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## Research Article

### ANTI-BACTERIAL ACTIVITY OF CLOVE OIL AGAINST STAPHYLOCOCCUS AUREUS

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#### ABSTRACT

Staphylococcus aureus has been well-documented as a human as well as animal pathogen. Cloves have been used by humans for medicinal applications for over 2000 years. It is widely used for relieving toothache or cavity problems and in aromatherapy and as an antiseptic in oral infections. Our study was intended to determine the antibacterial activity of clove oil against Staphylococcus aureus. The MIC of clove oil was appeared to be 0.125% for S. aureus. The clove oil was 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 has been well-documented as a human as well as animal pathogen. This organism is implicated in different infectious conditions such as furuncles or boils, cellulitis, impetigo, and postoperative wound infections of various sites. It has also been associated with serious and life-threatening infections such as bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, and meningitis<sup>[13]</sup>. Moreover, it is connected with toxin-related diseases. These isolates were found to be resistant to most of the available antibiotics due to intrinsic and extrinsic mechanisms<sup>[12]</sup>.

Essential oils are chemically terpenes that are one of the largest group of plant secondary metabolites. They are volatile, limpid, coloured and are soluble in lipids and organic solvents that have a lower density than water. They may be present in all plant organs of specific plant families, including buds, flowers, leaves, seeds, twigs, stems, flowers, fruits, roots, wood or bark and are generally stored by the plant in secretory cells, cavities, canals, glandular trichomes or epidermal cells. Essential oils of many medicinal plants have been used for evaluation of their antibacterial and anti-fungal activities<sup>[1,2]</sup>. Studies have shown that essential oils contain a wide series of compounds that can inhibit or slow the growth of bacteria, yeasts and moulds inhibiting their transmission. Essential oils have been widely used in dentistry as an antiseptic and local anesthetic<sup>[10]</sup>.

Clove oil and eucalyptus oils are two very familiar names in this category of essentials. Essential oils are potential sources of novel antibacterial compounds especially against bacterial pathogens<sup>[1]</sup>. The antibacterial property of essential oils from plants was used empirically for centuries, but only recently has it been studied scientifically. An increasingly body of research is being compiled on the antibacterial activity of various plant oil extracts and their specific components for possible application in fields ranking from food industry to dentistry<sup>[3,4]</sup>. Clove oil is extracted from *Syzygium aromaticum* of the Myrtaceae family. They are dried, the unopened inflorescence of about 1/2-3/4 inch in length. They contain 14-20% essential oil. Cloves are strongly pungent due to their high content of eugenol, which can be extracted by distillation to yield the essential oil<sup>[5,6]</sup>. Cloves have been used by humans for medicinal applications for over 2000 years. It is widely used for relieving toothache or cavity problems and in aromatherapy and as an antiseptic in oral infections. The health benefits of clove oil can be attributed to its antimicrobial, antifungal, antiseptic, antiviral, aphrodisiac and stimulating properties. The antimicrobial activity of the clove oil contributes in many application such as pharmaceutical, medicine and food preservative<sup>[11]</sup>.

It is a natural antibiotic<sup>[7]</sup> with broad antimicrobial activities against Gram-positive, Gram-negative, and acid-fast bacteria, as well as fungi<sup>[7,8]</sup>. It has been used as a histological clearing agent<sup>[3,4]</sup>. The intake of the oil provides carminative and anti-

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plasmodic properties. In the stomach, the effect is carminative, relaxing the gastric sphincter, and it encourage eructation. Patients suffering from arthritis, rheumatism and leg ulcers are advised to take clove oil<sup>[9]</sup>.

The aim of the study is to evaluate the antibacterial activity of clove bud oil against staphylococcus aureus. The antibacterial activity is carried out by agar well diffusion technique against the staphylococcus aureus and the zone of inhibition is measured in mm diameter. In the present study, clove oil was found to be equally effective against both gram-positive and gram-negative organisms. Thus, our study was intended to determine the antibacterial activity of clove oil against *Staphylococcus aureus*<sup>[11]</sup>.

## 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 4°C until further use.

### Antibiotic susceptibility 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. [16]

### Detection of antibacterial activity of clove oil against clinical isolates of *S. aureus*

Anti-bacterial activity of clove 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 oil 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 37°C for overnight. The lowest concentration of the essential oils that completely inhibited the growth of isolates was considered as MIC. [17]

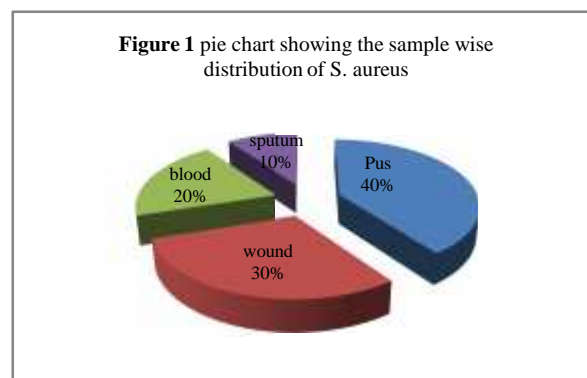
## RESULTS

### 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).

### Antibiotic susceptibility pattern

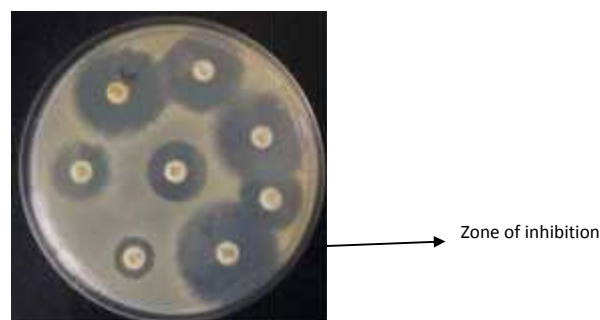
We have observed a varied pattern of sensitivity among one *S. aureus* isolates.



There was complete resistance observed for penicillin (100%), 9/20(45%) isolates were shown to be 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 clove oil against clinical isolates of *S. aureus*

We have observed that, clinical isolates of *S. Aureus* were inhibited from 0.125-1% of clove oil.

The MIC of clove oil was appeared to be 0.125% for *S. aureus*.

Dilutions of Clove oil	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
No. of organisms	0	0	8 (40%)	6 (30%)	3 (15%)	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. [17]

In contrast, in our study, we used clove oil against *Enterococcus* isolates. 40% of isolates were inhibited at 0.125%, 30% were at 0.25%, 15% were at 0.5% and 15% were at 1% of essential oil. Thus, the MIC of clove oil against *Enterococcus* isolates was found to be 0.125%.

## CONCLUSION

The clove oil was 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. Spices have been declared as powerful antibacterial agents and hence must be used appropriately. The use of spices in dentistry should be based on evidence of effectiveness and safety. The antibacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration<sup>[14]</sup>.

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