



## *Salmonella typhimurium* and *Listeria monocytogenes* Growth Inhibition by *Zataria multiflora* Essential Oil in Ground Meat

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### ABSTRACT

**Background:** *Zataria multiflora* boiss is a member of Lamiaceae family with antibacterial, antifungal, and antioxidant activity. The aim of this study was to evaluate chemical composition and antibacterial effect of *Zataria multiflora* essential oil against two foodborne pathogens in meat.

**Methods:** The inhibitory effect of *Zataria multiflora* essential oil (Minimum inhibitory concentration and Minimum bactericidal concentration) was evaluated against *Salmonella typhimurium* and *Listeria monocytogenes*, inoculated in ground beef meat after 3, 5, 7 and 9 days of storage.

**Results:** Result revealed 26 various compounds, representing 96.27% of total oil. Thymol was the most abundant compound among all constituents (29.2%). A significant reduction was observed in *Salmonella typhimurium* when adding 1 and 2% *Zataria multiflora* ( $p < 0.05$ ) compared with 0.5% and 1% concentrations during 9 days of storage. *Listeria monocytogenes* significantly decreased in treatments with 0.5%, 1 and 2% of *Zataria multiflora* essential oil.

**Conclusion:** *Zataria multiflora* has an inhibitory effect on the growth of *L. monocytogenes* and in higher concentrations on the growth of *S. typhimurium* and can be used as a natural preservative in order to retard the growth of food-borne pathogens.

## 1. Introduction

According to the World Health Organization (WHO) definition, food-borne diseases (FBD) are “diseases of infectious or toxic nature caused by,

or thought to be caused by the consumption of food or water” [1].

Over 250 food borne diseases have been

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Identified so far [2]. One such disease is listeriosis caused by *Listeria monocytogenes*, a short bacilli, gram positive, non-spore forming, catalase positive motile bacterium [3, 4]. Dairy products, vegetables and animal meat can be contaminated easily by this species and consequently the infection can be transferred to human [5]. Human listeriosis mortality rate is as high as 20-30%, indicating the great burden of disease. However, it has a low incidence [6]. The sensitivity of human strains of *L. monocytogenes* to a wide range of antibiotics such as erythromycin, penicillin, amoxicillin, ampicillin, co-trimoxazole, vancomycin, gentamicin, rifampicin, imipenem, and tetracycline has been reported by National Reference Center for Listeria (NRCL) [7]. However, most strains show natural resistance to current cephalosporins, especially third and fourth generation, current fluoroquinolones, oxacillin, lincosamides, and fosfomycin [8].

Salmonella is another common food-borne pathogen [9]. In the United States of America about 1.4 million cases develop salmonellosis, which results in nearly 600 deaths and 17000 hospitalizations annually [10].

*S. typhimurium* has various subtypes that are classified by phage typing. Similar to *Listeria*, *Salmonella* has shown resistance to various antibiotics such as ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol [11]. Due to the fact that antimicrobial resistance is growing [5] and the probable side effects they can cause [12], recent studies have focused on herbal essentials [13]. *Zataria multiflora* Boiss is a member of Lamiaceae family [14] and is a native plant of Iran which is consumed traditionally in foods, such as yoghurt as a flavouring, stimulant, condiment and carminative [15]. It is also used for alleviating pre-mature labor pains. *Zataria multiflora* essential oil (ZEO) has been used as an antitussive and antiseptic for respiratory tract infections. It has been also beneficial for managing irritable bowel syndrome (IBS) [16].

Anti-inflammatory effects of the extracts of aerial parts of *Zataria multiflora* against chronic and acute inflammations has also been reported [17]. Since phenolic compounds such as carvacrol and thymol constitute a great portion of ZEO, this oil can be used as an antibacterial, antifungal, and

antioxidant agent [15]. Ground and minced meat products are competent foods for bacterial growth. Oxidative rancidity and microbial growth are the main factors that affect shelf life of these products [18]. In order to extend the shelf life of such foods, a wide range of preservatives have been used [19]. Studies have shown that essential oils are appropriate substitutions for chemical preservatives [19, 20-21]. The aim of this study was to evaluate the antibacterial effects of ZEO on *L. monocytogenes* and *S. typhimurium* in ground meat.

## 2. Materials and Methods

### 2.1. Plant preparation and Chemical Analysis

The plant of *Zataria multiflora* Boiss was collected from local groceries of Urmia, Iran. Dried aerial parts of the plant were hydrodistilled for 3 h using a Clevenger-type apparatus in the Faculty of Agriculture and Natural Sciences, Urmia University, Urmia, Iran. Dehydrated oil was stored in a dark place at 4°C for further analysis according to Hashemi et al., [22].

Chemical composition of ZEO was analyzed with a gas chromatograph (Hewlett-Packard, Santa Clara, CA; 6890N) including a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 mm) and connected to a mass spectrometer (Hewlett-Packard 5973N). The gas chromatograph program was as follows: helium flow rate was 1.5 mL/min and temperature increased from 40 to 240°C with a gradient of 3°C/min. The initial and final temperature was hold for 6 min followed by an increase to 300°C for 15°C/min holding for 3 min. Injector port and detector temperature were 290° and 250°C, respectively. Mass spectral data available in the library (Wiley 2001 data software) were used for comparing the retention index with those of samples [22].

### 2.2. Preparation of bacteria

*L. monocytogenes* (PTCC 1163) and *S. typhimurium* (ATCC 13311) were obtained from culture collection of the Department of Food Hygiene, Faculty of Veterinary Medicine, University of Urmia, and Urmia, Iran. Bacterial

strains were transferred into 15 ml Brain Heart Infusion (BHI) broth and incubated overnight at 37°C [22]. The bacterial cells were centrifuged two times at 6000 rpm for 5 min followed by two washing steps with physiological saline.  $1 \times 10^8$  cfu/ml of each bacterial culture was determined by a spectrophotometer (Biotek Instrument Inc., Winooski, VT) at 600 nm.

### 2.3. Minimum Inhibitory Concentration Value of ZEO

Lowest concentration of the ZEO, inhibiting the growth of *L. monocytogenes* and *S. typhimurium* were measured according to the method described by Aliakbarlu et al., [24]. *L. monocytogenes* and *S. typhimurium* ( $10^5$  cfu/mL of each) were transferred to a 96-well microplate containing serial twofold dilutions of the ZEO (5,000-156.25 µg/mL). After incubation at 37°C for 24 h, the absorbance was measured at 600 nm by a spectrophotometer (Biotek Instrument Inc., Winooski, VT).

### 2.4. Inoculation of ground meat with ZEO and bacteria

Beef meat was ground in a meat grinder and homogenized with different concentrations of ZEO in a mixer under sterile condition.

Half of the meat samples containing different dilutions of ZEO (0.3, 0.5, 1 and 2 ml/100 g of ground beef) were inoculated with  $10^3$  CFU of *L. monocytogenes*/g and remaining of the samples were inoculated with  $10^3$  CFU of *S. typhimurium*.

After another homogenization step (Stomacher 400 Seward, London, England), meats were stored at 7°C for subsequent examination after 3, 5, 7 and 9 days of storage. In controls samples water was added instead of ZEO and all experiments were performed in triplicate.

### 2.5. Bacterial enumeration

Twenty five grams of meat was weighted and added into a plastic bag containing 225 ml of 1% peptone water. After homogenization for 1 min (Seward Stomacher 400), samples were pre

enriched at 35°C for 24 h. For enrichment step, 1 ml of previous step sample was added into two 9 ml tubes containing tetrathionate (TT) broth and Selenite Cystine (SC) broth and were incubated at 35°C for 24 h. Xylose lysine decarboxylase agar (XLD-Merck, Darmstadt, Germany) and bismuth sulphite agar (Merck, Darmstadt, Germany) were used for selective plating and incubated at 35°C for 24-48 h. Suspected colonies on plate agar were transferred onto Triple Sugar Iron agar (TSI) for confirmation. Suspected colonies were inoculated into triple sugar iron (TSI) agar and lysine iron agar (LIA) and incubated at 35°C for 24 h. The Voges-Proskauer-m ethyl red (VP-MR) tests were performed which Salmonella genus was VP negative and MR positive by this test.

Salmonella spp showed red on slant and yellow in the butt, with blackening of agar and on LIA, purple slant with black butt [25].

For enumeration of *Listeria monocytogenes* 5 g of ground meat was homogenized with 45 ml of peptone water (0.1%). Serial dilutions were prepared and 0.1 ml of each serial dilutions was spread on CHROMagar ÔListeria (CHROMagar Microbiology, France) incubating at 37°C for 24 h. Blue colonies with a white halo were considered as positive and used for enumeration of *Listeria* [26].

### 2.6. Statistical analysis

For statistical analysis PASW Statistics 18 (formerly SPSS Statistics) was used and all experiments were carried out in triplicates. For comparison of results among experimental groups analysis of variance (one-way ANOVA) was used.

Turkey's test was also used to compare the differences among mean values during the storage ( $P < 0.05$ ).

## 3. Results and Discussion

### 3.1. Chemical compositions of ZEO

The analysis of ZEO by GC-MS revealed 26 different compounds, representing 96.27% of total oil. Thymol was the most abundant compound

among all constituents (29.2%). Other major identified components were Carvacrol (19.64%), Burneol (6.62%), Thymol methyl ether (6.55%) and o-Isopropyltoluene (5.34%). The remaining components were 28.92% in total (Table 1).

### 3.2. Results of MIC and MBC

Results of MIC and MBC of the ZEO are shown in Table 2. *L.monocytogenes* growth was inhibited at the concentration of 625 µg/mL while bactericidal effects were observed at 1250 µg/mL.

### 3.3. Effects of ZEO on *L. monocytogenes*

The population of *L. monocytogenes* increased during the storage period in the control group. The number of bacteria in the samples treated with ZEO (0.3%), increased during 3 days of storage but showed a decreasing trend during the rest of storage period. However, in the samples treated with 0.5%, 1% and 2% of ZEO, the number of *L. monocytogenes* exhibited a decreasing pattern until the end of storage ( $P<0.05$ ).

Adding 0.3% and 0.5% of ZEO declined the population of bacteria significantly after 3 days of storage ( $P<0.05$ ). The number of *L. monocytogenes* reduced significantly from the first day of storage, when treating with 1 and 2% of ZEO ( $p<0.05$ ). Regarding various concentrations of ZEO, counting the number of bacteria indicated a greater reduction when adding 1 and 2% of ZEO compared with 0.3% and 0.5% concentrations ( $P<0.05$ ) (Table 3).

### 3.4. Effects of ZEO on *S. Typhimurium*

The count of *S. Typhimurium* in the control group increased during the storage period ( $P<0.05$ ). Addition of 0.3 and 0.5% ZEO caused a significant increase in the number of bacteria after 3 days of storage and a decrease after 7 days ( $P<0.05$ ). A significant reduction was observed when adding 1 and 2% ZEO ( $P<0.05$ ) after 7 days of storage compared with 0.5% and 0.3% concentration of ZEO ( $P<0.05$ ) (Table 4).

The most abundant compounds of ZEO were thymol (29.2%) and carvacrol (19.64%) in this study. Tajik et al. [15] reported thymol (64.87%)

and Sharififar et al., [27] reported thymol and carvacrol as the predominant components among other constituents.

In the current study the antibacterial activity of ZEO was determined at various concentrations against *L. monocytogenes* and *S. typhimurium* in minced beef meat. We found out that ZEO has an inhibitory effect on the growth of *L. monocytogenes* and in higher concentrations on the growth of *S. typhimurium*.

According to the results, the rate of *L. monocytogenes* and *S. typhimurium* growth was higher in the control group than the samples treated with ZEO ( $P<0.05$ ). Results also showed that ZEO had a better inhibitory effect on *L. monocytogenes* than *S. typhimurium* and this effect was more influential in higher concentrations (1% and 2%) on both bacteria.

In a study on raw buffalo patty, it was shown that 0.02% concentration of ZEO postponed *L. monocytogenes* growth [15]. Ekhtiarzade et al., [28] observed restriction effect of 0.4% concentration of ZEO on *L. monocytogenes* in salted fish fillets. The difference in inhibitory concentrations might be due to different compositions of ZEOs species or might be due to various proportions of phenolic compounds in the ZEOs. Basti et al., [29] also represented that ZEO had a significant inhibitory effect on *S. typhimurium* in the concentration of 0.06%.

The inhibitory effect of EOs could be explained by their effect on cell membrane. As our results showed, ZEO is mostly made up of thymol and carvacrol which are lipophilic in nature. These components can act on the cell membranes and disrupt them leading to the depletion of cell contents and subsequent death of the cell [30, 31].

Similar to our study, Yamazaki et al. [32] found significant effect of thymol and carvacrol on *L. monocytogenes*. The involvement of the hydroxyl group of carvacrol, in forming hydrogen bonds, can be an explanation for the high antibacterial activity of ZEO.

In Gram-positive bacteria like *L. monocytogenes*, the peptidoglycan layer plays role as a major permeability barrier [33],

**Table 1:** Chemical composition of *Z. multiflora* essential oil using GC/MS analysis.

Number	Component	Retention Time(Min)	Total (%)
1	$\alpha$ Thujene	17.32	0.21%
2	$\alpha$ Pinene	17.71	0.67%
3	Camphene	18.19	1.63%
4	$\beta$ pinene	19.17	0.42%
5	Myrcene	19.687	0.48%
6	$\alpha$ Phellandrene	20.31	0.27%
7	Terpinene-4-ol	20.69	0.73%
8	Burneol	21.17	6.62%
9	O-Isopropyltoluene	21.18	5.34%
10	Terpinolene	23.15	1.90%
11	Limonene	21.32	0.57%
12	Linalool	23.43	4.43%
13	$\delta$ -3-Carene	23.50	0.58%
14	Gamma terpinene	26.90	3.11%
15	Thymol methyl ether	27.82	6.55%
16	Thymol	30.19	29.2%
17	Sylvestrene	30.22	3.84%
18	Carvacrol	30.84	19.64%
19	Thymol acetate	31.92	1.28%
20	$\beta$ -Caryophyllene	33.62	0.35%
21	Aromadendrene	34.54	0.34%
22	$\alpha$ -Humulene	34.37	0.17%
23	3Methylresacetophenone	36.81	2.23%
24	Spathulenol	37.49	1.28%
25	Caryophyllene oxide	37.61	0.93%
26	1-Cycloheptene	38.23	2.78%
27	$\alpha$ Terpinene	44.78	0.72%
<b>Total Number</b>			<b>96.27%</b>

**Table 2:** ZEO minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (µg/mL) against *L.monocytogenes* and *S. typhimurium*.

EO		Bacteria	
		<i>L.monocytogenes</i>	<i>S. typhimurium</i>
<i>Z. multiflora</i>	MIC(µg/mL)	625	1250
	MBC(µg/mL)	1250	2500

**Table 3:** Antibacterial effect of *Zataria multiflora* essential oil on *L. monocytogenes* in ground meat.

Day Treatment	0	3	5	7	9
Control	5.00±0 <sup>Aa</sup>	5.23±0.25 <sup>Ba</sup>	5.40±0.30 <sup>Ba</sup>	5.73±0.25 <sup>Ca</sup>	5.87±0.25 <sup>Ca</sup>
0.3% ZME	5.00±0 <sup>Aa</sup>	5.17±0.12 <sup>Aa</sup>	4.70±0.10 <sup>Bb</sup>	4.30±0.10 <sup>Cb</sup>	4.13±0.15 <sup>Cb</sup>
0.5% ZME	5.00±0 <sup>Aa</sup>	4.80±0.20 <sup>Aa</sup>	4.63±0.12 <sup>Bb</sup>	4.13±0.15 <sup>Cb</sup>	3.83±0.12 <sup>Db</sup>
1% ZME	5.00±0 <sup>Aa</sup>	4.13±0.15 <sup>Bb</sup>	3.77±0.15 <sup>Bc</sup>	3.50±0.10 <sup>Cc</sup>	3.13±0.15 <sup>Dc</sup>
2% ZME	5.00±0 <sup>Aa</sup>	3.63±0.25 <sup>Bb</sup>	3.23±0.25 <sup>Bc</sup>	3.03±0.35 <sup>Cc</sup>	2.70±0.30 <sup>Dc</sup>

Different capital letters in each row indicate significant differences among each treatment during storage ( $P < 0.05$ ). Different small letters in each column indicate significant differences.

**Table 4:** Antibacterial effect of *Zataria multiflora* essential oil on *S. thyphimurium* population in minced beef.

Day Treatment	0	3	5	7	9
Control	5.00±0 <sup>Aa</sup>	5.67±0.15 <sup>Ba</sup>	6.27±0.35 <sup>Ca</sup>	6.60±0.10 <sup>Ca</sup>	6.73±0.15 <sup>Ca</sup>
0.3% ZME	5.00±0 <sup>Aa</sup>	5.47±0.35 <sup>Bb</sup>	5.70±0.10 <sup>Bb</sup>	5.43±0.15 <sup>Bb</sup>	5.20±0.30 <sup>Cb</sup>
0.5% ZME	5.00±0 <sup>Aa</sup>	5.27±0.45 <sup>Bb</sup>	5.37±0.25 <sup>Bc</sup>	5.40±0.20 <sup>Bb</sup>	4.77±0.15 <sup>Cc</sup>
1% ZME	5.00±0 <sup>Aa</sup>	4.80±0.30 <sup>Bc</sup>	4.63±0.15 <sup>Cd</sup>	4.37±0.25 <sup>Dc</sup>	4.40±0.50 <sup>Dd</sup>
2% ZME	5.00±0 <sup>Aa</sup>	4.27±0.35 <sup>Cd</sup>	3.90±0.30 <sup>De</sup>	3.70±0.10 <sup>Ed</sup>	3.47±0.35 <sup>Ee</sup>

Different capital letters in each row indicate significant differences among each treatment during storage ( $P < 0.05$ ). Different small letters in each column indicate significant differences between treatments at the same time ( $P < 0.05$ ).

While in Gram-negative bacteria such as *S. typhimurium*, the outer membrane of bacteria has such properties [34, 35]. Our results showed that ZEO had a lower suppressive effect on the growth of *S. typhimurium* which may be due to the hydrophilic properties of outer membrane of this gram negative bacterium. Salmonella typhi was found to be the most resistant bacteria (MICs of 0.8%) against ZEO in Fazeli et al., [36] study

while Bacillus cereus and Staphylococcus aureus showed higher sensitivity (MICs of 0.1%).

### 4. Conclusion

According to the results, the growth of *S. typhimurium* and specially *L. monocytogenes* was significantly affected by some specific concentrations of ZEO. We concluded that the

essential oil of this Iranian popular herb has promising inhibitory effects on the growth of bacteria; hence, this oil can be used practically as a natural preservative in order to inhibit the growth of food-borne pathogens and extend meat shelf life.

## Conflict of Interest

None.

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