Chemical Composition of Baobab Leaves and Fractionation of its Ethanolic Extract Using Column Chromatography

Running title: Baobab Leaves Composition and Ethanolic Extract Fractionation Using Chromatography

*Kayode Adebisi Arowora, Ojochenemi Ejeh Yakubu, Christopher Shaibu, Terungwa Joseph Iornenge, Kenneth Chinekwu Ugwuoke

1Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria

Abstract: Background and objective: This study investigated the nutritional and phytochemical composition, antioxidant characteristics of *Adansonia digitata* (baobab) leaves as well as fractionation of its Ethanolic extract using column chromatography. Materials and methods: Baobab leaves were collected from wukari, dried under shade and analyzed for proximate, minerals and phytochemical compositions. Results: Using the column chromatographic fractionation, the following ranges were observed: Total antioxidant capacity (355.56–2909mg/ml), Total phenolic content (2.92–365.42mg/ml), Total flavonoid content (0.41–3.05mg/ml), Metal chelating activity (361.54–5476.92mg/ml) and Beta carotene content (2.89–217.93mg/ml). Proximate composition of baobab leaves revealed the following levels: protein (19.84±0.022%), fat (3.72±0.014%), fibre (4.16±0.014%), ash (8.66±0.014%), Moisture (9.86±0.000%), and Carbohydrate (53.78±0.05%). The result of the Phytochemical analysis showed the presence of the following at indicated levels: Tannins (31.43±0.022mg/100g), Alkaloids (9.35±0.014mg/100g), Flavonoids (63.43±0.022mg/100g), Phenolics (124.36±0.05mg/100g), Glycosides (14.63±0.014mg/100g), Terpenes (12.65±0.02mg/100g) and steroids (6.13±0.022mg/100g). The mineral composition analysis revealed the presence of the following minerals: Ca (415.63±0.020mg/100g), Fe (10.93±0.014mg/100g), Mg (155.92±0.000mg/100g), K (345.41±0.000mg/100g), Na (21.43±0.022mg/100g), P (226.75±0.000mg/100g), Cr (1.06±0.02 µg/100g), S (0.17±0.022mg/100g), Zn (8.32±0.02mg/100g), Mn (35.16±0.05mg/100g), Cu (7.52±0.014mg/100g). Conclusion: This study has shown that *Adansonia digitata* leaves contain appreciable levels of nutrient components, useful minerals and phytochemicals such as cardiac glycosides and phenolics which are beneficial to heart and also have antioxidant properties for scavenging free radicals.

Keywords: Proximate composition, baobab leaves, phytochemicals, minerals

1. Introduction

*Adansonia digitata* (L.) called the baobab tree in English language, is very common to the Sahelian region and belongs to the Malvaceae family. The plant is a very massive tree with a very large trunk (up to 10 m diameter) which can grow up to 25 m in height and may live for hundreds of years. The plant is widespread throughout the hot and drier regions of tropical Africa. Baobab tree has multi-purpose uses and every part of the plant is reported to be useful.

The leaves are used in the preparation of soup. Seeds are used as a thickening agent in soups, but they can be fermented and used as a flavouring agent, or roasted and eaten as snacks. The pulp is either sucked or made into a drink while the bark is used in making ropes. The different parts of the plant provide food, shelter, clothing and medicine as well as material for hunting and fishing.

Baobab tree provides income and employment to rural and urban households. For instance, about 92,445 t of baobab leaf were produced in Burkina Faso in 1990, corresponding to a value of US$18.1 million. Previously published biochemical analyses revealed that the leaves, the seeds and the pulp from baobab are rich in nutrients.

Phytochemical (from Greek phyto, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators. Phytochemicals generally are regarded as research compounds rather than essential nutrients because proof of their possible health effects has not been established yet. Photochemical under research can be classified into major categories, such as carotenoids and polyphenols, which include phenolic acids, flavonoids, and stilbenes/ligans. Flavonoids can be further divided into groups based on their similar chemical structure, such as anthocyanins, flavones, flavanones, isoflavones and flavanols. Flavanolsare further classified as catechins, epicatechins and proanthocyanidins. The phytochemical category includes compounds recognized as essential nutrients, which are naturally contained in plants and are required for normal physiological functions, so must be obtained from the diet in humans. Some phytochemicals are known phytotoxins that are toxic to humans; some are compounds that could be toxic at high levels. Some phytochemicals are anti-nutrients that interfere with the absorption of nutrients. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts. Non-digestible dietary fibers from plant foods, often considered as a phytochemical, are now generally regarded as a nutrient group having approved health claims for reducing the risk of some types of cancer and coronary heart disease. Eating a diet high in fruits, vegetables, grains, legumes and plant-based beverages has long-term health benefits but there is no evidence that taking dietary supplements of non-nutrient phytochemicals extracted from plants similarly benefits health. Phytochemical supplements are neither...
recommended by health authorities for improving health, not approved by regulatory agencies for health claims on product labels.

As a result of its high natural vitamin C content, baobab fruit pulp has a well-documented antioxidant capability. Antioxidants could help prevent oxidative stress related diseases such as cancer, aging, inflammation and cardiovascular diseases as they may eliminate free radicals which contribute to these chronic diseases. The baobab fruit was found to have the highest content of vitamin C at 280 to 300 mg/100 g, out of all fruits investigated. This compared to a vitamin C content of 46 mg/100 g in oranges, a well-documented source of vitamin C.

Baobab fruit pulp was found to have interesting antioxidant properties; in particular, the Integral Antioxidant Capacity (IAC) value of baobab fruit pulp (11.1 mmol/g fresh weight) was higher than that of orange pulp (0.3 mmol/g fresh weight). The high vitamin C and antioxidant content of the fruit pulp may have a role to play in the extension of shelf-life for foods and beverages, as well as cosmetics. The food/beverage industry could introduce baobab fruit pulp into foods in order to act as a preserving ingredient by preventing oxidation of lipids in the food.

2. Materials and Methods

2.1 Sample Collection and Preparation

This research work was carried out in Wukari for about four months between May and September, 2018. The leaves of the Adansoniadigittata plant were collected within Wukari Local Government Area of Taraba state, Nigeria. The leaves were critically examined to be free of disease of any sort. Only healthy leaves were used for the analysis. The plant material was dried in the laboratory at room temperature for one week and pulverized using traditional mortar and pestle.

2.2 Ethanol Extraction

Exactly 200gram of the pulverized sample was soaked in about 1000ml of ethanol for 4 hours. The extract was first filtered using a filtered cloth of which the filtrate obtained was further filtered under reduced pressure using filter paper to obtain the final filtrate. The filtrates were concentrated at room temperature to obtain the desired concentrate.

2.2.1 Fractionation

The ethanol extract was subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while varying solvent combinations of increasing polarity were used as the mobile phase.

2.2.2 Packing of Column

In the packing of the column, the lower part of the glass column was stocked with glass wool with the aid of glass rod. 235 g of silica gel (G60-200 mesh size) was dissolved in 235 ml of n-Hexane to make the slurry. The chromatographic column (30 mm diameter by 400 mm height) was packed with silica gel and was allowed free flow of the solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed 24 h to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus.

2.2.3 Elution

The ethanol extract (2 g) was dissolved in 15 ml absolute ethanol and the solution was applied onto a chromatographic column (30 mm diameter by 400 mm height). Elution of the extract was done with solvent system of gradually increasing polarity, beginning from n-Hexane, ethyl acetate, ethanol, methanol and finally water. The following ratios of solvent combinations were sequentially used in the elution process: n-Hexane 100:00; n-Hexane: ethyl acetate 50:50; ethyl acetate: ethanol 100:00; ethyl acetate: ethanol 50:50; ethanol: methanol 100:00; ethanol: methanol 50:50; methanol: water 100:00; methanol: water 50:50; water 100:00. A measured volume of 100ml of each combination of solvent combination was poured into the column each at time using separator funnel. The eluted fractions were collected in aliquots of 50ml in each of the beakers.

2.3 Determination of Total Antioxidant Capacity (TAC)

DPPH radical scavenging activity was measured as per the procedure . The absorbance was measured in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg/ml Trolox equivalent (TE) using the regression equation from calibration curve.

2.4 Determination of Total Flavonoid Content

Flavonoids were determined using the aluminum chloride colorimetric method . Quercetin standard was used for derivation of the calibration curve. Total flavonoids were expressed as mg/ml quercetin equivalent (QE).

2.5 Determination of Total Polyphenol Content

Total phenolic content (TPC) of the extract was estimated following the phosphomolybdic/ phosphotungstic acid complex procedure of .

2.6 β-Carotene Bleaching Inhibition Assay

In this assay, antioxidant activity was determined by measuring the inhibition conjugated dieneydroperoxides arising from linoleic acid oxidation .

2.7 Metal Chelating Activity

The metal chelating activity was determined using 2mM iron chloride (FeCl3). 1.6ml of distilled water was added to a test tube, 0.05ml iron chloride was added also in the test tube containing distilled water. 0.5ml of the fraction
was added and was incubated for 10 minutes at 40°C. The absorbance was measured using spectrophotometer at 562nm. The concentration was estimated using calibration curve of iron (II) chloride.

The chelating activity of the extract at different concentrations was calculated as: % chelating activity = \((\frac{A_1 - A_2}{A_0}) \times 100\)

Where \(A_0 = \) Absorbance of the control (without extract); \(A_1 = \) Absorbance of reaction mixture and \(A_2 = \) Absorbance without FeCl₂.

### 2.8. Proximate Analysis

The proximate analysis (moisture, ash, crude lipid, crude protein, crude fiber, and carbohydrate) leaves of *Adansonia digitata* were determined using AOAC[31] methods. The moisture was determined using weight difference after oven drying at 100°C to a constant weight. Ash was determined using weight difference after being calcined in a furnace at 550°C for 8 hours. The nitrogen value which is the precursor for protein of a substance was determined by kjeldahl method. The nitrogen value was converted to protein by multiplying to a factor of 6.25. The solvent used was petroleum ether (boiling range 40 – 60 °C). For crude fiber, samples were digested in sulphuric acid and sodium hydroxide solutions and the residue calcined. The difference in weight after calcination indicates the quantity of fiber present. Carbohydrate was determined by difference method, by adding the proximate values of all the other parameters and subtracting it from 100[32,33].

### 2.9 Mineral Analysis

Mineral contents/composition of the *Adansonia digitata* leaves were determined using UNICAM solar969 Atomic Absorption Spectrophotometer for elements such as P, Fe, K, Na, Zn, Ca and Cu.

### 2.10 Determination of Phytochemical Composition:

Quantitative phytochemical analysis to determine the level of Alkaloids, Tannins, Saponins, Phlobatanin, Phenol, Anthraquinone, Steroids, Terpenoids, Flavonoid and Cardiac glycosides using standard methods as described by[34,35] were carried out.

### 2.11 Statistical Analysis

Mean of triplicate values were computed and Standard deviation (SD) of data obtained were determined (Mean ± SD).

### 3. Results

The result for Total Antioxidant Capacity (TAC) showed that fraction 6 has the highest antioxidant Capacity (2900 mg/ml) followed by fraction 7 (2410.46 mg/ml) and 5 (2350.98 mg/ml) with fraction 8 having the lowest Antioxidant Capacity (355.56 mg/ml) as shown below in Fig. 1.

![Figure 1: Total Antioxidant Activity](image)

Fraction 1=n-Hexane 100:00, fraction 2= n-Hexane 100:00, fraction 3 = n-Hexane: ethyl acetate 50:50, fraction 4= n-Hexane: ethyl acetate 50:50, fraction 5 = ethyl acetate: ethanol 100:00, fraction 6= ethyl acetate: ethanol 100:00, fraction 7 = ethyl acetate: ethanol 50:50, fraction 8 = ethyl acetate: ethanol 50:50, fraction 9 = ethanol: methanol 100:00, fraction 10 = ethanol: methanol 100:00, fraction 11 = ethanol: methanol 50:50, fraction 12= ethanol: methanol 50:50, fraction 13 =methanol: water 100:00, fraction 14 =methanol: water 100:00, fraction 15 =methanol: water 50: 50, fraction 16 = methanol: water 50: 50, fraction 17 = water 100:00, fraction 18 = water 100:00

The result for Total Phenolics Content revealed that fraction 8 (365.42mg/ml) had the highest Phenolics content followed by fraction 9 (302.92mg/ml) and fraction 6 (239.58mg/ml). The lowest Total phenolic content was observed fraction 12 (2.92mg/ml).
From the result obtained, fraction 6 (30.05mg/ml) had the highest flavonoid content just like in antioxidant activity. This is followed by fraction 7 (24.91mg/ml), while fractions 5 and 2 have approximately the same flavonoid content 23.63mg/ml and 23.82mg/ml respectively. The lowest flavonoid content was recorded for fraction 18 (0.41mg/ml) as shown below in figure 3.

Result for metal chelating activity revealed that fraction 7 (7261.54mg/ml) had the highest metal chelating activity followed by fraction 3 with a concentration of (5476.92mg/ml). The lowest metal chelating activity was observed in fraction 12 with concentrations of 361.54mg/ml.

Fraction 1= n-Hexane 100:00, fraction 2= n-Hexane 100:00, fraction 3 = n-Hexane: ethyl acetate 50:50, fraction 4= n-Hexane: ethyl acetate 50:50, fraction 5 = ethyl acetate: ethanol 100:00, fraction 6= ethyl acetate: ethanol 100:00, fraction 7 = ethyl acetate: ethanol 50:50, fraction 8 = ethyl acetate: ethanol 50:50, fraction 9 = ethanol: methanol 100:00, fraction 10 = ethanol: methanol 100:00, fraction 11 = ethanol: methanol 50:50, fraction 12= ethanol: methanol 50:50, fraction 13 =methanol: water 100:00, fraction 14 =methanol: water 100:00, fraction 15 =methanol: water 50:50, fraction 16 = methanol: water 50: 50, fraction 17 = water 100:00, fraction 18 = water 100:00

The result obtained from the beta carotene content (BCC) of *Adansonia digitata* leaves revealed that fractions 1 and 2 with the concentration of (217mg/ml) had the highest beta carotene activity followed by fraction 3 (155.07mg/ml). Fraction 5 (2.90mg/ml) was observed to have the lowest beta carotene activity.

Fraction 1= n-Hexane 100:00, fraction 2= n-Hexane 100:00, fraction 3 = n-Hexane: ethyl acetate 50:50, fraction 4= n-Hexane: ethyl acetate 50:50, fraction 5 = ethyl acetate: ethanol 100:00, fraction 6= ethyl acetate: ethanol 100:00, fraction 7 = ethyl acetate: ethanol 50:50, fraction 8 = ethyl acetate: ethanol 50:50, fraction 9 = ethanol: methanol 100:00, fraction 10 = ethanol: methanol 100:00, fraction 11 = ethanol: methanol 50:50, fraction 12= ethanol: methanol 50:50, fraction 13 =methanol: water 100:00, fraction 14 =methanol: water 100:00, fraction 15 =methanol: water 50:50, fraction 16 = methanol: water 50: 50, fraction 17 = water 100:00, fraction 18 = water 100:00

The result of nutritional evaluation of *Adansonia digitata* leaves revealed the presence of proximate components such as carbohydrate, protein, crude fat, moisture, ash and crude fibre. The results are as follows: soluble carbohydrates (53.78%), protein (19.84%), moisture (9.86%), ash (8.66%), fibre (4.16%) and fat (3.72%).
The phytochemical estimation of *Adansonia digitata* leaves showed that phenolics were predominantly present in the leaves of the plant at a concentration of 124.36mg/100g, followed by flavonoids with a concentration of 66.43mg/100g and then Tannins (31.43mg/100g) with the lowest phytochemical composition observed among glycosides, terpenes, alkaloids. The least concentration was observed in steroids (6.13mg/100g).

The minerals evaluation revealed the presence of Phosphorus (P), Copper (Cu), Zinc (Zn), Iron (Fe), Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg), Chromium (Cr), phosphorus (P) and Manganese (Mn). The result of the analysis showed that the Calcium content (415.63mg/100g) was the highest and Chromium content (1.06μg/100g) was the least.

4. Discussion

Antioxidant activity is an extremely significant activity which can be used as a preventive effect against disease. The Total Antioxidant capacity of ethanolic extract of *Adansonia digitata* leaves varies from 355.56-2900.00mg/ml. From previous research, it has been found that DPPH free radicals scavenging antioxidants capacity is as a result of their hydrogen donating ability. From the results of total antioxidant capacity obtained, it was observed that fraction 6 had the highest antioxidant value indicating that the solvent combination “ethyl acetate: ethanol” (100:00) is the most efficient solvent combination for determination of antioxidant activity of *Adansonia digitata* leaves.
ethanolic extract for Adansonia digitata leaves and fraction 8 showed the lowest extraction ability.

Phenolic compounds are the most active natural antioxidants in plants. They are very important plant constituents because their hydroxyl groups confer scavenging ability, because of the reactivity of the phenolic moiety of Adansonia digitata leaves have high content of phenols (365.42mg/100g). This was quite expected as many species of this genus are known for their high phenolic content. The presence of phenol is a clear indication that the plant; Adansonia digitata can be exploited in pharmaceuticals for the treatment of many disease conditions. The presence of phenols makes the plant a potential cancer therapy because phenols are well known for the enormous ability to combat cancer.

Aluminum chloride was used for the determination of the Total Flavonoid Content in Adansonia digitata leaf. Aluminum chloride formed acid stable complexes with C-4 keto groups and either the C-3 and C-5 hydroxide group of flavones and flavonoid. The Total Flavonoid content of Adansonia digitata leaves ranges from 0.41-30.05mg/ml

Result for metal chelating activity revealed that fraction 6 (7261.54mg/ml) has the highest metal chelating activity followed by fraction 2 with a concentration of (5476.92mg/ml) followed by fraction 8 (1630.77mg/ml). The lowest metal chelating activity was observed in fraction 11 with concentrations of 361.54 mg/ml.

β-carotene is one of the major carotenoids, and is one among them which had been known to exhibit provitamin A activity. Carotenoids also exhibit antioxidative properties due to their structural appearance; they have been found to reduce the risk of different diseases owing to the antioxidant effect. In the absence of antioxidants, beta carotene undergoes rapid bleaching and decolourisation from the formation of hydroperoxides by linoleic acid oxidation. The result obtained from the beta carotene content of A. digitata leaves revealed good antioxidant activity which ranges from (2.90mg/ml-217.39mg/ml). Fractions 1 and 2 with the concentration of (217mg/ml), had the highest beta carotene activity followed by fraction 3 (155mg/ml). Fraction 5 (2.90mg/ml) was observed to have the lowest beta carotene activity followed by fraction 13 with a beta carotene activity concentration of 4.35mg/ml.

The result of the phytochemical analysis of the pulverized Adansoniadigitata leaves showed the presence of flavonoids, alkaloids, tannins, terpenes, steroids, glycosides, phenolic and absence of anthraquinones, this observation is similar to the report by. Similar phytoconstituents in the crude extracts were recorded in various plants. Most alkaloids have a strong bitter taste and are very toxic and for these reasons they are used by plant to defend themselves against herbivores, and attacks by microbial pathogens and invertebrate pests. Steroids are very important compounds considering their relationship with compounds such as sex hormone, thus its importance and interest in pharmaceutical companies also mentioned that the analgesic activities were probably due to the presence of sterols. Cardiac glycosides are important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure. Adansonia digitata leaves have high content of phenols (33.02 mg/100g). This was quite expected as many species of this genus are known for their high phenolic content. The presence of phenol is a clear indication that the plant; Adansonia digitata can be exploited in pharmaceuticals for the treatment of many diseased conditions. The presence of phenols makes the plant a potential cancer therapy because phenols are well known for the enormous ability to combat cancer. The alkaloids content of Adansonia digitata leaves was relatively small (9.35 mg/100g), likewise the flavonoids content which is (66.43mg/100mg).

Proximate analysis is an important index to classify the nutritional component of a food material. Proximate analysis result of Adansonia digitata (baobab) leaves revealed that the leaves contained nutritional components that are good for human health; this observation was in accord with the findings.

The result of nutritional evaluation of Adansonia digitata leaves revealed the presence of carbohydrate, protein, crude fat, moisture, ash and crude fibre. The result of nutrients composition showed the following: carbohydrate, protein (53.78±0.05%), protein (19.84±0.022%), moisture (9.85±0.00%), ash (8.66±0.014%), fibre (4.16±0.014%) and fat (3.72±0.014%), this was agreement with the findings. It reported genetic diversity in nutritional traits in baobab leaves, which showed the presence of fat, ash, protein, fibre and carbohydrate contents in the ranges, 76.14-80.00%, 0.24-1.10%, 0.65-1.44%, 3.88-5.64%, 1.60-2.60%, 12.82-17.92% respectively. Also, based on the data of the leaves contained:13–15% protein, 4–10% fat. Calculated that, without considering the conversion factor or the effect of processing, the consumption of 20 g of dry leaf material would cover 10 to 16% of the protein recommended daily intake for children. The high carbohydrate content of seeds indicated that it was a good source of energy. A sample with high level of carbohydrates can regulate nerve tissue. Ash content in baobab leaves facilitate the metabolic processes, growth and development while moisture content reflects the amount of water in the sample which might influence its storability.

The mineral evaluation as showed in figure 8 revealed the presence of Phosphorus (P), Copper (Cu), Zinc (Zn), Iron (Fe), Calcium (Ca), Potassium (K), Sodium (Na), Magnesium (Mg), Chromium (Cr), and Manganese (Mn). The result of the analysis showed that Calcium content (415.63±0.02mg/100g) was the highest and Chromium content (1.05±0.02µg/g/100g) was the least. The presence of sodium and potassium to such extent show that the plant can be used in the management and treatment of diseases associated with the central nervous system (CNS) and also in the prevention of CNS associated disease condition. Thus, baobab leaves are a good source of zinc and calcium for children, pregnant woman and lactating women.

Volume 8 Issue 7, July 2019
www.ijsr.net
Licensed Under Creative Commons Attribution CC BY

Paper ID: ART20199083 10.21275/ART20199083 818
5. Conclusion

The results of the study revealed that baobab leaves contain appreciable levels of nutritional components that are good for human health and maintenance of the body cells. It reveals varying levels of phytochemicals capable of exhibiting free radical scavenging and antioxidant activity. Therefore, consumption of baobab will go along way in preventing diseases elicited by free radicals, oxidative stress and damage to the biopolymers of the body.

6. Significance Statement

This study discovered that the best solvent for exploiting total antioxidant in ethanol extract of baobab leaves was ethyl acetate. This observation can be beneficial in the production of nutraceuticals and functional foods. Also, this study has helped to uncover the solvents and solvents combination for determining the levels of flavonoids and other antioxidant properties which can also be exploited by pharmaceutical companies for drug production.

References


