

## The effectiveness of the combination of *Moringa oleifera* extract and propolis on *Porphyromonas gingivalis* biofilms compared to 0.7% tetracycline

Hansen Kurniawan, Widyastuti and Mery Esterlita Hutapea  
Department of Periodontics,  
Faculty of Dentistry, Universitas Hang Tuah,  
Surabaya – Indonesia

### ABSTRACT

**Background:** Periodontitis is an inflammatory disease that occurs in periodontal tissues. *Porphyromonas gingivalis* is also known as a bacterium commonly associated with the pathogenesis of periodontitis. Tetracycline is one of the antibiotics often used in periodontal tissue treatment. Propolis and *Moringa oleifera* are also known to have certain compounds assumed to be able to inhibit biofilm growth. **Purpose:** This study aims to understand the effectiveness of the combination of *Moringa oleifera* and propolis on *porphyromonas gingivalis* biofilms compared to 0.7% tetracycline. **Methods:** A biofilm inhibition activity test was performed using the broth micro dilution method. First, bacteria were prepared by making a suspension in brain heart infusion media and adjusting it to 0.5 McFarland I standard. Second, fifteen samples were divided into five groups; group K as control group (0.1% sodium carboxymethyl cellulose), T (0.7% tetracycline), and treatment groups with the combination of propolis and *Moringa oleifera* in various concentrations, such as P1(10%+20%), P2(10%+40%), and P3(10%+80%). Third, the result data obtained in the form of optical density (OD) was read by using an ELISA reader. Next, statistical analysis using analysis of the variance test was conducted ( $p < 0.05$ ). **Results:** There was no significant difference between group T and group P1 (0.075). Nevertheless, there were significant differences between group T and group P2 as well as between group T and group P3 (0.00) ( $p < 0.05$ ). **Conclusion:** The combination of 10% propolis and 40% *Moringa oleifera* as well as the combination of 10% propolis and 80% *Moringa oleifera* have better antibacterial effectiveness against *Porphyromonas gingivalis* biofilm than 0.7% tetracycline.

**Keywords:** biofilm; *Moringa oleifera*; *P. gingivalis*; propolis; tetracycline

Correspondence: Hansen Kurniawan, Department of Periodontics, Faculty of Dentistry, Universitas Hang Tuah. Jl. Arif Rahman Hakim No. 150 Surabaya, 60111 Indonesia. Email: hansen.kurniawan@hangtuah.ac.id

### INTRODUCTION

Periodontitis is an inflammatory disease that occurs in the supporting tissues of teeth caused by specific microorganisms triggering inflammation on periodontal ligament and alveolar bone.<sup>1</sup> Plaque and calculus bacteria are considered to be the main causes of periodontal disease. Plaque containing pathogenic microorganisms plays an important role in causing damage to the periodontal tissue.<sup>2</sup>

*Porphyromonas gingivalis* (*P. gingivalis*), moreover, is a bacterium commonly associated with periodontitis pathogenesis. Almost 40–100% of periodontitis cases

are caused by the opportunistic bacterial antigen of *P. gingivalis*. The presence of these bacteria in chronic periodontitis patients, according to a previous research on subgingival plaque as much as 85.75%.<sup>3</sup>

Furthermore, biofilm is a collection of microorganisms in which microbial cells attach to each other on a living or non-living surface, producing their own extracellular polymer matrix.<sup>4</sup> Biofilm consists of bacterial cells (5–25%) and glycocalyx matrix (75–95%).<sup>5</sup> In addition, biofilm is known as a microbial cell encased in a matrix of extracellular polymeric substances, such as polysaccharides, proteins and nucleic acids.<sup>6</sup> In the lower layer of biofilm, microbes are bound together in a polysaccharide matrix with other

organic and inorganic materials, while on the top layer, there is an amorphous layer extending to the medium around it. The fluid layer adjacent to the biofilm has a stationary and dynamic sub-layer.<sup>7</sup> Biofilm is also known to have an extracellular polymeric substance (EPS) matrix that is able to prevent the occurrence of antimicrobial diffusion, and, as a result, bacterial resistance to antibiotics increases while cell metabolic activity on the biofilm decreases.<sup>8,9</sup>

On the other hand, *Moringa oleifera*, also known as Moringa, is a plant widely used as a traditional medicine or for mystical therapy in Indonesia.<sup>10</sup> *Moringa oleifera* is also known to have anti-cancer, antibacterial and hypotensive properties that can inhibit bacterial and fungal activities.<sup>11</sup> According to Septiyani et al.<sup>12</sup> *Moringa oleifera* extract can be effective in inhibiting the growth of *P. gingivalis* bacteria at doses of 80% and 40%.

Similarly, propolis, according to previous research, has natural antibacterial and antibiotic properties.<sup>13</sup> Propolis, one of the natural products produced by honey bees, has been widely used not only as a medicine or supplement, mouth wash, anti-inflammatory and disease therapy, but also for accelerating the wound-healing process. In addition, propolis has many special benefits and potential since it has antibacterial and antiviral properties, so it can inhibit cancer growth.<sup>14,15</sup> According to Suryono et al.<sup>16</sup> 10% propolis even has an ability to inhibit *P. gingivalis* bacterial activities optimally.

Tetracycline is one of the oldest classes of broad-spectrum antibiotics in use. In addition to its antibacterial activity, tetracyclines are also known to have anti-inflammatory, anti-collagenase and wound healing properties, as well as the ability to reduce bone loss.<sup>17</sup> Tetracyclines, consequently, can be used as antibiotics for periodontitis cases. The biocompatibility of tetracyclines, mostly studied in the form of tetracycline gel with a concentration of 0.7%, is acceptable for tissue and can remove the smear layer and open dentinal tubules and the collagen matrix.<sup>18</sup>

Finally, it can be said that propolis and *Moringa oleifera* contain different secondary metabolites that have anti-bacterial activity. Meanwhile, tetracycline is one of the antibiotics that has been widely used for the therapy of periodontal disease. However, whether the combination of propolis and *Moringa oleifera* will be as effective as tetracyclines and whether propolis and *Moringa oleifera* have side effects is still debatable. Therefore, this research aims to explore the antibacterial effectiveness of the combination of propolis extract and *Moringa oleifera* leaf extract against *P. gingivalis* biofilms compared to 0.7% tetracycline.

## MATERIALS AND METHODS

This research is a true laboratory experimental research with the post only control group design. In this research, *Moringa oleifera* extract was prepared with a maceration

technique, in which 100 g of *Moringa oleifera* simplisa powder was put into erlernmeyer, immersed in 500 mL of 96% ethanol solution and covered with aluminium foil for five days, while being stirred occasionally. After five days, the sample was filtered using filter paper to produce filtrate 1 and residue 1. The residue was then added to 250 ml of 96% ethanol solution, covered with aluminium foil for two days, being stirred occasionally.<sup>11</sup>

After two days, the sample was filtered using filter paper to produce filtrate 2 and residue 2, then mixed into one and evaporated using a rotary evaporator to obtain *Moringa oleifera* extract. The *Moringa oleifera* extract obtained was kept in a water bath until all the ethanol solvent evaporated. The extract obtained was then weighed and stored in a closed glass container before used for testing. In total there were three test solutions made, namely *Moringa oleifera* extract at a concentration of 20% (0.2:0.8), *Moringa oleifera* extract at a concentration of 40% (0.4:0.8), and *Moringa oleifera* extract at a concentration of 80% (0.8:0.2).<sup>11</sup>

Afterwards, 800 g of propolis was cooled in a refrigerator, then put in an oven at 40°C for three days, and 2L of 70% ethanol liquid was added. To speed up the dissolving process, propolis was crushed with a stirrer and allowed to stand for a moment.<sup>18</sup> After that, the propolis was stirred every day and filtered, but the obtained filtrate was left to stand to precipitate substances that were not needed, but not dissolved in ethanol. The remaining filtrate was then put into a 90% ethanol solution. All of these steps were repeated three to five times, and the filtering process was also repeated three times until a thick propolis extract was obtained. Next, 0.1 g of the thick propolis extract was dissolved in 1 mL of 0.1% sodium carboxymethyl cellulose (Na CMC) so that the concentration of the propolis extract obtained became 10%.<sup>19</sup>

Subsequently, 0.7% tetracycline was prepared. First, tetracycline tablets were crushed. Afterwards, 175 mg of the crushed tetracycline was dissolved in 25 ml of aquadest.<sup>20</sup> Next, *Moringa oleifera* extract and propolis at various concentrations were mixed in a ratio of 1:1. Meanwhile, 0.1% Na CMC solution was divided into several groups. First, in the control group (K), 0.1 mL of *P. gingivalis* biofilm mixed with 0.1 mL of 0.1% Na CMC was put on a microtiter plate. Second, in the tetracycline (T) group, 0.1 mL of *P. gingivalis* biofilm mixed with 0.1 mL of 0.7% tetracycline was put on a microtiter plate. Third, in treatment group 1 (P1), 10% propolis extract mixed with 0.1 mL of 20% *Moringa oleifera* leaf extract was put on a microtiter plate. Fourth, in treatment group 2 (P2), 10% propolis extract mixed with 0.1 mL of 40% *Moringa oleifera* leaf extract was put on a microtiter plate. And, in treatment group 3 (P3), 10% propolis mixed with 0.1 mL of 80% *Moringa oleifera* leaf extract was put on a microtiter plate.

After that, a detection test for the formation of *P. gingivalis* bacterial biofilm was carried out using the congo red agar (CRA) method. First, *P. gingivalis* bacteria were inoculated on congo red agar and incubated for 48 hours at

37°C under anaerobic conditions. Congo red agar that can interact directly with polysaccharida intercellular adhesion formed a colour complex. After 48 hours, the plates were examined. If black colonies were formed, this indicated that the bacterial strain had produced biofilms. Meanwhile, if the colony showed a red colour, this illustrated that the strain had not formed any biofilm.<sup>21</sup>

In addition, a biofilm inhibition test was conducted by using the broth micro dilution method. First, bacteria were prepared by making a suspension in a brain heart infusion (BHI) media and adjusted to 0.5 McFarland I standard. Second, the equalized suspension of *P. gingivalis* bacteria was then diluted to a ratio of 1:100. Third, the bacterial suspension was inserted into a 96-well round bottomed plastic tissue culture plate (microtiter plate) with a total volume of 0.1 mL (100 µL) in each well using a micropipette. Fourth, observation was conducted on each test plate and each blank plate. Fifth, the test plate filled with a bacterial suspension was put on microtiter plate and then incubated at 37°C for 24 hours. Sixth, after 24 hours, the microtiter plate was removed from the incubator, and then the test extract solution was inserted into the microtiter plate filled with bacterial suspension, while the blank plate was filled with the test extract solution without bacteria. Seventh, the microtiter plate was put back into the incubator for 24 hours.<sup>22</sup> Eighth, the microtiter plate was removed from the incubator, and then the test solution was discarded and washed three times with 0.2 mL of phosphate buffer saline. Ninth, the microtiter plate was dried, and then 0.2 mL (200 µL) of 1% crystal violet was added to each well and left to stand for 15 minutes. Tenth, it was rinsed using distilled water and then dried for 15 minutes in an incubator at 37°C. In the last step, 0.2 mL of 2% 80 Tween was added to each microtiter plate.<sup>22</sup>

Next, an optical density (OD) test was performed. The values of density (OD) values obtained and were read using an ELISA reader at a wavelength of 515 nm. Afterwards, the inhibitory power of biofilm formation generated from the test solution was measured by the following formula:

$$\% \text{ Inhibition Power} = \left( 1 - \left( \frac{xODs - xODbs}{xODp} \right) \right) 100\%$$

Note:

- ODs : Optical density (515 nm) of tested sample
- ODbs : Optical density of blank sample
- ODp : (OD of test solvent - OD of blank solvent)

**Table 1.** The average (%) of *P. gingivalis* bacterial biofilm inhibition

Group	Mean (%)	Std. Deviation
K	0	0
T	31.8	3.76051
P1	36.4	0.71531
P2	57.8	3.40274
P3	72.4	2.10350

The reading results of the optical density values obtained with the ELISA reader were in the form of quantitative data. The data were then tested using the one-way analysis of variance (ANOVA) test.

## RESULTS

The research data were analysed descriptively to depict the distribution of data as well as summarise the characteristics of data in order to clarify the presentation of the data results. The data shown in Table 1 illustrates that the highest mean percentage of *P. gingivalis* biofilm inhibition was in the P3 group, with a value of 72.4, while the lowest mean percentage was in the K group, with a value of 0. Moreover, it also demonstrates that the mean percentage of *P. gingivalis* biofilm inhibition in the T group was 31.8, 36.4 in the P1 group, and 57.8 in the P2 group. Based on Table 2, the results of the LSD test showed that there was a significant difference in the percentage value of biofilm inhibition ( $p < 0.05$ ) in all groups, except between T and P1 ( $0.075 > 0.05$ ).

## DISCUSSION

The prevalence and severity of periodontal disease can be reduced by scaling root planning and antibacterial therapy. The antibacterial therapy often used to treat periodontal disease is tetracycline. Hence, this study aims to see the effect of *Moringa oleifera* extract at concentrations of 20%, 40% and 80% combined with 10% propolis on the biofilm formation of *P. gingivalis* bacteria strain ATCC 33277, compared with 0.7% tetracycline.

*Moringa oleifera* is known to have antibacterial ability in *P. gingivalis* at concentrations of 40% and 80%.<sup>12</sup> Similarly, propolis is also known to have antibacterial ability against *P. gingivalis* bacteria.<sup>23</sup> The inhibition ability of *Moringa oleifera* is known to be caused by a variety of active metabolic compounds it contains. Not only does *Moringa oleifera* contain great antibacterial properties consisting of saponins, triterpenoids and tannins, which work by damaging cells from bacteria, but also flavonoids as antioxidants playing a role in damaging the permeability of bacterial cell walls, microsomes and lysosomes as a result of the interaction between flavonoids and bacterial DNA.<sup>24</sup>

**Table 2.** The results of LSD Post-Hoc test

Group	T	P1	P2	P3
K	0.000*	0.000*	0.000*	0.000*
T		0.075	0.000*	0.000*
P1			0.000*	0.000*
P2				0.000*

\*  $p < 0.05$  (There are differences)

Propolis, on the other hand, is a natural material collected by honey bees from various plants, such as poplar, palm, pine, coniferous secretions, sap, resin, mucus and leaf buds. It is collected and carried by honey bees and used to seal the gaps in their hives. Initially, it acts as an antiseptic that prevents the beehive from microbial infection as well as the decomposition of intruders.<sup>25</sup> Propolis is known to have antibacterial, anti-viral, anti-fungal, antioxidant and anti-inflammatory functions that enhance the body's system.<sup>26</sup> Propolis is also known to contain amino acids, terpenoids and polyphenols (phenolic acids, esters and flavonoids).<sup>27</sup> Flavonoid is one of the important ingredients in propolis, and has antioxidant, anti-cancer, anti-inflammatory, allergy, antiviral and antibacterial effects.<sup>28–30</sup> Hence, flavonoids can eliminate the permeability of *P. gingivalis* bacterial cells.<sup>31</sup> Flavonoids can damage bacterial cells by lysing bacteria through protein binding so that bacteria will die.<sup>32</sup> According to the study of Asdar and Cindrakori, propolis contains apigenin and tt-farnesol which can decrease the amount of polysaccharides in the biofilm of microorganisms, so the biofilm formation can be stopped.<sup>33</sup>

Previous research conducted by Septiyani et al.<sup>12</sup> shows that *Moringa oleifera* extract at concentrations of 40% and 80% was able to reduce the biofilm formation of *P. gingivalis* bacteria. This previous research also reveals that 20% *Moringa oleifera* extract combined with 10% propolis even can inhibit the biofilm formation of *P. gingivalis* bacteria. Therefore, it can be said that based on this previous research, the combination of 10% propolis extract and 20% *Moringa oleifera* extract can be used for an alternative antimicrobial treatment as effective as 0.7% tetracycline. Meanwhile, the combination of 10% propolis and 40% *Moringa oleifera* as well as the combination of 10% propolis and 80% *Moringa oleifera* can be more effective than tetracycline 0.7%. Hence, two materials can be used synergistically to inhibit the biofilm formation of *P. gingivalis* bacteria.

Finally, it can be concluded that the combination of propolis extract and *Moringa oleifera* can generate antibacterial power to inhibit the biofilm formation of *P. gingivalis* bacteria. It is also known that 10% propolis and 20% *Moringa oleifera* have antibacterial power as great as 0.7% tetracycline. Nevertheless, the combination of 10% propolis and *Moringa oleifera* at concentrations of 40% and 80% has greater antibacterial power than 0.7% tetracycline. Besides, propolis and *Moringa oleifera* contain natural ingredients, so they have lower cytotoxicity than tetracyclines. Thus, it can be said the combination of propolis and *Moringa oleifera* is better than tetracyclines.

## REFERENCES

- Newman M, Takei H, Klokkevold P, Carranza F. Newman and Carranza's clinical periodontology. 13th ed. Philadelphia: Elsevier Saunders; 2018. p. 346–7.
- Quamilla N. Stres dan kejadian periodontitis (kajian literatur). J Syiah Kuala Dent Soc. 2016; 1(2): 161–8.
- Putri CF, Bachtiar EW. Porphyromonas gingivalis and pathogenesis of cognitive dysfunction: role of neuroinflammation cytokine (Literature review). Cakradonya Dent J. 2020; 12(1): 15–23.
- Jamal M, Tasneem U, Hussain T, Andleeb S. Bacterial biofilm: Its composition, formation and role in human infections. Res Rev J Microbiol Biotechnol. 2015; 4(3): 1–14.
- Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence. 2011; 2(5): 435–44.
- Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza's clinical periodontology. 12th ed. Philadelphia: Elsevier Saunders; 2015. p. 1779.
- Chandki R, Banthia P, Banthia R. Biofilms: A microbial home. J Indian Soc Periodontol. 2011; 15(2): 111–4.
- Gerits E, Verstraeten N, Michiels J. New approaches to combat Porphyromonas gingivalis biofilms. J Oral Microbiol. 2017; 9(1): 1300366.
- Chadha T. Bacterial biofilms: Survival mechanisms and antibiotic resistance. J Bacteriol Parasitol. 2014; 5(3): 190.
- Rockwood JL, Anderson BG, Casamatta DA. Potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by M. oleifera seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. Int J Phytotherapy Res. 2013; 3(2): 61–71.
- Dima LLRH, Fatimawali F, Lolo WA. Uji aktivitas antibakteri ekstrak daun kelor (*Moringa oleifera* L.) terhadap bakteri Escherichia coli dan Staphylococcus aureus. Pharmacon. 2016; 5(2): 282–9.
- Septiyani RI. Efektivitas ekstrak daun kelor (*Moringa oleifera* L.) dalam menghambat pertumbuhan bakteri Porphyromonas gingivalis. Thesis. Universitas Muhammadiyah Semarang: Semarang; 2019. p. 70–1.
- Kurniawati D. Uji aktivitas antibakteri propolis Trigona Spp. asal Bukit Tinggi pada tikus putih Sprague-Dawley. J Prog Kim Sains. 2011; 1(1): 25–31.
- Salatino A, Fernandes-Silva CC, Righi AA, Salatino MLF. Propolis research and the chemistry of plant products. Nat Prod Rep. 2011; 28(5): 925–36.
- Milani N, Bintari SH, Mustikaningtyas D. Pengaruh konsentrasi antibakteri propolis terhadap pertumbuhan bakteri Streptococcus pyogenes secara in vitro. Life Sci. 2016; 5(2): 95–9.
- Suryono S, Kusumawati I, Devitaningtyas N, Sukmawati A, Wijayanti P. Characteristic assay of incorporation of carbonated hydroxyapatite-propolis as an alternative for alveolar bone loss therapy on periodontitis: An in vitro study. J Int Oral Heal. 2020; 12(5): 463–9.
- Vagish Kumar LS. Pharmaceuticals: Tetracyclines and periodontal disease. Br Dent J. 2015; 218: 213.
- Sri Pradnyani IGA. Tetrasiklin HCL gel 0,7% meningkatkan jumlah sel fibroblas dan mempertebal ligamen periodontal pada sulkus gingiva tikus yang mengalami periodontitis. Intisari Sains Medis. 2017; 8(1): 14–8.
- Gani A, Sawal NW. Daya hambat ekstrak propolis Trigona Sp terhadap pertumbuhan bakteri Aggregatibacter Actinomycetemcomitans. Thesis. Makassar: Universitas Hasanuddin; 2013. p. 1–8.
- Perayil J, Menon KS, Biswas R, Fenol A, Vylloppillil R. Comparison of the efficacy of subgingival irrigation with 2% povidone-iodine and tetracycline HCl in subjects with chronic moderate periodontitis: A clinico microbiological study. Dent Res J (Isfahan). 2016; 13(2): 98–109.
- Purbowati R, Devi E, Rianti D, Ama F. Kemampuan pembentukan slime pada Staphylococcus epidermidis, Staphylococcus aureus, MRSA dan Escherichia coli. J Florea. 2017; 4(2): 1–9.
- O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp. 2011; (47): 2437.
- Amanda EA, Oktiani BW, Panjaitan FUA. Efektivitas antibakteri ekstrak flavonoid propolis Trigona Sp (*Trigona thoracica*) terhadap pertumbuhan bakteri Porphyromonas gingivalis. Dentin J Kedokt Gigi. 2019; 3(1): 23–8.
- Widowati I, Efiyati S, Wahyuningtyas S. Uji Aktivitas Antibakteri Ekstrak Daun Kelor (*Moringa Oleifera*) terhadap Bakteri Pembusukan Ikan Segar. Pelita. 2014; IX(2): 146–57.

25. Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, Bashir MA, Tahir M, Ansari MJ, Ghramh HA, Adgaba N, Dash CK. Composition and functional properties of propolis (bee glue): A review. *Saudi J Biol Sci.* 2019; 26(7): 1695–703.
26. Kaihena M. Propolis sebagai imunostimultor terhadap infeksi *Micobacterium tuberculosis*. In: *Prosiding FMIPA Universitas Pattimura*. Ambon: Universitas Pattimura; 2013. p. 71–2.
27. Pujirahayu N, Ritonga H, Uslinawaty Z. Properties and flavonoids content in propolis of some extraction method of raw propolis. *Int J Pharm Pharm Sci.* 2014; 6(6): 338–40.
28. Rismawati SN, Kimia JT, Jakarta U. Pengaruh variasi Ph terhadap kadar flavonoid pda ekstrasi propolis dan karakteristiknya sebagai antimikroba. *J Konversi.* 2017; 6(2): 89–94.
29. Grumezescu AM, Holban AM. *Therapeutic, probiotic, and unconventional foods*. St. Louis: Elsevier; 2018. p. 141–50.
30. Hermalinda R, Taufiqurrahman I, Helmi ZN. Total flavonoid content analysis flavonoid content analysis of ramania leaves' extract using ethanol, methanol and N-HEXANE as solvents. *Dentino J Kedokt Gigi.* 2019; 4(1): 60–3.
31. Karlina CY, Ibrahim M, Trimulyono G. Aktivitas antibakteri ekstrak herba krokot (*Portulaca oleracea L.*) terhadap *Staphylococcus aureus* dan *Escherichia coli*. *LenteraBio Berk Ilm Biol.* 2013; 2(1): 87–93.
32. Christianto CW, Nurwati D, Istiati. Efek antibakteri ekstrak biji alpukat (*Persea americana Mill*) terhadap pertumbuhan *Streptococcus mutans*. *Oral Biol Dent J.* 2012; 4(2): 40–4.
33. Asdar A, Cindrakori HN. Daya hambat gel propolis dari Sulawesi Selatan terhadap pertumbuhan bakteri *Porphyromonas gingivalis*. *B-Dent.* 2015; 2(2): 101–9.