CHEMICAL COMPOSITION AND NUTRITIONAL EVALUATION OF SANDBOX (*Hura crepitan)* SEED FLOUR FOR DOMESTIC CONSUMPTION AND INDUSTRIAL UTILIZATION IN NIGERIA

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ABSTRACT
Proximate, amino acids composition Hura Crepitan seed flour and physicochemical characteristics of oil were determined using standard analytical techniques. The mean values of various parameters for proximate composition (%) were: moisture (5.2±0.03), ash (31.06±0.06), crude fat (43.52±0.01), crude fibre (2.60±0.02) and carbohydrate (by difference) (10.06±0.016).The fatty acids were determined to be 34.82% and energy was 2306.56kJ/100g. Minerals(mg/100g) included: Zn(1.83), Fe(6.38), Mg(35.92), Na(71.94), Ca(117.17), K(62.17), P(24.60), Ni(0.30) and Cr(1.14) while Pb, Cd and Hg were not detected. Amino acid analysis revealed that Hura Crepitan seed flour contained nutritionally useful quantities of most of the essential amino acids. pH (5.92), density (0.77), free fatty acid (0.87),viscosity(39.55) and specific gravity (0.92).The seed oil was odourless at room temperature with light yellow colour. These results suggest that Hura crepitan seeds may be viable sources of oil going by their oil yield. However, the studied characteristics of all oils extracts in most cases compared favourably with other seed oil which is presently used for many domestic and industrial purposes in Nigeria.
Keywords: Hura Crepitan seed flour, chemical composition, physico-chemical properties**.**

INTRODUCTION
In recent times, the desire to conserve resources spent on importation of oil for domestic and industrial use gave renewed impetus in the search for novel sources to complement the traditional ones. There has been a focus on non utilized oil seeds for possible development and use [1]. Attention has therefore, been shifted to under-utilized local seeds for possible development and use as there are several of these under-exploited plant seeds in Nigeria. Oil seeds are important sources of nutritional oils, industrial and pharmaceutical importance. The properties of oils from different sources depend mainly on their composition. The study of these properties is important for their effective uses. *Hura crepitans* Linn is a tropical plant belonging to the family Euphorbiacea. In Nigeria, it is known as 'Odan Mecca' by the Kabba people of Kogi State and "Aroyin" by the Ijesha people of Osun State. Hura crepitans *is* a widely occurring self-regenerating ornamental plant in the tropics and often planted in towns and villages as a cover tree [2]. It has short, densely crowned spines on the trunk and branches, and the long-stalked leaves with prominent closely parallel pinnate nerves, the purple flower spikes and the large fluted flattened fruits are highly distinctive. This tree flowers usually at the beginning and again at the end of rainy season. A person who ate a seed of *Hura Crepitan* complained of burning in the throat, vomiting and purging, suffocating

and headache [3, 4]. It has been further stressed that people who had eaten the shells with the seeds were seized with violent vomiting and headache while those who ate the seeds without the shell suffered from nausea and violent pain in stomach, vomited once and had violent diarrhea. A milky juice that is present in all parts of the plant can cause blindness if applied, and oil of *Hura crepitans* can be used as a purgative. Despite of all these negative findings against *Hura crepitans* seed, the food potential of *H. crepitans* seeds were revealed. However, competitions for commercial edible plant oils which are obtainable from just about a dozen species of plants [5] have necessitated the search for oils from underutilized tropical plants such as *Hura crepitans* L. (Sandbox) (Euphorbiaceae). Despite the potential of this under-utilized species as a source of less consumed food and medicine, to our knowledge, scanty information is available on the chemical composition in Nigeria and whose chemical potential hitherto remains untapped. Therefore, the study is aimed at investigating the proximate, mineral and amino acid composition as well as the physicochemical characteristics of the lipid fraction of Hura *crepitan* seed produced in Nigeria. Such information may expand the scope of knowledge on the utilization and nutritional qualities of *H*ura *crepitan* seed flour.

MATERIALS AND METHODS

Sample preparation: Dry undehisced *H. crepitans* (Sandbox) pods were obtained from the farm of the Faculty of Agriculture of Federal University of Technology, Akure. The seeds were carefully removed from the pods and deshelled. The creamy white cotyledons of *H. crepitans (*sandbox) seeds were sun-dried for 2days. All chemicals used were of the analytical grade (British Drug House, London).

Chemical analysis: The proximate analyses of the samples for moisture, total ash and crude fibre were carried out in triplicate using the methods described in [6]. The nitrogen was determined by the micro Kjeldahl method described by [7] and the nitrogen was converted to protein by multiplying by a factor of 6.25 [8]. Carbohydrate content was estimated by difference of the mean values i.e. 100-(sum of percentages of moisture, ash, protein and lipids) [9, 10]. Data were expressed as per cent of dry weight.

Extraction of oil: 250g of the seed samples were pulverized in Monilex® blender into fine flour. The seed flour was transferred into a thimble and oil extracted using Soxhlet extractor using petroleum ether of Analar grade (British Drug Houses, London), boiling range 60-80^{0}C for 10h [11]. The extracting solvent was evaporated leaving the concentrated oil sample for analysis, extracted oil was quantified gravimetrically.

Mineral analysis: The mineral constituent present in the seeds were analyzed by dry-ashing at 550^{0}C to constant weight and dissolved in volumetric flask using distilled water with drops of concentrated hydrochloric acid. Sodium and potassium were determined by flame photometer (Model, 405, coming, UK) All other metals were determined by using atomic absorption spectrophotometer (Hitachi Z6100, Japan).

Amino acid analysis: 2.0g of Hura Crepitan seed flour was defatted with chloroform/methanol mixture using soxhlet extraction apparatus for 15 hours. 30mg of defatted sample was weighed into glass ampoule. 7ml of 6MHCl was added and oxygen expelled by passing nitrogen into ampoule. The sealed ampoule was put in oven for at 105 for 20hours and later allowed to cool before the content was filtered. The filtrate was evaporated to dryness at 40 under vacuum in a rotary evaporation. The residue was dissolved with 5ml acetate buffer (pH2.0). Amino acid analysis was carried by ion exchange chromatography [12].

PHYSICOCHEMICAL PROPERTIES DETERMINATION

Iodine value: For iodine value of sample, 0.20g of oil was dissolved in 15ml carbon tetrachloride in 100ml glass stoppered flask. 25ml of Wiji's solution was added; the flask stoppered and allowed to stand for 2hr in the dark at 25^0C. 20ml of 10% potassium iodide (KI) solution was added and the mixture titrated with 0.2N Sodium thiosulphate ($Na_2S_2O_3$) using starch indicator. A blank determination was carried out and the iodine value calculated using the formular:

Iodine value = $12.69N (V_1 – V_2) / W$

Where N = Normality of thiosulphate

V_1 = Volume (ml) of thiosulphate solution used in test

V_2 = Volume (in ml) of thiosulphate solution used in blank

W = Weight of sample

Saponification value: Saponification value of the oil samples were determined by dissolving 1g of oil in 12.5ml of 0.5% ethanolic KOH and the mixture refluxed for 30min. 1ml of phenolphthalein indicator was added and the hot soap solution titrated with 0.5NHCl. A blank determination was also carried out under the same condition and saponification value determined using the equation;

Saponification value = $56.1N (V_1-V_2) / W$

where N = Normality of Hydrochloric acid used

V_1 = Volume of Hydrochloric acid used in test 1

V_2 = Volume of Hydrochloric acid used in blank

W = Weight of oil used

Peroxide Value: For peroxide value (PV), 1g of oil sample was weighted into a 200ml conical flask, then 25ml of 2:1 v/v glacial acetic acid: chloroform solvent was added. 1ml of saturated potassium iodide was then added and mixture left in the dark for 1 minute. Next, 30ml of water was added and the mixture titrated with 0.02N thiosulphate solution using 5ml starch as indicator. A blank determination was similarly carried out. PV was calculated from the equation.

Peroxide value (PV) = $[100(V_1 – V_2)$ meg/Kg$] / W$

W = weight of sample

V_1 = Volume (ml) of thiosulphate used in test

V_2 = Volume (ml) of thiosulphate used in blank
N = Normality of thiosulphate ($Na_2S_2O_3$).

Acid Value: Acid Value of the oil sample was determined by dissolving 0.20g of oil in 2.5ml of 1:1 v/v ethanol: diethyl ether solvent and titrating with 0.1N sodium hydroxide while swirling using phenolphthalein as indicator.

Calculation is as follows:
Acid Value = [56.1 x N x V] / W
where N = Normality of NaOH used
V = Volume (ml) of NaOH used
W = Weight of sample used
Percentage free fatty acid (% FFA) (as oleic) was determined by multiplying the acid value with the factor 0.503. Thus % FFA = 0.503 x acid value.

Physical Characterization: colour and flavour of the oil samples was determined by observation using several independent competent individuals. Oil colour was correlated using colour chart while taste and odour were also determined. The refractive index of the oil samples at room temperature was determined using Abbe refractometer [8]. Specific gravity was determined at room temperature using Specific gravity bottle according to the method described by [8]. Viscosity was measured in centipoises (cp) which was performed using Ostwald kinematic viscometer with the aid of water bath at room temperature.

RESULTS

Table 1 presents results of the proximate composition of Hura crepitan seed flour. The moisture mean value of Hura crepitan seed flour is 5.20±0.03% dry weight. The value of protein content (31.06±0.06%). The ether extract (crude fat) with a mean value of 43.52±0.01%. The ash content mean value is 7.63±0.13. The carbohydrate content is 10.06±0.02% and crude fibre content was found to be 2.60±0.02%.

Table 1: Proximate composition (%) of Hura Crepitan seed flour

Composition	%
Moisture	5.20
Ash	2.60
Ether extract	43.52
Crude protein	31.06
Crude fibre	7.63
Carbohydrate (by difference	9.9
[a]Fatty acids	34.82
[b]Energy KJ/100g	2306.56

Values are mean ± standard deviation of triplicate determinations.
[a]calculated fatty acids (0.86× crude fat). [b]calculated metabolizable energy (KJ/100g)(Protein ×17+ Fat ×37+carbohydrate×17)

The mineral content (mg/100g) of Hura Crepitan seed flour is shown in table 2. Calcium was found to be present at significant levels (117.17 ± 0.2mg/100g). The mean value of potassium (62.17± 0.1mg/100g). Phosphorus mean value (62.9 ± 0.3mg/100g) is very close to that of sodium.

Table 2: Concentration of macro and microelement in of Hura Crepitan seed flour(mg/100g)

Mineral	mg/100g
Ca	117.17± 0.2
Na	71.94 ± 0.2
K	62.17 ± 0.2
Mg	35.92 ± 0.3
P	24.6 ± 0.2
Fe	6.38 ± 0.2
Zn	1.83 ± 0.1
Cr	1.14± 0.1
Ni	0.3± 0.1
Pb	ND
Hg	ND
Cd	ND
Na/K	1.2
Ca/P	4.8

Values are mean ± standard deviation of triplicate determinations. ND= Not detected

Table 3 present the amino acid analysis of the Hura Crepitan seed flour (g/100g protein). The major abundant amino acids were Glutamic acid, Arginine, Aspartic acid and Leucine with the values of 15.2, 5.8, 5.2 and 5.1 g/100g protein respectively.

Table 3: Amino acid composition of Hura Crepitan seed flour(g/100g) protein

Mineral	(Concentration g/100g)
Lysine*	2.6
Histidine*	2.1
Arginine*	5.2
Aspartic acid	5.8
Glutamic acid	15.4
Glycine	3.5
Valine*	3.2
Methionine*	2.3
Isoleucine*	4.5
Leucine*	5.1
Tyrosine	2.1
Phenylalanine*	4.3
Serine	3.7
Proline	2.26
Alanine	4.24
Threonine*	3.4

*Essential amino acids

Table 4 presents the physicochemical properties of oil. The colour of the oil was light brown, with specific gravity of 0.98. Viscosity value is 39.55. The saponification value of the oil was 210.56± 0.3 mgKOH/g and 267.84±0.3 mgKOH. The oil had high acid value of 3.65±0.4 mgKOH/g. The iodine value (4.82±0.1mg Iodine/g. The peroxide value is 2.47mg/g. The refractive index was found to be 1.470.

Table 4. Physicochemical Properties of oil Hura Crepitan seed flour

Parameters	Values
Colour	yellow
pH	5.51
Density	0.91
Acid value (mg KOH/g)	7.29
Free fatty acid (%)	3.93
Iodine value	4.82
Peroxide value	6.09
Saponification value	247.05
Unsaponifiable matter (%)	2.25
Specific gravity	0.98
Refractive index (25^0C)	1.48
Viscosity	39.55
State at room temperature	Liquid

Values are mean standard deviation of triplicate determinations

DISCUSSION

The moisture mean value of Hura crepitan seed flour is 5.20 ± 0.03% dry weight is some how low when compared with the mean value of moisture of legumes ranging between 7.0% and 11.0% reported by [13]. However this value is in close agreement with those reported by [14, 15] for fluted pumpkins seed of 5.0% and 5.50% respectively, but higher than the work reported by [16] (3.00± 0.13%) on Hura crepitan seed. The moisture content is an indication of good shelf life characteristic. The value of protein content (31.06±0.06%) is comparable with those reported for some conventional seeds [17] but higher than values obtained by [16] (22.20±0.60%) on Hura crepitan seed. The value is higher than the range found for cereal seeds, wheat and some low protein animal [18] but falls within the range for important legumes such as soybeans, cowpeas, pigean peas, melon, pumpkin and gourd seeds ranging between 23.1-33.0% [19]; chick beans 19.4% and lima bean, 19.8% [20] and Jack bean, 30.8%[21]. This seed could act as protein supplement though its suitability as food supplement depends on the presence of antinutritional factors and digestibility of its nutrients. The ether extract (crude fat) mean is high which shows that the processing of the oil for industrial or edible purposes will be economical. This value is higher than the value reported for the same seed [16] (37.78± 0.81%) and slightly higher than the value reported for the seed of groundnut and that of *Garcinia mangostona* [22]. Fat is important in diets because it promotes fat soluble vitamin absorption [23]. It is a high energy nutrient and does not add to the bulk of the diet. The ash content mean value is 7.63±0.13. It has been

recommended by [24] that ash contents of nuts, seed and tubers should fall in the range 1.5-2.5% in order to be suitable for animal feeds. The ash content of Hura crepitan seed does not fall within this range hence it cannot be recommended for animal feeds. The carbohydrate content is 10.06±0.02% and crude fibre content was found to be 2.60±0.02%.

These values are very low when compared with the report of [16] (27.18± 0.04%) and (6.7±0.20%) for carbohydrate and crude fibre respectively. These values suggest non suitability of compounding it in animal feed [25]. This result thus gave an indication that Hura crepitan seed flour is not a rich source of energy and is not capable of supplying the daily energy requirements of the body. The crude fibre of Hura crepitan seed is very low when compared with legumes, mean values ranging between 5-6% [21, 26]. Maintenance of internal distention for normal peristaltic movement of the intestinal tract is the physiological role of crude fibre. Okon [27] reported that a diet low in fibre is undesirable as it could cause constipation and associated diseases like piles, appendicitis and cancer. The mineral content (mg/100g) of Hura Crepitan seed flour is shown in table 3. Minerals are important in the diet because of their various functions in the body. They serve as cofactors for many physiological and metabolic functions. Calcium, an important mineral required for bone formation and neurological function, was found to be present at significant levels (117.17±0.2mg/100g). Calcium mean value of the present study is lower than melon, but higher than pumpkin and gourd seeds of 130.7, 72.3 and 54.9mg/100g respectively reported by [19]. Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C and D, chlorine and protein are all involved in bone formation [28]. Calcium is also important in blood clothing muscle contraction and in certain enzymes in metabolic processes. The mean value of potassium (62.17±0.1mg/100g) is very close to that of sodium. This is in close agreement with the observation of [29] and [30] that K was one of the most predominate mineral in Nigerian Agricultural Products. Sodium is a macronutrient and constitutes 2 percent of the total mineral content of the body. The mineral is vital in maintaining the body fluid volume, osmotic equilibrium and acid base balance. Deficiency of sodium occurs during hot weather. A significant consumption of tropical of the seed flour will provide the RDA of sodium as 1.37g is an adequate daily intake [31]. It has been reported that magnesium is an activator of many enzymes systems and maintains the electrical potential in nerves [32]. Phosphorus value is very close to that of sodium. Phosphorus is always found with calcium in the body both contributing to the blood. Low Ca/P ratio facilitates calcinations of calcium in the bone and helps to increase the absorption of calcium in the small intestine. Ca/P ratio of Hura Crepitan seed flour is greater than 1, indicating that it would serve as good source of mineral for bone formation. The ratio of sodium to potassium in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended [33]. The Na/K ratio for Hura Crepitan seed flour under consideration is more than one. This is an indication that consumption of Hura Crepitan would probably not reduced high blood pressure disease (Table 2).

Table 3 present the amino acid analysis of the Hura Crepitan seed flour (g/100g protein). The major abundant amino acids were Glutamic acid, Arginine, Aspartic acid and Leucine

with the values of 15.2, 5.8, 5.2 and 5.1 g/100g protein respectively. This observation is in close agreement with the work of [26, 34, 35, 36]. The sum of the aspartic acid and Glutamic amino acid was 21.2g/100gprotein. This value is lower than values obtained from selected oil seeds (melon, pumpkin, cashew nut flour and guort seeds) ranging between 24.2--29.5 [14, 19, 26]. Tryptophan was not determined. The total amino acid (TAA) of 34.8g/100g protein indicated that Hura Crepitan seed will contribute significantly to the supply of amino acid in diet This value is lower than that of melon, pumpkin and gourd seed of 53.4, 38.3 and 53.6g/100g protein respectively reported by [19]; soybean,44.4g/100gprotein[37]; Pigeonpeas,45.2g/100gprotein[38]. This is an indication that Hura Crepitan seed flour is a good source of essential amino acids. The amino acid requirements mg/kg/day for infants and 10–12-year-old children have been reported to be 31 for Isoleucine, 64 for lysine, 27 for sulfur amino acids, 37 for threonine and 14 for tryptophan [39]. There are also evidences that histidine is an essential amino acid for adults, and its dietary requirement in both normal and uremic men has been reported to be between 8 and 12 mg/kg/day [40, 41]. Table 4 presents the physicochemical properties of oil. The colour of the oil was light brown, with bland odour, tasteless and liquid form at room temperature. The specific gravity of oil indicates that it is less dense than water with refractive index of 1.48, which is in agreement with the value of 1.46 for B. Sapida oil[42]. This showed that the oil is less thick when compared with most drying oils whose refractive indices were between 1.475 and 1.485[43]. Viscosity value is 39.55 is similar with the work reported by [44,45], which can be used for driving stationary engines as been practiced in Mali. The oil could be used as lubricant in engine parts in the tropics if left overnight as solidification temperature of the oil is below -10^0C at any season [45]. The specific gravity and viscosity values of the oil were comparable with those obtained for Glycine max (soy bean) by[46]. Hence, they can be used as water evaporation retardants in arid regions where acute shortage is a menace. Also, since the oil is prone to faster biodegradation than fossil fuels, there is no problem of long term environmental pollution problems that may arise from their use. It may also find short term application in preservation of water for moulding blocks, watering seedlings.

The saponification value of the oil was 210.56± 0.3mgKOH/g at 0% and 267.84± 0.3mgKOH at 10% which is higher the values obtained for some vegetable oils ranging from 188-196mg KOH/g. However, there are some vegetables with higher saponification values such as coconut oil(253mgKOH/g), palm kernel oil (247mgKOH/g) and butter fat 225(mgKOH/g). It has been reported by [8] that oils with higher saponification values contain high proportion of lower fatty acids. Therefore the value obtained for Hura crepitan seed indicated that the oil contained high proportion of low fatty acids. The saponification value is high and this suggests the use of the oil in production of liquid soap, shampoos and lather shaving creams. The oil had high acid value of 3.65±0.4mgKOH/g at 0% and 24.95±0.4mgKOH/g at 10% when compared with the report of [16] (19.04± 0.41%); plukenetia conophora (11.5mgKOH/g) as reputed by [47] and low acid value when compared with benniseed (47.6%), by [48]. This can be used to check the level of oxidative deterioration of the oil by enzymatic or chemical oxidation. The acid value is expected to range from 0.00-3.00mgKOH/g oil before it can find application in cooking but the value is high for oil under

study. The acid value can be made fit by subjecting the oil to refining and this may also improve its quality for industrial purposes. The low saponification value and low acid value indicated that the oil may not be suitable for soap making. The iodine value (4.82±0.1mgIodine/g and13.70±0.1 mgIodine/g of Hura Crepitan seed oil is low when compared with the report of [16] (20.81± 0.20%); citrullus vulgaris with value 38.1±3% [49] and Hausa melon seed, 38.50 ±0.67% [50]. The low iodine value obtained suggests the presence of saturated fatty acid and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation and this paces the oil in the non drying oil groups. This oil may find application as a raw material in industries for the manufacture of vegetable oil based ice cream [51]. The peroxide value is 2.47mg/g oil at 0% to 44.04mg/g oil at 10% this value is within the range rancid oil.20.00 to 40.00 mg/g oil [16]. This shows that the oil is not rancid and considered stable [52]. The refractive index which is the ratio of the velocity of light in vacuum to the velocity of light in a medium was found to be 1.470 at 0% and 1.441at 10%.This refractive index is an indication of the level of saturation of the oil.

CONCLUSION

The present study indicated that Hura crepitan seed is rich in important food properties compared to some other oil seeds and nuts. The high quality protein of the seed showed a good source of protein and can be used as a protein supplement in compounding animal feed. The physicochemical properties of the oil indicated that if the oil is subjected to further refining, it could be useful in deep frying, emulsifier formulation, baking activities and as part of short cake and confectionary component as is a non drying oil. It may also find application industries for manufacture of vegetable oil based ice cream, liquid soap, shampoos and shaving cream. The oil could also serve as grease for engine parts without causing sticking problems both in the tropics and temperate region as the oil is free flowing at these temperatures.

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