

Antioxidant activity and total phenolic content of selected Jordanian plant species

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Abstract

There is currently an upsurge of interest in phytochemicals as new sources of natural antioxidants. The aim is to use them in foods and pharmaceutical preparations in order to replace synthetic antioxidants, which are being restricted due to their potential health risks and toxicity. The relative levels of antioxidant activity and the total phenolic content of aqueous and methanolic extracts of a total of 51 plant species of Jordanian origin have been determined using the improved ABTS^{•+} method and the Folin–Ciocalteu colorimetric method. The total phenolic content of aqueous and methanolic extracts of the investigated plant species ranged from 2.8 to 70.3 and from 2.6 to 59.6 mg GAE/g dry weight, respectively, while the total antioxidant capacity ranged from 12.9 to 731 and from 10.1 to 720 μmol TE/g dry weight, respectively. Based on our results, a number of plant species, namely, *Arbutus andrachne*, *Hypericum triquetrifolium* and *Rosmarinus officinalis*, were identified as among the best sources of free radical-scavenging compounds. There was positive linear correlation between antioxidant activity and total phenolic content for aqueous and methanolic extracts. Thus, it was concluded that phenolic compounds were the predominant antioxidant components in the investigated plant species.

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1. Introduction

Free radicals may be defined as any chemical species that are capable of existing with one or more unpaired outer shell electrons. They are extremely reactive and generally highly unstable (Martínez-Cayuela, 1995). Reactive oxygen species, such as superoxide radical (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl radical (OH[•]), and singlet oxy-

gen (¹O₂), are of the greatest biological significance (Martínez-Cayuela, 1995; Schöneich, 1999). They are extremely reactive and potentially damaging transient chemical species. In addition to exogenous sources of free radicals, such as ionizing radiation, tobacco smoke, pesticides, pollutants, and some medications, they are produced continuously in all cells, as metabolic byproducts by a number of intracellular systems: small cytoplasmic molecules, cytoplasmic proteins, membrane enzymes, peroxisomes, mitochondrial electron transport systems, and microsomal electron transport systems (Martínez-Cayuela, 1995).

All cellular components, proteins, polyunsaturated fatty acids, nucleic acids and carbohydrates, are prominent bio-

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logical targets of reactive oxygen species, giving rise to metabolic and cellular disturbances (Martínez-Cayuela, 1995). Fortunately, within biological systems, there are enzymatic systems and chemical scavengers: dietary antioxidants (α -tocopherol, β -carotene, ascorbic acid, glutathione, uric acid), some hormones (estrogen, angiotensin), and endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase), all of which are able to remove oxygen free radicals formed in cells and thus protect against oxidative damage (Halliwell & Gutteridge, 1990; Martínez-Cayuela, 1995). Tissue damage resulting from the imbalance between reactive oxygen species generating and scavenging systems (oxidative stress) has been implicated in the pathology of a number of disorders, such as atherosclerosis, ischemia-reperfusion injury, cancer, malaria, diabetes, inflammatory joint disease, asthma, cardiovascular diseases, cataracts, immune system decline, and could play a role in neurodegenerative diseases and ageing processes (Florence, 1995; Nakagami, Nanaumi-Tamura, Toyomura, Nakamura, & Shigehisa, 1995; Martínez-Cayuela, 1995; Schöneich, 1999; Young & Woodside, 2001).

Lipid peroxidation is one of the major causes of deterioration in foods that results in the formation of potentially toxic compounds. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butyl hydroquinone (TBHQ) and propylgallate (PG), are widespread food additives used to preserve against deterioration; however, their use is increasingly restricted, due to their potential health risks and toxicity. Moreover, there is a growing awareness among consumers regarding food additive safety (Moure et al., 2001).

There is currently an upsurge of interest in phytochemicals as potential new sources of natural antioxidants. The goal is to use them in foods and pharmaceutical preparations to replace synthetic antioxidants (Cai, Luo, Sun, & Corke, 2004; Katalinic, Milos, Kulisic, & Jukic, 2006; Wong, Leong, & Koh, 2006). Most antioxidants isolated from higher plants are polyphenols. In vascular plants, more than 4000 phenolic and polyphenolic compounds have been identified (e.g. phenolic acids, tannins, coumarins, anthraquinones, flavonoids) (Middleton & Kandaswami, 1994; Trease & Evans, 1989). A wide range of low and high molecular weight plant polyphenols with antioxidant properties has been studied (Hagerman et al., 1998). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have metal-chelating potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Moreover, phenolic compounds show different biological activities as antibacterial, anticarcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, and immune-stimulating agents (Larson, 1988).

The main aim of this research project was to screen some plant species in Jordan, with respect to their total phenolic content and antioxidant activity, as potential sources of natural antioxidants. The relationship between

phenolic content and antioxidant activity was also statistically investigated.

2. Materials and methods

2.1. General

The total antioxidant capacity assay was performed on MultiSpec-1501, SHIMADZU[®] photodiode diode array spectrophotometer (Kyoto, Japan), fitted with Julabo F40, Ultratemp 2000 temperature control. Total phenolic content assay was carried out using a Spectronic 601 spectrophotometer, (Milton Roy Company, USA). Aqueous and methanolic extracts were prepared using a KARL KOLB, water bath shaker (Scientific Technical Supplies, D-6072, Dreieich, Germany). The incubator was obtained from Binder GmbH, Bergstr., Tuttlingen. HPLC-grade methanol was obtained from Scharlau Chemie S.A. (Barcelona, Spain). HPLC-grade ethanol was obtained from Fisher Scientific UK limited, Bishop Meadow Road, Loughborough, Leicestershire LE11 5RG, UK. ABTS[®] was obtained from AppliChem GmbH, Ottoweg 10b, D-64291, Darmstadt; potassium persulfate and trolox[®] were obtained from Acros Organics, New Jersey, USA, Geel, Belgium. Folin–Ciocalteu reagent (2 N) was obtained from SDS Fine Chemicals, India. Gallic acid monohydrate was obtained from Janssen Chemica, B-2440, Geel, Belgium. Sodium carbonate, chemically pure, was obtained from Frutarom (UK) Ltd., Berkhamsted, Herts, UK.

2.2. Plant material

Wild plant materials were collected from different locations in Jordan. The collected materials were identified by M. G. Voucher specimens of collected plant species were deposited at the Herbarium Museum of the Faculty of Pharmacy, Jordan University of Science and Technology. The plant raw materials were cleaned and air-dried at room temperature. Plant parts were ground to a fine powder using a laboratory mill, passed through a 24 mesh sieve, to provide homogeneous powder for the analysis. Powdered materials were maintained at room temperature (22–23 °C), and protected from light until required for analyzes.

2.3. Extraction process

For aqueous extraction, a 250 mg aliquot of each dried and ground plant species was weighed into a test tube and extracted with 10 ml of deionized water at 80 °C for 1 h in a water bath shaker. After cooling, the extract was centrifuged at 3500 rpm for 10 min, and the supernatant was recovered and stored at 4 °C until used for the TEAC and total phenolic content assay. For methanolic extraction, a 250 mg aliquot was extracted with 10 ml of 80% methanol at 37 °C for 3 h in a shaking water bath. Other procedures were the same as in the water extraction

method. Two extraction replicates of the methanolic and water extracts were prepared for each plant species.

2.4. Assaying methods

2.4.1. Determination of total antioxidant activity

The antioxidant capacity assay was carried out using the improved ABTS^{•+} method, as described by Re et al. (1999). Briefly, ABTS^{•+} radical cation is generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate via incubation at room temperature (23 °C) in the dark for 12–16 h. The ABTS^{•+} solution was diluted with 80% HPLC-grade ethanol to an absorbance of 0.700 ± 0.040 at 734 nm and equilibrated at 30 °C. Plant extracts were diluted with distilled water or 80% methanol, such that after introduction of a 30 μ l aliquot of each dilution into the assay, it produced from 20% to 80% inhibition of the blank absorbance. To 3 ml of diluted ABTS^{•+}, 30 μ l of each plant extract solution were added and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6 min and the absorbance was recorded immediately at 734 nm. Trolox standard solutions (concentrations from 0 to 2.5 mM) in 80% ethanol were prepared and assayed using the same conditions. Appropriate solvent blanks were run in each assay. The percent of inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of trolox for the standard reference data. The absorbance of the resulting oxidized solution was compared to that of the calibrated trolox standard. Results were expressed in terms of trolox equivalent antioxidant capacity (TEAC, μ mol trolox equivalents per g dry weight of plant) (Re et al., 1999).

2.4.2. Determination of total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965), using gallic acid as a standard phenolic compound. Briefly, 50 μ l (two replicates) of the filtered extracts were mixed with 450 μ l of distilled water and 2.5 ml of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 ml of saturated sodium carbonate (75 g/l) were added. The absorbance of the resulting blue-coloured solution was

measured at 765 nm after incubation at 30 °C for 1.5 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of six points: 20, 100, 200, 300, 400, 500 mg/l of gallic acid in 80% methanol. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gramme of dry material.

2.5. Statistical analysis

For both assays, all data are shown as means \pm SD from two extraction replicates, each run in duplicate. Correlation and regression analysis of antioxidant activity (X) versus the total phenolic content (Y) was carried out using Microsoft Office Excel 2003. The student t -test was applied to test for significant differences between aqueous and methanolic extracts for antioxidant activity and total phenolic content.

3. Results and discussion

3.1. Antioxidant activity

The improved ABTS^{•+} method, as described by Re et al. (1999), was used to determine the antioxidant capacity for the plant species examined in this work. The concentration–response curves for ABTS^{•+}, as a function of five separately prepared stock solutions of trolox standards (0.25, 0.5, 1, 1.5, and 2 mM), are shown in Fig. 1a in terms of trolox equivalent antioxidant capacity (TEAC, μ mol trolox equivalents per g dry weight of plant). There was a large variation in the total antioxidant capacity of the aqueous and methanolic extracts of the plant species analyzed, as shown in Table 1. The values ranged from 12.9 to 731 and from 10.1 to 720 μ mol TE/g dry weight for aqueous and methanolic extracts, respectively. Highest levels of antioxidant activity of aqueous/methanolic extracts were obtained from *Arbutus andrachne* 731/720 μ mol TE/g dry weight, and *Hypericum triquetrifolium* 422/594 μ mol TE/g dry weight. The lowest levels of antioxidant activity were obtained from the aqueous/methanolic extracts of *Cicer arietinum* (15.3/10.1 μ mol TE/g dry weight) and the aque-

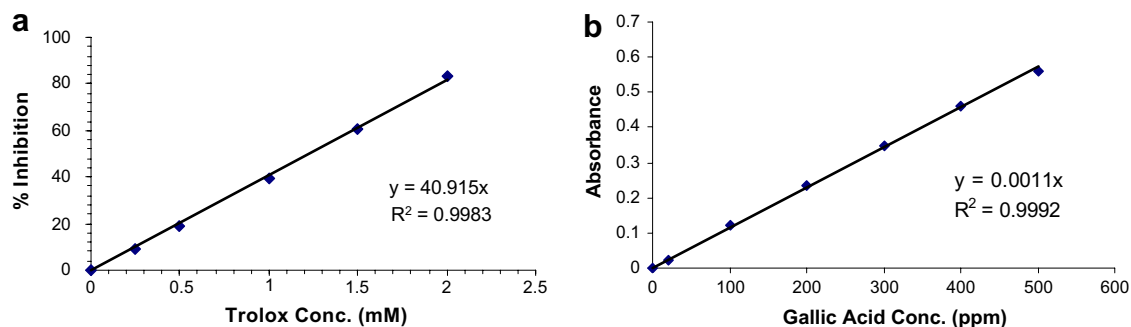


Fig. 1. (a) Concentration–response curve for the absorbance at 734 nm for ABTS^{•+} as a function of standard trolox solution. (b) Concentration–response curve for the absorbance at 765 nm for gallic acid standard.

Table 1
Antioxidant activity and total phenolic content of aqueous and methanolic extracts of 51 plant species from Jordan

Plant name	Antioxidant activity ($\mu\text{mol TE/g dry weight}$) ^a		Total phenolic content (mg GAE/g dry weight) ^b	
	Aqueous extracts	Methanolic extracts	Aqueous extracts	Methanolic extracts
<i>Achillea biebersteinii</i> Afan. (Asteraceae)	67.9 \pm 4.6	154 \pm 5.1	16.4 \pm 0.9	23.3 \pm 0.6
<i>Adonis palaestina</i> Boiss (Ranunculaceae)	73.9 \pm 7.2	60.2 \pm 3.7	18.0 \pm 1.9	14.1 \pm 0.4
<i>Anagallis arvensis</i> L. (Primulaceae)	146 \pm 4.9	63.8 \pm 2.8	24.7 \pm 1.0	19.5 \pm 0.7
<i>Anchusa italica</i> Retz. (Boraginaceae)	32.9 \pm 6.3	26.6 \pm 2.0	6.8 \pm 1.9	6.1 \pm 0.3
<i>Anthemis palestina</i> Boiss. (Asteraceae)	82.4 \pm 9.3	88.9 \pm 3.2	20.7 \pm 4.2	23.2 \pm 0.7
<i>Arbutus andrachne</i> L. (Ericaceae)	731 \pm 5.4	720 \pm 2.7	58.6 \pm 1.4	57.6 \pm 0.8
<i>Artemisia herba-alba</i> Asso (Asteraceae)	169 \pm 8.5	151 \pm 2.9	23.5 \pm 0.8	34.6 \pm 4.2
<i>Astragalus berytheus</i> Boiss. & Blanche (Papilionaceae)	63.2 \pm 3.1	43.9 \pm 1.4	14.2 \pm 1.1	13.5 \pm 0.3
<i>Astragalus peregrinus</i> Vahl (Papilionaceae)	53.9 \pm 5.9	38.7 \pm 1.2	11.7 \pm 0.4	14.6 \pm 0.3
<i>Calendula arvensis</i> L. (Asteraceae)	48.4 \pm 1.7	42.0 \pm 5.4	16.8 \pm 0.7	12.3 \pm 0.4
<i>Chrysanthemum coronarium</i> L. (Asteraceae)	143 \pm 5.9	224 \pm 7.1	27.4 \pm 0.5	59.6 \pm 4.1
<i>Cicer aretinum</i> ^c L. (Papilionaceae)	15.3 \pm 0.3	10.1 \pm 0.6	2.8 \pm 0.2	2.6 \pm 1.1
<i>Cichorium pumilum</i> Jacq. (Asteraceae)	86.4 \pm 0.1	73.1 \pm 0.4	19.5 \pm 1.1	15.7 \pm 0.8
<i>Citrus sinensis</i> ^{d,e} (L.) Osbeck. (Rutaceae)	90.2 \pm 1.7	55.8 \pm 2.8	23.4 \pm 1.0	13.9 \pm 0.0
<i>Cleome africana</i> Botsch. (Capparaceae)	46.8 \pm 2.6	40.0 \pm 1.6	14.0 \pm 0.1	12.6 \pm 0.4
<i>Convolvulus althaeoides</i> L. (Convolvulaceae)	64.3 \pm 3.3	70.2 \pm 3.5	18.9 \pm 0.3	17.6 \pm 0.7
<i>Crocus moabticus</i> Bornm. et Dinsm. (Iridaceae)	55.5 \pm 1.4	49.7 \pm 1.2	13.5 \pm 0.9	10.3 \pm 0.2
<i>Cyclamen persicum</i> Mill. (Primulaceae)	110 \pm 10.8	150 \pm 9.1	20.0 \pm 2.5	17.0 \pm 0.6
<i>Eryngium creticum</i> Lam. (Apiaceae)	49.3 \pm 7.5	56.4 \pm 0.6	11.6 \pm 0.2	13.3 \pm 0.5
<i>Fagonia arabica</i> L. (Zygophyllaceae)	75.1 \pm 1.3	30.1 \pm 0.9	18.0 \pm 0.4	11.3 \pm 0.1
<i>Fagonia bruguierii</i> DC. (Zygophyllaceae)	64.0 \pm 5.9	28.6 \pm 0.7	11.2 \pm 1.9	7.1 \pm 0.2
<i>Fumaria densiflora</i> DC. (Fumariaceae)	82.2 \pm 0.6	69.6 \pm 1.0	17.3 \pm 0.2	16.9 \pm 0.3
<i>Ginkgo biloba</i> ^c L. (Ginkgoaceae)	311 \pm 1.2	276 \pm 1.3	39.0 \pm 3.8	35.3 \pm 1.0
<i>Glaucium aleppicum</i> Boiss. & Hausskn. (Papaveraceae)	144 \pm 7.2	79.3 \pm 1.1	17.5 \pm 4.8	20.6 \pm 0.6
<i>Glaucium flavum</i> Crantz (Papaveraceae)	36.1 \pm 2.1	46.9 \pm 3.9	10.3 \pm 0.5	12.8 \pm 0.3
<i>Gundelia tournefortii</i> L. (Asteraceae)	57.3 \pm 2.7	63.9 \pm 2.0	13.2 \pm 5.0	14.7 \pm 0.2
<i>Haplophyllum buxbaumii</i> (Poir.) G. Don. (Rutaceae)	86.4 \pm 2.4	85.0 \pm 0.4	18.8 \pm 1.2	16.6 \pm 0.3
<i>Helianthemum ledifolium</i> (L.) Mill. (Cistaceae)	40.1 \pm 10.3	69.8 \pm 6.0	8.7 \pm 3.0	17.3 \pm 1.0
<i>Hypecoum dimidiatum</i> Delile (Papaveraceae)	97.5 \pm 8.7	72.7 \pm 9.8	19.1 \pm 0.2	13.9 \pm 0.3
<i>Hypericum triquetrifolium</i> Turra (Hypericaceae)	422 \pm 3.0	594 \pm 2.1	70.3 \pm 2.4	48.1 \pm 3.2
<i>Lavendula angustifolia</i> ^c P. Mill. (Lamiaceae)	84.6 \pm 3.7	32.8 \pm 0.3	18.1 \pm 0.4	8.3 \pm 0.0
<i>Linum pubescens</i> Banks & Sol. (Linaceae)	12.9 \pm 3.5	37.6 \pm 2.3	6.9 \pm 0.7	11.3 \pm 0.1
<i>Majorana syriaca</i> (L.) Kostel. (Lamiaceae)	92.5 \pm 1.5	165 \pm 3.7	19.3 \pm 1.3	22.1 \pm 3.2
<i>Malva nicaeensis</i> All. (Malvaceae)	21.8 \pm 1.5	24.4 \pm 1.1	6.2 \pm 0.6	6.7 \pm 0.7
<i>Mentha spicata</i> L. (Lamiaceae)	237 \pm 2.0	251 \pm 5.5	47.6 \pm 1.0	39.1 \pm 3.9
<i>Ononis natrix</i> L. (Papilionaceae)	82.0 \pm 1.5	76.7 \pm 2.0	16.9 \pm 0.4	21.1 \pm 0.7
<i>Onosma giganteum</i> Lam. (Boraginaceae)	32.9 \pm 10.6	27.3 \pm 1.8	4.3 \pm 2.0	4.6 \pm 0.2
<i>Paronychia argentea</i> Lam. (Caryophyllaceae)	79.0 \pm 5.0	85.7 \pm 3.8	15.9 \pm 0.3	15.5 \pm 1.0
<i>Peganum harmala</i> L. (Zygophyllaceae)	50.4 \pm 1.4	34.4 \pm 1.0	10.9 \pm 0.1	8.7 \pm 0.4
<i>Reseda alba</i> L. (Resedaceae)	36.5 \pm 7.9	29.9 \pm 2.3	13.6 \pm 0.9	12.0 \pm 0.4
<i>Reseda lutea</i> L. (Resedaceae)	70.8 \pm 6.0	116 \pm 13.6	17.7 \pm 1.3	15.1 \pm 0.3
<i>Rosmarinus officinalis</i> ^c L. (Lamiaceae)	324 \pm 2.0	274 \pm 1.4	48.9 \pm 2.3	39.1 \pm 3.6

(continued on next page)

Table 1 (continued)

Plant name	Antioxidant activity ($\mu\text{mol TE/g dry weight}$) ^a		Total phenolic content (mg GAE/g dry weight) ^b	
	Aqueous extracts		Aqueous extracts	
	Methanolic extracts		Methanolic extracts	
<i>Salvia ceratophylla</i> L. (Lamiaceae)	99.4 \pm 7.3	151 \pm 10.3	23.8 \pm 0.8	21.9 \pm 0.6
<i>Salvia fruticosa</i> Mill (Lamiaceae)	53.4 \pm 1.4	175 \pm 2.7	14.4 \pm 0.6	24.1 \pm 0.8
<i>Salvia hierosolymitana</i> Boiss. (Lamiaceae)	86.9 \pm 7.1	92.9 \pm 0.8	23.2 \pm 0.0	18.2 \pm 0.1
<i>Salvia spinosa</i> L. (Lamiaceae)	90.4 \pm 4.3	74.6 \pm 3.8	20.8 \pm 1.3	21.0 \pm 0.5
<i>Silene aegyptiaca</i> (L.) L.f. (Caryophyllaceae)	79.0 \pm 3.6	36.0 \pm 0.5	13.6 \pm 0.5	11.7 \pm 1.3
<i>Silybum marianum</i> (L.) Gaertn (Asteraceae)	51.0 \pm 1.1	79.7 \pm 3.4	11.1 \pm 0.4	18.3 \pm 1.8
<i>Tetragolobus palestinus</i> ^c Boiss. & Blanche (Papilionaceae)	23.5 \pm 3.4	24.4 \pm 1.7	4.8 \pm 0.8	3.6 \pm 0.0
<i>Tenarium polium</i> L. (Lamiaceae)	42.3 \pm 1.5	84.4 \pm 2.8	7.5 \pm 0.7	22.1 \pm 1.2
<i>Yarhemia iphionoides</i> Boiss. & Blanche (Asteraceae)	37.8 \pm 2.1	66.6 \pm 1.6	8.1 \pm 0.5	17.2 \pm 0.8

All data are shown as means \pm SD from two extraction replicates, each run in duplicate.

^a Data are expressed as $\mu\text{moles of trolox equivalents per g dry weight}$.

^b Data are expressed as mg of gallic acid equivalents (GAE) per g dry weight.

^c Only seeds were analyzed.

^d Only flowers were analyzed.

^e Cultivated.

ous extracts of *Linum pubescens* (12.9 $\mu\text{mol TE/g dry weight}$). The profound antioxidant activity of *H. triquetrifolium*, a grazing but toxic plant, could be attributed to known dianthrone (hypericin-like) and flavonoids (Couladis, Baziou, Verykokidou, & Loukis, 2002; Crockett, Schaneberg, & Khan, 2005). For *A. andrachne*, it is most likely due to polyphenol compounds that are known to be present in the *Arbutus* genus, primarily as condensed tannins and catechin gallate (Legssyer et al., 2004). Many plant extracts showed good levels of antioxidant activity when compared with *Ginkgo biloba*, a well known phytochemical source of antioxidant activity, which displayed values of 312 and 276 $\mu\text{mol TE/g dry weight}$ for aqueous and methanolic extracts, respectively.

3.2. Total phenolic content

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965), using gallic acid as a standard phenolic compound. A linear calibration curve of gallic acid, in the range 20–500 $\mu\text{g/ml}$ with r^2 value of 0.9992, was constructed (Fig. 1b). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gramme dry material. As shown in Table 1, there is large variation in the total phenolic content of the plant species investigated, ranging from 2.8 to 70.3 and from 2.6 to 59.6 mg GAE/g dry weight for those of aqueous and methanolic extracts, respectively. Many plant species showed remarkably high total phenolic content (GAE > 20 mg/g dry weight). For aqueous extracts, *H. triquetrifolium* and *A. andrachne* showed the highest total phenolic contents of 70.3 and 58.6 mg GAE/g dry weight, while, for methanolic extracts, *Chrysanthemum coronarium* and *A. andrachne* were the highest, with 59.6 and 57.6 mg GAE/g dry weight, respectively (Table 1). *C. arretinum* is the lowest in total phenolic content of both aqueous and methanolic extracts, at about 2.8 and 2.6 mg GAE/g dry weight, respectively. The vegetable edible plant garland chrysanthemum, *Chrysanthemum coronarium* L., which showed high levels of total phenolic content (59.6 mg GAE/g dry weight) is known to be rich in chlorogenic acid, 3,5-dicaffeoylquinic acid, 4-succinyl-3,5-dicaffeoylquinic acid, quercetin and its glycosides, rutin and isoquercetin, in addition to having high contents of ascorbic acid and carotenoids (Gins, Kolesnikov, Kononkov, Trishin, & Gins, 2000; Takenaka, Nagata, & Yoshida, 2000).

There was a positive linear correlation between antioxidant activity and total phenolic content for aqueous and methanolic extracts (coefficient $r = 0.892$ and 0.851 , respectively) (Fig. 2). These results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. These results were consistent with the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity (Cai et al., 2004; Zheng & Wang, 2001). There were no significant difference

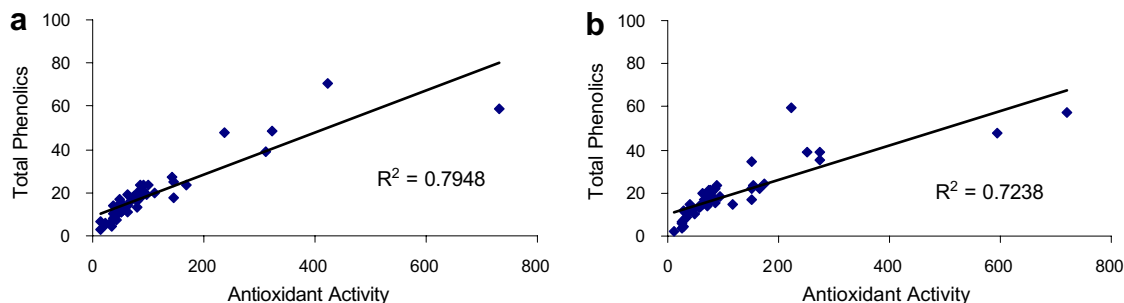


Fig. 2. Linear correlation between the total phenolic content and antioxidant activity (TEAC). (a) For aqueous extracts. Correlation coefficient (r) = 0.892. Coefficient of determination (r^2) = 0.795. (b) For methanolic extracts. Correlation coefficient (r) = 0.851. Coefficient of determination (r^2) = 0.724.

between aqueous and methanolic extracts for antioxidant activity or total phenolic content ($P > 0.05$).

The Folin–Ciocalteu assay gives a crude estimate of the total phenolic compounds present in an extract. It is not specific to polyphenols, but many interfering compounds may react with the reagent, giving elevated apparent phenolic concentrations (Prior, Wu, & Schaich, 2005). Moreover, various phenolic compounds respond differently in this assay, depending on the number of phenolic groups they have (Singleton & Rossi, 1965), and total phenolics content does not incorporate necessarily all the antioxidants that may be present in an extract. Hence, this may explain the equivocal correlation between total phenolic content and antioxidant activity of several plant species as appeared in Table 1. For example, although the total phenolic content of the aqueous extract of *H. triquetrifolium* (70.3 mg GAE/g dry weight) is higher than that of *A. andrachne* (58.6 mg GAE/g dry weight), the corresponding antioxidant activity of *A. andrachne* (731 $\mu\text{mol TE/g}$ dry weight) is higher than that of *H. triquetrifolium* (422 $\mu\text{mol TE/g}$ dry weight). Also, while the total phenolic content of the methanolic extract of *C. coronarium* (59.6 mg GAE/g dry weight) is higher than those of *H. triquetrifolium* (48.1 mg GAE/g dry weight) and *A. andrachne* (57.6 mg GAE/g dry weight), its corresponding antioxidant activity is lower (224 $\mu\text{mol TE/g}$ dry weight). Moreover, the total phenolic contents of the methanolic extract of *S. ceratophylla* (21.9 mg GAE/g dry weight) and *O. natrix* (21.1 mg GAE/g dry weight) are approximately the same, while the antioxidant activity of *S. ceratophylla* (151 $\mu\text{mol TE/g}$ dry weight) is approximately twice that of *O. natrix* (76.7 $\mu\text{mol TE/g}$ dry weight).

Interestingly, some plant species which showed good antioxidant activity in this study are being consumed among local populations in Jordan. For example, Horse-mint, *Mentha spicata*, which is known locally in Jordan as “Na’na”, is added to foods as a flavour; moreover, its decoction is used as a sedative, antirheumatic, and to relieve spasms and flatulence. Another example is the fruit of the Strawberry tree, *A. andrachne*, locally known as “Kaikub”. The decoction of Kaikub is used as a pectoral and antitussive agent, and to treat asthma. Rosemary, *Ros-*

marinus officinalis, known locally as “Hasalban” is used for its antiseptic and antispasmodic effects, and as a diuretic, carminative, and to stimulate bile flow (Karim & Quraan, 1986; Al-Khalil, 1995).

In the foreseeable future, identification and characterization of the active components from plant species which were identified in this research project with relatively high antioxidant activity, and which could provide potential natural sources of antioxidant compounds, will be undertaken. Special focus will be on those plants with high antioxidant activity and low phenolic content. Safety, edibility and *in vivo* efficacy studies on these potential plants will also be conducted.

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