

## DETERMINING THE COMPOUNDS IN ETHANOL EXTRACTS OF HELIOTROPIUM INDICUM USING GC-MS STUDY

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### ABSTRACT

The investigation was carried out to determine the chemical components of Ethanol extracts of *Heliotropium indicum* leaves using Perkin-Elmer Gas Chromatography–Mass Spectrometry. Ethanol extracts of *Heliotropium indicum* was used traditionally in India for the treatment of skin diseases. The ethanolic extract of the medicinal plant *H. indicum* have been investigated by GC-MS to identify compounds responsible for its therapeutical properties. The results of this study offer a platform of using *Heliotropium indicum* leaves as herbal alternative for various diseases. Phytochemical analysis of the extracts revealed that the activity of the plant material is due to the presence of various compounds.

### 1. Introduction

In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs and thus in many industrialized countries herbal medicines are gaining popularity. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. . Over 50% of all modern clinical drugs are of natural product origin (Stufness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995). They have been used as a source of medicine. The secondary metabolites of plants provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals(Koduru *et al.*, 2006). Plants are a rich source of secondary

metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fatima *et al.*, 2006). The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care and hence, *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Baliga *et al.*, 2003).

*Heliotropium indicum*, is one such very common medicinal plant in India with a long history of traditional medicinal uses in many countries in the world is popularly named as Indian heliotrope referenced under the symbol HEIN. It is a flowering herb, with or without the root. Even though It is very toxic herb from Boraginaeaceae family and are used very less number of present-day herbalists, it is anti-inflammatory (Ramamurthy *et al.*, 2010), antiulcer, diaphoretic, emmenagogue, febrifuge, oxytonic and stimulant (Kugelman *et al.*, 1976 and Srinivas *et al.*, 2000). The plant contains a complex of acids so called "organic acids" which stimulate white blood cell activity and speeds the healing of wounds if it is used in correct concentration. External usage of this plant is used in the treatment of slow-healing cuts, eczema, infected toe and fingernails etc. But internal consumption can cause damage to the kidneys and uterine bleeding.

In the present work alcoholic extract of *Heliotropium indicum* leaf extract was investigated to identify and characterize the compounds of therapeutic value extracted from *H. indicum*. Further, in this study the infusion prepared from this plant was characterized. The analytical method Gas Chromatography/Mass Spectrometry (GC/MS) was applied for the above said purpose.

## **2. Materials and Methods**

### **2.1 Plant materials:**

*Heliotropium indicum* leaves were collected from various places of Cuddalore District, Tamil Nadu, India. The leaves of *H. indicum* were washed with sterile distilled water. After, the leaves were shade dried and powdered using pestle and mortar. 20 gm powdered plant material is soaked in 50 ml of Absolute alcohol overnight and then filtered through whatmann filter paper No.

41 along with 2 gm 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 1 ml. The extracts were preserved at 5°C in airtight bottle until further use. The extracts were subjected to phytochemical analysis.

## 2.2 GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µMdf, composed of 100% Dimethylpolysiloxane), operating in electron impact mode at 70 eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

## 3. Results and Discussion

In the present study the compounds found in the leaves of *H. indicum* were studied by GC-MS. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. This study was performed on the alcoholic extracts of the plant leaves. The antimicrobial, anti-inflammatory compounds and some essential fatty acids identified from the extracts are listed in Tables.

**Table 1. Antimicrobial Compounds Identified in Prepared Extracts by GC-MS Study**

S.No	Antimicrobial Components	Formula
1	Benzene acetaldehyde	C <sub>8</sub> H <sub>8</sub> O
2	5H-1-Pyridine	C <sub>8</sub> H <sub>7</sub> N
3	2-Furan carboxaldehyde, 5-(Hydroxymethyl)-	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>
4	Benzene acetic acid	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>
5	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>
6	Phenol, 3-Isopropoxy-5-Methyl-	C <sub>10</sub> H <sub>14</sub> O <sub>2</sub>
7	3`-Acetyllycopsamine	C <sub>17</sub> H <sub>27</sub> NO <sub>6</sub>
8	Squalene	C <sub>30</sub> H <sub>50</sub>
9	Phytol	C <sub>20</sub> H <sub>40</sub> O

**Table 2. Fatty Acids Identified in Prepared Extracts by GC-MS Study**

S.No	Name of Fatty Acids	Formula
1	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>
2	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>
3	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
4	Hexadecanoic acid, Ethyl Ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
5	9,12-Octadecadienoic acid (Z, Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
6	9,12-Octadecadienoic acid, Ethyl Ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>
7	9,12,15-Octadecatrienoic acid, Ethyl Ester, (Z,Z,Z)-	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>

8	Octadecanoic acid, Ethyl Ester	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>
9	8,11,14 – Eicosatrienoic acid, (Z, Z, Z) -	C <sub>20</sub> H <sub>34</sub> O <sub>2</sub>
10	Docosanoic acid, Ethyl Ester	C <sub>24</sub> H <sub>48</sub> O <sub>2</sub>
11	Hexanoic acid, Ethyl Ester	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>

**Table 3. Antiinflammatory Compounds Identified in Prepared Extracts by GC-MS Study**

S. No	Anti-inflammatory Components	Formula
1	Phytol	C <sub>20</sub> H <sub>40</sub> O
2	9,12-Octadecadienoic acid (Z, Z)-	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
3	9,12-Octadecadienoic acid, Ethyl Ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>
4	Squalene	C <sub>30</sub> H <sub>50</sub>

It is found that the organic acid derivatives in the leaves are in very high concentration. In addition, it is found that the terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant.

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