EXPLORATION OF BIOACTIVE COMPOUNDS OF RAMBUSA (*Passiflora foetida L.*) ROOT EXTRACT FROM EAST KALIMANTAN COAL RECLAMATION LAND AS ANTIOXIDANT

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Abstract

The rambusa plant (*Passiflora foetida L.*) is a species utilized as a cover crop due to its capacity to undergo a natural regeneration process in the context of former coal mining operations in East Kalimantan. Rambusa plants have many phytochemical properties, one of which is as an antioxidant. Nevertheless, there is currently a paucity of scientific data regarding the potential antioxidant properties of bioactive compounds in Rambusa roots utilized as cover crop plants. The objective of this study is to investigate the composition of bioactive compounds in the root extract of Rambusa plants that are cultivated on land previously utilized for coal mining in East Kalimantan, with a particular focus on their antioxidant properties. In this study, the exploration was carried out by conducting phytochemical tests using three types of solvents, namely ethanol, ethyl acetate and n-hexane and antioxidant activity tests using the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) method on the extracted samples. Phytochemical screening results showed the presence of alkaloid, phenolic, flavonoid, terpenoid and saponin bioactive compounds in ethanol extract; alkaloid, phenolic and steroid bioactive compounds in ethyl acetate extract. At the same time, alkaloids and steroids are contained in the n-hexane extract. The DPPH test carried out on the three extracts showed very strong antioxidant activity with IC_{50} values respectively for ethanol, ethyl acetate and n-hexane extracts are 6.55 ppm, 3.51 ppm and 28.71 ppm. Based on previously reported antioxidant activity data, the antioxidant activity of Rambusa (*Passiflora foetida L.*) roots growing on coal reclamation land is proven to have much higher activity compared to Rambusa plants growing on fertile land. Thus, the root of the Rambusa plant (*Passiflora foetida L.*) has enormous potential as an antioxidant and natural medicinal raw material.

Keyword: *antioxidant, DPPH, Passiflora foetida L., reclamation*

Introduction

East Kalimantan is one of the largest coal-producing regions in Indonesia. In accordance with the aforementioned data, East Kalimantan is also the area with the largest reclaimed land in Indonesia. In 2022, the recorded coal reclamation area in East Kalimantan reached 10,869.5 hectares (Data KalTim, 2024). One of the reclamation activities undertaken is the revegetation of former coal mines with cover crop plants, with the objective of improving the soil content prior to the planting of woody species (Tampubolon *et al*., 2020). Cover crops are plants that are intentionally planted to protect soil from erosion, increase nutrient content and at the same time increase soil productivity on post-mining land. It is known that cover crop plants are pioneer and climax plants. This means that this type of plant can grow fast and has good

regeneration ability, has a root system that can symbiotic mutualism with certain types of fungi and bacteria and can grow continuously throughout the year (Yassir & Kumalaningsih, 2014; Yassir & Sitepu, 2014).

Rambusa (*Passiflora foetida L.*) is a plant species utilized as a cover crop on post-coal mine land in East Kalimantan. This plant is a type of liana that grows creeping and elongated and can grow in bushes and roadsides (Yassir & Sitepu, 2014). *Passiflora foetida L.* is a species from the genus *Passiflora*, which has around 600 flowering plants, of which 100 species are known to have edible fruits. In addition, the plant is an important component of traditional folk medicine in many regions. Few studies have been performed on the bioactive, pharmacological, and other properties of *Passiflora foetida L.* Several studies have found that leaf, fruit and stem extracts from Rambusa plants have several activities such as antioxidant, antibacterial, antiviral, antidiabetic and anticholesterol with the type of phytochemical content including alkaloid, flavonoid, polyphenol and steroid groups (Mulyani, 2019; Olla *et al*., 2020; Sari & Puspitasari, 2021; Chiavaroli *et al*., 2020; Dharmasiri *et al*., 2024; Fadeyi & Akiode, 2022; Song *et al*., 2020).

However, there is still not much scientific information about the potential content of bioactive compounds in the roots of Rambusa plants, both those growing on fertile land and those used as cover crop plants. The extreme habitat of cover crop Rambusa plants, allows the roots of these plants to contain different bioactive compounds, compared to Rambusa plants that grow naturally in fertile soil. Its ability to survive in extreme conditions on post-mining land, makes the roots of Rambusa plants a source of high antioxidant bioactive compounds and has the potential to become a promising medicinal raw material (Wardani *et al*., 2021). Plants possess antioxidant compounds to protect against external and internal stress. Some studies show that to adapt to extreme environments, some bioactive compounds in plants will be released in large quantities to deal with environmental stress (Chen *et al*., 2022; García-Caparrós *et al*., 2021; Pratyusha, 2022; Zahan Akhi *et al*., 2021). Therefore, it is necessary to explore the content of bioactive compounds in the root extract of Rambusa plants that grow on coal reclamation land and their activity as antioxidants.

Research Methods

Materials

The materials used in this study were the roots of Rambusa (*Passiflora foetida L.*) plants obtained from PT Ganda Alam Makmur coal mine reclamation land, East Kalimantan. Root samples have been determined by the Environmental and Forestry Instrument Standards Implementation Centre Samboja, East Kalimantan. The other materials were ethanol, n-hexane, ethyl acetate (Merck, Germany), reagents for the Dragendorff, Meyer and Wagner tests, reagents for the Lieberman-Burchard test, reagents for the Forth Test, Mg powder and HCl (Merck, Germany) for flavonoid tests and $FeCl_3$ (Merck, Germany) for phenolic tests and *1,1-diphenyl-2-picrylhydrazyl* (DPPH) (Sigma Aldrich) for antioxidant activity tests.

Procedure

Preparation of extracts

The root samples were washed and air-dried for 10 days. The next stage is the extraction of bioactive compounds using a single maceration method, using three different types of solvents, namely methanol, ethyl acetate and n-hexane which have polarity respectively polar, semi-polar and non-polar. It is, therefore, anticipated that all types of bioactive compounds present in the sample can be investigated. The results of maceration in the form of a solution are then filtered so

that the filtrate and residue are obtained. The filtrate obtained is then evaporated until the solvent separates from the extract and produces a thick extract.

Phytochemical screening

Phytochemical screening is one way to determine the content of bioactive compounds in a sample. Phytochemical screening carried out in this study includes alkaloid, flavonoid, phenolic, saponin, terpenoid and steroid tests.

Alkaloid test

Each thick ethanol, ethyl acetate and n-hexane extract was put in a test tube and tested for alkaloids using three different tests, namely the Mayer test, Dragendorff test and Wagner test. Positive results are indicated by a color change to white to yellowish for the Mayer test, reddish-orange for the Dragendorff test and brown precipitate for the Wagner test (Peni Pindan *et al*., 2021).

Flavonoid test

Each thick ethanol, ethyl acetate and n-hexane extract was put in a test tube, dissolved with the appropriate solvent and then added Mg powder and HCl solution. Positive results are indicated by the appearance of red, yellow and orange colors (Peni Pindan *et al*., 2021).

Phenolic test

Each thick ethanol, ethyl acetate and n-hexane extract was put in a test tube then added with $FeCl_3$ solution and boiled. Positive results are indicated by the appearance of green to black color (Peni Pindan *et al*., 2021).

Saponin test

Each thick ethanol, ethyl acetate and n-hexane extract was put in a test tube and tested for saponins using the Forth test. Positive results are indicated by the production of foam that remains in the test solution (Peni Pindan *et al*., 2021).

Terpenoid and steroid test

Each thick ethanol, ethyl acetate and n-hexane extract was put in a test tube and tested for Terpenoids and Steroids using the Lieberman-Burchard test. Positive results are indicated by the appearance of blue to green colour for steroids and red to purple colour for terpenoids (Peni Pindan *et al*., 2021).

Antioxidant activity test using DPPH method

Antioxidant activity test using *1-diphenyl-2-picrylhydrazyl* (DPPH) method was conducted by weighing 10 mg of each extract, then dissolved to various concentrations (2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm). Then each added 3.5 mL of DPPH. Next, it was vortexed and incubated at 37^{o}C. Absorbance was measured with a wavelength of 517 nm. Ascorbic acid was used as a comparator and positive control. A commonly used parameter to interpret the DPPH assay results is the IC_{50} value. The value indicates the concentration of the sample required to reduce the DPPH free radical activity by 50% (Rohmah *et al*., 2020; Gazali *et al*., 2019).

Data analysis

Data are expressed as mean ± SD on sample measurements. Calculation of the inhibition concentration value (IC_{50}) was obtained through a linear regression curve equation between % inhibition (y-axis) and extract concentration (x-axis) using a program on Microsoft Excel (Rohmah *et al*., 2020; Gazali *et al*., 2019).

Results and Discussion

The roots of Rambusa (*Passiflora foetida L.*) used in this study were obtained from the coal reclamation land of PT. Ganda Alam Makmur located in the Kaubun sub-district, East Kalimantan. The roots used consisted of taproots and fibrous roots attached to the tendrils of the Rambusa plant, as shown in Figure 1.

Figure 1. Rambusa (*Passiflora foetida L.*) plants as cover crop and root sample overview

Phytochemical screening

Phytochemical screening was conducted to explore the content of bioactive compounds in the roots of the Rambusa plant (*Passiflora foetida L.*) using three types of solvents with different polarity properties. The results of phytochemical screening on ethanol, ethyl acetate and n-hexane extracts showed that ethanol extracts contained alkaloid, phenolic, flavonoid, terpenoid and saponin bioactive compounds. This shows similarities in the content of bioactive compounds in methanol extracts of stems, leaves, flowers and fruit, except that the fruit does not contain saponins (Wardhani & Pardede, 2022a). The ethyl acetate extract contains bioactive compounds of alkaloids, phenolics and steroids, while the n-hexane extract contains alkaloids and steroids. The content of bioactive compounds of the three root extracts of the Rambusa plant is shown in Table 1.

Table 1. Phytochemical test results

Type of Test	Ethanol Extract	Ethyl Acetate Extract	n-Hexane Extract
Alkaloid			
1. Mayer	+	+	+
2. Dragendorff	+	+	+
3. Wagner	+	+	+
Phenolic	+	+	-
Flavonoid	+	-	-
Terpenoid	+	-	-
Steroids	-	+	+
Saponin	+	-	-

Based on the results of phytochemical screening in table 1, it is known that ethanol, ethyl acetate and n-hexane extracts of Rambusa plant roots contain different bioactive compounds. This is due to differences in polarity in each solvent used. The ethanol extract exhibited the widest range of bioactive compounds, including alkaloids, phenolics, flavonoids, terpenoids, and saponins. This broader spectrum reflects its ability to dissolve both polar and moderately non-polar compounds. The ethyl acetate extracts also contained alkaloids, phenolics, and steroids, indicating its intermediate polarity, which extracts medium-polarity bioactive compounds. The n-hexane extract was

more selective, primarily isolating alkaloids and steroids, which are generally non-polar.

Comparatively, a study on *Passiflora foetida L.* leaves found that leaves contain higher concentrations of total phenols and flavonoids, contributing to their stronger antioxidant properties. This difference may suggest that different parts of the plant produce unique phytochemicals depending on their biological roles.

Antioxidant activity test using DPPH method

Bioactive compounds that have antioxidant activity help the body overcome oxidative damage caused by free radicals. Free radicals are known to be a major factor in biological damage to living things. Thus, these compounds can help prevent various types of diseases. Terdapat beberapa metode yang dapat dilakukan untuk melihat kemampuan menghambat radikal bebas dari sediaan bahan alam, antara lain DPPH, FIC, FRAP, and ABTS (Rohmah, 2022). To test the antioxidant activity of ethanol, ethyl acetate and n-hexane extracts of Rambusa (*Passiflora foetida L.*) roots, a DPPH radical scavenging reaction was conducted. Purple free radical molecules can turn into stable yellow compounds when interacting with natural antioxidants. This is because compounds that have antioxidant activity give one electron to DPPH, thus causing free radical silencing. The antioxidant activity of the three extracts was analyzed using a UV-Vis spectrophotometer using a wavelength of 517 nm.

The results of DPPH silencing analysis by UV-Vis spectrophotometer for ethanol extract of Rambusa roots with Ascorbic acid as the comparative antioxidant are shown in Table 2. The results of DPPH silencing analysis by UV-Vis spectrophotometer for ethyl acetate extract of Rambusa roots with Ascorbic acid as the comparative antioxidant are shown in Table 3. The results of DPPH silencing analysis by UV-Vis spectrophotometer for n-hexane extract of Rambusa roots with Ascorbic acid as the comparative antioxidant are shown in Table 4.

Table 2. Antioxidant test results of ethanol extracts

x (ppm)	y (% Inhibition)	% Inhibition Ascorbic Acid
2	30.19	24.75
4	36.13	41.089
6	47.02	45.04
8	52.97	48.51
10	55.44	52.97

Table 3. Antioxidant test results of ethyl acetate extract

x (ppm)	y (% Inhibition)	% Inhibition Ascorbic Acid
2	45.54	24.75
4	51.98	41.089
6	58.41	45.04
8	73.76	48.51
10	93.56	52.97

Table 4. Antioxidant test results of n-hexane extract

x (ppm)	y (% Inhibition)	% Inhibition Ascorbic Acid
2	13.86	24.75
4	20.79	41.089
6	23.76	45.04
8	27.22	48.51
10	28.71	52.97

Based on the linear regression equation in each graph above, the comparison of each IC_{50} value describing the ability of each extract as an antioxidant is obtained as presented in Table 5. According to Molyneux, IC_{50} is the concentration of substrate solution or sample that can reduce DPPH activity by 50%. The smaller the IC_{50} value, the higher the antioxidant activity. Specifically, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 ppm (IC_{50} < 50 ppm), strong (50 ppm < IC_{50} < 100 ppm), moderate (100 ppm < IC_{50} < 150 ppm), weak (150 ppm < IC_{50} < 200 ppm), and very weak (IC_{50} > 200 ppm). Based on the results shown in Table 5, the greatest antioxidant activity respectively is the ethyl acetate extract, ethanol then n-hexane which is 6.55 ppm, 3.51 ppm and 28.71 ppm. Where for ethyl acetate and ethanol extracts have antioxidant activity that is classified as very strong even stronger than Ascorbic acid which is 8.357 ppm. Although still far below the strength of Ascorbic acid antioxidant activity, the n-hexane extract of Rambusa plant roots is still classified as a very strong antioxidant, because it has an IC_{50} of less than 50 ppm.

Several studies on the antioxidant activity of Rambusa (*Passiflora foetida L.*) plants growing in Indonesia have been reported previously. Some of the antioxidant activity studies were conducted on stems, leaves, fruits and flowers, and the antioxidant activity is shown in Table 6.

Table 5. IC_{50} values of Rambusa root extract and comparator compounds

Sample	IC_{50} (ppm)
Ethanol extract	6.55
Ethyl acetate extract	3.51
n-hexane extract	28.71
Ascorbic acid	8.357

Based on previously reported antioxidant activity data, the antioxidant activity of Rambusa (*Passiflora foetida L.*) roots growing on coal reclamation land is proven to have much higher activity compared to Rambusa plants growing on fertile land. This is due to the plant's ability to produce bioactive compounds in large quantities as a defence system in extreme growing conditions. The antioxidant properties of Rambusa root extract are largely attributed to the synergistic effects of its phytochemicals. These compounds work together to neutralize free radicals, which are responsible for oxidative stress. The presence of flavonoids, alkaloids, and phenolic compounds makes it an excellent candidate for further research and application in health supplements. The synergistic effects of these compounds enhance their antioxidant capacity, offering a promising avenue for preventing oxidative stress-related diseases. As interest in natural antioxidants continues to grow, Rambusa root extract stands out as a valuable botanical resource for promoting health and longevity.

Table 6. Antioxidant activity of Rambusa plant

Type of Extract	IC_{50} (ppm)
Flower (Rendowaty, 2024)	
Ethanol extract	71.22
Ethyl acetate extract	77.31
n-Hexane extract	72.31
Leaf (Triadisti, 2023)	
Methanol extract	97.453
Ethyl acetate extract	206.398
n-Hexane extract	129.035
Stem (Wardhani, 2022)	
Methanol extract	2581.932
Leaf (Wardhani, 2022)	
Methanol extract	349.5734
Fruit (Wardhani, 2022)	
Methanol extract	100.0751
Leaf (Fadillah, 2017)	
Methanol extract	237.68
Ethyl acetate extract	648.912
n-Hexane extract	105.840

Conclusions

The root of the Rambusa plant (*Passiflora foetida L.*) has been identified as a promising source of antioxidants, with the potential to be utilized as a raw material for pharmaceutical applications. This is indicated by the IC_{50} values of the three extracts, which were tested in ethanol, ethyl acetate, and n-hexanes. The results demonstrated that the ethanol and ethyl acetate extracts exhibited significantly higher antioxidant activity than Ascorbic acid or vitamin C.

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