# **Food and Pharmaceutical Sciences**

**Original** Article

## The Antibacterial Activity of Bajakah Tampala Extracts (Spatholobus littoralis Hassk.) Mouthwash Formulation Inhibited Dental Plaque against Streptococcus mutans

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Received: 25 July 2024; Revised: 9 August 2024; Accepted: 15 August 2024; Published: 23 August 2024

**Abstract:** Dental plaque is the main cause of dental caries caused by *Streptococcus mutans*, with a high prevalence in Indonesia. Currently, the mouthwash market contains high levels of alcohol, which can cause long-term side effects. Tampala bajakah root (*Spatholobus littoralis*) is used in traditional medicine for the Dayak community in Central Kalimantan. Bajakah Tampala root has antibacterial activity produced by flavonoids and phenolic compounds. The development of herbal cosmetics can be achieved by Bajakah Tampala mouthwash formulations to prevent dental plaque caused by *Streptococcus mutans* infection. In this study, ultrasound-assisted extraction (UBT) and infusion (IBT) derived the active compounds of Bajakah Tampala root extract. The various concentrations of UBT (20–80%) and IBT (10%) were evaluated for antibacterial activity higher than other extracts, with an inhibition zone of 14,01±2,70 mm. Based on these results, an effective mouthwash dosage formulation can be developed at 80% UBT. The formulation evaluation of mouthwash assessed viscosity, homogeneity, pH, and organoleptic test. The UBT mouthwash product has qualified formulation evaluation parameters. This research contributed to the innovation of herbal cosmetics by developing the potential of Indonesian medicinal plants.

Keywords: Bajakah Tampala, Antibacterial activity, Mouthwash, Herbal cosmetic, Streptococcus mutans.

### 1. INTRODUCTION

Dental plaque is the main cause of dental caries caused by *Streptococcus mutant* bacteria. The average prevalence of dental caries in Indonesian people is 88.8% of the total population [1]. The high prevalence rate is due to a lack of knowledge about dental and oral hygiene. Dental caries can be inhibited and treated with mouthwash formulations that are used for an antiseptic effect, control plaque, and have temporary antibacterial activity [2]. Mouthwash formula is a cosmetic product that performs effectively at cleansing areas of the mouth and teeth that are challenging to reach. Mouthwash formulations widely sold on the market contain high levels of alcohol, so they can cause uncomfortable sensations and have long-term effects such as cancer of the pharynx, mouth, and throat [3].

Natural ingredients are explored for Indonesia's biodiversity. One of the natural Indonesian products is the Bajakah Tampala root (*Spatholobus littoralis*). The root of Bajakah Tampala is a typical plant of Central Kalimantan that is commonly used by the Dayak community as traditional medicine. Bajakah root, or *Spatholobus littoralis* Hassk, is a typical herbal plant that grows in Kalimantan [4]. Bajakah root extract contains flavonoid, phenolic, and saponin components that have antioxidant, antiviral, antibacterial, anti-inflammatory, and anti-allergic activities [5]. Previous research reported that Bajakah Tampala extract has antibacterial activity against *S. aureus* and *E. coli* [6], [7]. Hamzah et al. (2024) reported also that Bajakah Tampala extract has antifungal activity against *Streptococcus mutants* was rarely carried out, so this study is interested in conducting research using these bacteria.

This research could contribute to the development of pharmaceutical products through various extraction methods. The ultrasound assisted extraction method (UAE) is one of the modern extraction methods for extracting active compounds using high-intensity sound waves [9]. The UAE method offers to enhance the mass transport of bioactive compounds, reduce times, and have a low temperature [10]. Bajakah tampala root extract was proven to have antibacterial activity using infusion and maceration method [11], [12], but no studies have been explained on the UAE method. The goals of research to develop a natural mouthwash formula from the Bajakah Tampala root extract. In addition, the potential of the mouthwash formulation of Bajakah Tampala root extract was accepted more easily by the community.

#### 2. MATERIALS AND METHODS

#### 2.1. Place and time of research

This research was conducted at the Biology Pharmacy Laboratory of Universitas Jenderal Ahmad Yani Yogyakarta. The research was conducted from May to July 2024.

#### 2.2. Tools and materials

The materials used include the Bajakah Tampala roots, water for injection (WFI), glacial acetic acid p.a, formic acid p.a, *Streptococcus mutant*, ethanol p.a., ethyl acetate p.a, glycerin, chloroform p.a, quercetin p.a, methanol p.a, Nutrient Agar (NA) media, n-butanol p.a, n-hexane p.a, sodium benzoate p.a, paper disc blank, amoxicillin paper disc, chloramphenicol paper disc, sorbitol, peppermint oil, and Total Care® Mouthwash. The tools used are Class II BSC, autoclaves, petri dishes, CAMAG® chambers, UV detectors, micropipette (Eppendorf), infuse pan, ultrasonic bath (Cole-Parmer), analytical balance (Ohaus), rotary evaporator, Brookfield viscometer, and other glasses.

#### 2.3. Sampling and Plant Determination

The Bajakah tampala roots (*Spatholobus littoralis*) were obtained from Central Kalimantan, Indonesia. The determination of samples was carried out at the Biology Learning Laboratory, Ahmad Dahlan University, with identification number 230/Lab.Bio/B/V/2024.

#### 2.4. Preparation and sample extraction of Bajakah Tampala roots

The Bajakah tampala roots were washed and dried for two days in sunlight, then reduced in particle size using a grinder and 40-mesh sieves.

#### 2.3.1. Infusion Method

10 g of root powder were added to 100 ml of aquadest in an Erlenmeyer. Samples were heated at 90 °C for 15 minutes. The infusion of Bajakah tampala (IBT) extract was filtered with filter paper [12].

#### 2.3.2. Ultrasound-Assisted Extraction (UAE)

100 g of root powder were extracted into 1000 mL of ethanol p.a., then sonicated with an extraction time of 60 minutes at the optimal temperature of 45 °C. The extract was evaporated with a rotary evaporator at a temperature of 40 °C until UAE of Bajakah tampala (UBT) extract was obtained [13].

#### 2.5. Qualitative Analysis of Flavonoid Content

The extracts were qualitatively tested using AlCl<sub>3</sub> and FeCl<sub>3</sub> reagents to determine the flavonoid and phenolic content. A few drops of 5% FeCl<sub>3</sub> were added, and then the colour changed to brown precipitation, as well as AlCl<sub>3</sub>. The saponin test was carried out with an extract dissolved in 10 mL of hot water and shaken for 30 seconds [14].

Thin-layer chromatography (TLC) involved a stationary phase on a 60 F254 silica gel plate and two different mobile phases with n-hexane: ethyl acetate (3:7) [7] and n-butanol: ethyl acetate: water (7:4:2), modified by [15]. 50 mg of root extract and 5 mg of standard quercetin were added to 5 mL of methanol p.a. The F254 silica gel plate was activated in an oven at a temperature of 100 °C for 30 minutes and was saturated with the mobile phase. The spots were observed under visible light, UV 254 nm, and UV 366 nm. A yellow spot indicated the presence of flavonoids in the samples under UV 366 nm light [16].

#### 2.6. Sterilization

The tools were sterilized in an oven at a temperature of 171 °C for 1 hour. The materials used were sterilized by autoclave at a temperature of 121 °C for 15 minutes. Nutrient agar (NA) media was dissolved in an aqueous solution and homogenized with a stirrer at a temperature of 100 °C until boiling. The NA was sterilized by an autoclave at 121 °C for 15 minutes. Continuously, the NA was cultured with *Streptococcus mutants*, modified by[17].

#### 2.7. Inoculation of Streptococcus mutant bacteria

In the Bio Safety Cabinet (BSC), *Streptococcus mutants* were inoculated by the zig-zag method on NA media and then incubated for 48 hours at 37 °C. *Streptococcus mutants* were suspended in a tube containing 5 mL of a 0.9% NaCl solution. Then McFarland standard 0.5 measured turbidity with a turbidimeter, modified by[17].

#### 2.8. Antibacterial Activity Test by Disc Diffusion Method

The serial concentrations of 10% of IBT and 20%, 40%, 60%, and 80% of UBT were dissolved in WFI. The blank paper disc was soaked in the UBT and IBT samples for 5 minutes. 100  $\mu$ L of bacterial suspension was inserted into a petri dish containing NA media. The petri dish media was divided into four parts, such as control and samples. As a positive control, paper discs containing amoxicillin and chloramphenicol. Blank paper discs contained WFI as negative controls. After that, the petri dish was incubated at 37 °C for 24 hours, and the clear zone formed was measured by their zone inhibition diameter. This test was repeated three times modified by [17].

2.9. Mouthwash Formulation of Bajakah Tampala Root Extract

The formulation of mouthwash was selected based on the optimal concentration of extracts in Table 1. Ingredients of glycerol, sorbitol, and sodium benzoate were added to the homogeneous. Peppermint oil and extract samples were mixed and milled back until homogeneous. This mixture was then supplemented with water until it reached a total volume of 100 mL, modified by [17].

Table 1. Formula Mouthwash			
Ingredients	Units	Quantity	
80% UBT Extract	mL	10	
Peppermint oil	mL	0.1	
Sorbitol	mL	0.25	
Sodium Benzoate	g	0.1	
Glycerol	mL	1.0	
Water	mL	ad 100	

2.10. Physical Evaluation of Bajakah Tampala Root Extract Mouthwash Formulation

#### 2.10.1. Organoleptic Test

Observations were made with the five observations directly, including aroma, colour, and taste [17].

#### 2.10.2. pH Test

pH paper was dipped in mouthwash for a few minutes and then matched with the colour of the indicator. Measurements are taken at room temperature. The pH of mouthwash is good at pH 5-7 [17]

#### 2.10.3. Homogeneity Test

It is done by observing the formulation that has been put into a clear bottle and given a white background. Good mouthwash formulations are homogeneous, not cloudy, and free from contamination and microbial growth [17].

#### 2.10.4. Viscosity Test

The viscosity of this formulation was measured using a Brookfield viscometer with the spindle rotation speed set at 100 rpm and using spindle no. 2. A total of 100 mL of samples were put into beaker glass (Sulistiyono et al., 2022). The standard viscosity of mouthwash on the market is  $\pm$  7.25 cP [18].

#### 2.11. Data Analysis Methods

The inhibition zone data at each concentration of extract samples can be analysed using the normality test, homogeneity test, and One-Way ANOVA method using SPSS software v.25 with a significant level of 95%. The normality test is carried out by the Shapiro-Wilk test if the number of samples is less than 50. The homogeneity test was used to determine whether the data obtained using Levene's test. The data is distributed normally and homogeneously using One-Way ANOVA with a significant (p) > 0.05 [13].

#### 3. RESULTS AND DISCUSSION

#### 3.1. Extraction of Bajakah Tampala roots

Bajakah tampala root powder is extracted using two extraction methods, namely UAE (UBT) and infusion (IBT). UBT extract evaluated % yield value was 15%, but IBT didn't give % yield value. A good yield value is not less than 7.2%. It can be concluded that the research yield is eligible [13].

#### 3.2. *Qualitative analysis*

#### 3.2.1. Phytochemical screening

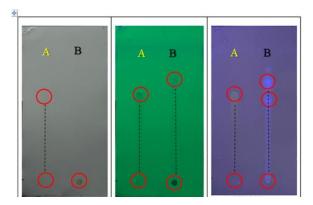
The results of phytochemical screening showed that flavonoids and phenolics were present in UBT and IBT extracts. UBT and IBT were given AlCl<sub>3</sub> to identify the content of flavonoids with their colour such as dark yellow. If contains saponins, foam 1–10 cm high is formed for no less than 10 minutes, and at the addition of 1 drop of HCl 2 N, the foam does not disappear. Meanwhile, saponins were not detected in the UBT and IBT extract [14].

Table 2. Phytochemical Screening Results			
No.	Active Compounds	Screening Result	
1	Flavonoid	+	
2	Phenolic	+	
3	Saponin	-	

Notes: (+) Positive; (-) Negative

#### 3.2.2. Thin-Layer Chromatography (TLC)

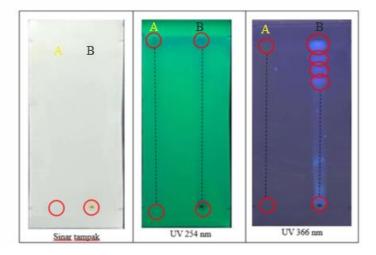
The results of the TLC test were used using a mobile phase of n-hexane: ethyl acetate with a ratio of 3:7 v/v and the stationary phase of the  $F_{254}$  silica plate with quercetin standard and UBT extract. The Rf (retention factor) value was calculated to be used as evidence in the identification of compounds, namely flavonoids. If the Rf value is the same, the compound has similar characteristics to the comparator (quercetin). The standard Rf result was 0.6, and the sample Rf was 0.7.



**Figure 1.** TLC results of UBT extract with n-hexane: ethyl acetate (3:7 v/v); A: Quercetin standard; B: UBT extract.

The results of the TLC test used a mobile phase of n-butanol: ethyl acetate: water with a ratio of 7:4:2 v/v/v and a stationary phase of silica plate F254 with quercetin standards and UBT extract.

The results of the UBT extract showed several fluoresced points that were visualized in UV 366 nm. The standard Rf result was 1, and the sample Rf was 0,7-1.



. Figure 2. TLC results of UBT extract with n-butanol:ethyl acetate:water (7:4:2 v/v/v); A: Quercetin standard; B: UBT extract

#### 3.3. Antibacterial activity using disk diffusion method

Antibacterial activity was evaluated the inhibition zone in various concentration of Bajakah Tampala with UAE (UBT) and infusion (IBT). According to the analysis of the inhibition zone, the concentration of 20% UBT and IBT extract could inhibit the growth of Streptococcus mutans bacteria in Figures 3 and 4. The inhibition zone wasn't formed optimally clear with an average diameter of 9.84±1.17 mm and 7.51±0.13 mm by 20% UBT and IBT extracts. The 40%, 60%, and 80% UBT-formed inhibition zones formed optimally clear with an average diameter of 10.78±1.17; 13.56±1.54; and 14.01±2.70 mm.

Treatments Concentration Replication Mean ±SD Interpretation Ι Π III UBT 20% 10.33 10.70 8.50  $9.84 \pm 1.17$ Medium UBT 40% 11.53 11.40 9.43  $10.78 \pm 1.17$ Strong UBT 60% 14.63 14.27 11.80  $13.56 \pm 1.54$ Strong UBT 80% 15.30 15.83 10.90 14.01±2.70 Strong IBT 7.43 10% 7.66 7.43 7.51 ±0.13 Medium Chloramphenicol 30.88 30.65 0 20.51±17.76 Powerful (+) Amoxicillin 30.40 26.53 24.00 26.97 ±3.22 Powerful (+) Negative Control 0 0 0  $0.00 \pm 0.00$ None (-)

Table 3. Inhibition Zone Diameter (mm) Evaluated Antibacterial Activity

Based on Table 3, the greater the concentration of the UBT root extract, the larger the clear zone formed in the petri dish. The selection of UBT concentrations was based on the strength of the diameter of the inhibition power at concentrations of 20% and 40%, which were included in the moderate category (5-10 mm), while concentrations of 60% and 80% were included in the strong category [19]. The higher the concentration of the Bajakah tampala root extract, the stronger the inhibitory power against *Streptococcus mutans*. Bajakah tampala extract has flavonoids that play an important role in inhibitory reactions to bacterial growth, especially *Streptococcus mutans*. Previous research conducted by [20] reported that papaya leaf and green tea infusion extracts have antibacterial activity against *Streptococcus mutans* bacteria due to their flavonoid content.

No	P (0,05)	
1	Shapiro-Wilk	0.061 (>0.05)
2	Levene test	0.015 (<0.05)
3	One-Way ANOVA	0.000 (<0.05)

The inhibition zone bacterial of Bajakah tampala root extract was evaluated normality test using the Shapiro-Wilk method that the value of significance of 0.061, shown in Table 4. The value obtained is greater than p > 0.05. These indicates that the data on the diameter of the inhibition zone of Bajakah tampala root extract against *Streptococcus mutans* was normally distributed. Furthermore, the results of the homogeneity test determined using Levene statistical test. The homogeneity test showed P<0.05, which means that data was not homogeneous. A One-Way ANOVA result could be performed that the data was normally distributed. The One-way ANOVA test obtained a result of 0.000, so the P value obtained is smaller than 0.05. The inhibition zone of Bajakah tampala root extract against *Streptococcus mutans* has a significant difference based on One-way ANOVA test. There is a difference in the diameter of the inhibition zone at each concentration of UBT, IBT, negative control and positive control (chloramphenicol and amoxicillin).



**Figure 3.** Inhibition zones formed on various concentrations of IBT, positive control and negative control against *S. mutans* 



Figure 4. Inhibition zones formed on UBT, positive control and negative control against S. mutans

#### 3.4. Mouthwash Formulations

In this study, one formula was made with the strongest concentration of extract for bacterial inhibition, which is at a concentration of 80%. The mouthwash made is composed of 80% UBT

extract (as an active substance), glycerol (as a humectant), sorbitol (a sweetener), sodium benzoate (as a preservative), peppermint oil (as a flavor enhancer or freshner), and water (as a solvent). The mouthwash that has been made must have the effectiveness of the active ingredient content, comfort when using, and viscosity of the solution. In addition, mouthwash must have a fresh taste when used for gargling.

#### 3.5. Mouthwash Evaluation

#### 3.5.1. Organoleptic and Homogeneity test

The organoleptic extract formulation has the appearance of a clear chocolate and peppermint fragrance with a mint taste. The homogeneity test on the mouthwash formula showed that there were no particles, it was not cloudy, and it was clear. The homogeneity test of the mouthwash formula obtained was acceptable.

	80% UBT	Total Care®
Fragrance	Peppermint fragrance	Mint
Taste	Mint	Mint
Color	Clear chocolate	Clear yellow
Texture	Liquid	Liquid



#### 3.5.2. pH Test

The pH value of mouthwash needs to be known so that it doesn't irritate the mouth or taste sour when used for gargling. The pH of mouthwash is neutral at pH 5-7. A mouthwash whose pH is acidic will cause irritation in the mouth, while a pH that is alkaline will trigger the growth of fungus that causes canker sores or aphthous stomatitis. From Table 6, the pH results of the mouthwash formula were similar to those of conventional mouthwash (Total Care).

l able 6.	Mouthwash	Evaluation

Formula	pН	Homogeneity	Viscosity
Mouthwash (80% UBT)	5	Clear	7.20 cP
Total Care ®	5	Clear	7.25 cP

#### 3.5.3. Viscosity test

Viscosity describes the amount of resistance that a liquid has to flow. The greater the viscosity value, the more difficult it is for a liquid to flow, and vice versa. Viscosity measurement is important to do because it can affect how easily the formulation flows out of the container so that it is easy to apply. The viscosity of mouthwash formulations is most important because it can affect the comfort of use. A good viscosity of Bajakah tampala mouthwash evaluated not too thick. The closer the viscosity of the mouthwash to the viscosity of the water, the better and more comfortable it is when used. The standard viscosity of mouthwash on the market is ± 7.25 cP (Rowe et al. 2009). The result of the viscosity observation in Table 6 showed that Bajakah Tampala mouthwash was similar value with conventional mouthwash (Total Care®) [17].

#### 3.6. Research limitations

The limitation of this study was that the raw material of Bajakah Tampala roots (*Spatholobus littoralis*) might not be easily available in several areas. Additionally, there were no saponins in the Bajakah Tampala extract in the results of the phytochemical screening test, which may limit the applicability of the research findings. The potential future of herbal mouthwash formulations is to optimize formulation, combination of other herbal medicines, stability of the product, and preclinic trials compared to other market mouthwashes.

#### 4. CONCLUSION

Studies revealed that 80% UBT had antibacterial activity greater than other extracts with an inhibition zone of 14,01±2,70 mm. These findings allowed for the development of an efficient mouthwash dose formulation at 80% UBT. The organoleptic, pH, homogeneity, and viscosity test were evaluated in the mouthwash formulation assessment. UBT mouthwash product qualified evaluation parameters. In conclusion, this study enhanced the potential of Indonesian medicinal herbs, which led to innovation in herbal mouthwash formulation.

**Funding:** This research was financed by Program Kreativitas Mahasiswa 2024 funding from Direktorat Jenderal Pembelajaran dan Kemahasiswaan Kementerian Riset, Teknologi, dan Pendidikan Tinggi Republik Indonesia.

Acknowledgments: We would like to express our gratitude to Bachelor Pharmacy, the Faculty of Health, Jenderal Achmad Yani University, and Direktorat Jenderal Pembelajaran dan Kemahasiswaan Kementerian Riset, Teknologi, and Pendidikan Tinggi Republik Indonesia for funding this research through the scheme "Program Kreativitas Mahasiswa 2024" funding. We also want to thank Dinas Kehutanan Barito Tengah, Kalimantan Tengah for sampling Bajakah Tampala roots.

**Conflicts of interest:** The authors declare no conflict of interest.

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