

Evaluation of Antibacterial Effects of Shiraz Oregano Essence (*Zataria Multiflora* Boiss) on *Salmonella typhi* and Comparing with Antibiotics

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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 *Salmonella typhi* Murium that is one of main causes of food poisoning in human, 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. Minimal Inhibitory Condensation (MIC) and minimal bactericidal condensation for Shiraz oregano plant were 312.5 µg mL⁻¹ and 652 µg mL⁻¹, respectively. The most sensitivity of *Salmonella typhi* Murium was to *Trimethoprim sulfamethoxazole* (TMP-SMX) and Chloramphenicol and it was severely resistant to other antibiotics. Activity of Shiraz oregano essence against *Salmonella typhi* Murium was more than Tetracyclin's activity, but in comparison with *Trimethoprim sulfamethoxazole* (TMP-SMX) and Chloramphenicol is lower. According to obtained results and limitations of increasingly usage of chemical antimicrobial substances there is a need to replacement of these substances with natural and herbal essences.

Key words: Shiraz oregano, *Salmonella typhi* Murium, antibacterial effects

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 (Baghalian 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. 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; Kamran *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 (Naghdibad and Malekizadeh, 2003; Gimpsey, 2007). *Salmonella typhi* Murium is one of important bacteria of Salmonella genus and among Salmonellae has high host prevalence and is isolating frequently from human and animal species as beef, sheep, bargain and poultry. This is the main cause of food poisoning in human. After all performed activities and health advancements, salmonellosis is yet a basic problem in health of human and livestock specially poultry industry (Dakhili, 1960; Dakhili *et al.*, 2006). Resistance of Salmonellas is increasing against current antibiotics, as in some of studies salmonella's resistance against antibiotics has been reported as 95% (Dakhili, 1960). Then finding a new antimicrobial agent especially an herbal one, is important, hence this study has performed to evaluate antibacterial effects of essence of oregano and comparison between its antimicrobial effects against *Salmonella typhi* Murium with current antibiotics.

MATERIALS AND METHODS

Shiraz oregano plant (*Zataria Multiflora* Boiss) 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 *et al.*, 2006; Bron and Finedgold, 1990).

We used antibiotics of companies available in market to compare current antibiotics, after that the powder of antibiotics was provided and amount of consumed powder and volume of needful fluid to thin antibiotic solution were determined.

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; Bron 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.

RESULTS

MIC and MBC of Essence of Shiraz oregano were 312.5 and 625 µg mL⁻¹, respectively. According to anti-biogram results (Table 1), the most sensitivity of *Salmonella Typhi* Murium was to *Trimethoprim*

Table 1: Comparison between MIC and MBC of studied groups on *Salmonella typhi* Murium

Antibiotic	MIC (µg mL ⁻¹)	MBC (µg mL ⁻¹)
Chloramphenicole	7.2	14.5
<i>Trimethoprim kulfamethoxazole</i>	12	24
Tetracycline	675	1350
Shiraz oregano	312.5	625

sulfamethoxazole (TMP-SMX) and Chloramphenicole but it was severely resistant to other antibiotics. The results of this research show that the activity of oregano's essence against *Salmonella Typhi* Murium was more than tetracycline and lower than *Trimethoprim sulfamethoxazole* (TMP-SMX) and Chloramphenicole.

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. 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⁻¹ 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 (Kim *et al.*, 1995). In another study, Karman et al 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 (Karaman *et al.*, 2001). Similar study by Rasooli *et al.* (2002) about bactericidal effects of Thymus pubescens 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 Baganboula *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.

Sensitivity of *Salmonella typhi* to used antibiotics

Antibiotic disks	T	SXT	NFX	E	C
Level of sensitivity	I	S	R	R	S

T: Tetracycline (30Mcg), SXT: Trimethoprim Sulfamethoxazole (1.25 Mcg)
 NFX: Enrofloxacin (5 Mcg), E: Erythromycin (15 Mcg), C: Chloramphenicol (30 Mcg), I: Intermediate S: Sensitive R: Resistant

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