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ANALYSIS OF FIXED OIL FROM *STERCULIA FOETIDA* LINN.

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Sterculia foetida L. (Sterculiaceae) seeds, GC-MS, FAME, Fatty acids, Fixed oil

Abbreviations:
C- Carbon, GC-MS- Gas chromatography-Mass spectroscopy, FAME- Fatty Acid Methyl Ester, M-Molarity

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ABSTRACT

Analysis of fatty acid composition in fixed oil extracted from *Sterculia foetida* Linn. (Sterculiaceae) seed was done with the help of GC-MS. The structural identification was done by preparing FAME of the fixed oil and then subjected to GC-MS wherein Flame Ionization Detector was used. The oil constituted of 7 fatty acids whose carbon chain length, degree and position of unsaturation were determined from the characteristic ionization and fragmentation of FAME resulting from GC-MS electron impact, chemical ionization modes. The fatty acids found in the oil were methyl esters of tetradecanoic acid (Myristic acid) (1.65%), hexadecanoic acid (Palmitic acid) (11.87%), 9-Octadecenoic acid (Oleic acid) (20.50%), 7, 10-Octadecadienoic acid (Linoleic acid) (20.50%), 8-(2-Octacyclopropen-1-yl)octanoic acid (Sterculic acid) (6.76%).

INTRODUCTION: The vegetable oils are important for agriculture and food industry. India is fortunate in having a wide range of oilseeds crops grown in its different agro climatic zones. Groundnut, mustard/rapeseed, sesame, safflower, linseed, castor are the major traditionally cultivated oilseeds. Soyabean and sunflower have also assumed importance in recent years.

Coconut is most important amongst the plantation crops for oil. Among the non-conventional oils, rice-bran oil and cottonseed oil are the most important. In addition, oilseeds of tree and forest origin, which grow mostly in tribal inhabited areas, are also a significant source of oils. These oils their production and use is based on a wide range of supporting sciences like physics, chemistry, biochemistry, agriculture, seed breeding, molecular biology, engineering, food science, nutrition, and medicine among others [1]. Although many of the plants have been extensively investigated for its fatty acid profile, the need still remains for investigation of fatty acid profile of under-studied plants containing minor oils.

Sterculia foetida L. is a tropical plant belonging to the Sterculiaceae family which is also called as 'Java-Olive', 'Bastard poon tree', '*Hazel sterculia*', 'Skunk tree', 'Poon tree' and 'Sam-rong' in Thai. In India it is known as 'Janglibadam' (Hindi, Bengali), 'Gorapu-badam' (Tamil). It is a large tree growing up to 4m in height and 3m in girth, with the branches arranged in whorls and spreading horizontally.

It is found tropical zone in Europe, Africa, and Asia. It is native in Australia, India, Bangladesh, Pakistan, Sri Lanka, Myanmar Indonesia, Kenya, Malaysia, Thailand, Philippines, Somalia, Tanzania and its a semi-autonomous part Zanzibar, Uganda, Yemen, Republic of Djibouti, Eritrea, Ethiopia in African continent and Ghana, Puerto Rico are the region where it grows exotically [2, 3]. In India, it is found usually in the western and southern parts of India. According to Germplasm Resources Information Network, Department of Agriculture United States thirty-three species are found in this family and basic distinguishing factor are seeds and leaves [4, 5].

The seeds of *Sterculia foetida* L. (Sterculiaceae) are of exalbuminous type which has starchy cotyledons and are straight with a small radical [6]. It is numerous (10-15), 3-4 inch long, ellipsoid, oblong, 1.5-1.8 cm slate-colored with yellow caruncle on one side at the base. The seeds have a pleasant taste and are sometimes eaten. Edible oil called Sterculia oil is present in the testa as well as the kernel. The total oil content is about 34%.

Sterculia foetida L. (Sterculiaceae) seed, its oil, leaves bark and gum have considerable medicinal value; the leaves of this plant are used as herbal medicine as aperient, diuretic and as insect repellant. Decoction of wood boiled with seed oil is said to be employed in rheumatism. A gum that resembles 'gum tragacanth' is obtained from the trunk and branches and is used for bookbinding and similar purposes. In Ghana, seeds are taken as a purgative. Oil from the seed is extracted on a local scale to be used in medicine internally in itches and other skin diseases and is applied externally as a paste [7, 8, 9]. It is used locally for illuminating purpose[2]. Seed oil also exhibits activities like antifungal[10], insecticide, antibiotic, antiviral, hormonal, carcinogenic or antitumoral. The defatted kernels are flavored like cacao, but are not bitter and are used to adulterate Cacao [2].

The seeds reported to contain fixed oil, proteins, saponins, carbohydrates, phytosterols, gums and mucilaginous substances [11]. *Sterculia foetida* oil has been found to contain 71.8% of sterculic acid and minor proportions of oleic, linoleic, and saturated acids [12]. The saturated component consists mostly of myristic and palmitic acids.

The oil consists of traces of a glyceryl ester of stearic acid, tristearin (0.8%) and tristerculin (31.4%) together with different amounts of the glycerides of the type GS~U, GS~2, and GU3 of other fatty acids. As the presence of cyclic fatty acids containing three carbons carbocyclic rings namely cyclopropane and cyclopropene fatty acids were reported in the previous work of Sterculia oil this study was, therefore, focused on characterization of fixed oil and its comparison with *Sterculia foetida* L. (Sterculiaceae) species of Thailand which is commonly known as *Sam-rong* seed [13, 14, 15].

MATERIALS AND METHODS:

Plant material: The plant specimen was collected near the areas of '*Peth taluka'*, Dist. Nashik. The specimen was then identified and authenticated at Botanical Survey of India, Koregaon Park, Pune. The herbarium was deposited with Reference No.VIJSTEF3 on 01/2011 as *Sterculia foetida* Linn. Family Sterculiaceae.

Extraction of Fixed Oil: Dried seeds of *Sterculia foetida* L. (Sterculiaceae) were cleaned and powdered. The powdered drug (50g) was then subjected to Soxhlet Extraction with Petroleum Ether 60-80 (200ml). Extraction was done till sufficient amount of oil (16.22g) was obtained [16].

Preparation of Fatty Acid Methyl Esters (FAMEs): 1g of oil was taken in 250 ml RBF, to which 12ml of 0.5M methanolic NaOH was added and subjected to reflux for 45-50 minutes. 15ml BF_3- Methanol was then added from top of the condenser and then again boiled for 20-25 minutes. 4-5ml of Petroleum Ether 60-80 was then added through the condenser, heated for 3-5 minutes, heating was stopped immediately and 12ml saturated NaCl was added. The mixture was shaken vigorously for 1 minute, additional NaCl was then added so that petroleum ether solution floats into the neck of flask. FAMEs were collected by means of syringe and kept in closely tight glass vial in refrigerator [17].

GC-MS analysis: Analysis of Fixed Oil obtained from *Sterculia foetida* L. (Sterculiaceae) was done by preparing FAME.

Operating Parameters: For GC-MS measurements, instrument Hewlett Packard G 1800 GC D Series with a capillary column HP-5. The carrier gas was helium (99.9), Flow rate: 1ml/min, Injection volume: 1µl

injector temperature was 250°C, interface heating was 280°C, ion-source heating 250°C, EI-mode was 70eV. Column oven temperature was initially maintained at 100°C and then programmed at the rate of 10°C up to 200°C for 3 minutes, then again programmed at rate of 10°C up to 250°C for 3 minutes and finally raised up to 280°C for 3 minutes. Injection mode was manual with temperature and pressure maintained at 200°C and 64.1 kPa respective with the split ratio of 20.0 Mass spectra correlations were done using NIST library with the help of HPCHEM software and published mass spectra [18].

RESULT: The Sterculia seed oil is a clear fluid, pale yellow in color with a faintly sweet nutty odor. The characteristics and fatty acid composition of the oil are presented in **Table 1**.

TABLE 1: ANALYTICAL DATA ON *STERCULIA FOETIDA* LINN. SEEDS AND OIL

Sr. no.	Property	Value
I	**Composition of seed**	
1	Moisture	9.2%
2	Oil	32.44%
II	**Oil characteristics**	
1	Acid value	7.42
2	Iodine value	142.96
3	Saponification value	154.78
4	Unsaponifiable matter	1.11%
III	**Fatty acid composition (Area %)**	
1	14:0	1.65%
2	16:0	11.87%
3	18:1	20.50%
4	18:2	12.86%
5	Sterculic acid	6.76%

The GC-MS analysis for FAME of *Sterculia foetida* L. (Sterculiaceae) seed oil showed that the oil consisted of 7 fatty acids (**Table 2**).

TABLE 2: LIST OF FATTY ACIDS PRESENT IN *STERCULIA FOETIDA* L. (STERCULIACEAE) SEED OIL BY GC-MS ANALYSIS

Saturated fatty acids	
Tetradecanoic acid, methyl ester (Myristic acid)	(1.65%)
Hexadecanoic acid, methyl ester (Palmitic acid)	(11.87%)
Heptadecanoic acid, 14-methyl, methyl ester (margaric acid)	(2.28%)
Unsaturated Fatty acids	
9-octadecenoic acid (Z), methyl ester (Oleic acid) is a monounsaturated omega-9 fatty acid	(20.50%)
7, 10-Octadecadienoic acid, methyl ester (Linoleic acid) is an unsaturated n-6 fatty acid	(12.86%)
Cyclopropene acids	
8-(2-Octacyclopropen-1-yl)octanoic acid (Sterculic acid)	(6.76%

Presence of Hexanedioic acid, 3, 4-dimethyl, ester (3R, 4S) (3, 4-Dimethyladipic acid dimethyl ester) and 6-Nonenal, (Z) were non fatty components.

DISCUSSION: Based on GC-MS chromatogram and library search data, the FAME of *Sterculia foetida* L. (Sterculiaceae) exhibited 9 compounds (**figure 1**). The major components were found to be tetradecanoic acid, methyl ester, hexadecanoic acid, methyl ester, 6-Nonenal, (Z), 9-Octadecenoic acid, methyl ester, 7, 10-octadecadienoic acid, methyl ester with retention times as 10.31, 10.40, 10.45, 11.77, 11.88 respectively (**Table 3**).

TABLE 3: RESULT OF FAME OF *STERCULIA FOETIDA* L. (STERCULIACEAE) BY GC-MS ANALYSIS

Name of compound	Mol. formula	Mol. wt	Retention time (min)	Peak area (%)
Tetradecanoic acid, methyl ester (Myristic acid),saturated fatty acid	$C_{15}H_{30}O_2$	242. 3975	10.310	1.646
Hexadecanoic acid, methyl ester, Palmitic acid, saturated fatty acid	$C_{17}H_{34}O_2$	270.4507	10.402	11.868
6- Nonenal, (Z) An ingredient of aroma. Odor description: powerful, fresh, fruity, melon.	$C_9H_{16}O_2$	140.2227	10.452	20.927
Hexanedioic acid, 3,4-dimethyl, ester 3R,4S)-3, 4-Dimethyladipic acid dimethyl ester	$C_8H_{13}O_4$	171.20	10.599	8.569
9-octadecenoic acid (Z), methyl ester. Oleic acid is a monounsaturated omega-9 fatty acid	$C_{19}H_{36}O_2$	296.4879	11.770	20.497
7,10-Octadecadienoic acid, methyl ester. Linoleic acid (LA) is an Dienoic unsaturated n-6 fatty acid.	$C_{19}H_{34}O_2$	294.4721	11.880	12.859
Heptadecanoic acid, 14-methyl, methyl ester. Margaric acid, is a saturated fatty acid	$C_{19}H_{36}O_2$	296.4879	12.070	2.278
1-Azuleneethanol, acetate	$C_{14}H_{14}O_2$	214	15.489	4.22
8-(2-Octacyclopropen-1-yl)octanoic acid. Sterculic acid. cyclopropene acids	$C_{19}H_{34}O_2$	294.47	14.59	6.76

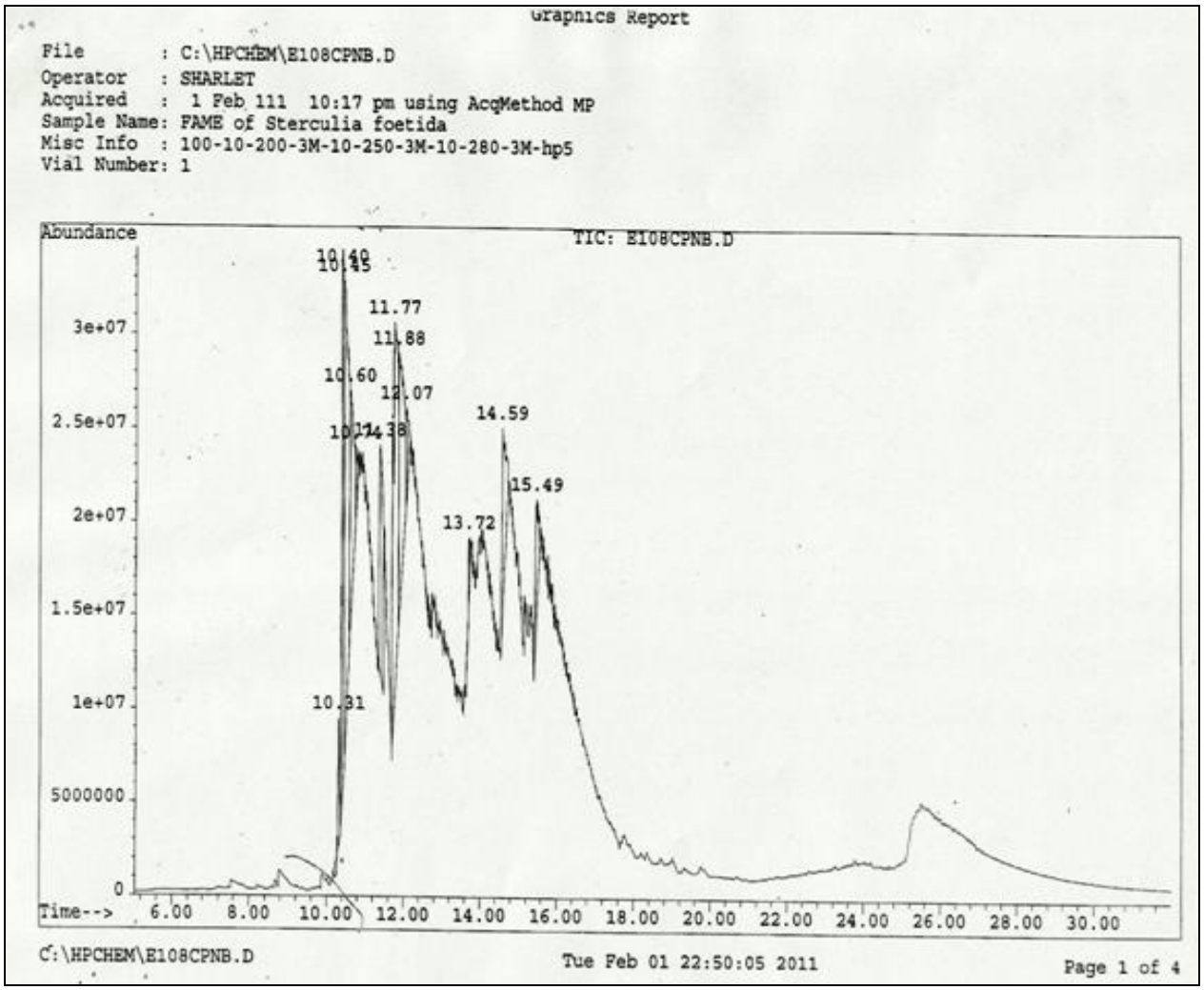

FIG. 1: CHROMATOGRAM OF FAME OF *STERCULIA FOETIDA* L. (STERCULIACEAE) SEED OIL

The oil exhibited marked tendency towards polymerization as GC-MS shows presence of Hexanedioic acid, 3, 4-dimethyl, ester (3R, 4S)-3, 4-Dimethyladipic acid dimethyl ester. Similarly, presence of 6- Nonenal, (Z) represents an azulene skeleton are found in nature as an ingredient of aroma with powerful, fresh, fruity, odor.

The major saturated fatty acid of *Sterculia foetida* L. (Sterculiaceae) seed oil was Hexadecanoic acid, methyl ester (Palmitic acid) (11.868%), followed by Heptadecanoic acid, 14-methyl, methyl ester (margaric acid), (2.278%), Tetradecanoic acid, methyl ester (Myristic acid) (1.468%) with retention time 10.40, 12.07, 10.31 respectively. The total saturated fatty acids amounted up to 15.80%

The major unsaturated fatty acid of *Sterculia foetida* L. (Sterculiaceae) seed oil was 9-octadecenoic acid (Z), methyl ester (Oleic acid) (monounsaturated omega-9 fatty acid) (20.497%) followed by 7, 10-Octadecadienoic acid, methyl ester (Linoleic acid) is an (Dienoic unsaturated n-6 fatty acid) (12.859%) with retention time 11.77, 11.88 respectively. The total unsaturated fatty acids amounted up to 33.3536%.

The ratio of total unsaturated fatty acids and saturated fatty acids was about 2.110.

Palmitic acid was reported as dominant fatty acid (52%) of *Sam-rong* seed oil but in Indian seed oil it was found to be 11.87%. Sterculic acid was found around 10% in *Sam Rong* seed oil while in Indian seed oil, it was 6.76%. Mass spectra of saturated fatty acids are especially interesting and that of palmitic acid (**Figure 2**). The most abundant peaks are at m/z = 87, the McLafferty rearrangement ion, and 74 in the lower molecular weight range.

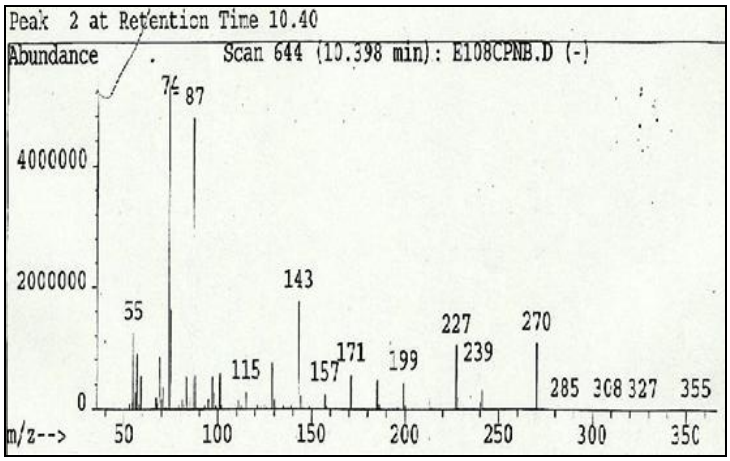

FIG. 3: MASS SPECTRUM FOR HEXADECANOIC ACID METHYL ESTER (PALMITIC ACID)

The peak m/e 74 which is characteristic of most methyl esters, and a series of peaks of ions corresponding to $(CH_2)_nCO_2CH_3^+$. The molecular ion is clearly abundant, and there are ions representing fragmentations between methylene groups of the form $[HOOC(CH_2)_n]^+$ from m/z = 115 to 270. An ion at m/z = 239 ($[M-17]^+$) presumably reflects a loss of OH^- from the carboxyl group.

The base peak of the spectrum of methyl palmitate (I) is m/e 74. This fragment results from a Mc-Lafferty rearrangement, which transfers a γ-hydrogen atom of the acid moiety to the carbonyl oxygen through a cyclic transition state, and cleaves the C_2-C_3 bond to give olefin II and ion III. The peak at M-43 in the spectra of fatty esters results from a loss of a propyl radical from the molecular ion to form a fragment of the type $(CH_2)_nCO_2CH_3^+$ (**Figure 3**) [20, 21].

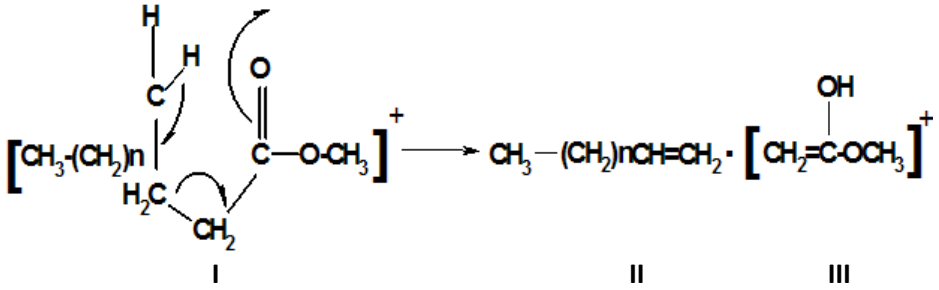

FIG. 3: FRAGMENTATION BY MC-LAFFERTY REARRANGEMENT

Relative ion abundances of observed ions from ionization of Palmitic acid:

57(95.7), 59(1.8), 73(100), 87(32.2), 97(14), 101(9.0), 115(14.0), 129(49.0), 143(7.5), 157(12.6), 171(13.7), 185(14.8), 199(3.9), 213(14.6), 227(2.2), 256(26.4)

Relative ion abundances of observed ions from ionization of Myristic acid:

55(100.0), 59(3.0), 60(71.5), 69(41.7), 73(80.5), 83(20.9), 87(79.8), 97(11.6), 101(9.4), 115(14.7), 129(92.2), 143(14.1), 157(6.1), 171(11.6), 185(35.4), 199(2.6), 228(19.6)

The spectra of unsaturated esters are complicated, as isomeric unsaturated esters have similar spectra except for α, β- unsaturated esters. The double bond in a long-chain unsaturated hydrocarbon or ester is also believed to be mobile upon electron impact; and therefore the mass spectrum cannot reveal the original site of unsaturation. In contrast to saturated fatty acids, in the oleic acid the ion representing the loss of the elements of water from the carboxyl group ([M-18]+, m/z = 264) is more abundant than the molecular ion.

The McLafferty ion at m/z = 60 is relatively small. The spectrum of linoleic acid is also dominated by hydrocarbon ions in the low mass range. By considering the high molecular weight part of the spectrum we could observe a good molecular ion (m/z = 369), useful confirmation that we have a C18 fatty acid with three double bonds. Then there is a uniform series of ions 14 atomic mass units (amu) apart, representing loss of each successive methyl and methylene group from the terminal end of the molecule, until we reach the ion at m/z = 298.

Then there is a gap of 26 amu for the carbons constituting the terminal double bond to m/z = 272, a further gap of 14 amu for the methylene group at carbon-11, then another gap of 26 amu between m/z = 234 and 258, a gap of 14 amu for the methylene group at carbon-8, and so forth.

The mass spectrum of 7, 10-Octadecadienoic acid, methyl ester (Methyl linoleate) (**Figure 4**) shows abundant molecular ion (m/z = 294) and prominent ions for loss of methanol and of the McLafferty ion (m/z = 263 and 220, respectively) [19]. There is little evidence for any ions that might serve to locate the double bonds, although it does appear that the ion at m/z = 150 is more abundant in methylene-interrupted polyenoic fatty acids with the (n-6) or ω6 structure [20].

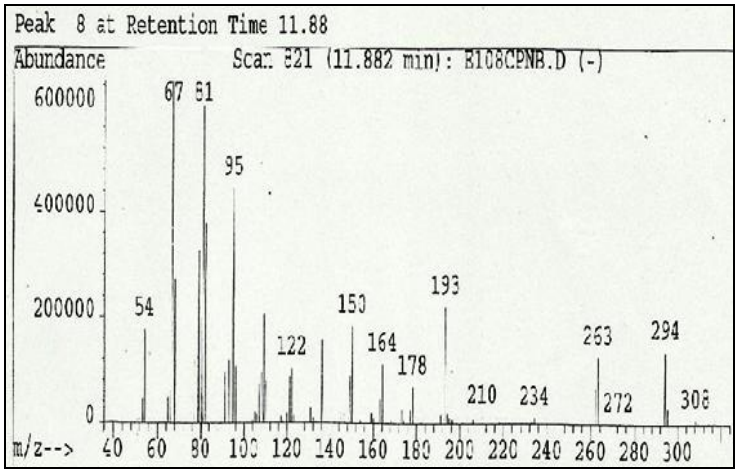

FIG. 7: MASS SPECTRUM FOR 7, 10-OCTADECADIENOIC ACID METHYL ESTER (LINOLEIC ACID)

GC-MS of the FAME gave the expected spectra for the diketo methyl esters from sterculic acids, respectively, but the mass spectrum for the Sterculic acid was inconclusive. The occurrence of Cyclopropenoid Fatty Acids (CPFA) in the oil was established by the Halphen test which gave a deep cherry-red color.

CONCLUSION: Polyunsaturated fatty acids of the n-9 family are less common in nature, but they are important biologically and especially in essential fatty acid deficiency in animals. As herbal products are expanding widely at faster rate in the market, it has become a basic need to study the plants, their constituents and thereby its use in a pharmaceutical and cosmeceutical field.

Therefore, this research work was performed in order to study the plant *Sterculia foetida* L. (Sterculiaceae) for which identification was done with the help of GC-MS. The report obtained by GC-MS analysis showed 7 fatty acids in the plant which may prove useful in future for preparation of medicinal, therapeutic or cosmetic product. Most of there components have prove to be useful for pharmaceutical and cosmeceutical purpose (Table 3). Considering this, a formulation of Hair Fixing Gel is prepared from the oil obtained out of *Sterculia foetida* L. seeds.

In conclusion, this work has presented the general properties of *Sterculia foetida* L. (Sterculiaceae) oil and its fatty acid profile. It turned out that *Sterculia foetida* L. (Sterculiaceae) oil could be a good source of natural oil rich in linoleic acid. This work might be useful for exploring the applications of *Sterculia foetida* L. (Sterculiaceae) bran and its oil.

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