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Physicochemical Characterization of *Cinnamomum Tamala* Seed Oil

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Abstract: ***Cinnamomum tamala seeds oil was extracted by an organic solvent 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 1.118 mg KOH/g oil, 0.101% and 120.32 gI_2/100 g respectively. The density and refractive index of the oil were found to be 0.8986 g/cm^3 and 1.4685 respectively. Gas chromatographic analysis of Cinnamomum tamala seeds oil detects the unsaturated fatty acids such as linoleic acid (47.01 wt.%) and oleic acid (26.32 wt.%) which together account for more than 70% of total fatty acids. The prominent saturated fatty acid present is palmitic acid (18.97 wt.%).***

Keywords: Cinnamomum tamala, unsaturated acid, Musa balbisiana Colla, transesterification

I. INTRODUCTION

Cinnamomum tamala belongs to family *Lauraceae*. It is also known as Indian Cassia and the leaves are commonly called as bay leaves. *Lauraceae* is an economically important family consisting mostly of trees or tree-like shrubs. The genus *Cinnamomum* is represented by about 350 species worldwide. *Cinnamomum tamala* is an evergreen tree, grown up to a height of 8 m and is native to northern western Himalaya, Sikkim, Assam, Mizoram and Meghalaya region [1]. Historically, it is one of the oldest known and used spices. Its dried leaves are used as a common ingredient of Indian cooking. The leaves of this tree have a clove like taste and a faintly pepper like odour. It is also used in Indian system of traditional medicines. In this paper, the physicochemical properties of *Cinnamomum tamala* seed oil is reported.



Fig.1. *Cinnamomum tamala* seed

II. EXPERIMENTAL SECTION

A. Materials

Cinnamomum tamala seeds were collected from Nalbari and Barpeta Districts of Assam, India during its availability of the season. The 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 extraction. Solvents and other chemicals used were of analytical grade, and they were procured from commercial sources and used as such without further treatment.

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B. 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 [2]. Iodine value was estimated by applying Wijs method [3, 4]. Moisture content was determined by oven drying a known quantity of the oil in the oven at 105^{o}C for 24 hours after which the percentage moisture was calculated as follows:

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

C. Oil Extraction

Solvent extraction technique was used for extraction of oil. Oil was obtained from 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.

D. Transesterification of Seed Oil

Transesterification of the purified oil was carried out at room temperature with methanol. The catalyst used for transesterification was prepared in the laboratory from the trunk of *Musa balbisiana* plant which is a reported heterogeneous catalyst [5]. A mixture of oil, methanol (10mL/g of purified oil) and catalyst (20wt% 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.

E. 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 *Cinnamomum tamala* seed oil is reported in Table 2.

III. RESULTS AND DISCUSSION

The results of the physical characteristics of oil obtained from the seeds of *Cinnamomum tamala* are shown in Table1.
The oil content of the seed is found to be 33.1%.
It is found that *Cinnamomum tamala* seed oil has a very low moisture content (0.101) which is indicative of a long storage life for the seed oils. Besides, it also indicates that the oil is of good qualities and is not easily subjected to contamination. A higher value of moisture has a negative effect on the transesterification of glycerides [6, 7, 8].

Table 1 : Some physicochemical properties of *Cinnamomum tamala* seed oil

S/N	Parameters	Observed values
1	Colour	Light yellow
2	Oil content (%)	33.1
3	Density (g/cm^3)	0.8986
4	Acid value (mg KOH/g)	1.118
5	Iodine value (gI_2/100 g)	120.32
6	Saponification value	189.34
7	Refractive index	1.4685
8	Moisture (%)	0.101

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A higher acid value (1.118) was observed in *Cinnamomum tamala* oil which indicates a high fatty acid content in the oil. This also suggests that these oils are not favourable source for production of biodiesel as high free fatty acid (FFA) content causes formation of soap during transesterification reaction of glycerides with alcohol and makes it extremely difficult to separate the products [9, 10].

The iodine value of *Cinnamomum tamala* seed oil was found to be 120.32. This implies that seed oil is highly unsaturated.

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 [11, 12, 13]. 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 [14].

Fatty acid profile of the FAME from *Cinnamomum tamala* 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 *Cinnamomum tamala* seed oil are presented in Table 2. The results show that *Cinnamomum tamala* seed oil is mainly dominated by unsaturated fatty acids namely linoleic acid (47.01 wt.%) and oleic acid (26.32 wt.%). The saturated fatty acids detected are palmitic acid(18.97 wt.%) and stearic acid (7.70 wt.%).

Table 2 : Fatty acid profile of FAME from *Cinnamomum tamala* seed oil

Retention time (min)	FAME	Molecular ion peak (m/z)	wt.%
16.61	Methyl palmitate	270	18.97
19.85	Methyl linoleate	294	47.01
19.96	Methyl oleate	296	26.32
20.05	Methyl stearate	298	7.70

The mass spectra of methyl palmitate, methyl linoleate, methyl oleate and methyl stearate are shown in Figs 2.a to 2.d and their molecular ion peaks are observed at 270, 294, 296 and 298 respectively.

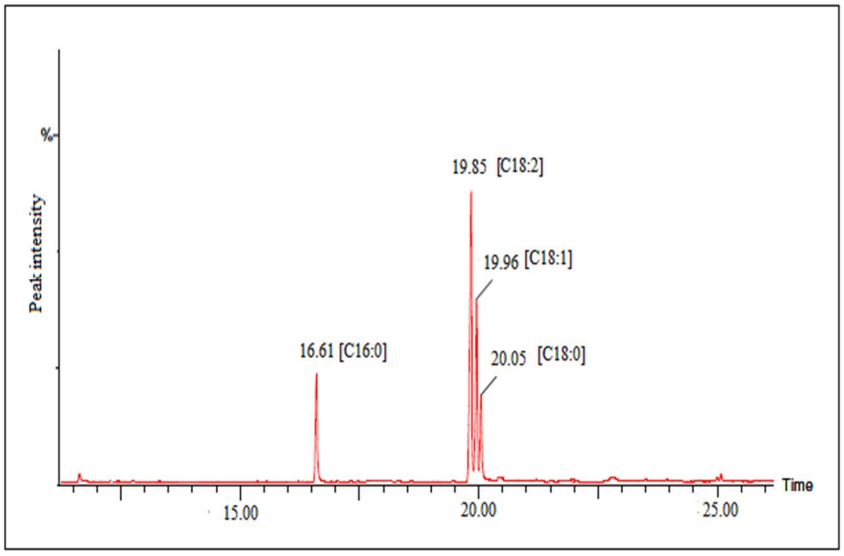


Fig. 2. Gas Chromatogram of FAME from *Cinnamomum tamala* seed oil

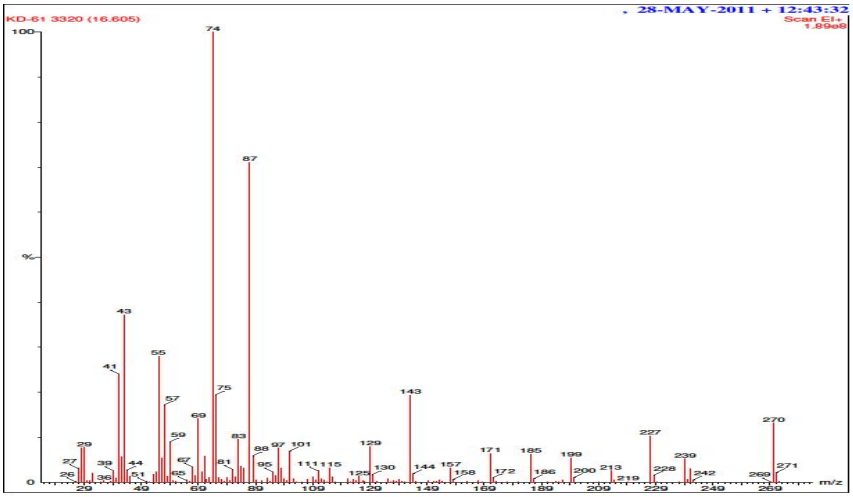


Fig. 2a. Mass spectrum of methyl palmitate

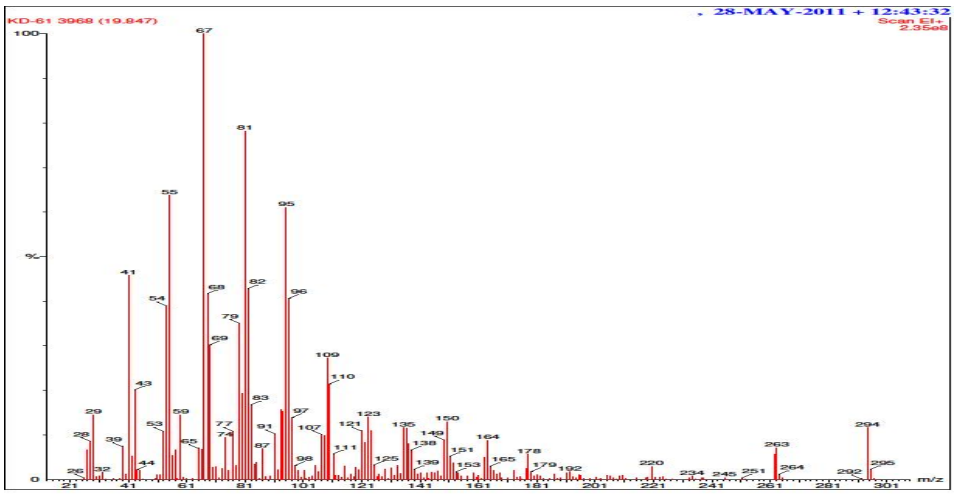


Fig. 2b. Mass spectrum of methyl linoleate

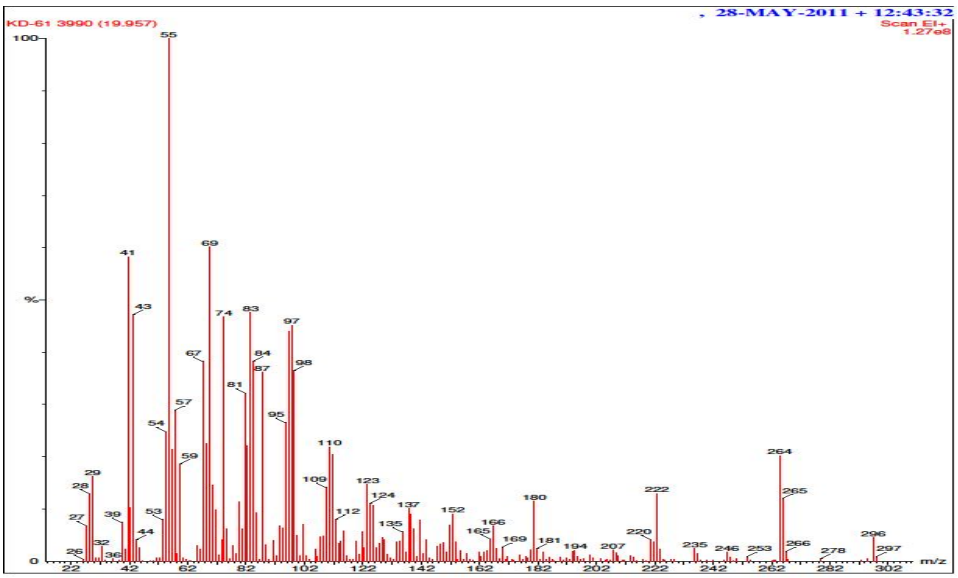


Fig. 2c. Mass spectrum of methyl oleate

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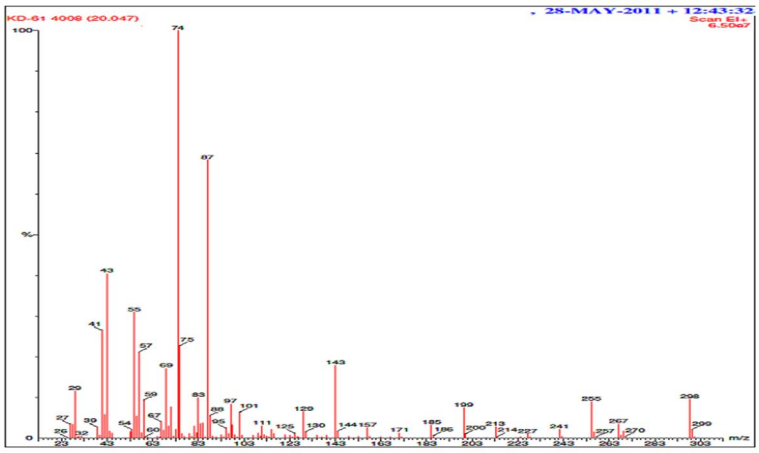


Fig. 2d. Mass spectrum of methyl stearate

The ^{1}H NMR spectrum of the FAME from *Cinnamomum tamala* seed oil is shown in Fig. 3. A multiplet appears at 5.32–5.36 ppm indicating the presence of olefinic protons (-CH=CH-). The singlet signal at δ 3.64 ppm represents methoxy protons of the ester functionality of the FAME. The triplet at δ 2.29 ppm (t, ^{3}J=7.5 Hz) indicates the α-methylene protons to ester (-CH_2-CO_2Me). The α-methylene protons to double bond (-CH_2-C=C-) appears as a multiplet at δ 1.98–2.04 ppm. The singlet signals at δ 1.23 and 1.28 ppm are due to the protons of backbone methylenes of the long fatty acid chain. The terminal methyl protons (C-CH_3) at δ 0.83–0.87 ppm appear as a multiplet.

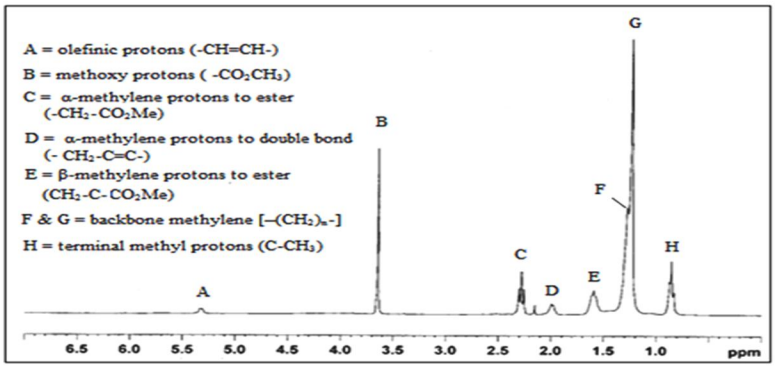


Fig. 3. ^{1}H NMR spectrum of FAME from *Cinnamomum tamala* seed oil

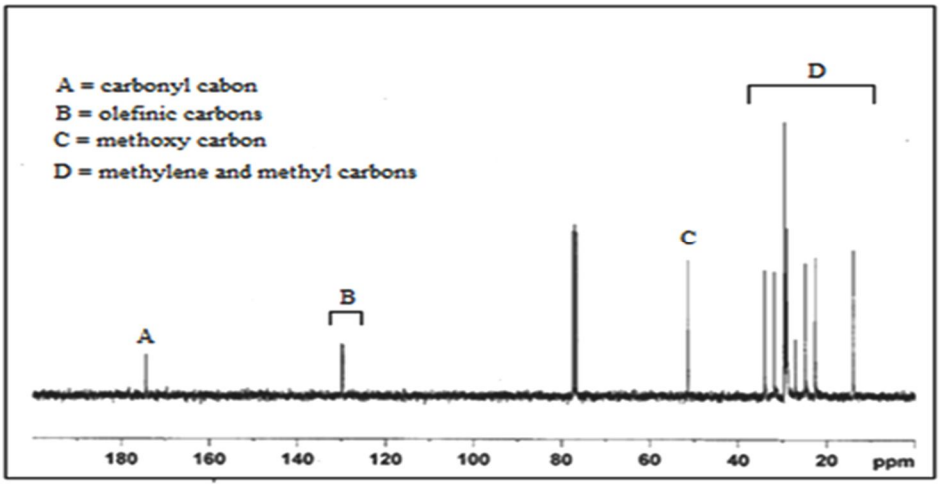


Fig. 4. ^{13}C NMR spectrum of FAME from *Cinnamomum tamala* seed oil

The ^{13}C NMR spectrum of FAME from *Cinnamomum tamala* seed oil is shown in Fig 4. The signal at δ 174.28 ppm indicates the carbonyl carbon of the ester molecules and the olefinic carbons appear at δ 127.83, 127.97, 129.68, 129.94 and 130.12 ppm.. The signal at δ 51.28 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 14.09 to 33.39 ppm.

The IR spectrum of FAME from *Cinnamomum tamala* seed oil is shown in Fig 5. A signal at 1744 cm^{-1} in the IR spectrum indicates the C=O stretching band of methyl esters while C-O stretching bands appear at 1170, 1198, and 1248 cm^{-1}. A weak signal is seen at 1625 cm^{-1} which may be due to C=C stretching frequency. Strong and sharp signals at 2854 and 2923 cm^{-1} indicate C-H stretching frequencies. The observation of an absorption peak at 723 cm^{-1} indicates the CH_2 rocking.

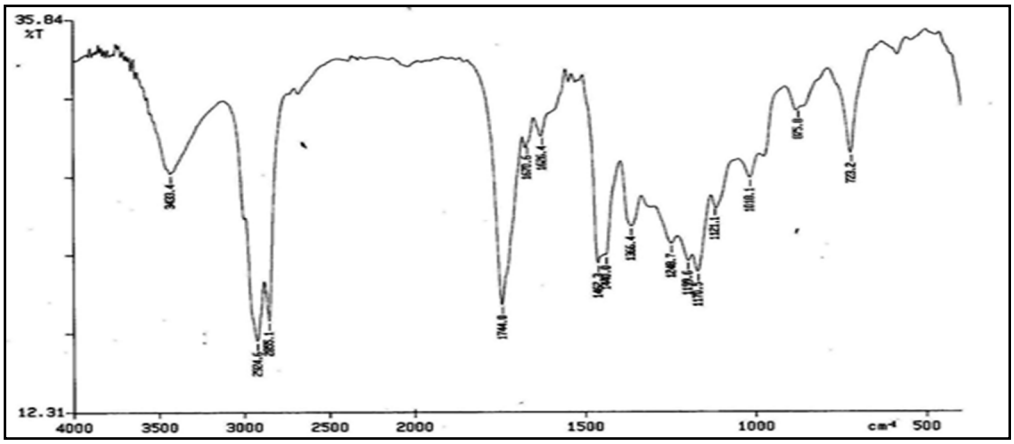


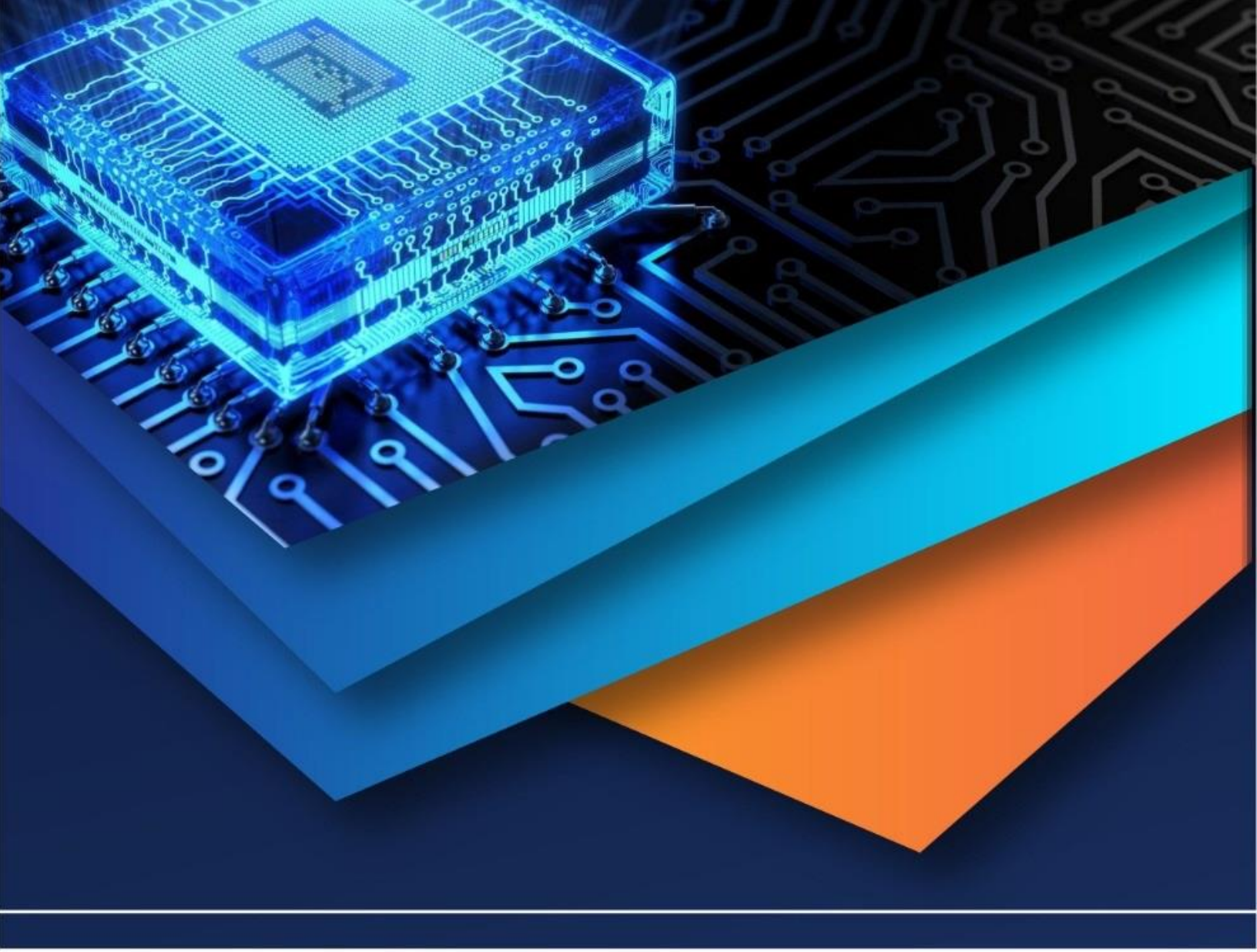
Fig 5. _The IR spectrum of FAME from *Cinnamomum tamala* seed oil

IV. CONCLUSION

In this study, the physicochemical properties of *Cinnamomum tamala* seed oil was investigated. The study revealed that unsaturated fatty acids such as linoleic and oleic acids are dominant fatty acids in *Cucumis sativus* L. seed oil. Linoleic acid is the major fatty acid found with 47.01 wt.% followed by oleic acid (26.32 wt.%) and palmitic acid (18.97 wt.%). A high fatty acid content (acid vale of 1.118) was observed for the seed oil. Iodine value, density and refractive index of the oil were found to be 120.32 gI_2/100 g, 0.8986 g/cm^3 and 1.4685 respectively.

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