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Natural Defence Potential of Syzygium aromaticum Oil Against Microbial and Fungal Pathogens

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Abstract: The increasing prevalence of antimicrobial resistance (AMR) among pathogenic microorganisms poses a serious threat to global public health and necessitates the development of effective alternative antimicrobial agents. Plant-derived essential oils have emerged as promising candidates due to their broad-spectrum activity and multi-target mechanisms of action. The present study evaluated the antibacterial and antifungal potential of Syzygium aromaticum (clove) essential oil against selected pathogenic microorganisms. Clove oil was extracted from dried flower buds by hydro-distillation and tested against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram-negative bacteria (Escherichia coli, Salmonella typhi), and pathogenic fungi (Candida albicans and Aspergillus niger). Antimicrobial activity was assessed using agar well diffusion and broth microdilution methods, following Clinical and Laboratory Standards Institute guidelines. The essential oil exhibited significant, concentration-dependent antibacterial and antifungal activity, with greater efficacy against Gram-positive bacteria. Staphylococcus aureus showed the highest susceptibility, with a minimum inhibitory concentration (MIC) of 0.125 mg/mL, whereas E. coli displayed comparatively higher resistance (MIC: 0.25 mg/mL). Pronounced antifungal activity was observed against Candida albicans. The antimicrobial efficacy of clove oil is attributed primarily to its high phenolic content, particularly eugenol, which disrupts microbial membrane integrity, increases permeability, and interferes with key metabolic processes. The multi-target mode of action reduces the likelihood of resistance development. These findings support the potential application of Syzygium aromaticum essential oil as a natural antimicrobial agent in pharmaceutical, food preservation, and cosmetic formulations, warranting further in vivo and toxicological investigations.

Keywords: Syzygium aromaticum; clove essential oil; eugenol; antimicrobial activity; antifungal activity; minimum inhibitory concentration; natural antimicrobials

I. INTRODUCTION

The rapid escalation of antimicrobial resistance (AMR) among pathogenic microorganisms represents a major global health challenge, significantly compromising the therapeutic efficacy of existing antibiotics and antifungal agents. Resistant bacterial strains and opportunistic fungi are increasingly implicated in persistent and recurrent infections, leading to prolonged illness, elevated healthcare costs, and increased mortality worldwide [1]. This growing threat has intensified the search for alternative antimicrobial strategies that are effective, safe, and environmentally sustainable.

Natural products, particularly plant-derived essential oils, have gained considerable attention as potential antimicrobial agents due to their complex chemical profiles and broad-spectrum biological activities. Essential oils are composed of diverse bioactive secondary metabolites that act through multiple cellular targets, including microbial membranes, metabolic enzymes, and genetic material. This multi-target mechanism reduces the probability of resistance development compared to conventional single-target synthetic drugs [2]. Moreover, essential oils obtained from culinary and medicinal plants are often regarded as safe for human use, further supporting their potential application in pharmaceutical, food, and cosmetic industries [3].

Syzygium aromaticum (L.) Merr. & Perry, commonly known as clove, is an aromatic spice belonging to the family Myrtaceae and is widely cultivated in tropical regions. Traditionally, clove has been utilized for its analgesic, antiseptic, anti-inflammatory, and antimicrobial properties. The essential oil extracted from clove buds is rich in phenolic compounds, predominantly eugenol, which constitutes approximately 70–85% of the oil and is recognized as the primary contributor to its antimicrobial activity [4].

Previous investigations have reported potent antibacterial activity of clove essential oil against both Gram-positive and Gram-negative bacteria, including clinically relevant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* species [5]. Eugenol exerts its antibacterial effect mainly by disrupting cytoplasmic membrane integrity, increasing permeability, and causing leakage of vital intracellular components, ultimately leading to cell death. Additionally, clove oil has demonstrated inhibitory effects on biofilm formation and microbial virulence mechanisms.

Clove essential oil has also shown significant antifungal efficacy, particularly against *Candida* species. Its antifungal action is primarily associated with interference in ergosterol synthesis, membrane destabilization, and inhibition of fungal growth and morphogenesis [6].

In view of the increasing demand for natural antimicrobial agents and the limitations of synthetic drugs, the present study aims to evaluate the antibacterial and antifungal potential of *Syzygium aromaticum* essential oil against selected microbial pathogens, thereby providing scientific evidence for its possible application as a natural antimicrobial agent.

II. MATERIALS AND METHODS

1) Plant Material and Essential Oil Procurement: Dried flower buds of *Syzygium aromaticum* (clove) were obtained from a certified herbal supplier and taxonomically authenticated. The plant material was cleaned and stored in airtight containers at room temperature. Essential oil was extracted by hydro-distillation using a Clevenger-type apparatus for 3 h, following standard procedures [2]. The oil was dried over anhydrous sodium sulfate, filtered, and stored in amber-colored vials at 4 °C until analysis.
2) Chemicals and Reagents: All chemicals and culture media used were of analytical grade. Mueller–Hinton agar and broth, Sabouraud dextrose agar and broth, dimethyl sulfoxide (DMSO), and standard antibacterial and antifungal agents were procured from certified suppliers. Eugenol (≥98% purity) was used as a reference compound for comparative analysis [4].
3) Test Microorganisms: Antibacterial activity was evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*, while antifungal activity was assessed against *Candida albicans* and *Aspergillus niger*. All microbial strains were obtained from a recognized culture collection and maintained at 4 °C on appropriate media [7].
4) Preparation of Inoculum: Bacterial cultures were grown in Mueller–Hinton broth at 37 °C for 18–24 h, and fungal cultures were incubated in Sabouraud dextrose broth at 28 °C for 48 h. Inoculum density was adjusted to the 0.5 McFarland standard prior to testing [7].
5) Antimicrobial Activity Assays: Antibacterial and antifungal activities were determined using the agar well diffusion method [8]. Sterile agar plates were inoculated with microbial suspensions, wells were filled with different concentrations of clove essential oil dissolved in 1% DMSO, and plates were incubated under suitable conditions. Zones of inhibition were measured in millimeters. Standard drugs served as positive controls, while solvent controls acted as negatives.
6) Minimum Inhibitory Concentration (MIC): MIC values were determined by the broth microdilution method using serial twofold dilutions of clove essential oil. The lowest concentration showing no visible microbial growth was recorded as the MIC [7].
7) Statistical Analysis: All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical significance was analyzed using one-way ANOVA, with $p < 0.05$ considered statistically significant.

III. RESULTS AND DISCUSSION

A. *Antibacterial Activity of Syzygium aromaticum Essential Oil*

The antibacterial efficacy of *Syzygium aromaticum* essential oil was evaluated against selected Gram-positive and Gram-negative bacterial pathogens using the agar well diffusion and MIC assays. The results demonstrated a clear **dose-dependent antibacterial activity**, with increasing concentrations of clove oil producing significantly larger zones of inhibition ($p < 0.05$).

Table 1. Antibacterial activity of clove essential oil against *Staphylococcus aureus*

Concentration (mg/mL)	Zone of Inhibition (mm)	Activity Level
0.25	10 ± 0.5	Moderate
0.50	15 ± 0.6	Good
1.00	20 ± 0.8	Strong
2.00	26 ± 1.0	Very strong

The strongest antibacterial effect was observed at 2.00 mg/mL, indicating high susceptibility of *S. aureus* to clove oil. This enhanced activity is consistent with previous reports highlighting the sensitivity of Gram-positive bacteria to phenolic-rich essential oils [4]. The relatively thinner peptidoglycan layer and absence of an outer membrane in Gram-positive bacteria may facilitate easier penetration of eugenol into the cell membrane.

Minimum inhibitory concentration (MIC) analysis further confirmed the potent antibacterial potential of clove oil.

Table 2. MIC values of clove essential oil against selected bacteria

Microorganism	MIC (mg/mL)
S. aureus	0.125
E. coli	0.25

Lower MIC values against *S. aureus* compared to *E. coli* indicate greater resistance among Gram-negative bacteria, likely due to the protective outer membrane rich in lipopolysaccharides, which restricts diffusion of hydrophobic compounds [5].

The antibacterial mechanism of clove oil is primarily attributed to eugenol, which disrupts cytoplasmic membrane integrity, increases permeability, and induces leakage of vital intracellular components such as proteins and nucleic acids, ultimately resulting in cell death [9].

B. Antifungal Activity of Syzygium aromaticum Essential Oil

Clove essential oil exhibited pronounced antifungal activity against *Candida albicans*, as evidenced by substantial growth inhibition in agar diffusion assays. The antifungal efficacy can be attributed to the ability of eugenol to interfere with ergosterol biosynthesis, disrupt membrane structure, and inhibit spore germination and hyphal development [6].

The lipophilic nature of eugenol enables effective interaction with fungal cell membranes, causing structural destabilization and functional impairment [3]. These findings support the potential application of clove oil as a natural antifungal agent, particularly against opportunistic yeast infections.

C. Comparative Mechanism of Action

Phenolic compounds such as eugenol exhibit stronger antimicrobial effects than monoterpenes due to their ability to denature proteins, inhibit key metabolic enzymes, induce oxidative stress, and disrupt microbial membranes [2]. The multi-target mode of action of clove essential oil significantly reduces the likelihood of resistance development, enhancing its suitability as an alternative to conventional antimicrobials.

IV. CONCLUSION

The present study demonstrates that *Syzygium aromaticum* essential oil possesses significant antibacterial and antifungal activities against selected pathogenic microorganisms. The oil exhibited a clear concentration-dependent inhibitory effect against both Gram-positive and Gram-negative bacteria, with *Staphylococcus aureus* showing greater susceptibility than *Escherichia coli*, as evidenced by larger zones of inhibition and lower MIC values. This differential sensitivity can be attributed to structural differences in bacterial cell envelopes, particularly the protective outer membrane present in Gram-negative bacteria.

In addition to its antibacterial efficacy, clove essential oil showed pronounced antifungal activity against *Candida albicans*, supporting its potential use against opportunistic fungal infections. The strong antimicrobial performance of clove oil is largely attributed to its high phenolic content, especially eugenol, which exerts its effect through multiple mechanisms including disruption of cell membrane integrity, increased membrane permeability, leakage of intracellular components, inhibition of key metabolic enzymes, and interference with ergosterol biosynthesis in fungi. The multi-target mode of action of clove essential oil is particularly advantageous, as it reduces the likelihood of resistance development compared to conventional synthetic antimicrobial agents. These findings reinforce the growing interest in plant-derived essential oils as effective and sustainable alternatives to synthetic antibiotics and antifungals. Overall, the results of this investigation provide scientific evidence supporting the potential application of *Syzygium aromaticum* essential oil as a natural antimicrobial agent in pharmaceutical formulations, food preservation systems, and cosmetic products. However, further studies focusing on toxicity evaluation, formulation optimization, and in vivo efficacy are necessary to fully validate its safety and clinical applicability.

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