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Research Article

GC–MS ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF BIOACTIVE COMPONENTS OF *HYBANTHUS ENNEASPERMUS*

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ABSTRACT

Hybanthus enneaspermus extracts were used to investigate the bioactive compounds by using GC–MS analysis. It revealed the existence of more than 10 compounds such as D-mannitol, tetradecanediol, phytol, 2-piperdinone, cedarn-diol, 2-mono linoleo glycerol trimethyl silyl ether, and silane. In addition, we evaluated the anti-microbial activity of ethanolic and aqueous extracts of *H. enneaspermus* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella shigae*, *Salmonella typhi*, *Proteus vulgaris*, *Candida albicans,* and *Aspargillus niger* by using agar disc diffusion method. Ethanolic extracts of *H. enneaspermus* were more active against microbes than aqueous extracts.

Keywords: *Hybanthus enneaspermus*, GC–MS, Anti-microbial activity, Agar-disc diffusion Method.

INDRODUCTION

To overcome health problems, the tribes of developing countries primarily rely on herbal medicines, which are giving beneficial effect to humans[1]. The herbs are constantly being screened for their biological and pharmacological activities such as anti-diabetic, anti-oxidant, anti-microbial, laxative, and anti-cancer activities[2]. The tribal communities of many countries are still using medicinal plants to cure sickness [3-6]. *Hybanthus enneaspermus* (Linn) F. Mull is a violaceae family known as Sthalakamala in ayurveda which is distributed in the tropical and sub tropical regions in the world. It is a woody troches herb present in warmer parts of India. It grows 15–30 cm in height with ascending nature[7]. The plant possesses anti-convulsant , and also used to treat diarrhea, dysuria, urinary tract infections, and male sterility[8,9]. In some part of India, the plant is used to treat diabetes and is also having anti-oxidant property and free radical scavenging activity[10]. Bio-active components of plants generally like phenol, saponins, alkaloids, amino acids, and flavanoids, which are possessing biological activities including anti-cancer, anti-fungal, and anti-inflammatory activities[11,12].

Plant Material and Preparation of Extract

Whole plants of *H. enneaspermus* were collected in the month of November and December from PRIST University Campus, Thanjavur, Tamil Nadu, India. The collected plants were identified and authenticated by a Botanist Prof. Dr. K. Singaravadivel, Department of Microbiology, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India. The collected plants were open-air-dried under the shade, pulverized in to a moderately coarse powder (using pestle and mortar). Three-hundred grams of the powered plants were extracted with ethanol (70%) using soxhlet apparatus for 48 h. A semi-solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The extract contains both polar and non-polar phytocomponents.

Gas chromatograph - Mass spectrometer (GC–MS) analysis

GC–MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and GC–MS instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1 µM df, composed of 100% dimethyl poly diloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature at 250°C; ion-source temperature at 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 36 min.

Phytochemical analysis

To find out the anti-microbial activity of *H. enneaspermus*, the total phenol and flavonoid contents were determined. The total phenol content of *H. enneaspermus* was determined using Folin-Ciocalteu method where as total flavonoid content was determined by using method of Kumaran and Karunakaran[13-16].

Preparation of test microorganisms

The pathogenic microbial strains namely *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 2065), *Pseudomonas aeruginosa* (NCIM 2036), *Shigella shigae* (NCIM 2024), *Salmonella typhi* (NCIM 2023), *Salmonella para typhi* (NCIM 2022), *Proteus vulgaris* (NCIM 2027), *Candida albicans* (NCIM 3102), and *Aspergillus niger* (NCIM 105) were obtained from National Chemical Laboratory (NCL), Pune and maintained by periodical sub-culturing on nutrient agar and sabouraud dextrose agar medium.

Evaluation of anti-microbial activity

The anti-microbial assay of different solvent extract was performed by agar disc diffusion method[17,18]. By inoculating a loopful culture in the nutrient broth (30 ml), the bacterial strain was activated where as the fungal strain was incubated for 6 h to maintain McFarland standard turbidity (108 cells/ml). A 0.1 ml of inoculums was inoculated into the molten Muller Hinton agar media (Hi-Media) and PDA media, spread uniformly into the Petri plate. The test compound (40 µl) was introduced on the disc (6 mm) (Hi-Media) and allowed to dry. Then the disc was impregnated on the seeded agar plate. Dimethyl sulfoxide (DMSO) was used as a negative control where as ciprofloxacin and nystadin were used as a positive control. The plates were done in triplicates and incubated for 24 h at 37°C for anti-bacterial activity where as the plates were incubated for 48–72 h at 28°C for anti-fungal activity.

RESULTS AND DISCUSSION

The ethanolic extract of *H. ennespearmus* was subjected to GC–MS analysis. Interpretation on mass spectrum GC–MS was conducted by using the database of National Institute Standard and Technology (NIST) which is having more than 62,000 patterns. The name, molecular weight, and structure of the components of the test materials were ascertained. GC–MS results shown that at least 13 compounds were present in ethanolic extraction of *H. ennespermus* (see Fig. 1).

The compounds of *H. ennespermus* was identified through mass spectrometry attached with gas chromatography. The unknown spectrum components were compared with the known spectrum components, which are stored in the NIST library and the data are given in Table 1.

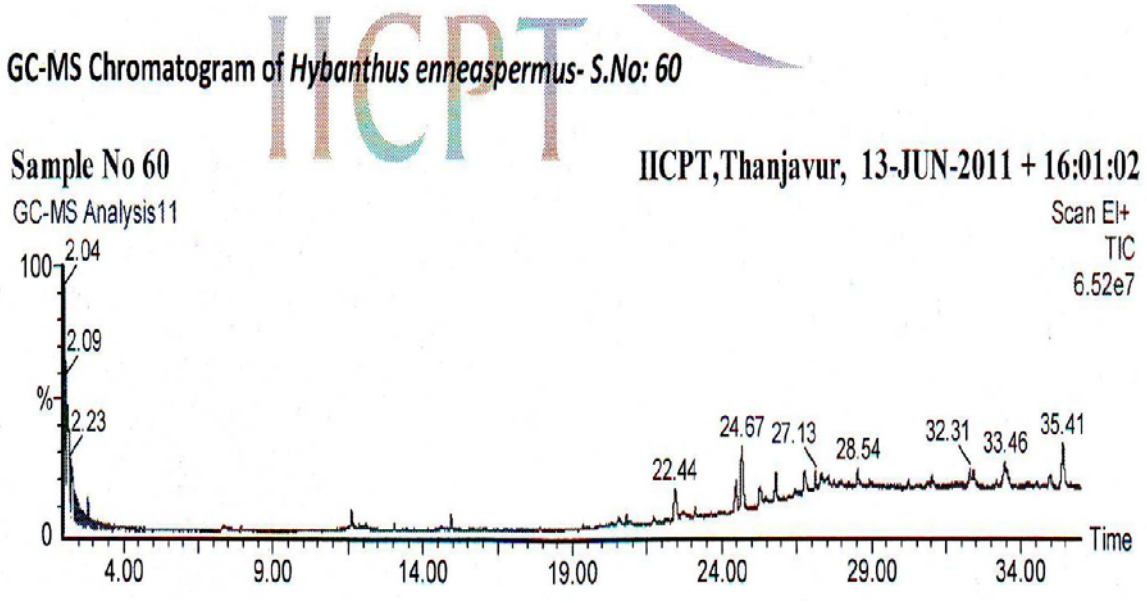


Fig. 1: GC-MS Chromatogram of *hybanthus enneaspermus*

Table 1: Phyto-components of the ethanolic extracts of *Hybanthus enneaspermus* by GC–MS

S. No.	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	2.81	Propane,1,1,3-triethoxy-	$C_9H_{20}O_3$	176	6.11
2	7.94	Phenol,4,6-di(1,1-dimethyl)-2-methyl-	$C_{15}H_{24}O$	220	0.64
3	11.62	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	5.79
4	12.1	7-Octen-3-ol, 2,6-dimethyl-	$C_{10}H_{20}O$	156	2.25
5	13.06	1,3-Dioxolane-2heptanenitrile, à-methyl-ë-oxo-2-pheny-	$C_{17}H_{21}NO_3$	287	0.8
6	14.94	Phytol	$C_{20}H_{40}O$	296	2.41
7	21.73	2-Pepridionne, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233	2.41
8	22.44	Cedran-diol, 8S, 14-	$C_{15}H_{26}O_2$	238	13.02
9	23.12	d-Mannitol, 1-decylsulfonyl-	$C_{16}H_{34}O_7S$	370	2.41
10	24.67	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	20.9
11	25.26	1-Monolonoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	498	8.36
12	33.46	Silane, 1,4-phenylenebis [trimethyl-	$C_{12}H_{22}O_4Si_2$	222	16.56
13	35.41	2,2-Dimethyl-6-methyl-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	$C_{14}H_{24}O_4$	256	18.33

Table 2: Qualitative phytochemical analysis of *Hybanthus enneaspermus*

Phytochemicals	Ethanolic extracts	Aqueous extracts
Alkaloids	+	+
Steroids	+	+
Phenols	+	+
Tannins	+	+
Flavones	+	+
Anthroquinones	+	+
Amino acids	+	+

The qualitative phytochemical analysis of *H. ennespermus* was performed by using Folin-Ciocalteu method and Kumaran, and the results are shown in Table 2.

H. ennespermus is used in treat the various diseases including diabetes. It contains majorly phenol and flavonoid, which are the main factors that inhibit the microbial activity[10,19]. Previously reported that the presence of alkaloids, flavanoids, tannins, cardio glycosides, saponins, and terpenoids like compounds in *H. enneaspermus* play a major role to cure common sickness[20]. Hence, the present study attempted to evaluate the anti-microbial activity of ethanolic extract of *H. enneapearmus* using disc diffusion method. This method is widely used to investigate the anti-microbial activity of plant substances. Ethanolic extract was shown more anti-microbial action than aqueous extract (see Fig. 2).

The test microorganisms of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. shigae*, *S. typhi*, *S. para typhi*, *P. vulgaris*, *C. albicans*, and *A. niger* were obtained from NCL and given in Table 3.

The effect produced by the sample was compared with the effect produced by the positive control. In this study, we were used ciprofloxacin 5 µg/disc for bacteria and nystatin 100 units/disc for fungi as standard control. The results showed most potent anti-microbial activity against *B. subtilis* and *P. aeruginosa* than *E. coli* and *S. aureus* (see Figs. 2 and 3).

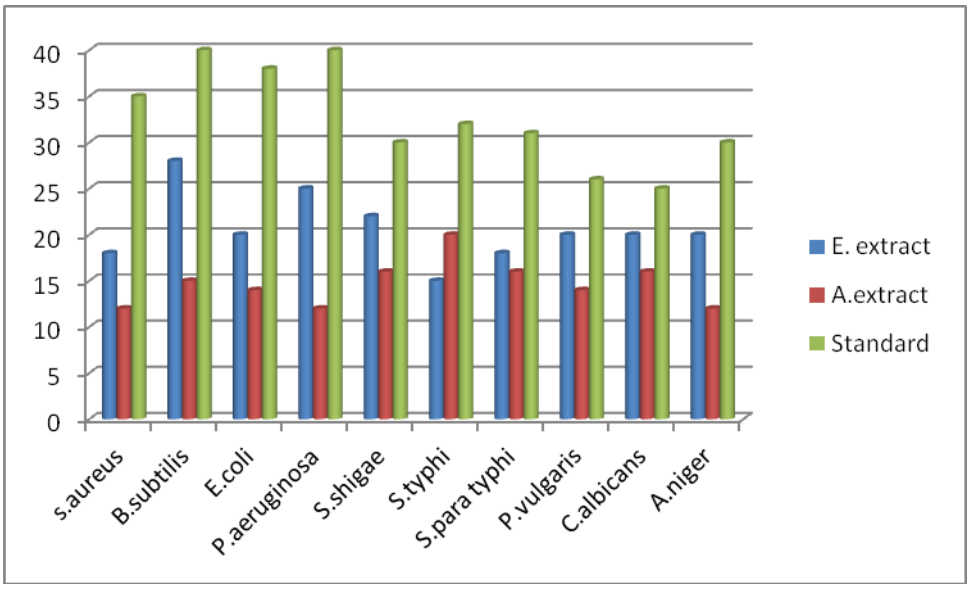


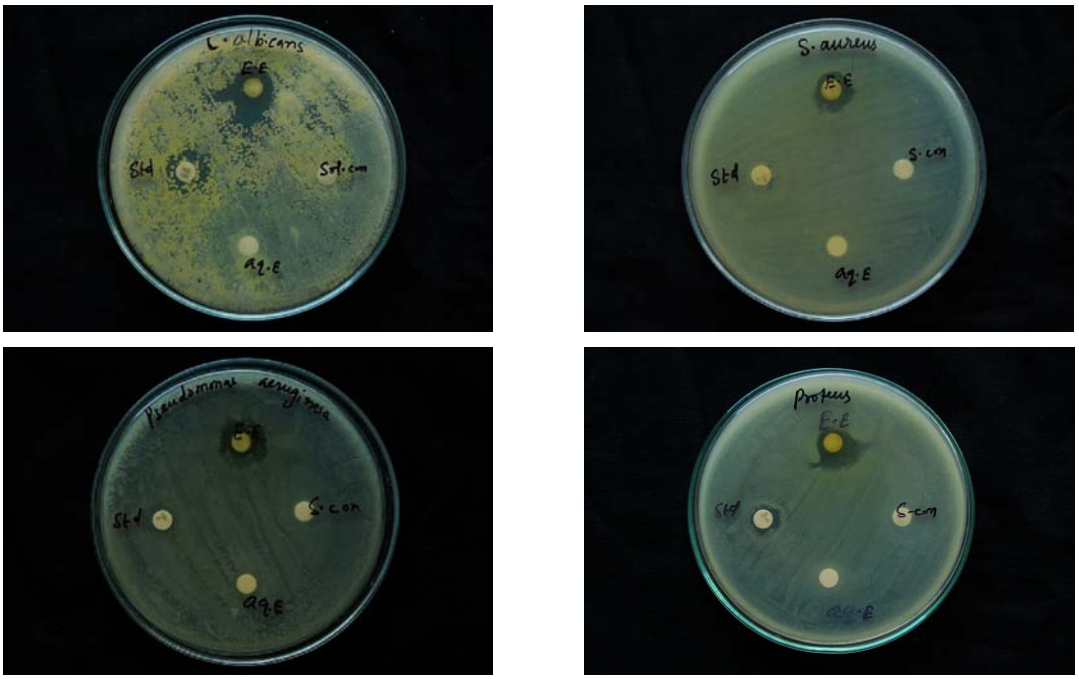
Fig. 2: Comparison of zone inhibition of ethanolic and aqueous extracts of *Hybanthus enneaspermus* with standard drugs against selected microbes

Table 3: Anti-microbial activity of ethanolic and aqueous extracts of *Hybanthus enneaspermus*

S. No.	Name of the microorganisms	Zone of inhibition (in mm)			
		Ethanolic extracts	Aqueous extracts	S.C	Standard
1	*Staphylococcus aureus* (NCIM 2079)	18	12	_	35
2	*Bacillus subtilis* (NCIM 2063)	28	15	_	40
3	*Escherichia coli* (NCIM 2065)	20	14	_	38
4	*Pseudomonas aeruginosa* (NCIM 2036)	25	12	_	40
5	*Shigella shigae* (NCIM 2024)	22	16	_	30
6	*Salmonella typhi* (NCIM 2023)	15	20	_	32
7	*Salmonella para typhi* (NCIM 2022)	18	16	_	31
8	*Proteus vulgaris* (NCIM 2027)	20	14	_	26
9.	*Candida albicans* (NCIM 3102)	20	16	_	25
10	*Aspergillus niger* (NCIM 105)	20	12	_	30

Standard– Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 units/disc for fungi.

S.C - Solvent control (solvent used DMSO)



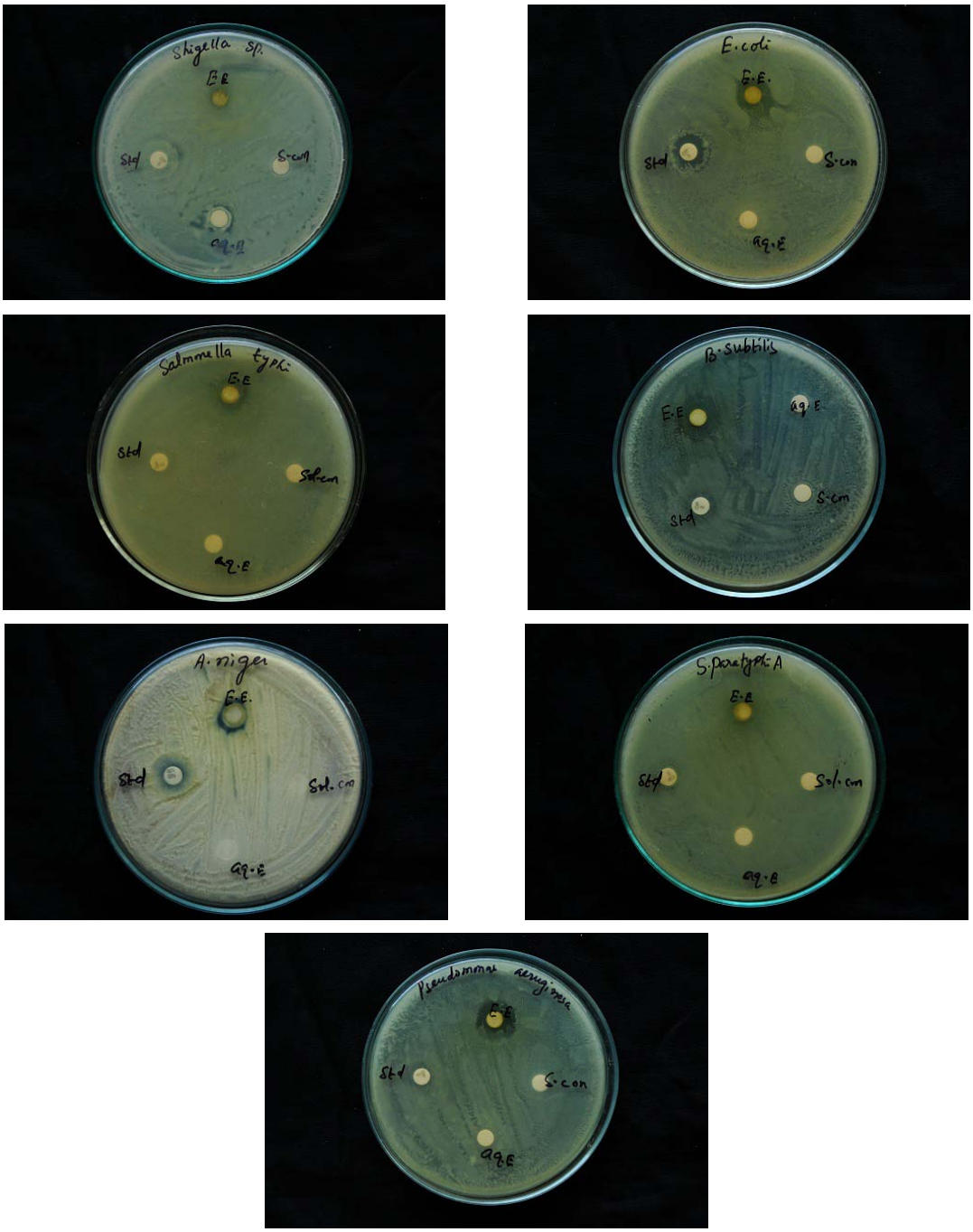


Fig. 3: Comparison of antimicrobial activity of aqueous and ethanolic extracts of *Hybanthus enneaspermus*

Aqueous extract also were shown moderate anti-bacterial and anti-fungal activities. The extract of this plant contains alkaloids and flavonoids, which are having biological activities. These alkaloids and flavonoids may be responsible for anti-microbial activity.

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