

Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq

Abdul-Lateef Molan^{1,*}, Abbas Mohamad Faraj², Abdulkhaliq Saleh Mahdy³

¹ Institute of Food, Nutrition and Human Health, Massey University, Private Bag 11 222, Palmerston North, New Zealand.

² College of Pharmacy, Hawler Medical University, Iraqi Kurdistan, Erbil, Iraq.

³ College of Agriculture, Diyala University, Diyala, Iraq.

*Corresponding Author: Email: A.L.Molan@massey.ac.nz

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Abstract

Several herbal plants have been used as therapeutics in Iraqi traditional medicine and phenolic content and antioxidant activity may contribute to their pharmacological effects. The total phenolic contents (TPC) and the antioxidant activities of water and ethanol extracts of 14 medicinal plants which had been used in Iraqi traditional medicine were investigated. The TPC were measured by Folin-Ciocalteu method. The antioxidant activity was assessed by ferric reducing antioxidant power (FRAP) assay and the scavenging activity towards 2, 2'-diphenyl-1-picryl hydrazyl (DPPH)-radical. Antioxidant activities as measured by FRAP and the scavenging activity towards DPPH radical was significantly correlated with TPC among all the plants studied. In most of the plants the TPC and the antioxidant activity of the ethanol extracts were significantly higher ($P < 0.05$) than that of water alone extracts. The results suggest that phenolic compounds are the significant contributors to the antioxidant activity of the medicinal plants studied. This study shows that the studied plants are good sources of free-radical scavenging compounds and may explain their traditional medicinal application. Therefore, ingestion of extracts from these plants may help to prevent *in vivo* oxidative damage associated with diseases and illnesses, for which the local traditional healers used some of these plants.

Keywords: Antioxidant activity; phenolic content

Introduction

In many parts of the world, medicinal plants are used as a source of phytochemicals to cure various illnesses such as urinary infections, cervicitis vaginitis, skin infections, blood infections, and gastrointestinal disorders (Caceres *et al.*, 1990; Bratner and Grein, 1994; Meyer *et al.*, 1996). In general, medicinal plants are the backbone of the traditional medicine (Farnsworth, 1994).

Plants are a good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated disorders (Hausladen and Stamler, 1999; Lee *et al.*, 2000). It is well known that free radicals are the major cause of various chronic and degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Scalbert *et al.*, 2005). Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants.

In Iraq, medicinal plants have not received much attention in terms of quantifying their antioxidant activity. The fourteen traditional Iraqi medicinal plants and herbs tested in this study were selected according to the folk medical treatment by the local traditional healers (men and women) living in the rural and mountain districts in Kurdistan region-Northern Iraq. The people living in this area still believe that the treatment by herbal medicinal plants is more effective and safe remedy. According to the folk experts in this field, there are so many cases that have benefited from treatment by these herbal medicinal plants after they lost their faith in treatment by the medicament.

Several methods have been developed to assay the antioxidant activity of herbal and plant extracts. The most common methods involve the determination of the ability to scavenge free radicals using DPPH assay, ferric reducing antioxidant power (FRAP) assay and ferrous-ion chelating assay (Singh and Rajini, 2004; Benzie and Strain, 1996; Chan *et al.*, 2007; Molan *et al.*, 2008, 2009). The main objectives of this study were to determine the total phenolic contents of 14 selected traditional Iraqi medicinal plants, to assay their antioxidant activities and to investigate the inter-relationship between phenolic content and antioxidant activity.

Materials and methods

Chemicals and reagents

DPPH, gallic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and ferric chloride were obtained from Sigma Chemical Inc., Australia. All other reagents and chemicals used were of analytical grade procured from local sources.

Medicinal plants

Fourteen medicinal plants commonly used in Northern Iraq were obtained from various locations in the Kurdistan District in April 2008, each with a Herbarium voucher specimen prepared and deposited at the herbarium (Salahaddin University, Erbil, Iraq). The scientific names, voucher numbers and medicinal applications are detailed in Table 1.

Sample collection and extraction

About 200-400 g of dry leaves and stems from each plant has been brought from Iraq to New Zealand in January 2009 in sealed, airtight, foil bags for analysis. The leaves and ste-

Table 1. Scientific names, local names, and medicinal uses of the medicinal plants.

S.No.	Botanical name	Local name	Voucher Number	Medicinal use
1.	<i>Potentilla supina</i> L.	Sazab	SU6700	Helminthiasis, colon cancer, asthma
2	<i>Xanthium brasiliicum</i> Vell.	Mosnak	SU6705	Migraine, allergies
3	<i>Scrophularia rimarum</i> Bornm.	Koshat	SU6712	Leukaemia, hypertension, obesity
4	<i>Colutea cilicica</i> Boiss. & Balansa	Q- Chiyaye	SU6709	Helminthiasis, hypertension
5	<i>Stachys lavandulifolia</i> Vahl.	Saqmonya	SU6720	Malaria, snake bite, insect bite
6	<i>Polygonum aviculare</i> L.	Wardena	SU6718	Helminthiasis, dysentery, haemorrhoids
7	<i>Achillea vermicularis</i> Trin.	Bozhan	SU6715	Helminthiasis, dysentery
8	<i>Gentiana olivieri</i> Griseb.	Sobry	SU6711	Helminthiasis, diarrhoea
9	<i>Aethionema grandiflorum</i> Boiss. & Hohen.	Shawena	SU6722	Meningitis, bacterial infections, typhoid
10	<i>Artemisia herba-alba</i> Asso.	Gyaband	SU6725	Snake bite, bacterial infections, diabetes
11	<i>Daucus muricatus</i> L.	Safandoleun	SU6730	Hypertension, sexual impotence in men and women
12	<i>Thymus syriacus</i> Boiss.	Jatra	SU6727	Kidney stone, gastrointestinal ulcers
13	<i>Ocimum basilicum</i> L.	Reahan	SU6740	Gastrointestinal disorders
14	<i>Thalictrum minus</i> L.	Mirany	SU6731	Kidney problems, sexual impotence in men and women

ms were grinded and then extracted either in water alone (water extracts) or in 50% ethanol-water (ethanol extract). Briefly, 20 g of dry sample was grinded in a domestic blender and 5 g of the ground sample was extracted for 6 h at room temperature with 250 ml of a mixture of ethanol and water (1: 1). A similar sample of the ground sample was extracted with 250 ml of boiling water. The sample suspensions were centrifuged (10,000 g for 15 min at 10°C) and the supernatant was filtered through a 0.45 µm Millipore filter and the filtrates were used for assessing the total phenolic contents and antioxidant activity of the plants. The filtrates stored at -20°C till analysis.

Assaying methods

Total phenols contents (TPC)

The total phenolic content (TPC) in the plant extracts was determined according to the method of Molan et al. (2009). Briefly, an aliquot of 12.5 µl of each plant was mixed with 250 µl 2% sodium carbonate solution in 96-well microplate and allowed to react for 5 min at room temperature. Then 12.5 µl of diluted Folin-Ciocalteu phenol reagent (1:1 with water) and allowed to stand for 30 min at room temperature before the absorbance of the reaction mixture was read at 650 nm using a microplate reader. Calibration was achieved with an aqueous gallic acid solution. The TPC of the extracts was expressed as mg gallic acid equivalent (GAE) per gram of each plant on dry basis and all determinations were performed in triplicate in two separate experiments.

Ferric reducing antioxidant power assay (FRAP)

The capacity to reduce ferric ions was determined using the Ferric Reducing Antioxidant Power (FRAP) assay as described by Benzie and Strain (1996), with slight modifications (Molan *et al.*, 2009). Briefly, an aliquot of 8.5 µl of each plant extract was added to 275 µl of diluted FRAP reagent (1:1 with water) using a microplate and the plates were incubated at 37°C. Standard curve was prepared using different concentrations (0.5-5 mmol/L) of FeSO₄.7H₂O. The antioxidant capacity of the extract was expressed as mmol FeSO₄ equivalents per litre of water and 50% aqueous-ethanol extracts. The absorbance was read at 595 nm using a microplate reader. All determinations were performed in triplicate in two separate experiments.

Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

The scavenging activity of the extracts was determined based on DPPH-scavenging assay described by Molan *et al.* (2009). Briefly, 25 µl of each extract was allowed to react with 250 µl of 0.2 mM DPPH in 95% ethanol in a 96-well microplate. The plate was then incubated in dark at room temperature for 30 min and the absorbance (A) was measured at 550 nm using a microplate reader and all determinations were performed in triplicate in two separate experiments. Trolox was used as a positive control.

The antiradical activity was calculated as a percentage of DPPH decolouration relative to a negative control using the following equation:

$$\text{Free-radical scavenging activity (\%)} = \frac{A_{(\text{blank})} - A_{(\text{extract})}}{A_{(\text{blank})}} \times 100$$

Statistical analysis

All experiments were carried out in three replicates and presented as mean ± standard error (SE) using SAS version 9.1. One-way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test for any significant differences between the means; the mean values of antioxidant activities between two extracts were analysed by *t*-test. Correlations were obtained by Pearson correlation coefficient. The level of statistical significance was set at $P < 0.05$.

Results

Description of the medicinal plants used in this study

Table 1 contains the information of the plants used in the present study. In Iraqi traditional medicine, when these herbs are used for patients, parts (mainly leaves) are boiled in water and infusions prepared are given to the patients orally. For each plant, a serial number was given and this order was followed throughout the paper.

Total phenolic content (TPC)

The total phenolic content (TPC), expressed as mg of gallic acid equivalent (GAE)/g of dry weight, is shown in Table 2. The TPC values showed a wide range (3.5-41.2 mg GAE

Table 2. Antioxidant activity and total phenolic content (TPC) of water and ethanol extracts of 14 medicinal plants used in Diyala Province, Iraq. Antioxidant activities were assessed by the ferric reducing antioxidant power (FRAP) assay and by 2,2'-diphenyl-1-picryl hydrazyl (DPPH) method. The data represent the mean \pm SEM of triplicate determinations of two experiments. Number given to each plant is the serial number shown in Table 1.

S. No.	TPC (mg of GAE/g DW) ^a		FRAP values (mmol/L) ^b		DPPH-scavenging activity (%) ^c	
	Water	Ethanol	Water	Ethanol	Water	Ethanol
1	30.5 \pm 0.24	32.3 \pm 0.2	13.4 \pm 0.1	5.0 \pm 0.7	25.2 \pm 0.15	35.97 \pm 0.2
2	18.12 \pm 0.3	30.9 \pm 0.12	8.8 \pm 0.6	14.1 \pm 0.01	14.3 \pm 0.5	23.13 \pm 0.3
3	32.76 \pm 0.1	29.4 \pm 0.01	14.4 \pm 0.04	13.0 \pm 0.15	28.02 \pm 0.2	40.4 \pm 0.8
4	21.6 \pm 0.12	17.5 \pm 0.04	8.4 \pm 0.01	14.2 \pm 0.17	3.4 \pm 0.2	8.5 \pm 2.1
5	26.5 \pm 0.01	29.2 \pm 0.31	12.9 \pm 0.2	12.3 \pm 0.07	9.99 \pm 0.2	14.6 \pm 0.2
6	18.3 \pm 0.09	29.8 \pm 0.14	11.9 \pm 0.02	13.1 \pm 0.1	16.6 \pm 1.2	22.8 \pm 1.1
7	19.3 \pm 0.06	24.7 \pm 0.12	6.9 \pm 0.04	13.0 \pm 0.1	6.7 \pm 1.1	13.1 \pm 0.2
8	24.5 \pm 0.16	25.0 \pm 0.01	12.7 \pm 0.04	13.0 \pm 0.08	11.8 \pm 1.3	27.24 \pm 1.7
9	32.7 \pm 0.13	33.2 \pm 0.05	12.8 \pm 0.06	13.6 \pm 0.03	22.7 \pm 1.1	34.03 \pm 0.9
10	28.1 \pm 0.12	28.4 \pm 0.24	13.4 \pm 0.01	13.6 \pm 0.13	17.7 \pm 1.1	32.7 \pm 0.8
11	13.6 \pm 0.12	29.2 \pm 0.02	10.6 \pm 0.03	13.3 \pm 0.03	1.49 \pm 1.3	7.1 \pm 1.9
12	15.1 \pm 0.03	16.6 \pm 0.04	9.7 \pm 0.01	13.4 \pm 0.23	7.52 \pm 0.4	12.6 \pm 0.2
13	41.2 \pm 0.06	38.5 \pm 0.4	15.7 \pm 0.28	9.6 \pm 0.17	31.5 \pm 0.7	44.7 \pm 1.1
14	9.7 \pm 0.04	3.5 \pm 0.02	5.0 \pm 0.01	14.3 \pm 0.01	0.745 \pm 0.7	0.15 \pm 0.6

^a Total phenolic content [TPC; mg Galic Acid Equivalent (GAE) per gram dry weight].

^b Ferric reducing activity (expressed as mmole of FeSO₄ equivalent/litre of extract).

^c Scavenging activity towards DPPH [expressed as the % DPPH-radical scavenging activity relative to the control incubations (without plant extracts)].

/g dry weights) and *Ocimum basilicum* L (Lamiaceae) showed the highest (41.2 mg GAE/g dry leaves) followed by *Scrophularia rimarum* Bornm (Scrophulariaceae), *Aethionema grandiflorum* Boiss and Hohen (Brassicaceae), *Potentilla supine* L (Rosaceae), *Artemisia herba-alba* Asso (Asteraceae), *Stachys lavandulifolia* Vahl (Lamiaceae), *Gentiana olivieri* Griseb (Gentianaceae), *Colutea cilicica* Boiss and Balansa (Leguminosae), *Achillea vermicularis* Trin (Asteraceae), *Polygonum aviculare* L (polygonaceae), *Xanthium brasiliense* Vell (Asteraceae), *Thymus syriacus* Boiss (Lamiaceae), *Daucus muricatus* L (Apiaceae) while *Thalictrum minus* L (Ranunculaceae) showed the lowest TPC value (Table 2).

The water and ethanol extracts from the same plant showed different TPC values. Ethanol extracts from 10 out of 14 plants showed significantly higher TPC values ($P < 0.05$) than their counterparts extracted with water alone. In contrast, the ethanol extracts from the leaves of *Scrophularia rimarum*, *Colutea cilicica*, *Ocimum basilicum* and *Thalictrum minus* showed significantly lower TPC values ($P < 0.0001$) than water extracts from the same plants. Only in *Artemisia herba-alba*, no significant difference in TPC values was found between water and ethanol extracts.

Antioxidant activity as assessed by ferric reducing antioxidant power (FRAP) assay

Antioxidant activity as measured by FRAP assay showed a wide range of variation among the plants studied as well as among the extracts used (Table 2). *Ocimum basilicum* showed the highest FRAP activity while *Thalictrum minus* showed the lowest FRAP value

(Table 2). As with TPC, the water and ethanol extracts from the same plant showed different antioxidant activity. In all plants, except three (*Artemisia herba-alba*, *Thymus syriacus* and *Ocimum basilicum*), the ethanol extracts showed significantly higher ($P < 0.05$) FRAP values than water extracts (Table 2).

In general, there was a significant positive correlation ($P < 0.01$) between TPC and FRAP values in both water and ethanol extracts (R^2 values being 0.71 and 0.79 with water and ethanol extracts, respectively).

Antioxidant activity as assessed by DPPH-radical scavenging assay

DPPH assay detects scavenging of free radicals by the tested samples through the scavenging activity of the stable 2, 2 diphenyl-1-picrylhydrazyl (DPPH) free radicals. When the original extracts (2% water or ethanol extracts) were diluted 1, 2, and 4 folds, most of the extracts showed 100% scavenging activity towards DPPH-radical and therefore, we decided to use 8-fold diluted extracts in order to compare between different plants. The scavenging activities of the plant extracts toward DPPH-radical are shown in Table 2.

As in FRAP and TPC assays, DPPH-scavenging activity was also the highest in *O. basilicum*, while *T. minus* showed the lowest activity. However, the scavenging activity towards DPPH radical of the ethanol extract was significantly higher ($P < 0.05$) than that of water extract in all plants except in *T. minus* (Table 2). A significant positive correlation ($P < 0.05$) was found between TPC and DPPH- scavenging activity in both water and ethanol extracts.

Discussion

The purpose of this study was to investigate the antioxidant activity and phenolic contents of some local medicinal plants and herbs used commonly in the traditional medicine in Northern Iraq in order to evaluate the scientific base of their application. All the shrubs and herbs tested in this study showed antioxidant activities.

Among the 14 tested medicinal plants, *O. basilicum* showed the highest TPC and antioxidant activity. This plant is used in traditional medicine, as a culinary herb and a well-known source of flavouring principles. A number of phenolic compounds with strong antioxidant activity have been identified in this herb (Nakatani, 1997). Javanmardi *et al.* (2003) screened 23 Iranian basil varieties for their total phenolic contents (TPC) and antioxidant activities and found that the TPC ranged from 22.9 to 65.5 mg gallic acid equivalent/g dry weight. The authors found a linear positive relationship between the antioxidant properties and total phenolic content of the tested basil varieties. Recently, Gulcin *et al.* (2007) investigated the antioxidant activity of water and ethanol extracts of Turkish basil (*O. basilicum*) and found that ethanol extracts showed higher antioxidant activity than water extracts.

The results of the present study showed that the TPC and antioxidant activity of *O. basilicum* are significantly higher than *Thymus syriacus* which belongs to the same family, Lamiaceae. Similarly, Javanmardi *et al.* (2003) found that the TPC in all tested Iranian basil

varieties was higher than the other Lamiaceous plants such as *Thymus vulgaris* (Kahkonen *et al.*, 1999).

Artemisia herba-alba was reported as a traditional remedy of enteritis, and various intestinal disturbances, among the Bedouins in the Negev desert (Friedman *et al.*, 1986) and *A. herba-alba* based teas were used in Iraqi folk medicine for the treatment of diabetes mellitus (Al-Waili, 1986). In the present study, *A. herba-alba* showed very high antioxidant activity which may support the traditional medicinal application of this plant.

Water and ethanol extracts from *Achillea vermicularis* showed very high TPC and strong scavenging activity toward DPPH radical. Nickavar *et al.* (2006) studied the free radical scavenging activity of six Iranian *Achillea* species and found that aqueous-ethanol extracts of *A. vermicularis* showed high flavonoid content and potent free radical scavenging activity.

Polygonum aviculare is a diuretic herb that is used mainly in the treatment of complaints such as dysentery and haemorrhoids and its diuretic properties make it useful in removing kidney stones (Grieve, 1984). In this study, this herb showed very high TPC and potent antioxidant activity which may support the traditional uses of this herb.

Some pharmacological studies showed that extracts or components of plants belonging to the genus *Stachys* exert significant antibacterial (Skaltsa *et al.*, 1999) and anti-inflammatory (Zinchenko *et al.*, 1981; Maleki *et al.*, 2001) effects. The potent antioxidant activity of *Stachys lavandulifolia* showed by the present study may support the biological activities mentioned above.

Although *T. minus* showed the lowest TPC and antioxidant activity, some studies showed that methanolic extract from this plant showed a potent antibacterial activity (Lotfipour *et al.*, 2008). The presence of antioxidant components other than polyphenolic compounds in the extracts may be responsible for the antimicrobial activity.

Our observation that very high values of antioxidant activity and phenolic content were found in ethanol extracts when compared with water extract is in line with reported data (Cheung *et al.*, 2003; Gulcin *et al.*, 2007). Further, the TPC content of some plants investigated in this study are in the range reported in literature (Javanmardi *et al.*, 2003).

In general, there was a good positive correlation between the TPC and antioxidant activity as assessed by FRAP and DPPH-scavenging assays among the water and ethanol extracts from the plants tested in this study. These findings suggest that polyphenols are important contributors to the antioxidant and free-radical scavenging activities of water and aqueous-ethanol extracts. Our results are in line with other studies conducted on other medicinal plants and herbs grown in other countries (Kevers *et al.*, 2007; Sreeramulu and Raghunath, 2010).

However, the lack of correlation between TPC and antioxidant activity reported in some studies (Mariko *et al.*, 2005) could be due to the different antioxidant activity parameters determined in these studies, different responses of phenolic compounds in

different bioassays used to determine the antioxidant activity (Kahkonen *et al.*, 1999) and/or the chemical structure of the phenolic compounds present in different plants. Moreover, the total phenolic content estimated by the Folin-Ciocalteu reagent may overestimate TPC because it is known to react with other components present in the plant extracts such as minerals and ascorbic acid (Matthaus, 2002; Deepa *et al.*, 2006). Consequently, the antioxidant/free-radical scavenging activities of the plant extracts cannot be predicted on the basis of their TPC alone, but also requires proper characterization of individual phenolic components. Hagerman *et al.* (1998) reported that the high molecular weight phenolics (tannins) have potent scavenging activity toward the free radicals and that the activity depends on the molecular weight, the number of aromatic rings and nature of hydroxyl groups.

The results of the current study have shown that most of the studied plants are potentially a good source of free-radical scavenging compounds and support the traditional medicinal application of some of the tested plants. The phenolic compounds make a significant contribution to the antioxidant activity in these extracts as evidenced by the positive correlation between phenolic contents and antioxidant activities. Therefore, ingestion of extracts from these plants may help to prevent *in vivo* oxidative damage associated with diseases and illnesses, for which the local traditional healers used some of these plants.

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