

## Antimicrobial and Anti-oxidative Effects of Methanolic Extract of *Dorema aucheri* Boiss.

M. Mianabadi<sup>1\*</sup>, M. Hoshani<sup>1</sup>, and S. Salmanian<sup>2</sup>

### ABSTRACT

*Dorema aucheri* Boiss. (Apiaceae) is an endemic plant growing in Iran. This plant is used as food and its extracts are recommended for the treatment of a wide range of diseases. To conduct this study, leaf, stem, and flower of *D. aucheri* were collected from near Yasouje, Iran. Antioxidant and antimicrobial effects of methanol extracts were measured. Further, total phenolic, flavonoid, anthocyanin, carotenoid, soluble sugar, gallic acid, chlorogenic acid, caffeic acid, and *p*-coumaric acid contents of plant methanol extracts were also determined. The results showed that total phenolic, anthocyanin, and soluble sugar in the stem of *D. aucheri* were 22.72 mg GAE g<sup>-1</sup> dW, 19.33 mg g<sup>-1</sup> dW, and 6.45 mg g<sup>-1</sup> dW respectively, greater than those of the other samples tested. Also, phenolic acids were identified by RP-HPLC and chlorogenic acid was the predominant phenolic compound in the samples. The highest amount of flavonoid (1.95 mg QE g<sup>-1</sup> dW) was observed in the flower. All of the different extracts exhibited a good antioxidant activity based on inhibition of fatty acid oxidation assay. The maximal inhibition was observed in the leaf (48.52%) and flower (54.24%) of *D. aucheri*. Ferric reducing antioxidant power (FRAP) was also high in the stem and flower. In addition, the results showed that leaf, stem, and flower extracts had inhibitory activity against four bacteria tested. The highest antimicrobial activity was obtained with flower extract. These results suggest that the methanolic extracts from different parts of *D. aucheri* are a valuable source of effective compounds. The antioxidant and antimicrobial activity of extracts was correlated with *p*-coumaric and caffeic acid content.

**Keywords:** Antioxidant, Antimicrobial activity, *Dorema aucheri*, Phenolic compounds.

### INTRODUCTION

Antioxidants play an important role in inhibiting oxidation reactions in nutritional, pharmaceutical, and cosmetic compounds and in the prevention of oxidative stress-related diseases (Moure *et al.*, 2001). Use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) during food processing causes unpleasant side effects, among which are cancer and liver enlargement. Therefore, finding natural antioxidants that inhibit chain reaction of free radicals and decelerate lipid oxidation and

protect the human body against diseases is crucial at the present time (Ebrahimabadi *et al.*, 2010). Microbial contamination is another problem that food, beverage, cosmetic, and pharmaceutical industries are faced with. Producers and researchers of food industry are concerned about increase in food-borne diseases by pathogens or their enterotoxins. Various species of *Escherichia*, *Staphylococcus*, *Salmonella*, *Yersinia*, and *Clostridium* lead to gastro-intestinal disorders such as diarrhea and vomiting. Moreover, some of these microorganisms are associated with economic losses through food spoilage (Demirci *et al.*, 2008). Therefore, much

<sup>1</sup> Department of Biology, Faculty of Science, Golestan University, Gorgan, Islamic Republic of Iran.

\* Corresponding author; e-mail: m.mianabadi@gu.ac.ir

<sup>2</sup> Faculty of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Islamic Republic of Iran.



research was conducted to find antimicrobial compounds, especially natural antimicrobial agents, during the last decade. In this regard, plants and herbal products such as essences have been greatly studied (Burt, 2004). Phenolic compounds are a class of plant secondary metabolites that have drawn attention in recent years due to their numerous biological functions. Antimicrobial and anti-oxidative activity of phenolic compounds is proven. Mechanisms poisoning microorganisms through phenolic compounds include adsorption, cell membrane breakdown, reactions with enzymes, and reduction of metal ions necessary for bacteria. Thus, the use of phenolic compounds in food not only protects them and increases their shelf life, but also controls the biological resources-related diseases properly (Majhenic *et al.*, 2007).

*Dorema aucheri* Bioss from Apiaceae family grows in southern provinces of Iran, in the early spring (Zargari, 1997). This is herbaceous, perennial, hair-covered and rooty plant. It has petioles with lining sheath, and stem and lower leaves are trifold or have three leaflets. Its corymb inflorescence is like a simple spherical umbrella without collar or its collar leaves are flimsy which fall very soon. White or yellow flowers are integrated as a wide cluster in inflorescence (Ghahreman, 1993). In the past, gum obtained from the plant was used in traditional medicine. It was used also in preparing local foods, but today it is applied as a condiment. Some believe that the plant extract is beneficial in lowering blood pressure (Wollenweber *et al.*, 1995). Research shows that consumption of *D. aucheri* lowers cholesterol and triglycerides (Hsiao *et al.*, 2003). Studies show that this plant has a protective effect on liver injury (Sadeghi *et al.*, 2007). Moreover, *D. aucheri* is stimulant, antispasmodic, and expectorant and is used in chronic bronchitis and asthma (Zargari, 1997).

Thus, since *D. aucheri* is edible and medicinal plant and the anti-oxidative effects of different parts of the plant have not been reported, we decided to measure some non-enzymatic anti-oxidant compounds and anti-oxidant and antimicrobial properties of methanolic

extract of its organs, in order to allow economic exploitation of the plant.

## MATERIALS AND METHODS

### Materials

Leaves, flowers, and stems of *D. aucheri* were collected from around Yasuj (30° 32', 54" N, 51° 42', 51" E, and altitude of 1,400 m), at flowering (end of June). The plant was identified at the Herbarium of Golestan University. All chemicals and reagents used in this study were of analytical grade and were purchased from Fluka, Merck and Sigma Chemical Companies.

### Preparation of the Plant Extract

Different parts of the plant were dried in the shade and grinded. Later, 1 g of each sample was soaked in 50 mL of 80% methanol and kept at room temperature for 48 hours. Then, the extracts were filtered and the solvent was evaporated on a rotary device at 40°C. The remaining was kept in refrigerator at 4°C for further tests (Pourmorad *et al.*, 2006). The remaining extracts were dissolved in distilled water for the next assays.

### Measurement of Total Phenolic, Flavonoid, Anthocyanin, and Carotenoid

To measure the total phenol content, 2 mL of 2% sodium carbonate, 2.8 mL of distilled water and 100 µL of 50% Folin-Ciocalteu's phenol reagent were added to 100 µL of plant extract. After half an hour, their absorbance was recorded at 720 nm compared to the control. Gallic acid was used as the standard for drawing the standard curve. The total phenolic content of the extract was reported based on milligrams of gallic acid equivalent per gram of dry weight of plant (Meda *et al.*, 2005).

Total flavonoid measurement was performed by adding 1.5 ml of 80% methanol,

100  $\mu$ L of 10% aluminum chloride solution, 100  $\mu$ L of potassium acetate 1M, and 2.8 mL of distilled water to 500  $\mu$ L of each extract. After 40 minutes, absorbance of the mixture was measured at 415 nm compared to the control. Quercetin was used to construct the calibration curve. The total flavonoid content of the extract was reported in milligrams of quercetin per gram of dry weight (Chang *et al.*, 2002).

To measure the total anthocyanin content, 0.02 g of dried plant tissue was pulverized with 4 mL of 1% methanol solution of hydrochloric acid in a porcelain mortar. The resulting solution was kept in the refrigerator for 24 hours. Then, the solution was centrifuged for 10 min at 13,000 $\times$ g. The supernatant was removed and absorbance of the solutions was measured at 530 and 657 nm against the control (1% methanolic solution of hydrochloric acid). Anthocyanin content of each extract was calculated using the following equation (Mita *et al.*, 1997).

$$A = A_{530} - (0.25 \times A_{657})$$

Where, *A* denotes absorbance of the solution (subscripts indicate the wavelength at which the absorbance is measured).

Measurement of total carotenoids was done through homogenization of 0.05 g fresh plant tissue with 5 mL acetone in a porcelain mortar in ice-cold bath. Then, 1 g of anhydrous sodium sulfate was also added to the homogenate and filtered with filter paper. The filtrate was brought to 10 mL volume with acetone and centrifuged at 2,600 $\times$ g for 10 minutes. Supernatant was removed and the solution absorbance was observed at 662, 645, and 470 nm against the control. Carotenoid content of each extract was calculated using the following equations (Lichtenthaler, 1987).

$$C_a 11.24 = A_{662} - 2.04 A_{645}$$

$$C_b 20.13 = A_{645} - 4.19 A_{662}$$

$$C_t 1000 = A_{470} - 1.9 A_{645} - 63.14 C_a / 214$$

Where, *C<sub>a</sub>*: Chlorophyll a level, *C<sub>b</sub>*: Chlorophyll b level, *C<sub>t</sub>*: Total carotenoid level, *A<sub>470</sub>*: Absorbance at 470 nm (for carotenoids), *A<sub>645</sub>*: Absorbance at 645 nm (for chlorophyll a), *A<sub>662</sub>*: Absorbance at 662 nm (for chlorophyll b).

### Determining Phenolic Compounds in Extracts

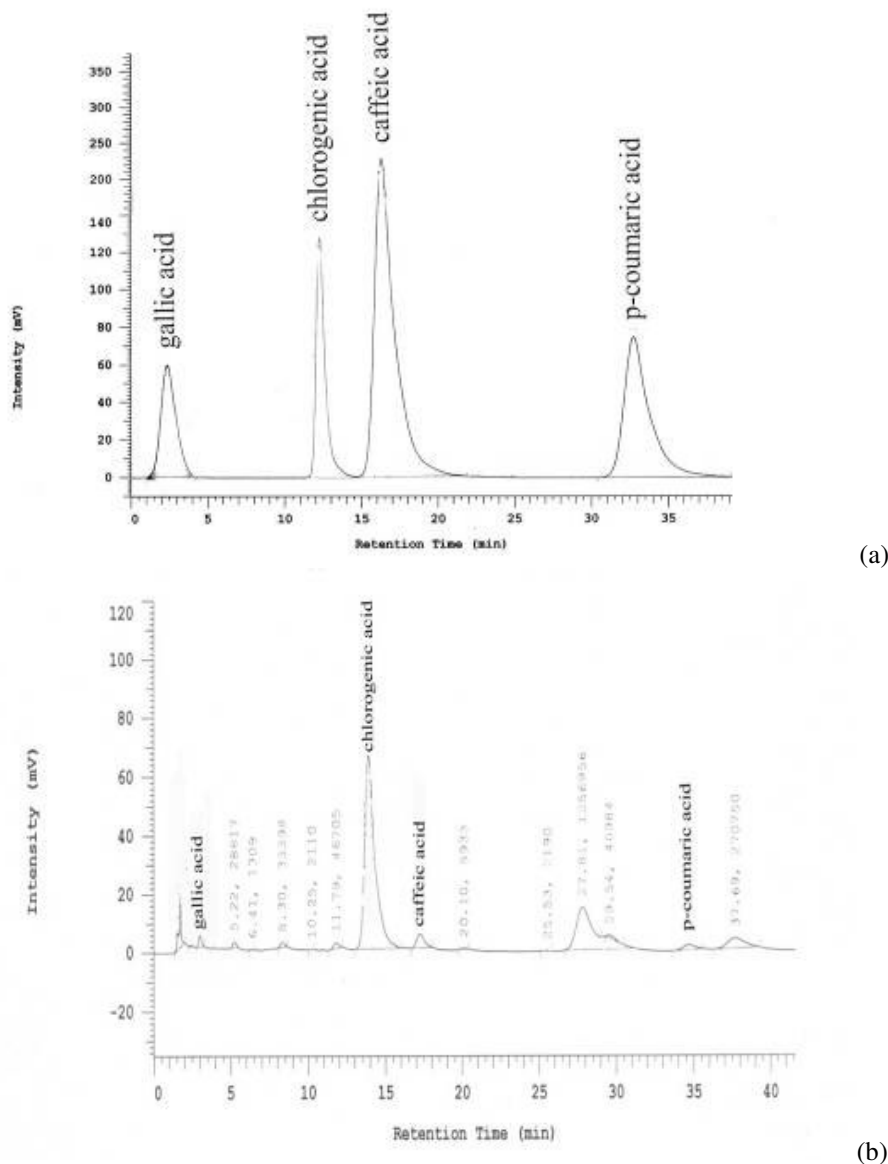
The type of phenolic compounds present in the plant extracts was determined with high performance liquid chromatography (HPLC) (Arabshahi and Urooj, 2007).

### HPLC Analysis

Merck Hitachi HPLC device with a UV/VIS detector and a C18 reversed-phase column of Lichrospher 100 with 4 $\times$ 250 mm dimensions and 5  $\mu$ m particle size were used to determine the phenolic compounds. The mobile phase consisted of water, methanol, and acetic acid (2:18:80) and the flow rate was 1 mL per minute. Chromatography was performed for 40 minutes. Spectra were recorded at 330 or 280 nm. In order to identify the phenolic compounds, gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid were used as standards. Of each compound, 0.125 g was dissolved in 25 mL HPLC grade methanol. Later, 0.2, 0.5, 1, 2, and 8 mL of each solution was diluted to 10 mL. Then, 20 mL of each sample was injected into the HPLC column and the standard curve was plotted to show the surface area of peak against concentration. Then, 1 g of each plant sample was extracted in 50 mL of methanol 80% (48h), the solvent was concentrated to dry at 40 $^{\circ}$ C in a rotary evaporator. The samples were filtered with 0.2  $\mu$ m filter paper and 20  $\mu$ L was injected into HPLC column [Figure 1, (a and b)].

### Measurement of Soluble Sugars

Soluble sugars were measured through phenol-sulfuric acid method (Kochert, 1978). Five mL of 70% ethanol was added to 0.05 g of dry plant in a test tube and was kept in refrigerator for a week. Then, the extracts were centrifuged at 10,000 $\times$ g for 15 minutes at room temperature. Supernatant was used for measurement of soluble sugars. The volume of 0.5 mL plant extract was



**Figure 1.** HPLC chromatogram of (a) Standard phenolic acids and (b) Chromatogram of *D. aucheri* extract.

brought to 2 mL with distilled water in a test tube. Then, 1 ml of 5% phenol and 5 mL of concentrated sulfuric acid were added to each tube. The mixture was well stirred and held at room temperature for 30 minutes. The absorbance of these solutions was measured at 485 nm compared to the control. Glucose was used as the standard to draw the standard curve. Soluble sugar content of the extract was

reported in milligrams per gram of plant dry weight.

### Antioxidant Activity Assays

#### FRAP Assay

In this method, the electron donating feature of antioxidants at low pH leads to

reduction of ferric cation to ferrous. Hence, they can convert colorless ferric tripyridyl triazine complex to blue ferrous tripyridyl triazine which absorbs at 593 nm. In order to measure this feature, 0.1 g of frozen plant tissue was homogenized with 5 mL of distilled water in a porcelain mortar in ice bath. The homogenate was filtered using Whatman filter paper grade No. 1. Then, 50  $\mu$ L of the yielded extract was added to 1.5 mL of FRAP reagent (300 mM sodium acetate buffer pH 3.6, ferric tripyridyl S-triazine 10 mM, and ferric chloride 20 mM). The mixture was vortexed and incubated at 30°C for 4 minutes. Absorbance of the solutions was read at 593 nm against the control (containing 50  $\mu$ L distilled water with 1.5 mL of FRAP reagent). Ferrous ammonium sulfate was used as the control for comparison (Ten *et al.*, 2003).

### $\beta$ -carotene Bleaching Assay (BCB)

Antioxidant activity of *Dorema aucheri* methanolic extracts for preventing oxidation of linoleic acid was studied through  $\beta$ -carotene-linoleic acid method (Kumazawa *et al.*, 2002). An amount of 100  $\mu$ L of the plant extract was diluted with methanol, then 3 mL of  $\beta$ -carotene-linoleic acid reagent was added and the absorbance of the solution was measured at time zero at 470 nm. The solution was incubated in a 50°C water bath for 60 minutes. Then, the absorbance of samples was recorded in the previous wavelength. The control sample contained methanol instead of plant extract. Breakdown rate of  $\beta$ -carotene (DR) and antioxidant activity of extracts (AOX%) were calculated using the following formula:

$$DR = \ln(A_{initial}/A_t)/60$$

$$AOX\% = [(DR_{Control} - DR_{Sample})/DR_{Control}] \times 100$$

Where,  $A_{initial}$  and  $A_t$  are absorbance of samples at baseline and after 60 minutes, respectively.

### Evaluation of Antimicrobial Activity

#### Preparation of Bacterial Strains

The microorganisms used in this study consisted of gram-positive bacteria,

including *Staphylococcus aureus* and *Bacillus cereus* and gram-negative bacteria, including *Escherichia coli*, and *Salmonella enterica*. Pure strains of these bacteria were purchased from the Iranian Center of Industrial Fungi and Bacteria Collection.

#### Determination of Minimum Inhibitory Concentration (MIC)

The MIC of methanolic extract of different parts of *Dorema aucheri* (leaf, stem, and flower) was studied using microbroth dilution. For this purpose, 96-chamber sterile plates were used. The dried plant extract was dissolved in a drop dimethyl sulfoxide and then different concentrations of extracts (0.15-40 mg mL<sup>-1</sup>) were provided through dilution of this solution with Mueller-Hinton broth medium. The day before the test, bacteria were cultured on nutrient agar at 37°C. To prepare bacterial suspension (10<sup>6</sup> cfu mL<sup>-1</sup>), 0.5 dilution of McFarland was used. After filling the wells, microplates were incubated at 37°C for 24 hours, and then their turbidity was read by ELISA reader at 630 nm. The first chamber lacking turbidity was considered as MIC (NCCLS, 2000).

#### Determination of Minimal Bactericidal Concentration (MBC)

Five mL of suspension from the chambers lacking turbidity was transferred to solid medium (Mueller-Hinton agar) and was kept overnight at 37°C. The first concentration lacking growth was considered as MBC (Oroojalian *et al.*, 2010).

### Statistical Analysis

All measurements were repeated 3 times and all data were reported as mean $\pm$ SD. One-way analysis of variance (ANOVA) was used to compare the means. Whenever the difference was significant, Duncan's test



was used for paired comparisons. SPSS software was used to calculate the correlation coefficient between characteristics. The results with a  $P < 0.05$  were considered significant.

## RESULTS

### Total Phenolic, Flavonoid, Anthocyanin, Soluble Sugar and Carotenoid

Comparison of the data on total phenol, total anthocyanin, and total soluble sugar in methanol extracts of different parts of *D. aucheri* showed that the plant stem had the highest total phenolic, total anthocyanin, and total soluble sugar at a level of  $22.72 \pm 0.851$  mg GAE  $g^{-1}$  dW, and  $19.33 \pm 0.616$  and  $6.45 \pm 0.013$  mg  $g^{-1}$  dW, respectively (Table 1). The results of flavonoid content measurement in *D. aucheri* extract showed that total flavonoid content was lowest in the stem and the highest in the flower extract. The results also revealed that the highest levels of gallic and chlorogenic acid were observed in *D. aucheri* stem, while the highest level of caffeic acid and *p*-coumaric acid were obtained in the flower extract

(Table 2). Comparison of means (Table 1) showed that the highest amount of carotenoids was observed in the leaf ( $1.58 \pm 0.07$  mg  $g^{-1}$  dW).

### Ferrous Reducing Antioxidant Power

The results of the FRAP assay showed that different parts of *D. aucheri* had a significant effect on anti-oxidative activity, so that the highest anti-oxidative activity was observed in the stem and flower of *D. aucheri*, yielding to  $4.33 \pm 0.079$  and  $4.22 \pm 0.114$  mM ferrous ion per gram of dry tissue, respectively (Figure 2-a).

### Inhibition of Lipid Peroxidation (BCB)

Comparative analysis showed that the impact of different parts of *D. aucheri* on inhibition of lipid oxidation was significant. The results revealed good anti-oxidative activity of all extracts, so that the highest percentage of inhibition of lipid peroxidation belonged to leaf and flower of *D. aucheri* (Figure 2-b).

**Table 1.** The amount of non-enzymatic antioxidant compounds in leaf, stem and flower of *D. aucheri*.<sup>a</sup>

Plant samples	Total phenol (mg GAE $g^{-1}$ dW)	Total flavonoid (mg QE $g^{-1}$ dW)	Total anthocyanin (mg $g^{-1}$ dW)	Total carotenoid (mg $g^{-1}$ dW)	Soluble sugar (mg $g^{-1}$ dW)
Leaf	$0.85^c \pm 8.36$	$1.62 \pm 0.099^b$	$1.49^c \pm 8.72$	$0.07^a \pm 1.15$	$1.88 \pm 0.017^b$
Stem	$0.537^a \pm 22.72$	$0.014^c \pm 1.024$	$0.616^a \pm 19.33$	$0.001^b \pm 0.66$	$6.45 \pm 0.113^a$
Flower	$0.55^b \pm 13.1$	$0.013^a \pm 1.95$	$0.581^b \pm 16.65$	$0.002^b \pm 0.736$	$1.53 \pm 0.014^c$

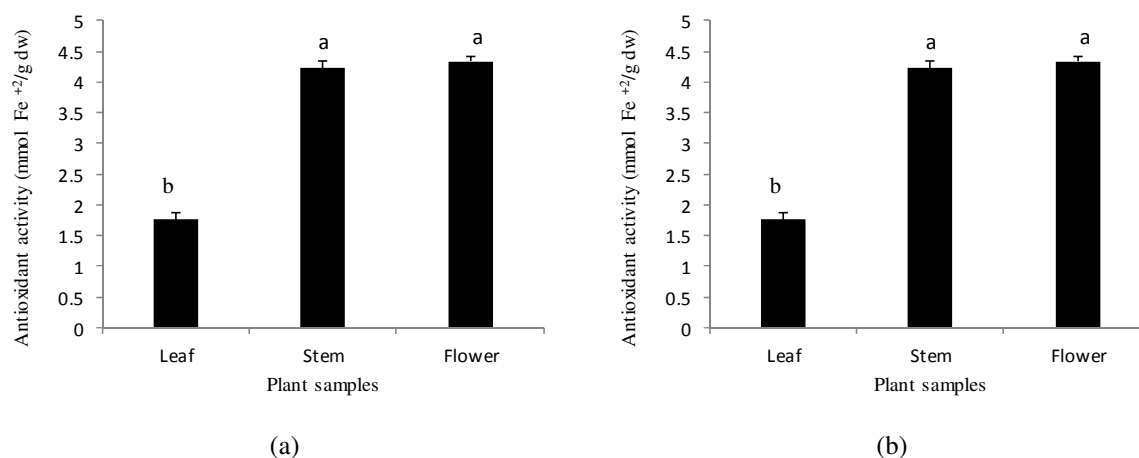
<sup>a</sup> Data are expressed as means  $\pm$  SD. Similar upper case letters indicate no significant differences (Duncan test,  $P < 0.05$ ).

**Table 2.** The amounts of phenolic compounds in Leaf, Stem and Flower of *D. aucheri*.<sup>a</sup>

Plant samples	Gallic acid (mg $g^{-1}$ dW)	Chlorogenic acid (mg $g^{-1}$ dW)	Caffeic acid (mg $g^{-1}$ dW)	<i>p</i> -coumaric acid (mg $g^{-1}$ dW)
Leaf	$0.174 \pm 0.002^c$	$1.14 \pm 0.019^b$	$0.055 \pm 0.0008^c$	$0.245 \pm 0.0001^b$
Stem	$0.663 \pm 0.0001^a$	$19.45 \pm 0.0005^a$	$0.767 \pm 0.000007^b$	*0.0
Flower	$0.195 \pm 0.0004^b$	$0.462 \pm 0.004^c$	$1.65 \pm 0.002^a$	$0.528 \pm 0.005^a$

<sup>a</sup> Data are expressed as means  $\pm$  SD. Similar upper case letters indicate no significant differences (Duncan test,  $P < 0.05$ ).

\* Stem of *D. aucheri* lacks *p*-coumaric acid.



**Figure 2.** Antioxidant activity of *D. aucheri* extracts by (a) FRAP method (b) BCB method. Data are expressed as means $\pm$ SD. The similar letters above the bars indicate no significant differences (Duncan's test  $P < 0.05$ ).

### Antimicrobial Activity

Antimicrobial activity of methanolic extracts of flower, leaf, and stem of *D. aucheri* was studied on some pathogenic bacteria including *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus*. The tested bacteria were those causing food contamination and spoilage, and food poisoning. The results are shown in Table 3 as MIC. Despite antimicrobial activity of all extracts, different microorganisms showed various responses. In general, gram-positive bacteria were more sensitive than gram-negative bacteria. Extracts did not show any inhibitory effect on *E. coli*. Among the studied bacteria, *Bacillus cereus* was more sensitive to extracts and flower extracts showed the highest antimicrobial activity.

### DISCUSSION

The most important factor which affects the anti-oxidative activity of phenolic compounds is their chemical structure. Ability to donate electron or hydrogen, form complexes with metals and antiradical activity of these compounds are linked to their hydroxyl groups in aromatic ring and their positions. In addition, the presence of other groups such as acetyl and methoxyl and their positions relative to hydroxyl groups are also important factors in the evaluation of anti-oxidative activity of phenolic compounds (Sroka and Cisowki, 2003). Chlorogenic acid, *p*-coumaric acid, and caffeic acid are derivatives of cinnamic acid, and their difference from hydroxy

**Table 3.** Minimum inhibitory concentration (MIC) of leaf, stem and flower extracts.

Bacteria	PTCC no.	Bacteria type	MIC		
			Leaf extract (mg ml <sup>-1</sup> )	Stem extract (mg ml <sup>-1</sup> )	Flower extract (mg ml <sup>-1</sup> )
<i>Bacillus cereus</i>	11778	G <sup>+</sup>	10	20	10
<i>Staphylococcus aureus</i>	1112	G <sup>+</sup>	20	40	20
<i>Escherichia coli</i>	1330	G <sup>-</sup>	–	–	–
<i>Salmonella enterica</i>	1639	G <sup>-</sup>	40	40	20



benzoic acid derivatives (including gallic acid) is the presence of a carboxyl group in their structure. Antioxidative activity of cinnamic acid is generally greater than hydroxy benzoic acid. The antioxidative power of caffeic acid is related to the presence of two hydroxyl groups in its structure, and *p*-coumaric acid, which has a hydroxyl group, can play an important role in scavenging free radicals, while its antioxidative power is lower than caffeic acid (Andreasen *et al.*, 2001).

Antimicrobial activity of extracts showed that the methanolic extract of *D. aucheri* flowers had the highest antimicrobial properties compared with other parts (Table 3). Antimicrobial activity of the flower extract can be attributed to the high amounts of phenolic compounds, such as *p*-coumaric and caffeic acid. These phenolic acids also increased the anti-oxidative activity of the flower determined by FRAP method (Table 4). Gram-negative bacteria have a peptidoglycan layer over the outer membrane of their cell wall. Hydrophilic surface of this membrane, rich in lipopolysaccharide molecules, operates as a barrier against antibiotics. Moreover, enzymes in the periplasmic space can break down incoming molecules from outside, but in gram-positive bacteria, antimicrobial agents easily destroy the cell wall and cytoplasmic membrane and lead to cytoplasmic leakage and cell lysis (Duffy and Power, 2001; Negi *et al.* 2003). This is why the MIC of plant methanol extracts on gram-negative bacteria more than gram-positive bacteria. Other mechanisms include damage to enzymes involved in energy production and cell structure compounds, and inactivation of genetic materials (Kotzekidou *et al.*, 2008). The number and position of hydroxyl groups is the key factor in the antimicrobial activity of phenolic compounds. With the increase in the number of hydroxyl groups, the antimicrobial activity increases as well (Cowan, 1999). Antimicrobial activity of flavonoids results from the ability to form complexes with bacterial cell wall, preventing the growth of

**Table 4.** Statistical analysis for correlation between the parameters evaluated in leaf, stem and flower of *D. aucheri*.

	Total phenol content	Total flavonoid content	Total anthocyanin content	Total carotenoid content	<i>P</i> -coumaric acid content	Caffeic acid content	Chlorogenic acid content	Gallic acid content	Soluble sugar content	Antioxidant activity (BCB)	Antioxidant activity (FRAP)	Antimicrobial activity
Antimicrobial activity	0.588*	0.537*	0.158 <sup>ns</sup>	-0.127 <sup>ns</sup>	0.579*	0.581*	0.281 <sup>ns</sup>	<sup>ns</sup> 0.126	0.48 <sup>ns</sup>	0.311 <sup>ns</sup>	0.531*	1
Antioxidative activity (FRAP)	0.738**	0.595*	**0.937	-0.820**	0.579*	0.850**	0.437 <sup>ns</sup>	*0.531	0.405 <sup>ns</sup>	-0.246 <sup>ns</sup>	1	
Antioxidant activity (BCB)	-0.711**	-0.776**	-0.405 <sup>ns</sup>	0.583*	0.850**	0.240 <sup>ns</sup>	-0.847**	-0.833**	-0.839**	1		

\*\* Correlation is significant at 0.01 levels; \* Correlation is significant at 0.05 levels; <sup>ns</sup> Correlation is not significant.



microorganisms. To apply their antimicrobial activity, phenolic compounds inhibit enzyme activity through reacting with their sulfhydryl groups or through nonspecific interactions with protein. With respect to antibacterial mechanism of plant extracts, it is shown that polyphenols are capable of forming heavy soluble complexes with proteins, thus binding to bacteria and destroying bacterial cell surface acceptors. Tannins form irreversible complexes with proline-rich proteins that lead to inhibition of cell wall protein synthesis. Consequently, using natural products as antibacterial substances is a proper method for controlling pathogenic bacteria and increasing food shelf life (Almajano *et al.*, 2008).

Comparison of findings of different studies seems complicated because the results are affected by factors such as incubation temperature and time, pH and type of culture medium, microbial growth phase, and volume of medium. Also, chemical composition, type, and mechanism of action of phenolic compounds of each extract are among factors which make difference in the results of the antimicrobial activity of the solvents (Wen *et al.*, 2003). Further, Kilani *et al.* (2005) and Salmanian *et al.* (2014) have pointed out that the antibacterial activity of *Cyperus rotundus* and *Crataegus elbursensis* were due to presence of phenolic compounds found in these plants. Antimicrobial activity of oak (*Quercus brantii*) shell was attributed to the presence of tannin compounds (Khosravi and Behzadi, 2006). Shahidi *et al.* (2004) studied the antimicrobial activity of methanolic extract of another species of oak (*Quercus acerifolia*) at a concentration of 20 mg mL<sup>-1</sup>. But, they reported no inhibition zone for *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli* (lack of antimicrobial activity of extracts). On the basis of investigation on the antimicrobial activity of various European teas, some polyphenols of tea extract demonstrated antimicrobial effects (Almajano *et al.*, 2008). In fact,

extracts with high antioxidant activity showed high antimicrobial activity and such antimicrobial activities were less attributed to the total amount of the extracts' polyphenols. In general, gram-negative bacteria are more resistant than gram-positive bacteria to herbal polyphenols, probably due to the different chemical composition of their cell wall (Negi *et al.*, 2003).

## CONCLUSIONS

The results of the study show that *D. aucheri* contains significant amounts of antioxidant compounds such as total phenolic, total flavonoids, total anthocyanins, *etc.* The presence of phenolic acid compounds such as gallic, caffeic, chlorogenic, and *p*-coumaric acid are associated with various anti-oxidative and antimicrobial activity of *D. aucheri* extracts. Therefore, the methanolic extract of *D. aucheri* flower had acceptable antimicrobial effect on *Bacillus cereus* and *Staphylococcus aureus* which were correlated with its high level of polyphenols.

## REFERENCES

1. Almajano, M. P., Carbo, R., Jimenez, J. A. and Gorden, M. H. 2008. Antioxidant and Antimicrobial Activities of Tea Infusions. *Food Chem.*, **108**: 55-63.
2. Andreasen, M. F., Landbo, A. K., Christensen, L. P., Hansen, A. and Meyer, A. S. 2001. Antioxidant Effects of Phenolic Rye (*Secale cereale* L.) Extracts, Monomeric Hydroxyl Cinnamates, and Ferulic acid Dehydrodimers on Human Low Density Lipoproteins. *J. Agric. Food Chem.*, **49**: 4090-4096.
3. Arabshahi, S. and Urooj, A. 2007. Antioxidant Properties of Various Solvent Extracts of Mulberry *Morus indica* L. Leaves. *Food Chem.*, **102**: 1233-1240.
4. Burt, S. 2004. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods: A Review. *Int. J. Food Microbiol.*, **94**: 223-253.



5. Chang, C., Yang, M., Wen, H. and Chern, J. 2002. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. *Food Drug Anal.*, **10**: 178-182.
6. Cowan, M. M. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Review*, **12**: 564- 582.
7. Demirci, F., Guven, K., Demirci, B., Dadandi, M. Y. and Baser, K. H. C. 2008. Antibacterial Activity of Two Phlomis Essential Oils against Food Pathogens. *Food Control*, **19**: 1159-1164.
8. Duffy, C. F. and Power, R. F. 2001. Antioxidant and Antimicrobial Properties of Some Chinese Plant Extracts. *Int. J. Antimicrob. Ag.*, **17**: 527-529.
9. Ebrahimabadi, A. H., Ebrahimabadi, E. H., Jafari-Bidgoli, Z., Jookar Kashi, F., Mazoochi, A. and Batooli, H. 2010. Composition and Antioxidant and Antimicrobial Activity of the Essential Oil and Extracts of *Stachys inflata* Benth from Iran. *Food Chem.*, **119**: 452-458.
10. Ghahreman, A. 1993. *Iranian Chromophytes*. First Edition, University Press, PP. 668-769.
11. Hsiao, G., Shen, M. Y. and Lin, K. H. 2003. Antioxidant and Hepatoprotective Effect of *Andropogon camphoratus* Extract. *J. Agric. Food Chem.*, **51**: 3302-3308.
12. Khosravi, A. D. and Behzadi, A. 2006. Evaluation of the Antibacterial Activity of the Seed Hull of *Quercus branti* on Some Gram Negative Bacteria. *Pak. J. Med. Sci.*, **22**: 429-432.
13. Kilani, S., Abdelwahed, A., Ammar, R. B., Hayder, N., Ghedira, K., Chraief, I., Hammami, M. and Chekir-Ghedira, L. 2005. Chemical Composition, Antibacterial and Antimutagenic Activities of Essential Oil from (Tunisian) *Cyperus rotundus*. *J. Essent. Oil Res.*, **17**: 695-700.
14. Kochert, A. 1978. Carbohydrate Determination by Phenol-sulfuric Acid Method. In: "Handbook of Physiology and Biochemical Methods", (Eds.): Hellebust, J. A. and Craige, J. S.. Cambridge University Press, London, PP. 95-97.
15. Kotzekidou, P., Giannakidis, P. and Boulamatsis, A. 2008. Antimicrobial Activity of Some Plant Extracts and Essential Oils against Foodborne Pathogens *In vitro* and on the Fate of Inoculated Pathogens in Chocolate. *LWT*, **41**: 119- 127.
16. Kumazawa, S., Taniguchi, M., Suzuki, Y., Shimura, M., Kwon, M. and Nakayama, T. 2002. Antioxidant Activity of Polyphenols in Carob Pods. *Agric. Food Chem.*, **50**: 373-377.
17. Lichtenthaler, H. K. 1987. Chlorophylls and Carotenoids; Pigments of Photosynthetic Membranes. *Methods Enzymol.*, **148**: 350-382.
18. Majhenic, L., Skerget, M. and Knez, Z. 2007. Antioxidant and Antimicrobial Activity of Guarana Seed Extracts. *Food Chem.*, **104**: 1258-1268.
19. Meda, A., Lamien, C. E., Romito, M., Millogo, J. and Nacoulma, O. G. 2005. Determination of the Total Phenolic, Flavonoid and Pralin Contents in Burkina Faso Honey, as well as Their Scavenging Activity. *Food Chem.*, **91**: 571-577.
20. Mita, S., Murano, N., Akaike, M. and Nakamura, K. 1997. Mutants of *Arabidopsis thaliana* with Pleiotropic Effects on the Expression of the Gene for Beta-amylase and on the Accumulation of Anthocyanin Those are Inducible by Sugars. *Plant J.*, **11**: 841-851.
21. Moure, A., Cruz, J. M., Franco, D., Dominguez, M., Sineiro, J. and Dominguez, H. 2001. Natural Antioxidants from Residual Sources. *Food Chem.*, **72**: 145-171.
22. NCCLS: National Committee for Clinical Laboratory Standards. 2001. *Performance Standards for Anti-microbial Susceptibility Testing: Eleventh Informational Supplement*. Document M 100-S11, National Committee for Clinical Laboratory Standard, Wayne, PA, USA.
23. Negi, P. S., Jayaprakasha, G. K. and Jena, B. S. 2003. Antioxidant and Antimutagenic Activities of Pomegranate Peel Extracts. *Food Chem.*, **80**: 393-397.
24. Oroojalian, F., Kasra-Kermanshahi, R., Azizi, M. and Bassami, M. R. 2010. Phytochemical Composition of the Essential Oils from Three *Apiaceae* Species and Their Antibacterial Effects on Food-borne Pathogens. *Food Chem.*, **120**: 765-770.
25. Pourmorad, F., Hosseinimehr, S. J. and Shahabimajd, N. 2006. Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medicinal Plants. *Afr. J. Biotechnol.*, **5**: 1142-1145.
26. Sadeghi, H., Ghaitasi, I., Mazrooghi, N., and Sabzali, S. 2007. The Hepatoprotective Effects of *Dorema aucheri* on Carbon

- Tetrachloride Induced Liver Damage in Rats. *J. Shahrekord University. Med. Sci.*, **6(1)**: 38-43. (in Persian)
27. Salmanian, S., Sadeghi Mahoonak, A. R., Alami, M. and Ghorbani, M. 2014. Phenolic Content, Antiradical, Antioxidant, and Antibacterial Properties of Hawthorn (*Crataegus elbursensis*) Seed and Pulp Extract. *J. Agr. Sci. Tech.*, **16**: 343-354.
28. Shahidi, B. G., Aghighi, S. and Karimi Nik, A. 2004. Antibacterial and Antifungal Survey in Plants used in Indigenous Herbal-medicine of South East Regions of Iran. *J. Biol. Sci.*, **4**: 405-412.
29. Sroka, Z. and Cisowski, W. 2003. Hydrogen Peroxide Scavenging, Antioxidant and Antiradical Activity of Some Phenolic Acids. *Food Chem. Toxicol.*, **41**: 753-758.
30. Ten, M., Szentmihaly, K., Sarkozi, A. and Varga, I. S. 2003. Examination on Antioxidant Activity in Greater Celandine (*Chelidonium majus* L.) Extracts by FRAP Method. *Acta Biol.*, **47**: 115-117.
31. Wen, A., Delaquis, P., Stanich, K. and Toivonen, P. 2003. Antilisterial Activity of Selected Phenolic Acids. *Food Microbiol.*, **56**: 305-311.
32. Wollenweber, E., Dorr, M. and Rustaiyan, A. 1995. *Dorema aucheri* the First Umbelliferous Plant Found to Produce Exudates Flavonoids. *Phytochem.*, **38(6)**: 1417-1427.
33. Zargari, A. 1997. *Pharmaceutical Plants*. Tehran University Press, PP. 212-221. (in Persian).

### فعالیت ضد میکروبی و آنتی اکسیدانی عصاره متانولی بیلهر *Dorema aucheri* Boiss.

م. میان آبادی، م. هوشنی، ش. سلمانیان

#### چکیده

گیاه بیلهر با نام علمی *Dorema aucheri* Boiss. (خانواده جعفری) به صورت بومی در ایران می‌روید. این گیاه دارای ارزش غذایی بوده و عصاره آن برای درمان بیماری‌های متعددی توصیه می‌شود. برای انجام این مطالعه، برگ، ساقه و گل گیاه بیلهر از حوالی شهر یاسوج، ایران جمع‌آوری شد و اثرات آنتی‌اکسیدانی و ضد میکروبی عصاره متانولی آن اندازه‌گیری شد. همچنین، میزان فنل کل، فلاونوئید کل، آنتوسیانین، کاروتنوئید، قند محلول، گالیک اسید، کلروژنیک اسید و p-کوماریک اسید عصاره متانولی گیاه نیز اندازه‌گیری شد. نتایج نشان داد که میزان فنل کل، آنتوسیانین و قند محلول عصاره ساقه بیلهر از سایر نمونه‌ها بیشتر بود و به ترتیب  $۱۹/۳۳ \text{ mg/g dw}$ ،  $۲۲/۷۲ \text{ mg GAE/g dw}$  و  $۶/۵۴ \text{ mg/g dw}$  بدست آمد. اسیدهای فنلی عصاره‌ها توسط RP-HPLC بررسی گردید، و مشخص شد که کلروژنیک اسید ترکیب فنلی غالب نمونه‌ها است. بالاترین مقدار فلاونوئید ( $۱/۹۵ \text{ mg QE/g dw}$ ) در گل مشاهده شد. تمامی عصاره‌ها فعالیت آنتی‌اکسیدانی خوبی در سنجش مهار اکسیداسیون اسید چرب از خود نشان دادند، بیشترین اثر مهار در برگ ( $۴۸/۵۲\%$ ) و گل ( $۵۴/۲۴\%$ ) بیلهر مشاهده شد. قدرت احیاکنندگی آهن (FRAP) نیز در ساقه و گل بالا بود. علاوه بر این، نتایج نشان داد که عصاره‌های برگ، ساقه و گل فعالیت مهار قابل ملاحظه‌ای بر علیه چهار باکتری مورد بررسی از خود نشان دادند. بیشترین فعالیت ضد میکروبی در عصاره گل مشاهده شد. نتایج نشان داد که عصاره‌های



متانولی بخش‌های مختلف گیاه بیلهر منبع غنی از ترکیبات موثر است. فعالیت آنتی‌اکسیدانی و ضد میکروبی عصاره‌ها با محتوای p- کوماریک اسید و کافئیک اسید همبستگی دارد.