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Differences of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Moringa Leaf Extract (*Moringa Oliefera L.*) on Bacteria *Aggregatibacter Actinomycetemcomitans* and *Porphyromonas Gingivalis*

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Abstract

Background: Moringa leaves (*Moringa oliefera L.*) has many nutrients that contain bioactive components such as tannins, flavonoids, and saponins, as antimicrobials. The growth of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas Gingivalis* must be inhibited so that they do not become pathogens and cause periodontitis. **Objective**: The general purpose of this study was to determine the Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) and inhibitory power of moringa leaf extract *(Moringa oliefera L.)* against bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. **Method**: The type of research used was laboratory experimental research. The design of this study is *post test only control group design* using the Kirby Bauer dilution method. With treatment extract concentration of 15%, 20%, 25%, 30%, 35% 100%, and positive control (Metronidazole). The measuring instrument in this study uses a caliper with millimeters (mm). **Results**: The results of the study of bacteria *Porphyromonas gingivalis* obtained from MIC by 20%, MBC 25% and bacteria *Aggregatibacter actinomycetemcomitans* obtained by MIC by 30% and MBC 35%. Kruskall Wallis test results showed that the value of $p<0.05$ so it can be concluded that there are significant differences in the inhibition zone of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. **Conclusion:** The extract of leaves of Moringa *(Moringa oliefera* L.) were able to inhibit bacterial growth *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

***Keywords**: Moringa leaves extract (Moringa oliefera L.), inhibitory power, bacteria Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis.*

Introduction

Periodontal disease is one of the most common dental and oral health problems in the community. Based on Indonesia's health profile in 2011, in 2010 there were 92,979 people who visited public hospitals belonging to the health ministry and local governments due to periodontal disease. Periodontal disease is an infection in the oral cavity that affects periodontal tissues. The main cause of this disease is microorganisms that colonize the surface of the tooth (bacterial plaque). Microorganism culture (bacteria) found in plaque shows the presence of certain gram negative bacteria in periodontitis.[1,2,3]

Periodontitis is an inflammation that affects the supporting tissues of a tooth, caused by microorganisms and can cause progressive damage to the periodontal ligament, alveolar bone and is accompanied by pocket formation.[4] Periodontitis causes permanent tissue

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destruction characterized by chronic inflammation, migration of the fused epithelium to the apical, loss of connective tissue and loss of alveolar bone.[5]

The main causes of periodontal disease are the presence of microorganisms that colonize the dental plaque. Dental plaque is a structured, soft, yellow substance, which is attached to the tooth surface. The content of dental plaque is various types of microorganisms, especially the remaining bacteria are fungi, protozoa and viruses. Plaque containing pathogenic microorganisms plays an important role in causing and exacerbating periodontal infections.[6] An increasing number of gram-negative organisms in subgingival plaques such as *Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, Tannerela forsythia* and *Treponema denticola* infect periodontal infection.[7] *Porphyromonas gingivalis* is a gram-negative anaerobic bacteria involved in the pathogenesis of periodontitis and other inflammatory diseases that destroy dental support tissue. These bacteria can invade the periodontal tissues locally and survive the host defense mechanism by utilizing a panel of virulence factors that cause deregulation of innate immune and inflammatory responses.[8] *Aggregatibacter actinomycetemcomitans* is a gram negative bacterium found in the oral cavity and one of the etiologies of aggressive periodontitis. This bacterium has the ability to produce leukotoxins which can cause damage to the periodontal tissues and one type of bacteria that is considered a periodontal pathogen.[9] Inappropriate and excessive use of antibiotics can result inbacteria being *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* resistant to antibiotic drugs that have been given. The resistance of these bacteria to antibiotic drugs allows the use of herbal medicines from natural ingredients to be one of the other alternatives in the treatment of periodontitis. The use of herbal medicines from natural ingredients is generally considered safer than the use of modern medicine, because herbal medicines as traditional medicines have relatively fewer side effects than modern drugs.[1]

Natural ingredients used as herbal medicines one of which is Moringa leaves or known as the Latin name *Moringa Oliefera*. Moringa plants are efficacious as anti-cancer, anti-bacterial, hypotensive, inhibiting the activity of bacteria and fungi. This is related to the chemical content contained in it, which is rich in vitamin A and vitamin C, gluconic compounds and isothiocinates.[10] Kelor leaves contain phytochemicals that make this plant capable of carrying out self-defense mechanisms. Phytochemicals contained include catechol tannins, galia tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, alkaloids, and reducing sugars.[11,12] The compound has the ability as a drug, its benefits are as antibiotics, skin care, anti-inflammatory, blood pressure, diabetes, and anemia.

Based on the description above, researchers are interested in conducting research to find out "The effect of Moringa leaf extract on bacterial growth, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* cause periodontitis.

Materials and Method

Type of research used in this study was a laboratory experimental study. The design of this study is *post test only control group design* using methods agar dilution and diffusion. This dilution method is used to determine the MIC (Minimum Inhibition Concentration) value, the lowest concentration that can inhibit the growth of test microbes. The dilution method is done by mixing the sample, the microbial test, and the inoculation media with several variations of dilution. The diffusion method to be used to determine the activity of antimicrobial agents or often also called the inhibitory test. This method uses disk paper that has contained Moringa leaf extract and then put it into a culture medium.

The Kirby Bauer method was carried out in observing certain inhibitory zone diameters and producing a good batch-to-batch, resulting in satisfactory growth of the most pathogenic bacteria. The treatment was carried out 4 times with a concentration of 15%, 20%, 25%, 30%, 35% 100%. The positive control used is Metronidazole. The research tools used are petri dishes, round oases, autoclaves, bunsen, erlenmeyer flasks, suction pipettes, filter devices, rotary evaporators, filter paper, sterile cotton swabs, stationery.

The materials used in the research were (*Moringa oleifera L.*), test bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Obtained from the Laboratory of Hasanuddin University Faculty of Medicine, sterile distilled water, 96% ethanol, Metranidazole, *Mueller Hinton Agar* (MHA), label paper and aluminum foil.[9] Samples of (*Moringa oliefera L.*) leaves were cleaned from the remaining dirt. After cleaning the moringa leaves are dried by air. After

drying, the sample is kept in a closed glass container. Oyster mushroom extract is obtained by maceration. The samples were in a closed container and then soaked with 96% ethanol solution and left for 5 days. After 5 days, the soaked sample is filtered using filter paper. The results of the filter are then evaporated using a rotary evaporator, so that a thick extract from the moringa leaf is obtained. The resulting thick extract is inserted into the vaporized container until all ethanol solvents evaporate.

Process of Testing Antimicrobial Effects of Diffusion Method

- 7 sterile test tubes were provided and Metranidazole. Dilution of the extract with sterile Aquadest was obtained to obtain concentrations of 15%, 20%, 25%, 30% and 35%, respectively as much as 5 mL.

- Each test tube is filled with the following conditions:

  Tube 1: 2.5 mL 15% extract + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 2: 2.5 mL 20% extract + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 3: 2.5 mL extract 25% + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 4: 2.5 mL 30% extract + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 5: 2.5 mL 35% extract + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 6: 2.5 mL extract 100% + 2.5 mL suspension *Porphyromonas gingivalis*

  Tube 7: 500 mg Metronidazole + suspension *Porphyromonas gingivalis* (positive control)

- Each test tube is filled with the following conditions:

  Tube 1: 2.5 mL 15% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 2: 2.5 mL of 20% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 3: 2.5 mL of 25% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 4: 2.5 mL 30% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 5: 2.5 mL of 35% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 6: 2.5 mL 100% extract + 2.5 mL suspension *Aggregatibacter actinomycetemcomitans*

  Tube 7: 500 mg Metronidazole + suspension *Aggregatibacter actinomycetemcomitans* (positive control)

Research Result

Minimum inhibitory effect of leaf extract Moringa *(Moringa oleifera L.)* against bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitan.*

The result of the tube dilution test is to see the turbidity level to determine the minimum inhibitory level. Tube dilution test results can be observed in Figures 1 and 2.

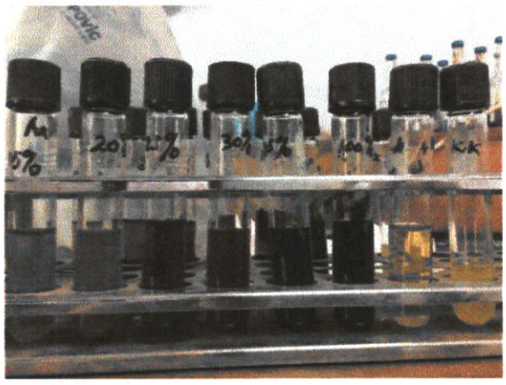

Figure 1. Dilution results of the tube *aggregatibacter actinomycetemcomitans*

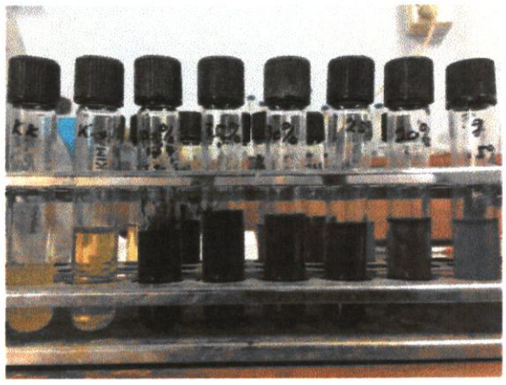

Figure 2. Dilution results oftubes *porphyromonas gingivalis*

From the results of tube dilution test observations in Figure 1 the Minimum Inhibitory Level (MIC) can be determined in 30% tubes starting to look clear and Figure 2 Minimal Inhibitory Levels (MIC) can be determined on the tube 20% starts to look clear.

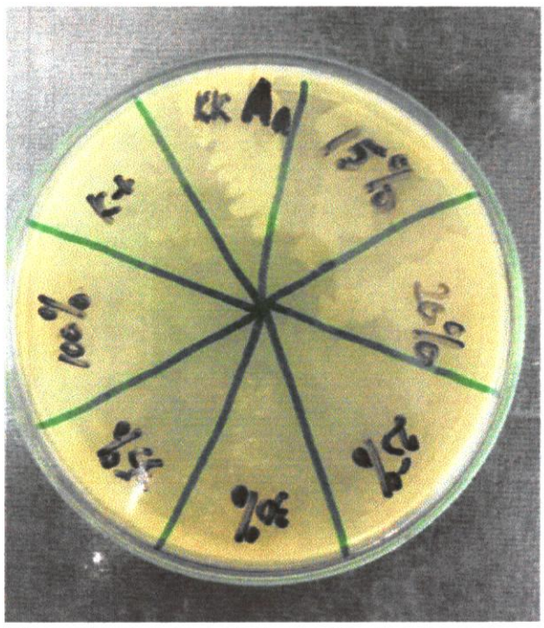

Figure 3 bacterial growth *Aggregatibacter actinomycetemcomitan*

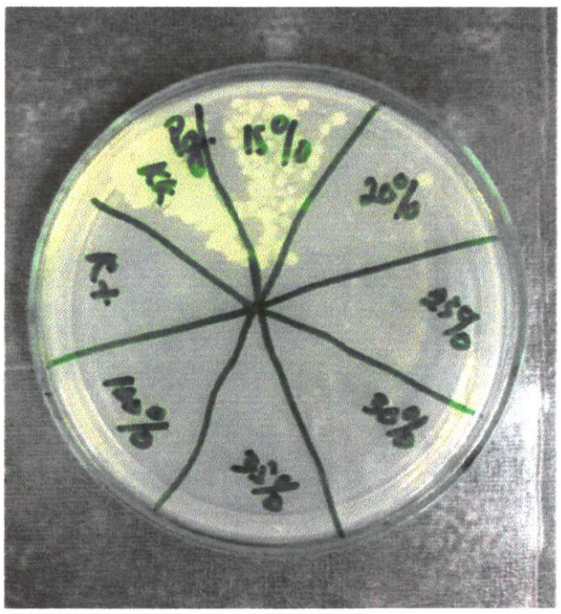

Figure 4 growth ofbacteria *Porphyromonas gingivalis*

Minimal Bactericidal Concentration of leaf extract *Moringa oleifera L.* against *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans.* From the results of the diffusion test observations in Figure 3, the Minimal Bactericidal Concentration (MBC) seen at a concentration of 35% shows that there is no growth of bacteria *Aggregatibacter actinomycetemcomitan* and in Figure 4 the Minimal Bactericidal Concentration (MBC) can be determined at a concentration of 25% .

Conclusion

The conclusions of this study are:

Extract (*Moringa oliefera L.)* can inhibit the bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

Minimal Inhibitory Concentration (MIC) of leaf extract (*Moringa oliefera L.) Moringa* which can inhibit bacteria *Porphyromonas gingivalis* is 20% with an average inhibition zone formed is 12.9 mm and for bacteria *Aggregatibactre actinomycetemcomitans* is 30% with an average zone inhibition of 10.2 mm.

The Minimal Bactericidal Concentration (MBC) of leaf extract killing (*Moringa oliefera L.)* which can inhibit bacteria *Porphyromonas gingivalis* is 25% with the average inhibition zone formed is 13.3 mm and for bacteria *Aggregatibacter actinomycetemcomitans* is 35% with zone average inhibition of 11.02 mm.

Suggestions

Need to be carried out further research onleaf extract (*Moringa oliefera L.)* with different methods to inhibit the growth of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans.*

Conflict of Interest: There is no conflict of interest in this study.

Source of Funding: Domestic government

Ethical Clearance: This study obtained a label of ethics escaped by the number: 0082/PL09/KEPKFKG - RSGMUNHAS/2018 and register number UH 17120068 on Oktober 9, 2018.

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