Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(2): 33-45
Received: 15-01-2022
Accepted: 21-02-2022

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Terpenoids in Gulguluthikthakam Kashayam: An ayurvedic formulation

Girija PV, Renuka NK and Vijayan KK

Abstract
Gulguluthikthakam kashayam is an excellent medicine for all types of inflammations especially pertaining to connective tissues, bones and joints. There are no prescription drugs that specifically target chronic inflammation. Now a days used nonsteroidal anti-inflammatory drugs carry side effects. Present study aims to identify the terpenoids present in the Gulguluthikthakam kashayam in order to confirm its efficacy. Volatile oil isolated from the kashayam using Clevenger apparatus and its chemical components determined with the help of GCMS. The compounds identified from the volatile oil of Gulguluthikthakam kashayam are Carvone, Beta-caryophyllene, Valencene, Cubenol, ar-Curcumene, Cedrol, Guaiol, Cembrene, Thunbergol, Guggulusterone, Mukulol and [6]-Paradol. The identified terpenoids found to have wide spectrum of biological activity so the synergistic effect between the different components present in the volatile oil may add its biological activity towards inflammation related diseases.

Keywords: GCMS, Gulguluthikthakam kashayam, inflammation, terpenoids, ayurvedic formulations, volatile oil, polyherbal

Introduction

Inflammation is a mechanism in which the body's immune system is stimulated against damage and act against toxic agents like bacteria, virus or fungus. It is a part of the host defence mechanisms that are known to be involved in the inflammatory reactions such as release of histamine, bradykinin & prostaglandins [1]. Inflammation is either acute inflammation or chronic inflammation. An acute inflammation is one that starts rapidly and becomes severe in a short span of time. Acute inflammation occurs after a cut on the knee, a sprained ankle or a sore throat. It's a short term response with localised effects. Chronic inflammation can have long term and whole body effects. Chronic inflammation is also called persistent or low grade inflammation because it produces a steady, low level of inflammation throughout the body. Chronic inflammation leads to over activation of macrophages, which ultimately causes over production of prostaglandins, leukotrienes, and cytokines and thereby induces chronic degenerative diseases like rheumatism, asthma, cancer, cardiovascular problems and atherosclerosis [2].

There is no specific remedy for chronic inflammation. However non-steroidal anti-inflammatory drugs are prescribed to suppress inflammation. Long term uses of these drugs also carry side effects like gastric lesions, cardiovascular, renal failure [3] and gastrointestinal [4, 5] implications.

Now there is a need for the safe, potent, nontoxic or less toxic anti-inflammatory drug. In Ayurveda there are polyherbal and herbal preparations to suppress the inflammation, to improve the immunity and remove the unwanted substance from the body efficiently.

Covid 19 has been conquered the world and lost lakhs of people's life due to the lack of specific medicine to treat. The studies reported, Abhijeet *et al*. [6] and Girija [7] highlighted importance of Ayurveda, traditional medicine, in managing the Covid 19 without serious complications. Whole world relay with the Ayurveda as medicine to treat the Covid 19, to boost the immunity and to subdue the post Covid 19 complications. The growing interest in Ayurvedic medicine and its efficacy in certain diseases, where modern medicine fails to act, has necessitates the chemical analysis of Ayurvedic medicines. The present study is on Gulguluthikthakam Kashayam, a polyherbal formulation used in the treatment of inflammatory conditions and related diseases. It is an excellent medicine for all types of inflammations especially pertaining to connective tissues, bones and joints.

The terpenoids present in the kashayam identified with the help of GCMS. In our knowledge this is the first report on terpenoids in Gulguluthikthakam Kashayam.

Materials and Methods

Chemicals and Apparatus used: Quantification of volatile oil had done with the Clevenger apparatus. Terpenoids present in the volatile oil had identified with the Gas Chromatography – Mass Spectrometry.

Gulguluthikthakam Kashayam is prepared from 29 well known plants [Table 1] as per Ashtanga Hridayam, a compendium of the Ayurvedic System by Vagbhata considered as "Heart or Essence of all the Eight Branches of Ayurveda," is one of the primary ancient root texts of Ayurveda. The Ashtanga Hridayam continues to serve as a root source for Ayurvedic philosophy and protocol, providing clear guidelines in all aspects of health.

Table 1: Each 5mL kashayam is made out of

SI No.	Botanical Name	Parts used	Form	Quantity (gm)
1.	Adhatoda vasica	Root	As it is	0.344
2.	Azadirachta indica	Stem	As it is	0.344
3.	Acorus calamus	Rhizome	As it is	0.344
4.	Aconitum heterophyllum	Tube root	As it is	0.344
5.	Alpinia calcarata	Rhizome	As it is	0.344
6.	Anetham graveolens	Fruit	As it is	0.344
7.	*Commiphora mukul*	Resin	Purified	0.344
8.	Celastrus paniculatus	Seed	As it is	0.344
9.	Cedrus deodara	Wood	As it is	0.344
10.	Cuminum cyminum	Fruit	As it is	0.344
11.	Cyprus rotundus	Rhizome	As it is	0.344
12.	Curcuma longa	Rhizome	As it is	0.344
13.	Cyclea peltata	Tube root	As it is	0.344
14.	Embelia ribes	Fruit	As it is	0.344
15.	Holarrhena antidysenterica	Seed	As it is	0.344
16.	Piper longum	Root	As it is	0.344
17.	Piper nigrum	Fruit	As it is	0.344
18.	Piper brachystachyum	Root	As it is	0.344
19.	Picrorhiza kurroa	Root	As it is	0.344
20.	Plumbago zeylanica	Rhizome	Purified	0.344
21.	Solanum indicum	Root	As it is	0.344
22.	Rubia cordifolia	Stem	As it is	0.344
23.	Saussura lappa	Root	As it is	0.344
24.	Scindapsus officinale	Fruit	As it is	0.344
25.	Tinospora cordifolia	Stem	As it is	0.344
26.	Trichosanthes dioica	Whole Plant	As it is	0.344
27.	Trachyspermum roxburghianum	Fruit	As it is	0.344
28.	Zingiber officinale	Rhizome	As it is	0.344
29.	Semecarpus anacardium	Seed	Purified	0.344

Quantification of Volatile oil: Gulguluthikthakam Kashayam was purchase from the Ayurveda Institute, The Arya Vaidya pharmacy, Coimbatore.

It is the decoction form of Guggulu Ghrita. All plant, except Plumbago zeylanica, *Commiphora mukul* and Semecarpus anacardium, are used without purification for the kashayam preparation.

200 mL of kashayam was taken into a 1000 mL volume flask. It is connected directly to the Clevenger apparatus and heated with the heating mantle for 3Hrs.The yield of volatile oil obtained (%) is calculated by the following formula:

% yield of volatile oil= (Volume of oil obtained/volume of kashayam taken) x100

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis: The sample was analysed by thermo scientific ISQ QD, Trace 1300 GC. Helium was used as the carrier gas 99.99%with a flow rate of 1 mL/minute. The GC oven temperature program was 60°c and held for 5 min. at first stage, then to 180°c at a rate of 4°c/min. Held for 5 min. The temperature was ramped to 280°c at a rate of 10°c/min. and kept for 2 min. The Mas Spectrum was taken in EI mode. The column used was TG5MS (30x0.25 mmx0.25 micrometre) MS transfer line temperature was 290°c and ion source temp was 230°c with a mass range of 40_600 amu. The database used is NIST MS.

Results and Discussions

Determination of volatile oil: On detailed literature survey the plants used for the preparation of kashayam contain high amounts of different volatile constituents. However, the total amount of volatile oil obtained is only 0.05% due to the vaporization of compounds at the preparation temperature of the kashayam.

Mass spectrum of the volatile oil showed several peaks indicating the presence of many phytochemical constituents (see the ion chromatogram, Fig.1). The mass spectra of the constituents compared with the NIST MS libraries and the twelve phyto constituents were characterized and identified (Table 2). The plant source of the identified constituents was also listed in the table.2.

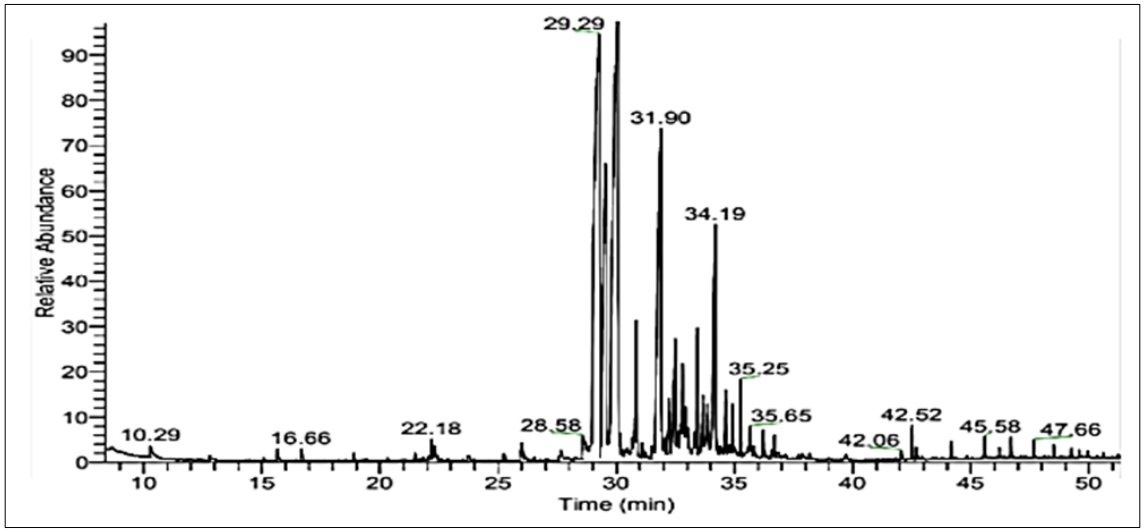

Fig 1: TIC of the volatile oil

The peak with retention time of 10.29 min.is identified as Carvone with a molecular ion peak at m/z 150.13 ($C_{20}H_{32}$ 150.10, monoisotopic). It is a monoterpene, 2-methyl-5-prop-1 -en-2-yl cyclohex-2-en-1-one. It is the constituent of Anetham graveolens [8] used in the kashayam. It produced a base peak at m/z 82.04 and other fragments at m/z 54.04 and 93.04[Figure 2]. The fragment pattern are given in the Figure 3.

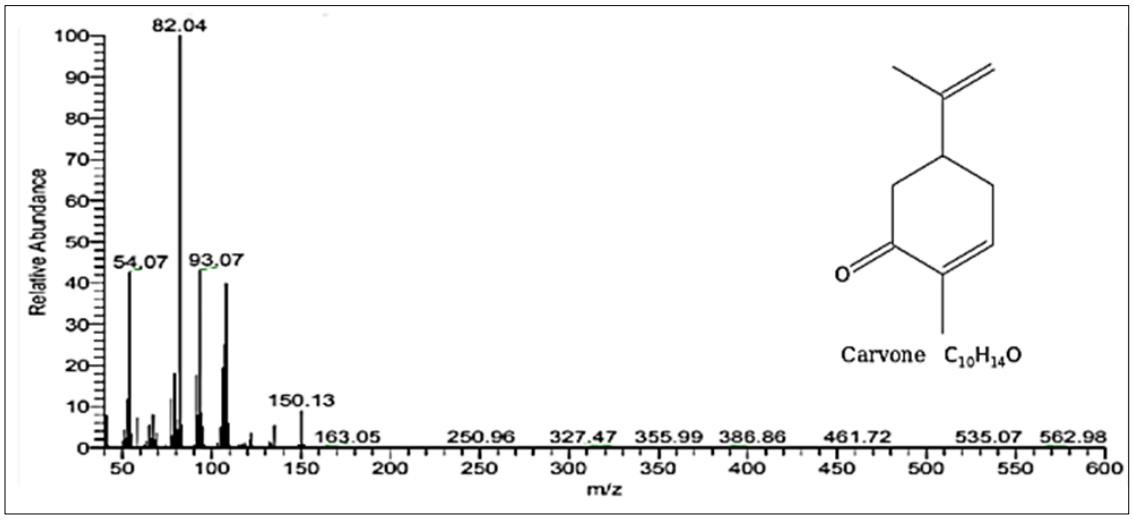

Fig 2: Mass spectrum of Carvone

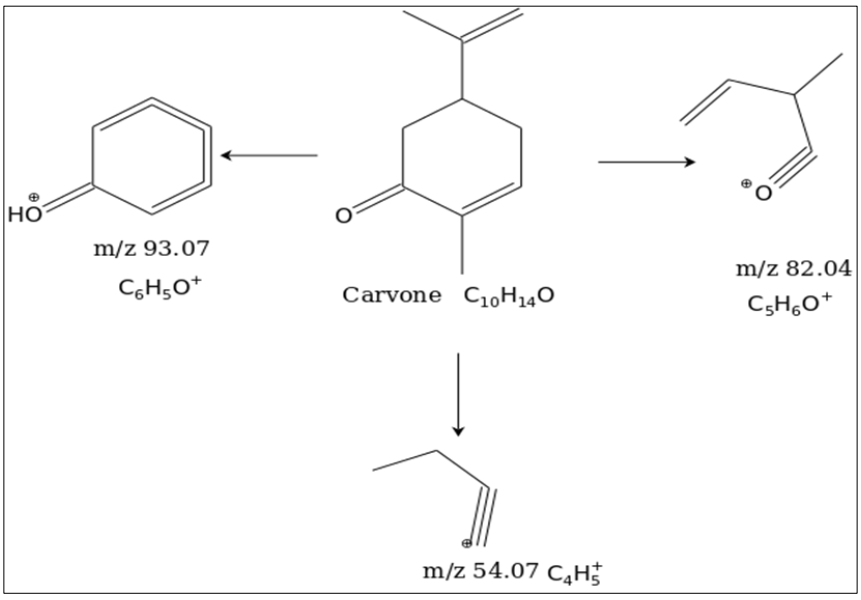

Fig 3: Fragment pattern of Carvone

The mass peak with retention time 15.65 min is identified as Beta-caryophyllene with the molecular ion at m/z 204.18($C_{15}H_{24}$ 204.187, monoisotopic). It gave the base peaks at m/z 133.11 and other fragments at m/z 79.06 and 91.07[Figure 4]. It is a sesquiterpenoid, (1*R*,4*E*,9*S*)-4, 11,11-trimethyl-8-methylidenebicyclo [7.2.0] undec-4-ene. This compound is the constituent of Piper nigrum [9], Curcuma longa [10] and Cuminum cyminum [11] used in the preparation of kashayam. Mass fragments are given in the Figure 5.

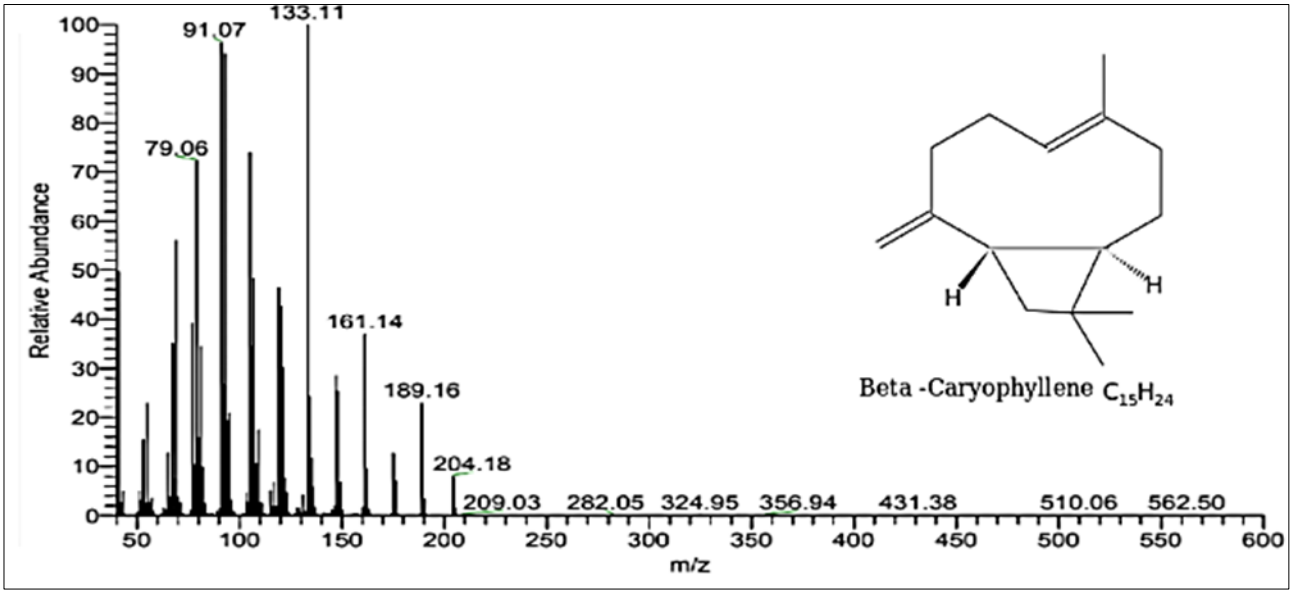

Fig 4: Mass spectrum of Beta-Caryophyllene

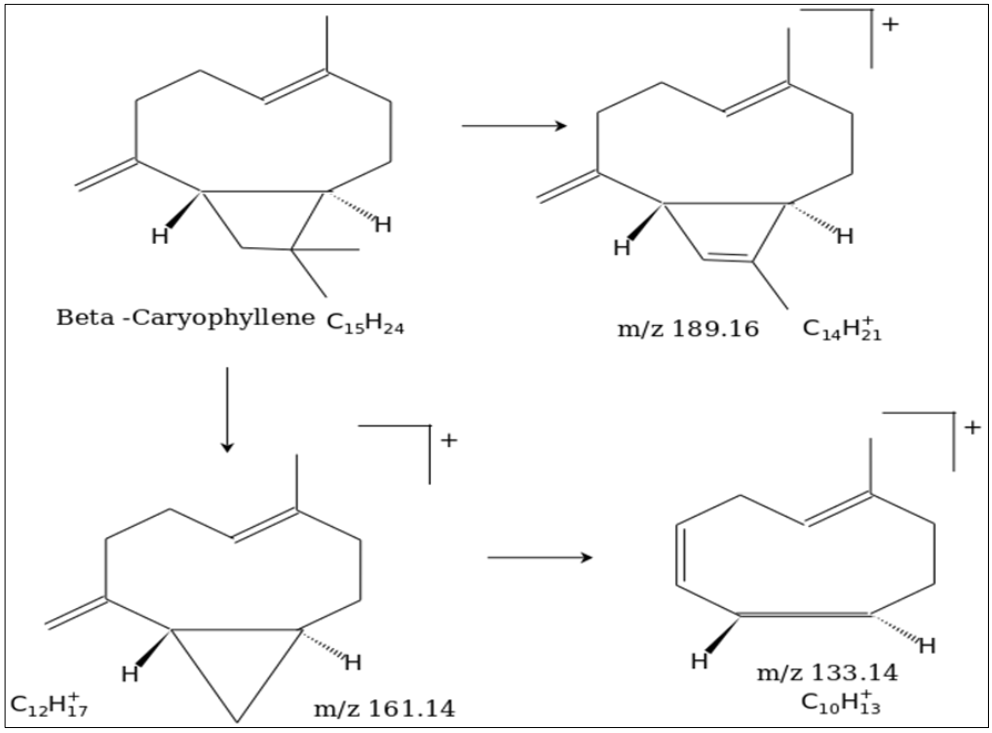

Fig 5: Fragment pattern of Beta -Caryophyllene

The peak with retention time 16.66 min was identified as Valencene, $C_{15}H_{24}$,204.19 (204.187, monoisotopic). It gave a base peak at m/z 93.08 and other fragments at m/z 80.07[Figure 6]. It is sesquiterpenoid, (3*R*,4*a S*,5*R*)-4*a*,5-dimethyl-3-prop-1-en-2-yl-2,3,4,5,6,7-hexahydro-1*H*-naphthalene. It is the constituent of Cyperus rotundus [12] and Zingiber officinale [13] used in the kashayam. Mass fragments leading to the confirmation tion are given in the figure 7.

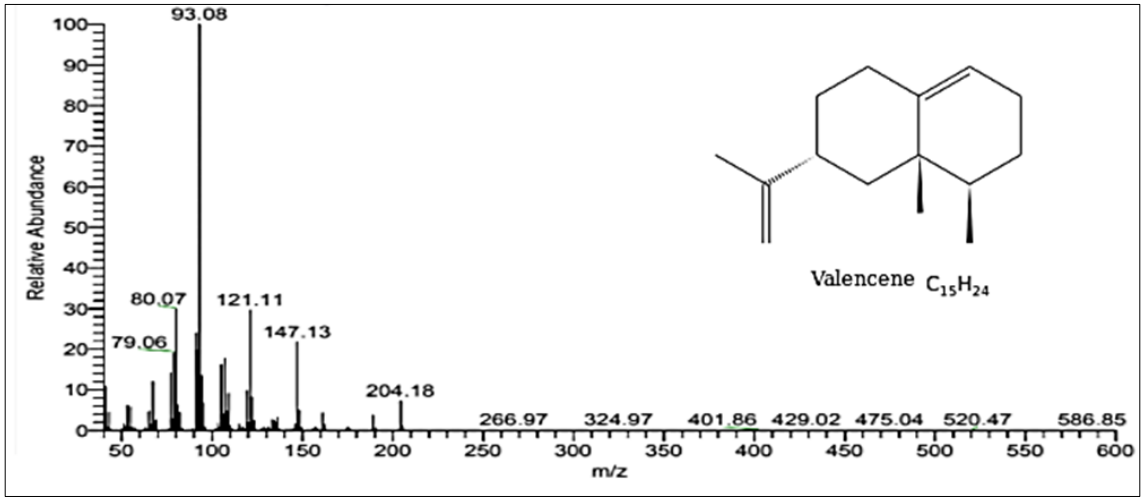

Fig 6: Mass spectrum of Valencene

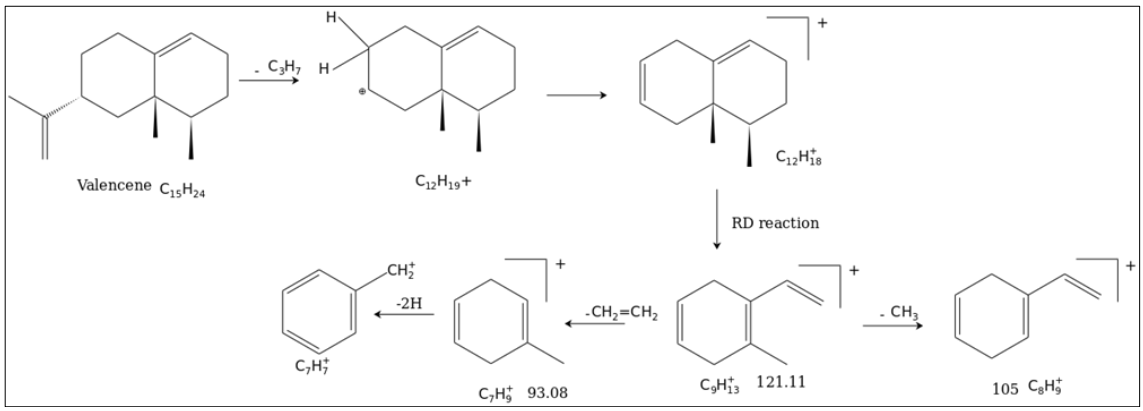

Fig 7: Fragment pattern of Valencene

The peak with retention time 18.89 min was identified as Cubenol according to the mass spectrum [Figure 8] and its fragmentation pattern [Figure 9]. Its molecular ion peak was found to be at m/z 222.18($C_{15}H_{26}O$, 222.198, monoisotopic). It produced a base peak at m/z 161.14 and other fragments at m/z 105.08,119.08 and 204.19. It is a sesquiterpenoid, (1*S*,4*R*,4*aR*,8*aR*)-4,7-dimethyl-1-propan-2-yl-2,3,4,5,6,8*a*-hexahydro-1*H*-naphthalen-4*a*-(2H) ol. This compound is the constituent of Zingiber officinale [13] used in the kashayam.

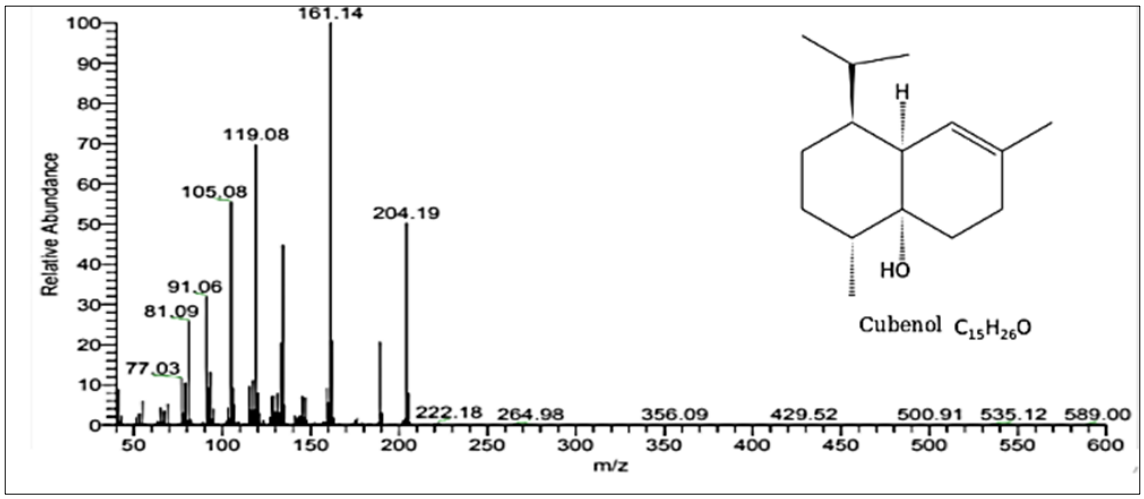

Fig 8: Mass spectrum of Cubenol

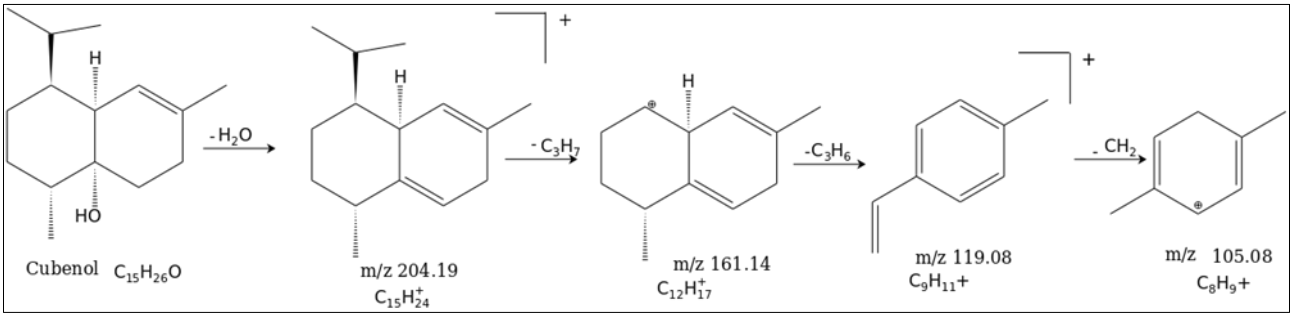

Fig 9: Fragment pattern of Cubenol

The mass peak with retention time 21.47 min was identified as Ar-Curcumene, it gave a molecular ion at m/z value of 202.17(C15H22, calculated for 202.341 monoisotopic). It is a sesquiterpenoid, 1-methyl-4-(6-methylhept-5-ene-2-yl) benzene. This molecule is a constituent of the medicinal plant Zingiber officinale [13], Piper nigrum [9], Piper longum [14] and Curcuma longa [10] used in the Kashayam. It gave the base peaks at m/z 119.10 and other major peaks at m/z 159.14,132.10, 91,59 [Figure 10]. The mass fragments are given in the Figure 11.

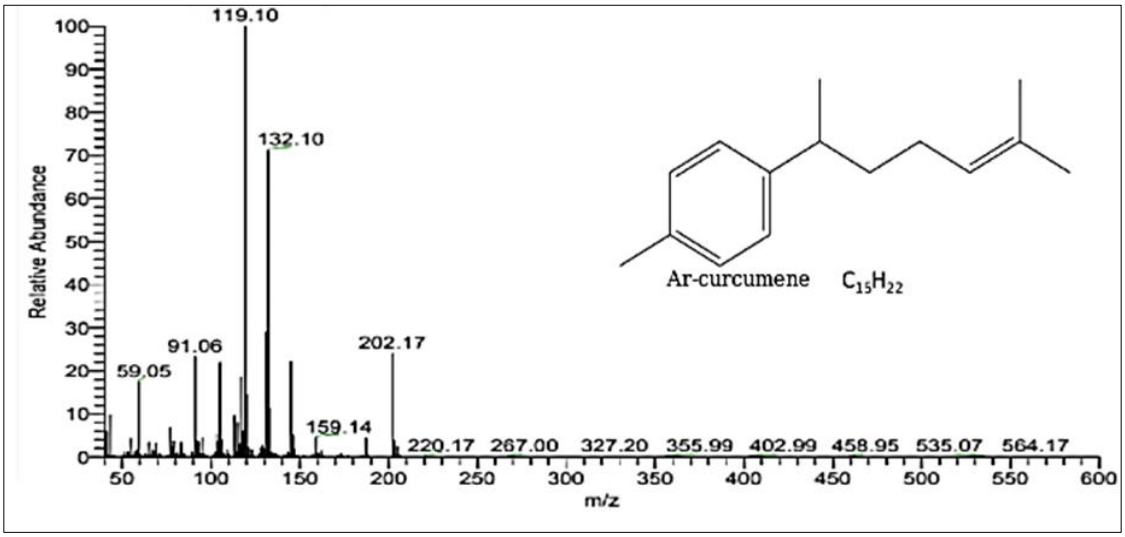

Fig 10: Mass spectrum of Ar-Curcumene

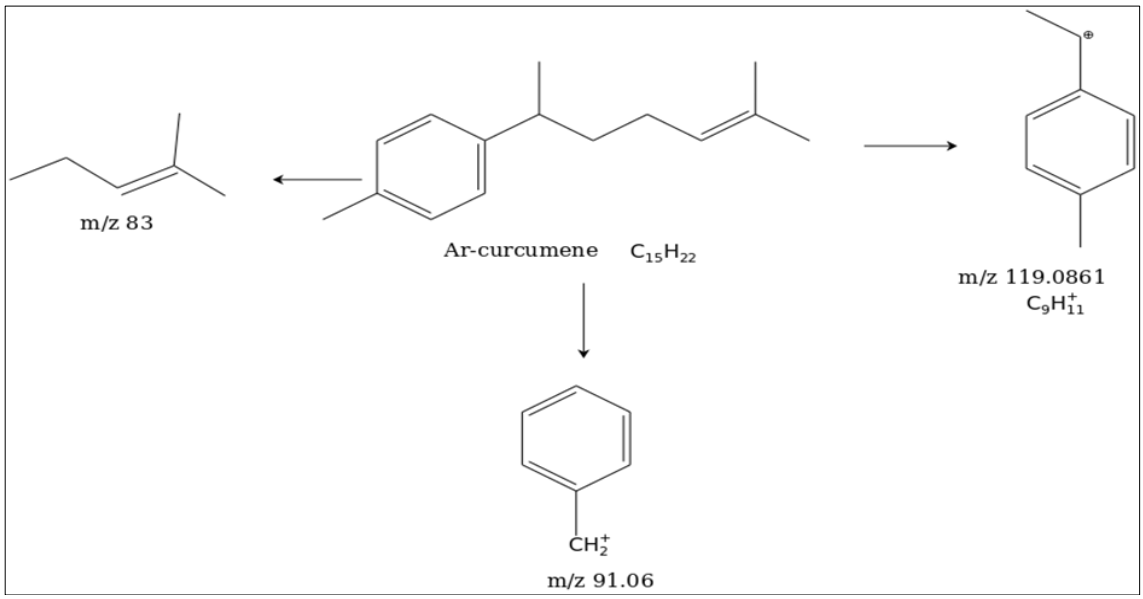

Fig 11: Fragment pattern of Ar-Curcumene

The mass peak with retention time 22.18 min was identified as Cedrol with the molecular mass 222.17($C_{15}H_{26}O$,222.198, monoisotopic mass). It gave a base peak at m/z 149.13 and other fragments at m/z 59.05 [Figure 12]. It is a sesquiterpenoid alcohol, (1*S*,2*R*,5*S*,7*R*,8*R*)-2,6,6,8-tetramethyl tricyclo [5.3.1.01,5] undecan-8-ol.It is a constituent of the medicinal plant Zingiber officinale [13], Piper nigrum [9] and Piper longum [14]. The mass fragments leading to the confirmation of Cedrol are given in the Figure 13.

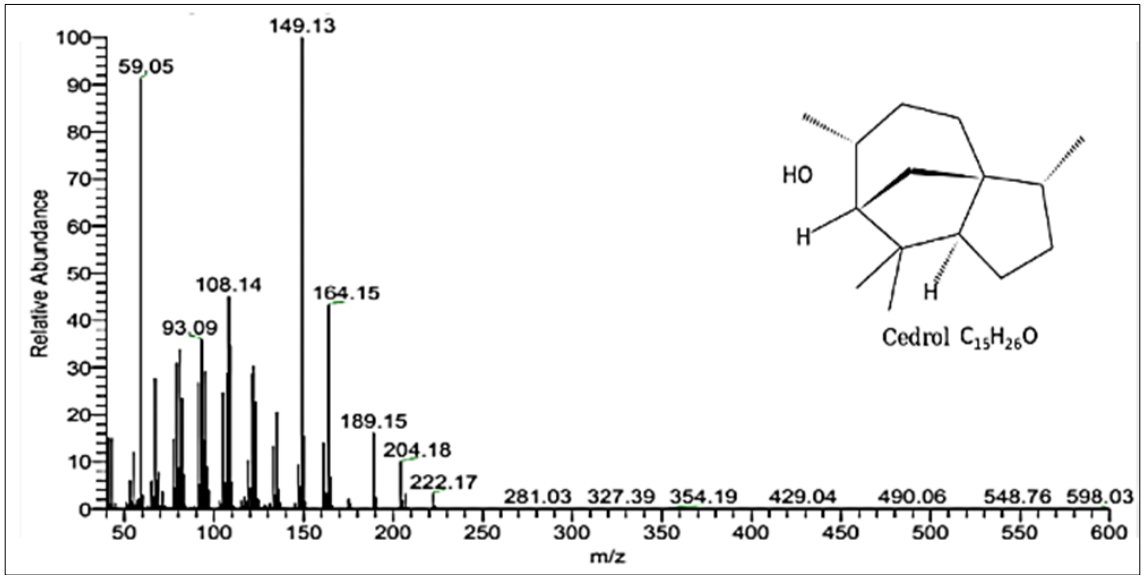

Fig 12: Mass spectrum of Cedrol

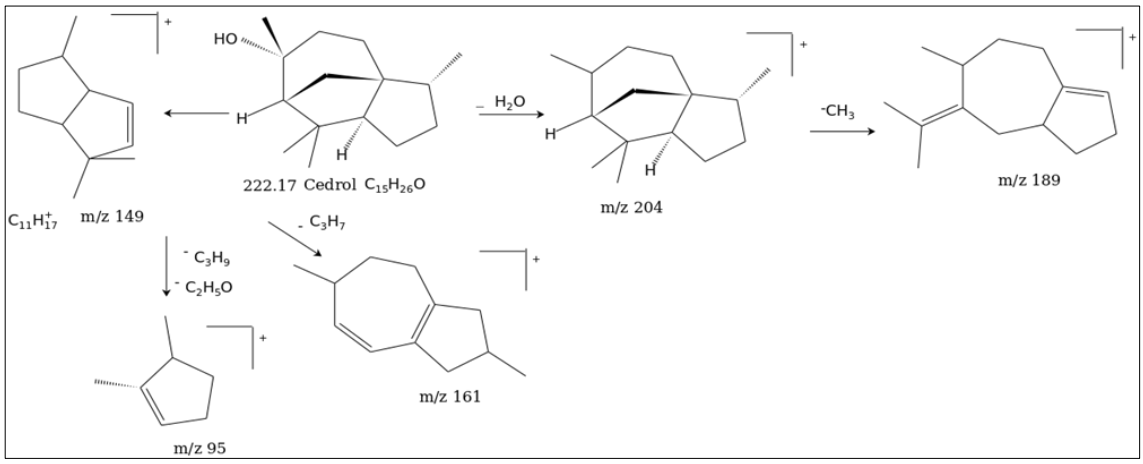

Fig 13: Fragment pattern of Cedrol

The peak with retention time 22.31 min was identified as Guaiol with the molecular mass 222.14($C_{15}H_{26}O$,222.198, monoisotopic mass) It is a sesquiterpenoid alcohol,2-[(3*S*,5*R*,8*S*)-3,8 -dimethyl-1,2,3,4,5,6 ,7,8- octahydroazulen-5-yl] propan-2-ol. This molecule is a constituent of the medicinal plant Zingiber officinale [13] used in the Kashayam. It gave the base peaks at m/z 149.11 and other major peaks at m/z 161.11,189.15,107.10,91.06 and 59.06[Figure 14]. The mass fragments are given in the Figure 15.

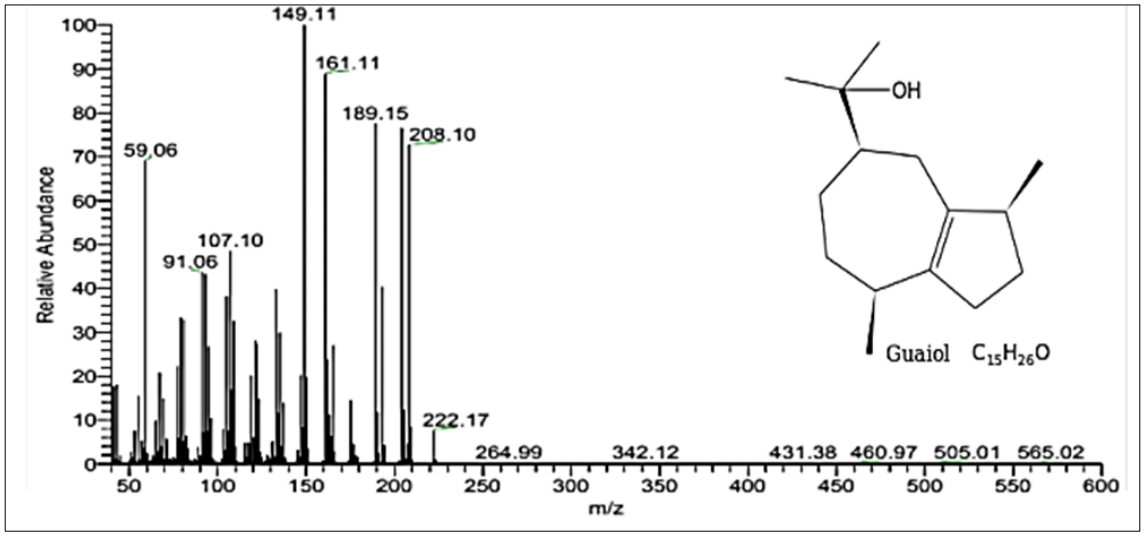

Fig 14: Mass spectrum of Guaiol

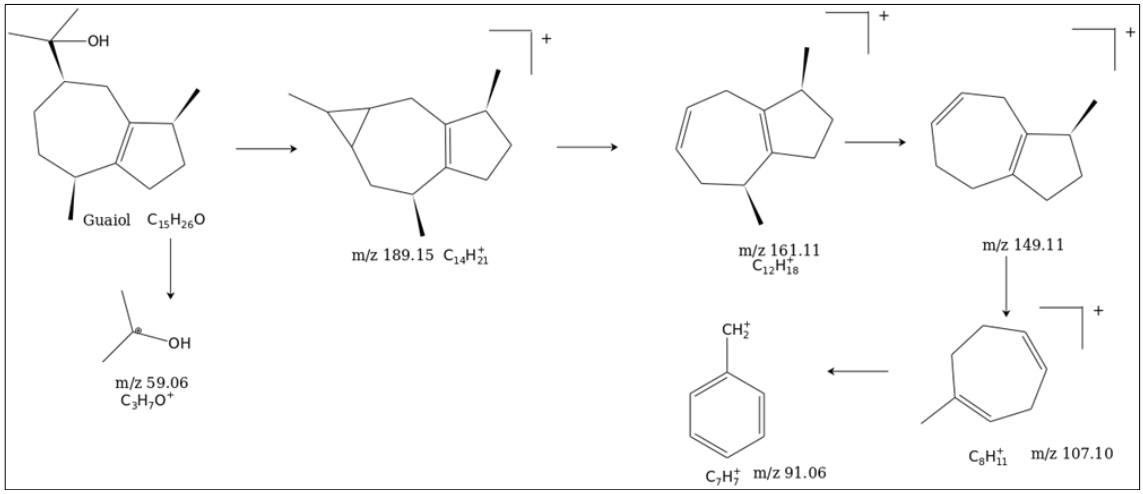

Fig 15: Fragment pattern of Guaiol

The peak with retention time 30.07 min was identified as Cembrene [see the figure16 and 17] with the molecular mass 272.23($C_{20}H_{32}$,272.25, monoisotopic mass). This is a diterpenoid, (1E,3Z,6E,10E)-3,7,11-trimethyl-14-propan- 2-ylcyclotetradeca-1,3,6,10-tetraene. This molecule is a constituent of the medicinal plant Zingiber officinale [13] and *Commiphora mukul* [15] used in the Kashayam. It gave the base peaks at m/z 93.09 and other major peaks at m/z 67.02 and 161.14.

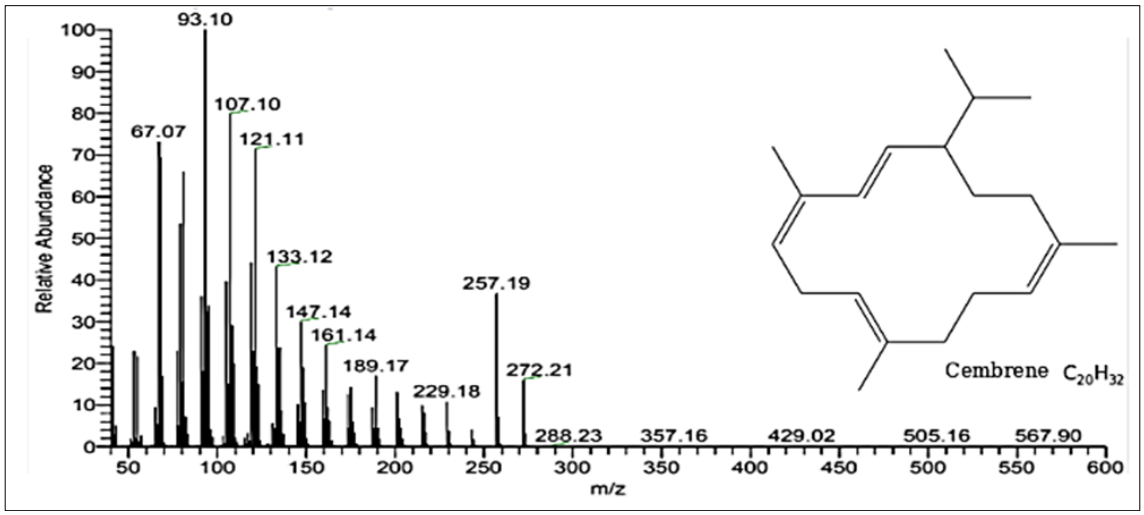

Fig 16: Mass spectrum of Cembrene

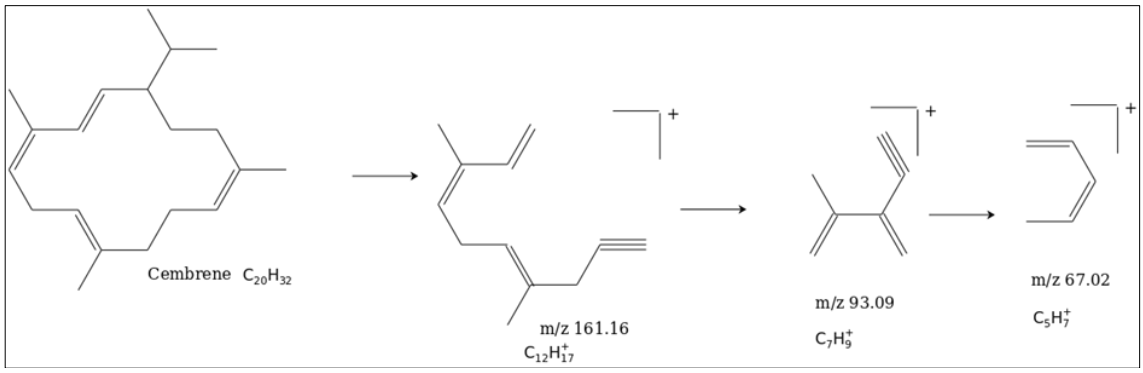

Fig 17: Fragment pattern of Cembrene

The mass peak with retention time 31.90 min was identified as Thunbergol with the molecular mass 290.25 ($C_{20}H_{34}O$,290.260, monoisotopic mass). This is a diterpenoid, (2*E*,7*E*,11*E*)-1,7,11-trimethyl -4-propan-2-ylcyclo tetra deca - 2,7,11-trien-1-ol. This molecule is a constituent of the medicinal plant Zingiber officinale [13], Anetham graveolens [8], Piper nigrum [9] and *Commiphora mukul* [15] used in the Kashayam. It gave the base peaks at m/z 81.08 and other major peaks at m/z 272,229.17,189 and 121.11. The mass spectrum [Figure 18] and fragments patter [Figure 19] are given.

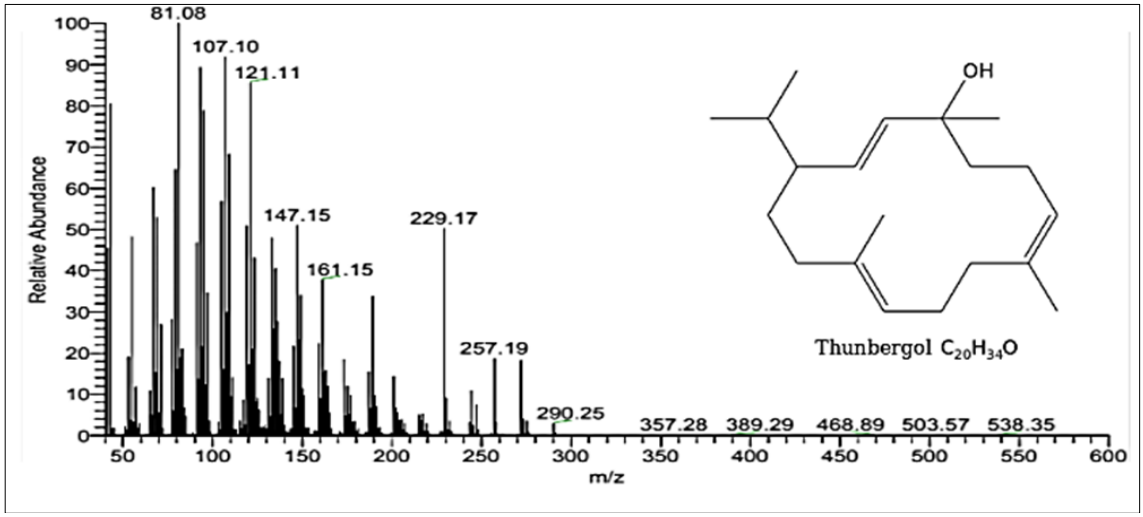

Fig 18: Mass spectrum of Thunbergol

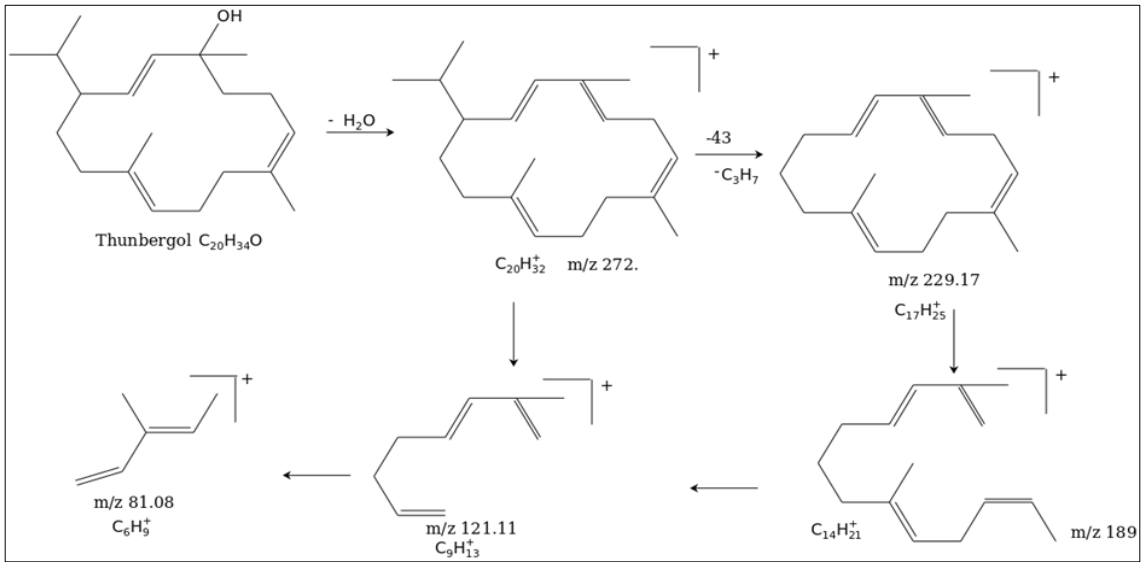

Fig 19: Fragment pattern of Thunbergol

The peak with the Retention Time of 33.42 min. gave a molecular ion at m/z value of 290.24 ($C_{20}H_{34}O$ calculated for 290.260, monoisotopic). This is identified as –Mukulol, (1*R*,2*Z*,6*Z*,10*Z*,14*S*)-3,7,11-trimethyl-14 -propan-2-ylcyclo tetradeca-2,6,10-trien-1-ol. This molecule is a constituent of the medicinal plant *Commiphora mukul* [15] used in the Kashayam. It gave the base peaks at m/z 109.12 and other major peaks at m/z 272.22 ,257.22,229.18,189.19 and 81.08. The mass spectrum [Figure 20] and its fragment pattern [Figure 21] are given.

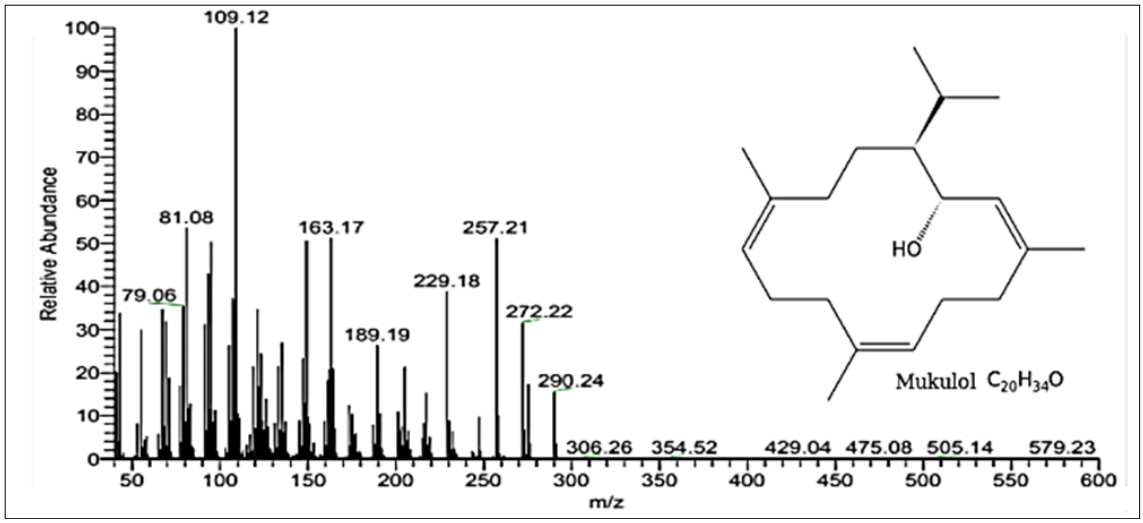

Fig 20: Mass spectrum of Mukulol

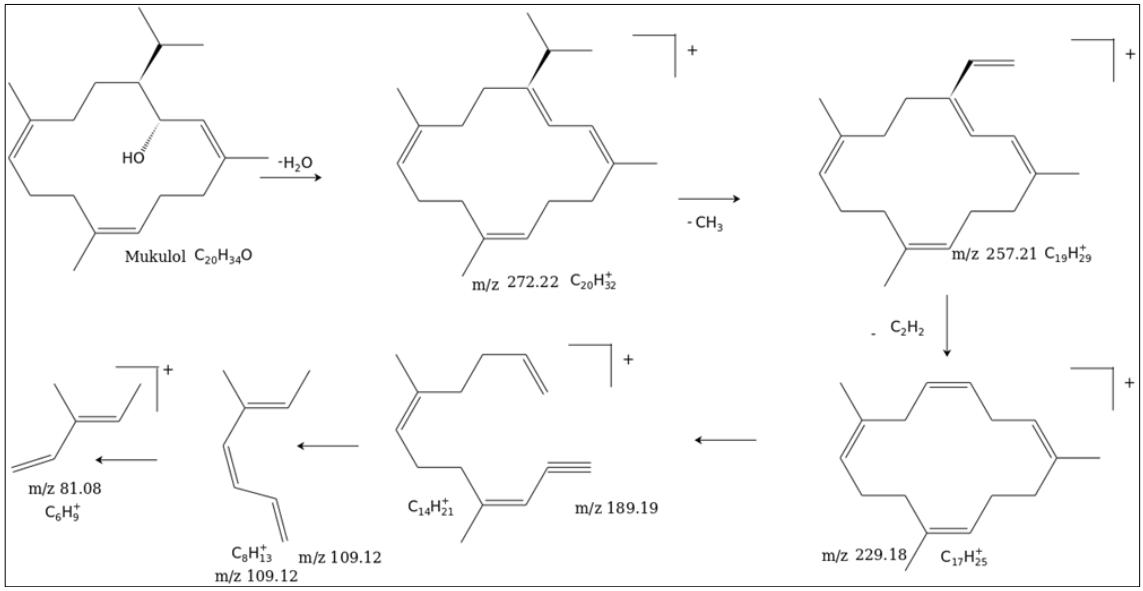

Fig 21: Fragment pattern of Mukulol

The peak with the Retention time 35.65 min. gave a molecular ion at m/z value of 278.16($C_{17}H_{26}O_3$ calculated for 278.18, monoisotopic). This is identified as – [6]-Paradol, a monomethoxy benzene, 1-(4-hydroxy-3-methoxyphenyl) decan-3-one. This molecule is a constituent of the medicinal plant Zingiber officinale [13] used in the Kashayam. It gave the base peaks at m/z 137.05 and other major peaks at m/z 179.07, 119.05 and 91.06[Figure 22]. The fragment pattern are given in the Figure 23.

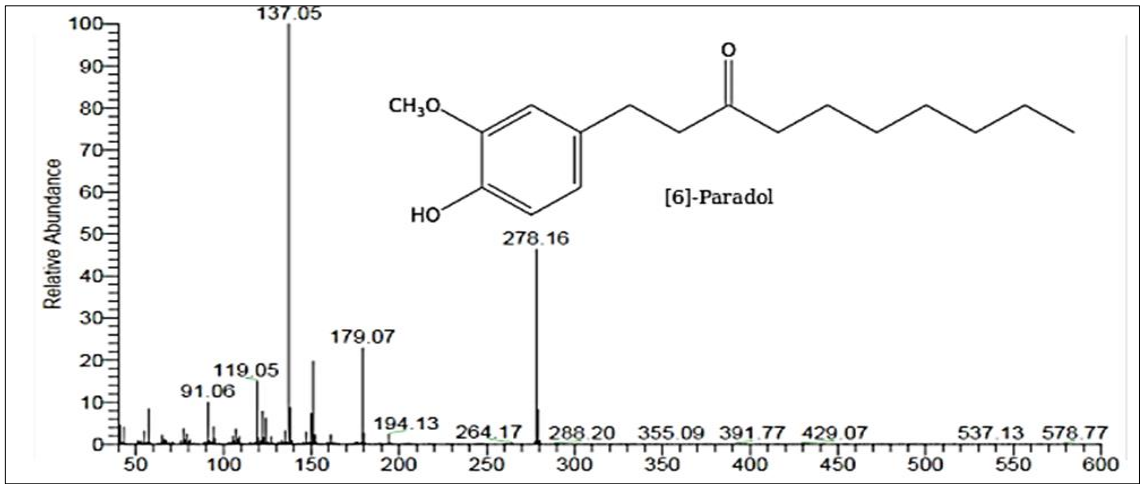

Fig 22: Mass spectrum of [6]-Paradol

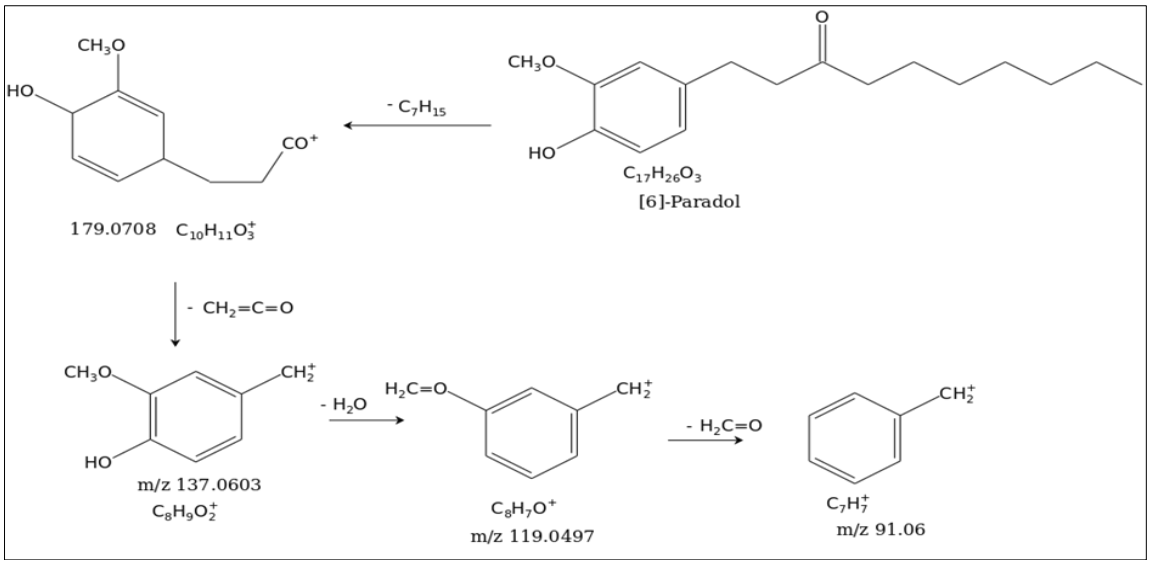

Fig 23: Fragment pattern of [6]-Paradol

The mass peak with the Retention time of 42.67 min. gave a molecular ion at m/z value of 316.23($C_{19}H_{24}O_4$ calculated for 278.18, monoiso topic). This is identified as – Guggulusterone, hormone. This molecule is a constituent of the medicinal plant *Commiphora mukul* [15] used in the Kashayam. It gave the base peaks at m/z 190.17 and other major peaks at m/z207.13,135.13,108.12 and 79.08[Figure 24]. The mass fragments are given in the Figure25.

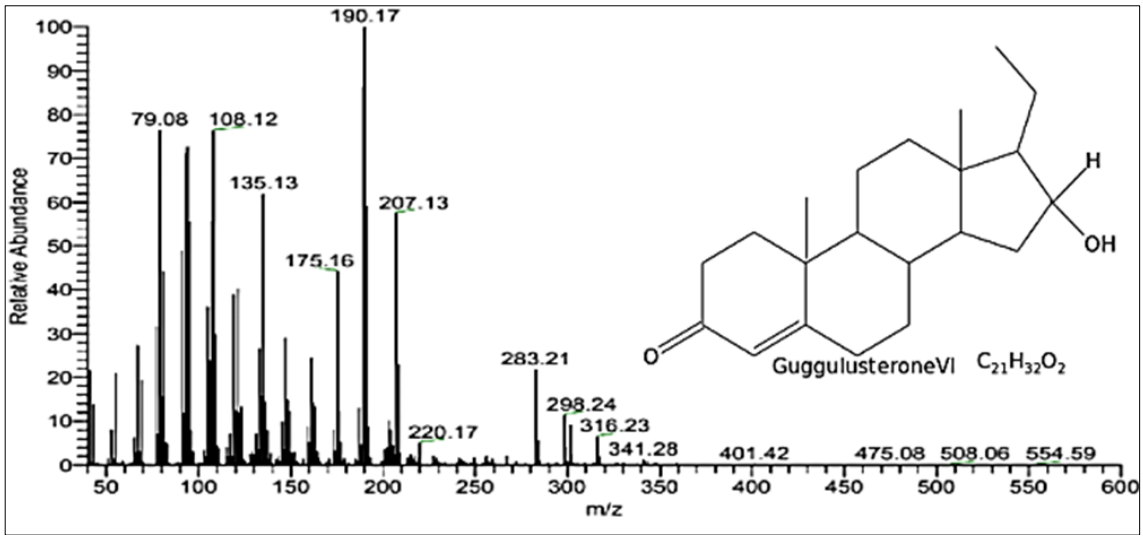

Fig 24: Mass spectrum of Guggulusterone VI

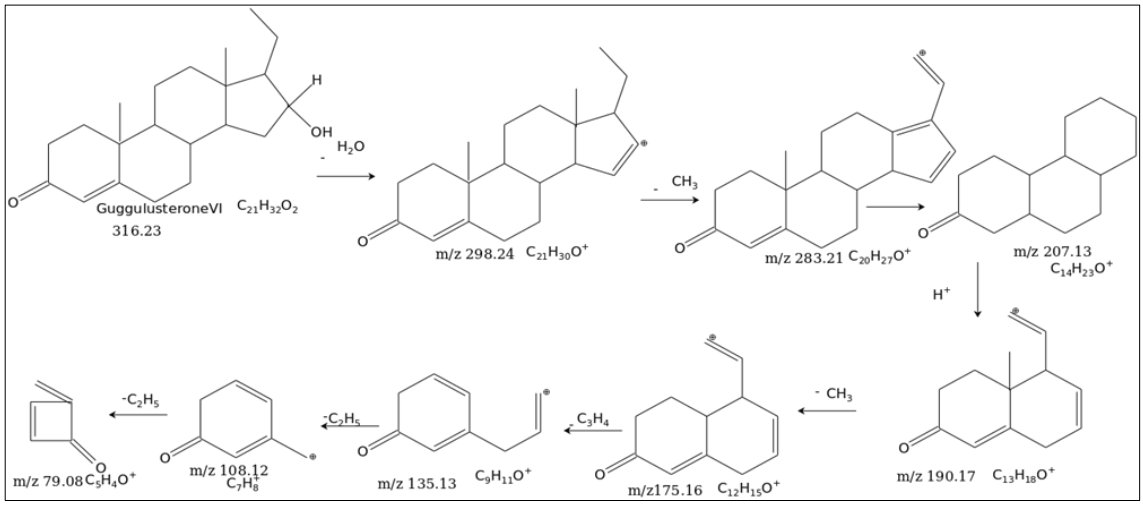

Fig 25: Fragment pattern of Guggulusterone VI

All the identified compounds had been reported as antioxidant, anti-inflammatory, analgesic, antipyretic, nephroprotective, anticholinesterase, antiarthritic, antispasmodic, antibacterial and immunostimulant. The results are presented in Table.2.

Table 2: Pharmacology of identified compounds

SI No.	Name of the compounds	Pharmacological properties
1.	Carvone	Antispasmodic [16], Antioxidant and free radical scavenging [17]
2.	Beta caryophyllene	Anti-inflammatory and analgesic effect on different inflammatory conditions [18]. Attenuates oxidative stress and neuroinflammation [19].
3.	Valencene	Inhibits inflammatory signalling process mediated by NF-kB. Antipyretic [20] and Anti-Inflammatory [21]
4.	Cubenol	Anti-inflammatory, Antioxidant and Antimicrobial [22].Anti-inflammatory and anticholinesterase activity [23]
5.	Ar-Curcumene	Anti-inflammatory, Antioxidant [24], Anti-inflammatory, Antinociceptive [25]
6.	Cedrol	Inhibits oxidative stress and inflammation [26], Reduces inflammation in Rheumatoid arthritis [27]
7.	Guaiol	Anti-inflammatory [28, 29]
]8.	Cembrene	Anti-inflammatory, Antioxidant [30], Treatment of Rheumatism [31]
9.	Thunbergol	Anti-inflammatory, Antioxidant [32], Nephroprotective [33]
10.	[6]-Paradol	Attenuation of neuroinflammatory process [34], Enhances Drug metabolism [35]
11.	Mukulol	Antiarthritic and Anti-inflammatory [36], Treatment for osteoarthritis inflammation [37]
12.	Guggulusterone VI	Anti-inflammatory and Antiarthritic [38], Anti-inflammatory [39]

Conclusion

The amount of volatile oil extracted from the Kashayam was found to be 0.05%. The identified terpenoids have wide spectrum of biological activities and are very specific to treat inflammation related diseases. It is concluded that Gulguluthikthakam kashayam does possess the medicinal

properties to support its function as a potent drug for which it is prescribed.

Acknowledgement
We thanks to department of chemistry Calicut University for providing the facility to do the experiment. We thanks to Mr Sanu, GCMS section School of environmental sciences, in this occasion, who had helped for GCMS analysis.

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