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

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A B S T R A C T

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 natural compounds (6-trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-o-riburonic acid (1), (6-cis-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-o-riburonic acid (2a), (6-trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-2-deoxy-o-riburonic acid methyl ester (3), and (6-trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-(5-acetyl)-2-deoxy-o-riburonic acid (4), having the rare deoxy-o-riburonic acid moiety, were isolated. Their structures were elucidated by UV, MS, 1H and 13C 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.

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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 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 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 contact with natural sources more than the urban ones.

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 lancelate 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...
acquired in CD$_3$OD 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 $\omega_1$ 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 an Xcalibur software. Column chromatography (CC) was performed over Sephadex LH-20 (3 × 100 cm) with MeOH as eluent at flow rate 0.8 mL/min. TLC analyses were performed using a Dani GC 1000 instrument on aL-CP-Chira-Val column (0.32 mm × 25 m).

2.2. Chemicals

Sodium acetate trihydrate, 2,4,6-tripryridyl-s-triazine (TPTZ), iron (III) chloride (FeCl$_3$·6H$_2$O), 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$_3$, CH$_2$Cl$_2$–MeOH (7:3) 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$_2$O. 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 results. Fractions B (508.2 mg) and C (122.8 mg) were separately purified by RP-HPLC with MeOH–H$_2$O (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 Isola column chromatography (25 g silica SNAP cartridge) eluting with CH$_2$Cl$_2$ 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), hydradmacrophyllol B (11), thunberginol F 7-β-δ-glucopyranoside (12), hydrangenol 4′-O-β-D-apiofuranosyl-(1→6)-δ-glucopyranoside (13), hydramacrophyllol A (14), hydrangelon (15), hydrangenol 8-β-δ-glucopyranoside (16), and 35-hydrangelon 4′-O-α-L-rhamnopyranosyl-(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)-3-0-β-δ-glucopyranosyl-2-deoxy-o-riburonic acid (1)

Amorphous powder; UV (MeOH) $\lambda_{max} (log\varepsilon) = 229 (3.83), 314 (4.01)$ nm; HRESIMS: $m/z$ 481.1335 [M+Na]$^+$, calcd. for C$_{20}$H$_{25}$O$_{12}$ $m/z$ 485.1424; ESIMS: $m/z$ 481 [M+Na]$^+$, 457 [M–H]$^–$, 325 [M–H–132]$^–$; $^1$H and $^{13}$C NMR data (CD$_3$OD, 600 MHz) see Table 1.

2.4.2. (6-Cis-p-coumaroyl)-3-0-β-δ-glucopyranosyl-2-deoxy-o-riburonic acid (2a)

Amorphous powder; UV (MeOH) $\lambda_{max} (log\varepsilon)$ 230 (3.85), 312 (4.01) nm; HRESIMS: $m/z$ 481.1340 [M+Na]$^+$, calcd. for C$_{20}$H$_{25}$O$_{12}$ $m/z$ 485.1424; ESIMS: $m/z$ 481 [M+Na]$^+$, 457 [M–H]$^–$. $^1$H and $^{13}$C NMR data (CD$_3$OD, 600 MHz) see Table 1.

2.4.3. (6-Cis-p-coumaroyl)-3-0-β-δ-glucopyranosyl-2-deoxy-o-riburonic acid methyl ester (3)

Amorphous powder; $\lambda_{max} (log\varepsilon) +17.3 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ 230 (3.80), 315 (4.05) nm; HRESIMS: $m/z$ 495.1490 [M+Na]$^+$, calcd. for C$_{21}$H$_{26}$O$_{13}$ $m/z$ 472.1581; ESIMS: $m/z$ 495 [M+Na]$^+$, 471 [M–H]$^–$, 325 [M–H–146]$^–$; $^1$H and $^{13}$C NMR data (CD$_3$OD, 600 MHz) see Table 1.

2.4.4. (6-Trans-p-coumaroyl)-3-0-β-δ-glucopyranosyl-2-deoxy-o-riburonic acid methyl ester (4)

Amorphous powder; UV (MeOH) $\lambda_{max} (log\varepsilon)$ 230 (3.80), 315 (4.05) nm; HRESIMS: $m/z$ 523.1443 [M+Na]$^+$, calcd. for C$_{22}$H$_{27}$O$_{13}$ $m/z$ 500.1530; ESIMS: $m/z$ 499 [M–H]$^–$, 325 [M–H–174]$^–$; $^1$H and $^{13}$C NMR data (CD$_3$OD, 600 MHz) see Table 1.
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 N₂. The residue was partitioned between H₂O and CHCl₃. The CHCl₃ layer was analyzed by GC using a L-CP-Chirasil-6H₂-leic acid bleaching method (BCB) (Russo et al., 2012).

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 FeCl₃ and 500 mM acetate buffer in distilled water pH 4.0 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: \( \frac{A_{\text{β-carotene after 180 min}} - A_{\text{initial β-carotene}}} {A_{\text{initial β-carotene}}} \times 100 \) (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 a sodium carbonate aqueous solution (10% w/v). 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. 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

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Table 1

| 1H and 13C NMR data of compounds 1–4 (CD3OD, 600 MHz).\(^a\) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( ^1 \)H   | \( ^1 \)C      | \( ^1 \)H      | \( ^1 \)C      | \( ^1 \)H     | \( ^1 \)C     | \( ^1 \)H     | \( ^1 \)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\(^b\)      | 3.53\(^b\)      | 3.60\(^b\)      | 3.71\(^b\)      |              |              |              |              |
| COCH₃         | 2.12 s          | 20.6            |
| COOCH₂        | 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) | 64.2          | 4.29 dd (12.0, 5.0) | 64.2          | 4.33 dd (12.0, 5.0) | 64.2          | 4.34 dd (12.0, 5.0) | 64.2          |
| \( p \)-Coumaroyl | 127.6          | 128.0           | 127.9           | 126.7           |
| 1’           | 127.6           | 132.5           | 131.1           | 130.9           |
| 2’/6’         | 7.48 d (8.0)    | 130.9           | 7.66 d (8.0)    | 133.5           | 7.48 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           |
| \( \alpha \)  | 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         |
| \( \beta \)   | 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           |

\(^a\) 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.
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 CHCl₃ (CL) and the CHCl₃/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: CHCl₃ aerial parts (CL); CHCl₃/MeOH aerial parts (CML); *n*-BuOH aerial parts (BuL); *n*-BuOH roots (BuR).](image-url)

![Fig. 2. RACI of *S. papposa* fractions coming from: (a) CHCl₃/MeOH aerial parts extract (CML); (b) *n*-BuOH aerial parts extract (BuL); (c) *n*-BuOH roots extracts (BuR).](image-url)
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 failed due to the presence of high polymerized polyphenols. The phytochemical study of \textit{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_{24}O_{12} (HRESIMS at \textit{m/z} 481.1335 \textit{[M + Na]}). The H and \textsuperscript{13}C NMR spectra (Table 1) showed typical signals of a trans-double bond [H-\textit{x} (\textit{\delta} 3.62, 1H, \textit{d}, \textit{J} = 16.0 Hz), H-\textit{b} (\textit{\delta} 7.66, 1H, \textit{d}, \textit{J} = 16.0 Hz), C-\textit{\alpha} (\textit{\delta} 114.7), C-\textit{\beta} (\textit{\delta} 146.6)]. The presence of a symmetrical 1,4-disubstituted phenyl group was deduced from the proton signals at \textit{\delta} 6.81 (2H, \textit{d}, \textit{J} = 8.0 Hz, H-3\textsuperscript{a}/H-5\textsuperscript{a}) and 7.48 (2H, \textit{d}, \textit{J} = 8.0 Hz, H-2\textsuperscript{a}/H-6\textsuperscript{a}). Furthermore, the HMBC correlations of H-2\textsuperscript{a}/C-1\textsuperscript{a}, C-4\textsuperscript{a}, C-5\textsuperscript{a}, and H-\textit{b}/COO, C-2\textsuperscript{a}, C-3\textsuperscript{a} revealed the presence of a trans-p-coumaroyl moiety. The 

![Chemical structure of compounds isolated from \textit{S. papposa} and \textit{S. judaica}](image)

The chemical structure of compounds isolated from \textit{S. papposa} and \textit{S. judaica} is shown in Fig. 3. The chemical structures of compounds 1–4 are depicted in (1), (2), (3), and (4), respectively.

### 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 5–9 did not dem-
Antioxidant activity of pure compounds using BCB and FRAP assays and RACI.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test</th>
<th>DPPH mg TE/g</th>
<th>BCB %AA</th>
<th>FRAP mg TE/g</th>
<th>Folin mg GAE/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃ aerial parts (CL)</td>
<td></td>
<td>5.2 ± 0.7</td>
<td>40.4 ± 2.4</td>
<td>10.3 ± 0.9</td>
<td>54.5 ± 3.5</td>
</tr>
<tr>
<td>CHCl₃/MeOH aerial parts (CML)</td>
<td></td>
<td>37.0 ± 0.24</td>
<td>57.9 ± 3.7</td>
<td>11.2 ± 1.2</td>
<td>65.3 ± 4.7</td>
</tr>
<tr>
<td>n-ButOH aerial parts (BUl)</td>
<td></td>
<td>58.3 ± 0.15</td>
<td>52.4 ± 2.6</td>
<td>53.2 ± 5.7</td>
<td>94.0 ± 8.1</td>
</tr>
<tr>
<td>n-ButOH roots (BuR)</td>
<td></td>
<td>261.5 ± 10.5</td>
<td>59.6 ± 3.2</td>
<td>158.9 ± 9.8</td>
<td>101.2 ± 8.7</td>
</tr>
</tbody>
</table>

**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 ± 1.9 | 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 | 275.9 ± 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 | 5.6 ± 0.6 | 51.1 ± 3.4 |
C | 1186.3 ± 71.7 | 61.5 ± 3.9 | 836.0 ± 48.2 | 1480.1 ± 81.5 |
D | 1680.5 ± 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.
** Milligrams of gallic acid equivalents per g of extract/fraction.

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 litera-

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Test</th>
<th>BCB %AA</th>
<th>FRAP mg TE/g</th>
<th>RACI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td>43.6 ± 3.1</td>
<td>11.6 ± 0.9</td>
<td>-0.27</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>46.1 ± 2.9</td>
<td>82.6 ± 5.1</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>47.7 ± 2.5</td>
<td>60.9 ± 4.5</td>
<td>0.12</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>42.8 ± 2.7</td>
<td>26.9 ± 1.9</td>
<td>-0.23</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>30.4 ± 1.7</td>
<td>16.0 ± 1.3</td>
<td>-0.71</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>18.9 ± 1.2</td>
<td>51.9 ± 1.9</td>
<td>-0.93</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>52.7 ± 4.1</td>
<td>383.8 ± 11.2</td>
<td>1.93</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>62.4 ± 4.1</td>
<td>165.0 ± 7.5</td>
<td>1.16</td>
</tr>
<tr>
<td>12</td>
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<td>51.8 ± 2.4</td>
<td>130.4 ± 5.2</td>
<td>0.62</td>
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<tr>
<td>13</td>
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<td>53.3 ± 2.7</td>
<td>74.0 ± 5.0</td>
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<tr>
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<td>46.2 ± 2.5</td>
<td>19.0 ± 0.9</td>
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<tr>
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<td>40.5 ± 2.1</td>
<td>9.2 ± 0.7</td>
<td>-0.40</td>
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<tr>
<td>16</td>
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<td>28.3 ± 2.0</td>
<td>80.9 ± 5.1</td>
<td>-0.46</td>
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<tr>
<td>17</td>
<td></td>
<td>11.6 ± 1.1</td>
<td>0.3 ± 0.0</td>
<td>-1.31</td>
</tr>
</tbody>
</table>

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

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-ribo-uronic 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 Tanimohardjo (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 phthalides and dihydroisocoumarins suggest these classes of compounds for further investigations.

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References


