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RESEARCH ARTICLE

GC-MS ANALYSIS ON ETHANOLIC LEAF EXTRACTS OF *VIBURNUM PUNCTATUM* BUCH. - HAM. EX D. DON

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Abstract

Viburnum punctatum Buch.-Ham. ex D. Don is a medicinally important plant belongs to Caprifoliaceae family also known as "Konakaram" commonly in Tamil. It is a small evergreen tree, commonly found in sholas and moist forests, above 1200m in South east Asia. Many of these species are recognized for their medicinal properties from very early times of this century. The leaves of *Viburnum punctatum* were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti periodic effect in the present study the ethanolic extract of *Viburnum punctatum* leaves has been subjected to GC-MS analysis. GC-MS analysis of the ethanolic extract revealed existence of eight peaks presented and the chemical constituents were identified. The major compounds are 2,3,3a,4,5,6,7,7a - Octahydro - 1H - cyclopenta [a] pentalen-7-ol (18.62%, RT - 16.67), Cyclohexene, 4 - isopropenyl -1 - methoxymethoxymethyl- (11.52%, RT - 14.55), 11-Desacycloxytetrahydrovaltrate (10.75%, RT - 26.50) and Copper(II) meso - (2-Formylvinyl) octaethylporphyrin (6.09%, RT 30.38).

Introduction

India has a wide and rich heritage of using medicinal plants and hosting thousands of medicinally valuable plants belonging to hundreds of families. One cannot assure that all these plants possess a long recorded history, although they have been reported to contain medicinally valuable phytochemical - pharmaceuticals and subjected to formulate, ayurvedic, siddha, unani and the Chinese system of medicine (Kathiresan Prabhu *et al*, 2011). Medicinal plants are widely used for the treatment of common animal and human infectious diseases since antiquity (Rios *et al*, 2005) and the plant products have been used with varying success to prevent and cure diseases throughout history (Raskin *et al*, 2002 and Arzu Ucar Turker *et al*, 2014).

According to an analysis by World Health Organization, nearly 80% of the world population are depending on herbal medicines for their health care problems (Farnsworth *et al*., 1985). The plants medicinal use is actually due to the presence of some active components that are effective for human body in many ways (Akinmoladun *et al*., 2007). Flavonoids, alkaloids, tannins, terpenoids, saponins, essential oils, and phenolic compounds are some of the chemical constituents responsible for bioactivity of plants (Edeoga *et al*., 2005; Tan *et al*., 2006). Synthesis of these such compounds are responsible for the ability of plants to resist themselves from predators as well as to destroy pathogenic microorganisms (Mans *et al*., 2000 and Yamin Bibi *et al*, 2010).

Viburnum punctatum Buch.-Ham.ex D.Don (*Viburnum acuminatum* Wall) is a medicinal plant belonging to Caprifoliaceae family, under the order Dipsacales (Prabhu *et al*., 2009). It belongs to the monotypic genus *Viburnum*, native to India, Bhutan, Nepal, Thailand, Cambodia, Vietnam, Indonesia and China. It is a shrub or medium sized tree, growing at an altitude not less than 1500 m; profusely with other plants in Nilgiri, Himalaya and Coimbatore. The leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti-periodic effect. Several workers have investigated the different parts of the plant phytochemically and

found to contain sugars, glycosides, sterols, terpenoids and phenolic compounds. The plant also reported to contain triterpenes, saponins in root, mucilage, tannin and lignin in leaf, saponins, starch grains and tannins in stem and glycoside, terpenoid and sterols in leaves (Renjith Alex *et al*., 2014). The present investigation have been carried out to determine the possible chemical components from *Viburnum punctatum* leaves by GC-MS.

MATERIALS AND METHODS:

Plant material collection :

Viburnum punctatum leaves were collected from The Nilgiri Hills, Tamil Nadu, India. The plant specimen was identified in Botanical Survey of India, Southern Region Centre, Coimbatore, India.

GC – MS analysis*:*

Freshly collected leaves of *Viburnum punctatum* were shade dried and then powdered using a mechanical grinder. 10 grams of pulverized leaf material were soaked in 100 ml of ethanol and kept on a rotary shaker for 24 h. The extract was filtered through a Whatman No. 1 Filter Paper and the process was repeated until all soluble compounds had been extracted. The extract was concentrated under reduced pressure in a rotary evaporator. The plant ethanolic extract was used for the GC-MS analysis.1 µl of the ethanolic leaf extract of *Viburnum punctatum* have been employed for GC-MS analysis (Gopalakrishnan *et al*., 2011 and Muthuchelian *et al*., 2011).

Instrument and chromatographic condition:

The GC-MS analysis was carried out on a Thermo GC - Trace Ultra Version: 5.0, Thermo MS DSQ II interfaced to the mass spectrometer (GC-MS) instrument and employing the following conditions: column ZB 5 - MS capillary standard Non - polar column, in a RD operator at the dimension of 30 minutes, ID: 0.25 mm and Film: 0.25 µm. Helium (99%) was used as the carrier gas at a constant flow of 1ml/min and an injection volume of 1 microliter was employed. The temperature of oven was programmed from 70°C and raised to 260°C at 6 C/ min. The Mass spectrum was taken from low mass (m/z): 50 to high mass (m/z): 650.

Component identification:

Interpretation on the GC-MS mass spectrum was done using the database of National Institute Standard and Technology (NIST).The NIST have more than 62,000 patterns. Mass spectrum of the unknown component was compared with the spectrum of the known components and stored in the NIST library. The compound name, molecular weight and structure of the components of the test materials were ascertained (Muthuchelian *et al.*, 2011 and Abirami *et al.*, 2012).

RESULT AND DISCUSSION

GC - MS analysis: GC-MS chromatogram of the ethanolic leaf extract of *Viburnum punctatum* (Figure 1) showed 8 peaks indicates the presence of eight phytochemical constituents. The eight phytoconstituents were characterized and identified on comparison of mass spectra of the constituents with the NIST library. (Table 1).

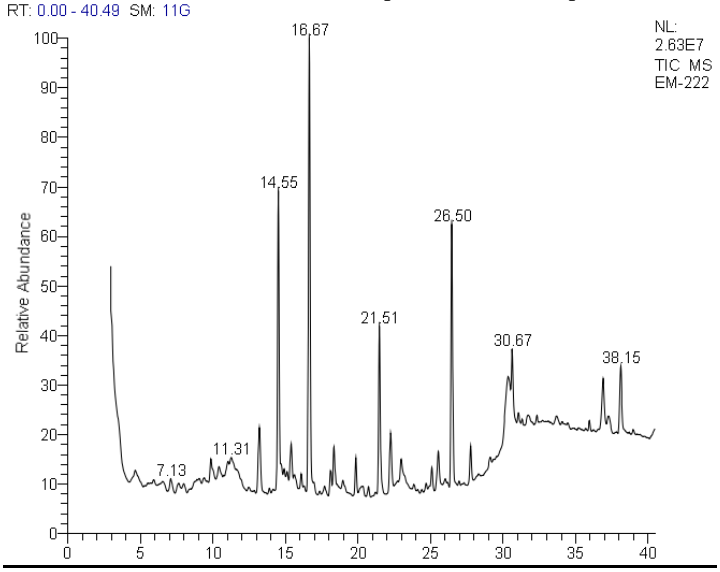


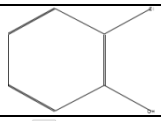
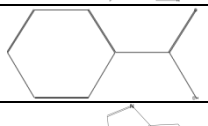
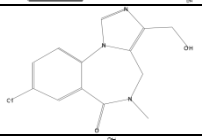
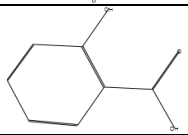
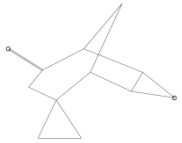
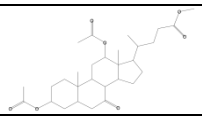
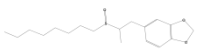
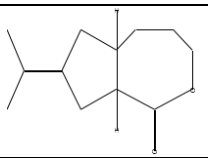
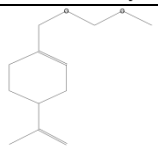
Figure.1

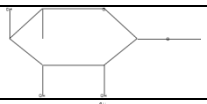
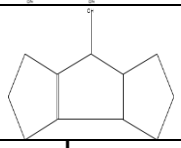
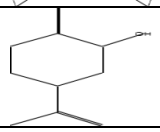
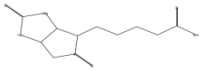
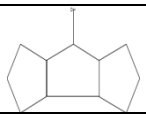
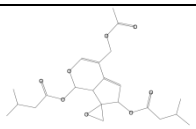
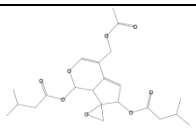
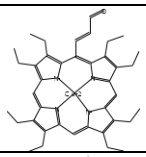
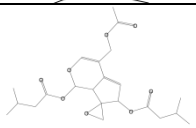
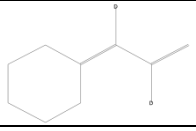
GC-MS Chromatogram of ethanolic leaf extract of Viburnum punctatum

Four major phytochemical constituents in mass spectra are presented in Figure 2 - Figure 5. They were identified as 2,3,3a,4,5,6,7,7a-Octahydro-1H-cyclopenta[a]pentalen-7-ol (18.62%, RT - 16.67), Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl- (11.52%, RT - 14.55), 11-Desacycloxytetrahydrovaltrate (10.75%, RT - 26.50) and Copper(II) meso-(2-Formylvinyl) octaethylporphyrin (6.09%, RT 30.38) respectively.

TABLE 1

***Phytocomponents identified in the ethanolic leaf extract of Viburnum punctatum by GC-MS*.**

No.	Retention Time	Compound Name	Molecular Formula	MW	Peak Area (%)	Structure
1	4.69	Phenol, 2-chloro- (CAS)	C6H5ClO	128	1.57	
2	6.58	3-Benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylonitrile	C11H7F4NS	261	0.77	
3	7.13	Benzoic acid, silver(1+) salt (CAS)	C7H6AgO2	229	0.79	
4	7.68	8-Chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-methyl alcohol	C13H12ClN3O2	277	0.85	
5	9.09	Benzoic acid, 2-hydroxy- (CAS)	C7H6O3	138	0.89	
6	9.90	7'-Oxaspiro[cyclopropane-1,4'-tricyclo[3.3.1.0(6,8)]nonan-2'-one]	C10H12O2	164	1.03	
7	10.45	Cholan-24-oicacid,3,12-bis(acetyloxy)-7-oxo-, methylester, (3à,5á,12à)- (CAS)	C29H44O7	504	0.80	
8	11.31	1,3-Benzodioxole, 5-[2-(octylsulfinyl)propyl]- (CAS)	C18H28O3S	324	3.70	
9	13.23	3-Oxabicyclo[5.3.0]decan-2-one,9-isopropylidene-, cis--	C12H18O2	194	3.53	
10	14.55	Cyclohexene,4-isopropenyl-1-methoxymethoxymethyl-	C12H20O2	196	11.52	

11	15.43	2H-Pyran-3,4,5-triol,tetrahydro-2-methoxy-6-methyl-	C7H14O5	178	1.85	
12	16.67	2,3,3a,4,5,6,7,7a-Octahydro-1H-cyclopenta[a]pentalen-7-ol	C11H16O	164	18.62	
13	18.36	cis-p-mentha-1(7),8-dien-2-ol	C10H16O	152	2.10	
14	18.46	Biotin Sulfoxide	C10H16N2O4S	260	2.02	
15	19.89	Neophytadiene	C20H38	278	1.46	
16	20.39	3,7,11,15 - Tetramethyl - 2 - hexadecen - 1 - ol	C20H40O	296	1.08	
17	21.51	2,3,3a,4,5,6,7,7a-Octahydro-1H-cyclopenta[a]pentalen-7-ol	C11H16O	164	6.61	
18	22.27	Hexadecanoic acid (CAS)	C16H32O2	256	2.74	
19	22.99	Hexadecanoic acid, ethyl ester (CAS)	C18H36O2	284	4.46	
20	25.12	Phytol	C20H40O	296	0.92	
21	25.56	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	278	2.19	
22	26.06	Octadecanoic acid (CAS)	C18H36O2	284	1.10	
23	26.50	11-Desacycloxytetrahydrovaltrate	C20H32O6	368	10.75	
24	27.80	11-Desacycloxytetrahydrovaltrate	C20H32O6	368	1.31	
25	30.38	Copper(II)meso-(2-Formylvinyl)octaethylporphyrin	C39H46CuN4O	649	6.09	
26	30.65	11-Desacycloxytetrahydrovaltrate	C20H32O6	368	2.76	
27	31.75	2,3-Diderterio-1,1-pentamethylene-1,3-butadiene	C9H12D2	122	0.74	

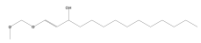
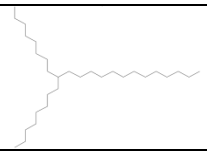
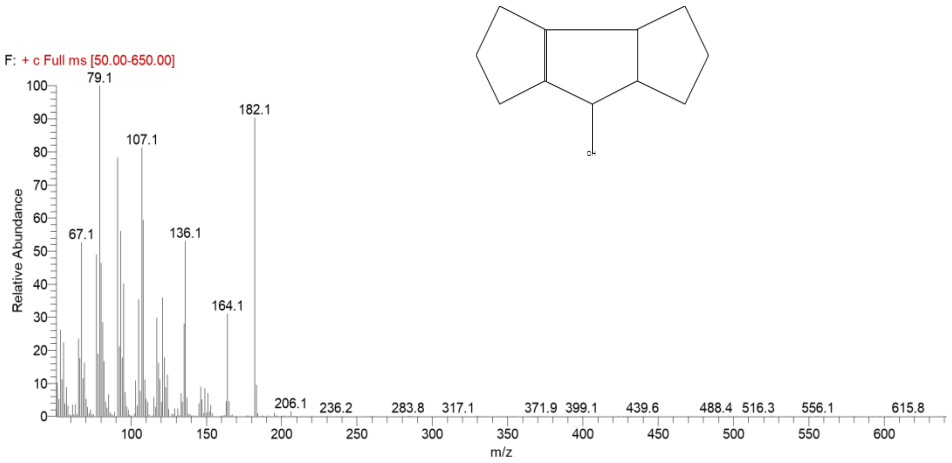
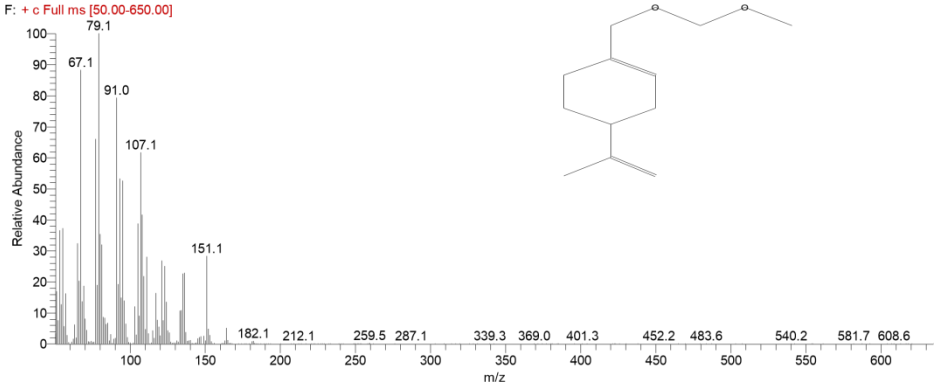
28	36.92	(E)-1-(Methoxymethoxy)-1-tetradecen-3-ol	C16H32O3	272	3.15	
29	37.31	[1-Pyridyl-4-(hydroxymethyl)-1-phenylpyrazol-3-yl]-(1,2,4)triazolo[4,3-a]qinoxaline	C24H17N7O	419	1.39	-
30	38.15	Docosane, 9-octyl- (CAS)	C30H62	422	3.19	

Figure.2

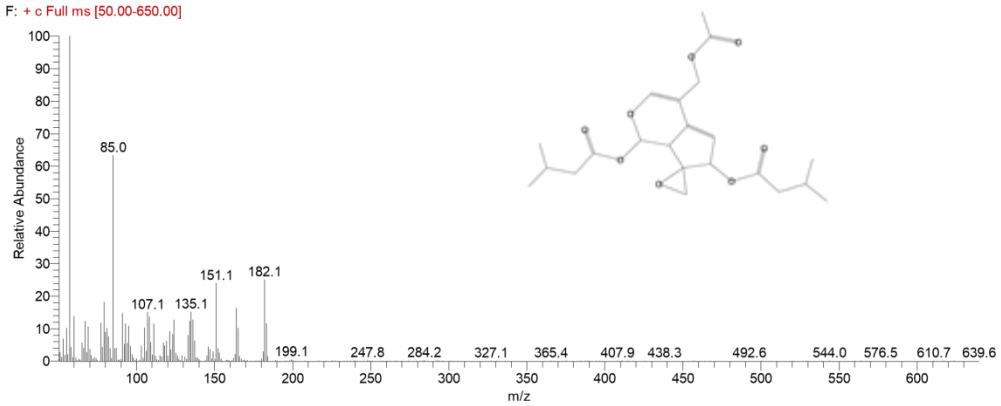


Mass spectrum of 2,3,3a,4,5,6,7,7a-Octahydro-1H-cyclopenta[a]pentalen-7-ol (18.62%, RT - 16.67)
Figure.3



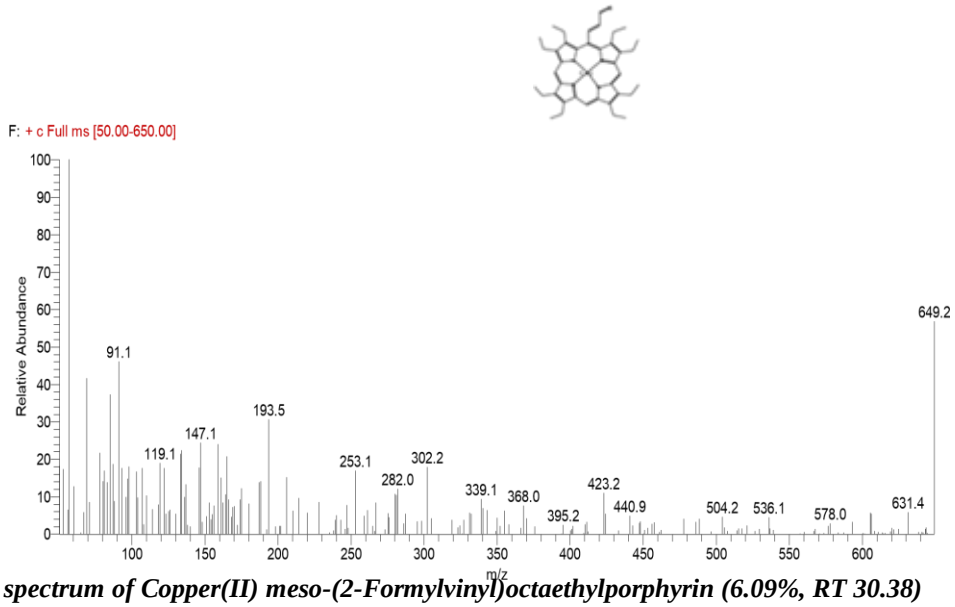
Mass spectrum of Cyclohexene, 4-isopropenyl-1-methoxymethoxymethyl- (11.52%, RT - 14.55)

Figure.4



Mass spectrum of 11-Desacycloxytetrahydrovaltrate (10.75%, RT - 26.50)

Figure.5



Mass spectrum of Copper(II) meso-(2-Formylvinyl)octaethylporphyrin (6.09%, RT 30.38)

CONCLUSION

In this study, eight chemical constituents have been identified from ethanolic leaf extract of *Viburnum punctatum* by Gas Chromatogram - Mass spectrometry analysis (GC-MS). The presence of various bioactive components justified the use of plant for various ailments by the traditional practitioners. However the isolation of individual phytochemical constituents and subjecting it them to biological activity will definitely give fruitful results. The result could be concluded that *Viburnum punctatum* contains various bioactive compounds. So it be

recommended as a plant of phytopharmaceutical importance. However, further studies are need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on ecosystem and the agricultural products.

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