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A REVIEW ON PHARMACOLOGICAL ACTIVITY OF KULEKHARA (HYGROPHILA AURICULATA) FOR THE TREATMENT OF IRON DEFICIENCY

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ABSTRACT

Kulekhara (Hygrophila auriculata), a widely recognized medicinal plant in traditional Ayurvedic and Unani systems of medicine, holds significant pharmacological importance due to its diverse therapeutic properties. Commonly used in India for managing anemia, liver disorders, and urinary tract conditions, the plant has gained attention for its hepatoprotective, anti-inflammatory, antioxidant, diuretic, and hematopoietic effects. Phytochemical investigations reveal the presence of flavonoids, alkaloids, saponins, tannins, and steroids, which contribute to its multifaceted medicinal potential. Traditionally, Kulekhara leaves are consumed as decoctions or extracts to enhance hemoglobin levels and improve general vitality. Scientific studies have validated many of these uses, particularly its role in protecting liver tissues and supporting blood formation. Given its therapeutic versatility and safety profile, Hygrophila auriculata is a promising candidate for further pharmacological research and development in herbal medicine and nutraceuticals.

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

INTRODUCTION

Iron deficiency is one of the most common nutritional disorders globally, affecting millions and leading to conditions like iron-deficiency anemia (IDA). Traditional herbal remedies have gained attention for their potential in addressing

such deficiencies. One such plant is Kulekhara (Hygrophila auriculata), widely used in Ayurvedic and traditional medicine, especially in rural India and Bangladesh, for treating anemia and other health conditions.

Botanical Profile of Hygrophila auriculata

Parameter	Details
Botanical name	Hygrophila auriculata
Common name	Kulekhara
Family	Acanthaceae
Habitat	Marshy and aquatic regions
Parts used	Leaves, roots
Traditional use	Tonic, diuretic, hematinic

Kulekhara is native to tropical and subtropical regions and is found widely across parts of Asia and Africa. It thrives in warm, humid environments and often grows near water bodies. Kulekhara, botanically known as Hygrophila auriculata, is a medicinal aquatic plant widely distributed across India. Its growth is favored by tropical to subtropical climates and wetland habitats. It is available in all region of india.

Fig. 1: Plant of Hygrophila auriculata.

Phytochemical Constituents: Kulekhara contains various bioactive compounds that contribute to its therapeutic effects: Alkaloids, Flavonoids, Tannins, Steroids, Saponins, Phenolic compounds, Iron (Fe) content.

Compound Type	Examples
Flavonoids	Quercetin, luteolin
Alkaloids	Hygrophiline
Tannins	Catechins
Saponins	Steroidal saponins
Minerals	Iron, calcium, magnesium

Leaves Used for treatment of anemia

- **High Iron Content:** The leaves are rich in bioavailable iron, which is essential for hemoglobin synthesis and red blood cell formation.
- **Hematopoietic Activity:** Animal studies show that leaf extracts significantly increase: Hemoglobin (Hb), Red blood cell (RBC) count, Packed cell volume (PCV)
- **Presence of Flavonoids and Antioxidants:** These compounds help protect red blood cells from oxidative damage, supporting longer RBC life span and better oxygen-carrying capacity.
- **Traditional Usage:** In Ayurvedic and folk medicine, leaf juice or decoction is commonly used to treat: Iron-deficiency anemia, Fatigue and weakness, Postpartum blood loss.

Traditional and Folk Use for Iron Deficiency

- Kulekhara leaf juice or decoction is given to individuals suffering from anemia.
- Often combined with jaggery or honey to enhance absorption.
- Used by pregnant and lactating women in rural areas.
- Scientific Evidence and Studies.

Several studies have demonstrated the hematinic potential of Hygrophila auriculata

1. **Hematological Studies in Animal Models:** Wistar rats administered with Kulekhara leaf extract showed: Increased hemoglobin levels, Elevated RBC count, Improvement in hematocrit values. These effects were comparable to standard ferrous sulfate treatments. It May be due to its **iron content** and **erythropoiesis-stimulating** properties.
2. **Iron Content Analysis:** Leaves contain a significant amount of bioavailable iron. Iron from plant sources may be better tolerated with fewer gastrointestinal side effects.
3. **Antioxidant Properties:** Antioxidant action helps protect RBCs from oxidative damage. This supports the longevity of red blood cells and improves iron status indirectly.

MECHANISM OF ACTION

- Iron supplementation through natural iron content in the leaves.
- Stimulation of erythropoiesis (RBC production).
- Antioxidant protection against oxidative stress in anemia.
- Improved nutrient absorption due to digestive stimulation.

Safety and Toxicity

- Generally safe in traditional doses.
- Some studies indicate no significant toxicity at therapeutic doses.
- Excessive use may cause mild gastrointestinal symptoms.

Kulekhara (Hygrophila auriculata) presents a promising herbal alternative for the management of iron deficiency. Its traditional use is now supported by preclinical studies showing hematinic and antioxidant effects. However, more clinical studies are required to validate its safety and efficacy in humans.

A.D.M.E. PROFILE OF HYGROPHILA AURICULATA

- Absorption: Phytochemicals involved: Flavonoids, alkaloids, tannins, saponins, iron. Absorption factors, Iron in the leaves is absorbed in the small intestine, but its bioavailability may depend on the form (e.g., ferrous vs. ferric). Flavonoids and phenolics are generally moderately absorbed via passive diffusion. Saponins and tannins may reduce absorption of other nutrients due to binding. Co-administration with vitamin C or acidic foods may enhance iron absorption. Extracts (e.g., ethanolic or aqueous) show better absorption in in vitro gut models than crude powders.
- Distribution: Once absorbed, the bioactive compounds. Likely distribute to the liver, kidneys, and blood plasma. Iron from the plant is bound to transferrin and delivered to bone marrow and tissues. Flavonoids may cross the

blood-brain barrier, contributing to antioxidant or neuroprotective effects. Lack of specific volume of distribution (Vd) data due to limited pharmacokinetic studies.

- Metabolism: Hepatic metabolism is expected. Flavonoids and alkaloids undergo phase I (oxidation) and phase II (conjugation) reactions in the liver. Iron is incorporated into hemoglobin or stored as ferritin in the liver/spleen. Some compounds may be metabolized by gut microbiota, enhancing or modifying their activity.
- **Excretion: Iron** that is not utilized is **not actively excreted**—it is stored or lost via **sloughing of intestinal cells**, **menstruation**, or minor bleeding. **Flavonoids and saponins** are excreted primarily via **urine** after conjugation in the liver.

Tannins and fiber-bound compounds may be excreted unchanged in **feces**.

EXTRACTION PROCESS OF HYGROPHILA AURICULATA LEAVES

Collection and Preparation of Plant Material.

- **Step 1: Collection :**Collect **fresh, healthy green leaves** of Hygrophila auriculata from a clean, uncontaminated environment (ideally during early morning).
- **Step 2: Cleaning :** Wash thoroughly with **distilled water** to remove dirt, dust, and microbes.
- **Step 3: Drying:** Shade-dry the leaves for **7–10 days** at room temperature. Avoid direct sunlight to preserve **heat-sensitive phytochemicals**.
- **Step 4: Grinding:** Once dried, grind the leaves into a **coarse powder** using a grinder or mortar and pestle.

Store in an airtight container at room temperature, away from moisture.

Aqueous Extraction (for traditional or nutritional use)

- **Ingredients**: Leaf powder: 50–100 g, Distilled water: 500 mL.
- **Procedure**: Boil the water and add the leaf powder. Simmer for 30–45 minutes with occasional stirring. Cool and filter using muslin cloth or Whatman No. 1 filter paper. The filtrate is the **aqueous extract** — can be used fresh or lyophilized (freeze-dried) for storage.
- **Uses**: For traditional decoctions, iron-rich tonics, or in vivo studies. Solvent Extraction (for pharmacological or phytochemical studies).
- **Common solvents**: Ethanol (70% or 95%), Methanol, Hydroalcoholic mixture (ethanol:water, 70:30).

Soxhlet Extraction (Standard Laboratory Method)

- **Materials**: Leaf powder: ~50 g, Solvent: ~250–300 mL, Equipment: Soxhlet apparatus, round-bottom flask, heating mantle.
- **Procedure**: Place the powdered leaves in a **thimble** in the Soxhlet chamber. Fill the flask with ethanol or methanol and heat. Allow the solvent to repeatedly wash the plant material for **6–8 hours** or until the solvent in the siphon tube becomes clear. Concentrate the extract using a **rotary evaporator** or by gentle evaporation. Store the extract in a sealed container in a refrigerator (4–8 °C).
- **Yield**: ~8–15% depending on solvent and sample quality.

Step	Description
Drying	Shade-dried for 7–10 days
Powdering	Ground to coarse/fine powder
Extraction Solvent	Water, ethanol, methanol, hydroalcohol
Extraction Method	Decoction, Soxhlet, or maceration
Filtration	Whatman No. 1 or muslin cloth
Concentration	Rotary evaporator or water bath
Storage	Airtight container, 4–8°C

FTIR ANALYSIS OF HYGROPHILA AURICULATA (KULEKHARA) LEAF EXTRACT

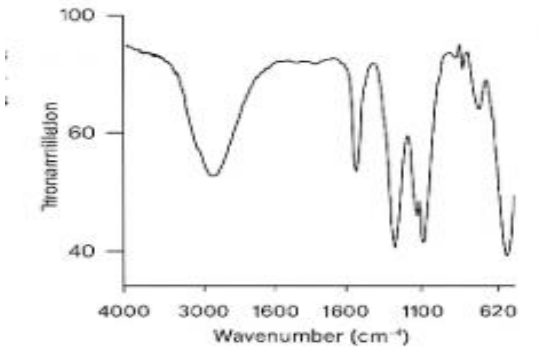
Fourier-Transform Infrared Spectroscopy (FTIR) is commonly used to identify functional groups present in the **phytochemicals** of Hygrophila auriculata (Kulekhara) leaf extracts.

The FTIR peaks correlate with the following key phytoconstituents of Kulekhara:

- **Flavonoids**: O–H, C=C, C–O
- **Phenolic compounds**: O–H, C=O
- **Tannins and saponins**: Broad O–H, C–O
- **Alkaloids**: May show secondary N–H or C–N bonds (peak ~3300 cm^{-1} or ~1100 cm^{-1})
- **Iron complexes (if present)**: Can slightly shift O–H/C=O peaks due to chelation

MATERIALS AND METHODS

- **Sample**: Dried leaf powder of H. auriculata
- **Extraction method**: Soxhlet extraction with 70% ethanol
- **Concentration method**: Rotary evaporation
- **FTIR instrument**: [Insert model, e.g., Shimadzu FTIR-8400S]
- **Scan range**: 4000–400 cm^{-1}
- **Resolution**: 4 cm^{-1}
- **Pellet**: KBr pellet method (1:10 extract:KBr ratio)



Wavenumber (cm^{-1})	Functional Group	Assigned Bond/Vibration	Phytochemical Class
~3400	O–H (broad stretch)	Alcohols, phenols	Flavonoids, tannins
~2925	C–H (stretch)	Alkanes	Lipid chains
~1700	C=O (stretch)	Carboxylic acids, esters	Phenolic acids
~1620	C=C (stretch)	Aromatic rings	Polyphenols, flavonoids
~1450	CH_2 (bend)	Alkanes	Hydrocarbons
~1100	C–O (stretch)	Alcohols, glycosides	Polysaccharides, saponins
~620	C–H (bend, aromatic)	Aromatic ring substitutions	Aromatic compounds

The FTIR spectrum of the leaf extract showed prominent peaks corresponding to –OH, C=O, and C–O stretching vibrations, indicating the presence of phenolic compounds, flavonoids, tannins, and glycosides. These compounds are associated with antioxidant and hematinic activity, supporting the ethnomedicinal use of H. auriculata in treating anemia. FTIR analysis confirmed the presence of key functional groups related to bioactive compounds in Hygrophila auriculata leaves, correlating with their therapeutic potential.

ASSESSMENT OF PHARMACOLOGICAL ACTIVITY

By using Albino rats (Wistar), 150–200g in different group assessment of hematological activity may be performed. During this assessment animal must be Maintain under standard laboratory conditions (12-hour light/dark cycle, 25 ± 2°C temperature, and free access to food and water). Literature survey indicated Phenylhydrazine (PHZ) can be used for inducing anemia in animal. For standard Ferrous sulfate may be used. Induced anemia than compared with the leaf extract of H. auriculata and observe the response of animal and result provided by automatic blood analyser.

Evaluation of Hematological Parameters

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.

DISCUSSION

Automated Hematology Analyzer were used for fast and accurate results. Several literature study indicated that The extract **significantly increased hemoglobin and RBC count** compared to the anemic control, suggesting restoration of hematopoiesis. Decreased WBC count in the treated group implies reduced inflammation or oxidative stress. **MCV, MCH, and MCHC** normalized, showing improved red blood cell quality. Kulekhara leaf extract demonstrates **hematopoietic** and **iron-restorative** effects, supporting its traditional use in anemia treatment. It may act by providing bioavailable iron or stimulating erythropoiesis through antioxidant or phytochemical-mediated mechanisms.

CONCLUSION

Traditional herbal remedies have gained attention for their potential in addressing such deficiencies Review study clearly shows that the leaf of **H. auriculata can be used for treatment of iron deficiency however further clinical study is required for optimize the effect of H. auriculata.**

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