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THE IN-VITRO ANTIBACTERIAL ACTIVITY OF SOME IRANIAN ESSENTIAL OILS ON *Bacillus cereus* AND *Staphylococcus aureus*

M. Mohsenzadeh[1], H. Aroiee[2]*, R. Rezaian Doloei[3], S. Mosapor, H. Karimzadeh and Noghteh Atf Eng.Co.[4]

[1]Department of Food Hygiene and Technology,
Ferdowsi university of Mashhad, Mashhad-Iran
[2]Department of Horticulture, Ferdowsi university of Mashhad, Mashhad-Iran
[3]Department of Agriculture, Azad university of Mashhad, Mashhad, Iran
[4]Noghtehatf@hotmail.com

ABSTRACT

Medicinal plants contain numerous biologically active compounds, many of which have been shown to have antibacterial properties. In this study, the antibacterial effect of the essential oils of thyme (*Thymus vulgaris* L.), peppermint (*Menthae piperita* L.), fennel (*Foeniculum vulgar* MILL) and caraway (*Carum carvi* L.) were studied for the activity against *Bacillus cereus* and *Staphylococcus aureus*. The essential oils of thyme (*Thymus vulgaris* L.) inhibited the growths of *Staphylococcus aureus* and *Bacillus cereus*, with MIC ranging from 25 to 50 μg/ml.

KEYWORDS: Essential oil, *Staphylococcus aureus, Bacillus cereus*, Antibacterial activity, MIC

1. INTRODUCTION

In many parts of the world it has been a tradition that herbal medicines are used for the treatment of many infectious diseases. These infections may be locally within the dermis and some can subsequently become generalized as a blood infection. Because of the side effect and the resistance of pathogenic microorganisms against antibiotics, much attention has recently been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants may offer a new source of antibacterial agents for use. In many parts of the world medicinal plants are used for antibacterial, anti fungal and antiviral activities [1].

Food industry tends 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 natural-green alternatives for the maintenance or extension of product shelf life [2]. Incidences of foodborne illnesses are still a major problem, even in developed countries. It has been estimated that 6 to 81 million cases of illnesses and up to 9000 deaths annually were attributed to foodborne pathogens in the USA alone [3]. In fact, food poisoning is still a threat for both consumers and the food industry despite the use of preserving processes. Meanwhile, consumers are concerned about the safety of foods containing preservatives. Therefore, there

*Corresponding author. Tel: 00985118795613-17. E-mail: aroiee_h@yahoo.com

has been a growing interest in new and effective techniques to reduce cases of foodborne illnesses. Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties [4]. Many spices and herbs may be served as potential alternatives since their essential oils possess antimicrobial activity. Although the antibacterial activity of essential oils from spices has been recently reviewed [2], 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 [5-6] 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. 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, Staphylococcus aureus* and *Bacillus cereus* are of great importance.

Furthermore, essential oils of many aromatic plants have been shown to possess antimicrobial activities [7-8]. For example, oils from basil, bay, clove, thyme and rosemary have bactericidal activities against *Listeria monocytogenes* and other pathogens [9-10]. The aim of the present study was to evaluate the antibacterial activity of essential oils from some edible plants against common foodborne pathogens.

2. MATERIALS AND METHODS

2.1 Extraction of essential oil

Plant species evaluated in the study are listed in Table 1. The essential oils of the plants were extracted by Clevenger instrument, via water distillation. The plant material was placed in water, heated to a boil and the steam carrying the essential oil was condensed and collected in a receiver flask.

2.2 Bacterial strains and inoculum preparation

Staphylococcus aureus ATCC 25923 and *Bacillus cereus* ATCC 11778 were used in this study. A loopful of bacteria from brain heart infusion (BHI) slants was transferred into nutrient broth (10 ml) followed by incubation at 37 °C for 24 h. The optical density of each 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), and incubated at 37 °C for 24 h. Stock cultures were maintained at -80 °C in litmus milk containing 5% sterile glycerol.

2.3 Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) test

Dilutions of each tested plant essential oil were prepared in 1.0 ml volumes of sterile brain heart infusion broth (BHI) to give a range of concentrations from 50 to 3.125 µg/ml. 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 growths were then examined. MIC was defined as the lowest extract concentration, showing no visible bacterial growth after incubation time. The nutrient agar (Oxoid) plates was spread with 0.1 ml of the broth from tubes that showed no visible growth and incubated for 24 h at 37 °C for the determination of MBC. Absolute alcohol was used as a control.

3. RESULTS AND DISCUSSION

Essential oils were tested at various concentrations, ranging from 5 to 50 µg/ml, and the evaluated MIC values are reported in Table 2. Thyme and mint essential oils at concentration of 25 µg/ml could inhibit the growth of *Staphylococcus aureus* and the minimum bactericidal concentration for *Staphylococcus aureus* was 30 and 35 µg/ml of thyme and mint essential oils respectively. Thyme essential oil at concentration of 15 µg/ml and mint and caraway essential oils at concentration of 50 µg/ml inhibited the growth of *Bacillus cereus* and the minimum bactericidal concentration for *Bacillus cereus* was 25 µg/ml of thyme essential oil and more than 50 µg/ml of mint and caraway essential oils (Table 2). Fennel essential oil had no inhibitory effect on both microorganisms tested at all concentrations. Natural substances that extracted from plants have applications in controlling pathogens in foods [11-12]. The investigations of Valero and Salmeron [13] showed that the essential oils of cinnamon, oregano and thyme were most effective on controlling *B. cereus*. Essawi and Srour [1] reported that some of the 15 plant extracts tested (*Salvia officinalis*, *Teucrium polium*, *Majorana syriaca*, *Thymus origanium*, *Thymus vulgaris*, *Commiphora opobalsamum Foeniculum vulgare*, and *Rosmarinus officinalis* exhibited an antibacterial effect against some of the eight tested bacteria (both gram-positive and gram-negative bacteria). Medicinal plants appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as chemotherapy [14]. 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 natural phenolic compounds or free hydroxyl group, which are classified as active antimicrobial compounds [1, 15]. Camporese *et al.* [16] reported that various concentrations of the plants extracts showed activity to some extent against *Escherichia coli* and *Pseudomonas aeruginosa*. A recent study demonstrated an antibacterial activity of rosmarinic acid [17] against *Escherichia coli* and *Staphylococcus aureus*. Sagdic *et al* [18] reported that between extracts of seven spices, thyme and oregano showed higher activity than others on the growth of *Escherichia coli* 0157:H7. Cummin extract was the least active against *E. coli* 0157:H7.

In conclusion, the essential oil of thyme (*Thymus vulgaris* L.) was the most effective compound against *Staphylococcus aureus* and *Bacillus cereus*. Results reported in this study showed that *Staphylococcus aureus* is more sensitive to essential oils tested. Some of these herbal drugs showed also a topical anti-inflammatory activity together with the antibacterial properties and they have been traditionally used as wound healing in Central America. Besides, the confirmation of the popular use and the obtained results demonstrate that these herbal drugs could represent a new source of antimicrobial agents with less expensive than the imported drugs [16].

Table 1 Plants used in the experiments

Name	Scientific name	Family
Thyme	Thymus vulgaris *L.*	Lamiaceae
Peppermint	Menthae piperita	Lamiaceae
Fennel	Foeniculum vulgar *MILL.*	Apiaceae
Caraway	Carum carvi *L.*	Apiaceae

Table 2 The in vitro antibacterial activity of essential oils

Plant essential oil	*Staphylococcus aureus* ATCC 25923		*Bacillus cereus*	
	Mean MIC (µg/ml)	Mean MBC (µg/ml)	Mean MIC (µg/ml)	Mean MBC (µg/ml)
Thymus vulgaris L	25	30	15	25
Foeniculum vulgar MILL	>50	>50	>50	>50
Menthae piperita L	25	35	50	>50
Carum carvi L	>50	>50	50	>50

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