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Estimation of Toxic Phorbol Ester in *Jatropha curcas* Oil, Cake and Biodiesel

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Biodiesel is a promising fuel to eradicate energy crisis of the present world. Among various non-edible oil seed crops *Jatropha curcas* is widely used for biodiesel production. The seed was collected from Gujarat, India and oil content of the seed kernel was determined by soxhlet apparatus. The oil content of the kernel was 57 %. The oil was subjected to biodiesel preparation by twin step method of acid esterification followed by alkali transesterification. The yield of biodiesel after reaction was 98.05 % from ^{1}H NMR studies. Toxic phorbol ester fraction was extracted from both oil and dehulled cake whereas biodiesel was used directly. The phorbol ester content of oil, cake and biodiesel was determined by high performance liquid chromatography (HPLC, Waters). The phorbol ester content of the oil was more (3.17 ± 0.04 mg g^{-1}) than the cake (1.59 ± 0.01 mg g^{-1}) but phorbol ester peak was not detected in biodiesel.

Key Words: Biodiesel, Jatropha oil, Phorbol ester, Transesterification.

INTRODUCTION

The ever increasing energy demand of the world for industries, transport, agriculture, *etc.*, the pollution problems of fossil fuel burning and the day-by-day depleting finite oil reserves are the major driving forces of searching alternative fuels of smaller environmental impact derived from renewable resources. One of such alternatives is biodiesel which is easily-biodegradable, renewable and has low pollutant emissions. Biodiesel is the monoalkyl ester of long chain fatty acids derived from transesterification of vegetable oils and animal fat with short chain mono-hydric alcohols in presence of catalyst. The oilseed crops used for biodiesel production are sunflower, soybean, rapeseed, linseed, cottonseed, canola, *etc.*[1], majority of which are edible in nature, thus brings in the food *versus* fuel controversy. Non-edible oils from various plants like Jatropha (*Jatropha curcas*), Karanja (*Pongamia pinnata*), Mahua (*Madhuca indica*), Neem (*Azadirachta indica*), Simarouba (*Simarouba glauca*) *etc.,* are feed stocks available for biodiesel production[2]. Among

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these *Jatropha curcas* is a wild oilseed plant of the tropics is now being credited as a most promising biofuel crop, ideally suited for growing in the wastelands of the country.

Jatropha curcas L. (Euphorbiaceae) is an oil-bearing shrub bearing 40-60 % oil[3] in the kernel with a fatty acid composition[4] similar to that of oils used for human nutrition. The seed is rich source of oil but can't be utilized for nutritional purposes due to its toxic nature and mostly used for biodiesel production. The oil of *J. curcas* can serve as a fuel for diesel engines[5] indicating its potential as a renewable energy source and can be used without modification of engine infrastructure. Besides being a source of oil, *Jatropha* also provides a meal (cake), which is rich in crude proteins (50-58 % depending on the residual oil) but is presently used only as a fertilizer[6]. Both the seed and oil of Jatropha have been found to be toxic. The seed cake may serve as highly nutritious protein supplement in animal feed but the presence of high level of antinutrients prevents to do so[7]. Phorbol esters are the major toxic constituents present in the aerial parts of the plant especially in seeds, which make the plant unpalatable and toxic to some vertebrates, insects and snails[3]. As per Ministry of Environment and Forests, Government of India reports, 25 children had admitted to hospital at Ahamadabad after eating *J. curcas* fruit at Kadi on September 29, 2007.

The prime objective of the study is estimation of toxic phorbol ester in oil, cake and biodiesel of *J. curcas*.

EXPERIMENTAL

Procurement of sample: *Jatropha* seed was collected from Gujarat, India for the study. The sample was cleaned manually to remove all foreign materials. The cleaned seeds were sun dried and then dried in hot air oven at 80 °C till free from moisture. The seeds were decorticated manually to obtain kernel.

Oil content determination and characterization of oil: About 50 g of kernel was grinded for 1 min, sieved through a 2 mm sieve. For oil content determination[8] the grinded kernel was extracted in soxhlet apparatus using petroleum ether (boiling point 60-80 °C). The extract was concentrated in rotavapour. The residual oil was cooled and weighed. The physicochemical properties (density, viscocity, acid value, saponification value, unsaponification matter and iodine value) of the oil were determined.

The fatty acid composition was determined by gas chromatographic analysis of the fatty acid methyl esters as follows[9]. GC analysis was carried out against standard fatty acid methyl esters (Sigma make) on a Varian CP-3800 gas chromatograph equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm WCOT column coated with 0.25 mm film thickness of polyethylene glycol supplied by J and W (carbowax column). Helium was used as the carrier gas at a flow rate of 1 mL/min at a column pressure of 22 KPa. 0.2 µL of sample was injected into the injection port of the GC using a split ratio of 50:1. Compound separation was achieved following a linear temperature program of 160 °C (1 min), 160-240 °C

(4 °C/min), 240 °C (23 min), so the total run time was 45 min. Percentage composition was calculated using peak normalization method assuming equal detector response. The peaks were identified by co-eluation of standard methyl ester samples procured from Sigma-Aldrich and analysed in the same GC conditions. The GC/MS analysis was carried out on a Varian Saturn 2200 GC/MS fitted with the same column and temperature programmed as above. MS parameters: ionization voltage (EI) 70 eV, peak width 2 s, mass range 40-500 amu and detector voltage 1.5 volts. Peak identification was confirmed by comparison of the mass spectra with mass spectra available on NIST-1 and NIST-II libraries.

Transesterification of *Jatropha* oil: Transesterification or alcoholysis is the displacement of one alcohol from an ester by another alcohol in the processes similar to hydrolysis; except that an alcohol is used instead of water. This process has been widely used to reduce the high viscosity of triglycerides.

Jatropha oil methyl ester (JOME) was prepared from seed oil. A two step procedure (acid catalyzed reaction and alkali catalyzed reaction) was followed due to high acid value of the oil[10]. The yield of product biodiesel was determined by ^{1}H NMR (nuclear magnetic resonance) spectroscopy on a Brucker 300 MHz instrument (Brucker DPX 300, Rheinstetten, Germany).

Extraction of toxic fraction: Extraction of toxic fraction[11] from *Jatropha* oil was done by solvent-solvent extraction method. The seed oil was extracted in methanol-water (9:1) mixture using separating funnel. The combined methanol-water layer was concentrated in a rotary evaporator and a viscous oily fraction separated from the aqueous solution after concentration. That was again extracted with diethyl ether. The combined ether layer was washed with water and evaporated. A brown viscous mass was obtained and thin layer chromatography (TLC) of that fraction was done for qualitative determination of toxic constituents. The extraction of toxic fraction from cake after oil extraction was done by the above process. The toxic sample of oil and cake was dissolved in tetrahydrofuran (THF) but biodiesel was dissolved in methanol for determination of phorbol ester by HPLC.

Determination of phorbol ester in oil, cake and biodiesel: The phorbol ester content of *Jatropha* oil, cake and biodiesel was quantified by HPLC[3]. Phorbol ester of *Jatropha curcas* seed oil, cake an biodiesel was determined by using high performance liquid chromatography (Waters 600 HPLC system) equipped with a reverse phase C_{18} column (Waters Spherisorb, 5 μm, 250 mm × 4 mm i.d.) waters 2998 photodiode array detector, waters 600 HPLC quaternary pump, waters inline degasser and empower software. The column temperature was controlled at 25 ºC and the flow rate was 1.3 mL min^{-1}. The solvents used were 1.75 mL ortho-phosphoric acid (85 %) in 1 L distilled water (A) and acetonitrile (B). All solvents were filtered and degassed by waters inline degasser. The gradient used was as follows: 0-10 min, 60 % A and 40 % B; 10-40 min, 50 % A and 50 % B; 40-55 min, 25 % A and 75 % B; 55-60 min, 100 % B and then the column was adjusted to the starting condition (60 % A and 40 % B). The phorbol esters peaks appeared between 41 and 48 min. The peaks

were integrated at 280 nm and the results were expressed as equivalent of phorbol-12-myristate 13-acetate (Sigma Chemicals), whose peak appeared at 50 min. Each analysis was conducted in triplicate.

RESULTS AND DISCUSSION

Oil content determination and characterization of oil: The oil content of the kernel of the seed collected from Gujarat is 57 % which is within the range of oil content of *Jatropha* seed kernel[3] 40-60 %. The physico-chemical properties of the oil were given in Table-1. The predominant fatty acids found were oleic acid, linoleic acid, palmitic acid and stearic acid. Palmitoleic acid is found to a lesser extent and linolenic acid was not detected (Table-2). Oleic acid (43.42 %) was the predominant fatty acid followed by linoleic acid (35.97 %) and is the highest percentage of total fatty acid.

TABLE-1
PHYSICO-CHEMICAL PROPERTIES OF JATROPHA OIL

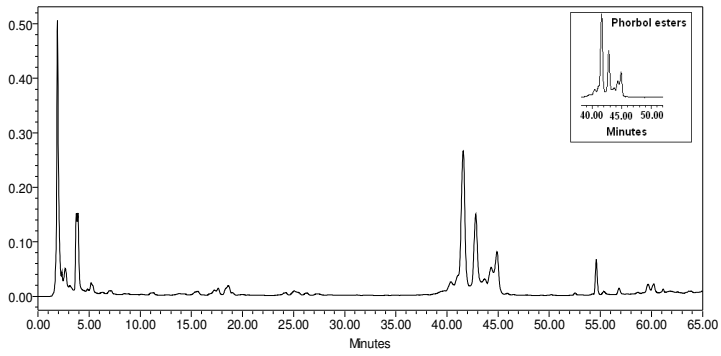
Properties	Jatropha oil
Acid value (mg KOH/g)	10.5
Saponification value (mg KOH/g)	189.2
Unsaponifiable matter (%, w/w)	1.59
Iodine value (gI_2/100 g)	97
Density (gm/cc)	0.91
Viscosity at 40 °C (cSt)	34.3

TABLE-2
FATTY ACID COMPOSITION OF JATROPHA OIL

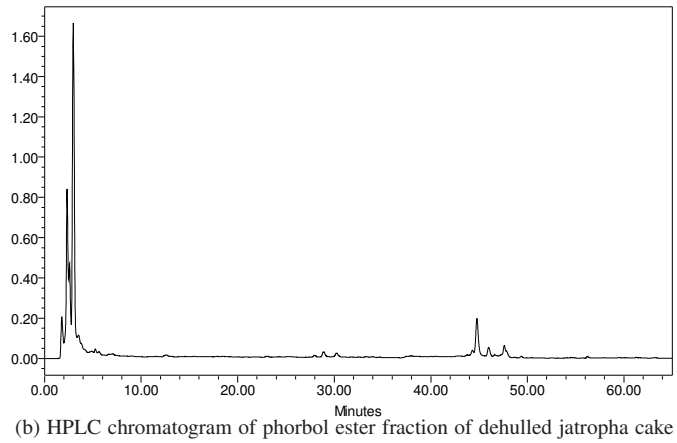
Fatty acid	Amount (%)	Identification
Palmitic acid (C16:0)	13.38	Co-eluation, MS
Palmitoleic acid (C16:1)	0.88	Co-eluation, MS
Stearic acid (C18:0)	6.36	Co-eluation, MS
Oleic acid (C18:1)	43.42	Co-eluation, MS
Linoleic acid (C18:2)	35.97	Co-eluation, MS
Linolenic acid (C18:3)	–	Co-eluation, MS
Saturated fatty acids	19.74	–
Unsaturated fatty acids	80.26	–

Transesterification of Jatropha oil: The acid catalyzed pretreatment reduced acid value of oil to an extent, that the oil is suitable for alkali-catalyzed reaction. Then the alkali used for alkali-catalyzed reaction compensates the acidity of pretreated oil and remaining part act as transesterification catalyst. Transesterification reaction for preparation of jatropha oil methyl ester was effectively carried out, which resulted in the desired products biodiesel and glycerol layer. The yield of methyl ester was 98.05 %, calculated from the ^{1}H NMR results.

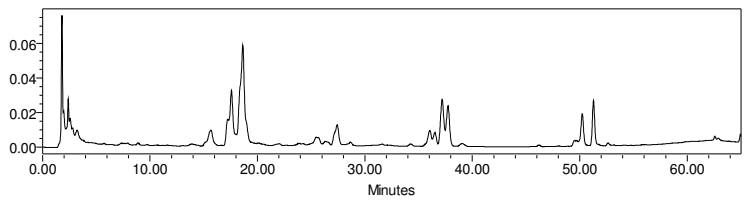
Determination of phorbol ester in oil, cake and biodiesel: Phorbol esters were detected in oil and cake (at retention time 41-48 min) but not detected in biodiesel (Fig. 1). The quantity of phorbol ester was amounted to 3.17 and 1.59 mg/g in oil and dehulled meal, respectively (Table-3). Thus oil bears more phorbol ester than that of cake. The phorbol ester content has been found to vary from 0.87-3.32 mg g^{-1} in different varieties of *Jatropha* seed[3]. Hass and Mittelbach[12] reported 0.31 % of phorbol ester in *J. curcas* oil and Aregheore *et al.*[13] reported a level of 0.13 mg phorbol esters g^{-1} in *J. curcas* meal.



(a) HPLC chromatogram of Phorbol ester fraction of Jatropha oil



(b) HPLC chromatogram of phorbol ester fraction of dehulled jatropha cake



(c) HPLC chromatogram of biodiesel

Fig. 1. HPLC chromatogram of (a) oil, (b) cake and (c) biodiesel

TABLE-3
PHORBOL ESTER CONTENT OF JATROPHA OIL, CAKE AND BIODIESEL

Samples	Phorbol ester content (mg/g)
Jatropha oil	3.17 ± 0.04
Dehulled cake	1.59 ± 0.01
Biodiesel	Not detected

Conclusion

Jatropha curcas is a potential oil seed crop for biodiesel production due to its high oil content and good quality of resulting biodiesel. Although both the oil and cake contain toxic phorbol ester the biodiesel was free from it. As *Jatropha* oil is a potential source for biodiesel preparation, huge amount of oil and cake will be generated and hence need to be handled carefully. Precautions must be taken when handling the oil to avoid skin contact and ingestion due to the presence of toxic constituents. Due to the presence of toxic phorbol ester the oil and other by-products require high safety level for use.

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