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The Effect of Ultrasound-Microwave Assisted Extraction (UAE-MAE) Method on the Antioxidant Activity of Ethyl Acetate Extract of Bajakah Roots (Spatholobus Littoralis Hassk) Using DPPH Method

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A B S T R A C T

The roots of Spatholobus littoralis Hassk, obtained from Central Kalimantan, were extracted using a combined ultrasound-microwave-assisted extraction method with ethyl acetate as the solvent. The results demonstrated potent antioxidant activity, with an IC_{50} value of 43.18 µg/mL, which correlates with the total flavonoid content (26.85 ± 0.059 mgQE/g) and total phenolic content (145.152 ± 0.04 mgGAE/g). Although vitamin C exhibited higher activity (IC_{50} 2.71 µg/mL), S. littoralis roots still hold significant potential as a natural antioxidant source.

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INTRODUCTION

Indonesia, with its abundant natural resources, possesses a wealth of medicinal plants, including bajakah tampala (Spatholobus littoralis Hassk). This root, traditionally used in Kalimantan, has demonstrated potent antioxidant activity in previous studies (Wahyudina, 2019). While prior research has employed maceration, reflux, and percolation for extraction (Wahyudina, 2019; Ayucecharia & Saptera, 2018; Latu, 2023; Lisa & Gabena, 2022).

This study utilizes a novel approach: ultrasonic microwave-assisted extraction (UAE-MAE). Given the limited research on ethyl acetate extraction of S. littoralis, this study investigates the total phenolic content, total flavonoid content, and antioxidant activity (using the DPPH method) of the resulting extract. These findings will contribute valuable data for future research on S. littoralis and its potential applications.

THEORETICAL REVIEW

Plant Collection and Extraction

Fresh roots of Spatholobus littoralis Hassk, a 30-year-old plant characterized by reddish-brown, smooth-surfaced roots, were collected from Muara Teweh, North Barito Regency, Central Kalimantan. A sequential extraction was employed using solvents of increasing polarity (n-hexane, ethyl acetate, and 96% ethanol) with a combined ultrasonic-microwave assisted extraction (UMAE) method. Each solvent extraction involved a 60-minute ultrasonic extraction at 30°C followed by a 15-minute microwave-assisted extraction. The resulting filtrates were concentrated using a rotary evaporator. The ethyl acetate extract was then analyzed for total phenol content, total flavonoid content, and antioxidant activity.

Phytochemical screening

Phytochemical screening is a preliminary stage in phytochemical research aimed at providing an overview of the compound groups contained in the plant being studied (Minarno, 2015).

Alkaloid

We started by weighing 500 mg of powdered plant material or a fresh plant sample. $CHCl_3$ was added to extract the alkaloids, followed by NH_4OH to basify the solution. After filtration, the extract was acidified with 1 mL of 2N HCl. The acidic layer was collected and divided into three test tubes. We then added Dragendorff's reagent, Mayer's reagent, and a blank to the respective tubes for further analysis.

Flavonoid

500 of dried or fresh plant material was weighed and then 10 mL of distilled water was added. The mixture was heated and filtered to obtain a filtrate. Concentrated HCl and 2 mg of magnesium metal were added to the filtrate. A red color indicated the presence of flavonoids. The mixture was then cooled and amyl alcohol was added, followed by shaking. A persistent red color confirmed the presence of flavonoids. (Andasari et al., 2020).

Triterpenoid

Three drops of the Liebermann-Burchard reagent (concentrated H_2SO_4 and glacial acetic acid) were applied to two milliliters of the sample solution. The development of a purple or red hue indicates a positive test for triterpenoids, whereas the development of a blue or green color indicates a positive test for steroids. (Rahayu et al., 2024).

Fenolic

A phytochemical screening for phenolic compounds was performed by treating the tampala bajakah extract with 1% ferric chloride solution. The development of a darker color relative to a pure extract served as a positive indication for the presence of phenolics.

Saponin

2 mL of the sample solution were taken and vigorously shaken. One drop of concentrated HCl was then added if foam appeared. The development of foam that lasted for 15 minutes and had a height of 1-3 cm indicated a positive saponin test. (Rahayu et al., 2024.

Total Phenol Content (TPC)

The Folin-Ciocalteu method, with gallic acid as a standard, is employed to quantify total phenolic content. This method relies on the reduction of phosphomolybdic-phosphotungstic acid to a blue-colored molybdenum-tungsten complex by phenolate ions in an alkaline environment (Grubesic et al., 2005). The intensity of the blue color, measured through absorbance readings, is directly proportional to the concentration of phenolic compounds, as higher concentrations lead to increased phenolate ion formation and a greater reduction of the Folin-Ciocalteu reagent (Wirasti, 2019). Gallic acid, a simple phenolic acid derived from hydroxybenzoic acid, serves as an appropriate standard for calibration (Kupina et al., 2019).

Total Flavonoid Content (TFC)

The total flavonoid content was assessed using a colorimetric assay with aluminum chloride (AlCl3). This method relies on the formation of a complex between AlCl3 and the carbonyl group at C-4 and the hydroxyl group at C-3 or C-5 of flavonoids and flavonols. Quercetin, a flavonol, was used as a standard, producing a purple color upon complexation with AlCl3 (Wirasti, 2019).

Antioxidant Analysis

Antioxidants are compounds that can donate electrons, protecting cells from oxidative damage. They neutralize harmful free radicals and reactive oxygen species (ROS) by donating electrons, thus preventing damage (Sayuti & Yenrina, 2015; Agustina, 2017).

The DPPH assay relies on the presence of stable, deep purple DPPH radicals, characterized by a centralized organic nitrogen (Dehpour et al., 2009).

When reduced by antioxidants, these radicals lose their color, turning yellow (Yahya & Nurrosyidah, 2020). The degree of color change is used to calculate the percentage inhibition of a substance against free radicals, which is then used to determine the IC50 value. The IC50 value represents the concentration of antioxidant needed to reduce the initial DPPH concentration by 50% (Prawitasari, 2019). The DPPH assay is favored for its simplicity, speed, and sensitivity in assessing the antioxidant activity of various compounds and plant extracts (Koleva et al., 2002).

METHODOLOGY

This research commenced with the dehydration of simplicia using a dehydrator instrument, followed by sequential polarity extraction utilizing the Ultrasound-Microwave Assisted Extraction (UAE-MAE) method. Subsequently, the ethyl acetate extract of Spatholobus littoralis Hassk underwent testing for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant activity.

RESEARCH RESULT

Phytochemical Screening

The results of phytochemical screening on the thick extract of Bajakah Tampala roots with ethyl acetate solvent can be seen in the table below:

Table 1. Phytochemical screening of *Spatholobus littoralis* Hassk root

Compound	Result	Color
Alkaloid	(+)	Red-Brown
Flavonoid	(+)	Chocolate
Triterpenoid	(+)	Green-blue
Fenolic	(+)	Green-black
Saponin	(+)	Persistent foam

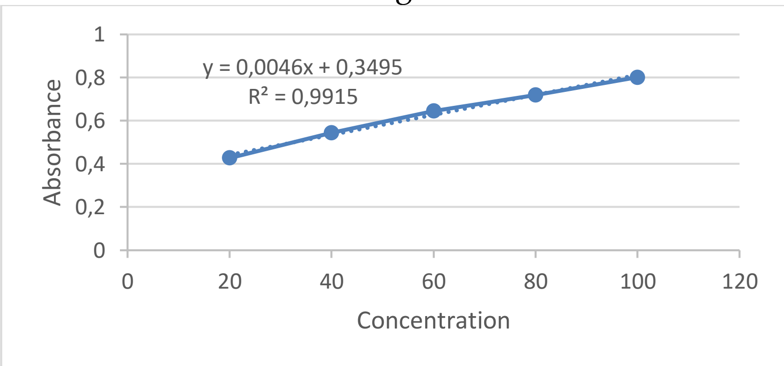
Total Phenol Content (TPC)

The total phenolic content of the tampala bajakah root extract was quantified using the Folin-Ciocalteu method, as described by Chun et al. (2003). This widely employed method is favored for its simplicity and relies on the reaction between phenolic compounds and the Folin-Ciocalteu reagent to produce a measurable colorimetric response.

Using UV-Visible spectrophotometry, the wavelength of the standard gallic acid solution was first measured within the 600–800 nm range before the total phenolic content was ascertained. A maximum wavelength of 766 nm was attained.

The absorbance of the standard gallic acid solution at various concentrations of 20, 40, 60, 80, and 100 µg/mL was measured at the previously obtained maximum wavelength. The absorbance measurements of the standard gallic acid solution were used to construct a calibration curve showing the relationship between concentration (C) and absorbance (A), resulting in a linear regression equation. The linear regression equation obtained was y = 0.0046x + 0.3495 with a correlation coefficient (r) of 0.9915, as shown in Figure 1

Figure 1. Graph of the relationship between concentration and absorbance of gallic acid solution



The Folin-Ciocalteu assay, employing gallic acid as a standard, was utilized to quantify phenolic content. This assay relies on the reaction between phenolic compounds and the Folin-Ciocalteu reagent under alkaline conditions (provided by Na2CO3), resulting in the formation of a blue-colored molybdenum-tungsten complex. The intensity of this color, measurable via spectrophotometry, is directly proportional to the concentration of phenolic compounds. This is attributed to the reduction of the phosphomolybdotungstate heteropoly acid by phenolic ions, yielding a more intense blue color with increasing phenolic concentration.

The total phenolic content in the bajakah tampala root extract was determined using a spectrophotometric method at 1000 ppm and measured at the maximum wavelength. As shown in Table 1, the total phenolic content was 145.152 mg gallic acid equivalents (GAE) per gram of extract. This high phenolic content is likely attributed to the use of ethyl acetate as the extraction solvent, which effectively extracts phenolic compounds due to its medium polarity, matching the polarity of the hydroxyl groups attached to the benzene rings of phenolic compounds (Naczk and Shahidi, 2004). A standard curve was used to calculate the total phenolic content based on the linear regression equation y = 0.0046x + 0.3495.

Table 2. Results of Determination of Total Phenolic Content in Bajakah Tampala Root Extract

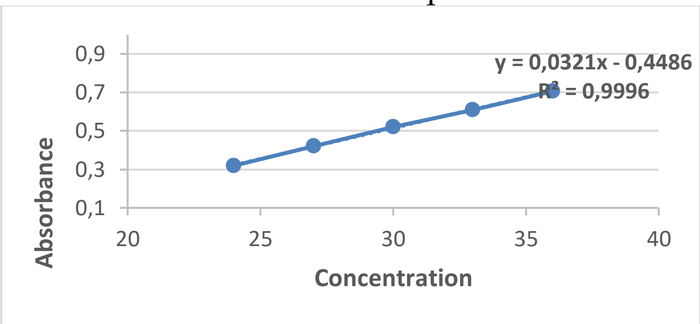
Sample	Concentration (μg/mL)	TPC (mgGAE/g)	SD	TPC ± SD (mgGAE/g)
Bajakah tampala root extract	1000	145,152	0,04	145,152 ± 0,04

Total Flavonoid Content (TFC)

Test of total flavonoid levels was carried out on the acetate extract of Bajakah Tampala roots using the UAE and MAE methods. Quercetin solution is used as a standard for testing total flavonoid levels. In this study, a standard solution of 50 µg/mL quercetin was measured at a wavelength of 400-500 nm using UV-Vis spectrophotometry and a maximum wavelength of 421 nm was obtained.

Total flavonoid content testing was carried out using the colometry method using $AlCl_3$ as a reagent. The results of measuring the quercetin calibration curve with concentrations of 24, 27, 30, 33, 36 µg/mL at a wavelength of 421 nm with an incubation time of 30 minutes can be seen in the table below:

Figure 2. Graph of the relationship between concentration and absorbance of quercetin



The total flavonoid content was determined spectrophotometrically at 421 nm using a quercetin calibration curve. The absorbance values were converted to quercetin equivalents (QE) using the linear regression equation derived from the standard curve. Each sample was analyzed in triplicate, and the average flavonoid content was calculated. The results of the flavonoid analysis are presented below.

Table 3. Results of Determination of Total Flavonoid Content in Bajakah Tampala Root Extract

Sample	Concentration (µg/mL)	TFC (mgQE/g)	SD	TFC ± SD (mgQE/g)
Bajakah tampala root extract	1000	26,85	0,059	26,85 ± 0,059

The total flavonoid content was calculated using the standard curve linear regression equation, namely y = 0.0321x + 0.4486 so that the average total flavonoid content in bajakah tampala root ethyl acetate extract was 26.85 mgQE/g, meaning that in every gram of root ethyl acetate extract Bajakah Tampala contains flavonoids equivalent to 26.85 mg of quercetin.

Antioxidant Analysis

This study investigated the antioxidant activity of ethyl acetate extract from Bajakah Tampala (Spatholobus littoralis Hassk) roots. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was employed, utilizing ascorbic acid as a standard.

Antioxidant activity was assessed using a UV-Vis spectrophotometer at a wavelength of 516 nm. The DPPH assay is based on the reduction of DPPH radicals by antioxidants, resulting in a color change from purple to yellow. The IC50 value, representing the concentration of extract required to inhibit 50% of DPPH radicals, was determined using a linear regression equation derived from a calibration curve of sample concentration versus percentage inhibition.

The linear regression equation obtained was $Y = 0.0028x + 0.2537$, with an R^2 value of 0.9931. The IC50 value was then calculated by substituting 50 for 'y' in the equation. All measurements were performed in triplicate. The results of the antioxidant activity test are presented below.

Table 4. Results Antioxidant activity Ethyl Acetate of Bajakah Tampala Root

Concentration (µg/mL)	(%) Inhibition	IC50 (µg/mL)	Category
10	2,58	43,18	Very Strong
20	3,17		
30	3,62		
40	4,10		
50	4,45		

From table 3 it can be seen that the % inhibition of antioxidant activity increases with increasing extract concentration. Percent inhibition is related to the absorbance obtained. The greater the absorbance of the sample, the smaller the percent inhibition obtained. Likewise with increases percent inhibition is caused by a decrease in sample absorbance. A decrease in the absorbance of the sample is caused by increasing the sample concentration of each plant extract. Thus it can be interpreted, the higher the concentration in the sample, the lower the absorbance and resulting percent inhibition which is getting higher. The smaller the absorbance means that this plant has strong antioxidant activity.

DISCUSSION

In the alkaloid test using Dragendorff's reagent, the nitrogen atom in the alkaloid forms a coordinate covalent bond with K^+, which is a metal ion, resulting in a precipitate. The results obtained from the extract of bajakah tampala root were positive for alkaloids.

Flavonoids are a group phenolic compounds which are tendency to bind proteins so it can disrupt metabolism bacteria. Positive extract contains flavonoids with the formation of precipitates chocolate. This is due to flavonoids has a benzene ring that has hydroxy groups that form precipitates chocolate.

The phenol test was conducted to confirm the presence of phenolic compounds in the root extract of Tampala bajakah. A positive result for the phenol test was indicated by a color reaction between the phenolic compounds and ferric chloride, forming a green, purple, or blue colored complex. The obtained result showed a dark green color, which is a positive indication of the presence of phenols.

Steroid and triterpenoid groups, which are nonpolar, and glycosyl groups, which are polar, are both found in saponins. Saponins can form micelles, where the polar groups face inside and the nonpolar groups face outward, when shaken with water because compounds with both polar and nonpolar groups will be surface active. In these circumstances, saponins will show up as foam.

From the test results it was found that the IC50 value of Bajakah Tampala root ethyl acetate extract was 43.18 μG/mL. This value is included in the very strong group because the IC50 value is less than 50 μG/mL
The choice of extraction method also greatly influences the process of extracting secondary metabolites from Bajakah Tamala roots. The combination method between ultrasonics and microwaves has a different mechanism from several existing methods.

Ultrasound-Microwave Assisted Extraction (UAE-MAE) is a modern extraction method that combines the power of ultrasonic waves and microwaves to increase the efficiency of extracting active compounds from natural ingredients. These two methods work with different but complementary mechanisms.

In the UAE method, ultrasonic waves produce small bubbles in the solvent which then collapse suddenly. The collapse of these bubbles creates very high pressures and temperatures locally, causing cell wall disintegration and increasing cell membrane permeability. Ultrasonic vibrations accelerate the movement of solvent and sample particles, thereby increasing contact between the solvent and the compound to be extracted. Meanwhile, in MAE, microwaves heat polar molecules in the sample directly and quickly. Water molecules in the sample will absorb microwave energy and produce heat. This heat causes an increase in temperature and pressure in the cells, thereby speeding up the extraction process. The increase in temperature due to microwaves increases the kinetic energy of molecules, thereby accelerating the process of compound diffusion from the sample matrix into the solvent.

The combination of UAE and MAE provides an excellent synergistic effect in the extraction process. Ultrasonic waves help break down cell walls and increase membrane permeability, while microwaves speed up the heating and diffusion processes. This method allows the extraction of active compounds in a shorter time using a smaller amount of solvent compared to conventional extraction methods. By adjusting operating parameters such as ultrasonic power, microwave power, extraction time, and solvent type, we can selectively optimize the extraction of target compounds.

CONCLUSIONS AND RECOMMENDATIONS

Based on the results and discussion, it can be concluded that secondary metabolite compounds are contained in the ethyl acetate extract of Bajakah Tampala roots are alkaloids, flavonoids, triterpenoids, phenolics and saponins. Bajakah tampala root ethyl acetate extract had total phenol content and total flavonoid content of 145,152 ± 0.04 mgGAE/g and 26.85 ± 0.059 mgQE/g. Bajakah

Tampala root ethyl acetate extract has an IC50 value of 43.18 μg/mL so it can be categorized as a very strong antioxidant.

FURTHER STUDY

This research was only conducted using ethyl acetate solvent and on the roots of the bajakah tampala plant, so this research can be continued by using solvents with different polarities and parts of the plant such as leaves and stems of the bajakah tampala plant.

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