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Research Article

Antibacterial and Antifungal Properties of Citronella oil Against Streptococcus mutans and Candida albicans by In Vitro Study

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Research Article

Antibacterial and Antifungal Properties of Citronella oil Against *Streptococcus mutans* and *Candida albicans* by In Vitro Study

Sifat Antibakteri dan Anti-fungal Minyak Serai terhadap *Streptococcus mutans* dan *Candida albicans* Secara In Vitro

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ABSTRACT

Streptococcus mutans and *Candida albicans* are the main microorganisms that cause dental cavities. It can cause infection, damaged tissue around teeth, abscesses, and focal infection to other organs in the body. Natural products are currently widely used as products or as additives in the prevention of dental caries which have more anti-bacterial and anti-fungal activities than antibiotics that can cause resistance. Citronella (*Cymbopogon citratus*) is abundant and easy to grow. This study was conducted to determine the effectiveness of citronella (*Cymbopogon citratus*) oil on the growth of *S. mutans* and *C. albicans* microorganisms by Minimum Inhibitory Content (MIC) and Minimum Bactericidal Content (MBC). The method of MIC is broth microdilution by making chlorhexidine concentration levels of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 0.2% with 4 replications each. The MIC value was determined based on absorbance spectrophotometry and the MBC value was determined from the agar plate using the spread method. Biofilm eradication test was conducted by crystal-violet staining and measuring the absorbance. The results of MIC and MBC were obtained on *S. mutans*, namely at concentrations of 25% and 100%, respectively. Meanwhile, the results of MIC and MBC on *C. albicans* were obtained at concentrations of 50% and 100%, respectively. Furthermore, the citronella oil has antibacterial and antifungal activities.

Keywords: Antimicrobial, *Candida albicans*, Minimum Bactericidal Content, Minimum Inhibitory Concentration, *Streptococcus mutans*

ABSTRAK

Streptococcus mutans dan *Candida albicans* merupakan mikroorganisme utama penyebab gigi berlubang. Hal ini dapat menyebabkan infeksi, kerusakan jaringan di sekitar gigi, abses dan infeksi fokal ke organ tubuh lainnya. Produk alami saat ini banyak digunakan sebagai produk atau sebagai bahan tambahan dalam pencegahan karies gigi yang memiliki aktivitas anti bakteri dan anti jamur dibandingkan antibiotik yang dapat menyebabkan resistensi. Citronella (*Cymbopogon citratus*) melimpah dan mudah tumbuh. Penelitian ini bertujuan untuk mengetahui efektivitas minyak serai wangi (*Cymbopogon citratus*) terhadap pertumbuhan mikroorganisme *S. mutans* dan *C. albicans* berdasarkan *Minimum Inhibitory Content* (MIC) dan *Minimum Bactericidal Content* (MBC). Metode MIC adalah mikrodilusi kaldu, dengan membuat kadar konsentrasi klorheksidin 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, dan 0,2% dengan masing-masing 4 ulangan. Nilai MIC ditentukan berdasarkan spektrofotometri absorbansi dan nilai MBC ditentukan dari lempeng agar menggunakan metode sebar. Uji eradikasi biofilm dilakukan dengan pewarnaan kristal-violet dan diukur absorbansinya. Hasil MIC dan MBC diperoleh pada *S. mutans* yaitu pada konsentrasi masing-masing 25% dan 100%, serta hasil MIC dan MBC pada *C. albicans* diperoleh pada masing-masing konsentrasi 50% dan 100%. Selain itu, minyak serai memiliki aktivitas antibakteri dan antijamur.

Kata Kunci: Antimikroba, *Candida albicans*, Kandungan Bakterisida Minimum, Konsentrasi Hambat Minimum, *Streptococcus mutans*

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INTRODUCTION

Dental cavities in people have been linked to *Streptococcus mutans* as the main causative agent. The bacterium's capacity to create the biofilm known as dental plaque on teeth surfaces is a crucial aspect of its pathogenicity (1). This organism also has surface proteins that work together to form tooth plaque and cause dental cavities, including glucosyltransferases, numerous glucan-binding proteins, protein antigen C, and collagen-binding protein (2). *Candida albicans* colonization caused significant microbial dysbiosis characterized with distinct microbial composition and structure as compared to the biofilm in the absence of *C. albicans*. These findings are in agreement with previous studies showing that *C. albicans* influenced the microbial composition of saliva biofilms (3). The primary bacterial pathogen in tooth caries, especially in early-childhood caries (ECC), is frequently identified as *S. mutans*. *Candida albicans* cells are commonly found in plaque biofilms from ECC-affected infants, along with heavy infection by *S. mutans*, suggesting that *S. mutans* may not act alone. Exopolysaccharide (EPS) synthesis is increased by the presence of *C. albicans*, causing conspecific biofilms to accumulate more biomass and contain more live *S. mutans* cells than single-species biofilms (4).

Nowadays, the demand for natural preservatives is solely related to the increasing interest in healthy lives and health issues. Additionally, the use of antibiotics leads to the development of multidrug resistant microorganisms, so research into these natural components has gained more focus in the scholarly community (5). Numerous useful natural compounds derived from various plant species have been found to possess antimicrobial qualities (6). Among them, some essential oils (EOs) have the potential to be useful food stabilizers, making them appealing substitutes for manufactured compounds (7). Additionally, EOs are not too difficult to obtain, ecologically favorable (they degrade rapidly in soil and water), and not too harmful to people (8).

Tropical and subtropical areas of the globe are home to *Cymbopogon citratus*, more commonly known as "Lemon Grass" (9). *C. citratus* is native to Asia (Indochina, Indonesia, and Malaysia), Africa, and the Americas, but is widely cultivated in temperate and tropical regions of the world (10). Alpha citral (geranial), beta citral (neral), and myrcene are the three primary constituents of this *C. citratus* essential oil. While studies have proven the antibacterial activity of alpha and beta citral against gram-positive and gram-negative bacteria as well as fungistatic activity against *Candida* species, myrcene has not exhibited any antimicrobial activity (11). The therapeutic actions of *C. citratus* include anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal, and anti-inflammatory effects. Antimalarial, antimutagenic, antimycobacterial, antioxidant, hypoglycemia, and neurobehavioral benefits, among others, have also been researched (12). With all of the benefits of *C. citratus* stated above, this study was to determine the effectiveness of citronella (*C. citratus*) oil on the growth of *S. mutans* and *C. albicans* microorganisms as antibacterial and antifungal activity.

METHOD

Preparation of Citronella Oil

The extraction was carried out at the Pharmaceutical Company PT Indesso Aroma, Baturaden, Purwokerto, Central Java with CoA No Batch. 895850017. The extraction was carried out for the plan to develop standardized herbal medicines from natural ingredients consisting of Citronella extract as an anti-bacterial and anti-fungal. The extraction method started by washing the lemongrass and cutting them to ¼cm x ¼cm x ¼cm. Methanol was used as maceration solvent with ratio of 1:10 between solute and solvent. Maceration was carried out for 10 hours (13). The resulting extract is in the form of oil.

Research on the MIC and MBC tests of citronella oil on the growth of *S. mutans* and *C. albicans* was carried out at the Aretha Medika Utama Bandung Biomolecular and Biomedical Laboratory, with work procedures using the 2012 CLSI (Clinical Laboratory Standard Institute) method with modifications. This research is pure experimental laboratory in vitro by comparing the sample groups containing citronella oil with concentrations 100%, 50%; 25%; 12.5%; 6,25%, and 3.125% and positive control in the form of 0.2% chlorhexidine (Minosep) with 4 replication. Citronella oil dilution was carried out by adding n-hexane. Chlorhexidine was acted as the positive control because it was reported that Chlorhexidine was an antiseptic that could go averse to both types of bacteria, viruses, and facultative aerobes and anaerobes (14). The growth control was used as the negative control.

Antibacterial and Antifungal Assay

The isolates of *S. mutans* and *C. albicans* were obtained by sub culture in MHA media at 37°C for 24 hours. Some of the colonies were inoculated to 10ml MHB. McFarland 0.5 standard was used to adjust the turbidity. Inoculum was obtained by conducting dilution to the solution with physiological solution. Media MHB with ratio of 1:20 was then used to conduct dilution. The inoculum was then obtained in the range of 1-5 x 10⁵ CFU/ml. The MIC of Citronella oil against *S. mutans* and *C. albicans* was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) M07-A9.

At the final concentration, 100µL of Citronella oil was added into 96-well microplate. For the positive control we added 100µl of 0.2% chlorhexidine (Minosep) to the well. The sample of each bacterial and fungal suspension (24h incubated *S. mutans* and *C. albicans*) respectively with a concentration of 1.5 x 10⁶CFU/mL with Mueller Hinton Broth (Himedia, M403-500G) were also added to the wells. The microplate was incubated for 24 hours at 37°C in anaerobic condition then measured the absorbance with Spectrophotometry (Multiskan GO Thermo Scientific 51119300) at 415nm for *S. mutans* and 510nm for *C. albicans*. The MIC was determined as the lowest concentration of Citronella oil in which visible bacterial and fungi growth was inhibited. Also, the quantitative evaluation of microbial growth was determined based on the previous study (15).

The MBC was considered to be the lowest concentration of Citronella oil which prevented the growth and reduced the

inoculum by >99.9% within 24 hours, irrespective of counts of survivors at higher sample concentrations. For this purpose, 20 μ L of contents of the well determined as the MIC and also the wells with a concentration of 2×10^3 CFU/ml were cultured in spread inoculation in the Mueller Hinton Agar by pour plate method (Himedia, M096-500G) and incubated for 24 hours in anaerobic condition with the temperature of 37°C and 5% CO₂ (15). Colony counter was used to count the total colony. The optical density (OD) measurement utilized microplate reader at wavelength of 625 nm. Treatments' OD were compared to Blank's OD.

RESULT

The measurement of optical density of Citronella oil against *S. mutans* results are presented in the table I, that showed Citronella oil 3.125% has the highest average value of absorbance with 0.2613 and lowest in the chlorhexidine control with mean values absorbance 0.0011. On the results of various measurements extract treatment, showed that the average value the highest corrected absorbance is found in the concentration of Citronella oil 100% with the absorbance value is 0.0023, This shows that *S. mutans* can be inhibited by Citronella oil.

The measurement of optical density of Citronella oil against *C. albicans* results are presented in the Table 1. The data showed that Citronella oil 3.125% has the highest average value of absorbance with 0.1192 and lowest in the chlorhexidine control with mean values absorbance 0.0012. Based on the measurement result of various extract treatments, Citronella oil 100% exhibited the highest absorbance value, which is 0.0022. It means that *C. albicans* could be inhibited by Citronella oil. The result of MBC *S. mutans* and *C. albicans* were shown in Table II. It was showed that both Chlorhexidine 0.2% and Citronella oil 100% resulted in zero total colony (0 CFU/ml). It means that Citronella oil 100% exhibited the same result as the positive control. Meanwhile, the highest total colony count came from Citronella oil 3.125% with average 253250 CFU/ml. In *C. albicans*, it is shown that the lowest total colony count at Citronella oil 100% with 0 CFU/ml and the highest at Citronella oil 3.125% with 61750 CFU/ml.

Table 1. Table of optical density of *S. mutans* and *C. albicans*

Samples	Optical Density (OD)	
	<i>S. mutans</i>	<i>C. albicans</i>
Chlorhexidine 0.2%	0.0012 \pm 0.0001	0.0012 \pm 0.0014
Citronella oil 100%	0.0023 \pm 0.0002	0.0012 \pm 0.0004
Citronella oil 50%	0.0415 \pm 0.0002	0.0337 \pm 0.0004
Citronella oil 25%	0.0939 \pm 0.0002	0.0567 \pm 0.0010
Citronella oil 12.5%	0.1917 \pm 0.0003	0.0775 \pm 0.0002
Citronella oil 6.25%	0.1961 \pm 0.0002	0.1057 \pm 0.0007
Citronella oil 3.125%	0.2613 \pm 0.0004	0.1192 \pm 0.0002

Note: *Data provided as mean \pm STD

Table 2. Table of total colony count of *S. Mutans* and *C. albicans*

Samples	Total Colony (CFU/ml)	
	<i>S. mutans</i>	<i>C. albicans</i>
Chlorhexidine 0.2%	0 \pm 0	0 \pm 0
Citronella oil 100%	0 \pm 0	0 \pm 0
Citronella oil 50%	20250 \pm 957	4500 \pm 1290
Citronella oil 25%	103750 \pm 2500	28750 \pm 2217
Citronella oil 12.5%	139500 \pm 2380	43500 \pm 4434
Citronella oil 6.25%	191500 \pm 5447	53500 \pm 5322
Citronella oil 3.125%	253250 \pm 2217	61750 \pm 4645

Note: *Data provided as mean \pm STD

Concentration of an antibacterial agent in the lowest value that could inhibit an organism development visually is called MIC (16). In this research, MIC value of Citronella oil against *S. mutans* and *C. albicans* was taken by the concentration of Citronella oil 25% with 75.08% and 53.66% respectively. MBC value was determined by concentration that could inhibit the growth of 99.9% organism (17). In this research, the MBC value of Citronella oil against *S. mutans* and *C. albicans* was taken by the concentration of Citronella oil 100% with 99.38% and 99.04%, respectively (Figure 1).

DISCUSSION

Terpenes, alcohols, ketones, aldehydes, and esters make

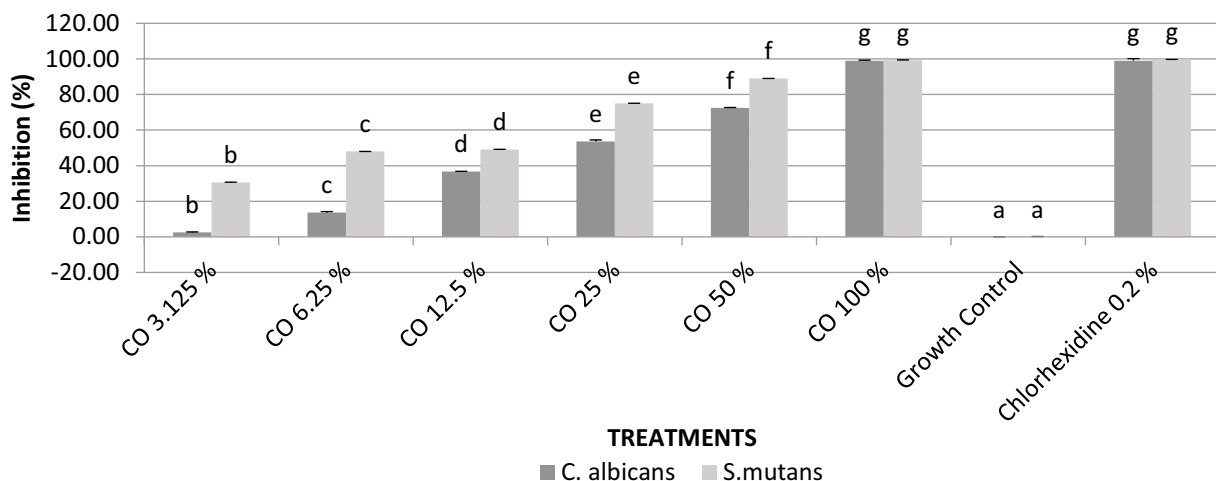


Figure 1. Citronella oil (CO) inhibition against *S. mutans* and *C. albicans*

Note: *Data was presented in mean \pm STD. Difference letters (a,b,c,d,e,f,g) were shown significantly difference among treatments in each sample ($p < 0.05$) based on Tukey Post Hoc Test.

up the majority of the substances found in *Cymbopogon citratus*. Essential oils that contain Citral, Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrcene, and Terpinol Methylheptenone are a few of the documented phytoconstituents. The plant is also said to contain phenolic substances like luteolin, isoorientin 2'-O-rhamnoside, quercetin, kaempferol, and apigenin as well as flavonoids. Numerous pharmaceutical actions, including anti-amoebic, antibacterial, antidiarrheal, antifilarial, antifungal, and anti-inflammatory characteristics, have been linked to *Cymbopogon citratus*, according to studies. Antimalarial, antimutagenic, antimycobacterial, antioxidant, hypoglycemia, and neurobehavioral benefits, among others, have also been researched (12).

Citronellal compounds, citronellol, geraniol, geranyl acetate, and citronellyl acetate were terpenoids. In order to reduce the permeability of the bacterial cell wall and cause the cells to lack nutrients, inhibit bacterial growth, or die, terpenoid compounds damage the membranes of lipophilic compounds by forming strong polymer bonds and harming porins (18). Monoterpene substances work as an antibiotic by having a toxic impact on the composition and operation of bacterial cell membranes. Since monoterpenes are lipophilic, they transition from the polar phase to the non-polar bacterial cell membrane structure (19).

Luteolin's ability to block DNA topoisomerase I and II activity, which led to a reduction in the production of nucleic acids and proteins, is what gives it its antibacterial properties (20). An important flavonoid compound, called quercetin has a wide range of medicinal effects. Numerous studies examine its antibacterial effects and potential mechanisms of action. It has been demonstrated that quercetin prevents the development of various Gram-positive and Gram-negative bacteria, fungus, and viruses. The method of its antimicrobial action involves mitochondrial failure, cell membrane injury, altered membrane permeability, suppression of protein and nucleic acid synthesis, downregulation of virulence factor expression, and prevention of biofilm formation. A powerful antimicrobial agent against drug-resistant strains, quercetin, has also been shown to suppress the development of a number of drug-resistant

microorganisms (21). Kaempferol targets the stability of the bacterial membrane to produce its antibacterial properties (22).

Secondary compounds of plants with antifungal action include flavonoids by denaturing the extracellular proteins of the fungus cell walls, flavonoids cause harm to the fungal cell walls and block the activity of enzymes. Denatured proteins disturb the process of how cells are made, resulting in modifications to the protein structure and an increase in cell membrane porosity. This rise results in intracellular material leaks, cellular ATP shortage, metabolism disturbance, growth suppression, and cell lysis (23). Although the exact method by which terpenoid compounds prevent the growth of fungus is still unknown, the existence of lipophilic or hydrophobic characteristics in terpenoid compounds causes cell coagulation and cytoplasmic membrane damage in fungal cells (24).

Based on the antibacterial and antifungal assay, Citronella oil 100% showed the best inhibition than any other concentrations. In addition, Citronella oil showed better inhibition to *S. mutans* in overall concentrations. However, the MIC obtained at concentration of 25% for both *S. mutans* and *C. albicans*. Previous study showed that *C. nardus* essential oil exhibited *S. mutans* activity at 25% (25). Meanwhile, inhibition of *C. albicans* was showed at concentration of 6.25%. This means that the result of this study is comparable to prior study. This study was hindered by some limitations, one of which is that the characterization of citronella oil has not been able to be conducted, thus restraining the ability to know the exact compound that is responsible for these bioactivity.

Citronella oil has the potency for antibacterial and antifungal treatment, through inhibit *S. mutans* and *C. albicans* growth.

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