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Research Article

Phytochemical Profile and Antioxidant Activity of Bajakah Kalalawit Leaf Extract

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ABSTRACT

Natural products have long been recognized for their significant role in supporting human health, with natural ingredients playing an essential part in disease treatment and wellness across generations. The bajakah species, particularly bajakah kalalawit (*Uncaria gambir* Roxb.), has gained attention in recent research due to its potential bioactive compounds. Especially, its antioxidant properties from any part of plant has potential to explore. This study aims to determine the various secondary metabolite compounds possessed by bajakah kalalawit leaves and test their antioxidants. Bajakah kalalawit leaves were extracted using reflux with aqueous solvents, checked for phytochemical profiles using GC-MS, and conducted antioxidant tests using the DPPH method. Based on the phytochemical test, there are 22 predicted compounds in the extract and the antioxidant test results showed an IC_{50} value of 14.44 ppm. In conclusion, bajakah kalalawit leaf aqueous extract has antioxidant activity and is supported by bioactive compounds.

Keywords: bajakah; reflux extraction; GC-MS; DPPH; bioactive compound

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INTRODUCTION

Natural products continue to be excellent in supporting various aspects of human health. From ancient times and for generations, natural ingredients cannot be separated from people's lives regarding healthiness and disease treatment. One of the natural ingredients that has become the focus of recent research and is widely researched is the bajakah species. Bajakah can be defined as a type of plant in the form of roots that grow in the forests of Kalimantan. Bajakah is widespread in areas such as Central Kalimantan, with hundreds of species that have not been comprehensively studied for their efficacy and bioactive compound content. Through testing the activity and content of bioactive compounds, scientific data will be obtained about secondary metabolite compounds responsible for pharmacological activity and the development of active compounds as pharmaceutical products (Twaij and Hasan 2022).

Bajakah species such as *Spatholobus suberectus* Dunn. have antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities (Zhang et al. 2022; Nguyen-Ngoc et al. 2022). One of the main activities possessed by natural materials is antioxidants, which have been widely studied using various approaches (Hunyadi 2019). Antioxidants are the initial gateway to supporting various other pharmacological activities. Many secondary metabolites from natural materials play a role in antioxidant mechanisms, both directly and indirectly, in efforts to treat degenerative diseases, and cancer, as well as in cosmetics (Hrelia and Angeloni 2020; Chaudhary et al. 2023).

Bajakah is one plant that is being widely studied for its antioxidant activity (Almeida et al. 2022). There are many utilizations of various types of bajakah as antioxidants (Amiani et al. 2022; Randan et al. 2023; Yudiane et al. 2023; Hartanti et al. 2022). The bajakah kalalawit (*Uncaria gambir* Roxb.) is one of the most abundant bajakah species in Sebangau National Park, Palangka Raya (Hastari and Octavianus 2021). Pharmacological activity research shows that bajakah kalalawit stem extract has potential as an antihypertensive (Devi et al. 2023). In another study, the stem and root extracts of bajakah kalalawit have antioxidant activity (Indriyah et al. 2023; Salsabila et al. 2023). Therefore, it is very challenging to investigate and further complement the various pharmacological activities of various parts of the bajakah kalalawit plant originating from Palangka Raya, especially the leaf extract, which has not been studied for its secondary metabolite compounds and antioxidant activity.

Based on the background and path of study on bajakah kalalawit, it is vital to investigate the pharmacological activity connected with the content of bioactive substances. This study utilizes bajakah kalalawit leaf extraction methods that have not been done and are based on the use of leaf extracts by local communities so that parts are used to explore secondary metabolite compounds and their antioxidant activity.

MATERIALS AND METHODS

Material

Bajakah kalalawit leaves (Palangka Raya, Indonesia), aquadest (PT. Brataco, Tangerang, Indonesia), ethanol pro analysis (PT. Merck Chemicals and Life Sciences, Jakarta, Indonesia), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (PT. Merck Chemicals and Life Sciences, Jakarta, Indonesia).

Method

Extraction and Phytochemistry Analysis

The leaves of the bajakah kalalawit plant were collected in Sebangau National Park, Palangka Raya. The bajakah kalalawit species was determined by the Botany Team from the Biology Department of Universitas Palangka Raya. The plant sample was extracted using the reflux method with aquadest (Gunawan et al. 2024). Furthermore, phytochemical testing was carried out using GC-MS instruments to see the secondary metabolite in bajakah kalalawit leaf water extract. Bajakah kalalawit extract was determined by Gas Chromatography (Agilent 6890 series) coupled with an HP-5MS column mass spectrometer at a column temperature of 50°C and was heated to 280°C at 10°C for 15 min. The helium was used as a carrier gas at 1.3 mL/min. The compounds of the extract were identified by matching their mass spectra and retention time with those obtained from NIST spectra libraries (NIST 2.0) and using literature data (Ezez et al. 2023; Nandika et al. 2019).

Antioxidant Analysis

Preparation of DPPH Solution

The DPPH compound weighed as much as 1.5 mg and was dissolved into 25 mL of ethanol p.a. to obtain a concentration of 0.15 mM, and the solution was covered with aluminum foil. A DPPH solution of 0.15 mM as much as 1 mL was put into a test tube, 1.5 mL of ethanol p.a. was added, and a vortex shaker was shaken until homogeneous. The solution was incubated for 30 minutes in a dark room. The solution was poured into a cuvette, and the absorbance value was measured using a UV-Vis spectrophotometer with a maximum wavelength of 516 nm. Modified method from Ezez et al. (2023) and Irawan et al. (2022).

Preparation of Sample Solution

The bajakah kalalawit extract, weighing as much as 5 mg, was dissolved into distilled water, as much as 50 mL, to obtain a concentration of 100 ppm. The sample solution was heated for ± 30 minutes at 30-50°C until completely

dissolved. Then, the solution was cooled in a cold water bath for about 10-15 minutes, and after cooling, it was put into a 50 mL volumetric flask.

The concentration variation of kalalawit bajakah extract is made by diluting each bajakah extract by taking as much as 1, 2, 3, 4, and 5 mL of 50 ppm bajakah extract using a volume pipette, then putting each into a 10 mL volumetric flask and adding distilled water to the limit mark. A solution of bajakah extract concentration variation of 5, 10, 15, 20, and 25 ppm was obtained (Ezez et al. 2023; Irawan et al. 2022).

Antioxidant Activity Testing

Sample solutions with concentrations of 5, 10, 15, 20, and 25 ppm were taken as much as 1 mL, put into a test tube, 0.5 mL of ethanol pro analysis, and 1 mL of 0.15 mM DPPH solution. The mixture was then shaken with a vortex shaker, covered with aluminum foil, and allowed to stand for 30 minutes. Absorbance readings using a UV-Vis spectrophotometer at a maximum wavelength of 516 nm. The absorbance value obtained was then calculated as % inhibition with the equation:

$$\%\ \text{inhibition} = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control}\ x\ 100\%$$

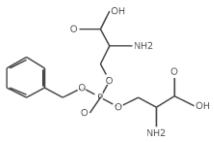
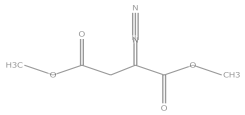
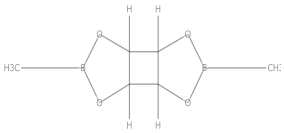
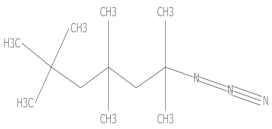
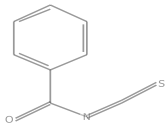
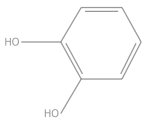
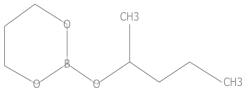
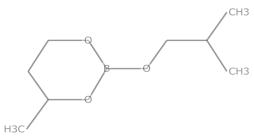
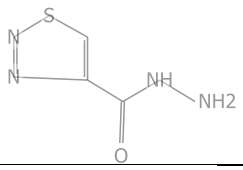
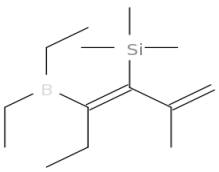
Antioxidant activity is determined from the inhibition concentration value of 50% (IC_{50}). Each known value of % inhibition and concentration is made a linear equation curve y = bx + a by replacing the y value with 50 from the linear line equation. Then, the x value, which is the IC_{50} value, will be obtained (Ezez et al. 2023; Irawan et al. 2022).

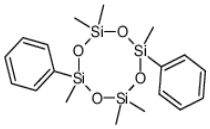
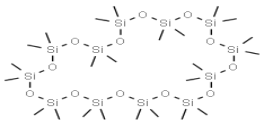
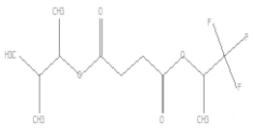
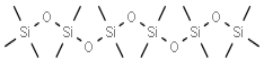
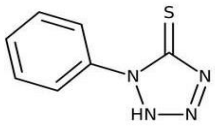
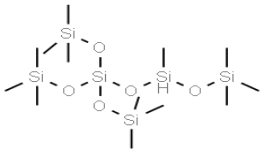
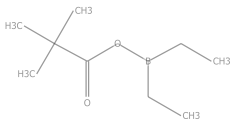
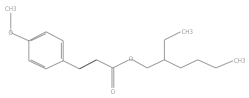
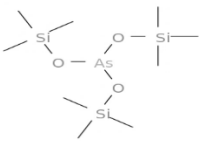
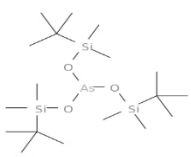
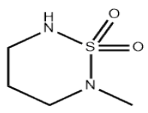
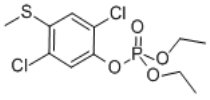
RESULTS

Phytochemistry Analysis

Based on the results of phytochemical testing using GC-MS, 33 peaks were obtained, including 22 compounds predicted using the NIST 2.0 library. This is in accordance with the phytochemical testing approach of *Uncaria gambir* using instruments by previous studies data (Ezez et al. 2023; Nandika et al. 2019). The phytochemical profile of the extracts showed a wide variation in chemical identity and structure. The results of GC-MS analysis of bajakah kalalawit leaf extract can be seen in Table 1.

Table 1. GC-MS Analysis for Phytochemistry Profile of Extract

No.	RT	Area (%)	Molecule Prediction (NIST 2.0)	Structure	MW (g/mol)
1	3.705	1.23	Benzyl-diseryl phosphate		362.27
2	4.752	0.8	Butanedioic acid, diazo-, dimethyl ester		172.14
3	5.805	1.09	Cyclobutane, 1,2:3,4-di-O-ethylboranediyl-		195.8
4	**6.995**	**23,42**	**2-Azido-2,4,4,6,6-pentamethylheptane**		**211.35**
5	8.655	0.57	Benzoyl isothocyanate		163.19
6	**10.154**	**1.53**	**Catechol**		**110.11**
7	12.975	1.24	1,3,2-Dioxaborinane, 2-(1-methylbutoxy)-		172.03
8	14.52	0.8	2-Isobutoxy-4-methyl-1,3,2-dioxaborinane		172.03
9	**17.232**	**8.99**	**1,2,3-Thiadiazole-4-carbohydrazide**		**144.16**
10	17.707	0.95	1,3-Hexadiene, 4-diethylboryl-3-trimethylsilyl		236.278

No.	RT	Area (%)	Molecule Prediction (NIST 2.0)	Structure	MW (g/mol)
11	18.365	5.23	2,2,4,6,6,8-Hexamethyl-4,8-diphenylcyclotetrasiloxane		420.755
12	19.487	0.91	Tetracosamethyl-cyclododecasiloxane		889.8
13	19.801	0.82	Succinic acid, 1,1,1-trifluoroprop-2-yl 3-methylbut-2-yl ester		284.27
14	20.934	0.46	Hexasiloxane, tetradecamethyl-		458.99
15	21.352	0.79	1H-tetrazole-5-thiol, 1-phenyl		178.21
16	22.262	0.4	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane		443.96
17	22.422	0.44	Propanoic acid, 2,2-dimethyl, anhydride diethyl borinic acid (Diethylboryl Pivalate)		170.06
18	**22.702**	**2.3**	**2-Ethylhexyl 4-methoxycinnamate**		**290.4**
19	22.874	0.61	Arsenous acid, tris (trimethylsilyl) ester		342.49
20	24.533	1,49	Tris(tert-butyldimethylsilyloxy)arsane		468.73
21	26.422	4.82	2-Methyl-1,2,6-thiadiazinane 1,1-dioxide		150.2
22	26.92	0.81	Chlorothiophos O-analog 2,5-isomer		345.18

Antioxidant Analysis

The bajakah kalalawit leaf extract was tested as an antioxidant using the DPPH method by analyzing the results of the IC_{50} value and the category of antioxidant activity. The results of the analysis in Table 2 show the inhibition of oxidation as seen from the increase in extract concentration, making the inhibition value higher. In addition, the calculation of the IC_{50} value is included in the small value, and the ability of a small concentration can provide as much as 50 percent inhibition.

Table 2. Antioxidant activity of leaf extract

Concentration (ppm)	Absorbances	Percentage of Inhibition ± SD	IC_{50} ± SD (ppm)
Control	0.670	0.00	**14.44** ± 0.07
5	0.583	13.06 ± 0.23	
10	0.409	39.03 ± 0.77	
15	0.289	56.87 ± 0.57	
20	0.228	65.97 ± 0.22	
25	0.106	84.25 ± 0.22	

DISCUSSION

We identified and explored the compounds contained in bajakah kalalawit leaves through phytochemical analysis. Based on the solvent used in the extraction, polar compounds can be extracted and identified through GC-MS instruments. The data in Table 1 shows the presence of several compounds that have dominant abundances, such as 2-Azido-2,4,4,6,6-pentamethylheptane (23.43%) and 1,2,3-Thiadiazole-4-carbohydrazide (8.99%). Furthermore, other compounds from bajakah kalalawit were identified as having potential as bioactive compounds mainly responsible for antioxidant activity.

Antioxidant analysis is the ability of bajakah kalalawit leaf extract to inhibit oxidation while confirming the presence of antioxidant bioactive compounds. Antioxidants are compounds that can inhibit the oxidative process. Antioxidant activity categories are based on IC_{50} values with values < 50 ppm (very strong), 50-100 ppm (strong), 101-150 ppm (moderate), and >150 ppm (weak) (Irawan et al. 2022). The ability of the extract to inhibit oxidation in Table 2 shows IC_{50} values < 50 ppm as very strong activity. This cannot be separated from the role of secondary metabolites present in bajakah kalalawit leaf extract. Derivative compounds of 1,2,3-Thiadiazoles show potential as antioxidants such as 1,2,3-Thiadiazole-4-carbohydrazide (Irfan et al. 2021). The compound isolate 2-ethylhexyl 4-methoxycinnamate (OMC) is known to have been used as a sunscreen mixture (Damiani et al. 2006) and responsible for antioxidant activity (Rachman et al. 2021). Other compounds such as cathecol also have antioxidant activity (Justino et al. 2006; Nuñez-Figueredo et al. 2018; Kim et al. 2020; Smolyaninov et al. 2022; De-kui et al. 2022).

Based on the results of the literature search and review of several kinds of literature on each compound molecule, it can be predicted that the compounds are responsible for the antioxidant activity of bajakah kalalawit leaf extract. Bajakah is one of the plants being widely studied for its antioxidant activity. In addition, the phytochemical profile data of bajakah kalalawit can be used as developed for the prediction of pharmacological activity through in silico tests and increase the potential for other pharmacological activities.

CONCLUSIONS

The results of phytochemical profile analysis showed that bajakah kalalawit leaf water extract contained 22 chemical compounds and had antioxidant activity in a very strong category. Furthermore, this research can be a guide in the search for other pharmacological activities, isolation of bioactive compounds, and molecular mechanism approaches.

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