Chemical Composition and Antioxidant Capacity of Extracts from the Wood of *Berberis vulgaris* Stem

Seyyed Khalil Hosseinihashemi,[a,*] and Hamed Aghajani [a]

Extracts from the woody stem of *Berberis vulgaris* were analyzed for their antioxidant capacity using the 2,2-dipheny-1-picrylhydrazyl (DPPH) method and compared with ascorbic acid (AA) and butylated hydroxytoluene (BHT). The most active extracts were analyzed for their chemical composition using gas chromatography-mass spectrometry. Acetone extract was found to be moderately active as an antioxidant agent at 47.6%, which was lower than the value of vitamin C (84.8%) at the concentration of 0.16 mg/mL. The major components identified in the acetone extract of the stem wood were 4-vinylguaiacol (75.5%) and D-mannoheptulose (8.8%). The dissolved water:methanol (1:1 *v/v*) partitioned from acetone extract afforded 12 fractions; among which, fraction F11 was found to have good antioxidant capacity (64.3%) at the concentration of 0.16 mg/mL. The major compounds identified in F11 were 3,4-dimethylthioquinoline (37.7%), methyl (1,2-dihydro-2-oxo-1-acenaphthylidene)acetate (19.9%), and α-cyclohexyl-3-furanmethanol (15.3%). It can be suggested that extracts of *B. vulgaris* wood may have a potential source of antioxidant compounds useful for new drugs from the natural basis.

Keywords: Berberis vulgaris stem wood; Antioxidant capacity; Acetone extract; Fraction; Gas chromatography-mass spectrometry
*Contact information: a: Department of Wood Science and Paper Technology, Karaj Branch, Islamic Azad University, Karaj, Iran; *Corresponding author: hashemi@kiau.ac.ir*

INTRODUCTION

The *Berberis vulgaris* (barberry) plant, which belongs to family Berberidaceae, is a shrub, growing in Asia and Europe, approximately 1 to 3 m tall, with spiny, yellow wood, and obviate leaves bearing pendulous yellow flowers succeeded by oblong red berries (Zargari 1983; Amin 1991; Ciulei *et al.* 1993; Dewick 2002; Damaschin and Analiza 2006). In Iranian traditional medicine, the root, bark, leaf, and fruit, of the plant have been used extensively for medicinal purposes as antiarrhythmic and sedative effects (Fatehi *et al.* 2005; Javadzadeh and Fallah 2012). The fruits are used as a food additive, and the juice is recommended to cure cholecytitis (Zargari 1983), and it was macerated with *Foeniculum vulgare* and used in Ancient Egypt to ward off pestilent fevers (Chevallier 2001). Different alkaloids were extracted from different parts of the plant (Končić *et al.* 2010; Javadzadeh and Fallah 2012).

The antioxidant properties, organic acid contents and phenolic compound contents of *Berberis vulgaris* fruits naturally grown in Gevaş Region (Van Province/Turkey) were determined by Gundogdu (2013). In the study of organic acids, malic acid content was found as 1.862 g kg^{-1}, citric acid content as 1.253 g kg^{-1}, tartaric acid content as 0.702 g kg^{-1}, and succinic acid content as 0.086 g kg^{-1}. The content of chlorogenic acid was the highest among phenolic compounds, which was found as 0.752 g kg^{-1}. The antioxidant capacity of the barberry fruits was also determined as 8.73 μmol TE g^{-1}. Due to their high

antioxidant capacity and content of biochemical compounds, barberries are considered as valuable fruits.

Berberine, the isoquinoline alkaloid, which is present in roots, rhizome, and outer bark, has been shown to have pharmacological, biochemical, and anti-cancer effects and for the treatment of hepatic oxidative stress, Alzheimer's disease, and idiopathic male factor infertility (Abd El-Wahab *et al.* 2013). It is present in plants of the *Berberis* genus (*B. aquifolium*, *B. vulgaris*, *B. aristata*, *etc*.) (Marinova *et al.* 2000; Kim *et al.* 2003; Mahata *et al.* 2011; Wu *et al.* 2011).

Different compounds isolated from the extracts of the plant exhibit good biological activities; anthocyanins from fruits extract show inhibitory effects on capillary permeability (Cohen-Boulakia *et al.* 2000) and anticholinergic and antihistaminergic effects (Tomosaka *et al.* 2008). Anthocyanins and carotenoid pigments have antioxidant and cytoprotective activities (Freile *et al.* 2003; Mahady *et al.* 2003; Kuo *et al.* 2004; Han and Lee 2005; Tomosaka *et al.* 2008). Phenolic compounds and other isolated compounds from different bark and fruits exhibit antibacterial antifungal and antioxidant activities (Jain and Kar 1971; McCartney 1989; Huffman 2003; Parekh and Chanda 2005; Özgen *et al.* 2012; Ghareeb *et al.* 2013; Mahmoudvand *et al.* 2014). Sesquiterpene, epi-ligulyl oxide, and derivatives of ammoxidized hydroquinone, such as 1,2-bis(trimethylsiloxy)ethane, were detected in barberry bark extracts (Hosseinihashemi *et al.* 2015). The epi-ligulyl oxide compound has been characterized in agarwood and eaglewood oil as a novel compound (Naf *et al.* 1992; Mei *et al.* 2008).

There have been several studies related to the antioxidant activities of extracts from different parts of *B. vulgaris*. Roots, twigs, and leaves ethanolic extracts provide good antioxidant activities that have been correlated with the content of main plant antioxidants, phenols, and flavonols (Hadaruga *et al.* 2010; Končić *et al.* 2010).

According to our survey, there have been no reports on the chemical composition of the woody stem extracts of *B. vulgaris*. Therefore, this work was aimed to evaluate the antioxidant capacity of extracts from woody stem of *B. vulgaris* and to compare them with ascorbic acid (AA) and butylated hydroxytoluene (BHT). The most active extract was analyzed for its chemical composition using gas chromatography-mass spectrometry (GC/MS).

EXPERIMENTAL

Plant Materials

Fresh stems of *B. vulgaris* were collected from Siahbishe, Chalous, and Mazandaran, North of Iran in May of 2013. The plant material was obtained from Islamic Azad University, Karaj, Iran. The bark was separated from the woody stem and air-dried to 8.0 percent moisture content.

Extraction and Fractionation

The wood of the stems was chopped into small pieces and crushed to wood flour using a laboratory electrical rotary mill. The flour size was between 40 and 60 mesh. The same extraction protocol as our previous work (Hosseinihashemi *et al.* 2015) was used in this study. Briefly explained, approximately 165 g of this flour was placed into the 3 extraction thimbles, then three samples were extracted independently using pure acetone (300 mL in a 500-mL balloon) and a Soxhlet-type apparatus for 8 h. The combined

extract was concentrated using a Heidolph Laborota 4001 rotary-evaporator apparatus (at 40 °C to reach total solvent evaporation) for approximately 15 min. Then, the extracts were collected, dried over anhydrous sodium sulphate, and stored at 4 °C until further analysis. The solid extractive weight was 4.15 g. Subsequently, 3.20 g of the solid extractives were dissolved in water:methanol (1:1 *v/v*) where the residue was discarded. Then the supernatant poured into a separatory funnel, followed by the addition of 50 mL of *n*-hexane. The mixture was shaken by hand for 10 min. Half a gram of the water:methanol aqueous supernatant extractive was used for column chromatography with silica gel, Merck KGaA 64271 Darmstadt, Germany. The 12 fractions were labeled F1 to F12 (Fig. 1). The 3 × 10 mL eluent volume was used in the chromatographic separation for each solvent.

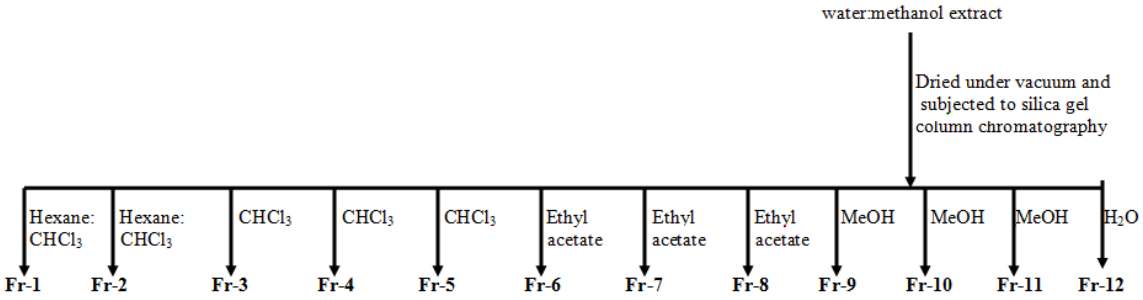

Fig. 1. Isolation scheme of active constituents of *B. vulgaris* from the water:methanol extract of woody stem

Free Radical Scavenging Activity by DPPH Assay

The free radical scavenging activities of the acetone and water:methanol extracts, as well as the fractions from F1 to F12 of the stem wood powders, were determined using the 2,2-dipheny-1-picrylhydrazyl (DPPH) method (Karau *et al.* 2013). The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers (Lewis 2012). For this method, a stock solution was prepared by dissolving 2.4 mg of DPPH powder in 100 mL of methanol. The stock solution was stored at 20 °C. The working solution was prepared by dissolving 1 mg of extract powder in 1000 µL methanol (1 mg/mL) into the main vial. The schematic of preparation process of working solution are shown in Fig. 2.

Serial dilutions were carried out with the stock solution and the working solution of tested extract to obtain concentrations of 0.16, 0.08, 0.04, 0.02, 0.01, and 0.005 mg/mL (Fig. 2). The experiment was performed in two replicates, and the average absorbance was recorded for each concentration. The reaction mixture was mixed for 10 s and left to stand at room temperature in a dark place for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer.

Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as the reference standards and were dissolved in methanol to make the reference standard solutions with the same concentration (1 mg/mL). The control sample (blank) was prepared with the same volume of solution (250 µL methanol + 1250 µL stock solution), without test compounds and the reference standards. Pure methanol (Sigma-Aldrich, Germany) was used to make the control sample. The UV scanning spectrophotometer device was first calibrated and adjusted with pure methanol.

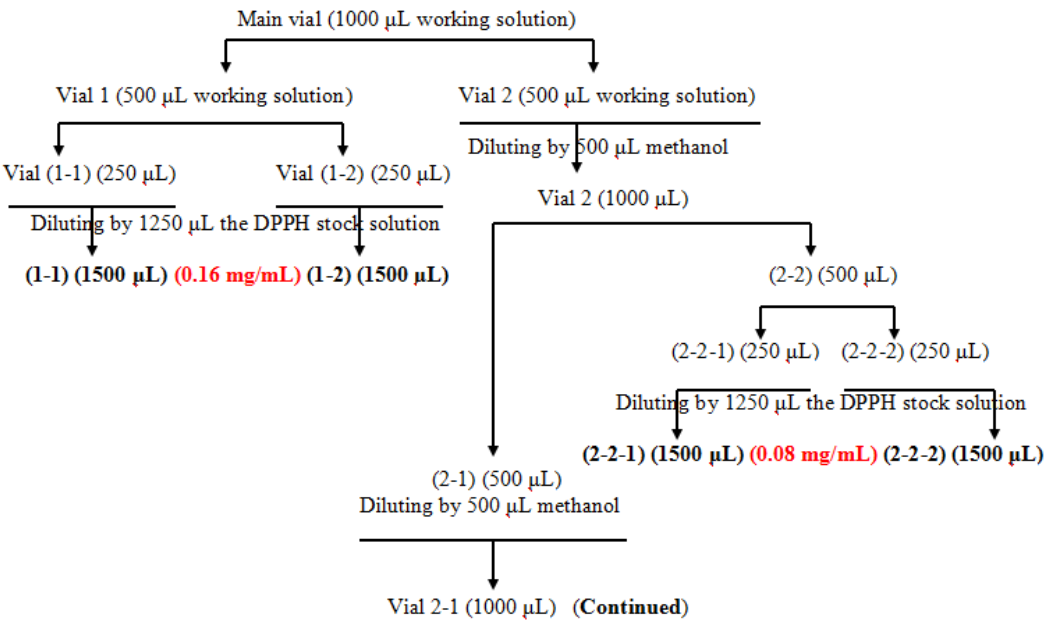

Fig. 2. Preparation process of working solutions.

The DPPH free radical scavenging activity (%) was calculated using the following equation,

$$\text{DPPH free radical scavenging activity (\%)} = 100(A_c - A_s)/A_c \quad (1)$$

where the percentage DPPH free radical scavenging activity value was calculated from the absorbance of the control or blank, A_c, and of the sample, A_s.

The controls contained all the reaction reagents except for the extract or positive control substance. The values are presented as the means of two replicate analyses.

Silylation Method

The acetone and F11 extracts of barberry wood were separated and concentrated using a Heidolph Laborota 4001 rotary-evaporator apparatus (at 40 ºC to reach total solvent evaporation) for approximately 15 min, separately. Then, the extracts were collected, dried over anhydrous sodium sulphate, and stored at 4 ºC until further analysis. In order to identify the components present in the extracts, about 1 mg (powder) extract was selected, mixed with 30 micro liter N,O-bis (trimethylsilyl) triflouroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) reagent and about 15 micro liter pyridine in test tube, then the test tube was placed in a water bath at 70 ºC for one hour.

Analysis of Extracts

Gas chromatography-mass spectrometry (GC/MS) analysis of the acetone and F11 extracts were performed using split mode (30:1 and 10:1) injection. One microlitre of the silylated extract was run on a HP 6890 (Hewlett Packard, USA) gas chromatograph fitted with a cross-linked 5.0% PH ME siloxane HP-5 capillary column (dimensions: 30 m x 0.25 mm, 0.50 µm coating thickness) and coupled with a model 5975B mass detector.

The GC/MS operation conditions were as follows: injector temperature 250 °C; transfer line 290 °C; oven temperature program 50 to 250 °C (5 °C/min); carrier gas: He at 1.4 mL/min; mass spectra: electron impact (EI+) mode 70 eV with a mass range of 40

to 450 *m*/*z*; and ion source temperature at 250 °C. Individual components were identified using Wiley 275 L and NIST05 mass database matching and by comparing the retention times and mass spectra of constituents with published data (Julian and Konig 1988; Adams 1995, 2001). Retention indices (R_I) were determined with reference to a homologous series of normal alkanes, using the following formula (Kovats 1958),

$$R_I = 100\,[(n + (N\text{-}n) \times \log t_{1R}\,(x) - \log t_{1R}\,(C_n)/\log t_{1R}\,(C_N) - \log t_{1R}\,(C_n)] \quad (2)$$

where R_I is the retention index of the compound of interest, t_{1R} is the net retention time (t_R-t_0), t_0 is the retention time of solvent (dead time), t_R is the retention time of the compound of interest, C_n and C_N are the number of carbons in the n-alkanes eluting immediately before and after the compound of interest, and N and n are the number of carbon atoms in the n-alkane eluting immediately before and after the compound of interest.

Statistical Analysis

Data of antioxidant capacity were statistically analyzed using the SPSS program.

RESULTS AND DISCUSSION

Antioxidant Capacity

Statistically, there were significant differences among the treatments (F1→F12, acetone, water:methanol, *n*-hexane, BHT, vitamin C, and their concentrations) (Tables 1 and 2).

The acetone extracts exhibited low antioxidant capacity overall (Fig. 3). At the concentration of 0.005 mg/mL, the lowest and highest antioxidant capacity was observed in F2 (1%) and the acetone extract (18.95%), respectively, which was lower than the value for vitamin C (77.74%). The lowest and highest antioxidant capacity came from F4 (33.25%) and F11 (64.29%) at 0.16 mg/mL, respectively, which was also lower than that of vitamin C (84.84%) at the same concentration. The same trend was observed with the reference, BHT.

Table 1. Statistical Analysis of the Effect of Treatments and Concentrations on the Antioxidant Capacity of Wood Extractives of *B. vulgaris*

Variations Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	176301.214a	101	1745.557	238.032	0.000
Intercept	220605.507	1	220605.507	30082.825	0.000
Fraction	108991.026	16	6811.939	928.909	0.000
Concentration	56395.461	5	11279.092	1538.071	0.000
Fraction * Concentration	10914.728	80	136.434	18.605	0.000
Error	1495.987	204	7.333		
Total	398402.709	306			
Corrected Total	177797.202	305			

Table 2. Mean±SD of the Antioxidant Capacity (%) as Affected by Different Treatments of Wood Extractives of *B. vulgaris* Compared with BHT and Vitamin C

Treatment	Concentration (mg/mL)					
	0.005	0.01	0.02	0.04	0.08	0.16
F1	1.97 ±0.33	4.10 ±0.82	11.31 ±1.80	15.57 ±4.43	23.28 ±12.79	49.51 ±2.30
F2	1.00 ±0.33	3.17 ±0.17	3.17 ±1.84	7.00 ±4.00	20.17 ±2.84	35.33 ±4.34
F3	3.84 ±0.17	12.58 ±0.00	16.89 ±1.00	26.16 ±2.65	36.42 ±1.00	44.54 ±2.16
F4	3.22 ±1.86	6.78 ±2.37	9.66 ±0.85	21.86 ±1.19	32.20 ±1.70	33.25 ±1.70
F5	2.73 ±0.16	6.11 ±0.97	12.86 ±1.93	19.13 ±0.81	22.85 ±2.73	41.32 ±2.41
F6	2.14 ±1.43	5.00 ±1.43	6.79 ±0.36	9.82 ±0.18	33.57 ±1.43	46.96 ±7.68
F7	1.15 ±0.17	2.62 ±0.00	5.41 ±2.13	6.72 ±0.49	21.80 ±6.40	39.34 ±1.64
F8	6.92 ±2.62	9.54 ±1.23	16.62 ±2.77	24.77 ±0.15	30.00 ±10.62	40.00 ±3.08
F9	2.79 ±1.15	3.93 ±1.64	6.23 ±0.00	14.26 ±1.81	25.57 ±3.28	55.25 ±0.50
F10	4.47 ±1.60	6.55 ±0.80	6.39 ±4.47	17.41 ±0.16	25.72 ±2.08	57.03 ±2.40
F11	4.25 ±1.19	5.27 ±0.17	12.41 ±1.54	23.47 ±2.72	34.86 ±2.21	64.29 ±1.02
F12	2.54 ±0.85	7.12 ±1.70	10.17 ±1.70	19.66 ±2.37	35.59 ±3.39	57.29 ±1.36
Acetone	18.95 ±4.21	23.68 ±3.16	29.74 ±1.85	31.84 ±0.27	36.32 ±1.06	47.63 ±0.79
water:methanol	1.50 ±1.50	8.25 ±0.75	10.50 ±0.50	19.50 ±0.50	34.75 ±6.25	58.00 ±2.00
n-hexane	4.46 ±1.50	16.43 ±5.17	13.85 ±0.23	23.71 ±1.64	46.24 ±2.12	49.30 ±0.94
BHT	63.45 ±0.00	69.66 ±0.00	72.41 ±0.00	72.76 ±0.00	73.10 ±0.00	73.45 ±0.00
Vitamin C	77.74 ±0.00	80.32 ±0.00	84.19 ±0.00	84.35 ±0.17	84.52 ±0.00	84.84 ±0.00

Additionally, among the assayed fractions, F11 and F12 showed high antioxidant capacity at 64.29% and 57.29%, respectively, when the concentration was 0.16 mg/mL. Looking at Figs. 1 and 3, the more polar compounds seem to have the higher antioxidant capacity, because following the extractions with non-polar solvents the fraction F11 retains an antioxidant capacity comparable to acetone fraction.

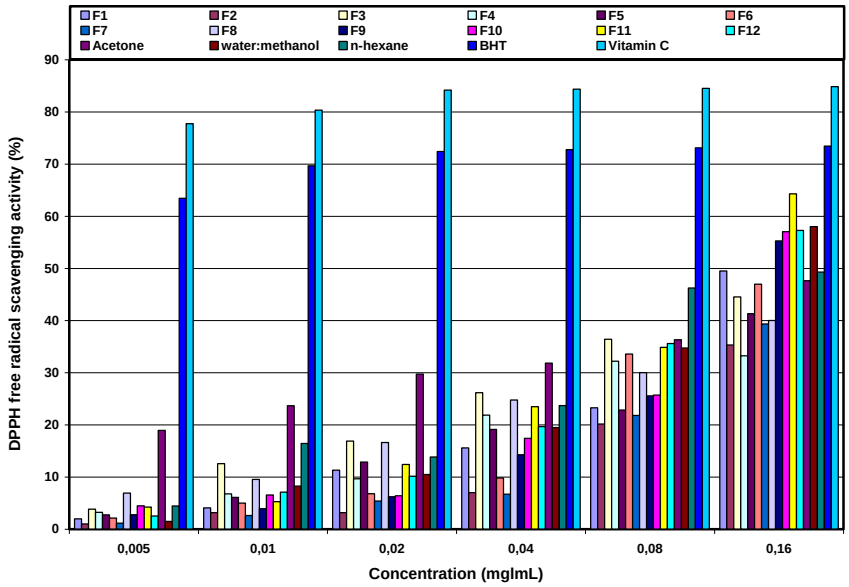

Fig. 3. The antioxidant capacity of extracts from the wood of *B. vulgaris*.

Wood Extract

The suggestable chemical constituents identified in the acetone extract are presented in Table 3. The acetone extract of the fresh woody stem of *B. vulgaris* exhibited a yellowish extract with a pleasant aroma, and a yield of 3.0% (v/w). Five components, comprising 99.25% of the woody stem extract, were identified. The major components of the woody stem extract were 4-vinylguaiacol (75.49%) and D-mannoheptulose (8.83%).

Table 3. The Suggestable Chemical Composition of Acetone Wood Extract of *B. vulgaris*

Components	Retention Time (min)	Retention Indices	Area (%)
4-Vinylguaiacol	21.304	1352	75.49
D-Mannoheptulose	26.821	1573	8.83
Benzothiazole, 2-methyl	33.245	1870	6.17
Tetradecanoic acid	35.147	1967	5.60
Di-(2-ethylhexyl)phthalate	44.992	2545	3.16
Total	-	-	99.25

The suggestable chemical constituents identified in F11 are presented in Table 4. The major compounds were 3,4-dimethylthioquinoline (37.73%), methyl (1,2-dihydro-2-oxo-1-acenaphthylidene)acetate (19.89%), and α-cyclohexyl-3-furanmethanol (15.28%).

The major compounds presented in F11 (Table 4) was not detected in the acetone extract. The likely reason is that the compounds presented in the acetone extract (Table 3) might have been extracted with miscellaneous solvents during the process of isolation by separatory funnel and purification by column chromatography.

Table 4. The Suggestable Chemical Composition of F11 Wood Extract of *B. vulgaris*

Component	Retention Time (min)	Retention Indices	Area (%)
3,4-dimethylthioquinoline	6.309	832	37.73
α-cyclohexyl-3-Furanmethanol	29.66	1685	15.28
Methyl (1,2-dihydro-2-oxo-1 acenaphthylidene)acetate	31.414	1780	19.89
5,5,8a-trimethyl-4a,5,6,7,8,8a-hexahydro-N-{2',2'-dimethyl-4'-phenyl-1',3'-dioxanyl)pyrrolo[3,4-a]naphthalene	33.549	1885	9.68
Total	-	-	82.58

Even though there have been no reports explicitly about the antioxidant capacity of extracts from wood of *B. vulgaris*, there have been several studies about the antioxidant capacity of different extracts from different parts of *B. vulgaris*. The hexane fraction of the crude methanol extract was the most active fraction, with an IC_{50} value of 72 µg/mL from the shade dried plant material (Kolář *et al.* 2010). Berberine, the active compounds of *B. vulgaris* extract, showed a significant antioxidant abilitiy through a reduction in the concentration of an oxidizing agent having reactive species, especially on hydroxyl and DPPH radicals (El-Sayed *et al.* 2011) and Cannabisin G and (±)–lyoniresinol were responsible for the antioxidant activity root bark extract (Tomoska *et al.* 2008). Recently, Hosseinihashemi *et al.* (2015) found that acetone extract of inner bark of *B. vulgaris* was the most active as an antioxidant agent at 98.61%, and the activity was higher than the value of vitamin C (93.03%) at the concentration of 0.16 mg/mL.

The identified compound; 4-vinylguaiacol (2-methoxy-4-vinylphenol), a simple phenolic compound, was reported as a major component in the ethanolic extract of stem bark from *Zanthoxylum Tetraspermum* (Ravikumar *et al.* 2012), *Dalbergia bariensis* wood (Yang *et al.* 2015), ethanol extracts of whole plant of *Leptadenia reticula* (Rajeswari *et al.* 2014), ethanolic extracts of the whole plant of *Mussaenda rondosa* (Gopalakrishnan *et al.* 2011), methanolic extract of leaves and petioles of *Malva sylvestris* (Tabaraki *et al.* 2012), and in grapes (*Vitis vinifera* L.) (Perestrelo *et al.* 2012).

Previously, the compound 4-vinylguaiacol showed antioxidant, antimicrobial, and anti-inflammatory activity (Ravikumar *et al.* 2012). 2-Methoxy-4-vinylphenol and 2,6-dimetoxy-4-vinylphenol, which are found in coffee (Flament and Bessiere-Thomas 2002), possess antioxidant activity as well as anti-inflammatory activity (Tabaraki *et al.* 2012). 2-methoxy-4-vinylphenol is a possible principle of antioxidant activity of various extracts obtained from the *Trametes versicolor* mushroom (Kamiyama *et al.* 2013). 2-methoxy-4-vinylphenol is component of persimmon peel (*Diospyros kaki* THUNB); it exhibits high antioxidant activity on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the SOD (superoxide dismutase) assay (Fukai *et al.* 2009). Hosseinihashemi *et al.* (2015) found that tetracosanoic acid-methyl ester, followed by phthalic acid-diisooctyl ester were the major compounds in acetone extract of inner bark of *B. vulgaris*; also fraction F11 from the dissolved water:methanol (1:1 *v/v*) partitioned from acetone extract had N-methyl-4-(hydroxybenzyl)-1,2,3,4-tetrahydroisoquinoloine and 9-α-hydroxy-17β-(trimethylsilyl-oxy)-4-anderostene-3-methyloxime as major compounds. Previous reports have shown the isolated compounds from different parts of *B.* vulgaris, *i.e.*, root or stem bark extract had protoberberines, bisbenzyl-isoquinoline alkaloids (berbamine, tetrandrine, chondocurine berberine, (-)-tejedine, jatrorrhizine,

columbamine, berberubine, oxicanthine, palmatine, vitamin C, resin, and tannins, and flavonoids (quercetin and kaempferol) (Damaschin and Analiza şi 2006; Fatehi *et al.* 2005; Akhtar *et al.* 1978; Akhter *et al.* 1979; Dewick 1993; Ivanovska and Philipov 1996; Suau *et al.* 1998; Aghbashlo *et al.* 2008).

CONCLUSIONS

1. The extracts from the wood of *Berberis vulgaris* were evaluated for their antioxidant capacity. The results exhibited moderate antioxidant capacity of *Berberis vulgaris* extracts having values lower than the standard antioxidant compounds (vitamin C and BHT).
2. The major components identified in the acetone extract of the woody stem were 4-vinylguaiacol and D-mannoheptulose. The dissolved water:methanol (1:1 *v/v*) partitioned from acetone extract afforded 12 fractions; among them, fraction F11 was found to have good antioxidant capacity at the concentration of 0.16 mg/mL.
3. The major compounds identified in F11 were 3,4-dimethylthioquinoline, methyl (1,2-dihydro-2-oxo-1-acenaphthylidene)acetate, and α-cyclohexyl-3-furanmethanol.

ACKNOWLEDMENTS

The authors would like to thank Dr. Irani, Dr. Ghare-vaysi, Dr. Mirabi, and Mr. Miri (Research of Islamic Azad University, Ghaemshahr Branch) for the use of their laboratory equipment, and special thanks to Mrs. Azimi (operator of GC/MS apparatus) for helping with the injection of test samples.

REFERENCES CITED

Abd El-Wahab, A. E., Ghareeb, D. A., Sarhan, E. E. M., Abu-Serie, M. M., and El Demellawy, M. A. (2013). "In vitro biological assessment of *Berberis vulgaris* and its active constituent, berberine: Antioxidants, anti-acetylcholinesterase, anti-diabetic and anticancer effects," *BMC Complement Alternat. Med.* 13, 218.

Adams, R. P. (1995). *Identification of Essential oil Components by Gas Chromatography/Mass Spectrometry*, 1st Edition, Allured Publishing Corp., Carol Stream, IL.

Adams, R. P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, 3rd Edition, Allured Publishing Corp., Carol Stream, IL.

Aghbashlo, M., Kianmehr, M. H., and Samimi-Akhijahani, H. (2008). "Influence of drying conditions on the effective moisture diffusivity, energy of activation and energy consumption during the thin-layer drying of Berberis fruit (Berberidaceae)," *Energy Convers. Manag.* 49(10), 2865-2871.

Akhtar, N., Naqvi, H., and Hussain, F. (1978). "Biochemical inhibition (allelopathy) exhibited by *Cenchrus ciliaris* and *Chrysopogon aucheri*," *Pakistan J. For.* 28(4), 194-200.

Akhter, M. H., Sabir, M., and Bhide, N. K. (1979). "Possible mechanism of antidiarrheal effect of berberine," *Indian J. Med. Res.* 70, 233-241.

Amin, G. H. (1991). *Popular Medicinal Plants of Iran*, Health Ministry Press, Tehran, Iran.

Chevallier, A. (2001). *The Encyclopedia of Medicinal Plants: A Practical Reference Guide to More Than 550 Key Medical Plants & Their Uses*, Reader's Digest, Pleasantville, NY.

Ciulei, I., Grigorescu, E., and Stănescu, U. (1993). *Plante Medicinale*, Fitochimie şi Fitoterapie (Medicinal herbs, Phytochemistry and Phytotherapy), vol. I, II, Ed. Medicală, Bucureşti, pp. 384-390.

Cohen-Boulakia, F., Valensi, P. E., Boulahdour, H., Lestrade, R., Dufour-Lamartinie, J. F., Hort-Legrand, C., and Behar, A. (2000). "In-vivo sequential study of skeletal muscle capillary permeability in diabetic rats: Effect of anthocyanosides," *Metabol.* 49(7), 880-885.

Damaschin, N., and Analiza şi, V. V. (2006). *Standardizarea unor forme farmaceutice homeopate. (Standardization of homeopathic medicinal forms)*, Institutul Naþional de Farmacie, Chiºinău, p. 25-32.

Dewick, P. M. (1993). "Isoflavonoids," in: *The Flavonoids. Advances in Research since 1986*, Harborne, J. B. (ed.), Chapman and Hall, London, pp. 117-238.

Dewick, P. M. (2002). *Medicinal Natural Products: A Biosynthetic Approach*, John Wiley & Sons, Chichester, West Sussex, UK.

El-Sayed, M., Ghareeb, D. A., Sarhan, E. M., and Khalil, A. A. (2011). "Therapeutic bio-screening of the bioactive extracted ingredients of *Berberis vulgaris*, berberine," *Func. Plant Sci. Biotechnol.* 5(Special Issue 1), 63-68.

Fatehi, M., Saleh, T. M., Fatehi-Hassanabad, Z., Farrokhfal, K., Jafarzadeh, M., and Davodi, S. (2005). "A pharmacological study on *Berberis vulgaris* fruit extract," *J. Ethnopharm* 102(1), 46-52.

Flament, I., and Bessiere-Thomas, Y. (2002). *Coffee Flavor Chemistry*, John Wiley & Sons, Ltd.: New York.

Freile, M. L., Giannini, F., Pucci, G., Sturniolo, A., Rodero, L., Pucci, O., Balzareti, V., and Enriz, R. D. (2003). "Antimicrobial activity of aqueous extracts and of berberine isolated from *Berberis heterophylla*," *Fitotera.* 74(7-8), 702-705.

Fukai, S., Tanimoto, S., Maeda, A., Fukuda, H., Okada, Y., and Nomura, M. (2009). "Pharmacological activity of compounds extracted from persimmon peel (*Diospyros kaki* THUNB.)," *J. Oleo Sci.* 58(4), 213-219.

Ghareeb, D. A., Abd El-Wahab, A. E., Sarhan, E. E. M., Abu-Serie, M. M., and El Demellawy, M. A. (2013). "Biological assessment of *Berberis vulgaris* and its active constituent, berberine: Antibacterial, antifungal and anti-hepatitis C virus (HCV) effect," *J. Med. Plants Res.* 7(21), 1529-1536.

Gopalakrishnan, S., and Vadivel, E. (2011). "GC-MS analysis of some bioactive constituents of *Mussaenda frondosa* linn," *Inter. J. Pharma. Bio. Sci.* 2(1), 313-320.

Gundogdu, M. (2013). "Determination of antioxidant capacities and biochemical compounds of *Berberis vulgaris* L. fruits," *Advances in Environmental Biology* 7(2), 344-348.

Hosseinihashemi, S. K., Anooshei, H., Aghajani, H., and Salem, M. Z. M. (2015). "Chemical composition and antioxidant activity of extracts from the inner bark of *Berberis vulgaris* stem," *BioRes.* 10(4), 7958-7969.

Hadaruga, D. I., Hadăruga, N. G., Rivis, A., Costescu, C., Ordodi, V. L., and Ardelean, A. (2010). "*Berberis vulgaris* extract/beta-cyclodextrin nanoparticles synthesis and characterization," *Rev. de Chimi.* 61(7), 669-675.

Huffman, M. (2003). "Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants," *Proceed of the Nutri. Soci.* 62(2), 371-381.

Ivanovska, N., and Philipov, S. (1996). "Comparative study on the immunological activity of a series of isoquinoline alkaloids," *Phytothera. Res.* 10(1), 62-65.

Jain, S. R., and Kar, A. (1971). "The antibacterial activity of some essential oils and their combinations," *Planta. Medica.* 20(2), 118-123.

Javadzadeh, S. M., and Fallah, S. R. (2012). "Therapeutic application of different parts *Berberis vulgaris*," *Inter. J. Agric. Crop Sci.* 4(7), 404-408.

Julian, D., and Konig, W. A. (1988). *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*, E. B. Verlag, Hamburg, Germany.

Kamiyama, M., Horiuchi, M., Umano, K., Kondo, K., Otsuka, Y., and Shibamoto, T. (2013). "Antioxidant/anti-inflammatory activities and chemical composition of extracts from the mushroom *Trametes versicolor*," *Inter. J. Nutri. Food Sci.* 2(2), 85-91.

Karau, G. M., Njagi, E. N. M., Machocho, A. K., Wangai, L. N., and Kamau, P. N. (2013). "Phytonutrients, minerals and in vitro antioxidant capacity of leaf and stem bark powders of *Senna spectabilis*," *J. Pharmacog. Phytochem.* 2(2), 51-59.

Kim, T. S., Kang, B. Y., Cho, D., and Kim, S. H. (2003). "Induction of interleukin-12 production in mouse macrophages by berberine, a benzodioxoloquinolizine alkaloid, deviates CD4+ T cells from a Th2 to a Th1 response," *Immun.* 109(3), 407-414.

Kolář, D., Wimmerová, L., and Kádek, R. (2010). "Acetylcholinesterase and butyrylcholinesterase inhibitory activities of *Berberis vulgaris*," *Phytopharma.* 1(1), 7-11.

Končić, M. Z., Kremer, D., Karlović, K., and Kosalec, I. (2010). "Evaluation of antioxidant activities and phenolic content of *Berberis vulgaris* L. and *Berberis croatica* Horvat," *Food Chemi. Toxic.* 48(8-9), 2176-2180.

Kovats, E. (1958). "Characterization of organic compounds by gas chromatography, Part 1. Retention, indices of aliphatic halides, alcohols, aldehydes, and ketones," *Helv. Chimi. Act.* 41(7), 1915-1932.

Kuo, C., Chi, C., and Liu, T. (2004). "The anti-inflammatory potential of berberine in-vitro and in-vivo," *Canc. Lett.* 203(2), 127-137.

Lewis, M. J. (2012). "Natural product screening: Anti-oxidant screen for extracts," Protocol No. 15.3b, Version 2.

Mahata, S., Bharti, A. C., Shukla, S., Tyagi, A., Husain, S. A., and Das, B. C. (2011). "Berberine modulates AP-1 activity to suppress HPV transcription and downstream signaling to induce growth arrest and apoptosis in cervical cancer cells," *Molecul. Canc.* 10, 39.

Mahady, G. B., Pendland, S. L., Stoia, A., and Chadwick, L. R. (2003). "In vitro susceptibility of *Helicobacter pylori* to isoquinoline alkaloids from *Sanguinaria canadensis* and *Hydrastis Canadensis*," *Phytothera. Res.* 17(3), 217-221.

Mahmoudvand, H., Mousavi, S. A. A., Sepahvand, A., Sharififar, F., Ezatpour, B., Gorohi, F., Dezaki, E. S., and Jahanbakhsh, S. (2014). "Antifungal, antileishmanial, and cytotoxicity activities of various extracts of *Berberis vulgaris* (Berberidaceae) and its active principle berberine," *ISRN Pharma.* 2014, 302-436.

Marinova, E. K., Nikolova, D. B., Popova, D. N., Gallacher, G. B., and Ivanovska, N. D. (2000). "Suppression of experimental autoimmune tubulointerstitial nephritis in BALB/c mice by berberine," *Immunopharma.* 48(1), 9-16.

McCartney, A. C. S. (1989). *Practical Medical Microbiology*, 14th Edition, J. G. Collee, A. G. Fraser, B. P. Marmion, and A. Simmons (eds.), Churchill Livingstone, New York, pp. 161-181.

Mei, W. L., Zeng, Y. B., Wu, J., Cui, H. B., and Dai, H. F. (2008). "Chemical composition and anti-MRSA activity of the essential oil from Chinese eaglewood," *J. Chinese Pharmac. Sci.* 17(3), 225-229.

Naf, R., Velluz, A., Busset, N., and Gaudin, J. M. (1992). "New nor-sesquiterpenoids with 10-epi-eudesmane skeleton from agarwood (*Aquilaria agallocha* Roxb.)," *Flav. Frag. J.* 7(6), 295-298.

Özgen, M., Saraçoğlu, O., and Geçer, E. N. (2012). "Antioxidant capacity and chemical properties of selected barberry (*Berberis vulgaris* L.) fruits," *Horticul. Envir. Biotechnol.* 53(6), 447-451.

Parekh, J. N. R., and Chanda, S. (2005). "Preliminary screening of some folkloric plants from Western India for potential antimicrobial activity," *Indian Pharma. J.* 37(6), 408-409.

Perestrelo, R., Caldeira, M., Barros, A. S., Rocha, S. M., and Câmara, J. S. (2012). "Solid phase microextraction as a reliable alternative to conventional extraction techniques to evaluate the pattern of hydrolytically released components in of *Vitis vinifera* L. grapes," *Talant*. 95, 1-11.

Rajeswari, J., and Rani, S. (2014). "GC-MS analysis of whole plant of *Leptadenia Reticulata*," *Inter. J. Pharm. Tech. Res.* 6(7), 2043-2050.

Ravikumar, V. R., Gopal, V., and Sudha, T. (2012). "Analysis of phytochemical constituents of stem bark extracts of *Zanthoxylum tetraspermum* Wight & Arn," *Res. J. Pharma. Biolog. Chem. Sci.* 3(4), 391-402.

Suau, R., Rico, R., Lopez-Romero, J. M., Najera, F., and Cuevas, A. (1998). "Isoquinoline alkaloids from *Berberis vulgaris* subsp. *Australis*," *Phytochem.* 49(8), 2545-2549.

Tabaraki, R., Yosefi, Z., and Gharneh, H. A. A. (2012). "Chemical composition and antioxidant properties of *Malva sylvestris* L.," *J. Res. Agric. Sci*. 8(1), 59-68.

Tomosaka, H., Salim, A. A., Keller, W. J., Chai, H., and Kinghorn, A. D. (2008). "Antioxidant and cytoprotective compounds from *Berberis vulgaris* (barberry)," *Phytothera. Res.* 22(7), 979-981.

Wu, Y., Li, J. Q., Kim, Y. J., Wu, J., Wang, Q., and Hao, Y. (2011). "In vivo and in vitro antiviral effects of berberine on influenza virus," *Chinese J. Integ. Med.* 17(6), 444-452.

Yang, L., Jiang, T., Liu, H., and Li, K. (2015). "Effects of different drying treatments on preservation of organic compounds in *Dalbergia bariensis* wood," *BioRes.* 10(4), 7092-7104.

Zargari, A. (1983). *Medicinal Plants*, Tehran University Press, Tehran, Iran.

Zhou, H., and Mineshita, S. (2000). "The effect of berberine chloride on experimental colitis in rats in vivo and in vitro," *J. Pharma. Experim. Therap.* 294(3), 822-829.

Article submitted: December 23, 2016; Peer review completed: February 21, 2017; Revised version received and accepted: March 25, 2017; Published: March 29, 2017.