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Research Article

GC-MS Analysis of Terpenes from Hexane Extract of Lantana camara Leaves

J. Mariajancyrani [1], G. Chandramohan[1] , P. Brindha [2], P. Saravanan[3].

[1]Department of Chemistry, A.V.V.M. Sri Pushpam College,

Thanjavur, Tamil Nadu, India--613 503.

[2]Centre for Advanced Research in Indian System of Medicine,

SASTRA University, Thanjavur, Tamil Nadu, India-613 401.

[3]Department of Chemistry, Kings College of Engineering,

Punalkulam, Thanjavur, Tamil Nadu, India.

ABSTRACT

Objective: To explore the terpenes present in the hexane extract of Lantana Camara leaves. The present study was to isolate and identify terpene fraction from hexane extract of Lantana camara leaves. The terpene fraction was separated from hexane extract and the identification of bio-active terpenes was investigated by using Gas chromatography and Mass spectrometry. The mass spectra of these terpenes were matched with the database available at National Institute of Standard and Technology (NIST) library. The Gas chromatography and Mass spectrometry analysis of terpenes lead to identification of 8 compounds. This analysis revealed that the terpene fraction contains mainly 10-Hydroxy-2, 4a, 6a, 6b, 9, 9, 12a-heptamethyl - 1, 2, 3, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 12b, 13, 14b-eicosahydropecene-2-carboxylic acid (Katonic acid). This is the first report to identify the above compound present in the leaves of lantana camara. The research reveals the potential of hexane extract of Lantana camara leaves as a good source of terpenes that justify the use of this plant for its various ailments by traditional practitioners.

Key words: Katonic acid, GC-MS, terpenes, Lantana camara.

INTRODUCTION

Medicinal plants are very high priced for human to exploiting biological activities, cost effectiveness and lesser side effects [1]. The rich sources of secondary metabolites present in medicinal plants with exciting biological activities. In these secondary metabolites are an outstanding source with a variety of structural arrangements and properties [2]. The crude extracts of lantana camara used for protection of cabbage against the aphid Lipaphis erysimi. The Lantana camara leaves having a lots of medicinal activity such as anti-inflammatory, analgesic, anti-tumor, antibacterial, sedative, fungicide and antimicrobial[3,4].Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature[5-8]. A large number of medicinal plants and their chemical constituents have shown beneficial therapeutic potentials, A majority of a Indian medicinal plants are evaluated for such properties[9]. In India, Lantana camara leaves and twigs are used in green mulch. The ash of lantana camara leaves is rich in potassium and manganese, which is very useful for manuring coconut trees.[10]. With this background, the present study was carried to investigate terpenes present in lantana camara leaves using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

The leaves of the plant Lantana camara collected from Thanjavur District in the month of July, 2010 and authenticated by Dr. John Britto, Rapinet Herbarium, ST.Joseph's College, Tiruchirappalli.

4kg of leaves were cleansed with running tap water and dried in shadow . The shade dried leaves were crushed into uniform powder using pulverizer. The powdered sample was stored in closed container or air tight polythene bags until use.

Extraction of plant material

The powdered sample was extracted with 95% ethanol by using cold method extraction in room temperature for one week. The 95% extract was further fractionated successively with petroleum ether, n-hexane, chloroform, ethyl acetate, ethanol, n-butanol and methanol. The solvents were recovered under reduced pressure. The hexane soluble part was filtered through whatmann -1 filter paper, distilled and concentrated to obtain the solid reddish residue. The final weight was noted and stored.

Separation of terpenes

5g of crude hexane extract was subjected to column chromatography using 60-120 silica gel mesh, that was packed using wet packing method in hexane with increasing polarity of hexane to chloroform .(100, 75:25, 50:50, 25;75, 100). From that 50% hexane fraction was rechromatographed with thin layer chromatography . Thin layer chromatography (TLC) was carried out using silica gel G. The thickness of the chromatographic plate coated with silica gel was 0.2 mm. All chromatograms were developed in a glass chamber (20 X 10cm^2) at room temperature. Among different solvent systems tested by micro-TLC, hexane: chloroform (9:1) was found to be suitable for good separation and, therefore, this solvent system was chosen for preparative TLC. The non-polar constituents are moving on the top of the TLC and then the top layer was scraped and separated out. The separated constituents were subjected to GC-MS study.

GC-MS analysis

Instrument Details

Make: PerkinElmerClarus 500
Software: Turbo mass ver5.2.0
Column Type: Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane)
Column length: 30m
Column id: 250µm

GC Conditions

Oven Program : 50ºc@7ºc/min to 220ºc(2min)@7ºc/min to 270ºc(10min)
Injector temp : 270ºc
Carrier gas : Helium @ flow rate 1ml/min
Split ratio : 1:20

MS Conditions

Mass Range : 40-600amu
Type of Ionization: Electron Ionization (EI)
Electron Energy : 70ev
Transfer line and source temperature: 200ºc, 180ºc
Library : NIST 2005
Sample Injected : 2.0 microlitres.

Identification of terpenes

5ml of TLC scraped fraction was mixed in 2ml of chloroform, and 3ml of conc. Sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids[11]. Interpretation on mass spectrum GC-MS was predicted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown compounds was compared with the spectrum of the component stored in the NIST library version (2005), software, Turbomas 5.2.

RESULTS AND DISCUSSION

The results pertaining to GC-MS analysis led to the identification of number of terpenes from hexane:chloroform (50:50) fraction of the hexane extract of Lantana camara leaves. GC-MS chromatogram showed 8 peaks, indicating the presence of 8 compounds and the structure of katonic acid were shown in (Fig 1, 2, 3). The percentage of peak area represents the quantity of individual terpenes present in the particular fraction.

The active principles with their retention time, molecular formula, molecular weight and concentration (%) in the scraped TLC fraction of Lantana camara leaves are presented in Table 1. These were collected from literature survey and Dr. Dukes Phytochemical and Ethnobotanical databases. The most prevailing major compounds Bicyclo[4.1.0]heptan-3-ol,4,7,7-trimethyl-(1á,3á,4á,6á,)-, Katonic acid (24.0), Germacrene D (14.3), ç -Elemene (11.4), Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (11.1) and minor components were Isoterpinolene (3.6), Santolina triene (3.4), ë-Cadinene (3.4).

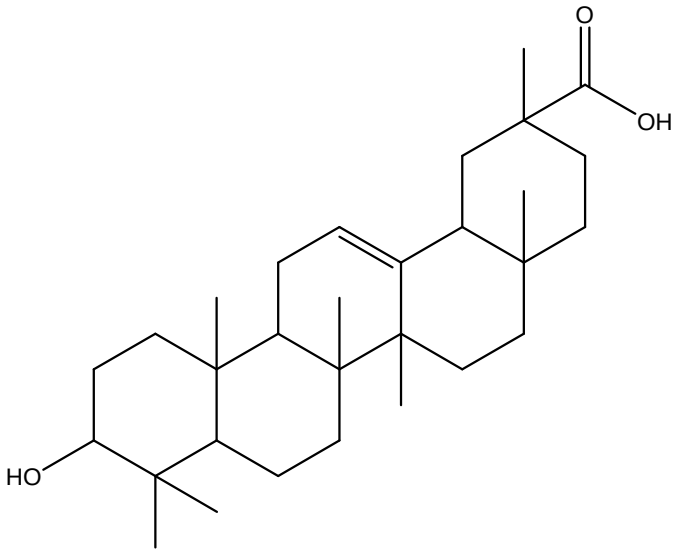

Fig-1 Structure of katonic acid

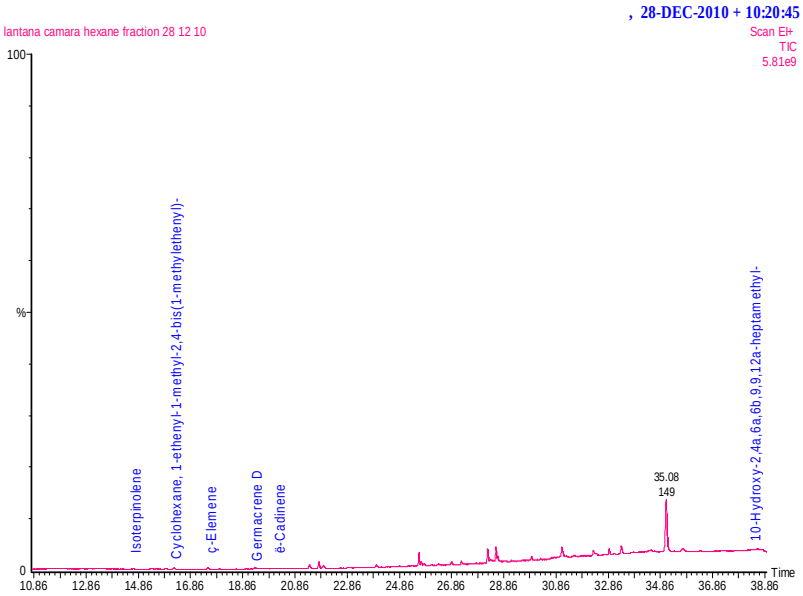

Fig-2 GC-MS Chromatogram of TLC fraction

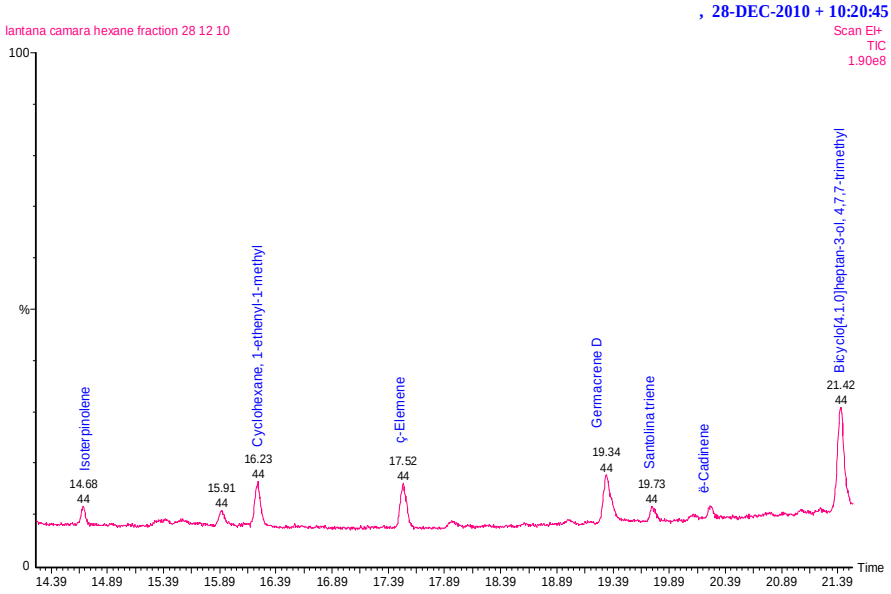

Fig-3 Enlarged GC-MS Chromatogram of TLC fraction

Table 1.Terpenes identified in the hexane:chloroform fraction of Lantana Camara Leaves by GC-MS.

Name of the compound	RT	PEAK AREA	M.F	MW	Nature of the Compound
Isoterpinolene	14.68	3.6	$C_{10}H_{16}$	**136**	Monoterpene
Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methyl ethenyl)-	16.23	11.1	$C_{15}H_{24}$	204	Sesquiterpene
ç -Elemene	17.52	**11.4**	$C_{15}H_{24}$	**204**	Sesquiterpene
Germacrene D	19.34	**14.3**	$C_{15}H_{24}$	**204**	Sesquiterpene
Santolina triene	19.73	**3.4**	$C_{10}H_{16}$	**136**	Monoterpene
ë-Cadinene	20.26	**3.4**	$C_{15}H_{24}$	**204**	Sesquiterpene
Bicyclo[4.1.0] heptan-3-ol,4,7, 7-trimethyl-(1á, 3á,4á,6 á,)-	21.42	28.9	$C_{10}H_{18}O$	**154**	Terpenealcohol
Katonic acid	38.57	24.0	$C_{30}H_{48}O_3$	**456**	Triterpenoid

The above terpenes have some biological activity such as Anti tumor, nematicide, analgesic, antibacterial, anti inflammatory, sedative, fungicide, pesticide, insecticide nematicide chemopreventive insectifuge hypocholesterolemic antiacne. Katonic acid is a type of triterpenoid and it was different from oleanolic acid and ursolic acid. Ursolic acid and oleanolic acid were already isolated in the lantana camara leaves but katonic acid was not identified till date. So, this is the first report to identify the katonic acid from hexane extract of lantana camara leaves. The present study results were confirmed by the many essential oils have been reported from Lantana camara, these essential oils are rich in terpenes and also supported and then supplemented the previous observations[11-18].Several Preliminary phytochemical screening studies have been carried out in different extract of the world using GC-MS[11-21].

CONCLUSION

GC-MS analysis is the first step to find the terpenes and understanding the nature of active principles in this medicinal plant. In this type of study will be helpful to isolate the individual terpenoid may proceed to find a novel drug and evaluation of pharmacological activity in the 50% hexane fraction is in progress. This study is only a preliminary study of the occurrence of certain properties of hexane extract an in-depth study will provide a good concrete base for all the biochemical and

phytochemical functions mentioned above. New scientific strategies for the evaluation of natural products with specific biological activities require the implementation of large screening process.

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