

Antibacterial Effects of *Zataria Multiflora* Boiss (*Shiraz oregano* essence) on Enterobacteriaceae Species

¹N. Abbasgholizadeh, ²G.H. Ettehad, ¹R. Arab, ¹A. Nemati, ²M. Barak, ³A. Pirzadeh and ¹M. Zarei

¹Department of Basic Science, Ardabil University of Medical Sciences, Ardabil, Iran

²Department of Pediatrics, Infectious Pediatric Ward, Imam Khomeini Hospital, Ardabil University of Medical Sciences, Ardabil, Iran

³ Department of ENT, Ardabil University of Medical Sciences, Ardabil, Iran

Abstract: According to increased bacterial resistance to common antibiotics, tendency toward using herbal drugs is increasing. Many researches have been executed about antibacterial and preservative effects of herbal essence like essences extracted from plants of Lamiaceae family (like *Shiraz oregano* herb). In this study, antibacterial effects of *Shiraz oregano* on Enterobacteriaceae species, is evaluating. To provide essence 50 g of dried ground leaf of plant beside 700 mL of distilled water were poured into flask and with 1 mL min⁻¹ velocity distillation the essence was extracted. Determination of microbial sensitivity was performed in the Kirby Bauer method. Among Enterobacteriaceae including *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* MIC varied between 78 and 624 µg mL. According to obtained results from this research and increasing limitations of chemical antimicrobial usage like side effects and drug resistance, there is need to replacement of these substances with natural ones and herbal essences and this issue can pave the way for studies about replacement of mentioned substances, to preserve alimentary substances and controlling of human's disease.

Key words: Antibacterial effects, *zataria multiflora*, enterobacteriaceae, bacterial resistance, *Shiraz oregano*

INTRODUCTION

Lamiaceae family is from biggest vegetable's families that have global distribution (except North and South Pole) and has 200 genus and 2000-5000 species of aromatic bush and short shrubs. Most of Lamiaceae produce Terpens and other types of compound that are stored in epidermal gland of leaves, stalks and generative organs (Baghlian and Naghdibadi, 2000; Naghdibadi and Malekizadeh, 2003) Many researches have been executed about antibacterial and preservative effects of herbal essence like essences extracted from plants of Lamiaceae family. *Zataria Multiflora* (*Shiraz oregano*) is from this family that is aboriginal of Iran, Afghanistan and Pakistan. This bush plant has multiple, thin, hard and very forked stalks with 40-80cm height. It is green to white and aromatic. Its leaf is short, with short leafstalk and can be round or elliptical. Stamens are 4 and each two of them are equal. Corolla is white and a little higher than calyx (Akhoondzadeh *et al.*, 2005; Karman *et al.*, 2001; Valero and Salmeron, 2003). Oregano is used in drug, alimentary, hygienic and cosmetic industries. Oil of oregano has properties like anti spasm, anti flatulence, anti fungi, anti rheumatism and expectorant. Essence of oregano is from 10 famous essences that have

antibacterial, anti fungal, antioxidant, natural preserver of food and retardant of mammalian's oldness and has a special place in word trade, somehow Europe and America are from major markets of oregano. Economical statistics show that America import 1000 tons oregano per year and 90% of oregano oil is producing in Spain (Naghdibadi and Malekizadeh, 2003; <http://www.crop.cri.n2/broadshe/thyme.htm>). Resistance of Enterobacteriaceae is increasing against current antibiotics. Then finding a new antimicrobial agent especially an herbal one, is important, hence this study has performed to evaluate inhibitory effects of essence of oregano on Enterobacteriaceae species.

MATERIALS AND METHODS

Zataria Multiflora Boiss (*Shiraz oregano* plant) was grinded. To provide essence 50 g of dried ground leaf of plant beside 700 mL of distilled water were poured into flask and with 1 mL min⁻¹ velocity distillation the essence was extracted. Determination of microbial sensitivity was performed in the Kirby Bauer method, in one-first dilution in number 1 of Mac Farland tube and tarnish of tube containing microbe culture was compared with it to obtain 1.5×10⁵ microbe condensation.

After providing microbe suspension, the plates were inoculated for 5 min with sterile swab smeared with microbe suspension and disk placement was performed beside flame by sterile clip. The interval between disks and plate's wall and interval between disks were determined 5 and 25 mm, respectively. Plates were conserved for 18-24 h at 37°C. After lapsing of necessary time the diameter of no growth aureole was measured with caliper (Dakhili, 1996; Dakhili *et al.*, 2006; Baron and Finedgold, 1990).

To determine MIC, different rarities of Shiraz oregano essence were provided and from each of rarities, 50 µL was added to sterile tubes containing 3 mL of bacteria and culture medium and after mixing for 18-24 h incubation (37°C). Results were determined according to tarnish of tubes and then the MIC was determined. Then samples from tubes with no tarnish were cultured in Muller Hinton medium and MBC of samples was calculated (The least of antimicrobial agents, which in less than 0.1% of primary inoculation was remained, was regarded as MBC) (Dakhili *et al.*, 2006; Baron and Finedgold, 1990).

To provide different rarities of essences two solvents (Ethanol and Tween 80) were used. Inhibitory effect (MIC) and bactericidal effect (MBC) of mentioned solvents was evaluated to ensure results that have no effect on growth of studied organism in selected rarities (0.1, 0.2, 0.4, 0.6, 0.8, 1 and 2%). Antimicrobial effect of essences was studied in disk plate method in two time stages, fresh essence and after 3 month, that suggests mentioned time lapsing had no effect on antimicrobial ability of essence. All microbes have been isolated from clinical samples (children with septicemia hospitalized at Imam Khomeini and fatemi hospitals).

RESULTS

In this study, inhibitory effects of Zataria Multiflora Boiss (*Shiraz oregano essence*) on Enterobacteriaceae species are tested. Among Enterobacteriaceae including *Escherichia coli*, *Klebsiella Pneumonia* and *Proteus Mirabilis* MIC varied between 78 and 624 µg mL (Table 1).

Table 1: MIC and MBC of Zataria Multiflora Boiss (*Shiraz oregano essence*) for Enterobacteriaceae Species

Species	Tested types numbers	MIC (µg mL)	MIC (µg mL)
<i>Escherichia coli</i>	8	78-156	156-312
<i>Klebsiella Pneumonia</i>	8	156-312	312-624
<i>Proteus Mirabilis</i>	5	156-624	312-1248

DISCUSSION

Comparison between results reported about antibacterial effects of different essences is very difficult, that differences in various methods for evaluating of antibacterial effects of different essences, resources of essences and different genus of used bacteria are from its reasons. Various studies has been performed about antibacterial effects of essences of herbs belong to Lamiaceae family (that our studied herb is one of them) and some of important compound in essences of this family among Carvacrol and Thymol (Basti and Razavilar, 2004; Akhoondzadeh *et al.*, 2004). In the study performed by Kim *et al.* (1995) antibacterial effects and calculation of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of Carvacrol on Salmonella Typhi Murium and its Rifampicin-resistant genus in Tryptic Soy Agar medium (using paper disks smeared with Carvacrol's different concentrations and determining the region of growth inhibition) and in Tryptic Soy Broth (according to measurement of growth tarnish by spectrophotometer in 540 nanometer wavelength and then culture on Tryptic Soy Agar) has been executed. They showed that Carvacrol has forceful antibacterial effects against both genuses with 250 µg mL MIC. In this research Carvacrol with 3 % concentration in 1% Tween 20, showed powerful bactericidal effect against Rifampicin-resistant genus in a sample of fish food. In another study, Karman *et al.* (2001) showed powerful bacteriostatic effect of Thymus Revolutus essence on gram-positive bacteria among Staphylococcus aerous. They illustrated high amount of Carvacrol in essence as possible reason of this effects. Similar study by Rasooli and Mirmostafa (2004) about bactericidal effects of Thymus pubescus essence (with high amount Carvacrol) on gram-positive bacteria, Staphylococcus aerous, gram-negative and *E. coli* was executed and like previous study, high amount of Carvacrol in essence was mentioned as reason of powerful bactericidal effect of studied essence. Similar results by Bagamboula *et al.* (2004) were obtained, in study about effects of Thyme and compounds of Carvacrol and Thymol on Shigella Sonei and Shigella Flexeneri. According to obtained results from this research and increasing limitations of chemical antimicrobial usage like side effects and drug resistance, there is need to replacement of these substances with natural ones and herbal essences and this issue can pave the way for studies about replacement of mentioned substances, to preserve alimentary substances and controlling of human's disease.

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