



## Identification of fatty acid methyl esters in biodiesel from *Pithecellobium monadelphum* seed oil

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### ABSTRACT

*Pithecellobium monadelphum* Kosterm oil was extracted and transesterification was carried out using methanol as the solvent in presence of a catalyst to produce biodiesel. Fatty acid methyl esters in biodiesel prepared from *Pithecellobium monadelphum* seed oil was analyzed by IR, NMR and GC-MS. The biodiesel consists of 29.44 wt.% of methyl palmitate (C16:0), 38.91 wt.% of methyl linoleate (C18:2), 18.77 wt.% of methyl oleate (C18:1), 6.85 wt.% of methyl stearate (C18:0), 2.87 wt.% of methyl arachidate (C20:0) and 3.13 wt.% of methyl behenate (C22:0).

**Key words:** Biodiesel, *Pithecellobium monadelphum*, transesterification, *Musa balbisiana* Colla.

### INTRODUCTION

Because of the limited reserves of fossil fuels, increasing prices of crude oils and environmental concerns, researchers are working hard to find a new energy resource that is renewable, clean, reliable and yet economically feasible as a substitution to the current fossil fuels. In this context, recently, biodiesel derived from non-edible vegetable oil has been shown to be a viable alternative to the conventional transport fuel, petrodiesel for diesel engines and is receiving worldwide attention because of its renewability, biodegradability, nontoxicity and carbon neutrality [1-4]. Biodiesel, mixture of fatty acid methyl esters (FAME), is generally produced from biological resources such as vegetable oils, animal fats or even waste cooking oils by transesterification with methanol in presence of a catalyst [1, 5, 6].

The biodiesel is quite similar to conventional diesel fuel in its physical characteristics and can be used alone or mixed in any ratio with petroleum based diesel fuel in most existing modern four-stroke combustion ignition diesel engines with very few technical adjustments or no modification. Biodiesel as a neat can be used as a direct substitute for petrodiesel and is technically called B100. The preferred ratio of mixture ranges between 5% (B5) and 20% (B20). The blending ratio has been investigated by various authors on CI engines. Up to 20% blending of biodiesel with diesel has shown no problems in engine parts [7-9].

Currently, more than 95% of the biodiesel is made from edible oil and biodiesel production from conventional sources (soybean, rapeseed, sunflower, palm, etc.) increasingly has placed strain on food production, price and availability [5, 10-13]. Taking these factors into consideration, non-edible oils definitely have the advantage over edible oils as biodiesel feedstock. Therefore, search for alternative biodiesel feedstocks like non-edible vegetable oils should be an important objective.

*Pithecellobium monadelphum* Kosterm (called *Maj* in Assamese, Fig. 1) is a huge woody tree which may grow as high as 50 metres or even more. The tree produces circular and flat seeds (Fig. 2) which are small in size but large in

number. The present study deals with the synthesis of biodiesel from *Pithecellobium monadelphum* seed oil by transesterification with methanol and determination of composition of the biodiesel formed by employing various instrumental techniques.



**Fig. 1.** *Pithecellobium monadelphum* tree



**Fig. 2.** *Pithecellobium monadelphum* seed

## MATERIALS AND METHODS

### Materials

*Pithecellobium monadelphum* Kosterm seeds were collected from Bongaigaon District of Assam, India during its availability of the season. The seeds were dried in sunlight and the kernel crushed using a grinder prior to oil extraction. Methanol used was of analytical grade (Merck, Mumbai, India). All other solvents and chemicals used were of analytical grade, and they were procured from commercial sources and used as such without further treatment.

### Oil Extraction

Extractability of oil was evaluated by solvent extraction of the crushed kernel. Crushed kernel in petroleum ether (bp 40-60 °C, 10 mL/g) was magnetically stirred at room temperature (22-23 °C) for 3 h, solvent was removed at 45 °C using a rotary vacuum evaporator to yield the 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. The oil was purified prior to transesterification done, by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent.

### Transesterification of Seed Oil

The purified oil was transesterified to fatty acid methyl esters (FAME) using a heterogeneous catalyst derived from the trunk of *Musa balbisiana* Colla [14, 15]. A mixture of oil in methanol (10 mL/g of oil) and the catalyst (20 wt.% of oil) was magnetically stirred at room temperature (32 °C) and the conversion was monitored by TLC. The reaction mixture was filtered under vacuum pump and the residue washed with petroleum ether and the combined filtrate was partitioned between water and petroleum ether. The organic phase was washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under vacuum to yield the crude product which was further purified by column chromatography over silica gel using 20:1 petroleum ether and ethyl acetate as the eluent. The purified product was further subjected to high vacuum to remove the last traces of solvents to yield pure biodiesel (FAME).

### Analysis of FAME

Fatty acid methyl esters (FAME) in biodiesel prepared from *Pithecellobium monadelphum* seed oil were estimated using Perkin Elmer Clarus 600 GC-MS. The column used was Elite 5 MS with dimension 30.0 m x 250 µm. The oven temperature was initially held at 140 °C for 5 min, increased to 240 °C at 4 °C/min, and then held for 5 min. The injector, transfer and source temperatures were 250 °C, 200 °C and 150 °C respectively. Carrier gas was helium and total scan time 35 min. Gas Chromatogram of the biodiesel is shown in Fig. 3. EI mode of ionization was applied and mass scan was from 20 to 400 Da. For identification of FAME library search was carried out using NIST, NBS and Wiley GC-MS library. Fatty acid profile of biodiesel from *Pithecellobium monadelphum* seed oil is reported in Table 1. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> at 300 and 75 MHz respectively using Bruker Avance III 300 MHz/54 mm NMR spectrometer. IR spectrum was recorded with a Perkin Elmer RX I FT-IR spectrometer as a thin film on KBr plate. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 0.85-0.88 (m), 1.24 (s), 1.30 (s), 1.59-1.63 (m), 1.99-2.05 (m), 2.29 (t, 3J=7.5 Hz), 2.76 (t, 3J=5.7 Hz), 3.65 (s), 5.31-5.36 (m). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 13.92, 13.96, 22.42, 22.54, 24.78, 25.45, 27.04, 28.94, 28.96, 29.00, 29.11, 29.17, 29.19, 29.30, 29.37, 29.43, 29.54, 29.61, 30.77, 31.37, 31.77, 32.35, 33.93, 51.29, 127.73, 127.87, 129.58, 129.83, 129.87, 130.03, 174.20. FT-IR (thin film): 735, 880, 1018, 1171, 1197, 1245, 1362, 1437, 1459, 1508, 1540, 1651, 1742, 2854, 2925, 3008 cm<sup>-1</sup>. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of biodiesel from *Pithecellobium monadelphum* seed oil are depicted in Fig. 4 & 5.

## RESULTS AND DISCUSSION

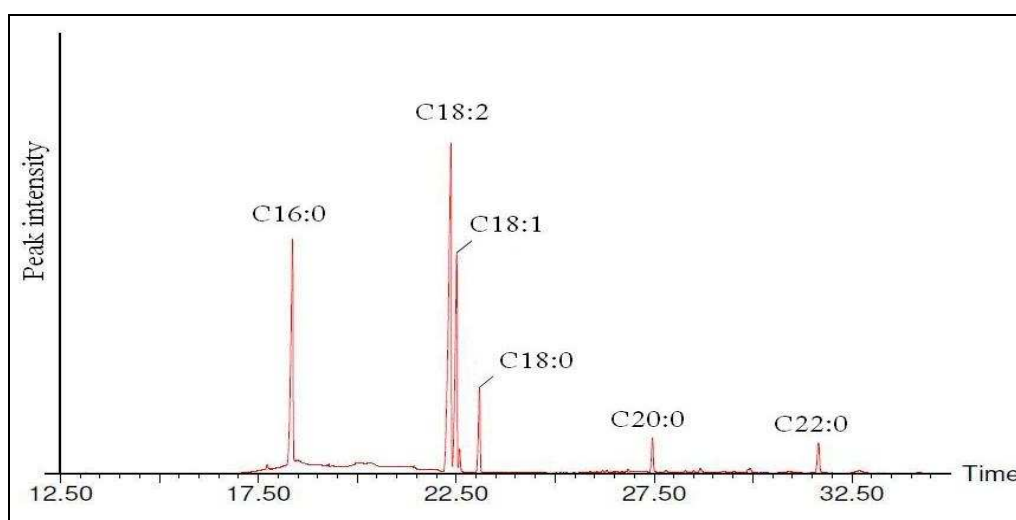
*Pithecellobium monadelphum* Kosterm is a huge woody tree which grows as high as 50 metres or even more. The seed had thin kernel covers which could not be dehusked easily. As the weight of the covers appeared negligible, it was considered appropriate to estimate their oil content without dehusking. The tree produces circular and flat seeds which are small in size but large in number. Oil content of the seeds with skiny cover is estimated at about 10 wt.% only. Free fatty acid from oil sample was removed by column chromatography before transesterification. Transesterification of seed oil to biodiesel was carried out using methanol as the solvent in presence of a catalyst derived from the trunk of *Musa balbisiana* Colla [14, 15]. The yield of biodiesel from *Pithecellobium monadelphum* seed oil was 93 wt.% at room temperature (32 °C) within 3.5 h. The transesterified products were purified by column chromatography and analyzed. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded in CDCl<sub>3</sub> at 300 and 75 MHz respectively using Bruker Avance III 300 MHz/54 mm NMR spectrometer. IR spectra were recorded with a Perkin Elmer RX I FT-IR spectrometer as a thin film on KBr plate. Composition of FAME mixture was estimated using Perkin Elmer Clarus 600 GC-MS.

**Analysis of FAME of biodiesel from *Gmelina arborea* seed oil**

Fatty acid profile of the biodiesel prepared from *Pithecellobium monadelphum* seed oil was determined by GC-MS analysis. The individual peaks of the gas chromatogram Fig. 3 were analyzed and the fatty acids were identified using MS database. Relative percentage of fatty acid esters was calculated from total ion chromatography by computerized integrator and results are presented in the Table 1. Biodiesel consists of 29.44 wt.% of methyl palmitate (C16:0), 38.91 wt.% of methyl linoleate (C18:2), 18.77 wt.% of methyl oleate (C18:1), 6.85 wt.% of methyl stearate (18:0), 2.87 wt.% of methyl arachidate (C20:0) and 3.13 wt.% of methyl behenate (C22:0). The linoleic acid is the major fatty acid followed by palmitic acid and oleic acid. Stearic, arachidic and behenic acids are present as minor constituents.

**Table 1. Fatty acid profile of biodiesel from *P. monadelphum* seed oil**

Retention time (min)	FAME	wt. %
18.37	Methyl palmitate	29.44
22.37	Methyl linoleate	38.91
22.52	Methyl oleate	18.77
23.09	Methyl stearate	06.85
27.46	Methyl arachidate	02.87
31.66	Methyl behenate	03.13

**Fig. 3. Gas chromatogram of biodiesel from *P. monadelphum* seed oil**

The mass spectra of biodiesel from *P. monadelphum* seed oil are shown in Figs. 3a to 3f. Molecular ion peaks and base peaks of the FAME are shown in Table 2 and they are in the expected values. The molecular ion peaks of methyl palmitate, methyl linoleate, methyl oleate, methyl stearate, methyl arachidate and methyl behenate were observed at 270, 294, 296, 298, 326 and 354 respectively as expected. It is interesting to note that all the saturated FAMES detected in the biodiesel from *P. monadelphum* (methyl palmitate, methyl stearate, methyl arachidate and methyl behenate) show  $\text{CH}_3\text{OC}(=\text{OH}^+)\text{CH}_2$  fragment and appears at  $m/z = 74$  as the base peak (100%) which is the result of McLafferty rearrangement [16, 17] during the MS analysis due to a six-member ring structure of an intermediate. Methyl linoleate shows  $[\text{CH}_2=\text{CHCH}=\text{CHCH}_2]^+$  fragment which appears at  $m/z = 67$  as the base peak (100%). Methyl oleate shows  $[\text{CH}_2=\text{CHCH}_2\text{CH}_2]^+$  fragment which appears at  $m/z = 55$  as the base peak (100%).

**Table 2. Molecular ion and base peaks of FAME from *P. monadelphum* seed oil**

FAME	Molecular ion peak (m/z)	Base peak (m/z)
Methyl palmitate	270	74
Methyl linoleate	294	67
Methyl oleate	296	55
Methyl stearate	298	74
Methyl arachidate	326	74
Methyl behenate	354	74

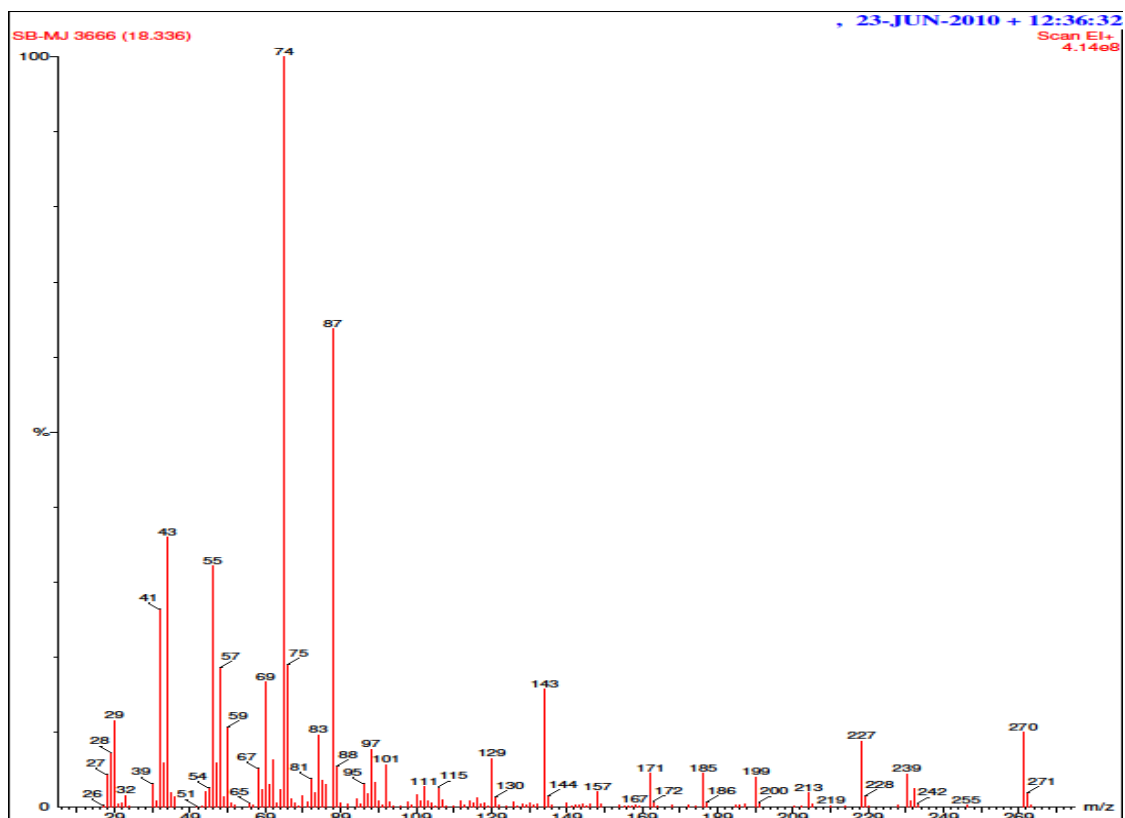


Fig. 3a. Mass spectrum of methyl palmitate

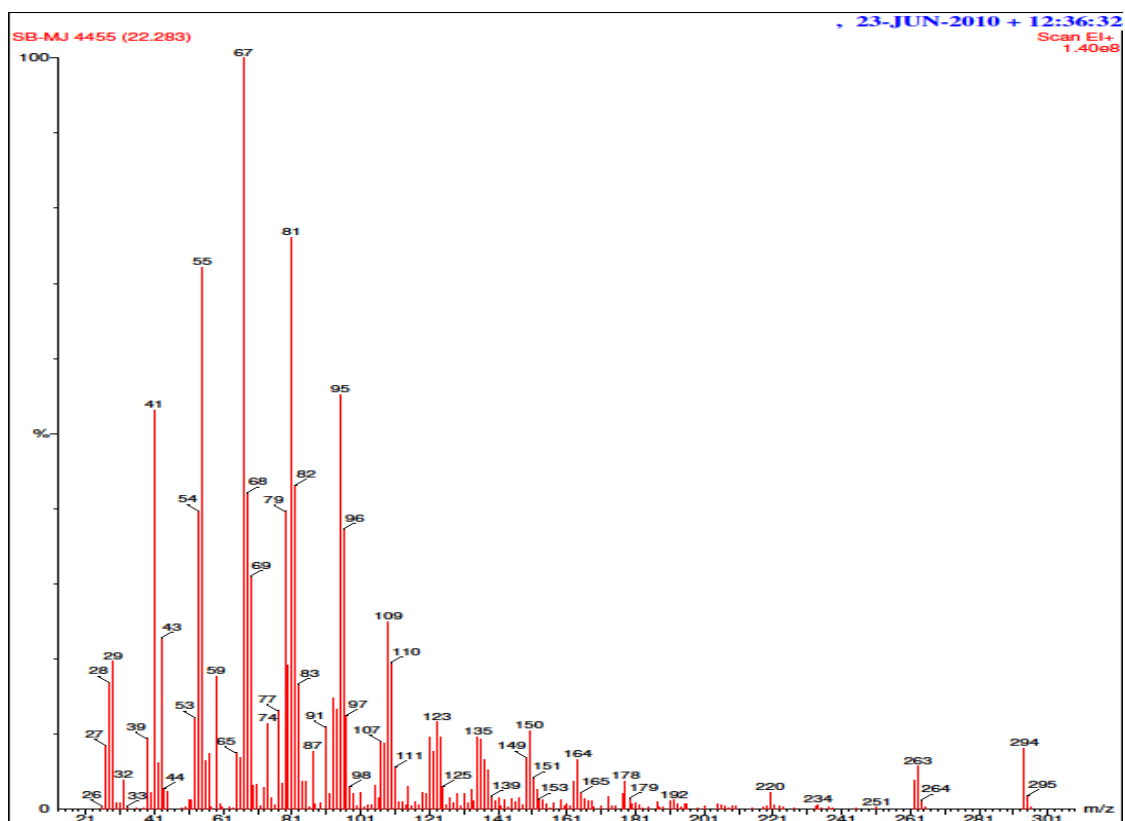


Fig. 3b. Mass spectrum of methyl linoleate

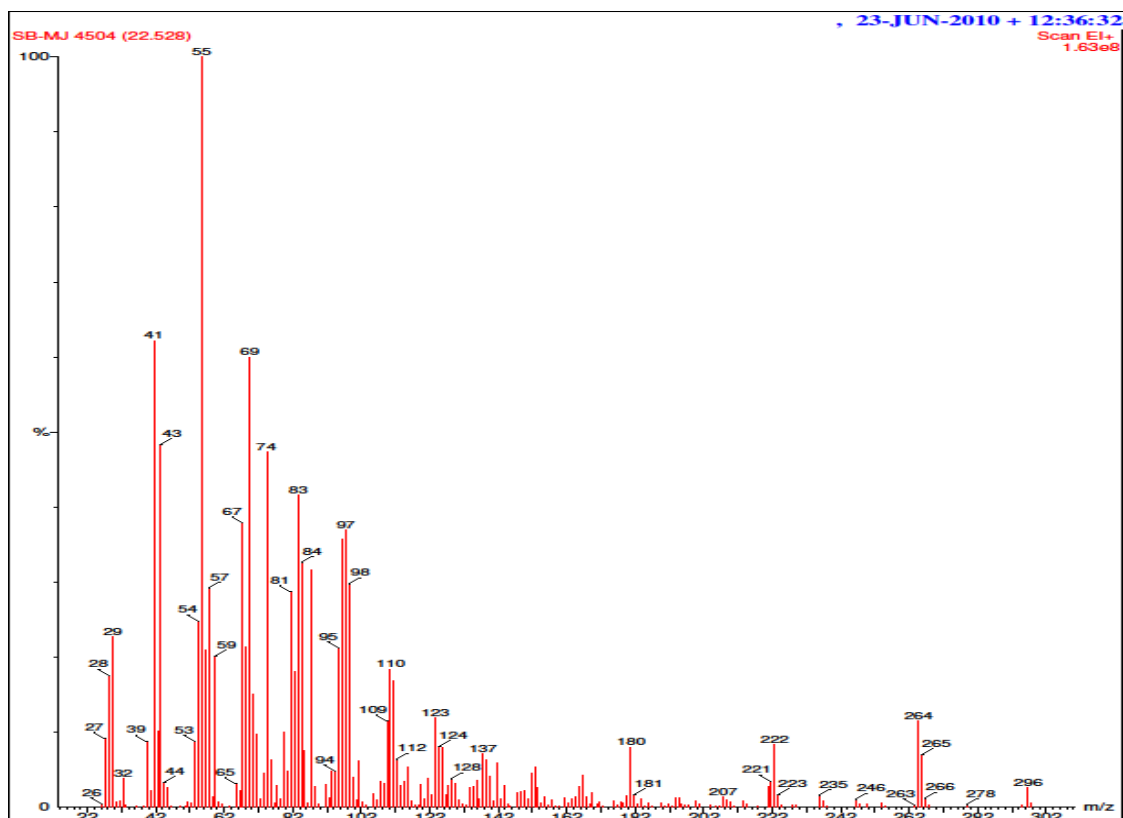


Fig. 3c. Mass spectrum of methyl oleate

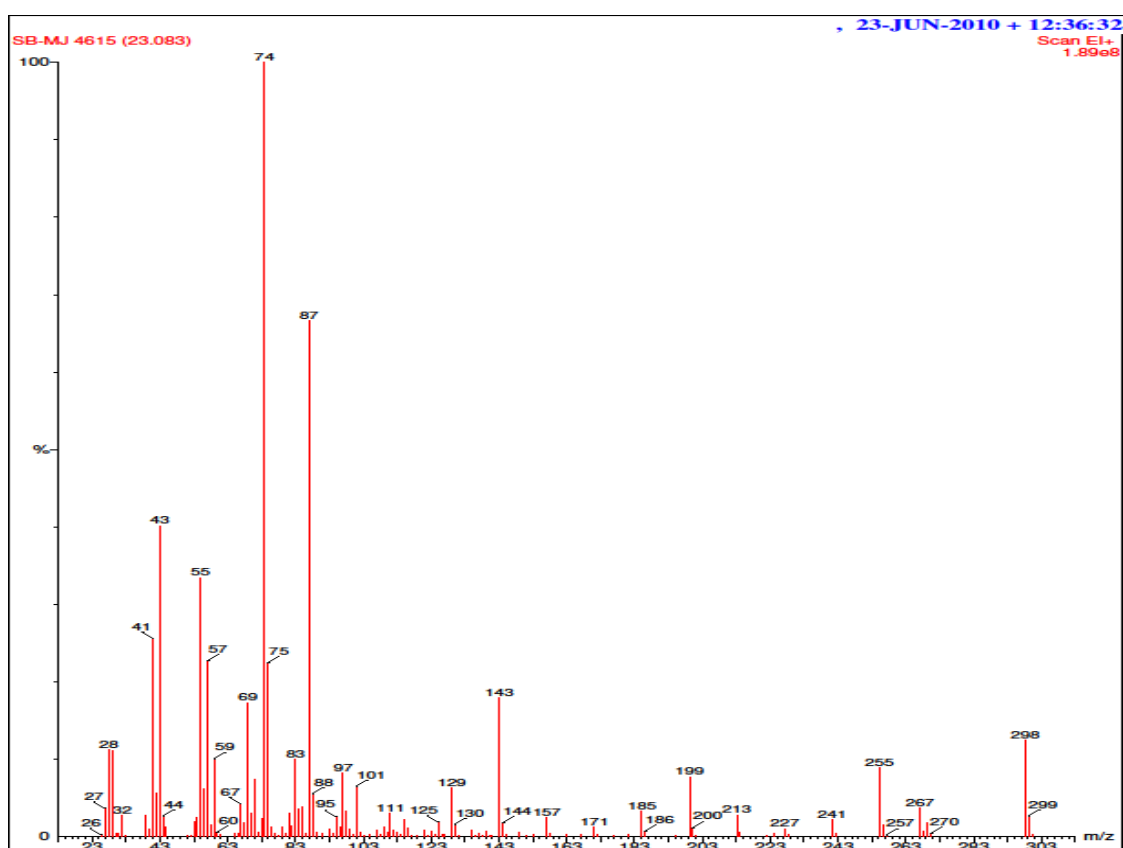


Fig. 3d. Mass spectrum of methyl stearate

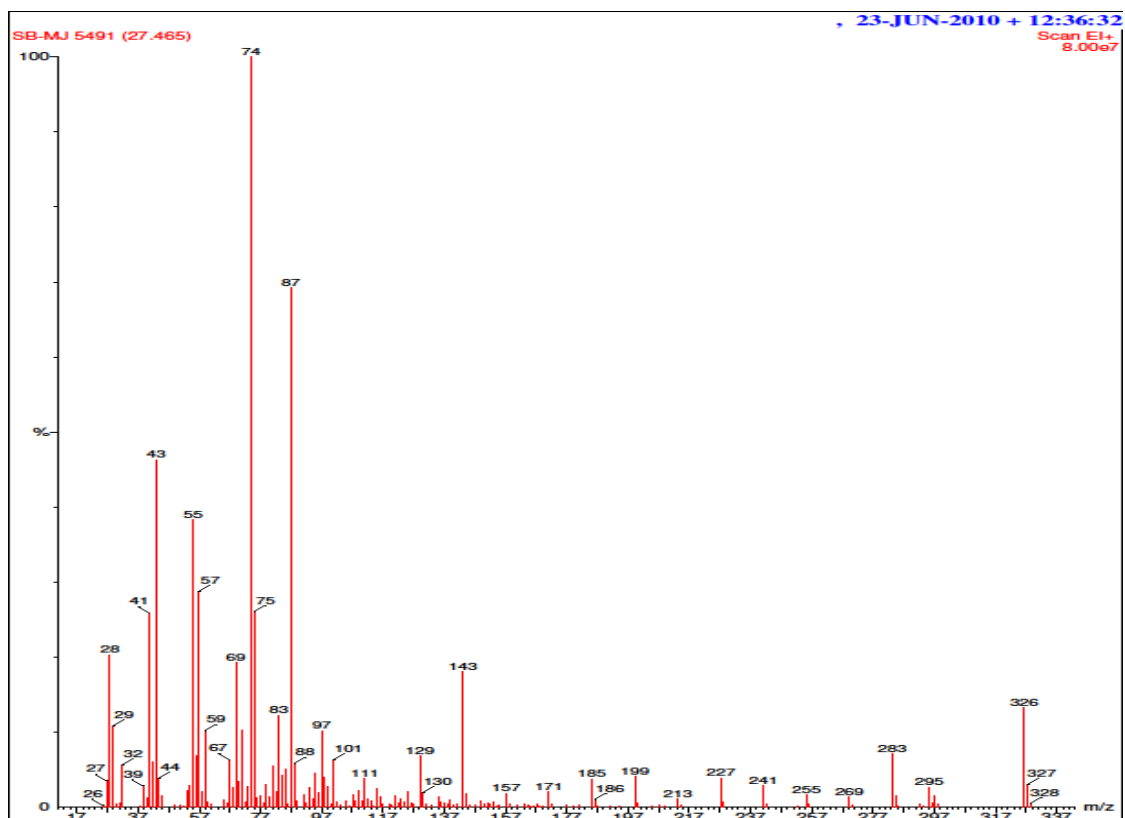


Fig. 3e. Mass spectrum of methyl arachidate

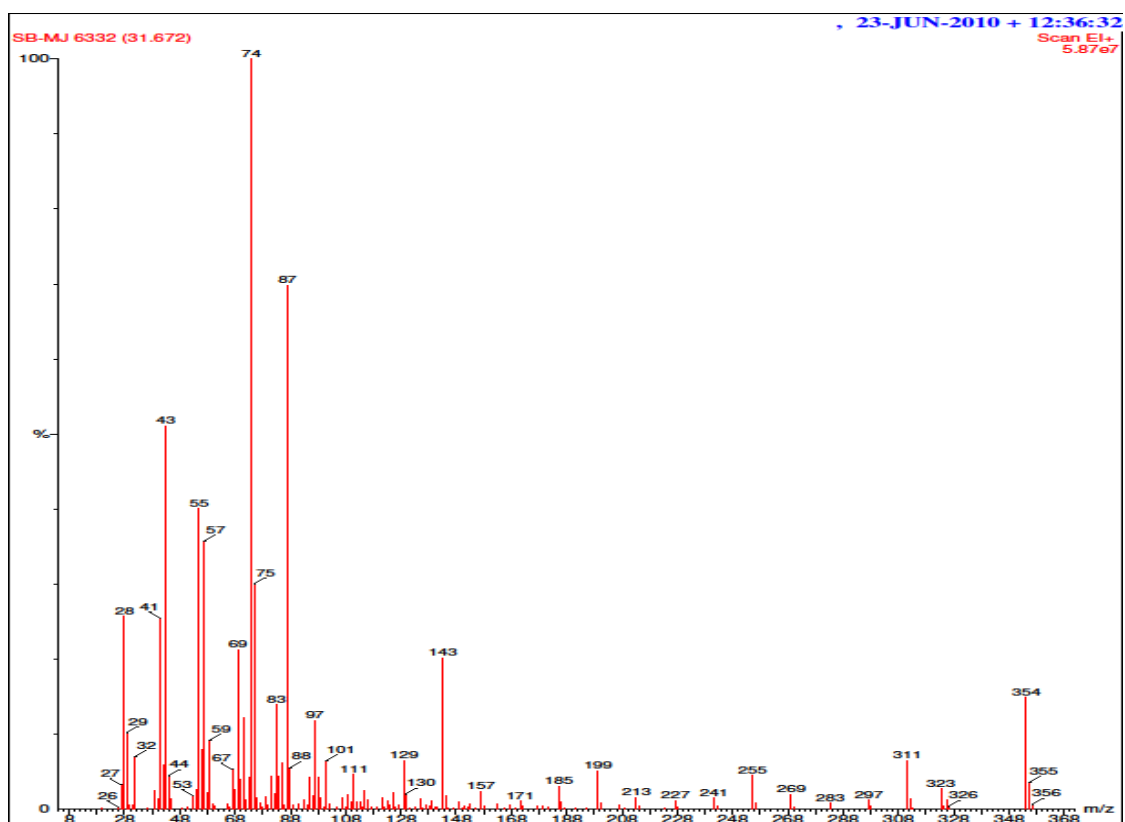
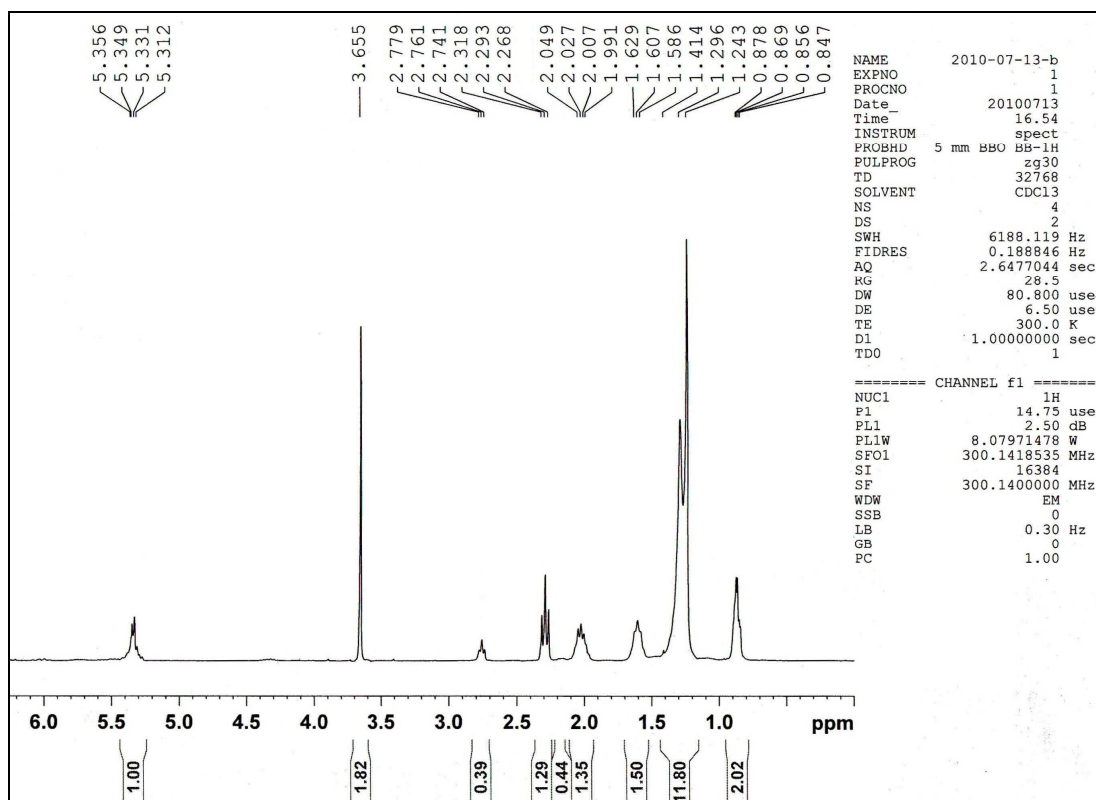
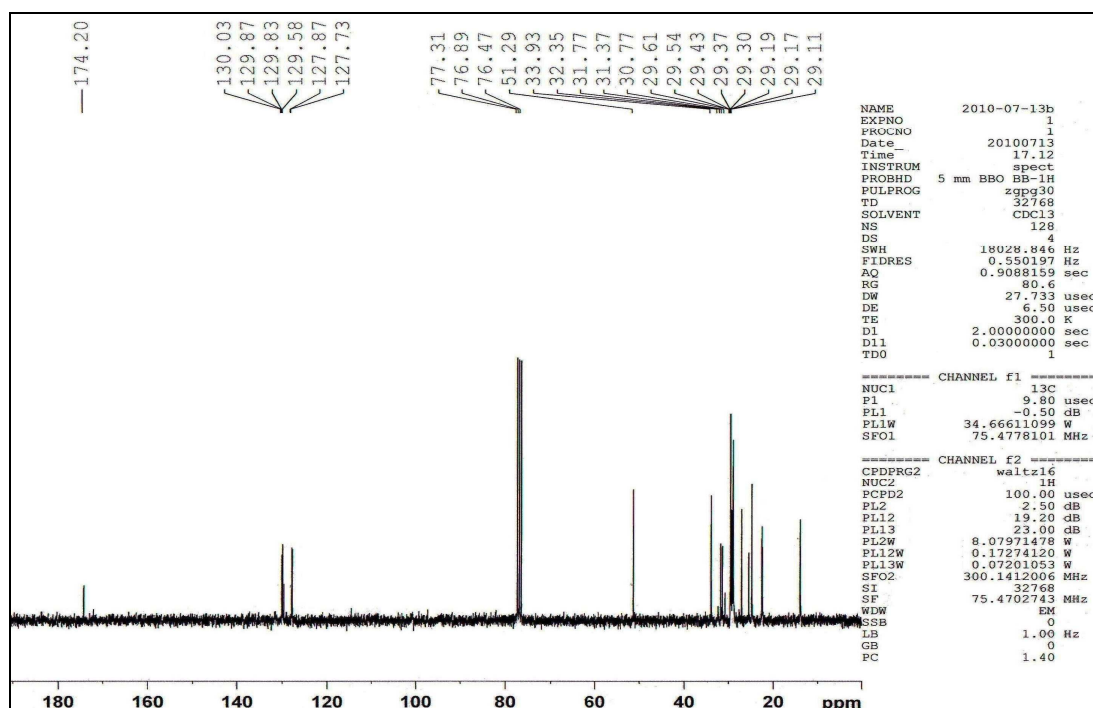


Fig. 3f. Mass spectrum of methyl behenate

Fig. 4.  $^1\text{H}$  NMR spectrum of biodiesel from *P. monadelphum* seed oilFig. 5.  $^{13}\text{C}$  NMR spectrum of biodiesel from *P. monadelphum* seed oil

The  $^1\text{H}$  NMR spectrum of biodiesel from *P. monadelphum* seed oil is shown in Fig. 4. The multiplet at  $\delta$  5.31-5.36 ppm represents the olefinic protons ( $-\text{CH}=\text{CH}-$ ). A singlet signal at  $\delta$  3.65 ppm is representing methoxy protons of the ester functionality of the biodiesel. The bis-allylic proton signal of polyunsaturated fatty acid (like linoleic acid) generally appears around at  $\delta$  2.8 ppm [3, 18]. So, the triplet at  $\delta$  2.76 ppm ( $t$ ,  $^3J=5.7$  Hz) indicates the bis-allylic protons ( $-\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}-$ ) of the unsaturated fatty acid chain. The triplet at  $\delta$  2.29 ppm ( $t$ ,  $^3J=7.5$  Hz) represents the  $\alpha$ -methylene protons to ester ( $-\text{CH}_2-\text{CO}_2\text{Me}$ ). The  $\alpha$ -methylene protons to double bond ( $-\text{CH}_2-\text{C}=\text{C}-$ ) appear as a



multiplet at  $\delta$  1.99-2.05 ppm. The  $\beta$ -methylene protons to ester ( $\text{CH}_2\text{-C-CO}_2\text{Me}$ ) also appear as a multiplet at  $\delta$  1.59-1.63 ppm. The singlet signals at  $\delta$  1.24 and 1.30 ppm are expected for the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons ( $\text{C-CH}_3$ ) at  $\delta$  0.85-0.88 ppm appear as a multiplet.

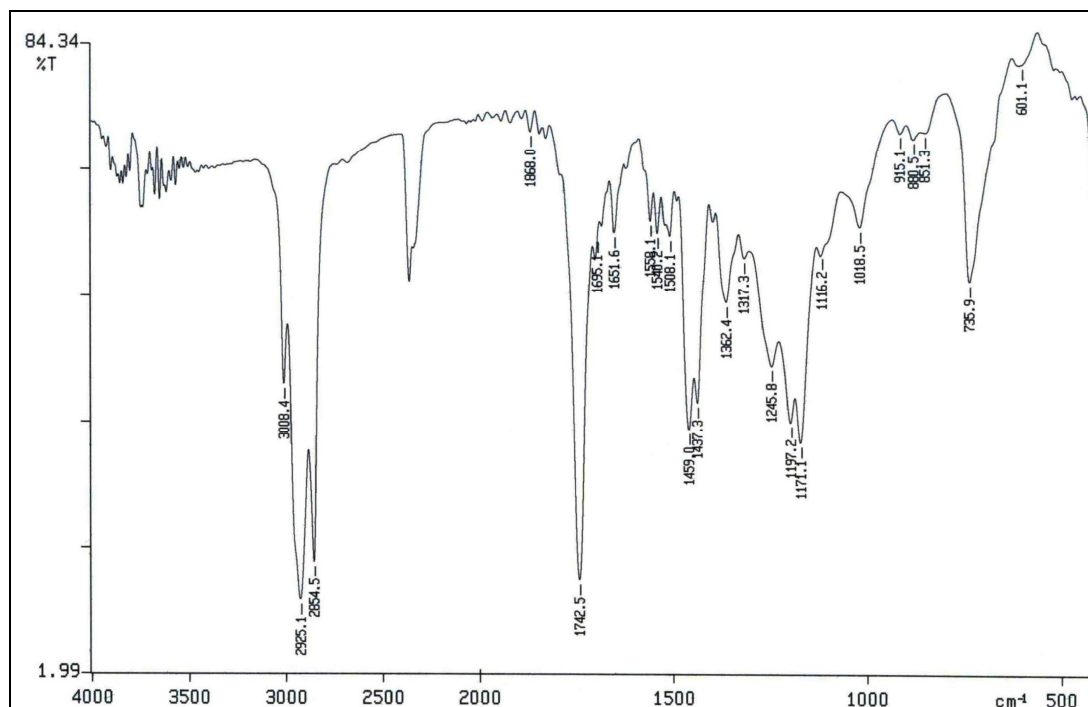


Fig. 6. IR spectrum of biodiesel from *P. monadelphum* seed oil

The  $^{13}\text{C}$  NMR spectrum of biodiesel from *P. monadelphum* seed oil is shown in Fig. 5. The signal at  $\delta$  174.20 ppm represents the carbonyl carbon of the ester molecules of biodiesel and the olefinic carbons appear at  $\delta$  127.73, 127.87, 129.58, 129.83, 129.87 and 130.03 ppm. The signal at  $\delta$  51.29 ppm in the  $^{13}\text{C}$  NMR spectrum of biodiesel is due to the methoxy carbons of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from  $\delta$  13.92 to 33.93 ppm.

The IR spectrum of biodiesel from *P. monadelphum* seed oil is shown in Fig. 6. IR spectrum of biodiesel showed a  $\text{C=O}$  stretching band of methyl esters at  $1742\text{ cm}^{-1}$  and  $\text{C-O}$  stretching bands at  $1171$ ,  $1197$  and  $1245\text{ cm}^{-1}$ . The weak signal at  $1651\text{ cm}^{-1}$  may due to  $\text{C=C}$  stretching frequency. Strong and sharp signals at  $2854$  and  $2925\text{ cm}^{-1}$  are due to  $\text{C-H}$  stretching frequencies. The absorbance at  $3008\text{ cm}^{-1}$  indicates the  $=\text{C-H}$  stretching frequency. The observation of an absorption peak at  $735\text{ cm}^{-1}$  suggested the  $\text{CH}_2$  rocking.

## CONCLUSION

The *Pithecellobium monadelphum* Kosterm seed oil biodiesel was synthesized by using a heterogeneous catalyst derived from the trunk of *Musa balbisiana* Colla with methanol and was characterized by FT-IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR analysis. The chemical composition of biodiesel was determined by GC-MS analysis. The six fatty acid methyl esters were identified in the biodiesel from *Pithecellobium monadelphum* seed oil and consists of 29.44 wt.% of methyl palmitate (C16:0), 38.91 wt.% of methyl linoleate (C18:2), 18.77 wt.% of methyl oleate (C18:1), 6.85 wt.% of methyl stearate (18:0), 2.87 wt.% of methyl arachidate (C20:0) and 3.13 wt.% of methyl behenate (C22:0). The linoleic acid is the major fatty acid followed by palmitic acid and oleic acid. Stearic, arachidic and behenic acids are present as minor constituents. The oil may or may not be useful as a source for the production of biodiesel but it may find application for other purposes. Therefore, more research is needed to explore its potentials for future industrial oilseeds crop.

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