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Evaluation of antibacterial potential of clove and rosemary essential oils against multidrug-resistant pathogens

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Abstract

Background and Objective: The rapid emergence of multidrug-resistant (MDR) bacterial strains has become a significant challenge in clinical treatment worldwide. Natural compounds, particularly essential oils, are being explored as potential alternatives to conventional antibiotics. This study aimed to evaluate and compare the antibacterial activity of essential oils extracted from clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) against MDR bacteria.

Methods: Essential oils were obtained via hydro-distillation and tested against four clinically isolated MDR bacteria—*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*—as well as two standard ATCC strains. The agar well diffusion method was employed to assess antibacterial activity, and the minimum inhibitory concentrations (MICs) were determined using two-fold serial dilution.

Results: Both clove and rosemary essential oils exhibited inhibitory effects against all test organisms. Clove oil demonstrated slightly stronger antibacterial activity compared to rosemary oil. The MIC for clove oil ranged from 0.312% to 1.25% (v/v), while that for rosemary oil ranged from 0.312% to 5% (v/v). Among the tested organisms, *A. baumannii* showed the highest sensitivity.

Conclusion: The findings suggest that clove and rosemary essential oils possess significant antibacterial properties and may serve as effective natural agents against MDR bacteria. Clove oil, in particular, exhibited greater potency and could be further investigated for therapeutic or preservative applications.

Keywords: Antibacterial activity, Multidrug-resistant bacteria, Minimum inhibitory concentration (MIC), Natural antimicrobials

Introduction

Antibiotics have long been the cornerstone for managing bacterial and fungal infections. Initially, their discovery was believed to signal the beginning of the end for infectious diseases. However, the frequent and excessive use of these agents has significantly contributed to the rise and spread of multidrug-resistant (MDR) bacterial strains. Notably, organisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Acinetobacter baumannii* have become major concerns in clinical settings due to their resistance to multiple antibiotics [1,2].

These MDR pathogens are not only prevalent in hospital-acquired infections but are increasingly being isolated from community sources as well. Despite the ongoing development of new antibiotics, many of them display limited effectiveness against resistant bacteria. This has intensified the search for novel antibacterial agents from alternative sources, particularly natural products [2,3].

Medicinal plants have gained attention for their diverse pharmacological properties, including antimicrobial potential. Essential oils derived from plants are complex mixtures of bioactive compounds, many of which possess antibacterial, antifungal, and antioxidant activities. These characteristics make them promising candidates for developing alternative antimicrobial treatments [3,4].

Clove (*Syzygium aromaticum*), belonging to the Myrtaceae family, is widely known for its use as a spice and traditional remedy. Its essential oil is rich in compounds like eugenol, which have demonstrated notable antimicrobial and antioxidant effects. Traditionally, clove oil has been employed for treating respiratory conditions, digestive issues, and as a topical analgesic in dental care [4,5].

Similarly, rosemary (*Rosmarinus officinalis L.*), a member of the Lamiaceae family, is a common culinary herb with medicinal applications. Its essential oil contains bioactive constituents such as 1,8-cineole, α-pinene, and camphor, which contribute to its antibacterial, antifungal, and antioxidant properties. Rosemary oil has also been studied for its potential anticancer activity [5,6].

The present study was designed to assess and compare the antibacterial effects of essential oils extracted from clove buds and rosemary leaves against multidrug-resistant bacterial isolates obtained from clinical samples.

Materials and Methods

A study was conducted from March 2018 to February 2019 at Department of Microbiology, Madha Medical College, Chennai to evaluate and compare the antibacterial activity of essential oils extracted from clove (*Syzygium aromaticum*) and rosemary (*Rosmarinus officinalis*) against MDR bacteria.

Plant Collection and Identification

Dried flower buds of clove (*Syzygium aromaticum*) and dried leaves of rosemary (*Rosmarinus officinalis*) were procured from a local market. The plant specimens were authenticated

Extraction of Essential Oils

Essential oils were extracted using a steam distillation process with the help of a Clevenger-type apparatus, following the methodology described by Harborne. For each plant, 130 grams of dried, ground material was placed in a round-bottom flask and covered with distilled water, filling approximately three-quarters of the vessel. Upon heating, steam carried the volatile components through a condenser where they were liquefied. The oil phase was collected, dried over anhydrous sodium sulfate to eliminate residual moisture, and stored appropriately. Oil density was calculated based on weight-to-volume (w/v) measurements [6].

Bacterial Strains and Culture Conditions

Four multidrug-resistant bacterial isolates—*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*—were selected for the study. These strains were originally isolated from clinical specimens such as wound swabs, sputum, throat swabs, ear discharges, and stool samples. In addition, two standard reference strains, *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853, were used as controls. All bacteria were sub-cultured on nutrient agar plates and incubated at 37°C for 24 hours before testing [7].

Antibacterial Testing Procedure

The antibacterial activity of each essential oil was assessed using the agar well diffusion technique. Bacterial suspensions were prepared at a concentration of approximately 10^6 CFU/mL, adjusted using the 0.5 McFarland standard. A volume of 100 µL of each suspension was evenly spread on Mueller-Hinton agar plates. After drying, wells with a diameter of 6 mm were made in the agar using a sterile cork borer. Fifty microliters (50 µL) of each oil dilution (ranging from 10% to 0.312% v/v in DMSO) were dispensed into the wells in triplicate. Plates were left at room temperature for about one hour to allow proper diffusion of the oils, followed by incubation at 37 °C for 24 hours. A clear zone around the well measuring greater than 6 mm was considered evidence of antibacterial activity. DMSO was used as the negative control [8].

Minimum Inhibitory Concentration (MIC) Determination

The MIC of each essential oil was established as the lowest concentration capable of completely inhibiting visible bacterial growth after 24 hours of incubation. A series of two-fold dilutions of the oils (ranging from 10% to 0.039% v/v) were prepared in sterile DMSO. A volume of 50 µL from each dilution was introduced into wells on Mueller-Hinton agar plates previously inoculated with standardized bacterial suspensions (10^6 CFU/mL). After 24-hour incubation at 37 °C, the lowest concentration showing a clear zone of inhibition was recorded as the MIC. All tests were carried out in triplicate to ensure reproducibility [9].

Results

The extracted essential oils from clove buds and rosemary leaves appeared yellow in color. The calculated densities were 1.04 mg/mL for clove oil and 1.07 mg/mL for rosemary oil, respectively. The antibacterial evaluation revealed that both essential oils exhibited measurable inhibitory effects against all tested bacterial strains, including multidrug-resistant (MDR) clinical isolates and standard reference organisms. The inhibition was assessed using agar well diffusion, and zone diameters were recorded across a series of decreasing concentrations.

Rosemary essential oil showed the strongest inhibition against *Acinetobacter baumannii*, producing a zone of 34.2 ± 1.3 mm at a concentration of 10% (v/v). This was followed closely by *Pseudomonas aeruginosa* (33.1 ± 2.3 mm) and *Enterococcus faecalis* (30.9 ± 2.1 mm) at the same concentration. However, when the concentration was lowered to 5%, inhibition zones declined significantly. No inhibition was recorded for several strains, including *P. aeruginosa* and *S. aureus*, at concentrations below 5%. Notably, *A. baumannii* remained sensitive even at 0.312%, showing a zone of 13.7 ± 2.4 mm. The standard strains (*S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853) exhibited moderate sensitivity to rosemary oil, with inhibition zones ranging from 15.3 mm to 24.3 mm depending on concentration.

Clove essential oil demonstrated consistent antibacterial effects across all tested organisms. At 10% (v/v), *A. baumannii* showed the widest inhibition zone (27.5 ± 2.1 mm), followed by *Enterococcus faecalis* (24.1 ± 2.3 mm) and *P. aeruginosa* ATCC 27853 (24.2 ± 1.3 mm). Notably, even at lower concentrations such as 0.625% and 0.312%, several strains including *S. aureus*, *E. faecalis*, and *A. baumannii* remained responsive, with measurable inhibition zones ranging from 6.3 mm to 13.5 mm. However, some isolates showed no detectable inhibition at concentrations below 0.625%.

Comparative analysis of minimum inhibitory concentrations (MICs) revealed that clove oil exhibited lower MICs, ranging from 0.312% to 1.25% (v/v), indicating stronger antibacterial potency at lower concentrations. For rosemary oil, MICs ranged from 0.312% to 5% (v/v), with reduced effectiveness at lower concentrations except against *A. baumannii*. Overall, the results suggest that clove essential oil is more potent across a wider range of concentrations, whereas rosemary oil demonstrates high efficacy primarily at higher doses. The zone of inhibition patterns aligns with the MIC data, reinforcing the superior broad-spectrum antibacterial potential of clove oil against MDR bacterial strains.

Table 1: Antibacterial Activity of Rosemary Essential Oil

Bacterial Isolates	Zone of Inhibition (mm) at Various Oil Concentrations (v/v)						
	10%	**5%**	**2.5%**	**1.25%**	**0.625%**	**0.312%**	**DMSO**
Acinetobacter baumannii	34.2 ± 1.3	19.8 ± 1.5	17.9 ± 1.7	17.5 ± 1.6	15.8 ± 2.0	13.7 ± 2.4	0
Pseudomonas aeruginosa	33.1 ± 2.3	17.6 ± 2.0	-	-	-	-	0
Staphylococcus aureus	29.5 ± 1.6	19.3 ± 3.0	-	-	-	-	0
Enterococcus faecalis	30.9 ± 2.1	20.7 ± 3.2	-	-	-	-	0
P. aeruginosa ATCC 27853	29.2 ± 2.4	15.3 ± 1.4	6.5 ± 3.2	6.8 ± 1.5	-	-	0
S. aureus ATCC 29213	24.3 ± 3.1	21.1 ± 2.0	-	-	-	-	0

Table 2: Antibacterial Activity of Clove Essential Oil

Bacterial Isolates	Zone of Inhibition (mm) at Various Oil Concentrations (v/v)						
	10%	**5%**	**2.5%**	**1.25%**	**0.625%**	**0.312%**	**DMSO**
Acinetobacter baumannii	27.5 ± 2.1	23.1 ± 1.4	20.4 ± 2.0	14.7 ± 1.2	13.6 ± 0.9	13.5 ± 0.6	0
Pseudomonas aeruginosa	16.2 ± 3.1	14.2 ± 1.5	11.3 ± 1.0	9.7 ± 2.1	-	-	0
Staphylococcus aureus	19.3 ± 2.6	17.1 ± 1.4	14.6 ± 2.0	12.1 ± 1.1	9.4 ± 2.1	-	0
Enterococcus faecalis	24.1 ± 2.3	21.3 ± 2.1	18.5 ± 3.1	14.2 ± 1.4	12.7 ± 1.3	6.3 ± 1.4	0
P. aeruginosa ATCC 27853	24.2 ± 1.3	20.5 ± 3.3	18.4 ± 1.1	19.1 ± 1.6	15.4 ± 2.2	15.2 ± 1.2	0
S. aureus ATCC 29213	21.6 ± 1.4	18.9 ± 2.1	14.2 ± 2.1	11.4 ± 2.0	-	-	0

Discussion

The increasing incidence of multidrug-resistant (MDR) bacterial infections, particularly in hospital environments, has posed a major challenge for effective treatment strategies. As conventional antibiotics lose their efficacy due to resistance, there is growing interest in alternative therapeutic approaches. One promising avenue involves the use of plant-derived bioactive compounds such as essential oils, which are known to possess broad-spectrum antimicrobial activity [10,11].

In this study, essential oils extracted from clove and rosemary demonstrated inhibitory effects against a panel of MDR clinical isolates and standard strains. Notably, *Acinetobacter baumannii* exhibited the greatest susceptibility to both oils, particularly at higher concentrations. Rosemary oil at 10% (v/v) produced an inhibition zone of 34.2 mm, while clove oil at the same concentration showed a slightly lower inhibition zone of 27.5 mm against this organism. These findings are consistent with the notion that *A. baumannii* is more responsive to essential oils, possibly due to its outer membrane permeability and oxidative stress sensitivity [11,12].

At reduced concentrations, rosemary oil displayed diminished activity across most tested strains. For instance, no inhibition was observed below 5% against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis*. Only *A. baumannii* remained responsive down to 0.312% (v/v), albeit with a smaller inhibition zone. In contrast, clove oil retained a broader spectrum of effectiveness even at lower concentrations. For example, *E. faecalis* and *S. aureus* exhibited measurable inhibition at 0.625%, while *P. aeruginosa* responded up to 1.25%. This enhanced low-concentration efficacy is likely attributed to the high content of eugenol and tannins in clove oil, both known for their strong antibacterial properties [13,14].

Comparison with previously reported data suggests a general consistency in antibacterial trends, though differences in inhibition zone diameters and MIC values are evident. These variations could be due to differences in plant chemotypes, geographical sources, extraction techniques, or the resistance profile of the tested clinical isolates. The results also indicate that rosemary oil, while effective, shows more selective activity that may be enhanced by synergy with other agents. The major active constituents in rosemary oil, such as 1,8-cineole and camphor, have demonstrated antimicrobial potential but may require higher concentrations or combinatory effects for broad efficacy. Clove oil, on the other hand, displayed relatively stable performance across multiple strains, supporting its use as a more potent single-agent antimicrobial [15,16].

These findings are in agreement with earlier studies on the antimicrobial activities of essential oils, but the present study adds value by focusing on MDR clinical isolates rather than standard laboratory strains alone. Given the global rise in antimicrobial resistance, the consistent inhibitory activity observed here supports the continued exploration of essential oils, particularly clove oil, as natural antibacterial agents [17].

Further chemical characterization, safety assessments, and *in vivo* studies are recommended to validate these *in vitro* findings and facilitate potential therapeutic or preservative applications

Conclusion

This comparative analysis demonstrates that both clove and rosemary essential oils possess significant antibacterial properties against multidrug-resistant (MDR) bacterial strains. Among the two, clove oil showed slightly superior effectiveness across the tested isolates. The results support the potential application of these natural oils as alternative antimicrobial agents in treating infections caused by resistant pathogens. Further research and clinical trials are necessary to validate their efficacy and safety for therapeutic or preservative use.

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Nil.

Conflict of interest

None.

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