Evaluation Of *Thevetia Peruviana* Seed Oil As Sustainable Biodiesel Feedstock From Manipur

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Abstract

The present study investigates the potential of Thevetia peruviana seed oil as a sustainable non-edible feedstock for biodiesel production in Manipur, North-East India. Recognizing the rich floral diversity of the region and the underutilization of non-edible oil-bearing plants, this work aims to promote value addition and biodiversity conservation through renewable energy applications. Seed oil was extracted using petroleum ether solvent extraction and purified by column chromatography. Physicochemical properties, including density (0.8925 g/cm³), refractive index (1.4720), acid value (0.405 mg KOH/g), iodine value (72.0 g I_2/100 g), saponification value (195.81 mg KOH/g), moisture content (0.102%), and oil yield (57.75 wt%), were determined following standard analytical procedures. Transesterification of purified glycerides was carried out using a bio-based catalyst derived from banana peel ash (Musa balbisiana), yielding 93.1 wt% fatty acid methyl esters (FAME) at room temperature. The fatty acid profile, determined by GC–MS, revealed the predominance of methyl oleate (58.63 wt%), followed by methyl palmitate (23.25 wt%), methyl stearate (14.05 wt%), methyl arachidate (2.81 wt%), and methyl behenate (1.27 wt%). Structural confirmation of biodiesel was achieved using ^{1}H and ^{13}C NMR and FT-IR analyses. Theoretical calculations of iodine value, saponification number, and cetane index based on fatty acid composition indicated favorable fuel properties. The findings demonstrate that Thevetia peruviana seed oil is a promising non-edible biodiesel resource capable of supporting sustainable energy production while contributing to regional biodiversity conservation and rural economic development.

Keywords: *Thevetia peruviana, non-edible oil, biodiesel, transesterification, fatty acid methyl esters (FAME)*

Introduction

The state of Manipur, located in North-East India, is recognized for its rich and diverse flora, distributed across fertile valleys and forested hill regions. This ecological wealth includes numerous oil-bearing plant species whose seeds contain substantial quantities of non-edible oils. Although these plants possess significant biochemical and industrial potential, many remain underexplored and are often regarded as having little direct economic value. Consequently, they receive minimal attention from cultivators, researchers, and policymakers. The gradual neglect of such species not only limits their commercial utilization but also contributes to the steady erosion of regional biodiversity. Scientific exploration and value addition of these non-edible oil resources, particularly for biodiesel production, could simultaneously foster biodiversity conservation and rural economic development. The growing global concern over depletion of fossil fuels and environmental degradation has intensified interest in renewable energy alternatives. Biodiesel has emerged as one of the most promising substitutes for conventional petro-diesel due to its renewability, biodegradability, low toxicity, and potential to reduce greenhouse gas emissions. Early foundational work by Srivastava A and Prasad R (2000) highlighted the feasibility of triglyceride-based fuels as sustainable diesel substitutes. Later, Zhang Y et al. (2003) provided comprehensive process design and technological assessment for biodiesel production from waste cooking oils, demonstrating the practicality of large-scale transesterification. Further advances were reviewed by Leung DYC et al. (2010), who emphasized the efficiency of catalysed transesterification using acid, base, or enzymatic catalysts. In addition, Knothe G (2010) extensively investigated the relationship between fatty acid composition and fuel properties, underscoring the importance of detailed chemical characterization.

Despite its advantages, the production of biodiesel from edible oils has raised serious concerns regarding food security and price volatility in agricultural markets. The diversion of edible oils for fuel can create imbalances between food supply and energy demand, particularly in developing countries. For this reason, non-edible oil-bearing plants have gained increasing attention as alternative feedstocks. Utilizing such resources ensures that edible oils remain available for nutritional and industrial uses while supporting sustainable energy initiatives. In this context, systematic identification and characterization of fatty acid constituents in seed glycerides are crucial. The fatty acid profile directly influences important fuel parameters such as cetane number, oxidative stability, viscosity, and cold flow properties. Therefore, comprehensive investigation of non-edible oil-bearing plants of Manipur is scientifically imperative and socio-economically valuable, offering a pathway toward sustainable biodiesel production and conservation of regional plant diversity.

Literature Review

Over the past two decades, biodiesel research has expanded considerably, focusing on feedstock diversification, catalytic innovations, and fuel quality assessment. Early work by Srivastava A and Prasad R (2000) established the technical feasibility of triglyceride-based fuels as viable substitutes for petro-diesel, outlining their combustion characteristics and environmental advantages. Subsequently, Zhang Y et al. (2003) presented a detailed technological assessment of biodiesel production from waste cooking oil, emphasizing process design, economic considerations, and large-scale applicability. Catalytic transesterification has been widely recognized as the most efficient method for biodiesel synthesis. Leung DYC et al. (2010) reviewed acid, base, and enzyme (lipase) catalysed systems, highlighting their respective merits and limitations in terms of conversion efficiency and by-product formation. Similarly, Zabeti M, Ashri WM, Daud WMAW, and Aroua MK (2009, 2010) investigated solid base catalysts such as alumina-supported calcium oxide, demonstrating enhanced reusability and improved operational stability. Li E and Rudolph V (2008) further explored MgO-functionalized mesoporous catalysts, reporting promising catalytic activity for vegetable oil conversion.

Feedstock quality significantly influences biodiesel properties. Moser BR et al. (2009) evaluated biodiesel derived from field pennycress oil and observed satisfactory fuel performance. Knothe G (2010, 2014) extensively analysed the relationship between fatty acid composition and fuel behaviour, demonstrating that oils enriched with specific fatty acids, such as palmitoleic acid, could improve oxidative stability and cold flow properties. These findings underscore the importance of detailed fatty acid profiling in feedstock selection. Analytical evaluation of free fatty acid (FFA) content is essential for determining appropriate processing routes. Satyarthi JK, Srinivas D, and Ratnasamy P (2009) introduced ^{1}H NMR spectroscopy as a rapid and reliable method for FFA quantification in oils and biodiesel. Standardized procedures compiled by AOAC International have further strengthened quality control and reproducibility in fuel analysis. Considerable attention has also been directed toward non-edible oils. Aliyu A, Godwin O, and Hamza A (2011, 2012) demonstrated successful biodiesel production from waste soybean and mahogany seed oils using NaOH-catalysed transesterification. Additionally, Deka DC and Basumatary S (2011) highlighted the potential of regionally available oil-bearing species for decentralized biodiesel production systems.

Objectives

The primary objective of this study is to evaluate the potential of *Thevetia peruviana* as a sustainable non-edible oil-bearing feedstock for biodiesel production in Manipur, North-East India. Specifically, the work aims to extract and purify seed oil from locally available plant material and determine its physicochemical properties, including density, refractive index, acid value, iodine value, saponification value, moisture content, and oil yield, in accordance with standard analytical procedures. A further objective is to convert the purified glycerides into fatty acid methyl esters (FAME) through transesterification using a bio-based catalyst derived from banana peel ash and to assess the conversion efficiency. The study also seeks to characterize the fatty acid composition of the resulting biodiesel using GC–MS, NMR, and FT-IR analyses, and to correlate the fatty acid profile with fuel-relevant properties. Ultimately, the research intends to demonstrate the suitability of *Thevetia peruviana* seed oil as an alternative, non-edible biodiesel resource that supports energy sustainability and biodiversity conservation.

Materials and Methods

Mature seeds of *Thevetia peruviana* were collected during the peak fruiting season (November to April) from the Manipur University Campus, Canchipur, Imphal West, Manipur (24°37′48.00″ N, 94°01′12.00″ E). The collected seeds were thoroughly cleaned to remove extraneous materials and subsequently sun-dried for 5–6 days to reduce moisture content. After drying, the seeds were manually deshelled, and the kernels were separated and finely ground using a mechanical grinder to obtain a uniform powdered sample suitable for solvent extraction. Analytical grade methanol (Mark, Mumbai, India) was employed for transesterification reactions. All other chemicals and solvents used in the study were of analytical grade, procured from standard commercial suppliers, and used without further purification.

Oil was extracted from crushed and powdered kernel of *Thevetia peruviana* seeds in petroleum ether (bp 40-60°C) (10ml/g) by stirring magnetically at room temperature using solvent extraction technique (25°C) for 4:30 hours. The solvent was removed at 45°C using a rotary vacuum evaporator (BUCHI Rotavapour 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 mash) using a mixture of petroleum ether and ethylacetate (20:1) as the eluent prior to transesterification is done.

$$\%\ Oil\ content = \frac{Weight\ of\ oil}{Weight\ of\ powdered\ seeds} \times 100 \quad \ldots\ldots\ldots (1)$$

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 (Horwitz, 2010) and these results are reported (Table 1).

Acid value (mg KOH / g) = $\frac{56.1 \times V \times N}{W}$ ………. (2)

where, V = titre value (mL)

N = normality of KOH solution (determined by standardizing KOH solution with oxalic acid).

W = weight of test sample taken in g.

Refractive indices of purified seedoils were determined by using the Abbe Refractometer (AW-24) at room of temperature, only two or three drops of oil was required. Densities of the purified oils were determined at room temperature (32°C). For this, a clean and empty plastic centrifuge tube was taken and weighed. Accurately 1000 μL (= 1 mL) of the liquid sample was transferred into the tube with the help of a syringe and then weighed again. Then the density is determined based on mass per unit volume of oil.

Iodine value = $\frac{12.69 \times N \times (V_B - V_S)}{W}$ ………. (3)

where, VB = Volume of sodium thiosulphate solution used for the blank (mL)

VS = Volume of sodium thiosulphate solution used for the oil sample (mL),

N = Normality of sodium thiosulphate solution used,

W = Weight of oil sample taken in g

Saponification value = $\frac{56.1 \times M \times (V_B - V_S)}{W}$ ………. (4)

where, VB = Volume of 0.5 M HCl solution used for the blank (mL)

VS = Volume of 0.5 M HCl solution used for the oil sample (mL)

M = Molarfity of HCl used W = Weight of oil sample taken in g

% Moisture = $\frac{W_1 - W_2}{W_1}$ x 100 ………………. (5)

where, W1 = Initial weight of oil,

W2 = Final weight of oil

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). 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 (25°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 (bp 40-60°C). 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 & evaporated to dryness on a rotary 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 Pockin Elmer Clarus 600 GC-MS. The column used was Elite 5 MS with initially held at 140°C for % min, increased to 240°C at 4°c/min, and then held for 5 min. The injector transfer and source temperatures were 250°C and 150°C respectively. Carrier gas was helium and total scan time 35 min. El mode of ionization was applied and mass gas was from 20 to 400 Da. For identification of FAME library search was carried out using National Institute of Standards and Technology (NIST), National Bureau of

Standards (NBS) and Wiley GC-MS library. Fatty acid profile of biodiesel from *Thevetia peruviana* seed oil is reported in Table 2. The ^{1}H & ^{13}c NMR spectra were recovered in Carbon Deutrium Trichloride (CD Cl_3) at 300 MHz /5mm. NMR spectrometer and IR spectrum were recorded with a Perkin Elmer RXIFT-IR spectrometer as a thin film on KBr plate.

Fatty acid composition of the FAME prepared from *Thevetia peruviana* seed oil was determined by GC-MS analysis. Each peak of the gas chromatogram (Figure 2) was analysed 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 Discussion

The yield of the extracted and purified glycerides from *Thevetia peruviana* seedoil was found to be 57.75 wt % at the room temperature (25°C) within 4:30 hours while the yield of transetrified glyceride known as Fatty Acid Methyl Ester (FAME) was 93.1 wt % at the room temperature (28°C) within 4:15 hours. The light yellow colour of the *Thevetia peruviana* seed oil was due to the presence of natural pigments like tocopherols, carotenoides and their derivatives. The yield of the oil was moderate. Density and iodine value of *Thevetia peruviana* seed oil were found to be 0.8925 g/cm^3 and 72.0 gI_2/100 respectively which are comparable to those of soya bean oil and sunflower oil. The acid value of this oil was found to be 0.405 mg KOH/g which is within the limit for industrially useful oil. Saponification value was 195.81 mg KOH/g whose value is suitable for soap making and cosmetic industries. Refractive Index of this oil was 1.4720 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 0.102% (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.

Analysis of FAME of Thevetia peruviana

^{1}HNMR (300 MHz, CD Cl_3): δ 5.31 -5.35 ppm, δ 3.65 ppm, δ 2.8 ppm, δ 2.8 ppm, δ 2.26-2.31 ppm, δ 2.01-2.02 ppm, δ 1.59-1.63 ppm, δ 1.25-1.29 ppm, δ 0.87-0.90 ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ 174.13 ppm, δ 129.57 ppm, δ 130.02 ppm, δ 51.26 ppm, δ 27.05 -33.92 ppm.

FT-IR (thin film): 1743.4 cm^{-1}, 2925.7 cm^{-1}, 2954.4 cm^{-1}, 1651.6 cm^{-1}, 3006.0 cm^{-1}, 1196.8 cm^{-1}, 1170.6 cm^{-1}, 1118.5 cm^{-1}, 1019.4 cm^{-1}, 722.7 cm^{-1}.

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 *Thevetia peruviana* consists of 23.25wt% of methyl palmitate (C16:0), 58.63wt% of methyl oleate (C18:1),14.05wt% of methyl stearate (C18:0), 2.81wt% of methyl arachidate (C20:0) and 1.27wt% of methyl behenate (C22:0). The mass spectra of methyl palmitate, methyl oleate, methyl stearate, methyl arachidate, methyl behenate are shown in Figure 3a-3e. The molecular ion peaks and base peaks are presented (Table 3). The ^{1}H NMR spectrum of the biodiesel in shown in Figure 4. The multiplet at δ 5.31-5.35 ppm indicates the olefinic protons (–CH=CH–). A singlet signal at δ 3.65 ppm suggests methoxy protons of the ester functionality of the biodiesel. It is interesting to note that no signal is observed at about δ 2.8 ppm which suggests the absence of bis-allylic protons ($–C=C–CH_2–C=C–$) of the unsaturated fatty acid chain. The bis-allylic protons / signal of poly unsaturated fatty acid (like linoleic acid) generally appears at δ 2.8 ppm. The multiplet at δ 2.26-2.31 ppm is due to the methylene protons to ester ($–OH_2–CO_2CH_3$). The α-methylene protons to double bond ($–CH_2–C=C–$) is seen as a multiplet at 2.01-2.02 ppm. The β-methylene protons to ester ($CH_2–C–CO_2CH_3$) also appear as a multiplet at δ 1.59-1.63 ppm. The singlet signals at δ 1.25-1.29 ppm indicates the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons ($C–CH_3$) at δ 0.87 - 0.90 ppm indicates as a multiplet.

The ^{13}C NMR spectrum of the biodiesel is shown in Figure 5 the signal at δ 174.13 ppm suggests the carbonyl carbon of the ester molecules and the olefinic carbons appear at δ 129.57 and 130.02 ppm. The signal at δ 51.26 ppm in the ^{13}C NMR spectrum indicates methoxy carbon of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from δ 27.05 to 33.92 ppm. The IR spectrum of the biodiesel in Figure 6 shows a sharp signal at 1743.4 cm^{-1} is an indicative of strong absorption by ester carbonyl stretching frequencies. Strong and sharp signal at 2925.7 and 2954.4 cm^{-1} suggests C–H stretching frequencies. The weak signal at 1651.6 cm^{-1} represents C=C stretching frequency. The signal at 3006.0 cm^{-1} represents =C–H stretching frequency. The bands at 1196.8, 1170.6, 1118.5 and 1019.4 cm^{-1} represent C–O–C stretching frequencies. The signal at 722.7 cm^{-1} indicates the CH_2 rocking.

Experimental & Theoretical determination of IV, SN and CI of FAMEs

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 IV. The IV, SN and CI of FAMEs were calculated using equations (6), (7) and (8) respectively.

Results are shown in Table 4.

$$IV = \sum(254 \times D \times Ai)/MWi \quad (6)$$

$$SN = \sum(560 \times Ai)/MWi \quad (7)$$

$$CI = 46.3 + \frac{5458}{S} - 0.225R \quad (8)$$

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)

Conclusion

The yield of the extracted and purified glycerides from *Thevetia peruviana* seed oil was found to be 57.75wt % at the room temperature (25°C) within 4:30 hours while the yield of transetrified glyceride known as Fatty Acid Methyl Ester (FAME) was 93.1 wt % at the room temperature (28°C) within 4:15 hours. The colour, density, acid value, iodine value, saponfication number, refractive index and moisture of the *Thevetia peruviana* seed oil were found to be light yellow, 0.8925 g/cm^3, 0.405 mg KOH/g, 70.20 gI2/100 g, 195.81 mg KOH/g, 1.4720 and 0.102 % respectively. The biodiesel from *Thevetia peruviana* seed oil, 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 *Thevetia peruviana* seed oil consists of 23.25 wt. % of methyl palmitate (C16:0), 58.63 wt. % of methyl oleate (C18:2), 14.05 wt.% of methyl stearate (C18:0), 2.81 wt. % of methyl arachidate (C20:0) and 1.27 wt. % of methyl behenate (C22:0). The molecular ion peak of methyl palmitate, methyl oleate, methyl stearate, methyl arachidate and methyl behenate were observe at 270, 296, 298, 326 and 354 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 50.31 (g/100 g), 191.52 (mg KOH/g) and 63.48 respectively.

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Table 1: Physical parameters of *Thevetia peruviana* seed oil calculated using equation (1-5).

Sl. No.	Parameters	Observed Values
1	Colour	light yellow
2	Oil content (wt. %)	57.75
3	Density (g/cm^3)	0.8925
4	Acid Value (mg KOH/g)	0.405
5	Iodine value (gI2/100g)	70.20
6	Saponification value (mg KOH/g)	195.81
7	Refractive Index	1.4720
8	Moisture (%)	0.102

Table 2: Composition of biodiesel from *Thevetia peruviana* seed oil

Entry	Retention time (mm)	FAME	wt%
1	18.55	*Methyl palmitate*	23.25
2	22.81	*Methyl oleate*	58.63
3	23.26	*Methyl stearate*	14.05
4	27.56	*Methyl arachidate*	2.81
5	31.75	*Methyl behenate*	1.27

Table 3: Molecular ion and base peaks of FAME from *Thevetia peruviana* seedoil

Entry	FAME	Molecular ion peak (m/z)	Base peak (m/z)
1	*Methyl palmitate*	270	74
2	*Methyl oleate*	296	55
3	*Methyl* stearate	298	74
4	*Methyl arachidate*	326	74
5	*Methyl behenate*	354	74

Table 4. Experimentally and theoretically as calculated IV, SN, CI of FAME Profile Thevitia Peruviana plant using equation No. 6-8.

Name of the oil plant	IV (g/100g)	$SN\,(mg\ KOH\ /\ g)$	CI
Thevitia Peruviana	50.31	191.52	63.48

Figure 1: ***Thevitia peruviana*** **plant and seeds**

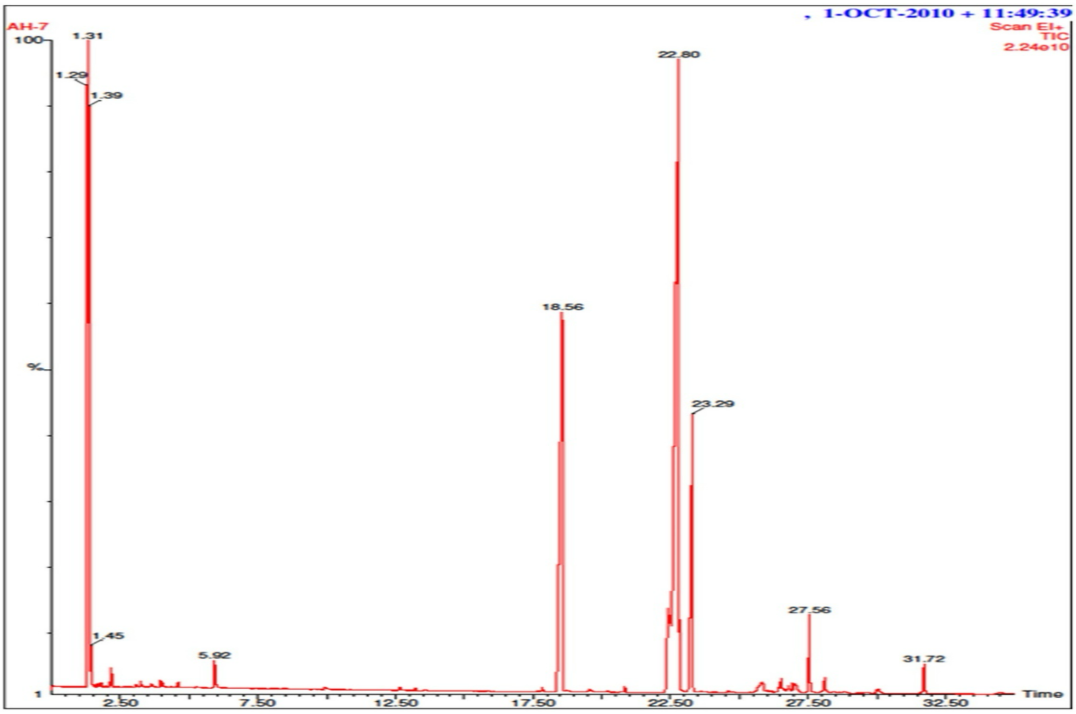

Figure 2: Gas chromatogram of biodiesel from ***Thevetia peruviana*** **seedoil**

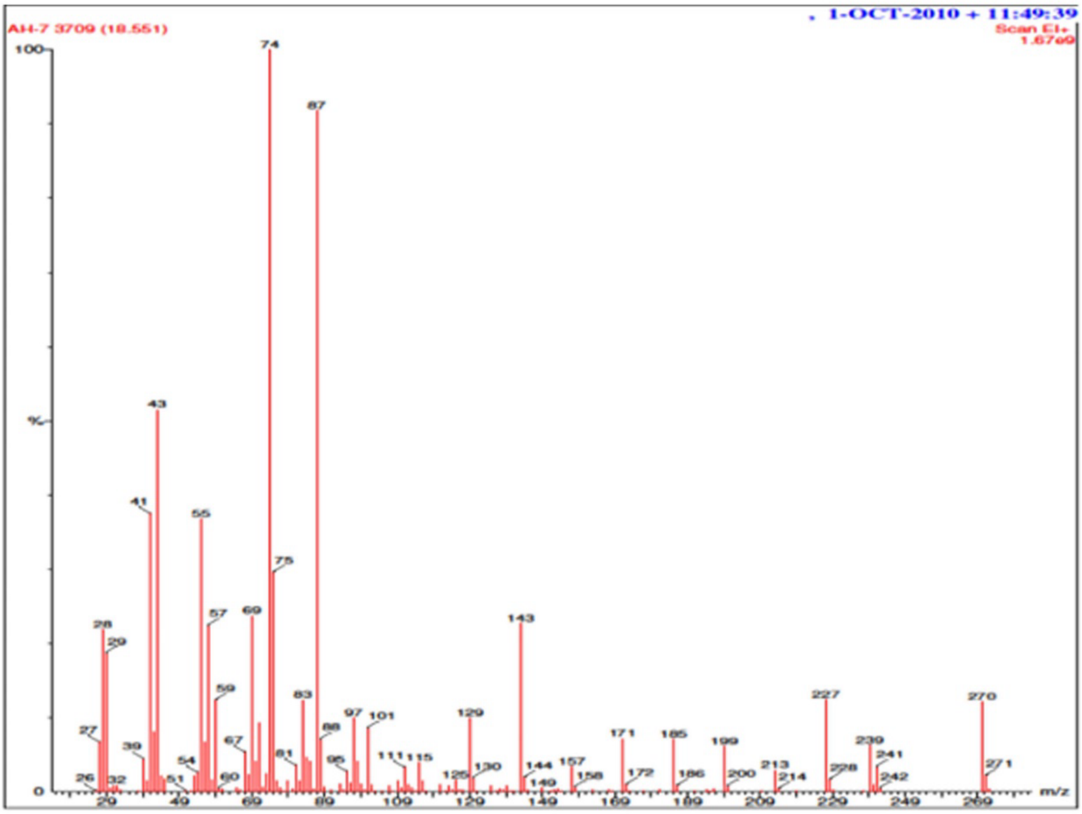

Figure 3 (a): Mass spectrum of methyl palmitate

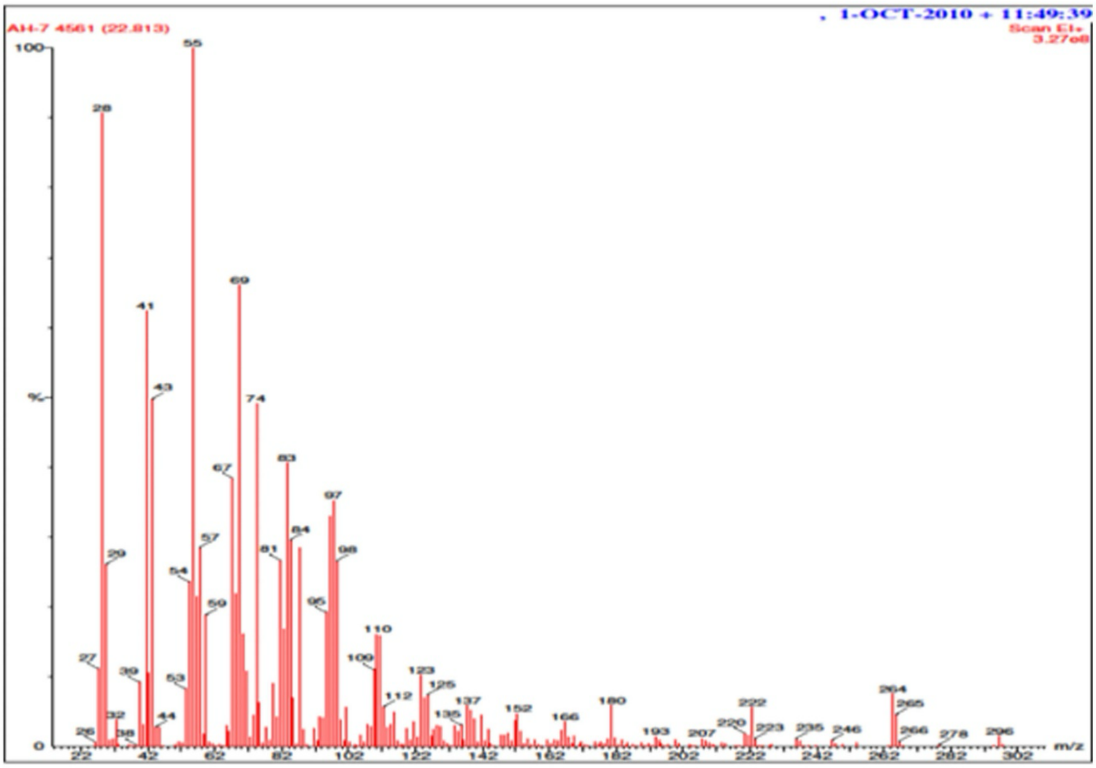

Figure 3 (b): Mass spectrum of methyl oleate

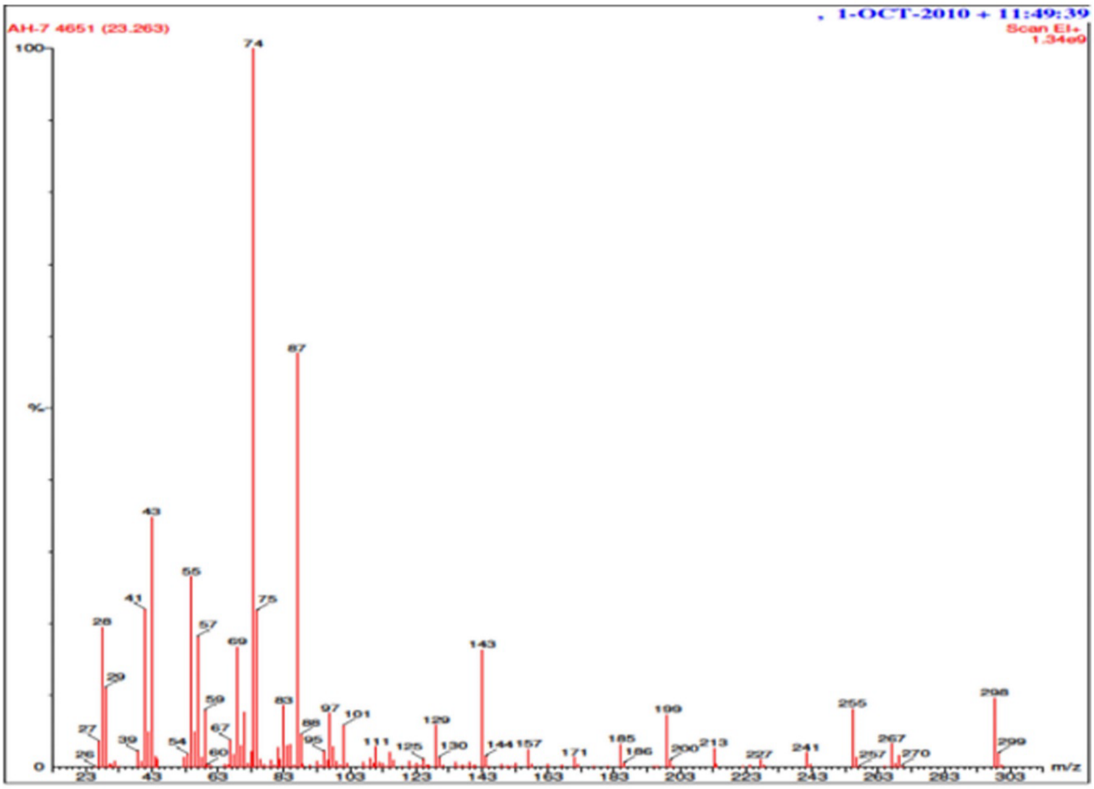

Figure 3(c): Mass spectrum of methyl stearate

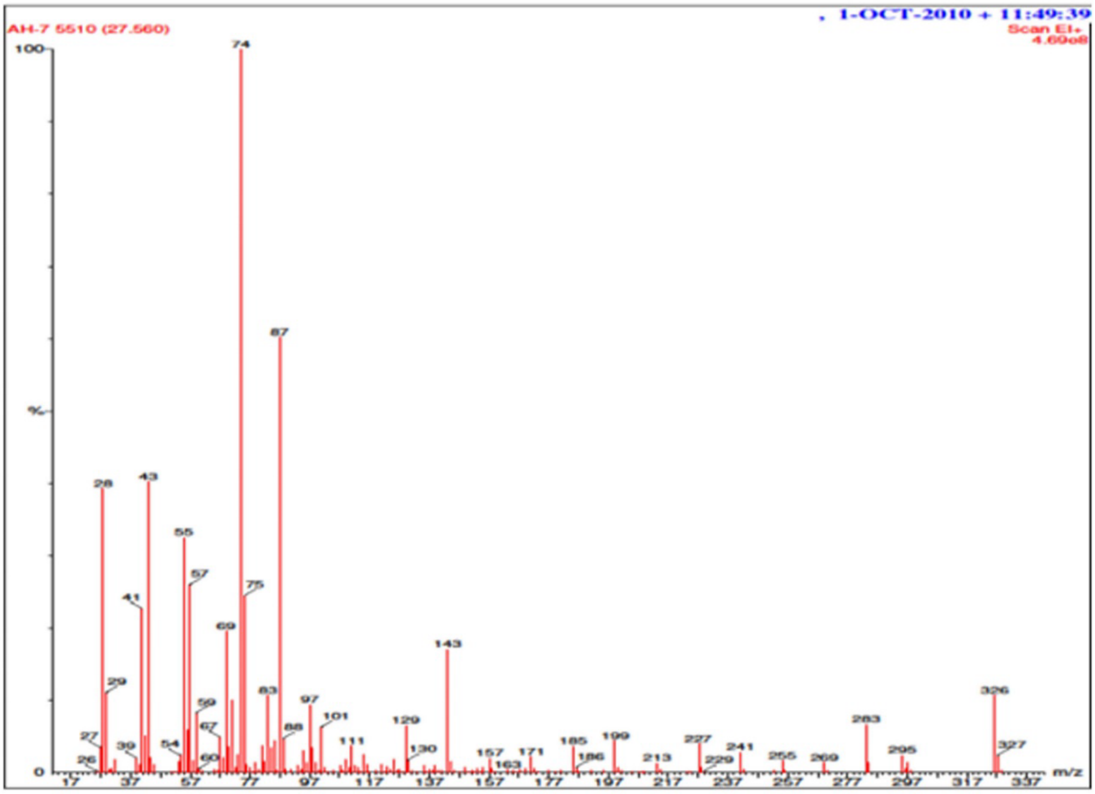

Figure 3(d): Mass spectrum of methyl arachidate

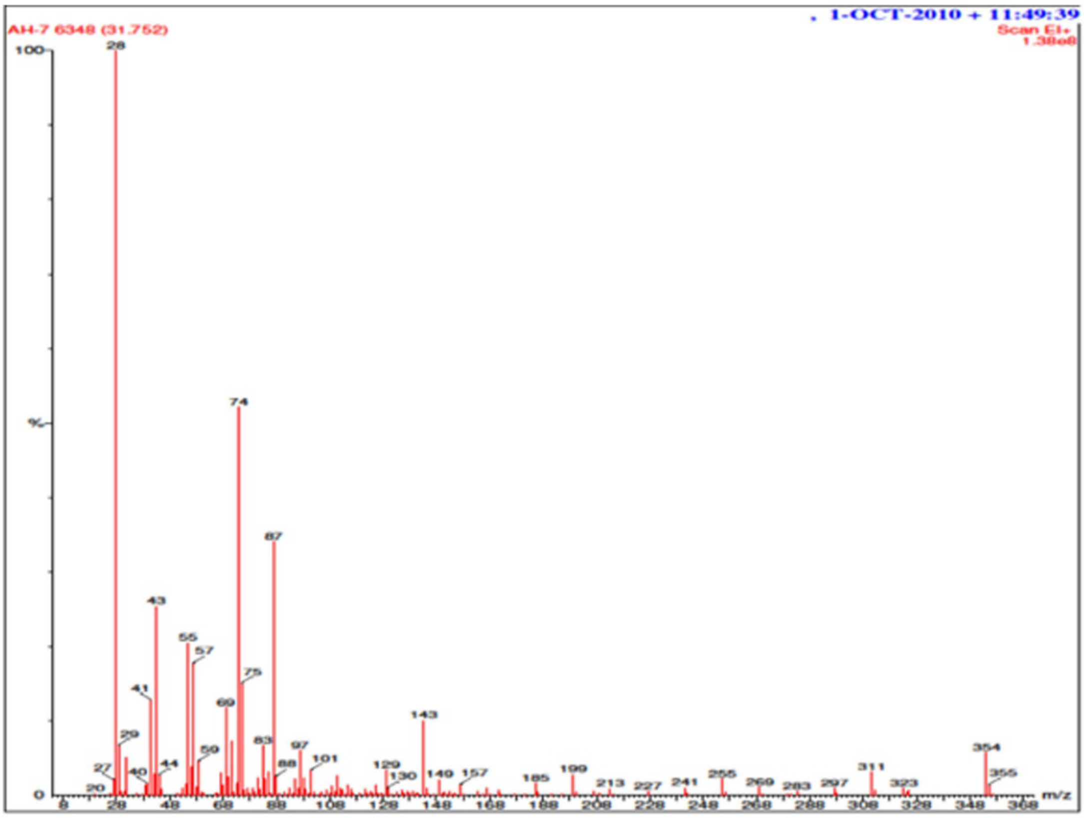

Figure 3(e): Mass spectrum of methyl behenate

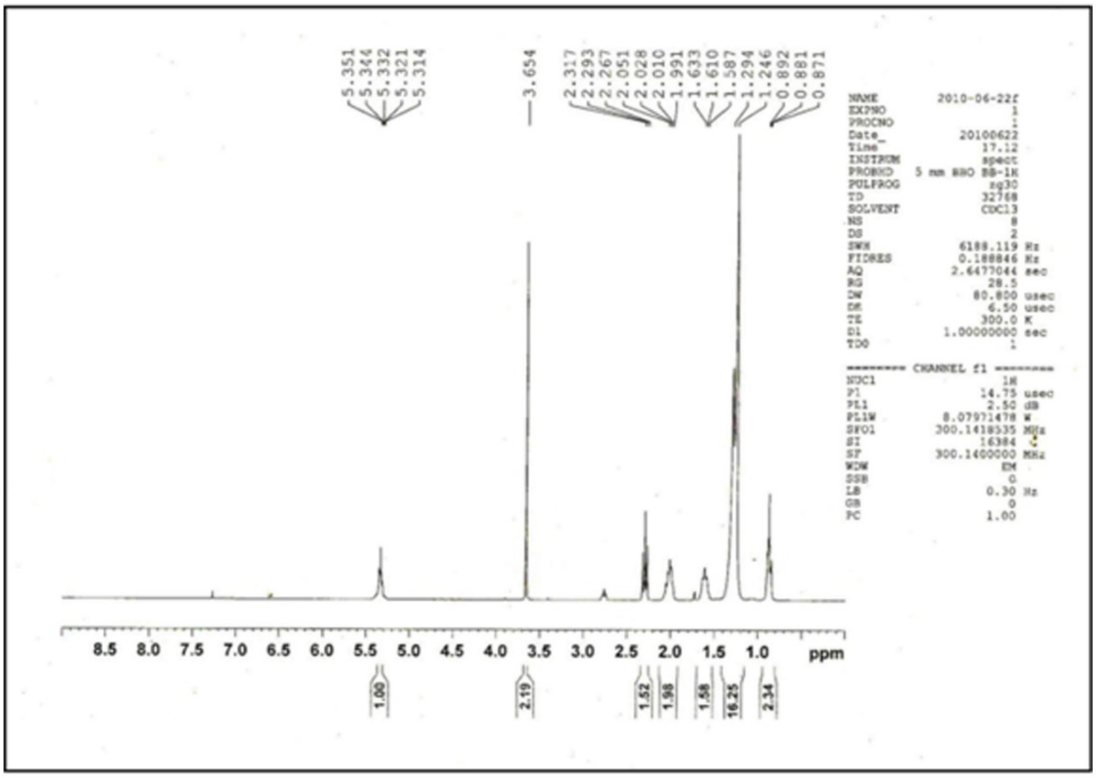

Figure 4: ^{1}H NMR Spectrum of biodiesel from *Thevetia peruviana* seedoil

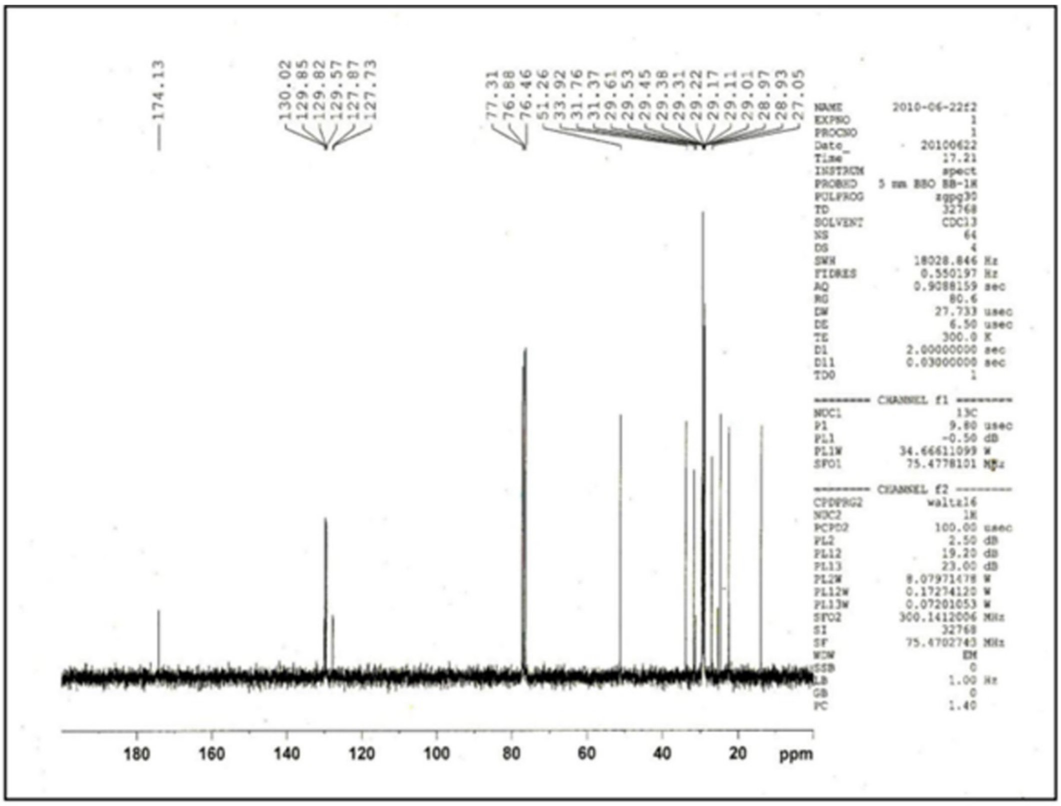

Figure 5: ^{13}C NMR Spectrum of biodiesel from *Thevetia peruviana*

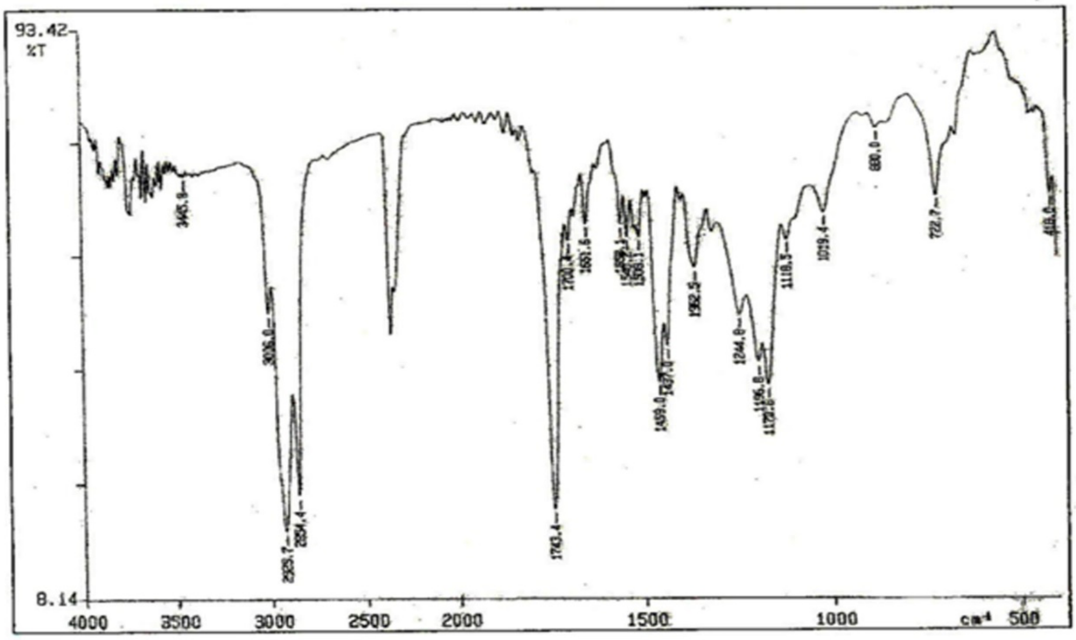

Figure 6: IR Sepectrum of biodiesel from *Thevetia peruviana* seedoil