

Inhibitory Effectiveness of Jatropha Leaf (*Jatropha curcas* L.) Extract against *Porphyromonas gingivalis* Bacteria

Mardiana Adam¹, Harun Achmad², Ainun Isnaeni Ilham³

¹Lecturer of the Department of Periodontics, Faculty of Dentistry, Hasanuddin University, Indonesia

²Lecturer of the Department of Pediatric, Dentistry Faculty of Dentistry, Hasanuddin University, Indonesia

³Student of the Faculty of Dentistry, Hasanuddin University, Indonesia

Abstract: Introduction: Gambier (*Uncaria gambier* Roxb) contains catechins which are functional compounds in the class of polyphenol compounds that have potential as antioxidants and antibacterials. Several factors cause periodontitis, one of which is bacteria and the predominant cause is *Porphyromonas gingivalis* (*P. gingivalis*). Objective: The purpose of this study was to determine the effect of gambier extract (*Uncaria gambier* Roxb.) on the inhibitory growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*) and determine the Minimal Inhibitory Concentration (MIC) of gambier extract (*Uncaria gambier* Roxb) on the growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*) and determine the minimal inhibitory concentration (MIC) of gambier extract (*Uncaria gambier* Roxb) *Porphyromonas gingivalis*. Method: This type of research method used in this study is experimental laboratory research. The research design used is post-test only control group design using the dilution method and disk diffusion method, 50%, 75%, 100%, negative control (distilled water), and positive control (metronidazole). The measuring instrument used in this study is the millimeter (mm) united calipers. Results: The results showed that 15% MIC began to form inhibitory zones at the growth of *Porphyromonas gingivalis* of (8.00 ± 0.95 mm). The results of the Post Hoc LSD test for the zone of inhibition between treatment groups on the average *Porphyromonas gingivalis* bacteria showed a significant value ($p < 0.05$). Conclusion: Gambier extract (*Uncaria gambier* Roxb) has an inhibitory effect on the growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*).

Keywords: gambier extract (*Uncaria gambier* Roxb.), Inhibitory power, periodontitis, *Porphyromonas gingivalis* bacteria

1. Introduction

Tooth and mouth disease is the sixth-highest health problem that is often complained about by the people of Indonesia.¹ The 2013 Riskesdas data states that provinces with high enough dental and mouth problems reaching more than 35% are South Sulawesi, South Kalimantan, and Central Sulawesi provinces.²

Periodontal disease is one of the dental and oral diseases that are often found in the world community, especially in Indonesia.³ In Indonesia, periodontal disease has a high enough prevalence that affects many humans almost all over the world and reaches 50% of the adult population.⁴

Periodontal disease is an inflammation that occurs in the supporting tissues of teeth, including alveolar bone and periodontal ligaments. Periodontal disease that is often found in gum inflammation or gingivitis and periodontitis.⁵ Several factors cause periodontitis, one of which is bacteria and the predominant cause is *Porphyromonas gingivalis* (*P. gingivalis*).⁴

One of the efforts to prevent periodontitis is through the development of natural ingredients by using gambier extract (*Uncaria gambier* Roxb). Gambier extract is a product of the gambier plant (*Uncaria gambier* Roxb), containing functional compounds that are included in the group of polyphenol compounds in gambier, especially catechins which have the potential as antioxidants and antibacterials.⁶

Based on several studies of gambier, its relation to antibacterial properties by Magdalena et al (2015) states

that gambier extract has the ability to inhibit *Escherichia coli* bacteria at 100% extract concentration, *Salmonella typhimurium* at 90% extract concentration, *Staphylococcus aureus* at 90% extract concentration and *Bacillus cereus* at an extract concentration of 80%.⁷ Lucida et al (2010) stated that the 7% gambier extract contained in toothpaste has optimal antimicrobial power in inhibiting the growth of *S. mutans* bacteria as a cause of dental plaque formation.¹ But the role of gambier extract in inhibiting *Porphyromonas gingivalis* bacteria have not been described in these studies. Based on the description above, the writer wants to know the effect of gambier extract (*Uncaria gambier* Roxb) on the inhibitory growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*).

2. Method and Material

This type of research used in this study is experimental laboratory research. The research design used is the post-test only control group design using the dilution method and disk-diffusion method. The research was conducted at the Phytochemical Laboratory of the Faculty of Pharmacy, Hasanuddin University and the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University, from May to July 2019.

Making gambier extract with various concentrations. Gambier extract was made using maceration and rotary evaporator methods. Gambier obtained from the market is broken into small pieces and then weighed as much as 300g. Erlenmeyer flask is washed and sterilized with ethanol, then weighed gambier is put into the Erlenmeyer

flask and 1 L ethanol is added, after that, it is stored for 1 week. During storage, stir 2 times in 1 day.

After storing, 2 times of filtering were then obtained 1500 ml of the results in the screening of gambier. Macerated gambier was filtered and then rotary evaporated at 60°C for \pm 1 hour until thick extract was obtained. After that, leave it for 2 hours up to the extract is being dried.

The weighed gambier extract was then dissolved with 5 mL of a 10% DMSO solution so that a concentration of 15% was obtained; 25%; 50%; 75%; 100%. Then the concentration of gambier extract is put into a vial bottle and labeled according to its concentration.

Preparation of Porphyromonas gingivalis Suspension Bacteria.

Porphyromonas gingivalis pure cultures were obtained from the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University. The bacterial suspension was made in a test tube containing 9% NaCl by expressing the turbidity with Mc. Farlands 0.5 - 0.65 uses densiCHECK turbidity.

Determination of Minimal Inhibitory Concentration (MIC) of Porphyromonas gingivalis bacteria

Seven test tubes were prepared and filled with 5 mL BHIB medium. Then 0.2 mL of Porphyromonas gingivalis is inserted in each tube. After that, gambier extracts were put into 15%, 25%, 50%, 75%, 100% gambier extracts as much as 5 mL, one subsequent tube was put in positive control (Metronidazole) as much as 5 mL, and one more tube was filled with sterile aqua dest as a negative control. All tubes were incubated at 37°C for 24 hours and then examined for the presence or absence of bacterial growth of Porphyromonas gingivalis which is characterized by the occurrence of turbidity in the tube. The Minimal Inhibitory Concentration is determined by paying attention to the first clear-looking concentration. Tubes that look turbid indicate still bacterial growth. After determining the MIC, the inhibition test is ready to be carried out on a Petri dish using a paper disc.

Antibacterial activity test.

Tests carried out by the disk-diffusion method or the Kirby-Bauer diffusion method using discs. On the media which has been dense is spread as much as 0.2 mL of bacteria, which has been adjusted to the standard 0.5 Mc Farland evenly using a spreader bar. The first MHA media was divided into seven sections, each placed on a disc containing gambier extract, metronidazole, and negative control. The treatment is carried out up to four times. Then incubated at 37°C for 1x24 hours and bacterial growth was observed. The length of the inhibition zone formed is measured using the calipers in millimeters.

3. Result

Based on the research conducted, it is found that at a concentration of 15% the growth of Porphyromonas gingivalis begins to appear clearly which shows the formation of an inhibition zone. After obtaining the Minimum Inhibitory Concentration (MIC), further research

on the inhibitory test of gambier extract (*Uncaria gambier* Roxb) obtains that the measurement results of the inhibition zone diameter of *Porphyromonas gingivalis* bacteria as presented in **Table 1**.

Table 1: Measured diameters of the inhibition zone

Kind of Intervention	Concentration (%)	Power of Inhibitory (mm)				
		I	II	III	IV	Mean
Extract of gambir (<i>Uncaria gambier</i> Roxb.)	15%	6,7	8,1	8,2	9	8.00 \pm 0.95
	25%	8,1	9,2	9,6	10,7	9.40 \pm 1.07
	50%	9	10	10	12	10.25 \pm 1.25
	75%	10	10,1	10,6	12,5	10.80 \pm 1.16
	100%	11,5	11,2	11	13,9	11.90 \pm 1.34
Control (+) Metronidazole		10,2	11,9	12,3	13,8	12.05 \pm 1.47
Control (-) Aquadest		5,6	5,6	5,6	5,6	5.60 \pm 0

Based on the Shapiro-Wilk statistical test, the results are to determine the normality value which is resulted in p value > 0.05. It means the data is normally distributed so the test can be continued to the parametric test namely One-way ANOVA. Based on the One-way ANOVA statistical test it is found that the significance value is 0.000 (p < 0.05) which meant that there are significant differences between treatment groups.

4. Discussion

Minimal Inhibitory Concentration (MIC) is seen from the minimum concentration of experimental material that can inhibit bacteria after 24 hours incubation and does not show any macroscopic growth of bacteria that can be seen from the results of culture on tubes that begin to turn clear by using the dilution method. From the observations showed that at a concentration of 15% the growth of *Porphyromonas gingivalis* began to appear clear which showed that the inhibition zone began to form.

In the study of the inhibitory test using the extract of gambier (*Uncaria gambier* Roxb) on the growth of *Porphyromonas gingivalis*, it was seen that the inhibition zone that was formed increased in proportion to the increase in extract concentration. These catechins are flavonoids that can be found in green tea, black tea, gambier, grapes and other food plants such as fruits and cocoa.⁸ The mechanism of action of phenols is based on denaturation and deposition of bacterial cell proteins and deactivates enzymes.^{9,10,11,12}

Flavonoids are the largest phenol compounds in nature and have been known to have biological activities as antioxidants, anti-melanogenic and anti-mutagenic. Flavonoids are found in plant tissues such as fruits and vegetables that are consumed every day. Catechins are flavonoid compounds that can be found in green tea, black tea, grapes and other food crops such as fruits and cocoa.^{10,13,14}

The antibacterial properties of gambier are inseparable from the components it contains, namely catechins and gallic acid (tannins). Catechins can inhibit the growth of several types of bacteria and act as anti-carcinogenic, while tannins have properties as algicides, antibacterial and antifungal. The mechanism of action of phenols as an

antibacterial compound in controlling bacteria, including changing cell walls, changing cell permeability, denaturing cell proteins, inhibiting the work of enzymes and inhibiting protein-nucleic acid synthesis. The mechanism is according to the group of antibacterial materials used.^{9,15,16}

Catechins are weak acids, difficult to dissolve in water and are very unstable in the open air. However, if the catechin is warmed up long enough or heating with an alkaline solution will easily become catechin Tannat, because it condensates itself and becomes easily dissolved in cold water or hot water. Catechins are easily oxidized at near-neutral pH (pH 6.9) and more stable at lower pH (2.8 and 4.9). Catechins are also easily decomposed by light with a greater reaction rate at low pH (3.45) than pH 4.9; 3.2.^{17,18,19}

This research proves that gambier extract (*Uncaria gambier Roxb*) has antibacterial power against *Porphyromonas gingivalis* with a Minimum Inhibitory Concentration (MIC) of 15% with an average inhibition zone diameter of 8.00 ± 0.95 and the largest inhibitory zone formed in gambier extract (*Uncaria gambier Roxb*) 100% concentration with an average diameter of 11.90 ± 1.34 mm. Thus the research hypothesis is accepted.

5. Conclusions and Suggestions

Based on the results of research that has been done, it can be concluded that the extract of gambier (*Uncaria gambier Roxb*) has an inhibitory effect on the growth of bacteria that cause periodontitis (*Porphyromonas gingivalis*) with a Minimum Inhibitory Concentration (MIC) of 15% with an average inhibition zone diameter formed at 8.00 ± 0.95 and the largest inhibitory zone was formed in extracts of gambier (*Uncaria gambier Roxb*) concentration of 100% with an average diameter of 11.90 ± 1.34 mm.

Further research needs to be done on gambier extract (*Uncaria gambier Roxb*) more thoroughly and using different methods for the bacteria *Porphyromonas gingivalis* and also other bacteria.

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