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Antibacterial and Antioxidant Properties of *Scrophularia Striata* Boiss. Methanolic Extract

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Scrophularia striata belongs to the Scrophulariaceae family and widely grows in the several regions throughout the world especially Iran, Turkey and Azerbaijan. The aims of the present study were to evaluate antibacterial activity of the S. striata methanolic extract collected from west part of Iran by micro-broth dilution and agar disk diffusion assays, and also determine its antioxidant properties using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) methods. The most antibacterial activity was observed against Bacillus cereus, followed by B. subtilis, S. aureus. Listeria monocytogenes, Salmonella typhimurium and Escherichia coli O157:H7, respectively. Moreover, the scavenging properties on DPPH radical scavenging and TBA of S. striata methanolic extract were found to be 0.92 ± 0.21 and 7.98 ± 0.23, respectively. The strong in vitro antibacterial and antioxidant activities of S. striata methanolic extract supports its traditional application in the treatments and/or prevention of different diseases.

Keywords: Scrophularia striata; Antibacterial; Antioxidant; Iran.

In the third world and developing countries, consumption of foods contaminated with some microorganisms such as *Listeria monocytogenes*, *Salmonella spp.*, *Escherichia coli* O157:H7, *Bacillus spp.* and *Staphylococcus aureus* represents serious health risks to humans [1]. In addition, the subsistence and growth of microorganisms in foods usually result in spoilage, toxin production and quality deterioration of vulnerable food products such as raw meat, fish, shrimp and salad vegetables [2,3]. Since ancient times, medicinal plants and spices have been incorporated to different types of food not only to improve the organoleptic properties (aroma, flavor and taste), but also as food preservatives [4]. In general term, essential oils and extracts are active compounds, which are often concentrated in a particular organ of plants including bud, seed, root, leave, stem, wood, bark and flower [5]. An estimated more than 3000 essential oils and extracts are recognized, which approximately 300 are commercially important for the flavor and fragrances markets as well as medicinal properties [6]. In the last decades, the essential oils and the herbal extracts from various species of edible and medicinal plants have attracted a great deal of scientific interest due to their potential as a source of natural agents to increase the safety and shelf life of foods and of natural biologically active compounds [7]. Especially, the antimicrobial activity of plant extracts have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [8,9].

Scrophularia striata Boiss. belongs to the family of *Scrophulariaceae* and widely grows as wild in the several regions all over the world especially Iran, Turkey and Azerbaijan [10]. It has square stems, opposite leaves and open two-lipped flowers forming clusters at the end

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of their stems [11]. In Iran, the species commonly known as "Tashneh dari" is abundant in the Zagros Mountainous Range [12]. Different parts of this plant has been used as Iranian folk remedies because of its medicinal properties for treatment of various diseases like scrophulas, scabies, tumors, eczema, psoriasis, rheumatics and chronic inflammatory diseases [11,13,14]. Earlier studies are focused on its application as a natural anticancer agent and indicated that methanolic extract of *S. striata* might contain various polar compounds that inhibit tumor invasion, metastasis and angiogenesis [11,14]. In addition, it has been reported that the oil extracted by steam distillation of leaves could inhibit numerous inflammatory factors such as PGE-2, leukotriene B4, NO, IL-1², IL-4, INF-³, but did not have any effect on the production of IL-10 [15].

The biological effects of the plant extracts and essential oils can vary greatly depending upon its chemical compositions which depends on the geographical and climate conditions, variety of species and genotypes of the plant [1,16,17]. Therefore, studying the biological properties of *S. striata* collected from each endemic area may have an important role in identification and introduction of new germplasm in order to be used in food and pharmaceutical industries [18]. Based on our findings, there is no comprehensive study on the antioxidant and antibacterial properties of *S. striata* collected from Kermanshah, west part of Iran. Therefore, the aims of the present study were to evaluate antibacterial activity of the *S. striata* methanolic extract by broth-micro dilution and agar disk diffusion assays, and also determine its antioxidant property using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) methods.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *S. striata* plant was collected from Kermanshah, west part of Iran during full flowering stage in April 2016. The plant was identified as *S. striata* Boiss. by a botanical taxonomist (Dr. Seyed Mohammad Masoumi, Faculty of Agriculture, Razi University, Kermanshah, Iran). Then, the fresh leaves of collected plant was washed with distilled water and air-dried indoor in a shady place at room temperature for twelve days.

Preparation of plant extract

To prepare *S. striata* methanolic extract, 5 g of fine-powdered plant was dissolved in 20 ml methanol and extracted with a shaker at room temperature for 24 h. The extract was filtered through Whatman filter paper no. 3, concentrated in a rotary evaporator at 40 ºC and stored at refrigerated temperature till further use [19,20].

Bacterial strains

The antibacterial activity of *S. striata* methanolic extract was studied against six pathogenic bacteria including *S. aureus* (ATCC 6538), *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 11774), *L. monocytogenes* (ATCC 19118), *S. typhimurium* (ATCC 14028) and *E. coli* O157:H7 (ATCC 10536). All aforementioned bacterial strains were purchased from the Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The strains were cultured overnight at 37 °C in Brain Heart Infusion broth (BHI), adjusted to a final density (5 log CFU/ml) using a spectrophotometer at 600 nm and used as an inoculum dose.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The MIC of the methanolic extract of *S. striata* was determined using micro-broth dilution assay descried by Shahbazi et al. (2015) [21] with some minor modifications. Different concentrations of dried plant extract ranging from 0.05 to 10 mg/ml were set up in Brain Heart Infusion (BHI; Merck, Darmstadt, Germany) broth containing dimethyl sulfoxide (DMSO; 0.5% v/v) and filtered by 0.45 µm filters for sterilization. The 96-well sterile micro-titer plate was prepared by pouring 180 µl BHI broth medium containing specified concentrations of the plant extract and 20 µl of fresh overnight bacterial cultures (5 log CFU/ml) into each well. Parallel positive (BHI broth containing inoculum without the tested materials) and negative controls (BHI broth containing the tested materials) were maintained in the last well of each strip. The microplates were shaked at 300 rpm for 20 s and incubated at 37 ºC for 24 h. The MIC was defined as the lowest concentration of the plant extract that completely inhibited the growth of microorganisms. Referring to results of the MIC, 20 µl of each well without any invisible growth was sub-cultured on BHI (Merck, Darmstadt, Germany)

agar plates, incubated at 37 °C for 24 h. MBC was determined as the highest dilution at which no growth occurred on the plates [1].

Agar disk diffusion assay

For agar disk diffusion assay, 100 µl of each bacterial suspension (8 log CFU/ml) was uniformly spread on BHI agar medium using a sterile cotton swab. Then, the sterile paper discs with 6 mm in diameter were impregnated with 10 µl of each designated concentration of plant extract and placed on the surface of the inoculated BHI agar plates. These plates were incubated for 24 h under appropriate cultivation temperature (37°C). The area of the inhibition zone (millimeter) was calculated as Àr² [20]. All tests were performed in triplicate.

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The antioxidant activity of the *S. striata* extract was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay through a UV-vis spectrophotometer [22,23]. An aliquot of 50 µl of various concentrations of the *S. striata* extract was added to 5 ml of methanolic DPPH solution and the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging activity of *S. striata* extract was calculated as follows [20,23]:

$$I\ (\%) = \frac{[Ab - AS]}{Ab} \times 100$$

Where I% is the capability to scavenge the DPPH radical or to inhibit free radicals, A_b is the absorbance of the control reaction (containing all reagents except the *S. striata* extract), and A_s is the absorbance of the *S. striata* extract sample.

Thiobarbituric acid reactive substances (TBA) assay

The thiobarbituric acid reactive substances (TBA) value, a secondary product of lipid peroxidation, of *S. striata* extract was evaluated according to the method of Singh *et al.*, (2010). The TBA value (Meq of malondialdehyde/g) of *S. striata* extract was calculated as following formula [4,24]:

$$\text{TBA value} = \frac{[50 \times (A - B)]}{M}$$

Where A is the absorbance of test sample, B is the absorbance of reagent blank and M is the mass of the sample (mg).

Table 1. Antibacterial activity of *S. striata* extract indicated as Minimum Inhibitory/Bactericidal Concentrations-MIC/MBC (mg/ml)

Bacteria	Extract		Tetracycline	
	MIC	MBC	MIC	MBC
S. aureus	0.8	0.8	2	2.5
B. subtilis	0.4	0.4	2.5	2.5
B. cereus	0.1	0.1	2	2
L. monocytogenes	2	2	2.5	2.5
S. typhimurium	4	8	2.5	2.5
E. coli O157:H7	8	10	2.5	2.5

RESULTS AND DISCUSSION

Bacteria are the most common cause of foodborne diseases and exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus, highly heat-resistant (*e.g. Bacillus subtilus, Bacillus cereus*). Some are capable of producing heat-resistant toxins (*e.g. Staphylococcus aureus, Clostridium botulinum*). Overall, these outbreaks caused 45,874 cases of illness (209 more than 2014), 3,892 hospitalisations (2,546 less than 2014) and 17 deaths (10 less than 2014). The overall reporting rate of food-borne outbreaks in the EU was 0.95 per 100,000 population, which represents a slight decrease compared with data provided for 2014 [25]. Epidemiological studies have consistently shown that there is a clear significant positive association between the intake of medicinal plants and a reduced rate of food borne diseases [19]. In the present study, antibacterial and antioxidant activities of the *S. striata* methanolic extract was examined using broth-microdilution assay, agar disk diffusion method, DPPH and TBA.

The antibacterial effects of *S. striata* extract against common food-borne pathogenic bacteria including *S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7 are exhibited in Table 1 and 2. Based on our findings, the most antibacterial activity was observed against *B. cereus*, followed by *B. subtilis*, *S. aureus*, *L. monocytogenes, S. typhimurium* and *E. coli* O157:H7, respectively. Indeed, Gram-negative bacteria (*S. typhimurium* and *E. coli* O157:H7) were more resistant to the presence of methanolic *S. striata* extract than Gram-positive bacteria (*S. aureus*, *B. subtilis*, *B. cereus* and *L. monocytogenes*). It can be attributed to the hydrophobic outer membrane surrounding the cell wall of Gram-negative bacteria, which restricts diffusion of lipophilic compounds such as essential

oils and extracts [26,27]. In agreement with our findings, Mahboubi and Haghi (2008) examined antibacterial effects of the essential oil and extract of *Mentha spicata,* and reported Gram-positive bacteria including *S. aureus*, *L. monocytogenes*, *B. cereus* were more sensitive than Gram-negative including *E. coli* O157:H7 and *Yersinia enterocolitica* [28]. In another study, Mahboubi et al., (2013) evaluated antibacterial activity of *S. striata* Boiss extract against some pathogenic and spoilage microorganisms and reported that *Staphylococcus epidermidis, Streptococcus sobrinus, Klebsiella pneumoniae, B. subtilis* and *B. cereus* had more sensitivity to aqueous extract of *S. striata* Boiss [11], which is good in agreement with our findings. Monsef-Esfahani et al., (2010) demonstrated that cinnamic acid, quercetine, isorhamnetin-3-O-rutinoside, nepitrin, phenyl propanoid glycoside (acteoside 1) are the most abundant compounds isolated from *S. striata* extract [10]. These data also are in agreement with Abbasi et al. (2007), using the same antimicrobial method, they have shown that *S. striata* extract was effective against *S. aureus* and *Pesudomonas aeroginosa* [29]. In agreement with our results are those of Sharafati-Chaleshtori and Rafieian-kopaei, (2014) who reported that *S. striata* ethanolic extract had inhibitory effect against the *E. coli* O157:H7 in two methods of sink diffusion and macrodilution [30]. The action mode the phenolic compounds is related to their hydroxyl group of the phenolic ring which plays a significant role in the formation of hydrogen bonds and also in the presence of delocalized electrons and subsequently dissipate the pH gradient over the bacterial cytoplasmic membrane [31]. It disturbs the proton motive force (PMF), depletes the amount of intracellular ATP (ATPin) pool and leads to impairment of essential process in the bacterial cell [3,32].

The scavenging properties on DPPH radical and TBA of *S. striata* methanolic extract were found to be 0.92 ± 0.21 and 7.98 ± 0.23, respectively (Table 3). DPPH scavenging ability of the *S. striata* methanolic extract was significantly higher than that of synthetic antioxidant BHT, indicating the presence of specific bioactive components in this plant that can be responsible for its antioxidant activity. Similar results were also found in a previous study where reported that all parts of *S. striata* had high antioxidant activities [33]. According to these results, the IC_{50} of *S. striata* extracts were ranged 0.98 and 0.99 mg/ml, which is good in agreement with our findings. The anti-inflammatory, antioxidant and immunomodulatory activities of some species of *Scrophularia* have also been reported by other researchers [12,34]. Differences in the results of antioxidant activities of *Scrophularia spp.* extracts among these studies could be mostly explained by variations in the phenolic concentrations of plant and used antioxidant method [34]. In accordance to the antibacterial property, the antioxidant effect of

Table 2. Antibacterial effect of *S. striata* extract by agar disk diffusion assay

	Inhibition zone (mm)					
	S. aureus	*B. subtilis*	*B. cereus*	*L. monocytogenes*	*S. typhimurium*	*E. coli* O157:H7
Extract	8.4 ± 0.1	10.1 ± 0.1	10.9 ± 0.2	7.8 ± 0.5	3.1 ± 0.0	3.1 ± 0.0
Tetracycline	10.2 ± 0.1	11.5 ± 0.7	14 ± 0.0	13 ± 0.1	12 ± 0.0	10 ± 0.2

Table 3. Antioxidant activity of *S. striata* extract (mg/ml; mean ± SD)

	Extract	BHT
DPPH radical-scavenging activity (IC_{50}[a])	0.92 ± 0.21	0.19 ± 0.12
TBA (EC_{50}[b])	7.98 ± 0.23	0.001 ± 0.000

[a] IC50, concentration (g/l) for a 50% inhibition.
[b] EC50, concentration (g/l) for a 50% inhibition.

S. striata extract is due to phytochemical contents especially compounds, including flavonoids, cinamic acid, phenylpropanoid, nepitrin, flavonoid glycoside, acteoside1 and phenylpropanoid glycoside [10]. In confirmation with our findings, Mahboubi et al., (2013) [11] and Monsef-Esfahani et al., (2010) [10] reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample and remarkable positive correlation between antioxidant property of *S. striata* extracts and its phenolic compounds. The antioxidant activity of the phenolic compounds were attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties [31,35]. However, further investigations about the total phenols (TP), total flavonoids (TFO) and total flavan-3-ols (TFL) contents of *S. striata* extract is required.

CONCLUSION

The present study indicated that *S. striata* methanolic extract showed remarkable antibacterial activity against common food-borne bacteria associated with outbreaks (*S. aureus*, *B. subtilis*, *B. cereus*, *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7) and also antioxidant property. Further research is required to evaluate the combination of *S. striata* extract with other antibacterial constituents such as nisin, lysozyme, monolaurin and other essential oils. The strong *in vitro* antibacterial and antioxidant activities of *S. striata* methanolic extract supports its traditional application in the treatments and/or prevention of different diseases.

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