

Original Research Article

Fatty Acid Profile and Physicochemical Properties of *Landolphia owariensis* P. Beauv Stringy Seed Oil

Tochukwu JN Okonkwo^{1*}, Wilfred O Obonga² and Chinedu JO Okonkwo³

¹Pharmaceutical and Medicinal Chemistry Department, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Port Harcourt, ²Pharmaceutical and Medicinal Chemistry Department, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, ³Biochemistry Department, Faculty of Chemical Sciences, College of Science, University of Port Harcourt, Port Harcourt, Nigeria

*For correspondence: **Email:** tjokonkwo@yahoo.com; **Tel:** +234-(0)-806-710-1645

Received: 20 February 2014

Revised accepted: 23 June 2014

Abstract

Purpose: To elucidate lipid profile of *Landolphia owariensis* P. Beauv stringy seed oil, the bulk physicochemical properties of its neutral lipid (NL), and also to ascertain its nutritional properties, safety and industrial applications.

Methods: The neutral lipid was recovered by macerating 500 g of *L. owariensis* stringy seed pulp in 1 L of petroleum ether (40 – 60 °C boiling range) for 48 h. Methyl esters of the inherent fatty acids were generated by transmethylation while the physicochemical properties of the NL was determined by official methods of the Association of Official Analytical Chemists (AOAC).

Results: The acid, iodine, saponification and peroxide values were 2.81 ± 0.01 mg KOH/g, 67.26 ± 1.05 mg I/100 g, 219.38 ± 2.69 mg KOH/g and 6.00 ± 0.28 mEq/kg, respectively. Thiobarbituric acid reactive substance (TBARS) value (6.10 ± 0.24 mg MDA/kg) of the NL further confirmed the stability of NL (neutral lipid) against oxidative rancidity. Gas chromatographic analysis of the methyl ester mix of *L. owariensis* stringy seed pulp NL indicate hexadecanoic acid (palmitic acid) as the principal fatty acid with an absolute proportion of 54.38 %. Palmitoleic acid (18.54 %) and linoleic acid (9.56 %) ranked second and third, respectively, while the saturated/unsaturated fatty acid ratio was 2.53.

Conclusion: The neutral lipid of seed oil exhibited strong stability to ketonic and oxidative rancidity, and is safe for edible purposes. Its relatively high unsaturated fatty acid proportion (28.29 %) indicates that *L. owariensis* stringy seed pulp fixed oil possesses good nutritional qualities.

Keywords: *Landolphia owariensis*, Stringy seed pulp, Lipid profile, Fatty acids, Palmitic acid, Palmitoleic acid, Linoleic acid

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Neutral lipids (NLs) are important elements in human nutrition. Apart from reducing the bulkiness of foods in the mouth, NLs are essential in the bio-synthesis of corticoids and steroidal hormones. Dietary, unsaturated fatty acids are essential for optimum health; as they are endogenous precursors of membrane polyunsaturated fatty acids (PUFs) and

eicosanoids — which comprised of prostaglandins, prosthacyclins, leukotrienes and thromboxanes. Fatty acids, sourced from NLs, are as well important bio-energy molecules, and at times play leading roles in the reduction of body cholesterol levels; as indicated by omega-3 fatty acids. Furthermore, fatty acids are important matrix in drug delivery and targeting [1].

Plants are the major suppliers of fatty acids, as their neutral lipids consisted mainly of fatty acids. The neutral lipids of many tropical plants are under exploited [2], because their fatty acid profiles and bulk (physicochemical) properties are yet to be fully elucidated [3]. The scenario became worsened, as the specific applications of lipids depend exclusively on their fatty acid profiles and bulk properties. Thus accelerated elucidation of lipid properties became imperative. This need cannot be over emphasized, as it is pivotal to the development of economical and sustainable schemes for the exploitation and utilization of plant-derived lipids.

Landolphia owariensis P. Beauv is native to Africa and is economically important for latex/rubber and folklore medicine [4,5]. It is commonly known as white rubber vine or vine rubber, and is widely used in the treatment of many ailments. Vast majority of its ethno pharmacological uses have been documented [6–11].

The folkloric use of *L. owariensis* as antimicrobial agent has been validated [12–14]. Its other medicinal and nutritional uses have as well been confirmed [2,5,15–17]. Akubugwo and Ugbogu reported the physicochemical properties of *Landolphia owariensis* seed oil [18].

In our continued, efforts to highlight the medicinal, nutritional and industrial potential of *L. owariensis* P. Beauv. [19,20], schematic procedures were adopted to elucidate the fatty acid profile of its stringy seed pulp. Because, LOSP is freely eaten by plants and animals from time immemorial. The findings should be in ascertaining the risk/benefit ratio of *L. owariensis* as well as its possible industrial applications.

EXPERIMENTAL

L. owariensis stringy seed pulp

The seeds of *Landolphia owariensis* P. Beauv were collected in March 2007, and authenticated by Mr CA Ugwuozor of the Department of Botany, University of Nigeria, Nsukka, Nigeria. A voucher specimen (no. UNH/133) was deposited at the department's herbarium. *L. owariensis* seeds were air-dried at ambient temperature and the stringy seed pulp separated and pulverized to a fine powder. *Landolphia owariensis* P. Beauv stringy seed pulp (LOSP) was macerated in 1.00 L of petroleum spirit (40 - 60 °C) for 48 h. The extract was dried *in vacuo* at less than 40 °C in a rotating evaporator. The neutral lipid (petroleum

spirit extract) of the stringy seed pulp was used to prepare methyl esters.

Preparation of LOSP methyl ester (Trans-esterification)

Two grams of the neutral lipid was transferred into a volumetric flask, dissolved in 50 ml chloroform and made up to 100 ml with more aliquots of chloroform. The chloroform solution was evaporated at room temperature to near dryness and the residue, re-dissolved in 1 ml of trans-esterification reagent (equi-volume mixture of 20 % benzene and 55 % methanol). The reaction mix was placed in a sealed round bottom flask and heated at 100 °C in a water bath for 30 min. After trans-esterification, 2 ml of a 1:1 mixture of n-hexane and water was added into the reaction bath and shaken vigorously for 2 min to extract the methyl esters. The extract was centrifuged, and 0.5 ml of the n-hexane phase transferred into a test tube for GC analyses.

Specific gravity

A dried and tarred 50 ml pyrometer bottle was filled with distilled water and weighed. The water was discharged and the bottle dried; after which it was filled with LOSP neutral lipid and weighed. The specific gravity (Sg) of LOSP neutral lipid was evaluated as the ratio of the weight of 50 ml of NL to the weight of an equal volume of water.

Refractive index

The oil sample was smeared on the lower prism of an Abbe refractometer with a compensator (water at 20 °C), a light passed by the angled mirror was made incident on the sample and the reflected light appeared in a form of a dark background. Using the fine adjustment, the telescope tubes was moved until black shadow appeared central in the cross wire indicator. Then, the refractive index was read off.

Flash point

A 10 ml aliquot of LOSP NL was placed in an evaporating dish and a thermometer suspended at the centre of the dish with the bulb deeping into the oil without touching the bottom of the dish. The temperature of the oil was raised; and the temperature at which the oil started flashing on to the thermometer stem, without supporting combustion was recorded as the flash point.

Free fatty acid (FFA) value

One millilitre of 1 % phenolphthalein solution (in 50 % aqueous alcohol) was added to 50 ml equi-

volume mixture of diethyl ether and alcohol, and the reagent carefully neutralized with 0.1 M KOH. Two gram of LOSP NL was dissolved in 20 ml of the neutral mixed solvent and titrated with 0.1 M KOH to a persistent light pink end-point. The FFA value (mgKOH/g) was evaluated as in Eq 1.

FFA value = $5.61T/W$ (1)
where T is the titer (in mL) and W is the weight of the of the sample.

Acid value

The acid value of LOSP NL was evaluated as $2(FFA)$.

Saponification value

Two grams of LOSP NL was dissolved in 25 ml of 0.5 M alcoholic KOH, and transferred completely into a round bottom flask with ground glass joint. The reflux condenser was attached, and the reaction system heated for 1 h in boiling water bath with continuous stirring. One ml of 1 % phenolphthalein was added to the reaction mix, and the system titrated hot against 0.5 M hydrochloric acid. A blank titration was done, and the saponification value (Sv) deduced as as in Eq 2.

$Sv = \{(b-a)28.05\}/(\text{weight of sample})$ (2)
where b and a are the respective titre values of the blank and test titrations.

Saponification equivalent

Saponification equivalent (Seq) is an index of the average acyl chain of lipids. It could be, loosely, taken to denote the average molecular weight of fatty acid mix of any neutral lipid. That is, it indicates the major fatty acid of fats and oils. Mathematically, it is related to saponification value as in Eq 3.

$Seq = 56100/\text{saponification value}$ (3)

Iodine value

Two grams of LOSP NL was dissolved in 10 ml of carbon tetrachloride and transferred into a glass-stoppered bottled. Thereafter, 20 ml of Wiji's solution was poured into the bottle, the stopper replaced (which was previously moistened with potassium iodide solution) and allowed to stand in the dark for 30 min with intermittent shaking. After 30 mins, 15 ml of 10 % potassium iodide solution and 100 ml of distilled water was added; and the system titrated against 0.1 M thiosulphate solution using starch indicator. A blank titration was carried out, and

the iodine value (Iv, mg I/100 g) evaluated as in Eq 4.

$Iv = \{(a-b)1.269\}/\text{sample weight}$ (4)
where b and a are the respective titre values of blank and test titrations.

Peroxide value

One gram of LOSP NL was weighed into a boiling tube, followed by addition of 1 g of KI and 20 ml of 2:1 (v/v) glacial acetic acid/chloroform mixture. The tube was placed in a boiling water bath such that the content boiled within 30 s, and continued boiling vigorously for more than 30 s. The reaction mix was quickly transferred into a flask containing 20 ml of 5 % KI solution, and the boiling tube rinsed twice with 25 ml of distilled water, each time. Using starch indicator, the system was titrated against 0.002 M sodium thiosulphate solution. A blank titration was carried out, and peroxide value (mEq/kg) computed as in Eq 5.

$\text{Peroxide value} = (a-b)2$ (5)
where a and b are titre value (expressed in ml) for test and blank titration.

Thiobarbituric acid (TBA) number/value

Ten gram of LOSP was macerated in 50 ml of distilled water for 2 min, and washed into a distillation flask containing 47.5 ml of distilled water. After which, the pH was adjusted to 1.5 with 2.5 ml of conc. HCl, and antifoam and anti-bump were added. The flask was heated in an electric mantle 50 ml of its content within the first 10 min of boiling. A 5-ml aliquot of the distillate was transferred into a glass-stoppered tube, and then 5 ml of TBA reagent (0.2883 g TBA/100 ml 90 % glacial acetic acid) was added, shaken and heated in boiling water for 35 min. A blank was similarly prepared with 5 ml of distilled water, against 5 ml of the distillate. Both tubes were cooled in water for 10 min, and the visible light absorbance (A) of the test solution measured against the blank at 538 nm. Thiobarbituric acid reacting substance (TBARS, mg MDA/kg) was evaluated as Eq 6.

$TBARS = 7.8 \cdot A$ (6)
where MDA denotes malondialdehyde and A is the absorbance of the test solution.

Viscosity, conductivity and pH determination

The viscosity, conductivity and pH of LOSP NL were determined with viscometer, conductivity meter and pH meter, respectively. The instruments were calibrated, as stated in the

respective manufacturer's manual, before taking measurements.

GC analysis of LOSP methyl esters

Agilent 6890 gas chromatograph equipped with an on-column automatic injector, flame ionization detector, HP 88 capillary column (100 m × 0.25 μm film thickness) was used for the GC analyses. Injection temperature was maintained at 220 °C with an integrator chart speed of 2 cm/min, while the detector operated at 250 °C. The column oven temperature was set at 180 °C and maintained constantly at 15-min analysis period. One microlitre of the sample was injected onto the column, eluted and the chromatogram recorded as per cent relative intensity against elution peak. The identity of the eluted peaks was obtained by matching against the retention parameter of a standard mixture of methyl esters.

Statistical analysis

Where applicable, results were expressed as mean ± SEM. Means were compared for statistical significant difference by one-way analysis of variance (ANOVA) using Duncan post-hoc test with the aid of GraphPad Prism® statistical software version 5.0. Effects were considered significant at $p < 0.05$ in all cases.

RESULTS

Table 1 shows the physicochemical properties of LOSP NL. The table indicated that the average molecular weight of LOSP NL corresponds to the dominance of palmitic acid in neutral lipids. Table 2 presents the fatty acid profile of LOSP NL.

DISCUSSION

The relatively high smoke and flash points of the lipid indicated that the acyl chains of LOSP NL fatty acids are of long saturated carbon chains. This was confirmed by the solid physical state of

the lipid at room temperature. Since long chain saturated fatty acids, are usually solids at room temperature. Thus the lipid will express high thermal stability; and will serve effectively as cooking (frying) oil with low potential to yield low molecular weight carcinogenic pyrolysis products.

Table 1: Physicochemical properties of LOSP NL

Parameter	Test value
Specific gravity	0.92 ± 0.01*
Refractive index	1.52 ± 0.03*
Smoke point (°C)	55.00 ± 0.87
Flash point (°C)	85.00 ± 1.01
Viscosity (pa/s)	1.54 ± 0.12
Conductivity (μs/cm)	137.40 ± 1.57*
pH	6.60 ± 0.06*
FFA value (mgKOH/g)	1.40 ± 0.01
Acid value (mgKOH/g)	2.81 ± 0.01
Saponification value (mgKOH/g)	219.38 ± 2.69*
Saponification equivalent	255.72 ± 2.62*
Iodine value (miliEqu./100 g)	67.26 ± 1.05
Peroxide value (miliEqu./Kg)	6.00 ± 0.28
TBARS value (mgMDA/Kg)	6.10 ± 0.24

*Significant at $p \leq 0.05$

The specific gravity (Sg), 0.92 ± 0.01, lay between 0.90 – 0.94; expected of neutral lipids. As Sg tends towards 0.90, it appears that the fixed oil of LOSP constituted majorly of fatty acids of high mean molecular weight. This was confirmed by the saponification value, saponification equivalent and the GC chromatogram of LOSP NL. In this regard, the saponification equivalent approximately tallied with the molecular mass of palmitic acid; which was indicated as the dominant fatty acid by the GC chromatogram. The moderate iodine value of the neutral lipid indicated that it is non-drying oil. It is because, semi-drying and drying oils usually possessed iodine values much greater than 80 mg/100 g. It further confirmed that LOSP NL glycerides and contain saturated acyl chains in high proportions.

Table 2: Fatty acid profile of LOSP NL and content of fatty acids

Carbon chain	Fatty acid	Retention time (min)	Peak height (cts)	Content (%)
C15	Pentadecanoic acid	12.3148	2060.49	1.85
C16:1	Palmitoleic acid	20.8408	20631.68	18.54
C16:0	Palmitic acid	26.6125	60519.72	54.38
C:10	Capric acid	35.9160	547.42	0.49
C:11	Undecanoic acid	39.4491	3788.48	3.41
C12	Lauric acid	42.4602	3962.08	3.56
C14	Myristic acid	45.7820	2156.65	1.93
C18:2	Linoleic acid	54.8729	11490.97	9.56
C17	Heptadecanoic acid	59.9773	1650.31	1.48
C18:3	Linolenic acid	65.8430	209.44	0.19
C18	Stearic acid	67.7085	5114.40	4.59

Stability of LOSP NL, as measured by the peroxide, TBARS, free fatty acid and acid values, were quite high. In actual fact peroxide value is used to monitor the development of rancidity through the evaluation of the quantity of peroxide (initiation product of oxidative rancidity) generated in the lipid. During storage of most fats, the peroxide value shows little increase in the early stages, known as induction period, after which there is then a marked increase. Peroxide value of oil is a measure of its content of oxygen, and fresh oils usually have peroxide values below 10 mEq./Kg. A rancid taste begins to show up when the peroxide value is between 20 and 40 mEq./Kg.

TBA value/number estimates the amount of MDA present in a lipid sample. MDA has been demonstrated to be an end product of oxidative rancidity of neutral lipids; and reacts with TBA to produce red pigment that can be estimated spectrophotometrically. Thus low TBARS values indicate low rate of lipid peroxidation and high stability against oxidative rancidity.

FFA and acid values of lipids measure the extent to which the glycerides in neutral lipids have been decomposed by lipase action, releasing free fatty acids. The determination is often used as a general indication of the condition and edibility of oils. As rancidity is usually accompanied by free fatty acid formation; and values in excess of 2 and 4 mgKOH/g, respectively, indicate ketonic rancidity. The pH of the sampled lipid revealed very weak acidity, which correlated well with the very low acid value of the sample. This further confirmed the stability of LOSP NL to enzymatic hydrolysis, could initiated by micro-organisms and inherent lipases in the neutral lipid.

Saponification value is inversely proportional to the mean molecular weight of fatty acids in the glyceride present. It indicates the potential of neutral lipids as raw materials in the manufacture of soapy detergents. As lipids with moderate to high saponification values are important raw materials in the manufacture of soapy detergents. The observed saponification value of LOSP NL is moderately high, expressing the ability of the neutral lipid to yield large amount of soap per unit weight.

The GC chromatogram of LOSP NL indicated a number of fatty constituents, out of which 11 were identified by standard comparison. It revealed hexadecanoic (palmitic) acid as the dominant fatty acid; while the mono-unsaturated

C16 fatty acid, palmitoleic ranked second in abundance. The doubly unsaturated C18 linoleic acid was, also, fairly abundant; representing third ranking in the relative fatty acid composition. Both unsaturated fatty acids are important elements in human and animal nutrition. As animal species are incapable synthesizing unsaturated fatty acids. Therefore, all their unsaturated fatty acid needs must be supplied in the diet. These fatty acids, otherwise known as essential fatty acids, are important precursors in the synthesis of bio-functional long chain poly-unsaturated fatty acids, which are the building blocks of eicosanoids and phospholipids. Also, they play leading roles in the reduction of low density lipoprotein-cholesterol (LDL-cholesterol).

CONCLUSION

The findings obtained demonstrate that LOSP neutral lipid consists majorly of the long chain C16 saturated and unsaturated fatty acids, namely, palmitic and palmitoleic acids, respectively. Since its saturated/unsaturated fatty acid ratio of 2.53 indicates high proportion of unsaturated fatty acids, the lipid is recommended for use as food additive/nutraceutical for the mitigation of hypercholesteremia.

ACKNOWLEDGEMENT

The authors are most grateful to Mr Alfred Ozioko, Bio-resources Development and Conservation Programme, Nsukka, Nigeria for collection of plant material.

REFERENCES

1. Okorie O, Okonkwo TJN, Nwachukwu N, Okeke I. Potentials of *Detarium microcarpum* (Guill and Sperr) Seed Oil as a Matrix for the Formulation of Haloperidol Injection. *Int J Pharm Sci Rev & Res* 2010; 5(1): 1–4.
2. Ihekoronye AI, Ngoddy PO. *Integrated Food Science and Technology for the Tropics*. London: Macmillian Publishers Ltd, UK; 1985. pp. 82–93.
3. Okonkwo TJN, Okonkwo CJO. Antioxidant Properties of *Diospyros preussi* (Ebenaceae Gurke) Seed Oil. *Trop J Pharm Res*, 2009; 8(6): 551–555.
4. Pichon M. *Monographie des Lardophées (Classification des Apocynacees, XXXV)*. *Me Ins Franc Afrique Noire* 1953; 35: 272–302.
5. Bolch N. *Crops and rubbers*. *Earthwatch* 1990; 10: 14–21.
6. Bouquet A. *Feticheur et Medicines Traditionelle du Congo (Brazzaville)*. *Mem O R S T O M* 1969; 32: 25–27.

7. Gill LS. *Ethnomedicinal uses of Plants in Nigeria*. 1st Ed. Benin City: Uniben Press, 1992; p 145.
8. Lewis WH, Elvin-Lewis MP. *Medical Botany*. 1st Ed. New York: John Wiley and Sons, 1977; pp. 21–22.
9. Irvine FR. *The Woody Plants of Ghana*. 2nd Ed. London: Oxford University Press, 1961; pp 26–28.
10. Anthony CB. *Natural Preservatives from Landolphia owariensis*. *Afr Dev J* 1995; 2: 21–22.
11. Dalziel JM. *Useful Plants of West Tropical Africa*. 2nd Ed. London: Crown Agents for the colonies, 1937. p. 374.
12. Ebi GC, Ofoefule SI. *Investigation into the Folkloric Antimicrobial Activities of Landolphia owariensis*. *Phytother Res* 1997; 11: 149–151.
13. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. *Evaluation of Extracts of the Roots of Landolphia owariensis for Antimicrobial Activity*. *J Ethnopharmacol* 2001; 78: 119–127.
14. Nwogu LA, Alisi CS, Ibegbulem CO, Igwe CU. *Phytochemical and Antimicrobial Activity of Ethanolic Extract of Landolphia owariensis leaf*. *Afr J Biotech* 2007; 6: 890–893.
15. Owoyele BV, Olaleye SB, Oke JM, Elegde RA. *Anti-inflammatory and Analgesic Activities of Leaf Extract of Landolphia owariensis*. *Afr J Biomed Res* 2001; 4: 131–133.
16. Oke JM, Hamburger MO. *Screening of some Nigerian Medicinal Plants for Antioxidant Activity using 2, 2 – diphenyl-picryl-hydrazyl radical*. *Afr J Biomed Res* 2002; 5: 77–79.
17. Olaleye SB, Owoyele VB, Odukanmi AO. *Antiulcer and Gastric Antisecretory Effects of Landolphia owariensis Extracts in Rats*. *Niger J Physiol Sci* 2008; 23(1-2): 23–26.
18. Akubugwo IE, Ugbogu AE. *Physicochemical Studies on Oils from Five Selected Nigerian Seeds*. *Pak J Nutr* 2007; 6: 75–78.
19. Okonkwo TJ, Osadebe PO. *Hepatoprotective Effect of Landolphia owariensis P. Beauv Seed against CCl₄-induced Hepatopathy in Rats*. *Port Harcourt Med J* 2010; 4: 307–312.
20. Okonkwo TJN, Osadebe PO. *Isolation and Characterization of Potential Bioactive Compounds from Landolphia owariensis P. Beauv Stringy Seed Pulp*. *Int J Appl Res Nat Prod* 2013; 6(3): 28–38.