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Title of Thesis

"Sustainable use and practical application in the industrial sector of permanent crops biomass: Orange tree (*C. sinensis* L.), Apricot tree (*P. armeniaca* L.) and Olive tree (*O. europea* L.)"

Scientific Disciplinary Sector

"AGR/06 Wood Technology"

PhD course Coordinator

Prof. Fabio Napolitano

Ph.D Student

Dr. Maria Roberta Bruno

Tutor

Prof. Luigi Todaro

Co-Tutor

Prof. Luigi Milella

Cycle XXXIII

… A me, alla forza di andare avanti nonostante tutto e

a chi lungo il cammino mi tende la mano.

Preface

The Ph.D. thesis borned due to the grant of Region Basilicata-Italy to develop a partnership between the University of Basilicata and the industrial sector, through the "Industrial 4.0" Ph.D. scholarships. My team guided by Prof.Todaro, have built the basis of the research on how to apply the principle of *circular economy* in order to use the secondary metabolites of agro-forestry biomass, in different industrial sectors such as pharmaceutical, cosmetic, agriculture, and others, to create new eco-sustainable industrial products. The statistical survey has shown as in Europe, in Italy, and in the Basilicata region, the permanent crops occupy relevant surfaces. The management of these crops leads to generate a huge quantity of biomass without an economic value that often is burned in the field or used to generate heat, energy, or in the best case used as compost for the cultivations. To avoid this loss of material, following the EU line guides for the next years, it was chosen to analyse the wooden residue of the tree most relevant species in the permanent crops or orange tree (*Citrus sinensis* L.), apricot tree (*Prunus armeniaca* L.) and the olive tree (*Olea europea* L.). The wooden material of these three species was collected in 2018 in the Basilicata region. Then, it was selected four different extraction techniques maceration, ultrasound assistant, accelerated solvent extraction and autoclaving and two different solvents as ethanol, water and ethanol and water (v/v 70:30) to analyzed the extraction yield, TPC, antioxidant activity, LC-MS, and GC-MS to calculate the difference between the different extraction techniques and different solvents. Most of the studies were made in the science laboratories of the university thanks Prof.Milella's team and Prof.D'Auria's team. The LC-MS analysis was made during the six months in France, to Nancy in the LERMAB laboratory thanks to Prof.Gerardin's team. These different analyses and studies have shown the potential of the extractives due to the antioxidant capacity and the presence of interesting molecular compounds moreover antioxidant compounds i.e. naringenin, catechin, scopoletin, oleuropein, ligustroside.

It was not possible to realize an industrial product using the different extract so it was chosen the apricot bark extracted with the maceration ethanol/water (70:30 v/v) who the best antioxidant capacity and the within the antioxidant compound.

These encouraging results have led to the development of the prototype of face cream with the support of Dr. Alessandra Miraglia and her ecocosmetic laboratory in which was made the formulation tests to produce the prototype.

Afterward, the cytotoxicity effect of both the extract and the cream prototype was studied in the laboratory of Science. These are preliminary tests, to whom are necessary to add tests and deepen researches to have a commercial product. The last period characterized

by the pandemic emergency has created several difficulties to continue the tests on the cosmetic product. The prototype cream is a start point from which to continue, to have on the cosmetic market a natural, sustainable, green product. Further analyses and studies may lead to the use of these vegetable extractives in other industrial sectors. The aim of the innovative industrial Ph.D. was achieved with the union of the university research with the industrial sector.

Introduction and Aim

In the Italian Mediterranean regions, excluding the islands, fruit tree crops occupy an area of approximately 1,059,048 ha (1). This area is occupied by 3% by orange tree (*Citrus sinensis* L.), 1% by apricot tree (*Prunus Armenica* L.), and about 66% by olive groves (*Olea Europea* L.). The area destined for agricultural production in Basilicata is equal to 833,847 ha (Istat, 2018), and of these 50,281 ha are destined for tree crops, 12% from orange groves, 9% from apricots, and 58% of tree crops are olive groves. These data show the importance of tree crops in the regions of southern Italy and Basilicata. Tree crops require canopy management that requires at least one pruning per year. Furthermore, some of these crops, especially apricots, have a life cycle that lasts about 15/16 years, after which they are explanted. Pruning and harvesting of orchards generate significant amounts of wood, which are often burned or sometimes shredded and left on the ground to increase organic matter. Burning this type of residue generates various problems, the first is the production of CO_2 in the atmosphere (2), the second is due to the legislation that has set limits to this practice, so it is not always possible to burn these residues. Due to these problems related to the management of wood residues, possible alternative uses of these materials are being sought in industrial sectors such as the chemical, pharmaceutical, nutraceutical, agricultural sectors. Several studies have focused on the extraction of secondary metabolites from waste wood materials. Secondary metabolites are chemical compounds produced by different parts of plants (wood, leaves, root, bark, etc.) which have various medical properties such as anticancer, antibacterial, anti-inflammatory, and other properties (3) (4). The extracts obtained from plants are non-structural compounds and are formed by both organic and inorganic compounds. The main organic compounds present are aliphatic, alicyclic, and phenolic compounds. From the studies conducted so far, the multiple potentialities of extracts of plant origin have emerged, furthermore, the reuse of production waste is perfectly suited to the concept of "circular economy", according to which it is necessary to achieve "zero waste" and exit from the current economic perspective take, make and dispose ” (Ellen MacArthur Foundation, 2010). Besides, the EU in the 7th General Program of Action of the Union in the field of the environment based on a clear long-term vision reports that: - "*In 2050 we will live well in respect of the ecological limits of our planet. Prosperity and a healthy environment will be based on a waste-free circular economy, in which natural resources are managed sustainably and biodiversity is protected, valued and restored in such a way as to strengthen the resilience of our society. Our growth will be characterized by reduced carbon emissions and*

will have long been decoupled from the use of resources, thus setting the pace for a secure and sustainable global society".

The research aims to enhance the biomass of these tree species which today is mostly burned. The compounds extracted from these tree species were analyzed, through the identification, characterization, and study of molecules.

In addition, an extract from one of the tree species has been added to a cosmetic cream formulated compatibly with the extract used.

The exploitation from a quantitative and qualitative point of view of the secondary metabolites obtained from the wood waste of the major fruit species of the Basilicata region will lead to:

- Reduce agricultural practices with high environmental impact such as waste incineration;
- Recovery of a waste product;
- Make a by-product with an economic value equal to zero a raw material with a specific economic value;
- Replace synthetic molecules with natural molecules that will be used in various industrial sectors such as the chemical, pharmaceutical, nutraceutical, and above all agricultural sectors;
- Compare the extraction techniques and molecule characterization techniques with the Italian-French partnership.
- Use practically the extractive compounds in a Basilicata cosmetic company.

In a historical moment in which environmental issues have assumed a key role in the economic and social planning of all States, the doctoral work aims at the research and development of useful compounds obtained from waste material, which can be used in different sectors industrial.

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Abstract

Of the Earth's surface the 30% is occupied by forest and almost 50% is occupied by agricultural crops. Both the forest and agricultural besides producing food, feed, fiber, and a wide range of necessary products like shelter, packaging, clothing, and communications, produce a huge quantity of biomass about 956 Mt of dry matter for agriculture and the forest 18,600 Mt dry weight. Much of forest biomass is used to produce energy and heat while most of the agriculture biomass remains in the field or is burned. The recent EU Directives have encouraged state members to follow circular economy guidelines on waste reuse, especially for waste originating from wood agro-forest biomass. The biorefinery that used the lignocellulosic material has increased the production of biobased material, due to the composition of the biomass. Within the biomass are present lignin, polysaccharides, and extractives. The extractives have a huge potential in the industrial sectors. Orange trees, apricot trees, and olive trees are typical Mediterranean crops and a major feature of the heritage in the Mediterranean basin, where they play an important environmental and economic role. Given the potential of biomass in the different industrial sectors, the research was focused on the valorization of biomass of orange trees, apricot trees, and olive trees. The biomass coming of these trees was selected and bark was separated from the wood. After that different extraction techniques were applied, including maceration, ultrasound-assisted extraction, accelerated solvent extraction, and autoclaving. The extractives obtained were evaluated of the antioxidant capacity with the measurement of total polyphenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), β-Carotene bleaching assay (BCB). Integrating the antioxidant capacity values generated from the different *in vitro* methods above mentioned was determined the relative antioxidant capacity index **(**RACI). Results demonstrated the potential antioxidant activity of the bark and wood, not investigated before. Then it is moved on to analyze the molecular compound present in these extract through the LC-MS analyses and GC-MS analyses. The chemical characterization showed the presence of different natural compounds, including polyphenols, alkaloids, and flavonoids. These analyses were necessary to build an industrial product. The extract with the best antioxidant activity and the antioxidant compound was the apricot bark extract through the maceration. Studying the market of cosmetic products it was chosen to make a prototype of face cream. It was necessary to make a cytotoxicity assay both for the extract and for the cream plus the extract. These preliminary assays have demonstrated the possibility of using the extracts from orchards and olive grove in the industrial fields. Therefore, the development of innovative applications that use biomass derivatives could lead

to their possible use in the market as a commodity for the chemical or cosmetic industries, giving new added value to the current use of biomass from agricultural practice. Through multi-criteria analysis, it was possible to recognize the sustainability of these cropping models and their ecological function, turning into the preservation of environmental resources, environmental quality, and quality of life.

Keywords: biomass, circular economy, biorefinery, biological and chemical analysis, natural cosmetic

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List of abbreviations

OCH	Orchard
OL	Olive Tree
VY	Vineyard
TG	Grape Table
MC	Moisture Content
W	Wood
B	Bark
OR	Orange Tree
AP	Apricot Tree
ME	Maceration Extraction
UAE	Ultrasound-Assisted Extraction
ASE	Accelerated Solvent Extraction
AT	Autoclaving
TPC	Total Polyphenolic Content
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
TE	Trolox Equivalents
SD	Standard Deviation
BCB	β-Carotene Bleaching Assay
BHT	Butylated Hydroxytoluene
LC–MS	Liquid Chromatography-Mass Spectrometry

U-HPLC	Ultra High Performance Liquid Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
PCA	Principal Component Analysis
RACI	Relative Antioxidant Capacity Index
GWP100	Global Warming Potential
OTP	Olive Tree Pruning
GMP	Good Manufacturing Practice
HepG2	Human Hepatocellular Carcinoma Cell Line
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
SWOT	Strengths Weakness Opportunities Threats

READING GUIDE

A brief guide on what is reported in each chapter is summarized to facilitate the reading of this thesis.

CHAPTER 1 provides a brief overview of the potential of biomass produced in the World, in Europe, and Italy. The chemical composition of biomass is also mentioned, with particular attention to extractives and their use in various industrial sectors.

CHAPTER 2 reports a botanical, dimensional, and productive evaluation of the species under study such as the orange tree (*C. sinensis* L.), apricot tree (*P.armeniaca*), and olive tree (*O.europaea* L.).

CHAPTER 3 shows all the materials and methods used for the chemical and biological analyzes to which the samples under study were subjected.

CHAPTER 4 illustrates the results obtained in the various tests carried out on the three species of this study. Specifically analyzes of TPC, DPPH, FRAP, BCB, RACI, GC-MS and LC-MS.

The analyzes have shown the potentiality of the woody biomass of orange tree, apricot tree and olive trees.

CHAPTER 5 illustrates the practical application of the apricot bark extract extracted through maceration, chosen for having shown interesting chemical and biological characteristics, for the realization of a prototype of cosmetic cream. The realization of the prototype involved cytotoxicity analysis of the product and the extract, LC-MS analysis of the extract and qualitative analysis of the cream.

CHAPTER 6 provides a brief overview of the results obtained and a SWOT analysis on the use of woody extracts in industrial products.

CHAPTER 1
OVERVIEW

1.1 Earth land cover

The Earth surface is about 510,065,285 km^2 of this, about 70% is occupied by water (oceans, sea, lakes, rivers). According to the data presented in the FAO databases, the world total land area is about 127,343,220.031 (1). The term land cover refers to the attributes of a part of the Earth's land surface and immediate subsurface, including biota, soil, topography, surface and groundwater, and human structures (2). Through the elaboration of the moderate resolution imaging spectrometer (MODIS), the land cover of the earth, excluded the urbanized areas, is divided into five main macro-categories: herbaceous crops, woody crops, grassland, tree-covered areas and shrub-covered areas. The data (Fig. 1) showed as the Americas have the higher land cover about 3,607,223 x 10^3 ha, followed by Asia 2,182,048 x 10^3 ha, Africa 1,925,846 x 10^3ha, Europe 1,837,580 x 10^3 ha and Oceania 831,112 x 10^3 ha. Most of the area is occupied by the tree-covered in America and Asia, respectively 2,077,269 x 10^3 ha and 1,617,705 x 10^3 ha. Grassland as it was conceivable, is mainly found in the Americans with 933,369 x 10^3 ha, while Asia has the largest area covered by herbaceous crops about 490,176 x 10^3 ha. Oceania has the largest shrub-covered areas about 443,703 x 10^3 ha. The woody crops are mostly present in Asia with 71,438 x 10^3 ha. It is necessary to note that Africa, even if in third place as for extension, in refers to macro-categories, does not have the widest regions. This is probably due to environmental factors as well as social conditions in the continent.

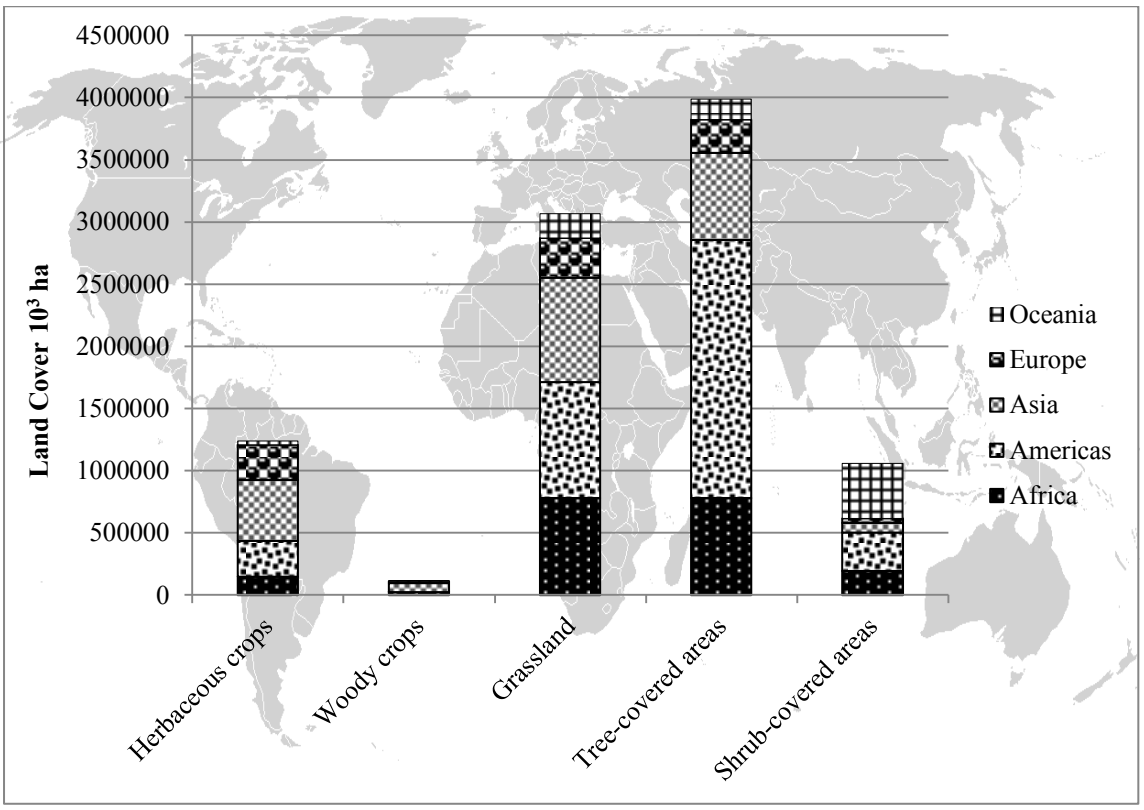

Figure 1 Land cover of the Earth, divided into five main macro-categories: Herbaceous Crops, Woody Crops, Grassland, Tree-Covered Areas, and Shrub-Covered Areas (FAOSTAT, 2017)

The European total surface (10,180,000 km^2) is 1/4 of the Asia (44,580,000 km^2) and Americas (42,550,000 km²). Subdividing the five macro-categories into detailed categories as agricultural land, croplands, land under perm. meadows and pastures and forest land, it can be noted in the world as the forest land occupies 31% of the land areas whereas agricultural and cropland occupy 38% and 12% respectively (Fig. 2). The data demonstrated that the European percentage of land area (Fig. 3) is in opposition to those of the world land area and the European forest land is about 45% of the total land while the agricultural is 31% and cropland is about 13%.

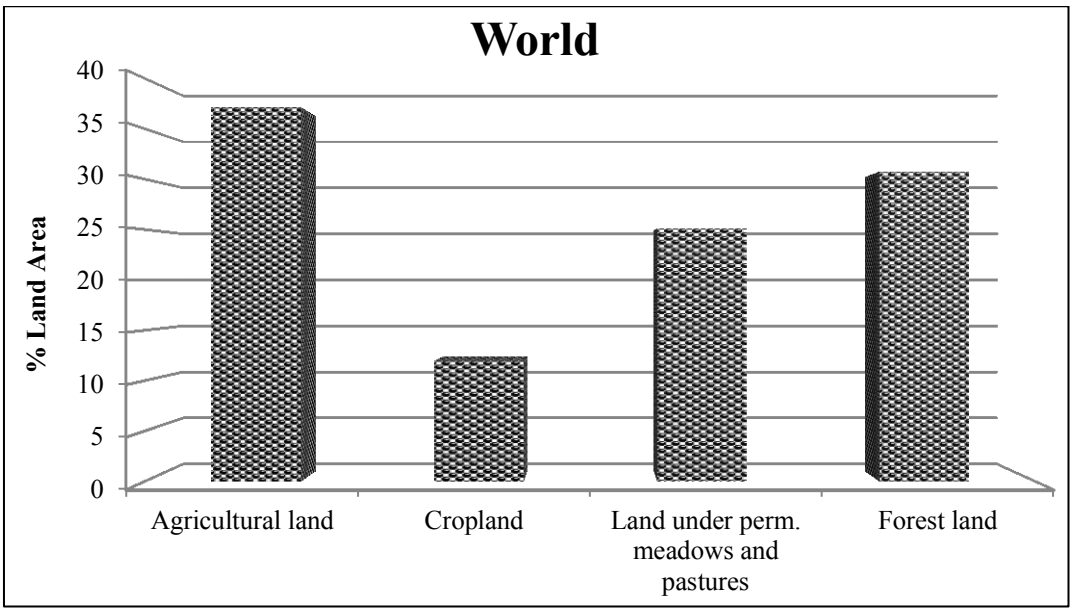

Figure 2 World land categories: agricultural land, croplands, land under permanent meadows and pastures and forest land (FAOSTAT, 2017)

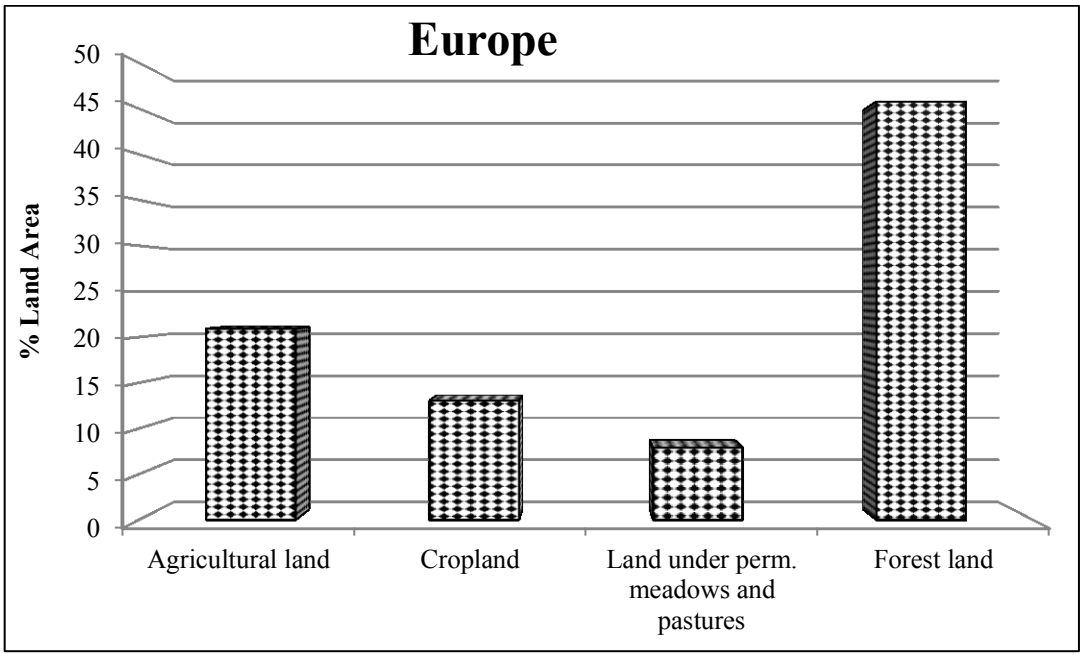

Figure 3 Europe land categories: agricultural land, croplands, land under permanent meadows and pastures and forest land (FAOSTAT, 2017)

Considering just the EU-28 according to the Eurostat databases land surface is 2,213,323.59 ha, of this about 1,015,482.48 ha are occupied by forests and 462,855.94 ha by agriculture crops. In the European states (Fig. 4), Sweden is the country with the largest forest area of 30,505 (10^3 ha) followed by Spain and Finland, 27,626,65 and 23,019,00 (10^3 ha) respectively. Italy is the 7th country in Europa for forest surface about 11,110,00 (10^3 ha). The EU-28 total Utilised Agricultural Area (UAA) the largest part (59.8 %) consisted of arable

land, close to one third was occupied by permanent grassland, and 6.6% by permanent crops (3).

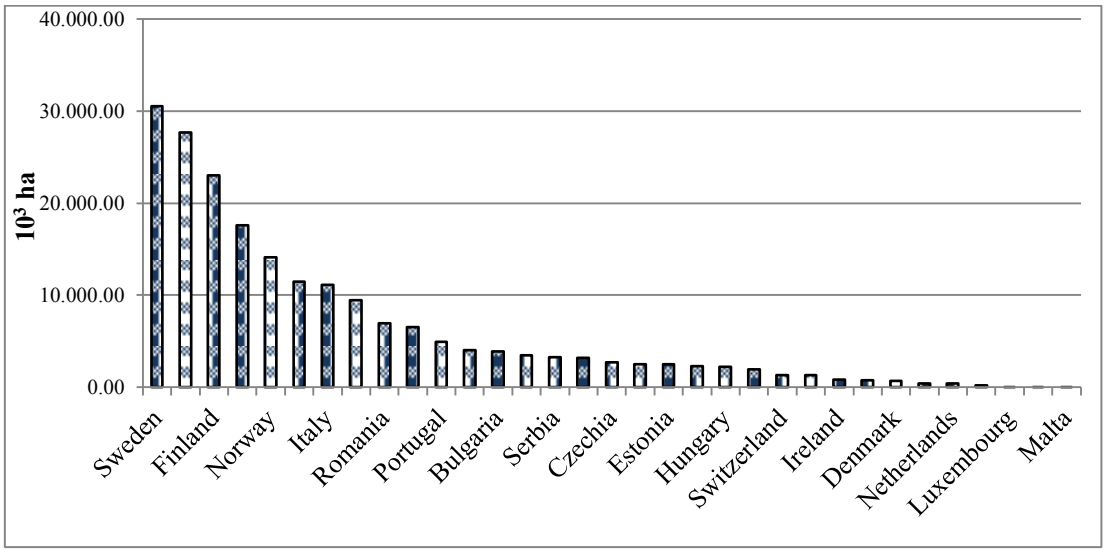

Figure 4 Forest land of EU-28 in hectares-ha (EUROSTAT, 2018)

Focusing on the permanent crops, according to the EU definition of these are part of *all fruit trees, all citrus fruit trees, all nut trees, all berry plantations, all vineyards, all olive trees and all other permanent crops used for human consumption (e.g. tea, coffee or carobs) and for other purposes (e.g. nurseries, Christmas trees or plants for plaiting and weaving such as rattan, or bamboo). Permanent crops are usually ligneous crops, meaning trees or shrubs, not grown in rotation, but occupying the soil and yielding harvests for several (usually more than five) consecutive years*.

Figure 5 are reported the hectares of the orchards, olive tree, vineyard and grape table in the European countries. Spain is the country with the largest number of hectares (6,400,631) dedicated to these permanent crops, followed by Italy (3,151,068) and Greece (2,213,994).

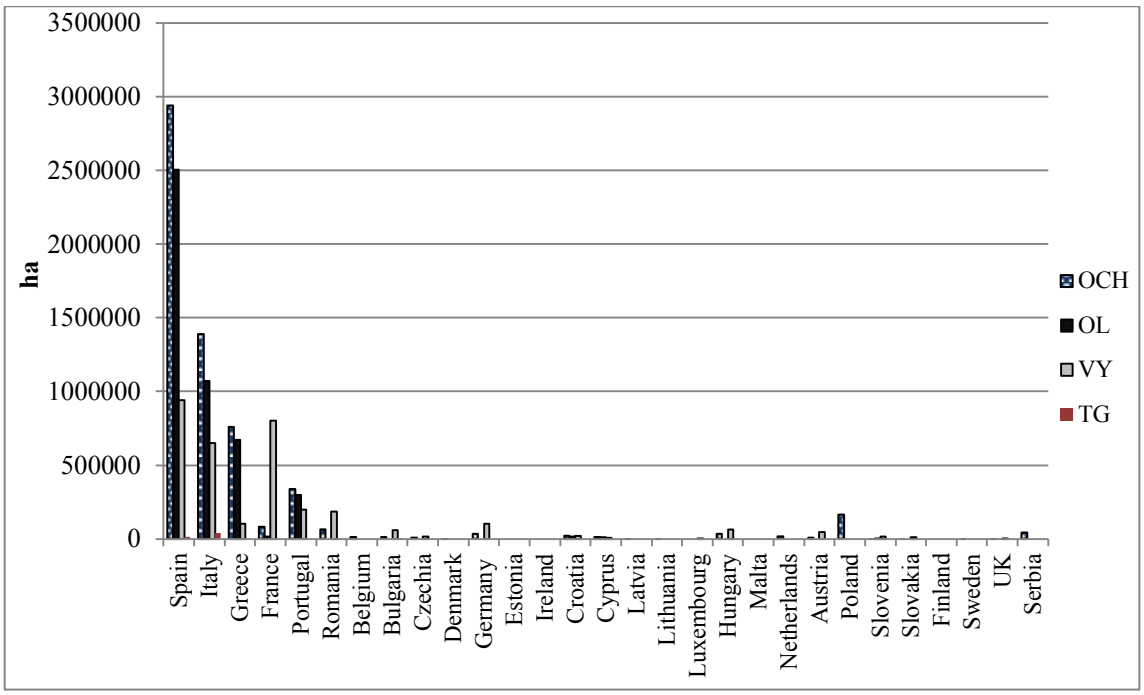

Figure 5 Hectares of the orchards (OCH), olive tree (OL), vineyard (VY) and grape table (TG) in the European countries (EUROSTAT, 2018).

According to the European Commission, the agricultural and forest sector are primary sectors for human life. Agriculture and forest products industries provide food, feed, fiber, and a wide range of necessary products like shelter, packaging, clothing, and communications. Next to this primary product, an important secondary product is represented by biomass (4). Biomass is also a source of a large variety of chemicals and materials, and electricity and fuels (5).

1.2 Biomass

The meaning of biomass according to McKendry (6) is the plant material derived from the reaction between CO_2 in the air, water, and sunlight, via photosynthesis, to produce carbohydrates that form the building blocks of biomass. According to the report published by the EU "*Biomass production, supply, uses and flows in the European Union"* (7), biomass is the core of the bioeconomy. Hall et al. (61), declared approximately 50% of the world's biomass (about 600 quads worldwide) is used by humans for food, construction and fuel. The primary use of this biomass is bioenergy**.** According to Camia et al. (7), energy use accounts for almost half (48%) of the total uses of woody biomass on the EU-28 level. Agriculture crops generate a quantity of biomass about 956 Mt of dry matter while the forest 18,600 Mt of dry weight.

Forestry waste is now recognized as an important source of biomass fuel but until now little attention has been paid to the 25 million tonnes of prunings produced each year by Europe's orchards, vineyards and olive groves (8).

1.3 The role of biomass in the industrial production

Enduring research and industrial development plus increased drivers to use in industrial sectors beyond energy have seen the focus of the biomass markets widen to include value chains for bio-based chemicals, pharmaceuticals, and other materials. The shift from petroleum- to biomass-derived materials appears to be the plausible long-term pathway to ensure a sustainable supply of carbon feedstock for the energy and chemical industry (9).

Recent studies and work in the S2Biom project (10) supported by the European Commission provided robust scientific evidence that at least 1 billion tonnes of lignocellulosic biomass will exist in Europe on an annual basis across the various supply sectors (agriculture, forestry, biowastes and dedicated perennial crops). Currently, the major shares of innovative bio-based products such as polymers, plastics, chemical building blocks, lubricants, solvents and surfactants in Europe are primarily based upon oil, sugar and starch sources with only a small share of natural fibers from wood. The annual consumption of agriculture-based lignocellulosic biomass for non-food and feed uses is currently quite small and estimated at 15

million tonnes (dry) although information relies on individual studies that are not harmonized across EU (11).

There are several studies on possible uses of the orchard biomass from wood residues. Most of them have studied the possibility of using biomass especially pruning biomass, to produce energy (12,13,14). According to Sherwood et al. (15), the use of this product to produce energy was encouraged by the availability of the huge material of low-value waste. This use does not promote the circular economy, which is based on the reuse of waste to minimize waste and generate additional value. For Szabó (16), agriculture being a multifunctional activity has a central role in the economy, especially in the bioeconomy by producing food as well as delivering public goods and services. The National Research Strategy BioEconomy 2030, declared that: *"The concept of the bioeconomy covers the agricultural economy and all manufacturing sectors and associated service areas that develop, produce, process, handle, or utilize any form of biological resources, such as plants, animals, and microorganisms. This spans numerous sectors, such as agriculture, forestry, horticulture, fisheries and aquaculture, plant and animal breeding, the food and beverage industries, as well as the wood, paper, leather, textile, chemicals and pharmaceutical industries, and aspects of the energy sector. Biobased innovations also provide growth impetus for other traditional sectors, such as in the commodity and food trade, the IT sector, machinery and plant engineering, the automotive industry, environmental technology, construction, and many service industries* (Fig.6)*."*

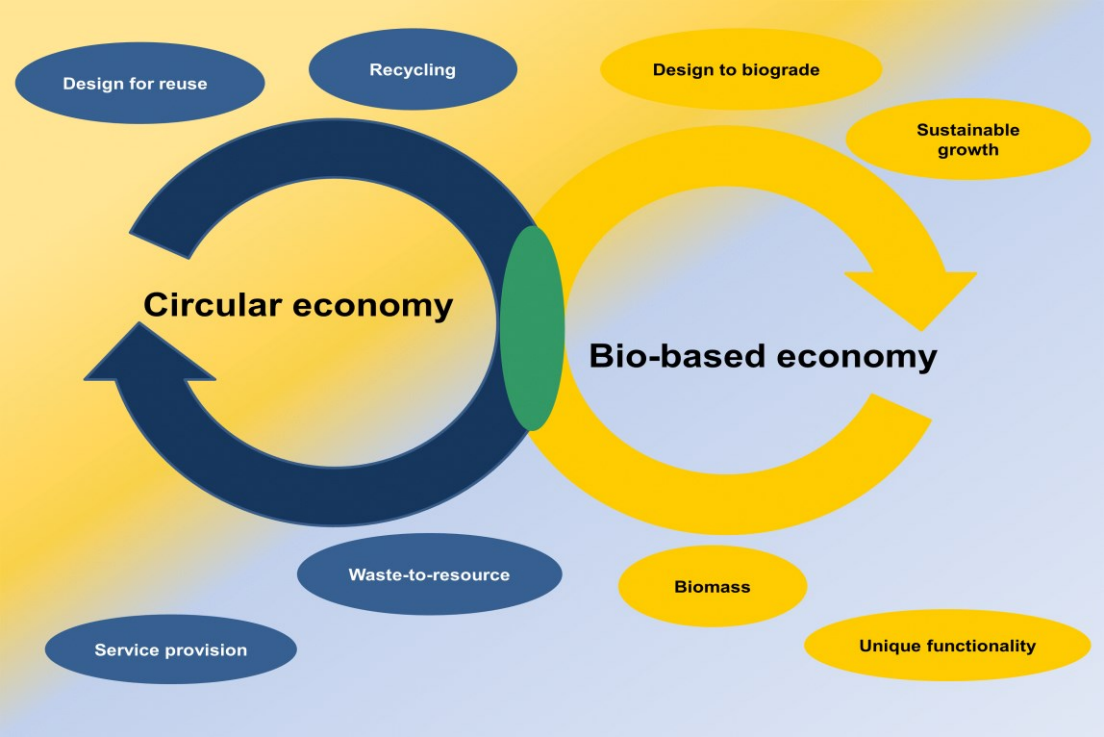

Figure 6 Link between the circular economy and bio-based economy

To justify the practice of energy recovery there must not be the possibility to use the biomass for any other processes other than being burned. To avoid the release of long cycle carbon into the atmosphere, contributing to increasing GHG emissions, only 100% bio-based products are suitable from the point of view of material recirculation. Recently, more studies have been carried out on the use of the wood and bark mainly coming from forests, to use the woody biomass and fiber for raw materials and buildings (17). Tree bark is a still largely underutilized side stream of the pulp and paper industries and wood works. In the production of wood pulp, for example, the felled timber is trimmed and cut to specific lengths, and its bark is then removed, either mechanically (depending on the size and shape of the trunk) or by use of water jets. The separated bark is then dewatered and used on-site as fuel while some of it goes into horticultural use. In the case of pulp production, as bark contains cellulose (less than wood) and a large variety of phenolics and sometimes sands, it requires close-to-complete removal before pulping and/or extraction. The bark could be also chemically utilized at least to some extent (Figure 7).

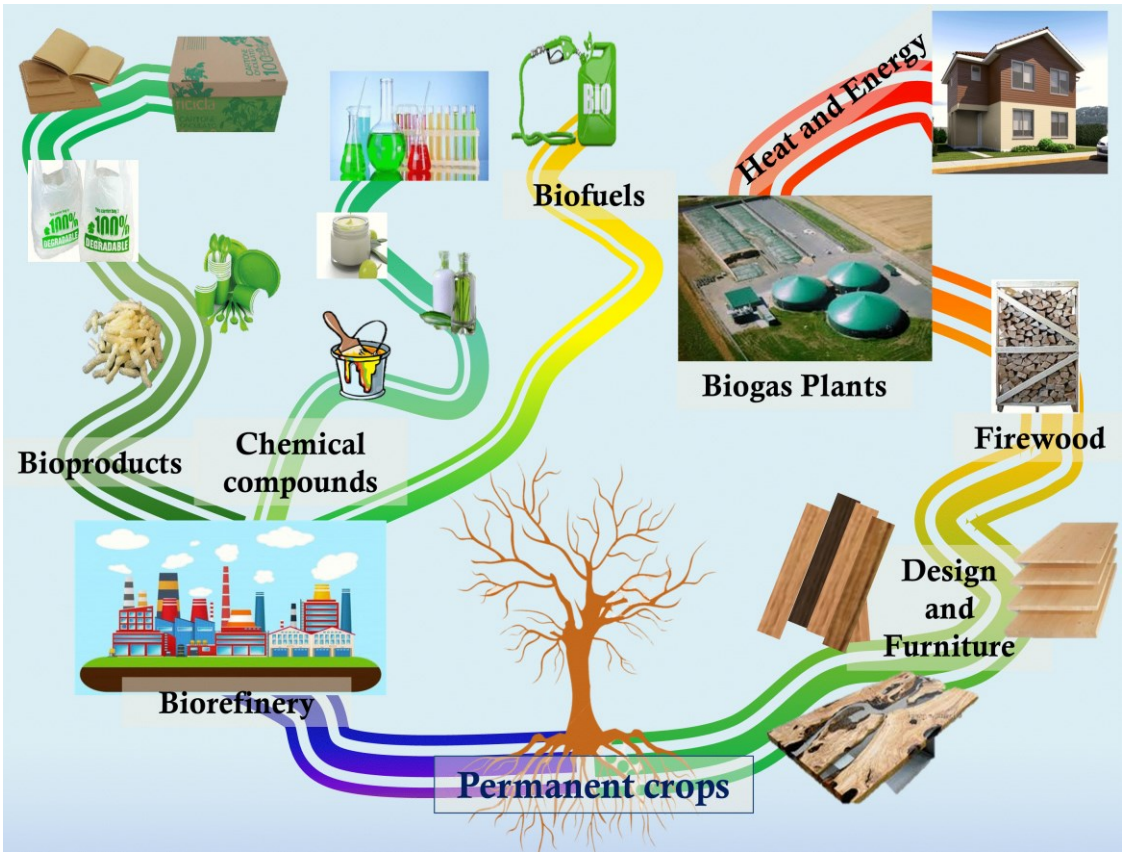

Figure 7. The use of permanent crops biomass in the industrial sector

However, the combustion of bark represents typical integrated production of bioenergy since it is normally burned in the on-site bark-burning furnace. On the other hand, the high share of extractives in bark would constitute a potential source of interesting and valuable compounds to be used in segments such as pharmacy and cosmetics, with a higher added value. However, such utilization is impeded by the need to extract these compounds from the bark matrix, which requires sometimes the use of organic solvents. Both extraction and separation are technically possible anyway.

The woody material from orchards is poorly investigated. The studies (18,19,20,21) about orchards are mainly concentrated on the evaluation of the fruit juices, parts of the fruits (peel, seeds, etc.) and sometimes on the leaves but rarely on the physical and chemical proprieties of the wood.

In contrast to past approaches, the agricultural waste derived from orchards should be considered as an optional source of income, rather than a cost. Orchards could also be "exploited" as starting material through extracting secondary metabolites with high intrinsic value, due to their wide range of biological activities. For instance, several studies have demonstrated the potential uses of the secondary metabolites derived from agricultural wood extracts, including antioxidant compounds and the radical scavenging properties. The secondary metabolites present in the wood and bark, their extraction using different techniques (22) and their possible use in different industrial sectors, especially in chemistry (23, 24).

European Union regulations state that waste materials should be reused and recycled as much as possible to create new circular economies to avoid wasting natural resources. Thus, it is important to analyze woody materials to determine the potential of secondary metabolites for use in various industries. Secondary metabolites are organic molecules that do not contribute to the normal growth and development of an organism, with their functions remaining largely unknown. The non-structural, secondary metabolites of wood cells can usually be removed with neutral solvents. Exudates are extracellular secondary metabolites that are formed by trees growing under certain conditions or after damage by fire, insect, fungal, or mechanical sources (25). These metabolites appear to be involved in organism defense (26). Extracts of these metabolites, thus, could include compounds with antibiotic, antiviral, antitumor, anti-inflammatory, and antioxidant activity properties. These compounds could be used to improve the health of humans and animals (27).

1.4 Chemical composition of agro-forest biomass

The wood dry biomass (100%) is composed of lignin (25%), polysaccharides (70%), organic materials (5%). Between the polysaccharides, there are cellulose (40%) and hemicellulose (30%). While in the organic materials (5%) the 3.5 % is represented by extractives (28). According to Demirbaş, (29) the proportion of these wood constituents varies between species, and there are distinct differences between hardwoods and softwoods. Hardwoods have a higher proportion of cellulose, hemicelluloses, and extractives than softwoods, but softwoods have a higher proportion of lignin. In general, hardwoods contain about 43% cellulose, 22% lignin, and 35% hemicelluloses while softwoods contain about 43% cellulose, 29% lignin, and 28% hemicelluloses (on an extractive free basis).

1.4.1 Lignin

It is a recalcitrant and complex phenolic macromolecule comprising three phenylpropanoids (cinnamyl alcohol), namely, coniferyl alcohol, p-coumaryl alcohol, and sinapylalcohol. Thus the lignin is considered the largest source of aromatic compounds, and therefore, the advancement in its valorization process has become a popular topic of research (30). Lignin is an amorphous three-dimensional natural polymer, consisting of phenyl-propane units linked through the ether and C-C bonds. These phenyl-propane units are sources of phenolic compounds (31). Lignin represents a feedstock for aromatic chemicals since accounts for 10–35% by weight of the lignocellulosic biomass (32). In particular, vanillin, besides aroma application, can be obtained through a lignin depolymerization process (33) and can be the starting material for the production of new polymers (34).

1.4.2 Polysaccharides

According to Amidon et al. (35), polysaccharides are constructed from monosaccharides, they revert to monosaccharides by acid or enzymatic hydrolysis. The polysaccharides include cellulose and hemicellulose.

1.4.3 Cellulose and Hemicellulose

Cellulose is a long-chain linear polymer with a typically crystalline structure. The cellulose polymers are arranged in microfibrils that are organized in fibrils, and these are

combined into cellulose fibers, which form the basic layer of the wood cell wall are responsible for the fibrous nature of wood cells. Hemicelluloses are natural carbohydrates of shorter chains and molecular weight and they are branched to form amorphous polymers of five or six-carbon sugars (36). Together with lignin, hemicellulose forms the matrix in which the cellulose fibrils are embedded. Thus, the hemicellulose acts as a connector between cellulose and lignin. A typical structure of wood, illustrating its complexity, is presented in Figure 8.

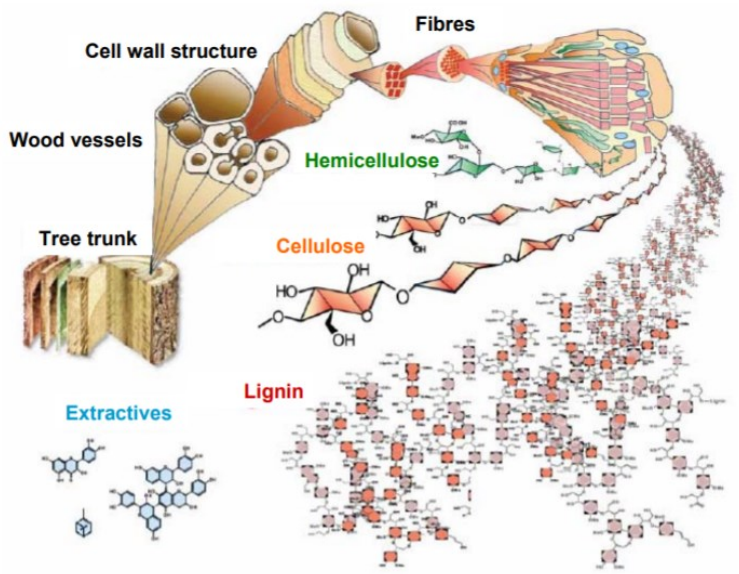

Figure 8 The chemical composition of wood biomass at different levels. Source: De Wild et al. (36).

1.4.4 Extractives

With the name "*extractives*" is indicated the chemicals in the woods that can be extracted by using different solvents, for this reason sometimes are classified under the solvent used. Extractives can be extracted from wood by diverse methods and solvents, and accurate methods have been applied to influence the yield and type of extracted composite. The quantity of extractive compound disbanded in each solvent may vary, and the choice of the suitable extraction techniques will depend on the plan of the final product wanted. In scientific research, the extractives are generally obtained by sophisticated, expensive and time-consuming laboratory methods (*e.g.* using Soxhlet apparatus). Depending on the species, wood can contain levels of extractible material ranging from 0.5% to about 20%. Furthermore, various parts of the same tree, such as stem, branches, roots, bark and needles, can differ depending on age, seasonality, and location (37). The extractives comprise both inorganic and organic components, called secondary metabolites. Organic extractives can be

classified into two different groups: aliphatic and alicyclic compounds and terpenes and terpenoids, esters of fatty acids, sugars and waxes (38). In general, softwood has a higher extractive content than heartwood (39) and contributes to wood proprieties such as color, odor and taste (40). Different types of extractives are necessary to maintain the diverse biological functions of the tree. For example, traces of certain metal ions are present as functional parts of the enzymes which are needed as catalysts for biosynthesis, fats constituent the energy source of the wood cells, whereas lower terpenoids, resin acids, and phenolic substances protect the wood against microbiological damage or insect attacks. The chemistry of wood extractives explains the effect that these compounds may have on wood industry development or other different industrial applications. Several researchers showed how different biomass sources could provide interesting results to exploit the economic and industrial potential of the biomass refinery (41). Wood extractives affect, among others, the manufacture of pulp and paper, paint and varnish films, and adhesives. There is evidence that certain amounts of some components are necessary to enable the living tree to resist diseases and insect attacks, and this could be very important when selecting silvicultural treatments or particular species or hybrids for the establishment of the timber crops of the future. The importance of biomass as a source for bio-active pesticide substances was recently highlighted by Villaverdeet al. (42). Several recent studies were focused on the characterization of bark extractives (43),(44) and the antioxidative properties of the bioactive compounds (FIGURE 9). Several studies (45) (46) analyzed the amount of extractives from wood wastes. Wood extracts possessed strong antioxidant activities and also suggest the contribution of phenolic to antioxidant activities. Can be observed that the potential of antioxidant activity increases together with the total amount of phenols in the wood extracts.

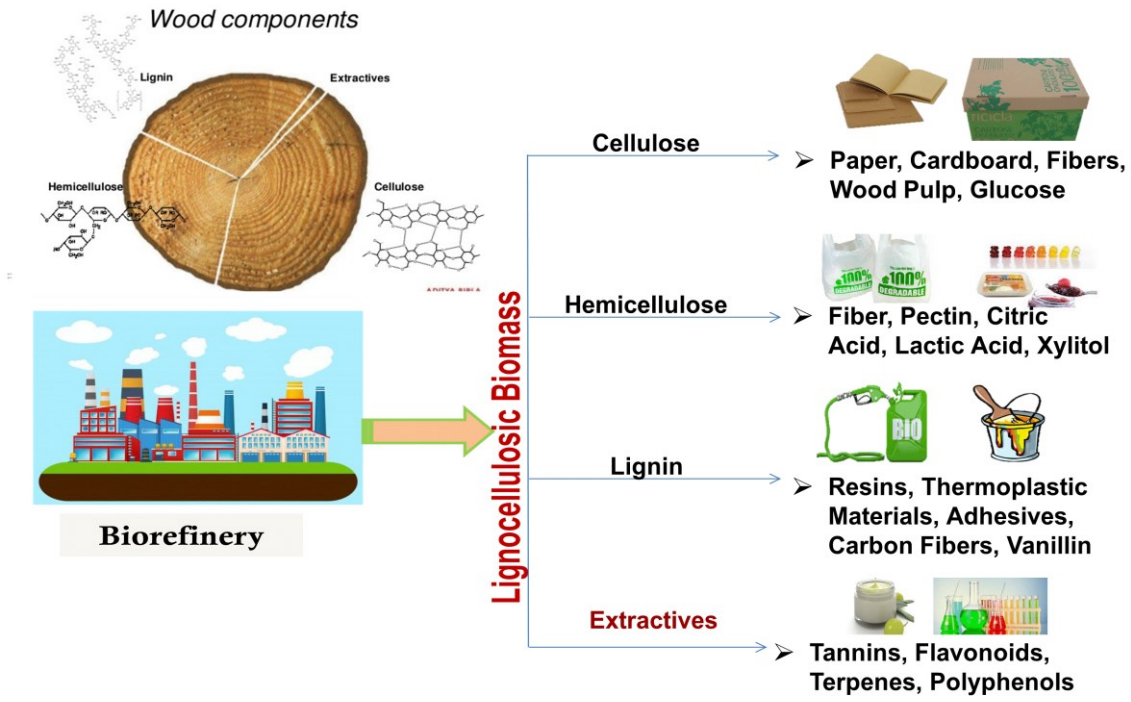

Figure 9 The use of wood chemical compounds in the biorefinery industry

1.5 Extractives in permanent crops

According to Gallezot (47) apart from providing food, feed and energy, biomass was employed throughout recorded history to extract valuable products such as medicinal drugs and flavors and fragrances. There are several studies regarding the extractive also from agricultural wooden biomass. The analyses on the compounds extracted from grapevine wood (48), in particular the stilbene compounds and their possible uses to contrast the development of *Plasmopora viticola* in the leaves. Furthermore, the stilbenoids have important antioxidant activities and anti-inflammatory activity (49). The wooden residue of grapevine resulting from the pruning, suffering from *Esca disease*, was also investigated and phenols and stilbene polyphenols were found (50). The results of this study underlined how these compounds are mainly present in the wood with the disease than the health wood, this demonstrates the potentialities of all types of residues of the grapevine, included those with diseases. The studies of the use of wood orange residues have concerned different topics. Concerning the extractives, by using different solvents (acetone, ethanol:toluene, dichloromethane and water) 221 different chemical components were extracted and 33 components were identified in the extracts using gas chromatography coupled with mass spectrometry (51). Between these compounds, there were acetic acid, furfural and vanillin. The components founded have

different potential use in different sectors as food, chemical, pharmaceutical. Further study has analyzed the possible application of orange extractives in the medical sector, specific for diabetic treatment (52). Through this study, in the stem bark of orange wood, is founded the antioxidant compounds (alkaloids, flavonoids, polyphenols) that have efficacy to keep under control diabetic pathology. Bruno et al. (53) showed as the pruning orange biomass has antioxidant properties and different useful compounds for the industrial sectors. The researchers have analyzed the antioxidant activity of olive wood residues (54) through the compounds extracted. These compounds have been characterized and the group predominant are *secoiridoids* (55), which have potential applications in the food, cosmetic and pharmaceutical industries. In addition to the study of the phenolic compounds in olive wood, other researches are focused on antifungal activities of these compounds (56) and have demonstrated how oleuropein, hydroxytyrosol and tyrosol have good anti-bio activity. Cara et al. (57) stated that olive tree pruning biomass is an interesting raw material for oligosaccharide production. Other, an interesting range of oligosaccharide fractions for several applications, namely for the growth of probiotic bacteria is obtained. Based on all the mentioned studies, the main properties of extractives cover a wide range of uses and applications, from human health to leather production or other types of industrial use (Figure 10).

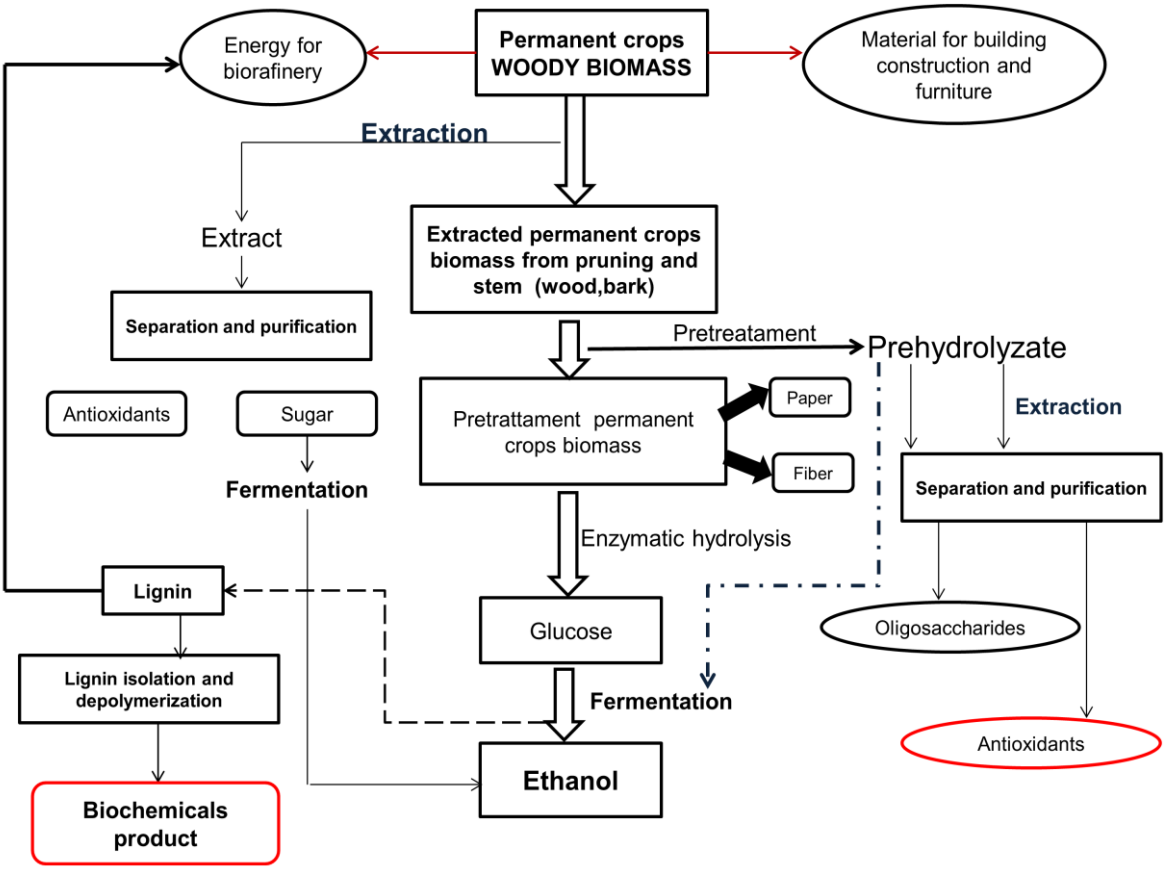

Figure 10 Flow diagram of biorefinery based on the permanent crops. Source: (58) (59) (60)

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CHAPTER 2
TREE SPECIES OBJECT OF THE THESIS PROJECT

Figure 5 showed as Italy is the second country in Europe for permanent crop surface. According to ISTAT, 2020 in Italy the surface of the **orange** tree is about 81,583 ha, 19,302ha of the **apricot** tree and **olive** tree 1,164,201 ha. These tree species complexities represent 54% of the total permanent crops in Italy.

According to Camia (1), the higher quantity of biomass in the permeant crops on the EU-28, coming from the olive tree is about 10.35 Mt, vineyards 4.66 Mt, apples 2.,29, citrus fruit 2.27 Mt and other perm crops 2.34 Mt. Pari et al (2), have estimated that in Italy around 6 million tons (over dry basis) of pruning biomass is available from the main orchards each year, (including uprooted biomass).

Given the importance that these crops play in Italy and the amount of biomass they generate, it was decided to focus our studies on the biological and chemical properties of wood residues.

2.1 Orange tree (*Citrus sinensis* L.)

The orange tree (Figure 11) is a fruit tree belonging to the *Rutaceae* family, whose fruit is orange. It is a plant that varies in size from a small shrub to a tree 4-5 m high. It has a round crown and branches with delicate and flexible thorns. The leaves are oval rounded at the base and sharp at the apex, the margin is whole to slightly notched; the petiole has a small wing on both sides. The flowers, isolated or gathered in small racemes at the axil of the leaves, have five greenish sepals and five pure white petals; they give off a pleasant but not strong scent. The fruit, which varies in shape from ovoid to rounded slightly depressed at the poles, is orange or yellow; the surface is usually smooth or sometimes slightly wrinkled, the juice is abundant and sweet.

Figure 11 Orange Tree **Source:** www.giardinaggio.it

The orange tree cultivation is largely presented in America and Asia, the country of origin of these species (Figure 12). In Europe, the area harvested is 272,287 ha with a production of about 6,451,581 t (Figure 13-Figure 14). The countries in Europe with the larges number of hectares and production are Spain and Italy respectively 139,626 ha and 81,015 ha and 3,639,853 t and 1,522,213 t (Figure 13-Figure 14).

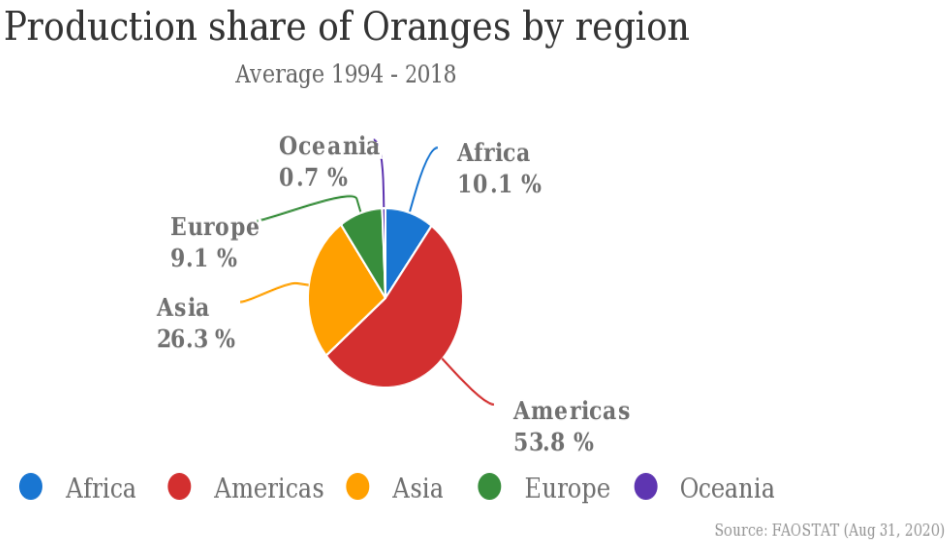

Figure 12 Average of orange production in the years 1994-2018 in the five continents. FAOSTAT, 2018

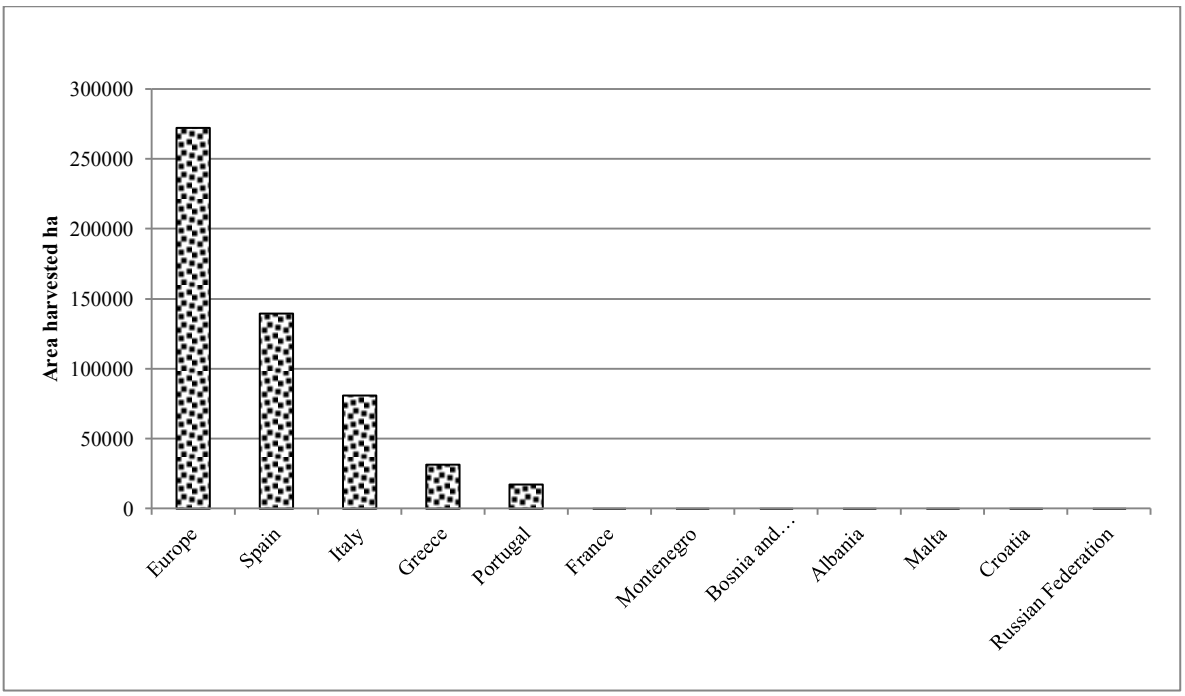

Figure 13 Orange tree area harvested (ha) in Europe and European countries. FAOSTAT, 2018

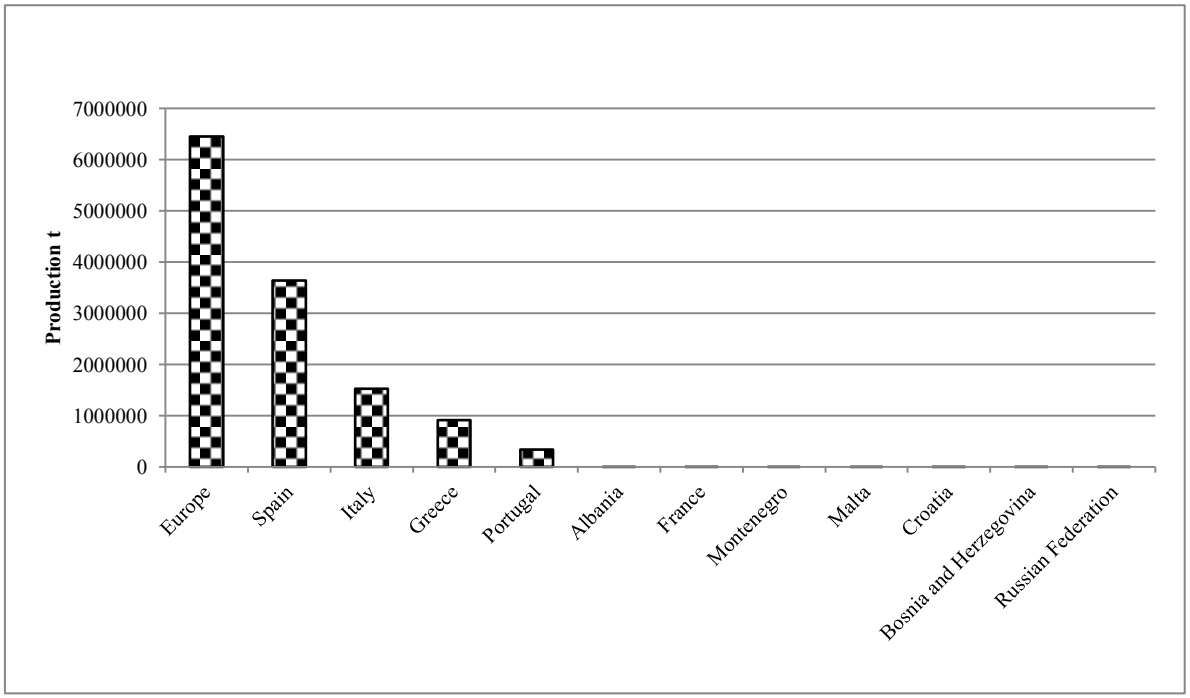

Figure 14 Orange tree production (t) in Europe and European countries. FAOSTAT, 2018

2.2 Apricot tree (*Prunus armeniaca* L.)

Apricot (Prunus armeniaca L.) (Figure 15) belongs to the *Rosaceae* family (subfamily: Prunoidae). The genus Prunus is largely cultivated in warm temperate regions. It is a deciduous plant of medium size, 3 to 7 meters high. However, the cultivated plants rarely reach 3 meters, to facilitate harvesting operations. The leaves are heart-shaped with a doubly serrated edge. The flowers have a calyx and pentamer corolla, they are white-pink, single, or paired. Flowering occurs, as in all Prunus, before foliation. The fruits are velvety drupes of dark yellow-orange color, with a tendency to faded red in the more mature fruits. Like all fruit plants, the latter is green, hard, and difficult to detach from the tree in conditions of little or no ripening of the drupe.

Figure 15 Apricot tree Source: www.giardinaggio.net

Asia is the world's largest producer of apricots followed by Europe (Fig. 16). In Europe, 102,602 ha of orchards are harvested, and apricot is one of the most important cultivated fruit trees in Spain, covering 20,567 ha terrestrial area but also in Italy with 17,809 (Fig. 17) (FAOSTAT, 2018). In Europe the production is about 946,126 t, it is interesting to note that Italy, despite being second for the harvested area, has the largest production of apricot about 229,020 t compared to Spain that has a production of about 176,289 t (Fig. 18).

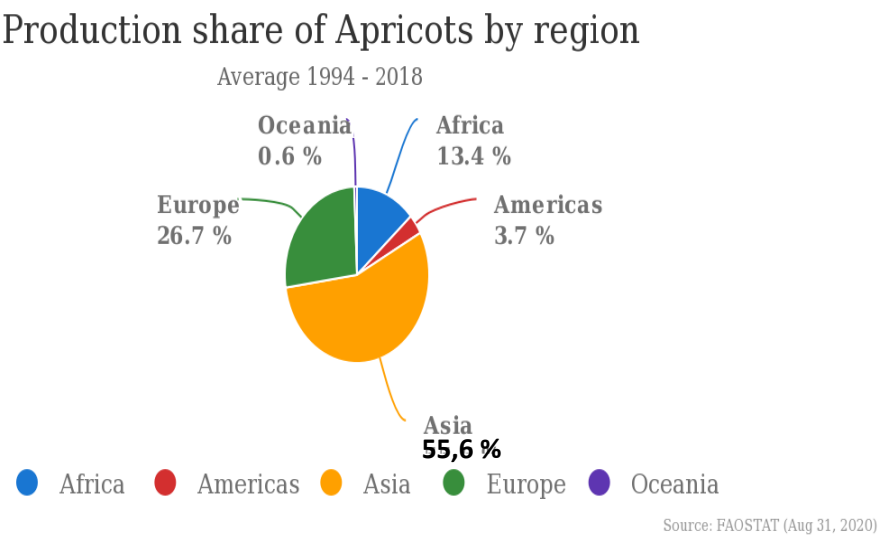

Figure 16 Average of apricot production in the years 1994-2018 in the five continents. FAOSTAT, 2018

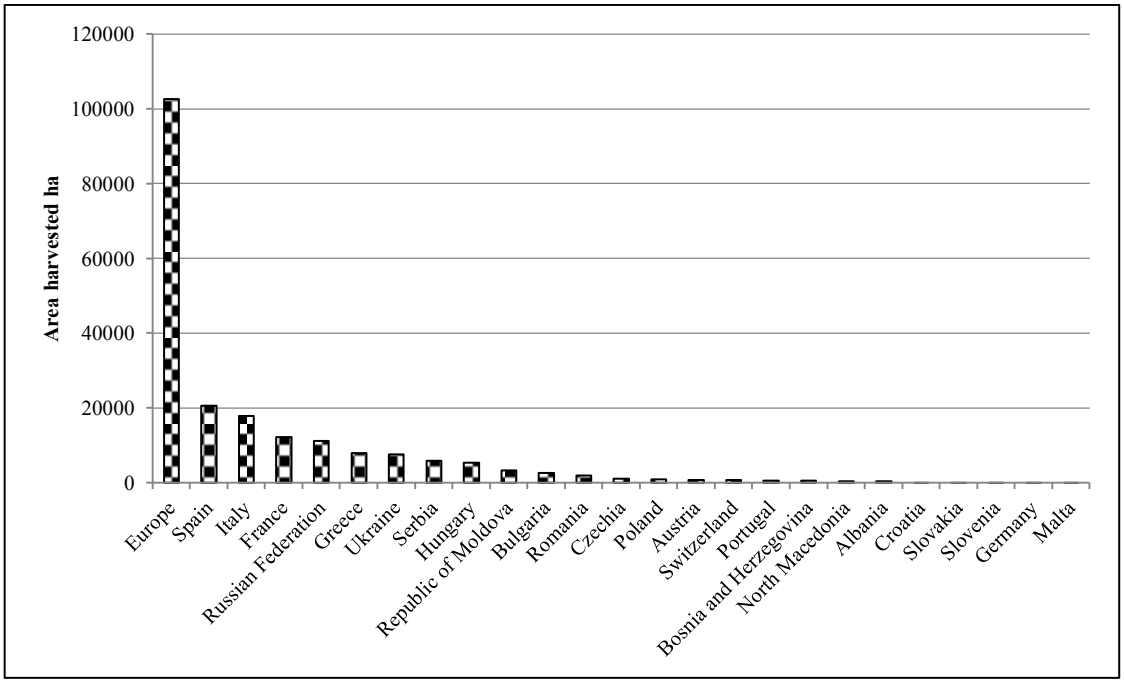

Figure 17 Apricot tree area harvested (ha) in Europe and European countries. FAOSTAT, 2018

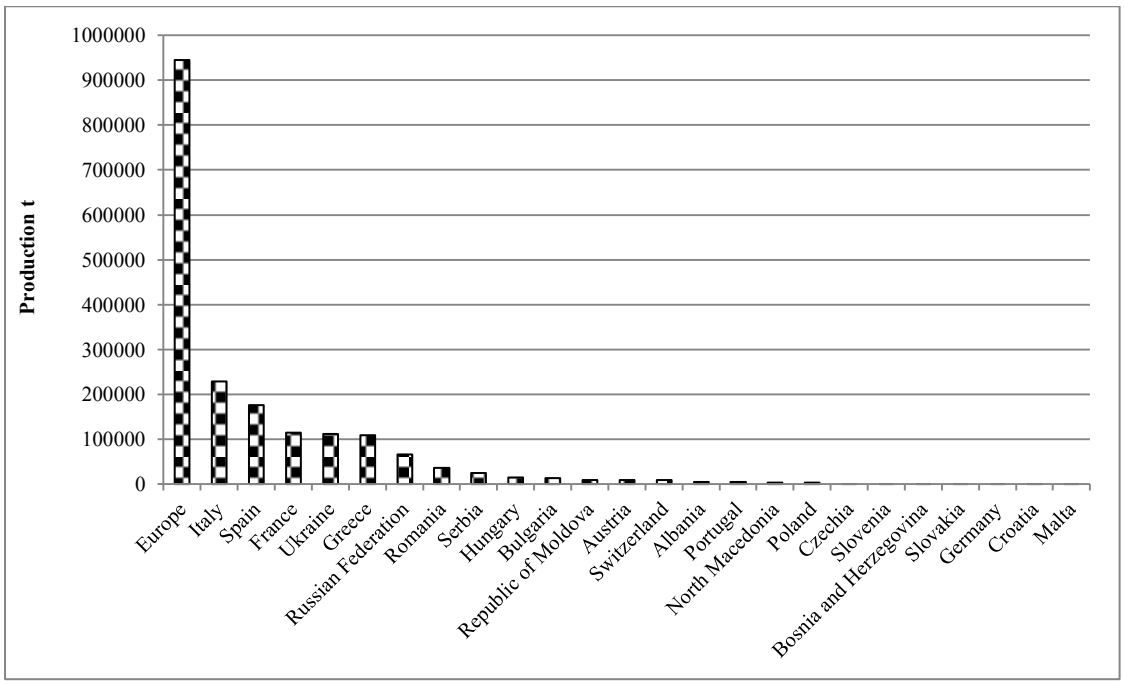

Figure 18 Apricot tree production (t) in Europe and European countries. FAOSTAT, 2018

2.3 Olive tree (*Olea europaea* L.)

The olive tree (*Olea europaea* L.) (Fig. 19) belongs to the *Oleaceae* family and has an ancient tradition of cultivation in the Mediterranian region. The olive tree (Olea europaea, Oleaceae) is a traditional symbol of abundance, glory, and peace, and its leafy branches were historically used to crown the victorious in friendly games and bloody war (3). The olive tree is an evergreen tree and a broad-leaved tree. It has slow growth and is very long-lived: in favorable climatic conditions, it can become millenary and reach heights of 15-20 meters. The plant begins to bear fruit 3-4 years after planting. The stem is cylindrical and twisted, with grey or dark grey bark and hard and heavy wood. The leaves are opposite, leathery, simple, whole, elliptic-lanceolate, with short petiole and entire margin, often revolute. The flower is hermaphroditic, small, with a calyx of 4 sepals and a corolla of white petals. The actual flowering takes place, according to the cultivars and areas, from May to the first half of June. The fruit is a globose, ellipsoidal, or ovoid drupe, sometimes asymmetrical. It consists of a "fleshy" part (pulp) that contains oil and a woody and wrinkled core. October-December is the harvest period, which depends on the crops and the use to be made: whether from oil or from the table.

Figure 19 Olive Tree **Source:** www.giardinaggio.net

According to Sofo et al. (4) the olive tree, like many other Mediterranean species adapted to semi-arid climates, and can tolerate the low availability of water in the soil through morphological, physiological and biochemical adaptations acquired in response to periods of

water shortage often lasting throughout the spring-summer period. The data coming from FAO was analyzed and it turned out that (Fig. 20) the total harvested area of olive trees in the world in the period 1994-2018. The 67% of the olive trees in the world are cultivated in Europe and the total harvested area is 5,141,214 ha (Fig. 21), mostly cultivated in Spain, Italy and Greece respectively with 2,579,001 ha, 1,147,505 ha and 963,120 ha. In Figure 22 the data showed that the production in Europe is about 13,701,251 t, mainly in Spain, Italy and Greece (9,819,569 t - 1,877,222 t -1,079,080 t).

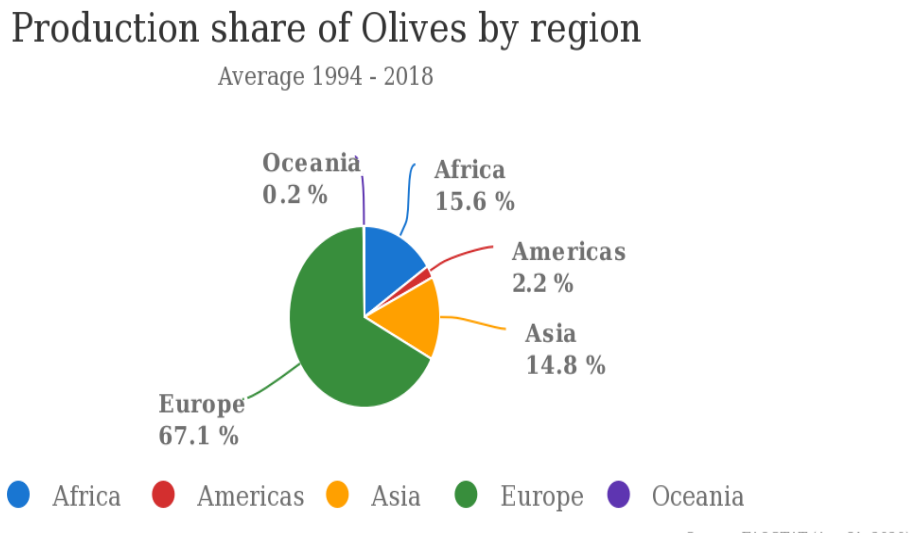

Figure 20 Average of olive production in the years 1994-2018 in the five continents. FAOSTAT, 2018

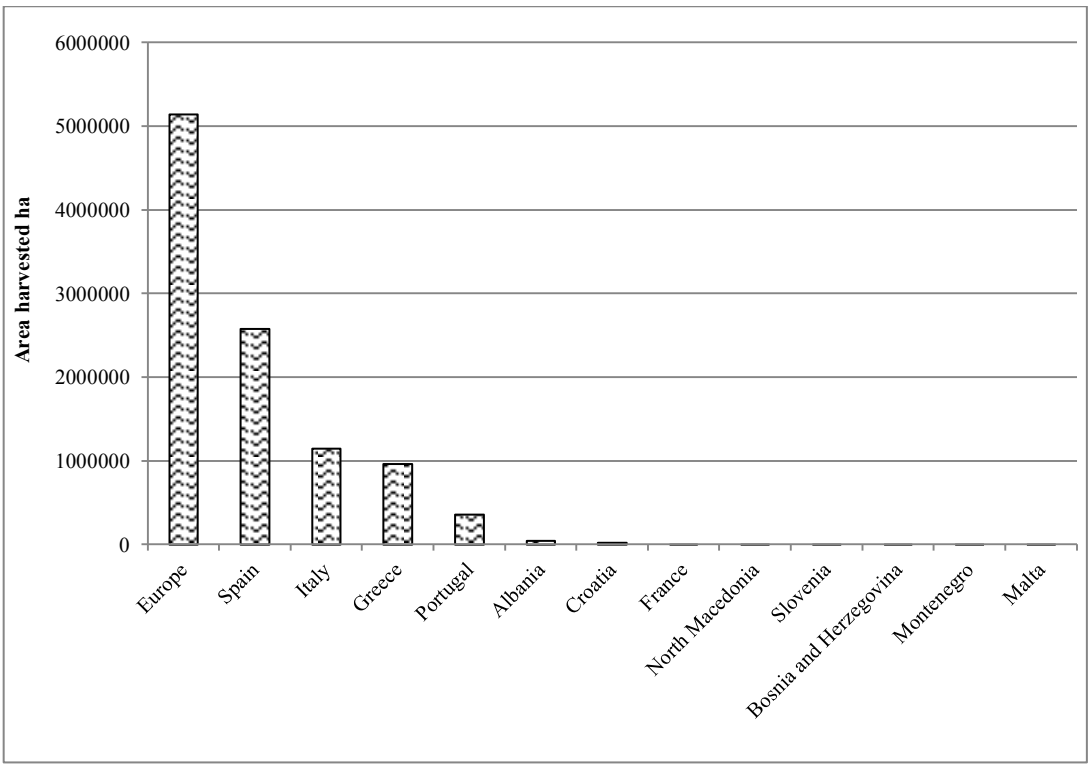

Figure 21 Olive tree area harvested (ha) in Europe and European countries. FAOSTAT, 2018

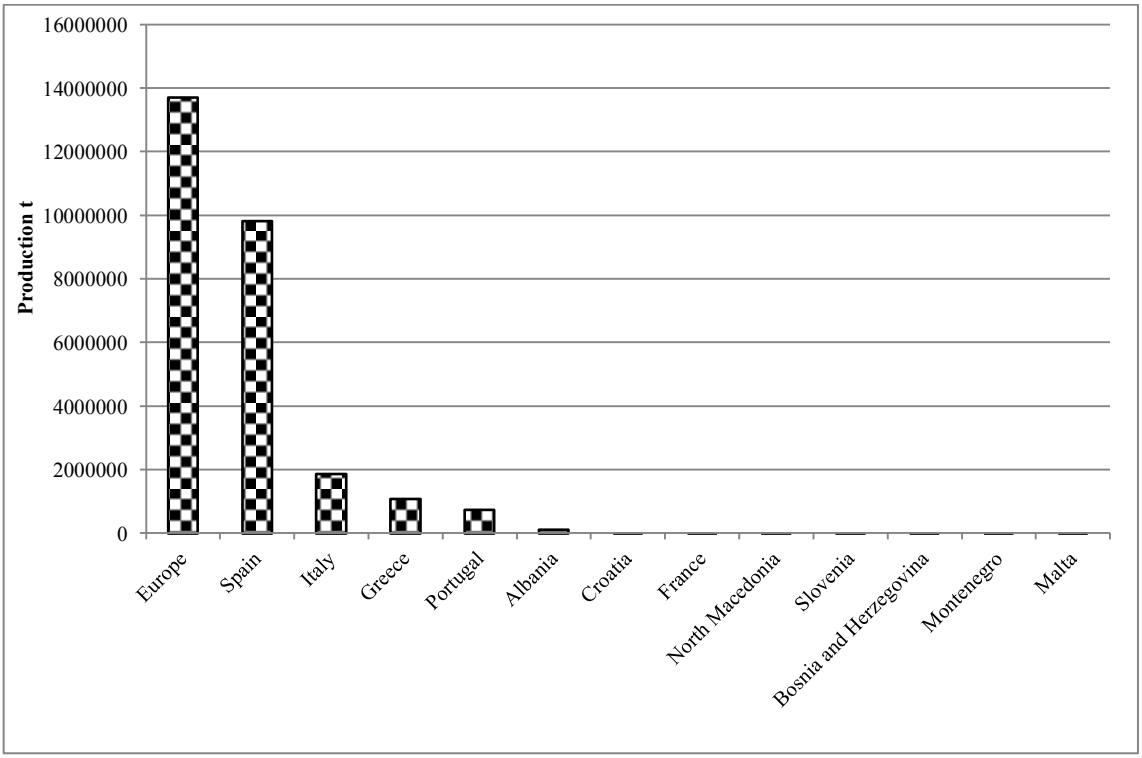

Figure 22 Olive tree production (t) in Europe and European countries. FAOSTAT, 2018

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CHAPTER 3
ANTIOXIDANT ACTIVY AND CHEMICAL CHARACTERIZATION

3.1 Materials And Methods

3.1.1 Site of study

The experimental area was located in Bernalda (Matera), southern Italy (Basilicata Region) (Lat.: 40°25'16.0"N ; Long.: 16°45'24.0"E), at 127 meters above sea level (Figure 23-Figure 24).

Figure 23 The image of Italy by Google Earth, subdivided into 21 regions. In red the Basilicata Region (1,2)

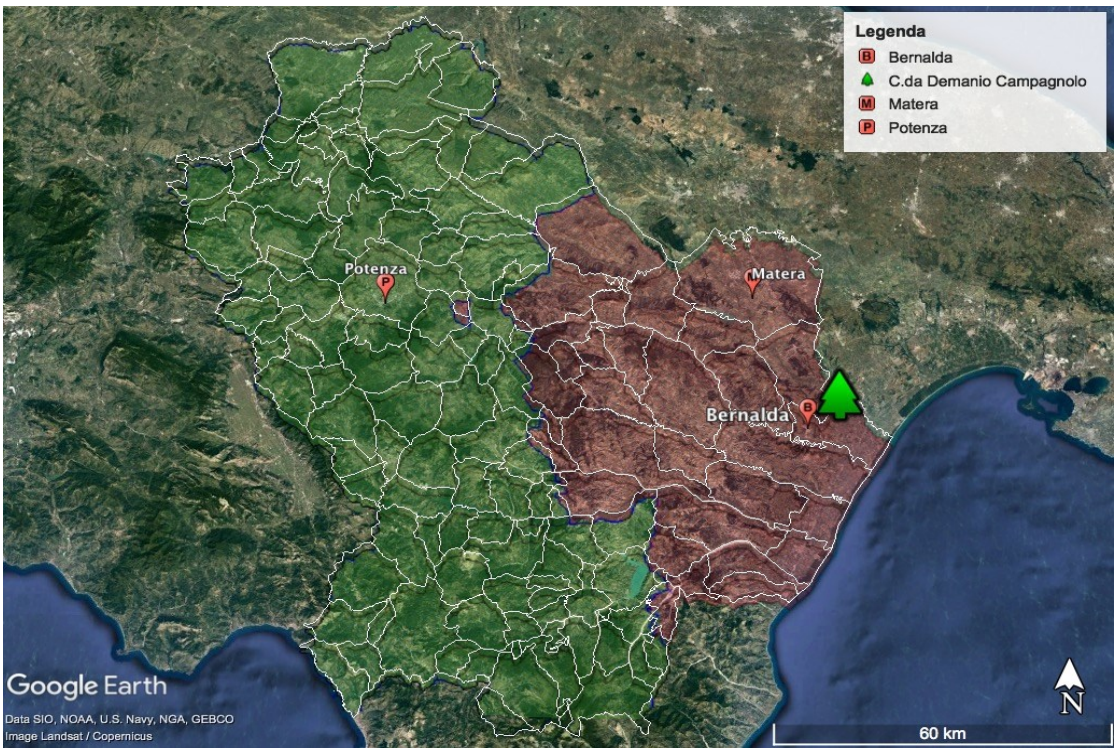

Figure 24 Image by Google Earth of the Basilicata region, subdivided in the two provinces. In green Potenza's province and in red Matera's province (1,2)

Specifically, the woody material from the pruning and removal of orange, apricot and olive trees was collected in an area called Campagnolo district. In the Figure 25-Figure 26 are indicated the Basilicata land cover. It is noted that the sampled area is mainly cereal and wine and fruit growing.

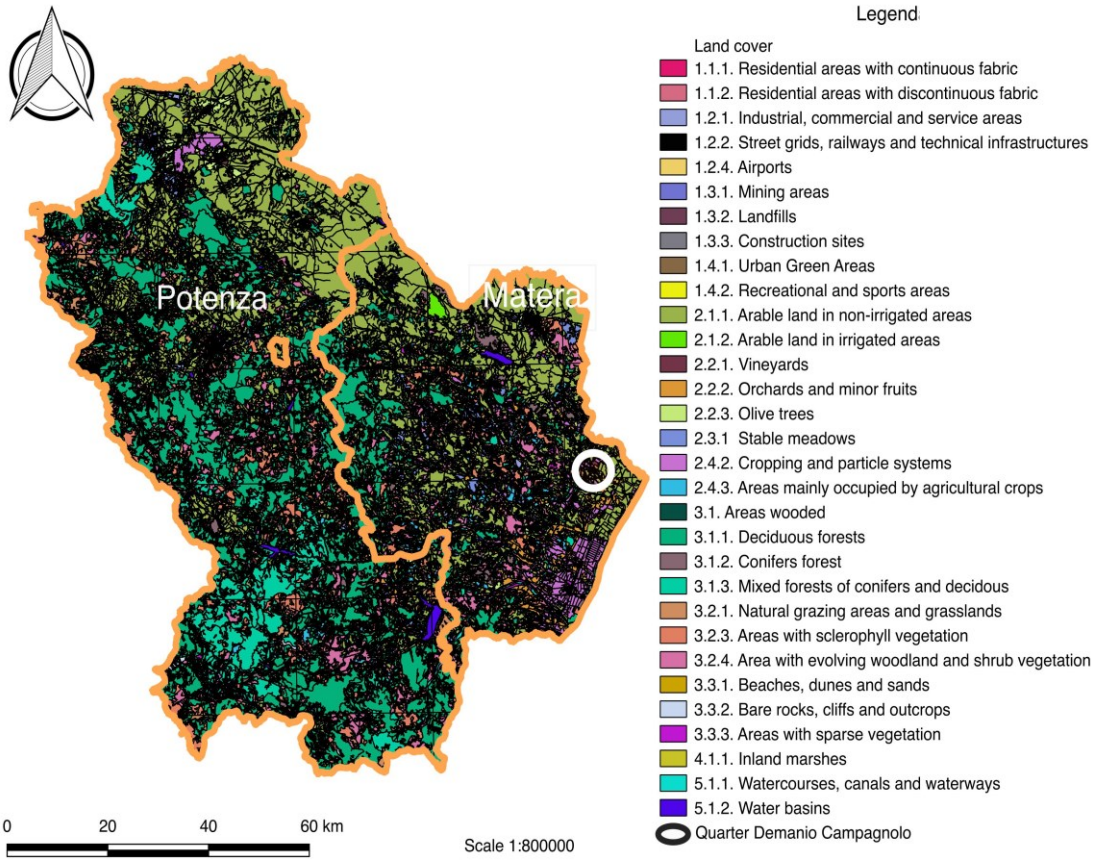

Figure 25 Basilicata Land Cover. Source: Rsdi.Regione.Basilicata.it (1)

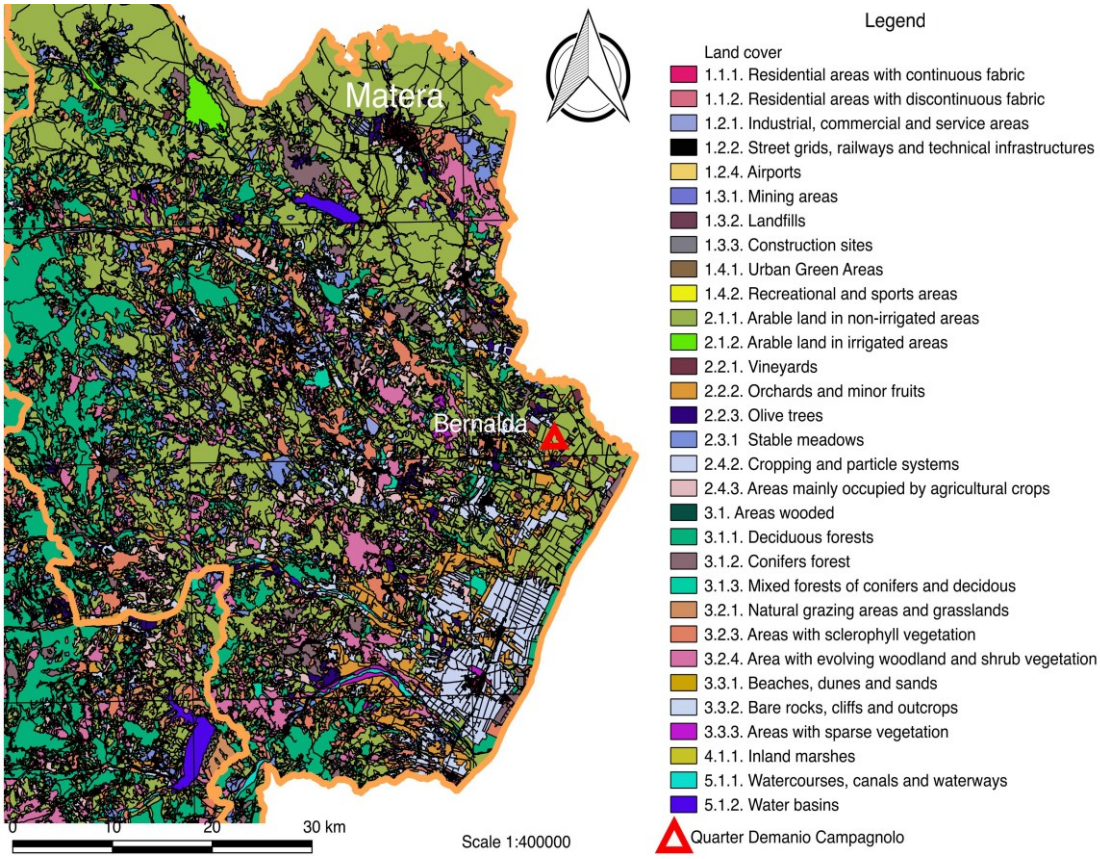

Figure 26 Particular of Basilicata Land Cover. Source: Rsdi.Regione.Basilicata.it(1)

3.1.2 Biomass Material

The woody pruning residues were taken from trees with a variable age: 12 years for the orange tree, 8 years for the apricot tree and 20 years for the olive tree. A square planting scheme was used with 5-m distance between plants. Five 1000 m^2 randomized plots were used in the experiment. In all plots, the diameter at half the height of all tree trunks below branches was measured, along with total height. Total wood volume (m^3 ha^{-1}) below the branches was calculated.

To evaluate the physical and mechanical characteristics of wood, 10 trees (two per plot) were felled. From each tree that was cut, the basal logs of short length were obtained and successively sawn into 30 mm thick boards. After sawing, the boards were conditioned in a climate room at T = 20 °C and φ = 65% to obtain a moisture content (MC) of about 12%. The boards were then cut to obtain samples without defects. Moisture content and wood density were determined according to UNI-ISO 3130 (1985) and 3131 (1985). Tangential, radial and total shrinkage were tested and calculated according to UNI-ISO 4469 (1985) and 4858 (1988). Mechanical tests consisted of the load needed for axial compression rupture (parallel to grain) according to UNI-ISO 3787 (1985).

3.1.3 Wood and bark extracts

Samples from pruning were collected from felled trees. The samples were randomly selected, separated into bark and wood, and were milled to powder through a 40-mesh sieve in a milling machine (Retsch GmbH, Germany). A drive power of 1.5 kW and rotor speed of 1500 min^{-1} guaranteed rapid size reduction. The milled wood for both sample types (bark and wood) was used in four different extraction techniques (Fig. 27a-b-c-d): maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclaving (AT).

Figure 27. a) Maceration Extraction (ME), b) Ultrasound-Assisted Extraction (UAE), c) Accelerated Solvent Extraction (ASE) and d) Autoclaving (AT).

3.1.4 Extraction techniques

As reported (3) for all extraction techniques, 10 g of small pieces of tree bark and wood were extracted using ethanol:water mixture (70:30 v/v) as a solvent. However, only water was used for AT, as reported (4)(5). Three experiments were completed for each extraction.

ME was carried out at room temperature by stirring the sample for 1 h in the solvent at a sample-to-solvent ratio of 1:5 (w/v). In comparison, UAE was carried out using an ultrasonic bath (Branson 1800, Danbury, Connecticut) under the same conditions used for ME. Extraction using an ASE system (ASE 150, Dionex Corporation, Sunnyvale, CA) was carried out at 100 °C and 1500 psi for 3 cycles of 5 min each. AT extracts of bark and wood were obtained using a Vapor Matic 770 sterilization autoclave, following the autoclave cycle:

121 °C, 1 atm, 20 min.

After extraction, all extract solutions were filtered (Fig. 28), and the solvent was removed under vacuum with a rotary evaporator at 37 °C.

Figure 28 Some samples after the extraction and filtering

After solvent removal, samples were suspended in water and freeze-dried (Heto Drywinner DW3/RV12, Edwards High Vacuum International, Crawley, UK) for 56 h, at -48 °C and 0.58 Pa (Fig. 29a-b).

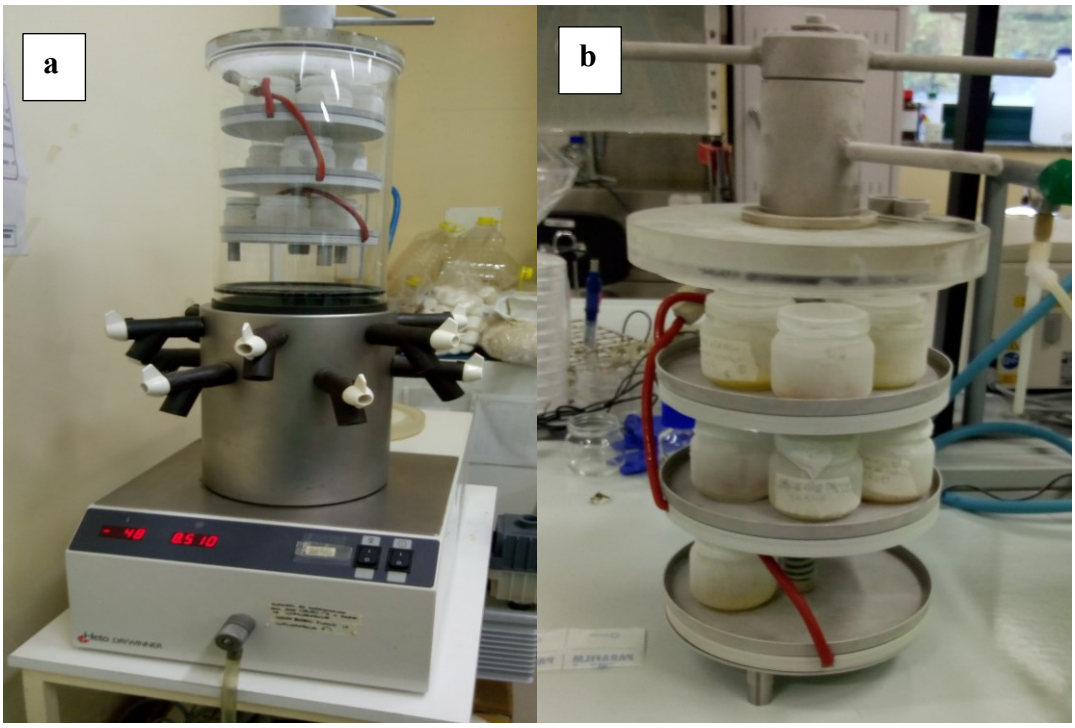

FIGURE 29a-b The samples in the freeze-dried machine

The samples were then stored in the dark at room temperature(Fig. 30). Extraction yields were calculated according to the following formula:

$$\% = \frac{dried\ extracts\ (g)}{milled\ wood\ (g)} \times 100$$

Figure 30 Samples after the freeze-drying

3.1.5 Total polyphenolic content (TPC)

The TPC was determined by the Folin–Ciocalteu reagent method (4) (5). An aliquot of extract (75 µL) was mixed with 500 µL of Folin–Ciocalteu reagent and 500 µL of Na_2CO_3 solution (10×) and, finally, water were added to reach a final volume of 1.5 mL. After incubation for 1 h in the dark at room temperature, the absorbance of the mixture was read at 723 nm. The TPC of the extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract ± standard deviation (SD).

3.1.6 Antioxidant activity

3.1.6.1 Radical scavenging activity

Radical scavenging ability was measured using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH);200 µL of 100 µM DPPH methanol solution was added to 50 µL of extract at different concentrations in 96-well plates and kept in the dark for 30 min. Absorbance at 515 nm was recorded. Lower absorbance of the reaction mixture indicates higher DPPH free radical scavenging activity(6,7). The radical scavenging activity of each

sample was expressed as milligrams of Trolox equivalents (TE) per gram of dried sample ± SD.

3.1.6.2 Ferric reducing antioxidant power (FRAP)

The FRAP method is based on the reduction of Fe^{3+} to Fe^{2+} by the action of electron-donating antioxidants. At low pH, in the presence of TPTZ, ferric–tripyridyltriazine (Fe^{3+}–TPTZ) complex is reduced to the ferrous form (Fe^{2+}–TPTZ). The reduction is monitored by measuring the change of absorbance at 593 nm. Increased absorbance of the reaction mixture indicates an increase of reduction capability(6,8,9). As reported by Todaro *et al.*,(12) FRAP reagent was prepared daily with 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride and 10 mM TPTZ (in 40 mM hydrochloric acid) at a ratio of 10 : 1 : 1. FRAP reagent (225 µL) was added to 25 µL of extract or methanol (for the blank) in a 96-well plate and incubated at 37 °C for 40 min. Trolox was used as standard. Results were expressed as milligrams of TE per gram of dried extract ± SD.

3.1.6.3 β-Carotene bleaching assay (BCB)

The inhibition of lipid peroxidation of wood extracts was assayed by the BCB method (13). Butylated hydroxytoluene (BHT) was used as a positive control of the reaction. *β*-Carotene (0.20 mg in 0.20 mL chloroform), linoleic acid (20 mg) and Tween 20 (200 mg) were transferred into a round flask. Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator, and 50 mL of distilled water was added. Extract (50 µL) at an initial concentration of2 $mgmL^{-1}$ was added to 950 µL of *β*-carotene emulsion, and then the solution (250 µL) was transferred into a 96-well plate. The absorbance was read every 30 min (0, 30, 60, 90, 120, 150 and 180 min) at 470 nm. Inhibition of lipid peroxidation was expressed as a percentage of antioxidant activity (% AA) ± SD using the following formula:

$$\%AA = \left[1 - \left(\frac{\text{A sample T0}' - \text{A sample T180}'}{\text{A blank T0}' - \text{A blank T180}'}\right)\right] \times 100$$

3.1.6.4 Determination of Relative Antioxidant Capacity Index (RACI)

According to Sun and Tanumihardjo (12) and Russo *et al.*(15) RACI is determined by integrating the antioxidant capacity values generated from different *in vitro* methods and allows a better comprehensive comparison. In RACI, the standard score was calculated using the following formula:

$$(x - \mu)/\sigma$$

where x is the raw data, μ is the mean, and σ is the SD. Standard scores have a mean of 0 and an SD equal to 1(16).

3.1.7 LC–MS analysis

U-HPLC analysis of extracts was carried out using Shimadzu LC–MS-8030 apparatus equipped with anSPDM20A diode array detector(Fig.31) (Fig.32). The separation was carried out in thermostatic conditions at 40 °C with a reversed-phase column (Phenomenex® Luna 3μm C18). Elution was carried out with a binary solvent system consisting of water with 0.1% formic acid and acetonitrile with 0.1% formic acid, running at the flow rate of 0.4 $mLmin^{-1}$. The injection volume was fixed at 1.0μL. Detection was carried out with a UV detector set at a wavelength of 280nm and under selected ion monitoring by negative- and positive-mode ESI-MS. The operating parameters for MSdetection were as follows: nebulizing gas (N_2) flow 3.0 $Lmin^{-1}$, dryinggasflow15 $Lmin^{-1}$, interface voltage 4.5 kV, gas pressure 230 kPa, DL temperature 250 °C, block heater temperature 400 °C.

Figure 31 The UHPLC-MS machine used in the analysis

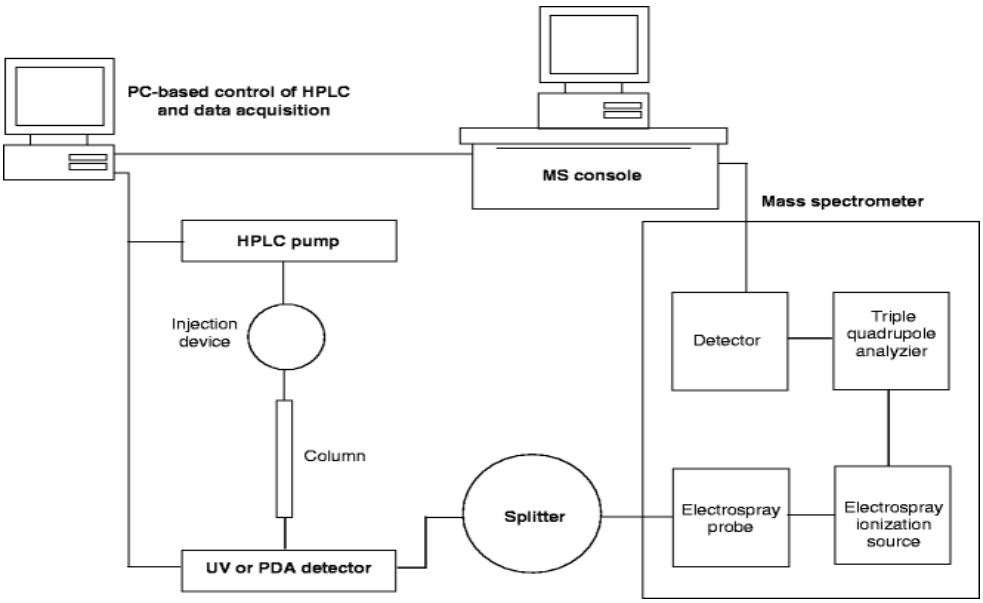

Figure 32 Schematization of a U-HPLC (17)

3.1.8 Gas chromatography-mass spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) was used for the qualitative and quantitative determination of organic compounds in bark and wood extracts of the orange tree, apricot tree and olive tree (Fig.33-Fig.34). Regarding the orange tree samples, there were problems with the results so the dataset was not analyzed. Each type of extract was injected into a gas chromatographic system consisting of a capillary column passing through a thermostat oven, crossed by a stream of helium. The various chemical species in a given sample were separated in

the column and detected by the mass spectrometer. Separation and detection of the analytes were achieved using an HP 6890 GC system equipped with an HP 5963 MS selective detector and a capillary column (HP-5MS, 30 m × 0.25 mm I.D., 0.25 µm film thickness; J&W Scientific, CA, USA). Samples were injected directly into the column at 80 °C. After injection, the temperature was held at 80 °C for 3 min, and then heated to 250 °C at a rate of 20 °C min^{-1} and held for 20 min (5). Compounds were identified based on computer matching mass spectra with the NIST11 library.

Figure 33 The GC-MS machine used in the analysis

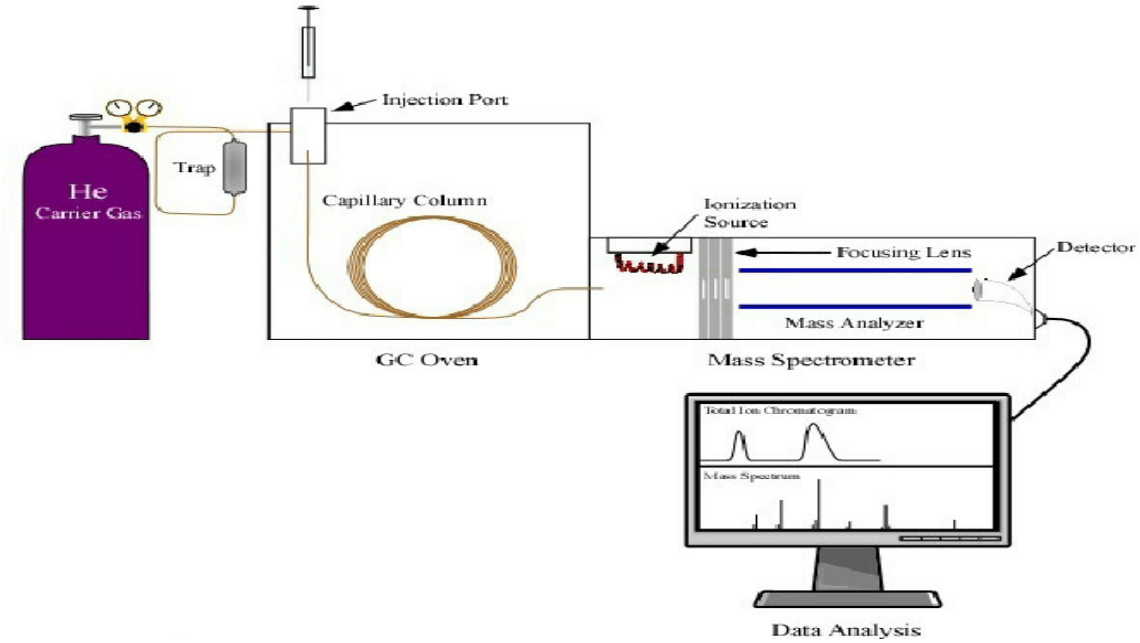

Figure 34 Schematization of a GC-MS **(18)**

3.1.9 Statistical analysis

Results are expressed as the mean ± SD of three independent experiments performed in triplicate. Principal component analysis (PCA), an unsupervised multivariate statistical tool that analyses data sets consisting of a large number of variables, was also used. It can develop a new and easier model with a smaller number of artificial variables that account for most of the variance in the normalized data set. To verify the correlations among antioxidant methods and chemical compounds, the Pearson correlation coefficient was determined. The relationship between compounds (present in at least three samples) and antioxidant activity results obtained from each test was considered. PCA and Pearson coefficients were computed using the R statistical software environment (19).

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CHAPTER 4
RESULTS AND DISCUSSION OF ANTIOXIDANT ACTIVITY AND CHEMICAL CHARACTERIZATION

4.1 ORANGE TREE

4.1.1 Wood characteristics

Fernández-Puratich et al.(1) have measured the mean of stem volume of orange trees to be 0.006 m^3/tree and the total biomass volume including canopy branches equal to 0.043 m^3/tree for orange tree. Stem mean diameter is equal to approximately 14.9 cm. This cultivation needs accurate management of trees, including pruning at least once per year. The life cycle of these orchards ranges from 16 years for intensive cultivations to more than 40 years for extensive cultivations, but when trees became unproductive the farmers pull them out. The pruning and explants produce a huge quantity of biomass. The pruning biomass of orange trees is estimated to be about 1.8 t $ha^{-1}year^{-1}$ (2). Aguado et al. (3), have declared that the main (wood) fraction of orange tree pruning can be used to obtain soda pulp of acceptable quality. The residual fraction (young stems and leaves) of orange tree pruning is suitable for cheap energy production by combustion. According to González et al. (4), the orange wood pruning contains the elements described in the table 1.

Table 1 The orange tree wood component % of dry matter

Wood component % of dry matter	
Carbon	45.45
Hydrogen	6.16
Nitrogen	0.41
Sulfur	0.01
Holocellulose	73.20
Lignin	19.95
Extractives	3.57
Ash	3.37
Volatile	78.79
Fixed compost	17.84

The gross power heating of orange trees of dry matter was calculated at 17.4 MJ(2). Underlying how it is a waste of material to use orange tree wood just to produce heat, Kongsager et al. (35), have calculated that the orange tree orchard sequestrates carbon in

measure 76 tC/ha. Recent studies about orange wood have investigated its technical characteristics for use as wood flooring (5), assessing it as a good material for manufacturing a high-quality product. In this study can be found the main physical properties of the orange wood as density 827 kg/m^3, basic density 666 kg/m^3, volumetric shrinkage 15.56%, longitudinal shrinkage 0.56%, radial shrinkage 5.20%, tangential shrinkage 10.52% and Brinell hardness about to 4.8 kg/mm^3.

Tichi et al. (6) have analyzed the dimension *C. sinensis* tree fiber in terms of length, lumen diameter, diameter, and cell wall thickness 0.76 mm, 23.64 μm, 9.23 μm, and 14.41 μm, respectively. Very few available studies on extractives from orange woody sources analyzed just some aspects such as the possible use of orange wood extractives in the medical sector especially for diabetic treatment (7), the potentialities of essential oils extracted from orange tree branches and their antibacterial activity (8), and their analysis by GC–MS technique (9).

4.1.2 Extraction yield

The extraction yield of pruning residue from *C.sinensis* bark and wood (Fig. 35) showed that extraction efficiency increased in the following order: ASE>UAE>ME>AT for wood and ASE>ME>UAE>AT for bark. Extractions performed with ASE led to the highest extraction yields (7.10% for wood and 12.50% for bark) independent of the nature of the plant matrix, whereas AT showed the lowest values, 2.80%for wood and 4.60% for bark.

As reported by Dai and Mumper (10), high temperature improves the viscosity and surface tension, enhancing the capacity of the solvent to penetrate the matrices and increasing the extraction yield, but the solvent mixture also affects the recovery of compounds. Despite the high temperature involved in the AT technique, the utilization of water as a unique solvent could explain this lower extraction yield. A previous study (11) reported that the mixture of water and ethanol is more appropriate for extracting chemical compounds from plant materials with biological activity and, according to Horvath (12), the mixture of solvents has a higher extraction capacity than the pure solvent.

All extraction techniques showed greater yield from bark than wood, in accordance with a previous study (13), showing that the bark contains more extractives than the wood as a consequence of its main biological functions to protect

the tree's essential living systems from extreme temperatures as well as from attacks from fungi, insects and animals, explaining its high extractive content.

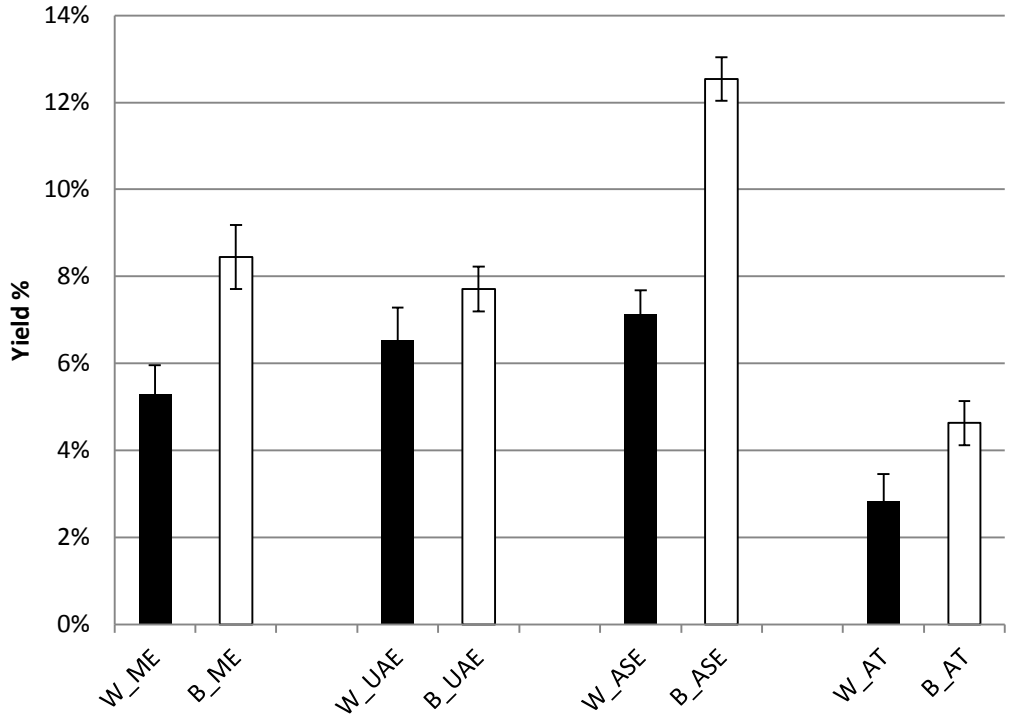

Figure 35.Extraction yield (%) fororange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

4.1.3 Total polyphenol content (TPC)

The effectiveness of different extraction techniques was evaluated in terms of TPC and antioxidant activity. As reported in Figure 36, higher TPC was found for bark extracts than for wood. TPC ranged from 79.42 ± 1.43to 57.03 ± 1.09 mgGAEg^{-1}in bark extracts and from 50.49 ± 3.45to 35.95 ± 0.41 mg GAE g^{-1}in wood. ASE was the extraction technique with the highest TPC, followed by ME, UAE and finally AT.

These results are comparable with the extraction yields, and the solvent probably affected the extraction. It has been previously demonstrated that polyphenols are more soluble in methanol and ethanol than in water, and our results are congruent with previous data (10). Moreover, the amount of phenolic compounds is influenced by the extraction time and temperature (13), but if the solubilization can be improved, degradation due to oxidation and hydrolysis can be accelerated and *vice versa* (14). However, as reported by Sulaiman *et al.*(15), the nitrogen gas in ASE can reduce the oxidation of the compounds at high temperatures.

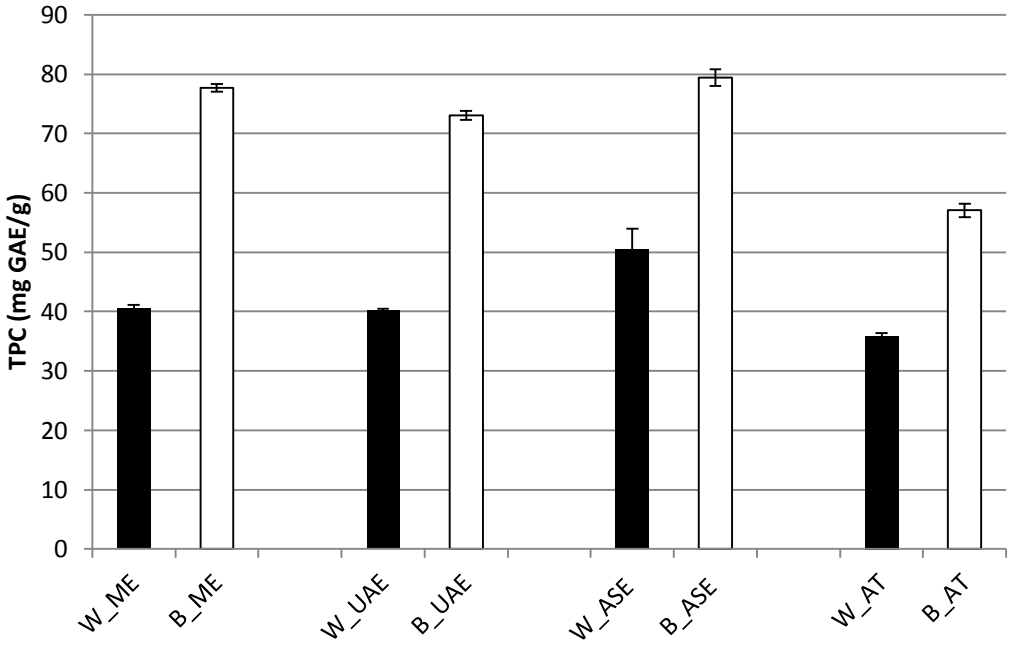

Figure 36 Total polyphenolic content (TPC) of orange tree wood (W) and bark (B). ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction;AT, autoclaving.

4.1.4 Antioxidant activity

As reported by Tuyen*et al.*,(16) polyphenol compounds possess antioxidant activity. All orange tree wood and bark extractives were analyzed for their antioxidant capacity using three different tests – DPPH, FRAP and BCB – to measure their radical scavenging activity, reducing power and lipid peroxidation inhibition, respectively.

According to TPC values, higher radical scavenging activity and reducing power were found for bark extracts than for wood. DPPH scavenging activity ranged from 60.51 ± 2.12 $mgTEg^{-1}$ (B_ASE) to 22.07 ± 1.93 $mgTEg^{-1}$ (W_ME), whereas reducing power varied from 181.88 ± 10.66 $mgTEg^{-1}$ (B_UAE) to 61.69 ± 5.62 $mgTEg^{-1}$ (W_ME). No significant differences were observed for bark extracts obtained by ASE, UAE and ME, which showed similar values (Fig.37 and Fig. 38). Wood extract derived from AT extraction had higher DPPH scavenging activity (35.35 ± 3.60 $mgTEg^{-1}$) and reducing power (87.31± 6.63 $mgTEg^{-1}$) than other wood extractives (Fig.37 and Fig. 38).

The BCB assay is a common test used for the evaluation of lipid peroxidation. It is a colorimetric method based on disappearance of the yellow colour of β-carotene due to its reaction with radicals generated by linoleic acid oxidation in an emulsion

(16). According to Da Pozzo *et al.*(17), the presence of antioxidants minimizes the oxidation of *β*-carotene.

Unlike the results for reducing power and DPPH scavenging activity, similar or higher inhibition of lipid peroxidation was observed for wood extracts than for bark extracts, for all extraction techniques except for AT.

Generally, the different behaviour of extracts in the BCB assay is due to the phenomenon called the 'polar paradox'(18–20). The polar paradox states that polar antioxidants are more effective in less polar media (bulk oil) than nonpolar ones, whereas nonpolar antioxidants are more effective in relatively more polar media (oil-in-water emulsions or liposomes) than their polar counterparts. However, the greatest inhibition of lipid peroxidation was found for bark and wood extracts obtained using the ASE technique, 70.52 ± 0.81% and 70.26 ± 1.53% AA, respectively (Fig.39), followed by W_UAE and W_ME extracts.

It is interesting to note that B_AT also showed greater inhibition of lipid peroxidation than W_AT, contrary to that observed for the other extraction techniques. Recent evidence shows that not all antioxidants behave in a manner proposed by this hypothesis in oil and emulsion, suggesting that antioxidant effectiveness depends also on several factors not exhaustively known nor controlled (21,22).

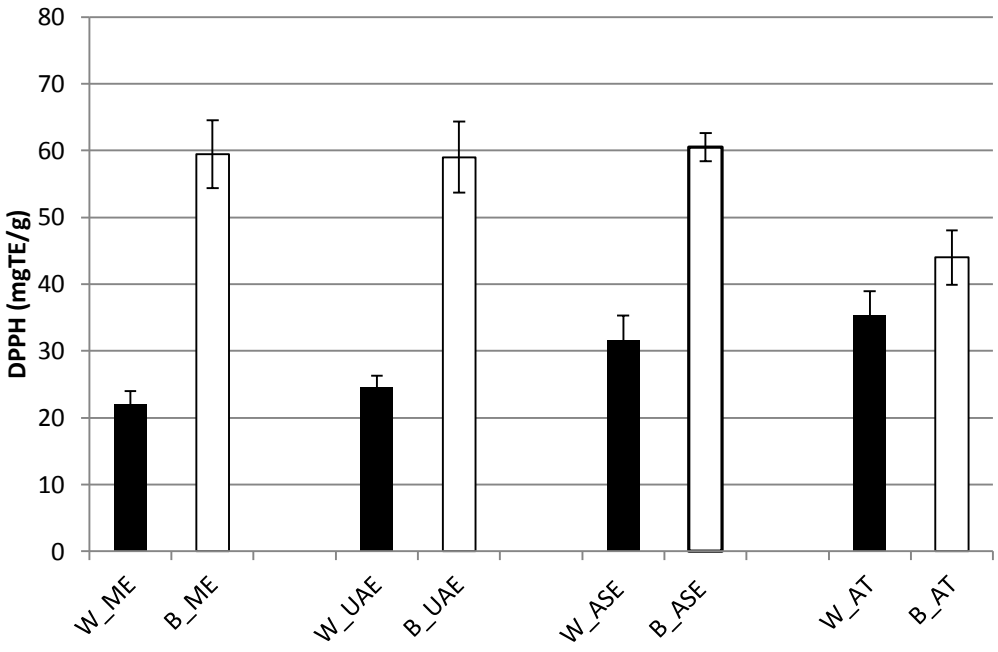

Figure 37 DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

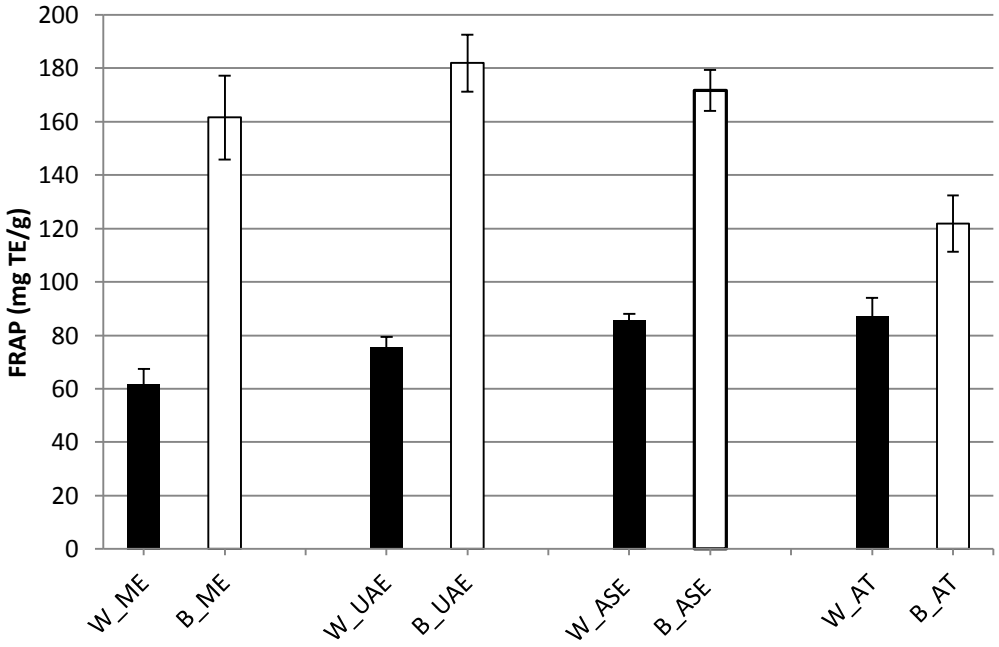

Figure 38 Ferric reducing antioxidant power (FRAP) of orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

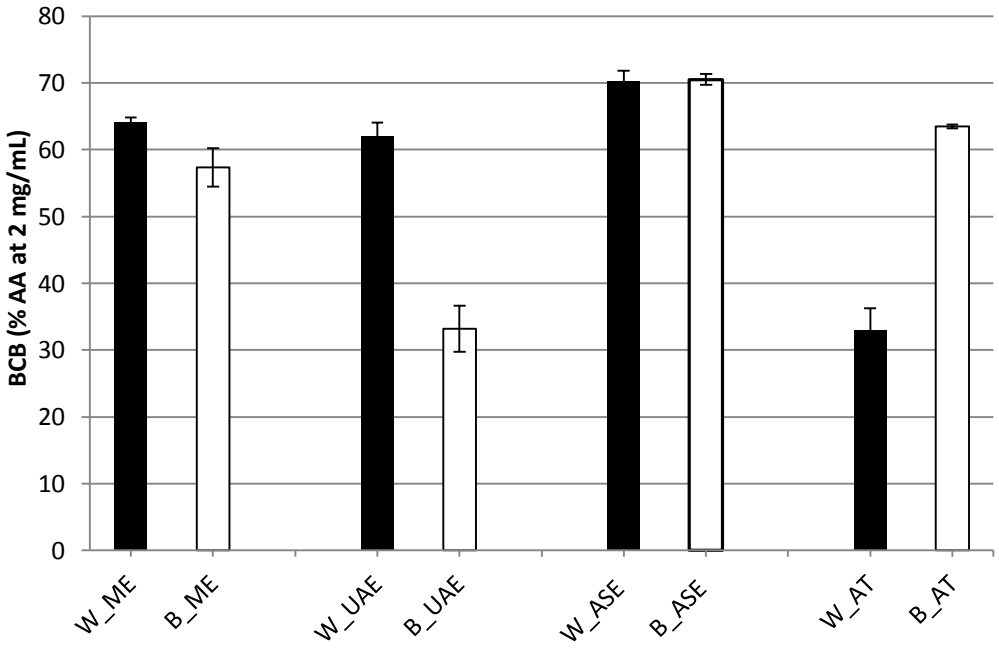

Figure 39 *β*-Carotene bleaching (BCB) assay on orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction;AT, autoclaving.

4.1.4 Relative Antioxidant Capacity Index (RACI)

The RACI is a hypothetical concept (23) to evaluate the relative antioxidant capacity of diverse extracts. In this study, results obtained from different antioxidant tests (DPPH, FRAP and BCB methods) along with TPC were used for RACI calculation. According to previous data, the RACI ranking (Fig.40) showed that bark extracts had a higher RACI than wood extracts, and the ASE technique had the highest value (1.11) followed by ME (0.80) and UAE (0.44). The lowest RACI(−0.95) was observed for W_AT.

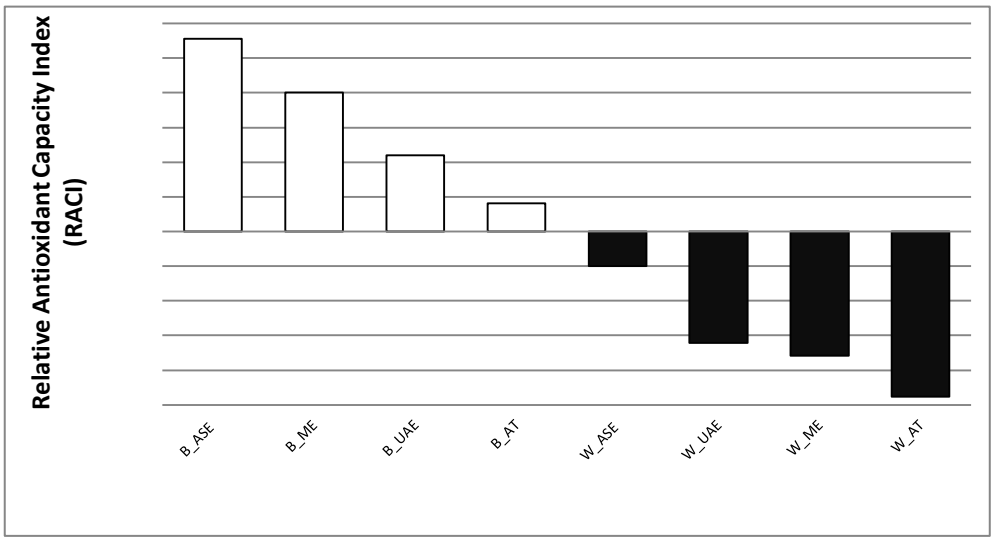

Figure 40 Relative Antioxidant Capacity Index (RACI) values obtained for orange tree wood (W) and bark (B) extractives using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

4.1.5 Statistical analysis

4.1.5.1 Pearson coefficient

To evaluate the correlation among TPC and the antioxidant assays, Pearson values were calculated among the mean of each variable (Table 2). The outcomes show that there is a positive correlation between all methods and TPC, but the strongest correlation was found to be between TPC and FRAP (r=0.95) and radical scavenging activity (r=0.94). A low correlation between TPC and BCB could be explained by BCB involving not only phenolic compounds but also lipophilic compounds, as mentioned previously.

TABLE 2 Pearson coefficient calculated between total polyphenolic content(TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power(FRAP) and inhibition of lipid peroxidation (BCB).

	TPC
DPPH	**0.940**
FRAP	**0.948**
BCB	**0.076**

4.1.5.1 Principal component analysis (PCA)

PCA was carried out on the data set after standardization of the antioxidant assays and TPC, and on the different extracts from the orange tree wood and bark. PCA (Fig.41a and Fig.41b) explained 99.23% of the data set's total variance. The first component (PC1) explained 73.38% of the total variance in the data set while PC2 explained 25.85%. Fig.41a and Fig.41b explain the relationships of antioxidant assays and TPC with the samples. In Fig.41a, the results indicate high positive antioxidant activity from B_ASE and B_ME, and negative antioxidant activity from W_AT. Furthermore, all the bark samples are located on the right while the wood samples are located on the left; this shows the significant difference between wood and bark. B_AT is situated in the middle of the PC1 zero point, quite a distance from the other samples, indicating that this sample is significantly different from the others. The location of B_ASE in the top right of PC1 can be explained by its high TPC value. In contrast, the wood samples are located in the left quadrant, demonstrating less TPC and at the same time better results in the BCB test, except for W_AT, due to the polar paradox explained in section 4.1.4. In Fig.41b, DPPH and FRAP are overlapping, closer to TPC, and on the right side of the plot, on the opposite side to BCB. This means that DPPH and FRAP are significantly correlated with TPC as the Pearson correlation has shown, whereas BCB is not correlated with the other antioxidant tests or TPC.

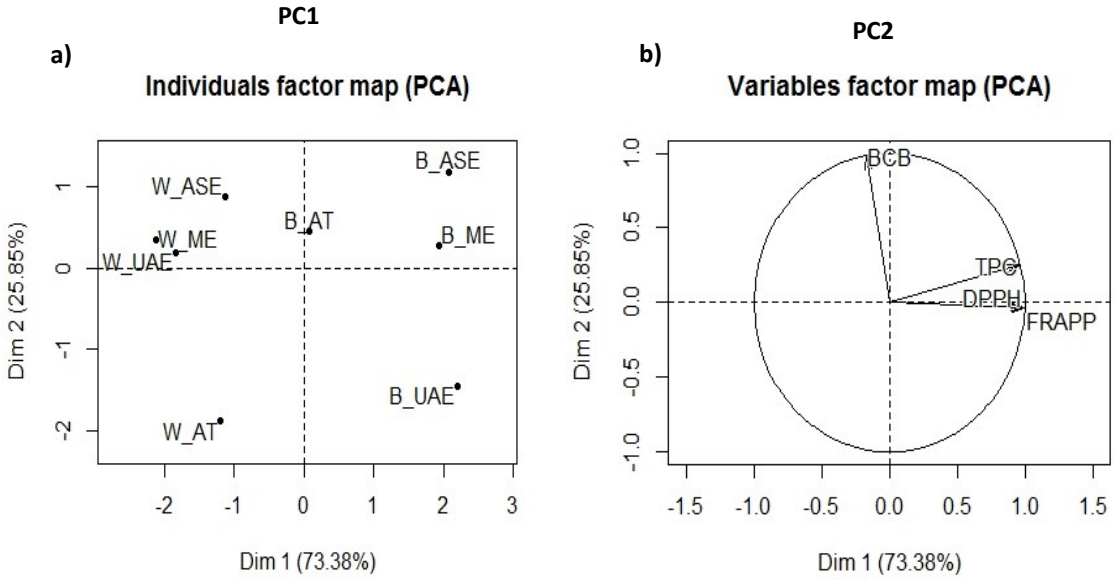

Figure 41 Principal component analysis (PCA) plots. (a) PCA scores from orange tree wood (W) and bark (B) pruning extracts using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving; (b) PCA scores for antioxidant activity (DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB)) and total polyphenolic content (TPC).

4.1.6 LC–MS analysis

In the LC–MS analysis (Table 3), caffeic acid was detected in all samples. As reported by Magnani *et al.*(24), caffeic acid is representative of cinnamic acid derivatives, also called phenylpropanoids. Caffeic acid (3,4-dihydroxycinnamic acid) is one of the hydroxycinnamate and phenylpropanoid metabolites more widely distributed in plant tissues. Hydroxycinnamic acid is the major subgroup of phenolic compounds (25). The properties of this compound have been widely investigated and it is known to act as a carcinogenic inhibitor, to possess antioxidant and antibacterial activity in vitro, and to contribute to the prevention of atherosclerosis and other cardiovascular diseases (24).

Flavonoids and alkaloids were detected, as was caffeic acid, in wood and bark samples. Flavonoids have different functions, from regulating plant development, pigmentation and UV protection, to an array of roles in defence and signalling between plants and microorganisms (26). Flavonoids also present numerous healthy effects. One of these is the antioxidant activity that prevents the risk of developing age-related vascular disease (17).These compounds have applications in food stabilization due to their ability to protect against peroxidation of oxygen-sensitive foods(27). Alkaloids were present in all bark extracts, especially B_UAE, while of the wood extracts they were present only in W_UAE. The known functions of alkaloids are related to protection and to regulation of plant growth

(28). Alkaloids are used in pharmacology as analgesics, antispasmodics and bactericidals; in particular, alkaloids have effects on the nervous system(29). Also, several potent anticancer drugs have been developed from plant alkaloids(30). Despite careful and deep bibliographic investigations, it was difficult to identify the nature of several detected peaks. Particularly, the unknown at 1.27 min which has a very important percentage area of ESI(-) TIC, it didn't exhibit any UV absorbance and, as shown by its Retention Time, seemed to be very polar, thus its structure was probably a disaccharide with M-H =341 Da (2 hexoses 2x 180 Da - 1 equivalent of water 18 Da = 342 Da). Future researches will effort to identify these unknown peaks.

As reported in the chapter 2, the importance of orchard cultivation is reflected in the number of hectares present in Basilicata Region (50,281.00). Identifying valuable alternative uses for biomass, which nowadays is mainly burned, represents for both researchers and landowners a challenge and an opportunity. As stated by Moncada and Aristizábal (31),biorefinery processes can draw benefits from the different biomass components (extractives holocellulose and lignin) and maximize the value derived from the raw material.

The advantages can be achieved mainly by implementing and developing integrated biorefinery processes of course taking into account the size of company and the relative economic possibilities. In this respect, the creation of an association of producers might facilitate the entire process system. One of the fundamental characteristics of a producer group is integration of the enterprises in a district to maximize their business efficiency and competitive ability. Through this strategy, both greater profitability and environmental sustainability can be achieved.

The scale of a biorefinery is a crucial point because its dimension is related to the final products, from a small scale in the case of added-value products (antioxidants) to a large scale in the case of bioenergy or food products (i.e. sugar) (32). In fact, biorefineries are very sensitive to the production scale(31,33,34).

Moncada and Aristizábal(31) reported several case studies and overall suggested that the decision of the scale dimension should be analysed for every biorefinery case during the preliminary design stages because several parameters should be taken into account: raw material, degree of development of the area, etc.

TABLE 3 Relative area percentage of orange wood (W) and bark (B) extractives by LC–MS in total ion chromatogram in negative mode (TIC^-).

Bark						
Ret. Time	***m/z***	**Product**	**Area (%)**			
			ME[a]	**UAE[b]**	**ASE[c]**	**AT[d]**
1.20	180	Caffeicacid	18.90	19.35	14.32	17.57
1.27	341	Unknown	23.48	21.60	18.09	18.983
3.64	264	Glycosylated compounds	1.86	1.90	2.06	0.61
4.47	594	Flavonoids	4.21	7.42	2.52	5.33
4.60	548	Glycosylated compound A	0.93	2.13	0.70	n.d.
4.71	386	Compound A without sugar	2.02	4.20	1.74	4.80
5.63	501	Alkaloid	2.91	5.04	3.30	5.33
6.30	728	Unknown	1.63	1.81	1.72	5.33
9.30	323	Alkaloid	7.04	11.43	9.16	1.36

Wood						
Ret. Time	***m/z***	**Product**	**Area (%)**			
			ME	**UAE**	**ASE**	**AT**
1.40	180	Caffeicacid	54.41	57.31	69.72	36.34
1.80	192	Glycosylated compounds	10.78	11.22	15.06	10.43
3.26	384	Lignan	n.d.[e]	1.01	n.d.	1.03
4.5-5	290	Catechin or isomer	11.56	10.31	14.35	10.45
6.44	330	Unknown	1.09	1.14	1.23	1.20
7.67	323	Alkaloid	n.d.	4.15	n.d.	n.d.

[a]Maceration extraction.

[b]Ultrasound-assisted extraction.

[c]Accelerated solvent extraction.

[d]Autoclaving (AT).

[e]Not detected.

4.1.7 Conclusions

The objective of this study was to analyse the wood and bark extractives from orange orchard tree pruning residue to find a sustainable use for this waste material, in accordance with the EU waste policy. From the data, it has emerged that the bark has higher TPC and antioxidant activity than the wood. This is confirmed for all extraction techniques (ME, UAE, ASE, AT), under the same extraction conditions. Of all samples, bark submitted to ASE has the highest TPC and antioxidant activity, probably due to the mixture of solvent (MeOH/H_2O 70:30 v/v) and temperature (100 °C). In the LC–MS analyses, important natural compounds such as caffeic acid, flavonoids and alkaloids were detected in all samples, even those with a quantitative difference. Caffeic acid is present in many food sources and several medications in popular use, mainly based on propolis(24). This confirmed the potential of caffeic acid for use in cosmetics and pharmaceuticals. While flavonoids are plant secondary metabolites that have several properties, the most important is great antioxidant activity. Alkaloids have important biological properties and practical applications in human health. The analyses and tests conducted on the pruning residue from orange orchards have demonstrated that the waste product could be used in industrial sectors such as nutraceutical, chemical, pharmacy and cosmetic sectors. The results obtained are encouraging and lead us to continue the study of these materials, to better understand the compounds present in the pruning biomass from orange orchards and their possible uses.

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4.2 APRICOT TREE

4.2.1 Wood characteristics

According to Akhmedov et al. (1) and Burg et al. (2) the content of apricot tree wood dry biomass is described in Table 4:

Table 4 The Apricot tree wood component % of dry matter

Wood component % of dry matter	
Carbon	47.2
Hydrogen	6.75
Nitrogen	0.62
Sulfur	0.21
Oxygen	42.47
Ash	3.37

Table 5 presents the main characteristics of wood material. Although the research was based on a relatively small number of trees, it provided useful information on the main characteristics of round wood.

Table 5 The main characteristics of the samples of Apricot tree wood

Parameters	No. of tests	Average value
Volume single tree (m^3)	200	0.016 ± 0.01
Volume (m^3 ha^{-1})	200	6.3 ± 0.59
Height of trunk (cm)	200	63.9 ± 2.56
Diameter of trunk (cm)	200	17.7 ± 0.83
Wood density$_{12\%}$ (g cm^{-3})	70	0.84 ± 0.07
Wood density$_{dry\ state}$ (g cm^{-3})	70	0.80 ± 0.06
Moisture content (%)	70	65.25 ± 10.62
Tangential shrinkage (%)	70	8.97 ± 1.64
Radial shrinkage (%)	70	4.02 ± 1.35
Total shrinkage (%)	70	13.61 ± 2.05
Compression strength (N mm^{-2})	30	43.32 ± 3.50

The results are comparable with those of others Rosaceae wooden materials. As expected, the volume of the base-trunk was low (due to the shape of the crown of the orchard and the young age), and was 1.47 m^3 for the tree and 6.40 m^3 ha^{-1} for the experimental area. However, in the only Basilicata Region, the total surface area of apricot cultivation is estimated at 3,765 ha (3). Thus, the availability of raw material at the end of fruit production is of potential economic interest.

Apricot wood was characterized by high density (0.84 g cm^{-1}). This characteristic was similar to that of many industrially valued hardwood trees in Europe. Apricot wood also showed high shrinkage (8.97, 4.02 and 13.61% for tangential, radial and total shrinkage, respectively). However, these disadvantages did not seem to affect the dimensional stability of the wood, due to the technical reasons reported in Berti *et al.*(4). The tangential/radial shrinkage ratio of several wood species ranges from just over 1 to nearly 3. Our study showed a T/R ratio of about 2.2. This value means that quarter sawn boards are more stable than flatsawn boards. With the first choice, the thickness of the wood board influences most of the wood movement (shrinking or swelling). In comparison, the surface (radial direction) of the wood board shows low change, which is crucial for flooring applications. Apricot is a highly decorative wood, which, due to the dense structure and fine texture, should be considered for use in designs where natural luster and reddish warm colours are sought.

Apricots must also be pruned at least twice a year, which generates about 2 q ha^{-1} biomass (5). These quantities of pruning generate energy about 3244.86 TJ (2). Large quantities of generated apricot wood residue are underused, with no suitable identified usage, particularly as trees must be replaced every 10–15 years (6). Most of the created biomass is burned in fields or used to produce heat for farm needs.

To the best of our knowledge, published information on apricot orchards is only available regarding the property of the fruits (7,8). Only a small number of studies have been conducted on the potential use of apricot tree biomass to produce heat (9) and fuel (10), despite potential interest and economic implications. Some research has been conducted on the chemical and anatomical characteristics of P. armeniaca fiber harvested in north Iran (6). The authors suggested that the fibres could be used as material for the pulp and papermaking industry. Subsequently, Tutuş, et al. (11) demonstrated that apricot tree wood is a good raw material for use in the paper industry field. There are some studies dedicate to the Gummi Armenicaea exedutate

from *A. armeniaca,* an alternative version of the Gummi Arabicae. Gum exudates are induced by infection, insect attack, mechanical and chemical injury, water stress, and some other environmental stresses (12). The polysaccharide complex fraction of Gummi Armeniaca is composed from the rest of α-L-arabionopyranose, β-L-arabionopyranose, α-D-galactopyranose, β-D-galactopyranose, α-D-glucopyranose, β-D-glucopyranose. In the gum, simple phenols were registered as catechol` hydroquinone, pyrogallol (13).

For fruit production, the length of the trunk of fruit trees is kept very short; however, the increased demand for wood resources and scarcity in wood availability make it important to verify the potential application of all underused wood material. In parallel, modern and efficient wood machine processing allows interesting material to be obtained from any piece of wood. For instance, Rosacea wood has interesting intrinsic characteristics that make it suitable for carving, veneer, mosaic, and other artisanal uses. Passialis and Grigoriou (14), showed that the dry density of wood ranged from 0.71 to 0.75 g cm-3. This species has porous rings with heterocellular rays, with mean cell dimensions of 0.61 ± 0.91 mm for fiber length, 0.25 ± 0.40 mm for vessel length and between 0.03 ± 0.05 mm for vessel width. Thus, apricot wood has shorter cell dimensions compared to peach, apple, pear and cherry. Evaluation of the wood anatomy of five Rosacea family species showed it could be potentially be used at the end of fruit production (15).

4.2.2 Yield Extracts

The extraction yield of wood and bark from apricot tree residue obtained with different extraction techniques (ME, UAE, AT, ASE) is shown in Figure 42. The extraction yield of wood (W) and bark (B) increased in the following order: ASE > UAE > ME >AT. Extraction was higher in B_ASE (13.51 ± 0.90 %) and W_ASE (9.68 ± 0.95 %), and lower in B_AT (4.67 ± 0.96 %) and W_AT (1.65 ± 0.93 %). Based on Péres *et al.*(16) ASE extraction techniques facilitate the extraction of samples by improving solvent accessibility to secondary metabolites trapped in the matrix pores. One of the most important factors influencing yield is extraction conditions, whereby more aggressive conditions (such as increased temperature) increase yield (17). In addition, independent of extraction technique, yield is higher in bark, due to more extracts being present in the bark, leaves, and roots compared to stem wood (18).

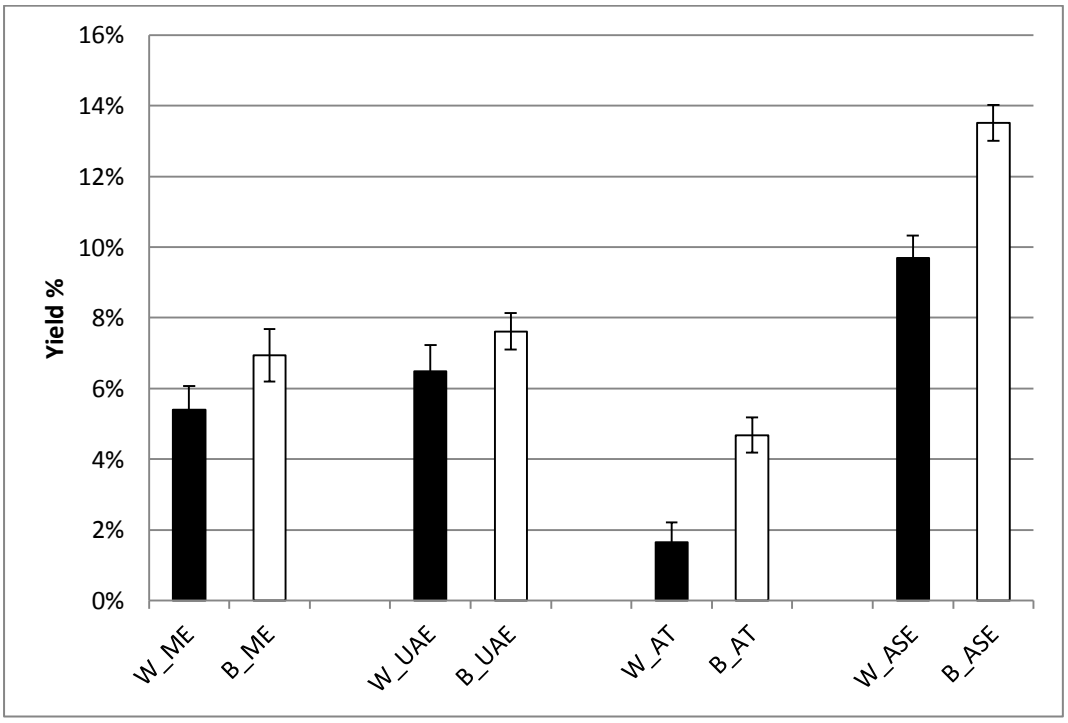

Figure 42 Yield (%) of wood (W) and bark (B) from apricot trees extracts obtained by using different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclave.

4.2.3 Total Polyphenol Content (TPC)

The highest total polyphenol content (TPC) (Fig. 43) was recorded for apricot bark under ASE (274.57 ± 14.30 mg GAE g^{-1}), and the lowest was recorded under autoclaving

(162.31 ± 10.23 mg GAE g^{-1}). Thus, pure water was not a very good solvent for phenolics (19). The highest TPC in the wood was obtained under W_ASE (188.00 ± 10.02 mg GAE g^{-1}). Thus, ASE extraction had the greatest quantity of polyphenols. The bark is considered to be rich in secondary metabolites, such as the tannins and other phenolics, which function to protect living tissues against biological attack (20) (21). In general, samples processed with W_UAE (153.29 ± 4.88 mg GAE g^{-1}) had the lowest TPC content. The TPC of the extract significantly increased with a rise in the extraction temperature, which reflected existing publications, due to an increase in phenolic solubility, diffusion rate, mass transfer, and extraction rate, as well as a reduction in solvent viscosity and surface tension (22,23).

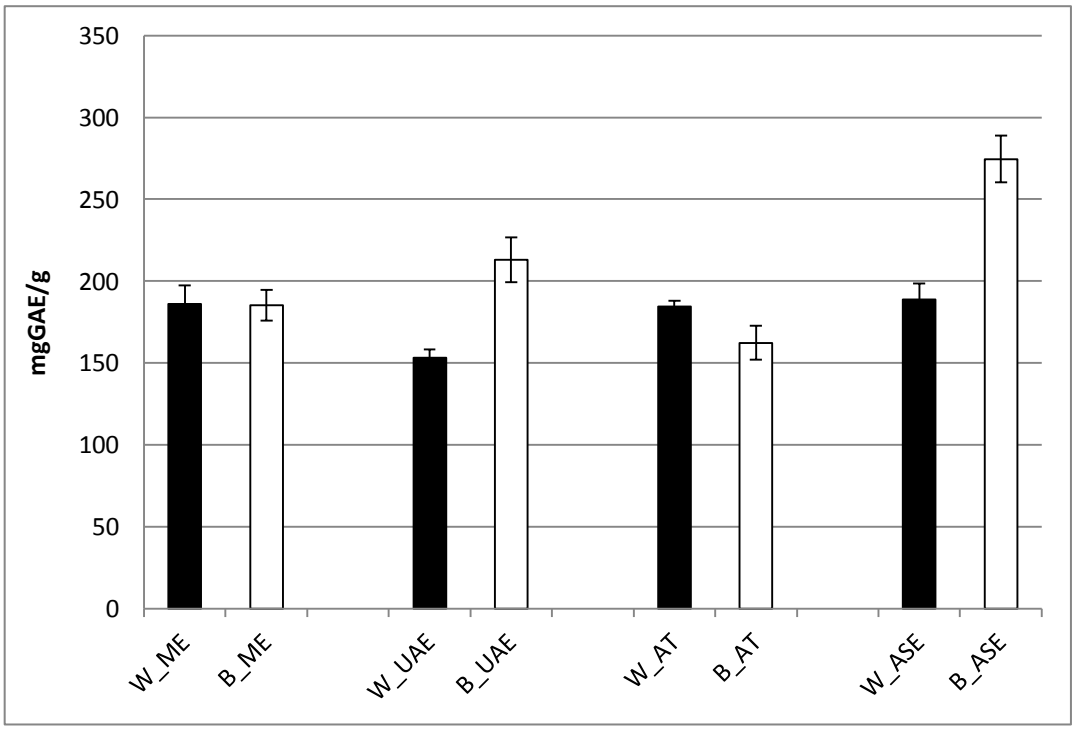

Figure 43 Total polyphenolic content (TPC) of wood (W) and bark (B) of apricot trees. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclave. Data are expressed as mean ± standard deviation of three values in mg of gallic acid equivalents per gram (mg GAE/g) of dried sample.

4.2.3 Antioxidant activity

Samples with the highest phenolic content tend to have the highest antioxidant activity (24,25). A fast, convenient, and universal method for quantifying the antioxidant efficacy of natural materials is useful. However, total antioxidant activity should be reported using more than one chemical assay. Despite this, many *in vitro* tests have been published on the antioxidant activity of natural samples (26). Thus, this study evaluated the antioxidant capacity of the extracts from the wood and bark samples of apricot trees using three tests

(DPPH, FRAP, and BCB). DPPH scavenging activity and FRAP (Fig.44 and Fig.45) were similar, with higher values being obtained for bark extracted with ME (5440.37 ± 185.56 and 1212.63 ± 114.03 mg TE g^{-1}, respectively). The lowest value was measured in wood ASE extracts when using DPPH (1597.58 ± 77.63 mg TE g^{-1}). For FRAP (Fig.45), the lowest values were obtained for AT antioxidant values of apricot wood (302.85 ± 27.70 TE g^{-1}). These results support previous TPC results. No significant differences were obtained for bark extracts obtained by ASE, UAE, and ME, which had similar values. High-temperature treatment produced extracts with higher polyphenol content and higher antioxidant activity (27).

The BCB assay (Fig.46) is frequently used to evaluate lipid peroxidation. It is a colorimetric method based on the disappearance of the yellow colour of *β*-carotene, due to its reaction with radicals generated by linoleic acid oxidation in an emulsion(28). As with DPPH and FRAP, the highest values were obtained in B_ME and W_ME in the BCB assay (67.81 ± 2.28 and 56.20 ± 2.79% AA). Of note, the lowest values were obtained for bark and wood extracted through ASE in the BCB assays (39.23 ± 5.04 and 27.45 ± 3.70% AA). This phenomenon was also reported by Bruno *et al.*(29), and was attributed to the 'polar paradox', whereby the nonpolar antioxidants mainly act in *β*-carotene emulsion, differing to the other two tests.

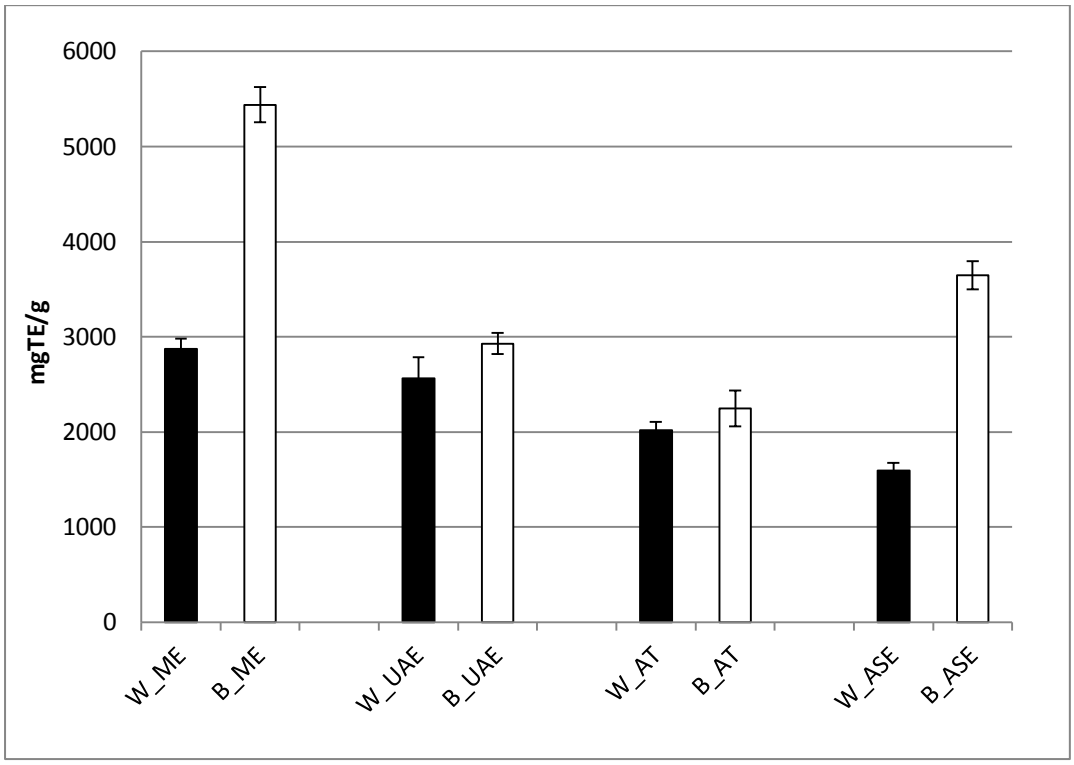

Figure 44 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of wood (W) and bark (B) from three extracts under different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclave. Data are expressed as mean ± standard deviation in milligrams of Trolox equivalent per gram (mg TE/g) of dried sample.

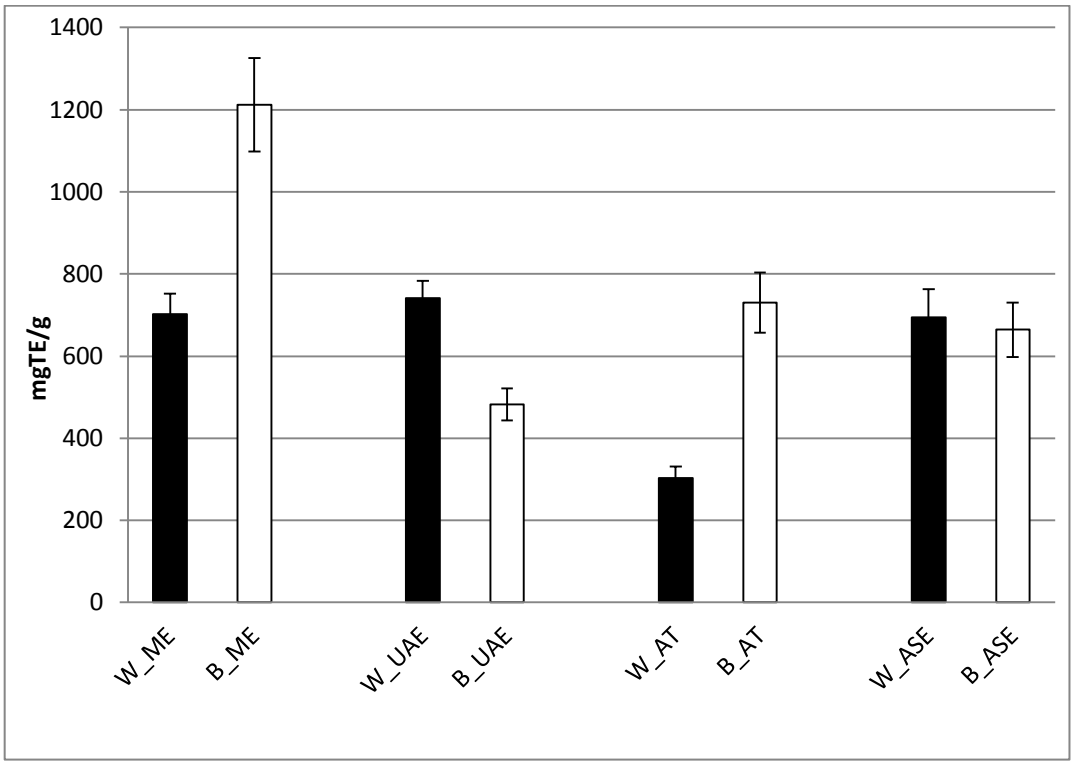

Figure 45 Ferric Reducing Antioxidant Power (FRAP) of wood (W) and bark (B) of apricot tree extracts obtained from different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclave. Data are expressed as mean ± standard deviation in milligrams of Trolox equivalent per gram (mg TE/g) of dried sample.

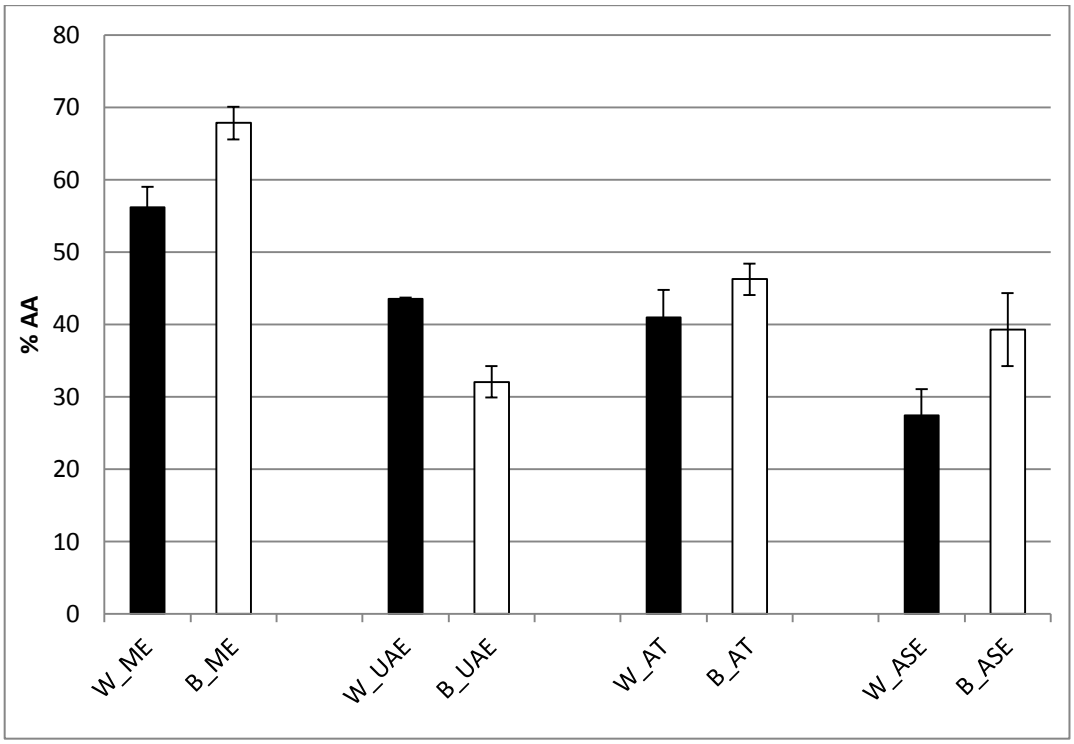

Figure 46 *β*-Carotene bleaching (BCB) assay of wood (W) and bark (B) of apricot tree extracts obtained using different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE,

accelerated solvent extraction; AT, autoclave. Data are expressed as mean ± standard deviation in percentage of antioxidant activity (% AA) at an initial sample concentration of 2 mg/mL.

4.2.4 Relative Antioxidant Capacity Index (RACI)

By integrating the antioxidant capacity (DPPH, FRAP, and BCB methods) results derived from different chemical methods, the Relative Antioxidant Capacity Index (RACI) of all tested extracts could be calculated (Fig.47), along with TPC. The highest RACI was obtained for B_ME (1.43), followed by B_ASE (0.57) and W_ASE (0.19). The lowest RACI was obtained for W_AT (-0.68). While ASE generated the highest extract yield, low RACI was obtained for the wood. Of note, all bark extracts had RACI values higher than the respective wood extracts, supporting a previous study on (29). These results could be explained by bark and wood having different phytochemical compositions. For example, Gao *et al.*(20), suggested that phenolic compounds with strong hydrogen-donating ability can reduce DPPH radicals better than other compounds. The authors also confirmed that, while inner bark and sapwood are rich in nutrients (e.g. glycosides and sucrose), the outer bark is normally deficient in these nutrients, but is very rich in secondary metabolites, which have the function of protecting living tissues against biological attack. Thus, the highest level of antioxidant activity likely occurs in the inner bark. Similar results were obtained in a study of apple trees (30).

Although polyphenol content was not high under the ME technique, both bark and wood extracts had high RACI values. Thus, the highest phenol content does not necessarily reflect the highest antioxidant capacity (31). Moreover, the high temperatures used for the extraction positively affect yield, but not antioxidant activity (32).

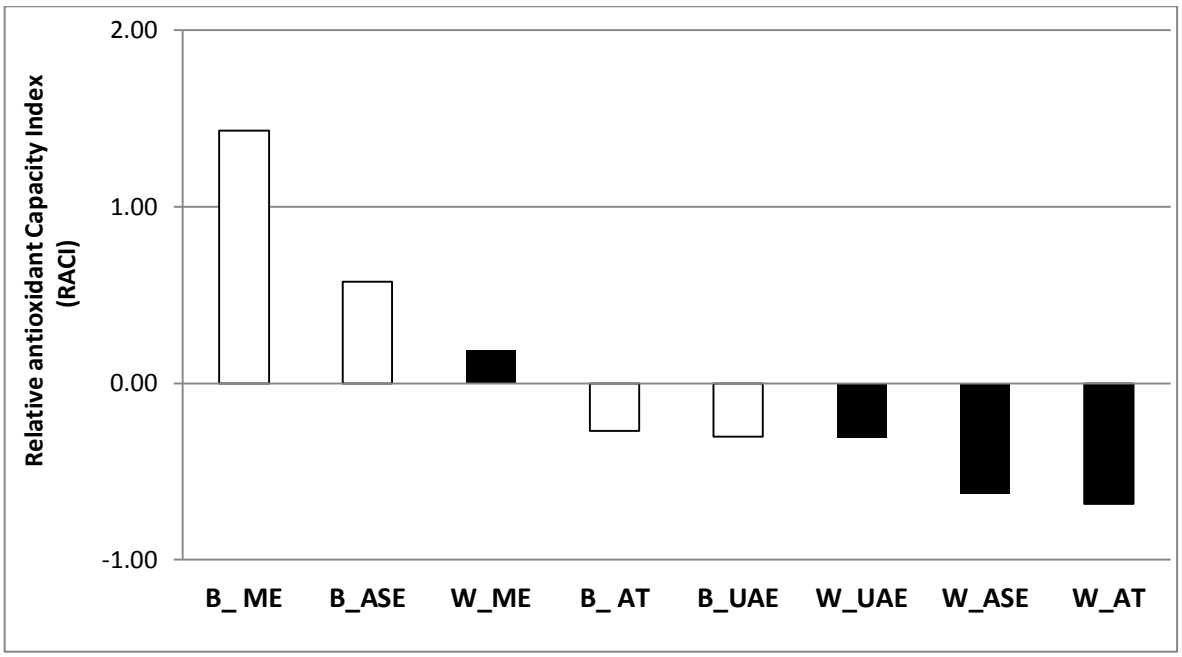

FIGURE 47 Relative Antioxidant Capacity Index (RACI) of apricot tree wood (W) and bark (B) extracted using different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

4.2.5 Statistical analysis

4.2.5.1 Pearson coefficient

TPC was weakly correlated with DPPH, FRAP and BCB (Table 6) were weak ($r = 0.28$, -0.15, and -0.25, respectively). The FRAP and BCB assay were both negatively correlated, with the assay decreasing with higher TPC. For BCB, the negative correlation was attributed to the 'polar paradox.' For FRAP, the negative correlation was attributed to phenolic compounds other than flavonoids influencing the test (23). Chew et al (33)., reported that most studies show no correlation between TPC and BCB. The two methods assayed different antioxidants. TPC indicates the total levels of both lipophilic and hydrophilic compounds. In contrast, BCB indicates only lipophilic compound levels (34).

TABLE 6 Pearson coefficient calculated for total polyphenolic content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB).

	TPC
DPPH	**0.28**
FRAP	**-0.15**
BCB	**-0.25**

4.2.5.2 Principal component analysis (PCA)

PCA was carried out on the data set after antioxidant assays and TPC were standardized for the different extracts from the apricot tree wood and bark. PCA (Fig.48a and Fig.48b) explained 90.44% of total variance in the data set. The first component (PC1) explained 61.11% of total variance in the data set, while PC2 explained 29.33%. Fig.48a and Fig.48b show how the antioxidant assay and TPC were correlated to the samples. Antioxidant activity from B_ME and W_ME (Fig.48a) reflected that reported in the previous tests. These two samples demonstrated better results in DPPH, FRAP and BCB assay, which were located in the same position, in the right quadrants (Fig.48b). The rest of the samples on the left (Fig.48a), showed no significant difference between bark and wood. B_ASE was situated in the middle of the PC1 zero point (top-right position), which was quite distant from the other samples; thus, this sample was significantly different from the others. The location of B_ASE and B_UAE in the top left of PC1 was explained by their high TPC value. In contrast, the wood samples together with B_AT (Fig.48a) were located in the bottom left quadrant, with less TPC activity. In Figure 48b, BCB and FRAP were almost overlapping, closer to DPPH, and were positioned on the right side of the plot, which was the opposite side to TPC. Thus, FRAP and BCB were weakly correlated, while TPC was not correlated with the other antioxidant tests as shown in the Pearson correlation.

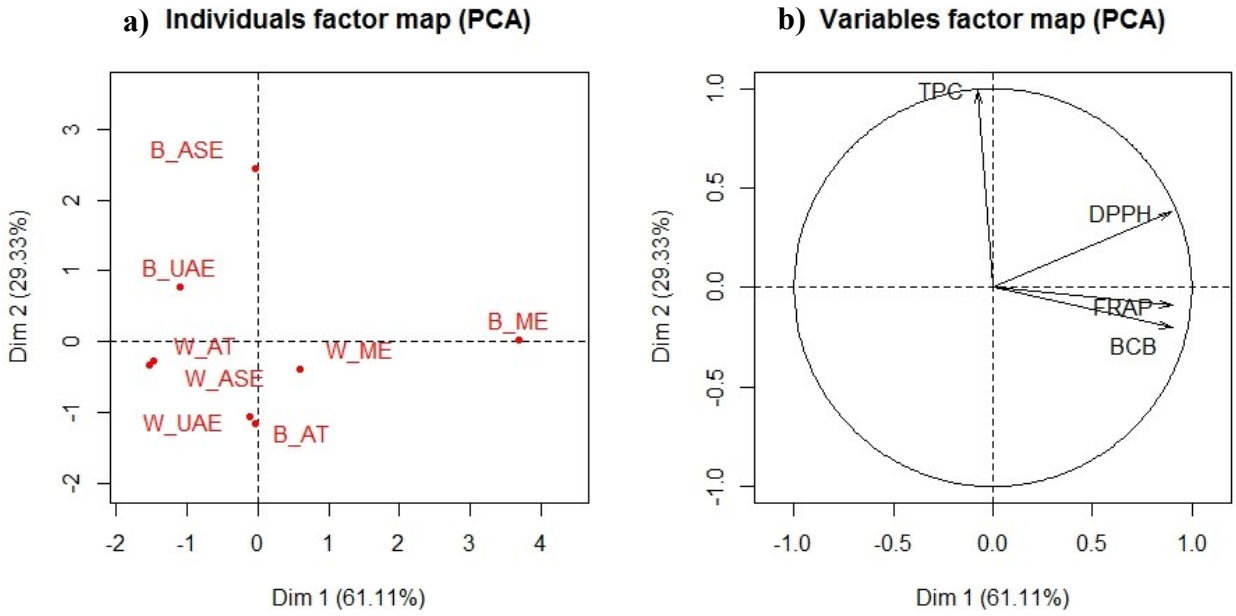

Figure 48 Principal component analysis (PCA) plots. (a) PCA scores of apricot tree wood (W) and bark (B) apricot pruning extracts using different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving; (b) PCA scores for antioxidant activity DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB) and total polyphenolic content (TPC).

4.2.6 GC-MS analysis

The GC-MS analysis (Table 7) of the extracts showed that the extracts differed depending on the method and material used. Both wood and bark produced relevant amounts of palmitic and linoleic acid. In contrast, only AT of apricot wood produced notable quantities of benzoic acid, 1,6-anhydro-*β*-d-glucopyranose, conyphenyl alcohol, and 5-*tert*-butylpyrogallol. ASE extraction of bark also produced a notable amount of glupyranose derivative. Furthermore, both autoclave and ME extraction produced notable amounts of 5-*tert*-butylpyrogallol. Wood is the main source of the most prevalent compound present in these samples, scopoletin. Scopoletin is a coumarin derivative that regulates blood pressure (35). It exhibits bacteriostatic activity against various species of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp., *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. It also exhibits anti-inflammatory activity (36,37,38). Furthermore, scopoletin regulates the hormone serotonin (39). Only the UAE of bark produced a notable amount of scopoletin. Bark contained stearic acid, whereas this compound was only extracted from wood when using ME. Bark also contained naringenin (ASE and UAE) and sitosterol. Naringenin is an important antioxidant compound that is an active chelator of metallic ions

and inhibitor of the enzyme xanthine oxidase(40,41). Surely, the presence of compounds as scopoletin and naringenin could explain the interesting antioxidant activity of investigating samples, but further LC-MS/MS analyses are needed to identify the compounds determining the activity found *in vitro* tests and other compounds with high molecular weight that could not be detected by the GC-MS technique.

Table 7 Gas chromatography-mass spectrometry (GC-MS) analysis of wood (W) and bark (B) of apricot pruning extracts using different extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving. Results are presented as percentage area.

			Area%							
Compound	**r.t. [min.]**	**Retention Index**	**W ASE**	**W AT**	**W ME**	**W UAE**	**B ASE**	**B AT**	**B ME**	**B UAE**
Ethyl acetate	3.79	613				0.29		2.26	0.52	
(*Z*)-2-heptenal	4.29	931					1.13		0.53	0.65
Glycerin	4.47	940	0.44		0.70					1.18
2,4-Dihydroxy-2,5-dimethyl-3(2*H*)-furan-3-one	4.58	977	0.68			2.10				
2-Pentylfuran	4.69	982								0.82
2-Furancarboxylic acid	5.59	999						0.30	0.14	0.07
2-Acetyl-5-methylfuran	5.89	1042		0.38						
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	6.44	1107			1.36		0.19		1.29	0.17
Benzoic acid	6.63	1131	0.72	4.07	0.87	0.75	0.15	0.38	1.02	0.52
Catechol	7.01	1160	0.95	1.85	1.02	0.73	0.31	0.50	0.97	0.76
3-Methoxyacetophenone	7.93	1277		1.53			0.64	0.65		0.46
2,6-Dimethoxyphenol	8.31	1348	1.35	1.64	0.91	1.61	0.30		0.23	
Orcinol	8.44	1369		0.92				1.69	0.82	
Vanillin	8.62	1386		1.33			0.69	1.28		0.30
1,6-Anhydro-*β*-d-Glucopyranose	9.29	1480		4.13			3.53			
2-Hydroxy-1-(1'-pyrrolidinyl)-1-buten-3-one	9.41	1523			0.23					

			Area%							
Compound	**r.t. [min.]**	**Retention Index**	**W ASE**	**W AT**	**W ME**	**W UAE**	**B ASE**	**B AT**	**B ME**	**B UAE**
5-Methoxyresorcinol	9.48	1542					1.64	3.42		
4-Hydroxy-3-methoxybenzoic acid	9.67	1566		2.23	0.78		1.02	1.46	1.49	0.87
3,4,5-trimethoxyphenol	9.89	1599		2.07	1.78	0.89	0.57			
Azelaic acid	10.03	1629							0.83	
1,3,5-Benzenetriol	10.15	1640					2.01			3.60
Tetradecanoic acid	10.69	1735	0.62			0.41				0.41
4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.75	1740	0.85	3.00	1.31	0.91	0.61			
9-Ethylphenanthrene	10.79	1780						0.36		0.32
5-*t*-Butylpyrogallol	11.00	1810		4.13				8.72	7.16	
4-Hydroxy-3,5-dimethylbenzoic acid	11.12	1830	0.98	1.11	1.60		1.01	1.78		
d-Mannitol	11.67	1910				0.95	0.87			0.60
Hexadecanoic acid	11.74	1942	1.45	2.25	2.16	1.83	6.39	1.23	2.49	7.38
d-*L*-Glucitol	11.97	1950				3.11			3.79	
Scopoletin	12.06	1974	6.75	9.87	3.97	5.71				9.99
5,7-Dihydroxy-2-methyl-4*H*-1-benzopyran-4-one	12.17	1990								10.43
Heptadecanoic acid	12.30	2022					1.38			1.13
(*Z*,*Z*)-9,12-Octadecadienoic acid	12.79	2130	4.22	0.45	9.27	8.71	9.72	0.89	5.71	0.53
Octadecanoic acid	12.95	2170			3.13		2.17	0.69	1.97	2.11
Nonadecanoid acid	13.70	2236								0.18
(Z,Z,Z)-9,12,15-Octadecatrienoic acid	13.94	2240					0.34			
(*Z*)-9-Octadecenamide	14.74	2375	0.13	0.27			0.89			0.19
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	16.50	2498	1.63							
Glycerol 1-palmitate	16.52	2510						1.71	1.85	
Docosanoic acid	17.24	2567					0.26			0.43

			Area%							
Compound	**r.t. [min.]**	**Retention Index**	**W ASE**	**W AT**	**W ME**	**W UAE**	**B ASE**	**B AT**	**B ME**	**B UAE**
(*Z*)-9,17-octadecadienal	19.79	2610			0.20		0.09			0.10
2,3-Dihydroxypropyl octadecanoate	20.40	2681	0.38	0.81	0.62	0.14			0.38	
Tetracosanoic acid	21.63	2760								0.15
Narigenin	24.73	3020					3.57	0.82	0.35	4.42
Sitosterol	29.92	3203						7.51	0.37	0.49

4.2.7 Conclusions

This study demonstrated that raw apricot wood material could be used for high added value industrial purposes. Apricot tree wood is considered a renewable raw material, despite its limited availability for manufacturing high-quality products, such as crafts, floor elements, toys, children's furniture, picture frames, boxes, carved wood, sculpture, and kitchen products. The extracts of apricot pruning residues exhibited *in vitro* antioxidant capacity, due to the presence of different chemical compounds that have potential health benefits. Extracts from wood and bark were rich in complex natural products. RACI index highlights how the bark samples extracted using ME and ASE showed the best antioxidant capacity. Moreover, the identification in B_ME and in AT extracts of 5-tert-butylpyrogallol, known antifungal compound, could be interesting for their future investigation as antifungal agents (42). Based on the data obtained in the present work, the most suitable extraction methods for bark and wood of apricot, both in terms of extraction yield but especially for the antioxidant activity shown, are ASE and ME. Mechanical, antioxidant, and chemical analysis of pruning residues demonstrated the potential use of this waste material by industrial sectors. This suggestion is in line with an existing EU policy encouraging the reuse of waste materials. Future studies will be focused on a complete phenolic elucidation and the analysis of the practical application of apricot tree extracts in industrial, agricultural, cosmetic and nutraceutical products with a view towards a circular bioeconomy.

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4.3 OLIVE TREE

4.3.1 Wood characteristics

The management of the olive tree involves annual or at least biennial pruning of the crown. According to Velázquez-Martí et al. (1), there are no differences in biennial pruning, reaching average residual biomass of 33 kg tree^{-1}. This means that in Mediterranean areas the residual biomass from olive pruning reaches an average of 1.31 t ha^{-1} in annual pruning and 3.02 t ha^{-1} in biennial pruning. Fernández-Puratich et al. (2) demonstrate that the mean stem volume for olive trees to be 0.027 m^3/tree, the total biomass volume including canopy branches is 0.066 m^3/tree for the olive tree, mean stem diameter 26.9 cm. Di Blasi et al. (3), have analyzed the olive wood component (Table 8)

Table 8 The Olive tree wood component % of dry matter

Wood component % of dry matter	
Ash	0.6
Extractives	7.5
Lignin	21
Holocellulose	71
Cellulose	34.4
Xylan	16
Arabin	1.9
Acetyl group	1.8
Galactoglucomannan	1.0

According to Negro et al. (4) in a lot of olive tree pruning (OTP) includes leaves (around 25% by weight), thin branches (around 50% by weight), and thick branches or wood (25% by weight). Proietti et al. (5), have calculated the carbon footprint in the olive tree wood for 11 years. In the Figure 49 are reported the resulted of this study that showed as the annual average GWP100 value for the first 11 years of an olive grove was 1.507 tCO_2-eq/ha per year, so the olive cultivation could be eligible for future investments related to the carbon credit market.

Permanent tree component	Dry weight (kg)	C (kg)	$CO_{2\text{-eq}}$ (kg)
Roots	8.23	4.11	15.22
Root collar	12.02	6.01	22.26
Trunk	13.94	6.97	25.81
Branches	16.32	8.16	30.22
Twigs	10.00	5.00	18.51
Leaves	6.72	3.36	12.44
Total	67.23	33.61	124.48

Figure 49 The annual average GWP100 value for the first 11 years of an olive grove (5)

There are several studies about the use of these types of pruning especially to produce heat and energy (6, 7, 8), or to produce pulp and paper (9, 10, 11). In the Negro et al. (4), research is been made on the fractionation of olive tree pruning followed by enzymatic saccharification. At the best conditions, an overall yield of 21.01 g glucose/100 g olive tree pruning and 9.54 g xylose/100 g olive tree pruning was obtained. Therefore, as an alternative, olive tree biomass has been proposed as feedstock for ethanol and the production of other chemicals. Toledano et al. (12), have estimated the cost of olive tree pruning collection around 45 $ ton-^{1}. Susmozas et al. (13), through the economic analysis, have calculated the potential of OTP. The amount of products expected for a 120 t day^{-1} of OTP plant (10% moisture content) is 13.1 m^{3} of ethanol per day of the main product, as well as 5.2 and 3.3 t per day of antioxidants and xylitol. These values correspond to a total yield of 108.8 l of ethanol, 42.9 kg of antioxidants and 27.2 kg of xylitol per ton of OTP. The production costs of ethanol, xylitol and antioxidants were also calculated using an economic allocation approach to distribute the operating cost: 0.09 (ethanol), 0.14 (xylitol) and 0.77 (antioxidants), and this leads to estimated production costs of 0.24, 1.48 and 5.12 € kg^{-1}, respectively. Accordingly, ethanol, xylitol and antioxidants have positive profit margins within an enzyme cost range of 0.1–4 € kg^{-1}. Some studies have deepen investigated the radical scavenging of olive tree wood residues and the molecular compounds present at their inside (14,15,16) and the antifungal activity (17). The analyses on the molecular compounds showed that the olive wood are present several secondary metabolites as i.e. oleuropein, ligustroside and other molecular derived by these. These compounds have different properties including antioxidant, anti-inflammatory, anti-atherogenic, anti-cancer activities, antimicrobial activity, antiviral activity, hypolipidemic and hypoglycemic effect (18).

4.3.2 Yield Extracts

In the Figure 50 was reported the extraction yield obtained through the four different selected extraction techniques. The extraction yield for the wood (W) showed that ASE and UAE had the same yields, following by ME and the last is AT. The extraction yield for the bark (B) increase into the following order: ASE > UAE > AT > ME. The higher extraction yield was relevant in the ASE with B (21%) and W (9%). Instead, the lower yield for the wood was in AT (3%) and for the bark was in ME (8%). In the literature, there were many studies about the higher quantity of the extractives in the bark than in the wood (19,20,21,22) and it is explained because the bark is most subjected to biotic and abiotic stresses affecting plants and to defend itself from them, it produces a high amount of secondary metabolites.

According to Vázquez et al.(23), the extraction yield was increased significantly when using organic solvents together compared to extractions with the organic solvents alone. Moreover, the higher extraction yield in the ASE techniques was due to the higher solubility of analytes insolvent and higher diffusion rate as a result of higher temperature (24).

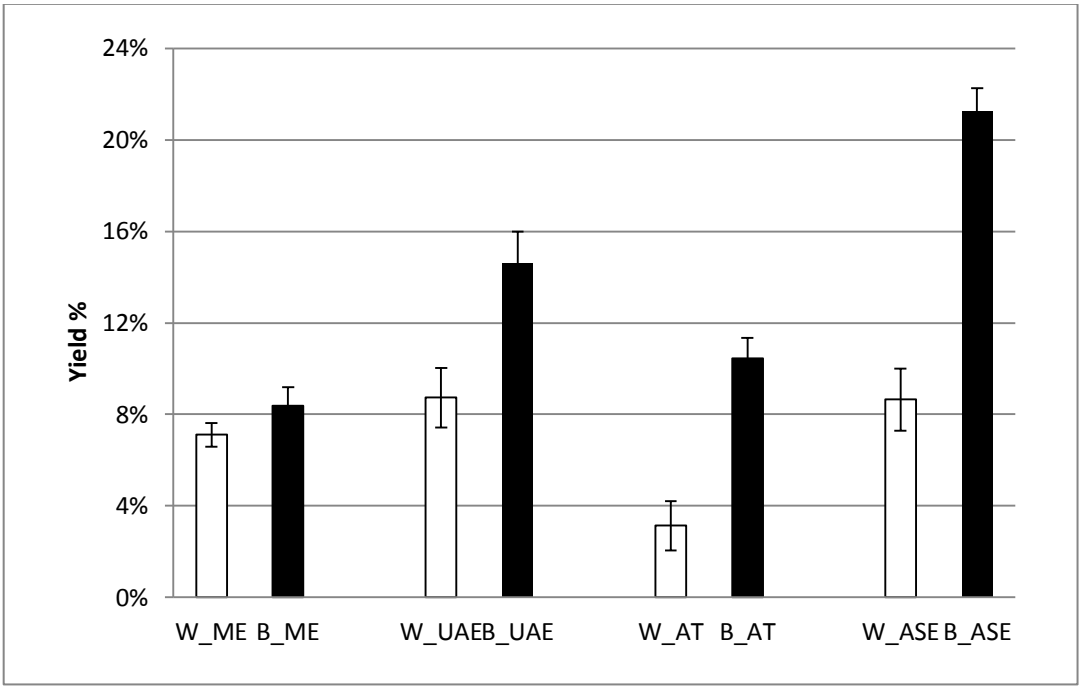

Figure 50 Yield (%) of wood (W) and bark (B) of olive trees extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT).

4.3.3 Total Polyphenolic Content (TPC)

The total polyphenolic content of the olive tree pruning samples was shown in Figure 51. The highest value of TPC was measured in the W_UAE (156.04 ± 4.42 mg GAE g^{-1}) following by B_ASE (144.63 ± 1.76 mg GAE g^{-1}). and B_AT (143.00 ± 1.66 mg GAE g^{-1}). According to Bouras et al.(25), the ultrasound extraction assistant through the internal superheating leads to cell disruption which facilitates leaching out the antioxidant compounds from the solid matrix into the surrounding solvent. The bark samples extract through the autoclaving indicated as hydrothermal processing, caused a disruption of the cell membranes, cell walls and made phenolic compounds more available by hydrolyzing the cell wall components (26). The bark is rich in secondary metabolites such as the tannins and other phenolics that function to protect the living tissues against biological attack (27). Whereas the lowest quantity of TPC was relevated in the W_AT (101.45 ± 2.85 mg GAE g^{-1}) and W_ASE (101.60 ± 2.90 mg GAE g^{-1}). According to Gao et al.(27), generally, the inner bark and sapwood are rich in nutrients such as sucrose and glycosides, not in phenolic compounds.

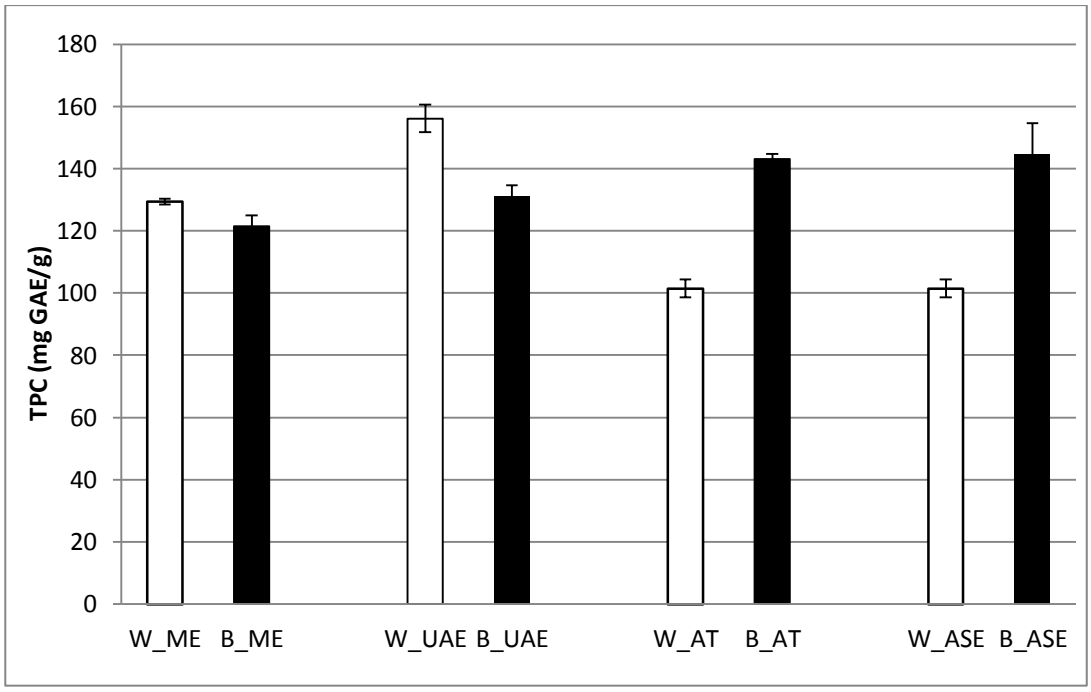

Figure 51 Total polyphenolic content (TPC) of wood (W) and bark (B) of olive trees. Where: maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT). Data are expressed as means ± standard deviation from three experiments in mg Gallic Acid Equivalents per gram of dried sample (mg GAE/g).

4.3.4 Antioxidant activity

The antioxidant capacity of samples was analysed utilizing three different *in vitro* assays as DPPH, FRAP and BCB.

The DPPH test (Fig. 52) showed as the higher value was present in W_ME sample (188.84 ± 20.6 mg TE g^{-1}) followed by W_UAE (164.17 ± 13.8 mg TE g^{-1}); while the lowest value was determined in the W_AT (26.40 ± 0.64 mg TE g^{-1}). This latter result is in accordance with the value obtained in TPC.

The data from FRAP (Fig. 53) showed the higher values in the W_UAE (408.80 ± 38.23 mg TE g^{-1}) and B_ASE (400.31 ± 26.0 mg TE g^{-1}) as in the TPC data; also in these case; the lowest data were obtained in the W_AT (74.87 ± 6.0 mg TE g^{-1}). According to Roby et al. (28), extracts with higher polyphenol content showed also higher antioxidant capacity. It can be concluded that the extracts obtained using higher polarity solvents were more effective radical-scavengers than were those obtained using less polar solvents. In the olive pruning samples, the data from TPC not always were in accordance with the DPPH and FRAP. Ku et al. (29) about this, declared that many studies have failed to find a significant correlation between total phenolic content and antioxidant activity of plant extracts, due to the sensitivity of Folin-Ciocalteu reagent against a wide range of substrates, which are easily oxidized; instead, the DPPH free radical exhibits different sensitivity to various antioxidants depending on their kinetic reactions with the DPPH free radical.

The β-carotene in BCB assay (Fig. 54) is an indicator that degrades during the oxidation process when linoleic acid turns to hydroperoxides during the high incubation temperature (30). In the BCB assay, the highest value is registered in the B_UAE and W_AT (62.74 ± 5.56 and 62.53 ± 1.00 % AA). The lowest BCB assay was in the samples bark, extracted through ASE and AT (17.39± 1.03 and 33.21 ± 2.62 % AA). Apparently, these data are opposite respect the DPPH and FRAP data, in particular regarding the W_AT. Diouf et al. (31) explained this behavior of BCB in contrast with the other antioxidant assays because the extraction will not only solubilise the phenolics, but also the sugars and some mineral constituents which are likely to also contain the transition metal cations. Transition metals enhance the rate of oxidation of edible oils by increasing the rate of generation of free radicals from fatty acids or hydroperoxides. Another explanation about the difference of the data obtained from BCB concerning the other antioxidant essays was the “polar paradox”. The ‘‘polar paradox” postulates that hydrophilic antioxidants are more effective than lipophilic

antioxidants in bulk oil, whereas lipophilic antioxidants show greater activity in emulsions (32).

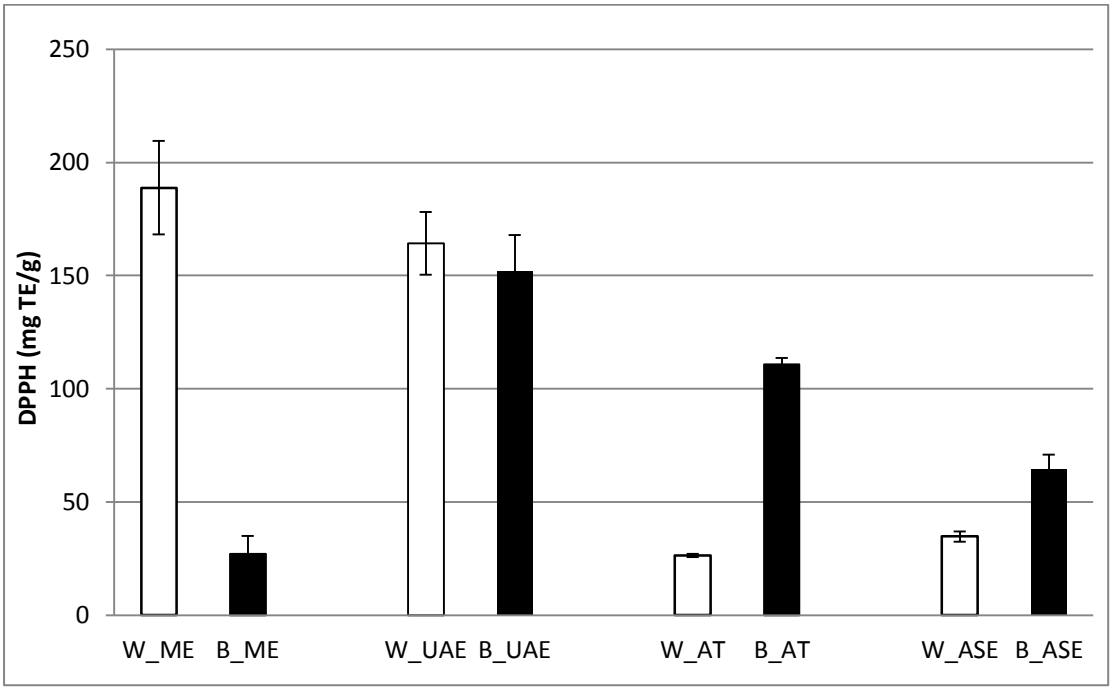

Figure 52 DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of wood (W) and bark (B) of olive tree extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT). Data are expressed as means ± standard deviation from three experiments in mg Trolox Equivalents per gram of dried sample (mg TE/g).

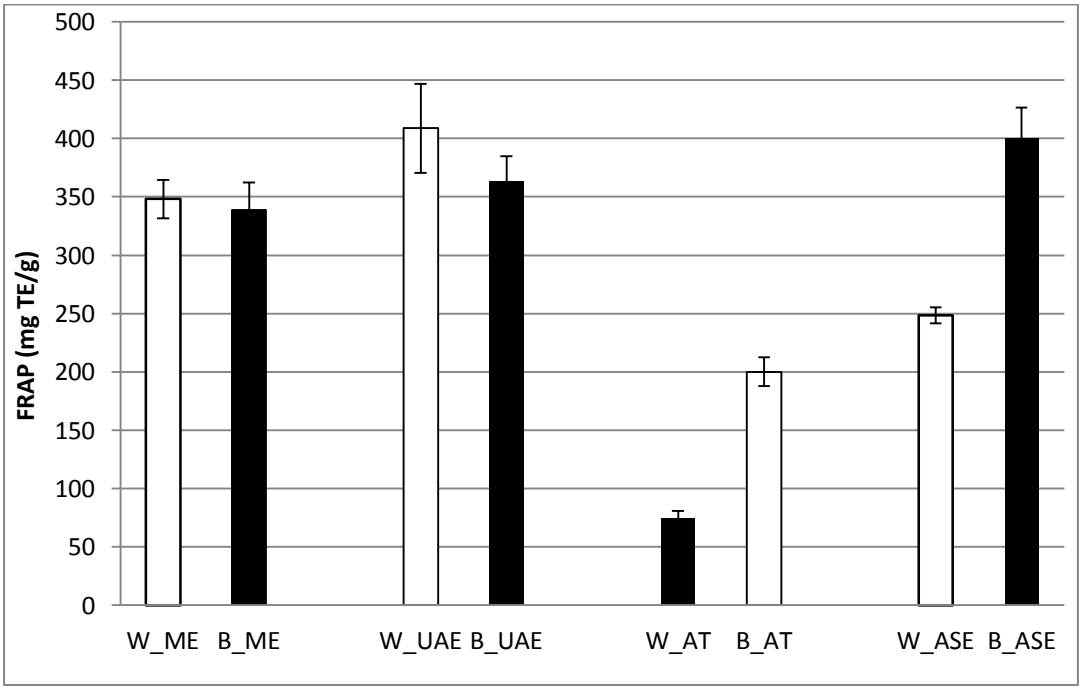

Figure 53 FRAP (Ferric reducing antioxidant power) of wood (W) and bark (B) of olive trees extractives obtained by using various extraction techniques. Where: maceration extraction (ME), ultrasound-assisted extraction (UAE), accelerated solvent extraction (ASE) and autoclave (AT). Data are expressed as means ± standard deviation from three experiments in mg Trolox Equivalents per gram of dried sample (mg TE/g).

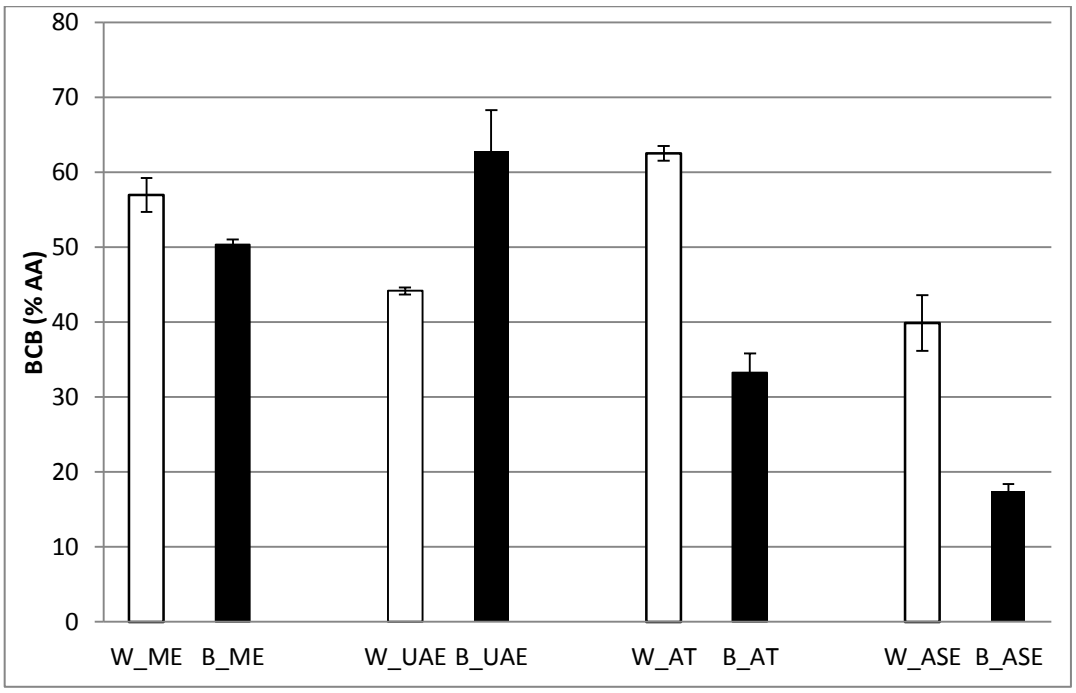

Figure 54 β-Carotene bleaching (BCB) assay on orange tree wood (W) and bark (B) extractives obtained using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving. Data are expressed as means ± standard deviation from three experiments in the percentage of antioxidant activity (% AA) at the initial concentration of at 2 mg/mL.

4.3.5 Relative Antioxidant Capacity Index (RACI)

The RACI index allows us to compare the antioxidant activity of plant complexes estimated by different chemical methods providing a more comprehensive determination of the entire antioxidant capacity (DPPH, FRAP and BCB methods) of the plant systems (33); TPC was also included. In the Figure 55, as predictable by the previous tests, the highest RACI was in B_UAE, while the lowest was in the W_AT. The data showed that in all samples, except for the samples extracted through autoclaving, there is an antioxidant activity. The less antioxidant activity in the AT extraction can be due to the combinations of solvents that are supposed to improve the extraction of phenolic glycosides, in according to Chirinos et al. (34). In fact, it has been previously demonstrated that polyphenols are more soluble in methanol and ethanol than in water, and our results are congruent with previous data (35).

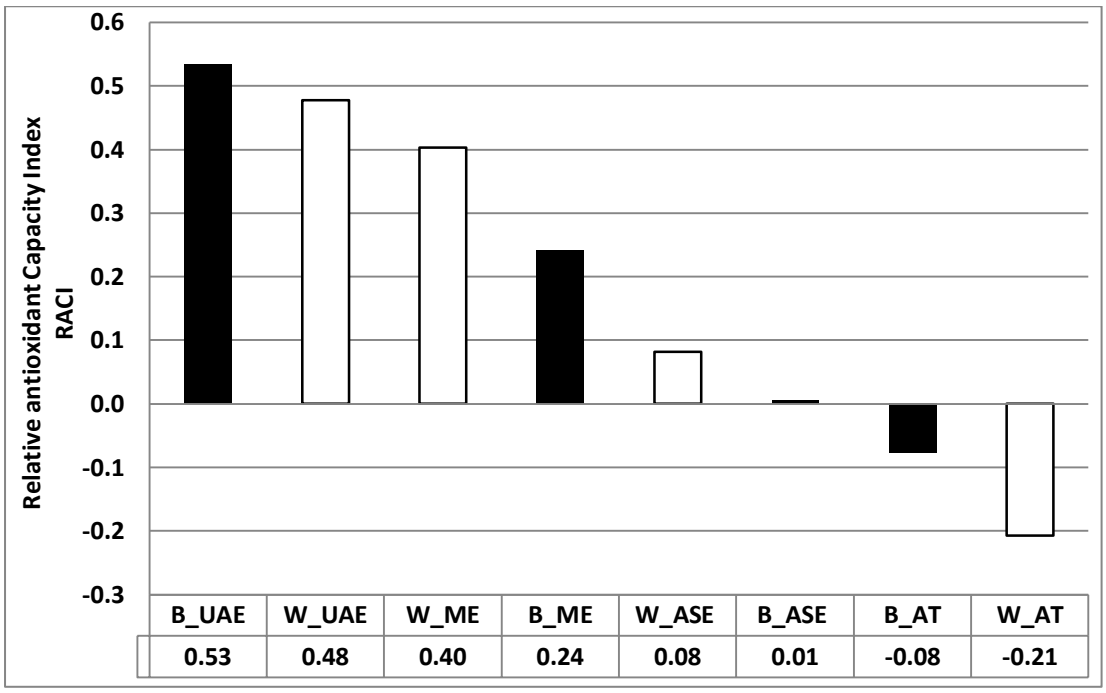

Figure 55 Relative Antioxidant Capacity Index (RACI) values obtained for olive tree wood (W) and bark (B) extractives using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving.

4.3.6 Statistical analysis

4.3.6.1 Pearson coefficient

The Pearson's coefficient between total polyphenolic content and the antioxidant assays (Table 9) showed a moderate positive correlation among TPC, DPPH and FRAP with r= 0.633, 0.661, respectively. These results suggest that the antioxidant activity of some extracts might be attributed to the presence of nonphenolic compounds (36). According to Mamat et al. (37), the moderate correletionships might suggest that phenolic compounds were not the only components responsible for the antioxidant activity. Regarding the correlation between TPC and BCB, the coefficient is negative with r= -0.440; this data can be explicated due to the “polar paradox”. The statistical analysis for the correlation suggested the need to perform more than one type of antioxidant activity measurement to consider the various mechanisms of antioxidant action and the limitation of each assay (38).

Table 9 Pearson coefficient calculated between total polyphenolic content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB).

	TPC
DPPH	**0.633**
FRAP	**0.661**
BCB	**-0.440**

4.3.6.2 Principal component analysis (PCA)

Principal components analysis (PCA) transforms the original, measured variables into new uncorrelated variables called principal components. The first principal component (Dim 1) covers as much of the variation in the data as possible. The second principal component (Dim 2) is orthogonal to the first and covers as much of the remaining variation as possible, and so on (39,40). PCA was applied to the after standardization of values of the antioxidant assays and TPC and in the different extracts from the olive tree wood and bark. PCA (Fig.56a and Fig.56b) explained 86.75 % of the data set's total variance. The first component (Dim 1) explained 57.06% of the total variance in the data set while Dim 2 explained 29.69%. Figure 56a and Figure 56b explain the connections of antioxidant assays and TPC with the samples. In Figure 56a, on the top right, there are the samples W_ME, W_UAE and B_UAE that had a high value of DPPH, FRAP assay and TPC; in fact, in the Figure 56b, it is found in the same position of these samples. In the Figure 56a, in the downright quadrant, were reported B_AT and B_ASE, which are in the same position of the TPC (Fig. 56b), to indicate that these samples had a high quantity of phenolic compounds. On the opposite side respect B_AT, there is W_AT (Fig. 56a), in the same quadrant of BCB (Fig. 56b) to demonstrated that had the highest BCB activity. B_ASE is situated on the right down of Fig. 56a, quite distant from the other samples, indicating that it is significantly different from the others. Instead, W_ASE (Fig. 56a) is on the left down quadrant because demonstrated low quantity in TPC and the antioxidant tests. In Figure 56b, FRAP and TPC are almost overlapping, on the right side of the plot, quite far from DPPH and on the opposite side to BCB. This means that FRAP and TPC are correlated to each other more than DPPH, while BCB is not correlated with the other antioxidant tests, as showed in the Pearson correlation.

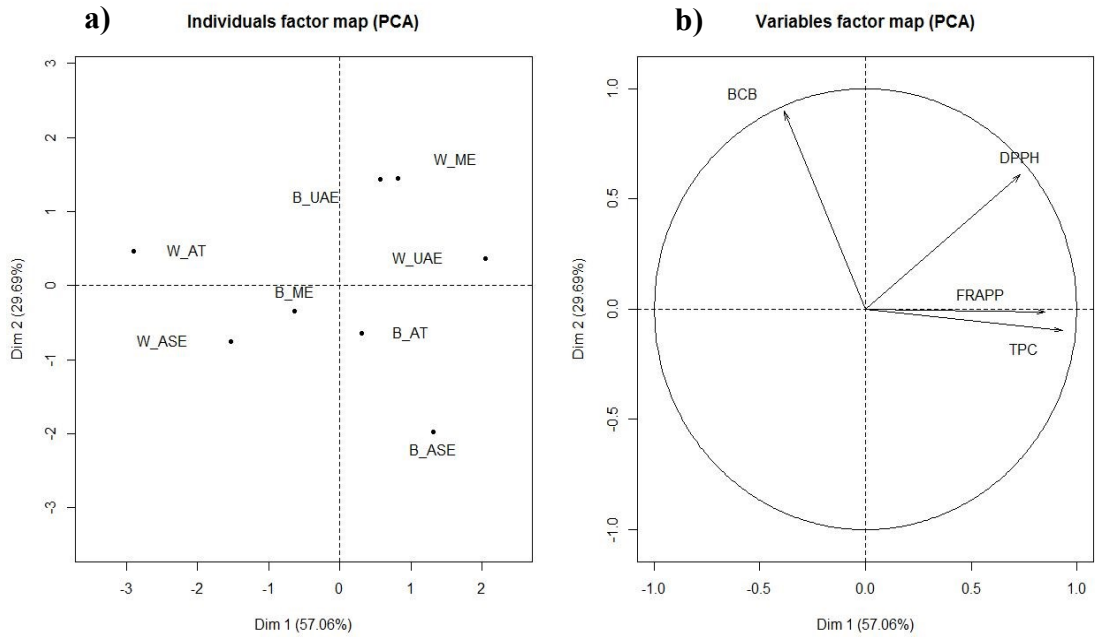

Figure 56 Principal component analysis (PCA) plots. (a) PCA scores from olive tree wood (W) and bark (B) pruning extract using various extraction techniques. ME, maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving; (b) PCA scores for antioxidant activity (DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric reducing antioxidant power (FRAP) and inhibition of lipid peroxidation (BCB)) and total polyphenolic content (TPC).

4.3.7 GC-MS analysis

The GC-MS analysis of the olive tree extracts showed the presence of several compounds (Table 10). The extraction of olive residues obtained using maceration showed the presence of 4-hydroxybenzeneethanol, 3,4-dihydroxybenzeneacetic acid, hexylresorcinol, 4-(3-hydroxy-1-propenyl)-2-methoxyphenol, 1,3,5-benzenetriol as main components. When the extraction was performed through sonication, the main components were hexylresorcinol, 1-methyl-*N*-vanillyl-2-phenethamine, and allopurinol. The use of water in autoclave to perform the extraction gave 4-ethoxy-2,5-dimethoxybenzaldehyde, 3,4-dihydroxybenzeneacetic acid, and 4-(3-hydroxy-1-propenyl)-2-methoxyphenol. Instead, ASE extraction allowed to obtain 3,4-dihydroxybenzeneacetic acid, 3,5-dimethoxy-4-hydroxyphenylacetic acid, and 4-(3-hydroxy-1-propenyl)-2-methoxyphenol. All these compounds are phenolic compounds deriving from lignin. It is interesting to note the presence of scopoletin, a coumarin derivative with interesting biological activities (41). The extraction of the cortex gave quite similar results, where mainly phenolic compounds were extracted.

Table 10 GC-MS analysis of olive residues extracts

Compound	r.t. [min.]	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % [± 0.03]							
Benzaldehyde	3.34	0.69	0.06		0.37	1.03	0.50		0.06
2-Methoxyphenol	3.37			0.10	0.55				
(*E*)-2-Heptenal	4.24		0.22						
Catechol	4.28			0.51					
Glycerin	5.81								1.04
Dihydro-4-hydroxy-2(3*H*)-furanone	5.90							1.18	
3-(2-furanyl)-2-propenal	6.06								
2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	6.47	0.57	0.34	0.80			0.75	1.35	0.79
3-Hydroxy-butanoic acid	6.59		0.04						
5-Hydroxymethylfurfural	6.91	0.78	0.90	0.34		0.88	1.40	3.60	1.05
1,6-Anhydro-β-d-glucopyranose	7.03			1.85				1.32	
1,2,3-Propanetriol-1-acetate	7.17								
Dianhydromannitol	7.44						0.23		
Methyl 2-oxo-2*H*-pyran-5-carboxylate	7.78	0.14		0.63	0.12	0.33	0.26		
2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	7.87			2.90					
2-Methoxy-4-vinylphenol	8.20			0.82	0.40	0.22	0.12	0.33	0.13
5-Formylsalicylaldehyde	8.51	0.27	0.23	0.28	0.19	0.69	0.42		0.56
4-Hydroxy-2-methylacetophenone	8.60				0.26				
Methyl 4-hydroxy-3-methoxybenzeneacetate	8.61			1.81					
2,6-Dimethoxyphenol	8.74		0.16	0.65	0.75	0.28	0.27		
Methyl 4-formylbenzoate	8.96	0.68	0.36	2.45			1.35	1.45	0.28
Methyl 2-formylbenzoate		0.79	0.46		1.78	2.53			0.56
2-Methylene-4-pentenal	9.29					0.64			
Vanillin	9.44		0.22	0.61	0.63	0.55	0.61		0.49

Compound	r.t. [min.]	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % [± 0.03]							
Homovanillinic alcohol	9.52	1.66		1.87	1.74				
4-(1-methylethyl)benzoic acid	9.65					0.39	0.24	0.25	
4-Hydroxybenzeneethanol	9.89	3.40	3.74	1.82	1.91	1.24			
2-Ethylphenol	9.92							1.54	
2-Hydroxy-5-methylbenzaldehyde	9.96								2.51
3-Methoxybenzaldehyde	9.98						2.67		
4-Ethoxy-2,5-dimethoxybenzaldehyde	10.03			5.72					
4-(4-Hydroxyphenyl)-2-butanone	10.07				1.93	1.45	0.72	1.21	
Eugenol	10.09		0.42	0.63	2.55				
Methyl mandelate	10.10					0.51	0.31	0.55	1.29
2-Methyl-6-methylene-7-octen-2-ol	10.11	3.42							
3-Formylbenzoic acid	10.27		1.63		0.97	2.01	2.40		2.77
1,4-Anhydro-d-mannitol	10.31	3.08							
3,4-Dihydroxy-benzeneacetic acid	10.48	5.61		5.12					
3,4-Dihydroxyphenyl-2-propanone	10.54	3.96							3.09
Hexylresorcinol	10.58	6.68	7.64						
2,6-Dimethyl-4-pyrimidinamine	10.61		7.87						
1-Methyl-*N*-vanillyl-2-phenethanamine	10.75		10.27						
4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	10.78	9.47		5.57					
1-(2,4,6-Trihydroxy-3-methylphenyl)-1-butanone	10.92	1.68	1.81						
d-Allose	10.99				0.32				
Allopurinol	11.02	8.06	11.36						
1,3,5-Benzenetriol	11.19						1.54	4.35	2.58
4-Acetyl-1-methylcyclohexene	11.27							7.35	
4-(Hydroxyacetyl)-1,1'-biphenyl	11.38		2.08						
N-Formyltyramine	11.53		1.67		1.68				2.05

Compound	r.t. [min.]	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % [± 0.03]							
4-Ethoxy-2,5-dimethoxybenzaldehyde	11.69	0.57	0.61						
3-Hydroxy-4-methoxybenzoic acid	11.73		1.22			0.91	0.39	0.83	
Apocynin	11.85					0.61	1.10	0.66	
4-Propyl-1,3-benzenediol	12.27					2.62			
2-Methyl-6-methylene-7-octen-2-ol	12.42				3.88		9.06		3.28
4-Hydroxy-3,5-dimethoxybenzaldehyde	12.73				0.84				
3,4-Dihydroxybenzeneacetic acid	13.15				10.04		8.36	6.82	13.64
3,4-Dihydroxyphenyl-2-propanone	13.18					9.70		4.42	
Mannitol	13.20	1.75	5.58	0.94			1.02		
3,5-Dimethoxy-4-hydroxyphenylacetic acid	13.22				6.74	7.88			
Sorbitol	13.41	1.71					2.94		
β -(4-Hydroxy-3-methoxyphenyl)propionic acid	13.64					3.39	3.32		3.37
4-Methoxy-4',5'-methylenedioxybiphenyl-2-carboxylic acid	13.67			1.02					
4-((1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol	13.71				17.40	3.28			2.22
Galactilol	13.94	3.01		8.43					
1-(2,4,6-Trihydroxyphenyl)-2-pentanone	14.02				1.76				
Desaspidinol	14.04						3.15	0.48	
Methyl 3-formyl-4,6-dihydroxy-2,5-dimethylbenzoate	14.14					1.67	1.63	8.30	6.49
2,4,5-trimethoxybenzaldehyde	14.75							2.51	
4-(Hydroxyacetyl)-1,1'-biphenyl	14.88								1.22
Methyl-α -(acetyloxy)-2-methoxybenzeneacetate	15.11								0.28
Hexadecanoic acid	15.77	0.75	0.90	1.03	1.18	0.87	0.41		0.74
Scopoletin	16.12	3.83	6.02		2.85	0.98	1.38	0.40	
3,5-Dimethoxy-4-hydroxycinnamaldehyde	16.18					1.52		0.44	
2'-Formyl-2,3,4,4'-trimethoxy-1,1'-biphenyl	16.20			0.72					

Compound	r.t. [min.]	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % [± 0.03]							
Glycerol 1-palmitate	16.54	0.70	0.97	4.92	1.24		1.08	1.04	
Heptadecanoic acid	16.70					0.63			
(*Z*,*Z*)-9,12-Octadecadienoic acid	17.39					0.22			0.19
Oleic acid	17.43	1.02	2.02		1.17	1.63			0.42
Xylitol	17.47						0.16		
Octadecanoic acid	17.62	0.12		2.55	0.28	0.76			0.27
Glucitol	17.84						1.33		
Hexadecanamide	17.87								0.21
Arabitinol	18.48						2.06		
Diaveridine	18.74								0.17
Eicosanoic acid	19.35		0.54		0.28	0.18			0.19
(*Z*)-9-Octadecenamide,	19.43								0.14
2-Hydroxy-1-(hydroxymethyl)ethyl hexadecanoate	20.60								1.42
Docosanoic acid	20.96		0.22		0.04	0.26			0.33
Tricosanoic acid	21.53		0.05		0.06				
4-Methoxy-4',5'-methylenedioxybiphenyl-2-carboxylic acid	21.94				0.97	0.33			
Stigmast-4-en-3-one	22.50		0.23	0.72					0.86
4,4'-Methylenebis[2,6-dimethoxyphenol	22.57				0.19				
7,9-Diethylbenz[a]anthracene	23.88				0.13				
4-(1,1-Dimethylallyl)-9-methoxy-7H-Furo[3,2-g][1]benzopyran-7-one	24.10		0.07						
Methyl 3,4-dimethoxymandelate	24.36								3.08
4-[[4-(Acetyloxy)-3,5-dimethoxyphenyl]methoxy]-3-methoxybenzaldehyde	24.41					3.05			
3-Methyl-1-(2,4,6-trihydroxy-3-methylphenyl)-1-butanone	25.98		2.90						

Compound	r.t. [min.]	Wood				Bark			
		ME	UAE	AT	ASE	ME	UAE	AT	ASE
		Area % [± 0.03]							
2-Phenylnaphthalene	31.46					1.86			
γ -Sitosterol	32.60			0.22	2.30	0.40	0.15		0.64
20-Hydroxvoaluteine	39.25				0.13				

4.3.8 Conclusions

Olive woody pruning biomass separated in wood and bark was analyzed utilizing four different extraction techniques. In the extractives obtained were measured the TPC, the antioxidant activities by three *in vitro* assays, and were determined the present compounds by GC-MS analysis. These measurements showed how the in terms of the extraction yield the better results were in the olive tree samples extracted through accelerated solvent extraction, while, in terms of the antioxidant activities, were UAE. ASE is an environmentally friendly and alternative green process to extract bioactive compounds from different sources; in this extractive method, the temperature plays a relevant effect on yield extraction.

The phenolic compounds were better extracted by UAE although with a lower yield based on extracted material whereas ASE offer higher yield but lower concentration of phenolic compound compared by UAE.

Regarding the analyses of molecular compounds, the GC-MS showed as in the olive tree sample are presented different phenolic compounds deriving from lignin with several biological activities.

The Annex 1 of the MD 10th August 2018 of the Italian Ministry of Health, according to the Directive (UE) 2015/2283, the bark of the surculi of *Olea Europea* L, is admitted in the food supplement, together at bud, flower, fruit, and oil. The present work add to the previous studies, the possibility to use waste material, as the olive tree pruning residues, in a concrete way in the different industrial sector as pharmacologic, cosmetic and agriculture thanks the interesting biological activity demonstrated *in vitro*. How is seen in the legislation is already possible to use a different part of the olive tree in the

food supplement, through the same passage it can be thinking to add the bark since this part is been demonstrated that has several proprieties.

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CHAPTER 5
INDUSTRIAL PROTOTYPE

5.1 Introduction

Antioxidant activity is fundamental to contrast oxidative stress. Oxidative stress has been implicated in various degenerative diseases in aging (1). It is good to explain clearly and concisely what is meant by oxidative stress (Fig.57). The cells within them maintain a REDOX balance. Inside the human body, there are free radicals and ROS. These are oxygen-containing molecules that possess one or more unpaired electrons. These free radicals try to disrupt the cellular balance by taking electrons from a healthy cell to stabilize. In doing so, they damage the cell and trigger the oxidative itself. In defense of the cells, antioxidants intervene in the body to counteract the action of free radicals, which couple with free radicals by "lending" them an electron and neutralizing them before they can cause cell damage.

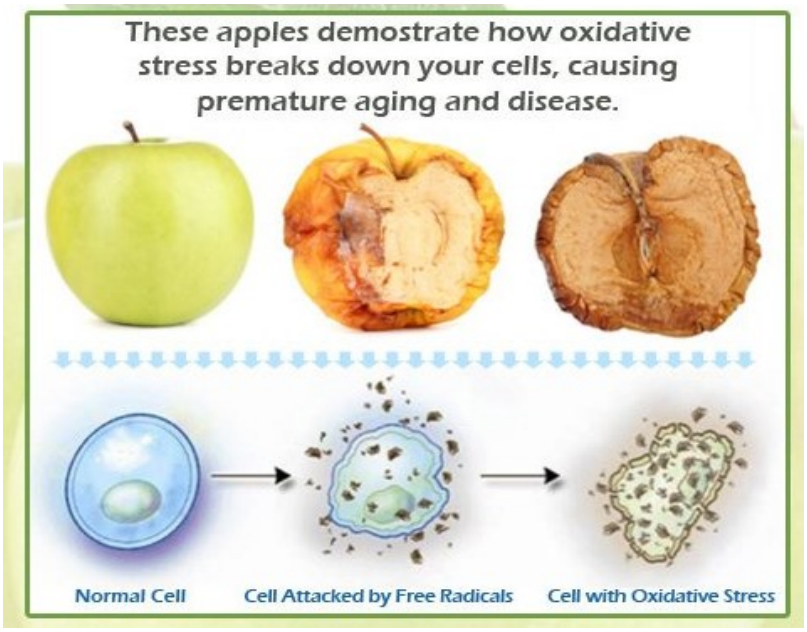

FIGURE 57 Representation of cellular oxidative stress Source: www.Alzheimer-riese.it

The antioxidant activity avoids the so-called "cellular aging" due to the oxidation of our cells caused by external agents (i.e. sun radiation, smog, etc.) and an unhealthy lifestyle (2). Cellular oxidation leads to cell death, with very serious consequences in the human body (3). Aging is the major risk factor for several life-threatening diseases. (4).

The skin plays an extremely important role, providing a vast physical barrier against mechanical, chemical, and microbial factors that may affect the physiological status of the body (5), so it is necessary to use the specific product to protect the skin from the environmental stress it is subjected to every day. The protective function is performed by a wide range of cosmetic products, especially creams, oils, lotions and tonics.

A huge part of the population uses cosmetic preparations containing synthetic ingredients for their instant effects. They show some advantages as follows s: less time consuming, ease of application, high aesthetic appeal, ease of store, easy to carry, etc., but also limitations such as sporadically deterioration, more unwanted after effects, skin allergies and cost-effectiveness (6). Between these synthetic ingredients, it found petrolatum (derived from petrol), parabens (potentially carcinogenic) and silicones (derived from silicon). In the modern age of Herbal cosmetic botanical extracts that support the health, texture and integrity of skin and hair are widely used in commercial cosmetic formulations. Plant materials, from which these extracts are prepared, have a long history of tradition. Historically, plants were the main source of cosmetic ingredients until methods for synthesizing substances with similar properties were discovered (7).

According to Joshi and Pawar (8) more consumers prefer natural cosmetics because they demand more natural products with traceable and more natural ingredients, free from harmful chemicals and with an emphasis on the properties of botanicals.

World demand for herbal products has been growing at a rate of 10% - 15% per annum (7). Europe and the United States are the two major herbal products markets in the world, with a market share of 41 percent and 20 percent respectively (9). The Beauty Report 2018 (10) reported that in Europe, Italy is the fourth economic system of cosmetics after Germany, France and the United Kingdom.

In the cosmetic sector, the main natural raw materials used as cosmetic ingredients are mostly represented by oils, fats, surfactants, emulsifiers, derived from vegetable oils, natural dyes, clays, essential oils extracted from plants for fragrances.

Among all these raw materials, the organic material par excellence is missing until now: wood.

Additionally, different research had introduced lignin-containing wood powders themselves as a natural sunscreen ingredient but using a cream already present in the commerce (NIVEA©) (11). The evaluation of functional properties of lignin samples revealed that the antioxidant behavior of lignins makes them suitable for use in cosmetic and topical formulations showing similar values to those found for commercial antioxidants (12). In add, also lignins from hazelnut and walnut shells were evaluated as potential bioactive ingredients for cosmetic products (13).

It is good to specify how the extractives object of this thesis work would fall within the so-called "botanicals".

For botanicals, therefore, we mean:

- a plant ingredient, or the "plant drug" the plant as a whole or its parts (whole, in pieces or cut) in untreated form, generally dried;

- a vegetable preparation is obtained by subjecting the vegetable ingredient to various treatments (such as extraction, distillation, pressing, fractionation, purification, concentration, fermentation, grinding and pulverization) (14).

The "botanicals" in relation to all potential uses (supplements, functional foods, additives for paints, etc.), compared to the sector of natural supplements, generate 50% of the turnover achieved by the sector, which in the 12 months between May 2018 and April 2019 recorded overall sales of 1,764 million euros (15).

These extracts as well as being added to a cosmetic product could also be added to non-cosmetic products, such as food products, paints, pesticides etc.

For these reasons as the conclusion of my Ph.D. project, it was realized a prototype of cosmetic face cream.

5.2 Regulations for cosmetic cream added with a vegetable extract

The creation and marketing of a cosmetic product is a process that follows certain legislative rules.

Before analyzing the legislation concerning the production of a cosmetic product, it is good to analyze the legislation regarding the pruning residues originating from orchards and olive groves. The Consolidated Environment Law n. 156/2016 indicates pruning, mowing as by-products and no longer as waste (therefore no longer usable). This law has undergone an integration through law n.37/2019 for which mowing and pruning are not considered waste under the following conditions: 1)for the maintenance of the public parks of the municipalities; 2) are not dangerous; 3) are used in agriculture,

forestry or for the production of energy from biomass, even outside the place of production or with transfer to third parties, through processes or methods that do not harm the environment or endanger human health. The burning of small piles is allowed for small piles not exceeding 3 cubic meters per hectare, per day. It is important to underline how pruning products not being configured as waste can lead to applications and uses in various industrial sectors not only to produce energy, obviously in a regulated manner.

Extractives from pruning, as already mentioned, fall into the botanicals category. This type of product is governed by the Ministerial Decree of 10 August 2018. Annex 1 of this Ministerial Decree, containing the list of admitted plants and related parts, accompanied, where appropriate, by additional provisions for use. In the annex 1, there are various plant species whose parts can be used in products, cosmetics, nutraceuticals and the like. Within this document, there are also the olive tree, orange tree and apricot tree species. Specifically, today it is possible to use leaves from olive tree, as well as fruits, buds, surculi, oil. Of the orange tree flowers can be used, leaves, pericarp, fruits or essential oil. Finally, you can use the apricot tree leaves, fruits without stones, oil. Of these three species at the moment in Annex 1 neither the bark nor the wood is present while for example, the bark of the peach tree is present. Therefore substances, preparations and extracts obtained from the plants listed but without a significant consumption history are configured as a novel food under Regulation (EU) 2015/2283. By novel food, we mean all those food products and substances without a history of "significant" consumption on May 15, 1997 in the EU, and which, therefore, must be subject to an authorization, to assess their safety, before being placed on the market. One of the novelties of Regulation (EU) 2015/2283 is the centralization of the authorization request, which must be submitted directly to the European Commission. The request, prepared following the guidelines published by the food safety authority (EFSA - European Food Safety Authority), must contain scientific data in support of the safety of the substance subject of the application for authorization. The safety assessment is done by EFSA. The Commission issues the authorization through the inclusion of the "authorized novel food" in the Union list together with all the specifications provided, including any food types in which it may be contained, the doses and other characteristics (16).

In the ministerial decree n. 10/2018 it is also admitted among the working processes physical processes, for obtaining "natural ingredients", maceration, which is the technique used in our case to extract the secondary metabolites from the bark of apricot wood by-products.

The EU regulation n. 1223/2009 regulates the production, packaging, distribution and sale of cosmetics, in order to protect the consumer. Furthermore, the definition of cosmetic is reiterated: *"a cosmetic product is any substance or mixture intended to be applied on the external surfaces of the human body (epidermis, hair system and hair, nails, lips, external genital organs) or the teeth and on the mucous membranes of the mouth in order, exclusively or mainly, to clean them, perfume them, modify their appearance, protect them, keep them in good condition or correct body odors ".*

To ensure product safety and better protect the health of consumers, among other aspects, the regulation provides that the production and packaging of cosmetic products are carried out according to good manufacturing practices (GMP). The harmonized standard that describes the specific GMP (Good Manufacturing Practice) requirements for the cosmetics sector is UNI EN ISO 22716: 2008 "Cosmetics - Good Manufacturing Practices (GMP) - Guidelines on Good Manufacturing Practices". This document, specially designed for the cosmetic industry, provides guidelines for the production, control, storage and shipping of cosmetic products.

According to Regulation (EC) No 1223/2009, "a cosmetic product made available on the market shall be safe for human health when used under normal or reasonably foreseeable conditions of use". For certain cosmetics ingredients with concerns for human health (UV filters, colorants and preservatives), safety evaluation is conducted by SCCS. The industry is responsible for the evaluation of other cosmetics ingredients, to be included in a cosmetic product safety report (CPSR) as part of a product information file (PIF) (17). The Scientific Committee on Consumer Safety (SCCS), endorsed in their recently revised "Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation" specific needs for cosmetic ingredients that have to be fulfilled. Sufficient information on the skin sensitization potential of individual chemicals/ingredients is always mandatory (18).

These are the main rules to which you must comply when you want to produce a cosmetic product with vegetable raw materials inside.

5.3 Extractive

In the previous chapters biological and chemical proprieties of the pruning residues of *Citrus sinensis* L., *Prunus Armeniaca* L., *Olea Europea* L. have been analysed. Pruning apricot bark extract (AP_B_ME) showed the best antioxidant activity by reporting the highest RACI value (Fig.58). According to Bonesi et al. (19) the RACI a reasonably accurate ranking of the antioxidant ability of investigated samples.

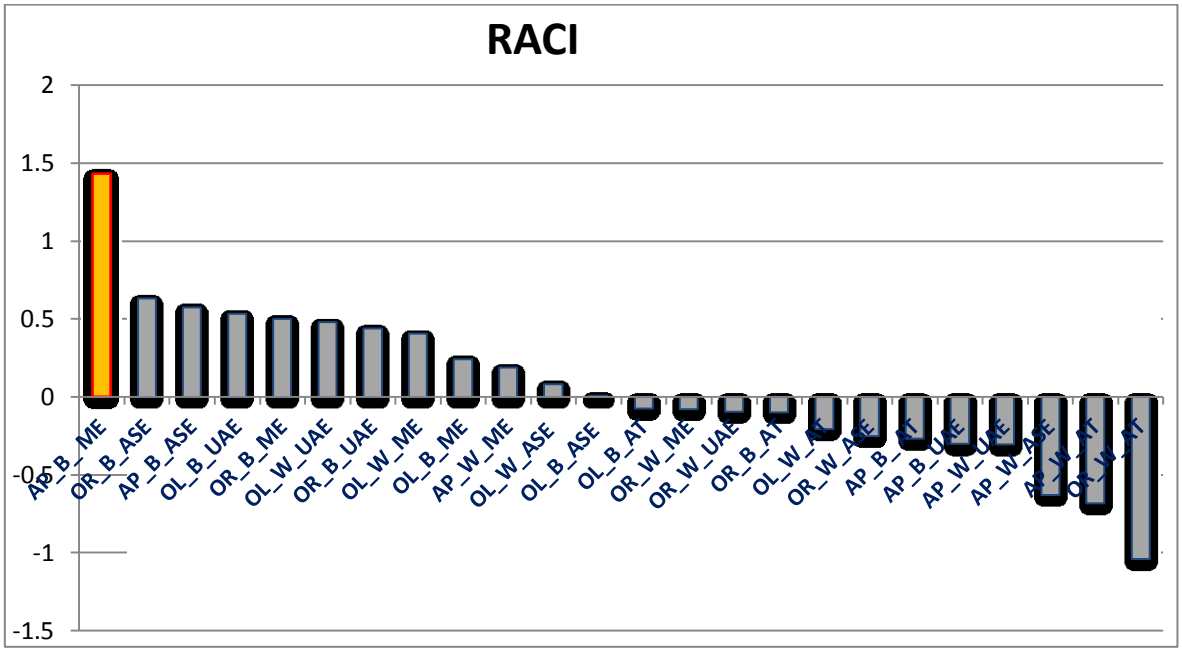

FIGURE 58 Relative Antioxidant Capacity Index (RACI) values obtained for orange tree (OR), olive tree (OL), apricot tree (AP); wood (W) and bark (B) extractives using various extraction techniques. ME,maceration extraction; UAE, ultrasound-assisted extraction; ASE, accelerated solvent extraction; AT, autoclaving. In yellow the highest value of RACI.

Through the LC-MS analysis of the wooden extractives of apricot tree, it emerged as inside the extractives there are several secondary metabolites with antioxidant activity as cathechine, epicatechine, narigenin etc. Catechin is responsible for antioxidant activity, anti-aging properties and cardiac health maintenance (20).

To avoid animal experiments in investigating the toxicity of cosmetic products, the current preliminary study has been carried out through the exam in vitro on the HepG2 cells, measured with MTT assay. HepG2 cells were chosen as representative of human-derived cell lines (21). Human HepG2 cells are an in vitro model system suitable for various studies. HepG2 cells and their derivatives are also used as a model system for studies on the detection of environmental and dietary cytotoxic and genotoxic agents (and thus cytoprotective, anti-genotoxic and conotoxin), and drug targeting studies (22). For these reasons and over that

the extraction through the maceration is less expensive and more green with a low input of the energy (23), it was selected as extractive AP_B_ME to realize a prototype of face cream able to counteract the aging of the skin.

5.4 Materials and methods

5.4.1 Materials

The extractive utilized is the apricot bark extract through the maceration (ME) in ethanol/water (70:30 v/v) as reported in the paragraph 3.1.4.

5.4.2 LC–MS analysis

U-HPLC analysis of extracts was carried out using an LC–MS-8030 Shimadzu apparatus equipped with a Diode array detector SPDM20A. The separation was carried out in thermostatic conditions at 40 °C with a reversed-phase column (Phenomenex® Luna 3µm C18). The elution was carried out with a binary solvent system consisting of water with 0.1% formic acid and acetonitrile with 0.1% formic acid, running at the flow rate of 0.4 mL/min. The injection volume was fixed to 1.0 µL. The detection was carried out with a UV detector set at the wavelength of 280 nm and under selected ion monitoring by negative and positive mode ESI-MS. The operating parameters for MS detection were as follows: nebulizing gas (N_2), flow 3.0 L/min, drying gas flow 15 L/min, interface voltage 4.5 kV, gas pressure 230 kPa, DL temperature: 250 °C, block heater temperature 400 °C.

Data were processed and analyzed with LabSolution software (5.42SP4, Shimadzu). Peaks were numbered according to their mass spectra and tentatively identified compare their mass with literature, commercially available libraries (MassBank of North America –MoNA, MassBank Europe, PubChem), and standards of (+) catechinehydratate and (-) epicatechin. The standards were obtained from Sigma–Aldrich, St. Louis, USA. The quantity of polyphenolic compound represented by each peak is expressed in catechin equivalent weights as described in (24).

5.4.3 Cell Culture and Drug Treatment

Human hepatocellular carcinoma cell line (HepG2) cells were cultured in DMEM (supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) and maintained at 37°C in a humidified atmosphere containing 5% CO_2. AP_B_ME extract and cream were dissolved in DMSO (100mg/mL) and different concentrations were tested (0.25-0.002 mg/mL)The final DMSO concentration in the culture was 1% (v/v), which did not affect cell growth when compared with the vehicle-free controls. DMSO-treated cells were used as control (CTRL) in all the experiments.

5.4.4 Cytotoxicity Assay

Cytotoxicity assay was performed to determine the extract concentration to be incorporated into the cream. Cell viability was evaluated on HepG2 cells used as a model by MTT assay, a colorimetric assay based on the conversion of the yellow tetrazolium salt MTT into purple insoluble formazan by succinate dehydrogenase enzyme of viable cells (25,26).

Cells were seeded in 96-well plate (10^4 cells/well), incubated overnight and treated with different concentrations of extract (0.25-0.002 mg/mL) for 24, 48 and 72 h. After removal of the medium, the cells were washed with phosphate buffer solution (PBS) and incubated with 0.75 mg/mL of MTT solution in PBS for 4 h. Then, the solution was removed, the cells were lysed using a solubilization solution (1:1 DMSO:isopropanol). The solubilized formazan product is spectrophotometrically quantified at 560 nm using Multiskan Go (Thermo Scientific).

The extract was incorporated in the cream (0.05% v/v) and an MTT assay was performed with the same procedure explained before.

5.4.5 Cream formulation

All chemicals used in cream formulations (Table 11) were purchased from Ecocosmesicreativa di A.M. Distilled water was used throughout the experiments.

Table 11 Reported the ingredients used in the cream formulation shared in three phases A-B-C

	Ingredients	**Intervals Of Concentration**
	PHASE A	
A1	WATER	≥75%-≤100%
A2	XANTHANE GUM	≥0.1%-≤1%
A3	GLYCERINE	≥1%-≤5%
A4	LACTIL (Sodium PCA, Glycine; Fructose, Urea, Niacinamide, Inositol, Lactic Acid)	≥1%-≤5%
A5	SODIUM CITRATE	≥0.1%-≤1%
A6	SODIUM DEHYDROACETATE	≤0.1%
A7	PHYTIC ACID	≥0.1%-≤1%
	PHASE B	
B1	RICE OIL	≥1%-≤5%
B2	OLIVE OIL	≥1%-≤5%
B3	SHEA BUTTER	≥1%-≤5%
B4	SUNFLOWER OIL	≥1%-≤5%
B5	OLIVEM 1000 (Cetearyl Olivate, Sorbitan Olivate)	≥1%-≤5%
B6	APEROXD TLA (Lecithin, Tocopherol, AscorbylPalmitate, Citric Acid)	≥0.1%-≤1%
B7	VITAMIN AND ACETATE	≥0.1%-≤1%
	PHASE C	
C1	DERMOSOFT ECO 1388 (Glycerin, Aqua, SodiumLevulinate, Sodium Anisate)	≥1%-≤5%
C2	DRY APRICOT EXTRACT BARK MACERATION (AP_B_ME)	≥0.1%-≤1%

5.4.6 Preparation of cream

In a suitable stainless steel, container was poured, while was stirred (Fimar - Italy), in order water and glycerine. Heated these on a plate (Falc - Italy) at a controlled temperature up to 70 ° C and add was stirred: xantana gum, lactil, sodium citrate, Sodium Dehydroacetate and Phytic Acid (Phase A). In another vessel put Rice Oil, Olive Oil, Shea Butter, Sunflower Oil, Olivem 1000, AperoxdTla and Vitamin E Acetate (Phase B). Heated on a plate, the phase B at a controlled temperature up to 70 ° C. Was poured phase A into phase B and emulsify. Finally, when cold (30 ° C) add while stirred: Dermosoft Eco 1388 and the apricot bark extract (ME).

5.4.7 Cream quality check

The samples were analysed for pH values, viscosity, stability and microbiological analyses by PH-meter. Viscometer (PCE – Germany), Centrifuge(Orma -Italy) the Contact Slide 2 and 4 (Liofilchem – Italy). The centrifuge was used to evaluate the stability of the emulsion subjected to mechanical stress (phase separation). Contact Slide 2 is a ready-to-use device with two different media coated into plastic support used for the microbial monitoring of surfaces and liquids even in the presence of residues of disinfectants. The selective medium allowed the isolation and enumeration of yeasts and moulds. The other medium is used for the enumeration of bacteria. Contact Slide 4 is a flexible ready-to-use device with two media solidified onto plastic support for Pseudomonas, yeasts and moulds detection for surfaces bacteriological control with inactivation of disinfectants. Quantitative and qualitative limits are based on the European Standard EN ISO 17516:2014 Cosmetics – Microbiology – Microbiologicallimits. The European Standard EN ISO 17516:2014 was approved by CEN on 9 August 2014 and at present is widely used by the cosmetics industry as an international standard.

5.6 Results and discussion

5.6.1 LC-MS analysis

In the Figure 59 are reported the tentatively peak identification based on the literature. In the

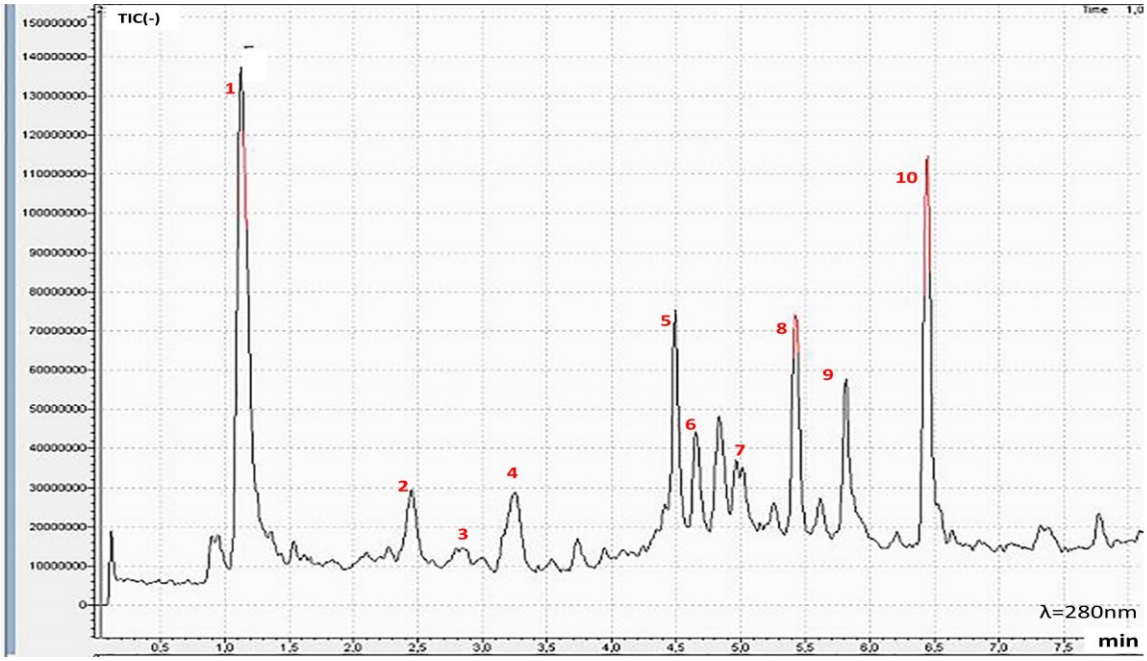

Figure **59** HP-LC chromatogram of AP_B_ME indicating the retention positions of the 10 compounds tentativly investigated

Table 12 are described different molecular compound identify, except for catecheine and epicatechin, the others were tentatively identified. According to Yilmaz (27), catechin and epicatechin are the major flavonol with dietary importance for human health. Catechine and epicatechin exhibited good antioxidant and antimutagenic activity (28). The others most frequent peaks identified were,*m/z* 505 [Flavonoid+H]$^-$, *m/z* 343 [Hypoprotocetraricacid+H]$^-$, *m/z* 575 [procydin dimer A+H]$^-$,*m/z* 191 [Scopoletin+H]$^-$, *m/z* 559 [Phenolic glycosides +H]$^-$,*m/z* 559 [Phenolic glycosides +H]$^-$, *m/z* 271 [Naringenin+H]$^-$. According to Bodet et al. (29), naringenin holds promises as a therapeutic agent for treating inflammatory diseases such as periodontitis. Regarding scopoletin Shaw et al. (30) affirmed that the ability of scopoletin to scavenge superoxide anion may have to promise in further usage in slowing or preventing diseased conditions related to oxidative damage. The others detected peaks (m/z 505, m/z 575, m/z 559) were polyphenolic compounds that have proprieties as antioxidant, antithrombotic, anti-inflammatory, and vasodilatory linked to preventing several diseases (31).

The peaks unknown as *m/z* 181 and *m/z* 287 mean that are necessary further investigations to identify them.

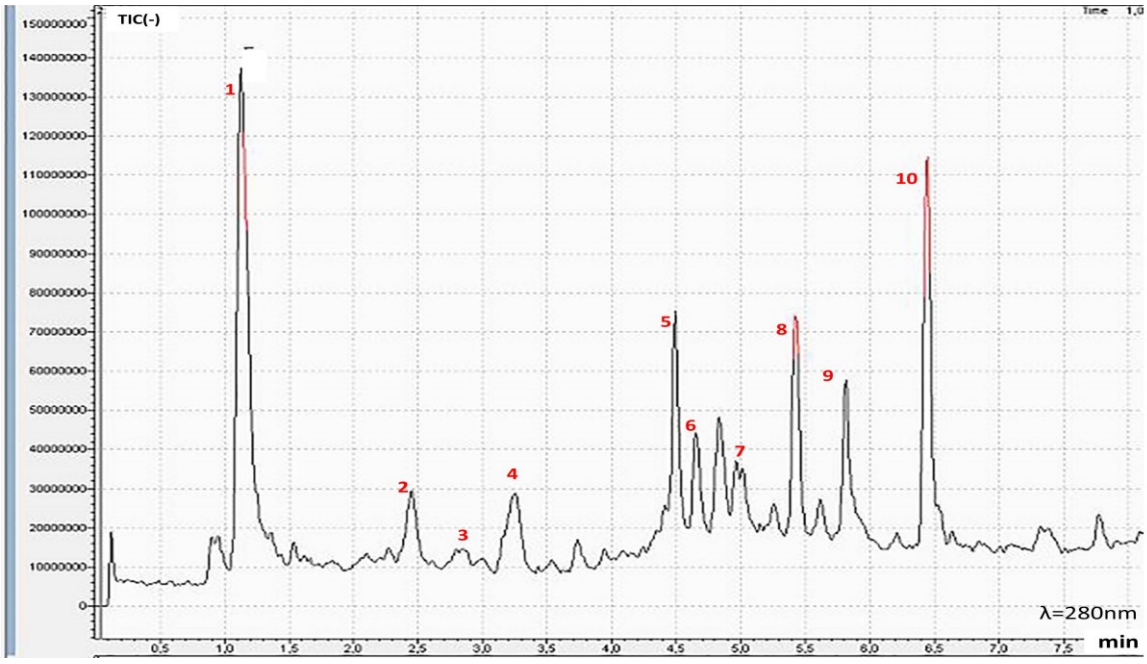

Figure 59 HP-LC chromatogram of AP_B_ME indicating the retention positions of the 10 compounds tentativly investigated

Table 12 Tentative identified components in tested Apricot tree samples

Samples	Peaks	Primary m/z fragment negative ion [M − H]−	Tentative assignment	Polyphenolic mg/g
Apricot Tree Bark Maceration (AP_B_ME)	1	181	Unknown	–
	2	289	Catechine	8.8
	3	289	Epicatechine	19.3
	4	505	Flavonoid	19.3
	5	343	Hypoprotocetraric Acid	–
	6	575	Procyanidin Dimer (Type A)	21.5
	7	559	Phenolic Glycosides	25.6
	8	287	Unknown	–
	9	191	Scopoletin	35,2
	10	271	Naringenin	60.9

5.6.2 Cytotoxic Effect of the apricot bark maceration extract on HepG2 Cells

HepG2 cell lines used as models were treated with different concentrations of AP_B_ME extract for 24, 48 and 72h to select the dose to incorporate in the cream. As shown in Figure 60, only the highest dose of the extract reduced significantly cell viability after 24, 48 and 72h. The extract showed no cytotoxic effect at other tested doses. On the contrary, the extract exhibited a proliferative effect on cultured cells at concentrations ranging from 0.100 to 0.006 mg/mL especially after 24 and 48h of treatment (Fig.60). Probably, the secondary metabolites found are responsible for this activity. As reported by Takano et al. (32), catechin and epicatechin stimulated the proliferation of myeloid cells, moreover procyanidin B-2 has been shown to promote the growth of hair epithelial cells after 5days (33).

The crude extract was incorporated into skin cream with 0.5% ratio based on *in vitro* cytotoxicity assay. As shown in Figure 61 the cream reported no cytotoxic effect at all tested doses after 24, 48 and 72h.

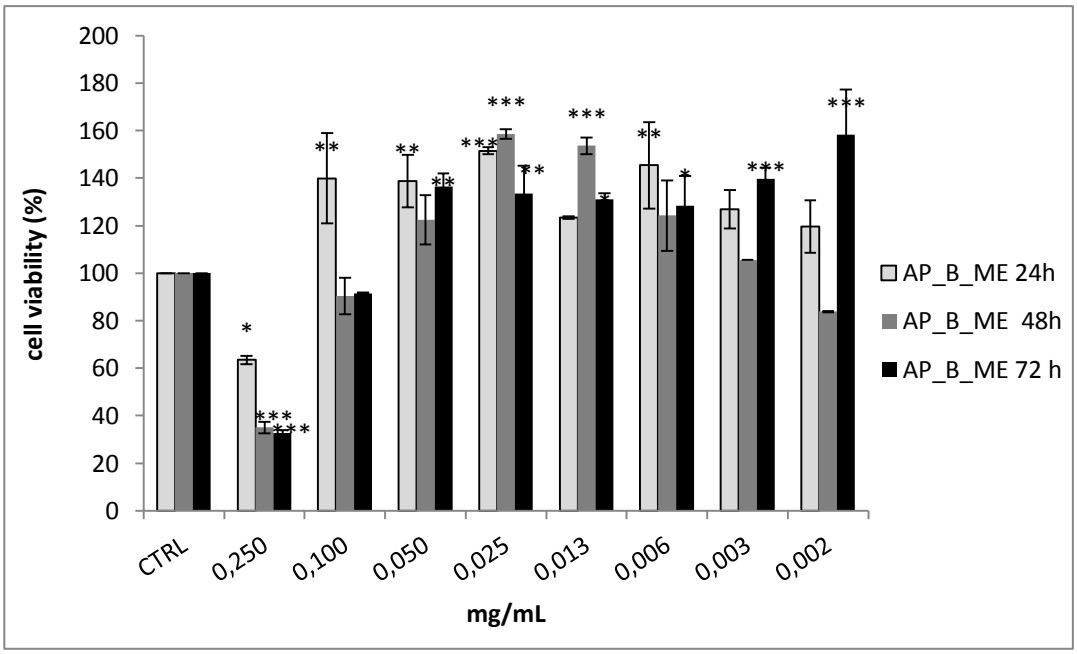

Figure 60 Cell viability, evaluated by MTT assay, of HepG2 cells treated for 24, 48 and 72h with different concentrations of AP_B_ME crude extract. Data are expressed as the mean ± SD of three independent experiments (n=3). ***p<0.001 , **p<0.01, *p<0.05 *vs* CTRL

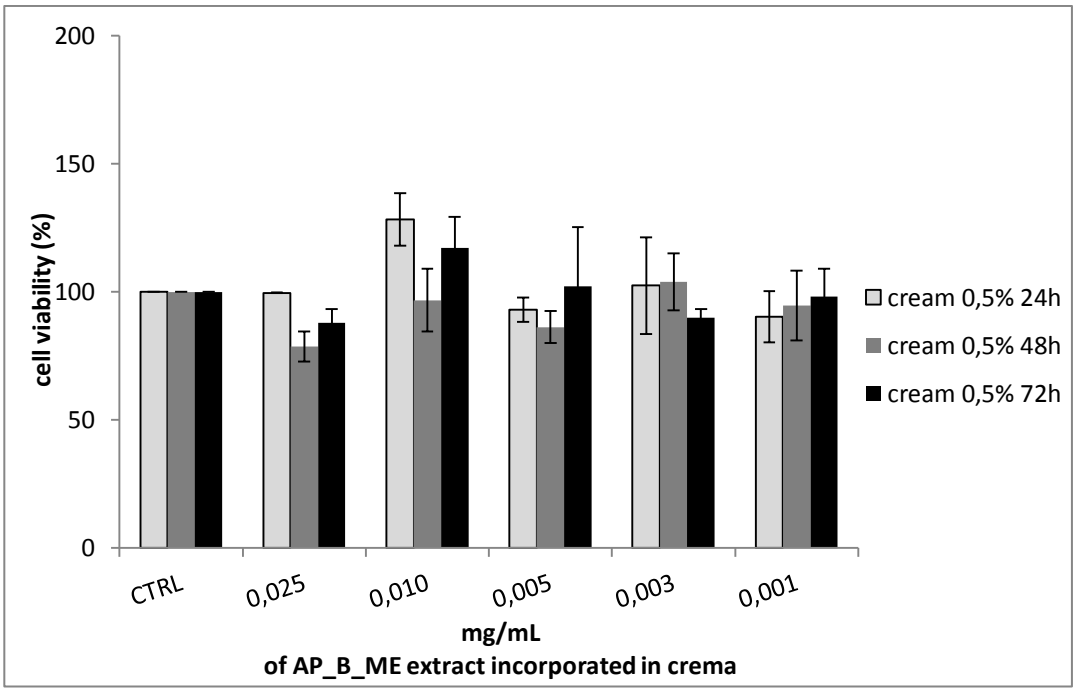

Figure 61 Cell viability, evaluated by MTT assay, of HepG2 cells treated for 24, 48 and 72h with AP_B_ME crude extract incorporated into skin cream (0,5% v/v) (b). Data are expressed as the mean ± SD of three independent experiments (n=3).

The preliminary study on the cytotoxicity cellular utilizing HepG2cell on the extract and the cream added with the extract showed positive results, but are necessary other cytotoxicity evaluation on cultures of human skin fibroblasts and keratinocytes, because both cell types have important roles in the skin (34). Also, HeLa cell is suitable to test the cytotoxicity of cosmetic ingredients (35) or SIRC-NRU test is particularly useful for the evaluation of non-irritants or weak irritants currently used in cosmetic formulations (34). The other possible test to measure the cell cytotoxicity is Episkin epithelia (36). As reported in the literature the MTT assay is used in many areas, especially in the identification of new drugs; however, this assay has limitations (37). Alone it is not sufficient to determine the cytotoxicity of a plant compound, other tests must be added to it.

The cytotoxicity measurement by MITT can be considered a starting point to which other tests as the neutral red test, lactate dehydrogenase (LDH) (38) can be added to accurately verify the cytotoxicity.

5.6.3 Quality check

In the Table 13 was reported the quality check analyses following the EU directive 1223/2009

The analyses visually evaluated the appearance of the cream, the consistency, and the phase separation. As for the color, the evaluation is done on sight while the smells analyzed directly. The pH is measured which must have a range between 4,8-5,8. Density was measured through a viscosimeter. The microbial analyses consisted of putting a few mg of cream on contact slides 2 and contact slide 4 and after 24 hours proceeded to count bacteria, molds, yeast and pseudomonas spp. The images (Fig. 62 – Fig. 63) show that no pathogens harmful to health have been detected.

Table 13 Cream Quality Check

PARAMETER	
ASPECT: Presence of lumps Consistency Phase separation	**Emulsion** No Emulsion No
COLOR (visual evaluation)	Ivory
ODOR (directed from the jar)	Characteristic
pH ± 0,5	5,3
VISCOSITY (mPS) 20 ° impeller RPM 20	11100
DENSITY (g/cm3)	n.a.
MICROBIOLOGICAL ANALYSIS Total bacterial count Yeasts and molds Pathogens	 Absent Absent Absent

Contact slide-2
(conta batterica totale+lieviti e muffe)

Figure 62 Microbial analyses-Contact slide 2

Contact slide-4
(Pseudomonas spp + lieviti e muffe)

Figure 63 Microbial analyses-Contact slide 4

5.7 Conclusions

The study preliminary on the prototype of cosmetic cream showed the feasibility of being able to produce a cream with apricot bark vegetable extract inside but further investigations are necessary. The LC-MS permitted to identify tentatively the compound present in the extract and due to the injection standard was possible to quantify the content of the polyphenols. *In-vitro* penetration studies showed that although catechins from plant extracts penetrate the horny layer of the skin into deeper layers of the epidermis and sometimes even into the dermis, no evidence of a systemic effect of the catechins was obtained (39). According to Bae et al. (40) catechins increase the penetration and absorption of healthy functional foods and bio cosmetics into the body and the skin, thus improving their utility.

Without injecting standard is almost complicated precise identification of compounds due to the co-elutions of the compounds. The injection of the others standard could permit to calculation of the total quantity and characterize the compounds present in the extract.

Regarding the production of the face cream plus the extract, the preliminary quality check has shown encouraging results, as the cream has not undergone physical, chemical and biological processes that could alter its stability.

The face cream prototype (Fig. 64) resulting from the thesis project differs from the eco-friendly and organic cosmetic products already on the market because they can complete the "circle" of the circular economy. In fact, starting from what is a by-product material (pruning residues and explant residues), we are able to extract a raw material for a new production. Normally cosmetic products that use natural molecules are produced using raw materials grown specifically for cosmetic productions. Instead, we will use an already existing raw material, without subtracting productive agricultural areas.

The prototype (Fig. 64) developed thanks to the thesis project marks a starting point to continue the process that will lead to the marketing of the finished cosmetic product.

Figure 64 The Face Cream Prototype

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CHAPTER 6
CONCLUSION AND RECOMMENDATION

The Ph.D. research aimed to give importance to agricultural by-products from orchards and olive groves. A large amount of woody biomass from these permanent crops was mostly burned or left in the field. Through the thesis work, we tried to enhance these by-products through the chemical and biological analyzes of these materials. Furthermore, given the industrial characterization of the Ph.D., an attempt was made to create an industrial prototype that included within it an extract from the biomass subject to enhancement.

The use of 4 different extraction techniques ME (maceration), UAE (ultrasound extraction), ASE (accelerated solvent extraction) and AT (autoclaving), with organic solvents such as ethanol and water, solvents with a low risk to health and the environment, has shown how the percentage of extractive yield in the different samples varies from:

- 3-12% for the orange tree;
- 1-13% for the apricot tree;
- 3-21% for the olive tree.

The highest yields were recorded in the bark using the two solvents together at the same time and using ASE technique as pressure and temperatures play a fundamental role in increasing the extraction yield.

Analyzes concerning the polyphenol content have shown that antioxidant activity is present in all species, especially in the extracts of:

- B_ASE in orange tree sample equal to 79 $mgGAE^{-1}$,
- B_ASE in apricot tree sample equal to 274 $mgGAE^{-1}$,
- B_ASE in olive tree sample equal to 144 $mgGAE^{-1}$.

The study on antioxidant activity showed how:

- in the orange samples, the greatest antioxidant activity is present in B_ASE,
- in apricot samples, the greatest antioxidant activity is present in B_ME,
- in the olive samples, the greatest antioxidant activity is present in B_UAE

The analysis of the molecular compounds of the different extracts showed that within these there are interesting molecular compounds, including:

- caffeic acid and flavonoids in the orange tree,
- scopoletin and naringenin in the apricot tree,
- phenolic compounds deriving from lignin and scopoletin in the olive tree.

From what was highlighted by the results obtained from the various analyses, in addition to the market analyses relating to the use of natural products in cosmetics, it was reached the prototyping of antioxidant face cream, the starting point for further research.

In Figure 65 the SWOT analysis on the extracts from the species subject of the thesis is reported to underline the future prospects of these products.

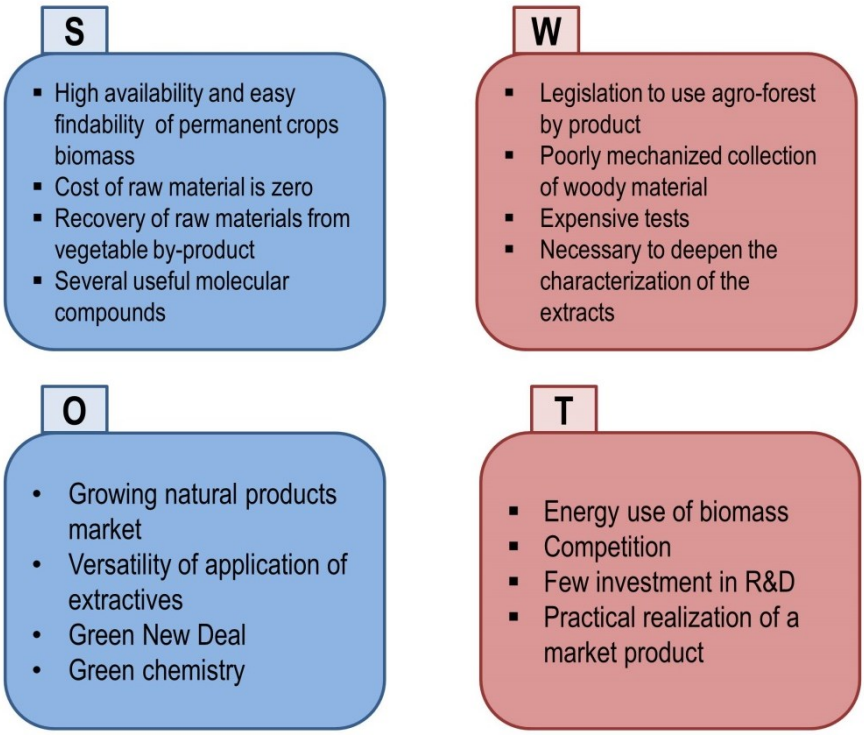

FIGURE 65 Strengths(S)-Weakness(W)-Opportunities (O)-Threats (T) diagram related to use of extractives obtained by biomass by-products.

Among the strengths, it is important to highlight how the large availability of wood material at no cost is an advantage over using synthetic products, which often have considerable costs. As a weakness, the rather complicated and cumbersome legislation for the use of these products could slow down the use of these products.

Between the opportunities, it is worth pointing out that national and international politics are pushing more and more so that there is a green conversion in all sectors and especially in the chemical sector.

The threats are the use still made of these biomasses to produce energy or heat. This if in some cases it can be a solution to produce energy from non-fossil fuels, on the other hand, however, it helps to release a large amount of CO_2 stored within the biomass in a few minutes.

The studies and researches of extracts from biomass remain important but even more important is the concrete application of these industrial residues, which, as seen in the thesis work, can concretely contribute to creating industrial products with ingredients and compounds of vegetable origin.

THE EDITOR

Dr. Maria Roberta Bruno

Maria Roberta Bruno has a Master Degree in Agricultural Sciences and Technologies. After graduation, she carried out activities related to the world of agriculture and environmental protection. She carried out activities in the field of experimental research in the CREA research center in Metaponto (Matera-Italy), where she analyzed different eco-sustainable agricultural systems.

In the Ph.D. project at the University of Basilicata, she has analysed from a chemical-biological point of view agricultural waste to create new eco-sustainable industrial products such as a prototype of cosmetic cream.

SCIENTIFIC PRODUCTION

Details of contributions or publications form part and/or are included in the research presented in these details

Poster and oral presentation to national conference:

Add Value Of Wood In The Future "*The perspectives of wood secondary metabolites: characterization and sustainable use*"- **Maria Roberta Bruno** - Firenze, 27 Febbraio 2018 –Oral Presentation

IV Congresso Nazionale di Selvicoltura:-" *Sustainable use of pruning biomass from Olive (Olea Europea L.) and Orange Trees (Citrus sinesis L.): the bark and wood extractives*"-**Maria Roberta Bruno**, Luigi Todaro, Antonella Giannone, Luigi Milella, Philippe Gerardin - Torino 5-9 Novembre 2018 - Poster.

XII Congresso Nazionale SISEF, "*Underutilised species resources in Italy for particleboards manufacture"* - Valentina Lo Giudice, Luigi Todaro, **Maria Roberta Bruno**, Paola Cetera, Octavia Zeleniuc - Palermo, 12-15 Novembre 2019 - Poster.

XII Congresso Nazionale SISEF, *"Estrattivi da biomassa di Arancio (Citrus Sinensis L.) e Albicocco (Prunus Persicae L.): nuove possibilità di sviluppo sostenibile per le Regioni del Mediterraneo"* - **Maria Roberta Bruno**, Luigi Todaro, Valentina Lo Giudice, Paola Cetera - Palermo, 12-15 Novembre 2019 -Poster.

Poster and oral presentation to international conference:

International Conference Wood Science and Engineering (ICWSE) 2019, *"Secondary metabolites of apricot trees (prunus armeniaca) pruning: possible sustainable use in the industrial sectors"* - **Maria Roberta Bruno**, Paola Cetera, Valentina Lo Giudice, Luigi Todaro, Luigi Milella -Brasov, Romania, 07–09 November 2019 –Oral Presentation

International Conference Wood Science and Engineering (ICWSE) 2019, *"Underutilised species resources in Italy for particleboards manufacture"* - Valentina Lo Giudice, Luigi Todaro, **Maria Roberta Bruno**, Paola Cetera, Octavia Zeleniuc - -Brasov, Romania, 07–09 November 2019 – Poster.

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Online conference

28th European Biomass Conference&Exhibition(EUBCE), *"From biomass of poplar utilizations to byproducts sector"* Paola Cetera, Nicola Moretti, Maurizio D'auria, Immacolata Faraone, Daniela Russo, **Maria Roberta Bruno**, Marco Fioravanti, Luigi Milella, Luigi Pari, Luigi Todaro - 6-10 Luglio 2020 –Poster

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115° Congresso della Società Botanica Italiana: "*From the woods, not just firewood: innovative use of Quercus cerris L. biomass*"- Immacolata Faraone, Giovanni Forte, Daniela Russo, **Maria Roberta Bruno**, Sonia Nardozza, Fabiana Labanca, Luigi Todaro, Luigi Milella - 9-11 Settembre 2020- Poster

Paper ISI

Bruno, M. R., Russo, D., Cetera, P., Faraone, I., Lo Giudice, V., Milella, L., Todaro, L., Sinisgalli, C., Fritsch, C., Dumarcay, S., Gérardin, P. (2020). *Chemical analysis and antioxidant properties of orange-tree (Citrus sinensis L.) biomass extracts obtained via different extraction techniques*. *Biofuels, Bioproducts and Biorefining***,** *14*(3), 509-520.

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In Press:
Valentina Lo Giudice, Paola Cetera, Teresa Lovaglio, **Maria Roberta Bruno** (2021). The different levels of valorization of lignocellulosic material obtained

from agroforestry activities in a view of circular economy, iForest - Biogeosciences and Forestry.

University Contest and Prizes

- Potenza, 19 giugno 2019: Ideas Contest: "*Shell InventaGiovani-Royal Contest*" – Presentazione idea "*Tree Green Power*" – Second award.
- Potenza, 11 ottobre 2019: Partecipazione finale Start Cup Basilicata- Presentazione Contest: *"Extra-attivi- Creazione crema viso da rifiuti agricoli"* – Money Award for the participation in the national competition.