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.\[1,2,3\]

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.\[4-7\]

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.\[8\]

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°C-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 the solvent of an extractor become colorless (approximately 4hr). First cycle has taken
30 min and subsequently 17 cycles were done, where each cycle took approximately 8 min. After that the extract was dried and solvent was recollected using rotavapor. 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,[9-14]

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% diphenyl 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⁻¹; and 300°C, where it was held for 6 min.

The mass detector conditions were maintained: 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.

| TEST FOR STEROID AND TRITERPENOIDS | PROCEDURE |
|------------------------------------|-----------|
| **Libermann’s- Burchard test:**    | 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. |
| **Salkowski reaction:**            | Extract was treated in chloroform with few drops of concentrated H₂SO₄, shaken well and allow standing for some time. |

| TEST FOR TANNINS AND PHENOLIC COMPOUNDS | PROCEDURE |
|-----------------------------------------|-----------|
| **Ferric chloride test:**               | Extract was treated with FeCl₃. |
| **Lead acetate solution:**              | Extract treated with few drops of lead acetate. |

| TEST FOR FLAVONOIDS | PROCEDURE |
|---------------------|-----------|
| **Shinoda test:**   | 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. |

| TEST FOR GLYCOSIDES | PROCEDURE |
|--------------------|-----------|
| **Foam test:**     | Shake the extract or dry powder vigorously with water. |

| TEST FOR PROTEINS | PROCEDURE |
|-------------------|-----------|
| **Biuret test (general test):** | To 3 ml of test solution, 4% NAOH and few drops of 1% CuSO₄ solution was added. |
| **Millons test:** | Extract treated with 2ml of Millons reagent (mercuric nitrate in nitric acid containing traces of nitrous acid). |

| TEST FOR AMINO ACIDS | PROCEDURE |
|----------------------|-----------|
| **Ninhydrin test:**  | Extract was heated with 5% solution of ninhydrin solution (indane-1,2,3 trionehydrate) in boiling water bath for 10 min. |

| TEST FOR CARBOHYDRATES | PROCEDURE |
|------------------------|-----------|
| **Molisch test:**      | Extract treated with few drops of alcoholic α-naphthal. 0.2ml of concentrated H₂SO₄ was added slowly through the side of the test tube. |
| **Benedicts test:**    | Extract treated with few drops of benedict reagent (alkaline solution containing cupric citrate complex). |

| TEST FOR ALKALOIDS | PROCEDURE |
|-------------------|-----------|
| **Mayer’s test:** | Extract was treated with Mayer’s reagent (Potassium mercuric iodide solution). |
| **Wagner’s test:** | 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 FOR STEROID AND TRITERPENOIDS | OBSERVATION | RESULT |
|------------------------------------|-------------|--------|
| Libermann’s- Burchard test:         | 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. | Presence of Steroid and triterpenoids |
| Salkowski reaction:                | Red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids. | |

| TEST FOR TANNINS AND PHENOLIC COMPOUNDS | OBSERVATION | RESULT |
|----------------------------------------|-------------|--------|
| Ferric chloride test:                  | Blue-black colour. | Presence of tannins and phenolic compounds |
| Lead acetate solution:                 | White precipitate. | |

| TEST FOR FLAVONOIDS | OBSERVATION | RESULT |
|---------------------|-------------|--------|
| Shinoda test:       | Orange, pink red to purple colour appears. | Presence of flavonoids |
|                     | Yellow coloured precipitate is formed. | |

| TEST FOR GLYCOSIDES | OBSERVATION | RESULT |
|---------------------|-------------|--------|
| Foam test:          | Absence of persistent foam. | Absence of glycosides. |
|                     | Absence of blue or green fluorescence. | |

| TEST FOR PROTEINS | OBSERVATION | RESULT |
|-------------------|-------------|--------|
| Biuret test ( general test ): | Absence of violet or pink colour. | Absence of proteins. |
| Millions test:    | Absence of white precipitate. | |

| TEST FOR AMINO ACIDS | OBSERVATION | RESULT |
|----------------------|-------------|--------|
| Ninhydrin test:      | Absence of purple or bluish colour. | Absence of amino acids |

| TEST FOR CARBOHYDRATES | OBSERVATION | RESULT |
|------------------------|-------------|--------|
| Molisch test :         | Absence of purple to violet colour at the junction. | Absence of carbohydrate. |
| Benedict’s test:       | Upon boiling on water bath reddish brown precipitate doesn’t forms, therefore reducing sugars are absent. | |

| TEST FOR ALKALOIDS | OBSERVATION | RESULT |
|-------------------|-------------|--------|
| Mayer’s test :    | Absence of cream colour precipitate. | Absence of alkaloids. |
| Wagner’s test:    | Absence of reddish brown precipitate. | |

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     | SULFURIOUS ACID, OCTADECYL 2-PROPYL ESTER | C21H44O4S         | 376              | 25.353         | 325,175.2 | 1.306  |
| 2     | 1,6;3,4-DIANHYDRO-2-DEOXY-BETA.-D-RIBO-HEXOPYRANOSE | C6H6O3         | 128              | 25.553         | 382,412.1 | 1.536  |
| 3     | 17-PENTATRIACONTENE                      | C35H70           | 490              | 26.093         | 4,471,202.5 | 17.961 |
| 4     | DOCOSANOIC ACID, DOCOSYL ESTER          | C44H88O2         | 648              | 26.583         | 6,323,849.0 | 25.404 |
| 5     | HEXATRIACONTANE                          | C36H74           | 506              | 26.763         | 6,055,150.5 | 24.324 |
| 6     | 17-PENTATRIACONTENE                      | C35H70           | 490              | 27.324         | 1,216,819.6 | 4.888  |
| 7     | CYCLOHEXANE, 1-(1,5-DIMETHYLHEXYL)-4-(4-METHYLPENTYL)- | C28H42          | 280              | 27.364         | 1,078,786.8 | 4.334  |
| 8     | 17-PENTATRIACONTENE                      | C28H42           | 490              | 27.499         | 2,166,417.0 | 8.703  |
| 9     | 11-TRICOSENE                             | C22H46           | 322              | 28.069         | 1,918,735.1 | 7.708  |
| 10    | 1-PENTACONTANOL                          | C25H70           | 490              | 28.159         | 596,499.4  | 2.396  |
| 11    | CYCLOHEXANE, 1-(1,5-DIMETHYLHEXYL)-4-(4-METHYLPENTYL)- | C28H42          | 280              | 29.644         | 358,503.8  | 1.440  |
Table 4: Nature and the biological activities of phytoconstituents of ethanolic 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[15]                                                 |
| 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[16,17] |
| 4    | 26.583         | 25.404      | Docosanoic Acid, Docosyl Ester | Emollient and skin conditioning[18,19]                                   |
| 5    | 26.763         | 24.324      | Hexatriacontane      | Anti inflammatory, analgesic activity, Radical scavenger and Antioxidant activity[20-26] |
| 6    | 27.324         | 4.888       | 17-Pentatriacontene  | Anti inflammatory, Anti cancer, Anti bacterial and Anti arthritic and Anti microbial activity[16,17] |
| 7    | 27.364         | 4.334       | Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)- | Anti bacterial and Anti cancer activity[27,28]                           |
| 8    | 27.499         | 8.703       | 17-Pentatriacontene  | Anti inflammatory, Anti cancer, Anti bacterial Anti arthritic and Anti microbial activity[16,17] |
| 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[27,28]                           |

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 | ![Molecular structure](image1) | ![Hit spectrum](image2) |
| 2     | 1.536       | 1,6;3,4-Dianhydro-2-Deoxy-.Beta.-D-Ribo-Hexopyranose | ![Molecular structure](image3) | ![Hit spectrum](image4) |
| 3     | 17.961      | 17-Pentatriacontene          | ![Molecular structure](image5) | ![Hit spectrum](image6) |
| 4     | 25.404      | Docosanoic Acid, Docosyl Ester | ![Molecular structure](image7) | ![Hit spectrum](image8) |
|   |   |                  |          |
|---|---|------------------|----------|
| 5 | 24.324 | Hexatriacontane | ![Graph](image1) |
| 6 | 4.888 | 17-Pentatriacontene | ![Graph](image2) |
| 7 | 4.334 | Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)- | ![Graph](image3) |
| 8 | 8.703 | 17-Pentatriacontene | ![Graph](image4) |
| 9 | 7.708 | 11-Tricosene | ![Graph](image5) |
| 10 | 2.396 | 1-Pentacontanol | ![Graph](image6) |
| 11. | 1.440 | Cyclohexane, 1-(1,5-Dimethylhexyl)-4-(4-Methylpentyl)- | ![Graph](image7) |

**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, anti-cancer, 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.

REFERENCES

1. Muhammad Ilyas et al., Biological Potential and Phytopharmacological Screening of Gomphrena Spei., International Journal of Pharma Research & Review, 2014; 3(1):58-66.
2. Ignacimuthu, S. and M. Ayyanar, Plants used for non-medical purposes by the tribal people in kalakad Mundanthurai Tiger reserve, Southern India. Indian Journal Traditional of Knowledge, 2009; 9(3):515-518.
3. Dias, D.A., R.M.X. De Moura, P.S. Pereira, A.H. Janeiro and S.C. Franca, Antimicrobial screening and quantitative determination of benzoic acid derivative of Gomphrena celosioides by TLC-Densitometry. Chem. Pharm. Bull. 2004; 52(11):1342-1344.
4. P Yamuna et al, Qualitative phytochemical analysis of Gomphrena globosa Linn. and Gomphrena decumbens Jacq., International Journal of Biology Research, 2017; 2(3):20-22.
5. Krishnasah D, Sarbatly R, Bono A; Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev, 2007; 1:97-104.
6. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry. 1997; 44:1185-1236.
7. Herouart D, Sangwan RS, Fliniaux MA, Sangwan-Norree BS. Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of *Datura innoxia*. Planta Med.; 1988; 54:14-17.
8. P Yamuna et al., GC-MS analysis of bioactive compounds in the entire parts of ethanolic extract of Gomphrena globosa Linn., International Journal of Research in Pharmacy and Pharmaceutical Sciences, 2017; 2(4):57-64
9. Khandelwal KR. Chapter 25, Evaluation. In: Practical Pharmacognosy. 12th Edition. Pune: Nirali Prakashan, 2010; 25.1-25.8.
10. Harborne JB. 1973. Phytochemical methods. A guide to modern Techniques of plant Analysis. Chapman and Hall, London, 267-270.
11. Khandelwal KR. Chapter 41, Evaluation. In: Practical Pharmacognosy. 19th Edition. Pune: Nirali Prakashan, 2008:157-158.
12. Raman N. Chapter 5, Qualitative Phytochemical Screening. In: Phytochemical Techniques. New Delhi: New India Publishing agency, 2006: 19-24.
13. P Yamuna et al., Qualitative Phytochemical Analysis of Gomphrena globosa Linn. and Gomphrena decumbens Jacq., International Journal of Biology Research, Volume 2017; 2(3):20-22.
14. Ayesha Siddiqua and Srirama Mittapally, Formulation and Evaluation of ethanolic extract of Cissus quadrangularis herbal gel, International Journal of Pharmaceutical and Biosciences, 2017; 4(4): 9-29.
15. Babu et al., Phytochemical Analysis of Ficus arnottiana (Miq.) Miq. Leaf Extract Using GC-MS Analysis, International Journal of Pharmacognosy and Phytochemical Research; 2017; 9(6):775-779.
16. Dinesh Kumar et al., GC-MS analysis of bioactive compounds from ethanolic leaves extract of Eichhornia crassipes (Mart) Solms. and their pharmacological activities, The Pharma Innovation Journal; 2018; 7(8):459-462.
17. Shubhangi Nagaro Ingle, Phytochemical Analysis of Ficus Religiosa L. (Moraceae) by GC-MS method, International Research Journal of Natural and Applied Sciences, 2017; 4(3):55-63.
18. Sunita Arora and Ganesh Kumar, Phytochemical screening of root, stem and leaves of Cenchrus biforous Roxb, Journal of Pharmacognosy and Phytochemistry; 2018; 7(1):1445-1450.
19. Pratima Rayya et al, Analysis of Medicinally Important Phytocompounds from Adina cordifolia Leaves, International Journal of Current Microbiology and Applied Sciences; 2018; 7(11):3007-3019.
20. Selvan et al, Bioactive compound identification, phytochemical estimation, In-Vitro Anti-Inflammatory and Antioxidant activity of Papulaca Lappacea, International Journal of Pharmacognosy; 2014; 19(9):596-604.
21. Pillai and Nair, GC-MS analysis of chloroform extract of Cleome Burmanni W. and A. (Cleomaceae), International Journal of Pharmaceutical Sciences and Research; 2013; 4(5):1930-1933.
22. Pandurangan A, Khosa RL and Hemalatha S, Anti-inflammatory and analgesic activity of Ichnocarpus frutescens, Pharmacologyonline; 2008; 1:392-399.
23. Sunita Arora and Sonam Meena, GC-MS Profiling of Ceropedia bulbosa Roxb. var.bulbosa, an endangered plant from Thar Desert, Rajasthan, The Pharma Innovation Journal; 2017; 6(11):568-573.
24. Shashikala A. et al., GC-MS analysis of phytocomponents in the methanolic extract of Propolis of Honey Bee, Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry. 2016; 4(2):74-78.
25. Premjani N, Jayanthi C. Antimicrobial activity of Diethyl Phthalate: An Insilico Approach, Asian Journal of Pharmaceutical and clinical Research, 2014; 7(4):141-142.
26. Ashwathanarayana R and Raja Naiika, Anti-Inflammatory properties of Pavetta Crassicaulis Bremek. leaf and flower crude extracts and its pure compounds collected from Western Ghats, Karnataka, India, Asian Journal of Pharmaceutical and Clinical Research, 2018; 11(9):72-90.
27. Balasubramanian et al., GCMS And FTIR analysis on the methanolic extract of Coriandrum Sativum leaves, European Journal of Pharmaceutical and Medical Research; 2018; 5(3):454-460.
28. Aasha Bharadwaj, Najam A. Shakiil, Vidyanath Jha, Rajinder Kumar Gupta. "Screening of nutritional, phytochemical, antioxidant and antibacterial activity of underutilized seeds of Scirpus articulatus: the basis of Khubahi Ramdana industry". Journal of Pharmacognosy and phytochemistry; 2014; 3(4):11-20.