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The Potential of Combining Lemongrass Leaf Extract (*Cymbopogon citratus*) and Basil Leaf Extract (*Ocimum sanctum*) to Inhibit the Growth of *Streptococcus mutans*

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Abstract

Dental caries is a bacterial infection caused by *Streptococcus mutans*, which forms plaque and produces acids that demineralize tooth enamel. The increasing prevalence of caries has encouraged the development of natural antibacterial agents that are safer and more biocompatible than synthetic materials. Lemongrass (*Cymbopogon citratus*) and basil (*Ocimum sanctum*) contain bioactive compounds such as flavonoids, alkaloids, saponins, and tannins that demonstrate antibacterial properties and may exert synergistic effects when combined. This study evaluated the inhibitory effect of combined lemongrass and basil extracts on *S. mutans* and determined their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using an in vitro design with serial dilution and colony-counting methods. Cultures of *S. mutans* in Brain Heart Infusion Broth were treated with extract concentrations of 100%, 50%, 25%, and 12.5%, alongside chlorhexidine and negative controls; data were analyzed using the *Kruskal–Wallis* test followed by the *Mann–Whitney post hoc* test. The results showed effective antibacterial activity, with an MIC of 12.5% and an MBC of 25%, and significant differences between treatment and control groups ($p = 0.005$), although no significant differences were observed among extract concentrations. These findings indicate that combined *C. citratus* and *O. sanctum* extracts possess synergistic antibacterial effects and hold potential as natural agents for the prevention of dental caries.

Keywords: *Cymbopogon citratus*; *Ocimum sanctum*; *Streptococcus mutans*; Antibacterial; MIC; MBC

1. Introduction

Dental caries is a chronic infectious disease that damages the hard tissues of the teeth due to bacterial activity, primarily *Streptococcus mutans,* which ferments carbohydrates and produces acids that trigger demineralization[85]. High consumption of cariogenic foods, such as candy, chocolate, bread, instant foods, and high-carbohydrate diets, also increases the risk of caries across various age groups [82]›[35]›[89].

Globally, more than 514 million children experience dental caries[87]. The highest prevalence is recorded in Southeast Asia, including Indonesia. Geographical factors, such as proximity to the equator, are also associated with low vitamin D levels and specific dietary patterns that increase caries risk[7]. In Indonesia, the SKI 2023 report states that 82.8% of the population experiences dental caries, with particularly high proportions among children aged 3–9 years and young adults[31].

Oral health problems in Indonesia are influenced by low awareness of maintaining oral hygiene and limited access to dental care services. In fact, preventive efforts—such as education, routine dental check-ups, and improved oral hygiene—are highly necessary[75]. Additionally, the rise of bacterial resistance to antibiotics has encouraged the search for natural alternatives that are safer, more affordable, and more accessible[69].

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Medicinal plants are one such alternative because they contain bioactive compounds such as alkaloids, flavonoids, tannins, and essential oils that can disrupt the metabolism and biofilm formation of *Streptococcus mutans*. Lemongrass (serai dapur) contains various phytochemicals including flavonoids and saponins[43], while basil (kemangi) is traditionally used as an antimicrobial agent and contains essential oils and other active compounds[26]. Basil extract is effective in inhibiting bacterial growth at concentrations of 80–100%[12], whereas lemongrass extract shows increased antibacterial activity with rising concentrations[81].

The therapeutic mechanism of combining both extracts is presumed to be complementary: flavonoids disrupt cell membranes, alkaloids inhibit bacterial metabolism, and saponins enhance solubility and bioavailability of other bioactive compounds, thereby increasing overall antibacterial effectiveness[27]›[46]. Based on these considerations, this study was conducted to evaluate the potential of combining lemongrass and basil extracts in inhibiting the growth of *Streptococcus mutans* as an alternative method for controlling caries-causing bacteria.

2. Material and methods

2.1. Preparation of Lemongrass Extract

Lemongrass preparation began by washing all plant parts thoroughly under running water to remove contaminants such as soil or mud. The cleaned plant material was thinly sliced with a sharp knife to increase surface area and facilitate drying. The slices were dried in an oven at 50°C until moisture content significantly decreased and the texture became brittle, indicated by easy breakage. Once adequately dried, the material was ground using a blender into coarse powder, then sieved to obtain finer and more uniform particles as raw material for extraction

2.2. Preparation of Basil Extract

Basil preparation followed similar steps: washing the plant thoroughly, slicing it thinly to facilitate drying, and oven-drying at 50°C until most moisture evaporated and the texture became brittle. After drying, the material was ground into coarse powder using a blender and sieved to obtain fine and homogeneous powder as extraction material [29].

2.3. Extraction of Lemongrass and Basil

For extraction, 500 grams of each powdered plant material were weighed and macerated in 5 L of 96% ethanol for three days. After maceration, the ethanol filtrate was evaporated using a rotary evaporator and further dried in an oven until a thick extract was obtained. The extract was collected in an Erlenmeyer flask and stored at room temperature for further use [65].

2.4. Preparation of *Streptococcus mutans* Culture

Preparation began with sterilizing tools and materials to prevent contamination. Stock cultures of *S. mutans* were obtained by inoculating one loopful of bacteria into Mueller Hinton Agar (MHA) and incubating for 24 hours at 37°C under anaerobic conditions. After incubation, one loopful of the culture was mixed with 0.9% NaCl physiological solution to form a suspension, and turbidity was adjusted to McFarland standard 0.5 (equivalent to 1.5 × 10[8] CFU/ml) through visual comparison against a black-striped background. Adjustment was made by adding bacterial colonies if too dilute or adding NaCl solution if too dense[8].

2.5. Lemongrass–Basil Extract Combination Test Against *Streptococcus mutans*

Seven test tubes were prepared: the first containing bacterial suspension (negative control); the second containing 10 ml of 100% extract combination; tubes three to five were serially diluted to obtain 50%, 25%, and 12.5% concentrations. The sixth tube contained BHIB as a media control, while the seventh contained BHIB plus chlorhexidine as a positive control. A total of 0.1 ml of *S. mutans* suspension was added to tubes two through seven, except tube six. All treatments were conducted in triplicate. Tubes were incubated for 24 hours at 37°C, after which 0.1 ml from each tube was plated onto MHA using a spreader and incubated again at 37°C for 2 × 24 hours. Colony counts were performed using a colony counter and analyzed to evaluate the antibacterial effect of the extract combination[47].

3. Results and discussion

The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests showed that the combination of lemongrass (*Cymbopogon citratus*) and basil (*Ocimum sanctum*) extracts was effective in inhibiting the growth of *Streptococcus mutans*. At concentrations of 100%, 50%, and 25%, as well as in the positive

control, no bacterial colonies were observed on nutrient agar. At the 12.5% concentration, a reduction in colony counts was recorded compared with the negative control. From these observations, the lowest concentration capable of killing the bacteria (MBC) was identified as 25%, while 12.5% demonstrated growth inhibition without bactericidal activity, establishing it as the MIC.

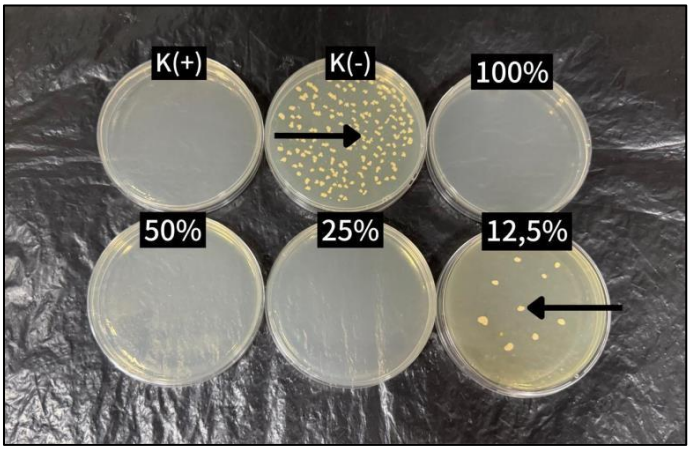

Figure 1 Colony counting assay in the first replication

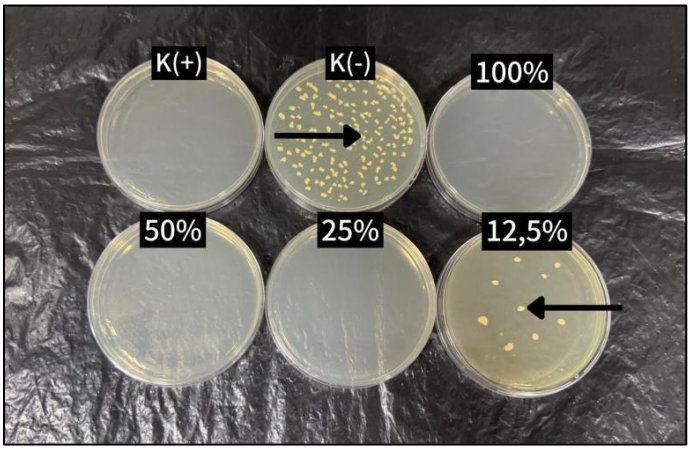

Figure 2 Colony counting assay in the second replication

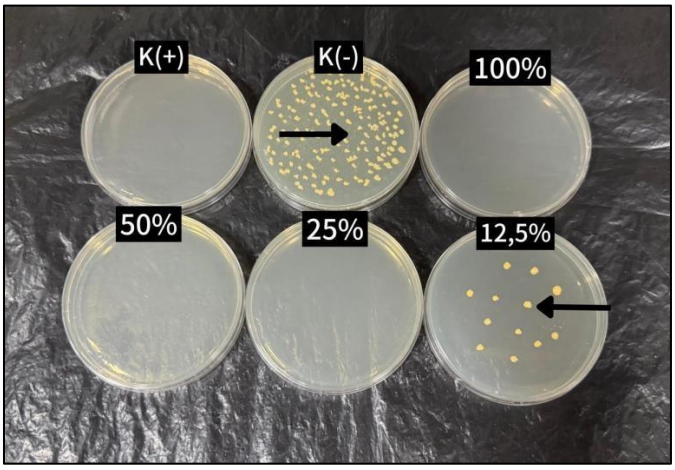

Figure 3 Colony counting assay in the third replication

Figure 4 Serial dilution results in the test tubes

The colony count results support these findings, where the negative control group showed an average of 173.33 ± 9.07 CFU (range 165–183), whereas extract-treated groups exhibited an average of 9.67 ± 2.08 CFU (range 8–12). Statistical analysis using the *Shapiro–Wilk* test indicated that the data were normally distributed ($p > 0.05$), but *Levene's* Test showed non-homogeneous variances between groups ($p = 0.006$). Thus, further analysis used the non-parametric *Kruskal–Wallis* test, which revealed significant differences among treatment groups ($p = 0.005$). Post hoc analysis using the *Mann–Whitney* test showed that the negative control differed significantly from all extract concentrations (100%, 50%, 25%, and 12.5%) at $p < 0.05$, but no significant differences were found among the extract concentrations ($p > 0.05$). These results confirm that the combination extract effectively reduces *S. mutans*, although increasing the concentration does not significantly enhance antibacterial effects despite showing a decreasing trend. Furthermore, the use of mucoadhesive gingival patches for the The antibacterial effect of the extract combination is believed to originate from synergistic interactions among phytochemical compounds present in both plants: alkaloids and saponins in lemongrass, and flavonoids and tannins in basil. Alkaloids interfere with peptidoglycan synthesis in bacterial cell walls, intercalate DNA, and inhibit key enzymatic activities, leading to bacterial cell death[39]›[83]. Saponins damage cell membranes by reducing surface tension and increasing membrane permeability, resulting in cytoplasmic leakage that compromises bacterial survival[61]. Flavonoids inhibit energy metabolism, membrane function, and the biosynthesis of essential macromolecules by forming complexes with extracellular proteins, thereby suppressing bacterial growth[71]. Tannins deactivate enzymes, disrupt microbial adhesion, inhibit protein transport, and interfere with DNA synthesis, significantly reducing bacterial proliferation (Sunani & Hendriani, 2023)

Table 1 Colony counting test results

No.	K(+)	K(-)	100%	50%	25%	12,5%
1	-	165	-	-	-	9
2	-	172	-	-	-	8
3	-	183	-	-	-	12

The synergism among these compounds increases the overall antibacterial effectiveness of the extract combination. Saponins from lemongrass compromise membrane integrity, enabling flavonoids, tannins, and other active compounds from basil to penetrate more easily into bacterial cells, enhancing antibacterial action[59]›[53]. This finding aligns with previous studies showing that basil extract alone is effective at 80% concentration and lemongrass extract at 50%, while the combination of both extracts in this study showed significant activity at a lower concentration of 25%, indicating synergistic effects[12]›[81].

Additionally, the use of chlorhexidine as a positive control demonstrated high antibacterial effectiveness against S. mutans. However, long-term use of chlorhexidine is known to have side effects such as tooth staining and disruption of normal oral flora[79]›[40]. The lemongrass–basil extract combination exhibited antibacterial effects approaching those of chlorhexidine at higher concentrations but without associated adverse effects, offering a safer, natural, and

biocompatible alternative. By inhibiting the growth of *S. mutans*, this extract combination has the potential to prevent plaque formation and enamel demineralization, which are the initial stages of dental caries development (Abdullah, 2022).

Furthermore, the study reinforces that increasing the extract concentration increases its inhibitory ability against *S. mutans*, due to the higher availability of bioactive compounds that interact with bacterial cell walls and metabolic systems. Therefore, the combination extract not only effectively inhibits bacterial growth but also provides a multi-targeted mechanism that enhances antibacterial performance. Based on these findings, the extract combination has potential for development as a herbal mouthwash or other oral health product, serving as a natural and effective alternative for preventing dental caries. Overall, this study supports the use of natural materials rich in active compounds as antibacterial agents against *S. mutans*, with MIC and MBC values demonstrating promising effectiveness [22]›[50].

4. Conclusion

The present study demonstrated that the combination of *Cymbopogon citratus* and *Ocimum sanctum* leaf extracts exhibits substantial antibacterial activity against *Streptococcus mutans*. The extract mixture achieved a Minimum Inhibitory Concentration (MIC) of 12.5% and a Minimum Bactericidal Concentration (MBC) of 25%, confirming its ability to inhibit and eliminate bacterial growth effectively. The enhanced activity observed is attributed to the synergistic interaction between the phytochemical constituents of both plants, which resulted in greater antibacterial efficacy than either extract alone.

Overall, the findings highlight the potential of this plant-based combination as a natural, safe, and accessible alternative for reducing oral bacterial load and preventing dental caries, providing societal benefit through improved oral health and offering a promising direction for future development of herbal oral-care products.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare that they have no conflict of interest.

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