JURNAL KEDOKTERAN DIPONEGORO
Online : http://ejournal3.undip.ac.id/index.php/medico
E-ISSN : 2540-8844
JKD, Volume 11, Nomor 3, Mei 2022

EFFECT OF LEMONGRASS STEM INFUSION (*CYMBOPOGON CITRATUS*) ON GROWTH OF *STREPTOCOCCUS MUTANS*

Azzadian Octa Kusuma[1*], Endang Sri Lestari[2], Tyas Prihatiningsih[3], Nadia Hardini[3]
[1]Undergraduate Program, Department of Dentistry, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia
[3] Department of Dentistry, Faculty of Medicine Universitas Diponegoro, Semarang, Indonesia
*Corresponding Author : Email : azzadianok@alumni.undip.ac.id

ABSTRACT

Background: One of the bacteria that plays an important role in the process of caries is *Streptococcus* mutans. Herbal plants have been widely used for maintenance and healthcare, one of which is lemongrass. Lemongrass has natural antibacterial properties which indicate that it suppresses the growth of *Streptococcus mutans.* **Objectives:** To determine the effect of lemongrass infusion on the growth of *Streptococcus mutans* at various concentrations. **Methods:** This study used *post test only control group design* with total sample is 9 samples, concentration 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, 1,56%, positive control, and negative control with 3 replications. The research uses dilution method by looking at the turbidity which can be marked as MIC, for MBC used pour plate method was determined by looking at the presence or absence of bacterial growth on the plate. Data analysis was performed using the spss application using Kruskal-Wallis test with significance level if the result of p<0,05. **Results:** The results of the sample group were 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, 1,56% in the MIC test showed turbidity in all samples, then the MBC test showed growth bacteria in all samples. The assessment of the probability test p value of 1,000 (>0,05) indicates that there is no significant difference between research groups. **Conclusion:** There was no effect of lemongrass stem *infusion* with various concentrations on the growth of *Streptococcus mutans.*

Keywords: *Lemongrass stem infusion, Streptococcus mutans, MIC, MBC*

INTRODUCTION

General problems of oral health according to WHO (World Health Organization) are caused by dental caries and periodontal disease.[1] Based on Riskesdas 2018, it was stated that 57.6% of Indonesian people have dental and oral problems.[2] Dental caries is tooth decay that occurs due to loss of tooth minerals due to carbohydrate fermentation by bacteria.[3] The onset of caries is characterized by demineralization of enamel and dentin minerals, followed by destruction of their organic materials.[4] Caries is the most common dental disease.[5]

Bacteria in the oral cavity are factor in the process of caries.[6] One of the bacteria that plays an important role is *Streptococcus mutans*, because it can make saliva more acidic by fermenting sucrose.[7] Metabolism of *S. mutans* produces a critical pH of 4,4-5,5 which is required for the onset of tooth demineralization.[8]

The prevalence of dental caries is quite high, giving an alternative preventive action which is a priority effort to reduce the prevalence of dental caries.[9] Prevention of dental caries includes improving nutrition, reducing consumption of cariogenic diets, improving oral hygiene, administering systemic and topical fluoride, and using traditional medicines.[10]

Traditional medicine is an ingredient in the form of plant material, animal material, mineral material, extract (*galenic*), or a mixture of these materials which have been used for procedures, and can be applied in accordance with the prevailing norms in society. Used medicinal plants is generally considered safer, easier to obtain and has a relatively cheap.[12] Thousands of plant species are estimated to be indicated as useful for treatment, one of which is lemongrass (*Cymbopogon citratus*).[13]

Lemongrass is an aromatic plant of the *Poaceae* (*Gramineae*) family that is cultivated in tropical and sub-tropical areas throughout the world.[3,12,13] Lemongrass leaves and stems contain flavonoids, geranyl acetate, phenolic compounds, steroids and saponins that function as antimicrobial, antibacterial, anti-inflammatory agents. inflammatory, and antifungal.[14,15] Previous studies have shown several benefits of lemongrass.[15] In this study, researchers conducted a study on the effect of infusion of lemongrass stems on the growth of *S. mutans* bacteria as a mouthwash.

METHODS

Research design

This experimental laboratory research with *post test only control group design* research was

conducted at the Microbiology Laboratory, Central Laboratory of the Diponegoro National Hospital in December 2020.

Sterilization Materials

The tools used were sterilized in an oven at a temperature of 180°C-200°C for 1 hour, while the media were sterilized by autoclave at a temperature of 200°C for 15 minutes.

Lemongrass Infusion

Lemongrass plants are cleaned with running water and then dried by drying in the sun, then dry cut into small pieces. After that, weighed 100 grams added 100 ml of distilled water to obtain a concentration of 100%. Erlemeyer is placed in a beaker filled with water and heated on a hot plate for 15 minutes starting at 90°C. Then the infusion solution was filtered using a glass funnel lined with filter paper.

Bacterial Rejuvenation

S. mutans (ATCC 25175) obtained from the Research Laboratory of the Faculty of Dentistry, Gadjah Mada University, Yogyakarta were transferred to new media. One osse of bacteria was taken and then streaked on BHI-A media and incubated at 37°C for 18-24 hours.

***S. mutans* standard 0,5 McFarland**

Pure culture of *S. mutans* which had been rejuvenated was suspended in 5 mL of 0.9% NaCl using an osse so that the turbidity was comparable to that of a 0,5 McFarland suspension.

Procedure

This study used the dilution method and was divided into 9 groups according to their respective concentrations, 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, positive control, and negative control. . Each tube was given 1mL of infusion and 0.1mL of *S. mutans* was repeated 3 times (9 tubes). All tubes were incubated at 37°C for 18-24 hours, then observed, compared with controls. The smallest concentration that can inhibit bacterial growth is determined as MIC. To determine the MBC infusion of lemongrass stems against *S. mutans*, further tests were carried out by taking 1 mL of the MIC test solution, grown on BHI-A media by pour plate, then incubated at 37°C for 18-24 hours. MBC was determined by the presence or absence of colony growth on the plate. The data was processed using *spss* statistics software. This study used the *Kruskal Wallis* non-parametric test to see a significant difference.

RESULT

The results of the analysis of the Minimum Inhibitory Concentration (MIC) test on 9 samples with each concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, positive control, and negative control with 3 replications determined using the dilution method by looking at the clarity of each infusion sample of each concentration that has been incubated for 18-24 hours. Assessment of Minimum Inhibitory Concentration (MIC) can be seen by determining the clarity of the sample. The clarity assessment was carried out by 3 observers. Assessment of Minimum Bactericidal Concentration (MBC) is determined by looking at the growth or absence of bacteria on BHI-A media at each concentration that has been incubated for 18-24 hours.

The results of the research that has been carried out to determine the MIC test on lemongrass stem infusion on the growth of *S. mutans* in the treatment group with concentrations of 100%, 50%. 25%. 6.25%. 3.125%. 1.56% showed that there was growth of *S. mutans* which was the same as the control treatment (+), except in the control treatment group (-) which showed no bacteria growth. Determination of the Minimum Bactericidal Concentration (MBC) in the treatment groups with concentrations of 100%, 50%, and 25% there was bacterial growth.

Table1. Minimum Inhibitory Concentration of Lemongrass Stem Infusion

Replication	K+	K-	P1 100%	P2 50%	P3 25%	P4 12.5%	P5 6.25%	P6 3.125%	P7 1.56%
I	Clear	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
II	Clear	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid
III	Clear	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid	Turbid

Table2. Lemongrass Stem Infusion Minimum Bactericidal Concentration

Replication	K +	K-	P1 100%	P2 50%	P3 25%	P4 12.5%	P5 6.25%	P6 3.125%	P7 1.56%
I	-	+	+	+	+	+	+	+	+
II	-	+	+	+	+	+	+	+	+
III	-	+	+	+	+	+	+	+	+

Note :
(-) No bacterial growth, (+) There is bacterial growth

Kruskal-Wallis test was carried out to see the difference/significance. The results of the *Kruskal-Wallis* test obtained a *p-value* of 1,000 (> 0.05), which means it can be concluded that there is no significant or significant difference in both MIC and MBC.

DISCUSSION

The results of the research that have been carried out show that at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56% there is color turbidity and bacterial growth in all groups of experimental samples. The results of the calculations that have been carried out on the Kruskal-Wallis statistical test produce a probability *p-value* of 1,000 (> 0.05) which can be concluded that the results are not significant or the hypothesis is rejected. The results showed that the infusion of lemongrass stems could not inhibit the growth of *S. mutans*. This does not support the theory that lemongrass has antibacterial activity that is effective in inhibiting the viability of bacteria, one of which *is S. mutans*.

The theory that has been put forward states that lemongrass has antibacterial, antimicrobial, antifungal, and anti-inflammatory active compounds.[13] In the fraction contained in antibacterial active compounds, the active compound fraction will attack bacterial cell components that have nucleic acid proteins, enzymes, membranes. semipermeable, and cell walls.[16] If the active fraction compounds attack one of the cell components, it will cause damage to bacterial cells, causing inhibition of bacterial growth.[16] Phytochemical analysis of lemongrass showed the presence of alkaloids, carbohydrates, saponins, reducing sugars, steroids, tannins, glycosides, proteins, flavonoids, resins, oils and terpenoids.[14,17,18,19] Flavonoids and terpenoids has inhibiting the growth of bacterial viability.[11, 19]

The content of specific flavonoids such as kaempferol and apigenin flavones are inhibitory effects on Gtfs in *S. mutans*.[20] Apigen provides activity against *streptococcal* membranes by increasing proton permeability and inhibiting acid production by *S. mutans* in biofilms.[21] Terpenoids have antibacterial effects that show inhibitory activity.[11,18,19] Terpenoids have a general structure consisting of patchouli alcohol (PA), andrographolide, and oleanolic acid.[22] Patchouli Alcohol exhibits a bactericidal effect of PA depending on time and dose under different pH conditions, and MIC.[22] Andrographolide is a diterpene lactone compound that has a significant inhibitory effect on *P. aeruginosa* biofilms and a synergistic antibacterial effect with azithromycin,in addition it also showed potential antibacterial activity against most of the Gram-positive bacteria, of which the most sensitive to the growth of *S. aureus*.[22] The acid showed that oleanolic acid had a certain inhibitory effect on *S. aureus* and *S. mutans*.[22]

Infusion stems of *C. citratus* did not show antibacterial activity against bacterial isolates.[23] This is in line with phytochemical screening and plant extract where it was stated that plant extraction using other organic solvents tended to show more consistent antibacterial activity compared to using water as a solvent for extraction.[23] Water-soluble phenolics exhibit antioxidant properties rather than antibacterial properties.[23] The low of phytochemicals in *C. citratus* indicates that the active constituents contribute to the antibacterial activity of *C. citratus* cannot be extracted with water, which is different from methanol extracts which have antibacterial properties.[9,23]

ETHICAL APPROVAL

This study received approval from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine, Diponegoro University with No.117/EC/H/FK-UNDIP/XI/2020.

CONCLUSION

The results of research conducted on the effect of lemongrass infusion on the growth of *S. mutans*, that lemongrass infusion did not affect the

growth of *S. mutans*, so it can be concluded that the lemongrass infusion does not contain phytochemicals that have an antibacterial effect.

SUGGESTION

Suggestions if further research is carried out, it is necessary to extract with other solvents that can bring out the antibacterial phytochemical content and there should be research on the phytochemical test of the content of the infusion of lemongrass stems qualitatively and quantitatively, so that the compounds and levels of compounds contained can be known.

REFERENCES

1. Pratiwi, N. L. The Trend Analysis of The Availability of Dental Caries and Dental Health Personnel in Indonesia. Dentistry. 2016; 6(360):1-5.
2. Badan Penelitian dan Pengembangan Kesehatan. Hasil Utama Riskesdas 2018. Jakarta: Kementerian Kesehatan Republik Indonesia. 2018;93-96.
3. Mitrakul, Kemthong, Ratchapin Srisatjaluk, Vimol Srisukh, Pattamapan Lomarat, Kadkao Vongsawan, and Theerachai Kosanwat. *Cymbopogon Citratus* (*Lemongrass Oil*) Oral Sprays as Inhibitors of *Mutans Streptococci* Biofilm Formation. J Clin Diagn Res. 12. 2018 Desc 1; 12(12):6-10.
4. Veiga, Nelio, Daniela Aires, and Filipa Douglas. Dental caries: A review. J Dent Oral Hyg 3.1. 2016; 2(5);1-2..
5. Yon, M. J. Y., Gao, S. S., Chen, K. J., Duangthip, D., Lo, E. C. M., and Chu, C. H. Medical Model In Caries Management. Dent J (Basel). 2019; 7(2);37:2-8.
6. Alejandra, Bojanich María, and Orlietti Mariano Daniel. Virulence Factors of *Streptococcus mutans* Related to Dental Caries. IntechOpen. 2020 Mar 11; 1-17.
7. Dianawati, Nur, Wahyu Setyarini, Ira Widjiastuti, Rini Devijanti Ridwan, and K. Kuntaman. The Distribution of *Streptococcus mutans* and *Streptococcus sobrinus* in Children With Dental Caries Severity Level. Dent J. 2020; 1(53): 36-39.
8. Adi, Prasetyo, Ambar Puspitasari, and Murni Ukhuwah. Pengaruh Konsentrasi Rebusan Kelopak Bunga Rossella terhadap pH Saliva Buatan. Majalah Kedokteran Gigi Indonesia 1.2. 2015; 156-160.
9. Adiguna, Prananda, and Oedijani Santoso. Pengaruh Ekstrak Daun Serai (*Cymbopogon citratus*) pada Berbagai Konsentrasi Terhadap Viabilitas Bakteri *Streptococcus mutans*. Jurnal Kedokteran Diponegoro. 2017; 6(4): 1-9.
10. Ortega-Cuadros, Mailen, Adriana Patricia Tofiño-Rivera, Luciano José Merini, and María Cecilia Martínez-Pabón. Antimicrobial Activity of *Cymbopogon citratus* (*Poaceae*) on *Streptococcus mutans* Biofilm and its Cytotoxic Effects. Revista de Biología Tropical 66. 2018; 1-4.
11. Izah, Sylvester Chibueze, and Ebinyo R. Aseibai. Antibacterial and Synergistic Activities of Methanolic Leaves Extract of Lemon Grass (*Cymbopogon citratus*) and Rhizomes of Ginger (*Zingiber officinale*) against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. Microbiology. 2018; 1: 26-30.
12. Aćimović, Milica, Ivana Čabarkapa, Mirjana Cvetković, Jovana Stanković, Biljana Kiprovski, Sonja Gvozdenac, and Nikola Puvača. *Cymbopogon citratus* (DC.) STAPH: Chemical Composition, Antimicrobial and Antioxcidant Activities, Use In Medinical And Cosmetic Purpose. Departement of Engineering Management in Biotechnology Faculty of Economics and Engineering Management University Business Academy Serbia. 2019 Aug 15; 2(6): 344-360.
13. De Silva, B. C. J., Won-Gi Jung, Sabrina Hossain, S. H. M. P. Wimalasena, H. N. K. S. Pathirana, and Gang-Joon Heo. Antimicrobial Property of Lemongrass (*Cymbopogon citratus*) Oil Against Pathogenic Bacteria Isolated From Pet Turtles. Laboratory animal research 33. 2017; 2: 84-91.
14. Lin, Xibin, Azhar Rafique, Tuba Fayyaz, Waqas Bashir, M. Luqman, Faisal Maqbool Zahid, and Kebin Zhou. Appraisal of *Cymbopogon citratus* (Lemon grass) for Antibacterial Activity of Against Uropathogens. 2020; 1-5.
15. Tofiño-Rivera, A., Mailen Ortega-Cuadros, D. Galvis-Pareja, Hedilka Jiménez-Rios, Luciano J. Merini, and María Cecilia Martínez-Pabón. Effect of Lippia Alba and *Cymbopogon citratus* Essential Oils on Biofilms of *Streptococcus mutans* and Cytotoxicity in CHO cells. J Ethnopharmacol. 2016; 194:749-754.

16. Erlyn, Putri. Efektivitas Antibakteri Fraksi Aktif Serai Terhadap Pertumbuhan Bakteri Streptococcus mutans. Fakultas Kedokteran Universitas Muhammadiyah Palembang. 2016: 6(2); 111-125
17. Oladeji, Oluwole Solomon, Funmilayo Enitan Adelowo, David Temitope Ayodele, and Kehinde Abraham Odelade. Phytochemistry and Pharmacological Activities of *Cymbopogon citratus*: a review. Scientific African 6. 2019; 6: 2-4.
18. Darmaga Campus, I. P. Potential of Lemongrass Leaves Extract (*Cymbopogon citratus*) as Prevention for Oil Oxidation. J Chem Pharm Res 7. 2015; 10: 55-60.
19. Garcia, R., Ferreira, J., P. Costa., Santos, T., Branco. F., Caramona. M., and Figueiredo, I. V. Evaluation of Inflammatory and Analgesic Activities of *Cymbopogon citratus* in Vivo-Polyphenols Contribution. Res J of Med Plant 9.1. 2015: 1-13.
20. Ren, Zhi, et al. "Inhibition of Streptococcus mutans polysaccharide synthesis by molecules targeting glycosyltransferase activity." Journal of oral microbiology 8.1. 2016: 31095; 4.
21. Gutiérrez-Venegas, Gloria, et al. "Effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque." Heliyon 5. 2019: 12; 1-5.
22. Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., and Yu, X. "Advances in Pharmacological Activities of Terpenoids." Natural Product Communications 15. 2020: 3; 1-13
23. Subramaniam, Geetha, Xin Ying Yew, and Lalita Ambigai Sivasamugham. Antibacterial activity of *Cymbopogon citratus* against clinically important bacteria. J Chemi Eng. 2020:29.