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LIPID COMPOSITION AND MOLECULAR SPECIATION OF THE TRIACYLGLYCEROL OF THE OIL OF *PICRALIMA NITIDA*

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Abstract
The seed of *Picralima nitida* was analyzed for the proximate and mineral composition while its oil was chemically evaluated. The crude protein content was found as 10.20±0.50 % while the carbohydrate content was 74.53±0.50 %. The iodine and saponification values were found as 136.40±1.00 and 198.25±0.30 mg KOH/g, respectively. Potassium (114.00±0.20 ppm) was the dominant mineral in the seed while calcium (91.00±0.20 ppm) was the dominant mineral in the oil. C18:1 (50.65±0.2 %) was the major fatty acid while the neutral lipids (96.30±0.50 %) were the predominant lipid class. The molecular speciation of the triacylglycerol revealed the presence of molecular species with equivalent carbon chain number C_{38} (20.38±0.1 %) as the most abundant species. Monogalactosylmonoacylglycerol (67.95±0.10 %) and phosphatidyl choline (66.30±0.10 %) were the main glycolipids and phospholipids found in the oil. Sterols and hydrocarbons were identified in the unsaponifiable matter of the oil using GC-MS.

Keywords: Fatty acids, Lipid classes, mineral, Picralima nitida, triacylglycerol

PICRALIMA NITIDA YAĞININ LİPİT KOMPOZİSYONU VE TRİGLİSERİT YAPISI

Özet
Picralima nitida tohumunun bileşim ve mineral analizleri yapılmış ve elde edilen yağlarının kimyasal bileşimleri araştırılmıştır. Tohumda ham protein % 10.20±0.50 olarak tespit edilirken, karbohidrat içeriği % 74.53±0.50 oranında bulunmuştur. İyot ve sabunlaşma sayıları sırasıyla 136.40±1.00 ve 198.25±0.30 mg KOH/g değerlerindedir. Tohumun temel minerali potasyum iken (114.00±0.20 ppm), yağda temel mineral kalsiyumdur (91.00±0.20 ppm). Yağda nötral lipit % 96.30±0.50 oranında hesaplanırken, temel yağ asidi olaik asittir (% 50.65±0.2). Eşdeğer karbon sayısına göre analiz edilen trigliseritlerde C_{38} trigliseridi en yüksek orana sahiptir (% 20.38 ±0.1). Yağın temel glikolipidi monogalatozilmonogliserol iken (% 67.95±0.10), temel fosfolipidi ise fosfotidil kolindir (% 66.30±0.10). Sterol ve hidrokarbonlar ise GC-MS kullanılarak belirlenmiştir.

Anahtar kelimeler: Yağ asitleri, lipit sınıfları, mineral, Picralima nitida, trigliserit

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INTRODUCTION

Seed oils are mainly triglycerides which are one of the cheapest raw materials that can be processed. Their characteristics depend mainly on their composition and no oil from a single source can be suitable for all purposes (1). The utilization of oils depends mainly on their properties and these compositions (2).

Lack of information on the composition and utilization of the many and varied lesser known underutilized seed oils indigenous to the tropics are more of problem than the real shortage of oils (3). This is also particularly valid for the Nigerian flora which has one of the most extensive floras in continental Africa. To achieve the most economical and efficient utilization of these lesser known underutilized seed oils, information on their properties and compositions is required. There are many seeds, which are underutilized due to lack of information on their composition and utilization. One of these underutilized seeds includes; *Picralima nitida*. *Picralima nitida* is a tree of about 25 m high. The wood is pale yellow and hard. The bark is used as a febrifuge while the root is used in the treatment of fever and pneumonia (4).

A number of minerals are required by human body in order to maintain good health. Some of these essential minerals are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment. It has also been reported that trace metals can be detected in plants and food stuffs (5). In response to these needs, *Picralima nitida* seed and seed oil from Nigeria were investigated for their proximate and mineral composition, physicochemical properties, fatty acid composition, phospholipids, glycolipids and molecular speciation of the triacylglycerol.

MATERIALS AND METHODS

Materials

The seed sample was obtained from Sorobale in Ondo state, Nigeria. It was identified at the herbarium unit, Botany and Microbiology Department, University of Ibadan. The whole seed was subsequently ground in a laboratory mill (Gallenkamph, 82942, Brit. Pat, England) and stored in a plastic bag at 4 °C prior to analysis. Silica gel (60-120 mesh) was purchased from Acme Synthesis Chemicals, Mumbai. This was further activated by heating in an air oven at 110 °C for 2 h. before being used for column chromatography. All solvents and chemicals used in this study were of analytical grade and were purchased from S.D. Fine Chemicals, Mumbai. Silica coated TLC plates (20 x 20 cm) were procured from Sigma-Aldrich, Hyderabad, India.

Extraction and physicochemical analysis of the oil of Picralima nitida.

Oil was extracted from *Picralima nitida* seed using soxhlet extractor with *n*-hexane for 10 h. The extracted oil was analyzed for iodine value, peroxide value, saponification value, refractive index, specific gravity, free fatty acid and unsaponifiable matter by method described by the Association of Official Analytical Chemist (6). The refractive index of the oil (at 25 °C) was determined with Abbe refractometer and the specific gravity measurement was also carried out at 25 °C using gravity bottle (7).

Proximate analysis of the seed of *Picralima nitida.*

Proximate analysis of the seed of *Picralima nitida* was carried out as described by the Association of Official Analytical Chemist (8).

Fatty acid composition of the oil of *Picralima nitida.*

Fatty acid methyl esters of the oil were prepared by refluxing the sample at 70 °C for 3 h. in 2% sulphuric acid in methanol. The esters were extracted into ethyl acetate, washed free of acid and passed over anhydrous sodium sulphate. The ethyl acetate extract was further concentrated using a rotary evaporator. The fatty acid composition was analyzed using an Agilent 6890 N series gas chromatography equipped with FID detector on a split injector. A fused silica capillary column (DB-225, 30x0.32 m i.d., J & W Scientifics, USA) was used with the injector and detector temperature

maintained at 230 °C and 250 °C respectively. The oven temperature was programmed at 160 °C for 2 min and finally increased to 230 °C at 4 °C/min. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The area percentages were recorded with a standard Chemstation Data System.

Mineral determination of the seed and seed oil of *Picralima nitida*.

Metals determined were lead, cadmium, copper, zinc, iron, magnesium, calcium, sodium, potassium and manganese. The seed (0.5 g) as well as the extracted seed oil (0.5 g) of *Picralima nitida* were taken separately for analysis. This was achieved by digesting the samples using 5 mL (2:1) of nitric acid (70 % concentration) and perchloric acid (90 % concentration) (9). These metals were analyzed by atomic absorption spectrophotometry (Perkin -Elmer, GMBH, Ueberlingen, Germany).

Separation of lipid classes of the seed oil of *Picralima nitida*.

Lipid classes of the oil of *Picralima nitida* were separated on a 1 g scale into neutral lipids, glycolipids and phospholipids by silica gel column chromatography using a glass column 20 cm x 2 cm OD packed with 30 g activated silica gel (60-120 mesh). Neutral lipids, glycolipids and phospholipids were eluted successively using chloroform, acetone and methanol respectively. The lipid fractions were screened by TLC for the identification of components using hexane - ethyl acetate (90:10, v/v) as developing solvent for neutral lipids, chloroform - methanol - water (65: 25 : 4, v/v/v) for glycolipids and phospholipids (10). The eluted spots were identified using different spray reagents such as iodine vapors for neutral lipids, ammonium molybdate - perchloric acid for phospholipids and α-naphthol for glycolipids (11). The individual fractions were pooled, distilled under vacuum to remove solvent and weighed for quantification. The individual lipid fractions were converted into fatty acid methyl esters by refluxing with 2% sulphuric acid in methanol for 3 h. The esters were extracted into ethyl acetate, washed with distilled water and dried over anhydrous sodium sulphate and the fatty acid profile was analyzed using GC as described above.

Molecular speciation of the triacylglycerols of *Picralima nitida*.

The reversed phased HPLC analysis was performed on HP-1100 series HPLC equipped with an Evaporative Light Scattering Detector (ELSD) 2000 (Alltech ELSD 2000, Alltech Associates Inc, USA). Triacylglycerols were isolated from the neutral lipids using column chromatography (Christie, 1982). About 25 µL of the triacylglycerols (1 mg/mL) was injected in the SGERP-column (250 SS 4.6-W5C18-RS). The molecular species of the triacylglycerols were eluted within 10 min using an isocratic mobile phase of 95:5 (v/v) of acetone/isopropanol at a flow rate of 1 mL/min. The molecular species were identified by their Equivalent Carbon Numbers (ECN), by injecting reference triacylglycerols mixture and also by comparing with the literature data. The operating conditions for ELSD are: drift tube temperature 30 oC, flow of nitrogen 1.5 L/min with impactor "on" mode.

Quantification of phospholipids present in the oil of *Picralima nitida*.

The phospholipids isolated from the oil using column chromatography were quantified with normal phase HPLC equipped with a quaternary pump and an evaporative light scattering detector (ELSD 2000, Alltech, Deerfield, IL). The operating temperature of the ELSD was 50oC and nitrogen was used as the nebulizing gas at a flow rate of 1.5 L/min. HPLC separation were made on a LiChrosorb Si 60 (5 µm, 20 x 3.0 mm i.d., Merck, Darmstadt, Germany) at a solvent flow rate of 1 mL/min. A binary gradient system composed of eluant A [chloroform/ methanol/ ammonium hydroxide (80:19.5:0.5, v/v/v)] and eluant B [chloroform/ methanolammonium hydroxide/ water (60:34:0.5:5.5, v/v/v/v)] was used following the solvent elution profile: eluant A for 10 min; followed by linear increase in eluant B to 100% and held for 15 min. Identification of the phospholipids was carried out by comparing them with the retention time of respective commercial standards. Calibration curves for each phospholipid was drawn by injecting different concentrations and these were used to quantify the individual phospholipids as described by Avalli and Contarini (12).

Determination of the glycolipids present in the oil of *Picralima nitida*.

The glycolipids were quantified on a reversed phase HP-1100 series HPLC equipped with Evaporative Light Scaterring Detector (ELSD, 2000, Alltech Associates Inc., USA). About 25µL of the glycolipids fraction (1.0 mg/mL) was injected in the SGERP-Column (250 SS 4.6-W 5C18-RS). The components of the glycolipids were eluted within 15 min using a mobile phase composing of chloroform and methanol (95:5 v/v) at a flow rate of 1.0 mL/min. The ELSD (Alltech) was maintained at an evaporation temperature of 60 °C and gas (Nitrogen) flow rate of 2.7 L/min.

Identification of unsaponifiables of the seed oil of *Picralima nitida*.

Oil (2 g) was dissolved in 25 mL of 2 M ethanolic potassium hydroxide and refluxed for 1 h. The reaction mixture was later diluted to 150 mL with distilled water and transferred into a separating funnel. The unsaponifiable matter was then extracted three times with 50 mL diethylether. The ether extract was first washed with 100 mL aqueous solution of 0.5 M potassium hydroxide in order to remove any residual fatty acids. This was further washed with distilled water until it was free of potassium hydroxide, dried over anhydrous sodium sulphate and concentrated using a rotary evaporator (13). The unsaponifiables were identified by GC-MS analysis using Agilent (Palo Alto, USA) 6890N gas chromatography equipped with an HP-1 MS capillary column connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 ev; m/z 50-550; source temperature 230 °C and quadruple temperature 150 °C). Structural assignments were made based on interpretation of mass spectrometric fragmentation and confirmation by comparism of retention time as well as fragmentation pattern of authentic compounds and the spectral data obtained from the Wiley and NIST libraries.

RESULTS AND DISCUSSION

Proximate composition of the seed of *Picralima nitida*

The proximate composition of the seed of *Picralima nitida* is presented in Table 1. The crude fat content of the seed was found to be 5.02±0.30% while the crude protein content was 10.20± 0.50%. The ash content is low (0.88±0.20%). The crude fibre and moisture content were 5.64±0.50 and 3.73±0.30%, respectively. The seed is a rich source of carbohydrate which was 74.53 ±0.50%.

Table 1. Proximate composition (%) of the seeds of *P. nitida*

Assay	*P. nitida*
Crude fat	5.02±0.30
Crude protein	10.20±0.50
Crude fibre	5.64±0.50
Ash	0.88±0.20
Moisture	3.73±0.30
Carbohydrate	74.53±0.50

Values are mean ± standard deviation of triplicate determinations.

Physicochemical properties of the seed oil of *Picralima nitida*

The physicochemical properties of the oil of *Picralima nitida* is shown in Table 2. The oil is brownish in colour and liquid at room temperature. The free fatty acid content was 1.41±0.50 %. The iodine value which indicates the degree of unsaturation was found to be 136.40±1.00 while the refractive index was 1.4500 ±0.10. The saponification value was 198.25±0.30 mg KOH/g. The peroxide value and unsaponifiable matter were 4.59±0.20 meqO2/kg Oil and 1.90 ±0.50%, respectively.

Table 2. Physicochemical characterization of the oils from *P. nitida*

Parameter	*Picralima nitida*
Colour	Brown
Free fatty acid (%)	1.41±0.50
Saponification value(mg KOH/g)	198.25±0.30
Iodine value	136.40±1.00
Unsaponifiable matter (%)	1.90±0.50
Peroxide value (meq O_2/kg Oil)	4.59±0.20
Refractive index (25 °C)	1.4500±0.10
Specific gravity (25 °C)	0.9600±0.10
State at room temperature	Liquid

Values are mean ± standard deviation of triplicate determinations.

Mineral composition of the seed and oil of *Picralima nitida*

The mineral composition of the seed and oil of *Picralima nitida* is presented in Table 3. K has the highest concentration in the seed (114.00±0.20 ppm) while Ca has the highest in the oil (91.00±0.20 ppm) of *Picralima nitida*. Na was found as 41.60±0.50 ppm in the seed and 40.10±0.20 ppm in the oil. These minerals are essential in human nutrition (14). Mg was found to be 13.20±0.50 ppm in the seed and 6.70±0.10 ppm in the oil while Fe was 5.50±0.70 ppm in the seed and 4.60±0.30 ppm in the oil. Mn (0.10 ±0.10 ppm in the seed and 0.10±0.30 ppm in the oil), Cr (0.18 ±0.10 ppm in the seed and 0.05 ±0.10 ppm in the oil) and Co (0.12±0.01 in the seed and 0.10±0.00 ppm in the oil) were found in small amount in both the seed and oil.

Table 3. Metal composition (ppm) of the seeds and oils of *Picralima nitida*

Metal	Seed *Picralima nitida*	Oil *Picralima nitida*
Na	41.60±0.50	40.10±0.20
K	114.00±0.20	36.80±0.10
Ca	92.00±0.10	91.00±0.20
Mg	13.20±0.50	6.70±0.10
Fe	5.50±0.70	4.60±0.30
Cu	6.62±0.10	4.99±0.20
Zn	3.08±0.30	3.00±0.50
Mn	0.10±0.10	0.10±0.10
Cr	0.18±0.10	0.05±0.10
Co	0.12±0.01	0.10±0.00

Average concentration ± standard deviation of triplicate determinations (ppm) (mg/kg)
ND = Not detected.

Fatty acid composition of the oil of *Picralima nitida*

C18:1 (50.65±0.2%) is the major fatty acid found in the oil of *Picralima nitida* as shown in Table 4. C18:2 was found to be 20.79±0.4% while C18:3 was 1.75±0.1%. The oil also contains C12:0 (0.66±0.5%) and C14:0 (0.51±0.1%). The presence of C20:0 and C22:0 were recorded as 1.36±0.3 and 1.34±0.2%, respectively. The unsaturation of the oil was 73.19% while the saturation level was found as 26.81%.

Table 4. Fatty acid compositions (wt %) of *Picralima nitida*

Fatty acids	*Picralima nitida*
12:0	0.66±0.5
14:0	0.51±0.1
16:0	17.84±0.4
18:0	5.10±0.5
18:1	50.65±0.2
18:2	20.79±0.4
18:3	1.75±0.1
20:0	1.36±0.3
22:0	1.34±0.2
Unsaturated	73.19
Saturated	26.81

Values are mean ± standard deviation of duplicate determinations.
ND= Not Detected.

Lipid classes and distribution of fatty acids in the lipid classes

The lipid class of the oil is shown in Table 5 while the distribution of the fatty acids is shown in Table 6. Neutral lipids (96.30±0.50 %) were the major lipid class in the oil. Glycolipids were found as 3.40±0.40% while the phospholipids were found to be 0.30 ±0.20%. C12:0 was higher in the phospholipids (0.92±0.2%) than in the glycolipids (0.20±0.2%) and neutral lipids (0.70±0.1%). C14:0 was not detected in the glycolipids but in the neutral lipids (0.55±0.1%) and phospholipids (0.70±0.2%). C16:0 was 17.40 ±0.2% in neutral lipids, 14.80±0.1% in glycolipids and 19.50±0.3% in phospholipids. C18:1 has the highest concentration all the lipid classes. Its value was 51.30±0.2% in the neutral lipids. The glycolipids contained it as 49.20±0.2% while it was found as 48.50±0.1% in the phospholipids. C20:0 had the highest concentration in phospholipids (3.8±0.5%) while C22:0 had the highest in the glycolipids (7.35±0.5%). The neutral lipids were most unsaturated (73.55±0.3%) lipid fraction when compared with other classes (Glycolipids and phospholipids) of lipids in the oil.

Table 5. Lipid classes (wt %) of the oils from *Picralima nitida*

Lipid class	*Picralima nitida*
Neutral lipids	96.30±0.50
Glycolipids	3.40±0.40
Phospholipids	0.30±0.20

Values are mean ± standard deviation of duplicate determinations.

Table 6. Fatty acid compositions (wt %) in the lipid classes of *Picralima nitida*

Fatty acids	NL	GL	PL
12:0	0.70±0.1	0.20±0.2	0.92±0.2
14:0	0.55±0.1	ND	0.70±0.2
16:0	17.40±0.2	14.80±0.1	19.50±0.3
18:0	4.95±0.1	5.30±0.3	8.20±0.1
18:1	51.30±0.2	49.20±0.2	48.50±0.1
18:2	20.25±0.1	19.35±0.1	17.5±0.1
18:3	2.0±0.1	0.60±0.2	0.35±0.3
20:0	1.10±0.1	3.2±0.1	3.8±0.5
22:0	1.75±0.1	7.35±0.5	0.53±0.2
Unsaturated	73.55±0.3	69.15±0.2	66.35±0.3
Saturated	26.45±0.2	30.85±0.2	33.65±0.2

Values are mean± standard deviation of duplicate determinations.
ND= Not Detected, NL= Neutral lipids, GL= Glycolipids,
PL= Phospholipids

Triacylglycerol molecular species composition of the oil of *Picralima nitida*

Molecular species with equivalent carbon chain number C38 (20.38±0.1%) were predominantly present in the oil of *Picralima nitida* as shown in Table 7. C40 (11.70±0.3%), C42 (13.37±0.1%), C44 (14.30±0.1%) and C46 (15.76±0.3%) molecular species were also found in the oil. C36 (7.74±0.1%) and C48 (7.07±0.2%) molecular species were found in almost equal concentration in the oil. C50 and C52 species were 9.38±0.1 and 0.30±0.2%, respectively.

Table 7. Triacylglycerol molecular species composition (wt %) of *Picralima nitida*

ECN	Expected molecular species	*Picralima nitida*
C36	LnLnLn	7.74±0.1
C38	LLnLn	20.38±0.1
C40	LLLn/LnLnP/LnLnO	11.70±0.3
C42	LLL/LLnO/PLLn/SLnLn	13.37±0.1
C44	LnOO/OLL/SLLn/PLL/PLnP	14.30±0.1
C46	POL/OOL/PLP	15.76±0.3
C48	SLnS/OOO/POP	7.07±0.2
C50	BLL/BOLn	9.38±0.1
C52	BOL/BPL/SLB	0.30±0.2

ND= Not Detected
Ln= linolenic acid, L= linoleic, O= oleic acid, P= palmitic acid,
S= steric acid, B= behenic acid

Glycolipids and phospholipids composition of the oil of *Picralima nitida*

Monogalactosylmonoacylglycerol is the major glycolipids found in the oil of *Picralima nitida* at a concentration of 67.95±0.10% as shown in Table 8. Monogalactosyldiacylglycerol was found as 14.29±0.20% while digalactosyldiacylglycerol was 10.57±0.10%. Digalactosylmonoacylglycerol has the least concentration of 7.19±0.40%. The phospholipid composition is presented in Table 9. Phosphatidyl choline was the dominant phospholipid found in the oil with a concentration of 66.30 ±0.10%. Phosphatidyl ethanol amine was found to be 15.60 ±0.50% while phosphatidyl inositol was 18.10±0.30%.

Table 8. Glycolipids composition (%) of the oil of *Picralima nitida*

Seed oils	MGDG	DGDG	DGMG	MGMG
Picralima nitida	14.29±0.20	10.57±0.10	7.19±0.40	67.95±0.10

MGDG= Monogalactosyldiacylglycerol,
DGDG= digalactosyldiacylglycerol,
DGMG= Digalactosylmonoacylglycerol,
MGMG= Monogalactosylmonoacylglycerol

Table 9. Phospholipids composition (%) of the oil of *Picralima nitida*

Seed oils	PC	PE	PI
L. cylindrica	66.30±0.10	15.60±0.50	18.10±0.30

Values are mean ± standard deviation of triplicate determinations.
ND= Not detected, PC= Phosphatidyl choline,
PE= Phosphatidyl ethanol amine
PI= Phosphatidyl inositol

Unsaponifiable matter composition of the oils of *Picralima nitida*

The unsaponifiable matter of the oils were isolated and identified using GC-MS. Hydrocarbons found in the oil includes; hexadecane, octadecane, eicosane, docosane, octadecene, hexadecene, heptadecane and pentadecane. Other compounds are stigmasterol, sitosterol and beta tocopherol.

CONCLUSIONS

The seed of *Picralima nitida* was subjected to proximate analysis while the oil was evaluated for its fatty acid composition, lipid classes, fatty acid distribution in the lipid fractions and molecular speciation of the triacylglycerols. The result revealed the seed of Picralima nitida to be a good source of carbohydrate. C18:1 fatty acid as the dominant fatty acid in the oil of Picralima nitida. Neutral lipids were predominantly present in this oil while molecular species with equivalent carbon chain number C38 was majorly present in the oil. Monogalactosylmonoacylglycerol was the dominant glycolipids while phosphatidyl choline was the main phospholipids found in the oil of *Picralima nitida*.

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