Advances in Research

21(12): 1-15, 2020; Article no.AIR.64332
ISSN: 2348-0394, NLM ID: 101666096

Nutritional and Phytochemical Properties of *Pachira aquatica* Seed Grown in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ASS and IOG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EED, AAA and IOJ managed the analyses of the study. While authors ASS, SRA and IOJ managed the literature searches data and produced the initial draft. All authors read and approved the final manuscripts.

Article Information

DOI: 10.9734/AIR/2020/v21i1230275
Editor(s):
(1) Prof. Antonio Mastino, University of Messina, Italy.
Reviewers:
(1) Martina Andriani, Sebelas Maret University, Indonesia.
(2) Meite Alassane, Universite Felix Houphouet-Boigny, Cote D'ivoire.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64332

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

ABSTRACT

The present study investigated the nutritional and phytochemical properties of seeds oils and flours from *Pachira aquatica* seed cultivated in Nigeria. Proximate composition and phytochemical composition were determined by standard methods while mineral content of the seed flours and oil was determined using Thermo Scientific iCAP 6200 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The oil which was extracted using Soxhlet extraction method had a yield of 44.43%. The proximate composition of the flour gave carbohydrate 18.12%; moisture 9.74%; protein 19.90%; ash 4.11%; fat 44.43; fibre 3.70%; while phytochemicals showed that phenol was 4.90 mg/100 g; Tannins 0.76 mg/100g; flavonoids 0.68 mg/100 g; glycosides 17.54%; saponins 6.03% and

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alkaloids 10.68%. Macro-element concentrations from the seed oil and seed flour respectively were 63.55 and 10611.74 mg/Kg K; 22.38 and 628.44 mg/Kg Mg; 67.55 and 4007.86 mg/Kg P; 52.65 and 1387.00 mg/Kg Ca; 17.95 and 35.23 mg/Kg Na. Micro-elements were 4.18 mg/Kg and 24.59 mg/Kg Cu; 4.43 mg/Kg and 40.16 mg/Kg Fe; 1.50 and 9.61 mg/Kg Mn; 2.92 mg/Kg and 25.68 mg/Kg Zn; 2.74 mg/Kg and 15.40 mg/Kg Al; 0.82 (mg/Kg103) and 2.27 (mg/Kg103) Ni; 1.11 (mg/Kg103) and 3.30 (mg/Kg103); 3.55 (mg/Kg103) and 17.19 (mg/Kg103) B; 0.20 (mg/Kg103) and 0.40 (mg/Kg103) Mo; 0.01 (mg/Kg103) and 0.03 (mg/Kg103) Se.
Toxic elements recorded 0.17 and 0.85 µg/Kg Cr; 0.18 and 0.23 µg/Kg Co; 0.01 and 0.01 µg/Kg As; 0.01 and 0.02 µg/Kg Cd; and 0.09 and 0.11 µg/Kg Pb for the seed oil and seed flour respectively. The investigation showed that *Pachira aquatica* seed is safe and nutritious for domestic purposes and industrial applications. The energy value indicates that the seed is a good alternative source of energy and could be taken when energy given food is required as it falls within the recommended energy dietary allowances especially for children. The bioactive properties of *Pachira aquatica* seed shows that it has medicinal potential worth exploring for pharmacological purposes.

Keywords: Pachira aquatica seed; seed oil; proximate; phytochemical; minerals composition; Nigeria.

1. INTRODUCTION

Pachira aquatica is a tree belonging to the Bombacaceae family. It is commonly known by different names such as Malabar chestnut, Brazil nut, Brown nut, Wild cocoa, French peanut, Guinea peanut, Money tree, Lucky tree and Epa igi (Yoruba language). *Pachira aqautica* is believed to have originated from Brazil but has sporadically extended to almost all parts of the tropics and subtropics while being a component of urban forestry in the temperate regions [1,2,3]. Uses of the plant is wide including being used as foods, medicines, fibre, dyestuff, wood etc. It is now being intentionally cultivated, and has become naturalized in many areas of the tropics for its edible seeds. In some parts of Africa, the paste from the ground seeds are used as a thickener in stews and sauces while the roast seed is sometimes used to make a beverage and taste like cocoa. The seeds can also be ground into a flour and used to make a bread. In a few communities, oil is extracted from the seeds and used for cooking [4,5,6]. Studies have shown that the plant is possess hypoglycaemic effect [7] and is used to ameliorate different ailments including stomach ache, ulcers, diabetes, bacterial infections, skin rashes and sores, and used as blood purifier [8].

Many plants with enormous bioactive potentials exist and containing beneficial phytochemicals but only a few of the known plants have been characterized and used in pharmaceutical studies, impacting the healthcare system in positive ways [9,10,11]. The use of phytochemicals for chemotherapy has assumed a very central stage more especially as alternatives to synthetic drugs; and so there are ongoing studies to look for natural alternatives for drug use and development [12]. Hence, the present study investigated the nutritional and bioactive compounds of *Pachira aquatica* seed oil and extracts. This will add value to its utility and possibly promote its inclusion in human nutrition, livestock feeds and as a natural or bioactive component in food supplements.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples were collected from Irewolede estate in Ilorin, Kwara State; identified at the herbarium of the Department of Plant Biology (Voucher number UILH/001/2019/1383), Faculty of Life Sciences, University of Ilorin, Kwara State. The mature pods of *Pachira aquatica* were plucked from the trees and dried in oven at 50^{o}C for 5 hours. The seeds were removed from the pods and dried a second time. The seed coat of the seeds was mechanically removed and stored in sealed containers in a dry and cool place for further analysis.

2.2 Proximate Analysis

The proximate compositions (moisture, ash, fat, fibre and protein) of *Pachira aquatica* seed flour was determined according to standard methods of AOAC [13] on the ground sample, was carry out at Nigerian Stored Products Research Institutes, Ilorin (NSPRI). Proximate analyses were carried out on the samples using standard AOAC methods [14]. Moisture content was determined using a hot air oven, by drying the

sample at 105°C± 2°C until a constant weight was obtained. Total fat was determined by Bligh and Dyer method using chloroform/methanol (1/1, v/v). Crude protein content was determined by converting the nitrogen content obtained by Kjeldahl's digestion method (N = 6.25). Ash content was determined after combustion for 20 h at 550°C. Total carbohydrate was determined by difference [15].

2.3 Minerals/Metal Determination

Pachira aquatica seed flour of 1.0 g was weighed into separate conical flasks and wet digested using HNO_3, H_2SO_4 and H_2O_2 mixture [16]. The micro, macro and toxic metals content was determined using Thermo Scientific iCAP 6200 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) and Agilent 7900 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) were used according the method describe by AOAC [13] carried out at ICP – MS Laboratory, Central Analytical Facilities Stellenbosh University, South Africa.

2.4 Phytochemical Screening

Phytochemical tests were carried out on the *Pachira aquatica* seed flour, and *Pachira aquatica* seed oil extracts using standard procedures to identify the constituents as described by Sofowara [17], Trease and Evans [18] and Edeoga et al. [19].

2.4.1 Test for tannins

About 0.5 g of each samples were boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.4.2 Test for saponin

About 2 g of each sample were boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of the filtrate was then mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.4.3 Test for flavonoids

A portion of each samples were heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow colouration indicated a positive test for flavonoids.

2.4.4 Test for steroids

2 mL of acetic anhydride was added to 0.5 g of each sample with 2 mL H_2S0_4. The colour change from violet to blue-green indicated the presence of steroids.

2.4.5 Test for terpenoids (salkowski test)

5 mL of each sample were mixed in 2 mL of chloroform, and concentrated H_2S0_4 (3 mL) then carefully added to form a layer. A reddish-brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.4.6 Test for Glycosides (Keller-Kiliani Test)

To 0.5 g of each sample, 5 mL water was added with 2 mL of glacial acetic acid containing one drop of ferric chloride solution and 1 mL of concentrated sulphuric acid was added. A brown ring at the interface indicated the presence of glycosides.

2.5 Phytochemical Quantitative Analysis

2.5.1 Total phenolic content determination

The total phenolic content of the samples was determined by taking 20 µL of the extract in a screw capped 11-mL test tube, together with 1.6 mL distilled water and Folin-Ciocalteu reagent (100 µL). All were mixed with each other, then 300 µL of 20% Na_2CO_3 solution added and well shaken in a shaking water bath at 40°C for 30 min and total phenolic content determined from the standard curve plotted by using gallic acid as standard at 760 nm according to the method of Anwar et al. [20].

2.5.2 Total flavonoid content determination

The total flavonoid (TF) content of the sample and extract was quantified according to the method described by Dewanto *et al.* [21] and the results determined as catechin equivalents (mg/100 g of dry weight). At a concentration of 1 mg/mL, the extracts were diluted with 4 mL of water in a 10 mL volumetric flask. Initially, 0.3 mL of 5% $NaNO_2$ solution was added to each volumetric flask; at 5 min, 0.3 mL of 10% $AlCl_3$

was added; and at 6 min, 2 mL of 1.0 mol/L NaOH was added before 2.4 mL water was added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm.

2.5.3 Tannins determination

The method described by Wahab and Elabor [14] was used. 0.2 g of sample and extract was measured into a 50 mL beaker. 20 mL of 50% methanol added and covered with paraffin and placed in a water bath at 77-80°C for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 mL volumetric flask using 50% methanol to rinse. This was then made up to mark with distilled water and thoroughly mixed. 1 mL of sample extract was then pipette into 50 mL volumetric flask; 20 mL distilled water, 2.5 ml Folin-Denis reagent and 10 mL of 17% Na_2C0_3 then added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min till a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 mL of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated using the formula:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x } 10{,}000} \quad (1)$$

2.5.4 Alkaloids determination

Five (5 g) of the samples and extract were placed in a 250mL beaker and 200mL of 10% acetic acid (CH_3CO_2H) in ethanol (C_2H_5OH) added. The mixture was then covered and allowed to stand for 4 hours at room temperature. It was then filtered with filter paper and the filtrate concentrated on a water bath until it reached a quarter of its original volume. Concentrated NH_4OH was then added drop wise until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute NH_4OH. The precipitate, alkaloid, was then dried and weighed. The percentage alkaloid is calculated by difference according to Obdoni and Ochuko [22] and Ifemeje et al. [23].

$$\% \text{ Alkaloid} = \frac{W_2\text{-}W_1}{W_t \text{ of sample}} \times 100 \quad (2)$$

where, W_1 = Weight of empty filter paper

W_2 = Weight of filter paper + Alkaloid

2.5.5 Total saponins determination

Five (5 g) of the sample and extract was put into 20 % acetic acid in ethanol and allowed to stand in a water bath at 50^0C for 24 hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was then added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [13,23].

$$\% \text{ Saponin} = \frac{W_2\text{-}W_1}{W_t \text{ of sample}} \times 100 \quad (3)$$

where, W_1 = Weight of filter paper
W_2 = Weight of filter paper + residue

2.5.6 Glycosides determination

To 1 g of extract in a beaker, 5 ml of aqueous methanol was added and mixture allowed standing for 10 minutes. From the mixture 1 mL of the extract was taken into a 100 mL beaker and 1 mL of 2% solution of 3,5-DNS (Dinitro salicylic acid) and 1 mL of 5% aqueous NaOH added. It was boiled for 2 minutes in a water bath at 95–100°C until brick red precipitate was observed. An empty Whatman filter paper No. 42(125 mm) was weighed and used to filter the boiled sample. The filter paper with the absorbed residue was dried in an oven at 50^0C till dryness and the filter paper reweighed. The percentage cardiac glycoside was calculated as described by Ifemeje et al. [23].

$$\% \text{ Glycoside} = (\text{Wt of filter paper+ Residue}) - (\text{wt of filter paper})/ \text{Wt of sample} \times 100 \quad (4)$$

3. RESULTS

The result showed the proximate composition of the *Pachira Aquatica* seed flour, minerals element and phytochemical composition present in the seed flour and the seed oil.

Table 1. Proximate composition of *Pachira aquatica* seed flour

Sample	Moisture content (%)	Protein content (%)	Ash content (%)	Fat content (%)	Fibre content (%)	Carbohydrate content (%)	Energy (Kcal/100g)
seed flour	9.74±0.12	19.90±0.26	4.11±0.03	44.43±0.70	3.70±0.18	18.12±0.40	547.14±0.32

Values are means ± standard deviation of duplicate determination. Means with the different superscripts in the same rows are significantly (P< 0.05) different

Table 2. Macro element presents in *Pachira aquatica* seed flour and seed oil

Minerals	Pachiral seed oil (mg/kg)	*Pachira aquatica* seed flour (mg/kg)
K	63.55±0.21	10611.74±1.88
Mg	22.38±0.54	628.44±1.86
P	67.55±0.64	4007.86±0.48
Ca	52.65±0.08	1387.00±0.17
Na	17.95±0.33	35.23±0.81

Values are means ± standard deviation of duplicate determination. Means with the different superscripts in the same rows are significantly (P< 0.05) different

Table 3. Trace micro element presents in *Pachira aquatica* seed flour and seed oil

Minerals	Pachira seed oil (mg/kg)	*Pachira aquatica* seed flour (mg/kg)
Cu	4.18±0.04	24.59±0.26
Fe	4.43±0.03	40.16±1.36
Mn	1..50±0.09	9.61±0.57
Zn	2.92±0.17	25.68±1.53
Ni (10^3)	0.82±0.31	2.27±0.10
Al	2.74±0.30	15.40±0.56
Ba (10^3)	1.11±0.06	3.30±0.67
B (10^3)	3.55±0.07	17.19±0.30
Mo (10^3)	0.20±0.00	0.40±0.01
Se (10^3)	0.01±0.00	0.03±0.00

Values are means ± standard deviation of duplicate determination. Means with the different superscripts in the same rows are significantly (P< 0.05) different

Table 4. Toxic metal element presents in *Pachira aquatica* seed flour and seed oil

Minerals	Pachira seed oil (µg/Kg)	Pachira seed flour (µg/Kg)
Cr	0.17±0.00	0.85±0.02
Co	0.18±0.01	0.23±0.01
As	0.01±0.00	0.01±0.00
Cd	0.01±0.00	0.02±0.00
Pb	0.09±0.01	0.11±0.01

Values are means ± standard deviation of duplicate determination. Means with the different superscripts in the same rows are significantly (P< 0.05) different

Table 5. Phytochemical screening test of *Pachira aquatica* seed flour

Test/sample	*Pachira aquatica* seed flour
Phenol	+
Tannin	+
Flavonoid	+
Saponin	+
Alkaloid	+
Steroid	+
Glycoside	+

+ means positive

Table 6. Phytochemical quantitative determination of *Pachira aquatica* seed flour

Sample	*Pachira aquatica* seed flour
Phenol (mg/100 g)	4.90±0.01
Tannins (mg/100 g)	0.76±0.00
Flavonoid (mg/100 g)	0.68±0.01
Glycosides (%)	17.54±0.55
Saponins (%)	6.03±0.22
Alkaloids (%)	10.68±0.47

Values are means ± standard deviation of duplicate determination. Means with the different superscripts in the same rows are significantly (P< 0.05) different

4. DISSCUSSION

4.1 Proximate Composition of *Pachira aquatica* Seed Flour

The results of the proximate composition of the dried seed powder of *Pachira aquatica,* on wet matter basis is given in Table 1. It is observed that dried seed powder of *Pachira aquatica* is a good source of lipid (44.43%). The crude fat values obtained for *Pachira aquatica* seed flour are similar to those reported for Amazonian seed (44.1%) [24] but slightly lower than the result reported for pumpkin seed (47.0-49.2%) [25]

conophor seeds (47.9 –51.1%) [26] and those reported for *Pachira aquatica* seed (53.90%) [27]. The crude protein value was lower than the one reported for Cashew nut flour (25.3%) [28], but close to the one reported for pigeon pea (21.6%) [29] and slightly higher than that of *Pachira aquatica* seed reported by Jorge and Luzia, [30] (11.86%) and Oliveira et al. [27] (12.9%). However, the difference in the protein and lipid contents observed between this work and other author cited may be explained by the difference in the moisture content of the seed powder, agricultural practices and geographical region. The moisture content was 9.74% which was higher than the values reported by Jorge and Luzia, (3.89%) [30]; however, it is within the recommended limit for plant flours of 15% established by law [31], which ensures higher quality, because the drier the flour, the higher its microbiological stability. The ash content value obtained in this study agrees with the acceptable ash range values of legumes at 2. 4 - 5.0% [32]. However, the fibre (3.70%) did not meet recommended dietary allowance (RDA) values for children, adults, pregnant and breast-feeding mothers of 19-25%, 21-38%, 28% and 29% respectively [33]. Therefore, *Pachira aquatica* fruit seed is not a good source of dietary fibre for humans, however, it could be recommended for low fibre feed formulation or supplementation. The *Pachira aquatica* seed flour exhibited energy value (547.14 Kcal/100 g) which indicates that the seed is a good alternative source of energy and could be taken when energy given food is required and falls within the recommended energy dietary allowances for children [34] while the extracted residues can be a good source of pretentious food for animal feed or any other food formulation.

4.2 Macro Element Presents in *Pachira aquatica* Seed Flour and *Pachira aquatica* Seed Oil

Mineral compositions and concentrations of the seed are shown in Table 2. High macro-mineral contents including potassium, phosphorus, magnesium and calcium was present; indicating that the seed could significantly contribute to the mineral intake in humans.

The potassium content was between 63.55 and 10611.74 mg/kg and showed a significant ($p<0.05$) difference. The highest potassium value was recorded by for *Pachira aquatica* seed flour and the lowest for *Pachira aquatica* seed oil. Potassium is an essential nutrient and has an important role in the synthesis of amino acids and proteins [35]. High intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risks [36]. This shows that *Pachira aquatica* seed flour can be used for animal feed or other food formulation. The amount of potassium content founds in this experiment were lower than the one reported for *Terminalia catappa* seed (4116.30 mg/100 g dry weight) by Monnet et al. [37] and the values reported for *Pachira aquatica* seed flour are higher than the 536.00 mg/100 g reported in Baobab (*Adansonia digitata*) seed flour by Adubiaro et al. [38].

Sodium content ranged from 17.95 to 35.23 mg/kg with *Pachira aquatica* seed oil having the lowest value and *Pachira aquatica* seed flour the highest value, with an observed significant ($p<0.05$) difference. The result found in all the samples were lower than the 8.42 mg/100g reported in Baobab (*Adansonia digitata*) seed flour by Adubiaro et al. [38]. Sodium content in combination with potassium is involved in proper maintenance of acid-base balance and nerve transmission in the body system. The variation of sodium to potassium in this work is of significant importance particularly for hypertensive patients [39]. Na/K ratio of less than one is recommended whereas 0.282 and 0.003 were obtained in this study for *Pachira aquatica* seed oil and *Pachira aquatica* seed flour implying that these could be suitable in ameliorating sodium-related health risk issues.

Pachira aqutica seed oil has the lowest value of 67.55 mg/kg for phosphorus and falls below the recommended daily intake of phosphorus (700 – 1250 mg/kg); while the *Pachira aquatica* seed flour was above the recommended daily intake. The phosphorus content found were higher than *Moringa oleifera* seed powder (0.619 mg/kg) as indicated by Kawo *et al*. [40] but lower than baobab (*Adansonia digitata*) seed flour (480 mg/100 g) and chicha seed (701.44 mg/100 g) as reported by Adubiaro *et al*. [38] and Fráguas et al. [41] respectively.

The Calcium content were significantly ($p<0.05$) different from each other and ranged from 52.65 to 1387.00 mg/kg. These values were lower than those obtained for *Bischofia javanica* seed (710 mg/100 g) by Indra et al. [42]. Calcium content for *Pachira aquatica* seed flour values fall within the Recommended Dietary Allowance (RDA) [33] of 800-1300 mg/kg while for *Pachira aquatica* seed oil was below the RDA. Calcium is one of

the major components of bones and teeth. It is necessary for blood clotting and muscle contraction.

Phosphorus is related to calcium in activity for bone, teeth and muscles growth and maintenance [39]. The availability of calcium in the body depends on calcium to phosphorus ratio and the presence of antinutritional factors. For good calcium intestinal absorption, Ca:P ratio of 1:1 is required [39]. Ca:P ratio obtained for the edible part of *Pachira aquatica* seed oil and *Pachira aquatica* seed flour, were 1:1.3 and 1:3 which indicates that the seed required to be supplemented with calcium rich diet to maintain Ca/P balance and to prevent mineral and osmotic imbalance.

Magnesium is an element which has a connection with circulatory diseases and calcium metabolism in the bones [43], it is also involved in bone mineralization, the building of protein, enzyme action, normal muscular contraction and transmission of nerve impulses. The magnesium content in this study are from 22.38 and 628.44mg/kg and they were significantly ($p<0.05$) different from each other. *Pachira aquatica* seed oil falls within the recommended Dietary Allowance (RDA) for magnesium 30-350 mg/kg. The value obtained in this study were lower than *Bischofia javanica* seed (610 mg/100 g) as reported by Indra *et al*. [42] and chicha seed (277.32 mg/100 g) by Fráguas *et al*. [41].

4.3 Trace Micro Element Presents in *Pachira aquatica* Seed Flour and *Pachira aquatica* Seed Oil

The results of micro elements were shown on the Table 3. The concentration of copper in the samples were below the recommended safe limit of World Health Organization/Food and Agricultural Organization (WHO/FAO) [40], Codex alimentary commission of 10.00 mg/kg but falls within the maximum safe level given by Standard Organization of Nigeria (SON) (20 mg/kg). The Recommended Dietary Allowance (RDA) for copper is given as between 0.9-2.0 mg/kg [44]. The crop can be used as supplement in food where copper is deficient. *Pachira aqutica* seed oil has the lower value of 4.18±mg/kg for copper and *Pachira aquatica* seed flour has highest value 24.59mg/kg. The results found were lower than those reported for Baobab (*Adansonia digitata*) seed flour (4.26 mg/100 g) by Adubiaro et al. [38], and chicha seed (2.93 mg/100 g) as reported by Fráguas et al. [41] The lowest concentration value were in *Pachira aquatica* seed oil due to low solvent polarity hexane solvent during extraction of the oil.

Zinc is a ubiquitous essential trace element necessary for normal growth of animals and is present in a host of enzymes in the human body and foods. The zinc content of the samples varied significantly from 2.92 and 25.68mg/kg which is within the safe limit of 100mg/Kg recommended by WHO for spices [45]. Unsafe levels of Zn can lead to respiratory system damage, stress and inhibition of normal growth and maturation [42]. The result also showed that the samples in whatever form were a good source of Zn and is capable of supplying the Recommended Dietary Allowance for Zn which is put at between 8-11 mg/kg.

The iron content present in the samples were significantly ($p<0.05$) different from each other. Iron is an essential micro nutrient for haemoglobin formation, normal functioning of central nervous system (CNS) and in oxidation of carbohydrate, protein and fat [46]. The iron content of most of the samples falls within the safety limit (300 mg/kg) [45]. The values found were lower than those reported for *Pachira glabra* leaves (128.71 mg/100 g) by Oni et al. [2], but higher than the one for *Bischofia javanica* seed (2.33 mg/100 g) by Indra *et al*. [42]. The Recommended Dietary Allowance for Iron is in the range of 8-15 mg/kg. The samples had appreciable amount of iron, its consumption could be encouraged for menstruating and lactating women.

Manganese content from Table 3 showed a significant difference ($p<0.05$) between sample concentrations ranging from 1.50 and 9.61 mg/kg with *Pachira aquatica* seed oil having the lower value while *Pachira aquatica* seed flour highest value. The amount of manganese found in chicha seed (0.66 mg/100 g) as reported by Fráguas et al. [41] was lower than the present study, however, *Pachira aquatica* seed oil was lower than it. Manganese is a component of the antioxidant enzyme superoxide dismutase (SOD), which is present in all aerobic cells, where it is required for the de-toxification of oxygen metabolites. Manganese functions in the body normal skeletal growth and development, normal connective tissue growth and development, glucose utilization and protein and nucleic acid metabolism [47].

Nickel is an essential element in animals [48] and has been speculated to play a role in the maintenance of membrane structure, control of prolactin, nucleic acid metabolism or as a cofactor in enzymes. It appears that most dietary intakes would provide sufficient amounts of this element [49]. Nickel (Ni) containing < 0.05ppm developed increases skin pigmentation of the legs, swollen hocks and thickening of the legs near the joints. These signs are not apparent in chicks fed the same diet supplemented with 2-5 ppm nickel [48]. The Nickel content in this study were lower than the one found in *Adansonia digitata* seeds (0.002 mg/g) by Abubakar *et al*. [50]

Aluminum content was 2.74 mg/Kg for *Pachira aquatica* seed oil and 5.5 mg/Kg in *Pachira aquatica* seed flour with a significant ($p<0.05$) different from each other. The result found in all the samples were lower than the values found in *Moringa oleifera* seed powder (144mg/kg) as indicated by Kawo *et al.* [40].

The other micro elements, in descending order by quantity, were Boron (3.55 and 17.19µg/kg), Barium (1.11 and 3.30 µg/kg), Molybdenum (0.20 and 0.40 µg/kg) and Selenium (0.01 and 0.03 µg/kg) for *Pachira aquatica* seed oil and flour respectively.

These trace microelements are essential components of biological structures and play a key role in a variety of the processes necessary for life by mediating in vital biochemical reactions. Excessive levels higher than needed for biological functions, of these elements can be toxic for the body health. Therefore, it has been found that the imbalances in the optimum levels of trace elements may adversely affect biological processes and are associated with many fatal diseases, such as cancers. In this study the concentrations of the essential elements appear to be lower which is within safety limit according to WHO [51].

4.4 Toxic Metal Elements in *Pachira aquatica* Seed Flour and *Pachira aquatica* Seed Oil

Table 4 shows Pb accumulation in the samples was found to below the maximum limits of WHO/FAO [52], 0.5ppm and below the maximum limit of 1ppm recommended by the Standard Organization of Nigeria (SON). The Pb accumulation in the samples were 0.09 µg/kg in *Pachira aquatica* seed oil and 0.11µg/kg in the seed flour with a significant ($p<0.05$) difference. The result found were lower than that obtained in *Pachira glabra* leaves (1.35 mg/100g) by Oni *et al*. [2]. Lead is not essential in normal functioning in human and is a known metal that damages the liver, kidneys, brain, central nervous and reproductive systems of man [53]. According to the Agency for Toxic Substances and Disease Registry (USA), Pb exposure has been attributed to consumption of contaminated fruits and grains [54].

Other heavy metal in this study including Cd (0.50 mg/kg), Cr (1.0 mg/kg), Ar (0.10 mg/kg) and Co (1.0 mg/kg) were detected below maximum limit of WHO/FAO [40], however, presence of heavy metals could possibly be due to environmental and soil factors. However, the levels of heavy metals found in the results were within the regulatory limits, suggesting that they may not impose any health risk.

4.5 Phytochemical Screening Test of *Pachira aquatica* Seed Flour

The *Pachira aquatica* seeds flour was screened for the presence of the following secondary metabolites: alkaloids, glycosides, flavonoids, tannins, steroids, phenols and saponins. The results of the phytochemical screening showed the presence of all the secondary metabolites analyzed in the sample shown on Table 4.

The presence of these phytochemicals in the sample is quite instructive as this lends credence to the use of the plant for medicinal purposes. A lot of plants contain non-toxic glycosides that can be hydrolyzed to give phenolic compounds that are toxic to microbial pathogens [55,56]. The saponin content was found to be present in the sample. Saponins possess the property of precipitating and coagulating red blood cells [57]. It also foamed in aqueous solution and has hemolytic effect and can also bind on cholesterol sites. These properties make saponins present in the plant to exhibit medicinal properties [57] and this therefore supports the findings in this present study that extracts of the plants may be useful in chemotherapy of mycotic infections which the antimicrobial studies revealed [58]. Alkaloids found can be corroborated with literature reports which indicate that naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities [59]. They exhibit marked physiological activity when administered to animals. Classes of alkaloids

are among the major powerful poisons known and despite being poisonous; some of the alkaloids are known to be useful in correcting renal disorders [60]. The use of some plants for medicinal purpose, in the traditional treatment of diseases is due to the presence of flavonoids and saponins [61,62]. The presence of flavonoid was evident to show that plants containing this can be used as diuretic, laxative, emollient and poultice [63] therefore; the use of plants rich in saponins and other Hyptis species in traditional medicine lends credence to the medicinal potentials of the plant. Tannins in some medicinal plants have been found to be responsible for the antiviral and antibacterial activities exhibited by such plants [64,65]. Therefore, *Pachira aquatica* seed could probably be a source of phytochemicals for the treatment of bacterial infections. Phenolic compounds like tannins present in plant cells are inhibitors of many enzymes (proteolytic and hydrolytic) used by plant pathogens. Other compounds such as saponins have antifungal properties [56,66]. Therefore, these phytochemicals detected in this study may be responsible for the antimicrobial potency and also lend credence to the claims of traditional application of the plant as remedies for various ailments.

4.6 Phytochemical Quantitative Determination of *Pachira aquatica* Seed Flour

Phytochemicals possess many ecological and physiological roles and they are widely distributed as plant constituents [67]. As a consequence of their antioxidant properties, phytochemicals exhibit a wide range of biological effects. Plants contain many bioactive chemical substances that produce definite physiological and biochemical actions in the human body. These bioactive constituents include alkaloids, tannin, flavonoids and phenolic compounds [68].

The result of the *Pachira aquatica* seed flour is presented in Table 5 based on wet matter shows that the alkaloids (10.68%) present in the result is higher than 6.40 % reported in Baobab (*Adansonia digitata*) seed flour by Adubiaro *et al*. [69] and also higher than the values reported by Okwu and Ezinne [70] on *Brachstegia eurycoma* seed (0.50 mg/100g) and *Mucuna flagellipes* seed (0.77 mg/100g). Pure, isolated plant alkaloids and their synthetic derivatives are used as a basic medicinal agent for its analgesic, antispasmodic and bacterial effects [71,59]. They exhibit marked physiological activity when administered to animals. Most plant parts used in the cure of diseases have been reported to contain traces of alkaloids. For instance, *Azadirachta indica* used in the cure of malaria contains alkaloids. According to Okwu and Omodamiro [72], Alkaloids at low concentration are therapeutically significant natural plant product owning to their analgesics, antispasmodic and anti-bacterial properties.

The result of the saponins content 6.03% is higher than 2.43% reported in *Ceiba Pentandra* by Chisom *et al*. [73] and 2.06% reported in Baobab (*Adansonia digitata*) seed flour by Adubiaro *et al*. [38] and Chisom *et al*. [73]. Saponins have bitter taste which could be associated with pharmacologic potentials, including hemolytic activities and beneficial effect on blood cholesterol levels, bone health, cancer and the stimulation of immune system [57]. Properties of saponins include formation of foams in aqueous solution, hemolytic activity and cholesterol binding properties and bitterness. Saponins natural tendency to ward off microbes makes it a good candidate for treating fungal and yeast infections. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion [58]. These compounds also appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells, particularly lung and blood cancers [57]. They also lower blood cholesterol there by reducing heart disease. The most outstanding and exciting prospects for saponins are how they inhibit or kill cancer cells. They may also be able to do it without destroying normal cells on the process, as is the mode of some cancer fighting drugs. [59] reported that cancer cells have a more cholesterol type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus interfere with cells growth and division.

The result of the cardiac glycoside content in *Pachira aquatica* seed flour (17.54%) is higher than the value reported by Chisom *et al*.[73] (14.25%) for *Bombax Buonopozense* leaves and higher than the value 0.32% reported in Baobab (*Adansonia digitata*) seed flour by Adubiaro *et al*.[38].

The flavonoid content reported for the samples were lower than the values obtained by Okoronkwo *et al*.[74] for utu (*Icacina*

Senegalensis)(2.82%) and Sycamore (*Ficus Sycomorus*)(3.63%) seeds, but slightly higher than the value obtained in Baobab (*Adansonia digitata*) seed flour (0.65 mg/100 g) by Adubiaro *et al*. [38]. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors [62]. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen for example; flavonoids inhibit estrogen synthesis, an enzyme that binds estrogen to receptors in several organs [61]. Some flavonoids behave as a powerful protective agent against inflammatory disorders.

Tannins content obtained in the sample was lower than the value obtained in Baobab (*Adansonia digitata*) seed flour (2.05 mg/100g) by Adubiaro *et al*. [38]. The presence of tannins implied that the samples have astringent and anti-microbial properties [64] and may be considered for treating a wide variety of ailments including inflammation, liver injury, kidney problems, hypertension, stomach problems and inhibition of reactive oxygen species [65].

In baobab seeds flour, the same family of *Bombacaceae* the amount of polyphenol obtained is 0.32 mg/100g by Adubiaro *et al*. [38] which is lower than the obtained in the present study (4.90mg/100g). Phenols, which are commonly found in both edible and non-edible plants, are one of the main secondary metabolites present in plants and have been reported to have multiple biological effects, including antioxidant activity. They are essential for the plant growth and reproduction, and are produced as a response for defending injured plant against pathogens [56]. According to reports from studies [66], the phenol in the samples could indicate their apparent antimicrobial potential, which could be considered in the treatment of typhoid fever and other bacterial infection.

The differences in the result from this study and those obtained by other researchers can be due to difference in seed sample, limited studies on *Pachira aquatica* seed flour and geographical location of the sample plant.

5. CONCLUSION

The study showed that *Pachira aquatic* is economically viable because of the high oil yield (44.43%). The energy value indicates that the seed is a good alternative source of energy and could be taken when energy given food is required as it falls within the recommended energy dietary allowances for children. The seeds could be used for food and nutraceutical applications. The bioactive properties of *Pachira aquatica* seed have great potential and are worth exploring for pharmacological purposes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lorenzi H. (). Brazilian Trees. Volume 1. 4th Edition. Institute Plantarum De Estudos Da Flora; Brazil; 2002. ISBN85-86714-17-8
2. Oni PI, Malomo AO. Adekoyeni OO. Preliminary evaluation of the ecology, economic importance and nutritional potentials of Pachira aglabra (Pasq.); a neglected fruit tree in Nigeria. International Journal of Current Microbiology and Applied Sciences. 2015;4(2):1030-1036.
3. Cheng LY, Liao HR, Chen LC, Wang SW, Kuo YH, Chung MI et al. Naphthofuranone derivatives and other constituents from *Pachira aquatica* with inhibitory activity on superoxide anion generation by neutrophils. Fitoterapia. 2017;117:16-21.
4. Yeboah SO, Mitei YC, Ngila JC, Wessjohann L, Schmidt J. Compositional and structural studies of the oils from two edible seeds: Tiger nut, Cyperus esculentum, and asiato, Pachira insignis, from Ghana. Food Research International. 2012;47(2):259-266.
5. Ogunlade I, Ilugbiyin A, Osasona AI. A comparative study of proximate composition, anti-nutrient composition and functional properties of Pachira glabra and Afzelia africana seed flours. African Journal of Food Science. 2011;5(1):32-35.
6. Wickens GE, Edible nuts. Non-wood forest products 5. FAO, Rome, 198; 1995. ISBN : 92-5-103748-5
7. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. Journal of Ethnopharmacology. 2005;99(3):325-348.
8. Pietsch J, Koch I, Hermanns-Clausen M, Hüller G, Wagner R, Dressler J. Pediatric plant exposures in Germany, 1998–2004. Clinical Toxicology. 2008;46(7):686-691.

9. Naczk M, Shahidi F. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis. 2006;41(5):1523-1542.
10. Suffredini IB, Sader HS, Gonçalves AG, Reis AO, Gales AC, Varella AD et al. Screening of antibacterial extracts from plants native to the Brazilian amazon rain forest and Atlantic forest. Brazilian Journal of Medical and Biological Research. 2004;37(3):379-384.
11. Boots AW, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. European Journal of Pharmacology. 2008;585(2-3):325-337.
12. Doughari JH, Human IS, Bennade S, Ndakidemi PA. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 2009;3(11):839-848.
13. AOAC Official methods of analysis. 18th Ed. American association of analytical chemists, Inc. Washington; 2010.
14. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences. 2002;8(2):203-208.
15. Wahab OM, Elabor QC. Variation in the Phytochemical Constituents of Seeds, Mature and Immature leaves of *Moringa oleifera* Lam. Growing in five local government areas of Oyo State, Nigeria. Journal of Natural Sciences Research. 2016;6(10):54-60.
16. Ihekoronye AI, Ngoddy PO. Integrated food science and technology for the tropics. macmillan. 1985;165-257
17. Aydin FA, Soylak M.. Separation preconcentration and inductively coupled plasma-mass spectrometric (ICP-MS) determination of thorium (IV): titanium (IV): iron (III), lead (II) and chromium (III) on 2-nitroso-1-naphthol impregnated MCI GEL CHP20P resin. Journal of hazardous materials. 2010;173(1-3):669-674.
18. Sofowara A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993;289.
19. Treatise GE, Evans WC. Pharmacognosy. 11th edn. Brailliar Tiridel Can. Macmillian publishers; 1989.
20. Anwar F, Siddiq A, Iqbal S, Asi MR. Stabilization of sunflower oil with *Moringa oleifera* leaves under ambient storage. Journal of Food Lipids. 2007;14(1):35-49
21. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry. 2002;50(10):3010-3014.
22. Ifemeje JC, JO ECE, Ezebuo FC. Determination of the anti-nutrient composition of Ocimum gratissimum, Corchorus olitorius, Murraya koenigii Spreng and Cucurbita maxima. International Journal of Innovation and Scientific Research. 2014;3(2):127-133.
23. Ige MM, Ogunsua AO, Oke OL Functional properties of the proteins of some Nigerian oilseeds: conophor seeds and three varieties of melon seeds. Journal of Agricultural and Food Chemistry. 1984;32(4):822-825.
24. Oliveira JTA, Vasconcelos IM, BezeiraL CM, Silveira SB, Monteiro ACA, Moreira RA.) Composition and nutritional properties of seeds from *Pachira aquatica* Aubl*, Sterculia striata* St. Hil et Naud and *Terminalia catappa Linn*. *Food Chemistry*. 2000;70(2):185-191.
25. Aremu MO, Olonisakin A, Bako DA, Madu PC. Compositional studies and physicochemical characteristics of cashew nut (Anarcadium occidentale) flour. Pak. J. Nutr. 2006;5(4):328-333
26. Oshodi AA, Esuoso KO, Akintayo ET. Proximate and amino acid composition of some underutilised Nigerian legume flour and protein concentrates. *Rivista Italiana Delle Sostanze Grasse*. 1998;75(8-9):409-412.
27. Jorge N, Luzia DMM. Characterization of seed oil *Pachira aquatica* aublet for food utilization. *Acta amazonica*. 2012;42(1):149-156.
28. Agência Nacional De Vigilância Sanitária (ANVISA). Resolução RDC n° 263, de 22 de setembro de 2005. Regulamento técnico para produtos de cereais, amidos, farinhas e farelos. Disponível em; 2005. Available:<http://www.suvisa.rn.gov.br/contentproducao/aplicacao/sesap_suvisa/arquivos/gerados/resol_263_set_2005.pdf>. Acess in: 12 August 2018.
29. FAO. Utility of tropical Foods: Tropical beans. Food and agricultural organization publication. 1989;22-26.

30. Dietary Reference Intakes (RDA): The essential guide to nutrient requirements. The national academics of sciences engineering medicine. Washington, DC 20001; 2006.
Retrieved: July, 2018.
31. FAO. Handbook on Human Nutritional Requirements, Rome, FAO, Food and Nutrition. 1974;Series No,5
32. Lake RCA, Pereira DA, Siqueira FAR, Szpiz RR, Oliveira JP Preliminary study of seed and oil from five species of amazonian. Acta Amazonica. 1987;16:369-376.
33. Fagbemi TN, Oshodi AA. Chemical composition and functional properties of full fat fluted pumpkin seed flour. Nigeria Food Journal. 1991;9:26-32.
34. Malik CP, Srivastava AK. Text book of plant physiology; 1979.
35. Ayoade GW, Amoo IA, Jabar JM, Ojo AM, Maduawuchi CO. Proximate, Minerals and Amino Acid Profile of (Canarium Schweinfurthii) Seed Pulp. International Journal of Science and Technology. 2017;6(1):657-661.
36. Monnet YT, Gbogouri A, Koffi PKB, Kouamé LP. Chemical characterization of seeds and seed oils from mature Terminalia catappa fruits harvested in Côte d'Ivoire. International Journal of Biosciences. 2012;2(10):110-124.
37. Adubiaro HO, Olaofe O, Akintayo ET, Babalola OO. Chemical composition, cacium, zinc and phytate interrelationships in baobab (Adansonia digitata) seed flour. Advance Journal of Food Science and Technology. 2011;3(4):228-232.
38. Umar KJ, Hassan LG, Ado Y. Mineral composition of Detarium microcarpum grown in Kwatarkwashi, Zamfara state, Nigeria. International Journal of Pure and Applied Science. 2007;1(2):43-48.
39. Kawo AH, Abdullahi BA, Sule MS, Hayatu M, Dabai YU. Comparative Analysis of the Phytochemical, Proximate and Elemental Composition of *Calotropis Procera* (Ait.F.) Ait.F. Latex and *Moringa oleifera* (Lam) seed powder. Life Journal of Science. 2013;15(3):555-563.
40. Fraguas RM, Simao AA, Lima RA, Rocha DA, Queiroz ER, Braga MA, Cesar et al. Chemical constituents of chichá (*Sterculia striata* St. Hil. et Naud.) seeds. African Journal of Agricultural Research. 2015;10(9):965-969
41. Indra R, Bachheti RK, Archana J. Chemical composition, mineral and nutritional value of wild *Bischofia javanica* seed. International food research journal. 2013;20(4):1747-1751.
42. Ishida H, Suzuno H, Sugiyama N, Innami S, Tadokoro T, Maekawa A. Nutritive evaluation on chemical components of leaves stalks and stems of sweet potatoes (Ipomoea batatas poir). food chemistry. 2000;68(3):359-367.
43. Alasalvar C, Shahid F, Amaral JS, Oliveira BP. Compositional characteristics and healths of hazelnut (Corylus avellana L): an overview. In alasalvar. CShahidi, F. (Eds): Tree nuts; compositions, phychemicals, and health effects Boca Ratoy FL: CRC Press Taylor and Francis Group; 2009;185-214.
44. Adeyeyei EI, Otokiti MKO. Proximate composition and some nutritionally valuable minerals of two varieties of capsicum annuml. (Bell and Cherry Peppers). Discovery and Innovation, 1999;11(1):75-82.
45. Biolab medical unit. Nutritional and Environmental Medicine, London England; 2015.
Available:https://www.biolab.co.uk/docs/manganese.pdf
Retrieved September 2018.
46. Nielsen FH. Essentiality and function of nickel. In International Symposium on Trace Element Metabolism in Animals. University Park Press, Baltimore; 1974.
47. Hurley LS. Manganese and other Trace Elements. in: Present Knowledge in Nutrition (Nutrition Reviews '): 4th ed. The Nutrition Foundation, in New York, Washington. 1976;65
48. Abubakar S, Etim V, Bwai D, Afolayan M. Nutraceutical evaluation of baobab (Adansonia digitata l.) seeds and physicochemical properties of its oil. Annals of Biological Sciences, 2015;3(2):13-19.
49. WHO. Trace elements in human nutrition and health. Geneva; 1996.
50. WHO/FAO. "The Role of food safety in health and development"- A report of a Joint FAO/WHO Expert Committee on Food Safety; 1984.
51. Lovei M, Levy BS. Lead exposure and health in central and Eastern Europe. Evidence from hungary, Poland and

Bulgaria. World Bank, Washington DC; 2000.

52. Nkansan MA, Amoako CO. Heavy metal content of some common spicies available in markets in the in the Kumasi metropolis of Ghana. American Journal of Scientific and Industrial Research. 2010;192:158-163.
53. McNamara L. Lead in our food? Now that's a heavy meal; 2008.
Available:http://:mcnamaraupdates.blogspot.com/2008/06/lead-in-our-food-now-that's-heavy-meal.html, Accessed: 27/09/2018
54. Gökay O, Kühner D, Los M, Götz F, Bertsche U, Albert K. An efficient approach for the isolation, identification and evaluation of antimicrobial plant components on an analytical scale, demonstrated by the example of Radix imperatoriae. Analytical Bioanal Chemisty; 2010.
DOI 10.1007/s00216-010-4153-2
55. Abaoba OO, Efuwape BM. Antibacterial properties of some Nigerian species. Biol. Res. Comm. 2001;13:183-188.
56. Sodipo OA, Akanji MA, Kolawole FB, Odutuga AA. Saponin is the active antifungal principle in Garcinia kola, heckle seed. Biosci. Res. Commun. 1991;3:171.
57. Ladan Z, Amupitan JO, Okonkwo EM, Aimola IA, Habila N. Antimicrobial potency of Hyptis spicigera leaf extracts against some pathogenic microorganisms. Journal of Medicinal Plants Research. 2009;3(11):902-905.
58. Okwu DE, Okwu ME. Chemical composition of Spondias mombin Linn plant parts. Journal of Sustainable Agriculture and Environment. 2004;6(2):140-147.
59. Konkwara JO. Medicinal plants of East Africa. Literature Burea, Nairobi. 1979;3-15.
60. Othira JO, Onek LA, Deng LA, Omolo EO. Insecticidal potency of Hyptis spicigera preparations against Sitophilus zeamais (l.) and Tribolium castaneum (herbst) on stored maize grains. African journal of agricultural research. 2009;4(3):187-192.
61. Zwadyk P. Enteriobactericeae in Zinsser Microbiology, 20th Ed. Gerogthieme Verlag, Stuggart. 1992;87.
62. Baba-Moussa F, Akpagana K, Bouchet P. Antifungal activities of seven West African Combretaceae used in traditional medicine. Journal of Ethnopharmacology. 1999;66(3):335-338.
63. Ladan Z, Amupitan JO, Oyewale OA, Ayo RG, Temple E, Ladan EO. Phytochemical screening of the leaf extracts of Hyptis spicigera plant. African Journal of Pure and Applied Chemistry. 2014;8(5):83-88.
64. Elegami AA, El-Nima EI, Tohami ME, Muddathir AK. Antimicrobial activity of some species of the family Combretaceae. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2002;16(6):555-561.
65. Mohanta TK, Patra JK, Rath SK, Pal DK, Thatoi HN. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of Semicarpus anacardium Lf. Scientific Research and Essays. 2007;2(11):486-490.
66. Okwu DE, Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology. 2006;5(4):357-361.
67. Hill AF. Economic Botany. *A Textbook of Useful Plants and Plant Products*. 2nd ed. New York: McGraw-Hill book company Inc. 1952;1-560.
68. Adubiaro HO, Olaofe O, Akintayo ET, Babalola OO. Chemical composition, cacium, zinc and phytate interrelationships in baobab (Adansonia digitata) seed flour. Advance Journal of Food Science and Technology. 2011;3(4):228-232.
69. Okwu DE, Ezinne O. Phytochemical composition of *Brachstegia eurycoma* and *Mucuna flagellipes* seeds. Medicinal and Aromatic Plant Science and Biotechnology. 2007;1(1):103-106.
70. Edeoga HO, Okwu DE, Mbaebie BO.. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685-688.
71. Okwu DE, Omodamiro OD. Effects of hexane extract and phytochemical content of *Xylopia aethiopica* and Ocimum gratissimum on the uterus of guinea pig. Bio-research. 2006;3(2):40-44.
72. Chisom IF, Okereke CN, Okeke CU. Comparative phytochemical and proximate analyses on Ceiba pentandra (L) Gaertn. and Bombax buonopozense (P) Beauv. International Journal of Herbal Medicine. 2014;2(2):162-167.
73. Okoronkwo CU, Ogwo PA, Udensi EA, Agu RO. Nutritional and Phytochemical

Composition of Utu (Icacina Senegalensis) and sycamore (Ficus Sycomorus) seeds. Journal of Environmental Science, Toxicology and Food Technology. 2014; 8(7):49-53.

74. Lake RCA, Pereira DA, Siqueira FAR, Szpiz RR, Oliveira JP. Preliminary study of seed and oil from five species of amazonian. Acta amazonica. 1987;16:369-376.

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