Bioactive compounds determination using GC-MS and TLC analysis of threatened medicinal plant *Hybanthus travancoricus* (Bedd.) Melch.

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ABSTRACT

The present investigation was carried out to determine the possible bioactive compounds from *Hybanthus travancoricus* by using acetone extract .The GC-MS analysis of acetone extract was performed using JEOL GCMATE II GC-MS with data system is a high resolution double focusing instrument. Ten compounds were identified from *Hybanthus travancoricus* showed highest peak area Trimethyl(4-tert-butylphenoxy)silane (18.90%) molecular weight (222.39 g/mol)and lowest peak area Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl (3.73%) molecular weight (207.23g/mol).TLC analysis with acetone solvent revealed three spot R_f values 0.79,0.82 and 0.91.The Maximum RF value was showed by spot-3 and minimum was observed in spot-1.

Keywords: *Hybanthus travancoricus*,GC-MS, Bioactive compound and TLC.

INTRODUCTION

Hybanthus travancoricus (Family–Violaceae) a perennial herb known as Orithazthamarai. The whole plant has aphrodisiac activity and it is used as rejuvenating herb in Siddha system of Medicine (Ramamorthy *et al*., 2011). The plant is popularly called "Ratanpurus" by the local Yanadi and Santal tribes, villagers and herbalists. This ethnobotanical herb is known to have unique medicinal properties (Prakash *et al.*,1999).

The medicinal herbs were gaining much importance in recent years due to wide applications of its bioactive molecules (Joselin *et al*., 2012, 2013, 2014; Sukumaran *et al*., 2014; Princy *et al*., 2022a,b; Mariyammal *et al*., 2023a,b). Different strategies have been developed for the selection of particular herbs for the study. The herb selected was screened for the active phytoconstituents. The specific compound present in the herb was subjected to isolation with different analytical techniques. The analogues of isolated molecules are characterized and structural modification has been done to enhance the desired activity and

minimize the unwanted side effects (Sharangouda *et al*., 2014). Herbal plants synthesize lower molecular weight organic compounds called secondary metabolites which possess various biological activities (Kiruba et al., 2011a,b; Mithraja et al., 2011; Rajan et al., 2011; Jeeva and Marimuthu, 2012; Anitha et al., 2012; Okenwa and Felicia, 2014). The present study bioactive compounds GC-MS and TLC analysis using acetone extract of *Hybanthus travancoricus* .

MATERIALS AND METHODS

Extraction of plant materials

The selected plant material *Hybanthus travncoricus* was shade dried and pulverized into powder, using a mixer grinder. Required quantity of the powder were weighed, transferred to the flask, treated with acetone until the powder was fully immersed, incubated overnight and filtered through a What man No.1 filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas in to the solution.

The acetone extract contains nonpolar component of the plant material and 2ml of sample solution was employed in GC-MS for analysis of different compounds.

Gas chromatography-Mass spectrometry (GC-MS analysis)

GC-MS analysis was performed using the JEOL GCMATE II GC-MS With data system is a high resolution double focusing instrument. Maximum resolution:6000 maximum calibrated mass:1500 Daltons equipped with a Elite-5MS (5%diphenyl/ 95%dimethyl poly siloxane) fused silica capillary column (30×0.25mm ID×0.25µm df).For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70ev.Helium gas (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1 injected temperature 250^0C; ion-source temperature 200^0C, the oven temperature was programmed from 110^0C (isothermal for 2 min), with an increase of 10^0C/min, to 200^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds from 40 to 450 Da. The solvent delay was 0 to 2 minutes, and the total GC-MS running time was 36 min. the relative percentage mount of each compound was calculated by comparing its average peak area to the total areas (Pana Michaela, 2011).

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the data base of national institute standard and technology (NIST) having more than 62,000 patterns. The spectrum of the known component was combined with the spectrum of the component stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dukes, 2013).

Thin Layer chromatography

Mobile phase was prepared by dissolving the Methanol: Dichloromethane in the ratio of 5:5 for the detection of Polar based compounds. And about 10µl of acetone (*Hybanthus travancoricus*) extract of samples were dropped on TLC sheet 2cm above from the bottom. Allow the chromatographic chamber for the separation of compounds as individual bands. Then chromatogram was developed and visualized under UV at 366 nm. After chromatogram was developed, the Rf (Retention Factor) was calculated by using the formula (Biradar and Rachetti,2013;Bhawani *et al*.,2010;Handrini and Tunjung,2015).

RESULTS

Active compounds using acetone extract of *Hybanthus travancoricus* (Bedd.) Melch.

The result on GC-MS analysis of *Hybanthus travancoricus* using acetone extract was presented in table 1 and figure1 .

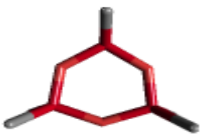
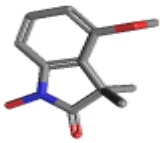
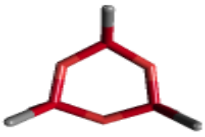
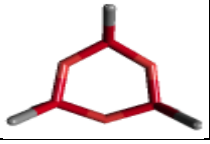
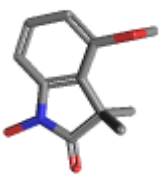
At the retention time was 16.639, 16.771 and 16.894 the compound Cyclotrisiloxane, cyclotrisiloxane and hexamethyl was identified and its peak area(9.27,8.11%,14,67%), molecular weight was 222.46 g/mol. The compound Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl- was identified at the retention time 16.686 and16.922 their peak area(8.46%,3.73%)and molecular weight at 207.23 g/mol. At the retention time 16.970,the compound 2-Ethylacridine and its peak area (6.71%) and molecular weight was 207.27 g/mol. The compound Trimethyl (4-tert-butylphenoxy) silane was identified at the retention time 17.140 , their peak area (18.90%) and molecular weight 222.399 g/mol. At the retention time 17.329, the compound tert-Butyl(5-isopropyl-2-methylphenoxy) dimethylsilane and their peak area(9.89%) and molecular weight 264.478 g/mol respectively. The compound 2-Methyl-7-phenylindole was identified at the retention time 17.379 and 17.470, peak area (10.08, 10.19%) and molecular weight 207.27 g/mol was identified.

TLC *Hybanthus travancoricus* (Bedd.)Melch.using acetone extract

The selected plant (*Hybanthus travancoricus*) parts were subjected to maceration extraction in acetone for 5-7 days. After extraction the aqueous layer of solvent was filtered with Whatman No.1 filter paper and the filtered plant extracts were tested for the presence of polar based phytocompounds using thin layer chromatography with the mobile phase of Methanol and Dichloromethane (5:5 ratio). The chromatogram was developed using UV light at the wavelength of 325nm and interpreted the chromatographic bands.

Hybanthus travancoricus acetone extract of showed three spots, Rf 0.79 and 0.82 revealed the presence of phenolic compounds in spot-1 & 2 while, spot-3 (0.91 Rf) has been detected as steroid. The Maximum RF value was showed by spot-3 and minimum was observed in spot-1.(Table.2 and Figure.2)

Table 1. Active compounds using acetone extract of *Hybanthus travancoricus*

S. No	chemical compound	Retention Time	Molecular Formulae	Molecular weight	Peak %	Molecular structure	Biological activity
1	Cyclotrisiloxane, hexamethyl-	16.639 min	$C_6H_{18}O_3Si_3$	222.46	9.27		Antimicrobial
2	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	16.686 min	$C_{11}H_{13}NO_3$	207.23	8.46		Antimicrobial, antioxidative
3	Cyclotrisiloxane, hexamethyl-	16.771 min	$C_6H_{18}O_3Si_3$	222.46	8.11		Antimicrobial
4	Cyclotrisiloxane, hexamethyl-	16.894 min	$C_6H_{18}O_3Si_3$	222.46	14.67		No activity
5	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	16.922 min	$C_{11}H_{13}NO_3$	207.23	3.73		Antimicrobial, antioxidative

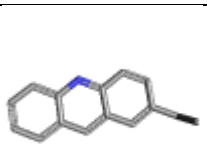
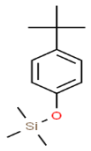
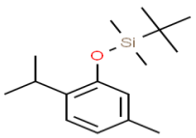
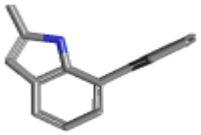
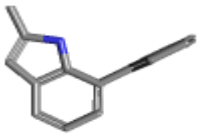
6	2-Ethylacridine	16.970 min	$C_{15}H_{13}N$	207.27	6.71		Antimicrobial
7	Trimethyl(4-tert-butylphenoxy) silane	17.140 min	$C_{13}H_{22}$	222.39	18.90		Antimicrobial
8	tert-Butyl(5-isopropyl-2-methylphenoxy)dimethylsilane	17.329 min	$C_{16}H_{28}$	264.47	9.89		Antitumor, antiinflammatory
9	2-Methyl-7-phenylindole	17.376 min	$C_{15}H_{13}N$	207.27	10.08		Anticancer,antidiabetic
10	2-Methyl-7-phenylindole	17.480 min	$C_{15}H_{13}N$	207.27	10.19		**No activity**

Figure 1. Active compounds using acetone extract of *Hybanthus travancoricus*

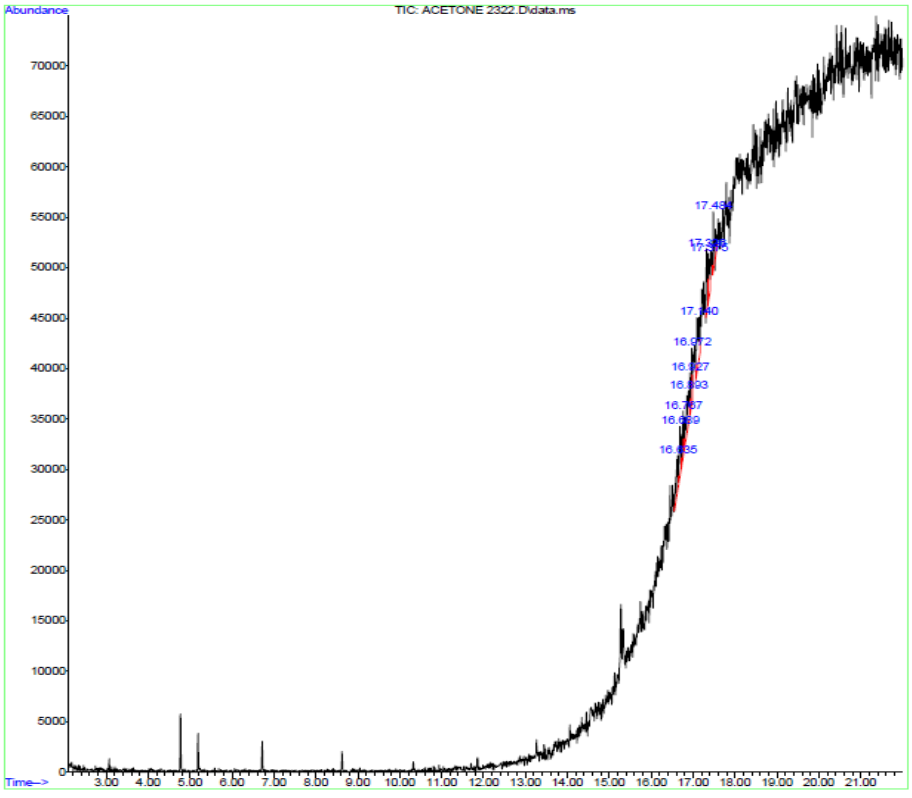


Table2. ***Hybanthus travancoricus*** **(Bedd.) Melch. using acetone extract**

S. No	Sample Code		Distance of Solvent	Distance of Solute	Band Colour		Rf Value
					UV	Visible	
1.	*Hybanthus travancoricus*	Spot -1	6.7	5.8	Blue	-	0.79
		Spot-2	6.7	5.5	Pink	Green	0.82
		Spot-3	6.7	6.1	Slight pink	-	0.91

Figure: 2 Retention factor values for the detection of Steroids

DISCUSSION

Plants produce a different mixture of secondary metabolites which commonly not essential for plant growth and reproduction but they play various roles in food and pharmaceutical industry (Balakumar et al., 2011; Mithraja et al., 2012a,b,c; Mary et al., 2012; Rajan et al., 2015). Liebler *et al*. (1996), Aysal *et al.* (2007) and Ibrahim and Abd-El-Aal (2008) discussed *Hybanthus enneaspermus* the herbs are having numerous bio active components which are identified by using GC or LC-MS. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used because of its simplicity, cost-effective and rapid tests for detecting phytocomponents.

Velayuthum *et al.* (2015) and Suman *et al*. (2016) interpreted the other compounds present in dried plant sample were rhodopin, carotene, milbemycin, lycoxanthin, astaxanthin etc. also recorded similar compounds through GC-MS except for stigmasterol. Analysis of the cell extract through GC-MS, showed the presence of desulphosinigrin and tetraacetyl-dxylonic nitrile (percentage peak area: 13.03) as major compounds. Desulphosinigrin possesses antiepileptic and anticancerous properties (Saravanan *et al.*, 2014).

CONCLUSION

The investigation concludes that acetone extract of *Hybanthus travancoricus* have active constituents responsible for many biological activities. Further, investigation needs to make this some as drug for many ailments.

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