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Antibacterial Effectiveness Test of South Kalimantan Bajakah Wood Extract against the Bacteria Staphylococcus Aureus Atcc 25923

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ABSTRACT

Introduction: Root canal treatment is a treatment that removes necrotic pulp tissue, biofilm and debris from the root canal system so that it can trigger the formation of healthy periapical tissue. Staphylococcus aureus is one of the important resistant microorganisms that is often isolated from root canal treatment. Bajakah wood (Spatholobus littoralis Hassk) is known to contain flavonoids, saponins, tannins and polyphenols.

Material and Methods: This research uses the well method. The number of samples was 20 samples with Bajakah wood extract as the test material. This research uses a posttest only control group design.

Results and Discussions: Data analysis using the Kruskal-Wallis test showed that there were significant differences ($p<0.05$) between the treatment groups.

Conclusion: There is no resistance to South Kalimantan Bajakah wood extract in concentrations of 50%, 75%, 100% against Staphylococcus aureus bacteria, which means there is no antibacterial activity.

KEYWORDS: Bajakah wood, staphylococcus aureus, root canal treatment.

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INTRODUCTION

Root canal treatment is a treatment that removes necrotic pulp tissue, biofilm and debris from the root canal system so that it can trigger the formation of healthy periapical tissue, in a tooth that can still be restored and has sufficient periodontal support.[1,2] Root canal treatment can be predicted with a success rate of 86 – 98%. However, failure of root canal treatment can also occur, one of the main causes is re-infection caused by bacterial persistence.[3] According to Irene et al. (2018) There are nine groups of bacteria identified in cases of apical periodontitis, namely Porphyromonas gingivalis, Streptococcus, Actinobacillus actinomycetem comitans, Pseudomonas, Staphylococcus, Klebsiela pneumonia, Escherichia colli, Alkaligines fascialis dan Enterobacter aerogenus.[4]

Staphylococcus aureus (S. Aureus) is one of the important resistant microorganisms frequently isolated from root canal treatment, playing a major role in the etiology of primary endodontic infections as long as root canals are left open.[5] S. Aureus is a gram-positive bacterium that is cocci-shaped and tends to be arranged in clusters described as "grapes". These microorganisms can grow aerobically or anaerobically (facultatively) and at temperatures between 18°C and 40°C.[6]

The goal of root canal treatment is to mechanically and chemically remove all necrotic vital tissue and microorganisms from the root canal system.[7] The root canal cleaning stage is root canal irrigation which is carried out using a chemical disinfection solution so that bacteria cannot reproduce. Irrigation is an important part of root canal treatment because it can remove microorganisms and debris, so that root canal obturation can be performed.[8]

A good irrigation material must be able to dissolve pulp tissue, smear layer and biofilm with low levels of toxicity.[8] The commonly used irrigation material is sodium hypochlorite (NaOCl). NaOCl has antibacterial properties and the ability to quickly dissolve necrotic tissue, vital pulp tissue, as well as organic components of dentin and biofilm.[9] However, NaOCl has disadvantages, namely a strong odor, unpleasant taste and cannot remove inorganic components

from the smear layer.[8] Current irrigation materials still have many shortcomings, therefore, more biocompatible herbal materials can be an alternative root canal irrigation material which is rich in antioxidants, antimicrobials and anti-inflammatory.[10]

Bajakah tampala (Spatholobus littoralis Hassk) is one of the plants that grows widely in the Central Kalimantan area, but this plant has not been widely used.[11] Bajakah plants contain saponins, flavonoids, phenolics and tannins. Bajakah wood extract has active compounds including flavonoids, phenolics, steroids, saponins, terpenoids and alconoids.[12] The stem and bark of Bajakah wood have quite high levels of flavonoids.[13]

Based on research conducted by Chaerul et al. (2022), it is known that Bajakah ethanol extract has good antibacterial activity.[12] The above statement is strengthened by research conducted by Saparuddin et al. (2023) which states that Bajakah wood ethanol extract has good antibacterial activity with the smallest extract concentration, namely 10%.[14] However, this is different from research conducted by Bayu (2019) showing that Bajakah wood extract has an antibacterial effect against Staphylococcus aureus at a concentration of 100%.[15]

MATERIALS AND METHODS

Tools and Materials

The tools used in the research are as follows:[14]

1. Water Bath (BUCHI®, BUCHI, Swiss)
2. Autoclave (BUCHI®, BUCHI, Swiss)
3. Incubator (Jisico®, Jisico, Korea)
4. Rotary Evaporator (BUCHI®, BUCHI, Swiss)
5. Maceration Vessel (Duran®, Schott, Jerman)
6. Petri dishes
7. Beaker
8. Test Tubes
9. Test Tube Rack
10. Ose needle
11. Pipette Volume
12. Measuring Cup
13. Bunsen
14. Container
15. Calipers
16. Glass Funnel
17. Wooden Stirrer
18. 6 mm Disc Paper
19. Sterile cotton
20. UV-Vis spectrum spectrophometer 25%T wavelength 583 nm

The materials used in the research are as follows:

1. Staphylococcus aureus ATCC 25923 bacterial culture
2. Bajakah wood which comes from Haruai District, Tabalong Regency, South Kalimantan
3. NaOCl 2,5%
4. DMSO
5. Ethanol 96%
6. Brain Heart Infusion (BHI) Media

Method

This research uses laboratory experimental research. The research design uses a posttest only control group design. This research was conducted at the BIOCORE Laboratory at Trisakti University and the MiCORE Laboratory at Trisakti University. Extract preparation was carried out in March 2024, and antibacterial testing was carried out in April 2024. The sample used in this research was South Kalimantan Bajakah Tampala wood extract (Spatholobus littoralis Hassk). The sample population used in this study was the bacterial population Staphylococcus aureus ATCC 25923. In this study the sample was calculated using the Federer formula, where based on this calculation the total number of samples was 25 samples.

RESULTS AND DISCUSSION

Results

The results of the research carried out aimed to determine the antibacterial activity of Bajakah wood extract (Spatholobus littoralis Hassk.) on the growth of Staphylococcus aureus ATCC 25923 bacteria using the agar well diffusion method. The drag diameter is indicated by the formation of a clear zone around the well hole which can be measured using a digital caliper. The antibacterial test treatment was divided into four groups, namely South Kalimantan Bajakah wood extract with concentrations of 50%, 75%, 100% and a positive control using 2.5% NaOCl. The measurement results are collected and recorded to proceed to the data analysis stage.

All data that has been collected is then analyzed statistically using IBM SPSS 29 statistical data processing software. The values from the hardness measurement results are first tested for normality of data distribution using the Shapiro-Wilk test. Based on the results of the normality test, all significance values are smaller than 0.01 or $p<0.05$, so it can be concluded that all data is distributed abnormally. Because the data is not normally distributed, data processing was carried out non-parametrically using the Kruskal-Wallis test which can be seen in table 1.

Table 1. Mean, Standard Deviation, and Results of the Kruskal-Wallis Test for Antibacterial Activity in Each Treatment Group

Experimental Group	Staphylococcus aureus (mm)					Mean (mm) ± SD	Sig
	1	2	3	4	5		
Bajakah Wood	0,00	0,00	0,00	0,00	0,00	0,00	**<0,01**

Extract 50% Bajakah Wood Extract 75%	0,00	0,00	0,00	0,00	0,00	0,00
Bajakah Wood Extract 100%	0,00	0,00	0,00	0,00	0,00	0,00
NaOCl 2,5%	12,12	12,97	13,12	13,20	13,21	12,92 ± 0,45

Based on table 1, data from antibacterial activity testing results can be seen that the highest DDH was in the positive control group with an average of 12.92 mm with a standard deviation of 0.45 mm. Based on the Bajakah wood extract treatment, there was no increase in the diameter of the resistance so the standard deviation was 0 mm.

DISCUSSION

Antibacterial is a substance that can disrupt or even kill the growth of bacteria by interfering with the metabolism of harmful microbes. Microorganisms can cause disease in other living creatures because they have the ability to infect, ranging from mild infections to severe infections and even death.[16] The formation of the inhibitory zone is due to the extract of Bajakah wood (Spatholobus littoralis Hassk) diffusing out to inhibit the growth of Staphylococcus aureus bacteria in the medium around the paper disk. The higher the concentration of the extract used, the higher the diameter of the inhibition zone.[17] The ability of Bajakah wood extract (Spatholobus littoralis Hassk) as an antibacterial is most likely because Bajakah wood contains flavonoid compounds. Flavonoids contain phenol groups which can cause protein denaturation and damage bacterial cell membranes causing damage to the permeability of bacterial cell walls, microsomes and lysosomes as a result of the interaction between flavonoids and bacterial DNA.[17]

From the results of this research, no inhibitory power was formed from several concentrations of bajakah wood. There are several factors that influence the diameter of the inhibition zone, one of which is the thickness of the agar media. The effective thickness of the agar medium is around 4 mm, if it is less then the diffusion of the extract will be faster, whereas if it is more then the diffusion of the extract will be slower. Apart from that, another possibility that can influence the diameter of the inhibition zone is the limited diffusion of the extract into the medium.[17] The higher the concentration, the lower the solubility, thereby causing a decrease in the rate of diffusion of active extract metabolites into the medium. This results in limitations of extracts with high concentrations in inhibiting the growth of S. aureus ATCC 25923 bacteria on BHI agar media.[17]

Research conducted by Weldy et al. (2022) showed that the average inhibition zone of Bajakah Wood ethanol extract with concentrations of 5%, 15%, 25%, 50% and 100% tested against Pseudomonas aeruginosa bacteria using the disc method was 11.1 mm; 13.3mm; 16.7mm; 20.0mm; and 22.4mm. The results obtained indicate that Bajakah Wood ethanol extract has antibacterial effectiveness against Pseudomonas aeruginosa.[11] Other research has also been conducted by Saputera et al. (2019), namely testing Bajakah Wood ethanol extract with a concentration of 3.12%; 6.25%; 12.5%; 25% and 50% against Eschericia coli bacteria using the well method produced an average inhibition zone of 9.8 mm; 15.83 mm and 20.32 mm.[18]

Another factor that influences the diameter of the inhibition zone in this study compared to previous studies is the type of bacteria used. In this study, the bacteria used was S. aureus ATCC 25923. S. aureus bacteria are gram-positive bacteria, which are gram-positive bacteria that have a thicker cell wall structure compared to gram-negative bacteria.[19] Gram-positive bacteria have a thickness of 20-80 nm with a peptidoglycan layer of > 50%, while gram-negative bacteria only have a thickness of 10 nm with only 10-20% peptidoglycan. Peptidoglycan is the main component of cell walls which are rigid and maintain cell integrity and determine cell shape. Due to the differences in cell wall structure, it is more difficult for secondary metabolite compounds in the extract to enter the bacteria. Therefore, this could influence the effectiveness of Bajakah Wood (Spatholobus sp.) ethanol extract in inhibiting the growth of S. aureus ATCC 25923.[16]

The result of an inhibition zone not being formed could be because the bajakah wood extract made in this study was still a crude extract, so it is possible that it still contains other organic materials that could interfere with the contact of the active compound with bacterial cells and reduce its antibacterial activity.[20] The solvent used also has an influence on the antibacterial ability of an extract. The extraction process is carried out by adding solvents that have different polarities to bind active compounds that also have different polarities. Bajakah wood contains flavonoids, tannins and saponins which are polar and semipolar polyphenols. Based on research conducted by Pujiastuti regarding the comparison of antibacterial effectiveness between 70% and 96% ethanol solvents, it was found that the 70% solvent was more polar than the 96% ethanol solvent and could attract antibacterial compounds better. In this study, the solvent used was 96% ethanol which is polar, which may have resulted in less active antibacterial compounds in Bajakah wood being extracted, resulting in no antibacterial activity on Staphylococcus aureus.[21] Research conducted by Diah Wulandari, et al (2021) states that the effective extraction temperature for bajakah

wood in the evaporation process using a rotary evaporator is 50 ºC – 60 ºC.[22]

After carrying out the antibacterial activity test and measuring the inhibition zone, a normality test was then carried out using the Shapiro-Wilk method to fulfill the requirements of the One-Way ANOVA test. Based on the normality test, the data is normally distributed with the condition p=0.05. The results of the normality test show that the measurement of the diameter of the inhibition zone is not normally distributed. Because the data obtained did not meet the One Way ANOVA requirements, the test was carried out non-parametrically using the Kruskal-Wallis test and the results obtained were that there was a significant difference in South Kalimantan Bajakah wood extract concentrations of 50%, 75%, 100% and the positive control. Even though the test results showed that there were differences between several concentrations, there was no increase in power resistance. Therefore, based on the resistance force which did not increase around the well hole, this research hypothesis was rejected, namely that there was no antibacterial activity of South Kalimantan bajakah wood extract (Spatholobus littoralis Hassk) against the bacteria Staphylococcus aureus ATCC 25923.

CONCLUSIONS

Based on the results of research regarding the antibacterial effectiveness test of Bajakah wood extract from South Kalimantan (Spatholobus Littoralis Hassk) against the bacteria Staphylococcus aureus ATCC 25923, it can be concluded that there is no antibacterial activity in Bajakah wood extract (Spatholobus littoralis Hassk) from South Kalimantan at concentrations of 50%, 75%, 100% against the bacteria Staphylococcus aureus ATCC 25923. The results of the antibacterial activity test can be seen that the inhibitory power was only present in the 2.5% NaOCl positive control group.

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