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Proximate Analysis And Phytochemical Screening Of Breadfruit (*TreculiaAfricana*) Seed Oil.

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----------ABSTRACT----------
African breadfruit (Treculiaafricana) seeds were purchased from Onitsha main market, Anambra State, Nigeria. Extraction, Proximate analysis and phytochemical screening of oil from the seeds of Treculiaafricana were carried out using standard methods. The sample was milled into flour of small particle size. Oil extraction was carried out using petroleum ether anddiethyl ether as solvents with a yield of 10.7% and 14.0% respectively, indicating that diethylether extracts gave a higher percentage of oil as comparedwith petroleum ether. This also indicates that breadfruit seed is a good source of vegetable oil and energy. Proximate analysis of the flour of breadfruit seedrevealed that the sample contained 4.89% moisture content, 1.90% ash, 8.51% crude fat, 5.61% crude fibre, 27.94% crude protein and 51.15% carbohydrate (CHO). Phytochemical screening shows the presence of secondary metabolites such as flavonoids, phenols, essential oil, saponins, tannins and amino acids except for alkaloids and sugar, indicating that the plant would have good medicinal applications as these metabolites have been reported to be good antifungal, antibacterial and good oxidant enriching food with nutrients.

Keywords*: Extraction, Proximate analysis, Phytochemical screening, Breadfruit, Seed, Treculia Africana*

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I. INTRODUCTION:

Oils from vegetable sources are receiving increasing attention and prominence over animal fat. The importance of vegetable oil is posited on its health benefits over animal fat in human nutrition (Wilcox, 2006). The low or complete absence of cholesterol, high content of unsaturated free fatty acids, low cost, purity, stability of vegetable oils among others food processing benefits are important nutritional considerations in food processing. Superior texture flavor profiles and vitamin (A, D, E and K) delivery underscore the preference for oils of vegetable origin. The desired processing attributes of vegetable oil are influenced by its physio-chemical characteristics and invariably dictates its usefulness in various industrial applications (Wilcox, 2006).

Oil is a reserve of high energy food for use by the germinating seed, and large amounts of oil are associated with large amount of protein. Over the last 20years, there has been considerable progress in utilization of vegetable oils and their derivatives in the formulation of bio-lubricants (Lea, 2003). The versatility of vegetable based fluids and downstream esters is now recognized in research projects in many areas where a number of applications may not have been previously possible, but where modification of the equipment or process designs themselves can enable potential advantages for users (Lea, 2003). The products offer low toxicity, low evaporation rates, low emissions and rapid biodegradability. The health and nutritional benefit of vegetable oil (e.g. coconut oil) especially as an antiviral, antibacterial and antiprotozoal monoglyceride used by humans or animals to destroy lipid-coated viruses such as HIV had been reported since the oil is metabolized in the body to release monolaurin (Rethinam, 2003). Its importance in food industries as biodiesel had also been reported (Rethinam, 2003).

Nigeria has the potential of leading the world, and sustains its leading role in Africa in the area of oleo chemical and biodiesel production from vegetable oil.

Breadfruit seed is a cheap and readily available legume with an annual production in metric tons. It is a member of the taxonomic family *Moraceae*, genus *Treculia*(Baiyeri and Mbah, 2006 a; Enibe, 2001)and a multipurpose tree crop of Southeast Nigeria. It can be found as forest trees in some tropical rain forests of west and central Africa (Nzekwe and Amujiri, 2011).

African breadfruit (*Treculiaafricana*) locally referred to as "Ukwa" in Igbo language, "afon" (Yoruba), "barafuta" (Hausa) "Ize" (Benin) "eyo" (Igala) and "Edikang" (Efik). It is one of the many treasured economical plants. The fruits are usually used as an economical substitute for yam. A matured seed of the crop is made up of the inner endosperm, an outer covering and the husk. The cooked seeds are a valuable food among the "Igbos" in particular and the Efiks, Kalabaris, Edos and the Ika Igbos in Delta State and most tribes of the Southern part of Nigeria (Okonkwo and Ubani, 2007). The seeds are roasted and are useful as thickeners in soups and are

eaten as snacks. The seed is a rich protein source (25-35%) therefore, among the plants consumed in the world it is one of the richest in terms of its benefits (Giami*et al*., 2004).

The de-fated seed contains 20% protein, which is higher than that for cereals and comparable to most pulses. It is primarily high in aromatic amino acids, hence making it a feasible method to obtain good quality protein. The raw seed contains 40 - 45% carbohydrates and also a good amount of vitamins and minerals (Oyetayo and Omenwa, 2006). It is also a good source of vegetable oil 15 – 20%. The oil yield of the seed compares well with that of cotton seeds, palm kernel and sunflower seeds. The fat and oil content of the seed makes it probable industrial raw materials in producing pharmaceutical drugs, vegetable oils, soaps, paints and perfumes (Nwabuezee*t al*., 2008). The seeds are found to have an excellent polyvalent dietetic value with biological value of its proteins exceeding that of vegetable cowpea and soybean (Enibe, 2001). To the best of our knowledge, little or no work has been done on African breadfruit (*Treculiaafricana*) seed and oil in this part of the world. This research is aimed at knowing the nutritional value and possible application of the oil from the seed of African breadfruit through the extraction of oil from the Breadfruit seed, proximate analysis, as well as qualitative and quantitative determination of secondary metabolites present in the oil.

II. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

African breadfruit (*Treculiaafricana*) seeds were purchased from Onitsha main market, Anambra State. They were cleaned by hand picking to rid them of any contaminating stones or extraneous and organic materials before being parboiled at 100^{o}C for 15min. Parboiled and drained seeds were then threshed in a commercial attrition mill and manually dehulled to recover the kernels. The kernels were sun dried for about 17 hours and then milled in a blender to fine flour (2 mm particle size). The flour was preserved in a tight polyethylene bag at room temperature (28± 20^{0}C) from which samples were collected for different analyses.

2.2 Extraction of Oil from African Breadfruit Seed

The method of extraction of oil as reported by Okene and Evbuomwan, (2014) was used. 100g of the grounded African breadfruit seeds were weighed into a thimble (semipermeable membrane) and placed into the soxhlet extractor with 200ml of petroleum ether solvent for the first run. The solid particles were removed by filtration to get the extracted lipids. The apparatus was placed on the heater and was left to run for 2hrs. Extracted oil was transferred into a conical flask and kept for further analysis. This same procedure was repeated for diethyl ether.

2.3Proximate Analysis

After bringing the samples to uniform size, they were analyzed for moisture, ash, crude fat, crude fibre, crude protein and carbohydrate (CHO) using standard methods of AOAC (2003).

Determination of moisture: Moisture was determined by oven drying method. 8.94g of well-mixed sample was accurately weighed in clean, dried crucible (W_1). The crucible was allowed in an oven at 100-105oC for 6-12 hours until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 min to cool. After cooling it was weighed again (W_2). The percentage moisture content was calculated by following formula:

$$\% \text{ Moisture} = \frac{W1 - W2}{\text{Wt of Sample}} \times 100$$

Where: W_1 = Initial weight of crucible + Sample, W_2 = Final weight of crucible + Sample
Wt = Weight of sample.

Ash Determination

An empty dried silica dish was weighed and labeled as W_1. 7.313g of the sample was weighed into the silica dish and reweighed as W_2. This was then placed in a furnace for 8hrs. it was then transferred into a desiccator and allowed to cool, the weight was then taken as W_3. The percentage ash content was calculated.

$$\% \text{ Ash} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where: W_1= Weight of empty silica dish, W_2= Weight of sample and dish before drying
W_3 = Weight of sample and dish after drying

Fat Determination

Using ether as the solvent for extraction, the soxhlet extractor was fit up with a reflux condenser and a small flask which had been previously dried in the oven and weighed. 2gm of sample was weighed and transfer to a fat-free extraction thimble, plugged lightly with cotton wool, and the thimble was placed in the extractor and 150cm^3 of petroleum ether (B.P. 60-80^{0}C) was added into the flask until it siphons over once. More ether was added until the barrel of the 100ml extractor was half filled, the condenser was replaced and the joints tightened and placed on the water bath or electro thermal heating mantle. The source of the heat was adjusted so

that the ether can boil gently and leave it to siphon over for at least 8 hours and finally watched until the ether was just short of siphoning over, then detached from the flask and the contents of the barrel of the extractor was siphoned into the ether stock bottle and drained well before removing the thimble and then dried in the oven. The condenser was replaced and the ether was distilled continuously until the flask was practically dried. The flask was detached (which now contains all the oil) and the exterior cleaned and dried in the oven to constant weight. The extracted residue was kept for "fibre" determination.

$$\% \text{ Crude fat} = \frac{\text{wt of ether extract}}{\text{wt of sample}} \times 100$$

Crude Protein Determination

The nitrogen of protein and other compounds were converted to ammonium sulphate by acid digestion with boiling sulphuric acid. About 150mg of the sample was placed in Kjeldahl flask and 200 milligram of catalyst mixture was added.

$10.0cm^2$ of concentrated sulphuric acid was added to the content of the flask and gently heated for few minutes until frothing ceased. The heat was increased to digest for 3 hours and allowed to cool and made to a $100cm^3$ volume with distilled water.

$10.0cm^3$ aliquot of the dilute solution of the digest was distilled by pipetting the volume into distillation chamber of micro Kjeldhal distillation apparatus. $10.0cm^3$ of 40% sodium hydroxide solution was added and steam distil into $10.0cm^3$ of 2% boric acid containing mixed indicator (note colour from red-green) and titrated with standard 0.2N hydrochloric acid to grey end point.

$$\% \text{ N} = \frac{(a-b)\times 0.01\times 14.0057\times c\times 100}{d\times e}$$

Where: a = Titre value for the sample, b = Titre value for the blank,

c = Volume to which digest is made up with distilled water, d = A liquor taken for distillation (5ml), e = Weight of dried sample (mg)

% Crude Protein = 6.25* x %N (*. Correction factor)

Determination of crude fiber:

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute H_2SO_4 and then with dilute KOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fiber.

Reagents:

Solution of sulphuric acid (0.128M) 7.1 ml, 96% per 1000 ml of distilled water

Solution of Potassium hydroxide (0.223M) 12.5g per 1000 ml of distilled water

Acetone (foam suppresser)

Procedure: Weighed 0.153 g sample (W_0) weighed and transferred to porous crucible. Then placed the crucible into Dosi-fiber unit and kept he valve in "OFF" position. After that added 150 ml of preheated H_2SO_4solution and some drops of foam-suppresser to each column. Then opened the cooling circuit and turned on the heating elements (power at 90%). When it started boiling, reduced the power at 30% and left it for 30 min. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from sample.

The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. Dried the sample in an oven at 150oC for 1 hour. Then allowed the sample to cool in a desiccator and weighed (W_1). Keep the sample crucibles in muffle furnace at 55oC for 3-4 hrs. Cooled the samples in desiccator and weighed again (W_2).

Calculations were done by using the formula:

$$\% \text{ Crude Fibre} = \frac{W1-W2}{W0} \times 100$$

Where: W_1 = weight of sample + crucible before drying, W_2 = weight of sample + crucible after drying, W_0 = weight of sample.

2.4 Phytochemical Screening

Phytochemical screening was carried out to determine the presence of secondary metabolites as described byOyoyede (2005). The crude aqueous extract of the seeds were screened for alkaloids, saponins, tannins, flavonoids, sugar, amino acid, essential oils and phenols. The procedures are described briefly as;

Tannin: 0.2g test solution was made with distilled water to which 0.01g lead acetate was added. The development of a white turbidity in the precipitate represented the presence of tannin.

Alkaloids: 0.2g of the test solution was made with 2N HCl. The aqueous layer was decanted. To the lower layer 2 drops of Mayer's reagent was added. Development of a white turbidity in the precipitate represented the presence of alkaloids.

Flavones: 0.2g of the test solution was mixed with 100µ1of ethanol, 0.02g of paradimethy1 amine benzaldehyde and two drops of conc. HCl. Development of red or pink colour indicate the presence of flavones

Sugar: 0.5g of the test solution was made in a clean test tube, to which 0.01g of anthrone and 3drops of conc. H_2SO_4 were added. The solution was heated for 1 to 2 minutes. Change of green to purple colour was noted to detect the presence of sugar in the sample.
Phenoplic group: An alcoholic plant extract was prepared in a test tube. Two drops of 1M ferric chloride were added. Appearance of intense color indicated the presence of phenolic groups.
Saponin: 0.2g of the test solution with distilled water (2 drops) was prepared in a test tube. The development of a foamy lather indicated the presence of saponin.
Amino acid: 0.2g of test solution made with two drops of 1% ninhydrine in alcohol was prepared in a test tube. Blue or violet colour development indicated the presence of amino acid.
Essential oil: 0.2g of the test solution made with two drops of 1M alcoholic $K_2Cr_2O_7$ and 3 drops of phenolphthalein was prepared in a clean test tube. Soap formation indicated the presence of essential oil.

III. RESULTS AND DISCUSSIONS

3.1 Extraction of Oil

The result of extraction of oil from the seeds of African breadfruit (Table 1) gave 14.0% yield with diethylether which was higher compared with 10.7%obtained from petroleum ether, this is an indication that diethylether is a better solvent for extraction than petroleum ether. This also shows that breadfruit seed is a good source of vegetable oil. The fat and oil content of the seed makes it probable industrial raw materials in producing pharmaceutical drugs, vegetable oils, soaps, paints and perfumes (Nwabueze*et al*., 2008).

Table1: Yield of Oil extracted from African breadfruit seed

Solvent Used	Yield (%)
Petroleum ether	10.7
Diethyl ether	14.0

3.2 Proximate Analysis

Proximate Analysis of *Treculia Africana* seed flour (Table 2) gave a moisture content of 4.89% which is lower compared to 8.19% for the boiled-undehulled seed and 8.12% for the boiled dehulled seed given by Tukura and Obliva (2015). The moisture content was however similar to the results reported for breadfruit seeds from a rainforest in Calabar (Elinge*et al.*, 2012), but lower than the values reported for Artocarpuscommunis (Aremu*et al.*, 2012). The moisture level of food is usually a measure of stability and susceptibility to microbial contamination. High level of moisture content is indicative of its high perishability. Variation in the moisture content could be attributed to the stage of maturity at which the crop was harvested, environmental and storage factors at the time of harvest. Low moisture content of food sample remains an asset in storage and preservation of the nutrients (Arawande *et al.*, 2009).

The percentage ash content was 1.90%,this according to Oyoyede, (2005) is partly a reflection of the mineral composition of the seed, indicating low inorganic matter content in the sample. The crude protein content was 27.94%.This shows that the removed outer coats contain cellulose, thereby shooting up the crude protein and reducing the carbohydrate content of dehulled seed (Ezigbo *et al.*, 2010).

The fat content obtained as 8.51% was similar to that gotten by Tukura and Obliva (2015) varied in the order of raw (7.48%) > boiled-dehulled (7.11%) > boiled- undehulled (7.25%), while crude fibre (5.61%) were similar the boiled-undehulled (5.38%) and boiled-dehulled (5.01%). However, according to Fasasi (2009) lower crude fibre (1.20 %) was reported and high crude fat content (8.20 %). The crude fibre has a useful role in providing roughage that aids digestion (Ezigbo*et al.*, 2010). High fat content will result to high energy value of the seed (Abiodun and Umeonuorah, 2013).

The total carbohydrate content of 51.15% was observed to be the highest of the nutritional components of the sample. Therefore, *Treculiaafricana* may be ranked as carbohydrate rich due to its high carbohydrate contents. Thus, it could serve as a good source of energy. The seeds may also serve as a raw material for production of snacks cookies (Osabor, *et.al*, 2009).

Table 2: Proximate Analysis of *Treculia Africana* seed flour

Components	Percentage (%)
Moisture	4.89
Ash	1.90
Crude fibre	5.61
Crude protein	27.94
Fat	8.51
Carbohydrate	51.15

3.3 Phytochemical Screening

The qualitative phytochemical screening of *Treculiaafricana* oil extract (Table 3) confirmed the presence of flavonoids, phenolic group, saponins, tannins, essential oil, and amino acids in both petroleum and diethyl ether extracts; and the absence of alkaloids and sugars in both solvents. The results also indicated higher amount of the phytochemicals in petroleum ether extract compared with the extract of diethyl ether. The presence of secondary metabolites such as flavonoids, alkaloids, phenolic group, saponins, tanins, sugars, essential oil and amino acids have been proven to be useful as antimicrobial agent and also use in the synthesis of complex chemical substances (Akrout et al. 2010).Ushie, *et.al* (2016) reported that flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and inhibit tumor growth. Saponins causes complexation with cholesterol to form pores in cell membrane bilayers, e.g., in red cell (erythrocyte) membranes, where complexation leads to red cell lysis (hemolysis) on intravenous injection (Francis *et al.*, 2002). Tannins can be used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, haemorrhoiods, and diarrhea, and as antidote in heavy metal poisoning (Ogunleye and Ibitoye., 2003).

Table 3: Qualitative Determination of *Treculiaafricana*of oil extract

Components	Petroleum ether extract	Diethyl ether extract
Alkaloids	-	-
Flavonoids	+	+
Phenolic group	++	+
Saponin	++	+
Tannins	++	+
Sugars	-	-
Essential oil	++	+
Amino acids	++	+

KEY: + = Present in little quantity, ++ = present in moderate quantity and - = Negative (absent)

IV. CONCLUSION

The extraction of oil from the breadfruit seed using petroleum ether and diethyl ether gave good yield which is an indication that the seed is a good source of vegetable oil. The presence of phytochemicals indicates it medicinal value. Further studies are going on these plants in order to quantify the detected phytochemicals, isolate, identify, characterize and elucidate the structure of the bioactive substances.The high carbohydrate and crude protein contents obtained shows that breadfruit seed can be a good source of energy and also help in body building.

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