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Formulation of Lemongrass Extract Mouthwash (Cymbopogon Citratus) as a Non-Pharmacological Effort in Inhibiting The Growth of Bacteria That Cause Dental Caries

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Abstract: Caries is a dental disease characterized by damage ranging from enamel, dentin, and pulp. Caries is caused by bacteria that stick to the teeth. The purpose of this study was to prove the effectiveness of lemongrass in inhibiting the growth of bacteria that cause dental caries. The pretest-posttest laboratory and field experiment consisted of a lemongrass mouthwash intervention group with concentrations of 33%, 36%, 39% and a chlorhexidine control group, each subject gargled for 2 minutes. Saliva was collected before and after gargling. The variables studied were the number of colonies and bacterial inhibition. The paired test of 36% (p=0.017) and 39% (p=0.006) concentration intervention groups was significantly different, while the chlorhexidine control group (p=0.091) was not significantly different. The organoleptic test results of the 36% lemongrass mouthwash had a dark brown color, slightly thick, the distinctive smell of lemongrass stems was still present, and the taste of mint. Based on this test, the most acceptable lemongrass mouthwash concentration is 36%. Giving a 39% lemongrass mouthwash formulation for 2 minutes effectively reduces the number of bacterial colonies that cause dental caries and the inhibition is better than the chlorhexidine control, while the 36% concentration is not significantly different from chlorhexidine.

Keywords: Antibacterial, Lemongrass Extract Mouthwash, Caries-Causing Bacteria

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INTRODUCTION

Oral health is very important for every individual. Problematic oral health can cause impaired speech function, chewing function, and aesthetic function which will definitely have an impact on one's activities. According to Federation Dentaire International (FDI), around 90% of the world's population experiences oral health problems ranging from dental caries, dental supporting tissue disease and even oral cancer (Hontong et al., 2016).

The oral cavity is one of the places in the body that contains microorganisms with the highest diversity compared to other places. The most abundant microorganism in the oral cavity is Streptococcus sp which plays a role in the beginning of the dental caries process. In addition, the bacterial colonies found at the beginning of plaque formation are Streptococcus mutans bacteria which have an important role in the development of dental caries in animals and humans (Juliantoni & Wirasisya, 2019).

Teeth are hard organs found in the mouth that are used to process food when eating, with that teeth have a function to tear, chew and smooth a food before the food enters the throat. Therefore, it is necessary to care for dental care so that the teeth are maintained and intact (Hafizah, 2021).

If you don't care about dental health, it can cause diseases that can damage the tooth coating, namely dental caries. Dental caries is a disease that affects many children and adults, both in milk teeth and permanent teeth. Dental caries is a dental tissue disease characterized by tissue damage, starting from the tooth surface, namely from enamel, dentin, and extending towards the pulp (Afrinis et al., 2020).

Dental caries is an infectious disease that affects almost 95% of the population in the world. The dental morbidity rate ranks as the 6th most common disease. Oral disease in Indonesia caused by dental caries ranks 10th highest with a prevalence of 45.68%. Dental caries is the most common disease in the oral cavity (Darsono, 2020).

Cariogenic bacteria are bacteria that have the ability to cause dental caries. These bacteria include Actinomyces, Lactobacillus, Streptococcus mutans, Streptococcus sanguinis, etc. The main bacteria causing dental caries are Streptococcus mutans and Lactobacillus acidophilus. S. mutans is a normal inhabitant of the oral cavity that can turn into a pathogen when the living environment of the bacteria is favorable and there is an increase in population. Streptococcus mutans and Lactobacillus acidophilus in the oral cavity produce lactic acid from fermented sugars, causing the pH of the plaque to decrease, if the decrease in pH occurs continuously, it will cause demineralization of the tooth root surface. These bacteria are resistant to acid, therefore they can survive in a medium with a high level of acidity. The bacteria attach to the tooth surface which can metabolize carbohydrates to produce organic acids which cause a decrease in oral pH, causing demineralization of tooth enamel. Based on the results. Based on the results (Riskesdas, 2018) the prevalence of

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dental caries in Indonesia is still relatively high at 57.6% and the proportion of cavities in Indonesia is 45.5% and in Central Java province is 43.4%.

A healthy oral cavity without plaque accumulation can be achieved by mechanical means such as brushing teeth. Cleaning actions by brushing teeth are often unable to reach the entire surface of the teeth, so other efforts are needed such as utilizing antibacterial materials (Pujoraharjo & Herdiyati, 2018). Mouthwash can be used to help clean the oral cavity from plaque and microorganisms that can cause damage to teeth and supporting tissues. The antibacterial properties of mouthwash are determined by the content of its active ingredients. Chlorhexidine is a gold standard mouthwash from the bisbiguanid group that can be effective as an anti-plaque and anti-gingivitis ingredient. Chlorhexidine has the ability to inhibit S. mutans bacteria (Fauzia et al., 2021). urrently, chlorhexidine is routinely used by doctors in the treatment of patients with periodontal cases, orthodontia and maxillofacial surgery. Chlorhexidine is available as mouthwash, soap, gel, spray, toothpaste and varnish in different strengths. Lower strengths have been used as mouthwashes (0.12%, 0.2%, 0.1%). Many studies have shown a correlation of efficacy and the strength of chlorhexidine used, unfortunately higher concentrations show an increase in side effects i.e. staining of teeth and restorations and decreased taste. Long-term use of chlorhexidine can cause several side effects such as causing tooth discoloration, impaired taste, and is an allergen if in constant contact14. These effects will occur if routine use is carried out continuously for more than 2 weeks within 2 times a day and in the long term for more than 2 years or if the use does not follow the correct rules. This is in accordance with previous research that after using chlorhexidine for 3-6 months, many extrinsic steins were found on the tooth surface. In cases of periodontitis, chlorhexidine is one of the antibiotics used in the treatment of periodontal disease, but there is a problem of bacterial resistance when antibiotics are used continuously. Microorganism resistance to antibiotics has an impact on high medical costs, infections are now back as a problem in the world of medicine. Efforts to overcome this resistance are carried out by finding new alternatives in inhibiting microorganisms.

Oral health is a fundamental part of general health and affects overall well-being. Having optimal oral health can help a person maintain the function of their oral cavity, and can make a person feel better and more confident. Oral health is considered very important because the oral cavity is a place for bacteria to grow, if the oral cavity is not cleaned properly, food debris will stick to the surface of the teeth and will form plaque. This plaque is a gathering place for bacteria that cause dental and oral diseases (Fatmawati, 2015). Oral health is one of the factors that support a healthy paradigm and is a national development strategy to realize health development for socially and economically productive human resources. Therefore, every individual must have the awareness, willingness, and ability to

improve their health to the highest level (Susilawati & Damayanti, 2020).

Maintenance of oral hygiene is one of the efforts in improving health. The mouth functions not only for the entrance of food and drink but more than that and not many people realize the magnitude of the role of the mouth for health and well-being. Therefore, oral health plays a very important role in supporting one's health (Ratih & Yudita, 2019). Oral health is sometimes the umpteenth priority for some people, even though oral and dental diseases have a serious impact on general health, because teeth and mouth are the entry points for germs and bacteria so that they are likely to interfere with the health of other organs.

MATERIALS AND METHODS

The conceptual framework in this study consists of independent variables, namely lemongrass extract mouthwash formulations, dependent variables, namely the growth of bacteria that cause dental caries.

The type of research used is laboratory and field experiments, pure experiment with pretest-posttest with control group design consisting of 2 (two) groups, namely the intervention group and the control group. The sampling method used was purposive sampling.

The population used was junior high school students. While the research sample was 35 students

maximum age of 15 years who have caries teeth. The sampling technique in this study was non probability sampling, which is a sampling technique that does not provide equal opportunities for each member of the population to be selected as a sample on the basis of sample selection considerations according to the researcher's judgment. The non probability sampling method used in this study is purposive sampling.

The tools used are beaker glass, maceration vessels, porcelain cups, funnels, glassware, and glassware.

porcelain, funnel, measuring cup, Hot plate, needle ose, filter paper, Laminar air flow, microscope spirit lamp, mortar, stamper, analytical balance (Sarltorius), glass object, wooden clamp, test tube, stirrer, glass, petri dish, erlenmeyer, volume pipette, stir bar, scales, test tubes, Durham tubes, tube racks, autoclave, transpipettes, dropper pipettes, inkas, incubator, oven, parchment paper, cotton wool, pH meter, vernier, knife, plastic pot, horn spoon, Vacum Rotary Evaporator, Brookfield viscometer, waterbath and mouthwash container.

Research on lemongrass extract mouthwash formulations, and bacteria that cause dental caries was conducted at the Microbiology and Parasitology Laboratory of the Faculty of Medicine, UNISSULA Semarang to test the effectiveness of lemongrass extract mouthwash formulations against the growth of bacteria that cause dental caries, this research was conducted in June 2022.

RESULTS AND DISCUSSION

The study was conducted in June 2022 at SMP Negeri 12 Semarang and the Laboratory of the Faculty of Medicine

Unissula Semarang which is located at Jalan Raya Kaligawe Km. 4, Semarang, Central Java. The materials used were lemongrass extract (Cymbopogon citratus), 90% ethanol, glycerin, benzoic acid, xylitol, oleum menthe, and distilled water. The research process began with the extraction of lemongrass plants and the formulation of mouthwash preparations carried out at the Laboratory of the Faculty of Medicine, Unissula Semarang. The tools used were blender, laboratory glassware (Pyrex), test tubes (Pyrex), digital scales, oven, viscometer, drop pipette, spatel, stirring rod and rotary evaporator. The lemongrass stems that have been collected are cleaned of dirt then washed thoroughly using running water then chopped and dried using an oven. The research subjects were obtained from the saliva of respondents who experienced dental caries at SMP Negeri 12 Semarang, then the saliva was taken to the Laboratory of the Faculty of Medicine, Unissula Semarang for culture and began the research process of the number of bacterial colonies and bacterial inhibition tests. The bacterial culture process is carried out on nutrient media to obtain bacteria that cause dental caries. After the bacteria grow on the media, gram staining is done to identify gram-positive and gram-negative bacteria. After gram staining, the type of bacteria is known, then proceed with planting bacteria with agar media to find bacterial species.

The cultured bacteria are then rejuvenated or purified by inoculating 1 ose of pure culture on Nutrient Agar media and then incubated at 37°C for 48 hours. In this research sample, the bacteria that cause dental caries found are Streptococcus mutans bacteria.

The concentrations of lemongrass extract mouthwash formulations used in this research are 33%, 36%, and 39% concentrations.

Bacterial testing was carried out on each treatment group using lemongrass extract mouthwash formulations at concentrations of 33%, 36%, 39%, and chlorhexidine mouthwash control.

The incubated bacteria were diluted by mixing 1 ose of bacteria into a test tube containing 1 ml of NaCl, then homogenized using a vortex and the turbidity was standardized with McFarland concentration so that the number of bacteria was eligible for the test. The growth inhibition test of Streptococcus mutans bacteria was carried out by dilution, namely by putting 1 ml of bacteria and 20 ml of Nutrient Agar in 5 enlenmeyer tubes with a volume of 50 ml containing lemongrass extract mouthwash formulations with each concentration of 33%, 36%, and 39% and chlorhexidine control. All tubes were incubated at 49°C to observe bacterial inhibition by looking at the turbidity of the tubes compared to the chlorhexidine control. Then the tubes were poured into Petri dishes and planted on Nutrient Agar, then incubated at 37°C for 48 hours. The minimum zone of inhibition formed was measured by counting bacterial colonies using markers and lights. Markers are used as stationery to write markers on the number of bacteria on petri dishes, lamps are used as lighting aids when counting bacterial colonies assisted by a microscope.

1. Data Normality Test

Normality tests are carried out using the Shapiro-Wilk statistical test which shows the distribution of normally distributed or abnormally distributed data.

Table 1. Table of Normality of Data on the Number of Bacterial Colonies

Bacteria	Group	Shapiro-Wilk
		Sig.
Streptococcus mutans	33% concentration solution	0.337
	36% concentration solution	0.301
	39% concentration solution	0.118
	Chlorhexidine	0.082

Based on Table 1, it shows that with the Shapiro-Wilk normality test, a p> 0.05 value is obtained so that the ability to reduce the number of bacterial colonies is normally distributed.

2. Parametric Test

The ability of the treatment group and the control group to Streptococcus mutans bacteria had a normal data distribution so that this study used a paired parametric test with the Paired T-Test and an unpaired test with the One Way Annova test.

a. The Paired T-Test was used to determine whether there was a difference in the average killing power of Streptococcus mutans in each group.

Table 2. Ability of Each Group in Reducing the Number of Bacterial Colonies

Group	Mean±SD Pretest	Mean±SD Posttest	Delta±SD	P-values
Concentration 33%	248.17±185.67	106.33±53.836	141.83±229.40	0.190
Concentration 36%	297.60±176.10	80.80±97.374	216.80±122.34	0.017
Concentration 39%	302.33±147.65	10.50±13.293	291.83±155.33	0.006
Chlorhexidine	364.00±338.31	288.00±257.36	136.00±159.15	0.091

Based on table 2, the lemongrass extract mouthwash formulation with a concentration of 36% and a concentration of 39% has a p value <0.05 indicating that these concentrations are more capable of reducing bacteria compared to the lemongrass extract mouthwash formulation with a concentration of 33%, and chlorhexidine which has a p value > 0.05. The highest ability to reduce the number of bacterial colonies was shown in the formulation of lemongrass extract mouthwash with a concentration of 39%. Based on the table, a graph was created that illustrated the ability of each treatment group to reduce the number of bacterial colonies.

b. One Way Annova test

One Way Annova test was conducted forlook at the differences between each group.

Table 3. Intergroup Difference Test in Reducing the Number of Bacterial Colonies

Pretest ± SD		
Group	**Mean±SD**	**P-values**
Lemongrass extract concentration 33%	284.17±185.670	0.852
Lemongrass extract concentration 36%	297.60±176.102	
Lemongrass extract concentration 39%	302.33±147.659	
Chlorhexidine	364.00±338.314	
Posttest ± SD		
Lemongrass extract concentration 33%	106.33±53.836	0.097
Lemongrass extract concentration 36%	80.80±97.374	
Lemongrass extract concentration 39%	10.50±13.293	
Chlorhexidine	228.00±257.360	
Delta(Δ)±SD		
Lemongrass extract concentration 33%	141.83±229.404	0.385
Lemongrass extract concentration 36%	216.80±122.349	
Lemongrass extract concentration 39%	291.83±155.339	
Chlorhexidine	136.00±159.153	

Based on table 3, obtain a value of p> 0.05 which indicates nosignificant differences between groups in reducing the number of bacterial colonies.

Based on research on the inhibition of lemongrass extract mouthwash formulations against Streptococcus mutans bacteria, the results obtained were the results of bacterial inhibition data in the form of the number of bacterial colonies. Bacterial testing was carried out in each treatment group, namely lemongrass extract mouthwash formulation concentration of 33%, lemongrass extract mouthwash formulation concentration of 36 %, formulation of lemongrass extract mouthwash concentration of 39%, and control of chlorhexidine mouthwash. The inhibition test of Streptococcus mutans bacteria obtained the following results:

1. Normality test

Table 4. Bacterial Inhibition Zone Data Normality Test

Group	Shapiro-

	Wilk Sig.
33% concentration solution	0.091
36% concentration solution	0.097
39% concentration solution	0.074
Chlorhexidine	0.075

Based on Table 4, it shows that with the Shapiro-Wilk normality test, a p> 0.05 value is obtained so that the inhibition ability of bacteria is normally distributed.

2. Parametric Test

   The inhibition zones of the treatment group and the control group for Streptococcus mutans bacteria had a normal data distribution, so this study used a parametric test with the One Way Annova test.

The One Way Anova test was carried out to see differences in the inhibition of bacteria in each group. Based on the One Way Annova test, the following results are obtained:

Table 5. Differences in Inhibitory Power of Bacteria in Each Group

Group	**Mean±SD**	**P-values**
Lemongrass Extract Concentration 33%	14.44±0.427	0.0001
Lemongrass Extract Concentration 36%	14.20±0.005	
Lemongrass Extract Concentration 39%	16.20±0.003	
Chlorhexidine	14.20±0.004	

Based on table 5, it shows that the concentration of the lemongrass extract mouthwash formulation and the control group obtained a p-value of 0.0001 (p <0.05) which indicates a significant difference between groups. For this reason, a Post Hoc test is needed.

The results showed that the formulation of lemongrass extract mouthwash was able to inhibit the growth of bacteria that cause dental caries. The results of previous studies showed that lemongrass leaf extract had the ability to inhibit bacterial growth. Kitchen lemongrass (Cymbopogon citratus) has a variety of ingredients including nutrients, minerals, and phytochemicals. The nutritional content of lemon grass extract (Cymbopogon citratus) includes carbohydrates, protein, and fiber. The minerals contained in it include phosphorus, calcium, magnesium, iron, and zinc. The phytochemical content in lemongrass stem extract includes flavonoids such as quercetin, alkaloids, saponins, tannins, anthraquinones, steroids, phenolic acids, and flavone glycosides and some of them have been shown to have antibacterial activity (Tanjung et al., 2022). Lemongrass extract has antibacterial activity because it contains flavonoids which can damage cell membranes and denature bacterial cell proteins so that they can inhibit bacterial growth (Atnawanty et al., 2020).

The mechanism of action of flavonoids as antibacterial compounds is divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism. In inhibiting

the synthesis of nucleic acids, flavonoid compounds play an important role in the process of interaction or hydrogen bonding by accumulating nucleic acid bases thereby inhibiting the formation of DNA and RNA. The results of the interaction of flavonoids will also cause damage to the permeability of the cell wall. In inhibiting the function of the cell membrane, flavonoids will form complex compounds from extracellular and dissolved proteins so that the cell membrane will be damaged and intracellular compounds will come out. Whereas in inhibiting energy metabolism by inhibiting the use of oxygen by bacteria,

Saponin compounds as active substances can increase the permeability of bacterial cell membranes, causing cell lysis. When saponins react with bacteria, they cause the bacteria to lyse or break.

While alkaloids have a mechanism of action as an antibacterial by inhibiting the constituent components of peptidoglycan in bacterial cells so that the cell wall does not form intact. This will cause cell death (Agustina et al., 2018).

Tannin compounds interact by forming complex polysaccharide compounds which can damage the bacterial cell wall so that the permeability of the bacterial cell becomes disturbed. The disturbed bacterial cell permeability causes the cell to be unable to carry out living activities, as a result the bacterial growth will be inhibited and cause the bacteria to die. Besides being able to damage the bacterial cell wall, tannins can also denature proteins and inhibit components of bacterial nucleic acid synthesis.

The results of the paired test in calculating the number of bacteria tested using the Paired T-Test showed that the formulation of lemongrass extract mouthwash with a concentration of 39% (p=0.006) and 36% (0.017) had a significant difference with the formulation of lemongrass extract mouthwash with a concentration of 33% (p =0.190), and chlorhexidine (p=0.091).

In the unpaired test using the One Way Annova test, it showed no significant difference before treatment with a value of p = 0.852 (p> 0.05), after treatment it produced a value of p = 0.097 (p> 0.05) which means there was no difference significant between groups after treatment, and the results of the delta test obtained a value of p = 0.385 (p> 0.05) which indicated that there was no significant difference between groups. Then to see comparisons between groups, a follow-up test was carried out using the LSD Post Hoc test. The results obtained from this test were that there was a significant difference between the 39% concentration of lemongrass extract mouthwash and chlorhexidine (p=0.016).

Lemongrass is a plant that contains citronellal, geraniol, and citronol which can inhibit bacterial activity. The content of lemongrass consists of caryophyllene which has antibacterial, anti-inflammatory, antitumor, antifungal and anesthetic properties (Rahayu et al., 2021). Citronellal is one of the aldehyde monoterpene groups that has antibacterial potential where the mechanism of action is by eliminating membrane proteins resulting in changes in cell membrane permeability. This group acts as a protein dehydrator at

low doses and at high doses as a protein denaturator. The alcohol and phenol groups present in lemongrass cause the rupture of the cytoplasmic membrane and damage to the bacterial cell wall.

The lemongrass extract mouthwash contains glycerin, xylitol, benzoic acid, oleum menthae, and distilled water. Glycerin as a humectant which functions to retain moisture thereby reducing irritation when in contact with the skin, the advantage of glycerin as a humetan is that it has the property of increasing the spreadability of preparations94. Glycerin which is used as an additional ingredient in mouthwash does not affect the inhibition of bacterial growth.

Xylitol is an organic polyalcohol chemical element which is widely used as an alternative sugar because it has the same level of sweetness as sucrose95. Xylitol is a type of sugar alcohol which has antibacterial properties and cannot be metabolized by S. mutans bacteria to become energy. Xylitol is commercially prepared from beech wood and is not fermentable by cariogenic bacteria96. S. mutans bacteria will try to metabolize xylitol but will not produce energy so that eventually the bacteria will run out of energy and then die which is marked by a decrease in the number of S. mutans bacteria (Rasyadi, 2018).

Benzoic acid is a white crystalline solid and is the simplest aromatic carboxylic acid. This weak acid and its derivatives are used as food preservatives. In mouthwash preparations, benzoic acid is used as a pH buffer in dosage formulations. Sodium benzoate is a preservative that has bacteriostatic and fungistatic properties under acidic conditions. Its mechanism as a preservative begins with the absorption of benzoic acid into the cell, then the intracellular pH of the cell becomes acidic. If the intracellular pH of cells decreases to 5 or even lower, the anaerobic fermentation of glucose decreases drastically which causes cell growth and development of microorganisms to be inhibited.

Based on the results of the pretest and posttest paired tests, the intervention group with lemon grass extract mouthwash formulations at concentrations of 36% (p=0.017) and 39% (p=0.006) had a significant difference, whereas in the control group chlorhexidine (p=0.091) there was no significant difference. The results of the different test for bacterial inhibition showed that the 36% concentration of lemon grass extract mouthwash compared to chlorhexidine did not have a significant difference with a p value = 0.990, while the 39% concentration compared with chlorhexidine had a significant difference with a p = 0.0001. In the organoleptic test results, the formulation of a lemongrass extract mouthwash with a concentration of 36% had a dark brown color, slightly viscous, the characteristic odor of lemongrass stalks was still present, a minty taste, and did not cause a burning taste. While the concentration of 39% has a more viscous solution, dark brown color with a very distinct smell from lemon grass stalks, spicy and minty taste, and does not cause a burning taste on the tongue. Based on the pH test, it showed that each concentration of lemongrass extract mouthwash formulation had a normal pH.

Then the viscosity test showed that the 36% concentration of lemon grass extract mouthwash was closer to the standard viscosity of mouthwash compared to the 39% concentration which had a higher viscosity. Based on these tests, the most acceptable concentration of lemongrass extract mouthwash formulation is 36%. Then the viscosity test showed that the 36% concentration of lemon grass extract mouthwash was closer to the standard viscosity of mouthwash compared to the 39% concentration which had a higher viscosity. Based on these tests, the most acceptable concentration of lemongrass extract mouthwash formulation is 36%. Then the viscosity test showed that the 36% concentration of lemon grass extract mouthwash was closer to the standard viscosity of mouthwash compared to the 39% concentration which had a higher viscosity. Based on these tests, the most acceptable concentration of lemongrass extract mouthwash formulation is 36%.

CONCLUSIONS

The results of this study indicate that the formulation of lemongrass extract mouthwash with a concentration of 33% in inhibiting the growth of bacteria that cause dental caries is no better than 0.2% chlorhexidine. Formulation of lemon grass extract mouthwash concentration of 36% in inhibiting the growth of bacteria that cause dental caries has no difference with 0.2% chlorhexidine. Formulation of lemon grass extract mouthwash concentration of 39% in inhibiting the growth of bacteria that cause dental caries is better than 0.2% chlorhexidine. The mouthwash formulation that has the highest antibacterial concentration is 39%. The formulation of the most comfortable lemongrass extract mouthwash to use based on the results of the stability test of the mouthwash and its ability to inhibit bacterial growth was a concentration of 36%.

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