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Antibacterial and Antioxidant Properties of Methanolic Extracts of Some Native Edible Plants Collected from Kermanshah, Western Iran

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HIGHLIGHTS

- The most antibacterial effectiveness was found for *Mentha longifolia* extract.
- Gram-negative bacteria were more resistant to the presence of methanolic plant extracts than Gram-positive bacteria.
- The highest antioxidant activity was found in *M. longifolia* extract.
- *Falcaria vulgaris*, *Allium rotundum*, *Tragopogon graminifolius*, and *M. longifolia* can be used for food preservation.

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Acronyms and abbreviations
BHT=Butylated Hydroxytoluene
BHI=Brain Heart Infusion
MIC=Minimum Inhibitory Concentration
MBC=Minimum Bactericidal Concentration
CFU=Colony Forming Unit
DPPH=2, 2-diphenyl-1-picrylhydrazyl hydrate

ABSTRACT

Background: There is growing demand to improve physicochemical, microbiological, and sensory properties of fresh foods using natural herbal antimicrobial and antioxidant compounds. The aim of the present study was to investigate antioxidant and antibacterial properties of some native edible plants of Kermanshah, Western Iran.

Methods: The methanolic extracts of leaves of *Falcaria vulgaris*, *Allium rotundum*, *Tragopogon graminifolius*, and *Mentha longifolia* plants were prepared. The antibacterial effects of these four plant extracts were determined on *Salmonella typhimurium*, *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 using micro-broth dilution and agar disk diffusion assays. Also, the 2, 2-diphenyl-1-picrylhydrazyl hydrate assay was used for determination of antioxidant properties of the plant extracts. The analysis was performed using SPSS 16.0 (Chicago, IL, USA) software package.

Results: The most antibacterial effectiveness was significantly (p<0.05) found for *M. longifolia* extract. The following sequence inhibition effect on investigated bacterial strains was observed: *M. longifolia*>*T. graminifolius*>*A. rotundum*>*F. vulgaris*. Moreover, Gram-negative bacteria were more resistant to the presence of methanolic plant extracts than Gram-positive bacteria. The highest antioxidant activity (based on IC_{50}) was significantly (p<0.05) found for *M. longifolia* (0.88±0.12 mg/ml); as well as these rates for *T. graminifolius*, *A. rotundum*, and *F. vulgaris* extracts were 0.45±0.78, 0.26±0.07, and 0.14±0.23 mg/ml, respectively.

Conclusion: The studied edible plants had antimicrobial and antioxidant activities that recommend as potential preservatives in food products. However, methanolic extract of *M. longifolia* had the best antibacterial and antioxidant properties *in vitro*.

Introduction

It has been reported that garlic, onion, basil, cinnamon, curry, mustard, ginger, and also other spices exhibit antimicrobial and antioxidant properties (Alzoreky and Nakahara, 2003; Benkeblia, 2004; Hussain et al., 2008; Park et al., 2008; Sakunpak and Panichayupakaranant, 2012). Also, antimicrobial and antioxidant compounds derived from natural sources like plants have been tradi-

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tionally used for treatment of various diseases especially cancers as well as gastroenteritis (Runyoro et al., 2010). Epidemiological studies have indicated that there is a remarkable positive association between the high intake of fresh fruits and vegetables and a reduction rate of heart disease, mortality, different types of cancers, and other degenerative diseases (Conforti et al., 2009; Pal et al., 2012; Schinella et al., 2002; Shahbazi et al., 2015; Yang et al., 2016).

Food-borne diseases have serious negative effects on public health and food security (Shahbazi and Shavisi, 2016). Moreover, there is growing demand to improve physicochemical, microbiological, and sensory properties of fresh foods using natural herbal antimicrobial and antioxidant compounds (Shahbazi et al., 2016). The use of antioxidants in food owing to their ability to control fat oxidation as well as increase the shelf life of the food is highly appreciated in food industries. butylated hydroxyanisole and Butylated Hydroxytoluene (BHT) are the most common synthetic antioxidants which is used in food products (Shahbazi et al., 2015). Despite the effectiveness and good stability, the use of these compounds due to their toxicity is limited in modern preservation methods (Alzoreky and Nakahara, 2003). Therefore, a particular tendency to use a healthy compounds such as edible plants and vegetables in food has arisen (Pal et al., 2012). Because of strong antioxidant and antimicrobial activities which are related to high levels of flavonoids and phenolic acid, some edible plants and vegetables are recommended as potential additives for food preservation (Sakunpak and Panichayupakaranant, 2012).

Previous studies indicated that extracts of various edible plants have strong antibacterial and antioxidant activities in food models as well as *in vitro* (Asekun et al., 2007; Gulluce et al., 2007; Jaberian et al., 2013; Mkaddem et al., 2009; Monfared et al., 2012; Shafaghat, 2010). However, a study on the antibacterial and antioxidant activities of *Falcaria vulgaris* (Paghazeh), *Allium rotundum* (Sir-e-Kouhi), *Tragopogon graminifolius* (Sheng), and *Mentha longifolia* (Pouneh) collected from Kermanshah, West of Iran has not been carried out so far. Therefore, the aim of the present study was to investigate antioxidant and antibacterial properties of methanolic extracts of some native edible plants of Kermanshah, Western Iran.

Materials and methods

Collection of plant materials

The fresh leaves of *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and *M. longifolia* plants were collected from Kermanshah, West of Iran during full flowering period (March to April 2016). The fresh leaves were air-dried in a shadow at 25±1 ºC for 14 days. Authentications of the plants were done by Faculty of Agriculture, Razi University, Kermanshah, Iran. The voucher numbers of *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and *M. longifolia* plants were 7163, 87981, 9160, and 3289, respectively.

Preparation of plant extracts

Five g of each fine-powdered plant was dissolved in 20 ml methanol and extracted with a shaker at 25±1 ºC for 24 h. The extract was then filtered by Whatman filter paper no. 3, concentrated in a rotary evaporator (IKA, Germany) at 40 ºC and stored at refrigerator (4±1 ºC) till next analysis (Motamed and Naghibi, 2010).

Preparation of bacterial strains

Salmonella typhimurium (ATCC 14028), *Bacillus subtilis* (ATCC 6633), *B. cereus* (ATCC 11774), *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 19118), and *Escherichia coli* O157:H7 (ATCC 10536) were obtained from the culture collection of the Iranian Research Organization for Science and Technology, Tehran, Iran. The standard bacterial strains were incubated at 37 °C for 24 h after inoculation in Brain Heart Infusion (BHI) broth medium, adjusted to a final density of 10^5 Colony Forming Unit (CFU)/ml as an inoculum dose (Carović-Stanko et al., 2010).

Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC)

The MIC values of methanolic plant extracts were determined using micro-broth dilution assay. Different concentrations of extracts ranging from 0.5 to 10 mg/ml were set up by serial dilution in BHI (Merck, Darmstadt, Germany) broth containing dimethyl sulfoxide (0.5% v/v). The 96-well sterile micro-titer plates were prepared by dispensing 180 µl BHI broth medium containing specified concentrations of the antibacterial agents and 20 µl bacterial inoculum (5 log CFU/ml) into each well. BHI broth containing inoculum without the analyzed materials (as positive control) and BHI broth containing the analyzed materials (as negative control) were evaluated in the last wells of each strip. The microplates were shaked at 300 rpm for 20 s and incubated at 37 °C for 24 h. The lowest concentration that completely inhibited the growth of microorganisms was defined as MIC value. To determine MBC, 20 µl of the content of each well with no invisible growth was sub-cultured on BHI (Merck, Darmstadt, Germany) agar and incubated at 37 °C for 24 h. The concentration of each extract in those wells that

yielded plates with no visible colonies was considered to be the MBC (Alves-Silva et al., 2013; Carović-Stanko et al., 2010; Shahbazi and Shavisi, 2016).

Agar disk diffusion assay

For agar disk diffusion assay, 0.1 ml of each bacterial suspension (10^8 CFU/ml) was uniformly spread on BHI agar medium using a sterile cotton swab. Then, the sterile paper disks (6 mm in diameter) incorporated with 10 µl of the highest concentration of each plant extracts (10 mg/ml) was placed on the surface of each BHI agar. The plates were incubated for 24 h at 37 °C. In order to evaluate antibacterial activity, the diameter of inhibition zone was measured, then disk diameter (6 mm) was deducted and the inhibition zone was reported as πr^2 (π= 3.14 and r=radius of inhibition zone which described previously by Ghasemi Pirbalouti et al. (2016).

Antioxidant analysis of methanolic plant extracts

The 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay was used for determination of antioxidant properties of plant extracts. Stock solutions (100 mg/ml) of each plant extract as well as BHT (Merck, Darmstadt, Germany) as the synthetic standard antioxidant were prepared in methanol. Selected diluted concentrations were mixed to 1 ml DPPH methanol solution. The mixtures were then shaken and allowed to stand for about 30 min at 25±1 °C in the dark. Tests were conducted for investigated extracts and BHT (as synthetic antioxidant). The ultraviolet absorbencies of these solutions were measured at 517 nm using visible light spectrophotometer. Finally, the DPPH radical scavenging activity was evaluated as follow (Wannes et al., 2010):

$$DPPH\ (I\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} is the absorbance of the blank sample and A_{sample} is the absorbance of the plant extract. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the curve of radical scavenge activity (I%) against sample concentration.

Statistical analysis

All experiments were carried out in triplicate. The analysis was performed using SPSS 16.0 for Windows (Chicago, IL, USA) software package. The significance level was considered $p<0.05$ in all experimental data obtained in this research.

Results

The antibacterial effects of *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and *M. longifolia* extracts against common food-related pathogens, including *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 are exhibited in Tables 1 and 2. As it can be seen, the most antibacterial effectiveness was significantly ($p<0.05$) found for *M. longifolia* extract, which inhibition zone and MIC were in the range of 3.14-12.45 mm and 2-10 mg/ml, respectively. The following sequence inhibition effect on investigated bacterial strains was observed: *M. longifolia*>*T. graminifolius*>*A. rotundum*> *F. vulgaris*.

Moreover, Gram-negative bacteria (*S. typhimurium* and *E. coli* O157:H7) were more resistant to the presence of methanolic plant extracts than Gram-positive bacteria (*S. aureus*, *B. subtilis*, *B. cereus*, and *L. monocytogenes*). *B. cereus* was significantly ($p<0.05$) the most sensitive bacterium for *M. longifolia*, *T. graminifolius*, *A. rotundum*, and *F. vulgaris* extracts with inhibition zones of 12.45±0.03, 10.56±0.01, 7.04±0.01, and 4.24±0.01 mm, respectively.

Based on the results of the present study, the highest IC_{50} was significantly ($p<0.05$) found in *M. longifolia* (0.88±0.12 mg/ml); these rates for *T. graminifolius*, *A. rotundum*, and *F. vulgaris* extracts were 0.45±0.78, 0.26±0.07, and 0.14±0.23 mg/ml, respectively.

Discussion

In general, it is assumed that the considerable antibacterial activities of *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and *M. longifolia* extracts could be related to the phenolic compounds; the hydroxyl groups in phenolic compounds are thought to lead inhibitory action as these groups can interact with bacterial cell membrane to disrupt membrane structures and cause the leakage of cellular components. This will lead to the collapse of the proton motive force and depletion of the ATP pool and ultimately resulting in cell death (Gyawali and Ibrahim, 2014; Jay et al., 2005). On the other hand, the significant difference in the antimicrobial action of plant extracts is probably due to variability in the nature and concentration of main groups of chemical compositions (Lv et al., 2011). Moreover, we found that Gram-negative bacteria were more resistant to the presence of investigated methanolic plant extracts than Gram-positive bacteria. It may be related to the hydrophobic outer membrane surrounding the bacterial cell wall that restricts diffusion of lipophilic materials like extracts (Shahbazi and Shavisi, 2016). Some similar investigations on the *in vitro* antibacterial activities of *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and *M. longifolia* extracts have been conducted (Alzoreky and Nakahara, 2003; Sakunpak and Panichayupakaranant, 2012; Shafaghat, 2010). Jaberian et al. (2013) collected *F. vulgaris* from

Table 1: Antibacterial effect of methanolic extracts of *Falcaria vulgaris, Allium rotundum, Tragopogon graminifolius*, and *Mentha longifolia* indicated as Minimum Inhibitory/Bactericidal Concentrations (MIC/MBC; mg/ml)

Bacteria	*M. longifolia*		*T. graminifolius*		*A. rotundum*		*F. vulgaris*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	4±0.0^{b}	4±0.0^{b}	8±0.0^{a}	8±0.0^{a}	8±0.0^{a}	10±0.0^{a}	>10^{a}	>10^{a}
B. subtilis	2±0.0^{b}	4±0.0^{b}	6±0.0^{b}	6±0.0^{b}	8±0.0^{a}	8±0.0^{a}	8±0.0^{a}	10±0.0^{a}
B. cereus	2±0.0^{b}	2±0.0^{b}	6±0.0^{b}	6±0.0^{b}	8±0.0^{a}	8±0.0^{a}	8±0.0^{a}	10±0.0^{a}
L. monocytogenes	8±0.0^{a}	10±0.0^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}
S. typhimurium	10±0.0^{a}	10±0.0^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}
E. coli O157:H7	10±0.0^{a}	10±0.0^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}	>10^{a}

* The following concentrations were used in the present study: 0.5, 1, 2, 4, 6, 8 and 10 mg/ml
* Columns representing different values are labeled with different letters (p<0.05)

Table 2: Antibacterial effect of methanolic extracts of *Falcaria vulgaris, Allium rotundum, Tragopogon graminifolius*, and *Mentha longifolia* using agar disk diffusion assay

Bacteria	Inhibition zone (mm)			
	M. longifolia	*T. graminifolius*	*A. rotundum*	*F. vulgaris*
S. aureus	8.88±0.01^{b}	4.26±0.05^{b}	3.14±0.00^{b}	3.14±0.00^{a}
B. subtilis	12.31±0.01^{a}	9.36±0.06^{a}	6.11±0.06^{a}	3.14±0.00^{a}
B. cereus	12.45±0.03^{a}	10.56±0.01^{a}	7.04±0.01^{a}	4.24±0.01^{a}
L. monocytogenes	7.05±0.06^{b}	3.14±0.07^{b}	3.14±0.05^{b}	ND
S. typhimurium	3.14±0.02^{c}	3.14±0.01^{b}	3.14±0.02^{b}	ND
E. coli O157:H7	3.14±0.01^{c}	3.14±0.05^{b}	3.14±0.03^{b}	ND

* ND: Not detected (the inhibition zone was not observed)
* Columns representing different values are labeled with different letters (p<0.05)

Hamedan province of Iran and examined its antibacterial effect by disk diffusion method. These authors found that methanolic extract of *F. vulgaris* (concentration of 10 mg/ml) had inhibition zone of 7-10 mm against various food-borne pathogens such as *B. cereus*, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Bacillus megateriu*. Moreover, Rasooli and Rezaei (2002) reported that *M. longifolia* essential oil had inhibition zone of 8-12 mm against *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7, which is in agreement with our findings. Also, these researchers showed high antibacterial activities of these extracts on Gram-positive and Gram-negative bacteria, which are in agreement with the results of the present study. However, reported different antibacterial activities of plant extracts could be due to the different bacterial strains, media, and chemical composition of the extracts (Hyldgaard et al., 2012; Lv et al., 2011).

The antioxidant activity of plant leave extracts is mostly attributed to the active compounds of phenolic fraction present in them (Akrami et al., 2015; Wannes et al., 2010). In the present research, all studied plants were obtained at the same time during the flowering stage in order to prevent climatic, edaphic as well as developmental influence on the antioxidant activity. As previously reported, antioxidant activity of *M. longifolia* extract could be related to its phenolic content like phenolic acid, rosmarinic acid, and polyphenols (Asekun et al., 2007; Gulluce et al., 2007; Mkaddem et al., 2009). Moreover, the remarkable difference in the antioxidant property of the investigated plant extracts is probably due to variability in the nature and concentration of main groups of chemical compositions (Lv et al., 2011). Similar with our findings, Gulluce et al. (2007) found that IC_{50} of methanolic extract of *M. longifolia* was 0.81 mg/ml. In another study by Mimica-Dukic et al. (1999), the antioxidant activity of *M. longifolia* extract was reported 0.85 mg/ml. Assadpour et al. (2016) found that the antioxidant activity of *A. rotundum* extract was 0.24 mg/ml, which is good in agreement with our findings. On the other hand, the remarkable antioxidant activity of methanolic *T. graminifolius* extract which encompasses a key role on various medicinal properties of this plant, including protective and healing function on peptic and duodenal ulcer and also wound healing and skin repairing activity (Farzaei et al., 2014a, b). Indeed, phenolic compounds are one of the main constituents of *Tragopogon* species, which has critical role in their antioxidant activities. Farzaei et al. (2014b) showed high content of total phenol (0.560±0.85 mg/g gallic acid equivalents) in ethanolic extract from *T. graminifolius* aerial part. The potential antioxidant activity of the methanolic extracts of *F. vulgaris* showed a high effective free radical scavenging in the DPPH assay, which is good in agreement

with previous studies (Jaberian et al., 2013; Monfared et al., 2012; Shafaghat, 2010). Jaberian et al. (2013) collected *F. vulgaris* from Hamedan province in the West of Iran and examined its antioxidant property by DPPH assay. Based on their findings, the IC_{50} value of the methanolic extract of *F. vulgaris* was 0.1273 mg/ml. Monfared et al. (2012) reported that IC_{50} in DPPH, reducing power, and total antioxidant capacity of methanolic extract of *F. vulgaris* were 97.36±0.29, 200.118, and 0.147 µg/ml, respectively.

Conclusion

Based on the results of the present study, *F. vulgaris*, *A. rotundum*, *T. graminifolius*, and also *M. longifolia* had antimicrobial as well as antioxidant activities that recommended to be used as preservatives in food products. However, methanolic extract of *M. longifolia* had the best antibacterial and antioxidant properties *in vitro*. Further researches are required to identify the detail phytochemical compounds of these plant extracts responsible for their antibacteial and antioxidant characteristic.

Conflicts of interest

There is no conflict of interest in this study.

Acknowledgments

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