

Physicochemical characteristics and triglyceride composition of *Mimusops elengi* seed oil

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ABSTRACT

*The lipid fraction of *Mimusops elengi* seeds was extracted and analyzed for their chemical and physical properties such as acid value, iodine value and density as well as refractive index, colour and moisture. The fatty acid composition of the extracted lipid was revealed using the gas chromatography mass spectrometry (GC-MS) method. Palmitic acid (53.55%) and oleic acid (28.52%) were detected as the dominant fatty acids in the *Mimusops elengi* seed oil. The TAG profile of the lipid was detected using reverse phase HPLC. The major TAGs found are OLLn(29.23%), PLL (19.30%), OOL(17.24%) and OOO(13.93%).*

Key words: *Mimusops elengi*, lipid, palmitic acid, *Musa balbisiana* Colla

INTRODUCTION

Plant oils are used in food, medicine, cosmetics and as fuels. They are consumed directly, or are used as ingredients in the preparation of food [1]. Fats and oils are the most concentrated kind of energy that humans can use [2, 3, 4]. They provide 9 kilocalories per gram of oil while the other two types of energy that human can use i.e. carbohydrates and proteins provide 4 kilocalories per gram each [5, 6, 7]. The world yearly production of oils exceeds 120 million tons of which about 80% are devoted to food uses, the remainder is used for non food uses, for example in making animal feeds, soap, oleochemicals [8, 9]. Plant seeds are important sources of oils of nutritional, industrial and pharmaceutical importance. The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid (FA) composition [10, 11].

Triacylglycerol (TAG) are the main constituents of vegetable oils. All oils consist of fatty acids bonded to a backbone structure, often glycerol [12]. Chemically, they are tri-ester of glycerol [13, 14].

Mimusops elengi is a medicinal plant belonging to family Sapotaceae. It is a small to large evergreen tree up to 15 m in height. The bark, flowers, fruit and seeds have great medicinal value [15]. *Mimusops elengi* seed is used traditionally for curing piles, headache, constipation, spermicidal etc [16]. The fragrant flowers bloom from January to March. It starts bearing fruits from Jan to May. The fruit is a berry, yellow in colour and ovoid in shape (ca. 2.5 cm long). It encloses one (rarely two) seed [17].



Fig.1. *Mimusops elengi* seed

This investigation was carried out to determine the composition and physicochemical characteristics of the oil extracted from *Mimusops elengi* seeds.

MATERIALS AND METHODS

Materials

Mimusops elengi seeds were collected from Nalbari and Barpeta Districts of Assam, India. The seeds were cleaned, de-shelled and the kernel was dried at a temperature of 100-105 °C for 35 min. The dry kernel was grounded using grinder prior to extraction. Solvents and other chemicals used were of analytical grade, and they were procured from commercial sources and used as such without further treatment.

Instruments used

¹H and ¹³C NMR spectra were recorded in CDCl₃ at 300 and 75 MHz, respectively using Bruker Avance III 300 MHz/54 mm NMR spectrometer. FT-IR spectra were obtained on a Perkin Elmer RX I FT-IR spectrometer. The colour of the oil sample was determined by observation using several independent competent individuals. Oil colours were correlated using colour charts. Refractive index was determined by using the Abbe Refractometer (AW-24) at room temperature (28 °C). The acid value was determined following established procedure of AOAC [18]. Iodine value was estimated by applying Wijs method [19, 20]. The saponification value was determined according to PORIM Official Test Method 1995. Moisture content was determined by oven drying a known quantity of the oil in an oven at 105 °C for 24 hours after which the percentage moisture was calculated as follows:

$$\% \text{ Moisture} = \frac{\text{Initial weight of oil} - \text{Final weight of oil}}{\text{Initial weight of oil}} \times 100$$

Oil Extraction

Extraction of oil was done by solvent extraction technique on the crushed kernel using petroleum ether as the solvent. Crushed kernel in petroleum ether (bp 40-60 °C, 10 mL/g) was magnetically stirred at room temperature (28-29 °C) for 3 h, solvent was removed at 45 °C using a rotary vacuum evaporator to yield the crude oil. The process was repeated twice 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

Transesterification of the purified oil was carried out at room temperature with MeOH. The catalyst used for transesterification was prepared in the laboratory from the trunk of *Musa balbisiana* plant [21, 22]. A mixture of oil,

methanol (10 mL/g of purified oil) and catalyst (20 wt% of oil) was stirred magnetically in a round bottom flask at room temperature (30-32 °C). Reaction was monitored by TLC. After completion of the reaction, the product mixture was partitioned between water and petroleum ether and the combined organic layers was washed with brine, dried over anhydrous Na₂SO₄ and the solvent removed under vacuum to yield the crude FAME mixture. The product 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. The purified product was further subjected to high vacuum to remove the last traces of solvents to yield pure FAME.

Analysis of FAME

The fatty acid methyl esters were identified using Perkin-Elmer Clarus 600 GC-MS analyzer. The column used was Elite 5 MS with dimension 30.0m x 250µm. The oven temperature was initially held at 140 °C for 5 minutes, increased to 240 °C at 4 °C/min and finally held for 5 min at 240 °C. The injector, transfer and source temperatures were 250 °C, 200 °C and 150 °C respectively. Helium was used as the carrier gas. The mass spectrum was scanned 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 FAME from *Mimusops elengi* seed oil is reported in Table 2.

TAGs composition

TAGs profile of the studied seed oil was determined by using high-performance liquid chromatography (HPLC) from Perkin-Elmer Series 200 equipped with UV detector. The TAGs of the oil was separated using commercially packed C18 column, 4.6µm×150.00mm from Waters. The mobile phase was a mixture of acetone:acetonitrile (63:37) set at a flow rate of 1 ml/min. Sample preparation involved sample dilution with acetone:acetonitrile (63:37) mixture before 20 µl of the sample being injected into HPLC with total running time of 60 min. TAG peaks were identified based on the retention time of available commercial TAGs standard.

RESULTS AND DISCUSSION

The physical characteristics of the oil obtained from the seeds of *Mimusops elengi* are shown in Table 1.

It is found that the kernel of *Mimusops elengi* seed contains 17.9% of oil.

The moisture content of *Mimusops elengi* seed oil is low (0.112) which shows that the oil is of good quality and could not be easily subjected to contamination/rancidity [23]. Besides, moisture play a significant role on the transesterification of glyceride [24].

Table 1: Some of the physicochemical properties of *Mimusops elengi* seed oil

S/N	Parameters	Observed values
1	Colour	Light green
2	Oil content (%)	17.9
3	Density (g/cm ³)	0.8573
4	Acid value (mg KOH/g)	1.107
5	Iodine value (gI ₂ /100 g)	90.31
6	Saponification value	132.21
7	Refractive index	1.4571
8	Moisture (%)	0.112

The acid value is the measure of quantity of fatty acids in the oil. A higher fatty acid value (1.107) was observed in *Mimusops elengi* oil. This reflects the high fatty acid content of the oil.

Iodine value measures the unsaturation of fats and oils. The iodine value of *Mimusops elengi* seed oil was found to be 90.31.

Mimusops elengi seed oil shows very low saponification value (132.21).

The refractive index (1.4571) of the oil is in the range with the values obtained for some conventional oils such as palm oil (1.449-1.451), soya bean oil (1.466-1.470) etc [25]. Since the refractive index of the oil is greater than that of water (1.330) at room temperature, this property suggests that the oil can be used in studies relating to optics [26].

Fatty acid profile of the FAME from *Mimusops elengi* seed oil was determined by GC-MS analysis. The most abundant fatty acid is the palmitic acid (53.55%) followed by oleic acid (28.52%), stearic acid (10.26%) and linoleic acid (7.65%). Both palmitic and stearic acid are the two major saturated fatty acids which together account for more than 60% of total fatty acids in *Mimusops elengi* seed oil. The unsaturated fatty acids, oleic and linoleic acids, are found to be present in 28.52% and 7.65% respectively.

Table 2 : Fatty acid profile of *Mimusops elengi* seed oil

Retention time (min)	FAME	wt. %
18.34	Methyl palmitate	53.55
22.15	Methyl linoleate	7.65
22.40	Methyl oleate	28.52
22.98	Methyl stearate	10.26

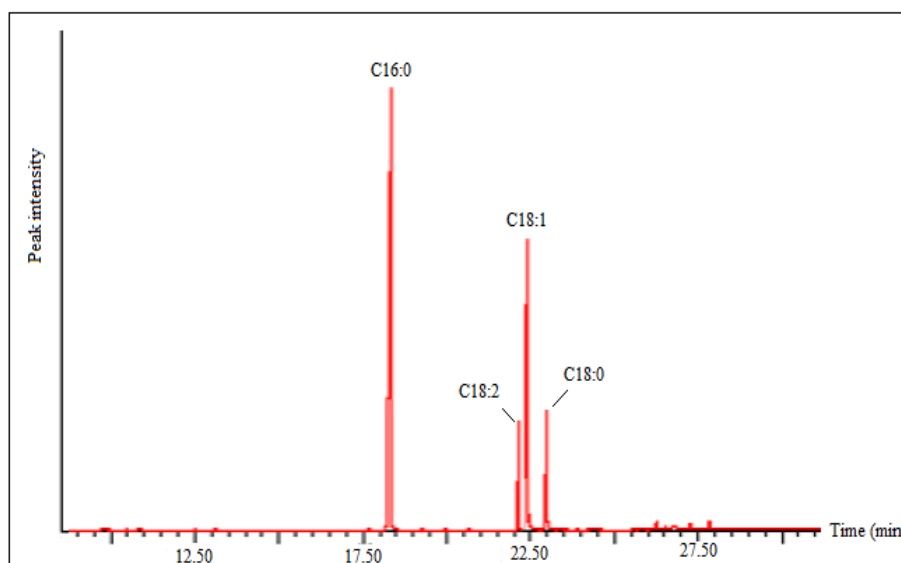


Fig.2. Gas Chromatogram of FAME from *Mimusops elengi* seed oil

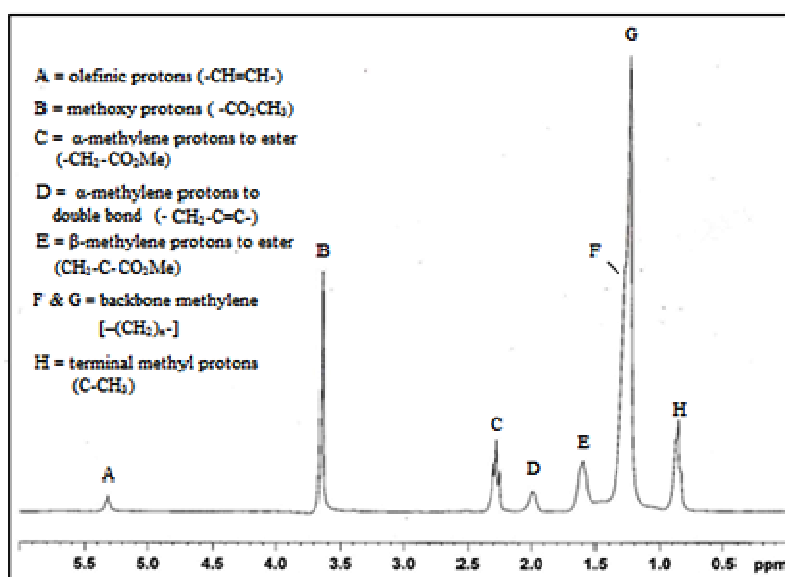


Fig.3. ¹H NMR spectrum of FAME from *Mimusops elengi* seed oil

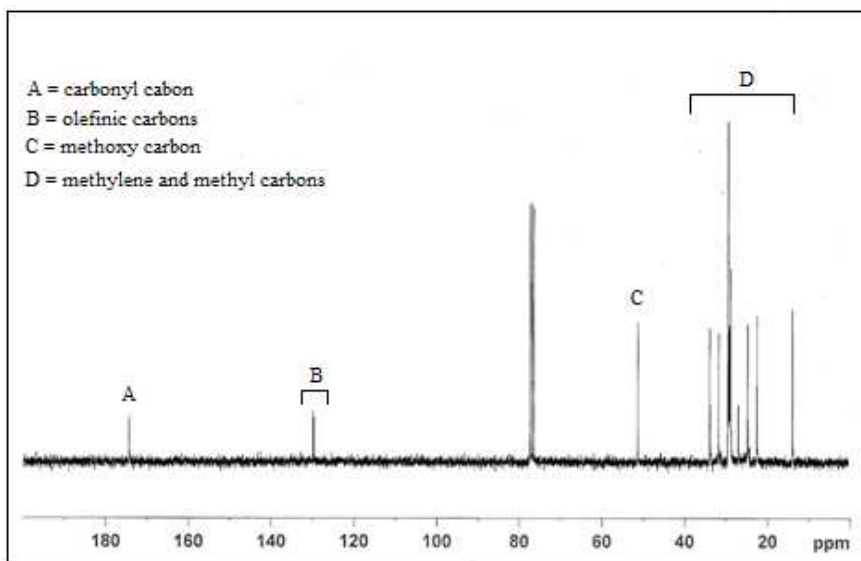


Fig.4. ^{13}C NMR spectrum of FAME from *Mimusops elengi* seed oil

The ^1H NMR spectrum of the FAME from *Mimusops elengi* seed oil is shown in Fig. 3. The multiplet at δ 5.32-5.33 ppm indicates the olefinic protons ($-\text{CH}=\text{CH}-$). A singlet signal at δ 3.64 ppm represents methoxy protons of the ester functionality of the FAME. The triplet at δ 2.28 ppm (t , $^3J=7.5$ Hz) represents the α -methylene protons to ester ($-\text{CH}_2-\text{CO}_2\text{Me}$). The α -methylene protons to double bond ($-\text{CH}_2-\text{C}=\text{C}-$) is seen as a multiplet at δ 1.98-1.99 ppm. The β -methylene protons to ester ($\text{CH}_2-\text{C}-\text{CO}_2\text{Me}$) also appear as a multiplet at δ 1.46-1.59 ppm. The singlet signals at δ 1.27 and 1.23 ppm are due to the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons ($\text{C}-\text{CH}_3$) at δ 0.83-0.87 ppm appear as a multiplet.

The ^{13}C NMR spectrum of FAME from *Mimusops elengi* seed oil is shown in Fig 4. The signal at δ 174.33 ppm indicates the carbonyl carbon of the ester molecules and the olefinic carbons appear at δ 129.64 and 129.89 ppm. The signal at δ 51.36 ppm in the ^{13}C NMR spectrum of FAME represents methoxy carbons of esters. The methylene and methyl carbons of fatty acid moiety appear in the range from δ 13.92 to 34.02 ppm.

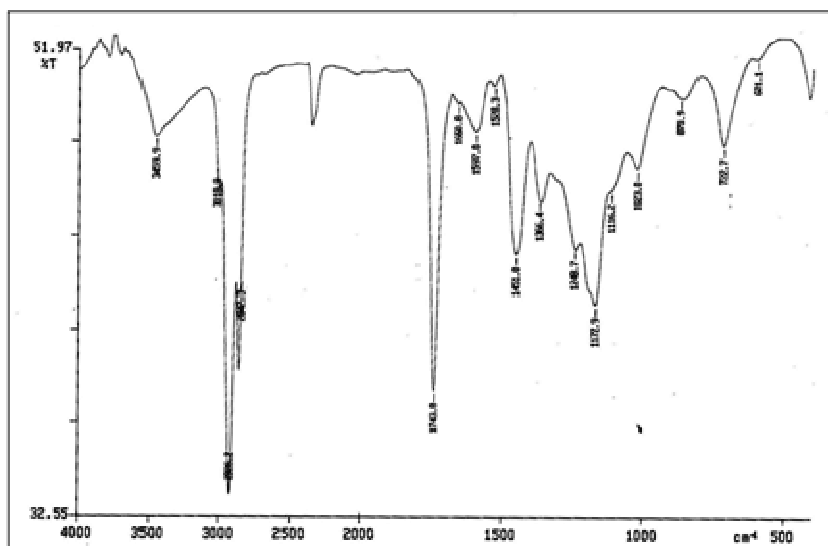


Fig.5. The IR spectrum of FAME from *Mimusops elengi* seed oil

The IR spectrum of FAME from *Mimusops elengi* seed oil is shown in Fig 5. IR spectrum of the FAME shows a C=O stretching band of methyl esters at 1744 cm^{-1} and C-O stretching bands at 1116 , 1173 and 1249 cm^{-1} . The weak signal at 1661 cm^{-1} may due to C=C stretching frequency. Strong and sharp signals at 2842 and 2926 cm^{-1} indicate C-H stretching frequencies. The absorbance at 3460 cm^{-1} is due to the =C-H stretching frequency. The observation of an absorption peak at 723 cm^{-1} suggested the CH_2 rocking.

The ^1H NMR spectrum of *Mimusops elengi* seed oil is shown in Fig. 6. The multiplet at δ : 0.82 - 0.94 ppm indicates the terminal methyl protons ($\text{C}-\text{CH}_3$). The backbone methylene protons [$-(\text{CH}_2)_n-$] is seen as a multiplet at δ 1.24-1.40 ppm. A multiplet at δ 1.58-1.63 ppm represents the β -methylene protons to ester ($\text{CH}_2-\text{C}-\text{CO}_2\text{Me}$). The α -methylene protons to double bond ($-\text{CH}_2-\text{C}=\text{C}-$) appears as a multiplet at δ 1.99 - 2.03 ppm. The α -methylene protons to ester ($-\text{CH}_2-\text{CO}_2\text{Me}$) occurs as a triplet at δ 2.30 ppm. A triplet at δ 2.81 ppm represents bis-allylic protons ($-\text{C}=\text{C}-\text{CH}_2-\text{C}=\text{C}-$). A multiplet at δ 4.14 ppm indicates methylene protons at C1 & C3 of glycerides ($-\text{CH}_2-\text{CO}_2\text{R}$). The methine proton at C2 of glycerides ($-\text{CH}-\text{CO}_2\text{R}$) occurs as a multiplet at δ 4.29 ppm. A multiplet at δ 5.30 - 5.32 ppm indicates olefinic protons ($-\text{CH}=\text{CH}-$).

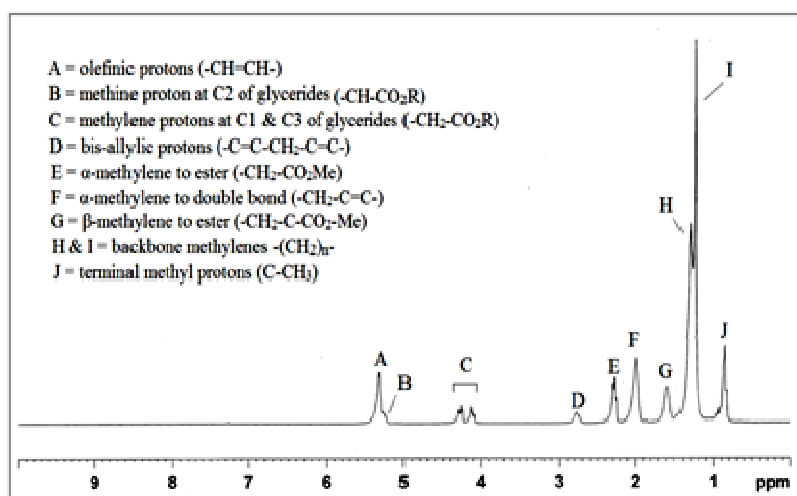


Fig.6. ^1H NMR spectrum of *Mimusops elengi* seed oil

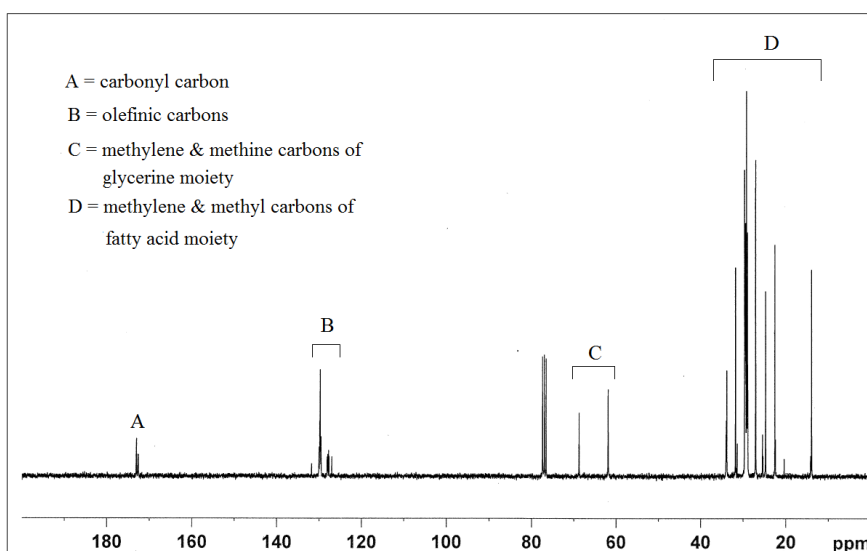


Fig.7. ^{13}C NMR spectrum of *Mimusops elengi* seed oil

The ^{13}C NMR spectrum of *Mimusops elengi* seed oil is shown in Fig. 7. The carbonyl carbon of the ester molecules appear at δ 172.55 and 172.96 ppm. The olefinic carbons appear in the range from δ 127.74 to 129.96 ppm. The signals in the range from δ 61.91 to 68.76 ppm represent methylene and methine carbons of glycerine moiety. The methylene and methyl carbons of fatty acid moiety appear in the range from δ 13.98 to 34.01 ppm.

Table 3: TAGs composition of *Mimusops elengi* seed oil

TAGs	ECNs	Relative composition (%)
LnLnL	38	10.15
LLLn	40	2.23
LLL	42	0.78
OLLn	42	29.23
PLL	44	19.30
OOL	46	17.24
POL+SLL	46	0.67
PPL	46	2.97
OOO	48	13.93
SOL+POO	48	0.47
PPP	48	2.36
SOO	50	0.33
POS	50	0.33

Ln: α -linolenic acid, L: linoleic acid, O: oleic acid, P: palmitic acid, S: stearic acid, ECNs: equivalent carbon number.

The TAGs profile of *Mimusops elengi* seed oil was characterized by reversed phase HPLC where the mechanism in separating the TAGs involves the chain length and degree of unsaturation of the fatty acids [27]. Due to the limitation in TAGs standard available commercially, the identified TAGs were concluded by comparing the retention time of standard TAGs peak. Some of the peaks were identified by comparing with the HPLC chromatographs of linseed and palm oil [28]. From the chromatograph obtained, major TAG peaks in the studied oil were the polyunsaturated TAGs of OLLn with 29.23% followed by PLL (19.30%), OOL (17.24%), OOO(13.93%) and LnLnL(10.15%). The oil was found to contain 2.97% of monounsaturated TAG namely PPL while saturated TAG was PPP with 2.36%. *Mimusops elengi* seed oil showed a significant content of monoglycerols (MAG) and diglycerols (DAG) whose peaks were observed before the first TAG peaks appeared at 6 min.

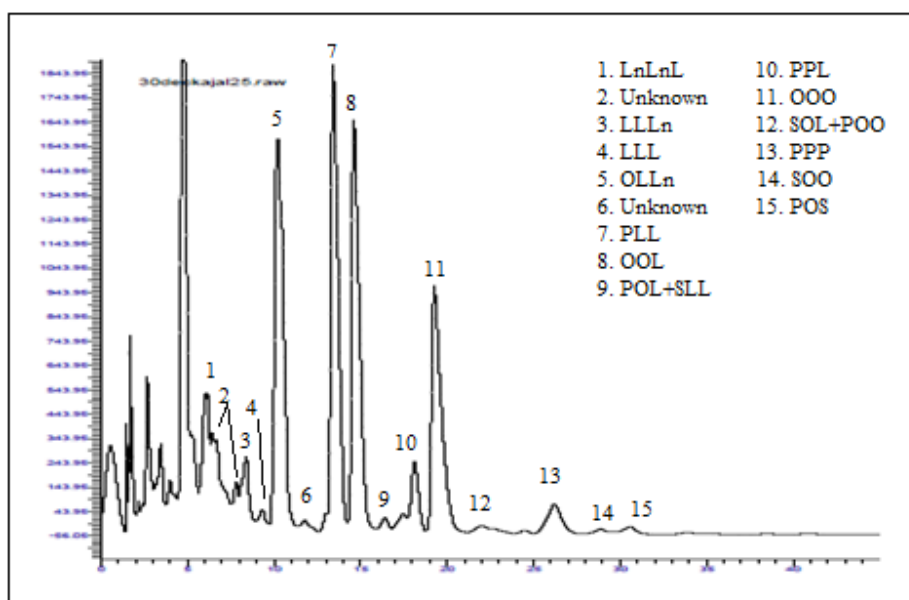


Fig.8. TAGs profile of *Mimusops elengi* seed oil

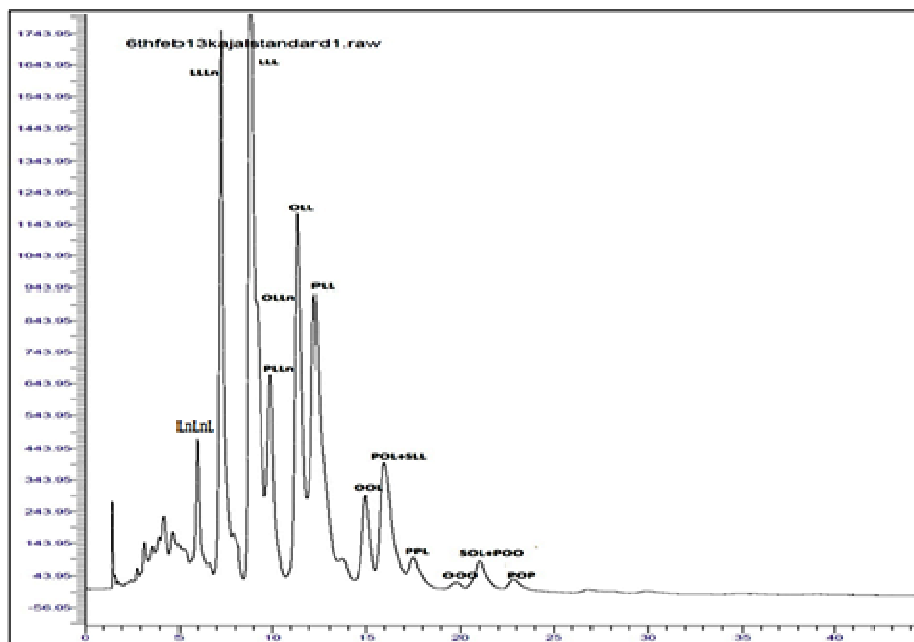


Fig.9. TAGs profile of soybean seed oil ((as standard)

CONCLUSION

The study reveals that palmitic acid (53.55%) and oleic acid (28.52%) are the major fatty acids in *Mimusops elengi* seed oil. The dominant TAGs found in the seed are OLLn(29.23%), PLL (19.30%), OOL(17.24%) and OOO(13.93%). The acid value, moisture content and iodine value were 1.107 mg KOH/g oil, 0.112% and 90.31 gI₂/100 g respectively. The density and refractive index of the oil were found to be 0.8573 g/cm³ and 1.4571 respectively.

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