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**ANTIFUNGAL ACTIVITY OF RAMANIA LEAF EXTRACT (*Bouea Macrophylla Griff*)  
 AGAINST *Candida albicans***

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**ABSTRACT**

**Background:** Root canal infection is a polymicrobial infection that causes pulpitis or localized pulp inflammation. Root canal infection occurs due to the presence of pathogenic microorganisms in the root canal. One of the most common pathogenic microorganisms found in root canals is the fungus *Candida albicans*. Treatment that can be done to treat pulp tissue infection is endodontic treatment. Chlorhexidine gluconate 2% is one of the irrigation materials that can be used in root canal treatment. Ramania leaf extract contains compounds such as triterpenoids, phenols, flavonoids, steroids, saponins, alkaloids, and tannins that can function as antifungals. **Purpose:** Measuring and analyzing the antifungal activity of ramania leaf extract (*Bouea Macrophylla Griff*) on the growth of *Candida albicans*. **Method:** This study is a true experimental study with a post-test-only design with a control group design consisting of 7 treatment groups and repeated 4 times. The antifungal activity was assessed from the inhibition zone formed on Sabouraud Dextrose Agar media by the diffusion method. **Results:** Based on the Mann-Whitney test showed that there was only 1 pair of groups that did not have a significant difference, namely 100% concentration of ramania leaf extract with 2% chlorhexidine gluconate while the other groups had significant differences from each other. **Conclusion:** Ramania leaf extract concentrations of 6.25%, 12.5%, 25%, 50%, and 100% had antifungal activity in inhibiting the growth of the fungus *Candida albicans*.

**Keywords:** Antifungal Activity, *Candida albicans*, Chlorhexidine gluconate 2%, Ramania Leaf Extract.

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**INTRODUCTION**

Dental and oral disease is one of the most common diseases in society. Based on the 2018 Riskesdas report, 57.6% of Indonesians experienced dental and oral health problems, namely caries or cavities and periodontal disease. Caries that are not treated immediately can lead to root canal infections. Root canal infection is a polymicrobial infection that causes pulpitis or localized pulp inflammation.<sup>1,2</sup> Infection in the root canal occurs because of the presence of pathogenic microorganisms in the root canal. One of the most common pathogenic microorganisms found in root canals is the fungus *Candida*. Based on the percentage found that *Candida albicans* causes root canal treatment failure, that is 36.7%.<sup>3</sup>

Eliminating *Candida albicans* is the main focus of root canal treatment because *Candida albicans* is one of the fungal species that can cause pulp disease and failure of root canal treatment. Pulp tissue that has experienced infection can be treated with a treatment consisting of three stages or also called the endodontic triad. The three stages of root canal treatment are biomechanical preparation accompanied by irrigation, disinfection or sterilization of the root canal, and

obturation or filling of the root canal.<sup>4</sup> The purpose of irrigation in root canal treatment is to dissolve necrotic tissue, clean the root canal and remove the smear layer to facilitate the filling of root canal treatment materials and eliminate or kill microorganisms in the root canal.<sup>3,5</sup> Using irrigants in root canal treatment should be used irrigants that have antiseptic properties, inhibit the metabolism or reproduction of microorganisms, sterilize root canals, dissolve the smear layer, do not change tooth color, have no odor, have low toxicity, and are economical.<sup>6</sup>

One of the root canal irrigation materials commonly used in root canal treatment is 2% Chlorhexidine gluconate with a concentration of 2%. 2% Chlorhexidine gluconate is used as a root canal irrigation solution because the solution has the advantage of being able to adhere to the root canal wall which lasts a long time and has good antimicrobial.<sup>7</sup> Another advantage possessed by 2% chlorhexidine gluconate is that it does not cause erosion so it does not irritate the periapical tissue and also has low toxicity. The disadvantages of 2% Chlorhexidine gluconate irrigation material are that it is less able to dissolve necrotic tissue and remove biofilm in root canals and

also long-term use will cause tooth discoloration.<sup>1,6,7</sup> Therefore, alternative supporting materials are needed that can eliminate microorganisms and have no side effects on root canals by using medicinal plants.

One of the plants that can be used as an antifungal alternative in root canal treatment is ramania leaf which in Latin is called *Bouea Macrophylla* Griff.<sup>8,9</sup> Ramania leaves contain compounds that have antifungal properties. Phytochemical test research states that secondary metabolites contained in ramania leaf extract are triterpenoids, saponins, alkaloids, phenols, flavonoids, steroids, and tannins. The content of the compounds formed besides having the ability as antifungal also shows activities such as antihistamines, diuretics, antihypertensives, antivirals, anti-radicals, insecticides, antioxidants, antibacterials, and anti-inflammatory.<sup>10</sup>

The results of the bacterial test activity of ramania leaf extract (*Bouea Macrophylla* Griff) against the growth of *Staphylococcus aureus* bacteria at concentrations of 10% and 15% had a moderate response with the results of inhibition zone diameters of 10.70 mm and 10.83 mm while concentrations of 20%, 25%, 40%, 80%, and 100% had a strong response in inhibiting the growth of *Staphylococcus aureus* bacteria resulting in the diameter of the inhibition zone formed at the highest concentration of 100% at 16.07%.<sup>11</sup> this study aimed to analyze the antifungal activity of the extract of ramania (*Bouea Macrophylla* Griff) leaves at concentrations of 6.25%, 12.5%, 25%, 50% and 100% against the fungus *Candida albicans*.

## MATERIALS AND METHODS

This research was conducted at the Biomedical Laboratory of the Faculty of Dentistry, Lambung Mangkurat University. This study begins with an ethical feasibility procedure, namely by submitting it to the ethics committee at the Faculty of Dentistry, Lambung Mangkurat University, and has been declared eligible based on the letter of ethical feasibility number: 012/KEPKG-FKGULM/EC/III/2022. This research uses a true experimental research method with a post-test only with a control group design. The samples used in this study were isolates of the fungus *Candida albicans* (ATCC 10231) and ramania leaf extract (*Bouea Macrophylla* Griff). with concentrations of 6.25%, 12.5%, 25%, 50%, and 100%. The control used in this study was 2% chlorhexidine gluconate as a positive control and distilled water as a negative control. The number of samples used in this study was based on an unpaired numerical comparative formula with more than two treatment groups consisting of 4 repetitions in each treatment group.

### Ramania Leaf Extract

Ramania leaf extract (*Bouea Macrophylla* Griff) was prepared using the maceration method. Ramania leaves were taken in Banjarbaru City, South

Kalimantan, and selected green leaves, were not damaged, not wilted, and free of pests. The 4th leaf from the top to the 5th leaf before the bottom nags as much as 1.<sup>12</sup> Furthermore, cleaning is carried out and the leaves are cut and then dried using a memmert tool, then grinded to a powder and weighed. The powder was then macerated in a container with 96% ethanol solvent until all the powder was completely submerged for 3 days. The extract resulting from the maceration was collected together and then evaporated. Evaporation was carried out using a memmert device at a temperature of 30-400C so that a thick ramania leaf extract was obtained. Then, the dilution was carried out at each concentration using the formula:

$$V_1 \cdot N_1 = V_2 \cdot N_2$$

V1 = Initial volume

N1 = Initial Concentration

V2 = Final Volume

N2 = Final Concentration

### Diffusion Method Inhibitory Activity Test

The diffusion test was carried out by applying the *Candida albicans* fungus which had been standardized with Mc Farland using a sterile cotton swab on SDA media. Soak the blank paper disc into each concentration of 6.25%, 12.5%, 25%, 50%, 100%, 2% chlorhexidine gluconate and distilled water for 3 hours. Place the soaked paper disc on the Sabouraud Dextrose Agar (SDA) media containing the fungus using tweezers and incubate the SDA with a temperature of 370C for 24 hours in the incubator and measure the inhibition zone for *Candida albicans* growth formed around the paper disc using a calliper measuring instrument.

## RESULTS

The results of the research on the antifungal activity of ramania leaf extract concentrations of 6.25%, 12.5%, 25%, 50%, 100%, chlorhexidine gluconate 2%, and aquadest against the fungus *Candida albicans* with the diffusion method obtained an average value average as follows:

**Table 1.** Average Results of Inhibition Zone Treatment Group Against *Candida albicans*

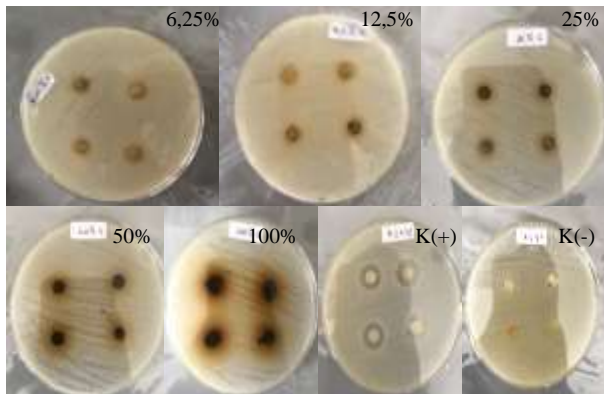
Treatment Groups	N	Mean ± Std. Deviation (mm)
EDR 6,25%	4	5,8 ± 0,25
EDR 12,5%	4	6,8 ± 0,25
EDR 25%	4	8,5 ± 0,40
EDR 50%	4	10,7 ± 0,95
EDR 100%	4	14,1 ± 1,03
CHX 2%	4	13,8 ± 1,31
Aquadest	4	0,0 ± 0,0

Description:

EDR: Ramania Leaf Extract

CHX: Chlorhexidine Gluconate

The results of the average diameter of the inhibition zone formed varied in each treatment group. Based on these results, it can be seen that the highest average diameter of the inhibition zone was found at 100% EDR, which was 14.1mm and the lowest average diameter of the inhibition zone was the negative control of distilled water. The results of this study indicate that there is an activity in each concentration of ramania leaf extract (*Bouea Macrophylla* Griff) on the growth of the fungus *Candida albicans*.



**Figure 1.** Inhibitory Zone Formed From Each Treatment With 4 Repetitions.

The data obtained from each treatment were then analyzed statistically using SPSS 26.0. First, the data normality test was carried out using the Shapiro-Wilk test. The results of the normality test of the data were obtained ( $p < 0.05$ ), which means that the data was not normally distributed, then continued with the non-parametric Kruskal Wallis test. Based on the results of the Kruskal Wallis test, it was obtained ( $p < 0.05$ ) which means that there was a significant difference in the average diameter of the inhibition zone for each treatment group, then the Post Hoc Mann Whitney test was continued to determine which group gave a significant difference. The results of the Mann-Whitney test showed that there was only 1 pair of groups that did not have a significant difference, namely the 100% concentration of ramania leaf extract with 2% Chlorhexidine gluconate. While the other groups have significant differences or significant differences from each other.

**Table 2.** Mann Whitney Test Results Antifungal Activity of Ramania Leaf Extract (*Bouea Macrophylla* Griff) against *Candida albicans*.

Perlakuan	EDR 6,25%	EDR 12,5%	EDR 25%	EDR 50%	EDR 100%	K+	K-
6,25%		0,015*	0,017*	0,017*	0,017*	0,017*	0,011*
12,5%			0,017*	0,017*	0,017*	0,017*	0,011*
25%				0,019*	0,019*	0,019*	0,013*
50%					0,019*	0,019*	0,013*
100%						0,642	0,013*
K+							0,013*
K-							

The results of the Mann-Whitney test showed that there was only 1 pair of groups that did not have a significant difference, namely 100% concentration of ramania leaf extract with 2% Chlorhexidine gluconate, which means that the largest inhibition zone, namely 100% concentration, contained compounds equivalent to chlorhexidine gluconate 2% so that Ramania leaf extract can be used as an alternative to supporting irrigation materials in root canal treatment.

## DISCUSSION

Based on the results of the study, it was shown that the concentration of ramania leaf extract (*Bouea Macrophylla* Griff) was 6.25%, and 12.5%, 25%, 50%, and 100% had antifungal activity in inhibiting the growth of *Candida albicans*. The results of research conducted on 100% concentration of ramania leaf extract had the strongest antifungal activity against the growth of *Candida albicans* because it had an average diameter of the inhibition zone of 14.1 mm which exceeded the ramania leaf extract at concentrations of 6.25%, 12.5%, 25%, 50%, 100%, and 2% chlorhexidine gluconate as positive controls.

The inhibition zone category according to David-Stout consists of 4 categories. Weak category if the inhibition zone formed is  $< 5$  mm. Medium category if the resulting inhibition zone of 5 to 10 mm. The strong category is 11 to 20 mm, and if  $> 20$  mm the category is very strong.<sup>13</sup> Based on this category, the inhibitory activity of 6.25%, 12.5%, and 25% ramania leaf extract concentrations were classified as moderate. The concentrations of 50%, 100%, and 2% chlorhexidine gluconate are classified as strong categories.

Based on the results of the research, the concentration of 6.25%, 12.5%, 25%, 50%, and 100% of ramania leaf extract increased the inhibitory power. This can be influenced by one of them because there are components of active compounds contained in the extract of ramania leaves, where the higher the

concentration used, the greater the components of secondary metabolites contained in an extract so the resulting inhibitory power will be even greater.<sup>11,14</sup>

Secondary metabolites contained in ramania leaves are triterpenoids, saponins, alkaloids, phenols, flavonoids, steroids, and tannins.<sup>15</sup> The mechanism of action of triterpenoids as antifungals is by inhibiting the growth and development of the cytoplasmic membrane of fungal spores.<sup>16</sup> Another mechanism of triterpenoid compounds in damaging fungal cells is by interfering with nutrient transport activities so that the cell membrane will lack nutrients and cell lysis or death occurs.<sup>16,17</sup>

The mechanism of action of saponins as antifungals is by breaking down fats in their cell membranes. This happens because saponins are polar surfactants. Lipids that break will result in disruption of the permeability of the fungal cell membrane, resulting in the diffusion process of materials or substances needed by the fungus can be disrupted, as a result, the fungal cells will burst. Damage to the cell membrane also causes cell leakage and will release important components in fungal cells such as proteins, nucleic acids, and nucleotides.<sup>18</sup> This saponin compound has the same mechanism as Chlorhexidine gluconate as an antifungal where Chlorhexidine gluconate also works by breaking down lipids in the cell membrane so that when it binds to the fungal cell membrane it will cause changes in the integrity of the cell wall. Changes in the integrity of the cell wall cause the function of the fungal cell membrane to be lost and lysis occurs.<sup>19,20</sup>

The mechanism of action of alkaloids as antifungals is by inhibiting the growth of protein formation, interfering with cellular respiration, and causing fungal cells to lyse or die.<sup>21</sup> Alkaloids can cause damage and death of fungi because they have a strong bond with ergosterol causing leakage of cell membranes.<sup>22</sup>

The mechanism of phenol as an antifungal is by inhibiting the synthesis of chitin cells for the formation of cell walls and damaging fungal cell membranes.<sup>22</sup> The mechanism of action of flavonoids as antifungals is by damaging the permeability of cell wall membranes and fungal extracellular proteins.<sup>21</sup> The mechanism of action of steroids as antifungals is by damaging the lipid membrane so that liposomes.<sup>16</sup> is by damaging the lipid membrane so that liposomes.<sup>23</sup> The conclusion of this research is There was the antifungal activity of ramania leaf extract (*Bouea Macrophylla* Griff) at concentrations of 6.25%, 12%, 25%, 50%, and 100% against the growth of the fungus *Candida albicans*.

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