



Calophyllum Inophyllum Linn (“honne”) Oil, A source for Biodiesel Production

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Available online at: www.isca.in, www.isca.me

Received 1st May 2013, revised 20th August 2013, accepted 10th November 2013

Abstract

An increasing demand of fossil fuels has being a critical problem for us. The natural resources of fossil fuel are dwindling day by day. Biodiesel that may called natural fuel may be a good source or substitute for fossil fuel in future. Biodiesel can be produced from non edible oil like *Jatropha curcus*, *pongamia pinnata*, *Madhuca indica*, *Gossypium arboreum*, *Simarouba glauca* etc. and more. There is a best source as a raw material that is *Calophyllum inophyllum* (honne) oil for biodiesel production. As it is an evergreen tree and grows along the coastal area. Our study is focused on the collection of seeds and oil extraction then proceed for biodiesel production with molar ratio 8:1, KOH were 1.2wt%, temperature 65°C, reaction time 90minutes were used and testing of parameters as per ASTM 6751 standards. The physical properties like acid value, density, Calorific value, Flash point, Fire point and Moisture, Viscosity shows of *calophyllum methyl esters* were 0.702, 892gm/cc, 37.18MJ/Kg, 176°C, 182°C and 0.01% . The physico-chemical parameters showed that *Calophyllum* may works as a sustainable feedstock for biodiesel production that is equivalent to fissile fuel as per ASTM 6751.

Keywords: Calophyllum oil, free fatty acid, esterification, transesterification etc.

Introduction

The concept using vegetable oil as a fuel dates back to 1895 when Dr. Rudolf Diesel developed the first diesel engine to run on vegetable oil. Rudolf Diesel Stated: “The use of vegetable oil for engine fuels may seem insignificant today. But such oil may become in source of time as important as petroleum and the coal tar products of the present time Biodiesel is a natural and biodegradable fuel defined as mixture of fatty acid methyl or ethyl esters derived from vegetable oil or animal fats and it is used in diesel engines and heating systems. Thus this fuel could be considered as mineral diesel substitute that having an advantage of reduction greenhouse gases because it is renewable resource¹. Mostly biodiesel is prepared from oils like soyabean, sunflower, rapeseed etc. throughout the world. Depending on the weather and soil conditions, different nations looking into various feedstock or vegetable oils for diesel fuel substitute; soyabean oil in USA, sunflower and rapeseed oil in Europe, palm oil in Malaysia and coconut oil Philippines are being considered as a substitutes for diesel fuel seed oil .As the extracted oil could not be used directly in diesel because of its higher viscosity. High viscosity of pure vegetable oil would reduce the fuel atomization and increase the fuel spray penetration, which would be responsible for high engine deposits and thickening of lubricating oil. The use of chemically altered vegetable oil that is biodiesel does not require modification in engine or injection system or fuel lines and is directly possible in any diesel engine. Biodiesel can be produced from vegetable oils or animal fats via transesterification. The transesterification is the reaction between oil and fat, with a short chain alcohol (methanol,

ethanol, and propanol) in the presence of suitable catalysts, as they give high production yield².

Most of researchers have worked feedstock having higher FFA levels using alternative processes like pyrolysis, micro emulsion, supercritical one. But there are certain exceptional cases wherein direct trans-esterification cannot be performed. Such cases appear in raw vegetable oils or in non edible oils like karanja³, *Jatropha*⁴, mahua⁵, castor⁶, *simaroubae*⁷ and nagchampa i.e. *calophyllum inophyllum*. As these non edible oils possess high free fatty acids (FFA). For determining whether the raw vegetable oils can be trans-esterified directly, the acid value is the most important property that must be known. After FFA determination, we can convert the oil into ester that is biodiesel with two step chemical process that are esterification and transesterification. In the first step, the oil is treated by an acid dissolved in methanol to reduce FFA content, whereas in the second step the preheated esterified oil is transesterified with methanol in the presence of base catalyst to form ester and glycerol⁸.

Why Calophyllum Inophyllum L.: *Calophyllum inophyllum* linn is a species of family Guttifereae (Clusiaceae), native to India, East Africa, Southeast Asia, Australia and South Pacific. Commonly it is called as ‘Indian laurel’, Alexandrian Laurel, Beach *calophyllum*, Beauty leaf, Pannay tree, Sweet Scented *Calophyllum* (in English), Pongnyet, Burmese, Hawaii, Kokani, Nagachampa, (in Marathi), Sultan Champa, Surpan (in Hindi), Nagam, Pinmai, Punnagam, Punnai, Pinnay, Namere (in Tamil), It is a broad leaved evergreen tree occurring as a littoral species

along the beach crests, although sometimes occurring inland⁹ and adjacent lowland forest. It has been widely planted throughout the tropics and is naturalized in the main Hawaiian Islands. The tree is valued for its hardiness and beauty as an ornamental tree. Oil from the nuts has been traditionally used for medicine and cosmetics and is today being produced commercially in the South Pacific. The tree grows best in direct sunlight, but grows slowly. Annual yield of 20-100 kg/tree of whole fruits have been reported^{10,11}. Trees begin to bear significantly after 4-5 years. The nut kernel contains 50-70% oil and the mature tree may produce 1-10 kg of oil per year depending upon the productivity of the tree and the efficiency of extraction process¹².

Although wildings occur, it can be moderately difficult to propagate. Its slow growth and large seeds make it unlikely that the tree will become an invasive weed if introduced into new areas¹⁴. Tree grow to height of 8-20m (25-65ft), sometimes reaching up to 35cm (11ft). Canopy width is often greater than the tree's height when the tree is grown in open location. It has a broad spreading crown often with large, gnarled, horizontal branches. The light gray bark shows deep fissures alternating with flat ridges.



Figure-1
Calophyllum seedling



Figure-2
Matured tree of Calophyllum



Figure-3
Flowers of calophyllum



Figure-4
Prematured fruit bunch



Figure-5
Dried fruits



Figure-6
Fresh fruit with shell

Uses: The total Calophyllum inohyllum linn tree has got excellent medicinal properties.

Wood: The beautiful wood has a fine lustrous texture that shows a distinctive interlocked grain. It is white and red when cut and ages to a reddish brown.

Because of this interlocked grain, swan surfaces tend to be woolly. The wood is moderately dense, specific gravity 0.6-0.8 and is somewhat difficult to work due to interlocked grain. The wood is particularly useful for food platters and calabashes, as it imparts no taste to the food. It is also prized for handicrafts because of its beauty. It is traditionally used in boat making. Elsewhere it is also used for general furniture, construction. It has however, been variously, described as vulnerable or resistant to termite attack¹⁵. The bark acts as an antiseptic and disinfectant. Rubbed with water lime juice, it makes a usefull application on armpits, groins and feet in bromidrosis. The bark taken internally acts as an expectorant and is useful in chronic bronchitis. The resin is mixed with strips of bark and leaves steeped in water and the oil which rises to the surface is a household application for sore eyes.

Oil: The greenish yellow oil obtained from the calophyllum seeds was used as alternative to candlenut oil in lamps. It may be used for hair oil. It was also used to furnish wooden bowls¹⁶ and for cosmetic and topical applications for healing of burns and skin diseases. As calophyllum oil is a significant topical healing agent with skin healing, anti-inflammatory¹⁷, antimicrobial properties¹⁸. The oil can also used for soap making.

Oil contains benzoic and oxi-benzoic acids. Small amount of vitamin F and phosphor-aminolipids come along with glycerides and saturated fatty acids. The plant contains free fatty acids, glycerides and steroids (canophyllal, canophyllol. Canoophylllic acids).

Filtered calophyllum oil is applied to wounds possesses the capacity to promote the formation of new tissue, thereby accelerating healing and growth of healthy skin. This process of forming new tissue is known as 'ciatrisation'¹⁹ The oil is a widely used as a traditional topical aid. In costal area some peoples uses calophyllum oil for applying to cuts, scrapes, soriasis, diabetic sores, anal fissures, sunburn, dry or scaly skin, blisters and to relieves sore throat when it is applied topically to the neck. The oil also demonstrates pain relieving properties and has been used traditionally to relieve neuralgin, rheumatism and sciatica.

The calophyllum oil also demonstrates anti-inflammatory activity which has 4-phenycoumarin calophylloidea²⁰ and a group of xanthenes including dehydrocycloguanandin, calophyllin-B, jacareubin, mesuaxanthone-A, mesuaxanthone-B and euxanthone. These all xanthenes explains reductions of rashes, sores, swelling and abrasions with topical applications of the oil²¹.

Xanthenes and coumarins in calophyllum oil demonstrate anti-oxidant properties, specifically inhibiting lipid peroxidation. The antioxidant property of oil helps to protect skin cells from damage by reactive oxygen species.

Leaves: The opposite leaves are dark green, shiny and hairless with broadly elliptical blades 10-20cm long 6-9cm wide. Both the tip and base of leaf are round.

The leaves are soaked in water yield bluish colour and natural scent is applied to inflamed eyes²². They contains friedlein and triterpenes of the friedlin group namely calophyllal, calophyllol and calophyllic acid.

De oiled Cake: It constitutes flavonoids, uranoflavonoids, and furan derivatives and is used in treating skin diseases and in bio pesticide.

The meal cake can be used as fertilizer, pesticide and used for organic farming. Seed shells can be used as combustibles.

Gum: The gum extracted from the plant (from wounded bark) is emetic and purgative but also has use for the treatment of wounds and ulcers.

Material and Methods

Seed Material: The fresh seeds are collected from wild region of Kokan (Dapoli, Ratnagiri, Harihareshwar) of Maharashtra state, India. The seeds are selected according to their conditions where damaged seeds are discarded and the good conditioned seeds are cleaned. De-shelled and dried at higher temperature at 100-105°C for 30min in oven. Then seeds are proceed for oil extraction through mechanical expeller at room temperature.

Pretreatment: Filtered calophyllum oil if first taken to remove moisture. As water content of the feedstock is critical parameter and should be kept below 0.06% w/w for better conversion of oil to esters.²³ Hence the raw oil is kept in an oven at 105°C for 2-3hrs to remove the water content from the oil.

After demoiature, detoxification with 1% hydrochloric acid (HCL) is carried out. The parameters present in trace quantity like carbon residue, unsaponifiable material and fiber etc. are removed. The oil was the processed for property testing are were shown in table-1

The free fatty acid content of raw oil and products after reactions were determined by standard titrimetry methods (ASTM-664). The fatty acid composition of calophyllum inophyllum oil was obtained from gas chromatography-flame ionization detector (GC-FID) and is shown in table -2.

Esterification: Calophyllum oil contains 19.58% free fatty acids. The methyl ester is produced by chemically reacting Calophyllum oil with an alcohol (methyl), in the presence of catalyst. A two stage process is used for the transesterification of Calophyllum oil. The first stage (acid catalyzed) of the process is to reduce the free fatty acids (FFA) content in Calophyllum oil by esterification with methanol (99% pure) and acid catalyst sulfuric acid (98% pure) in one hour time at 57°C in a closed reactor vessel. The Calophyllum oil is first heated to 50°C then 0.7% (by wt. of oil) sulfuric acid is to be added to oil and methyl alcohol about 1:6 molar ratio (by molar mass of oil) is added. Methyl alcohol is added in excess amount to speed up the reaction. This reaction was proceeding with stirring at 650 rpm and temperature was controlled at 55-57°C for 90 min with regular analysis of FFA every after 25-30 min. When the FFA is reduced upto 1%, the reaction is stopped. The major obstacle to acid catalyzed esterification for FFA is the water formation. Water can prevent the conversion reaction of FFA to esters from going to completion²⁴. To achieve acceptable percentage of FFA, we performed this stage two times. After dewatering the esterified oil is fed to the transesterification process²⁵.

Experimental set up: The experimental set up is shown in figure 7. A 2000 ml three necked round-bottom flask was used as a reactor. The flask was placed in heating mantle whose temperature could be controlled within ± 2 °C. One of the two side necks was equipped with a condenser and the other was used as a thermo well. A thermometer was placed in the thermo well containing little glycerol for temperature measurement inside the reactor. A blade stirrer was passed through the central neck, which was connected to a motor along with speed regulator for adjusting and controlling the stirrer speed.

1000ml of esterified Calophyllum oil was measured and poured into a 2000 ml three necked round bottom flask. This oil was heated upto 60°C. In 250ml beaker a solution of potassium methoxide was prepared using 0.5 wt.% sodium hydroxide pellet with 1:6 molar ratio of oil to methanol. The solution was

properly stirred until the potassium hydroxide pellet was completely dissolved. The solution was then heated upto 60 °c and slowly poured into preheated oil. The mixture was stirred vigorously for one and half hour. Finally FFA was checked and mixture was allowed to settle for 24 hours in a separating funnel. Thereafter, upper layer biodiesel was decanted into a separate beaker while the lower layer which comprised glycerol and soap was collected from the bottom of separating funnel. To remove any excess glycerol and soap from the biodiesel, hot water was used to wash it and then allowed it to remain in separating funnel until clear water was seen below the biodiesel in the separating funnel. The P^H of biodiesel was then checked. The washed biodiesel sample was then dried by placing it on a hot plate and excess water still in the biodiesel removed²⁶. The process flow chart is shown in scheme-1.

To achive highest yield of Calophyllum Oil Methyl Ester (COME) we have carried out some experiments with variation in catalyst concentration like 0.5%, 1.0% and 1.2%. The yield results are shown in graph-1.

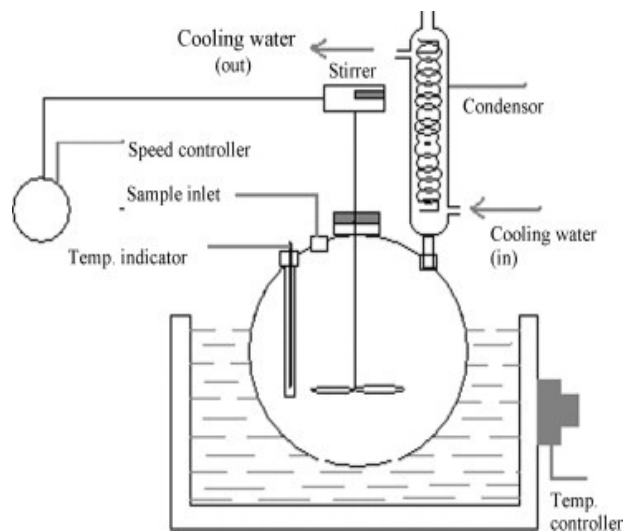
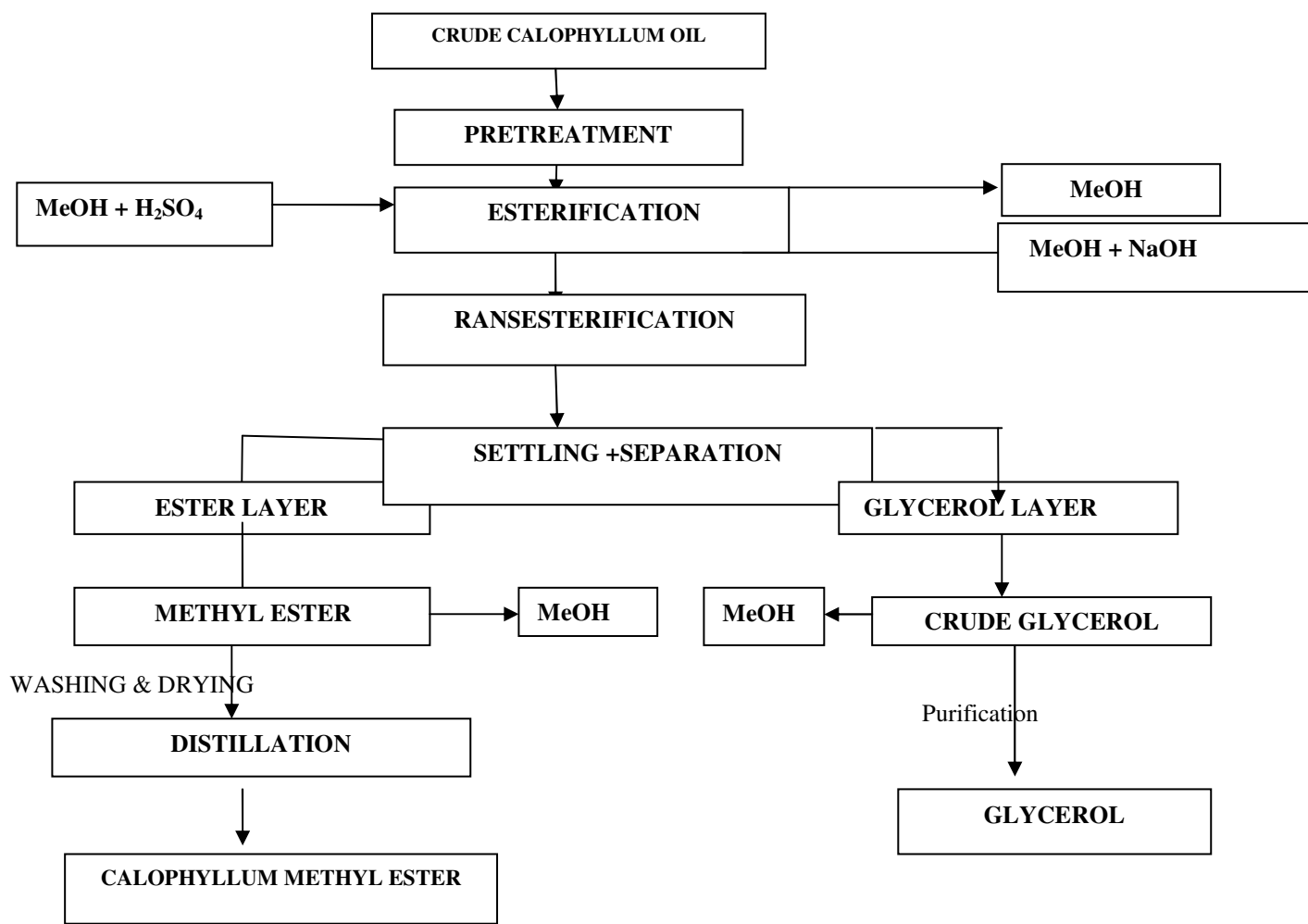


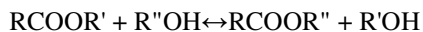
Figure-7
 Experimental Set up for transesterification of calophyllum crude oil



Scheme-1
 Experimental setup for preparation of Calophyllum methyl ester (Biodiesel)²⁷

Transesterification Reaction: Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except an alcohol is used instead of water.²⁷

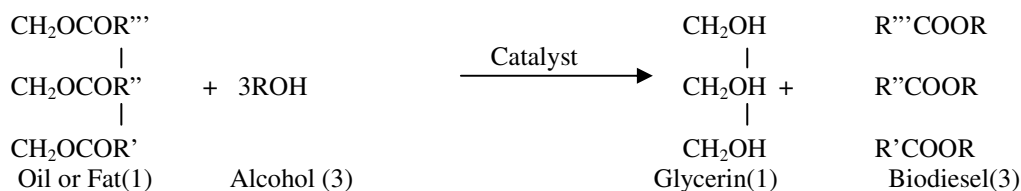
This process has been widely used to reduce the high viscosity of triglycerides. The transesterification reaction is represented by the general equation as below



Scheme-2

General equation of transesterification

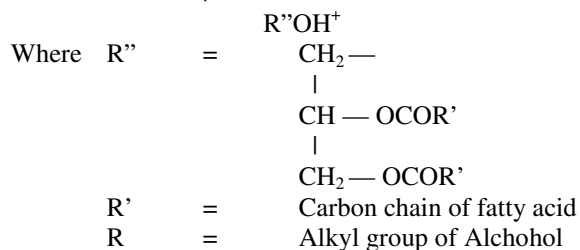
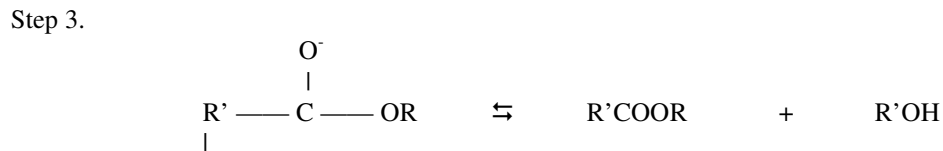
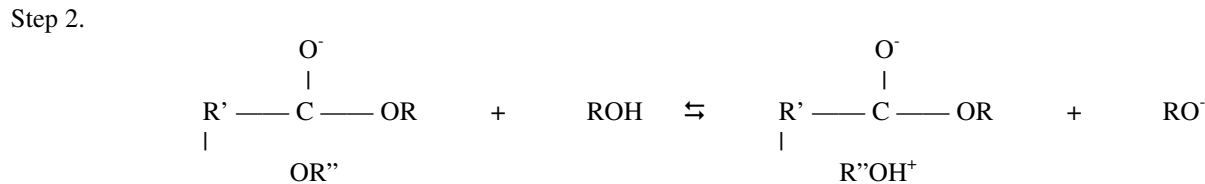
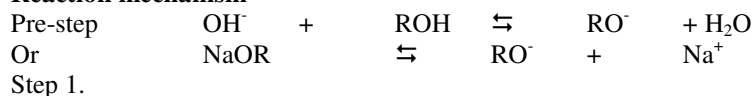
Some feedstock must be pretreated before they can go through the transesterification process. Feedstock with less than 5 % Free Fatty Acid, may not require pretreatment. When an alkali catalyst is added to the feedstock's (With FFA > 5 %), the Free Fatty Acid react with the catalyst to form soap and water as shown in the scheme-3.



Scheme-3

General equation for methanolysis of triglycerides

Reaction mechanism –



Scheme-4

Mechanism of base catalyzed transesterification

If methane is used in this process it is called methanolysis. Methanolysis of triglyceride is represented:

Transesterification is one of the reversible reactions. However, the presence of a catalyst (a strong acid or base) accelerates the conversion. In the present work the reaction is conducted in the presence of base catalyst²⁸. The mechanism of alkali-catalyzed transesterification is described below. The first step involves the attack of the alkoxide ion to the carbonyl carbon of the triglyceride molecule, which results in the formation of tetrahedral intermediate. The reaction of this intermediate with an alcohol produces the alkoxide ion in the second step. In the last step the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride. The same mechanism is applied to diglyceride and monoglyceride. The reaction mechanism of transesterification is shown in scheme-4.

Results and Discussion

Seed characterization: Fresh seeds contains moisture 12% .

Oil Percentage: The available oil percentage in calophyllum seeds is 55-75%. As per our practical trial, we recorded 52% of oil.

Physico- chemical Properties: The fresh extracted crude oil is greenish yellow and it get darkened during the storage. The oil having disagreeable odor and bitter taste. All properties are given in table-2 and were carried out as per American Standards' For Testing and Material (ASTM)- 6751.

Table-1

Physico-chemical Properties of Calophyllum inophyllum oil

Property	Unit	Value
Color	-	Greenish yellow
Odor	-	Disagreeable
Density at 15°C	gm/cc	910
Kinematic Viscosity at 40°C	cst	38.17
Free Fatty Acid	mgKOH/g	28.16
Moisture	%	12%
Saponification Value	-	203
Calorific Value	MJ/KG	32.50
Specific Gravity	-	0.908
Flash Point	°C	224
Fire Point	°C	253

The compressibility effect of the vegetable oil causes an earlier injection of fuel into the engine cylinder as compared to diesel fuel²⁹. This earlier injection does not play an important role, as the injection advance difference is at maximum 1°CA even for the neat vegetable oil³⁰.

The major difference occurs in atomization process, i.e. the mean droplet size of vegetable oil is much higher than diesel fuel³¹. This is because high viscosity (38.17Cst) and low volatility of vegetable oils lead to difficulty in atomizing the fuel and in mixing it with air.

Free fatty acid present in Calophyllum oil: Extracted oil consisted of pure triglyceride and rests were free fatty acids and lipid associates, which is the measure of Unsaponifiable matter.

Yield: Alkaline transesterification converts triglycerides in the oil to their respective methyl ester. Parameters that are to be optimized for alkaline transesterification are molar ratio and amount of catalyst. The rate of stirring, temperature and time was kept constant that are 650rpm, 65°C and 90min. Molar ratio employed during alkaline transesterification were 4:1, 6:1, 8:1 and 10:1. Out of the different molar ratios tried, 8:1 molar ratio gives high yield of biodiesel from calophyllum oil with 1.2% catalyst concentration. The used catalyst, potassium hydroxide which was separated easily from the mixture owing to soft nature of potassium soaps. The KOH amount was varied as 0.5wt%, 1.0wt% and 1.2wt%. The amount of KOH was needed

as a catalyst was 1.2wt% for calophyllum oil (figure-1). Potassium hydroxide, as a catalyst has an advantage over sodium hydroxide as the former is easily soluble in methanol. It is also suggested that waste stream occurring from the biodiesel purification (brine solution) while using KOH as catalyst may act as a fertilizer for soil due to potassium content.³² Temperature of 65 ±0.5°C was optimum for the best conversion of biodiesel. 650 rpm was found to be sufficient for mixing of methanol and oil phase resulting in better conversion. 90 minutes reaction time resulted in completion reaction with high conversion of fatty acid methyl esters of calophyllum oil. Beyond this value, no significant increase in conversion was observed.

Table-2

The Fatty acid composition of Calophyllum oil

Fatty acid name	Carbon number	Composition (%)
Lauric Acid	C12:0	0.75
Myristic Acid	C14:0	0.75
Palmitic Acid	C16:0	14.400
Heptadecanoic Acid	C17:0	0.110
Stearic acid	C18:0	15.570
Palmitoleic Acid	C16:1	0.246
Cis-10 Heptadecanoic Acid	C17:1	0.038
Oleic acid	C18:1n9c	34.410
Cis- 11 Ecosenoic Acid	C20:1	0.794
Linoleic Acid	C18:2n6c	28.343
Alpha- Linolenic Acid	C18:3n3	0.150
Gamma- Linoleic Acid	C18:3n6	0.238
Cis-11, 14, 17- Eicosatrienoic Acid	C20:3n3	0.249
Cis- 5,8,11,14,17- Eicosapentaenoic Acid	C20:5n3	0.066
Cis- 13,16- Docosadienoic Acid	C22:2	0.422

Properties of Calophyllum Methyl Ester: Table-3 shows the fuel properties of biodiesel determined as per ASTM standards. Among the general parameters for biodiesel, the viscosity controls the characteristics of the injection from the diesel injector. The viscosity of vegetable oil derived biodiesel can go to very high levels and hence it is important to control it within acceptable level to avoid negative impact on fuel injector system performance. Therefore viscosity specifications proposed are nearly same as that of the diesel fuel. It is further reduced with increase in petroleum diesel amount in the blend.

Table-3

Properties of Calophyllum Methyl Ester as Per ASTM 6751 Standard

Parameter	Calophyllum Oil Methyl Ester (COME)
Density (gm/cc),15°C	0.892
Kinematic viscosity (cSt), 30°C	3.87
Flash Point (°C)	176
Fire Point (°C)	182
Acid value	0.702
Moisture (%)	0.01
Gross Calorific value (MJ/KG)	37.18

The density of biodiesel from calophyllum oils meets the requirements of ASTM biodiesel is 0.892 gm/cc. Although the density of COME is slightly higher, but still included in the ASTM standard biodiesel making biodiesel produced viable. Differences related to the density of fatty acid composition and degree of purity of the biodiesel.

Flash point of a fuel is the temperature at which it ignites when exposed to a flame or spark. The flash point of biodiesel is higher than the petro diesel, which is safe for transport purpose.

The above listed fuel properties from experimental results indicate that the Calophyllum oil methyl ester (COME) is the best suited as per American Standards for Testing & Material (ASTM) norms for using as biodiesel in pure as well as in blending form.

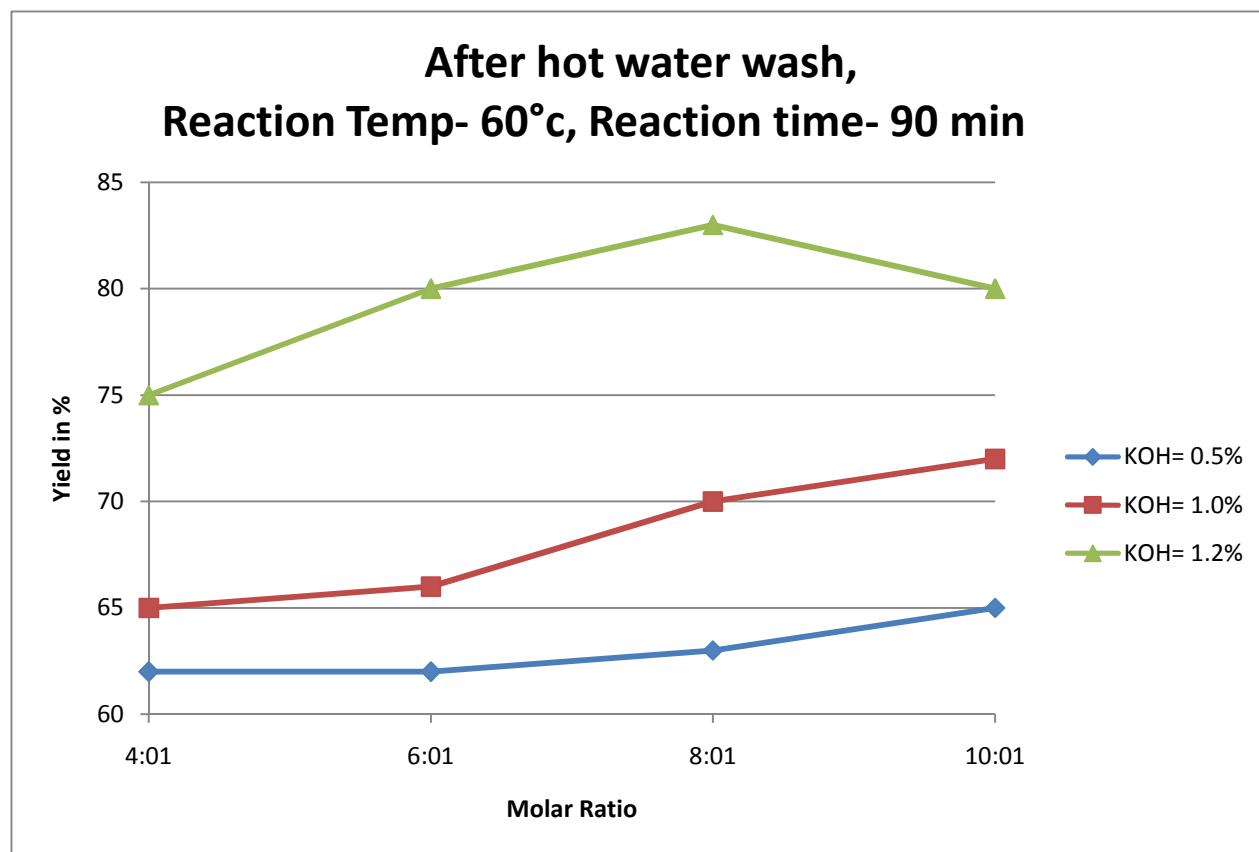
Conclusion

The Calophyllum oil exhibited good physico chemical properties and could be used as a biodiesel feedstock and as a

industrial application. The way of reducing the biodiesel production costs is to use less expensive feedstock containing free fatty acids, such as non edible oils. With no competing food uses, this characteristic turns attention to calophyllum inophyllum linn which grows in costal area of in our country. The production of biodiesel from this oil may provide a valuable local, regional and national benefit. Calophyllum tree can be planted as an ornamental tree like Pongamia pinnata in the gardens, on road sides, railway track. IBDC, Baramati is working for plantation of these non edible oil trees (diesel trees) through NGO. To develop biodiesel into an economically important option in India, it is required to work on biological innovations to increase the yield & minimize the gestation period of Calophyllum inophyllum linn tree.

Acknowledgment

The authors are acknowledge Indian Biodiesel Corporation, Baramati for their laboratory and field support as well as for financial support.



Graph-1

Graph represents observed yield of COME with variation in molar ratio and KOH concentration

References

1. Zaltica J.P., *Fuel*, **87**, 3522 (2008)
2. Felizardo P., Neiva Correia M.J., Raposo I., Mendes J.F., Berkemeier R. and Bordado J.M., *Waste Manage*, **87**, 26 (2004)
3. Bobade S.N. and Khyade V.B., Preparation of Methyl Ester (Biodiesel) from Karanja (Pongamia Pinnata)Oil, *Res. J. Chem. Sci.*, **2(8)**, 43-50 (2012)
4. Bobade S.N., Kumbhar R.R. and Khyade V.B., Preparation of Methyl Ester (Biodiesel) from Jatropha Curcus Linn Oil, *Res. J. A. F. Sci.*, **1(2)**, 12-19 (2013)
5. Puhan S., Vedraman N., Rambrahman B.V. and Nagrajan G., Mahua (Madhuca indica) seed oil: A source of renewable energy in India, *J. Sci.Ind. Res.*, **64**, 890-896 (2004)
6. Deshpande D.P., Urunkar Y.D. and Thakare P.D., Production of biodiesel from castor oil using acid and base catalysts, *Res. J. Chem. Sci.*, **2(8)**, 51-56 (2012)
7. Mishra S.R., Mohanty M.K., Das S.P. and Pattanaik A.K., Production of biodiesel (Methyl Ester) from Simarouba Gauca oil, *Res. J. Chem. Sci.*, **2(5)**, 66-71 (2012)
8. El-Mashad H.M., Zhang R. and Roberto J.A., *Biomass Engineering*, **99**, 220, (2008)
9. Kadambi K., The silviculture of Calophyllum inophyllum Linn, *Indian For.*, **83**,559-562 (1957)
10. National Council of Applied Economic Research (1965)
11. Venkanna B.K., Venkataramana Reddy, Biodiesel production and optimization from Calophyllum (hanne oil)-A three stage method., *Biosource Tech.*, **100**, 5122-5125 (2009)
12. Adeyeye A., Stadies on seed oils of Garcinia kola and Calophyllum inophyllum, *Journal of Science of food and Agriculture*, **57**, 441-442 (1991)
13. Hemavathy J. and Prabhakar J.V., Lipid composition of calophyllum inophyllum in kernel. *Journal of Americal Oil Chem. Soc.* **67(12)**, 955-957 (1990)
14. Kadambi K., The silviculture of Calophyllum inophyllum Linn, *Indian For.*, **83**, 559-562 (1957)
15. Mueller-Dombois D., Fosberg F.R, *Vegetation of the Tropical Pacific Island*, Springer-Verlag, New York (1998)
16. Little E.L. Jr. and Skolmen R.G, *Common forest trees of Hawaii (Native and Introduced) Agricultural Handbook No 679*. USDA Forest service, (1989)
17. Uphof J.C., *Directory of economic Plants*, Verlag von, J. Cramer, Lehre, Gremany (1968)
18. Arora R.B., Mathur C.N. and Seth S.D., Calophylloide, a complex coumarin anticoagulant from Calophyllum inophyllum linn, *Journal of Pharmacy and Pharmacology*, **14**, 534 (1962)
19. Sundaram B.M., Gopalkrishnan C, and Subramanian S., Antibacterial acitivity of xanthones from calophyllum inophyllum linn., *Arogya journal of health science*, **12**,48-49, (1986)
20. Petard P., Tahiti-Palynesian medicinal plants and Tahitian remedies, Noumea, New Caledonia, South Pacific Commission, (1972)
21. Bhalla T.N., Saxena R.C., Nigam S.K., Kisra G. and Bhargava K.P., Calophylloide, a new nonsteriodal anti-inflammatory agent, *Indian J. Med. Res.*, **72**, 762-5 (1980)
22. Nandkarni K.M., Nandkarni A.K., *Indian material Medica-with Aurvedic, Unani-Tibbi, Siddha, Allapathic, Homeopathic, Naturopathic and Home Remedies.* **2**, Popular Prakashan Private Ltd, Bombay, India (1999)
23. Givindchari T.R., Viswanathan N., Pai B.R., Rao R. and Srinivasan M., Triterpenses of calophyllum inophyllum linn, *Tetrahedron*, **23(4)**, 1901-1910, ISSN 0040-4020 (1967)
24. Bryan R.M., Biodiesel production, properties and feedstocks:Invited review, In vitro cell Div. Biol. Plant, **45**, 229-226
25. Sanz S., Nogh G.C., Rozita Y, An overview on Transesterification of natural oils and fats, *Biotechnology and Bioprocess Engineering*, **15**, 891-904 (2010)
26. Gerpen J.V., Biodiesel production and fuel quality University of Idaho, Moscow,1-12, (2003)
27. Tapasvi D., Wisenborn D., Gustafson C., Process model for biodiesel production from various feedstocks, *Trans, ASAE*, **48 (6)**, 2215-2221, (2005)
28. Keim G.I. and Newark J.N.,Treating the fats and fatty oils, US patents No- 2383601 (1945)
29. Varde K.S., Bulk modulus of vegetable oil-diesel fuel blends, **63**,713-5, (1984)
30. Rakopoulos C.D., Antonopoulos K.A., Rakopoulos D. C., Comparative performance and emissions study of a direct injection diesel engine using blends of diesel fuel with vegetable oils or bio-diesel of various origins, *Energy Conversion and Management*, **47**, 3272-3287, (2006)
31. Kamimoto T, Matsuoka S., Prediction of spray evaporation in receiprocating engine, *SAE*, (1977)
32. Tremblay A.Y., Cao P., Dube M.A., 'Biodiesel production using ultra low catalyst concentrations, *Energy and Fuels* (22) 2748-2755 (2008)