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Research Article

Phytochemical Characterization of Hexane Extract of *Hygrophila Auriculata* Leaves Through GC–MS Analysis

Nishvanth F.*[1], Aswini B.[2], Monika S.[3], Kalaiselvi G.[4], Jeyaprakash G.[5]

[1]*Assistant Professor cum Research Scholar, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur.*
[2]*Assistant Professor, Department of Pharmacology, Adhiparasakthi College of Pharmacy, Melmaruvathur-603319.*
3Assistant Professor, Department of Pharmaceutical Chemistry, Adhiparasakthi College of Pharmacy, Melmaruvathur.
[4]*Assistant Professor, Department of Pharmaceutical Chemistry, Karpaga Vinayaga Institute for Pharmaceutical Sciences, Chengalpat.*
[5]*Assistant Professor, Department of Pharmacology, Surya School of Pharmacy, Vilupuram*

Hygrophila auriculata (Schumach.) Heine is a medicinal herb widely employed in traditional systems of medicine owing to its diverse pharmacological properties. The present study aimed to characterize the phytochemical composition of the hexane leaf extract of *H. auriculata* using Gas Chromatography–Mass Spectrometry (GC–MS). GC–MS analysis revealed the presence of more than 60 phytochemical constituents, representing a broad spectrum of bioactive compounds, including phenolics, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, phytosterols, and quinone derivatives. The major constituents identified included carvacrol (Phenol, 2-methyl-5-(1-methylethyl)), *(Z,Z)*-α-farnesene, trans-α-bergamotene, acetyleugenol, copaene, longifolene, caryophyllene, α-humulene, neophytadiene, β-sitosterol, β-amyrone, 9,19-cycloergost-24(28)-en-3-ol, lup-20(29)-en-3-one, lupeol, stigmasta-3,5-diene, α-tocopherolquinone, and campesterol. These compounds have been reported to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, hepatoprotective, immunomodulatory, and cardioprotective effects. The abundance of terpenoids and phytosterols in the hexane extract highlights the effectiveness of non-polar solvent extraction in recovering lipophilic bioactive metabolites. In particular, the presence of pharmacologically important compounds such as lupeol, β-sitosterol, campesterol, caryophyllene, α-humulene, and carvacrol suggests that the extract possesses considerable therapeutic potential. The GC–MS fingerprint generated in this study provides a comprehensive chemical profile of *H. auriculata* leaves and offers scientific evidence supporting their traditional medicinal use. The identification of over 60 phytochemicals demonstrates the chemical richness of the hexane extract and underscores its potential as a valuable source of natural bioactive compounds for pharmaceutical, nutraceutical, and functional food applications. Further studies focusing on the isolation, purification, quantitative estimation, and biological evaluation of the major constituents are warranted to validate their pharmacological efficacy and explore their therapeutic applications.

Keywords: GC-MS analysis, *Hygrophila auriculata*, Phytochemicals, *Hygrophila spinosa*, Hexane extract.

INTRODUCTION

For thousands of years, medicinal plants have played a central role in human healthcare, providing a major source of therapeutic molecules and constituting the basis of traditional medical systems across the world[1]. Ancient cultures including Egyptian, Greek, and Chinese civilizations, as well as Indigenous groups in Africa, the Americas, and Asia, have long exploited the healing potential of plants to manage various diseases[2]. Owing to their diverse bioactive constituents, these plants have been employed against common disorders, infectious diseases, and severe conditions, establishing themselves as a vital component of healthcare worldwide. Their relevance extends beyond history, as medicinal plants continue to be a dynamic and evolving area influencing contemporary traditional and modern medicine.[3] While medicinal plants have been used in healthcare for centuries, scientific scrutiny of their properties is a relatively recent development. The progress of modern pharmacology has highlighted the importance of validating traditional knowledge and investigating the intricate phytochemical profiles of plants with long histories of medicinal use.[4] Despite its promising therapeutic profile, *Hygrophila auriculata* remains an underexploited medicinal species requiring further systematic investigation. A member of the Acanthaceae family, it is widely distributed in South and Southeast Asia including Nepal, India, Myanmar, Malaysia, and Sri Lanka.[5] The plant is a rich source of phytochemicals such as glycosides, polyphenols, phytosterols, fatty acids, alkaloids, proanthocyanidins, enzymes, amino acids, and terpenoids. It demonstrates a broad spectrum of pharmacological effects including cardioprotective, anti-stress, diuretic, anti-inflammatory, anticancer, hepatoprotective, nephroprotective, and neuroprotective activities. In addition, its traditional use in Bengali culinary preparations underscores its potential for translation into functional foods and nutraceuticals that integrate traditional wisdom with modern science.[6]

Vernacular names:[7]

Marathi : Kolshinda Talimkhana
Sanskrit : Kokilaksa
Bengali : Kuliyakhara
Gujrati : Ekharo
Hindi : Talmakhana
Kannada: Kolavali
Marathi : Talikhana Kalsunda
Tamil : Golmidi, Neermulli
Urdu : Talmakhana.

Taxonomy[8]

- Kingdom : Plantae
- Class : Magnoliopsida
- Subkingdom : Viridiplantae
- Superorder : Asteranae
- Infrakingdom : Streptophyta
- Order : Lamiales
- Superdivision: Embryophyta
- Family : Acanthaceae
- Division : Tracheophyta
- Genus : *Hygrophila* R. Br.
- Subdivision : Spermatophyta
- Species : *Hygrophila auriculata*

Traditionally, various parts of the plant including roots, leaves, and seeds have been utilized in Indian medicine as diuretic agents and for managing conditions such as jaundice, dropsy, rheumatism, anasarca, and urinogenital tract diseases. Ayurvedic literature describes *kokilaksha* as an effective remedy for arthritis. The plant is also credited with improving strength and appetite and with therapeutic efficacy against edema, ascites, excessive thirst, bladder calculi, ophthalmic disorders, and dysentery. Its root decoction exhibits diuretic properties, and a formulation containing its leaves and roots with flowers of *Stuea frondosa* is employed for leucorrhoea. Additionally, it is traditionally used to address impotence, spermatorrhea, and seminal weakness.[8,9] GC–MS analysis of the hexane extract of the leaves of *Hygrophila auriculata* was performed to identify the phytochemical constituents present in the extract.

MATERIALS AND METHODS

Collection of Plant material

Hygrophila auriculata leaves were collected in the month of April 2025 from Tirunelveli District, Tamil Nadu. The whole plant was authenticated by Dr. M. Syed Ali Fathima, Assistant Professor and Head, Sadakathullah Appa Arts and Science College, Tirunelveli. (Specimen Number: SAC/BOT/2025).

Figure 1: Leaves of *Hygrophila auriculata*

Extraction of Plant material

The leaves of *Hygrophila auriculata* was dried and powdered. The dried leaf powder extracted with n-hexane using Soxhlet apparatus. Finally, it was concentrated by vacuum evaporator.

GC-MS Analysis

The phytochemical composition of the hexane leaf extract of *Hygrophila auriculata* was analysed using Gas Chromatography–Mass Spectrometry (GC–MS) with an Agilent 7890B Gas Chromatograph coupled to an Agilent 5977A Mass Selective Detector (MSD). Separation was achieved using a fused-silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. A 1 µL aliquot of the sample was injected using a split ratio of 10:1. All samples were analysed in triplicate to ensure the reproducibility and reliability of the analytical results. Quality assurance procedures included instrument calibration before analysis, solvent-blank injections to detect potential contamination, and periodic analysis of a standard reference mixture to verify retention-time consistency, mass accuracy, and overall instrument performance throughout the analytical sequence. The injector and transfer-line temperatures were maintained at 250 °C and 280 °C, respectively. The oven temperature programme was initiated at 60 °C and held for 2 min, followed by a temperature ramp of 10 °C min^{-1} to 280 °C, where it was maintained for an additional 10 min. The mass spectrometer was operated in electron ionization (EI) mode at an ionization energy of 70 eV, with mass spectra acquired over an *m/z* range of 40–600. Compound identification was performed by comparing the acquired mass spectra with those available in the National Institute of Standards and Technology (NIST) and Wiley mass spectral libraries. The relative abundance of each identified compound was determined from its percentage peak area in the total ion chromatogram (TIC), providing a semi-quantitative estimate of the phytochemical composition of the extract[10].

RESULTS AND DISCUSSION:

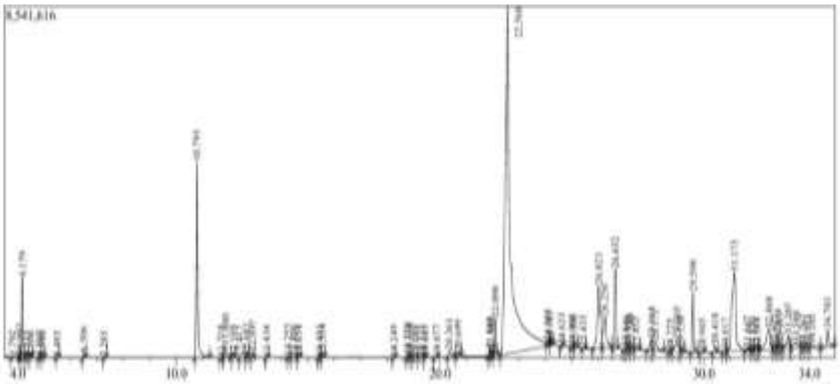


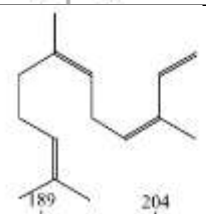
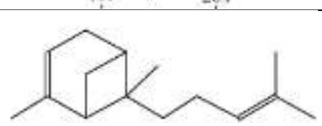
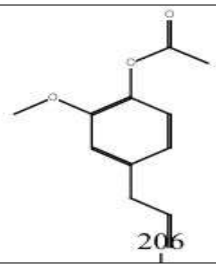
Figure 2: Chromatogram of Hexane extract of *Hygrophila auriculata*

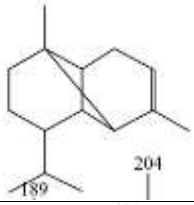
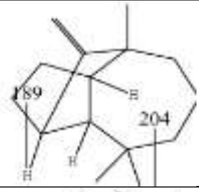
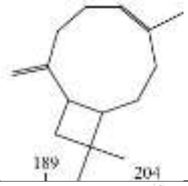
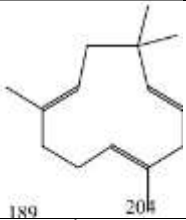
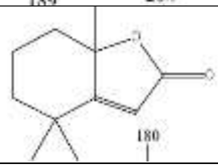
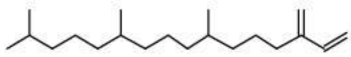
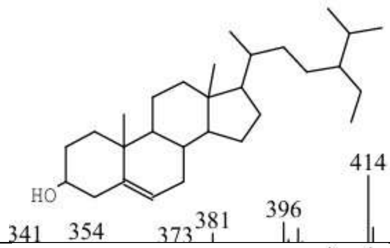
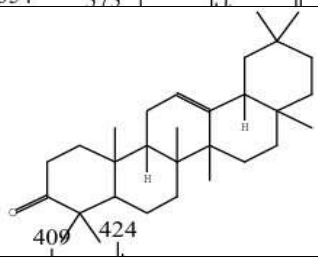
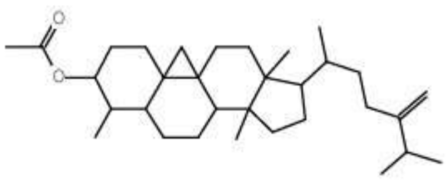
Table 1: Peak report of *Hygrophila auriculata*

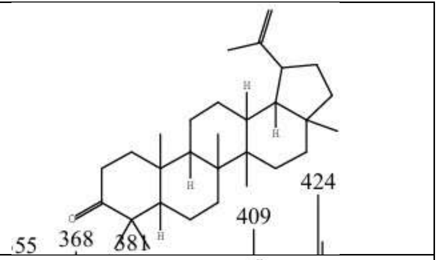
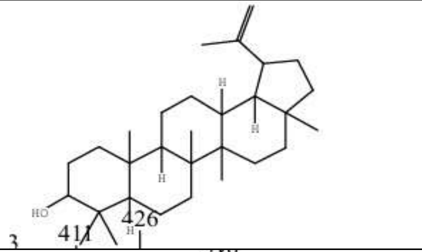
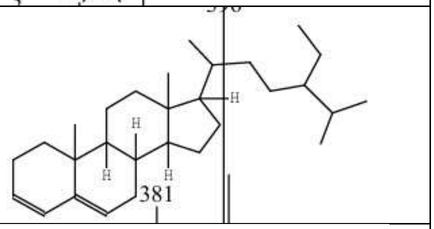
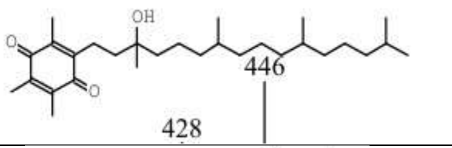
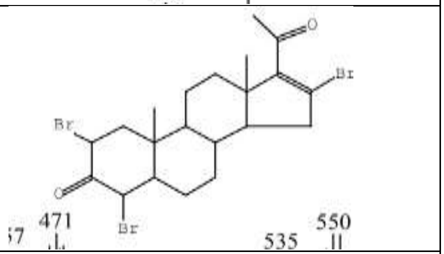
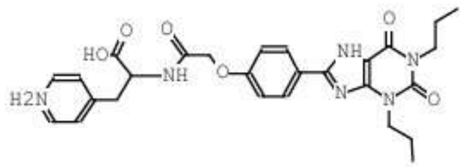
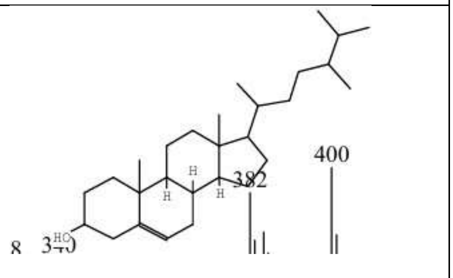
Peak	Retention time	Area	Peak area %	Height	Name of the compound
1	3.792	136691	0.06	19415	p-Xylene
2	4.092	563275	0.23	191192	Silane, ethenyldiethylmethyl-
3	4.179	5940997	2.45	1942861	Ethyl 3-ethoxyacrylate
4	4.333	81060	0.03	17450	3-Heptanol, 2,4-dimethyl-
5	4.506	60617	0.03	20242	Butane, 1,1,3-trimethoxy
6	4.805	70347	0.03	24550	Silane, ethenyldiethylmethyl-
7	4.948	68939	0.03	23351	Isopropyl acetate
8	5.493	60135	0.02	18045	Decane
9	6.506	413136	0.17	129875	2-Propanol, 1-[1-methyl-2-(2-propenyloxy)eth
10	7.285	44244	0.02	14502	Tridecane
11	10.793	18234004	7.53	4760606	Phenol, 2-methyl-5-(1-methylethyl)-
12	11.719	47337	0.02	14778	(Z,Z)-.alpha.-Farnesene
13	11.880	1338171	0.55	287209	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
14	12.169	70421	0.03	18385	Copaene
15	12.417	64356	0.03	14019	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-t
16	12.687	197511	0.08	62509	Longifolene
17	12.897	462137	0.19	145776	Caryophyllene
18	13.438	54828	0.02	19274	Humulene
19	14.253	165101	0.07	49625	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-o
20	14.496	107472	0.04	25341	cis-muurola-3,5-diene
21	14.654	62072	0.03	17578	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4
22	15.431	131271	0.05	34064	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-t
23	15.554	239713	0.1	52006	Diethyl Phthalate
24	18.249	52122	0.02	17891	Sulfurous acid, 2-ethylhexyl isohexyl ester
25	18.770	233033	0.1	72745	Neophytadiene
26	18.866	267965	0.11	77335	2-Pentadecanone, 6,10,14-trimethyl-
27	19.088	59460	0.02	19276	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
28	19.315	111022	0.05	26006	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
29	19.445	54760	0.02	18805	Cyclohexene, 3-methyl-6-(1-methylethylidene
30	19.877	132036	0.05	29847	Pentadecanoic acid, 14-methyl-, methyl ester
31	20.361	1283448	0.53	168300	n-Hexadecanoic acid
32	20.699	529307	0.22	100244	Hexadecanoic acid, ethyl ester
33	21.889	588571	0.24	173464	9,12-Octadecadienoic acid, methyl ester
34	21.960	511661	0.21	158376	6-Octadecenoic acid, methyl ester, (Z)-
35	22.098	3036379	1.25	906891	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
36	22.568	125716579	51.94	8444092	cis-9-Hexadecenal
37	24.085	172312	0.07	46682	Octacosane, 2-methyl-
38	24.183	54668	0.02	19621	(4,6,8,9-Tetramethyl-3-oxabicyclo[3.3.1]non-6
39	24.623	95454	0.04	30867	3-methyl-5-(2,6-dimethylheptyl)-1,5-Pent-2-en
40	25.008	146897	0.06	27449	Octane, 1-propoxy-
41	25.101	187478	0.08	51287	2-Bromotetradecane
42	25.435	132640	0.05	38264	Oleoyl chloride
43	26.023	12285209	5.08	1540446	beta-Sitosterol
44	26.256	6338442	2.62	784870	beta-Amyrone
45	26.652	6614348	2.73	1936958	Bis(2-ethylhexyl) phthalate
46	27.046	288175	0.12	65776	Heneicosane
47	27.133	420669	0.17	73809	Olean-12-en-3-ol, acetate, (3.beta.)-

48	27.249	627974	0.26	107429	.beta.-Amyrone
49	27.432	318374	0.13	41747	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimeth
50	28.014	1883358	0.78	288783	Pentatriacontane
51	28.171	2263940	0.94	238946	Lup-20(29)-en-3-one
52	28.725	111442	0.05	20584	2-Isopropyl-5-methylcyclohexyl methylphosph
53	29.005	3297194	1.36	324515	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
54	29.150	335057	0.14	97106	Eicosane
55	29.598	5745547	2.37	1469296	Squalene
56	29.945	279905	0.12	58886	Sulfurous acid, 2-propyl tridecyl ester
57	30.418	673568	0.28	171924	Tetratetracontane
58	30.817	246117	0.1	55501	Tetratetracontane
59	31.173	20533449	8.48	1928206	Olean-12-en-3-ol, acetate, (3.beta.)-
60	31.647	408937	0.17	57800	Heptacosane, 1-chloro-
61	31.850	109909	0.05	22720	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9
62	32.008	154314	0.06	28950	Tetrapentacontane, 1,54-dibromo-
63	32.468	5706068	2.36	521786	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
64	32.671	494642	0.2	92842	(1R,4aR,5S)-5-[(E)-5-Hydroxy-3-methylpent-
65	32.815	1116585	0.46	226690	Tetracontane
66	32.917	879989	0.36	138301	.beta.-Sitosterol acetate
67	33.207	2607122	1.08	341905	Cholesterol
68	33.500	2044484	0.84	148547	.alpha.-Tocopherolquinone
69	33.767	441610	0.18	59707	erythro-9,10-Dibromopentacosane
70	33.933	361972	0.15	49164	2.beta.,4.beta.,16.alpha.-Tribromoallopregn-16
71	34.101	697589	0.29	71311	Dotriacontane, 1-iodo-
72	34.703	2764291	1.14	438654	Campesterol

Table 2: Structure, molecular weight, molecular formula for some important compounds

Compound Name	Mol. Formula	Mol. weight	Structure
Phenol, 2-methyl-5-(1-methylethyl) or Carvacrol	C10H14O	150	
(Z,Z) alpha Farnesene	C15H24	204	
Trans- alpha-Bergamotene	C15H24	204	
Phenol, 2-methoxy-4-(2-propenyl)-, acetate or Acetyleugenol	C12H14O3	206	

Copaene or Tricyclo dec-3-ene	C15H24	204	
Longifolene	C15H24	204	
Caryophyllene or Bicyclo undec-4-ene	C15H24	204	
Humulene or alpha Caryophyllene	C15H24	204	
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	C11H16O2	180	
Neophytadiene or 7,11,15-Trimethyl-3-methylenehexadec-1-ene	C20H38	278	
beta-Sitosterol	C29H50O	414	
beta-Amyrone	C30H48O	424	
9,19-Cycloergost-24(28)-en-3-ol	C32H52O2	468	

Lup-20(29)-en-3-one	C30H48O	424	
Lupeol	C30H50O	426	
Stigmasta-3,5-diene	C29H48	396	
Alpha-Tocopherolquinone	C29H50O3	446	
2.beta 4.beta 16.alpha Tribromoallopregn-16-ene-3	C21H27Br3O2	548	
Xanthine, 8-[[4-[2-(4-aminophenyl)-1-carboxy] ethylamino] carbonyl] methoxyphenyl]-1,3-dipropyl	C28H32N6O6	548	
Campesterol	C28H48O	400	

CONCLUSION:

The present study successfully characterized the phytochemical composition of the hexane leaf extract of *Hygrophila auriculata* using Gas Chromatography–Mass Spectrometry (GC–MS). More than 60 phytochemical constituents were identified, demonstrating the chemical diversity of the extract. The detected compounds included phenolics, sesquiterpenes, diterpenes, triterpenoids, phytosterols, and quinone derivatives, with notable constituents such as carvacrol, *(Z,Z)*-α-farnesene, trans-α-bergamotene, acetyleugenol, caryophyllene, α-humulene, neophytadiene, β-sitosterol, β-amyrone, lupeol, campesterol, stigmasta-3,5-diene, and α-tocopherolquinone. These compounds are known to possess a broad spectrum of biological activities, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anticancer, and immunomodulatory properties. The predominance of terpenoids and phytosterols indicates that the hexane solvent efficiently extracted lipophilic bioactive metabolites, highlighting the therapeutic potential of the plant. The comprehensive GC–MS profile generated in this study provides valuable baseline information for the chemical standardization and quality assessment of *H. auriculata* leaf extracts. Furthermore, the findings scientifically support the traditional medicinal use of this species and emphasize its potential as a promising source of bioactive natural products for pharmaceutical, nutraceutical, and functional food applications. Future investigations should focus on the isolation and purification of the major phytochemicals, quantitative analysis, and in vitro and in vivo pharmacological studies to elucidate their mechanisms of action and validate their therapeutic potential.

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