



Received on 24 October, 2015; received in revised form, 09 December, 2015; accepted, 22 January, 2016; published 01 April, 2016

GC-MS ANALYSIS OF ETHYL ACETATE EXTRACT OF *ELEUTHERINE BULBOSA* (URBAN) MILLER (IRIDACEAE)

V. S. Rani * and Bindu R. Nair

Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram-695 581, Kerala, India.

Key words:

Eleutherine bulbosa, GC-MS, ethyl acetate extract, retention time

Correspondence to Author:

V. S. Rani

Research Scholar
Department of Botany
University of Kerala, Kariyavattom,
Thiruvananthapuram- 695 581,
Kerala, India.

Email: ranisankar28@gmail.com


ABSTRACT: The present study was carried out to detect the phytoconstituents present in the bulbous part of *Eleutherine bulbosa* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The mass spectrum of the crude ethyl acetate extract was compared with the available library sources (NIST08LIB, WILEY 8 and FAME). A total of 24 compounds were identified. Most of the major constituents were phenolic in nature. A phenolic compound detected to be present in highest concentration (34.20%) was, 1, 8-Naphthalenediol, 2, 7-diacetyl-3, 6-dimethyl. Carboxylic and sterol compounds were present in minute quantities.

INTRODUCTION: Plant substances continue to serve as viable sources of drugs for the human population and several plant-based drugs are in extensive clinical use¹. Medicinal plants now comprise a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals^{2,3}.

The World Health Organization (WHO), points out that the medicinal plants would be the best source to obtain natural compounds. The plant derived natural compounds such as alkaloids, phenolic compounds, quinones, tannins, saponins, terpenoids and flavonoids are reported to play a vital role in human health as these are utilized for the preparation of a variety of drugs^{4,5,6,7}.

The Iridaceae is an ornamental plant family of about 85 genera and 1500 species, which has an almost worldwide distribution⁸. Even though small in size, the family Iridaceae is particularly rich in phytoconstituents, especially of the phenolic type⁹. The genus *Eleutherine* consists of two species, *Eleutherine bulbosa* and *E.latifolia* distributed in Mexico, West Indies, Central and South America. The more common is *E. bulbosa* (syn. *E. plicata*), which is widely cultivated throughout the world especially in Africa and Asia (Philippines and Indochina) for its medicinal properties and is now naturalized there^{10,11}.

Eleutherine bulbosa is an important element of American Indian pharmacopoeia and is one of the few Neotropical Iridaceae with known medicinal uses¹². It is interesting to note that even though *E.bulbosa* is an exotic ornamental plant, the local traditional healers in Kerala have identified its medicinal properties and the bulb is being used as a cure for various ailments. But there were only a few reports about the phytochemical constitution of the plant from Kerala. Phytochemicals such as phenolic compounds, flavonoids, quinones,

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.7(4).1729-33
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(4).1729-33	

saponins and tannins were present in the ethyl acetate extract of bulbs of *E.bulbosa* and exhibited bioactivity¹³. The aim of the present study is therefore to conduct GC-MS analysis of the ethyl acetate extract of *E.bulbosa* bulbs.

MATERIALS AND METHODS:

Plant Material:

The plant material was collected from Kariyavattom, Thiruvananthapuram. It was identified and authenticated by the Curator, Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram. The plant material was also verified by comparison with specimens of *E. bulbosa* from Botanical Survey of India, southern circle, Coimbatore. A voucher specimen (Accession Number - KUBH 5802) has been deposited at the Kerala University Botanical Herbarium.

Preparation of plant extract:

From the collected plants, underground bulbs were removed, cleaned and air dried in room temperature. The dried bulbs were homogenized using a mechanical grinder to obtain fine powder. About 15gm of the bulb powder was extracted with 150ml ethyl acetate (76-77°C), 12h using the Soxhlet apparatus. The extract was concentrated to dryness under vacuum in a rotary evaporator at 45°C. The concentrated crude ethyl acetate extract was stored at 4°C in sterile air tight bottles.

GC-MS Analysis:

A 1µl aliquot of acetone solution of the ethyl acetate extract from *E.bulbosa* was injected into the GC-MS (QP-2010, Shimadzu, Japan). The VF-5MS column was 30.0m in length, 0.25mm in diameter and 0.25µm in thickness. The carrier gas was Helium. The GC oven temperature program was 70°C. The fraction was held for three minutes, raised at 10°C/minute to 300°C and again held for nine minutes. The injector temperature was set at 240°C. The mass spectrometer was electron impact mode, electron energy was -70ev, source temperature: 200°C and the scan range was 40-1000m/z.

Identification of the components:

The identity of the components in the ethyl acetate extract was assigned by the comparison of their retention time similarity indices and mass spectra fragmentation patterns with NIST08 LIB, WILEY 8 and FAME.

RESULTS: The bioactive crude ethyl acetate extract was subjected to GC-MS analysis. GC-MS analysis revealed the presence of 24 compounds. The results indicated that the ethyl acetate extract is rich in phenolic compounds. The GC-MS chromatogram of ethyl acetate extract of *E.bulbosa* bulbs is presented in **Fig.1**.

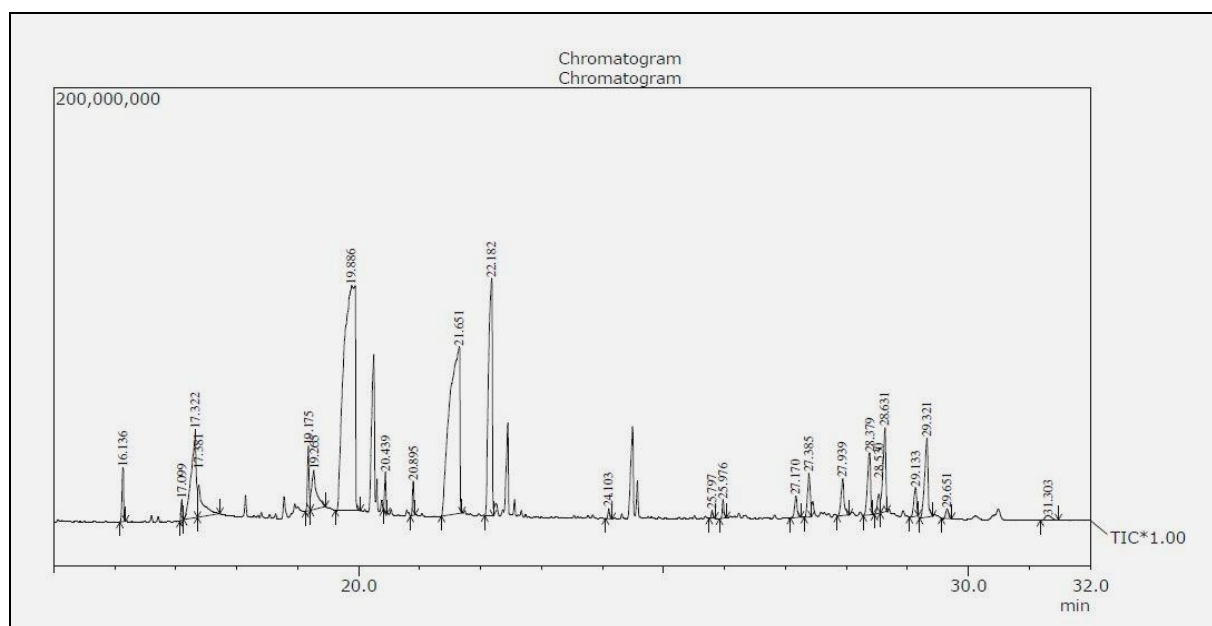


FIG. 1: GC-MS CHROMATOGRAM OF CRUDE ETHYL ACETATE EXTRACT OF *E. BULBOSA* BULBS

The phytoconstituents in the ethyl acetate extract of the plant, their retention time (RT), concentration in percentage, molecular formula and molecular weight is provided in the **Table 1**.

TABLE 1: PHYTOCONSTITUENTS OF CRUDE ETHYL ACETATE EXTRACT OF *ELEUTHERINE BULBOSA* BULBS

Peak	Compound	RT	Percentage	Molecular formula	Molecular weight(Da)
1	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	16.136	1.19	C ₁₆ H ₂₂ O ₄	278
2	Dibutyl phthalate	17.099	0.37	C ₁₆ H ₂₂ O ₄	278
3	N-hexadecanoic acid	17.322	5.75	C ₁₆ H ₃₂ O ₂	256
4	Hexadecanoic acid, ethyl ester	17.381	2.20	C ₁₈ H ₃₆ O ₂	284
5	Palmitic acid, butyl ester	19.175	1.44	C ₂₀ H ₄₀ O ₂	312
6	Octadecanoic acid	19.265	2.52	C ₁₈ H ₃₆ O ₂	284
7	1,8-naphthalenediol, 2,7-diacetyl-3,6-dimethyl	19.886	34.20	C ₁₆ H ₁₆ O ₄	272
8	Oxymetazoline	20.439	0.72	C ₁₆ H ₂₄ N ₂ O	260
9	N-butyl stearate	20.895	0.67	C ₂₂ H ₄₄ O ₂	340
10	Propanedinitrile,[(3,4,5-trimethoxyphenyl)methylene	21.651	22.53	C ₁₃ H ₁₂ N ₂ O ₃	244
11	Benzenedicarboxylic acid di-n-octyl ester	22.182	11.97	C ₂₄ H ₃₈ O ₄	390
12	Acenaphthylene, 1,2-dihydro-5-pentadecyl	24.103	0.21	C ₂₇ H ₄₀	364
13	Methyl linolenate	25.797	0.17	C ₁₉ H ₃₂ O ₂	292
14	Solanesol	25.976	0.40	C ₄₅ H ₇₄ O	630
15	Ergost-5-en-3-ol	27.170	0.76	C ₂₈ H ₄₈ O	400
16	Stigmasterol	27.385	1.57	C ₂₉ H ₄₈ O	412
17	Stigmast-5-en-3-ol, (3.beta.)	27.939	1.49	C ₂₉ H ₅₀ O	414
18	Cholest-4-en-3-one	28.379	2.50	C ₂₇ H ₄₄ O	384
19	9,19-cyclolanost-23-ene-3,25-diol, (3.beta.,23e)	28.530	0.72	C ₃₀ H ₅₀ O ₂	442
20	24(s)-ethyl-3.alpha.,5.alpha.-cyclocholest-22(e)-en-6-one	28.631	3.19	C ₂₉ H ₄₆ O	410
21	24-methylenecycloartan-3-one	29.133	1.08	C ₃₁ H ₅₀ O	438
22	Delta.4-sitosterol-3-one	29.321	3.62	C ₂₉ H ₄₈ O	412
23	Beta.-saccharostenone	29.651	0.34	C ₂₉ H ₄₆ O	410
24	Delta.(sup4)-cholestene-3,6-dione	31.303	0.39	C ₂₇ H ₄₂ O ₂	398

A phenolic compound detected to be present in highest concentration (34.20%) was, 1, 8-Naphthalenediol, 2, 7-diacetyl-3, 6-dimethyl, showed 19.883min as RT and a SI (Similarity Index) of 65 with NIST 08 LIB. The next major compound was Propanedinitrile, [(3, 4, 5-trimethoxyphenyl) methylene (22.53%), with RT 21.550min and SI 77 with NIST08.LIB. The next compound is Benzene dicarboxylic acid di-n-octyl ester (11.97%) [Common name -Di-n-octyl phthalate (DOP)] having RT of 22.182min and a SI 91 with Wiley 8 Library. Another compound namely n-hexadecanoic acid (5.75%) with RT 17.38min and showed 95% SI with NIST08 Library. Those compounds which were below five per cent level (concentration) include several carboxylic acid and phytosterol compounds.

DISCUSSION: Gas Chromatography is a technique used to separate compounds that might be present in a sample. GC-MS analysis can identify pure compounds present at less than 1mg¹⁴. The time it takes for any given compound to travel the length of the column is referred to as its retention time (RT). The RT for a given compound is an identifying characteristic. The detector for the

GC is the Mass Spectrometry (MS) detector. As a drug exits the end of the GC column it is fragmented by ionization and the fragments are sorted by mass to form a fragmentation pattern. Like the retention time (RT), the fragmentation pattern for a given drug is unique and therefore is an identifying characteristic of the drug. It is so specific that it is often referred to as the molecular fingerprint.

GC MS analysis of the present study revealed the presence of 24 compounds in ethyl acetate extract of *E.bulbosa*. Presence of 1, 8-Naphthalenediol, 2, 7-diacetyl-3, 6-dimethyl has not been reported elsewhere and therefore the present report appears to be the first report of the presence of this compound. Literature perusal showed that the family of 1, 8 naphthalene diol and their derivatives act as potent antioxidants¹⁵.

Propanedinitrile, [(3, 4, 5-trimethoxyphenyl) methylene has not been reported from this plant previously. Nitriles occur naturally in a diverse set of plant and animal sources. Over 120 naturally occurring nitriles have been isolated from terrestrial and marine sources. Propane nitrile compounds,

showed antimicrobial, antitumour activity and cytotoxic activity^{16, 17}. Benzene dicarboxylic acid di-n-octyl esters or Di-n-octyl phthalates (DOP) reported to have antibacterial activity^{18, 19, 20}. Usually, the presence of DOP in plants is from environmental pollution, such as the release of DOP from plastic films. *Eleutherine bulbosa* bulbs have 11.97% of DOP, which is probably a high quantity. It appears that the bulbs of the plant possess a relatively stronger ability to accumulate DOP compared to the other plants and therefore can possibly act as agents for phytoremediation. N-hexadecanoic acid is present in the crude ethyl acetate extract. N-hexadecanoic acid (palmitic acid) possesses antifungal, antioxidant, nematocidal, haemolytic, antimicrobial and antimalarial properties^{21, 22}.

CONCLUSION: The present study is the first report on the GC-MS analysis of ethyl acetate extract of *Eleutherine bulbosa*. About 24 chemical compounds were identified from the same. Most of the compounds detected from GC-MS analysis of ethyl acetate extract of *E. bulbosa* exhibited several bioactivities including antibacterial and antitumor potentialities. Further purification and isolation of pure compounds is needed to find out which is the actual compound/s responsible for the bioactivity.

ACKNOWLEDGEMENT: The authors are thankful to Dr. P.M. Radhamany, Professor and Head, Dept. of Botany, University of Kerala, Kariavattom, for providing the required facilities for the conduct of this research work

REFERENCES:

- Cragg GM, Newman DJ: Natural Products: A continuing source of novel drug leads. *Biochim Biophys Acta* 2013; 1830(6): 3670-3695.
- Sinhababu A, Banerjee A: Ethnobotanical Study of Medicinal Plants Used by Tribals of Bankura Districts, West Bengal, India 2013; 1(3):98-104.
- Shaik G, Sujatha N, Mehar SK: Medicinal plants as source of antibacterial agents to counter *Klebsiella pneumonia*. *Journal of Applied Pharmaceutical Science* 2014; 135-147.
- Lima GPP, Vianello F, Corrêa, CR, da Silva Campos RA, Borguini MG: Polyphenols in Fruits and Vegetables and Its Effect on Human Health. *Food and Nutrition Sciences* 2014; 5: 1065-1082.
- Griffiths K, Wilson DW, Singh RB, Meester FD: Effect of dietary phytoestrogens on human growth regulation: imprinting in health and disease. *Indian J Med Res* 2014; 140:82-90.
- Imran S, Suradkar SS, Koche DK: Phytochemical analysis of *Leonotis nepetifolia* (L.) R. Br. a wild medicinal plant of Lamiaceae. *Bioscience Discovery* 2012; 3(2): 197-196.
- Rajamurugan R, Deepa V, Sivashanmugam M, Raghavan CM: Phytochemistry, antioxidant and antibacterial activities of medicinal plants- a comparative study. *International Journal of Current Research and Review* 2013; 5(2): 08-19.
- Mabberley DJ. *The plant book: A portable dictionary of the vascular plants*. Cambridge University Press, Cambridge. Second Edition, 1997.
- Nath S, Saha PS, Jha S: Medicinal Bulbous Plants Biology, Phytochemistry and Biotechnology. In: (Ramawat KG and Merillon JM (eds)). *Bulbous Plants: Biotechnology*. CRC Press, India. 2013 pp. 338-369.
- Baker JG: *Handbook of the Irididae*, George Bell and Sons, London, 1892.
- Quisumbing E: *Medicinal Plants of the Philippines*. Philippines Department of Agricultural and Natural Resources Technical Bulletin, Manila, 1951.
- Schultes RE, Raffauf RF: *The Healing Forest: Medicinal and Toxic Plants of the Northwest Amazonia*. Dioscorides Press, Portland, US, 1990. 218-219.
- Rani VS, Bindu RN. Antimicrobial effects of crude extracts of *Eleutherine bulbosa*. *Journal of Medicinal and Aromatic Plant Sciences*. 2011; 33(1): 46-52.
- Liebler DC, Burr JA, Philips L, Ham AJL: Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products. *Analytical Biochemistry* 1996; 236:27-34.
- Foti MC, Jhonson ER, Vigvist MR, Wright JS, Barclay LR, Ingold KV: Naphthalene diols: a new class of antioxidants intramolecular hydrogen bonding in catechols, naphthalene diols and their aryloxy radicals. *The Journal of Organic Chemistry* 2002; 67(5): 5190-5196.
- Hussain AZ, Kumaresan A: GC-MS analysis and antibacterial evaluation of *Acalypha indica*. *Asian Journal of Plant Science and Research* 2013; 3(6):46-49.
- Pojarová M, Kaufmann D, Gastpar R, Nishino T, Reszka P, Bednarski PJ, Angerer EV: [(2-Phenylindol-3-yl) methylene] propanedinitriles inhibit the growth of breast cancer cells by cell cycle arrest in G₂/M phase and apoptosis. *Bioorganic and Medicinal Chemistry* 2013; 15(23): 7368-7379.
- Philip D, Kaleena PK, Valivittan K: GC-MS analysis and antibacterial activity of chromatographically separated pure fractions of leaves of *Sansevieria roxburghiana*. *Asian Journal of Pharmaceutical and Clinical Research* 2011; 4:130-133.
- Senthilkumar G, Madhanraj P, Panneerselvam A: Studies on the compounds and its antifungal potentiality of fungi Isolated from paddy field soils

- of Jenbagapuram Village, Thanjavur District, and South India. Asian Journal of Pharmaceutical and Clinical Research 2011; 1:19-21.
20. Shafaghat A, Farshid S, Vahidmani-Hooshyar A: A phytochemical and antimicrobial activity of *Lavandula officinalis* leaves and stems against some pathogenic micro organisms. Journal of Medicinal Plants Research 2012; 6:455-460.
 21. Pietro Z, Maurizio S, Maurizio B, Antonella M, Sergio, R, Carmen F, Felice S: Essential oil composition of stems and fruits of *Caralluma europaea* N.E.Br. (Apocynaceae). Molecules 2010; 15:627-638.
 22. Dhanalakshmi R, Manavalan R: Bioactive Compounds in Leaves of *Corchorus trilocularis* L. BY GC-MS Analysis. International Journal of PharmTech Research 2014; 6 (7):1991-1998.

How to cite this article:

Rani V S and Nair BR: GC-MS Analysis of Ethyl Acetate Extract of *Eleutherine Bulbosa* (Urban) Miller (Iridaceae). Int J Pharm Sci Res 2016; 7(4): 1729-33.doi: 10.13040/IJPSR.0975-8232.7(4).1729-33.

All © 2013 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)