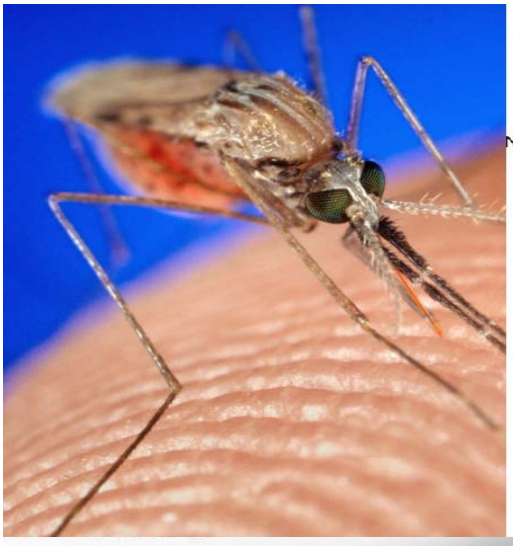
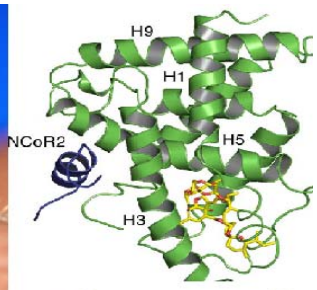
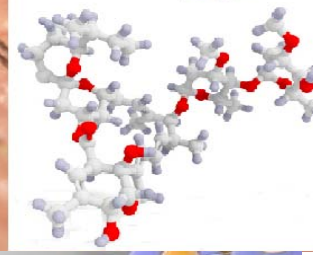
PhytoChem & BioSub Journal

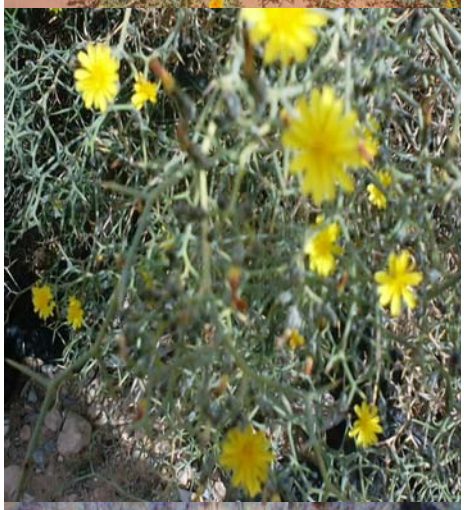
Peer-reviewed research journal on Phytochemistry & Bioactives Substances

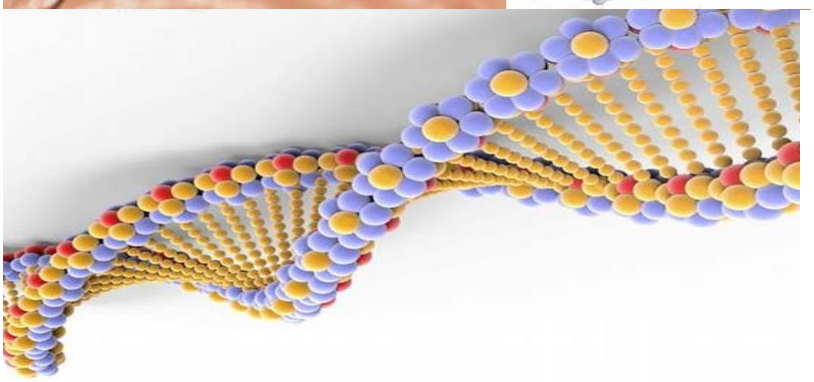
ISSN 2170 - 1768











PCBS Journal

Volume 10 N° 3 **2016**

ISSN 2170-1768

PhytoChem & BioSub Journal

ISSN 2170 – 1768

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

CAS Source Index (CODEN: PBJHB3)

Editor in Chief

Pr *Abdelkrim* CHERITI

Phytochemistry & Organic Synthesis Laboratory

08000, Bechar, Algeria

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PhytoChem & BioSub Journal Vol. 10(3) 2016
ISSN 2170-1768
CAS-CODEN:PBJHB3

PCBS Journal
ISSN 2170-1768

PhytoChem & BioSub Journal

2016
Vol.10 No. 3

Antioxidant activity, phenolic and flavonoid content of wild *Alhagi maurorum* root plant extracts

Fuad AL-RIMAWI [1*], Mohammad A. NEJADA [1], Mahmoud F. KHALID [2] & Ismail WARAD [3]

[1] Chemistry Department, Faculty of Science and Technology, Al-Quds University
P.O. Box 20002, Jerusalem, Palestine
[2] Al Quds-Bard College, Al-Quds University, Abu Dies, East Jerusalem, Palestine
[3] Department of Chemistry, Science College, An-Najah National University
P.O. Box 7, Nablus, Palestine

Received: September 14, 2016; Accepted: October 25, 2016
Corresponding author Email: falrimawi@science.alquds.edu

DOI:10.163.pcbsj/2016.10.3.80

Abstract. *Alhagi maurorum, belonging to family Leguminosae, is a highly branched spiny shrub. Roots may reach up to the depth of 15 meters. Alhagi maurorum is used in folk medicine, as a purgative, diaphoretic, expectorant and diuretic used to treat piles, migraine, warts and rheumatism. Samples of the root of Alhagi maurorum plant grown wild in Palestine were extracted with different solvents; water, 80% ethanol, and 100% ethanol. The extracts were analyzed for their total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA). Four different antioxidant assays were used to evaluate AA of the extracts: two measures the reducing power of the extracts (ferric reducing antioxidant power (FRAP) and Cupric reducing antioxidant power (CUPRAC)), while two other assays measure the scavenging ability of the extracts (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH)).The results revealed that the polarity of the extraction solvent affects the TPC, TFC, and AA. It was found that both TPC and AA are highest for plant extracted with 80% ethanol, followed by water, and finally with 100% ethanol. TFC however was highest in the following order: 80% ethanol >100% ethanol > water.*

***Key Words*:** *Alhagi maurorum, antioxidant activity, phenolic content, flavonoid content, DPPH, ABTS, FRAP.*

1. Introduction

There are more than 35 000 plant species which are used in different human cultures around the world for medicinal purpose. According to recent and previous investigators, polyphenols and flavonoids are natural antioxidant products of plants and they are present in different concentrations mostly in medicinal plants [1-4]. *Alhagi maurorum*is the single species of genus *Alhagi* found in Palestine, locally known as Aquol. It is a spiny-branched shrub, where it is used as a traditional herbal medicine. *Alhagi* species have proportionally the deepest root system of any plants. A 1 m high shrub may have a main root more than 15 m long due to their deep root system.

Alhagi species are drought avoiding plants that utilize ground water, adapting in that way perfectly to the hyper-arid environment [5].
In local communities, this plant is found to be beneficial against rheumatoid, liver disorders, infections of urinary tract, and stomach and intestinal disorders. Every part of plant can be drunk for the treatment of hemorrhoids [6].
Concerning the medicinal effects of *Alhagi maurorum*, Atta et al. (2004) reported that alcoholic extract of *Alhagi maurorum* exhibits anti-diarrheal activity *in vivo* at the oral dose of 200 and 400 mg/kg [7]. In folk medicine, *Alhagi maurorum* is considered to be effective for the cure of different aliments like bilharzias, rheumatism, liver diseases, urinary and digestive disorders [8]. Its flowers and leaves oil are used for treatment of piles, migraine, warts and rheumatism. Water extracts of its roots are used to enlarge the ureter and to remove kidney stones locally [9-11].
*Alhagi maurorum*is used also in veterinary medicine and animal nutrition [12-14]. Phytochemical screening of *Alhagi maurorum* extract revealed the presence of flavonoids, glycosides, alkaloids, saponins, tannins, steroids, and anthraquinone [15-16]. Pharmacological researches on the root extracts of this plant exhibited the activity of these extracts; therefore, extensive efforts were made to know that compound is the active one [5].The phenolic fraction from *Alhagi maurorum*acts as an antioxidant and can be useful as the natural factor protecting against diseases associated with oxidative stress [17]. *Alhagi maurorum* can be used as possible natural antioxidant, antimicrobial and an effective therapeutic agent in the management of acute promyelocytic leukemia [18].
To date, the scientific literature does not report about the antioxidant activity and phenolic content or flavonoid content of *Alhagi maurorum* plant from Palestine. Therefore, a study of the Palestinian *Alhagi* constitutes a valuable addition to the available literature. Abundant literature dealing with total phenolic and flavonoids content, biological activities, as well as antioxidant activity was published from different countries [5-11, 15-18], including those of the Middle East [19]. The main objective of the this study is therefore to quantitate total phenolic and flavonoid content, radical scavenging and antioxidant potential of different aqueous and ethanolic extracts of *Alhagi maurorum* plant growing wild in Palestine. Antioxidants contents were assayed using FRAP, CUPRAC, DPPH, and ABTS colorimetric methods. TPC and TFC of the extracts were evaluated using Folin-Ciocalteau, and aluminum chloride colorimetric methods, respectively.

2. Materials and methods

2.1 Plant material

The roots of Alhagi plant was collected from West Bank, Palestine in March 2015. The roots were air-dried in dark at room temperature for two weeks, then milled to a powdered plant material, and then stored in fridge until extraction.

2.2 Chemicals and reagents

2,4,6-tripyridyl- S-triazine (TPTZ), hydrochloric acid 37% (w/w), sodium hydroxide, ferric chloride trihydrate, ferrous sulfate heptahydrate, potassium persulphate, sodium acetate, sodium carbonate, sodium nitrite, aluminum chloride, methanol, folin-ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, cupper chloride, neocuproine, 99.9% ethanol, ammonium acetate, DPPH, methanol,ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)), and potassium persulphate were all obtained from Sigma-Aldrich, Germany. All chemicals and reagents were of analytical grade.
FRAP reagent was prepared according to Benzie and Strain (1999) [20] by the addition of 2.5 mL of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20mM $FeCl_3.6H_2O$ and 25 mL of 0.3M acetate buffer at pH3.6. Acetate buffer (0.3M) was prepared by dissolving 16.8 g of acetic acid and 0.8g of sodium hydroxide in 1000 mL of distilled water.

2.3 Extraction of the roots

Dry powder of roots (ten grams) was extracted separately with 100 ml of three extraction solvents (water, 80% ethanol, and 100% ethanol) in water bath at 37 ◦C for three hours. The extracts were then filtered and the filtrate was stored at 4 ◦C until used for analysis (TPC, TFC, and AA).

2.4 Measurement of Antioxidant Activity

2.4.1 FRAP assay

The antioxidant activity of the extracts was determined using a modified method of the assay of ferric reducing/antioxidant power (FRAP) of Benzie and Strain (1999) [20]. Freshly prepared FRAP reagent (3.0 mL) was warmed at 37°C and mixed with 40 µl of the extract and the reaction mixtures were later incubated at 37°C. Absorbance at 593 nm was read with reference to a reagent blank containing distilled water which was also incubated at 37 °C for upto 1 hour instead of 4 min, which was the original time applied in FRAP assay. Aqueous solutions of known Fe^{+2} concentrations in the range of 2-5 mM were used for calibration, and results were expressed as mmol Fe^{+2} /g.

2.4.2 Cupric reducing antioxidant power (CUPRAC assay)

The cupric ion reducing antioxidant capacity of the extracts was determined according to the method of Apak, Guclu, Ozyurek, Celik(2008) [21]. 100µl of sample extract was mixed with 1ml each of 10 mM of cupper chloride solution, 7.5 mM of neocuproine alcoholic solution (99.9% ethanol), and 1 M (pH 7.0) of ammonium acetate buffer solution, and 1ml of distilled water to make final volume 4.1ml.After 30 min, the absorbance was recorded at 450 nm against the reagent blank. Standard curve was prepared using different concentrations of Trolox.The results were expressed as µmol Trolox/g.

2.4.3 Free radical scavenging activity using DPPH (DPPH assay)

DPPH assay is based on the measurement of the scavenging ability of antioxidants towards the stable DPPH radical, and the procedure was done according to Brand-Williams, Cuvelier, and Berset (1995) [22]. A 3.9 mL aliquot of a0.0634 mM of DPPH solution in methanol (95%) was added to 100 µl of each extract. The mixture was vortexed for 5-10 sec. The change in the absorbance of the sample extract was measured at515 nm for 30 min till the absorbance reached a steady state. The percentage inhibition of DPPH of the test sample and known solutions of Trolox were calculated by the following formula:

$$\%\ \text{inhibition} = \frac{(A^o - A)}{A^o} \times 100$$

Where A^o is the absorbance of a solution of 100µl methanol 95% and 3.9 ml of DPPH at 515 nm, and A is the absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank. Standard curve was prepared using different concentrations of Trolox. The results were expressed as µmol Trolox/g.

2.4.4 Free radical scavenging activity using ABTS (ABTS assay)

A modified procedure using ABTS (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid)) as described by Pellegrini, Proteggente, Pannala, Yang, Rice-Evans (1999) [23] was used. The ABTS stock solution (7 mM) was prepared through reaction of 7 mM ABTS and 2.45 mM of potassium persulphate as the oxidant agent.The working solutionof $ABTS^{+\cdot}$ was obtained by diluting the stock solution in 99.9% ethanol to give an absorption of 0.70 ± 0.02 at 734 nm.200µl sample extract was added to 1800µl of $ABTS^{+\cdot}$ solution and absorbance readings at 734 nm were taken at 30°Cexactly 10 min after initial mixing (A).The percentage inhibition of $ABTS^{+\cdot}$of the test sample and known solutions of Trolox were calculated by the following formula:

$\%\ \text{inhibition} = \frac{(A^o - A)}{A^o} \times 100$, Where A^o is the absorbance of a solution of 200µl of distilled water and 1800µl of $ABTS^{+\cdot}$ at 734 nm, and A is the absorbance of the test sample at734 nm. The calibration curve between %inhibition and known solutions of Trolox (50–1000µM) was then established. The radical-scavenging activity of the test samples was expressed as Trolox equivalent antioxidant capacity TEAC (µmol Trolox/g sample).

2.5 Total phenolic content (Folin-Ciocalteau assay)

Total phenolics were determined using Folin-Ciocalteau reagents [24]. *Alhagi maurorum* plant extracts or gallic acid standard (40 µl) were mixed with 1.8 mL of Folin-Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 mL of sodium bicarbonate (7.5%, w/v) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Aqueous solutions of known gallic acid concentrations in the range of 10 - 500 mg/L were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/ g sample.

2.6 Total flavonoid content

The determination of total flavonoids was performed according to the colorimetric assay of Kim, Jeong, and Lee (2003) [25]. Distilled water (4 mL) was added to 1 mL of the extract in a test tube. Then, 0.3 mL of 5% sodium nitrite solution was added, followed by 0.3 mL of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 5 minutes, 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 mixed using test tube shaker and the absorbance of the pink color developed was determined at 510 nm. Aqueous solutions of known catechin concentrations in the range of 50 - 100 mg/L were used for calibration and the results were expressed as mg catechin equivalents (CEQ)/ g sample.

2.7 Statistical analyses

Three samples of *Alhagi maurorum* plant were independently analyzed and all of the determinations were carried out in triplicate. The results are expressed as means ± standard deviations.

3. Results and discussion

3.1. Total Phenolic Contents (TPC)

Contents in the plant extracts are usually evaluated using Follin-Ciocalteu's reagent (FC reagent). On reduction by hydroxyl moieties of phenolic compounds yellow color of FC reagent is reduced to blue colour proportionate to the amount of phenolic compounds present in the sample. This change in the colour is monitored spectrophotometrically at 765 nm. TPC of *Alhagi maurorum* plant extracts using three different solvents is shown in Table 1. As it is obvious from this table, the extraction solvent has an effect on the TPC of the plant extracts where significant differences ($p < 0.05$) between the TPC of the three extracts are indicated by different small letters (a, b, and c).The highest TPC was found for the plant material when extracted with 80% ethanol (329.1± 4.2mg/g), followed by plant material extracted with water (181.2± 2.9 mg/g) and finally with 100% ethanol (117.5± 2.0mg/g). These results show that TPC were only 55% and 36% when the plant material was extracted by distilled water and 100% ethanol, respectively as compared with the TPC extracted with 80% ethanol indicating the higher solubility of the phenolic compounds in 80% ethanol.

Table 1. Total phenolic content (TPC as mg Gallic acid/g DW*), total flavonoids contents (TFC as mg catechin/g DW), FRAP (mmol Fe^{+2}/g DW), CUPRAC (µmol Trolox/g DW), DPPH (µmol Trolox/g DW), ABTS (µmol Trolox/g DW), DPPH % inhibition, and ABTS % inhibition of *Alhagi maurorum* plant extracted with water, 80% ethanol, and 100% ethanol.

	TPC** (mg/g)	TFC (mg/g)	FRAP (mmol/g)	CUPRAC (µmol/g)	DPPH (µmol/g)	ABTS (µmol/g)	DPPH % inhibition	ABTS % inhibition
Water	181.2^{b} ± 2.9	5.1^{c} ± 0.7	4.0^{b} ± 0.8	1950^{b} ± 28	176^{c} ± 1.1	16.1^{c} ± 1.9	77.2^{c} ± 1.3	77.4^{c} ± 1.4
Ethanol (80 %)	329.1^{a}± 4.2	16.4^{a}± 0.8	9.3^{a}± 0.7	5280^{a}± 50	276^{a}± 1.4	31.4^{a}± 1.6	90.2^{a}± 2.4	86.1^{a}± 1.2
Ethanol (100 %)	117.5^{c} ± 2.0	10.7^{b} ± 0.1	2.7^{c} ± 0.3	1901^{c} ± 15	206^{b} ± 4.1	26.2^{b} ± 0.4	87.4^{b} ± 1.6	80.1^{b} ± 2.0

* DW: Dry weight
** Results are expressed as average of three samples of *Alhagi maurorum* shoots. Different small letters within column indicate significant difference ($p < 0.05$, $n = 3$).

3.2 Total Flavonoid Content (TFC)

The results of ferric chloride colorimetric test for determining flavonoids content are presented in Table 1.The same statistical analyses as for TPC were performed for total flavonoids content (TFC), and the results (Table 1) showed that significant differences between total flavonoids content of the plant materials extracted with the three solvents were obtained, where significant differences ($p < 0.05$) indicated by small letters (a, b, and c).The highest TFC was found for the plant material when extracted with 80% ethanol (16.4± 0.8mg/g) which is about 1.5 times significantly higher than that extracted with 100% ethanol (10.7± 0.1mg/g) and the later was significantly about two times higher than the TFC extracted with water(5.1 ± 0.7mg/g).Different trend of solvent effect on TFC and TPC was obtained where the highest content of TPC and TFC was obtained when the plant was extracted with 80% ethanol, while the lowest was with 100% ethanol for TPC and water for TFC, see Table 1. Apparently, mixed solvents of intermediate polarities (80% ethanol) are the most suitable extracting solvent for recovering the highest amounts of phenolic and flavonoid compounds which have both polar and non polar functional groups.

3.3 Antioxidant Activity

Antioxidant Activity accounts for the presence of efficient oxygen radical scavengers, such as phenolic compounds [26]. The antioxidant activity of phenolics is mainly due to their redox properties, which make them acting as reducing agents, hydrogen donors, and singlet oxygen quenchers [26]**.**

There are two types of antioxidant assays used to determine antioxidant activity of plant extracts. The first type measures the potential of extracts to reduce ions or oxidants (to act as reducing agents) like ferric ion, cupric ion. The main two assays of this antioxidant activity category are FRAP (measures the reduction potential of ferric to ferrous ion), and CUPRAC (measures the reduction of cupric to cuprous ion). The second category of antioxidant activity measures the ability of plant extracts to scavenge free radicals. DPPH and ABTS assays (where DPPH and ABTS are stable free radicals) are the two main examples of this category. These assays are used because they are quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidants present.

3.3.1 Reducing potential of plant extracts

3.3.1.1 FRAP assay

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}–

TPTZ). The reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom. The reduction of Fe^{3+}–TPTZ complex to blue-colored Fe^{2+}–TPTZ occurs at low pH.

The antioxidant test based on FRAP assay of *Alhagi maurorum root* extracts using three different solvents are presented in Table 1 (expressed as mmol Fe^{+2}/g of dry plant material). Statistical analyses showed that there are significant differences between FRAP values as a function of extraction solvent (Table 1), where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c).

Table 1 revealed that antioxidant activity (FRAP) of the *Alhagi maurorum* plant increased as the polarity of solvent changes (80% ethanol >water>100% ethanol), where FRAP values were found to be about two and three times significantly higher when extracted with 80% ethanol compared to water and 100% ethanol, respectively.

The trend of extraction solvent on the FRAP values was found to be the same as for TPC but different from TFC. This suggest that there is a correlation between AA (expressed as FRAP) and TPC,reflecting the fact that total phenolics are the major determinant of AA.

As in the case of TPC and TFC,80% ethanol gives higher amounts of AA (FRAP) compared with water or ethanol as extraction solvent of *Alhagi maurorum*.

3.3.1.2 CUPRAC assay

Although FRAP antioxidant assay has been very popular among researchers, CUPRAC assay is a relatively new assay developed by Apak, Guclu, Ozyurek, and Celik (2008) [21]. It utilizes the copper(II)–neocuproine [Cu(II)–Nc] reagent as the chromogenic oxidizing agent and is based on the cupric reducing ability of reducing compounds to cuprous.

Table 1 shows the CUPRAC antioxidant activity (expressed as µmole Trolox/g) of *Alhagi maurorum* plant extracts using three different solvents.Statistical analyses showed that there are significant differences between AA using the three extraction solvents, where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c).

Results showed that CUPRAC antioxidant activity of the *Alhagi maurorum*plant increased in the following order: 80% ethanol >water>100% ethanol which is the same trend as FRAP antioxidant activity, and TPC but different from TFC, which suggests that there is a correlation between CUPRAC and TPC.

3.3.2 Free radical scavenging ability of plant extracts

3.3.2.1 DPPH assay

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples [27]. It is a stable free radical with a characteristic absorption at 517 nm that was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. Antioxidants, on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, thus neutralizing its free radical character [28]. The color changed from purple to yellow and the absorbance at wavelength 517 nm decreased. DPPH assay is based on the ability of the stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenolics. The bleaching of DPPH solution increases linearly with increasing amount of extract in a given volume.

Table 1 shows the % inhibition of DPPH free radicals by the *Alhagi maurorum* plant extracted with the three solvents. Statistical analyses showed that there are significant differences between % inhibitions using the three extraction solvents, where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c), see table 1.

Table2 shows the % inhibition of DPPH at different concentrations of the crude extract (from 10 to 100µg/mL). This data shows that the extracts exhibited a dose dependent scavenging activity (a linear relationship between % of DPPH inhibition and concentration ($y = 0.88x + 2.1$, with R^2 of

0.9968), where y is the % of inhibition and x is the concentration). From this linear relationship, IC50 which is the concentration required to quench 50% of the DPPH free radicals was determined and was found to be54µg/mL.

DPPH antioxidant activity of *Alhagi maurorum* plant extracts using three different solvents was expressed as µmole Trolox/g (Table 1). Statistical analyses showed that there are significant differences between AA using the three extraction solvents, where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c).Results showed that DPPH antioxidant activity of the *Alhagi maurorum* plant increased in the following order: 80% ethanol >100% ethanol> water which is the same trend as TFC, but different from TPC, FRAP, and CUPRAC antioxidant activity.

Table 2:% inhibition of DPPH and ABTS free radicals by different concentrations of *Alhagi maurorum* plant extract.

Concentration of DPPH (µg/mL)	% inhibition of DPPH *	Concentration of ABTS (µg/mL)	% inhibition of ABTS *
10	11.4 ± 0.9	10	9.8 ± 0.8
30	29.2 ± 0.9	30	28.1 ± 1.2
50	43.2 ± 1.4	50	42.9 ± 2.2
70	65.4 ± 1.6	70	60.1 ± 1.4
100	90.1 ± 1.8	100	84.1 ± 2.1

*Results are expressed as Average ± standard deviation of three samples.

3.3.2.2 ABTS assay

The ABTS decolorization assay was applied to evaluate *in vitro* radical scavenging activity of ethanolic and aqueous extracts of *Alhagi maurorum.*ABTS radical cation, produced *in vitro* conditions, has the reduction potential comparable to that of hydroxyl radical which is produced *in vivo* during metabolic reactions and stressconditions [27-28]. All the antioxidative components lower in reduction potential than that of the ABTS radical cation, can reduce ABTS radical cation to its native form proportionate to their amount on a time scale. Trolox equivalent antioxidant capacity values were obtained by comparing the percentage inhibition values of extract samples with the standard trolox curve [27-28].

Table 1shows the % inhibition of ABTS free radicals by the plant extracted with the three solvents. Statistical analyses showed that there are significant differences between AA using the three extraction solvents, where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c), see Table 1.

Table 2 shows the % inhibition of ABTS at different concentrations of the crude extract (from 10 to 100µg/mL). This data shows that the extracts showed a dose dependent scavenging activity (a linear relationship between % of ABTS inhibition and concentration ($y = 0.8203x + 2.3451$, with R^2 of 0.9993), where y is the % of inhibition and x is the concentration). From this linear relationship, IC50 was determined and was found to be about 58µg/mL.

ABTS antioxidant activity of *Alhagi maurorum root* extracts using three different solvents was expressed as µmol Trolox/g (Table 1). Statistical analyses showed that there are significant differences between AA using the three extraction solvents, where significant differences ($p < 0.05$) are indicated by different small letters (a, b, and c).

Results showed that ABTS antioxidant activity of the *Alhagi maurorum* plant increased in the following order: 80% ethanol >100% ethanol> water which is the same trend as DPPH, but different from FRAP, CUPRAC, and TPC antioxidant activities.

It is interesting to note that same trend of DPPH and ABTS was found as TFC (80% ethanol > 100% ethanol > water). This may suggest that free radical scavenging ability of *Alhagi maurorum* root extract is correlated with TFC.

Furthermore, a positive correlation has been shown between antioxidant activities assays (FRAP and CUPRAC) and total phenolics, indicating that these compounds are more likely to contribute to the antioxidant potential (reduction potential) ofthe investigated plant extracts [29-30]. Thus, the phenolic and flavonoid compounds might contribute directly to antioxidative action of plant extracts [31].

Conclusions

Plant phenolic compounds can act as singlet oxygen quenchers and scavenge free radicals, so the presence of substantial amounts of these compounds in *Alhagi maurorum* root extract promotes it as an important source of antioxidants which reduce risk associated with degenerative diseases and provide health promoting advantage. Extraction solvent has an important role in determining the amounts of phenolics and flavonoids and consequently the antioxidant activity of *Alhagi maurorum* root extract. This extract is rich with phenolic compounds and flavonoids. Total phenolics and flavonoid contents as well as antioxidant activities are highest for plant extracted with 80% ethanol. Mixture of ethanol and water (80% ethanol) are the best solvent for extraction of phenolic and flavonoid compounds. There is a correlation between total phenolic content and FRAP and CUPRAC, and a correlation between TFC and DPPH and ABTS. *Alhagi maurorum* plant constitutes a natural source of potent antioxidants that may prevent many diseases and could be potentially used in food, cosmetics, and pharmaceutical industries.

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