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GCMS based metabolite profiling of *Tabernaemontana heyneana* Wall. An endemic plant of Western Ghats

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Abstract

Tabernaemontana heyneana Wall. *(Apocyanaceae)* is an endemic plant of Western Ghats used in traditional medicine in the management of several human diseases such as skin and venereal diseases, respiratory problems, nervous disorders, inflammatory diseases etc. These therapeutic properties of the plant are attributed to the presence of various bioactive principles. Many reports are available on the presence of various chemical constituents in different species of genus Tabernaemontana. However, information is sparingly available to delineate the phytochemical profiling of *T. heyneana*. The current study is devoted to investigate the various bioactive constituents present in the methanolic leaf extract of the plant. Methanolic leaf extract was subjected to GC-MS based metabolite profiling (Thermo Scientific, USA). GC-MS analysis of leaf extract allowed the identification of 9 compounds. The main constituents were Flavone, 9-Hexadecen -1 –ol [z]-, Cyclopentaneundecanoic acid, methyl ester, 6-Octadecenoic acid, methyl ester, 4h -1- benzopyran-4-one,2-[3,4-dimethoxyphenyl]-7-hydroxy, 7,10-Octadecadienoic acid,methyl ester, Corynan- 17-ol,18,19-didehydro-10-methoxy-, Estra 1,3,5-[10] 6-tetraene-3,17-diol,diacetate,[17a]-, Squalene. Future investigations could focus on the biological activity of identified phytochemicals from the leaves in order to provide scientific evidence for the observed pharmacological properties.

Keywords: *Methanolic leaf, pharmacological properties, Tabernaemontana heyneana, T. heyneana,*

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1. Introduction

The Western Ghats (80^0 20^0 - 20^0 40^0 N and 73^0 - 77^0 E) crossing from Tapti in Gujrat to Kanyakumari in Tamilnadu, which runs parallel along the west coast of the Indian peninsula, and forming an essentially uninterrupted relief for about 1600 km excluding Palghat gap, is highly admired as one of the 35 'Hot spots' [1]. Western Ghats is considered as one of the UNESCO World Heritage site which harbors about 1500 spp of medicinal plants [2]. Because of its complexity, heterogeneous landscapes and significant levels of biodiversity, it puts a platform for bioprospecting, particularly chemo prospecting of medicinal plants. *Tabernaemontana heyneana* Wall. (Apocyanaceae) is an endemic medium sized small tree to shrub that is distributed in the Western Ghats region of Karnataka, India. The plant possess numerous therapeutic properties against gonorrhea, respiratory problems, venereal

diseases, diabetes, nervous disorders, chronic bronchitis, rheumatism, cardiotonic ailments, burning sensation of eyes, improve vision and snakebite [3, 4]. The plant has been worked for the isolation and identification of alkaloids, flavonoids and other therapeutically significant phytochemicals by various researchers [5, 6]. Hitherto, the identification of several volatile metabolites remain undiscovered in the plant. Hence the contemporary study was intended to explore the possible volatile metabolites by formulating the methanolic extract from leaves and separation and identification of the compounds by subjecting it to GC-MS analysis.

2 Materials and Methods

2.1 Preparation of leaf crude extract

The fresh mature leaves were picked from healthy plants situated in and around Mangalore university campus, Karnataka, India. The collected leaves were washed 2-3 times thoroughly with running tap water, shade dried; coarsely powdered using a domestic grinder and the powder was kept in polythene bags with proper labelling in refrigerator until used.

The portion of the dried powdered leaf samples (150 g) was subjected to soxhlet extraction using methanol (300ml) for 36 h at the temperature not exceeding the boiling point of the solvent. The extracts obtained was filtered using Whatman filter paper No.1 and evaporated to dryness in flash evaporator. The extract was stored in the refrigerator until further use. The extract obtained was weighed and the yield was expressed in terms of percentage using the formula given below:

$$\text{Yield (\%)} = \frac{\text{Weight of the extract}}{\text{Weight of the sample}} \times 100$$

3. Results and Discussion

3.1 GC-MS analysis of methanol leaf extracts of *T. heyneana*.

After the successful completion of conventional soxhlet extraction we got 26 % of yield of extract. GC-MS investigation of the crude methanol extract of leaf has resulted in the identification of 9 compounds (Table 1) corresponding to 9 peaks obtained on the GCMS chromatogram (Fig 1). Fig 2 showed the percentage of each identified compound identified in the leaf extract with regard to their peak percentage. Flavone, 9-Hexadecen -1 –ol [z]-, Cyclopentaneundecanoic acid, methyl ester, 6-Octadecenoic acid, methyl ester, 4h -1-benzopyran-4-one,2-[3,4-dimethoxyphenyl]-7-hydroxy, 7,10-Octadecadienoic acid,methyl ester, Corynan- 17-ol,18,19-didehydro-10-methoxy-, Estra 1,3,5-[10] 6-tetraene-3,17-diol,diacetate,[17a]-, Squalene are the identified compounds. The mass spectra are the fingerprint of each identified compound shown in Figure 3.

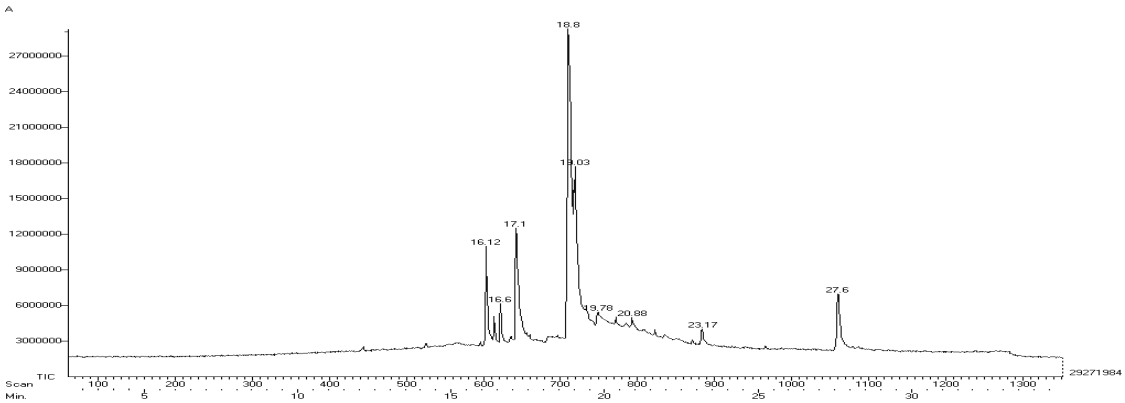

Figure 1. GC-MS Chromatogram of methanol leaf extract of *T. heyneana*

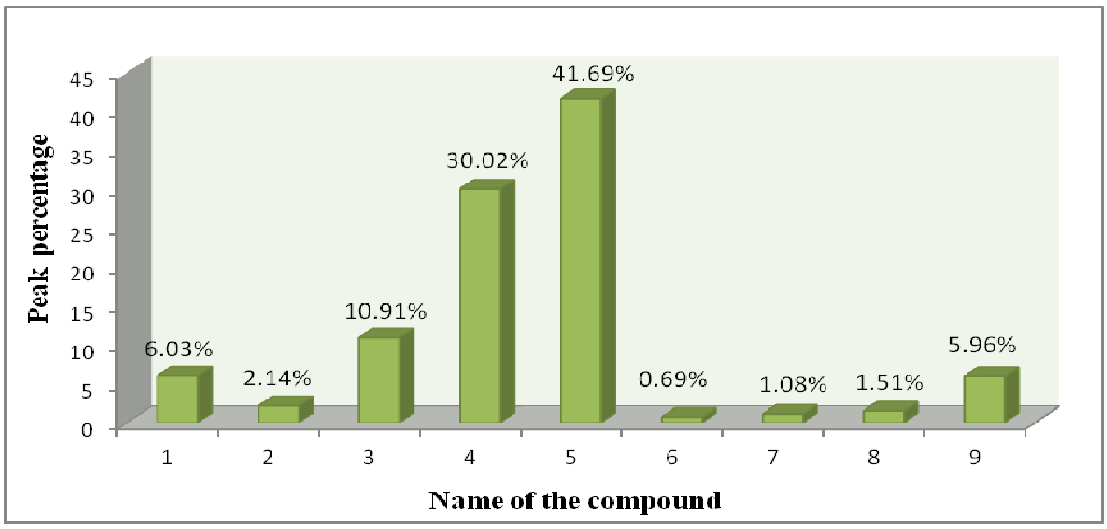

Figure 2. Graph showing the abundance of compounds in leaf extracts of *T. heyneana* in terms of their peak percentage**; (Where** 1) Flavone; 2) 9-Hexadecen -1 –ol [z]-; 3) Cyclopentaneundecanoic acid, methyl ester ; 4) 6-Octadecenoic acid, methyl ester; 5) 4h -1- benzopyran-4-one,2-[3,4-dimethoxyphenyl]-7-hydroxy; 6) 7,10-Octadecadienoic acid,methyl ester; 7) Corynan- 17-ol,18,19-didehydro-10-methoxy-; 8) Estra 1,3,5-[10] 6-tetraene-3,17-diol,diacetate,[17a]-; 9) Squalene.)

Table 1. Compounds identified by GCMS analysis of methanol extract of leaf of *T. heyneana*

Sl No.	RT	Name of the compound	Molecular Weight	Molecular formula	Activity	Reference
1	16.12	Flavone	222.243	$C_{15}H_{10}O_2$	Anti-inflammatory, antiulcer, antiviral, anti-cancer, anti-diabetic and cytotoxic,	[8]
2	16.6	9-Hexadecen -1 –ol [z]-	240.424	$C_{16}H_{32}O$	Not known	
3	17.1	Cyclopentaneundecanoic acid, methyl ester	268.441	$C_{17}H_{32}O_2$	Alpha amylase inhibitory activity, antimicrobial	[9, 10]
4	18.8	6-Octadecenoic acid, methyl ester	296.4879	$C_{19}H_{36}O_2$	Antioxidant, antimicrobial	[11]
5	19.03	4h -1- benzopyran-4-one,2-[3,4-dimethoxyphenyl]-7-hydroxy	298.294	$C_{17}H_{14}O_5$	Not known	
6	19.78	7,10-Octadecadienoic acid,methyl ester	294.479	$C_{19}H_{34}O_2$	Not known	
7	20.88	Corynan- 17-ol,18,19-didehydro-10-methoxy-	368.47	$C_{22}H_{28}N_2O_3$	Diabetic retinopathy treatment, lipid metabolism regulator, G-protein-coupled receptor kinase inhibitor	[12]
8	23.17	Estra 1,3,5-[10] 6-tetraene-3,17-diol,diacetate,[17a]-	354.43944	$C_{22}H_{26}O_4$	Not Known	

9	27.6	Squalene	410.718	$C_{30}H_{50}$	Anticancer, antimicrobial, antioxidant, chemo preventive, pesticide, anti-tumor, sunscreen	[13]

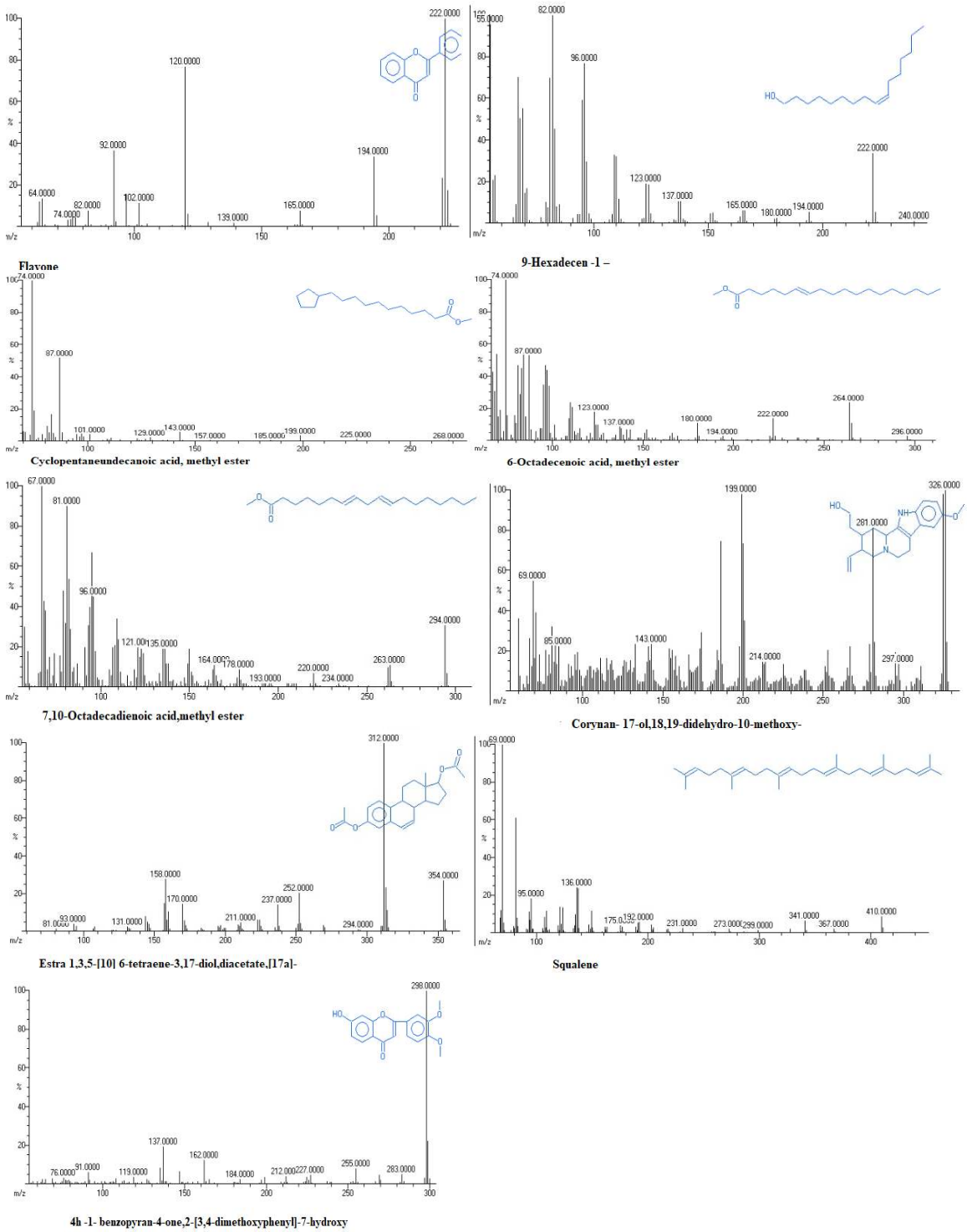

Figure 3. Mass spectrum and structure of metabolites identified by GC-MS in the methanolic extracts of *T. heyneana.*

GCMS is a sensitive and specific technique for the separation and identification of fatty acids in plants. Numerous metabolites have been identified by various researchers from different species of Tabernaemontana which possess important therapeutic attributes. Rutin is a type of flavone detected in the leaves of *T. divaricata* by HPTLC method which possess potent anti oxidant and free radical scavenging activity [14] 9, 12-Octadecadienoic acid (fatty acid) was also reported from the seeds *T. cymosa* [15]. Basumatary et al [16] reported the presence of 9- octadecanoic acid; 9, 12, 15-Octadecatrienoic acid, ethyl ester; 9, 12-Octadecadienoic acid; 11-octadecanoic acid; palmitic acid (hexadecanoic acid) and squalene in *T. divaricata* found to exhibit anti oxidant, antimicrobial, anticancer, hypocholesterolemic activity and antiulcerogenic acitivity.

Biodiesel (mixture of fatty acid methyl esters) was prepared from the seeds of *T. divaricata* and subjected to GCMS analysis to study the fatty acid profile revealed the presence of oleic acid and palmitic acid (Hexadecenoic acid); which are the useful sources for the production of biodiesel [16]. Baskar et al [17] reported the presence of myristic acid, palmitic acid, oleic acid and linolenic acid in *T. heyneana* used to treat the inflammation of cornea and in the treatment of cancer. The fatty acid analysis of *Cissampelos andromorpha*, *Rauwolfia grandiflora* and *Tabernaemontana flavicans* revealed the presence of oleic acid [18]. The present investigation revealed the identification of 9 fatty acids which are reported to exhibit a wide array of therapeutic properties (Table 1). Additional examination of the plant extract may lead to isolation, identification and their structural elucidation of phytoconstituents. Screening of pharmacological activity will be helpful for further drug development.

4. Conclusion

GCMS based metabolite profiling of methanolic leaf extract revealed the detection of 9 bioactive compounds of *T. heyneana*. The current investigation validates the use of entire plant for several disease treatments by traditional practitioners. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biologic and pharmacologic studies.

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