

Chemical profile of Leaves of Magana and NASE3 Varieties of *Manihot esculentum* Crantz Harvested from Bukedea District, Eastern Uganda

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ABSTRACT

Cassava leaves are widely consumed worldwide, particularly in the Bukedea district of Uganda, for food, medicine, and fodder. The existing correlation between high cassava leaf consumption and organ toxicity and failure remains poorly established within the Bukedea district. This investigation aimed to evaluate the phytochemical composition and toxicity profiles of cassava leaves consumed in the Bukedea District. An ethnobotanical survey was conducted among 60 participants selected from three villages in Bukedea, using a pretested questionnaire and an interview guide. The qualitative and quantitative phytochemical compositions of the ethanolic and aqueous extracts of NASE3 and Magana varieties were determined by High-Performance Liquid Chromatography (HPLC). Results were analyzed using R version 4.3.2 and presented as mean \pm SD in tables, Graphical HPLC reports, and figures generated by GraphPad Prism @9.00. An ethno-botanical survey revealed that NASE3 and Magana are the most commonly consumed varieties of cassava leaves, and highlighted their side effects. Qualitatively, Magana had significantly more phytochemicals than NASE3, which mainly consisted of toxic compounds ($p < 0.0001$). According to quantitative HPLC graphic reports, Magana had higher phytochemical concentrations than NASE3 ($p < 0.0001$). No significant difference ($p = 0.62-1.00$, $p > 0.0001$) in the mean concentration of the same phytochemical ingredient between the two extracts of each cassava leaf variety was exhibited. In conclusion, communities in Bukedea District are vulnerable to toxic phytochemicals from consuming NASE3 and Magana cassava leaves. Further studies on sub-acute and sub-chronic toxicity, the development of non-toxic cassava varieties, and chemical characterization are warranted.

INTRODUCTION

Manihot esculenta Crantz (cassava) is a major tropical root crop with numerous applications in food, feed, green energy, and industrial sectors (Byju and Suja, 2020). Cassava is widely grown and consumed in many regions worldwide, including Latin America, Indonesia, the

Philippines, Malaysia, Senegal, Mozambique, Tanzania, Sierra Leone, Nigeria, and Guinea, as well as Uganda (Okareh *et al.*, 2021). Cassava leaves are consumed as a vegetable in approximately 60% of countries in Sub-Saharan Africa and some Asian countries, including Indonesia (Frediansyah, 2024). Cassava leaves are widely consumed for their nutritional value,

particularly as a source of protein and vitamins, and for their medicinal properties in treating various diseases, including ulcers (Henneh *et al.*, 2022; Okareh *et al.*, 2021). Cassava leaves are widely consumed for food and medicine due to their ready availability, affordability, and, above all, perceived safety (Frediansyah, 2024). Despite their medicinal and food values, the toxicity of the leaves has been reported in some parts of the world, attributed to higher concentrations of cyanogenic glucoside, and anti-nutritional factors such as tannins and cyanides that reduce nutrient levels, bioavailability, uptake, and digestibility, limiting their potential use (Latif *et al.*, 2019). Furthermore, they have been linked to the development of several physiological disorders and organ failure (Latif *et al.*, 2019). Cassava leaf antinutrient and toxicity levels have been reported to be dependent on consumption levels and duration (Oresegun *et al.*, 2016). A pilot study conducted in the Teso sub-region of Eastern Uganda revealed that residents use cassava leaves as both a vegetable and a medicinal treatment for various ailments. However, the toxicity profile of these leaves has not been characterized, despite reports of toxicity elsewhere in the world, making investigation warranted.

Manihot esculentum is the most important species of cyanogenic plants containing a wide range of compounds with nutritional values, and antinutritional to high toxicity profiles (da Silva Santos *et al.*, 2020). Among the most critical phytochemical compounds found in cassava leaves are tannins, cyanides, phytates, nitrates, nitrites, polyphenols, oxalates, and saponins, which have been associated with a reduction in nutrient bioavailability, interference with the processes of digestion, absorption, and, in extreme conditions, may lead to organ failures (Henneh *et al.*, 2022). Alongside these deleterious compounds, cassava leaves contain a variety of mineral nutrients such as calcium, copper, manganese, potassium, zinc, lipids, carbohydrates, proteins, and vitamins (da Silva Santos *et al.*, 2020). Furthermore, high consumption of cassava leaves has been linked to mutagenic, carcinogenic, and teratogenic potential (da Silva Santos *et al.*, 2020). Fresh cassava leaves are highly toxic due to the

presence of free cyanides that are released when their cyanogenic glucosides, such as linamarin, are hydrolyzed (Zekarias *et al.*, 2019).

The phytochemical composition of cassava leaves varies significantly with the cultivation conditions (Oresegun *et al.*, 2016). Tannin concentration, for example, in the leaves influenced by water stress conditions (drought), stage of maturity, time of collection, processing methods, and cultivar or variety in question (Frediansyah, 2024). The Teso subregion of Uganda experiences prolonged drought. Thus, the possibility of high concentrations of toxic phytochemicals cannot be ruled out. Many households in the Teso sub-region primarily consume cassava leaves, assuming they are safe to eat. Despite the higher consumption rate of cassava leaves as a vegetable, the phytochemical composition of the commonly consumed cassava varieties has not been investigated. Thus, the current study aimed to determine the phytochemical composition and toxicity profiles of the tender leaves commonly consumed from cassava varieties.

METHODS

Study Site Description

Location

The study sites for collecting plant material were located in the Bukedea District, within the Teso subregion, 35 km and 292 km from Mbale City and Kampala, the Capital City of Uganda, respectively. They are located at 1.3557° N & 34.1087° E, at an average elevation of 1072 meters (3517.06 feet) above sea level (Google Maps, 2020). Kachumbala sub-county is located with geographical coordinates of 1.2136° N & 34.1191° E and at an average elevation of 1155.00 meters (3789.37 feet) above sea level.

Soils

The Teso sub-region has predominantly sandy loam soils that vary from sandy to loamy, depending on the terrain, and are less acidic, with a slightly alkaline pH (Kabasindi *et al.*, 2021). This type of soil, combined with the previously mentioned climatic conditions, fosters cassava growth and production, making it one of the most abundant crops in the area (Kabasindi *et al.*, 2021).

Climate

The average rainfall is 477.07 millimeters (18.78 inches), with 312.72 rainy days, and the temperature is 21°C (Google Maps). According to research, the cassava plant thrives best when rainfall is evenly distributed throughout the growing season at 1000-1500 mm, in less acidic or slightly alkaline soils, and an optimum temperature range of about 25-32° C, all of which are found in the Teso sub-region (Anikwe & Ikenganyia, 2018).

Demographic Characteristics

The locals are predominantly Iteso, Uganda's fifth-largest ethnic group, who are primarily peasants, often married with extended families, and of low socioeconomic status. As a result, reliance on locally available materials, such as cassava leaves, to supplement their food sources, increase their income, and treat their ailments cannot be ruled out.

Research Design

A quantitative, randomized, experimental, laboratory-based design was adopted.

Sampling Technique

Bukedea District was represented by residents of the three villages of Kongunga, Komurakere, and Kongoidi. Both snowball and purposive sampling techniques were employed to select participants, and the redundancy criterion was used to determine a sample size of 60.

The District Agricultural Officer (DAO) assisted in identifying the various cassava leaf varieties presented to him by the investigators and, as needed, consulted breeders or experts from the Serere Center. During these interviews, the investigators asked questions on cassava leaf varieties, processing techniques, stages of consumption, favorite varieties, and side effects. Participants were invited to accompany us on a guided field walk, and we took photographs with their mutual consent. The most popular cassava varieties were identified and selected for further studies on their photochemistry and toxicity profiles. The two commonly consumed plant varieties in Nigeria, Yellow and Nigeria Magana, were subsequently pressed in a plant press and taken to Serere Agricultural and Animal

Research Institute (SAARI) for identification. Nigeria Yellow was identified as NASE3, and Nigeria Magana as Magana.

Sample Size Determination, Exclusions, and Inclusions

The two most widely consumed cassava leaf varieties in Nigeria, Yellow and Nigeria Magana, were subsequently pressed in a plant press and taken to Serere Agricultural and Animal Research Institute (SAARI) for identification. Nigeria Yellow was identified as NASE3, and Nigeria Magana as Magana. These were selected for further study in photochemistry.

Data Collection

Ethnobotanical data

A pretested questionnaire administered by the researcher was used to collect data, along with interviews, supervised field trips, and observations. The questionnaire included semi-structured, open-ended, and closed-ended questions. Biodata, cassava leaf varieties used, and side effects of cassava leaf consumption were some of the topics covered in the question items. The collected data were both quantitative and qualitative.

Collection and Drying of Cassava Leaves

The young and tender cassava leaves of NASE3 and Magana were collected from the villages of Kamurakere, Kongunga, and Kongoidi in Kachumbala sub-county, Bukedea district. The leaves were spread on tables to dry under natural airflow for three days in the biology laboratory at room temperature (21 °C–22 °C) at the main campus of the Islamic University of Uganda, Mbale, to prevent fermentation. They were then transported to the Bio-analytical and Nutritional Laboratory at the National Crops Resources Research Institute (NaCRRI), where phytochemical analysis experiments were conducted.

Preparation and Extraction of the Crude Extract

Serial extraction methods were used according to the protocol developed by de Moura et al. (2009) and Kasolo *et al.*, with amendments. Plant leaves were collected, cleaned, and air-dried in a forced-air circulation

oven at 30 °C for 10 min, then ground to a coarse powder. The coarse powder was first soaked in ethanol (1:3 w/v) at 21 °C to prevent degradation of the heat-sensitive active compounds. This was done for 24 hours. Precisely 50 g of the powdered material was steeped in 150 ml of ethanol in an Erlenmeyer flask for 72 hours to extract the active compounds (Kasolo *et al.*, 2012). The mixture was shaken every 2 hours during the daytime to enhance extraction. It was then filtered, and the residue was dried. A rotating evaporator (G3 Heidolph Rotavapor, Germany) was then used to concentrate the filtrate. The yield of the crude extract was calculated after air-drying. The exact process was then repeated with distilled water as the second solvent to dissolve the active ingredients, after the residue was air-dried. This was performed at a 1:10 (w/v) ratio at 21 °C for 24 hours to prevent degradation of the heat-sensitive active compounds. The aqueous extract was heated to 96 °C in a water bath overnight to avoid fungal contamination. After 48 hours, the extract was concentrated using a freeze-drier, and the yield was calculated. All samples were placed in airtight containers and refrigerated throughout the experimental study.

Qualitative and Quantitative Analysis

Preparation of Standard (Stock solutions) and Calibration

The experimental trials were conducted in accordance with the method previously established by Okoro *et al.* (2019), with modifications. The laboratory was maintained at a temperature below 22°C to ensure reproducible findings. Solid samples of Magana and NASE3 cassava leaves were crushed into a coarse powder using a wood blender. Fifty grams of each compound was dissolved in a liter (50 g/l) of acetonitrile or water, where a stock solution of phenol, flavonoids, saponin, carotenoids, tannins, and terpenoids was formulated. Before HPLC analysis, the solution was mixed thoroughly and then stored in a 25-ml screw-capped vial (refrigerated at -20°C). To prepare a series of working solutions with concentrations of 0.25, 0.5, 1.25, 2.5, 6.25, 12.5, and 25.0 g/mL, the stock solutions of each analyte were further diluted with 10% methanol

(v/v). By diluting the respective mixed working solutions 10 times with blank (distilled water), calibration standards were generated.

Phytochemical Analysis of the Extracts for Selected Toxic Compounds with Isocratic Elution Systems

The supernatant was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter media into an amber 1.5 mL vial. Thereafter, the filtered and clarified supernatant was placed in a 1.5 mL amber HPLC vial to prevent isomerization upon light exposure, and then loaded into an HPLC system for separation and identification of the target analytes. The plant extracts were analyzed by HPLC (Shimadzu, Kyoto, Japan) using a SIL-20A HT system equipped with an SPD-20A Prominence UV/VIS Detector, an LC-20AD pump, a DGU-20A 5R degasser, and a CTO-10AS VP HPLC column forced-air oven. Chromatographic separation was performed under isocratic elution conditions, using a Zorbax Eclipse XDB C18 column (4.6 mm i.d. × 150mm, particle size 5µm, USA) from Agilent, which was maintained at 35°C. While using an isocratic elution of Matrix composition of water: acetonitrile: ethanol: acetic acid: ethyl acetate, Matrix ratio of 77.5:18:2.0:0.5:2.0 (v/v), elution time was 12.5 minutes while the column head pressure was set at 30psi as a maximum. A 10 µL injection volume of the analyte was used to identify the target analytes. In an isocratic elution condition, the specific wavelength at which the chromatogram was related to mill absorbance units was set at 278nm, in accordance with (Bajkacz and Kycia-Slocka, 2020; Bajkacz *et al.*, 2020), as previously reported in the analyses and graphical reports.

Data Analysis

The data were first cleaned through cross-checking and completeness checks. It was analyzed using R version 4.3.2, and graphs were generated using GraphPad Prism (Amin *et al.*, 2010). Shapiro-Wilk's test, also known as the D'Agostino test, indicated that the data were normally distributed. A one-way ANOVA was used to test for significance, and Tukey's test was used to locate the difference.

RESULT AND DISCUSSION

Different Varieties of Cassava Leaves Consumed by Residents in the Teso Sub-region.

The Teso sub-region was found to produce and consume five different types of cassava leaves **Figure 1**. As per **Figure 2**, the most popular kind of cassava leaves consumed were Nigeria Yellow (Aporu agoru) (53%), Magana (25%), Mfumbachai (10%), Eyok (10%) and the least was Kampala (2%) Nigeria yellow was identified as NASE3 yet Nigeria Magana was identified as Magana by the plant breeders at Serere Agricultural and Animal Research Institute. Consumption preferences were for cultivars with early maturity, flavor, sweetness, disease and drought resistance, and high yields, among other attributes. It is highly fascinating that the study findings were similar to those of Nakabonge *et al.*, (2018), who reported that in eastern Uganda, the most commonly grown

cassava varieties were Mfumbachai and Magana, for the same reasons noted above.

Qualitative Phytochemical Analysis

Overall, as shown in **Table 1**, the results indicate that the leaves of NASE3 and Magana contain several compounds; however, variation exists across the various types and extracts. For instance, terpenoids were absent in both the ethanol and aqueous extracts of NASE3, but were significantly present in the ethanol extracts of Nigeria Magana. Furthermore, aqueous and ethanol extracts of NASE3 did not contain phytosterol, although they were significantly present in NASE3. Moreover, high levels of phenols, flavonoids, and tannins were observed in both extract varieties. High and moderate concentrations of saponins and glycosides were detected in the ethanol extracts of NASE3 and Nigeria Magana, respectively. Alkaloids and phytosterols were detected at trace, insignificant levels in both cultivars and extracts.

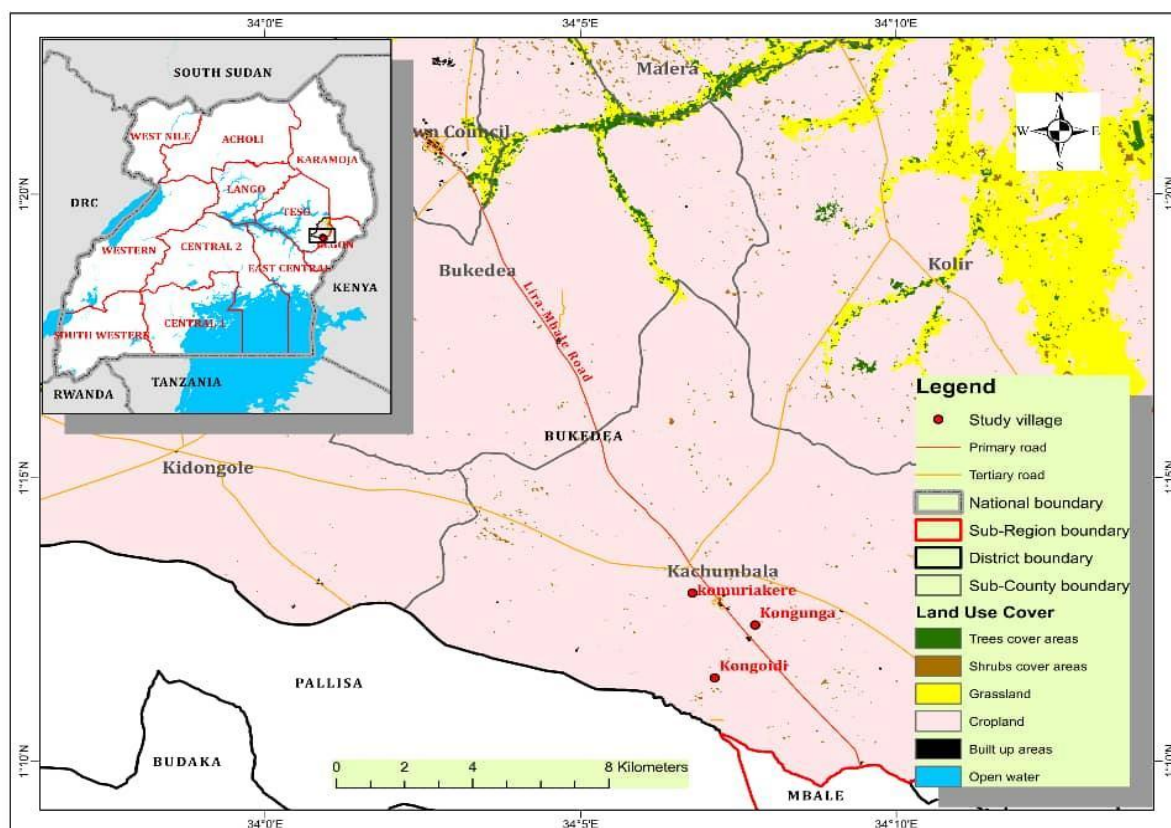


Figure 1. A map of Bukedea District as designed by the researcher indicating Sub-County and Villages where the study was conducted.

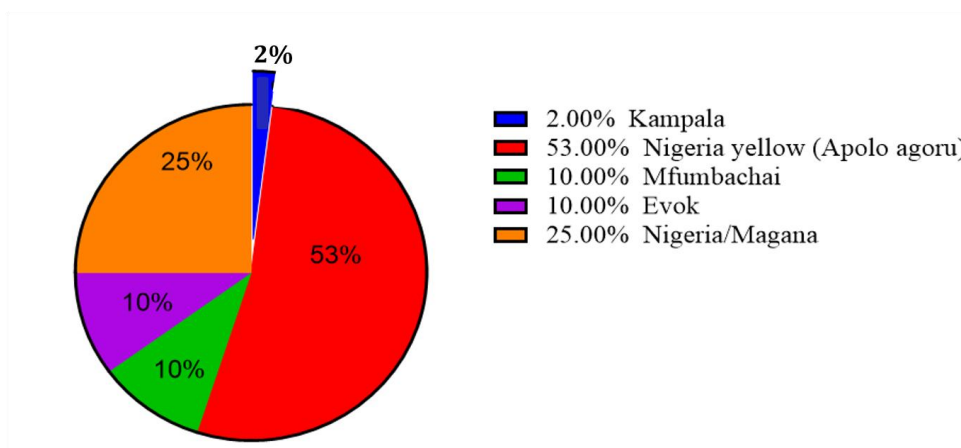


Figure 2. Cassava Leaf Varieties consumed.

In addition, saponins and glycosides were present in moderate concentrations. *Manihot esculenta* Crantz was revealed to contain several chemicals, including tannins, flavonoids, cyanogenic glycosides, saponins, and polyphenols (Mohidin *et al.*, 2023). Unlike the current investigation, which found significant amounts, the methanol extracts showed moderate concentrations of flavonoids, alkaloids, and saponins, which were attributed to habitat conditions (Mohidin *et al.*, 2023).

For instance, although ethanol was used in the current trial, it was applied to cultivars grown under hotter conditions than in the prior study. The results of this study show several toxic phytochemicals present in the leaves of cassava, which were similar to the phytotoxic chemicals such as phenols, tannins, flavonoids, terpenoids, alkaloids, steroids, and saponins that were discovered by Taupik *et al.*, (2023) during his study.

Table 1. Shows the qualitative phytochemical analysis of ethanol and aqueous extracts.

Phyto-compound	Qualitative test	NASE3		Magana	
		Water	Ethanol	Water	Ethanol
Alkaloids	Drangendroff's	+	+	+++	+++
Saponins	Froth	+	+++	+	++
Flavonoids	Alkaline reagent	+++	+++	+++	+++
Phenolic acids	Lead acetate	+++	+++	+++	+++
Phytosterols	Liberman-Burchard's	-	-	+	+
Terpenoids	Salkowski's	-	-	-	+
Glycosides	Borntrager's	+	+++	+	++
Tannins	Ferric chloride	+++	+++	+++	+++

Note: – absent, +: mild concentration, ++: moderate concentration, and +++: high concentration.

Side Effects of Consumption of Cassava Leaves

Figure 3 indicates that consumption of cassava leaves is not without side effects: approximately 10% reported stomachache, 12% diarrhea, and 37% vomiting; however, although the highest, 40% reported none.

These side effects, to a small extent, indicate that cassava leaf extract may be toxic, and this could manifest with frequent and prolonged consumption. Additionally, observations by (2022) showed that herbal extracts exhibited several side effects that were more likely to be

toxic than those of other agents. Also, observations by Başaran *et al.* (2022) showed that herbal extracts with several side effects were likely associated with toxicity, whereas other side effects, such as vomiting and nausea, were more pronounced than stomachache. The above gastrointestinal symptoms exhibited when cassava leaves are consumed are due to the presence of high levels of tannins, polyphenols, and fiber. These phytochemicals can reduce digestibility and nutrient uptake, potentially contributing to abdominal discomfort or diarrhea in sensitive individuals (Mombo, 2017).

Cassava leaves naturally contain linamarin and the enzyme linamarase in separate cell compartments. When leaves are chewed or digested, these two interact, releasing hydrogen cyanide (HCN), a potent toxin and an irritant to

the gastrointestinal tract. This irritation frequently manifests as stomach pain and nausea, which are classic early signs of acute cyanide intoxication (Mombo, 2017).

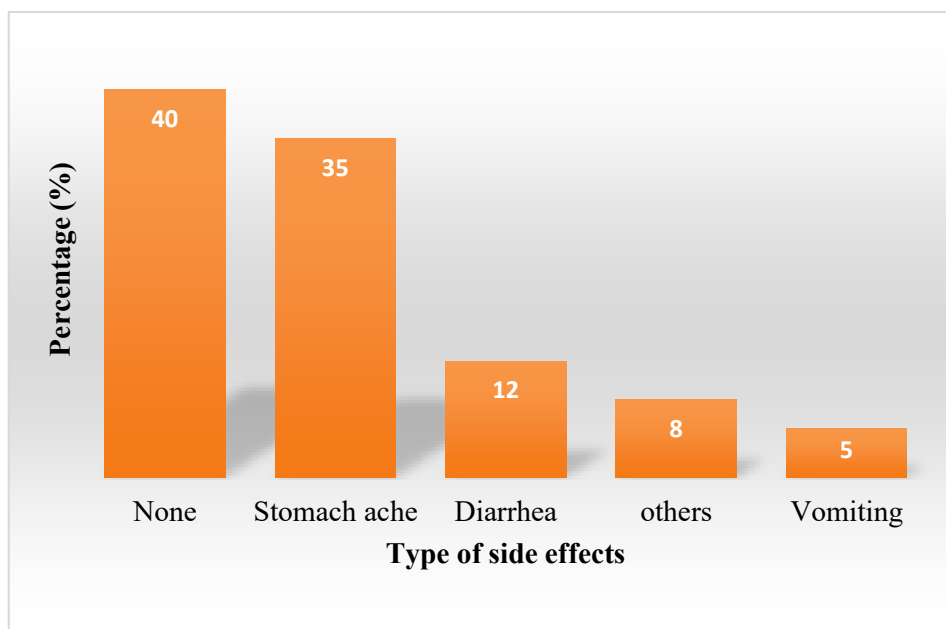


Figure 3. Side effects of the consumption of cassava leaves

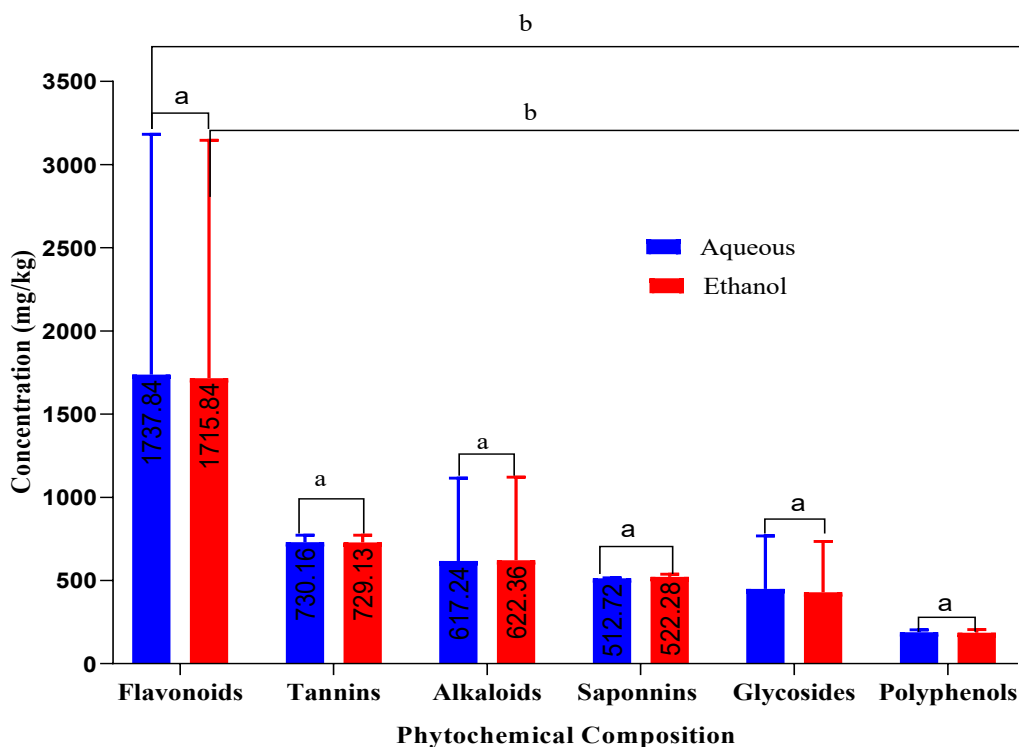


Figure 4. Shows the concentration of phytochemical compounds of ethanol and aqueous extracts of NASE3 and Magana. Where "a" is not a significant difference, and "b" is a significant difference.

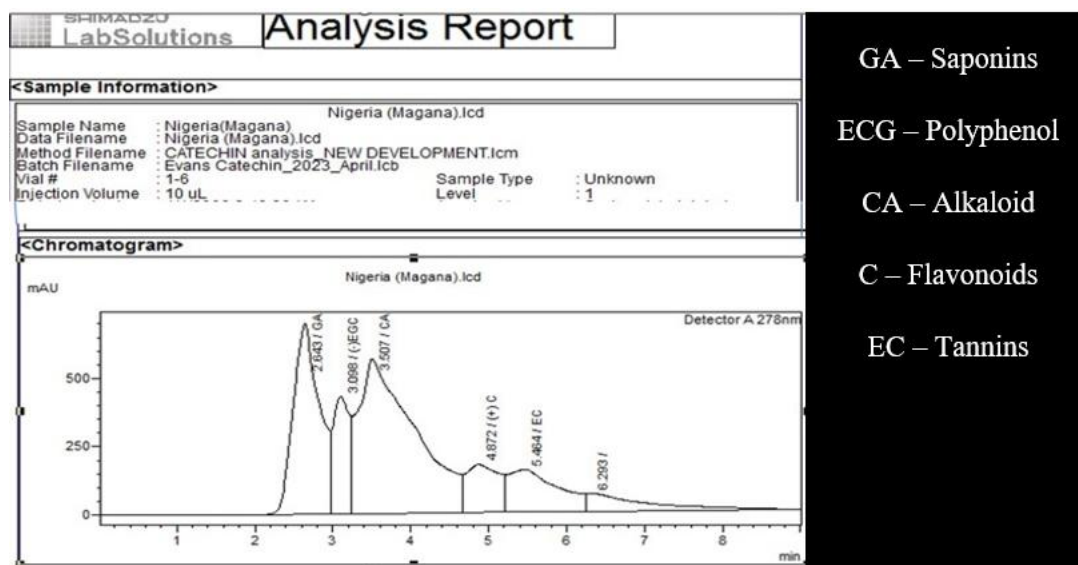


Figure 5. is an HPLC chromatogram showing the phytochemical compounds in the ethanol and aqueous extracts of Magana.

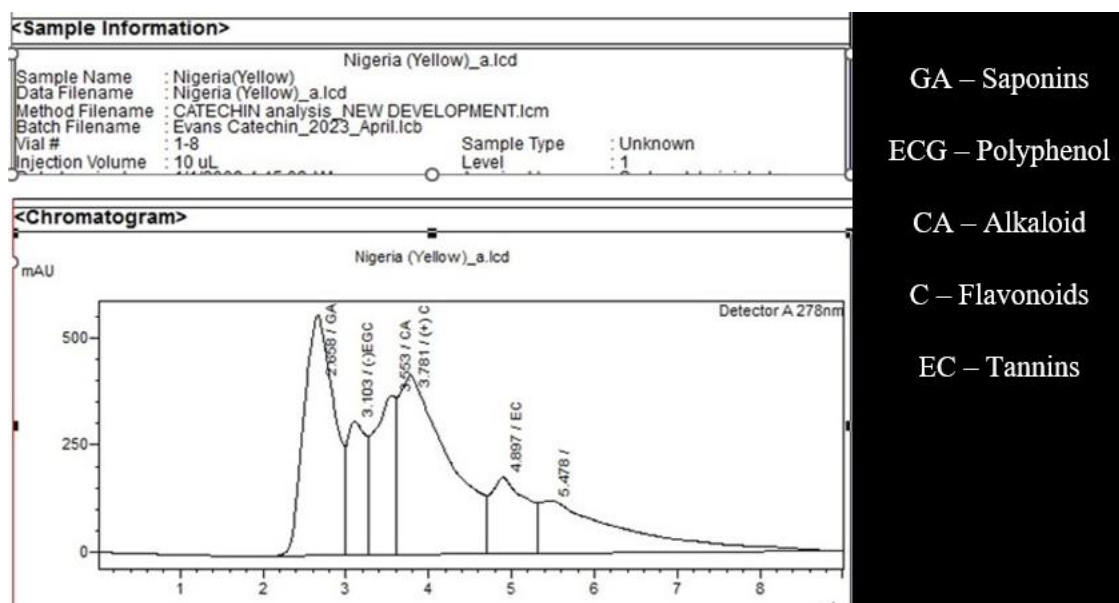


Figure 6. is an HPLC chromatogram showing the phytochemical compounds present in the ethanol and aqueous extracts of NASE3 (Nigeria Yellow).

Quantitative Phytochemical Analysis

Both the aqueous and ethanol extracts had significantly higher mean flavonoid concentrations than the remaining components in the investigation ($p < 0.0001$). There was no significant difference ($p=0.62-1.00$) in the mean concentration of the same phytochemical ingredient between the two extracts and cassava varieties, as exhibited in **Figure 4**. The difference between the ethanolic and aqueous

extracts was generally insignificant ($p = 0.533$). The HPLC chromatogram (**Figures 5** and **Figure 6**) showed that Magana had significantly higher phytochemical concentrations than NASE3.

In this investigation, the mean polyphenol concentration ($189.54 \pm 14.36 \text{ mg/kg}$) was found to be lower than previously reported (300 mg/kg) (Mohidin *et al.*, 2023). Remarkably, flavonoids, phenolics, and cyanogenic chemicals were found in cassava leaves (Laya & Koubala, 2020). Their average levels of cyanogenic

compounds (0.40), flavonoids (2.71), and phenolics (10.88) were, however, much lower than those reported for polyphenols (189.39 ± 14.36), flavonoids (1737.84 ± 1443.91), and glycosides (449.38 ± 318.35). The insignificant difference in the mean concentration of ethanolic and aqueous extracts is attributed to the fact that cassava leaves have less non polar compounds whose extraction was targeted by ethanol (Fioroni *et al.*, 2023; Okoro, 2020). The remaining polar compounds were to be fully extracted with water. These findings, however, are in line with those of Okoro *et al.*, (2019) whose findings showed that nearly identical amounts of ethanolic and aqueous compounds from cassava leaves were extracted. Water is readily available, and ethanol extracts a broad spectrum of phytochemicals. The chromatographs in **Figures 5** and **Figure 6** were not perfectly separated; this could have been due to the complexity of the sample matrix and the potential for similar chemical properties among the diverse compounds present in the extracts (Hopmans *et al.*, 2016). The Teso sub-region of Uganda and Malaysia both have distinct ecological conditions, extraction techniques due to different cultural setups, and cassava varieties, which account for the lower mean concentration (Laya & Koubala, 2020, Kudamba *et al.*, 2022).

CONCLUSIONS

Bukedea district communities are vulnerable to toxic phytochemicals that showed harmful side effects on consumption, whose quantities were revealed during phytochemical analysis of NASE3 and Magana. Recommendations are made for further studies on sub-acute and sub-chronic toxicity, the development of less toxic cassava cultivars, and the characterization and isolation of bioactive compounds responsible for cassava leaf toxicity.

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AUTHORS' CONTRIBUTIONS

The first author, Joweria Kayendeke, was an MSc student on this research project and was responsible for implementing the study, including sampling, data processing, and manuscript preparation. The second co-author, Dr. Cabral B. Kibedi, and the third co-author, Dr. Abdul Walusansa, co-supervised this research and reviewed the manuscript: the fifth and sixth co-authors, Prof. Erindyah R. Wikantyasning, and Assoc. Prof. Morita Sari provided a range of consultancy services to address research challenges, including concept formulation, phytochemical analysis, and methods in community health studies. Dr. Jamilu Ssenku served as the principal supervisor, responsible for the overall research direction.

CONFLICT OF INTERESTS

The authors have no conflict of interest in this publication.

ETHICAL CONSIDERATIONS

This study was in vitro and did not involve human subjects or living animals, so it did not require ethics committee approval. All procedures were carried out in accordance with the ethical standards of laboratory research and followed the guidelines in force at the institution where the study was conducted.

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