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# Phytochemical compounds and antioxidant capacities of *Abelmoschus manihot* leaf extracts using different solvents

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**Abstract.** *Winata GM, Hardinsyah H, Marliyati SA, Rimbawan R, Andrianto D. 2024. Phytochemical compounds and antioxidant capacities of* Abelmoschus manihot *leaf extracts using different solvents. Biodiversitas 25: 942-949. Gedi* (*Abelmoschus manihot* (L.) Medik) leaves are popularly consumed as a green vegetable by Eastern Indonesians. This work aimed to carry out the phytochemical compounds and antioxidant capacities of five extracts, i.e., water, 70% ethanol, absolute ethanol, ethyl acetate, and hexane of *Abelmoschus manihot* leaf. Qualitative analysis was done as a preliminary screening for flavonoid, tannin, saponin, alkaloid, triterpenoid, steroid, and quinone. Quantitative analysis determined the flavonoid, phenolic, tannin, quercetin, and steroid content. In-vitro antioxidant analysis was conducted to determine free radical scavenging activity and lipid peroxidation inhibitory activity. The results showed that flavonoid was only found in 70% ethanol extract; steroid was obtained in all extracts, while alkaloid, saponin, tannin, quinone, and triterpenoid were absent in all extracts. The results of the quantitative analysis showed that the highest flavonoid was in 70% ethanol extract (39.2 mg GAE/g), phenolic in ethyl acetate extract (34.792 mg QE/g), tannin in 70% ethanol (1.395 mg/g), quercetin in absolute ethanol extract (0.246 mg/g), and steroid content in hexane extract (201.948 mg/g). Half maximal inhibitory concentration ($IC_{50}$) of each extract to inhibit malondialdehyde (MDA) formation was lower than free radical scavenging activity. The $IC_{50}$ values of MDA formation in absolute ethanol and hexane extracts were 6.77 ppm and 7.67 ppm, respectively. The $IC_{50}$ of vitamin E was 3.67 ppm. Further study is needed to determine the mechanism of *Abelmoschus manihot* leaf extracts as antioxidants used as a functional food.

**Keywords:** *Abelmoschus manihot*, antioxidant, free radical scavenging, *gedi*, lipid peroxidation, phytochemical

**Abbreviations**: $IC_{50}$: half maximal inhibitory concentration

## INTRODUCTION

Gedi or aibika plant (*Abelmoschus manihot* (Linn.) Medik) is an annual flowering herbal plant that grows in tropical areas, especially in Asia and the Pacific Islands (Luan et al. 2020). It is commonly found in India, Southern and Eastern China, and Southeast Asia. Plant cultivars are diverse in Papua New Guinea, Solomon Islands, Vanuatu, and Eastern Indonesia (Preston et al. 1998). *A. manihot* is usually grown in a houseyard without adequate care, which causes suboptimal growth (Prabawardani et al. 2016). Morphologically, it has variations in shape and color due to genetic variations during adaptation over a very long period (Mandey et al. 2014). The leaf is finger-shaped, with varying numbers between three and nine fingers (Figure 1). Significant variations are seen in the shape and size of the leaves, the color of the petioles and stems, the branches, and the characteristics of the flowers. Observations at four locations in Papua showed that the morphological diversity of *A. manihot* plants did not only occur in different populations or locations but also the same population (Prabawardani et al. 2016). Differences in environmental conditions affect the nutritional and non-nutritional content of *A. manihot* plants (Warongan et al. 2017).

*Abelmoschus manihot* leaf is popularly consumed as a green vegetable by Indonesian people, similar to cassava, papaya, spinach, kale, and other leaves, especially in Eastern Indonesia such as Manado (North Sulawesi), Halmahera (North Maluku), and Papua (Mapanawang et al. 2016). It contains high protein and micronutrients, such as Ca, Fe, K, Mg, Mn, Na, Zn, Cu, folate, and β-carotene (Rubiang-Yalambing et al. 2016). In North Sulawesi, the leaves are processed into vegetables by adding coconut milk or boiled with other special spices as a mixture for Manado "tinutuan" porridge.

At least 128 active phytochemical components have been isolated from various parts of the *A. manihot* plant (Luan et al. 2020). Most were isolated from the flower, and some were from the seed, stem, and leaf. The main phytochemical compounds found in the plant are flavonoids, amino acids, nucleosides, polysaccharides, organic acids, steroids, and volatile oils with activities as

antiinflammation, antioxidant, antidepression, antitumor, immune modulation, cardioprotection, and hepatoprotection. Most active chemical compounds were isolated from the flower part as the most commonly used as a medicine.

A qualitative study showed that 70 and 96% ethanol extracts of *A. manihot* leaf contain flavonoids, alkaloids, and tannins (Pranowo et al. 2015). Flavonoid was found in ethyl acetate extract (Theodora et al. 2019), while flavonols were found in methanol extract (Pan et al. 2017). Alkaloids and steroid were found in ethanol extract (Hendrawati et al. 2020). Steroids are also obtained in benzene, ethanol, chloroform (Selvaraj et al. 2020), and methanol/n-hexane extracts (Mamahit 2009). Terpenes were found in ethanol, benzene, chloroform, and methanol extracts (Selvaraj et al. 2020). Polysaccharides were found in water/ethanol extract (Pan et al. 2018). Therefore, the *A. manihot* leaf has the potential to be a nutraceutical at the proper harvest time to get the highest amount of active components (Pan et al. 2017). This work aimed to carry out comprehensive qualitative and quantitative phytochemical compounds and antioxidant capacities study of five different leaf extracts using different polarity of solvents from a single source of *A. manihot* plant. No study explored comprehensive phytochemical compounds and antioxidant capacities of water, 70% ethanol, absolute ethanol, ethyl acetate, and hexane leaf extracts from the *A. manihot* plant.

## MATERIALS AND METHODS

### *Abelmoschus manihot* leaf extract preparation

The *A. manihot* leaves were collected from UKBB (*Unit Konservasi Budidaya Biofarmaka*), Trop BRC (Tropical Biopharmaca Research Centre), IPB University, Dramaga, Bogor, Indonesia. Previously, botanical species identification of *A. manihot* leaf was performed by a botanist at the Botanical Characterization Laboratory, National Research and Innovation Agency (BRIN), Cibinong, Indonesia. The young leaves were harvested in the morning, washed, oven-dried, ground, and sieved. The leaf powder was extracted using five different polarities of solvents, i.e., water, 70% ethanol (Merck, Germany), absolute ethanol (Merck, Germany), ethyl acetate (Merck, Sweden), and hexane (Merck, Germany). All solvents used were of analytical grade and were used without any purification.

Extraction using water as a solvent: the leaf powder was added with 60°C hot water (Farooq et al. 2013), continuously stirred until completely dissolved, and then underwent ultrasonic extraction at 59°C for 30 minutes (Wang et al. 2018) in a sonication cleaning bath (JAC, Korea) at the wavelength set at 40 kHz (Hendrawati et al. 2020). After that, the extract was squeezed through muslin cloth. The filtrate was collected and freeze-dried.

Extraction using 70% ethanol, absolute ethanol, ethyl acetate, and hexane solvents: the leaf powder was dissolved with the respective solvent and then underwent ultrasonic extraction at 40°C for 30 minutes in a sonication cleaning bath with the wavelength set at 40 kHz (Hendrawati et al. 2020). The sonicated solution was centrifuged at 2000 rpm for 5 minutes (Tafzi et al. 2017), filtered, condensed in a rotary vacuum evaporator (Winlab, Australia), and freeze-dried. All extracts were stored in vacuum-packed petri dishes at 4°C for further use (Pan et al. 2017).

### Qualitative phytochemical screening

Qualitative screening of the extract was carried out on all *A. manihot* leaf extracts (water, absolute ethanol, 70% ethanol, ethyl acetate, and hexane). It was conducted according to Harborne (1984) with some modifications.

*Flavonoid content*

One hundred mg of extract was boiled with distilled water for 5 minutes and then filtered. Magnesium powder, sodium chloride/ethanol (1:1), and amyl alcohol were added to the filtrate, forming an amyl alcohol layer. An orange coloration indicated the presence of flavonoids.

*Tannin content*

One hundred mg of extract was boiled with distilled water for 5 minutes and then filtered. After that, three drops of 10% iron(III) chloride were added to the filtrate. A green-black coloration indicated the presence of tannin.

*Saponin content*

One hundred mg of extract was boiled with distilled water for 5 minutes and then filtered. The filtrate was shaken vigorously. A stable frothing indicated the presence of saponin.

*Alkaloid content*

A few drops of ammonia were added to 100 mg of extract and then mixed until smooth. The solution was added with 5 mL of chloroform and then filtered. The filtrate was added with 2 M sulphuric acid. The acid solution was divided into 3 parts, each added with Dragendorff, Mayer, and Wagner reagents. Respectively, orange, white, and brown precipitates formed in the solution added with Dragendorff, Mayer, and Wagner reagents, indicating the presence of alkaloids. Madagascar periwinkle (*Catharanthus roseus)* leaf was the standard due to its high alkaloid content.

**Figure 1.** *Abelmoschus manihot* plant

*Triterpenoid or steroid content*

One hundred mg of extract was mixed with hot ethanol and then filtered. The filtrate was heated to dry, and 1 mL of diethyl ether was added to homogenize it. Then, 1 drop of concentrated sulfuric acid and 1 drop of anhydrous acetic acid were added. Green or blue indicated the presence of steroid, and red or purple indicated the presence of triterpenoid.

*Quinone content*

One hundred mg of extract was added with methanol and then heated and filtered. The filtrate was added with 3 drops of 10% sodium hydroxide. Red coloration indicated the presence of quinone.

**Quantitative phytochemical analysis**

Quantitative phytochemical content of the extract was carried out on all *A. manihot* leaf extracts (water, absolute ethanol, 70% ethanol, ethyl acetate, and hexane).

*Flavonoid content*

Flavonoid content was determined based on the formation of the flavonoids and aluminum complex with maximum absorptivity at 425 nm as described in *Monografi Ekstrak Tumbuhan Obat Indonesia* (Badan POM RI 2004) with some modifications. An amount of 200-300 mg of extract was added to a volumetric flask, added with 1 mL of HMT (Hexamethylenetetramine) solution, 20 mL of acetone, and 1 mL of the hydrochloric acid solution, hydrolyzed by refluxing for 30 minutes. The mixture was filtered with cotton, and the filtrate was put into a 100 mL volumetric flask. The residue was refluxed again with 20 mL of acetone for 30 minutes, filtered, and the filtrate was added to the 100 mL volumetric flask. The filtrate mixture was added with 100 mL of acetone. 20 mL of the filtrate was taken to a separatory funnel, added with 20 mL of water, and extracted with 15 mL of ethyl acetate thrice. The ethyl acetate fraction was collected and added with ethyl acetate up to 50 mL volume in a volumetric flask, the so-called stock solution.

Ten mL of stock solution was added with 5% v/v glacial acetic acid solution in methanol up to 25 mL in a volumetric flask, a so-called blank solution. Ten mL of stock solution was added with 1 mL of 2% aluminum chloride in glacial acetic acid solution up to 25 mL volume in a volumetric flask, the so-called sample solution. The measurement was done 30 minutes after adding aluminum chloride using a spectrophotometer at a wavelength of 425 nm with quercetin as the standard. The total flavonoid content was calculated as follows.

$$\% = \frac{Cp\ (As - Abs)}{Ap - Abp} \text{ x } 6.25 \text{ x } \frac{100}{Sample\ weight}$$

Where: %: total flavonoid levels calculated as comparison flavonoid as shown in the monograph, Cp: concentration of comparison solution, As: absorption of sample solution with aluminum chloride solution, Abs: absorption of blank solution, Ap: absorption of comparison solution with aluminum chloride solution, Abp: absorption of comparison solution without aluminum chloride solution, 6.25 = constant factor

*Phenolic content*

Phenolic content was determined using the Folin-Ciocalteu reagent described in *Monografi Ekstrak Tumbuhan Obat Indonesia* (Badan POM RI 2004) with some modifications. Ten mg of extract was added to a 25 mL volumetric flask and dissolved with methanol. Gallic acid was used as the standard. A stock solution was 500 ppm gallic acid. Standard solutions of 0, 10, 30, 50, 70, and 100 ppm were made in a 25 mL volumetric flask.

One mL of extract solution and each standard solution was placed in a test tube and added with 5 mL of 7.5% Folin Ciocalteu reagent, vortexed, and incubated in a dark room for 8 minutes each. After that, 4 mL of 1% sodium hydroxide was added, vortexed, and incubated in a dark room for 1 hour. The measurement was done using a spectrophotometer at a wavelength of 730 nm.

*Quercetin content*

Quercetin content was conducted according to Hertog et al. (1992) with some modifications. A 0.1 to 1 gram of extract was added with 20 mL of 62.5% methanol (2.5 mg/L TBHQ) and 5 mL of 6 M HCl, then refluxed for 2 hours at 90°C. After that, the solution was put into a 25 mL volumetric flask, 62.5% methanol was added to the line, filtered with 0.45 µm filter paper, and injected into HPLC. The stationary phase used was the Zorbac Eclipse-C18 column. The mobile phase was acetonitrile and $KH_2PO_4$ (pH 2.4) (25:75). Each injection's total run time was 30 minutes. The measurement was done at a wavelength of 370 nm.

*Tannin content*

The tannin content was determined as described in *Materia Medika Indonesia* (Departemen Kesehatan Republik Indonesia 1980) with some modifications. A 0.2 g of extract was heated with 500 mL of boiling water for 30 minutes with continuous stirring. The mixture was filtered with cotton, and the filtrate was put into a 250 mL volumetric flask. The remaining residue was re-heated, filtered, and placed into the same volumetric flask. The filtration process was repeated several times until tannin was not detected in the reaction of the filtrate and ammonium iron(III) sulfate. The solution was cooled and added with water to 250 mL volume. A 25 mL of the solution was placed into a 1000 mL volumetric flask, added with 750 mL of water and 25 mL of indigo sulfonic acid LP, then titrated with 0.1 N potassium permanganate until the solution turned yellow (0.1 N potassium permanganate equals 0.004157 g of tannin). The same procedure was done for the blank solution.

*Steroid content*

Steroid content was determined using Liebermann-Burchard reagent as described in Adu et al. (2019) with some modifications. A stock solution of the working standard, 1 mg/mL cholesterol, was made by dissolving 0.01 g of cholesterol with chloroform up to 10 mL in a

volumetric flask. The solution was kept at 2-8°C for later use. Standard solutions for method development and validation were prepared by diluting solutions in 0.02 to 0.08 mg/mL concentrations. The maximum wavelength for cholesterol identification was measured using 2.5 mL of 0.04 mg/mL cholesterol solution, added with 0.5 mL of Liebermann-Burchard reagent, and incubated in the dark for 90 minutes. A control solution of chloroform and Liebermann-Burchard reagent was also prepared. Aliquots of the final solutions of cholesterol and blank were scanned within a wavelength range of 200-800 nm using a UV-Vis Spectrophotometer. Maximum wavelength was recorded.

A 0.5 mL of 4 mg/mL extract was added with 4.5 mL chloroform. 2.5 mL of the mixture was added with 0.5 mL of Liebermann-Burchard reagent and incubated in the dark for 90 minutes. After that, the absorbance of the sample was measured at the maximum wavelength. The steroid content was estimated using a linear calibration model generated from standard solutions of the working standard and inserting recorded absorbances of the samples into the model.

**In vitro antioxidant activity**

The leaf extracts were screened for their in vitro antioxidant activities by two spectrophotometric methods, i.e., 2,2-diphenyl-1-picryl-hydrazyl (DPPH) for free radical scavenging activity and Thiobarbituric Acid Reactive Substances (TBARS) for lipid peroxidation inhibitory activity.

*Free radical scavenging activity*

DPPH scavenging activity assay was conducted according to Salazar-Aranda et al. (2011) with some modifications. 100 µL of extract and a positive control vitamin C were separately put into a microplate and added with 100 µL of DPPH. Meanwhile, 100 µL of ethanol was prepared as a negative control. The extract, vitamin C, and negative control were incubated at room temperature in the dark for 30 min. The measurement was determined using an ELISA kit at 517 nm wavelength. The capacity to scavenge DPPH radical was calculated as follows.

$$\text{Radical scavenging activity (\%)} = \frac{(A-B)}{A} \text{ x } 100$$

Where: %: radical scavenging activity, A: absorbance of the negative control, B: absorbance of the extract, vitamin C

The correlation between each concentration and its scavenging concentration was plotted, and the $IC_{50}$ was calculated by interpolation. The activity was expressed as $IC_{50}$, the inhibition concentration of each sample to scavenge 50% of DPPH radicals.

*Lipid peroxidation inhibitory activity*

Thiobarbituric Acid Reactive Substances (TBARS) assay was conducted as described in Chairunisa (2022). All solvents and reagents used were of analytical grade and were used without any purification. *A. manihot* leaf extract concentrations were made in 0.5 to 50 ppm. One mL of extract solution was added with 2 mL of 0.1 M phosphate buffer pH 7 and 2 mL of 50 mM linoleic acid in 99.8% ethanol. A mixture of 1 mL of 200 ppm α-tocopherol, 2 mL of 0.1 M phosphate buffer pH 7, and 2 mL of 50 mM linoleic acid in 99.8% ethanol was used as a positive control. A mixture of 1 mL of each solution (extract and positive control solution), 2 mL of 20% Trichloroacetic acid (TCA) solution, and 2 mL of 1% TBA solution in 50% acetic acid, was put in a dark bottle and incubated at a 40°C water bath for the time determined during linoleic acid incubation as described in Chairunisa (2022). The reaction mixture was placed in a boiling water bath for 10 min and centrifuged at 3000 rpm for 15 minutes after cooling. The measurement was done at a wavelength of 532 nm. One mL of a mixture of 3 mL of 99.8% ethanol, 2 mL of 0.1 M phosphate buffer pH 7, 2 mL of 20% TCA, and 2 mL of 1% TBA in 50% acetate was used as a blank solution. The blank solution was placed in a boiling water bath for 10 min and after cooling was centrifuged at 3000 rpm for 15 minutes. Antioxidant activity was calculated as follows:

$$\text{\% inhibition} = \frac{[MDA]\ Negative\ control - [MDA] Sample}{[MDA]\ Negative\ control} \text{ x } 100\%$$

## RESULTS AND DISCUSSION

**Qualitative phytochemical screening**

All *A. manihot* leaf extracts, i.e., water, 70% ethanol, absolute ethanol, ethyl acetate, and hexane extracts, were qualitatively screened for the presence of flavonoid, alkaloid, tannin, saponin, quinone, steroid, and triterpenoid. Table 1 presents the phytochemical content of the gedi leaf extracts. In general, solvents with high polarity index extracted compounds that have polar properties, e.g., sugars, amino acids, acids, bases (Houghton and Raman 1998), some flavonoid glycosides (Houghton and Raman 1998; Seidel 2012), tannins, and some alkaloids (Seidel 2012). Medium polarity solvents can solubilize volatile oils (Houghton and Raman 1998), alkaloids, flavonoid aglycones and glycosides (Houghton and Raman 1998; Seidel 2012), and moderately polar compounds with low molecular weight (Lin et al. 2009). Low and nonpolar solvents extract mostly lipophilic compounds, e.g., some flavonoid aglycones (Houghton and Raman 1998), alkaloids, waxes, fats, oils, volatile oils (Houghton and Raman 1998; Seidel 2012), alkanes, pigments, sterols, some terpenoids, and coumarins (Seidel 2012).

Alkaloid, saponin, tannin, quinone, and triterpenoid were not detected in any of the extracts. Flavonoid was only observed in 70% ethanol extract. Pranowo et al. (2015) also reported the absence of saponin in 70% and 96% ethanol extract. In contrast, Pranowo et al. (2015) reported the presence of flavonoids, alkaloids, and tannins in 70% and 96% extracts. Different varieties, environmental conditions, and extraction methods (Waris et al. 2016) might cause some differences in the phytochemical content of the leaf.

All extracts with different solvents contained steroids. The steroid is a nonpolar compound, so it was extracted by a nonpolar solvent,i.e., hexane. Although steroids are a

nonpolar compound, they may have a hydroxyl chain to dissolve in high and medium-polar solvents such as water, 70% ethanol, absolute ethanol, and ethyl acetate.

**Quantitative phytochemical content**

Quantitative phytochemical analysis was done to determine phenolic, flavonoid, tannin, quercetin, and steroid contents of all *A. manihot* leaf extracts, i.e., water, 70% ethanol, absolute ethanol, ethyl acetate, and hexane solvents. Phenolic compounds are commonly found in plants and are gaining interest due to their antioxidant properties and potential benefits for human health (Shahidi and Ambigaipalan 2015). Their hydroxyl groups and radical scavenging activities may contribute directly to their potent antioxidant capacity (Chang and Kim 2018). Plant phenolic compounds are the primary source of natural antioxidants, including flavonoids and tannins. Quercetin is the most ubiquitous flavonol, one of a class of natural flavonoids (Shahidi and Ambigaipalan 2015). Tannin exhibits radical scavenger activities, anti-superoxide formation (Jo et al. 2015), and antilipid peroxidation (Ajah et al. 2021). Phytosterols are natural plant steroids that may interact with steroid receptors and steroid metabolizing enzymes and affect the endocrine and reproduction system depending on their structure, concentration, receptor, and cell type (Dean et al. 2017).

The results of the quantitative study are presented in Table 2. Phenolic, tannin, flavonoid, and steroid were present in all *A. manihot* leaf extracts with different polarity solvents. Quercetin was present in water, 70% ethanol, absolute ethanol, and hexane extracts but absent in ethyl acetate extract. The phenolic content ranged from 3.7 mg GAE/g to 39.2 mg GAE/g. The highest phenolic content was in 70% ethanol extract (39.20 mg GAE/g), followed by absolute ethanol extract (9.90 mg GAE/g), water extract (7.00 mg GAE/g), hexane extract (5.30 mg GAE/g), and ethyl acetate extract (3.70 mg GAE/g), respectively. Previously, Sudewi et al. (2017) reported the flavonoid content in 96% ethanol extract of gedi leaf from two regions in North Sulawesi (Indonesia), Tomohon and Kotamobagu, was 46.679 mg/g and 61.763 mg/g, respectively. Pranowo et al. (2015) reported the flavonoid content in 70 and 96% ethanol extracts of gedi leaf from Cianjur, Jawa Barat (Indonesia) was 27.19 mg/g and 37.29 mg/g, respectively. Pine et al. (2015) reported the flavonoid content of gedi leaf of water, 70 and 96% ethanol extracts from Palu, Gorontalo, and Makassar ranged from 0.04-0.05 mg/g, 2.07-3.75 mg/g, and 23.63-41.56 mg/g, respectively.

The tannin content ranged from 0.411 mg/g to 1.395 mg/g. The highest tannin content was in 70% ethanol extract (1.395 mg/g), followed by water extract (0.837 mg/g), absolute ethanol extract (0.465 mg/g), hexane extract (0.451 mg/g), and ethyl acetate extract (0.411 mg/g), respectively. The flavonoid content ranged from 4.432 mg QE/g to 34.792 mg QE/g. The highest flavonoid content was in ethyl acetate extract (34.792 mg QE/g), followed by absolute ethanol extract (31.207 mg QE/g), hexane extract (29.296 mg QE/g), 70% ethanol extract (23.337 mg QE/g), and water extract (4.432 mg QE/g), respectively. The quercetin content ranged from 0.015 mg/g to 0.246 mg/g. The highest quercetin content was in absolute ethanol extract (0.246 mg/g), followed by hexane extract (0.051 mg/g), 70% ethanol extract (0.018 mg/g), and water extract (0.015 mg/g), respectively. The steroid content ranged from 27.571 mg/g to 201.948 mg/g. The highest steroid content was in hexane extract (201.948 mg/g), followed by ethyl acetate extract (171.244 mg/g), absolute ethanol extract (107.777 mg/g), 70% ethanol extract (93.992 mg/g), and water extract (27.571 mg/g), respectively.

**Free radical scavenging activity**

DPPH is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction by either hydrogen or electron-donating (Saklani et al. 2017). Therefore, DPPH is a valuable reagent for investigating phenolic compounds' free radical scavenging activity (Molole et al. 2022). The antioxidant reacts with DPPH and converts it to 1,1-diphenyl-2-picryl hydrazine. The reduction of DPPH absorption indicates the capacity of a substance to scavenge free radicals and is therefore considered an antioxidant (Shahidi and Zhong 2015).

**Table 1.** Qualitative phytochemical compounds of various *Abelmoschus manihot* leaf extracts

| Phytochemical compound | Indicator | *Abelmoschus manihot* leaf extract using a solvent of | | | | |
|---|---|---|---|---|---|---|
| | | Water | 70% ethanol | Absolute ethanol | Ethyl acetate | Hexane |
| Flavonoid | Yellow/orange | - | ++ | - | - | - |
| Alkaloid | | | | | | |
| Mayer | White precipitate | - | - | - | - | - |
| Wagner | Brown precipitate | - | - | - | - | - |
| Dragendorf | Orange precipitate | - | - | - | - | - |
| Tannin | Greenish black | - | - | - | - | - |
| Saponin | Stable frothing | - | - | - | - | - |
| Quinone | Red | - | - | - | - | - |
| Steroid | Green/blue | +++ | +++ | +++ | +++ | +++ |
| Triterpenoid | Red /purple | - | - | - | - | - |

Note: +++: very strong positive, ++: strong positive, -: negative

**Table 2.** Quantitative phytochemical compounds of various *Abelmoschus manihot* leaf extracts

| Phytochemical compound | Unit | *Abelmoschus manihot* leaf extract | | | | |
|---|---|---|---|---|---|---|
| | | Water | 70% ethanol | Absolute ethanol | Ethyl acetate | Hexane |
| Phenolic | mg GAE/g | 7.00 | 39.20 | 9.90 | 3.70 | 5.30 |
| Flavonoid | mg QE/g | 4.432 | 23.337 | 31.207 | 34.792 | 29.296 |
| Tannin | mg/g | 0.837 | 1.395 | 0.465 | 0.411 | 0.451 |
| Quercetin | mg/g | 0.015 | 0.018 | 0.246 | ND | 0.051 |
| Steroid | mg/g | 27.571 | 93.992 | 107.777 | 171.244 | 201.948 |

**Table 3.** DPPH scavenging activity of vitamin C and *Abelmoschus manihot* leaf extracts at $IC_{50}$

| Sample | $IC_{50}$ (ppm) |
|---|---|
| Vitamin C | $5.10 \pm 0.02^{a}$ |
| Water extract | $1908.48 \pm 46.47^{b}$ |
| 70% ethanol extract | $340.09 \pm 1.02^{b}$ |
| Absolute ethanol extract | $430.92 \pm 0.28^{b}$ |
| Ethyl acetate extract | $610.11 \pm 2.90^{b}$ |
| Hexane extract | $955.16 \pm 17.73^{b}$ |

Note: Data represents mean ± SD. Values in the same column followed by a different letter $^{(a-b)}$ were significantly different ($p < 0.05$) with vitamin C

**Table 4.** MDA formation inhibition of vitamin E and *Abelmoschus manihot* leaf extracts at $IC_{50}$

| Sample | $IC_{50}$ (ppm) |
|---|---|
| Vitamin E | $3.67 \pm 0.18^{a}$ |
| Water extract | $9.76 \pm 0.41^{b}$ |
| 70% ethanol extract | $9.12 \pm 0.16^{b}$ |
| Absolute ethanol extract | $6.77 \pm 0.38^{b}$ |
| Ethyl acetate extract | $9.10 \pm 0.51^{b}$ |
| Hexane extract | $7.67 \pm 0.33^{b}$ |

Note: Data represents mean ± SD. Values in the same column followed by a different letter $^{(a-b)}$ were significantly different ($p < 0.05$) with vitamin E

Based on half maximal inhibitory concentration ($IC_{50}$) values in Table 3, the highest DPPH radical scavenging effect among the *A. manihot* leaf extracts was detected in 70% ethanol extract (340.09 ppm), followed by absolute ethanol extract (430.92 ppm), ethyl acetate extract (610.11 ppm), hexane extract (955.16 ppm), and water extract (1908.48 ppm). The highest antioxidant property of 70% ethanol extract compared to other *A. manihot* leaf extracts might be due to the higher solubility of active compounds in 70% ethanol extract compared to other extracts. All *A. manihot* leaf extracts had a significantly lower ($p < 0.05$) scavenging activity than standard vitamin C (5.10 ppm). Pranowo et al. (2015) reported that the $IC_{50}$ values in 70% and 96% ethanol gedi leaf extracts were 625.14 ppm and 512.41, respectively. In addition, Pine et al. (2015) reported the $IC_{50}$ values of gedi leaf extract from Palu, Gorontalo, and Makassar were 575 ppm, 1340 ppm, and 1496 ppm, respectively.

The highest antioxidant activity in 70% ethanol extract might relate positively with the highest phenolic content in this extract (39.2 mg GAE/g). The result was supported by the highest tannin content in 70% ethanol extract (1.395 ppm). Tannin is a polyphenol compound with a high molecular weight (500 Da to 20 kDa). In-vitro studies showed that the antioxidant capacity of tannin is linked to the prevention of cardiovascular diseases, cancer, and osteoporosis, even though further pharmacological investigation has not been done as thoroughly as the simpler polyphenol (Fabbrini et al. 2022). The radical scavenging activity of the water extract was the lowest among other extracts. It suggests that the antioxidants in the water extract were weak radical scavengers and required high concentrations to have a significant effect.

**Lipid peroxidation inhibitory activity**

Lipid peroxidation is a major biomarker of oxidative stress, which results from the formation of highly reactive and unstable lipid hydroperoxides (Boligon et al. 2014). Malondialdehyde (MDA), one of the major components of Thiobarbituric Acid Reactive Substances (TBARS), is generated during lipid peroxidation and results in DNA damage that causes genotoxic and mutagenic effects (Gentile et al. 2017). The ability of the leaf extract to inhibit MDA formation and lipid peroxidation was determined using TBARS assay. At high temperatures and low pH, MDA, a secondary product of polyunsaturated fatty acids oxidation, binds TBA to form a pink complex that can be measured at 532 nm (Boligon et al. 2014; Ajah et al. 2021).

Based on the 50% inhibitory concentration ($IC_{50}$) values in Table 4, the highest MDA formation inhibition was detected in absolute ethanol extract (6.77 ppm), followed by hexane extract (7.67 ppm), ethyl acetate extract (9.10 ppm), 70% ethanol extract (9.12 ppm), and water extract (9.76 ppm). The result suggested that the antioxidant effect of *A. manihot* leaf extract was mainly due to the highest quercetin content (0.246 mg/g) in the absolute ethanol extract compared to other extracts. Quercetin is one of the most abundant dietary flavonoids in fruits, green leafy vegetables, and seeds. It has been used as a nutritional supplement and may be beneficial against metabolic and inflammatory disorders. It is quite soluble in alcohol and lipids (Anand et al. 2016). Meanwhile, the second highest antioxidant capacity in hexane extract was mainly due to its highest steroid content (201.948 mg/g) compared to other extracts. All *A. manihot* leaf extracts had a significantly lower ($p < 0.05$) scavenging activity than standard vitamin E (3.67 ppm).

**Discussion**

No previous study has explored a comprehensive study of the phytochemical compounds and antioxidant activities of the five different *A. manihot* leaf extracts using TBARS and DPPH assays. The current study showed that all *A. manihot* leaf extracts contained various concentrations of phytosteroids. The TBARS assay was more sensitive than the DPPH assay in determining the antioxidant activity of *A. manihot* leaf extracts because the $IC_{50}$ value of all extracts in inhibiting MDA formation was lower than the $IC_{50}$ of all extracts to exhibit free radical scavenging activity. Absolute ethanol and hexane extracts showed strong antioxidant activity by inhibiting MDA formation compared to standard vitamin E, ethyl acetate, 70% ethanol, and water extracts. In addition, absolute ethanol and hexane extracts contain a noticeable amount of quercetin and steroids, which may play a significant role in controlling oxidation. The result showed that *A. manihot* leaf extract has the potential to be a source of natural antioxidants for the treatment of oxidative stress-induced diseases. However, the quercetin and steroid components responsible for the high antioxidant capacity in *A. manihot* leaf absolute ethanol extract and hexane extract have not been studied previously. Therefore, further study is needed to determine the mechanism of the antioxidant activity of *A. manihot* leaf extracts, which may be used as functional food, pharmaceutical, and natural plant-based products.

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