WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 4, 1361-1372. Research Article ISSN 2277– 7105

PHYTO-CHEMICAL SCREENING, FT-IR AND GC-MS ANALYSIS OF *HYBANTHUS STELLARIOIDES* (DOMIN) P. I. FORSTER (VIOLACEAE)

Lingaraj Kulkarni*[1], Pradeep Bhat[2] and Ganesh Hegde (Rtd)[3]

*[1]KLES, G. H. Arts, Sci, & Com. College, Haveri, Karnataka, India.

[2]Indian Council of Medical Research, Belgum, Karnataka, India.

[3]P.G. Department of Studies in Botany, Karnatak University, Dharwad, Karnataka, India.

Article Received on
09 Feb. 2022,

Revised on 29 Feb. 2022,
Accepted on 19 March 2022

DOI: 10.20959/wjpr20224-23569

***Corresponding Author**
Lingaraj Kulkarni
Asst. Prof. of Botany, Dept. of Botany, KLES, G. H. Arts, Sci, & Com. College, Haveri-581110, Karnataka, India.

ABSTRACT

The purpose of the current study is to monitor the phytochemical constituents in ***H. stellarioides*** plant extract. Phytochemical screening of the sequential extracts were used for analysis which showed the presence of bioactive compounds like alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and carbohydrates. Ultra violet visible spectroscopy was used to recognize quantitative determination of different analytes by using wavelength and absorbance values. FT- IR analysis was used to identify the functional groups of the compounds and confirmed the presence of –OH, C-H, C=C (Alkene) and C-O (Ether) in methanol extract. GC-MS analysis for methanolic plant extract was done. The compound 3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5- dihydroxy; 3-trifluoroacetoxydodecane; cyclopenta (c) furo (3'-2'-h) (1) benzop; 3-hexadacycloxylocarbonyl-5-(2-hydroxy); 1,3-butanediol, diacetate; cholest-7-en-3 beta, 5 alpha- diol-6 alpha benz and 2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl-. are possessed analgesic, anti-inflammatory and antipyretic activities, antimicrobial and antioxidant activities. Natural Chemical compounds of the medicinal plants have provided numerous clinically useful drugs in the treatment of chronic or acute ailments and still remain as an essential component in the search for new medicines.

KEYWORDS: *Hybanthus stellarioides*, phytochemical screening, UV-Vis-spectroscopy, FT-IR, GC-MS analysis.

INTRODUCTION

The scientific studies of medicinal plants are carried out all over India since Vedic time 3000BC to 1000 BC.[1] India is the largest producer of medicinal herbs hence it called the botanical garden of the world. Recently medicinal plants are receiving great attention throughout the globe for its wide scope of medicinal application and their utilization. They play important role on the health of individuals and communities. Within these plants the medicinal value lies in the form of some chemical substances that play a define role on physiological action on the human body. The medicinal plants are excellent chemical factories. They are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances. Certainly more efficient than the modern laboratories in many respect. Without plants no one can survive on this earth and they are the part of life. Many people believe in Ayurveda or Unani because they do not show any side effects compared to allopathic medicines. The medicinal plants are used in current medicine throughout the biosphere to prevent or to cure diseases, but scientific information in terms of modern medicine is lacking in most of the cases. About 80% of the world population use traditional medicines, which are predominantly based on plant materials (WHO; 1993)*. However, nowadays it is necessary to deliver scientific proof, whether to justify the use of plant or its dynamic principles. Indian traditional medicine is one of the richest medicinal organizations between those obtainable around the world.

The medicinal plants are regularly being observed for their biological and pharmacological activities such as anti-diabetic, anti-oxidant, anti-microbial, laxative, and anti- cancer activities[2], The medicinal plants are having numerous bio active components which are identified (at less than 1ng) 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.

Phytochemical screening were carried out for the analysis of presence of bioactive compounds, FT-IR is one of the widely used methods to identify the chemical constituents (Liu et al., 2006)[3] and GC-MS analysis is a breakthrough in analysis of phytoconstituents and structure elucidation of the compounds as they have a sensitivity of detecting compounds in the plant (Liebler et al., 1996).[4]

***Hybanthus stellarioides* (Domin) P.I.Forster.** (Violaceae). A small erect, seasonal unbranched or rarely branched herb, growing to a height up to 30cm on dry hills of rocky

crevices, covered with spreading hairs, flower solitary, curved peduncle and drooping orange.[6,7,8] ***H. stellarioides*** is a sub species of *H. enneaspermus.*[5]

Hybanthus enneaspermus (Linn) F. Mull is also known as Sthalakamala in ayurveda which is distributed in the tropical and sub tropical regions in the world. The plant possesses anti-convulsant[9] 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 flavonoids.[10] 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.[11]

H. stellarioides possessing many similar biological activities of *H.enneaspermus.*[12] Hence this study creates a platform to screen many bioactive components to treat many diseases.

MATERIALS AND METHODS

H. stellarioides plant was collected from the crevices of sand stones on the rocky flat hill tops (near Navilatirtha Dam) of Saudatti in Belgaum District, Karnataka State and taxonomically authenticated. The voucher specimen is deposited in the museum at Department of Botany, Karnatak University, Dharwad (LCK/GRH-13, 2015).

The collected plant material was cleaned, open air shade dried and coarsely powdered. Hot percolation method was used for extraction by using Soxhlet apparatus, sequentially in petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water. All the extracts were dried at 40^{o}C and stored refrigerator for further use. The extracts preliminary phytochemical screening was done.[13] The extract taken for FTIR and GC-MS analysis to observe the presence of bioactive compounds.

Extraction and phytochemical screening

Plant samples (Whole plant) were cleaned; shade dried and powdered using a mixer blender to make fine powder hot percolation method was used for extraction using Soxhlet apparatus. A known amount of powdered material (50g) was refluxed sequentially in petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water (250ml each), in the order of increasing dielectric constant of the solvents, for 6-8 hours. All the extracts were dried and stored at 4^{o} C for further use. The extracts were subjected to preliminary phytochemical screening as per standard protocols (Horborne, 2007[13], Kokate, 1994[14], and Khandelwal, 2007.[15]).

Calculation of % Extractive value

Five gram coarse powder of the plant was refluxed in soxhlet apparatus with petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water (100ml each) sequentially. Each extract was evaporated to dryness in a China dish and weight was noted. The percentage extractive value with reference to the air-dried drug was calculated using the formula:

$$\% \text{ Extractive value} = \frac{Weight\ of\ the\ extract\ obtained}{Weight\ of\ powdered\ sample} \times 100$$

Fluorescence analysis

The plant extracts and the plant powder treated with different chemical reagents were observed under visible light as well as UV light (254 nm and 366 nm), as per the methods of Chase and Prett (1949)[16] for the Fluorescence analysis.

FTIR Spectroscopic analysis

The FTIR analysis was achieved using Perkin Elmer Spectrophotometer system, which was used to notice the typical peaks and their functional groups. FT-IR (Fourier Transform Infrared spectrophometry) is conceivably the most controlling tools for recognizing the kinds of chemical bonds (functional groups) present in compounds. The methanol extract was used for FTIR investigation (20).[3]

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 extract sample is diluted to 1:10 with the corresponding solvent and the extract was scanned in the wavelength ranging from 300-1100 nm using Perkin Elmer Spectrophotometer to detect the characteristic peaks and their functional groups. The peak values were recorded and the analysis was repeated twice for the spectrum confirmation.

GC-MS analysis

The GC-MS plays major role in the analysis of unidentified constituents of plant origin. The crude methanol extract containing different compounds of ***H. stellarioides*** was subjected for (GC-MS) analysis.

GC-MS analysis of the extract, fractions and sub fractions were performed using a Shimadzu Japan (QP20105 system), equipped with a Rtx-5MS, fused silica capillary column of 5% diphenyl and 95% dimethylpolysiloxane (30m × 0.25mm) ID × 1µm. Turbo mass 5.2.

Software was adapted to handle mass spectra and chromatograms. For GC-MS detection, an electron ionization system with ionizing energy of 70eV was used. The extract was dissolved in HPLC grade methanol and 2µl was injected for the determination. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 1µl was employed (split ratio of 10:1). The injector temperature and ion source temperature was set at 250°C and 280°C respectively. The oven temperature was programmed from 110°C, with an increase in 20°C/min up to 280°C. Mass spectra were taken at 70 eV with a scan interval of 0.5 seconds and fragments from 40 to 500 Da, and total GC running time was 36 min. Interpretation on mass spectrum was conducted using the database of National Institute of Standard Technology (NIST-05) library having more than 62,000 patterns. The amount of each component and its relative % was calculated by comparing its average peak area to the total areas. The spectrum of unknown component compared with the spectrum of the known components stored in the NIST library. The molecular weight and name of the components of the test materials were ascertained.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of successive extracts revealed the presence of different phytoconstituents **(Table-1).** Petroleum ether extract showed positive result only for steroids whereas, benzene extract showed presence of steroids and alkaloids. Chloroform extract was devoid of all the phytochemicals tested and acetone extract showed positive results for carbohydrates, tannins and phenolic compounds. Both methanol and ethanol extracts showed the presence of all the tested phytochemical constituents except proteins, amino acids and triterpenoids. However, water extract tested positive for carbohydrates, glycosides, saponin glycosides, tannins and phenolic compounds.

Table 1: Phytochemical investigation of extracts of *H. stellarioides* plant.

Sl. No.	Phyto- chemicals	Extracts						
		Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Ethanol	Aqueous
1	Carbohydrates	-	-	-	+	+	+	+
2	Proteins	-	-	-	-	-	-	-
3	Amino acids	-	-	-	-	-	-	-
4	Steroids	+	+	-	-	+	+	-
5	Triterpenoi ds	-	-	-	-	-	-	-
6	Glycosides	-	-	-	-	+	+	+
7	Saponin glycosides	-	-	-	-	+	+	+

8	Flavonoids	-	-	-	-	+	+	-
9	Tannins & Phenolic compounds	-	-	-	+	+	+	+
10	Alkaloids	-	+	-	-	+	+	-

(+) = Present, (-) = Absent)

(Two types of metabolites were present in plant cells. They are primary and secondary metabolites. Growth and metabolism of plants were directly linked with primary metabolites (carbohydrates). Secondary metabolites were considered as products of primary metabolites and are usually not involved in metabolic activity (Phenol, alkaloids, terpenoids, sterols, flavonoid, lignins and tannins etc,). The major uses of secondary metabolites are food seasoning, perfumes, pharmaceuticals, and pesticides10. Reducing sugars and alkaloids, terpenoids and flavonoids in ***H. stellarioides*** **plant** has anti-diuretic, anti-cancer, anti-viral, antianalgesic, antimalarial and antibacterial activities, due to the occurrence of secondary metabolites 11. The well-known alkaloids have antimicrobial 12 and antidiabetic 13 activities. Steroidal alkaloids are used as medicine.)

UV-Analysis

The ***H. stellarioides*** plant powder and the extract was observed under visible light as well as UV light (254 nm and 366 nm) and compared with the colour under visible light. It showed the variation in colour.

FTIR spectroscopic analysis

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 **(Fig. 1).** The results of FTIR peak values and functional groups were represented in **Table 2**. FTIR spectrum confirmed the presence of -OH C-H, C=C (Alkene) and C-O (Ether) in methanol extract of ***H. stellarioides*** **plant.**

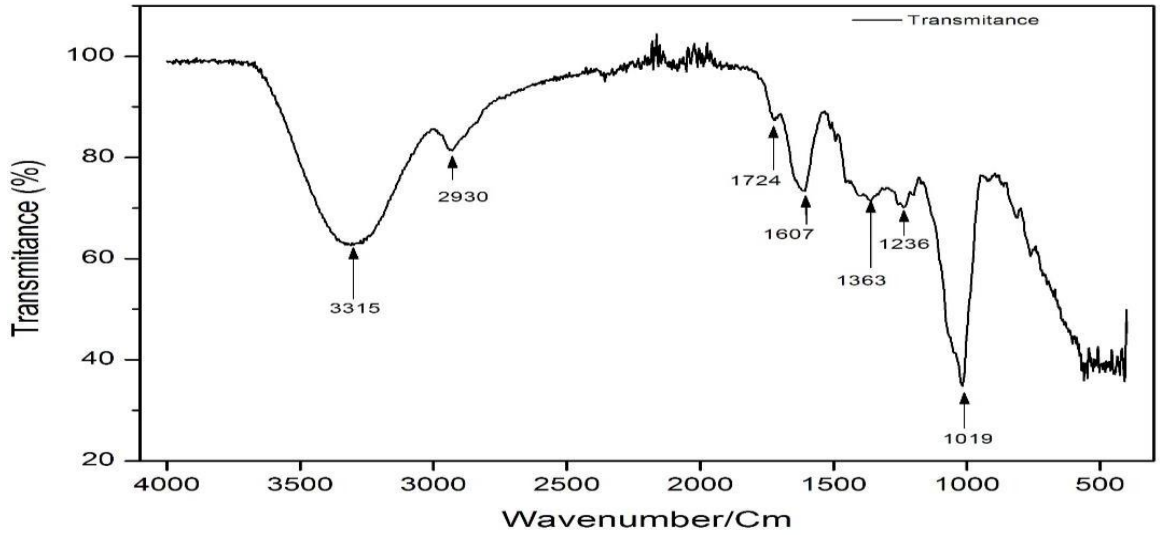


Fig. 1. FTIR spectrum and functional groups present in methanolic extract of *H. stellarioides* plant.

Table 2: Transmittance peaks along with functional groups.

Frequency (cm^{-1})	Functional group involved in transmittance
3315	V-OH/stretching (w)
2930	VC-H/stretching (w)
1607	VC=C, Alkene (w)
1363	α-CH3/bending (m)
1236	VC-O, Ether linkage (m)
1019	VC-O, Ether linkage (m)

GC-MS analysis of crude methanol extract

Methanol extract of the ***H. stellarioides*** **plant** GC-MS analysis was carried out to find out the presence of major bioactive constituents. Interpretation on mass spectrum GC–MS was conducted using the database of National Institute Standard and Technology (NIST-05) with more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were observed. The GC-MS chromatogram of crude methanol extract **(Fig.2)** revealed the presence of seven bioactive compounds. On comparing the values of mass spectra with the MS library (NIST-05) these compounds were present in ***H. stellarioides***. They could be identified as 3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydroxy; 3-trifluoroacetoxydodecane; cyclopenta(c)furo(3'-2'-h)(1)benzop; 3-hexadacycloxylocarbonyl-5-(2-hydroxy); 1,3-butanediol, diacetate; cholest-7-en-3 beta, 5 alpha-diol-6 alpha benz and 2-cyclopentene-1-one, 5-hydroxy- 2,3-demethyl-. Detailed properties of these compounds such as their retention time (RT), molecular formula, molecular weight, peak area in percentage and biological activities are provided in **Table-3 &**

4. It was evident from the GC–MS profile that, among seven compounds 1,3-butanediol, diacetate have the largest peak with 26.02% area.

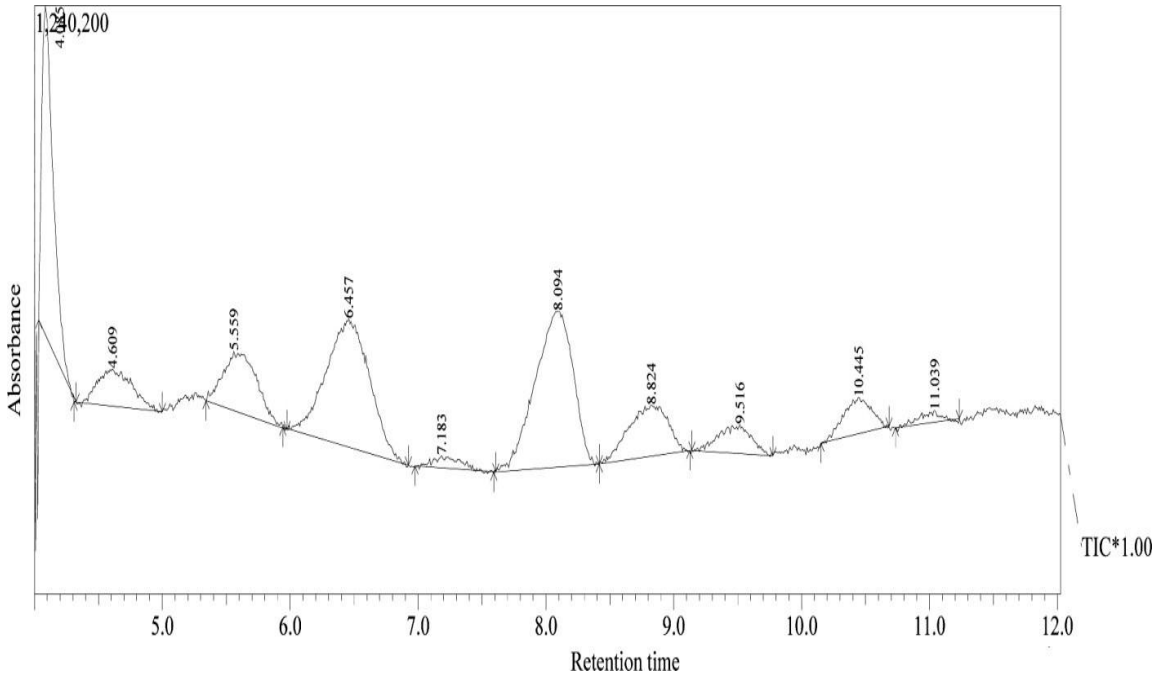


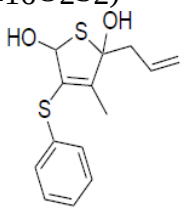
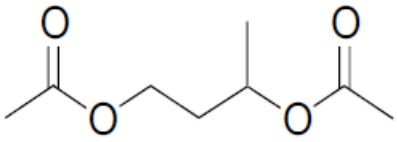
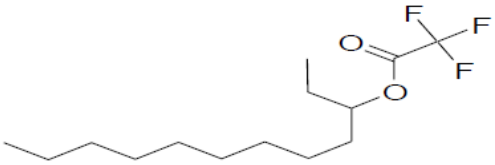
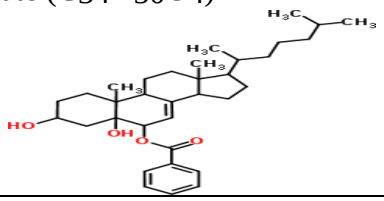
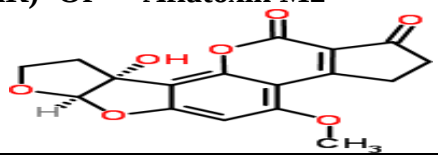
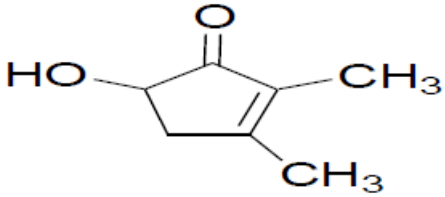
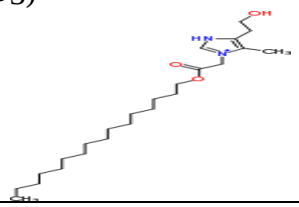
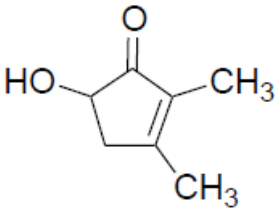
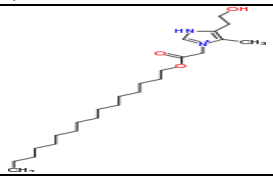
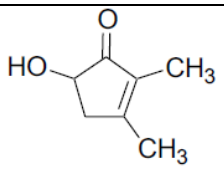
Fig. 2. GC-MS chromatogram and Mass spectra of *H. stellarioides* methanol extract.

Table 3: Components of crude methanol extract of *H. stellarioides* plant identified by GC- MS analysis.

Sl. No.	Retention time (in minutes)	Name of the compound	Molecular formula	Mol. weight (g/mol)	Peak area %
1.	4.085	3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydroxy	$C_{14}H_{16}O_2S_2$	280	16.20
2.	4.609	3-trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	282	5.44
3.	5.559	**Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-1,11-dione, 2,3,6a,8,9,9a-hexahydro-9a-hydroxy-4-methoxy-, (6aR,9aR)- Or Aflatoxin M2**	$C_{17}H_{14}O_7$	330	8.84
4.	6.457	3-[2-(Hexadecyloxy)-2-oxoethyl]-5-(2-hydroxyethyl)-4-methyl-1H-imidazol-3-ium. ($C_{24}H_{45}N_2O_3$)	$C_{24}H_{45}N_2O_3$	409	24.05
5.	7.183	3-[2-(Hexadecyloxy)-2-oxoethyl]-5-(2-hydroxyethyl)-4-methyl-1H-imidazol-3-ium. ($C_{24}H_{45}N_2O_3$)	$C_{24}H_{45}N_2O_3$	409	1.33
6.	8.094	1,3-butanediol,diacetate	$C_8H_{14}O_4$	174	26.02
7.	8.824	3,5-Dihydroxycholest-7-en-6-yl benzoate ($C_{34}H_{50}O_4$)	$C_{34}H_{50}O_4$	522	8.79
8.	9.516	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl-	$C_7H_{10}O_2$	126	4.00

9.	10.445	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl-	C7H10O2	126	4.39
10.	11.039	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl-	C7H10O2	126	0.94

Table 4: Components of crude methanol extract of *H. stellarioides* plant identified by GC- MS analysis. Name of the compound & Structure.

Sl. No.	Name of the compound & Structure	Sl. No.	Name of the compound & Structure
1	3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydroxy. (C14H16O2S2)  2-Allyl-3-methyl-4-phenylsulfanyl-2,5-dihydro-thiophene-2,5-diol	6	1,3-butanediol,diacetate (C8H14O4)  1,3-butanediol diacetate
2	3-trifluoroacetoxydodecane (C14H25F3O2)  3-trifluoroacetoxydodecane	7	3,5-Dihydroxycholest-7-en-6- yl benzoate (C34H50O4) 
3	**Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-1,11-dione, 2,3,6a,8,9,9a-hexahydro-9a-hydroxy-4-methoxy-, (6aR,9aR)- Or Aflatoxin M2** 	8	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl- (C7H10O2) 
4	3-[2-(Hexadecyloxy)-2-oxoethyl]-5-(2-hydroxyethyl)-4-methyl-1H-imidazol-3-ium. (C24H45N2O3) 	9	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl- (C7H10O2) 
5	3-[2-(Hexadecyloxy)-2-oxoethyl]-5-(2-hydroxyethyl)-4-methyl-1H-imidazol-3-ium. (C24H45N2O3)	10	2-cyclopentene-1-one, 5-hydroxy-2,3-demethyl-
			

DISCUSSION

The Genus *Hybanthus* Jacq. With 100 species is distributed throughout tropics (Mabberley, 2008) and it is represented by six species in India (Banerjee and Pramanik, 1993[5]; Reddy, 2001[17]; Sasi *et al*., 2011[18]; Ramana *et.al.* 2011[19]; Francisca *et al.*, 2013[20];). The species grows on the rocky flat hill tops in the crevices of sand stones (Kulkarni, *et.al*, 2016).[8] It is a seasonal herb but is a very good forage plant for sheep and goats.

The phytochemical study revealed the presence of carbohydrates, steroids, glycosides, saponin glycosides, flavonoids, tannins and phenolic compounds and alkaloids in *H. stellarioides*. In general saponin acts as antibacterial and anti-neoplastic. Flavonoids are reported to have anti-allergic, anti-inflammatory and anti-cancer properties. The alkaloids possess a good analgesic, anti-inflammatory and anti-oxidant activity (Pradhan, *et.al*, 2013).[21] Thus it is possible that *H. stellarioides* is also having the similar medicinal properties as that of *H. enneaspermus* which has to be further confirmed through various comparative biological activities.

The results of FTIR analysis clearly indicates the presence of -OH C-H, C=C (Alkene) and C-O (Ether) functional groups in methanol extract. Further, from GC-MS analysis it is observed that the methanol extract contain 7 bioactive constituents and probably these might have played a major role in the effective antimicrobial and antioxidant capacity of *H. stellarioides* methanol extract.

CONCLUSION

This preliminary analysis of crude extract from *H. stellarioides* proves that, the plant contains various bioactive phytochemical constituents contribute towards the biological applications of this plant which are identified in previous reports. Separation of individual phytochemical constituents and biological activity study will certainly give successful results. Thus, *H. stellarioides* is suggested as a plant of phyto pharmaceutical significance.

ACKNOWLEDGEMENT

The authors would wish to acknowledge to Prof. G.R. Hegde (Rtd), Karnatak University, Dharwad for his valuable guidance for this research work. I would like to thank Dept. of SAIF/CIL, Panjab University, Chandigarh and USIC Karnatak University, Dharwad for providing FTIR and GC-MS analysis results to carry out this research work.

REFERENCES

1. Dahanukar SA, Thatte UM, Nrmala Rege N. Immunostimulants in Ayurveda medicine. Immunomodulatory agents from plants, 1999; 289-323.
2. Khandelwal K. Pratical pharmocognosy, Nirali Prakashan, Pune, 2007.
3. Liu HX, Sun SQ, Lv GH, Chen KK. Study on Angelica and its different extracts by Fourier Transform Infrared Spectroscopy and two dimensional correlations IR Spectroscopy. Mol. Biomol. Spec., 2006; 64: 321-326.
4. Liebler DC. Burr JAPhilips L, Ham AJL. Gas chromatography – mass spectrometry analysis of vitamin E and its oxidation products. Anal. Biochem, 1996; 236: 27-34.
5. Banerjee SP. Pramanik BB. Violaceae. In: Sharma BD, Balakrishnan NP, Sanjappa M. (eds.). Flora of India, 1993; 2: 342-347.
6. Forster, PI. Hybanthus stellarioides new combination (Violaceae), a widespread species from eastern Australia and Papua New Guinea. Muelleria, 1993; 8(1): 17-19.
7. Ramana VM, Prasanna PV, and Venu P. Hybanthus stellarioides (Violaceae), a new record for India. Rheedea, 2011; 21(1): 10-12.
8. Kulkarni LC, Bhat P, Hegde GR. Hybanthus stellarioides (Domin) P. I. Forster (Violaceae) - a new record for Karnataka. Zoo's Print, 2016; 31(6): 6-7.
9. Mabberley DJ. Mabberley's Plant-Book, A portable dictionary of plants, their classification and uses. 3rd Edition. Cambridge University Press, Cambridge, 2008.
10. Ragavendran P, Sophia D, Raj CA, Gopalakrishnan V. Functional group analysis of various extracts of Aerva lanata (L.,) by FTIR spectrum. Pharmacologyonline, 2011; 1: 358-364.
11. Singh RP, Padmavathi B, Rao AR. Modulatory influence of Adhatoda vesica (Justicia adhatoda) leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. Mol. Cell Biochem, 2000; 213(1-2): 99-109.
12. Amutha priya D, Ranganayaki S, Suganya Devi P. Phytochemical screening and antioxidant potential of Hybanthus enneaspermus: A rare ethno botanical herb. Journal of Pharmacy research, 2011; 4(5): 1497-1502.
13. Horborne JB. Phytochemical methods, a guide to modern techniques of plant analysis, 3rd edn. Chapman and Hall, London (UK), 2007.
14. Kokate, CK. Practical pharmacongosy. Vallabh Prakashan, New Delhi, India, 1994.
15. Khandelwal K. Pratical pharmocognosy, Nirali Prakashan, Pune, 2007.
16. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Ameri. Pharma. Asson, 1949; 38: 324-331.

17. Reddy CS. Hybanthus vatsavayii (Violaceae): A new species from Andhra Pradesh, India. J. Econ. Taxon. Bot., 2001; 25: 219 – 220.
18. Sasi R, Sivalingam R, Rajendran A. Hybanthus puberulus M. Gilbert (Violaceae)-A new record for India. ZOO's PRINT, 2011; 26(12): 30-31.
19. Ramana VM, Prasanna PV, and Venu P. Hybanthus stellarioides (Violaceae), a new record for India. Rheedea, 2011; 21(1): 10-12.
20. Francisca GA, Rajendran, Parthipan M. Hybanthus verticillatus (Ortega) Baill.-Violaceae: A new record for India. ZOO's PRINT, 2013; 28(3): 16-17.
21. Pradhan D, Ojha V, Pandey AK. Phytochemical analysis of Tinospora cordifolia stem of varied thickness, Int. J. Pharmaceu. Sci. Res., 2013; 4(8): 3051-3056.