

Inhibitory Effect of Lemongrass Extract (*Cymbopogon citratus*) in Supragingival Plaque Bacterial Growth for Gingivitis Patient: A Research Study

Robiatul Firdaus¹, Carera I. Harryadi², Shafira Kurnia², Agung Krismariono²

¹Faculty of Dental Medicine, ²Department of Periodontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Aim: Gingivitis is a type of periodontal disease characterized by the inflammation of the gingival. Generally, gingivitis therapy is done with scaling and root planning and antibiotic medications. However, inappropriate use of antibiotic may lead to antibiotic resistance. An alternative therapy needs to be developed; lemongrass extract (*Cymbopogon citratus*) has an inhibitory effect on supragingival plaque bacterial growth in gingivitis patients. **Materials and Methods:** The lemongrass was extracted using the maceration procedure. Eight tubes containing lemongrass extract in different concentration were taken. The other two tubes contain supragingival plaque bacteria suspension as a positive control and brain heart infusion broth media as a negative control. Ten tube samples were tested for its inhibitory effect on the growth of supragingival plaque bacteria and measured using spectrophotometry in the wavelength of 570 nm. The absorbance percentage of a spectrophotometer is used to determine the minimum inhibitory concentration (MIC). The data were analyzed using SPSS for normality test (Shapiro–Wilk test), homogeneity test (Levene’s test), and assumption one-way Analysis of Variances (ANOVA) (Welch test) that continued with *post hoc* test (Games-Howell test). **Results:** The absorbance percentage of supragingival plaque bacterial colonies in lemongrass plant extract at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78% was 78.8%, 74.6%, 70.5%, 68.8%, 45.5%, 27.3%, 16.1%, and 5.1% of the positive control, respectively. The significance of the statistical test results was of 0.00 ($P < 0.05$; $df = 3$; confidence interval 95%). **Conclusions:** The growth of supragingival plaque bacteria in gingivitis patients is inhibited by extracts of lemongrass (*Cymbopogon citratus*), with a MIC of 12.5% with 68.8% inhibition.

Keywords: Gingivitis, Human and Health, Lemongrass, Minimum Inhibitory Concentration (MIC), Supragingival Plaque Bacteria

Received: 28-08-2021, **Revised:** 22-04-2022, **Accepted:** 11-05-2022, **Published:** 28-06-2022.

INTRODUCTION

Dental plaques based on gingival margin position consist of supragingival plaques in the coronal of the gingival margin and subgingival plaques below the crest of the gingival margin. Gingivitis is a minor form of periodontal disease characterized by gingival inflammation and clinical symptoms such as erythema, bleeding on probing, bulbous papillae, and edematous gingiva with a lack of stippling.^[1] Local factors in gingivitis are colonization of microorganisms in dental plaque on the surface of the teeth as a biofilm, attached to a matrices host polymers and main bacteria.^[2]

Gingivitis therapy management generally comprises scaling and root planning (SRP) and antibiotic medications. However, it can cause problems in the gingiva if the SRP is not conducted correctly. In addition, prolonged use of antibiotics may lead to antibiotic resistance that affects the prognosis of gingivitis.^[3] Data obtained from World Health Organization (WHO) show that 10%–15% of the

Address for correspondence: Dr. Shafira Kurnia,
Department of Periodontology, Faculty of Dental Medicine, Universitas
Airlangga, Jl. Major General Prof. Dr. Moestopo No. 47 Surabaya 60132,
East Java, Indonesia.
E-mail: shafira-k-s@fkg.unair.ac.id

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How to cite this article: Firdaus R, Harryadi CI, Kurnia S, Krismariono A. Inhibitory effect of lemongrass extract (*Cymbopogon citratus*) in supragingival plaque bacterial growth for gingivitis patient: A research study. *J Int Oral Health* 2022;14:324-30.

Access this article online

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DOI:
10.4103/jioh.jioh_226_21

world's population suffers from periodontal disease.^[4] The prevalence of Indonesians experiencing dental and oral health problems, including caries and periodontal diseases, according to Riset Kesehatan Dasar 2018, is around 57.6%, whereas the prevalence of periodontitis in Indonesia shows a percentage of 74.1.^[5] Uncontrolled bacterial growth in the oral cavity can cause various dental and oral health problems, especially gingivitis.^[6]

The lemongrass plant (*Cymbopogon citratus*) has active compounds, such as essential oils, consisting of citronella, citronellol, and geraniol that can disrupt the metabolism or death of bacterial cells.^[7,8] Leaves and lemongrass roots contain alkaloids, saponins, tannins, polyphenols, and flavonoids. Saponins and essential oils are a significant group of chemicals that provide activity to microbial destruction.^[9] In previous research, lemongrass leaf extract was found to have an antibacterial impact on the growth of *Streptococcus mutans*. The result was an average value of 3.96 mm.^[10]

Based on these studies, the antibacterial effect of lemongrass extract active compound had a potential to be tested in supragingival plaque bacterial growth for gingivitis patients using spectrophotometer measurement. The rationale for supragingival bacteria as a studied population because supragingival bacteria have a significant role in gingivitis pathogenesis, dominated by gram-positive coccus and short rods bacteria. This study sought to determine the inhibitory effect of lemongrass extract (*Cymbopogon citratus*) and to know the minimum inhibitory concentration (MIC) in supragingival plaque bacterial growth for gingivitis patients. This study aligns with the third goal of the 2030 sustainable development goals, ensuring a healthy life and improving the welfare of all people of all ages. It is hoped that it can be an alternative therapy for lemongrass extract (*Cymbopogon citratus*) in supragingival plaque bacterial growth for gingivitis patients through the use of mouthwash products or SRP companion therapy.

MATERIALS AND METHODS

Ethical approval and informed consent

This study was equipped with ethical approval that certifiably to be cleared and appropriate according to seven WHO 2011 to use research sample and conduct research. The approval number of this certificate is 323/HRECC.FODM/VII/2020. The date of approval is July 15, 2020.

Setting and design

The type of study conducted in this research is experimental laboratory research. In this investigation, some materials such as lemongrass (Surabaya, Indonesia), ethanol 96% (Merck), brain heart infusion broth (BHIB) (BD Biosciences, Bedford, MA), and mixed bacteria samples (Research Center, Faculty of Dental Medicine, Universitas Airlangga) were used. This research was started in July and ended in September 2020. The design

of this research is about the inhibition of lemongrass extract for gingivitis bacteria. Research sites are located in two locations as follows:

- Pharmaceutical Formulation and Technology Laboratory of Natural Ingredients, Widya Mandala: manufacture of lemongrass extract;
- Research Center Faculty of Dental Medicine, Airlangga University: supragingival plaque in gingivitis patients: sampling and inhibition test using spectrophotometry.

Sampling criteria

Sampling criteria in this study are supragingival plaque bacteria in gingivitis patients that had been bred at Research Center of Faculty of Dental Medicine, Airlangga University. Supragingival plaque bacterial stock was cultured using BHIB in a test tube. The sample population was calculated using Federer Formula (1977):

$$(n-1)(r-1) \geq 15$$

$$(8-1)(r-1) \geq 15$$

$$7r \geq 22$$

$$r \geq 3.142$$

with r is the number of samples or replication required, n is the number of treatments, and the number of replications required is three per treatment.

Methodology

The extraction of lemongrass is carried out in the Laboratory of Pharmaceutical Formulation and Technology of Natural Materials, Widya Mandala, Surabaya, Indonesia. The stem part of the lemongrass plant is washed and then cut thinly. The lemongrass pieces are dried in 50°C and crushed until a fine powder is obtained. Lemongrass powder is weighed 300 g and then diluted with 2.5 L ethanol 96% for 2 days. The 96% ethanol filtrate was evaporated using a rotary evaporator. Then the lemongrass extract was dried in an oven and stored at room temperature in an Erlenmeyer flask. The final volume of lemongrass extract obtained is 49 g.

Mixed bacteria for this research have been bred at the Research Center of Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia. The research was conducted three times handling ($n = 3$). Lemongrass extract was tested for its inhibitory effect on the growth of supragingival plaque bacteria using serial dilution and a spectrophotometer.

Sample preparation

Tube no. 1 contains a suspension of gingivitis patients' supragingival plaque bacteria prepared as the positive control. Test tube no. 2 contains 100% lemongrass extract,

10mL. The other eight test tubes contain only 5mL of BHIB. 5mL extract of lemongrass plant is inserted into test tube no. 3 and then stirred. After that, 5mL of liquid from no. 3 is taken and inserted into test tube no. 4 and stirred. The process is repeated until the liquid is inserted into test tube no. 9. Then, 5mL of liquid from tube no. 9 is removed, so each tube has the same volume of liquid. So, there were various lemongrass extract concentrations, 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. Ten test tubes are prepared. The suspension of supragingival bacteria as much as 1 mL is inserted into the test tube no. 2 to the test tube no. 9. The treatment was repeated three times to get an adequately large sample. The test tube is incubated at a temperature of 37°C for 24h.

Variable outcome

There are three variables in this study: independent variable, dependent variable, and controlled variable. Independent variables of this study were lemongrass extracts with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, and 0.78%. The dependent variable of this study is supragingival plaque bacteria’s growth in gingivitis patient. Controlled variables consist of BHIB, material and media used during study, incubation temperature, incubation time, and spectrophotometry.

Data measurement

Each of ten test tubes will be carried out with measurement numbers of bacteria with spectrophotometry with an optimization wavelength about 570nm to obtain maximum absorbance. Blanks in the first cell and the solution examined in the second cell are used as comparison solutions. The photocell space is enclosed in a “zero” galvanometer using the current dark button. The light file passed on the blank, and the galvanometer “zero” is obtained by rotating the sensitivity button. The transmission button used for a large setting is made at 100%. The light file is passed on to the solution to be analyzed. The absorbance scale indicates the absorbance of the sample solution. Repeat on the next test tube until the overall absorbance value of each solution in the test

tube is obtained. The formula for the percentage of live bacteria absorbance in spectrophotometry is calculated as follows:

$$\% A = \frac{OD_{treatment} + OD_{negative\ control}}{OD_{positive\ control} + OD_{negative\ control}} \times 100\%$$

with % A is percentage absorbance, and OD is optical density. OD value is the mean of absorbance from measurement data using a spectrophotometer.

Statistical analysis

Data management and analysis were performed using SPSS 24.0 (IBM, USA). The processing of data using statistical analysis test is as follows:

- a. Shapiro–Wilk test for normality test to determine if the data are normally distributed;
- b. Levene’s test for the homogeneity test of variance to test the similarity of variance in several samples;
- c. Welch test for the one-way Analysis of Variances (ANOVA) alternative difference test to determine whether there was a significant difference between the number of bacterial colonies in all treatment groups and continued with the *post hoc* test (Games-Howell test).

Statistical significance was analyzed using *P* value < 0.05%, degree of freedom is 3, and confidence interval is 95%.

RESULTS

The results obtained from observations and readings of absorbance values with different concentrations based on predetermined independent variables from concentrations of 100%, 50%, 25%, 12.5%, 6.25 %, 3.12%, 1.56% until 0.78%, where each concentration was repeated three times so that the following results were obtained [Table 1, Figure 1]. Table 2 and Figure 2 present the experimental data on determining the inhibitory effect of lemongrass plant extract (*Cymbopogon citratus*) in the growth of supragingival plaque bacteria for gingivitis patients were

Table 1: Absorbance value of each concentration

Group	Number of replications			Mean
	1	2	3	
Negative control	0.114	0.120	0.108	0.114
Positive control	0.996	0.989	0.992	0.992
Lemongrass extract’s concentration (%)	100	0.121	0.124	0.119
	50	0.168	0.162	0.174
	25	0.205	0.214	0.221
	12.5	0.242	0.231	0.225
	6.25	0.482	0.489	0.496
	3.12	0.688	0.679	0.692
	1.56	0.818	0.826	0.802
	0.78	0.972	0.944	0.902

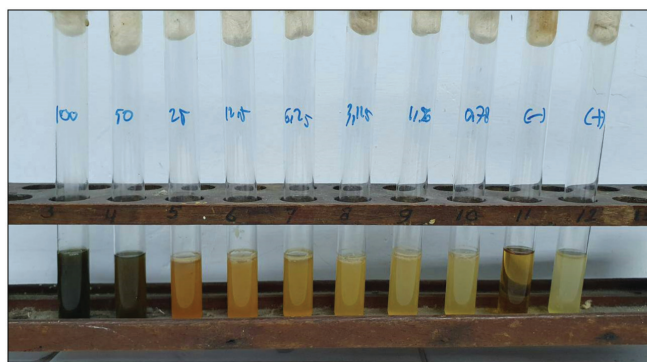


Figure 1: Various concentration of lemongrass extract

Table 2: Absorbance percentage		
Concentration (%)	Bacteria's life (%)	Bacteria's inhibition (%)
100	21.2	78.8
50	25.4	74.6
25	29.5	70.5
12.5	31.2	68.8
6.25	54.5	45.5
3.12	72.3	27.3
1.56	83.9	16.1
0.78	94.9	5.1

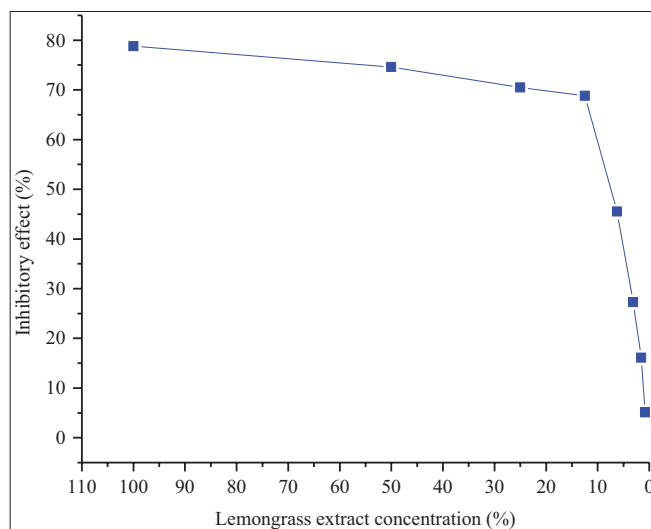


Figure 2: Graphic of bacteria's growth inhibitory effect

read absorbance level using a spectrophotometer in each treatment group according to the research variables. The absorbance level on spectrophotometry showed the ability of lemongrass extract to inhibit the growth of supragingival plaque bacteria in gingivitis patients. It is known that the more turbid the liquid in the test tube, the higher the optical density or, the greater the absorption value, and vice versa [Figure 1].

Based on data analysis through SPSS, the normality test with Shapiro-Wilk test [Table 3] obtained sample data from a normally distributed population. The homogeneity

Table 3: Normality test with Shapiro–Wilk test		
Concentration	Significance	Description
Negative control	1	Normal
Positive control	0.843	Normal
100%	0.78	Normal
50%	1	Normal
25%	0.862	Normal
12.5%	0.679	Normal
6.25%	1	Normal
3.12%	0.583	Normal
1.56%	0.637	Normal
0.78%	0.78	Normal

Table 4: Homogeneity test with Levene's test		
Levene statistic	Significance	Description
3.10	0.017	Nonhomogeneous

Table 5: Alternative difference test ANOVA with Welch test		
Welch test	Significance	Description
9752.19	0.000	There is a significant difference

test variant with Levene's test obtained a research sample group, which is not homogeneous or not similar [Table 4]. Based on both tests, the data in the study did not meet any of ANOVA's one-way assumptions. To find out if there are differences in each sample, an alternative ANOVA difference test is required using Welch test or unequal variance *t* test, and it is obtained that there is a mean difference that means the average on the variable is accessible to bound variables [Table 5]. Furthermore, *post hoc* tests were conducted using equal variances, not assumed group tests, namely the Games-Howell test [Table 6].^[11] Based on the comparative data, there is a significant difference between two variables being compared. Almost all data have meaningful differences between variables from one to another, from 100%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, negative control, and positive control. However, there are intervariable comparison results without significant differences, such as 100% with negative controls, 25% with 12.5%, and 1.56% with 0.78%. This shows that three intervariable comparison results do not have huge difference in research result.

DISCUSSION

An initial objective of the project was to identify the inhibition of lemongrass extract to the growth of supragingival plaque bacteria in gingivitis. A possible explanation for this might be that lemongrass extract had potential as an alternative therapy for gingivitis without antibiotic resistance effect. This is proven by the active ingredient in lemongrass. 600 g of lemongrass

Table 6: Post-hoc test with Games-Howell test

Concentration	Positive control	100%	50%	25%	12.5%	6.25%	3.12%	1.56%	0.78%	Negative control
Positive control	-	0.00	0.000	0.000	0.000	0.000	0.000	0.004	0.494	0.00
100%	0.000	-	0.013	0.007	0.006	0.000	0.000	0.000	0.003	0.669
50%	0.000	0.013	-	0.018	0.007	0.000	0.000	0.000	0.003	0.004
25%	0.000	0.007	0.018	-	0.341	0.000	0.000	0.000	0.003	0.001
12.5%	0.000	0.006	0.007	0.341	-	0.000	0.000	0.000	0.003	0.001
6.25%	0.000	0.000	0.000	0.000	0.000	-	0.000	0.000	0.008	0.000
3.125%	0.000	0.000	0.000	0.000	0.000	0.000	-	0.004	0.027	0.000
1.56%	0.004	0.000	0.000	0.000	0.000	0.000	0.004	-	0.102	0.000
0.78%	0.494	0.003	0.003	0.003	0.003	0.008	0.027	0.102	-	0.002
Negative control	0.000	0.669	0.004	0.001	0.001	0.000	0.000	0.000	0.002	-

plants (*Cymbopogon citratus*) contains active ingredients in essential oils 2.05% with geraniol 8.13%, flavonoids 3.66%, tannins 1.06%, and saponins 2.16%. The stem part of the lemongrass plant contains 85.73% essential oil citronella.^[12] The root part of the lemongrass plant contains approximately 0.52% alkaloids of 300 g of plant material. Its composition can be extracted to use as an inhibitor of bacterial growth.^[13]

Lemongrass extract dilution was done through serial dilution to determine the most effective concentration inhibiting bacterial growth.^[14] In addition, this method has higher sensitivity compared with diffusion techniques. However, this method requires thoroughness in its research. The inhibitory effect test in this study used spectrophotometry to determine the value of absorbance or OD with a wavelength of 570 nm because the cells can absorb at this wavelength.

The absorbance value indicates the amount of light absorbed by the solution sample contained in the tube with each concentration of lemongrass plant extract and supragingival plaque bacteria. Spectrophotometer has several advantages better than agar plate method. Spectrophotometry is an analytical instrument that is very easy to operate in short duration, fast, and cheap, with high analytical sensitivity and small repeat error because of the same wavelength control.^[15] However, this method has systematic errors that often occur in analysis using a spectrophotometer that causes errors in determining the absorbance value or OD, such as absorption by solvents, absorption by cuvettes, selectivity in distinguishing samples with other particles, or contaminants that absorb light in the same wavelength, and the measurement of the wavelength of the spectrophotometer is not appropriate. However, the weaknesses of this spectrophotometry can be overcome.^[15]

Based on the study results through spectrophotometry, the concentration of lemongrass plant extract by 0.78% to 100% concentration in the test tube showed an increased inhibitory effect in supragingival plaque bacteria's growth. This indicates that the concentration of lemongrass plant extract with the active compound significantly inhibits supragingival plaque bacteria's growth. MIC is the

lowest concentration of antimicrobials that inhibit visible bacteria or bacteria growth, determining resistance levels and hints of antimicrobial use.^[16]

MIC in this study was determined based on the reference journal written by Arthington-Skaggs *et al.*, who researched to determine the MIC endpoint of fluconazole and itraconazole through spectrophotometry against candida isolate.^[17] Through the study results, MIC was at a concentration of 12.5% with a percentage of inhibitory effect in supragingival plaque bacteria's growth, causing gingivitis by 68.8% with a limit of at least 50%. This is in accordance with the rational of the study, which is to determine the inhibitory effect of lemongrass extract (*Cymbopogon citratus*) and to know the MIC on supragingival plaque bacteria growth in gingivitis patients. However, not all data have significant difference of inhibition values between one variable and another. There are several variables, such as 100% with negative controls, 25% with 12.5%, and 1.56% with 0.78%, that have been measured in such a way with spectrophotometric measurements show no significant difference or there is a small difference inhibition effect. This can be proven by statistical analysis.

MIC of lemongrass extract in supragingival bacterial growth differs from one condition to another. In healthy patients, there is no growth of supragingiva plaque bacterial in groups of 100%, 50%, 25%, 12.5%, 6.25%, and 3.12%. The growth of supragingiva plaque bacterial colonies was only seen at 1.56% concentration and increased the number of bacterial colonies at 0.78%.^[18] Chronic periodontitis patients with *Porphyromonas gingivalis* bacteria as dominant bacteria have MIC from 200 µL of extract of lemon grass caused 50.2% reduction in number of colonies of *Porphyromonas gingivalis*.^[19] Patients with aggressive periodontitis have predominance of *Aggregatibacter actinomycetemcomitans* bacteria, MIC carried out 150 µL of extract caused 54.2% reduction and 200 µL of lemon grass oil and 59.2% reduction in number of colonies of *Aggregatibacter actinomycetemcomitans*.^[20] The difference in MIC results was influenced by the dominant type of bacteria in supragingival plaque bacteria under certain conditions. In patients with gingivitis, it is dominated by gram-positive coccus and short rods bacteria.

The higher concentration of lemongrass plant extracts directly increases the inhibition of supragingival plaque bacteria's growth for gingivitis. It is influenced by the number of active compounds contained in lemongrass extracts, such as essential oils, flavonoids, saponins, tannins, and alkaloids, which have an antibacterial function. The active compound of lemongrass extract is an essential oil that consists of citronella, citronellol, and geraniol, which can inhibit bacterial activity by denaturing and inactivating proteins such as enzymes. Therefore, bacterial cell walls will be damaged because of decreased permeability that allows the disruption of the transport of critical organic ions that will enter the bacterial cells, thus disrupting the metabolism or death of bacterial cells.^[8]

Flavonoids are antibacterial by forming complex bonds with cell walls, damaging bacterial cell membranes, and inhibiting the synthesis of bacterial cell macromolecules.^[21] Flavonoids work with several mechanisms of action, including inhibiting the synthesis of nucleic acids, inhibiting the function of cytoplasmic membranes, and inhibiting the metabolism activity of bacteria so that bacterial growth will be disrupted.^[22]

Saponin is a compound that provides a firm surface voltage reduction effect and works by disrupting the stability of bacterial cell membranes, thus causing lysis.^[10] Another activity of saponins is to reduce the efficiency of glucose utilization in microorganisms, affect growth and proliferation, reduce the activity of critical enzymes in physiology metabolism, and suppress protein synthesis, leading to bacterial cell death.^[21]

Tannin compounds can inhibit bacterial growth through their ability to bind to bacterial cell walls and protease activity. At low concentrations, tannins can inhibit bacterial growth, whereas at high concentrations, tannins are antibacterial. Tannin toxicity has the potential to harm bacterial cell membranes. Tannin astringent chemicals can cause complex bonding compounds against enzymes or microbial substrates and a complex of tannin, which binds against metal ions, which can exacerbate tannin toxicity.^[21]

Alkaloids may operate as an antibacterial mechanism by disrupting peptidoglycan constituent components in bacterial cells and lead to the failure of the cell wall formation, resulting in cell death.^[23] The ability of alkaloids in inhibiting bacterial growth is associated with the ability to interact with DNA to inhibit DNA synthesis and reverse transcriptase and release lipoteichoic acid adhesin from the cell surface, thus disrupting the permeability of bacterial cell membranes.^[21]

In addition to the content of lemongrass plant extracts that affect the growth of supragingival plaque bacteria, the structure of supragingival plaque bacteria also has a significant role in the inhibitions of bacterial growth. This condition is caused by the supragingival plaque

bacteria that is located in the coronal gingiva margin, which is dominated by gram-positive coccus and short rods bacteria on the surface of the teeth.^[6] Antibacterial components tend to be more sensitive to gram-positive bacteria because of the simpler structure of bacterial cell walls, making it easier for antibacterial chemicals to enter cells and discover targets for activity. On the other hand, the cell wall of gram-negative bacteria is a complex structure with triple-layer membrane, which consists of lipoproteins, outer membranes of phospholipids, and polysaccharides. The content of polysaccharides, lipids, and proteins in gram-positive bacteria is only 1%–4%, whereas it reaches 11%–22% in gram-negative bacteria. Thus, gram-negative bacteria's cell walls are more difficult to penetrate by the active ingredient in antibacterial. However, that does not mean that gram-negative bacteria's growth in supragingival plaques cannot be inhibited, only in more significant duration and concentration.^[24–26]

The cell viability test, which is a test to measure cell life's ability by calculating the number of living cells, supports the inhibitory impact of lemongrass extract against supragingival plaque bacteria in gingivitis patients. It is exposed to the material and to be tested by Methyl Thiazolyl Tetrazolium (MTT) assay method read using Enzyme-Linked Immunoassay (ELISA) Reader absorbent. High absorption values indicate many living cells, whereas low absorbance values indicate a small number of living cells.^[26,27] In 2018, cell viability experiments revealed that a 100% concentration of lemongrass extract with a live cell percentage of 100.41% could preserve the viability of fibroblasts Baby Hamster Kidney-21 (BHK-21) cells. The research of lemongrass plant extract and viability of living cells are expected to be a reference for further research.

However, minimum bactericidal concentration (MBC), which is the lowest concentration of antimicrobial agent required to kill 99.9% of bacteria in the inoculum after 24h of incubation under suitable conditions, cannot be determined through this spectrophotometric test because there are no related journals that can be used as a reference to determine MBC by spectrophotometry. Generally, MBC is determined by subcultured samples from test tubes, non-selective agar disks for colony counting or other bactericidal test methods. For further research, it is necessary to carry out tests that can show the MBC and toxicity test of lemongrass extracts on the growth of supragingival plaque bacteria in gingivitis patients. It is hoped that it can be an alternative therapy from lemongrass extract (*Cymbopogon citratus*) on supragingival plaque bacteria in gingivitis patients through the use of mouthwash products or SRP companion therapy.

CONCLUSION

The growth of supragingival plaque bacteria in gingivitis patients is inhibited by extracts of lemongrass stem

(*Cymbopogon citratus*), with a MIC of 12.5% and 68.8% inhibition. This antibacterial effect of lemongrass stem extract is exerted by several bioactive components such as essential oils, flavonoids, saponins, tannins, and alkaloids.

Acknowledgment

None.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Author contribution

Concept: SK, CIH, AK; design: SK, RF; definition of intellectual content: RF, CIH, AK; laboratory experiment and interpretation data: SK, RF; and manuscript writing: SK, CIH, RF, AK.

Ethical policy and institutional review board statement

This research has ethical approval and is certified to be ethically cleared.

Patient declaration of consent

Not applicable.

Data availability statement

Not applicable.

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