Indonesian Journal for Health Sciences
Vol. 9, No. 2, September 2025, pp. 111-117
ISSN 2549-2721 (Print), ISSN 2549-2748 (Online)

EFFECT OF EXTRACTION METHODS ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF BAJAKAH ROOT (*Spatholobus littoralis*)

Julia Then Agustin[1], Michael[2], Pretty Falena Atmanda Kambira[3], Deka Prismawan[4*]
[1,2,3,4] Department of Pharmacy, Atma Jaya Catholic University of Indonesia, Jakarta, Indonesia

Article History:
Submitted: 07/03/2025
Accepted: 11/08/2025
Published: 25/09/2025

Keywords:
Bajakah Root,
Extraction Methods,
Phenolic Content,
Antioxidant Activity,
Reflux Method

ABSTRAK

Abstract:
The Bajakah root (*Spatholobus littoralis*), a native plant of Kalimantan, is recognized for its phenolic content and antioxidant activity. This study aims to compare the effects of three extraction methods—maceration, soxhlet, and reflux—on the total phenolic content and antioxidant activity of Bajakah root extracts. The plant material was extracted using 96% ethanol, followed by phenolic content analysis via the Folin-Ciocalteu method and antioxidant activity evaluation using the DPPH assay. Results revealed that the reflux method produced the highest phenolic content (17.6 ± 0.1 mg GAE/g extract) and the strongest antioxidant activity (IC_{50} of 26 ± 1 µg/mL). The soxhlet method yielded a phenolic content of 17.4 ± 0.2 mg GAE/g extract with an IC_{50} of 30 ± 0 µg/mL, while maceration resulted in the lowest phenolic content (14.6 ± 0.7 mg GAE/g extract) and an IC_{50} of 34 ± 2 µg/mL. The heating processes involved in the reflux and Soxhlet methods likely facilitated the degradation of glycosides, thereby enhancing phenolic content. All methods demonstrated exceptionally strong antioxidant activity (IC_{50} < 50 µg/mL). This study underscores the significant influence of extraction methods on the phenolic quality and antioxidant capacity of Bajakah root extracts.

***Abstrak*:**
Akar Bajakah (Spatholobus littoralis), tanaman asli Kalimantan, dikenal karena kandungan fenolik dan aktivitas antioksidannya. Penelitian ini bertujuan untuk membandingkan efek dari tiga metode ekstraksi—maceration, soxhlet, dan refluks—terhadap kandungan fenolik total dan aktivitas antioksidan ekstrak akar Bajakah. Bahan tanaman diekstraksi menggunakan etanol 96%, kemudian dilakukan analisis kandungan fenolik menggunakan metode Folin-Ciocalteu dan evaluasi aktivitas antioksidan dengan uji DPPH. Hasil penelitian menunjukkan bahwa metode refluks menghasilkan kandungan fenolik tertinggi (17,6 ± 0,1 mg GAE/g ekstrak) dan aktivitas antioksidan terkuat (IC50 sebesar 26 ± 1 µg/mL). Metode soxhlet menghasilkan kandungan fenolik sebesar 17,4 ± 0,2 mg GAE/g ekstrak dengan IC50 sebesar 30 ± 0 µg/mL, sedangkan metode maserasi menghasilkan kandungan fenolik terendah (14,6 ± 0,7 mg GAE/g ekstrak) dan IC50 sebesar 34 ± 2 µg/mL. Proses pemanasan yang terjadi pada metode refluks dan soxhlet kemungkinan memfasilitasi degradasi glikosida, sehingga meningkatkan kandungan fenolik. Semua metode menunjukkan aktivitas antioksidan yang sangat kuat (IC50 < 50 µg/mL). Penelitian ini menegaskan bahwa metode ekstraksi memiliki pengaruh signifikan terhadap kualitas fenolik dan kapasitas antioksidan ekstrak akar Bajakah.

Corresponding Author:
Deka Prismawan
Department of Pharmacy,
Atma Jaya Catholic University of Indonesia,
Jakarta, Indonesia.
Email: deka.prismawan@atmajaya.ac.id

How to Cite:
J.T. Agistin, Michael, P.F.A., Kambira, D. Prismawan, "Effect of Extraction Methods on Phenolic Content and Antioxidant Activity of Bajakah Root (*Spatholobus littoralis*)", Indonesia. J. Heal. Sci., vol. 9, no. 2, pp. 111-117, 2025.

INTRODUCTION

Bajakah root (*Spatholobus littoralis*) is a tropical plant native to Indonesia, commonly found in tropical forests. It is distributed across regions such as Java, Kalimantan, Sabah, Sarawak, and the Philippines. Notably, this plant is abundant in Central, West, East, and North Kalimantan [1]. Bajakah has garnered significant attention for its potential anticancer properties. Traditionally utilized by indigenous communities for various medicinal purposes, Bajakah has recently been the subject of scientific investigations aiming to validate its therapeutic efficacy, particularly in oncology. In recent years, its prominence increased following its pharmacological benefits by certain group of people in Kalimantan who highlighted its potential as a treatment for breast cancer through the consumption of its boiled water extract [2]. However, the stakeholders in Indonesia have emphasized that the research on Bajakah Root's anticancer properties is still in its preliminary stages, and its effectiveness in eliminating cancer cells has yet to be confirmed. Further investigation is therefore essential [3].

The cytotoxic effects of Bajakah Root are attributed to its secondary metabolites, particularly the phenolic compounds. Phenolics, or polyphenols, are well-known secondary metabolites with cytotoxic properties, capable of damaging cancer cells and inhibiting the growth of abnormal cells [4]. Additionally, Bajakah Root exhibits antioxidant activity, which helps protect the body from oxidative reactions that can damage cells, thus potentially preventing oxidative stress [5] .

Various techniques are available, each with distinct advantages and disadvantages [6]. Factors such as the nature of the source material, interactions between compounds, solvent type, temperature, contact time, and particle size play crucial roles in determining extraction efficiency. Consequently, the entire extraction process—from method selection to operational conditions could strongly influences the quality and quantity of the resulting compounds [7]. Maceration is a simple, conventional method that relies on passive diffusion at room temperature, minimizing the risk of thermal degradation but often resulting in lower efficiency. In contrast, heat-assisted methods like Soxhlet and reflux extraction use elevated temperatures to increase solvent viscosity and enhance compound solubility, potentially increasing yield and reducing extraction time. However, the heat can also risk degrading thermolabile compounds. Therefore, a comparative evaluation is crucial for process optimization [8]**.** Comparing these three methods provides a comprehensive understanding of the trade-off between extraction efficiency, simplicity, and the preservation of bioactive compounds in *Spatholobus littoralis.*

While traditional use and some preliminary studies have highlighted the antioxidant potential of *Spatholobus* species, to our knowledge, no systematic study has been conducted to compare the effectiveness of maceration, Soxhlet, and reflux extraction on the phenolic content and antioxidant capacity of Bajakah root. This research aims to fill this scientific gap by comparing these different extraction methods to determine their effects on the phenolic content and antioxidant activity of Bajakah root extracts. The findings are intended to identify the most effective method for producing extracts with high potency for potential use in nutraceuticals and pharmaceuticals.

RESEARCH METHOD

Bajakah root samples were collected from West Kalimantan, Indonesia, and prepared for analysis by cleaning, chopping, and sun-drying. The dried roots were ground into a fine powder and stored in airtight containers until further use. All chemicals used in this study were bought from Merck, Germany and of analytical grade. Key instruments included are UV-Vis Spectrophotometer (Shimadzu UV-

1800), rotary evaporator (Heidolph Hei-VAP Core), Soxhlet apparatus, reflux apparatus, and maceration setup (IWAKI Glassware).

Three extraction techniques which is maceration, Soxhlet extraction, and reflux extraction were employed to extract bioactive compounds from the Bajakah root powder using ethanol as the solvent. For maceration, the powdered sample was immersed in ethanol and left at room temperature for 24 hours, with the solvent replaced after the first day. Soxhlet extraction was carried out by continuously cycling ethanol through the sample at solvent's boiling point (75 ± 3°C) for 15 heating cycles. While the reflux extraction involved heating the sample with ethanol at solvent's boiling point (75 ± 3°C) under continuous stirring of 400 rpm for 8 hours. After extraction, the solutions were filtered to remove residual plant material, and the filtrates were concentrated using a rotary evaporator to obtain a viscous extract. The extracts were stored at 4°C until further analysis.

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu method [9]. A gallic acid standard curve was prepared by diluting a stock solution to various concentrations. The optimal reaction time was determined by measuring the absorbance of a gallic acid solution mixed with Folin-Ciocalteu reagent and sodium carbonate over a period of time. For sample analysis, the extract was diluted and mixed with Folin-Ciocalteu reagent and sodium carbonate. After incubation, the absorbance was measured, and the TPC was calculated using the gallic acid calibration curve. Results were expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g).

The antioxidant activity of the extracts was evaluated using the DPPH free radical scavenging assay [10]. A DPPH solution was prepared, and its maximum absorption wavelength was determined. Serial dilutions of the extract and a vitamin C standard were prepared for the assay. The extract solutions were mixed with the DPPH reagent and incubated in the dark. After incubation, the absorbance was measured, and the percentage of free radical inhibition was calculated. The IC_{50} value, representing the concentration required to scavenge 50% of the DPPH free radicals, was determined using linear regression analysis. A lower IC_{50} value indicates higher antioxidant activity.

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Statistical analysis was conducted using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to identify significant differences between the methods, with $p < 0.05$ considered significant.

RESULT AND DISCUSSION

1. Extraction Efficiency and Yield

The extraction of Bajakah root in ethanol using maceration, Soxhlet, and reflux methods resulted in reddish-brown extracts with distinct aromas. Ethanol was employed due to its safety and wide range of polarity which is effective in extracting variety of bioactive compounds from plants [11]. Among these methods, Soxhlet extraction provided the highest yield (3.5%), followed by maceration and reflux (both 3.4%) (Table 1, Figure 1). However, these differences were not statistically significant. This aligns with recent studies that have shown Soxhlet extraction to be highly efficient for extracting non-polar and semi-polar compounds due to its continuous solvent recycling and prolonged extraction time [12]. However, the high yield does not always correlate with the quality or concentration of bioactive compounds, as prolonged heating may degrade thermolabile compounds [13].

2. Total Phenolic Content

The total phenolic content, measured using the Folin-Ciocalteu method, showed that the reflux method produced the highest concentration (17.6 ± 0.1 mg GAE/g

extract), closely followed by Soxhlet (17.4 ± 0.2 mg GAE/g extract), while maceration yielded the lowest (14.6 ± 0.7 mg GAE/g extract) (Table 1, Figure 1). Statistical analysis revealed that the phenolic content from both reflux and Soxhlet methods was significantly higher ($p < 0.05$) than that from maceration. The superior performance of reflux extraction can be attributed to its controlled heating, which enhances the solubility of phenolic compounds without causing excessive degradation [13]. These values are notably higher than those reported for other related species, such as *Spatholobus suberectus*, suggesting that *S. littoralis* is a particularly rich source of phenolic compounds[14].

In contrast, maceration, which relies on passive diffusion, is less efficient for extracting phenolic compounds due to its reliance on solvent penetration and time [12]. From a practical standpoint, these results indicate that for producing potent, phenolic-rich extracts for nutraceuticals, reflux is the most advantageous method.

Table 1.
Yield, Total Phenolic Content, and Antioxidant Activity of Bajakah Root Extracts from Different Extraction Methods

Extraction Method	Yield (%)	Total Phenolic Content (mg GAE/g extract)	Antioxidant Activity (IC_{50}, µg/mL)
Maceration	3.4	14.6 ± 0.7	34 ± 2
Soxhlet	3.5	17.4 ± 0.2	30 ± 0
Reflux	3.4	17.6 ± 0.1	26 ± 1

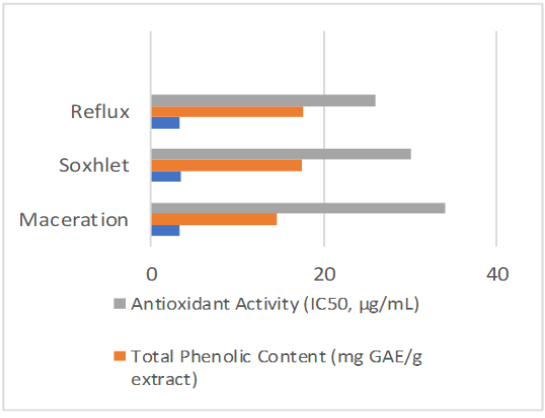

Figure 1. Bar Graph of Yield, Total Phenolic Content, and Antioxidant Activity of Bajakah Root Extracts from Different Extraction Methods.

3. Antioxidant Activity, Correlation and Mechanism

Antioxidant activity, analyzed using the DPPH method, indicated that reflux extraction resulted in the lowest IC_{50} value (26 ± 1 µg/mL), signifying the strongest antioxidant potential. While Soxhlet extraction followed with an IC_{50} of 30 ± 0 µg/mL, whereas maceration had the highest IC_{50} (34 ± 2 µg/mL), indicating the lowest antioxidant efficiency between the 3 methods (Table 1, Figure 1).

These results of enhanced antioxidant activity correlate well with the high phenolic content obtained via reflux and although Soxhlet extraction produced the highest crude yield (3.5%), this did not translate to the highest potency. This highlights a critical concept in natural product extraction of maximizing crude yield is not always the optimal strategy, as harsh or prolonged conditions can degrade sensitive compounds, thereby reducing the extract's specific activity [18].

It is well-established that flavonoids and tannins, often co-extracted with phenols, can exhibit powerful synergistic antioxidant activity, which may explain the very strong potency ($IC_{50} < 50$ µg/mL) observed in all extracts [19].

The strong antioxidant capacity is primarily attributed to the phenolic compounds' ability to donate hydrogen atoms [15], [16]. However, other bioactive compounds likely contribute to the overall effect. The reason why Soxhlet and reflux has higher antioxidant activity is due to thermal processes that may also break down glycosidic bonds, releasing free, more active phenolic aglycones. The reflux method appears to strike the best balance, enhancing solubility and compound release

while minimizing the thermal degradation seen in prolonged Soxhlet cycles [13].

The results show a strong correlation between total phenolic content and antioxidant activity, where extracts with higher phenolic content (such as from reflux) exhibited lower IC_{50} values, indicating stronger radical scavenging ability. Phenolic compounds are known to donate hydrogen atoms or electrons to neutralize free radicals, which explains their antioxidant potential [17]. However, other bioactive compounds, such as flavonoids and tannins, may also contribute to antioxidant activity [18], [19], [20]. Further analysis could help identify which specific compounds are most responsible for the observed effects. For instance, studies have shown that flavonoids and tannins exhibit synergistic effects with phenolic compounds, enhancing overall antioxidant activity [13].

The strong correlation between phenolic content and antioxidant activity is consistent with findings from other studies, which have demonstrated that phenolic compounds are major contributors to antioxidant activity due to their ability to donate hydrogen atoms or electrons to neutralize free radicals [17]. These findings are significant for practical applications, as reflux extracts of Bajakah root could be developed as effective natural antioxidants for the food preservation or cosmetic industries, or as active ingredients in health supplements aimed at mitigating oxidative stress.

4. Limitations and Future Research

While this study provides insight into the impact of extraction methods on phenolic content and antioxidant activity, some limitations exist. The correlation between total phenolics and antioxidant activity, while strong, does not exclude the contribution of other non-phenolic anti-oxidant compounds (e.g., certain terpenes or alkaloids) that were not quantified in this study but are known to possess antioxidant properties [21]. Future research could explore optimization of extraction parameters (e.g., temperature, solvent ratio) and assess the bioavailability of phenolic compounds in biological systems. Additionally, comparing different solvent systems could help determine the most effective method for maximizing bioactive compound retention [22].

These findings suggest that reflux extraction is the most effective method for obtaining high phenolic content and antioxidant activity in Bajakah root extract. However, further studies are needed to refine the process and explore potential applications in pharmaceuticals and nutraceuticals.

CONCLUSION

This study demonstrates that the choice of extraction method significantly influences the phenolic content and antioxidant activity of Bajakah root extracts. Reflux extraction yielded the highest phenolic content (17.6 ± 0.1 mg GAE/g extract) and the strongest antioxidant activity ($IC_{50} = 26 \pm 1$ µg/mL), outperforming Soxhlet and maceration methods. While Soxhlet extraction provided the highest yield, its prolonged heating may have caused partial degradation of phenolic compounds. These findings highlight reflux extraction as the most effective method for maximizing phenolic content and antioxidant potential in Bajakah root. Further research is recommended to optimize extraction parameters and explore the bioavailability of bioactive compounds for potential pharmaceutical and nutraceutical applications.

ACKNOWLEDGEMENTS

Authors express their gratitude to Atma Jaya Catholic University of Indonesia for the support in this research.

REFERENCES

[1] H. Hamzah, S. Pratiwi, A. Jabbar, A. Hafifah, B. Al-Fajri, and N. Nurhalisah, "Bioactivity Tracing of the Ethanol Extract of Bajakah Tampala (Spatholobus littoralis Hassk.) Typical Plant of Kalimantan Island as Antibiofilm of Staphylococcus aureus," *Open Access Maced J Med Sci*, vol. 11, no. A, pp. 8–14, 2023.

[2] Tempo, "Viral Penemuan Bajakah, Ratusan Orang Serbu SMA 2 Palangka Raya." [Online]. Available: https://tekno.tempo.co/read/1236417/viral-penemuan-bajakah-ratusan-orang-serbu-sma-2-palangka-raya?TeknoUtama&campaign=Tekno Utama_Click_4

[3] Kemenkes RI, "Kemenkes Terus Dukung Penelitian Akar Bajakah." [Online]. Available: https://www.kemkes.go.id/id/rilis-kesehatan/menkes-terus-dukung-penelitian-akar-bajakah

[4] J. Dai and R. J. Mumper, "Plant phenolics: Extraction, analysis, and their antioxidant and anticancer properties," *Molecules*, vol. 15, no. 10, pp. 7313–7352, 2010, doi: 10.3390/molecules15107313.

[5] K. Yamagata, "Do Coffee Polyphenols Have a Preventive Action on Metabolic Syndrome Associated Endothelial Dysfunctions? An Assessment of the Current Evidence," *Antioxidants*, vol. 7, p. 26, 2018.

[6] Q.-W. Zhang, L.-G. Lin, and W.-C. Ye, "Techniques for extraction and isolation of natural products: a comprehensive review," *Chin Med*, vol. 13, p. 20, Apr. 2018, doi: 10.1186/s13020-018-0177-x.

[7] U. Roy, B. Nielsen, and J. Milledge, "Antioxidant production in Dunaliella," *Applied Sciences (Switzerland)*, vol. 11, 2021.

[8] C. Bitwell, S. S. Indra, C. Luke, and M. K. Kakoma, "A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants," *Scientific African*, vol. 19, p. e01585, Mar. 2023, doi: 10.1016/j.sciaf.2023.e01585.

[9] V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent," *Methods in Enzymology*, vol. 299, pp. 152–178, 1999, doi: 10.1016/S0076-6879(99)99017-1.

[10] R. L. Prior, X. Wu, and K. Schaich, "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 10, pp. 4290–4302, 2005, doi: 10.1021/jf0502698.

[11] Munira, D. Amalia, W. Khazanah, and M. Nasir, "Aktivitas antibakteri ekstrak etanol daun kelor (*Moringa oleifera* Lamk) berdasarkan perbedaan waktu panen," *Indonesian Journal for Health Sciences*, vol. 5, no. 2, pp. 69-76, 2021, doi: https://doi.org/10.24269/ijhs.v5i2.3640.

[12] F. Chemat, M. A. Vian, and G. Cravotto, "Green extraction of natural products: Concept and principles," *International Journal of Molecular Sciences*, vol. 20, no. 13, pp. 1–25, 2019, doi: 10.3390/ijms20133300.

[13] O. R. Alara, N. H. Abdurahman, and C. I. Ukaegbu, "Extraction of phenolic compounds: A review," *Current Research in Food Science*, vol. 4, pp. 200–214, 2021, doi: 10.1016/j.crfs.2021.03.004.

[14] Md. Mohibbullah *et al.*, "The medicinal herb Spatholobus suberectus with promising in vitro antioxidant and anti-inflammatory potentials and its phytochemical characterization by RP-HPLC analysis," *Journal of Food Biochemistry*, vol. 42, no. 2, p. e12480, 2018, doi: 10.1111/jfbc.12480.

[15] J. Kruk, B. H. Aboul-Enein, E. Duchnik, and M. Marchlewicz,

"Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise," *The Journal of Physiological Sciences*, vol. 72, no. 1, p. 19, 2022, doi: 10.1186/s12576-022-00845-1.

[16] B. Chen and M. Xu, "Natural Antioxidants in Foods," in *Encyclopedia of Food Chemistry*, L. Melton, F. Shahidi, and P. Varelis, Eds., Oxford: Academic Press, 2019, pp. 180–188. doi: 10.1016/B978-0-08-100596-5.21599-0.

[17] B. Gullón, M. E. Pintado, J. A. Pérez-Álvarez, and M. Viuda-Martos, "Assessment of polyphenolic profile and antibacterial activity of pomegranate peel extracts obtained by different extraction techniques," *Food and Bioprocess Technology*, vol. 10, no. 5, pp. 1–12, 2017, doi: 10.1007/s11947-017-1944-x.

[18] F. Cosme, A. Aires, T. Pinto, I. Oliveira, A. Vilela, and B. Gonçalves, "A Comprehensive Review of Bioactive Tannins in Foods and Beverages: Functional Properties, Health Benefits, and Sensory Qualities," *Molecules*, vol. 30, no. 4, Art. no. 4, Jan. 2025, doi: 10.3390/molecules30040800.

[19] R. Novalia Rahmawati Sianipar *et al.*, "The role of selected flavonoids from bajakah tampala (*Spatholobus littoralis* Hassk.) stem on cosmetic properties: A review," *Saudi Pharmaceutical Journal*, vol. 31, no. 3, pp. 382–400, Mar. 2023, doi: 10.1016/j.jsps.2023.01.006.

[20] S. Skrovankova and J. Mlcek, "Antioxidant Potential and Its Changes Caused by Various Factors in Lesser-Known Medicinal and Aromatic Plants," *Horticulturae*, vol. 11, no. 1, Art. no. 1, Jan. 2025, doi: 10.3390/horticulturae11010104.

[21] R. Guan *et al.*, "A review of dietary phytochemicals and their relation to oxidative stress and human diseases," *Chemosphere*, vol. 271, p. 129499, May 2021, doi: 10.1016/j.chemosphere.2020.129499.

[22] N. N. Azwanida, "A review on the extraction methods use in medicinal plants, principle, strength, and limitation," *Medicinal & Aromatic Plants*, vol. 4, no. 3, pp. 1–6, 2015, doi: 10.4172/2167-0412.1000196.