Iranian Journal of Pharmaceutical Research (2011), 10 (1): 43-48
Received: October 2008
Accepted: September 2009

Original Article

The Essential Oils Activity of *Eucalyptus polycarpa*, *E. largiflorence*, *E. malliodora* and *E. camaldulensis* on *Staphylococcus aureus*

Yunes Panahi[a*], Morteza Sattari[b], Ahmad Pour Babaie[c], Fatemeh Beiraghdar[d], Reza Ranjbar[e], Ali Hedaiat joo[c] and Mohsen Bigdeli[f]

[a]*Research Center of Chemical Injuries, Baqyiatallah Medical Sciences University, Tehran, Iran.* [b]*Department of Microbiology, Tarbiat Moddares University, Tehran, Iran.* [c]*Department of Microbiology, Faculty of Sciences, Islamic Azad Unversity, Qom, Iran.* [d]*Nephrology and Urology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.* [e]*Molecular Biology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.* [f]*Aricultural Research and Training Center, Agriculture Ministry, Iran.*

Abstract

Because of its resistance to antibiotics, *Staphylococcus aureus* causes many of problems in hospital and society. As one of the main reasons of clinical infections it can cause to serious surgical and cutaneous infections and pneumonia. The inhibitory effect of the essential oils include; *Eucalyptus largiflorence, E. camaldulensis, E. malliodora* and *E. polycarpa* as a natural and herbal antimicrobial substances on *Staphylococcus aureus* ATCC 25923 and other antibiotic resistant series separated from clinical samples were evaluated. The minimum inhibition concentration (MIC) of the *Eucalyptus* essential oils was appointed by the dilution method in the tube and the results revealed that the values for the *E. camaldulensis* 1/256 v/v, *E. largiflorence* 1/512 v/v, *E. malliodora* 1/256 v/v and *E. polycarpa* 1/128 v/v were 3.9 µg/mL, 1.95 µg/mL, 3.9 µg/mL and 7.8 µg/mL, respectively. Eucalyptol is the major compound of the *Eucalyptus* essential oil. The MIC of the pure eucalyptol appointed by the dilution method in the tube was equal to 1.95 µg/mL. In conclusion, anti *Staphylococcus* activity of the *Eucalyptus* essential oils suggested it's clinically useful potentials, although further studies are required.

Keywords: *Eucalyptus polycarpa*; *Eucalyptus largiflorence*; *Eucalyptus malliodora*; *Eucalyptus camaldulensis*; *Staphylococcus aureus*; Eucalyptol.

Introduction

Staphylococcus aureus, an important pathogen in human, causes serious infections. This bacterium is known as one of the most resistible agent against typical antibiotics. It also causes plenty of problems in hospitals and society. Therefore finding of new and effective antimicrobial agents derived from new resources for such resistant bacteria is of an over riding importance.

Nowadays, scientists and clinicians are looking for more efficient drugs, derived from natural and herbal resources, against microbial and viral infections (1). Plants are considered as rich sources of antibiotic treatment medications and eucalyptus leaf with its antimicrobial properties have been used in the treatment of infectious diseases formerly in ancient medicine (2-5).

Several compounds have been extracted

* Corresponding author:
E-mail: yunespanahi@yahoo.com

from *Eucalyptus* as biological sources such as uglobals, grandinol, macrocarpals, eucalymin, robostadial, 1, 8-eineole and eucalypton with antimicrobial and remedial activities (1, 6-9).

The major compound of *Eucalyptus* is 1, 8-cineole with antimicrobial specifications (3, 5). According to recent studies carried out by Pattnaik *et al*. (10) *Eucalyptus* leaf and its essential oil showed a great antimicrobial activity. They used dilution method to measure the MIC of eucalyptus oil on the *E. coli*-sp11 bacterium and estimated value was about 2.5 µL/mL (10). Study by Adebolla *et al*. designed to evaluate the antimicrobial effect of five different eucalyptuses via diffusion in agar revealed that the obtained MIC for *Candida albicans* and gram positive and negative bacteria was 5 mg/mL (5).

Antimicrobial activity of *Eucalyptus* oil on 22 bacteria and 12 fungi via disc diffusion method conducted in a study by Pattnaik *et al*. (11) and results showed that the *Eucalyptus* oil is effective against 22 kinds of bacteria and 11 kinds of fungus. The MIC of bacteria was between 0.16 to 20 µL/mL where it was 0.25 to 10 µl/mL for fungus. Another study by Farah *et al*. (12) also evaluated the antimicrobial activity of the *E. camaldulensis* leaf oil. They mentioned that diluted 1/100 v/v oil is necessary to prevent microbes' activities. *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus* are all sensitive to the dilution 1/500 v/v and *Bacillus subtillis* was sensitive to the dilution 1/250 v/v (12). Pasi *et al*. (13) studied the antimicrobial oil of the Greek *Eucalyptus* and used gram positive bacteria *Staphylococcus aureus* and gram negative ones such as *Escherichia coli*, *Klebsiella* and *Pseudomonas aeruginosa*. The results showed that the *Eucalyptus* oil reacted against all bacteria and fungi effectively and the MIC was estimated 0.3 to 0.6 mg/mL (13).

Currently the antimicrobial extract and oil of *Eucalyptus* have been registered in the pharmacies of england, france, germany, india, japan and united state. According to recent studies *Eucalyptus* has shown an enormous antimicrobial activity and it also applied to produce new chemical compounds form other antibiotics (1).

The aim of present study was to evaluate antimicrobial activity *of E. camaldulensis, E. polycarpa, E. malliodora* and *E. largiflorence* on the *Staphylococcus aureus* ATCC25923 and other clinical resistant series.

Experimental

The ratification of bacterium series

To ensure the materials and kinds of the supplied bacteria, gram stain, oxidase experiments, coagolase, DNAse, catalase, movement in the semi-solid environment, the fermentation of mannitol, hemolysin, sensitivity to basitracin and culture in the liquid medium with 15% salt were carried out.

Essential oil extraction and diagnosis of it's chemical compounds

Eucalyptus leaves supplied from Fadak garden in Dezfoul city (southwest of Iran) in July 2003. We cut 50 g of the dried *Eucalyptus* into pieces and oil extracted by distillation apparetus in 200 mL of distilled water. The distillation velocity was between 2 to 3 mL/min and the process lasted 3 h. Acquired essential oil was injected to GC/MS instrument in order to separation. Finally compound analyzed after absorption on anhydrate sodium sulfate (14).

The GC-MS instrument (Hewlett-Packard 5973 Bston HP-5MS) was kept at 60°C for 3 min after injection where it can increase the temperature up to 220°C and keep at this temperature for 5 min. The velocity of gas carrying helium was 1 mL/min and analysis gas chromatography was 70 ev/min, in which the compounds were separated and analyzed.

The estimation of MBC and MIC by the dilution method in the tube and the second culture in agar

Dilution method was used to measure MIC. Colony made from 24 h culture of bacterium inoculated to Mooler Hinton Berath culture medium. This suspension was inoculated at 37°C for about 4 to 6 h in order to get the bacteria to the dynamic level and compared to Macfarland 0.5 standard at last. As a result the suspension contains 10 bacteria in each mL. Microbial suspension was diluted to the proportion of 1/100 in order to reach 10^6 bacteria in each mL.

To measure the MIC, 1 mL of Mooler Hinton

Table 1. The obtained results from the ratification of *Staphylococcus aureus* series.

Staphylococcus aureus series	Salt15	Hemolysin	Mannitol	Catalase	Coagulase	Oxidase
ATCC25923	+	*β*	+	+	+	-
Clinical samples						
Sputum	+	*β*	+	+	+	-
Wound	+	*β*	+	+	+	-

Berath culture was poured in 10 tubes and mixed right after adding 1 mL of the essential oil to the first tube. One mL of first tube was added to the second and 1 mL of the second to the third tube respectively. Thus the dilutions of 1/2 to 1/1024 are obtained. Then 1 mL of the microbial suspension was added to each tube to make 1/4 to 1/2048 concentrations. The tubes were incubated at 37°C and MIC was appointed by the growth or non-growth of the bacterium in the tubes. MBC was also measured by the second culture in Mooler Hinton Agar medium (1, 6, 18, 19).

The measurement of the zone of inhibition of bacterium adjacent to the eucalyptus essential oils

The intended bacterium is distributed on Mooler Hinton Agar medium with concentration of 0.5 macfarland and then some small holes were made on them. 50 µL of the essential oils were added to each hole and the plate was incubated at 37°C for 24 h. Finally, after 24 h, the diameter of the zone of inhibition were measured (1, 15-17).

Results

Relatively simple biochemical tests (*e.g.*, positive reactions for coagulase, nuclease, alkaline phosphatase and Mannitol fermentation) can be used to differentiate *S. aureus*. Other staphylococci were summarized in table 1. Results obtained from the chemical analysis of *Eucalyptus* essential oils by GC/MS has been showed in Table 2.

Minimum inhibitory concentration (MIC) and the Minimum Bactericide Concentration (MBC) of the essential oils *Eucalyptus* spp by dilution method in the tube and the second culture in agar were summarized in Table 3. Each of the oils was tested at concentrations ranging from 1.95 to 62.4 µg/mL and 1/512 to 1/16 v/v. Minimum Inhibition Concentration (MIC) of the eucalyptus essential oils for *E. camaldulensis* 1/256 v/v was equal to 3.9 µg/mL, for *E. largiflorence* 1/512 v/v was 1.95 µg/mL, for *E. malliodora* 1/256 v/v was 3.9 µg/mL and for *E. polycarpa* 1/128 v/v was 7.8 µg/mL.

Antibacterial activity of the essential oils *E. largiflorence, E. malliodora, E. polycarpa* and *E. camaldulensis* (zone of inhibition in mm) were 26, 22, 18 and 20 mm, respectively.

Discussion

The antibiotic resistance is a very common matter nowadays. *Staphylococcus aureus* is also considered as one of the resistant sample to drugs and pathogen agents in human, therefore it seems necessary to find out antimicrobial materials from alternative sources such as plants.

Eucalyptus is one of the herbal drugs with antimicrobial effects. In present study MIC of the essential oils of *E. malliodora*, *E. polycarpa*, *E. camaldulensis* and *E. largiflorence* were evaluated and estimated 3.9, 3.9, 1.95 and 7.8 µL/mL, respectively.

The diameter of the zone of inhibition adjacent to essential oils was estimated 20, 22, 26 and 19 mm respectively. The results showed that these essential oils can strongly prevent the growth of *Staphylococcus aureus* ATCC 25923 and other clinical resistant bacteria. The obtained results are in line with other studies. However, MIC of the *E. largiflorence* essential oils and diameter of zone of inhibition in comparison to other essential oils showed potent antimicrobial activity and inhibitory effect. This suggested *E. largiflorence* as an effective antimicrobial agent among all kinds of *Eucalyptus* (1).

In addition in present study, the antimicrobial activity of Eucalyptol was examined that showed a potent inhibitory effect on *Staphylococcus*

Table 2. The results obtained from the chemical analysis of *Eucalyptus* essential oils by GC/MS.

Chemical compounds	E. polycarpa %	E. mlliodora %	E. largiflorence %	E. camaldulensis%
α -Terpineol	-	0.74	1.19	-
Spathulenol	-	0.62	-	-
Eudesmol	1.03	-	-	-
á - Pinene	8.02	18.58	15.46	13.24
â- Pinene	-	0.82	0.66	0.48
â -Myrcene	-	-	-	0.22
1,8-Cineol	50.12	67.65	70.32	54.37
Terpinolene	-	0.074	1.09	0.21
E-Pinocarveol	1.68	0.45	0.55	1.85
Aromadendrene	4.18	1.00	1.78	6.18
Viridifelorol	-	5.79	4.78	10.96
Globulol	5.11	-	0.54	1.48
ã -Terpinene	-	-	-	0.01
Isoamyl	-	-	-	0.21
Pinocarveol	-	-	-	1.95
(+)-Isomenthol	-	-	-	0.3
Terpinen-4-ol	-	-	-	0.75
Myrtenol	-	-	-	0.39
E-(+)-carveol	-	-	-	0.29
Calarene	-	-	-	0.41
Allo-aromadendrene	-	-	-	1.63
â-Selinene	-	-	-	0.57
Ledene	-	-	-	0.53
ä-gurjunene	-	-	-	1.05
á-gurjunene	-	-	-	0.38
Eremophilene	-	-	-	0.55
Benzaldehyde	-	-	-	1.43

aureus ATCC 25923. Eucalyptol acts as a main substance of the *Eucalyptus* oil (14). On the other hand 1, 8-Cineol and α-pinene present in the chemical compounds of all four kinds of *Eucalyptus*, where *E. largiflorence* essential oil has the most antimicrobial activity comparing to others. Antibacterial activity of these essential oils were decreased when 1, 8-Cineol and α-pinene were less than normal levels.

Consequently α-pinene and 1, 8-Cineol are both considered as the most important antimicrobial substance of the *Eucalyptus* oil (13). Last studies showed that Eucalyptus oil has a widespread antimicrobial effects against the positive and negative gram bacteria (1, 5). Also there are other studies proved antimicrobial compounds in *Eucalyptus* and indicated the relation between traditional reported consumption and experiments have done in laboratories (6, 11). Therefore, extract of the *Eucalyptus* oil as an effective antimicrobial substance can be used in treatment of bacterial infections like skin injuries, acnes and lung infections (1, 2, 5, 6, 11).

Table 3. Minimum inhibitory concentration (MIC) and the minimum bactericide concentration (MBC) of the essential oils *Eucalyptus* spp by the dilution method in the tube and the second culture in agar.

Staphylococcus aureus series	*E. polycarpa*		*E. mlliodora*		*E. largiflorence*		*E. camaldulensis*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
ATCC25923	1.95 (1/51)	3.9 (1/256)	3.9 (1/256)	3.9 (1/256)	7.8 (1/128)	15.6 (1/64)	3.9 (1/256)	7.8 (1/128)
Clinical samples								
Sputum	3.9 (1/25)	7.8 (1/128)	7. 8 (1/128)	7.8 (1/128)	31.2 (1/32)	62.4 (1/16)	3.9 (1/256)	7.8 (1/128)
Wound	3.9 (1/25)	7.8 (1/128)	3.9 (1/256)	7.8 (1/128)	7.8 (1/128)	15.6 (1/64)	3.9 (1/256)	3.9 (1/256)

Each oils were test for MIC and MBC concentration were presented as µg/mL (v/v)

Eucalyptus has been showed a wide spectrum of antimicrobial and anti-cancer activities and used in design of new antibiotics (1, 2). It was concluded that the essential oils of *Eucalyptus* is effective against *Staphylococcus aureus in-vitro*, although more detailed studies require using these compounds in clinic and infectious disease.

References

(1) Srinivasan D, Nathan S and Suresh T. Antimicrobial activity certain indian medicinal plants used in folkoric medicine. *J. Ethnopharmacol*. (2001) 74: 217-220.

(2) Takasaki M, Konoshima T and Etoh H. Cancer chemopreventive activity of euglobal-G1 from leaves of *Eucalyptus grandis*. *Cancer Lett.* (2000) 155: 61-65.

(3) Siddiqui B and Sultana I. Triterpenoidal constituents from *Eucalyptus camaldulensis* var. *obtusa*. *Phytochem.* (2000) 54: 861-865.

(4) Ito H, Koreishi M, Tokuda H and Nishino H. Cypellocarpins A-C, phenol glycosides esterified with oleuropic acid, from *Eucalyptus cypellocarpa*. *J. Nat. Prod.* (2000) 63: 1253-1257.

(5) Sefidkon F, Assareh MH, Abravesh Z and Barazandeh MM. Chemical Composition of the Essential Oils of Four Cultivated *Eucalyptus* Species in Iran as Medicinal Plants (*E. microtheca*, *E. spathulata*, *E. largiflorens* and *E. torquata*). *Iranian J. Pharm. Res*. (2007) 6: 135-140.

(6) Nakayama R, Murata M and Homma S. Antibacterial compounds from *Eucalyptus perriniana. Agric. Biol. Chem*. (1990) 54: 231-232.

(7) Bennet-Jenkins E and Bryant C. Novel sources of anthelmentics. *Int. J. Parasitol.* (1995) 26: 937-47.

(8) Tarasova GD, Krutikova NM, PekLi FF and Vichkanova S. Experience in the use of eucalymine in acute inflammatory ENT disease in children. *Vestn. Otorinolaryngol.* (1998) 6: 48-50.

(9) Yamakoshi Y, Murata M, shimizu A and Homma S. Isolation and characterization of macrocarpals B-G antimicrobial compounds from *Eucalyptus macrocarpa*. *Biosci. Biotechnol. Biochem.* (1992) 56: 1570-6.

(10) Pattnaik S, Subramanyam VL and Rath CC. Effect of essential oils on the viability and morphology of *E. coli* (SP-11). *Microbios* (1995) 84: 195-199.

(11) Pattnaik S, Subramanyam VR and Kole C. Antimicrobial and antifungal activity of ten essential oils *in-vitro. Microbios* (1996) 86: 237-46.

(12) Farah A, Satrini B, Fechtal M, Chaouch A and Talbi M. Chemical composition and antimicrobial activity of essential oils from leaves of *Eucalyptus camaldulensis* and its natural hybrid (clone 583). *Acta Bot. Gallica* (2001) 148: 183-190.

(13) Pasi S, Harvala E, Kretsi O, Chinou E and Chinou I. *In-vitro* antimicrobial activity of natural products from *Eucalyptus* growing in Greece and mechanism of action. *Clin. Microb. Infection* (2001) 7: 391-394.

(14) Penfold AR and Willis JL. *The Eucalypts, Botany, Cultivation, Chemistry and Utilization*. Interscience Publishers, New York (1961) 551.

This article is available online at http://www.ijpr-online.com