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GC MS ANALYSIS OF TERPENES FROM ETHYL ACETATE EXTRACT OF CHRYSANTHEMUM INDICUM LEAVES

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ABSTRACT

Plants put together many different types of secondary metabolites, which have been subsequently exploited by humans for their beneficial role in a varied range of applications. Research interest towards the study of terpenes was mainly due to its pivotal role in our environment. The phytochemical screening process revealed the presence of appreciable quantity of the terpenoids. The thin layer chromatography (TLC) process confirms the possible presence of terpenoids by revealing the development of brown coloured band when treated with perchloric acid the results pertaining to GC-MS analysis led to the identification of number of terpenes from TLC fractions of ethyl acetate extract of *Chrysanthemum indicum* leaves. GC-MS chromatogram showed 24 peaks, indicating the presence of 24 compounds. The percentage of peak area represents the amount of individual terpenes present in the specified fraction. The most prevailing major compounds are α-pinene, Terpinene, Sabinene, santolina triene, Limonene, 3-Thujanol, Neophytadiene, Phytol.

KEYWORDS

Chrysanthemum indicum L, TLC and GC-MS.

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INTRODUCTION

Medicinal plants offer very high value for human to develop biological activities, cost effectiveness and lesser side effects (Naikgh *et al*, 2003)[1]. The rich sources of secondary metabolites present in medicinal plants proved exciting biological activities. Most of the Indian medicinal plants are evaluated for such properties. The availability of abundant medicinal plants and their chemical constituents revealed their favorable therapeutic potentials. Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from the plants

for specific diseases (Khan *et al*, 2009[2], Khan *et al*, 2010a[3], Khan *et al*, 2010b, Sahreen *et al*, 2010[4]). One such is the terpenes. Terpenoids, also known as is oterpenoids constitute the largest group of herbal secondary metabolites (Bruneton, 1999)[5]. Terpenoids are involved in defense, wound scaling and thermo tolerance of plants as well as in the pollination of seed crops (Heirich *et al*, 2004)[6]. They are also dependable for the flavor of fruits, the fragrance of the flowers and the quality of agricultural products. Massive quantity of terpenes are released by trees in warmer weather, the clouds reflect sunlight, allowing the forest to normalize its temperature (Mdee 2009)[7].

Terpenoids are classified as monoterpenes (C10), sesquiterpenes (C15), diterpene (C20), triterpenes (C30) and tetraterpenes (C40) on the basis of the number of isoprene units. (Banthorpe, 1991[8], Bruneton, 1999[5], Heirich *et al*, 2004[6], Gurib-Fakim, 2005[9]). Monoterpens and sesquiterpenes are the main components of essential oils and are commonly found in plant families Labiatae, Myrtaceae, Pinaceae, and Rutaceae (Harborne, 1998[10], Heirich *et al*, 2004[6]). Diterpenes include resin acids and plant hormones (gibberellins) (Harborne, 1998)[10].

Many of the diterpenes are toxic, but some, for example, forskolin (from gymnosperms), taxol (from the Pacific yew) and ginkgo ides (from Ginkgo biloba) are used in modern medicine for the treatment of hypertension, cancer and memory loss respectively (Brunet on, 1999[5], Heirich *et al*, 2004[6], Gurib-Fakim, 2005[9]). Triterpenoids are the most abundant plant terpenes, they include plant steroids and are components of saponins and steroidal glycosides (Harborne, 1998[10], Bruneton, 1999[5]) The most common tetraterpanoids are the carotenoids which are responsible for most of the yellow and orange plant pigments (Heirich *et al*, 2004)[6]. Tetraterpenoids also include the xanthophylls found in many yellow fruits and flowers (Bruneton, 1999)[5]. Terpenoids are in general soluble in common organic solvents. However, low molecular weight terpenoids such as essential oils, are thinly soluble in water. Hence terpenoids are generally extracted with nonpolar solvents and the volatile essential oils can be steam distilled (Satyajit *et al*, 2006).

A major contradicting task is to extraction, screening, isolation and purification of the compounds from the plants TLC forms the basic method for screening the terpenoid constituents, and further isolation of the separated compound has been carried out and confirmed by the GC-MS.

Chrysanthemum indicum is a perennial plant, branching herb, grows up to 25-100cm in height with yellow daisies is native to Asia and Northeastern Europe. The plant products are conventionally used for the treatment of cancer, pneumonia, colitis, stomatitis, sore, and fever. *Chrysanthemum indicum* plant is renowned to possess antibacterial, antioxidant, and oxidative DNA damage preventive activity (Jung 2009[11], Debnath *et al*, 2013[12]). The flower of *Chrysanthemum indicum* has strong aroma, the previous learning declares that the focus is towards the study on essential oil obtained from this plant (Shen *et al*, 2004[13], Wang *et al*, 2006[14], Ye and Deng, 2009[15]). Modest information about the Terpenoids obtained from *Chrysanthemum indicum* was reported and Chemical composition of this plant was shown to be depending on the soil and climate factors and where it is grown (Shen *et al*, 2004)[13]. With this information, the present study was carried out to investigate terpenes present in *Chrysanthemum indicum* leaves using GC-MS analysis.

MATERIAL AND METHODS

Collection of Chrysanthemum indicum L

Chrysanthemum indicum plants were collected from koripallalum vaigai river bed, Madurai district, Tamil Nadu, India. The plants were brought to Thiagarajar College, Botany laboratory for proper identification.

Drying and pulverizing

Fresh leaves of *Chrysanthemum indicum* were collected, cleaned and shade dried. It was powdered in a mixer. The powder was sieved and kept in a closed container in a dry place.

Preparation of Ethyl acetate extracts of *Chrysanthemum indicum*

The ground plant material was subsequently extracted with ethyl acetate. 5gm of fine powered sample was weighed and soaked with 100ml of ethyl

acetate and allowed to stand for 7 days at ambient room temperature. The soaked plant powder was filtered by passing through a whatman No.1 filter paper and used as crude extract. Crude extracts obtained from *Chrysanthemum indicum* were stored in a refrigerator and it is screened for the presence of phytochemicals.

Screening of terpenoids

Phytochemical Screening

The initial screening of the terpenoids in the ethyl acetate extracts was carried out with the basic qualitative test for terpenoids, where 2ml of the extract was mixed with 2ml of chloroform to this 1ml of Concentrated H2SO4 was added along the side of the tube and formation of reddish brown colour indicates the presence of terpenoids.

Thin layer chromatography analysis

Thin layer Chromatographic analysis of the extract obtained from *Chrysanthemum indicum* using ethyl acetate was carried out using modified method given by Wagner and Bladt (1996). The solvent system was selected as, ethyl acetate and benzene (1:1). In the TLC Screening procedure, TLC Silica Plate was taken and impregnated with the fine drop of extract. The plate was then dried using hot air oven and kept in the chromatographic chamber contain solvent system and the extract was allowed to run. Then it is allowed to dry and it was subjected to perchloric treatment by spraying over the TLC silica plate or, the plate was examined under the UV Chamber at 366nm. The presence of terpens constituent was confirmed by the presence of brown band while applying perchloric acid as an overlay.

Gas Chromatography - Mass Spectrometry (GC/MS) analysis

Sample preparation for GC-MS Analysis

The dichloromethane (DCM) was used as a solvent in GC-MS analysis. Fractions obtained from TLC were subjected to Gas Chromatography - Mass Spectrometry (GC/MS) analysis for the identification of secondary metabolites extracted from *Chrysanthemum indicum L* .

Identification of volatile compounds by GC-MS

The sample was fractionated and chemical compounds were identified by Gas Chromatography-linked Mass Spectrometry (GC-MS; QP-5050, Schimadzu, Japan) (Rameshkumar *et al*, 2000[16], Achiraman and Archunan, 2005). The 2 µl of extract was injected into the GC-MS system on a 30 m glass capillary column with a film thickness of 0.25µm (30m×0.2mm i.d. coated with UCON HB 2000) using the following temperature programme: initial oven temperature of 40°C for 4 min. increasing to 250°C at 15°C/ min and then held at 250°C for 10 min. The GC-MS (Schimadzu GC 15A) was set through FID detector coupled to an integrator. The relative amount of each component was reported as percent of the ion current. The GC-MS was under the computer control at 70 eV using ammonia as reagent gas at 95 eV carried out chemical ionization. Identification of unknown compounds was done through by probability-based matching using the computer Library built within the NICT 12 system.

RESULTS AND DISCUSSION

To search biologically-active materials from *Chrysanthemum indicum*, the leaves were extracted with ethyl acetate. The extracts were sequentially partitioned with ethyl acetate. The phytochemical screening process revealed the presence of appreciable quantity of the terpenoids (Figure No.1). The thin layer chromatography (TLC) process confirms the possible presence of terpenoids by revealing the development of brown coloured band when treated with perchloric acid (Figure No.2). The results pertaining to GC-MS analysis led to the identification of number of terpenes from TLC fractions of ethyl acetate extract of *Chrysanthemum indicum* leaves. GC-MS chromatogram showed 24 peaks, indicating the presence of 24 compounds (Figure No.3) and the structure of Monoterpenes and Ditrpenes were shown in (Figure No.4 and No.5). The percentage of peak area indicates the quantity of individual terpenes present in the specified fraction. The most prevailing major compounds are α-pinene, Terpinene, Sabinene, santolina triene, Limonene, *3*-Thujanol, Neophytadiene, Phytol. The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the scraped TLC fraction are presented in (Table No.1).

Discussion

The results relating to GC-MS analysis showed the way to the identification of number of terpenes from TLC fractions of ethyl acetate extract of *Chrysanthemum indicum* leaves. GC-MS chromatogram showed 24 peaks, indicating the presence of 24 compounds.

A broad spectrum of α-pinene, Terpinene, Sabinene, santolina triene, Limonene, 3-Thujanol, Neophytadiene, *Phytol* were noted. As per the literature study the presence of above said terpenes have showed some biological activity such as Anti-inflammatory, Anti-tumor, nematicide, analgesic, antibacterial, sedative, fungicide, pesticide, insecticide nematicide. Apart from that anti-carcinogenic (e.g. Taxol and perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity supplements its medicinal values.

Pinene ($C_{10}H_{16}$) is a bicyclic monoterpene chemical compound. Alpha-pinene is the most widely encountered terpenoid in nature and is highly repellant to insects (Nerio LS, Olivero-Verbel J, Stashenko E 2010[17]). A-Terpinene is a monoterpene found in the essential oils of aromatic plant sand this flavoring chemical were used in the cosmetics and food industries. It's used in both the pharmaceutical by showing anti-fungal and anti-oxidant properties confirmed to be valuable (Dewick, P. M. 2009)[18]. Sabinene contribute spiciness to black pepper occurred naturally as bicyclic monoterpene with the molecular formula $C_{10}H_{16}$. Phytol a branched - chain fatty alcohol, is an acyclic diterpene, obtained from the degradation of chlorophyll that act as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1. (Netscher, Thomas, 2007)[19]. Chlorophyll and phytol derivatives obtained from food hinder carcinogenesis and teratogenesis. Conversion of phytol to phytanic acid in humans is restricted to certain limit so it is largely extrapolated from animals by experimental trails. In ruminants, gut bacteria digest the ingested plant materials and liberates phytol, a branched - chain fatty alcohol constituent a part of chlorophyll pigment, which is then converted to phytanic acid and stored in fats (Daines *et al*, 2003)[20], (Van Den Brink D. M, Wanders, R. J. A, 2006)[21]. The plant extracts have significant insecticidal properties and could be commenced as botanical insecticides. Phytol and its metabolites chemical deterrents against predation. These compounds originate from host plants (Vencl, Fredric V, Morton, Timothy C. 1998)[22], (Watkins *et al*, 2010)[23]. The preliminary study reveals the presence of possible terepenes from the flower extract of *C.indicum* to be isolated. In future it would also be studied to check the bioactivity of such terepenes isolated from plant extracts.

Table No.1: Terpenes identified in the *leaves* extract of *Chrysanthemum indicum*

S.No	Compound name	R, time	Formula	Mol. wt
1	Benzoic acid	-	$C_{13}H_{22}O$	282
2	2, 5-Trimethylbenzaldehyde	1578	$C_{13}H_{22}O$	282
3	Naphthalene	1231	$C_{10}H_8$	128
4	Azulene	-	$C_{10}H_8$	128
5	4-Heptanol	-	C_8H_{18}	130
6	Phenol	2128	$C_{19}H_{24}O$	268
7	n-Hexadecanoic acid	1968	$C_{16}H_{32}O_2$	256
8	Pentadecanoic Acid	1869	$C_{15}H_{30}O_2$	242
9	Octadecanoic acid	2167	$C_{18}H_{36}O_2$	287
10	2-Chloroethyl Palmitate	2203	$C_{18}H_{35}ClO_2$	318
11	Tetradecanoic acid	-	$C_{16}H_{32}O_3$	272

Figure No.1: Preliminary phytochemical screening of terpenoids-Salkowiski test

Figure No.2: TLC of terpenoid fractions with R*f* values of 0.63, 0.79, 0.92 when a solvent phase of Benzene: Ethyl acetate (1:1) was used

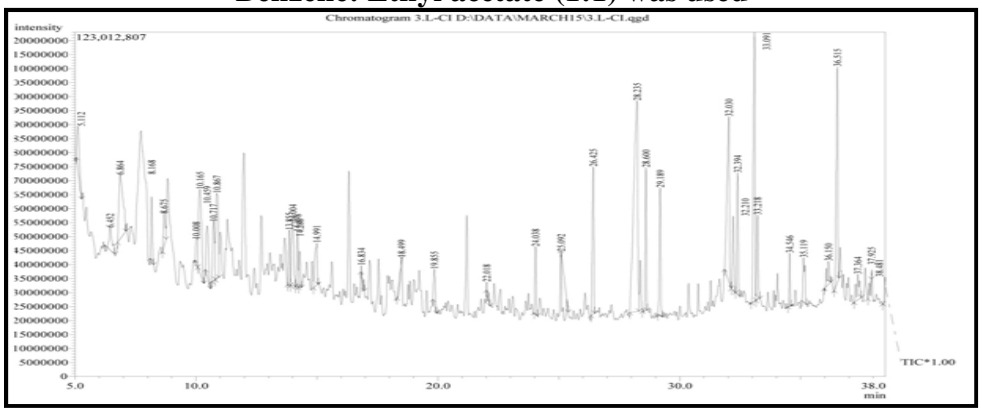

Figure No.3: GC-MS analysis of TLC fractions obtained from ethonolic extract of *Chrysanthemum indicum leaves*

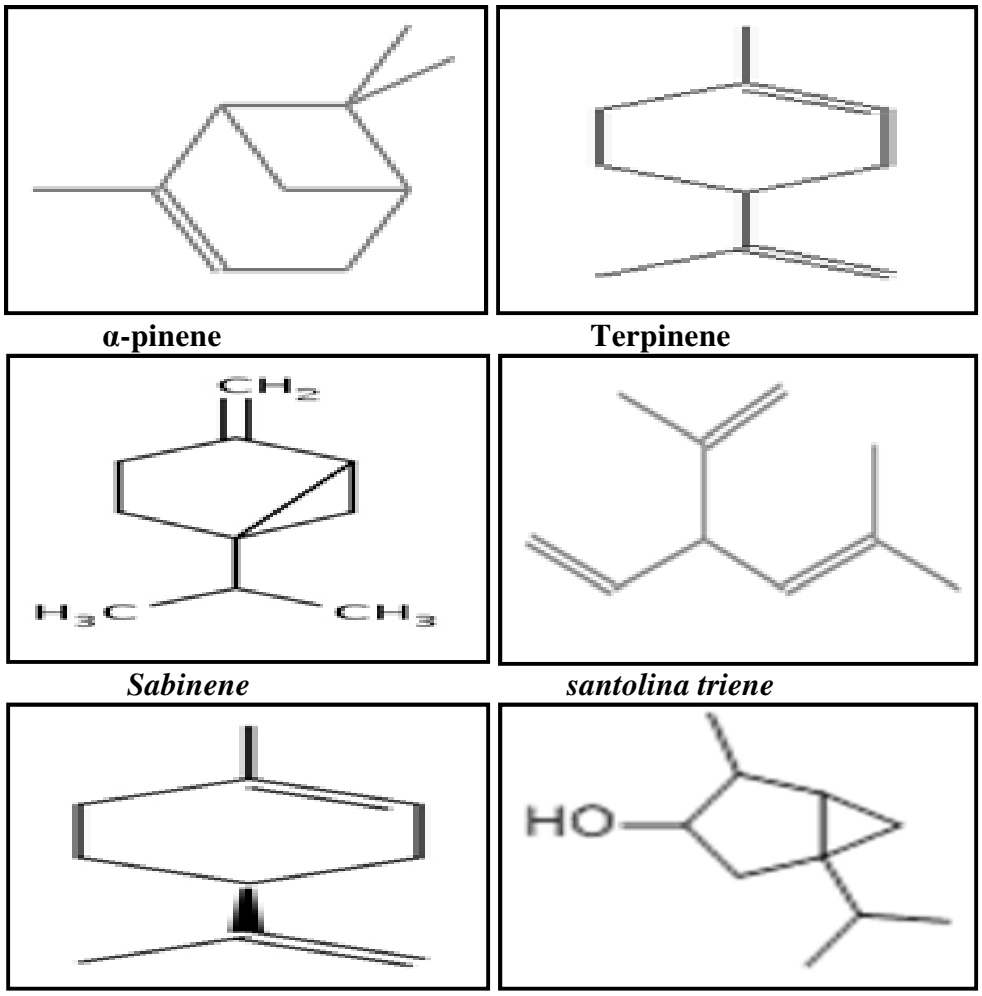

Figure No.4: Monoterpenes

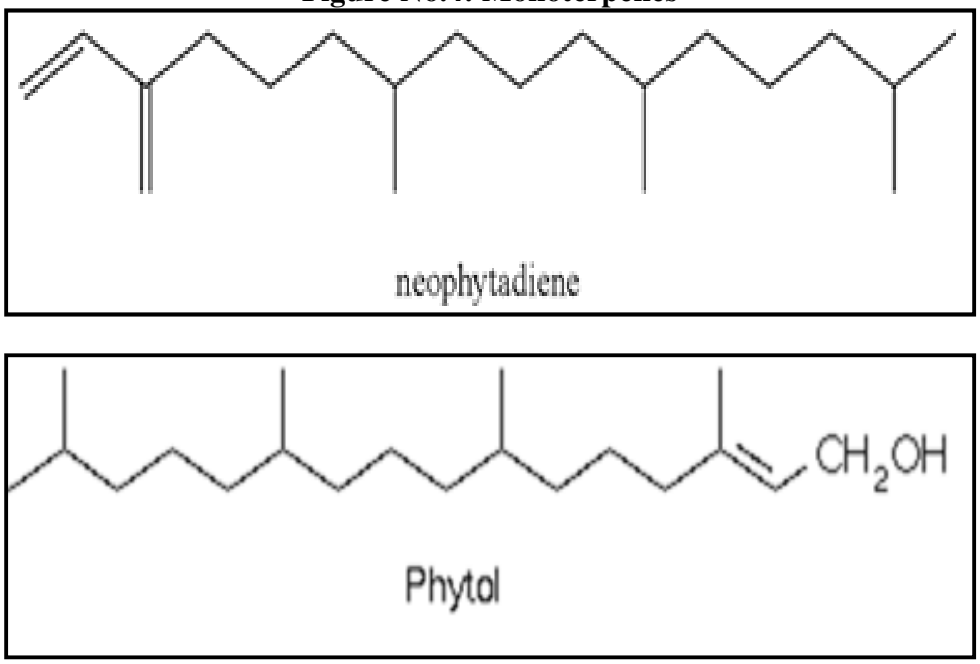

Figure No.5: Diterpenes

CONCLUSION

GC-MS analysis is used to analyse the terpenes and understanding the nature of active principles behind. This type of study will be useful to isolate the individual terpenoid and forms a root cause for the finding of novel drug and leads to assessing pharmacological activity. This is only a preliminary study of the occurrence of certain properties of ethyl acetate extract. Such scientific strategies for the costing of natural products with specific biological activities require the implementation of large screening process.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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