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Physicochemical properties of *Baccaurea remiflora* (Letuk) seed oil

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ABSTRACT

Baccaurea remiflora seeds oil was solvent extracted and analyzed for its physicochemical properties i.e. acid value, iodine value, moisture content, density and refractive index. The acid value, moisture content and iodine value were 1.127 mg KOH/g oil, 0.103% and 80.32 gI_2/100 g respectively. The density and refractive index of the oil were found to be 0.8674 g/cm^3 and 1.4672 respectively. Gas chromatographic analysis of Baccaurea remiflora seeds oil showed that saturated fatty acids such as palmitic acid (33.67%), stearic acid (19.38%) and arachidic acid (9.69%) account for more than 60% of total fatty acids judging by its low iodine value. Oleic (24.48%) and 11-trans-eicosenoic (12.75%) acids are the unsaturated fatty acids found in Baccaurea remiflora.

Key words: *Baccaurea remiflora*, physicochemical properties, unsaturated acid, *Musa balbisiana* Colla.

INTRODUCTION

One of the important uses of plants is in the production of oil. Oil is very important resource, much in demand everywhere in the world and is used in a variety of ways [1-3]. The sources of oils and fats are diminishing, this means therefore that there is a growing need for the search of new sources of oil as well as exploiting sources that are currently unexploited in order to supplement the existing ones [4]. Furthermore, the price of edible oil is increasing due to the effects of turning edible oil into energy sources [5]. Similarly the biodiesel market is growing [6]. The oil for nutritional uses and derivative products like soaps, cosmetics and medicinal products have become more unaffordable for most of the people because the sources of these products are small and limited [7, 8].

The world yearly production of oils exceeds 120 million tons of which about 4/5 are devoted to food uses, the remainder is used for non food uses, for example in making animal feeds, soap, oleochemicals [6]. 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 [9]. No oil from any single source has been found to be suitable for all purposes because oils from different sources generally differ in their FA composition [10, 11].

Fig. 1. *Baccaurea ramiflora* seed

Baccaurea ramiflora (Lour.), (family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Penninsular Malaysia, Tibet and Andaman islands [12]. It is a evergreen tree reaching a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diameter with leathery pericarp, three seeded arillus embedded in pinkish white pulp [13]. The leaf is simple, alternately arranged, with petiole. It is ovate to ovate-lanceolate in shape and 10-20 × 4-9 cm in size. The petiole is 1-8 cm long with lanceolated and fimbriated stipules. It grows in evergreen forests on a wide range of soils. The common names include Latkan or Bhubi (Bengali), Letuk (Assamese), Leteku (Hindi), Mafai (Thai) and Burmese grape (English) [14].

EXPERIMENTAL SECTION

Materials

Baccaurea ramiflora seeds were collected from Nalbari and Barpeta Districts of Assam, India during its availability of the season. The seeds were selected according to their condition where damaged seeds were discarded before seeds in good condition were cleaned, de-shelled and dried at high temperature of 100-105 ^{o}C for 35 min. Seeds were grounded using grinder prior to oil 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

^{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. 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 ^{o}C). The acid value was determined following established procedure of AOAC [15]. Iodine value was estimated by applying Wijs method [16, 17]. Moisture content was determined by oven drying a known quantity of the oil in the oven at 105 ^{o}C for 24 h after which the percentage of 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 ^{o}C, 10 mL/g) was magnetically stirred at room temperature (28-29 ^{o}C) for 3 h, solvent was removed at 45 ^{o}C using a rotary vacuum evaporator to yield the crude oil. The 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

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 [18-23]. 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^{o}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 Na_2SO_4 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 30.0 m x 250 μm. 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 *Baccaurea ramiflora* seed oil is reported in **Table 2.**

RESULTS AND DISCUSSION

The results of the physical characteristics of oil obtained from the seeds of *Baccaurea remiflora* are shown in **Table 1**. The oil content of the seed is found to be 25 wt%. The low moisture content (0.103) of *Baccaurea remiflora* seed oil shows that the oil is of good quality and could not be easily subjected to contamination/rancidity [24]. Besides, moisture plays a significant role on the transesterification of glyceride with alcohol using catalyst [25].

Table 1. Some physicochemical properties of *Baccaurea remiflora* seed oil

Sl. No.	Parameters	Observed values
1	Colour	Light brown
2	Oil content (wt%)	25
3	Density (g/cm^3)	0.8674
4	Acid value (mg KOH/g)	1.127
5	Iodine value (gI_2/100 g)	80.32
6	Refractive index	1.4672
7	Moisture (%)	0.103

The acid value is the measure of quantity of fatty acids in the oil. A higher fatty acid value (1.127) was observed in *Baccaurea remiflora* oil. This reflects the high fatty acid content of the oil. The acid value of oil is higher than ASTM value and the effect of esterification is to reduce the fatty acid level to the lowest value. Iodine value measures the unsaturation of fats and oils. The iodine value of *Baccaurea remiflora* seed oil was found to be 80.32. The refractive index (1.4685) of the oil is in close range with the values obtained for some conventional oils such as palm kernel oil (1.449-1.451), Soya bean oil (1.466-1.470) etc. [26]. Since the refractive index of the oil is greater than that of water (1.330) at room temperature, this property suggests the use of the oil in studies relating to optics.

Fatty acid profile of the FAME from *Baccaurea remiflora* seed oil was determined by GC-MS analysis. The individual peaks of the gas chromatogram (**Fig. 2**) were analyzed and the fatty acids were identified using MS data base. Relative percentage of fatty acid esters were calculated from total ion chromatography by computerized integrator. The fatty acid composition of *Baccaurea remiflora* seed oil is presented in **Table 2**. The saturated fatty acids account for more than 60% of total fatty acids. The major saturated fatty acids found in *Baccaurea remiflora* seed oil are palmitic acid (33.67%), stearic acid (19.38%) and arachidic acid (9.69%). Oleic (24.48%) and 11-*trans*-eicosenoic (12.75%) acids are the unsaturated fatty acids detected in *Baccaurea remiflora*.

Table 2. Fatty acid profile of FAME from *Baccaurea remiflora* seed oil

Retention time (min)	FAME	wt.%
16.67	Methyl palmitate	33.67
20.00	Methyl oleate	24.48
20.48	Methyl stearate	19.38
23.52	Methyl 11-*trans*-eicosenoate	12.75
23.96	Methyl arachidate	9.69

The mass spectra of methyl palmitate, methyl oleate, methyl stearate, methyl 11-*trans*-eicosenoate and methyl arachidate are shown in **Figs. 2a to 2e**, and their molecular ion peaks were observed at 270, 296, 298, 324 and 326 respectively (**Table 3**).

Table 3. Molecular ion and base peaks of FAME from *Baccaurea remiflora* seed oil

FAME	Molecular ion peak (m/z)	Base peak (m/z)
Methyl palmitate	270	74
Methyl oleate	296	55
Methyl stearate	298	74
Methyl 11-*trans*-eicosenoate	324	55
Methyl arachidate	326	74

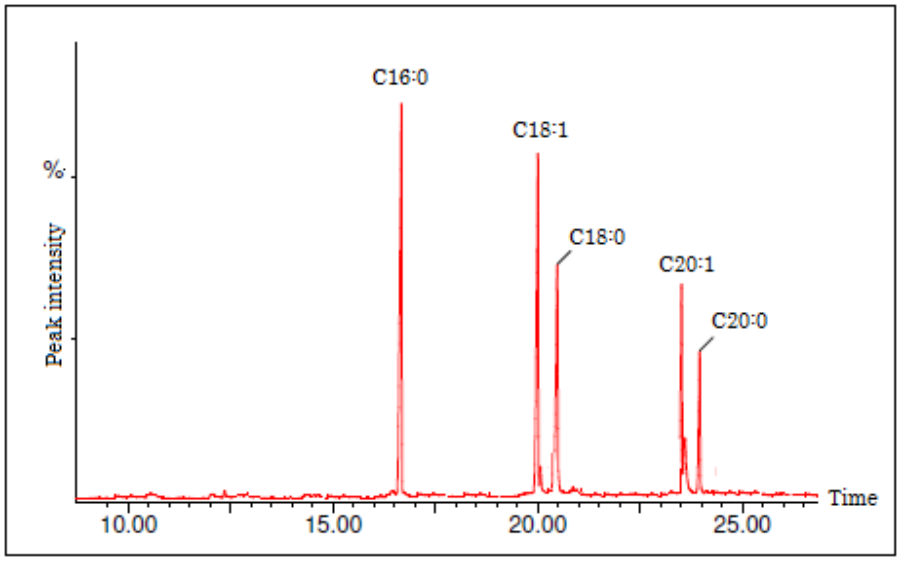

Fig. 2. Gas Chromatogram of FAME from *Baccaurea remiflora* seed oil

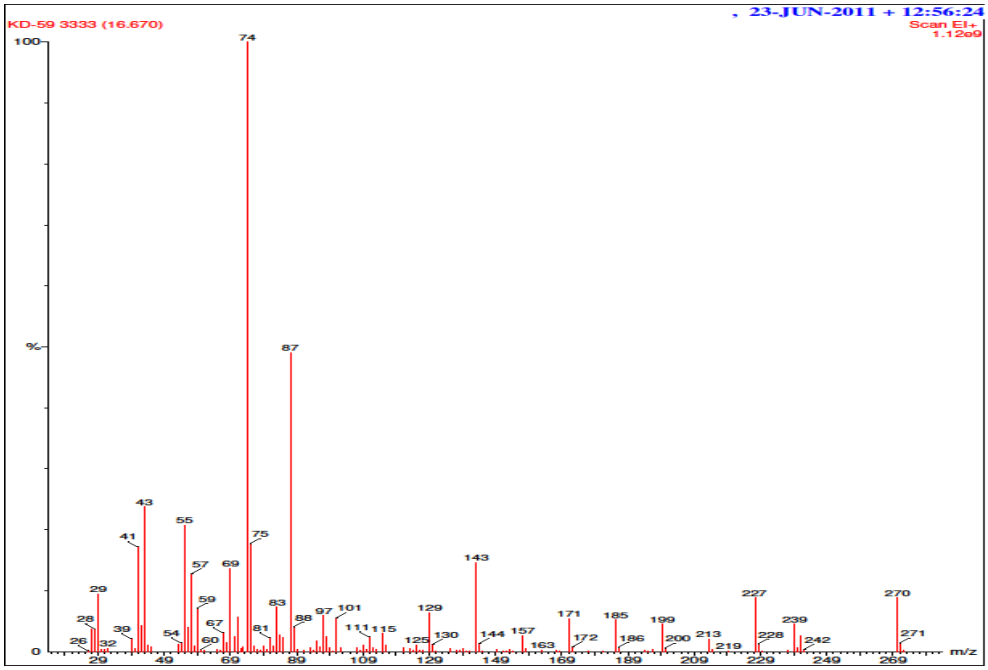

Fig. 2a. Mass spectrum of methyl palmitate

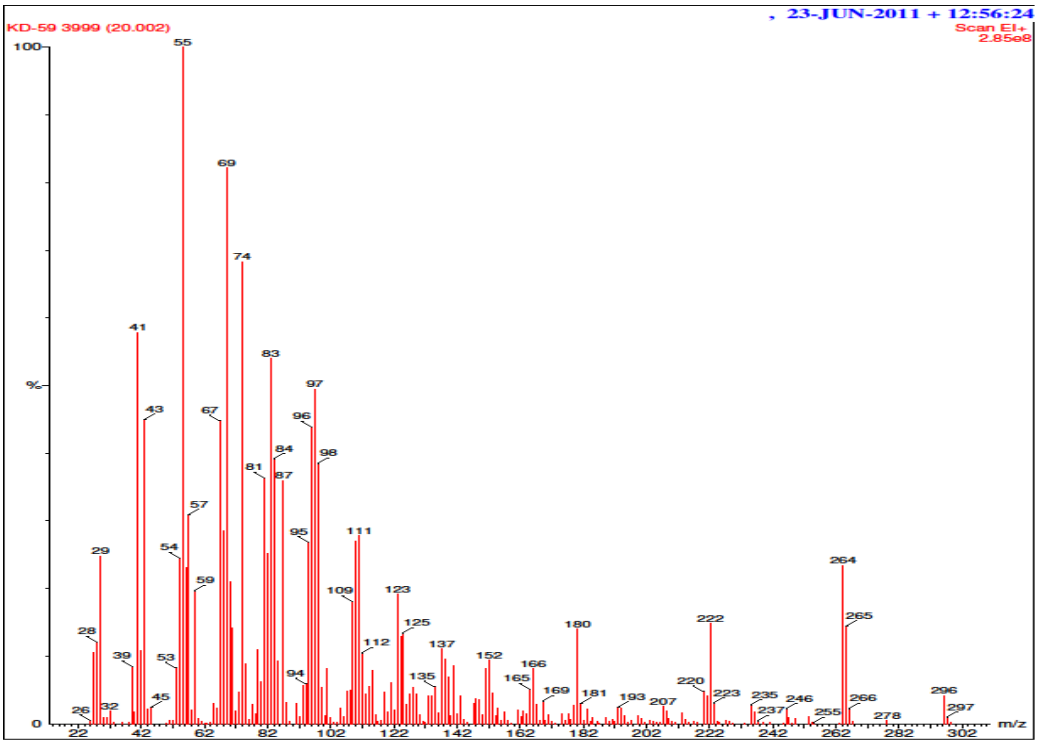

Fig. 2b. Mass spectrum of methyl oleate

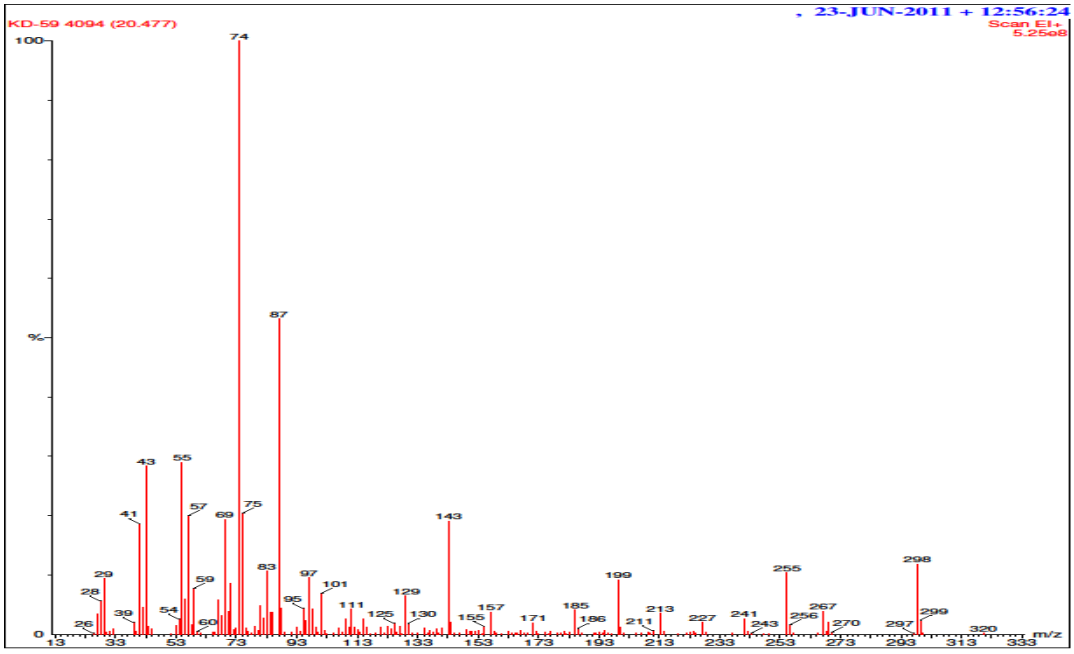

Fig. 2c. Mass spectrum of methyl stearate

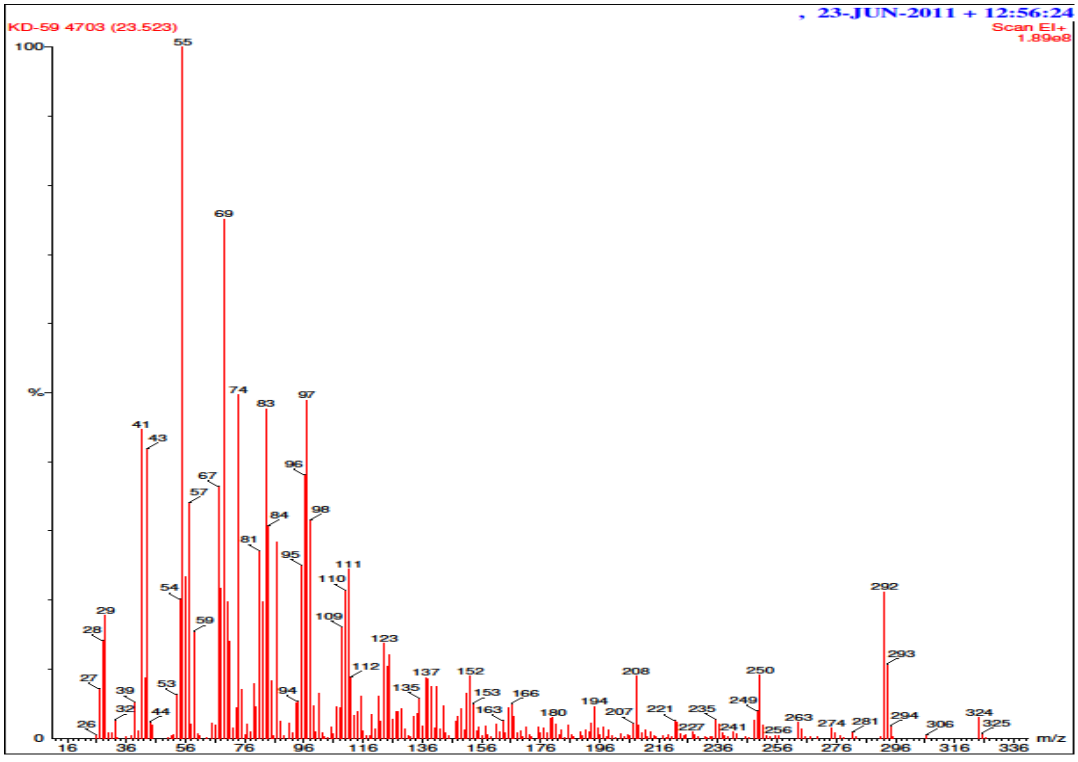

Fig. 2d. Mass spectrum of methyl 11-*trans*-eicosenoate

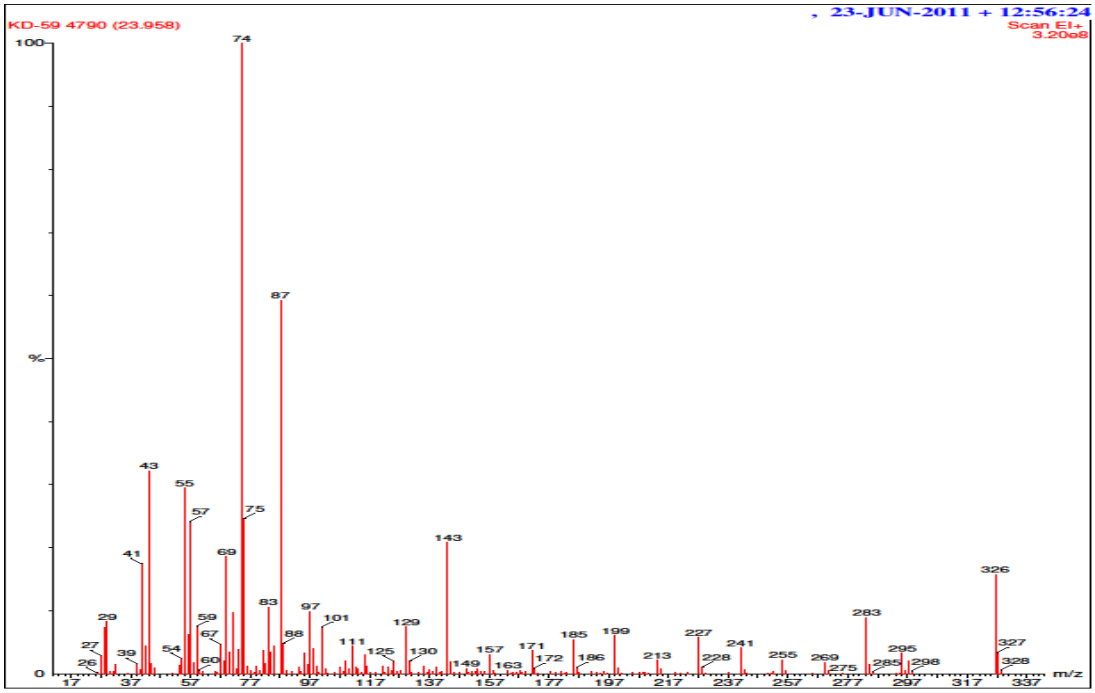

Fig. 2e. Mass spectrum of methyl arachidate

The ^{1}H NMR spectrum of the FAME from *Baccaurea remiflora* seed oil is shown in **Fig. 3.** The multiplet at 5.27-5.30 ppm indicates the olefinic protons (-CH=CH-). A singlet signal at δ 3.61 ppm represents methoxy protons of the ester functionality of the FAME. The triplet at δ 2.27 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 1.95-2.01 ppm. The β-methylene protons to ester (CH_2-C-CO_2Me) also appear as a multiplet at δ 1.54-1.59 ppm. The singlet signals at δ 1.25 and 1.21 ppm are due to the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons (C-CH_3) at δ 0.81-0.84 ppm appear as a multiplet.

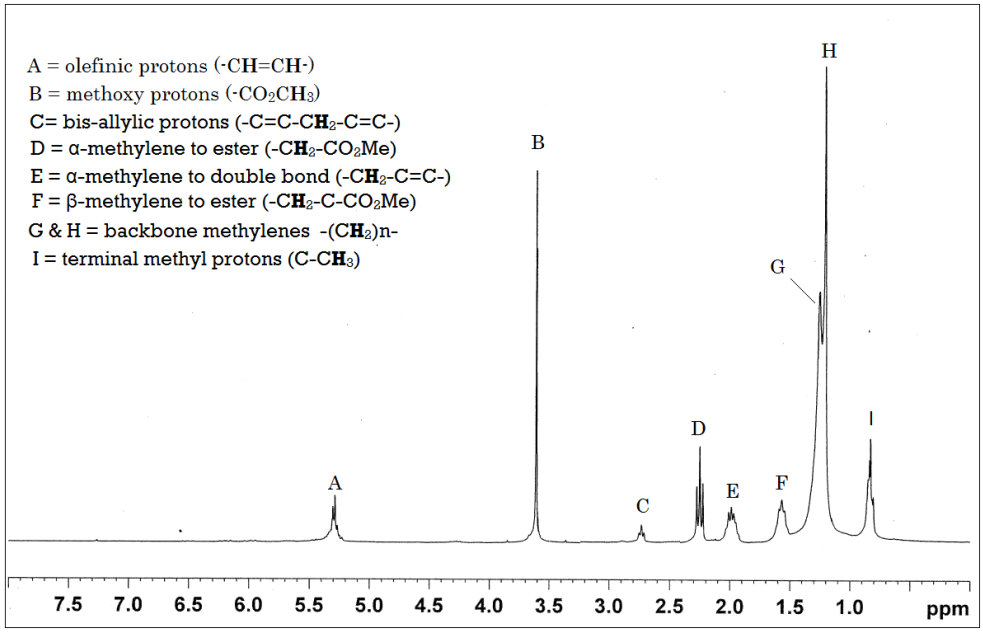

Fig. 3. ^{1}H NMR spectrum of FAME from *Baccaurea remiflora* seed oil

The ^{13}C NMR spectrum of FAME from *Baccaurea remiflora* 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.

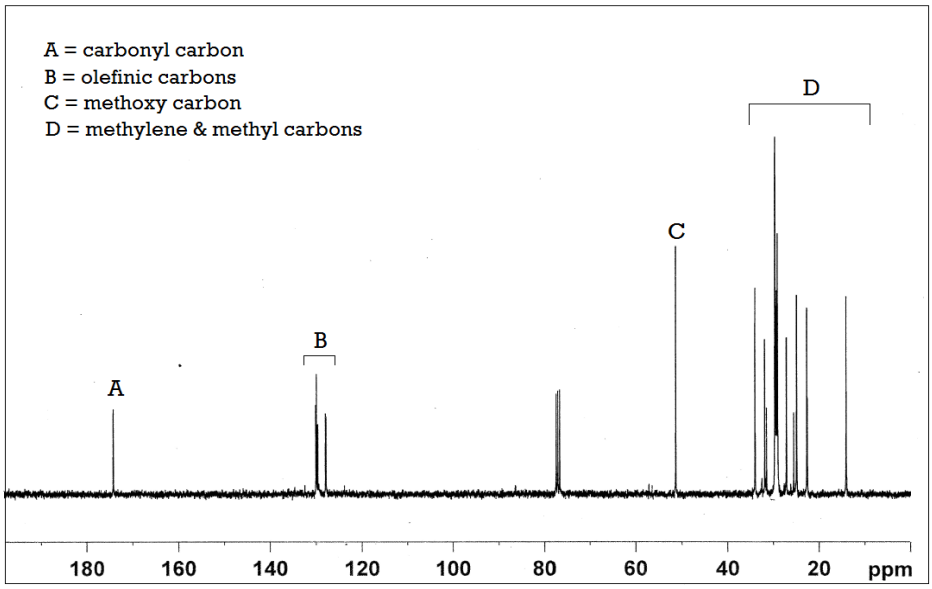

Fig. 4. ^{13}C NMR spectrum of FAME from *Baccaurea remiflora* seed oil

The IR spectrum of FAME from *Baccaurea remiflora* seed oil is shown in **Fig. 5.** IR spectrum of the FAME shows a C=O stretching band of methyl esters at 1743 cm^{-1} and C-O stretching bands at 1114, 1171 and 1246 cm^{-1}. The weak signal at 1659 cm^{-1} may due to C=C stretching frequency. Strong and sharp signals at 2843 and 2926 cm^{-1} indicate C-H stretching frequencies. The absorbance at 3462 cm^{-1} is due to the =C-H stretching frequency. The observation of an absorption peak at 721 cm^{-1} suggested the CH_2 rocking.

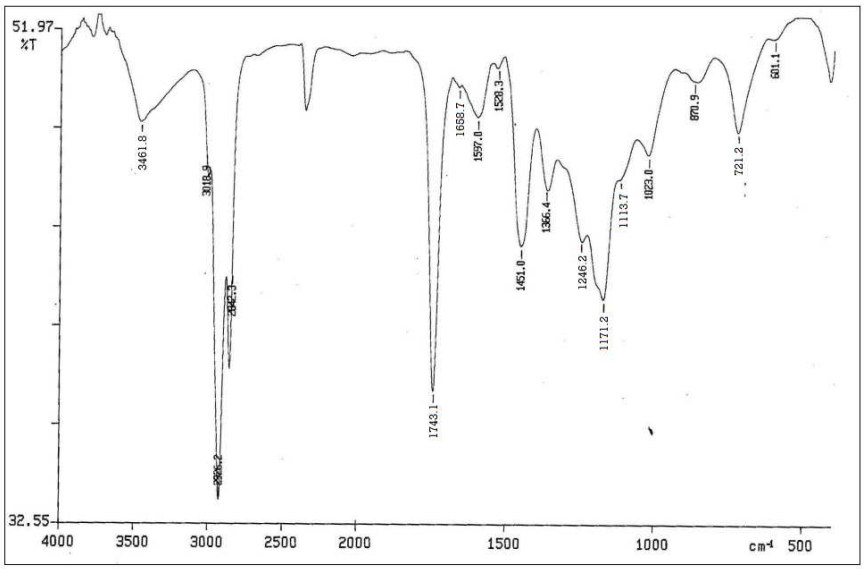

Fig 5. The IR spectrum of FAME from *Baccaurea remiflora* seed oil

CONCLUSION

In this study, the physicochemical properties of *Baccaurea remiflora* seed oil were investigated. The study revealed that saturated fatty acids such as palmitic acid (33.67%), stearic acid (19.38%) and arachidic acid (9.69%) account for more than 60% of total fatty acids while oleic (24.48%) and 11- *trans*-eicosenoic (12.75%) acids are the unsaturated fatty acids found in *Baccaurea remiflora*. The acid value, moisture content and iodine value were 1.127 mg KOH/g oil, 0.103% and 80.32 gI_2/100 g respectively. The density and refractive index of the oil were found to be 0.8674 g/cm^3 and 1.4672 respectively.

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