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Evaluation of Antibacterial Activity of Selected Iranian Essential Oils Against *Staphylococcus aureus* and *Escherichia coli* in Nutrient Broth Medium

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Abstract: The antibacterial effect of different concentrations (0.01 to 15%) of thyme (*Thymus vulgaris*), peppermint (*Mentha piperita* L.) caraway seed (*Carum carvi*), fennel (*Foeniculum vulgare*), tarragon (*Artemisia dracunculoides*) and pennyroyal (*Mentha pulegium*) essential oils on the *Staphylococcus aureus* and *Escherichia coli* was studied in nutrient broth medium. The MIC values of peppermint, fennel, thyme, pennyroyal and caraway essential oils against *Escherichia coli* were 0.5 ± 0.03 , 1 ± 0.03 , 0.3 ± 0.01 , 0.7 ± 0.03 and $0.6 \pm 0.02\%$ and in contrast, for *Staphylococcus aureus* were 0.4 ± 0.01 , 2 ± 0.13 , 0.1 ± 0.01 , 0.5 ± 0.02 and $0.5 \pm 0.02\%$, respectively. The MBC values of peppermint, fennel, thyme, pennyroyal and caraway essential oils for *Escherichia coli* were 0.7 ± 0.02 , 2 ± 0.05 , 0.5 ± 0.02 , 1 ± 0.02 and 0.8 ± 0.02 and for *Staphylococcus aureus* were 0.5 ± 0.02 , 4 ± 0.26 , 0.3 ± 0.02 , 0.7 ± 0.02 and 0.6 ± 0.01 , respectively. Statistical evaluation of the results indicated that the essential oils of thyme (*Thymus vulgaris*) showed the broadest spectrum of action ($p < 0.05$). Essential oils of peppermint (*Mentha piperita*), caraway seed (*Carum carvi*), pennyroyal (*Mentha pulegium*) and fennel (*Foeniculum vulgare*) had moderate effect against tested microorganisms and in contrast, tarragon essential oil were less effective against tested microorganisms. In conclusion, essential oils of edible plants could be a potential source for inhibitory substances for some foodborne pathogens. Natural substances that extracted from plants have applications in controlling pathogens in foods.

Key words: Essential oil, antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*, MIC, MBC

INTRODUCTION

The food industry has tended to reduce the use of chemical preservatives in their products due to increasing pressure from consumers or legal authorities, to either completely remove or to adopt more herbal alternatives for the maintenance or extension of product shelf life (Nychas, 1995). Medicinal plants may offer a new source of antibacterial agents for use. In many parts of the world medicinal plants are used for antibacterial, antifungal and antiviral activities (Essawi and Srour, 2000; Nevas *et al.*, 2004; Pina-Vaz *et al.*, 2004; Proestos *et al.*, 2005). Medicinal plants contain numerous biologically activity compounds, many of which have been shown to have antibacterial properties.

There is considerable interest in the possible use of these compounds as food additives, to delay the onset of food spoilage or to prevent the growth of foodborne pathogens. Among these pathogens *Escherichia coli* and *Staphylococcus aureus* are of great importance.

Although the antibacterial activity of Essential Oils (EOs) from spices has been recently reviewed (Nychas, 1995), their mechanism of action against the microorganisms has not been studied in great detail. Only

a few studies have focused on the mechanism by which spices or their essential oils inhibit microorganisms. Since essential oils consist of terpenes (phenolics in nature), it would seem reasonable that their mode of action might be related to those of other phenolic compounds. According to Conner and Beuchat (1984a and b) the antimicrobial action of essential oils may be due to impairment of a variety of enzyme systems including those involved in energy production and structural component synthesis.

The major antimicrobial components of spices and their essential oils are, for example, eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, carvacrol and thymol in oregano and thyme and vanillin in vanilla beans. The essential oil shows antioxidant (Dhuley, 1999), antibacterial (Nevas *et al.*, 2004; Friedman *et al.*, 2004) antifungal (Chami *et al.*, 2004; Wang *et al.*, 2005) and some other therapeutic activities. However, there are often large differences in the reported antimicrobial activity of oils from the same plant.

The antimicrobial activity of some essential oil components against foodborne pathogens, including mycotoxin-producing fungi, has also been tested (Kim *et al.*, 1995; Thompson, 1996; Cox *et al.*, 2000; Delaquis *et al.*, 2002; Benkeblia, 2004). More recently,

plant extracts have been developed and proposed for use in foods as natural antioxidants (Curvelier *et al.*, 1996; Basaga *et al.*, 1997; Dhuley, 1999) and/or antimicrobials (Del Campo *et al.*, 2000; Hsieh, 2001; Mejlholm and Dalgaard, 2002; Burt and Reinders, 2003).

Tests of antimicrobial activity can be classified as diffusion, dilution or bioautographic methods (Rios *et al.*, 1988; Koutsoumanis *et al.*, 1998). The principles and practice of these test methods are explained in the literature (Barry, 1976; Davidson and Parish, 1989) but it appears that no standardised test has been developed for evaluating the antibacterial activity of possible preservatives against food related microorganisms, although the need for such has been indicated (Davidson and Parish, 1989). The NCCLS method for antibacterial susceptibility testing, which is principally aimed at the testing of antibiotics has been modified for testing essential oils (NCCLS, 2000). The Minimum Inhibitory Concentration (MIC) is cited by most researchers as a measure of the antibacterial performance of EOs. The definition of the MIC differs between publications and this is another obstacle to comparison between studies. In some cases, the Minimum Bactericidal Concentration (MBC) or the bacteriostatic concentration is stated, both terms agreeing closely with the MIC.

The aim of the present study was to evaluate the antibacterial activity of several Iranian essential oils against the *Escherichia coli* and *Staphylococcus aureus* in broth media. It is well known that the activity of antibacterial varies according to the pathogen strain and may be influenced by the storage temperature of foods.

MATERIALS AND METHODS

Essential oils: The commercial and scientific names of the investigated plants are given in the Table 1. All essential oils used in this study were kindly provided by Dr. H. Aruei, Department of Horticulture, Ferdowsi University of Mashhad. The extractions were carried out by the method described by Helander *et al.* (1998), basically, plant materials (100 g) were cut into small pieces and placed in a flask (2 L) with double distilled water (1.5 L). A steam distillation continuous extraction head was attached to the flask. After steam distillation (3 h), the oils were isolated and dried over anhydrous sodium sulphate.

Strains, preparation of inocula: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were used in this study both kindly supplied by Mashhad University of Medical Sciences, Mashhad and Iran Medical Laboratory. Each culture was activated by transferring a loopful from brain heart infusion (BHI, Oxoid, CM0375) slants into nutrient broth (Oxoid, CM0001) followed by incubation at 37°C for 24 h. The optical density of each active culture was adjusted to 0.1 at 625 nm using fresh broth to give a standard inoculum of ca. 10⁶ Colony Forming Units (CFU) per ml. Bacterial counts were confirmed by plating out on Standard plate count agar (Oxoid, CM0325), plates incubated at 37°C for 24 h.

Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) test:

Dilutions of each plant essential oil to be tested were prepared in 1.0 mL volumes of sterile brain heart infusion broth to give a range of concentrations from 0.01 to 15%. After preparation of suspensions of test microorganisms (ca. 10⁶ organisms per ml), one drop of suspension (0.02 mL) was added to the extract broth dilutions. After 24 h incubation at 37°C, the tubes were then examined for growth. MIC was defined as the lowest concentration of essential oil, showing no visible bacterial growth after incubation time (Hammer *et al.*, 1999; NCCLS, 2000; Delaquis *et al.*, 2002). MBC was defined as the lowest concentration of essential oil at which no visible bacterial growth is observed after subculturing into fresh broth (Onawunmi, 1989 NCCLS, 2000). 0.1 mL from tubes that showed no visible growth added and spread on the nutrient agar medium (Oxoid, CM0003) and incubated for 24 h at 37°C for determination of MBC of essential oils. Oxacillin and norfloxacin (Sigma) served as positive controls for *Staphylococcus aureus* and *Escherichia coli*, respectively. Methanol (or water) were included in every experiment as negative controls.

Statistical analysis of data: All experiments were conducted in triplicate and tests were duplicated (two extractions). Experiment was conducted twice and data were averaged and analysed statistically using SPSS software (Version 10.0.5). The results are expressed as mean values±SE.

Table 1: Essential oils of plants investigated for antibacterial activity

Name	Botanical name	Family	Part used for essential oil extraction
Thyme	<i>Thymus vulgaris</i> Linn.	Labiatae	Leaves, Herb
Peppermint	<i>Mentha piperita</i> Linn.	Labiatae	Leaves
Pennyroyal	<i>Mentha pullegium</i> Linn.	Labiatae	Leaves, Herb
Tarragon	<i>Artemisia dracunculus</i> Linn.	Compositae	Leaves, Herb
Fennel	<i>Foeniculum vulgare</i>	Umbelliferae	Seeds
Caraway	<i>Carum carvi</i> Linn.	Umbelliferae	Seeds

Table 2: The *in vitro* antibacterial activity of essential oils from plants against bacteria

Name	Botanical name	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>	
		Mean MIC±SE (%)	Mean MBC±SE (%)	Mean MIC±SE (%)	Mean MBC±SE (%)
Thyme	<i>Thymus vulgaris</i> Linn.	0.1±0.01	0.3±0.02	0.3±0.01	0.5±0.02
Peppermint	<i>Menthae piperita</i> Linn.	0.4±0.01	0.5±0.02	0.5±0.03	0.7±0.02
Pennyroyal	<i>Mentha pullegium</i> Linn.	0.5±0.01	0.7±0.02	0.7±0.03	1.0±0.02
Tarragon	<i>Artemisia dracunculus</i> Linn.	7.0±0.40	9.0±0.55	6.0±0.16	8.0±0.21
Fennel	<i>Foeniculum vulgare</i>	2.0±0.13	4.0±0.26	1.0±0.03	2.0±0.05
Caraway	<i>Carum carvi</i> Linn.	0.5±0.02	0.6±0.01	0.6±0.02	0.8±0.02

RESULTS AND DISCUSSION

Because of *Staphylococcus aureus* and *Escherichia coli* are important bacteria among foodborne pathogens, they were selected for antibacterial assay of the essential oils (Table 2). The Minimum Inhibitory Concentration (MIC) values of peppermint, fennel, thyme, pennyroyal and caraway essential oils for *Escherichia coli* were 0.5±0.03, 1±0.03, 0.3±0.01, 0.7±0.03 and 0.6±0.02% and in contrast, for *Staphylococcus aureus* were 0.4±0.01, 2±0.13, 0.1±0.01, 0.5±0.02 and 0.5±0.02%, respectively. The Minimum Bactericidal Concentration (MBC) of peppermint, fennel, thyme, pennyroyal and caraway essential oils for *Escherichia coli* were 0.7±0.02, 2±0.05, 0.5±0.02, 1±0.02 and 0.8±0.02 and for *Staphylococcus aureus* were 0.5±0.02, 4±0.26, 0.3±0.02, 0.7±0.02 and 0.6±0.01, respectively (Table 2). Statistical evaluation of the results showed that thyme, caraway, peppermint and pennyroyal essential oils proved to be more effective in inhibiting the bacterial growth *in vitro* than fennel and tarragon essential oils (p<0.001).

The investigations of Valero and Salmeron (2003) showed that the essential oil of cinnamon, oregano and thyme were most effective on controlling of *B. cereus*. Essawi and Srour (2000) reported that *Salvia officinalis*, *Teucrium polium*, *Majorana syriaca*, *Thymus origanium*, *Thymus vulgaris*, *Commiphora opobalsamum*, *Foeniculum vulgare* and *Rosmarinus officinalis* exhibited an antibacterial effect against some of both Gram-positive and Gram-negative bacteria.

In our present study, it is deduced that the Essential oils of thyme (*Thymus vulgaris*) significantly showed the broadest spectrum of action (p<0.05). Essential oils of peppermint (*Mentha piperita*), caraway seed (*Carum carvi*), pennyroyal (*Mentha pullegium*) and fennel (*Foeniculum vulgare*) had moderate effect against tested microorganisms and in contrast, tarragon essential oil were less effective against tested microorganisms.

Determination of MIC and MBC of tested essential oils against tested bacteria showed that *Staphylococcus aureus* were significantly more susceptible to the essential oils than *Escherichia coli*. In accordance with previous findings, it seems that the antibacterial action of the essential oils is more pronounced on Gram-positive than on Gram-negative bacteria and these findings

correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plants showed activity against Gram-positive strains only (few are active against Gram-negative bacteria) (Kelmanson, 2000; Ali *et al.*, 2001). The resistance of Gram-negative bacteria towards antibacterial substances is related to lipopolysaccharides in their outer membrane (Gao *et al.*, 1999).

In literature, it has been indicated that the antibacterial activity is due to different chemical agents in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, which are classified as active antimicrobial compounds (Rojas *et al.*, 1992; Essawi and Srour, 2000). Camporese *et al.* (2003) reported that various concentrations of the plants species extracts showed activity to some extent against *Escherichia coli* and *Pseudomonas aeruginosa*, while *Aristolochia trilobata* leaves and bark *Syngonium podophyllum* leaves and bark were active also against *Staphylococcus aureus*. The highest activity was shown against the Gram-positive bacterium *Staphylococcus aureus*. A recent study demonstrated an antibacterial activity of rosmarinic acid against *Escherichia coli* and *Staphylococcus aureus* (Teng *et al.*, 2000). Sagdic *et al.* (2002) reported that between extracts of seven spices, thyme and oregano showed higher activity than others on the growth of *Escherichia coli* 0₁₅₇:H₇ strain.

CONCLUSIONS

In conclusion, edible plants could be a potential source for inhibitory substances for some foodborne pathogens. Natural substances that extracted from plants (specially, medicinal, aromatic and spice plants) have applications in controlling pathogens in foods (Davidson, 1997; Bowles and Juneja, 1998). Since the medicinal plants studied appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy.

The essential oils of thyme (*Thymus vulgaris*) showed the broadest spectrum of action against *Staphylococcus aureus* and *Escherichia coli* (p<0.05). Results reported in this study showed that essential oils of peppermint (*Mentha piperita*), caraway seed (*Carum*

carvi), pennyroyal (*Menthae pullegium*) and fennel (*Foeniculum vulgare*) had moderate effect and in contrast, tarragon essential oil were less effective against tested microorganisms ($p < 0.001$). Recently, some of these herbal drugs showed also a topical anti-inflammatory, activity that, together with the antibacterial properties supports their traditional use as wound healing in Central America. Furthermore, beside the confirmation of the popular use, the obtained results demonstrate that these herbal drugs could represent a new source of antimicrobial agents, less expensive than the imported drugs (Nychas, 1995).

The ability of edible plant extracts in inhibiting pathogenic bacteria in food matrices and the impact of plant materials on organoleptic properties of food require further studies.

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