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# GCMS ANALYSIS OF AN UNIQUE BIHERBAL EXTRACT

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## ABSTRACT

*In this study, traditionally significant medicinal plants Ficus religiosa (Linnaeus.) and Ficus racemosa (Linnaues.) were identified and authenticated. Stem powder of Ficus religiosa (Linn.) and bark powder of Ficus racemosa (Linn.) were mixed together to form a raw bi herbal powder. Using ethanol, extraction of raw biherbal powder was done for seven consecutive days by steady state cold maceration method. Residue obtained due to rotaevaporation was subjected to Gas Chromatography Mass Spectrometry (GCMS) analysis. Fourteen compounds were identified with their respective retention times and percentage of Probability. In the light of the earlier literature on the bioassays of the above individual explants of bi-herbal extract, future research and application on this bi-herbal extract or its purified fractions can be beneficial.*

## KEY WORDS

*Ficus religiosa L., Ficus racemosa L., Bi-herbal extract, Maceration, GCMS, anti-microbial activity.*

*Abbreviations:* **BA**, Bhagavathi Ana; **°C** , degree centigrade; **Fig,** Figure; **g,** gram; **HP-5MS,** High Performing- (5%phenyl- 95% methylpolysiloxane; **Linn,** Linnaeus; **m,** meter; **µm,** micrometer; **µl** , microliter; **min,** minutes; **ml,** milliliter; **mL/min,** milliliter per minute; **NI,** Nitrogen; **NIST**, National Institute of Standards and Technology; **psi**, pounds per square inch; **UGC MRP**, University Grants Commission Major Research Project; **ver.,** Version 2.

## 1. INTRODUCTION

Traditional knowledge is employed to mean knowledge, innovations and practices of indigenous and local communities (niscair.res.in 2018). It has been a predominant practice that as new chemical entities become hard to find, scientists across the world, often goes back to earlier substances to find novel properties (Sulekha and Savitha 2011). Several members of the genus *Ficus* (family: Moraceae) are being used traditionally in a wide variety of ethno medical remedies (Veerapur et al., 2009). According to Indian Traditional Knowledge the plant species governing the planet Jupiter is *Ficus religiosa* and Venus is *Ficus racemosa,* as referred in a traditional Sanskrit verse '*Aswatham Brihaspathaye, Audumbarag Shukraya*'(Chandrakanth et al.,1999).In Vedic culture these two plants are medicinally potential and their representing planets are mythologically very significant celestial entities. The *Ayurvedic* literature '*Sarangdhara Samhita*' highlighted the concept of polyherbalism to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects. When combining the multiple herbs in a particular ratio, it will give a better therapeutic effect and reduce the toxicity (Subramani P et al., 2014). Gas chromatography (GC) is a procedure in which the volatile components of a mixture are separated by partitioning between a moving, inert gas (carrier gas) and a non-volatile liquid (the stationary phase). Mass spectrometer is an instrument which creates and records weights and relative abundance of the fragments (Stephen,1980). In this article a biherbal extract made of above plants which was not reported earlier, was subjected to Gas Chromatography – Mass Spectrometry analysis for identification of compounds. Also, the type of GCMS

column employed was for the first time used for analysis of such biherbal extract.

## 2. MATERIALS AND METHODS

### 2.1 Materials:

*Ficus religiosa L., (Figure 1.0, 1.1 and 1.2)*; *Ficus racemosa* L. (Figure 2.0, 2.1 and 2.2) were identified at the field using standard keys and descriptions.

**Fig 1.0 *Ficus religiosa* L.**

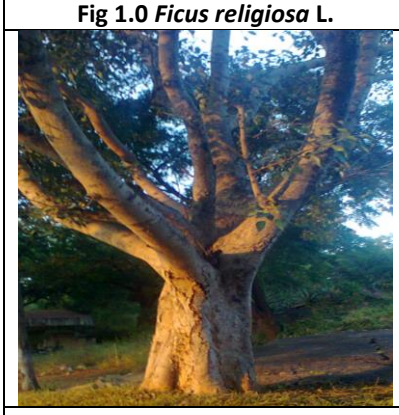


**Fig 1.1 *Ficus religiosa* L. (Stem explant)**

**Fig 1.2 *Ficus religiosa* L. (Stem powder)**

**Fig 2.0 *Ficus racemosa* L.**

**Fig 2.0** *Ficus racemosa* L. (Stem bark explants)

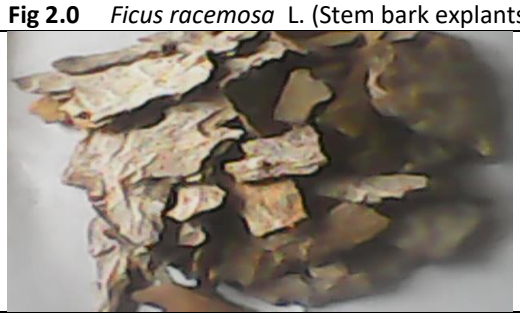


**Fig 2.1 Ficus *racemosa* L. (Bark powder)**

**2.2 Method of sample preparation, extraction and evaporation:**

Explants were cleaned, washed in running water and then in distilled water (Ganis, India) and naturally shade dried for a week and pulverized using mortar and pestle. 5.0 g each of the homogenized explants was weighed using electronic balance (Shimadzu AUX220, Japan) and mixed together forming a biherbal powder which was soaked in closed sterile, flat-bottom glass container (Borosil, India) containing whole 100 ml of analytical grade Ethanol (Hymankimia, United Kingdom), a selective solvent known as menstrum . This non-flowing system of menstrum and biherbal powder was kept in contact with each other and incubates at room temperature, with vigorous shaking at regular intervals for seven days**.** At the end of seventh day of maceration -after attaining equilibrium-the solution was filtered through muslin cloth (DS Enterprises, India). The liquid extract thus expressed was known as macerate. The remained inert fibrous, insoluble and damp solid residue, called marc, was strained to recover as much occluded macerate as possible. The expressed and strained liquids are called miscella. The miscella was further filtered through a, Whattmann no.1 filter paper (Whatman,UK) was evaporated under reduced pressure in vaccuo , at below 40°C using rotary evaporator (Supervac R/185, India), which yielded a gummy residue, the analyte.

**2.3 Metabolomic research using GCMS technology:**

GCMS analysis was carried out at BA Laboratory, Hyderabad, Telangana State, India using Agilent gas chromatograph model 6890 NI coupled to an Agilent 5973 NI mass selective detector. The GC was fitted with a HP-5MS capillary column of 30 m X 250 µm X 0.25 µm. The temperature program was as follows: injector temperature 280°C, initial oven temperature was at 60°C, then 60 °C for 1 min, 40 °C/min to 170 °C for 0 min, 10 °C/min to 310 °C for 3 min. Helium was used as carrier gas at 8.2317 psi pressure with flow 1 mL /min. Samples were solved in chloroform and 2 µl aliquot were injected automatically. Measurement of peak areas and data processing was carried out by GCMS solution ver.2. The spectrum of the unknown/test component was compared with the spectrum of the known components stored in the NIST library. The name and retention time, molecular weight and structure of the compounds in the biherbal extract were ascertained.

## 3. RESULTS

GCMS technique

GCMS running time for ethanol extract was 20.75 minutes. The total numbers of compounds identified were 14 (Table 1 and Fig 3 (Fig 3.1.0-3.14.1)).

**Table 1: Fourteen compounds in ethanol extract of biherbal powder**

| Retention time | Compounds | Probability |
|---|---|---|
| 10.264 | Isopropyl Palmitate | 9.92 |
| 10.454 | Hexadecanoic acid, ethyl ester | 35.6 |
| 11.031 | Curan-17 oic acid- 19,20 –dihydroxy-, methyl ester, (19s) | 9.58 |
| 11.735 | 9,12 – Octadecadienoic acid (z,z)- | 16.5 |
| 11.772 | Oleic acid | 9.60 |
| 11.915 | Linoleic acid, ethyl ester | 4.80 |
| 12.146 | Octadecanoic acid, ethyl ester | 21.7 |
| 13.555 | Eicosanoic acid | 38.2 |
| 14.719 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester | 18.4 |
| 15.125 | Docosanoic acid | 24.4 |
| 15.325 | Hexadecanoic acid, ethyl ester | 20.6 |
| 16.075 | Tetratetracontane | 6.50 |
| 16.080 | Tetratetracontane | 11.6 |
| 17.586 | Octacosane | 7.88 |

**Fig 3 GCMS chromatogram and compounds in bi-herbal ethanol extract**

**Fig 3.1.0: Isopropyl Palmitate**

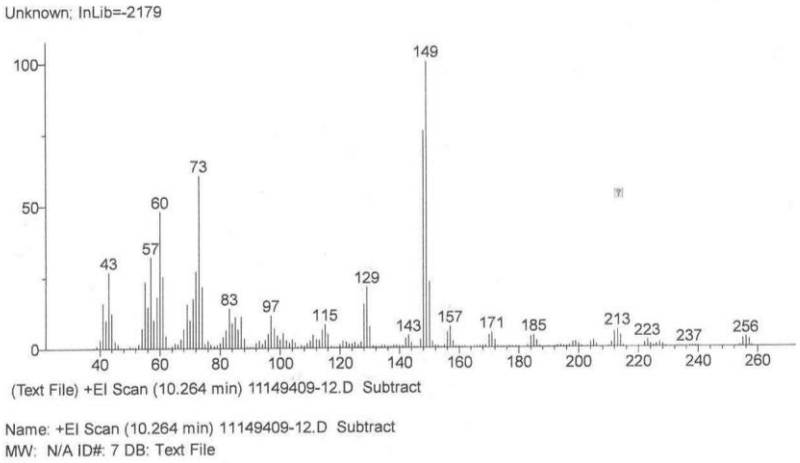



**Fig 3.1.1**

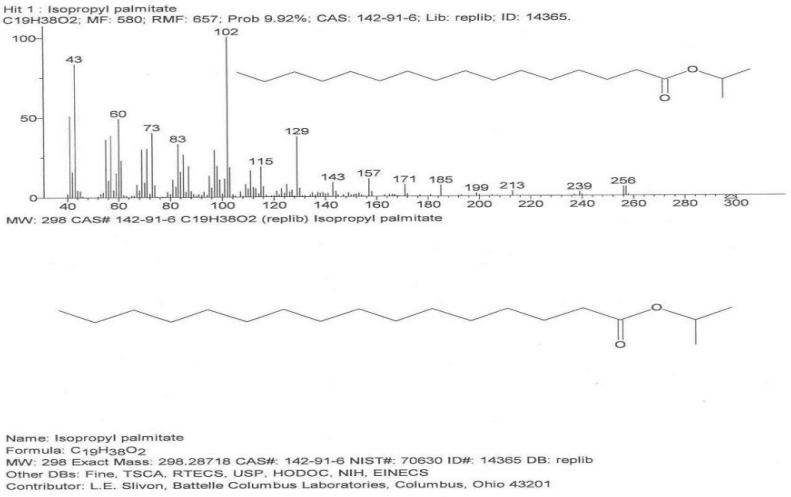



**Fig 3.2.0 Hexadecanoic acid, ethyl ester**

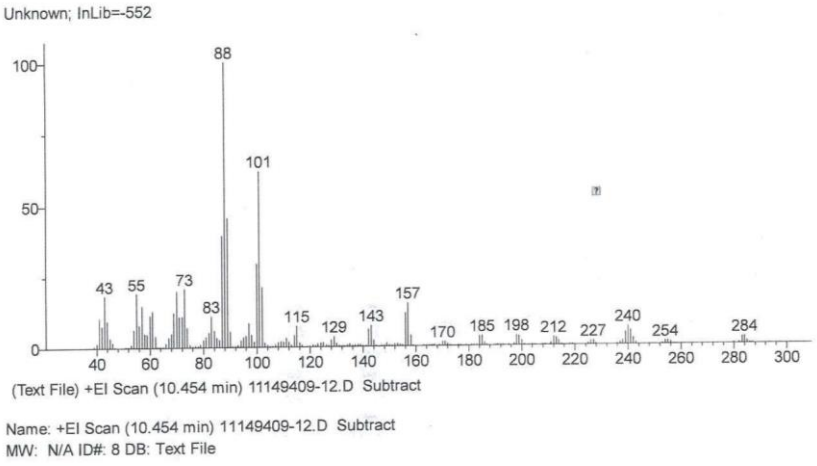


**Fig 3.2.1**

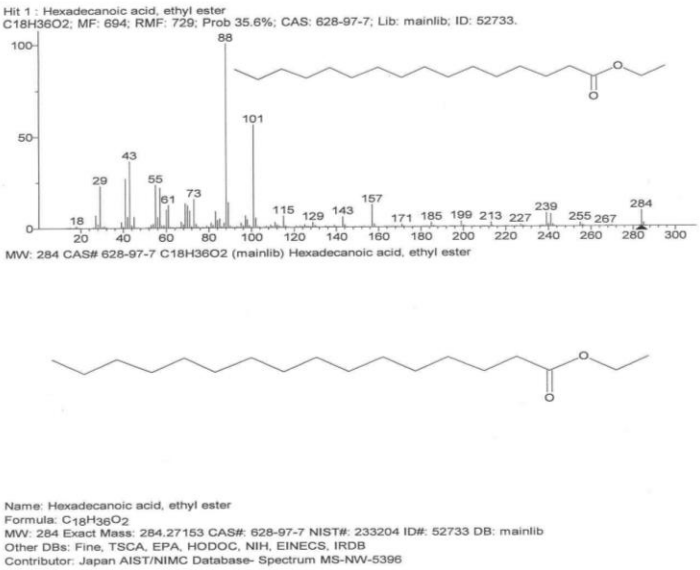



**Fig 3.3.0: Curan-17 oic acid- 19,20 –dihydroxy-, methyl ester, (19s)**

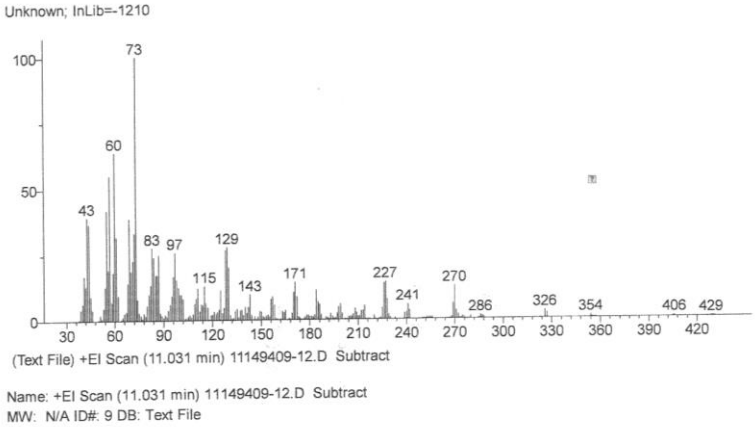



**Fig 3.3.1**

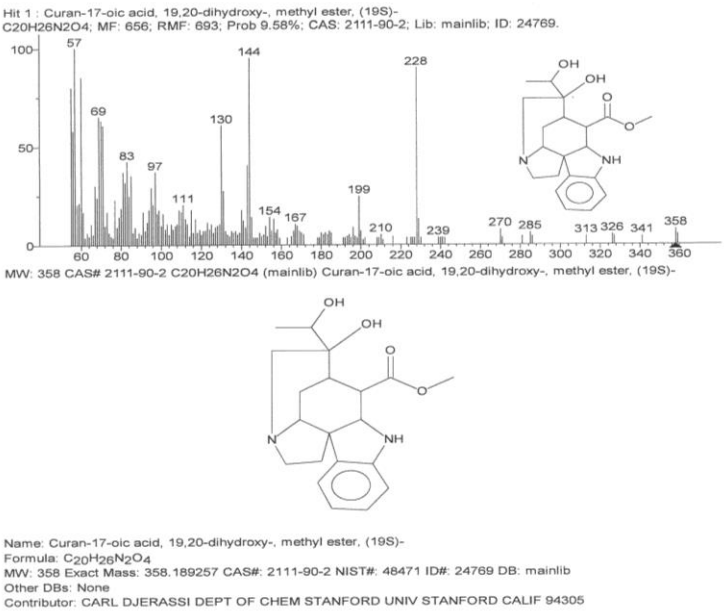


**Fig 3.4.0:9, 12 – Octadecadienoic acid (z,z)-**

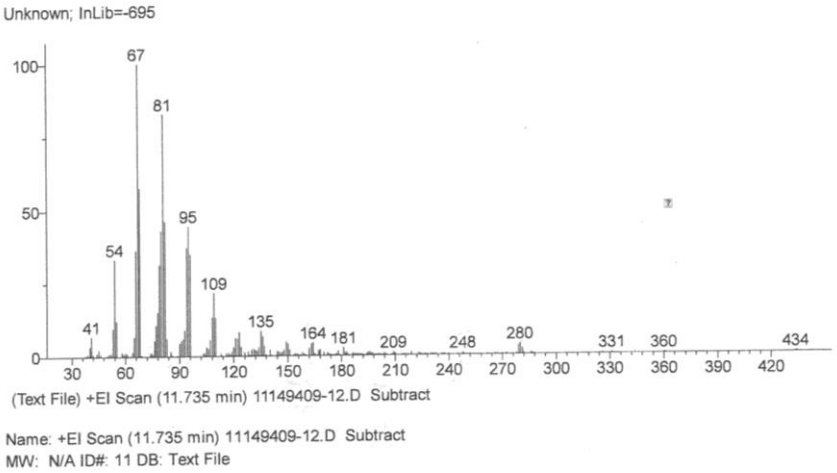



**Fig 3.4.1**

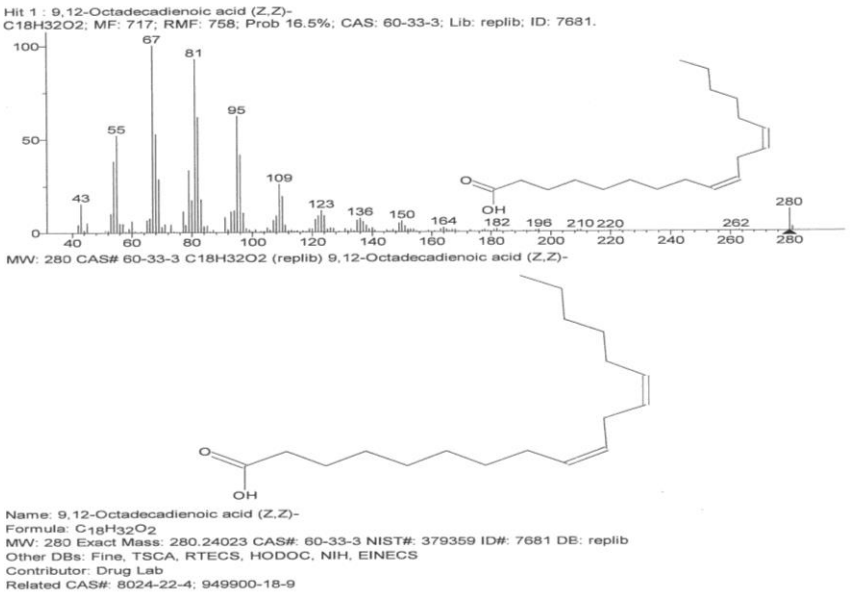



**Fig 3.5.0 Oleic acid**

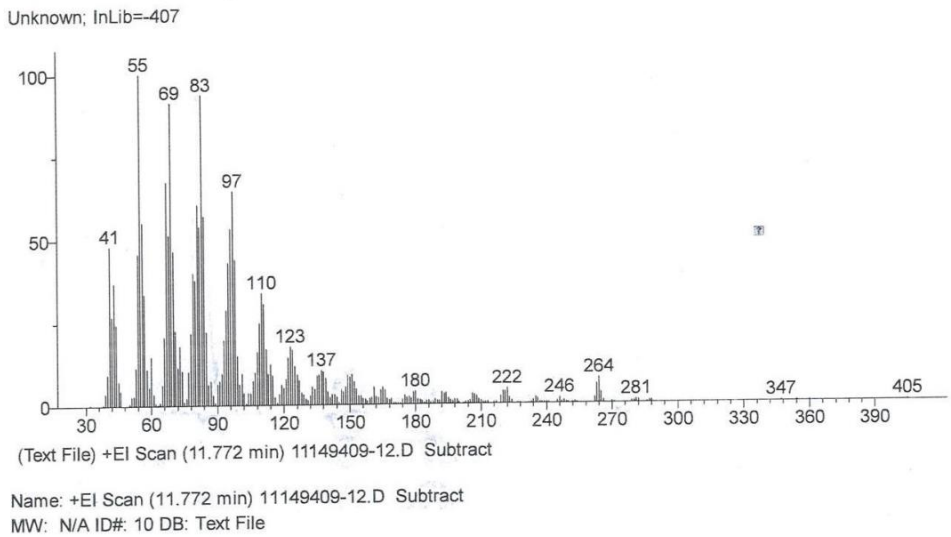


**Fig 3.5.1**

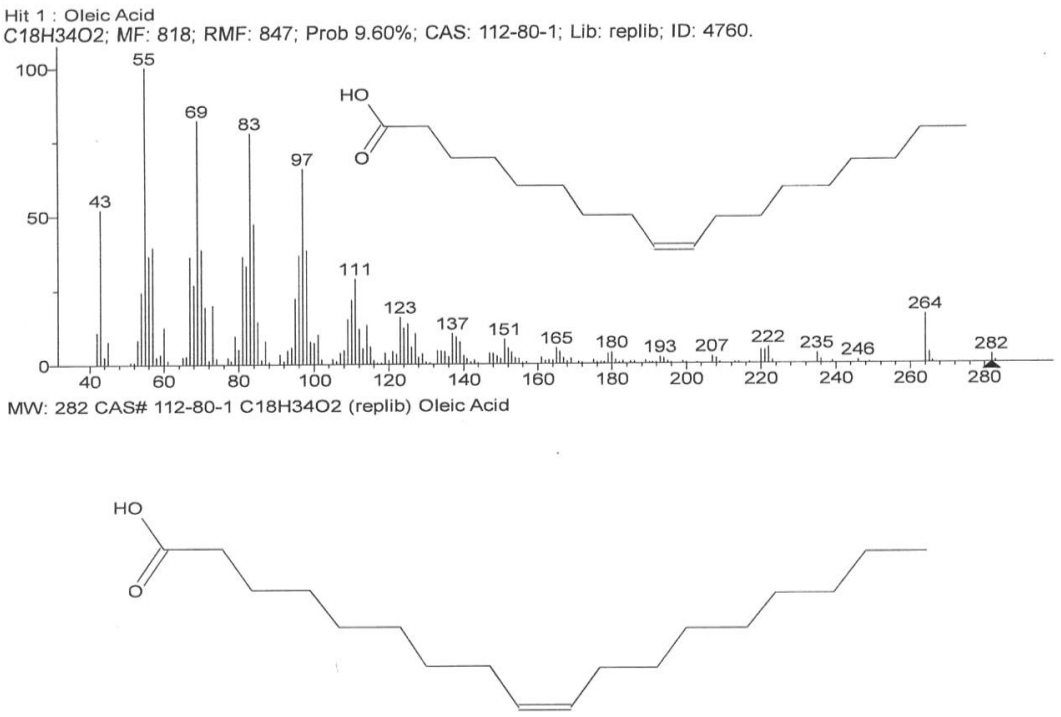



**Fig 3.6.0 Linoleic acid, ethyl ester**

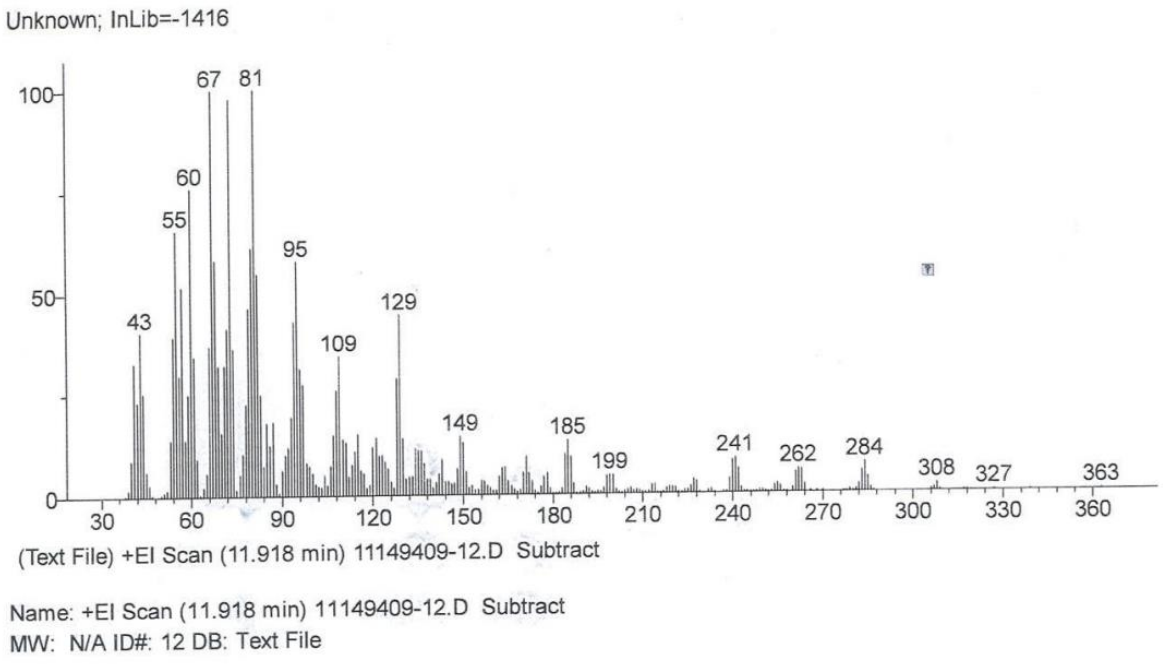


**Fig 3.6.1**

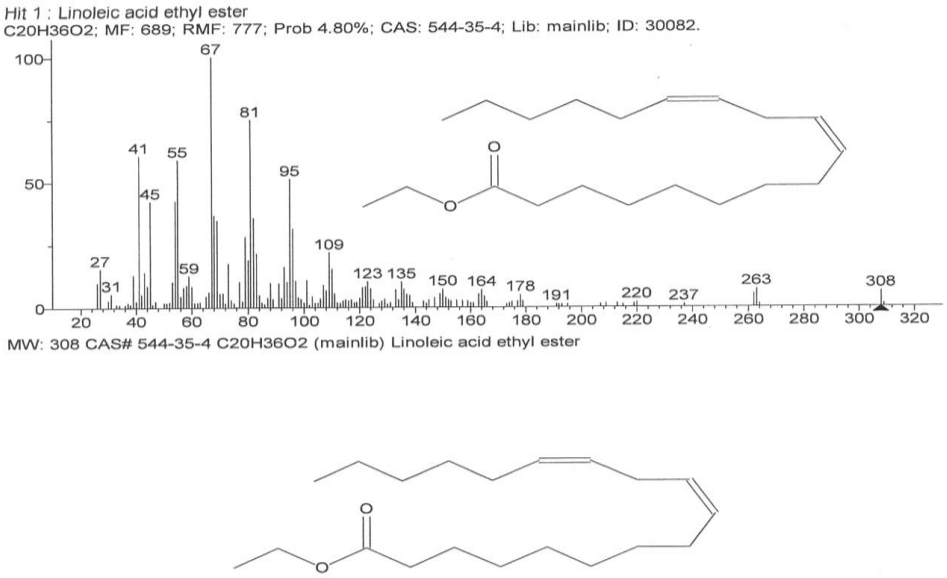



**Fig 3.7.0 Octadecanoic acid, ethyl ester**

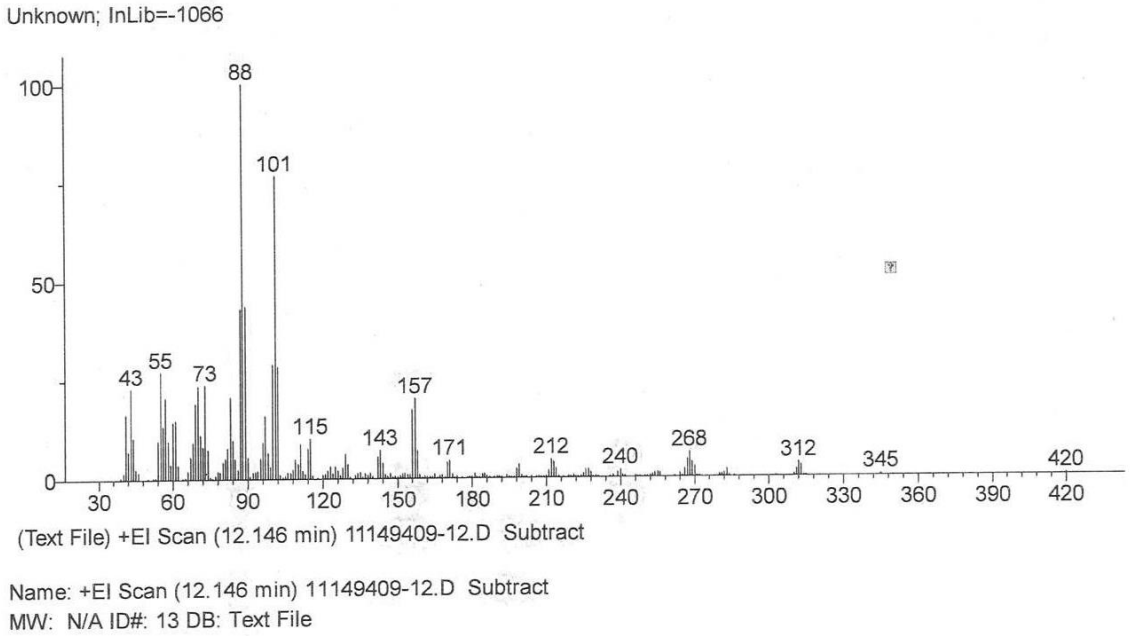


**Fig 3.7.1**

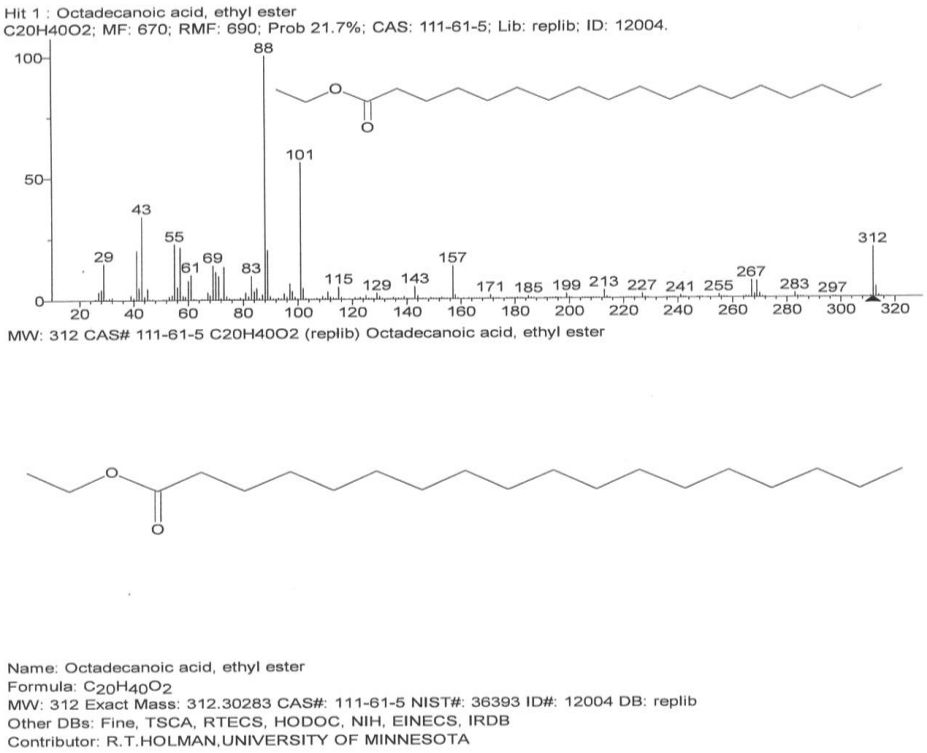



**Fig 3.8.0 Eicosanoic acid**

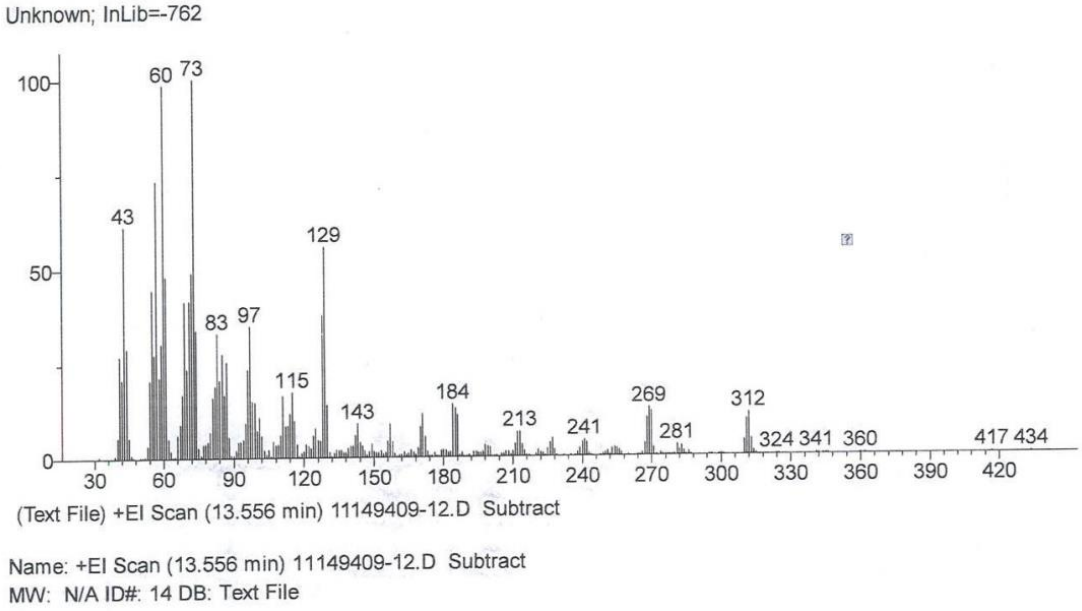


**Fig 3.8.1**

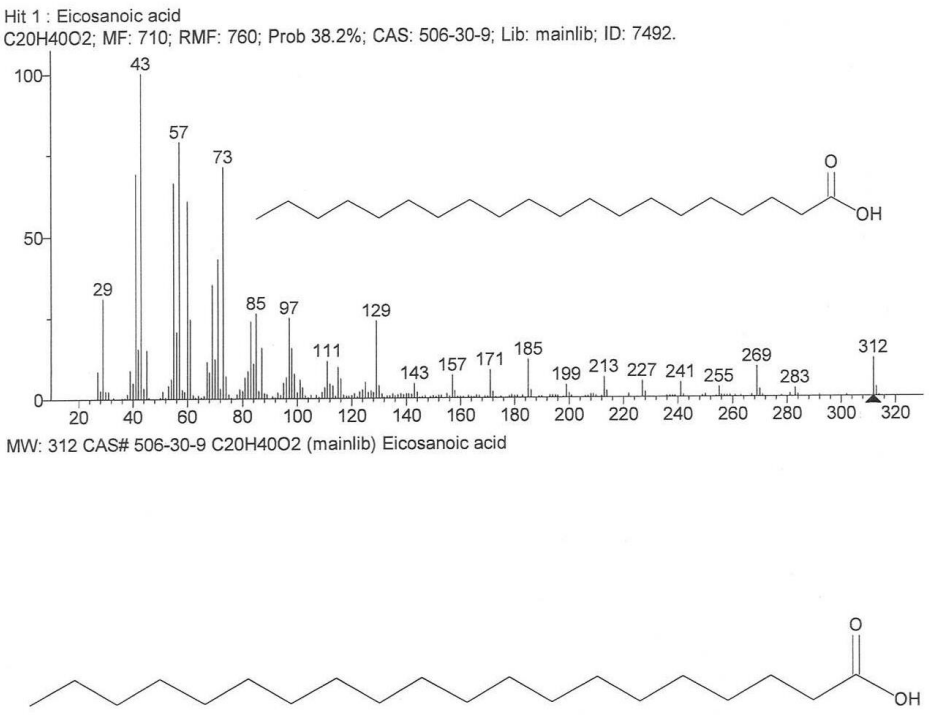



**Fig 3.9.0 Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester**

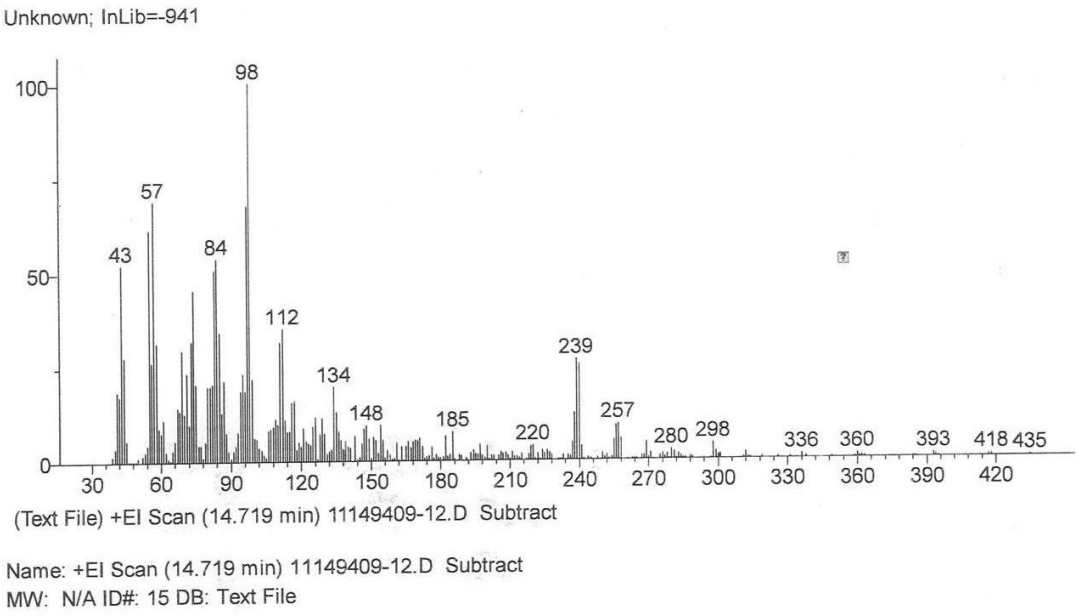


**Fig 3.9.1**

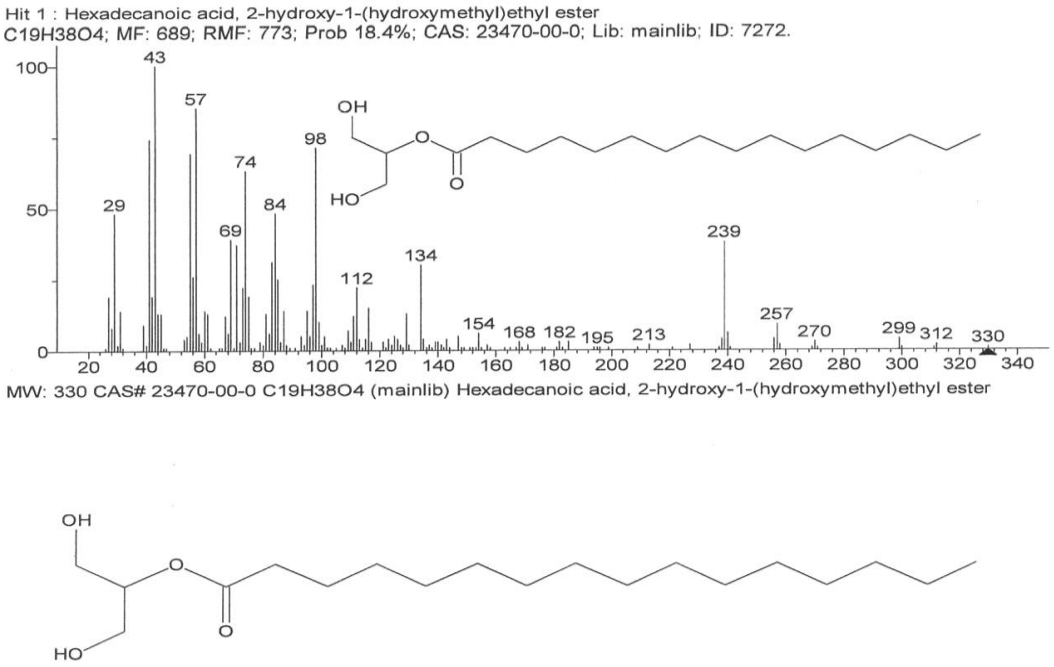



**Fig 3.10.0 Docosanoic acid**

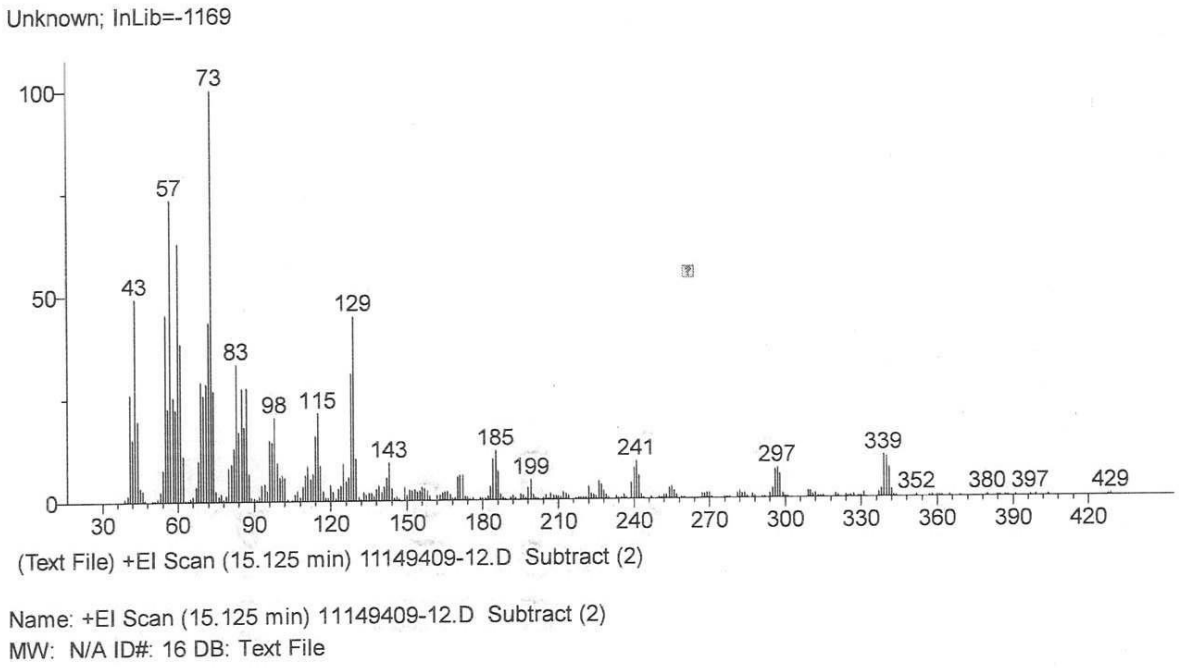


**Fig 3.10.1**

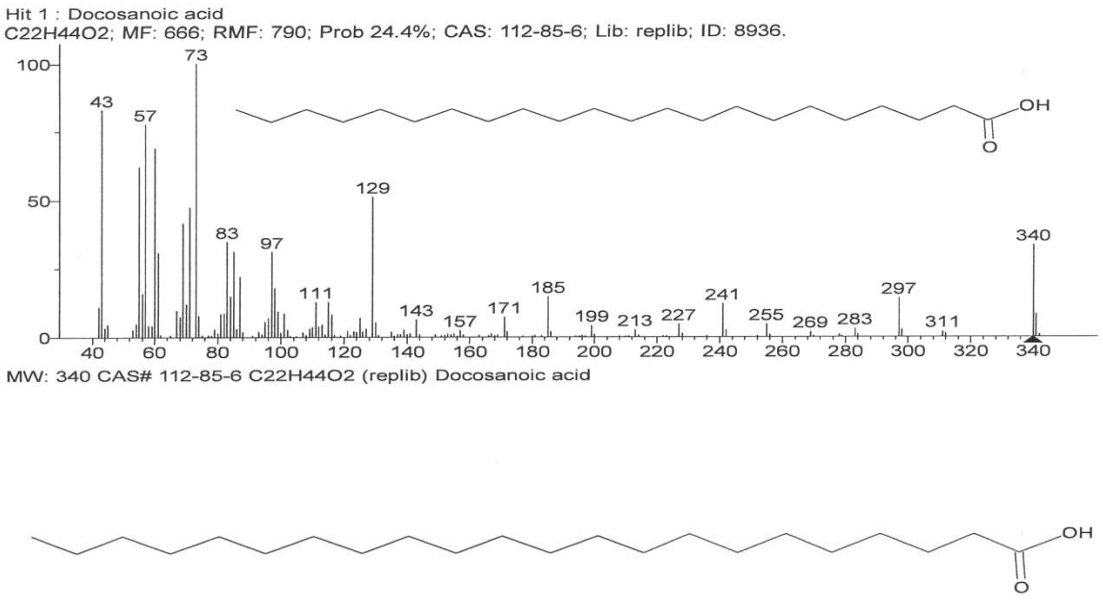



**Fig 3.11.0 Hexadecanoic acid, ethyl ester**

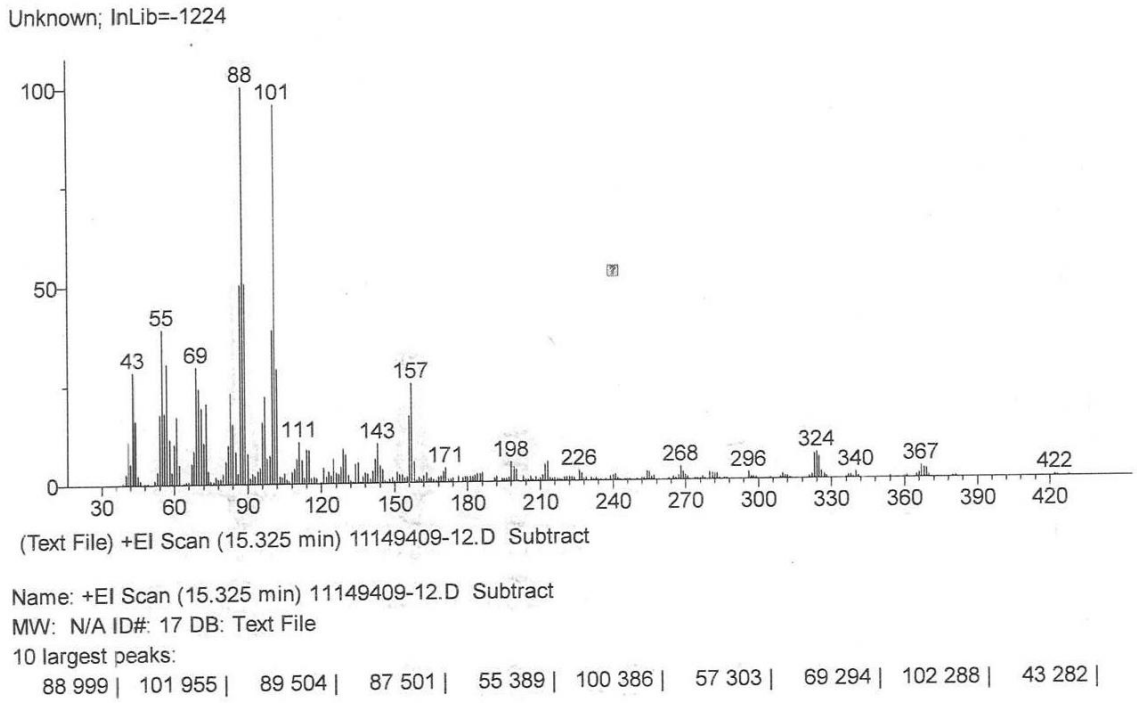
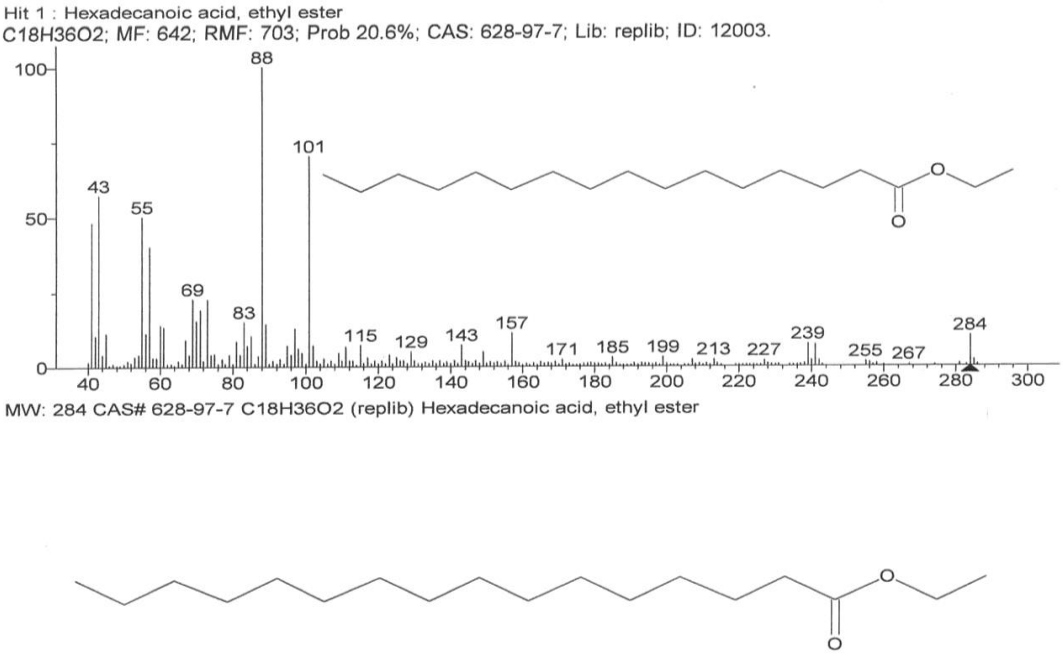
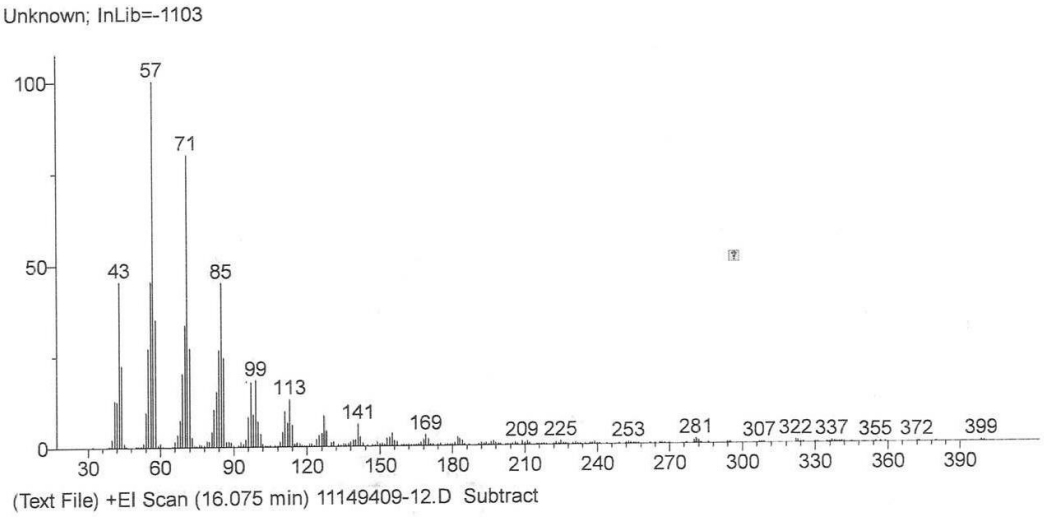


**Fig: 3.11.1**




Name: Hexadecanoic acid, ethyl ester
Formula: $C_{18}H_{36}O_2$
MW: 284 Exact Mass: 284.27153 CAS#: 628-97-7 NIST#: 43659 ID#: 12003 DB: replib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: ACIR ALIP ETHY SE30 05 1980 E M.HORN
10 largest peaks:
88 999 | 101 700 | 43 570 | 55 500 | 41 480 | 57 400 | 69 226 | 73 224 | 71 190 | 70 152 |

**Fig 3.12.0: Tetratetracontane**




Name: +EI Scan (16.075 min) 11149409-12.D Subtract
MW: N/A ID#: 18 DB: Text File
10 largest peaks:
57 999 | 71 798 | 43 453 | 56 453 | 85 449 | 58 349 | 70 333 | 55 270 | 72 270 | 84 265 |

**Fig 3.12.1**

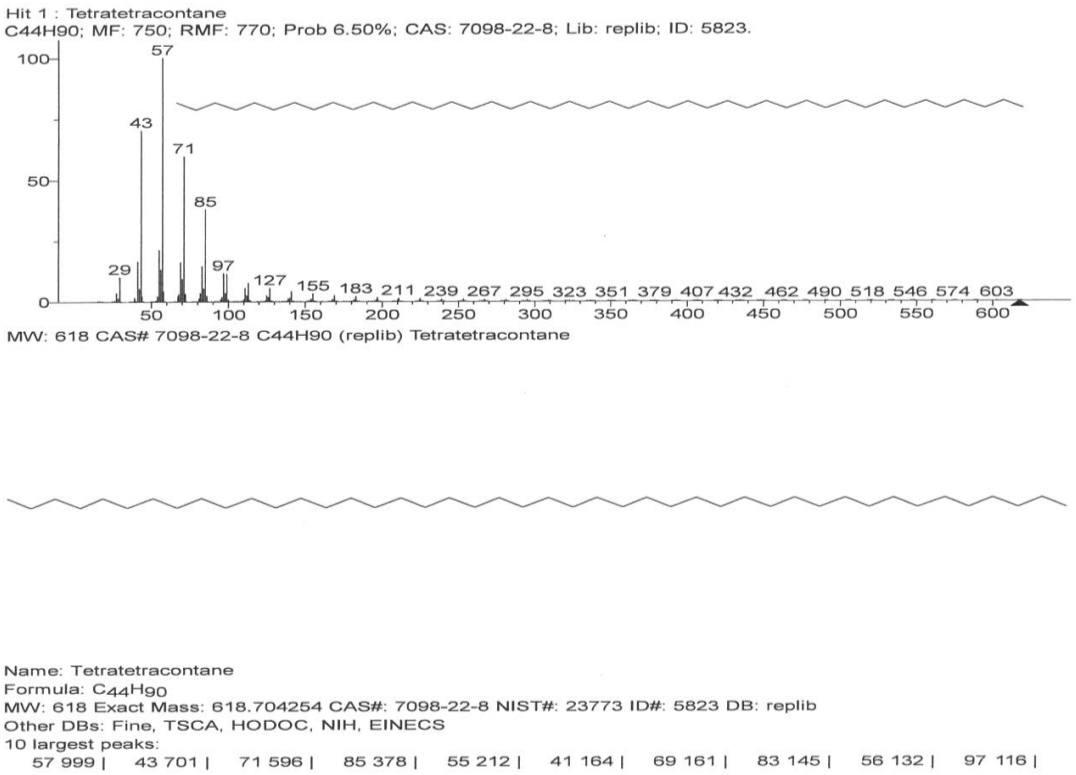



**Fig 3.13.0: Tetratetracontane**

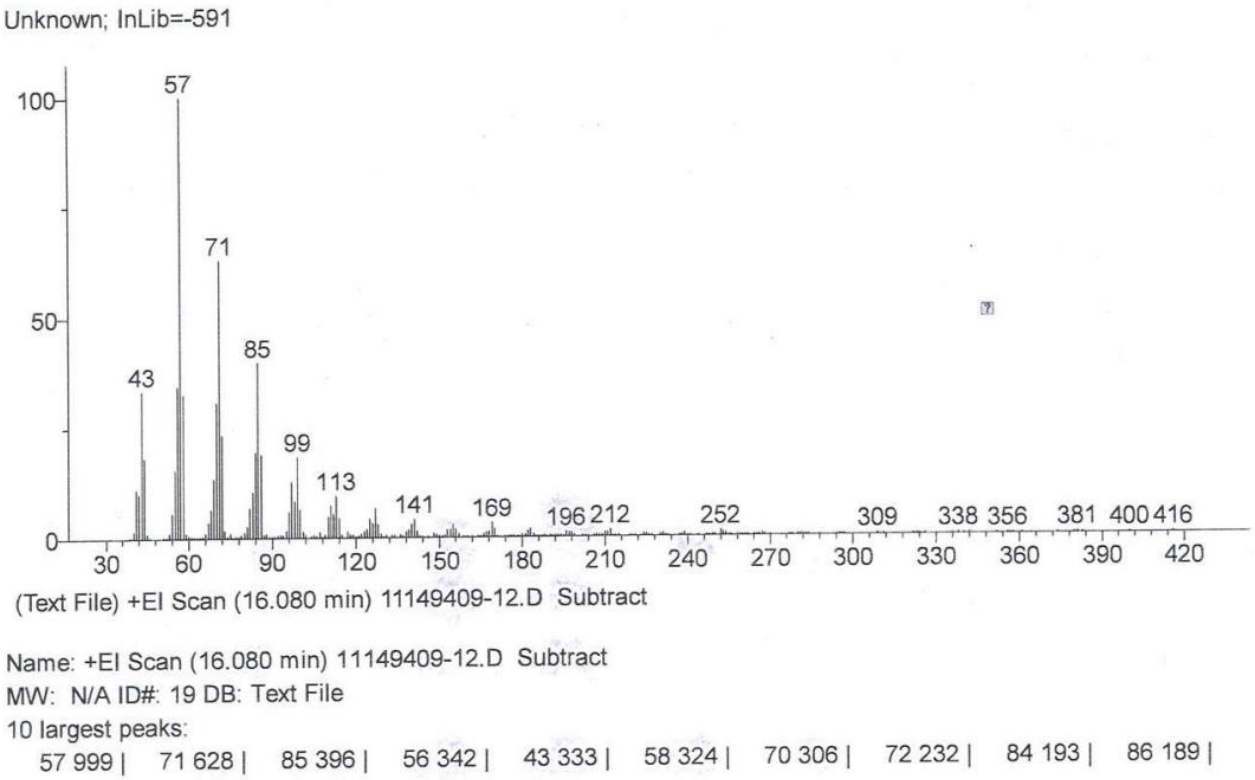
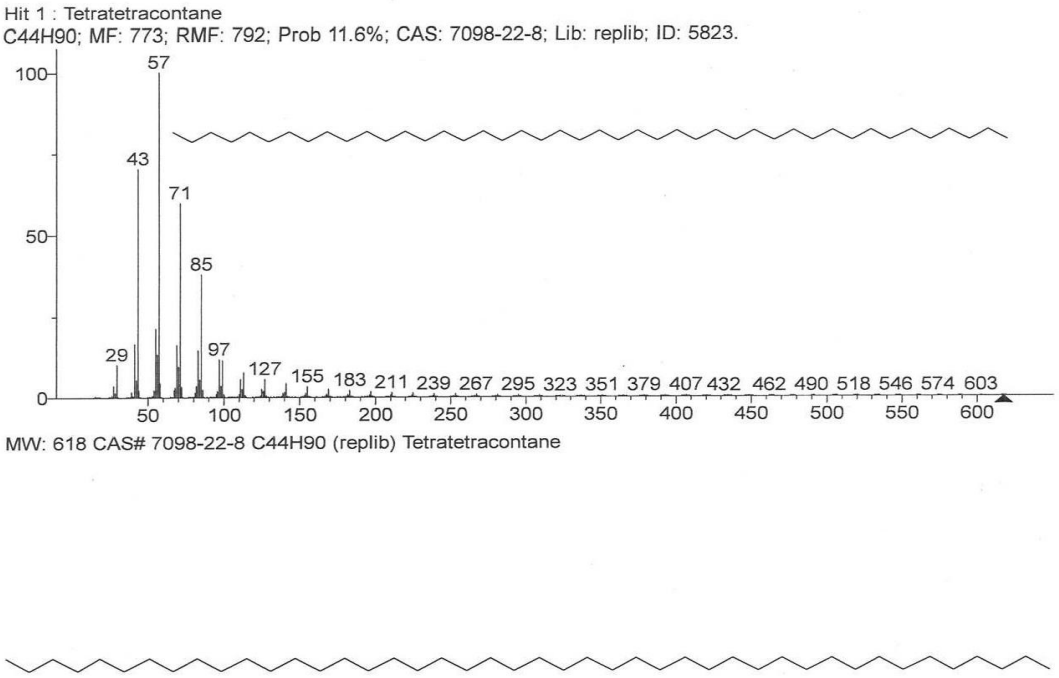
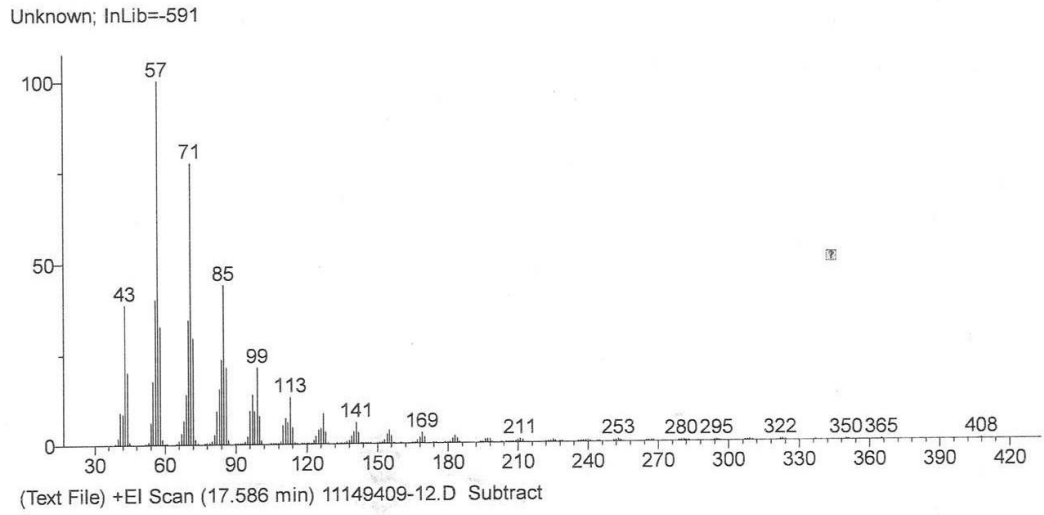


**Fig 3.13.1**




Name: Tetratetracontane
Formula: $C_{44}H_{90}$
MW: 618 Exact Mass: 618.704254 CAS#: 7098-22-8 NIST#: 23773 ID#: 5823 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS
10 largest peaks:
57 999 | 43 701 | 71 596 | 85 378 | 55 212 | 41 164 | 69 161 | 83 145 | 56 132 | 97 116 |

**Fig 3.14.0: Octacosane**




Name: +EI Scan (17.586 min) 11149409-12.D Subtract
MW: N/A ID#: 20 DB: Text File
10 largest peaks:
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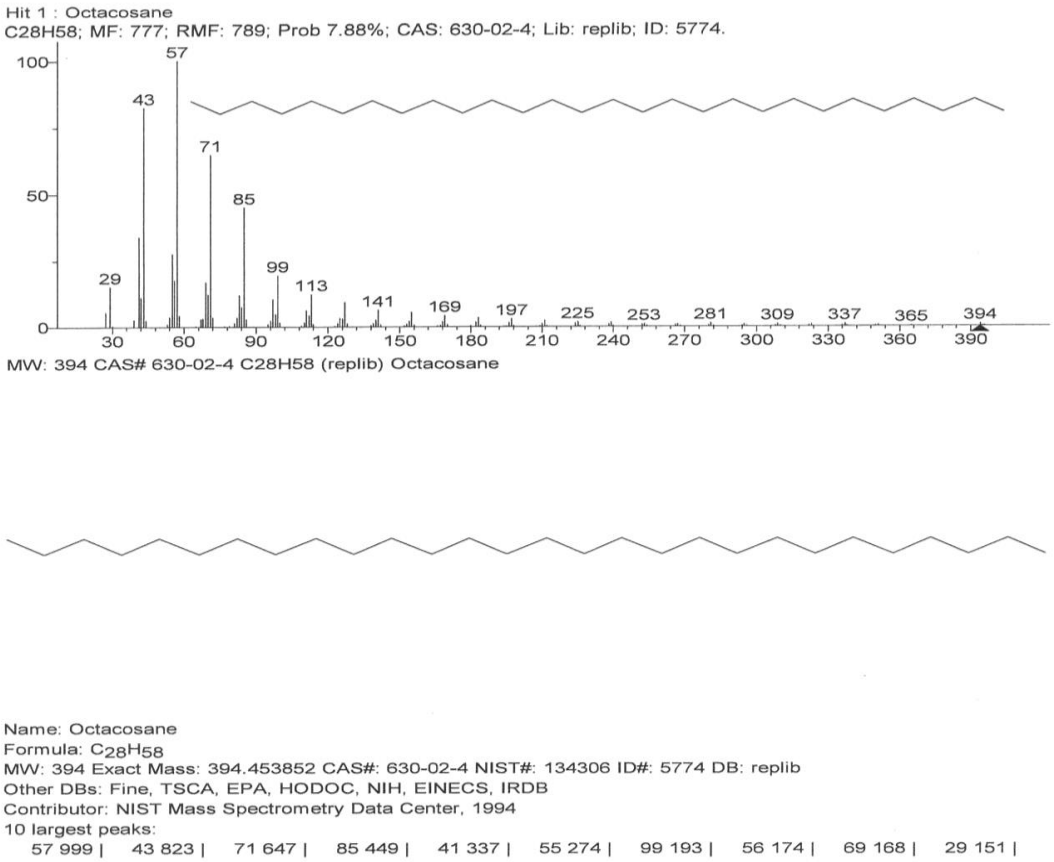
**Fig 3.14.1**




## 4. DISCUSSION

The ethanol extracts of stem bark of *Ficus racemosa* Linn. Showed wound healing (Biswas TK et al., 2003), anti-inflammatory (Li RW et al., 2003), anti-diarrhoeal (Mukherjee PK et al., 1998) and analgesic activity (Malairajan P et al., 2006). The bark of *Ficus racemosa* Linn. is highly efficacious in threatened abortion and also recommended in urological disorders, diabetes, hiccough, leprosy, dysentery, asthma and piles (Padmaa MP et al., 2009). The stem and bark extracts of *F. religiosa* proved lethal to *Ascaridia galli in vitro* (De Amorin A et al., 1999). *Ficus religiosa* stems are known to have medicinal properties and usually used separately or as a whole plant and may contains various bioactive compounds of medicinal importance (Manorenjitha MS et al., 2013). In the light of above information, this crude ethanol bi-herbal extract or its purified fractions can be tested *in-vitro* for bioassay's. Also, this raw biherbal powder can be extracted with other selected solvent(s) for identifying yet another set of compounds using GCMS technique. Anti-microbial record of each compound in this set of compounds can be known from chemdb NIAID database. If Anti-microbial activity of compounds - if any - found recorded in chemdb NIAID database, there may be a hope for *in vitro* anti-microbial efficacy of either crude or purified fractions of the biherbal extract made of selected solvent.

## ACKNOWLEDGMENTS

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