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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF EUCALYPTUS OIL AGAINST CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS

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ABSTRACT

Staphylococcus aureus is one of the important bacterial pathogen causing a wide spectrum of infections. Eucalyptus species are well known as medicinal plants due to their biological and pharmacological properties. The aim of the present study was to determine the antibacterial activity of eucalyptus oil against clinical isolates of Staphylococcus aureus. The MIC of eucalyptus oil was appeared to be 0.06% for S. aureus. The eucalyptus oil is found to have antibacterial activity against S. aureus. However, its irritant properties has been evaluated before it is formulated for medicinal purpose.

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INTRODUCTION

Staphylococcus aureus is one of the important bacterial pathogen causing a wide spectrum of infections (Arciola *et al.,* 2001). Many studies have been conducted to explain the structures and pathogenic mechanisms by which S. aureus is able to cause serious infections (O'Neill *et al.,* 2007). The ability of S. aureus to produce biofilm enables this organism to withstand the host immune response and is considered to be the cause of many chronic or persistent infections, as the biofilm creation protects bacteria from phagocytosis and antimicrobial agents (Foster, 2005). Another concern related to this pathogen is increasing resistance to oxacillin and many other antibiotics, but also circulation of multidrug resistant isolates within the hospital environment (Martín-López *et al.,* 2002). Staphylococcal pathogenesis is multifactorial, involving a combination of adherence and biofilm formation (Klug *et al.,* 2003). Eucalyptus is one of the very important and most widely planted genera across the world (Akin *et al.,* 2010). It is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively planted in many other countries (Mubita *et al.,* 2008). It was introduced in Algeria in 1854 by Ramel (Boulekbache-Makhlouf *et al.,* 2010). Eucalyptus species are well known as medicinal plants due to their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, however, is Eucalyptus globulus (E. globulus) which is the main furnisher of essential oils. These essential oils have different applications as anesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, hemostat, inhalant, insect repellant, preventitive, rubefacient, sedative yet stimulant, vermifuge, for a folk remedy for abscess, arthritis, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, leprosy, malaria, mastitis, miasma, pharygnitis, phthisis, rhinitis, sores, sore throat, spasms, trachalgia, worms, and wounds. Sometimes their demand is also high in the soap and cosmetic industries (Bajaj, 1995). Thus, the aim of the present study was to determine the antibacterial activity of eucalyptus oil against clinical isolates of Staphylococcus aureus.

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MATERIALS AND METHODS

Bacterial isolates

A total of 20 clinical isolates of *S. aureus* were collected from different clinical specimens of patients attending Saveetha Medical Collage and hospital. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy medium and stored at 4ºC until further use.

Antibiotic susceptability test: Antibiotic susceptibility testing was determined for these isolates to the following antibiotics

such as penicillin, erythromycin, clindamycin, ciprofloxacin, tetracycline, cotrimoxazole and linezolid. These antibiotics were procured from Himedia, Mumbai. This was performed by Kirby-bauer disc diffusion method as per CLSI guidelines (Clinical Laboratory Standards Institution, 2015).

Detection of antibacterial activity of eucalyptus oil against clinical isolates of S. aureus

Anti-bacterial activity of eucalyptus oil was tested against S. aureus isolates by minimum inhibitory concentration method. Mueller Hinton broth was supplemented with 0.002% (V/V) tween 80 (HiMedia, Mumbai) to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oils such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in Mueller Hinton Agar (MHA).

Media containing various concentrations of essential oils were poured over the sterile petridishes and allowed to dry. Media without essential oil was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC (Gopinath Prakasam *et al.,* 2014).

RESULTS

Sample wise distribution of clinical isolates of S. aureus

Of 20 clinical isolates of S. aureus, 8/20 (40%) were obtained from pus, 6/20 (30%) were from wound, 4/20 (20%) and 2/20 (10%) were from blood and sputum respectively (Figure 1).

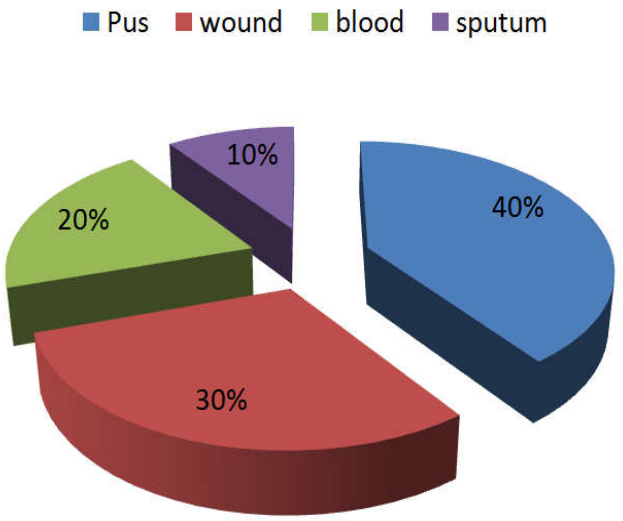

Figure 1. Pie chart showing the sample wise distribution of S. aureus

Antibiotic susceptibility pattern

We have observed a varied pattern of sensitivity among one *S.aureus* isolates. There was complete resistance observed for penicillin(100%), 9/20(45%)isolates were shown to the resistant to erythromycin,6/20(30%) were to cotrimoxazole,4/20(20%)were to linezolid followed by 3/20(15%) were resistant to ciprofloxacin and clindamycin respectively (Table 1) (Figure 2).

Table 1. Results of antibiotic susceptibility pattern of S.aureus

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Penicillin	0	0	20(100)
Erythromycin	14(70)	4(20)	2(10)
Clindamycin	15(75)	2(10)	3(15)
Ciprofloxacin	9(45)	8(40)	3(15)
Tetracyclin	14(70)	4(20)	2(10)
Cotrimoxazole	10(50)	4(20)	6(30)
Linezolid	10(50)	6(30)	4(20)

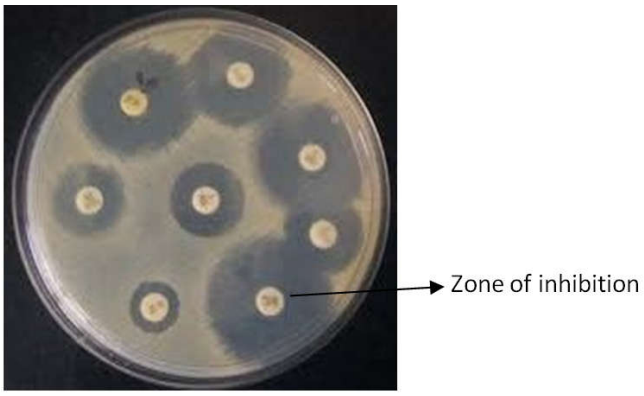

Figure 2. Representative picture showing antibiotic sensitivity pattern of *S. aureus*

Result of antibacterial activity of eucalyptus oil against clinical isolates of S. aureus

We have observed that, clinical isolates of S. aureus were inhibited from 0.06-1% of eucalyptus oil. The MIC of eucalyptus oil was appeared to be 0.06% for S. aureus**.**

Dilutions of eucalyptus oil	0.03 %	0.06 %	0.125 %	0.25 %	0.5%	1%	2%
No. of organisms	0	7 (35%)	7 (35%)	1 (5%)	2 (10%)	3 (15%)	0

DISCUSSION

Study conducted by Prakasam *et al.* from Chennai in 2014 demonstrated that, Acinetobacter strains were inhibited from 0.06 to 0.25%, 0.25-1% and 0.125-1% for clove, peppermint and eucalyptus oils respectively. In clove oil, 14/50 (28%) isolates were inhibited at 0.06%, 25/50 (50%) at 0.125% and 11/50 (22%) at 0.25% of clove oil. In peppermint oil, 34/50 (68%) isolates were inhibited at 0.25%, 12/50 (24%) and 4/50 (8%) were at 0.5% and 1% concentrations of peppermint oil respectively. In eucalyptus oils, 10/50 (20%) isolates were inhibited at 0.125%, 18/50 (36%) at 0.25%, 16/50 (32%) and 6/50 (12%) were at 0.5% and 1% respectively. Thus, the MIC of clove oil was found to be 0.06%, 0.25% for peppermint oil and 0.125% for eucalyptus oil (Gopinath Prakasam *et al.,* 2014). In contrast, in our study, we used eucalyptus oil against S. aureus isolates. 35% of isolates were inhibited at 0.06%, 35% were at 0.125%, 5% were at 0.25%, 10% were at 0.5% and 1% were at 15% of essential oil. Thus, the MIC of eucalyptus oil against S. aureus was found to be 0.25%.

Conclusion

The eucalyptus oil is found to have antibacterial activity against S. aureus. However, its irritant properties has been evaluated before it is formulated for medicinal purpose. Due to the extended drug resistance in S. aureus, it can be used as an alternative medicine.

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