

## Effects of *Zataria Multiflora* Boiss on Common Pathogenic Gram-Positive Cocci and Gram-Negative Bacilli

R. Arab and G.H. Ettehad

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 the one 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 one mL min<sup>-1</sup> velocity, distillation the essence was extracted. Determination of microbial sensitivity was performed in the Kirby Bauer method. Minimal Inhibitory Concentration (MIC) was 156 µg mL<sup>-1</sup> for 6 of 8 *Staphylococcus aureus* and it was 132 µg mL<sup>-1</sup> for other two. MIC varies from 78-624 for Enterobacteriaceae. Inhibitory effect of garlic extract for *Pseudomonas aeruginosa* was lower in comparison with other gram-negative bacteria. 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, gram-positive cocci, gram-negative bacilli

### 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 leafs, 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 the one essences extracted from plants of Lamiaceae family. Shiraz oregano (*Zataria Multiflora*) is from this family that is aboriginal of Iran, Afghanistan and Pakistan. This bush plant has multiple, thin, hard and very forked stalks with 40-80 cm 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; Mcgimpsey, 2008). 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

Shiraz oregano plant (*Zataria Multiflora* Boiss) was grinded. To provide essence 50 gram of dried ground leaf of plant beside 700 mL of distilled water were poured into flask and with one mL min<sup>-1</sup> 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  $1.5 \times 10^5$  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 Baron EJ and Finedgold 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 tamish 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 2 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 hospital).

## RESULTS

In this study inhibitory effects of garlic extract on common bacteria that produce infections including: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were tested. All microbes have been isolated from clinical samples (children with septicemia hospitalized at Imam Khomeini hospital). From 8 staphylococcus, among 6 types MIC was 156 µg mL<sup>-1</sup> while in 2 other types MIC was 312 µg mL<sup>-1</sup> MIC was lower in *Staphylococcus epidermidis* than *Staphylococcus aureus*. Among eight type of *Staphylococcus epidermidis* MIC varied between 19.5 and 78 µg mL<sup>-1</sup> (Table 1). Among Enterobacteriaceae including *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* MIC varied between 78 and 624 µg mL<sup>-1</sup>. Inhibitory effect of garlic extract against

Table 1: Minimal Inhibitory Concentrations (MIC) of garlic extract for *staphylococcus aureus* and *staphylococcus epidermidis*

Species	Tested types numbers	Dilution	MIC (µg mL <sup>-1</sup> )
<i>Staphylococcus aureus</i>	8	1/3200 - 1/6400	156-312
<i>Staphylococcus epidermidis</i>	8	1/3200 - 1/12800	19.5-78

Table 2: Minimal Inhibitory Concentrations (MIC) of garlic extract for gram negative bacteria species

Species	Tested types numbers	Dilution	MIC (µg mL <sup>-1</sup> )
<i>Escherichia coli</i>	8	1/3200 - 1/12800	78-156
<i>Klebsiella pneumoniae</i>	8	1/3200 - 1/6400	156-312
<i>Proteus mirabilis</i>	5	1/1600 - 1/6400	156-624
<i>Pseudomonas aeruginosa</i>	8	1/12 - 1/50	20-80

*Pseudomonas aeruginosa* was lower in comparison with other Gram-negative bacteria. Among 8 studied *Pseudomonas aeruginosa* in 1 type MIC was 20 µg mL<sup>-1</sup> while in other seven types MIC was 80 µg mL<sup>-1</sup> (Table 2). This study show that garlic extract has antimicrobial effect against tested bacteria either Gram-positive and Gram-negative bacteria, with this fact that among Gram-negative bacteria MIC for *Pseudomonas aeruginosa* was 1000 times lower in comparison with other tested Gram-negative Bacilli. This result show high resistance of these bacteria versus antimicrobial agents.

## 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, 2005).

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<sup>-1</sup> 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.* (2004) 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 Rasoli 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), was 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.

#### REFERENCES

- Akhoondzadeh, B.A., A. Misaghi and S. Gheybi, 2005. Effect of oregano oil on growth of *Bacillus cereus* in Heart and brain broth. Herbs Q., 4 (16): 48-55.
- Akhoondzadeh, B.A., V. Razavilar, A. Misaghi, B. Radmehr, R. Abbasifar, D. Yazdani, B.S.H. Akhoondzadeh, 2004. Effect of Shiraz oregano (*Zataria Multiflora* Boiss) on growth probability of *Staphylococcus aerous* in Heart and brain broth. Herbs Q., 10: 53-60.
- Bagamboula, C.F., M. Uyttendaele and J. Deberere, 2004. Inhibitory effect of thyme and basil essential oils, Carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol., 4 (21): 33-34.
- Baghlian, K. and H. Naghdibadi, 2000. Plants Contain Essence, 1: 103-104.
- Baron, E.J. and S.M. Finedgold, 1990. Diagnostic Microbiology. 8th Edn. Mosby Company Philadelphia. USA, pp: 84-171.
- Basti, A.A. and V. Razavilar, 2004. Growth response and Modeling of the effects of selected factors on the time-to-detection and probability of growth initiation of *Salmonella typhi* Murium. Food Microbiol., 21: 431-438.
- Dakhili, M., 1996. Finding source of poultry isolated Salmonellae by plasmid method in Chicken industry of Ghom. Thesis of Microbiology (Msc). Azad University, 55: 132-136.
- Dakhili, M., S.T. Zahraee, G.M. Torabi, A. Khavari, 2006. Evaluation of Antimicrobial effects of four herbal essences on *Salmonella typhi* Murium and comparison with current antibiotics in veterinary. Herbs Q., 20: 21-26.
- Karman, S., M. Digark, V. Ravid and A. Ilcim, 2001. Antibacterial and antifungal activity of essential oils of Thymus Revolutus Celak from Turkey. J. Ethnopharmacol., 76: 183-186.
- Kim, J.M., M.R. Marshal, J.A. Canell, J.F. Preston and C.I. Wel, 1995. Antibacterial activity of Carvacrol, citral and Geraniol against *Salmonella typhi* Murium in culture medium and fish cube. J. Food Sci., 60: 1364-1368.
- Mc gimpsey, J., Thyme-Thymus Vulgaris. <http://www.crop.cri.n2/broadshe/thyme.htm>
- Naghdibadi, H. and T.M. Malekizadeh, 2003. A review on oregano plant (*Thymus vulgaris* L). Herbs Q., 2 (8): 1-12.
- Rassoli, I. and S.A. Mirmostafa, 2004. Antibacterial properties of thymus pubescens. Herbs Q., 9: 44-48.
- Valero, M. and M.G. Salmeron, 2003. Antibacterial activity of essential oils against *Bacillus cereus* in tyndallized carrot broth. Int. J. Food Microbiol., 85: 73-81.