Project Completion Report

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Project Title: *Assessment and analysis of the plant oil feedstock available in North-East India for biodiesel production.*

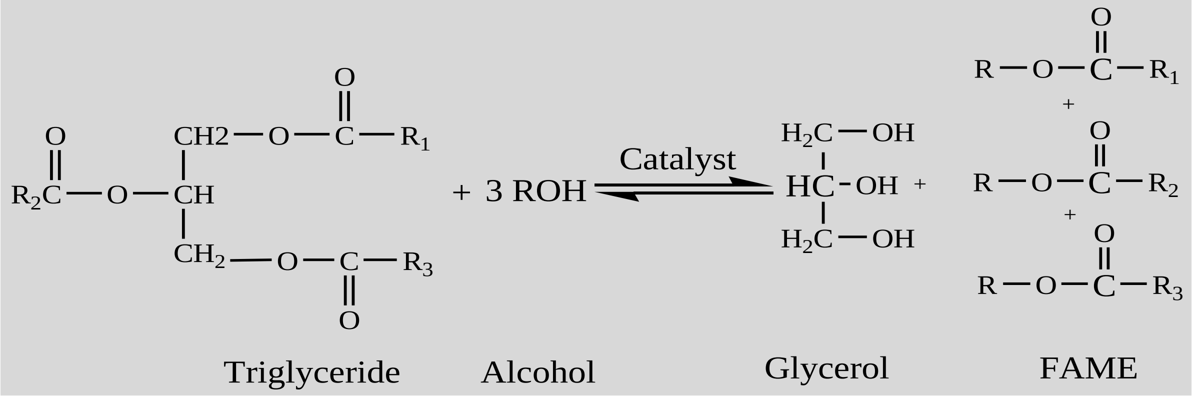
Introduction

Depleting supplies of fossil fuel and increasing environmental concerns have stimulated the intense search for alternative renewable fuels that are capable of fulfilling an increasing energy demand[1,2]. The energy supply of the world has relied heavily on non-renewable crude oil for more than two centuries. Because the production demand gap of fossil fuel is fluctuating worldwide, the price of conventional fossil fuel continues to rise, and the economies of importing nations suffer significant disruption. From an environmental perspective, combustion of petroleum fuels such as carbon dioxide (CO_2), carbon monoxide (CO), nitrogen oxides (NOx), and sulfur-containing residues are the principal causes of global warming. These concerns have driven significant investment in identifying and channeling renewable (biomass) raw materials into the manufacture of liquid fuel alternatives because the development of such biomass-based power would ensure that new technologies are available to keep pace with the need of society for new renewable power alternatives in the future. Biodiesel (fatty acid methyl esters) derived from the transesterification of vegetable oils or animal fats with methanol is a potential substitute for petroleum-based diesel fuels, due to its nontoxic, sulfur- and aromatic-free, biodegradable, and renewable features[3,4].

Vegetable oil is a promising alternative because it is renewable, environ-friendly and can be produced easily in rural areas[5]. But, direct use of vegetable oil (triglyceride) as fuel in diesel engine creates problem in engine performance due to its high viscosity and free fatty acid content as well as gum formation[6]. Transesterification of triglycerides with alcohol in presence of alkali catalyst can produce a new ester called biodiesel which can be effectively used as substitute for petroleum diesel in pure form or as blend. Engine performance tests of biodiesel have shown little problem having environmental benefits [7].

Biodiesel is a proven alternative to petrodiesel as a clean-burning renewable alternative fuel. Due to the continuous declining of limited petroleum reserves and the growing environmental concerns, the use of biodiesel in recent years as fuel in existing diesel engines has gained much importance [8]. The key issues of current biodiesel research are feedstock and conversion technology [9].

Different routes are available for biodiesel production from vegetable oils which include dilution, pyrolysis, microemulsification and transesterification. Out of these transesterification has been identified as the most viable process due to its high efficiency and simplicity [10]. In the reaction, 1 mol of triglyceride reacts with 3 mol of monohydric alcohol (usually methanol due to its low cost) in presence of a catalyst (acid, base or enzyme) **scheme 1.** The main product is fatty acid methyl ester (FAME), Known as biodiesel, and the by-product is glycerol. Usually, excess methanol is applied to enhance the conversion of triglyceride.



Scheme-1 Biodiesel Production Route

The major obstacle to widespread use of biodiesel is the cost involvement in comparison to its mineral counterpart mainly input costs of biodiesel production that is the feedstock, which is about 80% of the total operating cost. The high price of biodiesel is in large part due to the high price of the feedstock. Economic benefits of a biodiesel industry would include value added to the feedstock, an increased number of rural manufacturing jobs, an increased income taxes and investments in plant and equipment. These developments necessitates an urgent need to identify most potent low cost feedstock for biodiesel production and their characterization for cataloguing them for immediate use and also for future crop improvement.

The right choice of raw materials for biodiesel production must be always based on technical and economic aspects. For example, in developing countries like India, use of edible vegetable oils as feedstock is not an economically viable option as we are not self- sufficient in edible oils. India has rich and abundant forest resources with wide range of oil seed growing plants and many of these oil seeds can serve as potential feedstocks for biodiesel production. Several such feedstocks such as *Jatropha curcas* [11] , *Mesua ferrea* L., Cotton seed oil (*Gossypium hirsutum*), Karanja (*Pongamia glabra*) [12], field pennycress (*Thlaspi arvense* L.) [13], Rubber (*Hevea brasiliensis*) [14], Terminalia (*Terminalia belerica* Robx.) [15], Mahua (*Madhuca indica*) [16] etc. have already been identified. Therefore, it becomes inevitable to explore viability of new, unexplored non-edible oils in biodiesel production to meet feedstock requirements. Identification of new feedstocks will increase feedstock diversity and improve the economic value of that plant species

The production of quality biodiesel by transesterification reactions depends on types of feedstock, catalyst formation, catalyst concentration, reaction temperature, methanol-to-oil ratio along with free fatty acid compositions and moisture content of the vegetables oils used as feedstock for biodiesel production. The production of fatty acid methyl esters (FAME) from crude tobacco seed oil (TSO) having high free fatty acids (FFA) was achieved 91%

conversion in 30 minutes by two steps conversions [17]. Vicente et al (2006)[18] studied the methanolysis (alcoholysis with methanol) of 21 different vegetable oils using potassium hydroxide as a catalyst. They reported that biodiesel yield and ester content decreased when the vegetable oil acid value increased due to the neutralization of the free fatty acid content in the oil. Though the attempts have been made to get higher biodiesel yield from vegetable oil mixtures of cotton, Soybean, and Castor Oils, it is reported that methanolysis of mixtures of castor oil with soybean oil or cottonseed oil reaches average values that are comparable to those observed for the individual oils, but improves the purification process of the biodiesel produced[19].

The behavior of vegetable oils with different alkali catalysts were also reported by different researchers. Freedman et al. (1986)[20] showed that $NaOCH_3$ is a more effective catalyst formulation than NaOH and almost equal oil conversion was observed at 6:1 alcohol-to-oil molar ratio for 1%wt NaOH and 0.5%wt $NaOCH_3$; whereas Ma et al.(1998)[21] observed NaOH was a better catalyst than $NaOCH_3$ in transesterification of beef tallow. Vicente et al. (2004)[22] reported higher yields with methoxide catalysts, but the rate of reaction was highest for NaOH and lowest for $KOCH_3$ at 65^0C, a methanol-to-oil ratio of 6:1 and a catalyst concentration of 1%wt. Thus, characteristics differences of the vegetable oils extracted from different oil sources may influence the biodiesel properties. Therefore, it is urgent need to analysis the different vegetable oils for exploration of their characteristics differences on biodiesel production.

A large number of oilseed bearing plants, shrubs species and oil seeds plants are available and are remained unexplored in the existing forests and other localities of North East India. Very little study is reported in identification and characterization of these plants and shrubs species for their oil content, seed bearing capacities, growth, genetic variability and quality biodiesel production. Recently, the comprehensive analysis of fuel properties of biodiesel from the seed oil of *Pongamia glabra* (Vernacular Name-Koroch) and *Mesua ferrea* L. (Vernacular name- Nahar) available in the forest of this region were reported [23]. Chakraborty et al (2009)[15] reported that the prospect of terminalia oil (oil extracted from kernel of *Terminalia belerica* Robx available in North-East India) for biodiesel production is very high.

Keeping all above in view, the present investigation has been undertaken to facilitate the assessment and analysis the oil seeds of North East India to evaluate the best suitable plant oil feedstock for biodiesel production.

Objectives of the project

(a) to survey and screen the indigenous oil seed bearing plant species /plant oil feedstock available in the forest and other localities of North East India.
(b) to determine oil content and oil quality as well as the physicochemical characteristic of the vegetable oil and biodiesel produced from collected seeds of the plant species.
(c) to examine the methanolysis of different vegetable oils using variety of alkali catalysts giving special focus on higher biodiesel yield, purity and storage stability.

Materials and Methods

a) Oil seed collection: A survey was conducted and Oil seeds from six oil bearing tree species trees were collected from the forest and different localities of Assam and Arunachal Pradesh. They were *Gmelina arborea* (local name-Gomari), *Sterculia villosa* Roxb, (local name-Odal), *Acer laurinum* Hasskarl. (local name-Maple tree), *Biscofia javanica* Blume (local name-Uriam), *Thevetia peruviana* (local Name-Karabi), and *Mesua ferrea* Linn. (local Name-Nahar). Descriptions of selected oil seed bearing plant species are given in Table 1. The images of the selected plant species are given in Figure-1. The images of seeds of *Gmelina arborea, Sterculia villosa, Acer laurinum, , Biscofia javanica, Thevetia peruviana and Mesua ferrea* are given in Figure 2 (a,b), Figure 3(a,b), Figure 4 (a,b), Figure 5 (a,b), Figure 6 (a,b) and Figure 7 (a,b) respectively. Collected seeds were allowed to dry in sunlight to remove any traces moisture prior to removal of the kernels and oil extraction.

b) Chemicals: Synthesis-grade methanol (≥99% assay and ≤0.2% water content), $NaHCO_3$, NaOH (99% pure), KOH (85% pure), H_2SO_4 (98% pure), CaO(Analytical grade), Na_2SO_4, n-Hexane, Petroleum Ether 40-60°C, Petroleum Ether 60-80°C, were all purchased from Merck India Limited, Mumbai. All reagents were used without further purification.

c) Oil extraction: Collected seeds were subjected to dehulling, separating hull from nut, separating nut shell from kernel, drying and grinding followed by oil extraction. Oil extractions from the crushed kernels were done using soxhlet extraction system according to the AOAC official method (AOAC 963.15., 1976). The oil extractability was measured using three different solvents Petroleum ether (b.p 40-60 °C), Petroleum ether (b.p 60-80 °C) and commercial grade n-hexane. Excess solvent was removed with a Steroglass rotary vacuum evaporator (STRIKE202). After removing the solvent, the oil was filtered to remove particulate matter. The filtered oil was then stored in glass bottles for further experiments. Oil content for each of the individual samples was expressed in weight percentage (with triplicates). Oil content in the kernel was determined as follows (equation 1).

$$\text{Oil content in the kernel (wt. \%)} = \frac{\text{Weight of the oil extracted from kernel}}{\text{Weight of the kernel before oil extraction}} \times 100\% \text{ ------(1)}$$

Table 1. Description of the selected oil seed bearing plant species

Plant Species	Sampling point/Coordinates	Family	Distribution	Uses	Flowering	Seeding & Fruiting	Seed yield per annum per tree (mature)	Ref.
Gmelina arborea Roxb. Local name: Gamari	Tezpur University campus/ 26.6993° N, 92.8335° E	Verbenaceae	Found all over Assam and lower Nagaland	Wood use as fuel and building material; Medicinal use; ornamental tree	January – March	April – late June	15-18 Kg	24, 25
Sterculia villosa Roxb Local Name: Udal	Koliabor, Nagaon, 26˚32'44"N-92˚55'44"E	Malvaceae	Fairly common in Sibsagar, Lakhimpur,Mikir Hills , Darrang, lower Nagaland.	Wood is light, soft, white, used for tea boxes, packing boxes, scantling, fire wood etc. Bark yields a fiber used for elephant ropes, coarse canvas etc. Seeds are eaten roasted.	February-March	March - June	10-12 Kg	26
Acer laurinum Hasskarl. Local Name: Maple tree	Kohora forest range, 26˚33'25"N-93˚23'54"E	Sapindaceae	Asia, countries of southern hemisphere	No use is reported (non-edible)	January – March	April-October	5-10 Kg	27,28
Biscofia javanica Blume Local name: Uriam	Pasighat, Arunachal Pradesh 28.0619° N, 95.3259° E	Euphorbiaceae	Found in Sibsagar, Nowgong, Darrang,Kamrup and Arunachal Pradesh	Use for house building, bridges, mining posts, railway sleepers, boats, furniture making, carving etc.	August-November	December-March	15-20 kg	29
Thevetia peruviana S Local Name: Karabi phool	Madanpur, Kamrup 26.3175° N, 91.7271° E	Apocyanaceae	America, Asia, Africa	Fuelwood, ornamental tree	Throughout the year	Throughout the year	20-30kg	30,31
Mesua ferrea Linn.	Tezpur University campus/ 26.6993° N, 92.8335° E	Guttiferae	Upper Assam, Lower Nagaland, Darrang,N.C.Hills	One of the strongest and hardest woods in Assam, use as house post, bridges, boats, beams,walking stick etc.	April-May	August-October	10-15Kg	32

Figure-1: Images of selected plant species.

Figure 2: Images of seeds of *Gmelina arborea* (a)seeds, (b) Kernels

Figure 3: Images of Seeds of *Sterculia villosa* (a) seed with coat, (b) seed

Figure 4: Images of seeds *of Acer laurinum* (a) Seeds attached to wings (b) seeds removed from

Figure 5: Images of seeds of *Biscofia javanica* (a) seed coat, (b) seeds

Figure 6: Images of seeds of *Thevetia peruviana* (a) seeds , (b) kernels

Figure 7: Images of seeds of *Mesua ferrea* (a) seeds , (b) kernels

d) Biodiesel production: Biodiesel was produced by the two step process, pretreatment (i.e. acid catalyzed esterification of the Free Fatty acids to corresponding methyl esters) followed by transesterification due to the high % of FFAs<2% in extracted oil to avoid saponification (Sahoo et al.)[33]. In a typical reaction, 250 ml of preheated oil was added to a 500 ml two neck round bottomed flask containing 100 ml methanol with 2% H_2SO_4 (w/w) as catalyst equipped with a magnetic stirrer, reflux condenser and a thermometer on a oil bath maintained at 100°C for 2 h. After completion of the reaction excess methanol was recovered using a rotary evaporator, excess catalyst neutralized with $NaHCO_3$ solution for base catalysed reactions while for H_2SO_4 catalysed reactions distilled water was used for this purpose. The resulting oil and ester mixture was dried in an oven maintained at 110 ° C for 3 h prior to transesterification. Transesterification reactions were performed in a 150 ml two neck round bottomed flask equipped with a magnetic stirrer, reflux condenser and a thermometer on a oil bath maintained at 60°C using methanol:oil:catalyst (molar ratio) of 6:1:(0.15- 0.5). Since, catalysts also play crucial role in the overall process by influencing quality, yield as well as economic aspects (Freedman et al., 1984; Lam et al., 2010). Therefore methanolysis reactions were carried out utilizing all the major industrially important catalysts (NaOH, KOH, CaO and H_2SO_4) under similar conditions to get an overview of activity of the different catalysts. In a typical reaction about 50 ml of preheated oil was charged into the flask containing methanol and catalyst. Oil conversions were measured from methyl esters content of oil at different time intervals using ^1H NMR (Gelbard et al.)[34] as follows (equation 2):

$$Yield_{Ester} \quad = \quad \frac{2A_{Me}}{3A_{CH2}}\, X\; 100\;\% \qquad \text{---} \; (2)$$

Where,

A_{Me} = integration value of the methoxy protons of the methyl esters and

A_{CH2}= integration value of α-methylene protons

For determining final yield (equation 3), on completion of the reaction, the resulting product was cooled to room temperature and transferred to a separatory funnel for biodiesel and glycerin separation. It was left overnight to allow gravity separation, after two phases have separated, the upper phase was collected and the excess alcohol removed using a vacuum evaporator operated at 80 °C. The resulting biodiesel obtained was purified by successive washing with warm deionized water to remove residual catalyst, glycerol, methanol and soap. A small quantity of $NaHCO_3$ solution was used in the second washing to neutralize remaining soaps and catalyst.

$$Yield = \frac{\text{Weight of methyl ester produced}}{\text{Weight of oil taken}}\; \mathrm{X}100\;\% \; \text{-----------------------------------} \; (3)$$

e) Determination of oil properties: Major physicochemical properties of extracted oil such as kinematic viscosity, density, calorific value, acid value (%FFAs), iodine value, saponification value, oxidation stability, were determined using standard methods. The oxidation stability in terms of induction period was determined by the Rancimat model 743 following the EN 14112 method. Each 3 g of biodiesel sample is aging at 110°C with 10 l/h airflow rate.

The cetane number of biodiesel (FAME) was calculated according to the equation (4) (Krisnangkura,) [35]:

$$\mathrm{CN} = 46.3 + (5458/\mathrm{SAP}) - (0.225 \times \mathrm{IV}) \text{ -------------------------------- } (4)$$

Where,

CN=Cetane number, SAP= Saponification value (mg KOH g−1) and

IV=Iodine value (mg I2 g−1) of FAME

Fatty acid composition of the oil was determined from GC-MS of the corresponding methyl ester using standard procedure. GC-MS was performed using Perkin Elmer Claurus 600 equipped with TCD detector and elite wax column. The column temperature was programmed from 50 °C to 250 °C and the rate of increase was 5°C/min. The carrier gas used was helium and the flow rate was maintained at 1 mL/min. ^{1}H NMR, ^{13}C NMR of the oil was recorded in $CDCl_3$ at 300 and 75 MHz, respectively using JOEL 400 MHz/54 mm. MS was scanned from 20 to 400 Da. For identification of esters in methyl ester (FAME), library search was carried out using NIST, NBS and Wiley GC-MS library.

Results and Discussion

The oil seeds with corresponding percentage (%) of oil content have been illustrated in Table 2. In the various extraction studies carried out with different solvents (Petroleum Ether 40-60 °C, Petroleum Ether 60-80 °C and n-hexane), n-hexane showed best extractability.

Table -2: Oil content of the selected oil seeds

Sl..No.	**Local Name**	**Scientific name**	**Kernels obtaining**	**Oil content**
1.	Gamari	*Gmelina arborea*	Hard	60-62%
2.	Udal	*Sterculia villosa*	Easy	10-15%
3.	Maple	*Acer laurinum*	Easy	45-50%
4.	Uriam	*Biscofia javanica*	Hard	10-12%
5.	Karabi Phool	*Thevetia peruviana*	Moderate	62-63%
6.	Nahor	*Mesua ferrea*	Easy	73-75%

From Table-1, it is seen that oil content of Udal and Uriam seeds are less than 15%. But, other seeds have oil content in the range of 45-75%. Seeds with higher oil content (*Gmelina arborea, Acer laurinum, Thevetia peruviana and Mesua ferrea)* were taken for further investigation to produce biodiesel.

Fatty acid profile is of prime concern in determining the potential of feedstock form biodiesel production. Important fuel parameters like cetane number, calorific value, kinematic viscosity, oxidation stability, iodine number etc of vegetable oil based fuels like biodiesel are directly affected by the fatty acid composition of the feedstock oil [36]. The fatty acid compositions of the investigated non-edible oils determined by GC-FID are presented in Table 3. Gas Chromatogram of *Gmelina arborea* oil is presented in Figure-8. ^{1}HNMR was not used for fatty acid profiling as it cannot be used to quantify the saturated acids individually and also gives erroneous results in the presence of mono- or di-acylglycerols in oil [37].

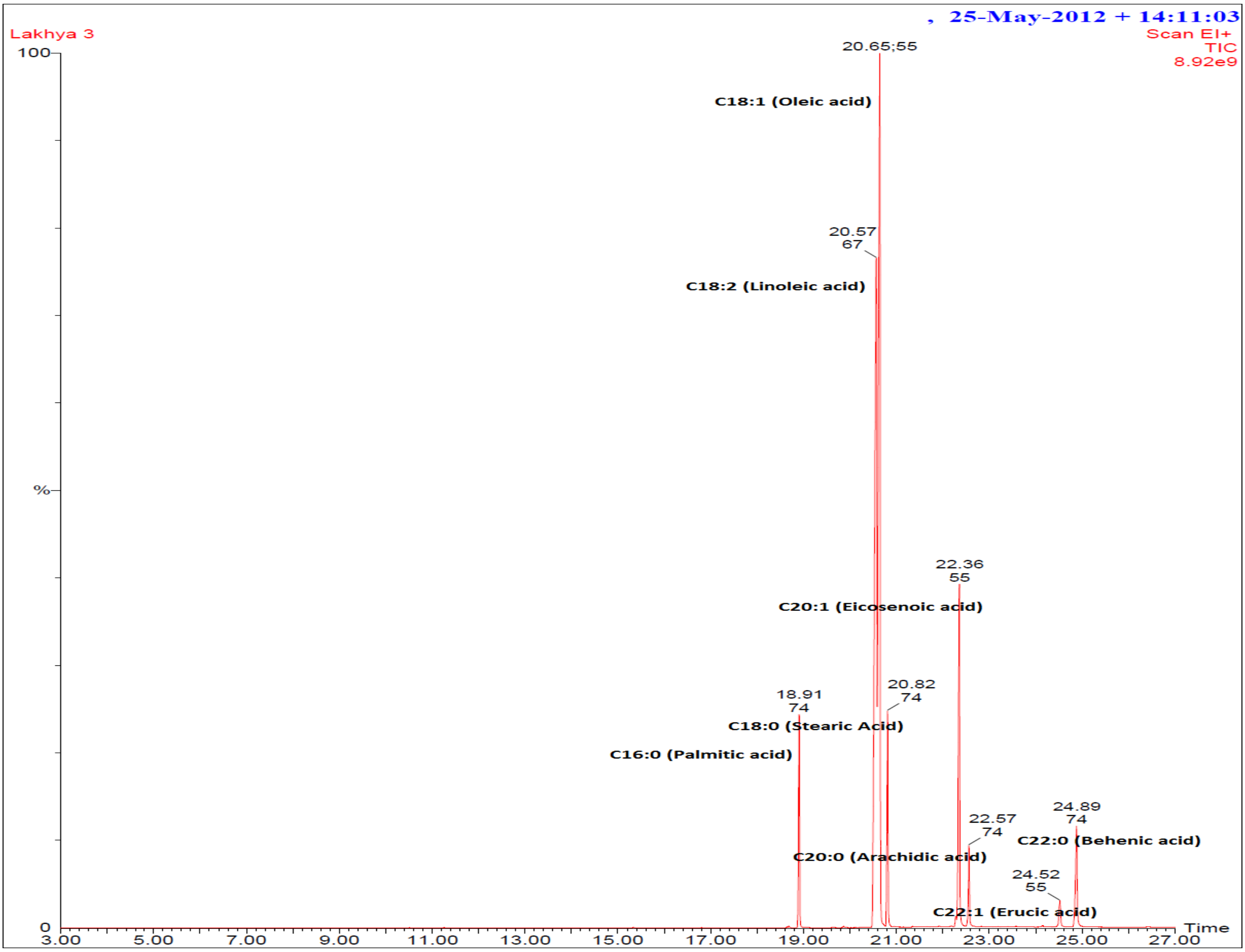


Figure-8: Gas chromatogram of *Gmelina arborea* oil methyl esters

Table-3: Fatty acid composition of the selected species

Fatty acid	*Gmelina arborea*	*Acer laurinum*	*Thevetia peruviana*	*Mesua ferrea*
Myristic (C14:0)	-	0.35	0.07	-
Palmitic (C16:0)	5.62	27.11	21.15	12.85
Palmitoleic (16:1)	-	1.48	0.19	0.15
Stearic (C18:0)	5.38	2.49	7.87	11.81
Oleic (C18:1)	33.99	12.96	43.44	49.3
Linoleic (C18:2)	36.03	34.69	25.33	21.17
Linolenic (C18:3)	-	-	-	-
Arachidic (C20:0)	2.35	1.18	1.16	0.9
Gadoleic (C20:1)	10.84	-	0.15	0.58
Behenic acid (C22:0)	4.64	0.35	0.46	0.27
Erucic acid (C22:1)	1.10	-	-	2.84
Ligoceric (C 24:0)	--	0.098	0.17	0.12
*CPFA		21.0		
Saturated (wt %)	18.01	31.58	30.88	25.95
Unsaturated (wt %)	81.98	68.42	69.11	74.04
Polyunsaturated (wt %)	36.03	34.69	25.33	21.17
Others	0.01	-	0.01	0.01

*CPFA (cyclopropene fatty acid) was determined using ^{1}H NMR as % CPFA = 150 × ($A_{-CH2\_}$ /A_{-CH2-})

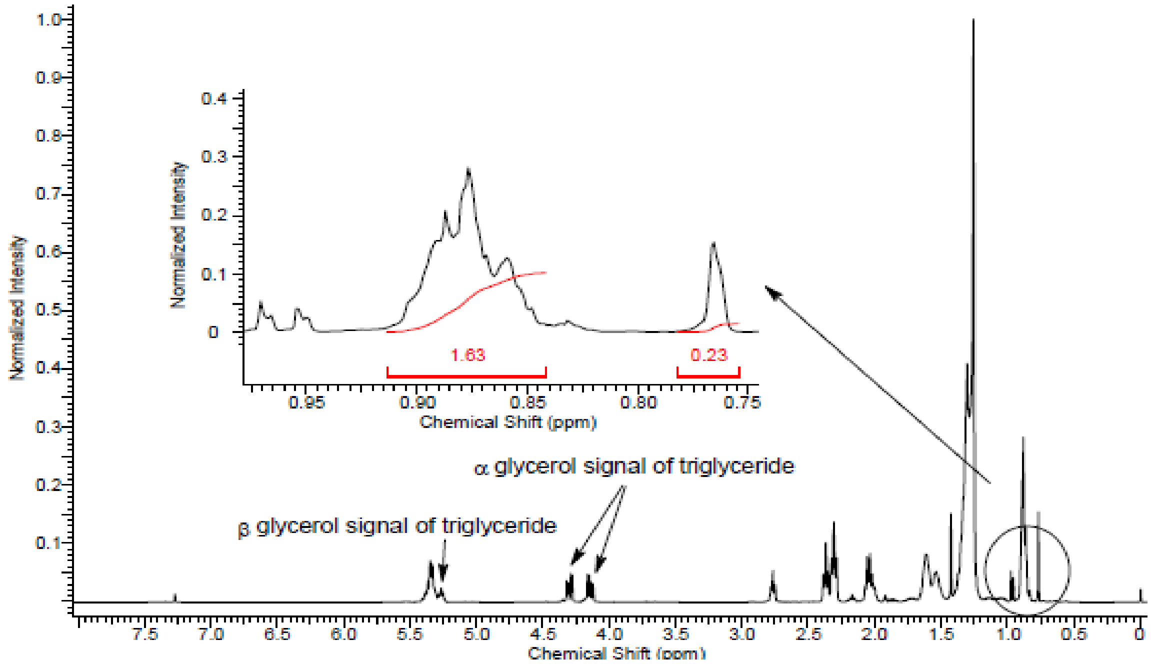


Figure 9: ^{1}H NMR patterns of *Acer laurinum*

The physicochemical characteristic of the non-edible oil from the seeds of selected plant species has been given in Table-4.

Table 4: Physicochemical properties of crude vegetable oil

Properties	Test Method	*Gmelina arborea*	*Acer laurinum*	*Thevetia peruviana*	*Mesua ferrea* L.
Gross Calorific Value (Kilojoules/g)	Auto Bomb Calorimeter	39.452	39.08	39.43	38.83
Density (kgm^{-3} @ 15°C)	ASTM D287	0.886	0.899	0.885	0.935
Kinematic Viscosity at 40°C (cSt)	ASTM D445	21.32	24.73	27.06	38.34
Acid value (mg KOH/g)	ASTM D 664	16.13	5.17	13.34	16.1
Oxidation stability @110°C (h)	EN14112	1.86	6.15	6.45	3.22
Saponification value (mg KOH g^{-1})	AOCS Official Method Cd 3-25	194.65	170.10	181.52	201.64[a]
FFA (%) (w/w)		8.10	2.56	6.70	8.09
Iodine value (mg I_2 g^{-1})	AOAC Official Method 993.20	105.16	98.25	101.23	87.42[a]

From Table-4, it is seen that little variations of Gross Calorific value and density are observed in oils of the selected plant species but kinematic viscosity was found in the range of 21.32 - 38.34 cSt at 40^0C. The kinematic viscosity of the oils in the descending order is as follows: *Mesua ferrea*> *Thevetia peruviana* > *Acer laurinum* > *Gmelina arborea***.** The viscosities of respective vegetable oils varied due to differences in their fatty acid composition. Generally, the presence of high amounts of unsaturated fatty acids such as oleic, linoleic and linolenic acids results in lower viscosities while more saturated oils are solid at room temperature, e.g. lard, butter, coconut oil. The low viscosity of *Acer laurinum* may be attributed to the presence of CPFA and unsaturated fatty acids as major constituents. In *Gmelina arborea,* due to the presence of high amounts of unsaturated fatty acids it was found to be highly susceptible to oxidation (induction period=1.86 h). This also represented by the high iodine value 105.16 mg I_2 g^{-1} indicating the presence of high amounts of double bond containing fatty acids.

The fuel properties of methyl esters produced from the oil of the above four species are presented in Table-5.

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Table 5: Fuel properties of methyl esters of selected plant species in comparison to similar non-edible oil methyl esters and Petrodiesel

Properties	Test methods	*G. arborea*	*A. laurinum*	*T. peruviana*	*M. ferrea*	*Jatropha Carcus*[38]	*Pongamia glabra*[15]	Petro Diesel	ASTM D 6751	EN 14214
Calorific Value (Kilojoules/g)	Auto Bomb calorimeter	39.779	39.45	39.01	41.11	39.65	43.42	45-48	NA	NA
Cetane number	ASTM D613	50.32	58.74	62.5	53.85	52.3	55	38-40	47 (min)	52 (min)
Density (g/cm^3 @ 15°C)	ASTM D287	0.886	0.88	0.875	0.897	0.875	0.903	0.848	0.88	0.86-0.90
Kinematic Viscosity (cSt at 40°C)	ASTM D445	4.2	4.73	4.37	5.8	5.11	6.13	1.3–4.1	1.9-6.0	3.5-5.0
Flash point (°C)	ASTM D93	115.0	113.0	108.0	112.0	186.0	95.0	52.0	130 (min)	120 (min)
Cloud point (°C)	ASTM D2500	6.0	5.0	4.0	5.0	4.0	3.5	-15 to 5	NA	NA
Poor point (^{o} C)	ASTM D 97	3.0	1.0	-2.0	1.0	NA	3.0	-35 to 15	-15 to 10	NA
Saponification Value (mg KOH g^{-1})	AOCS Official Method Cd 3-25	185.55	177.99	162.43	191.92	202.6	184.90	----	NA	NA
Acid value (mg KOH g^{-1})	ASTM D664	0.003	0.053	0.03	0.01	0.27	0.0	----	0.50 (max.)	0.50 (max)
Iodine value (mg I_2 g^{-1})	AOAC Official Method 993.20	112.89	80.934	77.33	92.77	93-106	89.01	----	NA	120 (max)
Oxidation stability @110°C (h)	EN14112	2.09	6.15	7.55	2.23	3.23[a]	2.35[a]	-----	3 (min)	6 (min)

NA = not available, a = Adapted from (Sarin et al. 2007)[39],

Cetane Number= Calculated from saponification and iodine value according to Krisnangkura, 1986[35].

In Table-5, it is seen that calorific value of all methyl esters of oils are less than the petro diesel. This may be due to presence of oxygen in the methyl esters. Cetane numbers of all methyl esters are found higher than petrodiesel and in the range of the value of ASTM D 6751 and EN 14214. However, *Thevetia peruviana* has shown higher cetane number (62.5) than other species under investigation (Table-5). Kinematic viscosities (cSt at 40^0C) of methyl esters of all species were found in the range of 4.2- 5.8, which are less in value in comparison to the kinematic viscosity of vegetable crude oils (21.32-38.34 cSt at 40^0C) as shown in Table -4. From the Table -5 , it is seen that methyl ester of *Mesua ferra* is more viscous than other species. Values of flash point, cloud point and Pour point of all methyl esters are found in the range of the values of ASTM D 6751 and EN 14214 (Table-5). Acid value of methyl esters are also found very lower (Table-5) comparing to the crude vegetable oil values (Table-4). After production of biodiesel, oxidation stabilities of the samples have been increased in comparison to crude vegetable oil. Overall, biodiesel produced from the oil seeds of selected plant oil species meet the required fuel quality standard as per ASTM D 6751 and EN 14214.

The results of transesterification studies on *Gmelina arborea* oil in methanol to *Gmelina arborea* oil Fatty Acid Methyl Ester (GOME) as representative test with different catalysts have been presented in Figure 10.

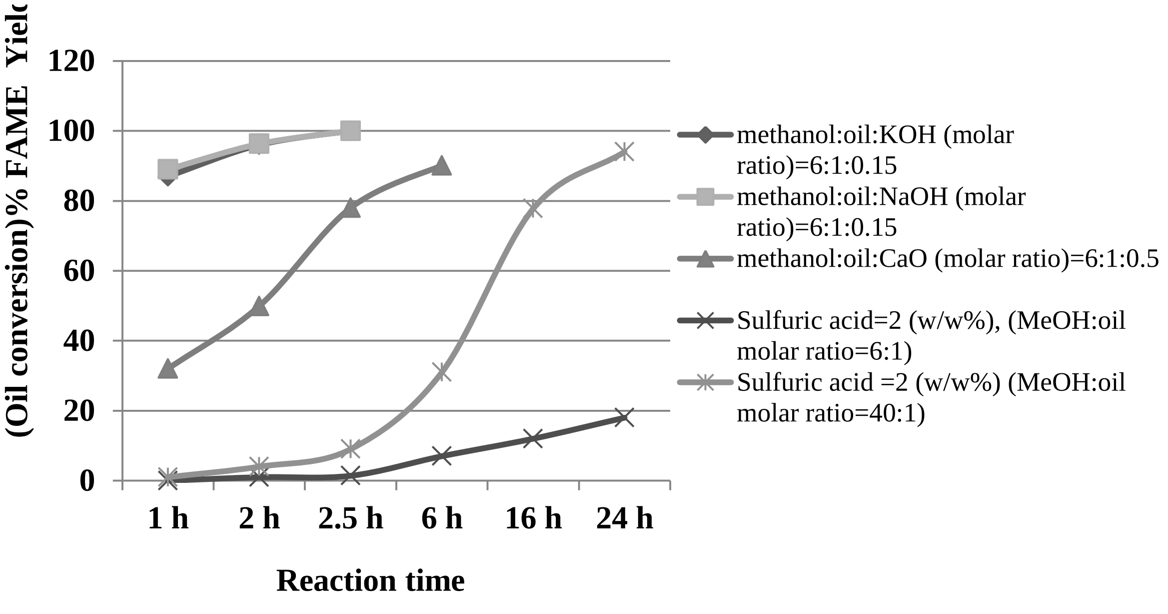


Figure -10: Effect of catalysts in FAME conversion of GOME

As expected our experiments shows that the more basic alkali metal hydroxide catalysts showed better activity. With, NaOH and KOH giving 100% conversion followed by CaO (78%) in 2.5 h with a 6:1:0.15 methanol:oil:catalyst (molar ratio). The use of 2% (w/w) H_2SO_4 as catalyst, reaction could reach high conversion in 24 h (94%) only when a very high 40:1 methanol:oil (molar ratio) was used; however with the ratio of (6:1) only 18% conversion was achieved. The use of higher catalyst loading (except H_2SO_4) resulted in soap forming side reactions, reducing biodiesel yield and causing difficulty in glycerine separation. The effect of catalyst loading on biodiesel yield and soap formations has been summarised in Table 6. Therefore in the present study NaOH and KOH were concluded to be the most effective catalyst for biodiesel production from *Gmelina arborea* oil. However, FAME yield was reduced to 95.2% following washings and neutralisation for NaOH catalysed process under optimal reaction conditions.

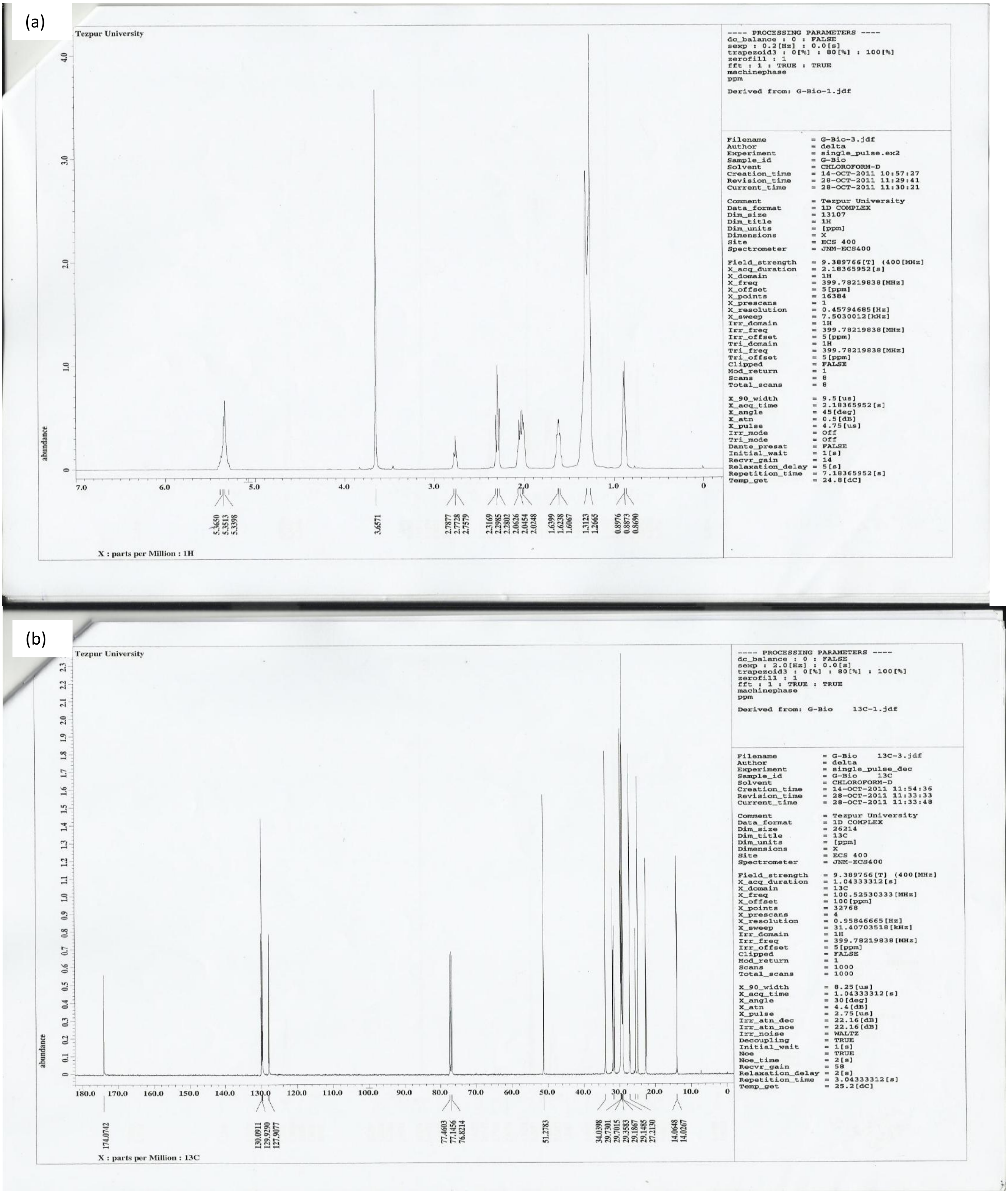
Table-6: Effect of catalyst loading on GOME yield

Catalyst	methanol:oil:catalyst (molar ratio)	Methyl ester yield (%)	Soap
NaOH	6:1:0.15	100	Not detected
	6:1:0.30	97.88	Detected
	6:1:0.50	88.99	Detected
KOH	6:1:0.15	100	Not detected
	6:1:0.30	95.88	Detected
	6:1:0.50	86.78	Detected
CaO	6:1:0.15	67.09	Not Detected
	6:1:0.30	79.88	Detected
	6:1:0.50	90.12	Detected
*H_2SO_4	6:1:(2% w/w)	18	Not detected
	40:1:(2% w/w)	94	Not detected

*Transesterification with H_2SO_4 was carried out with 2 (w/w %) Catalyst for 24 h

Characteristic study of *Gmelina arborea* oil methyl ester (GOME) with ^{1}HNMR and ^{13}CNMR was also carried out in this investigation. The formation GOME was confirmed by ^{1}HNMR shown in Figure 11. The appearance of characteristic methyl esters peak ($-OCH_3$) at 3.6648 ppm and disappearance of glyceridic protons peaks at 4.1 and 4.2 in the ^{1}HNMR spectra of biodiesel is indicative of the conversion.

From Table 5, it is seen that all major fuel properties such as density, viscosity, cetane number etc of methyl esters of selected plant species were within acceptable limits of ASTM D 6751, EN 14214 specifications except iodine value and oxidation stability. The Kinematic Viscosity of GOME was found to be lower than biodiesel of other plant species due to the presence of larger amounts of unsaturated fatty acid methyl esters. The low oxidation stability (IP= 1.86 h) of GOME was due to the presence of large amounts (36.03 %) of polyunsaturated fatty acid methyl esters of (Linoleic acid (C18:2)); therefore the produced methyl esters were highly susceptible to autoxidation. The reason for autoxidation is mainly due to the presence of unsaturated fatty acid chains, especially those with bis-allylic methylene moieties (Knothe et al., 2007)[40]. This low oxidative stability may be accounted for by blending with methyl esters of higher stability or by addition of antioxidants [40]. However, due to the presence of such high amounts of unsaturated, low melting fatty acid methyl esters, the produced biodiesel exhibited in good cold flow properties Pour point (3 °C) and cloud point.



```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 0.2[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Derived from: G-Bio-1.jdf
```

```
Filename          = G-Bio-3.jdf
Author            = delta
Experiment        = single_pulse.ex2
Sample_id         = G-Bio
Solvent           = CHLOROFORM-D
Creation_time     = 14-OCT-2011 10:57:27
Revision_time     = 28-OCT-2011 11:29:41
Current_time      = 28-OCT-2011 11:30:21

Comment           = Tezpur University
Data_format       = 1D COMPLEX
Dim_size          = 13107
Dim_title         = 1H
Dim_units         = [ppm]
Dimensions        = X
Site              = ECS 400
Spectrometer      = JNM-ECS400

Field_strength    = 9.389766[T] (400[MHz]
X_acq_duration    = 2.18365952[s]
X_domain          = 1H
X_freq            = 399.78219838[MHz]
X_offset          = 5[ppm]
X_points          = 16384
X_prescans        = 1
X_resolution      = 0.45794685[Hz]
X_sweep           = 7.5030012[kHz]
Irr_domain        = 1H
Irr_freq          = 399.78219838[MHz]
Irr_offset        = 5[ppm]
Tri_domain        = 1H
Tri_freq          = 399.78219838[MHz]
Tri_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 8
Total_scans       = 8

X_90_width        = 9.5[us]
X_acq_time        = 2.18365952[s]
X_angle           = 45[deg]
X_atn             = 0.5[dB]
X_pulse           = 4.75[us]
Irr_mode          = Off
Tri_mode          = Off
Dante_presat      = FALSE
Initial_wait      = 1[s]
Recvr_gain        = 14
Relaxation_delay  = 5[s]
Repetition_time   = 7.18365952[s]
Temp_get          = 24.8[dC]
```

```
---- PROCESSING PARAMETERS ----
dc_balance : 0 : FALSE
sexp : 2.0[Hz] : 0.0[s]
trapezoid3 : 0[%] : 80[%] : 100[%]
zerofill : 1
fft : 1 : TRUE : TRUE
machinephase
ppm

Derived from: G-Bio    13C-1.jdf
```

```
Filename          = G-Bio    13C-3.jdf
Author            = delta
Experiment        = single_pulse_dec
Sample_id         = G-Bio    13C
Solvent           = CHLOROFORM-D
Creation_time     = 14-OCT-2011 11:54:36
Revision_time     = 28-OCT-2011 11:33:33
Current_time      = 28-OCT-2011 11:33:48

Comment           = Tezpur University
Data_format       = 1D COMPLEX
Dim_size          = 26214
Dim_title         = 13C
Dim_units         = [ppm]
Dimensions        = X
Site              = ECS 400
Spectrometer      = JNM-ECS400

Field_strength    = 9.389766[T] (400[MHz]
X_acq_duration    = 1.04333312[s]
X_domain          = 13C
X_freq            = 100.52530333[MHz]
X_offset          = 100[ppm]
X_points          = 32768
X_prescans        = 4
X_resolution      = 0.95846665[Hz]
X_sweep           = 31.40703518[kHz]
Irr_domain        = 1H
Irr_freq          = 399.78219838[MHz]
Irr_offset        = 5[ppm]
Clipped           = FALSE
Mod_return        = 1
Scans             = 1000
Total_scans       = 1000

X_90_width        = 8.25[us]
X_acq_time        = 1.04333312[s]
X_angle           = 30[deg]
X_atn             = 4.4[dB]
X_pulse           = 2.75[us]
Irr_atn_dec       = 22.16[dB]
Irr_atn_noe       = 22.16[dB]
Irr_noise         = WALTZ
Decoupling        = TRUE
Initial_wait      = 1[s]
Noe               = TRUE
Noe_time          = 2[s]
Recvr_gain        = 58
Relaxation_delay  = 2[s]
Repetition_time   = 3.04333312[s]
Temp_get          = 25.2[dC]
```

Figure 11: (a) ^{1}HNMR and (b) ^{13}CNMR of GOME

Conclusion: In this present investigation, six oil seeds from six different oil seed bearing tree species viz. *Gmelina arborea* (local name-Gomari), *Sterculia villosa* Roxb, (local name-Odal), *Acer laurinum* Hasskarl. (local name-Maple tree), *Biscofia javanica* Blume (local name-Uriam), *Thevetia peruviana* (local Name-Karabi), and *Mesua ferrea* Linn. (local Name-Nahar) were collected from different localities and forest areas of Assam and foot hill of Arunachal Pradesh. After oil extraction form the collected oil seeds, it was found that the oil content of *Sterculia villosa* Roxb, and *Biscofia javanica* Blume were not in the accepted range. Therefore, vegetable oil from *Gmelina arborea*, *Acer laurinum* Hasskarl., *Thevetia peruviana* and *Mesua ferrea* Linn. were taken for further investigation to explore the possibility of quality biodiesel production. Out of these, two species viz. *Gmelina arborea* and *Thevetia peruviana* were recognized as potential feedstock for biodiesel production grown in this locality.

Future works:

Though fuel characteristic studies of the plat oils of above plant species showed their suitability as feedstock for biodiesel production, the project team recommends for engine performance and emission study of the methyl esters produced from the oils.

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