GC-MS Analysis of Ethyl Acetate Extract of Whole Plant of Rostellularia diffusa

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ABSTRACT

Introduction: To identify the various phyto constituents present in the unexplored plant *Rostellularia diffusa* (Willd.) by using gas chromatography and mass spectrometry. **Methods:** The whole plant of *Rostellularia diffusa* was extracted with ethyl acetate at room temperature for 72 h. The concentrated extract was subjected to GCMS analysis to detect the phyto constituents. **Results:** Totally 40 compounds were identified and the chromatograph showed 40 peaks with 40 individual compounds. The major constituents were identified in the extract were 16-Hentriacontanone (22.59%), Hexadecanoic acid (11.23%), Stigmast-5-en-3-ol (6.78%), 9-Octadecenoic acid (6.30%) and many other compounds were identified as low level. This preliminary study gives an idea to isolate the major active constituents present in the plant and also helps to develop potential pharmacologically active compounds.

Key words: *Rostellularia diffusa*, Bioactive compounds, Gas chromatography, Mass spectrometry, phytosterol.

INTRODUCTION

Phyto medicines are important component derived from various parts of the plants with various applications in pharmaceutical and herbal industry. Development of herbal remedies is more popular now a days due to less side effects and easy availabilities of medicinal plants.^{1,2} Presences of bioactive secondary metabolites in the medicinal plants are more responsible to cure several diseases in mankind.³

Rostellularia diffusa (Willd.) is a traditional herb belongs to acanthaceae family. The whole plants are used as brain tonic in traditional practice. Recently, anti-stress activity of *Rostellularia diffusa* was documented.⁴ as per the literature survey there is no other scientific reports are available regarding pharmacological and phyto chemical analysis of any part of this plant. In the present study, we evaluated the phyto chemical constituents of ethyl acetate extract of *Rostellularia diffusa* by gas chromatography and mass spectrometry (GC-MS), to provide the scientific information to develop potential phyto medicine.

MATERIALS AND METHODS

Plant material & Preparation of extract

The whole plant of *Rostellularia diffusa* was collected from the rock crevices in Tirumala forests, Tirupati, A.P, India in the month of August 2012 and was authenticated by Dr. K. Madhava Chetty, Professor and Head, Department of Botany, S. V. University, Tirupati and voucher specimen number was lodged (ANCP-MP-COL-01/13) and preserved in the herbarium, which was retained in our lab for future reference. Whole plant of *Rostellularia diffusa* was shade dried and coarsely powdered. The 500 g of the powdered plant material was defatted with petroleum ether (60-80°C) using a soxhlet extractor and then it is successively extracted with ethyl acetate for 72 h and the extract obtained was filtered and concentrated using rota evaporator (Medika Instrument). The yield of the extracts was found to be 9.8%.

GCMS analysis

The ethyl acetate extract of *Rostellularia diffusa* was subjected to GCMS detection. The detection was carried out with 6890 N Agilent gas chromatograph attached with a JMS 600 H JEOL mass spectrometer. The phyto constituents present in the extract was separated on a fused capillary SPBI column, 30 m 0.32 mm, 0.25 μ m film thicknesses in a temperature program initially 50 to 256°C with a rate of 4°C/ min with 2 min hold. The injector was at 260°C and the flow speed of the carrier gas helium, was 1 mL/min. The EI mode JMS 600 H JEOL mass spectrometer had ionization volt of 70 Ev, electron emission of 100 Ma, ion source temperature of 250°C. Ethyl acetate extract was

Cite this article : Sheik Uduman MST, Rathinam P, Karuru Y, Obili G, Chakka G, Janakiraman AK. GC-MS Analysis of Ethyl Acetate Extract of Whole Plant of Rostellularia diffusa. Pharmacog J. 2017;9(1):70-2.

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History

- Submission Date: 22-08-2016
- Review completed: 10-09-2016;
- Accepted Date: 12-09-2016.

DOI: 10.5530/pj.2017.1.13

Article Available online

http://www.phcogj.com/v9/i1

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Table 1: Phyto constituents of ethyl acetate extract of Rostellularia diffusa

Peak	Retention time	Area	Area%	Name of the phyto constituents
	4.738	191471	0.49	1,2,3-Propanetriol, monoacetate
	6.462	184807	0.48	Vanillin lactoside
	6.714	1291406	3.32	Benzeneethanol
	7.570	50652	0.13	Delta-cadinene
	7.871	115686	0.30	Dodecanamide
	7.929	1412789	3.63	Tyramine
	8.157	113584	0.29	1,2-benzenedicarboxylic acid, diethyl ester
	9.524	340689	0.88	Tetradecanoic acid
	10.126	196755	0.51	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-E]]
	11.039	4364196	11.23	Hexadecanoic acid (CAS) palmitic acid
	11.246	747478	1.92	Hexadecanoic acid, ethyl ester
	11.712	136931	0.35	Hexadecanoic acid (CAS) margaric acid
	12.058	429477	1.10	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-E]]
	12.209	448286	1.15	9,12-Octadecenoic acid (Z,Z)
	12.252	2448330	6.30	9-Octadecenoic acid (Z)
	12.385	620225	1.60	Octadecenoic acid
	12.421	146722	0.38	(Z,Z)-6,9-cis-3,4-epoxy-nonadecadiene
	12.708	305995	0.79	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
	13.058	1053793	2.71	Dihydrolinderalactone
	13.724	481596	1.24	3-oxatricyclo[20.8.00E7,16]Triconta-1(22), 7(16), 9,13,23,29-Hexaen
	14.166	196741	0.51	Eicosamethylcyclodecasiloxane
	15.496	359039	0.92	Tetratetracontane
	16.063	255023	0.66	Tetratetracontane
	16.182	468379	3.78	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22,-tetracosahexaene
	16.313	412897	1.06	1H-Purin-6-amine, [(2-flurophenyl)methyl]
	16.696	569762	1.47	Tetracosane
	17.145	436548	1.12	1H-Purin-6-amine, [(2-flurophenyl)methyl]
	17.779	684839	1.76	Nonadecan, 1,2-Epoxi-
	18.133	436139	1.12	Iron, monocarbonyl-1,3-butadiene-1,4-dicartonic acid
	18.247	1146605	2.95	Tetratetracontane
	18.332	493657	1.27	1-Eicosanol
	18.626	345119	0.89	Alpha-Tocopherol beta-D-mannoside
	19.136	935572	2.41	Z-2-Acetoxy-12-tetradecenitrile
	19.361	1282327	3.30	13-tertadecen-1-ol acetate
	19.809	1299082	3.34	Ergot-5-en-3-ol, (3-beta)
	19.913	631069	1.62	Wuweizisu C
	20.107	1232434	3.17	Stigmasta-5,22-dien-3-ol
	20.275	8779637	22.59	16-Hentriacontanone (Palmitone)
	20.411	185274	0.48	Docosane
	20.881	2635976	6.78	Stigmast-5-en-3-ol

injected 1 μL manually in split mode with the ratio of sample in split mode was 20:1.

GC-MS detection of phytoconstituents of ethyl acetate extract of *Rostellularia diffusa* was based on the computer evaluation of mass spectra of samples through National Institute Standard and technology (NIST), through comparison of peaks and retention time and computer matching as well as by following the characteristic fragmentation patterns of the mass spectra of particular class of compounds.

RESULTS AND DISCUSSION

GCMS is one of the most precise methods to identify various secondary metabolites present in the plant extract.⁵⁻⁷ The crude ethyl acetate extract of *Rostellularia diffusa* was analyzed by GCMS to detect various compounds with the help of NIST library.⁸ Totally 40 compounds were identified which have been listed in Table 1. The chromatograph showed 40 peaks with 40 individual compounds (Figure 1). The major constituents were identified in the extract were 16-Hentriacontanone (22.59%), Hexadecanoic acid (11.23%), Stigmast-5-en-3-ol (6.78%), 9-Octadecenoic acid (6.30%) and many other compounds were identified as low level.

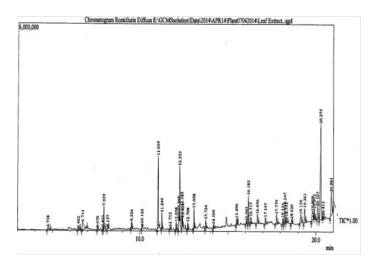


Figure 1: GCMS chromatograph of ethyl acetate extract of Rostellularia diffusa.

CONCLUSION

This is the preliminary study to identify the phyto constituents from the plant *Rostellularia diffusa*. Based on these report future study want to be design to isolate the major constituents from the *Rostellularia diffusa* to develop therapeutic herbal medicine.

ACKNOWLEDGEMENT

The author's thanks to management and principal, Annamacharya College of Pharmacy, Rajampet for the facility provided to carry out the research work.

CONFLICT OF INTEREST

None declared.

Source of support: Nil.

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