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Research Article

The Potential Of Citronella (*Cymbopogon nardus* (L.) Rendle) Essential Oil In Inhibiting The Formation Of *Escherichia coli and Candida albicans* Biofilms

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Abstract (in English)

Indonesia is a tropical country that has very high biodiversity, one of which is the citronella plant *(Cymbopogon nardus (L.) Rendle)*. Citronella leaves have a strong distinctive aroma and essential oils are odorous compounds found in the plant. This study aims to identify the components of compounds in essential oils in citronella oil and the potential for antibiofilm against *Escherichia coli and Candida albicans*. Essential oils were obtained through a distillation process. Furthermore, they were tested in four concentration series (0.125%; 0.25%; 0.5%; 0.1%) to measure the activity of the ability to inhibit the formation of *E. coli* and *C. albicans* biofilms using the microdilution method. The results of the antibiofilm test were analyzed based on optical density to calculate the percentage of inhibition. Citronella essential oil with the highest concentration of 1% showed inhibition of biofilm formation against *E. coli* and *C. albicans* of 84% and 79% respectively. Citronella essential oil can inhibit the formation of *E. coli* and *C. albicans* biofilms.

Keywords: *Biofilm, Citronella, E. coli, C. albicans*

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1 Introduction

Indonesia has a very large forest and consists of various natural resources that can be used as a source of traditional medicine. According to WHO, the use of traditional medicine and natural medicines by people in developed countries reaches 65%, and about half of the population in Indonesia still uses traditional medicine in the form of herbal medicine. One type of natural ingredient that is often used as a traditional medicine is citronella (*Cymbopogon nardus*) because it contains Geraniol, citronellal, and citronellol which can provide antibacterial effects [1],[2],[3].

The citronella plant (*Cymbopogom nardus*) is a geminus variety and is often called Citronella grass. Essential oils of lemongrass roots and leaves show significant activity against bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* and fungi such as *Candida albicans*, *Microsporum canis*, and *Trichophyton mentagrophytes*. Differences in antibacterial power of lemongrass can cause differences in the levels of components in the resulting lemongrass oil [4]. Biofilms are a health problem related to infection control, often found in cases of chronic infected wounds and in the use of implanted medical devices such as catheters and endotracheal tubes. Multispecies and dual-species biofilms are often less susceptible to antimicrobial agents than monospecies biofilms [3],[5],[6],[7],[8]

The multispecies community of microorganisms involved in biofilm formation and causing several diseases in humans includes Gram-positive bacteria (*Staphylococcus epidermidis and Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa and Escherichia coli*) and several from the genus Candida sp, especially *C. albicans* [4],[9],[10].

In this study, the effect of citronella essential oil (*Cymbopogom nardus*) on monospecies biofilms consisting of *E. coli, and C. albicans* fungi will be tested.

2 Method

2.1 Biofilm Formation Inhibition Test

A microtiter plate was utilized for the biofilm assay. Each well was filled with 100 µL of microbial suspension, containing approximately 10^7 CFU/mL. Subsequently, 100 µL of media with citronella oil at concentrations of 1% v/v, 0.5% v/v, 0.25% v/v, and 0.125% v/v was added to each well. The negative control consisted of a microbial suspension in the media, while chloramphenicol and nystatin served as positive controls. The plates were incubated at 37°C for 24 hours to observe the intermediate stages of biofilm formation. After rinsing the plates three times with distilled water, they were air-dried at room temperature for 5 minutes to remove excess water. Next, 125 mL of crystal violet solution (0.1% in distilled water) was added, and the plates were incubated at room temperature for 15 minutes. The wells were then gently rinsed three times with running water, and ethyl alcohol was added to each well. The optical densities (OD) were measured at 595 nm. The assays were performed in triplicate [7][11].

3 Result and Discussion

A biofilm inhibition test was performed to assess the impact of citronella essential oil concentration (*Cymbopogon nardus L.)* as an antibiofilm agent against *E. coli*, and *C. albicans*. The findings are illustrated in Figures 1 to 2. Chloramphenicol was used as the bacterial control, and nystatin served as the fungal control, both demonstrating biofilm inhibition of over 80% against *E. coli*, and *C. albicans*. According to the data in Figures 1 to 3, citronella essential oil exhibited the highest MIC_{80} at a concentration of 1% b/v. The average MIC value was nearly identical to that of the comparative control (chloramphenicol), while for C. albicans, the highest MIC_{80} was also observed at a concentration of 1% b/v.

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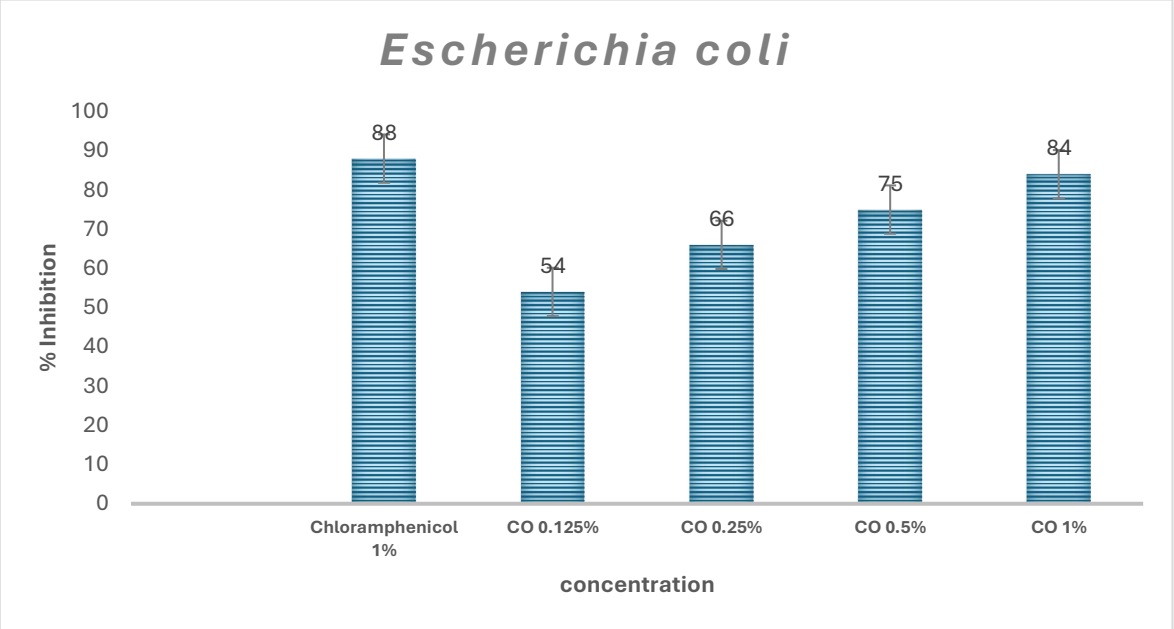

Figure 1. Inhibition of Biofilm Formation of *E. coli*

Figure 1 Citronella essential oil can disrupt cell membranes and inhibit cytoplasmic metabolism, making it effective against both Gram-negative and Gram-positive bacteria. This oil has been shown to inhibit the growth of *E. coli*, *Bacillus subtilis, Salmonella enterica typhimurium, Staphylococcus aureus, and Klebsiella pneumoniae* [13][14].

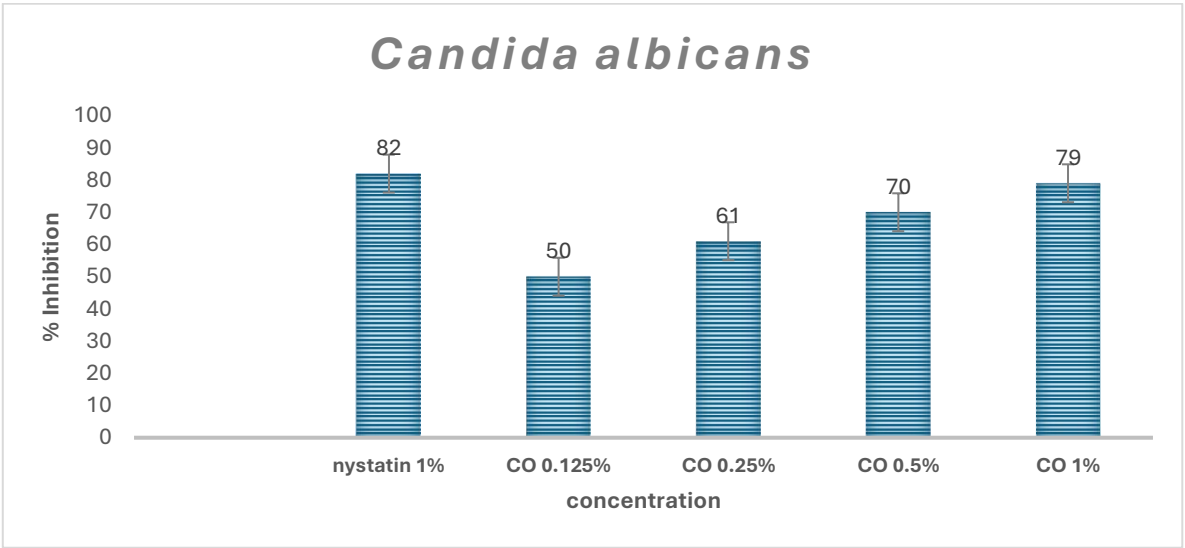

Figure 2. Inhibition of Biofilm Formation of *C. albicans*

Figure 2 illustrates that citronella essential oil exhibits inhibitory activity against the formation of *C. albicans* fungal biofilm, with concentrations ranging from 0.125% to 1%, showing the highest inhibition of 79% at a 1% concentration. This is similar to the inhibition observed with the nystatin control, which inhibited 82% of *C. albicans* biofilm formation. Several studies have also indicated that citronella essential oil is capable of inhibiting and killing the growth of various species within the Candida spp genus [1][15]. Citronella essential oil likely interacts with the cell membrane, but not with the cell wall, demonstrating strong antifungal and antibiofilm activity in vitro against *C. albicans* [11].

Essential oils, including those derived from plants such as *Cymbopogon nardus* (citronella), can inhibit the formation of biofilms in bacteria and fungi through several mechanisms [16]. One mechanism is by

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disrupting cell membranes, as essential oils generally contain compounds like terpenoids and phenols that can interact with the lipid layers of microbial cell membranes [17]. This interaction damages the membrane integrity, leading to leakage of cellular contents, which ultimately reduces the microorganism's ability to form and maintain biofilms. Additionally, essential oils can inhibit quorum sensing systems in bacteria and fungi[11] [18]. Quorum sensing is a communication process among microorganisms that coordinates biofilm formation and other behaviors [19]. By blocking this communication, essential oils can prevent the initiation or development of biofilm formation[20]. Essential oils can also alter microbial metabolic processes, including the production of extracellular substances (EPS) that are crucial for the structural stability of biofilms[19]. By inhibiting these pathways, essential oils reduce the ability of bacteria and fungi to form mature biofilms. Some essential oils also possess antioxidant properties that help reduce oxidative stress in microorganisms, weakening their ability to thrive in biofilm-forming environments [21].

4. Conclusion

This study concludes that citronella essential oil is effective in inhibiting the formation of biofilms by both bacteria and fungi. The oil demonstrated a strong inhibitory effect during the early stages of biofilm formation for *E. coli, and C. albicans.* These results are comparable to those of the control agents, chloramphenicol and nystatin, which also inhibited biofilm formation.

5. Declarations

5.1 Acknowledgements

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5.2 Author contributions

The authors contributed to this study as follows: Mentarry Bafadal conceptualized the research, conducted data analysis, and drafted the manuscript. Hasyrul Hamzah carried out the data collection and experimental procedures. Supriatno Salam validated the data and critically reviewed the manuscript content. Fajar Prasetya conducted the literature review and contributed to the refinement of the final manuscript.

5.3 Conflict of Interest

The authors declare that there is no conflict of interest related to the conduct and publication of this research

5.4 Funding Statement

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