Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI - 10.26479/2016.0203.03**

DETERMINATION OF BIOACTIVE COMPOUNDS IN *EVOLVULUS ALSINOIDES* LEAF EXTRACT USING GC-MS TECHNIQUE

T. K. Sujayil, T. S. Dhanaraj*
PG and Research Department of Biochemistry, Marudupandiyar College, TamilNadu, S. India

ABSTRACT: The aim of this study was to investigate the bioactive compounds from methanolic extract of *Evolvulus alsinoides* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GC-MS analysis of methanolic extract was done by standard protocol using the equipment 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. The GC-MS analysis revealed the presence of various compounds like n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, Benzoic acid, D-Allose and Cytidine in the methanolic extract of *Evolvulus alsinoides*. These findings support the traditional use of *Evolvulus alsinoides* in various disorders.

KEYWORDS: Gas Chromatography and Mass Spectroscopy, *Evolvulus Alsinoides,* Phytochemistry

***Corresponding Author: Dr. T. S Dhanaraj** Ph. D.
PG and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu, S. India
*Email Address: ramanisethuu@yahoo.com

1. INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.According to

OPS a *medicinal plant* is [1] any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process, or [2] any plant employed as a source of drugs or their precursors. A *phytopharmaceutical preparation* or *herbal medicine* is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation [3].Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. In order to understand the biological activity of a plant, be it medicinal, poisonous, or nutritive, it is necessary to know its chemical constituents. Thus, they are plant secondary and primary metabolites (e.g. alkaloids, terpenoids, phenolics, gums, mucilages, carbohydrates, amino acids, proteins, fatty acids, glycolipids, etc.) that organize medicinal plants[3].Knowledge of plant bioactivity has been accumulated by experimentation over centuries by people living in intimate association with their environment. Therefore, phytochemical research is very useful in drug discovery and development [4]. Within a decade, there were a number of dramatic advancesin analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identificationand structural determination of phytochemicals.Gas Chromatography Mass Spectroscopy, a hyphenated systemwhich is a very compatible technique and the most commonly usedtechnique for the identification and quantification purpose. Theunknown organic compounds in a complex mixture can bedetermined by interpretation and also by matching the spectra withreference spectra [5].The aim of this study is to determine the bioactive compounds present in *Evolvulus alsinoides*L. (Family: Convolvulaceae) leaf extract with the aid of GC-MS technique, which may provide an insight in its use in tradition medicine.

2. MATERIALS AND METHODS

Plant materials:

The Evolvulus alsinoides leaves were collected in January 2015 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts: The collected Evolvulus alsinoides leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

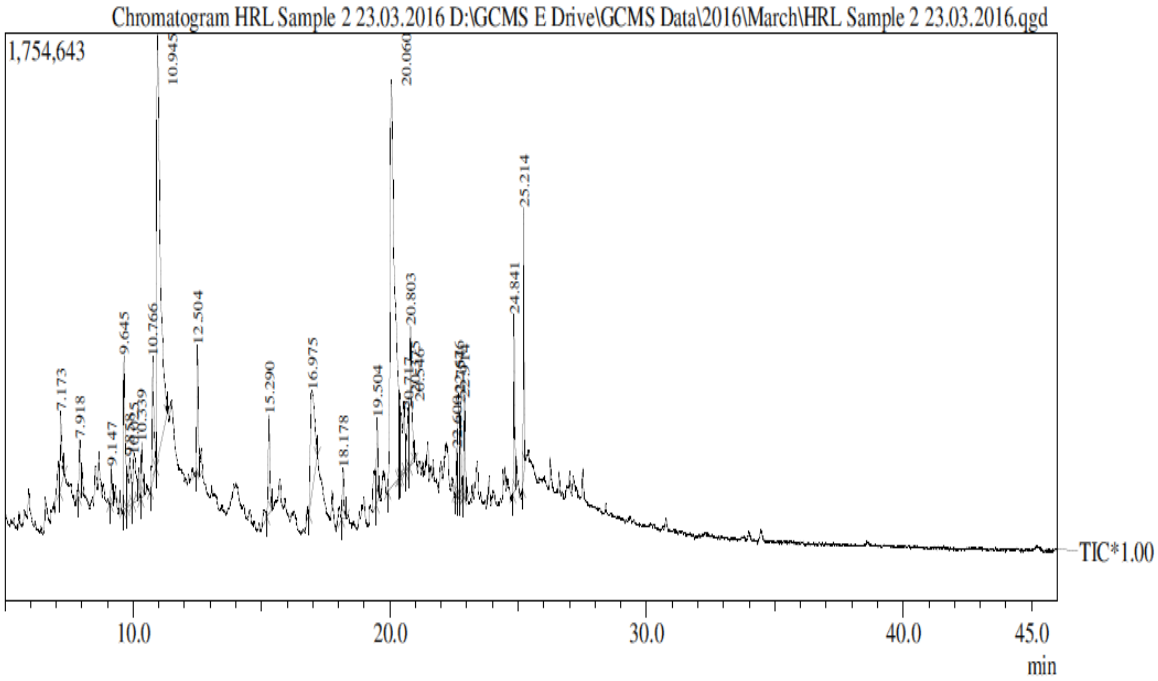
GC –MS analysis : GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm IDx 1μMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 μI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. 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 [6].

3. RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistryand identification of unknown samples. Additionally, it can identify trace in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system [7].

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[8].

GC-MS Analysis: In the present study twenty-five chemical constituents have been identified from the extract of Leaf by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds were n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, Benzoic acid, D-Allose and Cytidine (Table 1 and Fig 1). The presence of various bioactive compounds justifies the use of plant extract for various ailments by traditional practitioners. The biological activity of Leaf extract represented in the table 2.



Karpagasundariand Kulothungan [9] screened the bioactive components of *Physalis minima* leaves have been evaluated using GCMS. GC/MSanalysis of extract of *Physalis minima* leaves revealed the existence of Heneicosanoic acid (25.22),Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9,12-dienoic acid (32.02). This study supports our finding compounds.Prabhadevi*et al*[10] explored the phytochemical constituents present in *Allamanda cathartica (A. cathartica)* L. using GC-MS. The GC-MS analyses determined the presence of 28 different phytochemical compounds in the ethanolic leaf extract of *A. cathartica*. The major phytoconstituents were 9,12,15-octadecatrienoic acid (Z,Z,Z)- (16.39%), n-hexadecanoic acid (14.08%), 3-O-methyl-d-glucose (11.03%) and 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z)- (10.58%). The ethanolic stem extract of *A. cathartica* showed the presence of 26 different bioactive compounds. The major ones are 3-O-methyl-d-glucose (29.86%), 2-furancarboxaldehyde 5-(hydroxymethyl)- (14.87%), n-hexadecanoic acid (9.13%) and 9,12,15-octadecatrienoic acid (Z,Z,Z)- (7.34%).Similar types of compounds were agreement with our study. The investigation concluded that the stronger extractioncapacity of methanol could have been produced number ofactive constituents responsible for many biologicalactivities. So that those might be utilized for the development of traditional medicines and furtherinvestigation needs to elute novel active compounds fromthe medicinal plants which may be created a new way totreat many incurable diseases including cancer.

Table 1: Phytocompounds of Evolvulus alsinoides leaf extract identified by GC MS analysis

Peak	R.TIME	AREA%	NAME OF COMPOUND	MOLECULAR FORMULA	MOLECULAR WEIGHT
1	7.173	1.82	2,4-Imidazolidinedione, 3-Methyl	$C_4H_6N_2O_2$	114
2	7.918	1.34	4-Heptanone, 2-Methyl-	$C_8H_{16}O$	128
3	9.147	0.78	Levoglucosenone	$C_6H_6O_3$	126
4	9.645	3.46	4H-Pyran-4-one, 2,3-Dihydro-3,5-Dihydroxy-6-	$C_6H_8O_4$	144
5	9.858	2.30	Benzoic Acid	$C_7H_6O_2$	122
6	10.025	2.00	Cytidine	$C_9H_{13}N_3O_5$	243
7	10.339	1.03	4-Methyl-2-Oxopentanenitrile	C_6H_9NO	111
8	10.766	3.13	2,3-Dihydro-Benzofuran	C_8H_8O	120
9	10.945	20.92	5-Hydroxymethylfurfural	$C_6H_6O_3$	126
10	12.504	2.53	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150
11	15.290	2.47	1,1'-Bicycloheptyl	$C_{14}H2_6$	194
12	16.975	6.00	D-Allose	$C_6H_{12}O_6$	180
13	18.178	1.18	Cyclohexene, 1-Methyl-3-(Formylmethyl)	$C_9H_{14}O$	138
14	19.504	1.51	Diethyl phthalate	$C_{12}H_{14}O_4$	222
15	20.060	26.50	Beta.-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194
16	20.375	1.61	Tricyclo[2.2.1.0(2,6)]Heptan-3-OL	$C_7H_{10}O$	110
17	20.546	3.06	Benzoic Acid, 2,6-Bis Trimethylsil	$C_{16}H_{30}O_4Si_3$	370
18	20.717	2.09	Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis-	$C_9H_{20}O_2Si$	188
19	20.803	3.88	2-Cyclohexen-1-OL, 2,4,4-Trimethy	$C_{16}H_{24}O_2$	248
20	22.600	0.68	N-[3-(5-Furan-2-YL-[1,3,4]Oxadiazol-	$C_{26}H_{19}N_3O_3$	421
21	22.676	2.03	2(4H)-Benzofuranone, 5,6,7,7A-tetr	$C_{11}H_{16}O_3$	196
22	22.754	1.71	6-(3-Hydroxy-But-1-Enyl)-1,5,5-Trim	$C_{13}H_{22}O_3$	226
23	22.914	1.70	2-Cyclohexen-1-one, 4-Hydroxy-3,5,5-Trimeth	$C_{13}H_{18}O_3$	222
24	24.841	2.91	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256
25	25.214	3.38	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O_2$	284

Table 2: Biological Activity of some of the phytocomponents identified in the methanolic *Evolvulus alsinoides* leaf extract by GC-MS

Sr.No.	Compound name	Nature of Compound	Biological activity**
1	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic nemaicide, pesticde, Anti-androgenic flavor, hemalytic, 5- Alpha reductase inhibitor
2	Hexadecanoic acid, methyl ester	Fatty acid ester	Antioxidant, Antimicrobial Hypocholesterolemic, Antiandrogenic, Hemolytic, Alpha Reducatase inhibitor.
3	Benzoic acid	Benzen	Arachidonic acid-Inhibitor, Increase Aromatic Amino Acid Decarboxylase Activity and Inhibit Production of Uric Acid
4	D-Allose	Aldohexose sugar	Alcohol-Dehydrogenase-Inhibitor, Anticancer (Duodenum), Antidote (Diazepam), Antidote (Digoxin), Antileukotriene-D4, Circulatory-Depressant, CNS-Depressant and Coronary-Dilator
5	Cytidine	Nucleoside molecule	Glutamatergic antidepressant drug

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

4. CONCLUSION:

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Thus, the identification of bioactive compound in *Evolvulus alsinoides* was done by GC-MS analysis which shows the presence of 25 compounds. Among the identified compounds, n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, D-Allose and Cytidine have the role in antioxidant, antimicrobial and anti-inflammatory effects. From this study it can be concluded that the *Evolvulus alsinoides* may serve as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds

CONFLICT OF INTEREST

The authors declared that they have no competing interests.

REFERENCES

1. VelavanS., (2015) Phytochemical techniques. World Journal of Science and Research.1(2); 80-91.
2. Balunas, M.J. and Kinghorn, A.D., (2005) Drug discovery from medicinal plants. *Life Sci.*, 78,431-441..
3. R.Croteau, T. M. Kutchan and N. G. Lewis, B. Buchanan, W. Gruissem and R. Jones (eds) pp. 1250-1318. American Society of Plant Physiologists, Rockville, MD, USA,Natural products secondary metabolites (2000).
4. Heinrich M. & S. Gibbons. (2001). Ethnopharmacology in drug discovery: an analysis of its role and potential contributions. *J. Pharm. & Pharmacol.* 53: 425-432.
5. Ronald Hites A.,(1997). Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry., 609-611.
6. Srinivasan K and Ramarao P. (2013) Animal models in type 2 diabetes esearch: An overview. *Indian J Med Res* 125: 451-472.

7. SharmaP.C., YelneM.B., DennisT.J.,(2009) Database on medicinal plants used in Ayurveda. Delhi: Documentation and Publication Division, Central Council for Research in Ayurveda and Siddha, 3, 404-424,.
8. Duke's. Phytochemical and EthnobotanicalDatabases.www.ars-gov/cgi-bin/duke/. 2013.
9. Karpagasundari C and Kulothungan S. (2014) Analysisof bioactive compounds in Physalisminima leave using GC MS, HPLC, UV-VIS and FTIR techniques. Journal of Pharmacognosy and Phytochemistry, 3(4): 196-201.
10. Prabhadevi V, SahayaSathish S, Johnson M, Venkatramani B and Janakiraman N. (2012) Phytochemical studies on *Allamandacathartica* L. using GC-MS. *Asian Pacific Journal of Tropical Biomedicine*, 550-554.