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Proximate and Phytochemical Analysis of the Ethanol Extract of *Abelmoschus manihot* Leaves Collected from Tinoor Dua Village, North Tomohon, North Sulawesi

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Abstract

The flowers of *Abelmoschus manihot* have been broadly studied in China for their phytochemical content and biological activities; however, the leaves have been explored only to a limited extent. In Manado, Sulawesi, Indonesia, the leaves of this plant are the most important ingredient in tinutuan porridge. The total phenols (TPC) and total flavonoids (TFC) in the leaves collected in Bogor, Manado, Tomohon, and Kotamobagu have been previously reported. This study aimed to provide data on the proximate and phytochemical composition (TPC, TFC, and quercetin levels) of the ethanol extract of *A. manihot* leaves collected from Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia. Extraction was carried out at room temperature using 70% ethanol. The phytochemical screening test was conducted according to a standard procedure for plant extracts. The proximate composition of the extract was analyzed in accordance with SNI 01-2891-1992. The TPC and TFC were determined by following the procedures outlined in SNI 01-2891-1992. Additionally, the TPC and TFC were also determined in accordance with SNI 01-2891-1992, and the procedures outlined in the Indonesian Herbal Pharmacopoeia were followed. Quercetin levels were analyzed using HPLC. Proximate analysis revealed 35.60% of total ash, 28.56% of carbohydrate, 18.31% of water, 11.01% of protein, and 6.53% of fat, whereas the TPC was 1350 mg GAE/100 g extract, the TFC was 2670 mg QE/100 g extract, and quercetin in the extract was 373.7 ± 0.18 mg/L. These findings will support further exploration for the development of *A. manihot* leaves as a nutritional food or nutraceutical supplement.

Keywords: abelmoschus, flavonoids, gedi leaves, malvaceae, nutritional food, polyphenols, proximate analysis, quercetin

Introduction

Abelmoschus manihot L. Figure 1, commonly called sunset muskmallow, is a tropical shrub native to Southeast Asia. Particularly in Manado, Sulawesi, Indonesia, this plant is known by the local name of *gedi*. The leaves of this plant are edible, and it

has been reported that people in Manado have folklorically utilized the leaves as the most important ingredient in tinutuan porridge.[1] *Tinutuan* porridge, a traditional culinary dish which consists of eight ingredients, such as *gedi* leaves, yellow pumpkin, potatoes, cassava leaves, spinach, basil, and lemongrass, was announced for its effects in preventing chronic energy deficiency and anemia in pregnant women, when consumed routinely for 30 days.[2]

Figure 1. *Abelmoschus manihot* leaves, collected at the Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia. This picture was taken by the first author

Interestingly, phytochemicals contained in the flowers of *A. manihot* have been reported in several articles,[3–11] whereas those in the leaves have been explored only to a limited extent.[12–16] The leaves of *A. manihot* collected in Nanjing, China, were confirmed to contain flavonoids, such as quercetin, rutin, myricetin, isoquercitrin, hyperoside, and hibifolin.[12] A TPC ranging from 370 mg GAE/100 g in the ethyl acetate extract to 3920 mg GAE/100 g in the 70% ethanol extract was reported to be found in the leaves collected in Bogor, Indonesia,[15] and quercetin was identified in the leaves.[14] These phytochemicals may contribute to the plant's biological activities, such as antioxidant,[12–16,] and anti-inflammatory activities.[17,18] Considering the limited scientific data for the development of *A. manihot* leaves as a nutritional food, this work aimed to provide the proximate and phytochemical compositions of *A. manihot* leaves collected from Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia.

Method

Instrument and Glassware

Instruments and glassware were an HPLC (Waters Alliance e2695; https://www.waters.com/nextgen/us/en.html) with an ultraviolet detector, an ultraviolet-

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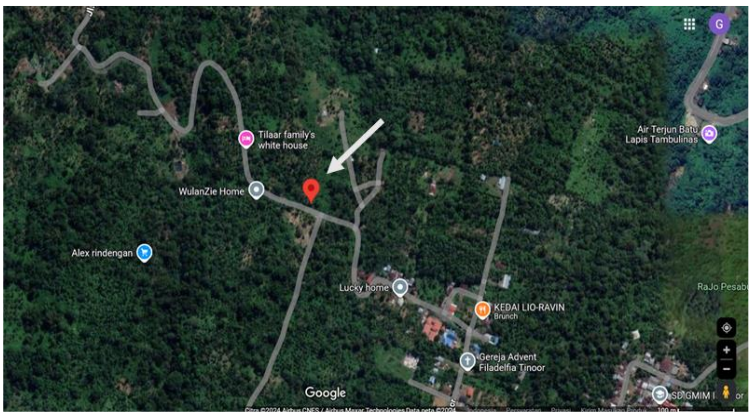
visible spectrophotometer (PerkinElmer Lambda 35; https://www.manualslib.com/manual/1363694/Perkinelmer-Lambda-25.html), a Fourier-transform infrared (FTIR) (Thermoscientific), analytical digital balance (Ohaus; https://asiapacific.ohaus.com/en-ap/products/balances-scales/analytical-balances), rotary evaporator (IKA RV 8 V), drying oven (Memmert), moisture analyzer (Ohaus), muffle furnace (SNOL 3/1100), Kjeldahl apparatus, Soxhlet extractor/fat analyzer (BUCHI), and Pyrex glassware for analytical laboratory.

Plant Material, Determination, and Extract Preparation

An approximate weight of 20 kg of fresh gedi leaves was harvested from Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia Figure 2.



(a)

(b)

Figure 2. (a) Google map of the location of the plantation where the *A. manihot* leaves were collected (shown by a white arrow) with coordinates at 1.3927010694292143, 124.82195873796563, Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia; (b) harvesting the leaves.

The plant sample was taxonomically determined by Arifin Surya Dwipa Irsyam, S. Si., M. Si. (e-mail: arifin@sith.itb.ac.id; Research Gate: https://www.researchgate.net/profile/Arifin_Irsyam) at the School of Life Sciences and Technology ITB, Jl. Let. Jen. Purn. Dr (HC) Mashudi No. 1, Kecamatan Jatinangor, Sumedang, West Java, 45363, Indonesia, and was confirmed as *Abelmoschus manihot*

L., with a document letter No. 8641/IT1.C11.2/TA.00/2024, signed on 28 November 2024.

In brief, 20 kg of fresh leaves were sorted, washed, spread in bamboo containers covered with a black cloth, and sun-dried for 8 hours per day for seven consecutive days. The dried leaves (2.360 kg) were crushed into small pieces, and soaked in 70% ethanol (1:10) at a room temperature of 24 ± 2 ^{o}C for 3 × 24 hours, then filtered using Whatman paper. The filtrate was rotary-evaporated at 40 ± 2 ^{o}C for 6 hours to a thick consistency.

Proximate Analysis

A Proximate analysis for water, ash, protein, fat, and carbohydrate content in the extract was carried out by following the Indonesian National Standard SNI 01-2891-1992 for Food Analysis.[19] The proximate analysis was carried out at the Central Laboratory of Universitas Padjadjaran. Due to the limited sample size, the analysis was conducted using a one-point measurement without replication.

Phytochemical Screening

Phytochemical screening is a rapid and economical color test procedure used to identify various types of phytochemicals, or secondary metabolites such as polyphenols, tannins, flavonoids, alkaloids, saponins, sterols, monoterpenes and sesquiterpenes, and quinones in plant extracts. The screening was conducted according to a standard procedure for plant extracts.[20]

Total Phenol Content (TPC)

The total phenol content (TPC) in the extract was determined using the Folin-Ciocalteu method.[21,22] The test solution involved weighing approximately 300 mg of the extract, dissolving it in 10 mL of methanol, and stirring for 30 min with a magnetic stirrer. 0.3 mL of the solution was reacted with 1.5 mL of Folin-Ciocalteu, and then incubated at room temperature for 3 minutes. Afterward, 1.2 mL of 5% sodium carbonate was added. The absorbance of the reaction mixture was measured at 750 nm. The TPC was quantified from a gallic acid curve prepared with gallic acid standards at concentrations ranging from 20 to 60 µg/mL, expressed as mg of gallic acid equivalent (GAE) per 100 g of dry extract weight, with an average of triplicate ± SD.

Total Flavonoid Content (TFC)

Determination of the total flavonoid content (TFC) in the extract was carried out by reacting the extract with 0.5 mL of 10% aluminum chloride in ethanol and 0.1 mL of 5% sodium acetate solution. The reaction mixture was incubated at room temperature for 20 minutes, and the absorbance of the mixture was measured at 415 nm. The TFC was quantified from a quercetin curve prepared with quercetin standards at concentrations ranging from 20 to 60 µg/mL, expressed as mg of quercetin equivalent (QE) per 100 g dry weight of the extract, as an average of triplicate ± SD.[23]

Determination of Quercetin in the Extract by HPLC

The determination of quercetin in the extract was performed using a reversed-phase HPLC system. Approximately 10 mg of standard quercetin was dissolved in 10

mL of HPLC-grade methanol. The quercetin standard solution was diluted to obtain increasing concentrations of 0.5, 1.0, 5.0, 10, and 50 mg/L for the standard curve. Similarly, approximately 0.156 g of the extract was dissolved in 10 mL of HPLC-grade methanol. 10 µL of the standard and the extract were injected into a C18 column, with an isocratic elution of acetonitrile and 2% v/v acetic acid, a flow rate of 1 mL/min, a running time for 30 minutes, and detection at 370 nm. The procedure was done in triplicate. Data were expressed as average ± SD.

Result

Extraction of *A. manihot* Leaves and the Proximate Composition of the Extract

Of the 20 kg fresh *A. manihot* leaf, after sun-drying the leaves for one week, yielded 2360 g of dried materials (11.8%). The 2360 g of dried leaves was macerated with 70% ethanol for 3 × 24 hours, resulting in 119.88 g of thick extract (5.07%), as shown in Figure 3.

Figure 3. The thick ethanol extract of *A. manihot* leaves cultivated in Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia.

The proximate composition of the ethanol extract from *A. manihot* leaves cultivated in Tomohon, North Sulawesi, Indonesia, resulted in a high total ash content of 35.60%, a high carbohydrate content of 28.56%, water content of 18.31%, protein content of 11.01%, and fat content of 6.53%.

Phytochemical Screening

Phytochemical screening test of the ethanol extract of *A. manihot* leaves revealed positive results for the presence of phenols, flavonoids, quinones, saponins, monoterpenes and sesquiterpenes, sterols, and alkaloids. The color test for tannins was found to be negative Table 1.

Table 1. Phytochemical Screening of the Ethanol Extract of *A. manihot* Leaves

Secondary Metabolite	Screening Test Result
Phenols	[+] Detected
Flavonoids	[+] Detected
Quinones	[+] Detected
Tannins	[-] Not detected

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Table 1. (Extension)

Secondary Metabolite	Screening Test Result
Saponins	[+] Detected
Monoterpenes and sesquiterpenes	[+] Detected
Sterols	[+] Detected
Alkaloids	[+] Detected

Total Phenol Content (TPC) and Total Flavonoid Content (TFC)

The TPC of the ethanol extract of *A. manihot* leaves was calculated using the gallic acid standard curve, resulting in 1350 ± 23.87 mg GAE/100 g dry weight extract. The total flavonoid content (TFC) was calculated using the quercetin standard curve, resulting in 2670 ± 16.79 mg QE/100 g dry weight extract.

Determination of Quercetin in the Extract by HPLC

The quercetin standard curve has a regression equation of $y = 28372x + 5850.8$, with an R^2 of 0.9998 (Figure 4), resulting in a quercetin level of 373.7 ± 0.18 mg/L.

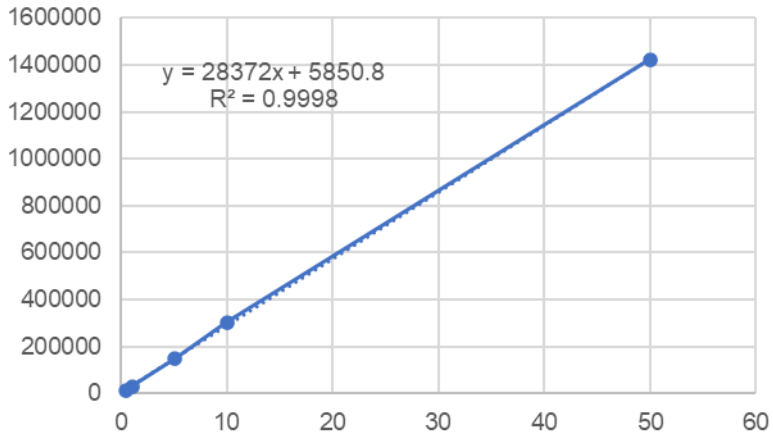

Figure 4. Quercetin standard curve, obtained by plotting the concentrations of quercetin standard (0.5, 1.0, 5.0, 10, and 50 mg/L) against the area under the curve (AUC) in a reversed-phase HPLC system.

Discussion

In the present study, an approximate weight of 20 kg of fresh gedi leaves (*Abelmoschus manihot*) harvested from Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia, after being sun-dried for one week, resulted in 2360 g (11.8%) of dried materials, of which 119.88 g of thick extract (5.07%) was yielded.

This yield was lower compared to a recent study reported by Sihotang et al., which was 10.46%.[23] In their study, the *A. manihot* leaves were obtained from the botanical garden of Universitas Prima Indonesia in Medan, North Sumatra, Indonesia, and were extracted using 96% ethanol.[22] Another study on the leaves of this plant reported the yield of their extraction, which was 9.86 %.[24]

Our phytochemical screening test revealed the presence of phenols, flavonoids, quinones, saponins, monoterpenes and sesquiterpenes, sterols, and alkaloids.

In comparison, the leaves of *A. manihot* collected from Tropical Biopharmaca Research Centre, IPB University, Bogor, Indonesia, which were extracted using five

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different solvents: water, 70% ethanol, absolute ethanol, ethyl acetate, and hexane, detected the presence of phenols, tannins, flavonoids, and steroids in all tested extracts. Quercetin flavonoid was found to be present in the water extract, 70% ethanol, absolute ethanol, and hexane extracts, but it was not detected in the ethyl acetate extract.[15]

In the present study, the proximate analysis demonstrated a high total ash level of 35.60%, a high carbohydrate content of 28.56%, a water content of 18.31%, a protein content of 11.01%, and a fat content of 6.53%, with a TPC of 1350 ± 23.87 mg GAE/100 g dry weight extract and a high TFC of 2670 ± 16.79 mg QE/100 g dry weight extract.

Compared to our study, the leaves of *A. manihot* collected from Bogor, Indonesia, resulted in a TPC ranging from 370 mg GAE/100 g in the ethyl acetate extract to 3920 mg GAE/100 g in the 70% ethanol extract, which is comparatively higher than that originating from North Tomohon, North Sulawesi.[15] Moreover, Sudewi et al. reported that a higher value of the TFC in the 96% ethanol extract of *A. manihot* leaves obtained from Tomohon and Kotamobagu, which were 4667.9 mg/100 g and 6176.3 mg/100 g, respectively.[16] The extract of the leaves collected in Manado, Sulawesi, Indonesia, was reported to contain 0.64% protein, 1.27% carbohydrate, 9.26% total energy, 7.1% water, and 0.64% ash.[24] *A. manihot* leaves provided by Suzhong Pharmaceutical Groups, China, showed 13.83% ash, 27.14% protein, and 41.68% carbohydrates, with a TPC value of 1616 ± 35 mg GAE/100 g extract.[25]

Moreover, HPLC analysis of quercetin in the extract yielded a concentration of 373.7 mg/L. According to our study, quercetin was also detected, in the leaves of *A. manihot* collected in Nanjing, China.[11–13] Quercetin at a low level of 0.6417 ± 0.00132 mg/L was found to be present in *A. manihot* flowers collected in Yongan Town, Chengdu, China.[11]

The high total ash content in plant extracts indicates the presence of numerous minerals. The high total ash content in plant extracts suggests the presence of numerous minerals. The High total ash content in plant extracts indicates the number of minerals contained in the extracts. Ash refers to the inorganic residue remaining after complete oxidation of organic matter in the plant extracts. During the ashing process, water and volatile compounds are vaporized, organic substances are burned in the presence of oxygen, and minerals are oxidized to oxides, sulfates, phosphates, chlorides, and silicates.[26] Based on our study, the ethanol extract of *A. manihot* leaves cultivated in North Tomohon, North Sulawesi, Indonesia, contained high minerals, carbohydrates, proteins, fats, phenolic compounds, and flavonoids; thus, it may have the potential to be developed as a nutritional food or nutraceutical supplement.

Conclusion

This study investigated the proximate and phytochemical composition of the ethanol extract of *Abelmoschus manihot* leaves, cultivated in Tinoor Dua Village, North Tomohon, North Sulawesi, Indonesia. Proximate analysis revealed that 35.60% of the total ash, 28.56% of carbohydrate, 18.31% of water, 11.01% of protein, and 6.53% of fat. The TPC of 1350 ± 23.87 mg GAE/100 g dry weight extract was comparatively lower than that originating from Bogor, West Java, under similar extraction conditions. The TFC of 2670 ± 16.79 mg QE/100 g dry weight extract was comparatively lower than that obtained from Tomohon and Kotamobagu, under different extraction conditions. The quercetin content in the extract was found to be 373.7 mg/L, relatively higher than that in *A. manihot* flowers collected in Yongan Town, Chengdu, China. These findings contribute scientific data for the development of *A. manihot* leaves as a nutritional food or nutraceutical supplement.

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