Atomic Spectroscopy

Article

The Extraction and analysis of Glycerides by GCMS and IR in Oil seeds from the *Sesbania sesban* plant in Manipur

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ABSTRACT: The oil obtained from Sesbania sesban seeds was extracted using a solvent extraction method with petroleum ether as an extracting solvent. To ensure purity for subsequent analysis, the oil underwent a purification process using column chromatography with a silica gel (60-120 mesh) column and an eluent composed of a mixture of petroleum ether and ethyl acetate (20:1 ratio). After this purification, the oil was subjected to transesterification, resulting in the production of biodiesel also referred to as fatty acid methyl ester (FAME).

The composition of fatty acid methyl esters (FAME) in Sesbania sesban seed oil was determined through NMR, IR and GCMS analysis. The fame profile of Sesbania sesban oil comprises of 5.21wt.% of methyl palmitate [C16:0], 93.17 wt.% of methyl linoleate [C18:2] and 1.61 wt.% of methyl stearate [C18:0].

Keywords: Chuchurangmei, Sesbania sesban, transesterification, non-edible vegetable oil, Biodiesel.

INTRODUCTION

The reduced use of nonedible oilseed products in the 20th century was mainly due to widespread availability of relatively cheap fossil fuel derived oils [1,19]. While there is already a wealth of data on the physical and chemical properties, as well as the fatty acid composition of more traditional oilseeds, this information is lacking for non-conventional oilseeds [20]. Nevertheless, ongoing efforts to explore and extract oils from non-conventional oilseeds are expected to help control the prices of expensive conventional oils, making them more affordable, especially in developing countries.

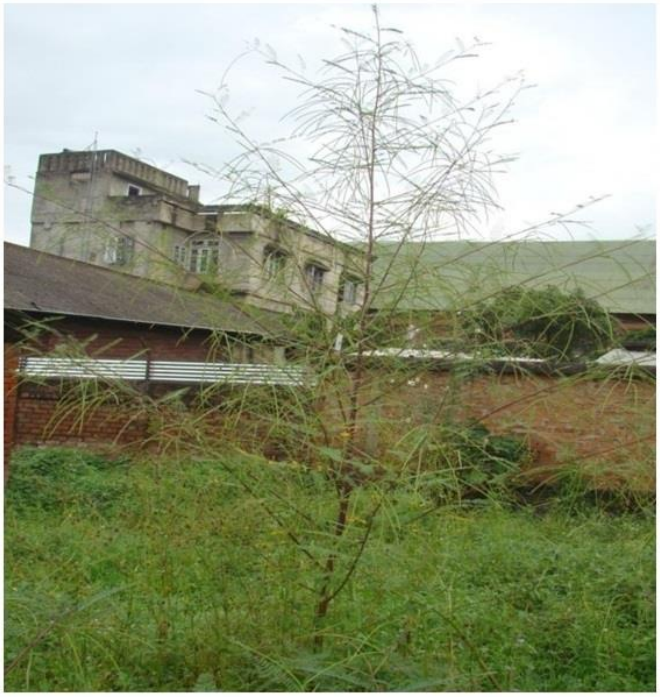
The North-East region of India, including Manipur is renowned for its rich plant diversity with numerous plant varieties bearing fruit seeds rich in non-edible oils thriving in both the plains and hilly areas of Manipur. Unfortunately, most of these plants have little no apparent economic value, leading to limited commercial use of thee oils they produce. Consequently, these plants are gradually disappearing from the landscape as they are considered unimportant by farmers, the government and both public and private sectors. These decline in plant diversity is a concerning trend.

Additionally, there is a growing concern that large scale production of biodiesel from edible oils may disrupt the global food supply and demand balance [2, 9-13, 20]. Therefore, using non-edible oils as the raw material for biodiesel industries could help reserve edible oils for other food related industries [2, 9-13, 20]. Biodiesel is gaining prominence due to its renewability, biodegradability, carbon neutrality and nontoxic nature and it is being adopted globally by countries such as Brazil, Indonesia, UK, Malaysia, Germany and Canada [2, 20]. This shifts towards biodiesel is crucial for ensuring self-reliance in our country [3-5]. In this context, identifying the fatty acid constituents in glycerides becomes imperative [3-5].

Biodiesel is primarily composed of methyl esters of long chain fatty acids and it is produced from nontoxic biological resources like vegetable oils and animal fats through transesterification with methanol in the presence of catalyst [2,5]. These catalysts can be acid, base or enzymes like lipase [2-5]. Biodiesel offers numerous advantages and can play a pivotal role in addressing global warming and energy challenges [15-17]. Thus, non-edible vegetable oils can serve as an alternative source for biodiesel production [6-8].

Sesbania sesban, known locally as Chuchurangmei in Manipuri, belongs to the *Fabaceae* family. This nitrogen fixing tree has potential for alley cropping and is widely cultivated in tropical regions of Africa and Asia. It is small, often multi-steamed tree, reaching heights of 4-8 meters. Its pods are semi-cylindrical, straight curved, measuring up to 30 cm in length and 5 mm in width, containing about 10-15 seeds. Dried leaves are used in some countries to make a tea known for its antibiotic, anti-tumour and contraceptive properties. The tree is also valued for its high biomass production in a short time and relatively smokeless nature, making it popular for fuelwood. *Sesbania sesban* leaves are a good source of protein for cattle and sheep. People also consume its leaves, flowers and seeds with young leaves and fruits are used for salads. After purification through column chromatography, it yields approximately 18.79 wt.% of oil.

Sesbania sesban seeds were gathered from Kshetrigao, Imphal East, located at coordinates (24.63° N, 94.02° E) in Manipur, India, during their seasonal availability, which typically falls between September and October. The collected seeds underwent an initial cleaning process and were then sun-dried for approximately 5 to 6 days. Subsequently, the seeds were deshelled, and their kernels were crushed using a grinder to facilitate oil extraction. Analytical grade methanol from Mark Mumbai, India, was employed in the extraction process, along with other analytical grade solvents and chemicals sourced from commercial suppliers, without any further treatment.



a. *Sesbania sesban* plant with fruits

b. *Sesbania sesban* seeds

Figure 1: *Sesbania sesban* plant with matured fruits.

MATERIALS AND METHODS

To extract the oil, the crushed and powdered *Sesbania sesban* seed kernels were subjected to solvent extraction in petroleum ether (with a boiling point of 40-60°C) at a ratio of 10 ml per gram of seed material. The extraction process involved magnetic stirring at room temperature (approximately 26°C) for a duration of 4 hours and 30 minutes. The solvent was subsequently removed at 45°C using a rotary vacuum evaporator (BUCHI Rotor vapour R-200), resulting in the production of crude oil. This extraction process was repeated 2 to 3 times on the seed cake, utilizing fresh solvent in each repetition to maximize oil extraction. The obtained oil was further dried using a vacuum pump.

Prior to the transesterification process, the oil underwent purification through column chromatography using silica gel (mesh size: 60-120) with a mixture of petroleum ether and ethyl acetate (in a 20:1 ratio) as the eluent. % of oil content in the oil seeds can be determined by the following formula [14],

% oil content= (Weight of oil / Weight of powdered seeds) *100…… (1)

The glyceride parameters including density, colour, refractive index, acid value, iodine value, saponification value were experimentally determined in accordance with the protocols outlined in the Association of Official Analytical Chemical Procedures [1].

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

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

Where, V_B = Volume of sodium thiosulphate solution used for the blank (mL)

V_S = 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

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

where, V_B = Volume of 0.5 M HCl solution used for the blank (mL)
V_S = Volume of 0.5 M HCl solution used for the oil sample (mL)
M = Molarity of HCl used
W = Weight of oil sample taken in g

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

where, W_1 = Initial weight of oil,
W_2 = Final weight of oil

The results of the experiments are presented in table 1. Refractive indices of the purified seed oils were measured with an Abbe Refractometer (AW-24) at room temperature, with only a few drops of oil required. Densities of the purified oils were determined at room temperature (32°C) by weighing a clean and empty plastic centrifuge tube, then transferring precisely 1 mL of the liquid sample into the tube using a syringe and weighing it again. The density was then calculated based on the mass per unit volume of the oil.

The pure oil was subjected to a transesterification process to convert it into fatty acid methyl esters (FAME) using a catalyst referred to as Athia, derived from the ashes of banana peels from *Musa balbisiana* variety, constituting 20% of the oil [18, 21]. This conversion involved vigorously stirring a mixture of the oil in methanol (at a ratio of 10 ml per 1 gram of oil) and the catalyst (comprising 20% of the oil) under magnetic agitation at room temperature (approximately 26°C) [21]. The progress of the reaction was monitored using Thin Layer Chromatography (TLC).

Upon completion of the reaction, the resulting mixture was extracted with petroleum ether (with a boiling point of 40-60°C). The organic layer was washed with brine, then left to dry over anhydrous Na_2SO_4 overnight, and the solvent was subsequently removed under vacuum, resulting in the production of a crude product. This crude product was further refined through column chromatography over silica gel, employing a mixture of petroleum ether and ethyl acetate (in a 20:1 ratio) as the eluent. The purified product was concentrated and evaporated to dryness using a rotary evaporator, which was followed by additional drying using a vacuum pump to eliminate any remaining traces of solvents, yielding pure biodiesel (FAME).

The composition of the FAME mixture was determined using a Parkin Elmer Clarus 600 GC-MS. The column used was the Elite 5 MS, initially held at 140°C for a specific duration, then increased to 240°C at a rate of 4°C per minute, and held at that temperature for 5 minutes. The injector transfer and source temperatures were set at 250°C and 150°C, respectively. Helium was used as the carrier gas, and the total scan time was 35 minutes. Electron ionization mode was applied, and the mass spectrum ranged from 20 to 400 Da. To identify the FAME, a library search was conducted using databases such as the National Institute of Standards and Technology (NIST), National Bureau of Standards (NBS), and Wiley GC-MS library. The fatty acid profile of biodiesel obtained from *Sesbania sesban* seed oil is presented in Table 2. Additionally, the ^1H and ^{13}C NMR spectra were recorded in Carbon Deuterium Trichloride (CD Cl_3) at 300 MHz using a 5mm NMR spectrometer, and the IR spectrum was captured on a Perkin Elmer RXIFT-IR spectrometer as a thin film on a KBr plate.

Table1: Physical parameters of *Sesbania sesban* calculated using equation (1-5)

Sl. No.	Parameters	Observed Values
1.	Colour	pale yellow
2.	Oil content (wt. %)	18.79
3.	Density (g/cm^3)	0.8498
4.	Acid Value (mg KOH/g)	0.670
5.	Iodine value ($gI_2/100g$)	123.81
6.	Saponification value (mg KOH/g)	167.28
7.	Refractive Index	1.4680
8.	Moisture (%)	0.104

RESULTS AND DISCUSSION

The fatty acid composition of the FAME, prepared from *Sesbania sesban* seed oil, was determined through GC-MS analysis. Each peak on the gas chromatogram (Fig. 2) was analysed, and the fatty acids were identified by comparing with MS database. Each peak represented a distinct fatty acid methyl ester. The presence of three peaks in the gas chromatogram indicated the existence of three different fatty acid methyl esters. The rightmost peak in the mass spectrum of any fatty acid methyl ester provided the molecular weight of the fatty acid, known as the molecular ion peak. Retention time denoted the time taken for a peak to develop. The base peak represented the tallest peak in the mass spectrum and indicated the ion with the highest relative abundance. The peak with the greatest m/z value was likely the molecular ion peak.

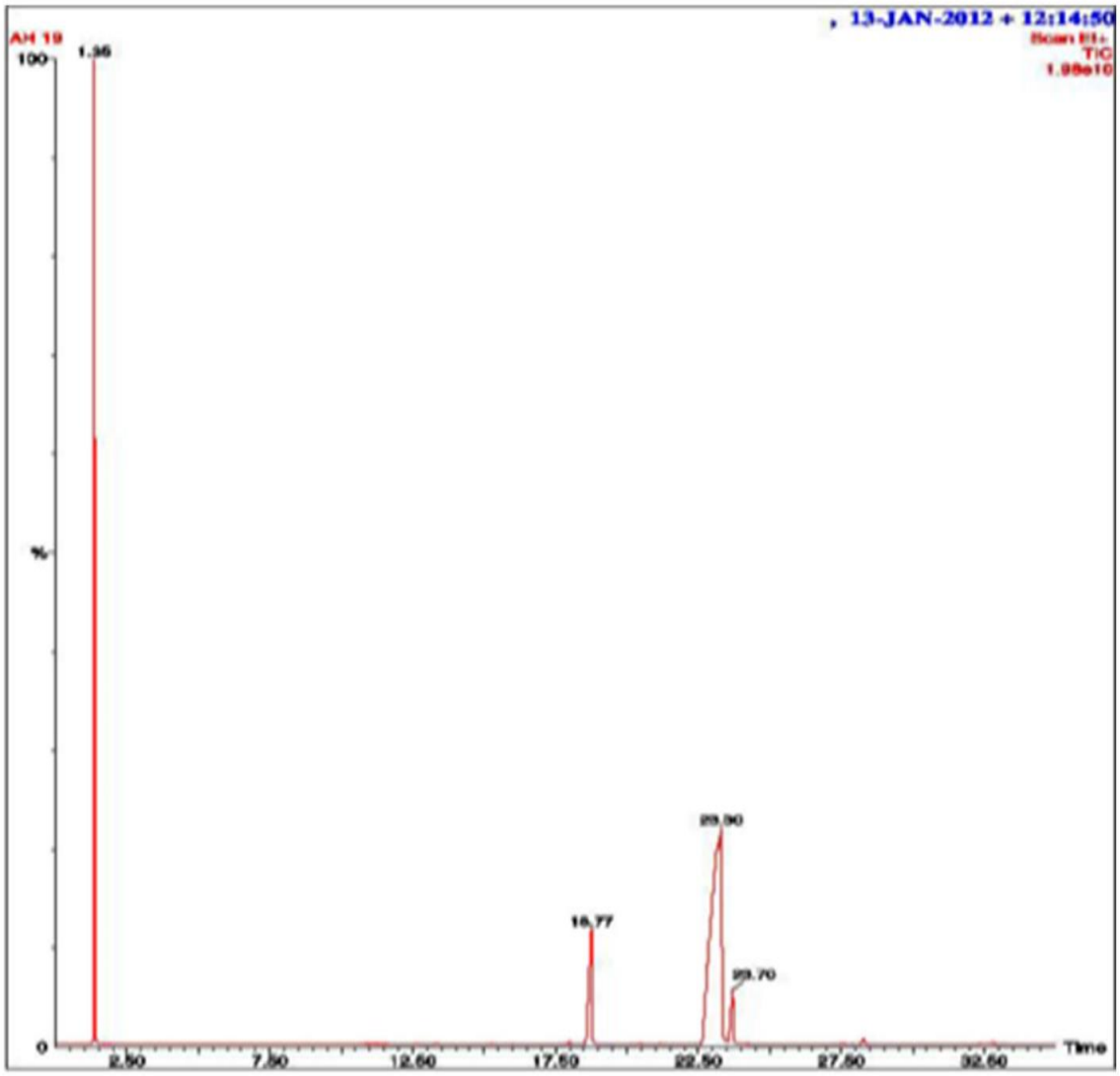


Figure 2: Gas Chromatogram of biodiesel from *Sesbania sesban* seed oil

The obtained and purified glycerides from *Sesbania sesban* seed oil had a yield of 18.79% at room temperature (26°C) within a duration of 4 hours and 30 minutes. In contrast, the yield of the trans esterified glyceride, known as Fatty Acid Methyl Ester (FAME), was 94.5% at room temperature (25°C) within 4 hours and 40 minutes.
The light-yellow hue of the *Sesbania sesban* seed oil was attributed to the presence of natural pigments such as tocopherols, carotenoids, and their derivatives. The oil yield was moderate. The density and iodine value of *Sesbania sesban* seed oil were measured at 0.8498 g/cm³ and 123.81 $gI_2/100$, respectively, and these values were comparable to those of soybean oil and sunflower oil. The acid value of this oil was determined to be 0.670 mg KOH/g, falling within the acceptable range for industrial-grade oil. The saponification value was calculated to be 167.28 mg KOH/g, a value suitable for applications in soap making and cosmetic industries. The refractive index of this oil was 1.4680, which did not significantly deviate from the values recorded for conventional seed oils like palm oil (1.445-1.451), cotton seed oil (1.468-1.472), safflower oil (1.473-1.476), and soybean oil (1.4728) at 25°C. The moisture content was determined to be 0.102%, a low value, which is favourable for maintaining high-quality standards and preventing contamination. Low moisture content is a vital criterion for commercial oil.

3.1: Analysis of FAME in *Sesbania sesban*:
^{1}HNMR (300 MHz, CD Cl_3): δ 5.304 -5.36 ppm, δ 3.66 ppm, δ 2.8 ppm, δ 2.30 ppm, δ 2.01 ppm, δ 1.64 ppm, δ 1.25 -1.30 ppm, δ 0.86 -0.91 ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ 174.37 ppm, δ 127.89 ppm, δ 128.03 ppm, δ 51.48 ppm, δ 24.95 -34.11 ppm.

FT-IR (thin film): 1739.79 cm^{-1}, 1546.91 cm^{-1}, 2926.01 cm^{-1}, 2854.65 cm^{-1}, 1240.23 cm^{-1}, 1170.79 cm^{-1}, 1103.28 cm^{-1}, 736.81 cm^{-1}, 3051.39 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 *Sesbania sesban* consists of 5.21wt% of methyl palmitate (C16:0), 93.17wt% of methyl linoleate (C18:2), and 1.61wt% of methyl stearate (C18:0). The mass spectra of methyl palmitate, methyl linoleate and methyl stearate are shown in Fig.3a – 3c. The molecular ion peaks and base peaks are presented (Table 3).

Table 2: Composition of biodiesel from *Sesbania sesban* seed oil

Entry	Retention time (mm)	FAME	wt.%
1	23.65	Methyl palmitate	5.21
2	23.21	Methyl linoleate	93.17
3	18.69	Methyl stearate	1.61

Table 3: Molecular ion and base peaks of FAME from *Sesbania sesban* seed oil

Entry	FAME	Molecular ion peak (m/z)	Base peak (m/z)
1	Methyl palmitate	270	74
2	Methyl linoleate	294	67
3	Methyl stearate	298	74

The ^{1}H NMR spectrum of the biodiesel derived from *Sesbania sesban* seed oil is presented in Figure 4. In this spectrum, the multiplet observed in the range of δ 5.304 to 5.36 ppm corresponds to the olefinic protons (–CH=CH–). There is a singlet signal at δ 3.66 ppm, which represents the methoxy protons of the ester functional group within the biodiesel. Another signal is noticeable at around δ 2.8 ppm, indicating the presence of bis-allylic protons (–C=C–CH_2–C=C–) in the unsaturated fatty acid chain. The triplet at δ 2.30 ppm (t, 3J = 7.5 Hz) may be attributed to the α-methylene protons adjacent to the ester (–CH_2–CO_2Me). The α-methylene protons adjacent to the double bond (–CH_2–C=C–) are observed as a multiplet at δ 2.01 ppm. Additionally, the β-methylene protons adjacent to the ester (CH_2-C-CO_2Me) also appear as a multiplet at δ 1.64 ppm. The multiplet ranging from δ 1.25 to 1.30 ppm corresponds to the protons of the backbone methylene in the long fatty acid chain. Lastly, the terminal methyl protons (C–CH_3) at δ 0.86 to 0.91 ppm manifest as a multiplet.

The ^{13}C NMR spectrum of the biodiesel obtained from *Sesbania sesban* seed oil is illustrated in Figure 5. In this spectrum, the signal at δ 174.37 ppm signifies the carbonyl carbon of the ester molecules, while the olefinic carbons are observed at δ 127.89 and 128.03 ppm. The signal at δ 51.48 ppm corresponds to the methoxy carbons present in the ester group. The methylene and methyl carbons of the fatty acid moiety are observed in the range between δ 24.95 and 34.11 ppm.

In the IR spectrum of the biodiesel from *Sesbania sesban* seed oil (Figure 6), a sharp signal at 1739.79 cm^{-1} indicates a strong absorption by the ester carbonyl stretching frequency. There is a weaker signal at 1546.91 cm^{-1}, which is likely attributed to the C=C stretching frequency. Two strong and sharp signals appear at 2926.01 and 2854.65 cm^{-1}, corresponding to C–H stretching frequencies. The bands at 1240.23, 1170.79, and 1103.28 cm^{-1} are associated with C–O–C stretching frequencies. The presence of an absorption peak at 736.81 cm^{-1} suggests CH_2 rocking, and the absorbance at 3051.39 cm^{-1} indicates the =C–H stretching frequency.

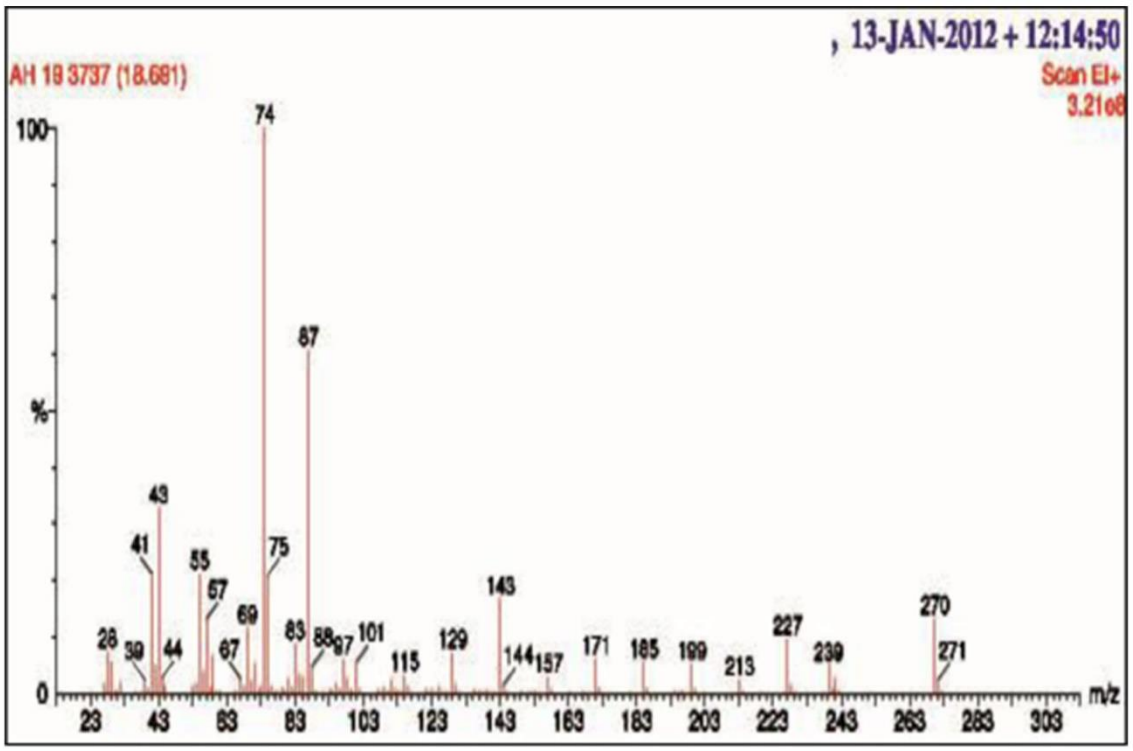


Figure 3 (a) Mass spectrum of Methyl palmitate

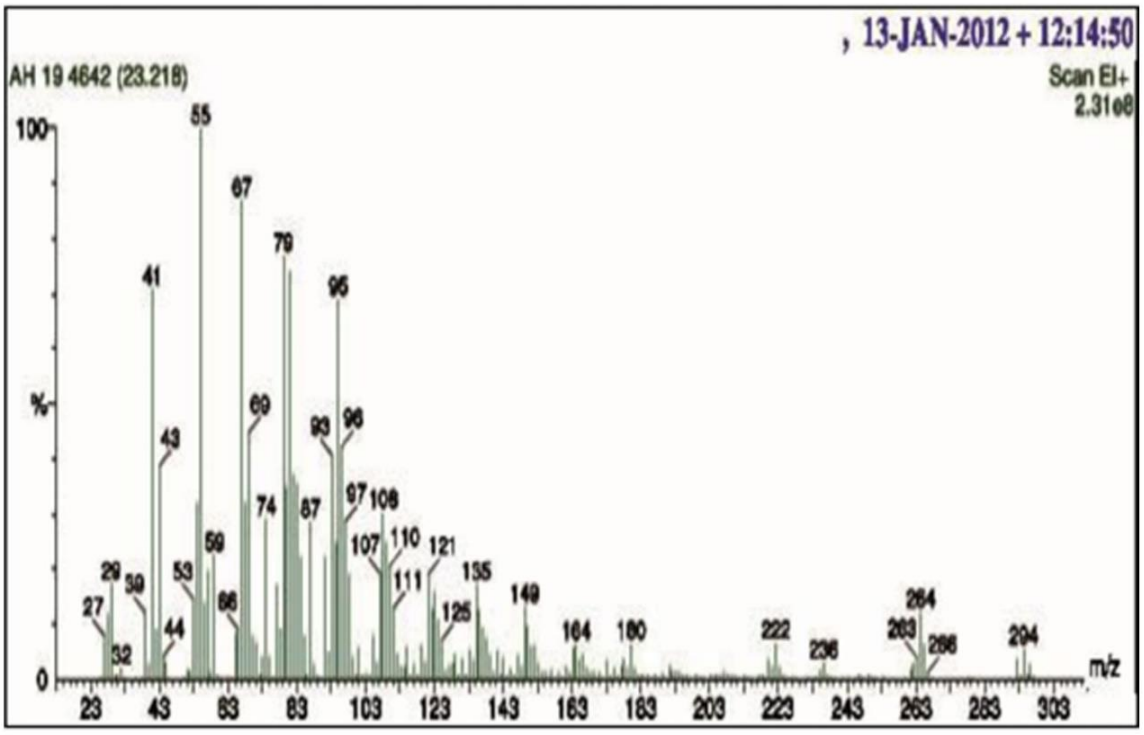


Figure 3 (b) Mass spectrum of Methyl linoleate

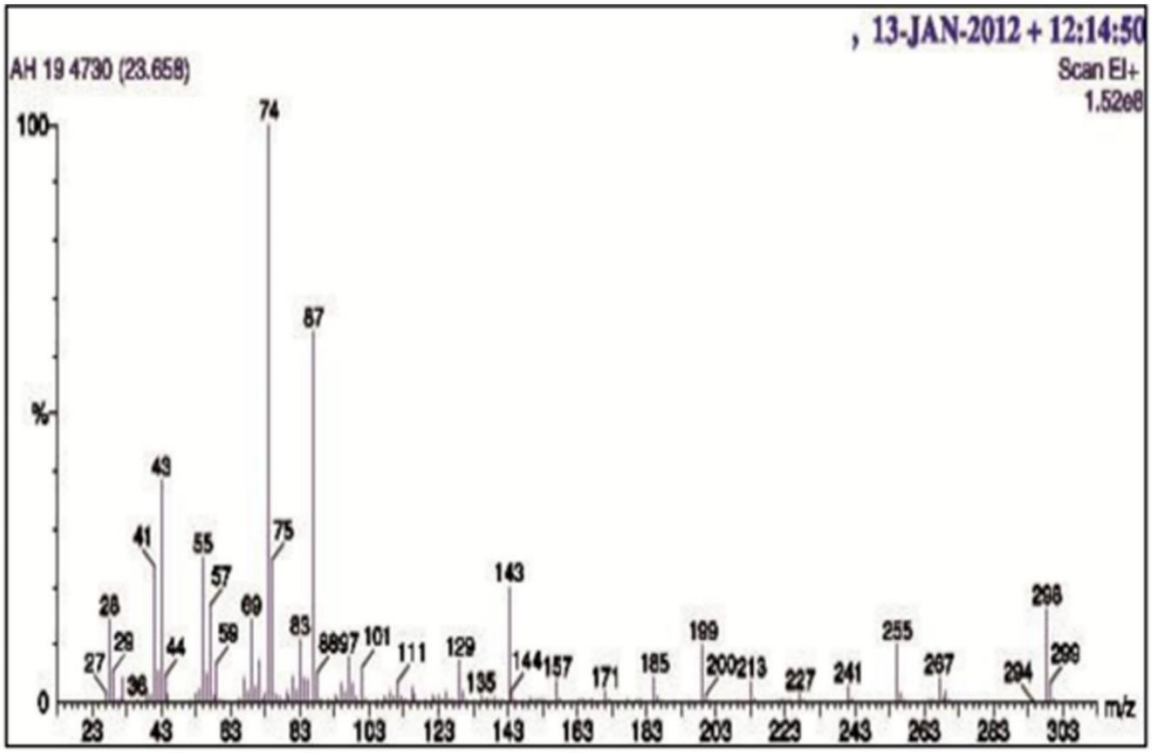


Figure 3 (c): Mass spectrum of Methyl Stearate

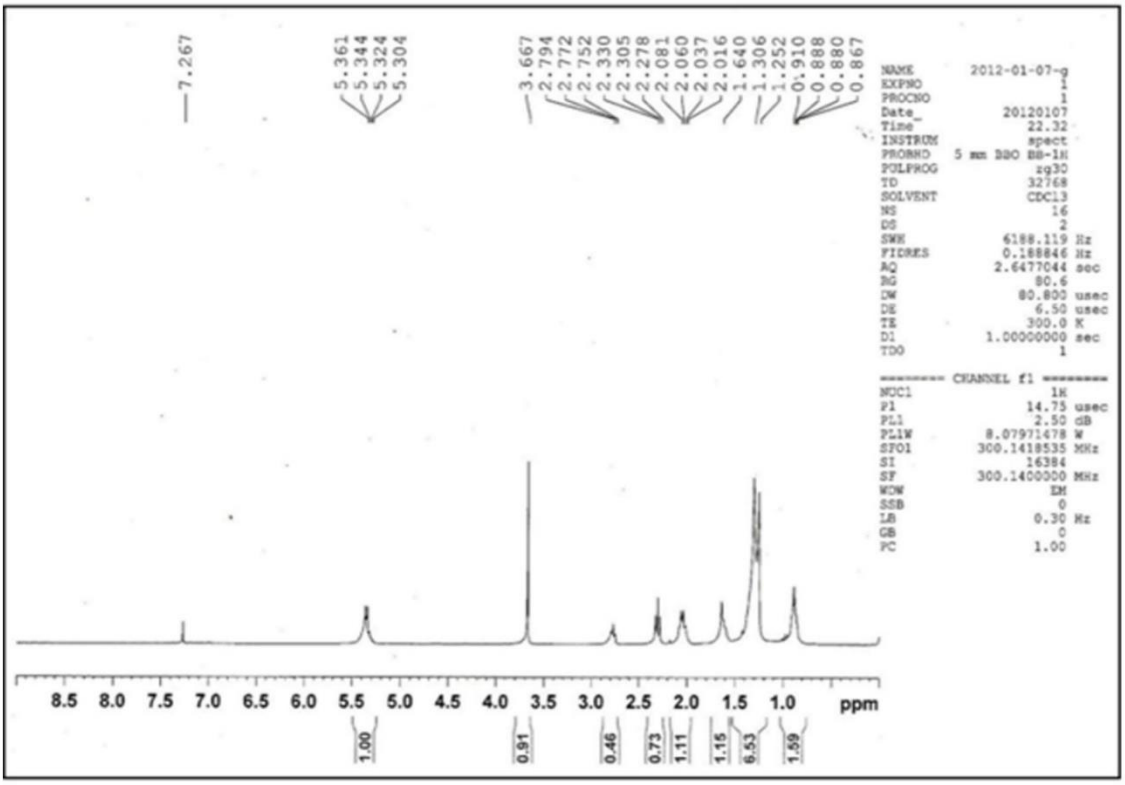


Figure 4: ^{1}H NMR spectrum of biodiesel from *Sesbania sesban* seed oil

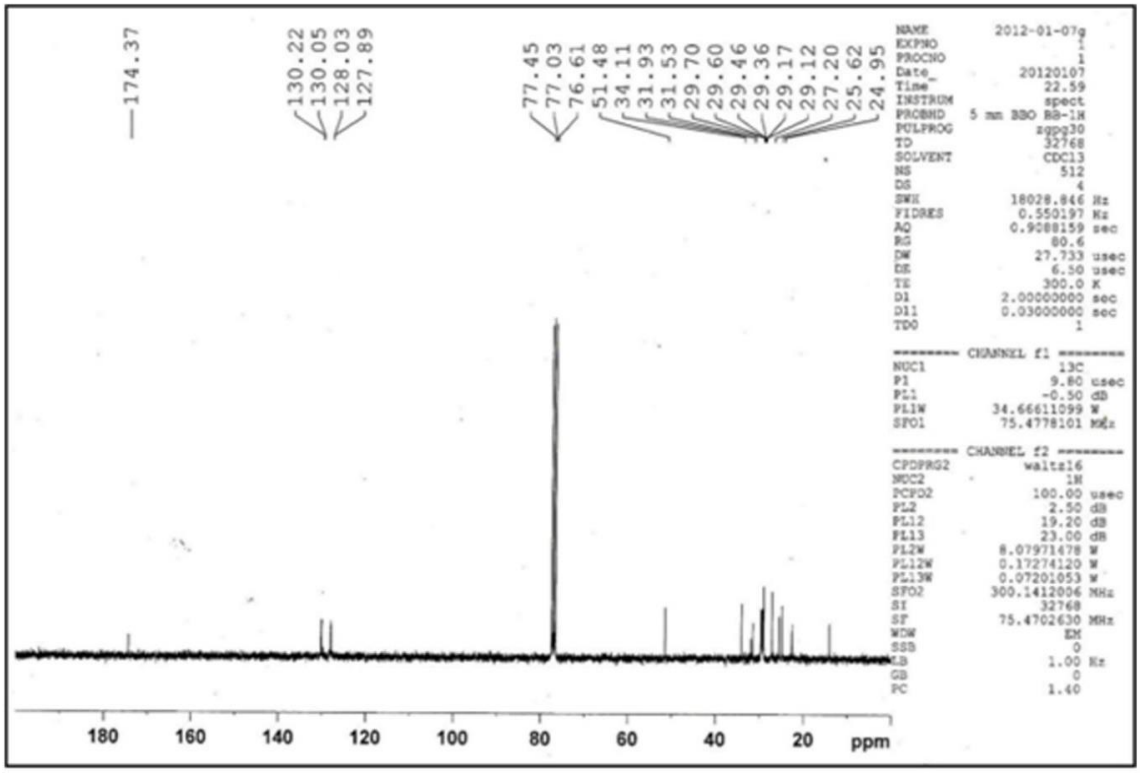


Figure 5: ^{13}C NMR spectrum of biodiesel from *Sesbania sesban* seed oil

Furthermore, the Iodine value (IV), Saponification number (SN), and Cetane Index (CI) were calculated both experimentally and theoretically, yielding values of 69.95 (g/100 g), 192.08 (mg KOH/g), and 58.98, respectively.

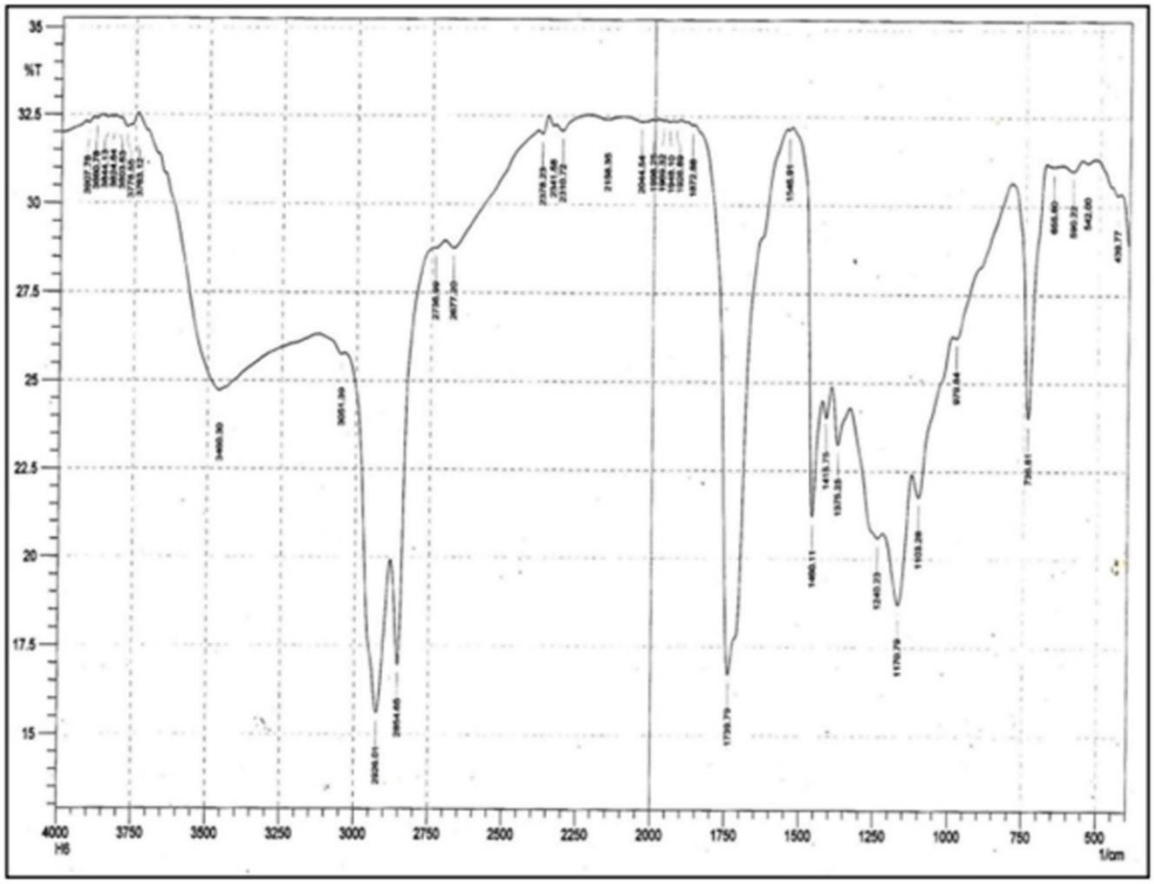


Figure 6: IR spectrum of biodiesel from *Sesbania sesban* seed oil

3.2: 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 [16] and the results are shown in Table 4.

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

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

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

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 of *Sesbania sesban* plant using equation No. 6-8.

Name of the oil plant	IV (g/100g)	SN(mg KOH / g)	CI
Sesbania sesban	69.94	192.08	58.98

CONCLUSION

Regarding the properties of *Sesbania sesban* seed oil, it had a pale-yellow colour and exhibited a density of 0.8498 g/cm³. The acid value was measured at 0.670 mg KOH/g, the iodine value at 123.81 gI_2/100 g, the saponification number at 167.28 mg KOH/g, the refractive index at 1.4680, and the moisture content at 0.104%. After the extraction and purification process using column chromatography, biodiesel was prepared from *Sesbania sesban* seed oil through a heterogeneous transesterification procedure. The composition of its fatty acid methyl esters (FAME) was analysed using IR, NMR, and GC-MS techniques. The investigation revealed that FAME derived from *Sesbania sesban* seed oil comprised 5.21 wt.% of methyl palmitate (C16:0), 93.17 wt.% of methyl linoleate (C18:2), and 1.61 wt.% of methyl stearate (C18:0). As expected, the molecular ion peaks for methyl palmitate, methyl linoleate, and methyl stearate were observed at 270, 294, and 298, respectively. The iodine value (IV), saponification number (SN) and cetane index (CI) of the biodiesel were determined and was found to be 69.94 (g/100g), 192.08 (mg KOH/g) and 58.98 respectively.

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