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The Antioxidant and Food value of *Chrysophyllum albidium* G. Don

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Abstract: The antioxidant activities, antioxidant phenolic compounds and food value analyses of African star apple (*Chrysophyllum albidum*) fruit were carried out using standard methods. The results showed that antioxidant activity is 92.5± 0.39%. The antioxidant phenolic compounds were revealed as total phenol (3146 ± 1.22mg/100g), flavonoid (840± 0.55mg/100g), anthocyanin (1.25 ± 0.04mg/100g), proanthocyanidin (0.003 ± 0.01mg/100g). The proximate compositions were shown as moisture (71.85 ± 0.25%), crude fibre (0.40 ± 0.10%), fat (0.82 ± 0.02%), ash (0.66 ± 0.02%), protein (0.59 ± 0.09%) and carbohydrate (25.68 ± 0.11%). Though the food value of *C. albidum* is generally low, the antioxidant in *C. albidum* is high enough to protect man from ailments caused by harmful free radicals in the body.
Keywords: Antioxidant activity, *Chrysophyllum albidum*, proximate composition, phenol

INTRODUCTION

Fruits and Vegetables are a good source of natural antioxidants, containing many different antioxidant components which provide protection against harmful free radicals which have been implicated in the etiology of several human ailments such as cancer, neural disorders, diabetes, arthritis and cardiovascular disorder [1-3]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia & reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [4-5]. Oxidation process is one of the most important routs for producing free radicals in food, drugs and living systems [6]. Catalase and hydropenxidase enzymes convert hydrogen peroxide and hydro peroxide to non-radical firms and function as natural antioxidants in human body. Due to depletion of immune system, consuming antioxidants as free radical scavengers is necessary [7-9].

The diet and food based approach in combating micronutrient malnutrition is essential for its role in increasing the availability and consumption of micro nutrient rich foods [10]. The food and agricultural organization of the United Nations (FAO) [31] reported that the countries of West and Central Africa sub-regions have a large number of under-utilized indigenous edible plant species that are important to the livelihoods of local population. Numerous scientific studies have shown the importance of indigenous edible plants in the nutrition of the rural human population in Africa[11-15]. All over Africa, these traditional food plants have been major sources of nutrients for rural dwellers that cannot pay for milk, egg and meat [16, 14]. One of such indigenous food is *Chrysophyllum albidium*.

Chrysophyllum albidium locally called "agbalumo" in south western Nigeria is usually available during the dry season (late December to March) each year. The fruits are gathered from the wild. Information is scantily available in literature on its chemical composition especially the antioxidant. This study was therefore designed to study the antioxidant profile and the proximate content of *C. albidium*.

MATERIALS AND METHODS

Plant Material

50 fully ripe fruits of Albidium were gotten from a popular market in Ile-Ife, Osun State Nigeria during its season in 2010. The fruits were thoroughly washed with distilled water. Half of the fruits were analyzed for the proximate on a wet weight basis while the remaining half was dried in the oven at 50^0C for 24hours. It was then milled and the resultant powder refrigerated and later analyzed for the antioxidants.

Antioxidant Assay

The hydrogen donating or radical scavenging of the extract was determined using the stable radical DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) according to the method described by Brand-Williams [17]. DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517 nm. 1 mg/ml of extract solution was prepared. 1 ml of methanol was then pipetted into the cuvette followed by addition of 1 ml DPPH reagent in the spectrophotometer and kept in the

dark for 30 minutes. The absorbance was read at 517 nm. This served as control. The absorptions of sample test containing 1ml of extract solution and 1ml of DPPH was prepared, kept in the dark for 30 minutes and read at 517 nm.

Determination of Total Phenols Content

Total phenol content was determined by the method of Singleton and Rossi [18] using the Folin – Ciocalteau reagent in alkaline medium. To 0.1 ml of extract, 0.9 ml of distilled water was added. 0.2 ml of Folin's regent was subsequently added and vortexed. After 5 minutes, 1 ml of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to 2.5 ml and incubated for 90 minutes at room temperature. Then, absorbance was read at 750 nm against reagent blank. For the standard solution, a stock solution of gallic acid 1 mg/ml was prepared. Then aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and each was made up to a total of 2 ml. From this range of concentrations, 0.1 ml was taken into different test tubes. The standard curve was prepared using 100, 200, 300, 400, 500 µg/ml solutions of gallic acid in methanol:water (70:30, v/v). Total phenol values was expressed in terms of gallic acid equivalent (mg/g of sample), which is a common reference compound.

Determination of Total Flavonoid Content

Total flavonoid content was determined using $AlCl_3$ method as described by Lamaison and Carnet [19]. To 0.1 ml of extract, 0.4 ml of distilled water was added, followed by 0.1 ml of 5% $NaNO_2$. After 5 minutes, 0.1 ml of 10% $AlCl_3$ was added, and then 0.2 ml of 1M NaOH and the volume was made up to 2.5 ml with distilled water. Absorbance was measured against reagent blank at 510 nm. For the standard solution, a stock solution of quercetin 1 mg/ml was prepared. Then aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and made up to a total of 2 ml. From this range of concentrations, 0.1 ml was taken into different test tubes. The standard curve was prepared using 100, 200, 300, 400, 500 µg/ml solutions of quercetin acid in methanol:water (70:30, v/v). Total flavonoid values was expressed in terms of quercetin equivalent (mg/g of sample), which is a common reference compound.

Determination of Total Proanthocyanidin Content

The proanthocyanidin content was determined using a modified method of Porter *et al*. [20] using the AlCl / Butan – 1-0l assay method. Extracts were diluted to provide spectrophotometric readings between 0.10 and 0.80 absorbance units. A 1.0 ml sample aliquot of adequately diluted extract was added to 9.0 ml of concentrated hydrochloric acid in n-butanol (10/90, v/v) in a screw top vial. The resulting solution was mixed by vortexing at a mid-range setting (5 to 6) for 10 to 15 seconds. Samples were heated for 90 minutes in an 85ºC water bath (Haake W19; Haake, Berlin, Germany) and then cooled to 15 to 25ºC in an ice bath. The absorbance at 550 nm was measured on a UV-visible spectrophotometer. A control solution of each extract was prepared to account for background absorbance due to pigments in the extracts. The control solution consisted of the dilute extract prepared in the hydrochloric acid/n – butanol solvent without heating.

Determination of Total Anthocyanin Content

The total anthocyanin content of the test samples was determined using the pH differential method of Fuleki and Francis [21] as described by Guisti and Wrolstad [22]. A pH 1.0 buffer solution was prepared by mixing 125 ml of 0.2 N KCl with 385 ml of 0.2 N HCl and 490 ml distilled water. The pH of the buffer was adjusted to pH 1.0 with 0.2 N HCl. A pH 4.5 buffer solution was prepared by mixing 440 ml 1.0 M sodium acetate with 200 ml 1.0 M HCl and 360 ml distilled water. The pH of the solution was measured and adjusted to pH 4.5 with 1.0 M HCl. 2 ml of anthocyanin extract was diluted to 50 ml in each of the pH 1.0 and 4.5 buffers and was allowed to equilibrate in the dark for 2 hours. The absorbance of the samples at 512 (A512 nm) and 700 nm (A700 nm) was measured on a Shimadzu 265 UV – visible spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD).

Proximate composition analysis

Crude protein, Carbohydrate, Ash, Crude fibre, Ether extract and Moisture contents were determined using the routine chemical analytical methods of Association of Official Analytical Chemists (AOAC, 1995)[23].

Moisture content

About 2 g samples of the extracted pulp were measured out in triplicate and oven-dried at 90^0C for 72 hours when constant weight constituted the moisture content. The difference expressed as a percentage of the initial weight was the percent moisture content.

Crude fibre content

To about 2 g homogenized pulp was added 1.25% hot tetra-oxo-sulphate (IV) acid in a 500 ml beaker. The content was covered and refluxed for 20 minutes. Filtration was done using vacuum pump and trap. The residue was washed thrice with hot water to remove the acid traces. The filtrate was then returned to the beaker and 200 ml hot 1.25% sodium hydroxide was added and refluxed for 30 minutes. Further filtration was done and the residue was washed, first with hot water followed with butan-1-ol and finally with hot tetra-oxo-sulphate VI acid. The residue was collected into a crucible, oven dried at 100ºC for 24 hours, cooled in a dessicator and weighed to obtain the weight of the crucible plus fibrous residue. The crucible was then transferred into a furnace set at 660 ºC for three hours for ashing. The content was thereafter cooled, and weighed to the obtain weight of crucible plus ash. Thereafter, fibre content and % crude fibre content were calculated

Crude protein

About 0.5 g of the homogenized pulp was weighed into Kjedahl flask. To the sample was added a 1: 2 mixture of perchloric acid and H_2SO_4. The sample was left to settle for 20 minutes and then transferred to a digester for one hour till sample turned colourless. The sample was then left to cool under fume-cupboard. The digest was distilled in a 500 ml flask using 100 ml distilled H_2O, 50 ml NaOH and selenium tablet. The flask was immediately covered and placed in a condenser. Attached to the condenser was a conical flask containing 50 ml boric acid which changed from red to green and volume was made up to 100 ml. The solution was then titrated using phenophtalein indicator against standardized sulphuric acid till the colour turned to red. From the entire procedure, the % Nitrogen was calculated.

Ether Extract/Fat content

About 2 g of sample was transferred into a fat free extraction thimble. The soxhlet extractor with a reflux condenser and a small flask was oven-dried, weighed and fitted up into a complete soxhlet apparatus. The fat free extraction thimble containing the sample was plugged lightly with cotton wool, petroleum ether was added and the thimble was placed in the soxhlet extractor. Additional petroleum ether was added until the barrel of the 300 ml extractor was half full. The condenser was replaced tightly and then placed on hot plate. The content was allowed to boil for 2 hours ensuring that the ether siphon until the siphoning was no longer noticed. The flask was detached and the contents of the extractor barrel was siphoned into ether stock bottle. The material was well drained. The content of the extractor thimble is removed and dried. The condenser and flask are replaced and then dried until the flask is practically dried. The flask is detached, cleaned and dried to constant weight. The difference between the initial flask weight and the final weight was ether extract (fat) content. The percent ether extract was then calculated.

Ash

About 2 g of the sample was weighed into a crucible (Platinum), transfer into a murffle furnace. Ignite at 600ºC for 3 hours. It was removed and allowed to cool in a desiccator and final weight was taken, using analytical weighing balance.

RESULTS

Results of antioxidant activities and antioxidant phenolic compounds are presented on Table1.

The results showed that antioxidant activities in *C. albidium* is very high (92.5± 0.39%). This indicates that its radical scavenging ability is very strong. The total phenol concentration in *C. albidium* fruit is (3146 ± 1.22mg/100g), while flavonoid concentration is (840± 0.55mg/100g).

Anthocyanin in *C. albidium* fruit is (1.25 ± 0.04mg/100g) while the proanthocyanidin concentration in *C. albidium* fruit is very low (0.003 ± 0.01mg/100g). These values were generally low.

The proximate laboratory analysis results are presented in Table 2. The proximate compositions were shown as moisture (71.85 ± 0.25%), crude fibre (0.40 ± 0.10%), fat (0.82 ± 0.02%), ash (0.66 ± 0.02%), protein (0.59 ± 0.09%) and carbohydrate (25.68 ± 0.11%). These values were generally low.

TABLE 1: Antioxidant activity and phenolic antioxidants of *Chrysophyllum albidum* (Dry weight basis)

Antioxidant activities (%)	Phenol (mg/100g)	Flavonoid (mg/100g)	Anthocyanin (mg/100g)	Proanthocyanidin (mg/100g)
92.5±0.39	3146±1.22	840±0.55	1.25±0.04	0.003±0.01

Mean of three determinations ±SD (standard deviation)

TABLE 2: Proximate Compositions of *Chrysophyllum albidum* (wet weight basis)

Crude protein	Crude fibre	Ether extract g/100g	Ash	Moisture	Carbohydrate
0.59±0.09	0.40±0.1	0.82±0.02	0.66±0.02	71.85±0.25	25.68±0.11

Mean of three determinations ±SD (standard deviation)

DISCUSSION

Food security and poverty alleviation in rural communities can be improved by diversifying the existing farming systems. Between the cropping seasons, farmers rely on alternative food products that include trees, fruits, animals and sale of non-wood products to earn a living especially during drought. Where commercial exploitation of indigenous fruit occur, they have shown great potential as much as exotic fruits in providing food and nutritional security and income generation. Weak and exploitive marketing channels and difficulties in entering into trade, all

present major hindrances to farmers wishing to develop the commercial potential from underutilized fruits [24].

The protein content of 0.59% is low, though, Ige and Gbadamosi[25] , recorded 8.2% for *Chrysophyllum albidium*. Different factors such as year effect and time of harvesting may be responsible. For instance the moisture contents differ, it was 71.9% in this study while Ige and Gbadamosi[25] and Ureigho and Ekeke[26] reported 69.9% and 70% respectively, hence the difference in other analyzed parameters. The moisture content of the fruit is generally low compared to other tropical fruits such as orange 85%, tomato 95%, banana 75% [27].

Fruits and vegetables are rich sources of natural antioxidants which have been established to promote health by acting against oxidative stress related disease such infections as; diabetics, cancer and coronary heart diseases [28]. Studies have shown a diminished risk of chronic diseases in populations consuming diets high in fruits and vegetables and it has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect [7]. Generally, food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions. The antioxidant activities i.e. free radical scavenging ability of *C. albidium* is very high and compared favourably with those present in exotic and other tropical fruits that are more common. Abdullah[29] recorded 83%, 76.1% and 98.4% radical scavenging activities for *Carica papaya*, *Ananas comosus* and *Guajava psidium* respectively.

According to Okoli and Okere [30] the flavonoid concentrations (mg/100g) in *Chrysophyllum albidium* plant parts: leaf is 15.3, root is 15.15, seed is 45.80, while stem is 14.30. These values were lesser than the flavonoid concentration got from its fruit (840). *C. albidium* has been found to have the highest content of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times of that of guava or cashew [32].

CONCLUSION

The food value of *Chrysophyllum albidium* fruit is not very high, however, the fruit is very high in antioxidants. The total phenols and flavonoid concentrations which are much stronger in antioxidant capacities than those of vitamins C and E are especially high. Popularizing the utilization of such fruits through research could be very rewarding especially health wise.

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