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GC-MS Determination of Bioactive Constituents of *Heliotropium indicum* Leaf

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The investigation was carried out to determine the chemical components of *Heliotropium indicum* leaves using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of ethanolic extract of *Heliotropium indicum* leaves revealed the existence of Squalene (23.84), Phytol (14.38) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (20.14). The results of this study offer a platform of using *Heliotropium indicum* leaves as herbal alternative for various diseases.

Keyword: *Heliotropium indicum*, GC/MS, Bioactive components.

1. Introduction

In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies. Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions. 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^[1].

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^[2]. Distinguished examples of these compounds include flavonoids, phenols and

phenolic glycosides, saponins and cyanogenic glycosides^[3]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes^[4]. It has been shown that *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^[4].

Pure drugs that are technologically produced or isolated from plants may be chosen for their high activity against a human disease, but they have disadvantages. They rarely have the same level of activity as the crude extract at parallel dose or concentrations of the active component^[5]. This phenomenon is attributed to the absence of interacting substances present in the extract. Furthermore, many plants contain substances that

inhibit multi-drug resistance (MDR). A further disadvantage is that pure drugs are often more expensive to produce and distribute, and so are often unavailable and/or unaffordable to the poorest populations in remote areas who need them most. In contrast, herbal medicines can sometimes be grown and produced locally, at lower cost, by or close to those who need them^[6]. Since there is no report on the phytoconstituents of ethanolic fraction of *Heliotropium indicum* leaves extract it was chosen as the subject of this study. The aim of this study is to determine the organic compounds present in the active fraction of *Heliotropium indicum* leaves extract with the aid of GC-MS Technique, which may provide an insight in its use in traditional medicine.

2. Material and Methods

2.1 Plant materials:

The fully mature *Heliotropium indicum* leaves were collected in April 2013 from Vandayar Iruppu, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil nadu, India. A Voucher specimen has been deposited at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, Tamil nadu, India.

2.2 Plant sample extraction

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 extract contains both polar and non-polar phytocomponents.

2.3 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

A phytochemical is any substance that is derived from a plant source. Sometimes the term phytonutrient or phytoprotection is substituted for the word phytochemicals. Phytochemicals may relate to any one of a number of vitamins, minerals or bioactive compounds produced by the plant. Besides vitamins and minerals, there are other phytochemicals which are known to be bioactive and may be useful in the fight against various diseases cancer, cardiovascular, arthritis etc. These include the allium compounds, dithiolthiones and isothiocyanates, terpenoids, phytoestrogens, flavonoids, phenolic compounds, protease inhibitors, phytic acid, glucosinolates and indoles, plant sterols, saponins and chemicals found in various botanicals^[7].

3.1 Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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. The biological activities listed (Table 2) are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3.2 GC-MS Analysis

Free radicals play a crucial role in the development of tissue damage in pathological events. The extraction method presented is simple, rapid and inexpensive, with reduced solvent consumption. 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. The acidic fractions were silylated and subjected to GC-MS investigation. It is evident from the table 2 that all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples

and library data of corresponding compounds. Four compounds were identified in *Heliotropium indicum* leaves by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were Squalene (23.84) and Phytol (14.38). The biological activities of prevailing compounds are summarized in table 2.

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

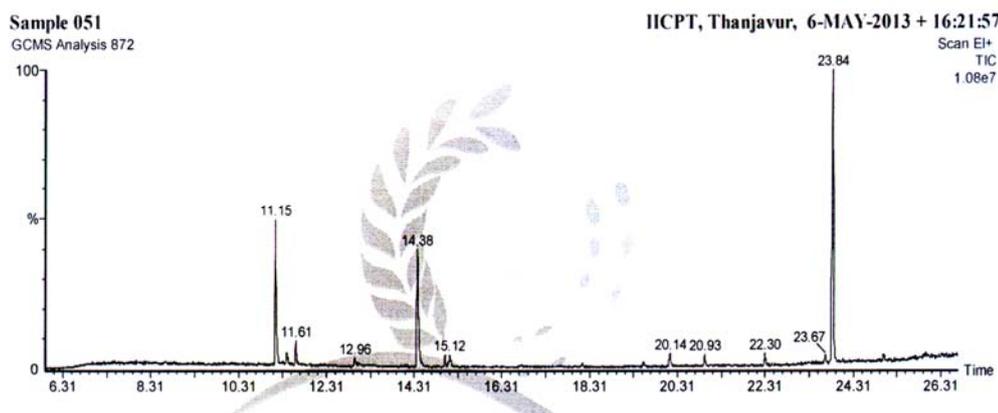


Figure 1: Chromatogram obtained from the GC/MS with the extract of *Heliotropium indicum* leaves

Table 1: shows the components identified in ethanolic extract of *Heliotropium indicum* leaves (GC MS study)

No.	RT	Name of the compound	Molecular formula	MW	Peak area %
1	11.15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	21.18
2	14.38	Phytol	C ₂₀ H ₄₀ O	296	23.53
3	20.14	1,2-Benzenedicarboxylic acid, diundecyl ester	C ₃₀ H ₅₀ O ₄	474	2.35
4	23.84	Squalene	C ₃₀ H ₅₀	410	52.94

Table 2: Activity of phyto-components identified in the ethanolic extracts of the *Heliotropium indicum* leaves by GC-MS.

Sl. No.	Retention Time	Name of the Compound	Biological activity**
1	14.38	Phytol	Cancer-Preventive
3	20.14	1,2-Benzenedicarboxylic acid, diisooctyl ester	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.
4	23.84	Squalene	Antibacterial, Antioxidant, Antitumor, Cancer-Preventive, Chemopreventive, Immunostimulant and Lipoxygenase-Inhibitor.

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

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