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Antibacterial effect and physicochemical properties of essential oil of *Zataria multiflora* Boiss

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ABSTRACT

Objective: To evaluate the antibacterial effect and physicochemical properties of essential oil of *Zataria multiflora* Boiss. **Methods:** Antibacterial activity of essential oil of *Zataria multiflora* Boiss was assessed by agar disc diffusion and minimal inhibitory concentration (MIC) was tested by E test. **Results:** The essential oil of *Zataria multiflora* Boiss was effective on pathogenic bacteria particularly *Staphylococcus aureus* (*S. aureus*). The MIC values of the target cultures ranged from 0.39 mg/mL to 1.56 mg/mL. The physicochemical properties like effects of pH, temperature, detergents, and enzymes on the activity of essential oil from *Zataria multiflora* Boiss were also determined. The essential oil was quite stable to temperature as tested against *S. aureus* and *Escherichia coli* (*E. coli*). The essential oil was very stable over a wide range of pH. The antibacterial activity of essential oil was insensitive to various protein–denaturing detergents (Such as Tween 80, Tween 20, Triton 100, etc.) and enzymes (namely proteinase K, trypsin, lipase, and lysosyme). **Conclusions:** A potential use of the essential oil from *Zataria multiflora* Boiss is suggested. More studies including further purification, mass spectra, nuclear magnetic resonance (NMR) and evaluation of toxicity are needed for confirmation of this suggestion.

1. Introduction

Research directed towards the development of new antimicrobial agents is necessary for several reasons like the emergence and rapid spread of drug resistant bacterial pathogens and raising the spectra of untreatable disease[1]. Moreover, experts rightly warn that if a new antimicrobial agent is not continued we will return to the pre–antibiotics era[2]. It is therefore imperative to find/develop safer and more potent compounds for the future that are not toxic to host.

Apart from the drug design, another relatively simpler but extremely useful approach is to screen local medicinal plants in search of suitable antimicrobial substances. Plant–derived products are present in 14 of the 15 therapeutic categories of pharmaceutical preparations that are currently recommended by medical practitioners and they form an

important part of the health–care system in the western world[3]. It is estimated that there are 2 500 000 species of higher plants and the majority of these have not been examined in details for their activities extensively[4].

The antimicrobial activity of certain Iranian medicinal plants was reported based on folklore information[5]. With respect to this subject, several consistent lines of evidence that essential oils are among the most important active constituents of plants have been generated over the past[6]. *Zataria multiflora* is a plant owned by the Labiatae family that is distributed only in Iran, Pakistan and Afghanistan. It is greatly used for medicinal and condimental purposes in these countries. This plant with the vernacular name of Avishan Shirazi in Iran has several traditional uses such as antiseptic, anesthetic and antispasmodic[7,8]. Most aspects of their medicinal use are related to the essential oil which contains various levels of thymol and/or carvacrol, phenolic derivatives with strong and wide–spectrum antimicrobial activity[9,10]. *Zataria multiflora* Boiss is a perennial herbaceous plant, distributed in central and southern Iran.

Although essential oil content, composition and antimicrobial properties of some species have been previously studied[11,12], however, we could found scanty

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information about antimicrobial activity and physico-chemical properties of *Zataria multiflora* Boiss. Thus, in the present study, antibacterial effect and physico-chemical properties of the essential oil of *Zataria multiflora* Boiss was determined.

2. Materials and methods

2.1. Plant material

Leaves of *Zataria multiflora* Boiss were purchased from local herbal medicinal shop from Ahwaz, Iran in June 2009. Identification of the species was confirmed by School of Sciences, Department of Botany, Chamran University, Ahwaz, Iran. To provide essential oil, 50 g of the dried leaves was added to 500 mL sterile distilled water and poured into flask to distillate the essences using Clevenger-type apparatus. The yield of essential oil was 1 g from 50 g dried leaves of this plant.

2.2. Microorganisms and their maintenance

Antibacterial activities of essential oil of *Zataria multiflora* Boiss was assessed by agar disc diffusion, using a panel of laboratory control strains obtained from the Persian Type Culture Collection. All bacteria were stored in trypticase soy broth containing 25% (v/v) glycerol. Standard strains including *Staphylococcus aureus* (*S. aureus*) PTCC 1113, *Escherichia coli* (*E. coli*) PTCC 1533, *Streptococcus pyogenes* PTCC 1447, *Streptococcus pneumoniae* PTCC 1240, *Proteus mirabilis* PTCC 1076, *Bacillus cereus* PTCC 1015, obtained from the Persian Type Culture Collection.

2.3. Determination of minimal inhibitory concentration by E test

Bacterial suspensions of freshly grown cultures were prepared in sterile saline and adjusted to a density of 10⁶ cell/mL. The cultures media (Mueller Hinton Agar (MHA), Hi Media) were inoculated by dipping a sterile cotton swab into the cell suspension and streaking it across the surface of the agar in three directions.

The essential oil was serially diluted in methanol, and five discs were soaked in 50 μ L of each dilution, kept at room temperature overnight. Six discs were placed on agar surface in a line and incubated for 24 hours at 37 °C. The minimal inhibitory concentration (MIC) values were read as the antibacterial concentration at the point where dense colonial growth intersected the disc. The test was performed in quadruplicate for each culture. Disc containing 10 μ L of methanol were kept as control.

2.4. Physico-chemical properties of essential oil from *Zataria multiflora* Boiss

2.4.1. Determination of shelf life

Essential oil of *Zataria multiflora* Boiss was stored at room temperature for 45 days. At different time intervals 50 μ L of the essential oil was removed and residual antibacterial activity was determined weekly against *S. aureus* and *E. coli*. The test was performed in triplicate.

2.4.2. Thermal stability of antibacterial activity of essential oil of *Zataria multiflora* Boiss

To determine the effect of temperature on the stability of the essential oil, screw capped ampoules, containing 100 μ L essential oil were kept at 20, 40, 60, 80 and 100 °C for one hour in water bath. The tubes were cooled to room temperature and volumes were brought to the original and the residual antibacterial activity was determined against *S. aureus* and *E. coli*. The tests were performed for triplicate.

2.4.3. Effect of pH on the antibacterial activity of essential oil of *Zataria multiflora* Boiss

Aliquots 100 μ L essential oil was mixed with 100 μ L of phosphate buffer of different pH, incubated for one hour and residual antibacterial activity for each aliquot was measured against *S. aureus* and *E. coli*. The tests were performed for triplicate.

2.4.4. Effect of detergents on activity of essential oil of *Zataria multiflora* Boiss

Susceptibility of the essential oil of *Zataria multiflora* Boiss to denaturation by various detergents (Tween 80, Tween 20, Toluene, Cetrimide, Triton 100, SLS and SDS) was studied. Detergents were added to the essential oil at the concentration of 0.01 g per mL. The mixture was incubated for three hours at room temperature and then tested for residual antibacterial activity against *S. aureus* and *E. coli*. Detergents added to distilled water were used as control to check the effect of detergents themselves on tested bacteria.

2.4.5. Effect of enzymes on antibacterial activity of the essential oil

The sensitivity of essential oil of *Zataria multiflora* Boiss to enzymes proteinase K (pH 8.5), trypsin (pH 8.1), lipase (pH 7) lysozyme (pH6.1) was tested. All enzymes were dissolved in distilled water at concentration of 1 mg/mL. 100 μ L of essential oil was mixed with 100 μ L enzyme and incubated at room temperature for three hours. 50 μ L of the mixture was added to five sterile discs and antibacterial activity was tested against *S. aureus* and *E. coli*.

3. Results

3.1. MIC Value

Essential oil of *Zataria multiflora* Boiss tested showed good antibacterial activity against tested microorganisms. The MIC values were ranged from 0.39 mg/mL to 1.56 mg/mL. The *S. aureus* was more sensitive than other tested bacteria (0.39 mg/mL). Similarly *Streptococcus pyogenes*

(1.56 mg/mL) and *Proteus mirabilis* (1.56 mg/mL) were most resistant bacteria. And *Streptococcus pneumoniae*, *E. coli* and *Bacillus cereus* had the same MIC as 0.78 mg/mL.

3.2. Heat stability of essential oil

Since the activity of essential oil was quite stable (100%) at room temperature for seven weeks (Table 1), experiments were also conducted to see the effect of elevated temperature on stability of essential oil. The effect of temperature and pH on antibacterial activity of essential oil against *S. aureus* and *E. coli* was analyzed in term of residual activity (Table 2). The antibacterial effect of essential oil was absolutely stable at the various temperatures. It is worth to note that the extract was quite stable even at 1 000 °C against both the target cultures.

After the incubation of the essential oil at pH in the range of 5–8, more than 92.8% residual activity was still showing on *S. aureus* and *E. coli* (data not shown).

3.3. Effect of detergents on activity of essential oil from *Zataria multiflora* Boiss

The antibacterial activity of essential oil alone showed 13.5 and 12 mm inhibition zone diameter against *S. aureus* and *E. coli* respectively; while no loss of antibacterial activity of essential oil was observed upon treatment with any of the detergents (Table 3).

Table 1

Shelf life of essential oil of *Zataria multiflora* Boiss at room temperature.

Incubation (week)	Inhibition zone dia (mm)		Residual activity(%)	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
First	18	16	100.0	100.0
Second	18	16	100.0	100.0
Third	18	16	100.0	100.0
Fourth	18	16	100.0	100.0
Fifth	18	16	100.0	100.0
Sixth	17	16	94.4	100.0
Seventh	17	16	94.4	100.0

Table 2

Effect of temperature and enzyme on essential oil fom *Zataria multiflora* Boiss.

Factors		Inhibition zone (mm)	
		<i>S. aureus</i>	<i>E. coli</i>
Temperature (°C)	20	14	13
	40	14	13
	60	14	13
	80	14	13
	100	14	13
	Enzyme	Trpsin	12
	Proteinase	13	11
	Lipase	13	10
	Lysozyme	12	11
	No enzyme	14	12

Table 3

Effect of detergents on antibacterial activity of essential oil from *Zataria multiflora* Boiss against *S. aureus* and *E. coli*.

Detergents	IZD (mm)		RA(%)	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
Tween 80	13.0	11.5	96.29	95.83
Tween 20	13.0	12.0	96.29	100.00
Triton 100	13.0	12.0	96.29	100.00
SDS	13.0	11.5	96.29	95.83
Cetrimide	13.5	11.5	100.00	95.83
SLS	13.0	11.5	96.29	95.83
Toluene	13.0	11.0	96.29	91.66
Essential oil	13.5	12.0	100.00	100.00

IZD: Inhibition zone diameter; RA: Residual activity.

3.4. Effect of enzymes on activity of essential oil from *Zataria multiflora* Boiss

Upon treatment of essential oil with proteinase K, trypsin, lipase, and lysozyme it gave 12–14 mm wide zone of inhibition with *S. aureus* and *E. coli* just as the zone of inhibition obtained with essential oil doubly diluted to compensate for the dilution caused by enzyme solution (Table 2).

4. Discussion

For hundreds, if not thousands of years, plants have been recognized for their therapeutic properties^[13]. Our study shows the antibacterial activity methanolic extract from *Zataria multiflora* Boiss against tested bacteria. The methanolic extract exerted varying levels of antibacterial effects against the target cultures. According to the data of Ali *et al* and Arab *et al*, the *Zataria multiflora* Boiss leaves showed different antimicrobial activities^[14,15]. It is known that genetic constitution and environmental conditions influence the yield and antimicrobial effect produced by plants.

Various studies has been performed about antibacterial effect of essences of herbs belong to Lamiaceae family and some important compound in essences of this family among Carvacrol and Thymol^[16–18].

The antibacterial activity of essential oil of *Zataria multiflora* Boiss leaves was quite stable at room temperature as well as to various temperatures. Proteolytic treatment of essential oil of *Zataria multiflora* Boiss leaves did not abolish its antibacterial activity against *S. aureus* and *E. coli*. It is therefore, tempting to propose the presence of non proteinic compound, which has potent antibacterial activity. Also treatment of antibacterial activity from essential oil to various detergents provided full recovery of antibacterial activity.

A potential use of essential oil from *Zataria multiflora* Boiss need more studies including further purification,

mass spectra, nuclear resonance (NMR) and evaluation of toxicity are needed and in progress for confirmation of this suggestion.

Conflict of interest statement

We declare that we have no conflict of interest.

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