

GC MS study on *siddha* formulation *Kizhanelli Thylam*

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Abstract

The present study deals with GC MS analysis of one siddha medicine formulation *KIZHANELLI THYLAM* which is prescribed for pitha related diseases such as kirukiruppu(giddiness),vayil neerural(excessive salivation),kai kal erichal(peripheral neuritis),kannerichal(burning eyes) and pitha paithiyam(psychiatric diseases).It is used as external bath oil. *KIZHANELLI THYLAM* prepared as per siddha literature Patharthaguna villakkam (Meteria Medica).*KIZHANELLI THYLAM* underwent standard procedure of GC MS analysis.GCMS analysis revealed the presence of 14 preliminary chemical components present in *kizhanelli thylam*.These components are D-Limonene, Ocimene, 3-Carene, Cyclohexonal,5-methyl-2-(1-methylethenyl), Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, Caryophyllene, Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-(1s-cis) and Caryophyllene oxide.The relationship between the claimed medicinal roles and the presence of biomolecules, as indicated by GC MS seems encouraging.

Keywords: *KIZHANELLI THYLAM*; Bath oil; GC MS Analysis; Pitha Diseases

1. Introduction

Siddha system is the most ancient and indigenous system of indian medicine.*Thylam* is important siddha formulation used as medicine in both internal and external application.The medicated oils are used as bath oil.Oil bath is one of the imperative practice.Regular practice of oil bath relaxes and rejuvenates us both physically and mentally.It is believed by the people of ancient time taking oil bath will improve and keep their good health.

According to *siddha* literature *Padhartha Guna Vilakkam* " Meteria Medica(vegetable kingdom)" *KIZHANELLI THYLAM* is one of the important *thylam* used as bath oil to treat *pitha* related diseases such as *kirukiruppu*(giddiness),*vayil neerural*(excessive salivation),*kai kal erichal*(peripheral neuritis),*kannerichal*(burning eyes) and *pitha paithiyam*(psychiatric diseases).However,the characteristics of traditional *siddha* medicine are systematize,multitarget and multichannel due to their complex chemical constituents and there is no study about the significant evidence of biomolecules present in it.This needs to be studied and scientifically understood. So,I took this study to find out the chemical components of *KIZHANELLI THYLAM*.

2. Material and methods

Ingredients of *KIZHANELLI THYLAM*:

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- *Kizhanelli (Phyllanthus amarus)*
- *kadukkai (Terminalia chebula)*
- Neem resin (*Azadirachta indica*)
- Gingelly oil (*Sesamum indicum*)
- Cow's Milk
- water
- Country sugar(*nattu sarkarai*)

2.1. *Phyllanthus amarus* (kizhanelli)

2.1.1. Therapeutic actions

Kizhanelli plant has many pharmacological activities like antioxidant, antiviral, Hepatoprotective and Nephroprotective.

2.2. *Terminalia chebula* (kadukkai)

2.2.1. Therapeutic actions

The extracts of kadukkai plant exhibit Anti-inflammatory, Anthelmintic and antioxidant activities.

2.3. Neem Resin

2.3.1. Therapeutic action

Neem resin have Antimicrobial Activity

2.4. Sesame oil (gingelly oil)

2.4.1. Therapeutic actions

Sesame oil have various pharmacological activities such as Antiaging, Demulcent, Anticancer, antioxidant, Antimicrobial and Vasorelaxant.

2.4.2. Authentication of raw drugs

Plant origin drug were identified and authenticated by the experts in PG Gunapadam Department, Government Siddha Medical College, Palayamkottai.

2.4.3. Procedure

The whole plant of *phyllanthus amarus* was collected and dried. 875gm of plant was cut into small pieces and boiled with 5.2 liter of water in the mud pot till the decoction concentrated to 1.3 liter and filtered. Each 17.5gm of *terminalia chebula* and neem resin were grounded with sufficient quantity of milk and made into paste. The paste, 1.3 liter of gingelly oil and decoction mixed together. Then it was boiled well until the confection in the oil converted into the waxy stage (*mezhu padham*). In that stage 17.5 gm of country sugar was sprinkled over the oil. Then this oil was filtered and cooled. Finally filtered oil was stored in closed container.

GC MS Analysis of prepared sample of *Kizhanelli Thyam* was conducted at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Chennai.

2.4.4. GC MS ANALYSIS

For the oil 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 0.45µm membrane filter to a vial.

2.4.5. 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).

3. Results and discussion

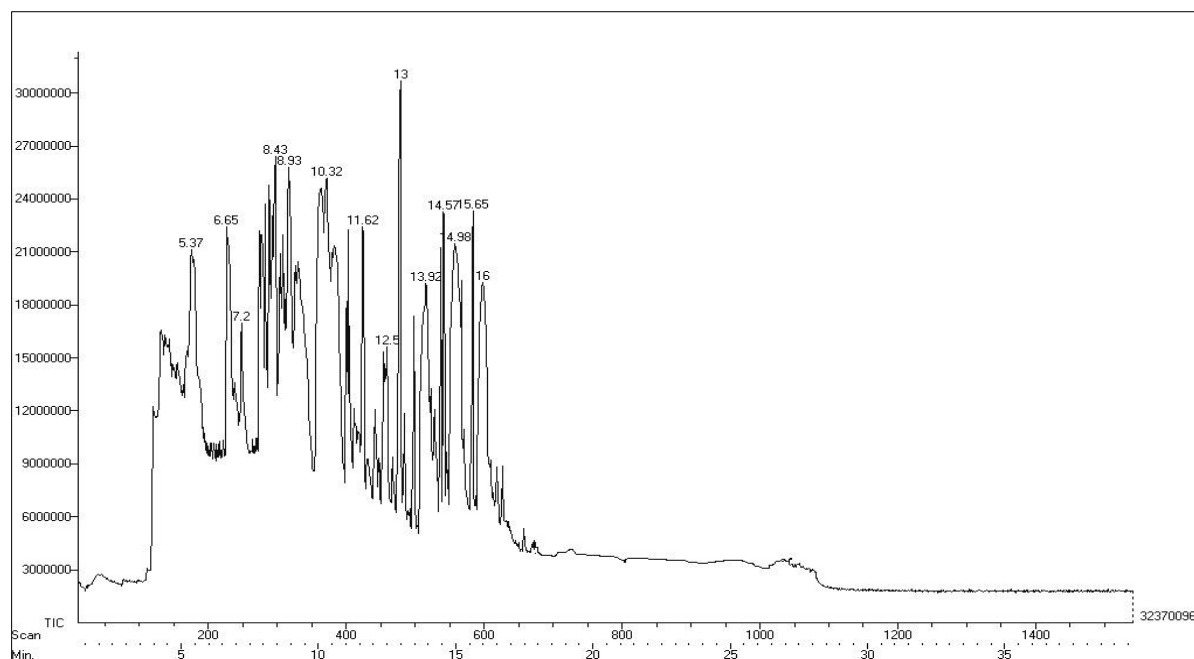
In the present study 14 chemical compounds have been identified. They were D-Limonene, Ocimene, 3-Carene, Cyclohexonal, 5-methyl-2-(1-methylethenyl), imonene-1,2-epoxide(fr.1), Bicyclo(3.1.1)hept-3-en-2-ol, 4,6,6-trimethyl, Limonene-1,2-epoxide(fr.1), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, Caryophyllene, Dimethyl phthalate, Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-(1s-cis), 1,2-benzenedicarboxylic acid ethyl methyl ester, Caryophyllene oxide and 1,4-benzenedicarboxylic acid diethyl ester. The active principles with their retention time and peak area % were tabulated in Table 1. Components and their medicinal roles were also tabulated in Table 2. Some of them namely D-Limonene shows Anti-inflammatory, anticancer activity. Ocimene shows Antiviral, anti-inflammatory, antifungal, anticonvulsant activities. 3-Carene shows Anti-inflammatory, antifungal, sedative, Antispasmodic activities and Cyclohexonal, 5-methyl-2-(1-methylethenyl) shows Anti-epileptic, Anti-anxiety, Anti-inflammatory, Antiviral, anesthetic activity. GC MS graphs were given in Figure 1-15.

Table 1 GC MS Profile Of KIZHANELLI THYLAM

S.NO	RETENTION TIME	PEAK AREA PERCENTAGE	Name of the Molecules Identified with NIST Library
1.	5.37	4.5	D-Limonene
2.	6.65	6.8	Ocimene
3.	7.2	3.0	3-Carene
4.	8.43	4.5	Cyclohexonal, 5-methyl-2-(1-methylethenyl)
5.	8.94	4.5	Limonene-1,2-epoxide(fr.1)
6.	10.32	9.1	Bicyclo(3.1.1)hept-3-en-2-ol, 4,6,6-trimethyl
7.	11.62	7.6	Limonene-1,2-epoxide(fr.1)
8.	12.5	5.3	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-
9.	13	14.5	Caryophyllene
10.	13.92	6.8	Dimethyl phthalate
11.	14.57	8.4	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-(1s-cis)
12.	14.98	8.8	1,2-benzenedicarboxylic acid ethyl methyl ester
13.	15.65	9.1	Caryophyllene oxide
14.	16	6.1	1,4-benzenedicarboxylic acid diethyl ester

Table 2 Medicinal Role Of Components

S.NO	Name of the Components	Promising Medicinal Roles
1.	D-Limonene	Anti-inflammatory, anticancer activity, Used in obesity.
2.	Ocimene	Antiviral, anti-inflammatory, antifungal, anticonvulsant activities.
3.	3-Carene	Anti-inflammatory, antifungal, sedative, Antispasmodic activities.
4.	Cyclohexanal,5-methyl-2-(1-methylethenyl)	Anti-epileptic, Anti-anxiety, Anti-inflammatory, Antiviral, anesthetic activity.
5.	Limonene-1,2-epoxide(fr.1)	Not known
6.	Bicyclo(3.1.1)hept-3-en-2-ol,4,6,6-trimethyl	Not known
7.	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	Antitumor activity
8.	Caryophyllene	Anti-inflammatory, Used in osteoarthritis, diabetes mellitus, anxiety, and liver fibrosis.
9.	Dimethyl phthalate	Not known
10.	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-(1s-cis)	Anti-inflammatory, antibacterial, anti tumor, antiviral, antifungal activities.
11.	1,2-benzenedicarboxylic acid ethyl methyl ester	Not known
12.	Caryophyllene oxide	Antifungal, Antiplatelet aggregation activity.
13.	1,4-benzenedicarboxylic acid diethyl ester	Not known

**Figure 1** GC Profile of Kizhanelli Thylam

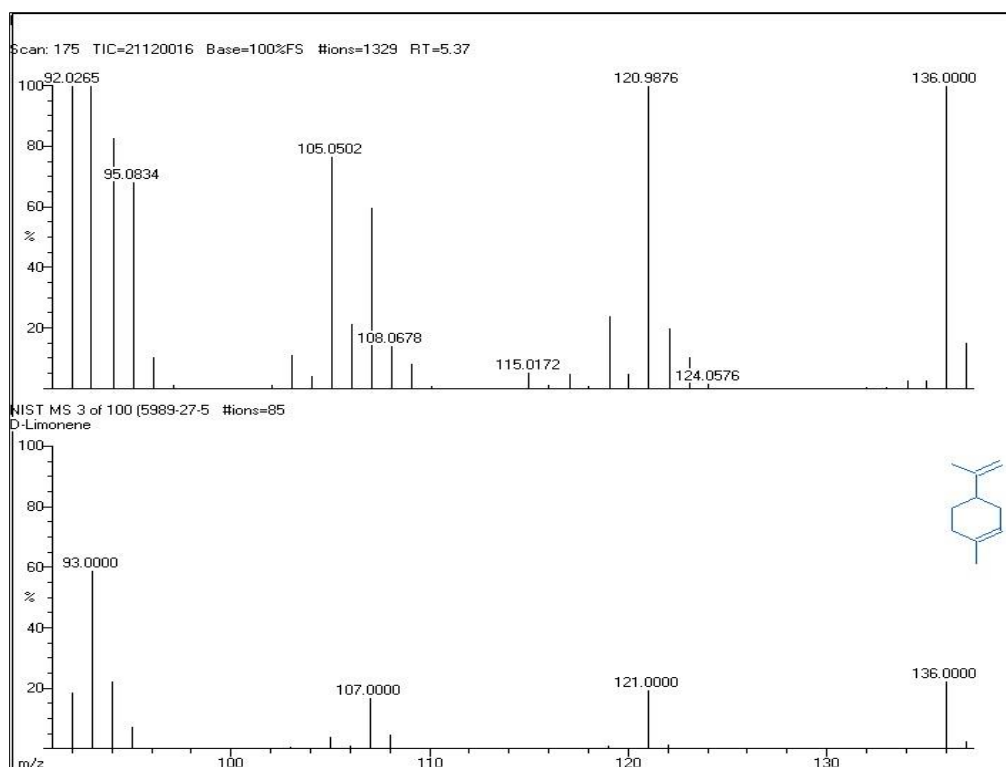


Figure 2 D-Limonene

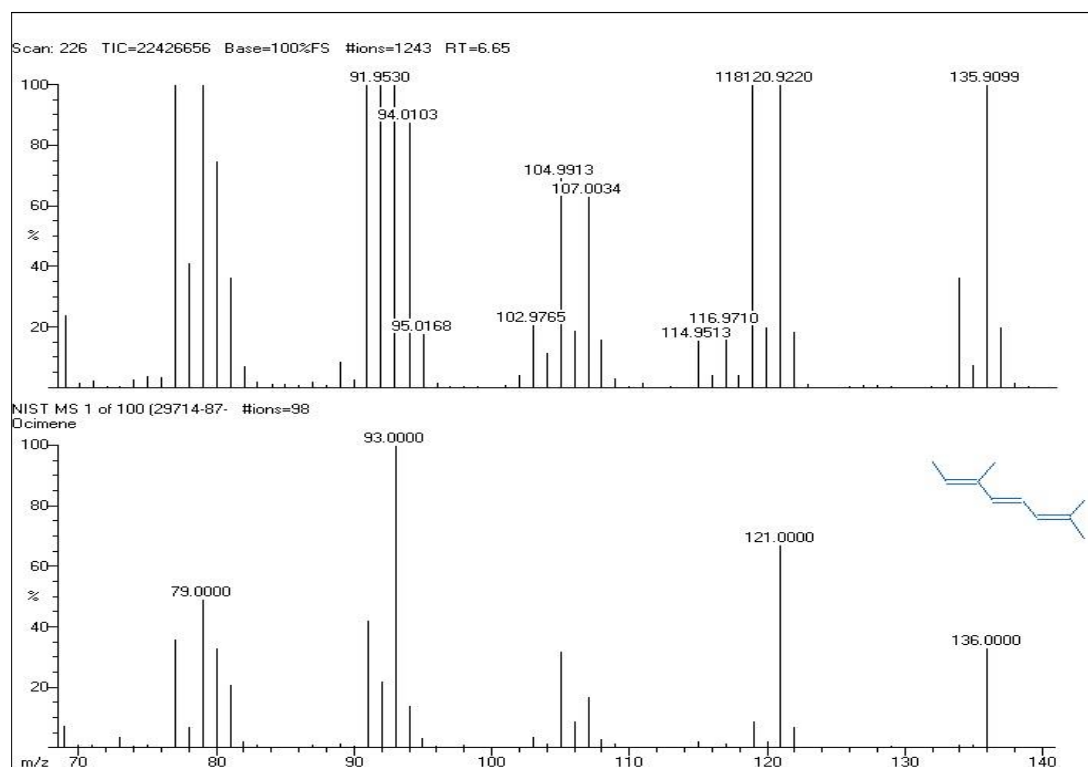


Figure 3 Ocimene

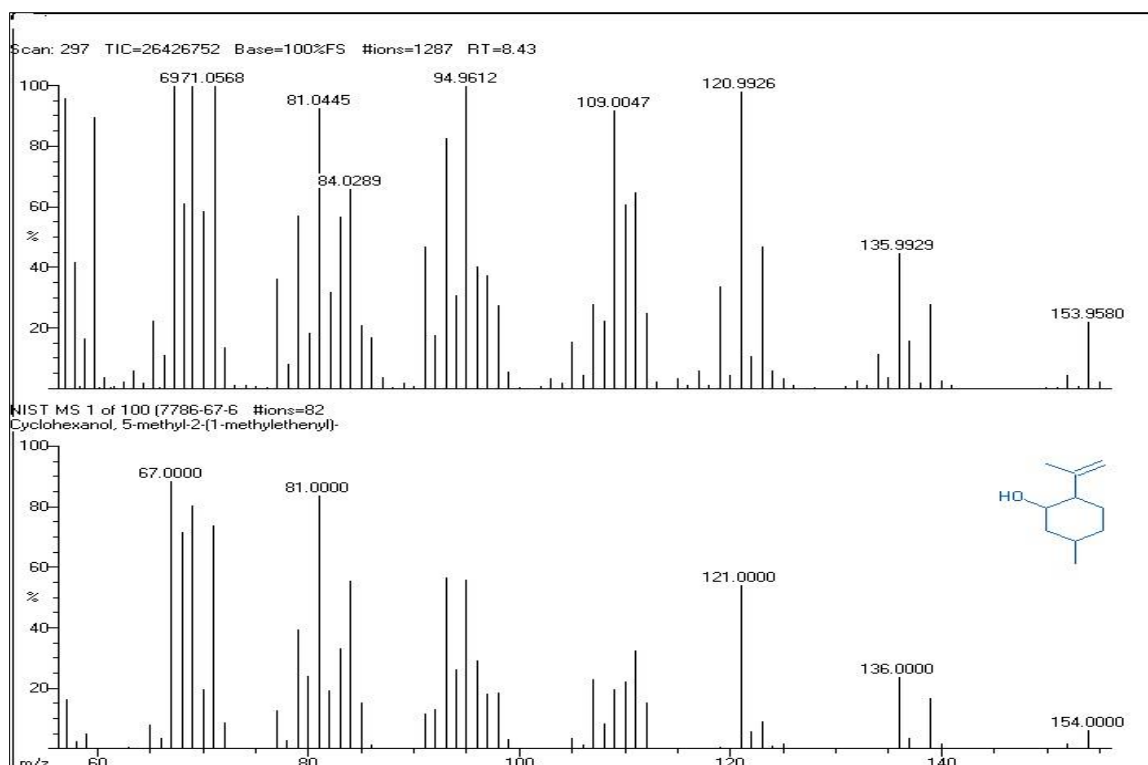


Figure 5 Cyclohexonal,5-methyl-2-(1-methylethenyl)

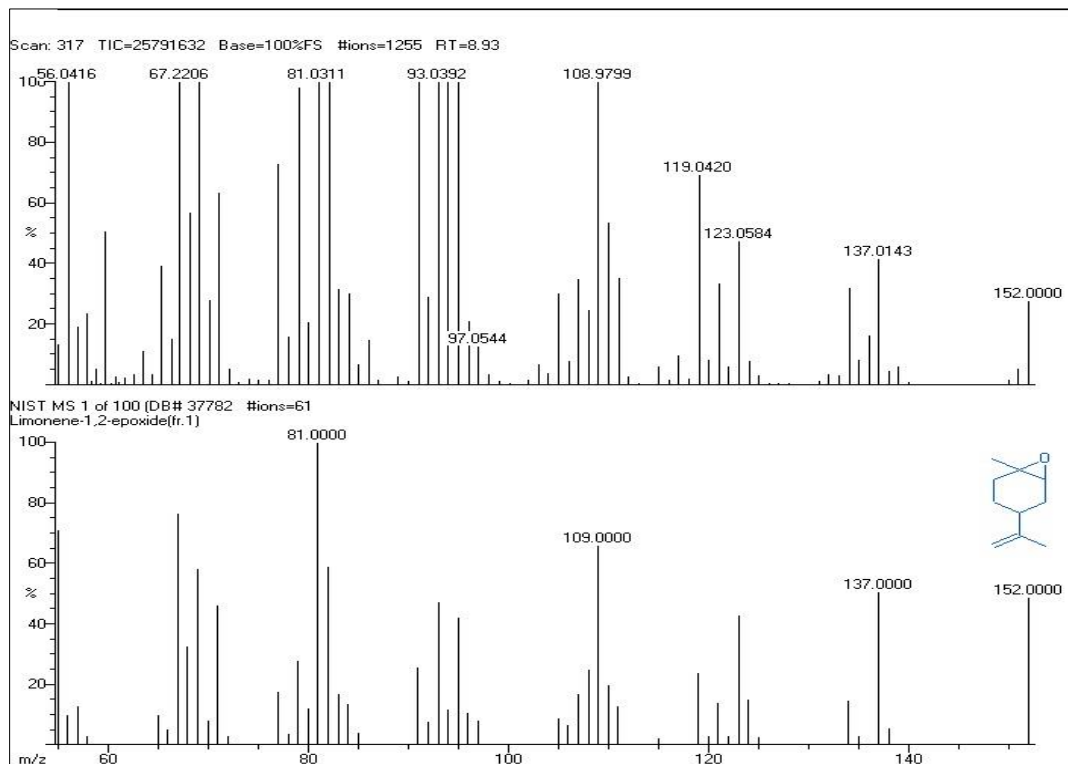


Figure 6 Limonene-1,2-epoxide(fr.1)

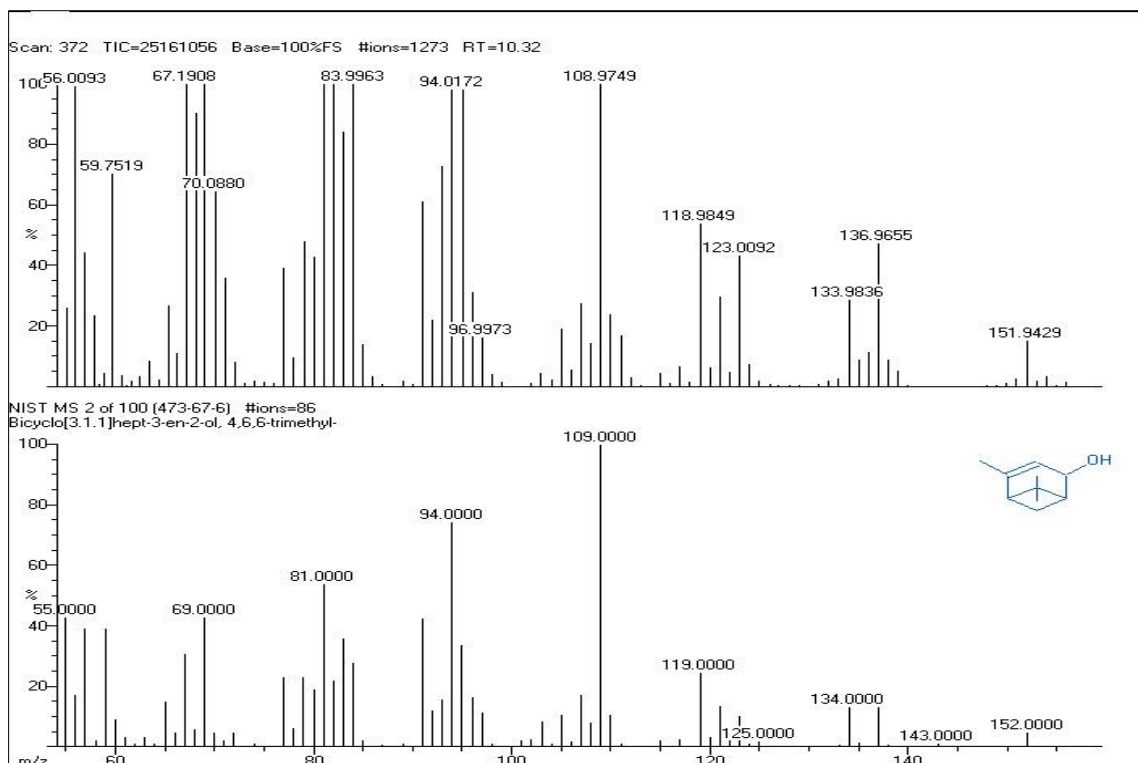


Figure 7 Bicyclo(3.1.1)hept-3-en-2-ol,4,6,6-trimethyl

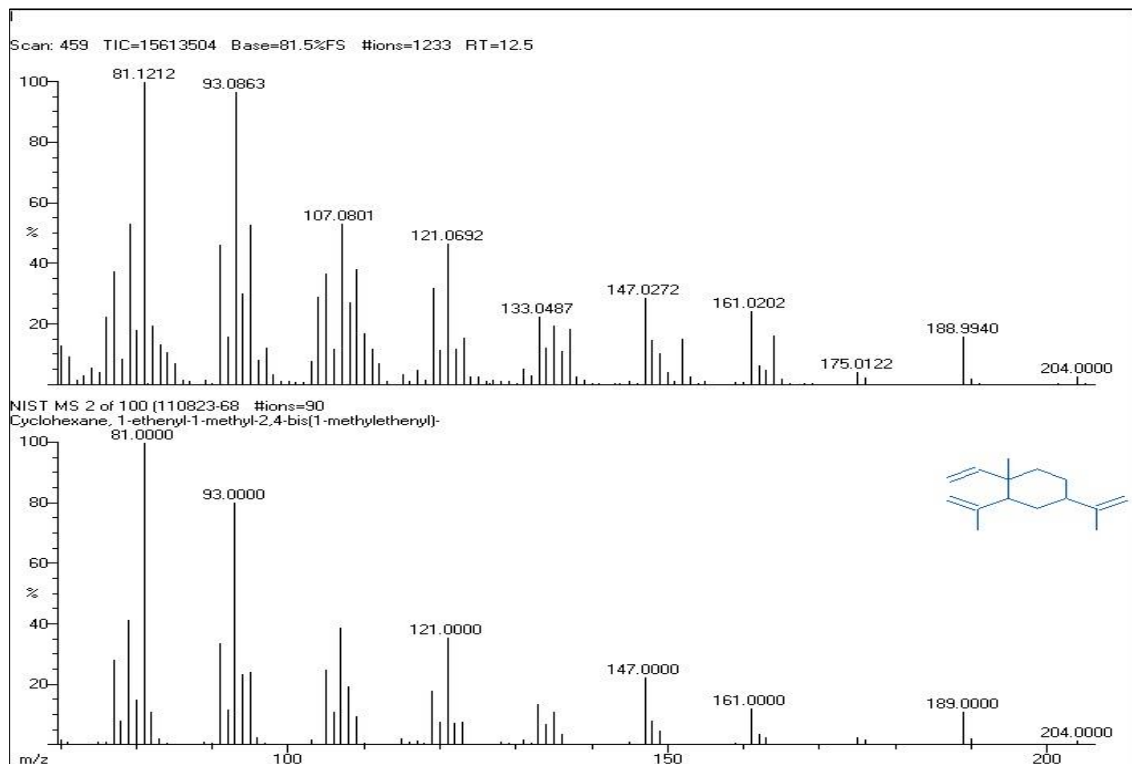


Figure 8 Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-

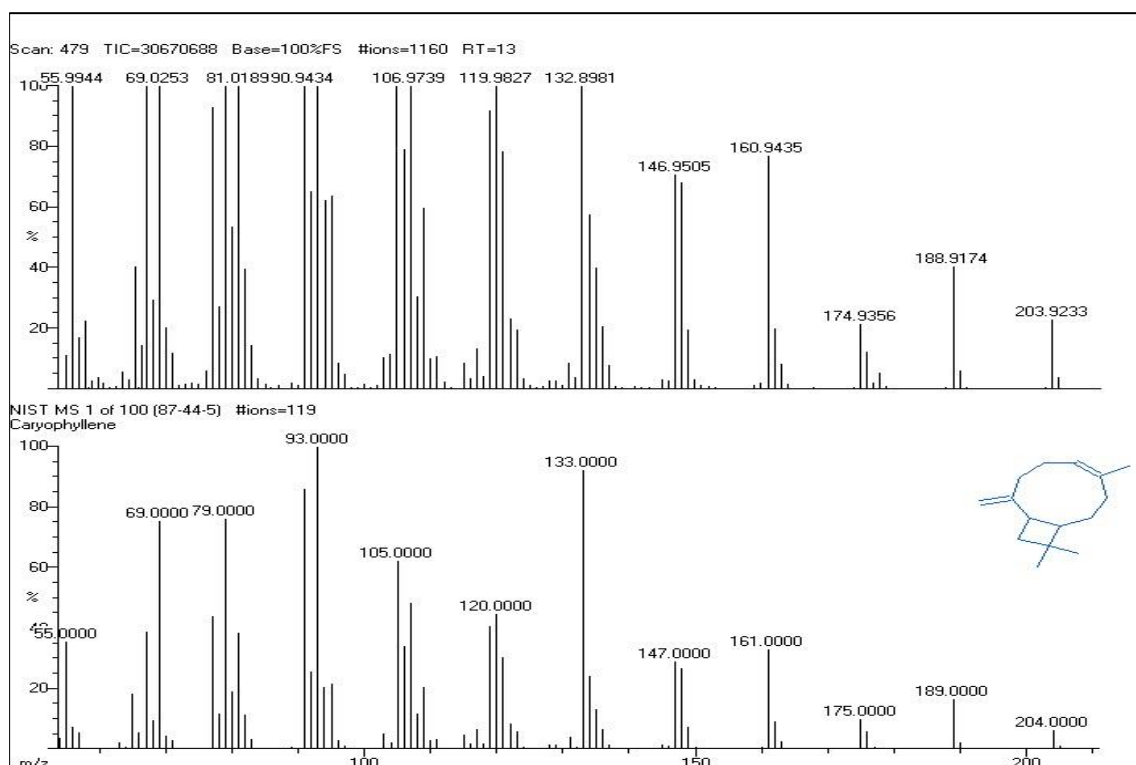


Figure 9 Caryophyllene

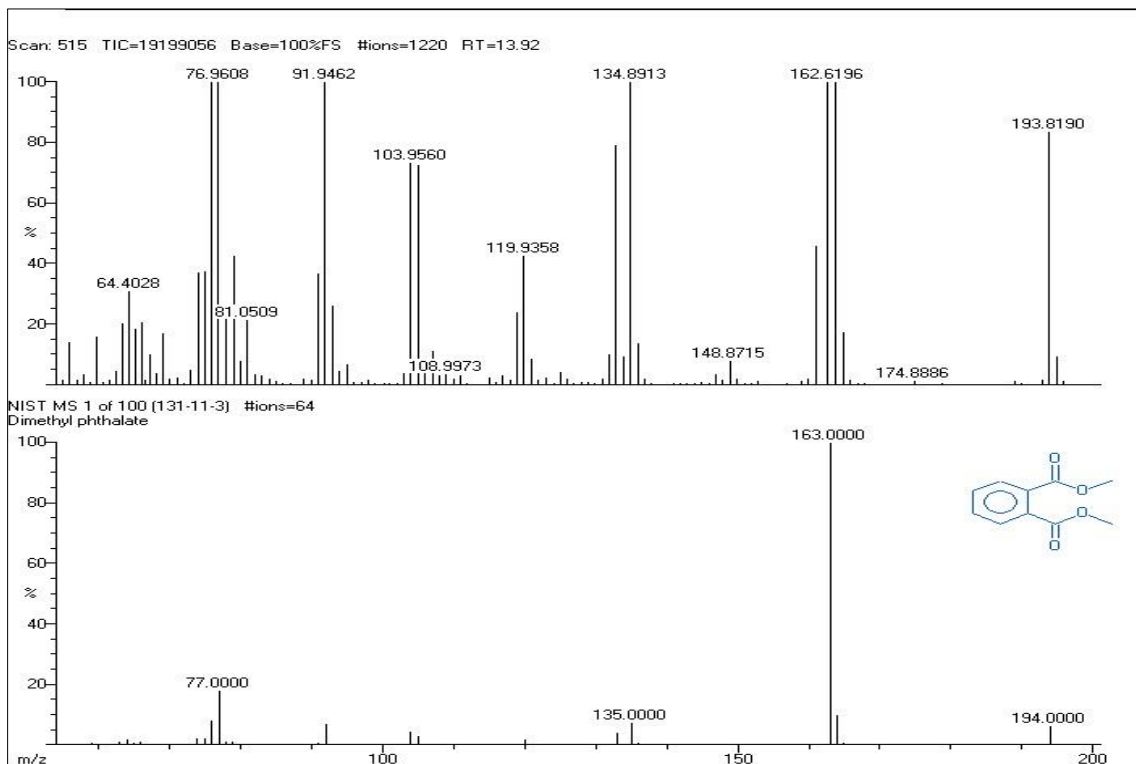


Figure 10 Dimethyl phthalate

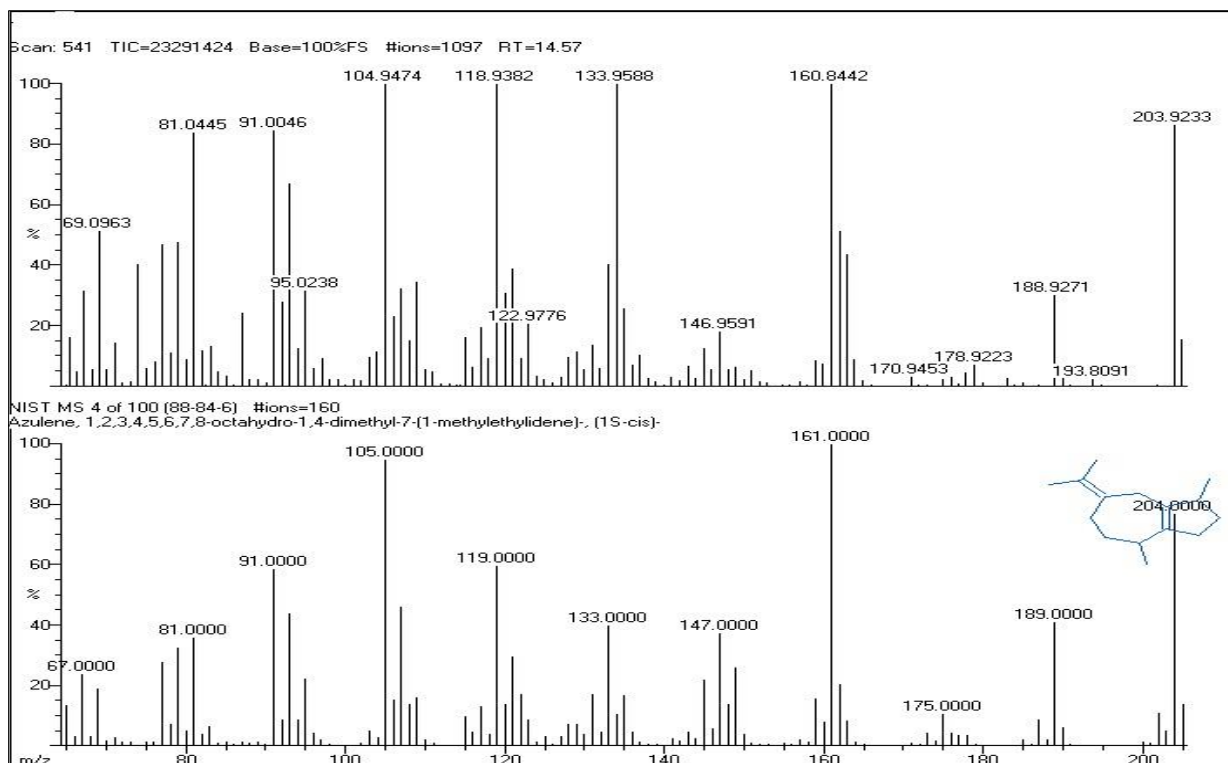


Figure 11 Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-(1s-cis)

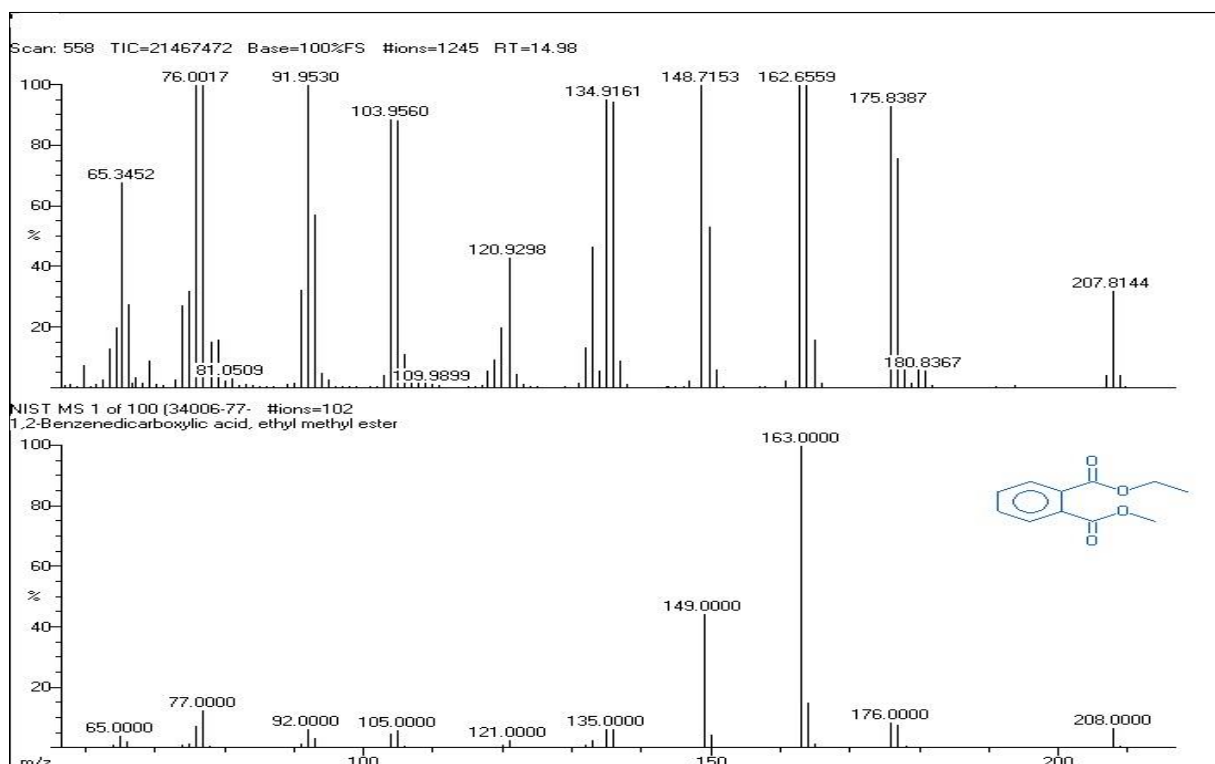


Figure 12 1,2-benzenedicarboxylic acid ethyl methyl ester

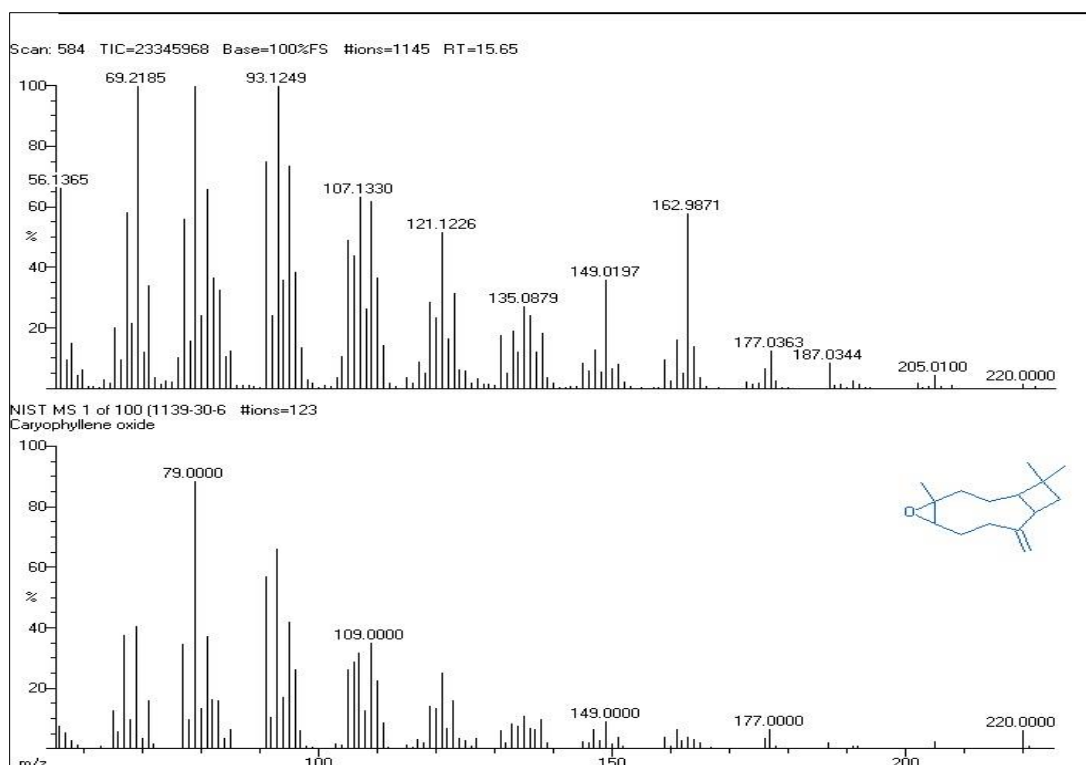


Figure 13 Caryophyllene oxide

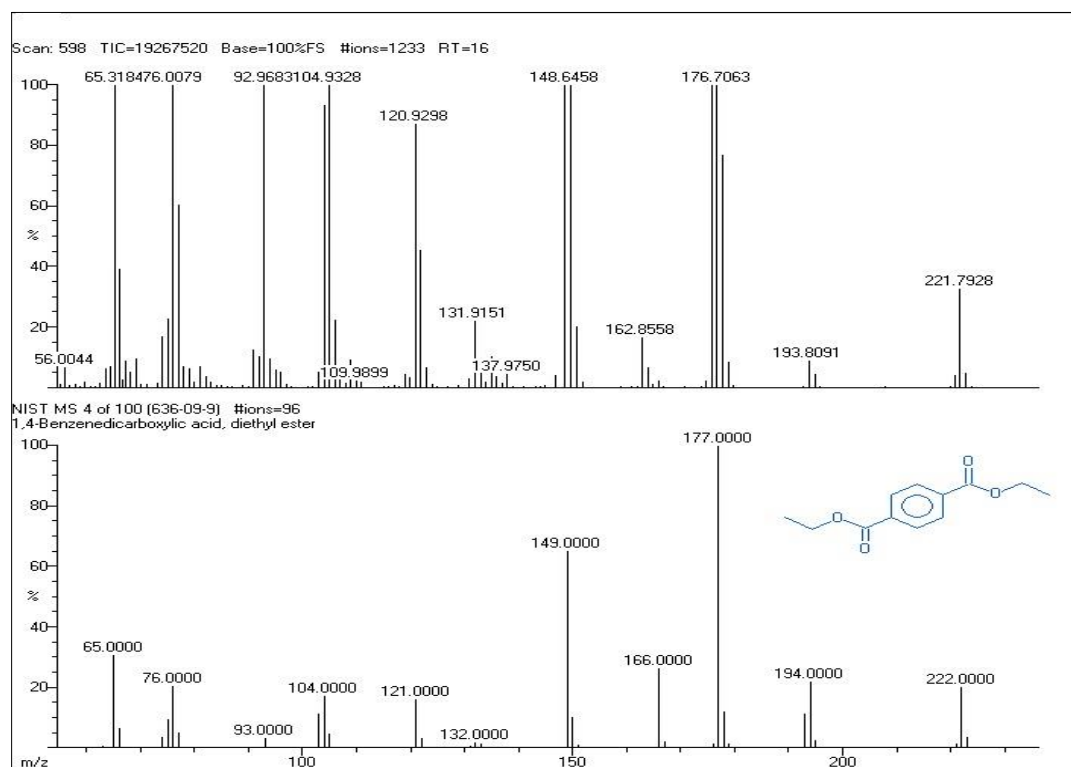


Figure 14 1,4-benzenedicarboxylic acid diethyl ester

4. Conclusion

From the above study results and discussion, it is clear that the medicinal role of the molecules present in *KIZHANELLI THYLAM* augurs well with its claimed role in *siddha* for the more of *pitha* related diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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