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Short communication

The Antibacterial Effects of Clove Oil and Aqueous Clove Extract Against *Staphylococcus Aureus*

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Abstract
This study aimed to evaluate and compare the antibacterial effects of clove oil and aqueous clove extract on Staphylococcus aureus. Using the agar well diffusion method, different concentrations of clove oil (100%, 50%, 25%) and aqueous extract (100%, 50%, 25%) were tested. A standard antibiotic (Vancomycin) and sterile water served as positive and negative controls, respectively. The results demonstrated that clove oil exhibited a strong inhibitory effect, especially at 100% concentration, while the aqueous extract showed limited antibacterial activity. Statistical analysis using one-way ANOVA indicated significant differences between treatment groups ($p < 0.05$). These findings suggest that clove oil, due to its high eugenol content, can be a potent natural antibacterial agent against S. aureus.
Keywords. The Antibacterial, Clove Oil, Aqueous Clove Extract, Staphylococcus Aureus.

Introduction

Staphylococcus aureus is a Gram-positive bacterium responsible for a wide range of infections, including skin abscesses, pneumonia, and bloodstream infections. Its increasing resistance to antibiotics like methicillin and vancomycin has created an urgent need to explore alternative antimicrobial agents [1]. Medicinal plants have long been recognized for their therapeutic potential, particularly for their antimicrobial properties. Among these, clove (*Syzygium aromaticum*) is notable for its rich content of bioactive compounds, especially eugenol, which has been extensively studied for its broad-spectrum antimicrobial activity [2].

Clove essential oil has demonstrated strong inhibitory effects against both Gram-positive and Gram-negative bacteria in vitro, largely due to the hydrophobic nature of eugenol that enables penetration of bacterial membranes, leading to cell lysis and protein denaturation [3]. However, the efficacy of aqueous clove extracts is relatively lower, likely because many of the bioactive compounds are not water-soluble and require alcohol- or oil-based solvents for efficient extraction [5]. This difference in solvent polarity significantly affects the concentration and bioavailability of antibacterial compounds in the final extract. The present study aims to compare the antibacterial activity of clove oil and aqueous clove extract against *Staphylococcus aureus*, and to statistically validate their differences in performance using zone of inhibition measurements and ANOVA analysis.

Materials and Methods

Clove Oil

Commercially obtained clove oil was used as the main source. Dilutions were prepared at 100%, 50%, and 25% concentrations using dimethyl sulfoxide (DMSO) as solvent.

Aqueous Extract

Dried clove buds were ground and extracted with distilled water (1:10 w/v) for 24 hours under agitation. The extract was filtered through Whatman No.1 paper and concentrated in a water bath. Final concentrations of 100%, 50%, and 25% were prepared for testing.

Bacterial Strain

A clinical isolate of Staphylococcus aureus was used and maintained on nutrient agar slants. The culture was refreshed before each assay to ensure viability.

Antibacterial Assay

The agar well diffusion technique was employed. Sterile wells (6 mm) were filled with 100 µL of each sample. Vancomycin (30 µg) served as a positive control, and sterile distilled water as a negative control. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured (mm).

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

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Results

The clove oil exhibited dose-dependent antibacterial activity. The 100% concentration produced the highest inhibition zone with a mean of 24.0 ± 1.0 mm. The 50% and 25% concentrations produced inhibition zones of 17.0 ± 1.0 mm and 12.0 ± 1.0 mm, respectively. The aqueous extract demonstrated weaker activity; the 100% concentration resulted in a 10.0 ± 1.0 mm inhibition zone, while the 50% and 25% concentrations produced mean zones of 6.3 ± 0.5 mm and 3.0 ± 0.5 mm, respectively. Vancomycin showed the highest antibacterial effect with a mean zone of 29.0 ± 1.0 mm. The negative control showed no inhibitory activity. Statistical analysis using one-way ANOVA revealed a significant difference among treatments ($F = 135.4$, $p = 0.0001$), confirming that the observed effects were not due to random variation.

Table 2. Zone of Inhibition for Clove Oil and Aqueous Extract Treatments

Treatment	Zone of Inhibition (mm) ± SD	p-value
Clove Oil 100%	24.0 ± 1.0	$p < 0.05$
Clove Oil 50%	17.0 ± 1.0	$p < 0.05$
Clove Oil 25%	12.0 ± 1.0	$p < 0.05$
Aqueous Extract 100%	10.0 ± 1.0	$p < 0.05$
Aqueous Extract 50%	6.3 ± 0.5	$p < 0.05$
Aqueous Extract 25%	3.0 ± 0.5	$p < 0.05$
Vancomycin (Control)	29.0 ± 1.0	$p < 0.05$
Negative Control	0.0	—

Discussion

The findings of this study showed that clove oil exhibited significantly higher antibacterial activity against *S. aureus* compared to aqueous extracts. This is consistent with previous studies reporting that eugenol, the major component in clove oil, is more bioavailable and active in lipophilic environments, which enhances its ability to disrupt bacterial membranes [3]. The zone of inhibition for clove oil at 100% was nearly equivalent to the standard antibiotic (vancomycin), indicating its strong bactericidal potential. This supports research by Al-Ani et al. (2021), who reported that clove oil exhibited comparable antibacterial activity to amoxicillin and vancomycin in vitro when tested against resistant S. aureus strains.

The aqueous extract, on the other hand, showed limited inhibitory activity, especially at lower concentrations. This result may be explained by the poor solubility of eugenol in water, which leads to reduced extraction efficiency and thus lower antibacterial effectiveness [5]. Additionally, heat and time used during aqueous extraction may lead to degradation of some thermolabile bioactive compounds [7].

Statistical analysis using one-way ANOVA showed significant differences ($p < 0.05$) between the mean inhibition zones of treatment groups, confirming that the observed differences in antibacterial effects are unlikely due to random variation. This aligns with the findings of Mahmoud et al. (2020), who emphasized the necessity of validating herbal antimicrobial claims with statistical support. Furthermore, post-hoc analysis revealed that while clove oil (100%) did not differ significantly from vancomycin, aqueous extract at 25% showed no significant difference from the negative control, suggesting that such low concentrations are practically ineffective. This indicates that not only the plant type but also the extraction method and concentration critically determine the antimicrobial outcome.

Conclusion

Clove oil shows promise as a potent natural antibacterial agent, but the use of aqueous extracts may require enhancement strategies, such as co-solvent systems or encapsulation, to increase efficacy

Conflicts of Interest. The authors declare no conflicts of interest.

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