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# EFFECTS OF THE ESSENTIAL OILS OF IRANIAN MEICINAL PLANTS ON THE GROWTH OF SOME PATHOGENIC BACTERIA

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## ABSTRACT

The antimicrobial activities of the essential oils of four different medicinal plants (cumin, thyme, mint and fennel) were investigated on *Escherichia coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeroginosa*. The antimicrobial activity of the essential oils was evaluated by disc diffusion method. The results showed that the most effective oils were thyme (*Thymus vulgaris* L.) and peppermint (*Mintae piperita*). The use of these essential oils, should be considered in the food preservation.

**KEYWORDS:** Essential oil, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa*, Antibacterial activity

## 1. INTRODUCTION

The interest in the substitution of traditional food preservatives, as antimicrobial and antioxidants, by natural compounds has fostered research on vegetable sources and the screening of plant materials in order to identify new compounds. It has been known since ancient times that medicinal, aromatic and spice plants and their essential oils (active substances) have varying degrees of antimicrobial activities [1-14]. The major antimicrobial substances of these plants 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 [14-15]. Furthermore, essential oils of many aromatic plants have shown to possess antimicrobial activities [16-17]. For example, oils from basil, bay, clove, thyme and rosemary showed bactericidal activities against *L. monocytogenesis* and other pathogens [18-19]. Incidences of foodborne illnesses are still a major problem, even in developed countries. 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 has been a growing interest in new and effective techniques to reduce cases of food borne illnesses. There is considerable

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interest in the possible use of these compounds as food additives, to delay the onset of food spoilage or to prevent the growth of food borne pathogens.

The aim of the present study was to evaluate the antibacterial activity of some edible and medicinal plant essential oils against common pathogens.

## 2. MATERIALS AND METHODS

### 2.1 Extraction of essential oil

The plants investigated in this study are listed in Table 1. The essential oils 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 is condensed and collected in a receiver flask and then essential oils separated from water by separator container.

### 2.2 Microorganisms

*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeroginosa* were used as test organisms. The bacteria were incubated on nutrient agar slants for 48 h at 37°C followed by inoculation in Mueller Hinton Agar medium. The bacteria were supplied by the Department of Microbiology, Medical Science university, Mashhad, Iran.

### 2.3 Antibacterial assay

Antibacterial activity was demonstrated using a modification of the method originally described by Kirby-Bauer (1966) which is widely used for the antibacterial susceptibility testing [20]. A loopful bacteria was taken from the stock culture and dissolved in 1 ml of brain heart infusion broth (BHI) and incubated at 35 °C. Final cell concentration were $10^6$-$10^7$ cfu/ml. One ml of this inoculum was added to each plate containing nutrient agar. All the tests were done by placing the paper discs (50 µl for 5 mm diameter filter paper disc) with various concentrations of essential oils on the Mueller Hinton Agar surface previously inoculated with tested microorganisms. Distilled water at a concentration of 50 µl/disc provided as a negative control. Plates were incubated at 37°C for 24 h and the formation of clear zone around the disc was observed.

## 3. RESULTS AND DISCUSSION

The essential oils, having antibacterial effect, against tested bacteria were shown in Table 2. The water (control) was ineffective. Essential oils of thyme (*Thymus vulgaris* L.) and peppermint (*Mentha piperita*) were more effective than others, and in contrast, fennel and cumin essential oils were ineffective against two tested bacteria, *Proteus vulgaris* and *Psedomonas aerginosa*. The results showed that the essential oil of peppermint was more effective than thyme essential oil in controlling *Klebsiella pneumoniae*, but for other pathogens the essential oil of thyme was more effective than other essential oils. The investigation of Valero and Salmeron (2003) showed that the essential oil of cinnamon, oregano and thyme were most effective on controlling *B. cereus* [15]. Essawi and Srour reported that *Salvia officinalis*, *Teucrium polium*, *Majorana syriaca*, *Thymus origanium*, *Thymus vulgaris*, *Commiphora opobalsamum Foeniculum vulgare*, and *Rosmarinus officinalis* exhibited an antibacterial effect against both gram-positive and gram-negative bacteria [21]. It has been indicated that the antibacterial activity of some medicinal and aromatic plants are due to different chemical agents in the extracts, 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 [21-22]. *Escherichia coli*, in comparison other pathogenic bacteria, is more influenced by the essential oils,but *Psedomonas*

*aerginosa* is less influenced by the essential oils. Camporese *et al.* reported that various concentrations of the plants 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* as well [23]. A recent study demonstrated an antibacterial activity of rosmarinic acid against *Escherichia coli* and *Staphylococcus aureus* [24]. Sagdic *et al.* reported that between extracts of seven spices, thyme and oregano showed higher activity than others on the growth of *Escherichia coli* 0157:H7 strain [14]. Natural substances that extracted from plants (especially, medicinal, aromatic and spice plants) have applications in controlling pathogens in foods [25-26]. Since the medicinal plants studied appear to have a broad anti microbial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy [27].

**Table 1** Essential oils of plants used in this experiment

| Name | Scientific name | Family | Part used for essential oil extraction |
|---|---|---|---|
| Thyme | *Thymus vulgaris* L. | Lamiaceae | Leaves + Stem |
| Peppermint | *Mentha piperita* | Lamiaceae | Leaves |
| Fennel | *Foeniculum vulgar* MILL | Apiaceae | Fruits (Seeds) |
| Caraway | *Carum carvi* L, | Apiaceae | Fruits (Seeds) |

**Table 2** Screening for antibacterial activity of essential oils.

| Plant essential oil | Diameter of the inhibition zone (mm) | | | |
|---|---|---|---|---|
| | ***Escherichia coli*** | ***Proteus vulgaris*** | ***Pseudomonas aeroginosa*** | ***Klebsiella pneumoniae*** |
| Thyme | 20 | 15 | 13 | 14 |
| Mint | 16 | 14 | 12 | 15 |
| Fennel | 8 | - | - | 6 |
| Cumin | 10 | - | - | 12 |

## 4. CONCLUSION

The essential oils of thyme (*Thymus vulgaris* L.) showed the broadest spectrum of action against *Escherichia coli.* The results that reported in this study showed that *Escherichia coli* is more sensitive to essential oils tested, and essential oils of fennel and cumin had no effect on *Psedomonas aerginosa* and *Proteus vulgaris.* The obtained results demonstrate that these herbal drugs could represent a new source of antimicrobial agents with less expensive than the imported drugs [23].

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