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# The GC MS Study of Poly Herbal Formulation Akkarakara Mezhugu Prepared as per Siddha Literature

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**ABSTRACT:**
AkkarakaraMezhugu is a polyherbal Siddha formulation traditionally used to treat various conditions, including Erigunmam, Valigunmam, Naavinsurasurappu, and Thosathisurangal. This study performed a phytochemical analysis of the formulation using Gas Chromatography-Mass Spectroscopy (GC-MS). GC-MS analysis of a concentrated ethanol extract identified seven primary bioactive constituents, primarily fatty acids, fatty acid esters, and steroidal compounds. The identified compounds Oleic acid,10-Octadecanoic acid methyl ester, n-Hexadecanoic, Hexadecanoic acid methyl ester, 8-Octadecanoic acid, methyl ester (E), Stigmastane 3,6-dione, and 3-Eicosene (E). Their presence likely contributes to the formulation's efficacy in managing gastrointestinal disorders like Gunmamnoi. Further clinical and toxicological studies are recommended to fully validate its safety and mechanisms of action.

**Keywords:** Akkarakaramezhugu, Siddha medicine, GC-MS, Peptic ulcer

## INTRODUCTION:

In Siddha system of medicine, 2 types of medicines (Internal and External) are used according to their mode of usages. Mezhugu is an internal medicine prepared by the way of grinding the ingredients with certain juices, extracts or honey till the soft waxy consistency is attained. Mezhugu the name is given for its waxy consistency. The semisolid consistency of this medicine is attained by the addition of mucilaginous or pectinaceous media, oils, fats, waxes or butter. It retains its potency for five years when stored in an airtight glass container. The formulation of Akkarakaramezhugu is given in the textbook of Chikicharathnadeepamennumvaithiyanool written by C. Kannusamypillai.

## MATERIALS AND METHODS:

**Ingredients of Akkarakaramezhugu:**

| S.NO | NAME OF THE DRUG | BOTANICAL NAME | ENGLISH NAME | PARTS USED |
|---|---|---|---|---|
| 1 | AKKARAKARAM | Anacyclus pyrethrum | Pellithry root | Root |
| 2 | THIPPILI | Piper longum | Long pepper | Fruit |
| 3 | KOSHTAM | Costusspeciosus | Costus root | Underground |

| | | | | |
|---|---|---|---|---|
| | | | | stem |
| 4 | SITHARATHAI | Alpiniaofficinarum | Lesser galanga | Underground stem |
| 5 | Kirambu | Syzygiumaromaticum | Clove | Flower |
| 6 | POORAM | | Hydragyrumsubchloride (calomel) | |
| 7 | THAEN | | Honey | |

**PROCEDURE:**

Akkakararam kalanju-10, Thippili kalanju-7, Koshtam kalanju-4, Sitharthai kalanju-8, Kirambu kalanju-7 are separately purified and powdered, add these powders into a stone mortar. Add two kalanju of fine powederedpuram and grind these drugs with honey. The mixture should be ground continously by two people, without taking break. Grind it for 3 days, at a rate of 2 samam per day (totally 6 hours grinding daily).

**AKKARAKARAM**

**THIPPILI**

**KOSHTAM**

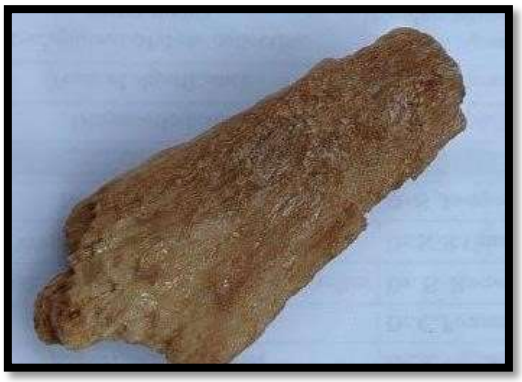
**SITHARATHAI**

**KIRAMBU**

**POORAM SUTHIKU MUN**

**POORAM PURIFICATION PROCESS**

**POORAM SUTHIKU PIN**



**VASTHIRAKAYAM**

**GAS CHROMATOGRAPHY- MASS SPECTROSCOPY ANALYSIS FOR THE DRUG AKM FOR PHYTOCHEMICAL ANALYSIS DERIVATIZATION PROCEDURE**

For the crude 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 _m). 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 sample-SA(diluted with methanol 1:10) were injected in the splitless mode. The percentage of composition of the samples were calculated by the GC peak areas. 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).

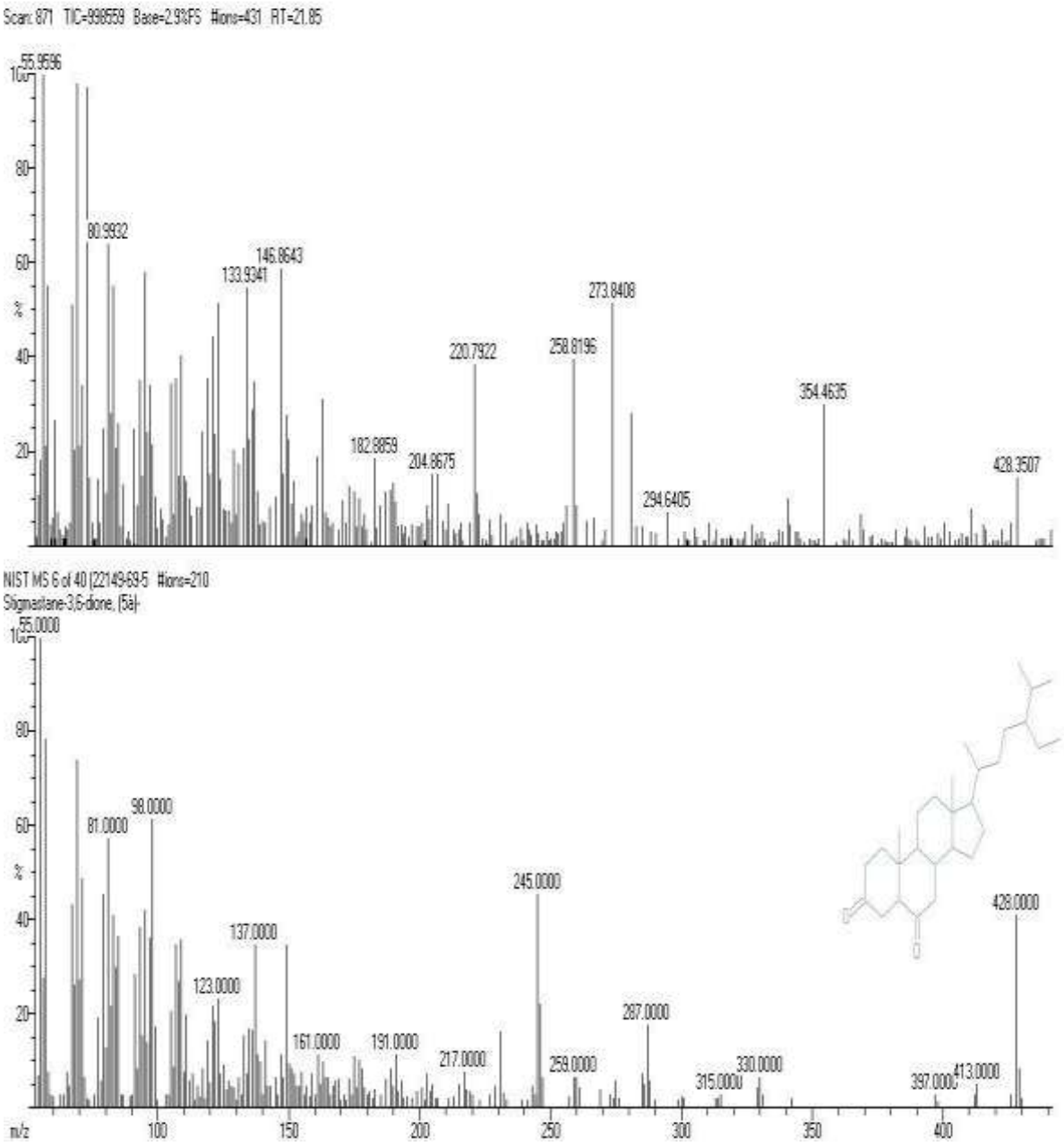
**RESULTS AND OBSERVATIONS**

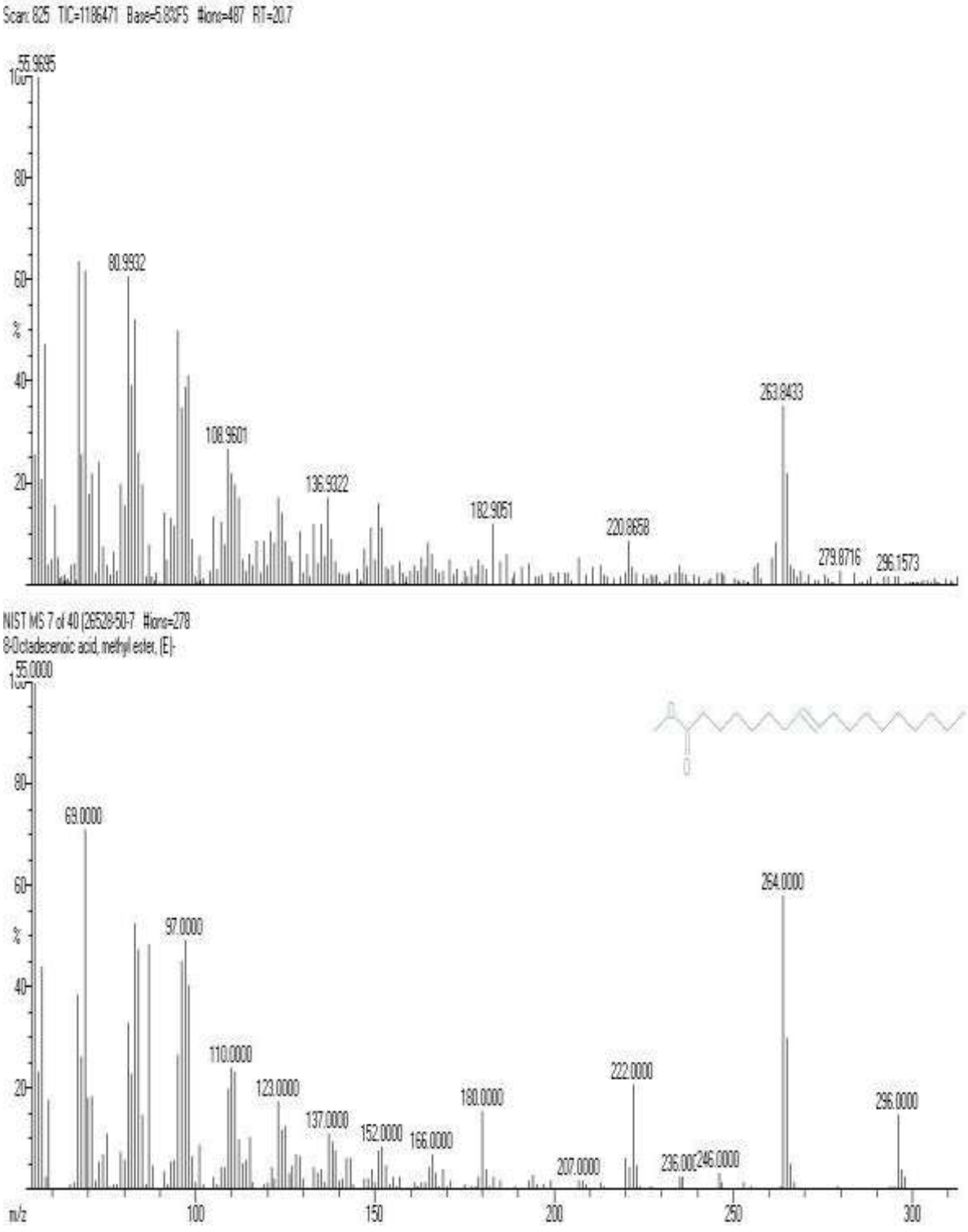
Gas chromatography mass spectroscopy analysis was carried out in crude extracts of the AKM such as ethanol extract. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed of GC-MS analysis of the extracts are given in figures. Phytochemical analysis by GCMS analysis of the AKM revealed the presence of different fatty acids, heterocyclic compounds etc.

**DISCUSSION**

The GCMS analysis of various compounds from AKM extracts was performed using JEOL GC-Mate-II with HP- 5 capillary column and typical total ion chromatograms (TIC) of each sample were given. The comparison of the mass spectrums with the data base gave more than 90% match as well as confirmatory compound structure match. The GCMS analysis of the concentrated ethanol extract resulted many compounds which have diverse in use.The concentrated ethanol extract contains a variety of fatty acids and derivative compounds.

## GC MS ANALYSIS REPORT:

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296.1573
NIST MS 7 of 40 (26528-50-7 #ions=278
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69.0000
97.0000
110.0000
123.0000
137.0000
152.0000
166.0000
180.0000
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222.0000
236.0000
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%
m/z
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150
200
250
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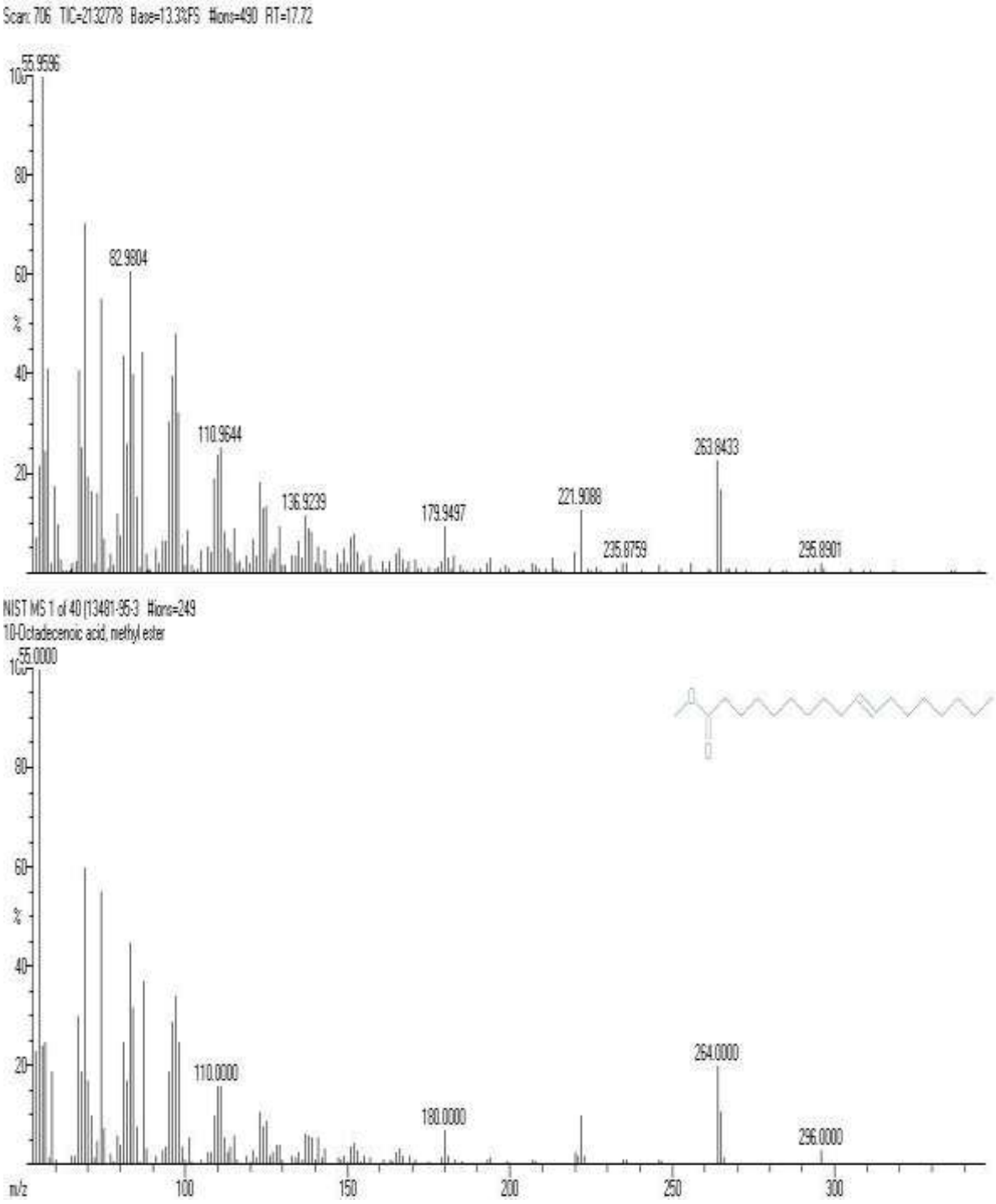
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m/z
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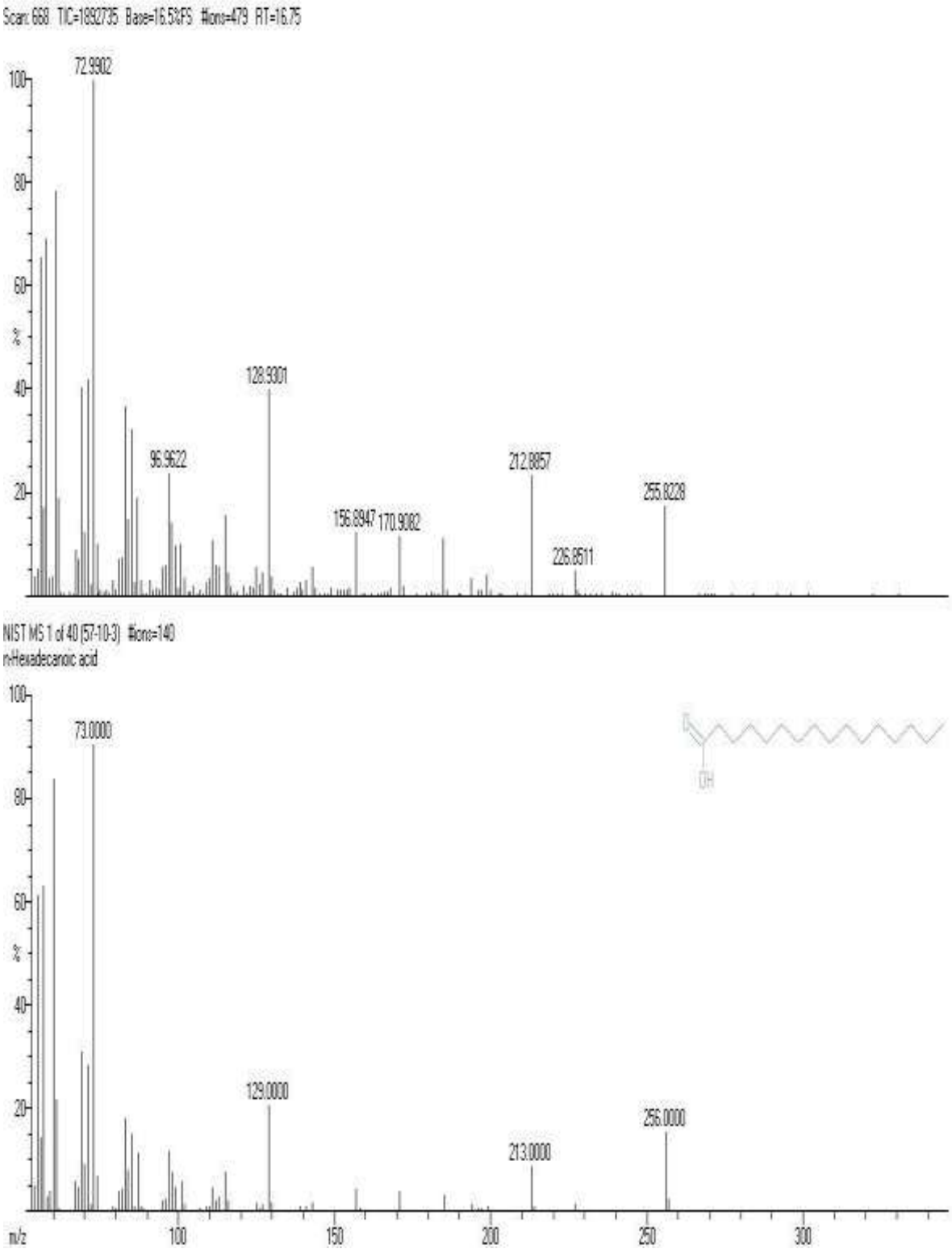
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256.0000
213.0000
m/z
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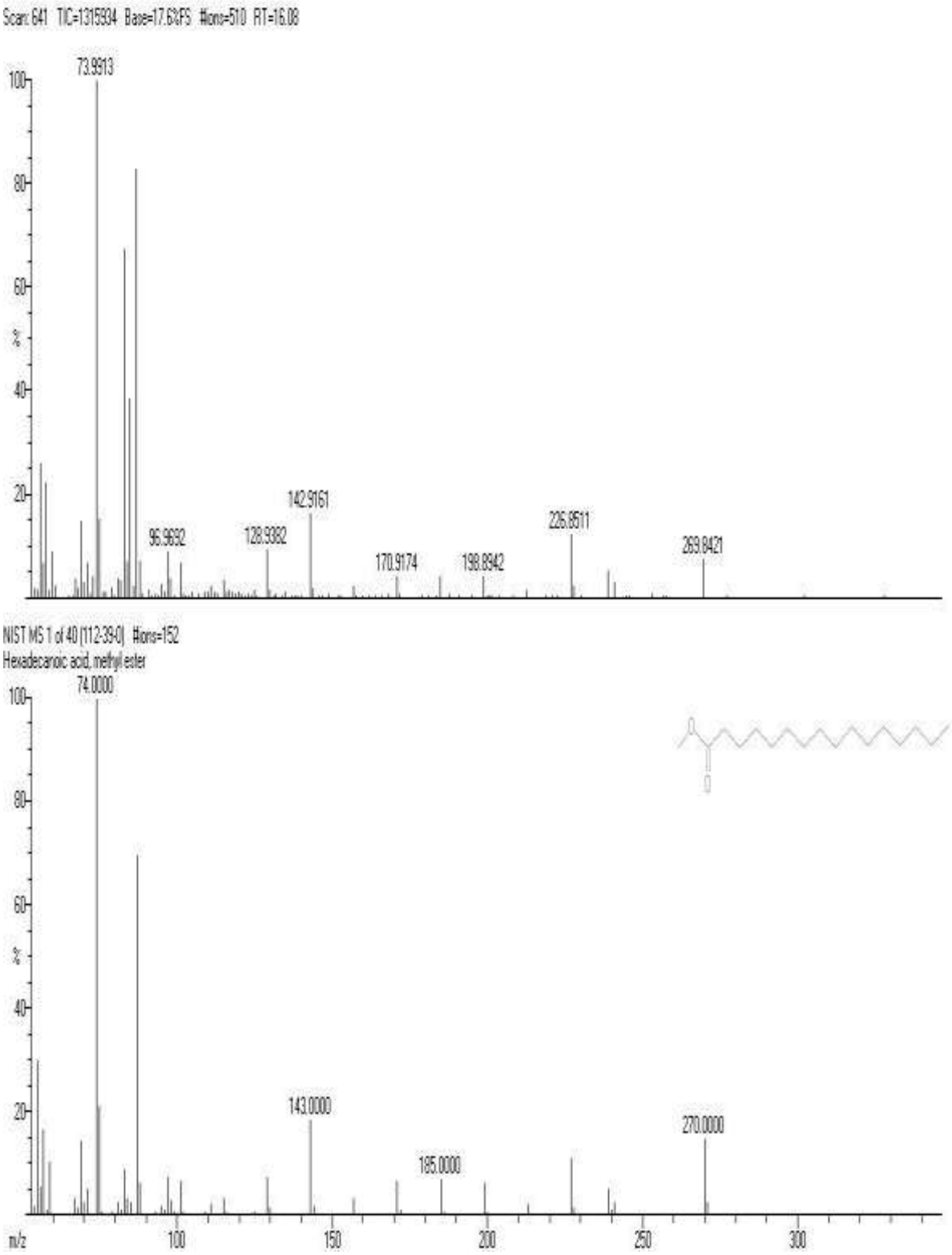
150
200
250
300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%
40
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m/z
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200
250
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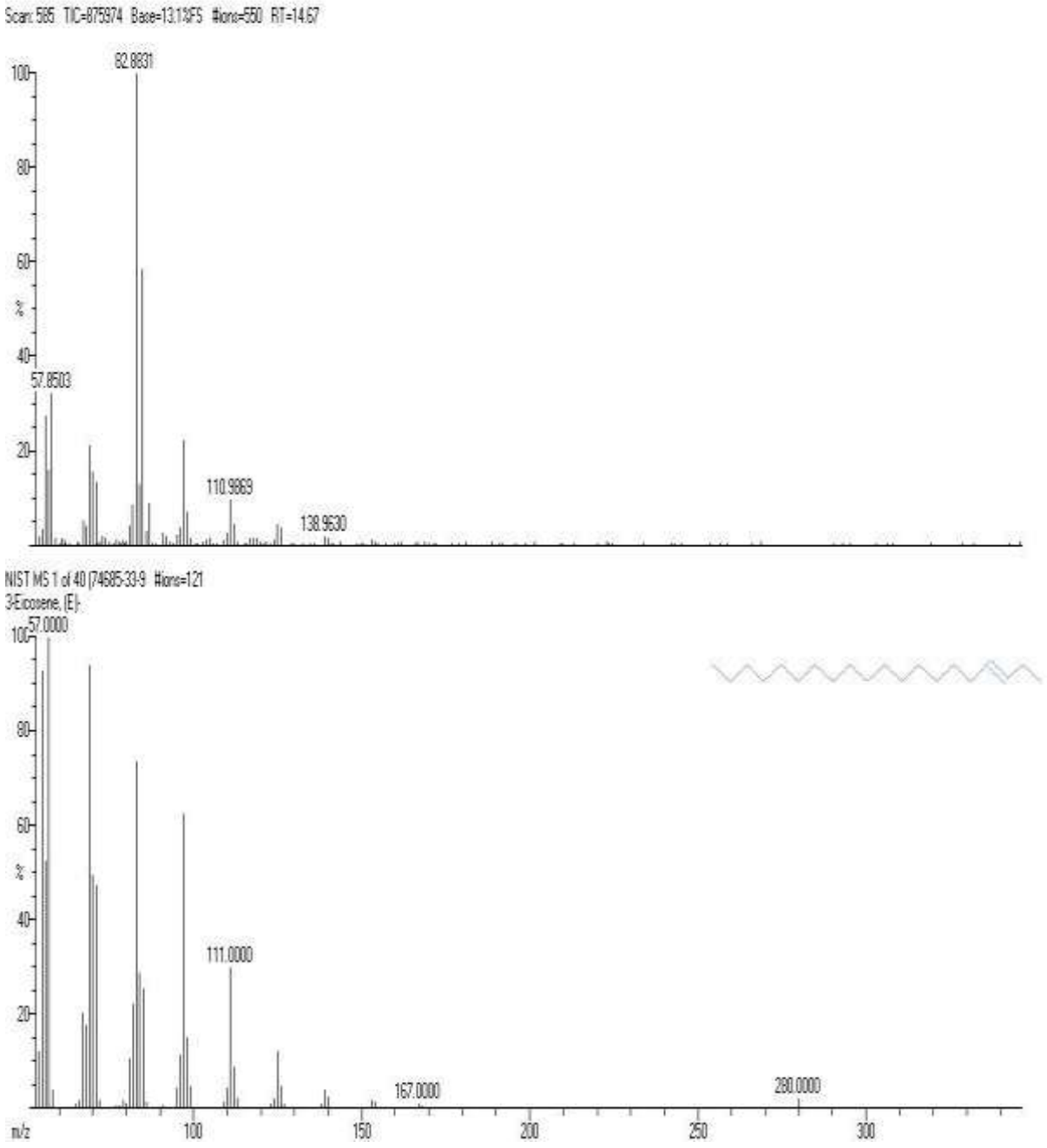


**GC MS PROFILE OF AKKARAKARA MEZHUGU:**

| S.NO | RETENTION TIME | PEAK AREA PERCENTAGE | Name of the Molecules Identified with NIST Library |
|---|---|---|---|
| **1.** | 14.67 | 3.70 | 3 – Eicosene (E) |
| **2.** | 16.08 | 11.0 | Hexadecanoic acid, Methyl ester |
| **3.** | 16.75 | 20.44 | n-Hexadecanoic acid |
| **4.** | 17.72 | 26.15 | 10-Octadecanoic acid, Methyl ester |

| | | | |
|---|---|---|---|
| **5.** | 18.43 | 28.30 | Oleic acid |
| **6.** | 20.70 | 8.50 | 8-Octadecanoic acid, Methyl ester (E) |
| **7.** | 21.85 | 4.70 | Stigmastane 3, 6 dione |

| S.NO | NAME OF THE COMPONENTS | PROMISING MEDICINAL ROLE |
|---|---|---|
| 1 | 3 Eicosene (E) | Antimicrobial,anti fungal antihyperglycemic, cytotoxic antioxidant, insecticidal &Pheromonal activity. |
| 2 | Hexadecanoic acid methyl ester | Antibacterial, antifungal, antioxidant, anti inflammatory, anti arthritic activity. |
| 3 | N-Hexadecanoic acid | Anti inflammatory, antibacterial, antioxidant, anticancer, neurotrophic effect, antifungal |
| 4 | 10-Octadecanoic acid methyl ester | Antibacterial, antifungal, antioxidant, decrease blood cholesterol |
| 5 | Stigmastane 3, 6 dione | Anti inflammatory, immunostimulant, antibacterial |
| 6 | 8-Octadecanoic acid | Anti inflammatory, antitumor, antibacterial |
| 7 | Oleic acid | Cardioprotective activity, Anti inflammatory, wound healing, Anti cancer |

**DISCUSSION:**

GC MS analysis of the ethanol extract of akkarakaramezhugu revealed the presence of several bioactive compounds, mainly fatty acids, fatty acid esters and steroidal compounds. The identified compounds included 3-Eicosene (E), Hexadecanoic acid methyl ester, n-Hexadecanoic acid, 10-octadecanoic acid methyl ester, oleic acid, 8-octadecaconoic acid methyl ester (E) and stigmastane 3,6-dione. These phytoconstituents may contribute to the therapeutic efficacy of the formulation.

Among the identified compounds, oleic acid showed the highest peak area percentage, indicating that it is one of the major constituents present in the formulation. Oleic acid is known for its anti-inflammatory and protective effects on the gastrointestinal mucosa, which may contribute to the therapeutic potential of akkarakaramezhugu in the management of gunmamnoi. Similarly, n hexadecanoic acid and hexadecanoic acid methyl ester possess significant anti-inflammatory and antioxidant properties that may help reduce gastric irritation and oxidative stress.

The presence of 10-octadecanoic acid methyl ester and 8-octadecanoic acid methyl ester suggests possible antimicrobial and cholesterol lowering activities. These compounds may support digestive health and improve metabolic balance. Stigmastane-3,6-dione identified in the extract is also reported to exhibit immunostimulant and antibacterial activities, which may enhance the overall efficacy of the formulation.

The synergistic action of these phytoconstituents may be responsible for the therapeutic effects of akkarakaramezhugu described in siddha literature. The grinding process with honey and prolonged trituration may also enhance the bioavailability and potency of the formulation.Thus, the study confirms that akkarakaramezhugu contains several biologically active compounds with medicinal importance.further pharmacological, toxicological, and clinical studies are required to validate its safety, efficacy, and mechanism of action in the management of gastrointestinal disorders.

**CONCLUSION:**

The preliminary phytoconstituents of Akkarakaramezhugu has been analysed by GCMS studies. GC-MS analysis successfully identified several bioactive constituents, primarily fatty acids, fatty acid esters, and steroidal compounds, such as Oleic acid and n-Hexadecanoic acid. The identified compounds possess antimicrobial, anti-inflammatory, antioxidant, and immunostimulant properties, which likely contribute to the medicine's efficacy in treating gastrointestinal disorders like Gunmamnoi.

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