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# In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss

F. Sharififar [a,*], M.H. Moshafi [b], S.H. Mansouri [c], M. Khodashenas [d], M. Khoshnoodi [e]

[a] *Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran*
[b] *Department of Pharmaceutical Science, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran*
[c] *Department of Microbiology, Faculty of medicine, Kerman University of Medical Science, Kerman, Iran*
[d] *Department of Botany of the Kerman Research Institute of Forests and Rangelands, Kerman, Iran*
[e] *School of Pharmacy, Kerman University of Medical Science, Kerman, Iran*

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**Abstract**

The present study was conducted to evaluate in vitro antibacterial and antioxidant properties of essential oil and methanol extracts from a unique and endemic plant, *Zataria multiflora* Boiss. The antibacterial test results showed that the essential oil of the plant strongly inhibited the growth of all of the microorganisms studied especially the Gram-negative strains. The polar fraction of methanol extract has been effective against Gram-positive strains, while the non-polar fraction has shown activity similar to essential oil. The antioxidant potential of the samples was evaluated using two separate methods, inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ammonium thiocyanate systems. Sub fractions of the methanol extract were able to reduce the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) with an $IC_{50}$ of $11.7 \pm 1.58$ and $16.2 \pm 1.61$ μg/ml, respectively for non-polar and polar ones, which the activity of the latter almost is equal to synthetic antioxidant BHA ($18.2 \pm 1.94$ μg/ml). Inhibition values of linoleic oxidation were calculated to be 82.4% and 80.3% for the polar and non-polar fractions, respectively. The essential oil to be showed more inhibition ($89.7 \pm 2.5$), similar to the synthetic antioxidants BHA ($97.8 \pm 2.94$) and ascorbic acid ($93.2 \pm 2.1$). The chemical composition of hydrodistilled essential oils of *Z. multiflora* was analyzed by GC/MS. A total of 25 compounds representing 99.78% of the oil were identified: thymol (37.59%), carvacrol (33.65%); *para*-cymene (7.72%), γ-terpinene (3.88%) and β-caryophyllene (2.06%) were the main components comprising 84.9% of the oil. Results here show that the essential oil and methanol extract of *Z. multiflora* possess antioxidant and antibacterial activity, and therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.


*Keywords:* *Zataria multiflora*; Antibacterial activity; Antioxidant activity

## 1. Introduction

Lipid peroxidation is a complex process occurring in aerobic cells and reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Formation of free radicals may play an important role in the origin of life and biological evolution, implying their beneficial effects on organisms (McCord, 2000). Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging of organisms and cancer promotion (Ashok & Ali, 1999). Reactive oxygen species are reported to be involved in asthma, inflammation, arthritis, neurodegeneration, Parkinson's disease, mongolism and perhaps dementia (Perry et al., 2000). They also exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells (Lander, 1997). However, free radicals and other relative species

* Corresponding author. Tel.: +98 341 322 0001; fax: +98 341 322 0799.
*E-mail address:* fsharififar@Kmu.ac.ir (F. Sharififar).

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cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA) which leads to cell injury and death (Ignaro, Cirino, Casini, & Napoli, 1999).

The exploration of naturally occurring antimicrobial for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Gould, 1995). Many spices and herbs exert antimicrobial activity due to their essential oil fractions Nychas reported antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against both bacteria and fungi (Nychas, 1995). Phenolic components, present in essential oils, have been known to possess antimicrobial activity and some are classified as Generally Recognized as Safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Singh, Singh, Bhunia, & Simmon, 2001). Avishane Shirazi is the Persian name for *Zataria multiflora* Boiss (*Z. multiflora*), belonging to the family Labiatae, and it is native to Iran. This plant is used traditionally in food, especially in yoghurt flavouring, as a stimulant, condiment, carminative and for treatment of pre-mature labor pains and rupture (Zargri, 1989) There are also commercial pharmaceuticals with formulae based on *Z. multiflora* essential oil. This oil has been used commonly in medicine for the treatment of respiratory tract infections as an antiseptic, antitussive and irritable bowel syndrome treatment (Aynehchi, 1991). Also, the extracts of aerial parts of *Z. multiflora* showed anti-inflammatory effects against acute and chronic inflammations in mice and rats (Hosseinzadeh, Ramezani, & Salmani, 2000). As far as our literature survey could ascertain, antibacterial activities of the essential oil of *Z. multiflora* have been previously published only against *Salmonella typhimurium* (Rasouli & Rezaei, 2001).

The aim of the present work was to study in vitro antioxidant and antibacterial activities of the essential oils and methanol extracts of *Z. multiflora* and to determine the chemical composition of its essential oil by GC/MS. Numerous techniques are available to evaluate the antioxidant activities of compounds and complex mixtures such as plant extracts. Despite the various methods, just one procedure cannot identify all possible mechanisms characterizing an antioxidant. Therefore, oil and methanol extracts were screened for their possible antioxidant activities by two complementary test systems, namely DPPH free radical-scavenging and ammonium thiocyanate methods.

## 2. Materials and methods

### 2.1. Plant material

*Zataria multiflora* Boiss. (Lamiaceae) tops at the full flowering stage (June and July) were collected from plants growing wild in the Firoozabad (in Fars province). The taxonomic identification of plant materials was confirmed by a senior plant taxonomist. Voucher specimens were deposited in the Herbarium of Kerman Faculty of Pharmacy, Kerman, Iran.

### 2.2. Preparation of the methanol extracts

Tops of the plant were dried in the shade, ground in a grinder with a 2 mm in diameter mesh, and about 200 g of dry powdered extracted with 85% methanol using percolation method for 48 h. Solvent removal carried out under vacuum afforded a semisolid mass with a yield of 13%. The resulting extract was fractionated with water and chloroform to give polar and non-polar sub fractions.

### 2.3. Isolation of the essential oil

The air-dried and ground herbal parts of the plant collected was submitted for 4 h to water-distillation using a British-type Clevenger apparatus (yield 2.8% v/w). The obtained essential oil was dried over anhydrous sodium sulphate, then stored at +4 °C until tested and analyzed.

### 2.4. Gas chromatography/mass spectrometry analysis

#### 2.4.1. Gas chromatography analysis

The essential oil was analyzed using a Shimadzu QP 5000 gas chromatograph equipped with a FID detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 160 °C at 3 °C/min, held for 10 min and finally raised to 240 °C at 3 °C/min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted sample (1/100 in acetone, v/v) of 1.0 μl was injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

#### 2.4.2. Gas chromatography/mass spectrometry analysis

GC–MS analysis of the essential oil was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC–MS system and literature data (Adams, 2001). Alkanes were used as reference points in the calculation of relative retention indices (RRI). GC and GC/MS analysis results are given in Table 1.

### 2.5. Antimicrobial activity

#### 2.5.1. Microbial strains

Sub fractions of the methanol extract and the essential oil were individually tested against a panel of