IJPSR (2014), Vol. 5, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 December, 2013; received in revised form, 16 February, 2014; accepted, 10 March, 2014; published 01 May, 2014

COMPARISON OF GC-MS ANALYSIS OF PHYTOCHEMICALS IN THE ETHANOLIC EXTRACTS OF *MARCHANTIA LINEARIS* LEHM & LINDENB. AND *MARCHANTIA POLYMORPHA* L. (BRYOPHYTA)

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Keywords:

GC-MS analysis, Bioactive compounds, *Marchantia linearis*, *M. polymorpha*, Ethanol extract.

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ABSTRACT: Bryophytes have unique aroma, taste and possess bioactive potential compounds with medicinal properties. Chemical studies of the bryophytes were limited in India due to the small size and expertise in identification of the species. Many of the bryophytes are used as folklore medicine by local tribes. The solvent extracts results poor yield leads to insufficient for testing biological activity. *In vitro* culture and suitable chemical synthesis was an alternative undertaken to overcome this issue. In the present study, the bioactive phytochemicals of *Marchantia linearis and M. polymorpha* have been analyzed using GC-MS. The phytochemical composition of the whole thallus ethanol extract was investigated using Perkin-Elmer Gas Chromatography - Mass Spectroscopy. GC-MS analysis of *M. linearis and M. polymorpha* ethanol extracts revealed the presence of the GC-MS chromatogram of the many peaks .The major compounds in *M. linearis and M. polymorpha* are n-hexadecanoic acid (28.13% and 18.72% respectively) followed by 1,2 benzene dicarboxylic acid (17.63% and 8.93% respectively). The medicinal potentiality of the liverworts may be due to the bioactive compounds present in the ethanolic extracts. Further studies are warranted to isolate and purify the lead molecules to evaluate its biological potentialities.

INTRODUCTION: Bryophytes are distributed along the tropical and temperate ecosystems and their ecological significances were well documented [1]. Although, bryophytes prefer damp or moist environments, they are resistant to pest and pathogenic attacks which suggest that they contain a pool of phytochemicals which are antimicrobials.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(5).1981-87

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(5).1981-87

Bryophytes comprises three groups liverworts (6,000 species), the hornworts (300 species), and mosses (15,000 species). This group represents the second largest group of land plants and pioneer in the evolution of land plants [2]. Bryophytes are with small image and are characterized by dominant gametophyte followed by a reduced or well developed sporophyte remains parasitic on gametophyte [3]. Jonathan Shaw *et al* [4] documented this group in many forest ecosystems and forms rich diversity along moist environments, wetland, and mountain ecosystems. Further, bryophytes are ideal environmental indicators and are important contributors to the nutrient and biogeochemical cycling.

Relatively, little is known about the secondary metabolites of bryophytes particularly at structural level and the information is very scattered [5]. The major limitations for this are the difficulties of proper identification, the limited number of the similar species available for subsequent analyses due to their inconspicuous position in the field and the instrumentation sophistication required.

However, during the last several years more than 400 lead molecules were isolated and structurally elucidated [6]. The major compounds are flavonoids, biflavonoids, terpenes, terpenoids, like diterpenoids, triterpenoids, lipophilic mono and disesquiterpenoids [5].

Characteristic scents of liverworts were due to the aromatic bibenzyls, benzoates, cinnamates or naphtalenes. Most of the phytochemicals in the bryophytes are biologically active substances. Phylogenetically, economically and ecologically these phytochemicals are likely involved in defense and protection against pest and microbial infections. Compared to the vascular plants, bryophytes have no thick walled cells to provide protection from abiotic or biotic stress.

Again, most bryophytes grow on forest floor in a close proximity to several biodegrading wastes and soil borne organisms. Hence, a defense against fungi or bacteria is vital for surviving in the moist, dampy habitat. Microbicidal activity against bacteria and fungi was reported from mosses [7] and the liverwort *Marchantia linearis* [8]. Since bryophytes are not well known to the common man, even to conservation biologists, it is necessary to highlight their role in nature and their therapeutical value.

The outputs thus provide a considerable potential for biotechnological and biopharmaceutical applications. The Kani tribes of South Kerala use *Marchantia linearis* for curing many skin borne allergies and tumors [9].

With this background the present study was aimed to identify and compare the phytochemicals present in *M. linearis and M. polymorpha* using GC-MS analysis.

MATERIALS AND METHODS:

Plant material: Fresh thallus of *Marchantia linearis* and *M. polymorpha* were collected from Kallar river floor of Ponmudi hills, Kerala, India (**Fig. 1 a & b**). Taxonomic identity was confirmed by comparing with authenticated herbarium specimen at Department of Botany Herbaria, University of Calicut, Kerala. Voucher specimens of the plants are kept in the herbarium of the institute.

FIGURE 1A & B: THALLUS OF *MARCHANTIA LINEARIS* AND *MARCHANTIA POLYMORPHA*

Extraction procedure: The shade dried powder of the thallus of *Marchantia linearis* and *M. polymorpha* (50 g) were separately extracted with ethanol (500 ml, 48 h) at temperature between 60-65°C by using Soxhlet extractor. The extracts were filtered in hot condition and concentrated in the vacuum under reduced pressure and dried in desiccators.

The extracts contain both variable polarity from highly polar to non-polar components and 0.2 µL sample of the solution was subjected to GC-MS for analysis for identifying the various phytochemicals.

GC-MS analysis: The GC-MS analyses of extracts were performed using a Perkin Elmer GC-MS equipped with a VF-5 MS fused silica capillary column (30 m x 0.25 mm i.d, film thickness 0.25 µm). GC-MS spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Pure helium gas was employed as a carrier gas at a constant flow rate of 1 mL/min. Mass transfer line and injector temperatures were set at 250°C. The oven temperature was programmed at 60°C for 2 min then increased to 300 °C for 6 min at the rate of 10°C/min. The samples were injected in split mode as 10:1.

Identification of phytochemicals: Interpretation on Mass-Spectrum GC-MS was carried out with reference to the software database of libraries like WILEY08, NIST08 and NIST08s. The name, molecular weight and percentage of unknown compounds were evaluated by software.

RESULTS AND DISCUSSION: In the present study, the GC-MS analysis of the ethanolic extracts of *M. linearis* and *M. polymorpha* showed the presence of seven and five compounds respectively **(Fig. 2a & b).**

The identified compounds of the thallus of *M. linearis*, their retention indices, compound, percentage composition, chemical structure and activities are given in **Table 1**.

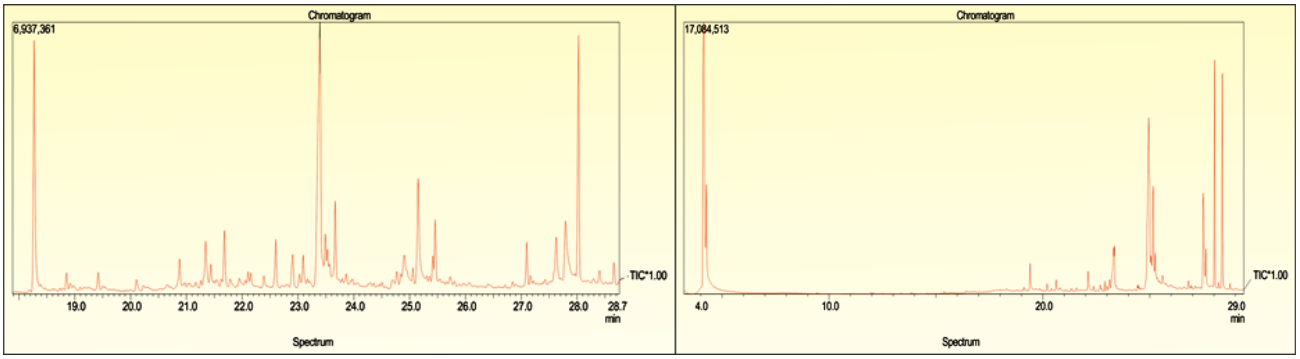
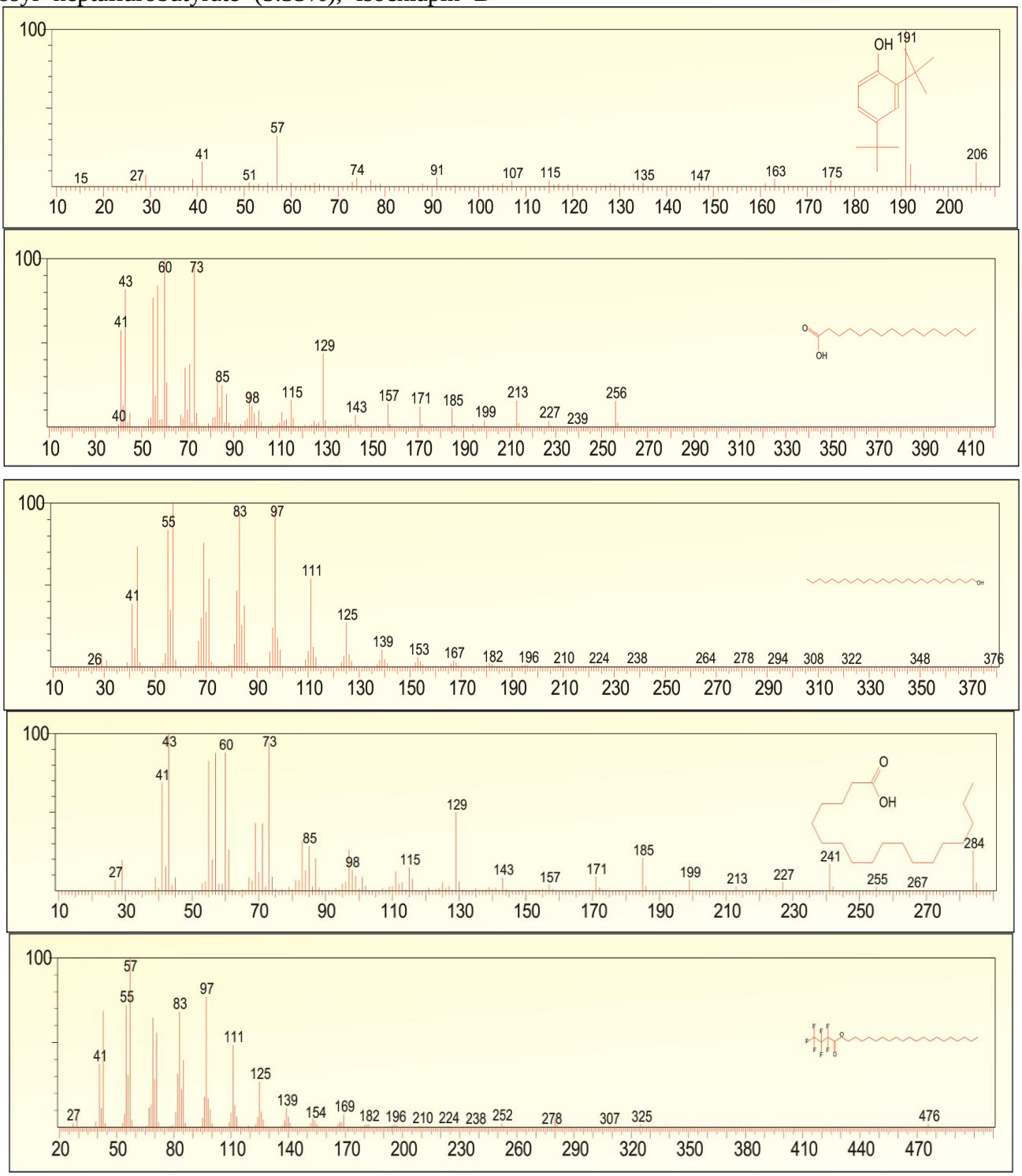


FIGURE 2A & B: GC MS CHROMATOGRAM OF ETHANOLIC EXTRACTS OF *M. LINEARIS* AND *M. POLYMORPHA*

TABLE 1: GC-MS CHROMATOGRAM ANALYSIS OF ETHANOLIC EXTRACTS OF *MARCHANTIA LINEARIS* AND *MARCHANTIA POLYMORPHA* SHOWING MAJOR PHYTOCHEMICALS

			Marchantia linearis	*Marchantia polymorpha*
Sl. No.	**Retention time**	**Compound name**	**Peak area %**	**Peak area %**
1	182367	Phenol 2,4-bis (1,1-dimethyl ethyl)	16.81	-
2	23.392	Hexadecanoic acid	28.13	18.72
3	23.667	1-heptacosanol	3.54	-
4	25.150	Octadecanoic acid	10.72	-
5	25.458	Eicosyl heptaflurobutyrate	5.35	-
6	27.100	Isochiapin B	2.53	-
7	28.025	1,2-benzene dicarboxylic acid	17.63	8.93
8	24.950	9-octadecanoic acid	-	25.34
9	27.500	Dichloro acetic acid 1-adamantyl methyl ester15.21	-	15.21
10		Ethyl adamantane	-	11.51

Biological activities of the compound are based on Phytochemical and Ethnobotanical Databases of Duke. The results in *M. linearis* showed the presence of hexadecanoic acid (28.13%), phenol, 2,4-bis(1,1-dimethyl ethyl) (16.81%), 1-heptacosanol (3.54%), octadecanoic acid (10.72%), eicosyl heptaflurobutyrate (5.35%), isochiapin B (2.53%), and 1,2-benzene dicarboxylic acid (17.63%). The spectrum profile of GC-MS confirmed the presence of 7 major components with retention time 18.267, 23.392, 23.667, 25.150, 25.458, 27.100 and 28.025 respectively **(Fig. 2a & b)** the individual fragmentation of the components is illustrated in **(Fig. 3 a-g).**



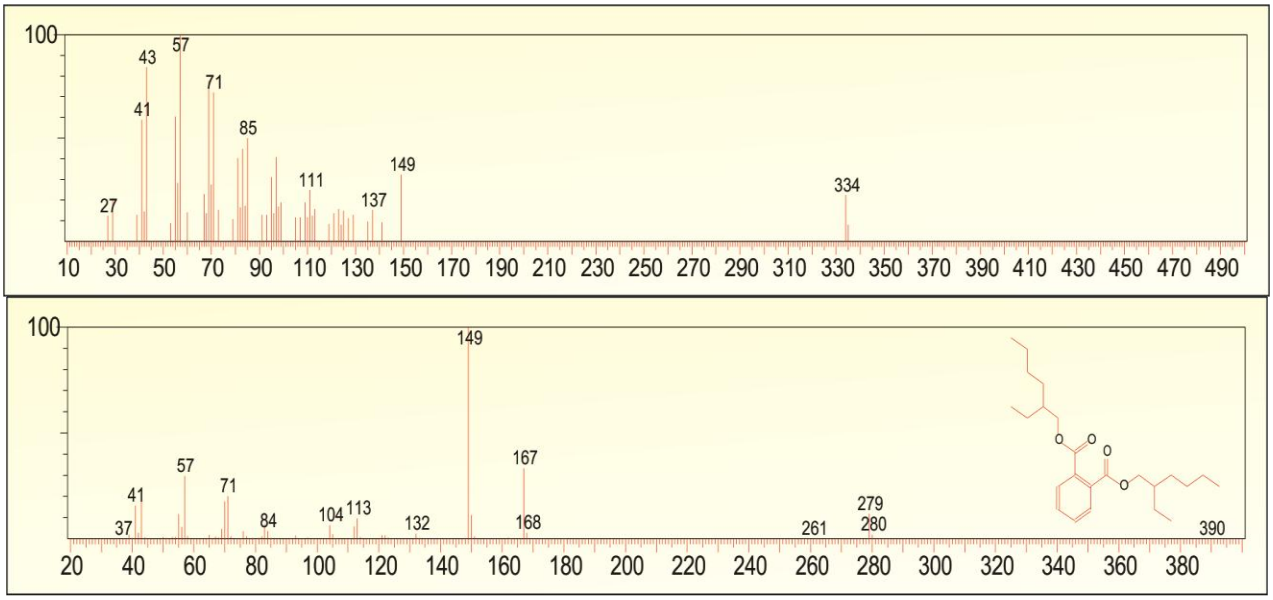
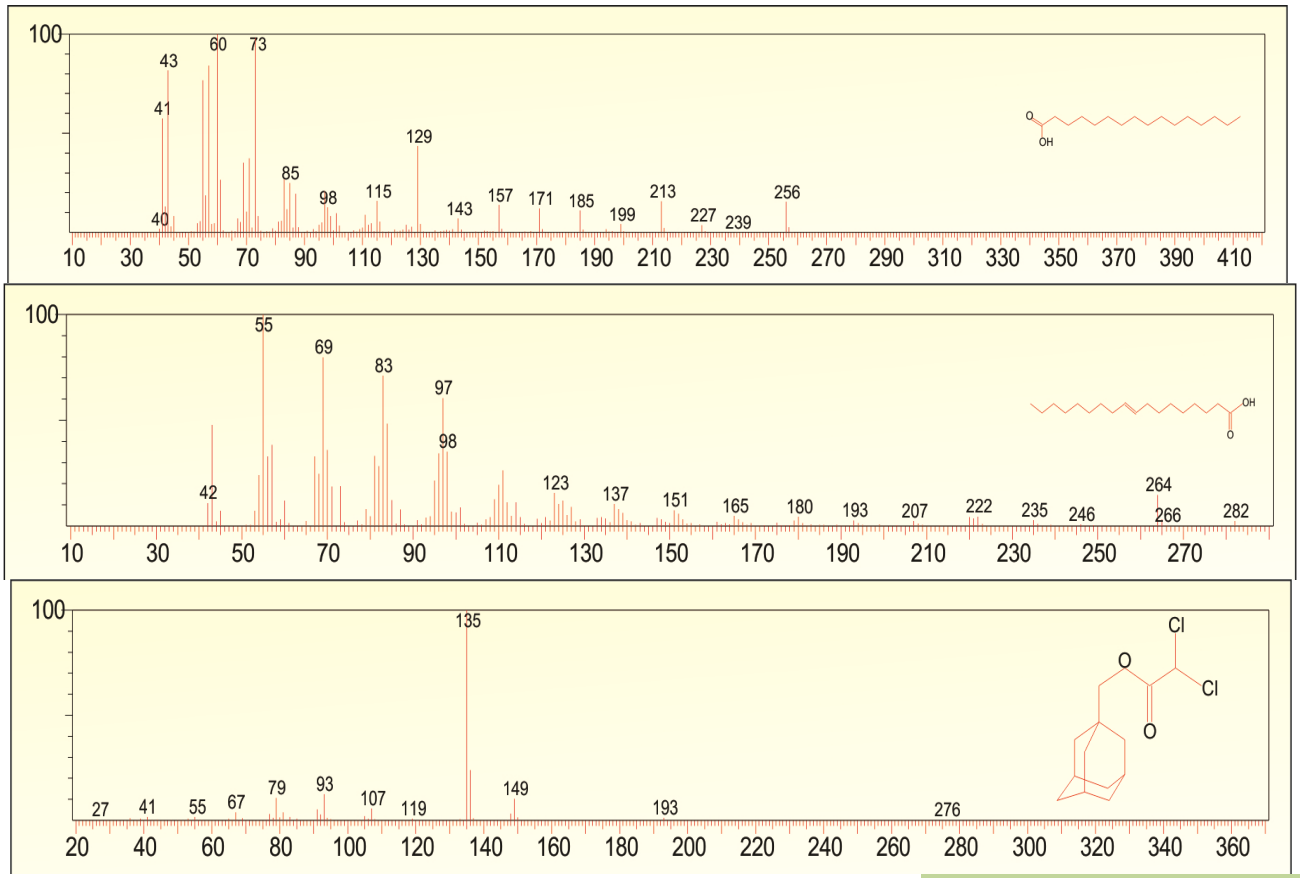


FIGURE 3: A, B, C, D, E, F & G: THE INDIVIDUAL FRAGMENTATION OF THE COMPONENTS OF GC MS CHROMATOGRAM OF *M. LINEARIS*

Similarly *M. polymorpha* displayed only 5 major compounds such as hexadecanoic acid (18.72%), 9-octadecanoic acid (25.34%), dichloroacetic acid 1- adamantyl methyl ester (15. 21%), 1,2-benzene dicarboxylic acid (8.93%) and 1-ethyl adamantine (11.51%). The corresponding retention times were 23.342, 24.950, 27.500, 28.033 and 28.400 respectively (**Fig. 4 a-e**). Hexadecanoic acid and 1, 2-benzene dicarboxylic acid is the common phytochemicals between the species but with different percentages.



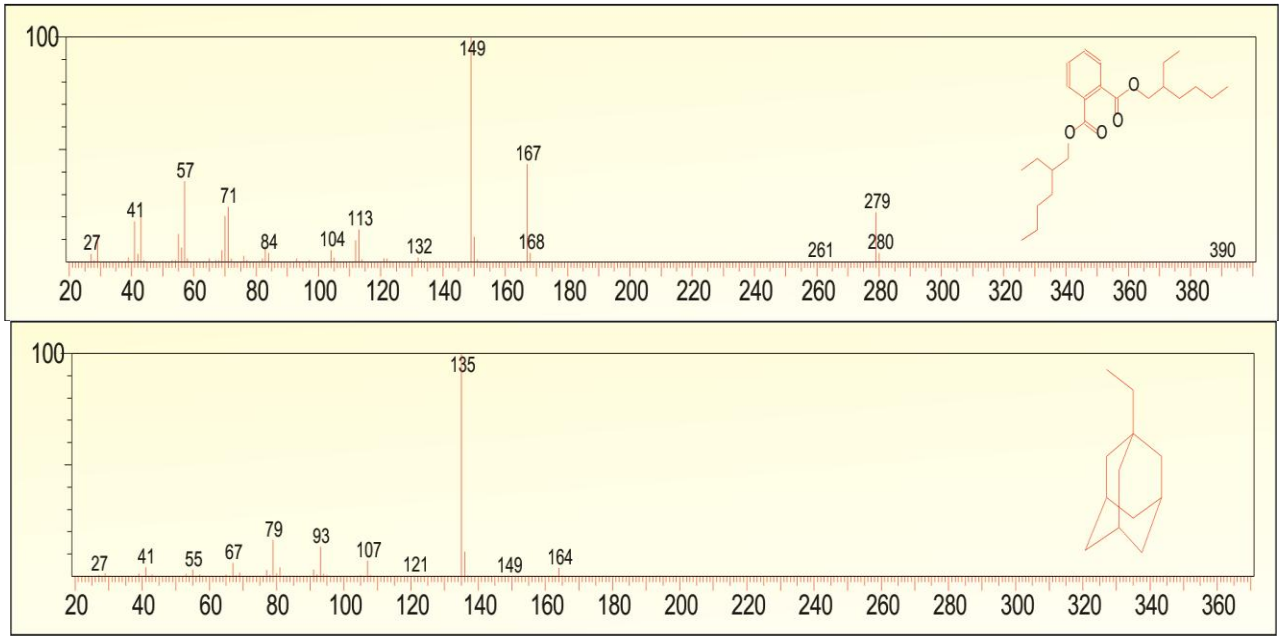


FIGURE 4: A, B, C, D & E: THE INDIVIDUAL FRAGMENTATION OF THE COMPONENTS OF GC MS CHROMATOGRAM OF *M. POLYMORPHA*

Aparna *et al* [10] attempted the structural and kinetics studies of n-hexadecanoic acid, is an inhibitor of phospholipase A(2) and confirmed as an anti-inflammatory compound. Phenol, 2, 4-bis (1, 1-dimethyl ethyl) derivatives are function as antioxidant.1-heptacosanol has the role of antioxidant and antimicrobial[11]. Isochiapin B has been identified by Chinese medicine to treat arthritis, tonsillitis, and other ailments. 1-ethyl adamantine was employed against viral fever and also to treat Parkinson's disease.

Derivatives include adapalene, amantadine, dopamantin, karmantadin, memantine, rimantadine, saxagliptin, tromantadine and vildagliptin as antiviral agents against HIV12. In addition to these play various pharmacological actions like anti-inflammatory, antibacterial, anti-helminthic activi¬ties for sesquiterpene lactones and anti-arthritic, pesti¬cide, antitumor, cancer preventive, anti-histaminic, hepatoprotective, hypocholesterolemic and anti-¬inflammation activities for fatty acids ester.

GC-MS analysis of the present study was more commendable than that of *Eupatorium odoratum* [11]; *Trichilia connaroides* (Meliaceae) [13]; *Epaltes divaricate* [14]; *Cassia italic* [15] and *Fagonia longispina* [16]. Selvamangai and Bhaskar [17] carried GC-MS analysis in *Eupatorium triplinerve* revealed the following components such as hexadecanoic acid (14.65%), 2,6,10-trimethyl, 14-ethylene14pentadecne(9.84%),bicycle[4.1.0]heptanes (2.38%), decanoic acid (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), octadecanoic acid (19.18%) and 2-hydroxy-3-[(9E)-9- octa decenoyloxy] propyl (9E)-9-octadecenoate (8.79%).

The percentages of the compounds are comparatively lower than *Marchantia* species.

There is growing importance in analyzing the secondary metabolites and their therapeutic potentialities. *Marchantia* is used in Chinese medicine however there are no reports on the thorough phytochemical analysis of the plant. This is the first report of the presence of some of the unique molecule resolved by GC-MS analysis and their medical utilities.

CONCLUSION: GC-MS analysis of phytochemicals in the ethanol extracts of thallus of *M.linearis and M.polymorpha* revealed 7 and 5 major compounds. The presence of various bioactive compounds justifies the use of the *Marchantia* species for curing various ailments by Chinese traditional practitioners.

Therefore, GC-MS method is a direct and accurate analytical method for identification of phytochemicals in the plant extracts. However, isolation of individual phytochemical constituents and subjecting it to medicinal property will definitely give promising results.

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How to cite this article:

Krishnan R and Murugan K: Comparison of Gc-Ms Analysis of Phytochemicals In The Ethanolic Extracts of *Marchantia Linearis* Lehm & Lindenb. and *Marchantia Polymorpha* L. (Bryophyta) *Int J Pharm Sci Res* 2014; 5(5): 1981-87. doi: 10.13040/IJPSR.0975-8232.5 (5).1981-87.

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