

## ARTICLE INFO

### AUTHOR'S AFFILIATIONS

Microbiology and Parasitology Department, Faculty of Medicine and Health Sciences, Warmadewa University<sup>1,2,3,4,5,6</sup>

Microbiology Department and Laboratory, Sanjiwani General Hospital, Gianyar, Bali, Indonesia<sup>7</sup>

Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland<sup>8</sup>

### CORRESPONDING AUTHOR

Marta Setiabudy, Microbiology and Parasitology Department, Faculty of Medicine and Health Sciences, Warmadewa University  
E-mail: [marta.sp.mk@gmail.com](mailto:marta.sp.mk@gmail.com)

### Article history

Received 04-06-2026  
Revised 05-08-2026  
Accepted 29-09-2026  
Available online

### Please cite this article in APA 7<sup>th</sup> edition style as:

Setiabudy, M., Indraningrat, A. A. G., Paramasatiari, A. A. A. L., Megawati, A. A. D., Widhidewi, N. W., Suryanditha, P. A., Suryawan, K., & Wedari, N. L. P. H. (2026). In vitro antibiofilm efficacy of citronella, lemongrass, ylang-ylang, and clove essential oils against clinical isolates of *Staphylococcus* spp.. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, 15(2), 190–201.  
<https://doi.org/10.30742/jikw.v15i2.5443>

## In Vitro Antibiofilm Efficacy of Citronella, Lemongrass, Ylang-Ylang, and Clove Essential Oils Against Clinical Isolates of *Staphylococcus* spp.

Marta Setiabudy<sup>1\*</sup>, Anak Agung Gede Indraningrat<sup>2</sup>, Anak Agung Ayu Lila Paramasatiari<sup>3</sup>, Anak Agung Dewi Megawati<sup>4</sup>, Ni Wayan Widhidewi<sup>5</sup>, Putu Arya Suryanditha<sup>6</sup>, Kadek Suryawan<sup>7</sup>, Ni Luh Putu Harta Wedari<sup>8</sup>

### Abstract

**Background:** Antibiotic resistance and biofilm formation highlight the need for alternative natural antimicrobial agents. Essential oils, including lemongrass, citronella, ylang-ylang, and clove oils, contain bioactive compounds with potential antibiofilm activity. **Objective:** The purpose of this study was to determine the antibiofilm activity of lemongrass, citronella, ylang-ylang, and clove essential oil against clinical *Staphylococcal* isolates. **Method:** This experimental study was conducted from June to October 2025 at the Warmadewa University Research Laboratory. A total of 36 clinical *Staphylococcus* spp. isolates from a regional hospital in Bali were tested. Biofilm formation was assessed using a microtiter plate assay with crystal violet staining after treatment with 9.1% (v/v) essential oil. Chloramphenicol and aquabidest were used as positive and negative controls, respectively, and optical density was analyzed using repeated-measures ANOVA. **Result:** This study showed that lemongrass, citronella, and ylang-ylang essential oils significantly reduced the formation of bacterial biofilms by 53%, 67%, and 47% compared to untreated group ( $p < 0.001$ ). Citronella was the most effective antibiofilm agent, followed by lemongrass and ylang-ylang, while cloves showed no significant effect. We hypothesize that these effects are due to cell membrane disruption, quorum sensing inhibition, and decreased biofilm matrix production. **Conclusion:** Citronella, lemongrass, and ylang-ylang essential oils significantly reduced biofilm formation by clinical isolates of *Staphylococcus* spp., with citronella showing the greatest reduction in biofilm biomass. These findings suggest that selected essential oils may have potential as natural agents for preventing or inhibiting biofilm formation and warrant further investigation, including studies evaluating their effects on established biofilms.

**Keywords:** Biofilm, Essential oil, *Staphylococcal* infection

## Original Research Article

### INTRODUCTION

*Staphylococcus* spp. are pathogenic microorganisms responsible for a wide range of infections, varying from minor skin conditions to serious, life-threatening diseases such as bacteremia, sepsis, pulmonary infections, and endocarditis. One of the principal virulence traits of *Staphylococcus* spp. is their ability to develop biofilms, which are organized bacterial communities that attach to both living tissues and

inert surfaces. Biofilm formation significantly enhances bacterial tolerance to antimicrobial agents and evasion of host immune defenses, thereby complicating infection eradication and contributing to the persistence of chronic infections and those associated with medical devices.

The widespread emergence of antibiotic resistance driven by biofilm formation has created an urgent need to identify alternative antimicrobial agents from natural sources. Essential oils have long been recognized for their antimicrobial properties, including their capacity to inhibit biofilm formation and disrupt established bacterial biofilms. Indonesia as a tropical country rich in biodiversity has various essential oil-producing plants, such as lemongrass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*), ylang-ylang (*Cananga odorata*), and clove (*Syzygium aromaticum*), which have the potential to be natural antibiofilm agents.

Essential oils are volatile substances derived from different plant parts, including leaves, flowers, wood, and seeds. Essential oils comprise a diverse array of bioactive compounds, including phenolic compounds, terpenoids, and aldehydes, which are well known for their antimicrobial activity (Sefriyanti et al., 2020; Wardani et al., 2024). The antibacterial mechanisms of essential oils include disruption of cell membranes, inhibition of metabolic enzymes, and inhibition of protein synthesis and bacterial nucleic acids. In addition, some essential oils also show antibiofilm activity by preventing bacterial cell adhesion, inhibiting biofilm growth, and damaging biofilms that have been formed (Kerekes et al., 2019)

Multiple studies have demonstrated that essential oils exhibit activity against *Staphylococcus aureus*, including antibiotic-resistant strains (Wardani et al., 2024). Therefore, essential oils from plants of Balinese origin, such as lemongrass, citronella, ylang-ylang, and clove, have the potential to be antibiofilm agents that can be used in the treatment of infections due to *S. aureus*.

Lemongrass essential oil is rich in citral, its major bioactive constituent, which has been reported to exhibit antibacterial activity against various pathogenic microorganisms, including *Staphylococcus aureus*. The antimicrobial action of citral is associated with disruption of bacterial cell membrane integrity, leading to increased membrane permeability and subsequent interference with essential cellular processes such as protein synthesis and nucleic acid metabolism, ultimately resulting in bacterial cell death (Zhou et al., 2023). In addition, citral may inhibit the activity of key metabolic enzymes, further contributing to its antibacterial effects. Beyond its antimicrobial properties, lemongrass essential oil has also been shown to possess anti-inflammatory activity, which may enhance its therapeutic potential in the management of biofilm-associated infections.

Citronella essential oil (*Cymbopogon nardus*) contains major bioactive compounds such as citronellal and geraniol, both of which demonstrate antibacterial activity against various pathogens, including *Staphylococcus aureus*. Citronellal inhibits the growth of *S. aureus* by disrupting bacterial cell membrane permeability, resulting in ion leakage and cellular dysfunction, while geraniol also exhibits significant antibacterial effects (Pontes et al., 2019). Several studies have shown that citronella oil can inhibit early stages of biofilm development and disrupt the structural integrity of mature biofilms. For instance, previous research demonstrated that citronella oil effectively inhibits the growth of oral pathogenic bacteria, highlighting its potential as a natural antibacterial agent (Ahmad Kamal et al., 2020).

Ylang-ylang essential oil (*Cananga odorata*) contains several natural bioactive constituents, including linalool, geraniol, and farnesol, which are recognized for their antibacterial and antifungal activities. Linalool has been reported to inhibit the growth of *Staphylococcus aureus*, primarily through disruption of cell membrane integrity and interference with protein synthesis. In addition, geraniol has demonstrated notable antibiofilm activity against *Candida albicans* and has been shown to downregulate the expression of fungal enzymes as well as host pro-inflammatory cytokines (Vasconcelos et al., 2024). Farnesol, a naturally occurring quorum-sensing molecule, is known to act as an anti-biofilm agent by interfering with communication between bacterial cells or the quorum sensing mechanism, which is important in the formation of biofilms. Research shows that farnesol can inhibit the formation of hyphae and biofilms in *Candida albicans*, as well as affect the production of farnesol and sterols (Lee et al., 2021) In addition, farnesol also shows effects on bacteria such as *Staphylococcus*

*aureus*, where exposure to farnesol results in a significant decrease in staphyloxanthin, an important virulence factor as oxidative stress resistance in *Staphylococcus aureus* (Kovács & Majoros, 2020). Thus, the compounds in ylang-ylang oil, such as linalool, geraniol, and farnesol, have great potential as antibacterial and antifungal agents, especially in inhibiting pathogen growth and biofilm formation.

Clove essential oil contains eugenol as its primary bioactive constituent, which exhibits broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Eugenol exerts its antibacterial effects by disrupting bacterial cell wall integrity, leading to ion leakage and impaired cellular function (Ashrafudoulla et al., 2020; Silva & Fernandes A., 2020). In addition to its antibacterial activity, eugenol has been reported to possess antibiofilm properties by inhibiting the expression of genes involved in *Staphylococcus aureus* biofilm formation (Zhou et al., 2023).

The antibiofilm activity of essential oils is thought to involve multiple mechanisms, including inhibition of bacterial adhesion during the initial stages of biofilm formation, disruption of quorum sensing-mediated cell-to-cell communication, and degradation of the biofilm extracellular matrix. Additionally, essential oils may suppress the metabolic activity of biofilm-embedded bacteria by interfering with the synthesis of enzymes and proteins essential for biofilm maintenance and growth. Collectively, these mechanisms suggest that essential oils derived from native Balinese plants hold promising potential as natural antibiofilm agents for the management of *Staphylococcus aureus*-associated infections.

*Staphylococcus* spp. are commensal microorganisms; however, they are also well-recognized as causative agents of a wide range of human infections, including skin and soft tissue infections, respiratory tract infections, osteoarticular infections, endovascular infections, and infections associated with immunocompromised conditions, medical devices, or implanted materials (Águila-arcos et al., 2018). *Staphylococcus aureus* remains a major global health concern, particularly methicillin-resistant *Staphylococcus aureus* (MRSA).

Humans and animals are colonized by numerous bacterial species belonging to the genus *Staphylococcus*; however, only a limited number have evolved the capacity to cause invasive disease. The acquisition or presence of specific virulence genes, such as those encoding fibrin-binding and agglutination factors, can transform commensal *Staphylococcus* spp. into invasive pathogens (Gibson et al., 2021).

Most *Staphylococcus* spp., including *Staphylococcus aureus*, possess the ability to form biofilms (Oliveira et al., 2021), a trait that significantly contributes to the development of severe and chronic infections. Biofilms are organized communities of microbial cells that are firmly attached to one another and to biotic or abiotic surfaces and are enclosed within a self-produced extracellular polymeric substance (EPS) matrix (Goel et al., 2021). Biofilm formation occurs as microorganisms establish a protected and favorable microenvironment, characterized by the secretion of extracellular polymers that promote adhesion. A defining feature of biofilm-associated infections is their marked resistance to antimicrobial agents and evasion of host immune responses.

Persistent infections related to biofilm formation are particularly challenging to manage due to their inherent resistance to many therapeutic agents. The limited efficacy of existing treatments and the potential toxicity of conventional antibiotics in vivo have driven interest in the exploration of effective natural antibiofilm agents. Natural product-based antibiofilm compounds have been reported to exhibit promising efficacy with fewer adverse effects compared to chemically synthesized antibiotics (Mishra et al., 2020).

The purpose of this study was to determine the antibiofilm activity of commonly used essential oils derived from local plants in Bali. Research and publication on the activity of antibiotics from natural ingredients in the form of essential oils are expected to be useful for the prevention and treatment of *Staphylococcus aureus* infection in the community in general and tourism actors in particular. This development is also a very attractive aspect for foreign tourists who are interested in natural ingredients as an alternative to therapy, for example in wounds.

## MATERIALS AND METHODS

The study was conducted at the Research Laboratory of Warmadewa University. This research employed an in vitro experimental design to evaluate the antibiofilm activity of selected essential oils against clinical isolates of *Staphylococcus* spp.

The target population consisted of clinical isolates obtained from patients with wound infections caused by *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. Clinical isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. were obtained from culture of patients treated at Sanjiwani Hospital. A total of 36 clinical isolates were included in the study and subjected to the biofilm formation assay.

Commercial essential oils of lemongrass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*), clove (*Syzygium aromaticum*), and ylang-ylang (*Cananga odorata*) were purchased from artisanal essential oil producer (traditional essential oil distiller) in Java Island. According to the manufacturer's specifications, the oils were obtained by steam distillation from lemongrass leaves, citronella leaves, clove flower buds, and ylang-ylang flowers traditionally. The oils were stored in amber glass bottles at room temperature until use. No additional chemical characterization, including gas chromatography–mass spectrometry (GC–MS), was performed prior to the antimicrobial and antibiofilm assays.

Once clinical ethics were approved and research permits were in place, the research was conducted. Ethical approval was obtained from the Ethics Committee of Faculty of Medicine, Warmadewa University (No: 105/Unwar/FKIK/EC-KEPK/IX/2025).

Thus, the research procedure:

### Sample preparation

Clinical specimens were cultured and incubated for 24 hours. Identification was done using VITEC 2® in Sanjiwani Hospital microbiology laboratory where the samples were obtained. The pure isolates that met the criteria were then collected and sent to Faculty of Medicine Warmadewa University Research Laboratory for further test.

Mueller–Hinton agar (MHA) plates were prepared according to the manufacturer's instructions. Isolates were subcultured and incubated for 24 hours.

### Bacterial Inoculum Preparation

Pure colonies of *Staphylococcus* spp. were harvested from overnight cultures and resuspended in phosphate-buffered saline (PBS; pH 7.0) supplemented with 1% glucose. The bacterial suspension was then standardized to a turbidity equivalent to 0.5 McFarland (approximately  $1 \times 10^8$  CFU/mL) using a Denscheck device, in accordance with CLSI guidelines.

### Biofilm Formation Assay

Biofilm formation was evaluated using the microtiter plate (MTP) assay with sterile 96-well polystyrene plates. Briefly, 200 µL of the standardized bacterial suspension was inoculated into each designated well (Čuvalová et al., 2018; Fitria, 2018). Experimental setups included appropriate controls. A positive control comprising bacterial suspension treated with chloramphenicol 20 ul (concentration of 0.01 gr/mL stock solution) in 200 ul suspension, and a negative control consisting of bacterial suspension with sterile distilled water in the absence of essential oil extract.

Lemongrass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*), ylang-ylang (*Cananga odorata*), and clove essential oil (*Syzygium aromaticum*) with volume 20 ul were added to the designated treatment wells at the base of the microtiter plates, followed by inoculation with the bacterial suspension. In every well received 20 µL of undiluted essential oil added to 200 µL of bacterial suspension, resulting in a final concentration of approximately 9.1% (v/v). The plates were incubated at 37°C for 48 hours under aerobic conditions to facilitate optimal bacterial growth and biofilm formation. The incubation temperature and duration were determined based on prior optimization experiments to ensure optimal bacterial growth and maximal biofilm formation under the experimental conditions.

Following inoculation, the microtiter plates were covered with the manufacturer's sterile lid and additionally sealed with plastic wrap during incubation to minimize evaporation and potential vapor-

phase transfer of volatile essential oil constituents. Treatment and control wells were assigned according to a predefined plate layout to reduce potential interference between adjacent wells.

#### **Biofilm Quantification**

After incubation, the medium in each well was carefully removed, followed by gentle washing twice with sterile distilled water to eliminate non-adherent cells. The adherent biofilm was then stained by adding 200 µL of 0.1% crystal violet solution to each well and allowing it to stand for 5 minutes (Kragh et al., 2019; O'Toole, 2019). Excess stains were removed through thorough washing, and the plates were left to air-dry. To quantify the biofilm biomass, the bound crystal violet was subsequently solubilized with 200 µL of 30% acetic acid per well and incubated for 5–15 minutes. The optical density of the resulting solution was then measured at 620 nm using a microplate ELISA reader.

#### **Data Analysis**

Data analysis was carried out in two phases. Initially, descriptive statistics were applied to characterize each study variable. Categorical variables were summarized as relative frequencies and reported in terms of counts and percentages.

Second, inferential analysis was performed to evaluate differences in biofilm formation between bacterial suspensions without essential oil treatment (no treatment/ untreated) and those treated with essential oil extracts. The normality of the biofilm optical density data was assessed using the Shapiro–Wilk test. Pairwise comparisons following repeated-measures ANOVA were performed using the Bonferroni adjustment. Biofilm formation categories were presented descriptively as frequencies and percentages and were not subjected to inferential statistical testing.

All statistical analyses were conducted using SPSS for Windows version 25 (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered indicative of statistical significance.

## **RESULTS**

Thirty-six biofilm-producing clinical isolates were included based on the availability of eligible isolates during the study period. Samples were obtained from Sanjiwani Hospital Microbiology laboratory. The age distribution of the patients involved showed that the age group of 46–65 years dominated (44.4%), followed by the age group >65 years (36.1%), while the young age group (<17 years) represented only 5.6% of the study population. This shows that the incidence of *Staphylococcus* spp. infection tends to increase in the adult to elderly age group as shown in table 1 below.

**Table 1.** Patient age distribution

| Age Category | Frequency (%) |
|--------------|---------------|
| <17          | 2 (5.6%)      |
| 17-45        | 5 (13.9%)     |
| 46-65        | 16 (44.4%)    |
| >65          | 13 (36.1%)    |

Blood was the most common specimen (55.6%), followed by wound base swabs (19.4%), sputum (13.9%), and small amounts from pus, tissues, and urine. This shows that *Staphylococcus* spp. is often isolated from systemic infections as well as infections in wounds and tissues (Linz et al., 2023; Neopane et al., 2018). The specimen types can be seen in table 2.

**Table 2.** Specimen type

| Specimen type | Frequency (%) |
|---------------|---------------|
| Blood         | 20 (55.6%)    |
| Sputum        | 5 (13.9%)     |
| Wound swab    | 7 (19.4%)     |
| Pus           | 2 (5.6%)      |
| Tissue        | 1 (2.8%)      |
| Urine         | 1 (2.8%)      |

In line with the specimen type, the most common clinical diagnoses of patients were pneumonia and other respiratory infections (33.3%), followed by cases of infected wounds/ abscesses/ cellulitis/ osteomyelitis (22.2%) and chronic diseases such as CKD (16.7%) and DM and its complications (13.9%).

The study included 36 clinical *Staphylococcus* isolates comprising *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. species, including *S. hominis* subsp. *hominis*, *S. haemolyticus*, *S. epidermidis*, and *S. capitis*. Species-level identification was performed for all isolates. However, the primary objective of the present study was to evaluate the overall antibiofilm activity of the essential oils against clinical *Staphylococcus* spp. rather than to compare responses among individual species. Consequently, the statistical analyses were performed using the combined *Staphylococcus* isolate collection.

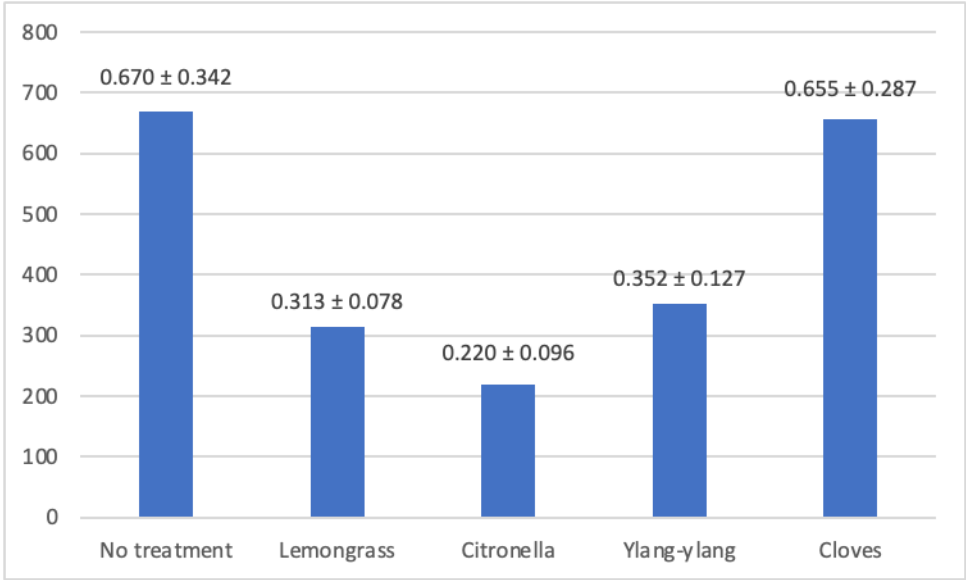


**Figure 1** Comparison of the mean biofilm formation values between the control group (without essential oil treatment) and groups treated with lemongrass, citronella, ylang-ylang, and clove essential oils. Biofilm formation was quantified using optical density measurements, with lower values indicating greater inhibitory activity against biofilm formation.

The distribution of biofilm optical density measurements was assessed using the Shapiro–Wilk test prior to repeated-measures ANOVA. The results were used to assess the suitability of the data for parametric analysis. Analysis of the antibiofilm assay demonstrated that lemongrass, citronella, and ylang-ylang essential oils significantly reduced biofilm biomass compared with the untreated control (all  $p < 0.001$ ). The untreated control exhibited the highest mean absorbance ( $OD_{620}$ ) of  $0.670 \pm 0.342$  AU, whereas citronella treatment resulted in the lowest mean absorbance of  $0.220 \pm 0.096$  AU. Lemongrass ( $0.313 \pm 0.078$  AU) and ylang-ylang ( $0.352 \pm 0.127$  AU) also showed lower biofilm biomass than the untreated control. In contrast, clove oil produced a mean absorbance of  $0.655 \pm 0.287$  AU, which was comparable to that of the untreated control and exhibited greater variability among the clinical isolates. Each treatment was performed in duplicate for every bacterial isolate, and the mean optical density value was used for statistical analysis.

In addition to quantitative measurements based on the average absorbance value, this study also groups the ability of biofilm formation into four categories, namely strong, moderate, weak, and none/ very minimal. Biofilm formation was classified according to the fundamental method criteria described by Stepanović et al in 2000. The optical density cut-off ( $OD_c$ ) was defined as the mean optical density of the negative control plus three standard deviations (0.342 AU). Isolates were classified as non-biofilm producers ( $OD \leq OD_c$ ), weak biofilm producers ( $OD_c < OD \leq 2 \times OD_c$ ), moderate biofilm producers ( $2 \times OD_c < OD \leq 4 \times OD_c$ ), and strong biofilm producers ( $OD > 4 \times OD_c$ ). The results of the observations show a clear difference in distribution between treatments, as shown in table 3 and figure 2 below.

**Table 3.** Categories of biofilm formation in each treatment group

| Biofilm formation  | No treatment | Lemongrass | Citronella | Ylang-ylang | Cloves |
|--------------------|--------------|------------|------------|-------------|--------|
| Strong             | 4            | 0          | 0          | 0           | 8      |
| Moderate           | 11           | 0          | 0          | 2           | 14     |
| Weak               | 18           | 3          | 3          | 13          | 9      |
| None/ very minimal | 3            | 33         | 33         | 21          | 5      |

Table 3 shows the distribution of biofilm biomass categories among the untreated and essential-oil-treated groups. In the untreated group, most isolates were classified as moderate (11/36) or weak (18/36) biofilm producers, while 4/36 isolates were classified as strong and 3/36 as none/very minimal. Following exposure to the essential oils, a marked shift toward the none/very minimal category was observed, particularly with lemongrass and citronella, with 33 of 36 isolates in each group classified as having none/very minimal biofilm biomass. The ylang-ylang group showed 21/36 isolates in the none/very minimal category, whereas the clove group showed 5/36. These descriptive findings demonstrate a shift in the distribution of observed biofilm biomass categories following essential-oil exposure, with the most pronounced shifts observed in the lemongrass and citronella groups.

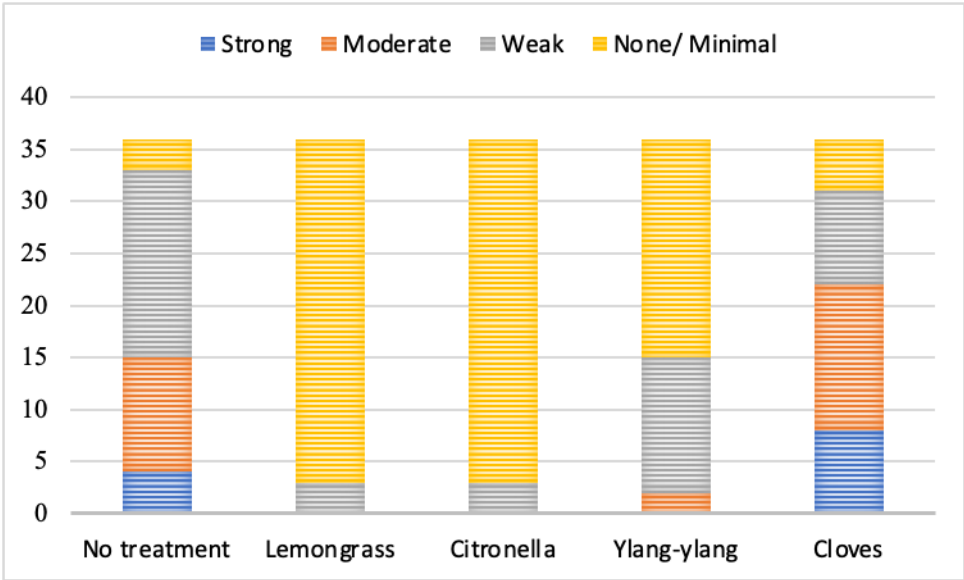


**Figure 2.** Distribution of biofilm biomass categories among the untreated control group and groups treated with lemongrass, citronella, ylang-ylang, and clove essential oils. Biofilm biomass was classified as strong, moderate, weak, or none/very minimal based on optical density values. The figure illustrates the observed distribution of biofilm biomass categories across the experimental conditions, with a marked shift toward the none/very minimal category in the lemongrass and citronella groups.

**DISCUSSION**

Demographic data of the study showed a dominance of the adult and elderly groups of age, which generally had comorbidities such as diabetes mellitus and chronic kidney disease. These conditions can increase the risk of recurrent infection and are recognized as risk factors for recurrent or more severe infections of infection by *Staphylococcus* spp. (Howden et al., 2023). Type of specimen and diagnosis also revealed consistent data.

The comparison graph from figure 1 showed citronella essential oil produced the greatest reduction in biofilm biomass, followed by lemongrass and ylang-ylang essential oils, whereas clove essential oil did not demonstrate a significant reduction under the present experimental conditions. These findings indicate that citronella, lemongrass, and ylang-ylang essential oils are associated with a

significant reduction in crystal violet-stained biofilm biomass compared with the untreated control. Although the present study did not investigate the underlying mechanisms or characterize the chemical composition of the essential oils, previous studies have suggested that the biological activity of essential oils is related to their hydrophobic nature and bioactive constituents. These properties facilitate interaction with the bacterial cell membrane, resulting in altered membrane permeability and disruption of membrane integrity (Elkenawy et al., 2023).

Citronella essential oil produced the greatest reduction in biofilm biomass, decreasing the mean OD value from 0.670 to 0.220, corresponding to an approximately 67% reduction compared with the untreated control. Previous studies have reported that *Cymbopogon nardus* essential oil commonly contains citronellal, citronellol, and geraniol as major constituents. Geraniol has been shown to inhibit *Staphylococcus aureus* biofilm formation, whereas citronellal has been reported to reduce biofilm thickness and bacterial metabolic activity (Ahmad Kamal et al., 2020; Pontes et al., 2019). In addition, geraniol has been demonstrated to inhibit the synthesis of major virulence factors, including staphyloxanthin and autolysins (Gu et al., 2022). However, because the chemical composition of the citronella essential oil used in the present study was not characterized, the observed reduction in biofilm biomass cannot be directly attributed to these specific constituents.

Similarly, lemongrass (*Cymbopogon citratus*) essential oil significantly reduced biofilm biomass, although to a lesser extent than citronella essential oil. Previous studies have reported that lemongrass essential oil is commonly rich in citral, a mixture of geraniol and neral, which has demonstrated antibacterial and biofilm-inhibitory activities. Citral has also been reported to reduce the microbial burden of pathogenic microorganisms and possesses antifungal, antiparasitic, antiproliferative, anti-inflammatory, and antioxidant properties in both in vitro and in vivo studies (Gutiérrez-Pacheco et al., 2023). Furthermore, citral has been associated with reduced expression of biofilm-related regulatory genes, including the *ica* genes involved in *Staphylococcus* biofilm formation. Nevertheless, as the essential oils evaluated in the present study were not chemically characterized, these previously reported mechanisms should be interpreted as potential explanations from the literature rather than mechanisms demonstrated in the present study.

Ylang-ylang (*Cananga odorata*) essential oil also produced a significant reduction in biofilm biomass compared with the untreated control ( $p < 0.001$ ), although its effect was less pronounced than that of citronella and lemongrass essential oils. Previous studies have reported that *C. odorata* essential oil contains various volatile compounds, with linalool commonly identified as one of its predominant constituents. Linalool has been widely recognized for its antimicrobial properties and has been reported to contribute substantially to the antibacterial activity of ylang-ylang essential oil (Elkenawy et al., 2023). In addition, linalool and benzyl benzoate have been associated with reduced bacterial adhesion and metabolic activity. Previous studies have also demonstrated the antibacterial activity of ylang-ylang flower extract against *Streptococcus mutans* and *Pseudomonas aeruginosa* (Purwa Hita et al., 2025). However, because the chemical composition of the essential oil used in the present study was not characterized, these previously reported constituents and mechanisms should be regarded as potential explanations from the literature rather than mechanisms demonstrated in the present study.

In contrast, clove (*Syzygium aromaticum*) essential oil did not produce a significant reduction in biofilm biomass compared with the untreated control ( $p = 0.291$ ). Previous studies have reported that clove essential oil commonly contains eugenol, a phenolic compound with well-documented antibacterial activity (Ashrafudoulla et al., 2020; Elbestawy et al., 2023; Markaoui et al., 2025). However, the biological activity of clove essential oil has been shown to vary depending on the target microorganism, chemical composition, concentration, and duration of exposure. For example, Rifai et al. (2024) reported that clove leaf essential oil exhibited inhibitory activity against *Candida albicans* biofilms, although the reduction was not statistically significant compared with the control group (Rifai et al., 2024). The absence of a significant effect observed in the present study may therefore reflect differences in experimental conditions, microbial species, or the chemical composition of the essential oil, which was not characterized in the present investigation.

The distribution of biofilm formation categories showed that citronella and lemongrass essential oils shifted a greater proportion of isolates from the strong or moderate biofilm-producing categories to the weak or non-biofilm-producing categories. These observations further support the ability of these essential oils to reduce crystal violet-stained biofilm biomass under the experimental conditions used in this study (Martínez et al., 2021).

The distribution of this category also illustrates the potential basis for further investigation of essential oils as a natural antibiofilm agent. For example, citronella and lemongrass may have potential for further investigation as active ingredients in antimicrobial coatings on medical devices, or in topical antiseptic formulations that target chronic infections due to biofilms.

The present findings provide preliminary evidence supporting the potential of selected essential oils as adjunctive candidates for biofilm control strategies. Nevertheless, the results were obtained from an in vitro model, and further investigations, including concentration-response studies, bacterial viability assays, cytotoxicity testing, and in vivo validation, are required before their potential clinical applications in medical devices, chronic wounds, or other healthcare settings can be established.

This study has several limitations. First, the commercial essential oils were not chemically characterized by gas chromatography–mass spectrometry (GC–MS); therefore, the chemical composition of each oil could not be confirmed, and the observed biological activities cannot be attributed to specific constituents. Second, biofilm formation was evaluated using the crystal violet microtiter plate assay, which quantifies total biofilm biomass but does not distinguish between inhibition of biofilm formation and reductions in biomass resulting from antibacterial activity. Because bacterial viability and planktonic growth were not assessed, the specific mechanism underlying the observed reductions in biofilm biomass could not be determined. Third, although all isolates were identified at the species level, the study was designed to evaluate the overall response of clinical *Staphylococcus* spp. isolates rather than species-specific susceptibility, and therefore comparisons among individual *Staphylococcus* species were not performed. Future studies incorporating chemical characterization of essential oils, complementary biofilm and bacterial viability assays, and species-specific analyses are warranted to further elucidate the mechanisms underlying the observed effects.

## **CONCLUSION**

This study demonstrated that lemongrass, citronella, and ylang-ylang essential oils significantly reduced crystal violet-stained biofilm biomass of clinical *Staphylococcus* spp. isolates compared with the untreated control. Among the tested oils, citronella produced the greatest reduction in biofilm biomass, followed by lemongrass and ylang-ylang, whereas clove essential oil did not show a significant effect.

These findings suggest that selected essential oils have potential as natural agents for reducing *Staphylococcus* biofilm biomass and may warrant further investigation as adjunctive approaches for biofilm control. However, because the chemical composition of the essential oils was not characterized and bacterial viability was not evaluated, the observed reductions in biofilm biomass cannot be attributed to specific chemical constituents or distinguished from antibacterial effects.

Future studies should include chemical characterization of essential oils, complementary assays to differentiate antibiofilm and antibacterial activities, dose-response and cytotoxicity evaluations, and in vivo studies to further assess their potential applications in infection prevention and wound management.

## **CONFLICT OF INTEREST**

The authors declare no conflict of interest/or conflict.

## **ACKNOWLEDGEMENTS**

The authors express their sincere gratitude to the Faculty of Medicine and Health Sciences, Warmadewa University, and to all individuals who contributed to the completion of this study. This research was financially supported by the Research Unit of the Faculty of Medicine and Health

Sciences, Universitas Warmadewa, Denpasar, Indonesia, under Decision No. 828/Unwar/FKIK/PD-13/VII/2025.

**REFERENCES**

Águila-arcos, S., Álvarez-rodríguez, I., & Garaiyurrebaso, O. (2018). *Biofilm-Forming Clinical Staphylococcus Isolates Harbor Horizontal Transfer and Antibiotic Resistance Genes*. 8(October 2017), 1–12. <https://doi.org/10.3389/fmicb.2017.02018>

Ahmad Kamal, H. Z., Tuan Ismail, T. N. N., Arief, E. M., & Ponnuraj, K. T. (2020). Antimicrobial Activities of Citronella (*Cymbopogon nardus*) Essential Oil Against Several Oral Pathogens and Its Volatile Compounds. *Padjadjaran Journal of Dentistry*, 32(1), 1. <https://doi.org/10.24198/pjd.vol32no1.24966>

Ashrafudoulla, M., Mizan, M. F. R., Ha, A. J. won, Park, S. H., & Ha, S. Do. (2020). Antibacterial and antibiofilm mechanism of eugenol against antibiotic resistance *Vibrio parahaemolyticus*. *Food Microbiology*, 91. <https://doi.org/10.1016/j.fm.2020.103500>

Čuvalová, A., Strapáč, I., Handrová, L., & Kmeť, V. (2018). Antibiofilm activity of mushroom extracts against *Staphylococcus aureus*. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*. <https://doi.org/10.24917/25438832.3supp.2>

Elbestawy, M. K. M., El-Sherbiny, G. M., & Moghannem, S. A. (2023). Antibacterial, Antibiofilm and Anti-Inflammatory Activities of Eugenol Clove Essential Oil against Resistant *Helicobacter pylori*. *Molecules*, 28(6). <https://doi.org/10.3390/molecules28062448>

Elkenawy, N. M., Soliman, M. A. W., & El-behery, R. R. (2023). In-vitro Antimicrobial Study of Non/irradiated Ylang-ylang Essential Oil Against Multi Drug Resistant Pathogens with Reference to Microscopic Morphological Alterations. *Indian Journal of Microbiology*, 63(4), 621–631. <https://doi.org/10.1007/s12088-023-01122-4>

Fitria, A. (2018). The Bactericidal and Antibiofilm Activity of Stem Bark of *Jatropha multifida* L. Against *Staphylococcus aureus* and MRSA. *EKSAKTA: Journal of Sciences and Data Analysis*, 42–55. <https://doi.org/10.20885/eksakta.vol18.iss1.art5>

Gibson, J. F., Pidwill, G. R., Carnell, O. T., Surewaard, B. G. J., Shamarina, D., ...& Foster, S. J. (2021). Commensal bacteria augment *Staphylococcus aureus* infection by inactivation of phagocyte-derived reactive oxygen species. *PLoS Pathogens*, 17(9). <https://doi.org/10.1371/journal.ppat.1009880>

Goel, N., Fatima, S. W., Kumar, S., Sinha, R., & Khare, S. K. (2021). Antimicrobial resistance in biofilms: Exploring marine actinobacteria as a potential source of antibiotics and biofilm inhibitors. In *Biotechnology Reports* (Vol. 30). Elsevier B.V. <https://doi.org/10.1016/j.btre.2021.e00613>

Gu, K., Ouyang, P., Hong, Y., Dai, Y., Tang, T., ...& Yin, L. (2022). Geraniol inhibits biofilm formation of methicillin-resistant *Staphylococcus aureus* and increase the therapeutic effect of vancomycin in vivo. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.960728>

Gutiérrez-Pacheco, M. M., Torres-Moreno, H., Flores-Lopez, M. L., Velázquez Guadarrama, N., Ayala-Zavala, J. F., ...& López-Romero, J. C. (2023). Mechanisms and Applications of Citral's Antimicrobial Properties in Food Preservation and Pharmaceuticals Formulations. In *Antibiotics* (Vol. 12, Number 11). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/antibiotics12111608>

Howden, B. P., Giulieri, S. G., Wong Fok Lung, T., Baines, S. L., Sharkey, L. K., ...& Stinear, T. P. (2023). *Staphylococcus aureus* Host Interactions and Adaptation. In *Nature Reviews Microbiology* (Vol. 21, Number 6, pp. 380–395). Nature Research. <https://doi.org/10.1038/s41579-023-00852-y>

Kerekes, E. B., Vidács, A., Takó, M., Petkovits, T., Vágvölgyi, C., ...& Krisch, J. (2019). Anti-biofilm effect of selected essential oils and main components on mono- and polymicrobial bacterial cultures. *Microorganisms*, 7(9). <https://doi.org/10.3390/microorganisms7090345>

Kovács, R., & Majoros, L. (2020). Fungal Quorum-Sensing Molecules: A Review of Their Antifungal Effect Against *Candida* Biofilms. In *Journal of Fungi* (Vol. 6, Number 3, pp. 1–13). MDPI AG. <https://doi.org/10.3390/jof6030099>

- Kragh, K. N., Alhede, M., Kvich, L., & Bjarnsholt, T. (2019). Into the well—A close look at the complex structures of a microtiter biofilm and the crystal violet assay. *Biofilm*, 1. <https://doi.org/10.1016/j.biofilm.2019.100006>
- Lee, J. H., Kim, Y. G., Khadke, S. K., & Lee, J. (2021). Antibiofilm and Antifungal Activities of Medium-Chain Fatty Acids Against *Candida albicans* Via Mimicking of The Quorum-Sensing Molecule Farnesol. *Microbial Biotechnology*, 14(4), 1353–1366. <https://doi.org/10.1111/1751-7915.13710>
- Linz, M. S., Mattappallil, A., Finkel, D., & Parker, D. (2023). Clinical Impact of *Staphylococcus aureus* Skin and Soft Tissue Infections. In *Antibiotics* (Vol. 12, Number 3). MDPI. <https://doi.org/10.3390/antibiotics12030557>
- Markaoui, I., Yahyaoui, M. I., Asehraou, A., Daoudi, A., Housni, B., & Bkiyar, H. (2025). Evaluation of the Antibiofilm Activity of Clove Essential Oil in the Development of Bioactive Coatings for Arterial Sampling Devices. *Microbiology Research*, 16. <https://doi.org/10.3390/microbiolres16120260>
- Martínez, A., Manrique-Moreno, M., Klais-Luna, M. C., Stashenko, E., Zafra, G., & Ortiz, C. (2021). Effect of Essential Oils on Growth Inhibition, Biofilm Formation and Membrane Integrity of *Escherichia coli* and *Staphylococcus aureus*. *Antibiotics*, 10(12). <https://doi.org/10.3390/antibiotics10121474>
- Mishra, R., Panda, A. K., De Mandal, S., Shakeel, M., Bisht, S. S., & Khan, J. (2020). Natural Anti-biofilm Agents: Strategies to Control Biofilm-Forming Pathogens. In *Frontiers in Microbiology* (Vol. 11). Frontiers Media S.A. <https://doi.org/10.3389/fmicb.2020.566325>
- Nazzaro, F., Coppola, F., Fratianni, F., Abdalrazeq, M., Ombra, M. N., ...& Zengin, G. (2025). Polyphenols Bioactive Metabolites, and Their Anti-Biofilm and Neuroprotective Potential. In *Foods* (Vol. 14, Number 22). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/foods14223976>
- Neopane, P., Nepal, H. P., Shrestha, R., Uehara, O., & Abiko, Y. (2018). In vitro biofilm formation by *Staphylococcus aureus* isolated from wounds of hospital-admitted patients and their association with antimicrobial resistance. *International Journal of General Medicine*, 11, 25–32. <https://doi.org/10.2147/IJGM.S153268>
- Oliveira, A., Pinheiro-Hubinger, L., Pereira, V. C., Riboli, D. F. M., Martins, K. B., ...& Cunha, M. (2021). Staphylococcal Biofilm on the Surface of Catheters: Electron Microscopy Evaluation of the Inhibition of Biofilm Growth by RNAIII Inhibiting Peptide. *Antibiotics*, 10(7). <https://doi.org/10.3390/antibiotics10070879>
- O'Toole, G. A. (2019). Microtiter dish biofilm formation assay. *Journal of Visualized Experiments*, (47), e2437.
- Pontes, E. K. U., Melo, H. M., Nogueira, J. W. A., Firmino, N. C. S., de Carvalho, M. G., ...& Cavalcante, T. T. A. (2019). Antibiofilm Activity of The Essential Oil of Citronella (*Cymbopogon nardus*) and Its Major Component, Geraniol, on The Bacterial Biofilms of *Staphylococcus aureus*. *Food Science and Biotechnology*, 28(3), 633–639. <https://doi.org/10.1007/s10068-018-0502-2>
- Purwa Hita, I. P. G. A., Budhi Setiawan, P. Y., Wintariani, N. P., & Anggarini, D. (2025). Antibacterial Activity of Cananga Extract (*Cananga odorata* (Lam.) Hook.F. & Thomson) Against *Streptococcus Mutans* and *Pseudomonas Aeruginosa* Bacteria. *Jurnal Farmasimed (JFM)*, 8(1), 122–130. <https://doi.org/10.35451/nyc2kg76>
- Putu Saraswati Kristina, N., Wayan Tanjung Aryasa, I., & Putu Risky Vidika Apriyanthi, D. (2023). Antibacterial Activity of Ethanol Extract of Tulak Leaves (*Schefflera elliptica* (Blume) Harms) Against *Staphylococcus aureus* and *Escherichia coli*. *Jurnal Tumbuhan Obat Indonesia*, 16(1), 41–51.
- Rifai, A., Endraswari, P. D., Setiawati, Y., & Koendhori, E. B. (2024). Effects of Clove Leaf Essential Oil (*Syzygium aromaticum*) in Inhibiting Biofilm Formation on *Candida albicans* Isolate. *JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga*, 15(2), 70–75. <https://doi.org/10.20473/juxta.v15i22024.70-75>

- Sefriyanti, S., Jayuska, A., & Hairil Alimuddin, A. (2020). Uji Aktivitas Antibakteri Minyak Atsiri Serai Wangi (*Cymbopogon bernadus* L.) Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Kimia Khatulistiwa*, 8(4), 1–4.
- Silva, N. C. C., & Fernandes A., Jr. (2020). Biological properties of eugenol: a review. *Food and Chemical Toxicology*, 135, 110850. <https://doi.org/10.1016/j.fct.2019.110850>
- Vasconcelos, P. G. S., Lee, K. M., Abuna, G. F., Costa, E. M. M. B., & Murata, R. M. (2024). Monoterpene Antifungal Activities: Evaluating Geraniol, Citronellal, and Linalool on *Candida* biofilm, Host Inflammatory Responses, and Structure–Activity Relationships. *Frontiers in Pharmacology*, 15. <https://doi.org/10.3389/fphar.2024.1394053>
- Wardani, E., Hariadi, P., & Puspita Yuliana, T. (2024). Uji Minyak Atsiri Daun Mengkudu (*Morinda citrifolia* L) terhadap Diameter Zona Hambat *Staphylococcus aureus*. *Jurnal Farmasi Klinis Dan Sains Bahan Alam*, 4(1), 50. <https://doi.org/10.29408/sinteza.v4i1.25237>
- Zhou, H., Chen, L., Ouyang, K., Zhang, Q., & Wang, W. (2023). Antibacterial activity and mechanism of flavonoids from *Chimonanthus salicifolius* S. Y. Hu. and its transcriptome analysis against *Staphylococcus aureus*. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.1103476>