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LETTER TO THE EDITOR

Phospholipid Composition of *Jatropha curcus* Seed Lipids

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Abstract *Jatropha curcus* L. oil has emerged as one of the most important raw materials for biodiesel production. However, no detailed study has been reported on characterizing the lipid constituents of jatropha oil. The present study revealed that the total oil content of jatropha seeds was 32% with a composition of 97.6% neutral lipids, 0.95% glycolipids and 1.45% phospholipids. The fatty acid composition of total lipids, neutral lipids, phospholipids and glycolipids was also determined and found to contain oleic acid (18:1) and linoleic acids (18:2) as major fatty acids. The phospholipids fraction was further characterized and quantified and found to contain phosphatidyl choline (PC) 60.5%, phosphatidyl inositol (PI) 24% and phosphatidyl ethanolamine (PE) 15.5%. The fatty acid composition and the positional distribution of the fatty acids of individual phospholipids were also reported.

Keywords *Jatropha curcus* · Fatty acid composition · Glycolipids · Phospholipids · Positional distribution of phospholipids

Sir

Jatropha curcus L. (popularly known as physic nut, pinoncillo, Habb-el-mueluk, purging nut, Barbados purging nut, ratanjyot etc.) oil is projected as one of the most important raw materials for biodiesel production. Countries like India, Nigeria and Kenya have initiated the planting of jatropha in an organized manner. Jatropha belongs to the Euphorbiaceae family. Jatropha fruit is used to treat diseases like dysentery, hemorrhoids, gonorrhea, coated tongue, infertility, small pox and other skin infections [1] and the oil is used for preparation of soap and cosmetics in many tropical countries [2]. Jatropha oil was traditionally known to be used for veterinary medicinal applications [3], to treat skin diseases and to give pain relief to the patients suffering from rheumatism [4]. Some components with anti-inflammatory and wound-healing properties have been isolated and characterized from jatropha leaves and latex [5, 6]. The molluscicidal, insecticidal and fungicidal effects of jatropha seeds and leaves have also been investigated [4]. Bhakare et al. [7] studied the fatty acid composition of phospholipid mixture isolated from jatropha oil and reported to contain palmitic (21.0%), stearic (12.2%), oleic (36.7%) and linoleic acid (30.0%) as major fatty acids. However, the composition of jatropha lipids and their phospholipid composition have not been reported.

The phospholipids present in the jatropha oil are generally removed during the degumming step before the preparation of biodiesel, as the amphiphilic nature of the phospholipids results in a strong emulsion and separation of glycerol and biodiesel layer becomes very difficult. As the phospholipids (a by-product of the degumming process) and their products are well known for use in different food and industrial applications like animal feed, cosmetics, pharmaceuticals, dietetics etc. [8], jatropha oil should also be exploited for the isolation of phospholipids to strengthen the economy of the biodiesel industry. In the present investigation, a detailed study was carried out on the phospholipid composition of jatropha oil. The fatty acid composition of jatropha phospholipids and their positional distribution in individual phospholipids were also reported.

Jatropha seeds were procured from a local market. Pre-coated TLC silica gel plates (20 × 20 cm silica

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gel 60 F_{254}, 0.25 mm thickness) were procured from Merck, Darmstadt, Germany. Silica gel (60–120 mesh) for column chromatography was purchased from Acme Synthetic Chemicals, Mumbai, India. L-α-Phosphatidyl inositol (PI) was procured from M/s Avanti Polar Lipids, Alabaster, Alabama, USA. The reference samples phosphatidyl choline (PC), phosphatidyl ethanolamine (PE); snake venom (*Naja naja* Atra, Formosan cobra) as phospholipase A_2 source and *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide (diazomethane precursor) were purchased from M/s Sigma Chemicals, St Louis, USA. HPLC grade solvents were procured from M/s Merck, Mumbai, India.

Total lipids were extracted from powdered jatropha seeds (50 g) with 250 mL of chloroform/methanol (2:1 v/v) by Folch's extraction method [9]. The crude jatropha lipid extract (16 g) was dried under reduced pressure. The jatropha lipids were fractionated into neutral lipids, glycolipids and phospholipids by silica gel column chromatography [10]. The column (60 × 4.5 cm) was packed with activated silica gel (60–120 mesh) and the crude lipid extract (10 g) was loaded on the column. Neutral lipids (9.76 g, 97.6%), glycolipids (0.095 g, 0.95%) and phospholipids (0.145 g, 1.45%) were eluted using chloroform, acetone and methanol, respectively. Glycolipids and phospholipids were qualitatively identified by developing TLC using chloroform/methanol/water (65:25:4 v/v/v) and spraying with α-naphthol [11] and ammonium molybdate [12] as spray reagents, respectively.

Fatty acid methyl esters (FAME) of total lipids, neutral lipids, glycolipids and phospholipids were prepared by refluxing the sample at 80 °C for 4 h in 2% sulfuric acid in methanol [13]. Analyses of the FAME by GC were carried out on a gas chromatograph (GC 6890 N, Agilent Technologies, USA) equipped with a flame ionization detector (FID) on a split injector. A fused silica capillary column (DB-225, 30 × 0.32 m i.d., J & W Scientifics, USA) was used for FAME analysis. The oven temperature was programmed at 160 °C for 2 min, increased to 180 °C at 5 °C/min, held at 180 °C for 2 min and finally increased to 230 °C at 4 °C/min. The injector and detector temperatures were held at 220 and 250 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The area percentages were recorded with a standard Chemstation Data System. The fatty acid composition of different lipid classes of jatropha total lipids is given in Table 1.

Oleic (18:1), linoleic (18:2) and palmitic acids (16:0) are the major fatty acids present in all the lipid classes. Fatty acid composition of the neutral lipid is similar to that of the total lipids, whereas an increase in palmitic acid and a decrease in oleic and linoleic acids were noticed in case of phospholipids. However, the amounts of palmitic and palmitoleic acids were considerably more abundant in the glycolipid fraction when compared to the total lipids (Table 1). Trace amounts of linolenic acid (18:3) were observed in all the lipid classes except in glycolipids.

Individual phospholipids 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 50 °C and nitrogen was used as the nebulizing gas at a flow rate of 1.5 L/min. HPLC separations were made on a LiChrosorb Si 60 (5 μm, 20 × 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/methanol/ammonium 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 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 (5–40 μg) and these were used to quantify the individual phospholipids following the method described by Avalli and Contarini [14]. The phospholipid composition of jatropha phospholipids was found to be; PC 60.5% ± 0.26;

Table 1 Fatty acid composition (wt.%) of jatropha total lipids, neutral lipids, glycolipids and phospholipids

Sample	Fatty acid composition (wt.%)[a]				
	16:0	16:1	18:0	18:1	18:2
Total lipids	16.6 ± 0.02	1.1 ± 0.01	6.9 ± 0.03	40.2 ± 0.01	35.2 ± 0.01
Neutral lipids	16.9 ± 0.06	1.2 ± 0.03	7.0 ± 0.03	41.3 ± 0.06	33.6 ± 0.11
Glycolipids	24.8 ± 0.14	7.1 ± 0.14	7.3 ± 0.00	31.8 ± 0.14	29.0 ± 0.14
Phospholipids	21.9 ± 0.06	0.3 ± 0.01	6.5 ± 0.06	39.8 ± 0.04	31.5 ± 0.06

Values are relative to total fatty acids in each sample

[a] 18:3 was found in traces in total lipids, neutral lipids and phospholipids

Table 2 Fatty acid distribution in individual phospholipid classes

Phospholipid	Positional distribution	Fatty acid composition (wt.%)[a]				
		16:0	16:1	18:0	18:1	18:2
PC	Total	14.5 ± 0.06	0.4 ± 0.06	6.4 ± 0.01	48.0 ± 0.11	30.7 ± 0.11
	sn-1	32.7 ± 0.03	0.7 ± 0.03	13.1 ± 0.21	31.6 ± 0.0	21.9 ± 0.07
	sn-2	4.9 ± 0.14	0.7 ± 0.07	1.2 ± 0.07	52.5 ± 0.07	40.7 ± 0.14
PI	Total	24.6 ± 0.11	0.7 ± 0.06	4.7 ± 0.02	34.1 ± 0.06	35.9 ± 0.11
	sn-1	49.3 ± 0.42	0.3 ± 0.02	11.7 ± 0.07	21.2 ± 0.35	17.5 ± 0.04
	sn-2	4.5 ± 0.02	0.3 ± 0.03	1.0 ± 0.03	38.8 ± 0.02	55.4 ± 0.03
PE	Total	23.6 ± 0.03	0.6 ± 0.0	4.4 ± 0.0	30.7 ± 0.01	40.7 ± 0.01
	sn-1	39.4 ± 0.35	0.7 ± 0.07	9.5 ± 0.04	23.8 ± 0.18	26.6 ± 0.06
	sn-2	6.8 ± 0.07	0.7 ± 0.07	1.8 ± 0.21	46.0 ± 0.35	44.7 ± 0.07

Values are relative to total fatty acids in each sample

[a] 18:3 was found in traces in all the phospholipid classes

PI 24% ± 0.21 and PE 15.5% ± 0.07, along with traces of lyso PE and lyso PC.

The phospholipid mixture was re-chromatographed on a silica gel column (60–120 mesh silica gel, 30 × 2 cm) to isolate individual phospholipids using gradient elution of methanol in chloroform at a range of 20, 40 and 50% for PE, PC and PI, respectively [15]. The purity of individual phospholipids isolated using silica gel column chromatography was determined by HPLC analysis and found to be 98.6% ± 0.07, 98.3% ± 0.03 and 97.2% ± 0.07 for PE, PC and PI, respectively.

The fatty acid composition and their distribution at the *sn*-1 and *sn*-2 positions of individual phospholipids of jatropha oil were also analyzed by GC as their methyl esters using reported methods [16, 17]. The fatty acid composition of individual phospholipids and the fatty acid distribution in *sn*-1 and *sn*-2 positions of PC, PI and PE are given in Table 2.

The major fatty acids in all the three phospholipids were found to be linoleic, oleic and palmitic acids. Linoleic acid content in PE was found to be the maximum (40.7%) compared to other phospholipids. It was found that PE and PI contained a higher amount of palmitic acid and a lower amount of oleic acid, whereas PC contains a lower amount of palmitic acid and a higher amount of oleic acid compared to that of the total phospholipids fraction of jatropha lipids. It was observed that the saturated fatty acids were mostly located in the *sn*-1 positions of all the three phospholipids, whereas the unsaturated fatty acids predominantly occupied the *sn*-2 positions.

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