Berita Biologi
LIPI
Jurnal ilmu-ilmu hayati

Berita Biologi 24(3): 459–467 (2025)
https://ejournal.brin.go.id/berita_biologi ISSN 2077 7019
DOI: 10.55981/berita_biologi.2025.11322

P-ISSN 0126-1754
E-ISSN 2337-8751

ARTICLE

ANTIOXIDANT ACTIVITY PROFILING OF RED BAJAKAH TAMPALA (*Spatholobus littoralis* Hassk.) FRACTIONS USING THE RADICAL SCAVENGING METHOD

(*Profiling Aktivitas Antioksidan dari Fraksi Bajakah Tampala Merah* (*Spatholobus littoralis* Hassk.) *dengan Metode Radical Scavenging)*

Nathasya Gracya Amanda[1], Ghani Nurfiana Fadma Sari[1], Mega Novita[2], Dian Marlina[1*]

[1]Faculty of Pharmacy, Universitas Setia Budi, Jl. Letjend Sutoyo Mojosongo, Surakarta, Central Java 57127, Indonesia
[2] Postgraduate Program of Natural Science Education, Universitas PGRI Semarang, Jl. Sidodadi Timur 24, Karang Tempel, East Semarang, Semarang, Central Java 50232, Indonesia

ABSTRACT

Indonesia's rich biodiversity harbors numerous plants with medicinal properties, including Red Bajakah Tampala (*Spatholobus littoralis* Hassk.), which has been used in traditional medicine for its therapeutic benefits for a long time. Despite its promising ethnomedicinal use, scientific validation of its antioxidant potential remains limited. This study aims to evaluate the antioxidant properties of Red Bajakah Tampala stem extract and to identify the major groups of bioactive compounds that may contribute to its antioxidant activity. The identification of these compounds was carried out through preliminary phytochemical screening to detect the presence of secondary metabolite groups, rather than isolating specific compounds. The antioxidant activity of the stem extract fractions was assessed using the DPPH assay, and the chemical composition was analyzed through phytochemical screening and thin-layer chromatography (TLC). The ethyl acetate fraction exhibited the strongest antioxidant activity, with an IC_{50} value of 12.87 ppm, followed by the n-hexane fraction (24.08 ppm) and the water fraction (57.23 ppm). Phytochemical screening revealed the presence of flavonoids, tannins, triterpenoids, phenols, and saponins, which are known contributors to antioxidant activity. These findings provide scientific evidence supporting the traditional use of Red Bajakah Tampala as a natural antioxidant, with implications for its potential use in therapeutic and cosmetic applications. Further research is needed on the molecular mechanisms and practical applications of Bajakah-based formulations.

Keywords: Red Bajakah Tampala; antioxidant activity; DPPH assay; flavonoids; phytochemical screening

Submitted: 2 May 2025; **Revision:** 8 August 2025; **Accepted:** 19 August 2025
***Corresponding author:** e-mail – marlina@setiabudi.ac.id

ABSTRAK

Indonesia memiliki keanekaragaman hayati yang kaya, termasuk tanaman yang dapat dimanfaatkan sebagai bahan obat karena manfaat terapeutiknya, termasuk Bajakah Tampala Merah (Spatholobus littoralis Hassk.), akan tetapi validasi ilmiah terhadap potensi antioksidannya masih terbatas. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dari ekstrak batang Bajakah Tampala Merah, serta mengidentifikasi golongan utama senyawa bioaktif yang berpotensi berkontribusi terhadap aktivitas antioksidannya. Identifikasi senyawa dilakukan melalui skrining fitokimia awal untuk mendeteksi keberadaan golongan metabolit sekunder. Aktivitas antioksidan dari fraksi ekstrak batang diuji menggunakan metode DPPH, sedangkan komposisi kimianya dianalisis melalui skrining fitokimia dan kromatografi lapisan tipis (KLT). Fraksi etil asetat menunjukkan aktivitas antioksidan terkuat, dengan nilai IC_{50} sebesar 12,87 ppm, diikuti oleh fraksi n-heksana (24,08 ppm) dan fraksi air (57,23 ppm). Skrining fitokimia mengungkapkan adanya flavonoid, tanin, triterpenoid, fenol, dan saponin yang diketahui dapat berkontribusi terhadap aktivitas antioksidan. Temuan ini memberikan bukti ilmiah yang mendukung penggunaan tradisional Bajakah Tampala Merah sebagai antioksidan alami, dengan implikasi untuk potensi penggunaannya dalam aplikasi terapeutik dan kosmetik. Penelitian lebih lanjut diperlukan untuk mengetahui mekanisme molekuler dan aplikasi praktis dari formulasi berbasis Bajakah Tampala Merah.

***Kata kunci**: Bajakah Tampala Merah; aktivitas antioksidan; uji DPPH; flavonoid; skrining fitokimia*

INTRODUCTION

Indonesia possesses a rich diversity of plants with medicinal properties, many of which remain underexplored from a scientific perspective. Numerous plants, known for their traditional uses, have not undergone rigorous scientific validation, making it essential for further research to confirm their efficacy and identify the active compounds responsible for their medicinal effects (Sianipar *et al*., 2023). Among these, antioxidant-rich plants hold particular promise due to their potential to mitigate oxidative stress and prevent diseases associated with free radical damage. Antioxidants play a crucial role in halting oxidation reactions by neutralizing free radicals, which are reactive molecules that can damage cells and tissues. Antioxidants are categorized into two types: endogenous antioxidants, which the body produces naturally, and exogenous antioxidants, which are derived from external sources and act quickly to neutralize free radicals (Hu *et al*., 2021). Research has shown that various antioxidants can significantly reduce the risk of oxidative stress-related diseases, including cancer, cardiovascular diseases, and neurological disorders (Muema *et al*., 2022). Among the plants studied for their antioxidant properties, Bajakah, particularly the Red Bajakah Tampala (*Spatholobus littoralis* Hassk.), has garnered attention due to its traditional use in ethnomedicine, especially among the Dayak tribe of Kalimantan. Various types of Bajakah, such as Tampala Bajakah, Lamei Bajakah, and Kalawit Bajakah, are often used in traditional medicine to treat ailments like cancer, tumors, diabetes, premature aging, and wounds (Hamzah *et al*., 2023). The application of Bajakah for medicinal purposes is deeply rooted in local knowledge and practices, passed down through generations. Recent studies have highlighted its potential as an antioxidant, with research utilizing methods such as the 1-diphenyl-2-picrylhydrazyl (DPPH) assay to evaluate the antioxidant activity of Bajakah extracts. This assay measures the ability of a sample to neutralize DPPH radicals, providing insight into the plant's capacity to protect against oxidative damage (Nurdyansyah *et al*., 2023). Such findings suggest that Bajakah holds considerable promise as a natural source of antioxidants with therapeutic potential.

Research on the exploration and scientific validation of the antioxidant potential of Red Bajakah Tampala (*Spatholobus littoralis* Hassk.) is necessary to provide scientific evidence for its traditional uses, especially those related to oxidative stress. The general solution to this problem lies in systematically evaluating the antioxidant properties of Bajakah through modern scientific methods, such as the DPPH assay, and determining the active compounds responsible for its bioactivity. Several studies have investigated the antioxidant properties of Bajakah and similar plants, providing a foundation for understanding their potential as therapeutic agents. A study by Yuniarti *et al*. (2021) demonstrated that Red Bajakah wood exhibited strong antioxidant activity, with an IC_{50} value of 26.29 ppm, which is categorized as very strong. This value is lower than that of vitamin C, which has an IC_{50} value of 30.74 ppm. It suggests that Bajakah is a potent antioxidant with therapeutic

benefits, similar to antioxidants like vitamin C. A previous study by Novanty *et al*. (2021) demonstrated that Bajakah Tampala can reduce Reactive Oxygen Species (ROS) levels in obese rats, thereby further reinforcing its antioxidant potential. Such findings underscore the promise of Bajakah as a natural source of antioxidants that could be harnessed for medicinal purposes. Further research on Bajakah's chemical composition has revealed the presence of bioactive compounds, such as phenolic compounds, flavonoids, tannins, and saponins, which contribute to its strong antioxidant activity (Imran and Alwahab, 2024). These compounds play a significant role in the ability of Bajakah to neutralize free radicals (Imran and Alwahab, 2024). Moreover, pharmacological studies on Bajakah have indicated the presence of alkaloids, flavonoids, saponins, tannins, and polyphenols, which are known for their cytotoxic, anti-inflammatory, and antioxidant properties (Rahmadiawan *et al*., 2025). This diverse range of bioactive compounds in Bajakah makes it a promising candidate for further scientific investigation, particularly in the development of natural antioxidant therapies.

Despite the promising findings on Bajakah's antioxidant properties, several gaps in the literature remain. While numerous studies have reported on the antioxidant capacity of Bajakah and related plants, there is a lack of comprehensive research that explores the specific mechanisms by which these antioxidants act on cellular systems. The studies conducted by Yuniarti *et al*. (2021) and (Novanty *et al*., 2021) primarily focused on the antioxidant activity, measuring IC_{50} values and ROS reduction in animal models. Still, they did not investigate the molecular pathways through which Bajakah's antioxidants exert their effects. Additionally, while the chemical composition of Bajakah has been partially characterized, more detailed studies are required to isolate and identify the exact compounds responsible for its antioxidant activity, particularly those that may have synergistic effects. Another gap in the literature is the limited research on optimizing Bajakah's antioxidant properties for practical applications. Studies such as those by Imran and Alwaha (2024) have shown promising results in terms of antioxidant activity; however, the formulation of these antioxidants into usable products, such as emulgel or other delivery systems, has not been thoroughly explored (Imran and Alwahab, 2024). The potential for developing Bajakah-based antioxidant formulations for therapeutic use remains untapped, mainly underscoring the need for further research on the practical applications of Bajakah in the medicinal and cosmetic industries.

The objective of this study is to evaluate the antioxidant properties of Red Bajakah Tampala (*Spatholobus littoralis* Hassk.) and to identify the major groups of bioactive compounds contributing to its activity through qualitative phytochemical screening and DPPH assay. This research aims to address part of the existing research gaps by providing scientific validation of its traditional use and by characterizing key antioxidant-related compounds. The novelty of this study lies in its integration of traditional ethnomedicinal knowledge with scientific analysis to support the use of Bajakah as a potential natural antioxidant. Although this study did not include molecular mechanism analysis or formulation studies, it provides a foundational basis for future research into therapeutic and cosmetic applications.

MATERIALS AND METHODS

Plant material and chemical

The stem of Red Bajakah Tampala (*Spatholobus littoralis* Hassk.), collected from Tamiang Layang, East Barito Regency, Central Kalimantan, was used for the study. Ethanol 96% was used for maceration and extraction, while DPPH (1-diphenyl-2-picrylhydrazyl) reagent was used to assess antioxidant activity (Baschieri and Amorati, 2021). Other chemicals included ethanol p.a., distilled water, N-hexane, ethyl acetate, quercetin (as a standard), saturated toluene, concentrated HCl, amyl alcohol, 2N HCl, $FeCl_3$, and Dragendorff reagent (Ewaldo *et al*., 2024;Freitas *et al*., 2022;Habisukan *et al*., 2024). The tools and equipment employed included knives, cutting boards, sieves, rotary evaporators, UV-VIS spectrophotometers, water baths, volumetric flasks and pipettes, desiccators, ovens, blenders, maceration containers, separating funnels, and thin-layer chromatography (TLC) plates.

Morphological Identification

The research was conducted through several stages, including morphological identification of the plant, drying and preparation of simplicia, maceration with ethanol, fractionation using liquid-liquid extraction, organoleptic testing, phytochemical screening, thin-layer chromatography, and antioxidant activity testing via the DPPH method.

Preparation and Extraction

The red bajakah tampala stems were sorted, washed, chopped, dried, and ground into a fine powder using a 40-mesh sieve. The drying shrinkage was tested according to the Indonesian Herbal Pharmacopoeia by heating a 2 g sample at 105°C and weighing at regular intervals until constant weight was achieved. Then, 900 g of powdered simplicia were macerated in 9,000 mL of 96% ethanol for 24 hours (with the first 6 hours of intermittent stirring). The resulting macerate was filtered and concentrated.

Fractionation and Solvent Evaporation

The extract was then fractionated sequentially using a liquid-liquid extraction method with N-hexane, ethyl acetate, and water using a separating funnel. Each fraction was collected and concentrated using a rotary evaporator (Ewaldo *et al*., 2024; Freitas *et al*., 2022). Experiments involving solvent evaporation were conducted using a rotary evaporator under reduced pressure, and drying shrinkage and water content were determined using a humidity balance and toluene distillation (Wołosiak *et al*., 2021).

Organoleptic Testing

Organoleptic properties (color, smell, taste, and texture) of the extract were recorded to characterize the physical qualities of the extract.

Phytochemical Screening and TLC Analysis

Phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, tannins, saponins, triterpenoids, and phenolic compounds, as determined by color reactions and spot observations on TLC plates under UV light.

Compound identification was performed using the TLC method with silica gel GF254 and various eluents for each class of phytochemical. Visualization was performed using UV light and color development reagents, such as $AlCl_3$ and Liebermann-Burchard (Habisukan *et al*., 2024).

Antioxidant Activity Test (DPPH Assay)

Antioxidant activity was assessed using the DPPH assay. A DPPH stock solution was prepared at a concentration of 158 ppm, and samples were tested at various concentrations. Each sample (1 mL) was mixed with 1 mL of DPPH and ethanol p.a. to a final volume of 5 mL, then incubated in the dark for 30 minutes at room temperature before measuring absorbance (Baschieri and Amorati, 2021).

The UV-VIS spectrophotometer was calibrated prior to use and operated in the 500–600 nm range for absorbance readings (Baschieri and Amorati, 2021). All sample identification and compound testing were verified in the Laboratory of the Faculty of Pharmacy, Universitas Setia Budi, Surakarta. The absorbance data from the antioxidant activity tests were used to calculate the IC_{50} values of the extract and fractions. The absorbance data from the antioxidant activity tests were used to calculate the IC_{50} values of the extract and fractions.

Statistical Analysis

Statistical analysis was performed using the one-way ANOVA method in SPSS (Statistical Package for the Social Sciences) to determine significant differences between groups. This analysis was critical for evaluating the antioxidant strength of each fraction compared to the standard quercetin.

RESULTS AND DISCUSSION

The identification of the plant sample by the Laboratory of Universitas Setia Budi, Surakarta, was confirmed as *Spatholobus littoralis* Hassk. (Red Bajakah Tampala stem), with identification number 094/DET/UPT-LAB/15.10.2024. A 9,030-gram dry weight sample yielded 26.02% powdered simplicial, surpassing the minimum standard of >10% (Kementerian Kesehatan RI 2017). The drying shrinkage rate was recorded at 4.3%, and the yield of the thick extract was 16%. The water content of the extract was approximately 5%, which is an acceptable level. The ethanol extract was fractionated using n-hexane, ethyl acetate, and water, with the ethyl acetate fraction yielding the highest amount of the extract. The use of several solvents in this study was intended to separate compounds based on their polarity, allowing for a more targeted identification of bioactive constituents responsible for antioxidant activity. Fractionation using a sequence of n-hexane, ethyl acetate, and water enables the selective extraction of non-polar, semi-polar, and polar compounds, respectively.

N-hexane, a non-polar solvent, typically extracts lipophilic substances such as fatty acids, terpenes, and some triterpenoids. These compounds may contribute to antioxidant activity through membrane stabilization and radical scavenging pathways. Ethyl acetate, a semi-polar solvent, is effective in extracting flavonoids, phenolic acids, and certain alkaloids many of which are well-known for their strong antioxidant properties due to their hydrogen-donating ability and radical stabilization capacity. Water, a polar solvent, extracts highly polar compounds including glycosides, saponins, and some sugars or amino acids. However, polar antioxidants often show weaker radical scavenging activity compared to semi-polar phenolic compounds, which may explain the relatively low antioxidant activity in the water fraction. Therefore, the strongest antioxidant activity observed in the ethyl acetate fraction can be attributed to its efficiency in extracting semi-polar compounds, especially flavonoids and phenolic derivatives, which were confirmed through both phytochemical screening and TLC analysis. Although organoleptic assessment was planned, this study did not include formal sensory evaluation or report the results, as the primary focus was on antioxidant and phytochemical profiling.

Phytochemical screening of the Red Bajakah Tampala stem extract (*Spatholobus littoralis* Hassk.) was conducted to identify the presence of major secondary metabolites using several qualitative tests with specific reagents (Table 1). The results revealed that the extract contains flavonoids, as indicated by a red color formation during the test. Tannins were also detected, confirmed by the appearance of a slightly blackish-green color, while phenols showed positive results characterized by a similar blackish-green coloration. The presence of triterpenoids was confirmed by the development of a red color after the addition of the Liebermann-Burchard reagent, and saponins were identified by the formation of stable foam that persisted for 10 minutes after shaking. In contrast, alkaloids were not detected, as no white, brick-red, or brown precipitate was observed when tested with Mayer's, Dragendorff's, or Bouchardat's reagents, respectively. These findings indicate that *Spatholobus littoralis* extract contains various bioactive compounds, particularly flavonoids, tannins, triterpenoids, phenols, and saponins, which may contribute to its potential pharmacological activities.

Table 1. Chemical compounds of Red Bajakah Tampala Stem Extract *(Spatholobus littoralis* Hassk.*)* based on phytochemical screening (*Senyawa kimia Ekstrak Batang Tampala Bajakah Merah (Spatholobus littoralis Hassk.) berdasarkan skrining fitokimia*).

Chemical Content (*Kandungan Kimia*)	**Description** (Deskripsi)	**Test Result Description** (*Deskripsi Hasil Tes*)	**Result** (*Hasil*)
Flavonoids	Positive results are indicated by a change in color to red (Yuniarti *et al*., 2021).	The red color was formed	(+)
Alkaloids	Mayer's reagent: positive results produce a white precipitate. Dragendorff's reagent: Positive results yield a brick-red color. Bouchardat's reagent yields positive results with a brown color (Yuniarti *et al*., 2021).	An orange-red precipitate	(−)
Tannins	Positive results are indicated by a change in color to blackish green (Yuniarti *et al*., 2021).	A slightly blackish green	(+)
Triterpenoids	Liebermann-Burchard reagent: positive results show a red color (Dewi *et al*., 2024).	Red color.	(+)
Phenols	Positive results show a color change to green, red-purple, blue, or black (Yuniarti *et al*., 2021).	Slightly blackish green.	(+)
Saponins	After shaking, the presence of remaining foam, if observed after 10 minutes, indicates a positive result (To'bungan *et al*., 2025).	Foam was formed and persisted for 10 minutes.	(+)

These findings were confirmed by the thin-layer chromatography (TLC) profile (Table 2), which reinforced the presence of active constituents, particularly flavonoids and saponins. The presence of flavonoids, indicated by the appearance of yellowish spots after spraying with 5% aluminum chloride. In addition, saponins were identified by the presence of multicolored spots (red, yellow, dark blue, purple, green, and yellow-brown) after spraying with the same reagent.

Table 2. Thin Layer Chromatography (TLC) Test Results of Extracts and Fractions of Red Bajakah Tampala Stem (*Spatholobus littoralis* Hassk.) (*Lapisan Tipis (TLC) Ekstrak dan Fraksi Batang Bajakah Tampala Merah (Spatholobus littoralis*).

No	**Chemical Content** (Kandungan Kimia)	**Procedure** (*Prosedur*)	**Spray Reagent** (Reagen semprot)	**Result** (*Hasil*)	**Reference** (*Referensi*)	**Description** (*Deskripsi*)	**Result** (Hasil)
1	Flavonoids	Mobile phase: Chloroform: Methanol (9:1), Quinoline as a standard	5% Aluminum (III) Chloride	Yellowish color	Chen *et al*., 2024	Positive results are indicated by the appearance of yellow spots after spraying with 5% Aluminum (III) Chloride.	(+)
2	Alkaloids	Mobile phase: Chloroform: Ethyl Acetate (6:4), Piperine as a standard	Dragendorff	Brown	Wołosiak *et al*., 2021	Positive results are typically marked by a yellow-orange coloration when sprayed with Dragendorff reagent.	(−)
3	Tannins	Mobile phase: Butanol: Acetic Acid (9:1) Comparator standard: Gallic Acid	1% $FeCl_3$	Black spot	Imran & Alwahab, 2024	The appearance of blackish-green spots characterizes positive results.	(+)
4	Triterpenoids	Mobile phase: Butanol: Ethyl	Liebermann-Burchard	Red spot	Sianipar *et al*., 2023	Positive results appear as purple-	(+)

No	Chemical Content (Kandungan Kimia)	Procedure (*Prosedur*)	Spray Reagent (Reagen semprot)	Result (*Hasil*)	Reference (*Referensi*)	Description (*Deskripsi*)	Result (Hasil)
		Acetate (12:8) Comparator standard: Stigmasterol				red spots after spraying with Liebermann-Burchard reagent.	
5	Saponins	Mobile phase: Chloroform: Methanol (17:3) Comparator standard: Sapogenin	Liebermann-Burchard	Various colors (red, yellow, dark blue, purple, green, yellow-brown)	Imran & Alwahab, 2024	Positive results show red, yellow, dark blue, purple, green, or yellow-brown spots when sprayed with Liebermann-Burchard reagent.	(+)

The DPPH antioxidant activity test showed a maximum wavelength at 517 nm, within the optimal detection range (Rumpf *et al*., 2023). The operating time (OT) for optimal reaction was 21 minutes. The IC_{50} values (Figure 1) revealed that the ethyl acetate fraction had the strongest antioxidant activity (IC_{50} = 12.87 ppm), followed by the n-hexane fraction (24.08 ppm), the extract (26.90 ppm), and the water fraction (57.23 ppm). These results categorize the ethyl acetate fraction as having very strong antioxidant activity with an IC_{50} value < 50 ppm (Rumpf *et al*., 2023). The IC_{50} values reported in this study for the extract and its fractions support the conclusions of Rumpf *et al*., 2023 regarding antioxidant classification, and further reinforce the idea that the ethyl acetate fraction has significant therapeutic potential. Quercetin, the reference compound, showed the lowest IC_{50} value (7.93 ppm), indicating its high antioxidant potential. These findings suggest that semi-polar compounds like flavonoids significantly contribute to the antioxidant properties of the Red Bajakah Tampala (Figure 1). Flavonoids are recognized for their ability to scavenge free radicals (Baschieri and Amorati, 2021).

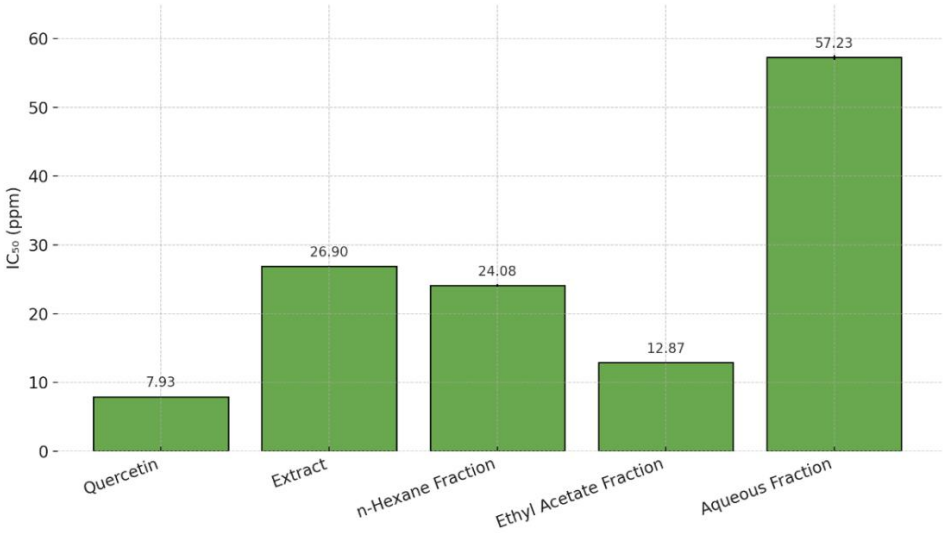

Figure 1. IC_{50} Values of Red Bajakah Tampala Extract and Fractions (DPPH Assay) *(Nilai IC_{50} Ekstrak dan Fraksi Bajakah Tampala Merah (Uji DPPH)*).

The Red Bajakah Tampala stem contains a range of bioactive compounds, especially flavonoids and triterpenoids, which have been related to antioxidant properties. The absence of alkaloids in both the tube tests and TLC analysis supports the specificity of the extract's phytochemical profile. Additionally, the positive results for saponins, phenols, and tannins indicate the multifaceted nature

of the antioxidant activity, which is not solely dependent on flavonoids. The high yield and low water content of the extract suggest that the drying and extraction processes were effective.

The powdered yield of 26.02% aligns with the findings of Hadanu *et al*. (2023), who reported a yield of 80% from fresh plant material (wet weight: 2.5 kg, dry weight: 2 kg), reflecting a similar dry-to-wet ratio. Similarly, the phytochemical findings of this study are in agreement with those reported by Yuniarti *et al*., 2021, Dewi *et al*., 2024, and To'bungan *et al*., 2025, confirming the consistency of chemical profiles and supporting the reliability of the phytochemical screening methods employed.

Future research should focus on the quantitative determination of active compounds using HPLC or LC-MS/MS to understand their contributions to antioxidant activity. This study acknowledges the limitation of relying solely on the DPPH assay. To fully address the mechanistic aspects of antioxidant activity, future studies should incorporate additional assays such as ABTS, FRAP, and ORAC, along with cellular or molecular approaches. In vivo or in vitro studies could explore therapeutic applications, including anti-inflammatory or cytoprotective effects. Moreover, exploration of formulation techniques such as nanoemulsions or encapsulation may enhance the stability and delivery of these bioactive compounds.

CONCLUSION

This study confirmed the antioxidant potential of Red Bajakah Tampala (*Spatholobus littoralis* Hassk.) through DPPH free radical scavenging analysis and qualitative phytochemical screening. The ethyl acetate fraction exhibited the strongest antioxidant activity (IC_{50} = 27.4 ppm), followed by the ethanol extract (IC_{50} = 42.5 ppm). Phytochemical screening revealed the presence of flavonoids, tannins, triterpenoids, phenols, and saponins, which are known contributors to antioxidant activity. These findings support the traditional use of Bajakah Tampala as a natural antioxidant and provide a scientific foundation for its potential application in the development of therapeutic or cosmetic products.

AUTHOR CONTRIBUTIONS

NGA: contributed to the research data collection and analyzed the data, GNFS: reviewer for this student research, MN: article preparation and manuscript revision, DM: corresponding author: conceptualized the research, helped the data analysis, helped draft the article, and finalized the manuscript.

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