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Enzymatic Synthesis of Biodiesel from *Jatropha tanjorensis* Ellis and Saroja and its Potential as Biodiesel Feedstock

K.P. ARUN*, N. RAVICHANDRAN, M. NITHYA DEVI, R. VAJRAI and P. BRINDHA

Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur-613 401, India

*Corresponding author: E-mail: arun@carism.sastra.edu

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21st century has almost become "Green century" with an increased demand for plant products (bioenergy-oil). Hence, to develop a natural biodiesel feedstock, common species of *Jatropha* in and around Thanjavur is selected based on its enriched monoalkyl ester content which could be a good source for generating biofuels. *Tanjorensis* seed oil were extracted and analyzed for its physico-chemical properties such as % free fatty acid, acid value iodine, peroxide, saponification values. *P. aeruginosa* crude lipase treated transesterification followed by GC-MS analysis of oil proved that oleic acid (37.3 %) and linoleic acid (33.3 %) were the principal fatty acids while palmitic acid and stearic acid being the major saturated fatty acids. Oil contents of *Tanjorensis* seed oil were comparable with the oil extracted from other species of *Jatropha* and were found to be close to oil from *Jatropha curcas*. *Tanjorensis* seed oil extracts could be useful in industrial applications and as a biodiesel feedstock.

Keywords: *Jatropha tanjorensis*, Biodiesel, *P. aeruginosa* crude lipase, Transesterification, Fatty acids.

INTRODUCTION

Consumption of fuel has been increasing constantly, which is approximately 96 million barrels of crude oil per day. The consumption in few years will rise by 7-10 % yearly [1]. Various vegetable oils have been used to extract biodiesel and lubricant [2]. Biodiesel (monoalkyl esters of fatty acids) extracted from animal fats or from various vegetable oil, is one among the clean and renewable fuel. Transesterification of animal fats or vegetable oils with methanol or ethanol with alkaline or acid catalysts is the usual method followed for the production of biodiesel [3,4]. Enzymatic (E.C. 3.1.1.3) transesterification process is less energy consuming and more efficient in separation and purification of biodiesel when compared to general transesterification. Therefore, the use of enzymes could be an appealing option because enzymatically produced biodiesel can be used directly without purification [5]. The transesterification of plant oils such as methyl or ethyl esters has shown approximately similar characteristics like cetane number, density, viscosity and calorific value as of mineral diesel [6,7]. Hence with a view to develop an ecofriendly biodiesel feedstock, common species of *Jatropha* in and around Thanjaavur is selected as these species are well known for their fatty acids with rich monoalkyl esters, which could be a good source for generating biofuels. In the present paper attempts have been made to study the composition of *Tanjorensis* seed oil obtained from the selected source *Jatropha tanjorensis* Ellis and Saroja and to evaluate its potential as biodiesel feedstock.

EXPERIMENTAL

Seeds of *Jatropha tanjorensis* Ellis and Saroja were collected from the surrounding areas of Thanjavur district, identified using standard floras [8] and authenticated with the help of specimen deposited at Raphinet herbarium (RHT 1291), St. Joseph College Trichy. The voucher specimens were also preserved in the herbarium of CARISM, Sastra University, Thanjavur [9]. The seeds were chosen as per their physical conditions. Seeds were de-shelled and kept for drying at high temperature of 100-105 °C for 35 min. De-shelled seeds were ground using bench top blender and used further for extraction.

***Pseudomonas* strain and culture conditions:** *Pseudomonas aeruginosa* excretes several enzymes, three of which possess lipolytic activity: an extracellular phospholipase C (heat-labile hemolysin), a membrane-bound esterase and an extracellular lipase. Phospholipase C [10] and esterase [11] has been purified to homogeneity and extensively characterized for its lipase activity. *Pseudomonas aeruginosa* culture was grown and maintained on a mineral based (MB) medium additionally supplemented with 1 % (v/v) cotton seed oil (as a solitary C-source) by repeated sub culturing. The mineral based broth

pH 7.5 contained: 0.3 % $NaNO_3$, 0.01 % K_2HPO_4, 0.05 % $MgSO_4 \cdot 7H_2O$, 0.001 % $FeSO_4 \cdot 7H_2O$ and 0.5 % yeast extract. The mineral based was sterilized at 1.1 bar pressure for 20 min at 121 °C. Culture was grown at 34 °C with continuous shaking (150 rpm) for 2 days, after which the cells were harvested and the supernatant was filtered and taken to extract the extracellular lipase produced by *Pseudomonas aeruginosa*. Crude protein was purified by salting out precipitation using ammonium sulfate at 80 % saturation, this was then centrifuged at 14,000 g for 25 min at 4 °C, thus pellet formed was discarded and supernatant was filtered through 0.45 µ syringe filter and concentrated to 10 mL (0.05 M citrate-phosphate buffer pH 7.0) using lyophilizer (Christ, Lyo A1-4). Protein content was measured using Lowry's method [12]. This filtrate is hereafter referred as crude lipase (E.C. 3.1.1.3).

Entrapment of crude lipase in polyacrylamide and assay of lipase activity: The polymerization mixture contains enzyme solution, 13.6 % (w/v) acrylamide, 5 % of cross-linker monomer (N,N-methylene-*bis*-acrylamide) and citrate-phosphate buffer (pH 7.0, 0.05 M) polymerization was made with the addition of 1 % N,N,N',N'-tetramethylethylenediamine (TEMED) and finally 10 % of ammonium persulphate was added to the polymerization mixture. Gel was washed with chilled citrate-phosphate buffer (pH 7.0, 0.05 M) and was cut into small fragments. Unbound enzymes were removed by washing these small gel fragments in the same buffer at 4 °C. Lipase activity for the above prepared immobilized crude lipase was assayed using *p*-nitrophenyl phosphate (*p*-NPP). Reaction mixture comprised of *p*-NPP stock solution (20 mM in iso-propanol), 15 % of immobilized crude lipase and *Tris* buffer (pH 8.5, 0.05 M) to make final volume of 3 mL, this mixture was incubated at 45 °C for 20 min in a water bath. The reaction was terminated with the addition of chilled 1:1 acetone:ethanol mixture. Control containing heat-inactivating (5 min in boiling water bath) enzyme was also included. The absorbance of heat-inactivating lipase was subtracted from the absorbance of the respective crude lipase. The absorbance (A_{410}) of *p*-nitrophenol released was measured (Perkin Elmer, UV/visible spectro-photometer). Reference curve of *p*-nitrophenol (2-20 µg/mL in pH 8.5, 0.05 M *tris* buffer) was generated and the unknown concentration of *p*-nitrophenol released was determined. Assay was performed in triplicate and the mean values were recorded. Enzyme activity was calculated as; 1 unit (IU) of crude lipase activity is defined as the release of *p*-nitrophenol (µmol) during the hydrolysis of *p*-NPP by 1 mL of enzyme at 45 °C under assay conditions (Table-1).

Oil extraction and enzymatic transesterification process: Defatting of mechanically ground seeds were carried out *via* Soxhlet apparatus, using *n*-hexane (b.p. 40-60 °C). The seed characteristics are shown in Table-1. Lipid extract was obtained and the hexane content was removed using rota-evaporator at 40 °C. For enzymatic biodiesel synthesis, nearly every source of triglycerides can be taken as enzyme substrates. Enzymatic transesterification reaction mixture contained 2 g of oil, 0.2 g of crude lipase (10 %) with 1:2 alcohol (methanol)-to oil molar ratio. The reaction was carried at 50 °C with constant stirring at 150 rpm. Optimization of the reaction was carried out with varying reaction time. Separate layer was observed with the repeated use of enzyme. This substrate/product layer on the surface of the enzymatic support can lead to the loss of enzymatic activity by hindering the diffusion of substrate and product [13], this was overcome by the use of non-polar solvent, which also helps in preserving the enzyme activity (Fig. 1). 500 µL of reaction mixture was taken and centrifuged to get the clear oil/biodiesel (upper layer), which is now separated from enzyme (pellet) and was analyzed by gas chromatography. The extracted seed oil was stored at -20 °C for successive physicochemical analysis. The percentage yield of selected seed oil is shown in Table-1.

TABLE-1
SEED CHARACTERISTICS AND PHYSICO-CHEMICAL PROPERTIES OF *Jatropha tanjorensis* SEED OIL

Seed characteristics (Value ± SD)	
Length (cm)	1.50 ± 0.2
Width (cm)	0.80 ± 0.1
Weight (g)	0.55 ± 0.1
Physico-chemical properties	
Oil yield (%)	62.23 ± 0.11
Acid value	0.82-1.98
Iodine value	92-104
Saponification value	180-200
Peroxide value	1.45 ± 0.02
Density at 20 °C (g/mL)	0.812-0.899
Crude lipase (mg/mL)	7.56
Crude lipase activity (mU/mL)	2450

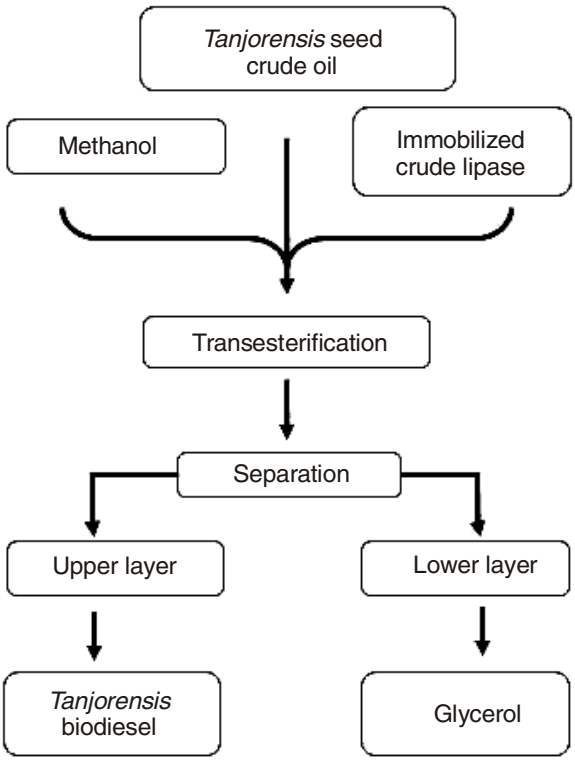

Fig. 1. Enzymatic biodiesel production

Oil content: The lipid content was determined in the oil extracted from 10 g of seed powder. Results are presented in Table-1 as the oil percentage present in seed powder.

Acid value, % free fatty acid: To 1 g of oil, 20 mL of neutral ethanol was pipette out into a 250 mL flask, mixed

well and added 3 drops of 0.5 % *m*-cresol purple indicator and this solution mixture was titrated against 0.05 N standard KOH solution. The end point is determined when purple colour appeared. The amount of KOH required for neutralizing the free fatty acid of the given sample is then calculated (ASTM, 2003). Results are given in Table-1.

Iodine value: *Jatropha tanjorensis* oil (0.5 g) was dissolved in 10 mL of chloroform in a dry iodine flask. 30 mL of the Hanus iodine solution was burette out. It was allowed to stand in the dark for 30 min and was shaken occasionally. Meanwhile, 10 mL of 15 % KI was taken and diluted to 100 mL with distilled water in a conical flask. This was then quickly titrated against 0.1 N sodium thiosulphate, the liberated iodine was determined with the formation of pale yellow colour. Further, 3 mL of freshly prepared starch solution was titrated to the end point, which is the disappearance of blue colour. Titration was performed in triplicates including blank without oil (Table-1).

Saponification value: Determination of saponification value was according to MPOB test method [14]. In brief, 1.5 g of *Jatropha tanjorensis* seed oil was taken into a 200 mL conical flask to which 25 mL of 0.5 mol/L potassium hydroxide ethanol was added and a cooling pipe was fixed to the flask. This was then gently heated for 30 min with occasional shaking. Heat was adjusted so as to prevent back flow of ethanol. It was then immediately chilled and titrated against 0.5 mol/L HCl before the *Jatropha tanjorensis* seed oil gets solidified. Procedure repeated three times to obtain mean values of titration volume.

$$\text{Saponification value (mg/g)} = (\text{B-TV}) \times 1.006 \times \text{C} \times \text{U} \times \text{Sample size} \quad (1)$$

where, B = Blank, TV = Titration volume, C = concentration conversion coefficient, U = unit conversion coefficient (Table-1).

Peroxide value: Peroxide value was determined as per the procedure of Cox and Pearson [15]. To 1 g of oil, 1 g of potassium iodide and 20 mL of 2:1 glacial acetic acid:chloroform mixture was added into a clean dry tube. Tube contents were then vigorously boiled in a water bath for 30 s. Contents were then quickly transferred to a conical flask containing 20 mL of 5 % KI solution. This was then washed twice with 25 mL water and titrated against N/500 sodium thiosulphate solution till yellow colour quenches. Further to this, starch solution was added and shaken vigorously and titrated carefully till the blue colour disappears. A blank was also set at the same time.

Calculation

$$\text{Peroxide value (milliequivalent peroxide/kg sample)} = \text{S} \times \text{N} \times 1000/\text{Weight of sample (g)} \quad (2)$$

where, S = mL of $Na_2S_2O_3$ (Test-Blank) and N = normality of $Na_2S_2O_3$ (Table-1).

Density: Density of the oil was determined at 25 °C using distilled water as reference (Table-1).

Fatty acid compositions: Fatty acid composition of seed oil was estimated *via* Perkin Elmer Clarus 500 series GC-capillary column 5 % phenyl 95 % dimethylpolysiloxane (30 m × 0.25 mm × 0.50 µm) equipped with MS Mass Range of 40-450 amu and Electron energy of 70 ev. About 100 µL of crude oil thus obtained is mixed with 1mL hexane from which 1 µL was introduced into the GC. The injector and detector temperature was programmed at 280 °C with a flow rate of 1.0 mL/min. (Oven program: 70 °C (1 min) @ 8 °C/min to 150 °C (1 min) @ 8 °C/min to 280 °C (10 min). Helium was used as carrier gas. The peaks obtained were characterized and composition of the oil seed was determined by comparing the retention times with that of authentic standards, which was also analyzed under the same conditions. Results are given in Table-2 (Fig. 2).

RESULTS AND DISCUSSION

The presence of methanol and the by-product glycerol (hydrophilic) is insoluble in the oil, which easily gets adsorbed onto the surface of the immobilized lipase thereby inhibits its transesterification activity. To prevent the above mentioned inactivation of immobilized lipase, the ration was kept below the theoretical oil-alcohol stoichiometric ratio of 1:3.

Chemical and physical properties: The *Tanjorensis* seed dimensions almost resembles with seed dimensions of *Jatropha* species given in Table-3. From the above data it is also noted that the yield percentage of oil collected from *Tanjorensis* seed was 62.23 ± 0.11 %. The oil content of *Jatropha tanjorensis* seed was almost nearer to that of *Jatropha curcas* (63.16 ± 0.35) [16,17]. This is highest when compared to linseed (33.33 %), soybean (18.35 %) and palm kernel oil (44.6 %) [18]. High level of oil in *Jatropha tanjorensis* possibly confirmed that it could be used as non-edible oil feedstock in oil industries. Currently, oil production from *Jatropha curcas* is 2000 L/ha oil per annum [19].

Table-4 presents the comparison of iodine value, Saponification value and density of oil obtained from *Tanjorensis* seed and other *Jatropha* species. A higher iodine value such as 92-104 indicates high level of unsaturation of fats and oils [20,21]. Europe's EN 14214 standard specification of iodine value for biodiesel is 120, a high iodine values of *Tanjorensis* seed oil could be because to the presence of high unsaturated fatty acids (oleic acid and linoleic acid) content, which could deposits or worsen the lubrication property [22]. High saponification value of *Tanjorensis* seed oil (180-200) indicated that oil contains triglycerides could be benefited for the manufacture of soaps. Present results indicate that *Tanjorensis* seed oil contains 0.82-1.98 % free fatty acid. The free fatty acid and moisture content can contribute in the transesterification of glycerides [23] and hence, determine the properties of biodiesel fuel.

Fatty acid composition: Each type of fatty acid and its quantity is yet another vital parameter that could determine the fuel nature of the oil under study. Physical characteristics of fatty acid and triglycerides are dependent on chain length and number of double bonds [24]. Fatty acid composition of the oil have its own vital role in some parameters of the biodiesel, like combustion quality (cetane number) and temperature load (cold flow) [25]. Generally triglyceride can be of saturated (Cn: 0) and unsaturated with one double bond (monounsaturated Cn: 1) or two or three double bonds (polyunsaturated comprising Cn: 2, 3). Table-5 gives the fatty acid composition comparison of *Jatropha tanjorensis* seed oil

TABLE-2
GC-MS ANALYSIS DATA ON *Tanjorensis* SEED OIL

S. No.	Peak name	m.f.	m.w.	Fatty acids	Retention time	Peak area	Peak area (%)
1	Decahydro-2,6-dimethyl-naphthalene	$C_{12}H_{22}$	166	Oleic acid	7.17, 7.98	1122827	1.5058
2	(E)-2-Decen-1-ol	$C_{10}H_{20}O$	156	Linoleic acid	7.38	664106	0.8906
3	Hexadecane	$C_{16}H_{34}$	226	Palmitic acid	10.81	686984	0.9213
4	2-Butyl-2-ethyl-5-methyl-3,4-hexadienal	$C_{13}H_{22}O$	194	Palmitic acid	11.95	260482	0.3493
5	2,6-*bis*(1,1-dimethylethyl)-4-methyl-methylcarbamate phenol	$C_{17}H_{27}NO_2$	277 (Terbucarb)	Linoleic acid	12.67	711420	0.9541
6	(Z)-3-Tetradecene	$C_{14}H_{28}$	196		13.22	200475	0.2689
7	2-methyl-decane	$C_{11}H_{24}$	156	Oleic acid	13.98	919652	1.2334
8	N-phenyl-benzenamine	$C_{12}H_{11}N$	169		14.72	342215	0.459
9	Oxalic acid allyl octadecyl ester	$C_{23}H_{42}O_4$	382	Oleic acid	15.35	73223	0.0982
10	2-Ethylhexyl ester benzoic acid	$C_{15}H_{22}O_2$	234		15.76	427127	0.5728
11	15-Methyl-methyl ester hexadecanoic acid,	$C_{18}H_{36}O_2$	284	Palmitoliate	15.82	149453	0.2004
12	Dicyclohexyl phosphine	$C_{12}H_{23}P$	198		16.44	143930	0.193
13	3,5-di-*tert*-Butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$	234		16.74	125019	0.1677
14	Eicosane	$C_{20}H_{42}$	282	Arachidic acid	16.83	518680	0.6956
15	methyl ester 10,13-Octadecadiynoic acid	$C_{19}H_{30}O_2$	290	Stearic acid	17.99	551532	0.7397
16	2,2a,4a,5,6,7-Hexahydro-2,2,4a-trimethyl-1*H*-cyclobuta[c]pentalen-5-one	$C_{13}H_{18}O$	190		18.08	321645	0.4314
17	Pivalate limonen-6-ol	$C_{15}H_{24}O_2$	236	Linoleic acid	18.15	1114325	1.4944
18	4b,5,6,7,8,8a,9,10-Octahydro-4b,8-dimethyl-2-isopropylphenanthrene	$C_{19}H_{28}$	256	Margaric acid	18.36	210466	0.2823
19	Methyl ester hexadecanoic acid	$C_{17}H_{34}O_2$	270	Palmitic acid, methyl ester	18.5	5286037	7.0892
20	Andrographolide	$C_{20}H_{30}O_5$	350		18.66, 18.86	5173585	6.9384
21	1,7,7-Trimethyl-3-phenethylidenebicyclo[2.2.1]heptan-2-one	$C_{18}H_{22}O$	254	Linolenic acid	18.99	421737	0.5656
22	6-Octyloctadecahydrochrysene	$C_{26}H_{46}$	358	Stearic acid	19.04	60353	0.0809
23	4-(4-Butylcyclohexyl)-4-butoxy-2,3-dicyanophenyl ester benzoic acid	$C_{29}H_{34}N_2O_3$	458	Linoleic acid	19.09	470539	0.6310
24	Methyl pimar-7-en-18-oate(phenanthrenecarboxaldehyde)	$C_{21}H_{34}O_2$	318	Linoleic acid	19.31	4765947	6.3917
25	1-(Methylsulfinyl)dodecane	$C_{13}H_{28}OS$	232	Oleic acid	19.41	1132621	1.5190
26	5-Methyl-2-(1-methyl-1-phenylethyl)-cyclohexanol	$C_{16}H_{24}O$	232	Palmitoliate	19.49	567016	0.7604
27	1,2,3,4,4a,9,10,10a-Octahydro-1,4a-dimethyl-7-(1-methylethyl)-[1S-(1à,4aà,10aá)]-1-phenanthrenecarboxaldehyde,	$C_{20}H_{28}O$	284	4-Epiabietal, dehydro-oleic acid	19.59, 20.07	8224866	11.0305
28	1,2,3,4,4a,9,10,10a-Octahydro-6-hydroxy-1,4a-dimethyl-[1S-(1à,4aà,10aá)]-1-phenanthrenecarboxaldehyde	$C_{17}H_{22}O_2$	258	Podocarpal, oleic acid	19.83	849280	1.139
29	10,18-Bisnorabieta-8,11,13-triene	$C_{18}H_{26}$	242	Linoleic acid	20.33	5403407	7.2466
30	Methyl ester (E,E)-9,12-octadecadienoic acid	$C_{19}H_{34}O_2$	294	Linolelaidic acid, methyl ester	20.6	7052463	9.4582
31	Methyl ester (Z)-9-octadecenoic acid	$C_{19}H_{36}O_2$	296	Oleic acid, methyl ester	20.67	6625911	8.8861
32	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	Myristic acid, methyl ester	20.94	2692484	3.6109
33	3,4'-Diisopropylbiphenyl	$C_{18}H_{22}$	238	Stearate	21.03	487378	0.6536
34	4'-Methoxy-2-hydroxystilbene	$C_{15}H_{14}O_2$	226	Oleic acid	21.21	1315308	1.764
35	1-Ethyl-4-methoxy-9H-pyrido[3,4-b]indole	$C_{14}H_{14}N_2O$	226	Linoleic acid	21.36	489128	0.656
36	Myristohydroxamic acid	$C_{14}H_{29}NO_2$	243	Myristic acid	21.44	350883	0.4706
37	Fluoranthene	$C_{16}H_{10}$	202		21.57	325350	0.4363
38	2-Methyl-6-(1-methyl-1-phenylethyl)phenol	$C_{16}H_{18}O$	226	Oleic acid	21.63	269106	0.3609
39	2,3,5-Trimethyl-phenanthrene	$C_{17}H_{16}$	220	Oleic acid	21.82	240936	0.3231
40	N-Isobutyl-(2E,4Z)-octadienamide	$C_{12}H_{21}NO$	195	Oleic acid	21.99	605465	0.812
41	Methyl ester 2-oxo-hexadecanoic acid	$C_{17}H_{32}O_3$	284	Palmitate	22.13	1124994	1.5088
42	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$	282		22.37	622637	0.835
43	4,6,2',6'-Tetramethyl-biphenyl-2,4'-diamine	$C_{16}H_{20}N_2$	240	Oleic acid	22.63	390129	0.5232
44	N,N-Diethyl-dodecanamide	$C_{16}H_{33}NO$	255	Diethyllauramide oleic acid	23.54	2132789	2.8603
45	Dehydroabietic acid methyl ester	$C_{21}H_{30}O_2$	314	Stearic acid	23.65	848617	1.1381
46	3,4-Dihydro-2-(3-methyl-2-thenylmethylene)-naphthalen-1(2*H*)-one	$C_{16}H_{14}OS$	254	Oleic acid	23.98	552549	0.741
47	Linoleoyl chloride	$C_{18}H_{31}Ocl$	298	Linoleic acid	24.23	4188849	5.6177
48	Podocarp-12-en-14-ol	$C_{17}H_{28}O$	248	Oleic acid	26.32	1100412	1.4758
49	*cis*-(2-Phenyl-1,3-dioxolan-4-yl)methyl ester-9-octadecenoic acid	$C_{28}H_{44}O_4$	444	Oleic acid	26.44	2250297	3.0179

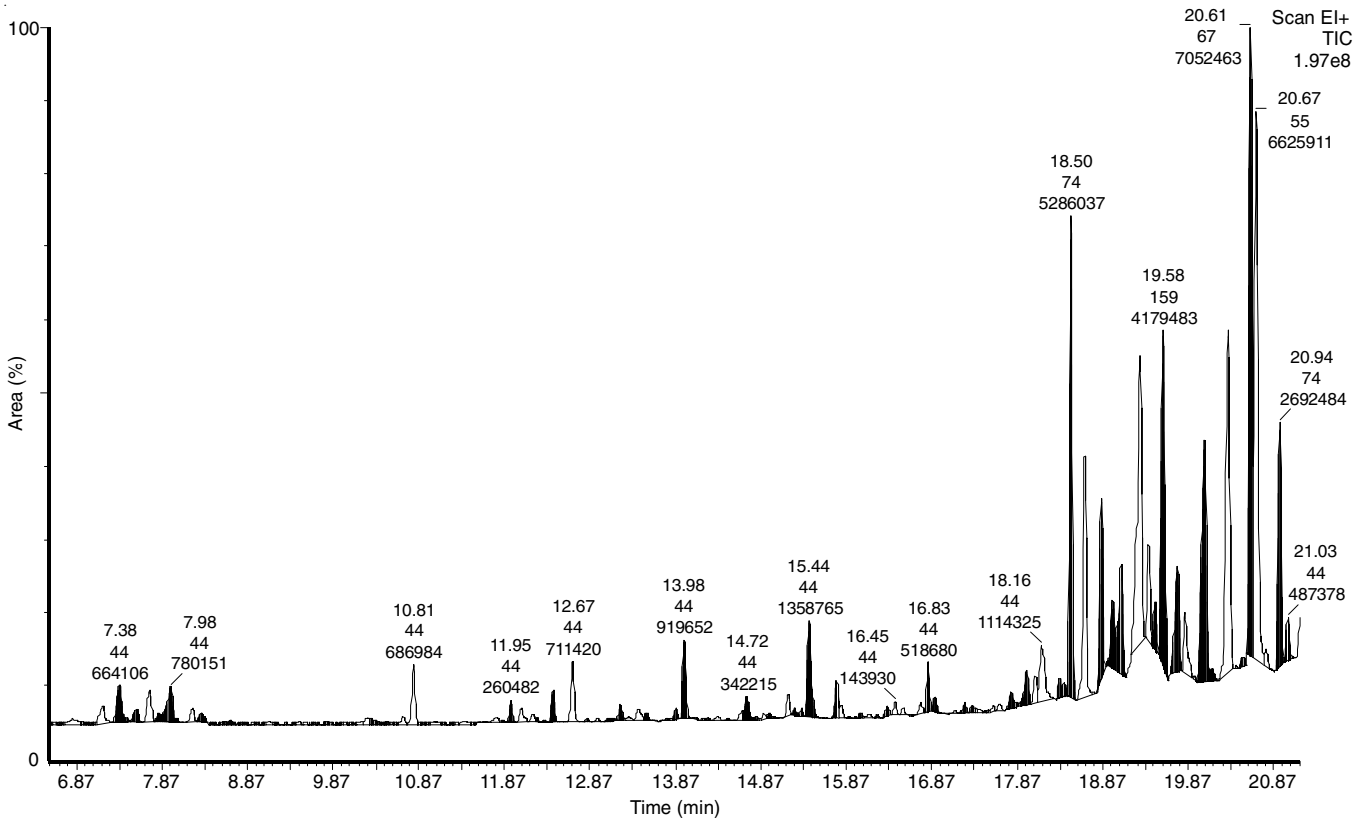

Fig. 2. GC-MS chromatogram of *Jatropha tanjorensis* seed oil

TABLE-3
COMPARISON OF SEED CHARACTERISTICS AND OIL YIELD IN *Jatropha* SPECIES

Jatropha species	Length ± SD (cm)	Width ± SD (cm)	Weight ± SD (g)	Oil yield % ± SD (n = 5)
Jatropha tanjorensis	1.5 ± 0.2	0.8 ± 0.1	0.55 ± 0.1	62.23 ± 0.11
Jatropha elbae	1.4 ± 0.1	1.3 ± 0.1	1.29 ± 0.3	55.25 ± 0.80 [Ref. 27]
Jatropha andrieuxii	0.9 ± 0.1	0.6 ± 0.1	0.07 ± 0.0	39.77 ± 1.15[Ref. 27]
Jatropha rzedowskii	0.9 ± 0.2	0.9 ± 0.2	0.41 ± 0.06	47.69 ± 2.20 [Ref. 27]
Jatropha curcas	1.7 ± 0.1	0.8 ± 0.1	0.54 ± 0.09	63.16 ± 0.35 [Ref. 26]

TABLE-4
CHEMICAL AND PHYSICAL PROPERTIES

Characterization	*Jatropha tanjorensis*	*Jatropha curcas* [Ref. 26]	*Jatropha andrieuxii* [Ref. 27]	*Jatropha elbae* [Ref. 27]	*Jatropha rzedowskii* [Ref. 27]
Free fatty acid as oleic acid (%)	0.82-1.98	0.92-6.16	0.3 ± 0.054	0.3 ± 0.047	0.3 ± 0.09
Iodine value	92-104	89-112	92.56 ± 14.36	76.11 ± 6.0	88.55 ± 6.85
Saponification value	180-200	188-209	202.5 ± 9.36	192.1 ± 6.82	193.6 ± 1.23
Density at 20 °C (g/mL)	0.812-0.899	0.860-0.933	0.922 ± 0.005	0.93 ± 0.004	0.929 ± 0.006

with other plant seed oils and with other mentioned *Jatropha species* [26,27]. Preferably plant oil supposed to have low level saturation and low polyunsaturation [28]. Soybean, sunflower oils are rich in polyunsaturated acids (linoleic acid and linolenic acid) (Table-2) and are liable to generate methyl ester fuels having less oxidation stability. Plant seed oils with a unsaturated fatty acids (*e.g.*, palm oil) generally have a higher freezing point and at low temperatures this makes a poor flow characteristic [28]. European standard has set a definite level of linolenic acid and other fatty acids containing four double bonds in their fatty acid methyl esters (FAMEs), which must not cross the level of 12 % and 1 % respectively. *Tanjorensis* seed oil contain 0.6 % linolenic acid and it is lower to that of soybean oil (Table-3) and is within the limit prescribed by FAME.

The principal fatty acid in *Tanjorensis* oil consists of saturated fatty acid of 21.6 %, monounsaturated fatty acids of 37 % and polyunsaturated fatty acid of 34 %. Monounsaturation of *Tanjorensis* seed oil is much greater than *Jatropha curcas* and other plant seed oils. The major fatty acid compositions of *Jatropha* seed oil are oleic acid, linoleic acid, palmitic acid, myristic acid and the stearic acid. Oleic acid with 37 % was of maximum percentage followed by 33 % of linoleic acid. Thus, *Tanjorensis* seed oil can be called as oleic-linoleic oil. When compare to others plant seed oils (Table-2), *Jatropha tanjorensis* seed oil has higher oleic acid content than other mentioned plant oils. *Tanjorensis* seed oil consists of lower level of 0.6 % linolenic acid when compared to soyabean oil (Table-5).

TABLE-5
COMPARATIVE PROPERTIES OF CRUDE OILS OF *Jatropha* SPECIES

Fatty acid	*Jatropha tanjorensis*	Palm kernel oil [Ref. 29]	Sunflower oil [Ref. 29]	Soybean oil [Ref. 29]	Palm oil [Ref. 29]	*Jatropha curcas* [Ref. 26]	*Jatropha andrieuxii* [Ref. 27]	*Jatropha elbae* [Ref. 27]	*Jatropha rzedowskii* [Ref. 27]
Oleic 18:1	37.0	15.4	21.1	23.4	39.2	37-49	24.0	26.0	22.0
Linoleic 18:2	33.0	2.4	66.2	53.2	10.1	35-44	38.0	34.0	48.0
Palmitic 16:0	11.0	8.4	–	11.0	44.0	10-15	7.0	10.0	12.0
Stearic 18:0	2.6	2.4	4.5	4.0	4.5	2-9	5.0	7.0	7.0
Palmitoliec 16:1	1.0	–	–	–	–	0.9-1.5	–	–	2.0
Linolenic 18:3	0.6	–	–	7.8	0.4	0.2			
Arachidic 20:0	0.7	0.1	0.3	–	–	0.2			
Margaric 17:0	0.3	–	–	–	–	0.1			
Myristic 14:0	4.1	16.3	–	0.1	1.1	0.1			
Caproic 6:0	–	0.2	–	–	–	–			
Caprylic 8:0	–	3.3	–	–	–	–			
Lauric 12:0	–	47.8	–	–	0.2	–			
Capric 10:0	–	3.5	–	–	–	–			
Saturated	17.1	82.1	11.3	15.1	49.9	21.6			
Monounsaturated	37.0	15.4	21.1	23.4	39.2	45.4			
Polyunsaturated	34.0	2.4	66.2	61.0	10.5	33.0			

The crude oil from *Jatropha tanjorensis* is comparable when compared to other three oils obtained from *Jatropha* species and with other vegetable oils (Table-5). This oil has showed much resembling features with *Jatropha curcas* from other part of world [25-27]. GC MS generated mass spectra of the *Tanjorensis* seed oil compositions were matched and assigned according to NIST 08 Mass Spectral Library. The crude oil from *Jatropha tanjorensis* had similar main components in similar proportions and resembled much with *Jatropha curcas* seed oil from Africa Asia and South America.

Conclusion

Physico-chemical properties and GC-MS data obtained from the seed oil of *Jatropha tanjorensis* presented. In this study suggested that this could be a potential biodiesel source as the seed oil composition revealed higher percentage of oleic acid, linoleic acid, palmitic acid and stearic acid. Presence of TAGs such as OLL and OOL and physicochemical properties such as iodine number and saponification value further suggested its biodiesel value. Also, this study shows the proficient transesterification of *Tanjorensis* oil is possible by *Pseudomonas aeruginosa* secreted extracellular lipases catalysis in presence of methanol as solvent. As this plant can be easily grown in barren conditions, tropical and subtropical regions across the developing world must be utilized for developing an ecofriendly biodiesel feedstock contributing to a healthy society.

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