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Comparative antioxidant activity of extracts from leaves, bark and catkins of *Salix aegyptiaca* sp.

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ABSTRACT

Leaves, bark and catkins of *Salix aegyptiaca* L. were extracted into solvents of increasing polarity from cyclohexane (non-polar), butanol, ethanol and water (polar) and analysed for their antioxidant capacity, total phenol and flavonoids. The highest antioxidant activity (19 μg/ml IC_{50} for inhibition of DPPH radical activity), total phenolic content (212 mg gallic acid equivalents/g of dried extract) and total flavonoid (479 mg catechin equivalents/g of dried extract) was observed in the ethanolic extract of bark. HPLC identification of phenolic compounds from the extracts indicated the presence of gallic acid, caffeic acid, vanillin and *p*-coumaric acid, myricetin, catechin, epigallocatechin gallate, rutin, quercetin as well as salicin. Our data indicates the presence of high amounts of phenols and flavonoids in different parts of *S. aegyptiaca* species and propose that extracts from this plant may be utilised as a source of health promoting antioxidants.

1. Introduction

Salicylates (salicylic acid and acetyl salicylic acid (ASA, Aspirin®) are a class of compounds which have been used throughout the world for centuries as analgesic, antipyretic, and anti-inflammatory drugs (Mahdi, Mahdi, & Bowen, 2006). Their anti-inflammatory function is thought to arise through the inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) leading to the inhibition of prostaglandin (PG) synthesis (Yu et al., 2002). Epidemiological studies have shown that patients under long term Aspirin® therapy have lower propensity to develop cardiovascular disease and colorectal cancer (Steinbach et al., 2000). Since salicylic acid and other salicylates are naturally present in various fruits and vegetables, consuming these components in the daily diet might greatly reduce the risk of colorectal carcinoma (Paterson & Lawrence, 2001). However, other studies have suggested that the beneficial effects of ASA may be through COX-independent pathways (Law et al., 2000).

The Willow family of plants, *Salix*, contains notable amounts of endogenous salicylate compounds. Willow bark has been used since ancient times due to the anti-inflammatory and anti-nociceptive functions of its active constituent salicin (Chrubasik, Kunzel, Model, Conradt, & Black, 2001). Specifically, the discovery of Aspirin® has diverted significant attention to this plant species (Jones, 2003). However, pharmacological studies have indicated that the fraction of total salicin is not adequate to explain the potency of willow bark (Fiebich & Chrubasik, 2004; Nahrstedt, Schmidt, Jäggi, Metz, & Khayyal, 2007). In addition, salicin and its metabolites don't have the acetylating potential of ASA. Therefore, there should be additional mechanisms to provide the anti-inflammatory potential of willow bark such as the antioxidant functions of other constituent phytochemicals (Hostanska, Jurgenliemk, Abel, Nahrstedt, & Saller, 2007).

The efficacy of plant derived phytochemicals on human ailments related to dietary habits such as cardiovascular diseases, type II diabetes, and several types of cancers has gained renewed interest in the recent past. These non nutrient bioactive compounds naturally available from plants have lower potency than 'allopathic' drugs, however, as they are traditionally consumed in significant amounts through the diet, they may have a long term physiological benefits without any harmful side effects (Espin, Garcia-Conesa, & Tomas-Barberan, 2007).

The purpose of this study was to evaluate the total antioxidant activity and to determine the phenols, flavonoids and salicin in extracts of leaves, bark and catkins of *Salix aegyptiaca* L. This hitherto unexplored species of *Salix* is endogenous to the Middle East and extracts from its catkins is traditionally consumed as a health drink in this part of the world. We report here that the parts of this plant extracted in solvents of increasing polarity have very high antioxidant content as indicated by their radical quenching ability as well as total phenol and flavonoid content. Of the individual phenols and flavonoids evaluated, significant amounts of catechin and rutin were found in the water extract of leaves and ethanolic extract of

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bark respectively. In addition, a considerable amount of myricetin was observed in ethanolic extract of bark and water extract of leaf. This species of plants is therefore an excellent source of antioxidants and can be consumed for its health promoting effects.

2. Materials and methods

2.1. Plant selection

Plant collection was carried out according to published guidelines (N'Guessan et al., 2007). Briefly, catkins, leaves and barks of *S. aegyptiaca* were collected from Ghaene ghom, Iran, during the 2007 harvest season. The plant materials were identified morphologically at the herbarium of the Medicinal Plants Research Institute of Shahid Beheshti University of Tehran, Iran. The fresh catkins (15 kg), leaves (2 kg) and barks (1 kg) were air dried at room temperature for 1 week giving 1.5 kg, 500 g and 250 g dried catkins, leaves and barks respectively. The samples were then pulverised to a powder form using a steel blender (Sinbo, Istanbul, Turkey) and stored in a desiccator at 4 °C in the dark until analysis.

2.2. Chemicals

Gallic acid, (+)catechin, epigallocatechin gallate, vanillin, myricetin, quercetin, rutin, caffeic acid, coumaric acid, Folin and Ciocalteu's phenol reagent and DPPH were obtained from Sigma Chemical Co. (Taufkirchen, Germany). All other chemicals used were purchased from Merck (Darmstadt, Germany) and were of chromatography grade. Distilled deionised water (ddH_2O) was used throughout the experiments.

2.3. Extract preparation

A total of eleven different extracts were prepared from different parts of the plant using the procedure of Gezer et al. (2006) and Kim, Jeong, and Lee (2003) with some modifications. Solvent selection was based on increasing polarity. The dried plant material from leaves, bark and catkins (10 g) were separately dissolved in cyclohexane (CycH) (non-polar), butanol (BuOH), ethanol (EtOH) and water (most polar) with the ratio of 1 to 10 (w/v) for barks and leaves and 1 to 40 (w/v) for catkins. As the catkins in dried powder form tended to float in all the solvents, it was necessary to use a higher volume of solvents in order to ensure the complete extraction of the active ingredients. The samples were extracted by sonicating for 20 min in a bath sonicator (Bandelin Sonorex Model RK 100H, Berlin, Germany) followed by incubation at 30 °C with shaking at 150 rpm for 24 h (Kim et al., 2003). The duration of sonication was maintained for short periods and the water of the bath was changed frequently to prevent any significant increase in the temperature of the extracts. Following this, they were filtered through a Whatman (No. 1) filter paper and centrifuged at 6861 × *g* for 10 min using an Eppendorf 5804R refrigerated benchtop centrifuge (Eppendorf, Wesseling-Berzdorf, Germany). The procedure was repeated for second time by adding fresh solvents. The supernatants were then collected and concentrated under vacuum at 45 °C using a rotary evaporator (Buchi Rotavapor R-200, Essen, Germany) and finally desiccated by lyophilisation (Labconco Freeze Drying System, Kansas City, MO, USA) and kept in the dark at −20 °C until analysis (Gezer et al., 2006; Kim et al., 2003).

2.4. Determination of antioxidant activity

Owing to the large number of extracts used in this study along with the necessary replications to make the data statistically relevant, all assays were scaled down to be accommodated in 96 well plates for absorbance measurements in a microplate reader. Flat bottom 96 well plates (Greiner, Frickenhausen, Germany) were used in order to accommodate a maximum volume of 350 μL without spillage.

2.4.1. DPPH Assay

The antioxidant activities of all the extracts were evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay according to Gezer et al. (2006) with some modifications. This assay is based on the ability of DPPH, a stable free radical, to be quenched and thereby decolorize in the presence of antioxidants resulting in a reduction in absorbance values (Gezer et al., 2006). Ten mg of the lyophilised powder for each of the eleven extracts was weighed in a precision balance (Adventurer Ohaus, Pinebrook NJ, USA) and dissolved in 1 ml methanol. This original stock solution (10 mg/ml) was further serially diluted with methanol to give a range of concentrations. Twenty five microliters from these stock solutions were then added to 325 μl of 0.005% methanolic DPPH solution in 96 well plates to give a final concentration range of 5–120 μg/ml at room temperature. The DPPH solution was prepared freshly every day. The decrease in absorbance was measured at 490 nm (Hwang et al., 2001) at an end point of 30 min and 1 h after incubation at 25 °C in the dark in a BioRad 680 microplate reader (BioRad, Hercules, CA, USA). The results were compared with quercetin and BHT as standards and methanol was used as a blank (Okusa, Penge, Devleeschouwer, & Duez, 2007).

The percentage of DPPH free radical quenching activity was determined from the formula:

$$\text{DPPH Scavenging Effect}(\%) = \left(\frac{A_{\text{DPPH}} - A_{\text{Extract}}}{A_{\text{DPPH}}}\right) \times 100$$

where A_{DPPH} refers to the absorbance value at 490 nm of the methanolic solution of DPPH and A_{Extract} stands for the absorbance value at 490 nm for the different extracts.

A chart was generated for the percentage activity to quench DPPH free radical of each concentration (ranging from 5–120 μg/ml) of the individual extracts using the equation above. The equation generated from this chart was used to determine the concentration of each extract required to quench 50% of the DPPH free radical activity (IC_{50} value). The antioxidant activity of the different extracts was also expressed in terms of quercetin equivalents (QE) and represented as mg of QE per gram of dried extract. Each sample was assayed at least six times.

2.5. Total phenol assay

Total phenolic constituents of the extracts were analysed by Folin and Ciocalteu method using gallic acid as standard as described by (Singleton, Orthofer, & Lamuela-Raventos, 1999) with some modifications.

For the preparation of gallic acid stock solution, 0.5 g of dry gallic acid was dissolved in 10 mL of ethanol and diluted to 100 ml with ddH_2O. Then 0, 1, 2, 3, 5, and 10 ml of the gallic acid stock solution was diluted to 100 ml with ddH_2O in volumetric flasks giving an effective concentration range of 0, 50, 100, 150, 250, and 500 mg/l gallic acid.

The extracts (3.5 μl) from a 1 mg/ml methanolic stock solution or standard solutions of gallic acid (3.5 μl) or ddH_2O as blank were added to separate test tubes and mixed thoroughly with 276.5 μl ddH_2O and 17.5 μl of Folin and Ciocalteu reagent. After 8 min 52.5 μl of 7% Na_2CO_3 solution was added, and mixed thoroughly by pipetting. The final concentration of the extracts in each well was 10 μg/ml. The solutions were incubated at 20 °C for 2 h and the absorbance versus blank (0 mg/l gallic acid) was read at

765 nm using a BioRad 680 microplate reader (BioRad, Hercules, CA, USA). In addition, a sample containing only methanol processed in the same manner to account for any background due to methanol. The total phenol content of the extracts was determined by comparing with a calibration curve of the gallic acid standard and represented as mg gallic acid equivalents (GAE)/g of dried samples. Each sample was assayed at least six times.

2.6. Total flavonoid assay

The total flavonoid content in the extracts was determined by an aluminium colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999) with some modifications. A standard solution of (+)catechin at different concentrations (20, 40, 60, 80 and 100 mg/l) was prepared by dissolving (+)catechin in ddH_2O. The extracts (35 μl) from a 1 mg/ml stock solution or standard (+)catechin solutions (35 μl) or ddH_2O (as blank) were mixed thoroughly by pipetting with 140 μl ddH_2O in a 96 well plate. Then 10.5 μl 5% sodium nitrite ($NaNO_2$) was added. The mixture was incubated for 5 min at 25 °C and 10.5 μl of 10% aluminium chloride ($AlCl_3$) was added to and then 6 min later, 70 μl 1 M NaOH was added to the wells. The total volume was made up to 350 μl by adding ddH_2O. The absorbance was read at 490 nm in a BioRad 680 microplate reader (BioRad, Hercules, CA, USA) using ddH_2O as a blank. Additionally, a blank sample containing only methanol was processed in the same manner to account for any background due to methanol. The total flavonoid content was expressed as mg of catechin equivalents (CE)/g of dried samples. Each sample was assayed at least 6 times.

2.7. Identification of salicin and individual phenolic compounds by HPLC

2.7.1. Chromatographic conditions

Salicin and phenolic compounds in the extracts were analysed by high performance liquid chromatography (HPLC) using a Varian ProStar HPLC with a Varian 330 PDA detector (Varian, Palo Alto, CA, USA). The columns used were: Varian Intersil ODS3 (150 × 4.6 mm, 5 μm) for salicin and Varian Pursuit C18 (150 × 4.6 mm, 5 μm) column for phenolic compounds. Samples (100 μl) were injected into the column by means of an autosampler and the flow rate was maintained at 1 ml/min. The column temperature was set at 30 °C for salicin and 35 °C for phenolic compounds. Chromatographic data were acquired and processed using the Varian Star Workstation accompanying the HPLC equipment. The chromatographic conditions for the determination of salicin were according to the procedure by Minakhmetov, Onuchak, Kurkin, Zapesochnaya, and Medvedeva (2002) and Poblocka-Olech et al. (2007). Briefly, the mobile phases used were A: a mixture of water and trifluoroacetic acid (TFA) (100:0.05 v/v) and B: acetonitrile. The mobile phase B (acetonitrile) was delivered in a linear gradient from 3% to 48% (with a decreasing gradient from 97% to 52% for mobile phase A) in 30 min. The detection wavelength was set at 268 nm. Individual phenolic compounds were detected according to Garcia-Falcon, Perez-Lamela, Martinez-Carballo, and Simal-Gandara (2007) with some modifications. The mobile phase consisted of A: formic acid in water (2.5% v/v) and B: methanol. The ratio of mobile phases A and B delivered to the column for elution were as follows: 100:0 for 0–7 min, 80:20 for 7–47 min, 60:40 for 47–72 min, and a final conditioning step at a ratio of 100:0 for 72–75 min. The detection wavelength was set at 280 nm for gallic acid, catechin, epigallocatechin gallate (EGCG) and vanillin, 320 nm for caffeic acid and coumaric acid and 360 nm for rutin, myricetin and quercetin.

2.7.2. Preparation of extracts and standards

The extracts from bark and catkins and leaves possessing the highest total phenolic and flavonoid contents were selectively analysed to further identify and quantify a range of phenolic and flavonoid compounds by HPLC. The dried extracts were dissolved in methanol at a concentration of 10 mg/ml, centrifuged at 11481 × *g* for 10 min, filtered through a 0.45 μm filter and injected in the column via an automated injection system. For salicin, a standard curve was generated by duplicate injections of different concentrations (10–0.5 mg/ml) of standard salicin in methanol. The standard compounds selected for the identification of phenolic compounds were gallic acid, vanillin, caffeic acid, coumaric acid, and the flavonoids catechin, EGCG, rutin, myricetin and quercetin. A standard curve for each compound was generated by duplicate injections of a serial dilution of a stock solution of 1 mg/ml in methanol to give a range of different concentrations between 2 and 100 μg/ml.

2.7.3. Determination of salicin content

Since the Willow family of plants is known for its salicin content and potential therapeutic benefits, we determined the salicin level in bark, leaves and catkins extracted in all four solvents (water, ethanol, butanol and cyclohexane). Each extract was injected at least 3 times and the retention time of salicin was ~10 min. Since the retention time of peaks can alter in discrete but small units between runs, a standard salicin sample (1 mg/ml) was injected after every sample injection to confirm the retention time of the salicin peak. In addition, the extracts were spiked with pure standard salicin for each run in order to confirm the retention time and help in the identification of the salicin peak in the extracts.

2.7.4. Determination of phenolic compounds

The phenolic compounds were identified on the basis of a comparison of their retention time with that of authentic standards. Each sample was injected at least 3 times and a mix of the standards was injected after every sample injection to confirm the retention time of each identified peak. In addition, for some phenolic compounds the extracts were spiked with the pure standard compound in order to further confirm the retention time and identity of the compound.

2.8. Statistical analyses

Data analysis and graphing was performed using the GraphPad Prism 5 software package (La Jolla CA, USA). In most cases, the mean of at least six experiments is plotted together with the standard deviation. Statistical analysis between experimental results was based on Spearman's non parametric correlation. Significant difference was statistically considered at the level of $P < 0.05$.

3. Results and discussion

3.1. Determination of antioxidant activity

The DPPH radical scavenging activity of the different extracts from catkins, leaves, bark of *S. aegyptiaca* was assayed as described in Materials and Methods. An extract with high antioxidant activity was capable of quenching the DPPH free radicals at a much lower concentration. The results shown in Fig. 1 indicate that the highest antioxidant activity was observed in the ethanolic extract of bark with an IC_{50} value of 19 ± 3 μg/ml, while the lowest activity was shown by the bark extract in cyclohexane (bark Cyc.H) with an IC_{50} value of 319 ± 4 μg/ml. The IC_{50} values of the standards were 3.1 and 26 μg/ml for quercetin and BHT, respectively.

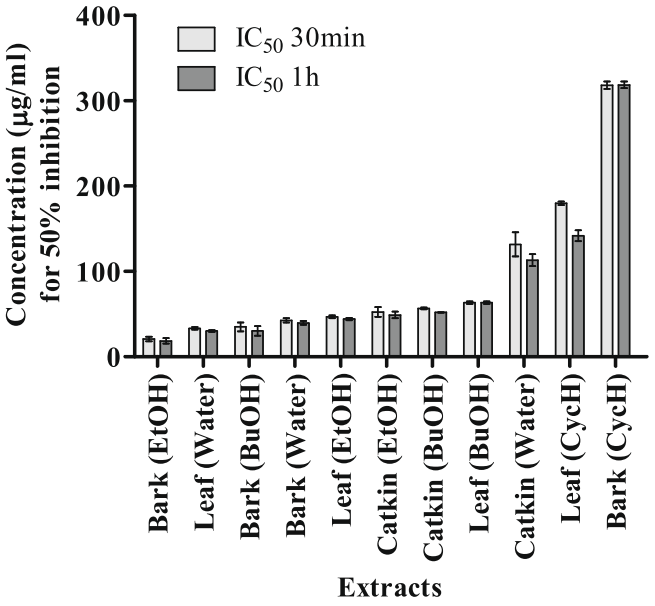

Fig. 1. DPPH free radical scavenging assay showing the concentration of extracts necessary for 50% inhibition of the free radical activity of DPPH at two time points (30 min and 1 h). The data are displayed with mean ± standard deviation of six replicates. The names in parentheses indicate the solvent into which the parts of the plants were initially extracted.

Since quercetin is a very potent antioxidant, when the DPPH free radical quenching data was represented as quercetin equivalents per gram of dried extracts, the ethanolic extract of the bark with 169 ± 28 mg QE equivalent/g dried sample showed the highest amount of quercetin equivalents (Table 1).

Owing to the phenolic structure of salicin, we also examined the free radical quenching ability of purified commercial salicin by the DPPH free radical scavenging assay. However, commercial salicin as well as acetyl salicylic acid did not show any free radical quenching activity (data not shown) which could indicate that the potent antioxidant capacity of the various parts of the plant bear no correlation with their salicin contents. This fact is also corroborated by the salicin content of the extracts as detected by HPLC (Table 1). When a statistical correlation analysis was conducted between the salicin content and the antioxidant activity of the extracts we could not detect any significant correlation (Spearman's $r = 0.4727$).

3.2. Determination of total phenol and total flavonoids

The total phenolic content of different extracts from catkins, leaves and bark of *S. aegyptiaca* was assayed by the Folin and Ciocalteu method using gallic acid as standard ($y = 0.0666x + 0.0634$, $R^2 = 0.9905$) as described in Materials and Methods. The data presented in Table 1 indicates that the highest total phenol content of 212 ± 4 mg GAE/g of dried sample was obtained in the ethanolic extract of bark while the lowest total phenol content of 4 ± 1 mg GAE /g of dried sample was obtained in cyclohexane extract of bark. The phenolic content of *S. aegyptiaca* bark extracts is nearly double the total phenolic content reported for black teas (80.5–134.9 mg/g) and green tea (65.8–106.2 mg/g) (Khokhar & Magnusdottir, 2002).

The total flavonoid content of different extracts from catkins, leaves, bark of *S. aegyptiaca* was assayed by aluminium colorimetric assay as described in Materials and Methods. (+)Catechin, which is a flavonoid found in high amounts in willow extracts (Juergenliemk, Petereit, & Nahrstedt, 2007), was used as a standard ($y = 0.0043x + 0.0305$, $R^2 = 0.9948$) and the total flavonoid content was expressed as mg of catechin equivalents (CE)/g of dried samples. The data presented in Table 1 indicate that the highest flavonoid content of 479 ± 63 mg CE/g of dried samples was observed in the ethanolic extract of bark and the lowest content was observed in the cyclohexane extract of the leaves (2 ± 1 mg CE/g of dried samples). For the determination of both total phenols and total flavonoids, the samples where the extracts were replaced by methanol did not show any background absorbance.

The total flavonoid content reported for black tea (34 mg epicatechin equivalents (ECE)/2 g serving) and green tea (47 mg ECE/2 g serving) (Lee, Kim, Lee, & Lee, 2003) all appear to be much lower than the flavonoid content of the different extracts under study here. Statistical analyses of the antioxidant activities indicated the presence of good correlation (Spearman's $r = 0.938$) between total flavonoid content and radical scavenging activity in quercetin equivalents at a high significance level ($p < 0.0001$) ($y = 0.2535x + 16.035$). A similar relationship (Spearman's $r = 0.942$) with high significance ($p = 0.0004$) was also obtained between total phenol content and radical scavenging activity in quercetin equivalents ($y = 1.4924x - 0.863$).

3.3. Determination of salicin and phenolic compounds by HPLC

The salicin content of the extracts was determined by using HPLC as described in Materials and Methods. Based on area under the curve calculations and comparison with a standard curve of different concentrations of salicin, the highest salicin content was obtained in the ethanolic extract of bark (3.1 ± 0.04 mg/ml) followed by the water extract of catkins (1.8 ± 0.1 mg/ml) and water and butanolic extract of leaves (Table 1). This was expected, since the bark of *Salix* species, particularly the ethanolic extracts, are known to harbour high amounts of salicin (Guvenc, Arihan, Altun, Dinc, & Baleanu, 2007; Young, 2004). In addition, we have shown here for the first time that water extract of catkins, which is widely marketed as a traditional health drink in parts of the Middle East, particularly in Iran, also has high salicin content.

Based on the highest total phenols, flavonoids and antioxidant activities, the ethanolic extract of bark and catkins and the water extract of leaves were selected for further identification and quantification of the individual phenols and flavonoids by HPLC. The phenolic compounds identified were gallic acid, *p*-coumaric acid, vanillin and caffeic acid and the flavonoids identified were catechin, epigallocatechin gallate, rutin, quercetin and myricetin (Table 2). Amongst these, catechin (8.3 ± 0.4 mg/g of dried extract) and myricetin (8.2 ± 0.95 mg/g) were the most abundant polyphenols in the water extract of leaves. Myricetin (5.9 ± 0.4 mg/g) and rutin (4.6 ± 0.3 mg/g) were the predominant flavonols in the ethanolic extract of bark, and catechin (0.99 ± 0.01 mg/g) and epigallocatechin gallate (0.98 ± 0.01 mg/g) were the major flavonols identified in the ethanolic extract of catkins. Previous reports indicate a similar profile whereby the most typical flavonoids in willow bark extracted in polar solvents were the flavan-3-ols catechin, epicatechin, epigallocatechin and the catechin-3-*O*-(1- hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester (Juergenliemk et al., 2007). Studies on the polyphenol content of leaves of six different *Salix* species have revealed that the major flavones were luteolin and apigenin and their derivatives while the major flavonols were myricetin and quercetin and their derivatives along with isorhamnetin-3-glucoside (Nyman & Julkunen-Tiitto, 2005).

Our studies have indicated that extracts with the highest antioxidant activity (such as the ethanolic extract of bark and the water extract of leaves) strongly correlate with total phenol and flavonoid content (Table 1). In addition, analysis and quantification of individual phenolic acids and flavonoids by HPLC have also indicated that these extracts are rich sources of phytochemicals, comparable to the traditionally rich sources such as tea and red wine

Table 1
Antioxidant activity, contents of total phenols and flavonoids and salicin in the different extracts of *S. aegyptiaca*. QE = quercetin equivalents GAE = gallic acid equivalents and CE = catechin equivalents. Numbers in parentheses represent the standard deviation.

Samples	Antioxidant activity (mg QE/g dried sample)	Total phenol (mg GAE/g dried sample)	Total flavonoid (mg CE/g dried sample)	Salicin content (mg/ml of extract)
Catkin BuOH	60 (± 0.3)	81 (± 1)	152 (± 6)	0.9 (± 0.1)
Catkin EtOH	64 (± 5)	107 (± 3)	351 (± 2)	0.2 (± 0.002)
Catkin Water	29 (± 2)	86 (± 1)	68 (± 15)	1.8 (± 0.1)
Leaf BuOH	49 (± 1)	36 (± 1)	125 (± 23)	1.3 (± 0.03)
Leaf EtOH	70 (± 2)	64 (± 1)	165 (± 3)	0.7 (± 0.002)
Leaf Water	103 (± 4)	163 (± 3)	280 (± 4)	1.4 (± 0.02)
Leaf Cyc.H	22 (± 1)	19 (± 1)	2 (± 1)	0.07 (± 0.01)
Bark BuOH	105 (± 20)	211 (± 3)	419 (± 64)	0.2 (± 0.02)
Bark EtOH	169 (± 28)	212 (± 4)	479 (± 63)	3.1 (± 0.04)
Bark Water	78 (± 4)	139 (± 1)	243 (± 10)	0.07 (± 0.01)
Bark Cyc.H	10 (± 0.1)	4 (± 1)	73 (± 5)	0.04 (± 0.01)

Table 2
Mean concentration of phenolic compounds in ethanolic extracts of catkins, leaf and bark of *S. aegyptiaca* detected and quantified by HPLC. The approximate retention time in minutes that the compounds were eluted is indicated.

Category	Compound	Retention time (min)	Catkins (mg/g)[a]	Leaf (mg/g)	Bark (mg/g)
Phenolics	Gallic acid	5.1	0.05 (±0.002)	0.04 (± 0.01)	0.69 (± 0.02)
	Caffeic acid	41.7	0.53 (± 0.01)	0.15 (± 0.01)	0.06 (± 0.01)
	Vanillin	47.1	0.34 (± 0.05)	0.52 (± 0.05)	1.53 (± .13)
	p-Coumaric acid	48.9	0.18 (± 0.03)	0.92 (± 0.08)	0.80 (± 0.04)
Flavonols	Myricetin	53.0	n.d.	8.2 (± 0.95)	5.87 (± 0.42)
	Catechin	38.8	0.99 (± 0.01)	8.3 (± 0.39)	0.93 (± 0.03)
	Epigallocatechin gallate (EGCG)	45.3	0.98 (± 0.01)	0.47 (± 0.02)	2.39 (± 0.29)
	Rutin(quercetin-3-rhamnosyl glucoside)	52.2	0.29 (± 0.01)	n.d.	4.59 (± 0.25)
	Quercetin	55.3	n.d.	n.d.	1.47 (± 0.16)
	Total (mg/g):		3.4	18.6	18.3

[a] Expressed in mg/g of dried extract ± SD.

grapes. The catechin content of the water extract of leaf (8.3 mg/g of dried extract) is much higher than that reported for green (1.3 mg/g) and black tea (1.7 mg/g) (Khokhar & Magnusdottir, 2002). Additionally, the EGCG content of the ethanolic extract of bark (2.4 mg/g), although considerably lower than that in green teas (23–26 mg/g) (Khokhar & Magnusdottir, 2002), was comparable to that of black teas (2.7–3.4 mg/g) (Khokhar & Magnusdottir, 2002). The quercetin content of the ethanolic extract of bark (1.5 mg/g) was also higher compared to that in the skin of red grapes (40–169 mg/100 g) (Iacopini, Baldi, Storchi, & Sebastiani, 2008). The rutin content in the ethanolic extract of bark (4.6 mg/g) was found to be more than 10 fold higher than that found in Portuguese red grape skin (0.03–0.2 mg/g) (Novak, Janeiro, Seruga, & Oliveira-Brett, 2008). Finally, the myricetin content of the water extract of leaves (8.2 mg/g) and the ethanolic extract of bark (5.9 mg/g) was also found to be much higher than that in red grape skins (44 μg/g) (Novak et al., 2008) and the ethanolic extract of *Fructus lycii* (247 μg/g) (Le, Chiu, & Ng, 2007).

It has been proposed that although willow extracts have been traditionally used as anti-inflammatory compounds for their salicin content, the presence of high amounts of phenolic compounds can contribute to the beneficial effects seen with the consumption of commercial willow extracts (Nahrstedt et al., 2007). This hypothesis is supported by the fact that a wide range of flavonoids such as myricetin, kaempferol, quercetin, rutin and luteolin have immunomodulatory and anti-inflammatory activities by inhibiting pro-inflammatory cytokine production and their receptors (Qin & Sun, 2005). The considerable myricetin, rutin and catechin content of willow extracts identified in the current study could potentially contribute to the anti-inflammatory functions of willow extracts. We therefore propose that extracts from this species of plants may provide substantial amounts of a combination of antioxidants and thereby provide health promoting benefits to the consumers.

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