RESEARCH PAPER

Antioxidant Activity and Inhibition of α-Glucosidase in Ethanol Extracts of Bajakah Tampala (Spatholobus littoralis Hassk), Bajakah Kalalawit (Arcangelisia flava (L.) Merr) and Bajakah Kuning (Uncaria gambir (W.Hunter) Roxb.

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ABSTRACT

Bajakah is a plant whose roots are used in traditional medicine and has many pharmacological properties in ethnomedicine. Bajakah contains secondary metabolites, namely flavonoids and phenols, which produce activities such as antioxidants, antidiabetics, anti-inflammatories, and anticancer agents. The objective of this study was to determine the total phenolic content, total flavonoid content, and α-glucosidase inhibitory activity of 96% ethanol extract of bajakah. This study began with phytochemical screening of crude drugs and extracts using thin-layer chromatography, total phenolic content using the Folin-Ciocalteru method, total flavonoid content using the AlCl3complex method, and antioxidant activity using the ABTS ((2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) method, and α-glucosidase inhibition was determined in vitro. The data on total phenolic content, total flavonoid content, and antioxidant activity (IC50) were analyzed using Pearson's correlation. α-glucosidase inhibition activity with IC50values.The results showed that the total phenolic and total flavonoid content in the three bajakah extracts, namely bajakah tampala, had values of 133.38 ± 6.33 mgGAE/g and 11.12 ± 0.24 mgGAE/g sample, bajakah kalalawit 112.24 ± 10.02 mgGAE/g and 7.28 ± 0.12 mgGAE/g sample, bajakah kuning 21.14 ± 1.55 mgGAE/g and 10.0 ± 0.50 mgGAE/g sample. Tampala bajakah extract had the strongest antioxidant activity with an IC50value of 46.32 ± 0.01 mL/L and α-glucosidase inhibition activity of 346.15 U/L. There was a moderate correlation between total phenol content and IC50value.

Keywords: Bajakah, Antioxidants, Total Phenolic Content, Total Flavonoid Content, α-glucosidase

How to cite this article: Damayantie W, Mahfudh N, Widyaningsih W. Antioxidant Activity and Inhibition of α-Glucosidase in Ethanol Extracts of Bajakah Tampala (Spatholobus littoralis Hassk), Bajakah Kalalawit (Arcangelisia flava (L.) Merr) and Bajakah Kuning (Uncaria gambir (W.Hunter) Roxb. Int J Drug Deliv Technol. 2026;16(17s): 259-270. DOI: 10.25258/ijddt.16.17s.32

INTRODUCTION

A plant that is often used empirically by the people of Kalimantan is Bajakah. Indonesians have a habit of using traditional medicine as an alternative treatment for various diseases. Degenerative diseases continue to be the leading cause of death in every country in the world. Degenerative diseases can be caused by the formation of free radicals that react with cells and body tissues(1) . Antioxidants are compounds that can neutralize free radicals in the human body so that cell damage can be prevented(2). Antioxidants are highly oxidizable or strong reducing agents, so they tend to react with free radicals before other molecules. There are two types of antioxidants: antioxidants produced naturally by the body (endogenous antioxidants) and exogenous antioxidants(3). If antioxidants are insufficient, the body needs exogenous antioxidants. Various plants in Indonesia can act as antioxidants due to their active compounds. One such plant is bajakah. Bajakah comes in various types, such as bajakah tampala, kalalawit, and kuning(4). Bajakah tampala has been proven to accelerate wound healing(5). The efficacy and pharmacological activity of Bajakah Tampala are thought to be due to its various phenolic compounds. Research shows that bajakah tampala (*Spatholobus littoralis* Hassk**)** contains alkaloids, flavonoids, phenols, saponins, and tannins(6). Antioxidants are essential for healing and treating degenerative diseases such as diabetes, liver damage, inflammation, cancer, cardiovascular disease, neurological disorders, and the aging process(7). Various

compounds found in bajakah play a role as antioxidants(8) . Research shows that bajakah wood has antioxidant activity (IC_{50}) of 26.29 ppm, which is classified as very strong activity. In fact, this antioxidant activity is higher than that of vitamin C, which has an IC_{50}value of 30.74 ppm(9). Researchers have discovered many chemical compounds that have the potential to act as free radical scavengers and antioxidants. Phenolic compounds are substances that have antioxidant properties that are essential in the recovery and treatment of various degenerative diseases, such as diabetes, liver damage, inflammation, cancer, cardiovascular disorders, neurological disorders, and aging(10) . This study aims to identify the relationship (correlation) between antioxidant activity, total phenolic content, total flavonoids, and the ability of bajakah extract to inhibit *the α-glucosidase* enzyme in vitro and to provide an overview of the therapeutic potential of this plant in the treatment of type 2 diabetes. Inhibition *of α-glucosidase* is expected to reduce glucose absorption in the digestive tract, thereby controlling blood glucose levels after meals(11). Furthermore, through multivariate correlation analysis of bajakah extract that simultaneously integrates phenolic, flavonoid, antioxidant, and α-glucosidase inhibition parameters, specific bioactivity patterns supporting the development of Kalimantan ethnobotanical-based diabetes phytopharmaceuticals that have not been previously explored are revealed.

MATERIALS AND METHODS

Materials

The main materials used in this study included Bajakah Tampala, Bajakah Kalalawit, and Bajakah Kuning, which were collected from the Balikpapan Botanical Garden Technical Implementation Unit (UPTD), Karang Joang, North Balikpapan, East Kalimantan, and determined at Gadjah Mada University Faculty of Pharmacy with number UN1/FA.2/BF/PT.01.06/2025. Additional materials used were 96% ethanol solvent *(Brataco)*, quercetin *(Sigma-Aldrich),* sodium nitrite *(SmartLab)*, aluminum chloride *(Thermo Scientific Chemicals),* distilled water, hydrochloric acid *(Labscan),* N-hexane *(Merck),* ethyl acetate *(Emsure),* methanol *(Emsure),* sodium hydroxide *(Sigma-Aldrich),* sodium carbonate *(Emsure)*, gallic acid (Sigma-Aldrich), Folin-Ciocalteu's reagent *(Merck)*, sodium carbonate *(SmartLab)*, and ABTS (2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) using UV-Visible Spectrophotometry, *α-Glucosidase* (*QuantiChromTM α-Glucosidase Assay Kit*)

The equipment used in this study included: *rotary evaporator* (Heidolph), analytical balance (Biobased), *water bath* (Memmert), autoclave, Whatman No. 1 filter paper, *moisture balance* (OHAUS MB 45), and UV-Vis spectrophotometer *(Thermo Scientific).*

Work procedure

Preparation of Bajakah Extract

Bajakah Tampala, Kalalawit, and Kuning were collected from the Balikpapan Botanical Garden, Karang Joang, North Balikpapan, East Kalimantan, and plant specimens were identified at Gadjah Mada University, Faculty of Pharmacy, with No. UN1/FA.2/BF/PT.01.06/2025. 510 grams of Bajakah stem powder was macerated with 1.5 L of 96% ethanol until the powder was completely submerged. The maceration process was carried out for 3x24 hours while stirring occasionally. The macerate was then separated from the residue using a . The maceration product was then evaporated using a rotary evaporator and concentrated using a water bath.

Fractionation.

Fractionation of bajakah ethanol extract was carried out in stages using N-hexane, ethyl acetate, and methanol solvents through liquid-liquid partition extraction to separate compounds based on polarity. This process produced pure fractions ready for testing of *total phenolic content* (TPC), *total flavonoid content* (TFC), and antioxidant activity. Five grams of ethanol extract was dissolved in 50 mL of n-hexane and stirred for 15 minutes, then separated into n-hexane and residue fractions. The residue was treated with 50 mL of ethyl acetate twice, producing an ethyl acetate fraction, followed by methanol for the final fraction. The partition fractionation procedure aims to gradually isolate active phenolic compounds and flavonoids(12) .

Phytochemical Screening

Bajakah powder was tested for its secondary metabolite content. Several tests were conducted, including alkaloid, quinone, saponin, flavonoid, phenolic, tannin, and steroid/triterpenoid tests. The flavonoid test was conducted by adding Mg and concentrated HCl to the bajakah tampala extract. The addition of concentrated HCl was used to hydrolyze flavonoids into their aglycones, namely by hydrolyzing

O-glycosyl. Glycosyl will be replaced by H^+ from acid due to its electrophilic nature. Reduction with Mg and concentrated HCl can produce red or orange complex compounds in flavonols, flavanones, flavanonols, and xanthones(13) . The alkaloid test was performed by pipetting 1 ml of the sample, mixing it with 1 ml of chloroform and 1 ml of ammonia, placing it in a test tube, heating it over a water bath, shaking it, and filtering it. The filtrate obtained is divided into two parts. Then, add 3 drops of H_2SO_4 2N to each part, shake, and let stand until the upper part of each filtrate separates and is tested with Mayer's and Dragendorff's reagents. A positive test result is obtained if a brown precipitate forms with the Dragendorff reagent and a white precipitate forms with the Mayer reagent(14) . Saponin test: 1 ml of extract is dissolved in 10 ml of distilled water, shaken, and if stable foam or bubbles form for less than 10 minutes, then upon addition of 1 drop of 2N HCl, the foam does not disappear(15) . Heat 5 ml of the sample extract, then add 1% $FeCl_{(3)}$.A positive result is indicated by a blue-black color change(15) . Terpenoid test: Add 1 ml of the sample extract to the Liebermann-Burchard reagent. The presence of terpenoids is indicated by the formation of a red color. Steroid Test: 1 ml of sample extract is added to Liebermann-Burchard reagent; the presence of terpenoids is indicated by the formation of a blue color(16) .

Determination of Total Phenol Content (TPC)

The total phenol content was tested using the *Folin-Ciocalteu* method. Weigh 10.0 mg of gallic acid standard or extract, add 7.5 mL of distilled water and 0.5 mL of *Folin-Ciocalteu* reagent, and shake until homogeneous. The resulting solution is clear yellow in color and is left to stand for 10 minutes. Then add 1.5 mL of Na_2CO_3 200g/L solution, shake until homogeneous. The solution is left to stand for 2 hours in a dark place. Next, measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 760 nm. The total phenol content is expressed in mg GAE (*Gallic Acid Equivalent*) per gram of sample(17) .

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined using aluminum chloride colorimetry. For TFC testing, 50 mg of each extract sample was taken and added to 2 mL of 4N HCl. Hydrolysis was performed in an autoclave at 110°C for 2 hours. The sample was filtered to obtain a clear filtrate, then ether was added to the filtrate for extraction. The upper ether phase layer was collected, and the ether phase was taken. The extraction was repeated three times. Next, the ether phases are combined. Dry to remove solvents and weigh 10.0 mg of standard quercetin gallic acid or extract, then add 0.3 mL of 5% sodium nitrite. After 5 minutes, add 0.6 ml of 10% aluminum chloride, wait 5 minutes, add 2 ml of 1 M sodium hydroxide. Then add distilled water to 10 ml with a measuring flask. Transfer to a cuvette and measure with a UV-Vis spectrophotometer at a wavelength of 510 nm(18).

Antioxidant Activity Test using the ABTS (*2,2-Azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid)* Method

The testing procedure was carried out by weighing 7.5 mg of ABTS, dissolving it in a 5 ml measuring flask of pro injection water in a tightly closed dark container, then left to stand for 12 hours. A total of 3.5 mg of potassium persulfate was dissolved in 5 ml of pro injection water. The two solutions were mixed and brought to a volume of 25 ml with sterile water for injection, then incubated in a dark room for 12-16 hours at a temperature ^{of} 22-24°C. One milliliter of the ABTS stock solution was pipetted and added to a 5 ml volumetric flask with sterile water for injection up to the mark.

The 1000 ug/ml extract stock solution was pipetted into separate tubes (100, 200, 300, 400, and 500 µl). Then, distilled water was added to a 5 ml measuring flask up to the mark, resulting in concentrations of 20, 40, 60, 80, and 100 µg/ml. Each extract was weighed at 25 mg and dissolved with 25 ml of injection water up to the mark to obtain a concentration of 1000 ppm. 1 ml of ABTS solution was taken and placed in a 5 ml measuring flask and left for 6 minutes in a dark room. The absorbance was then measured using an ultraviolet-visible spectrophotometer. Vitamin C was used as a reference, and measurements were performed three times(19) . The IC_{50}value indicates the percentage of inhibition, which can be calculated using the following formula:

$$Inhibisi = \frac{(\text{Abs Kontrol (ABTS)} - \text{Abs Sampel})}{\text{Abs Kontrol (ABTS)}}$$

x 100%

Then calculate using the linear regression equation y = ax + b

α-glucosidase **Inhibition Activity**

α-Glucosidase activity is determined according to modifications using the *QuantiChromTM α-Glucosidase* Assay Kit from *BioAssay Systems, which* is designed to measure *α-glucosidase* activity directly in biological samples without pretreatment. This improved method uses *p-nitrophenyl-α-D-glucopyranoside*, which is specifically hydrolyzed by *α-glucosidase* to form a yellow product (maximum absorbance at 405 nm). The reaction rate is directly proportional to enzyme activity. Samples must be processed quickly and mixing must be brief but thorough. The test can be performed at room temperature or 37°C. Reagents are prepared at room temperature and then mixed with 200 µL of Test Buffer and 8 µL of α-NPG substrate (final 1.0 mM) for each 96-well assay. Procedure using a 96-well plate: transfer 20 µL of distilled water (H_2O) to two wells on a clear-bottomed 96-well plate. Add 200 µL H_2O to one well and 200 µL Calibrator to the other well (total volume 220 µL). Then transfer 20 µL of sample to the other well. Transfer 200 µL Working Reagent only to the sample well. The final reaction volume in the sample well is 220 µL. Tap the plate briefly to mix. Then, read the OD at 405 nm (t = 0), and read again after 20 minutes (t = 20 minutes) on the plate reader. The calculation of the *α-glucosidase* activity of the sample (U/L) is:

$$\alpha\text{-}glucosidase = \frac{OD20 - OD0}{ODcalibrator - ODH2O} \; e\,x\; 250 \text{ (U/L)}$$

OD20 and OD0 are the OD405nm values of the sample at 20 minutes and 0 minutes, respectively. ODCALIBRATOR and ODH_2O are the OD405nm values of the Calibrator and H_2O at 20 minutes. Unit definition: one unit of enzyme catalyzes the hydrolysis of 1 µmol of substrate per minute at pH 7.0(20) .

RESULTS AND DISCUSSION

Results of 96% Ethanol Extraction of Bajakah Tampala, Kalalawit, Kuning

The ethanol extract of Bajakah wood was obtained by maceration using 96% ethanol, with a yield of 9.7% from 510 grams of simplisia. The extract obtained was thick and brownish after evaporation in a vacuum evaporator.

Phytochemical Screening Test of 96% Ethanol Extracts of Bajakah Tampala, Kalalawit, Kuning

Research on phytochemical screening aimed to determine the presence of secondary metabolite compounds in the three Bajakah extracts. The results of the study are summarized in **Table 1.**

Table 1 Phytochemical Screening of Bajakah Extracts

Type of test	Observation Results	Reference	Tampala	Kalalawit	Yellow
Flavonoid HCl P + Mg	Red	Red	+	+	+
Alkaloid Mayer's reagent	White precipitate	White	+	+	+
Dragendorff's reagent	Brown precipitate		+	+	+
Saponin Foam test (Shaking)	Foaming	Foaming	+	+	+
Tannin $FeCl_3$	Blue-black precipitate	Blue-black	+	+	+
Terpenoid Lieberman-Burchard reagent	Red	Red	+	+	+
Steroid Lieberman-Burchard Reagent	Blue	Blue	+	+	+

The results of this study are in line with the research conducted by Alexander *et al*. 2023, which found that Bajakah Kalalawit (Uncaria gambir (W.Hunter) Roxb) contains high levels of terpenoids, steroids, and tannins(21) . The screening results obtained

from the 70% ethanol extract of Bajakah Tampala stems showed positive results for phenolic and flavonoid compounds(5) , and the phytochemical screening results of the ethanol extract *of Uncaria gambir* (W.Hunter) Roxb showed the presence of chemical compounds, namely alkaloids, flavonoids, saponins, and tannins(22) .

***Total Phenolic Content (TPC)* and *Total Flavonoid Content (TFC)* of Bajakah Extracts and Fractions**

Determination of total phenolic content using the *Folin Ciocalteau* method. The basic mechanism of the *Folin Ciocalteau* method is an oxidation/reduction reaction with phenolic groups that are oxidized by tungsten and molybdenum oxide contained in the *Folin Ciocalteau* reagent, and metal ions are reduced, producing a blue color. The determination *of* total *flavonoid content* was determined by using the Farasat *et al* (2024) method, with quercetin as the standard(23) . Flavonoids containing hydroxyl groups can form coordination complexes with aluminum ions. The formation of these complexes causes a shift in the absorption wavelength towards longer wavelengths (bathochromic effect), which is significant in spectrophotometric analysis. To maintain the stability of this complex structure in the visible spectrum range, sodium acetate was added as a stabilizing agent. This approach is commonly applied in determining total flavonoid content using the *UV-Vis spectrophotometry* method to obtain accurate and scientifically reproducible results(24) . The results of the measurement of total phenolic and flavonoid content are presented in **Table 2.**

Table2 . Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Sample	**Total phenolic content mgGAE/g ± SD**	**Total Flavonoids mgGAE/g ± SD**	**IC_{50}(µg/ml) ± SD**
Bajakah Tampala Extract	133.38 ± 6.33	11.12 ± 0.24	46.32 ± 0.01
N-Hexane Fraction	0.94 ± 0.12	3.02 ± 0.75	2050.89 ± 27.73
Ethyl acetate fraction	1.92 ± 0.16	1.36 ± 0.22	1324.44 ± 44.16
Methanol Fraction	2.31 ± 0.52	1.59 ± 0.31	488.07 ± 0.99
Kalalawit Bajakah Extract	112.24 ± 10.02	7.28 ± 0.12	94.72 ± 0.02
N-Hexane Fraction	0.40 ± 0.01	0.43 ± 0.06	2224.98 ± 14.64
Ethyl acetate fraction	12.52 ± 1.29	1.26 ± 0.05	3633.28 ± 44.51
Methanol Fraction	68.68 ± 1.50	2.98 ± 0.10	56.28 ± 0.16
Yellow Bajakah Extract	21.14 ± 1.55	10.0 ± 0.50	68.69 ± 0.03
N-Hexane Fraction	0.40 ± 0.06	0.93 ± 0.14	6246.72 ± 78.64
Ethyl acetate fraction	0.39 ± 0.02	0.40 ± 0.08	1863.81 ± 18.26
Methanol Fraction	24.45 ± 1.49	2.54 ± 0.16	619.91 ± 0.77

Based on the results of the analysis of *total phenolic content* (TPC), *total flavonoid content* (TFC), and antioxidant activity in extracts of several types of bajakah with varying solvents. Solvent polarity has a significant effect on bioactive compound content and antioxidant capacity. The TPC value shows that bajakah extract with 96% ethanol solvent produces higher total phenolic content compared to more polar or non-polar solvents. This is due to the ability of 96% ethanol to dissolve both polar and semipolar phenolic compounds, resulting in more optimal extraction of phenolic acids and polyphenols. Tampala bajakah has the highest total phenolic value of 133.3 ± 6.33 mgGAE/g. A high total phenolic value indicates a higher content of phenolic compounds , so the antioxidant potential of tampala bajakah extract tends to be stronger than other bajakah with lower phenolic values.

This study is in line with previous research, finding that bajakah tampala stems with 96% ethanol extract have a total phenolic content of 10.11 mg GAE/g extract, which is higher than extracts with other solvents such as n-hexane and ethyl acetate(25) . Antioxidant activity determined using the ABTS method showed that extracts with high TPC and TFC values had stronger

antioxidant activity, as indicated by greater ABTS radical inhibition or lower IC_{50}values. This confirms the positive correlation between the content of phenolic compounds and flavonoids with antioxidant activity. The differences in antioxidant activity between bajakah types and solvents indicate that antioxidant effectiveness is not only determined by the amount of compounds, but also by the type and chemical structure of the active compounds extracted.

Phenolic compounds play an important role in inhibiting free radical activity that contributes to degenerative diseases through antioxidant mechanisms. Phenolic compounds have hydroxyl groups that can donate hydrogen atoms or electrons to neutralize free radicals, thereby converting highly reactive free radicals into more stable compounds that are less damaging to cells(25) . The results of the correlation between *total phenolic content* (TPC) and antioxidant extracts and fractions against antioxidant activity are presented in **Figure 1.** Correlation between *total phenolic content* (TPC) and *total flavonoid content* (TFC) of bajakah tampala against antioxidant activity.

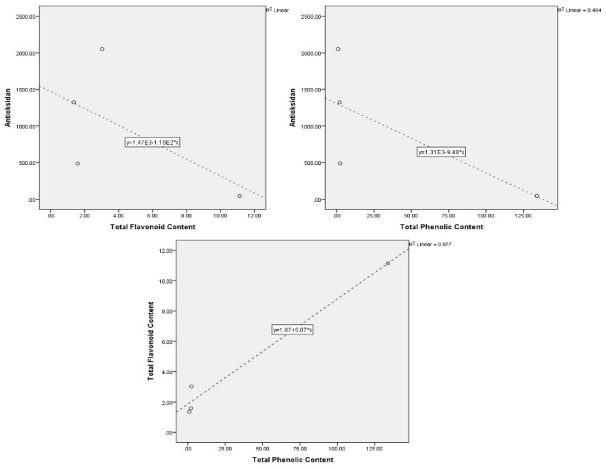


Figure 1. Correlation between a) TPC and TFC, b) TPC and IC50, c) TFC and IC50

Table 3. Pearson Correlation Analysis

Variable	TPC	IC_{50} µg/mL
TPC	**1**	**-.703**
TFC	**0.986***	**-.600**
IC_{50} µg/mL	**-.703**	**1**

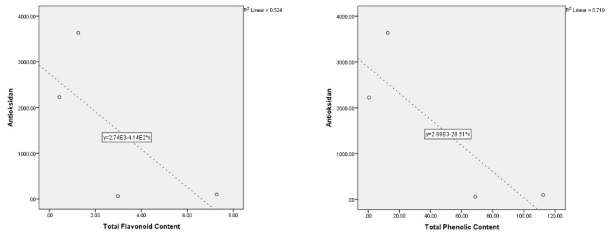
- Correlation is significant at the 0.05 level (two-tailed).

Total *phenolic content* (TPC) and total *flavonoid content* (TFC) of Tampala bajakah extract and fractions using *the Kolmogorov-Smirnov test* obtained a sig of 0.2>0.05, indicating that total phenolic and total flavonoid content are normally distributed. This was followed by a correlation test using the *Spearman* test, as the data was normally distributed. The analysis results showed that TPC and IC_{50} (r = -0.703) had a strong negative correlation. This negative correlation indicates that the higher the total phenolic content in the extract, the lower the IC_{50} value, which means that the antioxidant activity is stronger. Phenolic compounds have aromatic hydroxyl groups that can donate hydrogen atoms or electrons to free radicals, thereby stopping oxidative chain reactions.

TFC showed a very strong positive correlation and significance with TPC (r = 0.986; p< 0.05). This indicates that flavonoids are the main components of phenolic compounds in bajakah extract and contribute significantly to the total phenolic content, which is influenced by the level of flavonoids extracted.

Previous studies have stated that the total *phenolic,* flavonoid, and carotenoid content of each bajakah extract was significantly different (sig. < 0.05), indicating that ethanol has a greater influence on determining phenol and flavonoid content compared to the use of ethyl acetate and n-hexane solvents(26) .

The results of the study on the relationship between TPC and IC_{50} show a moderate negative correlation (r= -0.600), which falls into the moderate negative correlation category. This means that an increase in flavonoid levels tends to increase antioxidant activity, but not as strongly as total phenolic compounds as a whole.The results of the correlation between *total phenolic content* (TPC) and antioxidant extracts and fractions against antioxidant activity are presented in **Figure 2.** Correlation between *total phenolic content* (TPC) and *total flavonoid content* (TFC) of bajakah kalalawit against antioxidant activity.



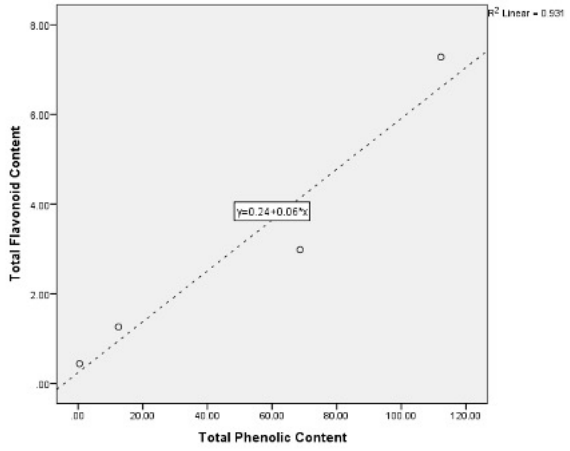


Figure 2. Correlation between a) TPC and TFC, b) TPC and IC50, c) TFC and IC50.

Table 4 . Pearson Correlation Analysis

Variable	TPC	IC_{50}µg/mL
TPC	**1**	**-.848**
TFC	**.965***	**-.724**
IC_{50} µg/mL	**-.848**	**1**

- Correlation is significant at the 0.05 level (two-tailed).

The total *phenolic content* and total *flavonoid content* of bajakah kalalawit extract and fractions using *the Kolmogorov-Smirnov test* obtained a sig of 0.2>0.05, indicating that the total phenolic and total flavonoid content is normally distributed. This was followed by a correlation test using the *Spearman* test, as the data is normally distributed. The results of the study obtained a correlation coefficient between *the* total *flavonoid content* (TFC) of bajakah kalalawit extract and fractions against *the total phenolic content* with a correlation value of (0.965) ($p < 0.05$), indicating a very strong and significant positive correlation. This very high correlation value indicates that the increase in TPC in Bajakah Kalalawit is largely due to the increase in flavonoid content, so that flavonoids play a role in determining the phenolic profile and bioactivity potential of the extract.

Previous studies have shown that antioxidant activity evaluated using the FRAP and DPPH methods shows a strong correlation between total phenolics and flavonoids with a significance value of $p<0.05$ with a low probability of occurring by chance. These results indicate a correlation between phenols and flavonoids and antioxidant activity in Bajakah kalalawit(27) .

The results of the study obtained a correlation coefficient of total *phenolic content* (TPC) against antioxidant activity (IC_{50}) of bajakah kalalawit extract and Pearson's correlation (-0.848), indicating a very strong negative relationship between TPC and antioxidant activity in bajakah kalalawit extract. Statistically, this value is close to -1, which means that the relationship between the variables is very close and consistent. The higher the TPC value, the lower the IC_{50}value,indicating stronger antioxidant power. The correlation value r = -0.724 shows a strong negative correlation between TFC and IC_{50}, which means that the higher the flavonoid content, the lower the IC_{50}value,or in other words, the stronger the antioxidant activity of the bajakah kalalawit extract. The results of the correlation between *total phenolic content* (TPC) and antioxidant activity of the extract and fractions are presented in **Figure 3.** Correlation between *total phenolic content* (TPC) and *total flavonoid content* (TFC) of yellow bajakah and antioxidant activity.

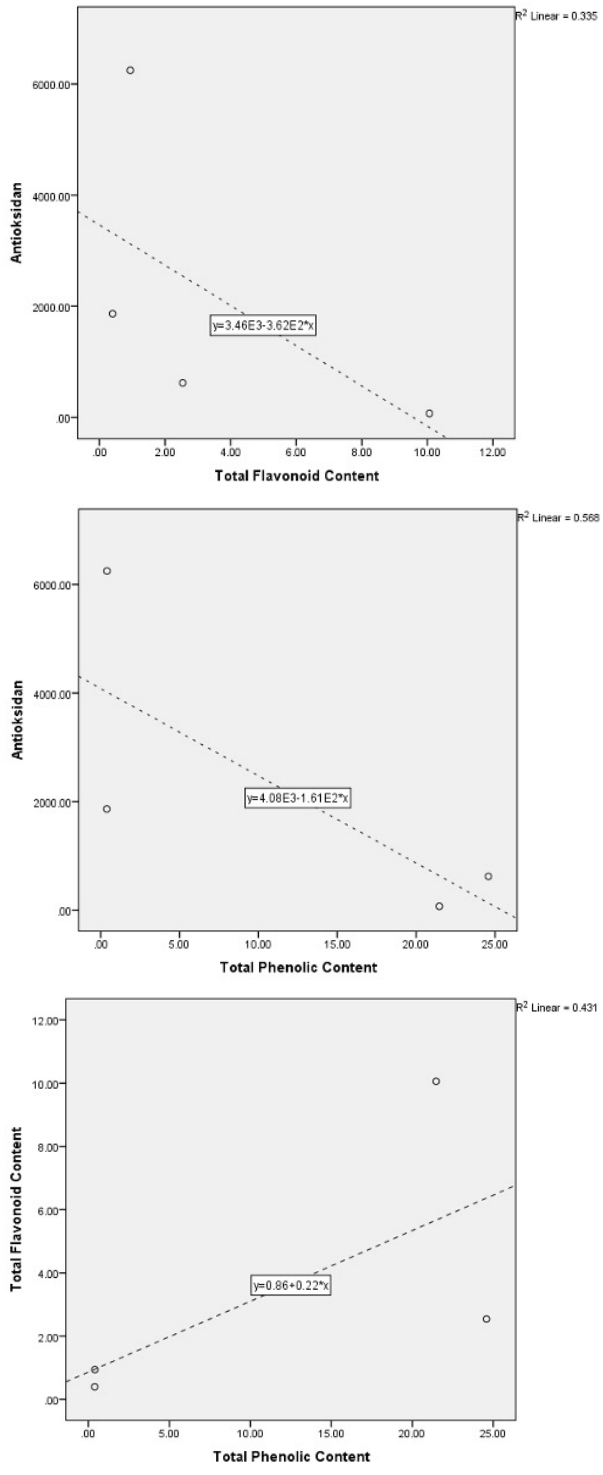


Figure 3. Correlation between a) TPC and TFC, b) TPC and IC50, c) TFC and IC50.

Table 5. Pearson Correlation Analysis

Variable	TPC	IC_{50}µg/mL
TPC	**1**	**-.754**
TFC	**.657**	**-.580**
IC_{50} µg/mL	**-.754**	**1**

- Correlation is significant at the 0.05 level (2-tailed).

Total *phenolic content* (TPC) and total *flavonoid content* (TFC) of yellow bajakah extract and fractions using *the Kolmogorov-Smirnov test* obtained sig 0.2>0.05, indicating that total phenolic and total flavonoid are normally distributed. This was followed by a correlation test using the *Spearman* test, as the data were normally distributed. The results of the study obtained a correlation coefficient of total *flavonoid content* and total *phenolic content* of yellow bajakah extract and fractions against antioxidant activity $IC_{50}$of (0.657), indicating that this correlation is significant at the 0.05 level. The value of 0.657 indicates a strong positive relationship. This means that when the flavonoid value increases, the total *phenolic* value also tends to increase with antioxidant activity.

The results of the study obtained a correlation coefficient between *the* total *phenolic content* and antioxidant content of yellow bajakah extract and a Pearson correlation coefficient (-0.754), indicating a moderate negative relationship. This indicates that as phenolic content increases, antioxidant activity tends to decrease (Sig. (*2-tailed)* p-value (0.245>0.05)). Although the correlation coefficient is high (-0.754), the relationship is not statistically significant. There is a 24.6% chance that this negative trend occurred by chance.

The correlation coefficient between total *phenolic content* and antioxidant activity of extracts and fractions (Pearson correlation -0.580) indicates a moderate negative relationship. As total *phenolic content* increases, antioxidant activity tends to decrease (Sig. (*2-tailed)* p-value (0.420>0.05)), so the results are not statistically significant. However, the value is closer to the significance threshold, indicating a moderate negative trend (although still not definitive).

From the data obtained, there is a strong positive correlation, indicating a strong and statistically significant positive relationship between flavonoids (TFC) and total phenolic content. There is a moderate negative correlation, indicating a moderate negative

relationship between the two types of content (TFC, TPC) and antioxidant activity. However, this relationship is not statistically significant with this sample size, meaning it could be due to chance.

Results of antioxidant activity testing using the ABTS method

The results of the antioxidant activity of bajakah extracts and fractions with ABTS free radical inhibition using a UV-Vis spectrophotometer are presented in **Table 5.**

Table 5. Results of antioxidant activity using the ABTS method for bajakah extracts and fractions

Sample	IC_{50} (µg/ml) ± SD
Vitamin C	3.20 ± 0.003
Bajakah tampala extract	46.32 ± 0.01
n-Hexane fraction of bajakah tampala	2050.89 ± 27.73
Ethyl acetate fraction of bajakah tampala	1324.44 ± 44.16
Methanol fraction of bajakah tampala	488.07 ± 0.99
Kalalawit bajakah extract	94.72 ± 0.02
n-Hexane fraction of bajakah kalalawit	2224.98 ± 14.64
Ethyl acetate fraction of bajakah kalalawit	3633.28 ± 44.51
Methanol fraction of bajakah kalalawit	56.28 ± 0.16
Yellow bajakah extract	68.69 ± 0.03
n-Hexane fraction of yellow bajakah	6246.72 ± 78.64
Yellow bajakah ethyl acetate fraction	1863.81 ± 18.26
Yellow bajakah methanol fraction	619.91 ± 0.77

Vitamin C as a positive control had the lowest IC_{50}value of approximately 3.20 ± 0.003 µg/ml, indicating very strong antioxidant activity. The antioxidant activity of the extract, bajakah fraction, and quercetin with a very strong category (IC_{50}<50 ppm) was obtained, namely the bajakah tampala extract at 46.32 ± 0.019 µg/ml, which was much higher than vitamin C, showing antioxidant activity with a strong category (IC_{50} 50-100 ppm) bajakah kalalawit extract, yellow bajakah extract, and bajakah kalalawit methanol fraction, respectively, obtained IC_{50} values of 94.72 ± 0.02, 68.69 ± 0.03, and 56.28 ± 0.166 µg/ml. These data support previous studies reporting that ethanol extract of bajakah tampala root has an IC_{50}value of 9.206 µg/mL, which is classified as very strong. Meanwhile, bajakah kalalawit has an IC_{50}value of approximately 70.81 µg/mL, which is classified as moderate to strong. (28)

Another study found that Bajakah tampala (*Spatholobus littoralis* Hassk) has very strong antioxidant activity with an IC_{50}value reported to be around 9.2 µg/mL, almost equivalent to the standard quercetin (IC_{50} 7.15 µg/mL), which indicates very high antioxidant potential(28) .

Phenolic compounds and flavonoids are the main components responsible for antioxidant activity. Bajakah tampala has a rich and diverse profile of phenolic compounds and flavonoids, which significantly increases its ability to neutralize free radicals(29) . Extraction with polar solvents such as ethanol or methanol can maximize the extraction of phenolics and flavonoids in bajakah tampala extracts, thereby increasing antioxidant activity. The low $IC_{50}$of bajakah tampala is due to its high phenolic and flavonoid content, environmental conditions that support secondary metabolite production, the plant parts used, and optimal extraction methods, all of which contribute to its very strong antioxidant activity(4) .

Results of antidiabetic activity of *α-glucosidase* inhibition

The results of testing the antidiabetic activity of bajakah extracts and fractions against *α-glucosidase* activity are presented in **Table 6.**

Table 6. Results of antidiabetic activity against *α-glucosidase* activity test of bajakah extracts and fractions.

Sample	Activity *α-glucosidase (U/L)*
Acarbose	100
Bajakah Tampala Extract	346.15
N-Hexane Fraction	76.92
Ethyl Acetate Fraction	379.80
Methanol fraction	67.30
Kalalawit Bajakah Extract	19.23
N-Hexane Fraction	211.5
Ethyl Acetate Fraction	1336.53
Methanol fraction	1177.88

Yellow Bajakah Extract	798.07
N-Hexane Fraction	-57.69
Ethyl Acetate Fraction	139.42
Methanol fraction	706.73

α-glucosidase activity is measured based on the rate of change in absorbance by substrate hydrolysis by the enzyme. The greater the decrease in absorbance, the higher the enzyme activity. The results obtained can be seen from the data, acarbose as a standard control shows an activity of around 100U/L. Acarbose is a compound that is well known and widely used as an *α-glucosidase* enzyme inhibitor, which functions to inhibit the enzyme to slow down the breakdown of carbohydrates into glucose, thereby helping to control blood sugar levels in diabetes. In the *α-glucosidase* activity test, acarbose is used as a standard comparison (positive control) because its effectiveness and characteristics are well known. The bajakah tampala extract has an activity of 346.15U/L, which is higher than the control, indicating that this extract increases *α-glucosidase* activity. The n-hexane fraction of yellow bajakah showed negative activity (-57.69U/L), which means that there was no *α-glucosidase* activity, while other samples showed varying activity values, from low (e.g., 19.2307U/L in kalalawit bajakah extract) to very high (>1000 U/L).

The *α-glucosidase* enzyme activity value from this absorbance data describes the sample's ability to affect the enzyme, either through inhibition (decreasing activity) or stimulation (increasing activity). To determine the specific inhibitory effect.

CONCLUSION

Based on the overall research results covering phytochemical screening, determination of total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (ABTS), statistical correlation analysis, and evaluation of α-glucosidase inhibition activity *in vitro.* The 96% ethanol extract of bajakah tampala showed the best bioactivity with a total *phenolic content* (TPC) test value of (133.38 ± 6.33 mgGAE/g) and *a* total *flavonoid content* (TFC) test showing that the bajakah tampala extract was relatively high at (11.12 ± 0.24 mgGAE/g) sample, which directly implies the strongest antioxidant activity (IC_{50} ABTS 46.32 ± 0.01 µg/mL). This relationship was reinforced by the results of Pearson's correlation analysis**,** which showed a strong negative correlation between TPC and IC_{50} ($r = -0.703$)**,** indicating that an increase in phenolic compound content contributes significantly to an increase in free radical scavenging capacity.

In kalalawit bark, although the TPC (112.24 ± 10.02 mgGAE/g) and TFC (7.28 ± 0.12 mgGAE/g) values were lower than those in tampala bark, the antioxidant activity was still classified as strong, especially in the methanol fraction (IC_{50} 56.28 ± 0.16 µg/mL). Correlation analysis showed a very strong negative relationship between TPC and IC_{50} ($r = -0.848$) and a strong negative correlation between TFC and IC_{50} ($r = -0.724$) that was statistically significant. This indicates that in kalalawit bajakah, flavonoids play a more direct role in antioxidant activity than in other bajakah, possibly influenced by the presence of characteristic flavonoids and isoquinoline alkaloids, which are known to have high biological activity.

Meanwhile, yellow bajakah exhibits different characteristics. Although it has a relatively high flavonoid content (TFC 10.0 ± 0.50 mgGAE/g), its overall TPC value is low (21.14 ± 1.55 mgGAE/g). The correlation between TPC and TFC with antioxidant activity shows a moderate negative trend, but not statistically significant, indicating that the antioxidant activity of yellow bajakah does not entirely depend on the total quantity of phenolics or flavonoids**,** but is likely influenced by the specific composition of bioactive compounds, such as catechins and condensed tannins, which have different antioxidant mechanisms and are not always optimally detected by conventional TPC methods.

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