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The inhibition of α-Glucosidase enzyme activity from Standardised Ethanol Extract of *Abelmoschus manihot* (L.) Medik Leaves

(Penghambatan Aktivitas Enzim α-Glukosidase oleh Ekstrak Etanol Terstandar Daun *Abelmoschus manihot* (L.) Medik)

ANDRI PRASETIYO[1]*, WIWI WINARTI[1], RINI AGUSTIA[1]

[1]Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Pancasila, South Jakarta, Jakarta, 12640, Indonesia

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Abstract: People have long utilised the leaves of the red gedi, *Abelmoschus manihot* (L.) Medik is an anti-diabetic medication. The study's objectives were to classify chemical constituent groups, assess extract quality, and evaluate the activity of α-glucosidase enzyme inhibitors. The extraction method employed kinetic maceration with a 70% ethanol solvent, identification was accomplished through phytochemical screening, a quality determination was based on both specific and non-specific parameters, and α-glucosidase inhibition was used in an in vitro antidiabetic activity test. Flavonoids, alkaloids, saponins, steroids, and triterpenoids were found in the phytochemical screening test results. The results of the quality parameter test obtained viscous extract, blackish green color, 70.58% water soluble extract, 49.46% ethanol soluble extract, 7.82% loss on drying, 4.19% water content, total ash content 6.97%, acid insoluble ash content 0.60%, water-soluble ash content 5.64%, residual solvent 0.76%, heavy metal content Pb 1.35 mg/kg, Cd 0.06 mg/kg, plate number total ≤10 colonies/g, yeast plate number 20.1995 colonies/g and total flavonoid content 1.20%. The α-glucosidase enzyme inhibitory activity test from the extract and acarbose (positive controls) obtained IC_{50} values of 81.84 µg/mL and 42.6 µg/mL, respectively. In conclusion, the α-glucosidase enzyme may be inhibited by the ethanolic extract of *A. manihot* (L.) Medik leaves.

Keywords: α-glucosidase, *A. manihot* (L.) Medik, antidiabetic, red gedi.

Abstrak: Daun gedi merah *Abelmoschus manihot* (L.) Medik telah banyak digunakan oleh masyarakat secara turun temurun sebagai obat antiabetes. Tujuan penelitian identifikasi golongan senyawa kimia, penetapan mutu dan uji aktivitas penghambat enzim α-glukosidase pada ekstrak. Metode ekstrak yang digunakan maserasi kinetik menggunakan pelarut etanol 70%, identifikasi dilakukan dengan penapisan fitokimia, penetapan mutu digunakan parameter spesifik dan non spesifik serta uji aktivitas antidiabetes dilakukan secara in vitro dengan penghambatan enzim α-glucosidase. Hasil uji skrining fitokimia menunjukkan adanya kandungan flavonoid, alkaloid, saponin, steroid, dan triterpenoid. Hasil uji parameter mutu diperoleh ekstrak kental, warna hijau kehitaman, kadar sari larut air 70,58%, kadar sari larut etanol 49,46%, susut pengeringan 7,82%, kadar air 4,19%, kadar abu total 6,97%, kadar abu tidak larut asam 0,60%, kadar abu larut air 5,64%, residu pelarut 0,76%, kadar logam berat Pb 1,35 mg/kg, Cd 0,06 mg/kg, angka lempeng total ≤10 koloni/g, angka lempeng khamir 20,1995 koloni/g dan kadar flavonoid total 1,20%. Uji aktivitas penghambatan enzim α-glucosidase dari ekstrak dan akarbosa (kontrol positif) diperoleh nilai IC_{50} masing-masing sebesar 81,84 µg/mL dan 42,6 µg/mL. Ekstrak etanol daun *A. manihot* (L.) Medik memiliki potensi untuk menghambat enzim α-glukosidase.

Kata kunci: α-glukosidase, *A. manihot* (L.) Medik, antidiabetes, gedi merah.

*Corresponding author
e-mail: andriprasetiyo@univpancasila.ac.id

INTRODUCTION

THE TROPICAL plant *Abelmoschus manihot* L. Medik, known as "gedi," was classified as a medicinal herb[1,2]. *Abelmoschus manihot* (L.) Medik can be a complementary treatment to reduce blood sugar and blood pressure and work as an anti-inflammatory[3]. It was reported to contain phenols, tannins, steroids, and flavonoids. Many pharmacological activities, including anti-inflammatory and anti-diabetic effects, have been linked to the class of naturally occurring polyphenolic secondary metabolites known as flavonoids[4]. *A. manihot* (L.) Medik leaf fraction of ethanol ethyl acetate extract has an antidiabetic effect, as evidenced by the reduction in blood glucose levels in test mice from 348 mg/dl when induced by peptone to 152.33 mg/dl after 21 days[5].

Diabetes was a metabolic and endocrine condition with disturbed lipid and carbohydrate metabolism that was growing at the fastest pace globally. Impaired insulin secretion, action, or both may be responsible for the defective metabolism. α-glucosidase inhibitors were a more effective class of anti-diabetic medications than α-amylase inhibitors at reducing hyperglycemia, particularly postprandial hyperglycemia[6]. A condition known as postprandial hyperglycemia after eating causes a sudden increase in blood glucose levels. The principal nutrients in the human diet were carbohydrates like sucrose and starch. The small intestine's epithelium contains the membrane-bound α-glucosidase enzyme. It expedites the conversion of oligosaccharides and disaccharides into simple glucose, which was then absorbed and circulated in the body[7]. The α-glucosidase enzyme can be inhibited to delay the digestion of carbohydrates and lower blood glucose levels by 0.61 mmol/L[8]. Acarbose and miglitol were two medications accessible as α-glucosidase inhibitors and prevented the gastrointestinal tract from absorbing carbohydrates.

The oldest kind of medicine known to humanity was the use of herbs, which all cultures throughout history have practiced. All medications should meet synthetic or herbal, safe, and efficient standards. The WHO refers to the procedures involved in the physicochemical evaluation of crude medicines, including aspects like standardisation and quality control of herbals. Herbal quality was also impacted by the techniques used for harvesting, drying, storing, moving, and processing (such as the extraction method and solvent's polarity, the stability of the ingredients, etc.).

Standardising herbal medicines was a quality control process to ensure that the marketed medicines contained the right active components to provide therapeutic effects[9]. Standardisation of extracts was a process of guaranteeing that the product had certain parameter values that were constant and had been determined. Specific and non-specific characteristics evaluate extract quality standards[10]. Standardising extracts can help makers of herbal medicines become more profitable[11,12].

A. manihot (L.) Medik leaves may contain a secondary metabolite that can be used as a source for standardised herbal medicines or phytopharmaceuticals, particularly for antidiabetics[1]. The extraction of raw materials was required for the standardisation of herbal medicine. This study uses α-glucosidase inhibition to assess the antidiabetic efficacy of an ethanol extract of *A. manihot* (L.) Medik leaves. Identification of phytochemicals, including compounds tested, were flavonoids, alkaloids, saponins, tannins, steroids, and triterpenoids. Extract quality parameters tested were specific and non-specific parameters. This research was expected to increase the use of *A. manihot* (L.) Medik leaves as a medicinal plant.

MATERIALS AND METHODS

MATERIALS. The ingredients include a 70% ethanol extract of *A. manihot* (L.) Medik leaves, ethanol (Merck), α-Glucosidase from *Saccharomyces cerevisiae*, 4-Nitrophenyl α-D-glucopyranoside, Acarbose, Quarcetin were purchased from Sigma-Aldrich.

Equipments. Research instruments included Atomic Absorption Spectrophotometry (Shimadzu-AA-6800, Japan), Gas chromatography (Shimadzu-GC-17A, Japan), UV-Vis spectrophotometry (Shimadzu, UV-1800, Japan), Karl Fischer, Rotary vacuum evaporator (Heidolf, Germany), oven, furnace, water bath, micropipette.

METHODS. Sample Collection. *A. manihot* (L.) Medik leaves samples were procured in Minahasa, North Sulawesi, Indonesia. The samples were in Cibinong Bogor, West Java, in the Research Centre of Biologi Bogoriense Herbarium.

Preparation Extracts. *A. manihot* (L.) Medik leaves were ground into a 500 g powder and extracted with 5 L of 70% ethanol by maceration. The final extract was dried in a rotary evaporator in a hot water bath to produce a maceration crude extract. Before analysis, raw extracts were kept at 40°C.

Test for Organoleptic. The goal of the organoleptic test was to use the sense of taste to reveal the qualities quickly, clearly, and objectively. Shape, odour, colour, and taste were some organoleptic characteristics of the extract of *A. manihot* (L.) Medik leaves that have been described. The test findings

were determined by observation after the substance had been exposed to air for 15 minutes. Once the container holding no more than 25 g had been opened, the material was carried out after about 25 g has been transferred to the 100 mL vaporizer plate and exposed to the air for 15 minutes[9].

Water Soluble Compound. Using 25 mL of water-chloroform in a plugged Erlenmeyer, 1 gram of *A. manihot* (L.) Medik extract was macerated for 24 hours while being shaken for the first 6 hours. Moreover, it was left to stand for 18 hours before being filtered. A cup was used to dry the filtrate steam. When the weight was consistent, the residue was heated at 105°C. The weight of the initial extracted material and the fraction of water-soluble chemicals were then calculated[10].

Ethanol Soluble Compound. One gram of *A. manihot* (L.) Medik extract was macerated for 24 hours while shaken for the first six hours in 25 mL of water chloroform in an Erlenmeyer flask with a stopper. Moreover, it was not filtered until it had stood for 18 hours. Drying the filtrate steam was done in a cup. The residue was heated at 105 °C once the weight was constant. Then, the weight of the initially extracted material and the percentage of water-soluble compounds were computed. Identifying non-specific extract parameters[3].

Water Content. The Karl Fischer titration determined the water content of A's 70% ethanolic extract. *A. manihot*[2].

Total Ash Content. Two grams of each of the three replicates of the extract were weighed before being added to the silicate exchange rate, which has been anchored and slowly spawned by raising the temperature to 600 ^{0}C until it was carbon-free. Once at room temperature, they were gradually chilled before being placed in the desiccator and repeatedly weighed until it attain a stable weight[2].

Acid-Insoluble Ash Content. Ash was obtained from the full ash rate setting, which was cooked for 5 mins in 25 mL of LP-diluted hydrochloric acid. The acid-insoluble portion was then collected, filtered out the remaining ash, rinsed with hot water, and heated at the exchange rate until they reached a consistent weight[2].

Metal Contamination (Pb and Cd). A 250 mL Erlenmeyer flask was filled with 2 grams of the extract after being metered out. The flask was then filled with 25 mL of HCl solution and left to boil for 5 minutes. The solution was transferred, chilled, and then quantitatively diluted with distilled water to mark the lines. Following a shake, Whatman filter No. 1 was used to filter it. The same reagent used to create the extracted material was also used to create a blank solution. Before samples were taken, the standard series solution of blanks should be examined for absorbance. A calibration curve was produced by combining the X as the concentration and the Y as the absorbance. Then determine how much metal was in the sample that was removed[2].

Test for Mould and Yeast Contamination. After being pipetted into Potato Dextrose Agar (PDA) medium using a 1 mL sterile pipette and implanted, the 10^{-4} dilution extract was incubated for five days at 25°C. The number of colonies observed and counted after growing for five days[2].

Test for Microbial Contamination (Total Plate Number). The ethanolic 70% extract of *A. manihot* (L.) Medik leaves was tested for total plate number using count plate methods[2].

Test for Total Flavonoid. The previous study stated that the total flavonoid was assessed using a slightly modified aluminium chloride colorimetric technique[11]. The calibration curve was created using quercetin. Quercetin was dissolved in ethanol 96% to a concentration of 10 mg, and the solution was subsequently diluted to 2, 4, 6, 8, and 10 g/mL. 1 mL of each sample solution, along with 3 mL of 96% ethanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water, was mixed with 1 mL of each standard solution concentration. For 10 minutes, the mixture was continually shaken at room temperature. The absorbance at 376 nm was compared to a blank without aluminium chloride using a Shimadzu UV-1800 UV-Vis spectrophotometer. Total flavonoids were presented using mean ±SD (n=3)[2].

Inhibitor of α-Glucosidase Assay[13]. The reaction mixture contained 25 µL of 4-nitrophenyl α-D-glucopyranoside (20 mmol), 50 µL of phosphate buffer 0.1 M at pH 7, 10 µL of the test sample at a series of concentrations, and 25 µL of α-glucosidase solution (0.2 units/mL) (50-1000 ppm). 100 µL of sodium carbonate solution 0.1 M was added to the reaction mixture following 30 minutes at 37°C of incubation. The inhibitory activity at 410 nm was monitored using the microplate reader. The inquiry uses acarbose as a positive control.

This study tested the activity of α-glucosidase inhibition using acarbose as a positive control and 70% ethanol extract of *A. manihot* (L.) Medik leaves. α-glucosidase is an enzyme that catalyses the cleavage of the α-1,4 glycosidic bond of the starch molecule. The working principle of this test was that the hydrolysis reaction that occurs on the substrate p-nitrophenyl-α-D glucopyranoside yields α-D-glucose, and p-nitrophenol was coloured yellow. The weaker yellow colour produced by p-nitrophenol indicates the magnitude of α-glucosidase inhibitory

activity. Based on the quantity of p-nitrophenol produced, the absorbance was measured at 405 nm[14].

Five different sample concentrations (Figure 1) were employed in the in vitro α-glucosidase inhibitor experiment of the *A. manihot* (L.) Medik leaf extract to produce a graph with an x-axis representing concentration and a y-axis representing percent inhibition. The IC_{50} of the extract was calculated using a regression equation from the graph. Five different sample concentrations were employed in the in vitro α-glucosidase inhibitor experiment of the *A. manihot* (L.) Medik leaf extract to produce a graph with an x-axis representing concentration and a y-axis representing percent inhibition.

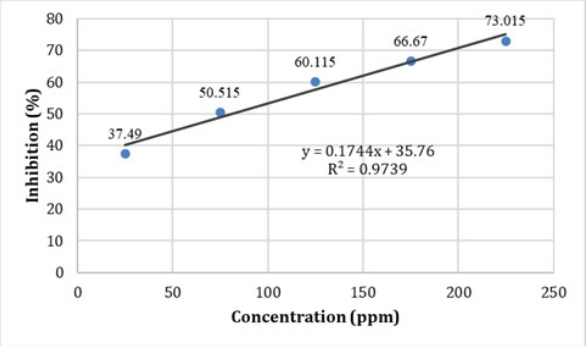

Figure 1. Percentage of α-glucosidase inhibition from ethanol extract of *A. manihot* leaves.

RESULTS AND DISCUSSION

The Spesific Extract Parameters. The specific parameters include phytochemical screening, water and ethanol-soluble compounds, and organoleptic testing. The results shown in Table 1; Show that phytochemical screening aimed to give a general overview of the extract's constituents. The analysis of the extract of *A. manihot* (L.) Medik in 70% ethanol revealed that it included alkaloids, saponins, flavonoids, steroids, and triterpenoids in the literature. It contains flavonoids and steroids which can provide antidiabetic activity. The amount (%) of compounds dissolved in water was greater than the levels of dissolved compounds in ethanol. This was because the water solvent can attract more polar compounds compared to non- and semi-polar compounds. Secondary metabolites that may be absorbed in water solvents aresaponins and flavonoids. The secondary metabolites that were absorbed in the ethanol solvent were thought to be saponins, steroids, triterpenoids, and flavonoids. Thus, utilising a polar solvent will be the most advantageous extraction method. The results of the organoleptic examination showed that the extract obtained had a blackish-green colour, a distinctive odour, and an astringent taste that met the standards.

The Nonspesific Extract Parameters. The nonspecific parameters that have been tested include water content, total ash content, acid-insoluble ash content, heavy metal contamination, mould, and yeast contamination, microbial contamination, and total flavonoid. Determining the extract's moisture level was crucial for assuring its quality because a high water content will increase humidity and raise the possibility of unwelcome microbe growth. A low moisture level indicates a good extract. The Indonesian Herbal Pharmacopoeia states that the allowed water content must be less than 10%. This study's ethanolic 70% extract of *A. manihot* (L.) Medik has a water content of 4.20%. Hence, it satisfies the required standards for quality.

Table 1. The outcome of standardising specific parameters in the 70% ethanolic *A. manihot* (L.) Medik extract.

Parameter	Outcome
Organoleptic	Colour: greenish black Odour: distinctive smell Taste: astringent
Content of water-soluble compounds (%)	70.5±1.67
Content of ethanol-soluble compounds (%)	49.46±1.94
Phytochemistry Content	Alkaloid, flavonoid, saponin, steroid, triterpenoid

Total ash attempts to give a general picture of the mineral composition both internally and externally, starting at the extraction stage. The ash content tests carried out were total ash content and acid-insoluble ash content. Total ash content was the ash produced from extracts fired in the furnace. The total ash content test aims to determine the content of physiological mineral compounds (K, Mg, and Ca) and non-physiological compounds (dust, pollutants, and soil) contained in the extract. The total ash content test results that have been carried out (Table 2) show a total ash content of 6.97%±0.42. The more ash there was in the extract, the more minerals were present in it. Acid-insoluble ash content was the residual ash obtained from determining the total acid-insoluble ash content. Acid insoluble ash content was determined to determine heavy metal compounds (Hg, Pb, and Cd) and silicates that were insoluble in acid. The results of the acid-insoluble ash content test that has been carried out (Table 2) show an acid-insoluble ash content of 0.60%±0.072. Previous studies have shown that the acid-insoluble ash content of *A. manihot* (L.) Medik leaf extract ranges from 0.5-0.83%[2,15].

Testing for heavy metal contamination was carried out to ensure that the extract did not contain certain heavy metals exceeding the specified value, which was harmful to health. Heavy metal contamination can come from the environment where these plants grow. The maximum limits for Pb and Cd metal content were less than 10 mg/kg and less than 0.3 mg/kg[16]. The results of the heavy metal contamination tests that have been carried out (Table 2) show that the contents of Pb and Cd metals in the extract were, respectively 1.35 mg/kg and 0.06 mg/kg. Based on this, the 70% ethanol extract of *A. manihot* (L.) Medik leaves met the established standards.

Table 2. The outcome of standardising nonspecific parameters in the ethanolic 70% extract of *A. manihot* (L.) Medik.

Parameter	Outcome
Water content (%)	4.2±0.42
Total ash content (%)	6.97±0.42
Acid-insoluble ash content (%)	0.60±0.072
Pb (mg/kg)	1.35
Cd (mg/kg)	0.06
Mold and yeast Contamination (colonies/g)	≤ 10
Total Plate Number (colonies/g)	≤ 100
Total Flavonoid as Quarcetin (%)	1.2

Many microbiological pollutants, such as bacteria, fungi, and viruses, have been linked to herbal medicinal plants. The environment, one of several factors that influence this microbiological growth, significantly impacts the quality of herbal remedies. Herbal medications frequently contain bacteria and fungi that were derived from the soil. Further contamination, such as the potential development of Escherichia coli or Salmonella spp., can also be brought on by harvesting practices, wet sorting, dry sorting, and inadequate storage. Even though many different types of bacteria and fungi were derived from the natural microflora, only aerobic spore-forming bacteria were frequently dominant. The presence of bacteria (Total Plate Number) and yeast moulds (Yeast Fungi Number) in each sample revealed the existence of microbial contamination. According to Table 2, the ethanolic 70% extract of *A. manihot* (L.) Medik leaves had 1.0 x 10 colonies per gr of mould yeast number and 1.0 x 100 colonies per gr total plate number. According to POM RI, the maximum number of mould colonies per gram was, therefore, 1 x 10^3 colonies; thus ethanol 70% extract of *A. manihot* (L.) Medik leaves can be assumed to have met the standards for microbial contamination and mould yeast number[16].

The result of IC_{50} of the extract was calculated using a regression equation from the graph. The result showed (Table 3) that extract *A. manihot* (L.) Medik leaves had lower inhibitory activity than acarbose, with IC_{50} values of 81.84±2.97 µg/mL and 42.6 ±1.21µg/mL. This study (Table 1) showed that ethanol extracts 70% *A. manihot* (L.) Medik contain flavonoids, alkaloids, saponins, steroids, and terpenoids. Flavonoids were the greatest class of compounds that showed α-glucosidase inhibitory activity[17,18]. Plants produce a wide variety of hydroxylated phenolic compounds known as flavonoids, which include the subclasses of isoflavonoids, flavanones, flavanols, flavonols, and flavones. Sesquiterpenoids, diterpenoids, and triterpenoids collectively make up the 95 terpenoids with α-glucosidase inhibitory action that have been reported[19]. Based on previous studies, 70% ethanol extract of *A. manihot* (L.) Medik leaves has activity as an α-glucosidase inhibitor due to the flavonoids and terpenoids in the extract.

Table 3. *A. manihot* extract's leaf of ethanol 70% IC_{50} α-glucosidase inhibitory activity.

Sample	IC_{50} (µg/mL)
Acarbose	42.6±2.97
Extract *A. manihot*	81.84±1.21

CONCLUSION

The ethanolic 70% extract of *A. manihot* (L.) Medik has been found to meet the requirements based on standardising particular and non-specific parameters**.** Screening results of a 70% ethanol extract of *A. manihot* (L.) Medik leaves contain alkaloids, flavonoids, saponins, steroids, and triterpenoids**.** *A. manihot* (L.) Medik leaf extract has a thick consistency, greenish-black colour, distinctive aromatic odour, and astringent taste. Levels of dissolved compounds in ethanol 49.46%; content of compounds dissolved in water 70.5%; water content 4.2%; ash content 6.97%; acid insoluble ash content 0.60%; heavy metal contamination Pb 1.35 ppm and Cd 0.06 ppm; microbiological contamination TPC <100 colonies/g and TYMC ≤10 colonies/g; total flavonoid as quercetin content 1.2%. In vitro test results of α-glucosidase enzyme inhibitory activity against 70% ethanol extract of *A. manihot* (L.) Medik leaves showed an IC_{50} value of 81.84 µg/mL and acarbose as a positive control with an IC_{50} value of 42.6 µg/mL. For the treatment of diabetes, *A. manihot* (L.) Medik may have α-glucosidase inhibitor action. The 70% ethanol extract of *A. manihot* (L.) Medik leaves has the potential to be developed as an antidiabetic herbal medicine. Further research was needed for in vivo studies and fractionation of *A. manihot* (L.) Medik leaf extract for better.

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