World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

REVIEW ON PHARMACOLOGICAL ACTIVITY OF ANTHRACLUS CADAMBA BASE EXTRACT IN PHENYL HYDRAZINE INDUCED ANEMIA

Omprakash B. Ukey[1]*, Amol Bondre[2], Rajesh Mujariya[3] and Manjeet Singh[4]

[1]Research Scholar of Institute of Pharmaceutical Science & Research, Balaghat (M.P.)
[2]Associate Professor of Institute of Pharmaceutical Science & Research, Balaghat (M.P.)
[3]Principal & Professor of Institute of Pharmaceutical Science & Research, Balaghat (M.P.)
[4]Executive Director of Institute of Pharmaceutical Science & Research, Balaghat (M.P.)

***Corresponding Author: Omprakash B. Ukey**
Research Scholar of Institute of Pharmaceutical Science & Research, Balaghat (M.P.)

Article Received on 18/04/2025 **Article Revised on 08/05/2025** **Article Accepted on 28/05/2025**

ABSTRACT
Anthocephalus cadamba, commonly known as Kadamba, is a medicinal plant belonging to the Rubiaceae family. Traditionally, it has been used in Ayurveda and folk medicine to treat various ailments, including fever, inflammation, digestive disorders, and skin diseases. This review aims to summarize the pharmacological activities of Anthocephalus cadamba-based extracts, emphasizing their therapeutic potential and future research directions. The plant has been studied for its different pharmacological activities, for example, antimicrobial, antidiabetic, sedative, antiepileptic, antioxidant, antitumor, analgesic, antipyretic, anti-inflammatory, diuretic, laxative, hepatoprotective, anthelmintic, wound healing and antidiarrhoeal. This article were focused on extraction of chemical constituent from fruit of kadamab and evaluated them against their antidiarrhoeal effect. This study cover the pharmacological activity evaluation process for HCT, RBC count, Hb, MCV, MCH, MCHC manually as well as by automatic analyzer. The major constituents of the plant are triterpenes, triterpenoid glycosides, flavanoids, saponins, indole alkaloids, cadamine, isocadambine. Here we have put forth an attempt to sum up all the phytochemicals and their significance to render the intrigue that would help in their commercialization.

KEYWORDS: Anthocephalus cadamba, Traditional uses, Pharmacological Effect, Chemical Constituents, Kadamba, HCT, RBC count, Hb determination.

INTRODUCTION

Anthocephalus cadamba 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: Flower of Anthocephalus cadamba.

Part usage of anthocephalus cadamba

Different parts of Anthocephalus cadamba are used for medicinal and therapeutic purposes:

Bark: Used for its anti-inflammatory, antimicrobial, and hepatoprotective properties.

Leaves: Known for antioxidant, analgesic, and wound-healing effects.

Flowers: Traditionally used in treating fever, infections, and as an aphrodisiac.

Fruits: Beneficial for digestive disorders and have potential antidiabetic properties.

Roots: Used in treating skin diseases and gastrointestinal issues.

Significance of kadamba tree

Anthocephalus cadamba is a bigger tree with a height of 20-45metres and a trunk diameter of 100-160 cm. It has a broad crown and a straight cylindrical bole. At the age of four, kadam may begin to flower. It blooms in India from July to December. Flowers are bisexual.

Bark: The bark of the young tree is smooth and light, whereas the bark of the older tree is tough. The bark is used to treat infections on the skin. When Anthocephalus cadamba bark is combined with water, honey and cumin, it is used to treat hoarseness of the throat (zeera). Orally, it is given to the patient. The use of freshwater for bathing, which keeps the skin smooth and free of infection.

Leaf: Leaves are glossy green, opposite, simple, more or less sessile to etiolate, ovate to elliptical and more or less sessile to etiolate (15-50 x 8-25 cm). Clustered inflorescence with terminal globose heads without bracteoles and sub sessile fragrant orange or yellow flowers; Flowers are bisexual and 5-merous, with a funnel-shaped calyx tube and a gamopetalous saucer-shaped corolla with a narrow tube and narrow lobes that imbricate in the bud. Stamens 5, filaments short and anthers basified, inserted on the corolla tube. Ovary inferior, binocular, sometimes 4-locular in the upper section, with a spindle-shaped stigma and style extruded. Fruits have four hollow or solid structures in their upper portions, allowing them to grow in numbers. Seeds that are trigonal or irregular in form.

Flower: The blooms are small, orange-colour and arranged in a globose head with a diameter of 3-5 cm. Flowers are bisexual and 5-merous, with a funnel-shaped calyx tube and a gamopetalous saucer-shaped corolla with a narrow tube and narrow lobes that imbricate in the bud. Stamens 5, filaments short, anthers basifixed, placed on the corolla tube. Ovary inferior, binocular, sometimes 4-locular in the upper section, with a spindle-shaped stigma and style extruded. Vegetables are made from flowers.

Fruit: Fruits are abundant, with four hollow or solid structures in their upper portions. When ripe, the fruits are meaty, orange, globose pseudocarps 5-7 cm in diameter and yellow.

Phytochemical constituents present in kadamb

Phytochemical investigations of Anthocephalus cadamba have identified several bioactive compounds:

- **Flavonoids:** 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.

Base extract Collection and Purification process

The collection and purification of Anthocephalus cadamba extract involve several steps to ensure the bioactive compounds are efficiently isolated and maintained in their active forms.

1. Collection and Preparation

- The selected plant parts (bark, leaves, flowers, or fruits) are harvested 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

- **Solvent selection:** Depending on the targeted bioactive compounds, different solvents such as ethanol, methanol, water, or hydroalcoholic mixtures are used.
- **Cold maceration:** The powdered plant material is soaked in the solvent at room temperature for 24-72 hours with occasional stirring.
- **Soxhlet extraction:** For higher efficiency, Soxhlet extraction is used with continuous refluxing of the solvent over an extended period.
- **Ultrasound-Assisted extraction (UAE):** Ultrasonic waves are applied to enhance cell wall rupture and improve yield.
- **Supercritical fluid extraction (SFE):** A high-efficiency method using supercritical CO_2 to extract non-polar bioactive compounds.

3. Filtration and Concentration

- 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.

4. Purification and Standardization

- **Liquid-Liquid partitioning:** Used to separate different classes of compounds based on polarity.

- **Chromatographic techniques**

Thin Layer Chromatography (TLC) for preliminary analysis.
Column Chromatography for large-scale purification.
High-Performance Liquid Chromatography (HPLC) for precise compound separation and quantification.
Freeze-Drying (Lyophilization): The purified extract is freeze-dried to enhance stability and shelf life.

Pharmacological activities

1. **Antiulcer activity:** Several studies have demonstrated the gastroprotective potential of Anthocephalus cadamba 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 Anthocephalus cadamba 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 Anthocephalus cadamba 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 Anthocephalus cadamba 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:** Cadamba'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 Cadamba 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 Cadamba's bark and leaves had antifungal activity against Aspergillus fumigatus and Candida albicans. They also discovered that the Cadamba 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 Anthocephalus cadamba 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.

Anti diarrhoeal activity evaluation procedure

- **Assessment Procedure of Hemoglobin (Hb) Levels (g/dL) from Retro-Orbital Plexus or Tail Vein Blood Collection**

The hemoglobin (Hb) level is a key parameter in assessing anemia and the efficacy of Anthocephalus cadamba extract in phenylhydrazine-induced anemia models. Below is a step-by-step procedure for blood collection, processing, and hemoglobin measurement.

1. **Blood collection methods:** Blood samples are collected either from the retro-orbital plexus (for rats and mice) or the tail vein (less invasive alternative).

A. Retro-Orbital Plexus Blood Collection (Common for Rats & Mice) Procedure

Animal preparation: Lightly anesthetize the rat/mouse using isoflurane or ketamine-xylazine mixture to minimize pain and distress. Place the animal in a restrainer, ensuring stability and head positioning.

Collection method: Use a sterile capillary tube (heparinized or non-heparinized). Insert the capillary tube at a 45° angle into the medial canthus (inner corner

of the eye). Apply gentle rotational pressure until blood appears in the tube. Collect 0.5 to 1 mL of blood into a pre-labeled EDTA-coated tube (to prevent clotting).

Post-Collection care: Apply gentle pressure with sterile gauze to stop bleeding. Return the animal to its cage and monitor until full recovery from anesthesia.

B. Tail vein blood collection (Less invasive alternative) Procedure

Animal preparation: Warm the tail by placing the animal under a heating lamp or dipping the tail in warm water (37-40°C for 1–2 min) to dilate blood vessels. Restrain the animal gently in a holding device.

Collection method: Use a 23G or 25G needle with a syringe or microhematocrit tube. Make a small lateral puncture on the tail vein (about 1 cm from the tip). Collect 0.3–0.5 mL of blood into an EDTA tube.

Post-Collection care: Apply sterile gauze or petroleum jelly to stop bleeding. Monitor the animal for signs of distress.

Hemoglobin (Hb) Measurement Methods

Cyanmethemoglobin Method (Standard Lab Method)

Principle: Hemoglobin (Hb) reacts with Drabkin's reagent to form a stable cyanmethemoglobin complex, which is measured spectrophotometrically.

Procedure

Reagents required: Drabkin's reagent (contains potassium ferricyanide and potassium cyanide)
Distilled water, Blood sample (collected in EDTA-coated tube)

Steps

- Take 5 mL of Drabkin's reagent in a test tube.
- Add 20 µL of blood sample.
- Mix well and let it stand for 5–10 minutes at room temperature.
- Measure absorbance at 540 nm using a UV-visible spectrophotometer.
- Compare absorbance values against a hemoglobin standard curve.

Calculation

Use the formula: Hb (g/dL) = (Absorbance of sample/Absorbance of standard)×Standard Hb

- **Assessment Procedure of Red Blood Cell (RBC) Count (×10^6/µL) from Retro-Orbital Plexus or Tail Vein Blood Collection**

The red blood cell (RBC) count is a crucial hematological parameter for evaluating anemia and the therapeutic potential of Anthocephalus cadamba extract in phenylhydrazine-induced anemia models. Below is a step-by-step guide to blood collection, processing, and RBC measurement.

RBC Count Measurement Methods

A. Manual RBC Count Using a Hemocytometer (Neubauer Chamber)

Principle: The diluted blood sample is placed in a Neubauer hemocytometer chamber, and RBCs are counted under a microscope.

Reagents & Materials

Hayem's or Dacie's RBC diluting fluid, Contains sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and mercuric chloride, $HgCl_2$), Neubauer hemocytometer, Microscope, Blood sample (EDTA tube)

Procedure

Dilution of blood sample

- Take 20 µL of whole blood**.**
- Add it to 4 mL of RBC diluting fluid (1:200 dilution).

- **Loading the Hemocytometer**
- Place a coverslip over the Neubauer chamber.
- Load 10 µL of diluted blood into the chamber using a micropipette.
- Allow cells to settle for 2 minutes.

Counting RBCs

- Use a light microscope (40× objective).
- Count RBCs in 5 large squares of the Neubauer grid.

Calculation

Use the formula: RBC (×106/µL)= Number of cells counted× Dilution factor× 10Area counted (mm2) × Depth (mm)

- **Assessment Procedure of Hematocrit (HCT/PCV, %) from Retro-Orbital Plexus or Tail Vein Blood Collection**

Hematocrit (HCT), also known as Packed Cell Volume (PCV), is a key hematological parameter used to evaluate anemia and erythropoietic activity. It represents the percentage of red blood cells (RBCs) in whole blood and is essential for assessing the effects of Anthocephalus cadamba extract in phenylhydrazine-induced anemia models.

Hematocrit (HCT/PCV) Measurement Methods

A. Microhematocrit Method (Gold Standard)

Principle: Blood is centrifuged in a capillary tube, separating RBCs from plasma. The RBC percentage in total blood volume represents HCT (%).

Reagents & Materials: Heparinized microcapillary tubes, Microhematocrit centrifuge, Hematocrit reader scale.

Procedure

Filling the capillary tube

- Fill a heparinized capillary tube with blood up to two-thirds of its length.

- Seal the tube's open end with clay or wax.

Centrifugation

- Place the tubes in a microhematocrit centrifuge.
- Centrifuge at 12,000 rpm for 5 minutes.
- Reading Hematocrit Value
- After centrifugation, three layers are visible:

Bottom layer → Packed RBCs
Middle layer (Buffy coat) → WBCs & platelets
Top layer → Plasma

- Measure the height of the RBC column using a hematocrit reader scale.
- Express results as HCT (%).

Calculation

HCT(%)=(Height of RBC column/
Total height of blood column)×100

Automated hematology analyzer (Faster & More accurate)

Manual method is time consuming. Modern hematology analyzers (e.g., Sysmex, Mindray, or Beckman Coulter) use impedance-based or laser flow cytometry for RBC counting.

Procedure

- Load 10–20 µL of whole blood into the analyzer.
- The machine provides RBC count, Hb, HCT, MCV, MCH, and MCHC within seconds.

Future prospects

Despite promising pharmacological evidence, further clinical studies are needed to validate the efficacy and safety of Anthocephalus cadamba-based extracts in humans. Standardization of extraction methods and identification of active compounds will be crucial for its therapeutic application.

CONCLUSION

Anthocephalus cadamba 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. Further research is warranted to explore its clinical applications and molecular mechanisms of action. Anthocephalus cadamba extract demonstrates promising hematopoietic and antioxidant effects in PHZ-induced anemic rats/mice. Manual method is time consuming hence now a days automated analyser is used to determine RBC count, Hb, HCT, MCV, MCH, and MCHC within seconds. 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.

REFERENCE

1. Dwevedi, Alka, Kuldeep Sharma, and Yogesh K. Sharma. "Cadamba: A miraculous tree having enormous pharmacological. implications." Pharmacognosy reviews, 2015; 9, 18: 107.
2. Bhumakar, H., and Dr Sailesh Naryan. "A Review on: Pharmacological Activity of Anthocephalus cadamba." World Journal of Pharmaceutical Research, 2018; 7, 16: 383-394.
3. Dubey, Atul, Satish Nayak, and D.C. Goupale. "A review on phytochemical, pharmacological and toxicological studies on Neolamarckia cadamba." Der. Pharm. Let, 2011; 3: 45-54.
4. Mondal, Sumanta, et al. "“Haripriya” god's favorite: Anthocephalus cadamba (Roxb.) Miq.- At a glance." Pharmacognosy Research, 2020; 12, 1: 1.
5. Hassan, Md. Asif, et al. “Evaluation of analgesic, anthelmintics and cytotoxic potential activity of barks of Anthocephalus cadamba.” International Journal of Innovative Pharmaceutical Sciences & Research, 2013; 1: 99 – 107.
6. Kumar, Vishnu. "Medicinal Properties of Anthocephalus indicus (Kadam): An Indigenous Medicinal Plant." Era's J Med Res, 2017; 4: 7-11.
7. Yadav, K. N., et al. "Herbal Remedy For Wound Management: A REVIEW." IJP, 2018; 5(8): 440-449.
8. Alam, Gulzar, Manjul Pratap Singh, and Anita Singh. "Wound healing potential of some medicinal plants." International journal of Pharmaceutical sciences Review and Research, 2011; 9, 1: 136-145.
9. Kumarasamyraja, D., N. S.Jeganathan, and R. Manavalan. "A review on medicinal plants with potential wound healing activity." Int J Pharm Pharm Sci, 2012; 2: 105-11.
10. Gurjar, Himanshu, et al. "Hypoglycemic effects of methanolic extract of Anthocephalus cadamba bark in alloxan induced diabetic rats (Rox B) Miq." Int. J. Pharm. Sci. Res, 2010; 1, 3: 79-83.
11. Singh, Himanshu Pratap, et al. "Phytochemical analysis, exploration of antidiabetic and antioxidant potential of Anthocephalus cadamba (Roxb.)." International Journal of Research and Development in Pharmacy & Life Sciences, (IJRDPL) 6: 2800-2805.
12. Alam, Mohammad A., et al. "Anthocephalus cadamba (Roxb.) Miq., Rubiaceae, extract shows hypoglycemic effect and eases oxidative stress in alloxan-induced diabetic rats." Revista Brasileira de Farmacognosia, 2011; 21, 1: 0-0.
13. Swarnkar, Ramji, et al. "Pharmacological investigation of leaves of Anthocephalus cadamba (Roxb) for hepatoprotective activity." International Journal of Research and Development in Pharmacy & Life Sciences (IJRDPL), 2016; 5: 2410-2413.
14. Dolai, Narayan, Aminul Islam, and Pallab Kanti Haldar. "Inhibitory effects of Anthocephalus cadamba stem bark fractions intercede anti-inflammatory and carbon tetrachloride induced hepatotoxicity in rats." Oriental Pharmacy and Experimental Medicine, 2015; 15, 2: 123-134.
15. Bachhav, R. S., V. V. Buchake, and R. B. Saudagar. "Analgesic and anti-inflammatory activities of

Anthocephalus cadambaroxb. leaves in wistar rats." Research Journal of Pharmacy and Technology, 2009; 2, 1: 164-167.
16. Ambujakshi, H. R., et al. "Analgesic activity of Anthocephalus cadamba leaf extract." Journal of Pharmacy Research, 2009; 2, 8: 1279-1280.
17. Sumanta, Mondal, G. K. Dash, and Acharyya Suman. "Analgesic, anti-inflammatory and antipyretic studies of Neolamarckia cadamba barks." Journal of Pharmacy Research, 2009; 2, 6: 1133-1136.
18. Chandrashekar, Kodangala Subraya, Kodangala Subraya Prasanna, and Borthakur Abinash. "Anti-inflammatory effect of the methanol extract from Anthocephalus cadamba stem bark in animal models." International Journal of Plant Biology, 2010; 1, 1: e6-e6.