Indian Journal of Experimental Biology
Vol. 50, July 2012, pp. 502-510

Biodiesel production from seed oil of *Cleome viscosa* L.

Rashmi Kumari[1, 2], Vinod Kumar Jain[1] & Sushil Kumar[*2]

[1] School of Environmental Sciences, Jawaharlal Nehru University (JNU) and [2]Genetic Genomics Laboratory, National Institute of Plant Genome Research (NIPGR), New Delhi 110 067, India

Received 21 November 2011; revised 9 April 2012

Edible oil seed crops, such as rapeseed, sunflower, soyabean and safflower and non-edible seed oil plantation crops Jatropha and Pongamia have proved to be internationally viable commercial sources of vegetable oils for biodiesel production. Considering the paucity of edible oils and unsustainability of arable land under perennial plantation of Jatropha and Pongamia in countries such as India, the prospects of seed oil producing *Cleome viscosa*, an annual wild short duration plant species of the Indogangetic plains, were evaluated for it to serve as a resource for biodiesel. The seeds of *C. viscosa* resourced from its natural populations growing in Rajasthan, Haryana and Delhi areas of Aravali range were solvent extracted to obtain the seed oil. The oil was observed to be similar in fatty acid composition to the non-edible oils of rubber, Jatropha and Pongamia plantation crops and soybean, sunflower, safflower, linseed and rapeseed edible oil plants in richness of unsaturated fatty acids. The Cleome oil shared the properties of viscosity, density, saponification and calorific values with the Jatropha and Pongamia oils, except that it was comparatively acidic. The *C. viscosa* biodiesel had the properties of standard biodiesel specified by ASTM and Indian Standard Bureau, except that it had low oxidation stability. It proved to be similar to Jatropha biodiesel except in cloud point, pour point, cold filter plugging point and oxidation stability. In view of the annual habit of species and biodiesel quality, it can be concluded that *C. viscosa* has prospects to be developed into a short-duration biodiesel crop.

Keywords*: Cleome viscosa* seed oil, Linoleic acid rich oil, Non edible biodiesel oil, Soybean/sunflower like oil

Fossil fuels have been the principal resource of energy for steering infrastructural and economic developments both in the developing and the developed world[1-3]. There has however been a depletion in fossil fuel reserves and a massive increase in fuel prices resulting in unequal availability of these resources between developing and developed nations of the world[1,3]. Total dependence on fossil fuels for energy requirements is no longer sustainable, and hence in the last few decades, research has been intensified for developing new renewable resources of energy[1,4.]

Among living organisms being explored as sources of renewable energy, two kinds of plant/microbial products seem promising: namely, alcohol produced as a product of fermentation from carbohydrates, and semi-synthesized biodiesel from vegetable oils[5,6]. Biodiesel production has been successfully experimented from both edible and non- edible vegetable oils[5,7-9]. For example, most oilseed crops including rapeseed, sunflower, soybean, safflower and cotton seed have been successfully used for biodiesel production [5,7,10]. Among non-edible oils, biodiesel has been produced from oils of *Jatropha curcas* and *Pongamia pinnata*[3,5]. *J. curcas* and *P. pinnata* plantations developed in some arable and non-arable land areas are already serving as bulk resources of non-edible oil for biodiesel production [5,11-13]. Experiments on many other plant seeds for production of biodiesel are actively going on[6,9]. However, presently biodiesel is produced mainly from oils of edible oilseed crops and from non-edible oilseed plantations.

In a densely populated country like India, urbanization and industrialization are eating up cultivable lands and as a result, edible oil is falling short in supply, necessitating large scale import[5,13,14]. The plantations of *J. curcas* and *P. pinnata* being perennial, any large scale cultivation of these non-edible oil plants in arable lands would negatively impact food security[5,11-13]. Under these circumstances, there is need for cultivation of alternate edible or non-edible seed oils as resource for biodiesel production, which fits into the crop rotation protocols practiced

*Corresponent author
Telephone: 91-11-26735177
Fax: 91-11-26741658
e-mail: sushil2000_01@yahoo.co.in
rashmikumarijnu@gmail.com
vkj0400@mail.jnu.ac.in

for intensive agriculture on arable lands. To meet the challenge of biodiesel production, one possibility is to increase the production of edible oils so that surplus quantities are spared for biodiesel production. Another possibility in sustainability terms is to cultivate new short duration crop plants for oils, which are economically suitable for conversion into biodiesel. Based on the latter supposition, attempts were made to explore some non-edible crop plants found in north and western India for biodiesel production.

A survey of the flora in Delhi region (India) identified several herbaceous plants whose seeds have been reported to be rich in oil[15-18]. Among these, the medicinal plant C*leome viscosa* has an oil whose fatty acid composition appears suitable for biodiesel production[17-27]. *C. viscosa*, a capparidaceae plant, is a short duration annually growing plant flourishing during the monsoon (rainy) season in India. It is found in the Aravali mountain ranges of north-western India encompassing parts of Gujarat, Rajasthan, Haryana and Delhi[16,28]. *C. viscosa* is also a casual weed plant found in many other semitropical/semitemparate regions of India[23,28-31]. Hitherto, *C. viscosa* seeds have served as a raw material for the extraction of coumarinolignoids, a valuable chemical entity needed by pharmaceutical industries for liver diseases and immunomodulation[21,22]. Under the extraction protocol of industrial production of coumarinolignoids from *C. viscosa,* the residue of the seeds oil is treated as disposable output. In view of the fatty acid composition of *C.viscosa* seed oil it was hypothesized that the oil could possibly be used for commercial production of biodiesel. The present study further explores the idea for utilization of industrial waste for biodiesel production. In this regard, (a) the seeds from natural population of *C. viscosa* found in the Aravali mountain range were used for oil extraction. After the preliminary characterization, the oil was converted into biodiesel; (b) the *C. viscosa* biodiesel was characterized and compared with *J. curcas* biodiesel as per the standards defined for biodiesel by institutions such as the Indian Standard Bureau (IS 15607) and American Society for Testing and Materials (ASTM D 6751).

Materials and Methods

In order to compare the quality of biodiesel of *C. viscosa* with other researched non-edible plant-based biodiesel, experiments were also conducted with *J. curcas*. The plant, *Cleome viscosa* was got identified at NISCAIR, New Delhi 110012 and plant specimen was deposited with ref. no. NISCAIR/RHMD/consult/-2011-12/1889/189.

Seed collection — For the seed collection, plants were sampled from the natural populations of *C. viscosa* growing among Aravali mountain ranges in the Indian states of Rajasthan, Haryana and Delhi. Plant populations growing in and around Jaipur, Ajmer, Faridabad and Delhi were sampled for mature pods. Pods were also collected from Nalanda district in the Bihar state of eastern India for the study of seed oil composition. The sample areas were visited many times between June and November in the year 2008. Bulk of the plants was observed to have grown in July after a few episodes of monsoon rain. These plants appeared to have completed their life cycle in about 13-15 weeks time. Altogether 35-40 kg of pods were collected from each of the sample sites. The pods were collected in muslin cloth bags, and dried-up by spreading them under the sun. Then the dried pods were stored in separate paper bags location-wise and according to the time of collection. Later, the pods, pooled location-wise, were thrashed to extract seeds. The seed yield varied between 22-26% of the fresh pod weight. Thus about 10-12 kg of dry seeds per sample-site became available for oil extraction.

Extraction of oil and biodiesel synthesis — First the seeds were mechanically crushed and oil was extracted in hexane using the Soxhlet apparatus. Oil was separated from the solvent by rotavapor at 65-70 ^{o}C. The percentage of oil was calculated in terms of the weights of seed and oil. Conversion of oil into bodiesel was carried out in a 300 mL reactor-flask equipped with a mechanical stirrer and reflux condenser, over a heating mantle at 65 ^{o}C, using 2% H_2SO_4[32,33]. A series of reactions were carried out at the same temperature and at same level of molar ratio of methanol and oil (40:1) for different time periods e.g. 5, 9, 13, 24 and 26 h[32,33]. The highest possible yield of biodiesel was obtained at 24 h. Aliquots of the reaction mixtures were collected at various intervals of time to check the completion process of biodiesel formation by a thin layer chromatography. After completion of the biodiesel formation process, glycerine was separated with the separating funnel. The biodiesel was then purified by washing with water and dried by rotavapour. The use of H_2SO_4 as a catalyst facilitated both transesterification and

esterification in the synthesis of biodiesel from the high acid value *C. viscosa* oil.

Characterization of oil and biodiesel—*C. viscosa* oil and its biodiesel were characterized by use of methods mentioned in Table 1 and 2. The properties of the biodiesel B100 biodiesel (neat biodiesel) were compared against the specifications for commercial biodiesel as described in ASTM - D 6751 and Indian Standard IS 15607: 2005 (Table 2).

Three methods were used to analyze the fatty acid composition of the oil from *C. viscosa* from the Delhi population. (a) AOAC 996.06, (b) IS :548 (Part iii)-1976 (Reaffirmed. 2000) and (c) ASTM methods. ASTM 2800 and ASTM 1983 were used for esterification of fatty acids and their gas chromatographic (GC) analysis respectively. The GC analysis deployed Chemito 8610HT equipment with polar packed SP-2340 (100% cyanoprophyl) column using flame ionization detector (FID) at 320 ^{o}C with oven at 150 ^{o}C to 250 ^{o}C by raising the temperature @ 6 ^{o}C/min for 20 min and using Helium as a carrier gas. The GC analysis, based on AOAC996.06 method, deployed Variant 3800 GC with FID, capillary column DB23 (50% cyanoprophyl and 50% methyl polysiloxane), injector temperature 225 ^{o}C, detector at 285 ^{o}C, oven at 100 ^{o}C, with rise in temperature @ 3 ^{o}C /min till 240 ^{o}C for 15 min and Helium as a carrier gas. The Indian Standard method was carried out on Agilent 6890 GLC with packed column of 5% DEGS (diethylene glycol succinate), with injector temperature at 220 ^{o}C, detector at 250 ^{o}C and isothermal condition at 170 ^{o}C using Nitrogen as a carrier gas.

Since the products of oil extraction by the three methods were found to be similar, subsequently ASTM method was routinely followed. All ^{1}H NMR analyses of oil and biodiesel were performed on a Bruker AV 300MHz 7.0 Tesla spectrometer at 24.85 ^{o}C, using a 5 mm inverse probe-head and spectral width 0.0 to 5 ppm. Sample(s) of 5% dilution (v/v) in deuterated chloroform ($CDCl_3$) solvent containing tetramethylsilane (TMS) as internal reference were used for analysis of ^{1}H-NMR. Mid-IR (FT-IR) spectra in the region between 4000–400 cm^{-1} were recorded on Perkin Elmer BX-2 FT-IR spectrophotometer equipped with deuterated

Table 1 — Properties of *C. viscosa* oils from three locations in the Indo-Gangetic plains

Serial no.	Parameter[a]	Unit	Oil from plant population of				Mean ± SD	Method and reference
			Delhi	Haryana	Rajasthan	Bihar		
1	Fatty acid	%						
	Palmitic acid (C16:0)		10	7	11	11	9.8 ± 1.9	ASTM D 1983 (1990)[b]
	Stearic acid (C18:0)		6	5	3	5	4.8 ± 1.3	
	Oleic acid (C18:1)		22	23	18	17	20.0 ± 2.9	ASTM D 2800 (1992)[b]
	Linoleic acid (C18:2)		62	65	68	67	65.5 ± 2.6	
	Saturated fatty acids		16	12	14	16	14.5 ± 1.9	
	Unsaturated fatty acids		84	88	86	84	85.5 ± 1.9	
2	Acid value	mgKOH/g	58.1	48.3	43.4	[c]	49.9 ± 7.5	ASTM D 664 (2006)
3	Viscosity at 40 ^{o}C	mm^2/sec	30	30	31		30.3 ± 0.6	ASTM D 445 (2006)
4	Density at 15 ^{o}C	g/cm^3	0.92	0.92	0.92		0.92 ± 0	ASTM D 4052 (2002)
5	Specific gravity at 15 ^{o}C	Ratio	0.92	0.92	0.92		0.92 ± 0	ASTM D 4052 (2002)
6	Refractive index at 20 ^{o}C	Ratio	1.47	1.47	1.47		1.47 ± 0	ASTM D 1218 (2002)
7	Carbon residue	% mass	0.46	0.48	0.50		0.48 ± 0.02	ASTM D 4530 (1993)
8	Lubricity	µm	92	92	139		107.7 ± 27.1	ISO 12156-1:(1997)
9	Saponification value	mgKOH/g	214	213	210		212.3 ± 2.1	ASTM D 94 (2002)
10	Calorific value	MJ/kg	39.5	39.6	39.6		39.6 ± 0.1	ASTM D240 (2002)

[a] Pooled oil of Delhi, Haryana and Rajasthan origins subjected to mass spectra analyses gave major peak of free fatty acid , diglyceride and triglyceride of linoleic acid at 280, 616 and 879 mass value respectively. Fourier transform infrared spectroscopy of the pooled oil revealed functional group at 1744.8cm^{-1} of C=O stretching of ester and at 1711.2 cm^{-1} of C=O stretching of free fatty acids. The Fourier transform nuclear magnetic resource spectra showed signal of ester, unsaturated protons, fatty acids having more than one double bond at 4.10-4.40, 5.0-5.4 and 2.81-2.84 ppm. These confirmed the predominance of unsaturated fatty acid, the linoleic acid.

[b] The results of fatty acid analyses of oils using Indian Standard (IS 15607: 2005), Association of Official Analytical Chemists (AOAC 996.06, 2005) and American Society for Testing and Materials (ASTM D 6751) were observed to be similar.

[c] not done.

Table 2 — A comparison of parameters of *C. viscosa* and *J. curcas* biodiesels, prepared in the study, with those specified by IS and ASTM for commercial biodiesel

S.No.	Parameter	Unit	*C. viscosa*[c]	*J. curcas*	IS 15607[(a)]	ASTM 6751[(b)]	Method and reference
1	Flash point (closed cup)	°C	173	144	≥ 120	≥ 93	ASTM D 93 (2006)
2	Kinematic viscosity at 40 °C	mm^2/sec	5.2	4.2	2.5-6.0	1.9-6.0	ASTM D 445 (2006)
3	Cloud point	°C	21.0	0.0	NR	NM	ASTM D 2500 (2005)
4	Oxidation stability	hours	1.0	4.0	≥ 6	≥ 3	EN 14112 (2003)
5	Carbon residue (100% sample)	% mass	0.04	0.03	≤ 0.05	≤ 0.05	ASTM D 4530 (1993)
6	Acid number	mgKOH/g	0.29	0.23	≤ 0.50	≤ 0.50	ASTM D 664 (2006)
7	Sulphur	% mass	0.0027	0.0007	≤ 0.0050	≤ 0.0015 (15 Grade) ≤ 0.05 (500 Grade)	ASTM D 5453 (1993)
8	Copper strip corrosion level	Number	1	1	≤ 1	≤ 3	ASTM D 130 (1994)
9	Cetane number		53.0	55.0	≥51	≥ 47	IP 498 (2004)
10	Free glycerine	% mass	0.0045	0.0012	≤ 0.02	≤ 0.02	ASTM 6584 (2000)
11	Total glycerine	-do-	0.0240	0.0048	≤ 0.25	≤ 0.24	ASTM 6584 (2000)
12	Ester content	-do-	96.5	97.0	96.5	NR	EN 14103 (2003)
13	Phosphorus content	-do-	< 10ppm	< 10ppm	≤ 0.001	≤ 0.001	ASTM D 5185 (97)
14	Sulfated ash	-do-	0.005	0	≤ 0.020	≤ 0.020	ASTM D 874 (1996)
15	Water and sediments	% vol	0; 0	0; 0	Water ≤ 0.05 (% weight) Total contaminants ≤ 0.0024(% weight)	≤ 0.05	ASTM D 2709 (1996)
16	Methanol content	% vol	0.0007	0.0003	≤ 0.20	≤ 0.20	EN 14110 (2003)
17	Ca+ and Mg, combined	ppm	<1	< 1	NM	≤5	ASTM D 5185 (97)
18	Na+ and K combined	ppm	<1	< 1	NM	≤ 5	ASTM D 5185 (97)
19	Density at 15 °C	g/cm^3	0.89	0.88	0.86- 0.90	NR	ASTM D 4052 (2002)
20	Iodine value	Number	116.3	91.3	NM	NR	[47]
21	Refractive index at 20 °C	Ratio	1.46	1.45	NR	NR	ASTM D 1218 (2002)
22	ASTM colour value	Number	<2	<2	NR	NR	ASTM D 1500(2004)
23	Specific density at 15 °C	ratio	0.89	0.88	NR	NR	ASTM D 4052 (2002
24	Calorific value	MJ/kg	39.9	39.5	NR	NR	ASTM D240 (2002)
25	Cold filter plugging point (CFPP)	°C	+ 9	-1	NR	NR	ASTM D 6371 (2005)
26	Lubricity	µm	204	212	NR	NR	ISO 12156-1,1997)
27	Fatty acid composition	%					
	C16:0		8.1	12.6	NR	NR	ASTM D 1983(2002)
	C18:0		4.5	3.6			
	C18:1		19.9	38.2			
	C18:2		67.4	45.5			
28	Pour point	°C	+ 3	-3	NR	NR	ASTM D 97 (2005)

[a] Indian standard (IS) 2005;
[b] ASTM (2009); [c]FT-NMR spectra of biodiesel showed the shift of glycerol $–CH_2OH$ signal from δ4.2 ppm of oil to $–OCH_3$ signal at δ 3.65 ppm. FT-IR spectral analyses showed esters bands at 1196.7cm^{-1}, 143.0cm^{-1} and 1743.1cm^{-1}. The mass spectral peak at 294 was for methyl esters of linoleic acid. FT-IR,NMR and mass spectra confirmed conversion of oil into biodiesel.
NR = Not reported in Specification by ASTM and IS;
NM = Not mentioned in the ASTM and IS specification

triglycerine sulphate (DTGS) detector at a resolution of $4cm^{-1}$; 50 scans were collected to obtain the average value. Micromass autospec ultima mass spectrometer instrument was used to analyze the composition of the oil and biodiesel based on field desorption mass spectrometry.

Dichloromethane solvent was used to prepare a 20% solution of oil and biodiesel. An aliquot (2 µL) of this solution was coated on the emitter wire and desorbed by applying electricity to it in the ionization chamber. The mass to charge ratio of all the compounds of the sample were measured in magnetic chamber and amplified by photon multiplier tube. Spectra of amplified charge to mass ratio were recorded into software. GPC analysis was carried out on Waters 515 HPLC equipment fitted with UV and RI detector. Stainless steel PL-gel column of 60 cm × 7.5 cm having pore size of 100Å was used with tetrahydrofuran as the mobile phase at a flow rate of 1mL/min. The sample was then passed through Rheodyne injector using 20µL loop. The chromatographic data of percentage of fats and esters in biodiesel and oil were processed through Millennium 32 HPLC software.

Procedures for Jatropha curcas—Seeds of *J. curcas* were collected from trees cultivated in the farm of the National Institute of Plant Genome Research, New Delhi. Conversion of biodiesel was carried out by using 6:1 molar ratio of methanol and oil but with the use of 0.5% NaOH as a catalyst[34].

The procedure for physico-chemical characterization of *J. curcas* was the same as in case of *C. viscosa*.

General—This study is based primarily on two important considerations, viz (i) development of short duration non-edible seed oil crops for biodiesel production is possible, and (ii) the unsaturated fatty acid rich oil of the short duration wild plant species *C. viscosa* is a suitable candidate for the desired development of a non-edible oilseed crop for biodiesel production. The second part of the theory has been experimentally tested in the present study.

Results

Quality of C. viscosa seed oil—As reported earlier, *C. viscosa* oil was extracted from the seeds collected from several locations in the Indian states of Delhi, Haryana, Rajasthan and Bihar. The oil content of the solvent-extracted oil in the seeds accessed from these four states was estimated as 24, 22, 23 and 21%, respectively. The quality of oil was investigated and analyzed in terms of their fatty acid composition and other physico-chemical properties. The fatty acid composition of the oil extracted from the Delhi population proved to be similar in all the three methods of analysis standardized by Indian Standard, AOAC and ASTM (Table 1). The Delhi oil sample contained unsaturated fatty acids and saturated fatty acids in 84 and 16% concentration, respectively. The main unsaturated fatty acids found were linoleic acid (62%) and oleic acid (22%). The concentrations of saturated fatty acids were: 10% palmitic acid and 6% stearic acid. The oils obtained from the seeds of Rajasthan and Haryana populations were of relatively better quality than the oils obtained from seeds of Delhi and Bihar because of the relatively higher concentration of unsaturated fatty acid in the former oil (Table 1). In the four samples of oils, the average concentration (%) of the linoleic, oleic, palmitic and stearic acids were 65.5, 20.0, 9.8 and 4.8, respectively. The physico-chemical properties of the oils extracted from the seeds of Delhi, Haryana and Rajasthan origins (Table 1) were generally similar except that lubricity in the Rajasthan oil sample was higher. The average values for acidity, viscosity, density (g/cm^3), specific gravity, refractive index, carbon residue, lubricity (µm), saponification (mg KOH/g) and calorific (MJ/kg) in each case were 49.9, 30.3, 0.92, 0.92, 1.47, 0.48, 107.7, 212.3 and 39.6, respectively. The results of FT-NMR, FT-IR and mass analyses are reported in Table 1. These observations confirm that the *C. viscosa* seed oil is rich in unsaturated fatty acid, free fatty acid and linoleic acid.

Comparison of properties of C. viscosa oil with other oils—The fatty acid composition of *C. viscosa* oil was compared with 13 other vegetable oils that are being resourced for biodiesel production (Table 3). These 14 oils roughly fall into three groups. One group comprises *Calophyllum inophyllum, Pongamia pinnata, Madhuca longifolia, Azadirachta indica*, *Gossypium hirsutum* and *Hibiscus sabdariffa* in which the concentration of unsaturated fatty acid is less than 75%. The second group comprises *C. viscosa, Ficus elastica, J. curcas* and *Glycine max* in which the concentration of unsaturated fatty acids is between 80 - 86%. The third group comprises *Helianthus annus, Carthmus tinctorius, Linum usitatissimum,* and *Brassica napus* which contain unsaturated fatty acids in concentrations ≥ 90%. In all the members of the

Table 3 — Comparison of physicochemical properties of *Cleome viscosa* oil with that of other biodiesel resource plants

Characteristic	Unit	Common and botanical name of resource plant															Mean ± SD	Reference(s)
		Wild mustard	Soybean[c]	Sunflower[d]	Safflower	Linseed[e]	Rapeseed	Cotton seed	Roselle	Polanga	Rubber	Neem[f,g]	Pongamia	Jatropha	Mahua[f]			
		Cleome viscosa,[a,b]	*Glycine max*	*Helianthus annuus*	*Carthamus tinctorius*	*Linum usitatissimum*	*Brassica napus*	*Gossypium hirsutum*	*Hibiscus sabdariffa*	*Calophyllum inophyllum*	*Ficus elastica*	*Azadirachta indica*	*Pongamia pinnata*	*Jatropha Curcas* b	*Madhuca longifolia*			
Fattyacid	%																	
C16:0		9.3	13.9	6.4	7.3	5.1	3.5	28.7	18.2	12	10.2	14.9	11.7	12.6	16.0	22.1	12.7±6.9	[4,8,35-38]
C18:0		4.7	2.1	2.9	1.9	2.5	0.9	0.9	4.1	13	8.7	19.2	7.5	6.6	6.5	22.6	7±6.8	
C18:1		21.0	23.2	17.7	13.6	18.9	64.1	13.0	33.3	34.1	24.6	55.5	51.6	49.8	43.5	1.7	30±18.3	
C18:2		65.0	56.2	72.9	77.2	18.1	22.3	57.4	38.2	38.3	39.6	9.1	16.5	31.0	34.4	46.0	42±21.5	
C18:3		ND	4.3	0	0	55.1	8.2	0	2.1	0.3	16.3	NR	2.7	ND	0.80	11.3	8.4±15.6	
Saturated fatty acid	%	14.0	16.0	9.3	9.2	7.6	4.4	29.6	22.3	25	18.9	37.6	19.2	19.2	22.5	44.7	19.9±11.5	
Unsaturated fatty acid		86.0	84.0	90.7	90.8	92.4	94.6	70.4	73.6	72.7	80.5	64.6	70.8	80.8	78.7	59.0	79.3±11.1	
Acid value	mgKOH/g	49.9	0.2	0.2	0.7	0.2	1.1	0.1	1.3[h]	44	34	52	5.1	5.4	3.8	38.0	16.5±21.4	[4,10,36-39]
Viscosity at 40 °C	mm^2/sec	30.3	32.9	32.6	31.2[i]	25.8	35.1	33.5	36.4	72	66.2	44	27.8	33.0	18.2[j]	24.6	37±14.5	[4,8,36-42]
Density at 15 °C	g/cm^3	0.92	0.92	0.92	0.92	0.92[k]	0.92	0.92[k]	0.92	NR	NR	0.92[k]	0.94[k]	0.92	0.94[k]	0.96	0.93±0.01	[8,10,36,37,39-42]
Calorific value	MJ/kg	39.6	39.6	39.6	39.5	39.3	39.7	39.5	NR	39.3	37.5	34.1	34.0	ND	38.5	36	38.2±2.1	[4,10,36,38,39]
Saponification value	mgKOH/g	212.3	220.8	191.7	190.2	188.7	197.1	207.7	126.2	NR	194.0	209.7	188.5	215.0	200.8	190.5	194.3±23.1	[8,35,43,44]
Oil content	%	23[l], 25-37 [m](31)[n]	15-20 (18)	25-45 (35)	20-35 (28)	40-44 (42)	38-46 (42)	18-25 (22)	19	NR	40-50 (45)	40-50 (45)	27-39 (33)	30	30-40 (35)	35-42 (39)	32.5±9.1	[8,38,42,45,46]

[a] Average of Delhi, Haryana, Rajasthan populations; [b] This study; [c]Soybean oil contains 0.3% C16:1;[d] Sunflower oil contains 0.1% C16:1; [e] Linseed oil contains 0.3% C16:1; [f]Average arrived at from the reported ranges of fatty acids; [g] Neem oil contains 1.4% C14:0 and 2.1% C20:0; [h] Acid value (2x 0.67) [39] ; [i] Average value of viscosity; [j] 34.0 [8]; [k] temperature not reported ; [l] mean of estimation on seeds from populations growing in Delhi, Haryana, and Rajasthan; [m] The range reported in literature; [n]The value in parentheses are averages ; ND – Not detected; NR – Not reported

second group, except *J. curcas*, the major fatty acid was linoleic acid. In *J. curcas*, oleic acid occurred at a higher concentration than linoleic acid. The oils of *F. elastica*, *B. napus* and *M. longifolia* possess linolenic acid concentration of 16.3, 8.2 and 11.3% respectively, sharing the property of linolenic acid richness with that of *L. usitatissium* oil.

Physico-chemical properties—The physico-chemical properties of *C. viscosa* oil were compared with those reported for 13 of the oils listed in Table 3. The comparative measures of viscosity (cSt), acid value (mgKOH/g), calorific value (MJ/kg), density (g/cm^3) and saponification value (mgKOH/g) for each are given in the Table 3. It was found that *C. viscosa* oil was similar to all the other oils in terms of calorific value, density and saponification properties. The *C. viscosa* oil was highly acidic like the non-edible oils of *C. inophyllum*, *F. elastica*, *M. longifolia* and *A. indica* used as resource for semisynthesis of biodiesel. In this respect soybean, sunflower and rapeseed edible oils and *P. pinnata* and *J. curcas* non-edible plantation oils have low acid value compared to *C. viscosa*. In terms of viscosity, *the C. viscosa* oil is like all the other oils except that the oils of *C. inophyllum* and *F. elastica* have very high viscosity.

Cleome viscosa biodiesel synthesis—The percentage yield of biodiesel was estimated to be 95% in terms of total weight of biodiesel and oil. This estimate was confirmed by the presence of esters content of 96.5% by the method EN 14103. Gel permeation chromatography (GPC) process further confirms the conversion rate from oil to biodiesel at 97%. The values of FT-NMR, FT-IR and mass spectra as reported in Table 1 and 2, also confirm the feasibility of conversion of the *C.viscosa* oil into biodiesel. The ester content of the biodiesel in a parallel experiment with the *Jatropha curcas* was found to be 97% (Table 2), a value which is also confirmed by measurement with GPC at 99%.

Comparative properties of biodiesels obtained from oils of C. viscosa and J. curcas—The *C. viscosa* and *J. curcas* biodiesels prepared during the present study were characterized for 28 properties and their observations are summarized in Table 2. The corresponding biodiesel critical parameters specified by ASTM and Indian Standards are also given in Table 2. Except for oxidation stability, the *C. viscosa* and *J. curcas* biodiesels fulfill the prescribed standards in respect of parameters such as flash point, kinematic viscosity, carbon residue, cetane number, free and total glycerine, esters content, phosphorus, sulfated ash and methanol contents, elemental contamination (Ca, Mg, Na, K and P), density, water and sediment contents. Among biodiesels, *J. curcas* biodiesel is found to be superior to *C. viscosa* biodiesel in terms of cloud point, pour point, cold filter plugging point and oxidation stability. The two biodiesels were similar in terms of calorific value, copper strip corrosion, refractive index, lubricity, content of fats, esters, water and sediments, ASTM colour value, and combined elemental contamination (Ca, Mg, Na, K and P content).

Discussion

The results show that the oil content of *C. viscosa* seed is about 23% comparaed to 30-33% in the seeds of *J. curcas* and *P. pinnata* and 18- 45% in edible oilseeds (Table 3). Further, the *C. viscosa* oil is similar in its richness of linoleic acid compared to the edible oil of sunflower and in its richness of unsaturated fatty acid to the oils of the rubber plant, *J. curcas*, soybean, sunflower, safflower, linseed and rapeseed. The biodiesel produced from the oil of *C. viscosa* fulfills the ASTM and Indian Standard requirements for vegetable oil-based biodiesels except in terms of oxidation stability. The *C. viscosa* biodiesel is largely similar to *J. curcas* biodiesel and shares with it the property of oxidation instability. Since *C. viscosa* plant population grows naturally in non-agricultural and degraded soils that occur in the Aravali mountain range, the present study indicates immense potential for development of *C. viscosa* as a short duration biodiesel crop of the rainy season. It is visualized that domestication of *C.viscosa* may lead to selection of lines with increased yield of seeds with higher levels of oil content. Work in this direction too is in progress in our laboratory.

In summery, the evaluation of the oil of *C. viscosa* population growing in the Aravali range of the Indo-Gangetic plains reveals that *C. viscosa* oil is rich in unsaturated fatty acids (86%). Also being rich in linoleic acid (65%), it is highly similar to sunflower oil. The *C. viscosa* oil shares the properties of viscosity, density, calorificity and saponification with Jatropha and Pongamia oils and differs from these oils in possessing a higher acid value. The biodiesel of *C. viscosa* fulfills the ASTM and IS specifications. It is comparable to *J. curcas* biodiesel in 24 out of 28 properties tested. It is marginally inferior to *J. curcas* biodiesel in cloud point, pour point and cold

filter plugging point, cold filter plugging point and oxidation stability. However, except for oxidation stability, other parameters are not mentioned as requirements in both the ASTM and IS specifications (Table 2). Almost all biodiesel derived from vegetable oils have poor oxidation stability[48]. Further work is required for the improvement of oxidation stability of biodiesel via treatment with antioxidant reagents[49]. The oil cake of *C.viscosa* could possibly be used as cattle feed and agricultural fertilizer and for the production of energy[50-52]. The oil cake of *C.viscosa* is considered safer than that of *J.curcas* which is poisonous[53] in view of the seeds of *C.viscosa* serving as traditional condiment, ingredient in traditional medicine and source of pharmaceuticals[18,21-24,]. Based on the above results, *C. viscosa* appears to be a potential short duration plant resource of non-edible seed oil for synthesis of biodiesel. It however, requires to be improved for oil yield by application of plant breeding procedures. *C. viscosa* may develop into a crop plant in future.

Acknowledgement

Grateful thanks are due to the Vice-Chancellor of JNU and Director(s) of NIPGR and Indian Oil Corporation (IOC, Faridabad, Haryana) for allowing the use of their institutional facilities, Director of the National Botanical Research Institute for the seeds of an improved accession of *J. curcas*, to S.K. Puri and Ravindra Kumar of IOC for their helpful advice in chemical analysis, Ajay Kumar for his proof reading help and laboratory colleagues for their cooperation and Vishakha Sharma and Anshika Tyagi for their help in the processing of the manuscript. The paper describes a part of the research for Ph.D degree of Jawaharlal Nehru University by RK, under the supervision of VKJ and SK.

References

1 Asif M & Muneer T, Energy supply, its demand and security issues for developed and emerging economies, *Renew Sust Energ Rev*, 11 (2007) 1388.

2 Gui M M, Lee K T & Bhatia S, Feasibility of edible oil versus non-edible oil versus waste edible oil as biodiesel feedstock, *Energy*, 33 (2008) 1646.

3 Medina I O, Garcia F E, Farfan J N & Figueroa M S, Does biodiesel from *Jatropha curcas* represent a sustainable alternative energy source?, *Sustainability*, 1 (2009) 1035.

4 Sahoo P K & Das L M, Process optimization for biodiesel production from Jatropha, Karanja and Polanga oils, *Fuel*, 88 (2009) 1588.

5 Biwas P K, Pohit S & Kumar R, Biodiesel from Jatropha: Can India meet the 20% blending target?, *Energ Policy*, 38 (2010) 1477.

6 Demirbas A, The importance of bioethanol and biodiesel from biomass, *Energ Source*, 3 (2008) 177.

7 Demirbas A, Importance of biodiesel as transportation fuel, *Energ Policy*, 35 (2007) 4661.

8 Karmakar A, Karmakar S & Mukherjee S, Properties of various plants and animals feedstocks for biodiesel production, *Bioresource Technol*, 101 (2010) 7201.

9 Pinto A C, Guarieiro L L N, Rezende M J C, Ribeiro N M, Torres E A, Lopes W A, Pereira P A de P & Andrade J B de, Biodiesel: An overview, *Brazilian Chem Soc,* 16 (2005) 1313.

10 Goering C E, Schwab A W, Daugherty M J, Pryde E H & Heakin A J, Fuel properties of eleven vegetable oils, *Trans Am Soc Agric Eng*, 25 (1982) 1472.

11 Achten W M J, Mathijs E, Verchot L, Singh V P, Aerts R & Muys B, Jatropha biodiesel fueling sustainability, *Biofuels Bioprod Bior*, 1 (2007) 283.

12 Kesari V & Rangan L, Development of *Pongamia pinnata* as an alternative biofuels crop – current status and scope of plantations in India, *Crop Sci Biotechnol,* 13 (2010) 127.

13 Openshaw K. A review of *Jatropha curcas*: An oil plant of unfulfilled promise, *Biomass Bioenerg*, 19 (2000) 1.

14 Patil P D, Gude V G & Deng S. Biodiesel production from *Jatropha curcas*, waste cooking, and *Camelina sativa* oils, *Ind Eng Chem Res,* 48 (2009) 10850.

15 Maheswari J K. *Illustrations to the flora of Delh*, 1st ed, (Council of Scientific and Industrial Research, New Delhi) 1966.

16 Maheswari J K. *The flora of Delhi*, 1st ed, (Council of Scientific and Industrial Research, New Delhi) 1976, 63.

17 Rukmini C, Chemical, nutritional and toxicological evaluation of the seed oil of *Cleome viscosa, Indian Med Res*, 67 (1978) 604.

18 Anonymous, *The Wealth of India*, A Dictionary of Indian Raw Materials and Industrial products, Vol. II, (Council of Scientific and Industrial Research, New Delhi) 1950, 231.

19 Aparadh V T & Karadge B A, Fatty acid composition of seed oil from some Cleome species, *Pharmacognosy J,* 2 (2009) 324.

20 Ayyanar M & Ignacimuthu S, Herbal medicines for wound healing among tribal people in Southern India: ethanobotanical and scientific evidences, *Int Applied Res Natl Products*, 2 (2009) 29.

21 Chattopadhyay S K, Srivastava S, Negi A P, Gupta A & Khanuja S P S, Hepatoprotective pharmaceutical composition comprising a mixture of coumarinolignoids process for preparation thereof, *United States Patent* 2004, US patent number 0191343A1, 30 September 2004.

22 Khanuja S P S, Pal A, Chattopadhyay S K, Darokar M P, Patel R P, Gupta A K, Negi A S, Kaur T, Tandon S, Kahol A P & Garg A, Immunomodulatory pharmaceutical composition for preparation thereof, *United states Patent* 2007, US patent number 0258989A1**,** 8 November 2007.

23 Maikhuri R K, Semwal RI, Rao KS, Nautiyal S & Saxena K G, *Cleome viscosa*, Capparidaceae: A weed or a cash crop?, *Econ Bot* , 54 (2002) 150.

24 Mali R G, *Cleome viscosa* (wild mustard): A review on ethnobotany, phytochemistry, and pharmacology, *Pharm Biol*, 48 (2010) 105.

25 Azam M.M, Waris A & Nahar N M, Suitability of some wildly grown seed oils for use as biodiesel, *Energ Source Part A: Recovery, utilization and environmental effects*, 32 (2010) 657.

26 Rao R P, Azeemoddin G, Ramayya D A, Rao S D T, Devi K S, Pantulu A J & Lakshminarayana G, Analysis and processing of *Cleome viscosa* seed and oil, *Fette, Seifen, Anstrichmittel,* 82 (1980) 119.

27 Williams L A D, Vasques E, Reid W, Porter R & Kraus W, Biological activities of an extract from *Cleome viscosa* L. (Capparaceae), *Naturwissenchaften*, 90 (2003) 468.

28 Bhandari M M, *Flora of the Indian Deseart*. 1st ed. (Scientific Publisher, Jodhpur) 1978, 42.

29 Duthie J F, *Flora of the upper Gangetic plain and of the adjacent Siwalik and Sub-Himalayan tracts*. 1st ed. Vol. 1 (Part i-ii) (M/s. Bishen Singh Mahendra Pal Singh Dehradun) 1973, 50.

30 Oommachan M, *The flora of Bhopal*. 1st ed. (J.K. Jain Brothers, Bhopal) 1977, 46.

31 Roxburgh W, *Flora Indica or description of Indian plants*. 1st ed. (Today and Tomorrow, New Delhi) 1832

32 Canakci M & Gerpen J V, Biodiesel production via acid catalysis, *Trans Am Soc Agri Eng (ASAE),* 42 (1999) 1203.

33 Formo M W, Ester reactions of fatty materials, *Am Oil Chemists' Soc*, 31 (1954) 548.

34 Freedman B, Pryde E H & Mounts TL, Varibles affecting the yields of fatty esters from transesterified vegetable oils, *Am Oil Chemists' Soc*, 61 (1984) 1638.

35 Demirbas A, Chemical and fuel properties of seventeen vegetable oils, *Energy Source*, 25 (2003) 721.

36 Ghage S V & Raheman H, Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids, *Biomass Bioenerg*, 28 (2005) 601.

37 Nakpong P & Wootthikanokkan S, Roselle (*Hisbiscus sabdriffa* L.) oil as an alternative feedstock for biodiesel production in Thailand, *Fuel* 89 (2010) 1806.

38 Ramadhas A S, Jayaraj S & Muraleedharan C, Biodiesel production from high FFA rubber seed oil, *Fuel*, 84 (2005) 335.

39 Sekhar M C, Mamilla V R, Mallikarjun MV & Reddy KVK, Production of biodiesel from neem oil, *Int J Eng Studies*, 1 (2009) 295.

40 Alonso J S J, Sastre J A L, Aviia C R & Lopez E, A note on the combustion of blends of diesel of soya, sunflower and rapeseed vegetable oils in a light boiler, *Biomass Bioenerg*, 32 (2008) 880.

41 Lopez Sastre J A, Guijosa L & Snaz J M, Los aseites vegetales como combustibles ecolo gicos (Vegetable oils as environmentally friendly fuels), *Energy,* 21 (1995) 71.

42 Ogut H, Eryilmaz T & Oguz H, Some Safflower (*Carthamus tictorius* L.) cultivars produced ozellikerini comparative study of biodiesel fuel. 1, *National Oil Seed Plants and Biodiesel Symposium Samsun,* held on 28-31 May (Turkey) 2007, 11.

43 Bamgboye A I & Adejumo O I, Physiochemical properties of Roselle oil, *Nutrition Food Sci*, 40 (2010) 186.

44 Ikwuagwu O E, Ononogbu I C & Njoku O U, Production of biodiesel using rubber [*Hevea brasiliensis* (Kunth. Muell.)] seed oil, *Ind Crop Prod*, 12 (2000) 57.

45 Langstraat A, Characteristics and composition of vegetable oil-bearing materials, *Am Oil Chem Soc*, 53 (1976) 241.

46 Mohamed R, Fernandez J, Pineda M, & Aguilar M, Roselle (*Hibiscus sabdariffa*) seed oil is a rich source of γ-tocopherol, *Food Sci*, 72 (2007) S207.

47 Sarpal A S, Kapur G S, Mukherjee S, Jayaparkas K C & Jain S K, Determination of iodine value of lubricating oils by nuclear magnetic resonance (NMR) spectroscopy, *Lubr Eng*, 51 (1995) 209.

48 Leung D Y C, Koo B C P & Guo Y, Degradation of biodiesel under different storage conditions, *Bioresource Technol*, 97 (2006) 250.

49 Dunn R O, Effect of antioxidants on the oxidative stability of methyl soyate (biodiesel), *Fuel Process Technol* 86 (2005) 1071.

50 Rukmini C & Deosthale Y G, Nutritive value of defatted seed cake of *Cleome viscosa*, *Am Oil Chem Soc,* 56 (1979) 503.

51 Chandra R, Vijay V K & Subbarao P M V, A study on biogas generation from non-edible oil seed cakes: potential and prospects in India, The 2nd joint international conference on sustainable energy and environment (SEE 2006), 21-23 Nov. 2006, Bangkok Thailand.

52 Balan V, Rogers C A, Chundawat S P S, Sousa L D C, Slininger P J, Gupta R & Dale B E, conversion of extracted oil cake fibers into bioethanol including DDGS, canola, sunflower,sesame, soy and peanut for integrated biodiesel processing, *J Am oil Chem Soc,* 86 (2009) 157.

53 Horiuchi t, Fujiki H, Hirota M, Suttajit M, Suganuma M, Yoslioka A, Wongcha V, Hecker E & Sugimura T, Presence of tumor promoters in the seed oil of Jatropha curcas from Thailand, *Jpn J Cancer Res,* 78 (1987) 223.