

# Phytochemical Analysis of *Hybanthus enneaspermus* using UV, FTIR and GC- MS

T. Anand<sup>1\*</sup>, K.Gokulakrishnan<sup>2</sup>

<sup>1</sup>Department of Biochemistry PRIST University, Vallam, Thanjavur 613 403, Tamil Nadu, India

<sup>2</sup>Department of chemistry PRIST University, Vallam, Thanjavur 613 403, Tamil Nadu, India

## ABSTRACT

The present study was carried out to characterize the bioactive constituents present in ethanolic extracts of *Hybanthus enneaspermus* using UV, FTIR and GC-MS. The crude extracts were scanned in the wavelength ranging from 300-1100 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. For GC-MS analysis, 20 g sample is extracted with 50 ml ethanol, filtered in ash less filter paper with 4 g sodium sulphate and the extract is concentrated to 1 ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver. 2.0 - Year 2005 library. The biological activities are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The UV profile showed different peaks ranging from 300-1100 nm with different absorption respectively. The FTIR spectrum confirmed the presence of phenols, alcohols, alkanes, alkyl halides, carboxylic acids, aromatics, nitro compounds and amines in ethanolic extract. The results of the GC-MS analysis provide different peaks determining the presence of phytochemical compounds with different therapeutic activities. The major phytoconstituents were (5E,13E)-5,13-Docosadienoic acid (20.90%) and Cedran-diol, 8S, 14- (13.02) which are possessing many biological activities. hence this study creates a platform to screen many bioactive components to treat many diseases.

**Keywords:** FTIR , GC-MS, *Hybanthus enneaspermus* , Phytochemical, UV-spectrometry

## 1. INTRODUCTION

In order 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 [2-7]. The herbs are having numerous bio active components which are identified ( at less than 1 ng ) 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.[8-10] *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 [11]. The plant possesses anti-convulsant [12,13], and also used to treat diarrhea, dysuria, urinary tract infections , male sterility and diabetes because which possess many bioactive components such as phenol,alkaloids and flavanoids[14,15]. In some part of India the plant is used to treat diabetes and which is also having anti-oxidant property and free radical scavenging activity [16].

## 2. Materials and Methods

### 2.1 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 open-air-dried under the shade, pulverized in to a moderately coarse powder (using pestle and mortar). Three-hundred grams (300 g) 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.

### 2.2 UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 300-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

### 2.3 GC–MS analysis

GC–MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC–MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1  $\mu$ M 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  $\mu$ l was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 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.

### 2.4 Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name and molecular weight of the components of the test materials were ascertained.

## 3. RESULTS AND DISCUSSION

The qualitative UV spectrum profile of *Hybanthus enneaspermus* L. ethanolic extract was selected at wavelength from 300 to 1100 nm due to sharpness of the peaks and proper baseline. The UV profile of ethanol extract of *Hybanthus enneaspermus* chosen wavelength of 300 to 400 nm and the profile showed the peaks at 338 and 365 respectively and another chosen wavelength of 900 to 1100 showed the peaks at 922 and 1042.(Fig.1).

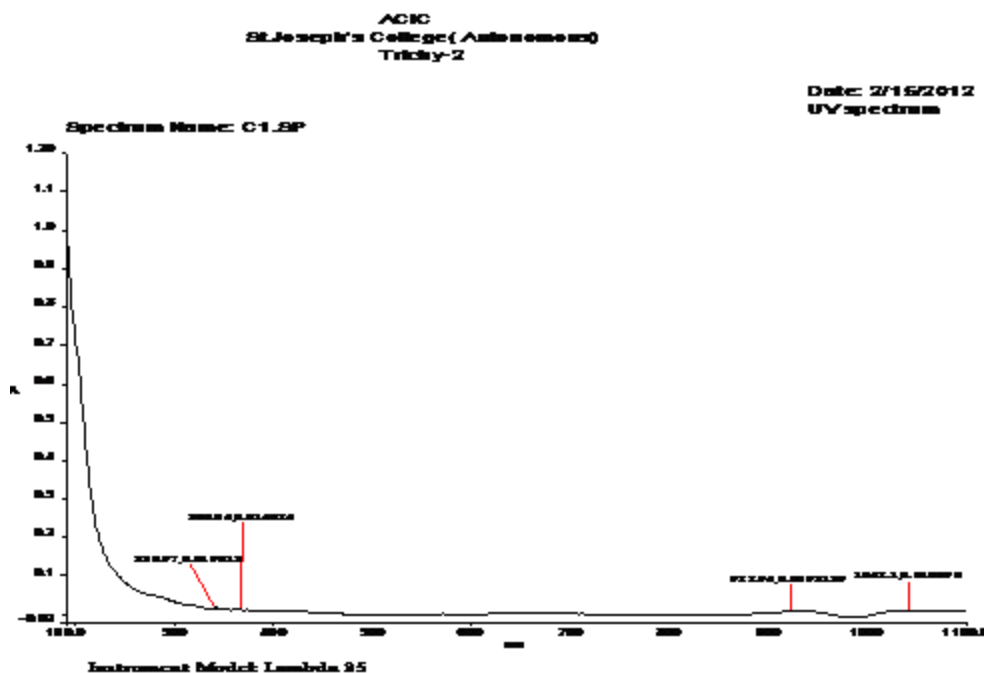


Figure 1: UV spectrum of ethanolic extract of *Hybanthus enneaspermus*

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 1. The FTIR spectrum profile was illustrated in the Fig. 2. FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in ethanol extract. Hence, the

crude extracts subjected to UV and FTIR analysis is used for the identification of chemical constituents present in *Hybanthus enneaspermus*. In addition, UV and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition.

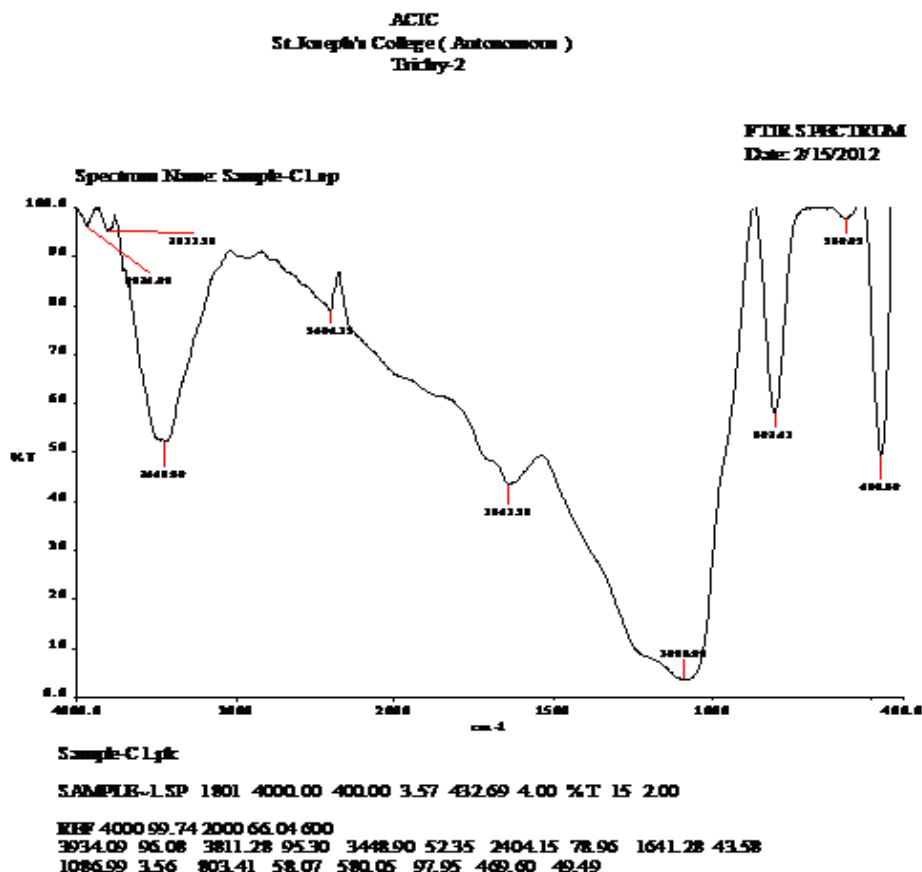


Figure2: FTIR spectrum of *Hybanthus enneaspermus*

**Table 1:** FTIR peak values and functional groups of different extracts of *Hybanthus enneaspermus*.

Peak values	Functional groups
3934	Unknown
3811	Unknown
3448	Alcohol (including phenol)
2404	Carboxylic acid
1641	Non-acid carbonyl
1086	Alcohol
803	Aromatic
580	Alkyl halides
469	Alkyl halides

The ethanolic extract of *H. enneaspermus* was subjected to GC-MS analysis. Interpretation on mass spectrum GC-MS was conducted 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 10 compounds were present in ethanolic extraction of *H. enneaspermus* (Fig. 3 and Table 2).

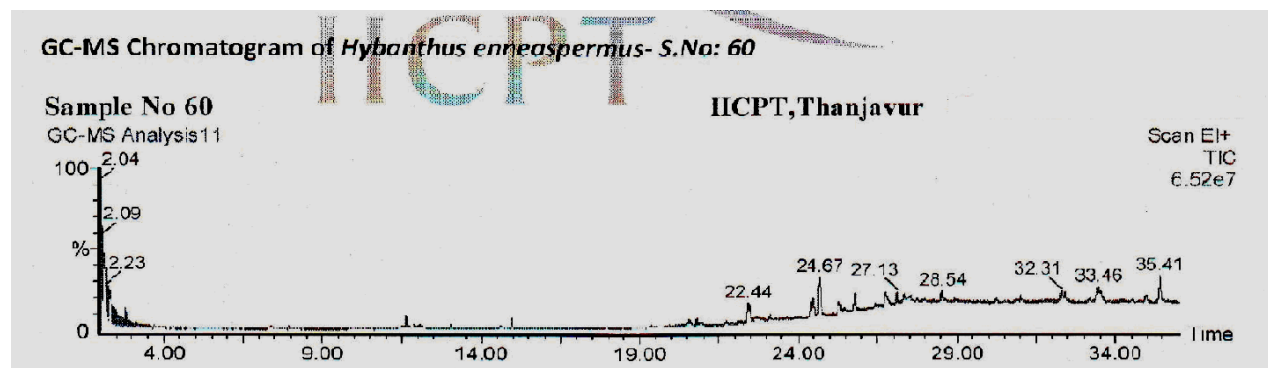


Figure 3: GC-MS of *Hybanthus enneaspermus*

The compounds of *H. enneaspermus* 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 is given Table 2. The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *Hybanthus enneaspermus*.

Table 2: phytochemical analysis of ethanolic extract of *Hybanthus enneaspermus*.

RT	Name of the compound	Molecular formula	MW	Peak area%	Compound nature	Biological activity
2.81	Propane,1,1,3-triethoxy-	C <sub>9</sub> H <sub>20</sub> O <sub>3</sub>	176	6.11	Ether compound	No activity
7.94	Phenol,4,6-di(1,1-dimethyl)-2-methyl-	C <sub>15</sub> H <sub>24</sub> O	220	0.64	compounds are soluble and chiral nature	inhibit bacterial, fungal, protozoan and parasite growth
11.62	1,14-Tetradecanediol	C <sub>14</sub> H <sub>30</sub> O <sub>2</sub>	230	5.76	Alcoholic compound	Antimicrobial
14.94	Phytol	C <sub>20</sub> H <sub>40</sub> O	296	2.41	Diterpene	Antimicrobial, Anti cancer, Anti-inflammatory, Hepatoprotective, Anti – androgenic
21.73	2-Pepridionne, N-[4-bromo-n-butyl]-	C <sub>9</sub> H <sub>16</sub> BrNO	233	2.41	Alkaloid	Antimicrobial Anti-inflammatory Antioxidant
22.44	Cedran-diol, 8S, 14-	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	238	13.02	Sesquiterpene alcohol	Antimicrobial Antiinflammatory
24.67	2H-Pyran, 2-(7-heptadecyloxy)tetrahydro-	C <sub>22</sub> H <sub>40</sub> O <sub>2</sub>	336	20.90	Flavonoid fraction	Antimicrobial Antiinflammatory Antioxidant

#### 4. CONCLUSION

The investigation concluded that the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of

traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

## 5. ACKNOWLEDGEMENT

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