta, M.; Alfa, M.; Dugo G.
Università di Messina - Dipartimento S.A.S.T.A.S., V.leF. Stagno d'Alcontres,31, 98166 Messina, Italy
**llatorre@unime.it*

Introduction

Ochratoxin A (OTA) is the main mycotoxin detected in grapes and wines and is considered one of the most harmful contaminants to human health. The presence of OTA in wine has been reported by several authors from the entire world since its first description in 1996 (Zimmerli *et al.*, 1996) and until now OTA and its related compounds are the only mycotoxins detected in wine (Remiro *et al.*, 2012).
In the present work we aimed to estimate the presence and levels of OTA in Sicilian wines made ÿ'3fÿ'3ffrom grapes of vines subjected to organic farming and produced according to standardized method of vinification that restricts the use of sulphites and that also provides precise strict rules on winemaking techniques and processing aids.

Method

A fast reversed-phase UPLC method was utilized for OTA determination in 55 different wine samples (red and white) produced in Sicily and elaborated from the most diffused grapes of vitis vinifera varieties. All the grapes come from vines bred spalliera, a vertical-trellised training system characterized by winter and green pruning, with frequent skim. The treatments against the vine diseases were biological: copper sulphate was used against powdery mildew until the closing of the bunch; against downy mildew it has used the Bordeaux mixture. The vinification was carried out for each wine sample, according to Implementing Regulation (EU) N. 203/2012, that established detailed rules on organic wine.
Analytical methods included the clean-up purification by using commercial immunoaffinity columns and an Aquity UPLC® system equipped with a RF detector; the sensitivity of the analytical method was 0.01 $\mu g\ L^{-1}$.

Results / Discussion / Conclusion

Our results indicate that the red wine samples proved to be more contaminated with OTA than the white wines, which is in accordance with previous reports from Europe for no biological wine (Bejaoui H *et al.*, 2006). The higher levels of OTA contamination in red wine compared with white wine is probably due to the characteristics of the winemaking process, especially because of the maceration of the grape. According to Lasram *et al.* (2008) this phase is when most of the OTA extraction from grapes into wine occurs, suggesting that grape skin is the main source of OTA. OTA was detected in all wine samples analyzed and the levels were always below the maximum tolerable EU limit (2 $\mu g\ L^{-1}$). The detected levels ranged from 0.021 to 0.112 $\mu g\ L^{-1}$ for the white wines (mean value 0.052 $\mu g\ L^{-1}$), and from 0.081 to 0.711 $\mu g\ L^{-1}$ (mean value 0.331 $\mu g\ L^{-1}$) for the red wines.
The incidence of OTA in wines produced according organic farming and winemaking was not greater than in normal commercial wines and the results of this study indicate a low risk of exposure to OTA by consumption of these biological wines. In addition, considering that the average wine consumption in Italy is 400 mL day^{-1}, that the average body weight of an Italian man is 70 kg and that the highest level of OTA, determined for these wines was 0.711 g L^{-1}, we can estimate a maximum intake of ochratoxin weekly equal to 1.96 g L^{-1} (daily 0.28 g L^{-1}), which represents only 23% of the weekly dose allowed.

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PO147
DETECTION OF AN ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF BENZIMIDAZOLES IN SHEEP AND CATTLE MILKSAMPLES

Pellegrino, E. [1]; La Scala, L.[1]; Alongi, A.[1]*; Polizzotto, G.[1]; Giaccone, V. [1]; Macaluso, A.[1]; Ferrantelli, V.[1]

[1] *Istituto Zooprofilattico Sperimentale della Sicilia, Via Gino Marinuzzi n.3, Palermo, Italy, Europe*
**alongi.angela@gmail.com*

Introduction

The benzimidazoles are broad-spectrum drugs used in the veterinary field having anti helminthic and antiprotozoalaction against intestinal and tissue parasites. They inhibit the tubulin assembly in the cytoplasmic microtubules,alter the absorption of glucose with decreased ATP, and inhibit fumarate reductase specification of worms. In thisstudy, milk samples of sheep and cattle in Sicily were tested by confirmatory method for the detection ofbenzimidazoles (albendazole, sulfoxid-albendazole, sulfon-albendazole, flubendazole, oxibendazole, febendazole,thiabendazole) and one benzimidazole metabolite (febantel) using Liquid Chromatography Tandem MassSpectrometry (LC-MS/MS).

Method

Sheep and cattle milk samples (n. 53) were extracted using QuEChERS. Samples were weighted into 50 ml centrifuge tubes. Internal Standard, albendazolo-d3, was added in all samples. Fortification of samples was carriedby addiction of mixed working standards solution. Acetonitrile was added and the samples were placed at -20°C for15 min. SPE Citrate Extraction Tube was added, shaken vigorously for 30 sec and then centrifuged for 10 min at3.500 rpm at +4°C. The supernatant was transferred into SPE Cleanup Tube, shaken vigorously for 30 sec and filtered through a 0.2 µm filter before LC-MS/MS analysis. The detector used was a triple quadrupole mass spectrometer, ESI Source.

Results / Discussion / Conclusion

53 milk samples of sheep and cattle have been tested. All samples were negative for benzimidazoles. The validation data has been evaluated in accordance with the Commission Decision 2002/657/EC. Since benzimidazoles are used in both prophylactic and therapeutic way against parasites, they can cause chemoresistance, aversion, vomiting, loss of appetite, increased thirst and variation volume of urine output. The use of veterinary drugs can cause the presence of residues in food of animal origin that, without adequate interruption periods, can become an hazard toconsumers.

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PO148
DETECTION OF NITROFURAN METABOLITES IN USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Alongi, A.[1]*; La Scala, L.[1]; Vella, A.[1]; Ferrantelli, V.[1]
[1]PhD/Prof/other, University/Institution, Address, City, State, Country
*alongi.angela@gmail.com

Introduction

In the industrial system of meat production the nitrofuran antibiotics are employed for the treatment of bacterial diseases in animals. They were banned from use in the European Union due to the carcinogenicity effects of their residues on humans European Community (Reg. 2377/90). Since nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoin) are rapidly metabolized, is more reliable the detection of their strongly tissue-bound metabolites. Thus the analysis of these compounds is based on the determination of their toxic metabolites: 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), semicarbazide (SEM) and 1-aminohydantoin (AHD). We have developed and validated an highly sensitive and specific analytical methods fordetecting of residues of nitrofuran metabolites in meat and eggs using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Method

Samples preparation required no longer exposure to direct light because sensivity of nitrofuran metabolites. The extraction procedure consists in hydrolysis and derivatization, neutralization and extraction with clean-up. Homogenized meat of different animal species and eggs was weighted into a 50 ml polypropylene dark tube. Internal standard (AMOZ-d5) was added in all of the samples while mixed working standards solution was added into fortified samples. HCl 0.25M and 2-NBA 0.1M fresly prepared in methanol were used for hydrolysis and derivatization steps. The samples were stored in the dark at 37°C overnight (16-18 hours). After cooling to room temperature, the pH were neutralized and etyl acetate was addictioned. After evaporation under nitrogen flow at 40°C, the extract was reconstituted and the autosample was charged. The analyses were performed on a ultraperformance liquid chromatography system. The mass spectrometer was a triple quadrupole set in the positive electrospay ionization (ESI) mode.

Results / Discussion / Conclusion

A sensitive and reliable method has been developed for detection of low level of nitrofuran metabolites in meat of different animal species and eggs (1 μg/Kg) using LC-MS/MS. It is a rapid method with a short chromatographic run time of six min for each sample. Since the carcinogenic effect to humans of these banned compounds, it's very important to monitor these residues according to requirements in Commission Decision 2002/657/EC. The procedure is simply and sensitive for routine analysis.

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PO149
DEVELOPMENT OF A RAPID METHOD FOR DETECTION OF NITROIMIDAZOLES IN ANIMAL BLOOD SERUM USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

Alongi, A.[1]*; La Scala, L.[1]; Lo Cascio, G.[1]; Macaluso, A.[1]; Ferrantelli, V.[1]
[1] *Istituto Zooprofilattico Sperimentale della Sicilia, Via Gino Marinuzzi n.3, Palermo, Italy, Europe*
**alongi.angela@gmail.com*

Introduction

The nitroimidazoles, which include metronidazole (MNZ), dimetrazole (DMZ), ronidazole (RNZ), ipronidazole (IPZ), are a group of drugs with activity against anaerobic bacteria and parasitic infections and have been used in livestock as effective therapeutic and prophylactic agents. Studies have shown a direct genotoxic effects in human for some nitroimidazoles therefore, their use in veterinary practice, is banned and regulated within the European Community (Reg. 2377/90) due to the suspected carcinogens and mutagens activity. We have developed a rapid and sensitive methods for detecting nitroimidazoles in animal blood serum by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Method

Bovine blood serum was fortified with the internal standards DMZ-d3 and RNZ-d3. Acetonitrile and NaCl were added. After centrifugation, hexane was added to the top organic layer for clean-up and then it was discarded. The extract was evaporated under nitrogen flow and reconstituted in 5% of methanol and filtered through 0.2 μm filters. An aliquot was injected into liquid chromatography column.The analyses were performed on a ultraperformance liquid chromatography system. LC separation was made using
0.1% formic acid in water (mobile phase A) and methanol (mobile phase B). The compounds were detected in the multiple reaction monitoring (MRM) mode in the MS/MS analyzer applying two ion transitions for a molecule. Mass spectrometer was a triple quadrupole set in the positive electrospay ionization (ESI) mode.

Results / Discussion / Conclusion

A multi-residue methods has been developed for a simultaneously identified six nitroimidazole compounds in animal blood serum using LC-MS/MS. It is a rapid method with a short chromatographic run time of six min for each sample. Since the carcinogenic and mutagenic effect to humans of these banned compounds, it's very important to monitor these residues in blood serum according to requirements in Commission Decision 2002/657/EC. The aim of this work to develop a rapid method for detection of nitroimidazoles has been successfully achieved, but further investigation is required to complete confirmatory validation method for animal blood serum matrix and to performe analysis for other different matrix.

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PO150
PROFILAXIS OF VULVOVAGINAL CANDIDIASIS FROM *Candida krusei*: IN VIVO ASSAY WITH *Syngonanthus nitens* EXTRACT INCORPORATED OR NOT INCORPORATED INTO NANOESTRUCTURED SYSTEM

Ramos, M. A. S.[1], Toledo, L. G.[3], Calixto, G. M. F.[1], Guioti, F.[1], Bonifácio, B. V.[1], Negri, K. M. S.[1], Silva, P.B.[1], Santos, L. C.[2], Almeida, M. T. G.[3], Chorilli, M.[1], Bauab, T. M.[1]

[1]*School of Pharmaceutical Sciences of São Paulo State University, Araraquara- SP, Brazil*
[2]*Chemistry Institute of São Paulo State University, Araraquara-SP, Brazil*
[3]*School of Medicine of São José do Rio Preto, SJRio Preto-SP, Brazil*
bauabtm@fcfar.unesp.br

Introduction

The number of cases of vaginal infections caused by Candida krusei is grows sharply which is worrying because of the intrinsic resistance of this species presents to drugs derived from azoles, such as fluconazole. In this sense, the search for alternatives that act preventively in the development of vulvovaginal candidiasis caused by this pathogen show to be important and necessary. The Syngonanthus nitens Bong. Ruhlland (Eriocaulaceae) is evident in the microbiological area, presenting important as antiulcerogenic and antimicrobial profiles [1]. The pharmaceutical nanotechnology that allow the improvement of pharmacological parameters of active principles synthetic and natural, as the use of precursors systems of liquid crystal (PSLC) [2]. The aim of this study was to realize an experimental *in vivo* assay of vulvovaginal candidiasis caused by C. krusei in order to verify the prophylactic potential of the methanol extract of S. nitens incorporated or not incorporated in a nanostructured precursor system of mucoadhesive liquid crystals.

Method

We used female rats Wistar (Rattus novergicus) with 8 weeks of age and weight of 150-200 g. The experimental groups (6) were established with four animals in each one: Group 1- negative infection, Group 2 - without immunosuppressive agent, Group 3 - with immunosuppressive agent, Group 4 - treated with amphotericin B, Group 5 - treated with the extract not incorporated (250 ug/mL), Group 6 - treated with extract incorporated in PSLC (250 ug/mL). The PSLC containing 50% of oleic acid as the oily phase, 10% of a polymer dispersion (Carbophol 974P 0.5% + 0.5% Policarbophyl-P) as aqueous phase and 40% of Procetyl AWS- as surfactant agent and mucoadhesive. Initially, the animals received their respective treatments 10 days before to infection and immunosuppression. The animals were treated with estradiol solution (20 mg/b.w. subcutaneously) twice daily for 5 days before the infection to induce estrus pseudo state. After 10 days of treatment the animals were immunosuppressed with cyclophosphamide (50 mg/b.w. intraperitoneally) and subjected to infection by a fungal suspension of C. krusei (ATCC 6258) 5x106 cell /mL vaginally. After 2 days of infection, was realized vaginal washes with sterile PBS and plated on Sabouraud dextrose agar with chloramphenicol for to determine whether there has been the establishment of infection. The animals were treated for 10 days and cultures of vaginal fluids were taken on days 2, 4, 6, 8 and 10. The treatments were performed with intervals of 12 hours during 22 days. At the end of the experiment all animals were euthanized in a CO2 chamber [3].

Results / Discussion / Conclusion

The animals treated with the extract incorporated into the PSLC not developed the infection and remained healthy throughout the experiment. Those treated with extract not incorporated developed the disease state and the remission of the disease was until the 6th day of analysis, where all were considered cured. We emphasize the promising prophylactic action exerted by the extract incorporated in PSLC as compared the action of the extract not incorporated, which are justified in which the incorporation in nanostructured systems enhances the biological activity of the plant, and in addition to its characteristic mucoadhesive, which promoted greater interaction of the drug with the route of administration.

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PO151
IN VITRO SYNERGISTIC AND ANTIMICROBIAL PROFILE OF THE *Astronium urundeuva* EXTRACT AGAINST *Helicobacter pylori*

Duarte, M. L. B.[1], Ramos, M. A. S.[1], Loureiro, R. M.[1], Bonifácio, B. V.[1], Negri, K. M. S.[1], Souza, L. P.[2], Vilegas, W.[2], Bauab, T. M.[1]

[1]*School of Pharmaceutical Sciences of São Paulo State University,Araraquara-SP, Brazil.*
[2]*Coastal Campus of São Vicente of São Paulo State University,São Vicente-SP, Brazil.*
bauabtm@fcfar.unesp.br

Introduction

The search for new drugs that have activity against Helicobacter pylori has observed over the past decade, due to the high rate of diseases caused by this microorganism, such as ulcers, gastric inflammation and stomach cancer. In this sense, the study of medicinal plants is attractive, due to the lower rates of toxic effects and the high level of resistance to conventional drugs. Astronium urundeuva (Allemão) Engl. (Anacardiaceae) is used in Brazilian traditional medicine for the treatment of various diseases, and has as its main phytochemicals constituents the essential oils and hydrolysable and condensed tannins. The study of this extract is promising for the discovery of new therapeutic potential as antibacterial and synergistic effect. [1]. The aim of this study was to determine the antibacterial and synergistic activity of the ethanol extract of leaves of Astronium urundeuva (AUL) against Helicobacter pylori.

Method

For the performance of the two in vitro experiments was employed H. pylori (ATCC 43504) strain at a concentration of 1.0 x 107 cells / mL. The minimum inhibitory concentration (MIC) was performed by microdilution technique [2] with modifications. Amoxicillin in 500 μg/mL was used as positive control. The synergistic effect of AUL was verifield with omeprazole (OMP - 5 μg/mL), clarithromycin (CLR - 125 μg/mL) and amoxicillin (AMX - 250 μg/mL) individually and in combination (triple drug), was evaluated by the microdilution technique modified for checkerboard assay [3]. The data obtained was determined the fractional inhibitory concentration index (FICI) being considered as synergistic values ÿ'3fÿ'3f≤'3d 0.5. The vegetal extract was used in concentrations from 2000 to 7.81 μg/mL in both experiments. Assays were performed in triplicate.

Results / Discussion / Conclusion

AUL showed effective activity against bacteria, with MIC of 1000 μg/mL. The checkerboard assay demonstrated the synergistic potential of AUL with AMX (Not combined: MIC of 1000 μg/mL - Combined: MIC of 7.81 μg/mL - FICI: 0.01), CLR (Not combined: MIC of 1000 μg/mL - Combined: MIC of 31.2 μg/mL - FICI: 0.03) and the triple drug (Not combined: MIC of 1000 μg/mL - Combined: MIC of 125 μg/mL - FICI: 0.12). No synergistic effect was observed with OMP. It is concluded that the results were significant in both trials, because of evidence that the AUL is active against bacteria and has synergistic profile when combined with conventional treatment using antibiotics associated to the control of H. pylori, collaborating with the discovery of new drug approaches in the area medical used as an adjuvant and also reducing the use of synthetic drugs, therefore decrease the side effects submitted by them.

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PO152
PIPERINE, A NATURAL BIOENHANCER, NULLIFIES THE ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF CURCUMIN IN STREPTOZOTOCIN-DIABETIC RATS

Arcaro, C. A.; Assis, R. P.; Gutierres, V. O.; Costa, P. I.; Baviera, A. M.; Brunetti, I. L.*
Department of Clinical Analysis, School of Pharmaceutical Sciences, UNESP, Araraquara, São Paulo, Brazil
**brunetti@fcfar.unesp.br*

Introduction

Curcumin, the yellow pigment isolated from Curcuma longa L. rhizomes, has several pharmacological properties, including the antidiabetic activity (Gutierres *et al.*, 2012); however, its low bioavailability is a limitation for its use as a therapeutic agent. Piperine, an alkaloid extracted from Piper nigrum fruits, has bioenhancer properties via inhibition of biotransformation processes and increases the bioavailability of compounds, including curcumin (Shoba *et al.*, 1998). In this context, we evaluated the antidiabetic and antioxidant activities of curcumin incorporated in yoghurt, alone and in association with piperine, in streptozotocin-diabetic rats.

Method

Male Wistar rats (15010g) received streptozotocin (STZ, 40mg/kg, i.v.) to induce diabetes and they were divided into 7 groups (10 rats/group): diabetic treated by gavage with yoghurt (DYOG, vehicle), 4U insulin (DINS), yoghurt incorporated with 90mg/kg curcumin (DC90), 20 and/or 40mg/kg piperine (DP20 and DP40) and association curcumin+piperine (DC90P20 and DC90P40), for 45 days. Body weight (g) and glycemia (mg/dL) were determined weekly; alanine (ALT, U/L) and aspartate (AST, U/L) aminotransferases and alkaline phosphatase (ALP, U/L) plasma activities were measured at every 15 days. At the end of the experiment, the liver were removed for the analysis of lipid peroxidation, malondialdehyde (MDA, M/g tissue), protein carbonyl groups (PCO, M/mg protein), reduced glutathione (GSH, mM/g tissue), superoxide dismutase (SOD, U/mg protein), glutathione peroxidase (GSH-Px, mmol/min/mg protein) and catalase (CAT, mmol/min/mg protein). Data were considered significant at $p<0.05$ (One-way ANOVA followed by Student-Newman-Keuls).

Results / Discussion / Conclusion

All groups beginning with similar glycemia (mg/dL) values (442±22) and, after 45 days of treatment, the glycemia levels were decreased in DINS (145±18), DC90 (330±77), DP20 (399±60) and DC90P20 (441±59) and unchanged in DP40 (570±30) and DC90P40 (620±42) when compared with DYOG (655±14). After 45 days, body weight was increased in DINS and DC90, without changes in the others groups. The beneficial effects of curcumin against liver toxicity were observed, since the activities of AST, ALT and ALP were lower in DC90, as well as in DINS, DP20, DP40 and DC90P20 groups when compared with DYOG. However, in DC90P40, the activities of AST and ALT were increased when compared with DYOG. Curcumin also reduced the oxidative stress in liver of diabetic rats, since DC90 showed decreased levels of MDA (M/g tissue) (DC90 114±5.2 vs DYOG 198±4.3) and PCO (M/mg protein) (DC90 0.42±0.05 vs DYOG 0.94±0.07) and increased activities of SOD, GSH-Px and CAT. DC90P20 group showed similar changes in MDA (130±4.3), PCO (0.47±0.05) and CAT as those of DC90, but GSH-Px and GSH levels were similar those of DYOG. DP20 and DP40 showed decreased levels of MDA and PCO, increased activities of SOD and CAT and unchanged GSH-Px, although GSH levels were elevated in DP20. All biomarkers of oxidative stress were similar between DC90P40 and DYOG. The blockade of the curcumin benefits when administered with a higher piperine dose evidences that the biotransformation of curcumin is essential to its antidiabetic and antioxidant activities, which may be related to a metabolite(s).

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PO153
CROTON SPECIES DIVERSITY IN DRY SCRUB IN SOUTH ECUADOR: A REVIEW OF THEIR TRADITIONAL USES AND THEN INFLUENCE OF INTRASPECIFIC VARIATION IN THEIR CHEMICAL COMPOSITION

Augusta Cueva[1], Nixon Cumbicus[1], Emma L. Ehret[2] and David Tank[2]
[1] *Universidad Técnica Particular de Loja, Departamento de Ciencias Naturales, Loja, Ecuador*
[2] *University of Idaho, College of Natural Resources, Department Forest, Moscow, EEUU*

Abstract
Croton is one of the largest angiosperm genera, globally containing between 1200-1300 species in the tropics and subtropics. Several species have a long role in the traditional use of medicinal plants and some of them had been well studied. Ecuadorian Croton species are not well studied, and classification schemes within the genus do not always agree, partly due to the vast amount of interrelationships to consider, reality that is deep in Ecuadorian dry ecosystems, the most poorly studied ecosystem in the country.
We conducted a study in dry scrub, where some Croton species are fund as keystone ones. The sampling was developed in the whole Loja province identifying five species: C. elegans, C. chimboracensis, C. ruisianus var. podadenius, C. marañonensis (Sp.Nov.) some of these species are new register for the Southern Ecuador. For two additional species: C. wagneri and C. thurifer that have classification problems using traditional taxonomy we developed a molecular study in order to know the genetic relationships of these two species within Croton and its intraspecific variation. The analysis was based on nuclear (ITS), chloroplastic (trn exon, trnL-trnF) and mithocondrial (rps3 gene) regions. The preliminary results of these genetic relationships and its intraspecific variation are present.

PO154
DETECTION OF POLYPHENOLS, FLAVONOIDS AND ANTHOCYANINS IN GRAPES AND WINES OF THE NERO D'AVOLA AND PERRICONE VARIETIES

Gottuso V.[1]*; Pumilia G.[1]; Squadrito M.[2]; Dugo G.mo.[1,3]
[1] *Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A.), via G. Marinuzzi, 3 Palermo, Italy.*
[2] *Unità Operativa Specializzata (U.O.S.) - Centro per l'innovazione della filiera vitivinicola, C.da Bosco, 421Marsala (TP), Italy.*
[3] *Università degli Studi di Messina, Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute (S.A.S.T.A.S), Viale F. Stagno d'Alcontres, 31, Messina, Italy*
**valentinagottuso@libero.it *gpumilia@unime.it*

Introduction

Research regarding the effects dietary polyphenols have on human health has developed considerably in the last decade. The antioxidant properties of polyphenols have been widely studied, but it has become clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress (Vinson, et. al., 1998). More recent interest has focused on the bioactive phenolic compounds in grapes. Anthocyanins, flavanols, flavonols and resveratrol are the most important grape polyphenols because they possess many biological activities, such as antioxidant, cardioprotective, anticancer, anti-inflammation, anti-aging and antimicrobial properties. The total phenolic content of grape skins varied with cultivar soil composition, climate, geographic origin, cultivation practices and level of maturation (Bruno. et. al., 2007). In this study, the total phenolic and anthocyanin contents were detected in Sicilian local grapes and wines Nero d'Avola and Perricone.

Method

Nero d'Avola and Perricone grape samples were detected in 4 different periods of maturation. After fermentation, we also analysed the Nero d'Avola and Perricone wines. (+)-Catechin, malvidin-3-O-glucoside were used as standards. Total Phenolic Content (TPC) was analyzed by a colorimetric assay using Folin-Ciocalteau's phenol reagent. TPC, flavonoids, and anthocyanins were detected using a UV-Spectrophometer at 750, 280 and 540 nm respectively (Di Stefano, *et al.* 1995). The results were expressed as mg/kg (fresh weight) for grapes and mg/l for wines. Furthermore the anthocyanin profile was analyzed using HPLC-DAD (Squadrito, *et al.* 2007).

Results and discussion

The TPC in grape samples increased during the maturation period with the highest concentrations of 2062,16±7,83 mg/kg for Nero d'Avola and 2266,1±59,39 mg/kg for Perricone varieties. Also the TPC in Perricone was higher than the Nero d'Avola wine. The results of the flavonoid content expressed as TPC showed a major concentration for Perricone grapes (3233,29±347,32 mg/kg) and wine (3193±145,66 mg/L) with respect to the Nero d'Avola variety. Furthermore the same trend was shown for the anthocyanin content in grape samples, but with lower values than flavonoids (792,09 ± 36,25 mg/kg for Perricone and 633,59 ± 15,55 mg/kg for Nero d'Avola). Results concerning the anthocyanin profile are expressed individually as a percentage. The most representative anthocyanins for Perricone grapes were malvidin and peonidin, whereas Nero d'Avola grapes indicated malvidin, petunidin and delphinidin. From these results the anthocyanin profile can be considered specific for the varieties analyzed. This study confirms that wines produced from Perricone and Nero d'Avola varieties contain high levels of polyphenols, flavonoids and anthocyanins and, therefore, validates their antioxidant and health properties.

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PO155
DECAY FOR SULPHUR DIOXIDE IN MEAT ON THE BASIS OF THE METHOD OF CONSERVATION (4 ± 2 °C REFRIGERATOR AND -20 ± 2 °C FREEZER)

Ferrantelli V.[1], Mammina L.M.[1], Baglieri S.[2], Fiorista B.C.[1], Palumbo S.[1], Porcarello C.[1], Chetta M.[1], Vella A.[1], Macaluso A.[1]

[1]*Istituto Zoo profilattico Sperimentale della Sicilia "A. Mirri"*
[2]*ASP Ragusa*
**claudiaporcarello@tiscali.it*

Introduction

Sulfites are commonly used chemical compounds that are widely used as food additives, in the category of conditionally permitted preservatives and antioxidants, although they are not allowed in the preparation of fresh meat. An Italian law (D. Lgs 209/1996) regulates permitted food additives in the preparation and preservation of foods; used or intended to be used as ingredients in the production stage or preparation of food products and still present in the final product. In this work it was evaluate the decay of sulfite concentration that occurs over time and according to the method of preservation: refrigerator and freeze. The analysis were carried out on positive samples result by ion chromatography.

Method

A number of 38 meat samples (15 sausages, 10 burgers and chopped 13) was examined for the determination of sulfur dioxide concentration in two different temperature (4 ± 2 ° C and -20 ± 2 ° C), by ICS 3000 ion chromatograph with suppressed conductivity (DIONEX). Samples were homogenised thawed at room temperature. A solution with 25 g of sample and 160 ml of HCl (2,3%) were placed in a test tube by distillation. It was poured the distillate in a 100 ml volumetric flask and dilute to volume with deionized water and injected into the ion chromatograph.

Result/discussion/conclusion

Obtained results showed an average loss of 73.50% in of the sulfite concentration in samples stored in a refrigerator method and an average loss of 16% in samples stored in freezer (-20 ± 2 ° C) (the data refer to a retention period of 7 days after the first analysis). A significantly lower decay has been found in some sporadic sample, this data does not correspond to the majority of the obtained results, it could be the result of a non-uniform distribution of the additive within the analyzed sample or by the presence of flavorings added to the matrix, as for example, resveratrol, alpha-tocopherol, ascorbic acid, polyphenols, omega 3-6, which are natural antioxidants that lower still more the decay. In conclusion, there is a decay of the sulfite concentration in meat, and meat stored in a refrigerator have a decay that is greater after 7 days compared to the decay of freeze method. So the low temperatures slow the decay process (this can be given by oxidation of these additives or instability of these increasing temperatures). However, the concentration of sulphites in both situations decreases in time, with cases in which concentrations are equal to zero even after a few days from the first analysis.

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PO156
DETECTION OF CADMIUM LEVELS IN ORGANS AND TISSUES OF CATTLE, PIGS, SHEEP AND EQUINES OF SICILY

Vella A.[1], Macaluso A.[1]; Graci S.[1], Buscemi M.D.[1], Cammilleri G.[1], Vaiana V.[3], Giangrosso I.E.[1], Sciandrone M.[1], Lo Dico G.[1]; Vazzana M.[2], Ferrantelli V.[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"*

[2]*Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF)- Università degli Studi di Palermo*

[3]*UVAC-PIF- Ministero della Salute*

**Stef.graci@gmail.com; Vincenzo.ferrantelli@izssicilia.it*

Introduction

Cadmium (Cd) is a naturally occurring heavy metal that can be emitted as part of industrial activities. It is an environmental contaminant that is readily taken up by plants and transferred to animals by feed intake. Actually there's no clear about its biological function in either animals or humans but seems to mimics the actions of other divalent metals that are essential to several biological functions (EFSA Panel on Contaminants in the Food Chain, 2009). In animals, Cd is not easily cleared by the cells, and the poor efficiency of cellular export systems explains the long residence time of this element in storage tissues such as the intestine, liver and kidneys (EFSA Panel on Contaminants in the Food Chain, 2009). In human body, Cadmium is transported in blood by a low molecular weight protein, metallothionein, that bound it. Ingestion of Cadmium by food intake can cause symptons like nausea, vomiting, abdominal cramp and headache within minutes of ingestion. Long-term ingestion of Cadmium results in serious disease of the kidneys as well as of the bone. Furthermore different studies have shown that assumption of Cd poisoning foods, such as animal products, constitute a risk factor for breast, endometrial, or ovarian cancers in postmenopausal women. The aim of this study was to determine the levels of cadmium in organs and tissues of cattle, pigs and sheep and equines commercialized in Sicily, in order to have an overview of the health status of Sicilian farm animals.

Method

A number of 294 muscle, adipose tissue and liver samples of cattle (Bos Taurus), pigs (Sus scrofa domesticus L.), sheep (Ovis aries) and equines (Equus caballus) came from different areas of Sicily, were examined in 2013 for the assessment of Cadmium concentration. The samples, received at the residues laboratory of the Chemestry and Food Technologies of the Zooprophylactic Institute of Sicily, underwent to an extraction process by the use of a Multiwave digester 3000 (Anton Paar), in a solution with 5 ml of 65% HNO3. The digestion product, brought to a 50 ml volume with deionized water, were placed to an atomic absorption spectrometer AAnalist 800 (Perkin Elmer) for the analysis.

Results / Discussion / Conclusion

Only one sample out totalling of 294 shows the presence of cadmium traces (0,13± 0,02 mg/kg). It is clear from the results that cadmium concentration in muscle, liver and adipose tissue of sheep, pigs, cattle and equines in Sicily were generally lower and also lower than maximum acceptable concentration by the European Commission (maximum acceptable concentrations in Reg. CE 1881/2006 is 0,05 mg/kg for muscle, 0,5 mg/kg for liver of cattle, pigs and sheep, 0,20 mg/kg for equines). Adopt policies for land irrigation with water that is not contaminated by heavy metals, remains an excellent tool for the prevention of animal welfare. It is always useful evaluate and monitor cadmium and other heavy metal levels in foodstuffs.

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PO157
DETECTION OF LEAD IN NOT PROCESSED VEGETABLES COMMERCIALIZED IN SICILY

Macaluso A.[1], Vella A.[1], Sciandrone M.[1], Cammilleri G.[1], Graci S.[1], Collura R.[1], Vazzana M.[2], Pinelli F.[2], D'Anna M.[3], Giangrosso I.E.[1], Ferrantelli V.[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" Via G.Marinuzzi,3 Palermo,Italy*

[2]*Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF)- Università degli Studi di Palermo Italy*

[3]*ASP6 Palermo*

**gaetano.cammilleri86@gmail.com;andrea.macaluso@izssicilia.it*

Introduction

Heavy metals are among the major contaminants of vegetables (Zaidi *et al.*,2005). Metals are intrinsic component of the earth crust; however, today soil contamination with heavy metals is an environmental problem on a global scale and it is becoming increasingly important as industrialization increases (Salvatore *et al.*, 2009). Heavy metals are not biodegradable, have long biological half-lives and have an accumulation potential in different body organs leading to un wanted effects (Nabulo *et al.*, 2011; Singh *et al.*, 2010; Jarup, 2003; Sathawara *et al.*, 2004). Most heavy metals are extremely toxic for their solubility in water. This property leads to a contamination of vegetables subject to irrigation (Arora *et al.*, 2008). Vegetables are an important part of a healthy and balanced diet.(A. Jafarian- Dehkordi 2013). Vegetables have a propensity to accumulate heavy metals. Accumulation of these molecules into vegetables is a health concern, because the excessive content of metals in food is associated with a lot of diseases such as cardiovascular, kidney, nervous and bone diseases (WHO, 1992, 1995; Steenland and Boffetta, 2000; Jarup, 2003, Powers *et al.*, 2003; Llobet *et al.*, 2003; Ikem and Egiebor, 2005; Yargholi and Azimi, 2008; Mitra *et al.*, 2009). It has been shown that contamination of vegetables is mainly by Pb, Cd, Cr and As (Gupta U.C *et al.*1998) In this study it was evaluated the concentration of lead in not processed vegetables commercialized in Sicily.

Method

A number of 243 samples (apricots, asparagus, beets, artichokes, lettuce, cherries etc.) from different areas of Sicily, were examined in 2013 for the detection of lead level. The samples, received at the residues laboratory of the Chemestry and Food Technologies of the Zooprophylactic Institute of Sicily, underwent to a extraction process by the use of a Multiwave digester 3000 (Anton Paar) in a solution with 5 ml of 65% HNO3. The digestion product, brought to a 50 ml volume with deionized water, were placed to an atomic absorption spectrometer AAnalist 800 (Perkin Elmer) for the analysis.

Results / Discussion / Conclusion

Standard curves of Pb showed a good linear relationship between concentrations of the heavy metals and respective absorbances. 31 of 243 samples examined revealed trace of lead that are below the limits described in Reg. CE 1881/2006. Only one sample of chicory and one of cauliflower were over the limit of 0.3 mg/Kg, described in Reg. CE 1881/2006. Peaches and lettuces were the examined vegetable that showed the greater number of samples with a concentration of lead that was reached by the used method of analysis (5 samples). Pb is not only detrimental to human health through the food chain but also become phytotoxic at high tissue concentrations. It is therefore important to control the Pb concentration in plants, especially in the edible parts of crops to ensure food safety. In order to limit the accumulation of soil Pb in crops, a good understanding of the transfer characteristics of Pb from soils to crops is required.

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PO158
DETERMINATION OF AFLATOXIN B1, B2, G1, G2 IN ALMOND MILK

Macaluso A.[1], Ferrantelli V.[1], Olibrio F.[1], Sposito P.[1], Rosa F.[1], Palumbo S.[1], Vitale M.[1], Porcarello C.[1], Calvacca F. P.[2], Vella A.[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri" Via G. Marinuzzi, 3, Palermo, Italy*

[2]*ASP 6 Palermo, Italy*

**antonio.vella@izssicilia.it*

Introduction

The aflatoxins are mycotoxins, synthesized as secondary metabolites by some species of fungi of the genus Aspergillus parasiticus and Aspergillus flavus, which have a toxic action to humans and animals. Their presence in food results in huge economic loss; it has been estimated that for food recalling and treatment of acute and / or chronic diseases by food poisoning, millions of euro are necessary every year. The European committee recommends the EC member states to implement a coordinated inspection program in the field, in order to verify the presence and concentration of mycotoxins. The aim of our work was to verify the presence of aflatoxins B1, B2, G1, G2 in 40 samples of almond milk in Sicilian territory using ELISA screening test (Enzyme-linked immunosorbent assay). The results have demonstrated the safety of the product under this point of view.

Method

Sampling: 40 almond milk samples of different brand were collected in bar and supermarket in different Sicilian provinces.

For the determination of aflatoxins, we used the screening by a ready to use direct competitive ELISA (Enzyme-linked immunosorbent assay) kit (Celer AFLA MA210, Tecna) following manufacture's instruction.

Result/Discussion/Conclusion

The analyzes performed by ELISA in 2013 on 40 samples of almond milk from companies and private individuals of the Sicilian territory resulted with values lower than 2 ug/ kg ÿ'3fÿ'3ffor total aflatoxins. These values ÿ'3fÿ'3fare lower compared to the reference limits which are referred on aflatoxin results from nuts. However, slightly difference under the minimum residual level were detected. The variability of the observed results might be attributable to the different percentage of almonds in the samples, that ranged from a low of 2.5% to a maximum of 12%. Additionally the samples were not homogenous because we analysed different commercial brands and homemade products. Two samples contained also other dried fruits such as pistachio that might have influenced the obtained values. Although not included in the list of PAT (typical agro-alimentary products), Sicilian almond milk is extremely widespread and typical in this region. Its organoleptic and nutritional composition makes it a drink healthier than most of the soft drinks available on the market. The results of our research reveal that the drink is also safe since aflatoxins B1, B2, G1, G2, were present in very low concentration.

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Development of ultrasensitive direct chemiluminescent enzyme immunoassay for determination of aflatoxin B1in food products.Feng-Yih Yu, AnastasiaV.Gribas, MarinaM.Vdovenko, IvanYu.Sakharov

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PO159
EVALUATION OF HISTAMINE CONCENTRATION IN TUNA COMMERCIALIZED IN SICILY IN 2013

Macaluso A., Lo Cascio G., Calvaruso E., Porcarello C., Cicero A., Giangrosso G., Chetta M., Cammilleri G., Ferrantelli V.

Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri" Via G. Marinuzzi, 3 Palermo, Italy
**vincenzo.ferrantelli@izssicilia.it; *gaetano.cammilleri86@gmail.com*
**enzacalvaruso@gmail.com*

Introduction

Histamine poisoning is one of the most common form of intoxication caused by seafood consumption. Histamine is formed in the flesh of fish by decarboxylation of histidine, a reaction catalyzed by histidinedecarboxylase which is found in some bacterial species. This occurs during the deterioration of fish, if kept in unsuitable conditions of temperature, even in absence of typical signs that would indicate the non-edibility of the product. More involved are fishes with rich free-form histidine meats such as mackerel (Scomber scombrus), sardine (Sardina pilchardus) anchovies (Engraulis encrasicolus) and Tuna fish (Thunnus thynnus). Recently in Sicily consumption of tuna has been subject of hundreds cases of food poisoning. The intake of high concentrations of histamine leads to an onset of symptoms called Scombroid Syndrome. This pathology manifest obvious symptoms which consists of skin redness, throbbing headache, burning mouth, abdominal cramps, nausea, diarrhea, palpitations, malaise and rarely hyperthermia or loss of vision. Symptoms usually appear within 10-30 minutes of ingestion of fish and are generally self-limiting. Physical signs may include diffuse pallor, rash, tachycardia, dyspnea, and hypotension or hypertension. A recent report of the European Autority of Food Safety (EFSA) described that the consumption of food containing higher amounts of toxic biogenic amine(s) may cause food intoxication and indicates the need for a better hygiene process and other controls. Member States informed the EFSA that findings of certain levels of toxic biogenic amines (BA) in fermented food could be of concern and reported a recent increase of biogenic amines content in some fermented foods. Presently, only high-performance liquid chromatography (HPLC)-based methods enable simultaneous and high sensitivity quantification of histamine in foods, hence are best suited for monitoring and control purposes. In this study it was evaluated the level of histamine in Sicilian tuna through the use of HPLC method.

Method

A number of 406 samples of Tuna was examinated in 2013 at the laboratories of Chemistry and Food Technology of the Zooprophylactic Institute of Sicily. The extraction method involves: weighing of samples (10±0.1g), addition of 6 ml of 6% perchloric acid and water up to 50 ml of volume, centrifugation and filtering. Obtained extract is injected into an Agilent UPLC 1290 for the for the detection of histamine concentration.

Results / Discussion / Conclusion

23 of the 406 examined tuna samples, were detected a concentrations of histamine that exceeding the limits of Reg. 2073/2005 and subsequent amendments. In the course of the year, the trend of the analyzes have found a significant increase of samples during the period from May to July, corresponding to the increased activity of fresh tuna fishing. This increase, combined with the high temperatures and bad storage techniques, have lead to an increase of histamine positivity cases. Consequently, there has been an increase of scombroid syndrome cases due to consumption of tuna. A special case of positivity was observed during December. The samples tested were from a batch of tuna caught in India. This positivity can be attributed to bad techniques of storage and transport of products.

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PO160
VALIDATION AND DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN CITRUS BY GC-MS COUPLED WITH MODIFIED QUECHERS SAMPLE PREPARATION PROCEDURE

Randisi B.[1], Macaluso A.[1], Lo Cascio G.[1], Amante C.[1], Calvaruso E.[1], Pantano L.[1], Vella A.[1], Alonzo E.[2], Ferrantelli V.[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia, via G.Marinuzzi 3, Palermo, Italy*
[2]*Servizio Igiene Alimenti, Sorveglianza e Prevenzione Nutrizionale (S.I.A.N.)Via Tevere 39 (fraz. Cerza) 95027 San Gregorio di Catania*
**randisi.barbara@gmail.com*
**liciapantano@gmail.com*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) form as a result of incomplete combustion of organic compounds and their primary source in air is the incomplete combustion of wood and fossil fuel. PAHs are also found in gasoline and diesel motor vehicle exhaust. A reliable and rapid method for the validation and determination of polycyclic aromatic hydrocarbons in citrus was developed using a QuEChERS based extraction procedure.

Method

This study aims at developing a method for the determination of 15 polycyclic aromatic hydrocarbons (PAHs) in citrus fruit samples by combining the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method and determination by gas chromatography–'2dmass spectrometry (GC-EI-MS). Quantification limits ranging from 1 to 5 µg kg-1 were obtained. Recoveries ranged from 70% to 120% for most of the 15 PAHs under analysis.

The acetate QuEChERS method was used for the extraction step.The procedure is based on a short mechanical agitation (vortex during 60 s) using a small volume of acetonitrile (10 ml) as extraction solvent. It is considered as asoft extraction method (less interfering compounds are extracted) which involves extraction with acetonitrile (ACN) followed by an optional clean up procedure by dispersive solid-phase extraction (d-SPE).

Chromatographic analyses were carried out by a Thermo Scientific apparatus, model GC–MS TRACE 1310 TSQ QUANTUM XLS, equipped with an autosampler and a mass spectrometric detector with a quadrupole mass filter.

Results / Discussion / Conclusion

This study has demonstrated that QuEChERS is a rapid and reliable method to determination of PAHs from citrus fruit. The Polycyclic aromatic hydrocarbons (PAHs)distribution and migration between peel and pulp were discussed.

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PO161
VALIDATION AND DETERMINATION OF PYRETHROID AND CARBAMATES PESTICIDES IN HONEY BY GC-MS COUPLED WITH MODIFIED QUECHERS SAMPLE PREPARATION PROCEDURE

Amante C.[1], Macaluso A.[1], Lo Cascio G.[1], Randisi B.[1], La Scala L.[1], Pantano L.[1], Vella A.[1], Iannolino G.[2], Ferrantelli V.[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia, via G.Marinuzzi 3, Palermo, Italy*
[2]*Dipartimento Attività Sanitarie e Osservatorio Epidemiologico- Assessorato Regionale della Salute– Via Vaccaro n° 5, Palermo, Italy*
**amante.claudia76@gmail.com*

Introduction

Honey is a wholesome natural product consumed worldwide. There is an increasing interest in monitoring honey for the presence of pesticides. A reliable and rapid method for the validation and determination of pyrethroid and carbamate pesticide residues in honey was developed using a QuEChERS (quick, easy, cheap, effective, rugged and safe) based extraction procedure.

Method

Gas chromatography tandem mass spectrometry (GC-MS) was used for the quantification and confirmation of pyrethroid and carbamate pesticide residues in honey. Different extraction procedures were studied and optimized in order to obtain better recoveries. The modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was applied for preparing samples. For the extraction procedure, 5.0 g honey was weighed in a polypropylene tube and 5 mL of water was added. The mixture was vortexed for 1 min. Afterwards, 10 mL acetonitrile was added and the sample was homogenized. After adding acetate extraction, the mixture was vortexed again for 1 min, and then it was centrifuged for 10 min at 3000 rpm. The acetate-buffered version gave higher and more consistent recoveries. The extraction method was followed by a clean-up procedure by dispersive solid-phase extraction (d-SPE).
The analytes were determined by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM).

Results/Discussion/Conclusion

The aim of this study was to investigate the potential interrelation to the exposure to pesticides. Thus honey samples from different areas of Sicily were analyzed for the presence of pesticide residues. This study has demonstrated that QuEChERS is a rapid and reliable method to determination of pesticides from honey.

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PO162
VALIDATION OF A CHROMATOGRAPHIC ANALYTICAL METHOD FOR QUALITATIVE ASSESSMENT OF FATTY ACID METHYL ESTERS PERCENTAGE IN PISTACHIO

Lo Cascio G., Macaluso A., Caldara G. F., Randisi B., Amante C., Alongi A., Migliazzo A., Ferrantelli V.
Istituto Zooprofilattico Sperimentale della Sicilia, via G.Marinuzzi 3, Palermo, Italy
**giovanni.locascio71@gmail.com*

Introduction
The opening of European Union market to other Countries has led to an uncontrolled invasion of food products. Sometimes this process and the poor quality of these foreign food products affects the loss value of those products with specific and unique characteristics. Concerning the pistachio, local producers have felt the need to obtain a specific certification in order to distinguish, and so protect, this product from the competition with foreign kind of pistachio with less unique properties and taste. For this reason a method for detection and analysis of fatty acids in pistachio has been performed and validated by Chemistry Laboratories of the Istituto Zooprofilattico Sperimentale of Sicily.

Method
Pistachio (Pistacia vera) nuts from Bronte, a city near Etna Vulcans in the East region of Sicily, were collected and analyzed in this study to determine the fatty acid profile of their oils by gas-chromatography. Nuts were finely ground using a grinder. Fat extraction is carried out using an Accelerated Solvent Extraction based system. The oil from the finely ground pistachio samples was extracted with acetone/petrol ether (7:3, v/v). The esterification of fatty acids to fatty acid methyl esters is performed using an alkylation derivatization reagent. Analysis of fatty acids methyl esters is performed using a gas-chromatic technique with a FID detector [Thermo Trace GC Ultra] using a 100% PoliethylenGlycole capillary column.

Results / Discussion / Conclusion
The results obtained have showed a specific percent composition pattern of identified linoleic, oleic, palmitic, and stearic fatty acids contained in the pistachio nuts. The total oil content of the nuts ranged from 50.4 to 55.8% (w/w). The most abundant monounsaturated fatty acid was oleic acid (C18:1), while linoleic acid (C18:2) was the most prevalent polyunsaturated fatty acid. Oleic acid was the main fatty acid presenting 72% of the total fatty acid content. The percentage values of the four detected fatty acids are the main characteristics of the pistachio subject of our study and they give us a specific identification of the most peculiar traits of Bronte's Pistachio as protected designation of origin product. In conclusion, the present data indicate that pistachio nuts are a good dietary source of unsaturated fatty acids, emphasizing that Bronte's pistachio is of high interest due to the high nutritive value and lipid content. Since this type of gas-chromatographyc analysis is been performed and validated, we affirm that this technique used by "Chemistry Laboratories of the Istituto Zooprofilattico Sperimentale of Sicily" can be used to measure the quality of Pistachios.

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PO163
EVALUATION OF TRACE METALS LEVELS IN TISSUES AND LIVERS OF COMMERCIAL FISH SPECIES FROM THE COASTAL WATER OF THE SICILY

Lo Dico G. M.[1], Vella A.[1], Lo Cascio G.[1], Giangrosso I. E.[1], Macaluso A.[1], Pistoia F.[2], Ferrantelli V.[1]
[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri"*
[2]*ASP 6 Palermo, Italy*
**gigilodico@gmail.com*

Introduction

This study is focused on evaluating of the levels of trace metals in tissues of various commercial fish species such as Xiphias gladius, hunnus thynnus, Gadus morhua, Sparus aurata, Scomber scombrus, Sardina pilchardus, Pleuronectes platessa and Lepidopus caudatus that were collected from the coastal water of the Sicily. The concentrations of Fe, Zn, Al, As, Cd and Pb in these muscles and livers samples of fish were quantified. Have been also examined the relationship between the concentrations of metals and the height and weight of the various species. In general, the iron has high concentrations in fish species. However, the analysis of Cd in the muscle of fish showed elevated levels that exceed international standards. Have been also recorded an interesting trend on the positive correlation between the fish weight and length and metals concentrations. Fortunately, the level of such concentrations of metals in fish has not exceeded the permitted level of international standards.

Method

Apparatus

In this study we have used Agilent Technologies model 7700X inductively coupled plasma mass spectrometry. After calibrating the instrument with standard solutions derived from commercial materials, it was optimized according to the manufacturing standards.

Samples Preparation

The specimens were dissected with sterilized stainless steel equipment. The homogenized samples (muscle and liver) were digested in triplicate in a microwave oven digestive system (Anton Paar multiwave 3000) with HNO3 (65% Merck) in Teflon vessels. The residues were filtered through a 0.45 µm filter and transferred to a 50mL volumetric flask than diluted to level with deionised water.

Result, Discussion and Conclusion

The precision and accuracy of the applied analytical method was validated by accurate analysis of standard reference material of marine biota samples (FAPAS freeze-dried mussel tissue).

Calibration curve of each element must be able to produce good correlation coefficient R2 = 0.999.

The LOQ values were found to be 2.87 for Al, 4.92 mg/Kg for Fe, 4.73 mg/Kg for Zn, 0,003 mg/Kg for As, 0,003 mg/Kg for Cd, and 0,004 mg/Kg for Pb.

The variability in heavy metal levels in different species depends on feeding habits, ecological needs, and metabolism, age, size, and length of the fish, and fish habitat. Concentrations of trace metals detected in the muscle, gill, and liver samples indicate different bioaccumulation potentials. Muscles seem to be a transitory tissue in the pathway of metal uptake and in metal storage, whereas the liver appears to be the tissue, specialized in metal storage and detoxification.

The relationships between body size and trace element concentrations in the two fish species were also investigated and significant positive correlations between the total fish length and weight and heavy metal concentrations. Particularly, the concentrations of some of the heavy metals of concern had positive, high correlations with fish weight and total length. On the other side, metal concentrations were more affected by fish weight than by length.

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PO164
CYTOTOXIC AND ANTI-PROLIFERATIVE ACTIVITIES OF EXTRACTS FROM TWO SPECIES OF THE GENUS *Tabebuia* (BIGNONIACEAE)

Jiménez-González, F. J.[1]; Lopera-Castillón, I. A.[2]; Melchor, J. J.[3]; Veloza, L. A.[4]; Sepúlveda-Arias, J. C.[5]*

[1]*PhD Candidate, Grupo Polifenoles/Universidad Tecnológica de Pereira, La Julita, Pereira, Colombia*
[2]*PhD Candidate, Grupo Infección e Inmunidad/Universidad Tecnológica de Pereira, La Julita, Pereira, Colombia*
[3]*BSc, Grupo Infección e Inmunidad/Universidad Tecnológica de Pereira, La Julita, Pereira, Colombia*
[4]*MSc, PhD, Grupo Polifenoles/Universidad Tecnológica de Pereira, La Julita, Pereira, Colombia*
[5]*MD, PhD, Grupo Infección e Inmunidad/Universidad Tecnológica de Pereira, La Julita, Pereira, Colombia*
**jcsepulv@utp.edu.co*

Introduction

Plant derived molecules, their semi-synthetic and synthetic derivatives are important sources of antitumor drugs and more than 50% of the drugs in clinical trials for anti-cancer activity were isolated from natural sources. For this reason and due to the fact that cancer and inflammatory diseases are public health problems around the world, the search of new anti-cancer drugs is one of the most studied subjects in the field of natural products research. The genus Tabebuia has been widely studied due to their anti-inflammatory and anti-cancer uses in traditional medicine, however, there are few reports concerning the phytochemical composition and biological activities of the species Tabebuia chrysantha and Tabebuia rosea, which are endemic species in the coffee growing area of Colombia. In this study we report the cytotoxic and anti-proliferative activities of the extracts obtained from the leaves and inner bark of T. chrysanta and T. rosea.

Method

Both, leaves (1.2 Kg) and inner bark (2.0 Kg) from T. chrysantha and T. rosea were submitted to percolation with methanol during six days at room temperature followed by liquid-liquid extractions with n-hexane, dicloromethane, chloroform, etyl acetate, butanol and water. Extracts were concentrated under reduced pressure and used for the in vitro assays. The cytotoxic and anti-proliferative activities of the different extracts were evaluated with the MTT assay using Hek-293 (human renal cell), Hep-G2 (human hepatocarcinoma), MCF7 (human breast adenocarcinoma), B16F10 (murine melanoma) and HeLa (human cervical adenocarcinoma) cell lines. The extracts were used at different concentrations (400, 200, 50, 10 and 1 μg/mL in 1% DMSO) for the assays.The selectivity index (SI) was calculated based on the CC50 and the IC50 values. A stimulation index (SI) ≥'3d2 indicates that the inhibitory effect of the extract is selective for the tumor cell line with no or little effect on the normal cell line.

Results / Discussion / Conclusion

The results showed that the dichloromethane extract obtained from the leaves of T. chrysantha has anti-proliferative activity against the MCF7 cell line (SI=2.09, IC50=48.68 ±1.2 μg/mL) whereas the chloroform extract obtained from T. rosea displayed anti-proliferative activity against B16F10 (SI=3.2, IC50=36.44± 1.7 μg/mL), MCF7 (SI=2.55, IC50=45.52 ±1.2 μg/mL), Hep-G2 (SI=5.50, IC50=21.05±1.4 μg/mL) and HeLa (SI=2.01, IC50=57.64±1.2 μg/mL) cell lines. The next step is the isolation and characterization of the compounds responsible for the anti-proliferative activity observed.

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PO165
EFFECT OF PRESSURE COOKING IN ANTIOXIDANT ACTIVITY IN SEEDS OF CHICKPEA

Sánchez, X. M.[1], Dávila, G.[2], Jiménez, C.[2]
[1]PhD student, [2]PhD. Escuela, Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala S/N, Casco de Santo Tomas
**crisjm_99@yahoo.com*

Introduction

Chickpea is one of the most ancient consumed legumes, for its storage the seeds are previously drying, then for its consume these are rehydrated by soaking and finally cooked, improving this digestibility, palatability and inactivating or reducing the non-nutritional compounds (Avola *et al.*, 2012). It has been reported that intake of chickpeas has health benefits because some of their compounds can help reduce the risk of chronic degenerative diseases (CDD), among other mechanisms it has been proposed that this effect partly is due.to its antioxidant capacity

Method

Chickpea seeds were obtained in a Sinaloa State. Market. The seeds were soaked in water at 1:10 (w/v) ratio for 12 h at room temperature, after this treatment, the soak water was discarded and the seeds were cooked in a pot pressure (15lb) for different time periods. Proximal chemical composition of chickpea flour and cooked seeds, were carried out according to the AOAC methods 2005. Phenolic compounds were determined following the method of Abdel -Aal and Hucl, 1999; the antioxidant activity was determined by DPPH, ABTS procedures and Fe and Cu chelation activities in a microplate assay.

Results / Discussion / Conclusion

During cooking chickpea procedure, the protein and lipid concentration increased 13 and 10% respectively compared with the original values. This increase is probably due to the losing of soluble solids during soaking and boiling. Furthermore, carbohydrates, fiber and ashes decreased 17.5, 13 and 1.5% respectively. Phenolics concentration diminished 46% after thermal and pressure treatment. This behaviour could be explained because several of these are heat labile and water soluble compounds (Nithiyanantham *et al.*, 2012).

The Chickpea seed phenolic compounds concentration, was measured and related to standards of gallic acid and quercetin The in vitro Antioxidant capacity measured as IC50, in chickpea raw extract was 10.22 g / uL and for cooked chickpea 13.34 g/ uL. The cooking procedure decreased significantly the antioxidant capacity ($p < 0.05$ (40%). The antioxidant activity in chickpea was 65 and 90 lower compared to gallic acid and quercetin and the obtained for cooked chickpea 74 and 92 % compared to the same standards. The decrease of DPPH inhibitory activity is attributed to lossing of soluble and thermolabile antioxidants respect to the trapping capacity of the ABTS radical and it was also affected by cooking decreasing 28% compared to the antioxidant activity of the raw chickpea seed extract. Both values ÿ'3fÿ'3fwere lower than those determined for gallic acid and quercetin, (6 and 9 times higher than the seed extracts). The chelating activity of Fe and Cu obtained for Raw and cooked chickpea seeds extracts were 66 and 55 % respectively in comparison with EDTA, which is a reference compound.

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PO166
INHIBITION OF THE MPO AND NAG ACTIVITIES EXPLAINS THE TOPICAL ANTI-INFLAMMATORY ACTION OF *Gossypium hirsutum* L. (MALVACEAE)

Santos, B. C. S.[2]; Silva, J. J. M.[2]; Ferreira, A. V. D.[1,2]; Pinheiro, R. P.[1]; Chibli, L. A.[1]; Rodrigues, K. C. M.[1]; Del-Vechio-Vieira, G.[2]; Alves, M. S.[1,2]; Sousa, O. V.[1,2*]

[1]*Graduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, 36036-330, Juiz de Fora, Brazil*

[2]*Faculty of Pharmacy, Federal University of Juiz de Fora, Brazil*

**orlando.sousa@ufjf.edu.br*

Introduction

Gossypium hirsutum L. (Malvaceae), known as "cotton", presents pharmacological activities as anti-inflammatory, cicatrizing, antimicrobial and antihemorrhagic. The present study evaluatedthe myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities in ear edema of mice treated with ethanol extract of *G.hirsutum* leaves.

Method

G. hirsutum was collected in Juiz de Fora, Minas Gerais, Brazil, and a voucher specimen (CESJ number 48.612) was deposited in the Herbarium of the Federal University of Juiz de Fora. Ethanol extract (EE) was obtained from driedand powdered leaves by static maceration. Croton oil-induced ear edema in Swiss mice (n = 5)(protocol number 049/2012)was performed according to Schiantarelli *et al.*, and the animals were treated with 0.10, 0.25 and 0.50 mg/ear of the extract and 0.10 mg/ear of dexamethasone (positive control).MPO activitywasassayedaccordingto Bradley *et al.* with modifications, while the NAG assaywasconducted in accordance withSanchez and Moreno's method. Statistical significance was determined by one-way analysis of variance followed by the Student-Newman-Keuls ($p< 0.05$).

Results / Discussion / Conclusion

The topical applicationofcrotonoilintensivelyincreased MPO and NAG activities, whichwere significantly ($p< 0.05$) inhibitedby topical applicationofthe extract anddexamethasone. EE inhibited the MPO activity in 31.54% (0.10 mg/ear), 36.67% (0.25 mg/ear) and 39.49% (0.50 mg/ear), while dexamethasone inhibited in 21.00%. After treatment, the NAG activity was inhibited in 37.35%, 41.36% and 46.38% at the concentrations of 0.10, 0.25 and 0.50 mg/ear, respectively. In this test, dexamethasone was active in37.61% of inhibition.These results indicate that the inhibition of the MPO and NAGactivities explains the topical anti-inflammatory action of*G. hirsutum*.

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PO167
TOPICAL ANTI-INFLAMMATORY ASSESSEMENT OF *Vernonia polyanthes* LESS. (ASTERACEAE) BASED ON MPO AND NAG PARAMETERS

Rodrigues, K. C. M.[1]; Santos, B. C. S.[2]; Chibli, L. A.[1]; Temponi, V. S.[2]; Ferreira, A. V. D.[1,2]; Del-Vechio-Vieira, G.[2]; Alves, M. S.[1,2]; Sousa, O. V.[1,2*]

[1]*Graduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Juiz de Fora, 36036-330, Juiz de Fora, Brazil*
[2]*Faculty of Pharmacy, Federal University of Juiz de Fora, Brazil*
** orlando.sousa@ufjf.edu.br*

Introduction

Vernoniapolyanthes Less. (Asteraceae), known as "assa-peixe", is a medicinal plant found in South America that has been intensively studied in function of the anti-hypertensive, diuretic, antinociceptive and anti-inflammatory properties. The present study quantified the myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities in ear edema of mice treated with extracts of *V. polyanthes* leaves.

Method

V. polyanthes was collected in Juiz de Fora, Minas Gerais, Brazil, and a voucher specimen (CESJ number 10.329) was deposited in the Herbarium of the Federal University of Juiz de Fora. Hexane (HE), ethyl acetate (EAE) and ethanol (EE) extracts were obtained from driedand powdered leaves by static maceration. Croton oil-induced ear edema in Swiss mice (n = 8)(protocol number 106/2012)was performed according to Schiantarelli *et al.*, and the animals were treated with 0.1, 0.5 and 1.0 mg of the extracts. MPO activitywasevaluatedaccordingto Bradley *et al.* with modifications, while the NAG assaywasconducted in accordance withSanchez and Moreno's method.Statistical significance was determined by one-way analysis of variance followed by the Student-Newman-Keuls ($p< 0.05$).

Results/Discussion/Conclusion

The topical applicationofcrotonoilintensivelyincreased MPO activity, whichwassignificantlyinhibitedby topical applicationoftheextractsanddexamethasone (0.1 mg/ear). HE was more effective on the MPO activity with inhibition of 54.52% (0.1 mg/ear), 50.53% (0.5 mg/ear) and 41.81% (1.0 mg/ear), while dexamethasone (positive control) inhibited 59.88%. The NAG activity was significantly blocked after topical application of the extracts and dexamethasone. HE inhibited the NAG activity in 66.02%, 73.50% and 49.70% at the concentrations of 0.1, 0.5 and 1.0 mg/ear, respectively. EE also showed inhibitory activityin 41.62% (0.1 mg/ear), 54.80% (0.5 mg/ear) and 43.12% (1.0 mg/ear) on the NAG. In this test, the inhibition of the dexamethasone was 66.02%. These results suggest that the inhibition of the MPO and NAGactivities is one of the mechanisms of action of the topical anti-inflammatory activity of*V. polyanthes*.

Acknowledgement: UFJF, FAPEMIG, CAPES and CNPq.

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PO168
PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *Umbilicaria calvescens* Nyl.

Cóndor Cuyubamba, Elena Alicia[1]*; Ramos Chávez, Judith[2]
[1]*M.Sc. Chemistry, National University of Engineering, Av. Túpac Amaru 210, Rímac*
[2]*Student of National University of Engineering, Av. Túpac Amaru 210, Rímac*
**econdor@uni.edu.pe*

Introduction

Lichens are organisms that live in different habitats, from cold areas to the tropics. Several studies have been made to determine the factors that affect their growth and distribution, for example, are very sensitive to air pollution which allows its use as bioindicators.

The Peru is a country with diverse ecosystems with a high diversity of lichens, with very few chemical, biological and physico-chemical studies at the level of their extracts and their secondary metabolites. However worldwide, is known of the use of the extracts to treat some diseases in traditional medicine, including renal, respiratory disorders and stomach. As for the isolated metabolites excelsa the large amount of type phenolic compounds, which have a large number of biological activities especially the antioxidant. Similarly, there are reports of applications at the industry level for the preparation of cosmetic products.

Within this group of organisms we have the genus *Umbilicaria*, which is characterized by the presence of a varied chemical composition, among which are the most common metabolite that is usnic acid and acid lecanorico, which occurs in concentrations different depending on the species.

This work was carried out the qualitative analysis from the lichen *Umbilicaria calvescens Nyl.*, which was collected towards Huaytapallana, province of Huancayo (Dep. Junín, 4500 msnm).

Method

The procedure used was the Rondina & Coussio (1969):

• 5 g of lichens was extracted in methanol (t.amb., 20 h). It was then extracted to reflux for 3 hours. Then it was filtered.

• 5 mL of methanol extract was separated (fraction for evidence of primary and/or secondary amino-grupos, free phenolic groups and tannins) and the rest was dry.

• The solid obtained previously was acidified (HCl 1% at 50 °C), then filtered* for subsequent treatment.

• The solid obtained in the previous step was dissolved with chloroform at 50° C and filtered. The organic phase was used for the testing of steroids, triterpenes, quinones and anthrones or antranoles.

• The acid solution* obtained above (ca. 20 mL) is added NH_3 (ca. 15 mL),then it was extracted with chloroform. The organic phase was used for testing of alkaloids, triterpenes and steroid.

• The aqueous phase was extracted with chloroform and methanol (3:2) (2x25 mL).The organic phase is used for the testing of flavonoids, alkaloids, leucoantocianidine and catechins, triterpenes and steroid.

• 1 g of dried and powdered sample was added to 10 mL of distilled water and heated for 15 minutes to 70^{0}C, then proceeded to filter and allow to cool at room temperature (fraction for evidence of primary or secondary aminogrupos and saponins).

Conclusion

The lichen *Umbilicaria calvescens Nyl.* contains Aminogrupos primary and/or secondary, free phenolic groups, tannins and triterpenoids and steroids.

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PO169
ETHNOMEDICINE AND FORESTS: PROMOTING HUMAN HEALTH

Boeri, P. [1]; Rangel Cano R.M.[2] and Sharry S.[3]
Universidad de Río Negro, Sede Atlántica. Viedma, Argentina
CINVESTAV, Unidad Irapuato, México.
Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina CICPBA.
ssharry@gmail.com

Introduction

The use of medicinal plants plays an important role in the lives of rural people, particularly in remote parts of developing countries which are poorly served with health facilities. Products derived from plants found in the forests are not only useful for traditional medicine, but also often have a considerable market value. The sale of raw materials for pharmaceutical purposes can be especially important for subsistence farmers. This work aims to contribute to the ongoing discussion about human health and biodiversity by concentrating on the relationships between forests and ethnomedicine. Forests play a crucial role in providing food security and human health. They are veritable storehouses of biological diversity, and forest products are the mainstay of households worldwide. As living systems, forests have a vital role in maintaining the ecological base for food and health security**.** Trees are sources for a wide variety of medicines (prescription drugs; traditional plant remedies and teas and herbs) Non-timber forest products include familiar things like mushrooms and ginseng, as well as more than 120 distinct chemicals derived from plants that are used in a variety of drugs (Landscape and Human Health Laboratory. 2001). Anti-cancer drugs based on taxol come from the Pacific yew (*Taxus brevifolia*) of the US Pacific Northwest, and the asthma drug theophylline comes from the plant *Theobroma cacao*, found from southeastern Mexico to the Amazon basin. The National Cancer Institute says that more than two-thirds of all cancer-fighting drugs come from rainforest plants. More discoveries are made each year. Just recently, scientists found a compound in the needles of the Eastern red cedar (*Juniperus virginiana*) that can be used to fight*methicillin-resistant Staphylococcus aureus* (MRSA), an infection that kills thousands of people every year (Landscape and Human Health Laboratory. 2001). Traditionally, the importance of forests and trees has been clearly recognized by cultures worldwide. Trees have featured throughout history: in religion and folklore and are often described as God's gift to humans. They are recognized for their regenerative nature and are associated with health, marital harmony and longevity. For these and other reasons, forests are carefully protected by traditional societies. Ethnomedicine is a prolific field of study in Argentina and Mexico and appear, as well as useful contributions to decision make about natural resources management for conservation. It is suggested that future studies include ecological issues, phytochemical, pharmacological and toxicological assessment of the species, and the strategies of use developed by communities, focusing in protection and conservation of species as well as human communities studied (Trillo et al,2011). The disappearance of the natural vegetation and even its replacement with artificially established forests of exotic species are changing the ecology of the environment with a consequent disappearance or change in occurrence of many medicinally useful species, thus depriving rural communities of their benefits. This review gives a short overview of the most important health benefits that forests provide to humans, and the risks that forests may pose to human health. Furthermore, it discusses the future challenges for the research on the links between forests and ethnomedicine, Forests represent rich natural pharmacies by virtue of being enormous sources of plant and microbial material with known or potential medicinal or nutritional value. Utilizing forests effectively in health promotion could reduce public health care budgets and create new sources of income. Main challenges to delivering health through forests are due to ecosystem and biodiversity degradation, deforestation, and climate change. In addition, major implementation of research results into practice is still lacking. Inadequate implementation is partly caused by insufficient evidence base and partly due to the lack of policy-makers' and practitioners' It is irresponsible for humans as a species to allow the amazing bounty of forests – and their potential for future discoveries – to be threatened because of agriculture, timber harvesting, or development for housing. Protecting these resources instead of destroying them could quite literally save our lives. There is therefore, a need to identify tree species which are capable of multiple uses, such as wood production as well as alternative products such as medicines. This would promote recognition of the value of particular species and result in their inclusion and consideration in forest management planning. Forests are good for you!.

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PO170
HPLC-DAD ANALYSIS OF PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF *Achillea grandifolia* FRIV.

Taşkın D.[1,2]*; Alkaya B. D.[1]; Dölen E.[1]
[1]*Marmara University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey*
[2]*Yeni Yüzyıl University, Faculty of Pharmacy, Department of Analytical Chemistry, Istanbul-Turkey*
**duygu.susuz@yeniyuzyil.edu.tr*

Introduction

The aim of this study was to determine the antioxidant capacity of ethanolic extracts from Achillea grandifolia Friv. flowers, leaves and stem by spectrophotometer and analyse phenolic compounds of these extracts using High Performance Liquid Chromatography with a diode-array-detector (HPLC-DAD).

Comparative analysis of antioxidant capacity was performed using Cupric reducing antioxidant capacity (CUPRAC), Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant capacity (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and metal chelating methods in ethanolic extracts of A. grandifolia flowers, leaves and stem. Also, total phenolic content of extracts were measured by the Folin-Ciocalteu method.

According to the results of other antioxidant capacity methods except for metal chelating method, flowers extract of A.grandifolia showed the highest antioxidant capacity. However, stem extract of A. grandifolia showed the highest antioxidant capacity in metal chelating method.

As a result of the analysis of RP-HPLC, ethanolic extracts of A. grandifolia contain rutine, quercetin-3-O-galactoside, luteolin, quercetin and quercetagetin 3,6-dimethyl ether. Also this plant contains phenolic acids such as chlorogenic acid, caffeic acid and their isomers.

The flowers extract of A. grandifolia contain more phenolic compounds than leaves and stem extracts as results of HPLC analysis. Hence, a significant linear relationship was found between antioxidant capacity and total phenolic content. On the basis of the obtained results, A. grandifolia was found to serve as a potential source of natural antioxidant due to their richness phenolic compounds.

Keywords: Achillea grandifolia Friv, phenolic compounds, antioxidant capacity, HPLC-DAD

PO171
PHENOLIC IDIOBLASTS LOCATION IN LEAVES OF *Kalanchoe fedtschenkoi* AND *Kalanchoe tubiflora*

Casanova, J. M.[1]; Da Silva, L. F. A.[1]; Nascimento, L. B. S.[1]; Leal-Costa, M. V.[3]; Costa, S. S.[2]; Tavares, E. T.[1]

[1]*Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Instituto de Biologia, Departamento de Botβnica, Cidade Universitária, 21.941-902, Rio de Janeiro, Brazil*

[2]*Universidade Federal do Rio de Janeiro, Núcleo de Pesquisas de Produtos Naturais, Cidade Universitária, 21.941-902, Rio de Janeiro, Brazil*

[3]*Instituto Federal de Educação, Ciência e Tecnologia Fluminense, Campos dos Goytacazes Rio de Janeiro, RJ, Brazil.*

**jamilecasanova@yahoo.com.br*

Introduction

Kalanchoe fedtschenkoi R. Hamet & H. Perrier and *Kalanchoe tubiflora* (Harvey) R. Hamet (Crassulacea) are ornamental species that have important pharmacological aspects.[1] Both have been studied for the isolation of bioactive substances.[2] The aim of this study is the location of phenolic compounds in leaves of both species at different development stages.

Method

Less expanded leaves were collected from the second node and complete expanded leaves from the fifth. The fresh material was cross sectioned with the Ranvier microtome at the base, middle third and top of leaf blade. Only the leaf blade margins of *K. fedtschenkoi* was sectioned. The sections were prepared with ferric chloride, assembled on 50% glycerin and sealed with colorless enamel.[3]

Results / Discussion / Conclusion

In *K. tubiflora*, phenolic idioblasts were observed in the second layer inner to epidermis. They are more continuous in the abaxial surface than in the adaxial one. They were also observed near and on the vascular bundles. In the principal vascular bundle, they were on the xylem parenchyma and bordering the phloem. The immature vascular bundles have not idioblasts. In *K. fedtschenkoi*, phenolic idioblasts were detected under the epidermis, varying from three to four layers on blade margin. Besides, they were also found following the vascular bundles, diffuse on the clorenchyma and under the epidermis of all analyzed sections. We observed no significant differences for the presence of idioblasts in different development stages of leaves from *K. tubiflora* and *K. fedtschenkoi*. The detection of phenolic compounds in leaves of both species is important because they could have medicinal properties, contributing to their biological activities. The next step of this study is the anatomical description of leaves from both species.

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PO172
REDUCTION OF SPENT COFFEE GROUND MASS AND PRODUCTION OF POLYPHENOLS BY *Bacillus subtilis*

[a]Peña, Jimena M.[1]; [a]Rangel, Paulina E.[1]; [a]Nares, C. Ameyalli[1]; Cerón, Anabelle[2]; Salazar, Juan R.[3]*

[1]Student, [2]Prof, [3]PhD/Prof. La Salle University, Benjamín Franklin 47 Colonia Hipodromo Condesa, Mexico City, Mexico
[a]The corresponding author certifies that three first authors contributed equitative to the proyect
**corresponding author *juan.salazar@ulsa.mx, union.gea@hotmail.com*

Introduction

According to Euromonitor International, in 2010 the consumption of coffee in Mexico was about 35 640 million kg; 54% of this was destined to ground coffee, which produced 19 246 ton of wastes from restaurants, fast-food and local coffee shops (Euromonitor International, 2012).

The coffee wastes composition is very complex such that a wide variety of chemical compounds are present as lignin, cellulose and hemicellulose. The lignin mostly contains methoxylated phenolic groups, and a few terminal aldehyde groups; this material is difficult to degrade because most of the hydrolytic enzymes can't get access to cellulosic fibers (Lew, 2013). Coffee naturally contains a variety of compounds including caffeine, xanthine and polyphenols (Gutiérrez, 2002). The genus Bacillus have the capacity to degrade lignocellulose components (Delfín *et al.*, 2012).

This work aims to characterize the effect of Bacillus subtilis on the reduction of spent coffee ground waste as well as the production of polyphenols as a suitable source of high value compounds in byproducts.

Method

Spent coffee grounds were collected from a local coffee shop. It was dried for 2 hrs at 105±5 °C and mixed to homogenize the sample. To prepare the experiment, 12.5g spent coffee and 25 ml of mineral medium, according to De la Garza, were transferred to six glass tubes, they were subsequently sterilized using a Harvey autoclave at 121°C for 15 min. Five of the samples were inoculated with B. subtilis ATCC 6635 (1x108 UFC/ml), finally all the tubes were incubated at 37 °C on a shaker at 100 rpm. The tubes were taken out one by one of the incubation at different intervals of time, and they were refrigerated at 10 ± 2°C until their analysis.

Physicochemical properties. Thus, for the spent coffee grounds characterization, some of the most important parameters were considered. These included the pH (according to the potentiometric titration) using a Conductronic pH meter and the reduction of the sample that was quantified in each interval of time when the sample´s dry weight was compared with the initial dry weight.

Microbial viability. The microbial viability was followed according to the Streak Method on Brain Heart Infusion Agar (BHI Agar) BD BioxonMR and the sole presence of B. subtilis was detected simultaneously by morphology.

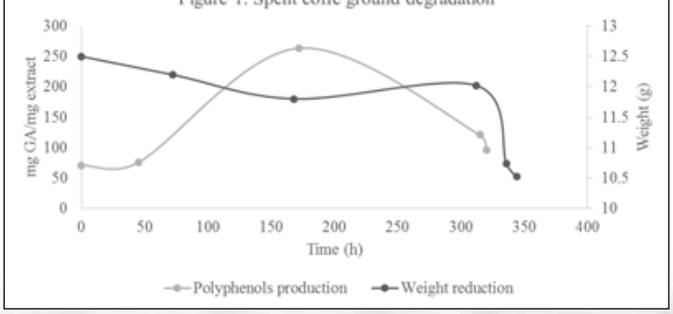
Total polyphenols content determination. The sample's supernatant was centrifuged for 5 min at 5 000 rpm, after they were collected. The method used for the determination was Follin-Chocalteu colorimetric analysis according to García. The samples were diluted with distilled water, obtaining a 1/10 dilution. Absorption determination for total polyphenols content was made using a BioTek® Epoch Microplate Spectrophotometer at 760 nm

Results / Discussion

Physicochemical properties. At the beginning the pH was 4.61, decreasing to 4.50 at the end of the experiment. The diminution of the spent coffee ground was about 1.97g, representing the 16% of reduction. Microbial viability. It was confirmed the only presence of B. subtillis through its colonial morphology.

Total polyphenols content determination. The production of polyphenols in the inoculated sample with B. subtillis increased about 4 times compared with the sample without inoculation. The production of polyphenols is proportional to the spent coffee grounds degradation (Figure 1).

Figure 1. Spent coffe ground degradation



Conclusion

The production of polyphenols can be observed during the spent coffee grounds degradation by B. subtillis, supplying us an alternative of an integral waste management. The FDA considers B. subtillis as an innocuous microorganism, therefore it can be used in these procedure without risk for the human health.

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PO173
TRADITIONAL MEDICINAL USES OF POT-HONEY BY MELIPONICULTORS FROM EL ORO PROVINCE IN ECUADOR

Vit, P.[1,2,3]; Vargas, O.[4]; López,T. V. [5]; Maza Valle, F.[6]

[1] *PhD/Prof, Centro de Investigaciones Agropecuarias (CIAP); Universidad Técnica de Machala, Machala, El Oro, Ecuador.*
[2] *Departamento Ciencia de los Alimentos, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida 5101, Venezuela*
[3]*Honorary Associate Biomedical Sciences, School of Medical Sciences, The University of Sydney, 75 East Street, Lidcombe, NSW 1825, Australia.*
[4]*VMDe/* [5]*VMD/Prof. Facultad de Ciencias Agropecuarias;*
[6]*MSc/Prof. Departamento de Planificación; Universidad Técnica de Machala/Av. Panamericana Km 5 ½ Via Pasaje, Machala, El Oro, Ecuador. vitolivier@gmail.com*

Introduction

Ecuador has a surface of almost 300.000 km² divided into 24 provinces with the highest biodiversity in the planet. The ecosystems represented by the volcanic mountains of Los Andes, the Guayaquil Gulph, the woody plains and the Amazonia, host different species of stingless bees, some of them are used in traditional meliponiculture. The Ecuadorian pot-honey was not included in the book Pot-honey: A legacy of stingless bees (Vit *et al.*, 2013), and here we initiate a contribution to add to the megabiodiversity of this country. In this work we study Ecuadorian meliponines and the different medicinal uses of their honey produced in cerumen pots, to retrieve the relation man-bee-environment. Although these pot-honeys are produced and used before Columbus, they are not yet considered in the honey regulations (Vit, 2008). The Universidad Técnica de Machala is located in El Oro Province, in the southwest of the country where Cantón Piñas, Balsas, Las Lajas and Zaruma visited in this work are located. An important effort is the current revision of the honey regulations in Ecuador NTE INEN 1572 (INEN, 1988) to consider if the pot-honey standards may be included.

Method

Stingless bee keepers –named meliponicultors in Latin America– were visited in different locations from Provincia El Oro in Ecuador. Questionnaires were used for personal identifications of meliponicultors and to inform the medicinal use of pot-honey by themselves and consumers. Ethnic names of the stingless bees were taken in each sampling, used for preliminary identification (Ramírez *et al.*, 2013). Pot-honeys and stingless bees were collected for further collaborative studies for their characterization. Stingless bees were sent to Dr. SRM Pedro, Universidade de São Paulo for identification.

Results / Discussion / Conclusion

Stingless bees kept by visited Ecuadorian meliponicultors in El Oro province are "abeja de tierra" *Geotrigona* spp., "bermeja" *Melipona mimetica*, "cananambo" *Melipona indecisa*, "catiana" or "catana" *Scaptotrigona* spp., "pirunga" *Paratrigona* sp., "piton" *Nannotrigona* sp. Pot-honey is widely used alone or mixed with medicinal plants to treat bruises, tumors, ocular cataracts, pterigium, inflammation, infections, varicose veins, after childbirth, kidney diseases, and soothing balm before sleeping. Their sensory qualities are appreciated. In a previous study, pot-honey collected by Achuar in the Amazonian forest of Ecuador to treat throat inflammation (Guerrini *et al.*, 2009). Further ongoing studies are of interest to identify the megabiodiversity of stingless bees in Ecuador, the traditional meliponiculture and medicinal uses of pot-honey. This joint effort besides the characterization of pot-honeys and its inclusion in the honey standards of the NTE INEN 1572 regulations (Vit *et al.*, unpublished), using the *Melipona favosa* pot-honey model (Vit, 2013), would increase its current value in the market up to USD 27/kg, promote the study of its medicinal properties and praise the activity of meliponicultors.

Acknowledgements
To Prometeo, Senescyt, Ecuador for the grant to Patricia Vit at Universidad Técnica de Machala, Provincia El Oro. To Mrs. Elizabeth Brito Administrative Assitant from the Planning Department, and Mrs. Esperanza Poma Bustos, Director of Public Relations, for the superb logistics, the Principal of UTMACH Eng. César Abad Quezada for the facilities provided for field work, and the drivers Francisco Saavedra, Jairo Riofrio and Wilson Ríos. To Prof. José Quevedo from UTMACH for the initial contact with pot-honey producers from Piñas. To meliponicultors Evita María Guairacocha Pereira, Ubertino Matamoro, María Torres, Andrés Torres, Graciela Pacheco, Segundo Feijó, Arturo Balareso, Oswaldo Ajila, Guillermo Feijoó, César Jaramillo, Segundo Lapo, Abelardo Román, Rosa and Marina Estrada, Daniel and José Zúñiga from Ecuador to keep the tradition beyond ancient times.

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PO174
COMPARISON OF ANTIOXIDANT ACTIVITY BETWEEN AN ETHANOL EXTRACT AND A SUPERCRITICAL FLUIDS EXTRACT OF *Vanilla planifolia* JACK *Orchidaceae*

Rojas-Ávila, Adrián[1]; Dávila-Ortiz, Gloria[1]; Galicia-Luna, Luis Alejandro[2]; Tapia Ochoategui A.P[1]; Sánchez-Mendoza, Noemi Amellalli[1]

[1]. Laboratorio de Graduados en Alimentos. Escuela Nacional de Ciencias Biológicas (IPN). Prolongación de Carpio y Plan de Ayala s/n. 11340 México, Distrito Federal. [2]. Laboratorio de Termodinámica. Escuela Superior de Ingeniería Química e Industrias Extractiva (IPN). Unidad Profesional Adolfo López Mateos, Gustavo A. Madero, 07738. México, Distrito Federal
Email: adr6102@gmail.com

Introduction

Obtaining vanilla extracts is performed in order to accomplish the characteristic aroma of this Mexican origin pod, which is subjected to curing process traditionally named "beneficio"; hence is achieved with lower humidity of pods to formation of new volatile compounds that give a special quality characteristics. Commercially and traditionally there are natural extracts which are obtained through the use of alcoholic solutions, however, complete extraction of vanillin (major compound) present in the pod is not completed, so it relies on the addition of synthetic vanillin allowing cover the requirements of quality standards. The supercritical fluid extraction (SFE) is an alternative technology to extract the majority aromatic compounds that provide strength and unique aroma profile, thus achieving higher efficiency of extraction of these molecules prized for their aroma.

This study shows the quantification of total phenolic compounds in ethanolic and supercritical extracts and both evaluation of antioxidant activity by DPPH and ABTS methods and compares differences and selective extraction capacity of Supercritical Fluids Extraction.

Method

Quantification of total phenolic compounds was determined according to the Folin-Ciocalteu method and tests for DPPH antioxidant activity and ABTS methods were performed according to the Brand-Williams (1995) and Arnao (2001) methods respectively.100g of vanilla pods were placed in 1L of ethanol 60% for 24 hours, thereby obtaining the ethanol extract. The supercritical extract was obtained at 150 bar, temperature in the extractor and separator 60 and 23 ° C respectively and CO2 flow was 3 g / min for 8 h. Once the extracts were obtained the antioxidant capacity was determined for ABTS and DPPH activity and results were reported as mg eq. of Trolox.

Results / Discussion / Conclusion

Trolox equivalents and gallic acid per mL in both extracts were similar, both total phenolic compounds and antioxidant activity are greater in ethanol extract per g of vanilla, mainly due to the selectivity of the extraction process using supercritical fluid which only allowed to extract volatile compounds and not those related to color, since the supercritical extract presents transparent appearance while ethanolic extract has brown color.

Vanilla extracts can be prized as well as aromatic characteristics and can also be an important source of antioxidants, tested by ABTS and DPPH radicals.

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PO175
EVALUATION OF PHYSICAL, NUTRITIONAL AND NON-NUTRITIONAL CHARACTERISTICS OF *Inga* spp. SEED

Sánchez-Mendoza, Noemi Amellalli[1]; Jiménez-Martínez, Cristian[1]; Dávila- Ortiz, Gloria[1]; Rojas-Ávila, Adrián[1]

[1]. *Laboratorio de Graduados en Alimentos. Escuela Nacional de Ciencias Biológicas (IPN). Prolongación de Carpio y Plan de Ayala s/n. 11340 D.F. México.*

Email: amellalli.sm@gmail.com

Introduction

Legumes can be eaten as fresh pods, tubers, flowers, leaves, seed coat and seeds of these plants. Legumes are important in developing countries, because are an essential source of protein. Consumption of local legumes contributes to the livelihoods of communities that collect and consume their own pods; however, nowadays there is not a real interest to disseminate agricultural knowledge as important growing. Inga (Inga spp.) are leguminous trees wildly distributed from Mexico to Argentina, produce pods which are commonly marketed as fruit because of its cottony pulp that cover its seeds, although seed are not consumed and usually discarded. Legume seeds are widely consumed due its content and quality protein in addition to presence of non-nutritional compounds that bring healthy benefits by consumption, associated with the limited research about this seed; it was made the nutritional and no nutritional characterization of Inga's seeds to be considered as source of such compounds.

Method

Pods and seeds of Inga spp. were collected from Ozumba de Alzate, Mexico State and the image analysis was determined. Seeds were separated from the pulp, washed and dried at room temperature and ground to flour. To quantify nutrient compounds (AOAC, 2005) and no nutritional compounds (Phenolic, Folin-Ciocalteu, saponins, trypsin inhibitors and Phytates,). Images of pods and seeds ware obtained by a scanner and processed using the Image J software to obtain data of major and minor axis. Washing was performed to eliminate cottony pulp residues and prevent fungus growth; water excess was removed with cloth. Fresh seeds were placed in 10 cm plastic sheeting and proceed in the same way as pods for its dimensions. The seeds were placed at room temperature for 24, 48, 72 and 96 h, reaching <8.5% moisture, then the dimensions of the dried seeds were determined as is described before. Dried seeds were ground, sieved and content of nutritional and no nutritional compounds was determined as described above.

Results / Discussion / Conclusion

Inga spp flour nutritional characterization was 0.3% ± 8.02 moisture, 2.41 ± 0.01% ash, 9.62 ± 1.5% ether extract, 21.59 ± 1.4 protein, 28.99 ± 37.39% carbohydrate and fiber, expressed on dry basis. For no nutritional compounds was determinated 1.54 mg phytic acid / g, 1.42 mg eq. gallic acid / g, 1.31 mg eq. + catechin / g, 23.39 mg eq. diosgenin / g, 86.86 TIA / g (mg pure trypsin inhibited / g sample) expressed on dry basis.

The seeds reduced its size by 32.4% by drying. The results of the nutritional composition of the seed show that may be feasible to use as protein source with food purposes; however, more depth studies are needed to determine protein quality such (as digestibility). The fiber content is high, which could involve benefits to health by consumption. The presence of non-nutritional compounds is important because it could cause harm if it is ingested causing an interference with the bioavailability of nutrients so it is convenient to identify them; in other hand, these same compounds may have an interesting functional activity, and could bring health benefits for the consumer. Activity of trypsin inhibitors is high compared to values reported in other legume species, and could interfere with of protein digestibility. Seed size was reduced in 32.4% after drying. Seed's Protein content suggested is a possible food source but more studies are needed to determine protein quality and digestibility to be considered a viable and safety option as food source. Non-nutritional compounds such as phenolic compound, phytates and tannins are present in low proportion compared with those reported in other legumes. Saponins and activity of trypsin inhibitors are present in high proportion compared with those reported in other legumes.

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PO176
EFFECTS OF CHRONIC ADMINISTRATION OF BSS-4 ON THE LONG-TERM SPATIAL MEMORY AND ACTIVITY OF ANTIOXIDANT ENZYMES OF RAT HIPPOCAMPUS

Gil-Lievana, E.[1], Martínez, I.[1], Luna, F.[1], Fernández-Herrera, M. A.[2], Sandoval-Ramírez, J.[2]
[1]Departamento de Farmacia, [2]Laboratorio de Síntesis y Modificación de Productos Naturales. Facultad de Ciencias Químicas. Benemérita Universidad Autónoma de Puebla. 72570 Puebla, México
elvigil30@gmail.com

Introduction

Experimental evidences have shown that the administration of diosgenin in rodents improves their learning skill, spatial memory, the antioxidant enzymes activity, and discloses a neuroprotector effect.1,2 The structure-activity relationship studies on diverse organic compounds has permitted to get better drugs, one of this is the 22-oxocolest-5-ene-3β,16β,26-triyl triacetate (BSS-4), a steroidal cholestanic compound which has been synthesized from diosgenin.3 BSS-4 and diosgenin differs only in the composition of the side chain. In our participation, we will expose the effect of chronic BSS-4 administration on the long-term spatial memory.

Method

The more appropriated method for conduct evaluation is the Barnes maze, due to the propensity of rodents to find out small holes in order to escape, and the natural preference for dark environments.4
To ascertain the role of BSS-4 vis à vis learning and memory, superoxide dismutase (SOD) and glutathione peroxidase (GPX) in hippocampus were evaluated.
The hippocampus plays a role in memory processing, recognition, acquisition, storage of the contextual details and temporal order of previous experiences, but especially for spatial memory.
BSS-4 was administrated to female Sprague-Dawley rats, weighing 200–260 g, at 0.5 and 1.0 mg/kg i.p. dose during 25 days. The data acquisition session was realized immediately after the last administration of BSS-4, and then 24 h later the knowledge retention ability, using the Barnes maze, was evaluated. The day after the animals were decapitated, the hole hippocampus recuperated, dissected, and the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPX) was quantified.

Results/Discussion/Conclusion

The over-all evaluation shows that the chronic administration of BSS-4 improved the data acquisition, the long-term spatial memory and the activity of SOD and GPX was increased. Those results reinforces the hypothesis that the steroidal cholestanic compound BSS-4 equally binds to the diosgenin receptor 1,25D3-MARRS,5 stimulating C-Fos expression, which is involved in the molecular mechanisms of learning and memory through the stimulation of neurotrophins FOXO3 and NRF2. These last modulate transcription of genes to increase the activity of SOD and GPX in the hippocampus. The hypothesis is based on the steroidal skeleton similarity of BSS-4 and diosgenin, even if they differs at the side chain.

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PO177
CHEMICAL PROFILE OF NON-POLAR FRACTION OF MARINE ALGAE *Bryothamnion seaforthii* (TURNER) KÜTZING

Silva, D. L.[1]*; Feltran, G. P.[2]; Lourenço, C. C.[2]; Santana, A. E. G.[1], Salvador, M. J.[2]; Araújo-Júnior, J. X.[1,3]

[1]*Chemistry and Biotechnology Institute, Federal University of Alagoas, Maceió, Alagoas, Brazil*
[2]*Department of Vegetal Biology, State University of Campinas, Campinas, São Paulo, Brazil*
[3]*Pharmacy and Nursing School, Federal University of Alagoas, Maceió, Alagoas, Brazil*
**danielliradasilva@gmail.com*

Introduction

Several studies have shown that algae are interesting for biological activities (Abou-Elela *et al.* 2009). For many algae species were described the use in popular medicine (Vieira *et al.* 2004). Previous reports identified many volatile compounds in red algae non-polar fractions: fatty acids, hydrocarbons, esters, alcohols and steroids. These non-polar fractions and volatile compounds presented several biological activities, trypanocidal, leishmanicidal and antifungal (Rocha *et al.* 2011). Bryothamnion seaforthii (Turner) Kützing is red algae, from family Rhodomelaceae. From this specie, several types of lectins and hemagglutinins were isolated before (Vieira *et al.* 2004). This work reports the identification of compounds by GC/MS for the marine algae B. seaforthii and the preliminary antimicrobial activity against Staphylococcus aureus.

Method

B. seaforthii sample was collected at Riacho Doce beach (Maceió-Alagoas), dried in an oven (37 ° C), pulverized and extracted with ethanol in Soxhlet apparatus. The crude extract was fractioned with non-polar (hexane and chloroform) and polar (ethyl acetate and methanol) solvents. The non-polar fraction obtained with chloroform was used in this work. GC/MS analysis was performed on a Shimadzu QP 2010 instrument using a RTX-5MS column (30 m x 0.32 mm i.d., 0.25 µm film thickness) and the operating conditions were: helium as the carrier gas; flow rate of 1.52 mL/min; column temperature 60 °C for 6 min then increasing at 25 °C /min from 60 to 300 °C; injector temperature, 300 °C; volume injected, 1 µL; splitless. MS were recorded in electron ionization (EI) mode, 70 eV; ion source temperature, 300 °C; solvent cut time, 3.00 min. Compounds were identified by comparison with data in the Wiley 7.0 and NIST libraries and Pherobase® data. Preliminary antimicrobial activity was realized using the method described by Salvador *et al.* (2005) in duplicate assay. Indicative strains were maintained as pure culture in Laboratório de Produtos Naturais e de Bioensaios in IB/DFV/Area Ciências Farmacêuticas/UNICAMP.

Results / Discussion / Conclusion

Thirty compounds were identified and their Kovats Indexes (KI) were calculated, including: pentadecane (1500); tridecanal (1515); dodecanoic acid (1570); tetradecanal (1617); tetradecan-1-ol (1681); heptadecane (1700); 2,6,10,14-tetramethylpentadecane (1757); tetradecanoic acid (1767); (-)-loliolide (1799); hexadecanal (1822); neophytadiene (1842); pentanoic acid (1867); heptadecan-2-one (1906); methyl hexadecanoate (1929); (9Z)-hexadec-9-enoic acid (1951); hexadecanoic acid (1972); ethyl hexadecanoate (1996); octadecan-1-ol (2093); phytol (2121); methyl octadecanoate (2130); (9Z)-dec-9-enoic acid (2153); octadecanoic acid (2169); bis(2-ethylhexyl) ester hexadecanoic acid (2406); tricosane (2600); 2,6,10-tetramethylhexadecane (2700); octacosane (2800); nonacosane (2900); 3β-Cholest-5-en-3-ol (3191). This non-polar fraction was effective against Staphylococcus aureus ATCC14458 strain (< 1.0 mg/mL).

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PO178
LABORATORY SCHOOL. MEDICINAL PLANTS AS A MODEL OF LEARNING

Basiglio Cordal, M. A.; Adema, M.; Briones, M. V.; Villarreal, B.; Bravo M. L.; Abedini, W.; Sharry, S.

Centro Experimental de Propagación Vegetativa (C.E.Pro.Ve), Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, CICPBA- CONICET, Diagonal 113 N° 469 (1900), La Plata, Buenos Aires, Argentina.

Introduction

Micropropagation is a laboratory technique that allows to propagate plants on a large scale in a short time and under controlled conditions. It is used for the production of medicinal plants worldwide. the Context of "Plan Estratégico Agroalimentario y Agroindustrial 2010- 2020" (PEEA, Argentina), the Faculty of Agricultural and Forestry Sciences, National University of La Plata (Buenos Aires, Argentina), sponsoring various agricultural schools, and through C.E.Pro.Ve implemented the project entitled "Plantas de Probeta. Dejemos descansar al Poroto". The objective of the project is to incorporate proposed model for teaching science directed research, considering the integration of different areas of knowledge and using plant biotechnology for the development of crops of interest to the community. With this strategy, teachers and students cease to be mere spectators of events to actively participate in solving real problems. Specific objective was raised to promote in (as) students the importance of the cultivation of medicinal plants in school and in the community. The first phase took place in the School of Agricultural Education No. 1 of San Antonio de Areco, Buenos Aires, and consisted of the development of a laboratory of plant tissue culture and teacher training. This methodology is simple and easy to apply and was used as a model tissue culture protocol in the following medicinal plants: Pelargonium graveolens, Erythrina crista- galli y Nicotiana tabacum. Pelargonium graveolens o Malva rosa, Is used as a cell regenerator anti-inflammatory, in cases of dermatitis, acne, oily skin, burns. Presents antiespasmolíticas properties (Lis - Balchin et al, 1998) and antioxidants (Sun W, et al, 2005). Erythrina crista -galli or Seibo is an astringent and sedative, is used to heal wounds, such as haemorrhoids and vaginal douching in cases of candidiasis (bark). Nicotiana sp. is stimulating salivary secretion, has an emetic effect, sedative and mild diuretic effect. Wet leaves are hemorrhoid and can be used as an antiparasitic. Has sedative and hypnotic properties (its infusion has varied alkaloids). The results enabled teachers and students see the importance of the use of medicinal plants, and we could provide theoretical - practical knowledge about the properties of these plants. This project will raise community awareness about the benefits of medicinal plants in their homes. Plants have been used for centuries by different human groups, as they represent the main source of natural products, which by their significant therapeutic effects are used in the human body, as in other animals, to treat various ailments. Therefore, it is effective to recover and reassess the traditional lore regarding the use of plants, use them as resource and enhance and analyze man - plant relationships, from an anthropological, ecological, botanical and medicinal view. Through this project, not only are trained to use advanced techniques, but the use and properties of many plants that are within our reach spread.

PO179
ANTI-STAPHYLOCOCCAL PROPERTIES OF PROPOLIS FROM AUSTRALIAN STINGLESS BEES *Tetragonula carbonaria*

C. Flavia Massaro[1], Mohammad Katouli[1], Eva Hatje[1], Tanja Grkovic[2], Hoan Vu[2], Ronald Quinn[2], Thomas Smyth[3], Franklin Smyth[4], Tim Heard, Helen M Wallace[1], Peter Brooks[1]

[1] *University of the Sunshine Coast,*
[2] *Eskitis Institute for Drug Discovery, Griffith University, Queensland, Australia*
[3] *Teagasc Food Research Centre, Dublin, Ireland*
[4] *University of Coleraine, Northern Ireland, UK*

Introduction

Bees collect plant resins and mix them with their beeswax to produce propolis. Bee propolis has been used as a traditional remedy in ancient civilisations and modern societies of Europe, America and Asia. Natural products of propolis have been reported for their antibacterial properties [1]. However, no antimicrobial investigations have been conducted on Australian propolis yet. This study aimed to assess the *in vitro* anti-staphylococcal properties and cytotoxicity towards Caco-2 and Vero cells of Australian stingless bees (*Tetragonula carbonaria*) propolis.

Method

Propolis was harvested from 14 beehives in Queensland. Ethanol crude extracts were analysed by liquid chromatography-mass spectrometry, and structure elucidation was performed using NMR and HR-ESI-MS. Propolis was fractioned in individual constituents that were purified by repeated column chromatography. Purified constituents and crude extracts were evaluated against 12 strains of *Staphylococcus aureus* including clinical strains of seven multidrug-resistant types and three methicillin-resistant of different clonal types. Antimicrobial activities were assessed using gel diffusion assay according to CLSI guidelines as well as broth dilution assay with MTT-tetrazolium dye to quantitate the bacterial production of formazan crystals at 570 nm. Positive and negative controls were phenol standard and ethanol, respectively. Bactericidal concentrations were identified as 99.9% growth inhibition after replating on fresh agar. Cell viability was tested on Vero and Caco-2 cells seeded at 104 cells/mL, and assessed by MTT and trypan blue assays using bright field microscopy.

Results / Discussion / Conclusion

Propolis constituents were *C*-methyl flavanones and phloroglucinol derivatives that originated from the fruit resins of *Corymbia torelliana* trees. The crude extracts and individual compounds exerted anti-staphylococcal effects at MIC values 6.9 - 511.1 μg/mL [2], while full cell viabilities were 7.0 - 40.0 μg/mL for different samples. Further investigations are warranted on the mode of action by propolis samples, and for the development of potential antimicrobials to treat infections caused by *S. aureus* strains.

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PO180
ANTI-INFLAMMATORY ACTIVITY OF *Pereskia aculeata* Miller LEAVES

Pinto, N. C. C.[1]; Silva, J. M.[1]; Machado, D. C.[2]; Conegundes, J. L. M.[2]; Scio, E.[3]*
[1]*MSc, Laboratory of Bioactive Natural Products. Department of Biochemistry. Institute of Biological Science of Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil*
[2]*Pharmacy student Laboratory of Bioactive Natural Products. Department of Biochemistry. Institute of Biological Science of Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil*
[3]*Prof/PhD, Laboratory of Bioactive Natural Products. Department of Biochemistry. Institute of Biological Science of Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais 36036 900, Brazil*
**elita.scio@ufjf.edu.br*

Introduction

Pereskia aculeata Miller is a climbing cactus naturally occuring from south to northeastern Brazil, where the people commonly use its succulent leaves as a vegetable with high nutritional value (Peterson, 2009). There are reports that leaves of P. aculeata are also used in Brazilian folk medicine as emollients, in skin wound healing, and to treat inflammation (Sartor *et al.*, 2010). Thus, this study aimed to evaluate the anti-inflammatory activity of the hexane fraction obtained from the crude extract of P. aculeata leaves.

Method

The dried leaves of P. aculeata were powdered and extracted by maceration with methanol. The extract was concentrated on a rotatory evaporator to obtain the crude extract, which was resuspended in water/methanol (8:2 v/v). A hexane partition was performed to obtain the hexane fraction. To evaluate the anti-inflammatory activity, the croton oil-induced ear edema test in mice (n = 8) was performed by the method described by Schiantarelli *et al.* (1982) with minor modifications. Edema was induced on the right ear by topical application of 20 μL of croton oil 2.5% (v/v) in acetone, which were immediately treated with 20 μL of the hexane fraction (1.0, 0.5, or 0.1mg/ear) or dexamethasone (0.1mg/ear). The left ear received 20 μL of the vehicle acetone. After 6 h, the animals were euthanized and 6 mm diameter ear punch biopsies were collected from both ears and weighed in analytical balance. The weight difference between right and left ears indicated the edema intensity.

Results / Discussion / Conclusion

The hexane fraction showed a remarkable anti-inflammatory activity at all doses tested. The dose of 1.0 mg/ear was more effective, inhibiting 75% of the edema intensity, while the doses of 0.5 and 0.1 mg/ear inhibited the edema by 46% and 54%, respectively. The reference drug dexamethasone showed an inhibition of 70%. The anti-inflammatory activity of the hexane fraction may be related to the blockade of some pro-inflammatory mediators, including eicosanoids, serotonin and histamine. In our preliminary studies, sterols were detected in high concentrations in this fraction and they may be responsible for these effects, as the anti-inflammatory activities of plant sterols are well described in the literature (Ling and Jones, 1995).
This study showed that the leaves of P. acuelata are endowed with chemical constituents with anti-inflammatory potential. However, further studies are needed to identify the substances responsible for this effect and to verify their mechanism of action.

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PO181
EVALUATION OF ANTITUMOR ACTIVITY OF EXTRACTS FROM *Licania tomentosa* AND MOLECULAR DOCKING STUDIES

Nóbrega, A. B.[1,2]*, Oliveira R.[3], Lessa, B. M.[2], Souza A. M. T.[6], Slana, G. B. C. A.[3,4]*, Paiva, S. R.[1,5]*

[1]*Pós-Graduação em Ciências Aplicadas a Produtos para a Saúde, UFF, Rua Mário Viana N□'3f 523, Santa Rosa, Niterói, RJ, Brasil.*
[2]*Farmanguinhos, Fiocruz, Rio de Janeiro, Rua Sizenando Nabuco 100, Rio de Janeiro, RJ, Brasil*
[3]*Programa de Produtos Bioativos e Biociência, UFRJ, Macaé, Brasil*
[4]*Instituto Nacional de Propriedade Industrial, INPI, Praça Mauá N, Centro, Rio de Janeiro, RJ, Brasil*
[5]*Setor de Botβnica, Departamento de Biologia Geral, Instituto de Biologia, UFF, Outeiro de São João Batista s/n, Niterói, RJ, Brasil.*
[6]*Faculdade de Farmácia, UFRJ, Macaé, Brasil.*
**abnobrega@gmail.com; glauciaslana@gmail.com; selmapaiva@id.uff.br*

Introduction

The family Chrysobalanaceae comprises 17 genera and some species of this family has interesting biological activity for the treatment of numerous diseases(Estrada O. *et al.*, 2011; Alvarado-Castilho C. *et al.*, 2012). Many Chrysobalanaceae species, in special *Licania* and *Parinari*, are widely used in folk medicine to treat several diseases as diabetes, stomach aches, malaria and herpes. Licania tomentosa, typical in the northeastern region of Brazil, is popularly known as "oiti" and is used as a hypoglycemic, anti herpes and diuretic (Braca A. *et al.*, 2003; Cartaxo S. L. *et al.*, 2010; Castilho R. O. *et al.*, 2005). Previous studies also reported antitumoral and antiviral activities (Fernandes J. *et al.*, 2003). Ovarian cancer is the fifth most common cancer among women and remains one of the deadliest gynecologic malignancies. Despite intensive studies, every year, more than 100,000 women die from this disease worldwide. The cell line of tumor ovarian, OVOCAR-3 there occurs a high expression of ERa and ERβ (Kang S. K. *et al.*, 2001). The treatment of OVCAR-3 cells with 17β-estradiol induced the growth of this type of tumor. The presence of tamoxifen, a classic estrogen receptor antagonist, prevented the effects of 17β-estradiol and, consequently, the tumor has not developed (Choi J. H. *et al.*, 2011; Kang S. K. *et al.*, 2001).

Method

Fractions of methanol and hexane extracts from leaves and fruits from Licania tomentosa were used for tests on the tumor cells. Molecular docking studies were performed to predict the mode of interaction of the molecules present in the extract with the line OVCAR-3.

Results / Discussion / Conclusion

Results showed that the hexane extract of the leaves have a significative response with IC50 of 194 mg / mL. The molecular docking carried out in order to observe the interaction of the molecules in the extract with the estrogen receptor corroborated with the experimental results and suggest a synergist action of the extracts components on the inhibition of the growth of the cell line OVCAR3.

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PO182
CHEMICAL INVESTIGATION OF THE MARINE FUNGUS *Penicillium vinaceum*

Ibrahim Asiri, Jihan M. Badr, Diaa T. A. Youssef
Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia
iasiri34@yahoo.com

Introduction

Macro and micro fungi have been part of human life for thousands of years. They were used as food, in preparation of alcoholic beverages, as medication in traditional medicine and for cultural purposes. Certainly, one of the most famous natural product discoveries derived from a fungus microorganism is penicillin. It was obtained from the fungus, Penicillium notatum discovered by Fleming in 1929. Marine-derived fungi have been reported to accumulate biologically active metabolites. Generally, marine-derived fungi are considered as a source of important chemical diversity which is reflected in the considerable number of literature records concerned with secondary metabolites revealed from marine fungi (Manimegalai *et al.*, 2013). Marine-derived fungi research bloomed in the 1990s, and especially since 1998. Although the number of new compounds recorded per year was highest in 1998 and 2000, generally, the trend demonstrates that the number of new compounds is still increasing (Bugni & Ireland, 2004). Studies on biological activities of marine fungal-derived metabolites focused mainly on the areas of anticancer and antibiotic properties although other selective activities are also included. Marine fungal-derived metabolites such as avrainvillamide, sargassamide, and halimide inhibited cancer cell lines selectively and showed *in vivo* activity in preclinical models (P-388 lymphocytic leukaemia), these are now in preclinical development (Sameh, 2012).

Method

The marine fungus Penicillium vinaceum was chosen for chemical investigation. Large scale cultivation was carried out using (14 x 2L) flasks for liquid cultures. The cultures were then incubated at room temperature. After 30 days, 250 mL of ethyl acetate were added to each flask containing 500 mL culture medium and left overnight to stop cell growth. Culture media and mycelia were separated by vacuum filtration using Buchner funnel. The filtrates were collected and extracted with ethyl acetate till exhaustion. The combined ethyl acetate phases were washed with water and then concentrated under reduced pressure to give a brown viscous crude extract. Fungal mycelia were separated from culture media and left in MeOH overnight for extraction. The extract was dried under reduced pressure to give a brown viscous extract. Different chromatographic techniques were used for isolation of pure compounds. This included column chromatography packed with either silica gel or Sephadex, preparative thin layer chromatography and high performance liquid chromatography.

Results / Discussion / Conclusion

The purity of the isolated compounds was checked using TLC and revealing was performed by UV radiation, conc. ammonia vapour, Dragendorff 's and anisaldehyde /conc. H2SO4 spray reagents as well as by HPLC. Finally, seven pure compounds were isolated. Five of the isolated compounds gave orange colour when sprayed with Dragendorff 's reagent indicating their alkaloid nature. The structures of the isolated compounds were established based on different spectroscopic data including MS, 1D (1H NMR and 13C NMR) and 2D NMR (COSY, HSQC, and HMBC). The compounds could be discriminated as an isocoumarine, five alkaloids belonging to the indole and diketopiperazine classes and a small peptide consisting of three amino acids. On reviewing the literatures, it was found that two out of the seven isolated compounds are new isolated for the first time from a natural source. The activities of the isolated compounds against different pathogenic bacteria and fungi were assessed. These results indicate the importance of marine fungi as a source of new and bioactive metabolites that could be effective drug candidates in future.

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PO183
EVALUATION OF WOUND HEALING POTENTIAL OF *Eugenia pruniformis* LEAVES

Albuquerque, R. D. D. G.[1]*; Angeli-Gamba, T.[2]; Esteves, R.[3]; Machado, D. E.[4]; Perini, J. A.[5]*; Oliveira, A. P.[6]*; Rocha, L. M.[7]*

[1]MSc, Universidade Federal do Rio de Janeiro, Programa de Pós Graduação em Biotecnologia Vegetal, Rio de Janeiro, RJ, Brazil
[2]Pharmacist, Centro Universitário Estadual da Zona Oeste do Rio de Janeiro, Laboratório de Pesquisa em Ciências Farmacêuticas, Rio de Janeiro, RJ, Brazil
[3]Pharmacy Student, Universidade Federal Fluminense, Laboratório de Tecnologia em Produtos Naturais, Niterói, RJ, Brazil
[4,5]Dr, Centro Universitário Estadual da Zona Oeste do Rio de Janeiro, Laboratório de Pesquisa em Ciências Farmacêuticas, Rio de Janeiro, RJ, Brazil
[6]Dr, Universidade Federal do Rio de Janeiro, Centro de Ciências da Saúde, Rio de Janeiro, RJ, Brazil
[7]Dr, Universidade Federal Fluminense, Laboratório de Tecnologia em Produtos Naturais, Niterói, RJ, Brazil
[1] ricardo-diego-cf@hotmail.com; [5]* jamilaperini@yahoo.com.br; [6]* adrianapassos@pharma.ufrj.br[7]* lean@vm.uff.br*

Introduction

Treatment of diabetic wounds, bed sores and ulcerations, characterized by poor healing of wounds, have always been a major challenge in healthcare (Góis *et al.*, 2010). Therefore, the search for new drugs with wound healing activity has englobed also the research of substances from natural source. Among the plants of Restinga de Jurubatiba National Park, in Rio de Janeiro State, the species Eugenia pruniformis (Myrtaceae), popularly known as "azeitoninha-da-praia", presents flavonoids as one of the main group of chemical compounds. Flavonoids are widely known to show medicinal activities as antimicrobial, antioxidant, anti-inflammatory and wound healing activities (Brunstein *et al.*, 2012). The aim of this study was to evaluate the wound healing potential of leaves from Eugenia pruniformis.

Method

Leaves of Eugenia pruniformis were collected in Restinga de Jurubatiba National Park, Rio de Janeiro State, Brazil. Fresh leaves were dried and after, ground and subjected to extraction by maceration with 96% ethanol during 15 days. The extract was partitioned with hexane and then with ethyl acetate. The ethyl acetate extract was subject to a thin layer chromatography analysis with specific eluents for glycosides and no glycosides flavonoids in comparison with flavonoids standards. HPLC-UV analysis of the ethyl acetate extract was made with mobile phase of acetonitrile:water (10 to 60% 30 minutes). To determinate wound healing activity was used the excision wound model in Wistar rats. The project was approved by the Ethics Committee for Animal Experimentation of the State University of West Zone of Rio de Janeiro. Wistar rats were topically treated with a solution of 5% leaf ethyl acetate extract in propylene glycol. Tissue samples were obtained on the 1th, 8th and on the 15th day after injury and wounds size were analyzed. Healing was assessed by the tissue morphological characteristics using Sirius red and Hematoxylin-Eosine and hydroxyproline dosage by the colorimetric study.

Results/Discussion/Conclusion

Ethyl acetate extract of E. pruniformis leaves presented a yield of 1,46% on the total weight of fresh leaves. TLC and HPLC-UV analysis were identified the presence of three flavonoids: hyperoside (major compound), quercetin and kaempferol. The histological examination indicated regression of the lesions with better epithelialization and more effective re-organization of the dermis from treated group. Furthermore, the extract was able to increase the concentration of hydroxyproline in days 8th and 15th compared to the control group. The relationship between flavonoids and the wound healing process improvement has been described for Eugenia genus that is in agreement with these results (Brunstein *et al.*, 2012). The presence of quercetin, kaempferol and hyperoside, the major compound of the extract by HPLC analysis, may be associated with increased levels of hydroxyproline and the process of tissue repair. In conclusion, the ethyl acetate extract was able to increase the levels of hydroxyproline and improve the tissue regeneration process of epithelial wounds in Wistar rats.

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PO184
NOVEL CHEMICAL CONSTITUENTS WITH ANTI-INFLAMMATORY ACTIVITYFROM A WIDELY USED BIOFERTILIZER PLANT *Sesbania aculeata*

Sharma, S.[1]*; Chattopadhyay, S. K.[1]; Singh, M.[2]; Bawankule, D. U.[2]; Kumar, S.[3]
[1]*PhD Scholar, Process Chemistry and Technology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot Road, Lucknow, Uttar Pradesh, India*
[1]*PhD/Ex-Chief Scientist, Process Chemistry and Technology Department, CSIR-Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot Road, Lucknow, Uttar Pradesh, India*
[2]*PhD Scholar, Molecular Bioprospection Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot Road, Lucknow, Uttar Pradesh, India*
[2]*PhD/ Scientist, Molecular Bioprospection Division, CSIR-Central Institute of Medicinal and Aromatic Plants, Near Kukrail Picnic Spot Road, Lucknow, Uttar Pradesh, India*
[3]*PhD/ Scientist, Directorate of Medicinal and Aromatic Plant Research, Boriavi, Anand, Gujarat, India*
[1]**shelly.sharma37@gmail.com*

Introduction
Sesbania aculeata (Family Fabaceae) has several synonyms like Sesbania cannabina and S. bispinosa in English, Jayanti and Dhunchi in Sanskrit and Hindi, and most commonly it is called as dhaincha in Oriya. Dhaincha belongs to the legume family and is an ideal green manure crop as it is quick growing, succulent and easily decomposable (The Wealth of India, 1972). The present research work is carried out on the leaves, stems, roots and seeds of Sesbania aculeata, which were not fully studied before for their important biologically active secondary metabolites. The investigation leads to the isolation of three novel compounds, compound 1 (ceramide type), 2 (cerebroside type) and 3 (triterpene acid 3-O-a-L-rhamnopyranoside) which were isolated for the first time from this species along with nine known compounds (Tricontanol, Lauric acid, Palmitic acid, Heptadecanoyl-1-tridecanoic acid, β-sitosterol, stigmasterol, poriferasterol glucoside, ononitol and pinitol). The anti-inflammatory potential of all these novel compounds were evaluated using in-vitro target based anti-inflammatory activity in LPS-stimulated macrophages. All compounds showed moderate to good anti-inflammatory activity.

Method
Systematic chemical investigation of the leaves of the plant S. aculeata using organic solvent extraction, fractionation and column chromatographic purification of different extracts afforded three novel chemical compounds. Their identification was done with the help of UV, IR, NMR and Mass. Pharmacological (in vitro) studies was also carried out by using primary cell culture model with ELISA kit and cell viability assays was also measured.

Results / Discussion / Conclusion
In conclusion, the present study established the first report on the presence of a new ceramide (1), a new cerebroside (2), in addition to a new triterpene acid 3-O-a-L-rhamnopyranoside (3) from the leaves of Sesbania aculeata along with nine known compounds. The anti-inflammatory potential of all the three novel compounds and the respective hexane and ethyl acetate extracts of the leaves were evaluated using in-vitro target based anti-inflammatory activity in LPS (lipo-polysaccharide)-stimulated macrophages. The results demonstrates that the new compounds (1-3) and hexane (HL) and ethylacetate (EAL) extracts, at a dose of 10 μg/mL showed significant ($P<0.001$) inhibition of TNF-a, and IL-6 ($P<0.05$), pro-inflammatory cytokines. Cell viability assay showed that all compounds were non toxic to normal cells. All these results therefore, confirmed the presence of bioactive compounds in the leaves of Sesbania aculeata and further line of research for this herb should be vitalized.

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PO185
COMPREHENSIVE GAS CHROMATOGRAPHIC (GC×GC) ANALYSIS OF *ARTEMISIA ARBORESCENS* L. (ASTERACEAE) FROM SOUTH ITALY

Costa, R.[1*]; Ragusa, S.[2]; Bonaccorsi, I.[1]; Zanotto, A.[1]; Franchina, F.A.[1]; Grasso, E.[1]; Mondello, L.[1,3,4]

[1] *University of Messina, Dipartimento di Scienze del Farmaco e deiProdotti per la Salute (SCIFAR), Viale Annunziata, Messina, Italy*
[2]*Dipartimento di Scienze della Salute, Università degli Studi 'Magna Graecia' di Catanzaro, Viale Europa, 88100 Catanzaro, Italy*
[3]*Chromaleont s.r.l. A start-up of the University of Messina, c/o Dipartimento SCIFAR, University of Messina, Viale Annunziata, 98168 Messina, Italy*
[4]*Università Campus Biomedico, Via Alvaro del Portillo, 21, 00128 Roma, Italy*
**costar@unime.it*

Introduction

Artemisia arborescens L. (Asteraceae) is an upright shrub, one meter tall, with gray-white hairy branches, finely divided leaves (3-5 cm long), and yellow flowerheads grouped in cobs. This species is typical of the Mediterranean flora and grows commonly along the coasts, on calcareous rocks or close to old walls (Pignatti, 1997). Previous investigations on this plant species have been directed toward the determination of volatile constituents and biological assay. The essential oil has been reported to possess anti-flogistic, emmenagogue, aperitif and digestive, antihistaminic, antielmintic, antiviral and antimicrobial properties. Chamazulene, beta-thujone and camphor have been listed among its predominant constituents (Lo Presti *et al.*, 2007). It is also used as rodenticide and moth repellent.

Method

In the present study, leaves and flowers of *A. arborescens* L. plants collected in Sicily (Brolo and Lipari, Messina) and in Calabria (Staletti, Catanzaro), in the period January-May 2014, have been subjected to extraction of the essential oil by conventional hydrodistillation. The distillates were preliminarly analyzed by monodimensional GC techniques, by means of both FID and MS detection systems. Successively, the same oils were analyzed through two-dimensional comprehensive gas chromatography (GC×GC), in order to obtain a metabolomic profile of the constituents. Comprehensive GC is based on the exploitation of an orthogonal system, with a set of two columns having different selectivities (Costa *et al.*, 2010). Consequently, the sample undergoes complete separation and single constituents are displayed in a bidimensional plot, characterized by a group-type pattern.

Results / Discussion / Conclusion

The GC and GC×GC analytical methodologies developed in the present research study allowed to evaluate in depth two variables affecting the volatile fingerprint of *Artemisia arborescens*, namely the plant part (leaves and flowers) and the geographical origin. Qualitative and/or quantitative differences were observed for the different samples investigated. The GC×GC analyses allowed to obtain a "comprehensive" screening of the essential oils, with the determination of compounds present at trace level and otherwise masked by co-elution phenomena.

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PO186
CAPACITY OF INHIBITION OF CHOLINESTERASE ENZYMES AND ANTIOXIDANT ACTIVITY OF PROPOLIS COLLECTED IN TWO REGIONS OF COLOMBIA

Rodríguez, Y. A.[1]*; Gutiérrez, M.[1,2]; Astudillo, L.[1,2]; Rojano, B.[3]; Gil, J.[3], Marín-Loaiza, J.[4]
[1]*Laboratory of Organic Synthesis, Institute of Chemistry of Natural Resources, Universidad de Talca, Talca, Chile.*
[2]*Interdisciplinary Excellence Research Program on Healthy Aging, Universidad de Talca, Talca, Chile*
[3]*Química de los productos naturales y los alimentos. Universidad nacional de Colombia. Medellín, Colombia.*
[4]*Grupo de investigación en Farmacognosia y Fitoquímica (GIFFUN), Universidad Nacional de Colombia, Bogotá, Colombia*
**yenuro30@gmail.com*

Introduction
A comparison of the physicochemical properties of propolis collected in the regions of Cundinamarca (D1) and Boyacá (D2) was conducted. The samples were sonicated in ethanol and the corresponding extracts were obtained. Content of phenolic and flavonoids totals, the antioxidant activity and the capacity of inhibition of cholinesterase enzymes were determinate. The values obtained in the physiochemical analysis and content of phenolics and flavonoids were compared with quality standards set internationally.

Method
The antioxidant activity of the extracts was assessed by the DPPH• radical scavenging ability using the methodology of Brand-Williams *et al.*, 1995. Quercetin was used as reference compound. The total phenolic content of the extracts was determined according to the Folin-Ciocalteu method (Singleton *et al.*, 1965). The total flavonoid content was determined spectrophotometrically based on the absorption of the complex AlCl3 – flavonoid formed (Zhishen *et al.*, 1999). Capacitiy of acetyl cholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition was determined according the methodology of Ellmann *et al.*, 1961, with some variations. The extracts were assayed in the dilution interval of 500 - 15 µg/mL. Galantamine served as the positive control. For the physicochemical characterization of crude propolis, methods are followed recommended in the Standard of the Ministry of Agriculture of Brazil (APACAME, 1999).

Results
The extracts obtained were evaluated as moderated AChE/BChE inhibitors, IC50 values were calculated from the regression equations prepared from the concentrations of the samples. The results indicate that the studied propolis showed variation in physicochemical properties. The values ÿ'3fÿ'3fof some of the estimated physicochemical parameters did not meet the quality standards set internationally; however, ethanolic extracts of propolis tested had a high content of phenols, which are related to the antioxidant properties found experimentally.

Discussion
The results revealed that propolis have low potential application in neurodegenerative disease. The values are not comparable to the reference inhibitor. With respect to the antioxidant activity; the results obtained for the sample D2 is higher when are compared to. This relates to the contents of flavonoids and total phenols, where D2 values are greater than D1.Total flavonoid content is an important parameter, because flavonoids are substances that gives greater biological activity to propolis (Bankova, 2005).

Conclusion
The variation in the composition of propolis may be due mainly to factors such as the geographic and climatic characteristics which were collected the samples.

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PO187
ANTI-INFLAMMATORY ACTIVITY OF GLUCOMORINGIN ISOTHIOCYANATE IN A MOUSE MODEL OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Lombardo, G. E.[1]; Galuppo, M.[2]; Giacoppo, S.[2]; Cirmi, S.[1]; Ferlazzo, N.[1]; De Nicola, G. R.[3]; Iori, R.[3]; Mazzon, E.[2];Bramanti, P.[2]; Navarra, M.[1]*

[1]*Dipartimento di Scienze del Farmaco e Prodotti per la Salute, Università degli Studi di Messina, V.le Annunziata, 98168, Messina, Italy*
[2]*IRCCS Centro Neurolesi "Bonino-Pulejo", Via Provinciale Palermo, contrada Casazza, 98124, Messina, Italy*
[3]*Consiglio per la Ricerca e la sperimentazione in Agricoltura, Centro di Ricerca per le Colture Industriali (CRA-CIN), Via di Corticella 133, 40128, Bologna,Italy*
**mnavarra@unime.it*

Introduction

Glucomoringin (4(a-L-rhamnosyloxy)-benzyl glucosinolate; GMG) is an uncommon member of glucosinolate group belonging to the Moringaceae family, of which Moringa oleifera Lam. is the most widely distributed. Bioactivation of GMG with the enzyme myrosinase forms the corresponding isothiocyanate (4(a-L-rhamnosyloxy)-benzyl isothiocyanate; GMG-ITC). The aim of this study was to test GMG bioactivated with myrosinase that provides the therapeutic natural agent GMG-ITC, in an animal model of experimental autoimmune encephalomyelitis (EAE), that mimics the physiopathology of the human multiple sclerosis (MS).

Method

C57Bl/6 male mice were injected with myelin oligodendrocyte glycoprotein 35–55 (MOG35–55) which is able to evoke an autoimmune response against myelin fibers. Mice were daily weighed and observed for signs of EAE. Clinical score was evaluated using a standardized scoring system. GMG-ITC (10 mg/kg GMG + 5 µl/mouse myrosinase) was daily i.p. administrated 1 week before the induction of EAE; after immunization, the treatment was daily protracted until the twenty-first day. At the end of the experiment, animals were sacrificed and spinal cord tissues were harvested and processed in order to evaluate parameters of disease. Bax, ERK 1/2 and phospho-ERK expression were detected by Western Blot analysis in the homogenates of biopsies. Myelin and phospholipids in histological sections were evaluated by Luxol Fast Blue (LFB) staining. Immunohistochemical localization of TNF-a, IL-10, NOS2, nitrotyrosine, Bax, and Bcl-2 was carried out by specific antibody. To test whether spinal cord damage was associated with apoptotic cell death, we performed TUNEL-like staining in the perilesional spinal cord tissue.

Results and discussion

Results of this study showed that GMG-ITC was able to counteract the inflammatory cascade that underlies processes leading to severe MS. In particular, the drug was effective to reduce the phosphorylation of ERK 1/2, known to be involved in many signaling pathway such as the gene transcription of pro-inflammatory cytokines including TNF-a, that resulted down-regulated by GMG-ITC. Moreover, decreased NOS2 and nitrotyrosine tissue expression suggest its capability in reducing oxidative stress. Finally, GMG-ITC diminished the levels of Bax and increased those of Bcl-2, that in turn counteract apoptotic cell death of EAE.
Taken together, this researchsupports the role of GMG-ITC as a useful drug for prevention or treatment of MS.

PO188
BERGAMOT JUICE EXTRACT REDUCES INFLAMMATION IN LPS-STIMULATED THP-1 CELLS

Risitano, R.[1]; Currò, M.[1]; Lombardo, G. E.[2]; Cirmi, S.[2]; Ferlazzo, N.[2]; Caccamo, D.[1]; Ientile, R.[1]; Navarra, M.[2]*

[1]*Department of Clinical and Experimental Medicine, University of Messina, Via Consolare Valeria, 1, 98125, Messina, Italy*
[2] *Department of Drug Sciences and Products for Health, University of Messina, V.le Annunziata, 98168, Messina, Italy*
**mnavarra@unime.it*

Introduction

Plant polyphenols exert anti-inflammatory activity through both anti-oxidant effects and modulation of pivotal pro-inflammatory genes. Recently, *Citrus bergamia* has been studied as a natural source of bioactive molecules with antioxidant activity, but few studies have focused on molecular mechanisms underlying their potential beneficial effects. Several findings have suggested that polyphenols could influence cellular function by acting as activators of SIRT1, a nuclear histone deacetylases, involved in the inhibition of NF-êB signaling. On the basis of these observations we studied the anti-inflammatory effects produced by the flavonoid fraction of the bergamot juice (BJe) in a model of LPS-stimulated THP-1 cell line, focusing on SIRT1-mediated NF-êB inhibition.

Method

In order to assess either LPS and BJe toxicity on THP-1 monocytes, preliminary experiments were carried out using MTT test. mRNA of pro-inflammatory cytokines IL-6, IL-1, TNF-a were analyzed by real-time PCR, while their secretion in the supernatants were assessed by ELISA. The role of NF-êB and SIRT1 were investigated by EMSA analysis of nuclear fractions and Western blot, respectively.

Results and Discussion

In this study we demonstrated that BJe is able to reduce significantly both transcription profile and protein levels of pro-inflammatory cytokines such as IL-6, IL-1, TNF-a. Moreover, our results provide evidence that BJe act by a mechanism involving the inhibition of NF-êB activation, likely via SIRT1 activation.
These results suggest that BJe may be useful for the development of alternative pharmacological strategies aimed at reducingthe inflammatory process.

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PO189
EVIDENCES OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF BERGAMOT ESSENTIAL OIL IN *IN VIVO* MODELS

Lombardo, G. E.[1]; Mannucci, C.[2]; Cirmi, S.[1]; Ferlazzo, N.[1]; Pergolizzi, S.[3]; Calapai, G.[2]; Navarra, M.[1]*

[1]*Department of Drug Sciences and Products for Health, University of Messina, V.le Annunziata, 98168, Messina, Italy*
[2]*Department of Clinical and Experimental Medicine, University of Messina, Via Consolare Valeria, 1, 98125, Messina, Italy*
[3]*Department Department of Environmental Sciences, Territorial, Food and Health Security, University of Messina, Viale F. Stagno d'Alcontres, 31, 98166, Messina, Italy*
**mnavarra@unime.it*

Introduction

Renewed interest in natural products as potential source of drugs led us to investigate on the anti-inflammatory and analgesic activities of bergamot essential oil (BEO). Citrus bergamia Risso et Poiteau (bergamot), is a small tree belonging to the Rutaceae family. To date, ninety-five percent of worldwide bergamot production occurs in the Ionic costs of Reggio Calabria (Calabria, Italy). Bergamot fruit is used mostly for the extraction of its essential oil from the peel, widely used in perfume industries. BEO comprises a volatile fraction (93–96% of total) containing monoterpene and sesquiterpene hydrocarbons (such as limonene, α- and β-pinene) and oxygenated derivatives (such as linalool and linalyl acetate). The non-volatile fraction (4–7% of total) consisting essentially of coumarins and psoralens, such as bergapten and bergamottine (Costa *et al.*, 2010). Because the toxicity of bergapten, our study has been performed using the BEO fraction deprived of bergapten (BEO-BF).

Method

Carrageenan-induced paw oedema in rats was used as a model of inflammation. In particular, paw volume was measured by plethysmometer, levels of cytokines and prostaglandin E2 (PGE2) were detected in the paw homogenate by ELISA assays, as well as nitrite/nitrate was determined in the exudates by Griess reaction. Histological examination of sections from rat paws was performed by staining with Masson trichrome and a rabbit polyclonal antibody against PGP 9.5. The antinociceptive activity of BEO-BF was examined in mice by two different pain models: the writhing and the hot plate tests, as models of inflammatory pain and supra-spinal analgesia, respectively. The antioxidant activity of BEO-BF was evaluated by cell-free tests, including DPPH, chelating activity and reducing power assays.

Results and Discussion

Treatment with 100 and 500 μl of BEO-BF (i.p.) 1 h before the subplantar injection of 1% carrageenan led to a significant inhibition of paw oedema associated with reduction of IL-6, IL-1β and TNF- production as well as PGE2 and nitrite/nitrate levels. The latter could be related to the antioxidant properties of BEO-BF. Moreover, histological examination of paw biopsies showed a reduction of pathological changes typically of oedema in BEO-BF treated rats.

The analgesic activity of BEO-BF showed in the writhing test strengthen its anti-inflammatory capability, while the results of hot-plate test suggest that the supra-spinal analgesia participates to the antinociceptive effect of BEO-BF.

In summary, our study indicate that BEO-BF exerts protective effects in carrageenan-induced paw oedema accompanied by a pronounced analgesic response, suggesting its potential role as anti-inflammatory drug.

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PO190
FUMIGANT EFFECT OF VENEZUELAN SPECIES ESSENTIAL OILS ON *Tecia solanivora* POVOLNY

Rosslyn, Ramírez[1]; Flor, Mora[1]; Ilka, Dominguez[2]; Nurby, Ríos[1]; María, Gualtieri[1]; Wilson, Ramírez[2]; Luis, Rojas[3]; Bladimiro, Silva[1]; Juan, Carmona[1]

[1]Departamento de Farmacognosia y Medicamentos Orgánicos, Facultad de Farmacia, [2]Laboratorio de Entomología del Instituto de Investigaciones Agropecuarias Facultad de Forestal, [3]Instituto de Investigaciones Facultad de Farmacia4. Universidad de Los Andes, Mérida, 5101, Venezuela.

rosslyn@ula.ve

Introduction

Essential oils have been widely used for their bactericidal, virucidal, fungicidal, parasiticidal, insecticide and larvicide properties. It has been observed that Tecia solanivora Polvolny is one of the most important and invasive pests of potato (Solanum tuberosum) crops. It is commonly known as Guatemalan Moth (Herrera, 1998). Searching alternatives to decrease levels of Tecia solanivora population is very important, therefore, the purpose of this work is to determine the fumigant action of essential oils of some species from Lamiaceae, Myrtaceae, Rutaceae, Verbenaceae, Rubiaceae and Graminea families. Fresh leaves of the species under study were subjected to hidrodestilación using a Clevenger trap. Separation and chemical identification of the components of these oils has been previously done. The fumigant bioassay was evaluated using the impregnated paper method (Mona, F Abd 2011) Volumes of 0.2 ml; 0.05 ml; 0.012 ml of each oil were evaluated. Larvae of the third and fourth instar of T. solanivora were used and assays were performed in duplicate. The mortality of essential oils was registered after 24 hours. The essential oils of Lamiaceae family were found to be the most effective. They produced around 50% of mortality with volumes of 0.012 ml of oil.

Keywords: Pope, Tecia solanivora, Bioassay fumigamte, Lamiaceae, Myrtaceae.

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PO191
IDENTIFICATION OF ALKALOIDS FROM *Stenomesson aurantiacum* (Kunth) Herb FROM THE ECUADORIAN ANDES

Acosta K.[1], Bastida J., Pigni N.
[1]*Prof, Escuela Superior Politécnica de Chimborazo, Panamericana Sur Km 1 1/2, Riobamba, Chimborazo, Ecuador*
**karen.acosta.leon@gmail.com*

Introduction

The plant family Amaryllidaceae has isoquinoline type alkaloids that have been the subject of active research for almost 200 years and, the last three decades, many of these compounds have been isolated and tested for different biomedical activities showing a great potential to a wide variety of diseases. Ecuador has 33 species of Amaryllidaceae and 12 of these are endemic, which are distributed especially in the Andean and Amazon. The phytochemical studies on these Ecuadorian species are absent, with the exception of this study, wich focuses on the identification of alkaloids present in Stenomesson aurantiacum from the Ecuadorian Andes. This species is located between southern Colombia and northern Peru at altitudes between 2,700 and 4,000 meters [1-2].

Methodology

The plant material was collected in the month March 2013 from Cuicococha (Imbabura, Ecuador) and dried for 48 hours at 60 °C and vacuum. The purified extract of alkaloids was obtained by means of acidification followed by basification, for each of the plant parts (roots, stems, leaves and flowers). The purified extract, dissolved in ethyl acetate and methanol was analyzed by gas chromatography and mass spectrometry, with the stationary phase of 5% phenylmethylsilicone.
For obtaining and analyzing the spectral data, 2.71 AMDIS (NIST) software was used, which allowed to verify the purity of the signals and calculating the Retention Indices (RI).

Results/Discussion/Conclusion

In this study, it has been proved the existence of fifteen different types of alkaloids in Stenomesson aurantiacum, being the most abundant, hemantamine, tazetine and lycorine. It is important to stress the biomedical importance of these alkaloids found in various studies. Hemantamine, at in vitro studies, has shown favorable effects especially against tumor cells of different types and antiparasitic properties against Plasmodium falciparum, Trypanosoma brucei rhodesiense and Trypanosoma cruzi [5-10]. Lycorine has an antitumor, antiviral, antifungal, antiparasitic and acetylcholinesterase activities, among others. Furthermore, tazetine has a slight anti-tumor, anti-hypertensive and anti-malarial activity [11-12].
The realization of this study is the begining of future research on Stenomesson aurantiacum relative to malaria and Chagas, so important diseases in Ecuador.

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PO192
EFFECT VANILLA MARC ON ANTIOXIDANT ACTIVITY AND DIETARY FIBER IN MUFFINS

Morato-Torres, C. A.[1]; Tapia-Ochoategui, A. P.[1]*; Jiménez-Martínez, C.[1]; Dávila-Ortiz, G.[1]*
[1]PhD, ENCB/IPN, Prolongación Carpio y Plan de Ayala S/N, Casco de Santo Tomás, Ciudad de México, D.F., México
*adriana3599@gmail.com; *gdavilao@yahoo.com

Introduction

There is an increasing trend worldwide toward using natural flavors. Vanilla has become an important food-flavoring agent; the demand for natural vanilla is increasing (Krushnamurthy *et al.* 2010). Nowadays it's been necessary to design new foodstuffs products that could cover the caloric needs meanwhile offering health benefits to the consumer (Barbosa-Canovas, *et al.* 2010). Vanilla marc (feed stock from the handcraft alcoholic extraction process of vanilla pods), contain residual phenolic compounds, resins, and mainly dietary fiber.The dietary fiber content in vanilla marc overcome the present in wheat bran, that is commonly used to enrich bakery products (°ahin 2011). However, the substitution of wheat flour for another ingredient in high quantities may compromise the structural integrity of the product (Gill *et al.* 2002). Hence the present study is an attempt to evaluate the utilization of vanilla marc as a source of flavor as well as an antioxidant and dietary fiber source in muffins.

Method

Muffins dough was prepared with 5, 10, 15, 20, and 25 % of vanilla marc. The values of the moisture, ash, protein, lipid, carbohydrate and total dietary fiber contents were determinate (AOAC, 1998). The vanilla marc and muffins were crushed, extracted with aqueous ethyl alcohol and screened for total polyphenol content and DPPH free radical scavenging activity. The total phenolic content was determined according to the Folin-Ciocalteu method of Waterhouse *et al.* (2002). The DPPH free radical scavenging activity was estimated using the method of Brand-Williams *et al.* (1995). The data were reported as the mean ± the standard deviation (SD) experiments run in triplicate and were analyzed used Graphpad Prism version 5.0c with a Tukey test and a Significance level $p < 0.05$.

Results / Discussion / Conclusion

The moisture content ranged between 31 and 37 % for 5, and 25 % substitutions with vanilla marc respectively, meanwhile the ash content has maintain around 3% for both substitutions. For 5 and 25 % substitutions, protein ranged between 4 and 3 % respectively and the lipid content was observed between 31 and 37 %. The carbohydrate content decreased ranging between 27 and 6 %, in contrast with dietary fiber that registered an increment between 5 to 17 % for 5 and 25 % of vanilla marc substitutions respectively. Therefore the 25 % substituted muffin covers 65 % of adult daily requirements of dietary fiber (Escudero-González, 2006). The total polyphenol content of vanilla marc was observed to be 16 ± 0.7 mg/g as gallic acid equivalents, and muffins with 25 % vanilla marc substitution showed polyphenol contents of 36 ± 1.0 mg/g as gallic acid equivalents. Vanilla marc and muffins with 25 % vanilla marc substitution, showed 41 % and 23 % free radical scavenging activity as against 93 % for BHA. This activity overcomes the one registered in commercial bread by nearly 9 % (Martinez, 2005) consequently giving a competitive advantage among other commercial bakery products.

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PO193
BETWEEN NATURE AND CULTURE IN AOSTA VALLEY (ITALY): FOLK USES OF PLANTS AT THE FOOT OF THE MONT BLANC

Vitalini, S.[1]*; Uva, C.[1]; Iriti, M.[1], Tomè F.[2], Puricelli, C.[3]
[1]*PhD/Dr/PhD, Università degli Studi di Milano/Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Via Celoria 2, Milano, 20133, Italy*
[2]*Dipartimento di Bioscienze, Università degli Studi di Milano, via Celoria 26, 20133 Milano, Italy*
[3]*Dr, Università degli Studi di Milano/Museo Astronomico - Orto Botanico di Brera, via Brera 28, 20121, Milano, Italy*
**sara.vitalini@guest.unimi.it*

Introduction

The Aosta Valley is a region characterized by a fully mountainous territory, with a high plant diversity (more than 40% of the total Italian flora) and numerous endemic alpine species. Although in recent years the ethnobotanical works in the Italian Alps have been increasing, this area remains little studied leaving room for investigation. In particular, the only two researches of this kind in the Aosta Valley date back to more than 30 years ago (Binel, 1972; Chimenti Signorini *et al.*, 1983). In order to take stock of situation and evaluate the real risk of a loss of the plant traditional knowledge, we decided to develop this project.

Method

The study gathered information on the medicinal and food plant species traditionally used in the north-west part of the region. Demographic characteristics of participants, vernacular names of the plants, their utilized parts and preparation methods were investigated and recorded. Field work was carried out through face-to-face interviews with native people in the area. It was obtained prior informed consent from all respondents and it was observed the Code of Ethics of the International Society of Ethnobiology. The local importance of the documented plants was analyzed and evaluated by calculating some ethnobotanical indices. Herbarium materials were prepared. Specimens were entitled and preserved at the Brera Botanical Garden (Milan State University).

Results / Discussion / Conclusion

Results and Discussion: The first data showed a total of 88 plant species belonging to 32 families known by respondents. Among them, 81 species were wild and 7 species were cultivated plant. The dominant families were Asteraceae (>21%), Lamiaceae (>10%), and Apiaceae (> 7%); the most frequent preparations were infusion and decoction. It was found that Peucedanum ostruthium W.D.J.Koch, Artemisia genipi Stechm., Chenopodium bonus-henricus L. were the most collected species. The local plants were considered helpful mostly for the treatment of abdominal and stomach pain (17%), cough and cold (12%), inflammations of different organ systems (9%), wounds (4%). Conclusion: Our results documented the traditional knowledge of medicinal plant species is mainly retained by elders, and most of them gained it as children from the parents and grandparents during their rural life. With changes in the lifestyle habits as well as environmental conditions, the knowledge and the uses of medicinal plants are progressively disappearing. In fact, informants believe that more plants were in use in the past than now, as a result of the modern health care system expansion based on synthetic medicines.

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PO194
HYPOLIPIDEMIC EFFECT OF A METABOLITE ISOLATED OF *Eryngium heterophyllum* IN A MODEL OF HYPERCHOLESTEROLEMIA IN MICE

Castro-Torres I.G.[1]*, De la O-Arciniega M.[2], Nieto-Camacho A.[1], Martínez-Vázquez M.[1]
[1]*Prof/PhD/Prof, Instituto de Química, Universidad Nacional Autónoma de México, Circuito exterior s/n, Ciudad Universitaria, Delegación Coyoacán 04510, México D.F., México*
[2]*PhD, Área Académica de Farmacia, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Ex-Hacienda la Concepción, 42160, Tilcuautla, Hidalgo, México.*
**ibrahim1002@hotmail.com*

Introduction

Hypercholesterolemia is a metabolic disorder characterized by an increase in plasma cholesterol levels (over 200 mg/dL) (Lee *et al.*, 2011). This alteration may be an important risk factor for development of cardiovascular diseases (atherosclerosis) and gallstones (Nelson, 2013). Statins are the most commonly used drugs for the treatment of hypercholesterolemia; however, these drugs produce many adverse effects. Eryngium heterophyllum is a plant of the Umbelliferae family widely used in the Mexican traditional medicine for inhibiting cholesterol and triglycerides levels in blood (Castro-Torres *et al.*, 2014). This plant is popularly known as "Hierba del Sapo" (toad grass) (Argueta *et al.*, 1994). Ethanolic and aqueous extracts of the aerial parts of the plant have been used in many regions of Mexico, but there is no current wide scientific support for this.

Method

Aerial parts of Eryngium heterophyllum were collected in July 2006 in Atlixco, Puebla, México, and identified by a taxonomist. Dried plant (1.0 kg) was extracted with a mixture of ethanol and water (7:3) using maceration method and then was concentrated it under reduced pressure (10.5 g). Metabolite was separated by column chromatography technique and identified by spectroscopic methods. We used male CD mice fed with a hypercholesterolemic diet for six weeks, which were treated with a metabolite isolated of the plant (D-mannitol) at doses of 10 mg/kg for three weeks. We performed histopathological studies of the liver and measured concentrations of cholesterol: total, HDL and LDL.

Results / Discussion / Conclusion

Hypercholesterolemic group developed cholesterol gallstones and its diet produced changes in hepatocytes, vacuolar degeneration and fibrosis. Our results showed that the metabolite significantly decreased serum cholesterol levels and LDL levels at the doses evaluated, compared with the group with hypercholesterolemia. There were no changes in HDL cholesterol levels as well as in the liver tissues; D-mannitol did no inhibit the formation of cholesterol gallstones in mice. This popular metabolite has properties for decreasing serum lipids levels and it is the major component of the hydroalcoholic extract of Eryngium heterophyllum.

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PO195
ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *Petiveria alliacea* L. (PHYTOLACCACEAE): AN ETHNOMEDICINAL PLANT

Luna-Cazáres, L. M.[1]*; Ortega-Correa, R.[1]; González-Esquinca, A. R.[1]
[1] *PhD/Profesor and Research Laboratorio de Fisiología y Química Vegetal, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas. Libramiento Norte-Poniente No. 1150. Colonia Lajas Maciel. CP. 29035. Tuxtla Gutiérrez, Chiapas, México.*
**lorena.luna@unicach.mx; lucaz58@hotmail.com*

Introduction

Therapeutic efficacy of many indigenous plants of Chiapas, México for several disorders has been described (González Esquinca *et al.*, 2013). At present, the plants used in traditional medicine to treat diseases of microbial origin are studied due to the reemergence of diseases that seemed already eradicated the resistance of microorganisms to antibiotics. P. alliacea called zorrillo, a very important medicinal plant in traditional Mexican and Latin American, is used alone or combined with other plants to address various ailments, including infectious type. As been used as an antirheumatic, against cough, fevers, gastroenteritis, cancer and inflammations (Pérez-Leal *et al.*, 2006), probably because it contains sulfur compounds.The aim of this study was to evaluate the in vitro antibacterial potentials of P. alliacea against Gram-negative and Gram-positive bacteria.

Method

Leaves, stems and roots of several individuals, all in bloom phenological stage were collected. The material was certificated by the Herbarium CHIP of Tuxtla Gutiérrez, Chiapas, Mexico, and assigned the registration number 41223. In the present study, the microbial activity of hexane, ethyl acetate and methanol extracts of leaves, stems and roots of P. alliacea was evaluated for potential antimicrobial activity against medically important bacteria. The antimicrobial activity was determined in the extracts using agar disc diffusion method (Bauer *et al.* 1966; Andrews, 2009). The antibacterial activities of extracts (2.5, 5, 10 mg/mL) of zorrillo were tested against two Gram-positive Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228) and four Gram-negative Escherichia coli (ATCC 25922), Salmonella Typhi (ATCC 6539), S. sonnei (ATCC 25931) and Pseudomonas aeruginosa (ATCC27853). Zone of inhibition of extracts were compared with that of standard gentamicina (Relative Percent Inhibition) (Rivero *et al.*, 1977).

Results / Discussion / Conclusion

The most active was the hexane of leaves extract to inhibit growth bacteria four, hexanic stems to prevent the growth of three strains. Seven extracts showed activity, but leaves and stems presented a broad-spectrum of activity. The antibacterial activities of the extracts increased linearly with increase in concentration of extracts. As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *S. epidermidis* were more sensitive as compared with *E. coli.* The larger the zone of inhibition was the ethyl acetate extract of the root of S. epidermidis (24.6 mm) this was also the highest (113.8%) of the same bacteria PIR. This study demonstrated that P. alliacea may be more effective in treating dermal conditions inthat gastrointestinal disorders.

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PO196
ASSESSMENT OF MEMBRANE ERGOSTEROL AS TARGET OF ANTIFUNGALACTIVITY OF CURCUMIN

Morais, S. E.[1]; Andrade, J. T.[1]; Ferreira, J. M. S.[1]; Araújo, M. G. F.[2]*
[1]*Universidade Federal de São João Del-Rei, Rua Sebastião Gonçalves Coelho, 400, Chanadour, Divinópolis, MG, Brazil*
**mgfaraujo@ufsj.edu.br*

Introduction

Curcumin is a polyphenol compound produced by the rhizome of Curcuma longa specie, which is widely used as a spice in Asian cooking. This compound possess a wide range of pharmacological activities like antioxidant, antibacterial, antimutagenic, antiinflamatory, among others (Cho *et al.*, 2005; Sharma *et al.*, 2009). The antifungal effect of curcumin was demonstrated against different fungal strains (Martins *et al.*, 2009). This work focused on the evaluation of curcumin antifungal activity against the reference specie of *Candida albicans* (ATCC 18804) and 6 clinical strains, as well as its ability to binding to exogenous ergosterol.

Method

The antifungal activity against *C. albicans* strains was performed according to a standard reference method (CLSI, 2008). Curcumin was dissolved in ethanol at initial concentration of 4000 mg/l and 0.1 ml of this concentration was added in each of a 96-well microtiter plate containing Sabouraud dextrose broth (SDB). The initial test concentration was serially diluted two-fold. Each well was inoculated with a suspension containing $2.5x10^3$ CFU/mL of yeast. The antifungal cetoconazole and ethanol were included in the assays as positive and negative controls, respectively. The microplates were incubated at 48 h at 37°C. The MIC of sample was detected following visual observation and the growth of yeast was visualized by turbidity of the media. MIC was defined as the lowest curcumin concentration showing no visible fungal growth after incubation time. A sample from each well that showed antifungal activity was plated on Sabouraud dextrose agar (SDA) for to determination of minimal fungicidal concentration (MFC). In all assays, the samples were processed by triplicate, and each experiment was carried out in triplicate. To determine if curcumin binds to the fungal membrane sterol, the MIC of this compound for *C. albicans* was determined with and without the addition of ergosterol. If the activity of curcumin was caused by binding to ergosterol, the exogenous ergosterol would prevent the binding to the fungal membrane's ergosterol. As a consequence, MIC value enhancement in the presence of exogenous ergosterol.

Results / Discussion / Conclusion

Antifungal activity of curcumin could be confirmed and the results showed that the MICs required to inhibit the growth of the *C. albicans* cell population was 500 mg/l for ATCC, while for clinical strains the MICs ranged between 250 to 2000 mg/l. Results showed that the MIC of curcumin showed enhancement in the presence of exogenous ergosterol (from 500 to 2000 mg/l), suggesting that this compound would act by binding to membrane ergosterol. In conclusion, curcumin is found to be active against all tested clinical and standard strains and showed the capability of binding to ergosterol membrane, under the present experimental conditions. Thus, curcumin can be considered a natural product with promising antifungal potential.

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PO197
ETHNOBOTANICAL SURVEY OF *Catasetum macroglossum* REICHB. F. (ORCHIDACEAE), "SUELDA CONSUELDA", IN THE CITY OF QUEVEDO, LOS RIOS, ECUADOR

Ramos Corrales, P. C.[1], Bruno, F.[2], Beltrami, F.[3], Rosella, M. A.[4]*
[1]*Ag.E. M.Sc., UTEQ, Km 1 ½, Via santo Domingo, Quevedo, Quevedo, Ecuador*
[2,3]*Lic., Farmacobotánica-LABRAM. Fac. Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina (CP 1900)*
[4]*Ph.D., Farmacobotánica-LABRAM. Fac. Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina (CP 1900)*
** marirosella@yahoo.com.ar*

Introduction
Quevedo is an urban-rural town where the main economic source is agricultural and livestock and citizens are directly or indirectly linked to such activities. It is very common that people go to medicine men or healers of plants to care for their ailments. C. macroglossum is an endemic species of Ecuador and the only orchid used for medicinal purposes, so it is interesting to conduct a survey on traditional uses, preparation and dosage forms of this species as a first step to rescue those traditional knowledge that at risk of being lost. (Caniago and Siebert 1998, Ramos Corrales et al, 2011).

Method
An anonymous semi-structured survey to ninety common people (men and women) was conducted. Simultaneously three healer's plants (2 females and 1 male) who were leaders about the community medicinal habits were interviewed. Surveys and interviews were done in the towns of Cuatro Mangas, Buena Fe y Valdramina. Collected data were arranged for later analysis using Alban's modified technique. In this technique the common names of plants are not taken in the native language, but rather they are translates and a set of prompt questions at the beginning of data collection is performed. From the data collected in the interviews it was possible to compile a list of medicinal plants, their vernacular names, their therapeutic applications, used parts, methods of preparation and administration.

Results / Discussion / Conclusion
Ethnobotanical knowledge is transmitted orally from one generation to another, mainly through women.
There is a tendency to promote the integration of traditional medicine and modern allopathic medicine. Often plants traditional healers or healers, who are highly respected and trust of the people, follow several training courses related to the activities they carry out, and do not refuse to advise their "patients" visit the doctor when the situation is out their knowledge.
There is also currently in Ecuador an increasing number of physicians who, as part of therapy, prescribe medicinal plants to their patients thus integrating traditional medicine and modern allopathic medicine.
C. macroglossum was the only orchid cited by interviewed healers, who used it to treat "inflammations, pains and broken bones" in three main ways, all topical, described by themselves as "Crush and place on the sick part coated with a newspaper's sheet", "Heat `papita` directly on a campfire and then crush it" and "Grandparents make 'butter' of `suelda consuelda". Most (98.6%) of respondents no-healers knew C. macroglossum by the name of "suelda consuelda" and the rest (1.4%) as "Papita" because the pseudo bulb is similar to green potato. Common people used it in a similar way either from their own farms, acquiring it in specific areas of supply markets or visiting traditional healers. Should be noted that sellers of medicinal plants do not appear to be involved in the prescription or recommendation of the plant. Nobody grows C. macroglossum and it grows wild in the wettest part of the high branches of old cacao's trees. So, the fact that this species grows and develops freely in a given tract ensures that there are conditions of humidity, light, etc., suitable for associated crops.

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PO198
THE FOLK MEDICINAL PLANTS OF ENEZ (EDIRNE - TURKEY)

Tuzlacı, E. [1]; Bulut, G.[1]*; Aksoy, M. M.[2]
[1]Prof. Dr., Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
[1]PhD, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
[2]Pharmacy student, Marmara University, Faculty of Pharmacy, Istanbul, Turkey
*gizem.bulut@marmara.com.tr

Introduction
This study was made to reveal the plants used as traditional folk medicine in Enez (Edirne) situated in European part of Turkey.

Method
The specimens of the plants used as folk remedies have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration, and the duration of treatment has been recorded. The ethnopharmacological information was obtained from the local people by personal interviews carried out face to face. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University.

Results
As a result of identification of the plant specimens, 37 species, used as a traditional folk medicine in Enez, have been determined. According to the majority of the informants, the plants are mostly used for gastrointestinal system diseases, cold, urinary system diseases, wound and diabetes.

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THE FOLK MEDICINAL PLANTS OF ŞANLIURFA (TURKEY)

Bulut, G.[1]*; Tuzlacı, E.[1]; Bilgin, E.[2]
[1]PhD, Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
[1]Prof. Dr., Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
[2]Pharmacy student, Marmara University, Faculty of Pharmacy, Istanbul, Turkey
*gizem.bulut@marmara.com.tr

Introduction
This study was made to reveal the plants used as traditional folk medicine in Şanlıurfa situated in southeast of Turkey.

Method
The specimens of the plants used as folk remedies have been collected and the information about the local names, the part(s) used, the ailments treated, the therapeutic effect, the preparation, the methods of administration, and the duration of treatment has been recorded. The ethnopharmacological information was obtained from the local people by personal interviews carried out face to face. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University.

Results
As a result of identification of the plant specimens, 34 species, used as a traditional folk medicine in Şanlıurfa, have been determined. According to the majority of the informants, the plants are mostly used for cold, diabetes, stomach diseases and shortness of breath.

PO200
ETHNOBOTANICAL SURVEY OF PLANTS KNOWN AS "BALSA" IN TWO NATURAL REGIONS OF ECUADOR

Ramos Corrales, P. C.[1], Moracci, L.[2], Menestrina, B.[3]; Del Valle, M. E.[4], Rosella, M. A.[5*]
[1]Ag.E. M.Sc., UTEQ, Km 1 ½, Via santo Domingo, Quevedo, Quevedo, Ecuador
[2,3, 4]Lic., Farmacobotánica-LABRAM. Fac. Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina (CP 1900)
[5]Ph.D., Farmacobotánica-LABRAM. Fac. Cs. Exactas, UNLP, 47 y 115, La Plata, Argentina (CP 1900)
* marirosella@yahoo.com.ar

Introduction

Different parts of *Ochroma pyra*midale (Ex. Cav. Lam.) Urb. (Bombacaceae) and other species called "balsa" are used in folk and traditional medicine for various purposes. This work aims to inform about the uses and craft and medicinal properties of plants known as "balsa" in the Amazon and Coast regions of Ecuador.

Method

Information about folk and traditional use, used parts, etc. of plants called "balsa" was collected in the cantons of Puyo (Ecuadorian Amazon region) where the traditional use of these plants are preserved and Quevedo (Ecuadorian Coast region) an agricultural-forest area with technically advanced crops of *O. pyramidale.*

The information was collected through interviews and semi-structured anonymous surveys on a total of 66 adult people (35-60 years) men and women in three population groups: the general public, traditional healers and members of the original peoples Shuar and Cofanes (Ramos *et al.*, 2011).

From the data collected in the interviews we compile a list of species known under the vernacular name of "balsa" or the equivalent term in the Kichwa language of the Sierra, craft purposes, its therapeutic applications, parts used,preparing and administering. (Ramos et al, 2011;. Reyes, 2013).

Results and Discussion

In the city of Quevedo (Coast region) the general public and traditional healers, known only as "balsa" to *O. pyramidale* and use it to make crafts, modeling, construction of marine vessels and housing. In the region of Puyo (Ecuadorian Amazon region) healers, general public and the Shuar people, name several plants as "balsa" due to the buoyancy of the wood.The most used is *O. pyramidale*, which in addition to those already mentioned the Shuar used for its medicinal properties: the sap of the bark mixed with water for constipation problems of cattle and kidney problems. Its fresh and infused leaves as both a poultice and baths to treat inflammation The infusion of the root against venereal diseases. They also called "balsa" species of several families: Araliaceae: *Schefflera pentandra* Harms, to make wooden utensils (spoons). Asteraceae: *Tessaria integrifolia* Ruiz & Pav. "Balsa" o "palo Bobo", "Nanavi waska" in Kichwa of the Sierra language, used by the Cofan people as fuel for campfire and for houses construction. Tiliaceae: *Christiana africana* DC, "real Balsa" has forestry use to prevent soil erosion. *Trichospermum galeottii* (Turcz) Kosterm, "Balsa" or "balsilla", for housing construction. Malvaceae: *Heliocarpus americanus* L., "white balsa" "male balsa" "balsilla" or "Huanbo sapan" in Kichwa Eastern language, is used for making charcoal, canoes, paddles, toys, crafts. Apocynaceae: *Tabernaemontana sana* Ruiz & Pav. "Balsa's dog", "Upiana" in Kichwa language, is used for building houses. Its fruit latex is given to dogs to improve their ability to hunt. The bark as blood purifier and the leaves as antiparasitic, for stomachache or vomiting; Bombacaceae: *Pachira trinitensis* Urb, "Balsa Jibara" as timber for fuel and construction; Euphorbiaceae: *Alchornea grandis* Benth, "male Balsa" or "soft stick" for the building of houses and fences; *Croton chocoanus* Croizat. "male Balsa" for construcción.

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PO201
ANTI-LYMPHOMA EFFECT OF PRODUCTS OBTAINED FROM *Decachaeta incompta* M. KING & H. ROB.

Pérez H.[1]; Calzada, F.[1*]; Barrientos-Salcedo, C.[2]; García-Hernández, N.[3]; Ordoñez, R. Ma.[3]; Bautista, E.[4]; Ortega, A.[4]

[1]*PhDr/Senior researcher C, Medical Research Unit in Pharmacology, Speciality Hospital, National Medical Center Siglo XXI, IMSS, Av. Cuauhtemoc 330, Col Doctores, Del. Cuauhtemoc. CP 06725, México, D. F., México.*

[2]*Faculty of Bioanalysis, Universidad Veracruzana, Veracruz, México.*

[3] *Medical Research Unit in Genética, Pediatric Hospital, National Medical Center Siglo XXI, IMSS, Av. Cuauhtemoc 330, Col Doctores, Del. Cuauhtemoc. CP 06725, México, D.F., México.*

[4]*Institute of Chemistry UNAM, México, D. F., México.*

**fercalber10@gmail.com*

Introduction

Chemotherapy is an effective treatment against various types of cancer either singly or in combination with surgery and/or radiotherapy. However, chemotherapeutic effects of most of the drugs showed limited efficacies due to the development of various side effects. This fostered our attempts to evaluate some plant products against cancer as they are less likely to cause serious side effects.

The aim of the present study is to evaluate the effect of extract and incomptines A-D obtained of leaves of Decachaeta incompta R. M. King & H. Rob. against lymphoma induced in Balb-c mice.

Method

Animals were divided into eight group's viz. G1 to G8 of six each one. For comparison, G1 designated as normal control group was used which was neither inoculated with cancer cells nor treated with natural products. TC-1 cells were injected intraperitoneally with 1 x 106 TC-1 cells/mouse to all the mice of the G2, G3, G4, G5, G6, G7 and G8 groups. As the groups G2 was reserved as cancer control, it was not treated with any natural product but only with water. On the next day (24 h after inoculation) the animals of G3 were treated with 1.25mg/kg of methotrexate while the mice of G4 to G8 were treated with 150mg/kg (extract) or 10 mg/kg (incomptines A-D), orally. The treatment was continued for 9 days. The effect on lymph nodes was determined comparing the results obtained in the test samples against those obtained from the batch of animals inoculated with cells and animals that received no treatment. The results are expressed as % effect on the lymph nodes.

Result/Discussion/Conclusion

Incomptine D was the best natural product, showed inhibition effect in male (86.3%) and female (182.1%) mice. The effect was comparable to methotrexate, used as a control drug. The effect shown by incomptine D isolated from Decachaeta incompta allows proposes it as a candidate for anticancer drug development.

Acknowledgments

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PO202
ETHNOPHARMACOLOGICAL SURVEY OF THE USE OF MEDICINAL PLANTS FOR DIABETIC PATIENTS, THE BAUXITE DISTRICT, OURO PRETO, BRAZIL

Marques, F. S.[1]; Santos, C. M.[1]; Maia, L. F.[1], Rodrigues-das-Dores, R. G.[2]*
Student of Pharmacy, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy/ Health Center, Ouro Preto, Minas Gerais, Brazil.
PhD/ Research/ Coordinator, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy/ Health Center, Ouro Preto, Minas Gerais, Brazil.
plantasmed@ufop.br/rosanagrd@gmail.com

Introduction
Ouro Preto, Brazilian city, cultural patrimony of humanity with an estimated 70,000 inhabitants. It's typical for its traditional cuisine, the cultural diversity, religiosity and preservation of primary orality.It constitutes a complex mosaic where different generations live together and share experiences about the use of herbal teas for different purposes. Diabetes is the second most prevalent disease in the city, affecting 1 in every 10 residents of the Bauxite district.

Method
Given this high prevalence, this paper proposed conduct the survey of ethnopharmacological use of medicinal plants and herbal medicines in hyperglycemic patients undergoing treatment, residents in Bauxite district (Ouro Preto, Brazil),through semi-structured interviews, using the method of "snowball", trying to correlate, guide, follow these respondents regarding the use of hypoglycemic drugs and medicinal/ herbal plants (CAAE: 0010.0.238.000-11).

Results/ Discussion/ Conclusion
100 patients enrolled in the program HIPERDIA (MS, Brazil) were interviewed, regardless of gender, constituting the number of respondents was 72.73% women and 27.27% men, aged 45-90 years.Of the respondents, 77% are patients with diabetes and 33% are hypertensive and diabetic. Diabetics (77%), the parameter measurement of blood glucose, 57.1% of the values were above 140 mg/dL, indicating that impairment of drug treatment, wrong diet or lack of physical activity. 59.7% said that use of medicinal plant as a complementary therapy. The prevalence of use is among women (84.8%). 39 species were related, with 133 citations, where 94% are used alone and 6% in associations (up to 3 species). The most cited plants were *Mentha* sp. (mint 10.53 %); *Matricaria recutit*a L. (chamomile8.27 %);*Leonurus sibiricus* L. (Macaé herb 8.27 %); *Cammelia sinensis*(L.)Kutntze (mate tea 6.02 % e green tea 1.5 %); *Cymbopogon citratus* (DC.) Stapf. (lemongrass 6.77 %); *Melissa officinalis* L (lemon balm 3.01 %), *Lippia* sp. (lippia 1.50 %); *Pimpinella anisum* L. (anise 5.26 %), *Malus domestica* L. (apple peels 3.76%); *Plantago* sp. (3.01%); *Citrus sinensis* L. (orange 3.01%); *Baccharis trimera*(Less) DC.*Bidens pilosa* L., *Phyllanthus* sp., *Cinnamomum zeylanicum* J. Presl. (2.26 %); *Mikania* sp. (guaco), *Passiflora edulis* Sims.; *Sechium edule* (Jacq.) Swartz. (chayote), *Bauhinia forficata* Link. (paw-of-cow), *Citrus limon* L. (lemon) (1.5 %). Other 17 species (12.78%) had just one quote (0.75%). The uses are ethnomedicinal muscle relaxation, as a sedative (50%), bile or choleretic (33%) and digestive (17%). The plants cited as co-adjuncts in the treatment of diabetes were *Bauhinia forficata* Link,*Baccharis trimera* (Less) D.C., *Passiflora edulis* L., *Cynara scolymus* L., *Cucumis sativus*L. e *Plectranthus barbatus* Andrews (Brazilian boldo). These species mentioned, only pharmacologically *B. forficata* e *B. trimera* possess hypoglycemic effect(Menezes *et al.*, 2007; Pepato *et al.* 2002), however, should not be used concomitantly with medications. Infusion is the way of preparation used by 24.68% of respondents; 2.6% use maceration and there were no reports of using decoction and in 85% of preparations, the leaves are the part used. 29.32% of the patients grow plants in urban gardens or sites and use them *in natura* (without drying process), 39.10% use sachets of teas processed (dried plant), 0.75% in pharmacy and the other 30.83% did not report how they acquire or acquire with neighbors and relatives.All respondents reported that they did not report the use of medicinal teas for prescribers of hypoglycemic medication.

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PO203
ANTICARIOGENIC ACTIVITY OF *Camellia sinensis* L.

Dores, R. G. R.[1]*; Braga, T. V.[2]; Ramos, C. S[1].; Evangelista, F. C. G.[3]; Tinoco, L. M. S.[4]; Varotti, F. P.[3]; Carvalho, M. G.[2]; Sabino, A. P.[2]

[1]*Federal University of Ouro Preto, Ouro Preto, MG, Brazil.*
[2]*Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.*
[3]*Federal University of São João Del Rei, Divinópolis, MG, Brazil.*
[4]*Faculty Pitágoras, Belo Horizonte, MG, Brazil.*
**rosanagrd@gmail.com*

Introduction

Camellia sinensis L. known as green tea, Theaceae family, is consumed in more than 160 countries due to its characteristics of aroma, flavor and medicinal properties. Streptococcus mutans, Streptococcus mitis e Streptococcus oralis are involved in the formation of dental caries. The process involves bacterial adhesion, biofilm formation and demineralization of dental enamel by the acid produced by the microorganisms (Chelli-Chentouf *et al.*, 2012).

Method

This study evaluated the antibacterial activity of the extract of Camellia sinensis L. front of Streptococcus mutans (ATCC 25175), Streptococcus mitis (ATCC 49456) and Streptococcus oralis (ATCC 10557). The C. sinensis plant drug was obtained from the supplier of natural products® and the extract prepared according to the general methods of Farmacopéia Brasileira V (2010). Evaluation of antibacterial activity was performed by disk diffusion method according to the Clinical and Laboratory Standart Institute protocol (CLSI, 2006). The paper disks impregnated with 10µL of the extract (50mg/mL) were performed in triplicate. The minimum inhibitory concentration (MIC) was determined according to the Clinical and Laboratory Standart Institute protocols (CLSI, 2003). This test was performed in sterile 96-well microplates. The extract was tested in serial dilution from 1000 µg/mL in triplicate.

Results and Discussion

The inhibitory halo greater was 12.5 mm ± 0.86 for S. mutans, 12.7 mm ± 0.58 for S. mitis and 12.2 mm ± 0.76 for S. oralis. In the evaluation of antibacterial activity by disc diffusion, the plant extract is considered active when there is formation of inhibitory halo greater than or equal to 7.0 mm. The MIC value for the extract was 500 µg/mL for S. mutans 1000 µg/mL for S. mitis and S. oralis.

Conclusion

These results suggested that Camellia sinensis L. may inhibit the caries-inducing properties of Streptococcus spp. and thus may be beneficial for the dental care.

Acknowledgements

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PO204
EFFECTIVENESS OF DRYING PROCESSES OF *Psidium guajava* L. LEAVES IN TWO STAGES OF MATURITY IN TERMS OF LEVELS OF FLAVONOIDS, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

Rodrigues-das-Dôres, R. G.[1]*; Trindade, R. S.[2]; Souza, C. S.[2]; Braga, T. V.[3]

[1] *PhD/Research/ Coordinator, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy, Health Center, Campus UFOP, Ouro Preto, Minas Gerais, Brazil*

[2] *Student, Federal University of Ouro Preto/ Laboratory of Medicinal Plants and Phytotherapy, Health Center, Campus UFOP, Ouro Preto, Minas Gerais, Brazil*

[3] *Doctorate Student, Federal University of Minas Gerais/ Program Post Graduate of Clinical Analyses, FAFAR, Campus UFMG, Belo Horizonte, Minas Gerais, Brazil*

**plantasmed@ufop.br ou rosanagrd@gmail.com*

Introduction

Leaves of *Psidium guajava* L. (guava) in Brazilian communities are used in cases of diarrhea, dysentery, flatulence and abdominal pain. There is no standardization of plant organ used, how to use and dosage. In the communities, the traditional population reports the use of leaves and bark in natura in teas (infusion and decoction) and beverages. With the expansion of Phytotherapy in Brazil, vegetable drug (dried leaves of guava) is available in the herbalists with extensive marketing, without having standardized quality and or determination of minimum levels of biomarkers.

Method

Seeking to establish a standard that meets the Brazilian market of medicinal plants this study aimed to validate methodologies for natural and artificial drying of the leaves of guava at different stages of maturity – young, in primary development and adult, fully expanded – depending on quantification of secondary compounds and antioxidant properties. In the botanical standardization was made macroscopic description of the leaves. In the processes of drying, 5 g of fresh leaves (young and old) were exposed to artificial drying - SMO (microwave), SEA (circulating air oven), SEC (conventional oven) and natural (SCD - drying thin layer, as the treatments (SMO1, SMO2, SEA1, SEA2, SEC1, SEC2, SCD1 and SCD2) to constant weight. After the drying process, methanol extracts (macerated) were made, flavonoids identified qualitatively by TLC (fingerprint), dosed the contents of phenolic compound and flavonoids, the antioxidant activity determined for validation of drying processes. All analyzes were performed with four replicates and the data from the experiments were subjected to ANOVA and mean comparison test (Tukey) at 5 % significance level.

Results / Discussion / Conclusion

The stage of leaf development did not influence the drying processes, for residual humidity. The organoleptic characteristics (color, odor, and flavor) do not changes in any treatment. The highest yield of extracts of dried leaves (both stages) was obtained in artificially dried process (microwave). The levels of phenolic compound, in the development stages, did not differed in function of artificial and natural drying, thus being both effective processes. The extracts obtained after drying in a ventilated oven (SEA) had a higher yield of flavonoids for both young and mature leaves. In all extracts of young leaves, the levels of flavonoids were higher than those of adult leaves, when compared against the same methodology drying. The content of flavonoids of extracts of young leaves was greater than that of mature leaves, when compared against the same methodology drying. The antioxidant activity of extracts from young leaves of guava obtained after drying in a conventional oven were superior to all other extracts, confirming that the phytomedication obtained with young leaves should have better pharmacological effect. In the TLC were detected in all treatments flavonoids and tannins. In function on the results obtained, the best drying process was in a ventilated oven for young leaves and mature leaves, regarding preservation of biocompounds.

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PO205
DETERMINATION OF FATTY ACID COMPOSITION IN SICILIAN EXTRA VIRGIN OLIVE OILS BY MEANSOF 1H NMR SPECTROSCOPY AND GAS CHROMATOGRAPHY

Cicero, N.[1]; Mallamace, D.[1]; Corsaro, C.[2]; Salvo, A.[1]; Rando R.[1]; Bartolomeo G.[1]; Dugo, G.[1]
[1]*Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute - University of Messina, Viale ferdinando Stagno d'Alcontres, 98166 Messina, Italy*
[2] *Department of Physics and Hearth Science - University of Messina, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy.*

Introduction

The fatty acid composition in extra Virgin Olive Oils (VOOs) is the major factor influencing their chemical and physical properties and consequently their overall quality in terms of organolepetic and nutritional factors.
In particular, health effects have been attributed to certain fatty acids such as oleic and linolenic acids [1]. Indeed a balanced proportion of fatty acyl chains in the diet is of primary importance for human health.
As it is well known, the composition of fatty acids in extra VOOs changes from cultivar to cultivar.
Our aim is to characterize the individual composition allowing the identification of the geographical origin of each cultivar. In doing so it is possible to determine the extra VOO authenticity and to prevent the adulteration of high-value extra VOOs [2]. We use two different but complementary experimental techniques such as Nuclear Magnetic
Resonance (NMR) spectroscopy and Gas Chromatography (GC) in order to determine the fatty acid composition of several Sicilian extra VOOs.
The big difference between the two techniques is that for GC experiments there is the need to "extract" the portion of interest within samples whereas for NMR experiments samples were only diluted in deuterated chloroform before the acquisition of the spectra. GC experiments were performed with a GC Shimadzu 2010 with FID detector. NMR experiments were conducted with a Bruker Avance 700 MHz spectrometer by means of the experimental setup known as High-Resolution Magic Angle Spinning (HR-MAS).
The synergic use of both techniques has allowed obtaining the fatty acid composition of different extra VOOs produced in Sicily in order to safeguard both producers and consumers by commercial frauds. For example, the development of precise experimental protocols would allow the protection of those extra VOOs accredited by European certificate such as Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO).

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PO206
BIOLOGICAL ACTIVITIES AND PHENOLIC CONTENT IN EXTRACTS OF *Brassica* AND *Chicorium* SPECIES

Russo, D.; Di Marsico, A.; Marino, L.; Scrano, L.; Candido, V.; Milella, L.[2*]
University of Basilicata, v.le dell'Ateneo Lucano, 85100 Potenza, ITALY
** luigi.milella@unibas.it*

Introduction

Fruit and vegetable-derived foods have become a very significant source of nutraceutical phytochemicals. Diets rich of these compounds are associated to a lower risk of cancer and cardiovascular diseases [1]. Some vegetables have gained attention due to their phenolic content, in particular anthocyanins, natural colorants with health-promoting properties [2]. The aim of this study was to evaluate total polyphenols content and antioxidant activity among different extracts from three common vegetables: cabbage, red cabbage and red chicory.

Method

Dried samples of two varieties of *Brassica oleracea* L. (cabbage and red cabbage) and *Chicorium intybus* L. (red chicory) were extracted by sequential maceration by using n-hexane, chloroform and acidified methanol. Methanol was acidified by 1% HCl to extract anthocyanins, phenolic compounds abundant in selected vegetables. Methanol extracts were partitioned by H_2O/buthanol. All extracts and fractions were tested to evaluate radical-scavenging activity, reducing power and inhibition of lipid peroxidation by three different assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing ability power) and BCB (beta-carotene bleaching), respectively [1]. Total polyphenols content was also investigated by Folin-Ciocalteu test [3], and anthocyanin content was also measured. Relative Antioxidant Capacity Index (RACI) was also calculated to compare the results obtained from different tests.

Results / Discussion / Conclusion

Results showed that buthanol fractions had the highest content of polyphenols related to their radical-scavenging and reducing power. Among investigated vegetables, *Cichorium inthybus* demonstrated the highest antioxidant activity related to the highest polyphenols content. The present work demonstrated that *C. intybus* is a most promising source of bioactive compounds with wide range of potential applications. Further investigations may be focused on characterization and isolation of secondary metabolites, responsible of antioxidant activity.

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PO207
MACAMIDES AND FATTY ACIDS CONTENT COMPARISON IN MACA CULTIVATED PLANT UNDER FIELD CONDITIONS AND GREENHOUSE

Melnikovova, I.[1*]; Havlik, J.[1]; Fernandez Cusimamani, E.[1]; Mecca, A.[2]; Frescura, D.[2]; Milella, L.[2*]
[1]*PhD, Czech University of Life Sciences Prague, Department of Crop Sciences and Agroforestry in Tropics and Subtropics, Prague, Czech Republic*
[2]*PhDBasilicata University, Department of Biology, Faculty of Pharmacy, v.le dell'Ateneo Lucano, 85100 Potenza, ITALY*
** E-mail addresses: melnikovova@its.czu.cz; luigi.milella@unibas.it*

Introduction

Maca (*Lepidium meyenii* Walp., Brassicaceae) is a Peruvian tuberous crop cultivated in the Andes mountains at altitudes above 3.500 m. It is grown for consumption of its nourishing hypocotyl and it is extensively used for nutritional and medicinal purposes. Maca is normally used to increase physical energy, support the immune system, and is traditionally considered to be an aphrodisiac which enhances fertility in humans and in domestic animals. The purpose of the study was to reveal how maca responds to diverse climatic conditions and what the prospects of its cultivation are out of its original area.

Method

Five samples of dried ground maca hypocotyls and one sample of dried ground leaves were compared collected from the same genotype cultivated in different climatic conditions.
One gram of each finely ground maca sample was extracted in petrolether in enclosed extraction vials for 4 hours. Each sample was stored dried and before the analysis each one was diluted to final concentration 0.5 mg/mL. Before extraction, internal standard linoleic acid methylester was added in concentration 1 mg/g of dried sample. Analysis was performed by the method described by McCollom *et al.* [1]and macamides and fatty acids were identified by retention time (RT) by comparison and UV spectra according to their publication. Acquired data were quantified according to the calibration curve with external standards. Analysis were repeated three times and results were expressed as mg/g of dried weight (DW).

Results / Discussion / Conclusion

The macamide (main quality marker of maca) content has been analyzed by HPLC-UV in plant material of various samples of maca of Peruvian origin and it was compared to content in samples of maca cultivated in the Czech Republic, under field conditions and in a greenhouse. There was a significantly lower concentration of macamides in the sample grown in the Czech Republic compared to the Peruvian samples. There were no macamides found in samples cultivated in the greenhouse. In fact this study proved that maca produced much less macamides under mild climatic conditions of the Czech Republic then in its original environment, wind-blown rocky areas in high plateau of Peru [2].If we admit that macamides are responsible for fertility enhancing properties of maca, we can conclude there is no possibility of cultivation for this maca genotype in the Czech Republic for this use.

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PO208
BIOLOGICAL ACTIVITY OF FROG SKIN SECRETIONS FROM *HYPSIBOAS CREPITANS* (ANURA: HYLIDAE)

Lamadrid-Feris, F.[1]; Valencia J., A.[3]; Sihler, W.[5]; Souza, M. L.[5]; Grossi-de-Sá, M. F. [5]; Franco, O. L.[4]; Arboleda V., J. W.[2,4]*

[1]*MSc/Profesora cátedra, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Biología, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

[2]*PhD/Profesor Asociado, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Biología, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

[3]*PhD/Profesor Asociado, Universidad de Caldas, Departamento de Fitotecnia, Manizales, Calle 65 # 26-10, Colombia.*

[4]*PhD/Profesor Titular, Universidade Católica de Brasília, Centro de Análises Proteômicas e Bioquímicas, SGAN 916, Campus II – Asa Norte – Brasília/DF – 70790-160 Brazil.*

[5]*PhD/Pesquisador, Embrapa-Recursos Genéticos e Biotecnologia, PqEB, W5 Norte Final, Brasília/DF, 70770-900, Brazil.*

**jwarboleda@gmail.com / jorgearboleda@mail.uniatlantico.edu.co*

Introduction

The nature exhibits great number of organisms that secrete substances through their skin as the first defense mechanism against pathogens, predators or competitors. The amphibians are known to possess many glands that produce compounds involved in those mechanisms (Rinaldi, 2002; Barra and Simmaco, 1995). Although the cutaneous secretions of amphibians contain a wide number of biologically active substances, those with antimicrobial activity have particular interest because they could represent novel and useful molecules with potential to be used in several areas of the human development such as: industry, crop protection and medicine among other. For example, peptides from the brown Russian frog, *Rana temporaria* have demonstrated similar inhibitory effect than antibiotic acting on gram-negative and gram-positive bacteria (Samgina *et al.* 2012*)*. Likewise, the toxin batrachotoxin obtained from frogs of the family Dendrobatidae, is an interestingly compound that exhibits biological activity against predators (Cortázares, 2011). The frogs of low lands as the white frog *Hypsiboas crepitans,* faces extreme climatic conditions due to the water stress and high temperatures characteristic of this ecosystem; which could affect the secretion of substances from physiologic repertoire not only to protect them from drying, but also against typical pathogens in places like the ponds used in the reproduction (Elkan, 1968; Pellis *et al.* 2010). In fact, are these conditions that allow *H. crepitans* be considered as an interestingly source of compounds with antimicrobial activity.

Method

Samples were taken from different individual selected at the natural habitat of the *H. crepitans,* it rubbing several parts (back, legs or head) with wet cotton swab. Then, extractions were realized dissolving them with double distilled water or with Tris-HCl 50 mM buffer solution, followed by centrifugation at 10000 rpm for 15 min. The bioassays of antibacterial and antifungal activity were carried out by using the microdilution protocol in broth described in the M7-A6 of the National Committee for Clinical Laboratory Standards (2006), against the bacteria *Escherichia*, *Klebsiella*, *Staphylococcus* and *Candida* sp., which are considered the main cause of infections at hospital level. In addition, tests of cytotoxic activity on insect cell line *S. frugiperda*: IPLB-Sf-21, mosquito larvicidal activity and hemolytic activity were performed by following the methodology described by Arboleda *et. al* (2011). All experiments were evaluated with 3 repetitions, including appropriate controls. Results were analyzed by using ANOVA and Tukey's mean comparison test using SPSS software (SPPS Inc., Chicago, Illinois).

Results and Discussion / Conclusion

In vitro test performed with the extracts of cutaneous secretions from different parts of the frog *H. crepitans,* have showed antimicrobial activity and significant statistical differences in all assessments when compared with the respective controls used Gentamicin or Nystatin. The inhibition rate ranged from 30% to 90% against *Escherichia* and *Staphylococcus,* but this assay showed no inhibitory activity against *Klebsiella*. It was observed that a minimum concentration of aqueous extract (1:25 v/v) was required to induce membrane rupture, vacuolization, decreased in growth rate and deformation of the insect cell line Sf-21. The cell viability ranged from 14% to 45%, showing a positive exposure-response relationship. Different authors worldwide have previously demonstrated the presence of molecules from frogs, with potential to biotechnological applications (Rinaldi, 2002; Samgina *et al.* 2012). Moreover, these results confirm the initial hypothesis of the presence of antimicrobial substances in the cutaneous secretions of the banana frog, which are of interest for pharmaceutical or agrochemical industry. In conclusion, a systematic bioprospection for identification of promising com-

pounds from animals of the Anuran families should be included in addition to the study of aspects related to feed and animal behavior

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PO209
CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF HAZELNUT SHELLS

Picerno, P.; Franceschelli, S.; Di Somma, A.; Esposito, T.; Sansone, F.; Del Gaudio, P.; Russo, P.; Aquino, R.; Mencherini, T.*
Department of Pharmacy, University of Salerno, via Giovanni Paolo II, 132, 84084, Fisciano (SA)
**tmencherini@unisa.it*

Introduction

Hazelnut (*Corylus avellana* L., Betulaceae family) is one of the most cultivated and consumed nut in the world, not only as whole fruit (raw or roasted) but also as an ingredient of manufactured food products.
A lot of hazelnuts by-products, including the green leafy cover, hard shell and skin, are produced during their harvesting and industrial processing. The hard shells account for a majority of this waste and represent a huge amount of discarded material available at very low cost. Nowadays, the hazelnuts shells are used for burning as a heat source, for mulching, and as a raw material for the furfural production in the dye industry (Stévigny C., *et al.* 2007). In the last years, great attention has been paid to the recovery and upgrading of agro-industrial residues as potential sources of bioactive compounds with beneficial effects on human health, and some papers have already showed the potential use of hazelnut wastes as natural antioxidants (Shaidi F., *et al.* 2007; Contini M., *et al.* 2008).
For the first time, in this research, it has been reported the chemical composition of a polar extract obtained from hazelnuts hard shells. Moreover, the antiproliferative activity of the extract and its constituents has been evaluated on A375 and SK-Mel-28 (melanoma cancer) and Hela (cervical carcinoma) cell lines.

Method

Hazelnut (var. Mortarella and San Giovanni in mixture) hard shells were kindly supplied by a local hazelnut processing industry (Hazelnuts South Italy Manufacturing s.r.l., Baiano, AV). The shells were defatted with *n*-hexane and chloroform and then extracted with methanol. This extract was purified by Sephadex LH-20 followed by RP-HPLC to give twelve compounds. Their structures were elucidated using spectroscopic methods including 1D- and 2D-NMR experiments as well as ESIMS analysis. The polyphenols content of the extract was examined by the Folin-Ciocalteau colorimetric assay (Picerno P., *et al.* 2011). The concentrations of the major compounds were determined by a RP-HPLC-DAD direct calibration method. The free-radical scavenging activity of both the extract and pure compounds was evaluated by DPPH-test (Picerno P., *et al.* 2011). The effects of hazelnut shells extract and its constituents on cell proliferation and apoptosis on human cancer cell lines were evaluated by the MTT bioassay and propidium iodide staining, respectively (Mencherini T., *et al.* 2011).

Results

The phytochemical investigation of the methanol extract of hazelnut shells led to the isolation and characterization of four neolignans, seven phenolic compounds, and a diarylheptanoid. C-veratroylglycol, lawsonicin and cedrusin resulted the major constituents of the extract (2.93, 1.98, and 1.79%, respectively, HPLC method). The polar extract showed a significant and concentration dependent free-radical scavenging activity (EC_{50} 31.87 ÿ'3fg/ml, with respect to ÿ'3f-tocopherol used as positive control EC_{50} 10.10 ÿ'3fg/ml) correlated to its high polyphenols content (193.80 ÿ'3fg/mg), mainly gallic acid and methyl gallate (EC_{50} 1.20 and 1.19 ÿ'3fg/ml, respectively). Moreover, the extract exhibited a signiÿ'3fcant and concentration-dependent inhibitory eÿ'3fect on tested cancer cell growth (IC_{50} 500 µg/mL), also increasing the percentage of hypodiploid cells at the same concentration. The activity seem to be correlated to the presence of pure compounds, balanophonin (a neolignan) and gallic acid, which showed a citotoxic effect on all tested cell lines (IC_{50} 150 ÿ'3fM). Furthermore, the neolignan cedrusin showed a citotoxic activity only on the A375 and HeLa cells.

Conclusion

Our results showed that hazelnut shells, a by-product of industry processing, can be consider a newsworthy source of bioactive compounds (neolignans, phenolics and diarylheptanoids) with good antioxidant and promising chemopreventive properties.

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PO210
TECHNOLOGICAL PROPERTIES OF A MICROENCAPSULATED MILK THISTLE (*SILYBUM MARIANUM*) EXTRACT

Francesca Sansone*[1], Teresa Mencherini[1], Patrizia Picerno[1], Tiziana Esposito[1], Paola Russo[1], Pasquale Del Gaudio[1], Franco Gasparri[2], Rita P. Aquino[1]
[1]*Department of Pharmacy, University of Salerno, via Giovanni Paolo II, 132, 84084, Fisciano (SA)*
[2]*Dermo-Cosmetic R&D Rottapharm Madaus Spa Via Valosa di Sopra, 20900 Monza, Italy*
**fsansone@unisa.it*

Introduction

Milk thistle (*Silybum marianum*) has been used for 2.000 years as an herbal remedy for a variety of ailments, particularly liver, kidney, and gall bladder problems. The active ingredient in milk thistle seeds is known as silymarin, a group of flavonoids (silibinin, silidianin, and silicristin), which are thought to help repair liver cells damaged by alcohol and other toxic substances. Silymarin also keeps new liver cells from being destroyed by these same toxins. It reduces inflammation (suggested for people with liver inflammation or hepatitis) and is a strong antioxidant. Most milk thistle products are standardized preparations made from the seeds of the plant containing 70 - 80% of silymarin. In this research we studied an industrial milk thistle extract (MTE) which is unique because its highest concentration (91%) of these powerful compounds, especially silibinin. Unfortunately, MTE has a very low water solubility and low permeability trough epitelial cells giving either practical difficulties for the successive manufacturing and reduced bioavailability. This reserch reports on the encapsulation of MTE by spray-drying in a Sodium carboxymethylcellulose coating/swelling matrix, and its technological characterization, to overcome the MTE limited industrial processability and bioavailability after administration.

Method

Milk Thistle Extract was supplied by Rottapharm s.p.a. (Monza, Italy), Sodium carboxy methyl cellulose (NaCMC, medium viscosity, E466) was supplied by Sigma Aldrich (Milan, Italy). A liquid solution (200 mL) containing 1:1 w/w NaCMC/MTE (2 g), was prepared using a H_2O/EtOH/Acetone 50/15/35 solvent ratio. NaCMC was dissolved in surfactant water with 0,05% w/v of SLS (Sodium Lauryl Sulfate); then, the organic solvents were slowly introduced and, finally, the MTE dried extract was added to the polymeric solution under continuous magnetic stirring. A 1% w/v total final concentration was kept. The liquid feed was spray dried in a Büchi B-191 Mini Spray Dryer (Büchi Laboratoriums-Tecnik, Flawil, Switzerland) under the following experimental conditions: inlet/outlet temperatures 100/65°C; spray flow feed rate 5ml/min; nozzle diameter 0.7 mm; drying air flow 600 l/h, air pressure 6 bar, aspirator 100%. Physicochemical and technological characteristics of the produced powder (MTE3sls) such as encapsulation efficiency, particle size (LLS analysis), solid state (DSC), morphology (SEM, FM), *in vitro* dissolution (USP II) and permeation (Frantz cells apparatus) properties were examined.

Results

The encapsulation efficiency obtained for MTE3sls and evaluated as silymarin content (ASC), was fairly high (91.2%). The functionality of MTE is correlated to the ASC, the higher is the ASC in the produced encapsulated powder form, the greater should be the final formulation functional activity, thus, the obtained encapsulation efficiency is a very interesting and promising characteristic. FM and SEM results indicated that the produced powder is characterized by microparticles in amorphous state. The MTE3sls encapsulated sample showed well formed, small (4.36μm) and trendy spherical microparticles with a smooth surface. Moreover, *in vitro* dissolution and basic permeation profiles, showed an enhancement of the dissolution rate in water (90% in 30 min) and a higher permeation (18.9 $\mu g/cm^2$ in 180 min) of MTE3sls with respect to MTE (1% dissolution rate and 3.6 $\mu g/cm^2$ of permeation, at the same times).

Conclusion

The spray-drying process led to a handling powder with improved technological characteristics, bioavailability, quality and safety of use, suitable as an ingredient for pharmaceutical, cosmetic or nutraceutical products.

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PO211
LEVELS OF CADMIUM IN WARTY CRAB (*Eriphia verrucosa*): PRELIMINARY RESULTS

Ariano A.[1], Lo Voi A.[2], Velotto S.[1], D'Ambola M.[1], Cacace D.[2], Severino L.[1]
[1]Department of Veterinary Medicine and Animal Production, Division of Toxicology, University of Naples Federico II, via Delpino 1, 80137, Naples, Italy;
[2]S.S.I.C.A. - Stazione Sperimentale per l'Industria delle Conserve Alimentari – Parma - Italy via Nazionale 121, 84012 Angri (SA)
lorella.severino@unina.it

Abstract

EU regulations establish the cadmium maximum residual levels (MLR) for crab taking into account only concentrations obtained for crab muscle, mainly from appendages, therefore excluding other organs and tissues.
The objective of the present study was to evaluate cadmium levels in appendages and also in digestive gland, well-known to be the most site for cadmium storage and detoxification. For this purpose, concentrations of cadmium were determined in Warty Crab (*Eriphia verrucosa*) collected from the southern Tyrrhenian Sea by means of microwave digestion and atomic absorption spectrometry.
Cd concentrations were found very low in all samples of muscle from crab appendages (< LOQ). Digestive gland showed Cd concentrations ranging between 0.931 and 4.612 mg kg^{-1} (mean value 3.107 mg kg^{-1}).
Therefore, preliminary results show that cadmium concentrations were largely below the MLR established by the European Commission for muscle from crab appendages (Reg CE 1881/2006). Digestive gland showed the highest metal concentration. The observed results highlighted that the consumption of organs and tissues included in crab body such as abdomen, gonads and, in particular, digestive gland, very probable in certain populations of Mediterranean region due to the traditional and unusual consumption of raw and whole crustaceans, substantially increased the cadmium intake up to alarming values.

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PO212
MICROELEMENTS IN BEANS FROM MEDITERRANEAN AND TROPICAL AREAS

Naccari C., Di Bella G., Bua G. D., Lo Turco V., Dugo G.mo
University of Messina, Departmentof Environment, Safety, Territory, Food and Health Sciences "Carmelo Abbate", VialeFerdinandoStagnod'Alcontres, N 31,Messina (ITALY); gdibella@unime.it

Introduction

Beans are the most important legumes for direct human consumption in the world (Broughton et al, 2003) andrepresent economically a very important food crop in many tropical and subtropical areas, where they are produced.Thisfood are commonly consumed in diet, often according to traditional recipes, for its nutritional properties, being rich of proteins, vitamins and minerals (Welch *et al.*, 2000).Micronutrients, essential elements in body development, potentially toxic when exceed the normal functional level, are easily adsorbed from soil through radical apparatus of plants.The aim of the present investigation was evaluate the content of essential and toxic microelements (Cr, Co, Ni, Se, Sb, V, Cd, Pb, As and Hg) in several bean samples, like Small Pinto (SP) and Green Eyed (GE) from Italy, Black Turtle (BT) from Mexico, Red Kidney (RK) from India, Red Adzuki (RA) from East Asia, Small Red (SR), Red Flat (RF) and White Eyed (WE) from Ghana.

Method

Beans samples were subjected to mineralization using a closed-vessel microwave digestion system(CEM Microwave™ Digestion System, Discovery SP-D, CEM Corporation Mathews, NC, USA).Chromium (Cr), Cobalt (Co), Nickel (Ni), Selenium (Se), Antimony (Sb), Vanadium (V), Cadmium (Cd), Lead (Pb) and Arsenic (As) concentrations were analyzed by Inductively Coupled Plasma-Mass Spectrometry iCAP Q (Thermo Scientific, Waltham, MA ICP-MS), equipped with an auto-sampler ASX520 (Cetac Technologies Inc., Omaha, NE, USA).For Mercury (Hg), instead, a Direct Mercury Analyzer (DMA-80, Milleston, USA) was used.Data are expressed as mean ± S.D. of at least three determinations.

Results

The analysis carried out showed the presence ofmicroelements studied in all bean samples. Among essential micro-elements, the concentrations observed were higher for Ni (range: 14.321 - 83.564 ng/g), intermediate for Cr (range: 0.450 - 3.339 ng/g), Co (range: 0.522 - 7.581 ng/g) and Se (range: 0.441 - 1.924 ng/g), smaller forSb (range: 0.028 - 0.075ng/g) and V (range:0.102-0.816 ng/g). Particularly,the biggest content for Ni was found inBlack Turtle (BT) (83.564±0.093 ng/g), Red Adzuki (RA: 74.306±0.010 ng/g) and Red Kidney (RK: 67.019±0.054 ng/g);for Co and V in Red Kidney (RK: 7.581±0.019 ng/g and 0.816±0.005ng/g) andfor Cr in Red Adzuki (RA: 3.339±0.010 ng/g). Small Pinto and White Eyed, instead, were richest of Se (SP: 1.924±0.002ng/g;WE: 1.309±0.002ng/g).Relating to toxic metals, Pb presented the highest residual levels (range: 4.084- 6.810ng/g), Hg intermediate (range:1.610 - 4.198ng/g), Cd and As lowest (range: 0.231-0.317 ng/g; range:0.033- 0.077ng/g). In details, Small Pinto, Green Eyedand White Eyedshowed highest concentrations of Cd (SP: 0.317±0.009; GE: 0.315±0.005; WE 0.316±0.001 ng/g) and Pb (SP: 6.810±0.016; GE: 6.665±0.017; WE: 4.717±0.006 ng/g); Small Red of As (SR: 0.077±0.001 ng/g), Red Adzuki,Black Turtleand Small Red of Hg (RA: 4.198±0.024; BT: 4.125±0.040; SR: 3.861±0.026ng/g).

Discussion andConclusion

The preliminary results of this study showed a different trend of microelements distribution among bean samples analyzed, particularly relating to Ni and Se. In fact, Ni concentrations were very low in beans from Ghana (SR, RF, WE), intermediate in beans from Italy (SP, GE) and significantly higher in those from India (RK), East Asia(RA) and Mexico (BT). For Se, instead, the highest content was found in Small Pinto from Italy and White Eyed from Ghana, possible due to the area of production more rich in selenium than others.
The microelements content, in fact, is directly influenced by the different uptake and transport of from soil to aerial parts of plant through its radical apparatus; consequently, environmental features (climate, soil, geology) and pollution, specific agricultural practices, in addition to genetic factors and different variety of beans, play a key role in the mineral distribution.Therefore, further investigation will be carried out on other varieties of beans samples from several tropical areas to analyze metals distribution according to origin, variety and cultivars, with potential use to food composition tables.

These results, however, confirmed a safe consumption of these legumes for the presence of significant content of essential microelements and low residual levels of toxic metals, less to specific MRL, according to CE regulation 1881/2006.

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PO213
POPSUPTAKE BY FOOD CHAIN: CASE STUDY OF LEGUMES FROM GHANA

G. Di Bella[a], I. Haddaoui[b], V. Lo Turco [a], R. Rando[a], G.Dugo [a]
[a]Department of Environment, Safety, Territory, Food and Health Sciences "Carmelo Abbate" University of Messina (Italy),Viale Ferdinando Stagno d'Alcontres n°3198166-S. Agata (Messina). *gdibella@unime.it*
[b] Department of Horticultural Systems Engineering and Environment, Higher Institute of Agronomy Chott Mariem, Sousse University, BP. 47, 4042 Sousse, Tunisia

Introduction
Persistent organic pollutants (POPs) are a wide class of chemical species with different physicochemical properties and toxicology.Pollution of food chain by POPs is one of the global problems that arearousing great attention.
The aim of this studywas to determine the uptake ofpolycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organo-chlorinated pesticides (OCPs) residues inlegumes fromKade,South Ghana.

Method
Five different varieties of legumes were the subject of this work.All subsequent determinations were carried out in triplicate.Extraction was done with an accelerated solvent extractor (ASE 350, Dionex), in two steps at two different temperature, followed bya clean up according to the EPA Standard Method 3620C (USEPA, 2007). Finally thesamples were analyzed by GC–MS–MS–SRM (TSQ Quantum XLS, Thermo Fisher Scientific).

Results / Discussion
Results revealed the uptake ofPOPs inlegumes samples,demonstrated by thepresence of residues of PAHs (concentration ranged from 18.81±22.86 to 88.83±26.18 μg kg^{-1}ww), PCBs (from 1.85±24.66 to 3.87±25.76 μg kg^{-1}ww) and OCPs (from 0.65±3.86 to 1.81±16.74 μg kg^{-1}ww). Low Molecular Weight (LMW) PAHs (such as, acenaphthylene, fluorene, phenanthrene, anthracene and pyrene) were the dominant congeners in legumes samples.We record concentration exceeding Europeans Union limitsfixed to 2 μg kg^{-1}(EU CR, 2005)for Benzo_a_antracene, Benzo_b_fluoranthene, Benzo_k_fluorantene, dibenzo-a,h-anthracene, indeno-1,2,3-cd-pyrene and Crisene.For PCBs we registered levelsover the legal limit of 3 μg kg^{-1} in two samples. DDE are the most detected molecules among the 16 sought OCPsand are also the most detected molecules among metabolites of DDT.It can be assumed thatasystem is stable with no new DDT inputsand DDT residues in legumesderivefrom historic contamination.

Conclusion
We can think that the possible impacts of miners activities on food chain are manifested in the detection of large number of POPs.In Ghana, environmental problems are associated with the mining of precious minerals that begin in the early 1900s. In fact, with this long period of mining in the region of Ghana a large numbers of persistent organic pollutants (POPs) can spread on water, air and agricultural soil and transferred to the human food.

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PO214
FATTY ACID CONTENT IN DIFFERENT BEAN VARIETIES

Ravenda P., Di Bella G., Potortì A. G., Lo Turco V., Dugo G.mo
University of Messina, Dipartimento di Scienze dell'Ambiente, della Sicurezza, del Territorio, degli Alimenti e della Salute (S.A.S.T.A.S.) "Carmelo Abbate", Viale Ferdinando Stagno d'Alcontres 31- 98166, Vill. S. Agata – Messina (Italy); gdibella@unime.it

Introduction

Beans are nutritionally and economically important food crop in each part of the world. Numerous cultivars of *Phaseolus vulgaris* have now been diffused, the seeds of which differ in size, shape, and color and are called by a variety of names, depending on where they are cultivated.
The aim of this research was to study the fatty acid content in beans of different varieties and geographical provenience, in order to evaluate the amount of linolenic and linoleic acids, the precursors of the long-chain n-3 and n-6 polyunsaturated essential fatty acids (EFA), which have an immense interest as good nutritional agents and nutraceuticals.

Method

Samples of dry beans from eight different cultivars of *Phaseolus vulgaris* were purchased randomly from sellers in the Italian or Ghanaian market in lots of one kilogram.
The eight varieties were: small pinto (SP), green eyed (GE), black turtle (BT), red kidney (RK), red adzuky (RA), small red (SR), red flat (RF) and white eyed (WE) beans.
The lipid fraction was extracted with *n*-exane and transesterified with 0.5M KOH in methanol. 1 μL of the upper layer was analyzed by GC-FID using a SLB-IL 100 (Supelco, Bellefonte, PA, USA) capillary column (60m x 0.25 mm i.d., 0.20 μm f.t.).
The obtained results were submitted to chemometric analysis using SPSS 21.0 software (SPSS Inc., IL).

Results / Discussion / Conclusion

The oil yields for the eight varieties ranged from 1.4% to 2.1%. The principal determined fatty acids in analyzed beans were palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6) and linolenic (18:3n-3), which represented about the 97% of total fatty acid content.
The Kruskal-Wallis test results showed significant differences at *p*-level below 0.01 for fatty acids previously cited, according to bean varieties. In particular, SP, GE, BT and RK bean varieties had a high predominance of linolenic acid (40.8-59.7 %), followed by linoleic (16.2-32.7 %); whereas linoleic and palmitic acids are the dominant in SR (respectively 41.1 and 33.3 %), RF (33.4 and 28.1 %) and WE (31.1 and 28.5%). Only RA showed the same amount of palmitic, oleic and linolenic (about 25 %). Stearic acid was present at the lowest concentration in all samples (1.2-6.4%).
Therefore, consumption of small red, red flat and white eyed bean varieties, can provide health benefits for human, since they contained a good amount of the n-3 and n-6 EFA, which reduce the risk of coronary heart disease.

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PO215
DETERMINATION OF HYPOGLYCEMIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *Justice chlorostachya* (PLANT INSULIN). IN MICE WITH INDUCED HYPERGLYCEMIA

Pazmiño, C.[1]; Contero, F.[1]; Vinueza, D.[1]
[1]*Profr, Escuela Superior Politécnica de Chimborazo/ Escuela de Bioquímica y Farmacia, Panamericana Sur Km 1 1/2, Riobamba, Chimborazo, Ecuador.*
**carlin_uno@hotmail.com*

Introduction

Diabetes is a metabolic disorder characterized by an increase in the concentration of blood glucose due to a defect in the secretion or action of insulin. (Moore & Dean., 2009) There two types of diabetes, type 1 and 2, of which diabetes mellitus type 2 (non insulin dependent) is the most prevalent and is major causes of premature illness and death worldwide (Wild *et al.*, 2004). WHO estimates that worldwide there are over 220 million people with type 2 diabetes mellitus, a risk factor that most likely, barring some intervention, will double by 2030 (WHO., 1998) Current therapy treatment groups as sulfonylureas biguadinas etc, has not been able to restore functional homeostasis in glycemic control of patients with diabetes. Medicinal plants with anti-diabetic activity can be an important source of new oral hypoglycemic compounds, either as first-line compounds in the treatment of diabetes, or as adjuncts to existing therapies (Li *et al.*, 2009; Sharma *et al.*, 2011; Ortiz *et al.*, 2012). For this reason is very important to demonstrate scientifically that the ethnoancestral knowledge of Ecuadorian Amazonian indigenous communities. The same as have used to Justice chlorostachya (plant insulin) as an essential arsenal phytotherapy for diabetes mellitus non-insulin dependent.

Method

The Hydro-alcoholic extracts (16, 32 and 64 mg/kg/day) of Justicia Chlorostachya were administered to both normal and Hyper-glicemic induced mouse BALB/c at defined time intervals. Blood glucose levels were measured at 0, 0.5, 1, 2 h. The blood glucose level was determined at different times by the glucose oxidase method. The dates obtained were statistically analyzed with ANOVA at one factor with clustered data a 95% confidence (a=0,05). The acute toxicity (OECD., 2008) and the histological analysis with Hematoxilin & Eosin Tintion were used to assess the gastric, hepatic and renal parenchyma.

Results / Discussion / Conclusion

The results clearly show the hypoglycemic effect of the hydro alcoholic extract of Justice chlorostachya, stressing that from the lowest dosage 16 mg/kg weight, it proved to be a substituting potential as efficient as the treatment with acarbose (0.7mg/Kg weight) with 1x10-14 p-value. Moreover it was evident that with the toxicological and hystopathological study the administered dosages proved to be atoxic, and histologically safe over the pharmacokinetic organs involved (Stomach, Liver, Kidney). It is concluded that the ethnobotanic use given to vegetal insulin, as a hypoglycemic agent, is scientifically testes with the present investigation, and that the protagonistic secondary metabolites in such a pharmacological capacity are phenolic compounds with flavonoid prototype.

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PO216
GASTROPROTECTIVE EFFECT OF CONCENTRATED AQUEOUS EXTRACT OBTAINED BY GRINDING AND CONCENTRATION UNDER CONTROLLED CONDITIONS FROM TUBERS OF *Smallanthus sonchifolius* ON INDOMETHACIN-INDUCED GASTRIC ULCER IN *Rattus norvegicus*

Vinueza, D.[1]*; Donoso, C.[1]; Andino, G.[1]; Abdo, S.[1]; Contero, F.[1]; Duque, O.[1]; Cuadrado, L.[2]; Villacrés, E.[2]

[1]*Prof, Escuela Superior Politécnica de Chimborazo, Panamericana Sur Km 1 1/2, Riobamba, Chimborazo, Ecuador*
[2]*Ing, Instituto Nacional de Investigaciones Agropecuarias, Panamericana Sur Km. 1, Mejía, Pichincha, Ecuador*
**drvinueza@espoch.edu.ec*

Introduction

According to research conducted to date is considered that gastric ulcer is a common disease in which discontinuity is observed in the gastric mucosa (Manonmani *et al.*, 1995). Gastric ulcer is pathology of wide recurrence within the population, which could be caused by a number of factors such as stress, drugs, alcohol, etc. (Mc Guigan, 1991). The conventional drugs used in the treatment of gastric ulcer include histamine (H2) receptor antagonists, proton pump inhibitors, antacids and anticholinergics; although they provide beneficial effects, most of these drugs contribute various undesirable side effects and drug interactions (Prakash *et al.*, 1998). Use of natural drugs in gastric ulcers is well documented. Most of these drugs augment the mucosal defensive factors, which are thought to be important for protection of gastric mucosa (Goel, 1986).

Since ancient times, many people have used plants as a resource to address their needs both from a nutritional standpoint as preventive and curative. Smallanthus sonhifolius belongs to the Asteraceae family and has been used as a traditional herbal medicine against a variety of diseases in some countries as Brazil and Perú. At contemporaneous time, some polysaccharides such as xylans are useful for lowering cholesterol levels, decreasing postprandial glucose absorption in the intestine, and promoting gastroprotective effects (Cipriani et al., 2008; Peng *et al.*, 2009). In this sense, the compositional characteristics of S. sonchifolius root tubers, rich in fructooligosaccharides (oligofructans) are polymers of D-fructose joined by β (2—>1) linkages and terminating with a sucrose molecule, taken important relevance. The singular configuration of linkages, making FOS indigestible (commonly called "dietary fiber") it has to show beneficial effects by many investigations associated to the Bifidobacteria proliferation. Bifidobacteria appear to benefit humans and animals by several mechanisms: immunopotentation, intestinal acidification of the large bowel/cecum through organic acid production, and competing with less desirable bacteria for both nutrients and sites of attachment to the intestinal wall (Rašiæ & Kurmann, 1983 in Modler,1994). The production of organic acids (acetic and lactic) limits the growth of putrefactive and pathogenic bacteria which are capable of producing undesirable enzymes such as β-glucuronidase, nitroreductase, azoreductase and β-glucosidase (Daly,1991; Kurmann & Rašiæ, 1991 in Modler, 1994). Curbing the growth of these undesirable organisms has additional benefits in terms of reducing the production of substances noxious to the host (vasoconstricting amines, phenols, steroid metabolites, bacterial toxins, etc.) and also reducing the release of N-nitroso compounds which are potential carcinogens. Reducing intestinal pH also reduces hepatic load by increasing the excretion of ammonia (Miller-Catchpole, 1989 in Modler, 1994).

Method

S. sonchifolius concentrated aqueous extract was donated from Instituto Nacional de Investigaciones Agropecuarias (INIAP) of Ecuador with a labeled amount of 30 % fructooligosaccharides (fractions GF2-GF-4), determined through of high performance liquid chromatography coupled a refraction index detector. The concentrated aqueous extract was obtained from tuber of S. sonchifolius (line ECU 1243) and later concentration of obtained juice on rotary evaporator. Ranitidine was suministrated from Nueva Industria Farmacéutica Asociada (NIFA) of Ecuador. Indomethacin was purchased from Sigma Aldrich (USA). All other reagents were of analytical or high-performance liquid chromatography grade as appropriate. S. sonchifolius concentrated aqueous extract was conveniently diluted with deionized water until get administration concentrations of 50, 100 y 200 mg of Fructooligosacharides (GF2-GF-4 fractions) by Kg of animal weight. Ranitidine was diluted with the same vehicle until get an administration concentration of 100 mg/Kg. Indomethacin was prepared to an administration concentration of 50 mg/Kg and was used how ulcerogenic rev.: 20.02.25 05.07.2014 agent. Male Wistar rats weighing 265±33 g were used. The animals were housed under standard laboratory

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conditions (12 h light/dark cycle, temperature 22±2 °C, relative humidity 55±10 %). Standard pellet food and H_2O were available ad libitum. Subsequently, the animals were deprived of food 24 h prior to the experiment, but were provided with free access to drinking water until 1 h before producing a gastric injury. All animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. The laboratory rats were used as experimental subjects, of 4 - 4.5 months old, obtained from the animal facility center of the Faculty of Sciences, ESPOCH. 30 rats were divided into 5 experimental groups of 6 rats each. Based on the protocol of Lee, et al (1971), 30 minutes before administration of ulcerogenic agent, were treated at the same day orally with deionized water (negative control), ranitidine solution 100 mg/Kg (positive control) or various concentrations of S. sonchifolius concentrated aqueous extract diluted each corresponding to 50, 100 and 200 mg/Kg. At the end of the experiment, euthanasia was carried up and it was obtained stomachs by laparotomy and determined the ulceration index. The stomachs were subject to histopathology analysis.

Results / Discussion / Conclusion

Administration of the medium doses of *S. sonchifolius* concentrated aqueous extract caused a significant decrease in ulceration index in comparison with the negative control. The ranitidine administration causes this effect, since ranitidine is a probed antagonist of histamine receptors (H2). No significant difference at the decrease in the ulceration was observed only in one experimental group treated with a 100 mg/Kg dose of S. sonchifolius concentrated aqueous extract, compared with positive control, which could indicate a significant decrease in the ulceration at this dose. The extracts tested could have effect on the microbiota, and short chain fat acids levels. The treatments with S. sonchifolius concentrated aqueous extract at low dose (50 mg/Kg) and high dose (200 mg/Kg), have not gastroprotective effect manifested in a very poor gastroprotective effect at low dose and a negative effect at high dose verified by the increase of the ulceration compared with negative and positive controls, maybe due to a overacidification at colonic level. The use of this species in traditional Andean medicine for ulcer treatment is partial justified, requiring further study to establish the appropriate dose.

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PO217
METABOLIC PROFILES OF *Bursera simaruba*-DERIVED ENDOPHYTIC BACTERIA FROM CASANARE, COLOMBIA

Fierro, J. E.[1]; Guerrero-Perilla, C.[2]; Jiménez, P.[3]; Coy-Barrera, E. D.[3]*
[1] *Master student, Universidad Militar Nueva Granada, Km. 2 Chia-Zipaquira route, Cajicá, Colombia*
[2] *Chemist, Universidad Militar Nueva Granada, Km. 2 Chia-Zipaquira route, Cajicá, Colombia*
[3] *PhD, Universidad Militar Nueva Granada, Km. 2 Chia-Zipaquira route, Cajicá, Colombia*
**ericsson.coy@unimilitar.edu.co*

Introduction

The Burseraceae family is known for being resinous plants as well as it has been characterized as a family of ethnobotany importance in different cultures, represented as the source of frankincense and myrrh (Daly *et al.* 2012). There are some studies about the chemical characterization on its metabolites, e.g. oleoresins (Siani *et al.* 2012), but some aspects of their biology and their associations with other organisms are still unknown. Endophytes have been reported as good sources of metabolites of great importance. Such cases are exemplified by the production of the biologically important metabolite Taxol by the fungus Pestalotiopsis sp. (Kumaran *et al.* 2010). Thus, as part of our research on metabolites from endophytes, the present work is related to the preliminary metabolic study of bacterial endophytes from Bursera simaruba belonging to the Burseraceae family, Colombia.

Method

Leaves of *B. simaruba* were collected in the region of Casanare, Colombia. The leaves were superficially disinfected with 70% EtOH for 1 min, 1% sodium hypochlorite for 3 min, and then several washes with sterile distilled water. Explants of ca. 2 mm2 were grown in Nutrient Agar (Oxoid) and cultured at 25°C. Bacteria were recovered from the media during the first 5 days, and were purified and preserved in NA+10% glycerol at -20°C. The bacteria were reactivated in NA (OXID) and a colony derived from a single cell was cultivated in 15 mL of NB (OXOID) for 15 days until the culture reach the maximum density. The cultures were centrifuged at 4500 rpm for 5 min and the supernatant (culture medium) was separated from the pellet (bacterial cells) in different vials. The supernatant was lyophilized and stored at -20°C. Both samples were subjected to an ethyl acetate extraction for 12 h. The ethyl acetate was separated from the original samples and let until evaporation. In addition, in order to chemically characterize, compare and highlight main components on each extract, a UFLC-DAD-based profiling were performed. All chromatographic data were correlated by principal component analysis (PCA).

Results / Discussion / Conclusion

Five bacteria were isolated form the B. simaruba explants. The UFLC profiles exhibited distinctive compounds that might be interesting in further isolation purposes. The chromatograms of the endophytes-derived extracts were also compared with the chemical profile of the leaf extract of B. simaruba in order to observe shared compounds. An interesting relationship between the profiles in B. simaruba and endophytes was observed. A PCA was performed with the chromatogram signals input derived by each extract. The PCA-derived score plots clustered those bacteria that possess similar composition in the case of bacterial cell-derived extracts. The analysis of the media extract, grouped the different extracts for the metabolites that the bacteria secreted to the media.

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PO218
ANTIOXIDANT CAPACITY OF COLORED KERNELS OF MAIZE VARIETIES (*Zea mays*) FROM BOGOTA PLATEU

Velásquez, Y.[1,2]*; Quiñonez, R.[2]; Coy-Barrera, E. D.[2]*
[1]*Licenciatura en Química, Facultad de Ciencias y Educación, Universidad Distrital Francisco José de Caldas, Bogotá D.C, Colombia*
[2]*Integrated Reasearch Group on Chemistry and Biology (InQuiBio), Facultad de Ciencias Básicas y Aplicadas, Universidad Militar Nueva Granada, Cajicá-Cundinamarca, Colombia*
**ericsson.coy@unimilitar.edu.co; yevela.e4@gmail.com*

Introduction

Maize (*Zea mays*) is one of the most important cereal crops in the world in terms of production, and the second one regarding area after wheat. Also, maize is one of the oldest foods with great benefits and many uses (Fenalce, 2011). Among consuming benefits is found the antioxidant capacity, conferred by compounds that retard or inhibit oxidative degradation of organic molecules, such as phenolic compounds, including flavonoids and anthocyanins. These type of commonly are found in colored kernels of maize. Antioxidants have been of interest because they may help to combat some degenerative diseases, considered those foodstuffs as nutraceuticals (Ramos F., González M. and García A., 2012). Therefore, this study aims to evaluate the total content of phenolic and flavonoids and determine the antioxidant capacity of colored kernels of maize (Zea mays)-derived extracts from Bogota plateau by methods DPPH, ABTS•+ and FRAP. All data was then analyzed by principal components analysis (PCA) in order to observe chemical variability and the correlation with the antioxidant activity.

Method

Twenty-five kernel samples of colored maize were random selected in the Bogota plateau. The total content of phenolic and flavonoids and the antioxidant capacity were evaluated by standardized colorimetric procedures, such as method Folin-Ciocalteau for phelolics, AlCl3 for flavonoids, and DPPH, ABTS•+ and FRAP for antioxidant capacity (Bernal F, Cuca L, Yamaguchi L, Coy E., 2013). Antioxidant capacity was determined as Trolox uM equivalents per g of dry sample. Each sample was analyzed in triplicate. The extracts were profiled by UFLC-UV-DAD after optimized conditions. All data were analyzed by principal component analysis.

Results / Discussion / Conclusion

The total phenolic content of each sample of colored maize was between 538 to 1663 mg/kg, and the total flavonoid content between 6 to 68 mg/kg. It was determined that there is a similar trend in the DPPH, ABTS•+ and FRAP methods, where DPPH method exhibited highest values. Data obtained of total phenolic content and antioxidant capacity by the three methods were correlated and it was determined that there is a proportional relationship. The PCA performed in combination with UFLC data shown that the samples are basically clustered into three groups related to the variability in its profiles, metabolite content and antioxidant capacity.

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PO219
DEVELOPMENT OF AN ALGORITHM TO OPTIMIZE THE PROCESS OF MILK ULTRAFILTRATION

Barba A. A., Calabrò V., Grasso A., Villecco F., Attianese P.
Dipartimento di Farmacia - DIFARMA, Università degli Studi di Salerno, 84084 Fisciano (SA)
Dipartimento di Ingegneria Industriale - DIIN, Università degli Studi di Salerno, 84084 Fisciano (SA)
Dipartimento di Ing. Informatica, Modellistica, Elettronica e Sistemistica – DIMES, Università della Calabria, Arcavacata (CS)
pattianese@unisa.it

Introduction

The wastewater treatment of dairy farms is of particular importance as it allows to transform, indeed, the waste by-products, with consequent benefits both from an environmental and economic point of view.

Among the treatment techniques, those of the membrane filtration are the most advantageous. They in fact have reduced energy consumption and allow the possibility of exploiting moderate operating conditions compared to consolidated separation processes with a better quality of the products obtained, which do not undergo thermal degradation and do not need to be subjected to purification operations. In particular the choice of the size and type of membrane allows to recover from the wastewaters, significant quantities of products with high biological value, such as serum proteins and lactose, which are, therefore, raw materials for different industrial sectors.

Such treatment can also be used for the purpose of concentration only, as the traditional techniques of evaporation / drying require the application of rather high temperatures that could irreversibly modify the constituents of the milk.

However, during filtration, the permeate flux decays inevitably due to the increase of the resistance to the transport of matter caused by the phenomenon of fouling. The aim of this work is to analyze the ultrafiltration process and identify the critical parameters with the intention to reach the definition of a method that can allow optimization of filtering operations.

Were selected materials, and has been organized the system on a laboratory scale. It has been proposed a method that uses the pressure oscillations to remove deposits accumulated on the membrane surface or penetrated into the pores in order to restore the functionality of the initial filter. It was developed the algorithm of the process and the controller is designed with fuzzy logic self-learner, necessary for the intensification of the process.

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PO220
FOOD INTOLLERANCE IN MIGRAINE

Pizza, V.[1]; Mainenti, M.[2]; Iannuzzi, S.[1]; Agresta, A.[1]; Cassano, D.[1]; Colucci d'Amato, C.[3]; Russo, L.[4]; Rastrelli, L.[4]; Capasso A.[4]
[1]Neurophysiopatology Service, Azienda Sanitaria Locale, Italy
[2]Endocrinology Service, Azienda Sanitaria Locale, Italy
[3]Neurosciences Department, Second University of Naples, Italy.
[4]Departmentof Pharmacy, University of Salerno, Italy.
*annacap@unisa.it

Introduction

Several factors can trigger migraine; among them, dietary factors play a very important role in the onset of migraine attacks. The aim of our study was to evaluate the incidence of food intolerances in a group of migraineurs, by using the Cytotoxic test.

30 consecutive patients suffering from migraine and coming to the Headache Center of S.Luca Hospital, Vallo della Lucania (SA) were examined. 23 were women, whose mean age was 28.2 years, range 13-47 years, 7 were men, whose mean age was 39.33 years, range 28-62 years. The Cytotoxic test is capable of identifying the presence of specific food intolerances by observing the appearance, the size, the shape or the integrity of leukocytes exposed to extracted food antigens or other materials derived from specific foods.

We found that: 12 women (52.17 %) and 3 men (42.85 %) were intolerant to tyramine. 3 women (13.05 %) and no man (0 %) were intolerant to milk 4 women (17.39 %) and 1 man (14.28 %) were intolerant to yeast. 4 women (17.39 %) and 1 man (14.28 %) were intolerant to Solanaceae. 5 women (21.74 %) and no man were (0 %) intolerant to coffee. 5 women (21.74 %) and no man (0 %) were intolerant to cocoa. 2 women (8.69 %) and no man (0 %) were intolerant to tea. 1 woman (4.35 %) and 1 man (14.28 %) were intolerant eggs. 1 woman (4.35%) and no man (0 %) were intolerant to pork. 1 woman (4.35 %) and no man were intolerant to sugar.

Our study showed a high incidence of food intolerance in migraineurs (in women more than in men). The dietary factors which gave more significant results were tyramine, yeast, solanaceae, coffee and cocoa. These results are in agree with those of other studies found in literature, proposing tyramine, coffee and cocoa as very important migraine-precipitating factors. Besides, there are few evidences about the comorbidity between migraine and intolerance to solanaceae. For this reason, further studies are requested to confirm this hypothesis.

Key words: migraine, food intolerance, cytotoxic test

PO221
EFFICACY AND TOLERABILITY OF A COMBINATION PRODUCT WITH L-TRYPTOPHAN, *GRIFFONIA SIMPLICIFOLIA*, VITAMIN PP AND VITAMIN B6 IN PEDIATRIC MIGRAINE PROPHYLAXIS: AN OPEN STUDY

Pizza, V.[1]; Busillo, V.[2]; Cassano, D. [3]; Agresta, A.[1]; Colucci d'Amato, C. [4]; Russo, L.[5]; Rastrelli, L.[5]; Capasso A.[5]

[1]Neurophysiopatology Unit, Headache Centre, S. Luca Hospital, Vallo della Lucania (SA), Italy
[2]Neurology Division, Headache Centre, Maria SS Addolorata Hospital, Eboli (SA), Italy
[3]Territorial Headache Centre, Distretto N. 60, Nocera Inf. (SA), Italy
[4]Neurosciences Department, Second University of Naples, Italy.
[5]Departmentof Pharmacy, University of Salerno, Italy.
annacap@unisa.it

Introduction

The aim of this study was to evaluate the efficacy and tolerability of combination product with L-tryptophan, Griffonia simplicifolia, Vitamin PP and Vitamin B6 in prophylaxis therapy of pediatric migraine.

Fifthy outpatients (32 F, 18 M), mean age 10.7 years (SD 5.8), range 4-18 years, suffering from ICHD-2 migraine without aura were enrolled. The mean duration of disease was 2.9 (SD 1.6) years, range 1-4 years. At baseline the mean frequency of attacks was 7.6/month (SD 3.3), range 4-12; the mean number of drugs intaking for acute attacks was 6.6 tablets/month (SD 2.2).

During the six month evaluation period the combination product with L-tryptophan, griffonia simplicifolia, vitamin PP and vitamin B6 was administered (at dose 100 mg, 480 mg, 18 mg and 1 mg/die, respectively). All patients filled a headache-diary card during the evaluation.

The basal frequency of attack was 7,6 (SD 3.3) and 4,2 (SD 2.6), 3,6 (SD 2.8), 2,2 (SD2.6), after 1, 3 and 6 months respectively [$P < 0.01$; $P < 0.01$; $P < 0.01$]. The basal value of intaking drugs for acute attacks was 7,6 (SD 3.3) and 2,1 (SD 2.5), 1,9 (SD 1.5), 1,4 (SD 2.7) after 1, 3 and 6 months respectively [$P < 0.01$; $P < 0.01$; $P < 0.1$] (T-test analysis). The combination product with L-tryptophan, griffonia simplicifolia, vitamin PP and vitamin B6 was well tolerated (11 patients complained somnolence, diarrhea and gastralgia but none patient withdrew the study).

These data showed a good efficacy in reduction of frequency and intensity of headache attacks, a good tolerability and a very good reduction of drugs intaking for acute attacks.

Our study suggests that the combination L-tryptophan, griffonia simplicifolia, vitamin PP and vitamin B6 could be an alternative therapy for pediatric migraine prophylaxis.

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PO222
TRACEABILITY OF INTERDONATO LEMON BY TRACE ELEMENT CONTENT AND CHEMOMETRIC ANALYSIS

Potortì A. G., Somma R., Mottese A., Bua D., Cicero N., Pollicino G. M., Salvo A., Dugo G.mo
Dipartimento SASTAS, Messina University, Messina, Italy

Introduction

In the last years, trace elements content was used as geographical "tracers" to determine the provenance of food. The Interdonato Lemon (hereafter IL) produced in the north-eastern Sicily had received from the European Community the label of Protected Geographical Indication (PGI). The aim of this research was to correlate the trace element content of IL pulp to its geographical origin to protect the IL PGI from others of different origin.

Method

The levels of 19 trace elements in 28 IL pulp samples were determined by Agilent 7500cx (Agilent Technologies, CA) ICP-MS spectrometer. Before instrumental analysis, the samples were digested in triplicate using a closed-vessel microwave digestion system (Ethos 1, Milestone, Italy). The obtained results were submitted to chemometric analysis using SPSS 21.0 software (SPSS Inc., IL). The starting multivariate matrix was constituted by 28 cases (lemon pulp samples under analysis) and 13 variables (concentrations of Cr, Ni, Pb, Mn, Al, Ca, K, Mg, Na, Fe, B, Cu and Zn determined in analyzed samples). Co, As, Se, Cd, Sb and V content was always < LOQ, so they were not entered in the statistical analysis. The data were subdivided into two groups, according to the origin of lemon fruit: the first one (22 samples) consisting of lemons from Messina farms producing PGI, and the second one (6 samples) consisting of Turkish provenience lemons. Initially, the non-parametric test of Mann-Whitney was applied to study the significances of differences. Successively, data set was normalized and Principal Components Analysis (PCA) was performed to try to differentiate samples coming from different areas according to concentrations of trace elements. Finally, Canonical Discriminant Analysis (CDA) was conducted to attempt to classify different lemon fruits.

Results and Discussion

The Mann-Whitney test results showed significant differences at p-level below 0.05 for K, Ca, Mg, Na, Zn, B, Ni, Cr and Pb: PGI IL samples were characterized by lowest levels of all these elements except for chromium and lead. Factor Analysis by Principal Components extraction was conducted considering only these 9 variables, previously normalized: three principal components (with eigenvalues equal to 4.816, 1.548 and 1.057), explained the 53.508%, 17.205% and 11.743% of total variance, were extracted. The Scatterplot relative to the first two components showed a good degree of separation between IL PGI and non-PGI samples: the last showed positive PC1 and were characterized by six elements which had the largest positive coefficients in PC1 (K, Ca, Mg, Na, Zn and B), while Cr and Pb were the variables with the greatest negative association with PC1 and they are characteristic elements in IL PGI. Starting from the initial classification of the samples as IL PGI or non-PGI, CDA was further conducted. The obtained canonical discriminant function showed high discriminative power with high correlation value (0.937) and low value of Wilks lambda (0.122). The analysis showed that boron (0.912), sodium (0.772) and magnesium (-0.537) were the best variables to discriminate between the two groups of samples. The classification matrix, resulting by applying the discriminant function [F=3.602·(B)+0.050·(Na)-0.010·(Mg)-2.739] to the data set, indicated that the 100% of total samples are correctly classified as IL PGI or non-PGI.

Conclusions

The statistical analysis performed on IL trace element concentration showed that a good classification between samples coming from different regions can be achieved. The developed procedures can be used as method to assess PGI IL traceability in order to preserve this peculiar product against frauds or commercial disputes.

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PO223
THE GEOCHEMISTRY OF RARE EARTH ELEMENTS (REES) IN THE PRODUCTION SOIL-PGI INTERDONATO LEMON SYSTEM (MESSINA, SOUTHERN ITALY)

Mottese, A.; Somma, R.; Bua, G. D.; Cicero, N.; Pollicino, G.; Potortì, A.G.; Salvo, A.; Dugo, Giacomo
Dipartimento SASTAS, Messina University, Messina, Italy

Introduction
The Interdonato Lemon (hereafter IL) is a protected geographical indication (PGI) citrus fruit cultivated in the area extending from Messina to Casalvecchio siculo (north-eastern Sicily). The aim of the present research is to investigate the distribution of REEs in soils and different parts of the Interdonato Lemon citrus fruit to find possible markers for its traceability.

Methods and materials
The concentration of 13 REEs in 94 specimens sampled in three Messina farms producing PGI IL and in one Turkey farm producing non-PGI IL was determined to reconstruct chemometric models able to protect the PGI IL from others of different origin. To better carry out this result, chemical and geochemical data have been integrated with mineralogical and textural analyses of production soils.
The REEs analysed by Inductively coupled plasma mass spectrometry (ICP-MS) were: La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Lu. This technique was applied on production soils, IL roots, leaves, and juice. For each element three replicates were provided. Sample digestion was realized using different solutions: 9ml of HNO_3 + 3ml of HCl for soils, 6ml of HNO_3 + 2ml of HCl for roots, 7ml of HNO_3 + 1ml of H_2O_2 for leaves, and 8ml of HNO_3 for juice.
X-ray diffractometry technique was used for the soil mineralogical analyses, whereas the soil texture was analysed by sieving for grains of soil fraction > 63 μm and laser diffraction for grains of soil fraction < 63 μm.

Results
It is noteworthy that REEs abundance decreases with increasing atomic number in both even- and odd-numbered sequences present in both soils and plants; a similarity in the proportion of REEs in the plants and the exchangeable REEs content in the soil is known as well. The results indicated that REE contents in different parts of the production soils-IL plant system followed the order: soil>root>leaf>lemon fruit. The analysed samples show decreasing concentrations of REEs proceeding from the light to the heavy REEs, being these latter less basic and less soluble elements. As concern the two most abundant light REEs (La and Ce) found in the PGI samples, they generally show a 1:2 ratio (except in the leaves). Consequently, this report makes that *Lanthanum* and *Cerium* found in the IGP lemon juice are 18000-19000 times lower than concentrations found in soil. The 1:2 ratio is not observable in the juice non-PGI samples from Turkey being characterized by analogous values.
Under a geological point of view, the studied soils are characterized by a grain size typical of gravels with sands or silty sands made up of clasts of silicates (quartz, chlorite, muscovite, and albite). They overlie silicate bedrocks made up of Paleozoic metamorphic rocks (phyllites) or Pleistocene sedimentary clastic deposits (Gravels and sands of the Messina Formation) deriving from silicate crystalline rocks.
According to most authors, the original source of REEs in soils is related to the underlying bedrock. Consequently, the REEs concentrations in the soils, in agreement with the mean values generally found in the soils, presumably derive from phyllosilicates (chlorite and muscovite) found in the studied soils.

Conclusions
According to the obtained chemical and geochemical analyses accomplished in the PGI soil-IL system sampled at Messina and in the non-PGI Turkey IL, a possible candidate for traceability of the PGI IL is represented by the ratio between the *Lanthanum* and *Cerium* concentrations. Further investigation on the non-PGI IL is indispensable to corroborate this preliminary conclusion.

PO224
THERMAL WATER IN ASSOCIATION TO DRUG THERAPY FOR THE TREATMENT OF PSORIASIS

V.Panico[1], L. Cerrato[2], B. Salvati[2], S. Morelli[2], L. Russo[2], L. Rastrelli[2]
[1]*Direzione Sanitaria Terme Capasso, Italy*
[2]*Dipartimento di Farmacia, Università degli Studi di Salerno, Italy*

Introduction
Psoriasis is an inflammatory disease of the skin, chronic and relapsing. It is not infectious nor contagious, affecting more than 100 million people in the world, in Italy are affected about 2 and a half million. It is generally characterized by reddened patches, covered with whitish scales, which are located in some typical sites such as elbows, knees, scalp. The lesions are stable and persist for long. It develops when the body's immune system gets a rapid growth of the epidermis resulting in flaking. At the base there is a genetic disease, which is transmitted by inheritance, with the intervention of environmental factors and psycho-emotional triggers. It comes in various forms and there is today a cure, but it can be kept under control with appropriate treatment strategies. There is no cure better than another, because each patient responds differently to therapy chosen by the medical specialist. It 's definitely a disease with a strong negative impact on the quality of life for the patient.

Materials and methods
At the spa "Terme Capasso" site in Contursi Terme (SA) were enrolled over a period of time between January 2013 and April 2014, 72 patients (42 M and 30 F) aged between 23 and 68 years of all ages suffering from mild to moderate psoriasis. The disease was diagnosed following specialist dermatological and classified according to the PASI score. This score is calculated by taking into account some features of psoriatic pathology such as the extension of the surface of the skin affected by the disease, inflammation, infiltration and desquamation. For each of these parameters is given a score by getting a final result between zero and seventy-two. A score of at least eight defines a mild psoriasis, a score of eight to twelve, and finally a moderate psoriasis values ÿ'3fÿ'3fexceeding twelve a severe psoriasis. The patients were divided into two groups: the first, consisting of 38 patients (27 M and 11 F) with PASI average of 11.2, treated for 20 days with calcipotriol + betamethasone topically and the second, of 34 patients (15 M and 19 F) with a mean PASI 11.8, treated for 20 days with calcipotriol + betamethasone topical and daily baths with sulfur water for 30 minutes. After the first twenty days of therapy only the first group continued for another twenty week period following, however, the therapeutic protocol of the second group.

Results
At the end of each treatment cycle lasting twenty days each patient underwent a skin examination to detect the results and calculate the PASI score. Below, in the figure, the results obtained will be highlighted.

Conclusions
The thermal therapy has proved particularly useful for the control of psoriasis, particularly in combination with a suitable pharmacological therapy administered topically. These results lead us to speculate that, in these patients with mild-to-moderate psoriasis, this combination therapy could really be decisive for optimal control of the disease with a drastic reduction in the use of other drugs burdened with numerous side effects. This would detect particularly useful for both patients and for the expense of the NHS. We hope that further studies with greater statistical power, can be implemented with the aim of developing a more complete and comprehensive treatment protocol.

PO225
TRADITIONAL KNOWLEDGE OF CARAPANAÚBA (*Aspidosperma* spp.) TRADE OF MEDICINAL PLANTS IN THE MUNICIPALITY OF MANAUS - AMAZONAS - BRASIL

Da Silva, F. J. P.[1*]; Fraxe, T. J. P.[2]
[1] *Mestranda em Ciência do Ambiente, Universidade Federal do Amazonas, Av. Rodrigo Octávio 3000, Manaus, Amazonas, Brasil*
[2] *Professora Drª Coordenadora do Núcleo de Socioeconomia, Universidade Federal do Amazona, Manaus, Amazonas, Brasil*
* tcheska_22@hotmail.com

Introduction
In the Amazon, the richness of its flora and popular knowledge passed down through the generations, allows medicinal plants to take root and be sold in free markets and popular (DUARTE, 2006) shows. Regarding this biodiversity countless species are relevant for traditional medicine. However, it stands Aspidosperma genre, known regionally for Carapanaúba and notoriously recognized in the Amazon for its potential therapeutic effect (AÑEZ, 2009). Be noted as well, in the last decades of the twentieth century, there was an increase of interest in this genre. In this perspective, the study proposes to develop trade in medicinal plants in the city of Manaus on gender Aspidosperma. Consequently, the aim of this work is the ethnoknowledge of this genus for medicinal purposes for medicinal purposes by checking their respective indications, as well as value added to the empirical knowledge herbalists.

Methodology
For the study, were selected the following locations of commercialization of the: "Manaus Moderna" and "Mercados Adopho Lisboa". Thus, the research was conducted in three phases:
-Visits to commercial outlets of medicinal plants;
-Interviews-spot through structured questionnaires to traders;
-Note the direct-marketing environment;
Finally, completion of the georeferencing of local marketing.

Results
With reference to commercialization of the Aspidosperma genus, three forms were found: sale in natura, capsule and semi-processed. The part of the plant used in this marketing was only the bark.
Regarding the indication for use, classified in accordance with the International Statistical Classification of diseases and Related Health Problems (ICD-10), more relevantly has been disease, symptoms and signs involving the digestive tract abdomen. The results showed the main indications for use, especially gastritis, inflammation of the uterus and malaria, prevailing water immersion as the form most frequently used. In relation to obtaining feedstock of the genre, it was found that the same is collected directly from extractive forest, which are resold for an average of $3.00 (three reais) for about 100 grams of bark.

Discussion
The record of the rich information in the trade of medicinal plants, about gender Aspidosperma in the city of Manaus, becomes important for the recovery of the processes used in folk therapy. Similarly, the marketplaces "Mercados Adopho Lisboa" and "Manaus Moderna" play a fundamental role in the conservation of popular knowledge, being necessary to have the record and the decoding of this ethnoknowledge, to be used simultaneously with studies more coherent to the genre Aspidosperma.

Conclusion
Information obtained from such studies can subsequently be applied in actions that target the orientation of merchants, as of the consumers of products of the Aspidosperma genre, providing relevant information on it to support studies of their management and conservation. It is noteworthy that there is need for further studies on trade in native medicinal plants, especially the genre Aspidosperma.

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PO226
THE REPRESENTATION OF ROSEHIP

Bicco R.[1]
[1]*PhD student of "Environment and structures representation, protection and safety and land management", SecondaUniversitàdegliStudi di Napoli _ Department of Architecture and Industrial Design Luigi Vanvitelli S. Lorenzo ad Septimum, Aversa (CE), Italy*
rossellabicco@live.it

Abstract
The wild rose (commonly known also as dog rose, rose hip, briar rose) is widely recognized for the beneficial properties that it holds for our organism and it's high content of active principals like vitamin A, C, E and K, bioflavonoids, tannins, pectins, organic acids, polyphenols and carotenoids; used both in herbal medicine and as a cosmetic, or like in Solvenia for the Cockta, a very popular soft drink, and also for it's great symbolic role during medieval times. It had so many popular meanings, religious and in literature where writers were asked to trace a semantic reading in the variables of form, color, perfume, the number of petals, the presence of thorns.
Tied to a circle, symbol of the sky and the sun, we can find an interesting stylization of the rose in rosettes, that together with windows and lateral fissures, brightened the vast and dark gothic cathedrals, as a portal of communication between man and the divine. The rose an allegoric symbol of immortality has been known since ancient times, we can in fact see it on Egyptian tombs. It is a recurring architectural symbol on the Via sacra in Varese, the rose hip is the symbol of regeneration because it does not need to be pollinated, so it is used as the symbol of virginity and the resplendent visage of the holy mother, it defines absolute perfection, a deed without fault. The same which we see in the twelve star crown of the immaculate also called Morning Star meaning the one who announces the dawn, in other words the coming of the lord. Uniting the tips of the star, a regular polygon forms: a pentagon, a shape whose particular geometric elements are well known to the builders of gothic cathedrals. In fact, we are often faced with the "golden effect" or "divine proportions", that part of a segment whose proportional average of the whole and the remaining part are the same, which, when it is adhered to, brings to mind our esthetic idea of beauty. And again in this case we find ourselves before an image of the divine, of beauty of itself, of perfection.

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PO227
ADD VALUE FEED PRODUCT OBTAINED FROM CITRUS WASTE BY CONTROLLED FERMENTATIVE PROCESSES

Gervasi T.*; Tropea A.; Di Bella G.; Potortì A. G.; Lo Turco V.; Cicero N.; Vadalà R.; Dugo G.mo
University of Messina Department S.A.S.T.A.S., V.leF. Stagno d'Alcontres,31, 98166 Messina, Italy
*tgervasi@unime.it

Introduction

Italy is one of the major producer of Citrus and its derivates, the main wastes from the citrus processing industry are available in large amounts, mostly in Sicily. These wastes can be dried to extract pectin and/or fermented to produce animal feed. The main component of this waste is the peel, which consists mostly of pectin, cellulases with D-galacturonic acid as the main monomer.
The aim of this work was to produce single cell protein (SCP) from bergamot wastes after pectin extraction through a controlled fermentative process by growing Saccharomyces cerevisiae and Phaffia rodozyma (De Gregorio *et al.*, 2002; Tropea *et al.* 2013). The use of the pigmented yeast could be interesting because the astaxanthin can be an ideal complement for animal feeding.

Method

S. cerevisiae and P. rodozyma were grown in 200ml of malt extract (1 g/l) medium in 200 ml baffled flasks at 30 °C and 21°C respectively for 3 days on a rotary shaker incubator at 80 rpm.
The bergamot wastes, provided by Simone Gatto srl, have been supplemented with (NH)2SO4 6,4%, NH4 H2PO4 0.7%, KCl 2.0%, Mg SO4 7 H2O 0,05% and vitamins (biotin 0,0833 mg/l, Ca- panthothenate and inositol 100mg/l).
Both the fermentations were carried out separately at different temperature, in a 25 l B. Braun Biotech International bioreactor containing 15 l of culture medium. Culture pH was initially adjusted at 5.05 by addition of 30% NaOH pH: 5.05 without any further correction; the mixing was set at 400 rpm trough two six-blade Rushton turbines set near air sparger (0.5mm diameter holes); air flow: 4 l/min; air sterilization: filtration through 0.2 mm Sartofluor filter and pressure: 200 mbar. Antifoam was added within the process to avoid excesses of foam production.
S. cerevisiae and P. rodozyma culture samples (200 ml) were daily collected by a Watson-Marlow 503U peristaltic pump, tested for sterility by spreading on PCA and MEA, and finally centrifuged. Cell pellets were washed three times with NaCl 0.8% and finally freeze-dried at 30°C for 24 h.
All samples collected from the two processes were analysed for SCP and total N determination by kijeldahal method, moreover samples obtained from P. rodozyma culture were assayed for astaxanthin determination by a Shimadzu liquid chromatography system equipped with a photodiode array detector and coupled with a MS detector (Shimadzu 2010 system).

Results / Discussion / Conclusion

The SCP production by S. cerevisiae in batch fermentation gave rise to a 38% protein in biomass whereas P. rodozyma 17%. The astaxanthin production was 0.89 mg/g and biomass yield 4.5g/l.
The SCP yields obtained by S. cerevisiae overpass the minimum value for the commercialization of this product as animal feed, however the SCP obtained during the second fermentation results very interesting because of the presence of astaxanthin.
Further studies are in progress to improve the SCP and carotenoids production by pigmented yeasts from food wastes.

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PO228
BATCH AND FED-BATCH CAROTENOIDS PRODUCTION BY *RHODOTORULA*

Gervasi T.*; Tropea A., Lo Turco V., Potortì A. G.; Cicero N.; Di Bella G.; Dugo G.mo
University of Messina, Department S.A.S.T.A.S., V.leF. Stagno d'Alcontres,31, 98166 Messina, Italy
**tgervasi@unime.it*

Introduction

Carotenoids are ubiquitous pigments that can be found in high concentrations in plants, algae and microorganisms. These pigments represent a group of valuable molecules for the pharmaceutical, chemical, food and feed industries, not only because they can act as vitamin A precursors, but also for their colouring, antioxidant and possible tumour-inhibiting activity. The microbial production of carotenoids is of paramount interest if compared with the extraction from vegetables or chemical synthesis (De Haan A. *et al.* 1991).
The aim of this study was to compare two different fermentative processes, batch and fed batch, to observe the influence of nitrogen and carbon source in carotenoids production by *Rhodotorula*.

Method

Rhodotorula strain was obtained from in-house cloture collection. The strain was grown for 24 hours at 20°C in malt extract broth for 3 days on a rotary shaker incubator at 150 rpm.
The medium used during the fermentative processes was constituted by glucose 20 g/l; $(NH_4)_2SO_4$ 5 g/l; KH_2PO_4 1 g/l; $MgSO_4$ $7H_2O$ 0.5 g/l; $CaCl_2$ $2H_2O$ 0.1 g/l; yeast extract 1 g/l.
The two fermentation modes, batch and fed batch were carried out separately in a 25 l B. Braun bioreactor with a working volume of 15 l, at 21°C; pH: 5.05 without any further correction; mixing: 350 rpm with two six-blade Rushton turbines set near air sparger (0.5mm diameter holes); air flow: 4 l/min; air sterilization: filtration through 0.2 mm Sartofluor filter and pressure: 200 mbar.
The medium used for the batch fermentation was the one reported above; whereas during the fed-batch fermentation process the culture was feed with $(NH_4)_2SO_4$, KNO_3 and pepton,as nitrogen source, and glucose as carbon sources to observe their influence on carotenoids production.
Rhodotorula culture samples (300 ml) were daily collected by a Watson-Marlow 503U peristaltic pump and tested for sterility by spreading on PCA and MEA and finally centrifuged. Cell pellets were washed tree times with NaCl 0.8% and finally freeze-dried at 20°C for 24 h. Subsequently 5ml dimethylsulfoxide (DMSO) at 55°C for 15 min was added to 200 mg of dry yeast. Carotenoids were analyzed according procedure reported by Tropea *et al.* 2013 by a Shimadzu liquid chromatography system equipped with a photodiode array detector and coupled with a MS detector (Shimadzu 2010 system)

Results / Discussion / Conclusion

Studies indicates that the red yeasts of the genus *Rhodotorula* have a potential for industrial carotenoid production in addition to the well known astaxanthin producer *Phaffa rhodozyma* (Buzzini 2001).
During fermentation carried out using the batch mode, the highest pigment production was 0.8 mg/g and biomass yield 4.0 g/l., whereas during fed batch cultivation the highest carotenoids production (0.91 mg/g) and biomass yield (5.5 g/l) were observed after $(NH_4)_2SO_4$ and KNO_3 addition. Glucose and peptone feeding had no relevant effects on pigment production.
It was observed how ammonium source feeding allowed us to obtain a greater pigment production. Further studies are in progress currying out an enlightened fermentation to observe the role of light for the production of carotenoids.

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PO229
BIOETHANOL PRODUCTION FROM PINEAPPLE WASTE BY SUPPLEMENTING MEDIA

Tropea A.*; La Torre G. L.; Gervasi T.; Di Bella G.; Gentile F.; Lo Turco V.; Dugo G.mo
University of Messina, Department S.A.S.T.A.S., V.leF. Stagno d'Alcontres,31, 98166 Messina, Italy
**atropea@unime.it - Author for contact*

Introduction

There is great interest in producing bioethanol from biomass and there is much emphasis on exploiting lignocellulose sources, from crop wastes through to energy-rich crops (Goh, *et al.*, 2010). Some waste streams, however, contain both cellulosic and non-cellulosic sugars. Pineapple wastes are rich in intracellular sugars and plant cell walls which are composed mainly of cellulose, pectic substances and hemicelluloses suitable for ethanol production (Tropea, *et al.* 2014). The purpose of this study was to investigate the potential to transform such residues into ethanol after plant cell walls enzymatic saccharification, and fermentation of the resulting simple sugars using the *Saccharomyces cerevisiae* strain.

Method

Fermentation test was carried out in a 2.5 L batch fermenter (LH Fermentation 2000 Series). The fermenter was equipped with one four-bladed rushton turbine, and the common control systems: temperature and pH probes, CO_2 detector and gas mass flow meter.

Pineapple wastes, enclosing fruit skin and core, were homogenized in a fruit blender. The resulting homogenate, with a dry matter content of 14% (w/w), was diluted with water to a 9% dry matter, in a working volume of 1.5L and immediately treated at 100°'b0C for 10 min under continuous mixing to inactivate endogenous enzymes and reduce microbial spoilage. No further sterilization procedure was adopted. Before fermentation starting the media was supplemented by digestive enzymes, urea phosphate, Ca-panthothenate and biotin (Bohlscheid *et al.*, 2007).

Fermentation parameters were: 30°C, pH4.5 and constant stirring at 200 rpm. CO_2 evolution was measured during the fermentation and representative samples were taken at regular intervals for ethanol, glycerol, soluble and insoluble sugars determination by GC and HPLC methods; moreover protein and lignin determination were carried out by Kijeldahal and Klason methods respectively.

Results / Discussion / Conclusion

Substrate initial fibers and soluble sugars were 24 and 41% respectively; the main sugars were Glucose and Xilose.

By 21 h soluble sugars and fiber utilization by the yeast, as well as ethanol production stopped. The highest ethanol production was 4%, reaching a 97% of the Theoretical Yield (TY). Though the alcohol concentration obtained appears rather low, due of course to the low sugar content, this study could be attractive because TY, calculated on dry matter loss, was really high making these wastes an excellent raw material for ethanol production by *S. cerevisiae*. Moreover substrate resulting from the fermentation process was enriched in protein, rising up to the 21.3%, and lignin making this biomass suitable, after separation, for animal feed and energy production respectively.

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PO230
WASTES FROM FOOD-GRADE PECTINE PRODUCTION: SOURCES OF SCP AND ETHANOL

Tropea A.*; Gervasi T.; Di Bella G.; Potortì A. G.; Cicero N.; Lo Turco V.; Dugo G.mo
University of Messina, Department S.A.S.T.A.S., V.leF. Stagno d'Alcontres,31, 98166 Messina, Italy
**atropea@unime.it - Author for contact*

Introduction

Citrus peel extraction and refining of food-grade pectin give rise to two main wastes, exhausted peel and molasses, which can be used in sequence for the production of feed, ethanol and biogas.
Citrus peel was used to produce SCP by growing *Trichoderma viride* while *Saccharomyces cerevisiae* was used to produce ethanol from molasses (Tropea *et al.* 2014). The spent N-rich liquid residual from ethanol fermentation by yeast was also used as medium for SCP production by *T. viride* (Zhang *et al.*, 2008; Boluda-Aguilar *et al.*, 2010).
The aim of this research was to verify the exploitation of wastes from a pectin-producing factory, mainly exhausted citrus peel and molasses, using batch and continuous fermentations in a laboratory 30-litres B. Braun fermenter.

Method

Microbial strains were obtained from in-house culture collection and were grown in malt extract broth at 30°C for 3 days on a rotary shaker incubator at 150 rpm.
Fermentations were carried out in batch and continuous modes, at 30°C, pH 5.05 without any further correction, mixing 200 rpm with two six-blade Rushton turbines set near air sparger (0.5mm diameter holes), air flow 4Lmin^{-1}, air sterilization by filtration through 0.2 mm Sartofluor filter and pressure 200 mbar. Fermentations were followed also by monitoring, through a continuous IR monitor, CO_2 concentration in exhaust air. Dissolved oxygen tension was monitored as well by a pO_2 probe. To estimate production rates the following determinations were carried out. All samples collected during fermentations were filtered through paper, obtaining a solid residue and a liquid. On lyophilized residue were tested: SCP production (gL^{-1} medium), amount of protein in SCP (g/100g SCP). On liquids were determined: total N (mg/100 ml) and dry matter (g/100ml).
Ethanol production from the fermentation of molasses resulting from the concentrated washing waters of virgin lemon peels before pectin extraction, was determined by HPLC.

Results / Discussion / Conclusion

The first results showed that the SCP production by *T. viride* in batch fermentation gives rise to a 27.6% protein in biomass, whereas in continuous fermentation SCP production was ranging about 28.6% protein.
Production of ethanol from molasses showed to reach about 5% after 4 days. The amount of protein in the yeast SCP of course is higher than in mould SCP, about 43%. The liquids coming from fermentation processes, being yet rich in C-sources like celluloses, hemicelluloses and pectins, finally could be used to produce biogas.
In conclusion, the proposed study could allow, by using the wastes from the food-grade pectin production process, to obtain several benefits: SCP production of different protein content can be used as feed for ruminants and pets, an interesting ethanol production and virtually avoid the cost of a further processing of the waste liquids from fermentation which could be used for biogas production.

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PO231
ANTIVIRAL EFFECTS OF GREEN TEA (*Camellia sinensis*) AGAINST PATHOGENIC VIRUSES OF HUMAN AND ANIMALS

Muhammad Shahid Mahmood[1], José L. Martinez[2], Azhar Aslam[1], Azhar Rafique[3], Iftikhar Hussain[1], Silvia Vargas-Rueda[4], Ahrar Khan[5], Claudio Laurido[6], Shahid Ali[1] & Rao Zahid Abbas[7]

[1]Institute of Microbiology, University of Agriculture, Faisalabad, 38040-Pakistan
[2]Vicerrectory of Investigation, Development and Innovation, Universisity of Santiago of Chile
[3]Department of Zoology, Wildlife and Fisheries GC University Faisalabad, 38040-Pakistan
[4]Facultad de Quimica Ambiental, Universidad Santo Tomas, Bucaramanga, Colombia.
[5]Department of Pathology, University of Agriculture, Faisalabad, 38040-Pakistan
[6]Faculty of Comical and Biology, University of Santiago of Chile, Santiago, Chile
[7]Department of Pathobiology, University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur
joseluis.martinez@usach.cl or shahiduaf@gmail.com

Introduction

Tea is the second most addictive drink globally, following water and various formulations of soda water. Green tea is manufactured from the leaves of plant *Camellia sinensis* (Chacko *et al.,* 2010). In the repertoire of traditional Chinese medicines from ancient times green tea served as a helpful and important beverage. The use of green tea has been claimed to have a lot of pharmacological and physiological health benefits. Green tea is considered to posses both anti-bacterial and antiviral effects

Method

This report is based on a bibliographic survey of the effects of *Camellia sinensis*. Databases included in this search were, among others, Pubmed, Thomson Reuters, Ebsco, Google Academic.

Results / Discussion / Conclusion

Beneficial effects of green tea are mainly attributed to the presence of polyphenols, which are known as catechins with several isomers, including (–)-epigallocatechin gallate (EGCG), (–)-epicatechin, (–)-epicatechin gallate, (–)-epigallocatechin and (+)-catechin, the EGCG being a major component (Jin, 2013).
The green tea catechins posses a wide range of antiviral activities against a variety of viruses on which they act by interfering with their replication cycle (Araghizadeh *et al.,* 2013). This minireview would endeavour to present detailed information regarding antiviral activity of green tea against a number of different viruses and demonstrate the existence of a promising future for their continual use not only as a popular drink but as a beneficial therapeutic agent as well.

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PO232
EFFECT OF THE DIETARY ISOTHIOCYANATE ERUCIN ON PRO-INFLAMMATORY BIOMARKERS IN HUMAN VASCULAR ENDOTHELIAL CELLS

Melchini, A.[1]; Catania, S.[2]; Costa, C.[3*]
[1] PhD, Food and Health Programme, Institute of Food Research, Norwich, United Kingdom
[2] ChD, Dept. SASTAS, University of Messina, via C. Valeria 1, Messina, Italy
[3] PhD, MD, Dept. SASTAS, University of Messina, via C. Valeria 1, Messina, Italy
*ccosta@unime.it

Introduction

A growing body of evidence from human intervention trials, cell and animal models indicates a protective effect of isothiocyanates (ITCs) against chronic diseases. ITCs are bioactive compounds delivered in our body through the consumption of cruciferous vegetables such as broccoli, cauliflower, cabbage, rocket, etc. The emerging evidences indicate that ITCs are able to modulate phase I, II and III detoxification enzymes, regulate cell growth by induction of apoptosis and cell-cycle arrest, and target anti-oxidant and inflammatory pathways (Wagner *et al.*, 2013). Erucin (ER) is an ITC obtained from enzymatic hydrolysis of glucoerucin, found at high levels in rocket salad species (*Eruca ssp.* Mill, *Diplotaxis ssp.* L.) and has recently been reported to have anti-inflammatory properties in various cells models (Cho *et al.*, 2013). In this study, we investigated the anti-inflammatory effects of ER using lipopolysaccharide (LPS)-stimulated human umbilical vein endothelial cells (HUVECs) as an *in vitro* model of inflammation.

Method

HUVECs were obtained from Cambrex Bio Science (Wokingham, England). The experiments were performed with HUVECs in both an unstimulated state and under inflammatory conditions induced by LPS. HUVECs were pre-treated with 0-5 μM ER for 20 hours and then challenged with 500 ng/ml LPS for 4 hours. Cells were treated with other dietary ITCs (sulforaphane SF, iberin IB) following the same experimental condition in order to compare the biological activity of ER with other ITCs. IL-6 concentration was determined on supernatant using a commercially available immunoassay kit (R&D system). Cell lysates were assayed at the final concentration of 0.5mg/ml by using the PathScan® Inflammation Multi-Target Sandwich ELISA (Cell Signaling Tech.). ANOVA followed by Dunnett's test were used for statistical analysis.

Results / Discussion / Conclusion

Treatment with ER reduced IL-6 release by HUVECs in a dose-dependent manner ($p<0.01$ at 0.5 μM; $p<0.001$ at 2 and 5 μM). LPS remarkably increased IL-6 levels compared to basal conditions ($p<0.001$). When inflammation was induced after pre-treatment with ER, the isothiocyanate significantly reduced the levels of this inflammatory cytokine compared to LPS alone ($p<0.01$ at 0.5 μM; $p<0.001$ at 2 and 5 μM). A similar effect has been observed following the pre- treatment of HUVECs cells with the other isothiocyanates SF and IB. Treatment of HUVECs cells with LPS induced differential phosphorylation of NF-êB, JNK and Stat3 as detected by the multi-target ELISA; when cells were pre-treated with ITCs the level of phosphor-NF-êB, JNK and Stat3 remained unchanged. LPS is an exogenous ligand of toll-like receptor-4 (TLR-4) and is found circulating at elevated levels in patients with chronic inflammatory conditions (Stoll *et al.*, 2004). Recent findings have suggested that TLR4 signalling may represent a molecular target responsible for anti-inflammatory actions of ITCs (Koo *et al.*, 2013). In our study we showed that ER is able to significantly suppress LPS-induced cytokine production (IL-6) and modulate pro-inflammatory targets in human endothelial cells. The ability of dietary compounds, such as ER, to modulate molecular mechanisms that affect endothelial inflammation can be a key point of the potential preventive action exerted by functional foods (Melchini *et al.*, 2010). Thus, further studies aimed to understanding the specific action of this molecule on inflammatory signaling pathways would be important to find a significant therapeutic application.

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PO233
METALLIC PHTHALOCYANINE AND PORPHYRIN PHOTOSENSITIZER AS ANTIMICROBIAL PHOTODYNAMIC THERAPY

Romero P., R. D.[2]; Quigua O., R. M.[2]; Arboleda V., J. W.[3]; Diaz U., C. E.[4].; Vallejo L., W. A.[4]; Valle-Molinares, R. H.*[1]

[1]*M.Sc,Profesor, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Biología, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

[2]*Estudiante, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Biología, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

[3]*PhD/Profesor Asociado, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Biología, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

[4]*PhD/Profesor Asociado, Universidad del Atlántico, Facultad de Ciencias Básicas, Programa de Quìmica, Km. 7 Antigua via a Puerto Colombia, Barranquilla, Atlántico – Colombia.*

**rogervalle@mail.uniatlantico.edu.co*

Introduction

The antimicrobial photodynamic therapy (APDT) studies have been increasing due to the promissory results1–4. This technique use a no toxic photosensitizer (PS) that produce reactive oxygen species (ROS) such as singlet oxygen (1O2) and hydroxyl radicals (HOÿ)5. ROS need not enter to the cell for inhibit o kill, avoiding the development of resistance mechanisms5. Important given the increasing number of resistant microorganisms to commercial antibiotic; routinely used for attend hospital infections; do necessary to develop alternative mechanisms for combat these germs. The phthalocyanine and porphyrin have been used in different researches due to your capacity for to produce ROS and these organic molecules can be coupled to different metals for increasing the ROS producing and to assess the effect on microbicide power of such modifications.

Method

The antimicrobial activity of PS was surveyed on the next strain: Staphylococcus aureus methicillin resistant and Klebsiella pneumonia (donated by Instituto Nacional de Salud-Colombia), Staphylococcus aureus wild type ATCC 25923, Escerichia coli ATCC 25922. For Minimal Inhibitory Concentration (MIC) determination of phthalocyanine and porphyrin, the microdilution technique was applied6. Briefly: using DMSO as solvent, a serial dilution set of each PS was prepared (0.30, 0.60, 1.20, 2.50, 5.00, 10.00, 20.00, 40.00, 75.00, 150.00 μg/mL). Each treatment consisted of nutrient broth, the indicated PS concentration and the strain. The inoculum of each strain consisted of a dilution of an overnight culture until reach an OD600 of 0.025. Each treatment was carried out by duplicate and exposed to visible light irradiation for photochemical activations of PS. Then cultures were incubating to 37°C and OD600 was read at 24 and 48 hours. Irradiation, negative and positive controls was used; the first containing all, strain, culture medium and PS but not submitted to irradiation; the negative control contained all except PS and submitted to irradiation and finally the positive control contained all except PS which was substituted by gentamycin as reference antibiotic.

Results / Discussion / Conclusion

A total of six Photosensitizer are contemplated which currently we have result of Zn associated phthalocyanine, Cu associated Phthalocyanine, no metal associated phthalocyanine, and no metal associated porphyrin on the four strain evaluated. Specifically for Staphylocuccus aereus methicillin resistant the results showing that in lower concentrations (0.30μg/mL) the no metal associated phthalocyanine produce inhibition closer to 40% and 100% of inhibition for no metal associated porphyrin. The inhibition percentages obtained are according to previous researches supporting the idea of potential use of porphyrin and phthalocyanine as an alternative antimicrobial therapy1–4,7. Results of with and without irradiation test no show significant differences, suggesting that PS can be stimulated to produce ROS by the light used normally to illuminate hospital rooms, eliminating the need of use special equipment to irradiate the molecules

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PO234
HMG-CoA REDUCTASE INHIBITORY ACTIVITY OF *ACHILLEA WILHELMSII*

Khazneh, E.[1*]; Hribova, P. [1]; Fleming, H. [2.]
[1]*PhD/research assistant, VFU, Palackeho 1-3, Brno, CZ*
[1]*Student, Univeristy of Strathclyde, 16 Richmond St, Glasgow G1 1XQ, UK*
**khaznehe@vfu.cz*
hribovap@vfu.cz
hannah.fleming.2013@uni.strath.ac.uk

Introduction

Hypercholesterolemia and its induced oxidative stress are now considered to be one of the major contributors in progression of atherosclerosis. Cholesterol synthesis is regulated by the enzyme ß-hydroxy- ß -methylglutaryl-CoA reductase (HMG-CoA reductase), the rate limiting enzyme of cholesterol pathway, and catalyzes the conversion of HMG-CoA to mevalonic acid. Currently available drugs that lower cholesterol level mainly work by inhibiting the HMG-CoA reductase activity. Nonetheless, these oral medications have certain limitation and are associated with side effects. Therefore, naturally derived agents are in high demand for the treatment of hyperlipidemia. In this study we evaluated the HMG-CoA reductase inhibitory activity of fractions isolated from *Achillea wilhelmsii* C. Koch.

Method

The HMG-CoA reductase assay kit was purchased from Sigma. The plants extracts were prepared and separated into chromatographic fractions using column chromatography. The fractions were thoroughly analyzed using TLC, HPLC/DAD/MS. The isolated fractions were evaluated for their inhibitory activity. The method of assay was adapted to the 96 well plate ELISA reader. All the procedures were conducted according to the specifications provided by the kit's manufacturer.

Results / Discussion / Conclusion

4 out of 12 factions showed very promising inhibitory activity. One fraction showed a comparable inhibitory activity to the standard provided in the kit (pravastatin). The analyzed fractions showed the presence of 2 sesquiterpene lactones and 2 methoxylated flavonoids.
Achillea wilhelmsii which belongs to the family Asteraceae has a great potential in the management of hyperlipidemia. The plant has long been used by the locals in many countries in the Middle East to manage diseases like diabetes and hypertension, and this study supports its use to treat hyperlipidemia as well.

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PO235
"COSMOETICHE ACTIONS" OF LANDESIGN

Sabina Martusciello[1*], Dolores Maria Morelli[1], Luca Rastrelli[2]
[1]*Department of Architecture and Industrial Design "Luigi Vanvitelli", Second University of study, Naples*
[2]*Department of Pharmacy, University of study, Salerno*
[*]*sabina.martusciello@unina2.it*

Introduction

The Applied Research Project "landesign / Ali-ment-action" [1](scientific supervisors Prof. Sabina Martusciello, Prof. Dolores Maria Morelli, Department of Architecture and Industrial Design "Luigi Vanvitelli" Second University of study, Naples with Prof. Luca Rastrelli, Department of Pharmacy, University of study Salerno) structuring, since 2010, a permanent dialogue with the young graduates, college students, schools, institutions, municipalities, businesses and the green economy tourism, agro-food and pharmaceutical industries has expanded the traditional meaning of cosmetics (science for the maintenance and daily care of the body) in Cosmoetica or projects, actions and products for the beauty and balance of the environment and the green spaces of our earth (landesign) and man.

The "cosmoetiche actions" of Applied Research Project "landesign / Ali-ment through the process-action-action-ment Ali (Ali = componte creative, scientific mind = ingredient; action = ingredient production) reformulate, thanks to the skills multidisciplinary, the relationship between man and the environment, food and health, land and design, content and container, research and scientific innovation, the development of products and services in a Mediterranean character (cosmetics, nutraceuticals, food, design, fashion).

The "cosmoetiche actions" the supply chain in-training, training, training-with structured among School-University-Enterprises with the slogan E-DUCO / PRO-DUCO, (E-DUCO in the double meaning of the Latin breed, feed, food and pull out, pull out, draw and PRO-DUCO, meaning to promote, generate, build), on the themes of recovery of the territory and its redesign through local knowledge, care, culture / architecture and culture of the land and its products have achieved, to date, the following results: recovery of 110000 square meters of outdoor space abandoned; opening of 82 gardens in schools bells of every order and degree; prototyping of 240 objects and products foodesign, activemodesign, pharmafoodesign and giocodesign that meet the 6 criteria: function, form, feasibility, economics, ecology and emotion.

All the artifacts resulting from "cosmoetiche actions" abide by the "good manners of the project" choice; travel check; recognizability of form and content; environmental security; careful and continuous research on the knowledge and interest in the themes of place to detail with the use of substances, forms and local materials, the close correspondence between the functional components and the cooprogettazione with end users of the products.

[1] The Research Project, in relevance to the core values ??of the BIE (peace, tolerance, dialogue, etc..) Has the 3rd prize of the contest sponsored by the Italian Foundation Accenture, reporting from MIBACT and is testimonial of the Project School / University EXPO 2015

PO236
ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LEAVES EXTRACTS OF FIVE *JUNIPERUS* L. (CUPRESSACEAE) SPECIES INCLUDED IN *JUNIPERUS* SECTION FROM TURKEY

Miceli, N.[1*]; Marino, A.[1]; Köroğlu (Güvenç), A.[2]; Taviano, M. F.[1]
[1]*PhD, University of Messina/Department of Scienze del Farmaco e Prodotti per la Salute, S.S. Annunziata, 98168 Messina, Italy*
[2]*Prof, Ankara University/ Department of Pharmaceutical Botany, Tandoğan 06100, Ankara, Turkey*
**nmiceli@unime.it*

Introduction

The genus *Juniperus* L. (Cupressaceae) comprises about 60 monoecious or dioecious evergreen species, trees or shrubs distributed throughout the Northern hemisphere. The genus is usually divided into three distinct sections or subgenera: *Juniperus* (syn: *Oxycedrus*, 9 or 10 species), *Caryocedrus* (one species, *J. drupacea* Labill.), and *Sabina* (approximately 50 species). However, concerning this genus, Flora of Turkey reports only two sections, *Juniperus* and *Sabina* Spach., and *J. drupacea* is included in *Juniperus* section (Taviano *et al.*, 2011). In Turkish traditional medicine, the species under the *Juniperus* section are more frequently employed as a folk remedy. *Juniperus* L. female cones (improperly called "berries") and leaves have been used by Anatolian people since ancient times as antihelmintic, diuretic, stimulant and antiseptic (Oztürk *et al.*, 2011). In continuation of our studies on *Juniperus* L. species under *Juniperus* section from Turkey, this work was designed to evaluate the antioxidant and antimicrobial potential of methanol leaves extracts of *J. communis* L. var. *communis* (*Jcc*), *J. communis* L. var. *saxatilis* Pall. (*Jcs*), *J. drupacea* Labill. (*Jd*), *J. oxycedrus* L. ssp. *oxycedrus* (*Joo*), *J. oxycedrus* L. ssp. *macrocarpa* (Sibth. & Sm.) Ball. (*Jom*).

Method

The total polyphenol content of *Juniperus* L. spp. leaves extracts was determined spectrophotometrically by the Folin-Ciocalteau reagent. The antioxidant properties were examined by different *in vitro* methods: DPPH test, reducing power assay, Ferrous ions (Fe^{2+}) chelating activity and TBA assay. Further, the correlation between TPC and antioxidant activity was evaluated. The antimicrobial potential of *Juniperus* L. spp. extracts against bacteria and yeasts was evaluated by standard methods. The inhibition of biofilm formation was quantified using a microplate-based screening assay (Miceli *et al.*, 2011).

Results / Discussion / Conclusion

The total polyphenol content (TPC) ranged from 71.77 ± 0.13 mgGAE/g (*Jcc*) to 133.28 ± 1.74 mgGAE/g (*Joo*). In the DPPH test the extracts exhibited strong scavenging activity (IC_{50} from 0.092 ± 0.006 mg/mL *Joo* to 0.403±0.043 mg/mL *Jcc*). The extracts showed good reducing power (ASE/mL from 2.56 ± 0.06 *Joo* to 9.66 ± 0.16 *Jd*). In the Ferrous ion-chelating assay *Jd* displayed the strongest effect (IC_{50} 2.65 ± 0.37 mg/mL), whereas *Joo* and *Jom* did not manifest any activity. In the TBA assay the extracts showed anti-lipid peroxidation activity (IC_{50} from 4.39 ± 0.47 µg/mL *Jcs* to 71.42 ± 3.01 µg/mL *Jom*). A strong positive correlation between TPC and both DPPH test and reducing power assay was highlighted (R^2>0.9); no correlation with Ferrous ion-chelating and TBA assays was found. All the extracts have shown bacteriostatic activity exclusively against *S. aureus* in the range of 19.53-78.12 µg/mL, and *Jd* resulted the most effective. The extracts had no significant effect on the *S. aureus* biofilm formation at sub-minimum inhibitory concentration (*MIC*) levels.

The obtained results give support to the ethnopharmacological use of these Turkish *Juniperus* L. species, as well as demonstrating the potential of these plants as sources of natural antioxidant and antimicrobial compounds. Bioassay-guided fractionation procedures are necessary to characterize and isolate the active constituents.

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PO237
DISEASE CELIAC IN ARGENTINA

Teresita Di Bernardi[1*], Mónica Bufor[1], Etile Spegazzini[2]
[1]*Carrera de Farmacia, Facultad de Ciencias Exactas y Naturales, Universidad de Belgrano. Villanueva 1324, Ciudad Autónoma de Buenos Aires. C.P. C1426DQG - Arg.*
[2] *ACOFAR[1] (Coop. Farmacéutica Azul), Guido Spano 664, Azul. C.P. 7300. Bs. As. - Arg.*
**dibernardi808@hotmail.com*

Introduction

The celiac disease (1) is the permanent intolerance to gluten, a set of proteins present in wheat, oat, barley and rye (TACC) and their derivatives of these four cereals. Found in both children and adults. Currently, the incidence is higher in women than in men.
The proteins are classified into two groups, prolamins and glutenins. Prolamines have different names depending on the cereal of origin:

Wheat:(**T**rigo) Triticum spp. L. "wheat" = gliadin
Oat:(**A**vena) Avena sativa L. "oat" = avenin (TACC)
Barley: (**C**ebada) Hordeum vulgare L. "barley" = hordein
Rye: (**C**enteno)Secale cereale L. "rye" = secalin

The gluten-grains is the most popular format of toxic prolamins to celiac. Gliadin causes the main damage-since it is frequently used in the food industry.
Oat apparently not harmful but in industrialization process, it can be contaminated with grains of wheat, barley or rye.
This intolerance produces a characteristic lesion of the intestinal mucosa causing atrophy of the villus of the small intestine, which alters or reduces the absorption of nutrients from food.
Also features associated with autoimmune and genetic diseases can be discovered in asymptomatic patients.
It is said that celiac disease is an autoimmune condition.

Materials and methods

The micrographic feature is one of the first test of identification, which can even found fragmented vegetable, power or processed food cereals
These descriptions are considered "authority" and may be the first source characterization, microscopic feature.
The micrograph is then be a different micro analytical chemical method micro analytical methods can reach, in some cases, overcome the latest in terms of speed and ease of execution and, more importantly, in terms of their ability resolution. Wheat, oats, barley and rye: In this paper the histological diagnostic elements of each of the species under study is established. Histological sections of each of the samples to establish the protein layer and the histological diagnosis are identifed

Conclusion

The presence of the aleurone layer (gluten) and starch layer, will be detected in different species of TACC.
This paper offers an overview of celiac disease, legislation in Argentina and a method for detecting gluten in flour samples and / or processed products containing any of the cereal TACC.
Argentina is estimated that 1 in 100 people can be celiac.
Argentina has a National Law No. 26.588 - Resolution No. 1560-1507 and No. 18.284 Argentine Food Code Act - Joint Resolution 120/03 and 516/03 of Dr. Eduardo Cueto Rua was one of the main authors.
(1) www.celiaco.org.ar

PO238
EFFECTS OF *FAGOPYRI HERBA* ON CHOLESTEROL AND BILE ACID METABOLISM IN HIGH-FAT FED RATS

Aleksandra Mišan[1], Ivan Milovanoviæ[1], Maja Đurendiæ-Brenesel[2], Miljan Miliæ[3], Vladimir Pilija[2], Nebojša Iliæ[1], Anamarija Mandiæ[1]

[1]*Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[2] *Institute of Forensic Medicine, Clinical Center Vojvodina, Hajduk Veljkova 7-9, 21000 Novi Sad, Serbia*
[3]*Oncology Institute of Vojvodina, Put dr Goldmana 4, 21204 Sremska Kamenica, Serbia*
E-mail of corresponding author: aleksandra.misan@fins.uns.ac.rs

Introduction

High-fat food intake is connected with increase in associated cardiometabolic risk factors, such as increased serum cholesterol levels. *Fagopyri herba*, dried areal parts of common buckwheat, has been used in the treatment of vascular diseases and reported to possess medicinal properties. The present work was undertaken to investigate the effect of *Fagopyri herba* consumption on bile acid and cholesterol excretion in male Wistar rats fed a standard and a hyperlipidemic diet for 8 weeks.

Methods

A total of of 25 male Wistar rats were randomly divided into five groups: a standard diet group (I), a 5% *Fagopyri herba* supplemented diet group (II), a high-fat diet group (III), a high-fat 5% *Fagopyri herba* supplemented diet group (IV), a group eating the same diet as III group for 7 weeks and the same as group IV for 1 week (V). Feces of the rats was collected and bile acid and cholesterol content was analysed by means of liquid chromatography (HPLC) with diode array (DAD) and light scattering detection (ELSD).

Results/Discussion/Conclusion

High-fat fed rats showed higher total levels of cholesterol and bile acids excreted in feces. Levels of cholic, ù- and β-muricholic acid were found to be significantly higher in *Fagopyri herba* supplemented high-fat diet groups (IV and V), compared to group which received only high-fat diet (III). Levels of cholesterol were found to be significantly higher in group V in comparison with other groups, which suggests that postponed *Fagopyri herba* introduction into the high fat diet can ameliorate the effects of prolonged high fat diet consumption in rats.

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PO239
HERBAL DRUG *FAGOPYRI HERBA* AS A SOURCE OF BIOACTIVE PHENOLIC COMPOUNDS

Aleksandra Mišan, Bojana Šariæ, Ivan Milovanoviæ, Pavle Jovanov, Nebojša Iliæ, Anamarija Mandiæ, Marijana Sakaè
Institute of Food Technology, Bulevar cara Lazara 1, Novi Sad, Serbia
nebojsa.ilic@fins.uns.ac.rs

Introduction
Due to a high content of rutin (2-10%), *Fagopyri herba* has been shown to be efficient in the treatment of vascular diseases. As such, it can be considered as a source of bioactive compounds for functional food formulations. The effects of ethanol to water ratio and temperature on the extraction efficiency and phenolic composition of *Fagopyri herba* extracts were investigated.

Methodology
Extracts were obtained by maceration with water and ethanol/water mixtures, with the ratio of raw materials to ethanol solution of 1:50, for 24 h at room temperature and subsequent extraction in an ultrasonic bath for 10 min. These extracts were compared with corresponding extracts which were previously brought to boiling. Identification of the phenolic compounds in extracts was carried out by LC-DAD-ESI-MS/MS, and quantification of the identified compounds was carried out by rapid resolution reverse phase HPLC/DAD method (Mišan *et al.*, 2011).

Results / Discussion / Conclusion
According to obtained results, extraction with boiling ethanol/water (80:20) has been shown to be the most efficient method for achieving the highest yield of rutin (4.99%). Comparing the results of rutin yield obtained by different extraction procedures, it has been concluded that temperature exhibited the highest influence on water extraction.

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PO240
Astronium sp: ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY

Negri, K. M. S.[1*]; Bonifácio, B. V.[1]; Ramos, M. A. S.[1]; Silva, P. B.[1]; Assis, R. P.[1]; Souza, L. P.[2]; Vilegas, W.[3]; Brunetti, I. L.[1]; Bauab, T.M[1]

[1]*School of Pharmaceutical Sciences of Araraquara, São Paulo State University, Rodovia Araraquara-Jaú Km 1, 14801-902, Araraquara, São Paulo, Brazil*

[2]*Institute of Chemistry, São Paulo State University, Prof. Francisco Degni Street, 55, Quitandinha, 14800-060, Araraquara, São Paulo, Brazil*

[3]*UNESP - São Paulo State University - Coastal Campus of São Vicente, Infante Dom Henrique Square, s/n, Parque Bitaru, 11330-900, São Vicente, São Paulo, Brazil*

**kamilafarm@gmail.com*

Introduction

Plant species constitute a fascinating topic of academic research and development, once it has shown a promising source of compounds that can be used to develop new herbal. *Astronium* sp (Anacardiaceae) is a neotropical genus of tree species that are typical of savannas of North and Northeast of Brazil; among them we can highlight the following species: *Astronium urundeuva* (aroeira-do-sertão), *Astronium graveolens* (guaritá) and *Astronium fraxinifolium* (gonçalo-alves), which have anti-inflammatory, antimicrobial, anti-ulcerogenic and healing properties(1).

Method

The minimum inhibitory concentration (MIC) was determined by the microdilution technique against the strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella* sp (ATCC 19196) and *H. pylori* (ATCC 43504). The plant extracts were tested from 1000µg/mL to 7.81µg/mL and the inoculum contained 10^7 UFC/mL(2). Amoxicillin (100µg/mL) was used as positive control for *H. pylori* and ampicillin (5µg/mL) for the other bacteria; the resazurin (0.01%) was used as a developer. The antioxidant activity was determined using the reactive species hypochlorous acid (HOCl), being the principle of this assay the oxidation reaction of 3,5,3',5'-tetramethylbenzidine (TMB) with the increase in absorbance at 652 nm. Another method was the taurine-chloramine system obtained by the HOCl (40 µM) by reaction with the taurine (Tau - 5mM) during 5 minutes in 50 mM sodium phosphate buffer, pH 7.4. The samples were added to the mixture following the incubation for 10 minutes and addition of TMB (2.8 mM). The absorbance reading was taken at 652 nm(3).

Results / Discussion / Conclusion

Only the leaves of *A. graveolens* and *A. urundeuva* showed antibacterial activity against *S. aureus* (MIC's 250 and 125µg/mL, respectively). The other species of *Astronium* sp did not show antibacterial activity at the tested concentrations against the other bacteria. The extracts showed different behaviors against different bacteria, showing that the mechanism of action may be dependent on the ultrastructure of the microorganism, which would limit the access to the site of action of molecules present in different extracts. Samples were effective on kidnapping HOCl and showed IC_{50} values very close to each other, but *A. urundeuva* leaves (IC_{50} 1.09µg/mL) was the most efficient. The lower *scavenger* potential of HOCl was played by the leaf extract of *A. fraxinifolium* (IC_{50} 1.40µg/mL). The extracts of *Astronium* sp species were considered effective kidnappers of taurine chloramine. The extracts of the stem showed a better activity than the leaves, being *A. graveolens* (IC_{50} 29.33µg/mL) the most efficient. The leaf extract of *A. urundeuva* (IC_{50} 76.82µg/mL) was the less efficient. Therefore, the extracts proved to be more effective sequestrants for HOCl than the taurine.

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PO241
(E)-2-(2-nitrobenzylidene)-1,1-diphenylhydrazine

Aguirre Cabrera C.[1], Cabrera-Vivas B. M.[2*], Meléndrez-Luévano R.[2*], Ramírez Juan C.[2], Ortiz Márquez J. A.[2], Sansinenea Royano E.[2], Toledano M. Y.[3]

[1]Bachelor´sdegreeStudentin Chemistry,Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.

[2]PhD/Facultad de Ciencias Químicas, Edificio 179, 18 sur y Av. San Claudio. Benemérita Universidad Autónoma de Puebla, C. P. 72001, Col. San Manuel, Puebla, México.

[3]PhD/Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, Departamento de Inmunología. Av. Universidad 3000, Distrito Federal, C.P. 04510.

carla.aguirre27@gmail.com

Introduction

Hydrazides and hydrazones are present in many of the bioactive heterocyclic compounds that are of wide interest because of their diverse biological and clinical applications. This created interest in researchers who have synthesized variety of hydrazide-hydrazones derivatives and screened them for their various biological activities viz anticancer[1], anti-HIV[2], antimycobacterial[3], anti-inflammatory[4], antidiabetic[5], antimicrobial[6], as well antimalarial activities[7].

Method

228 mg (1.24 mmol) diphenylhydrazine were dissolved in ethanol and acetic acid (0.5 mL) were slowly added into this solution while stirring, 300 mg (1.24 mmol) of 2-Nitrobenzaldehyde we added drop by drop into the above solution strongly stirring and the resulting mixture was kept at room temperature until it became orange transparent solution. After one and a half hours the orange solution precipitated. The reaction was monitored by TLC, aluminum AlugramSil G/UV254. The mixture was separated with filtration in *vacuo* system and the precipitate was washed three times with cold methanol. Recrystallization was performed three times with ethanol, to obtain orange crystals for X-ray analysis andcharacterization was performed by m. p., U.V, I.R. ^{1}H NMR, ^{13}C NMR.

Results / Discussion / Conclusion

Yield 91%, orange crystals, mp. 133-135°C. FT.IR (film): (cm^{-1}): 3026v(C-H), 1577 v(C=N), 1334 v(NO_2). ^{1}H NMR (400 MHz, $(CD_3)_2CO$: δ/ppm, *J*/Hz): 8.28 (dd,1H,C3), 7.91 (dd,1H,C5), 7.72 (m,1H,C4), 7.60 (s,1H,C=N), 7.52(d, *J* = 1.44, 1H, C6), 7.48 (m, 4H,C2'), 7.25 (m,6H,C4', C2'). ^{13}C NMR (400 MHz, $(CD_3)_2CO$): (δ/ppm):143.16 (C2), 132.99 (C1'), 132.97 (C4), 130.41 (C=N), 130.13 (C6), 129.99 (C3'), 128.40 (C1), 127.72 (C3), 125.26 (C4'), 124.49 (C5), 122.41 (C2'). MS-EI: m/z 317.12. $C_{19}H_{15}N_3O_2$.
Our work group has made an experimental approach with the evaluation of synthetized hydrazones as (*E*)-2-(2-nitrobenzylidene)-1,1-diphenylhydrazinepresented IC_{50} of 0.96 μM, that represent a 7 fold increase in the potency of cell growth inhibition with respect to metronidazole (IC50 = 6.8 μM).

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&

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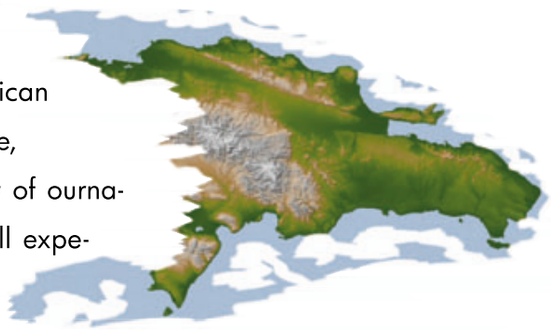

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