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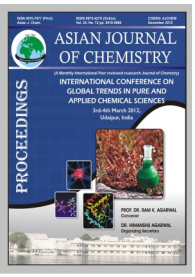


# Minimum Inhibitory Concentration of *Zataria multiflora* Boiss. Essential Oil on *Sallmonella enteritidis, Listeria monocytogenes, Escherichia coli* 0157: H7 and *Staphylococcus aureus*†

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The objective of this study was to determine the minimum inhibitory concentration of the essential oil of *Zataria multiflora* Boiss. in inhibiting *Salmonella enteritidis, Listeria monocytogenes, Escherichia coli* 0157: H7 and *Staphylococcus aureus*. The essential oil of *Zataria multiflora* was extracted with a Clevenger-type apparatus using hydro-distillation method for 2 h. The effect of *Zataria multiflora's* essential oil on growth of mentioned pathogens were determined by using microtiter plate. The minimum inhibitory concentration of *Zataria multiflora* Boiss. for *S. enteritidis* and *E. coli* were enumerated at 6250 ppm. The minimum inhibitory concentration of antimicrobial agent for *L. monocytogenes* and *St. aureus* were found at 3125 ppm.

**Key Words: Essential oil of *Zataria multiflora* Boiss., Minimum inhibitory concentration, *Salmonella enteritidis*, *Listeria monocytogenes, Escherichia coli* 0157: H7, *Staphylococcus aureus*.**

## INTRODUCTION

The increasing occurrence of food-borne disease combines with the resulting social and economic problems means there is a steady determination to produce new antimicrobial agents[1]. Concerns for the safety of some chemical preservatives and negative customer feedbacks over their use have encouraged increasing concentration in more natural green alternatives for the extension of product shelf-life[1]. According to Dillon and Board[2] spices have been used for a long time as food flavouring agents as well as in herbal medicines. Particular attention has been focused on the application of plant's extract[1,3]. This is as a result of reports that some spices and their essential oils have antimicrobial activities, such as basil, garlic, mint, onion, sumac and *Zataria multiflora* Boiss.[4].

*Zataria multiflora* Boiss. is a plant that belongs to the *Laminaceae* family and grows only in Iran, Pakistan and Afghanistan[5]. It is used as a traditional cure for its antiseptic, analgesic and carminative specifications. In addition because of its strong taste and nice aroma, it is widely used as flavouring in the wide range of foods. The antimicrobial activities of the plant are also well established against a wide variety of bacteria[1,6] and fungi[6]. However the antimicrobial activity of the plant is attributable to the phenolic compounds such as carvacrol and thymol[5,7].

Fazeli *et al.*[8] studied the antimicrobial activities of *Zataria multiflora* against some food borne bacteria. The effect of the extract of *Zataria multiflora* was examined against several Gram positive and Gram negative bacteria by using disc and diffusion methods. In this study minimum inhibitory concentration (MICs) as well as minimum bactericidal concentrations (MBCs) of the extract was considered. The results showed that this antimicrobial agent has inhibitory effect on food borne bacteria and can therefore be used as a natural preservative. Furthermore *Zataria multiflora* Boiss. Essential oil and nisin were studied by Misaghi and Akhondzadeh[9] to judge the effect on the growth and survival of *Bacillus cereus* ATML 11778. No growth was seen in combination Essential oil >= 0.005 %, Nisin >= 1.5 μg $mL^{-1}$, temperature <= 30 ºC and pH <= 7.4 in 43 days of storage.

## EXPERIMENTAL

**Preparation of essential oil:** *Zataria multiflora* Boiss. was purchased from Soha4 company, Iran. The dried plant material was employed in all extractions of essential oils. Fifty g dried leaves of *Zataria multiflora* was placed into a flask

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and the essential oil was extracted with a Clevenger-type apparatus using hydro-distillation method for 2 h (until no more essential oil was obtained). The essential oil was collected, dried with anhydrous sodium sulphate and stored at 4 ºC and kept for further usage.

**Inoculum preparation:** *Salmonella enteritidis, Listeria monocytogenes, Escherichia coli* 0157:H7 VT negative and *Staphylococcus aureus* were obtained from Microbiology Research Unit, Microbiology Research Unit, Shahid Beheshti University, Tehran, Iran. These were maintained on nutrient agar (Oxoid CM0003) slopes at 5 ºC.

The above mentioned micro-organisms were transferred from the cultures to the slopes and incubated for 24 h in 37 ºC. After that the procedure for the three sub-cultures was carried out to make sure the organisms were active and vital. The organisms were subcultured three times on consecutive days in nutrient broth (Oxoid CM0001) incubated at 37 ºC at precise 24 h intervals, followed by streaking on nutrient agar (NA) incubated at 37 ºC to check purity.

The inoculum was prepared using the third subcultured, which contained *ca*. $10^8$ cfu/mL. Decimal dilutions were made to give a concentration of *ca*. $10^6$ cfu/mL. From this a volume of 500 µL was used to inoculate the broth/avishan combinations to give a final concentration of *ca*. $10^4$ cfu/mL.

**Determination the effect of *Zataria multiflora* essential oil on growth of pathogens by using microtiter plate:** Five mL of dimethyl sulfoxide 10 % was provided by adding 4.45 mL distilled water to 0.55 mL dimethyl sulfoxide. Then the mixture of DMSO 10 % was added to 0.2 g of the essential oil of *Zataria multiflora* Boiss. to achieve $10^5$ ppm of the stock of agent.

Essential oil was prepared in sterile BHI broth. Two mL of the BHI broth was added to 10. An initial sample was taken from the stock by using the sterile syringe and needle to withdraw almost 2 mL of the sample that was transferred to the first well.

160 µL of BHI broth added to each well of the micro plate then put in 20 µL of each dilution of the essential oil and 20 µL of the $10^5$ cfu/mL. Decimal dilutions up to 97 ppm were prepared and incubated at 37 ºC for 24 h.

## RESULTS AND DISCUSSION

There are different studies about the antimicrobial activities of the essential oils of *Lamiaceae* family. *Zataria multiflora* belongs to this family as well. It shows they have two very important phenolic components such as carvacrol and thymol.

Table-1 shows the minimum inhibitory concentration of the essential oil of *Zataria multiflora* that will inhibit the visible growth of the *Salmonella enteritidis, Listeria monocytogenes, Escherichia coli* 0157: H7 and *Staphylococcus aureus* after overnight incubation.

TABLE-1
MINIMUM INHIBITORY CONCENTRATION OF *Zataria multiflora's* ESSENTIAL OIL ON FOOD-BORNE BACTERIA

| Bacteria | *MIC (ppm) |
|---|---|
| *E. coli* | 6250 |
| *S. enteritidis* | 6250 |
| *St. aureus* | 3125 |
| *L. monocytogenes* | 3125 |

The antimicrobial activity of the *Zataria multiflora's* essential oil is more effective on *L. monocytogenes* and *St. aureus* (Gram positive) than *S. enteritidis* and *E. coli* (Gram negative).

Various antimicrobial agents act by interfering with cell wall. Gram positive bacteria have a thick cell wall, high in peptidoglycan and techoic acid. Gram negative bacteria have a thinner cell wall composed mainly of a phospho-lipid bilayer. In addition, Gram negative bacteria contain an outer membrane and a periplasmic space, which contains peptidoglycan, protein ingredients[10].

Therefore the essential oil of the *Zataria multiflora* may be able to spread easily through the loose outer wall of Gram positive bacteria, but must go throughout the narrow channels of the Gram negative outer membrane.

In this project there has been two Gram positive and two Gram negative bacteria. *Salmonella enteritidis* and *Escherichia* coli are Gram negative, *Listeria monocytogenes* and *Staphylococcus aureus* are Gram positive bacteria. So two different types of behaviour of the two groups of the bacteria can possibly be explained by their different cell walls' structure. As mentioned above for Gram negative bacteria, it may be difficult for the extract to pass through the porin channels on the cell wall. Whereas for Gram positive bacteria, *L. monocytogenes* and *St. aureus*, the essential oil can get through the cell wall without difficulty. For this reason, the essential oil is more effective on *L. monocytogenes* and *St. aureus*.

To conclude the essential oil of *Zataria multiflora* might help to decrease the risk of food borne illness related to these microorganisms.

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