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Physicochemical Property and Some Vitamin Contents of *Telfaria occidentalis* Seeds Oil

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ABSTRACT: Fluted pumpkin, a member of the *Cucurbitaceae* family, is a versatile fruit and is used for various food processing application in Nigeria. As a result of its versatility, this study was conducted to ascertain the physiochemical characteristics and some vitamins content of its seeds oil. The pumpkin pods were gotten from a local farm in Benin metropolis, Edo state, Nigeria. The extraction of oil was conducted by the use of Seed-2-oil ® hot press machine. Various other standard methods were employed in the study. The physicochemical characteristics showed that the light yellow oil had a refractive index of $1.471^0C \pm 0.001$ and melting point of $19.67^0C \pm 1.80$ while the chemical properties revealed acid value (0.64 mg KOH/ g of oil ± 0.05), peroxide value (0.99 Meq of O_2/Kq of Oil ± 0.01), saponification value (189.73 mg KOH/g of oil ± 5.20) and p-anisidine (0.09 ± 0.01) respectively. The findings for the vitamins revealed vitamin A (781IU ± 1.35), D (1361IU ± 0.14) and K_3 (8.145IU ± 0.36) respectively. The findings from this study revealed that pumpkin seed oil can be a good nutritive enrichment of foods and food products and a possible utilization in pharmaceuticals.

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Many human diseases are caused by oxidative stress which is usually initiated by free radicals. These free radicals react with macromolecules such as DNA, proteins and lipids, thereby damaging them with consequences resulting in diabetes, hypertension, atherosclerosis, cancer among others (Potterat, 1997). Endogenous antioxidants such as super oxide dismutase, catalase, glutathione reductase, ascorbic acid and tocopherol protect the body against the damaging effects of free radicals. Certain pathologic situations may disrupt the protective effects of these endogenous antioxidants. To protect man from the destructive effects of free radicals in such situations the administration of exogenous antioxidants is required (Yadav *et al*., 2010). Since ancient times, many herbal medicines in different formulations have been recommended for the treatment of various diseases (Al-Rowais, 2002). For such reasons, traditional and complementary medicines have seen an upsurge in their popularity for the treatment of different diseases. Herbal medicine development is one of the main subjects of studies in the National Center for Complementary and Alternative Medicines, Bethesda, USA which was established in 1998 by the US Government (Edwards *et al*., 2005; Fan, 2005). *T. occidentalis* is wide cultivated for its palatable and nutritive leaves. The leaves in comparison with alternative tropical vegetables have high alimentary price. Its supermolecule content (21 %) is on top of those of alternative normally used leafy vegetables. The leaves area unit wealthy in vitamins and minerals like Ca, P, Fe etc. The seed is additionally eaten as food. The oil obtained from the seed is employed in change of state (Akoroda, 1990). The study aims to assess vitamins A, D and K contents and the physicochemical properties of the *T. occidentalis* seed oil.

MATERIALS AND METHODS

Pumpkin pods were obtained from a local farm situated in Benin City, Edo State, Nigeria. The fresh seeds were authenticated at the department of Plant Biology and Biotechnology of University of Benin. They were cleaned to avoid surface contamination. The seeds were extracted and dehusked and then dried at room temperature.

Seed oil extraction: The oil was extracted from the dried seeds using the seed-2-oil ® extraction machine (India) at a controlled temperature and the oil stored in a dry container and sealed until needed for analysis.

Determination of physicochemical properties of the pumpkin seed oil: Peroxide value: Rancidity is brought about by the action of air (oxidative) or by

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microorganisms in oil. In oxidative rancidity, oxygen is taken by the oil or fat with the formation of peroxide. Peroxide value is a measure of the peroxide contained in the oil. The peroxide present are determined by titration against the presence of K1 using starch as indicator (A.O.A.C, 2000). Five grams of each oil sample was weighed in 250 mL of conical flask; then, 30 mL of acetic acid and chloroform solvent mixture (ratio 3:2) was added to the oil sample. Then, 1 mL of potassium iodide solution was added to the solution. The mixture was then left in the dark cupboard for 1 min with intermittent stirring after which, distilled water (30ml) was then added. Slowly, titrate liberated iodine in 0.01 N sodium thiosulphate solution until vigorously shaking yellow color was gone and after that 1 mL of starch solution indicator was added and we continued titration by vigorous shaking to release all from chloroform layer until blue color disappeared. *Saponification Value:* Saponification value is the amount (mg) of alkali required to neutralize a definite quantity of an oil. This value is necessary for comparative study of the fatty acid chain length of oil. A known quantity of oil is refluxed with excess amount of alcoholic KOH. After saponification the remaining KOH is estimated by titrating it against a standard acid (A.O.A.C, 2000). Into 250 mL Erlenmeyer flasks was added 2gm (w/v) along with a solution of potassium hydroxide dissolved in alcohol (25ml) while same procedure was done for the blank. The samples flask and the blank flask were connected with air condensers and boiled gently in the water bath, steadily until the saponification was completed, indicated by absence of oily matter and the appearance of clear solution. Clarity was achieved in half hour boiling. After the flask and the condenser cooled, inside of the condensers was washed down with about 10 mL of ethanol and then 1 mL of phenolphthalein indicators was added to the solution. The potassium hydroxide in excess was then titrated against 0.5N hydrochloric acid to form a cloudy solution. *Iodine Value:* Iodine value is a measure of the degree of unsataturation in oil. The higher the unsaturation the greater the possibility of the oil to go rancid. Oil contain both saturated and unsaturated fatty acids. Iodine gets incorporated into the fatty acid chain wherever the double bond exists. Hence the measure of iodine by an oil, gives the degree of unsaturation. Iodine value/number is defined as the 'g' iodine absorbed per 100g of the oil (A.O.A.C, 2000). Five grams of the oil sample was weighed in 250 mL conical flasks and then 25 mL of carbon tetra chloride was added to the oil sample and content was mixed well. To the resulting solution, Hanus reagent (25ml) was then added and mixed by swirling which was then to incubate for 30mins in the dark. After standing, 15 mL of potassium iodide solution was added and then 100 mL of distilled water was added into the mixture and 1 mL starch indicator solution was added to the sample solution. The discharged iodine was appraised with sodium thiosulphate solution (0.01N) to give a colourless solution. The blank determination was carried in the same manner as test sample but without oil.

P-Anisidine Value: The anisidine value (AV) method measures thye secondary oxidation compounds, primarily 2-alkenal and 2,4 alkadienals generated due to hydroperoxide decomposition and it is more sensitive to unsaturated aldehydes (Gordon, 2001). Since the anisidine value represents the content of secondary oxidation products, it is used instead of, or together with the peroxide value (PV) to evaluate fats and oils. For example, anisidine value is considered to be a more reliable indicator of oxidative rancidity than peroxide value in a long-term storage stability study of palm olein oil (van der Merwe, et al 2004). One gram of fat is reacted with 100mL of a solution (p-methoxyaniline) in iso-octane and the amount of reaction products determined spectrophotometrically at 350nm in a 10mm cell. The anisidine value is defined as 100 times the absorbance of the solution resulting from this reaction.

Acid Value: The free fatty acid is estimated by titrating it against potassium hydroxide (KOH) using phenolphthalein as indicator. The acid value is mg KOH required to neutralize the free fatty acid present in one gram of sample. It is expressed as oleic acid (octadec-9-enoic acid) equivalent. Three grams of each cooled oil sample was weighed in 250ml of conical flask and 25ml of freshly neutralized ethyl alcohol was added to the sample and then shaken well to dissolve sample. The sample solution was boiled for about five minutes and cooled then 1ml of phenolphthalein indicator was added to the sample solution. The solution was cured with 1N sodium hydroxide solution to give a pink colored appearance.

D*etermination of vitamin A acetate (Retinol) content:* Vitamin A acetate was determined using Ashok and Kumar method, (2011). This is a chemically, 15-apo-B-carotene-15-ol, 3, 7-dimethyl-a-(2, 6, 6-trimethylcyclohex-1- enyl) nona-2, 4, 6, 8-tetraen-1-ol. It is a yellowish powder. Sample 0.15mg was weighed and taken into a round bottom flask. In the bottom flask, 2ml of potassium hydroxide solution, 10ml of glycerol, and 50ml methanol was added and

mixed very well, then reflexed for 45 minutes in boiling water and allowed to cool. The flask was washed with distilled water and taken washing in separator and then extracted with 4×25ml diethyl ether, combined ether extract washed with water. The water layer was then discarded then the ether layer was taken in a dry 100ml volumetric flask by passed through anhydrous sodium sulphate and up to 100ml of diethyl ether was made and mixed well. Its absorbance was recorded at 325nm against blank. The equation was used.

$$IU = \frac{S_A \text{ x } 1830 \text{ x } 100}{100 \text{ x SD x SW x AW}}$$

Where IU = Amount of unit; S_A = Sample Absorbance; 1830 = Factor; SD = Sample Dilution; SW = sample weight; AW = Average weight

Determination of vitamin D3 (cholecalciferol) content: It is chemically, (5z, 7e) - (3s)-a, 10-secoholesta-5, 7, 10(19)-triene-3-ol, it is a white crystalline compound. It was determined using Ashok and Kumar method, (2011). Both standard and sample were prepared. In Standard preparation, 0.025gm of vitamin D3 working standard was weighed accurately, 25ml of solution mixture (chloroform and methanol in ratio 1:9) was mixed in a volumetric flask, dissolved and diluted with the solution mixture. In the sample preparation, 0.001g of vitamin D3 sample was taken in 25ml volumetric flask with solution mixture (chloroform and methanol in ratio 1:9). It was made up to the mark and mixed well. Its absorbance was recorded at 264nm against blank.

Determination of vitamin k3 content: Vitamin k3 chemically, 2-methyl-1,4-naphthaquinone. It is a pale-yellow crystalline powder. It is a fat-soluble dietary principle required for the synthesis of clothing factors. Vitamin k3 was determined using Ashok and Kumar method (2011). In standard preparation, 0.025g of menadione working standard was weighed and taken into 100ml volumetric flask and 50ml of chloroform was added to dissolve. After it was filtered and 1ml was taken into 50ml volumetric flask made up to the mark with chloroform. In sample preparation, 250mcg of sample was taken into a separator. In separator, 5ml of water was added, mixed well and extracted with 4×10ml chloroform. The water layer was discarded and chloroform was taken in dry 50ml volumetric flask by passed through anhydrous sodium sulphate and made up to 50ml with chloroform. Procedure, 5ml of the standard, sample and blank solution was taken into test tubes. In each test tube, 2ml of 0.2% solution of 2,4-dinitrophenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) and mixed well. After that it was heated in a water bath until almost dryness and cooled at room temperature. 15ml solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube. Its absorbance was recorded at 635nm against blank.

RESULTS AND DISCUSSION

Tables 1 and 2 below show the physicochemical analysis and some vitamins content of pumpkin seed (*T. occidentalis*) seeds oil. The high ratio of this oil looks to substantiate the high variety of carbon atoms in their fatty acids (Falade; *et al* 2008). Ratio conjointly will increase because the covalent bond will increase (Eromosele and Paschal, 2003). The iodine worth may be alive of the degree of unsaturation in oil and will be wont to quantify the quantity of double bonds gift within the oil that reflects the status of oil to reaction. The iodine worth is high and this reflected the presence of high proportion of unsaturated fatty acids within the seed oil. The iodine worth may be a very little above100 so it may be classified as semi animal oil. The iodine worth compared favorably to iodine value of each white and red *benni*, seeds (Mohammed and Hamza, 2008). Aremu, *et a*l. (2006) reportable that the lower the iodine worth the lesser the amount of unsaturated bonds; thus, the lower the status of such oil to aerophilous rancidity.

Table 1. Physicochemical and chemical parameters of *T. occidentalis* seed oil

Measured Parameters	Values obtained
Melting Point (^{0}C)	19.67 ± 1.80
Refractive Index (25^{0}C)	1.471 ± 0.001
Iodine Value (g of Iodine/100g of oil)	60.72 ± 3.56
Peroxide Value (Meq of O_2/Kq of Oil)	0.99 ± 0.01
p-anisidine Value	0.09 ± 0.01
Acid Value (mg KOH/ g of oil)	0.64 ± 0.05
Saponification Value (mg KOH/g of oil)	189.73 ± 5.20

Therefore, non-drying oils don't seem to be appropriate for ink and paint production thanks to their non-drying characteristics however could also be helpful within the manufacture of soaps (Kochhar, 1998) and might be considered liquid oil. A decent animal oil ought to have iodine worth of one hundred thirty and higher than. Thus, pumpkin seed oil may be classified as drying oils. High iodine worth may be a pointer to the presence of high proportion of unsaturated fatty acids within the seed oil; per se quantity of iodine that may be absorbed by the unsaturated acids would be higher (Iko *et al*., 2012) and oils with such characteristic could so be notice helpful as raw materials within the manufacture of vegetable oil-based frozen dessert (Oderinde, *et a*l 2009). The definite quantity is low, definite quantity of zero.00 to 3.00 mgKOH/g oil is suggested for oil to seek out application in cookery (Bazongo et al., 2014). Thus, the seed oil of *T. occidentalis* may be

appropriate for cookery. The free carboxylic acid worth of one.74 waterfall at intervals the utmost limit of fifty for free of charge fatty acids in high grade vegetable oil in African nation (Tanko *et al*., 2017). Peroxide worth is AN index of rancidity, so low peroxide worth indicates resistance of the oil to peroxidation throughout storage. The peroxide worth of *T. occidentalis* is low (0.990 mEq/Kg) compared to the utmost acceptable worth of ten milliequivalent KOH/g set by the Codex Alimentarius Commission for groundnut seed oils (Lazos, 1986). The oil is so stable and wouldn't simply go rancid. A rise in chemical reaction worth in oil will increase the volatility of the oils. It enhances the standard of the oil as a result of it shows the presence of lower relative molecular mass parts in one g of the oil which is able to yield a lot of energy on combustion (Engler, et al 1983). The low chemical reaction worth is a sign that the oil might not be appropriate for soap creating, oil-based ice-cream and shampoos. It's been reportable by (Magaia and Skog, 2017) that oils with high chemical reaction values contain high proportion of lower fatty acids. Oils with iodine worth but a hundred gI_2/100g of oil square measure non-drying oil.

Table 2. Some Vitamins content of *T. occindentalis* seed oil

Vitamins	Concentration
Retinol (Vitamin A Acetate)	781 ± 1.35 IU
Cholecalciferol (Vitamin D3)	1361 ± 0.14 IU
Menadione (Vitamin k3)	8.145 ± 0.36 IU

Vitamin A (retinol) is an important nutrient required in little amounts by humans for the traditional functioning of the sensory system, growth and development and maintenance of animal tissue cellular integrity, immune functions and replica (Amitromach *et al*., 2009; Zeng *et al*., 2016). Protaminenoids square measure found in inexperienced foliaceous vegetables (spinach, amaranth and young leaves from varied sources), yellow vegetables (example pumpkin, squash and carrots), and yellow and orange non citrus fruits (example mango, apricots and papaya) (Begum et al., 2002). Vitamin D is needed to take care of traditional blood levels of metal and phosphate, that square measure successively required for the traditional mineralization of bone, contraction, nerve conductivity, and general cellular operate altogether cells of the body (Karanja *et al*, 2013). vitamin D conjointly modulates the transcription of cell cycle proteins that decreases cell proliferation and increase cell differentiation of variety of specialized cells of the body (eg osteoclastic precursors, enterocytes, keratinocytes) (Karanja *et al*, 2013). Vitamin K is an important fat-soluble substance that is required for a singular for a post translational chemical modification during a little cluster of proteins with metal binding properties, conjointly called vitamin K dependent proteins (Walsh *et al*., 2005; Stafford, 2005). Thus far, the sole unequivocal role of vitamin K in health is within the maintenance of traditional curdling. In this study, the A, D and k content of *T. occidentalis* seed oil had a high degree showing that the seed oil is suitable for consumption. Vitamins have numerous organic chemistry functions, together with functions as hormones (vitamin D), inhibitor (vitamin E), and mediators of cell signaling and regulators of cell and tissue growth and differentiation (vitamin A) (Wiseman *et al*., 2017).

Conclusion: This study showed that *T. occidentalis* seed is a good source of edible oil. Its fatty acids composition is comparable to that of some conventional oils. The seed oil is of unsaturated type and can be classified in the oleic – linoleic acid group. Furthermore, it contains sufficient amount of vitamins A, D and B and could serve as dietary supplements for these vitamins.

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