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Research Article

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Identification of chemical composition of biodiesel from *Tabernaemontana divaricata* seed oil

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ABSTRACT

Tabernaemontana divaricata Linn 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 Tabernaemontana divaricata seed oil was analyzed by IR, NMR and GC-MS. The composition of the biodiesel is estimated as 27.0 wt.% methyl palmitate (C16:0), 10.54 wt.% methyl linoleate (C18:2), 56.23 wt.% methyl oleate (C18:1) and 6.20 wt.% methyl stearate (C18:0).

Key words: Biodiesel, *Tabernaemontana divaricata* Linn, transesterification, *Musa balbisiana* Colla.

INTRODUCTION

Due to depletion of petroleum reserves and increase in environmental problems, the worldwide demand for alternative energy to petroleum diesel is increasing continuously. Recently, biodiesel has received more and more attention as a promising alternative fuel because of its renewability, biodegradability, nontoxicity and carbon neutrality [1-5]. Biodiesel, mixture of fatty acid methyl esters (FAME), is obtained from renewable sources such as vegetable oils, animal fats or even waste cooking oils by transesterification with methanol in presence of a catalyst [6-10]. As the demand for edible vegetable oils has increased tremendously in recent years, it is impossible to produce biodiesel from edible oils which leads to food crisis. Moreover, these oils could be more expensive to use as biodiesel. Hence, more and more research needs to be done on non-edible tree seed oils which are considered as economically viable sources for the production of biodiesel.

Tabernaemontana divaricata Linn (*Kathanda* in Assamese), belongs to the *Apocynaceae* family, is a flowering small woody tree which grows to about 2 to 3 metres in height (**Fig. 1**). The plant flowers through out the year in the climatic condition of Assam but heavy flowering can be seen only in autumn season. The present study deals with the synthesis of biodiesel from *Tabernaemontana divaricata* seed oil by transesterification with methanol and determination of composition of the biodiesel formed by employing various instrumental techniques.

EXPERIMENTAL SECTION

Materials

Tabernaemontana divaricata Linn 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.

Fig. 1. *Tabernaemontana divaricata* plant

Fig. 2. *Tabernaemontana divaricata* seed

Oil Extraction

Extractability of oil was evaluated by solvent extraction of the crushed kernel. Crushed kernel in petroleum ether (bp 40-60 ºC, 10 mL/g) was magnetically stirred at room temperature (22-23 ºC) for 3 h, solvent was removed at 45 ºC 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 [2, 11]. 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 ºC) 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_2SO_4 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 *Tabernaemontana divaricata* 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 90 °C for 5 min, increased to 130 °C at 10 °C/min, held for 5 min, increased to 210 °C at 10 °C/min and then held for 15 min. The injector, transfer and source temperatures were 220 °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 *Tabernaemontana divaricata* seed oil is reported in **Table 1**. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ 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. ^{1}H NMR (300 MHz, $CDCl_3$): δ 86-0.89 (m), 1.26 (s), 1.30 (s), 1.59-1.64 (m), 2.00-2.06 (m), 2.30 (t, ^3J=7.5 Hz), 2.77 (t, ^3J=6.0 Hz), 3.67 (s), 5.33-5.36 (m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.95, 22.42, 22.53, 24.79, 25.46, 27.00, 27.05, 28.93, 28.97, 29.00, 29.10, 29.17, 29.30, 29.37, 29.43, 29.52, 29.61, 31.37, 31.75, 33.94, 51.27, 127.74, 127.88, 129.58, 129.84, 130.05, 174.16. FT-IR (thin film): 723, 880, 1019, 1121, 1170, 1197, 1245, 1362, 1437, 1458, 1508, 1540, 1651, 1744, 2854, 2925, 3005 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of biodiesel from *Tabernaemontana divaricata* seed oil are depicted in **Figs. 4 & 5**.

RESULTS AND DISCUSSION

Tabernaemontana divaricata Linn is a flowering small woody tree which grows to about 2 to 3 metres in height (**Fig. 1**). The plant flowers through out the year in the climatic condition of Assam but heavy flowering can be seen only in Autumn season. The oil content of the seed (**Fig. 2**) is estimated at 24 wt.% crude oil which loses about 3 wt.% after column chromatography. 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 [2, 11]. The yield of biodiesel from *Tabernaemontana divaricata* seed oil was 95 wt.% at room temperature (32 °C) within 3 h. The transesterified products were purified by column chromatography and analyzed. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ 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 *Tabernaemontana divaricata* seed oil

Fatty acid profile of the biodiesel prepared from *Tabernaemontana divaricata* 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**. From GC-MS analysis, the composition of the biodiesel is estimated as 27.0 wt.% methyl palmitate (C16:0), 10.54 wt.% methyl linoleate (C18:2), 56.23 wt.% methyl oleate (C18:1), and 6.20 wt.% methyl stearate (C18:0). The oleic acid is the major fatty acid followed by palmitic acid comprising of about 66 wt.% unsaturated and 33 wt.% saturated fatty acids.

Table 1. Fatty acid profile of biodiesel from *T. divaricata* seed oil

Retention time (min)	FAME	wt.%
22.82	Methyl palmitate	27.00
25.50	Methyl linoleate	10.54
25.69	Methyl oleate	56.23
26.17	Methyl stearate	06.20

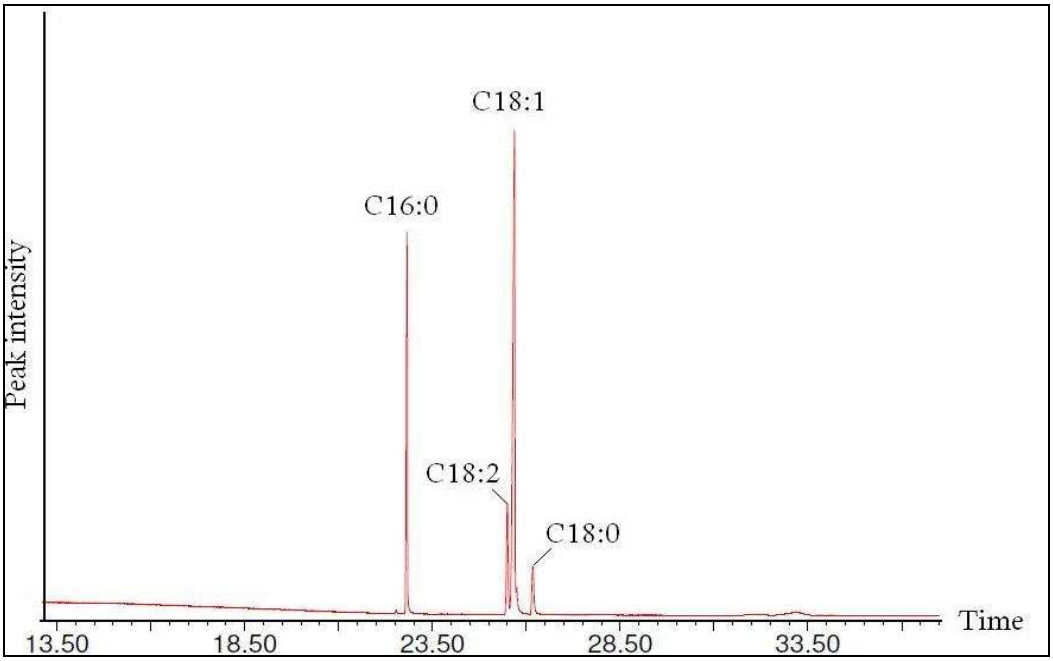

Fig. 3. Gas chromatogram of biodiesel from *T. divaricata* seed oil

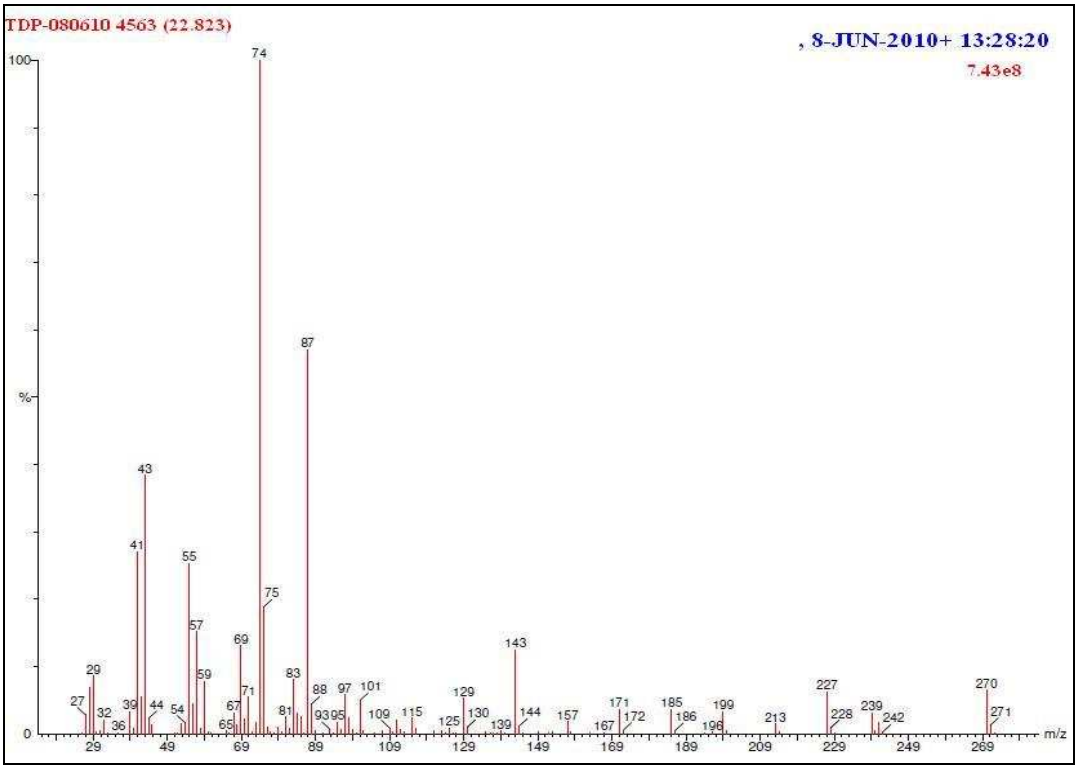

Fig. 3a. Mass spectrum of methyl palmitate

The mass spectra of biodiesel prepared from *T. divaricata* seed oil are shown in **Figs. 3a to 3d**. Molecular ion peaks and base peaks of the FAME are shown in **Table 2** and they are at the expected m/z values. The molecular ion peaks of methyl palmitate, methyl linoleate, methyl oleate and methyl stearate were observed at 270, 294, 296 and 298 respectively as expected. It is interesting to observe that the saturated FAMEs detected in the biodiesel from *T. divaricata* (methyl palmitate and methyl stearate) show $CH_3OC(=OH^+)CH_2$ fragment and appears at m/z = 74 as the base peak (100%) which is the result of McLafferty rearrangement [12, 13] during the MS analysis due to a six-member ring structure of an intermediate. Methyl linoleate shows $[CH_2=CHCH=CHCH_2]^+$ fragment which appears at m/z = 67 as the base peak (100%). Methyl oleate shows $[CH_2=CHCH_2CH_2]^+$ fragment which appears at m/z = 55 as the base peak (100%).

Table 2. Molecular ion and base peaks of FAME from *T. divaricata* 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

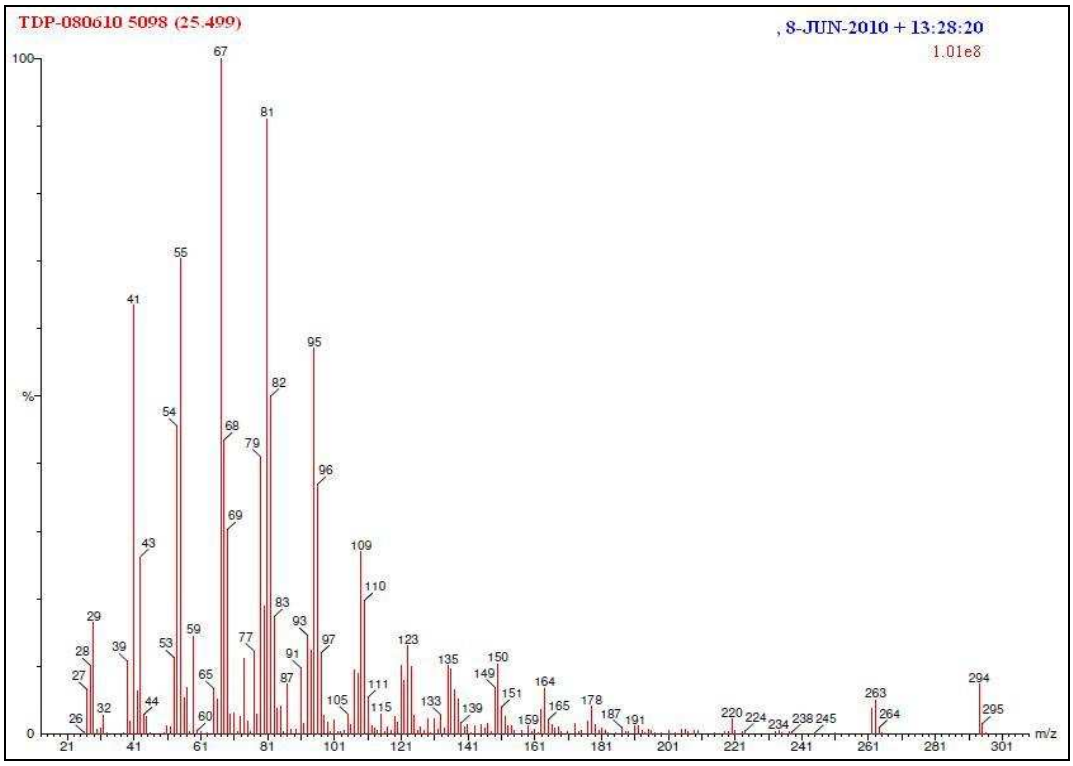

Fig. 3b. Mass spectrum of methyl linoleate

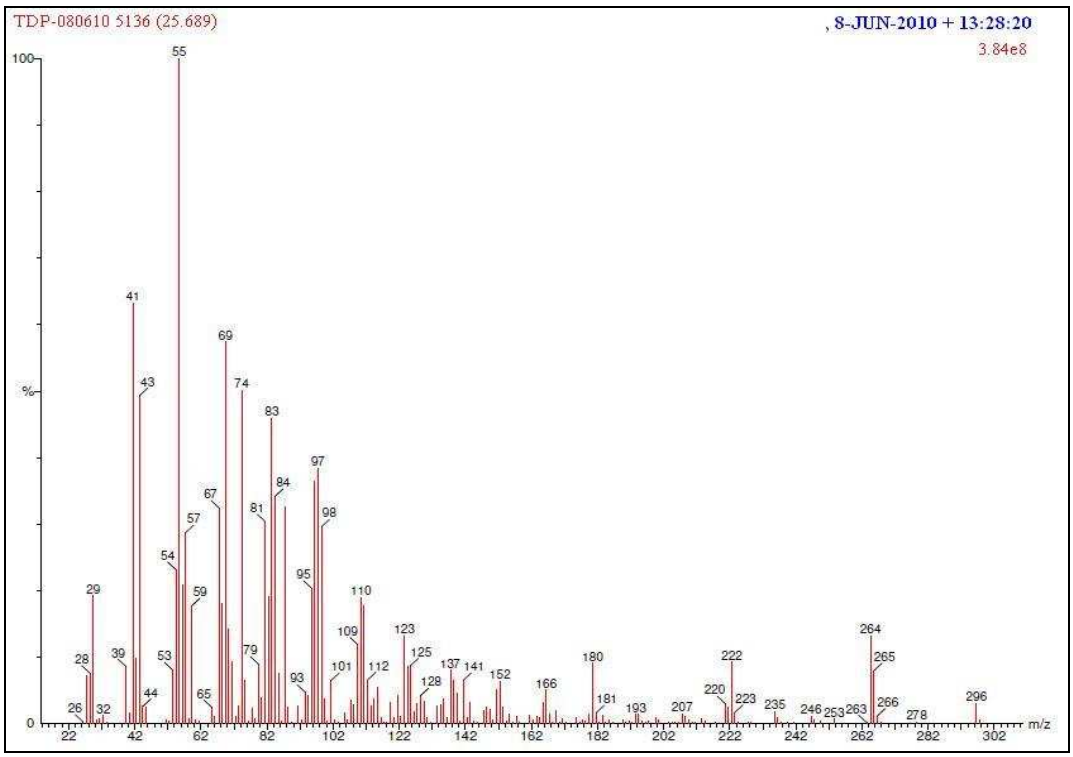

Fig. 3c. Mass spectrum of methyl oleate

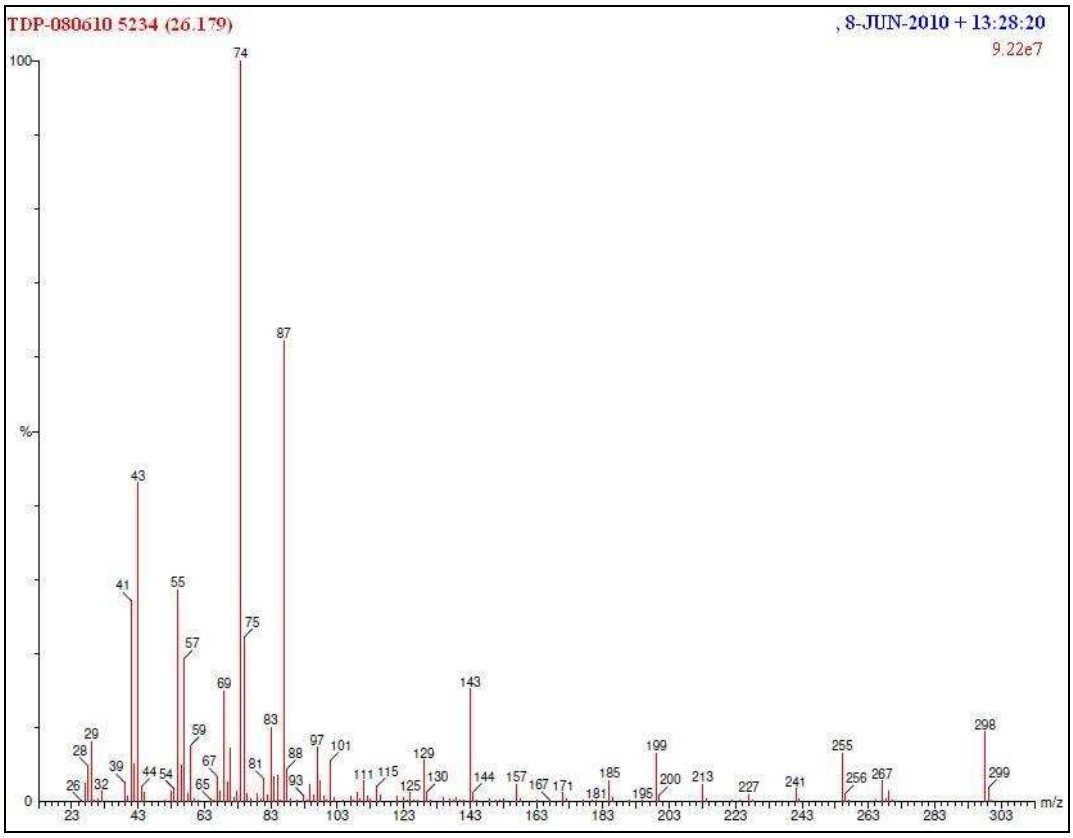

Fig. 3d. Mass spectrum of methyl stearate

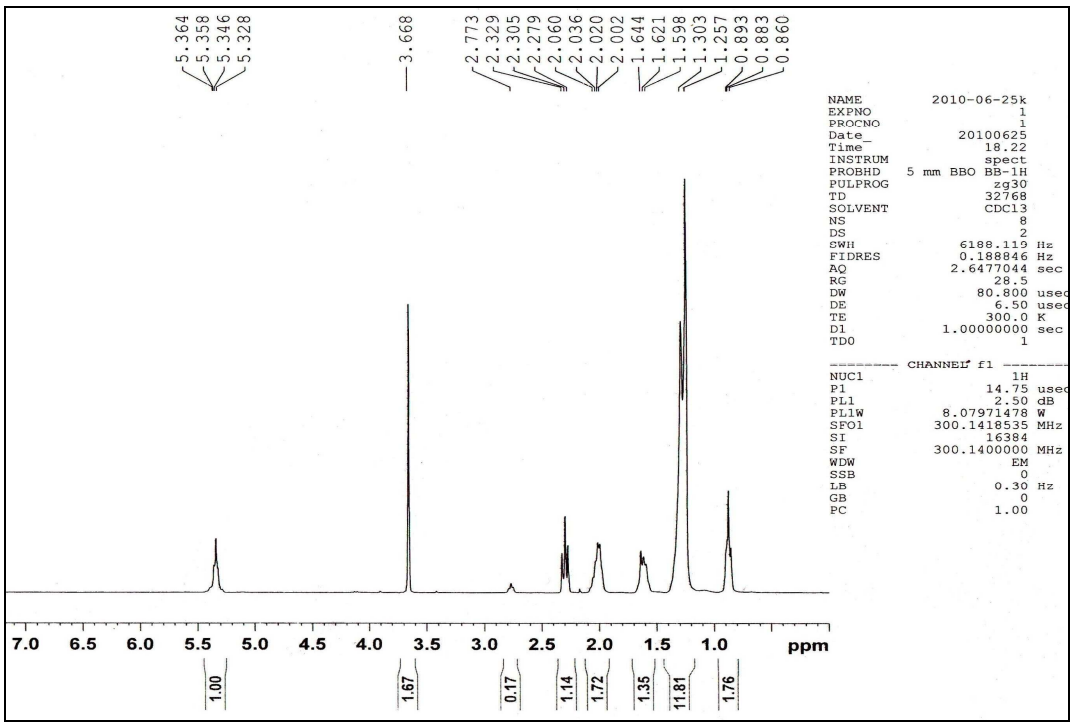

Fig. 4. ^{1}H NMR spectrum of biodiesel from *T. divaricata* seed oil

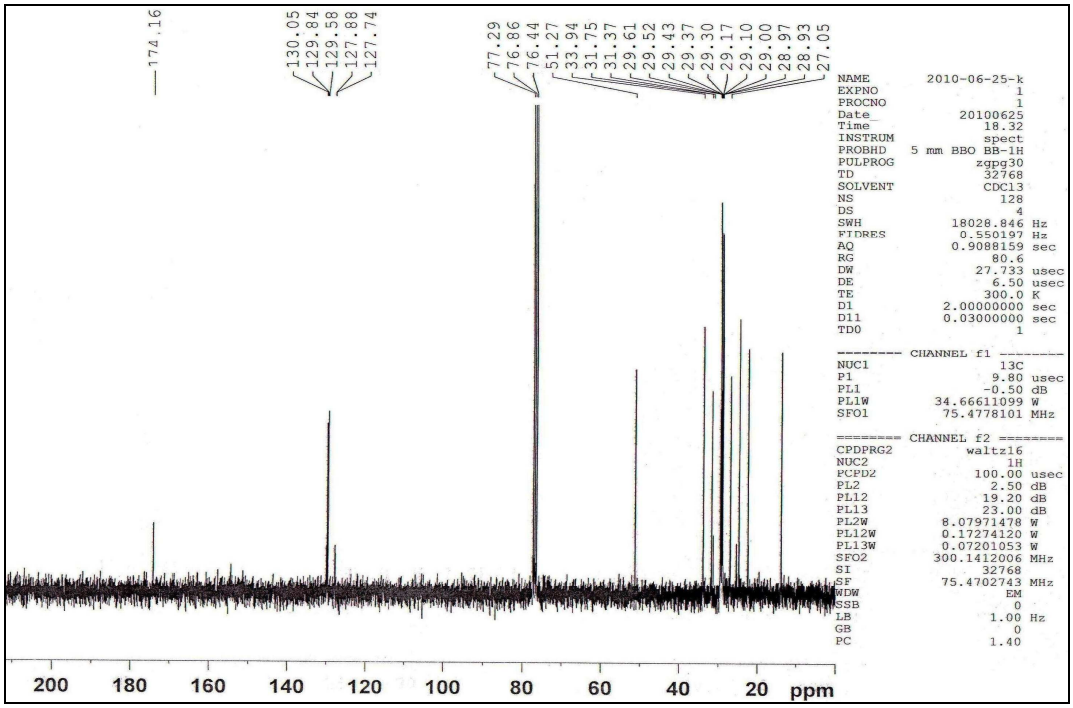

Fig. 5. ^{13}C NMR spectrum of biodiesel from *T. divaricata* seed oil

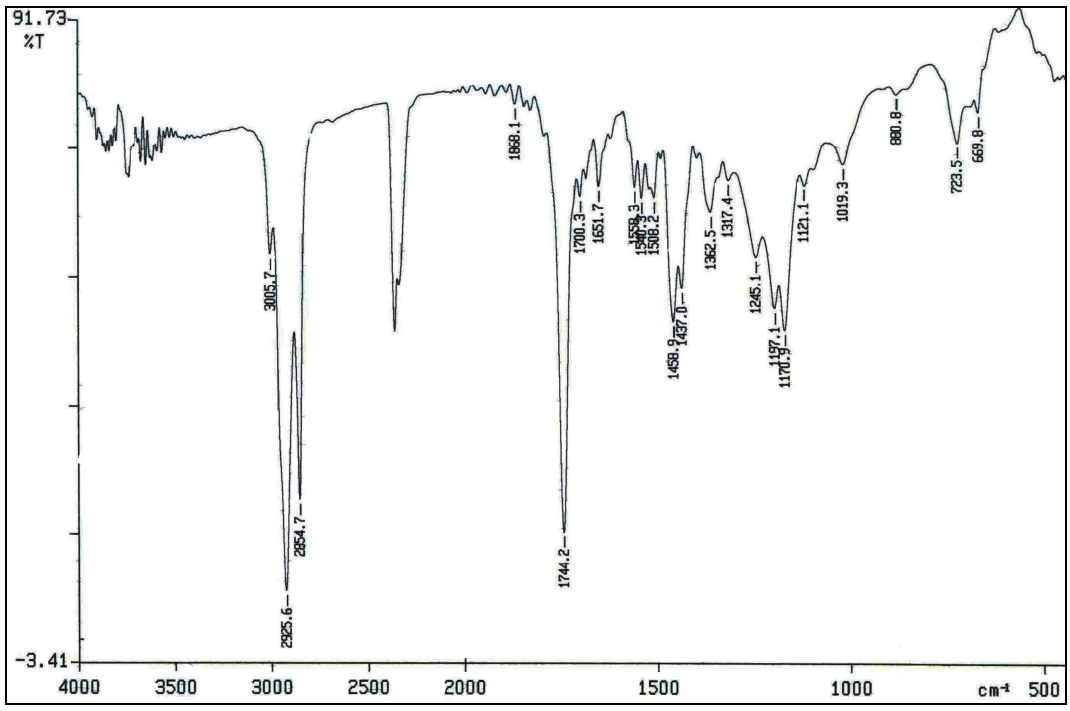

Fig. 6. IR spectrum of biodiesel from *T. divaricata* seed oil

The ^{1}H NMR spectrum of biodiesel from *Tabernaemontana divaricata* seed oil is shown in **Fig. 4**. The multiplet at δ 5.33-5.36 ppm represents the olefinic protons (-CH=CH-). A singlet signal at δ 3.67 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 δ 2.8 ppm [14, 15]. So, here the triplet which appears at δ 2.77 ppm (t, ^{3}J=6.0 Hz) indicates the bis-allylic protons (-C=C-CH_2-C=C-) of the unsaturated fatty acid chain. The triplet at δ 2.30 ppm (t, ^{3}J=7.5 Hz) represents the α-methylene protons to ester (-CH_2-CO_2Me). The α-methylene protons to double bond (-CH_2-C=C-) is seen as a multiplet at δ 2.00-2.06 ppm. The β-methylene protons to ester (CH_2-C-CO_2Me) also appear as a multiplet at δ 1.59-1.64 ppm. The singlet signals at δ 1.26 and 1.30 ppm are due to the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons (C-CH_3) at δ 86-0.89 ppm appear as a multiplet.

The ^{13}C NMR spectrum of biodiesel from *T. divaricata* seed oil is shown in **Fig. 5**. The signal at δ 174.16 ppm represents the carbonyl carbon of the ester molecules and the olefinic carbons appear at δ 127.74, 127.88, 129.58, 129.84 and 130.05 ppm. The signal at δ 51.27 ppm in the ^{13}C NMR spectrum of biodiesel is due to methoxy carbons of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from δ 13.95 to 33.94 ppm.

The IR spectrum of biodiesel from *T. divaricata* seed oil is shown in **Fig. 6.** IR spectrum of biodiesel showed a C=O stretching frequency of methyl esters at 1744 cm^{-1} and C-O stretching bands at 1121, 1170, 1197 and 1245 cm^{-1}. The weak signal at 1651 cm^{-1} may due to C=C stretching frequency. Strong and sharp signals at 2854 and 2925 cm^{-1} are due to C-H stretching frequencies. The absorbance at 3005 cm^{-1} indicates the =C-H stretching frequency. The observation of an absorption peak at 723 cm^{-1} suggested the CH_2 rocking.

CONCLUSION

The *Tabernaemontana divaricata* Linn 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}H and ^{13}C NMR analysis. The chemical composition of biodiesel was determined by GC-MS analysis. The four fatty acid methyl esters were identified in the biodiesel from *Tabernaemontana divaricata* seed oil and consists of 27.0 wt.% methyl palmitate (C16:0), 10.54 wt.% methyl linoleate (C18:2), 56.23 wt.% methyl oleate (C18:1) and 6.20 wt.% methyl stearate (C18:0). The oleic acid is the major fatty acid followed by palmitic acid comprising of about 66 wt.% unsaturated and 33 wt.% saturated fatty acids. The oil may be useful as a source for the production of biodiesel or for other purposes. Therefore, more research is needed to explore its potential for future industrial oilseeds crop.

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