ORIGINAL ARTICLE

Antibacterial test of *Peperomia pellucida* (L.) Kunth extract against *Porphyromonas Gingivalis* as a potential herb for periodontitis: a laboratory experiment

Dewi Lidya Ichwana Nasution[1,5*]
Sri Tjahajawati[2]
Ratna Indriyanti[3]
Amaliya Amaliya[4]
Rina Putri Noer Fadilah[1,6]
Rahman Mutiara[5]

[1]Doctoral Program Study of Dentistry, Universitas Padjadjaran
[2]Departement of Oral Biology, Faculty of Dentistry, Universitas Padjadjaran
[3]Departement of Pedodontia, Faculty of Dentistry, Universitas Padjadjaran
[4]Departement of Periodontology, Faculty of Dentistry, Universitas Padjadjaran
[5]Department of Periodontology, Faculty of Dentistry, Universitas Jenderal Achmad Yani
[6]Department of Public Health, Universitas Jenderal Achmad Yani

* Correspondence:
Dewi.ichwana@lecture.unjani.ac.id

Received: 25 July 2023
Revised: 07 November 2023
Accepted: 27 November 2023
Published: 30 November 2023
DOI: 10.24198/pjd.vol35no3.47856

p-ISSN 1979-0201
e-ISSN 2549-6212

Citation:
Nasution, DLI. Tjahajawati, S.Indriyanti, R. Amaliya, A. Fadilah, RPN. Mutiara,R. Antibacterial test of *Peperomia Pellucida* (L.) Kunth extract against *Porphyromonas Gingivalis* as a potential herb for periodontitis: a laboratory experiment. Padj J Dent, November. 2023; 35(3): 181-186.

ABSTRACT

Periodontitis is a multifactorial inflammatory disease which is generally caused by plaque accumulation. Many studies have shown that *Porphyromonas gingivalis* (Pg) is the main etiological agent that contributes to chronic periodontitis. Scaling and root planing (SRP) is the gold standard for periodontitis treatment. The use of antibiotics as additional agents accompanying the SRP procedure has limitations that can cause resistance to subgingival periodontal pathogens. *Peperomia pellucida* (L.) Kunth *i.e* betel leaf is a natural ingredient that contains anti-inflammatory, antibacterial and antioxidant properties. This study aimed to analyze the inhibition of *Peperomia pellucida* (L.) Kunth extract against Pg bacteria. **Methods**: The type of study used was an experimental laboratory with a Post-Test Control Group Design research design which was divided into 6 treatment groups using the disk diffusion method with concentrations of *Peperomia pellucida* (L.) Kunth extract 25, 50, 75 and 100%, sterile aquades as a negative control and Chlorhexidine as a positive control. Data analyses of One Way Anova and Post Hoc Tukey were used **Results:** *Peperomia pellucida* (L.) Kunth extract concentrations of 25, 50, 75, and 100% had an effect on reducing the growth of *Porphyromonas gingivalis* ($p \leq 0,05$), the average inhibition response was 14.40 mm at 25% concentration, 16.58 mm at 50% concentration and 19.30 mm at 75%, 21.88 mm at 100% concentration.**Conclusion**: *Peperomia pellucida* (L.) Kunth extract has an antibacterial effect against *Porphyromonas gingivalis* which has the potential to be used as a periodontitis herb.

KEYWORDS

Betel leaf extract *Peperomia pellucida* (L.) Kunth, periodontitis, *Porphyromonas gingivalis*

INTRODUCTION

Oral health is a part of general health that needs to be considered.[1] The main problem of dental and oral disease is caries and periodontal disease. According to Basic Health Research (RISKESDAS) in 2018, the prevalence of periodontitis in Indonesia was 74.1%.[2] From the results of this research, it appears that most of Indonesia's population has dental and oral health problems. Periodontal disease is a disease of the oral cavity whose prevalence and intensity is still high.[3] Periodontal tissue is a tooth supporting network consisting of soft and hard tissues. Periodontal tissue consists of the gingiva, cementum, periodontal ligament, and alveolar bone that supports the teeth.[4,5] The most common types of periodontal disease are periodontitis and gingivitis.[6] Gingivitis is an inflammation that affects the soft tissue around the teeth, namely the gingival tissue.[7] Periodontitis is a periodontal tissue disease that attacks tooth supporting tissues due to plaque accumulation.[8]

Several pathogenic bacteria cause periodontitis, including *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*, *Tannerella forsythia, Prevotella intermedia,* and *Treponema denticola*.[9] One of the pathogenic bacteria which is a normal flora is *Porphyromonas gingivalis* which is known to have a dominant prevalence of 96.2% in chronic periodontitis.[10] This bacterium can be found in the oral cavity in the area of the tongue, tonsils, subgingival plaque and gingival sulcus area.[11] *P. gingivalis* are rod-shaped obligate anaerobic gram-negative bacteria that produce black colonies on blood agar. *P.gingivalis* is the main etiology that is very influential in the severity of periodontal disease, especially chronic periodontitis.[12-15] Chronic periodontitis occurs due to colonization of bacterial subgingival plaque which can regulate the immune cell response due to the presence of virulence factors from these bacteria. *Porphyromonas gingivalis* has a virulence factor by

communication with other organisms that causes tissue damage and can cause inflammation. Attachment to the subgingival sulcus is stronger than that of other bacteria in patients with chronic periodontitis.[7,12,14]

Scaling and root planing are treatments that can be performed to eliminate pathogens of periodontal disease. This treatment has become the gold standard for the treatment of periodontal disease. Scaling and root planing (SRP) are sometimes not optimal because it is difficult to reach the surface of the tooth anatomy under the gums and only certain pathogens can be removed.[16,17]

The use of locally administered antimicrobials is a relatively recent addition in the management of periodontitis. Currently, additional therapy is given with chlorhexidine mouthwash which is often used by the people because it has anti-bacterial and anti-inflammatory effects and is also used for periodontal treatment.[11,18-23]

Chlorhexidine gluconate (CHX) is an antibacterial agent that has long been used as a topical antiseptic and antibacterial agent in medicine.[20,24] Prolong time usage will cause an unpleasant taste, sensation, give brown color to the teeth, increase the formation of calculus, and ulcers in the mucosa.[25]

Peperomia pellucida (L.) Kunth, which has long been known as a medicinal plant, may have the potential to have the antibacterial ability of P. gingivalis. Betel plant originated from America but is commonly found in Southeast Asia.[26] *Peperomia pellucida* is a wild plant that grows around the yard or in damp areas. This plant has anti-inflammatory, antibacterial, antioxidant, and analgesic properties. Antibacterial and antiseptic properties of betel leaves, namely saponins, flavonoids, tannins, alkaloids, and triterpenoids.[27] *Peperomia pellucida* (L.) Kunth has broad-spectrum antimicrobial activity on *Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans.*[28]

It has been widely used as an anti-inflammatory and antibacterial agent, but so far, no research has been found on the antibacterial ability of this plant against *P. gingivalis.*[28] Based on the description above, this study aimed to analyze the inhibition of *Peperomia pellucida* (L.) Kunth extract against Pg bacteria.

METHODS

This study was a laboratory experiment and the Post-Test Control Group Design. This research was conducted at the Microbiology Lab, Faculty of Dentistry, Airlangga University, Surabaya. The objects used were betel plant extracts made by the Biochemistry Laboratory Team of the Faculty of Dentistry, Jenderal Achmad Yani University.*Porphyromonas gingivalis* (ATCC 33277) obtained from the Microbiology Lab, Faculty of Dentistry, Airlangga University, Surabaya. Determination of the number of samples used the Federer formula.

This study was conducted to determine whether there were differences in the growth of *Porphyromonas gingivalis* given betel leaf extract (*Peperomia pellucida* (L.) Kunth with concentrations of 25, 50, 75 and 100%. This study used 6 treatment groups, namely, group 1: the negative group, only treated with *Aquades*; group 2: positive group, treated with 0.2% Chlorhexidine; group 3: treatment group with 25% betel leaf extract; group 4: treatment group with 50% betel leaf extract; group 5: treatment group with 75% betel leaf extract; group 6: treatment group with 100% betel leaf extract. Based on the calculations obtained from the formula, the number of repetitions needed was 4 repetitions for each treatment group.

The materials used in this study were betel leaf extract, *P.gingivalis* bacteria, BHI agar and BHIB, petridish, 70% ethanol, 70% alcohol, 0.9% NaCl, hermin, vitamin K, immersion oil, crystal violet, lugol, *Aquadest*, and 0.2% *Chlorhexidine* (CHX). This study used betel leaves obtained from the Percikan Iman Arjasari plantation, Bandung Regency, West Java. Then a determination test of betel leaves was carried out at the National Research and Innovation Agency, Cibinong Bogor. As much as 250 grams of leaves, stems and flowers were taken and then dried at room temperature (not exposed to direct sunlight) for ± 2 days to reduce the water content. 70% ethanol extract of betel leave (*Peperomia pellucida* (L.) Kunth) was prepared by maceration method, as follows:[29,30] Prepare 250 grams of betel leaves, then clean and rinse with water, then mash and put in the oven until dry; After drying, blend the betel leaves to a powder, then macerate the betel leaf powder using 70% ethanol solvent ; Leave it for 3 days; After 3 days, filter using filtration paper then evaporate using a rotary vacuum evaporator; Betel leaf extract is ready to use; The concentrations used in inhibition test were 25, 50, 75 and 100%; concentration was made with 2 ml of distilled water, positive control with 0.2% chlorhexidine, and negative control with distilled water.[31]

Steps: Prepare BHI agar enriched with hemin and vitamin K as preparation of agar media for *Porphyromonas gingivalis* bacterial culture. Mix BHI agar 4.7 grams with 100 mL *Aquadest* into an Enlenmeyer tube then add 50 µl hermin and µl vitamin K. Then cover the media using aluminum foil and sterilize using autoclave at 121°C for 15 minutes. After that, put it in a sterilized petri dish with a diameter of 10 cm and a thickness of 4 mm, and wait for it to solidify.[32]

The bacterial suspension was prepared according to the turbidity standard of 0.5 Mc Farland (1,5 x 10^8 CFU/ml) where 1 loop of *Porphyromonas gingivalis* bacteria was taken in a sterile loop. It was dissolved in 2 ml of BHIB liquid medium in a test tube. Steps: Put the test tube into the desiccator and incubate at 37°C for 24 hours. Dilute by adding distilled water and homogenize in a centrifuge. Measure the absorbance and match with a standard turbidity of 0.5 McFarland with an absorbance of 0.05 and a wavelength of 560 nm using a spectrophotometer.[33]

Re Identification of *P. gingivalis* was carried out by gram staining.[34,35] Then it was marked with 3 types of labels, namely the code K+ for positive control (*Chlorhexidine* 0.2%), K- for negative control (*Aquades*), and betel leaf extract with concentrations of 25, 50, 75 , and 100%.

The inhibition test procedure on *P. gingivalis* was carried out using disk diffusion method.[9,36] A stock solution of extract was prepared by dissolving 0.1 g of extract with 100 mL of distilled water to produce a final concentration of 100 mg/mL. The stock solution was then diluted to concentrations of 25, 50,75 and 100 mg/mL of extract. 20 µl of each dilution was impregnated into sterile, blank disks 6 mm in diameter. 5 µl of extract was spotted alternately on both sides of the disks and allowed to dry before the next 5 μL was spotted to ensure precise impregnation. Aquades-loaded disks were used as negative controls and the positive controls used were Chlorhexidine gluconate 0,2%. All disks were fully dried before the application on the bacterial lawn. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the disks. The assay was repeated four times. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract. The statistical difference of the mean zone of inhibition of the extract was carried out by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test at a significance level of $p<0.05$.

RESULTS

To Determine the antibacterial activity of *Peperomia pellucida* (L.) Kuth extract to *P.gingivalis* the disk diffusion method was used. The results of the study can be seen in the table below.

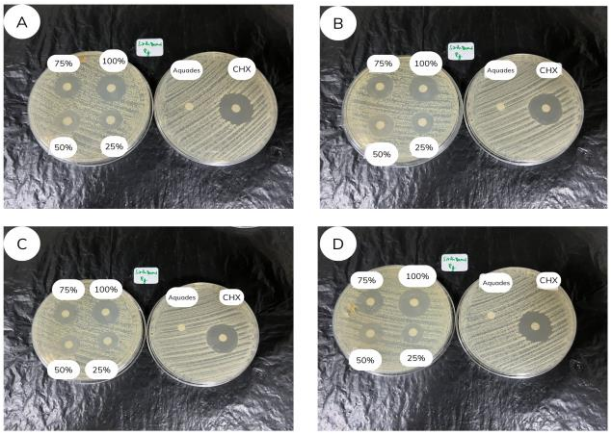

Figure 1: Zones of inhibition produced by *Peperomia pellucida* (L.) Kunth extract 25%, 50%, 75% and 100%, sterile aquades as a negative control and Chlorhexidine as a positive control against *P.gingivalis* in disk diffusion test. A-D: Repetition of the test from first to fourth

Table 1. Zone of inhibition (mm) against *P.gingivalis*

Group	Zone of Inhibition	Mean	SD	Min-Max
Aquades	0 0 0	0	0	0-0
Chlorhexidine	25.69 25.72 24.36 25.05	25.21	0.64	24.36-25.72
25% Concentration of extract	14.76 14.84 13.60 14.40	14.40	0.57	13.60-14.84
50% Concentration of extract	16.89 16.80 15.92 16.72	16.58	0.45	15.92-16.89
75% Concentration of extract	19.16 19.20 18.86 19.98	19.30	0.48	18.86-19.98
100% Concentration of extract	21.64 22.26 21.20 22.40	21.88	0.56	21.20-22.40

Based on the table above, for betel leaf extract, the highest average is at a concentration of 100%, which is 21.88 mm with a minimum value of 21.20 mm and a maximum value of 22.4 mm and the lowest average at a concentration of 25% that is equal to 14.40 mm with a minimum value of 13.60 mm and a maximum value of 14.84 mm. Meanwhile, the positive control for Chlorhexidine had an average of 25.21 mm and the negative control for Aquades had an average of 0.00 mm.

Table 2. Comparison between treatments with Post Hoc Test

Comparison	p-value	Interpretation
Aquades with chlorhexidine	0.000	There is a difference
Aquades with 25%	0.000	There is a difference
Aquades with 50%	0.000	There is a difference
Aquades with 75%	0.000	There is a difference
Aquades with 100%	0.000	There is a difference
Chlorhexidine with 25%	0.000	There is a difference
Chlorhexidine with 50%	0.000	There is a difference
Chlorhexidine with 75%	0.000	There is a difference
Chlorhexidine with 100%	0.000	There is a difference
25% with 50%	0.000	There is a difference
25% with 75%	0.000	There is a difference
25% with 100%	0.000	There is a difference
50% with 75%	0.000	There is a difference
50% with 100%	0.000	There is a difference
75% with 100%	0.000	There is a difference

The results of zone of inhibition analysis between each extract treatment for each concentration, positive and negative controls using one-way ANOVA showed that the probability value (p-value) was 0.000 (<0.05), which means there was a significant difference between each treatment group, to find out more about the comparison between concentrations of betel leaf extract, a post hoc tukey test was carried out which can be seen in Table 2. The post hoc test in comparing between concentrations of antibacterial power between betel leaf extract, the results showed that there was a significant difference between the treatment groups of betel leaf extract at concentrations of 25, 50, 75, and 100% and control (+) and control (-) ($p<0.05$) (Table 2.).

DISCUSSION

Peperomia pellucida (L.) Kunth has demonstrated broad-spectrum antibacterial activity against gram-positive bacteria*;* such as *Staphylococcus aureus, Bacillus subtilis,* and gram-negative bacteria; such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiellae pneumonae, Salmonellae typhi, Enterobacter aerogenes, Edwardsiella tarda, Flavobacterium sp., Aeromonas hydrophila, Vibrio cholera, Vibrio alginolyticus, and Vibrio parahaemolyticus.*[28]

From the research results (figure.1 and table.1)*, Peperomia pellucida* (L.) Kunth extract with a concentration of 25, 50, 75, and 100%, Chlorhexidine, Aquades was able to inhibit the growth of *P. gingivalis* bacteria, which is a gram-negative bacterium. It can be seen that the average inhibition response was 14.40 mm at 25% concentration, 16.58 mm at 50% concentration and 19.30 mm at 75%, 21.88 mm at 100% concentration, 25.21 mm for positive control (CHX) and 0.00 mm for negative control (table.1). The higher the concentration of the extract, the wider the inhibition zone formed. This study is in accordance with a study by Zubair et al.,[31] the inhibition of bacterial growth of all strains by the hexane, chloroform, ethyl acetate, ethanol, and water extracts of leaves of *Peperomia pellucida* (L.) Kunth was dose-dependent as evident by the higher zone of inhibitions at higher concentrations.

In addition, the results carried out by Edi et al.,[37] showed an average inhibition test using henna leaf extract at a concentration of 25%, namely 7.64 mm, 50% concentration, namely 9.24 m, 75% concentration, namely 12 .30 mm, and 100% concentration with 16.37 mm. Meanwhile, the positive control for Chlorhexidine 0.2% was 18.76 mm and the negative control was 0%. This indicates a strong inhibition zone against *P. gingivalis.*

The antibacterial ability of *Peperomia pellucida* (L.) Kunth can be caused by the compounds contained in this plant. The structure and pharmacological active principles of *Peperomia pellucida* (L.) Kunth leaves have been elucidated based on chromatographic and spectroscopic methods using Nuclear Magnetic Resonance (NMR) and Mass Spectroscopy (MS) techniques. The phytochemicals in this plant are alkaloids, including secolignans, tetrahydrofuranlignans, as well as dihydronaphthalenone, peperomins A, B, C, and E, sesamin, and isoswertisin. *Peperomia pellucida* (L.) Kunth also contains several essential oils, especially dillapiole, β-caryophyllene and carotol which have high larvicidal activity. Other compounds are flavonoids such as acacetin, apigenin, isovitexin, pellucidatin, and anthraquinone.[28,31]

The mechanism of action of flavonoids against cell damage is by destroying the proteins that make up the cytoplasm by affecting the permeability of the cell membrane and cytoplasm of bacteria, thus triggering ion imbalances in the cell. Tannins have the ability to inhibit transcriptase so that cells cannot synthesize proteins and then bacteria lyse and die.[38] Saponins in rambai leaf extract are able to disrupt the integrity of the cell membrane, causing a decrease in the tension in the cell wall structure which leads to cytoplasmic leakage and cell death. Alkaloids have a mechanism of action as

an antibacterial by disrupting the components that make up peptidoglycan in bacterial cells resulting in cell wall lysis in the bacterial cell wall layer.[38,39]

The content of triterpenoids of *P.pellucida* can cause damage to lipid membranes resulting in liposome leakage. Steroids have permeable characteristics to lipophilic compounds so that they are able to interact with phospholipid membranes which have an impact on decreasing cell integrity and changes in cell membrane structure which in turn causes *P. gingivalis* bacterial cells to lyse and die. The presence of several ingredients found in betel leaf *(Peperomia pellucida* (L.) Kunth) can inhibit the growth of gram-positive and negative bacteria.[40]

The Post Hoc test from table 2 showed that there was a significant difference between each treatment. This is in line with the research conducted by Sarwo, et al.,[40] which showed that the results of the inhibition of henna leaf extract 25, 50, 75, and 100% on the growth of *P.gingivalis* in vitro was a significant difference. There are several factors that can cause the formation of inhibition zone, namely the content of active compounds in the extract used, the concentration of the extract and the type of bacteria.[42]

Chlorhexidine gluconate 0.2% showed the ability to inhibit and kill bacterial growth, because it has the ability to damage the bacterial cell wall and then entering the cytoplasm and attacking the cell nucleus.[43,44] Lipopolysaccharide of Gram negative bacteria will bind to the cationic molecules of chlorhexidine thereby limiting and reducing its effectiveness.[45]

The present study showed the antibacterial efficacy of the *Peperomia pellucida* (L.) Kunth extract. Though, it requires further assessment whether the disk diffusion test method could be improved or whether susceptibility testing using an MIC-based method. *Peperomia pellucida* (L.) Kunth has a potential future direction in developing promising therapeutic agents for periodontitis chronic adjunctive therapy. Various periodontal pathogen bacteria types need to be investigated to confirm whether *Peperomia pellucida* (L.) Kunth is a potential material to deal with periodontitis treatment.

Peperomia pellucida (L.) Kunth in this study was extracted using the conventional maceration method which needs to be developed with other solvents or modern extraction techniques to obtain sufficient quality and quantity of bioactive molecules.

CONCLUSION

Peperomia pellucida (L.) Kunth extract has an antibacterial effect against *Porphyromonas gingivalis* which has the potential to be used as a periodontitis herb.

Acknowledgement: Our gratitude goes to LPPM UNJANI
Author Contributions: Conceptualization, D.N and RM.; methodology, D.N and R.M; software, R.F and R.M.; validation, D.N, R.F and R.M; formal analysis, R.F; investigation, D.N; resources, D.N.; data curation, D.N; writing original draft preparation, D.N writing review and editing, D.N.; visualization, D.N; supervision, R.F; project administration, R.M.; funding acquisition, D.N. All authors have read and agreed to the published version of the manuscript.", please turn to the CRediT taxonomy for the term explanation.
Institutional Review Board Statement: "The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Komisi Etik Penelitian Universitas Padjadjaran (KEP Unpad) with code No: 1338/UN6.KEP/EC/2022 for studies not involving humans or animals.
Informed Consent Statement: The research was "Not applicable" for studies not involving humans and animals.
Data Availability Statement: We provide details data supporting reported results can be found at https://drive.google.com/file/d/14rm11lcR1WsojGFqF_D3aFR-uZ7-Yi_u/view?usp=sharing
Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Anang A, Robbihi HI. Hubungan Pengetahuan dengan Perilaku Kesehatan Gigi dan Mulut. JI-KES 2021;4(2):55–9.
2. Badan Penelitian dan Pengembangan Kesehatan. Laporan Nasional RISKESDAS 2018. Lembaga Penerbit Badan Penelitian dan Pengembangan. 2018. p. 204.
3. Nariratih D, Rusjanti J, Susanto A. Prevalence and characteristics of aggressive periodontitis. Padj J Dent. 2011;23(2):97–104. DOI : 10.24198/pjd.vol23no2.14020
4. Velsko IM, Fellows Yates JA, Aron F, Hagan RW, Frantz LAF, Loe L, et al. Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. Microbiome.2019 Jul 6;7(1).DOI : 10.1186/s40168-019-0717-3
5. Kwon TH, Lamster IB, Levin L. Current concepts in the management of periodontitis. Int Dent J. 2021;71(6):462-76.DOI : 10.1111/idj.12630
6. Frédéric LJ, Michel B, Selena T. Oral microbes, biofilms and their role in periodontal and peri-implant diseases. Vol. 11, Materials. MDPI AG; 2018. DOI : 10.3390/ma11101802
7. Abdulkareem AA, Al-Taweel FB, Al-Sharqi AJB, Gul SS, Sha A, Chapple ILC. Current concept pathogenesis of periodontitis: from symbiosis to dysbiosis. J Oral Microl. 2023;15(1):2197779.DOI: 10.1080/20002297.2023.2197779
8. Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. Vol.84, Periodontology 2000. Blackwell Munksgaard; 2020. P.14-34 DOI: 10.1111/prd.12331
9. Hans VM, Grover HS, Deswal H, Agarwal P. Antimicrobial efficacy of various essential oils at varying concentrations against periopathogen Porphyromonas gingivalis. 2016;10(9):ZC16–ZC19. DOI: 10.7860/JCDR/2016/18956.8435
10. Saquib AS,AlQahtani NA, Ahmad I, Arora S, Asif SM, Javali MA, Nisar N. Synergistic antibacterial activity of herbal extracts with antibiotics on bacteria responsible for periodontitis. J Infect Dev Ctries. 2021 Nov 30;15(11):1685-1693. DOI: 10.3855/jidc.14904
11. Dwipriastuti D, Putranto RR, Anggarani W. Perbedaan Efektivitas Chlorhexidine Glukonat 0,2% Dengan Teh Hijau (Camellia Sinensis) Terhadap Jumlah *Porphyromonas Gingivalis*. ODONTO Dent J. 2017;4(1):50.DOI : 10.30659/odj.4.1.50-54
12. How KY, Song KP, Chan KG. *Porphyromonas gingivalis:* An overview of periodontopathic pathogens below the gum line. Front Microbiol. 2016;7(FEB):1–14.DOI : 10.3389/fmicb.2016.00053
13. Shaikh HFM, Patil SH, Pangam TS, Rathod KV. Polymicrobial synergy and dysbiosis: An overview. Vol. 22, Journal of Indian Society of Periodontology. Medknow Publications; 2018. p. 101–6. DOI: 10.4103/jisp.jisp_385_17
14. Palm E, Khalaf H, Bengtsson T. *Porphyromonas gingivalis* downregulates the immune response of fibroblasts. BMC Microbiol.

2013;13(1):5–7.DOI :10.1186/1471-2180-13-155
15. Kuboniwa M, Hendrickson EL, Xia Q, Wang T, Xie H, Hackett M, et al. Proteomics of *Porphyromonas gingivalis* within a model oral microbial community. BMC Microbiol. 2009;9:1–14.DOI: 10.1186/1471-2180-9-98
16. Marchesan J, Girnary Ms, Jing L, Miao MZ, Zhang S, Sun L, et al. An experimental murine model to study periodontitis. Nat rotoc. 2018 Oct 1; 13(10):2247-67. DOI: 10.1038/s41596-018-0035-4
17. Vyas M, Vyas S. Assessment of Tetracycline as an Adjunct to Scaling and Root Planing in Periodontitis Patients. Int J Contemp Med Res Int J Contemp Med Surg Radiol. 2018;3(1):126–8. Avalible https://www.ijcmsr.com/ijcmsr_3_1_58_v1_2.pdf
18. Komala ON, Suwandi T. Pengaruh Chlorhexidine Chip Pada Perawatan Periodontal: Sebuah Meta – Analisis. B-Dent J Kedokt Gigi Univ Baiturrahmah. 2021;8(3):226–34.DOI: 10.33854/jbd.v8i2.861
19. Siddarth M, Singh P, Gupta R, Sinha A, Shree S, Sharma K. Comparative evaluation of subgingivally delivered 2% curcumin and 0.2% chlorhexidine gel adjunctive to scaling and root planing in chronic periodontitis. Photodiagnosis Photodyn Ther. 2020;29:101665. https://pubmed.ncbi.nlm.nih.gov/32690830/
20. Sastravaha G, Gassmann G, Sangtherapitikul P, Grimm WD. Adjunctive periodontal treatment with Centella asiatica and Punica granatum extracts in supportive periodontal therapy. J Int Acad Periodontol. 2005;7(3):70–9. https://pubmed.ncbi.nlm.nih.gov/16022023/
21. Kim WJ, Soh Y, Heo SM. Recent advances of therapeutic targets for the treatment of periodontal disease. Biomol Ther (Seoul). 2021;29(3):263–7. DOI: 10.4062/biomolther.2021.001
22. Das S, Singh S, Garg S. Evaluation of Wheat Bran as a Biosorbent for Potential Mitigation of Dye Pollution in Industrial Waste Waters. Orient J Chem 2019;35(5).20;19(4):1079–91. DOI : 10.13005/bbra/3057
23. Lee, S., Kim, I. H., Hong, J., Jeon, B. J., Kim, S. S., Lee, J. W., Park, J. Y., Choi, S. H., Lee, T. K., Cha, J. K., & Won, M. H. (2022). In Vitro and In Vivo Anti-Inflammatory Effects of TEES-10®, a Mixture of Ethanol Extracts of Ligularia stenocephala Matsum. & Koidz. and Secale cereale L. Sprout, on Gingivitis and Periodontitis. Dentistry journal, 10(8), 143. DOI: 10.3390/dj10080143
24. Putranto RA. Peran Irigasi Klorheksidin Pada Perawatan Penyakit Periodontal. J Kedokt Gigi Terpadu. 2019;1(1):35–9.https://e-journal.trisakti.ac.id/index.php/jkgt/article/download/5155/3917
25. Siddarth M, Singh P, Gupta R, Sinha A, Shree S, Sharma K. Comparative evaluation of subgingivally delivered 2% curcumin and 0.2% chlorhexidine gel adjunctive to scaling and root planing in chronic periodontitis. Photodiagnosis Photodyn Ther. 2020;29:101665. https://pubmed.ncbi.nlm.nih.gov/32690830/
26. Sitorus E, Momuat LI, Katja DG. Aktivitas Antioksidan Tumbuhan Suruhan (Peperomia pellucida [L.] Kunth). J Ilm Sains. 2013;13(1):80.DOI: 10.35799/jis.13.1.2013.2116
27. Fatin A, Dwiyanti S, Lutfiati D. Pengaruh Proporsi Olive Oil Dan Tumbuhan Suruh Cina (Peperomia Pellucida L) Terhadap Hasil Jadi Clear Pads (Kapas Pembersih. e-Jurnal. 2020;09(4):117–24.https://ejournal.unesa.ac.id/index.php/jurnal-tata-rias/article/view/35861
28. Ahmad I, Hikmawan BD, Sulistryarini R, Mun'im A. *Peperomia pellucida (L.) Kunth* herbs: A comprehensive review on phytochemical, pharmacological, extraction engineering development, and economic promising perspectives. J Appl Pharm Sci, 2023; 13(01):001-009.DOI: 10.7324/JAPS.2023.130201
29. Yufiradani Y, Mayefis D, Marliza H. Uji Aktivitas Antibakteri Ekstrak Daun Suruhan (Peperomia Pellucida L. Kunth) Terhadap Propionibacterium Acnes Penyebab Jerawat. J Ris Kefarmasian Indonesia. 2020;2(1):35–41.DOI: 10.33759/jrki.v2i1.70
30. Sundari D, Almasyhuri A. Uji Aktivitas Antiseptik Ekstrak Etanol Daun Sirih (*Piper betle* Linn.) dalam Obat Kumur terhadap *Staphylococcus aureus* secara in Vitro. J Kefarmasian Indones. 2019;9(1):10–8.DOI:10.22435/jki.v9i1.351
31. Amarathunga, Dinithi & Kankanamge, Upekshi. a Review on pharmacognostic, phytochemical and ethnopharmacological findings of *Peperomia Pellucida (L.)* Kunth: Pepper Elder. Inter Res J of Phar. 2017;8(11):16–23. DOI:10.7897/2230-8407.0811211
32. Wunsch CM, Lewis JP. *Porphyromonas gingivalis* as a model organism for assessing interaction of anaerobic bacteria with host cells. J Vis Exp. 2015 Dec 17;(106):e53408. DOI : 10.3791/53408.
33. Pujiastuti P, Lestari S, Fakultas P, Gigi K, Jember U, Konservasi B, et al. Perbedaan Efektivitas Antibakteri Ekstrak Daun Sirih Merah (Piper crocatum) pada *Porphyromonas gingivalis* dan Streptococcus viridans. JKG Unej. 2015;12(1):1–4.
34. Hans VM, Grover HS, Deswal H, Agarwal P. Antimicrobial efficacy of various essential oils at varying concentrations against periopathogen *Porphyromonas gingivalis*. J Clin Diagn Res. 2016 Sep;10(9):ZC16-ZC19. DOI: 10.7860/JCDR/2016/18956.8435
35. Müller-Heupt LK, Vierengel N, Groß J, Opatz T, Deschner J, von Loewenich FD. Antimicrobial Activity of *Eucalyptus globulus*, *Azadirachta indica*, *Glycyrrhiza glabra*, *Rheum palmatum* Extracts and Rhein against *Porphyromonas gingivalis*. *Antibiotics*. 2022; 11(2):186. DOI: 10.3390/antibiotics11020186
36. Wayne, PA. Performance standards for antimicrobial disk susceptibility tests. 13th ed. CLSI standard M02. Clinical And Laboratory Standards Institute. 2018
37. Edi IS, Melodya CE, Hidayati S, Purwaningsih E. Perbedaan daya hambat ekstrak daun pacar kuku (lawsonia inermis) terhadap pertumbuhan bakteri porphyromonas gingivalis. JIKG. 2021;3(2):452-463
38. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control. 2019 Jul 16;8:118. DOI: 10.1186/s13756-019-0559-6.
39. Cankaya TII, Somuncuoglu EI. Potential and prophylactic use of plants containing saponin-type compounds as antibiofilm agents against respiratory tract infections. Evid Based Complement Alternat Med. 2021 Jul 23;2021:6814215. DOI: 10.1155/2021/6814215.
40. Mayefis D, Marliza H, Yufiradani. Uji Aktivitas Antibakteri Ekstrak Daun Suruhan (Peperomia pellucida L. Kunth) Terhadap Propionibacterium acnes Penyebab Jerawat. J Ris Kefar Indo. 2020;2(1):35–41.DOI:10.33759/jrki.v2i1.70
41. Eolia C, Syahputra A. Efektivitas antibakteri ekstrak etanol daun tin (Ficus carica Linn.) terhadap bakteri *Porphyromonas gingivalis* secara in vitro. J Ked Gig Univ Padja. 2019;31(3):171–7.DOI : 10.24198/jkg.v31i3.23639
42. Chitsazha R, Faramarzi M, Sadighi M, Pourabbas R, Pourhajibagher M, Firouzi N, Ghasemirad M. Evaluation of antibacterial effect of concentrated growth factor on *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. J Family Med Prim Care. 2022 Jun;11(6):2865-2869. doi: 10.4103/jfmpc.jfmpc_2065_21.
43. Silvia RM, Fiandita CV, Prihanti AM, Wahyukundari MA, Arina YMD. Effectiveness of Cacao leaves extract of Edel variety from Jember as antibacterial against Staphylococcus aureus and Porphyromonas gingivalis. Padj J Dent, July. 2023;35(2):106-110. DOI: 10.24198/pjd.vol35no2.46171
44. Noor DI, Krishnawan Firdaus IWA, Oktiani BW. Antibacterial Effectivity Test Of Ulin Bark Extract (Eusideroxylon Zwageri) On The Growth O*f Porphyromonas Gingivalis*. Dentino J Kedokt Gigi. 2021;6(1):37.DOI : 10.20527/dentino.v6i1.10638
45. Nørskov-Lauritsen N, Claesson R, Jensen AB, Åberg CH, Haubek D. Aggregatibacter Actinomycetemcomitans: Clinical significance of a pathobiont subjected to ample changes in classification and nomenclature. Pathogens. 2019;8(4). DOI: 10.3390/pathogens8040243