

CHAPTER-23

FLUORESCENCE, PHYTOCONSTITUENT SCREENING, TLC AND LC-MS ANALYSIS OF *HYGROPHILA AURICULATA* FROM AKOLA REGION (MS), INDIA

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Summary

Hygrophila auriculata is one of the important medicinal plants found in India, which is Acanthaceae family and is distributed in tropical and subtropical region of India. The this present study we have performed phytochemical analysis to validate their phytochemical constitution, Thin Layer Chromatography (TLC) analysis and Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis of the methanolic leaves and stem extract of *H.auriculata*. The fluorescence analysis under visible and ultra violet light for leaf and stem powder treated with various chemical reagents revealed different patterns of fluorescence effect. The preliminary phytochemical study showed that the plant is rich in phytochemicals and possess significant level of alkaloids, cardiac glycosides, terpenoids, reducing sugar, saponins, flavonoids and steroids. TLC of methanolic extract was executed for 2 essential phytoconstituents alkaloids and flavonoids. Further, the leaf extract of this plant was analysed using LC-MS. This report revealed the chemical compounds. *H. auriculata* possess various medicinal properties, so more research must be done on its traditional claims.

Keyword: Hygrophila auriculata, Fluroscence, TLC, Liquid Chromatography-Mass Spectroscopy, phytoconstituents.

Introduction

On a global scale, the role of traditional medicines in resolving health problems is invaluable. In both modern and traditional medicine, medicinal plant continues to provide valuable therapeutic agents (Kshirsagar et. al., 2010). Humans have looked to nature for cures for various illnesses. Similar to how

animals develop their therapeutic abilities, plants also started out on an innate level, since the beginning of time (Petrovska, 2012). Numerous active components found in medicinal plants are known to exist in large quantities and may one day be used to create new therapeutics (Pola et. al., 2018 and Singh, 2020). The World Health Organisation estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Antony, 1989). The plant *Hygrophila auriculata* (Schumach.) Heine, which belongs to Acanthaceae, has been traditionally used for the treatment of inflammation pain, urinary infection, edema, gout and as a diuretic. It is described in ayurvedic literature as Ikshura, Ikshugandha, and Kokilasha having eyes like Kokila or Indian Cuckoo (Chopra, 1956).

Hygrophila auriculata also known with few synonyms viz, *Asteracantha longifolia*, *Hygrophila spinosa*, *Hygrophila schulli*. In Kannada, it is called as *Nirmuli*, *Kolavanki gida* and an annual stout plant (Eazhisaivallabi, et. al., 2012).

In this present study, phytochemicals in plant will be identified using qualitative phytochemical screening, Thin-layer chromatography, and LC-MS. Then, the relationship between structure and function and bioactivity will be figured out. This will create a new road map for drug discovery by molecular docking.

Material and Method

Chemicals: The investigation was conducted using analytical-grade solvents and reagent.

Collection and Identification of Plant

The plant material of *Hygrophila auriculata* was collected during the winter season of 2019- 2020 from the Akola region (MS). After the collection of plant material, the plant specimen was identified taxonomically using the flora of Maharashtra (Karthikeyan and Singh, 2001), and a specimen herbarium was deposited in the Department of Botany, Shri Shivaji College of Arts, Commerce, and Science, Akola.

Preparation of plant material

The collected plant material was cleaned with running water to get rid of adherent dust and other foreign objects, and then they were shade dried for 15 days. The dried leaf and stem were individually homogenized to produce fine, coarse powder. The leaf and stem were extracted with methanol using a Soxhlet continuous extraction and the extract was filtered using What man No. 1 filter paper. The extract obtained was stored in a glass bottle for further analysis.

Fluorescence Analysis

The coarsely powdered samples of leaves and stem (1g each) were treated separately with different reagents (5ml each) such as acids and alkaline solutions along with other solvents inside clean test tubes and shaken well and allowed to stand overnight. The individual solutions were observed under visible light and UV light for their characteristics color and compared with a standard color chart

(Shirsat and Suradkar, 2017; Jasutkar *et al*, 2018) and all the observations were taken as per Aeri *et al*, (2020).

Preliminary phytochemical screening

Chemical tests for screening and identification of bioactive chemical constituents present in the methanolic extract of the leaf and stem of *H. auriculata* were carried out using the standard procedure (Harborne, 1998; Koche et al., 2010; Shirsat et al., 2014).

Thin Layer Chromatography

The sample was loaded. The plates were put in the glass chamber until the solvent was full. Then, the mobile phase was routed through the absorbent phase until it attain 3/4 of the way through the plate. TLC was performed for Alkaloids and Flavonoids. Once the phytoconstituent were separated, certain reagents were sprayed on it. The coloured spots formed on the stationary phase that were marked, identified and measured (Senguttuvan J. 2016). The sample solute is frequently described by the term Retention factor (Rf). It was determined using the following formulas,

$$\text{Rf value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Liquid Chromatography-Mass Spectrometry (LC-MS)

The LC-MS analysis of methanolic leaf and stem extract of *Hygrophila auriculata* was done from Bioanalytical services MIT Technology Business Incubation Centre, Pune.

Result and Discussion

The detailed observations on fluorescent analysis, qualitative phytochemical analysis, TLC and LC-MS analysis of methanolic extracts of *H. auriculata* are presented in the following paragraphs.

Fluorescence Analysis

The powdered material of shade dried leaves and stem of *H. auriculata* was analysed for its physical properties, especially its coloration; untreated as control and after addition of various routine laboratory chemicals under visible light as well as UV light. The results showed that there is drastic color change when the reaction mixture was exposed to both visible and UV light. It is an important criterion to decide the authenticity of the market available crude powdered drug of traditional medicinal plants. The fluorescence characters of leaves and stem powders of *H. auriculata* are noted in **Table 1**.

Qualitative Phytochemical analysis of Methanolic leaf and stem Extract of H. auriculata

The qualitative phytochemical analysis showed that the methanolic leaf and stem extracts of *H. auriculata* are rich in phytochemical composition. Leaf extract

Table 1: Fluorescence Analysis of Leaf and Stem of *H. auriculata*

Particulars		<i>Hygrophila auriculata</i>			
		Leaf		Stem	
Sr. No.	Chemicals	Visible light	UV light	Visible light	UV light
1	D. W	Colourless	Colourless	Colourless	Colourless
2	HCL	Dull green	Florescent green	Dull green	Light green
3	50%HCL	Colourless	Colourless	Colourless	Colourless
4	H ₂ SO ₄	Brown	Dark brown	Blackish brown	Black
5	50% H ₂ SO ₄	Dull green	Light green	Light brown	Florescent green
6	NaOH	Light yellow	Yellowish green	Light yellow	Light green
7	5% FeCl ₃	Light brown	Dull brown	Dull brown	Dull brown
8	50% HNO ₃	Light yellow	Florescent yellow	Light yellow	Light green
9	NaNO ₃	Colourless	Colourless	Colourless	Colourless
10	Hydroquinone	Brown	Florescent green	Dull brown	Green

revealed the presence of cardiac glycosides, terpenoids, reducing sugar, saponins, tannins, and steroids but gave negative result for alkaloids phenolics, and carbohydrates. While the stem extract showed the positive tests for alkaloids, cardiac glycosides, terpenoids, flavonoids, and steroids but gave negative results for reducing sugar, saponins, tannins, phenolics and carbohydrates (**Table- 2**).

Phytochemicals are the biologically active compounds having medicinal properties. The qualitative phytochemistry of plants indicates the basic bioactive compounds present in the plants. Earlier, several workers had presented the phytochemical analysis of different medicinal plants. Ashwathi Shrinivasan & Angayarkanni (2023) reported qualitative phytochemicals from 17 different therapeutically important plants from diverse habitats.

Table: 2. Qualitative phytochemical analysis of methanolic leaf and stem extracts of *Hygrophila auriculata*

Name of the test	Alkaloids	Cardiac glycoside	Terpenoids	Reducing Sugar	Saponins	Tannins	Flavonoids	Phenolics	Steroids	Carbohydrates	Protein and Amino acid
Leaf	-	+	++	+	+	+	-	-	++	-	-
Stem	+	++	+	-	-	-	++	-	+	-	-

Table: 3. Rf value obtained from TLC analysis of Methanolic extract:

Table: RF Values obtained from TLC analysis								
Methanolic Extract								
	No. of spot	1	2	3	4	5	6	7
Leaf	Rf	0.95	0.13	0.11	0.08	0.07	0.06	0.04
Stem	Value	0.16	0.13	0.11				

Thin Layer chromatography of Hygrophila auriculata

The TLC analysis was performed to identify the number of compounds extracted from the powder samples in their methanolic extract. The mobile phase was used as Petroleum ether: Acetone: Formic acid (70:20:10).

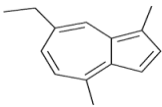
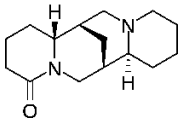
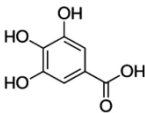
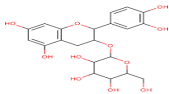
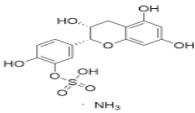
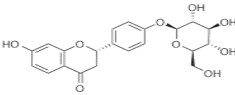
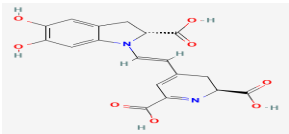
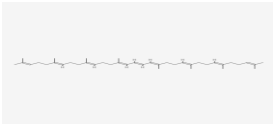
The extract of *H. auriculata* leaf and stem showed the 7 and 3 spot resp. in this stem showed less spot than leaf. Leaf and stem of *H. auriculata* showed two common compounds having Rf value 0.13 and 0.11 respectively (**Table 3**).

LC-MS Analysis of methanolic leaf extract of Hygrophila auriculata

The LC-MS examination of the methanolic leaf extract of *Hygrophila auriculata* leaves revealed a total of 08 compounds, each of which displayed distinct phytochemical properties. The chemical constituents with the retention time (RT), Molecular formula and Molecular weight (MW) were presented in Table 5. The major components present in leaves of *H. auriculata* are Chamazulene,

Lupanine, Digallic acid, (+)-Catechin 3-O-glucose, (-)-Epicatechin 3'-O-sulfate, Liquiritin, Isobetanidin and Phytoene.

Table: 4. The components revealed from the methanolic leaf extract of *Hygrophila auriculata*

Sr. No.	RT (min)	Compound Name	Structure of Compound	Molecular Formula	M.W g/mol	Peak Area %
1.	0.788	Chamazulene		C ₁₄ H ₁₆	184.27 g/mol	42.83
2.	1.253	Lupanine		C ₁₅ H ₂₄ N ₂ O	248.36 g/mol	15.06
3.	2.052	Digallic acid (gallic acid)		C ₇ H ₆ O ₅	170.12 g/mol	10.10
4.	2.416	(+)- Catechin 3-O-glucose		C ₂₁ H ₂₄ O ₁₁	452.4 g/mol	9.80
5.	2.438	(-)- Epicatechin 3'-O-sulfate		C ₁₅ H ₁₄ O ₉ S	370.3 g/mol	12.93
6.	2.753	Liquiritin		C ₂₁ H ₂₂ O ₉	418.4 g/mol	9.28
7.	1.023	Isobetanidin		C ₁₈ H ₁₆ N ₂ O ₈	388.3 g/mol	22.32
8.	2.146	Phytoene		C ₄₀ H ₆₄	544.9 g/mol	10.55

The presence of several bioactive metabolites in *Hygrophila auriculata* leaves support their use by traditional healers for a wide range of ailments.

Accordingly, isolating specific phytochemical components and applying them to biological action will produce an abundance of result.

Conclusion

The Fluorescent analysis of powder was unique and could be used as a marker to note the adulteration in market available crude drug powder. The present study reveals that the methanolic leaf and stem extract of *Hygrophila auriculata* rich in cardiac glycosides, terpenoids and steroids, according to the quantitative phytochemical study. It is evident from the findings of the phytochemical research that *H. auriculata* produces a large number of secondary metabolites with therapeutic significance. LC-MS method is the direct analytical approach for identification of phytocompounds with only few grams of plant extract. The LC MS analysis helped to identify 8 phytocompounds which are known to have antioxidant, anticancer, anti-inflammatory properties. Further, the Pharmacological investigation is essential to confirm the medicinal potential of plant powder and its identified phyto-components.

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References

- [1] Aeri, V., Anantha Narayana, D. B., and Singh, D. (2020). Microscopic identifying features of barks and leaves. In, *Powdered crud drug microscopy of leaves and barks* (Eds. Aeri et al). Elsevier Publication.
- [2] Antony, V. T. (1989). Systematic studies on the Flora of Kottatam District, Kerala. Ph. D. Thesis. Bharathiar University, Coimbatore. 1989.
- [3] Ashwathi Shrinivasan, C, V., and Angayarkanni, D. T. (2023). Qualitative phytochemical analysis of Medicinal Plants Selected from Temperate, Tropical and Hill Regions. *International Journal of Pharmaceutical and Bio-Medical Science*, 3(1): 11-17.
- [4] Chopra, R. N., Nayer, S. L., Chopra, I. C. (1956). Glossary of Indian Medicinal plants, National Institute of Science and Communication. *C. S. I. R Publication*: New Delhi: India:1956. P. 330-2.
- [5] Eazhisavallabi, D., Ambika, R., Venkatalakshmi, P. (2012). Preliminary phytochemical analysis and antimicrobial screening of *Hygrophila spinosa*. *International Journal of Pharm Tech Research*, 4(1): 466-468.
- [6] Gupta, M., Thakur, S., Sharma, A., and Gupta, S. (2013). Qualitative and Quantitative analysis of phytochemicals and pharmacological value of some dye yielding medicinal plants. *Oriental Journal of Chemistry*, 29(2), 475-481.
- [7] Harborne. J. B. (1998). Phytochemical Method: A Guide to Modern Techniques of Plant Analysis. 2nd Edition, *Chapman and Hall Publishers, London*, 1998.

- [8] Jasutkar, J. M., Shirsat, R. P., & Koche, D. K. (2018). Pharmacognostic study, chemical Analysis and Antioxidant Potential of *Leucas indica* L. (R. Br.). *Journal of herbal drugs*, 9 (1), 1-5.
- [9] Koche, D., Shirsat, R., Syed Imran, Bhadange, D. (2010). Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola District (MS) India. *International Journal of Pharma and Bioscience*, 1(4): B253- B256.
- [10] Kshirsagar, A. D., Ingale, K. G., Vyawahare, N. S. and Thorve, V. S. (2010). *Hygrophila spinosa*: A comprehensive review. *Pharmacognosy Reviews*, 4(8): 167.
- [11] Petrovska, B. (2012). Historical review of medicinal plants' usage. *Pharmacogn Rev.* 6(11):1.
- [12] Pola, S., Kadali, V. N. (2018). Medicinal Plants of West Godavari. In: Drug Discovery - Concepts to Market. *InTech*; Publications.
- [13] Senguttuvan, J. and Subramaniam, P. (2016). HPTLC Fingerprints of various Secondary Metabolites in the Traditional Medicinal Herb *Hypochaeris radicata* L. *J Bot.* 2016:1-11.
- [14] Shirsat, R., Suradkar, S. S. and Koche, D. (2014). Preliminary phytochemistry and antimicrobial activity of *Salvia plebeia* R. Br. and *Colebrookea oppositifolia* Smith. *International Journal of Pure and Applied Science and Technology*, 20(1): 21-24.
- [15] Singh, S. A., Gowri, K., Chitra, V. (2020). A Review on Phytochemical Constituents and Pharmacological Activities of the plant: *Aerva lanata*. *Research J. Pharm Technol* 13(3):1580.

