**EVALUATION OF THE MINERAL CONTENT, VITAMIN CONTENT AND PARTIAL OIL CHARACTERIZATION OF AFRICAN OIL BEAN (*Pentaclethra macrophylla Benth*) SEED**

**ABSTRACT**

**Background and Objective:** The African oil bean (*Pentaclethra macrophylla*) is a specie of the family Leguminosea and an indigenous of tropical Africa. The seed is widely consumed by Nigerians especially in Eastern part of Nigeria. It can be used as flavoring in food and also serves as a substitute for meat. This research work is aimed at evaluating the nutritional constituents of the African oil bean (*Pentaclethra macrophylla*) seed.  **Materials and Method:** The African oil bean seed was evaluated for vitamin, minerals and partial oil characterization using standard laboratory methods. **Results:** The results gotten from the evaluation of vitamins A, D, C, E and Folate were; 9.32%, 19.34%, 68.83%, 37.60% and 0.25% respectively and this shows that the seed is rich in vitamin C. The mineral analysis for Se, Co, Cu, Ca, Zn, Mn, Fe, Mg, K and Na were; 0.077%, 0.045%, 0.395%, 8.484%, 0.430%, 0.172%, 2.199%, 5.747%, 5.270% and 5.923% respectively and the result shows that the African oil bean seed is a rich source of calcium. The oil characterization analysis for saponification value, peroxide value, iodine value, acid value and free fatty acid value were; 134.64%, 12.5%, 89.93%, 7,641% and 3.821% respectively. The high iodine value of the seed oil shows that it cannot be classified as a non-drying oil and cannot be used for paint or polish production. However, the low free fatty acid value shows the oil is edible. The low saponification value shows that the oil contains low molecular weight fatty acid glyceride. **Conclusion:** The seed proves to be beneficial, not only as a food source but also a therapeutic agent for combating diseases.

**KEYWORDS**

African oil bean seed, mineral, vitamins, partial oil characterization.

**INTRODUCTION**

The African oil bean (*Pentaclethra macrophylla*) is a specie of the family Leguminosea and an indigenous of tropical Africa. The seed is widely consumed by Nigerians especially in Eastern part of Nigeria and it is locally called different names in different tribes for example; Apara in Yoruba, Ukpaka or Ugba (Igbo) and Ukana in Efik. It can be used as flavoring in food and also serves as a substitute for meat1.

The tree grows in the Eastern and Southern parts of Nigeria. It grows up to about 21m high and has a width of 6m. It is well branched, forming a crown-like canopy. It blossoms within March and April after which the pods (brown and woody when matured) explodes, scattering the seeds about and curls up. The seeds are dorsally flattened, hard and brown in color and are about 6cm in length and 3cm wide2.

The fleshy part of the African oil bean seed serves as food, can be eaten as snack or used as flavoring. It is prepared by boiling for about 12h, dehusked, sliced, wrapped in plantain leaves and left to ferment for about 2-4 days at surrounding temperatures. It can be mixed with palm oil, spiced and eaten with cooked finely sliced cassava locally called Abacha or eaten with roasted yam and vegetable. The African oil bean seed is reported to be rich in protein (48%), amino acids and fatty acids3.

The nutritional value, bioactive and antinutritional composition of *pentaclethra macrophylla Benth* contains high levels of flavonoid, phenols and prothocyanins4. It also contains high levels of lipid, protein, dietary fibre and vitamins5.

The African oil bean (*pentaclethra macrophylla Benth*)seed contains 47% oil6. The seed is used in producing ‘owala-oil’ (a lubricant) and ‘owala-butter’ and are also used in soap and candle production. The wood of African oil bean tree can serve as an ornament and a good source of shade and can be found along the old thoroughfare of Biafra land. The seed contains a toxic alkaloid, hence it is crushed and used as poison for hunting and warfare7.

*Pentaclethra macrophylla Benth* exhibits antimalarial and antidiabetic effect8. The effective part of the plant that fights against diarrhea and diabetes is attributed to the presence of antioxidants9.

**MATERIALS AND METHODS**

**Study area:** The study was carried out at Biochemistry Department, Biochemistry Department Research Laboratory, Faculty of Natural and Applied Sciences, Nnamdi Azikiwe University, Awka, Nigeria, from April to May, 2023. Docchy Analytical Laboratory and Environmental Service Limited, Awka, Nigeria conducted the physicochemical, mineral and vitamin composition analysis.

**Sample collection and preparation:** The sample of *Pentaclethra macrophylla Benth* seed used was purchased from Eke Awka market, Awka, Nigeria. The fresh sample was carefully selected to removed infested seeds. The selected seeds were dehusked using mortar and pestle then sun-dried and grinded with an electric blender and stored in an air-tight container away from sunlight and dust for subsequent use.

**PROCEDURE**

**ESTIMATION OF VITAMINS**

**Estimation of vitamin A**

Vitamin A was estimated by the method of Bayfield and Cole (1980)11 using the principle of which the assay is based on the spectrophotometric estimation of the color produced by vitamin A acetate or palmitate with TCA. The reagents used were Saponification mixture (2N KOH in 90% alcohol), Petroleum ether (40-60℃), Anhydrous sodium sulphate, Chloroform, Vitamin A palmitate, TCA reagent (60% TCA in chloroform) – prepared fresh.

All procedures were carried out in the dark to avoid the interference of light. 1g of sample was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60℃ in the dark. The tubes were cooled and 20ml of water was added and mixed well. Vitamin A was extracted twice with 10ml of (40-60℃) petroleum ether. The two samples were cooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60℃. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5g were pipetted out into a series of test tubes. The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as mg/kg.

**Estimation of vitamin E**

Vitamin E was estimated in the samples by the Emmerie-Engel reaction as reported by Rosenberg (1992)12 using the principle of Emmerie-Engel reaction which is based on the reduction of ferric to ferrous ions by Vitamin E which with 2,2'-dipyridyl forms a red color. Vitamin E and carotenes were first sampled with xylene and read at 460nm to measure carotenes. A correction was made for these after ferric chloride has been added and read at 520nm. The reagents used were Absolute alcohol, Xylene, 2,2'-dipyridyl (1.2g/L in n-propanol), Ferric chloride solution (1.2g/L in ethanol), Standard solution (D, L-α-Vitamin E, 10mg/L in absolute alcohol) and Sulphuric acid (0.1N). The sample (2.5g) was homogenized in 50ml of 0.1N sulphuric acid and allowed to stand overnight. The contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper. Aliquots of the filtrate were used for the estimation and poured into 3 stoppered centrifuge tubes containing 1.5ml of sample, 1.5ml of the standard and 1.5ml of water which were pipetted out separately. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed and centrifuged. Xylene (1.0ml) layer was transferred into another stoppered tube. To each tube, 1.0ml of dipyridyl reagent was added and mixed well. The mixture (1.5ml) was pipetted out into a cuvette and the extinction was read at 460nm.

**Estimation of vitamin C**

Vitamin C was analysed by the spectrophotometric method described by Roe and Keuther (1943)13. Absorbate was converted into dehydroascorbate on treatment with activated charcoal, which reacted with 2,4-dinitrophenyl hydrazine to form osazones. These osazanes produced an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm. The reagents used were TCA (4%), 2,4 dinitrophenyl hydrazine reagent (2%) in 9N H2SO4, Thiourea (10%), Sulphuric acid (85%) and Standard ascorbic acid solution: 100µg/ml in 4% TCA. Ascorbate was extracted from 1g of the sample using 4% TCA and the volume was made up to 10ml with the same. The supernatant obtained after centrifuging at 2000rpm for 10minutes was treated with a pinch of activated charcoal, shaken vigorously using a cyclomixer and kept for 5 minutes. The charcoal particles were removed by centrifugation and aliquots were used for the estimation.

Standard ascorbate ranging between 0.2 – 1.0ml and 0.5ml and 1.0ml of the supernatant were taken. The volume was made up to 2.0ml with 4% TCA. DNPH reagent (0.5ml) was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37℃ for 3 hours resulting in the formation of osazane crystals. The crystals were dissolved in 2.5ml of 85% sulphuric acid.

**Estimation of vitamin D**

Vitamin D was assayed according to the method of Brockmann*et al.* (1974)14 using the principle which is based on the formation of a yellow color by reaction of the vitamin with a chloroform solution of trichloroacetic acid. Vitamin D3 sample (25g) was weighed and 25 ml of working standard was placed in a volumetric flask with solution mixture (chloroform and methanol in ratio 1:9) which was dissolved and diluted with solution mixture to make up to the mark and properly mixed. Exactly 1.6ml of 0.25N HCL, 0.5ml of 15.0% trichloroacetic acid (TCA) and 0.5ml of 0.375% 0f thiobarbituric acid (TBA) was added and the absorbance was read at 464nm against blank.

**PARTIAL OIL CHARACTERIZATION**

**Acid value**

The procedure involved mixing of 25ml diethyl ether with 25ml alcohol and 1ml phenolphthalein (1%). The sample was carefully neutralized with 0.1M NaOH. 1-10g of the oil and was dissolved in the mixed neutral solvent and titrated with aqueous 0.1M NaOH shaking constantly until pink color which persists for 15 seconds was obtained.

Calculation: $Acid value=\frac{titre\left(ml\right)x 5.61}{weight of sample used}$

The FFA figure is usually calculated as oleic acid (1ml 0.1M sodium hydroxide = 0.0282g oleic acid), in which case the acid value = 2x FFA. For most oils acidity begins to be noticeable to the palate when the FFA calculated as oleic acid is about 0.5- 1.5 %, For palm oil as palmic (1ml 0.M NaOH = 0.0256g) and palm kernel, coconut and similar lauric acid (1ml 0.M NaOH =0.0200g).

**Iodine value**

The iodine value was determined by pouring the oil onto a small beaker and a small rod was placed in the beaker. A suitable quantity of the sample was weighed by difference into a dry glass -stoppered bottle of about 250ml capacity. The approximate weight in (g) of the oil to be taken was calculated by dividing 20 by the highest expected iodine value. Then 10ml of carbon tetrachloride was added to the oil and dissolved. 20ml of wijis’ solution was added, and the stopper previously moistened with potassium iodine solution was inserted and allowed to stand in the dark for 30 minutes. 15ml of potassium iodine solution (10%) and 100ml water was added and mixed, then titrated with 0.1M thiosulphate solution using starch as indicator just before the end-point (titration = aml). A blank was carried out at the same time commencing with 10ml of carbon tetrachloride (titration = bml).

Calculation: $iodine value=\frac{\left(b-a\right) x 1.269}{wt. \left(g\right)of sample}$

Note: if (b-a) is greater than b/2 the test must be repeated using a smaller amount of the sample. It should be noted also that the less unsaturated fats with low iodine values are solid at room temperature, or conversely, oils that are more highly unsaturated are liquid (showing there is a relationship between melting points and the iodine value).

**Preparation of wijis’ solution**

Wijis' solution was prepared by dissolving 8g iodine trichloride in 200ml glacial acetic acid and then dissolving 9g iodine in 300ml carbon tetrachloride. The two solutions were mixed and diluted to 1000ml with glacial acetic acid.

**Peroxide value**

The procedure for estimating peroxide value involved weighing 1g of oil into a clean dry boiling tube and while the oil is still liquid, 1g powdered potassium iodide and 20ml of solvent mixture (2 vol glacial acetic acid + 1 vol chloroform) was added to the oil. Then the tube was placed in boiling water so that the liquid boils within 30 seconds and then allowed to boil vigorously for not more than 30 seconds. The contents were poured quickly into a flask containing 20ml of potassium iodide solution (5%), the tube was washed twice with 25ml water and titrated with 0.002M sodium thiosulphate solution using starch. The blank was performed at the same time.

**Saponification value**

The saponification value was calculated by weighing 2g of the oil into a conical flask, exactly 25ml of the alcoholic potassium hydroxide solution was added. A reflux condenser was attached and the flask heated in boiling water for 1hr, shaking frequently. The flask and condenser was allowed to cool before washing the inside of the condenser with a little distilled water. Exactly 1ml of phenolphthalein (1%) solution was added and titrated with the hot excess alkali and 0.5N hydrochloric acid until the pink color of the indicator disappeared. The blank was determined using the same procedure.

Saponification value= $\frac{56.1N \left(Vb-Vs\right)}{wt. \left(g\right)of sample}$

Where Vb = volume in milliliters of the hydrochloric acid solution used for the blank.

 Vs= volume in milliliters of the hydrochloric acid used for the determination of sample

 N= normality of the hydrochloric acid

**Thiobarbituric acid value (Tba)**

The procedure for determination of thiobarbituric acid value involved softening 10g of the fatty sample with 50ml water for 2minutes and washed into a distillation flask with 47.5ml water. Then 2.5ml hydrochloric acid was added to bring the PH to 1.5, followed by an antifoam preparation and a few glass beads. The flask was heated by means of an electric mantle so that 50ml distillate is collected in 10 minutes from the time boiling commences. Exactly 5ml distillate was pipetted into a glass-stoppered tube and 5ml TBA reagent (0.2883g/100ml of 90% glacial acetic acid) stopper was added, shaked and heated in boiling water for 35minutes. The blank was prepared similarly using 5ml water reagent. Then the tubes were cooled in water for 10 minutes. The absorbance (D) was measured against the blank at 538nm using 1cm cells. TBA no. (as mg malonaldehyde per kg sample) = 7.8D

**Specific gravity**

The density bottle was used to calculate the specific gravity of the oil. A 25ml bottle with a clean, dry stopper was weighed (W0) then filled with oil and reweighed to determine W1. The bottle was cleaned and dried and the oil was replaced with distilled water, the result was weighed to produce W2.

Specific gravity= w1-w2

 w2-w0

**Refractive index**

The Abbe refractometer was reset with a light compensator, then the oil sample was seared on the lower prism of the instrument and closed. A light was passed by means of the bangled mirror, the reflected light appeared in form of a dark background. The telescope tubes were moved using a fine adjustment until the shadow appeared central in the cross wire indicator. The reading of the refractive index was taken.

**Smoke and fire point**

A portion of the oil (10ml) was poured into an evaporating dish. A thermometer was suspended at the centre of the dish ensuring that the bulb just dips inside the oil without touching the bottom of the dish. The temperature of oil was raised gradually using hot plate. Then the temperature at which the oil sample gives off a thin bluish smoke continuously was notted as the smoke point. Similarly, the temperature at which the oil started flashing without supporting combustion was equally noted as the flash point. The temperature at which the oil starts supporting combustion was recored as the fire point.

**MINERAL ANALYSIS**

**Methods for the Mineral Analysis**

Heavy metal analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophometer according to the method of APHA 199515 (American Public Health Association). The working principle for the atomic absorption spectrometer is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element was used, making the method relatively free from spectral radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

**Sample Digestion**

Approximately 2g of the dried sample was weighed into a digestion flask and 20ml of the acid mixture (650ml conc. HNO3; 80ml perchloric acid; 20ml conc. H2SO4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100ml mark Adrian (1973)15.

**Preparation of reference solutions**

A series of standard metal solutions in the optimum concentration range were prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/litre.  A calibration blank was prepared using all the reagents except for the metal stock solutions.

**RESULTS AND DISCUSSION**

**Elemental (Mineral) Analysis**: The result for the mineral analysis of the African oil bean (*Pentaclethra macrophylla* Benth) seed shows that the seed is a rich source of calcium. Calcium had the highest concentration of all the elements that were analyzed.

**Table 1a: Showing the macromineral composition of African oil bean seed**

|  |  |
| --- | --- |
| Macro mineral  | Concentration (ppm) |
| MagnesiumSodiumCalciumPotassium | 5.7475.9238.4845.270 |

**Table 1b: Showing the micromineral composition of African oil bean seed**

|  |  |
| --- | --- |
| Micro mineral  | Concentration (ppm) |
| IronZincSeleniumCopperCobaltManganese | 2.1990.4300.7770.3950.0450.172 |

**VITAMIN ANALYSIS**

**Table 2: showing the vitamin composition of African oil bean seed**

|  |  |
| --- | --- |
| Vitamins | Concentration (mg/kg) |
| Vitamin AVitamin CVitamin DVitamin EFolate | 9.32± 0.00268.83± 0.02719.36± 0.03737.599±0.030.25±0.153 |

This result on the pie chart shows that the African oil bean is a rich source of vitamins. It is high in Vitamin C and Vitamin E compared to other vitamins.

Figure 1: Chart showing the vitamin composition present in African oil bean seed.

**PARTIAL OIL CHARACTERIZATION**

The result from the partial oil characterization of African oil bean seed shows high acid value indicates hydrolysis and oil rancidity and the low value for free fatty acid indicates the oil is edible.

**Table 3: Showing the partial oil characterization parameter of African oil bean seed**

|  |  |
| --- | --- |
| Parameters | Concentration |
| Saponification valuePeroxide valueIodine valueRefractive indexAcid valueFree fatty acidViscositySpecific gravityCloud pointFlash pointBoiling pointMelting point | 134.643±0.200012.5± 0.115089.933± 0.10001.413± 0.00057.641± 0.05803.821± 0.5600 104.90± 2.1100 0.9302± 1.360013.13± 0.1500266 ± 2.0000325.67± 4.51007.3± 0.3100 |

**DISCUSSION**

African oil bean seed is rich in minerals, vitamins, and healthy fats, incorporating these seeds into your diet can provide numerous health benefits and contribute to overall well-being. In addition to its nutritional value, it is found that African oil bean contains latent therapeutic properties. The seed contains compounds that possess properties that make it a prospect for advancement of new drugs and treatments. Research has shown that these properties include anti-inflammatory, antioxidant and antimicrobial effects.

In this study, the Vitamin content, Mineral content and Partial oil characterization of the African oil bean seeds was evaluated. The analysis of Vitamin shows that African oil bean seed is rich in Vitamin C and Vitamin E.

According to Enujiagha and Akanbi (2009), the estimation of mineral content of African oil bean showed high values for calcium (8.16%) which has a similar percentage with the value of calcium (8.48%) gotten through this study (Table 1). Calcium helps blood vessels move blood throughout the body and helps release hormones that affects many functions in the body.

The evaluation of partial oil characterization of the African oil bean seed (Table 3) was found to have a relatively low value for free fatty acid (3.821%) compared to Enujiugha and Ayodele-Oni (2003)16 result that showed 32.9% free fatty acid, this shows that the oil is edible. Abighor *et al* (1997)17 recorded a high saponification value of 171.1 compared to the result (134.6) obtained through this study.

**CONCLUSION**

African oil bean seed is a rich source of vitamins, minerals and healthy fats. The tree is beneficial for both medicinal and economic purposes. The oil in the seeds can be extracted and used in the production of soaps, candles and cooking oil. The shell is used in decorations and in the production of beads, dancing costumes, bags and necklaces. The wood are sources of fuel for cooking and the bark are used in herbal medicines for treating worms due to their anthelmintic properties. There has also been interest in the use of African oil bean as a biofuel feedstock. The seed contains a high percentage of oil, which can be extracted and processed into biodiesel. This could provide a sustainable source of energy for communities in the region, while also reducing dependence on fossil fuels.

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