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RESEARCH ARTICLE

GC-MS ANALYSIS OF BIOACTIVE CONSTITUENTS OF *MAGNOLIA NILAGIRICA* (ZENKAR) FIGLAR. (MAGNOLIACEAE)AN ENDEMIC MEDICINAL PLANT TO THE WESTERN GHATS, INDIA

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ABSTRACT

The present study was carried out to determine the possible bioactive components of methanolic leaf extract of *Magnolia nilagirica* by using GC-MS analysis. The GC-MS analysis provides different peaks determining the presence of 55 compounds were identified from the leaf extract of *M. nilagirica*. The main compounds in the plant extract were identified as 1Butanol, 3 – Methyl (13.25 %), 2H-Cyclohepta[b]furan-2-one (11.11 %), 5-O-Methyl-d-gluconic acid dimethylam (7.50%), Xanthanin (3.94%), 3.alpha.,6.alpha.-Dihydroxy-5.beta.-preAllopregnane-7.alpha.,11.alpha.-diol-3 (3.47%) and 2,5-Anhydro-1,6-Dideoxyhexo (3.29 %), 1,3-Cyclohexadiene (3.04%) 2,3-Dihydro-5-Hydroxy-6-Met4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd (3.04%). The presence of various bioactivecompounds confirms the application of *M. nilagirica* for various ailments by traditional practitioners. However, the isolation of individual phytochemical constituents may proceed to find a noval drug.

INTRODUCTION

In recent years the plant used in the management and curing of diseases has gained considerable importance.Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits and seeds Gordon (2001). It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources into developing medicines by medicinal plants. If we can come back to our nature, culture and tradition on use of medicinal plants it can bring up a bright and healthy new generation Kirtikar and Basu (1918). There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and Alzheimer's diseases (Sheeja and Kuttan, 2007; Mukherjee *et al.,* 2007). *Magnolia nilagirica* belonging to the genus *Magnolia* (Magnoliaceae) is a native to tropical and subtropical South and South-East Asia, including southern China. It is widely used in both Ayurveda and Homeopathic medicine.

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Flower buds of *M.champaca* is commonly used by many traditional healers in most of the herbal preparations for diabetes Srinivasan (2005) and kidney diseases (Soltan and Sirry, 2002). Traditionally, it is being used in fever, colic, leprosy, post-partum protection (Khan and Kihara, 2002) and in eye disorders Sobhagini *et al.* (2004). It has been reported to possess antipyretic, antiulcer and anti-inflammatory Vimala *et al.* (1997); Abdul Kaffoor *et al.* (2016) insecticidal Ulla *et al.* (1995) antioxidant, antimicrobial Chattopadhyay (2003) and leishmanicidal activities Takahashi (2004). The active constituents reported in this plant are alkaloids, tannins, saponins, sterols, flavonoids and triterpenoids (Khan and Kihara, 2002). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. With this background, the present study was aimed to identify the phytoconstituents present in *M. nilagirica* using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant materials

Magnolia nilagirica (Zenker) Figler. belongs to the family Magnoliaceae was collected from natural forest of Kotagiri, Niligiri Hills, Tamil Nadu. The specimen was identified by Dr. M. Murugesan, Scientist: B, Botanical Survey of India, Shillong. A voucher specimen was deposited in the Department

of Botany, Kongunadu Arts and Science College, Coimbatore (KASC/BOT/0017). The plants were thoroughly washed in running tap water with sodium chloride and then in sterile water before being shade dried for 20 days.

Solvent Extraction

Plant materials (whole plant) was collected and washed with distilled water and shade dried for a week. The dried sample were manually ground to fine powder using pulverizer and passed through 40 mesh sieve and stored in air tight containers. The coarsely powdered plant material was weighed to 250g and Soxhlet extracted with petroleum ether, chloroform, ethyl acetate and methanol separately for 12 hours. The filtrate was evaporated to dryness under reduced pressure using rotary vaccum evaporator and the solid mass obtained was stored at 4ºC until further use. The stored filtrate was used for the various phytochemical and biological studies.

GC-MS Analysis

Gas Chromatography (GC) analysis was carried out using Varian 5975 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatography was fit with VF 5 MS capillary column (30 m × 0.25 mm). The injector temperature was set at 240ºC, and the oven temperature was initially be at 70ºC then programmed to 300ºC at the rate of 10ºC/minute and finally held at 300ºC for 10min. Helium was used as carrier gas with the flow rate of 1.51ml/min. The percentage of composition of extract was calculated by GC peak areas. The compounds were identified based on comparison of their retention indices (RI), retention time (RT) and mass spectra.

GC-MS- identification of compounds

Identification was based on the molecular structure, molecular mass and calculated fragments.

Table 1. GC-MS analysis showing various compounds identified in methanolic leaf extract of *Magnolia nilagirica*

S. No.	Name	Retentions Time	Molecular weight	Molecular formula	Area%
1	2-Methyl-1-Propoxy Propane	5.619	64	$C_{13}H_{26}O$	0.74
2	2-Furanmethanol	6.713	98	$C_5H_6O_2$	1.60
3	OximeMethoxy-phenyl-	7.469	151	$C_8H_9NO_2$	2.51
4	Dihydro-2(3H)-Furanone	7.589	86	$C_4H_6O_2$	1.04
5	1,2-Cyclopentanedione	7.826	98	$C_5H_6O_2$	1.11
6	Methyl 6-O-[1-methylpropyl]-beta-d-g	8.885	250	$C_{11}H_{22}O_6$	0.12
7	2-Hydroxy-gamma-butyrolactone	9.413	102	$C_4H_6O_3$	0.32
8	7-Oxa-Bicyclo[]2.2.1]HEPT-5-EN-	9.507	110	$C_6H_6O_2$	0.14
9	7-Tridecanone	9.935	198	$C_{13}H_{26}O$	2.67
10	2,5-Anhydro- 1,6-Dideoxyhexo-3,4-DIULOSE	10.524	128	$C_6H_8O_3$	3.29
11	2,3-Dihydro-5-Hydroxy-6-Methyl-4H-Pyran-4-one	10.793	128	$C_6H_8O_3$	2.78
12	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	12.117	144	$C_6H_8O_4$	1.40
13	1-Butanol, 3-Methyl-	12.809	130	$C_7H_{14}O_2$	13.25
14	1,2-Benzenediol	13.525	110	$C_6H_6O_2$	2.46
15	2-Furancarboxaldehyde, 5-(H’	13.800	126	$C_6H_6O_3$	1.81
16	Phosphoric acid, methyl-, bis(trimethyl	14.084	240	$C_7H_{21}O_3Psi_2$	0.14
17	2,5-Hexanedione	14.357	114	$C_6H_{10}O_2$	0.14
18	Vitispirane	14.411	192	$C_{13}H_{20}O$	0.91
19	Octanoic acid, 7-oxo-, methyl ester	14.911	172	$C_9H_{16}O_3$	0.16
20	2-Methoxy-4-Vinylphenol	15.011	150	$C_9H_{10}O_2$	1.45
21	5-O-Methyl-d-glucomic acid dimethyl	16.253	237	$C_9H_{19}NO_6$	7.50
22	Benzeneethanol, 4-hydroxy-	17.722	138	$C_8H_{10}O_2$	1.28
23	3-Nitrobenzyl iodide	18.159	263	$C_7H_{61}NO_2$	2.53
24	Epoxy-linalooloxide	19.349	186	$C_{10}H_{18}O_3$	0.68
25	Beta-D-Glucopyranose, 1,6-A	20.376	162	$C_6H_{10}O_5$	0.36
26	DOET	20.954	223	$C_{13}H_{21}NO_2$	0.61
27	(-)-5-Oxatricyclo(8.2.0.0(4,6))DODECANE	21.774	220	$C_{15}H_{24}O$	1.30
28	2-Naphthalenemethanol, decahydro-alpha	24.538	222	$C_{15}H_{26}O$	1.27
29	11-Hexadecyn-1-ol	25.634	238	$C_{16}H_{30}O$	2.51
30	1H-Cycloprop(e)azulen-7-ol, decahydro	26.755	220	$C_{15}H_{24}O$	0.91
31	Cyclohexanol, 2-methyl-3-(1-methyleth	26.899	196	$C_{12}H_{20}O_2$	1.24
32	9,10-Dimethyltricyclo(4.2.1.1(2,5)) deca	27.238	196	$C_{12}H_{20}O_2$	1.04
33	4-((1E)-3-Hydroxyl-1-propenyl)-2-methoxyphenol	27.419	180	$C_{10}H_{12}O_3$	1.26
34	Widdrolhydroxyether	28.115	238	$C_{15}H_{26}O_2$	1.62
35	Pluchidiol	28.675	208	$C_{13}H_{20}O_2$	2.46
36	Ergost-25-Ene-3,5,6,12-Tetrol	28.914	448	$C_{28}H_{48}O_4$	1.76
37	1H-Cycloprop(E)Azulen-7-ol	29.783	220	$C_{15}H_{24}O$	-0.46
38	4-O-Methylmannose	30.518	194	$C_7H_{14}O_6$	1.07
39	2H-cyclohepta(b)furan-2-one, 3,3a,4,7	32.245	306	$C_{17}H_{22}O_5$	11.11
40	Kauren-19-yl-Acetate	33.553	330	$C_{22}H_{34}O_2$	0.20
41	Tricyclo(4.4.0.0(2,7))Dec-8-ene	34.092	220	$C_{15}H_{24}O$	0.62
42	Octadecanoic acid	34.418	284	$C_{16}H_{36}O_2$	0.93
43	9,12-Octadecadienoic acid (Z,Z)	34.686	280	$C_{18}H_{32}O_2$	1.45
44	9,12-Octadecadienoic acid (Z,Z)	35.189	294	$C_{19}H_{34}O_2$	1.03
45	Pregnane-3, 20-dione	35.629	316	$C_{21}H_{32}O_2$	1.40
46	1,3-Cyclohexadiene, 2,6,6-trimethyl-1-	35.946	188	$C_{14}H_20$	3.04
47	2(3H)-Naphthalenone, 4,4A,5,6,7	36.125	218	$C_{15}H_{22}O$	0.97
48	Xanthanin	36.287	316	$C_{18}H_{20}O_5$	3.94
49	Benzyl, beta-d-glucoside	36.442	270	$C_{13}H_{18}O_6$	1.46
50	2,5,5-Trimethyl-4-methylene	37.288	206	$C_{14}H_{22}O$	0.97
51	9,19-Cyclolanostan-3-ol, acetate, (3.beta)	38.467	470	$C_{32}H_{54}O_2$	0.88
52	3.alpha,6.alpha-Dihydroxy-5.beta-pre	39.291	334	$C_{21}H_{34}O_3$	3.47
53	Allopregnane-7.alpha.,1,1.alpha-diol-3.	39.958	348	$C_{21}H_{32}O_4$	1.04
54	4,4-DImethyl-3-(3-methylbut-2-enylide)	40.822	236	$C_{15}H_{24}O_2$	0.18
55	1,4,7,10,13,16-Hexaoxacyclooctadecane	41.496	264	$C_{12}H_{24}O_6$	0.32

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 name of the components of the test materials was ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the WILEY8 and NIST08 library version (2012) and turbo mass 5.2 software. This is done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and traditional use as a herbal medicine, in addition to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their biological or therapeutic relevance.

RESULTS

The GC-MS analysis of *Magnolia nilagirica* revealed that the presence of 55 compounds (phytochemical constituents) in methanolic leaf extracts that could contribute the medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area (%) and retention time (RT).

The first compound identified with less retention time (5.619 and 6.713 min) was Propane, 2-Methyl-1-Propoxy2-Furan Methanol Oxime-, Methoxy-phenyl, respectively. Whereas, 4,4-Dimethyl-3-(3-methylbut-2-enylide and 1,4,7,10,13,16-Hexaoxacyclooctadecane was the last compound which took longest retention time (40.822 and 41.496 min) to identify. The highest peak area for 1 Butanol, 3–Methyl (13.25 %), 2H-Cyclohepta[b]furan-2-one (11.11 %), 5-O-Methyl-d-gluconic acid dimethylam (7.50%), Xanthanin (3.94%), 3.alpha.,6.alpha.-Dihydroxy-5.beta.-preAllopregnane-7.alpha.,11.alpha.-diol-3 (3.47%) and 2,5-Anhydro-1,6-Dideoxyhexo (3.29 %), 1,3-Cyclohexadiene (3.04%) 2,3-Dihydro-5-Hydroxy-6-Met4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd (3.04%)were presented in (Table 1). Thus, GC-MS analysis is the first step towards understanding the nature of active principles in this plant extract and further investigations into the pharmacological importance of *M. nilagirica* and their detailed phytochemistry may add new knowledge to the information in the traditional medical systems for treating various diseases and its complications.

DISCUSSION

GC-MS analysis was done using the organic solvent ethyl acetate and it shows the presence of 55 chemical compounds present in *M. nilagirica*.

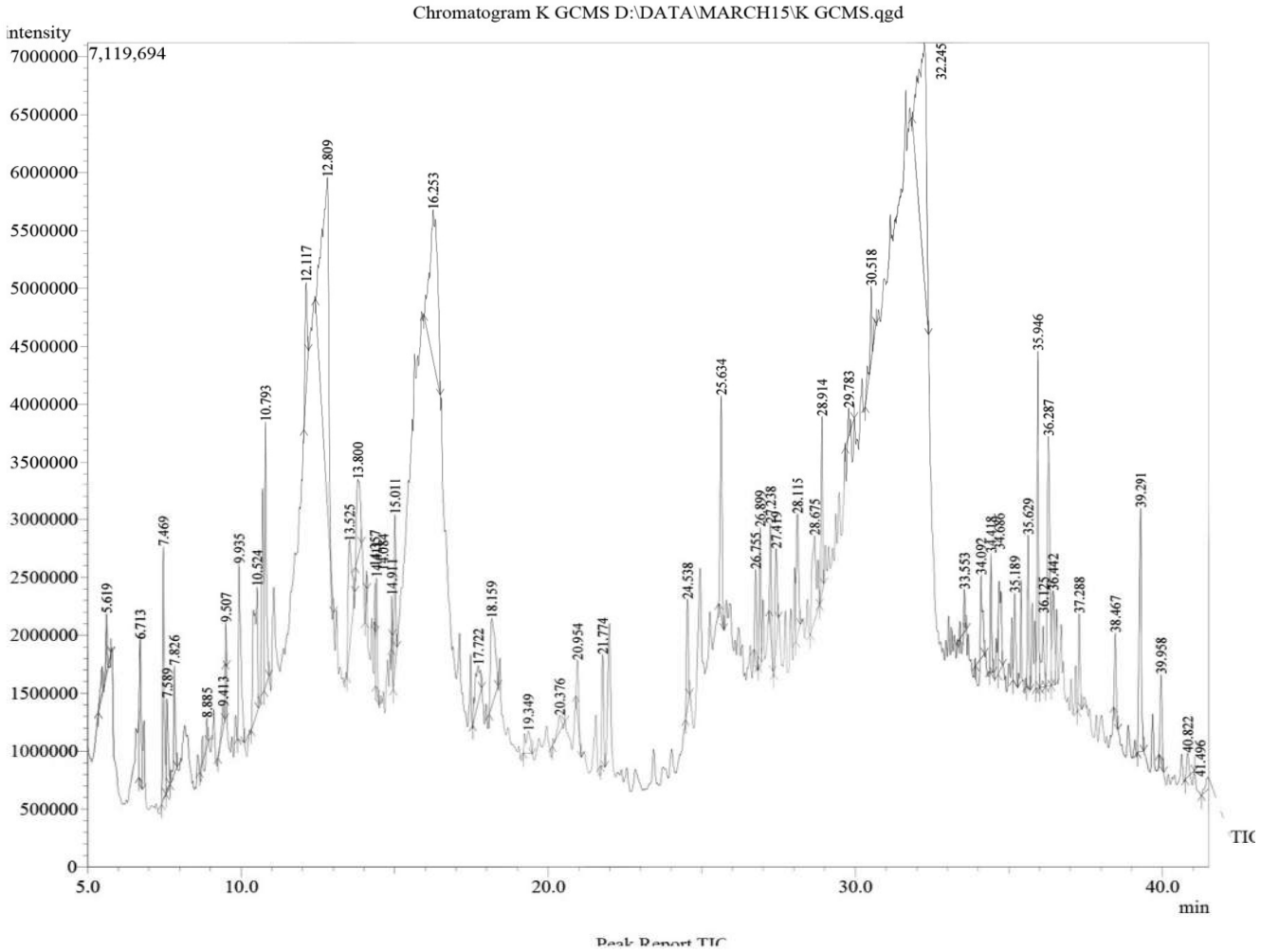

Fig. 1. GC-MS Chromatogram of Magnolia nilagirica

The sample was extracted with ethyl acetate because majority of the secondary metabolites were present in this solvent and antibacterial activity was higher in this solvent. GC-MS analysis also provides the spectrum for the ethyl acetate extract. The major compounds with highest peak area are shown in the (Fig.1). They are 1Butanol, 3-Methyl (13.25 %), 2H-Cyclohepta[b]furan-2-one (11.11 %), 5-O-Methyl-d-gluconic acid dimethylam (7.50%), Xanthanin (3.94%), 3.alpha.,6.alpha.-Dihydroxy-5. beta.-pre Allopregnane-7.alpha.,11.alpha.-diol-3 (3.47%) and 2,5-Anhydro-1,6-Dideoxyhexo (3.29 %), 1,3-Cyclohexadiene (3.04%) 2,3-Dihydro-5-Hydroxy-6-Met4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd (3.04%). Malathi and Rajan (2015) analysed the dry flowers of *M. champaca* using GC-MS techniques. They reported the presence of 12 different phytochemicals with carbamazepine epoxide found to be the compound with maximum peak percentage in methanol extract in GCMS. The bioactive components of *Physalis minima* leaves have been evaluated using GCMS, The chemical compositions of the extract of *P. 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) Karpagasundari and Kulothungan (2014).

Methanolic extract of *B. biternata* leaves was investigated using GC-MS results showed the presence of four important compounds present in the plants extract were identified as Fluorine, 5,9-Tetradecadienedioic acid, Pentadecanoic acid and 9-Octadecenoic acid. The presence of various phytochemicals contributed to the medicinal activity of this plant. The presence of bioactive compounds justified the use of *B. biternata* for various ailments. Many of the compounds identified using GC-MS have medicinal properties like antiinflammatory, ant cancerous, hepatoprotective activity etc. Amala *et al.* 2013. Tetradecanoic acid and octadecanoicacidare reported to have potential antibacterial, antifungal and antiinflammatory (McGraw *et al.,* 2002; Seidel and Taylor, 2004) properties which could be effective in the management of bacterial, fungal and viral infections.

Literature survey disclosed that *Michelia champaca* to contain michelia- A, liriodenine, parthenolide and guaianolides (Gupta *et al.,* 2005; Toshiyuki *et al.,* 1982). The plant could be an excellent supply of esters of carboxylic acid, benzaldehyde, group alcohol, isoeugenol and sesquiterpene lactones Mullaicharam and Kumar (2011). Polyphenolic compounds like gallic acid was isolated from the leaves and stem barkof *M. champaca*. Methyl linoleate, methyl anthranilate were different esters isolated from *M. champaca*. Stigmasterol and 3β-16α- dihydroxy-5-cholestene-21-al were additionally isolated from stem bark of *M. champaca* Makhija *et al.* (2010). Active constituents were (E)-3,7-dimethylocta-2,6-dien-1-ol, decahydro-3,5,8-trimethylazuleno[6,5-b]furan-2(3H)-one, 3, 5,7-trihydroxy-2-(3,4-ihydroxyphenyl)chroman-4one, methyl 2-aminobenzoate Raja and Ravindranadh (2014).

Conclusion

GC-MS method is a direct and fastest approach for identification of phytocomponents in the plant extracts. In this present study, there are about 55 compounds are identified in methanolic leaf extract of *Magnolia nilagirica*.

The results highlights the potentiality of these species which are responsible for the anticar cinogenic, antifertility, antipyretic, antioxidant and antimicrobial properties.Thus the plant studied can be used as a potential source of new useful drugs.

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