

Chemical Composition of *Hura crepitans* Seeds and Antimicrobial Activities of its Oil

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Abstract: *Seeds of Hura crepitans (Sandbox tree) were decorticated, milled and homogenized to flour from which oil was extracted using solvent extraction method. Proximate, mineral and fatty acid compositions, as well as, antimicrobial activity of Hura crepitans seed flour and oil were determined using standard test methods. Results of these determinations show that the seed has high crude fat content and is very rich in magnesium. Oleic acid is the most abundant fatty acid and contains more unsaturated fatty acids, but showed no observable antimicrobial activity against the test organisms; E. coli, P. aeruginosa, S. aureus and C. albicans.*

Keywords; *Hura crepitans*, proximate, minerals, antimicrobial

1. Introduction

Africa and indeed most tropical and sub-tropical countries are blessed with numerous seeds and nuts, many of which are yet to be fully exploited due to a dearth of information on their chemical, biological and industrial importance. One of such seed-bearing plants is *Hura crepitans*, commonly referred to as Sandbox tree.

This research work has as its objectives; to determine the proximate and mineral compositions of the kernel of *Hura crepitans* seed, as well as, fatty acid composition and potential antimicrobial activity of its oil. This is done with a view to ascertaining the nutritional value of the seeds and potential industrial applications to which they could be put.

2. Literature survey

Hura crepitans (Sandbox tree) is a tropical plant belonging to the family of *Euphorbiaceae* (spurge family). It is commonly planted in the cities and villages of the south-western part of Nigeria. In Nigeria, it is known as "Odan Mecca" by the Kabba people of Kogi State, Nigeria and as "Aroyin" by the Ijesha people of Osun State, Nigeria, [1]. The trees are about 9 meters tall on the average, with the masculine and feminine flowers on the same tree. The flowers of the masculine are of dark red colour arranged laterally on the small branches. The bark of the tree is covered with lots of short spines when it grows; the woody fruits resemble small pumpkin pods in which the seeds are housed with about 13 seeds in a pod [2]. The unripe seed pods are green in colour, but when ripe, they turn brown. On maturation and drying, the pods explode violently to liberate their seeds to the surrounding. The seeds are small and brown in colour, with an average diameter of about 1-5cm.

Oil extracted from the dry seeds is used as a purgative. The juice of *Hura crepitans* contains a volatile colorless liquid called "Hurin" which some researchers claimed could be

used for the treatment of elephantiasis and leprosy. The oil, though a vegetable oil, is not edible because it is poisonous when ingested and can cause cornea damage or partial blindness when brought in contact with the eye [3]. Such claims of therapeutic potency of this oil, serves as a motivation to evaluate its antimicrobial properties in this study.

3. Previous work

In 2002, Professor Isiaka Amoo, while working with his research team on *Hura crepitans* seeds, reported on the physicochemical properties of the oil [4]. Other scientists such as, Fowomola and Akindahunsi [1], as well as Oderinde et al. [5] had all worked on the physicochemical properties and fatty acid composition of the oil

4. Method / Approach.

4.1. Materials

Seeds that were used for this research work were collected from *Hura crepitans* trees around the campus of Auchi Polytechnic, Edo State, Nigeria, in the months of November and December, 2011. The seeds obtained were those dispersed by the explosion of the matured and dry pods. All solvents and reagents used were of analytical grade, most of which were obtained from the Chemical Store Department of Chemistry, Federal University of Technology Akure.

4.2. Sample Preparation

Seeds of *Hura crepitans* were decorticated, milled with a kitchen blender and homogenized to very fine flour. The resulting flour was put in an air-tight plastic container and stored in a refrigerator at 4°C throughout the duration of the research work.

4.3. Proximate Composition Analysis of Seed Flour

4.3.1. Moisture Content Determination

This was determined by drying the seed flour to constant weight at 105°C. Clean, dry, weighed evaporating dishes were used for this purpose. About 2g of the powdered sample was taken in the dish and placed in an oven to dry at 105°C. After 5 hours of drying, the sample was withdrawn, placed in a desiccator to cool down and weighed. The process of drying, cooling, and weighing was repeated until a constant weight was obtained. The weight of water present was found by difference and expressed as a percentage as follows:

$$\% \text{ Moisture Content} = \frac{(\text{weight loss due to drying}) \times 100}{(\text{weight of sample})}$$

$$\% \text{ Moisture Content} = \frac{(w_2 - w_3) \times 100}{(w_2 - w_1)} \quad (1)$$

4.3.2. Crude fat determination

About 2g of oven-dried seed flour was extracted with petroleum ether for 6 hours in a Soxhlet extractor. After removing the ether by evaporation, the oil residue obtained was dried at 105°C for 30 minutes, cooled in a desiccator and weighed. This alternate drying and weighing at 30 minutes interval was continued until a constant weight was achieved. The percentage fat content of the sample was calculated as below.

$$\% \text{ Crude Fat Content} = \frac{(\text{weight of extracted fat}) \times 100}{(\text{weight of sample})}$$

$$\% \text{ Crude Fat Content} = \frac{(w_2 - w_3) \times 100}{(w_1)} \quad (2)$$

4.3.3. Crude Protein Determination

The crude protein content was determined using microkjeldahl method as described by AOAC [6]. 0.20 g of sample was weighed into a long necked Kjeldahl flask, 1 tablet of Kjeldahl catalyst (Kjeltabs) was added along with 25cm³ conc. H₂SO₄. The flask was swirled to mix the contents. It was gently clamped in an inclined position and heated electrically in a fume cupboard until a clear solution was obtained. The solution was cooled, transferred to a 100cm³ volumetric flask and diluted up to the mark with distilled water. 10ml of the diluted solution was placed in a distillation flask and made alkaline with 0.1 M NaOH. The flask was connected to the distillation apparatus and 40% NaOH was added to the flask through a tap funnel. The flask was swirled to mix its content. Steam generated by heating water in a steam generator connected to the flask was passed in to liberate ammonia gas. Liberated ammonia gas was trapped in a conical flask containing boric acid solution. The conical flask was positioned such that the stem of the condenser dipped into the boric acid solution. After collecting about 50cm³ of the distillate, the receiver was lowered and the tip of the condenser was washed with distilled water, the ammonia solution in the distillate was titrated against 0.1M HCl. A blank determination was carried out using the same amount of the reagents in the absence of the sample.

$$\% \text{ Nitrogen Content} = \frac{(\text{Titre value} \times M \times 0.0014 \times Df \times Cf \times 100)}{(\text{weight of sample})} \quad (3)$$

where;

M = Molarity of HCl = 0.01M

Df = Dilution factor = 50

Cf = Correction factor = 10

% Crude protein = % Nitrogen x 6.25

% Nitrogen was converted to percent crude protein by multiplying with 6.25, the conversion factor. Most proteins contain 16% Nitrogen, hence, the conversion factor is 6.25 (100/16 = 6.25).

4.3.4. Determination of Crude Fibre

200ml of freshly prepared 1.25% H₂SO₄ (0.1275M) was added to 2g of seed flour, which had been defatted by extraction with ether, and brought to boil quickly. Boiling was continued for 30 minutes after which the mixture was filtered. The residue was washed free of acid with plenty of warm water. The residue was then transferred quantitatively into a digestion flask; 200ml of 1.25% NaOH (0.313M) was added and boiled for 30 minutes. The mixture was then filtered and the residue washed free of alkali with warm water. The residue was then washed thrice with methylated spirit, thrice with petroleum ether and was allowed to properly drain. The residue was then transferred to a dried, weighed silica dish and dried to a constant weight at 105°C. The organic matter of the residue was burnt off by igniting for 30 minutes in a muffle furnace at 600°C. The ash left behind was cooled and weighed. The loss in weight on ignition was reported as crude fibre [7].

4.3.5. Determination of Total Ash

5 grams of the sample was weighed into a previously dried, cooled and weighed silica crucible. The crucible and content was ignited, first gently over a low flame until charred, and then in a muffle furnace at 550°C until a white ash was obtained. The ash was moistened with distilled water, dried on steam bath and then on hot-plate and re-ashed at 550°C to constant weight. The weight of ash formed was obtained by difference and expressed as a percentage of the sample used [7].

4.3.6. Determination of Mineral Content of the Ash

The ash obtained from ash determination was dissolved in 10ml HCl (1vol water + 1vol conc. HCl) and transferred quantitatively to a 100ml volumetric flask, 10ml 5% lanthanum solution was added and diluted to the mark. The concentration of the metals in the solution was then determined using a BUCK Scientific Atomic Absorption/Emission Spectrophotometer, Model 210 VGP to analyze the sample [7].

4.3.7. Carbohydrate by Difference

The carbohydrate content was determined by difference i.e.

$$\% \text{ Carbohydrates} = 100 - (\%Mo + \%As + \%Cf + \%Cp) \quad (4)$$

where;

%Mo = Percentage moisture content

%As = Percentage ash content

%Cf = Percentage crude fat

%Cp = Percentage crude protein

4.4. Antimicrobial Analysis of *Hura crepitans* Seed Oil

Two samples of the oil were obtained and labeled A and B respectively. The disc diffusion method of [8] and the Agar-well (or "punch hole") diffusion methods described by Ogbolu [9] were used to determine the antimicrobial activity of the oil samples. The nutrient medium used was blood agar base with 2.5 mg methylene blue, to highlight any zones of inhibition, for strains of bacteria and enriched with 2% glucose for strains of *Candida albicans*. It was prepared by adding 18.5g of nutrient agar in 500ml deionised water and allowed to soak for 10 minutes. The mixture was swirled to mix, sterilized by autoclaving for 15mins at 121°C and cooled to 47°C. Defibrinated blood and 2.5mg methylene blue were added, mixed together and poured into sterile Petri-dishes to solidify.

4.4.1. Preparation of Cultures and Inoculation

Two strains each of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, isolated from urine and wound swab respectively, were utilized in this analysis. They were obtained from the Microbiology Laboratory Department of Irrua Specialist Hospital, Irrua, Edo State Nigeria and were identified and confirmed by the Head Microbiologist, Mr. Inyang N.J. The organisms were separately used to inoculate the Petri dishes by streaking the surface of the plates in a zigzag manner until all the surfaces were covered. The inoculated plates were incubated at room temperature for 24 hours.

4.4.2. Assay of the Oil

Disc Diffusion Method: Sterilized 6mm filter paper discs soaked in the oil samples A & B were placed on the inoculated plates and incubated at 37°C for 24 hours for bacteria and at 35°C for 48 hours for strains of *Candida albicans*. The plates were examined for clear zones of inhibition, the presence of which indicated activity.

Agar-Well Diffusion Method: 6mm diameter wells (or holes), made on the inoculated agar plates using a sterile glass punch, were filled with oil samples A and B with the aid of micropipette. The plates were then placed in an incubator maintained at 37°C for 24 hours for bacteria and at 35°C for 48 hours for strains of *Candida albicans*. The plates were later examined for any zones of inhibition.

4.5. Fatty Acid Analysis of *Hura crepitans* Oil

Hura Crepitans Seed Oil (HCSO) was extracted with n-hexane using soxhlet extraction technique [6]. The fatty acid constituents of the oil and the percentages in which they are present were determined by Gas-Liquid Chromatography.

5. Results and Discussion

5.1. Proximate Analysis of *Hura crepitans* Seed

Result of the proximate analysis of the *Hura crepitans* seed is presented in Table 1.

Table 1: Proximate Composition of *Hura Crepitans* Seed

Parameter	Percentage (%)
Moisture	3.10 ±0.30
Crude Fat	53.81 ±0.10
Crude Fibre	1.21 ±0.10
Crude Protein	37.64 ±0.20
Ash	3.55 ±0.10
Carbohydrate by difference	0.69 ±0.20

Results are means of duplicate determinations ± S.E

The low moisture content obtained in this determination is the same as that reported for the seed by Oderinde et al. [5] and is an indication that the seed will have a long shelf-life. The crude fat value (53.81%) is in close agreement with that obtained for the seed as reported by Amoo et. al. [4], Fowomola and Akindahunsi [1], but is higher than the figures reported for oil seeds, which have crude fat content ranging from about 18% in soyabean to as high as 43% in groundnut oil [11]. Therefore, *Hura crepitans* can be regarded as an oil seed. The crude protein content of the seed (37.64%) is comparable with that of *Albizia lebbek* [12], *Cucumeropsis edulis* [13] and *Telfaaria occidentalis* [14], but is higher than that of *Opuntia ficus indica* [15] and *Hura crepitans* [10]. This high protein value makes the seed a potential protein source which could be incorporated into animal feed production. The crude fibre content the seed (1.21%) is very low, lower than that reported for groundnut [16]. This low crude fibre value of the seed, in addition to its high protein content, makes it suitable for inclusion in the diets of non-ruminants like fish where low fibre content is desirable. The ash content of the seed (3.55%) is comparable with that of *Citrullus lanatus* [17] and falls within the range (3.0 – 4.8%) reported for some legumes [18]. The carbohydrate content of the seed is very low compared to the high values reported by for six varieties of dehulled African yam bean flour [19].

5.2. Mineral Composition of *Hura crepitans*

Ash obtained from the proximate analysis of *Hura crepitans* was analyzed for its mineral content using a BUCK Scientific Atomic Absorption/Emission Spectrophotometer, Model 210 VGP to analyze the samples and the results are as presented in Table 2.

Table 2: Mineral Composition (ppm) of *Hura crepitans*

Mineral	Concentration
Magnesium	119.65
Cobalt	0.05
Copper	2.67
Iron	50.70
Cadmium	0.06
Arsenic	0.20
Zinc	0.04
Chromium	0.28
Manganese	2.65
Mercury	ND
Lead	ND
Calcium	0.11

Sodium	2.15
Potassium	4.20

ND= Not detected

A total of fourteen minerals were determined in the seed. Magnesium was the most abundant, followed by iron, potassium, manganese, copper and sodium in that order. Mercury and Lead were not detected while the rest minerals were present in trace amounts. The values for sodium, calcium and potassium are comparable with those reported for the seed by Fowomola and Akindahunsi [1] but are by far lower than those obtained for *Citrullus lanatus* [17] and cashew nut meal [20]. The magnesium and iron contents of the seed are higher than those of the extra – cotyledonous deposit of Pride of Barbados [21] and of soyabean and palm kernel cakes [22]. Thus, the seed is a good source of magnesium and iron. Therefore, the seed cake can be a cheap source of these minerals in animal diet.

5.3. Fatty Acid Composition

Table 3 shows the fatty acid profile of *Hura crepitans* seed oil (HCSO).

Table 3: Fatty Acid Components of *Hura crepitans* Seed Oil

Fatty Acid	% Composition
<i>Saturated Fatty Acids</i>	
10:0 Capric	0.13
12:0 Lauric	2.38
14:0 Myristic	1.55
16:0 Palmitic	3.05
18:0 Stearic	3.54
20:0 Arachidic	2.58
22:0 Behenic	2.92
24:0 Lignoceric	2.77
Total Saturated Fatty Acids	18.92
<i>Unsaturated Fatty Acids</i>	
16:1 Palmitoleic	1.75
18:1 Oleic	23.87
18:2 Linoleic	0.03
18:3 Linolenic	2.97
Total Unsaturated Fatty Acids	28.62
<i>Unidentified Components</i>	52.46

From Table 3, it can be seen that the principal fatty acid in the oil is oleic acid (23.87%), followed by stearic acid (3.54%); the value for linoleic acid (0.03%) is the smallest. These values are in very close agreement with those reported by Fowomola and Akindahunsi [1], for *Hura crepitans* oil. The amounts of Myristic (1.55%) and Palmitoleic (1.75%) are comparable; the same can be said for lauric, linoleic, Arachidic, behenic and lignoceric acids. Five unidentified components whose individual concentrations range from 2.51 to 16.27% were detected in the sample. Disregarding these unidentified components, the proportion of unsaturated and saturated fatty acids are 28.62 and 18.92% respectively.

The oleic acids to linoleic acid (O/L) ratio as well as iodine value are both indicators of oil stability. High O/L ratio signifies longer shelf life [23]. From Table 3 the O/L ratio of HCSO is 7.96%. This value is very much higher than the

1.48% reported for peanut oil by Branch et al., [24]. The implication of this is that HCSO will have a long shelf life, longer than peanut oil, and may therefore be of valuable industrial application.

5.4. Analysis of Anti-Microbial Activity of *Hura Crepitans* Oil

Antimicrobial properties of mechanically extracted and solvent extracted *Hura* seed oil samples on two strains of clinically isolated *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were investigated using two different antimicrobial sensitivity tests – disc diffusion and agar – well diffusion methods. Results of the investigations are presented in Tables 4 and 5.

Table 4: Result of Agar-well Diffusion method, Using Neat Oil (100% HCSO)

Micro-organisms								
Oil samples	S ₁	S ₂	P ₁	P ₂	E ₁	E ₂	C ₁	C ₂
A	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-

Table 5: Result of Disc Diffusion Method Using Neat Oil (100% HCSO)

Micro-organisms								
Oil samples	S ₁	S ₂	P ₁	P	E ₁	E ₂	C ₁	C ₂
A	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-

A = Soxhlet extracted oil; B = Mechanically extracted oil; S = *Staphylococcus aureus*; P = *Pseudomonas aeruginosa*; E = *Escherichia coli*, C = *Candida albicans*; (-) signifies no zone of inhibition; Subscripts 1 and 2 represent different strains of the species of microorganisms obtained from urine and wound swab.

The Agar –well diffusion and disc diffusion methods are among the two basic conventional methods used to carry out antimicrobial analysis of most liquid (oil) samples. Using these methods, it is expected that the sample (oil) would diffuse from the well or disc into the nutrient medium inoculated with the organisms and create a “zone of inhibition”. Absence of any zone of inhibition implies that the sample (oil) does not have antimicrobial activity on the test microorganisms. The results in Table 4 showed no zone of inhibition for the Agar well diffusion method.

In the disc diffusion method, the oil that was impregnated into the discs placed on the cultured medium was expected to diffuse from the discs into the medium and exert its effect, if any, on the cultured organisms. However, it was observed after the incubation period that the cultured microorganisms grew around the discs; no zone of inhibition was seen using this method as shown in Table 5.

6. Conclusion

Results of this work show that *Hura crepitans* seed was found to possess appreciable levels of protein and some

nutritionally valuable minerals such as; Magnesium, Iron, Potassium and Sodium. It is a good source of non-edible oil which can be processed into biodiesel or soap. The predominant fatty acid in the oil is oleic acid and judging from the high O/L ratio, the oil will have a long shelf-life and will be suitable for industrial applications. However, the oil does not possess antimicrobial activities against the test organisms – *E. coil*, *P. aeruginosa*, *C. albicans* and *S. aureus*.

7. Future Scope

Further research work should focus on using other test organisms such as fungi, protozoan, etc. to study the antimicrobial activities of the oil. Special effort should be made to carry out simultaneous in-vivo and in-vitro tests, just in case such future studies reveal any promising prospect for its use as an antimicrobial agent.

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