ANTIOXIDANT ACTIVITY AND PHENOLIC PROFILING OF TWO EGYPTIAN MEDICINAL HERBS *Polygonum salicifolium* Brouss ex Wild AND *Polygonum senegalense* Meisn

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Abstract. *Polygonum salicifolium* Brouss ex Wild and *Polygonum senegalense* Meisn (polygonaceae), are two medicinal herbs, native to Egypt. The methanol extracts of both the plants were analyzed for total phenolic, total flavonoid, and antioxidant activity. Chemical investigations afforded eight phenolic compounds identified on the basis of chemical and spectroscopic tools. This study shows that the investigated taxa could be good sources of free-radical scavenging compounds and may explain their traditional medicinal application.

Keywords: *Polygonum*; biological activity; flavonoids; *C*-glycosylflavone.

INTRODUCTION

Antioxidants are vital substances with the ability to protect the body from damage caused by free radicals induce oxidative stress [22]. Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT), are suspected to prompt negative health effects [21], strong restrictions have therefore been placed on their application and there is a trend to substitute them with naturally occurring antioxidants that focused more on medicinal plants, especially spices and herbs [10].

Thirteen species of *Polygonum* L. were listed in Egypt by Täckholm [26] while Boulos [2] separated the genus according to the morphological characters into two different genera *viz Polygonum* L. and *Persicaria* (L.) Mill. *Polygonum senegalense* Meisn is used for removal of ectoparasites from livestock and managing other diseases in cattle [20]. Flavonol glycosides are predominant in *Polygonum salicifolium* Brouss ex Wild while flavones, flavonols, chalcones and dihydrochalcones were isolated from *P. senegalense* [3, 5, 11, 20]. The present work aims to evaluate the antioxidant activity of the methanol extracts of *P. salicifolium* and *P. senegalense* in addition to the isolation and identification of the phenolic constituents which may be responsible to the antioxidant activity of the investigated taxa.

MATERIALS AND METHODS

General

2,2-diphenyl-1-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, butylated hydroxytoluene (BHT), Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany). UV spectra with shift reagents were recorded on Shimadzu, 2401, PC UV-visible recording spectrophotometer. NMR spectra were obtained in DMSO-d6 on Jeol- EX-500 spectroscopy; 500 MHz (1H NMR) and 125 MHz (13C NMR) and Joel JNM-EX 270 spectroscopy; 270 MHz (1H NMR) and 67.5 MHz (13C NMR). EI-Mass spectra were obtained using Finnigan MAT SSQ 7000 mass spectrometer. ESI-MS: Micromass Quattro-LC. Triple quadrupole mass spectrometer equipped with a Z-Spray electrospray ion source.

Collection of plant materials

The investigated taxa were collected from different localities and voucher specimens are deposited in the herbarium of the National Research Centre (CAIRC). Details of the collected samples are given below, *Polygonum salicifolium* Brouss. ex Wild, Ismailia Canal and Nile-Delta region, Egypt, 15 Nov. 2009, leg. S.R.Hussein, s.n. 837. *P. senegalense* Meisn, El-Kanater city, Nile-Delta region, Egypt, 2nd October 2009, leg. S.R.Hussein, s.n. 829. The investigated taxa were identified according to the description reported by Täckholm [26] and Boulos [2].

Preparation of crude extract

The aerial parts of *P. salicifolium* and *P. senegalense* were air dried until did not form lumps when touched and then ground with a coffee grinder into a fine powder that would pass through a 0.4 mm screen. Each powder (10 g) was extracted by 100 ml of methanol (90 %) at 50 °C for 5 h under reflux in soxhlet apparatus according to Matkowski and Piotrowska [19]. The extracts were then filtered and concentrated under reduced pressure at 50 °C using a rotary evaporator (Buchi Rotavapor R-200) to obtain a methanol type of crude extract. The crude extracts were stored at 4 °C before being used for the next antioxidant analysis.

Determination of total phenolics content

Total phenolic content of each extract was determined by using the Folin-Ciocalteu reagent [25]. Methanolic extract (0.5 ml), 0.5 ml of Folin-Ciocalteu reagent, 10 ml of 7.5% sodium carbonate and distilled water were added to a final volume of 25 ml. After 1h, the absorbance of the mixture was measured at 725 nm against a blank by spectrophotometer (Thermo Scientific Evolution - Unicam UV 300). The measurement was compared to a standard curve

prepared with gallic acid solution (Sigma Chemical). The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAL g^{-1} dw).

Determination of total flavonoids

The determination of total flavonoids was performed according to the colorimetric assay of Chang *et al.* [4]. Distilled water (2.8 ml) was added to 0.5 ml of plant extracts. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance was determined at 510 nm. A calibration curve was prepared with quercetin and the results were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QU g^{-1} dw).

Ferric reducing antioxidant power (FRAP assay)

Ferric reducing power was determined following the method reported by Zhao et al. [30]. The diluted methanol, extract (100 µL) contains (800µg/ml) and (1300µg/ml) for *Polygonum salicifolium* and *Polygonum senegalense* respectively, was mixed with phosphate buffer (2.5 ml, 200 mM, pH 6.6) and 1% potassium ferricyanide (2.5 ml). Then the mixtures were incubated at 50 °C for 20 min. The quantity 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 5000 $_x$g for 10 min at room temperature. The upper layer of the solution (5 ml) was removed into new test tube and mixed with distilled water (5 ml) and 0.1% ferric chloride (1 ml). The absorbance of the reaction mixtures were measured at 700 nm. The final results were expressed as mg ascorbic acid equivalents per gram of dry weight (mg ASC g^{-1} dw).

Determination of free radical scavenging activity (DPPH assay)

Quantitative measurement of radical scavenging properties of both extract was carried out according to Tenpe *et al.* [27]. Briefly, 0.1 mM solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH˙) in 95% methanol was prepared and 1 ml of this solution was added to 3 ml of each methanolic extract at different concentration (50-200 µg/mL). Butylated hydroxytoluene (BHT) was used as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. The capacity to scavenge the DPPH˙ radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \text{ADPPH} - \text{AS} / \text{ADPPH} \times 100$$

where, ADPPH is the absorbance of the DPPH solution and AS is the absorbance of the solution when the sample extract is added.

Isolation and identification of phenolic compounds

The aerial part of *Polygonum salicifolium* and *Polygonum senegalense* were dried and grounded. Each powder (500 & 650 gm, respectively) was extracted twice with 70% methanol. The concentrated extract (61 & 86 gm, respectively) was partitioned with solvents of increasing polarity (petroleum ether, diethyl ether, chloroform, acetone, ethyl acetate, methanol and water). Isolation of compounds carried out using column chromatography (MN-polyamide 6S) and paper chromatography (Whatman no. 1 and 3MM) using butanol–acetic acid–water 4:1:5 (BAW upper phase), water and 15% AcOH (water–acetic acid 17–3) [8, 16]. The diethyl ether and chloroform fractions of *P. senegalense* were added to each other and chromatographed on PC using BAW as eluent to yield compounds **2** & **3**. The methanol fraction was subjected to a polyamide column (85 × 3 cm) and eluted with MeOH/H_2O mixtures of decreasing polarities to yield compounds **4** & **5** from its fractions. The water fraction yields compounds **7** & **8** after chromatographed on PC using BAW flowed by % AcOH as eluent. The methanol fraction of *P. salicifolium* was chromatographed on PC using BAW then % AcOH as eluent to yield compounds **1**, **5** & **6**. The compounds were further purified on a Sephadex LH-20 column with standard solvent systems [18]. Trace compounds were identified by co-chromatography with reference samples (reference samples were obtained from Department of Phytochemistry and Plant Systematics, National Research Centre, Egypt).

Compounds identified were determined by R_f values, colour reactions, UV spectrophotometry, ^{1}H- and ^{13}C-NMR spectrometery (DMSO-*d6*), EI- or ESI-MS spectrometery and co-chromatography with reference samples. Sugars of *O*-glycosides were identified by enzymatic hydrolysis (β-glucosidase) or acid hydrolysis followed by co-chromatography with reference standards. The structure of isolated compounds is represented in figure (1). Details of the isolated compounds (**1-3, 5, 6, 8**) were present in Hussein *et al.* [11] while compounds **4** and **7** are outlined below:

3-*O*-α-rhamnopyranoside 6, 7-dimethoxy kaempferol (**4**): R_f: 0.76 (BAW), 0.22 (H_2O), 0.54 (15%AcOH). UV/Vis λmax (MeOH): 272, 345; (+NaOMe): 278, 329, 399; (+$AlCl_3$): 274, 305sh, 352; (+$AlCl_3$+HCl): 280, 303sh, 351, 406sh; (+NaOAc): 278, 301sh, 354; (+NaOAc+H_3BO_3): 276, 346. ^{1}H-NMR in DMSO-*d6*: δ 7.97 (2H, d, *J*= 8.5Hz, H-2`, H-6`), δ 6.96 (2H, d, *J*= 8.5Hz, H-3`, H-5`), δ 6.91 (1H, s, H-8), δ 5.31 (1H, d, *J*= 2.5Hz, H-1``), δ 3.90 (3H, s, OCH_3), δ 3.78 (3H, s, OCH_3), δ 0.81 (3H, d, *J*= 5.5Hz , CH_3-rhamnose).

Ellagic acid (**7**): R_f: 0.48 (BAW), 0.00 (H_2O), 0.09 (15%AcOH). UV/Vis λmax MeOH): 255, 358. ^{1}H-NMR in DMSO-*d6*:δ 7.46 (2H, s, H-5, H-5`). EIMS: *m/z* 302 equivalent to molecular formula of $C_{14}H_6O_8$

Statistical analysis

All experimental results were expressed as means ± S.E. Analysis of variance was performed by ANOVA procedures. The results with $P < 0.05$ were regarded to be statistically significant. Data were statistically analyzed using Costate Statistical Package [1].

RESULTS

Total phenolics and total flavonoids content

In this study, the total phenolics content was determined by using the Folin-Ciocalteu reagent and expressed as mg Gallic acid (GAL) equivalents/g dry weight, the level of total phenolic of *P. salicifolium* (33.98 mg/g dw) is higher than that of *P. senegalense* (23.4 mg/g dw). In addition the content of flavonoids, (expressed as quercetin equivalents) varied from 12.1 mg/g dw for *P. salicifolium* to 5.9 mg/g dw for *P. senegalense*.

Antioxidant activity

Ferric reducing antioxidant power (FRAP)

The presence of reductant such as antioxidant substances in samples causes the reduction of the Fe^{3+} ferricyanide complex to Fe^{2+} ferrous form. The transformation of iron (III) to iron (II)-reducing activity in methanol extract of both *Polygonum* species was expressed as mg ascorbic acid equivalents/g sample based on dry weight. The ferric reducing power activity (7.3 mg/g d.w.) was found in *Polygonum salicifolium* comparing to the activity (6.2 mg/g d.w.) of *Polygonum senegalense*.

Free radical scavenging activity by DPPH

Measurements of free radical scavenging activity (DPPH) were carried out according to Tenpe *et al.* [27]. This method is based on the reaction of DPPH that is characterized as a preformed stable free radical with a deep violet colour and any substance that can donate a hydrogen atom to DPPH reduces it to a stable diamagnetic molecule as stated previously by Lee and Shibamoto [15]. The results of the DPPH in % inhibition (Fig. 1) revealed that the methanolic extract of *P. salicifolium* exhibited the highest radical scavenging activity with 93.02%±0.04 followed by BHT standard with 86.5%±0.09 and *P. senegalense* extract with 68.13% ±0.06 at 200µg/ml. The IC50 of *P. salicifolium* was found to be 27.46 ±0.81µg ml^{-1}, compared to *P. senegalense*, which showed an IC50 of 35.02 ±0.19 µg ml^{-1}, where the IC50 value of BHT was found to be 30.14 ±0.51 µg ml^{-1}. The IC50 values are negatively related to the antioxidant activity, as it express the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC50 value represents the higher antioxidant activity of the tested sample.

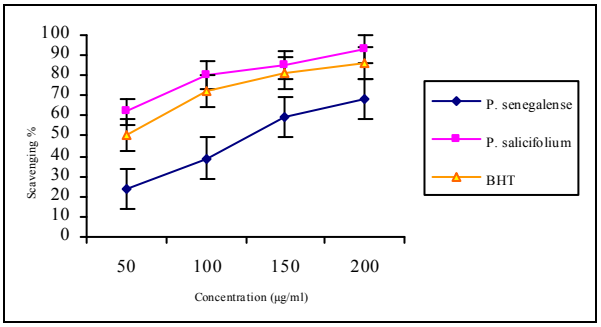

Figure 1. DPPH radical scavenging activity of *P. salicifolium* , *P. senegalense* and BHT standard. Each value is expressed as mean ± standard error (n = 3)

Phenolic investigations

Eight compounds (one flavone, six flavonols and two phenolic acids) were isolated from the studied taxa using chemical and physical investigations and identified as: Apigenin-6-*C*-arabinopyranosyl-8-*C*-glucopyranoside (**1**), 3,6-dimethoxy-kaempferol (**2**), 3,7,4`-tri-methoxy-kaempferol (**3**), 6,7-dimethoxy-kaempferol-3-*O*-α-rhamnopyranoside (**4**), quercetin-3-*O*-β-glucopyranoside (**5**), quercetin-3,7-di-*O*-glucopyranoside (**6**), ellagic acid (**7**) and gentisic acid 5-*O*-(6`-*O*-galloyl)-glucopyranoside (**8**). The structure of isolated compounds is represented in figure (2).

Three compounds (**1**, **5** and **6**) were identified in *P. salicifolium,* while six compounds (**2-5, 7**and **8**) were identified in *P. senegalense.* Compounds **4** and **7** were identified in *P. senegalense* for the first time while the remaining compounds were previously recorded by Hussein *et al.* [11]. The isolated compounds were identified by comparison of the spectral data with those reported in literature [11, 16, 23, 24] and/or by co-chromatography with authentic samples.

DISCUSSION

It has been found that phenolics contribute to quality and nutritional value in terms of modifying colour, taste, aroma, flavor and in providing beneficial health effects [28]. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports [6, 14]. In addition flavonoids show antioxidant activity and their effects on human nutrition and health are considerable, the action mechanisms of flavonoids are through the scavenging or chelating process [13].

In accordance with our work, genus *Polygonum* is a diuretic herb that is used mainly in removing of kidney stones, it used in the folk medicine as expectorant, tonic, insecticide, antihelmentic, astringent, heamostatic, and in treatment of gout and haemorroids [7, 29]. In this study, both herbs exhibited FRAP and antioxidant activity which may support the traditional uses of this herb. The reducing properties are associated with the presence of compounds such as phenolics and flavonoids, which exert their action by breaking the free radical chain through donating a hydrogen atom. Thus, the redox potential of phenolic compounds plays an important role in determining the antioxidant capacity [17]. The DPPH results agree with the in vitro results of Hsu *et al*., [9] who reported that *Polygonum cuspidatum* Siebold & Zucc. is an important source of superoxide radicals scavenging and clearly has antioxidant effects.

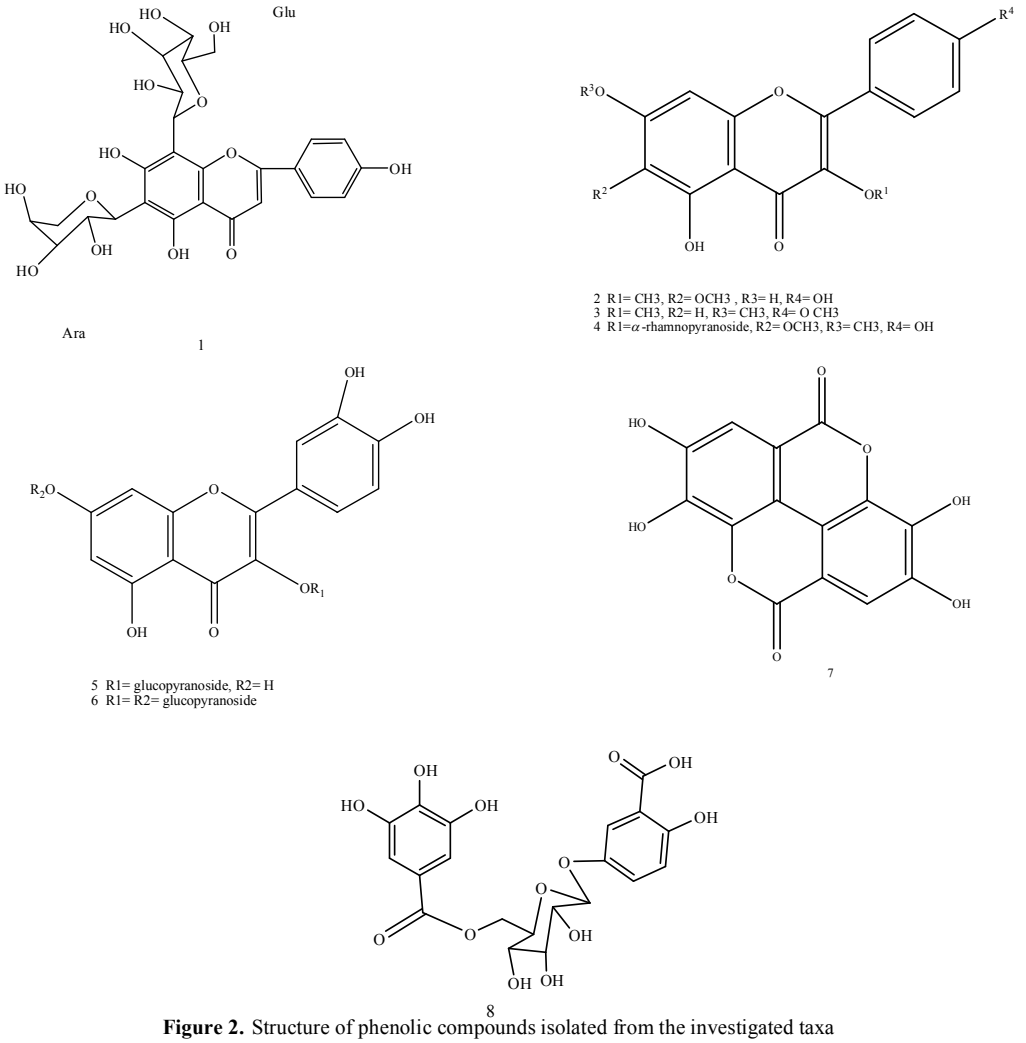

Figure 2. Structure of phenolic compounds isolated from the investigated taxa

From the phenolic and flavonoid profile of the studied taxa; *P. salicifolium* is characterized from *P. senegalense* in presence of apigenin-6-*C*-arabinopyranosyl-8-*C*-glucopyranoside (compound **1**), such nucleus may explain why the antioxidant activity of *P. salicifolium* is slightly stronger than the other taxa. This is in accordance with Jay *et al.* [12] who mentioned that plants rich in *C*-glycosylflavone compounds give high antioxidant effect relative to those containing quercetin. All the previous results indicated that the extracts of the studied taxa have a noticeable effect on the scavenging of free radicals and can be regarded as promising candidates for a plant-derived antioxidant compounds and reveal that Egyptian species offer an interesting source of new antioxidative plant extracts, such as those of *P. salicifolium* and *P. senegalense* there being a potential for their use in different fields (foods, cosmetics, pharmaceuticals) and may encourage their consumption for health protection.

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