International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 09 September 2024

Page No: 765-771

DOI: https://doi.org/10.47191/ijpbms/v4-i9-10, Impact Factor: 7.792

Testing the Inhibitory Activity of Bajakah Tampala (Spatholobus littoralis *Hassk)* Wood Extract from South Kalimantan on the Growth of Pseudomonas aeruginosa Bacteria

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ABSTRACT

Background: Dental root canal treatment aims to eliminate microorganisms that cause infection in the tooth root canal, one of which is *Pseudomonas aeruginosa* bacteria. Disinfection of dental root canals with 0.5% - 5.25% NaOCl irrigation solution is still constrained so it is necessary to look for alternatives. The bajakah tampala plant (*Spatholubus littoralis Hassk)* is known to contain antibacterial compounds, namely flavonoids, saponins, tannins, and polyphenols.

Objective: To evaluate the inhibitory activity of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) from South Kalimantan against the growth of *Pseudomonas aeruginosa* bacteria.

Method: *Post test-only with control group design*. The test groups were 100%, 75%, 50% bajakah tampala wood extract solution and positive control 2.5% NaOCL solution. Extraction using maceration method. Bacterial growth inhibition was tested by well diffusion method. Inhibition zone was measured using a caliper. **Results**: The growth inhibition from the largest to the smallest was shown by 75%, 100% and 50% bajakah wood extracts with mean inhibition zone diameters of 5.05 mm, 3.84 mm and 2.02 mm respectively, meanwhile the positive control was 16.14 mm. *One Way Anova* test showed significant difference in inhibition ($p<0.05$) in all groups. *Post hoc* test showed no significant difference in the inhibition of 75% bajakah tampala wood extract with 100% extract. The inhibition of 100% and 75% extracts was significantly greater than 50% extract. The inhibition of NaOCL 2.5% was significantly greater than all groups of test extracts.

Conclusion: Bajakah tampala wood extract has inhibitory activity against the growth of *Pseudomonas aeruginosa* bacteria.

ARTICLE DETAILS

Published On:
30 September 2024

Available on:
https://ijpbms.com/

KEYWORDS: Irrigation solution, bacterial inhibition, bajakah tampala wood extract, *Spatholobus littoralis Hassk*, *Pseudomonas aeruginosa.*

1. INTRODUCTION

Pulp necrosis is a condition of damage (non-vital) nerve tissue in the pulp chamber and tooth root canal which is generally caused by microorganisms as infectious agents in exposed pulpo-dentin tissue.[1,2,3] One of the opportunistic bacteria that cause pulp necrosis is *Pseudomonas aeruginosa*.[3,4] This bacterium was found in necrotizing pulp at a percentage of 2%. *Pseudomonas aeruginosa* is a gram-negative facultative anaerobic bacterium, which is round with gray-green colonies and is resistant to most antimicrobial agents. [3,5,6]

Treatment of pulp necrosis can be done through root canal treatment or endodontic treatment. This treatment is guided by the endodontic triad, namely cleaning and shaping, root canal irrigation and obturation. Endodontic treatment effectiveness depends on root canal irrigation or disinfecting procedures because at this stage the microorganisms, residual necrotic pulp tissue, smear layer and debris in the root canal are cleaned.[2]

The irrigation solution material that is widely used in dentistry today and has become the gold standard is sodium hypochlorite (NaOCl) 0.5% - 5.25% because it has a broad spectrum antimicrobial effect, is able to clean vital and inhibit

the development of smear layers that can cause failure in endodontic treatment. However, this NaOCl irrigation solution still has obstacles including causing toxicity to periodontal tissue, having an unpleasant odor and taste, and can cause structural changes in the dentin of the tooth root canal. [4,7, 8] Therefore, an alternative herbal-based irrigation solution material that does not cause toxicity to the tissue is needed. One of the herbal plants that is thought to have the potential to be used as an alternative material for dental root canal irrigation solutions is the bajakah tampala plant (*Spatholobus littoralis Hassk*), because this plant is known contains phytochemical components such as flavonoids, tannins, saponins, and polyphenols with antibacterial activity.[9,10,11] The bajakah tampala plant grows in lush forests with peatlands on the island of Borneo in Indonesia until the height of the plant reaches 50 meters[12] . This plant is widely used as a medicinal plant by the Dayak tribe. The most widely used part of the plant is the stem or wood.[13,14] The benefits of this plant are believed by the community to treat various diseases such as diarrhea, body aches, cancer, and can heal wounds or as anti-inflammatory drugs.[15]

Research by Abdulrahman *et al*. in 2021 has explained that the bajakah tampala plant contains secondary metabolite compounds of alkaloids, phenols, tannins, and flavonoids.[16] Further research by Latu S and Wahid S in 2023 and Rahman HA and Johannes E in 2022 has proven that bajakah tampala wood extract has antibacterial activity because it can inhibit the growth of Staphylococcus *aureus* bacteria at concentrations of 10% and 50%.[9,10] Research data related to the inhibitory activity of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) which grows in South Kalimantan against the growth of *Pseudomonas aeruginosa* bacteria, until now is still very limited. This study aims to evaluate the inhibitory activity of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) which grows in South Kalimantan with 96% ethanol solvent against the growth of *Pseudomonas aeruginosa* bacteria as an effort to develop alternative dental root canal irrigation solution materials.

2. MATERIALS AND METHODS

2.1 Research type and design

This research is a laboratory experimental research with posttest only with control group design.

2.2 Research materials (samples)

The research sample tested in this study was bajakah tampala wood extract (*Spatholobus littoralis Hassk*) which grows in South Kalimantan with concentrations of 100%, 75%, and 50%. The positive control used 2.5% NaOCl solution (ONEMED®). The total number of samples used was 20.

2.3 Sterilization of research tools

The tools to be used were washed thoroughly, dried and sterilized in an autoclave (BUCHI®, BUCHI, Switzerland) at 1 atm at 121^0 C for 15 minutes. For metal-based tools, sterilized using a bunsen flame for 1 minute.[17]

2.4 Sample preparation and plant extract preparation

Samples of bajakah tampala wood were washed thoroughly, dried and shaved into wood fibers and then dried in the sun in a place not exposed to direct sunlight until dry. The bajakah tampala wood shavings were then chopped using a blender and then sieved to become powder (simplisia).[17] The extraction procedure for the bajakah tampala plant was carried out at the BIOCORE Laboratory of the Trisakti Faculty of Dentistry, Jakarta using the maceration method with 96% ethanol solvent (1.00971.2500, EMPROVE®, Darmstadt Germany) with a powder and solvent ratio of 1: 5. Bajakah wood powder that has been soaked in a maceration vessel (Duran®, Schott, Germany) is placed in a place that is not exposed to sunlight for 3x24 hours, in every 15 minutes stirring is done. After three days the macerate was filtered. The filtrate was then evaporated with a rotary evaporator (BUCHI®, BUCHI, Switzerland) at 78^0 C, after which it was filtered using whatman paper until a thick extract was obtained. The bajakah wood extract was diluted by adding 5% Dimethyl sulfoxide (DMSO) + 1% of tween 20.[11,18]

2.5 Preparation of bajakah tampala wood plant extract solution

The procedure for making extract solutions and testing the inhibitory power of bajakah tampala wood extract was carried out at the MICORE Laboratory of the Trisakti Faculty of Dentistry, Jakarta. The concentration of 100% extract is made by dissolving 1 ml of bajakah tampala wood extract into 1 ml of DMSO 5% + 1% of *tween* 20. The concentration of 75% extract concentration was made by dissolving 0.75 ml of bajakah tampala wood extract into 1 ml of DMSO 5% + 1% of *tween* 20. The concentration of 50% extract concentration was prepared by dissolving 0.5 ml of bajakah tampala wood extract into 1 ml of DMSO 5% + 1% of *tween* 20.

2.6 Preparation of BHI Agar media

Brain Heart Infusion Agar (BHI-A) powder (M211, HIMEDIA®, Mumbai India) of 52 grams was put into 1000 ml of pure/distilled water and heated to boiling and dissolved completely. The BHI-A agar medium was then sterilized by autoclaving at 15 lbs

pressure (121^0 C) for 15 minutes. The BHI-A agar medium was then removed and cooled to 45-50^0 C and mixed well and poured into sterile petri dishes.

2.7 Preparation of BHI *Broth* media

Brain Heart Infusion Broth (BHI-B) powder (M210, HIMEDIA®, Mumbai India) of 37 grams was put into 1000 ml of pure water/distilled water and heated to dissolve the media completely then poured into bottles or tubes and sterilized by autoclaving at 15 lbs pressure (121^o C) for 15 minutes.

2.8 Preparation of *Pseudomonas aeruginosa* bacteria suspension

Preparation of Pseudomonas *aeruginosa* bacterial suspension was carried out by taking 1 ose of culture and then inserted in a tube containing 2 ml of Brain Heart Infusion Broth (BHI-B) then homogenized and incubated for 24 hours at 37^o aerobically. The turbidity of the bacterial suspension was measured with a microplate reader (SAFAS MP 96,Monaco) with a wavelength of 600 nm to the equivalent of the Mc Farland 0.5 standard ($1.5x10^8$ CFU/ml).

2.9 Inhibition activity test of *Pseudomonas aeruginosa* bacteria

The inhibition activity test of bajakah tampala wood extract against *Pseudomonas aeruginosa* bacteria was carried out using the well diffusion method.[17] The bacterial suspension was taken using a sterile ose and scraped thoroughly onto the surface of the BHI agar medium that had been prepared in a sterile petri dish. The Petri dish is then rotated 60^0 C to ensure even distribution of bacteria that have been inoculated on the BHI agar media. Making wells on the BHI Agar media was done using a cork borer. Each well was then dripped with 20 µl of 100%, 75%, and 50% bajakah tampala wood extract, as well as a positive control of NaOCl 2.5%. Petri dishes were incubated at 37^0 C for 24 hours. The diameter of the clear zone formed around the wells was measured by repeating 5 times for each test sample using a caliper in the vertical and horizontal directions. [19]

Data Analysis

The data of the study were analyzed using IBM SPSS 29 *software*. The normality test of the data of the study results was carried out using *Shapiro-Wilk. One-way* ANOVA test to see significant differences in all groups. *Bonferroni Post Hoc* test to see significant differences between pairs of treatment groups studied.

3. RESULTS

The zone of inhibition against *Pseudomonas aeruginosa* bacteria in the form of a clear zone formed around the wells on the BHI agar medium which is dripped with 100%, 75%, and 50% solution of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) and positive control (NaOCL 2.5%), can be seen in Figure 1.

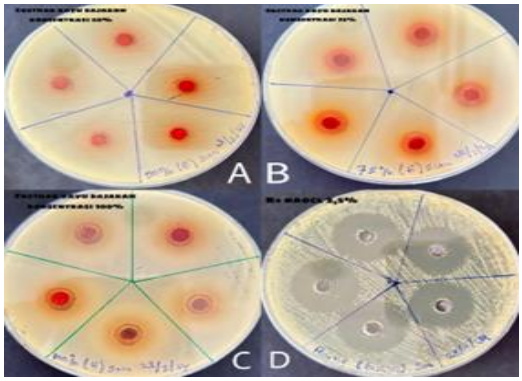

Image 1. Zone of Inhibition of Bajakah Tampala Wood Extract against *Pseudomonas aeruginosa* Bacteria. Concentration of 50% (A), concentration of 75% (B), concentration of 100% (C), NaOCl 2.5% (D).

The inhibitory zone has an average diameter of 100%, 75%, and 50% bajakah tampala wood extract (*Spatholobus littoralis Hassk*) and positive control (NaOCL 2.5%) against the growth of *Pseudomonas aeruginosa* bacteria can be seen in Table 1.

Table 1. Mean Value, Standard Deviation, and One-Way ANOVA Test Results of the Zone of Inhibition of *Pseudomonas aeruginosa* Bacteria in Each Treatment Group

Treatment Group	*Pseudomonas aeruginosa* inhibition zone diameter (mm)					Mean (mm) ± SD	Sig
	1	222	3	4	5		
50% Bajakah Wood Extract	1.80	2.00	1.65	2.35	2.30	2.02 ± 0,30	<0.001
75% Bajakah Wood Extract	5.40	5.10	5.00	5.00	4.75	5.05 ± 0,23	
100% Bajakah Wood Extract	4.40	3.45	2.60	3.10	5.65	3.84 ± 1,20	
NaOCl 2.5%	17.65	16.70	15.70	14.75	15.90	16.14 ± 1,09	

Based on table 1, the average diameter of the inhibition zone generated around the wells of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) at a 75% concentration is 5.05 mm which is the largest value compared to the 50% concentration which produces a diameter of 2.02 mm and 100% produces a diameter of 3.84 mm, while the 2.5% NaOCl as a positive control shows the largest inhibition zone because the average inhibition zone formed is 16.14 mm. A bar chart showing the difference in the average diameter of the inhibition zone of all treatment groups of bajakah tampala wood extract and positive control tested against *Pseudomonas aeruginosa* bacteria can be seen in Figure 2.

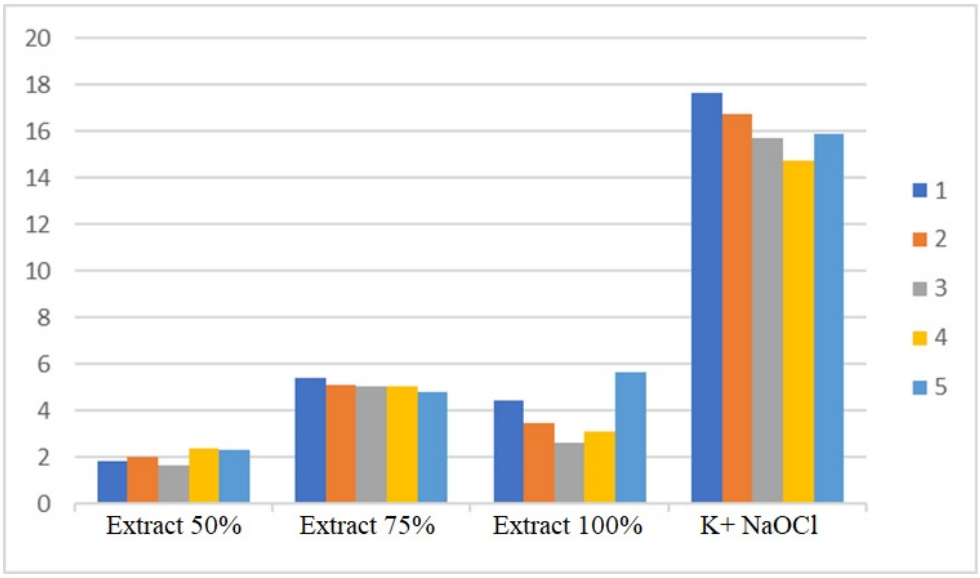

Image 2. Average Value of Inhibition Zone Diameter Treatment Groups of Bajakah Tampala Wood Extract and Positive Control Against *Pseudomonas aeruginosa* bacteria

The results of the *Shapiro-Wilk* normality test showed that all data on the average diameter of the inhibition zone of all treatment groups of bajakah tampala wood extract and positive control were normally distributed. with a value of $p>0.05$. The results of the *One-way* ANOVA test of all groups showed significant differences in bacterial inhibition between 50%, 75%, and 100% bajakah tampala wood extract and positive control with a value of P = <0.001 ($p<0.05$). The results of the *Post Hoc Bonferroni* test showed that the average value of the diameter of the inhibition zone of 100% bajakah tampala wood extract did not have a significant difference when compared to 75% extract (P = 0.217). The mean diameter of the inhibition zone of 100% (P=0.02) and 75% (P=<0.001) bajakah tampala wood extracts showed a significant difference when compared to the 50% extract. The positive control group (NaOCL 2.5%) showed a significantly greater mean value of inhibition zone diameter than all the test extract treatment groups (P=<0.001).

4. DISCUSSION

Effective root canal treatment (endodontics) aims to remove bacteria from the root canal system because the presence of bacteria and their metabolite products can lead to persistent infection and failure of root canal treatment.[8] Infection in the root canal can persist, when the dental pulp becomes necrotized due to caries, trauma, periodontal disease or due to iatrogenic factors,[20] The root canal irrigation procedure is one of the important aspects that play a role in the cleaning of the root canal system given the complexity of the anatomy of the human tooth root canal and the presence of bacteria in the form of biofilms adhering to the surface of the tooth root canal walls. as the main source of infection that causes dental pulp and periapical diseases. [8,21]

The selection of *Pseudomonas aeruginosa* bacteria tested for growth inhibition by bajakah tampala wood extract (*Spatholobus littoralis Hassk*) in this study, based on the results of research by Rostinawati T et al in 2017 which has explained that *Pseudomonas aeruginosa* bacteria are facultative anaerobic gram-negative bacteria that are able to live in conditions of depleted total oxygen and are proven to be found in the dental pulp of patients experiencing necrosis.[3] Although *Pseudomonas aeruginosa* is a facultative anaerobic bacterium that prefers to use aerobic respiration, its ability to undergo anaerobiosis can be due to the presence of nitrate (NO_3 -) which acts as the final terminal electron acceptor.[5] *Pseudomonas aeruginosa bacteria* are also known to be motile (have flagellum and pili as a means of movement) and can live in humid and aqueous conditions.[22,23] This bacterium was found in necrotizing pulp with a percentage of 2%.[4]

The choice of extraction by maceration method in this study was carried out because it is one of the selective methods to extract bioactive compounds contained in plants without causing damage to compounds that are susceptible to heat.[24] The maceration method as a process of extracting

simplisia, is carried out using a solvent by immersion and several times shaking or stirring at room temperature.[24,25] The liquid will penetrate the cell wall and enter the cell cavity containing the active substance that will dissolve, due to the difference in the concentration of the active substance solution inside the cell and outside the cell so that the solution is pushed out.[25] The use of 96% ethanol solvent in the extraction method in this study was chosen because it is non-toxic, good absorption and high solubilization ability so that it can extract compounds that are non-polar, semi-polar and polar, and easily penetrate into the cell wall of the sample to produce a concentrated extract.[26] The use of solvents that have the same polarity as the molecular polarity of the solute, in this maceration extraction method, will produce better extraction results.[24]

The selection of samples in the form of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) as an alternative irrigation solution from herbal ingredients in this study, because it is known to contain secondary metabolite compounds in the form of saponins, flavonoids, and tannins which function as antibacterial compounds based on research by Hazna LZ *et.al* in 2021.[25] Other literature also explains that the use of herbal alternatives in dental root canal treatment is currently becoming more popular because in addition to the advantages of property content, the availability of herbal ingredients is easy to find and without side effects.[26]

According to research by Hidayahtullah SH and Mourisa C in 2023, flavonoid secondary metabolites have a mechanism of action as antibacterial compounds by disrupting the integrity of bacterial cell membranes in forming complex compounds against extracellular proteins. In addition, flavonoids can work by denaturing bacterial cell proteins and damaging cell membranes. Polyphenols as phenol-derived compounds work by denaturing cell proteins through hydrogen bonds formed between phenols and proteins, causing damage to protein structures. Saponins work as antibacterial compounds by lowering surface tension resulting in increased cell permeability or leakage and resulting in intracellular compounds leaving the bacterial cell.[27]

The method of testing the inhibitory activity of *Pseudomonas aeruginosa* bacteria carried out in this study uses the well diffusion method because it has the advantage of being easier to measure the area of the inhibition zone formed. This is because the bacterial isolate in the well diffusion method is active not only on the upper surface of the agar medium, but can also move to the bottom of the agar medium in a Petri dish.[28]

Although the average value of the diameter of the inhibition zone of *Pseudomonas aeruginosa* bacterial growth by bajakah tampala wood extract (*Spatholobus littoralis Hassk*) 100% concentration appears smaller at 3.84 mm compared to 75% extract which is 5.05 mm, *Bonferroni post hoc* test statistical analysis of the results of this study concluded that there was no significant difference with a $p>0.05$ value. The average value of the diameter of the inhibition zone of bajakah tampala wood extract (*Spatholobus littoralis Hassk*) with a concentration of 100% which is smaller than the 75% extract in this study, may be due to the concentration of 100% extract having a higher viscosity than the 75% extract so that the ability of 100% extract to diffuse in the BHI agar medium in petri dishes is reduced.[29] According to Ariyanti et al cited by Anggita A et al [29], stating that differences in diffusion of antibacterial compounds in the agar medium as well as different types and concentrations of antibacterial compounds, can give different inhibition zone diameter results. According to Nurainy et al cited by Anggita A et al,[29] stated that the higher the concentration of the extract will affect the viscosity of the extract solution, the thicker the extract solution used, the more difficult it is for the extract solution to diffuse properly in the agar medium. The average value of the diameter of the inhibition zone of *Pseudomonas aeruginosa* bacterial growth by bajakah tampala wood extract (*Spatholobus littoralis Hassk*) concentrations of 100% and 75% in this study was further concluded to be greater than the average value of the diameter of the inhibition zone of the 50% extract which was only 2.02 mm. This can be explained because the higher the concentration of the extract, the more the content of antibacterial active ingredients so that the formation of the inhibition zone of bacterial growth will be greater.[29]

The strength of bacterial inhibition of 100% concentration bajakah tampala wood extract based on the diameter of the inhibition zone formed, which is 3.84 mm can be categorized as weak, while the 75% extract with an inhibition zone diameter of 5.05 mm has a moderate inhibition strength category. The strength of bacterial inhibition of 50% concentration bajakah tampala wood extract based on the diameter of the inhibition zone formed, which is 2.02 mm can be categorized as weak. According to Davis and Stout cited by Alouw GEC et al, [28] criteria for antibacterial power with an inhibition zone diameter of less than 5 mm is categorized as weak, 5-10 mm inhibition zone is categorized as moderate, 10-20 mm inhibition zone is categorized as strong and more than 20 mm inhibition zone is categorized as very strong.

Other research results on bajakah tampala plants (*Spatholobus littoralis Hassk*) that are in line, have also been conducted by Weldy S *et.al* in 2022 but using samples of bajakah tampala stem extracts from Central Kalimantan with the *disc diffusion* method.[30] Weldy S *et.al*'s research in 2022 explained that bajakah tampala stem extract from Central Kalimantan with concentrations of 5%, 15%, 25%, 50% and 100% had an average inhibition zone diameter of 11.1 mm, 13.3 mm, 16.7 mm, 20.0 mm and 22.4 mm respectively against the growth of *Pseudomonas aeruginosa* bacteria.[30] The positive control, cyprofloxacin antibiotic, showed an

average inhibition zone diameter of 50.1 mm against *Pseudomonas aeruginosa* bacteria.[30]

Furthermore, the use of 2.5% NaOCL solution was chosen as a positive control in this study because it is the gold standard irrigation solution that is most commonly used in dental root canal treatment and has broad antibacterial capabilities along with the ability to dissolve organic tissue.[1,8,21] This is evident from the average value of the diameter of the inhibition zone of the 2.5% NaOCL solution which is significantly greater at 16.14 mm compared to the average value of the diameter of the inhibition zone of all concentrations of bajakah tampala wood extract tested in this study. The antibacterial mechanism of 2.5% NaOCL solution can occur through hydrolyzation action to form hypochlorous acid which is then decomposed into a new oxygen ecology to contaminate bacterial proteins, disrupt oxidative phosphorylation of bacterial biofilms and disrupt bacterial DNA synthesis so as to cause bactericidal effects in a broad spectrum.[31]

5. CONCLUSIONS

Bajakah tampala (*Spatholobus littoralis Hassk*) wood extracts with concentration 100%, 75% and 50% from habitats in South Kalimantan were shown to have inhibitory activity against the growth of *Pseudomonas aeruginosa* bacteria.

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