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Research Article
Phytochemical Composition and Antioxidant Potential of Elephant Cassava (*Manihot esculenta* var. Gajah): A Promising Functional Food from Muna Island, Indonesia

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Abstract

Background and Objective: Functional foods containing abundant bioactive compounds have gained increasing attention for their contributions to health promotion and the prevention of degenerative disorders. Elephant cassava (*Manihot esculenta* var. Gajah), a local cultivar grown on Muna Island, Indonesia, is notable for its large tubers and high productivity. Therefore, this research was designed to investigate its phytochemical composition, antioxidant potential and cyanogenic safety of elephant cassava to evaluate its feasibility as a functional food source. **Materials and Methods:** Ethanol extracts of elephant cassava tubers were prepared by maceration and fractionated by solvent partitioning. Qualitative phytochemical screening, thin-layer chromatography (TLC), antioxidant assays using DPPH and FRAP methods and qualitative cyanide detection via alkaline picrate test were performed. Furthermore, LC-MS/MS analysis was employed to identify the major bioactive compounds, followed by the structural elucidation of the isolated molecules using FTIR, NMR and MS techniques. **Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics and steroids, while saponins, tannins and triterpenoids were absent. Antioxidant assays demonstrated strong radical scavenging and reducing capacities, with IC values of 20.9 µg/mL for the extract and 11.8 µg/mL for vitamin C, categorizing the extract as highly active. The FRAP assay further confirmed the comparable reducing power of this product to that of vitamin C. Cyanogenic glycosides were undetectable, indicating the product's safety for consumption. The LC-MS/MS analysis identified key metabolites, including myo-inositol, trehalose, nicotinamide, adenine, indoline, palmitic acid glycerol ester and 1-stearoylglycerol, all of which contribute to the nutritional and pharmacological potential of this compound. **Conclusion:** Elephant cassava tubers possess a rich phytochemical profile and exhibit strong antioxidant activity with no detectable cyanogenic toxicity. The presence of nutritionally valuable and bioactive compounds underscores its potential as a safe and promising candidate for the development of functional foods.

Key words: *Manihot esculenta* var. Gajah, functional food, phytochemicals, antioxidant activity, LC-MS/MS, cyanogenic glycosides

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Functional foods containing bioactive compounds have recently attracted considerable attention due to their proven significant health benefits and their role in preventing various degenerative diseases[1]. Although present in relatively small amounts, bioactive compounds in food can contribute to the regulation of biological mechanisms in the body, thereby playing an important role in both the prevention and treatment of metabolic disorders. Among the diverse groups of bioactives, antioxidants hold a particularly crucial role, as they inhibit oxidative processes, maintain biomolecular stability and reduce the risk of chronic diseases[2]. However, the endogenous antioxidant defense system of the human body has limitations, making dietary intake of exogenous antioxidants highly necessary. According to Boukhers *et al.*[3], consuming antioxidant-rich foods is effective in neutralizing excess free radicals and suppressing oxidative stress, a major contributor to cellular and tissue damage.

Plants are one of the primary sources of phytochemicals, which possess potential nutraceutical and therapeutic properties that exert beneficial effects on human health, both in disease prevention and treatment. Owing to their vast chemical diversity and wide range of biological activities, plants have become a global research focus[4]. Among tropical food crops, cassava (*Manihot esculenta*) holds strategic value, serving not only as a staple carbohydrate source but also as a critical commodity for food security in developing countries, particularly in tropical regions[5]. Cassava is known to contain a variety of secondary metabolites, including terpenoids, flavonoids, alkaloids and polyphenols, which play an important role in biological activities. This demonstrates cassava's potential as a natural source of antioxidants capable of neutralizing free radicals[6]. In addition, plant-derived polyphenols possess both antioxidant and anti-inflammatory properties, contributing to the prevention of metabolic diseases such as diabetes and cardiovascular disorders[7]. Therefore, the utilization of local plants rich in bioactive compounds, such as cassava, represents an important strategy in the development of functional foods based on biological resources.

One of the local cassava varieties that has not been extensively studied is elephant cassava (*Manihot esculenta* var. Gajah). This plant is known for producing exceptionally large tubers with high productivity, reaching approximately 50 kg/plant. The variety is widely cultivated on Muna Island, Indonesia; however, to date, no comprehensive scientific reports are available regarding its phytochemical profile or antioxidant capacity. Previous studies have primarily focused on common cassava varieties or on specific plant parts, such as leaves and peels, to investigate phenolic content and antioxidant activity[3]. Moreover, the safety aspects of this local variety have not been thoroughly evaluated, particularly with respect to its content of cyanogenic compounds, namely Hydrogen Cyanide (HCN), which is toxic if consumed without proper processing[5]. Therefore, investigations into the phytochemical composition, antioxidant activity and HCN levels of elephant cassava are essential to ensure both the potential and safety of this variety as a source of functional food.

The objective of this study is to identify the phytochemical profile and evaluate the bioactive properties of elephant cassava (*Manihot esculenta* var. Gajah), as well as to analyze its Cyanogenic Compound (HCN) content to ensure its safety. The findings of this research are expected to provide a scientific basis for the utilization and development of elephant cassava as a functional food.

MATERIALS AND METHODS

Study area: This research was conducted in Muna Regency, Southeast Sulawesi, Indonesia, from May to August, 2025. Muna Regency is part of the Sulawesi Islands and is characterized by a tropical monsoon climate, with an average annual temperature ranging between 26-34°C and relatively high humidity. The annual rainfall is relatively high but unevenly distributed throughout the year, with the peak rainy season occurring from November to May. The local community widely cultivates elephant cassava (*Manihot esculenta* var. Gajah) as a staple food due to its high productivity and strong adaptability to both dry and marginal lands.

Plant collection and identification: Elephant cassava (*Manihot esculenta* var. Gajah) was collected from the Muna Regency Area, Southeast Sulawesi, Indonesia. The plant material was subsequently transported to the Pharmacy Laboratory at Mandala Waluya University for identification and sample preparation. Botanical identification was performed using a morphological approach to confirm the authenticity of the specimen as *Manihot esculenta* var. Gajah. The tuber portions were thoroughly washed to remove any adhering soil and then sliced into thin sections. The slices were dried in an oven at a controlled temperature (±45°C) until completely dehydrated. The dried material was then ground into simplicia powder, which was subsequently used as the raw material for the extraction process[8].

Extraction using the maceration method: The extraction of bioactive compounds from elephant cassava (*Manihot esculenta* var. Gajah) was carried out using the maceration method with 96% ethanol as the solvent. A total of 530.7 g of simplicia powder was immersed in approximately 12 L of ethanol at room temperature. The maceration process was conducted in three cycles, each lasting 24 hrs, with periodic stirring to facilitate the diffusion of soluble compounds. At the end of each cycle, the mixture was filtered and the residue was re-extracted with fresh ethanol to maximize the yield of bioactive constituents. The combined filtrates from the three maceration cycles were then evaporated using a rotary vacuum evaporator until a thick green extract was obtained, with a total mass of 19.4 g. This value was used to calculate the extraction yield, which corresponded to approximately 3.7% of the dry weight of the simplicia[9].

Fractionation of the ethanol extract: The crude ethanol extract was subsequently fractionated to separate bioactive components based on differences in polarity. Fractionation was carried out using liquid-liquid partitioning with non-polar, semi-polar and polar solvents. In the initial step, the concentrated extract was dissolved in distilled water and extracted with n-hexane to obtain the non-polar fraction enriched with hydrophobic compounds. The remaining aqueous phase was then further extracted with ethyl acetate to yield the semi-polar fraction, while the residual aqueous phase was designated as the polar fraction. Through this stepwise partitioning approach, the ethanol extract was separated into three principal fractions: Non-polar (n-hexane), semi-polar (ethyl acetate) and polar (water), each of which was subsequently subjected to further analysis[10].

Phytochemical screening: Qualitative phytochemical screening was carried out on the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) to determine the classes of secondary metabolites present. Alkaloid detection was carried out using Mayer's and Dragendorff's reagents. A positive reaction was indicated by the formation of a white precipitate with Mayer's reagent or an orange-red coloration with Dragendorff's reagent. Flavonoids were tested using the Shinoda method, in which the addition of magnesium powder and concentrated HCl produced a red coloration as an indicator of flavonoid presence. Saponins were examined using the frothing test, where shaking the extract in water or dilute HCl yielded stable foam as a positive result. Tannins were detected using 1% $FeCl_3$ solution, with a blue-green or dark black coloration indicating their presence. Phenolic compounds were tested with 1 N NaOH solution, where the appearance of a red coloration confirmed a positive reaction. Terpenoids and steroids were evaluated using the Liebermann-Burchard reagent, in which the appearance of a green ring suggested triterpenoids, whereas a purple ring indicated steroids. All reactions were observed visually and the results were recorded as either positive or negative[11].

Thin-layer chromatography (TLC): The chromatographic profile of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) was analyzed using the thin-layer chromatography (TLC) method. Silica gel 60 F plates were employed as the stationary phase, while the mobile phase consisted of mixtures of organic solvents with varying polarity. The extract of *Manihot esculenta* var. Gajah was applied onto the baseline of the TLC plate and then developed in a closed chromatographic chamber containing the selected eluents. Upon completion of elution, the separated compound spots were observed under UV light at wavelengths of 254 nm, where compounds appeared as dark spots against a fluorescent background and at 366 nm, where certain spots exhibited characteristic fluorescence (e.g., blue, green or orange), depending on the type of compound.

The retention factor (Rf) values were calculated by comparing the migration distance of each compound spot with that of the solvent front. Low Rf values (0.1-0.3) generally indicated polar compounds, medium Rf values (0.3-0.7) suggested semi-polar compounds and high Rf values (0.7-0.9) were characteristic of non-polar compounds. Preliminary interpretation of compound classes was based on fluorescence patterns; for instance, bright blue spots often indicated the presence of alkaloids or indole derivatives, yellowish-green spots suggested flavonoids, whereas orange-redish spots were typically associated with anthraquinones or curcumin-like compounds[11].

Antioxidant activity assay: The antioxidant activity of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) was evaluated using two complementary methods: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay. In the DPPH method, the basic principle lies in the ability of antioxidant compounds to donate hydrogen atoms or electrons to neutralize DPPH free radicals. The DPPH exhibits a deep violet color in its radical form, but upon reacting with antioxidants, it is reduced to the non-radical form (DPPH-H), which appears pale yellow. This color change can be quantitatively measured using a UV-Vis spectrophotometer at 517 nm. The decrease in absorbance was calculated as the percentage of inhibition relative to the control and

dose-response data were used to determine the IC value, defined as the concentration of extract required to inhibit 50% of DPPH radicals. A lower IC value indicates stronger antioxidant activity. The FRAP method is based on a different principle, measuring the ability of antioxidant compounds to reduce Fe^{3+} to Fe^{2+} under acidic conditions. The reduction product forms a blue Fe^{2+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex with maximum absorbance at 593 nm. For the assay, the extract was mixed with freshly prepared FRAP reagent consisting of acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. The mixture was incubated for approximately 10 min at room temperature, after which absorbance was measured at 593 nm. Antioxidant activity was expressed in equivalents relative to a reference standard[12].

Qualitative test for cyanide (HCN): To detect the presence of cyanogenic compounds capable of releasing Hydrogen Cyanide (HCN), a qualitative test was performed using the alkaline picrate paper method. The extract sample was placed in a sealed container, followed by the addition of dilute HCl solution as a hydrolysis agent. The container was equipped with a filter paper strip impregnated with alkaline picrate solution, prepared from picric acid and sodium carbonate (Na_2CO_3). If the sample contained cyanogenic glycosides, such as linamarin or lotaustralin, acid hydrolysis would cleave these compounds, releasing HCN in a gaseous form. The liberated HCN then reacted with the yellow picrate paper, reducing the picrate to picramic acid, which caused the paper to change color from yellow to orange-red or brown. This color change was interpreted as a positive result, indicating the presence of free HCN or its cyanogenic precursors in the sample[13].

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis: The characterization of bioactive compounds from the ethanol extract and its fractions of elephant cassava (*Manihot esculenta* var. Gajah) was performed using high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Compound separation was carried out on a reversed-phase C18 column with a particle size of 1.7-5 µm. The mobile phase consisted of water containing 0.1% formic acid and acetonitrile with 0.1% formic acid, applied in a gradient system at a flow rate of 0.2-0.3 mL/min to achieve optimal resolution[13]. Detection was conducted using a high-resolution mass spectrometer equipped with an electrospray ionization (ESI) source. The instrument was operated in both positive and negative ionization modes, allowing for the identification of compounds with varying degrees of polarity. During chromatographic separation, peaks observed in the UV chromatogram (254 nm) were ionized and the mass spectra were recorded at different m/z ratios. Fragmentation data obtained from MS/MS analysis were then used to generate molecular fragmentation patterns. These patterns were compared against database libraries and relevant scientific literature, enabling the identification of the molecular structures of bioactive compounds[14,15].

Determination of active compound structure: The pure compounds successfully isolated from the active fractions were characterized using spectroscopic techniques, namely Fourier Transform Infrared (FTIR), Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS). The FTIR spectroscopy was employed to identify the principal functional groups within the molecule based on the absorption patterns of chemical bond vibrations. For instance, an absorption band around 3400 cm^{-1} typically indicates the presence of hydroxyl (-OH) groups, whereas a band near 1700 cm^{-1} suggests the presence of a carbonyl (C=O) group. These data provide preliminary information regarding the functional group composition of the molecule. Subsequently, NMR spectroscopy (^{1}H-NMR and ^{13}C-NMR), including two-dimensional techniques such as HSQC and HMBC, was applied to analyze the hydrogen and carbon frameworks of the molecule[16]. This method elucidates the number of atoms, their chemical environments and the connectivity between atoms, thereby enabling a detailed elucidation of organic compound structures. Mass spectrometry (MS) complemented the FTIR and NMR data by providing information on molecular weight, molecular formula and fragmentation patterns. MS/MS analysis was employed to examine molecular fragments, while electrospray ionization (ESI) enabled the detection of molecular ion peaks. The resulting fragmentation data were then compared with library databases to confirm compound identity[17].

RESULTS AND DISCUSSION

Yield and phytochemical composition of elephant cassava tuber extract: The extraction of elephant cassava (*Manihot esculenta* var. Gajah) tubers was performed using the maceration method with ethanol as the solvent, which is known to effectively dissolve a wide range of polar and semi-polar compounds, including phenolics, flavonoids, alkaloids and glycosides[18]. The yield of the elephant cassava extract was determined and the results are presented in Table 1.

Table 1: Percentage yield of elephant cassava tuber extract

Weight until dry (g)	Extract weight (g)	Percentage of yield
530.7	19.4	27.8

Table 2: Data from testing the compound content of elephant cassava

Chemical content	Reagent	Results	Remarks
Alkaloids	Mayer	White sediment	+
	Dragendorff	Orange-red	+
Flavonoids	Mg powder+HCl	Red	+
Saponins	HCl	Foam	-
Tannins	$FeCl_3$ (1%)	Blue-green	-
Phenols	NaOH (1N)	Red	+
Triterpenoids	Liebermann Burchard	Green ring	-
Steroids	Liebermann Burchard	Violet ring	+

Table 1 presents the percentage yield of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah). From 530.7 g of dried material, 19.4 g of thick extract was obtained, corresponding to an extraction yield of 27.8%. This yield is relatively high compared to previous reports on other cassava varieties, which typically yield between 10 and 20%[19]. The high percentage yield indicates that elephant cassava tubers contain abundant secondary metabolites, particularly polar compounds such as phenolics, flavonoids, alkaloids and glycosides, which are soluble in ethanol[20]. Furthermore, the 27.8% yield suggests that elephant cassava tubers are not only rich in carbohydrates and starch but also possess significant fractions of secondary metabolites with potential as raw materials for functional food development.

Subsequently, phytochemical screening was carried out on the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) to identify the classes of natural compounds present. The testing was conducted to provide a preliminary overview of the bioactive compound profile contained in the extract. The results of the phytochemical identification of elephant cassava are presented in Table 2.

The results of phytochemical screening of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) revealed the presence of several classes of secondary metabolites. The alkaloid test using Mayer's reagent produced a white precipitate, while Dragendorff's reagent yielded an orange-red coloration, both indicating the presence of alkaloids in the extract. The detection of alkaloids is noteworthy, as these compounds play a role in plant defense mechanisms and possess potential biological activities, including antimicrobial and anti-inflammatory properties[9]. In addition, the flavonoid test using the Shinoda method produced a red coloration, indicating the presence of flavonoids, particularly those belonging to the flavonol or flavanone groups. This positive result was further supported by the detection of phenolics, as confirmed by the NaOH 1N test, which produced a red coloration. Flavonoids and phenolic compounds are biologically significant due to their ability to donate electrons or hydrogen atoms, thereby neutralizing free radicals and contributing to the antioxidant activity of the extract[21].

In the tannin test using 1% $FeCl_3$ reagent, no blue-black or greenish coloration was observed, indicating that the tannin content in the extract was low. Similarly, the foam test for saponins yielded a negative result, suggesting that the saponin content was either very low or absent. This finding is consistent with previous reports indicating that cassava tubers generally contain lower levels of tannins and saponins compared to the leaves[9]. In contrast, the test for steroids and triterpenoids using the Liebermann-Burchard reagent produced a purple ring, which indicated a positive result for steroids, while triterpenoids were not detected. The presence of steroids is in line with literature reports that cassava contains phytosterols and triterpene derivatives, which are potential bioactive compounds with antimicrobial and antiviral activities[22].

Overall, the phytochemical profile of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) was characterized by the presence of alkaloids, flavonoids, phenolics and steroids. At the same time, saponins, tannins and triterpenoids were not detected. These findings are consistent with the report of Mohidin *et al.*[9], which stated that ethanol extracts of cassava contain alkaloids, flavonoids and phenolics but are very poor in saponins and tannins. Accordingly, this phytochemical composition may account for the biological activities observed, particularly the antioxidant potential of the elephant cassava extract.

DPPH and FRAP antioxidant activity: The antioxidant activity of the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) was evaluated using two approaches, namely the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay and the ferric reducing antioxidant power (FRAP) assay. These methods were selected because they represent two different

mechanisms of antioxidant action: Hydrogen atom transfer (HAT) in the DPPH assay and single electron transfer (SET) in the FRAP assay[23]. In the DPPH assay, the extract demonstrated a strong capacity to neutralize free radicals. The color change of the DPPH solution from purple to yellow, resulting from the donation of hydrogen atoms by antioxidant compounds, was used as an indicator of radical scavenging activity. The detailed results of the DPPH inhibition percentages at various concentrations are presented in Table 3.

In the DPPH assay, the extract of elephant cassava (*Manihot esculenta* var. Gajah) demonstrated a high efficiency in neutralizing free radicals. The color change of the DPPH solution from purple to yellow, resulting from the donation of hydrogen atoms by antioxidant compounds, was used as an indicator of radical inhibition. The radical scavenging percentage of the extract increased with rising concentrations; however, at higher concentrations, some color interference was observed, which may have affected the spectrophotometric readings. At concentrations ranging from 25 to 100 ppm, the tuber extract was able to reduce free radicals by 15-21%, which was comparable to the effectiveness of vitamin C used as a control (11-22%) within the same concentration range. To evaluate the intrinsic antioxidant potential, the IC_{50} value was determined by applying linear regression analysis to the dose-response curve, which represents the concentration required to achieve 50% inhibition of the antioxidant activity.

Based on Fig. 1a-b, it is evident that the slope of the vitamin C curve is steeper than that of the extract, indicating that each incremental increase in vitamin C concentration produces a sharper rise in radical scavenging activity compared to the elephant cassava extract. However, extrapolation of both curves shows that 50% inhibition was achieved at relatively low concentrations. This finding highlights the potential of elephant cassava extract to effectively neutralize radicals in lipid-rich environments. From the dose-response curve analysis, the IC_{50} value of the extract was determined to be 20.9 µg/mL, while that of vitamin C was 11.8 µg/mL. According to Molyneux's classification, IC values below 50 µg/mL indicate very strong antioxidant activity[23]. Thus, although slightly lower than vitamin C, the elephant cassava tuber extract still exhibits competitive antioxidant capacity. These results are consistent with previous reports on cassava leaves, which demonstrated very strong antioxidant activity with IC values in the range of 17-30 µg/mL[24]. Such consistency indicates that elephant cassava is rich in antioxidant compounds, particularly phenolics and flavonoids, which serve as the major contributors to its DPPH radical scavenging activity.

The FRAP assay was conducted to complement the understanding of the antioxidant mechanism of elephant cassava extract, specifically its ability to reduce Fe^{3+} ions to Fe^{2+} within the ferroin complex (Fe(III)-TPTZ to Fe(II)-TPTZ, which appears blue)[25]. Unlike the DPPH assay, which is based on hydrogen atom transfer, the FRAP assay evaluates antioxidant capacity through electron transfer. The FRAP results of elephant cassava extract in comparison with vitamin C are presented in Table 4.

Table 3: Results of DPPH method inhibition percentage analysis

Sample type	Concentration (ppm)	Absorbance blank	Absorbance sample	Inhibition (%)
Vitamin C	100	0.42	0.355	10.95
	75	0.42	0.354	14.76
	50	0.42	0.351	18.09
	25	0.42	0.331	21.90
Elephant cassava	100	0.42	0.374	15.47
	75	0.42	0.358	15.71
	50	0.42	0.344	16.42
	25	0.42	0.328	21.19

Table 4: Results of percentage inhibition analysis using the FRAP method

Sample type	Concentration (ppm)	Absorbance blank	Absorbance sample	Inhibition (%)
Vitamin C	20	0.714	0.355	50.26
	40	0.714	0.333	53.39
	60	0.714	0.312	56.32
	80	0.714	0.295	58.70
	100	0.714	0.272	61.88
	120	0.714	0.252	64.72
Elephant cassava	20	0.714	0.385	46.10
	40	0.714	0.368	48.53
	60	0.714	0.344	51.89
	80	0.714	0.324	54.69
	100	0.714	0.303	57.63
	120	0.714	0.282	60.47

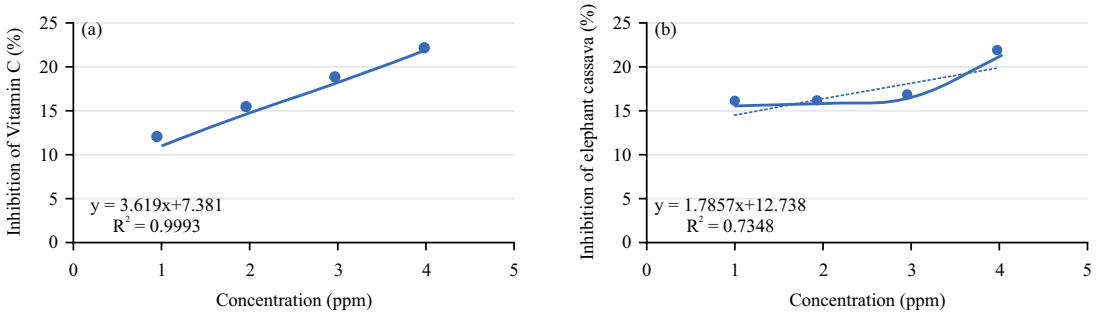


Fig. 1(a-b): Graph of concentration-dependent activity in the DPPH assay, (a) Vitamin C and (b) Elephant cassava (*Manihot esculenta* var. Gajah) extract

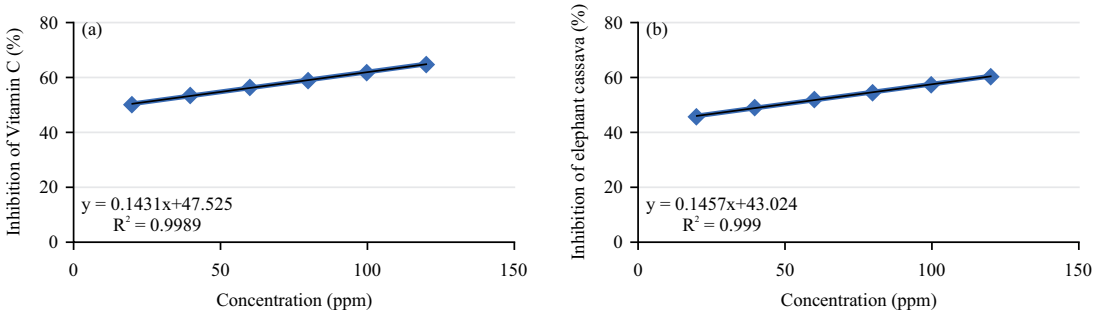


Fig. 2(a-b): Graph of concentration-dependent activity in the FRAP assay, (a) Vitamin C and (b) Elephant cassava (*Manihot esculenta* var. Gajah) extract

The results of the antioxidant activity test using the FRAP method on the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) tubers demonstrated a trend of increasing reduction capacity with rising sample concentrations. At the lowest concentration of 20 ppm, the extract achieved an inhibition of 46.10%, whereas vitamin C, as the positive control, reached 50.26%. This value continued to increase with higher concentrations, reaching a maximum of 120 ppm, at which the elephant cassava extract recorded 60.48%, only slightly lower than vitamin C, which reached 64.72%. These findings confirm that the elephant cassava tuber extract exhibits a strong electron-donating capacity, comparable to that of vitamin C.

The consistent increase in inhibition percentage from 20 to 120 ppm for both samples indicated a linear relationship between concentration and reduction activity. This phenomenon suggests the involvement of phenolic and flavonoid compounds present in the elephant cassava extract, which act as electron donors in the reduction of Fe^{3+} to Fe^{2+} [26]. The high antioxidant activity observed here is in agreement with the results of the previous DPPH assay, thereby strengthening the evidence that the elephant cassava extract operates through dual mechanisms-hydrogen atom donation and electron transfer-in neutralizing free radicals. To further illustrate the relationship between sample concentration and reduction capacity, the FRAP results were plotted as a linear regression curve, as presented in Fig. 2a-b.

Figure 2a-b illustrates the relationship between sample concentration and the reducing capacity of Fe^{3+} to Fe^{2+} as determined by the FRAP method, for both the positive control and the elephant cassava tuber extract. For vitamin C, the regression equation obtained was $y = 0.1431x+47.525$ with a coefficient of determination (R^2) of 0.9989. In comparison, the elephant cassava extract yielded a regression equation of $y = 0.1457x+43.024$ with an R^2 value of 0.999. This indicates a very strong correlation between the concentration of elephant cassava extract and its reducing capacity. Notably, the regression coefficient of elephant cassava was slightly higher than that of vitamin C, suggesting that bioactive compounds in cassava, particularly phenolics and flavonoids, contribute to its antioxidant activity. However, its effectiveness at lower doses is more limited compared to vitamin C[12]. Furthermore, the antioxidant potential was confirmed by calculating the IC_{50} value. In this study, vitamin C exhibited a relatively low IC_{50}

value, indicating very strong antioxidant activity. Conversely, the elephant cassava tuber extract demonstrated a higher IC_{50} value than vitamin C, yet still fell within the criteria for effective antioxidant activity. This finding confirms that phenolic and flavonoid compounds in elephant cassava tubers play a significant role in reduction activity, even though they are less effective than vitamin C at lower concentrations[27].

HCN content and safety implications of the extract: The safety of cassava consumption is closely related to its Hydrogen Cyanide (HCN) content, which is released from the hydrolysis of cyanogenic glycosides, primarily linamarin and lotaustralin, naturally present in cassava tissues[28]. The observed levels of HCN in elephant cassava tubers are presented in the following Table 5.

The HCN content in the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) was qualitatively evaluated using the picrate paper method. The results showed that the picrate paper, moistened with a mixture of 5% tartaric acid and 8% Na_2CO_3, exhibited no color change either before or after heating at 80°C. This indicates that HCN was not detected in the extract, with levels below the detection limit of the picrate method (1-2 mg per 100 g sample). This finding is highly significant from a food safety perspective. For comparison, raw cassava leaves have been reported to contain HCN in the range of 53-1300 mg/kg, thus requiring processing methods such as boiling or drying to reduce toxicity levels[29]. The negative result of this qualitative test demonstrates that the elephant cassava variety is safe for consumption, as its cyanide content lies far below the threshold of toxicity.

Characterization of active compounds by LC-MS/MS: The characterization of active compounds in plant extracts is a crucial step in elucidating their chemical composition and potential bioactivity. In this study, LC-MS/MS analysis was performed on the ethanol extract of elephant cassava (*Manihot esculenta* var. Gajah) to identify specific metabolites underlying its biological activities. Compound identification in the positive electrospray ionization mode detected several molecules representing different groups, including polar compounds (sugar alcohols, disaccharides, vitamins and nucleobases), indolic alkaloids and lipids in the form of fatty acid monoglycerides. This diversity reflects the multifunctional potential of elephant cassava extract from both nutritional and pharmacological perspectives, thereby reinforcing its position as a promising candidate for the development of functional foods[29]. The identified compounds in the ethanol extract of elephant cassava are presented in Table 6.

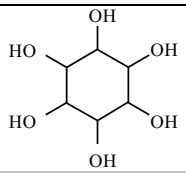
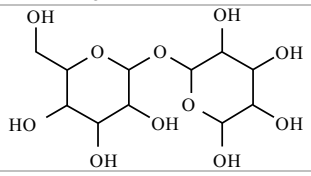
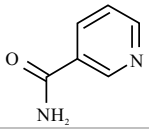
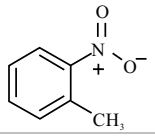
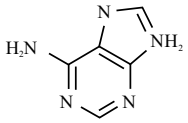
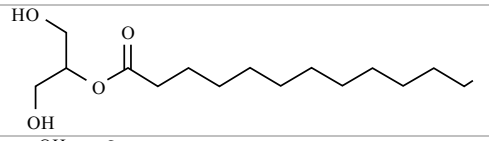
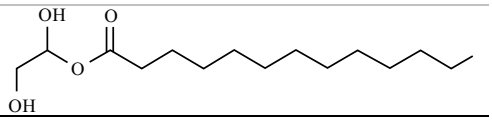
Table 6 shows seven major compounds identified in the elephant cassava extract. These include carbohydrate and sugar derivatives (myo-inositol and trehalose), a vitamin (nicotinamide), a nucleobase (adenine), an indolic alkaloid (indoline) and lipid monoglycerides (palmitic acid monoester and 1-stearoylglycerol). Myo-inositol is a cyclic polyol known as an essential component in various cellular processes. It plays a crucial role in cell growth and survival, contributing to the development of neural tissue, bone formation and reproductive functions. Myo-inositol is also involved in intracellular signal transduction as a second messenger for various hormones, neurotransmitters and growth factors, in addition to maintaining cellular osmotic balance. From a health perspective, myo-inositol has been widely studied as a nutritional supplement due to its ability to improve insulin sensitivity and modulate metabolic parameters[30]. Therefore, the presence of myo-inositol in the extract suggests potential metabolic benefits, such as supporting blood glucose regulation and hormonal function, thereby reinforcing the role of elephant cassava as a functional food candidate for the prevention of metabolic diseases.

The carbohydrate compound trehalose was also detected in the elephant cassava extract, contributing to its value as a functional nutrient. Trehalose is a non-reducing disaccharide composed of two glucose units linked by an α,α-1,1-glycosidic bond. This sugar is commonly found in legumes, seaweed and fungi and has been part of the human diet for a long time. Trehalose possesses a mild sweetness and hygroscopic properties that enable it to serve as a stabilizing agent, preventing premature aging in starch- and protein-based products. The consumption of trehalose has been reported to prevent various common health problems, including osteoporosis, metabolic syndrome and neurodegenerative diseases such as Alzheimer's. In animal model studies, trehalose has been shown to improve glucose tolerance and enhance insulin sensitivity while suppressing the enlargement of visceral adipocytes. A clinical study in healthy humans further reported that daily intake of 10 g of trehalose improved postprandial glucose response compared to sucrose[31]. Thus, the presence of trehalose in elephant cassava tubers enhances its functional value as a food capable of supporting glucose homeostasis and preventing metabolic complications.

Table 5: Qualitative analysis of HCN

Plant type	Before	After	Remark
Elephant cassava	Picrate paper yellow	Picrate paper yellow	Negative

Table 6: Identification of compounds in elephant cassava extract using Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

Compound	Formula	Observed RT (min)	Observed m/z	Structure
Myo-inositol	$C_6H_{12}O_6$	0.501	180.0639	
Trehalose	$C_{12}H_{22}O_{11}$	0.834	342.1162	
Nicotinamide	$C_6H_6N_2O$	1.332	122.0482	
Indoline	C_8H_9N	1.734	119.0735	
Adenine	$C_5H_5N_5$	3.009	135.0545	
Palmitic acid glycerol ester	$C_{19}H_{38}O_4$	13.695	330.2772	
1-stearoylglycerol	$C_{21}H_{42}O_4$	14.334	358.3089	

The identification of nicotinamide in the elephant cassava extract indicates that this plant contains an essential vitamin component. Nicotinamide is the active form of vitamin B3 and serves as a precursor of key coenzymes such as NAD^+/NADH and $NADP^+$/NADPH. This vitamin is required in hundreds of enzymatic reactions involved in energy production, DNA repair and various metabolic pathways. A deficiency of vitamin B3 leads to pellagra, whereas adequate intake protects cells against oxidative stress and the effects of aging. Nicotinamide supplementation has been shown to enhance cellular antioxidant status both directly, by scavenging free radicals and indirectly, by supporting endogenous antioxidant systems. Moreover, nicotinamide has been investigated for its anti-inflammatory and skin-protective effects, as well as its potential to prevent certain types of non-melanoma skin cancers. Overall, a sufficient intake of vitamin B3, in the form of nicotinamide, contributes to normal cellular signaling, DNA synthesis and repair and antioxidant defense in the body[32]. Therefore, the presence of nicotinamide highlights the nutritional value of elephant cassava, with its extract serving as a potential source of vitamin B3 to support metabolic health.

Adenine, identified in the extract, is one of the nucleotide bases that constitute nucleic acids and serves as an essential component of energy-related molecules. The presence of free adenine in the extract indicates the occurrence of endogenous nucleosides/nucleotides within the tuber. Numerous studies have shown that nucleotide supplementation supports immune function, enhances resistance to infections, accelerates intestinal cell recovery and improves hepatic metabolism. Additionally, dietary nucleotides function as prebiotics, helping to balance the gut

microbiota and promote gastrointestinal health[33]. In athletes, nucleotide consumption has been reported to reduce stress hormones, particularly cortisol, while maintaining immune function during intensive training[34]. Thus, although adenine itself is a simple molecule, its presence reflects the potential nutritional benefits of elephant cassava tubers as a source of nucleotides capable of supporting vital physiological functions, particularly in digestive and immune health.

In addition to the nutritional compounds described above, the elephant cassava extract also contained a heterocyclic alkaloid component, indoline. Indoline is a hydrogenated derivative of the indole ring. This structural class is widely distributed among plant secondary metabolites and is frequently associated with potent biological activities. In general, indole derivatives have been reported to exhibit a broad spectrum of pharmacological effects, including anticancer, antimicrobial, anti-inflammatory and antioxidant properties. The indole nucleus is considered an important pharmacophore in many bioactive compounds due to its ability to interact with diverse biomolecular targets within cells[35]. Tuber extracts of Amorphophallus paeoniifolius containing alkaloids exhibited antiallergic activity by inhibiting the release of histamine and serotonin. The detection of indoline in this analysis suggests that indole-based compounds in elephant cassava tubers may also contribute as bioactive components. Although indoline itself is a relatively simple molecule, it may serve as an indicator of the presence of other, more complex indole-derived alkaloids within the extract. The combination of indolic compounds may contribute to the antioxidant, anti-inflammatory, neuroprotective or even anticancer cytotoxic effects of elephant cassava extract, as has been documented for many medicinal plants rich in indole alkaloids.

The final two compounds identified were palmitic acid monoester glyceride and 1-stearoylglycerol, both of which belong to the monoglyceride class. Monoglycerides are lipids consisting of a single fatty acid esterified to glycerol, in this case, palmitic acid and stearic acid. Their presence reflects the lipid content in the tuber, partially released during the extraction process. Nutritionally, monoglycerides are known to be easily digestible and serve as an energy source for the body. Beyond their nutritional role, certain saturated monoglycerides have also been reported to possess natural antimicrobial activity. For example, medium-chain monoglycerides (C10-C14) can disrupt microbial cell membranes due to their amphiphilic properties, making them effective in inhibiting the growth of pathogenic bacteria and viruses. The most well-known example is monolaurin (C12), which has been widely used as a natural preservative due to its antibacterial and antiviral properties[36]. The presence of these two compounds in elephant cassava not only supports its antimicrobial potential but also enhances its application in the development of functional food products based on elephant cassava, as their emulsifying properties may facilitate the incorporation of the extract into food matrices.

Overall, the compound profile identified in the elephant cassava (*Manihot esculenta* var. Gajah) tuber extract demonstrates a synergy between nutritional content and pharmacological potential, supporting its role as a functional food. From a nutritional perspective, elephant cassava tubers provide essential components, including vitamins (nicotinamide), natural prebiotics (trehalose and myo-inositol) and nucleotides (adenine), which play crucial roles in metabolism. From a bioactive perspective, the extract contains phytochemicals such as indolic alkaloids (indoline) and monoglycerides (monopalmitin and monostearin), which are known to exhibit antimicrobial, anti-inflammatory, antioxidant and even anticancer activities. The combination of these compounds in elephant cassava aligns with previous research, which reports that *Amorphophallus paeoniifolius* exhibits diverse biological activities, including antibacterial, antioxidant, antitumor, analgesic and antidiarrheal effects. The identification of these bioactive constituents in elephant cassava reinforces the notion that this plant is not only a source of carbohydrates but also a promising functional food, with a diverse bioactive compound profile that can contribute to human health.

CONCLUSION

This study revealed that *Manihot esculenta* var. Gajah from Muna Island, Indonesia, possesses a diverse profile of bioactive compounds, including alkaloids, flavonoids, phenolics and steroids, with strong antioxidant activity as demonstrated by both DPPH and FRAP assays. The elephant cassava extract exhibited a high yield of 27.8% and a very strong antioxidant capacity, with an IC value comparable to that of vitamin C. The LC-MS/MS analysis successfully identified several important metabolites, such as myo-inositol, trehalose, nicotinamide, indoline, adenine and monoglycerides, which confer both nutritional and pharmacological value. Another important finding of this study is the absence of detectable Hydrogen Cyanide (HCN) in the qualitative analysis, indicating that this plant is safe for consumption. These findings emphasize that elephant cassava is not only a valuable source of carbohydrates but also holds significant potential for development as a functional food with nutraceutical benefits.

SIGNIFICANCE STATEMENT

This study provides comprehensive scientific evidence on the phytochemical profile, antioxidant activity and safety evaluation of elephant cassava (*Manihot esculenta* var. Gajah) from Indonesia. The discovery of diverse bioactive compounds with strong antioxidant potential, together with the absence of cyanogenic content, underscores its relevance as a safe and effective source of functional food. These findings strengthen the potential of elephant cassava as a plant-based functional food that not only contributes to the development of nutraceutical products but also supports the promotion of local varieties as part of sustainable and environmentally friendly dietary practices aimed at preventing chronic diseases associated with oxidative stress, particularly in tropical regions.

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