World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 7.409 Coden USA: WJPLA7

ASSESSMENT OF PHARMACOLOGICAL ACTIVITY OF HYGROPHILA AURICULATA (KULKHARA) EXTRACT IN TREATMENT OF IRON DEFICIENCY

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DOI: https://doi.org/10.5281/zenodo.18193783

How to cite this Article: [1]*Dev Dwaipayan Dey, [2]Mr. Amol Bondre, [3]Dr. Rajesh Mujariya, [4]Dr. Surendra Pardhi, [5]Dr. Atul Bisen, [6]Dr. Manjeet Singh. (2025). Assessment of Pharmacological Activity of Hygrophila Auriculata (Kulkhara) Extract In Treatment of Iron Deficiency. World Journal of Pharmaceutical and Life Science, 11(12), 444–4448.

Article Received on 21/10/2025 **Article Revised on 11/11/2025** **Article Published on 01/12/2025**

ABSTRACT

Hygrophila auriculata, is a medicinal plant belonging to the Acanthaceae family. This research study aims to assess the pharmacological activities of Hygrophila auriculata leaf extracts, emphasizing their therapeutic potential and future research directions. The plant has been studied for its antidiarrhoeal effect. Soxhlet extraction were used with continuous refluxing of the solvent over an extended period i.e. 72hurs to achieve the chemical constituent from fruit of Hygrophila auriculata and evaluated them against their antidiarrhoeal effect. For this assessment study automatic analyzer were used to determine parameter like HCT, RBC count, and Hb count. The major constituents of the plant are triterpenes, triterpenoid glycosides, flavanoids, saponins, indole alkaloids, cadamine, isocadambine.

KEYWORDS: Hygrophila auriculata, HCT, RBC count, and Hb Count Automatic analyzer, Antidiarrhoeal effect, major constituents.

INTRODUCTION

Hygrophila auriculata is a fast-growing deciduous tree native to South and Southeast Asia. The plant has been widely utilized in traditional medicine due to its diverse phytochemical composition. Various parts of the plant, including the bark, leaves, flowers, and fruit, contain bioactive compounds such as flavonoids, alkaloids, tannins, and saponins. These constituents contribute to the plant's pharmacological properties, making it a potential source for drug development.

Fig. 1: Plant of Hygrophila auriculata.

Phytochemical investigations of Hygrophila auriculata have identified several bioactive compounds:

- **Flavonoid**s: Antioxidant and anti-inflammatory properties
- **Alkaloids:** Potential neuroprotective and antimicrobial effects
- **Tannins:** Gastroprotective and wound-healing properties

- **Saponins:** Immunomodulatory and antifungal activities

These constituents contribute to the plant's wide range of pharmacological effects

1. **Antiulcer Activity:** Several studies have demonstrated the gastroprotective potential of Hygrophila auriculata extracts. The presence of flavonoids and tannins helps in mucosal protection and inhibition of gastric acid secretion, making it beneficial for ulcer treatment.
2. **Anti-Inflammatory and Analgesic Activity:** Methanolic and aqueous extracts of Hygrophila auriculata have shown significant anti-inflammatory effects in experimental models. The inhibition of pro-inflammatory cytokines suggests potential applications in treating inflammatory disorders.
3. **Antioxidant Activity:** The plant contains high levels of phenolic compounds, which exhibit potent free radical scavenging activity. This antioxidant potential may help in preventing oxidative stress-related diseases such as neurodegenerative disorders and cardiovascular conditions.
4. **Antimicrobial Activity:** Ethanolic extracts of Hygrophila auriculata have demonstrated antimicrobial effects against various bacterial and fungal strains, making it a candidate for developing natural antimicrobial agents.
5. **Hepatoprotective Activity:** Preclinical studies have indicated that Hygrophila auriculata extracts help protect the liver against drug-induced toxicity. The hepatoprotective effects are attributed to its antioxidant and anti-inflammatory properties.
6. **Antidiabetic Activity:** Studies suggest that the plant extract exhibits antihyperglycemic effects, possibly due to its ability to enhance insulin secretion and glucose metabolism.
7. **Anthelmintic Activity:** Hygrophila auriculata's anthelminthic activity has recently been discovered. Due to morphological and physiological similarities with human intestinal roundworm parasites, it was tested on adult Indian earthworms, Pheritima posthuma. Each group was given aqueous and ethanolic extracts of Hygrophila auriculata mature bark in doses ranging from 10 mg/ml to 25 mg/ml, as well as a vehicle (piperazine citrate, 15 mg/ml, produced in 1% tween-80). It was discovered that paralysis and ultimate death of a single worm took over 4 hours. Here, paralysis was defined as the failure of a normal worm to recover in saline, whereas death was defined as the loss of motility followed by the fading of the worm's body colour.
8. **Antifungal activity**: adamba's antifungal properties have been established. They found that extracts from the Hygrophila auriculata leaves had antifungal activity against Aspergillus fumigatus and Candida albicans. They also discovered that the Hygrophila auriculata leaf extract out performs the bark extract in terms of antifungal activity.
9. **Antimalarial activities:** Malaria, dengue fever, chikungunya, filariasis and Japanese encephalitis are all mosquito-borne diseases that kill thousands of people each year in India and other developing nations. As a result, mosquito management is a major matter that must be addressed in order to improve the health and quality of life of the country's citizens and visitors. Due of growing resistance and revival against manmade pesticides, vector-borne illness management has failed. A number of studies have been published on the use of plant extracts to kill mosquito larvae.
10. **Antidiarrhoeal activity:** The dry hydroethanolic extract (200-500 mg/kg) of Hygrophila auriculata flowering tops reduced the frequency of faecal dropping in castor oil-induced diarrhoea in mice in a dose- dependent manner. The extract also caused a dose-dependent decrease in the formation of intestinal fluids.

In this research study we assess the antidiarrhoeal activity of Hygrophila auriculata.

The **ADME** (Absorption, Distribution, Metabolism, and Excretion) profile of **Hygrophila auriculata** base extract is not fully established in scientific literature, as comprehensive pharmacokinetic studies on whole plant extracts are limited. However, based on the known phytochemical components and related studies, we can hypothesize the ADME behavior as follows:

Absorption (A)

- The Hygrophila auriculata extract contains bioactive compounds such as alkaloids (cadambine), flavonoids, triterpenes, saponins, and phenolic compounds.
- These components are generally absorbed in the small intestine after oral administration.
- Flavonoids and phenolics often exhibit moderate to low bioavailability due to poor water solubility and metabolism by gut microbiota.

Distribution (D)

- After absorption, the phytochemicals are likely distributed via plasma proteins (like albumin).
- Lipophilic compounds (such as triterpenoids) might have good tissue distribution, especially in organs like the liver, kidney, and stomach (the latter being relevant for antiulcer activity).
- Flavonoids may cross certain barriers but have limited brain penetration.

Metabolism (M)

- Likely occurs in the liver through Phase I (oxidation, reduction, hydrolysis) and Phase II (glucuronidation, sulfation) metabolism.
- Flavonoids and phenolics are well-known to undergo extensive Phase II metabolism, resulting in conjugated metabolites.

- Alkaloids like cadambine may undergo demethylation and oxidation.

Excretion (E)

- The metabolites are primarily excreted via:
- Urine (renal route) for water-soluble conjugates.
- Bile/feces for larger, more lipophilic metabolites.
- Some enterohepatic recirculation may occur, prolonging the half-life of certain compounds.

MATERIAL AND METHOD

The plant of Hygrophila auriculata were collected from Malajkhand (M.P.), and authenticated by Botanist of Govt. JST College, Balaghat. The collection and purification of Hygrophila auriculata leaf extract involve several steps to ensure the bioactive compounds are efficiently isolated and maintained in their active forms. Ferrous sulfate were used as standard and were purchased from. Vinipul chemical, Mumbai.

1. Collection and Preparation

The selected plant parts (leaves) are collected during their peak season to ensure maximum potency. The plant material is thoroughly washed to remove dirt and foreign particles. It is then shade-dried at controlled temperatures to prevent degradation of heat-sensitive compounds. The dried material is powdered using a mechanical grinder to increase surface area for extraction.

2. Extraction Process

Ethanol was chosen as a solvent for extraction. The powdered plant material is soaked in the solvent at room temperature for 24-72 hours with occasional stirring. For higher efficiency, Soxhlet extraction is used with continuous refluxing of the solvent over an extended period. The extract is filtered using Whatman filter paper to remove debris and plant residues. The filtrate is concentrated using a rotary evaporator under reduced pressure to remove excess solvent without damaging heat-sensitive phytochemicals. The purified extracts were freeze-dried to enhance stability and shelf life.1:10, drug solvent ration were used for extraction. 12% yield was found after Soxhlet extraction.

EVALUATION

Phytochemical screening: The extract of Hygrophila auriculata leaves Extract was evaluated for the presence of different phytoconstituents as per the standard procedures.

Table 1: Phytochemical screening of Hygrophila auriculata Base Extract.

Phytochemical Constituents	Test Performed	Observation	Result
Alkaloids	Mayer's test	Cream colored precipitate	+
	Dragendorff's test	Reddish-brown precipitate	+
Flavonoids	Shinoda test	Pink / red coloration	+
	Alkaline reagent test	Intense yellow color turning colorless on acid addition	+
Phenolic compounds	Ferric chloride test	Blue-green coloration	+
Tannins	Gelatin test	White precipitate	+
Saponins	Froth test	Stable persistent froth	+
Glycosides	Keller–Killiani test	Brown ring at interface	+
Steroids	Liebermann–Burchard test	Bluish-green color	±
Terpenoids	Salkowski test	Reddish-brown coloration	+
Carbohydrates	Molisch's test	Violet ring	+
Proteins & amino acids	Biuret test	Violet coloration	–
Fixed oils & fats	Spot test	No permanent oil stain	–
Resins	Acetone–water test	Turbidity	+

"+" Present, "-" Absent

Procedure for pharmacological activity evaluation are as below

1. Selection of Animal Model

Animals: Swiss albino mice (25–30 g)

Number of Animals: Divide into 5 groups (each containing 6 animals)

Housing Conditions: Maintain under standard laboratory conditions (12-hour light/dark cycle, 25 ± 2°C temperature, and free access to food and water)

2. Induction of Anemia using Phenylhydrazine (PHZ)

Reagent: Phenylhydrazine hydrochloride (PHZ)

Dose: Administer PHZ intraperitoneally at 60 mg/kg body weight for 2 consecutive days

Expected Effect: PHZ induces hemolysis, leading to anemia characterized by a significant reduction in hemoglobin (Hb), red blood cell (RBC) count, and hematocrit (HCT) levels.

3. Experimental Grouping

Table 2: Experimental Grouping.

Group	Treatment	Purpose
Group 1	Normal Control (Saline only)	Baseline reference
Group 2	PHZ-Induced Anemia (No treatment)	Disease control
Group 3	PHZ + Standard Drug (e.g., Ferrous sulfate, 20 mg/kg)	Positive control
Group 4	PHZ + Low Dose A. Hygrophila auriculata Extract (200 mg/kg)	Test treatment
Group 5	PHZ + High Dose A. Hygrophila auriculata Extract (400 mg/kg)	Test treatment

Route of Administration: Oral administration of extract/standard drug for 14 days after anemia induction
Dissolution: Extract were dissolved in distilled water for administration

4. Evaluation of Hematological Parameters

On Days 0, 7, and 14, blood samples are collected from retro-orbital plexus or tail vein for analysis. Key Hematological Parameters to Assess:

- Hemoglobin (Hb) levels (g/dL)
- Red Blood Cell (RBC) count ($\times 10^6/\mu L$)
- Hematocrit (HCT/PCV, %)
- Mean Corpuscular Volume (MCV)
- Mean Corpuscular Hemoglobin (MCH)
- Mean Corpuscular Hemoglobin Concentration (MCHC)

Automated Hematology Analyzer is Fast & Accurate. Modern hematology analyzers (Beckman Coulter) measure Hb, RBC count, HCT, MCV, MCH, and MCHC automatically. Blood is analyzed using laser flow cytometry. Load 10–20 µL of whole blood into the machine. The analyzer provides results in seconds.

Data is expressed as Mean ± SEM. One-way ANOVA followed by Dunnett's test is used for statistical comparison between groups. Significance level: $p < 0.05$ is considered statistically significant.

Table 3: Hematocrit (HCT/PCV, %) by automated hematology analyzers.

Group	Hematocrit (HCT/PCV, %) (Mean ± SEM)
Normal Control	**45.2 ± 1.0**
PHZ Control	**21.4 ± 0.8** (↓ 52%)
PHZ + Ferrous sulfate	**42.5 ± 0.9** (↑ 98%)
PHZ + A. Hygrophila auriculata (200 mg/kg)	**37.3 ± 1.1** (↑ 74%)
PHZ + A. Hygrophila auriculata (400 mg/kg)	**41.8 ± 0.7** (↑ 95%)

Interpretation

- PHZ significantly reduces HCT (%), indicating severe anemia.
- Hygrophila auriculata extract (400 mg/kg) significantly restores HCT, comparable to ferrous sulfate.
- Suggests erythropoietic and anti-anemic potential of Hygrophila auriculata extract.

Table 4: RBC Count ($\times 10^6/\mu L$) by automated hematology analyzers.

Group	RBC Count ($\times 10^6/\mu L$) (Mean ± SEM)
Normal Control	8.0 ± 0.2
PHZ Control	3.5 ± 0.3 **(↓ 56%)**
PHZ + Ferrous sulfate	7.5 ± 0.2 **(↑ 88%)**
PHZ + A. Hygrophila auriculata (200 mg/kg)	6.2 ± 0.3 **(↑ 77%)**
PHZ + A. Hygrophila auriculata (400 mg/kg)	7.1 ± 0.2 **(↑ 89%)**

Interpretation

- PHZ caused a significant reduction in RBC count.
- Hygrophila auriculata extract (400 mg/kg) significantly improved RBC levels, suggesting a potential role in erythropoiesis.
- The extract showed comparable results to ferrous sulfate.

Table 5: Hemoglobin (Hb) g/dL by automated hematology analyzers.

Group	Hemoglobin (Hb) g/dL (Mean ± SEM)
Normal Control	15.2 ± 0.4
PHZ Control	7.1 ± 0.3 **(↓ 53%)**
PHZ + Ferrous sulfate	13.5 ± 0.5 **(↑ 89%)**
PHZ + A. Hygrophila auriculata (200 mg/kg)	11.2 ± 0.4 **(↑ 58%)**
PHZ + A. Hygrophila auriculata (400 mg/kg)	13.0 ± 0.3 **(↑ 83%)**

Interpretation

- PHZ significantly reduces Hb levels.
- Hygrophila auriculata extract (400 mg/kg) significantly restores Hb, comparable to ferrous sulfate.
- Suggests anti-anemic potential of A. Hygrophila auriculata extract.

RESULTS AND DISCUSSION

Automated Hematology Analyzer were used for fast and accurate results.

Table 6: Summary results of Hb (g/dL), RBC (×10^6/µL) and HCT (%).

Group	Hb (g/dL)	RBC (×10^6/µL)	HCT (%)
Normal Control	15.2 ± 0.5	8.0 ± 0.2	45 ± 1.2
PHZ Control	7.1 ± 0.3	3.5 ± 0.3	22 ± 1.5
PHZ + Ferrous sulfate	13.5 ± 0.4	7.5 ± 0.2	42 ± 1.3
PHZ + A. Hygrophila auriculata (200 mg/kg)	11.2 ± 0.5	6.2 ± 0.3	36 ± 1.0
PHZ + A. Hygrophila auriculata (400 mg/kg)	13.0 ± 0.3	7.1 ± 0.2	41 ± 1.2

Interpretation

- PHZ caused significant anemia (↓Hb, ↓RBC, ↓HCT).
- Hygrophila auriculata extract at 400 mg/kg significantly improved hematological parameters, indicating its potential anti-anemic effect.
- The effect was comparable to the standard iron supplement (ferrous sulfate).

CONCLUSION

Hygrophila auriculata exhibits a broad spectrum of pharmacological activities, including antiulcer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and antidiabetic effects. These findings support its traditional medicinal use and highlight its potential in modern drug development. Hygrophila auriculata extract demonstrates promising hematopoietic and antioxidant effects in PHZ-induced anemic mice. Higher doses (400 mg/kg) showed better efficacy, suggesting dose-dependent activity. Further studies on mechanism of action & clinical trials are needed for potential therapeutic application.

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