Food Chemistry 160 (2014) 298–304

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant and free radical-scavenging activity of constituents from two *Scorzonera* species

Luigi Milella [a], Ammar Bader [b], Nunziatina De Tommasi [c,*], Daniela Russo [a], Alessandra Braca [d]

[a] Dipartimento di Scienze, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy
[b] Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, P.O. Box 13174, 21955 Makkah, Saudi Arabia
[c] Dipartimento di Farmacia, Università di Salerno, via Giovanni Paolo II 132, 84084 Fisciano, Italy
[d] Dipartimento di Farmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 17 March 2014
Accepted 19 March 2014
Available online 27 March 2014

Keywords:
Scorzonera papposa DC.
Scorzonera judaica Eig.
Glycosides
Antioxidant activity

ABSTRACT

The aim of this study was to investigate the secondary metabolites content of *Scorzonera papposa* DC., an edible plant eaten in the desert region of Jordan and to assess its antioxidant and free radical-scavenging activity. By using this bioassay-oriented approach nine compounds, including the new natural compounds (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid (**1**), (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid (**2a**), (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid methyl ester (**3**), and (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid (**4**), having the rare deoxy-D-riburonic acid moiety, were isolated. Their structures were elucidated by UV, MS, ^{1}H and ^{13}C NMR and 2D NMR. The antioxidant activity of the *S. papposa* pure compounds and of related derivatives isolated from another *Scorzonera* species (*S. judaica* Eig.) was also tested. The Relative Antioxidant Capacity Index (RACI) was applied as an integrated method to compare the antioxidant activities obtained using different chemical methods.

1. Introduction

Recently, wild edible plants attract a new attention since they represent an important source of food, beverages and natural remedies for several ailments. People of the rural societies are in contact with natural sources more than the urban ones. In the Middle East a narrow number of Bedouins, living in the desert and depending mainly on the pasture of goats and camels, have a good knowledge about edible (Dal Piaz et al., 2009; Malafronte et al., 2012), medicinal (Bader, Braca, De Tommasi, & Morelli, 2003) and aromatic plants (Flamini, Cioni, Morelli, & Bader, 2007). The genus *Scorzonera* includes about 170 species distributed worldwide; it belongs to the family Asteraceae which include many edible plants. This genus has attracted the attention of researchers due to the many chemical classes of its secondary metabolites, including dihydroisocoumarins, stilbenes, lignans, phenolic derivatives (Bader, De Tommasi, Cotugno, & Braca, 2011), phtalides (Sari et al., 2007), coumarins, kavalactones (Jiang, Wang, Lv, & Yue, 2007), sesquiterpenes (Zidorn, 2008), triterpenes (Wang, Li, Qui, & Guan, 2007), and flavonoids (Sareedenchai & Zidorn, 2010). *Scorzonera papposa* DC. is a perennial herb, with cylindrical root, lanceolate leaves with undulate margin, pink showy flowers (Al-Eisawi, 1998). It grows extensively in desert, semi-desert and mountain environments particularly after raining season; all parts of this plant are considered edible and they are eaten raw or cooked (Baily & Danin, 1981; Tukan, Takruri, & Al-Eisawi, 1998); however no previous phytochemical investigation on this species was carried out to date. Since the non economic crops constitute an important source for nutrition mainly in unfavorable condition, the aim of this study was to investigate the secondary metabolites content of *S. papposa* growing in Jordan and to evaluate their antioxidant and free radical-scavenging activities. On the basis of obtained results, some related compounds isolated by our group from another edible *Scorzonera* species (*S. judaica* Eig.) were also tested.

2. Materials and methods

2.1. General

Optical rotations were measured on a Perkin–Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. UV spectra were recorded on a Perkin–Elmer–Lambda spectrophotometer. NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K. All the 2D NMR spectra were

* Corresponding author. Tel.: +39 089 969754; fax: +39 089 969602.
E-mail address: detommasi@unisa.it (N. De Tommasi).

http://dx.doi.org/10.1016/j.foodchem.2014.03.097
0308-8146/

acquired in CD_3OD in the phase-sensitive mode with the transmitter set at the solvent resonance and TPPI (Time Proportional Phase Increment) used to achieve frequency discrimination in the ω_1 dimension. Standard pulse sequences and phase cycling were used for DQF-COSY, TOCSY, HSQC, and HMBC, experiments. HRESIMS were acquired in the positive ion mode on a Q-TOF premier spectrometer equipped with a nanoelectrospray ion source (Waters-Milford, MA, USA). ESIMS were obtained from an LCQ Advantage ThermoFinnigan spectrometer (ThermoFinnigan, USA), equipped with a Xcalibur software. Column chromatography (CC) was performed over Sephadex LH-20 (40–70 μM, Amersham Pharmacia Biotech AB, Uppsala, Sweden). HPLC separations were conducted on a Shimadzu LC-8A series pumping system equipped with a Shimadzu RID-10A refractive index detector and Shimadzu injector on a C_{18} μ-Bondapak column (30 cm × 7.8 mm, 10 μM waters, flow rate 2.0 mL/min). TLC analyses were carried out using glass-coated silica gel 60 F_{254} (0.20 mm thickness) plates (Merck). GC analyses were performed using a Dani GC 1000 instrument on a L-CP-Chirasil-Val column (0.32 mm × 25 m).

2.2. Chemicals

Sodium acetate trihydrate, 2,4,6-tripyridyl-s-triazine (TPTZ), iron (III) chloride ($FeCl_3$ $6H_2O$), Folin–Ciocalteu reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical, β-carotene, linoleic acid, Tween 20, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and gallic acid were purchased from Sigma–Aldrich (Milano-Italy). *n*-Hexane, chloroform, methanol, hydrochloric acid and glacial acetic acid were purchased from Carlo Erba (Milano-Italy). All spectrophotometric measurement were done on a CARY 1E UV–VIS spectrophotometer (Varian, Leini, Italy). All other chemicals and solvents used in this study were of HPLC grade.

2.3. Plant materials

The aerial parts and the tuberous roots of *S. papposa* were collected during the flowering stage in the Dab'a desert reserve (50 km South of Amman), Jordan, during April 2009 and were identified by one of the authors (A. Bader). A voucher specimen (number Jo-It 2009/2) is deposited in herbarium of the laboratory of Pharmacognosy, Umm Al-Qura University, Makkah, Saudi Arabia.

2.4. Extraction and isolation

The aerial parts (300 g) and the roots (250 g) of *S. papposa* were dried and sequentially extracted with solvents of increasing polarity, *n*-hexane, $CHCl_3$, $CHCl_3$–MeOH (9:1), and MeOH by exhaustive maceration (3 × 2 L), to give 4.1, 2.5, 2.7, 18.0 g of the respective residue for the aerial parts and 7.9, 4.6, 5.2, 24.6 g for the roots respectively. Both MeOH extracts were partitioned between *n*-BuOH and H_2O. The *n*-BuOH soluble fraction of aerial parts (1.8 g) was separated by CC using Sephadex LH-20 (3 × 100 cm) with MeOH as eluent at flow rate 0.8 mL/min. Collected fractions were combined into five groups (A–E), based on their TLC profiles. Fractions B (380.7 mg) and C (279.5 mg) were separately purified by RP-HPLC eluting with MeOH–H_2O (3:7) to give compounds **1** (12 mg, t_R = 27 min) and **3** (1.8 mg, t_R = 46 min), from fraction B, and compounds **2a**–**b** (1.0 mg, t_R = 21 min) and **1** (3.6 mg, t_R = 27 min), from fraction C. Fractions D (190 mg) and E (264.6 mg) were separately purified by RP-HPLC eluting with MeOH–H_2O (2:3) to give compounds **8** (1.0 mg, t_R = 13 min) and **9** (1.1 mg, t_R = 18 min), from fraction D, **7** (2.0 mg, t_R = 12 min) and **6** (14.6 mg, t_R = 13 min), from fraction E, respectively. The $CHCl_3$–MeOH extract of aerial parts (1.5 g) was separated by CC using Sephadex LH-20 (3 × 100 cm) with MeOH as eluent at flow rate 0.8 mL/min. Fractions were pooled into four groups (A–D) on the basis of their TLC results. Fractions B (508.2 mg) and C (122.8 mg) were separately purified by RP-HPLC with MeOH–H_2O (3.5:6.5) to give pure compound **4** (1.5 mg, t_R = 37 min), from fraction B and pure compounds **1** (14 mg, t_R = 12 min), **2a**–**b** (3.3 mg, t_R = 16 min), and **4** (3.0 mg, t_R = 37 min), from fraction C, respectively. The *n*-BuOH soluble fraction of roots (2.6 g) was separated by CC using Sephadex LH-20 (3 × 100 cm) with MeOH as eluent at flow rate 0.8 mL/min. Collected fractions were combined into four groups (A–D), based on their TLC profiles. Fraction C (358.8 mg) was subjected to Biotage Isolera column chromatography (25 g silica SNAP cartridge) eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$, at a flow rate 25 mL/min. Compound **5** (9.6 mg) was eluted with $CHCl_3$–MeOH 7:3.

Thunberginol F (**10**), hydramacrophyllol B (**11**), thunberginol F 7-*O*-β-D-glucopyranoside (**12**), hydrangenol 4′-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**13**), hydramacrophyllol A (**14**), hydrangenol (**15**), hydrangenol 8-*O*-β-D-glucopyranoside (**16**), and 3*S*-hydrangenol 4′-*O*-α-L-rhamnopyranoysl-(1→3)-β-D-glucopyranoside (**17**), were purified from *S. judaica* as reported in our previous paper (Bader et al., 2011).

2.4.1. (6-Trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-D-riburonic acid (**1**)

Amorphous powder; UV (MeOH) λ_{max} (log ε) 229 (3.83), 314 (4.01) nm; HRESIMS: *m/z* 481.1335 $[M+Na]^+$, calcd. for $C_{20}H_{26}O_{12}$ *m/z* 458.1424; ESIMS: *m/z* 481 $[M+Na]^+$, 457 $[M-H]^-$, 325 $[M-H-132]^-$; ^1H and ^{13}C NMR data (CD_3OD, 600 MHz) see Table 1.

2.4.2. (6-Cis-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-D-riburonic acid (**2a**)

Amorphous powder; UV (MeOH) λ_{max} (log ε) 230 (3.85), 312 (4.01) nm; HRESIMS: *m/z* 481.1340 $[M + Na]^+$, calcd. for $C_{20}H_{26}O_{12}$ *m/z* 458.1424; ESIMS: *m/z* 481 $[M + Na]^+$, 457 $[M-H]^-$; ^1H and ^{13}C NMR data (CD_3OD, 600 MHz) see Table 1.

2.4.3. (6-Cis-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-D-ribono-γ-lactone (**2b**)

Amorphous powder; UV (MeOH) λ_{max} (log ε) 230 (3.85), 312 (4.01) nm; HRESIMS: *m/z* 463.1225 $[M + Na]^+$, calcd. for $C_{20}H_{24}O_{11}$ *m/z* 440.1319; ESIMS: *m/z* 439 $[M-H]^-$; ^1H NMR (CD_3OD, 600 MHz): δ 2.74 (2H, overlapped signal, H_2–2), 3.21 (1H, dd, *J* = 9.0, 8.0 Hz, H–2′), 3.32 (1H, t, *J* = 9.0 Hz, H–4′), 3.36 (1H, t, *J* = 9.0 Hz, H–3′), 3.53 (1H, m, H–5′), 3.93 (2H, m, H_2–5), 4.30 (1H, dd, *J* = 12.0, 5.0 Hz, H–6′b), 4.40 (1H, d, *J* = 8.0 Hz, H–1′), 4.48 (1H, dd, *J* = 12.0, 3.5 Hz, H–6′a), 4.53 (1H, m, H-3), 4.57 (1H, m, H-4), 5.81 (1H, d, *J* = 12.0 Hz, H-α), 6.89 (1H, d, *J* = 12.0 Hz, H-β), 6.77 (2H, d, *J* = 8.0 Hz, H-3″/H-5″), 7.66 (2H, d, *J* = 8.0 Hz, H-2″/H-6″); ^{13}C NMR (CD_3OD, 600 MHz): δ 38.3 (C-2), 60.3 (C-5), 64.2 (C-6′), 71.2 (C-4′), 75.1 (C-2′), 75.4 (C-5′), 77.2 (C-3), 77.6 (C-3′), 84.6 (C-4), 105.1 (C-1′), 115.6 (C-3″/C-5″), 116.0 (C-α), 128.0 (C-1″), 133.5 (C-2″/C-6″), 145.2 (C-β), 159.8 (C-4″),168.5 (COO), 177.0 (C-1).

2.4.4. (6-Trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-D-riburonic acid methyl ester (**3**)

Amorphous powder; $[\alpha]_D^{25}$ +17.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.80), 315 (4.05) nm; HRESIMS: *m/z* 495.1490 $[M + Na]^+$, calcd. for $C_{21}H_{28}O_{12}$ *m/z* 472.1581; ESIMS: *m/z* 495 $[M + Na]^+$, 471 $[M-H]^-$, 325 $[M-H-146]^-$; ^1H and ^{13}C NMR data (CD_3OD, 600 MHz) see Table 1.

2.4.5. (6-Trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid (**4**)

Amorphous powder; UV (MeOH) λ_{max} (log ε) 230 (3.80), 315 (4.05) nm; HRESIMS: *m/z* 523.1443 $[M + Na]^+$, calcd. for $C_{22}H_{28}O_{13}$ *m/z* 500.1530; ESIMS: *m/z* 499 $[M-H]^-$, 325 $[M-H-174]^-$; ^1H and ^{13}C NMR data (CD_3OD, 600 MHz) see Table 1.

Table 1
^{1}H and ^{13}C NMR data of compounds **1–4** (CD_3OD. 600 MHz).[a]

	1		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		173.4		174.0		172.0		173.0
2	2.72[b]	39.0	2.74[b]	38.3	2.75[b]	38.2	2.74[b]	38.0
3	4.23 m	78.8	4.28 m	78.0	4.22 m	78.0	4.55 m	77.3
4	3.73 m	74.3	3.65 m	74.2	3.65 m	74.0	3.60 m	74.1
5a	3.67[b]	63.4	3.58[b]	64.3	3.70[b]	63.0	4.24[b]	63.4
5b	3.64[b]		3.55[b]		3.60[b]		3.71[b]	
OMe					3.55 s	51.9		
$\underline{COO}CH_3$								172.0
$COO\underline{CH_3}$							2.12 s	20.6
Glc 1′	4.48 d (7.8)	104.9	4.36 d (8.0)	105.1	4.44 d (8.0)	105.3	4.44 d (8.0)	105.0
2′	3.25 dd (9.0. 7.8)	74.5	3.20 dd (9.0. 8.0)	74.9	3.22 dd (9.0. 8.0)	75.0	3.22 dd (9.0. 8.0)	74.7
3′	3.39 t (9.0)	77.2	3.33 t (9.0)	77.5	3.37 t (9.0)	77.3	3.37 t (9.0)	74.4
4′	3.37 t (9.0)	71.4	3.34 t (9.0)	71.0	3.34 t (9.0)	71.4	3.32 t (9.0)	71.3
5′	3.50 m	75.2	3.49 m	75.0	3.47 m	75.1	3.54 m	75.2
6′a	4.51 dd (12.0. 3.0)	64.3	4.46 dd (12.0. 3.5)	64.2	4.46 dd (12.0. 3.5)	64.2	4.53 dd (12.0. 3.5)	64.2
6′b	4.35 dd (12.0. 5.0)		4.29 dd (12.0. 5.0)		4.33 dd (12.0. 5.0)		4.34 dd (12.0. 5.0)	
p-Coumaroyl								
1″		127.6		128.0		127.9		126.7
2″/6″	7.48 d (8.0)	130.9	7.66 d (8.0)	133.5	7.49 d (8.0)	131.1	7.48 d (8.0)	130.9
3″/5″	6.81 d (8.0)	116.4	6.77 d (8.0)	115.6	6.84 d (8.0)	116.6	6.84 d (8.0)	116.6
4″		160.2		159.8		160.2		161.1
α	6.38 d (16.0)	114.7	5.81 d (12.0)	116.0	6.36 d (12.0)	114.6	6.40 d (12.0)	114.5
β	7.66 d (16.0)	146.6	6.89 d (12.0)	145.2	7.66 d (12.0)	146.4	7.68 d (12.0)	146.0
COO		169.2		168.5		168.8		169.0

[a] *J* values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by DQF-COSY, 1D-TOCSY, HSQC, and HMBC experiments.
[b] Overlapped signal.

2.5. Acid hydrolysis of compounds *1–4*

A solution of each compound (2.0 mg) in 1 N HCl (1 mL) was stirred at 80 °C in a stoppered reaction vial for 4 h. After cooling, the solution was evaporated under a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.2 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution, the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was analyzed by GC using a L-CP-Chirasil-Val column (0.32 mm × 25 m). Temperatures of both the injector and detector was 200 °C. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5 °C/min. Peak of the hydrolysate was detected by comparison with retention times of authentic sample of D-glucose (Sigma Aldrich) after treatment with 1-(trimethylsilyl)imidazole in pyridine.

2.6. Antioxidant activity assays

2.6.1. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out as described by Russo, Bonomo, Salzano, Martelli, and Milella (2012) with slight modifications. The FRAP reagent was made fresh before each experiment, and it was prepared by mixing 300 mM acetate buffer in distilled water pH 3.6, 20 mM $FeCl_3$ $6H_2O$ in distilled water and 10 mM TPTZ in 40 mM HCl in a proportion of 10:1:1. For each sample 150 μL of appropriately diluted sample (150 μL of methanol for the blank) and 1350 μL of FRAP reagent was added and incubated at 37 °C for 40 min in the dark. Absorbance of resulting solution was measured at 593 nm. Trolox was used as a reference antioxidant standard. FRAP values were expressed as mg Trolox equivalents (mg TE)/g of sample. Each reaction was performed in triplicate.

2.6.2. β-Carotene bleaching assay

The antioxidant activity was evaluated by the β-carotene-linoleic acid bleaching method (BCB) (Russo et al., 2012). β-Carotene solution (0.2 mg of β-carotene dissolved in 0.2 mL of chloroform), linoleic acid (20 mg) and Tween 20 (200 mg) were mixed. Chloroform was removed by using rotary evaporator at room temperature. Distilled water (50 mL) was added with oxygen, then 9.5 mL of the emulsion were transferred into several tubes containing 0.5 mL of sample (the final concentration for all tested samples was 0.1 mg/mL) or methanol as blank. BHT was used as positive control. The tubes were placed at 50 °C for 3 h. The absorbance was measured at 470 nm at 0′, 30′, 60′, 90′, 120′, 150′ and 180′. Each sample was carried out in triplicate. Results were expressed as percentage of β-carotene bleaching inhibition and calculated as follows: $(A_{\beta\text{-carotene after 180 min}}/A_{\text{initial }\beta\text{-carotene}}) \times 100$ (AA%).

2.6.3. DPPH radical-scavenging activity

The DPPH assay was used to measure radical scavenging activity of extracts and fractions. The ability to scavenge the DPPH free radical was monitored according to the method reported by Fernandes et al. (2013) with slight modifications. All samples were tested individually at different concentrations by addition to a methanolic solution of DPPH radical (100 μM). For each measure 300 μL appropriately diluted sample was added to 1200 μL of DPPH reagent, the mixtures were stirred and allowed to stand in the dark at room temperature. In the control 300 μL of methanol, instead diluted sample, was added to 1200 μL of DPPH solution. The absorbance of the resulting solutions was measured at 515 nm after 30′. In all experiments Trolox radical scavenging activity was also determined and used as a reference. Sample activity was expressed as mg TE/g of sample (Padula et al., 2013). Each reaction was performed in triplicate.

2.6.4. Total polyphenols

Total polyphenolic content (TPC) was determined according to the Folin–Ciocalteu procedure (Milella et al., 2011) by adding 75 μL of the diluted samples (in the blank 75 μL of methanol) to 425 μL of distilled water, 500 μL of Folin–Ciocalteu reagent and 500 μL of a sodium carbonate aqueous solution (10% w/v). The

mixture was stirred and left in the dark for 60 min then absorbance was measured at 723 nm. Gallic acid was used as reference standard and TPC was expressed as mg gallic acid equivalents (GAE)/g of sample. Each reaction was performed in triplicate.

3. Results and discussion

3.1. Screening of extract antioxidant activity

The aerial parts (L) and roots (R) of *S. papposa* were sequentially extracted with solvent of increasing polarity yielding *n*-hexane, chloroform, chloroform–methanol 9:1, and methanol extracts, in the amounts of 4.1, 2.5, 2.7, 18.0 g for aerial parts and 7.9, 4.6, 5.2, 24.6 g for roots, respectively. The methanol residues were both partitioned between *n*-BuOH and water, obtaining a *n*-butanol fraction. All extracts were submitted to the BCB and FRAP test for a preliminary screening of antioxidant activity (data not shown). The extracts that demonstrated significant activities were the two *n*-butanol (BuL and BuR), the $CHCl_3$ (CL) and the $CHCl_3$/MeOH (CML) extracts of the aerial parts, while no significant effect was observed for all other extracts. Thus, to confirm and deepen the antioxidant activity of the four active extracts, BCB, FRAP, DPPH, and Folin Ciocalteu test were performed (Table 2). A new concept, Relative Antioxidant Capacity Index (RACI) was applied integrating antioxidant capacity data determined by several methods. The data of BCB, FRAP, DPPH, and TPC were used to calculate the RACI, that allows the comparison of phytocomplex antioxidant capacity derived from different chemical methods. TPC results were included in RACI calculation since phenolics can act with other mechanisms (not measurable with our tests) and can contribute significantly to phytocomplex health promoting value; moreover it was recently proposed that results obtained by Folin–Ciocalteu procedure could be also interpreted as an alternative way to measure the total reducing capacity of extracts as the reagent reacts with any reducing substance (Fernandes et al., 2013). In this way RACI provided a more comprehensive assessment of the whole antioxidant potential. Results (Fig. 1 and Table 2) showed BuR as the most active extract, followed by BuL and CML.

3.2. Bioassay-oriented fractionation of the active extracts

The three extracts that showed a significant activity were separated by Sephadex LH-20 column chromatography collecting four main fractions from BuR, five fractions from BuL, and four fractions from CML extracts, respectively. Fractions were evaluated for their TPC and antioxidant activities (Table 2). Results showed that among CML fractions, C demonstrated to be the one with the highest activity, with FRAP data four times higher than fractions A and B (Table 2). A single assay cannot determine completely the antioxidant activity of a phytocomplex, thus different approaches are needed to understand the biological activity of complex mixture of secondary metabolites (Gironés-Vilaplana et al., 2012). For this reason the antioxidant activity was tested by using two complementary systems: DPPH and β-carotene bleaching assays. Fraction C from CML was two times more active than fractions A and B in DPPH and BCB tests, confirming the FRAP results. Fraction D showed no significant activity (data not shown). RACI was used to compare the antioxidant potential of fractions A–C and as shown in Fig. 2a, fractions B and C, demonstrating values higher than −0.50, were submitted to HPLC separation. Five fractions were obtained from BuL extract. TPC was significantly different among fractions, ranging from 80.7 (fraction A) to 535.1 (fraction E) mg GAE/g (Table 2). Moreover, the obtained antioxidant activity from DPPH, FRAP, and BCB (Table 2) showed variability in the antioxidant capacities of BuL fractions. This variability could be due to different mechanism of action of each assay and to the variety of fraction chemical components. Thus, on the basis of the RACI (higher than −0.5, Fig. 2b) we selected the active fractions for further phytochemical analysis. Four fractions were obtained from BuR extract. Fraction C showed the highest DPPH value (1186.3 mg TE/g) and the highest FRAP and TPC values, while

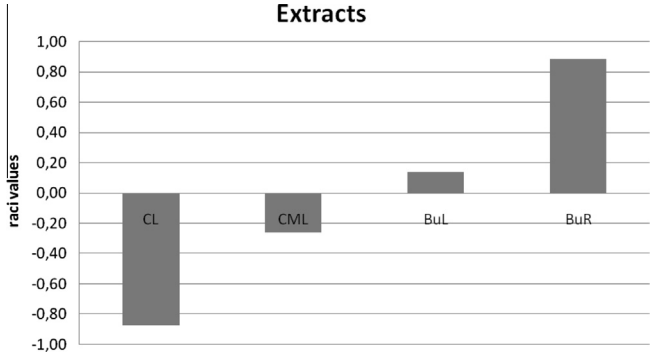

Fig. 1. RACI of *S. papposa* active extracts: $CHCl_3$ aerial parts (CL); $CHCl_3$/MeOH aerial parts (CML); *n*-BuOH aerial parts (BuL); *n*-BuOH roots (BuR).

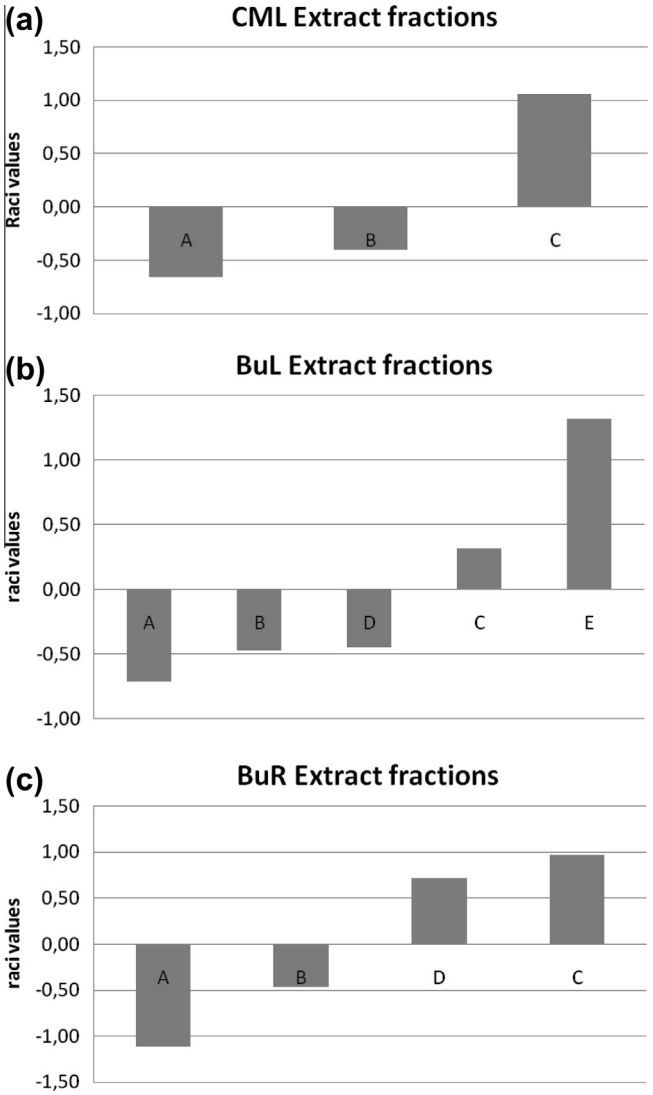

Fig. 2. RACI of *S. papposa* fractions coming from: (a) $CHCl_3$/MeOH aerial parts extract (CML); (b) *n*-BuOH aerial parts extract (BuL); (c) *n*-BuOH roots extracts (BuR).

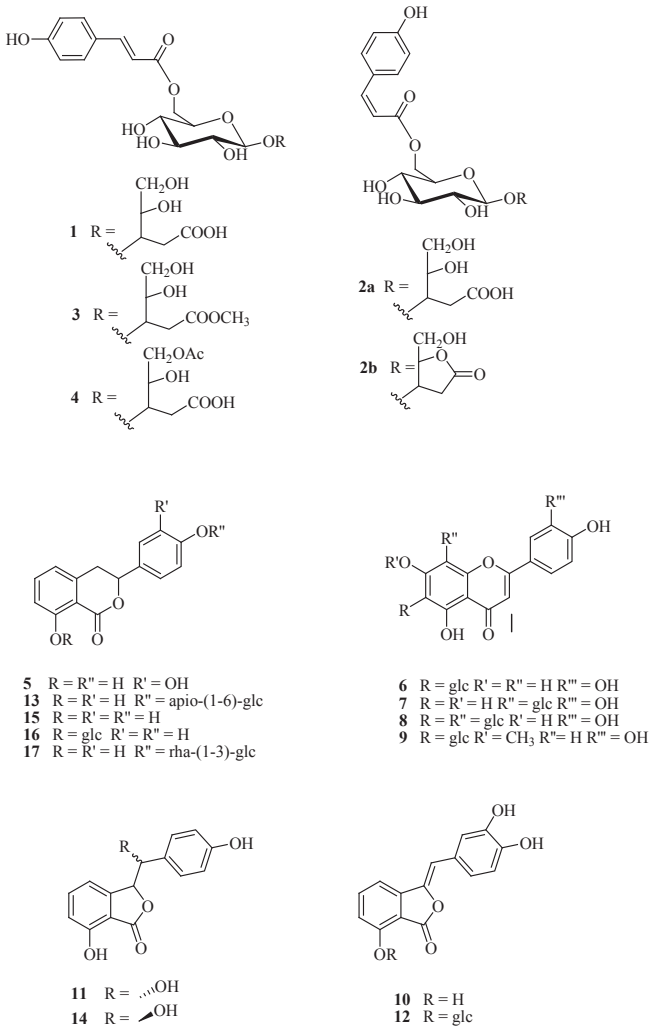

Fig. 3. Chemical structure of compounds isolated from *S. papposa* and *S. judaica*.

fraction D demonstrated the best results in BCB test (64.3%). On the basis of RACI (Fig. 2c) the active fractions C and D were submitted to chromatographic separation by RP-HPLC. Unfortunately, the chromatographic separation attempts performed to purify fraction D components failed due to the presence of high polymerized polyphenols. The phytochemical study of *S. papposa* active fractions yielded nine compounds (Fig. 3) of which four were new natural products (**1**–**4**).

3.3. Chemical compounds identification

Compound **1** NMR and MS data demonstrated the molecular formula $C_{20}H_{26}O_{12}$ (HRESIMS at *m*/*z* 481.1335 $[M + Na]^+$). The ^1H and ^{13}C NMR spectra (Table 1) showed typical signals of a *trans*-double bond [H-α (δ 6.38, 1H, d, *J* = 16.0 Hz), H-β (δ 7.66, 1H, d, *J* = 16.0 Hz), C-α (δ 114.7), C-β (δ 146.6)]. The presence of a symmetrical 1,4-disubstituted phenyl group was deduced from the proton signals at δ 6.81 (2H, d, *J* = 8.0 Hz, H-3″/H-5″) and 7.48 (2H, d, *J* = 8.0 Hz, H-2″/H-6″). Furthermore, the HMBC correlations of H-2″/C-1″, C-4″, C-5″, and H-β/COO, C-2″, C-3″ revealed the presence of a *trans*-*p*-coumaroyl moiety. The ^{13}C NMR spectrum of **1** (Table 1) showed 11 other carbon signals including one anomeric carbon signal at δ 104.9 suggesting the presence of a sugar moiety. 1D-TOCSY and DQF-COSY led to establish two spin systems: H-1–H_2-6 attributable to a hexose residue and H_2-2–H_2-5 suggesting a polyalchol moiety. Hydrolysis of **1** with 1 N HCl yielded D-glucose as determined by GC of its trimethylsilylated derivatives on a chiral column. The polyalchol moiety was elucidated as 2-deoxy-D-riburonic acid by studying the remaining ^1H and ^{13}C NMR signals and by the correlations from the HSQC and HMBC spectra; this was confirmed also by a peak observed in the ESIMS spectra at *m*/*z* 325 $[M–H-132]^-$ (Hiradate, Morita, Sugie, Fujii, & Harada, 2004). The substituent position was achieved from the HMBC correlations of H-6′/COO, H-1′/C-3, and H-4/C-1′ suggesting that the *trans*-*p*-coumaroyl moiety was attached at C-6′ and the β-D-glucopyranose was established at C-3 of 2-deoxy-D-riburonic acid. On the basis of the above evidence, the structure of **1** was elucidated as (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid.

Compound **2a** was assigned molecular formula $C_{20}H_{26}O_{12}$ (HRESIMS at *m*/*z* 481.1340 $[M + Na]^+$), resulting to be an isomer of **1**. The spectral data of compound **2a** indicated the same skeleton of **1**. Comparison of NMR spectra of **2a** with those of **1** showed that **2a** differed from **1** only in the signals due to the ester moiety linked at C-6′ being a *cis*-*p*-coumaroyl instead of a *trans*-*p*-coumaroyl group. Moreover in the NMR spectra of **2a** were present also signals due to a 2-deoxy-D-ribono-1,4-lactone (Table 1 and Section 2) (Dong, Shi, Wu, & Tu, 2007). The signals of the 2-deoxy-D-riburonic acid were present both in its open form and closed γ-lactone ring, indicating that both forms were in inseparable mixture. Thus, **2a** and **2b** were identified as (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid and (6-*cis*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-ribono-γ-lactone, respectively.

Compound **3** was obtained as an amorphous powder with molecular formula of $C_{21}H_{28}O_{12}$ by analysis of its HRESIMS spectrum (*m*/*z* 495.1490 $[M + Na]^+$). Comparison of its NMR spectra (Table 1) with those of **1** showed that **3** differed in the signals due to the 2-deoxy-D-riburonic acid moiety. Analysis of spectroscopic data showed the presence of an additional methoxyl group esterified at C-1. Thus, **3** was identified as (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-2-deoxy-D-riburonic acid methyl ester.

Compound **4** had the molecular formula $C_{22}H_{28}O_{13}$ (HRESIMS at *m*/*z* 523.1443 $[M + Na]^+$). The ESIMS spectrum showed an $[M–H]^-$ ion at *m*/*z* 499 $[M–H]^-$ that was 42 uma higher than that of **1** and a fragment ion at *m*/*z* 325 $[M–H-174]^-$ was also observed. These data were compatible with the presence of an additional acetyl group in **4**. Its ^1H NMR spectrum was very similar to that of **1** with the main difference being a downfield shift of H_2-5 (δ 3.71 and 4.24 in **4** versus δ 3.64 and 3.67 in **1**) and a signal at δ 2.12 (3H, s) for one acetyl group, indicating that **4** was esterified with an acetyl group at C-5 (Table 1). Therefore, the structure of **4** was determined to be (6-*trans*-*p*-coumaroyl)-3-*O*-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-D-riburonic acid.

Compounds **5**–**9** were identified as thunberginol G (**5**) (Mandal & Roy, 2008), isoorientin (**6**) (Kato & Morita, 1990), orientin (**7**) (Kato & Morita, 1990), isoschaftoside (**8**) (Xie, Veitch, Houghton, & Simmonds, 2003), and swertiajaponin (**9**) (Borøy, Rayyan, Fossen, Chalberg, & Andersen, 2009) by comparison of their NMR and MS literature data.

3.4. Antioxidant activity of pure compounds

Antioxidant activity and RACI of isolated compounds were measured (Table 3). Compound **5** showed the highest AA and FRAP value (46.1% and 82.6 mg TE/g, respectively) followed by **6** (47.7% and 60.9 mg TE/g, respectively). Among new natural compounds **1**–**4**, only **3** showed a weak activity on BCB test. It was expected that new compounds were not so efficient on the basis of their chemical structure, because it was previously demonstrated that on BCB test the affinity of the antioxidant for the lipid and thus the lipophilic nature of the molecules proved to be the determining factor (von Gadow, Joubert, & Hansmann, 1997). Compounds **8**–**9** did not dem-

Table 2
Total phenolic content and antioxidant activity of *S. papposa* extracts and fractions using DPPH, BCB, FRAP, and Folin assays.

Sample	Test			
Extracts	DPPH mg TE/g*	BCB %AA**	FRAP mg TE/g*	Folin mg GAE/g***
$CHCl_3$ aerial parts (CL)	5.2 ± 0.7	40.4 ± 2.4	10.3 ± 0.9	54.5 ± 3.5
$CHCl_3$/MeOH aerial parts (CML)	37.0 ± 02.4	57.9 ± 3.7	11.2 ± 1.2	65.3 ± 4.7
n-BuOH aerial parts (BuL)	58.3 ± 03.5	52.4 ± 2.6	53.2 ± 5.7	94.0 ± 8.1
n-BuOH roots (BuR)	261.5 ± 10.5	59.6 ± 3.2	158.9 ± 9.8	101.2 ± 8.7
BuL fractions				
A	15.8 ± 1.1	55.2 ± 2.9	20.7 ± 3.1	80.7 ± 5.4
B	64.9 ± 4.5	53.2 ± 2.4	60.9 ± 4.7	238.0 ± 9.8
C	171.6 ± 8.7	50.7 ± 2.6	205.0 ± 11.2	533.9 ± 21.5
D	249.4 ± 10.8	43.9 ± 1.9	157.7 ± 9.1	276.9 ± 12.5
E	439.5 ± 21.4	55.4 ± 3.1	418.8 ± 25.4	535.1 ± 25.8
CML fractions				
A	6.5 ± 0.5	34.0 ± 1.6	5.5 ± 0.5	51.1 ± 3.4
B	14.4 ± 1.1	32.6 ± 1.9	5.6 ± 0.6	38.9 ± 3.1
C	16.8 ± 0.8	50.6 ± 3.5	19.5 ± 1.1	116.1 ± 7.8
BuR fractions				
A	319.9 ± 23.4	60.2 ± 3.3	315.6 ± 18.7	724.8 ± 41.2
B	545.5 ± 41.2	49.9 ± 2.8	594.6 ± 30.9	573.9 ± 36.8
C	1186.3 ± 71.7	61.5 ± 3.9	836.0 ± 48.2	1480.1 ± 81.5
D	1080.5 ± 75.8	642.7 ± 29.9	570.9 ± 42.6	360.8 ± 18.5

Values are the mean of three determinations ($P < 0.05$).
* Milligrams of Trolox Equivalents per g of extract/fraction.
** Antioxidant activity.
*** Milligrams of gallic acid equivalents per g of extract/fraction.

Table 3
Antioxidant activity of pure compounds using BCB and FRAP assays and RACI.

Test			
Compound	BCB %AA*	FRAP mg TE/g**	RACI
3	43.6 ± 3.1	11.6 ± 0.9	−0.27
5	46.1 ± 2.9	82.6 ± 5.1	0.17
6	47.7 ± 2.5	60.9 ± 4.5	0.12
7	42.8 ± 2.7	26.9 ± 1.9	−0.23
8	30.4 ± 1.7	16.0 ± 1.3	−0.71
9	18.9 ± 1.2	51.9 ± 1.9	−0.93
10	52.7 ± 4.1	383.8 ± 11.2	1.93
11	62.4 ± 4.1	165.0 ± 7.5	1.16
12	51.8 ± 2.4	130.4 ± 5.2	0.62
13	55.3 ± 2.7	7.4 ± 0.5	0.45
14	46.2 ± 2.5	19.0 ± 0.9	−0.15
15	40.5 ± 2.1	9.2 ± 0.7	−0.40
16	28.3 ± 2.0	80.9 ± 5.1	−0.46
17	11.6 ± 1.1	0.3 ± 0.0	−1.31

Values are the mean of three determinations ($P < 0.05$).
* Antioxidant activity.
** Milligrams of Trolox equivalents per g of pure compound.

onstrate to be the major contributors to *S. papposa* extracts activity. It is evident that the potential of *S. papposa* pure compounds is definitely lower than the fraction from they come from (Tables 2 and 3). Due to the activity showed by compound **5** and few literature data on antioxidant activity of dihydroisocoumarins we also evaluated the activity of related compounds **10**–**17** isolated by our group from edible *S. judaica* (Bader et al., 2011). Compounds **10**–**13** showed the highest BCB values (AA higher than 50%), while compounds **10**–**12** were the most active in the FRAP test. On the basis of RACI the phtalides **10**–**12** showed the highest value followed by dihydroisocoumarins **5** and **13**.

4. Conclusions

The bioassay oriented protocol supported by Relative Antioxidant Capacity Index (RACI) allowed the isolation from *S. papposa* polar extracts of nine compounds, of which four were new. The structure of the new compounds presented a rare deoxy-D-riburonic moiety. The measured antioxidant activity showed different values among diverse methods in accordance with findings previously obtained (Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009) where it was demonstrated that different antioxidant methods can give back sensible differences even due to the multiple reaction mechanism and different phase locations involved in the measuring of antioxidant capacity of complex plant extracts and derivatives. According to Sun and Tanumihardjo (2007) we used a RACI as an approach to compare chemical antioxidant assays. The key advantage of RACI is that it is a numeric scale integrating multiple chemical method allowing comparison antioxidant capacity of food, extracts, fractions, and pure compounds. We could hypothesize that the antioxidant activity of *S. papposa* extracts and fractions may be due to the presence of a combination of compounds acting synergistically or as vehicle enhancing the biological activity. However, the antioxidant activity of phtalides and dihydroisocoumarins suggest these classes of compounds for further investigations.

Acknowledgement

Authors are grateful to Immacolata Faraone, Domenico Frescura and Giovanna Imbrenda for their skillful assistance.

References

Al-Eisawi, D. M. (1998). *Field guide to wild flowers of Jordan and neighbouring countries*. Amman: The National Library (p. 117).
Bader, A., Braca, A., De Tommasi, N., & Morelli, I. (2003). Further constituents from *Caralluma negevensis*. *Phytochemistry, 62*, 1277–1281.
Bader, A., De Tommasi, N., Cotugno, R., & Braca, A. (2011). Phenolic compounds from the roots of the Jordanian Viper's Grass *Scorzonera judaica*. *Journal of Natural Products, 74*, 1421–1427.
Baily, C., & Danin, A. (1981). Bedouin plant utilization in Sinai and the Negev. *Economic Botany, 35*, 145–162.
Borøy, Ø., Rayyan, S., Fossen, T., Chalberg, K., & Andersen, O. M. (2009). *C*-glycosylanthocyanidins synthesized from *C*-glycosylflavones. *Phytochemistry, 70*, 278–287.

Dal Piaz, V., Vassallo, A., Lepore, L., Tosco, A., Bader, A., & De Tommasi, N. (2009). Sesterterpenes as tubulin tyrosine ligase inhibitors. First insight of structure–activity relationships and discovery of new lead. *Journal of Medicinal Chemistry, 52*, 3814–3828.

Dong, C., Shi, S., Wu, K., & Tu, P. (2007). Chemical constituents from roots and rhizomes of *Clematis hexapetala* Pall. *Zeitschrift fur Naturforschung, 62B*, 854–858.

Fernandes, F., Ramalhosa, E., Pires, P., Verdial, J., Valentão, P., Andrade, P., et al. (2013). *Vitis vinifera* leaves towards bioactivity. *Industrial Crops and Products, 43*, 434–440.

Flamini, G., Cioni, P. L., Morelli, I., & Bader, A. (2007). Essential oils of the aerial parts of three *Salvia* species from Jordan: *Salvia lanigera*, *S. spinosa* and *S. syriaca*. *Food Chemistry, 100*, 732–735.

Gironés-Vilaplana, A., Valentão, P., Andrade, P. B., Ferreres, F., Moreno, D. A., & García-Viguera, C. (2012). Phytochemical profile of a blend of black chokeberry and lemon juice with cholinesterase inhibitory effect and antioxidant potential. *Food Chemistry, 134*, 2090–2096.

Hiradate, S., Morita, S., Sugie, H., Fujii, Y., & Harada, J. (2004). Phytotoxic *cis*-cinnamoyl glucosides from *Spiraea thunbergii*. *Phytochemistry, 65*, 731–739.

Jiang, T. F., Wang, Y. H., Lv, Z. H., & Yue, M. E. (2007). Determination of kava lactones and flavonoid glycoside in *Scorzonera austriaca* by capillary zone electrophoresis. *Journal of Pharmaceutical and Biomedical Analysis, 43*, 854–858.

Kato, T., & Morita, Y. (1990). *C*-glycosylflavones with acetyl substitution from *Rumex acetosa* L. *Chemical & Pharmaceutical Bulletin, 38*, 2277–2280.

Malafronte, N., Vassallo, A., Dal Piaz, F., Bader, A., Braca, A., & De Tommasi, N. (2012). Biflavonoids from *Daphne linearifolia* Hart. *Phytochemistry Letters, 6*, 621–625.

Mandal, S. K., & Roy, S. C. (2008). Titanocene (III) chloride mediated radical-induced synthesis of 3,4-dihydroisocoumarins: synthesis of (±)-hydrangenol, (±)-phyllodulcin, (±)-macrophyllol and (±)-thunberginol G. *Tetrahedron, 64*, 11050–11057.

Milella, L., Caruso, M., Galgano, F., Favati, F., Padula, M. C., & Martelli, G. (2011). Role of the cultivar for choosing Clementine fruits with high level of health-promoting compounds. *Journal of Agricultural and Food Chemistry, 59*, 5293–5298.

Padula, M. C., Lepore, L., Milella, L., Ovesna, J., Malafronte, N., Martelli, G., et al. (2013). Cultivar based selection and genetic analysis of strawberry fruits with high levels of health promoting compounds. *Food Chemistry, 140*, 639–646.

Russo, D., Bonomo, M. G., Salzano, G., Martelli, G., & Milella, L. (2012). Nutraceutical properties of *Citrus clementina* juices. *Pharmacologyonline SPL, 1*, 84–93.

Sareedenchai, V., & Zidorn, C. (2010). Flavonoids as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Biochemical Systematics and Ecology, 38*, 935–957.

Sari, A., Zidorn, C., Ellmerer, L. P., Özgökçe, F., Onganiac, K. H., & Stuppner, H. (2007). Phenolic compounds from *Scorzonera tomentosa* L. *Helvetica Chimica Acta, 90*, 311–317.

Sun, T., & Tanumihardjo, S. A. (2007). An integrated approach to evaluate food antioxidant capacity. *Journal of Food Science, 72*, R159–R165.

Tabart, J., Kevers, C., Pincemail, J., Defraigne, J.-O., & Dommes, J. (2009). Comparative antioxidant capacities of phenolic compounds measured by various tests. *Food Chemistry, 113*, 1226–1233.

Tukan, S. K., Takruri, H. R., & Al-Eisawi, D. M. (1998). The use of wild edible plants in the Jordanian diet. *International Journal of Food Sciences and Nutrition, 49*, 225–235.

von Gadow, A., Joubert, E., & Hansmann, C. H. (1997). Comparison of the antioxidant activity of Aspalathin with that of other plant phenols of Rooibos Tea (*Aspalathus linearis*), α-Tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry, 45*, 632–638.

Wang, B., Li, G. Q., Qui, P. J., & Guan, H. S. (2007). Two new oleane-type triterpene fatty esters from *Scorzonera mongolica*. *Chinese Chemical Letters, 18*, 708–710.

Xie, C., Veitch, N. C., Houghton, P. J., & Simmonds, M. S. J. (2003). Flavone *C*-glycosides from *Viola yedoensis* Makino. *Chemical & Pharmaceutical Bulletin, 51*, 1204–1207.

Zidorn, C. (2008). Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae. *Phytochemistry, 69*, 2270–2296.