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Nutritional Properties and Antioxidant Activity of *Chrysophyllum africanum* Leaves and Pulp

Elekwa Iheanyichukwu¹, Nwaogwugwu Joel Caleb^{1*}, Ikonne Okezie Uche², Ezekwa AS³ and Egege Amaka Nkechinyere¹

¹Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Nigeria

²Department of Statistics Faculty of Biological and Physical Sciences, Abia State University, Nigeria

³Department of Medical Biochemistry Imo State University, Owerri, Nigeria

*Corresponding authors: Nwaogwugwu Joel Caleb, Department of Statistics Faculty of Biological and Physical Sciences. Abia State University, Nigeria, Tel: +2348060539271; E-mail: ifesinachinwa@gmail.com

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Abstract

The aim of this study was to determine the chemical composition and antioxidant activity of methanol extract of *Chrysophyllum africanum* leaves and pulp. Results revealed that the leaves contain considerable amounts of minerals and vitamins as follows: 14.14 mg/100 g for iron, 2.37 mg/100g for potassium, 1434.38 mg/100 g for manganese, 82.71 mg/100 g for magnesium, 185.49 mg/100g for calcium, 28.33 mg/kg for ascorbic acid, 13.33 mg/kg for thiamine and 55.83 mg/kg for pyridoxine. Furthermore, it was found that both the leaves and pulps were also rich in the following: 297.49-304.94 mg QE/100 g for total flavonoids, 2086.98-2304.72 mg GAE/100 g for total phenolics, 1.6-96400 mg/kg for citric acid and 130.23-515.23 mg/100 g for tannins. Our results therefore demonstrate that *Chrysophyllum africanum* could serve as supplementary source of essential nutrients and antioxidant components with health benefits.

Introduction

Plant and fruits have been helpful in treatment and management of diseases, with most pharmaceutical drugs derived from them. Fruits constitute important parts of a balanced diet as they are natural sources of food nutrients namely: protein, carbohydrate, minerals and dietary fibre, needed by man and animals. African Star Apple (*Chrysophyllum africanum*), locally called "Udala" by the Ibos and "Agbalumo" by the Yorubas is found mostly in African Countries. Its distribution extends from Sierra Leone to the Congo region and Angola, found in rain forest and transitional formations, often planted for its edible fruits. Its distribution also extends to Sierra Leone to Spain, Guinea, extending to Sudan, Uganda, Kenya and Nyasaiano. It is also found in countries like Southern Nigeria, Cameroons, Ghana, Ivory Coast and Sierra Leone.

Chrysophyllum africanum is of the family "Sapotacea". Its habitat is usually on riverside in closed forest, and often planted in villages. *Chrysophyllum africanum* has different species, but *Chrysophyllum africamum* and *Chrysophyllum albidum* bear the same common name in Nigeria "UDALA" [1]. A medium sized, evergreen tree usually 70 ft to 100 ft high; bole straight, flitted, bark gray and riddget, slash thin, cale brown, darkening to orange, heartwood whitish when first felled, turning a pink buff to an olive yellow and finally a yellowish brown, not demarcated from the sapwood.

Texture fine to medium, grain straight to occasionally interlocked, luster rather low; wood contains a pale brown gum. *Chrysophyllum africanum* bears edible fruits with large berries containing five large flattened seeds. It is greenish in colour when unripe and pale orange when ripe. It is pointed at both ends. The fruits are large and more than 4 cm wide, shaped like orange or apple; it is often cultivated for its edible fruits and the pulp having a pleasant acid taste [2]. *Chrysophyllum africanum* (African Star apple propagation is by seed either by encouraging natural regeneration or plantation traditionally. The sapwood is pale yellow and takes a good polish. It is fine grained, hard and tough polishes well. It is used in carving and tourney. The seeds yield edible oil, which is sometimes used in Ashanti for making soap. The latex is used as birdlime, the back is also used medically, often sold in the market and the tree is usually grown for this purpose.

In parts of Anambra and Imo States, this tree (African Star Apple) forms the focal point or venue for a fertility rite, in which young girls, childless wives celebrates a festivity- eating, singing and dancing for the sole purpose of praying to the gods of birth, this is a gesture of charity, since children are freely entertained without discrimination or distinction. The African Star Apples are valuable sources of minerals such as protein, fats and oil, carbohydrates etc. [3].

Material and Methods

Extracts preparation

10 g of each sample was extracted by maceration in 50 ml of methanol for 3 days with frequent agitation at a speed of 280 rpm at 28°C in dark. Between extractions, the samples were centrifuged for 10 min with 2000 rpm. The combined supernatants were collected, filtered through Whatman No. 1 filter paper and evaporated to dryness. The residual crude methanol extract was weighed and stored at 4°C for further use.

Determination of total phenolics and flavonoids

Total phenol contents of the extracts were determined by the modified Folin-Ciocalteu method [4]. Gallic acid was used as the standard and a calibration curve in the linear range of 0-100 mg/ml.

The following formula was used to calculate the final total phenolic acid content:

TPC (mg GAE/kg) = [GAE (mg/l)*total volume of methanol extract (ml*10⁻³)*dilution factor]/[Sample weight (g)*10⁻³]

The total flavonoids content was estimated by aluminium chloride (AlCl₃) colorimetric method with some minor modification (Ozkok et al., 2010). Quercetin was used as standard for calibration in the linear range 0-100 mg/L. Total flavonoid content was calculated as quercetin equivalent (QE)).

TFC (mg GAE/kg) = [QE (mg/ml)*total volume of methanol extract (ml)*10^{-3*}dilution factor]/[Sample weight (g)*10⁻³]

Determination of the free radical scavenging activity in the 1, 1-diphenyl-2-picrylhydrazil radical (dpph) assay

The free radical scavenging activity of the plant extracts was analyzed by using 2, 2- diphenyl-1-picrylhydrazyl (DPPH) assay [5]. The determinations were performed in triplicate. Inhibition of DPPH by the extract was calculated with the formula below.

DPPF (%) = $(1-A_{sample}/A_{blank})*100$

Where A_{blank} is the absorbance of control (1.1-4 mol/L DPPH methanol solution), and A_{sample} is the absorbance of the test sample.

Total ascorbic acid

Total ascorbic acid content of the pulp and leaf of *Chrysophyllum africamum* were estimated using the method of Sadasivam and Manickam, 1996 [6]. 5 ml of the standard solution of ascorbic acid were taken into 100 ml conical flask. 10 ml of 4% oxalic acid was added and titrated against the dye. The appearance of pink colour was taken as end point as it persists for few minutes. The amount of dye consumed was equivalent to the amount of ascorbic acid. The sample was extracted in 4% oxalic acid and make up to a known volume (100 ml) and centrifuged. A volume of 5 ml of the supernatant and 100 ml of 4% oxalic acid were taken and titrated against the dye. Amount of ascorbic acid was calculated and expressed in µg Vit C Eg/100 g.

The reducing power of metal ion

The power of the extracts to reduce metal ion was evaluated according to the methodology described by Yildirim et al. [7]. In determining the reducing power of metallic ions, phenolic substances present in the extracts are reacted with ferricyanide ion $[Fe (CN)_6]^{3-}$ and are oxidized, while the $[Fe(CN)_6]^{3-}$ is reduced to ferrocyanide ion $[Fe (CN)_6]^{4-}$. This then reacts with ferric ion (Fe^{3+}) form ferric ferrocyanide or hexacyanoferrate III (Fe₄ [Fe (CN)₆]³), also known as Prussian blue. Hence, blue color formation measured at 700 nm is used to monitor the concentration of Fe²⁺ [8].

So, 1 ml of extracts was used at concentrations of 0.05, 0.1, 0.2 and 0.3 mg ml⁻¹ in ethanol. Solution of gallic acid at the same concentrations was used as standards. It was added to each sample extract and gallic acid, 1.0 mL of phosphate buffer 0.2 mol L⁻¹ (pH 6.6) and 1.5 ml of 1% potassium ferricyanide. Then the samples were incubated at 50°C for 30 min. After this time was added 1.5 ml of 10% trichloroacetic acid and the mixtures were centrifuged at 2500 rpm for 8 min. Then, 2.0 ml were removed from the top layer of each sample and added 2.0 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm in UV-Vis spectrophotometer. Tests were performed in triplicate and the reducing

power was considered directly proportional to the absorbance observed.

Statistical analysis

Descriptive statistical analyses for calculating the means and the standard deviation of the mean were performed using the Statistical Package for Social Sciences (SPSS) version 19. Results were expressed as a mean \pm standard deviation (SD). A value of p<0.05 was used to denote statistical significance.

Result s and Discussion

Mineral and vitamin contents

Results for the mineral and vitamin contents of the *Chrysophyllum africanum* are shown in Table 1. The pulp from *Chrysophyllum africanum* has shown to be a good source of minerals and vitamins as evidenced by the contents as follows: 14.15 mg/100 g for iron, 1436.38 mg/100 g for potassium, 2.36 mg/100 g for manganese, 82.71 mg/100 g for magnesium and 185.49 mg/100 g for calcium.

These mineral contents were found to be higher as compared to the values reported for *Chrysophyllum africanum* by Ibrahim [9] who reported the following values for different minerals: 7.66 μ g/100 g for iron, 10.03 mg/100 g for potassium, 135 μ g/100 g for manganese, 0.45 mg/100 g for magnesium and 100.00 mg/100g for calcium. The values for sodium content in leaves (17.03 mg/100 g of dry weight) and pulp (441mg/100 g of dry weight) were almost the same concentration. The calcium content of *Chrysophyllum africanum* pulp (185.49 mg/100 g) was significantly higher than seeds (64.33 mg/100 g). However, the mineral element in *Chrysophyllum africanum* fruit, such as calcium, sodium, potassium were found to be higher than the values reported in dabai fruits by Ibrahim, 2011 [9].

Additionally, it was observed that the amounts obtained for copper, iron, potassium, manganese, magnesium and calcium were almost 3fold greater in the pulp than in the leaves which demonstrate that it if properly utilized, it can adequately meet the nutritional needs of by *Chrysophyllum africanum*, consumers.

Results showed there were variations in amounts for different vitamins. For both leaves and pulp, it was observed that pyridoxine was the most abundant one followed by ascorbic acid and lastly thiamine (Table 1).

Mineral	Pulp	Leaves
Zinc (Zn) (mg/100 g)	0.88 ± 0.06	1.87 ± 0.06
Iron (Fe) (mg/100 g)	14.15 ± 0.02	3.33 ± 0.07
Managanese (Mn)(mg/100 g)	2.36 ± 0.01	0.17 ± 0.08
Copper (Cu) (mg/100 g)	0.29 ± 0.01	0.48 ± 0.03
Potassium (K) (mg/100 g)	1436.38 ± 0.01	411.23 ± 2.03
Sodium (Na) (mg/100 g)	17.03 ± 0.01	17.62 ± 0.07
Magnesium (Mg) (mg/100 g)	82.71 ± 0.01	45.78 ± 0.03
Calcium (Ca) (mg/100 g)	185.49 ± 0.1	64.33 ± 0.23
Phosphorus (P) (mg/100 g)	0.70 ± 0.21	1.56 ± 0.21

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Ascorbic acid (mg/kg)	6.83 ± 0.03	28.34 ± 0.03	
Thiamine (mg/kg)	0.02 ± 0.01	13.32 ± 0.03	
Riboflamin (mg/kg)	0.35 ± 0.01	0.54 ± 0.03	
Pyridoxine (mg/kg)	10.03 ± 0.15	56.82 ± 0.12	
Nicotinamin (mg/kg)	0.01 ± 0.01	0.02 ± 0.01	
Cyanocobalamin (mg/kg)	0.03 ± 0.03	0.05 ± 0.01	
Values are means ± standard deviation of three determinations (n=3)			

 Table 1: Mineral composition and vitamin content of the

 Chrysophyllum africanum pulp and leaf (dry weight basis).

The vitamin contents were found to be higher in leaves than in the pulp and the values were as follows: 28.33 mg/kg for ascorbic acid, 13.32 mg/kg for thiamine, 56.82 mg/kg for pyridoxine for leaves while the values for the pulp were as follows: 6.83 mg/kg for ascorbic acid, (0.02 mg/kg for thiamine and 10.03 mg/kg for pyridoxine.

The riboflavin content in seeds (0.52 mg/kg) was slightly higher than in pulp (0.35 mg/kg). The vitamin content ranges for nicotinamide and cyanocobalamin were 0.01-0.02 mg/kg and 0.02-0.03 mg/kg respectively for seeds and pulp.

It was interesting to note that thiamine; pyridoxine contents of *Chrysophyllum africanum* leaves, were very higher than Recommended Dietary Allowances (RDA) with their values exceeding the RDA of 1.3 mg/day for adult males and 1.1 mg/day for women- 1.5

mg/day pregnant/lactating of thiamine (RDA 2010). The values of B_1 (thiamine), B_2 (riboflavin) B_3 (Niacin), B_6 (Pyridoxine) obtained in *Chrysophyllum africanum* fruit were also higher than reported by Ismail et al. 2013 [10] in banana guava, okra, brinjal and spinach. These vitamins are recommended during pregnancy for lactating women and adolescents during growth and against chronic diseases such as cardiovascular disease, morning sickness, insomnia, anxiety, anti-tuberculosis medication and Post Menstrual Syndrome [11].

Total phenolic, total flavonoids and antioxidant activity

Results for the total phenolics and total flavonoid of extracts obtained from the pulp and seed samples are shown in Table 2. Results showed that the amounts of phenolic compounds (251.72 mg GAE/100 g of dry weight) and total flavonoids content 1308.89 mg GAE/100 g were higher in the pulp than in the aqueous leave extract, with values of (298.49 mg GAE/100 g of dry weight) and (298.43 mg QE /100 g of dry weight), respectively.

In fact, total polyphenol amount in *Chrysophyllum africanum* fruit, were higher than that of exotic fruit such as Fardh-Tamr (235 mgGAE/100 g), Khalas-Tamr (231 mgGAE/100 g), Khasab- Rutab (116 mg GAE/100 g) and Khalas-Rutab (81 mg GAE/100 g) [12] and pineapple (129 mg/100 g), mango (546 mg/100 g), guava (39 mg/100g) as reported by [13], and total flavonoids content in *Chrysophyllum africanum* fruit, were higher than that in whole fruit such as Fardh-Tamr (34 mg CEQ/100g) Khasab-Tamr (27 mg CEQ/100 g) and Khalas-Tamr (25 mg CEQ/100 g) Fardh-Rutab (66 mg CEQ/100 g) Khasab-Tamr (46 mg CEQ/100 g) and Khalas-Rutab (19 mg CE/100 g) [14].

Antioxidant	Pulp	Leaves		
Phenolics content GAE mg/100 ga	2305.83 ± 0.05	2097.97 ± 0.09		
Flavonoids content mg QE/100 gb	308.89 ± 0.04	298.49 ± 0.01		
DDPH radical-scavenging activity %c	65.93 ± 0.07	298.43 ± 0.02		
Ascorbic Acid content (AAC) Ug vitamin C Eq/mgd	64.90 ± 0.07	72.60 ± 0.04		
Values are means ± standard deviation of three determinations				
Values are means ± standard deviation of three determinations				
^a Results were expressed as mg of Gallic acid equivalents (GAE) 100 g of extract.				
^b Results were expressed as mg of quercetin equivalents (QE) /100 g of extract.				
^c Results were expressed as mg/mL of extract.				
^d Results were expressed as mg of quercetin equivalents (AAC) Ug vitamin C Eq/mgd.				

Table 2: Antioxidant Composition of the Chrysophyllum africanum Pulp and Leaves.

The antioxidant properties of *Chrysophyllum africanum* the fruit were evaluated by DPPH radical scavenging capacity, reducing power (Fe³⁺ into Fe²⁺) and the total monomeric anthocyanins. The antioxidant activity of the methanol extracts of both leaves and pulp of *Chrysophyllum africanum* are shown in Table 2. The seeds of monkey apple fruits presented the highest DPPH scavenging activity (65.93% of dry weight) and reducing power (298.43 mg/100 g of dry weight) activities, whereas the pulp presented the highest phenolics content and the lowest antioxidant activity for DPPH and reducing power assays.

With respect to total monomeric anthocyanin content, values obtained in this study (Table 2) was lower than those of other fruit, Acerola (Malpighia emarginata DC.) pulp (28.27 mg/kg, cyanidin-3-glucoside), squeezed (52.3 mg/kg, cyanidin-3-glucoside,) and Crushed fruit (49.7 mg/kg cyanidin-3-glucoside).

Conclusions

Results from this study have shown that the chemical composition and antioxidant activity of the pulp and seeds of *Chrysophyllum africanum* fruits differ significantly. The pulp of the *Chrysophyllum* *africanum* fruits was found to contain higher amounts for minerals, organic acids, flavonoids and phenolics compared to the amounts contained in the leaves. However, results have also shown that leaves contain higher amounts as compared to the pulp for crude fat, crude fiber, proteins, total energy, vitamins, carbohydrates, ant-nutrient factors and antioxidant activity. The results of this study have clearly demonstrated that the *Chrysophyllum africanum* fruit contains substantial amounts of antioxidants which if well exploited and promoted can address many nutritional related disorders and also be useful in food industry for production of a variety of value added products.

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