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Extraction and Characterization of Glycerides from Oilseed of *Benincasa Hispida* Plant Found in Manipur

Md. Abdul Halim Shah[1], Dr. Naorem Sarita Devi[2]

[1]Department of Chemistry, Dhanamanjuri University (D.M. College of Science) Imphal -795001, India
[2]Associate professor Department of Environmental Science

ABSTRACT

Extraction of seedoil (*Benincasa hispida*) was done by solvent extraction technique on the crushed kernel using petroleum ether as the solvent. The oil was purified prior to further analysis by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether and ethyl acetate (20:1) as the eluent. The fatty acid methyl ester (FAME) of the oil of *Benincasa hispida* consists of 11.29wt % of methyl palmitate(C16:0),82.64wt% of methyl linoleate(C18:2) and 6.06wt% of methyl stearate(C18:0).

Keywords: Ash gourd, *Benincasa hispida*, Transesterification, Non-edible vegetable oils, Athia, Biodiesel.

INTRODUCTION

North-East India including Manipur is known for its rich plant diversity. There are many plants which produce fruits with seeds highly rich in non-edible oils in both plain and hill areas of Manipur (India). Many of these plants have no apparent economic value. Thus such oil finds very limited commercial uses and plants producing such oils are slowly disappearing because such plants are considered not important by farmers, government, any public and private sectors. As a result plant diversity is dwindling. Moreover, it is believed that large scale production of biodiesel from edible oils may cause global imbalance to the food supply and demand market. Hence use of non-edible oils as the feedstock for biodiesel industries will spare edible oils for use in other industries of edible products [4],[5]. The use of biodiesel now-a-days has become important for diesel engines and is getting worldwide attention because of its renewability, biodegradability, non-toxicity and carbon neutrality [6]-[9]. The developed countries like Brazil, Indonesia, Malaysia, USA, UK, Canada and Germany have already started using biodiesel blended petrodiesel.

Biodiesel usually consists of methyl esters of long chain fatty acids and is made from nontoxic biological resources such as vegetable oils and animals fats by transesterification with methanol in presence of a catalyst [4],[8],[12]. The non-edible vegetable oils can be used as alternative feed stocks for the production of biodiesel [2],[3],[10],[13].Proper utilization of the available non-edible oils will open up enormous scope for rural development in terms of employment opportunity for youth and infrastructure development in North East region of India. The by-product of oil extraction from these seeds and biodiesel production process could also be utilized for organic fertilizer, bio gas production and soap making.

Benincasa hispida *is* locally called Torbot in Manipuri belongs to the family ***Cucurbitaceae*** (Fig: 1a-1b). It is a vine grown for its very large fruits, eaten as a vegetable when mature. The fruits is fuzz when young. It was originally cultivated in South East Asia as well. Ash gourd can typically be stored for 12 months and that is why some places including Manipur is used as one of the vegetables during winter. In Pakistan and Northern India, this vegetable is used to prepare a candy called Petha. Cooked and sweetened Ash gourd can help people suffering from heart ailments, anemia, body heat etc. Being low in calories, it is helpful for people maintaining a diet and people suffering diabetes. The seeds are cooked in milk and taken to increase “sperm count” and to improve sperm locomotion.

Fig.1a. Flowering stage with fruits of *Benincasa hispida* plant

Fig.1b *Seeds of Benincasa hispida* plan

MATERIALS AND METHODS

Benincasa hispida seeds were collected from the Phouden, Thoubal District (24.63 94.02)(24°37'48.00" N 94°01'12.00" E), Manipur (India) during its availability of the season, November-April. The seeds were first cleaned and dried for 5/6 days in the sunlight, deshelled and the kernel crushed using a grinder priorto oil extraction. Methanol used was analytical grade (Mark Mumbai, India). All other solvents and chemicals used were analytical grade and they are procured from commercial sources and used as such without to further treatment.

Benincasa hispida, the seeds have medium hard outer shell and can be easily dehusked before carrying out the estimation of the oil content. Kernel content is moderate (about 21.12 g Kernal in 25.50 g seed.

Oil was extracted from crushed and powdered kernel of *Benincasa hispida* seeds in petroleum ether (bp 40-60°C) (10ml/g) by stirring magnetically at room temperature using solvent extraction technique (22-23°C) for 4:30 hours. The solvent was removed at 45°C using a rotary vacuum evaporator (BUCHI Rotavapour R-200) to yield 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 which was further dried by using vacuum pump. The oil was purified by column chromatography over silica gel (60-120 mash) using a mixture of petroleum ether and ethylacetate (20:1) as the eluent prior to transesterificationis done.

$$\% \, Oil \, content = \frac{Weight \, of \, oil}{Weight \, of \, powdered \, seeds} \times 100$$

The parameters of glycerides such as density, colour, refractive index, acid value, iodine value and saponification value were experimentally determined in accordance with the Association of Official Analytical Chemical Procedures [11] and these results are reported(Table 1).

Acid value (mg KOH / g) = 56.1 x V x NW, 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.

Refractive indices of purified seedoils were determined by using the Abbe Refractometer (AW-24) at room of temperature, only two or three drops of oil was required. Densities of the purified oils were determined at room temperature (32 0C). For this, a clean and empty plastic centrifuge tube was taken and weighed. Accurately 1000 μL (= 1 mL) of the liquid sample was transferred into the tube with the help of a syringe and then weighed again. Then the density is determined based on mass per unit volume of oil.

Iodine value = 12.69 x N x (VB−VS)W
Where, VB = Volume of sodium thiosulphate solution used for the blank (mL)
VS = 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

Saponification value = 56.1 x M x (VB−VS)W
where, VB = Volume of 0.5 M HCl solution used for the blank (mL) VS = Volume of 0.5 M HCl solution used for the oil sample (mL) M = Molarfity of HCl used W = Weight of oil sample taken in g
% Moisture = W1 − W 2 W1 x 100 , where W1 = Initial weight of oil, W2 = Final weight of oil

Table 1: Physical parameters of Benincasa hispida seed oil.

Sl. No.	Parameters	Observed Values
1.	Colour	light yellow
2.	Oil content (%)	12.22
3.	Density (g/cm^3)	0.8987
4.	Acid Value (mg KOH/g)	0.790
5.	Iodine value (gI2/100g)	93.25
6.	Saponification value(mg KOH/g)	190.76
7.	Refractive Index	1.4652
8.	Moisture(%)	0.101

The purified oil was transesterified to fatty acid methyl esters (FAME) using a catalyst from the peels of Athia (*Musa balbisiana*), a banana plant [1]. A mixture of the oil in methanol (10 ml/1g of the oil) and the catalyst (20 wt % of the oil) was stirred vigorously magnetically at room temp (28°C) and the conversion completion of the reaction was monitored by Thin Layer Chromatography (TLC). After completion of the reaction, the product mixture was extracted with petroleum ether (bp 40-60°C). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 & the solvent was removed under vacuum to yield the crude product which was further purified by column chromatography over silica gel using petroleum ether & ethyl acetate (20:1) as the eluent. The product was concentrated & evaporated to dryness on a rotary

evaporator which was further dried using vacuum pump to remove the last traces of the solvents to yield pure biodiesel (FAME).

The composition of FAME mixture was estimated using Pockin Elmer Clarus 600 GC-MS. The column used was Elite 5 MS with initially held at 140°C for % min, increased to 240°C at 4°c/min, and then held for 5 min. The injector transfer and source temperatures were 250°C and 150°C respectively. Carrier gas was helium and total scan time 35 min. El mode of ionization was applied and mass gas was from 20 to 400 Da. For identification of FAME library search was carried out using National Institute of Standards and Technology (NIST), National Bureau of Standards (NBS) and Wiley GC-MS library. Fatty acid profile of biodiesel from *Benincasa hispida* seed oil is reported in Table 2. The ^{1}H & ^{13}c NMR spectra were recovered in Carbon Deutrium Trichloride (CD Cl_3) at 300 MHz /5mm. NMR spectrometer and IR spectrum were recorded with a Perkin Elmer RXIFT-IR spectrometer as a thin film on KBr plate.

Fatty acid composition of the FAME prepared from *Benincasa hispida* seed oil was determined by GC-MS analysis. The each peak of the gas chromatogram (Fig.2) was analysed and the fatty acid was identified using MS database. Each peak represents one fatty acid methyl ester. The three peaks in the gas chromatogram which means the presence of three different fatty acid methyl Easters. The peak at the farthest distance on the right side in mass spectrum of any fatty acid methyl ester gives the molecular weight of the fatty acid. This peak is known as molecular ion peak. Retention time is the time taken when any peak develops. Based peak means the tallest peak in the mass spectrum due to the ion with the greatest relative abundance. The peak with the greatest m/z value is likely to be the molecular ion peak.

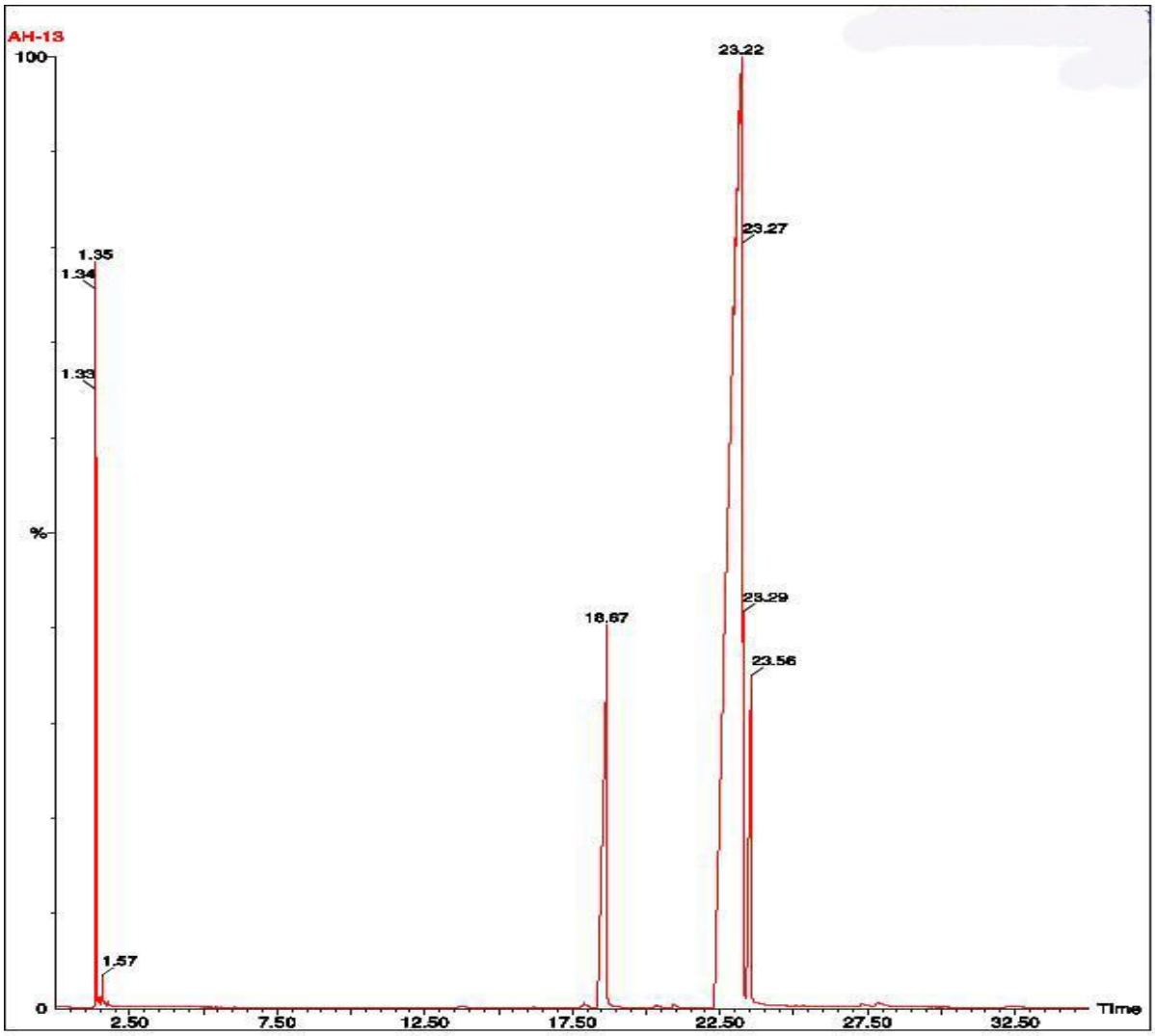

Figure: 2. Gas chromatogram of biodiesel from *Benincasa hispida* seed oil.

RESULTS AND DISCUSSION

The yield of the extracted and purified glycerides from *Benincasa hispida* seedoil was found to be 12.22wt % at the room temperature (27°C) within 4:30 hours while the yield of transetrified glyceride known as Fatty Acid Methyl Ester (FAME) was 57.9 wt % at the room temperature (28°C) within 4:15 hours.

The light yellow colour of the *Benincasa hispida* seed oil was due to the presence of natural pigments like tocopherols, carotenoides and their derivatives. The yield of the oil was moderate. Density and iodine value of *Benincasa hispida* seed oil were found to be 0.8987 g/cm^3 and 93.25 gI_2/100 respectively which are comparable to those of soya bean oil and sunflower oil. The acid value of this oil was found to be 0.790 mg KOH/g which is within the limit for industrially useful oil. Saponification value was 190.76 mg KOH/g whose value is suitable for soap making and cosmetic industries. Refractive Index of this oil was 1.4652 which is not very much different from those recorded for conventional seed oils such as palm oils (1.445-1.451), cotton seed oil (1.468-1.472), safflower oil (1.473-1.476) and soya bean oil (1.4728) at 25'C. Moisture was found to be 0.101% (low value) which is suitable good quality and contamination does not take place easily due to its low value of moisture. Low moisture content is an essential criterion for commercial oil.

Analysis of FAME of Benincasa hispida

^{1}HNMR (300 MHz, CD Cl_3): δ3.33-5.35 ppm, δ 3.65 ppm, δ 2.8 ppm, δ2.29 ppm, δ 1.99-2.00 ppm, δ 1.58-1.63 ppm, δ 1.24-1.29 ppm, δ0.84-0.89 ppm. ^{13}C NMR (75MHz, $CDCl_3$): δ 174.23 ppm, δ 129.67 and the δ 129.92 ppm, δ 51.37 ppm, δ27.15-34.03 ppm. FT-IR (thin film): 1724.36, 1436.97, 2933.73, 2767.85, 3047.53, 736.81,1251.80 and 1192.01 cm^{-}.

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 *Benincasa hispida* consists of 11.29wt% of methyl palmitate (C16:0), 82.64wt% of methyl linoleate (C18:1) and 6.06wt% of methyl stearate (C18:0).

Table 2: Composition of biodiesel from *Benincasa hispida* seed oil

Retention time (mm)	FAME	wt%
18.20	Methyl palmitate	11.29
22.40	Methyl linoleate	82.64
26.80	Methyl stearate	6.06

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 3: Molecular ion and base peaks of FAME from *Benincasa hispida* seedoil

Sl.No	FAME	Molecular ion peak (m/z)	Base peak (m/z)
1	*Methyl palmitate*	270	74
2	*Methyl linoleate*	294	55
3	*Methyl* stearate	298	74

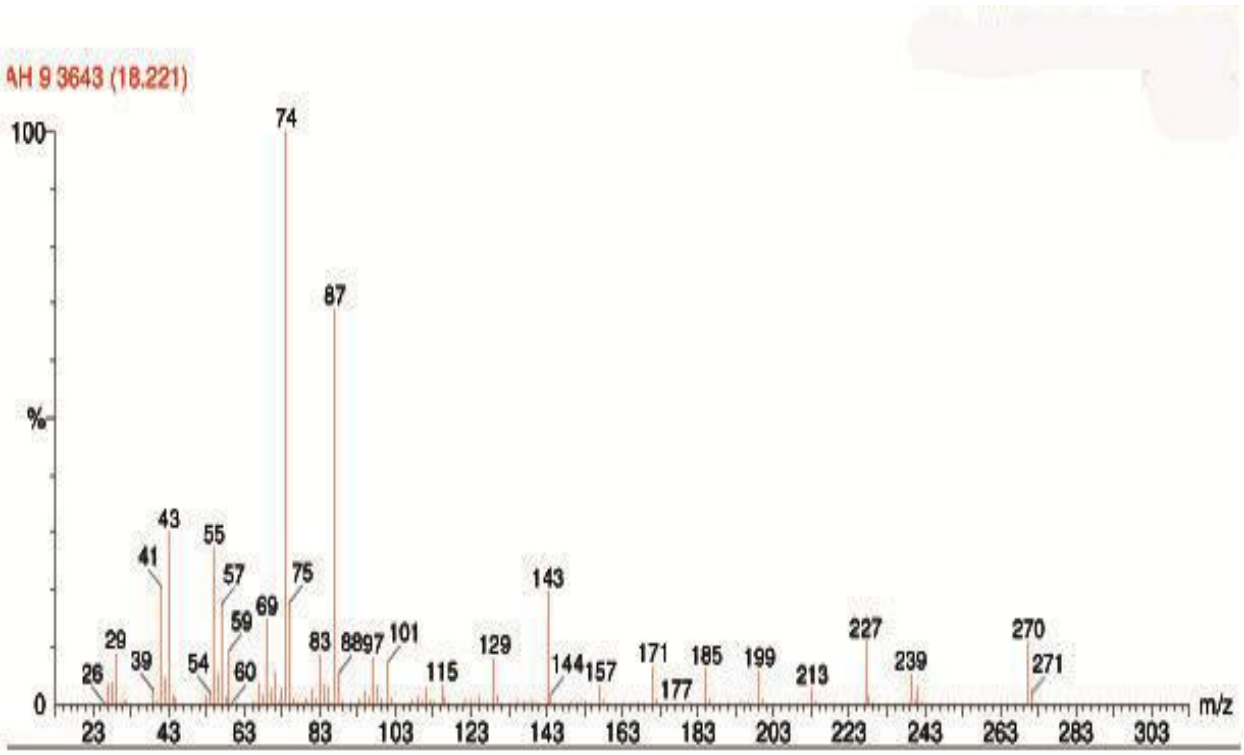

Fig.3a : Mass spectrum of methyl palmitate

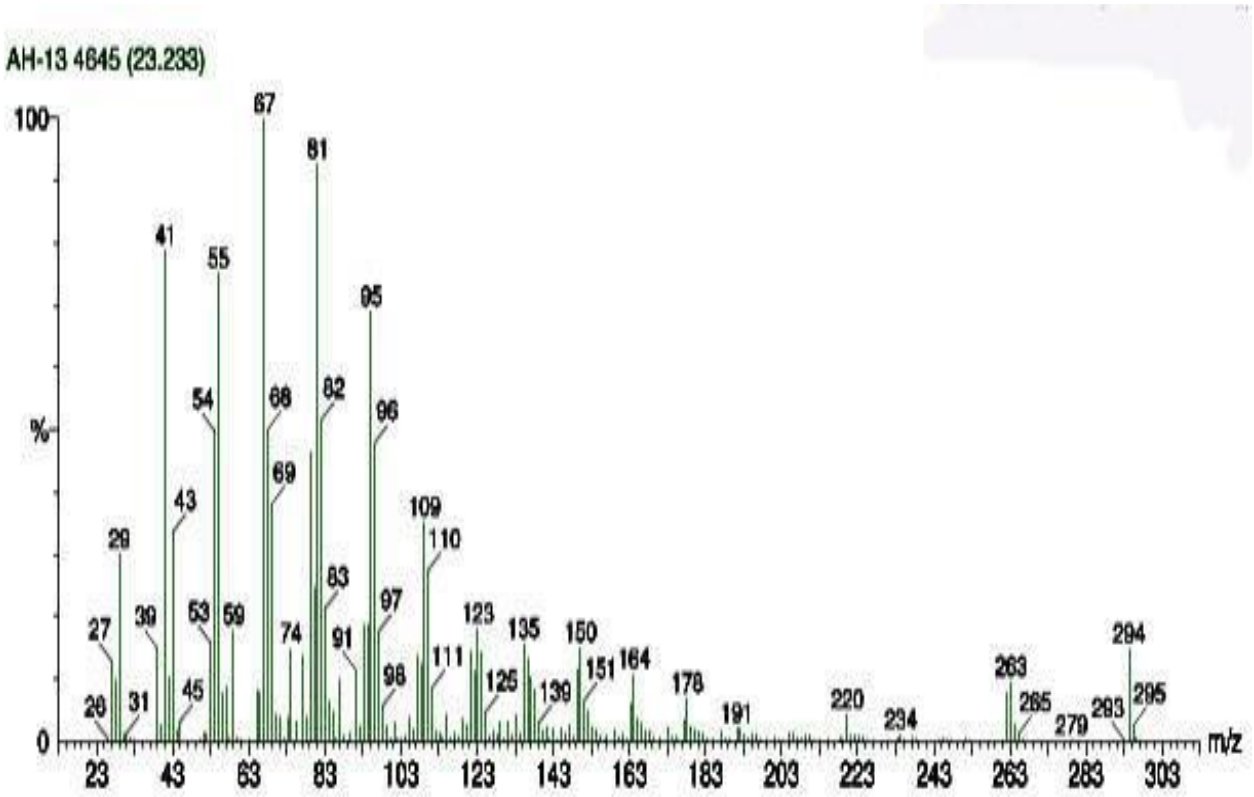

Fig.3b: Mass spectrum of methyl linoleate

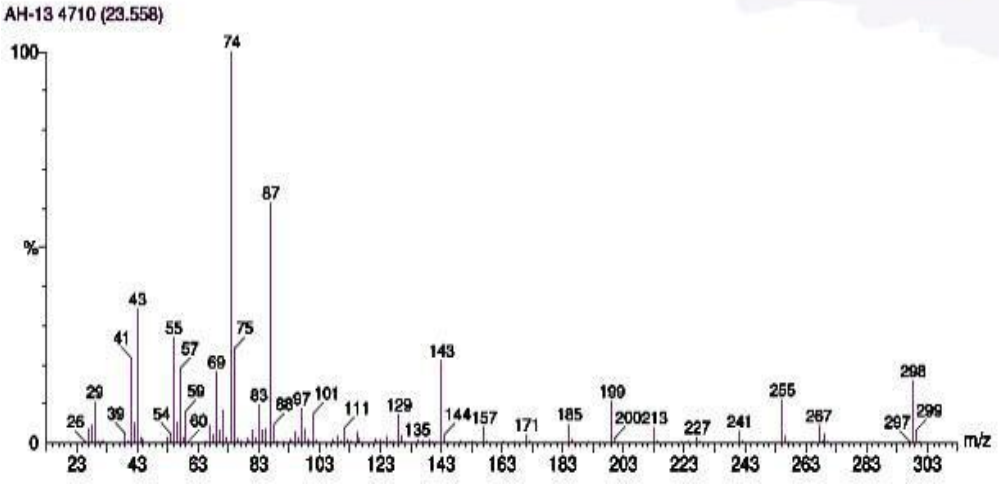

Fig. 3C:-mass spectrum of methyl stearate

The multiplet at δ 3.33-5.35 ppm indicates the olefinic protons (–CH=CH–)(Fig 4) A singlet signal at δ 3.65 ppm is indicating methoxy protons of the ester functionality of the biodiesel. The triplet signal at around δ 2.8 ppm suggests bis-allylicprotons(–C=C–CH2–C=C–) of the unsaturated fatty acid chain. The triplet at δ 2.29 ppm (t, 3J = 7.5 Hz) may be due to the ∝-methylene protons to ester (–CH2–CO2CH3). The ∝- methylene protons to double bond (–CH2–C=C–) is seen as a multiplet at δ 1.99 - 2.00 ppm. The β-methylene protons to ester (CH2–C–CO2CH3) also appear as a multiplet at δ 1.58 - 1.63 ppm. The multiplet at δ 1.24 - 1.29 ppm is due to the protons of backbone methylene of the long chain fatty acid chain. The terminal methyl protons (C–CH3) at δ 0.84 - 0.87 ppm appear as a multiplet.. The signal at δ 174.23 ppm suggests the carbonyl carbon of ester, the olefinic carbons appear at δ 129.67 and 129.92 ppm (Fig 5). The signal at δ 51.37 ppm in the 13C NMR spectrum of biodiesel is due to methoxy carbons of esters. The methylene and methyl carbon of fatty acid moiety appear in the range from δ 27.15 to 34.03 ppm. In IR spectrum of biodiesel from *Benincasa hispida* seed oil (Fig.6) a sharp signal at 1724.36 cm-1 suggests strong absorption by ester stretching frequency. The weak signal at 1436.97 cm-1 may be due to C=C stretching frequency. Strong and sharp signals at 2933.73, 2767.85 cm-1 are due to C–H stretching frequencies. The observance at 3047.53 cm-1 the =C–H stretching frequency. The observation of an absorption peak at 736.81 cm-1 indicates the CH2 rocking.

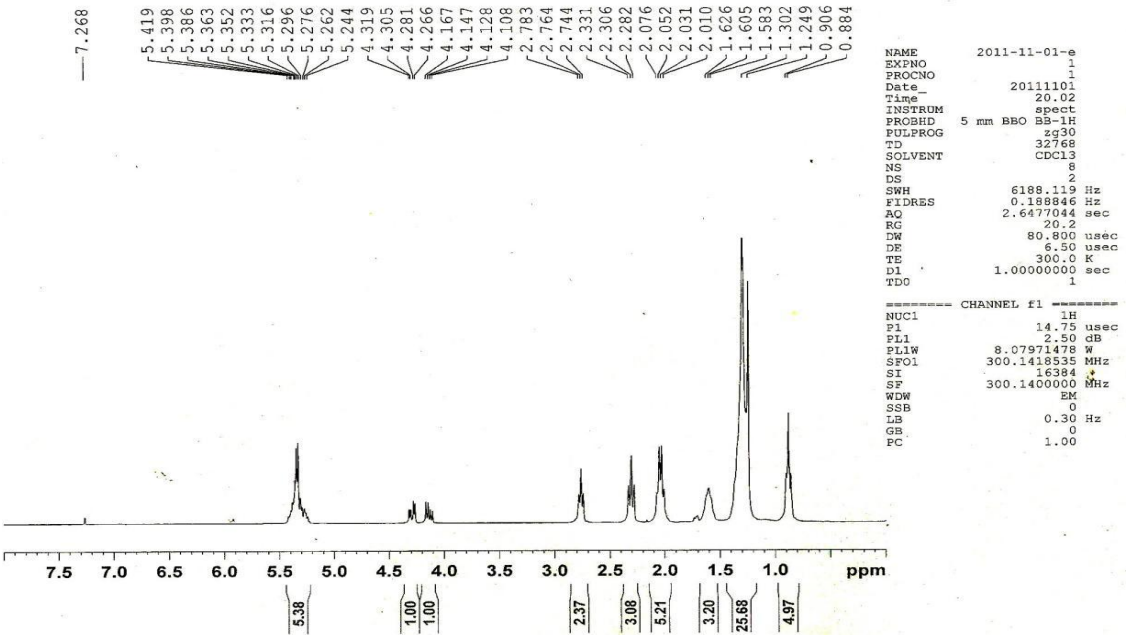

Fig 4:1H NMR spectrum of biodiesel from *Benincasa hispida* seed oil

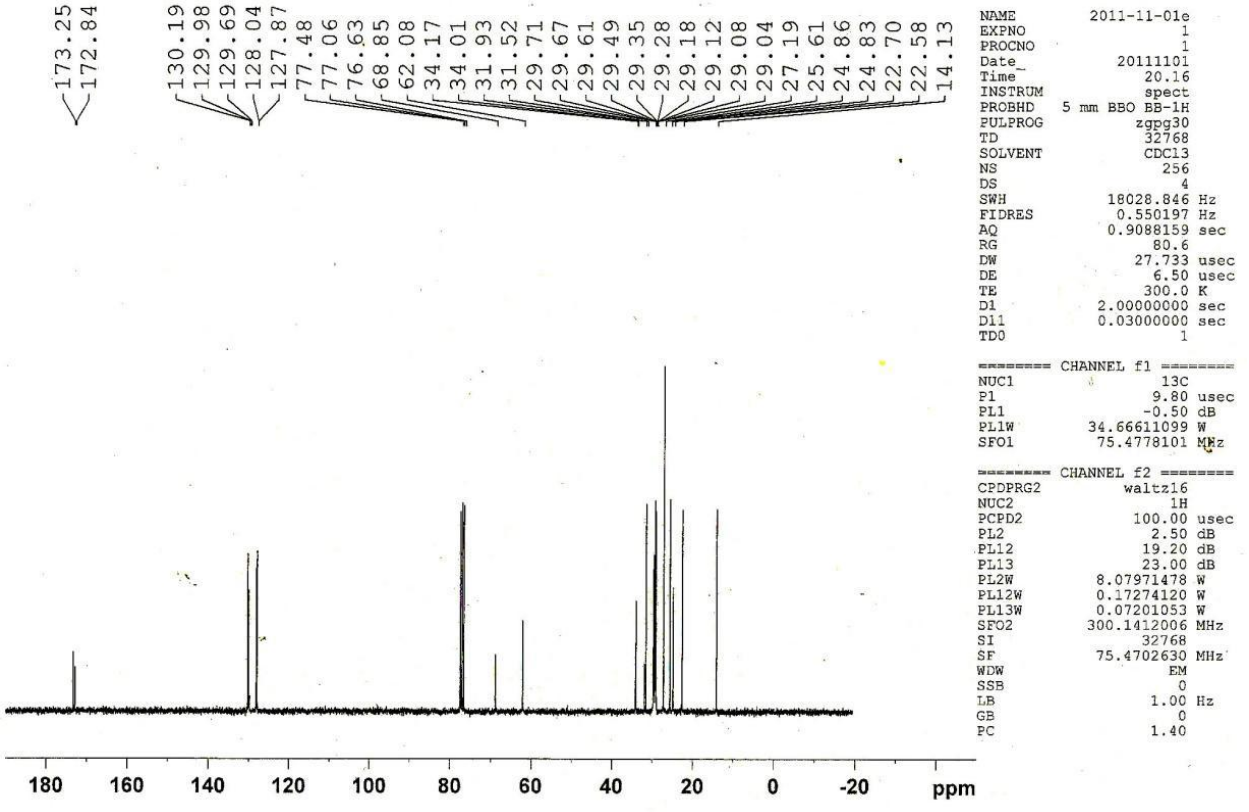

Fig.5: 13C NMR spectrum of biodiesel from *Benincasa hispida* seed oil

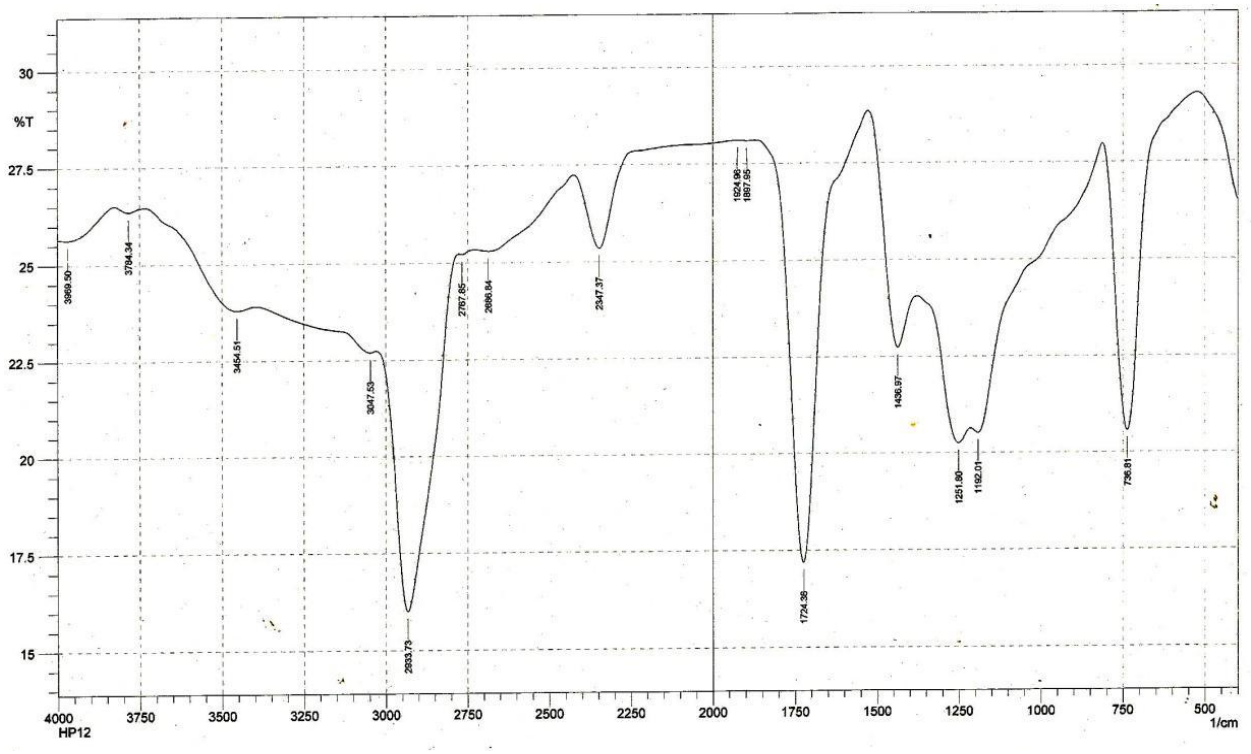

Fig.6: IR spectrum of biodiesel from *Benincasa hispida* seed oil

CONCLUSION

The yield of the extracted and purified glycerides from *Benincasa hispida* seed oil was found to be 12.22wt % at the room temperature (27°C) within 4:30 hours while the yield of transetrified glyceride known as Fatty Acid Methyl Ester (FAME) was 57.9 wt % at the room temperature (28°C) within 4:15 hours. The colour, density, acid value, iodine value, saponfication number, refractive index and moisture of the *Benincasa hispida* seed oil were found to be light yellow, 0.8987 g/cm^3, 0.790 mg KOH/g, 93.25 gI2/100 g, 190.76 mg KOH/g, 1.4652 and 0.101 % respectively. The biodiesel from *Benincasa hispida* seed oil, after extraction and purification by column chromatography, was prepared by heterogeneous transesterification process and analyzed for its fatty acid methyl esters composition using IR, NMR and GC-MS. This study found that FAME from *Benincasa hispida* seed oil consists of 11.29 wt. % of methyl palmitate (C16:0), 82.64 wt. % of methyl linoleate (C18:2) and 6.06 wt.% of methyl stearate (C18:0). The molecular ion peak of methyl palmitate, methyl linoleate and methyl stearate were observe at 270,294 and 298 respectively as was expected

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