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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT OF GALOBA DURIAN FRUIT PEEL (*Ammomum spp.*)

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Abstract

Background: Amomum spp. (red variant), An endemic plant widely found in Maluku, including Hatu Village, Ambon, is valued for its medicinal and economic roles.

Objective: This study aimed to evaluate the phytochemical content and antioxidant activity of Ammomum peel extract using the DPPH assay.

Methods: The fruit peel of Ammomum spp. (red variant) was extracted by maceration using ethanol as solvent and concentrated by evaporation at room temperature. The antioxidant activity was quantitatively and qualitatively assayed using the DPPH assay method and by observing the color change in the extract. Percent inhibition data were analyzed using linear regression.

Results: The analytical findings confirmed the presence of flavonoids, saponins, tannins, steroids, and phenolics as secondary metabolite content in the galoba fruit peel extract. The free radical scavenging activity exhibited a strong value of 59.04 µg/mL. Therefore, this study suggests using the red variant of Ammomum spp. fruit peel extract in the treatment of free radical-induced disorders.

Conclusions: Our study revealed that the peel extract of red Galoba durian contained secondary metabolites and strong antioxidant activity.

Cite this Article

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INTRODUCTION

Galoba durian is an endemic plant of Maluku. Consisting of two varieties based on the color of the fruit, namely red and green Galoba durian. The distribution of this plant in Ambon can be found in several places, such as in the highlands and coastal areas. Galoba durian belongs to the Zingiberaceae family genus Ammomum, comparising of 150 species (1,2). This plant is characterized by rhizomes close to the surface of the soil, a pseudostem, a leafy shoot, a whole ligule with a relatively short length, a petiole length that develops to a small size, and a lamina that is linear to narrowly oblong (3). The community has long known and utilized Ammomum spp. due to its potential therapeutic and culinary value in traditional practices. Research on the phytochemistry and pharmacology of Ammomum has been conducted, focusing on its secondary metabolite compounds, including volatile oils and non-volatile compounds, which have attracted significant attention for further study (4).

Many secondary metabolite compounds have been isolated from roots, aerial parts, seeds, fruits, and rhizomes of Ammomum, including benzaldehyde, cycloterpenaldehyde, diarylheptanoid, diterpenoid, flavonoid, monoterpenoid, phenylpropanoid, sesquiterpenoid, steroid, and other chemical groups. Flavonoids and diterpenoids are two of the most common compound components contained in Ammomum species (5). Flavonoids were successfully isolated from the fruits, while terpenoids were found in the roots, fruits, and rhizomes (6).

The secondary metabolite compounds of Ammomum species have been demonstrated to possess medicinal properties. The seed extract of Ammomum subulatum exhibited the highest antioxidant activity compared to the seed extract of Ammomum xanthoides, and had the highest antibacterial activity against Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter aerogenes, and Salmonella typhimurium (7). Some research indicates that the ethanol extract of Ammomum muricarpum rhizome showed high DPPH radical scavenging activities (8), and galoba fruit extract (Ammomum spp.) has a significant antioxidant activity effect, where this extract can reduce malondialdehyde (MDA) levels in serum hyperglycemia mice induced by streptozotocin (9). Moreover, extracts from Ammomum villosum seeds have a high content of phenolic compounds, including vanillic acid, catechin, epicatechin, protocatechuic acid, quercetin, and quercitrin (10). This extract represents excellent antitumor and antioxidant activity and can serve as the source of antioxidants and new antitumor drug candidates. The novelty of this research on the red variant of Ammomum spp. fruit has not been widely studied. Therefore, this study was conducted to evaluate the phytochemical content and antioxidant activity of Ammomum peel extract using the DPPH assay.

MATERIALS AND METHODS

Materials

The plant materials used, are red galoba durian collected from Hatu village (-3.6574 LS dan 128.04727 BT, and the chemical materials were distilled water, 96% ethanol Merck 1.2500, concentrated hydrochloric acid (HCl) Merck, Magnesium powder Merck, chloroform SmartLab, glacial acetic acid Merck 1.00063.2500, concentrated sulfuric acid (H2SO4) Merck 1.00731.2500, ammonia (NH3) Merck 105432, Dragendorf Merck, 2,2-diphenyl-1-picrylhydrazyl (DPPH) Sigma Aldrich, sodium chloride (NaCl) SmartLab, ferric

chloride (FeCl3) 10% Merck, Wagner's reagent Nitra Kimia, NaCl Merck, methanol Merck, and Vitamin C Merck 1. 00468.0500

Method

This study employed quantitative and qualitative experimental research methods, which consisted of: Fruit Peel Extract: The dried red galoba durian fruit peels was blended until smooth, 250 g was put into 750 ml of 96% ethanol, and macerated for one day. Maceration products were rotavaporated for two hours at a temperature of 45 °C.

Flavonoid Assay: A total of 2 mL of fruit peel extract was added to 0.1 mg of Mg powder, then HCl was added until a color change occurred. The formation of orange, yellow, and brick red colors indicates the presence of flavonoid content in the extract (11). Tannin and Polyphenol Assay: A total of 1 ml of solution of red galoba durian fruit peel extract is reacted with FeCl3 10%. Dark, blue, or greenish black color occurred, indicating the presence of tannins and polyphenols content (12). Saponin Assay: A total of 2 mL of red global durian fruit peel extract is put in a test tube, and 2 mL of distilled water is added and then shaken. Observed foam that persisted for 10 minutes indicates the saponin content (13)

Steroid Assay: A total of 2 mL of red galoba fruit peel extract was mixed with 2 mL of concentrated sulfuric acid (H2SO4). Steroid content is indicated by the formation of green or blue-green color (14).

Alkaloid Assay: A total of 2 mL red galoba fruit peel extract and 2 M HCl was added and heated while stirring, then cooled to room temperature. NaCl powder was added while stirred and reacted with 1-3 drops of Wagner reagent. The formation of a brownish-red precipitate indicates that the test sample contains alkaloid compounds (15).

Antioxidant Assay: A total of 40 g of DPPH solid was dissolved into 100 mL of methanol to obtain a concentration of 40 ppm DPPH (stored at room temperature in dark conditions). Determination of the wavelength is done by measuring the absorbance of 40 ppm DPPH solution at a wavelength of 400 - 800 nm using a UV-Vis spectrophotometer Apel (12,16).

Vitamin C was used as a comparison solution made with concentrations of 2, 4, 6, 8, and 10 µg/mL, respectively. Then, 1 mL of vitamin C solution was added to each test tube. Then, 100 µg/mL DPPH solution and methanol, as well as 3 mL, were added to each test tube. The mixture of solutions was shaken until homogeneous and incubated in the dark for 30 minutes. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of Apel 515 nm (17).

Determination of antioxidant activity was carried out by taking 1 mL of thick extract of red galoba fruit peel and preparing concentrations of 20, 40, 60, 80, and 100 µg/mL. Each 1 mL of extract solution from different concentrations was mixed with 1 mL of 100 µg/mL DPPH solution. Then, diluted with methanol to a total volume of 5 mL. The solution was incubated at room temperature in the dark for 30 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer Apel at a wavelength of 515 nm. The antioxidant activity of the extract was expressed as percent inhibition calculated by the formula:

% Inhibition = (Ao-Ai)/Ao x 100% (18)

Description:

I: Percentage of inhibition or inhibitory power (%)

Ao: Absorbance Blanko (Solvent + DPPH)

Ai: Sample Absorbance (Solvent + DPPH + Sample)

Data from the inhibition percentage and the absorbance measurement were then used to calculate the linear regression equation with the following formula:

Y = bx + a (18)
Description:
Y: sample absorbance
a: intercept (intersection)
b: slope
x: concentration (µg/mL).

RESULTS AND DISCUSSIONS

Extraction from Galoba durian fruit peel produced a thick extract of 0.83 grams. The fruit peel extract was further analyzed to determine the contained antioxidant compounds. The phytochemical profile of Galoba durian fruit peel antioxidants is shown in Table 1 and Figure 1.

In this study, we found that the peel extract of Galoba durian fruit contained secondary metabolite compounds such as flavonoids, saponins, tannins, steroids, and phenolics not found. In general, medicinal plants from the Zingiberaceae family contain secondary metabolite compounds like antioxidants (19). This was also reported by (20) that galoba fruit contains flavonoid, quinone, monoterpene, and sesquiterpene compounds, and has strong antioxidant activity (23.43 µg/mL). Antioxidants are secondary metabolites produced by plants as a defense against abiotic stress, by reducing the activity of free radicals that can damage the structure of plant cells, inhibit lipid peroxidation, and chelate metal ions (21–23). Antioxidants are also well known for their ability to prevent cell damage, thereby reducing the risk of various chronic diseases such as cancer (24).

Table 1. Phytochemical profile of the red variant of galoba durian peel extract

Target compounds	Presence in the extract	Color
Flavonoids	+	Brick red
Saponins	+	Oranges with foam
Tannin	+	Greenish black
Steroids	+	Green
Phenolic	-	Black

Description: (+) Positive: There are secondary metaboliteu compounds
(-) Negative: There are no secondary metabolite compounds

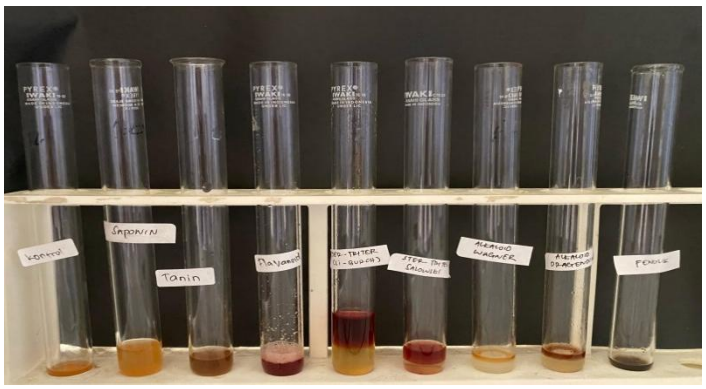

Figure 1. Visualization of color changes as an indicator of secondary metabolite content during the DPPH assay

The DPPH assay revealed antioxidant activity in the peel extract of Galoba durian. The absorbance values obtained from the DPPH assay at various sample concentrations, measured in triplicate. The average absorbance was used to calculate the percentage of DPPH radical inhibition, which reflects the antioxidant activity of the sample (Table 2). These data serve as the basis for the subsequent simple linear regression analysis to determine the relationship between sample concentration and % inhibition (Figure 2). The antioxidant activity is shown by the IC50 value. The results show that the peel extract of Galoba durian has an IC50 value of 59.04 µg/mL. IC50 value between 50-100 µg/mL exhibits strong antioxidant activity (25,26). Therefore, it can be concluded that the compounds found in the peel extract of Galoba durian have strong antioxidant activity.

Table 2. Percentage of DPPH Radical Inhibition at Various Sample Concentrations

Concentration (ppm)	Replicate			Average	% Inhibition
	1	2	3		
20	0.336	0.334	0.307	0.3257	22.09
40	0.331	0.249	0.294	0.2913	30.30
60	0.215	0.235	0.207	0.2190	47.61
80	0.189	0.147	0.136	0.1573	62.36
100	0.172	0.114	0.107	0.1310	68.66

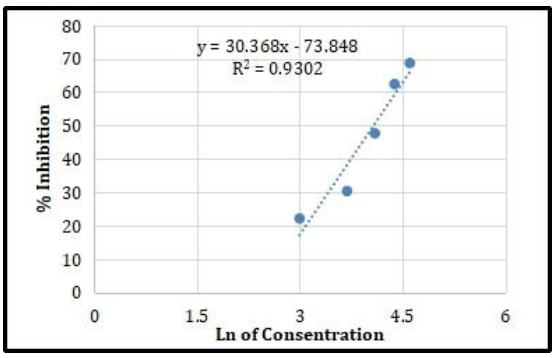

Figure 2. Antioxidant activity Galoba graph of durian fruit skin extract

The Zingiberaceae family, such as cardamom, turmeric, and ginger, have bioactive compounds with strong antioxidant activity (27). High antioxidant activity is caused by a high content of phenolic compounds. The addition of one type of Ammomum extract, such as cardamom, causes an increase in antioxidant activity in coffee extract (28,29). High antioxidant activity also functions as an anti-inflammatory, antimicrobial, and anticancer. Some research suggested that polyphenol compounds have anti-inflammatory effects that inhibit gene pathways activated by oxidative stress (30). Moreover, phenol compounds can act synergistically with other bioactive compounds as antimicrobials in breaking lipid bonds and lysing bacterial cell walls (31). Other work has also reported the antiaging and antibacterial activity against Escherichia coli (28,29).

CLINICAL IMPLICATION

The clinical implications of this study are as initial information about the secondary metabolite content of traditional plants that will be used as antioxidants and anti-inflammatories.

LIMITATIONS

The limitations of this study are that it does not use several solvents to extract samples so that it can compare which solvent can extract all secondary metabolite components from Ammomum spp, and does not use a larger number of samples to get more extracts.

CONCLUSIONS

This study revealed that the peel extract of red Galoba durian contains secondary metabolites and exhibits strong antioxidant activity. The data obtained in this study can be used as a potential database in the development of antioxidants as drug candidates from Galoba durian. The future development of this research is to use more accurate methods for the qualitative and quantitative content of secondary metabolites of Ammomum spp and whether it has the potential as an antibacterial and anti-inflammatory.

CONFLICT OF INTEREST

The research was conducted without commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

F.S. designed and conducted the research, analyzed and interpreted the data, and wrote the manuscript draft. B.S.M. conducted the research and wrote the draft of the manuscript. W. M designed the research, reviewed the manuscript draft, and supervised the process. K. L. P. designed and conducted the research, wrote the draft of the manuscript, reviewed the draft of the manuscript, and supervised the process. W. N. N and D. A. P. U conducted the research.

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