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ANTIBACTERIAL ACTIVITY OF RAMBAI LEAF EXTRACT (*Sonneratia caseolaris*) CONCENTRATION 25% 50% 75% AND 100% AGAINST BACTERIA *Enterococcus faecalis*

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ABSTRACT
Background*: The causative factor of failure in the treatment of the root canal is the presence of microorganisms, namely the bacterium Enterococcus faecalis. Rambai leaf extract (Sonneratia caseolaris) also contains secondary metabolite compounds, namely, triterpenoids, phenols, tannins, steroids, and flavonoids that are able to inhibit the growth of Enterococcus faecalis bacteria which have the potential to cause failure in root canal treatment.* ***Purpose:*** *This research has a purpose to see the antibacterial activity of Rambai leaf extract (Sonneratia caseolaris) concentrations of 25%, 50%, 75%, and 100% against Enterococcus faecalis bacteria. Method: This study is a true experimental study designed by Posttest Only with Control Group Design using six.* ***Material and methods:*** *Rambai leaf extract concentrations of 25%, 50%, 75%, 100%, positive control of chlorhexidine gluconate 2%, and negative control of aqua dest was repeated 4 times. Antibacterial activity can be seen from the magnitude of the inhibitory zone formed in (MHA) Mueller Hinton Agar and BHIB (Brain Heart Infusion) using the diffusion method.* ***Results:*** *Based on Mann Whitney's final test, there was a significant difference in the clear zones formed around the paper disk. The average clear zone of Rambai leaf extract (Sonneratia caseolaris) concentration is 25% at 10.0mm, 50% at 12.7mm, 75% at 17.0mm, and 100% at 18.3mm.* ***Conclusion:*** *There is activity of Rambai leaf extract (Sonneratia caseolaris) concentrations of 25%, 50%, 75%, and 100% against Enterococcus faecalis bacteria.*

Keywords: *Antibacterial Activity, Enterococcus faecalis, Rambai Leaf Extract*
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INTRODUCTION

Disease health teeth and mouth often experienced by the community is caries and periodontal disease. [1] Survey results according to Basic Health Research (Riskesdas) in 2018 occurred an enhancement percentage of problems in teeth and mouth from 25.9% to 57.6%. Damage teeth because caries makes teeth perforated and damaged the network of hard teeth. Carious teeth if not conducted care so will be damaged and resulted in happening pulp necrosis .[2]

Necrosis pulp is a state pulp that has been dead, flow vessels blood not there and nerves pulp already not working.[3] Development knowledge and technology in the field of health teeth and mouth in maintaining tooth that is Care Channel Root (PSA). one failure Care Channel The root (PSA) is existence microorganisms in channel root namely *Enterococcus faecalis*. *Enterococcus faecalis* is the most resistant bacteria found in channel infected roots inside the cavity mouth. That thing is caused because of factor virulence from *Enterococcus faecalis.*[3.4]

Existence factor virulence cause *Enterococcus faecalis* to have the ability to compete with other bacteria and also form a colony on the *host*, as well as resistant to mechanism defense *hosts*. *Enterococcus faecalis* live in small areas nutrition as in part channel root. Bacteria *Enterococcus faecalis* could remove with the use of solution irrigation that is *Chlorhexidine gluconate* 2%.[4.5]

Chlorhexidine 2% concentration is recommended as solution irrigation channel root, because have an effect broad antimicrobial and can last long with ability attached to the wall channel root. Deficiency *chlorhexidine* as ingredient irrigation that is no could dissolve microorganisms and tissues channel root, so that required something alternative ingredient easy irrigation obtained, able dissolve microorganisms and tissues channel root as well as an economical price that is with utilizing potency herbal plants in Indonesia.[6]

Herbal plants in the South Kalimantan area that are trusted by the community have many benefits as herbal medicine, one of them is Rambai (*Sonneratia caesolaris).* Rambai is a typical ordinary plant used Becomes from plant medicine in part fruit, skin, stem, and leaves. Plant this could utilize for ingredient food, ripe fruit old could be utilized as drinks, and sticks from tree Rambai could be utilized as wood burn. Utilization of leaves on tassel still very rare is used, while leaf Rambai has content compound more antibacterial tall compared with the fruit.[7]

Based on screening results phytochemicals from leaf Rambai (*Sonneratia caseolaris*) in it contain compound metabolites secondary namely: triterpenoids, flavonoids, phenols, steroids, and tannins. Leaf Rambai (*Sonneratia caseolaris*) has content of flavonoids highest compared to other parts of vines.[7] Compound nutritious tannins and flavonoids as antibacterial. Based on the results calculation rate of phytochemical extract leaf Rambai (*Sonneratia caseolaris*) shows that the rate highest found in extract leaf shoots Rambai, that is flavonoid content in leaves shoots Rambai of 5.0741 mg/ml EK, levels tannins in leaves shoots Rambai about 2.6667 mg/ml EC, while rate phenol in leaves shoots Rambai about 8,2500 mg/ml EAG.[8]

Study before Aniqah (2020) extract leaf Rambai (*Sonneratia caseolaris*) 20%, 40%, 60%, and 80% against bacteria *Streptococcus mutants* show existence inhibition of bacteria the with use method dilution, but research test with method diffusion is still very limited, while method diffusion is the method used for determining activity antimicrobial agent.[9] Based on the background behind that, a researcher interested for To do a study about activity extract leaf Rambai with use diffusion method.[9]

MATERIAL AND METHODS

Study this was carried out in the Faculty's Basic Laboratory University of Gastric Mathematics Mangkurat and Laboratory biomedical Faculty University of Gastric Dentistry Mangkurat Banjarmasin. Study this has to get permission ethics from Committee Ethics Faculty University of Gastric Dentistry Mangkurat No. 029/KEPKGFKGULM/EC/IV/2022. Study this is study experimental pure (*true experimental*) with design *Posttest Only with Control Group Design* using six treatments, including extract leaf Rambai (*Sonneratia caseolaris*) 25% concentration, extract leaf Rambai (*Sonneratia caseolaris*) 50% concentration, extract leaf Rambai (*Sonneratia caseolaris*) 75% concentration, extract leaf Rambai (*Sonneratia caseolaris*) concentration 100%, *Chlorhexidine gluconate* 2% as control positive, and aqua dest as control negative. Each treatment conducted repetition 4 repetitions using the formula comparative numeric no in pairs.

Extraction Leaf Rambai

Leaf Rambai has taken part leaf young counting 1-3 pieces from on shoots leaf as much one kilogram in research this leaf Rambai obtained from the banks of the Barito River, Village Anjir Serapat, district Anjir Muara, Barito Kuala district, and a determination test was conducted. The extraction process is carried out with the method of maceration. Leaf Rambai dried, next destroyed until shaped powder weighed as much as 100gr and macerated in receptacle glass with solvent ethanol 96% to whole powder submerged. Simplicity ready macerated with a soak to in solvent ethanol 96% to submerged for ± 3 days, then filtered with the paper filter. Residue return macerated again in the same way, as much as 2 times. Extracts that have been macerated evaporated for separate solvent with use tool *mem*bers so that obtained extract leaf Rambai thick. Extract tested free ethanol with add a few drops of acid sulfate thick and sour acetate. If the extract does not smell of ester (alcohol) then the extract is declared not to contain alcohol. Conducted dilution extract leaf Rambai with formula dilution.

Breeding Bacteria *Enterococcus faecalis*

Preparation bacteria with scratch *Enterococcus faecalis* in MHA media and silenced in incubator 37oC ^{for} 24 hours. After incubation, detected *Enterococcus faecalis* will be in the form of a colony round small and 1-2 m in diameter. Colony bacteria results growth suspended for 24 hours to in 0.5ml liquid BHI and done incubation for 24 hours at 37 o ^{C} addition aqua dest sterile on suspension bacteria on BHI so that turbidity in accordance standard concentration bacteria *Mc Farland* 0.5 is 1.5x108cfu/ml.

Test Activity Antibacterial Method Diffusion

Control positive used is *chlorhexidine gluconate* 2% which has been prepared as much as 2 ml of 2% *chlorhexidine gluconate* is taken using a micropipette and inserted into the tube. Doing diffusion test with method Smearing bacteria *Enterococcus faecalis* which has been in accordance with standard *Mc Farland* 0.5 is 1.5x108 with cotton stick sterile on MHA media. Soak *paper disk* empty with extract leaf Rambai (*Sonneratia caseolaris)* concentrations of 25%, 50%, 75%, 100%, *Chlorhexidine gluconate* 2%, and distilled water for 3 hours. Gluing *paper disk* that has been soaked extract leaf Rambai (*Sonneratia caseolaris)* concentration of 25%, 50%, 75%, 100%, *Chlorhexidine gluconate* 2% and distilled water on MHA media containing bacteria *Enterococcus faecalis*. Incubating MHA with 37^{o}C ^{for} 24 hours. Measuring zone of inhibition growth bacteria uses *caliper.*

RESULT

The results of the research " Activity " Antibacterial Extract Leaf Rambai (*Sonneratia Solaris*) Concentration of 25%, 50%, 75%, and 100% Against *Enterococcus faecalis* ". Based on the results measurement extract leaf clear zone from every concentration as follows:

Table 1. Results of the Table of Average and Standard Values Activity Diameter Deviation Antibacterial Extract Leaf Rambai (*Sonneratia caseolaris*) Concentration of 25%, 50%, 75%, 100%, *Chlorhexidine gluconate* 2%, and Aquades to Bacteria *Enterococcus faecalis.*

Kelompok Perlakuan	*Mean* ± Standar Deviasi (mm)
Kelompok 1	10,0 ± 0,81
Kelompok 2	12,7 ± 0,50
Kelompok 3	17,0 ± 0,81
Kelompok 4	18,3 ± 0,75
Kelompok 5	18,3 ± 0,47
Kelompok 6	0,0 ± 0,0

Extract leaf Rambai at each concentration has an average diameter of the clear zone that varies. The average diameter of clear zone group 1 extract leaf fringes at a concentration of 25% is 10.0mm. The average diameter of clear zone group 2 extract leaf fringes at 50% concentration is 12.7mm. The average diameter of clear zone group 3 extract leaf fringes at 75% concentration is 17.0mm. The average diameter of clear zone group 4 extract leaf fringes at 100% concentration is 18.3mm. The average diameter of clear zone group 5 control positive *Chlorhexidine gluconate* 2% is 18.3mm. Based on results the seen the average diameter of the clear zone was highest found in group 4 extract leaf Rambai at 100% concentration and control positive *Chlorhexidine gluconate* at 2%. The average diameter of the clear zone Lowest is group 6 control negative aqua dest. Research results from this show the existing activity of antibacterial extract leaf Rambai (*Sonneratia caseolaris*) against the bacteria *Enterococcus faecalis*.

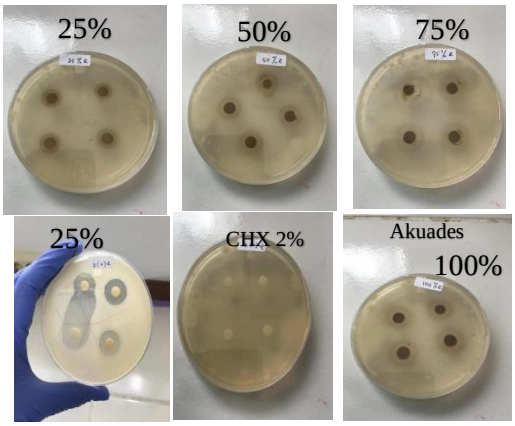

Figure 1. Inhibitory Zone Extract Leaf Rambai (*Sonneratia caseolaris*) against *Enterococcus faecalis* with 4 repetitions.

Data obtained then conducted analysis statistics using SPSS 26.0. Test the normality of the data using the *Shapiro Wilk* test because the amount sample is not enough 50 and obtained data that is not normally distributed ($p<0.05$), namely in the group extract leaf Rambai with the concentration of 25% so that non-parametric analysis test was performed *Kruskal Wallis* with level 95% confidence. Non- parametric analysis test results *Kruskal Wallis* is $p= 0.001 < 0.05$ means there is a significant difference in the mean diameter of the clear zone for every group treatment. So next with the *Mann-Whitney* test for knowing the group that gives the difference mean.

Table 2. *Mann Whitney.* Test Results Activity Antibacterial Extract Leaf Rambai (*Sonneratia caseolaris*) Concentration of 25%, 50%, 75%, 100% *Chlorhexidine gluconate* 2%, and Aquades To *Enterococcus faecalis.*

Perlakuan	EDR 25%	EDR 50%	EDR 75%	EDR 100%	CHX 2%	Akuade
EDR 25%		0,017*	0,019*	0,019*	0,019*	0,013*
EDR 50%			0,017*	0,017*	0,017*	0,013*
EDR 75%				0,056	0,037*	0,013*
EDR 100%					1,000	0,013*
CHX 2%						0,013*
Akuades						

Based on a table on could is known only there are 2 pairs of groups that do not different significant, that is 75% EDR group with 100% EDR and 100% EDR group with 2% CHX. Group other have difference significant one each other, so equal group with control positive that is, group extract leaf Rambai with 100% concentration.

DISCUSSION

Research results activity antibacterial extract leaf Rambai (*Sonneratia caseolaris*) concentrations of 25%, 50%, 75%, and 100% contained growth *Enterococcus faecalis* with use method diffusion show that extract leaf Rambai (*Sonneratia caseolaris*) concentrations of 25%, 50%, 75%, and 100% had activity antibacterial in hinder growth bacteria *Enterococcus faecalis*. Based on the results study on extract leaf Rambai concentration of 100% and *Chlorhexidine gluconate* 2% has activity most powerful antibacterial to bacteria *Enterococcus faecalis*, because has an average diameter of the clear zone by 18.3mm which exceeds extract leaf Rambai at a concentration of 25% is 10mm, 50% is 12.7mm, 75% is 17mm.

Based on the *Mann-Whitney* Test extract leaf Rambai at 100% concentration and 2%, *chlorhexidine gluconate* no there is a significant difference, so that the concentration the have power equivalent inhibition with control positive in hinder growth bacteria. That thing caused because extract leaf Rambai 100% concentration has compound metabolites more secondary tall compared with concentration other. According to Suciarti LK (2017) mentions the existence of a different zone of inhibition occurs because the existing rate of substance is different active from every concentration affected by series dilution. The more many substances dissolved active, then will the more the diameter of the inhibition zone formed. According to Ruhana Afifi (2018), the more and more tall concentration, then the more many content metabolites secondary contained in extract leaf Rambai which has the ability to hinder bacteria *Enterococcus faecalis*.[10]

Extract leaf Rambai has content metabolites secondary. Metabolites secondary is the resulting compound or synthesized in cells and groups taxonomy certain at the level certain growth .[11] Content metabolites secondary to extract leaf Rambai namely, phenols, flavonoids, triterpenoids, steroids, and tannins.[1] According to Winarti (2019) results from calculation rate phytochemical extract leaf Rambai (*Sonneratia caseolaris*) shows that rate highest found in extract leaf shoots Rambai, that is flavonoid content in leaves shoots Rambai of 5.0741mg/ml EK, levels tannins in leaves shoots Rambai about 2.6667mg/ml EC, while rate phenol in leaves shoots Rambai about 8,2500mg/ml EAG.[8] Study others who have content metabolites same secondary with leaf Rambai namely, Mariam F (2020) who researched about skin stem wood ironwood to bacteria *Aggregatibacter actinomycetemcomitans.* Skin stem wood ironwood has content metabolites secondary namely, flavonoids, phenols, tannins, steroids, and triterpenoids.[12]

In general, mechanism work flavonoids are divided into 3, namely, inhibiting the synthesis of sour nucleic acid, inhibiting function membrane cells, and inhibits energy metabolism .[13] Flavonoids is substance phenolic hydroxylated synthesized by plants as a response to infection microorganisms with mechanism hinder membrane cytoplasm and metabolism bacteria. According to Mulyani (2021) Flavonoids also have activity anti-inflammatory with mechanisms to prevent and weaken response inflammation and function as agent cardioprotective and neuroprotective. Flavonoids also have activity antioxidants because of their ability could catch radical hydroxyl, radical lipid peroxy, and superoxide anions. Flavonoids work as antifungals with the method of protein denaturation, interfere lipid layer, and result in the damaged cell wall. That thing could occur because the Lavonoid character lipophilic, so will tie phospholipids in the membrane cell mildew and annoying permeability cell membrane.[14]

Mechanism the action of triterpenoids is with method react with porins on the membrane outside wall cell bacteria, forming bond strong polymer, so that reduce permeability wall bacterial cells.[15] According to Rahmitasari (2020) Porin can be damaged causing a lack of permeability wall cell bacteria that will result in cell bacteria deficiency nutrition, so growth bacteria stagnate and die.

Tannins are component substances very complex organic from compound difficult phenolic separated and difficult crystallize. According to Liling (2020), Tannin has activity-related antibacterial with the ability to deactivate cell microbes and inactivate enzymes, as well as disturb protein transport in the layer in the cell.[16]

Compound phenol according to Sari (2018) works almost the same as flavonoids, namely could destroy and penetrate wall cell bacteria, precipitating cell proteins and microbes which are poison for proplasm. Compound phenol in kills bacteria there are 3 ways, namely denatures bacterial proteins, inhibits the synthesis of wall cells, and damages membrane bacterial cells.[17] Compound phenol denatures cell proteins bacteria with method shape bond hydrogen with bacterial proteins. That thing results in bacterial protein structure Becoming broken down and enzymes becoming inactive. Consequently, the denaturation of cell proteins bacteria, then all activity metabolism cell bacteria stopped, because all activity metabolism of cell bacteria is catalyzed by enzymes which are proteins.[17] According to Liling RD (2020) mechanism steroids work to hinder bacteria is to damage the bacterial plasma membrane, which causes the leak cytoplasm to go out next cell causing cell death. [16] Steroids can interact with membrane phospholipid cells that are permeable to compounds lipophilic so which causes integrity membrane decrease as well as morphology membrane cell change that causes cell brittle and brittle.[18]

Chlorhexidine gluconate 2% has a comparable mean zone of inhibition with extract leaf Rambai 100% concentration. Control positive on research this has an average clear zone of 18.3mm against bacteria *Enterococcus faecalis*. *Chlorhexidine gluconate* is an ingredient irrigation channel root spectrum wide and low toxic. According to Sari DP (2017) *Chlorhexidine*

gluconate, 2% can damage cell membrane bacteria that cause happening change in permeability membrane cytoplasm, changing balance osmotic mobile, annoying metabolism bacteria, division, and growth cell bacteria disturbed, so wall cell bacteria damaged, lysed, and finally died.[17]

Study this besides influenced from extract leaf Rambai and *Chlorhexidine gluconate* 2%, also influenced by properties and virulence bacteria *Enterococcus faecalis*. *Enterococcus faecalis* is bacteria facultative Cocci-shaped gram-positive anaerobes.[5] *Enterococcus faecalis* are capable endure life in an environment that does not support and survive as microorganisms in the root canal.[4] According to research conducted by Yuslianti (2021) *Enterococcus faecalis* is one common bacteria that cause disease infection secondary post care root canal.[19] *Enterococcus faecalis* could influence channel root and form colonies on the dentin surface with the help of *lipoteichoic acid*, while *aggregate substance* and *surface adhesion*, component another role in the attachment of collagen.[20]

Based on results overall on research extract leaf Rambai (*Sonneratia caseolaris*) has potency as an ingredient irrigation channel root, because has content compound antibacterial that can hinder bacteria *Enterococcus faecalis* which is a bacteria reason failure in care channel root. Need studies must continue conducted to Fulfill conditions made as alternative ingredients new solution irrigation channel root, likeability dissolve network organic and inorganic, stress surface low, and not character toxic. Based on the results study so could be concluded that there is the activity of antibacterial extract leaf Rambai (*Sonneratia caseolaris*) concentration 25%, 50%, 75%, 100% *chlorhexidine gluconate* 2% as control positive and aqua dest as control negative to bacteria *Enterococcus faecalis.*

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