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Extraction and Characterization of Three Tropical Seedoils: *Telfairia occidentalis, Hura crepitans* and *Cucumeropsis mannii*

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Abstract The aim of this study is to investigate the potential use of the selected seed oils. The oils were extracted using cold maceration method and the physicochemical characteristics of the oils determined using standard methods. The following results were obtained for the physicochemical parameters analysed: for *Telfaria ocidentalis* seed oil. Oil yield 12.32 ±0.04%, Acid value 10.396 ±0.2mg/g, Free fatty acid 5.198 ±0.24%, Specific gravity 0.910 ±1.0 Saponification value 154.92 ±1.24 mgKOH/g, Iodine value 10.67 ±0.64 I_2/100g, Refractive index 1.4635 ±0.1,Viscosity 0.058 ±0.3 Pa.s Moisture content 0.150 ±0.4% and Peroxide value 1.999 ±0.00 meq/kg. For *Hura crepitan* seed oil**.** Oil yield 12.45 ±0.3%, Acid value 12.795 ±0.1 mg/g, Free fatty acid 6.398 ±0.22%, Specific gravity 0.970 ±0.11, Saponification value 157.08 ±1.8 mgKOH/g, Iodine value 11.938 ±0.56 I_2/100g, Refractive index 1.4635 ±0.2, Viscosity 0.037 ±0.25Pa.s, Moisture content 1.515 ±0.00%, and Peroxide value 1.999 ±0.00 meq/kg. For *C. mannii* seed Oil. Oil yield 19.92 ±0.45%, Acid value 2.4 ±0 mg/g, Free fatty acid 1.2 ±0.00%, Specific gravity 0.900 ±0.00, Saponification value 168.3 ±1.7 mgKOH/g, Iodine value 11.684 ±0.2 I_2/100g, Refractive index 1.4641 ±0.0, Viscosity, 0.048 ±0.0 pa.s, Moisture content 0.155 ±0.00% and Peroxide value 1.999 ±0.00 meq/kg were obtained. From the obtained values of the determined parameters, the oils can be extracted from the three selected seeds in commercial quantities and that all the seedoils may be utilized in the industrial soap production and Biodiesel production. There is the need to carry out optimization test of the antioxidants to know the optimal values at which they will function optimally.

Keywords *Telfaria ocidentalis*, *Hura crepitan*, saponification value, specific gravity, iodine value

1. Introduction

Cucumeropsis mannii Naud (Melon) is popularly called "*egusi*" in West Africa. This is the true indigenous *egusi* of West Africa [1]. Melon is a cucurbit crop that belongs to the *Cucurbitaceae* family with fibrous and shallow root system. It is a tendril climber or crawling annual crop, mostly grown as a subsidiary crop interplanted with early maize and yam in some savanna belt of Nigeria [2]. Cucurbit species are among the economically most important vegetable crops and are grown in both temperate and tropical regions [3]. As reported by reference [4], the seeds have about 50% lipid. Most of their oil is made of non-saturated fatty acids. Conjugated fatty acids among some cucurbitaceae oils make them highly useful as drying oils, that is, they combine readily with oxygen to form elastic, water proof film. Seed oils are important sources of nutritional oils, industrial and pharmaceutical importance [5], and current emphasis on sustainable development has made it imperative to search for industrial raw materials from renewable sources.

The Sandbox tree (*Hura crepitans*; syn. *Hura brasiliensis* Wild), also known as Possum wood and Jabillo, is an evergreen tree of the spurge family (*Euphorbiaceae*), native to tropical regions of North and South America in the Amazon rain forest. The tree however extends to all regions of the world including Africa. In Nigeria, it thrives in the middle belt. Oils extracted from the derived seeds are also used as a purgative. The leaves are used against eczema. Its pale, yellow or brown soft wood is used for furniture under the name Hura. In summary, the Sandbox tree often can be found in nearly pure stands on mostly loam soil in the flat coastal region [6].

Telfairia occidentalis is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. Common names for the plant include fluted gourd, fluted pumpkin, and ugu in the Igbo language. *T. occidentalis* is a member of the Cucurbitaceae family and is indigenous to southern Nigeria. The fluted gourd grows in many nations of West Africa, but is mainly cultivated in [Igboland/southeastern Nigeria] and it is used primarily in soups and herbal medicines [7-8]. Although the fruit is inedible, the seeds produced by the gourd are high in protein and fat, and can, therefore, contribute to a well-balanced diet. The plant is a drought-tolerant, dioecious perennial that is usually grown trellised. *T. occidentalis* is traditionally used by an estimated 30 to 35 million people indigenous people in Nigeria, including the Efik, Ibibio, and Urhobo [9].

Lipid peroxidation is a major deteriorative change commonly encountered in oil and the extent of lipid peroxidation depends on different factors which include the fatty acid composition viz-a-viz level of unsaturation; packaging material and storage condition [10].

2. Materials & Methods

2.1. Materials

Telfaria occidentalis and *Cucumperosis mannii* seeds were purchased in Jalingo main market, while *Hura crepitans* seeds were obtained from Makurdi, opposite second gate of BSU. All the sample seeds were authenticated by a specialist, Mr. TedGabiu of Biological Sciences Department, Taraba state university Jalingo. All the chemicals used were of analytical grade and were purchased from Sigma and Aldrich.

2.2. Methods

Three feedstocks comprising two, non-edible oils (*Telfairia occidentals* Hook F, (TVO), and *Hura crepitians* L (HVO) and one edible oil (*Cucumeropsis manii* Naud (CVO) were extracted and characterized using standard methods. All the seeds were removed from the bulb and dried to a constant weight after which the mesocarps were removed by dehulling. The decorticated or dehulled dried seeds were grinded into flake using a Quasa blender and grinder. The cold-maceration extraction method was used for the extraction.

Cold maceration

One kg of each sample was extracted with 2 L of n-hexane in a 4-L extracting jar, i.e. 2 L of n-hexane was poured into a 4-L jar containing 1kg of powdered sample. The jar was covered and allowed to stay at room condition for a period of 72 hrs. After which the resulting solution was decanted off the jar and filtered using Whatman filter paper. The filtrate (oil + n-hexane) was then concentrated by distillation with the aid of a vacuum pump at room temperature to recover the oils. Percentage yield of each of the oils was calculated [11-12].

3. Physico-chemical characterisation of the oils

3.1. Determination of peroxide value

5g of each oil sample was weighed into 250 mL conical flask, and maintained at 298 K for 1 hour. 30 cm^3 of a solution mixture consisting of 60% glacial acetic acid and 40% of chloroform added to the oil sample. The flask was stirred to dissolve the solvent mixture. 5 cm^3 of saturated potassium iodide solution was then added. The solution was placed in a dark cupboard for 5 min and 3 cm^3 of distilled water added. 0.5 cm^3 of 1% starch solution was added and titrated with 0.1 M $Na_2S_2O_3$ with vigorous shaking. The titration was continued until the blue colour disappeared [13-15]. Thereafter, the peroxide value of the oils was calculated using equation 3.1

$$\text{POV}\left[\frac{\text{meq}}{\text{kg}}\right] = \frac{(V_1 - V_0)\text{ x }1000\text{ x T}}{\text{W}} \quad (3.1)$$

Where, POV is peroxide value, Vo and V_1 are 0.1 mol/L of sodium thiosulphate solution in the blank and main test respectively, T is titre value of the thiosulphate solution, W is the weighed portion of substance in grams.

3.2. Iodine value

0.1g of all the oil samples was dissolved in 15 mL of carbon tetrachloride. 25 mL of Wijs' solution was added from a burette. The components were thoroughly mixed, the flask stopped and the mixture allowed to stand in the dark for 2 h at room temperature. Thereafter 150 mL of water and 20 mL potassium iodide were added to the reaction mixture. The solution was titrated with sodium thiosulphate using starch indicator [15, 16-20]. Similar titration was also carried out with a blank sample. The iodine value was thereafter calculated using equation 3.2 below.

Iodine value (mg/g) = $(T_1\text{-}T_0)$ M × 12.7 (3.2)

3.3. Determination of acid value/FFA

25 mL of diethyl ether were mixed with 25 mL of ethanol and 1 mL of phenolphthalein indicator solution added. This was then neutralized with 0.1 M KOH solution. 1 g of each of the sample oils was dissolved in the neutralized solvent mixture. This was then titrated with 0.1 M NaOH with thorough shaking continuously until a pink colour which persisted for 115 seconds was obtained [21-22].

$$\text{Acid Value (mgKOH/g)} = \frac{40 \times \text{C} \times \text{V}}{\text{Weight of sample}} \quad (3.3)$$

The acid value was calculated using equation 3.3 above, where; C is concentration of KOH used, V is volume of mass of KOH;

FFA=AV/2.

3.4. p-anisidine value

The AOCS CD 18-90 method and the IUPAC2.504 Official Method for p-anisidine were used. The methods are based on a chemical reaction between the aldehydes in the oil or fat and p-anisidine which produces a colour that can be measured at 350 nm.

The carbonyl content in oils will be determined by standard method according to AOCS. It measures the reactiveness of the aldehydes carbonyl bond on the p-anisidine amine group forming a Schiff's base which absorbs at 350nm.

2g (W) of each sample was dissolved in 25 ml isooctane and absorbance A1 was measured at 350nm against a blank isooctane. An aliquot (5ml) of this solution, respectively 5ml of isooctane (as blank) was transferred to each of two test tubes of 10ml and 1ml anisidine solution (0.25% g/v glacial acetic acid) added to each. After 10 minutes the absorbance A2 was measured at 350nm against isooctane containing p-anisidine.

The p-AV was determined as;

p-AV =25 ×1.2 ×(A2-A1) / W (3.4)

where 1.2 = the correction factor for the dilution of the test solution with 1 mL of the reagent or glacial acetic acid.

3.5. Determination of refractive index value

The refractive index of the oil will be determined by using Abbe refractometer at 306 K after which temperature correction will be made to obtain the correct refractive index of the oil. The expression to be used to correct for temperature effect is shown in equation 3.5 below.

$RI_{correct}$= (T-20) K × 0.0000078=RI (3.5)

Where $RI_{correct}$ the refractive index is after temperature correction, RI is refracticve index obtained before temperature correction is made, T is temperature.

3.6. Specific gravity determination

The specific gravity of the oils was calculated as follows using equation 3.6 [23].

Specific gravity = 0.08475 + 0.003 × SV + 0.00014 × IV (3.6)
Where SV is Saponification value and IV is Iodine value.

3.7. Moisture content

2 g of the oil sample was weighed into a petri dish which was dried in an oven, cooled in desiccator and weighed. The petri dish with oil was then placed in an oven at a temperature of 373 K for 45 minutes; at the end of the drying period, the sample was removed and cooled in the desiccator for 15 minutes and weighed. The moisture content was calculated as follows using equation 3.7 [24].

Moisture Content (%) = $\frac{Ca-Cb}{W}$× 100% (3.7)

Where; *a* is the weight of petri dish and sample before oven drying, b is weight of petri dish and sample after oven drying, w is weight of sample.

3.8. Determination of Viscosity

100 cm^3 of oil sample was poured into the cup of "Clandom Viscometer, Model VT – 03 Viscometer", the lowest number spindle was selected and screwed into the underside of the viscometer, and the cup containing the sample was carefully locked into position so that the spindle cone was completely immersed in the sample. The machine was then switched on and the pointed deflection on the machine scale observed for about ten seconds and allowed to stabilize, after which the position of the pointer on the scale read; this gave the value of viscosity of the oil sample in centipoises, cP [23].

3.9. Determination of saponification value

1 g of each of the oils was measured and poured into a flask and 12.5 mL of alcoholic potassium hydroxide solution was added into the flask and placed on a boiling water bath for 40 minutes accompanied by shaking. After the time interval of 40 minutes, the flask was then removed and 0.4mL of the 1 % phenolphthalein indicator was added. Thereafter, while still warm, it was titrated with 0.5 M HCl, until the faint pink colour permanently disappeared and a titer value was obtained. Similar titration was also carried out with a blank sample. The saponification values for the various oils were calculated using equation 3.8 [24].

Saponification Value (mg/g) = $\frac{(Vb-Va)}{W}$ ×N × 56.1 (3.8)

Where, Vb is the value of HCl used in the blank, Va is the value of HCl used in titration with the oil, W is the weight of the oil used, N is the normality of HCl solution, 56.10 is the equivalent weight of potassium hydroxide.

4. Results and Discussion

Table 4.1: Physico-chemical properties of *Telfaria ocidentalis* seed oil, *Hura crepitan* seed oil and *C. mannii* seed Oil

S/NO	PARAMETERS	UNITS	TOO	HCO	CMO
1	ACID VALUE	mg/g	10.396 ±0.2	12.795 ±0.1	2.4 ±0
2	FREE FATTY ACID	%	5.198 ±0.24	6.398 ±0.22	1.2 ±0.00
3	SPECIFIC GRAVITY	-	0.910 ±1.0	0.970 ±0.11	0.900 ±0.00
4	SAPONIFICATION VALUE	mg/g	154.92 ±1.24	157.08 ±1.8	168.3 ±1.7
5	IODINE VALUE	mg/g	10.67 ±0.64	11.938 ±0.56	11.684 ±0.2
6	REFRACTIVE INDEX	-	1.4635 ±0.1	1.4635 ±0.2	1.4641 ±0.0
7	VISCOSITY	Pa.s	0.058 ±0.3	0.037 ±0.25	0.048 ±0.0
8	MOISTURE CONTENT	%	0.150 ±0.4	1.515 ±0.00	0.155 ±0.00
9	PEROXIDE VALUE	meq/kg	1.999 ±0.00	1.999 ±0.00	1.999 ±0.00
10	EXTRACTION YIELD	%	12.32 ±0.04	12.45 ±0.3	19.92 ±0.45
11	COLOUR	-	GOLDEN YELLOW	LIGHT BROWN	PALE YELLOW

Values are means ±SD of three determinations

NB: TOO=*Telfairia ocidentalis*seed oil, HCO= *Hura crepitans* seed oil, CMO= *Cumperopsis mannii* seed oil.

The physico-chemical properties of the seed oils are shown in Table 4.1 above.

Acid values

Acid value measures the presence of corrosive free fatty acids and oxidation products. This is actually an important variable in considering the quality of oil because the lower the free fatty acid, the better the quality of oil. The acceptable limit for edible oils is ≤10 [25]. Thus, from the results, CMO has actually passed the test of being edible with acid value of 2.4 *mg/g*. FFA concentrations of the oils were all higher than the maximum limit of 2.0% reported for high-grade Codex Alimentarius [26] except for MCO which was 1.2%.

Acid values of the nonedible oils were also very high and exceeded the ASTM standard of 0.8 mgKOH/g. Vegetable oils containing high free fatty acids have significant effects on the transesterification with methanol using alkaline catalyst. It also interferes with the separation of fatty acid ester and glycerols [27]. This indicates that the oils would be better converted to biodiesel using the two-stage process of esterification and transesterification [28].

Specific gravity

The specific gravity of TOO and HCO oil seeds were slightly higher than those reported for most conventional oilseeds which are about 0.9 while that of CMO was exactly 0.9 [29]. In addition, the viscosity values of TOO was in the range (50 to 100 mPas) of most vegetable oils [29]. While those of the remaining two were less. These results corroborate the fluid state of the studied oils at ambient temperature and this physical characteristic could be suitable for skin care products preparation [29]. The relatively low peroxide values of all the oilseeds indicate that they are less liable to oxidative rancidity at ambient temperature [30]. Therefore, these oilseeds could be suitable in combination with antioxidants for cosmetic formulations [31].

Saponification value

Saponification value (SV) is an index of average molecular mass of fatty acid in the oil sample. Saponification value is used in checking adulteration. The SV value obtained for the oil samples in Table 4.1 showed 154.92 mg KOH/g for TOO, 157.08 mg KOH/g for HCO and 168.3 mg KOH/g for MCO. The values are below the expected range of 195–205 mg KOH/g of oil for edible palm oils as specified by SON [32] and NIS [33]. The lower value of saponification values suggests that the mean molecular weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules did not interact with each other. In addition, the studied oilseeds could be recommended for soap making and in the manufacture of lather shaving creams due to their relatively higher saponification values [33].

Iodine value

Iodine value (IV) measures the degree of unsaturation in a fat or vegetable oil. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively [34, 35]. It was observed that measured iodine values for TOO, HCO and MCO are 10.67, 11.938 and 11.684g respectively. These low iodine values may have contributed to its greater oxidative storage stability. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils. The iodine values obtained for the three seed oils are lower than that of the other common seed oils such as safflower and soybean oil with iodine values of 145 g/100g and 132 g/100g [36] respectively. The iodine values obtained for the three seed oils are all less than 100gI2/100g which qualifies them as non-drying oils useful in soap manufacture.

Refractive index

The refractive indexes of the three oilseeds are within the range of those reported for edible oils. The study indicates that seed oils of the plants contain lower FFA and so, they can be recommended for salads seasoning and can be stored for longer period without deterioration [37, 38]. These values are very close to the values reported for other seed oils, that is, 1.47 for soybean oil, and 1.47 for corn oil [39]. The refractive index of oils actually depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation. Triacylglycerols

have higher refractive indices than do their constituent free acids. Values of refractive index for different oils generally vary between 1.447 and 1.482 [40].

Viscosity

Oils are mixtures of triglycerides (TGs) and their viscosity depends on the nature of the TGs present in the oil. The viscosity changed due to the different arrangement of the fatty acids on the glycerol backbone of the triglyceride molecule. Therefore, viscosity is related to the chemical properties of the oils such as chain length and saturation/unsaturation. Table 4.1 shows that at room temperature of 35 °C the viscosity is high in TOO than the remaining two seed oils. It explains that the viscosity and density decreases with an increase in unsaturation and increases with high saturation and polymerization, Viscosity also depends on sheer stress and temperature. Sheer stress does not have much effect on the storage of oils which are used for edible purposes but the temperature does affect it.

Moisture content

Moisture content was higher in HCO than in the other two oils. This is an indication that HCO will be more prone to hydrolytic rancidity than the other two oils.

Peroxide value

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage. It could be used as an indication of the quality and stability of fats and oils [41]. The relatively low peroxide values of all the seed oils are an indication that they are stable relatively at room temperature. Therefore, these oilseeds could be suitable in combination with antioxidants for cosmetic formulations [31]. High peroxide value is associated with high rancidity rate. Thus, with this fact, the low peroxide values obtained from these oils is simply an indication the oils are less liable to rancidity at room temperature.

5. Conclusion

This study on the extraction and characterization of three vegetable seed oils actually confirmed that all the oils are stable at ambient temperature going by their low peroxide and iodine values. Therefore, these oilseeds could be suitable in combination with antioxidants for cosmetic formulations. The oils were also characterized by high saponification values. Hence they could be recommended for soap making and in the manufacture of lather shaving creams due to their relatively higher saponification values.

Acid values of the nonedible oils were very high and exceeded the ASTM standard of 0.8 mgKOH/g. Vegetable oils containing high free fatty acids have significant effects on the transesterification with methanol using alkaline catalyst. It also interferes with the separation of fatty acid ester and glycerols. It is therefore recommended that the oils would be better converted to biodiesel using the two-stage process of esterification and transesterification. Optimisation of antioxidants to know the concentration of antioxidants and the correct combination to be used is also required to carried out.

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