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Comparison of Antibacterial Effects of Eucalyptus Essence, Mint Essence and Combination of Them on *Staphylococcus aureus* and *Escherichia coli* Isolates

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Abstract: Increasing resistance against antibiotics leads to trying to find new antimicrobial drugs. One source of new antimicrobial drugs is medicinal plants which are used in traditional medicine. Since ancient times medicinal plants were helpful against many diseases, these plants and their derivatives may be a new approach in case of resistant bacteria. Aim of this study was to evaluate antimicrobial effects of eucalyptus essence, mint essence and combination of them against *Staphylococcus areus* and *Escherichia coli* isolates. In this study mint and eucalyptus essence were obtained from Arasbazar pharmaceutical company and Staphylococcus areus P.T.C.C1112 and *Escherichia coli* P.T.C.C1330 were purchased from Iran industrial fungi and bacteria collection center. Disc diffusion test and well test were done to evaluate antibacterial effect of eucalyptus essence, mint essence and 1:1 ratio combination of them. Each test was done 3 times. Average diameter of inhibition zone in nutrient agar media was measured and antibacterial effects of these 3 were compared. Gentamicin (80µg/ disc) was used as reference antimicrobial material. Essence of eucalyptus in well method was the most effective against *Escherichia coli* while combination of eucalyptus and mint had the least effect against this isolate. Results of study on *Staphylococcus areus* showed that essence of mint in comparison to others had the most antimicrobial effect while combination of eucalyptus and mint had the least antimicrobial effect. In present study it was showed that eucalyptus essence had a good effect against *E. coli*. In present study surprisingly, synergistic effect was not seen in combination of mint and eucalyptus essence. Even this combination led to decrease in antimicrobial activity. Antimicrobial effect of combination 1:1 was less than mint and eucalyptus essence separately, a finding which had not been reported yet.

Key words: Eucalyptus essence · Mint essence · *Staphylococcus areus* · *Escherichia coli* · Antimicrobial effect

INTRODUCTION

Essences which are available in aromatic plants are one of the valuable compounds having different therapeutic characteristics including antimicrobial effect [1]. Increasing resistance against antibiotics leads to trying to find new antimicrobial drugs. One source of new antimicrobial drugs is medicinal plants which are used in traditional medicine. Since ancient times medicinal plants were helpful against many diseases, these plants and their derivatives may be a new approach in case of resistant bacteria [2].

Different species of eucalyptus contain essence. Genus of eucalyptus all around the world have more than 700 species, among them at least 500 species have essence. Essence or leaves of eucalyptus is used for relief of respiratory system inflammation in diseases such as bronchitis or diphtheria [3].

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Leaves or essence of some species of eucalyptus are used in treatment of some special fevers including fevers due to malaria or typhoid and also in treatment of some dermal inflammations and scars of burning. Also, hydro extract of some species of eucalyptus are used for treatment of bacterial melena, joint pains and relief of tuberculosis symptoms in western and eastern traditional medicine. Eucalyptus essence also has antioxidant and anti-inflammatory effects [4].

Essence of eucalyptus and its major component, 1 and 8 cineol is widely used in industry in manufacturing of softeners, pomades, antitussive syrups, toothpastes and also as flavor in many medicines. Also this component is used as fragrance in soaps, powders and other washing materials [5].

Several studies are done on antimicrobial properties of some eucalyptus species essence. In a study done on antimicrobial effect of eucalyptus essence on 4 gram positive and negative bacteria were studied. Results showed that eucalyptus essence poses moderate antimicrobial effect and in studied isolates, *Bacillus subtilis* had the most susceptibility. In another study, antimicrobial effects of eucalyptus essence on *Klebsiella*, *Pseudomonas*, *Proteus*, *Escherichia coli* and *Staphylococcus areus* were measured. Obtained data revealed that *Klebsiella* and *Escherichia* were more susceptible in comparison to others and susceptibility of *Pseudomonas* and *Proteus* were less than other isolates [6]. It was reported that this essence is also effective against methycyline resistant *Staphylococcus areus* and in treatment of local infections [7, 8].

Mint is one of the most important medicinal plants which have a wide use in pharmaceutical, food and hygienic industry [9, 10]. According to recent researches, its useful effect in control and treatment of irritable bowel syndrome are approved. Also this plant is used in treatment of inflammatory bowel diseases and gall bladder and liver insufficiencies [11]. Regarding performed studies, menthol is the major component of mint. Menthol is used as a tonic in gastric problems, fever reducer, antitussive and antiemetic and also as a disinfectant in pulmonary inflammations [12]. This component of mint is widely used in food, cosmetic and perfume industry, also in pharmaceutical products [11].

Menthol is known as a disinfectant with effective antimicrobial properties [13, 14]. In 2002, Aridogan *et al*., studied the antimicrobial effects of mint essence. They showed that mint essence is a potent antimicrobial agent against *Staphylococcus areus* and *Escherichia* [15]. In another study done by Iscan *et al.*, Antimicrobial effect of mint essence against 21 pathogen microorganisms was determined. Reported results showed that this essence strongly inhibits growth of pathogen microorganisms [16].

Based on available evidence and regarding to developing resistance of bacteria against antibiotics, using of antimicrobial agents derived from medicinal plants as new natural bacteriostat or bacteriocide agents against pathogens should be considered more. Present study aimed to evaluate antimicrobial effects of eucalyptus essence, mint essence and combination of them against *Staphylococcus areus* and *Escherichia coli* isolates.

MATERIALS AND METHODS

In this study eucalyptus and mint essences were obtained from Arasbazar pharmaceutical company, Amol, Iran and *Staphylococcus areus* P. T.C.C1112 and *Escherichia coli* P.T.C.C1330 isolates were purchased from Iran industrial researches center. Comparison of antimicrobial effects of these two essences and combination of them was done by disk diffusion and well methods. Gentamicin 80 µg/disc was used as reference antimicrobial agent.

Disk Diffusion Method: First a 0.5 Mc Farland suspension of bacterial isolates equal to 1.5× 10 was prepared, then 100µl of suspension was cultured uniformly on nutrient agar media. After that, sterile blank disks were put on microbial media and by a sampler disks were smeared with dilution of 10, 20, 30 and 40 of essences. A disk containing 80µl of gentamicin was used as positive control in all plates. Plates were incubated in 37°C for 24 hours and then diameter of inhibition zone were measured based on millimeters by a ruler.

Well Method: First, using a sterile Pasteur's pipit, 5 wells with defined distances were made on nutrient agar media. In second step 100 µl of suspension were uniformly cultured on media. Then, by a sampler dilution of 50, 60, 70, 80 and 90 of essences were poured in wells. Plates were incubated in 37°C for 24 hours and then diameter of inhibition zone around disks by ruler.

RESULTS

In disk diffusion method for bacteria of *Escherichia coli* for all of 3 agents in dilutions of 10 to 40 inhibition zone were not observed. For *Staphylococcus areus* in dilution of 10 for all 3 agents no inhibition zone was

Table 1: Results of antimicrobial effects of mint essence, eucalyptus essence and combination of them on Staphylococcus areus in disk diffusion test (based on mm)

Studied agent	10µl	20µl	30µl	40µl
Mint essence	---	10	12	13
Eucalyptus essence	---	7	10	11
Mint+ Eucalyptus	---	---	8	10
Gentamicin	35	---	---	---

Table 2: Results of antimicrobial effects of mint and eucalyptus essence and combination of them against *Escherichia coli* isolate in well method (based on mm)

Studied agent	50µl	60µl	70µl	80µl	90µl
Mint essence	12	13	15	16	17
Eucalyptus essence	11	12	14	15	16
Mint+ Eucalyptus	8	10	12	13	14

Table 3: Results of antimicrobial effect of mint essence, eucalyptus essence and combination of them on Staphylococcus areus isolate in disk diffusion test (based on mm)

Studied agent	50µl	60µl	70µl	80µl	90µl
Mint essence	26	27	29	31	33
Eucalyptus essence	23	24	25	26	28
Mint+ Eucalyptus	9	11	15	19	20

created and for combination of eucalyptus and mint essence (1:1) also in dilution of 20 inhibition zone was not observed. In combined essence inhibition zone was measurable from dilution of 30 and for mint essence and eucalyptus essence it was measurable from 20 (Table 1).

Well Method: In this method essence of eucalyptus was the most effective against *Escherichia coli* while combination of eucalyptus and mint had the least effect against this isolate (Table 2).

Results of study on *Staphylococcus areus* showed that essence of mint in comparison to others had the most antimicrobial effect while combination of eucalyptus and mint had the least antimicrobial effect.

DISCUSSION

Medicinal plants by different mechanisms from antibiotics inhibit bacterial growth; this makes additional researches in medicinal plants uses area essential [17].

Regarding bactericidal effects of essences it is mentioned that one of important characteristics of these components is their hydrophobic nature which leads to distribution of them in lipid parts of cell wall or bacterial mitochondria and cause disruption in their structure and permeation. Many ions and other vital cell components leave microbial cells and leads to cell death [18, 19].

In present study antimicrobial effects of mint and eucalyptus essence and combination of them on *Staphylococcus areus* and *Escherichia coli* was evaluated. Results showed that mint essence possessed good antimicrobial effect against gram positive bacteria of *Staphylococcus areus* but its effect on *Escherichia coli* was poor. In line with these results a study done by Mahboubi *et al*., reported that menthol showed good antimicrobial effect against gram positive bacteria, fungi and yeasts. But against *E. coli*, *S. typhi* and *P. aeruginosa* it was a poor antimicrobial agent [20].

Davoodi and coworkers in comparison of antimicrobial effect of pepper mint essence and tetracycline on *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhimorium* reported that this essence comparing to tetracycline had significant antibacterial effect on *Escherichia coli* and *Salmonella typhimorium* isolates. A finding which is not parallel with the results of present study [21].

In present study it was showed that eucalyptus essence had a good effect against *E. coli*. According to a research by Mahboubi *et al*., essence of eucalyptus showed good antimicrobial effect against *Aspegilus flavus, Vibrio cholera* and *Staphylococcus aureus* [20].

Dadgar *et al*., showed that among 20 species which were studied for antibacterial effect, ethanolic extract of eucalyptus showed a very good antibacterial effect against Staphylococcus areus resistant and susceptible to methycyline isolates [22].

Shan *et al*., 2007 in a study on in vitro antibacterial activity of dietary spice and medicinal herb extracts showed that, mint caused a 9.8 mm diameter of inhibition zone on *Staphylococcus areus* culture and 4.6 mm for *Escherichia coli*, which is in common with present study that inhibition zone of mint for *S. areus* was more than *E. coli* [23]. Gram positive are known to be more susceptible to essential oils than gram negative bacteria [24, 25]. The weak antibacterial activity against gram negative bacteria was ascribed to the presence of an outer membrane [26, 27] which possessed hydrophilic polysaccharide chains as a barrier to hydrophilic essential oils.

Inouye *et al*., 2001 reported that *E. coli* was the least susceptible bacteria to 14 different essential oils, including mint and eucalyptus. They also demonstrated that susceptibility of *Staphylococcus areus* to mint essence was more than *Escherichia coli* [28].

In present study surprisingly, synergistic effect was not seen in combination of mint and eucalyptus essence. Even this combination led to decrease in antimicrobial

activity. Antimicrobial effect of combination 1:1 was less than mint and eucalyptus essence separately, a finding which had not been reported yet. This finding might be due to some components in mint and eucalyptus essences which are antagonists and might neutralize each other and weaken their antimicrobial activity. Further studies are needed for precise describing of this phenomenon.

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