Antibacterial Effectivity Of Nanoemulgel *Phaleria Macrocarpa's* Leaf On The *Porphyromonas Gingivalis* ATCC 33277 Biofilm Thickness

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Received 3 May 2024; 1st revision 19 July 2024; 2nd revision 22 July 2024; Accepted 31 July 2024; Published online 31 July 2024

Keywords:

Patient safety; Patient safety culture; Educational Dental Hospitals

ABSTRACT

Background: *Porphyromonas gingivalis is an oppoturnistic microorganism in periodontal disease that is formed at the stage maturation of biofilm formation. One of the natural materials biofilm reduction is the Phaleria macrocarpa. Phaleria macrocarpa's leaf contains chemical compounds such as flavonoids, tannins, alkaloids, saponins that function as antibacterials.The aim of this research is to determine the effectiveness of the nanoemulsion of the gel Phaleria macrocarpa's leaf on the thickness of the P. gingivalis bacterial biofilm.*
Method: *The research design was carried out with a post test only with a total of 28 samples, then divided into 4 groups of treatment of Phaleria macrocarpa's leaf extract nanoemulsion gel preparations with variations in concentrations of 30%, 40%, and 50%, as well as a control group of 25% metronidazole gel. The reading of the anti-biofilm thickness test results was measured by OD (Optical density) using an ELISA-reader.*
Results: *Oneway Anova parametric test among obtained p<0.000 results showing the effect of Phaleria macrocarpa' leaf gel nanoemulsion extract on the decrease in the thickness of the Porphyromonas gingivalis bacterial biofilm.*
Conclusion: *It can be concluded that the nanoemulsion gel formulation of Phaleria macrocarpa's leaf extract concentrations of 30%, 40%, and 50% is effective, with a concentration 50% was most effective against reducing the thickness of the bacterial biofilm Porphyromonas gingivalis.*

doi: http://dx.doi.org/10.30659/odj.11.1.168-174
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Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: *Pratiwi et al*. Antibacterial Effectivity Of Nanoemulgel Phaleria Macrocarpa's Leaf On The Porphyromonas Gingivalis ATCC 33277 Biofilm Thickness. Odonto: Dental Journal, v.11, n.1, p.168-174, July 2024

INTRODUCTION

Periodontitis is an inflammatory condition present in the periodontium affecting soft tissue and supportive structures of the teeth. Periodontal disease is the most common oral health condition, but it often goes undiagnosed because it is painless. It caused by microorganisms, resulting in progressive damage to the periodontal ligament, cementum, and alveolar bone.[1,2,3] Periodontal disease can have serious consequences if not treated immediately, such as difficulty chewing, speaking and tooth loss.[4]

According to Riset Kesehatan Dasar (RISKESDAS) report in 2018 showed a high predominance of periodontitis (74.1%), with the highest rate in premature patients (77.8%) and decreased slowly in elderly patients (66.0%).[5] The World Health Organization (WHO) reports a prevalence of periodontal disease ranging from 40% to 75% in the population aged 35 to 44 years, with the highest prevalence among young and middle-aged adults.[4]

In response to new scientific evidence, the classification of periodontitis has been revised several times over the past 30 years. In 2017, the American Academy of Periodontology (AAP) published an updated classification of periodontal and peri-implant diseases and conditions.[6] The distinction between chronic periodontitis and aggressive periodontitis has been abandoned on the basis that there is little evidence from biological studies to suggest that chronic periodontitis and aggressive periodontitis are distinct entities, rather than variants of the same disease process.[7]

The etiology of periodontitis is primarily attributed to plaque accumulation, where plaque serves as the main etiological factor, and the presence of *Porphyromonas gingivalis* bacteria.[8] These bacteria release various pathogenic factors that invade the periodontal tissues and cause damage to the surrounding tissues.[9]

Among the bacteria considered to cause periodontal disease, *P. gingivalis* has been extensively studied due to its unique ability to evade the immune response. *P.gingivalis* is an oral anaerobic gram-negative bacterium considered a major pathogen causing periodontal disease due to the production of several virulence factors and extracellular proteases such as lipopolysaccharide, fimbria, gingipain, etc. leading to destruction of periodontal tissues. The different surface compositions of *P. gingivalis* allow the bacteria to interact with the external environment and simplify growth, nutrient supply, bacterial colonization, and protective biofilm formation. protect bacteria from host defenses.[10]

One of pharmacotherapeutic therapy for periodontal treatment is metronidazole gel, which can be employed as an adjunctive therapy to maximize treatment efficacy in the non-surgical phase.[11] However, the use of metronidazole gel is associated with side effects and is considered effective only against anaerobic bacteria when combined with amoxicillin as a broad-spectrum antibacterial agent to inhibit gram-negative anaerobic bacteria, the causative agents of periodontitis.[12] Therefore, the treatment approach for periodontal disease can be optimized through the utilization of herbal or natural herbs containing antibacterial properties.[13]

Natural substances playing a role as an antibiofilm agent is the leaf of *Phaleria macrocarpa* or “mahkota dewa”.[14] *Phaleria macrocarpa*’s leaf contains chemicals such as flavonoids, tannins,

alkaloids, and saponins. Flavonoids are phenols that possess antimicrobial properties and inhibit the enzymatic activity of microorganisms disrupting metabolic processes.[15]

METHODS

The following study was a laboratory experimental research conducted using a post-test only group design. The sample size consisted of 28 samples divided into 3 treatment groups, nanoemulsion gel formulations of *Phaleria macrocarpa*'s leaf extract with concentrations of 30%, 40%, and 50%, and 1 positive control group, which utilized 25% metronidazole gel. Each group comprised 7 samples, calculated using the Federer formula.

The research process commenced with the submission of ethical clearance to the Dental Ethics committee of Sultan Agung Islamic University, and this study received ethical approval with reference number 483/B.1-KEPK/SA-FKG/IX/2023.

The extract of *Phaleria macrocarpa*'s leaf was made using maceration method. A total of 250 grams of *Phaleria macrocarpa*'s leaf that have been cleaned using running water are cut into small pieces and then dried. The dried leaves were mashed using a blender. The leaf powder was soaked in 1000 ml in a 96% ethanol solution until completely submerged for 24 hours with shanker.

The extract of *Phaleria macrocarpa*'s leaf was filtered to separate the pulp and filtrate. Furthermore, the filtrate was evaporated using a rotary evaporator at a temperature of 50 C to evaporate all the ethanol to obtain extract of *Phaleria macrocarpa*'s leaf. The extract then made into nanoemulsion by mixing the *Phaleria macrocarpa*'s leaf extract with 3.6 grams of Tween 80 with 100 grams of aquadest until homogeneous using a homogenizer at a speed of 1000 rpm for 30 minutes.

Dissolve 0.1 grams of methyl paraben and 0.02 grams of propyl paraben into 10 grams of propylene glycol along with palm oil then add it to the previous mixture of ingredients and re-homogenize it using an over stirrer. Then unite the oil and water phases until homogeneous using an over stirrer at 1000 rpm for 1 hour.

The preparation of the gel base begins by mixing HMPC 4000 with aquadest until completely dispersed and homogeneous in the mortar. Let the mixture sit for 1 hour until a gel mass forms. After the gel mass was formed, 0.1 grams of methyl paraben, 2 grams were added. Propyl paraben and 2 grams of glycerin were then stirred until homogeneous. Mix the gel
base with the entire formulation of Phaleria macrocarpa's leaf gel nanoemulsion at room temperature using a homogenizer at 3000 rpm for 15 minutes.

The nanoemulsion gel formulations of *Phaleria macrocarpa*'s leaf extract were divided into 3 concentration groups, and then biofilm thickness testing was conducted. Biofilm thickness testing involved the inoculation of bacterial stock onto reaction tubes, followed by incubation at 37°C for 15 minutes. Subsequently, *Porphyromonas gingivalis* solution was added to each well of the microplate. Each solution in the microplate was then tested with *Phaleria macrocarpa*'s leaf extract compounds at concentrations of 30%, 40%, and 50%, as well as 25% metronidazole gel, followed by incubation at 37°C for 24 hours. Afterward, the samples were rinsed with PBS, stained with 1% crystal violet, and treated with 98% alcohol. Biofilm thickness was measured using Optical Density readings obtained from an ELISA reader.

RESULTS

The results of biofilm thickness readings obtained from this study were presented in Table 1. Based on Table 1, the mean value of the 50% concentration group was the lowest, with an average value of 1.88 compared to the other groups. The optical density values for the 50% concentration group indicate the lowest value, 1.88. The 50% concentration group showed highest effectiveness in inhibiting the thickness of *Porphyromonas gingivalis* bacterial biofilm.

The results of this research data were then subjected to statistical analysis between the treatment groups, comprising concentrations of 30%, 40%, 50%, and the positive control group. The results of these statistical tests were presented in Table 2.

The normality test results using the Shapiro-Wilk method indicate that $p > 0.05$, thus allowing the conclusion that the data for all groups are normally distributed. Subsequent data analysis proceeded with the parametric One-Way ANOVA test.

The results of the Bonferroni Post Hoc test showed significance between the positive control and the 30%, 40%, and 50% concentrations, with values of .016, .011, and .010 ($p < 0.05$) respectively. This signifies a significant difference between the positive control and the 30%, 40%, and 50% concentrations.

Table 1. Biofilm Thickness Results (nm)

Group	Sample	Mean
Treatment	Nanoemulsion gel of god's crown plant of 30%	1.91
	Nanoemulsion gel of god's crown plant of 40%	1.89
	Nanoemulsion gel of god's crown plant of 50%	1.88
Control	Gel *Metronidazole* 25%	2.50

Table 2. Results of Normality and Homogeneity Tests

Group	*Shapiro-Wilk*		*Levene's Test*
	Sig.	Notes	
25% Metronidazole gel	0.720*	Normal	
30% concentration	0.974*	Normal	
40% concentration	0.684*	Normal	0,553*
50% concentration	0.381*	Normal	

Table 3. Post Hoc Test Results

Group	25% *Metronidazole* gel	30% concentration	40% concentration	50% concentration
25% Metronidazole gel	-	.016*	.011*	.010*
30% concentration	.016*	-	1.000	1.000
40% concentration	.011*	1.000	-	1.000
50% concentration	.010*	1.000	1.000	-

DISCUSSION

The use of natural herbs as therapeutic agents has increased rapidly in recent years. One of the natural herbs that commonly used is the *Phaleria macrocarpa*'s. This plant can be used for both fruits and leaves. Previous studies have shown that this plant can be beneficial as an anti-bacterial, anti-inflammatory, antioxidant and an ingredient as an anti-cancer. The results of the toxicity test showed that the extract of *Phaleria macrocarpa*'s leaf is relatively safe.[12]

Side effects of using the *Phaleria macrocarpa*'s leaf as a local therapeutic agent have hardly been found.[13]

The results of the biofilm thickness test indicated that the nanoemulsion gel with a 50% concentration showed lowest biofilm thickness. It means 50% concentration group had the greatest antibacterial effect and exhibited a brighter color intensity compared to the 30% and 40% concentration groups in inhibiting *Porphyromonas gingivalis* bacterial biofilm.

According to Pratiwi in 2018 research, showed that a 30% concentration of *Phaleria macrocarpa*'s leaf extract had antibacterial properties in inhibiting the growth of *Staphylococcus aureus* bacteria, compared with 10% and 20% concentration. Furthermore, Radita in 2019 explained that *Phaleria macrocarpa*'s leaf extract was effective in inhibiting the growth of gram-negative bacteria. This is because, at high concentrations, *Phaleria macrocarpa*'s leaf extract demonstrates significant inhibition of the growth of biofilm-forming bacteria. The greater concentration of *Phaleria macrocarpa*'s leaf extract has more effective in inhibiting the growth of biofilm formation bacteria.[16]

Previous research was known to use the form of extract formulation, while in this study it used the form of gel nanoemulsion formulation.[17] Advances in science and technology and along with the development of the times introduced the stabilization of production in the form of gel nanoemulsions. Gel nanoemulsion is a gel-shaped preparation and has a relatively small size ranging from 50-1000 nm. Nanoemulsions gel have a very small size and have the advantage of facilitating the rate of absorption of drugs into the body.[14]

Gel nanoemulsion preparations are said to be better because they can increase absorption, helping to dissolve drugs which is lipophilic so as to increase bioavailability. Previous research stated that gel nanoemulsions are best used for topical administration of drugs.[18] Gel nanoemulsion is form of small particles which is effective to increase the stability of the active ingredient and easy to apply.[17]

Phaleria macrocarpa's leaf contains antibacterial compounds, such as flavonoids, tannins, alkaloids, triterpenoids, saponins, and polyphenols. Flavonoids, tannins, alkaloids, and saponins are antibacterial substances in *Phaleria macrocarpa*'s leaf. The antibacterial compound can bind proteins in the formation of bacterial cell walls, interfere with the arrangement of peptidoglycan bacterial cells, damage the cytoplasmic membrane which causes the permeability of the bacterial wall to be disturbed so that the bacterial cell wall is not formed completely and the bacteria is lysis. Several factors can inhibit the growth of *Porphyromonas gingivalis* bacteria, including variations in extract concentrations and the constituents present in *Phaleria macrocarpa*'s leaf.[19, 20]

CONCLUSION

Based on the conducted research, it can be concluded that *Phaleria macrocarpa*'s contain various chemical compounds with antibacterial properties. The results of the biofilm thickness test reveal differences in antibacterial effectiveness among the 30%, 40%, and 50% concentrations, with the 50% concentration being the most effective as an antibacterial agent.

REFERENCES

1. Ridwan, M.A. *Dkk.* (2021) "Sistem Pakar Diagnosa Penyakit Periodontal Menggunakan Metode Dempster – Shafer," *Simetris: Jurnal Teknik Mesin, Elektro Dan Ilmu Komputer*, 11(2), Hal. 594–607
2. Harapan, I.K., Ali, A. Dan Fione, V.R. (2020) "Gambaran Penyakit Periodontal Berdasarkan Umur Dan Jenis Kelamin Pada Pengunjung Poliklinik Gigi Puskesmas Tikala Baru Kota Manado Tahun 2017," *Jigim (Jurnal Ilmiah Gigi Dan Mulut)*, 3(1), Hal. 20–26. Tersedia Pada: Https://Doi.Org/10.47718/Jgm.V3i1.1430.
3. Hanum Nurma Azuro, Moch. Yunus, and Rara Warih Gayatri, (2021), "Early Detection of Periodontitis in Public Health Centres in Malang: An Overview" in The 2nd International Scientific Meeting on Public Health and Sports (ISMoPHS 2020), KnE Life Sciences, pages 134–148. DOI 10.18502/kls.v0i0.8875
4. Lamster, I.B. Dan Pagan, M. (2017) "Periodontal Disease And The Metabolic Syndrome," *International Dental Journal*, 67(2), Hal. 67–77. Tersedia Pada: Https://Doi.Org/10.1111/Idj.1226
5. Badan Penelitian dan Pengembangan Kesehatan. Laporan Nasional Riset Kesehatan Dasar 2018. Jakarta: Kementerian Kesehatan Republik Indonesia; 2018. p. 201–204
6. Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS, Mealey BL, Papapanou PN, Sanz M, Tonetti MS. A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. J Periodontol. 2018; 89(Suppl 1): S1–8
7. Neelam Das (2021). Critical Analysis of AAP 1999 and 2017 the World Workshop Classification Systems: An Insight. Saudi J Oral Dent Res, 6(7): 304-312
8. Lesouhaitier, O. *Dkk.* (2019) "Host Peptidic Hormones Affecting Bacterial Biofilm Formation And Virulence," *Journal Of Innate Immunity*, 11(3), Hal. 227–241. Tersedia Pada: Https://Doi.Org/10.1159/000493926
9. Putri, C.F. Dan Bachtiar, E.W. (2020) "Porphyromonas Gingivalis Dan Patogenesis Disfungsi Kognitif : Analisis Peran Sitokin Neuroinflamasi," *Cakradonya Den J;*
10. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Zamanian Azodi M. Study of *Porphyromonas gingivalis* in periodontal diseases: A systematic review and meta-analysis. Med J

Islam Repub Iran. 2017 Sep 12;31:62. doi: 10.18869/mjiri.31.62. PMID: 29445691; PMCID: PMC5804457
11. Needleman, I. (2016) "The Good Practitioner ' S Guide To Periodontology We ' Re A 1000 + Strong Membership," *British Society Of Periodontology*, 03(03), Hal. 1–52.
12. Pratiwi, R. *Dkk.* (2022) "Odonto Dental Journal. Volume 9. Special Issue 1. April 2022," *Odonto Dental Journal*, 9(1), Hal. 115–130.
13. Nikham Dan Erlinda, T. (2012) "Uji Bahan Baku Antibakteri Dari Buah Mahkota Dewa (Phaleria Macrocarpa (Scheff) Boerl .) Hasil Iradiasi Gamma Dan Antibiotik Terhadap Bakteri Patogen," Hal. 168–174.
14. Prajapati, B.G. Dan Jivani, Malay N. (2021) "Nanoemulgel Innovative Approach For Topical Gel Based Formulation," (August).
15. Taha, M. *Dkk.* (2017) "Aktivitas Antibakteri Ekstrak Buah Mahkota Dewa (Phaleria Macrocarpa) Terhadap Staphylococcus Aureus," Hal. 16–23.
16. Radita, D. C. and Widyarman, A. S. 2019. Mahkota Dewa (God's Crown) Fruit Extract Inhibits The Formation of Periodontal Pathogen Biofilms In Vitro. Journal of Indonesian Dental Association. 2(2). 57– 62. doi: 10.32793/jida.v2i2.404.
17. Kumar, Sandeep & Patil, Anasuya & Nile, Nilam & Varma, Ajit & Sahu, Dipansu & Vishwakarma, Dhaneshwar & Meena, Mukesh Kumar & Kar, Nihar & Kumar, Pavan. (2023). Formulation and characterization of Miconazole Nanoemulgel for Topical Delivery by Using Natural Oils Corresponding. Journal of Cardiovascular Disease Research. 14. 2167-2182. 10.48047/jcdr.2023.14.08.234.
18. Sari, A.I. *dkk.* (2017) "Review : Formulasi Nanoemulsi Terhadap Peningkatan Kualitas Obat," 16, hal. 247–254.
19. Tedjasulaksana (2016) "Metronidasol Sebagai Salah Satu Obat Pilihan Untuk Periodontitis Marginalis," Hal. 19–23.
20. Catherine *Dkk.* (2019) "Contribution Of Statins Towards Periodontal Treatment : A Review," 2019