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Research Article

In Vitro Anti-Inflammatory Activity of Bajakah Tampala Root (*Spatholobus littoralis* Hassk.)

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ABSTRACT: Bajakah tampala root (Spatholobus littoralis Hassk.) contains phenolic compounds, flavonoids, tannins, terpenoids and saponins. Flavonoid compounds have pharmacological effects, namely anti-inflammatory. The purpose of this study was to determine the anti-inflammatory activity of ethanol extract of bajakah tampala root by inhibiting protein denaturation in vitro using UV-Vis spectrophotometer and diclofenac sodium as positive control. The extract was prepared by maceration method using 96% ethanol solvent. The results showed that the ethanol extract of bajakah tampala root had a percentage of inhibition of 31.27% at the lowest concentration (10 ppm) and 43.28% at the highest concentration (50 ppm) with an IC_{50} value of 77.92 µg/mL and the ic50 value of diclofenac sodium is 12.26 µg/mL. The percentage of inhibition that is more than 20% is considered to have inhibitory power in the protein denaturation process. So, it can be concluded that the ethanol extract of bajakah root has potential as an anti-inflammatory.

KEYWORDS: Bajakah tampala root; extract; anti-inflammatory; protein denaturation

1. INTRODUCTION

Indonesia has various types of plants and abundant natural resources. Indonesia is one of the megabiodiverse countries because it has the second largest tropical forest in the world, has more than 20,000 species of medicinal plants, but only 1,000 species have been recorded and have been used for traditional medicine, with only about 300 species (Hariana, 2013). People in Indonesia have a habit of using traditional medicine as an alternative to treat various diseases. Traditional medicine uses natural ingredients derived from plants, which contain chemical compounds known as secondary metabolites.

Plant extracts have been widely studied for their anti-inflammatory properties, which refer to their ability to reduce or inhibit inflammation, a biological response to harmful stimuli such as pathogens, damaged cells, or irritants. While inflammation is essential for healing, chronic inflammation is associated with various diseases, including arthritis, cardiovascular disorders, and cancer. The anti-inflammatory effects of plant extracts are primarily due to their bioactive compounds, such as flavonoids, phenolics, alkaloids, and terpenoids, which act through several biological mechanisms. One major mechanism is the inhibition of pro-inflammatory mediators like cytokines (e.g., TNF-α, IL-1β, IL-6), prostaglandins (particularly PGE2), and nitric oxide (NO). These compounds often exert their effects by downregulating key enzymes such as cyclooxygenase (COX-1 and COX-2) and inducible nitric oxide synthase (iNOS).

Additionally, plant extracts modulate various intracellular signaling pathways involved in inflammation, including the nuclear factor-kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and Toll-like receptor (TLR) pathways. Another important mechanism is their antioxidant activity, which helps neutralize reactive oxygen species (ROS) that contribute to oxidative stress and inflammation. Plants rich in antioxidants, such as polyphenols and flavonoids, play a dual role by both reducing oxidative damage and suppressing inflammatory responses (Fawwaz et al., 2023).

Common examples of anti-inflammatory plant extracts include *Curcuma longa* (turmeric), where curcumin inhibits COX-2 and NF-κB; *Zingiber officinale* (ginger), which contains gingerol with COX and lipoxygenase (LOX) inhibitory activity; *Camellia sinensis* (green tea), whose catechins like EGCG suppress inflammatory cytokines; and *Nigella sativa* (black seed), where thymoquinone exhibits both anti-inflammatory and antioxidant effects. The efficacy of these extracts is often demonstrated through both in vitro studies, using cell lines like RAW 264.7 macrophages stimulated with lipopolysaccharides (LPS), and in vivo models such as carrageenan-induced paw edema, cotton pellet-induced granuloma, and the acetic acid-induced writhing test. Due to their broad-spectrum activities and lower risk of side effects compared to synthetic drugs, plant extracts hold great promise as natural alternatives or complementary agents in managing inflammatory conditions.

One of the plants empirically used by the people of inland Kalimantan as traditional medicine is bajakah tampala root (*Spatholobus littoralis* Hassk.) Based on qualitative preliminary tests conducted by Anshari (2012) bajakah tampala contains phenolics, flavonoids, tannins, terpenoids and saponins. The content of these secondary metabolite compounds can treat various degenerative diseases, such as diabetes, cancer, tumors, and others (Fawwaz et al., 2022). Active compounds that play a role in the pharmacological effects of a medicinal plant are flavonoid compounds. Flavonoid compounds have potential as wound and side healers, especially for gastric irritation that triggers gastric ulcers and kidney damage. As a result, the search for alternative inflammatory treatments with herbal plants is on the rise.

2. EXPERIMENTAL SECTION

2.1. Tools

Pipet tetes (Pyrex,Jerman), tabung reaksi, (Pyrex,Jerman), gelas beaker 10 mL, 50 mL, 100 mL, dan 1000 mL (Pyrex,Jerman) , Labu ukur 10 mL, 50 mL, 100 mL, 250 mL (Pyrex,Jerman), mikropipet (dragonlab, China), gelas ukur 50 mL, dan 100 mL (Pyrex,Jerman), sendok tanduk (ROFA, Indonesia), blender (Philips, Belanda), penangas air, spektrofotometer UV-Vis (thermoScientific Tipe Genesys 10s UV-Vis, Jerman), pH meter dan rotary evaporator (Buchi, Swiss),timbangan analitik (electronic balance, China) vortex (Krisbow, Indonesia).

2.2. Sample Preparation

Tampala bajakah root (S. littoralis Hassk.) from Bakungan Village, Loakulu District, Kutai Kartanegara Regency, Central Kalimantan Province and the sample used is ethanol extract of bajakah root (*S. littoralis* hassk). Samples of bajakah tampala roots (S. littoralis Hassk.) that have been washed using running water until clean. Next, the samples were dried and sorted dry and then mashed using a blender. The powdered simplisia is stored in a tightly closed container and

2.3. Extraction

The extraction procedure uses a cold extraction method, namely the maceration technique by means of 300 g of bajakah tampala (S. littoralis Hassk.) root simplisia powder extracted by maceration method using 96% ethanol for 3 x 24 hours and repeated remaceration until clear. Every day, stirring was occasionally done. The results of the bath were then filtered and concentrated using a rotary evaporator at 50°C to obtain a thick extract. Furthermore, the calculation of extract yield was carried out:

$$\%\ yields\ extract\ = \frac{total\ weight\ of\ the\ extract}{total\ simplisia\ powder\ wight}\ \text{x}\ 100\ \%$$

2.4 Qualitative Analysis

2.4.1. Flavonoid Test

100 mg ethanol extract of bajakah tampala root was added to 10 ml of hot water, boiled for 5 minutes and filtered. The filtrate obtained was added 0.05 grams of Mg powder, then 2 drops of concentrated HCl were added and shaken vigorously. The presence of flavonoid compounds is marked by the formation of orange to red or yellow color (Jannah et al., 2020).

2.4.2. Saponin Test

Weighed 100 mg ethanol extract of bajakah tampala root dissolved with ethanol solvent, then shake vigorously. Add 1 drop of concentrated HCl. The presence of saponin compounds is characterized by the formation of foam that is 1-3 cm high and will last for 15 minutes (Jannah et al., 2020).

2.4.3. Terpenoid Test

100 mg ethanol extract of bajakah tampala root was dissolved with 0.5 ml chloroform, added 0.5 ml CH3COOH, and dripped 2 ml H2SO4 through the tube wall. The presence of terpenoid compounds is marked by the formation of purple or red color (Jannah et al., 2020).

2.4.4. Phenolic Test

100 mg ethanol extract of bajakah tampala root was dissolved with ethanol solvent, shaken vigorously, and 3 drops of 1% FeCl3 solution were added. The presence of phenolic compounds is marked by the formation of red, green, blue, purple, or black colors (Jannah et al., 2020).

2.4.5. Tannin Test

100 mg ethanol extract of bajakah tampala root was dissolved with hot water, then boiled for 5 minutes and filtered. The filtrate obtained was added 1% gelatin. The presence of tannin compounds is characterized by the formation of a white precipitate (Jannah et al., 2020).

2.5. Anti-inflammatory Activity

2.5.1. Preparation of TBS (Tris Buffer Saline) Solution

TBS solution is made by dissolving 4.35 g of NaCl in 200 mL of distilled water, 605 mg of Triss buffer is added to 400 ml of distilled water. Then to adjust the pH, glacial acetic acid is added to obtain a pathological pH of 6.2-6.5 and then filled with distilled water to 500 ml in a volumetric flask (Reynaldi & Yani, 2021).

2.5.2. Preparation of 0.2% BSA (Bovine Serum Albumin)

Preparation of 0.2% BSA was done by dissolving 0.2 grams of BSA (Bovine Serum Albumin) with 100 mL of Triss buffer saline solution (Reynaldi & Yani, 2021).

2.5.3. Preparation of Negative Control Solution

A total of 500 µl of ethanol was added with 0.2% BSA (Bovine Serum Albumin) solution in TBS (Triss Buffer Saline) into a volumetric flask to a volume of 5 mL (Fitri Yanti, 2021).

2.5.4. Preparation of Diclofenac Sodium Solution as Positive Control

Preparation of diclofenac sodium solution as a positive control, 10 mg of diclofenac sodium was dissolved with 96% ethanol into a 10 mL volumetric flask and diluted with ethanol to 10 mL, so as to obtain a mother solution with a concentration of 1000 ppm. Then diluted to 100 ppm. Then a concentration series of 10; 12.5; 15; 17.5; 20 ppm was made by pipetting the stock solution as much as 0.5; 0625; 0.75; 0.875; 1 ml, respectively, then sufficient with solvent to the final volume of 5 ml. (Rahmawati, Widiastuti, & Sulistya, 2020).

2.5.5. Preparation of Anti-inflammatory Activity Test Solution of Ethanol Extract of Bajakah Root

A total of 10 mg of bajakah tampala root extract (S. littoralis Hassk.) was dissolved in extract solvent (ethanol) in a 10 mL volumetric flask and then diluted with solvent to a volume of 10 mL so as to obtain a mother solution with a concentration of 1000 ppm. Then diluted to 100 ppm. Then a concentration series of 10; 20; 30; 40; 50 ppm was made by pipetting the stock solution as much as 0.5; 1; 1.5; 2; 2.5 ml, respectively, then sufficient with solvent to the final volume of 5 ml (Rahmawati, Widiastuti, & Sulistya, 2020).

2.5.6. Anti-Inflammatory Activity Testing

500 µl of each sample solution, positive control solution, and negative control were taken, 0.2% BSA solution in TBS was added until the volume became 5 mL and incubated at 25°C for 30 minutes. Each mixture was then heated for 5 minutes at 100°C, then cooled by immersing in a container of water for approximately 10 minutes. After cooling, the solution was vortexed and absorbance measurements were taken using a UV-Vis spectrophotometer at a wavelength of 660 nm (Mulyani, T, 2023).

2.6. Data Analysis

2.6.1. Calculation of Protein Denaturation Inhibition Activity

Compounds that inhibit protein denaturation greater than 20% are considered to have anti-inflammatory activity.Protein denaturation inhibition activity was measured using the following formula:

$$\% \text{ of inhibition} = \frac{absorbance\ of\ negative\ control - absorbansi\ of\ test\ solution}{absorbance\ of\ negative\ control}\ x\ 100\%$$

2.6.2. Calculation of IC_{50} Value

The IC_{50} value of each sample concentration was calculated using the linear regression equation formula. Sample concentration as the x-axis and % inhibition as the y-axis of the equation Y = a + bx, where y = % of inhibition (50), x = contentration, a = intercept (intersection of lines on the axis Y), b = slope. To determine the IC_{50} value, it can be calculated using the formula:

$$IC_{50} = \frac{(50 - a)}{b}$$

3. RESULTS AND DISCUSSION

Bajakah tampala root was chosen as a sample because it contains flavonoids that have potential as anti-inflammatory (Panche et al., 2016), besides that bajakah tampala root also has antioxidants that can accelerate wound healing (Ginwala et al., 2019). **Table 1** showed that the yield of the extract obtained is 10.1%. This result shows a good percentage yield. According to the Indonesian Ministry of Health (2008), the optimal extract is one that has a yield of >10% (Indonesian Ministry of Health, 2008 in Ramadhani et al., 2020).

Table 1. Extraction result and % yield of ethanol extract of bajakah tampala root (*S. littoralis* Hassk.)

Extraction Methods	Solvent	Simplisia weight (g)	Solvent (mL)	Extract weight (g)	Extract yirlds (%)
Maceration	Ethanol 96%	300	2900	30.3	10.1

The qualitative analysis exhibited that all compounds tested was positive found in the extract as shown in **Table 2**. After the qualitative test was carried out, the anti-inflammatory activity test was continued. Inflammation is a response of the body's immune system to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation (Bare et al., 2019). One of the causes of inflammation is protein denaturation in tissues. So that the search for anti-inflammatory compounds can use the method of inhibiting protein denaturation (Shandy et al., 2023).

Table 2. Qualitative analysis of bajakah tampala (*S. Littoralis* Hassk.)

Compounds	Test Result	Description
Flavonoid	Orange to red in color	(+)
Saponin	Forms foam	(+)
Terpenoid	Purplish red	(+)
Phenolik	Black color	(+)
Tannin	White precipitate formed	(+)

Description: + = positive

The protein denaturation method was chosen because it is suitable for initial screening of anti-inflammatory activity testing in vitro. Protein denaturation inhibition testing uses bovine serum albumin (BSA) as a protein. Testing of anti-inflammatory activity against protein denaturation is carried out with the addition of Bovine Serum Albumin (BSA) solution, this is because to reduce the use of live specimens in the drug development process and when BSA is heated, denaturation will occur. According to Nasution (2019), compounds that can stabilize proteins from the protein denaturation process are compounds that have potential as anti-inflammatories. Where, there is an interaction between BSA and the active substance of the compound which results in the bonding of the active substance with tyrosine, threosine and lysine (Novika et al., 2021).

The positive control used is diclofenac sodium because it is an anti-inflammatory drug from the NSAID group, widely used to treat inflammatory symptoms, and its ingredients are easily available (Abidin et al., 2019). Additionally, it has the strongest anti-inflammatory properties. Anti-inflammatory activity of positive control shown in **Table 3,** and the calibration curve exhibited in the **Figure 1**.

Table 3. Anti-inflammatory activity of sodium diclofenac

Concentration (mg/L)	Absorbance	Inhibition (%)
Negative control	0.675	0.00
10	0.362	46.37
12.5	0.328	51.40
15	0.311	53.92
17.5	0.308	54.37
20	0.302	55.25

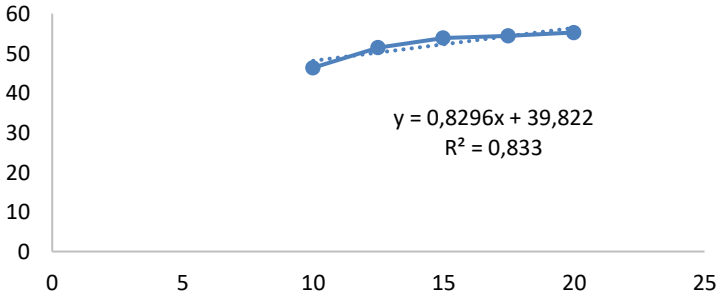

Figure 1. Linear regression of positive control

Based on the results of diclofenac sodium anti-inflammatory activity testing as shown in **Table 3**, the percentage of inhibition increases as the concentration of diclofenac sodium increases. The percentage of diclofenac sodium inhibition is above 20%, in the range of 43.37% - 55.25%. According to the literature, the percentage of inhibition of more than 20% is considered to have inhibitory power in the protein denaturation process (Novika et al., 2021).

The anti-inflammatory activity of ethanol extract of bajakah tampala root shows that there is an increase in the percentage of inhibition when the concentration is increased like diclofenac sodium. The percentage of inhibition of bajakah tampala root already has inhibitory power because it is more than 20% in the range of 31.27% - 43.28% (**Table 4**). Furthermore, the IC_{50} value was calculated based on the linier regression of inhibitory activity of extract (**Figure 2**). The IC_{50} value is a number that shows the concentration of a sample that can inhibit inflammatory activity by 50% (Sukandiarsyah, et al., 2023).

Table 4. Anti-inflammatory activity of bajakah tampala root

Concentration (mg/L)	Absorbance	Inhibition (%)
Negative control	0.908	0.00
10	0.624	31.27
20	0.599	34.03
30	0.587	36.35
40	0.561	38.21
50	0.515	43.28

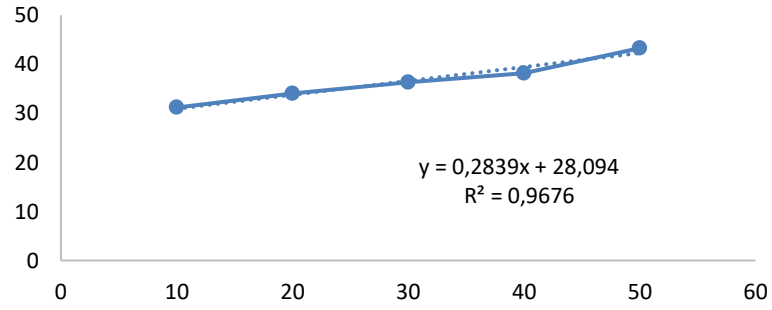

Figure 2. Linear regression of the ethanol extract of bajakah tampala root

The IC_{50} calculation uses a curve between the independent variable, namely solution concentration (X) and the dependent variable, namely % inhibition (Y) so that a linear regression equation is obtained. Linear regression is a statistical method used to form a model or relationship between one or more independent variables X with a response variable Y. Then the linear regression of diclofenac sodium is obtained y = 0.8296 + 39.822 where the R2 value = 0.833. The linear graph of ethanol extract of bajakah root is y = 0.2839 + 28.094, where the value of R2 = 0.9676. According to Sugiyono (2006) a very strong correlation coefficient is at 0.80-1.000 because the correlation value close to 1 has a stronger variable relationship and the line has a positive slope, the increasing concentration (X) the % inhibition value of the sample (Y) is increasing, so it can be continued to the calculation of IC_{50} (Sugiyono 2006 in Reynaldi and Yani, 2021).

Table 5. The comparison of the IC_{50} value calculation

Samples	IC_{50} Value
Diclofenac sodium	12.26 µg/mL
Ethanol extract of bajakah tampala root	77.92 µg/mL

From these results, it is known that there is potential anti-inflammatory activity in the bajakah tampala root sample. The anti-inflammatory activity is due to the presence of several chemical contents, namely flavonoids, tannins, and terpenoids in bajakah tampala root. According to Fridina (2012) flavonoid content inhibits the lipooxygenase pathway directly in inflammation which causes inhibition of eicosanoid biosynthesis and activates free radicals that can attract various inflammatory mediators. Tannin content can affect the inflammatory response with its activity as a free radical antidote. The terpenoid content can inhibit the release of prostaglandins from their source cells, so that the formation of histamine, prostaglandins and other chemical mediators that cause inflammation can be inhibited (Fridina, 2012 in Novika et al., 2021). From this description, it can be concluded that ethanol extract of bajakah tampala root has potential as an anti-inflammatory.

4. CONCLUSION

The percentage value of inhibition of ethanol extract of bajakah tampala root increases according to the concentration with the lowest percentage at a concentration of 10 mg/L which is 46.37% and the highest percentage at a

concentration of 20 mg/L which is 55.25% with an IC50 value of 77.92 µg/mL. These results indicate that bajakah tampala root has inhibitory power in the protein denaturation process because the percentage of inhibition is more than 20%. So it can be concluded that the ethanol extract of bajakah tampala root (Spatholobus littoralis Hassk.) tested in vitro through the protein denaturation method gives the result that the ethanol extract of bajakah tampala root has potential anti-inflammatory activity.

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