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Evaluation of the phenolic and flavonoid contents and radical scavenging activity of three southern African medicinal plants

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***Warburgia salutaris* (Bertol. F.) Chiov*s*, *Rhoicissus tridentata* (L.f.) Wild & Drum and *Terminalia sericea* (Burch. ex DC.), are widely used medicinal plants in southern Africa. The aim of the study was to determine the phenolic and flavonoid content and evaluate the antioxidant activity of the three medicinal plants. Total phenolic and flavonoid contents were determined spectrophotometrically as gallic acid and rutin equivalents, respectively. Individual phenolic acids were identified by means of gas chromatography-mass spectrometry. Antioxidant activities of the crude extracts were assessed using the TEAC assay. The highest phenolic content was detected in the crude methanol extract of the bark of *W. salutaris* and the highest flavonoid content was found in the crude methanol extract of the leaves of this plant. In all the studied plants the alkaline hydrolysable fraction yielded a greater variety of phenolic acids compared to the soluble/free phenolic acid fractions. The three medicinal plants investigated were found to be strong radical scavengers supporting the traditional use of these medicinal plants.**

Key words: Antioxidant activity, flavonoids, medicinal plants, phenolic acids, phenolic content, *Rhoicissus tridentata*, *Warburgia salutaris*, *Terminalia sericea*.

INTRODUCTION

The use of medicinal plants forms the basis of the traditional healing system in many developing countries. The plants investigated in this study, *Warburgia salutaris* (Bertol. F.) Chiovs (Canellaceae), *Rhoicissus tridentata* (L.f.) Wild & Drum (Vitaceae) and *Terminalia sericea* (Burch. ex DC.) (Combretaceae), are widely used medicinally. *W. salutaris* is commonly employed in the treatment of inflammatory diseases as well as coughs, colds and chest complaints (Watt et al., 1962; Hutchings, 1996). *R. tridentata* tubers are used for gynaecological purposes, stomach ailments, as well as kidney and bladder complaints (Watt et al., 1962; Hutchings, 1996; Brookes et al., 2006). *T. sericea* root decoctions are used to treat pneumonia, stomach disorders and diarrhoea (Watt et al., 1962; Hutchings, 1996).

Medicinal plants contain many compounds, amongst which are polyphenols that possess antioxidant properties against oxidative stress, a major cause and aggravating factor in a number of chronic diseases (Ray et al., 2000). Flavonoids have been reported as being able to

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scavenge basically all known reactive oxygen species (ROS) (de Beer et al., 2002). These compounds show antioxidative properties by a number of mechanisms, including scavenging of free radicals, chelation of metal ions such as iron and copper which are of major importance in the initiation of radical reactions, and inhibition of enzymes responsible for free radical generation (Ebenharder et al., 2003).

Phenolic acids are a subgroup of secondary metabolites that are commonly found in plants and are generally divided into two groups. Depending on the core structure they could be classified as either hydroxybenzoic acid derivatives (gallic, ellagic, vanillic and syringic acids) or hydroxycinnamic acid derivatives (*p*-coumaric, ferrulic, caffeic, and synaptic acids). The hydroxycinnamic acid derivatives are more potent antioxidants than their hydroxybenzoic acid counterparts. This is due to an increase in the possibilities for delocalisation of the phenoxyl radical by hydroxycinnamic acid derivatives (Chen et al., 1997). Not only do phenolic acids possess antioxidant activity because of their stable radical intermediates, they are also able to donate hydrogen and electrons to stabilise other free radicals. This is evident from the fact that an increased number of hydroxylated positions on the core structure usually corresponds with more potent antioxidant activities (Rice-Evans et al., 1996; de Beer et al., 2002).

Although *W. salutaris* (Bertol. F.) Chiov*s, R. tridentata* (L.f.) Wild & Drum and *T. sericea* (Burch. ex DC.) have been reported to be biologically active, the compounds responsible for the activity have not been well-researched. The aim of this study was to determine the total phenolic and flavonoid contents, identify the individual phenolic acids and assess the antioxidant activities of the three above-mentioned medicinal plants.

MATERIALS AND METHODS

Chemicals, solvents and reagents

All solvents and reagents from various suppliers were of the highest purity. Folin-Ciocalteu phenol reagent, gallic acid, rutin, methanol (99.9%, HPLC grade), ethyl acetate, diethyl ether and all phenolic acid standards; vanillic, ferulic, caffeic, syringic, *p*-coumaric, sinapic, protocatechuic, 3-hydroxybenzoic, 4-hydroxybenzoic, 2-hydroxycinnamic, 3-hydroxycinnamic, gallic acids; bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), 6-hydroxy-2,5-7-8-tetramethylchroman-2-carboxylic acid (Trolox), ethanol, 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and potassium persulfate were purchased from Sigma-Aldrich. Sodium carbonate (Na_2CO_3) and aluminum chloride ($AlCl_3$) were purchased from Merck. In all experiments, purified deionised water used was obtained from a MilliQ water purification system (Millipore).

Plant material

Bark of the *W. salutaris*, was purchased from the Mai Mai market, Johannesburg, South Africa, tubers of *R. tridentata* were a gift from Dr. Lynn Katsoulis (Department of Pharmacology, University of the Witwatersrand, South Africa) and roots of *T. sericea* were collected in Makhado, Limpopo Province, South Africa. The identities of the specimens were confirmed by a botanist and voucher specimens of the plants are deposited in the Soutpansbergensis herbarium in Makhado. Plant material was dried and ground to a fine powder using an Ika Analytical Mill (Staufen, Germany).

The ground material of the different parts of these plants were used to prepare crude methanol and water extracts and used for the determination of their total flavonoid and phenolic acid contents as well as for the assessment of their ability to scavenge the $ABTS^{\bullet+}$, the pre-formed radical monocation of ABTS**.** The ground material of these plants was also used to prepare free and bound phenolic acid extracts followed by their identification with gas chromatography-mass spectrometry (GC-MS).

Crude extracts

The extraction procedure for total phenolic compounds in methanol extracts was according to Velioglu et al. (1998) with minor modifications. One gram of the ground plant material was shaken for 4 h at 25ºC with 99.9% methanol (10 ml). The mixture was centrifuged (1 000 g; 15 min) and the supernatant filtered through 0.45 µm membrane filters (Millipore). Purified deionised water (15 ml) was added to the supernatant and the methanol evaporated at 30°C. Ethyl acetate (5 ml) was added to perform two liquid-liquid extractions of the aqueous phase. Ethyl acetate layers were combined and anhydrous sodium sulphate added to suppress water. The ethyl acetate layers were filtered, concentrated to dryness using a rotary evaporator and the residue re-dissolved in 5 ml ethanol. Yields were determined gravimetrically.

The method of Yu et al. (2001) was followed in order to extract total phenolic compounds from aqueous extracts. One gram of ground plant material was suspended in 10 ml of boiling purified deionised water, heated for 30 min, after which the mixture was allowed to cool to room temperature. The latter was centrifuged (10 000 g; 15 min) and filtered (0.45 µm). The filtrate was extracted three times with 5 ml ethyl acetate. All the ethyl acetate fractions were combined and anhydrous sodium sulphate added to suppress water. The ethyl acetate fraction was filtered, dried and the residue re-dissolved in 5 ml purified deionised water.

Quantitation of total flavonoid and phenolic compounds in methanol and water crude extracts

Total flavonoid content: Flavonoid content was determined using the method of Quettier-Deleu et al. (2000). A 1 ml of the undiluted extract was added to 1 ml of a 2% aluminium chloride reagent and incubated in the dark for 10 min. The absorbance was read at 430 nm using a Spectronic Genesys 5 spectrophotometer. Flavonoid content expressed in mg/g as rutin equivalent (RE) was calculated using the equation**:**

$$X = \frac{[\text{EX Abs} \times (\text{mg rutin in 1 ml ethanol}) \times \text{DilF.} \times \text{FW/DW}]}{[\text{ST Abs} \times \text{weight of plant extract}]}$$

Where: X = Flavonoid content, mg/g plant extract in rutin equivalent; DilF. = Dilution factor of the extract; FW/DW = the ratio between the fresh and dry plant weight (water percentage); EX Abs = Absorbance of the plant extract; St Abs = Absorbance of the rutin standard.

Total phenolic content: Total phenolic content in methanol and water extracts was determined using the Folin-Ciocalteu method of Djeridane et al. (2006). A gallic acid standard curve was established

(0.1 to 0.5 mg/ml). One hundred microlitres of the sample was dissolved in 500 µl of diluted (1/10) Folin-Ciocalteu phenol reagent and 1000 µl of purified deionised water. The solution was mixed and allowed to stand at room temperature for 3 min. Thereafter, a 1.5 ml of a 20 % Na_2CO_3 solution was added and the mixture was shaken and incubated for 2 h in the dark. Absorbance was read at 765 nm using a Spectronic Genesys 5 spectrophotometer. Total phenolic content of methanol and boiling water extracts, expressed as gallic acid equivalence (GAE) or milligram of gallic acid per gram dry weight of plant material, were then calculated using the following formula:

$C = (c \times V) / m$

where: C is the total phenolic content in mg/g plant extract, in GAE; c is the concentration of Gallic acid established from the calibration curve in mg/ml; V is the volume of extract in ml; m is the weight of plant methanolic or boiling water extract in g.

Antioxidant activity

Antioxidant activity was determined according to the method of Re et al. (1999). This method involves the production of the $ABTS^{\bullet+}$ radical through the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate. $ABTS^{\bullet+}$ was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm. $ABTS^{\bullet+}$ (2 ml) was added to 20 µl of different concentrations of plant extract (1 to 4 mg/ml), and incubated for 3 min, after which the absorbance was determined at 734 nm (PerkinElmer Lambda 25). Trolox was used as positive control. TEAC values were calculated according to Obón et al. (2005) where the ratio of the slope of the corresponding sample versus the calculated slope for Trolox was determined.

Phenolic acid extraction and identification

Extraction and hydrolysis was performed according to the method of Kim et al. (2006). Plant material was defatted twice with *n*-hexane at 4:1 ratio (v/w) to plant material, shaking for 1 h at 25ºC and filtering through a Whatman No. 1 filter paper. The defatted fraction was extracted twice with methanol for 5 h while shaking at 25ºC, filtered through Whatman No. 1 filter paper and the supernatants dried using a rotary evaporator. Soluble/free phenolic acids were extracted from the residue whereas the plant residue remaining on the filter paper was used to extract bound phenolic acids.

Free phenolic acids

The dried supernatant residue from the methanol extract was re-dissolved in acidified water (pH 2, using HCl) and partitioned twice for 1 h each with diethyl ether-ethyl acetate (1:1). The collected diethyl ether-ethyl acetate extracts were dehydrated with anhydrous sodium sulphate and filtered (0.45 µm). Sample solvent was evaporated under nitrogen stream and then derivatized by the silylation of the hydroxyl groups in plant phenolic acids using 250 µl BSTFA containing 1% TMCS at 80ºC for 30 min.

Bound phenolic acids

The residue remaining on the filter paper was hydrolyzed with 4 M NaOH for 4 h at 25ºC. The supernatants were collected and acidified (pH 2) using 6 M HCl and the solution extracted using diethyl ether-ethyl acetate (1:1). Sample solvents were dried and derivatized as described earlier.

Phenolic acid identification

A Trace GC 2000 was coupled to a Trace MS in the Electron Impact (EI) mode with the electron energy set at 70 eV and the mass range at *m/z* 25-700. The column used was a Zebron capillary column ZB-5MS (30 m × 0.25 mm, i.d.) with 0.25 µm film thickness (Phenomenex, USA). The injector temperature was set at 280ºC and the detector at 290ºC. Analysis was performed in the split-less mode with 1 min split-less-time. The gradient temperature program was set as follows: 100ºC held for 5 min, increasing 5ºC/min to a final temperature of 280ºC held for 5 min. The post run was 10 min at 100ºC. The flow rate of carrier gas (helium) was maintained at 1.2 ml/min. X-calibur software was used for data acquisition. Identification of individual phenolic acids in each plant extract was established by comparing their gas chromatographic retention times and silylated derivative mass spectra to those of purified standards.

Statistical analysis

Results are expressed as mean ± the 95% confidence interval as obtained from triplicates run on three independent analyses. The data was analysed using GraphPad Prism 4.0 and STATA software packages.

RESULTS AND DISCUSSION

Phenolic and flavonoid content of the crude water and methanol extracts of the three plants are provided in Table 1. With regards to the water extracts, roots of *T. sericea* contained the highest flavonoid and phenolic content whereas for methanol extracts, the bark of *W. salutaris* contained the highest total phenolic content. The total phenolic content in all plant extracts was found to be higher in methanol extracts than water extracts. *W. salutaris* has been shown to contain flavonoids and flavonols (Manguro et al., 2003; Frum et al., 2005) and plants from the Vitaceae family are known to contain numerous phenolic compounds (Dictonary of Natural Products, 1996).

Phenolic acids are a subgroup of secondary metabolites that are commonly found in plants, which occur in the free and bound forms. A list of free and bound phenolic acids identified in the three plants is provided in Table 2. It can be seen that in addition to the phenolic acids, organic acids (malic, succinic and fumaric acids) were detected in the free phenolic fraction and malonic and propanoic acids in the hydrolysable fraction of *R. tridentata.* Homovanillyl alcohol, a product of lignin hydrogenation, was also found in the soluble / free phenolic fraction of *R. tridentata*. The presence of both hydroxybenzoates (gallic and vanillic acids) and hydroxyl-cinnamates (ferulic acid) were confirmed in the bound phenolic fraction of all three plants. Only extracts of the roots of *T. sericea* contained hydroxycinnamates in the free phenolic fraction, whereas hydroxybenzoates were found in the free phenolic acid fractions of all extracts. Phenolics belong to a category of natural com-pounds, which possess antioxidant (Chen et al., 1997; Rice-Evans et al., 1996; Cai et al., 2006) and anti-inflammatory activities

Table 1. Total phenolic (TPC), total flavonoid (TFC) concentrations and antioxidant activities of standard hydroxybenzoic and hydroxycinnamic acids as well as crude methanol and water extracts of the three plants investigated. Slopes indicate those of the relevant percentage inhibition plots with the origin as intercept.

Compound	Slope (±95% CI)	Antioxidant potency relative to Trolox	TPC (mg/g)	TFC (mg/g)
Hydroxybenzoic acids				
Gallic acid	244.4 ±44.4	9.4	-	-
3,4-dihydroxybenzoic acid	191.6 ±85.5	7.4	-	-
Syringic acid	83.9 ±6.6	3.2	-	-
Hydroxycinnamic acids				
Caffeic acid	105.7 ±36.6	4.1	-	-
Sinapic acid	99.1 ±11.1	3.8	-	-
p-coumaric acid	96.7 ±9.3	3.7	-	-
Ferulic acid	31.7 ±7.7	1.2	-	-
2-hydroxycinnamic acid	11.8 ±2.2	0.5	-	-
3-hydroxycinnamic acid	0.7 ±1.4	0.0	-	-
Vanllic acid	n.d.	n.d.	-	-
Plant extracts				
W.salutaris (leaves) crude MeOH	98.9 ±5.8	3.8	12.6 ±0.1	3.6 ±0.1
W.salutaris (leaves) crude H_2O	48.2 ±3.7	1.9	6.0 ±0.1	1.0 ±0.1
W.salutaris (bark) crude MeOH	126.7 ±15.1	4.9	13.8 ±0.2	0.2 ±0.1
W.salutaris (bark) crude H_2O	74.2 ±3.9	2.9	6.9 ±0.1	0.6 ±0.1
R. tridentata (tubers) crude MeOH	135.3 ±24.8	5.2	11.4 ±0.1	0.2 ±0.1
R. tridentata (tubers) crude H_2O	105.0 ±12.4	4.0	6.4 ±0.1	0.4 ±0.1
T. sericea (roots) crude MeOH	133.9 ±27.2	5.2	12.5 ±0.1	1.2 ±0.1
T. sericea (roots) crude H_2O	128.8 ±18.6	5.0	9.0 ±0.1	6.0 ±0.1
Trolox	26.0 ±3.2	1	-	-

n.d. = not detected.

(Tunon et al., 2009). The antioxidant activity of crude methanol and water extracts of leaves and barks of *W. salutaris*, of tuber of *R. tridentata*, and roots of *T. sericea* are presented in Table 1. The crude methanol extracts of the roots of *T. sericea and* tubers of *R. tridentata* showed the highest antioxidant activity while the majority of phenolic acid standards had antioxidant activity better than Trolox. Antioxidant activity has previously been reported for the bark of *T. sericea* (Opoku et al., 2002; Steenkamp et al., 2004; Masoko et al., 2005), *W. salutaris* (Leshwedi et al., 2008) and tubers of *R. tridentata* (Naidoo et al., 2006) where the antioxidant activity of the latter plant has in part been ascribed to the compounds: catechin, epicatechin, gallic acid and epigallo-catechin-gallate (Naidoo *et al.*, 2006).

The radical scavenging activity of phenolic acids is related to the number and position of hydroxyl groups and methoxy substituents in the molecules (Cai et al., 2006). For example, gallic acid (3, 4, 5-trihydroxybenzoic acid, with the most hydroxyl groups, had the strongest radical scavenging activity (Table 1). This finding is supported by Rice-Evans et al. (1996) who reported that gallic acid has increased antioxidant activity relative to Trolox owing to its greater number of hydroxyl groups. Furthermore, gallic acid is a strong chelating agent and forms complexes of high stability with Fe^{3+} ions (Li et al., 2000). This also corresponds with some of the previously reported activities of gallic acid, which include anticancer (Chen et al., 2009) and antibacterial activities (Kang et al., 2008).

Structure-antioxidant activity of the studied phenolic compounds could be observed. For example, the anti-oxidant response in dihydroxybenzoic acids is reported to be dependent on the relative positions of the hydroxyl groups in the ring. Dihydroxylation in the *ortho* and *meta* or in the *meta* and *para* positions to the carboxylate group enhances antioxidant activity (Rice- Evans et al., 1996). From the results presented in Table 1, it can therefore be seen that 3,4 dihydroxybenzoic acid with hydroxyl groups positioned in the *meta* and *para* positions to the carboxylate group gave high TEAC values. On the other hand, methoxy substitution in

Table 2. Free and bound phenolic acids identified in the roots of *T. sericea,* tubers of *R. tridentata,* bark of *W. salutaris* and leaves of *W. salutaris.* Intensities of fragmentation ions are expressed as percentages of the base peak (In parentheses) [EI, 70 eV].

Herb	Free phenolic acid	Bound phenolic acid	Retention time (min)	Fragmentation ions used to confirm phenolic acids (intensities)
	Gallic acid		25.56	458.4 (98); 443.0 (51); 281.0 (97)
	Vanillic acid		21.44	312.0 (61); 297.0 (100); 267.0 (68); 223.1 (54)
	3,4-dihydroxybenzoic acid		22.63	370.1 (49); 355.1 (28); 281.0 (12); 193.1 (100)
	p-Coumaric acid		25.09	308.1 (9); 293.1 (17); 219.1 (14)
	Caffeic acid		28.86	396.1 (81); 381.1 (21); 307.1 (13); 219.1 (99)
		Gallic acid	25.57	458.5 (96); 443.1 (52); 281.1 (25)
		Vanillic acid	21.43	312.1 (61); 297.2 (100); 267.1 (66); 223.2 (50)
T. sericea (roots)		3,4-dihydroxybenzoic acid	22.62	370.2 (36); 355.2 (20); 193.1 (77)
		p-Coumaric acid	25.08	308.1 (62); 293.1 (81); 219.2 (75)
		Caffeic acid	28.85	396.2 (29); 219.2 (35)
		Ferulic acid	28.01	338.1 (100); 323.1 (62); 293.0 (39); 249.1 (53)
		Syringic acid	24.25	342.1 (53); 327.1 (80); 312.0 (60); 297.0 (52)
		4-hydroxycinnamic acid	18.35	282.2 (24); 267.1 (100); 223.2 (71); 193.1 (55)
		Hydrocinnamic acid	21.35	310.2 (26); 192.2 (74); 177.1 (21)
		Benzoic acid	26.2	428.2 (20); 325.1; (33) 236.2 (44)
		4-hydrophenylacetic acid	18.59	252.2 (35);192.2 (10);179.2 (100);131.1 (10)
	3,4-dihydroxybenzoic acid		22.63	370.1 (56); 355.1 (32); 281.1 (16); 193.1 (100)
	Vanillic acid		21.44	312.2 (51); 297.1 (92); 267.1 (65); 223.1 (57)
W. salutaris (bark)		Gallic acid	25.47	458.2 (37); 443.2 (14); 281.1 (59)
		Vanillic acid	21.47	312.1 (59); 297.1 (100); 267.1 (64); 223.1 (53)
		3,4-dihydroxybenzoic acid	22.67	370.1 (70); 355.1 (42); 281.1 (15); 193.1 (100)
		Syringic acid	24.27	342.1 (43); 327.1 (66); 312.0 (48); 297.0; (40)
		4-hydroxybenzoic acid	18.51	282.3 (10); 267.1 (37); 223.1 (18); 193.0 (17)
		Syringic acid	24.4	342.2 (27); 327.2 (46); 297.2 (24); 312.2 (31); 253.1 (15)
*W. salutaris (*leaves)	n.i.	*p*-Coumaric acid	25.21	308.2 (55); 293.1 (83); 219.1 (71)
		Ferulic acid	28.15	338.2 (100); 323.2 (63); 308.1 (62); 249.1 (54)
		Sinapic acid	30.87	368.2 (100); 353.2 (38); 338.2 (95);279.1 (16)
	Vanillic acid		21.44	312.2 (28); 297.3 (47); 267.2 (39)
R. tridentata (tubers)	3,4-dihydroxybenzoic acid		22.63	370.2 (37); 355.2 (21); 281.1 (9); 193.1 (100)
	Gallic acid		25.5	458.2 (74); 443.2 (47); 281.1 (87)
	Phthalic acid		19.81	310.3 (4); 295.2 (42); 221.2 (18);147.1 (100)

Table 2. Contd.

	Vanillic acid	21.44	312.3 (31); 297.3 (58); 267.2 (44)
	3,4-dihydroxybenzoic acid	22.64	370.2 (14); 355.2 (8); 281.1 (4); 193.1 (37)
	Gallic acid	25.51	458.2 (71); 443.2 (46); 281.1 (79)
	Ferulic acid	28.04	338.2 (39) ; 323.2 (25); 308.1 (22); 249.2 (23)
	p-coumaric acid	25.09	308.2 (36); 293.1 (52); 219.1 (56)

n.i. = none identified.

hydroxylbenzoates results in a decrease in antioxidant capacity when the 3 and 5 hydroxyl groups in the trihydroxylbenzoic acid (gallic acid) are replaced with methoxy groups in *p*-hydroxydimethoxybenzoic acid (syringic acid). Subsequently, it can be seen from this table that radical scavenging capacity of hydroxybenzoic acids were found to decrease in the following order: gallic acid > 3, 4 dihydroxybenzoic acid > syringic acid. For hydroxycinnamic acids, it was observed that dihydroxyl cinnamates (caffeic acid) were more active in scavenging radicals than monohydroxycinnamates (*p*-coumaric acid; 2-hydroxycinnamic acid). Sinapic acid with methoxy substituents on the 3,-5-hydroxyl was also more active than the monohydroxy and the hydroxymethoxy cinnamates (ferulic acid). An increase in antiradical capacity was noted in monohydroxycinnamic acid with the hydroxyl group on the *p*-position (*p*-coumaric acid) compared to the corresponding *m* and *o* positions (2- and 3- hydroxycinnamic acids). Besides the number and position of hydroxyl groups and methoxy substituents in hydroxycinnamates, the acrylic acid side chain (-CH=CH-COOH) in hydroxycinnamates is considered to be key for the significantly greater antioxidant efficiency than the carboxylic acid side chain (-COOH) in the hydroxybenzoates (Rice-Evans et al., 1996). An overall view of the antioxidant activity of phenolic acids indicates that hydroxycinnamic acids (e.g. sinapic acid), having the same hydroxyl and methoxy groups as hydroxybenzoates (e.g. syringic acid), are more effective in scavenging radicals. It has been reported that the conjugated double bond in the side chain of hydroxy-cinnamics contribute to their antioxidant activity by stabilizing radicals (Chen et al., 1997; Foti et al., 1996). Nearly all the phenolic acid standards tested for their radical scavenging activity were more potent antioxidants than Trolox, except for 2-hydroxycinnamic and 3-hydroxycinnamic acids. The average slope of 26.0 determined for Trolox during the current work correlates very well with the 26.5 determined for Trolox by Obón et al. (2005), indicating good reproducibility of the TEAC assay.

The presence of the hydroxybenzoic and hydroxycinnamic acids are confirmed in the three plant extracts (Table 2). Subsequently, the antioxidant activity of the crude methanol and water extracts may be attributable to their content of these compounds. Crude aqueous extracts showed a positive correlation between phenolic content and antioxidant activity with a correlation coefficient of $r = 0.66$, which is in agreement with literature (Velioglu et al., 1998; Shan et al., 2005). However, the correlation coefficient between the total phenolic content and antioxidant activity of crude methanol extracts was determined to be $r = 0.04$, indicating little correlation between phenolic contents and antioxidant activity in crude methanol extracts. This is in contrast to the observations by others who found a high correlation between antioxidant activities of plant materials/ products with their total content of phenolic compounds (Velioglu et al., 1998; Shan et al., 2005). The weak correlation may be explained by the fact that the antioxidant activity of plant materials not only depends on the concentration but also on the structure and synergistic interactions between antioxidants (Djerdane et al., 2006). Moreover, the F-C reagent is affected by several other interfering non-phenolic substances such as organic substances e.g. proteins and sugars in crude plant extracts (Box, 1983). Shan et al. (2005) also reported that it is difficult to obtain good correlations between phenolic content and antioxidant activity when the ranges of the values obtained for total phenolic content and antioxidant activity are very small.

In conclusion, the three medicinal plants investigated were found to be strong radical scavengers supporting the traditional use of these medicinal plants.

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Abbreviations

ABTS, 2,2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid; **BSTFA,** bis(trimethylsilyl)trifluoroacetamide; **DilF,** dilution factor; **EI,** electron impact ionization; **FW/DW,** ratio between the fresh and dry plant weight; **GAE,** gallic acid equivalence; **GC,** Gas chromatography; **MS,** Mass spectrometry; **RE,** rutin equivalence; **TEAC,** trolox equivalence antioxidant capacity; **TFC,** total flavonoid content; **TMCS,** trimethylchlorosilane; **TPC,** total phenolic content.

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