Fatty Acid Methyl Ester analysis of some oil plants found in Bihar, India: A Comparative study.

Abstract

Today's developmental world needs large amount of energy. Due to the limited fossil fuel source, there is need of some alternate fuel sources among which biodiesel from vegetable oil widely practiced. There is an increasing interest in India to search for suitable low cost alternative fuels that are Eco friendly. Biodiesel is a renewable, biodegradable and non toxic fuel. In this paper an attempt has been made to study and compare the oil percentage and FAME components of three non edible oil seed plants abundantly found in Bihar, India.

Oil from the seed kernel was extracted by solvent extraction technique through Soxhlet apparatus using n-hexane as solvent. Percentage oil content for Jetropha, Mahua and Castor are found around 76, 41 and 33 respectively. Further extracted oil were analysed by GC-MS for their fatty acid methyl ester (FAME) components. Palmitic, Linoleic, oleic are most common fatty acid found among three.

Keywords: *Biodiesel, Jetropha, Mahua, Castor, Soxhlet, FAME, GC-MS, Bihar.*

Introduction

The world is presently undergoing rapid development and thus requires a large amount of energy sources to meet the pace of development and there is need of alternate source of green renewable source of energy. Today, mankind is almost totally dependent on the fossil fuels (coals, petroleum etc.) to provide electricity and transport fuel etc. These sources are, however, non renewable and may run out in the near future. Recently fuel derived from biomass has been receiving increased attention due to the availability of raw materials especially in tropical and temperate zones of world. Bio fuels are gaining increased public and scientific attentions; this can be due to factors such as oil price hike, the need for increased energy security, and concern over greenhouse gas emissions from fossil fuels.

Biodiesel can be produced either from edible or from non edible oils. Most of the edible oils are produced from the crop land. The use of non edible oils for bio diesel production has recently been of great concern because they are eco-friendly and cheap. Disadvantages of using bio diesel produced from agricultural crops involve additional land use, as land area is taken up and various agricultural inputs with their environmental effects are inevitable. Switching to bio diesel on a

large scale requires considerable use of our arable area. If the same thing is to happen all over the world, the impact on global food supply could be a major concern. Currently, more than 95% of the world bio-diesel is produced from edible oil which is easily available on large scale from the agricultural industry. However, continuous and large-scale production of bio diesel from edible oil without proper planning may cause negative impact to the world, such as depletion of food supply leading to economic imbalance. A possible solution to overcome this problem is to use non-edible oil. As the demand for edible oils for food has increased tremendously in recent years, it is urgently required to justify the use of these non edible oils for fuel use purposes such as bio diesel production. Moreover, these oils could be less expensive to use as fuel. Hence, the contribution of non-edible oils such as Jatropha and karanja and Mahua will be significant as a non edible plant oil source for biodiesel production. Several studies have shown that there exists an immense potential for the production of plant based oil to produce biodiesel. Azam *et al*.(2005) studied the prospects of fatty acid methyl esters (FAME) of some 26 non-traditional plant seed oils including Jatropha to use as potential biodiesel in India. Among them, *Azadirachta indica*, *Calophyllum inophyllum*, *J. curcas* and *Pongamia pinnata* were found most suitable for use as biodiesel and they meet the major specification of biodiesel for use in diesel engine. Moreover, they reported that 75 oil bearing plants contain 30% or more oil in their seed, fruit or nut. Subramanian *et al* 2003 reported that there are over 300 different species of trees which produce oil bearing seeds. Thus, there is a significant potential for non-edible oil source from different plants for biodiesel production as an alternative to petro diesel.

- Jatropha, a member of the *Euphorbiaceae* family, is an indigenous plant found in tropical and sub tropical part of America, Africa and Asia (Divakara *et al* 2010).

Jatropha genus account for 175 species with 12 species reported in India. Depending on the geographic location, its common names are Barbados nut Black; vomit nut, Curcas bean, *Kukui haole* Physic nut or *Jungle Erandi* (in India), Purge nut, *Purgeerboontjie*, and Purging nut tree (Barceloux 2008). Among all species of Jatropha, *jetropha curcus* regarded as a nonedible oil crop which finds greater interest in biodiesel production. It is up to 8-15 feet tall tree. It is well adapted to both arid and semi-arid condition. Its oil content ranges from 35% in seed and 50-60% in kernel with oleic (C18:1) and linoleic (C18:2) as its major fatty acids. *Jatropha curcas* is a drought-resistant, pest and disease resistant, about 50 year life expectancy, can be grown in an adverse land situation, require minimum inputs for cultivation and contributes for eco-restoration on all types of wasteland(Siddharth *et al* 2010) .

It has small capsule like round fruit of 2.5-4 cm. Long which becomes dark brown when ripe, splitting of which release 2-3 black seeds of 2cm long (*phanerocotylar*).

Like other species of the Euphorbiaceae family, *J.curcas* also contain highly toxic poisonous substance curcin (a *phytotoxin- Toxalbumin*)(Felke J,1914).Cursing is a ribosome inactivating protein (RIP)(Barbieri *et al* 1993). It has Antihelminthic effect (Jummai *et al*, 2014).

According to National Biodiesel Mission (NBM) India, the nonedible oil seeds like Jatropha are most suited for biodiesel production in India, but unfortunately the seed yield from Jatropha tree is much more less than stipulated, then there is a need of alternate of Jatropha seed oil.

Mahua (*Madhuca indica*), a deciduous tree belongs to *Sapotaceae* family. It is found throughout the tropical and subtropical (mainly in central and north forest) region of the Indian subcontinent. It has socioeconomic values as about 30-40 percent of the tribal economy of India, primarily in northern India such as in Bihar, Madhya Pradesh and Orissa, are dependent on the Mahua flowers and seeds. Moreover, ***Madhuca indica*** **and** ***Madhuca longifolia*** is two important species of Mahua in India, whose seeds are used for extracting yellowish oil (Mahua butter) generally meant for soap production. Mahua flowers are edible, but largely used for producing countryside cheap alcoholic liquor in rural parts of India. Mahua Seed yield ranges from 20-200 kg per tree every year, where oil content is 30-45%.

Neem, Kusum, Pongamia, Mahua seeds are other inedible oil sources for biodiesel production in India. Amongst, Mahua seed oil valued most due to their large availability and potential growth with age, where the estimated production, according to KVIC is 5-lakh tons/year.

Castor / Palma Christi or arand (*Ricinus communis*) is a species that belongs to the *Euphorbiaceae* family. It is a non-edible, poor soil resistant, a perennial oilseed crop that can be grown in tropical, subtropical (wild or cultivated), arid, semiarid region and even on marginal lands, which are not competitive with food production lands of the globe. It can withstand in diverse climatic conditions such as long period of draught, but will thrive under higher rainfall. Castor oil plant, actually originated from Africa, but spread out in many countries of the globe.

At Present, India ranked one in the production as well as in export of Castor oil (60 to 70% of world trade) in the world followed by China and Brazil. In India, it is grown on 713,000 hectares of rain fed land and it yields 850,000 tons of Castor seeds per year. Although, Castor is growing in nearly all provinces of India, but equally a matter of their production, Gujarat (83%) passes over other states followed by south Indian states. India exports 200,000 to 225,000 tonnes of Castor oil and about 15000 tonnes Castor seeds per year Castor seed comprise about 50 to 60 % non edible oil.

Materials and Methods

Extraction of oil from seeds of Jatropha, Mahua and Castor

A. Pretreatment of seeds:-

It is the primitive stage of sample preparation where following steps had been taken in to account.

A. Seed collection:-

The seeds were locally collected from districts of Bihar. (Jatropha seeds from Purnia district, Mahua and Castor from Samastipur District of Bihar) for experimentation and extraction of oil.

B. Drying:-

Seeds were cleaned properly and kernels removed. Kernels dried in an electric oven at 35^0c to reduce moisture to reduce moisture.

C. Grinding:-

It was done by using a mortar pestle to rupture the cell wall so that solute release for direct contact with solvent.

D. Weighing :-

On an electric balance weighed before and after the drying process using Metller weighing machine model no. ML 204 /AO1.

B. Experimental Procedure:-

For the extraction of oil soxhlet apparatus was used.

Procedure

Pretreated fine grinded seed's kernels were put in a known weight of thimble made up of whatmann filter paper no.40. Thimble contains 26.43 gm, 29.47 gm and 16.11 gm respectively of Jatropha, Mahua and Castor grind seeds. Then the thimbles filled with sample were put in the appropriate place inside soxhlet apparatus. 300 ml of n-hexane as solvent was measured using measuring cylinder and then poured into each three round bottom flask of 500ml capacity. Set the temp at 60^0c and heated for 6 hours. After that oil was recovered by solvent evaporation. Then recovered oil were again heated at a low temp to complete evaporation of solvent, leaving behind the solvent **(**Singh *et al* 2009**).**

Characterization of extracted oil

Characterization of oil done using GCMS (Gas chromatography Mass Spectroscopy) **PERKINELMER USA** Model- CLARUS 600.

Determination of fatty acid composition

GC MS STUDY OF FATTY ACID COMPOSITION

Experiments were conducted to find out the fatty acid composition of Jatropha, Mahua and Castor seed oil extracted by soxhlet apparatus. The composition of oil collected were analysed by GC MS model **PERKINELMER USA** Model- CLARUS 600 that equited with different detectors and a capillary column. The fatty acid present in the oil of Jatropha Mahua and Castor were analysed and result were inlisted in table 1.

Result and Discussions

The following results were obtained from the solvent extraction of seed oil.

Table:1 oil percentage of different seeds during experimentation

S. no	Sample oil	Amount of seed taken	Amount of oil extracted	Percentage
1	Jatropha	26.43gm	20.3ml	76.80%
2	Mahua	16.11gm	6.7ml	41.58%
3	Castor	26.47 gm	9.8ml	33.25%

The following chromatogram of oil sample were obtained by using GC – MS analysis

A. **GC – MS analysis Jatropha Seed Oil:-**

the chromatogram of Jatropha seed oil shown two peaks in between 0-100% range. The maximum peak was recorded after 6.5 min of exposure showing peak height 6.3155 where as the other peak recorded in between 5.75-6.00 of exposure showing peak height 5. 3773.

The total number .of fatty acid recorded in chromatogram was 40 which were separated on the basis of their molecule weight.

The different composition of fatty acid in 0-100% range were recorded having molecular weight 54 to max.no.652.

In Table 2 the result of fatty acid composition of Jatropha seed oil having different composition were enlisted showing to 20 fatty acid which can be utilized for transesterification . In Table 2 and 3, the structural composition, chain and bond angles were shown.

Table 2. list of the important fatty acid present in Jatropha

S. No.	Name	M.W.	Formula
1.	Ascorbic acid2,6 Dihexadecanoate	652	$C_{38}H_{68}O_8$
2.	Hexadecanoic acid	568	$C_{16}H_{32}O_2$
3.	Eicosanoic acid	312	$C_{20}H_{40}O_2$
4.	Dimethyl spiro decane	166	$C_9H_{15}N_3O_2$

A. **GC-MS analysis of Mahua seed oil:-**

Mahua seed oil extracted were analysed for the fatty acid composition throught using GCMS model no **PERKINELMER USA** Model- CLARUS 600.

The chromatogram of Mahua seed oil shown three peak at different time interval ,the maximum peak of 6.2755 is between time interval of 6.25-6.75 were recorded which were 100%.the middle peak was recorded in between 5.85+6.00 range of time interval showing peak size 5.8773 approximately 68% and minimum peak point were recorded at the time interval of 6.51-6.55 having peak size of 6.3155.

The total number of fatty acid were recorded 40 Separated on the basis of molecular weight and number of carbon .The five major fatty acid recorded in Mahua oil were Ascorbic acid2,6 Dihexadecanoate , Tetracosanoic acid, Heptadecanoic acid, Tricosenyl formate and Octadecanoic acid having different molecular weight and formula.

Table 3. List of the important fatty acid present in Mahua.

S. No.	Name	M.W.	Formula
1.	Ascorbic acid2 ,6 Dihexadecanoate	652	$C_{38}H_{68}O_8$
2.	Tetracosanoic acid	368	$C_{24}H_{48}O_2$
3.	Heptadecanoic acid	270	$C_{17}H_{34}O_2$

4.	Tricosenyl formate	366	$C_{24}H_{46}O_2$
5.	Octadecanoic acid	884	$C_{18}H_{34}O_2$

A. **GC-MS analysis of Castor seed oil:-**

Experiments were conducted to find out fatty acid composition of Castor seed oil .

The running time of the sample in time scale was 0-7 m and the percentage of peaks of fatty acid were calculated in 0-100% scale .The several peaks were recorded in chromatogram of Castor seed oil. maximum peak of 6.755 was recorded showing 100% result in scanEL+TIC 8.5209 at terminal of 6.25-5.50m.just before that the minimum peak of 6.15398 was recorded at time interval of 6.15 to 6.25 time interval. The 2 peak were also recorded showing peak high approximately 30% time interval 5.75-6.00 and another at the time interval of 6.50-6.60 showing 1st peak height 5.8675 and another 6.3155.the fatty acid composition of Castor seed oil recorded 20 according to their molecular weight and carbon number.

Table 4 shows the major fatty acid present in Castor oil. The molecular weight and formula were also mentioned into the table.

Table 4. list of the important fatty acid present in Castor

S. No.	Name	M.W.	Formula
1.	Ascorbic acid 2,6 Dihexadecanoate	652	$C_{38}H_{68}O_8$
2.	Hexadecanoic acid	568	$C_{16}H_{32}O_2$
3.	Eicosanoic acid	312	$C_{20}H_{40}O_2$
4.	Dimethyl spiro decane	166	$C_9H_{15}N_3O_2$

Conclusion

Standard sampling and analytical techniques have been used to generate primary and secondary data by using instruments viz. Soxhlet apparatus and GC-MS. The data generated during this research work has been presented in to table 1-4 . Jatropha, Mahua and Castor seeds were used for extraction of oil in which the best result was obtained from Jatropha seed . The percentage oil extracted from Jatropha oil was 76.80% followed by Mahua where the oil percentage was

41.58% and the minimum oil percentage i.e .33.25% in Castor. Oils of different species under investigation when exposed to open air and sunlight for a long time would affect the fatty acid concentration. Most common acid among three investigated oil sample Ascorbic acid2,6 Dihexadecanoate was most prominent in terms of concentration followed by Tetracosanoic acid and Heptadecanoic acid.Among three oil samples, ricinoleic acid was only found in Castor oil.

Fatty acid composition of different species was studied by using GC MS .The important fatty acid produced in GC MS of Jatropha oil were Ascorbicacid2,6 Dihexadecanoate , Hexadecanoic acid, Eicosanoic acid, Dimethyl spiro decane having molecular weight 652,568,312 and 166 respectively. The important fatty acid produced by Mahua oil was Ascorbic acid2,6 Dihexadecanoate , Tetracosanoic acid, Heptadecanoic acid, Tricosenyl formate and Octadecanoic acid having molecular weight 652,368,270,366 and 884 respectively. Similarly the fatty acid composition of Castor oil was Ascorbic acid2,6 Dihexadecanoate, Tetracosanoic acid, Heptadecanoic acid, Tricosenyl formate, Octadecanoic acid having molecular weight 652,568,312 and 166 respectively.

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