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GC-MS PROFILING OF *HYBANTHUS ENNEASPERMUS* (L)F.MUELL FROM KANYAKUMARI DISTRICT OF TAMIL NADU, INDIA.

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ABSTRACT

Hybanthus enneaspermus (L)F.Muell is also known as spade flower, perennial herb belongs to the family Violaceae. In traditional medicines it is used for the treatment of diarrhea, urinary infections, leucorrhoea, dysuria, inflammation, cholera and sterility, general tonic for pregnant women, diuretic for gonorrhea. The plant has reported to have anti-bacterial, anti-arthritic, antidiabetic, anti-inflammatory, antitussive, antiplasmodial, anticonvulsant and free radical scavenging activity. The present work was done to profile the bioactive compounds of this medicinal plant collected from two different regions of Kanyakumari district Tamil Nadu. Sample A -Marthandam and sample B-Nagercoil. These areas are subjected to two different climatic regimes, soil profiles and Anthropogenic interactions. Sample A showed 14 peaks at retention time ranging from 13-21 minutes. Around 22 compounds were identified in the sample A whereas only 10 different compounds were identified in sample B at 5 peaks with the retention time varying from 16-20 minute were recorded. The medicinal properties of the compounds profiled are discussed.

Keywords : *Hybanthus enneaspermus,* GCMS, Bioactive compounds, Climatic regimes, Soil profiles, Anthropogenic interactions, Biological activities, Organic compounds, Microbicides, Fungicides, Herbicides, Pesticides

INTRODUCTION:

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavour, fertilizers. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, were herbal medicine has continuous history of long use. The development and recognition of medicinal and financial aids of these plants are on rise in both industrialized and developing nations (Dar *et al*.,2017). Medicinal plants are the ''backbone'' of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. Plants produce several chemical compounds that have biological functions, as well as provide defense against insects, fungi and anthophagous mammals. A minimum of 12,000 such compounds are isolated till now. Which is considered to be only accounting to 10% of

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the total availability (Patel *et al*.,2019).According to Fabricant and Farnsworth (2001) the goals of using plants as sources of therapeutic agents are 1,to isolate bioactive compounds for direct use as drugs; 2,to produce bioactive compounds of novel or known structures as lead compounds for semi synthesis to produce patentable entities of higher activity and or lower toxicity, to use agents as pharmacologic tools, to use the whole plant or part of it as a herbal remedy.

Hybanthus enneaspermus (L)F.Muell belongs to the family violaceae. It is a perennial herb or shrub distributed in the tropical and subtropical regions of the world in the warmer part of India (Sahoo *et al*., 2006). It is also distributed in Srilanka, tropical Asia, Africa, Australia. It is commonly found in riverbanks, open grass lands, sandy regions and waste lands. The genus consists of the world, often seen in mountainous region. It is also known as spade flower and pink ladies slipper (Patel *et al* .,2013).

This plant has been used for centuries in traditional medicine for the treatment of gonorrhoea, urinary infections, antitussive, vomiting, wandering of the mind, urethral discharges, blood disorders, asthma, anticonvulsant and for its tonic properties (Hemalatha *et al*.,2004). In addition to that, the drugs obtained from this plant are used for antiplasmodial, anti inflammatory, anti-inflammatory, anti-arthritic, anti-diabetic (Patel *et al.,*2011). The plant contains alkaloids, flavonoids, triterpenes, phenols, flavones, anthraquinone, L-dopa and diosgenin (Patel *et al*.,2013). According to (Shantha *et al*.,2001) among them, L- dopa has majorly used as a drug for the treatment of Parkinson's disease. The root infusion of this plant is used as a diuretic for gonorrhoea and urinary infections. The decoction of leaf and tender stalks are used as a demulcent. Leaves are traditional used as an external application for the treatment of wounds. Dried leaf powder is used to treat asthma. The fruit possess antivenom activity against snake and Scorpion sting. External application of the herb relieves ulcer and head ache. It increases libido and used to treat stress related disorder. (Shekhawat *et al*.,2014).

MATERIALS AND METHODS:

Two samples of *Hybanthus enneaspermus* chosen for the study were collected from Marthandam (sample A) and Nagercoil (sample B), Kanyakumari District, Tamil Nadu. The plant was identified taxonomically. Fresh plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water. Then it was shade-dried, powdered and used for extraction.

Fig:1.*Hybanthus enneaspermus* (L.) F.Muell

Sample A

Sample B

Preparation of aqueous leaf extracts:

The collected plant parts (10 g) have been washed and the adhering dirt's had been removed. Then it was cut into small pieces and shade dried. The powder of plant materials are macerated separately with 25 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatmann No.1 filter paper and heat sterilized at 120°C for 30 min. The extract was preserved aseptically in a brown bottle at 4°C until further use (Sukanya *et al*., 2009).

METHODOLOGY

Samples were prepared with respective solvent for GC-MS analysis. The analysis parameters are given below.

GCMS (Gas chromatography and Mass spectroscopic) (Kakimoto *et al* 2005):

GC-MS analysis was performed using the JEOL, GCMATE, GC-MS with data system is a high resolution double focusing instrument. Maximum resolution: 6000 Maximum calibrated masa: 1500 Dalton's equipped with an Elite-5MS (5%diphenyl/95% dimethyl poly siloxane) fused a capillary column (30×0.25µm ID ×0.25µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV . Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 M1/minute, and an injection volume of 2µ was employed (a split ratio of 10:1). The injector temperature was maintained at 250° C, the ion-source temperature was 200° C, the oven temperature was programmed from 110°(isothermal for 2minutes),with an increase of 10°C /minute to 200° C, then 5°C/minute to 280° C , ending with a 9 minutes isothermal at 280° C .Mass spectra were taken at 70ev; a scan interval of 0.5s and fragment from 45 to 450 Da. The solvent delay was 0 to 2minutes, and total GC/MS running time was time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The procedure of Kakimoto *et al,* 2005 was followed.

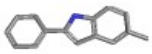
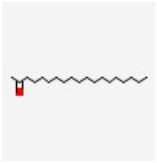
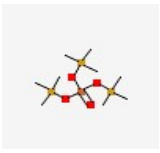
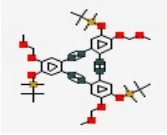
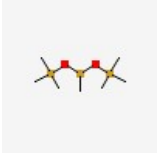
Identification of components was done by Interpretation of spectrum GCMS was conducting data base of National Institute Standard and Technology (NIST) and Wiley Spectra Libraries. The molecular weight, molecular formula and the number of hits was used to identify the name of the compound from NIST and Wiley Spectra Libraries.

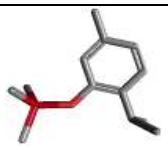
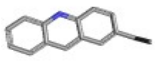
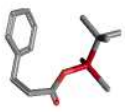
RESULT:

GCMS analysis of sample A showed 14 peaks at RT varying from 13 to 21 minutes. About 22 compounds were identified from these peaks.

Table 1a. GCMS Profile of *Hybanthus enneaspermus* (sample A)

Name of the compound	Retention Time	Molecular Structure	Molecular Formula	Biological activity
2-Isoxazoline, 3-phenyl-			C_9H_9NO	Antimicrobial activity (Kumar *et al*., 2014)

1-Octen-3-yne	13.528 min		C_8H_{12}	Antimicrobial activity (Gurusamy *et al*.,2021)
Methyl N-chloroacetylcarbamate	14.521 min		$C_4H_6ClN_3$	No biological activity
1H-Indole,5-methyl-2-phenyl			$C_{15}H_{13}N$	Antifungal activity (Mannaa and Kim 2018)
2-Nonadecanone			$C_{19}H_{38}O$	Antidiabetic activity (Tan *et al*.,2019)
1,2-Bis(trimethylsilyl)benzene	16.875 min		$C_{12}H_{22}Si_2$	Antioxidant Activity (Kavipriya and Chandran 2018)
Arsenous acid,tris(trimethyl)sil…			$C_9H_{273}Si_3$	No biological activity
Tris(tert-butyl dimethyl silyloxy)…	16.922min		$C_{18}H_{45}AsSi_3$	No biological activity
1,1,1,3,5,5,5Heptamethyltrisiloxane	17.102 min		$C_7H_{21}O_2Si_3$	Antimicrobial activity (Mala *et al*., 2020)
Trimethyl(4-(2-methyl-4-oxo-2-pe…			$C_{15}H_{24}Si$	Antibacterial, Anti-inflammatory activity

Silane,trimethyl(5-methyl-2-(1-…			$C_{13}H_{22}OSi$	(Manikandan *et al*., 2018) Coupling agent (Suman *et al*.,2013)
Silicic acid,diethylbis(trimethylsilyl) ester	17.168 min		$C_{10}H_{28}O_4S_3$	Antibacterial activity (Hema *et al*., 2011)
2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	17.206 min		$C_{13}H_{22}OSi_2$	Antioxidant activity (Devi *et al*., 2018)
4-(1,1-Dimethyl propyl phenoxy)silane	17.282 min		$C_{14}H_{24}OSi$	No biological activity
2-Ethylacridine Trimethyl (4-tert-butyl phenoxy)s	17.669 min	 	$C_{15}H_{13}N$ $C_{13}H_{22}OSi$	Antimicrobial and antitumor (Vijayakumari and Stephan Raj, 2018) Anti oxidant, Antimicrobial, anti inflammatory and anti convulsant activity (Manikandan *et al* 2018)
1,2-Benzisothiazol-3-amine tbdms Benzene propanoic acid,tert-buty…	17.802 min	 	$C_{13}H_{20}N_2Si$ $C_{15}H_{24}OSi$	Antioxidant Antimicrobial, Activity (Siham A.Salim.,2018) Antimicrobial and Antifungal Activity

				(Wikipedia 2021)
Methyltris(trimethylsiloxy)silane	17.924 min		$C_{10}H_{30}O_3S_4$	No biological activity
1,4-Bis(trimethylsily)Benzene	18.199min		$C_{12}H_{22}Si_2$	Anti tumor activity, Anti microbial, (Maheshwari and Vijyarengan 2020)
Tert-Butyl(5-isopropyl-2-methylp…	20.676 min		$C_{14}H_{22}O$	No biological activity
Tetrasiloxane,decamethyl…	21.726 min		$C_{10}H_{30}Si_4$	Antioxidant, Antimicrobial, Anti-inflammatory (Sushma *et al*., 2017)

At peak 1corresponding to RT 13.528 min two chemical compound were identified .2-Isoxazoline, 3-phenyl-, 1- octen-3-yne were observed (Table:1a). Isoxazoline, constituting an important family of five-membered heterocycles with one oxygen atom and one nitrogen atom at adjacent positions (Agrawal & Miahra,2018) 2-Isoxazoline,3-phenyl are versatile intermediates in organic synthesis can be found in numerous drug and natural products. A wide range of isoxazoles was used to fight against infectious diseases. The most commonly encountered products containing isoxazolins are some veterinary medicines used to prevent flea infestations in dogs. It has gained much importance in the field of medicinal chemistry as the anticancer agents. They are also reported to possess good antimicrobial, analgesic, anti-inflammatory activities (Alshamari *et al.,*2020).1- octen-3-yne also known as 1-octen-3-one, is the odorant that is responsible for the typical "metallic" smell of metals and blood coming into contact with skin It is used as flavoring agent, perfumery use, food and beverage. It is a natural product found in *Origanum hyperifolium, perilla frutescens* var. crispa (pub chem). Oct-1-en-3-one is the degradative reduction product of the chemical reaction of skin lipid peroxides . Around 3 chemical compounds were identified at RT 14.521 Methyl N-chloroacetylcarbamate, 1H-Indole, 5-methyl-2-phenyl-, 2-Nonadecacone at peak 2. Carbamates make structural and/or functional part of many drugs. (Matosevic and Bosak,2020). 5-Methylindole is used as an intermediate in the synthesis of compounds with a variety of pharmacological properties, such

as staurosporine-like bisindole inhibitors of protein kinases (Wikipedia,2020). It is used in the preparation of antifungal agents. It has the potential of antifungal agents.

At RT 16.875 min (peak 3) three compounds 1,4-Bis(trimethylsilyl) benzene, 1,2-Bis(trimethylsilyl)benzene, Arsenous acid, were identified (Table:1a). 1,4-Bis(trimethylsilyl) benzene (TMSB) can be used as a precursor for developing silicon carbide. Arsenous acid is the inorganic compound It is known to occur in aqueous solutions (Wikipedia,2021). The presence of Arsenous acid suggest that the soil in which the plant grows has some arsenic metal content, which would have been absorbed by the plant and converted to Arsenous acid. Tris(trimethylsil)silane is the organosilicon compound with the formula $(Me_3Si)_3SiH$. It is a colorless liquid that is classified as a hydrosilane since it contains an Si-H bond.

Around three compounds 1,2-Bis(trimethylsilyl)benzene, Arsenous acid,tris(trimethylsil…, Tris (tert- butyldimethylsilyloxy)… were identified at peak 4 at RT 16.922(Table:1a). Tris(tert-butoxy)silanethiol is a silicon compound containing three *tert*-butoxy groups and a rare Si–S–H functional group. This colourless compound serves as an hydrogen donor in radical chain reactions. (Wikipedia, 2021).RT 17.102 corresponding to peak 5 showed three compounds 1,1,1,3,5,5-Heptamethyltrisilo…, Trimethyl[4- (2-methyl-4-oxo-2-pe…, Silane, trimethyl[5-methyl-2-(1-…1,1,1,3,5,5,5-Heptamethyltrisiloxane is one of numerous organometallic compounds having application in , pharmaceuticals industry It has herbicides, insecticides, fungicides and plant growth regulators.. Trimethylsilanol (TMS) is an organosilicon compound and a colourless volatile liquid exhibiting antimicrobial properties as proved by Yun-mi Kim *et al*. (2006).

Silicic acid, diethyl bis(trimet…, Tris(tert-butyldimethylsilyloxy)…, tris(trimethylsil… were identified at RT 17.168 at peak 6 . Silicon protective role is as a biologically active element capable of triggering a broad range of natural defences. (Chérif *et al.*, 1994; Fawe *et al.*, 1998). RT 17.206 min showed compounds like 2,4,6-Cycloheptatrien-1-one,3,5…,Trimethyl [4-(2-methyl-4-oxo-2-pe, Arsenous acid, tris(trimethylsil… in peak 7 (Table:1a). Cycloheptatriene (CHT) is an organic compound . It is a closed ring of seven carbon atoms joined by three double bonds (as the name implies) and four single bonds. (Wikipedia,2020). It is a floral compound having the role of pheromones (The Pherobase, 2021). Peak 8 exhibited 3 chemical compounds 1,4-Bis(trimethylsily)benzene, 4-(1,1-Dimethylpropyl) phenol,tr…, 1,2-Bis(trimethylsilyl)benzene at RT 17.282 (Table:1a). 4- (1,1-Dimethylpropyl) phenol is a flammable, hardly ignitable, white solid with an aromatic odor, which is practically insoluble in water and is a floral compound(Wikipedia,2021:Pherobase, 2021). 2-Ethylacridine, Trimethyl(4- tert-butyl phenoxy)si…, Trimethyl[4-(2-methyl-4-oxo-2-pe… were identified at RT 17.669 in peak 9(Table:1a). Acridine is an organic compound and a nitrogen heterocycle Acridine and it derivatives are known to possess bacteriostatic activity against many gram positive and gram negative bacteria (Adolph *et al*., 2004) Acridine is an antimalarial drug.

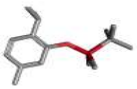
At RT 17.802 min corresponding to peak 10 - 1,2-Benzisothiazol-3-amine tbdms, Benznenepropanoic acid, tert-buty…, tris(trimethylsil…Benzisothiazol has a microbicide and a fungicide mode of action. It is widely used as a preservative as well (Wikipedia,2021). Phenylpropanoic acid or hydrocinnamic acid is a carboxylic acid with the formula $C_9H_{10}O_2$ belonging

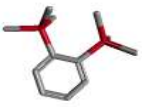
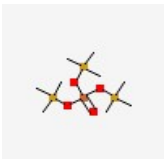
to the class of phenylpropanoids. Phenylpropanoic acid has a wide variety of uses including cosmetics, food additives, and pharmaceuticals (Hameed *et al.,* 2018). Around three compounds were identified at RT 17.924 Methyltris(trimethylsiloxy)silane, 2,4,6-Cycloheptatrien-1-one,3,5…, Trimethyl[4-(2-methyl -4-oxo-2-pe… at peak 11(Table:1a). Methyltrimethoxysilane is an organosilicon compound . It is a colorless, free-flowing liquid (Wikipedia 2020).This compound has been identified in the GCMS profile of *Ficus arnottiana* (Babu *et al* ., 2017). Cycloheptatriene (CHT) is an organic compound ..Trimethyl[4-(2-methyl -4-oxo-2-pe is an organic compound . It is an aromatic amine that is of commercial interest as a precursor to dyes (Wikipedia ,2021).

RT18.199 min showed compounds like 2,4,6-Cycloheptatrien-1-one,3,5…, 1,4-Bis (trimethylsilyl)benzene, Trimethyl (4- tert-butylphenoxy)si… in peak 12 (Table:1a). Bis(trimethylsilyl)benzene is the chemical compound .This compound is colourless, vile-smelling liquid is a useful aprotic source of "S^{2-}" in chemical synthesis. Trimethyl (4- tert-butylphenoxy)si which is widely used as an antioxidant in industrial applications. (Babu *et al*.,2017). Methyltris(trimethylsiloxy)silane, tert-Butyl95-isopropyl-2-methylp…, Arsenous acid, tris (trimethylsil… were identified at RT 20.676 in peak 13 (Table1a). tert-Butyl95-isopropyl-2-methylp is an organic chemical compound consisting of three isopropyl groups bound to a central nitrogen atom (Wikipedia 2020).Peak 14 exhibited 3 chemical compounds 1,4-Bis (trimethylsilyl)benzene, Tetrasiloxane, decamethyl-, Methyltris(trimethylsiloxy)silane at RT 21.726 (Table:1a). 1,4-Bis (trimethylsilyl)benzene is colourless, vile-smelling liquid is a useful aprotic source of "S^{2-}" in chemical synthesis. also called D_4,Tetrasiloxane is an organosilicon compound . It is a colorless viscous liquid. It is a common cyclomethicone. It is widely used in cosmetics(Wikipedia 2015).

GCMS profile of the sample B of *Hybanthus enneaspermus* showed 10 compounds distributed along 5 peaks (Table b)Benzene,2-[(tertbutyldimethyls…,1,2-Bensisothiazol-3-aminetms, Cyclotrisiloxane, hexamethyl were the new compounds seen in sample B compared to sample A.

Table 1b GCMS Profile of *Hybanthus enneaspermus* (Sample B

Name of the compound	**Retention Time**	**Molecular Structure**	**Molecular Formula**	**Biological activity**
Methyltris(trimethylsiloxy)silane			$C_{10}H_{30}O_3Si_4$	No biological activity
Tetrasiloxane,decamethyl	16.648 min		$C_{10}H_{30}Si_4$	Antioxidant, Antimicrobial, Anti-inflammatory (Sushma *et al*., 2017)
Benzene,2-[(tert-butyldimethyls…			$C_{16}H_{28}OSi$	No biological activity

1,2-Bis(trimethylsilyl)benzene	16.714 min		$C_{12}H_{22}Si_2$	AntioxidantActivity (Kavipriya and Chandran 2018)
1,2-Benzisothiazol-3-amine tbdms			$C_{13}H_{20}N_2SSi$	Antioxidant,Antimicrobial ,Activity(Siham A.Salim.,2018)
2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	16.970 min		$C_{13}H_{22}OSi_2$	Antioxidant activity (Devi *et al*., 2018)
1,4Bis(trimethylsilyl)benzene			$C_{12}H_{22}Si_2$	Anti tumor Activity, Anti microbial,Anti (Maheshwari and Vijyarengan 2020)
1,2-Bensisothiazol-3-amine tms	17.915 min		$C_{12}H_{22}Si_2$	No biological activity
Arsenous acid,tris(trimethylsil…			$C_9H_{273}Si_3$	No biological activity
Cyclotrisiloxane,hexamethyl-	20.903 min		$C_6H_{18}O3Si_3$	Antibacterial,Antioxidant activity (Momin K and Thomas S.K 2020)

Three phytocompounds viz: Methyl tris(trimethylsiloxy) silane, Tetrasiloxane, decamethyl-,Benzene,2-[(tert-butyldimethyls…were identified at RT 16.648corresponding to peak 1 (Table 1b).Methyltrimethoxysilane is an organosilicon compound . It is a colorless, free-flowing liquid. (Wikipedia,2020). At RT 16.714, peak 2 showed three chemical compounds 1,2-Bis (trimethylsilyl)benzene, Methyltris(trimethylsiloxy)silane, 1,2-Benzisothiazol-3-amine tbdms.) (Table :1b). 1,2-Benzisothiazol-3-amine tbdms is a organic compound, which is a white solid, it is structurally related to isothiazole. BIT is widely used as a preservative and has antimicrobial properties(Wikipedia,2021).

2,4,6-Cycloheptatrien-1-one,3,5…, 1,4-Bis(trimethylsilyl)benzene, 1,2-Bis(trimethylsilyl) benzene were identified RT 16.970 at peak 3.2,4,6-Cycloheptatrien-1-one,3,5…, is colourless liquid .It is ligand in organometallic chemistry and a building block in organic synthesis. Cycloheptatriene is not aromatic, as reflected by the nonplanarity of the methylene bridge (-CH_2-) with respect to the other atoms; however the related tropyliumcation.Bis(trimethylsilyl)Benzene is the chemical compound . Often abbreviated $(tms)_2S$, this colourless, vile-smelling liquid is a useful aprotic source of "S^{2-}" in chemical synthesis(Wikipedia,2020).

Peak 4 exhibited 3 chemical compound 1,2-Bis(trimethylsilyl)benzene, 1,2-Benzisothiazol-3-amine tdms, Arsenous acid, tris(trimethylsil…at RT 17.915 (Table"1b).1,2-Benzisothiazol-3-amine tdms. The anhydride form of arsenous acid, arsenic trioxide, is used as a herbicide, pesticide, and rodenticide(Wikipedia ,2021).

RT 20.903 showed compounds like Methyltris(trimethylsiloxy)silane, Arsenous acid, tris(trimethylsil…, Cyclotrisiloxane, hexamethyl- at peak 5 (Table:1b). Methyltris(trimethylsiloxy)silane On physical and chemical indexes: firstly, shall be indicated carbon atom distribution; secondly, shall be indicated average molecular weight., Used in cosmetics, should be test for harmful substances or furtherly test for microorganisms, according to local regulations and standards. Tris(trimethylsil…, Cyclotrisiloxane, hexamethyl- compound is used as a reagent to deliver hydrogen atoms. (Wikipedia,2021) It has antimicrobial, antioxidant and antibacterial properties.

SUMMARY AND CONCLUSIONS:

The GCMS analysis of this important medicinal plant has brought out the characterization of bioactive principles which can pave the way for in silver studies and dead to design drugs for combating emerging pandemics.

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