Inhibitorty Activity of Lemongrass (*Cymbopogon Nardus*, (L.) *Rendle*) Essential Oils on The Growth of Bacterial *Phorpyromonas Gingivalis* and *Aggregatibacter Actinomycetemcomitans* In Vitro

Kosno Suprianto, Emriadi**, Ade Ismail Abdul Kodir***, Dian Lestari*****

**Department of Periodontic, Faculty of Dentistry, Andalas University*
***Department of Chemistry - Andalas University*
****Department of Periodontic, Faculty of Dentistry, Sultan Agung Islamic University*
***** Dentistry Study Program, Faculty of Dentistry, Andalas University*

***Correspondence*: skosno@yahoo.com**

Received 17 July 2024; 1st revision 13 August 2024; 2st revision 10 December 2024; Accepted 31 December 2024; Published online 31 December 2024

Keywords:

Aggregatibacter actinomycetemcomitans, inhibitory power, lemongrass essential oils, Porphyromonas gingivalis

ABSTRACT

***Background**: Periodontal disease is one of oral and dental health problems that suffered by many people in the world and occurs in 50% of adult population. Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans are the main bacteria that cause periodontitis. One of the plants that is often used as an alternative antimicrobial agent is citronella (Cymbopogon nardus L.) which contains citronellal and geraniol compounds. This study aims to determine whether the essential oils of lemongrass leaves have inhibitory effect on the growth of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in vitro.*

***Method**: The production of lemongrass essential oils is carried out by the distillation method and 25%, 50%, and 75% concentration of essential oils are diluted using Tween 20 and distilled water. The inhibition test was carried out by the Kirby-Bauer disk diffusion method on Mueller Hinton Agar media.*

***Result**: The analysis showed that there was a significant difference between the inhibitory power produced by each concentration of essential oil of citronella leaf on the growth of Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.*

***Conclusion**: The conclusion of this study is the essential oils of lemon grass with concentrations of 25%, 50%, 75%, and 100% have different inhibitory properties in inhibiting the growth of Porphyromonas gingivalis bacteria and Aggregatibacter actinomycetemcomitans.*

doi: http://dx.doi.org/10.30659/odj.11.2.359-365
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Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: *Suprianto et al*. Inhibitorty Activity of Lemongrass (Cymbopogon Nardus, (L.) Rendle) Essential Oils on The Growth of Bacterial Phorpyromonas Gingivalis and Aggregatibacter Actinomycetemcomitans In Vitro. Odonto: Dental Journal, v.11, n.2, p. 359-365, December 2024.

INTRODUCTION

Periodontitis is a destructive inflammatory disease that occurs in the supporting tissues of the teeth due to the presence of specific microorganisms[1,2] . The severity of periodontal tissue damage is related to changes in the composition, potentially pathogens microorganisms of the plaque, host resistance, and the form of surrounding tissue[3]. Periodontitis occur from exposure to periodontal tissue by dental plaque bacteria. The most common types of bacteria found in periodontitis are *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Bacteroides forsythus*. *Porphyromonas gingivalis* is often found in chronic periodontitis, whereas *Aggregatibacter actinomycetemcomitans* is often found in aggressive periodontitis[4].

Management of periodontitis is performed with mechanical cleaning (scaling and root planning) to remove deposits in the form of plaque and subgingival calculus that cause damage to periodontal tissue[5]. The combination of antimicrobial therapy and mechanical therapy gives more effective results compared to mechanical therapy only. The application of metronidazole gel as an additional therapy after scaling and root planning provides effective results[6].

Natural ingredients such as extracts and plant essential oils can be used as alternative microbial agents[7]. One of the plants that is often used as an alternative antimicrobial agent is citronella (Cymbopogon nardus L.). This plant is often used for the treatment of neurological and gastrointestinal disorders, and as an antispasmodic, analgesic, antibacterial, and antipyretic[7,8]. Antibacterial compounds contained in essential oils of citronella are citronellal, geraniol, linalool, and α-terpineolIn a study conducted by Poelongan, it was found that essential oils of lemongrass leaves also showed inhibition zones to the growth of *E. coli* bacteria and *Staphylococcus aureus* at a concentration of 25% weight / volume[9]. The purpose of this study was to determine whether the essential oils of lemongrass leaves have inhibitory activity on the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* in vitro.

RESEARCH METHOD

This type of research is experimental laboratories (true experimental) research using a research design in the form of a posttest only control group design. The research samples were pure cultures of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* obtained from the Microbiology Laboratory of the Faculty of Medicine, Andalas University.

Making Lemongrass Essential Oil

The lemongrass leaves used in this study were obtained from Kuranji, Padang, West Sumatra. The procedure for making this essential oil is carried out by collecting 500 grams green lemongrass leaves that are 3-6 months old, then cleaned by washing them under running water to remove dirt attached to the leaves. Furthermore, the leaves are dried by leaving them in the open air without being exposed to direct sunlight for five days.

Prepare the *clevenger apparatus*. Put 100 grams dried lemongrass leaves and 200 ml water, into the distillation flask. Essential oils that come out are collected in glass bottles. Repeat the distillation stage until enough essential oil is obtained.

Making Lemongrass Essential Oil Concentration Series

Pure essential oils are distilled lemongrass oils with a concentration of 100%. Concentrations of essential oils are made with the formula:

$$V_1.C_1=V_2.C$$

V1: Volume of essential oil needed for dilution

C1: Initial concentration

V2: Volume of results

C2: Concentration of results

Essential oils are dissolved with sterile distilled aquades to obtain the concentration series of 25%, 50%, and 75%. So that essential oils can dissolve completely in distilled water, add 5µL of tween 20.

Antibacterial Test

This study uses paper discs diffusion method. Disc paper is made by 3 layers of Whatman filter paper which is glued and then cut it with 2.5 mm diameter paper punch to obtain a round paper discs, then sterilized using an autoclave at 121ºC for 15 minutes. Sterile paper disc was soaked with 10 µl citronella essential oils 25%, 50%, 75%, 100% concentration and Tween 20 as control, using a micropipette. Placed the paper discs on Mueller Hinton Agar which has been smeared by *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. In each variable group three replications were performed. Observations were made after the samples were incubated for 24 hours at 37 ℃ temperature to see the inhibition zone that formed around the paper disc, then the diameter of the inhibition zone was measured with calipers. Bacterial growth inhibition zone diameters are measured using the formula:

$$\left| D = \frac{(D_1 - D_3) + (D_2 - D_3)}{2} \right|$$

D = inhibition zone diameter (mm)

D_1 = diameter of vertical inhibition zone (mm)

D_2 = diameter of horizontal inhibition zone (mm)

D_3 = disc paper diameter (mm)

RESULTS

Essential oils of lemongrass leaves have inhibitory effect on the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Measurement results of the average diameter of the inhibited zone formed can be seen in table 1.

Table 1. Average diameter of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* inhibitory zones

		Inhibitory Zone Diameter (mm)	
Variable	**n**	***Porphromonas***	***Aggregatibacter***

		gingivalis	*actinomycetemcomitans*
Concentration 100%	3	8,42± 1,69	9,37 ± 0,78
Concentration 75%	3	4,77 ± 0,78	7,33 ± 1,70
Concentration 50%	3	3,50 ± 0,48	5,50 ± 1,55
Concentration 25%	3	1,92 ± 0,68	3,53 ± 1,13
Tween 20	3	0,43 ± 0,37	0,02 ± 0,03

According to Davis and Stout in 1971, the inhibitory properties of bacteria based on the diameter of the inhibition zone are grouped as follows:

1. Very strong; inhibitory zone diameter> 20 mm
2. Strong; inhibitory zone diameter between 10-20 mm
3. Moderate; inhibitory zone diameter between 5-10 mm
4. Weak; inhibitory zone diameter <5 mm

Based on the classification above, essential oils of lemongrass that form the largest inhibitory zone of the two bacteria are essential oils with concentration of 100%, which is included in the moderate group.
One way ANOVA parametric test was performed to see the difference in the average inhibition diameter of each concentration on the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

Table 2. One way ANOVA test results on each control group

	One way ANOVA		
	Df	**Mean**	**P**
Porphyromonas gingivalis	4	27,91	0,000
Aggregatibacter actinomycetemcomitans	4	38,72	0,000

The table above shows the value of p <0.05 which means there is a significant difference on each concentrations of citronella essential oils on inhibiting the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. The difference in inhibition of each group can be determined by conducting a Post-Hoc LSD analysis. The results of Post-Hoc LSD analysis can be seen in table 3.

Tabel 3. *Post Hoc LSD* test result

Group		**Citronella Essential Oil**				
Bacteria	**Concentration**	**Tween 20**	**25%**	**50%**	**75%**	**100%**
Porphyromonas gingivalis	Tween 20	-	0,078*	0,002	0,000	0,000
	25%		-	0,062*	0,004	0,000
	50%			-	0,124*	0,000
	75%				-	0,001

Aggregatibacter actinomycetemcomitans	Tween 20	-	0,005	0,000	0,000	0,000
	25%		-	0,072*	0,003	0,000
	50%			-	0,090*	0,003
	75%				-	0,064*

The results of Post-Hoc LSD test (Least Significant Difference) showed that there were significant ($p < 0.05$) differences on the inhibition zone in some groups and not significant ($p > 0.05$) differences in others. To compare the inhibitory properties of *Porphyromonas gingivalis* bacteria and *Aggregatibacter actinomycetemcomitans* bacteria at each concentration of lemongrass essential oils, the unpaired T test was conducted. The results of the unpaired T test can be seen in table 4.

Table 4. Results of the unpaired T test of the two bacteria on the same concentration

Concentration	Difference in Average	p
25%	1,616	0,101
50%	2,000	0,100
75%	2,567	0,076
100%	0,950	0,425

DISCUSSION

Table 1 shows that the essential oils of lemon grass with concentrations of 25%, 50%, 75%, and 100% have inhibitory activity on the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Tween 20 as control also has inhibitory activity on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, because it contains 50% lauric acid which has the ability to inhibit bacterial growth by damaging the cell membranes so that it disrupts the cellular metabolism of bacteria. However, the inhibition caused by lauric acid in gram-negative bacteria is weaker than in gram-positive bacteria[10].

The active compounds contained in essential oils of citronella leaf are citronellal, geraniol, linalool, and α-terpineol[11]. Citronellal and geraniol are terpenoid compounds composed of carbon (C), hydrogen (H) and oxygen (O) with chemical formulas of $C_{10}H_{16}O$ and $C_{10}H_{18}O$. The terpenoid compounds in essential oils of citronella disturb the bacterial metabolic system by damaging the structure of cell walls and interfering with the active transport of bacteria[12]. These active compounds will denaturate and activate proteins so that there is a decrease in the permeability of bacterial cell walls that disrupt the transport of organic ions and cause disruption of bacterial cell metabolism[13].

Inhibition zones formed by each concentration of essential oils of citronella have different averages (table 2). The higher the concentrations of essential oil, the greater inhibitory effect occur on the growth of the bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. This is due to the presence of a dose dependent activity on essential oils of citronella against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, which means an increase in antibacterial activity in accordance with the increase on the concentration of essential oils[14]. The results of this study are in line with the results of a Poeloengan (2009)

study on the inhibition of citronella leaf essential oils on the growth of bacteria that cause clinical mastitis on cattle[13],[15]. The essential oil of citronella was tested on *Staphylococcus aureus, Staphylococcus epidermis*, and *Streptococcus agalactiae* with concentrations of 6.25%, 12.5%, 25%, and 50%, which showed that the greater the concentration, the greater the inhibition of bacteria[16].

Table 1 shows that the inhibition zones formed by essential oils of fragrant lemongrass on *Aggregatibacter actinomycetemcomitans* are greater than *Porphyromonas gingivalis* bacteria. This happen because *Aggregatibacter actinomycetemcomitans* bacteria are more sensitive to linalool and α-terpineol compounds that was found in essential oils of citronella. In accordance with the research of[4]. about the antimicrobial effect of linalool and α-terpineol on periodontopathic and cariogenic bacteria which states that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of linalool and α-terpineol compounds on *Aggregatibacter actinomycetemcomitans* are lower than MIC and MBC on *Porphyromonas gingivalis* bacteria.

CONCLUSION

Essential oils of *Cymbopogon nardus* have inhibitory activities on the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* in vitro.

ACKNOWLEDGEMENT

The authors are grateful to Andalas University for funding and supporting this research.

REFERENCES

1. Sedghi, L. M., Bacino, M. & Kapila, Y. L. Periodontal Disease: The Good, The Bad, and The Unknown. *Front. Cell. Infect. Microbiol.* **11**, 1–26 (2021).
2. Butera, A., Gallo, S., Pascadopoli, M., Taccardi, D. & Scribante, A. Home Oral Care of Periodontal Patients Using Antimicrobial Gel with Postbiotics, Lactoferrin, and Aloe Barbadensis Leaf Juice Powder vs. Conventional Chlorhexidine Gel: A Split-Mouth Randomized Clinical Trial. *Antibiotics* **11**, (2022).
3. Kwon, T. H., Lamster, I. B. & Levin, L. Current Concepts in the Management of Periodontitis. *Int. Dent. J.* **71**, 462–476 (2021).
4. Naghsh, N., Yaghini, J., Narimani, T. & Khodarahmi, E. Antimicrobial efficacy of antiplaque agents of common toothpastes against Porphyromonas gingivalis and Streptococcus oralis: An in vitro study. *Dent. Res. J. (Isfahan).* **21**, 1–5 (2024).
5. Maddi, A., Standards, D. & Committee, C. Management of Periodontal Disease Introduction. *Clin. Guidel. Progr.* 1–23 (2023).
6. Zúñiga-Loor, D., Parise-Vasco, J. M. & Montesinos-Guevara, C. Mechanical debridement combined with amoxicillin and metronidazole compared with mechanical debridement alone for the treatment of chronic periodontitis: An overview of systematic reviews. *Dent. Med. Probl.* **61**, 439–446 (2024).
7. Aprilia, R., Nurlela, N. & Susanty, D. Effectiveness of Lemongrass Extract (Cymbopogon citratus) in Hand Sanitizer Formulation Against Bacteria. *J. Sains Nat.* **13**, 176–182 (2023).
8. Primasari, V. S. & Syaharani, C. F. Potensi Minyak Esensial Serai Terhadap Bakteri Patogen Periodontal. *e-GiGi* **11**, 86–92 (2023).
9. Carvalho, Ê. S. *et al.* Anticariogenic Activity of Three Essential Oils from Brazilian Piperaceae. *Pharmaceuticals* **15**, 1–11 (2022).
10. Firdaus, R., Harryadi, C. I., 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 Heal.* **14**, 324–330 (2022).
11. Anwar, Y. & Siringoringo, V. S. Fractionation of Citronella Oil and Identification of Compounds by Gas Chromatography-Mass Spectrometry. *Pharm. Sci. Res.* **7**, 138–144 (2020).
12. Chouhan, S., Sharma, K. & Guleria, S. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines* **4**, 58 (2017).
13. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R. & De Feo, V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* **6**, 1451–1474 (2013).
14. Das, P., Dutta, S., Begum, J. & Anwar, M. N. Antibacterial and Antifungal Activity Analysis of Essential

Oil of Pogostemon cablin (Blanco) Benth. *Bangladesh J. Microbiol.* **30**, 7–10 (2016).
15. Trang, D. T. *et al.* Essential Oils of Lemongrass (Cymbopogon citratus Stapf) Induces Apoptosis and Cell Cycle Arrest in A549 Lung Cancer Cells. *Biomed Res. Int.* **2020**, (2020).
16. Aiemsaard, J., Borlace, G. N., Thongkham, E. & Jarassaeng, C. Antibacterial efficacy of essential oil spray formulation for post-milking disinfection in dairy cows. *Vet. World* **16**, 1552–1561 (2023).