ANTIBACTERIAL EFFECT OF SOME IRANIAN ESSENTIAL OILS AGAINST *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN NUTRIENT BROTH MEDIUM

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ABSTRACT

The antibacterial activity of various concentrations (0.01 to 15%) of peppermint (*Mentha piperita L.*) fennel (*Foeniculum vulgar*), caraway seed (*Carum carvi*), thyme (*Thymus vulgaris*) pennyroyal (*Mentha pullegium*) and tarragon (*Artmesia dracunculus*) essential oils on the *Escherichia coli* and *Staphylococcus aureus* was studied in nutrient broth medium.The MIC values of peppermint, fennel, thyme, pennyroyal and caraway essential oils against *Escherichia coli* were 0.5, 1, 0.3, 0.7 and 0.6 % and in contrast, for *Staphylococcus aureus* were 0.4, 2, 0.1, 0.5 and 0.5 % respectively. The MBC values of peppermint, fennel, thyme, pennyroyal and caraway essential oils for *Escherichia coli* were 0.7, 2, 0.5, 1 and 0.8 and for *Staphylococcus aureus* were 0.5, 4, 0.3, 0.7 and 0.6 respectively. The results of the antibacterial assay indicated that the essential oils of thyme (*Thymus vulgaris*) showed the broadest spectrum of action and peppermint (*Mentha piperita*), caraway seed (Carum carvi), pennyroyal (Menthae pullegium) and fennel (Foeniculum vulgar) 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.

KEYWORDS: Essential oil; Antibacterial activity; *Escherichia coli*; *Staphylococcus aureus*, MIC, MBC

1. 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 [1]. Many spices and herbs may be served as potential alternatives since their essential oils possess antimicrobial activity [1]. 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 [2]. 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. The assessment of antimicrobial activity of plant essential oil has been based on somewhat subjective methods as the disc diffusion method or the less accurate turbidimetry [3].

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Although the antibacterial activity of essential oils from spices has been recently reviewed [1], their mechanism of action against the microorganismshas 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,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 antimicrobial activity of some essential oil components against foodborne pathogens, including mycotoxin-producing fungi, has also been tested [6-9]. More recently, plant extracts have been developed and proposed for use in foods as natural antioxidants [10-12] and/or antimicrobials [13-15].

The aim of the present study was to evaluate the antibacterial activity of several Iranian essential oils against the *E.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.

2. MATERIALS AND METHODS

2.1 Essential oils.

The plants investigated in this study are listed in Table 1.The commercial and scientific names of the plants are given in the table 1. The essential oils of the plants extracted by Clevenger instrument, via water distillation. The plant material is placed in water, heated to a boil and the steam carrying the essential oil is condensed and collected in a receiver flask and then essential oils separated from water by separator container.

2.2 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) slants into nutrient broth (10 ml) 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. 10^6 colony forming units (CFU) per ml. Bacterial counts were confirmed by plating out on Standard plate count agar (Difco), plates incubated at 37 °C for 24 h.

2.3 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 (BHI) to give a range of concentrations from 0.01 to 15%. After preparation of suspensions of test microorganisms (ca.10^6 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 extract concentration, showing no visible bacterial growth after incubation time. 0.1 ml from tubes that showed no visible growth added and spread on the nutrient agar medium (Oxoid) and incubated for 24 h at 37°C for determination of MBC of essential oils. Absolute alcohol was similarly used as a control.

3. RESULTS AND DISCUSSION

The essential oils were tested at various concentrations against *Escherichia coli* and *Staphylococcus aureus*, ranging from 0.01 to 15%, and the evaluated MIC and MBC values are reported in Table 2. The minimal inhibitory concentration (MIC) values of peppermint, fennel, thyme, pennyroyal and caraway essential oils for *Escherichia coli* were 0.5, 1, 0.3, 0.7 and 0.6 % and in contrast, for *Staphylococcus aureus* were 0.4, 2, 0.1, 0.5 and 0.5 % respectively. The minimum bactericidal concentration (MBC) of peppermint, fennel, thyme, pennyroyal and caraway essential oils for

Escherichia coli were 0.7, 2, 0.5, 1 and 0.8 and for *Staphylococcus aureus* were 0.5, 4, 0.3, 0.7 and 0.6 respectively (Table 2).

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*) showed the broadest spectrum of action and peppermint (*Mentha piperita*), caraway seed (Carum carvi), pennyroyal (Menthae 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 teseted 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) [17-19]. The resistance of Gram-negative bacteria towards antibacterial substances is related to lipopolysaccharides in their outer membrane [20,21].

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 [2,22]. 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* [24]. 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.

Table 1. Essential oils of plants investigated for antibacterial activity.

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

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

Name	Botanical name	Staphylococcus aureus		Escherichia coli	
		Mean MIC (%)	Mean MBC (%)	Mean MIC (%)	Mean MBC (%)
Thyme	*Thymus vulgaris* LINN	0.1	0.3	0.3	0.5
Peppermint	*Menthae piperita* LINN	0.4	0.5	0.5	0.7
Pennyroyal	*Mentha pullegium LINN*	0.5	0.7	0.7	1
Tarragon	*Artemisia dracunculus LINN*	7	9	6	8
Fennel	*Foeniculum vulgare*	2	4	1	2
Caraway	*Carum carvi LINN*	0.5	0.6	0.6	0.8

4. 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 [26,27]. 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 *Bacillus cereus*. Results reportrd in this study showed that *Staphylococcus aureus* is more sensitive to essential oils tested. 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 [1].

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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