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Research Article

Evaluation the Antibacterial Effects of Two Commercial Products of *Eucalyptus globulus* Against Common Microbial Causes of Respiratory Tract Infections

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ABSTRACT

Background: Recently, antimicrobial activity of medicinal plants have attained once more importance due to drug resistance of microbial isolates to common antibiotics as well as fewer side effects and low cost of herbal products comparing to chemical drugs. *Eucalyptus globules* (*E. globulus*) has been widely applied as a natural remedy in respiratory tract infections. The present study focused on the evaluation of antibacterial effect of two commercial products of *E. globulus* against common microbial causes of respiratory tract infections. To this end, two commercial products of *E. globules* including inhaler and oral soft capsule with standard expiration date, (in three different batch numbers) were purchased from the pharmacy stores of Tabriz city.

Methods: The antibacterial efficiency of these products were investigated using *Minimum Inhibitory Concentration* (MIC), *Minimum Bactericidal Concentration* (MBC) and disk diffusion methods against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomona aeruginosa*.

Results: Based on the obtained results, these commercial products of *E. globules* showed significant inhibitory effects against Gram-positive bacteria. The findings also indicated that the *Eucalyptus* inhaler products had more inhibitory effects than *Eucalyptus* oral soft capsule, however batch to batch variations were of concern.

Conclusion: This research presents optimistic result on using the *Eucalyptus* as an alternative antibacterial agent against respiratory tract pathogenic microorganisms.

Introduction

Medicinal plants have been recognized and applied throughout the human history. Some herbal extracts has reported to be efficient for the treatment of various diseases, mainly infectious diseases.[1-6] *Eucalyptus* is a diverse genus of flowering trees and more than 700 species of *eucalyptus* are mostly native to Australia[1.] *Eucalyptus globulus* (*E. globulus*) is the most widely cultivated species in subtropical and Mediterranean regions.[1] Great attention has been focused on the medical properties of this plant in recent years. Research data has confirmed that the extracts of *eucalyptus* revealed several biological effects including antibacterial, anti hyperglycemic, antioxidant effects as well as stimulating and antisepticise activities.[7-10] The results of a research by Takahashi et al showed that the *eucalyptus* extracts and three compounds from *eucalyptus maculate* had effective antimicrobial effects against microorganisms that cause food poisoning, acne and athlete's foot.[1] In another work, Dakov et al reported the antimicrobial activity for the essential oil of *E. globulus* against *Streptococcus pyogenes (S. pyogenes)*, *Escherichia coli (E. coli)*, *Candida albicans*, *Staphylococcus aureus (S. aureus)*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Their MIC results showed the lowest activity against *Pseudomonas aeruginosa (P. aeruginosa)* and *Salmonella infantis* (3.13 mg/ml) while the highest activity was against *S. aureus*, *E. coli*, and *S. pyogenes* (0.09 mg/ml).[9] It has also been reported by Yamakoshi et al that macrocarpals from *E. macrocarpa* were effective

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against Gram positive bacteria including *S. aureus* and *Bacillus subtilis.*[10]

In the present study, we investigated the antimicrobial activities of some commercial products of *E. globulus* including inhaler and oral soft capsule, with standard expiration date and in three different batch numbers, against respiratory tract pathogenic microorganisms *including S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa* using MIC, MBC and disk diffusion methods.

Material and Methods

Media and chemicals

All media [*Mueller-Hinton agar* (MHA), *Mueller-Hinton broth* (MHB) and Twin medium] were obtained from *Merck* (Darmstadt, *Germany).* Barij Eucalyptus Soft capsule containing 200mg Eucalyptus globulus essence and Eucalyptus inhaler of Barij containing 28 mg 1,8 cineole , 3mg thymol and 10mg menthol were purchased from pharmacies in Tabriz, Iran.

Microorganisms

The microorganisms used for antimicrobial assay were obtained from Iran's Biotechnology Institute of Scientific and Technical Research (Tehran, Iran) that were as follows: *Staphylococcus aureus* PTCC 1112, *Streptococcus pyogenes* PTCC 1447, *Escherichia coli* PTCC 1338, *P. aeruginosa* PTCC 1074.

Inoculum preparation

The bacteria were activated according to standard protocol and the cultures of bacteria were maintained in their proper agar media as the stock cultures. To prepare the inoculum, a single colony from the bacterial stock cultures was transferred into Mueller Hinton Broth and incubated overnight at 37 ˚C. Then, cells were collected by centrifugation (3000 rpm) for 10 min. Collected cells were washed twice and re-suspended with a sterile physiologic saline solution (0.9% (w/v) sodium chloride) to achieve the inoculum approximately equal to 10^6 CFU/mL^{-1}.[11]

Sample preparation of eucalyptus*'s commercial products*

1 ml of samples was added in the first tube and twofold serial dilutions were prepared using sterile buffer. For *eucalyptus's* inhaler, 1 ml of solution was used directly while for *eucalyptus*'s oral soft capsule, 1 ml of capsule's content was collected and used**.**

Determination of MICs and MBCs

The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of a bacterium, whereas the minimum bactericidal concentration (MBC) is the concentration that results in microbial death. MICs of the products were determined for most sensitive bacterial species. For MIC determination, 100 µl of bacterial inocula was transferred into the tubes and all tubes were incubated for 24 h at 35 ˚C. After 24 h incubation of dilution tubes, the first tube of the series with no sign of visible growth was considered as the MIC. This process has been done three times.

MBC was determined for each set of test tubes in the MIC assay as follow; a loop full of broth was collected from the tubes without any visible growth and cultured at 37°C for 24 h. The highest dilution that shows no colony formation on solid medium was considered as MBC.

Disc diffusion method

Disc diffusion method was performed using filter paper discs (about 6 mm in diameter). The discs were autoclaved and impregnated by 30 µl of solution of the antimicrobial products and placed on the Muller Hinton Agar plates. After 24 h incubation (37°C), inhibition zone diameters were read.

Results

MIC and MBC results

Based on the obtained results for MIC and MBC, the commercial products of *eucalyptus* exhibited inhibitory effects on both Gram positive and Gram negative bacteria. However, the inhibitory effects of these products were relatively stronger against Gram positives compared to those of Gram negatives. The results also showed that the *eucalyptus* inhaler had more inhibitory effects than *eucalyptus* oral soft capsule.

The results of MICs and MBCs against four selected bacteria are shown in Table 1. Also the streak cultures related to the MBC determinations are provided in figures 1 to 4.

Table 1. Results of MICs and MBCs against 4 selected bacteria.

Eucalyptus products	***Staphylococcus aureus***		***Streptococcus pyogenes***		***Escherichia coli***		***Pseudomonas aeruginosa***	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
Inhaler	1/32	1/16	1/16	1/16	1/16	1/8	1/16	1/8
Soft capsule	1/4	1/4	1/8	1/4	1/8	1/8	1/2	1/2

Table 2. Results of disk diffusion method against 4 selected bacteria.

Eucalyptus products	Mean zones of growth inhibition (mm)±SD* (n=4)			
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*
E*ucalyptus* inhaler	22±2	13±3	11±2	10±3
***Eucalyptus* soft capsule**	ND**	4±1	6±1	ND

*Standard Deviation
**Not Detected

Figure 1. streak cultures with eucalyptus on *Staphylococcus aureus* PTCC 1112.

Figure 2. Streak cultures with eucalyptus on *Streptococcus pyogenes* PTCC 1447.

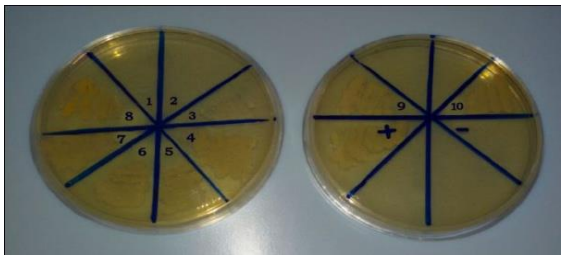

Figure 3. Streak cultures with eucalyptus on *Escherichia coli* PTCC 1338.

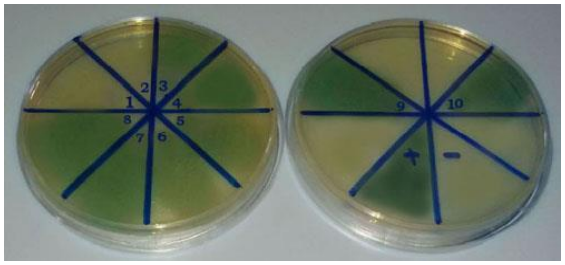

Figure 4. Streak cultures with eucalyptus on *Pseudomonas aeruginosa* PTCC 1074.

Disk diffusion method

The inhibition zone diameters obtained by disk diffusion method against four selected microorganisms are shown in Table 2. The results indicated that the *eucalyptus* inhaler had more inhibitory effects than *eucalyptus* oral soft capsule using the filter paper disc agar diffusion technique. The results also showed that the e*ucalyptus* inhaler had strong activity against *S. aureus* (inhibition zone 22 mm), moderate activity against *S. pyogenes* (inhibition zone 13 mm) and weak inhibition effect against *E. coli* (inhibition zone 11 mm) and *P. aeruginosa* (inhibition zone 10 mm). In the case of e*ucalyptus* oral soft capsule, overall findings showed weak activities against the tested microorganisms. *E. coli* with inhibition zone of 6 mm and *S. pyogenes* with inhibition zone of 4 mm are the susceptible microorganisms. Interestingly the applied *eucalyptus products* showed no activity against *S. aureus* and *P. aeruginosa* in disk diffusion method.

In this study, two commercial products of *eucalyptus* were used with three batch numbers; each experiment was carried out in four replications. The sample of experiment was shown in Figures (5 to 8).

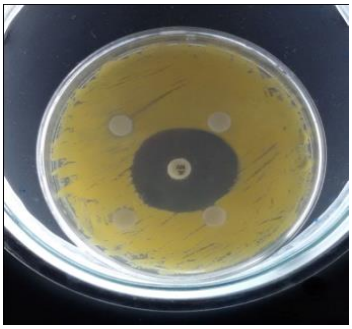

Figure 5. Disk diffusion method with Eucalyptus commercial product on *Staphylococcus aureus* PTCC 1112.

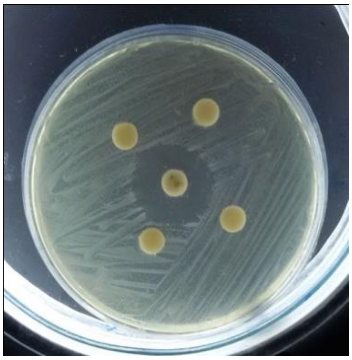

Figure 6. Disk diffusion method with Eucalyptus commercial product on *Streptococcus pyogenes* PTCC 1447.

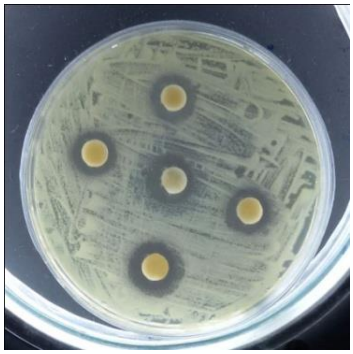

Figure 7. Disk diffusion method with Eucalyptus commercial product on *Escherichia coli* PTCC 1338.

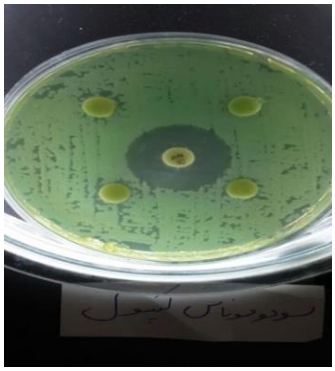

Figure 8. Disk diffusion method with Eucalyptus commercial product on *Pseudomonas aeruginosa* PTCC 1074.

Discussion

The present study aimed to evaluate the antibacterial effect of two commercial products of *E. globulus* including inhaler and oral soft capsule against common microbial causes of respiratory tract infections.

Barij Eucalyptus Soft capsule containing 200mg Eucalyptus globulus essence and Eucalyptus inhaler of Barij containing 28 mg 1,8 cineole , 3mg thymol and 10mg menthol with standard expiration date, (in three different batch numbers) were used. Based on the results of our experiments, Eucalyptus products both showed antimicrobial activity against the tested strains. However the activity was relatively stronger against Gram positives compared to those of Gram negatives. The results also showed that the *eucalyptus* inhaler had more inhibitory effects than *eucalyptus* oral soft capsule.

According to reports, the antibacterial activity of *eucalyptus* extracts has been due to the chemical components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, β- pinene, γ-terpinene, α- terpinol, alloocimene and aromadendrene.[12-14] Furthermore, it has been demonstrated that Gram positive bacteria are more susceptible to essential oils and herbal extracts than Gram negative bacteria.[15]

Nevertheless, *Eucalyptus* has shown antimicrobial effect against both Gram positive and Gram negative bacteria. The results of a work by Bachir et al showed that essential oil of the leaves of *E. globulus* has antimicrobial activity against Gram negative bacteria (*E. coli*) as well as Gram positive bacteria (*S. aureus*).[13] These results are also similar to the results reported by other researchers on the antimicrobial activity of essential oil of *E. globulus* leaves and other similar species.[15-17] Gram negative bacteria are *more resistant* against antimicrobial agents because of *their* impermeable *cell wall* owing to lipid and *lipoprotein* content that form a barrier to hydrophobic compounds.[18]

Sattari et al study showed that the aqueous and alcoholic extract of *Eucalyptus* had antibacterial activity of against *P. aeruginosa* and the inhibitory effect of this plant on *P. aeruginosa* is consistent with our study. This study also suggested that the effect of commercial products such as essence eucalyptus inhaler of *Eucalyptus* should be studied on bacteria.[19]

Jahan et al study shows that the phytochemicals of *Eucalyptus* had tannins, saponins, glycosides and steroids and growth inhibitory zone for *E. coli* in the form of *Estonia* to be 10 mm, in the form of methanol was 14 mm and 6 mm in aqueous form.[20]

In another study that was conducted by Gogte et al, using disk diffusion technique, the diameter of the growth of *eucalyptus* on *S. aureus* was 20 mm. The results of this study were consistent with our finding about essence eucalyptus inhaler and inhibition zone diameter eucalyptus on *E. coli* was 15 mm. Also the MIC for *S. aureus* was in dilutions of 1/8 V/V, which is partly consistent with *eucalyptus* oral soft capsule; of course antibacterial effect of capsule is weaker than the inhaler. In general, the results were in line with our results.[21]

In a study on the antimicrobial effect of *E. globulus* and several other plants *(thyme, Satureja khuzestanica, Origanum vulgare*) when combined, the antimicrobial effect of *eucalyptus* reduced.[22] In another study the antibacterial effect of eucalyptus and *mint*, when the two plants were used in combination, the antibacterial effect decreased.[23] Possible interactions between the ingredients may be a probable mechanism for decreasing the antimicrobial activity in combination.

Conclusion

The prepared solutions from two commercial products of *E. globulus* showed different degrees of antibacterial effect against four selected bacteria commonly isolated from respiratory tract infections. The results suggest that the products have significant growth inhibiting effects against both Gram positive and Gram negative bacteria. The results indicate that essence *Eucalyptus* inhaler

is far more powerful than *Eucalyptus* oral soft capsule in used concentration. The efficacy of these products may provide a new way for the prevention and treatment of infectious diseases caused by various bacteria that have developed resistance to antibiotics. As a common procedure, the incorporation of this herb into the antimicrobial drug formulations can also be recommended.

Conflict of interests

The authors claim that there is no conflict of interest.

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