

Gas Chromatography-Mass Spectrometry Analysis of Kandangaari Nei – A Polyherbal Siddha Drug

P. Robin Jude¹, B. Vedha², G. Esakkypandian³

^{1,2}PG scholar, Department of Gunapadam, Government Siddha Medical College, Palayamkottai.

³HOD & Professor, Department of Gunapadam, Government Siddha Medical College, Palayamkottai.

ABSTRACT:

Siddha system of medicine is the indigenous system of medicine practiced profoundly over southern part of India and Sri Lanka. This system of medicine includes wide variety of therapies comprising internal medicine, external therapies and surgeries. *Kandangaari nei* is one such drug, mentioned in *Agathiyar vaithiya kaaviyam* 1500, a drug endorsed for *shayakaasam* and *kaasavagaigal*. The study aims at identifying the bioactive components of the drug using Gas chromatography-mass spectrometry analysis, to correlate the literature with pharmacological evidence. The GC-MS analysis of the drug resulted in various compounds including dasycarpidan-1-methanol, n-hexadecanoic acid, heptadecanoic acid. Earlier studies on these components unveiled their clinical significance in the context of respiratory diseases, substantiating the science behind the formulation.

Keywords: Siddha, *kandangaari nei*, *Agathiyar vaithiya kaaviyam* 1500, Gas Chromatography-Mass Spectrometry, respiratory ailments.

INTRODUCTION:

Siddha system of medicine is the indigenous medical system which was mainly practiced over southern part of India and Sri Lanka. The traditional system of medicine comprises of treatment methods involving internal medicine, external therapies (including reduction techniques and trauma management) and surgeries. The science behind internal medicine is stupefying as there are 32 forms of them, each having their shelf life according to their form/moisture content. *Kandangaari nei* is one such medicine belonging to the form of *nei*, mentioned in *Agathiyar vaithiya kaaviyam* 1500(1), indicated for *Shayakaasam* and *kaasavagaigal*, relating to respiratory tract ailments(2). The route of administration is clearly mentioned in the literature and the medicine seems to be a Mouth Dissolving compound. This study aims at identifying bioactive compounds of the drug through GC-MS analysis for better understanding of the science behind the drug.

MATERIALS AND METHODS:

Collection and authentication of raw drugs:

Fresh plants were collected from local area of Palayamkottai, Tirunelveli. Raw drugs were bought from raw drug store. The drugs were identified and authenticated by experts in the Department of Gunapadam, Government siddha medical college, Palayamkottai.

Preparation of Kandangaari nei:**Ingredients of kandangaari nei:**

- Solanum xanthocarpum (kandankathiri)-juice of whole plant
- Phyllanthus emblica (nelli)- fruit
- Clerodendrum serratum (kandubaarangi)-root
- Alpinia officinarum (sittrathai)-root
- Wrightia tinctoria (vetpaalai) -seed
- Phyllanthus amarus (keezhanelli)-whole plant
- Trianthema decandra (chaaranai) -root
- Tribulus terrestris (nerunjil)-root
- Abies webbiana (thaalisapathiri)-leaves
- Tragia involucrata (sirukanjori)-root
- Cow's ghee

Procedure:

As mentioned in *Agathiyar vaithiya kaaviyam* 1500(1), the raw drugs and fresh plants were purified. 325ml of juice of *kandanakathiri* is taken in a vessel (*vanali*) and equal quantity of cow's ghee is added to it. The other drugs which are mentioned above (1 kalanju each) are made into powder, triturated together in the form of *karkam* and added to the vessel. The mixture is boiled until the confection in the oil is converted into *mezhu padham*. The ghee is then filtered and cooled and stored in airtight container.

GC MS ANALYSIS:

For the ethanol extracts, a small amount of concentrated sample was taken in a separating funnel and shaken by adding water and ethyl acetate in the ratio of 1:4. The upper layer was collected and concentrated in rotary evaporator to about 1.5 ml. Added 100µl N, O-Bis(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane(BSTFA+TMCS) and 20µl pyridine and heated at 60°C for 30 minutes.

For the layers which are separated from the crude extracts, a small amount of extract was taken and evaporated out totally. To this added acetonitrile and filtered into a conical flask. To the filtrate added 50µl BSTFA+TMCS and heated at 60°C in a water bath for 30 minutes. Filtered using 0.45µ membrane filter to a vial.

GC-MS Procedure

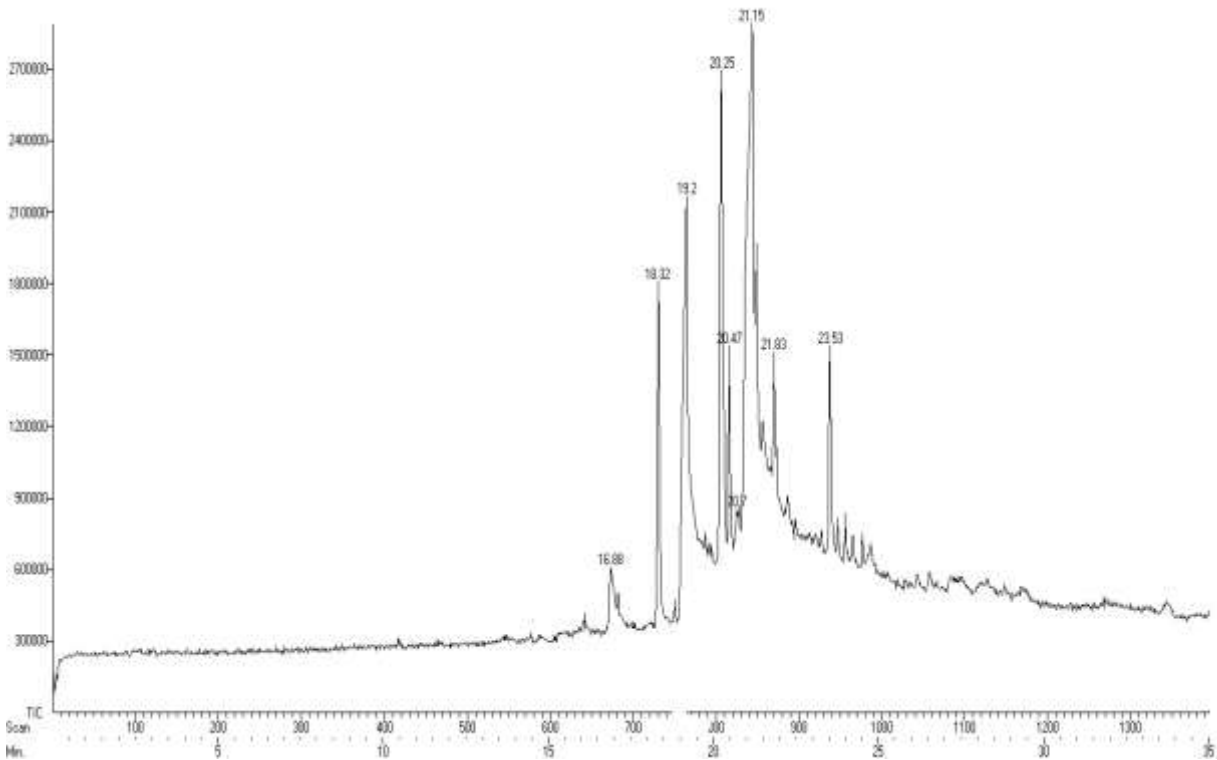
Gas chromatography (GC) analysis was carried out using Agilent 6890N gas chromatography equipped with photon multiplier tube as detector coupled to front injector type 1079. The chromatograph was fitted with HP 5 MS capillary column (30 m ×0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 250°C, and the oven temperature was initially at 70 °C hold for 4 mins then programmed to 200°C at the rate of 10°C/min and finally held at 200 °C for 13 min. Helium was used as a carrier gas with the flow rate of 1.5 ml/min. 0.2 microlitre of the Drug MC (diluted with methanol 1:10) were injected in the splitless mode. GC–mass spectrometry (GC–MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250°C. The mass spectra were obtained by centroid scan of the mass range from

50 to 600 amu. The compounds were identified based on the comparison of their retention indices (RI), retention time

(RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data (Adams, 2009).

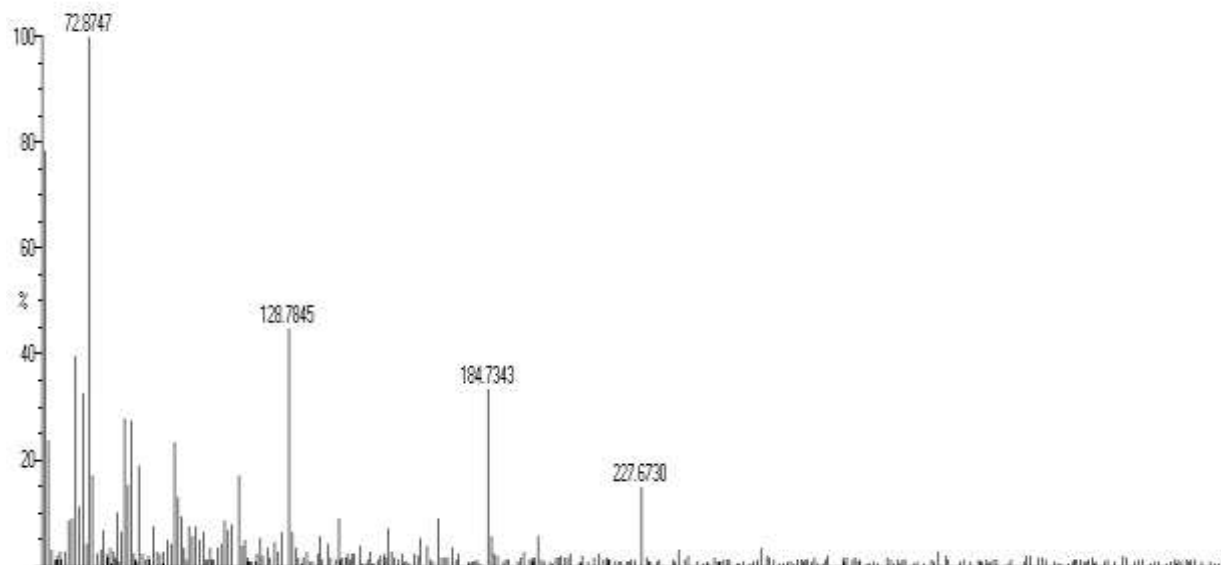
RESULTS AND DISCUSSION:

GC MS ANALYSIS REPORT:

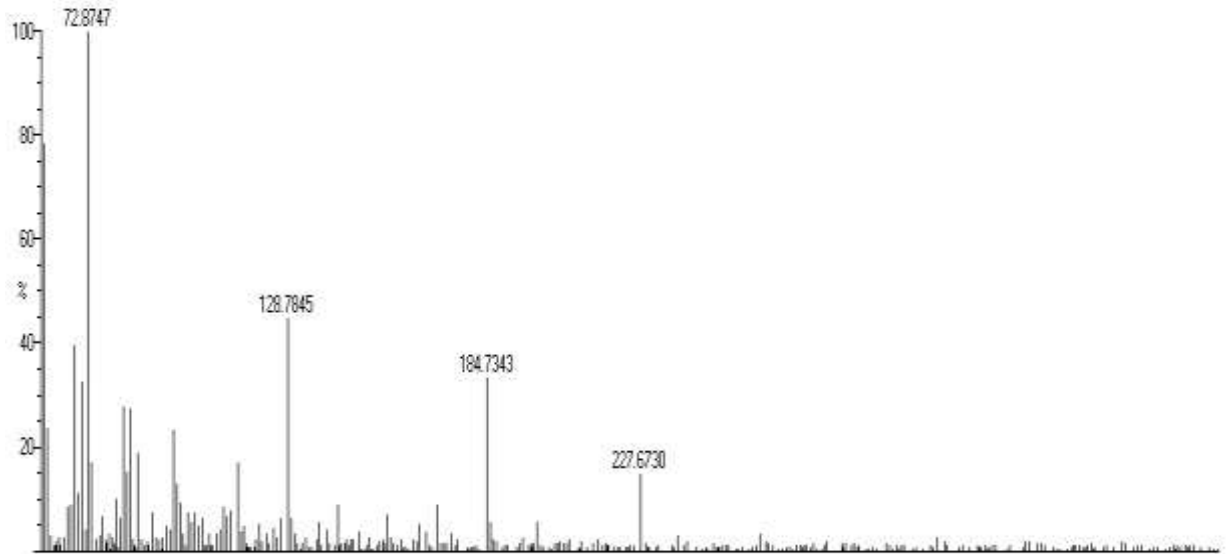


GC OF KANDANGAARI NEI

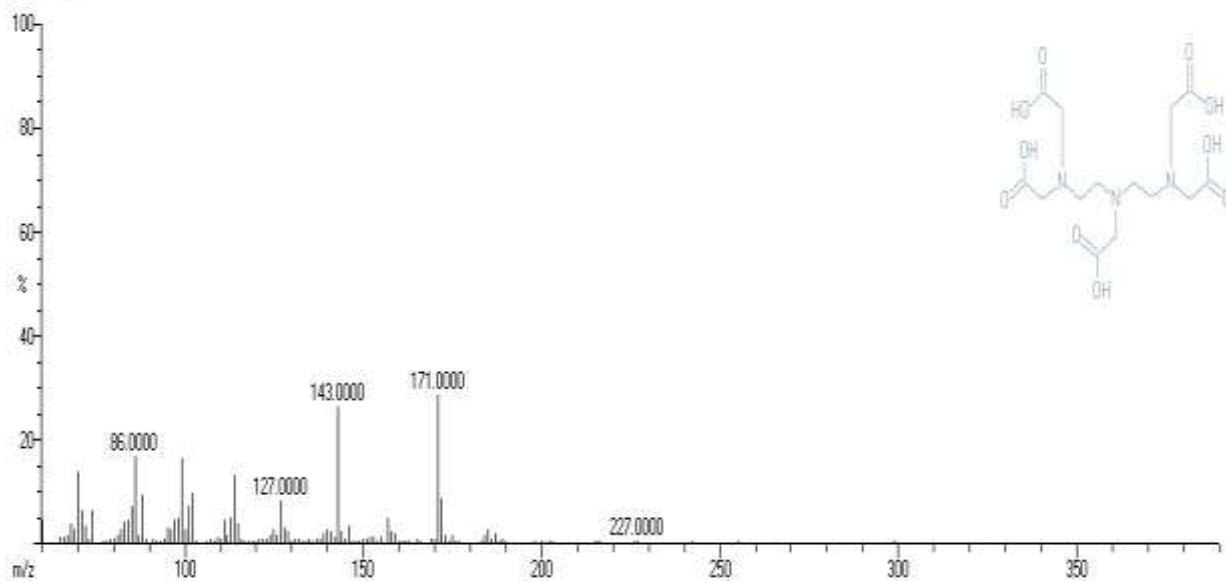
Scan: 673 TIC=607858 Base=3.7%FS #Ions=514 RT=16.68



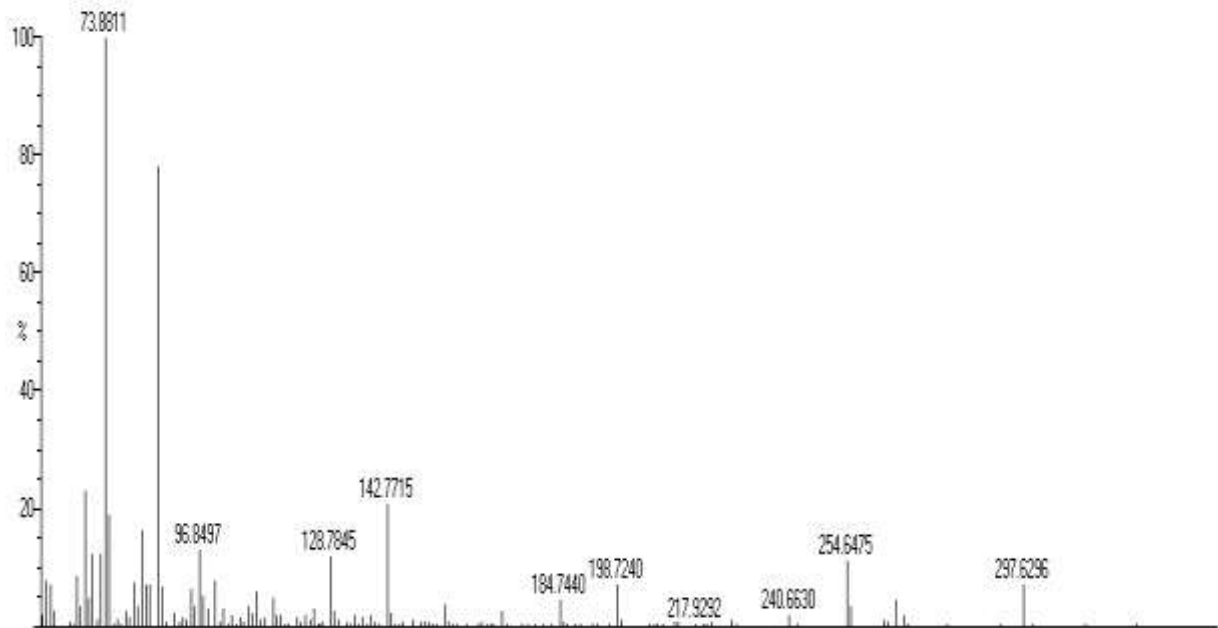
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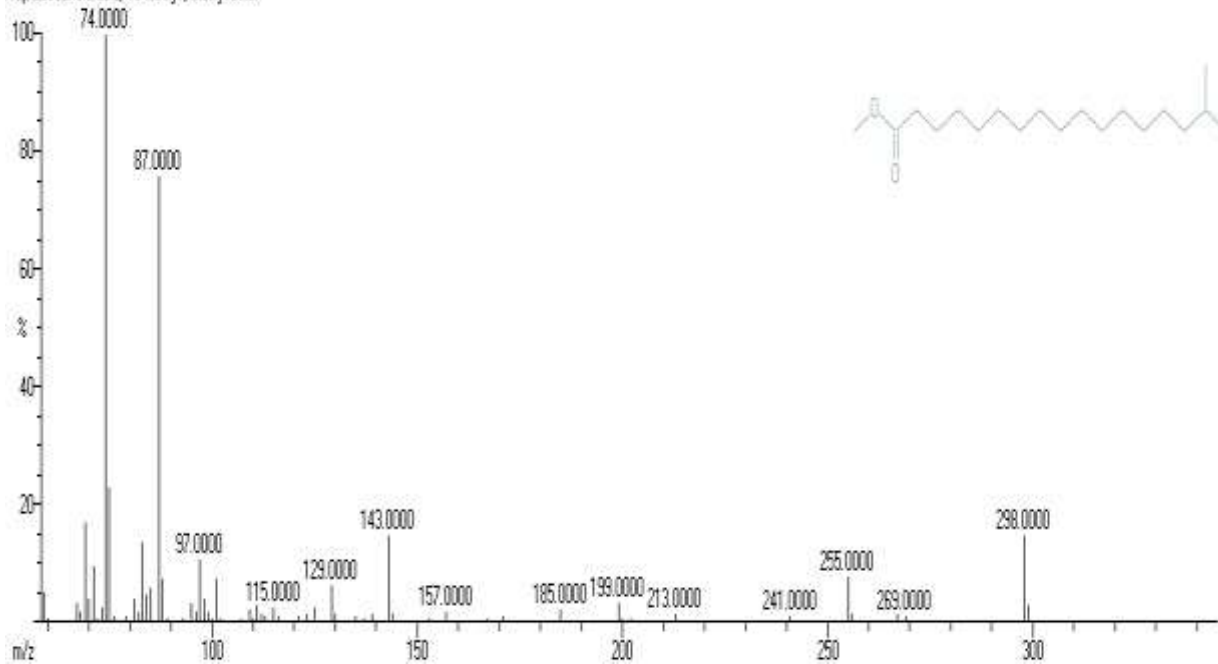
NIST MS 6 of 40 (67-43-6) #Ions=313
Pentetic Acid



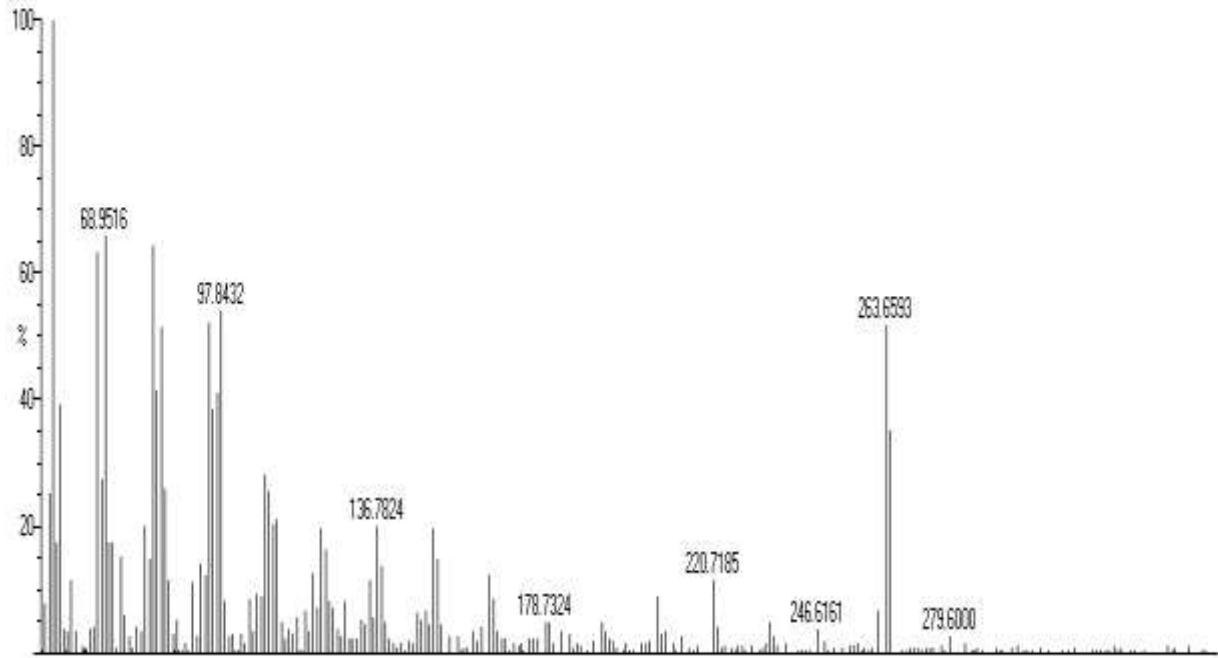
Scan: 816 TIC=1537053 Base=19.7%FS #Ions=493 RT=20.47



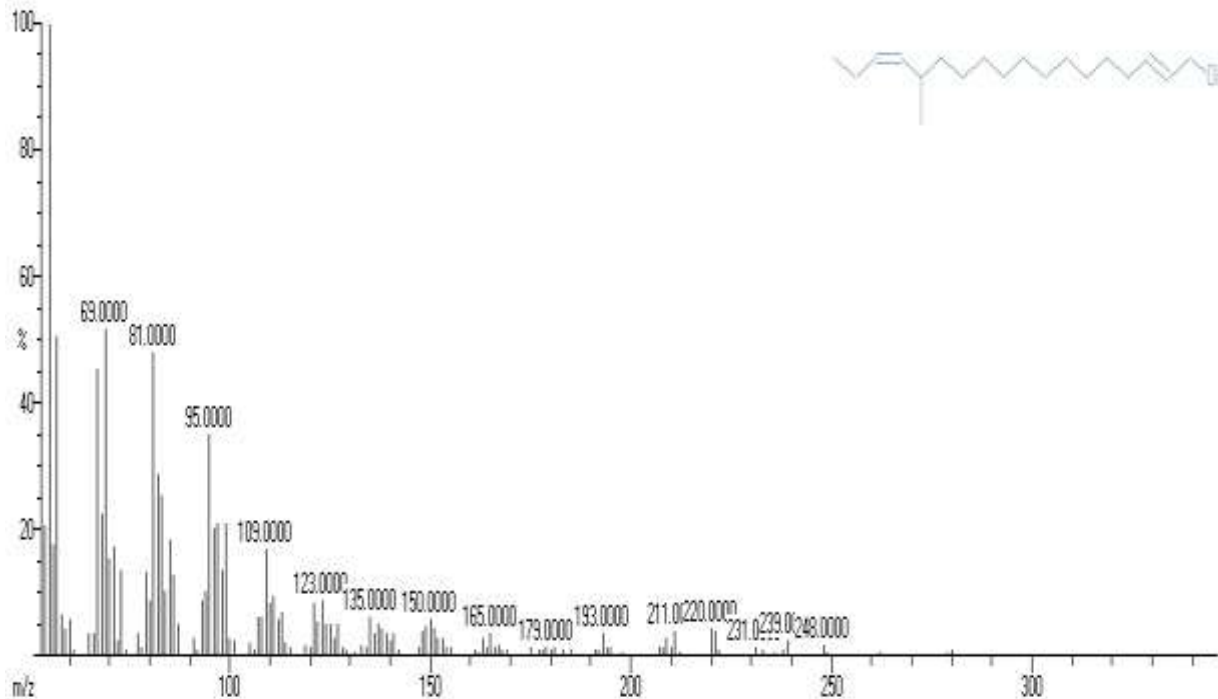
NIST MS 2 of 40 (5129-61-3) #Ions=165
 Heptadecanoic acid, 16-methyl-, methyl ester



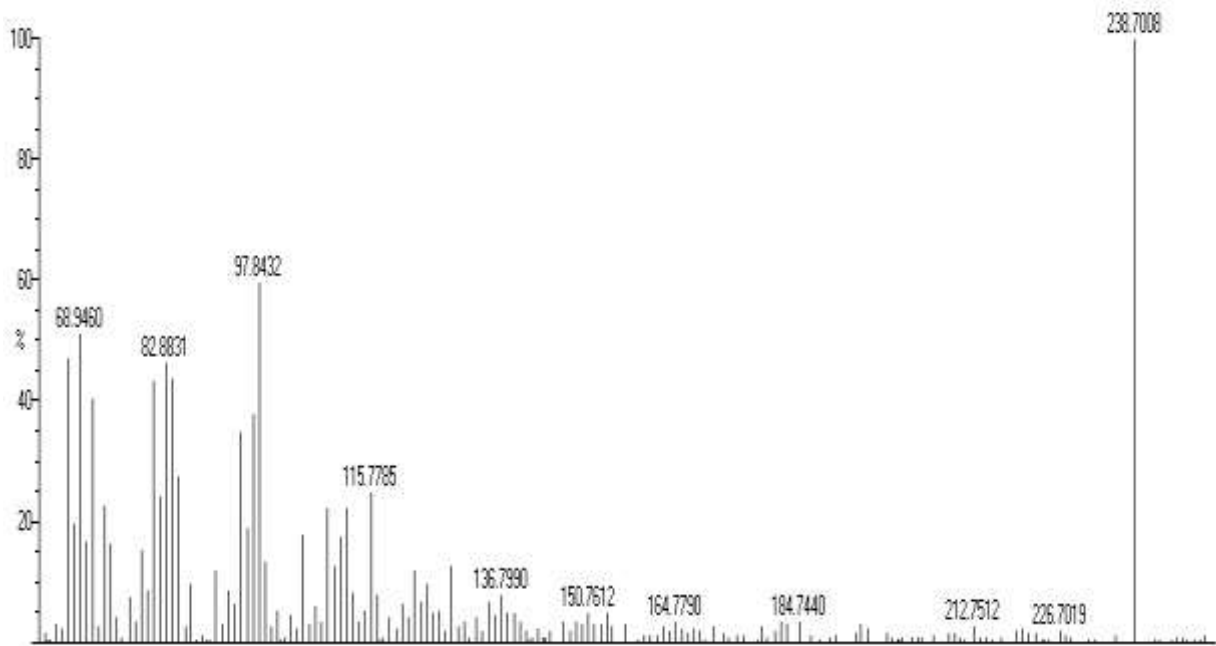
Scan: 938 TIC=1536390 Base=8.1%FS #Ions=470 RT=23.53



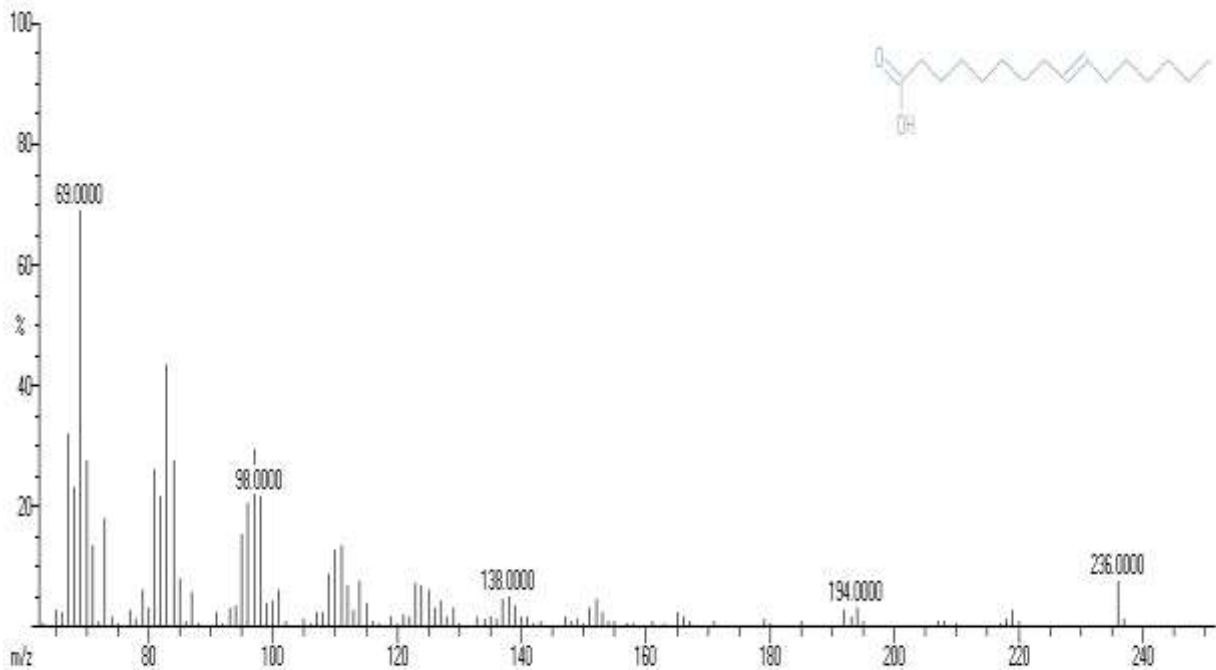
NIST MS 2 of 40 (DB# 15782) #Ions=156
12-Methyl-E,E-2,13-octadecadien-1-ol



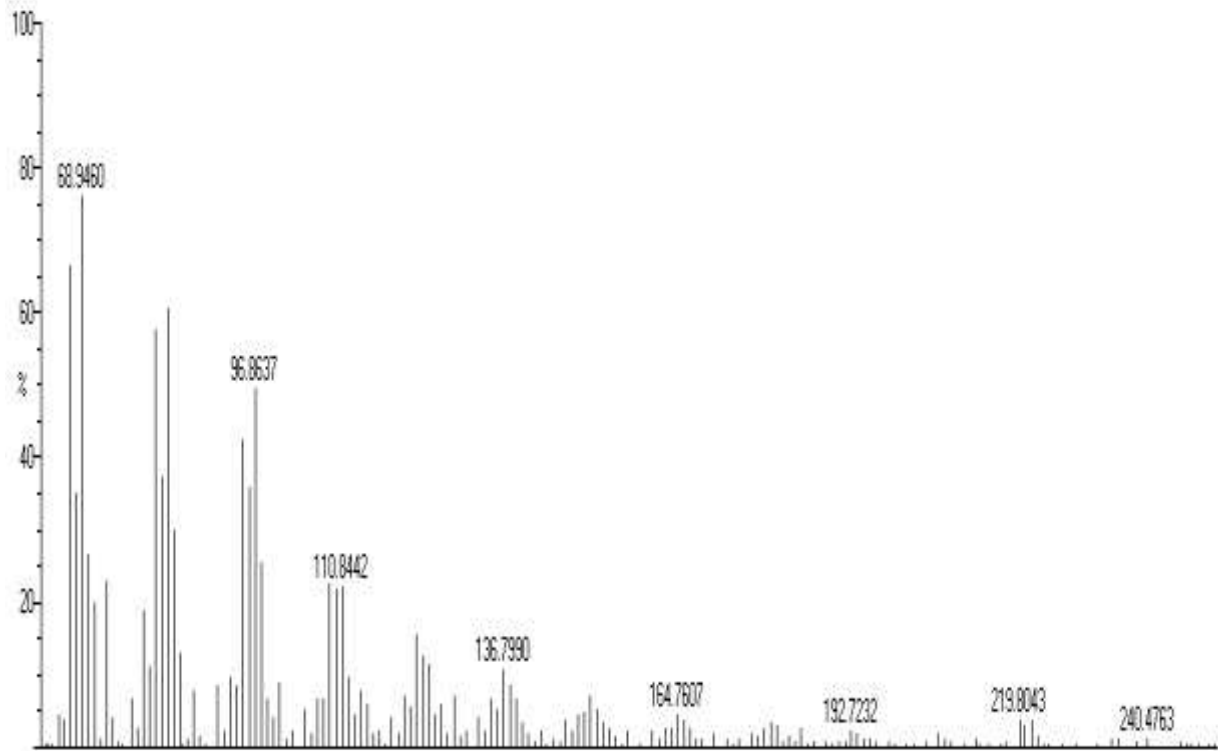
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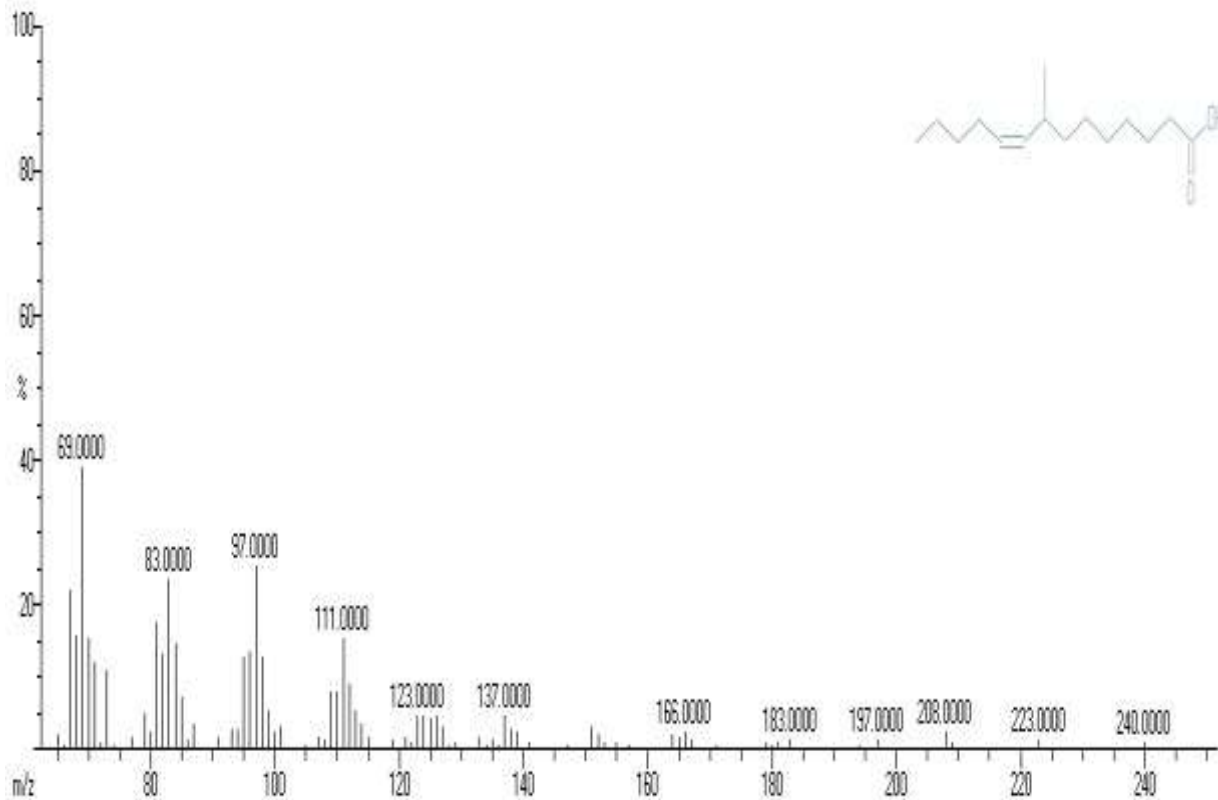
NIST MS 1 of 40 (2091-294) #Ions=227
9-Hexadecenoic acid



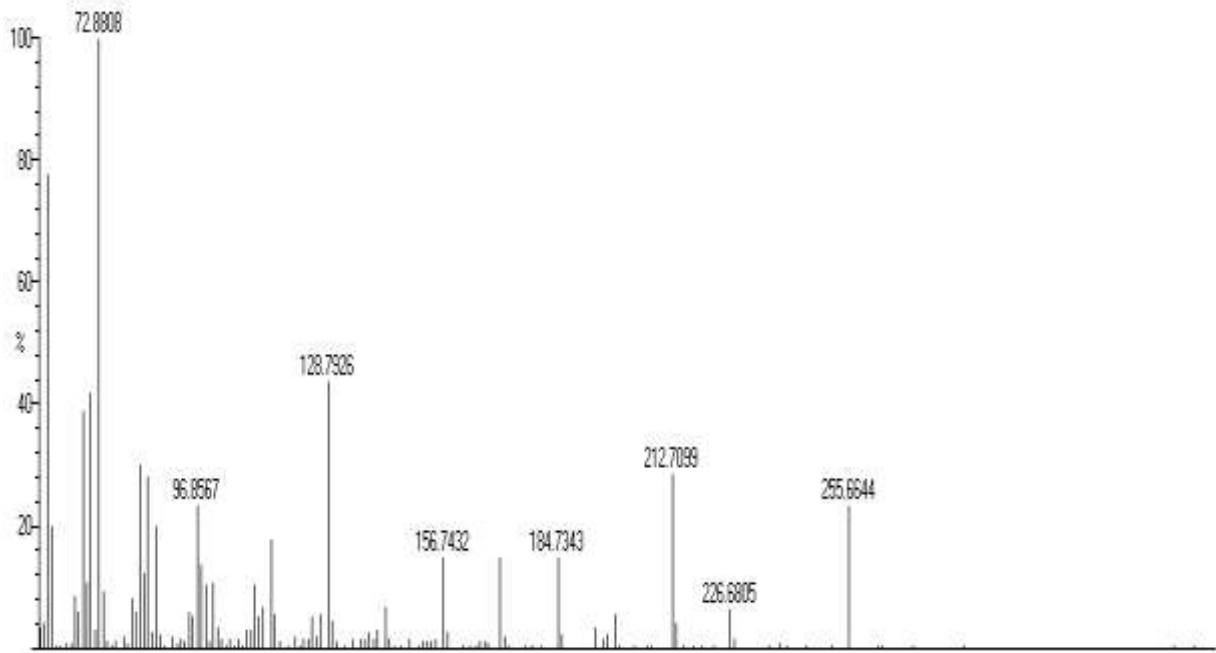
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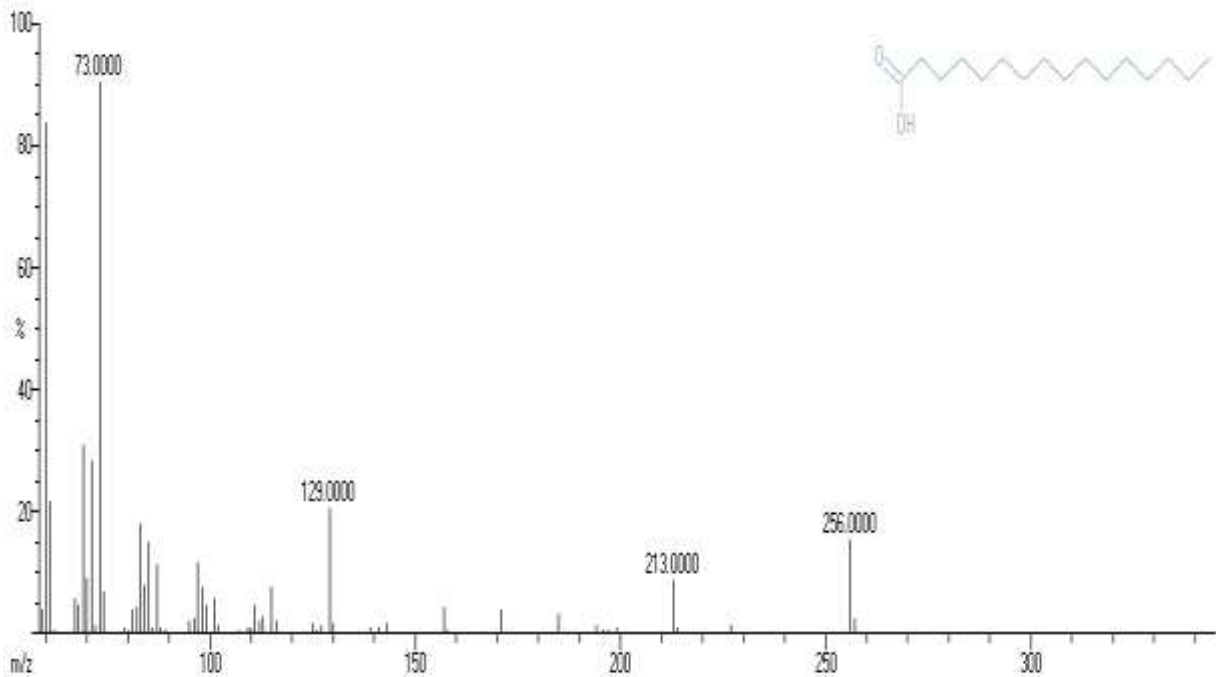
NIST MS 7 of 40 (DB# 15781) #Ions=168
Z-9-Methyl-9-tetradecenoic acid



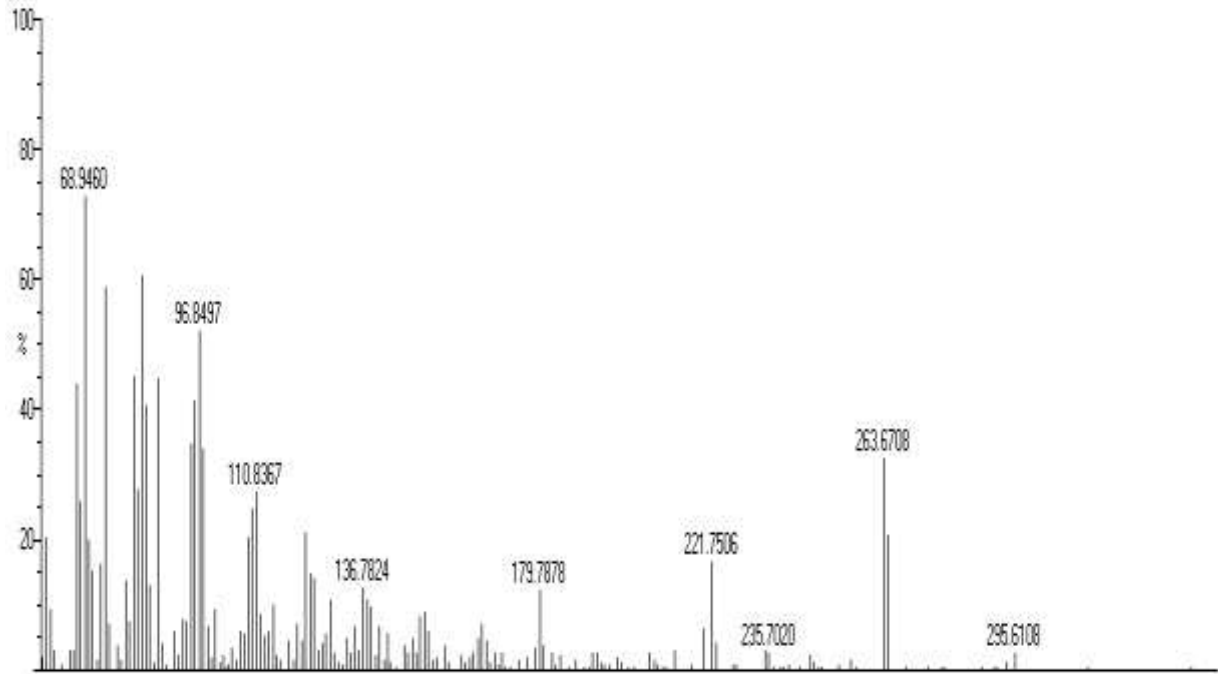
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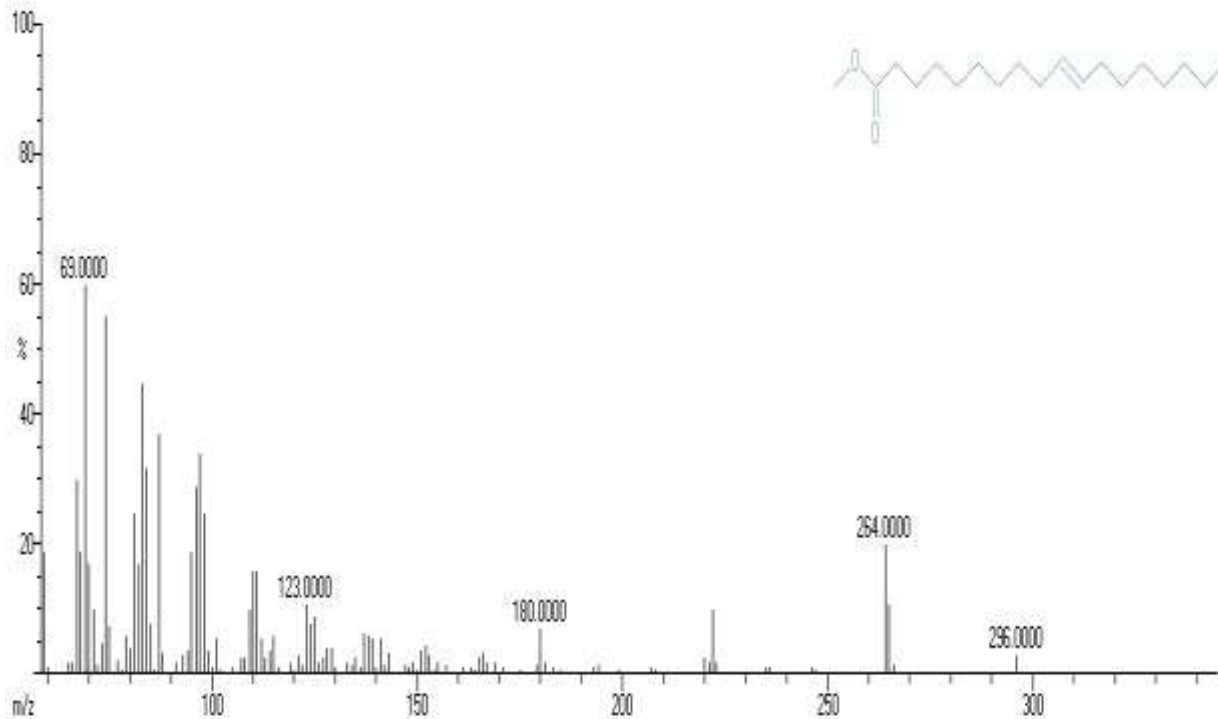
NIST MS 1 of 40 (57-10-3) #Ions=140
n-Hexadecanoic acid



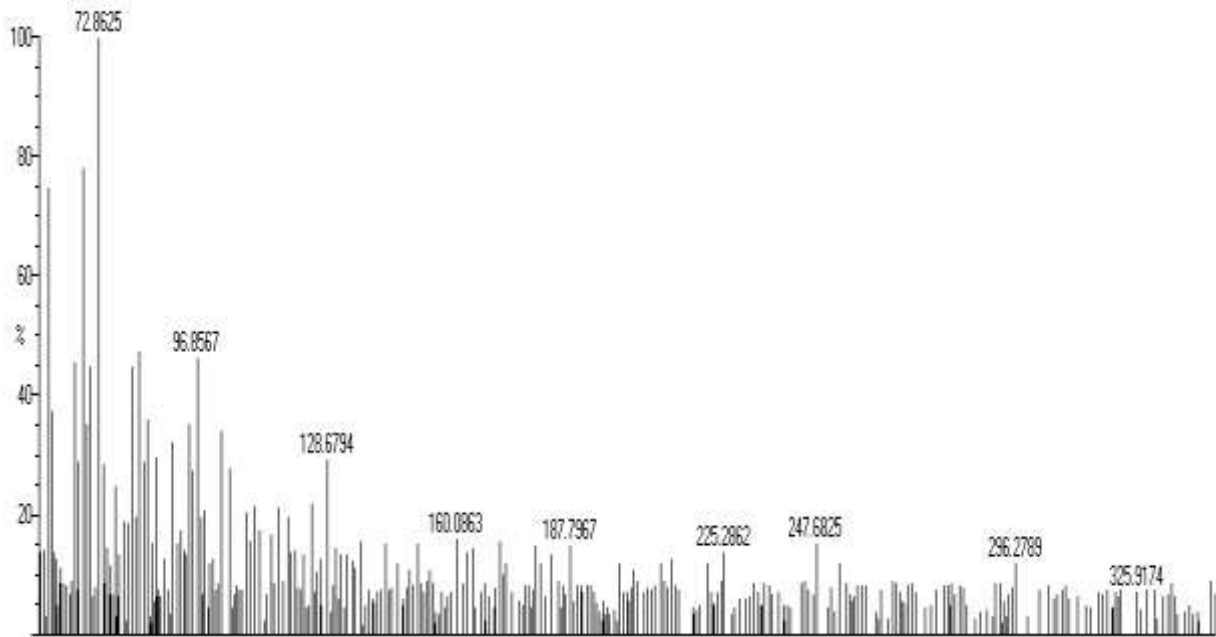
Scan: 807 TIC=2691621 Base=15.9%FS #Ions=485 RT=20.25



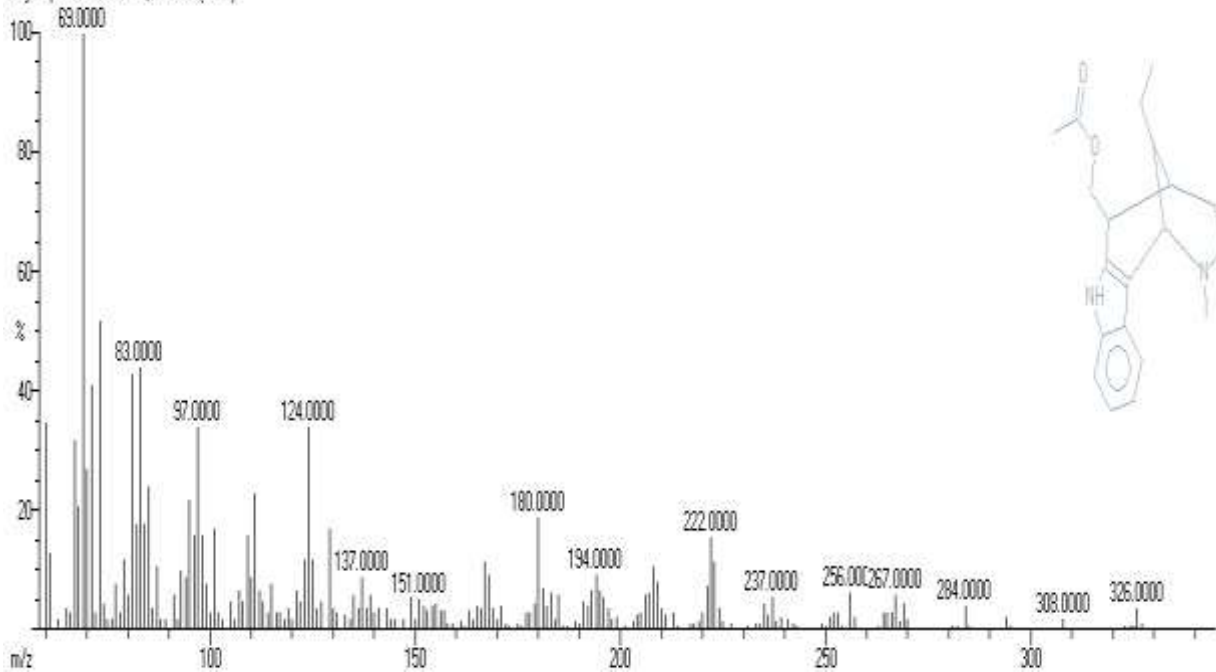
NIST MS 1 of 40 (13481-95-3) #Ions=249
10-Octadecenoic acid, methyl ester



Scan: 725 TIC=373133 Base=7%FS #Ions=541 RT=18.32



NIST MS 2 of 40 (56724-48-6) #Ions=223
Doxycycline-1-methanol, acetate (ester)



| S.NO | RETENTION TIME | PEAK AREA PERCENTAGE | Name of the Molecules Identified with NIST Library (3) |
|------|----------------|----------------------|--|
| 1. | 16.88 | 2.40 | Pentetic acid |
| 2. | 18.32 | 14.42 | Dasycarpidan-1-methanol,acetate |
| 3. | 19.20 | 18.02 | n-Hexadecanoic acid |
| 4. | 20.25 | 21.63 | 10-octadecenoic acid |
| 5. | 20.47 | 8.41 | Heptadecanoic acid, 16-methyl,methyl ester |
| 6. | 21.15 | 21.87 | Z-8-Methyl-9-tetradecenoic acid |
| 7. | 21.83 | 4.80 | 9-Hexadecenoic acid |
| 8. | 23.53 | 8.40 | 12-Methyl-E,E-2,13-Octadecadien-1-ol |

| S.NO | Name of the Components | Promising Medicinal Roles |
|------|--|---|
| 1. | Pentetic acid | Not known |
| 2. | Dasycarpidan-1-methanol,acetate | Anti-inflammatory, antifungal, antibacterial. |
| 3. | n-Hexadecanoic acid | Antibacterial, anti-inflammatory, antioxidant. |
| 4. | 10-octadecenoic acid | Antioxidant, antibacterial. |
| 5. | Heptadecanoic acid, 16-methyl,methyl ester | Anti-proliferative, inhibits cell proliferation in PC-9 non-small-cell lung cancer cells. |
| 6. | Z-8-Methyl-9-tetradecenoic acid | Antibacterial. |
| 7. | 9-Hexadecenoic acid | Antioxidant, anti-inflammatory. |
| 8. | 12-Methyl-E,E-2,13-Octadecadien-1-ol | Antioxidant. |

The Gas Chromatography-Mass Spectrometry analysis showed 8 components. Of these n-hexadecanoic acid (18.02%) has been studied for its significant antibacterial activity against 2 different strains of *Klebsiella pneumoniae* (DF30 & B40), *Pseudomonas aeruginosa*, *S.aureus*(3). Dasycarpidan-1-methanol (14.42%) has been studied for its antifungal activity. The molecular docking of the compound confirmed that Dasycarpidan-1-methanol gets successfully attached to fungi's 7EUS and 7EUT proteins, which play an important role in maintaining stability of cell structure(4). Studies on Heptadecanoic acid (8.41%) showed that the compound inhibits cell proliferation in PC-9 non-small cell lung cancer with acquired Gefitinib resistance(5).

CONCLUSION:

The GCMS analysis of the drug *Kandangaari nei* reveals several compounds, each having pharmacological activity relating to the indication of the drug. Studies on Compounds such as n-hexadecanoic acid, dasycarpidan-1-methanol, heptadecanoic acid have already shown their promising clinical roles, ensuring that the drug will be efficacious in treating respiratory ailments. As this is a preliminary study, further research must be addressed including preclinical/clinical studies for complete knowledge about efficacy of the drug.

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ent siddha medical college, Palayamkottai.

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