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Ultrasound-Assisted Extraction Enhances Phenolic, Flavonoid, and Antioxidant Profiles of *Spatholobus littoralis* Roots

Ekstraksi Berbantuan Ultrasonik Meningkatkan Profil Fenolik, Flavonoid, dan Antioksidan Akar *Spatholobus littoralis*

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ABSTRACT

Spatholobus littoralis Hassk. roots contain antioxidant-related compounds whose recovery may be influenced by extraction method and solvent polarity. This study compared ultrasound-assisted extraction and microwave-assisted extraction using n-hexane, ethyl acetate, and 96% ethanol to evaluate their effects on total phenolic content, total flavonoid content, and DPPH radical-scavenging activity. Among the tested conditions, 96% ethanol combined with ultrasound-assisted extraction produced the highest phenolic content, flavonoid content, and antioxidant activity, with values of 89.33 mg GAE/g, 40.10 mg QE/g, and IC_{50} 72.27 µg/mL, respectively. Strong negative correlations were observed between DPPH IC_{50} and both total phenolic content ($r = -0.961$) and total flavonoid content ($r = -0.941$). These findings indicate that ultrasound-assisted extraction with 96% ethanol was the most effective condition for obtaining antioxidant-rich *S. littoralis* root extract in this study.

Kata kunci:
Aktivitas antioksidan
Ekstraksi berbantuan ultrasonic
Fenolik
Flavonoid
Spatholobus littoralis

ABSTRAK

Akar *Spatholobus littoralis* Hassk. mengandung senyawa yang berkaitan dengan aktivitas antioksidan, dan perolehannya dapat dipengaruhi oleh metode ekstraksi serta polaritas pelarut. Penelitian ini membandingkan ekstraksi berbantuan ultrasonik dan ekstraksi berbantuan gelombang mikro menggunakan n-heksana, etil asetat, dan etanol 96% untuk mengevaluasi pengaruhnya terhadap kadar fenolik total, kadar flavonoid total, dan aktivitas penangkapan radikal DPPH. Di antara kondisi yang diuji, etanol 96% dengan ekstraksi berbantuan ultrasonik menghasilkan kadar fenolik, kadar flavonoid, dan aktivitas antioksidan tertinggi, masing-masing sebesar 89,33 mg GAE/g, 40,10 mg QE/g, dan IC_{50} 72,27 µg/mL. Korelasi negatif yang kuat ditemukan antara IC_{50} DPPH dan kadar fenolik total ($r = -0,961$) serta kadar flavonoid total ($r = -0,941$). Temuan ini menunjukkan bahwa ekstraksi berbantuan ultrasonik dengan etanol 96% merupakan kondisi paling efektif untuk memperoleh ekstrak akar *S. littoralis* kaya antioksidan dalam penelitian ini.

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1. INTRODUCTION

Excessive formation of free radicals can promote oxidative reactions in biological systems and contribute to oxidative stress, which is associated with cellular damage and several degenerative conditions (Sadiq, 2023). Antioxidants can reduce oxidative damage by neutralizing free radicals through electron or hydrogen atom donation (Halliwell & Gutteridge, 2015; Pisoschi & Pop, 2015). Plant-derived antioxidants are of particular interest because many medicinal plants contain secondary metabolites, especially phenolic compounds and flavonoids, that are closely related to radical-scavenging activity (Cai et al., 2004).

Spatholobus littoralis Hassk., locally known as bajakah tampala, has been studied as a potential source of natural antioxidant compounds. Previous studies have reported antioxidant activity from this plant, particularly in root extracts (Sianipar et al., 2023; Mahfudh et al., 2024). However, many earlier studies relied on conventional extraction methods, such as maceration, percolation, reflux, or related techniques, which may require longer extraction times or exposure to heat that can affect thermolabile bioactive compounds (Mahfudh et al., 2024; Hasna et al., 2021; Nastiti & Nugraha, 2022; Savić Gajić et al., 2019; Arsul et al., 2022; Nur et al., 2024).

Extraction method and solvent polarity are important factors that influence the recovery of phenolic and flavonoid compounds from plant materials. Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) have been widely used to improve extraction efficiency, reduce processing time, and facilitate the recovery of bioactive compounds compared with several conventional approaches (Yusoff et al., 2022; Savić Gajić et al., 2019; Lopeda-Correa et al., 2022). Nevertheless, comparative information on the effects of UAE and MAE, combined with solvents of different polarity, on the phenolic content, flavonoid content, and antioxidant activity of *S. littoralis* roots remains limited.

Therefore, this study aimed to compare UAE and MAE using n-hexane, ethyl acetate, and 96% ethanol as extraction solvents for *S. littoralis* roots. The extracts were evaluated for total phenolic content, total flavonoid content, and DPPH radical-scavenging activity to determine how extraction method and solvent polarity influence the antioxidant-related profile of the root extracts.

2. METHODS

2.1. Plant material and authentication

Roots of *S. littoralis*, locally known as bajakah tampala, were collected from Muara Teweh, North Barito Regency, Central Kalimantan, Indonesia. The plant material was authenticated at the Biological Research Center, National Research and Innovation Agency (BRIN), Cibinong, Bogor, West Java, Indonesia, to confirm its botanical identity, under document number B-1392/II.6.2/IR.01.02/5/2024.

2.2. Preparation of root powder

Fresh *S. littoralis* roots were cleaned to remove soil, stems, leaves, and other foreign materials. The roots were washed under running water, cut into smaller pieces, and dried at 45°C for 12 h using a dehydrator. The dried roots were ground into powder and sieved through a 40-mesh sieve. The powdered material was then used for extraction.

2.3. Extraction procedure

Powdered *S. littoralis* roots were sequentially extracted using ultrasound-assisted extraction and microwave-assisted extraction with solvents of increasing polarity, namely *n*-hexane, ethyl acetate, and 96% ethanol. For each extraction, 200 g of root powder was mixed with 1,000 mL of solvent at a 1:5 (w/v) ratio. Ultrasound-assisted extraction was performed at 38 kHz for 60 min at 30°C, whereas microwave-assisted extraction was conducted at 900 W and 45°C for 12 min (Chemat et al., 2017; Chemat & Cravotto, 2013). After extraction, each mixture was filtered, and the filtrate was concentrated at 45°C to remove the solvent and obtain the corresponding extract. The extracts were subsequently used to determine total phenolic content, total flavonoid content, and DPPH radical scavenging activity.

2.4. Determination of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with gallic acid as the reference standard (Singleton et al., 1999). Each extract solution was reacted with Folin–Ciocalteu reagent and sodium carbonate solution, followed by incubation in the dark. Absorbance was measured using a UV–Vis spectrophotometer, and total phenolic content was calculated from the gallic acid calibration curve. The results were expressed as mg gallic acid equivalent per gram of extract (mg GAE/g). Measurements were performed in triplicate.

2.5. Determination of total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the reference standard (Chang et al., 2002). Each extract solution was reacted with aluminum chloride and sodium acetate, followed by incubation at room temperature. Absorbance was measured using a UV–Vis spectrophotometer, and total flavonoid content was calculated from the quercetin calibration curve. The results were expressed as mg quercetin equivalent per gram of extract (mg QE/g). Measurements were performed in triplicate.

2.6. DPPH radical-scavenging activity assay

Antioxidant activity was evaluated using the DPPH radical-scavenging assay. The DPPH solution was prepared in methanol and protected from light prior to use (Kiromah et al., 2021). Extract solutions at concentrations of 100, 120, 140, 160, and 180 µg/mL were mixed with the DPPH solution and incubated in the dark. Absorbance was measured using a UV–Vis spectrophotometer. The percentage of DPPH inhibition was calculated from the absorbance values, and IC_{50} values were determined based on the relationship between extract concentration and inhibition percentage. A lower IC_{50} value

indicated stronger radical-scavenging activity (Brand-Williams et al., 1995; Molyneux, 2004). All measurements were performed in triplicate.

2.7. Statistical analysis

Data are presented as mean ± standard deviation. Differences among extract groups were analyzed using one-way analysis of variance followed by Tukey's honestly significant difference post hoc test. Pearson correlation analysis was used to evaluate the relationship between total phenolic content, total flavonoid content, and DPPH IC_{50} values. A *p*-value below 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Effect of extraction method and solvent on phenolic, flavonoid, and antioxidant profiles

The extraction method and solvent polarity clearly influenced the recovery of antioxidant-related compounds from *S. littoralis* roots. Across both extraction methods, 96% ethanol produced substantially higher total phenolic and flavonoid contents than ethyl acetate and n-hexane, indicating that the major phenolic- and flavonoid-related constituents in the roots were more efficiently extracted using a polar solvent system (**Figure 1A** and **1B**). This pattern is consistent with the chemical characteristics of many phenolic and flavonoid compounds, which contain hydroxyl groups and therefore tend to show better solubility in polar solvents (Dai & Mumper, 2010; Do et al., 2014). These results indicate that solvent polarity was a key determinant of phytochemical recovery from *S. littoralis* roots.

Ultrasound-assisted extraction produced higher total phenolic and flavonoid contents than microwave-assisted extraction under comparable solvent conditions. The highest total phenolic content was obtained from the 96% ethanol extract prepared by ultrasound-assisted extraction, with a value of 89.33 mg GAE/g, followed by the corresponding microwave-assisted ethanol extract at 83.24 mg GAE/g (**Figure 1A**). A similar pattern was observed for total flavonoid content, where ultrasound-assisted extraction with 96% ethanol yielded the highest value of 40.10 mg QE/g, compared with 35.72 mg QE/g for the microwave-assisted ethanol extract (**Figure 1B**). This finding suggests that ultrasound-assisted extraction was more effective in recovering phenolic and flavonoid constituents from *S. littoralis* roots under the conditions used in this study.

The higher recovery observed with ultrasound-assisted extraction may be related to acoustic cavitation, which can disrupt plant cell structures, improve solvent penetration, and enhance mass transfer of intracellular compounds into the extraction medium. In contrast, microwave-assisted extraction relies mainly on dielectric heating, which may accelerate extraction but can also expose thermolabile compounds to localized heating depending on the extraction conditions. Because phenolics and flavonoids may differ in thermal stability, controlled ultrasound treatment may offer an advantage for preserving antioxidant-related compounds (Vo et al., 2023). Thus, the present results support the suitability of ultrasound-assisted extraction for obtaining phenolic- and flavonoid-rich extracts from *S. littoralis* roots.

The antioxidant activity results followed the same general trend as the phenolic and flavonoid contents. The 96% ethanol extract obtained by ultrasound-assisted extraction showed the strongest DPPH radical-scavenging activity, with the lowest IC_{50} value of 72.27 µg/mL, followed by the microwave-assisted ethanol extract with an IC_{50} value of 81.09 µg/mL (**Figure 1C**). In contrast, n-hexane extracts showed the weakest antioxidant activity, with IC_{50} values of 304.84 µg/mL for ultrasound-assisted extraction and 357.29 µg/mL for microwave-assisted extraction. Because lower IC_{50} values indicate stronger radical-scavenging activity, these findings show that extraction conditions producing higher phenolic and flavonoid contents also tended to produce stronger antioxidant activity.

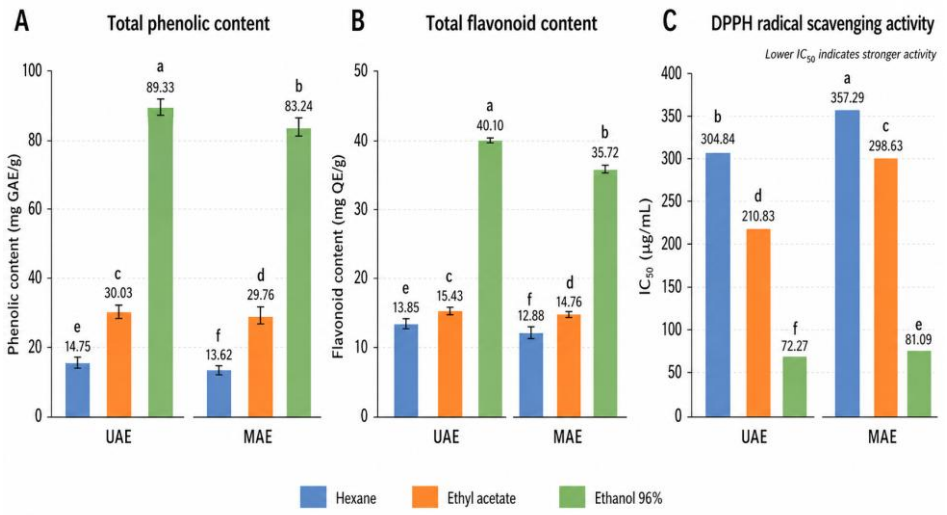

Figure 1. Total phenolic content (A), total flavonoid content (B), and DPPH radical-scavenging activity (C) of *Spatholobus littoralis* root extracts obtained using ultrasound-assisted extraction and microwave-assisted extraction. Data are presented as mean ± SD. Different letters indicate significant differences among groups based on Tukey's HSD test ($p < 0.05$). Lower IC_{50} values indicate stronger antioxidant activity.

3.2. Relationship between bioactive compound content and antioxidant activity

The correlation analysis further supports the contribution of phenolic and flavonoid compounds to the antioxidant activity of *S. littoralis* root extracts. Total phenolic content showed a strong negative correlation with DPPH IC_{50}, with Pearson's $r = -0.961$, $R^2 = 0.924$, and $p = 0.002$ (**Figure 2A**). Total flavonoid content also showed a strong negative correlation with DPPH IC_{50}, with Pearson's $r = -0.941$, $R^2 = 0.885$, and $p = 0.005$ (Figure 2B). These negative correlations indicate that extracts with higher phenolic and flavonoid contents tended to show lower IC_{50} values and, therefore, stronger radical-scavenging activity.

The strong relationship between phenolic and flavonoid contents and DPPH activity is chemically plausible because these compounds can contribute to antioxidant activity through hydrogen atom donation, electron transfer, and stabilization of free radicals. Phenolic and flavonoid compounds are widely recognized as important antioxidant constituents in plant extracts, although their activity depends on concentration, structural features, extraction conditions, and interactions with other metabolites (Prior et al., 2005; Brand-Williams et al., 1995; Pedisić et al., 2023). In this study, the ethanol extracts, particularly those obtained using ultrasound-assisted extraction, showed the most favorable combination of high phenolic content, high flavonoid content, and low IC_{50} values.

Overall, the results indicate that both solvent polarity and extraction method affected the antioxidant-related profile of *S. littoralis* root extracts. Among the tested conditions, 96% ethanol combined with ultrasound-assisted extraction produced the highest total phenolic and flavonoid contents and the strongest DPPH radical-scavenging activity. However, this conclusion is limited to the extraction conditions and antioxidant assay used in this study. Further studies using additional antioxidant assays, compound-level identification, and extraction optimization are needed to clarify the specific compounds responsible for the activity and to strengthen the application potential of *S. littoralis* root extracts.

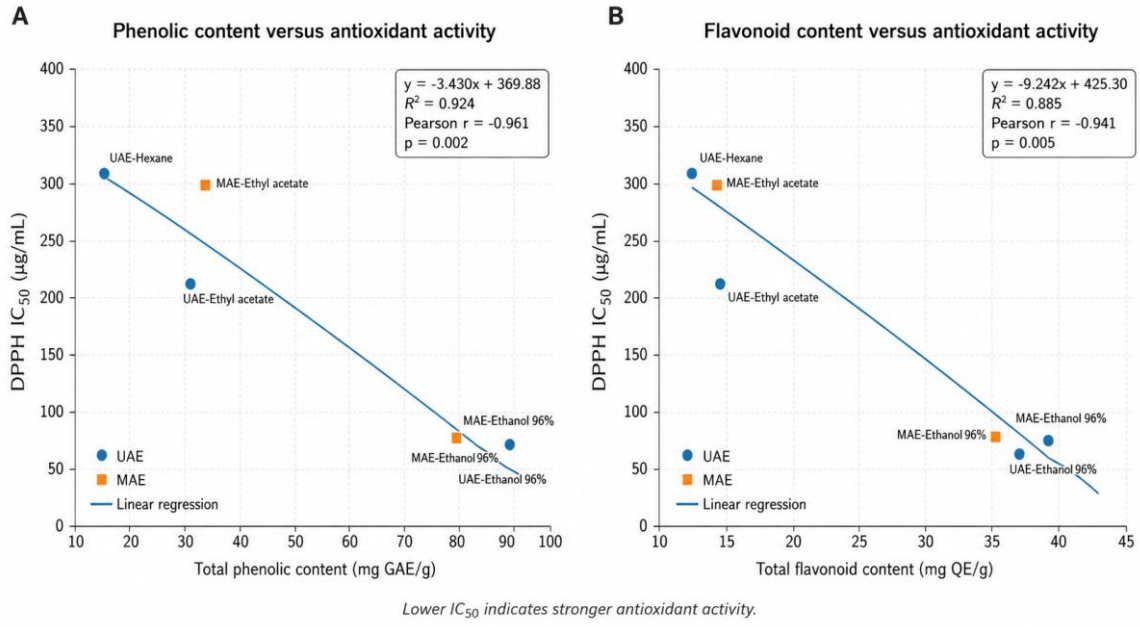

Figure 2. Correlations between bioactive compound contents and antioxidant activity of *Spatholobus littoralis* root extracts. (A) Total phenolic content versus DPPH IC_{50}. (B) Total flavonoid content versus DPPH IC_{50}. Solid lines represent linear regression; lower IC_{50} values indicate stronger antioxidant activity.

4. CONCLUSION

This study showed that extraction method and solvent polarity influenced the phenolic content, flavonoid content, and antioxidant activity of *S. littoralis* root extracts. Among the tested conditions, 96% ethanol combined with ultrasound-assisted extraction produced the highest total phenolic content, highest total flavonoid content, and strongest DPPH radical-scavenging activity, with values of 89.33 mg GAE/g, 40.10 mg QE/g, and IC_{50} 72.27 µg/mL, respectively. The strong negative correlations between DPPH IC_{50} and both total phenolic and flavonoid contents indicate that these compounds were important contributors to the antioxidant activity of the extracts. These findings provide useful comparative data for selecting extraction methods and solvents to obtain antioxidant-rich *S. littoralis* root extracts. Further studies using additional antioxidant assays and compound-level identification are needed to confirm the constituents responsible for the observed activity.

AUTHOR CONTRIBUTIONS

Conceptualization, M.R.G. and B.E.; methodology, M.R.G., B.E., and M.H.; validation, B.E., M.H., and H.S.; formal analysis, M.R.G. and H.S.; investigation, M.R.G.; resources, B.E. and M.H.; data curation, M.R.G.; writing—original draft preparation, M.R.G.; writing—review and editing, B.E., M.H., and H.S.; visualization, M.R.G.; supervision, B.E., M.H., and

H.S.; project administration, M.R.G. and B.E.; funding acquisition, B.E. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants, human biological materials, or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ROLE OF FUNDERS

The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, grammar, and readability of the text. After using this tool, the authors carefully reviewed, edited, and verified the content to ensure that it accurately represents their own data, analysis, and interpretation. The authors take full responsibility for the integrity, accuracy, and originality of the manuscript.

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