IJPSR (2015), Vol. 6, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 April, 2015; received in revised form, 29 June, 2015; accepted, 28 August, 2015; published 01 November, 2015

GC-MS ANALYSIS OF LEAF EXTRACT OF *GYNOCHTHODES UMBELLATA* (L.) RAZAFIM. AND B. BREMER (RUBIACEAE)

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Keywords:

Gynochthodes umbellata, *Morinda umbellata*, Ethanolic extract, GC-MS, Rubiaceae

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ABSTRACT: The present investigation was carried out to determine the presence of volatile, bioactive, phytocomponents in the leaf ethanolic extract of *Gynochthodes umbellata* (Syn. *Morinda umbellata*) belonging to the family Rubiaceae, using Gas Chromatography- Mass Spectrometry (GC-MS) analysis. *Gynochthodes umbellata* is an important medicinal plant widely used in traditional system of medicine. Literature survey revealed that till date, no work has been reported on GC-MS analysis of ethanol extract of *Gynochthodes umbellata* leaves. The mass spectrum of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC-MS analysis revealed the presence of 32 compounds. Sterols, stanols and Terpenoids were the most prominent compounds detected in leaf ethanolic extract of this plant. The major compounds detected were 9,19-Cyclolanost-24-en-3-ol (16.09%), 2,5,5-Triphenyl-4-methoxyimidazole (10.87%), β-Sitosterol (8.81%), Stigmast-4-en-3-one (7.26%), Phytol (5.62%). The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra.

INTRODUCTION: Since ancient times, people have been exploring the nature particularly plants in search of new drugs which resulted in the use of large number of medicinal plants to treat various diseases. The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments [1]. The study of plants continues principally for the discovery of novel secondary metabolites. Many higher plants are major sources of useful secondary metabolites which are used in pharmaceutical, agrochemical, flavour and aroma industries.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(11).4826-29

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(11).4826-29

These secondary metabolites can be derived from any part of the plant like bark, leaves, flowers, seeds, etc *i.e.*, any part of the plant may contain active components [2]. The screening of plant extracts is an innovative strategy to find medicinally important compounds, which will help to develop new drugs [3]. Gas chromatography (GC) is used to separates mixtures into individual components using a temperature-controlled capillary column. Mass spectrometry (MS) is used to identify the various components from their mass spectra. Gas Chromatography (GC) and Mass Spectroscopy (MS) associated with particular detection techniques has become a sophisticated means for analysis of various compounds [4].

Gynochthodes umbellata (Syn: *Morinda umbellata*) belonging to the family Rubiaceae, a rare medicinal plant was selected for the present study. *G. umbellata* is a pretty woody climber with bright

orange fruits having potent medicinal properties. Traditionally it is used for treating dysentery and diarrhoea. The leaves are used to expel intestinal worms. Literature survey revealed that till date, no work has been reported on GC-MS analysis of ethanol extract of *Gynochthodes umbellata* leaves. A preliminary phytochemical screening has shown that many phytochemicals are present in the ethanolic extracts of the different parts of the plant[5].Therefore, it is important to identify and characterize bioactive phytochemical compounds from ethanolic extract of the plant with the help of GC-MS.

MATERIALS AND METHODES:

Collection of plant material:

GC-MS analysis of leaf of *G. umbellata* was done in the present work. Fresh leaves of *G. umbellata* were collected from naturally growing plants in Kariyavattom Campus, University of Kerala, Thiruvananthapuram, Kerala. The plant was properly identified with the help of authentic literature and documented with their characteristic features. The voucher specimens are deposited in the herbarium of Department of Botany, University of Kerala, Kariyavattom (KUBH 7000).

Preparation of the plant extract:

The fresh leaves of *G. umbellata* were collected, washed and shade dried at room temperature. The dried leaves were pulverized to powder in a mechanical grinder and stored in airtight bottles until future use. 10gm of the leaf powder was subjected to Soxhlet extraction using 200ml ethanol. The extracts were concentrated under reduced pressure in a rotary evaporator (Superfit, Rotavap) and stored in the refrigerator until further use. Two microlitres of the extract was employed in GC-MS analysis.

GC – MS analysis:

The analysis of the extract was performed using GC-MS (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5ms fused silica capillary column of 30m length, 0.25mm diameter and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. The carrier gas was Helium (99.99%) used at a constant flow rate of 1.51 ml/min. injector and mass transfer line temperature were set at 200°C and 240°C respectively. The oven temperature was set from 70 to 220°C at 10°C/min, held isothermal for three minutes and finally raised to 300°C at 10°C/min. Two microlitres of the sample was injected in a split mode with a scan range of 40 – 1000 m/z. The total running time of GC-MS was 35 min. The relative percentage of the extract was expressed as percentage with peak area normalization.

Identification of the components:

Interpretation of mass spectrum GC-MS was conducted using data base of National Institute Standard and Technology [6] and Wiley Spectra Libraries [7]. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library.

RESULTS AND DISCUSSION:

The phytocomponents present in the leaf ethanolic extract of *G. umbellata* were identified by GC-MS analysis, GC-MS running time is 35min. The GC-MS chromatogram of ethanolic extract of leaf of *G. umbellata* is presented in **Fig.1**. The active compounds in the leaf ethanolic extract of the plant, their retention time (RT), molecular formula, molecular weight and concentration is provided in **Table 1.**

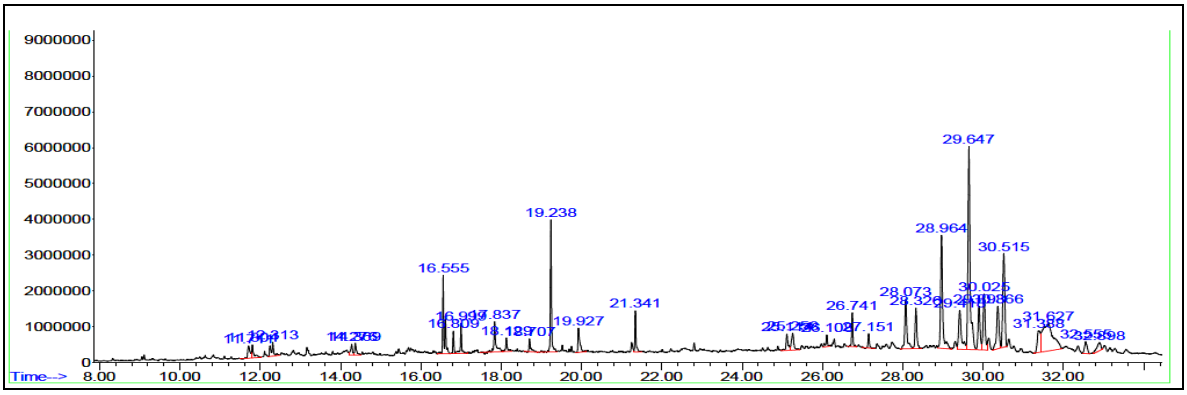

FIG. 1: GC-MS CHROMATOGRAM OF *G. UMBELLATA* LEAF ETHANOL EXTRACT

TABLE 1: PHYTOCOMPONENTS IN THE LEAF ETHANOLIC EXTRACT OF *G. UMBELLATA*

Sl.No.	Retention Time	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1.	11.711	Ethyl trans-2-pentenoate	$C_7H_{12}O_2$	128.1690	1.03
2.	11.808	(4-Fluorophenyl)acetone	C_9H_9FO	152.1660	0.98
3.	12.313	5-Hepten-2-one, 5,6-dimethyl-	$C_9H_{16}O$	126.2000	1.38
4.	14.274	Hexanal, 4,4-dimethyl-	$C_8H_{16}O$	128.2120	0.75
5.	14.371	9-Octadecene, 1,1-dimethoxy-, (Z)-	$C_{20}H_{40}O_2$	312.5304	0.77
6.	16.555	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.)	$C_{10}H_{18}$	138.2499	4.34
7.	16.807	6-Octen-1-ol, 3,7-dimethyl-, propanoate	$C_{13}H_{24}O_2$	212.3285	0.87
8.	17.000	Cyclohexanol, 2-(1-methylethyl)-	$C_{10}H_{20}O$	156.2652	1.46
9.	17.840	Tridecanoic acid	$C_{13}H_{26}O_2$	214.3443	2.02
10.	18.129	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.4772	0.88
11.	18.709	Phthalazin-1-one	$C_8H_6N_2O$	146.1500	0.77
12.	19.236	Phytol	$C_{20}H_{40}O$	296.5300	5.62
13.	19.927	Phenol, 4,4'-(1-methylethylidene)bis-	$C_{15}H_{16}O_2$	228.2863	1.74
14.	21.339	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324.5411	1.77
15.	25.112	Silane, diethylpentadecyloxy(3-phenylpropoxy)-	$C_{28}H_{52}O_2Si$	448.7968	1.26
16.	25.261	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltr idecyl)-, [2R-[2R*(4R*,8R*)]]-	$C_{27}H_{46}O_2$	402.6529	1.52
17.	26.108	Voachalotine pseudoindoxyl, 17-deoxy-6,17-epoxy-, (6.beta.)-	$C_{22}H_{24}N_2O_4$	380.4369	0.74
18.	26.739	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394.6755	1.51
19.	27.148	Vitamin E	$C_{29}H_{50}O_2$	430.7061	0.71
20.	28.076	Campesterol	$C_{28}H_{48}O$	400.6801	3.97
21.	28.329	Stigmasterol	$C_{29}H_{48}O$	412.6908	2.69
22.	28.960	.beta.-Sitosterol	$C_{29}H_{50}O$	414.7067	8.81
23.	29.421	Cholest-4-en-3-one	$C_{27}H_{44}O$	384.6377	4.06
24.	**29.644**	**9,19-Cyclolanost-24-en-3-ol**	**$C_{30}H_{50}O$**	**426.7120**	**16.09**
25.	29.896	Lanosterol	$C_{30}H_{50}O$	426.7100	3.24
26.	30.023	Acetamide, 2-(1-isoquinolinylthio) -N,N-dimethyl-	C_4H_9NO	087.1204	4.20
27.	30.364	Humulane-1,6-dien-3-ol	$C_{15}H_{26}O$	222.3660	3.84
28.	30.513	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.6908	7.26
29.	31.390	N-(6-Methoxy-1,3-benzothiazol-2-yl)-2,2,3,3,3-pentafluoropropanamide	$C_{11}H_7F_5N_2O_2S$	326.2420	2.45
30.	31.627	2,5,5-Triphenyl-4-methoxyimidazole	$C_{42}H_{30}N_4$	590.7144	10.87
31.	32.556	Urs-12-en-28-oic acid, 3-hydroxy-,methyl ester, (3.beta.)-	$C_{31}H_{50}O_3$	470.7269	1.17
32.	32.898	2-Ethylacridine	$C_{15}H_{13}N$	207.2704	1.23

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peaks indicate the relative concentrations of the components present in the plant [8]. GC-MS analysis revealed the presence of 32 compounds. Sterols, stanols and Terpenoids were the most prominent compounds detected. The major compounds detected were 9,19-Cyclolanost-24-en-3-ol (16.09%), 2,5,5-Triphenyl-4-methoxyimidazole (10.87%), Beta-Sitosterol (8.81%), Stigmast-4-en-3-one (7.26%), Phytol (5.62%), Bicyclo[3.1.1] heptane, 2,6,6-trimethyl-, (1.alpha.,2.beta.,5.alpha.) (4.34%), Acetamide, 2-(1-isoquinolinylthio) -N,N-dimethyl- (4.20%), Cholest-4-en-3-one (4.06%), Campesterol (3.97%), Humulane-1,6-dien-3-ol (3.84%). 9,19-Cyclolanost-24-en-3-ol or cycloartenol, is an important type of stanol found in plants. Cycloartenol is an important precursor of steroid biosynthesis and is a component of making K-liquid chlorophyll, which is a health beverage preparation, which helps detoxify and reduce toxins in the body. Drink supplements that contain efficacious cycloartenol also increase nutrient intake in the blood to increase oxygen in the blood helping the regeneration of red blood cell [9]. 2,5,5-Triphenyl-4-methoxyimidazole is an another important compound eluted, which possessing wide range of biological activities.

β-sitosterol is a waxy substance which is white in colour which possessing antimicrobial, anti oxidant, anti inflammatory, antiasthma, anti arthritic and hepato protective activities [10]. β-sitosterol present in plant oil is reported to lower down the cholesterol level in blood [11], this is due to the property of β-sitosterol to inhibit the absorption of cholesterol in the body [12]. Beta-sitosterol is used for heart disease and to reduce high cholesterol.

It is also used for boosting the immune system. Stigmast-4-en-3-one is an important steroid compound present in plants, which shows antimicrobial, antioxidant and antiarthritic properties [13]. It is important to note that steroids are found in large number with very high concentration as compared to other chemical constituents. Phytosterols, which cannot be synthesized by human and therefore all plant sterols and stanols in the human body are of diet origin [14]. Another important compound detected was phytol. It is a diterpene, which posses antibacterial and anti-inflammatory activities [15, 16].

This study revealed that this important woody climber have many important phytoconstituents which posses various biological activities. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

CONCLUSION: This is the first report on the GC-MS analysis of *G. umbellata*. From the GC – MS analysis result, it could be concluded that *G. umbellata* leaf contains various important bioactive compounds and therefore, it is recommended as a plant of phytochemical and pharmaceutical importance. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various diseases.

ACKNOWLEDGEMENT: The authors wish to thank Kerala State Council for Science Technology and Environment, Government of Kerala for funding this research work and express our thanks to Professor and Head, Department of Botany, University of Kerala, Kariyavattom, Thiruvananthapuram, Kerala for providing facilities for doing this work.

REFERENCES:

1. Savithramma N, Linga Rao M and Suhrulatha D: Screening of Medicinal Plants for Secondary Metabolites. Middle-East Journal of Scientific Research 2011;8 (3): 579-584
2. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H: Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia 2011;1(1): 98-106
3. Zhang A, Sun H and Wang X: Recent advances in natural products from plants for treatment of liver diseases. Eur J Med Chem 2013;63:570– 7.
4. Vinodh KS, Natarajan A, Devi K and Senthilkumar B: Chemical composition of aqueous leaf extract of *Murraya Koenigii*. Int J Pharm Biol Archiv 2013;4:493–7.
5. Anjusha S and Gangaprasad A: Pharmacognosy and phytochemical evaluation of *Gynochthodes umbellata* (L.) Razafim. and B .Bremer (Rubiaceae) a potent dye yielding medicinal plant. Journal of Pharmacy Research 2014; 8 (8): 1120-1125
6. Stein S: National Institute of Standards and Technology (NIST) Mass Spectral Database and Software.Version 3.02, USA,1990
7. Mc Lafferly FW: Registry of mass spectral data. Wiley New York, Edn. 5, 1989.
8. Lakshmi SP and Bindu RN: GC-MS analysis of chloroform extract of *Cleome burmanni* W. and A. (Cleomaceae).International Journal of Pharmaceutical Sciences and Research 2013;4(5):1930-1933
9. Matsuda SP, Darr LB, Hart EA, Herrera JB, McCann KE, Meyer MM, Pang J and Schepmann HG: Steric bulk at cycloartenol synthase position 481 influences cyclization and deprotonation. Org Lett. 2000; 2(15):2261-2263.
10. Rexlin Sujila SM, Rajadurai M, Rexlin Shairibhai SM: Hepatoprotective effect of β-sitosterol on lipid peroxidation and antioxidant status in ethanol-induced hepatotoxic rats International. Journal of Integrative sciences, Innovation and Technology 2014;3(4): 01-11
11. Birgit S, Sabine G, Karl-Heinz E and Pablo S: Phytosterol oxidation products in enriched foods: Occurrence, exposure, and biological effects. Mol. Nutr. Food Res. 2015; 00, 1–14
12. De Smet E, Mensink RP and Plat J: Effects of plant sterols and stanols on intestinal cholesterol metabolism: Suggested mechanisms from past to present. Molecular Nutrition & Food Research 2012;56 (7): 1058–1072
13. Ahmed JS and Kumar P: GC-MS Study of the *Excoecaria agollocha* Leaf extract from Pitchavaram, Tamilnadu , India. Researcher 2012;4(6)
14. Jong A, Plat J, and Mensink RP: Metabolic effect of plant sterols and stanols. J Nurt Biochem 2003; 14:362-369.
15. Mohammad TG, Mohammad HE, Ali J, Seyedhossein H, Mohammad M: Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal 2015;2(1): 13–16
16. Peter O, Malin H, Lars I. Hellgren and Rikard H: Phytol: A Chlorophyll Component with Anti-inflammatory and Metabolic Properties. Recent Advances in Redox Active Plant and Microbial Products 2014; 345-359.

How to cite this article:
Anjusha S, Gangaprasad A and Radhamany PM: GC-MS Analysis of Leaf Extract of *Gynochthodes Umbellata* (L.) Razafim. and B. Bremer (Rubiaceae). Int J Pharm Sci Res 2015; 6(11): 4826-29.doi: 10.13040/IJPSR.0975-8232.6(11).4826-29.