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Antioxidant Activity of Some Jordanian Medicinal Plants Used Traditionally for Treatment of Diabetes

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Abstract: Medicinal plants are being used extensively in Jordanian traditional medicinal system for the treatment of diabetes symptoms. Twenty one plant samples were collected from different Jordanian locations and used for antioxidant evaluation. The level of antioxidant activity was determined by DPPH and ABTS assays in relation to the total phenolic contents of the medically used parts. The most frequently used plant parts as medicines were fruit, shoot and leaves. The total phenolic contents of methanol and aqueous extracts, from plants parts, ranged from 6.6 to 103.0 and 3.0 to 98.6 GAE mg g^{-1} of plant part dry weight, respectively. DPPH-TEAC of the methanol extracts of plants parts were varied from 4.1 to 365.0 mg g^{-1} of plant dry weight versus 0.6 to 267.0 mg g^{-1} in aqueous extracts. Moreover, the mean values of $ABTS^{\bullet-}$ (IC_{50}) varied from 6.9 to 400.0 μg dry weight mL^{-1} ABTS in methanol extracts versus 9.8 to 580.5 μg mL^{-1} in aqueous extracts. According to their antioxidant capacity, the plants were divided into three categories: high (DPPH-TEAC ≥ 80 mg g^{-1}), (i.e., *Punica granatum* peel, *Quercus calliprinos* leave, *Quercus calliprinos* fruit, *Cinchona ledgeriana* and *Juniperus communis* leave), moderate (DPPH-TEAC range 20-80 mg g^{-1}) (i.e., *Salvia fruticosa* shoot, *Crataegus azarolus* stem, *Crataegus azarolus* leave, *Varthemia iphionoides* shoot, *Artemisia herba-alba* shoot, *Thymus capitatus* shoot, *Morus nigra* leaves and *Arum palaestinum* leaves) and low antioxidant plants (DPPH-TEAC<20 mg g^{-1}), (i.e., *Matricaria aurea* shoot, *Artemisia judaica* shoot, *Teucrium polium* shoot, *Pinus halepenss* pollen grains, *Sarcopoterium spinosum* root, *Crataegus azarolus* fruit, *Inula viscose* shoot and *Achillea fragrantissima* shoot). The antioxidant activity of these plant's extracts and their potential rule in radical scavenging agreed with their potential use by Jordanian population as a traditional anti-diabetic agents.

Key words: Antioxidant, DPPH, ABTS, trolox equivalent antioxidant capacity, polyphenol, gallic acid equivalents, Jordan

INTRODUCTION

Finding healing powers in plants is an ancient idea (Cowan, 1999). Herbal medicines are one of the important cultural and traditional parts of the of people. Today, most of the world population depend on herbal medicines for their health care needs (Manandhar, 1995).

Jordan has great variation in wild plants due to the geographical diversity and climatic circumstances. It is known to have more than 2000 plant species belonging to about 700 genera. Among these plants, there are more than 485 medicinal species from approximately 99 plant families (Oran and Al-Eisawi, 1998; Afifi and Abu-Irmaileh, 2000). Ethno-pharmacological survey of traditional drugs consumed in Jordan indicated that there are 236 plants origin, traditional drugs for treatment of different diseases (Lev and Amar, 2002). Many studies investigated the antimicrobial (Mahasneh and El-Oqlah, 1999), antidiabetic (Hamdan and Afifi, 2004), anticancer (Abuharfeil *et al*., 2000) and antiulcer (Alkofahi and Atta, 1999) activities of different extracts from these plants.

Several chronic diseases that are due to oxidative stress, such as atherosclerosis, cancer, diabetes, ageing and other degenerative diseases in humans are generated by over production of Reactive Oxygen Species (ROS) (Valko *et al*., 2004). The imbalance between the production of free radicals and antioxidant defense systems might cause damage to lipids, protein and nucleic acids (Poulson *et al*., 1998).

Diabetes Mellitus (DM) is a complex metabolic disorder characterized by high blood glucose level due to the inability of the body cells to utilize glucose properly. Although insulin treatment and other chemical therapies can control many aspects of diabetes, numerous complications are common incidents of the DM (Brownlee and Cerami, 1981). The oxidative stress may

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have a common pathway linking diverse mechanisms for the diabetes complications such as vascular dysfunctions, nephropathy, neuropathy and retinopathy (Baynes, 1991). Moreover, diabetes mellitus results in a reduction of endogenous antioxidants and an increase in oxidative stress in the human body. Antioxidants have been shown to reduce the risk of diabetes onset (Montonen *et al*., 2004), improve glucose disposal (Ylonen *et al*., 2003) and improve some of the associated complications (De Young *et al*., 2004).

Plants often contain wide variety of antioxidant molecules, such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones and tannins), tocopherols, carotenoids and ascorbic acid (Cai *et al*., 2004). These natural antioxidants are distributed in different parts of the plants such as wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds (Chanwitheesuk *et al*., 2005).

Antioxidants phenolic compounds may function as terminators of free radical chains or chelators of redox-active metal ions that are capable of catalysing lipid peroxidation (Schroeter *et al*., 2002).

Previous Epidemiological studies have shown that the intake of natural antioxidants is associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with aging (Yang *et al*., 2001). Recently, there has been a considerable interest in finding natural antioxidants to replace synthetic ones.

Information on antioxidant activity and phenolic compounds of traditional Jordanian medicinal plant is scarce. So far, no survey study of the antioxidant activity and phenolic compounds of these plants have been recorded. Also, these plants were recommended by herbal healers for treatments of diabetic symptoms and its complications Therefore, the main objectives of present study were to determine total phenolic compounds of the methanolic and aqueous extracts of 21 Jordanian medicinal plants as well as evaluating their antioxidant activity by using $ABTS^{\bullet-}$ and DPPH methods.

MATERIALS AND METHODS

Chemicals: Absolute ethyl alcohol and absolute methanol were purchased from HAYMAN, England. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet-}$), 6-hydroxy-2,5,7,8 tetramethyl chroman-2 carboxylicacid (Trolox), Gallic acid, Folin-Ciocalteu's phenol reagent, potassium persulfate were purchased from Sigma-Aldrich (Germany). All other reagents were of analytical grade.

Plant selection and collection: A direct questionnaire as a way of detection the most common plants that are used in traditional medicine as ant-diabetes was designed and forward directly to traditional medicinal plant markets. The questionnaire raised a question, what are the common used ant-diabetic plants? Sample study included 100 traditional herbs markets from different locations in north, south and middle of Jordan.

The selected medicinal plants (Table 1) were collected from south and north regions of Jordan during the spring and summer 2005. The taxonomic identification was authenticated by Dr. S. Al-Quran, biology department, Mutah University. The collected plants were cleaned, dried for 10 days under shade at room temperature, then grounded and extracted.

Quercus calliprinos fruit, *Q. calliprinos* leaves, *Thymus capitatus* shoot, *Artemisia herba-alba* shoot, *A. judaica* shoot, *Varthemia iphionoides* shoot, *Arum palaestinum* leaves, *Salvia fruticosa* shoot, *Morus nigra* leaves, *Juniperus communis* leaves, *Crataegus azarolus* fruit, *C. azarolus* stem, *C. azarolus* leaves, *Achillea fragrantissima* shoot, *Sarcopoterium spinosum* root, *Punica granatum* peel, *Teucrium polium* shoot, *Matricaria aurea* shoot, *Cinchona ledgeriana* leaves, *Pinus halepensis* pollen grain and *Inula viscose* shoot which have the highest frequency (>30%) of healers recommendations for further analysis were selected and prepared according to traditional healers recommendation and Lapornik *et al*. (2005).

Extracts preparation: The extracts were prepared according to Lapornik *et al*. (2005) with some modifications. Briefly, 1 g of plant part was extracted in 35 mL of 80% methanol or distilled water with shaking for 36 h at 30°C. The extract was filtrated through four layers of gauze. The final volume of the filtrate was brought to 50 mL. The filtrates were centrifuged at 4000 rpm for 10 min and the supernatants were either stored at -20°C until analysis or used directly for the following experiments.

Total phenols: The total polyphenol content of plants extracts was estimated by the Folin-Ciocalteau phenol reagent according to Hagerman *et al*. (2000). 0.1 mL of diluted extracts was transferred into test tubes and their volumes made up to 0.5 mL with distilled water. After addition of 0.25 mL Folin-Ciocalteu reagent and 1.25 mL 20% aqueous sodium carbonate solution, tubes were vortexed and the absorbance of blue colored mixtures was determined at 725 nm after 40 min against a blank solution containing 0.1 mL of the solvent instead of the tested sample. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in milligrams per gram of dry matter of sample, using a standard curve generated with different concentrations of gallic acid.

DPPH radical scavenging assay: Antioxidant activity of plants extracts was determined by Blois (1958), where 50 μL of different concentrations of plant extracts and trolox were taken in different test tubes, then 2.5 mL of 0.1 mM DPPH methanolic solution was added to these tubes and shaken vigorously. The tubes were allowed to stand at 25°C for 20 min. The control was prepared as above using methanol solvent instead of extract. The decrease in the absorbance of the formed blue to violet reagent was determined after 20 min at 517 nm and the percentage inhibition activity was calculated from the formula:

$$\frac{(A_0-A_1)}{(A_0)}\times 100$$

Where:
A_0 = Absorbance of the control
A_1 = Absorbance of the extract/standard

In this assay, the antioxidant activity was expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (mg trolox g^{-1} dry weight).

ABTS free radical scavenging assay: The free radical scavenging ability was determined using ABTS radical cation decolorization assay according to Re *et al.* (1999). Generation of radical cation ($ABTS^{\bullet}$) involves the reaction between ABTS and potassium persulfate and production of the blue/green $ABTS^{\bullet}$ chromophore with a maximum absorption at 734 nm. In presence of antioxidants, the pre-formed radical cation is reduced to ABTS, proportionally to the antioxidant activity.

Statistical analysis: All data analyses were performed using the statistical package of statistical product and service solution (SPSS) version 10. Results were reported as mean±SD.

RESULTS

Plant selections and frequency: The selected plant species with a frequency of herbal healers recommendations more than 30% and designated as medicinal plants used for the treatment of diabetes symptoms and its complications are shown in Table 1.

Total phenolic contents: The phenolic contents of methanol and aqueous extracts of different parts for selected plant were evaluated using the Folin-Ciocalteu reagent and expressed as mg Gallic Acid Equivalent (GAE)/g of dry weight (wt.). There was great variation between phenolic contents of these plants. In particular a significant difference ($p<0.01$) between methanolic and aqueous extracts was observed within same species except in *Punica granatum* peel and *Achillea fragrantissima* shoot. However, the phenolic contents of the methanolic extracts ranged from 6.6 to 103.0 GAE mg g^{-1} dry wt., with an average value of 32.9 GAE mg g^{-1} dry wt., while in aqueous extracts, these phenolic contents were ranged from 3.0 to 98.0 GAE mg g^{-1} dry wt. with average value of 18.4 GAE mg g^{-1} dry wt. (Table 2).

Table 1: Plants used for the treatment of diabetes and its complications in Jordan

Plant species used medicinally	Plant part medicinally used	Recommendation (%)*
Quercus calliprinos	Fruit	69
Quercus calliprinos	Leaves	57
Artemisia herba-alba	Shoot	62
Punica granatum	Peel	61
Crataegus azarolus	Fruit	58
Achillea fragrantissima	Shoot	58
Morus nigra	Leaves	56
Salvia fruticosa	Shoot	54
Teucrium polium	Shoot	54
Arum palaestinum	Leaves	52
Artemisia judaica	Shoot	46
Crataegus azarolus	Leaves	43
Varthemia iphionoides	Shoot	42
Ciuchona ledgeriana	Leaves	42
Sarcopoterium spinosum	Roots	41
Crataegus azarolus	Stem	39
Thymus capitatus	Shoot	36
Juniperus communis	Leaves	36
Matricaria aurea	Shoot	36
Inula viscose	Shoot	35
Pinus halepeusis	Pollen grain	31

*: Results expressed as percentage herb healer that elect the plant for diabetes treatment

Table 2: Total phenolic compounds in aqueous and methanol extract of tested plants

Plant species	Plant part	Total phenolic (GAE mg g^{-1} dry weight) Aqueous extract	Methanol extract
Quercus calliprinos	Fruit	21.7±1.3	48.0±2.0
Quercus calliprinos	Leaves	48.5±1.5	75.4±3.8
Thymus capitatus	Shoot	10.6±0.7	31.1±2.4
Artemisia herba-alba	Shoot	7.8±0.8	31.3±2.2
Varthemia iphionoides	Shoot	5.5±0.8	38.7±1.6
Artemisia judaica	Shoot	3.0±0.6	20.6±1.7
Arum palaestinum	Leaves	21.4±2.1	27.6±2.4
Salvia fruticosa	Shoot	18.5±2.3	46.7±2.9
Morus nigra	Leaves	12.4±1.1	24.7±2.4
Juniperus communis	Leaves	28.8±3.1	60.2±2.3
Crataegus azarolus	Fruit	8.8±1.0	12.2±1.3
Crataegus azarolus	Stem	9.4±0.7	22.1±1.1
Crataegus azarolus	Leaves	13.6±1.8	30.6±2.5
Achillea fragrantissima	Shoot	6.9±0.5*	6.6±0.4*
Sarcopoterium spinosum	Root	8.7±0.7	13.2±1.8
Punica granatum	Peel	98.6±6.7*	103.0±5.2*
Teucrium polium	Shoot	8.5±1.0	14.6±1.9
Matricaria aurea	Shoot	9.1±0.7	18.3±1.6
Ciuchona ledgeriana	Leaves	25.0±2.3	43.8±3.4
Pinus halepeusis	Pollen grain	7.1±0.3	13.1±0.6
Inula viscose	Shoot	4.0±0.7	7.5±0.4

Values expressed as mean±SD of three samples analysed separately. Values expressed as Gallic Acid Equivalents (GAE) mg g^{-1} of dry Wight; *: No significant differences between aqueous and methanol extracts determined at $p<0.01$

Table 3: The DPPH-TEAC and ABTS-IC_{50} values on different assay systems of high, moderate and low antioxidant extracts category

	Methanol extraction*		Aqueous extraction*	
Plants	[a]DPPH-TEAC (mg g^{-1})*	[b#]ABTS-IC_{50} (μg mL^{-1})	[a]DPPH-TEAC (mg g^{-1})*	[b#]ABTS-IC_{50} (μg mL^{-1})
High				
Quercus calliprinos/fruit	105.3±2.4	14.0±0.1	75.5±1.8	41.4±0.8
Quercus calliprinos/leaves	195.5±4.1	11.9±0.2	83.0±1.6	20.4±0.3
Punica granatum/peel	365.0±5.0	6.9±0.1	267.1±3.5	9.8±0.1
Juniperus communis/leaves	81.1±2.0	24.7±0.2	27.4±0.8	48.0±0.3
Cinchona ledgeriana/leaves	84.2±1.9	32.1±0.2	17.1±0.6	188.5±1.2
Moderate				
Thymus capitatus/shoot	30.4±0.9	68.2±0.8	4.6±0.4	144.0±1.0
Artemisia herba-alba/shoot	31.3±1.1	76.4±0.9	3.3±0.6	220.0±1.1
Varthemia iphionoides/shoot	40.6±1.4	52.3±0.6	3.5±0.5	250.5±1.5
Arum palaestinum/leaves	24.3±1.0	116.0±1.0	13.4±0.8	146.7±1.2
Salvia fruticosa/shoot	60.8±1.8	29.6±0.2	13.5±0.6	180.1±1.4
Morus nigra/leaves	26.7±0.9	124.2±0.9	3.4±0.8	330.0±1.4
Crataegus azarolus/stem	52.1±1.3	45.1±0.4	14.0±0.5	220.0±1.0
Crataegus azarolus/leaves	45.6±1.5	58.3±0.5	9.4±0.6	196.4±0.8
Low				
Artemisia judaica/stem	18.3±0.6	104.6±0.9	2.7±0.3	280.6±1.5
Crataegus azarolus/fruit	12.9±0.7	114.2±1.0	0.9±0.2	250.0±1.8
Achillea fragrantissima/shoot	4.1±0.4	400.0±1.5	0.8±0.2	450.0±2.0
Sarcopoterium spinosum/root	13.5±0.5	124.8±0.6	4.6±0.4	198.0±1.6
Tencrium polium/shoot	18.3±0.8	146.4±1.1	9.1±0.8	230.0±2.0
Matricaria aurea/shoot	19.6±0.5	120.3±0.9	5.7±0.7	230.1±1.3
Pinus halepeusis/pollen grain	17.7±0.9	126.9±0.6	4.1±0.3	490.7±1.9
Inula viscose/shoot	7.6±0.4	290.0±1.4	0.6±0.1	580.5±2.5

[a]: Trolox Equivalent Antioxidant Capacity (TEAC) (mg trolox per g dry matter): mean of triplicate assays,±SD; [b]: IC50 (Concentration that decrease 50% of ABTS) and expressed as μg of dry wight mL^{-1} of ABTS: mean of triplicate assays, ±SD; * and # Significant differences between aqueous and methanol extracts DPPH-TEAC and ABTS-IC_{50} at $p<0.01$, respectively

Antioxidant activity: The antioxidant activity of methanol and aqueous plant extracts was evaluated according to their ability for scavenging free radicals by using DPPH and ABTS assays and expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (mg trolox g^{-1} dry weight). IC_{50} of each extract was detected by using ABTS assay and expressed as μg dry weight mL^{-1} of ABTS. A significant differences ($p<0.01$) were found between antioxidant activity of methanol and aqueous extracts. According to TEAC of methanolic extracts in DPPH assay, the tested plants were classified into three categories: high (DPPH-TEAC = 80 mg g^{-1}), moderate (DPPH-TEAC range 20-80 mg g^{-1}) and low antioxidant plants (DPPH-TEAC<20 mg g^{-1} dry weight) as shown in Table 3.

Some plants showed large difference in their response to DPPH or ABTS method such as *Curcuma longa* (DPPH-TEAC = 13.3±1.2 mg g^{-1} vs ABTS-TEAC = 25.3±2.4 mg g^{-1}) while others showed no differences, such as *Matricaria aurea* (DPPH-TEAC = 19.6±1.8 mg g^{-1} vs. ABTS-TEAC = 20.3±2.7 mg g^{-1}).

High antioxidant plants: The high antioxidant group involved *Punica granatum* peel, *Quercus calliprinos* leave, *Quercus calliprinos* fruit, *Cinchona ledgeriana* and *Juniperus communis* leave. As shown in Table 3, the methanol extracts of these plants have DPPH-TEAC of more than 80 mg g^{-1} and ranged from 81.1 to 365 mg g^{-1}; while their ABTS-IC_{50} was ranged from 6.9 to 32.1 μg mL^{-1}. On the other hand, DPPH-TEAC and ABTS-IC_{50} of aqueous extracts for these plants were 17.1-267.1 mg g^{-1} and 9.8-188.5 μg mL^{-1}, respectively.

Moderate antioxidant plants: DPPH-TEAC and ABTS-IC_{50} for the methanol extracts of plants with moderate antioxidant capacity (i.e., *Salvia fruticosa*, *Crataegus azarolus* stem, *Crataegus azarolus* leave, *Varthemia iphionoides*, *Artemisia herba-alba*, *Thymus capitatus*, *Morus nigra* and *Arum palaestinum*) were 24.3-60.8 and 29.6-124.2 μg mL^{-1} respectively. While in aqueous extracts DPPH-TEAC were ranged from 3.3 to 14.0 mg g^{-1} and ABTS-IC_{50} ranged from 144.0 to 330.0 μg mL^{-1}.

Low antioxidant plants: plants with the low antioxidant capacity involved *M. aurea* shoot, *A. judaica* shoot, *T. polium* shoot, *P. halepensis* pollen grains, *S. spinosum* root, *C. azarolus* fruit, *I. viscose* shoot and *A. fragrantissima* shoot. The methanolic extracts of these plants have DPPH-TEAC ranging from 4.1 to 19.6 mg g^{-1}, while the ABTS-IC_{50} ranged from 104.6 to 400 μg mL^{-1}. The DPPH-TEAC and ABTS-IC_{50} of aqueous extracts were 0.6-9.1 mg g^{-1} and 198.0-580.5 μg mL^{-1}, respectively.

Correlation between antioxidant activity and total phenolic contents: The correlation coefficients between antioxidant activity and total phenolic content of the tested plants were calculated (Fig. 1a). There was a high

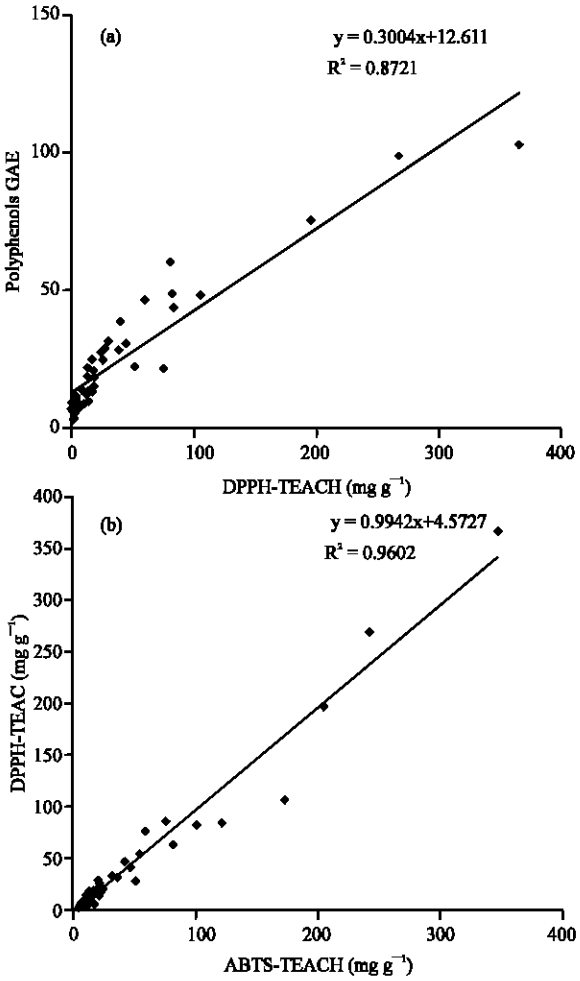

Fig. 1: Correlations analysis between DPPH-TEAC and total phenolic content of tested extracts (a) and between DPPH-TEAC and ABTS-TEAC (b). Results are expressed as mean of triplicate assays, ±SD

positive correlation between DPPH-TEAC and the total phenolic content of the tested extracts (R^2 = 0.87). Such high correlation (R^2 = 0.96) was also found between DPPH-TEAC and ABTS-TEAC in plant extracts as shown in Fig. 1b.

DISCUSSION

It has long been recognized that plants contain many natural substances. The phenolic compounds are widely distributed, sometimes present in surprisingly high concentrations, in plants and have an antioxidant activity (Lapornik *et al*., 2005). They have the ability to scavenge free radicals such as the Reactive Oxygen Species (ROS) which are determined by their reactivity as hydrogen- or electron donating agents (Fernandez-Pachon *et al*., 2006). In present study, the total antioxidant effect of methanol and aqueous extracts of some medicinal plants that are traditionally used in Jordan, were measured and compared to total phenolic contents. Two commonly accepted assays, DPPH and ABTS, were employed to evaluate the total antioxidant effects of tested plants. DPPH-TEAC and ABTS-IC_{50} were indicated for each extract.

Extreme variations in antioxidant activity and total phenols were found between tested extracts. It can be observed that the phenolic contents in the extracts highly correlate with their antioxidant activity, (R^2 = 0.87) (Fig. 1), confirming that phenolic compounds contribute significantly to the antioxidant activity of these plant extracts. The large variation in plant antioxidant activity may result from differences in total phenolic contents (Table 2). Such observation agreed with several previous findings (Velioglu *et al*., 1998; Zheng and Wang, 2001; Sun *et al*., 2002; Cai *et al*., 2004). Moreover, Singleton and Rossi (1965) noticed that various phenolic compounds have different responses to Folin-Ciocalteau assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented in ortho or para-position. Since these structural features of phenolic compounds are responsible for antioxidant activity (Katalinic *et al*., 2006). Thus, polyphenols measurements in extracts may be related to their antioxidant activities.

DPPH and ABTS methods have been used by many researchers to evaluate the free radical scavenging activity of antioxidant molecules and plant extracts. DPPH does generate strongly colored solutions with methanol which is eliminated in presence of antioxidants (Blois, 1958; Matsukawa *et al*., 1997; Yan *et al*., 1998, 1999). On the other hand, the decolorization of the ABTS radical cation also reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to deactivate these radical species (Pellegrini *et al*., 1999). The data obtained from the two radical scavenging methods suggest high accuracy and constancy between them.

Using DPPH or ABTS methods, some plants showed large difference in their TEAC values, whereas others showed little differences. This may be due to variation in types of phenolic compounds, that differ significantly in their reactivity towards $DPPH^{\bullet}$ and $ABTS^{\bullet}$ (Katalinic *et al*., 2006). Moreover, Campos and Lissi (1996) found a difference in reaction kinetics between

phenols and ABTS$^{\bullet}$ or DPPH$^{\bullet}$ over a similar range of concentrations. They established that the reactions of phenols with ABTS radical cation are usually rapid, but the reactions with DPPH radical differ from compound to compound. Furthermore, the affinities of different plants toward the two above radicals were sometimes significantly altered due to variation of ABTS and DPPH solubility in aqueous medium.

This study established that the methanol extracts were significantly higher in phenolic contents and antioxidant activity than aqueous extracts. However, it was found that methanolic plant extracts are the most effective scavenger of DPPH radical (Miliauskasa *et al*., 2004). Lapornik *et al*. (2005) ascribed higher values of total polyphenols and antioxidant activity of plant by-products methanol extracts vs. water extracts to the fact that methanol is less polar solvents than water. Hence, it was suggested that methanol is more efficient solvent for cell walls and seeds degradation, that have unpolar character causing the release of polyphenols from cells. In other literature (Moure *et al*., 2001), the decaying of polyphenols in water extract was ascribed to high temperature, though in our work all samples were extracted at room temperature. Other explanation for such decrease is ascribed to the activity of polyphenol oxidase, which degrade polyphenols in water extracts, but neutralized in methanol medium (Zhang *et al*., 2001). In present study, the decrease in antioxidant activity among the aqueous extracts was in accordance with the amount of plant phenolic contents. This verifies that the amount of phenolic compounds was responsible for their antioxidant activities. Therefore, the differences between antioxidant activities of methanol and aqueous extracts might reflect differences in polyphenolic contents of these extracts.

Punica granatum peel methanolic extract exhibited the highest polyphenolic contents and antioxidant capacity among all tested extracts. The total phenolic content of the *Punica granatum* peel methanol extract was 103.0 GAE mg g^{-1} and the DPPH-TEAC was 365.0 mg g^{-1}, while ABTS IC50 7.0 μg mL^{-1}. Previous work found that the HPLC analysis of the methanolic extract of *Punica granatum* peel have detected some polyphenols, like gallic acid (34.03%) and catechin (3.31%) (Murthy *et al*., 2004). These polyphenols present in the methanolic extract of *Punica granatum* peel may be responsible for its high antioxidant activity. Moreover, the antioxidant activity of ethanol, methanol and water extracts of *Punica granatum* peels and seeds have been reported in various *in vitro* models. Among all extracts, the methanol extract possessed the highest antioxidant activity in various models (Singh *et al*., 2002).

In conclusion, present study indicated that some Jordanian medicinal plants are promising sources of natural antioxidants. Total phenol content and total antioxidant capacity differs significantly among 21 selected plant extracts. There was significant linear correlation between phenolics concentration and antioxidant capacity of extracts. The antioxidant capacity and radical scavenging agreed with their uses as traditional ant-diabetic remedy.

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