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, Guggulsterone, 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.

Gulguluthikham Kashayam is prepared from 29 well known plants [Table 1] as per Ashta 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.

Quantification of Volatile oil: Gulguluthikham 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, Commpihora mukal 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.
The peak with retention time of 10.29 min. is identified as Carvone with a molecular ion peak at m/z 150.13 (C_{10}H_{14}O, monoisotopic). It is a monoterpen, 2-methyl-5-propyl-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.
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, (1R,4E,9S)-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.

![Mass spectrum of Beta-Caryophyllene](image1)

**Fig 4:** Mass spectrum 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, (3R,4aS,5R)-4a,5-dimethyl-3-prop-1-en-2-yl-2,3,4,5,6,7-hexahydro-1H-naphthalene. It is the constituent of Cyperus rotundus [12] and Zingiber officinale [13] used in the kashayam. Mass fragments leading to the confirmation are given in the figure 7.

![Fragment pattern of Beta-Caryophyllene](image2)

**Fig 5:** Fragment pattern of Beta-Caryophyllene

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, (1R,4E,9S)-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.

![Mass spectrum of Beta-Caryophyllene](image1)

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![Fragment pattern of Beta-Caryophyllene](image2)

**Fig 5:** Fragment pattern of Beta-Caryophyllene
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\textsubscript{15}H\textsubscript{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, (15,4\text{R},4\text{aR},8\text{aR})-4,7-dimethyl-1-propan-2-yl-2,3,4,5,6,8a-hexahydro-1\text{H}-napthalen-4\text{a}-(2\text{H})ol. This compound is the constituent of Zingiber officinale \cite{13} used in the kashayam.
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.

The mass peak with retention time 22.18 min was identified as Cedrol with the molecular mass 222.17(C15H26O,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, (1S,2R,5S,7R,8R)-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.
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-[(3S,5R,8S)-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.
The peak with retention time 30.07 min was identified as Cembrene [see the figure 16 and 17] with the molecular mass 272.23 (C_{20}H_{32}O, 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.

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, (2E,7E,11E)-1,7,11-trimethyl-4-propan-2-ylcyclo tetradeca-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 pattern [Figure 19] are given.
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, (1R,2Z,6Z,10Z,14S)-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.
The peak with the Retention time 35.65 min. gave a molecular ion at m/z value of 278.16(C₁₁H₁₆O₃ 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.
The mass peak with the Retention time of 42.67 min. gave a molecular ion at m/z value of 316.23(C\textsubscript{19}H\textsubscript{24}O\textsubscript{4} calculated for 278.18, monoiso topic). This is identified as Guggulusterone, hormone. This molecule is a constituent of the medicinal plant *Commiphora mukul*\(^\textsuperscript{15}\) used in the Kashayam. It gave the base peaks at m/z 190.17 and other major peaks at m/z 207.13,135.13,108.12 and 79.08 [Figure 24]. The mass fragments are given in the Figure25.

All the identified compounds had been reported as antispasmodic, antibacterial and immunostimulant. The results are presented in Table.2.

### Table 2: Pharmacology of identified compounds

<table>
<thead>
<tr>
<th>SI No.</th>
<th>Name of the compounds</th>
<th>Pharmacological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Carvone</td>
<td>Antispasmodic(^\textsuperscript{[14]}), Antioxidant and free radical scavenging(^\textsuperscript{[17]})</td>
</tr>
<tr>
<td>2.</td>
<td>Beta caryophyllene</td>
<td>Anti-inflammatory and analgesic effect on different inflammatory conditions(^\textsuperscript{[18]}). Attenuates oxidative stress and neuroinflammation(^\textsuperscript{[19]}).</td>
</tr>
<tr>
<td>3.</td>
<td>Valencene</td>
<td>Inhibits inflammatory signalling process mediated by NF-kB. Anti-inflammatory(^\textsuperscript{[20]}) and Anti-Inflammatory(^\textsuperscript{[21]}).</td>
</tr>
<tr>
<td>4.</td>
<td>Cubenol</td>
<td>Anti-inflammatory, Antioxidant and Antimicrobial(^\textsuperscript{[22]}), Anti-inflammatory and anticholinesterase activity(^\textsuperscript{[23]})</td>
</tr>
<tr>
<td>5.</td>
<td>Ar-Curcumene</td>
<td>Anti-inflammatory, Antioxidant(^\textsuperscript{[24]}), Anti-inflammatory, Antinociceptive(^\textsuperscript{[25]})</td>
</tr>
<tr>
<td>6.</td>
<td>Cedrol</td>
<td>Inhibits oxidative stress and inflammation(^\textsuperscript{[26]}). Reduces inflammation in Rheumatoid arthritis(^\textsuperscript{[27]}).</td>
</tr>
<tr>
<td>7.</td>
<td>Guaiol</td>
<td>Anti-inflammatory(^\textsuperscript{[28,29]})</td>
</tr>
<tr>
<td>8.</td>
<td>Cembrene</td>
<td>Anti-inflammatory, Antioxidant(^\textsuperscript{[30]}), Treatment of Rheumatism(^\textsuperscript{[31]})</td>
</tr>
<tr>
<td>9.</td>
<td>Thunbergol</td>
<td>Anti-inflammatory, Antioxidant(^\textsuperscript{[32]}), Nephroprotective(^\textsuperscript{[33]})</td>
</tr>
<tr>
<td>10.</td>
<td>[6]-Paradol</td>
<td>Attenuation of neuroinflammatory process(^\textsuperscript{[34]}). Enhances Drug metabolism(^\textsuperscript{[35]})</td>
</tr>
<tr>
<td>11.</td>
<td>Mukulol</td>
<td>Antiarthritic and Anti-inflammatory(^\textsuperscript{[36]}), Treatment for osteoarthritis inflammation(^\textsuperscript{[37]})</td>
</tr>
<tr>
<td>12.</td>
<td>Guggulusterone VI</td>
<td>Anti-inflammatory and Antiarthritic(^\textsuperscript{[38]}), Anti-inflammatory(^\textsuperscript{[39]})</td>
</tr>
</tbody>
</table>

### 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.

References


