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# ANTIBACTERIAL ACTIVITY OF RAMANIA LEAF EXTRACT (*Bouea Macrophylla Griff*) AGAINST THE GROWTH *Actinomyces spp*

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***ABSTRACT***
***Background:*** *One of the causes of tooth nerve death or pulp necrosis is microorganisms, namely is Actinomyces spp. Ramania leaf extract (Bouea Macrophylla Griff) contains secondary metabolites, namely triterpenoids, alkaloids, saponins, tannins, flavonoids, steroids, and phenolics which function to inhibit the growth of Actinomyces spp.* ***Purpose****: This study aims to analyze the antibacterial activity of ramania leaf extract (Bouea Macrophylla Griff) at concentrations of 6,25%, 12,5%, 25%, 50%, and 100% against the growth Actinomyces spp.* ***Methods:*** *This research is a pure experimental (true experimental), with Posttest Only with Control Group Design. Antibacterial activity test used a paper disc diffusion with seven treatments, namely ramania leaf extract (Bouea Macrophylla Griff) with a concentration of 6,25%, 12,5%, 25%, 50%, and 100%, Chlorhexidine gluconate 2% as a positive control and Aquades as a negative control of Actinomyces spp were repeated 5 times. Measuring the diameter of the clear zone formed on Mueller Hinton Agar (MHA) media using a caliper.* ***Results:*** *Based on the results of the Mann Whitney test, there was 1 pair of groups that did not differ significantly in inhibiting Actinomyces spp bacteria, namely the 25% concentration group with a positive control of 2% Chlorhexidine gluconate, while the other groups had significant differences from each other.* ***Conclusion:*** *There is antibacterial activity in ramania leaf extract (Bouea Macrophylla Griff) against Actinomyces spp.*

***Keywords****: Actinomyces spp, Antibacterial Activities, Chlorhexidine gluconate 2%, Ramania Leaf Extract.*
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## INTRODUCTION

Based on the Pusdatin of the Ministry of Health (2018), the prevalence of dental caries in Indonesia is 88.8%, with the prevalence of caries in the roots of teeth at 56.6%. According to data obtained by the Ministry of Health, it shows that the most prevalent dental and oral diseases in Indonesia are caries and periodontal disease. According to the Indonesian Ministry of Health, the prevalence of active dental caries is quite high, in Kalimantan around 80.2%, Sumatra 65.4%, and the lowest, 56.8%, is in Bali and Java.[1,2] Caries is an abnormality in the hard tissues of the teeth, involving the enamel, dentin, and cementum, caused by the acidic activity of bacteria and the fermentation of carbohydrates. Caries that are not treated immediately can cause reduced blood flow to the pulp, which can lead to nerve death or pulp necrosis. Treatment that can be done to treat pulp necrosis is root canal treatment.[3,4]

Root canal treatment is carried out to remove microorganisms present in the root canals of teeth or substantially reduce the work of microbes.[3] Irrigation is one of the important factors in achieving the success of root canal treatment. If irrigation is not carried out properly, reinfection and root canal failure will occur.1. One of the dominant bacteria in root canal infections is Actinomyces spp.[5]

Actinomycess spp. bacteria are the main focus in root canal treatment because these bacteria play an important role in the pathogenesis of necrotic teeth. Elimination of these bacteria can be carried out by root canal irrigation procedures.[5] The

ideal irrigation material has non-toxic properties, can inhibit the reproduction of microorganisms, remove the smear layer, can dissolve necrotic pulp tissue during the root canal preparation stage, and does not change tooth color.[3]

One of the ingredients for root canal irrigation is chlorhexidine gluconate 2%. Chlorhexidine gluconate 2% has good antimicrobial properties because it can bind to the tooth root canal wall. The disadvantages of chlorhexidine gluconate irrigation material are that it is not able to remove biofilm in tooth root canals, it is less able to dissolve organic and inorganic tissue, and the price is quite expensive, so an alternative root canal irrigation material is needed that is easily available, is able to remove biofilm in dental root canals, and is economical. namely, by utilizing the potential of medicinal plants in Indonesia.[6,7]

Ramania plant, also known as gandaria, is a swamp plant native to South Kalimantan.Utilization of ramania leaves is still very limited, because many do not know the content contained in ramania leaves. Based on the phytochemical test of the ethanolic extract of ramania leaves, it was found that the largest secondary metabolite content in ramania leaves is triterpenoids, flavonoids, saponins, phenols, alkaloids, and steroids. The content has antibacterial, antioxidant, antifungal, and anticancer benefits.[8,9]

The results showed that the antibacterial activity of ramania leaf extract (Bouea Macrophylla Griff) had a good response in inhibiting Staphylococcus aureus bacteria, with the largest average inhibition zone of 16.7 mm at a concentration of 100%.[10] Antibacterial activity using concentrations of 12.5%, 25%, 50%, and 100% resulted in an average inhibition zone of 10.79 mm, 11.60 mm, 13.75 mm, and 14.76 mm, respectively. The results of this study indicate that the higher the concentration used, the greater the antibacterial activity obtained.[11]

There is still no information regarding the antibacterial activity of ramania leaf extract (Bouea macrophylla Griff) against Actinomyces spp. So that it becomes the basis for conducting research on ramania leaf extract (Bouea macrophylla Griff) with concentrations of 6.25%, 12.5%, 25%, 50%, and 100% having antibacterial activity in inhibiting the growth of Actinomyces spp. predominant in pulp necrosis.

## MATERIAL AND METHODS

This research has been approved and authorized by the Ethics Commission of the Faculty of Dentistry, University of Lambung Mangkurat No. 013/KEPKG-FKGULM/EC/III/2022. This research method uses a pure experimental design with a post test design with control group design consisting of 7 treatments, including ramania leaf extract (Bouea Macrophylla Griff) with a concentration of 6.25%, 12.5%, 25%, 50%, 100%, Chlorhexidine gluconate 2% as a positive control and distilled water as a negative control. Each treatment group was repeated 5 times. The manufacture of ramania leaf extract and the antibacterial activity test using the diffusion method were carried out at the Biomedical Laboratory of FKG ULM.

This research uses tools such as oven, petri dish, autoclave, analytical balance, test tube, erlenmeyer, measuring cup, pipette, micropipette, sterile tube, WH10 filter paper, blender, mixing rod, magnetic stirrer, measuring flask, millimeter scale caliper, cotton sterile sticks, Bunsen lamp, Hanscoon, masks and markers. The main ingredient used in this research is ramania leaf (Bouea Macrophylla Griff) which comes from Banjarbaru, South Kalimantan. Bacteria Actinomyces spp (ATCC 19246), Chlorhexidine gluconate 2%, sterile distilled water, Brain Infusion Heart Broth (BHIB) culture media, Mueller Hinton Agar (MHA) medium, Mc Farland solution 0.5, acetic acid, concentrated sulfate and 96% ethanol solvent.

### Ramania Leaf Extraction

Preparation of extracts of ramania leaves (Bouea macrophylla Griff) using the maceration method. The selected leaves are dark green ramania leaves (4th leaf from the top to 5th leaf before the base of the twig) with a length range of 11-45 cm and a width of 4-13 cm.[12] The concentration of ramania leaf extract was obtained using the dilution formula:

$$V_1N_1 = V_2.N_2$$

**Information:**

$V_1$= Volume of solution to be diluted (ml)
$N_1$= The concentration of the ramania leaf extract available (%)
$V_2$= volume of solution water and extract which desider (ml)
$N_2$= The concentration of the ramania leaf extract will be created (%)

### Diffusion Method Antibacterial Activity Test

Soaking the blank paper disks into each tube containing ramania leaf extract (Bouea macrophylla Griff) with concentrations of 6.25%, 12.5%, 50%, 100%, and 2% chlorhexidine gluconate for 3 hours. The next step is to apply the Actinomyces spp. bacteria that have complied with the standard Mc Farland 0.5 (1.5x108) using a sterile cotton swab on the MHA media. After that, put a paper disk that has been soaked in ramania leaf extract (Bouea macrophylla griff) concentration 6, 25%, 12.5%,

50%, 100%, Chlorhexidine gluconate 2% and distilled water on Mueller Hinton Agar media containing Actinomyces spp. Mueller Hinton Agar (MHA) Incubated for 24 hours at 37ºC, after which the zone of inhibition of the growth of Actinomyces spp. bacteria was measured using a caliper.

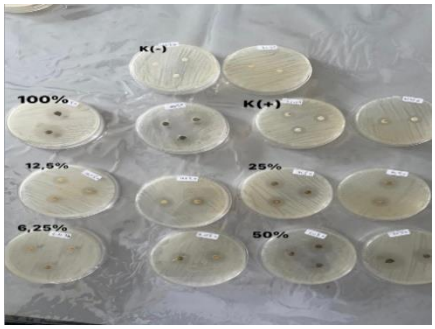
## RESULT

The results of the study on the average diameter of the inhibition zone of antibacterial activity of ramania leaf extract (Bouea macrophylla griff) against Actinomyces spp. bacteria from each treatment group can be seen as follows:

**Table 1.** Results Table Average Value and Standard Deviation of Antibacterial Avtivity of Ramania Leaf Extract (Bouea Macrophylla Griff), 2% chlorhexidine gluconate and aquadest against Actinomyces spp.

| Treatment Group | n | Mean ± *Standar Deviasi*(mm) |
|---|---|---|
| **6,25%** | 5 | 9,5 ± 0,50 |
| **12,5%** | 5 | 13,1 ± 0,54 |
| **25%** | 5 | 14,7 ± 0,97 |
| **50%** | 5 | 21,7 ± 0,67 |
| **100%** | 5 | 24,7 ± 1,60 |
| **CHX 2%** | 5 | 15,4 ± 0,82 |
| **Akuades** | 5 | 0 ± 0 |

Based on table 1, it was found that the ramania leaf extract at each concentration had an average diameter of the inhibition zone that varied. The average diameter of the inhibition zone of group 1 ramania leaf extract at a concentration of 6.25% was 9.5 mm. The average diameter of the inhibition zone of group 2 ramania leaf extract at a concentration of 12.5% was 13.1 mm. The average diameter of the inhibition zone of group 3 ramania leaf extract at a concentration of 25% was 14.7 mm. The average diameter of the inhibition zone of group 4 ramania leaf extract at a concentration of 50% was 21.7 mm. The average diameter of the inhibition zone of group 5 ramania leaf extract at 100% concentration was 24.7 mm. The average diameter of the inhibition zone of group 6 positive control chlorhexidine gluconate 2% was 15.4 mm. The average diameter of the inhibition zone of group 7 negative aquadest control was 0 mm. The results of this study indicate that there is antibacterial activity in each concentration of ramania leaf extract (Bouea Macrophylla Griff) against Actinomyces spp.




**Picture 1**. Inhibition Zone of Ramania (Bouea Macrophylla Griff) Leaf Extract, Chlorhexidine gluconate 2% and Aquades against Actinomyces spp bacteria with 5 repetitions.

The data obtained were then statistically analyzed using SPSS 26.0. The normality test of the data used the Shapiro Wilk test because the sample was <50. The results of the normality test of the data obtained p <0.05, meaning that the data was not normally distributed, then the data was continued with the Kruskal Wallis nonparametric test. The result of the calculation using the Kruskal Wallis nonparametric analysis test is p <0.05, meaning that there are differences in each treatment group, so that the Mann Whitney Post Hoc Test can be continued to determine which group shows a significant difference.

**Tabel 2.** Mann Whitney Test Results Antibacterial Activity of Ramania (Bouea macrophylla griff) Leaf Extract, 2% chlorhexidine gluconate and Aquades against Actinomyces spp.

| Perlakuan | EDR 6,25% | EDR 12,5% | EDR 25% | EDR 50% | EDR 100% | CHX 2% | Akuades |
|---|---|---|---|---|---|---|---|
| EDR 6,25% | | 0,008* | 0,008* | 0,008* | 0,008* | 0,008* | 0,008* |
| EDR 12,5% | | | 0,032* | 0,008* | 0,008* | 0,008* | 0,008* |
| EDR 25% | | | | 0,008* | 0,008* | 0,548 | 0,008* |
| EDR 50% | | | | | 0,032* | 0,008* | 0,008* |
| EDR 100% | | | | | | 0,008* | 0,008* |
| CHX 2% | | | | | | | 0,008* |
| Akuades | | | | | | | |

Based on the table above, it can be seen that there was only 1 pair of groups that did not differ significantly in inhibiting Actinomyces spp. bacteria (p>0.05), namely the 25% concentration group with a positive control of 2% chlorhexidine gluconate, while the other groups had significant differences from each other.

## DISCUSSION

The results of the antibacterial activity of 6.25%, 12.5%, 25%, 50%, and 100%

concentrations of ramania leaf extract against the growth of Actinomyces spp. bacteria using the diffusion test method showed that the ramania (Bouea Macrophylla griff) leaf extract concentrations of 6.25%, 12.5%, 25%, 50%, and 100% had antibacterial activity in inhibiting the growth of Actinomyces spp. Based on the results of the research that has been carried out, it was found that at the 100% concentration of ramania leaf extract, the average diameter of the inhibition zone was 24.7 mm, which exceeded the concentration of 6.25% of Ramania leaf extract by 9.5 mm, 12.5% by 13.1 mm, 25% by 14.7 mm, 50% by 21.7 mm, and chlorhexidine gluconate 2% by 15.4 mm as positive controls. This is in accordance with the higher concentration, the more secondary metabolites contained, so that the inhibition zone obtained is greater, so that 100% concentration of ramania leaf extract in inhibiting the growth of Actinomyces spp has an average inhibition zone that is higher than the concentrations of 6.25%, 12.5%, 25%, and 50%.[11]

The secondary metabolites in ramania leaves are flavonoids, saponins, phenols, alkaloids, steroids, and triterpenoids. The triterpenoid compounds contained in ramania leaves are 329,46 mg/g. The mechanism of triterpenoids that function as antibacterials is by the reaction of porins (trans membrane proteins) on the outside of the bacterial cell wall, resulting in strong polymer bonds and decreased cell wall permeability, which will affect the lack of nutrient intake into cells. This will result in damage to the cell wall pore and bacterial cell lysis.[13,14]

The mechanism of action of alkaloids that contain nitrogen in ramania leaves can bind to amino acid intracellular compounds that function to protect bacterial cell walls and bacterial DNA. This reaction causes the composition of the intracellular substance of amino acids to be damaged. Furthermore, the genetic balance in bacterial DNA and biosynthesis in bacterial cells is disrupted, resulting in the death of bacterial cells.[15]

The mechanism of saponins in inhibiting Actinomyces spp. bacteria is by causing protein leakage in bacterial cells.[13] Saponin compounds work by lowering the tension on the surface of the cell wall membrane, resulting in increased permeability and cell leakage so that important compounds spread out.[15] Substances that come out of the cell, such as amino acids, enzymes, organic ions, and nutrients, When organic ion enzymes leave the cell together with important substances such as water and nutrients, it can cause metabolism to be hampered, resulting in a decrease in ATP required for cell growth. Furthermore, cell proliferation is disrupted, which causes bacterial lysis.[16]

Tannin and phenol compounds have the ability to inhibit bacteria by eliminating the protein-binding structure of bacterial cells.[13] Flavonoids are compounds that can disrupt the cytoplasmic membrane and damage the hydrogen attachment originating from the DNA chain. This causes damage to the lipid structure of the DNA of the bacterial cell nucleus, resulting in inhibition of the development or growth of bacteria, and cell death.[7,17] Steroid compounds in ramania leaf extract function as antibacterials, namely by binding to cell phospholipid membranes that have permeable properties to lipophilic compounds, causing the membrane strength to decrease and causing damage to the cytoplasmic membrane of bacterial cells.[15,18] Similar to steroids, chlorhexidine gluconate 2% also has the ability to damage the cytoplasm of bacterial cells, namely by the interaction between the positive charge of the chlorhexidine molecules against the negatively charged bacterial cell wall, which then causes penetration into the cytoplasm. The cytoplasmic membrane becomes damaged and semipermeable, causing leakage of cell components so that the bacterial cell dies.[17] The conclusion of this study is that ramania leaf extract (Bouea macrophylla griff) has antibacterial activity against Actinomyces spp. bacteria, so it has the potential to be used as an alternative material to support root canal irrigation.

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