

## **GC-MS analysis of bioactive components of the methanol extract of *Hibiscus tiliaceus* Linn.**

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### **ABSTRACT**

*The present investigation was carried out to determine the possible chemical components from *Hibiscus tiliaceus* by GC-MS technique. This analysis revealed that the methanol extract contains N, N- Dimethylglycine (83.97%), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (2.94%) and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- (2.69%). The presence of some of these constituents in the plant extract provides the scientific evidences for the antimicrobial, anticancer, antioxidant and antidiabetic properties of the plant.*

**Key words:** Phytochemicals, GC-MS, *Hibiscus tiliaceus*, Herbal medicine

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### **INTRODUCTION**

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. In addition, the use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe [1]. In developing countries, a huge number of people lives in extreme poverty and some are suffering and dying for want of safe water and medicine, they have no alternative for primary health care. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development.

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytochemicals for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of chinese medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids [2]. Hence, the present study was carried out to determine the possible phytochemical components from *Hibiscus tiliaceus* by GC-MS analysis.

*Hibiscus tiliaceus* Linn (Belpata) is branched tree of 20 – 30 fit height belonging to the family Malvaceae. The plant is found along the eastern and western coast of India [3]. Plant has a large heart shaped green color, alternate petiolated leaves and bright yellow with crimson color center flower. Fruit are subglobular, capsule up to 2.5 cm long with numerous small seeds. The crude extracts of Belpata have use in treatment of skin conditions [4], constipation [5], contraceptive [6], morning sickness [7], diabetics [8], fracture [9]. Chemical constituents reported are gossypol, Mansonones – D and F, Gossypetin glycoside, Hibiscones, Hibiscoquinones-A & D [10], Lapachol, Quercetin, kampferol and p- coumaric acid are reported in different parts of *Hibiscus tiliaceus* Linn. [11].

## MATERIALS AND METHODS

### Collection of plant material

The leaves of *H.tiliaceus* were collected from the Pachamalai, Eastern Ghats of Tamilnadu, South India. They were identified and authenticated by the Rabinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamilnadu, India.

### Preparation of powder and extract

Leaves of *H.tiliaceus* (5g) was shade dried, powdered and extracted with methanol for 24 hours using cold maceration methods. The extract was then filtered through Whatmann filter paper No.41 along with 2g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1ml. The extract contains both polar and non-polar phytochemicals.

### GC-MS Analysis

The GC-MS analysis of *H.tiliaceus* powder leaves extract with in methanol, was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25 $\mu$ mdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min. and the injector was operated at 290°C and the oven temperature was programmed as follows; 50°C at 8°C/min to 200°C (5min) at 7°C/min to 290°C(10min).

### Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the (NIST), library. The name, molecular weight and structure of the components of the test materials were ascertained [12], [13].

## RESULTS AND DISCUSSION

Medicinal plants are being used as valuable sources of food and medicine for the prevention of illness and maintenance of human health. In India, many indigenous plants are widely consumed as food or home remedies especially in the treatment or management of common diseases. The compounds present in the methanolic extract of *H.tiliaceus* were identified by GC-MS analysis presented in figure 1. The active principle Molecular Weight (MW), Concentration (%), Molecular Formula (MF), and Retention Time (RT) is presented in Table 1. More than nineteen compounds were identified in the extract. The prevailing compounds were

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanol extract of *H.tiliaceus*. The compounds present in the methanol extract of *H.tiliaceus* identified by GC-MS analysis as shown in Fig. 5. It was found that the main constituents of leaves N, N-Dimethylglycine (83.97%), 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol (2.94%) (Synonym: Phytol) and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (2.69%) (Table 1 & Fig 1).

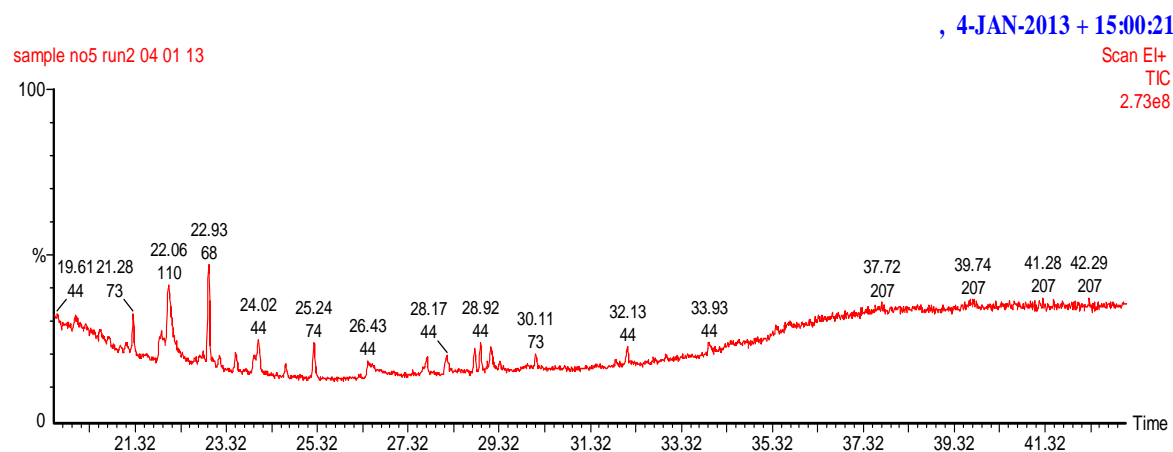
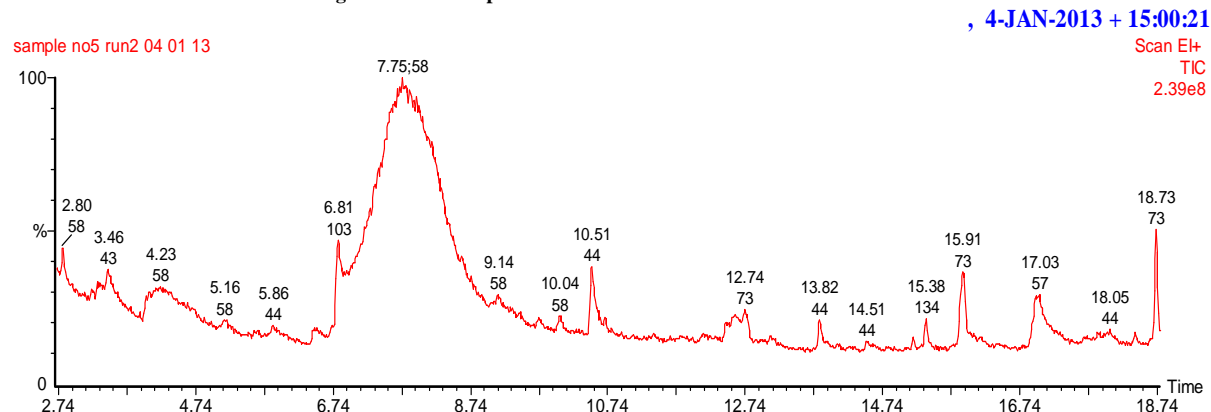
Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies. Phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells [14]. Phytol, Phenol, 2, 4-bis (1-phenylethyl) - which are all have medicinal properties. Dimethylglycine (DMG) is a tertiary amino acid involved in a variety of biological processes because it is an intermediary metabolite in the cellular metabolism of choline and betaine.

In the present study twenty three chemical constituents have been identified from methanolic extract of the leaves of *H.tiliaceus* by Gas Chromatogram-Mass spectrometry analysis. The presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results.

**Table 1: GC-MS analysis revealed the presence of phytochemical components in methanol leaf extracts of *Hibiscus tiliaceus***

| S. No. | Peak Name  | Retention time | Peak area | %Peak area |
|--------|--|----------------|-----------|------------|
| 1.     | Name: 2-Nitro-tertiary butanol<br>Formula: C <sub>4</sub> H <sub>9</sub> NO <sub>3</sub><br>MW: 119  | 2.80           | 990379    | 0.6862     |
| 2.     | Name: 2-Propanone, 1-(1-methylethoxy)-<br>Formula: C <sub>6</sub> H <sub>12</sub> O <sub>2</sub><br>MW: 116  | 3.46           | 1357485   | 0.9405     |
| 3.     | Name: 2-Cyclopenten-1-one, 2-hydroxy-<br>Formula: C <sub>5</sub> H <sub>6</sub> O <sub>2</sub><br>MW: 98   | 5.86           | 292059    | 0.2023     |
| 4.     | Name: N,N-Dimethylglycine<br>Formula: C <sub>4</sub> H <sub>9</sub> NO <sub>2</sub><br>MW: 103   | 7.75           | 121209376 | 83.9769    |
| 5.     | Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-<br>Formula: C <sub>6</sub> H <sub>8</sub> O <sub>4</sub><br>MW: 144                      | 10.51          | 3885271   | 2.6918     |
| 6.     | Name: Nonanenitrile<br>Formula: C <sub>9</sub> H <sub>17</sub> N<br>MW: 139  | 11.40          | 105898    | 0.0734     |
| 7.     | Name: 2-Furancarboxylic acid, tetrahydro-3-methyl-5-oxo-, methyl ester<br>Formula: C <sub>7</sub> H <sub>10</sub> O <sub>4</sub><br>MW: 158        | 13.11          | 108878    | 0.0754     |
| 8.     | Name: 2-Methoxy-4-vinylphenol<br>Formula: C <sub>9</sub> H <sub>10</sub> O <sub>2</sub><br>MW: 150   | 13.82          | 464095    | 0.3215     |
| 9.     | Name: 2,4-Dimethoxyphenol<br>Formula: C <sub>8</sub> H <sub>10</sub> O <sub>3</sub><br>MW: 154   | 14.51          | 312661    | 0.2166     |
| 10.    | Name: 1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethanone<br>Formula: C <sub>13</sub> H <sub>18</sub> O <sub>2</sub><br>MW: 206 | 15.18          | 253550    | 0.1757     |
| 11.    | Name: Benzenamine, N,N-diethyl-<br>Formula: C <sub>10</sub> H <sub>15</sub> N<br>MW: 149   | 15.38          | 816933    | 0.5660     |
| 12.    | Name: 4-Methyl-2,5-dimethoxybenzaldehyde<br>Formula: C <sub>10</sub> H <sub>12</sub> O <sub>3</sub><br>MW: 180                                     | 18.42          | 274576    | 0.1902     |
| 13.    | Name: Phenol, 2,6-dimethoxy-4-(2-propenyl)-<br>Formula: C <sub>11</sub> H <sub>14</sub> O <sub>3</sub><br>MW: 194                                  | 20.74          | 258781    | 0.1793     |
| 14.    | Name: 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol<br>Formula: C <sub>10</sub> H <sub>12</sub> O <sub>3</sub><br>MW: 180                          | 21.90          | 961801    | 0.6664     |
| 15.    | Name: Oxazole, 5-hexyl-2,4-dimethyl-<br>Formula: C <sub>11</sub> H <sub>19</sub> NO<br>MW: 181   | 22.06          | 1850007   | 1.2817     |
| 16.    | Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol<br>Formula: C <sub>20</sub> H <sub>40</sub> O<br>MW: 296  | 22.93          | 4254851   | 2.9479     |
| 17.    | Name: 11,13-Dimethyl-12-tetradecen-1-ol acetate<br>Formula: C <sub>18</sub> H <sub>34</sub> O <sub>2</sub><br>MW: 282                              | 23.53          | 833557    | 0.5775     |
| 18.    | Name: Hexadecanoic acid, methyl ester<br>Formula: C <sub>17</sub> H <sub>34</sub> O <sub>2</sub><br>MW: 270  | 25.24          | 1842187   | 1.2763     |
| 19.    | Name: 1(2H)-Naphthalenone, octahydro-4-hydroxy-, trans-  | 28.17          | 1021669   | 0.7078     |

|     |  |       |         |        |
|-----|--|-------|---------|--------|
|     | Formula: C <sub>10</sub> H <sub>16</sub> O <sub>2</sub><br>MW: 168   |       |         |        |
| 20. | Name: 9,12-Octadecadienoic acid, methyl ester<br>Formula: C <sub>19</sub> H <sub>34</sub> O <sub>2</sub><br>MW: 294    | 28.79 | 890217  | 0.6168 |
| 21. | Name: 11,14,17-Eicosatrienoic acid, methyl ester<br>Formula: C <sub>21</sub> H <sub>36</sub> O <sub>2</sub><br>MW: 320 | 28.91 | 1070652 | 0.7418 |
| 22. | Name: Phytol<br>Formula: C <sub>20</sub> H <sub>40</sub> O<br>MW: 296  | 29.14 | 1075575 | 0.7452 |
| 23. | Name: 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene<br>Formula: C <sub>25</sub> H <sub>42</sub><br>MW: 342      | 39.74 | 206067  | 0.1428 |

Figure 1: GC-MS spectrum of methanol extract of *Hibiscus tiliaceus*

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