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Research Article

Phytochemical Investigation of Essential Oils from Ethanolic Extract of *Polyalthia longifolia* by Gas Chromatography-Mass Spectroscopy

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ABSTRACT

Background: India has considered *Polyalthia longifolia* (PL) has one of the locally important plant. It has been known for its rich heritage with leaves and bark used in manufacturing of essential oil. The plant has its utility in skin diseases, diabetes, hypertension and helminthiasis as well as vitiated conditions of *vata* and *pitta*. Phytochemical screening of PL ethanolic extract of leaves has revealed the presence of alkaloids, glycosides, carbohydrates, steroids, flavonoids, saponins and tannins. Objective: The purpose of Gas Chromatography–Mass Spectroscopy (GC-MS) studies on PL leaves was to explicate phytoconstituents which may prove its effectiveness in near future in the treatment of various diseases. Materials and Methods: Selected GC-MS coupling technique helped us to recognize the phytoconstituents based on their retention time and verification of MS spectra by MS libraries from PL leaves extract. Results: The components present were Caryophyllene, Oxirane tetradecyl, 5-(7a-Isoprenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, Cedrandiol (8S,14) and γ-Gurjunenepoxide-(2) from PL ethanolic leaves extract. Conclusion: In future, PL leaves may be subjected to different extraction and chromatographic techniques in order to check the presence of some additional phytoconstituents present in maximum proportion and their corresponding ethnopharmacological activity.

Keywords: *Polyalthia longifolia*, Gas Chromatography–Mass Spectroscopy, Caryophyllene, 5-(7a-Isoprenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol, γ-Gurjunenepoxide-(2).

INTRODUCTION

Phytochemistry is one of the burgeoning fields of research which deals not only with chemical structure identification, biosynthesis and metabolism but is also concerned with the applications of immense biological activities[1]. In modern era, world researchers has made immense progress by using applications of hyphenated techniques to elucidate, isolate, identify and characterize numerous amounts of phytoconstituents present in the plants. Gas Liquid Chromatography (GLC) is one of the best identical techniques at par with High Pressure Liquid Chromatography (HPLC) in terms of its senstivity and ability to provide both quantitative and qualitative results in single operation. Dissimilarity between GLC and HPLC is in terms of operating temperature since GLC is capable of being subjected to possible thermal rearrangement during separation[2]. Mass spectroscopy, in recent years has completely provided a new vision to studies correlated to natural products and have paved the way for phytochemistry research. Its uniqueness of identifying a particular component based on molecular weight and fragmentation pattern has resulted its coupling to many other chromatographic techniques on the same grounds. Gas Chromatography- Mass Spectroscopy (GC-MS) is one of the most advanced and a sophisticated technique that is used in elucidation of structure[3]. Essential oil from terpenoids class comprises of one of the important basis of commercially available perfumes and puts forth its application in food industries in terms of flavouring agents and spices. Plant families rich in plethora of essential oils are Compositae, Labiatae, Myrtaceae, Pinaceae, Rosaceae and Umbelliferae. Terpenes are classified into two classes, *viz*; mono and sesquiterpenes. Sesquiterpenes are bifurcated according to the presence of basic carbon skeleton. The common ones are either acyclic (*e.g.* farnesol), monocyclic (*e.g.* γ-bisabolene) or bicyclic (*e.g.* Caryophyllene). Due to the volatile nature of Terpenes, the ideal method applicable for their separation would be definitely GLC which is considered to be one of the essential method for identifying essential oils from barks and leaves[1,4]. *Polyalthia longifolia* is a large shrub of genes including 120 species. India has nearly 14 species of *Polyalthia*. There are two distinct varieties of this species, both found in Maharashtra and elsewhere. One of them is with spreading a perpendicular branch that is generally known as the typical variety. The other variety is with drooping pendulous branches and is used as an avenue tree. Leaves are alternate, stipulate, mildly aromatic, shinning, glabrous, narrowly lanceolate, tapering to a fine

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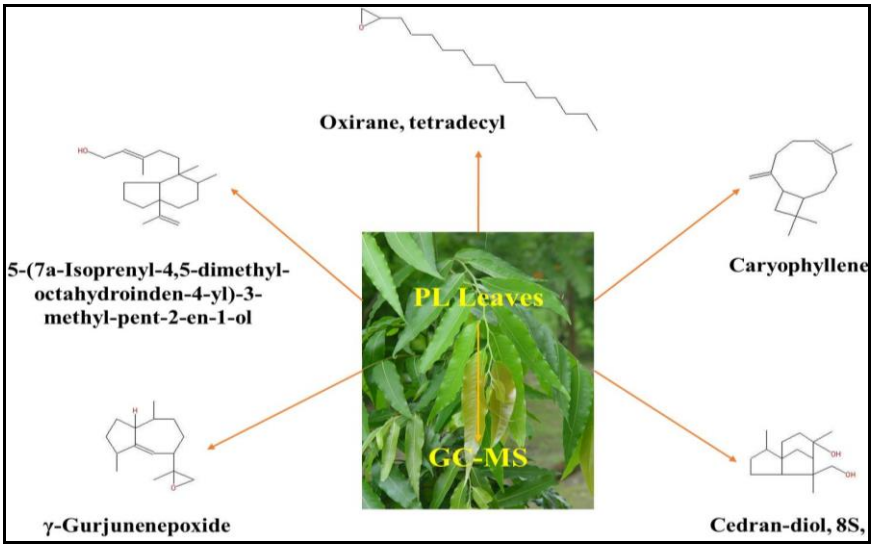

Figure 1:

Table 1: Various components and their fragments in EPL leaves extract

Sr. No.	Retention Time	Identity	Molecular formula	m/z	Synonyms	Figure No.
1	9. 55	(1)	$C_{15}H_{24}$	204	Bicyclol [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R,4E,9S)]; Bicyclol [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene-, (E)-(1R,9S)-(-)-; β-Caryophyllen; β-Caryophyllene; trans-Caryophyllene; L-Caryophyllene; Caryophyllene α + β mixture; Bicyclol [7.2.0] undec-4-ene, 8-methylene-4,11,11-trimethyl-, (E)-(1R,9S)- (-)-; 8-methylene-4,11,11-(trimethyl) bicyclol [7.2.0] undec-4-ene; 4,11,11-Trimethyl-8- methylene bicyclol[7.2.0]undec-4-ene.	3
2	27. 82	(2)	$C_{16}H_{32}O$	240	Hexadecane,1,2, epoxy-; Hexadecylene oxide; 1,2-Epoxyhexadecane; 1,2-Hexadecane oxide; 1,2-Hexadecane epoxide; 2-Tetradecyloxirane.	4
3	27. 99	(3)	$C_{20}H_{34}O$	290	No synonyms	5
4	29. 45	(4)	$C_{15}H_{26}O_2$	238	Cedrane-8,13-diol	6
5	29. 73	(5)	$C_{15}H_{24}O$	220	1,2-(3,8-Dimethyl-1,2,3,5,6,7,8,8a-octahydro-5-azulenyl-)-2-methyloxirane	7

acuminate apex, margin is markedly undulate pinnately veined, leatherly or subcoriaceous, shortly petiolate having petiole of 6 mm long[5-7]. Leaf oil has been reported to contain a vast number of sesquiterpene constituents such as alloaromadendrene, β-selinene, α-humulene and ar-curcumene *etc*.[8] The partition of volatile phytoconstituents is carried out mainly by GLC coupled with any other chromatographic techniques. Scientists and researchers has brought to our due considerations that complex mixture of oils can be detected and identified by high resolution of coupling of two hyphenated techniques[1]. In the current research article, we have identified and confirmed the structures of the constituents by GC-MS from the ethanolic extract of PL leaves.

MATERIALS AND METHODS

Part A: Collection, authentication extraction and preliminary phytochemical screening

Collection, authentication, extraction of PL leaves was done and subjected to preliminary phytochemical screening as stated in our previous studies[8].

Part B: Gas Chromatography–Mass Spectroscopy (GC-MS) studies on MECN leaves

Mobile phase: Ethanol

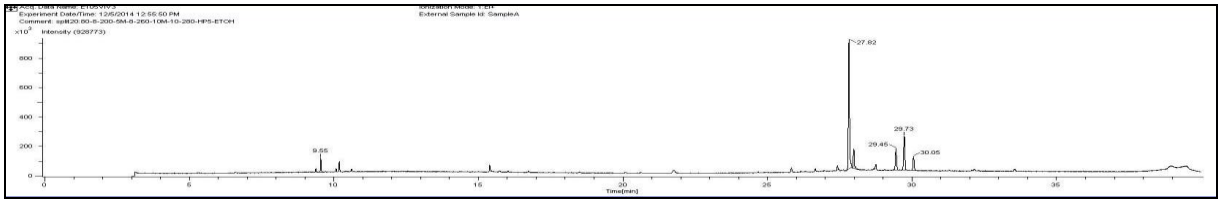

Figure 2: GC-MS spectrum of EPL leaves extract

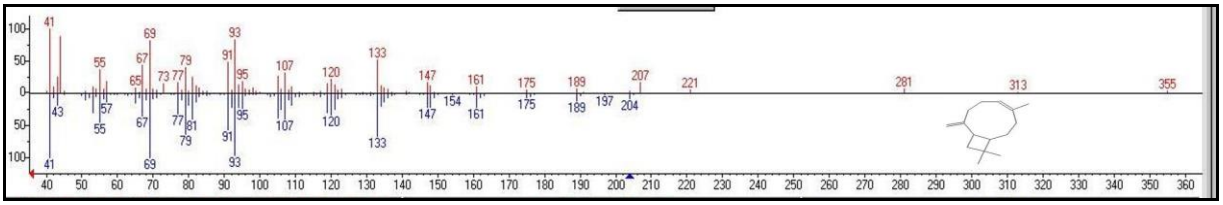

Figure 3: Mass spectrum showing presence of Caryophyllene (1) in EPL leaves extract

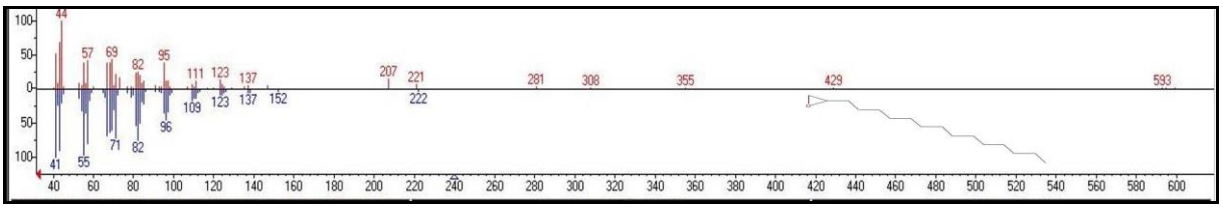

Figure 4: Mass spectrum showing presence of Oxirane, tetradecyl (2) in EPL leaves extract

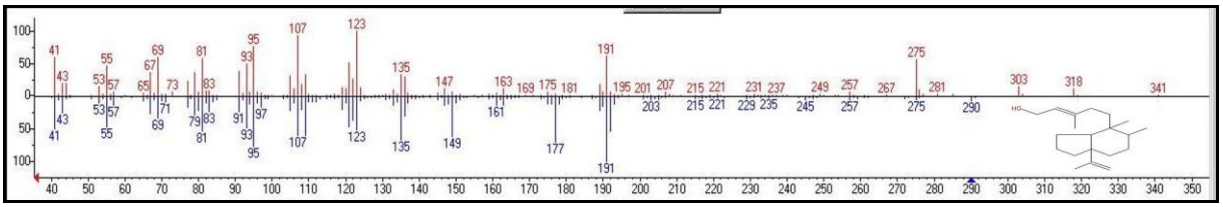

Figure 5: Mass spectrum showing presence of 5-(7a-Isoprenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol (3) in EPL leaves extract

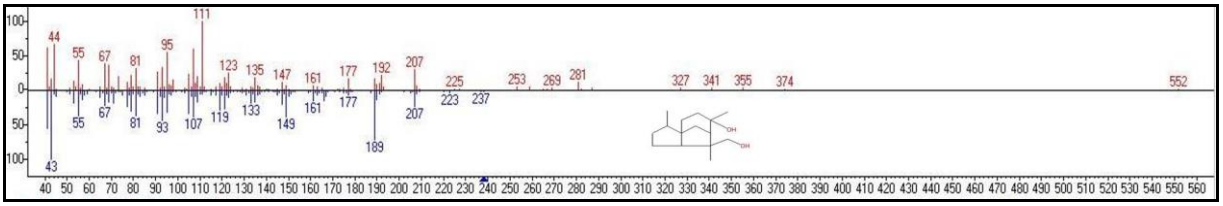

Figure 6: Mass spectrum showing presence of Cedran-diol, 8S, 14 (4) in EPL leaves extract

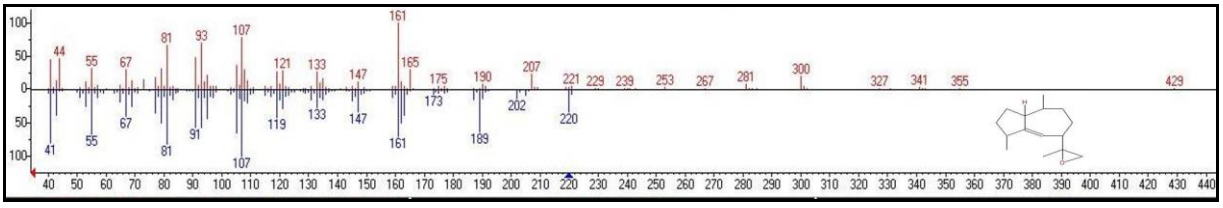

Figure 7: Mass spectrum showing presence of γ-Gurjunenepoxide (5) in EPL leaves extract

EI- MS Spectrum was scanned at 70 eV with instrument details as follows:
Model of MS: Joel
Model: Accu Time of Flight Analyzer (TOF) GCV
Specification: Mass range of 10-2000 amu and resolution of 6000.

Make of GC: Agilent 7890
Detector: Flame Ionization Detector (FID)
Run Time: 30 min
GC-MS analysis was performed by splitless injection (spilt 20:80-8-200-5M-8-260-10M-10-280-HP5-ETOH) of 1.0 µL of the sample in ethanol on a Hewlett Packard

6890 (USA) gas chromatograph fitted with a cross-linked 5% phenyl methyl siloxane HP-5 MS capillary column (30 m x 0. 25 mm x 0. 26 μm coating thickness), coupled with a mass detector. GC-MS operating conditions were as follows: injector temperature 200°C, transfer line 260°C, oven temperature programme 60–280°C with ramping 5°C min^{-1}, carrier gas: helium at 1.5 mL min^{-1}, Electron Impact, individual components were identified by NIST MS 2.0 structural library.

RESULTS AND DISCUSSION

Essential oils, being volatile are elucidated and identified based on their m/z charges by GC-MS. In the present study, caryophyllene was eluted in GC-MS studies which is as one of the bicylic sesquiterpenoids. Essential oils have vast number of applications ranging from major to minor ailments[1,8]. In the current research article, five components were identified by GC-MS technique as releaved in mass spectrum of PL extract. (Fig. no. 2). The components present in ethanolic PL leaves extract (EPL) are depicted with their retention time, m/z, formula and synonyms in Table no. 1. They were found to be Caryophyllene **(1)** with m/z 204 and fragment ions of 41, 55, 69, 79, 93, 107, 120, 133, 147, 161, 175, 189, 204; Oxirane tetradecyl **(2)** with m/z 240 and fragment ions of 41, 55, 71, 82, 96, 109, 123, 138, 222; 5-(7a-Isoprenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol **(3)** with m/z 290 with fragment ions of 41, 55, 69, 81, 95, 109, 123, 135, 149, 163, 177, 191, 205, 217, 229, 245, 257, 275, 290; Cedrandiol (8S,14) **(4)** with m/z 238 and fragment ions of 43, 55, 81, 93, 107, 119, 149, 161, 177, 189, 207, 223, 238 ; γ-Gurjunenepoxide-(2) and **(5)** with m/z 220 and fragment ions of 41, 55, 67, 87, 91, 107, 119, 133, 147, 161, 173, 189, 202, 220 (Fig. no. 3-7).

CONCLUSION

The article on EPL extract probably would help the phytochemistry field to focus more on identification, isolation and characterization of the essential oils which could have ethnopharmacological potential in future.

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