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ORIGINAL ARTICLE

Chemical composition of selected Saudi medicinal plants

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Abstract Medicinal plants are important in traditional medicine and modern pharmaceutical drugs; therefore, the interest in the analysis of their chemical composition is increasing. In this study, selected medicinal plants including *Achillea fragrantissima* (Forssk) Sch., *Amaranthus viridis* L., *Asteriscus graveolens* (Forssk.) Less., *Chenopodium album* L., and *Conyza bonariensis* (L.) Cronquist were collected from the rangeland of western regions (Bahra and Hada areas) of Saudi Arabia to study their chemical composition. Eight minerals (Mg, Ca, Cr, Mn, Fe, Co, Cu, and Zn), total phenolic contents, antioxidant activity, and free-radical scavenging ability were examined in order to evaluate the medicinal potential of these plants. All the plants were found to be rich sources of minerals and antioxidants, although there were significant differences ($p < 0.05$) in their chemical composition, which may provide a rationale for generating custom extracts from specific plants depending on the application. The findings of this study will thus facilitate herbalists in their efforts to incorporate these plants into various formulations based on their chemical composition.

1. Introduction

Medicinal plants have been used for centuries throughout the world, and many people still rely on indigenous medicinal plants for their safe or primary health care needs. Several researchers including Rahman et al. (2004), El-Ghazali et al. (2010) and Daur (2012) have revealed that Saudi Arabia has valuable medicinal plants and its natural stress conditions of drought and heat are considered as positive factors for medicinal plants. To date however, little attention has been focussed on the chemical analysis, conservation, and sustainable production of medicinal plants in the country (Sher and Alyemeni, 2011; Almehdar et al., 2012). Due to the increasing interest in herbal medicine, our objective in this study was to conduct a systematic chemical analysis of the medicinal plants in the Kingdom of Saudi Arabia.

The present study was designed to collect and analyze certain plants of Saudi Arabia (Af, Av, Ag, Ca, Cb) that are

Abbreviations: Af, *Achillea fragrantissima*; Av, *Amaranthus viridis*; Ag, *Asteriscus graveolens*; Ca, *Chenopodium album*; Cb, *Conyza bonariensis*

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mentioned with medicinal value in folk-care system by various researchers, such as McGaw and Eloff (2008), Gidaya et al. (2009), Jana and Shekhawat (2010).

2. Materials and methods

Medicinal plants (Af, Av, Ag, Ca, Cb) were collected from rangeland in Western Saudi Arabia that extends over 164,000 km^2 along the coast of the Red Sea, including Makkah and Jeddah regions (Makkah Province). Chemicals in the study HNO_3, H_2O_2 and multi-elemental standard were from Merck Chemical Ltd while methanol, Folin–Ciocalteu's Phenol Reagent (2 M), sodium carbonate (Na_2CO_3), gallic acid, anhydrous sodium acetate, deionized water, $FeCl_3{\cdot}6H_2O$, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine, HCl, $FeSO_4$ and DPPH (2,2-diphenyl-1-picrylhydrazyl) were from Sigma Aldrich Chemical Ltd.

2.1. Sample preparation

All the plant samples were oven-dried at 65 °C for 48 h and ground in a Wiley mill (1-mm screen). The following analyses were performed at least in triplicate for each plant sample.

2.2. Determination of minerals

The minerals were determined using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) by following the methodology of Momen et al. (2006) with little modifications. Briefly, for each sample 0.5 g of the powdered sample was taken in a beaker and 8 mL of 6:2 HNO_3 (65%)/H_2O_2 (30%) mixture was added; the mixture was kept covered for 12 h at room temperature (RT). The covered beaker was then placed on a hot plate and was heated for 2 h until digestion was complete. After cooling to RT, the contents of the tubes were transferred to 100-mL volumetric flasks, and the volumes of the contents were made up to 100 mL using deionized water. The wet digested solutions were transferred to plastic bottles and labeled. Eight minerals (Mg, Ca, Cr, Mn, Fe, Co, Cu, and Zn) were then quantified using ICP-OES. The operating conditions used for the ICP-OES determination were 1300 W RF power, 15 L min^{-1} plasma flow, 2.0 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, and 1.5 mL min^{-1} sample uptake rate. The calibration standards were prepared from multi-elemental standard after appropriate dilution.

2.3. Extract preparation

For antioxidant analysis, 1 g of each plant powdered material was extracted in 50 mL methanol (70%) by swirling for 1 h at RT in an orbital shaker. The extracts were then filtered by passing through Whatman filter paper (No. 1) and stored at 4 °C for the following analyses.

2.3.1. Total phenolic content

The total phenolic content was measured according to the method of Ghasemzadeh et al. (2010). Sample of plant extract or standards (0.5 mL) was mixed with 2.5 mL of Folin–Ciocalteu's Phenol Reagent (2 M). After 5 min, 2.0 mL of 20% sodium carbonate was added to the mixture and kept for 1 h; then the absorbance was measured at 750 nm by using a spectrophotometer (U-2001, model 121-0032 Hitachi, Tokyo, Japan). The amounts of total phenol in the samples were calculated using a gallic acid calibration curve. The curve was prepared using 0, 50, 100, 150, 200, and 250 mg L^{-1} solutions of gallic acid in methanol:water (50:50, v/v). The results were expressed as mg gallic acid/g of dry plant material.

2.3.2. Ferric-reducing ability of plasma assay (FRAP assay)

The FRAP assay, is a powerful tool for evaluating antioxidant activity, where ferric-tripyridyltriazine (TPTZ-Fe^{3+}) complex is reduced to TPTZ-Fe^{2+} in the presence of antioxidants. FRAP values were obtained for the plants by following the method of Benzie and Strain (1999) with slight modifications, plant extracts (150 µL) were mixed with 2.85 mL of FRAP solution that contains a mixture of acetate buffer (300 mM, pH 3.6), ferric chloride (20 mM, $FeCl_3{\cdot}6H_2O$), and TPTZ-solution of 10 mM [2,4,6-Tris(2-pyridyl)-s-triazine] in proportion of 4:1:1. After 30 min of reaction, the absorbance was measured at 593 nm. The results were calculated from a standard curve of µM $FeSO_4$ and were expressed as µM $FeSO_4$/g dry plant material.

Below is the detail of each reagent of FRAP solution mentioned above:

- Acetate buffer (300 mM, pH 3.6): 1.68 g of anhydrous sodium acetate was dissolved in 800 ml of deionized water, and 16 ml of acetic acid was added. The total volume was made to 1000 ml with deionized water. The pH was corrected with acetic acid or with 0.1 N NaOH, as required.
- Ferric chloride (20 mM, $FeCl_3{\cdot}6H_2O$): 270 mg $FeCl_3{\cdot}6H_2O$ was dissolved in 50 ml of deionized water.
- TPTZ-solution of 10 mM: 160 mg of TPTZ was dissolved in 50 ml of 40 mM HCl.

2.3.3. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The assay was carried out spectrophotometrically as described by Koleva et al. (2002). Briefly, 2.5 mL of each plant extract was added to 2.5 mL of methanolic solution of DPPH (100 M). After incubating at RT for 15 min, absorbance was read at 517 nm. The assay is based on the ability of the extract to scavenge the stable DPPH. The results were expressed as the percentage inhibition of the DPPH radical that was calculated using the following formula: DPPH Radical Scavenging Activity (%) = $[(A0 - A1/A0) \times 100]$, where A0 indicates absorbance of the control, and A1 absorbance of extract sample.

2.4. Statistical analysis

Data were analyzed statistically according to a completely randomized design using MSTATC software, and means were compared using the least significant differences test (Russell, 1986). Microsoft Excel (Microsoft Corp., Redmond, WA, USA) was used for calculating the standard deviation values.

3. Results and discussion

Table 1 shows the results of the mineral analysis for plants including *Achillea fragrantissima* (Forssk) Sch., *Amaranthus viridis* L., *Asteriscus graveolens* (Forssk.) Less, *Chenopodium*

Table 1 Mineral composition of selected Saudi medicinal plants.

Plants	Minerals (ppm)							
	Mg	Ca	Cr	Mn	Fe	Co	Cu	Zn
Achillea fragrantissima (Forssk) Sch	1200^{c} (±4.12)	14400^{b} (±6.01)	4.10^{c} (±0.05)	88.5^{c} (±0.08)	192^{d} (±0.22)	0.43^{b} (±0.01)	4.44^{d} (±0.14)	400^{b} (±0.28)
Amaranthus viridis L.	8255^{b} (±6.10)	15280^{a} (±5.80)	5.16^{c} (±0.05)	108.10^{b} (±0.08)	480^{a} (±3.20)	0.42^{b} (±0.11)	12.44^{b} (±0.10)	356^{c} (±0.22)
Asteriscus graveolens (Forssk.) Less.	1050^{c} (±5.17)	13200^{c} (±4.80)	8.4^{b} (±0.21)	107.10^{b} (±0.19)	204^{d} (±2.08)	0.63^{a} (±0.03)	26.8^{a} (±0.18)	544^{a} (±1.20)
Chenopodium album L.	8900^{a} (±6.80)	11500^{d} (±10.14)	18.73^{a} (±2.10)	148.00^{a} (±0.48)	380^{b} (±0.51)	0.68^{a} (±0.02)	14.44^{b} (±0.04)	21.20^{d} (±0.28)
Conyza bonariensis (L.) Cronquist	1092^{c} (±0.17)	10200^{e} (±4.48)	5.50^{c} (±0.11)	152.60^{a} (±0.14)	255.1^{c} (±0.05)	0.12^{c} (±0.01)	8.20^{c} (±0.22)	38.80^{d} (±0.10)

Each value in the table is the mean of three replicates (±standard deviation).
Mean values with different superscript letters in the same row differ significantly ($p < 0.05$).

Table 2 Total phenolic content, antioxidant activity and free radical scavenging activity of selected Saudi medicinal plants.

Test sample	Total phenolic content GAE[a] (mg/g)	Antioxidant activity FRAP (μM $FeSO_4$/g)	Free radical scavenging activity [DPPH inhibition (%)]
Achillea fragrantissima (Forssk) Sch	48.2 ± 3.00^{b}	1655.11 ± 44.20^{b}	32.02^{d}
Amaranthus viridis L.	36.0 ± 1.33^{c}	1488.20 ± 50.01^{c}	16.04^{c}
Asteriscus graveolens (Forssk.) Less.	35.0 ± 2.10^{c}	1544.30 ± 32.00^{bc}	25.00^{b}
Chenopodium album L.	45.0 ± 2.40^{b}	1765.50 ± 64.40^{ab}	28.01^{b}
Conyza bonariensis (L.) Cronquist	78.0 ± 2.10^{a}	1820.00 ± 38.10^{a}	58.04^{a}

Each value in the table is the mean of three replicates ± standard deviation.
Mean values with different superscript letters in the same row differ significantly ($p < 0.05$).
[a] GAE, gallic acid equivalents.

album L., and *Conyza bonariensis* (L.) Cronquist. We observed significant differences ($p < 0.05$) in the concentrations of Mg, Ca, Cr, Mn, Fe, Co, Cu, and Zn in the medicinal plants. This result is supported by the results of Pytlakowska et al. (2012), which also revealed differences in the mineral composition of several medicinal plants. We note here that our focus is not to compare but rather to explore the mineral composition in the selected plants and to discuss their importance in human health. Due to a deficiency of these minerals in human diet, most of these minerals are often taken as supplements (Agarwal et al., 2011) for their important role in human health; for example, magnesium is reported to have a curative effect in more than 300 health disorders, including headache and fatigue (Champagne, 2008; Sun-Edelstein and Mauskop, 2009; Rodriguez-Moran et al., 2011; Bor-Tsung et al., 2011; Dowling et al., 2012; Sylvia et al., 2013). Calcium is required for bone repair, vascular contraction, and many other metabolic functions (Straub, 2007; Zhao et al., 2011; Munaron, 2012). Cr ameliorates some of the symptoms of diabetes (Cefalu et al., 2010) and Mn plays a role in the function of connective tissue, bones, and blood clotting factors (Son et al., 2007). Similarly Cr, Mn, Fe, Co, Cu, and Zn have been reported as essential or beneficial to human health (Martínez-Ballesta et al., 2010).

Table 2 shows the total phenolic content, antioxidant activity, and free radical scavenging activity for all the plants in the current study. According to Krishnaiah et al. (2011) two-thirds of all the plant species in the world have medicinal value. This is largely due to their antioxidant potential, which can help prevent the cellular oxidative stress associated with the pathology of diseases such as cancer and cardiovascular or inflammatory diseases. The new plant (Af, Av, Ag, Ca, Cb*)* showed considerable antioxidant activity ranging from 1488.20 to 1820.00 μM FeSO4/g that is comparable to many valuable medicinal plants as reported by Katalinic et al. (2006). Similarly free radical scavenging activity is comparable to other important medicinal plants as reported by Padmanabhan and Jangle (2012). Our present study actually underscores the potential utility of the selected Saudi medicinal plants in home recipes or herbal medicine that may be of interest to consumers. As there are no previous investigations on chemical analysis of these medicinal plants neither in Saudi Arabia nor elsewhere so will this investigation be work globally as source of knowledge for other researchers. It may be worth to mention that in Table 2, lower absorbance of the reaction mixture [DPPH inhibition (%)] means higher free radical scavenging activity. In this study, significant differences ($p < 0.05$) were observed among the studied plants for each of the properties that were evaluated. Our results regarding differences in total phenolic content, antioxidant activity, and free radical scavenging activity are consistent with the findings of Singh et al. (2012) and Jaberian et al. (2013), who also observed differences in the antioxidant properties in other medicinal plants.

4. Conclusions

The current study of selected Saudi medicinal plants (Af, Av, Ag, Ca, Cb) may help in characterization and documentation of the medicinal plant diversity of the country. It is hoped that the study will generate an interest in the proper consumption, conservation, and sustainable production of these plants. The findings of the study are expected to be equally beneficial to everyone (beyond Saudi Arabia) dealing with medicinal plants across the world.

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