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The Inhibition of Moringa Leaves Extract (*Moringa oleifera* Lamk.) to the Growth of *Porphyromonas gingivalis*

By

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Abstract

Background: Periodontal disease is one of the oral health problems with a prevalence of 50 % of the number of adults worldwide. The most common periodontal disease is chronic periodontitis with the main cause being plaque bacteria in the gingival sulcus. The most dominant bacteria is Porphyromonas gingivalis. Moringa is a plant used as an herbal medicine, especially the leaves with antibacterial properties because they contain flavonoids, saponins, tannins, alkaloids, triterpenoids, and terpenoids. Objective: This study aimed to determine the presence or absence of the inhibitory ability of moringa leaf extract and the largest concentration of moringa leaf extract in inhibiting the growth of P. gingivalis. Material and Methods: The method used was the paper disc diffusion method. 24 samples were divided into 6 groups. The treatment group was given various concentrations (100%, 75%, 50%, and 25%) of moringa leaf extract, the positive control group was given 0.2% chlorhexidine gluconate and the negative control group was given aquadest. The disc which spilled by various concentrations of moringa leaf extracts, 0.2% chlorhexidine, and aquadest was placed on a medium which has been inoculated by P. gingivalis, then incubated at 37ºC for 24 hours. The inhibition zone was measured using a digital caliper. Results: Moringa leaves extract 25 %, 50 %, 75 %, and 100 % had different inhibitory power against P. gingivalis, with a Kruskal-Wallis test significance value of p=0,00. Conclusions: Moringa leaf extract has an inhibitory effect on P. gingivalis. 75 and 100% of moringa leaf extract has the highest antibacterial inhibition zone.

Keywords: *Inhibitory, Moringa Leaf, Periodontitis, Porphyromonas gingivalis*

INTRODUCTION

Periodontal disease is one of the oral health problems with a 50 % prevalence of worldwide numbers of adults. It is indicated by inflammation that attacks supporting tissues of the teeth, which are the gingiva, periodontal ligaments, cementum and alveolar bone deepening pocket, recession, and both.[1] *Porphyromonas gingivalis*, an outstanding element of oral microorganism communities, is the principle pathogen that causes periodontitis.[2] *P. gingivalis* has produced diverse traces of proof of the contribution of this anaerobe to the development of periodontal disease. There is powerful proof that factors to *P. gingivalis* because of the keystone species inside the improvement of continual periodontitis.[3,4]

P. gingivalis growth causing chronic periodontitis can be done with plaque control.[5] Mouthwash is an antibacterial meant to reduce the accumulation of plaque. Chlorhexidine is one of the synthetic mouthwashes that can cause xerostomia, altered flavor sensations, especially salt and bitter, burning sensations, desquamation of the oral mucosa, discolored or coated tongue, swelling of the parotid gland, and oral paraesthesia.[6,7] Since chlorhexidine has several side effects, an antibacterial alternative is essential to minimize them.

Herbal medicine is another alternative that has minimal side effects. The amount one of the plants used for medicines is *Moringa oleifera* Lamk. Moringa is known as a Magic Tree that contains antibacterial ingredients; for example,

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flavonoids, saponins, tannins, alkaloids, triterpenoids, and terpenoids that can be used to grow bacteria.[8,9] Accordingly, the authors are interested in analyzing the capacity of Moringa leaves to inhibit the growth of *P. gingivalis.*

MATERIAL AND METHODS

This research was a laboratory experiment with a post-test-only control group research design. The material used in this research was *M. oleifera* leaves collected from the backyard of one of the residents in Pesanggaran Village, Banyuwangi Regency, Indonesia. The research group consisted of leaves extracted concentrations of 100% (P4), 75% (P3), 50% (P2), and 25% (P1), sterile aquadest for the negative control, and chlorhexidine gluconate for positive control.

The stages of work in this research started with sterilizing the tool and then extracting *M. Oleifera* with a 96% ethanol solvent using the maceration method. The maceration stage was executed for forty-eight hours even stirring each eight hours the usage of a stirring rod after which it became regenerated for forty-eight hours. The filtrate became evaporated with a rotary evaporator at a temperature of 50ºC to gain concentrated extract with a concentration of 100%. Then the extract was diluted to a concentration of 75, 50, and 25%. The dilution outcomes were filtered using a syringe filter and inserted in the Eppendorf tube.

The subsequent level is the preparation of Brain Heart Infusion Broth (BHI-B) media and Brain Heart Infusion Agar (BHI-A). After making the media, followed by making a suspension of *P. gingivalis*. *P. gingivalis* used in this have been received from the Microbiology Laboratory of the Faculty of Dentistry University of Jember. That bacteria has been recognized as pure *P. gingivalis* ATCC 33277. One ointment of *P. gingivalis* from pure strains was put in a vacuum tube containing 2 mL media BHI-B. Vacuum tubes have been inserted into the desiccator and incubated at 37°C for twenty-four hours. Then dilution was accomplished by adding sterile distilled water and homogenized with a mixing vortex until the absorbance reached 0.5 Mc Farland measured using a densitometer. The disc diffusion technique was used for antibacterial activity. One hundred µL of bacterial suspension dripped on each culture medium. The suspension becomes flattened on the surface of the culture medium using sterile cotton swabs. 13µL of every dilution of moringa leaf extract (100%, 75%, 50%, and 25%) was impregnated into sterile, blank discs 6 mm in diameter. Aquadest sterile was used as the negative control and the positive control used was 0,2% chlorhexidine gluconate. All discs had been completely dried earlier than the application on the Petri dish which bacteria was inoculated. Paper discs are affixed to each surface of the culture media that has been inoculated with bacteria using sterile tweezers. The petridish was inserted into the desiccator after which incubated for twenty-four hours at 37ºC in an upside-down position to prevent water vapor from falling into the media in order that it does now no longer intrude with bacterial growth. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the discs using a digital shear term with 0.01 mm accuracy and recorded. The method of measuring the diameter of the inhibitory zone was by measuring the length diameter (a) plus the short diameter (b) and then dividing it into two. The results of the research data were Shapiro-Wilk and Levene statistical tests using Statistical Product and Service Solution (SPSS) software. The resulting data were normally distributed and not homogeneous ($p < 0.05$) so that non–-parametric Kruskal Wallis statistical analysis was carried out ($p < 0.05$), and developed with the Mann–Whitney statistical test ($p < 0.05$).

RESULT

The research results found that there were inhibition zones around the disc paper in the research group P2 (50 %), P3 (75 %), P4 (100 %), and (K^+) (figure 1) of the inhibition of *Moringa oleifera* Lamk leaves extract on *P. gingivalis* growth. Based on observation and measurement, the average diameter of the inhibitory zone of each research group presented in Table 1 was obtained.

Table 1. Average diameter of antibacterial inhibition of Moringa leaf extract against *P. gingivalis*

Experimental Group	N	Inhibition Zone Diameter (X ± SD) (mm)
P1	4	0 ± 0
P2	4	5.41 ± 0.03
P3	4	5.91 ± 0.29
P4	4	6.40 ± 0.16
K^+	4	12.13 ± 0.48
K^-	4	0 ± 0

* P1: Leaves extracted concentration of 25 %
P2: Leaves extracted concentration of 50 %
P3: Leaves extracted concentration of 75 %
P4: Leaves extracted concentration of 100 %
K^+: Positive control (chlorhexidine gluconate 0.2 %)
K^- : Negative control (Steril Aquades)
N: Total number of samples
X: Average
SD: Standard deviation

Inhibition zone diameter of growth *P. gingivalis* data obtained was analyzed statistically. The first statistical test performed was the Shapiro–Wilk normality test. The results of the normality test of the data in this research indicate that the data is normal with $p > 0.05$. Then the homogeneity test showed the similarity of data. The homogeneity test shows the results of $p < 0.05$ which states that the variety of data is not homogeneous. The results of data analysis using Kruskal–Wallis showed $p = 0.00$ ($p < 0.05$), this means that the inhibition of *P. gingivalis* had a significant difference between the entire experimental group. These results mean that Moringa leaf extract can inhibit the growth of *P. gingivalis*. Then the Mann–Whitney test was performed to see the differences between the two research groups. The results of the Mann–Whitney difference test showed that there were

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significant differences ($p<0.05$) between the experimental groups except between groups in 25 % Moringa leaves extract concentration with negative controls, and between 75 % and 100 % Moringa leaves extract concentration groups.

DISCUSSION

Based on the results of the research, it was found that Moringa leaf extract had an inhibitory effect on *P. gingivalis*. The results of the research are suitable with the hypothesis that Moringa leaf extract has an inhibitory effect on *P. gingivalis*. The inhibitory effect is due to the content material of bioactive compounds in Moringa leaf extract, which can harm the protein synthesis system, harm to the cell wall, which reasons lysis ensuing in cell wall damage which can intervene with the mechanism of bacterial cell wall synthesis.[10] The active compounds include flavonoids, saponins, tannins, alkaloids, terpenoids, and triterpenoids.[8,9] Each of these compounds has different mechanisms to inhibit bacterial growth.

The antibacterial mechanisms of flavonoids are specifically as follows: nucleic acid synthesis inhibition, alteration in cytoplasmic membrane function, power metabolism inhibition, reduction in cell attachment and biofilm formation, inhibition of the porin on the cell membrane, converting of the membrane permeability, attenuation of the pathogenicity cytoplasmic membrane damage (probable with the aid of using hydrogen peroxide).[11] Saponins bind with cholesterol inside the cell making the saponin-cholesterol complex which ends ultimately in the lysing of the cells. Saponins disturb the permeability of bacterial cells by binding to the outer membrane.[12] Saponins can impair the permeability of the bacterial outer membrane. About 90% of the surface of the Gram-negative bacteria cell wall outer membranes that do not comprise natural cholesterol are protected with lipopolysaccharide (LPS). Saponins can interact with the lipid A a part of Proteus LPSs, thereby increasing the permeability of the bacterial cell wall due to their detergent-like properties. Theoretically, this activity can facilitate the influx of antibiotics via the bacterial cell wall membrane.[13] The antibacterial effectiveness of tannins is defined by their ability to pass through the bacterial cell wall up to the internal membrane, interfere with the metabolism of the cell, and - as a result—destroy. Tannic acid inhibits the bacteria's attachment to the surfaces. A loss of bacteria adhesion to the surface results in bacteria cell death. Moreover, the sugar and amino acid uptake are inhibited by tannic acid which limits the bacteria growth.[14,15] Alkaloid has an antibacterial mechanism by disrupting the bacterial cell membrane, affecting the DNA function, and inhibiting protein synthesis.[16] Terpenoids run by inhibiting essential techniques which can be vital to microbial survival, this consists of oxygen uptake and oxidative phosphorylation.[17] Another compound, triterpenoid has bioactivity as antibacterial bioactive compounds, because they have lipophilic compounds that can damage bacterial membranes.[18,19] Terpenoid compounds can form relatively strong polymeric bonds, interacting with transmembrane proteins (porins) positioned at the outer membrane of the bacterial cell wall. These bonds are strongly able to adverse the porin, lowering the permeability of the bacterial cell wall, thereby inhibiting the absorption pathway of bacterial nutrients. Nutrients that are inhibited will have an effect on stopping bacterial growth and killing bacteria.[20]

Moringa leaf extract with 50%, 75%, and 100% concentrations have the ability to inhibit the growth of *P. gingivalis.* The inhibition of 50% Moringa leaf extract concentration was smaller when compared with 75% and 100% Moringa leaf extract concentrations. This is likely because the material with a greater concentration has more active substances, so the inhibitory zone formed will be greater.[21] In 75 % and 100% Moringa leaves extract concentrations matter of fact to have similar ability to inhibit the growth of *P. gingivalis.* These results are not in accordance with the hypothesis that the largest concentration in inhibiting the growth of *P. gingivalis* is 100%, this might be because the concentration of Moringa leaves extract of 100 % is too thick, so it is difficult to be absorbed into the disc and unable to diffuse maximally in the media.[22]

The inhibition zone of 25% Moringa leaf extract concentration is zero which means that it cannot restrain the bacteria growth. This phenomenon is assumed that an antibacterial addictive substance in that dilution is still too little to be able to inhibit *P. gingivalis* growth.[23] The inhibition zone has begun to be seen at a concentration of 50% and continues to increase until a concentration of 100%. Gram-negative bacteria like *P. gingivalis* exhibit a toxicogenic ability through freeing endotoxins, which might be the lipopolysaccharide component (LPS) available in its outer cell wall. Endotoxins are launched into the tissues inflicting harm after the cell is lysed. There are three well-described areas inside the LPS: one in all, the lipid A, has a lipidic nature, and the opposite two, the Core and the O-antigen, have a glycosidic nature, they all with independent and synergistic functions.[24] Those lipopolysaccharides deliver Gram-negative bacteria greater resistance against antibiotics that cannot penetrate them.[25] Any alteration inside the outer membrane by Gram-negative bacteria like converting the hydrophobic properties or mutations in porins and different factors, can create resistance.[26,27] The presence of biofilms produced by *P. gingivalis* can also affect the level of penetration of antibacterial agents. It is well known that Biofilms are aggregates of microorganisms adherent to every different and/or to a surface and encapsulated inside a self-produced matrix. These organized communities constitute a significant health risk because of their resistance to host protection mechanisms and their reduced susceptibility to conventional antimicrobials.[28] Bacteria will be protected from surfactant surfaces, antibodies, and antibiotics by building a "guard self" on the top of the biofilm that can neutralize antibiotics enzymatically or release antibiotics based on structural changes. Changes in the microchemical environment in biofilms can also counteract the action of antibiotics. Changes in biofilm osmotic through the relative changes in porine can reduce the permeability of envelope cells to antibiotics.[29]

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The results of this research point out that 75% and 100% concentrations of Moringa leaf extract have smaller inhibitory power compared to the positive control of chlorhexidine gluconate 0.2%. This is possible that the ability of 0.2% chlorhexidine gluconate which has an anti-microbial impact on bacteria, fungi, and viruses causative to some distinctive oral diseases.[30] *In vitro*, the anti-bacterial effects of chlorhexidine all relate to altered cell membrane permeability. At low concentrations (0.02%-0.06%) chlorhexidine causes displacement of Ca^{2+} and Mg^{2+} and lack of K^{+} from the cell wall, resulting in a bacteriostatic impact. At excessive concentrations (>0.1%) chlorhexidine causes leakage of all the primary intracellular additives out of the cell, resulting in a bactericidal (cell lysis and death) impact.[30,31]

Antibacterial strength can be divided into three classes, namely (1) the diameter of the inhibition zone which is more than 6 mm is assessed as strong, (2) the 3 mm to 6 mm inhibition zone is classified as moderate, and (3) the inhibition zone which is less than 3 mm is classified as weak. Therefore, it can be concluded that 0.2 % chlorhexidine gluconate is classified as strong, and the strength of the inhibitory power of Moringa leaves extract is classified as moderate.[32]

CONCLUSION

1. Moringa leaf extract has the ability to inhibit the growth of *P. gingivalis*.
2. The concentration of Moringa leaf extract which has the highest inhibitory power to the growth of *P. gingivalis* is 75% and 100%.

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