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# Glycerides extraction and characterization from the seed oil of Terminalia chebula plant found in Manipur

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## ABSTRACT

**Seed oil of *Terminalia chebula*was extracted by Solvent extraction technique on the crushed kernel using petroleum ether as the solvent. The oil was purified prior to further analysis by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent. The purified oil was further transesterified giving the final product known as biodiesel. The biodiesel is also known as the fatty acid methyl ester (FAME). Fatty acid methyl ester (FAME) composition of Terminalia chebula was determined by NMR, IR and GC-MS analysis. The FAME of Terminalia chebula consists of 69.79 wt.% of methyl palmitate (C16:O), 9.82 wt. % of methyl oleate (C18:1), 20.38wt.% of methyl stearate (C18:O).**

**Keywords: Biodiesel, Manahi, Non-edible, *Terminalia chebula*, transesterification, Yellow myrobalan**

## INTRODUCTION

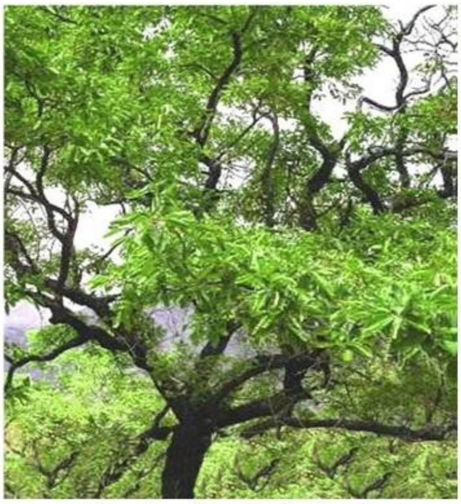
Most natural fats or oils consist of mixed triglycerides, which can be either saturated or unsaturated. Triglycerides find applications in various industries, including soap manufacturing, paint and varnish production, synthetic detergents, glycerol, high molecular weight acids, vanaspati ghee, oil cloth, printing inks, hair oil, candles, polishes, medicine, and drugs for conditions like heart diseases, strokes, and diabetes. These triglycerides serve as a storage form of energy and are crucial energy sources for transportation.

In the North-Eastern Indian state of Manipur, rich plant diversity is renowned. The region hosts numerous plant varieties producing fruits with seeds that are highly abundant in non-edible oils, growing in both plain and hilly areas. Unfortunately, many of these plants lack apparent economic value, leading to limited commercial use and their gradual disappearance. The neglect by farmers, government, and both public and private sectors contributes to the decline in plant diversity. Furthermore, there is a concern that large-scale biodiesel production from edible oils could disrupt the global food supply and demand balance. Thus, utilizing non-edible oils for biodiesel production could help preserve edible oils for other essential industries.

Biodiesel has gained global attention due to its renewability, biodegradability, carbon neutrality, and non-toxicity, making it a preferable alternative for diesel engines. The absence of sulphur in biodiesel contributes to lower air pollution levels. Its use has the potential to reduce pollutants, potential carcinogens, and promote a balance between agriculture, economic development, and the environment. Developed countries such as Brazil, Indonesia, Malaysia, USA, UK, Germany, and Canada have already adopted biodiesel blended with Petro-diesel. The identification of fatty acid constituents in glycerides is crucial in this context.

Biodiesel is typically composed of methyl esters of long-chain fatty acids, derived from non-toxic biological resources like vegetable oils and animal fats through transesterification with methanol and a catalyst. Catalysts can be acid, base, or enzyme (lipase). The by-products of oil extraction and biodiesel production can be repurposed for organic fertilizer, biogas production, and soap making. Proper utilization of these non-edible oils holds significant potential for rural development, providing employment opportunities for the youth and contributing to infrastructure development in Manipur.

*Terminalia chebula* (Yellow myrobalan) locally called Manahi / Bahera in Manipuri belongs to Combretaceae family. It is a medium to large sized tree with eye-shaped long leaves and flowers in dull white colour (Fig. 1). It is easily recognized by its small size fruits with the five distinct ribs on its body. Each fruit has a single seed that is light yellow in colour. This indigenous tree is widely distributed in Bihar, West Bengal, Assam, Central India and South India including Manipur. It is a deciduous tree growing to 30 m (98 ft) tall with a trunk up to 1m (3 ft.) in diameter. In Manipur fruits are used in chronic ulcers, in toothache and bleeding gums and bark is also diuretic. *Terminalia chebula* seeds give about 30.68 wt.% oil after purification by column chromatography.



**a. *Terminalia Chebula* plant**

**b. Matured fruit of *Terminalia Chebula* plant**

**c. *Terminalia Chebula* seeds**

**Figure: *Terminalia Chebula* plant and seeds**

## MATERIALS & METHODS

### A. Materials

*Terminalia Chebula* were collected from Phouden, Thoubal, Manipur (India) during its availability of the season of June and July. The seeds were first cleaned and dried for 5/6 days in the sunlight, deshelled and the kernel crushed using a grinder prior to oil extraction. Methanol used was analytical grade (Merck Mumbai, India). All other solvents and chemicals used were of analytical grade and they are procured from commercial sources and used as such without to further treatment.

### B. Methods

Oil was extracted from crushed and powdered Kernel in petroleum ether (b.p. 40-600C) (10ml/g) by stirring magnetically at room temp (270C) for 4:15 hours. The solvent was removed at 150C using a rotary vacuum evaporator (BUCHI Rotavapor R-200) to yield crude oil. This process was repeated 2-3 times with the seed cake using fresh solvent each time in order to extract most of the oil which was further dried by using vacuum pump. The oil was purified by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent prior to trans-esterification done. The parameters of glycerides such as density, colour, refractive index, acid value, iodine value and saponification value were experimentally determined in accordance with the Association of Official Analytical Chemical Procedures [18] and these results are reported (Table 1).

The purified oil was transesterified to fatty acid methyl esters (FAME) using a catalyst called Athia, a banana plants (ashes from the peels of banana fruits, variety used Musa balbisiana,20 wt% of the oil) [19]. A mixture of the oil in methanol (10 ml/1g of the oil) and the catalyst (20 wt% of the oil) was stirred vigorously magnetically at room temp (27°C) and the conversion completion of the reaction was monitored by Thin Layer Chromatography (TLC). After completion of the reaction, the product mixture was extracted with petroleum ether. The organic layer was washed with brine, dried over anhydrous $Na_2SO_4$overnight and the solvent was removed under vacuum to yield the crude product which was further purified by column chromatography over silica gel using petroleum ether and ethyl acetate (20:1) as the eluent. The product was concentrated and evaporated to dryness on a rotatory evaporator which was further dried using vacuum pump to remove the last traces of the solvents to yield pure biodiesel. (FAME).

The composition of FAME mixture was estimated using Perkin Elmer Clarus 600GC-MS. The column used was Elite 5MS with dimension 30.0m × 250mm. The oven temp was initially held at 1400C for 5 min, increased to 2400C at 40C/min, and then held for 5min. The injector, transfer and source temperatures were 2500Cand 1500C respectively. Carrier gas was Helium and total scan time 35 min. EI mode of ionization was applied and mass san was from 20 to 400Da. For identification of FAME library search was carried out using National Institute of Standards Technology (NIST), National Bureau of Standards (NBS) and Wiley GC-MS library. Fatty acid profile of biodiesel from *Terminalia Chebula* seed oil is reported in table 2. The $^{1}$H &$^{13}$C NMR spectra were recorded in Carbon Deuterium Trichloride (CDCl3) at 300 & 75 MHz respectively using Bruker Advance III 300MHz/54mm NMR spectrometer. IR spectrum was recorded with a Perkin Elmer RX1FT-IR spectrometer as a thin film on KBr plate.

Fatty acid composition of the FAME prepared from *Terminalia Chebula* was determined by GC-MS analysis. Each peak of the gas chromatogram (Figure 2) was analyzed and the fatty acid was identified using MS database. Each peak represents one fatty acid methyl ester. The three peaks in the gas chromatogram which means the presence of three different fatty acid methyl Easters. The peak at the farthest distance on the right side in mass spectrum of any fatty acid methyl ester gives the molecular weight of the fatty acid. This peak is known as molecular ion peak. Retention time is the time taken when any peak develops. Based peak means the tallest peak in the mass spectrum due to the ion with the greatest relative abundance. The peak with the greatest m/z value is likely to be the molecular ion peak.

## RESULTS AND DICUSSION

The yield of the extracted and purified glycerides from *Terminalia Chebula* was found to be 30.68wt% at the room temperature (270C) for 4.15 hours while the yield transesterified glyceride known as FAME was 87.34wt% at the temperature 300C for 4:25 hrs. The trans-esterified products were purified by column chromatography and analyzed.

The pale yellow colour of the *Terminalia Chebula* was due to the presence of natural pigments like tocopherols, carotenoids and their derivatives. The yield of the oil was moderate. Density and iodine value of *Terminalia Chebula* were found to be 0.8457g/cm3 and 80.31 gI2/100 respectively which are comparable to those of soya bean oil and sunflower oil. The acid value of this oil was found to be 0.410 mg KOH/g which is within the limit for industrially useful oil. Saponification value was 191.60 mg KOH/g whose value is suitable for soap making and cosmetic industries. Refractive Index of this oil was 1.4695 which is not very much different from those recorded for conventional seed oils such as palm oils (1.445-1.451), cotton seed oil (1.468-1.472), safflower oil (1.473-1.476) and soya bean oil (1.4728) at 25’C. Moisture was found to be 0.115% (low value) which is suitable good quality and contamination does not take place easily due to its low value of moisture. Low moisture content is an essential criterion for commercial oil.

**Table1: Physical parameters of Terminalia Cedula calculated using equation (1-5).**

| Sl No. | Parameters | Observed values |
|---|---|---|
| 1 | Colour | Pale yellow |
| 2 | Oil content (wt.%) | 30.68 |
| 3 | Density (g/cm3) | 0.8457 |
| 4 | Acid value (mg KOH/g) | 0.410 |
| 5 | Iodine value (g I2/100g) | 80.31 |
| 6 | Saponification value (mg KOH/g) | 191.60 |
| 8 | Refractive index | 1.4695 |
| 9 | Moisture (%) | 0.115 |

### C. Analysis of FAME of *Terminalia Chebula*:

Relative percentages of fatty acid esters were calculated from the total ion chromatography by computerized integrator and results are presented (Table 2). Fatty Acid Methyl Ester (FAME) from *Terminalia Chebula* consists of 69.79wt% of methyl palmitate (MP) (C16:0), 9.82 wt.% of methyl oleate (MO)(C18:1), 20.38wt.% of methyl stearate (MS) (C18:0).

The mass spectra of biodiesel from *Terminalia Chebula* are shown in figure 3a to 3c. Molecular ion peaks and the basic peaks of the FAME are presented in Table 3 and they are in the expected values. The molecular ion peaks of MP, MO, MS, and were observed at 270, 296, and 298, respectively as was expected.

**Table2: Fatty Acid profile of FAME from *Terminalia Chebula***

| Entry | Retention time (min) | FAME | wt.% |
|---|---|---|---|
| 1 | 18.22 | Methyl palmitate | 69.79 |
| 2 | 22.25 | Methyl oleate | 9.82 |
| 3 | 22.87 | Methyl stearate | 20.38 |

**Table 3: Molecular ion and base peaks of FAME from seed oil from *Terminalia Chebula***

| Entry | FAME | Molecular ion peaks (m/z) | Base peak (m/z) |
|---|---|---|---|
| 1 | Methyl palmitate | 270 | 74 |
| 2 | Methyl oleate | 296 | 55 |
| 3 | Methyl stearate | 298 | 74 |

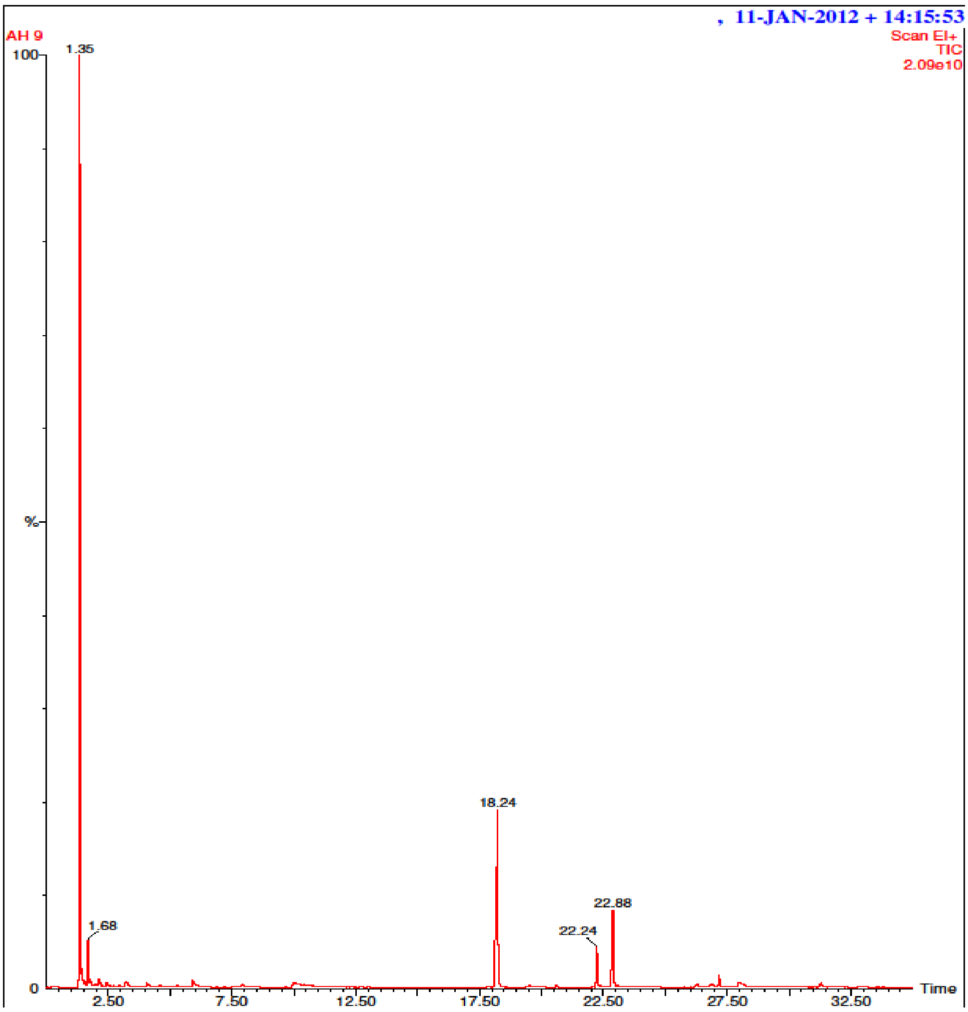



**Fig.2: Gas chromatogram of biodiesel from *Terminalia Chebula*seed oil**

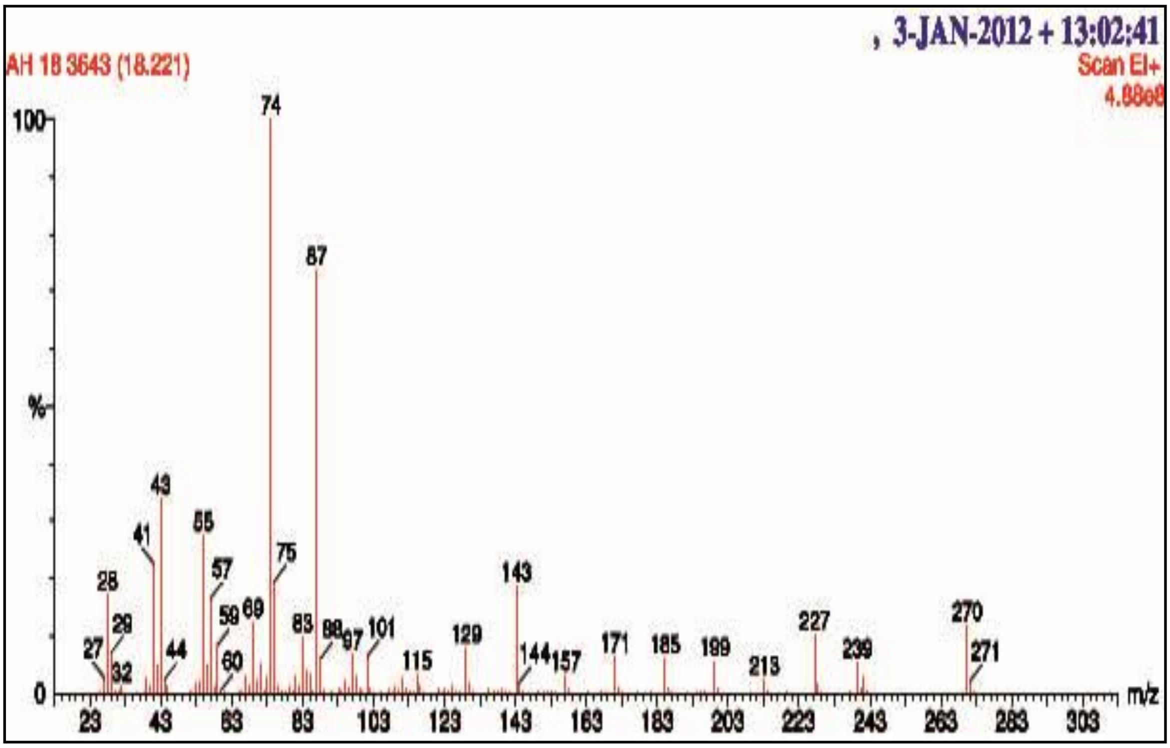



**Fig. 3a: Mass spectrum of methyl palmitate**

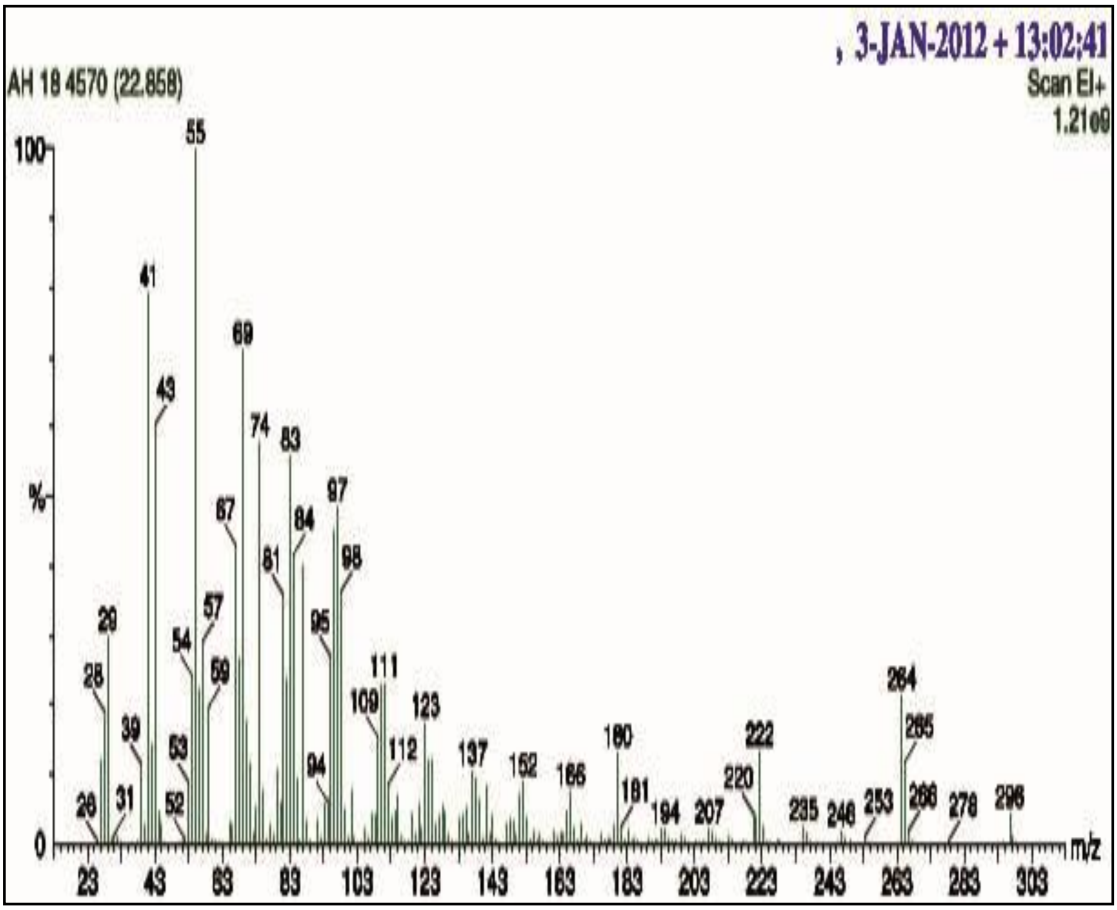



**Fig. 3b: Mass spectrum of methyl oleate**

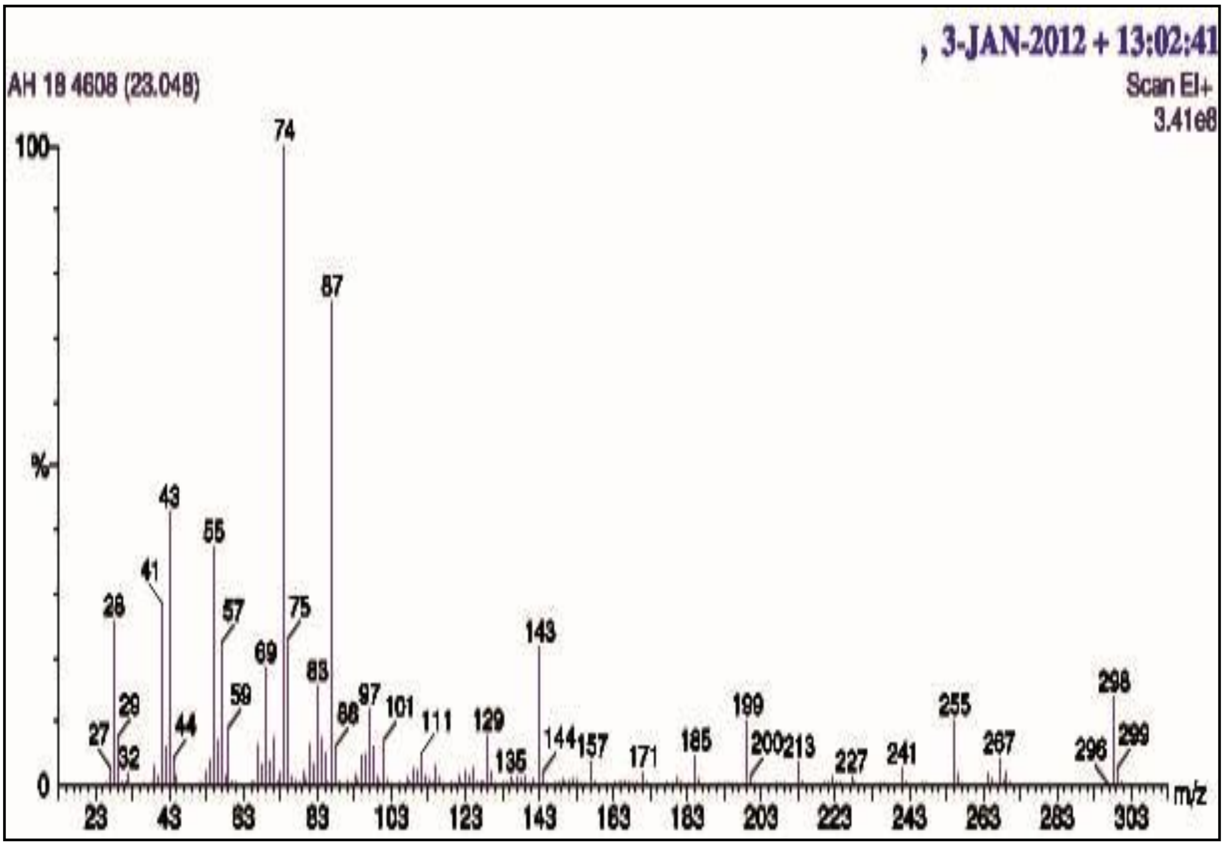



**Fig. 3c: Mass spectrum of methyl stearate**

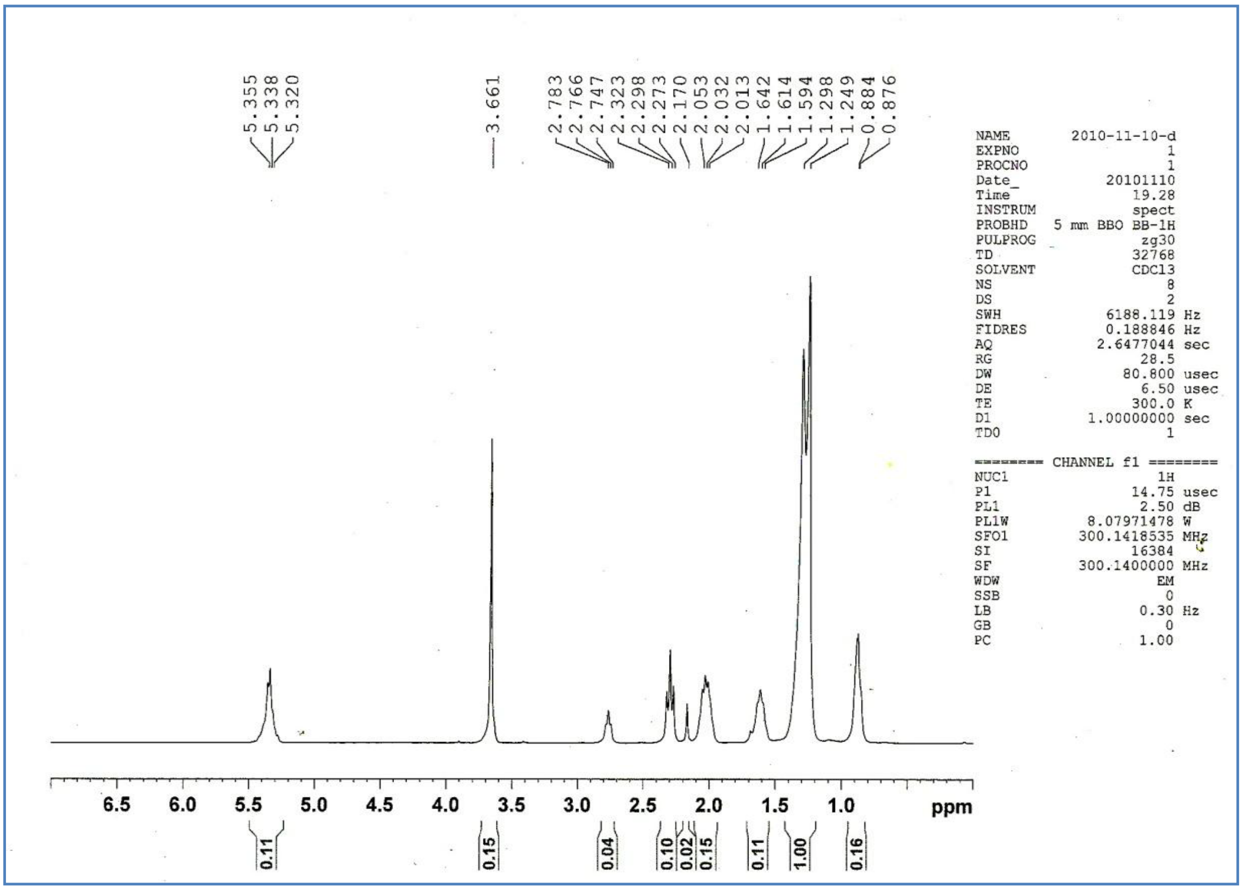



**Fig.4: [1]H NMR spectrum of biodiesel from *Terminalia chebula*seed oil**

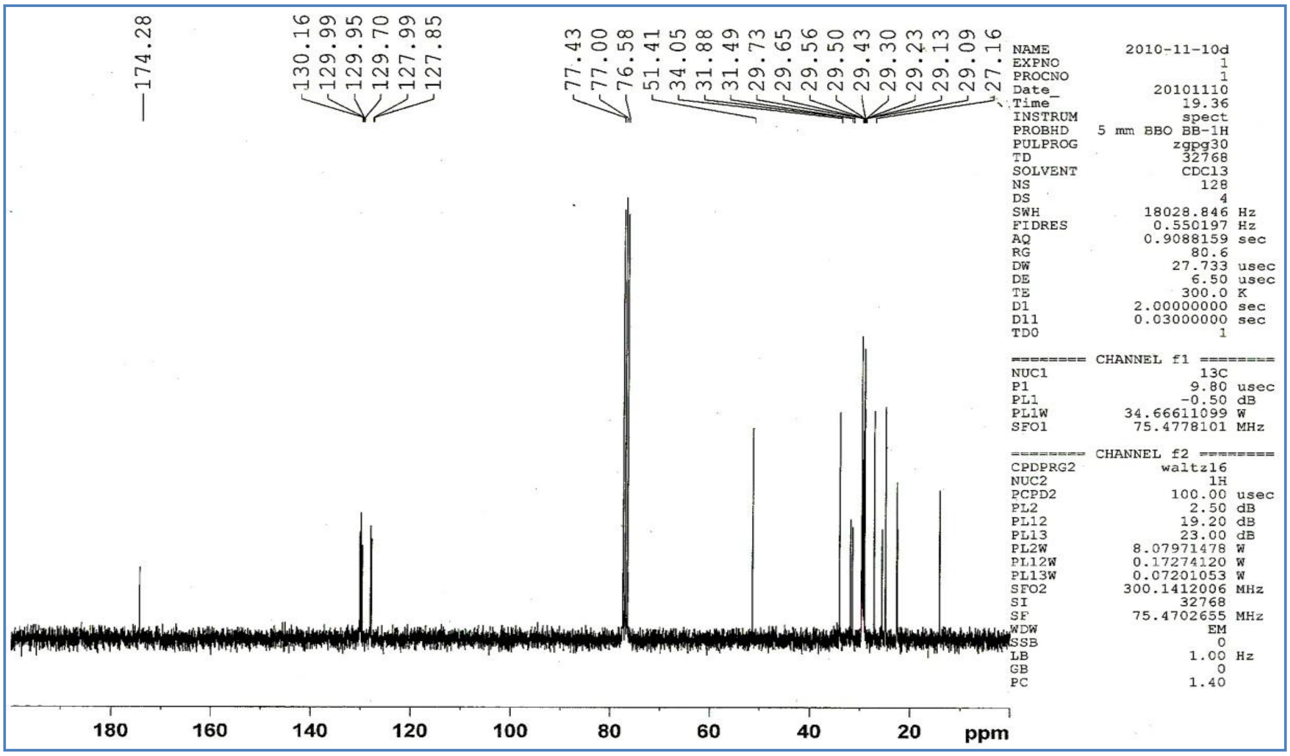



**Fig. 5: $^{13}$C NMR spectrum of biodiesel from *Terminalia Chebula* seed oil**

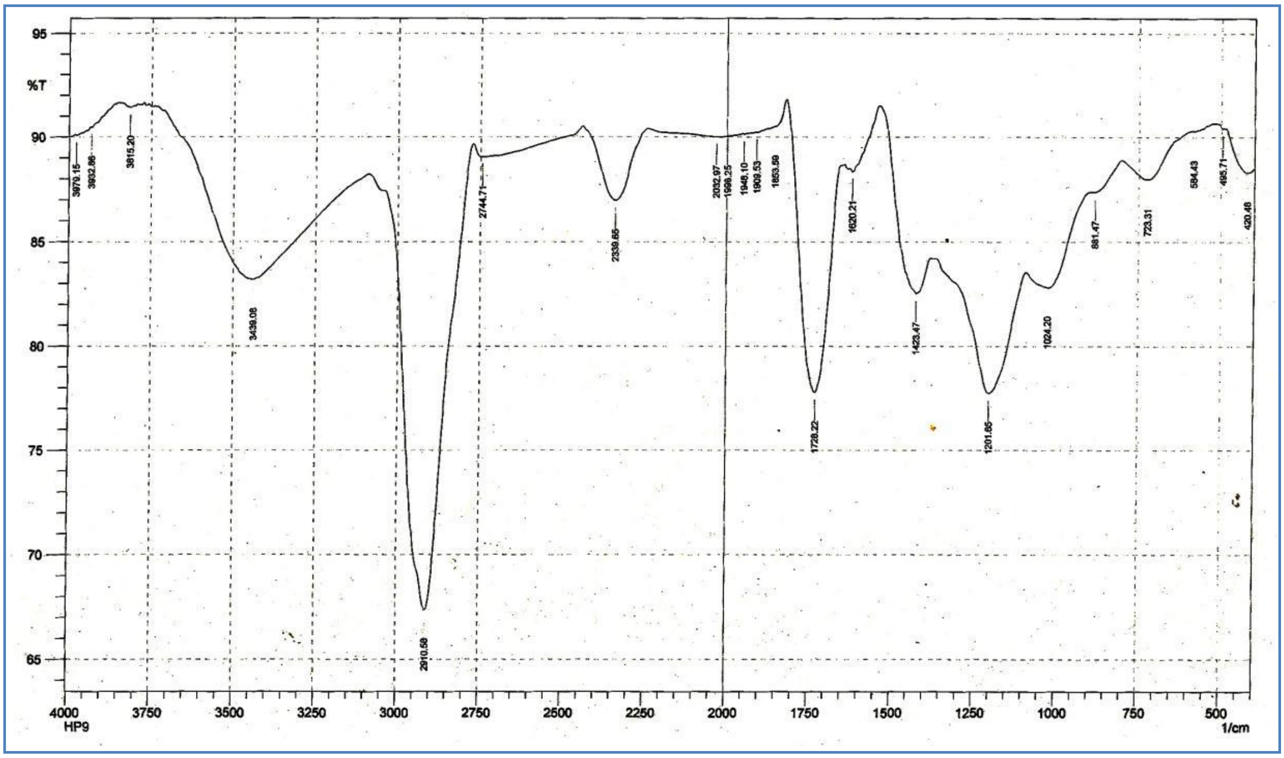



**Fig.6: IR spectrum of biodiesel from *Terminalia Chebula***

Fig 4. shows $^{1}$H NMR spectrum of biodiesel from *Terminalia chebula* seed oil. The multiplet at $\delta$ 5.32 - 5.36 ppm indicates the olefinic protons (–CH=CH–). A singlet signal at $\delta$ 3.66 ppm suggests methoxy protons of the ester functionality of the biodiesel. A signal at $\delta$ 2.78 ppm indicates the bis-allylic protons (–C=C–CH2–C=C–) of the unsaturated fatty acid chain. The triplet at $\delta$ 2.29 ppm. (t, 3J = 7.5 Hz) may be due to the $\propto$-methylene protons to ester (–CH2–CO2CH3). The $\propto$-methylene protons to double bond (–CH2–C=C–) is seen as a multiplet at $\delta$ 2.01 - 2.05 ppm. The $\beta$-methylene protons to ester (CH2–C–CO2CH3) also appear as a multiplet at $\delta$ 1.59 - 1.64 ppm. The multiplet at $\delta$

1.25 - 1.30 ppm is due to the protons of backbone methylene of the long fatty acid chain. The terminal methyl protons (C–CH3) at $\delta$ 0.88 - 0.89 ppm appear as a multiplet.

In Fig.5. shows the 13C NMR spectrum of biodiesel and the signal at $\delta$ 174.38 ppm to gives the carbonyl carbon of the ester molecules. The signals at $\delta$ 129.70, 129.99 and 130.16 ppm indicate the olefinic carbons. The methoxy carbons of esters appear at $\delta$ 51.41 ppm. The signal from $\delta$ 27.16 to 34.05 ppm. indicates the methylene and methyl carbons of fatty acid moiety.

In the Fig.6 the IR spectrum of the biodiesel is shown and a sharp signal at 1728.22 $cm^{-1}$suggests C=O stretching frequency. The weak signal at 1620.21 $cm^{-1}$indicates C=C stretching frequency. Strong and sharp signals at 2910.58 and 2744.71 $cm^{-1}$suggest C-H stretching frequencies. The signal at 3439.06 $cm^{-1}$indicates the =C–H stretching frequency. The band at 1201.65 and 1024.20 $cm^{-1}$represents C–O–C stretching frequencies. The signal at 723.31 $cm^{-1}$indicates $CH_2$rocking.

**D. Determination of IV, SN and CI of FAMEs theoretically and experimentally:**

Three important physical properties of biodiesel, viz. iodine value (IV), saponification number (SN) and cetane index (CI) were performed applying theoretical calculation based upon fatty acid profile shown in the Table 4. The IV, SN and CI of FAMEs were calculated using equations (6), (7) and (8) respectively. Results are shown in Table 4.

$$\mathrm{IV} = \sum(254 \times \mathrm{D} \times \mathrm{Ai})/\mathrm{MWi} \ldots\ldots\ldots\ldots\ldots\ldots (6)$$
$$\mathrm{SN} = \sum(560 \times \mathrm{Ai})/\mathrm{MWi} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$
$$\mathrm{CI} = 46.3 + \frac{5458}{\mathrm{S}} - 0.225\mathrm{R} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Where, D = number of double bonds in the ith component
Ai = percentange of the ith component in the chromatogram
MWi = molecular weight of the ith component of the FAME in the oil
S = saponification number (SN) as calculated by the equation [7]
R = iodine value (IV) as calculated by equation (6)

**Table 4. Experimentally and theoretically as calculated IV, SN, CI of FAME Profile *Terminalia Chebul*a plant**

| **Name of the oil plant** | ***IV* (g/100g)** | ***SN*(*mgKOH* / *g*)** | ***CI*** |
|---|---|---|---|
| *Terminalia Chebula* | 80.42 | 191.52 | 63.48 |

**E. Conclusion:**

The yield of the extracted and purified glyceride from *Terminalia Chebula* was found to be 30.68 wt% at the temperature 270C within 4:15 hours while the yield of transesterified glyceride known as fatty ester (FAME) was 87.34 wt% at temperature 300C within 7:25 hours. The colour, density, acid value, iodine value, saponification number, refractive index and the moisture of the *Terminalia Chebula* were found to be pale yellow, 0.8457g/cm3, 0.410 mg KOH/g, 80.31g I2/g, 191.60mgKOH/g, 1.4695 and 0.115 wt% respectively.

The biodiesel from *Terminalia Chebula* after extraction and purification by column chromatography was prepared by heterogeneous transesterification process and analyzed for its fatty acid methyl esters composition using IR, NMR and GC-MS. This study found that FAME from *Terminalia Chebula* consists of 69.79 wt.% of methyl Palmitate (C16:0), 9.82 wt.% of methyl Oleate (C18:1), 20.38 wt.% of Methyl SterateC18:0). The molecular ion peak of methyl palmitate, methyl oleate, methyl stearate, were observed at 270, 296 and 298 respectively as was expected. The Iodine value (IV), Saponification number (SN) and Cetane Index (CI) were calculated experimentally and theoretically and were found to be 80.42 (g/100 g), 191.52 (mg KOH/g) and 63.48 respectively.

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