

Chapter 3

Phytochemical and cytotoxicity analysis of flowers of *Hygrophila auriculata* (Schumach.) Heine

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Abstract: Globally, researchers are seeking new pharmacological agents from natural sources. Wetland plants remain largely unexplored in this regard. This study focused on *Hygrophila auriculata*, a wetland plant, to investigate its phytochemical composition and cytotoxicity activity. Phytochemical screening of the hydroethanolic extract of *H. auriculata* flowers revealed a diverse array of bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, and reducing sugars. Additionally, the extract exhibited toxicity against *Artemia salina*, indicating its potential pharmacological applications. These findings highlight the untapped potential of wetland plants like *H. auriculata* as a source of novel bioactive compounds.

Keywords: Natural products, pharmacological agents, wetlands

INTRODUCTION

The growing demand for nature-based medications stems from their minimal side effects, affordability, and chemical-free composition (Sinha *et al.*, 2023; Hossain *et al.*, 2025; Das *et al.*, 2025). However, the increasing prevalence of diverse health issues, the emergence of new diseases, drug failures, antimicrobial resistance, and climate change-related problems necessitate the exploration of novel pharmacological agents (Hebbbar *et al.*, 2024; Bhat *et al.*, 2024). In this context, wetland plants are relatively underexplored, offer immense potential

due to their medicinal, ecological, and food value (Devi *et al.*, 2023). Moreover, these plants play a crucial role in maintaining ecological balance and supporting other organisms. Therefore, investigating wetland plants can raise awareness about their importance and conservation. Therefore, in the present study, flowers of *Hygrophila auriculata* belonging to the Acanthaceae family (Figure 1) were selected for qualitative phytochemical screening and cytotoxicity analysis. *H. auriculata*, a wetland plant growing in marshy areas and wetlands. Traditionally, its plant parts are used for the treatment of jaundice, edema, gastrointestinal ailments, diarrhoea, dysentery, urinogenital disorder, gall stones, urinary calculi, kidney stone, leucorrhoea, rheumatism, tuberculosis, anaemia, body pain, constipation, skin disease, and as an aphrodisiac agent (Sethiya *et al.*, 2018). The present study highlights the importance of flowers for future drug formulation.

MATERIALS AND METHODS

The flowers of *H. auriculata* were collected from Cuttack, Odisha, India (Sethi *et al.*, 2024; Figure 2). The plant species was identified by the authors (Jena *et al.*, 2025). The phytochemical analysis was carried out using different solvents by using standard methods of phytochemical screening (Devi *et al.*, 2024; Jena *et al.*, 2024; Marndi *et al.*, 2024). The detection of nine secondary metabolites was conducted using standard methods (Marndi *et al.*, 2024).

Test for tannin: About 1 ml of the flower extract was taken. 3-5 drops of 10 % lead acetate solution were added to it. The formation of gelatinous precipitate confirmed the positive results for the presence of tannin.

Test for saponin: About 1 ml of the flower extract was taken, and 1 ml of distilled water was added and shaken well. The persistent froth formation confirmed the presence of saponin.

Test for flavonoids: About 1 ml of the flower extract was taken. 2 ml of 2% NaOH solution and then diluted HCl was added to it. The colour initially turned to an intense yellow with the NaOH solution and later became colourless. This colour-changing transformation confirmed the presence of flavonoids.

Test for terpenoids: About 1 ml of the flower extract was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then, 6 drops of concentrated sulphuric acid

were added. The appearance of a reddish-brown interface confirmed the presence of terpenoids.

Test for phenolic compounds: About 1 ml of the flower extract was taken, and a few drops of 5% ferric chloride solution was added to it. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the flower extract was taken, and 2-3 drops of Fehling's solution A and B were added, respectively. Then kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar.

Test for steroids: About 1 ml of the flower extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid were added to it. The appearance of 2 phases, with the upper red and lower yellow with green fluorescence, confirmed the presence of steroids.

Test for alkaloids: About 1 ml of the flower extract was taken, and 3 to 4 drops of Dragendroff's reagent were added. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of the flower extract was taken, and 3 to 4 drops of 2,4-dinitrophenylhydrazine (DNPH) reagent was added. The yellow crystal formation confirmed the presence of carbonyl compounds.



Figure 1: Flowers of *Hygrophila auriculata*

Toxicity to *Artemia salina*: For toxicity analysis, the hatching of brine cysts was the initial process. Standardization of the optimum was done at 2.0, 3.5, and 5.0 % saline concentration to check the optimum salinity for proper hatching of brine shrimp (*Artemia salina*) cysts (Kumar *et al.*, 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration at room temperature or between 28-35 °C and light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of the cysts, proper aeration, and light provided. Different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the flower extract were taken, and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5mg/ml) or potassium dichromate and 3.5% saline water, respectively, with a volume of 2 ml. The live larvae are highly mobile and thus differentiate from the unhatched cysts. The survivor nauplii were counted to estimate the death rate (Kumar *et al.*, 2012).



Figure 2: Collection of flowers of *Hygrophila auriculata* for experimental works

RESULTS AND DISCUSSION

The phytochemical analysis of *H. auriculata* flowers revealed the presence of diverse bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, and reducing sugars (Table 1). The hydro-ethanolic extract of the flowers exhibited a broad

spectrum of phytochemicals, indicating its potential as a source of natural remedies. The cytotoxicity analysis using *Artemia salina* showed that the extract exhibited significant toxicity, with a death rate of 100 % at concentrations of 25-200 mg/ml (Table 2). The presence of diverse bioactive compounds in *H. auriculata* flowers suggests that this plant could be a valuable source of natural remedies for various ailments. The cytotoxicity activity exhibited by the extract against *A. salina* further supports its potential as a pharmacological agent. The results of this study agree with the traditional uses of *H. auriculata* in folk medicine, where it is used to treat various diseases. Overall, this study provides evidence for the medicinal potential of *H. auriculata* flowers and highlights the need for further research to explore its pharmacological activities.

Table 1: Phytochemical analysis of flowers of *Hygrophila auriculata* using hydroethanolic extract

Bioactive compounds	Hydroethanolic extract
Tannin	+ve
Saponin	+ve
Flavonoids	+ve
Terpenoids	-ve
Phenolic compounds	+ve
Reducing sugar	+ve
Steroids	-ve
Alkaloids	-ve
Carbonyl compounds	-ve

(+ve: Presence; -ve: Absence)

Table 2: Cytotoxicity analysis of flowers of *Hygrophila auriculata* using hydroethanolic extract

Extract	Concentration (in mg/ml)	Initial number of Nauplii	Number of deaths of Nauplii (after 1 hour)	Death rates (%)
Hydroethanolic	25	10	10	100
	50	10	10	100
	100	10	10	100
	200	10	10	100
	3.5% Saline	10	0	0
	Vincristine sulphate	10	10	100

CONCLUSION

The present study reveals the phytochemical composition and cytotoxicity activity of *Hygrophila auriculata* flowers, highlighting their potential pharmacological applications. The presence of diverse bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, and reducing sugars, suggests that *H. auriculata* flowers could be a valuable source of natural remedies. The cytotoxicity activity exhibited by the extract against *Artemia salina* further supports its potential as a pharmacological agent. Future studies should focus on isolating and characterizing the bioactive compounds present in *H. auriculata* flowers, as well as evaluating their pharmacological activities in more detail. Additionally, clinical trials are necessary to confirm the safety and efficacy of *H. auriculata* flower extracts for various medicinal applications. Furthermore, conservation efforts should be made to protect *H. auriculata* and other wetland plants, which are facing threats due to habitat destruction and over-exploitation. By exploring the medicinal potential of these plants, we can not only discover new drugs but also contribute to the conservation of these valuable resources.

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