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Advances in Bioresearch

## SHORT COMMUNICATION

# Antimicrobial Effect of Aromatic Oils towards both *Staphylococcus aureus* and *Staphylococcus epidermidis*, a Microbe Obtained from Infections of the Mouth Interior

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**ABSTRACT**
*The increasing prevalence of antibiotic-resistant bacterial strains has necessitated the exploration of alternative antimicrobial agents, such as aromatic oils. This study investigates the antimicrobial efficacy of selected aromatic oils against Staphylococcus aureus and Staphylococcus epidermidis, both of which are implicated in oral infections. Using disc diffusion and broth dilution methods, the inhibitory effects of essential oils such as tea tree, eucalyptus, clove, and thyme were evaluated against clinical isolates obtained from intraoral infections. Results demonstrated significant antimicrobial activity, with clove oil exhibiting the highest potency against both bacterial species. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays further confirmed their bactericidal potential. These findings suggest that aromatic oils could serve as promising natural alternatives for managing oral bacterial infections, particularly in cases of antibiotic resistance. Further research is warranted to explore their clinical applications and mechanisms of action.*
***Keywords:*** *Essential oils, Staphylococcus aureus, Staphylococcus epidermidis, oral infection, antimicrobial activity, natural compounds, MIC*

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## INTRODUCTION

Oral and maxillofacial infections remain a significant clinical concern, often arising from the complex interplay between host factors and a diverse microbial ecosystem. Among the predominant pathogens associated with infections of the oral cavity and surrounding structures are *Staphylococcus aureus* and *Staphylococcus epidermidis*. While traditionally considered skin commensals, these Gram-positive cocci are frequently implicated in opportunistic infections, including periodontitis, peri-implantitis, post-extraction wound infections, and abscess formation within the oral cavity. Their presence in oral biofilms and resistance to conventional antibiotics underscore the need for novel antimicrobial strategies [1].

The emergence of antibiotic-resistant strains of *S. aureus*, particularly methicillin-resistant *S. aureus* (MRSA), and the biofilm-forming ability of *S. epidermidis* contribute to the limited effectiveness of standard antimicrobial therapies. These challenges have sparked renewed interest in plant-derived

compounds with antimicrobial properties, especially essential oils, which are complex mixtures of volatile, aromatic compounds synthesized by various plant species. Historically used in traditional medicine, these oils are now being scientifically validated for their antimicrobial, antifungal, and anti-inflammatory activities.

Essential oils such as those derived from *Thymus vulgaris* (thyme), *Cinnamomum zeylanicum* (cinnamon), *Melaleuca alternifolia* (tea tree), and *Syzygium aromaticum* (clove) have demonstrated broad-spectrum antimicrobial activity in vitro. Their mechanisms of action include disruption of microbial membranes, interference with enzymatic systems, and inhibition of quorum sensing. However, the efficacy of these oils against oral staphylococcal isolates—especially strains obtained directly from clinical oral infections—remains underexplored [2].

This study aims to investigate the antimicrobial potential of selected aromatic oils against clinical isolates of *S. aureus* and *S. epidermidis* derived from infections of the mouth interior. By assessing both zone of inhibition and minimum inhibitory concentrations (MICs), we aim to identify promising candidates for future development as natural antimicrobial agents in oral healthcare formulations [3].

## MATERIAL AND METHODS

### Collection and Identification of Microorganisms

Clinical samples were collected from patients presenting with intraoral infections (gingival abscesses, mucosal lesions, and periapical infections) at a District Hospital Raebareli dental Department Swab samples were obtained under aseptic conditions and immediately transferred to the microbiology laboratory for analysis. Samples were cultured on Mannitol Salt Agar and Blood Agar, then incubated at 37°C for 24–48 hours. Colonies exhibiting morphological characteristics of *Staphylococcus* spp. were subjected to Gram staining and standard biochemical tests (catalase, coagulase) for preliminary identification. Confirmatory identification of *Staphylococcus aureus* and *Staphylococcus epidermidis* was performed using the VITEK 2 compact system.

### Aromatic Oils and Preparation

The essential oils used in this study included:

- **Thyme oil (*Thymus vulgaris*)**
- **Cinnamon oil (*Cinnamomum zeylanicum*)**
- **Clove oil (*Syzygium aromaticum*)**
- **Tea tree oil (*Melaleuca alternifolia*)**

All oils were obtained from certified commercial suppliers (purity ≥99%) and stored in amber vials at 4°C. For antimicrobial assays, oils were emulsified in 1% dimethyl sulfoxide (DMSO) and prepared at varying concentrations ranging from 0.125% to 2% (v/v).

### Antimicrobial Susceptibility Testing

### Agar Well Diffusion Method

The antimicrobial activity of the essential oils was evaluated using the agar well diffusion method. Mueller-Hinton Agar plates were inoculated with standardized bacterial suspensions (0.5 McFarland standard, ~$1.5 \times 10^8$ CFU/mL). Wells of 6 mm diameter were punched into the agar and filled with 50 µL of each oil solution. Plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition (ZOI) was measured in millimetres using a digital calliper. DMSO was used as a negative control, while vancomycin (30 µg) served as a positive control.[4]

### Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method in sterile 96-well microtiter plates. Serial dilutions of the oils were prepared in Mueller-Hinton Broth to obtain final concentrations ranging from 0.125% to 2% (v/v). Each well was inoculated with 100 µL of bacterial suspension ($10^6$ CFU/mL). Plates were incubated at 37°C for 24 hours, and microbial growth was assessed via optical density readings at 600 nm. The MIC was defined as the lowest concentration that visibly inhibited bacterial growth.[5]

## RESULTS AND DISCUSSION

### Isolation and Identification of Oral Pathogens

From a total of 25 clinical swab samples collected from intraoral infections, 18 isolates were confirmed as *Staphylococcus aureus* and 12 as *Staphylococcus epidermidis* based on colony morphology, Gram staining, biochemical tests, and VITEK 2 system confirmation. Both pathogens were found to be prevalent in infections of mucosal lesions and peri-apical regions, with *S. aureus* slightly more dominant in acute abscesses.[6]

**Agar Well Diffusion Assay**

All tested essential oils demonstrated measurable antimicrobial activity against both *S. aureus* and *S. epidermidis* isolates. Table 1 summarizes the mean zone of inhibition (ZOI) for each oil [7].

| Essential Oil | *S. aureus* (mm) | *S. epidermidis* (mm) |
|---|---|---|
| Cinnamon oil | 23.4 ± 1.2 | 21.8 ± 1.0 |
| Thyme oil | 21.7 ± 1.1 | 20.2 ± 1.4 |
| Clove oil | 17.5 ± 0.9 | 16.2 ± 1.1 |
| Tea tree oil | 14.8 ± 0.8 | 13.6 ± 0.7 |
| Vancomycin (control) | 25.6 ± 0.9 | 24.9 ± 1.0 |

Table 1. Mean Zone of Inhibition (mm) ± SD for various essential oils and vancomycin against *Staphylococcus aureus* and *Staphylococcus epidermidis*

Cinnamon and thyme oils exhibited the most potent antimicrobial effects, producing larger inhibition zones compared to clove and tea tree oils. *S. aureus* showed slightly higher sensitivity to all oils than *S. epidermidis*, which aligns with previous studies suggesting the thicker biofilm matrix of *S. epidermidis* can confer enhanced resistance [8].

**Minimum Inhibitory Concentration (MIC)**

The MIC values further confirmed the antimicrobial potential of the oils, as shown in Table 2.

| Essential Oil | *S. aureus* MIC | *S. epidermidis* MIC |
|---|---|---|
| Cinnamon oil | 0.25 | 0.5 |
| Thyme oil | 0.5 | 0.5 |
| Clove oil | 1.0 | 1.0 |
| Tea tree oil | 1.5 | 2.0 |

**Table 2. Minimum Inhibitory Concentration (MIC) of different essential oils against *Staphylococcus aureus* and *Staphylococcus epidermidis***

Cinnamon oil demonstrated the lowest MIC against both bacterial species, indicating higher potency even at minimal concentrations. Thyme oil also showed strong activity, while clove and tea tree oils were less effective at lower concentrations [9].

**CONCLUSION**

The results of this study strongly suggest that selected aromatic oils, particularly cinnamon and thyme, possess significant antimicrobial properties against *S. aureus* and *S. epidermidis* isolated from oral infections. These findings are consistent with previous reports attributing antimicrobial activity of these oils to their high content of bioactive constituents such as cinnamaldehyde and thymol, which are known to disrupt bacterial cell membranes and interfere with metabolic processes.[10].

The slightly reduced sensitivity of *S. epidermidis* may be due to its known ability to form biofilms, which protect against antimicrobial penetration. Nevertheless, the activity of these oils was comparable to that of vancomycin in the agar diffusion test, highlighting their potential as alternative or adjunctive agents for managing staphylococcal oral infections. [11]. Moreover, the advantage of using natural essential oils lies in their lower tendency to induce resistance and their multifunctional bioactivity (antimicrobial, anti-inflammatory, antioxidant), which may be beneficial in complex oral infections.

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