Fatty Acid Methyl Ester analysis of some oil plants found in Bihar, India: A Comparative study.

Abstract

Today's developmental world needs large amount of energy. Due to the limited fossil fuel source, there is need of some alternate fuel sources among which biodiesel from vegetable oil widely practiced. There is an increasing interest in India to search for suitable low cost alternative fuels that are Eco friendly. Biodiesel is a renewable, biodegradable and non toxic fuel. In this paper an attempt has been made to study and compare the oil percentage and Fatty acid methyl ester (FAME) components of three non edible oil seed plants abundantly found in Bihar, India.

Oil from the seed kernel was extracted by solvent extraction technique through Soxhlet apparatus using n-hexane as solvent. Percentage oil content for *Jetropha, Mahua* and *Castor* are found around 76 %, 41% and 33% respectively. Further extracted oil were analysed by GC-MS for their FAME components. palmitic, linoleic, oleic are most common fatty acid found among three.

Keywords: *Biodiesel, Jetropha, Mahua, Castor, FAME, GC-MS, Bihar.*

1 Introduction

The world is presently undergoing rapid development and thus requires a large amount of energy sources to meet the pace of development and there is need of alternate source of green renewable energy. Today, mankind is almost totally dependent on the fossil fuels (coals, petroleum etc.) to provide electricity and transport fuel etc. These sources are, however, non renewable and may run out in the near future. Recently fuel derived from biomass has been receiving increased attention due to the availability of raw materials especially in tropical and temperate zones of world. Bio fuels are gaining increased public and scientific attentions; this can be due to factors such as oil price hike, the need for increased energy security, and concern over greenhouse gas emissions from fossil fuels.

Biodiesel can be produced either from edible or from non edible oils. Most of the edible oils are produced from the crop land. The use of non edible oils for bio diesel production has recently been of great concern because they are eco-friendly and cheap. Disadvantages of using bio diesel produced from agricultural crops involve additional land use, as land area is taken up and various agricultural inputs with their environmental effects are inevitable. Switching to bio diesel on a large scale requires considerable use of our arable area. If the same thing is to happen all over the world, the impact on global food supply could be a major concern. Currently, more than 95% of the world bio-diesel is produced from edible oil which is easily available on large scale from the agricultural industry. However, continuous and large-scale production of bio diesel from edible oil without proper planning may cause negative impact to the world, such as depletion of food supply leading to economic imbalance. A possible solution to overcome this problem is to use non-edible oil. As the demand for edible oils for food has increased tremendously in recent years, it is urgently required to justify the use of these non edible oils for fuel use purposes such as bio diesel production. Moreover, these oils could be less expensive to use as fuel. Hence, the contribution of non-edible oils such as Jatropha and karanja and Mahua will be significant as a non edible plant oil source for biodiesel production. Several studies have shown that there exists an immense potential for the production of plant based oil to produce biodiesel. Azam *et al*.(2005) studied the prospects of fatty acid methyl esters (FAME) of some 26 non-traditional plant seed oils including Jatropha to use as potential biodiesel in India. Among them, *Azadirachta indica*, *Calophyllum inophyllum*, *J. curcas* and *Pongamia pinnata* were found most suitable for use as biodiesel and they meet the major specification of biodiesel for use in diesel engine. Moreover, they reported that 75 oil bearing plants contain 30% or more oil in their seed, fruit or nut. (Subramanian *et al* 2005) reported that there are over 300 different species of trees which produce oil bearing seeds. Thus, there is a significant potential for non-edible oil source from different plants for biodiesel production as an alternative to petro diesel.

- Jatropha, a member of the *Euphorbiaceae* family, is an indigenous plant found in tropical and sub tropical part of America, Africa and Asia (Divakara *et al* 2010).

Jatropha genus account for 175 species with 12 species reported in India. Depending on the geographic location, its common names are Barbados nut Black; vomit nut, Curcas bean, *Kukui haole* Physic nut or *Jungle Erandi* (in India), Purge nut, *Purgeerboontjie*, and Purging nut tree (Barceloux, 2008). Among all species of Jatropha, *jetropha curcus* regarded as a nonedible oil crop which finds greater interest in biodiesel production. It is up to 8-15 feet tall tree. It is well adapted to both arid and semi-arid condition. Its oil content ranges from 35% in seed and 50-60% in kernel with oleic (C18:1) and linoleic (C18:2) as its major fatty acids (Ram *et al*, 2004). *Jatropha curcas* is a drought-resistant, pest and disease resistant, about 50 year life expectancy, can be grown in an adverse land situation, require minimum inputs for cultivation and contributes for eco-restoration on all types of wasteland(Siddharth *et al* 2010) .

It has small capsule like round fruit of 2.5-4 cm. Long which becomes dark brown when ripe, splitting of which release 2-3 black seeds of 2cm long (*phanerocotylar*).

Like other species of the Euphorbiaceae family, *J.curcas* also contain highly toxic poisonous substance curcin (a *phytotoxin- Toxalbumin*)(Felke J,1914).Cursing is a ribosome inactivating protein (RIP)(Barbieri *et al* 1993). It has Antihelminthic effect (Jummai *et al*, 2014).

According to National Biodiesel Mission (NBM) India,(Zhang.Y, *et al*, 2003) the nonedible oil seeds like Jatropha are most suited for biodiesel production in India, but unfortunately the seed yield from Jatropha tree is much more less than stipulated, then there is a need of alternate of Jatropha seed oil.

Mahua (*Madhuca indica*), a deciduous tree belongs to *Sapotaceae* family. It is found throughout the tropical and subtropical (mainly in central and north forest) region of the Indian subcontinent. It has socioeconomic values as about 30-40 percent of the tribal economy of India, primarily in northern India such as in Bihar, Madhya Pradesh and Orissa, are dependent on the Mahua flowers and seeds. Moreover, ***Madhuca indica* and *Madhuca longifolia*** are two important species of Mahua in India, whose seeds are used for extracting yellowish oil (Mahua butter) generally meant for soap production. Mahua flowers are edible, but largely used for producing countryside cheap alcoholic liquor in rural parts of India. Mahua Seed yield ranges from 20-200 kg per tree every year, where oil content is 30-45%.

Castor / Palma Christi or arand (*Ricinus communis*) is a species that belongs to the *Euphorbiaceae* family. It is a non-edible, poor soil resistant, a perennial oilseed crop that can be grown in tropical, subtropical (wild or cultivated), arid, semiarid region and even on marginal lands, which are not competitive with food production lands of the globe. It can withstand in diverse climatic conditions such as long period of draught, but will thrive under higher rainfall. Castor oil plant, actually originated from Africa, but spread out in many countries of the globe.

In India, it is grown on 713,000 hectares of rain fed land and it yields 850,000 tons of Castor seeds per year. Although, Castor is growing in nearly all provinces of India, but equally a matter of their production, Gujarat (83%) passes over other states followed by south Indian states. India exports 200,000 to 225,000 tonnes of Castor oil and about 15000 tonnes Castor seeds per year Castor seed comprise about 50 to 60 % non edible oil.

2 Materials and Methods

2.1 Extraction of oil from seeds of Jatropha, Mahua and Castor

(a) Seed collection:-

The seeds were locally collected from districts of Bihar. (Jatropha seeds from Purnia district, Mahua and Castor from Samastipur District of Bihar) for experimentation and extraction of oil.

(b) Drying:-

Seeds were cleaned properly and kernels removed. Kernels dried in an electric oven for 20 minutes at 65^{0}c to reduce moisture content(Mardhiah et al. 2017).

(c) Grinding:-

It was done by using a mortar pestle to rupture the cell wall so that solute release for direct contact with solvent.

(d) Weighing :-

On an electric balance weighed before and after the drying process using Metller weighing machine model no. ML 204 /AO1.

2.2 Experimental Procedure:-

For the extraction of oil soxhlet apparatus was used.

Procedure

Pretreated fine grinded seed's kernels were put in a known weight of thimble made up of whatmann filter paper no.40. Thimble contains 26.43 gm, 29.47 gm and 16.11 gm respectively of Jatropha, Mahua and Castor grind seeds. Then the thimbles filled with sample were put in the appropriate place inside soxhlet apparatus. 300 ml of n-hexane as solvent was measured using measuring cylinder and then poured into each three round bottom flask of 500ml capacity. Set the temp at 60^{0}c and heated for 6 hours. After that oil was recovered by solvent evaporation. Then recovered oil were again heated at a low temp to complete evaporation of solvent, leaving behind the solvent **(**Singh *et al* 2009)**.**

2.3 Characterization of extracted oil

Experiments were conducted to find out the fatty acid composition of *Jatropha, Mahua and Castor* seed oil extracted by soxhlet apparatus. Characterization of oil done using GCMS (Gas chromatography Mass Spectroscopy) PERKINELMER USA Model- CLARUS 600. GC/MS is the most popular chromatography mass spectrometry coupling technology, suitable for the analysis of vegetable oil feedstock for biodiesel as well as FAME analysis.

The Target substance enters into MS through GC and converted into gaseous ions through ionization source and then enter into the mass analyzer where ions with different e/m ratio, sequentially separated and enter into the electron multiplier, generating electrical signal, in order to give the 3D information of the target substances, making qualitative analysis more accurate by using ion fragment information. (Zhen Xue, *et al*, 2015).

3 Result and Discussions

The following results were obtained from the solvent extraction of seed oil.

Table: 1 Oil percentage of different seeds during experimentation

S. no	Sample oil	Amount of seed taken	Amount of oil extracted	Percentage
1	Jatropha	26.43gm	20.3ml	76.80%
2	Mahua	16.11gm	6.7ml	41.58%
3	Castor	26.47 gm	9.8ml	33.25%

The following chromatogram of oil sample were obtained by using GC – MS analysis

3.1 GC – MS analysis Jatropha Seed Oil:-

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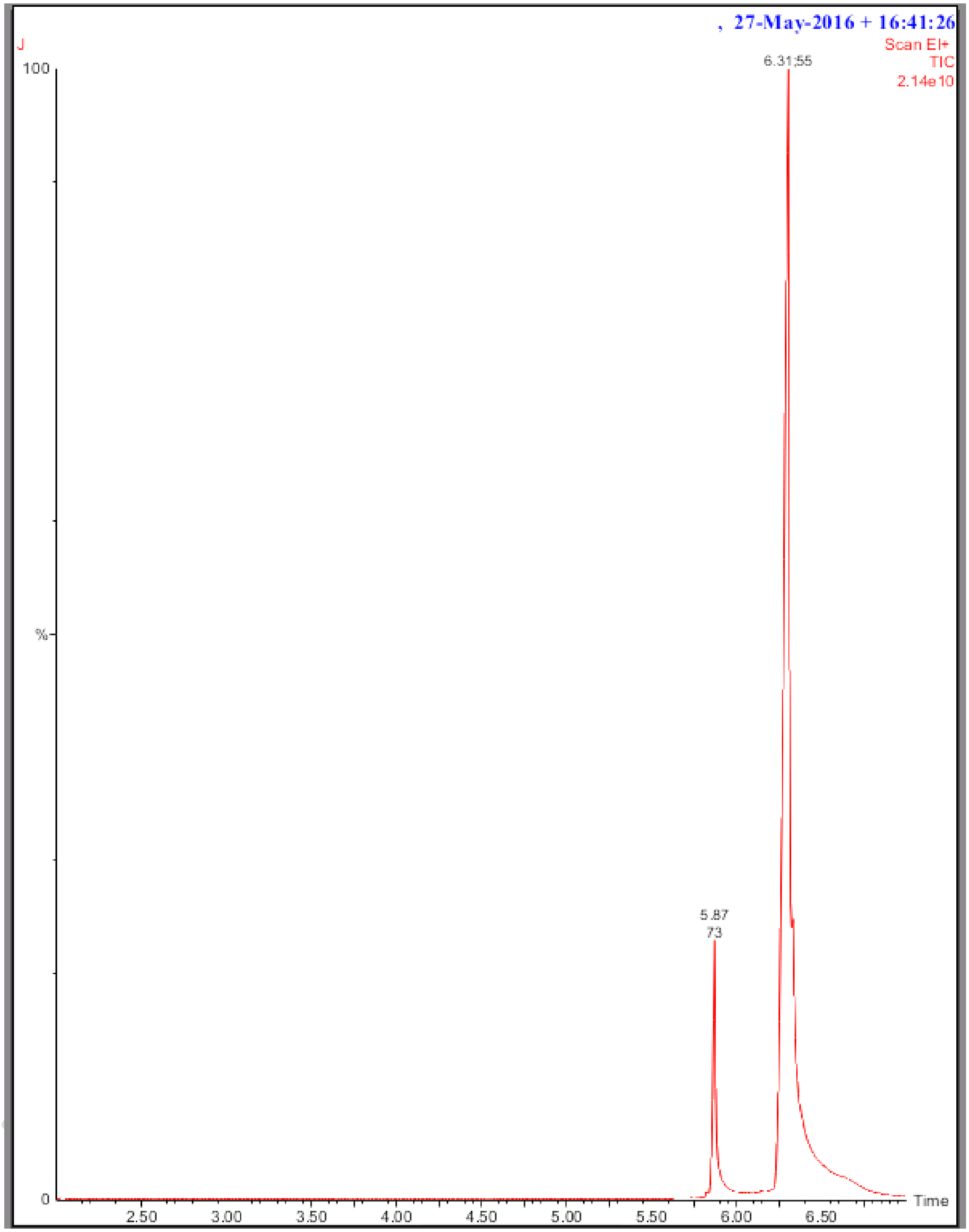
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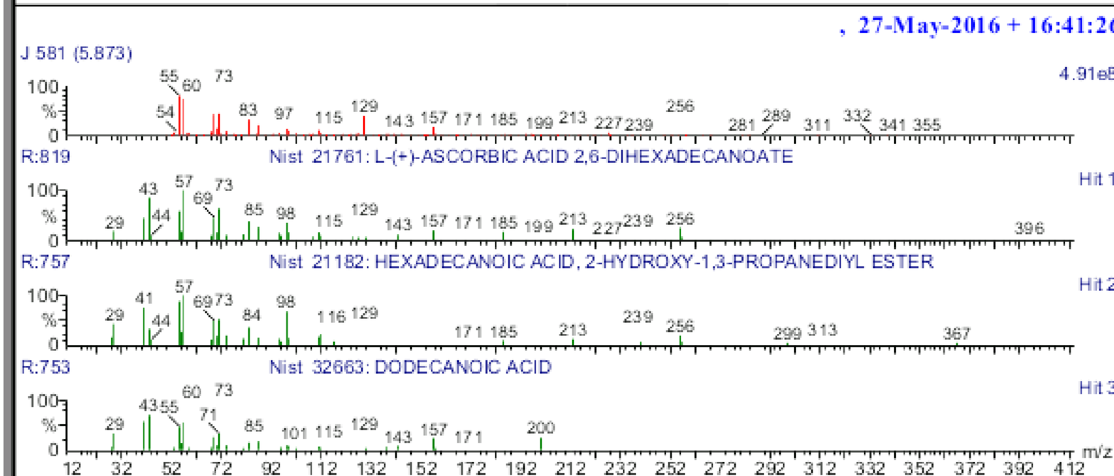
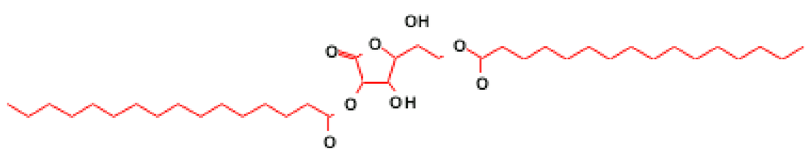
Hit	REV	for	Compound Name	M.W.	Form
1	819	776	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	652	C38H
2	757	689	HEXADECANOIC ACID, 2-HYDROXY-1,3-PROPANEDIYL ESTER	568	C35H
3	753	646	DODECANOIC ACID	200	C12H
4	750	625	UNDECANOIC ACID	186	C11H
5	748	667	EICOSANOIC ACID	312	C20H
6	746	603	PENTADECANOIC ACID, 15-BROMO-	320	C15H
7	745	650	TETRACOSANOIC ACID	368	C24H
8	741	647	HEPTADECANOIC ACID	270	C17H
9	734	582	11-BROMOUNDECANOIC ACID	264	C11H
10	728	604	NONANOIC ACID	158	C9H
11	726	627	TETRADECANOIC ACID	228	C14H
12	721	574	N-DECANOIC ACID	172	C10H
13	719	691	N-HEXADECANOIC ACID	256	C16H
14	718	640	OCTADECANOIC ACID	284	C18H
15	713	608	TRIDECANOIC ACID	214	C13H
16	712	568	PENTADECANOIC ACID, 14-BROMO-	320	C15H
17	705	534	12-BROMODODECANOIC ACID	278	C12H
18	701	634	HEXADECANOIC ACID, 1-(HYDROXYMETHYL)-1,2-ETHANEDIYL ESTER	568	C35H
19	685	615	PENTADECANOIC ACID	242	C15H
20	682	578	L-ASCORBIC ACID, 6-OCTADECANOATE	442	C24H





Hit	REV	for	Compound Name	M.W.	Form
1	848	776	(Z)-14-TRICOSENYL FORMATE	366	C24H
2	833	760	9-OCTADECENOIC ACID, 1,2,3-PROPANETRIYL ESTER, (E,E,E)-	884	C57H
3	831	783	OLEYL ALCOHOL	268	C18H
4	828	720	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	C37H
5	825	762	1,19-EICOSADIENE	278	C20H
6	825	700	9-HEXADECENOIC ACID, 9-OCTADECENYL ESTER, (Z,Z)-	504	C34H
7	812	693	ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-	312	C20H
8	809	711	13-DOCOSEN-1-OL, (Z)-	324	C22H
9	809	753	ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-	312	C20H
10	807	737	CIS-9-HEXADECENAL	238	C16H
11	806	716	1,10-HEXADECANEDIOL	258	C16H
12	805	756	CYCLODODECANEMETHANOL	198	C13H
13	801	739	13-OCTADECENAL, (Z)-	266	C18H
14	801	734	11-DODECEN-1-OL TRIFLUOROACETATE	280	C14H
15	797	732	TRICHLOROACETIC ACID, UNDEC-2-ENYL ESTER	314	C13H
16	796	722	Z-11-TETRADECEN-1-OL TRIFLUOROACETATE	308	C16H
17	794	677	OLEIC ANHYDRIDE	546	C36H
18	791	708	Z,E-2-METHYL-3,13-OCTADECADIEN-1-OL	280	C19H
19	790	717	11-DODECEN-1-OL DIFLUOROACETATE	262	C14H
20	788	706	1,21-DOCOSADIENE	306	C22H

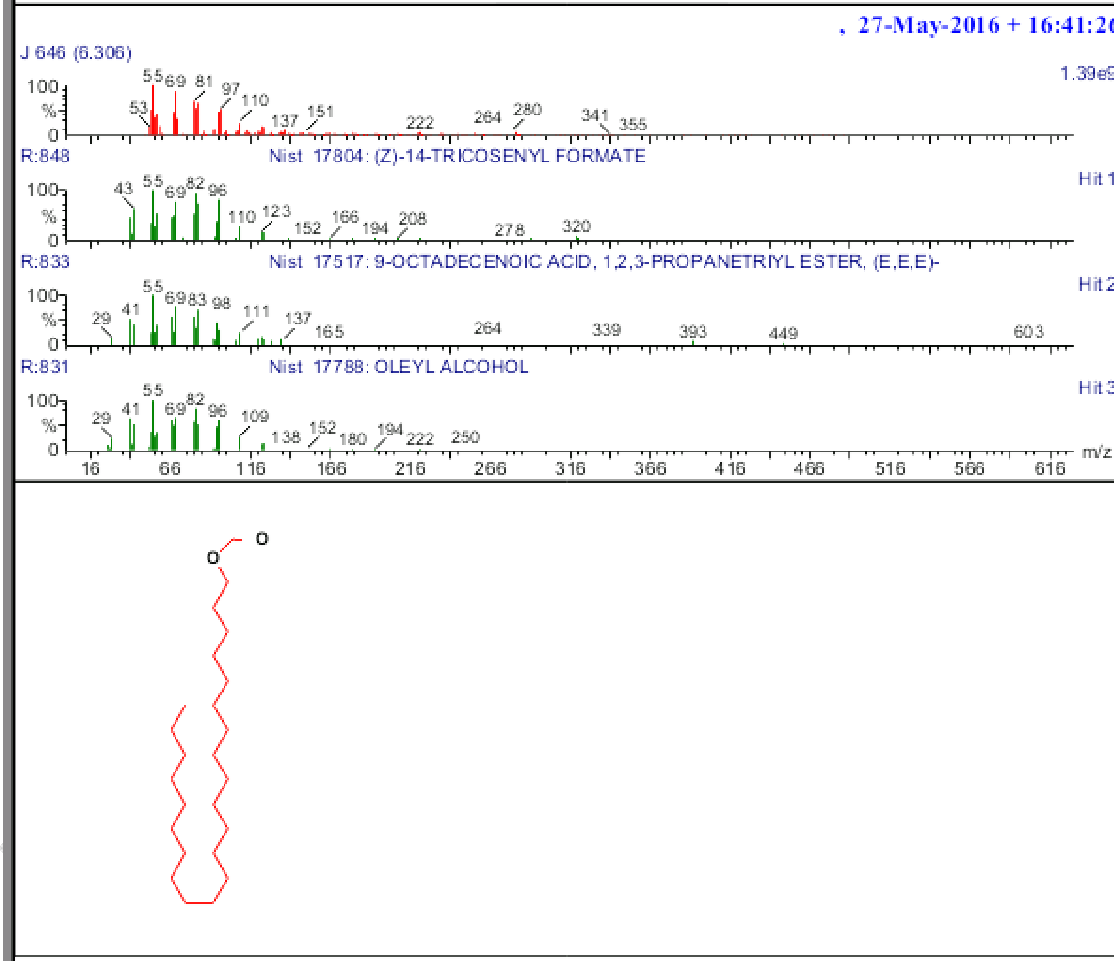
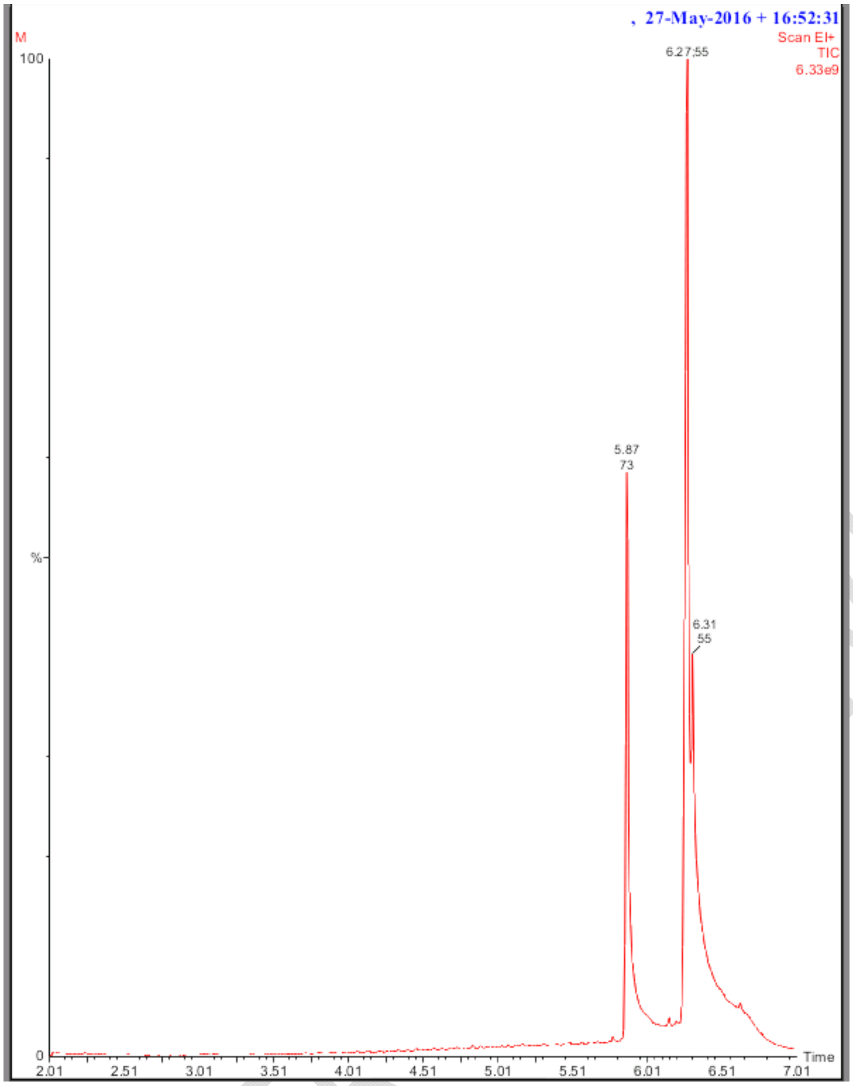


Fig 1, 2, 3: The Chromatogram of Jatropha seed oil.

3.2 GC-MS analysis of Mahua seed oil:-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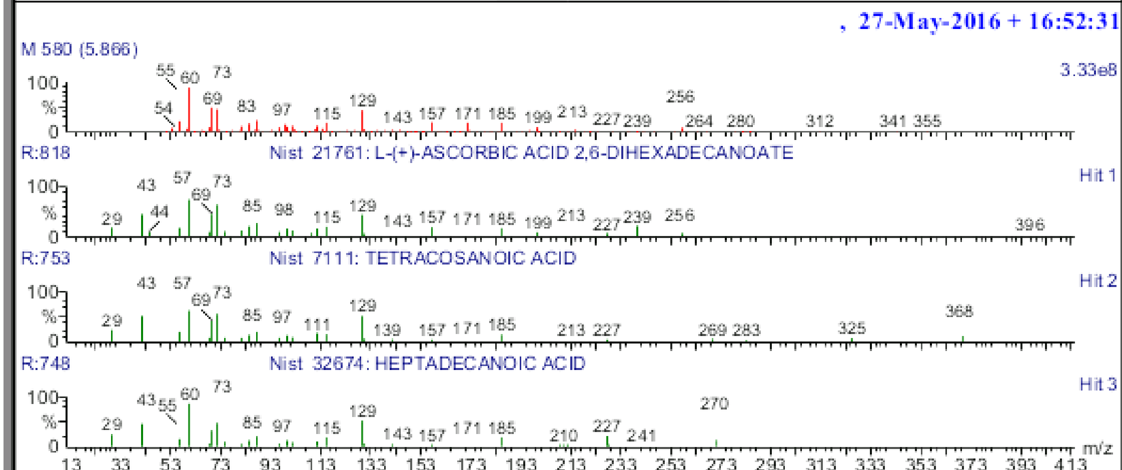
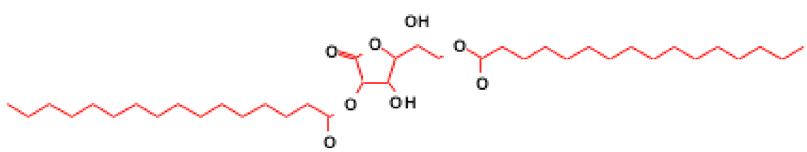
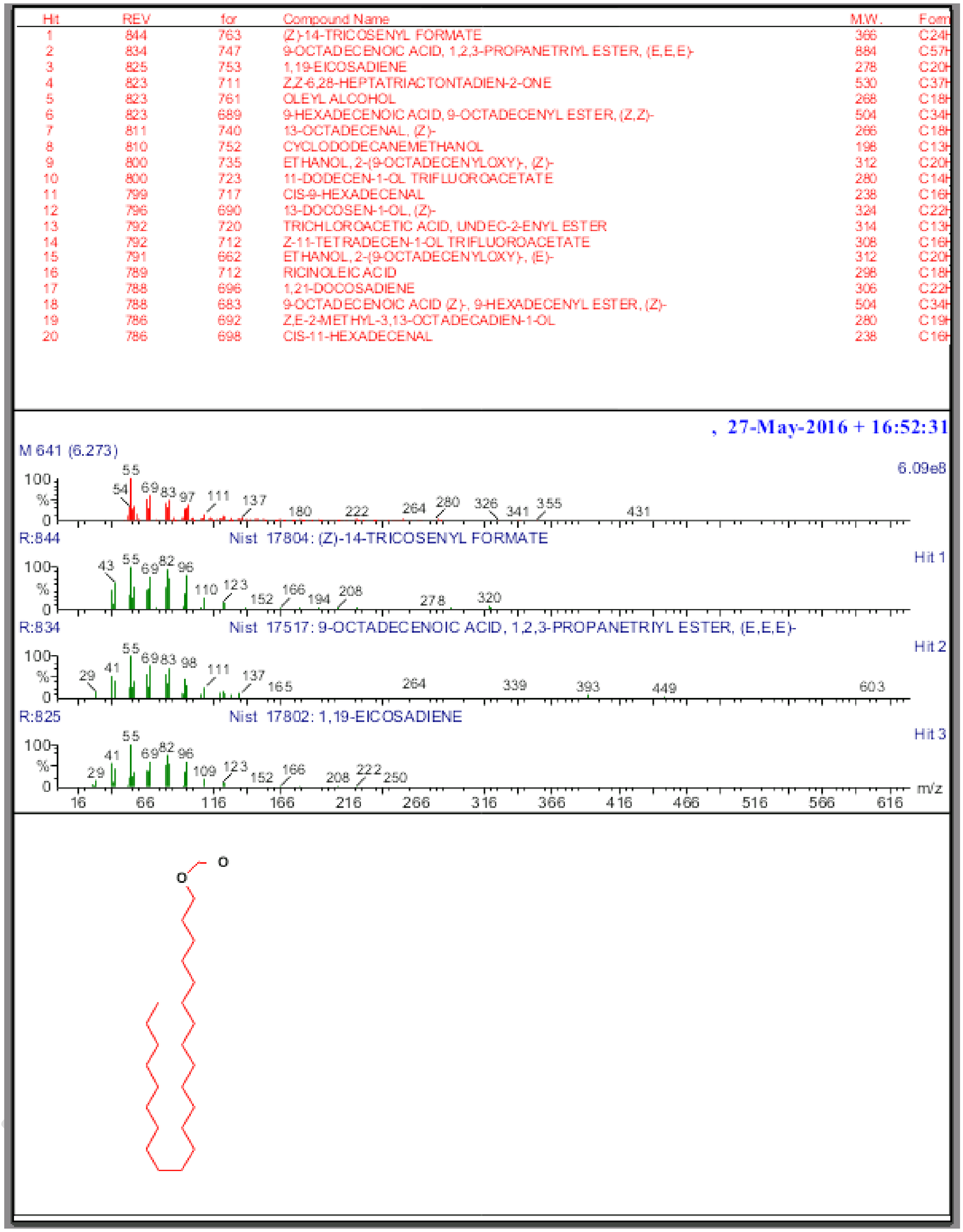
Hit	REV	for	Compound Name	M.W.	Form
1	818	773	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	652	C38H
2	753	653	TETRACOSANOIC ACID	368	C24H
3	748	649	HEPTADECANOIC ACID	270	C17H
4	748	659	EICOSANOIC ACID	312	C20H
5	745	594	PENTADECANOIC ACID, 15-BROMO-	320	C15H
6	740	618	DODECANOIC ACID	200	C12H
7	740	664	HEXADECANOIC ACID, 2-HYDROXY-1,3-PROPANEDIYL ESTER	568	C35H
8	735	594	UNDECANOIC ACID	186	C11H
9	722	559	11-BROMOUNDECANOIC ACID	264	C11H
10	721	610	TETRADECANOIC ACID	228	C14H
11	715	563	PENTADECANOIC ACID, 14-BROMO-	320	C15H
12	712	575	NONANOIC ACID	158	C9H
13	710	553	N-DECANOIC ACID	172	C10H
14	709	678	N-HEXADECANOIC ACID	256	C16H
15	708	616	OCTADECANOIC ACID	284	C18H
16	700	581	TRIDECANOIC ACID	214	C13H
17	692	509	12-BROMODODECANOIC ACID	278	C12H
18	691	618	PALMITIC ANHYDRIDE	494	C32H
19	683	571	L-ASCORBIC ACID, 6-OCTADECANOATE	442	C24H
20	679	605	HEXADECANOIC ACID, 1-(HYDROXYMETHYL)-1,2-ETHANEDIYL ESTER	568	C35H





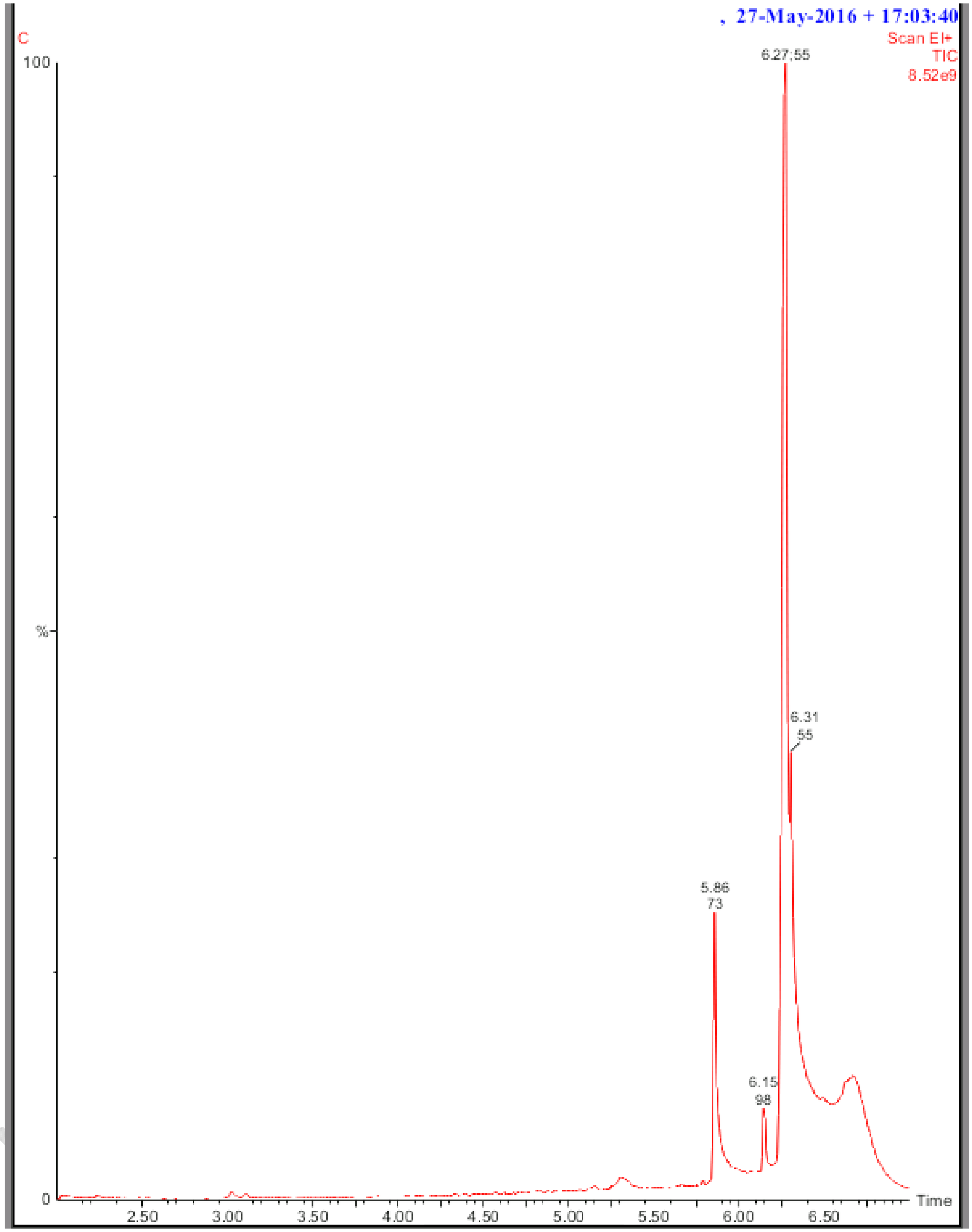


Hit	REV	for	Compound Name	M.W.	Form
1	844	763	(Z)-14-TRICOSENYL FORMATE	366	C24H
2	834	747	9-OCTADECENOIC ACID, 1,2,3-PROPANETRIYL ESTER, (E,E,E)-	884	C57H
3	825	753	1,19-EICOSADIENE	278	C20H
4	823	711	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	C37H
5	823	761	OLEYL ALCOHOL	268	C18H
6	823	689	9-HEXADECENOIC ACID, 9-OCTADECENYL ESTER, (Z,Z)-	504	C34H
7	811	740	13-OCTADECENAL, (Z)-	266	C18H
8	810	752	CYCLODODECANEMETHANOL	198	C13H
9	800	735	ETHANOL, 2-(9-OCTADECENYLOXY)-, (Z)-	312	C20H
10	800	723	11-DODECEN-1-OL TRIFLUOROACETATE	280	C14H
11	799	717	CIS-9-HEXADECENAL	238	C16H
12	796	690	13-DOCOSEN-1-OL, (Z)-	324	C22H
13	792	720	TRICHLOROACETIC ACID, UNDEC-2-ENYL ESTER	314	C13H
14	792	712	Z-11-TETRADECEN-1-OL TRIFLUOROACETATE	308	C16H
15	791	662	ETHANOL, 2-(9-OCTADECENYLOXY)-, (E)-	312	C20H
16	789	712	RICINOLEIC ACID	298	C18H
17	788	696	1,21-DOCOSADIENE	306	C22H
18	788	683	9-OCTADECENOIC ACID (Z)-, 9-HEXADECENYL ESTER, (Z)-	504	C34H
19	786	692	Z,E-2-METHYL-3,13-OCTADECADIEN-1-OL	280	C19H
20	786	698	CIS-11-HEXADECENAL	238	C16H

Fig 4, 5, 6: The chromatogram of Mahua seed oil.

3.3 GC-MS analysis of Castor seed oil:-

Experiments were conducted to find out fatty acid composition of Castor seed oil.



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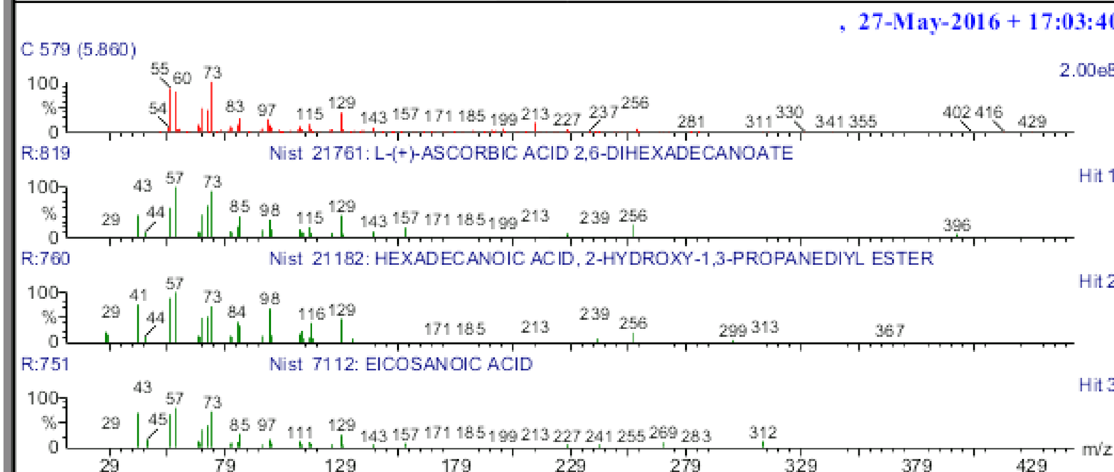
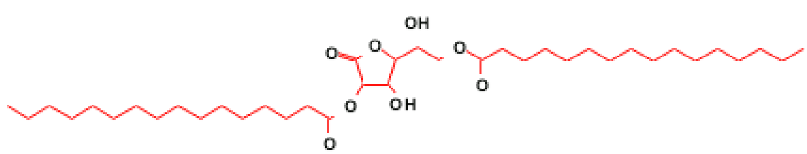
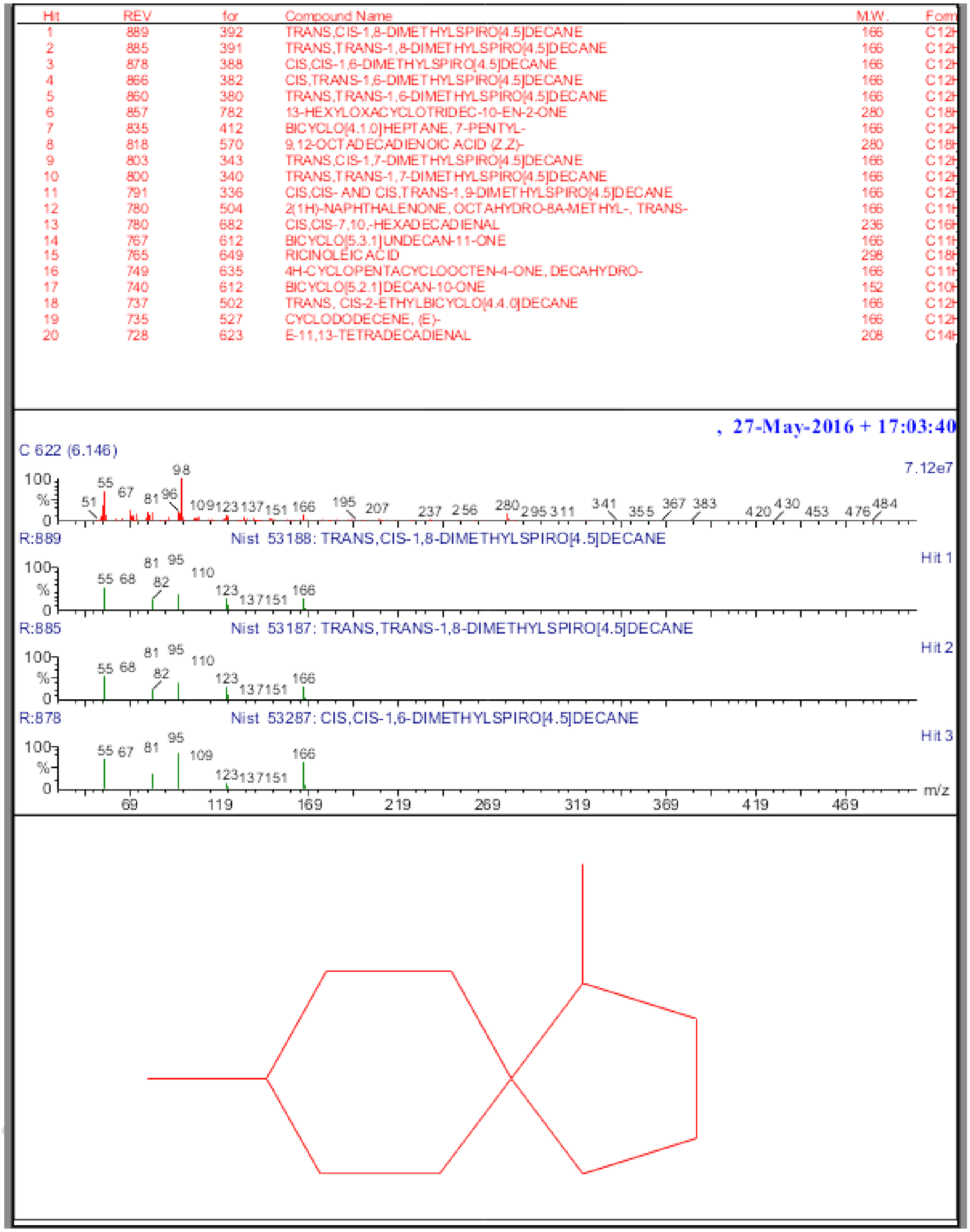
Hit	REV	for	Compound Name	M.W.	Form
1	819	769	L-(+)-ASCORBIC ACID 2,6-DIHEXADECANOATE	652	C38H
2	760	688	HEXADECANOIC ACID, 2-HYDROXY-1,3-PROPANEDIYL ESTER	568	C35H
3	751	669	EICOSANOIC ACID	312	C20H
4	750	644	DODECANOIC ACID	200	C12H
5	747	606	PENTADECANOIC ACID, 15-BROMO-	320	C15H
6	747	623	UNDECANOIC ACID	186	C11H
7	746	650	TETRACOSANOIC ACID	368	C24H
8	739	644	HEPTADECANOIC ACID	270	C17H
9	735	590	11-BROMOUNDECANOIC ACID	264	C11H
10	726	608	NONANOIC ACID	158	C9H
11	725	625	TETRADECANOIC ACID	228	C14H
12	721	690	N-HEXADECANOIC ACID	256	C16H
13	719	639	OCTADECANOIC ACID	284	C18H
14	717	575	N-DECANOIC ACID	172	C10H
15	716	578	PENTADECANOIC ACID, 14-BROMO-	320	C15H
16	712	606	TRIDECANOIC ACID	214	C13H
17	703	538	12-BROMODODECANOIC ACID	278	C12H
18	703	633	HEXADECANOIC ACID, 1-(HYDROXYMETHYL)-1,2-ETHANEDIYL ESTER	568	C35H
19	687	614	PENTADECANOIC ACID	242	C15H
20	683	576	L-ASCORBIC ACID, 6-OCTADECANOATE	442	C24H







Hit	REV	for	Compound Name	M.W.	Form
1	889	392	TRANS,CIS-1,8-DIMETHYLSPIRO[4.5]DECANE	166	C12H
2	885	391	TRANS,TRANS-1,8-DIMETHYLSPIRO[4.5]DECANE	166	C12H
3	878	388	CIS,CIS-1,6-DIMETHYLSPIRO[4.5]DECANE	166	C12H
4	866	382	CIS,TRANS-1,6-DIMETHYLSPIRO[4.5]DECANE	166	C12H
5	860	380	TRANS,TRANS-1,6-DIMETHYLSPIRO[4.5]DECANE	166	C12H
6	857	782	13-HEXYLOXACYCLOTRIDEC-10-EN-2-ONE	280	C18H
7	835	412	BICYCLO[4.1.0]HEPTANE, 7-PENTYL-	166	C12H
8	818	570	9,12-OCTADECADIENOIC ACID (Z,Z)-	280	C18H
9	803	343	TRANS,CIS-1,7-DIMETHYLSPIRO[4.5]DECANE	166	C12H
10	800	340	TRANS,TRANS-1,7-DIMETHYLSPIRO[4.5]DECANE	166	C12H
11	791	336	CIS,CIS- AND CIS,TRANS-1,9-DIMETHYLSPIRO[4.5]DECANE	166	C12H
12	780	504	2(1H)-NAPHTHALENONE, OCTAHYDRO-8A-METHYL-, TRANS-	166	C11H
13	780	682	CIS,CIS-7,10,-HEXADECADIENAL	236	C16H
14	767	612	BICYCLO[5.3.1]UNDECAN-11-ONE	166	C11H
15	765	649	RICINOLEIC ACID	298	C18H
16	749	635	4H-CYCLOPENTACYCLOOCTEN-4-ONE, DECAHYDRO-	166	C11H
17	740	612	BICYCLO[5.2.1]DECAN-10-ONE	152	C10H
18	737	502	TRANS, CIS-2-ETHYLBICYCLO[4.4.0]DECANE	166	C12H
19	735	527	CYCLODODECENE, (E)-	166	C12H
20	728	623	E-11,13-TETRADECADIENAL	208	C14H

Fig 7, 8, 9: The chromatogram of Castor seed oil.

4 Conclusion

Standard sampling and analytical techniques have been used to generate primary and secondary data by using instruments viz. Soxhlet apparatus and GC-MS. The data generated during this research work has been presented in to table 1-4. Jatropha, Mahua and Castor seeds were used for extraction of oil in which the best result was obtained from Jatropha seed. The percentage oil extracted from Jatropha oil was 76.80% followed by Mahua where the oil percentage was 41.58% and the minimum oil percentage i.e .33.25% in Castor. Oils of different species under investigation when exposed to open air and sunlight for a long time would affect the fatty acid concentration. Most common acid among three investigated oil sample Ascorbic acid2,6 Dihexadecanoate was most prominent in terms of concentration followed by Tetracosanoic acid and Heptadecanoic acid.Among three oil samples, ricinoleic acid was only found in Castor oil.

Fatty acid composition of different species was studied by using GC MS .The important fatty acid produced in GC MS of Jatropha oil were Ascorbicacid2,6 Dihexadecanoate , Hexadecanoic acid, Eicosanoic acid, Dimethyl spiro decane having molecular weight 652,568,312 and 166 respectively. The important fatty acid produced by Mahua oil were Ascorbic acid2,6 Dihexadecanoate , Tetracosanoic acid, Heptadecanoic acid, Tricosenyl formate and Octadecanoic acid having molecular weight 652,368,270,366 and 884 respectively. Similarly the fatty acid composition of Castor oil were Ascorbic acid2,6 Dihexadecanoate, Tetracosanoic acid, Heptadecanoic acid, Tricosenyl formate, Octadecanoic acid having molecular weight 652,568,312 and 166 respectively.

Oil from each origin has its own special characteristics. The oils with good physicochemical properties like Density, Specific gravity, refractive index, acid value, iodine value, saponification value will have potential to be biodiesel feedstocks.

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