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Research Article

## GC-MS Analysis of Bioactive Compounds and Phytochemical Evaluation of the Ethanolic Extract of *Gomphrena globosa* L. Flowers

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### ABSTRACT

*Gomphrena globosa* (L.) has high medicinal values. All parts are been used as folk medicines. The extract of whole plant / flowers has shown different phytochemical constituents with various clinical properties. These phytochemicals recognised are responsible for various activities such as anti-inflammatory, anticancer, antibacterial, analgesic and cytotoxic. The ethanolic extract of *Gomphrena globosa* L. flowers of biochemical test indicates the presence of sterols, triterpenoid, tannins, phenols and flavonoids, and on GC-MS analysis it has shown 11 phytochemical compounds with different pharmacological activities. The major bioactive compounds are Docosanoic Acid, Docosyl Ester (25.404%) and Hexatriacontane (24.324%), has proven anti-inflammatory activity.

**Keywords:** *Gomphrena globosa* L., phytochemical constituents, GC-MS and anti-inflammatory.

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### INTRODUCTION:

Natural products from plants are a rich resource used for centuries to cure various ailments. The use of bioactive plant-derived compounds is on the rise, because side effects of synthetic drugs can be even more dangerous than the diseases they claim to cure. In contrast, plant derived medicines contain natural substances that can promote health, alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. So, in respect of the healing power of plants and a return to natural remedies is an absolute requirement of present and future time.<sup>[1,2,3]</sup>

Medicinal plants are a major natural alternative to synthetic drugs and gaining a lot of attention. The potential therapeutic activities are attributed to the presence of phytochemical constituents. Phytochemicals are present in the medicinal plants, leaves, flowers, vegetables and roots. Phytochemicals can be primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities.

Alkaloids are used as anaesthetic agents and are found in medicinal plants.<sup>[4-7]</sup>

For identification, qualitative and quantitative estimation of phytochemicals, different highly accurate procedures like GC-MS and HPTLC are commonly used to obtain the chemical fingerprint of high quality.

On interpretation of the fingerprint, it would be easier to elucidate the pharmacological activities of the phytochemicals.<sup>[8]</sup>

### MATERIALS AND METHODS

#### I. Collection and identification:

The flowers of *Gomphrena globosa* L. of Indian origin were collected and spread on a plain paper and dried under shade at room temperature (22°-36°C) for about 10 days. The dried flowers are then used for further studies.

#### II. Soxhlet extraction with ethanol:

About 15gm of *Gomphrena globosa* L. dried flowers was uniformly packed into a thimble of soxhlet apparatus and extracted with 150ml of ethanol solvent and temperature was maintained at 50°C. The process of extraction continued till the solvent in siphon tube of an extractor become colorless (approximately 4hr). First cycle has taken

30min and subsequently 17 cycles were done, where each cycle took approximately 8min. After that the extract was dried and solvent was recollected using rota vaporator. Dried powder transfer into sterilized bottle and stored at 8°C for further analysis.

### III. Qualitative biochemical analysis:

The qualitative tests for various phytoconstituents as shown in table 1 was carried out by using ethanolic extract of *Gomphrena globosa* L. flowers.<sup>[9-14]</sup>

### IV. Gas-Chromatography-Mass Spectrometry

The GC-MS analysis of ethanolic extract of *Gomphrena globosa* L. flowers gives a fingerprint for the identification and quantification of phytochemical present. The GC-MS was performed at "VIT-SIF Lab, SAS, Chemistry Division for NMR and GC-MS Analysis" India using a GC-MS Model; clarus 680 equipped with a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60°C (2 min); followed by 300 °C at the rate of 10°C min<sup>-1</sup>; and 300°C, where it was held for 6 min.

The mass detector conditions were maintained as: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec

and scan interval of 0.1 sec. Fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

## RESULTS AND DISCUSSION:

### Qualitative biochemical analysis:

The qualitative tests for various phytoconstituents were carried out by using ethanolic extract of *Gomphrena globosa* L. flowers and the phytoconstituents present in it are shown in table 2.

### Chromatographical Analysis:

Chromatographic fingerprint analysis of Indian variety dried flowers of ethanolic extract of *Gomphrena globosa* L. using GC-MS has shown 11 peaks indicating the presence of 11 phytochemical compounds.

The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula as shown in table 3.

The phytochemical compounds recognized through GC-MS analysis showed many biological activities are listed in Table 4.

Individual phytochemical constituents in the ethanolic extract of *Gomphrena globosa* was given in table 5 as analysed by GC-MS analytical procedure.

**Table 1:** Qualitative biochemical analysis of ethanolic extract of *Gomphrena globosa* L. flowers

TEST	PROCEDURE
<b>TEST FOR STEROID AND TRITERPENOIDS</b> <b>Libermann's- Burchard test:</b>  <b>Salkowski reaction:</b>	Extract treated with few drops of acetic anhydride, boiled and cooled. Few drops of concentrated sulphuric acid is added from the side of the test tube. Extract was treated in chloroform with few drops of concentrated H <sub>2</sub> SO <sub>4</sub> , shaken well and allow standing for some time.
<b>TEST FOR TANNINS AND PHENOLIC COMPOUNDS</b> <b>Ferric chloride test:</b> <b>Lead acetate solution:</b>	Extract was treated with FeCl <sub>3</sub> . Extract treated with few drops of lead acetate.
<b>TEST FOR FLAVONOIDS</b> <b>Shinoda test :</b>	To dry powder or extract, 95% ethanol, few drops of conc. HCL and 0.5 g magnesium turning was added. To small quantity of residue, lead acetate solution was added.
<b>TEST FOR GLYCOSIDES</b> <b>Foam test:</b>	Shake the extract or dry powder vigorously with water.
<b>TEST FOR PROTEINS</b> <b>Biuret test ( general test ):</b>  <b>Millons test :</b>	To 3ml of test solution, 4% NAOH and few drops of 1% CuSO <sub>4</sub> solution was added. Extract treated with 2ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid).
<b>TEST FOR AMINO ACIDS</b> <b>Ninhydrin test :</b>	Extract was heated with 5% solution of ninhydrin solution (indane-1,2,3 trionehydrate) in boiling water bath for 10min.
<b>TEST FOR CARBOHYDRATES</b> <b>Molisch test :</b>  <b>Benedicts test :</b>	Extract treated with few drops of alcoholic α-naphthol. 0.2ml of concentrated H <sub>2</sub> SO <sub>4</sub> was added slowly through the side of the test tube. Extract treated with few drops of benedict reagent (alkaline solution containing cupric citrate complex).
<b>TEST FOR ALKALOIDS</b> <b>Mayer's test :</b> <b>Wagner's test :</b>	Extract was treated with Mayer's reagent (Potassium mercuric iodide solution). Extract was treated with Wagner's reagent (Solution of iodine in potassium iodide).

Table 2: Qualitative biochemical analysis of ethanolic extract of *Gomphrena globosa* L. flowers

TEST	OBSERVATION	RESULT
<b>TEST FOR STEROID AND TRITERPENOID</b> <b>Libermann's- Burchard test:</b>  <b>Salkowski reaction:</b>	<ul style="list-style-type: none"> <li>Brown ring at the junction of two layers and the upper layer turns green which shows the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.</li> <li>Red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids.</li> </ul>	Presence of Steroid and triterpenoids
<b>TEST FOR TANNINS AND PHENOLIC COMPOUNDS</b> <b>Ferric chloride test:</b> <b>Lead acetate solution:</b>	<ul style="list-style-type: none"> <li>Blue-black colour.</li> <li>White precipitate.</li> </ul>	Presence of tannins and phenolic compounds
<b>TEST FOR FLAVONOIDS</b> <b>Shinoda test :</b>	<ul style="list-style-type: none"> <li>Orange, pink, red to purple colour appears.</li> <li>Yellow coloured precipitate is formed.</li> </ul>	Presence of flavonoids.
<b>TEST FOR GLYCOSIDES</b> <b>Foam test:</b>	<ul style="list-style-type: none"> <li>Absence of persistent foam.</li> <li>Absence of blue or green fluorescence.</li> </ul>	Absence of glycosides.
<b>TEST FOR PROTEINS</b> <b>Biuret test ( general test ):</b> <b>Millons test :</b>	<ul style="list-style-type: none"> <li>Absence of violet or pink colour.</li> <li>Absence of white precipitate.</li> </ul>	Absence of proteins.
<b>TEST FOR AMINO ACIDS</b> <b>Ninhydrin test :</b>	<ul style="list-style-type: none"> <li>Absence of purple or bluish colour.</li> </ul>	Absence of amino acids
<b>TEST FOR CARBOHYDRATES</b> <b>Molisch test :</b>  <b>Benedicts test :</b>	<ul style="list-style-type: none"> <li>Absence of purple to violet colour at the junction.</li> <li>Upon boiling on water bath reddish brown precipitate doesn't forms, therefore reducing sugars are absent.</li> </ul>	Absence of carbohydrate.
<b>TEST FOR ALKALOIDS</b> <b>Mayer's test :</b> <b>Wagner's test :</b>	<ul style="list-style-type: none"> <li>Absence of cream colour precipitate.</li> <li>Absence of reddish brown precipitate.</li> </ul>	Absence of alkaloids.


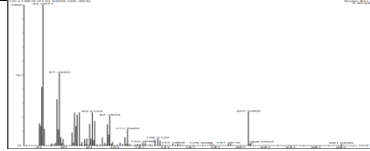
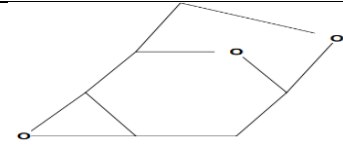
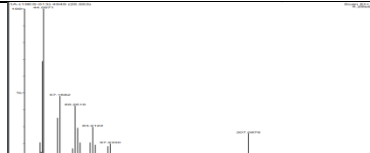
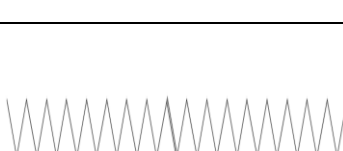
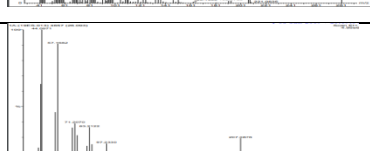
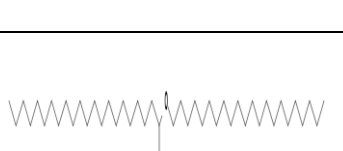
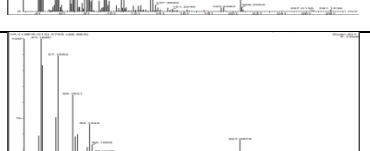
Table 3: GC MS analysis of ethanolic extract of *Gomphrena globosa* L. flowers.


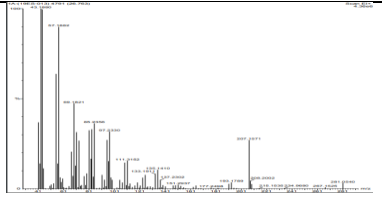
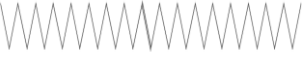
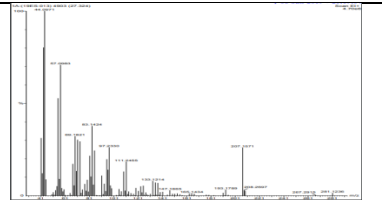
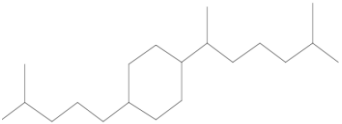
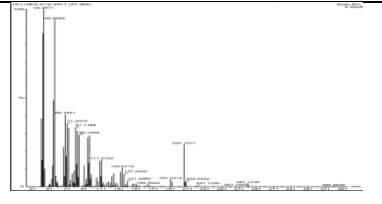

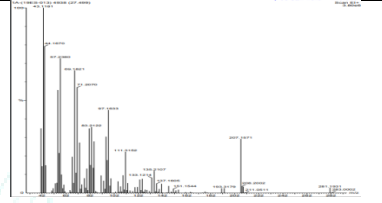

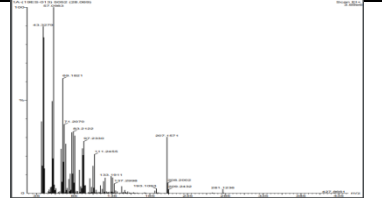

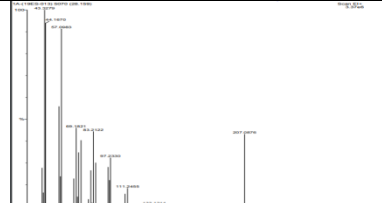
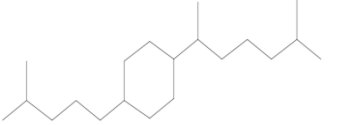
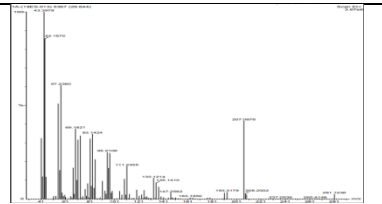
S. no	Peak name	Molecular formula	Molecular weight	Retention time	Area	% Area
1	SULFUROUS ACID, OCTADECYL 2-PROPYL ESTER	C <sub>21</sub> H <sub>44</sub> O <sub>3</sub> S	376	25.353	325,175.2	1.306
2	1,6;3,4-DIANHYDRO-2-DEOXY-.BETA.-D-RIBO-HEXOPYRANOSE	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	128	25.553	382,412.1	1.536
3	17-PENTATRIACONTENE	C <sub>35</sub> H <sub>70</sub>	490	26.093	4,471,202.5	17.961
4	DOCOSANOIC ACID, DOCOSYL ESTER	C <sub>44</sub> H <sub>88</sub> O <sub>2</sub>	648	26.583	6,323,849.0	25.404
5	HEXATRIACONTANE	C <sub>36</sub> H <sub>74</sub>	506	26.763	6,055,150.5	24.324
6	17-PENTATRIACONTENE	C <sub>35</sub> H <sub>70</sub>	490	27.324	1,216,819.6	4.888
7	CYCLOHEXANE, 1-(1,5-DIMETHYLHEXYL)-4-(4-METHYLPENTYL)-	C <sub>20</sub> H <sub>40</sub>	280	27.364	1,078,786.8	4.334
8	17-PENTATRIACONTENE	C <sub>35</sub> H <sub>70</sub>	490	27.499	2,166,417.0	8.703
9	11-TRICOSENE	C <sub>23</sub> H <sub>46</sub>	322	28.069	1,918,735.1	7.708
10	1-PENTACONTANOL	C <sub>35</sub> H <sub>70</sub>	490	28.159	596,499.4	2.396
11.	CYCLOHEXANE, 1-(1,5-DIMETHYLHEXYL)-4-(4-METHYLPENTYL)-	C <sub>20</sub> H <sub>40</sub>	280	29.644	358,503.8	1.440

**Table 4:** Nature and the biological activities of phytoconstituents of ethonolic extract of *Gomphrena globosa L.* flowers.

S.no	Retention time	Peak area %	Name of the compound	Activity
1	25.353	1.306	Sulfurous Acid, Octadecyl 2-Propyl Ester	Antibacterial activity <sup>[15]</sup>
2	25.553	1.536	1,6;3,4-Dianhydro-2-Deoxy-.Beta.-D-Ribo-Hexopyranose	-
3	26.093	17.961	17-Pentatriacontene	Anti-inflammatory, Anti cancer, Anti bacterial, Anti arthritic and Anti microbial activity <sup>[16,17]</sup>
4	26.583	25.404	Docosanoic Acid, Docosyl Ester	Emollient and skin conditioning <sup>[18,19]</sup>
5	26.763	24.324	Hexatriacontane	Anti inflammatory , analgesic activity, Radical scavenger and Antioxidant activity <sup>[20-26]</sup>
6	27.324	4.888	17-Pentatriacontene	Anti inflammatory, Anti cancer, Anti bacterial and Anti arthritic and Anti microbial activity <sup>[16,17]</sup>
7	27.364	4.334	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	Anti bacterial and Anti cancer activity <sup>[27,28]</sup>
8	27.499	8.703	17-Pentatriacontene	Anti inflammatory, Anti cancer ,Anti bacterial Anti arthritic and Anti microbial activity <sup>[16,17]</sup>
9	28.069	7.708	11-Tricosene	-
10	28.159	2.396	1-Pentacontanol	-
11.	29.644	1.440	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-	Anti bacterial and Anti cancer activity <sup>[27,28]</sup>

**Table 5:** The molecular structure, molecular formula and GC-MS spectrum of individual phytochemical components of the ethanolic extract of *Gomphrena globosa L.* flowers.

S. no	Peak area%	Name of the compound	Molecular structure	Hit spectrum
1	1.306	Sulfurous Acid, Octadecyl 2-Propyl Ester		
2	1.536	1,6;3,4-Dianhydro-2-Deoxy-.Beta.-D-Ribo-Hexopyranose		
3	17.961	17-Pentatriacontene		
4	25.404	Docosanoic Acid, Docosyl Ester		

5	24.324	Hexatriacontane		
6	4.888	17-Pentatriacontene		
7	4.334	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-		
8	8.703	17-Pentatriacontene		
9	7.708	11-Tricosene		
10	2.396	1-Pentacontanol		
11.	1.440	Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)-		

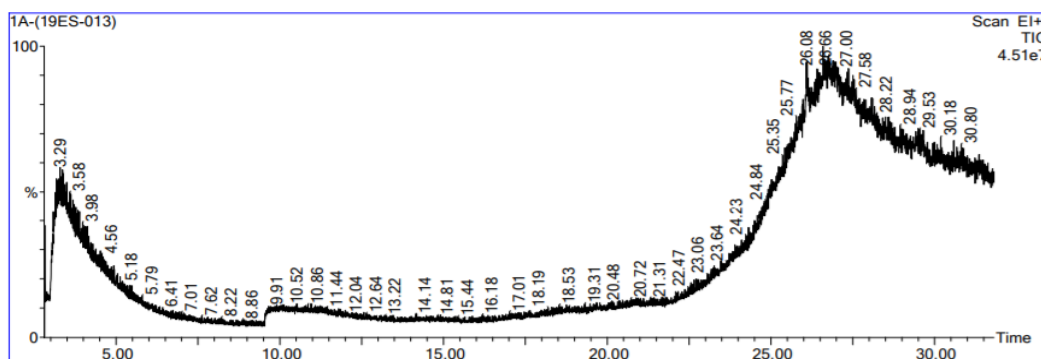


Figure 1: Chromatogram of ethanolic extract of *Gomphrena globosa* L. flowers.



## CONCLUSION

The plant *Gomphrena globosa* L. is highly valuable as traditional medicines in the treatment of various human ailments. Biochemical analysis of ethanolic extract of *Gomphrena globosa* L. has shown the presence of sterols, triterpenoid, tannins, phenols and flavonoids. On GC-MS analysis of ethanolic extract of *Gomphrena globosa* L. flowers of Indian origin has shown 11 prominent phytochemicals which are attributed with potential pharmacological activities such as antioxidant, anti-inflammatory, anticancer, anti arthritic and antibacterial. It was found that 7 phytochemicals have shown anti-inflammatory activity. Hence, it would be having a promising anti-inflammatory activity.

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