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Biodiesel production from *Madhuca indica*: A potential species for semi-arid lands of Hyderabad-Karnataka region

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Abstract
The increasing demand of fuels is getting vital importance in search of alternative fuels to the depleting fossil fuel is renewable fuels. The research on searching for renewable energy sources getting the more importance for energy security. Mahua a biodiesel species also gained role in the biodiesel production. The present investigation was carried out on Mahua species by oil extraction and its biodiesel conversion. The single stage transesterification process was used for the production of biodiesel. The results revealed that the FFA and oil content of the Mahua were found to be 2.86±0.20 and 37.34±0.57. The biodiesel and glycerine obtained by transesterification was found to be 889.9±1.43 and 203.5±0.83. The fuel properties of Mahua biodiesel was met the ASTM and BIS standards. The biodiesel used for running the local autos and cars and their opinion was good and mileage also enhanced compared with the normal fossil fuels. Seed cake used as manure for the crops development.

Keywords: *Madhuca indica,* Mahua, biodiesel, transesterification, glycerine, seed cake

Introduction
Biofuels is getting an ideal attention in worldwide as substitutes for the fossil fuels because of the scarcity and polluting nature. The term biofuel means liquid or gaseous fuel obtained from the biological material either from plant, animal or microbial process. The term biofuel includes biodiesel, bioethanol and biogas. Biodiesel is the first renewable fuel to succeed in the evaluation of emission results and a potential health effect submitted to the U.S. Environmental Protection Agency (EPA) and is the major reason to promote the biodiesel production in worldwide.
Biodiesel is a renewable energy for diesel engines which are produced by the transesterification process of vegetable oils or animal fat with the use of methanol or ethanol and a catalyst NaOH or KOH. The biodiesel production cost is more compared with the available fossil fuels because of the renewable resources feedstock (Naik *et al.*, 2010) [8]. But, the renewable sources are having more advantages than the fossil fuels such as reduction in carbon dioxide emission and global warming, good mileage, low consumption of biodiesel fuel (Demirbas, 2009; Selvakumar *et al.,* 2013 and 2016) [2, 6, 9]. Many researchers have worked on biodiesel production from TBOs such as *Pongamia*, Neem, *Jatropha* and other waste seeds in India (Kaul *et al.,* 2003; Doddabasawa *et al.,* 2014) [7, 3]. Mahua is also a best suitable tree in the semi-arid region of Hyderabad-Karnataka region and Mahua contribution was around 164200.43 mt (Ghadge *et al.,* 2005) [10]. The Mahua trees which are available in the north-Karnataka region and their seeds were purchased from the farmers and used for biodiesel production and its quality analysis.

Materials and Methods
Sample Collection
Mahua seeds were purchased from the local farmers of Hyderabad-Karnataka region and carried to the Biofuel Information and Demonstration centre, College of Agriculture, Bheemarayanagudi. Mahua seeds were used for the extraction of oil by oil expeller and solvent extraction method and biodiesel production by transesterification reaction and its quality analysis.

Oil Extraction
Mahua seed oil was extracted by both solvent extraction process and mechanical expeller which was manufactured by the Malnad Extraction Industries Shivamoga, Karnataka.

Solvent extraction

The cleaned and good quality Mahua seeds were first powdered using a mechanical grinder and then 100gms of sieved powder was packed in Whatman filter paper and kept in solvent extraction chamber. 350ml of petroleum ether (60-80 ^{0}C) was taken in a 500ml round bottom flask as a solvent. The oil extraction was done by heating the solvent @ 70 ^{0}C for 120 minutes. The oil content obtained was recorded and used for analysis of lipid components by TLC, FFA content and Biodiesel production.

Oil Expeller

Around 10kgs of Mahua seeds were crushed using the oil expeller (Manufactured by Malnad Biodiesel extraction Industries, Shimoga-Karnataka). The oil content and seed cake obtained were recorded and the seedcake was passed it again for 2 times to remove the oil completely from the cake. Then oil was filtered using micro-filters, further the oil used for the Free Fatty acid calculation and biodiesel production as per method of Doddabasawa *et al.*, 2014.

Thin layer chromatography (TLC)

Silica gel slurry was prepared using 0.02M sodium acetate buffer and spread on TLC plate having 250µm thickness. The TLC plate was activated by heating at 105° C for 30 minutes. 5µl of mahua seed oil sample extracted and olive oil as a standard for comparing the Rf values which were spotted on the TLC plates. The spots were dried in air and were kept in a TLC chamber containing Petroleum ether: Diethyl ether: Acetone (90:10:1). TLC plates were kept for lipid components separation for around 30-40 minutes and the spots were visualized by spraying the 0.2% Ninhydrin reagent and then the Rf values were calculated.

Biochemical Properties

Specific Gravity (AOAC 2000)

The *Mahua* oil which was extracted from oil expeller was filled in 500 ml measuring cylinder and the hydrometer was dipped in the oil and the density of oil was recorded. The specific gravity of the oil was calculated by using the following formula:

Specific gravity= density of oil /density of water.

Free fatty acid (FFA) content (AOAC, 2000)

The Mahua oil which was extracted from the oil expeller was titrated by using 0.1 N NaOH freshly prepared solution in a clean burette. Simultaneously, 30ml of isopropanol was taken in a clean 250ml conical flask and 3-4 drops of 0.1 N NaOH (neutralization) and then 10gms of oil sample was added to the flask and then heated to 50^{0}C and allowed the solution to cool and then 5-6 drops of phenolphthalein indicator was added and titrated against the 0.1 N NaOH until the colour turns from yellow colour to permanent pink colour (Doddabasawa *et al.*, 2014) [3]. Repeated the titration for another 2 times and mean burette reading was taken for the FFA calculation. The FFA content was calculated using the formula:

$$\frac{\text{FFA (\%)} = 28.2 \text{ X } 0.1 \text{ N NaOH X MBR}}{\text{gm of oil sample taken}}$$

Where, 28.2–molecular weight of standard oleic acid divided by 10, MBR-mean burette reading

For 0-FFA, NaOH used for preparation of methoxide solution was 3.5 gm (Table 1)

Table 1: FFA concentration and NaoH to be added for biodiesel production

FFA (%)	NaOH (gm)
0	3.5
1	4.5
2	5.5
3	6.5
4	7.5
5	8.5
6	9.5
7	10.5

Biodiesel Production

One litre of *Mahua* seed oil was taken in the 3-neck flask with reflex condenser and was heated to 65 ^{0}C. On the basis FFA percentage, the methoxide solution was prepared by using the formula:

Quantity of Methanol=2.25 X FFA=x value/0.7914=y ml/litre of oil

y ml Xdensity of oil= z ml of methanol/litre of oil

Methoxide solution was prepared by mixing the required quantity of NaOH and methanol as per calculation was added into the 3-neck flask. The transesterification process was done about 90-120 minutes at 65 ^{0}C to obtain biodiesel. The entire reaction mixture was transferred to separating funnel and allowed for settling. After 30-40 minutes two layers were observed. The lower layer was found to be glycerine and the upper layer was found to be biodiesel and later it was used for washing with warm water to remove untreated NaOH and methanol in biodiesel and later moisture content was removed by open drying at 110 ^{0}C for 15 minutes. Finally, the biodiesel was obtained recorded and was used for quality analysis as per ASTM and ES, 2003. The yield of biodiesel was calculated by using the following formula:

Yield of Methyl esters (Biodiesel) = (methyl ester produced in gms /oil used in the reaction in gms) X 100

Qualitative analysis of Biodiesel

Density by Hydrometer method

500ml of *Mahua* biodiesel was poured in 500ml measuring cylinder and allow the biodiesel to settle and it was done at 15 ^{0}C. Hydrometer was placed in the cylinder and the density was recorded at which the surface of the biodiesel touches the stem of the hydrometer.

Kinematic viscosity test at 40 ^{0}C (ASTM D445)

Around 3/4th of *Mahua* biodiesel was added in the cannon–fenske viscometer Tube no. 100 of the viscometer bulb and kept the tube in viscometer water bath and was heated to 40 ^{0}C. Then the temperature maintained for a period of 20-30 minutes till biodiesel attains 40 ^{0}C temperature. The biodiesel was sucked by using sucking bulb till the biodiesel reaches the starting mark in the right side vessel and slowly removed the bulb and simultaneously, stop watch was started and then stopped the watch once flow reaches the bottom mark in the right vessel in a bulb. The time taken from start point to end point was recorded in seconds. The viscosity was calculated by using the formula:

Kinematics viscosity (in cst) = (number of seconds) X Viscometer tube calibration factor

Flash point test (ASTM D93)

The biodiesel was poured in the flash point apparatus up to the mark indicated. The biodiesel was heated and stirred at regular intervals. The flash point was observed by introducing

the external fire. The flash point temperature was recorded.
Calculation:
Flash Point = Noted flash temperature

Copper strip corrosion test (ASTM D130)
Biodiesel was poured in the copper strip corrosion test bomb up to the mark in the bomb. Polished copper strip was immersed in test bomb containing biodiesel. Then biodiesel was kept in incubation for 3 hours and temperature was maintained for 50 ^{0}C ± 1 ^{0}C. After 3 hours the copper strip from the apparatus was removed from the bath and compared with ASTM copper strip corrosion standards.

Results and discussion

Oil Extraction
The percentage of oil obtained by the solvent extraction was found to be 44.46±0.55 for 100gms of seeds. The percent of biodiesel obtained from the commercial oil expeller was found to be 37.34±0.57. The ± values were standard error of the mean.
Thin Layer Chromatography:
The fatty acid profile present in the *Mahua* seed oil sample was identified by TLC method and was compared with standard Olive oil (Fig 1). With reference to the Rf value of the spots identified on the TLC plate saturated fatty acid like Linoleinic acid, Palmatic acid, Stearic acid and unsaturated fatty acid like Oleic acid were identified (Table 2). The Rf values were on par with standard Rf values of olive seeds and it clearly showed that *Mahua* can be used for biodiesel production.

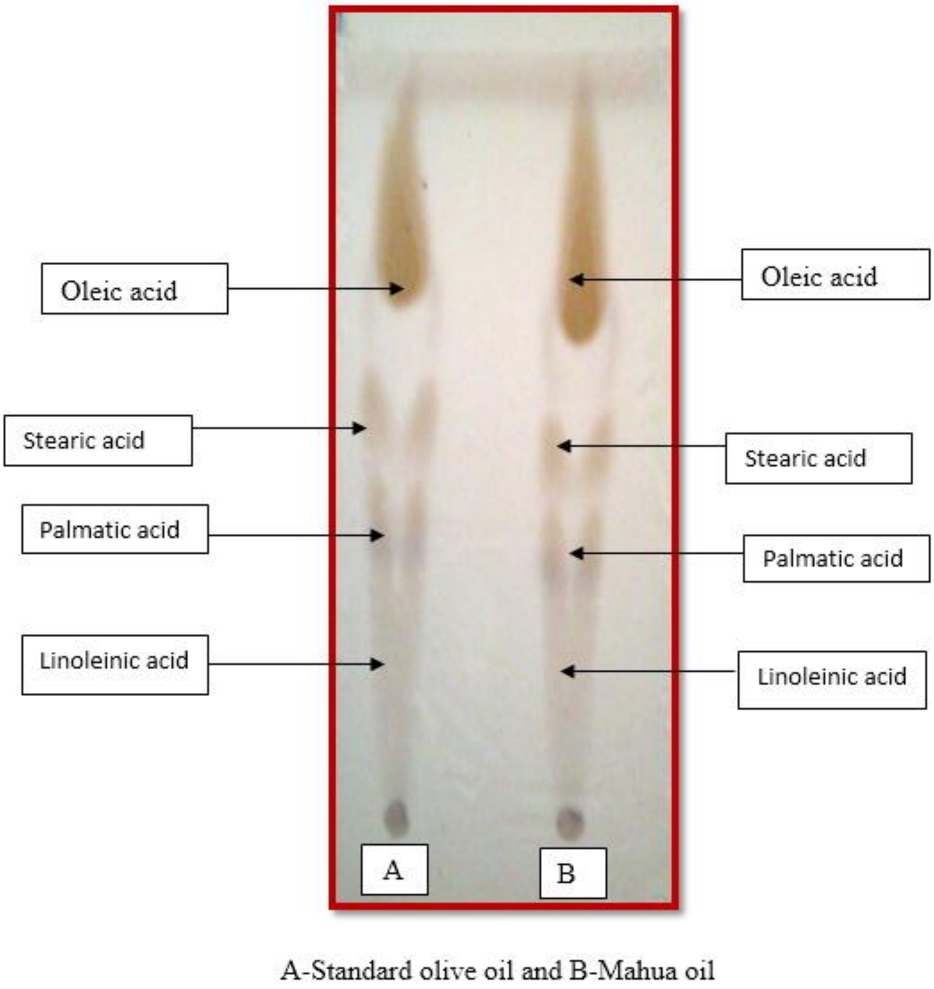

Fig 1: TLC plate showing the Fatty acids in Mahua and standard olive oil.

Table 2: TLC plate showing the fatty acids components present in the extracted oil of *Mahua* and standard olive oil

Spot No.	Fatty acids	R_f of Olive oil sample (Standard sample)	R_f Value of Mahua seed oil sample
1	Linoleinic acid	0.16	0.16
2	Palmatic acid	0.44	0.39
3	Stearic acid	0.73	0.70
4	Oleic acid	0.92	0.93

Physico-chemical properties
The physico-chemical properties of oil such as specific gravity, FFA and biodiesel yield and its quality analysis meets the ASTM and EN standards and were shown in the table 3.

Table 3: Mahua oil and Biodiesel characteristics

Sl No	Parameters	Unit	Mahua
1	Specific gravity of oil	kg/m^3	865.8±0.86
2	Density of Biodiesel at 15 ^{0}C	kg/m^3	824.6±1.07
3	FFA	mg/g	2.86±0.20
4	Oil yield by solvent Extraction unit	%	44.46±0.55
5	Oil yield by mechanical expeller	%	37.34±0.57
6	Biodiesel Yield per litre of oil	%	88.99±1.43
7	Crude glycerine yield per litre of biodiesel	%	20.35±0.83
8	Seed cake per kg of seeds	gm	0.51±0.02
9	Copper corrosion strip test	--	NO.1
10	Viscosity test	mm^2/sec	4.5±0.20
11	Flash point test	^{0}C	159.66±1.20

Conclusion

The utilization of raw materials from the local market or from the farmers plays vital for the generation of income and maintenance of the environmental conditions by utilizing their products and by-products. So, here a successful attempt was made by extracting the oil from *Mahua* seeds was used biodiesel production transesterification process. It is important to know that oil is much cheaper as compared with any other available feedstock for biodiesel. Mahua biodiesel have a better prospectus as its tree has abundant seeds and oil content is also very high. The present study provides vital role of utilization of locally available Mahua feed stocks and their conversion to biodiesel, finally the advantage of this study is income generation to famers and to the country.

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