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Antibacterial Effectiveness Test of Lemongrass Leaf Extract (*Cymbopogon citratus*) on the Growth of *Streptococcus sanguinis* on Removable Orthodontic Acrylic Bases

Davin[1*], Cindy Denhara Wijaya[2], Nora Fine Damayanti Sianipar[3]

[1,2,3] Dentistry Education Study Program, Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Indonesia

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Corresponding Author:
Author Name*: Davin
Email*: davin@unprimdn.ac.id

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ABSTRACT

The use of acrylic base as a removable orthodontic base plate has the disadvantage of being porous, thus increasing the risk of microorganism colonization, including Streptococcus sanguinis. Lemongrass leaves (Cymbopogon citratus L.) contain secondary metabolites that have antibacterial properties such as alkaloids, saponins, tannins, polyphenols. The purpose of this study was to determine the antibacterial effectiveness of lemongrass leaf extract (Cymbopogon citratus) 40% and 50% against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. This type of research is a true experimental with a post- test only control group design. The number of groups in this study was 4 (four), each consisting of 6 replications. The antibacterial testing of lemongrass leaf extract used the diffusion method. Data analysis used the Kruskal-Wallis and Mann-Whitney statistical tests. Based on the results of the study, the average number of Streptococcus sanguinis bacterial colonies on removable orthodontic acrylic bases from negative controls, positive controls, 50% and 40% lemongrass leaf extract was 1725±171,980 CFU/mL, 4.17±2,639 CFU/mL, 451.67±57,840CFU/mL, and 662.50±23,889 CFU/mL. The results of the Kruskal-Wallis test stated that there was a significant difference in the average number of colonies between all groups ($p=0.000$; $p<0.05$). The results of the Mann-Whitney test stated that there was a significant difference in antibacterial effectiveness between the two different groups ($p=0.004$; $p<0.05$). The conclusion of this study is that lemongrass leaf extract has antibacterial effectiveness against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases.

Keywords: removable orthodontics, lemongrass leaf extract, streptococcus sanguinis, acrylic base, antibacterial

INTRODUCTION

Malocclusion is a term that represents dental anomalies or occlusal conditions of the upper and lower jaw arches that deviate from ideal occlusion [1]. Common factors causing malocclusion include bad habits, heredity, history of trauma, environment, predisposing diseases, physical disabilities, nutrition, and posture [2], [3]. If left untreated, malocclusion can cause disorders of speech, facial harmony, masticatory function, and oral hygiene, which in turn negatively impact an individual's mental and physical health [2].

Removable orthodontic appliances are a common type of appliance used to treat malocclusion, alongside fixed orthodontic appliances [4]. These appliances can be removed and inserted by the patient themselves, offering simpler treatment compared to fixed appliances, as they are considered more functional and flexible. Removable orthodontic appliances affect various orofacial muscles and dentoalveolar development [5] and are expected to align teeth and improve facial symmetry [4].

Removable orthodontic appliances tend to be used by children and adolescents during the primary and mixed dentition periods, as bone growth and tooth eruption can be utilized during this stage to shift teeth and correct mild malocclusions. One of the main components of a removable appliance is the orthodontic acrylic baseplate, which is a broad plate designed for small tipping movements [6]. Orthodontic bases are made of acrylic resin, whose polymerization is classified into three types: heat-cured, light-cured, and self-cured. Self-cured or autopolymerizing acrylic resin, also called cold-curing resin, is a common material used in the manufacture of removable orthodontic plates [7]. Despite its widespread use, self-cured acrylic resin has the disadvantages of a low degree of polymerization and high monomer residue [6].

The use of acrylic resin as a base plate offers aesthetic advantages, but acrylic resin is porous. This porosity, along with the components embedded within it, increases the risk of retention of microorganisms and food particles [2], [8]. *Streptococcus sanguinis*, a gram-positive bacterium that is a pioneer colonizer in the oral

cavity, can adhere to the acrylic base and serve as an anchor for other oral microorganisms. This has the potential to form plaque, caries, periodontal disease, and biofilm on the surface of dentures, which can cause inflammation or infection of oral tissues, including denture stomatitis in removable orthodontic users [9]. Therefore, removable orthodontic acrylic bases need to be cleaned mechanically and chemically using antibacterial solutions.

Chlorhexidine is an effective bisbiguanide derivative with low toxicity, rapid action, and a broad spectrum. Chlorhexidine 0.2% has been shown to be effective against oral bacteria, is bactericidal and fungicidal at a concentration of 1.3%, and can reduce plaque by up to 80% due to its bacteriostatic phenol content at 0.1–1% concentration. The chlorine compound in chlorhexidine also acts as a high-level disinfectant, effective against various viruses, parasites, bacteria, fungi, and spores. The antiseptic effect of chlorhexidine is not only bacteriostatic but also adheres for a long time to the tooth surface, thus providing a bactericidal effect against Streptococcus sanguinis [10], [11]. Research by [11] shows that rinsing with chlorhexidine can suppress plaque accumulation and improve dental and oral hygiene status in orthodontic appliance users.

However, long-term use of chlorhexidine can cause side effects, such as changes in the sense of taste, changes in the color of teeth and tongue, and mucosal irritation [12]. Therefore, it is important to find natural alternatives with lower risks and without contraindications, for example, using lemongrass leaves (*Cymbopogon citratus*) which have antibacterial potential due to the content of active compounds such as saponins, flavonoids, tannins, alkaloids, polyphenols, and essential oils [2], [13]. Essential oils and saponins play an important role in antimicrobial activity, as seen from their effectiveness against *Salmonella typhimurium* and *E. coli* [14].

Previous research by [14] showed that lemongrass leaf extract with concentrations of 20%, 30%, 40%, and 50% had significant differences in inhibiting Streptococcus mutans ($p<0.05$). Lemongrass leaf extract was proven to be effective in inhibiting the growth of these bacteria, but there has been no research on its effectiveness against Streptococcus sanguinis on removable orthodontic acrylic bases. Therefore, this study aims to explore the effectiveness of lemongrass leaf extract in inhibiting the growth of Streptococcus sanguinis on removable orthodontic acrylic bases.

The formulation of the research problem is whether there is a difference in antibacterial effectiveness between lemongrass leaf extract (*Cymbopogon citratus*) concentrations of 40%, 50%, 0.2% chlorhexidine, and DMSO against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. The general objective of this study was to determine the antibacterial effectiveness of lemongrass leaf extract 40% and 50%, 0.2% chlorhexidine gluconate, and DMSO against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. The specific objectives of the study include: (1) determining the number of Streptococcus sanguinis colonies in the treatment group, (2) comparing the positive control (0.2% chlorhexidine) with 40%, 50%, and DMSO lemongrass leaf extract, (3) comparing 40% lemongrass leaf extract with 50% and DMSO, and (4) comparing 50% lemongrass leaf extract with DMSO on the growth of Streptococcus sanguinis on removable orthodontic acrylic bases.

RESEARCH METHODS

This research is a true experimental study with a post-test only control group design, aimed to test the antibacterial effectiveness of lemongrass leaf extract (Cymbopogon citratus) against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. The research was conducted in several laboratories at the University of North Sumatra, namely the Medanese Herbarium Laboratory for plant identification, the Cosmetics and Phytochemistry Laboratory of the Faculty of Pharmacy for extract preparation and testing, the Microbiology Laboratory of the Faculty of Pharmacy for bacterial suspension preparation, effectiveness testing, and colony counting, and the Medan Sei Sikambing Dental Laboratory for acrylic base plate production. The research was conducted from November to December 2025.

The study population consisted of pure culture isolates of S. sanguinis from the USU Medical Microbiology Laboratory. The study sample was a self-cured acrylic base measuring 10 x 10 x 2 mm. Based on the Federer formula, 24 samples were obtained which were divided into four groups, namely 40% lemongrass leaf extract (6 samples), 50% lemongrass leaf extract (6 samples), 0.2% chlorhexidine positive control (6 samples), and DMSO negative control (6 samples). Sample inclusion criteria included self-cured acrylic plates and fresh lemongrass leaves with intact leaves, while exclusion criteria included yellowed or rotten lemongrass leaves and porous or broken acrylic plates.

The independent variables of the study were 40% and 50% lemongrass leaf extract and 0.2% chlorhexidine, while the dependent variable was the number of S. sanguinis colonies. Controlled variables included the size and shape of the acrylic base, monomer-polymer ratio, soaking time, material concentration, media, temperature, culture time, plaster ratio, and stirring duration, while uncontrolled variables included lemongrass leaf morphology, ecosystem, and natural treatment during growth.

Research tools include vortex, test tubes, cuvettes, petri dishes, micromotors, scales, blenders, autoclaves, colony counters, incubators, and other general laboratory equipment. Research materials include S. sanguinis isolates, lemongrass leaves, chlorhexidine, 96% ethanol, acrylic resin monomers and polymers, bacterial growth media, plaster casts, PBS, artificial saliva, and supporting laboratory materials.

The research procedure began with the creation of a self-cured acrylic base by mixing polymer and monomer, pressed in a 10 x 10 x 2 mm mold, trimmed,

and polished. Lemongrass leaves were washed, dried, ground, and extracted by maceration using 96% ethanol, then diluted to 40% and 50% concentrations using DMSO. Phytochemical screening was carried out to detect alkaloids, flavonoids, terpenoids/steroids, tannins, and saponins. Sterilization of the equipment was carried out using an oven, heating, and autoclaving, while Nutrient Broth was prepared from sterilized nutrient broth and prepared according to standards. S. sanguinis bacterial suspension was prepared and its turbidity was adjusted to the McFarland 0.5 standard.

Acrylic base was soaked in synthetic saliva to form pellicle, then in bacterial suspension for 24 hours at 37°C. Samples were then soaked in each treatment (lemongrass leaf extract 40%, 50%, chlorhexidine 0.2%, and DMSO) for 8 hours, rinsed with PBS, vibrated using a vortex, and serial dilutions were performed up to 10^-2. The number of colonies was counted after being planted on Blood Agar and incubated at 37°C using a colony counter. All data were analyzed statistically using one-way ANOVA and post hoc LSD tests with a significance level of 95% if the data were normal and homogeneous, while abnormal or inhomogeneous data were analyzed using the Kruskal-Wallis and Mann-Whitney tests.

RESULT AND DISCUSSION

Phytochemical screening was conducted as an initial step to confirm the presence of active compounds that play a role in the mechanism of inhibiting microbial growth. The phytochemical screening results presented in Table 1 indicate that the ethanol extract of lemongrass leaves contains several active compounds, namely alkaloids, flavonoids, saponins, and tannins, which are known to play an important role in antimicrobial activity.

Table 1. Phytochemical Screening Results of Lemongrass Leaf Extract

Secondary Metabolites	Reagent	Result
Alkaloids	Dragendorf	+
	Bourchardat	-
	Mayer	+
Flavonoids	Mg Powder + Amyl Alcohol + HCl	+
Glycosides	Molish - H2SO4	+
Saponins	Shaken Hot Water	+
Tannins	FeCl3	+
Triterpenoids/Steroids	Lieberman-Bourchard	-

Description: (+): there is a group of secondary metabolite compounds; (-): there is no group of secondary metabolite compounds

Based on Table 1, the phytochemical screening results show that lemongrass (Cymbopogon citratus) leaf extract contains several groups of secondary metabolite compounds characterized by positive (+) reaction results. Alkaloid compounds were identified through tests using Dragendorff, Bouchardat, and Mayer reagents which showed positive reactions, indicating the presence of alkaloids in the extract. Flavonoid compounds were also detected positively through magnesium powder and amyl alcohol tests with the addition of HCl. In addition, glycoside compounds were identified using the Molisch–H_2SO_4 reagent, saponins were detected through a foam test with shaken hot water, and tannins were confirmed with the $FeCl_3$ reagent which gave a positive result. In contrast, triterpenoid/steroid compounds were not detected in lemongrass leaf extract, which was indicated by a negative (–) result in the Liebermann–Burchard test. These findings indicate that lemongrass leaf extract is rich in bioactive compounds that have the potential to support biological activity, especially as an antibacterial agent.

As a follow-up to the phytochemical screening results indicating the presence of bioactive compounds in lemongrass leaf extract, the test was continued with an analysis of the antibacterial effectiveness against the growth of Streptococcus sanguinis. Table 2 shows the average number of Streptococcus sanguinis colonies. Based on the results of the study, the number of Streptococcus sanguinis bacterial colonies on removable orthodontic acrylic bases from the negative control group, positive control, lemongrass leaf extract concentration of 50%, and 40% with an average of 1725±171,980 CFU/mL, 4.17±2,639 CFU/mL, 451.67±57,840 CFU/mL, and 662.50±23,889 CFU/mL, respectively.

Table 2. Average Number of Streptococcus sanguinis Colonies in All Groups

Group	Replication						*Mean*±SD
	1	2	3	4	5	6	
K-	1645	1650	1941	1649	1939	1531	1725±171.980
K+	4	4	3	9	4	1	4.17±2.639
50% Extract	407	427	429	473	560	414	451.67±57.840
40% Extract	685	668	624	679	643	676	662.50±23.889

Before conducting hypothesis testing to determine differences in effectiveness between treatment groups, prerequisite data analysis tests were first conducted, including normality and homogeneity tests. Table 3 shows the results of the normality test using the Shapiro–Wilk test and homogeneity test using

the Levene test. Based on the results, it can be concluded that the data were not normally distributed and not homogeneous, so data analysis was continued using nonparametric tests, namely the Kruskal–Wallis test and the Mann–Whitney test.

Table 3. Results of Normality and Homogeneity Tests

Group	*Shapiro-Wilk* *p-value*	*Levene test* *p-value*
K-	0.073	0.000
K+	0.143	
50% Extract	0.050	
40% Extract	0.257	

Description: *Significant (p < 0.05)

Based on Table 3, the results of the normality test using Shapiro–Wilk show that the p-value in each treatment group, namely the negative control group (K–), positive control (K+), 50% lemongrass leaf extract, and 40% lemongrass leaf extract, is at or below the significance limit (p < 0.05), so the data is declared not normally distributed. Meanwhile, the results of the homogeneity test using the Levene test show a p-value of 0.000 (p < 0.05), which indicates that the data between groups are not homogeneous. Thus, it can be concluded that the data does not meet the assumptions of the parametric test, so further statistical analysis was carried out using nonparametric tests, namely the Kruskal–Wallis test to see overall differences and the Mann–Whitney test for comparative analysis between groups. The results of the study on the antibacterial effectiveness of lemongrass leaf extract (Cymbopogon citratus) at concentrations of 40% and 50%, 0.2% chlorhexidine gluconate, and DMSO against the growth of Streptococcus sangunis on removable orthodontic acrylic bases (Table 4).

Table 4. Antibacterial Effectiveness of Lemongrass Leaf Extract (*Cymbopogon citratus*) 40% and 50% against the Growth of *Streptococcus sanguinis* on Removable Orthodontic Acrylic Base

Group	*Mean*±SD	*p-value*
K- (DMSO)	1725±171.980	0.000*
K+ (Chlorhexidine gluconate)	4.17±2.639	
50% Extract	451.67±57.840	
40% Extract	662.50±23.889	

Description: *Significant (p < 0.05)

Table 4 shows the Kruskal-Wallis statistical test. Based on the results of the study, it can be stated that there is a significant difference in the average number of colonies between all groups (p = 0.000; p <0.05). From the results obtained, there is an effectiveness of lemongrass leaf extract (Cymbopogon citratus) 40% and 50%, 0.2% Chlorhexidine gluconate, and DMSO on the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. The results of the study on the difference in the effectiveness of lemongrass leaf extract concentrations of 40% and 50%, 0.2% chlorhexidine gluconate, and DMSO on the growth of Streptococcus sangunis on removable orthodontic acrylic bases using the Mann-Whitney test can be seen in table 5 below.

Table 5. Difference in Antibacterial Effectiveness of Lemongrass Leaf Extract (*Cymbopogon citratus*) 40% and 50% against the Growth of *Streptococcus sanguinis* on Removable Orthodontic Acrylic Base

Group		p-value
K+ (Chlorhexidine Gluconate 0.2%)	K-(DMSO)	0.004*
	50% Extract	0.004*
	40% Extract	0,004*
Lemongrass Leaf Extract 40%	50% Extract	0,004*
	K-(DMSO)	0,004*
Lemongrass Leaf Extract 50%	K-(DMSO)	0,004*

Note: *significant (p < 0.05)

Table 5 shows the Mann-Whitney statistical test which was conducted to compare different treatment groups found significant differences in the Kruskal-Wallis analysis in the overall. Based on the results of the study, it can be stated that there is a significant difference in antibacterial effectiveness between the lemongrass leaf extract groups at concentrations of 40% and 50%, chlorhexidine gluconate 0.2% (positive control), and DMSO (negative control) against the growth of Streptococcus sangunis on removable orthodontic acrylic bases. So, it can be concluded that the use of lemongrass leaf extract at both concentrations has significantly different antimicrobial effectiveness compared to the positive control and negative control.

Currently, the use of herbal plant-based cleaning agents for removable orthodontic acrylic bases has begun to gain traction (Rahmi & Harahap, 2023). Lemongrass, or Cymbopogon citratus, is used in dentistry as a disinfectant for acrylic bases. Soaking the acrylic base in lemongrass leaf extract (Cymbopogon citratus) causes the compounds contained in it to be absorbed by diffusion and adhere to the cavities of the acrylic resin due to its water absorption and porosity [15]. This study aimed to determine the antibacterial effectiveness of 40% and 50% lemongrass leaf extract,

0.2% chlorhexidine gluconate, and DMSO on the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. Based on the results of the Kruskal Wallis test (p=0.000; p<0.05), there was a very significant difference in the average number of S. sangunis colonies between all treatment groups. In this study, 0.2% chlorhexidine gluconate served as the gold standard (positive control) because it is the most common and effective disinfectant agent used in dentistry to kill microorganisms. 0.2% chlorhexidine gluconate showed the lowest colony count of 4.17±2.639 CFU/mL, validating its role as a very strong antibacterial agent. Meanwhile, the negative control (DMSO) showed the highest average colony count (1725±171.980 CFU/mL), confirming that the solvent did not have an antibacterial effect. Lemongrass leaf extract at a concentration of 50% produced a significantly lower average colony count (451.67±57.840 CFU/mL) compared to lemongrass leaf extract at a concentration of 40% (662.50±23.889 CFU/mL).

Phytochemical screening results showed that the ethanol extract of lemongrass leaves is rich in secondary metabolites such as tannins, glycosides, saponins, flavonoids, and alkaloids, while triterpenoids/steroids were not found. Some of these active compounds are known to have broad antimicrobial potential. Flavonoids work by disrupting the function of the bacterial cytoplasmic membrane, resulting in leakage of vital intracellular components that can inhibit enzyme function. Tannins act as chelating agents and can form complexes with extracellular proteins and cell wall proteins, disrupting bacterial metabolism and cell integrity. Saponins are surface active (surfactants) and interact with bacterial cell membranes, causing lysis or membrane damage, while alkaloids can intercalate with bacterial DNA, disrupting the replication process.

The results of the Kruskal-Wallis statistical test in this study showed that there was a significant difference in the average number of colonies between all groups (p=0.000; p<0.05). Both 40% and 50% leaf extracts were effective against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases. Lemongrass extract with a concentration of 50% was more effective than the 40% concentration, indicating that 50% lemongrass extract was the most optimal in significantly reducing the growth of S. sangunis. This study underscores the existence of a dose-dependent relationship of lemongrass. This is supported by a study by [16] who examined lemongrass essential oil against oral bacteria, showing that increasing lemongrass concentration significantly increased its inhibitory power. The effectiveness of lemongrass is largely distributed in its main essential oil component, namely citral. Citral is able to disrupt cellular respiration, damage the lipid structure of the membrane and penetrate the bacterial cell membrane, similar to the study conducted by [17] confirmed that citral exhibited strong antibiofilm and antibacterial activity against Streptococcus spp., supporting the use of lemongrass extract in orthodontic disinfection applications.

This study examines the effectiveness of natural ingredients (lemongrass leaf extract) as an alternative to chemical disinfectants (Chlorhexidine gluconate 0.2%) to overcome the problem of bacterial contamination, especially Streptococcus sanguinis, on removable orthodontic acrylic bases. Bacterial contamination of orthodontic appliances can cause halitosis, gingivitis, and stomatitis. Therefore, the need for effective, safe, and easily accessible antibacterial agents is very high. Although Chlorhexidine gluconate 0.2% is the gold standard, in this study lemongrass leaf extract with concentrations of 40% and 50% has antibacterial effectiveness, but along with increasing the concentration of the extract, it is possible to conduct further research using higher concentration levels of the extract and can be another alternative for removable orthodontic acrylic base disinfectant products from natural ingredients, especially considering the potential side effects of 0.2% chlorhexidine gluconate in long-term use.

One limitation of this study is the use of the spreading method to count colonies, which has the potential to reduce the accuracy of the research results. Spreading inoculums with a spreader is prone to variability that can disrupt the consistency and accuracy of colonies on agar media. Additionally, other indicators, such as agar media thickness and incubation time, can cause errors in manual colony counting. To anticipate this, researchers used a digital colony counter that can provide more precise and stable results through automatic detection and calculation based on digital imaging. According to [18], this device can reduce manual errors, increase accuracy, and shorten the counting process, thereby minimizing data errors and strengthening the reliability of research findings.

CONCLUSION

Based on the results of the study, it can be concluded that the average number of Streptococcus sanguinis bacterial colonies on removable orthodontic acrylic bases treated with lemongrass leaf extract at concentrations of 50% and 40%, 0.2% chlorhexidine gluconate, and DMSO were 451.67±57.840 CFU/mL, 662.50±23.889 CFU/mL, 4.17±2.639 CFU/mL, and 1725±171.980 CFU/mL, respectively. Statistical analysis showed a significant difference in antibacterial effectiveness between the lemongrass leaf extract groups (40% and 50%), 0.2% chlorhexidine gluconate as a positive control, and DMSO as a negative control against the growth of Streptococcus sanguinis on removable orthodontic acrylic bases (p=0.000; p<0.05). Chlorhexidine gluconate 0.2% showed the highest antibacterial effectiveness and was significantly superior to lemongrass leaf extract at concentrations of 40%, 50%, and DMSO (p=0.004; p<0.05). In addition, lemongrass leaf extract at concentrations of 50% had significantly better antibacterial effectiveness than lemongrass leaf extract at concentrations of 40% and DMSO (p=0.004; p<0.05). However, both lemongrass leaf extract at concentrations of 40% and 50% were shown to have significantly better antibacterial

effectiveness in inhibiting the growth of Streptococcus sanguinis compared to DMSO as a negative control.

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