

The Inhibitory Power of Lemongrass Extract (*Cymbopogon citratus*) Against The Growth of Bacteria *Fusobacterium nucleatum*

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Abstract

Periodontal disease is a disease that affects the oral cavity and its surrounding tissues as well as the teeth. *Fusobacterium nucleatum* is one of the Gram-negative bacteria involved in periodontal disease. With its proteins and receptors on the outer membrane, this species is known to invade oral epithelial cells and aggregate with early and late colonizers. Lemongrass plant extract (*Cymbopogon citratus*) has been shown to inhibit the growth of supragingival plaque bacteria in gingivitis patients. The objective of this research was to find out what the minimum inhibitory concentration (KHM) of lemongrass extract is on the growth of *Fusobacterium nucleatum* bacteria. The study involved the extraction of lemongrass extract as well as phytochemical tests on the extract. The *Fusobacterium nucleatum* bacteria were then grown at each concentration of lemongrass plant extract used in this study: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78%. Furthermore, data analysis was performed on the obtained results. This study shows that lemongrass extract (*Cymbopogon citratus*) inhibits the growth of *Fusobacterium nucleatum* bacteria with a minimum inhibitory concentration (KHM) of 0.78% and a percentage of inhibition of 55.7%. This is due to the presence of many antibacterial ingredients in lemongrass plant extract, such as alkaloids, flavonoids, saponins, tannins, and phenols.

Keywords: Lemongrass extract, *Cymbopogon citratus*, *Fusobacterium nucleatum*, periodontal.

1. Introduction

Periodontal disease is a common oral disease that affects the tissues and teeth around the teeth. This disease, which includes gingivitis and periodontitis, has a global prevalence of 20% to 50% (Nazir et al., 2020). According to the WHO, nearly 19% of the global population has severe periodontal disease (World Health Organization, 2022). Periodontitis affects 74.1% of people in Indonesia, and gingivitis affects 13.9% of people aged 15 and up (Ministry of Health of the Republic of Indonesia, 2019). Periodontal disease develops as a result of a complex interaction between subgingival biofilms and the host immune inflammatory response in gingival and periodontal tissues. The process is a defense mechanism against periodontal disease-causing bacteria (Newman et al., 2019).

The pathogenesis of periodontitis is influenced by two major factors: subgingival microbiota virulence factors and the host immune-inflammatory response. Lipopolysaccharides (LPS), enzymes and harmful products, microbial invasion, fimbriae, and bacterial DNA are all virulence factors. Other factors include inflammatory mediators produced by the host, such as cytokines, prostaglandins, and matrix metalloproteinases (MMPs) (Newman et al., 2019).

Fusobacterium nucleatum is one of the Gram-negative bacteria involved in periodontal disease. With its proteins and receptors on the outer membrane, this species is known to invade oral epithelial cells and aggregate with early and late colonizers. Pathogenicity of *Fusobacterium nucleatum* is attributed to its role as a “bridge within the organism” that facilitates the integration of periopathogens in biofilms. *Fusobacterium nucleatum* is important in shifting the environment from Gram-positive to Gram-negative dominance. Under these conditions, the biofilm community changes and becomes more pathogenic, increasing the progression of periodontal disease (Tefiku et al., 2020). *Fusobacterium nucleatum* has two important virulence factors that can bind to other species: RadD for Gram-positive species and Fap2 for *Porphyromonas gingivalis* (Arenas Rodrigues et al., 2018; Newman et al., 2019). RadD and Fap2 are members of the autotransporter family of proteins. These proteins are derived from Gram-negative bacteria and have potent virulence factors. Adhesion, cell aggregation, biofilm formation, and invasion are all biological functions of autotransporters (Tefiku et al., 2020).

The first phase of treatment for patients with periodontitis is non-surgical scaling and root planing. These procedures are designed to remove plaque from the teeth and prevent the formation of periodontal pockets. If non-surgical treatment fails to heal the pockets, surgery is required to remove inflammatory tissue and reduce bone damage (American Dental Association, 2005). In periodontal cases, antibiotic therapy can also be used. However, antibiotic use increases the risk of bacterial resistance (Herawati, 2011).

Indonesia is a tropical country with an abundance of biological resources. This diversity, particularly the flora, provides numerous benefits. Lemongrass (*Cymbopogon citratus*) is one of the plants that can be used as medicine. This plant has a distinctive leaf blade that extends towards the base and tapers upwards, with a shiny green and smooth appearance. Alkaloid compounds, saponins, flavonoids, phenolic acids, and other compounds are found in lemongrass. Lemongrass essential oil has antimicrobial properties, particularly against Gram-positive bacteria and fungi (Khan and Abourashed, 2010).

Previous research has shown that lemongrass extract inhibits the growth of supragingival plaque bacteria in gingivitis patients. As a result, at a concentration of 12.5%, the minimum inhibitory concentration is 68.8% (Firdaus, 2021). The objective of this research was to find the minimum inhibitory concentration (KHM) of lemongrass extract (*Cymbopogon citratus*) on the growth of *Fusobacterium nucleatum* bacteria.

2. Material and Research Method

The Herbal Materia Medica Batu prepared the lemongrass extract (*Cymbopogon citratus*), the Organic Chemistry Laboratory FST Universitas Airlangga performed phytochemical tests, and the Dental Research Center FKG Universitas Airlangga performed an inhibition test of lemongrass extract (*Cymbopogon citratus*) against the growth of *Fusobacterium nucleatum* bacteria. Osse, test tube rack, test tube, erlenmeyer tube, spirit brander, petri dish, measuring cup, anaerobic incubator, micropipette, autoclave, sterile cabinet, rotary evaporator, filter paper, and drop plate were used in this study. While the materials used included sterile distilled water, 96% ethanol, magnesium powder, amyl alcohol, chloroform, Dragendorf reagent, Meyer reagent, Wagner reagent, anhydrous acetate solution, concentrated H₂SO₄ solution, FeCl₃ 1%, *Fusobacterium nucleatum* ATCC 25586 bacterial stock, Brain Heart Infusion Broth (BHIB) media, and Mueller-Hinton Agar (MHA) media.

Bacterial stocks used in this study were cultured in test tubes using Brain Heart Infusion Broth (BHIB). In this study, each group was subjected to three repetitions. Lemongrass plant extract concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% were used as independent variables. The dependent variable is the percentage of *Fusobacterium nucleatum* bacteria growth inhibition.

Lemongrass extraction was accomplished by thoroughly washing the lemongrass plant and then aerating it at room temperature (37°C). Moreover, lemongrass plants were cut into small pieces and dried in a 55°C oven until the weight of the lemongrass was constant. The dried lemongrass plants were then pulverized and filtered into powder using a blender. The powder was then placed in an erlenmeyer flask and macerated with 400 mL of 96% ethanol solvent for 1-2 days at room temperature. The sample was then filtered, and the filtrate and pulp were obtained. A rotary evaporator was used to evaporate the solvent at 55-63°C until a thick extract was obtained. Furthermore, quantitative phytochemical tests were carried out consisting of flavonoid tests, alkaloid tests, triterpenoid or steroid tests, tannin tests, and saponin tests.

Bacterial preparation was accomplished by planting 1 osse of bacteria from the stock in 5 mL of BHIB media and incubating at 37°C for 224 hours. Turbidity was matched to 0.5 McFarland (1.5108 CFU/mL). The dilution method was then used to test the inhibition of *Fusobacterium nucleatum* bacteria growth by lemongrass extract. Eight test tubes were prepared and labeled in total. The first tube contained 10 mL of 100% lemongrass plant extract, while the remaining seven tubes contained 5 mL of BHIB media. A total of 5 mL of lemongrass plant extract from tube 1 was transferred to tube 2 and homogenized. Then, 5 mL of liquid from tube no. 2 was homogenized in tube no. 3. The procedure was repeated until tube no. 8 was reached. Then, 5 mL of liquid from tube no. 8 was discarded, resulting in a uniform volume of liquid in each tube. Each test tube was filled with 0.1 mL of bacterial suspension. Two test tubes, nos. 9 and 10, were

prepared and labeled. Tube 9 contained only bacterial suspension as a negative control, and tube 10 contained only 5 mL of BHIB media as a media control. To obtain an adequate sample size, the treatment was repeated three times. Moreover, all test tubes were incubated for 224 hours at 37°C. Each test tube's suspension was diluted to 0.1 mL and spread onto MHA media before being incubated at 37°C for 224 hours. The number of colonies grown on MHA media was counted, and the resulting data was analyzed.

The data was analyzed using statistical tests that included a data normality test using the Shapiro-Wilk test to see if the data was normally distributed, a data homogeneity test using Levene's Test to test the similarity of variants of several samples, a difference test with the Kruskal Wallis test to determine if there is a significant difference in the number of bacterial colonies in all treatment groups, and a post hoc test (Mann-Whitney) to determine significant differences in each group.

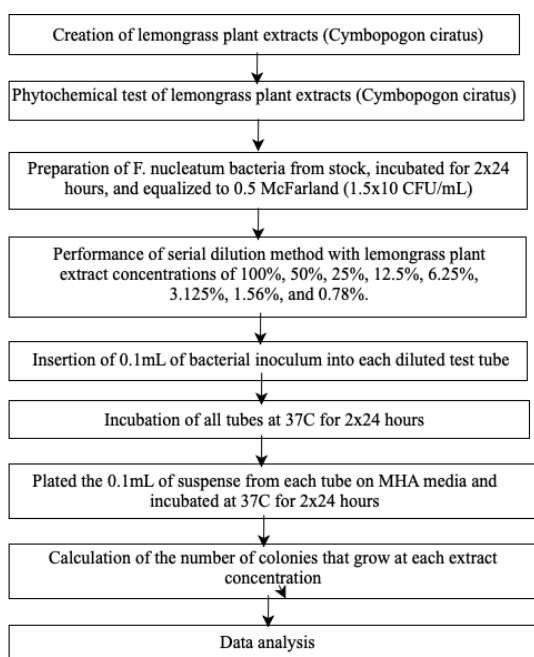


Fig. 1. (a) Research Procedure

3. Result and Discussion

According to the phytochemical test results from this study, saponin is the active ingredient with the highest content (7.2%). Alkaloids, flavonoids, tannins, and phenols are among the other active ingredients found in extract samples (Table 1).

Table 1. Phytochemical Test Result of Lemongrass Plant Extracts

Parameter	Result (%)	Sample mass (g)	Method
Saponins	7,2	1,25	Gravimetry
Alkaloids	4,39	2,5	Gravimetry
Flavonoids	2,05	1,12	UV – Vis Spectrophotometry
Phenol	1,25	1,09	Gravimetry
Tannis	0,69	1,09	UV – Vis Spectrophotometry

Lemongrass plant extract was tested using the dilution method to see if it inhibited the growth of *Fusobacterium nucleatum* bacteria, and the results were obtained using the colony count method. The following graph depicts the percentage inhibition of bacterial growth.

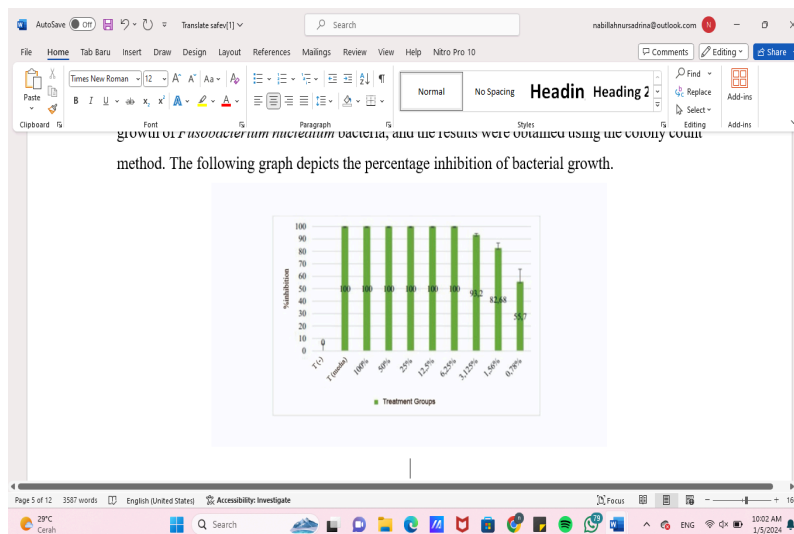


Fig. 2. (a) Chart of Percentage of

The normality test showed that the data were not normally distributed because the data in one of the sample groups, namely the 3.125% concentration group, had a significance value < 0.05 . In the negative control group, 100%; 50%; 25%; 12.5%; and 6.25% no significance value was produced because no bacterial colonies grew (Table 2).

Table 2. Result of Shapiro – Wilk Normality Test

Sample Group	Sig.	Description
Negative control	0,702	Normally distributed
Media control	-	-
100%	-	-
50%	-	-

25%	-	-
12,5%	-	-
6,25%	-	-
3,125%	0,000	Not normally distributed
1,56%	0,726	Normally distributed
0,78%	0,780	Normally distributed

The homogeneity test was then used to continue the data analysis. The significance value for the homogeneity test was 0.05, indicating that the data was not homogeneous. The Kruskal Wallis non-parametric difference test was then used because one of the data points in the sample group was not normally distributed. The difference test yields a significance value of 0.05, indicating that the data is not homogeneous. The Kruskal Wallis non-parametric difference test was then used because one of the data points in the sample group was not normally distributed. The difference test results produced a significance value of 0.05, which is 0.001, indicating that there is a significant difference between the sample groups. To see which groups were significantly different, the Mann-Whitney non-parametric post hoc test was conducted. Two groups with a significance value <0.05 are groups that have significant differences (Table 3).

Table 3. Result of Post Hoc Mann-Whitney Test

Kelompok	K (-)	K (media)	100%	50%	25%	12,5%	6,25%	3,125%	1,56%	0,78%
K (-)										
K (media)	0,037 *									
100%	0,037 *	1								
50%	0,037 *	1	1							
25%	0,037 *	1	1	1						
12,5%	0,037 *	1	1	1	1					
6,25%	0,037 *	1	1	1	1	1				
3,125%	0,046 *	0,034 *	0,034 *	0,034 *	0,034 *	0,034 *	0,034 *			
1,56%	0,049 *	0,037 *	0,037 *	0,037 *	0,037 *	0,037 *	0,037 *	0,046 *		
0,78%	0,049 *	0,037 *	0,037 *	0,037 *	0,037 *	0,037 *	0,037 *	0,046 *	0,049 *	

Lemongrass (*Cymbopogon citratus*) is a well-known medicinal plant in Indonesia. Lemongrass is well-known for its flavoring properties. Furthermore, this plant has medicinal properties. Lemongrass leaves have antibacterial, carminative, fungicidal, analgesic, antiseptic, astringent, bactericidal, and antidepressant properties (Magotra, Singh, and Singh, 2021).

Buzaina (2021) previously conducted a phytochemical test on lemongrass plant extract. There are some discrepancies between the findings of this study and this study. Saponins (2.01%), tannins (1.17%), and alkaloids (0.94%) were found to have the highest percentage of compound content in that study. Several factors influence the difference, including genetics, environment, post-harvest storage, and plant extract processing (Li, Tsao, and Deng, 2012). The lemongrass plants used in this study was obtained from UPT Laboratorium Herbal Mitra Medica Batu. The agency is in charge of conducting research on medicinal plants and developing traditional medicine. This location guarantees the quality of medicinal plants (UPT Laboratorium Herbal Materia Medica Batu, 2022).

An inhibition test is used to determine a material or compound ability to inhibit bacterial growth. KHM was obtained at a concentration of 0.78% with a percentage inhibition of 55.7% in this study. The minimal inhibitory concentration of an antibacterial agent defines the level of susceptibility or resistance of a specific bacterial strain in vitro. The determination of a reliable KHM value has a significant impact on the choice of a therapeutic plan, which can affect therapy efficiency (Kowalska-Krochmal and Dudek-Wicher, 2021). The KHM value was used in clinical settings to determine the most effective drug for the treatment of bacterial infections. In this study, KHM was found to inhibit bacterial growth by up to 50% at 0.78% concentration and 90% at 3.125% concentration. Lemongrass plant extract at a concentration of 0.78% was expected to be used as a preventive therapy for periodontal bacterial infection, whereas lemongrass plant extract at a concentration of 3.125% was expected to be used as a curative therapy because it had the potential to inhibit bacterial growth by up to 90%.

Lemongrass (*Cymbopogon citratus*) plants contain a variety of antibacterial compounds, including alkaloids, flavonoids, saponins, tannins, and phenols. Saponin is the most abundant compound in lemongrass extract, accounting for 7.2% of the total. Saponins work as antibacterials by lowering surface tension and increasing the permeability of bacterial cell walls. This causes the cell membrane to become unstable. Saponins then diffuse and cause the cytoplasm to exit the cell, resulting in cell death (Sudarmi, Darmayasa, and Muksin, 2017; Nugraha, Achmad, and Sitompul, 2019). Another mechanism involves decreasing the efficiency of glucose utilization by microorganisms, decreasing the activity of the major enzymes involved in metabolism, and suppressing protein synthesis (Mawan, Indriwati, and Suhadi, 2018).

With a percentage of 4.39%, alkaloids are the second most abundant compound. Alkaloids work as antibacterials by disrupting cell metabolism. Bacterial growth is inhibited as a result of this. Another

mechanism that is thought to occur is damage to the peptidoglycan components in the bacterial cell wall, resulting in cell death (Marfuah, Dewi, and Rianingsih, 2018). Alkaloids also work by inhibiting DNA synthesis and reverse transcriptase and releasing lipoteichoic acid adhesins from the cell surface (Firdaus et al., 2022).

Flavonoids have a percentage of 2.05% in lemongrass plant extracts that have been tested for phytochemicals. Flavonoids act as antibacterials by forming complex compounds that inhibit nucleic acid synthesis, alter cytoplasmic membrane function, inhibit energy metabolism, reduce cell attachment and biofilm formation, and alter membrane permeability. Bacterial growth is inhibited by these mechanisms (Farhadi et al., 2019).

Lemongrass extract also has a phenol content of 1.25%. Antioxidant activity is well known for phenolic compounds. Phenolic compounds are known for their antioxidant activity. According to some studies, phenols work by altering cell membrane permeability. Changes in some intracellular functions are caused by phenolic compound hydrogen bonding with enzymes or by modifying cell wall rigidity so that the cell wall loses its integrity. This causes irreversible damage to the cytoplasmic membrane and coagulation of cell contents, resulting in disruption of intracellular enzymes and cell damage (Bouarab-Chibane et al., 2019).

With a percentage of 0.69%, tannin is the compound with the lowest percentage in lemongrass extract. Tannins work as antibacterial agents by chelating iron away from bacterial cells, preventing bacteria from growing. Tannins can also inhibit bacterial cell wall synthesis by inactivating enzymes involved in the process or by directly binding to the cell wall. Tannic acid can also bind to peptidoglycan, compromising integrity of the cell wall. Another mechanism is to damage the bacterial cell membrane by increasing cell wall permeability. Because of the presence of lipopolysaccharides, some tannins have an effect on the outer membrane of Gram-negative bacteria. Lipopolysaccharides are bound by proanthocyanidin resulting in instability of the outer membrane integrity (Farha et al., 2020).

The mechanism of antibacterial compounds caused bacterial cell damage, causing the bacteria to be lysed and unable to act as “bridge microorganisms” in the biofilm. Because there was no bond between early and late colonizers, the biofilm lost its integrity under these conditions. This reduced the pathogenicity of the community in the biofilm, which slowed the progression of periodontal disease.

There are other studies that support the findings of Firdaus’s study (2022). The study found that lemongrass extract (*Cymbopogon citratus*) inhibited periodontal pathogenic bacteria, specifically supragingival plaque bacteria that caused gingivitis. KHM was obtained at 12.5% with a percentage of inhibition of 68.8% in this study. The percentage of inhibition was determined using UV-Vis spectrophotometer analysis equipment in the study. The spectrophotometer method is simpler to use but has lower sensitivity and selectivity than the colony count method because it cannot distinguish samples with

particles or other contaminants that absorb light at the same wavelength (Rambet, Waworuntu, and Gunawan, 2017). Furthermore, the study was carried out on mixed bacteria samples containing various types of periodontal pathogenic bacteria with varying characteristics. As a result, the KHM value in that study exceeded the KHM value in this study.

Susanto and Girsang (2020) conducted a similar study. The study analyzed the effectiveness of Aloe vera hydrogel against the *Fusobacterium nucleatum* bacteria. The findings showed that Aloe vera hydrogel could inhibit the growth of *Fusobacterium nucleatum* bacteria at 5% and 10% concentrations, with an average diameter of the inhibition zone greater than 10 mm. Because *Fusobacterium nucleatum* bacteria are less sensitive to concentrations below them, the two concentration groups were chosen (Susanto and Girsang, 2020). This supports the statement that lemongrass extract has greater potential for inhibiting the growth of periodontal pathogenic bacteria and could be used as an alternative ingredient in the treatment of periodontal disease.

This study had limitations because it was only conducted in vitro on a small number of treatment groups. Thus, it was expected that more in vivo and in vitro research involving larger samples will be conducted in the future to test the effectiveness of lemongrass (*Cymbopogon citratus*) on periodontal pathogens, particularly *Fusobacterium nucleatum* bacteria.

4. Conclusion

This study finds the inhibition of lemongrass extract (*Cymbopogon citratus*) against the growth of *Fusobacterium nucleatum* bacteria with a minimum inhibitory concentration (KHM) of 0.78% and a percentage of inhibition of 55.7%.

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Non to declare

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