GC-MS ANALYSIS OF METHANOLIC EXTRACT OF HYDROCOTYLE CONFERTA WIGHT (APIACEAE) – AN ENDANGERED PLANT SPECIES IN SOUTHERN WESTERN GHATS, INDIA

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ABSTRACT

Phytochemical constituents are responsible for medicinal activity of plant species. In the present study the bioactive compound of methanolic extract of *H. conferta* was analyzed using 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) and WILEY8 library. GC-MS analysis showed the existence of various compounds with different chemical structures. A total of 33 compounds of were identified from the methanolic extracts of *H. conferta.* However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Key words: GC-MS analysis, Bioactive compounds, *Hydrocotyle conferta,* methanol extract.

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INTRODUCTION

Natural plant product have been used various ailments since time immemorial. These can be derived from various part of the plant like bark, leaves, stem, flowers, roots, fruits, seeds etc.., (Cragg and David,2001). Phytochemicals constituents are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on (Kumar *et al*., 2009). Phytochemical constituents are the basic source for the establishment of some pharmaceutical drug industries. The phytochemical constituents are playing an important role of the identification of crude drugs (Savithramma *et al*., 2011). The present study revealed that the various phytochemical constituents of *H. conferta* which is used for antimicrobial ....etc.

Materials and methods

2.1 Preparation of plant sample

H. conferta plant was collected from Kodanadu, The Nilgiri Hills, The Western Ghats, Southern India, Tamil Nadu. The plant was identified and authenticated by a plant taxonomist SACON, Coimbatore. The plant material was dried in shade and then powdered using pulveriser and passed through 100 mesh sieve. About 100 g of dried plant powder was defatted with petroleum ether used for this study re-extracted with methanol. This extract after evaporation of methanol, the filtered residue was stored at 4 ^{0}C in refrigerator.

Gas Chromatography analysis

Gas Chromatography (GC) analysis of the methanol extract of *H. conferta* was performed 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 of compound was based on the molecular structure, molecular mass and calculated fragments. 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 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 turbomass 5.2 software.

RESULTS

The GC – MS analysis of the methanolic extract of *H. conferta* showed the presence of 33 compounds and their biological activity. Bioactive compounds of methanolic extract of *H. conferta* with retention time (RT), compound name, percentage of peak area, molecular formula, molecular weight and mark was represented in the Table 1and Fig 1.

The results revealed that 2-Furancarboxaldehyde, 5-(hydroxymethy (22.73%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd (13.83%), 2-Hydroxy-gamma-butyrolactone (8.23%), Cyclopropanecarboxylic acid, 3-ethenyl- 2,2-dimethyl- (5.83%), Nonanal dimethyl acetal (5.21%),2-Furanacetic acid, .Alpha.-H (4.42%), Ethanone, 1-4-Methoxypheny (4.19%), Pentanal (4.05%), 1,5-Decadiyne (3.59%), 2-Methoxy-4-Vinylphenol (3.04%), 2-Undecanone (2.80%), 2,5-Anhydro-1,6-Dideoxyhexo,3,4-Diulose (2.60%),4,4,5,8-Tetramethylchroman-2-ol (2.23%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-fura (2.27%), Methyl (Z)-5,11,14,17-eicosatetraenoate (1.79%), 2-Furancarboxaldehyde (1.73%), 2-Acetyl-2-Hydroxy-.Gamma.-Butyrolactone (1.93%), 2-Furanmethanol (1.17%), 9,12-Octadecadienoic acid (Z,Z)- (1.12%), 4-Hydroxy-2,5-Dimethyl-3(2H)- Furanone (1.11%), 2(3H)-Furanone, Dihydro- (1.04%), 2-Methoxy-6-(1-Methyl-2-proppenyl (0.74%), Oxime-, methoxy-phenyl- (0.74%), Phenol, 3-methyl- (0.70%), Phenol,2,4-BIS(1,1-dimethylet (0.71%), 1,6,10-Dodecatrien-3-ol, 3,7,11-trimeth (0.47%), Tetradecanoic acid (0.52%), 1H-Cycloprop[E]azulen-7-OL, Decahydro-1,1,7-Trimethyl (0.22%), 4-Acetyl-1-methylcyclohexene (0.08%), 2-(2-Ethylpiperidin-1-yl) acetonitrile (0.00%), 2,4,7,9-Tetramethyl-5-decyn-4,7-diol(-0.14) and 1-(Phenylthio)-2-Propylamine Hcl (-0.16%) respectively.

Discussion

In the present study, the GC-MS analysis of the methanolic extract of *H. conferta* showed the presence of 33 compounds. Gas chromatogram revealed the relative concentration of different phytochemical compounds that are eluted as a function of retention time. The relative concentration of bioactive compounds present in *H. conferta* was indicated by peak heights. The mass spectrometer used for the analysis of phytochemical compounds that was eluted at various times and used for the identification of natural structure of compounds. The separation of phytochemical compounds from large fragment to little fragment gave rise to peak appearance at various m/z ratios (Janakiraman *et al*.,2012). In various plant parts of the world several phytochemical studies was carried out using GC-MS (Wu *et al*., 2010; Sangeetha and Vijayalakshmi, 2011). A GC-MS instrument has been used for identification components that are present in natural and biological system (Oleszek and Marston, 2000; Philipson, 2007; Daffre *et al*., 2008). Kavisa Ghosh and Indra (2014) reported the ethanolic extract of *Centella asiatica* has been subjected to GC-MS analysis and chemical constituents have been identified. *H. conferta* used in various medicines however there are no reports on the thorough phytochemical analysis of the plant. 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.

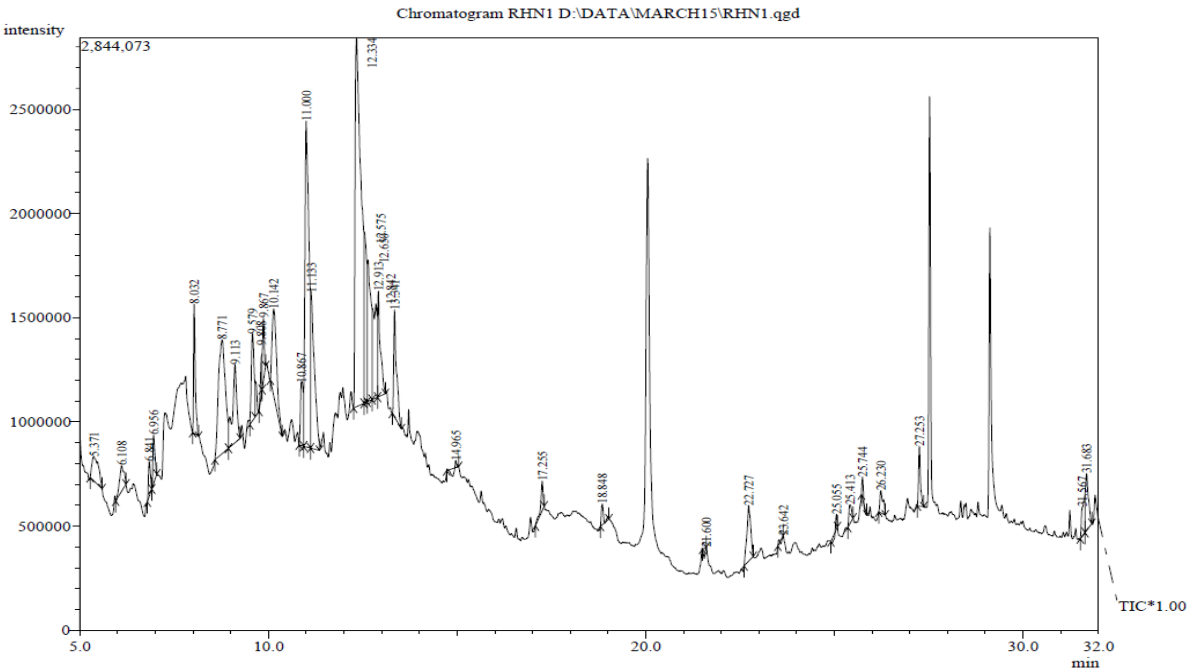
Table1: GC-MS analysis of methanolic extract of *H. conferta*

Sl. No	RT	Name of Compound	Molecular Formula	Molecular weight (g/mol)	Peak Area (%)	Mark
1	5.371	2-Furancarboxaldehyde	C6H6O3	126	1.73	MI
2	6.108	2-Furanmethanol	$C_5H_6O_2$	98	1.17	MI
3	6.841	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151	0.74	-
4	6.956	2(3H)-Furanone, Dihydro-	$C_4H_6O_2$	86	1.04	-
5	8.032	2,4-Dihydroxy-2,5-dimethyl-3(2H)-fura	$C_6H_8O_4$	144	2.27	-
6	8.771	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102	8.23	-
7	9.113	1,5-Decadiyne	$C_{10}H_{14}$	134	3.59	V

8	9.579	2,5-Anhydro-1,6-Dideoxyhexo, 3,4-Diulose	$C_6H_8O_3$	128	2.60	V
9	9.808	Phenol, 3-methyl-	C_7H_8O	108	0.70	-
10	9.867	4-Hydroxy-2,5-Dimethyl-3(2H)- Furanone	$C_6H_8O_3$	128	1.11	V
11	10.142	Pentanal	$C_5H_{10}O$	86	4.05	-
12	10.867	2-Acetyl-2-Hydroxy-.Gamma.-Butyrolactone	$C_6H_8O_4$	144	1.93	-
13	11.000	4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd	$C_6H_8O_4$	144	13.83	V
14	11.133	Cyclopropanecarboxylic acid, 3-ethenyl- 2,2-dimethyl-	$C_8H_{12}O_2$	140	5.83	V
15	12.334	2-Furancarboxaldehyde, 5-(hydroxymethyl	$C_6H_6O_3$	126	22.73	-
16	12.575	2-Furanacetic acid, .Alpha.-H	$C_6H_6O_4$	142	4.42	V
17	12.650	Nonanal dimethyl acetal	$C_{11}H_{24}O_2$	188	5.21	V
18	12.842	Ethanone, 1-(4-Methoxypheny	$C_9H_{10}O_2$	150	4.19	V
19	12.913	2-Undecanone	$C_{11}H_{22}O$	170	2.80	-
20	13.341	2-Methoxy-4-Vinylphenol	$C_9H_{10}O_2$	150	3.04	-
21	14.965	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	$C_{14}H_{26}O_2$	226	-0.14	MI
22	17.255	Phenol, 2,4-BIS(1,1-dimethylet	$C_{14}H_{22}O$	206	0.71	MI
23	18.848	1,6,10-Dodecatrien-3-ol, 3,7,11-trimeth	$C_{15}H_{26}O$	222	0.47	MI
24	21.600	1-(Phenylthio)-2-Propylamine Hcl	$C_9H_{14}ClNS$	203	-0.16	MI
25	22.727	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206	2.23	-
26	23.642	2-(2-Ethylpiperidin-1-	$C_9H_{16}N_2$	152	0.00	MI

		yl)acetonitrile				
27	25.055	4-Acetyl-1-methylcyclohexene	$C_9H_{14}O$	138	0.08	MI
28	25.413	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.52	MI
29	25.744	1H-Cycloprop[E]azulen-7-OL, Decahydro-1,1,7-Trimethyl	$C_{15}H_{24}O$	220	0.22	MI
30	26.230	2-Methoxy-6-(1-Methyl-2-proppenyl).	$C_{15}H_{16}O$	212	0.74	MI
31	27.253	4-Hydroxy-.beta.-ionone	$C_{13}H_{20}O_2$	208	1.22	-
32	31.567	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.12	-
33	31.683	Methyl (Z)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318	1.79	V

Fig1:GC-MS Chromatogram of the methanolic extract of *H. conferta*



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