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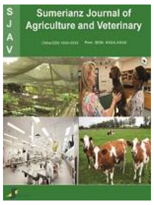

Original Article Open Access

Phytochemical and Trace Heavy Metal Composition of *Manihot Esculenta* (Crantz) and *Manihot Glaziovii* (Muell. Arg) Complex in Nigeria

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Abstract

The phytochemical and trace heavy metal composition of four varieties of *Manihot esculenta* (NR-8082, NR-8083, NR- 8212 and NR- 30572) and *M. glaziovii* were determined. Saponin, alkaloid, HCN and tannin content were investigated in the leaves, stems and tubers of plants studied. NR-8083 had the highest amount of the phytochemicals in the leaf (saponin, 5.6400^{a}; alkaloid, 1.5110^{a}; HCN, 68.6100^{a}; tannin, 1.2250^{a}). Saponin and tannin were found to be the least in the leaf of NR-8082(0.9860^{d} and 0.4760^{b}) respectively. Alkaloid and HCN were more in the stems of NR- 8212(1.6040^{a} and 8.9290^{a}) respectively, while saponin(0.3840^{c}) and alkaloid (0.5240^{c}) were least in the stem of *M. glaziovii*. Saponin and HCN were high in the tuber of NR- 8082(2.1400^{a} and 11.7500^{a}) respectively. Apart from Zn (85.7500^{b}), all the trace heavy metals were high in the leaf of NR-8082(Mn, 91.7500^{a}; Cu, 169.000^{a}; Fe, 142.2230^{a}). The highest mean content of manganese was found in the stem of NR-30572 (176.0000^{a}) while copper (149.0000^{a}) and Zinc (135.7500^{a}) were more in NR-8083 and NR-8082 respectively. All the trace heavy metals were also low in the tubers of NR-30572(Mn, 32.0000^{d}; Cu, 130.5000^{c}; Zn, 57.0000^{e}; Fe, 98.1260^{d}). The presence of tannin and alkaloids shows that they can be used not only for nutrition purposes but also for therapeutic, both as food and in drug formulation and production.
Keywords: Phytochemical; Trace heavy metal; *Manhot esculentum*; *Manihot glaziovii*; Tannin; Saponin.

1. Introduction

Phytochemicals refers to chemical that are found naturally in plants [1, 2]. Some important phytochemicals include alkaloids, tannins, resins, carbon compounds, hydrogen, gum etc. [3]. Alkaloids are organic acid occurring primarily in plants. The often have a powerful effect on animal physiology, and are of interest to the pharmaceutics industry for the development of drugs [4]. They play protective role in animals and possess therapeutic potency [5]. Foo [6] maintained that *Phyllanthus amarus* which is traditionally used to treat the dropsy, diabetes and jaundice contain hydrosable tannin. While screening some members of the Anacardiaceae, [7] observed that tannins were common among the various tubers of the family. Saponins are plant glycosides studied for their health benefits. They posses antitumor properties and have synergistic effects when combined in drug therapy. They act on the central nervous system with theurapetic effect [8, 9] The presence of saponin on different groups of plants has been determined by Carvalho and Seita [10] in *Sacuringa tinctoria*,Peng and Kobayshi [11] in *Allium* Liu, *et al.* [12] in Bulplenrum, Riaz and Chaidhary [13] in Centrum, Rahila, *et al.* [14] in some Euphorbiaceae, *Aspilia africana* [15], *Carica papaya* [16, 17] Curcurbitaceae [18] *Paninystalia macruceras* [19].

Phytochemicals are known to play active chemical role for sound health and are used in the treatment of diseases such as asthma, cold, cough, diabetes, diarrhea, dysentery and malaria [20-24]. Phyotochemicals have been found in several plant species use as food and feed. These plants may be fruits, vegetables, spices, herbs [25].

In *Manihot esculata* (Cassava), cultivars with root containing less than 50mg of HCN per kg are considered sweet. Analysis of about 100 cultivar for HCN content gave an average of 158 mg/kg fresh whole tuber with a maximum value of 438 mg/kg.

Trace heavy metals are necessary in maintaining the metabolism of the human body, though at higher concentrations, they can lead to poisoning. Trace heavy metals include copper, zinc, iron, manganese etc. Copper is a necessary substance in human health. But in high quantity it can cause anemia, stomach irritation, liver and kidney damage.

Cassava (*Manihot esculanta*), is a perennial shrub that grow to a height of 6-8 feet [26]. The large compound, dark green, reddish leaves are palmately divided into about seven leaflets [27]. The cone- shaped roots are starch storage organs. The plant is monoecious, meaning that there are separate male and female flowers borne on the same plant [27].

Manihot glaziovii is a small tree usually 10 meter high or less with a dense crown leaves and long petiole. Propagation of cassava is by cutting, cutting from older stem give higher yields than cutting from young stem [27].

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This work is aimed at comparing the different phytochemicals of the four varities of cassava and that of *Manihot glaziovii* and to investigate the different heavy metals of the four varieties of *Manihot esculenta* and those of *Manihot glaziovii*.

2. Materials and Methods

2.1. Sample Collection

Fresh and matured leaves, stem and tubers of four varieties of cassava (NR-8082 NR-8083, NR-8212 and NR-30572) were collected from plants grown in plots from National Root Crops Research Institute (NRCRI) Umudike Abia State were studied in comparism with *M. glaziovii* which was collected from the surrounding of the institute (NRCRI).

2.2. Preparation of Sample for Analysis

The leaves, stems and tubers were separated according to their varieties and species. They were put in sample envelopes and placed in an oven at 55^0C to dry. The dried samples were milled with Thomas Willey milling machine and stored in an air tight container for the various analysis.

2.3. Alkaloid Determination

5g of the sample were weighed into a 250ml beaker with 200ml of 20% acetic acid with ethanol added. It was covered and allowed to stand for 4 hours. This was filters and extract concentrated using a water bath to 25% of the original volume. Conc. ammonium hydroxide was introduced to the extract drop wise, until the precipitation was completed.

The solution was left to settle and the precipitate collected, filtered and weighed. The alkaloid was taken and expressed as percentage alkaloid [28].

2.4. Determination of Saponin

The method used by Obodoni and Ochuko [29] was employed. The samples were ground with 20g aqueous ethanol added. The samples were heated over a hot water with continuous stirring at about 60^0C for 4 hours. The mixture was filtered and the residue was extracted again with another 200ml 20% ethanol. The extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was put into a 250ml separator funnel with 200ml of diethylether added and shook vigorously. The aqueous layer was recovered while the ether layer was removed. The process of purification was repeated.

60ml of n-butanol was introduced. The extracts of n-butanol were washed twice with 10ml of 5% aqueous sodium chloride. The solution left was heated in a water bath. The samples were oven dried to a constant weight after evaporation. The saponin content was calculated as percentage.

2.5. Tannin Determination

100ml plastic bottle was used to weigh 500mg of sample. A mechanical shaker was used to shake the mixture after adding 50ml of distilled water. This mixture was filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipette out and mixed with 3ml of 0.1m $Fecl_3$ in 0.1 NHCl and 0.0008m potassium ferrocyanide tube at 120nm wavelength, within 10 minutes. A blank sample was made with the color developed and read at equivalent wavelength. A standard was made using tannin acid to get 100 ppm and measured [30].

2.6. HCN Determination

This was determined by the alkaline picrate method modified by Onwuka [31]. 5g of the sample was weighed into a plastic tube and 50ml of distilled water added and homogenized for about 30 minutes. This was allowed to incubate for about 18 hours. The solution was filtered into a 50ml volumetric flask, 2ml of the extract was pipette into a test tube and 4ml of alkaline picrate solution added and made up to 10ml with distilled water. It was heated up in a water bath for about 5 minutes. The optical density of the sample was read at 490nm using spectrophotometer. A set of cynide working standards were prepared in a test tube and the color developing solution added and made up to mark, read at 490nm.

3. Result

The results of the phytochemical and mineral content of the leaves, stems and tubers of the various varieties of *M. esculenta* and *M. glaziovii* were subjected to analysis of variance (ANOVA) with mean separation and are summarized in tables 1-6.

Table-1. Phytochemical composition of the leaves of the varieties of *Manihot esculenta* and *M. glaziovii* investigation

Phyto- Chemical	NR-8082	NR- 8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Saponin	0.9860^d	5.6400^a	3.5460^c	4.5840^b	3.440^c	0.5266	*
Alkaloid	0.8420^b	$1/5110^a$	0.5440^c	0.7680^{bc}	0.7620^{bc}	0.2302	*
HCN	66.2600^b	68.6100^a	55.9200^c	42.8570^d	31.5790^c	0.5388	*
Tannin	0.4760^b	1.2250^a	1.4760^a	1.7320^a	1.4330^a	0.1843	*

For the phytochemical composition in the leaves of the cassava cultivars and *M. glaziovii* in table 1, apart from NR- 30572 which had more tannin content, though not significantly different from the tannin in NR-8083, NR-8212 and *M. glaziovii*, NR-8083 had the highest of all the phytochemicals. Saponin and alkaloids were found to be least in the leaves of NR-8082 and NR-8212 respectively, while tannin was found to be less in NR- 8082.

Table-2. Phytochemical composition of the stem of the four varieties of *Manihot esculanta* and *M. glaziovii* investigation

Phyto- Chemical	NR-8082	NR-8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Saponin	0.4300^{c}	0.9800^{a}	0.740^{a}	0.5440^{c}	0.3840^{c}	0.5730	*
Alkaloid	0.8380^{b}	0.95266^{b}	1.6040^{a}	1.6260^{a}	0.6260^{c}	0.5504	*
HCN	7.9000^{b}	6.1100^{c}	8.9290^{a}	5.1690^{d}	5.2630^{d}	0.2846	*
Tannin	2.9500^{bc}	2.2940^{c}	3.5500^{ab}	3.9090^{a}	0.7360^{d}	0.2456	*

Alkaloid and HCN were found to be more in the stem of NR-8212 and least in *M. glaziovii*. Saponn was more in the stem of NR-8083 and least in *M. glaziovii* though this was not significantly different from NR-8082 and NR-30572 (table 2). Apart from tannin all the phytochemicals were high in the stem NR–8212.

Table-3. Phytochemical composition of the tubers of the four varieties of *Manihot esculenta* and *M. glaziovii* investigated

Phyto-Chemical	NR-8082	NR-8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Saponin	2.1400^{a}	1.3380^{b}	1.0380^{b}	2.2200^{a}	0.9860^{b}	0.2280	*
Alkaloid	0.2849b^{c}	0.4380^{b}	0.8100^{a}	0.4510^{b}	0.2000^{c}	0.5510	*
HCN	11.7500^{a}	11.0000^{a}	6.2030^{c}	8.0830^{b}	4.9000^{d}	0.3459	*
Tannin	0.1910^{c}	1.0520^{b}	1.1650^{b}	0.2210^{c}	3.4590^{a}	0.1897	*

In table 3, mean content of saponin and HCN were highest in the tubers of NR-30572 and NR-8082 respectively. The mean alkaloid content was highest in the tubers of NR-8212 and least in *M. glaziovii*, while tannin content was highest in *M. glaziovii* and least in NR-8082

Table-4. Trace heavy metal composition of the leaves of the four varieties of *Manihot esculanta* and *M. glaziovii* studied

Heavy Metal	NR-8082	NR-8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Mn	91.7500^{a}	18.0000^{e}	43.75000^{d}	75.5000^{b}	63.25500^{c}	0.2626	*
Cu	169.0000^{a}	139.0000^{d}	155.5000^{b}	124.0000^{c}	154.2500^{c}	0.3763	*
Zn	85.7500^{b}	59.5000a	96.5000^{a}	60.5000^{c}	61.0000^{c}	0.4024	*
Fe	142.2230^{a}	141.2230^{b}	130.0010^{c}	99.5859^{d}	100.2320^{d}	0.2081	*

For the mean trace heavy metal composition in the leaves of the cassava varieties and *M. glaziovii* studied, Mn, Cu and Fe were found to be highest in the leaf of NR-8082; Zn was more in MR-8212. Mn and Zn were least in the leaf of NR-8083 (table 4) Cu and Fe was lowest in MR-30572.

Table-5. Trace heavy metal composition of the stems of the four varieties of *Manihot esculenta* and *M. glaziovii* studied

Heavy Metal	NR-8082	NR-8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Mn	6.2500^{b}	37.7500^{b}	78.5000^{b}	176.0000^{a}	75.2500^{b}	0.26061	*
Cu	148.0000^{a}	149.0000^{a}	141.2500^{b}	143.0000^{b}	139.0000^{c}	0.6742	*
Zn	135.7500^{a}	135.5000^{a}	85.5000^{b}	65.5000^{d}	71.7500^{c}	0.2921	*
Fe	143.1560^{a}	127.5740^{b}	128.2320^{b}	99.5760^{c}	101.7250^{c}	0.7004	*

In table 5, the trace heavy metals in the stems of the plants investigated witnessed the highest mean content of Mn in NR-30572 and NR-8082. Cu and Zn were more in NR-8083 and NR-8082 respectively and were least in *M. glaziovii* and NR-30572 respectively. All the trace heavy metals were high in NR – 8082 except Mn.

Table-6. Trace heavy metal composition of the tubers of the four varieties of *Manihot esculenta* and *M. glaziovii* investigated

Heavy Metal	NR-8082	NR-8083	NR-8212	NR-30572	*M. glaziovii*	SEM	SIG
Mn	191.5000^{b}	190.7500^{b}	220.0000^{a}	32.0000^{d}	54.2466^{c}	0.2708	*
Cu	150.2500^{b}	153.0000^{a}	104.2500^{d}	130.5000^{c}	149.7500^{b}	0.4693	*
Zn	58.7500^{d}	88.5000^{b}	119.5000^{a}	57.0000^{e}	78.5000^{c}	0.4643	*
Fe	139.5360^{a}	127.5740^{b}	128.2320^{b}	98.1260^{d}	112.2850^{c}	0.4266	*

Considering the trace heavy metals in the tubers of the cultivars and species of Manihot studied, Mn and Zn were found to be high in the tuber of NR-8212. Mn and Cu were highest in NR- 30572 and NR-8083 respectively (Table 6).

4. Discussion

The level of phytochemicals in the leaves of the various varieties and species of Manihot examined is in line with the finding of Cerada and Mattos [32] according to him, tannin content of cassava in the leaf ranges from 1.5 to 4%. They are helpful in its usage as roughages for animal feed [23, 32]. Young and tender cassava leaves are good sources of dietary proteins and vitamin K. Cassava has established role in the treatment of Alzheimer's disease patients by limiting neuronal damage in the brain. Its adequate amount of potassium which is an important component of cell and body fluid that help regulate heart rate and blood pressure [33]. Consuming boiled cassava root (sweet variety) in the diet control cancers while a nested case control study conducted in India to identify the effect of dietary factors on causation of breast cancer using food frequency questionnaires has shown that consumption of tapioca is connected with a reduced risk of developing breast cancer [34] The cyanogenic glucosides (linamarin and lotaustralin) in cassava leads to the production of amygdaline or vitamin B-17 which is claimed to kill cancer cells [35]. The presences of saponin in the different parts of the plants were related with the other phytochemicals. The medicinal plant, *Euphorbia hirta* in used as purgative just as cassava, yet it contain alkaloids. This report is not strange since Rahila, *et al.* [14] observed the presence of alkaloid in some Euphorbia species. They also agreed that some of their Euphorbia species are used as purgative.Phytochemical works have been done in cassava [36] and in other plants; *Populus tremula* [37], *Neptunia oleracea* [38], *Stereospermum colais* [39], *Laportea aestuans* [40] and *Chrysophyllum albidum* [41].

The four varieties of cassava investigated fell under the sweet cassava as the HCN content were low in the tuber, though high in the leaves (table 1 and 2). Varieties with roots containing less than 50mg of HCN per kg are considered sweet since the estimate of protein is based on total nitrogen. The hydrolytic products of glycosides are incorporated into amino acids for protein synthesis in cassava. Therefore the occurrence of species with low HCN level is valuable discovery and can be considered a useful parent plant

The trace heavy metal distribution in the leaves, stems and tubers of the plants investigated were also significant. The heavy metals were relatively higher in the leaf, stem and tuber of NR-8082 compared with the other varieties. Their reduction in the part of the plant investigated is in line with the fact that they are trace element and are needed in smaller amount by plants. Furthermore, the phytochemical composition and trace heavy metal content in the leaves, stems and tubers of the plants considered were not only useful in its nutritional, medicinal and taxonomic importance, but were also botanically relevant.

The amounts of the different phytochemicals and trace heavy metals in the parts of the various varieties of cassava and *Manihot glaziovii* studied can serve as a guide and can further be explored both nutritionally and therapeutically.

5. Conclusion

In conclusion, findings from this work show that the phytochemical composition of *Manihot esculenta* and *Manihot glaziovii* reveal the presence of substances with established medicinal values like flavonoids, alkaloids, phenols, etc. However, some constituents like HCN and tannins were found present in the plants. But the levels of these antinutrients were well below critical level and therefore should not be of real concern. Also, these plants are not eaten raw traditionally and it was believed that normal food processing techniques may reduce the levels or even eliminate them completely. There is therefore need for the consumption of these plants for their therapeutic potential and nutritional value.

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