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PHARMACOLOGICAL EVALUATION OF IPOMOEA TRILOBA LEAF EXTRACT IN WISTAR RATS

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ABSTRACT

The present study was undertaken to evaluate the antioxidant and anti-ulcer potential of *Ipomoea triloba* leaf extract using in vitro and in vivo experimental models. Phytochemical screening of the methanolic extract revealed the presence of bioactive constituents such as phenolics, flavonoids, glycosides, tannins, and terpenoids. Quantitative analysis showed high total phenolic content (52.5 mg gallic acid equivalent/g extract) and total flavonoid content (27.5 mg rutin equivalent/g extract), indicating a rich antioxidant profile. The in vitro antioxidant activity assessed by the DPPH radical scavenging assay demonstrated significant free radical scavenging potential with an IC_{50} value of 48.61 µg/mL. Acute oral toxicity studies conducted as per OECD-423 guidelines showed no signs of toxicity or mortality up to a dose of 2000 mg/kg, confirming the safety of the extract. In the indomethacin-induced gastric ulcer model in Wistar rats, *Ipomoea triloba* extract exhibited dose-dependent gastroprotective effects. The higher dose (400 mg/kg) significantly reduced ulcer index, gastric juice volume, and free acidity while improving gastric pH, with effects comparable to the standard drug ranitidine. Macroscopic examination of gastric tissues supported the biochemical findings, showing reduced mucosal damage and enhanced tissue protection. These results validate the traditional use of *Ipomoea triloba* and suggest its potential as a safe, natural anti-ulcer agent, warranting further mechanistic and clinical investigations.

KEYWORDS: *Ipomoea triloba*; Anti-ulcer activity; Antioxidant activity; Phytochemical analysis; Indomethacin-induced gastric ulcer; Wistar rats.

Ayurveda is one of the oldest holistic healthcare systems, originating in India approximately 5,000 years ago and practiced extensively during the Vedic period. The term *Ayurveda* translates to the "Science of Life" and focuses on health promotion, disease prevention, and the maintenance of balance through natural therapies and lifestyle practices. Classical Ayurvedic texts such as the *Charaka Samhita* and *Sushruta Samhita* documented nearly 700 medicinal plants during the first millennium BC, reflecting the advanced knowledge of herbal therapeutics in ancient India. Today, Ayurveda forms a major component of the AYUSH system and is widely recognized as complementary and alternative medicine across the globe (Nahar & Sarker, 2019).

India possesses immense biodiversity, with approximately 7,500 plant species used in traditional healthcare practices, particularly in rural and tribal regions. Medicinal plants remain central to conventional and traditional medical systems due to their accessibility, affordability, and therapeutic potential. Globally, natural products contribute to more than 50% of drugs currently in clinical use, and nearly half of the drugs approved in the last three decades are derived directly or indirectly from natural sources such as plants, microorganisms, fungi, and animals (Dias et al., 2012; Li et al., 2018). The growing demand for herbal medicines, nutraceuticals, cosmetics, and phytopharmaceuticals highlights the increasing relevance of plant-based therapeutics.

Despite their popularity, herbal medicines face challenges related to quality, safety, and standardization. Issues such as contamination, adulteration, misidentification, and variability in phytochemical composition may compromise efficacy and safety. These concerns necessitate strict adherence to Good Agricultural and Collection Practices (GACP) and Good Manufacturing Practices (GMP), along with the

application of modern analytical and pharmaceutical techniques to ensure consistent quality of herbal products (Zhang et al., 2012).

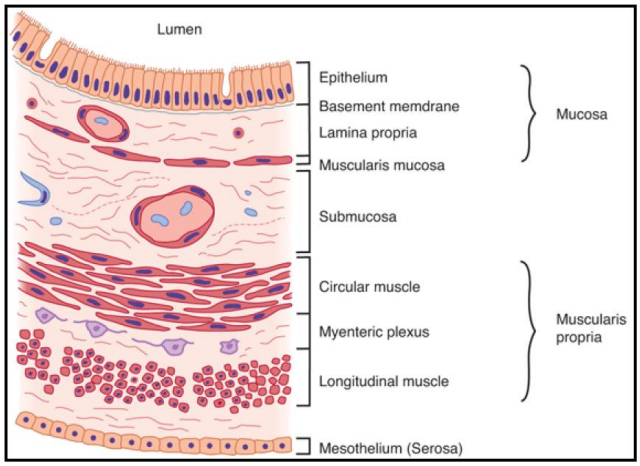


Figure 1: Organization of the wall of the intestine into functional layers.

Medicinal plants have gained considerable attention for their role in the management of gastrointestinal disorders, particularly peptic ulcer disease. Peptic ulcers result from an imbalance between aggressive factors, including gastric acid, pepsin, NSAIDs, *Helicobacter pylori*, and oxidative stress, and defensive mechanisms such as mucus, bicarbonate secretion, prostaglandins, nitric oxide, and mucosal blood flow (Patwardhan et al., 2004). Experimental studies have demonstrated that several medicinal plants possess antiulcer activity, largely attributed to secondary metabolites such as flavonoids and tannins, which exhibit antioxidant, cytoprotective, and anti-inflammatory properties (Gokhale & Kokate, 2008).

Figure 2: Symptoms of peptic ulcer.

The stomach plays a vital role in digestion through mechanical mixing, chemical breakdown of food, and secretion of gastric enzymes and acid. Its structural organization into mucosa, submucosa, muscularis externa, and serosa ensures efficient digestion and protection against injury. Disruption of gastric mucosal integrity can lead to ulcer formation and complications such as bleeding, perforation, and obstruction (Hosoda et al., 2019; Ogobuiro et al., 2023).

Peptic ulcer disease affects millions of individuals worldwide and remains a significant cause of morbidity and mortality. Epidemiological data indicate that approximately 10–20% of patients develop complications, with a notable proportion requiring surgical intervention (Lau et al., 2011). The disease is influenced by multiple etiological factors, including NSAID use, *H. pylori* infection, stress, smoking, dietary habits, and oxidative stress (Son et al., 2015). Current treatment strategies focus on *H. pylori* eradication using proton pump inhibitors and antibiotics, acid suppression, and preventive measures to minimize recurrence and complications (Lanas & Chan, 2017).

In conclusion, the integration of traditional medicinal knowledge with modern pharmacological research offers a promising approach for the development of effective and safer antiulcer therapies. Medicinal plants continue to represent valuable sources of bioactive compounds with significant therapeutic potential in gastrointestinal disorders.

PLANT PROFILE: Ipomoea triloba

Parameter	Details
Botanical Name	*Ipomoea triloba* L.
Family	Convolvulaceae
Common Names	Three-lobed morning glory, Littlebell morning glory
Vernacular Names	Hindi: Chhoti Kalmi
Synonyms	*Ipomoea triloba* var. *purpurea*
Taxonomical Classification	Kingdom: PlantaeDivision: AngiospermsClass: EudicotsOrder: SolanalesFamily: ConvolvulaceaeGenus: *Ipomoea*Species: *I. triloba*
Geographical Distribution	Tropical and subtropical regions including India, Southeast Asia, Africa, and the Americas
Habitat	Roadsides, agricultural fields, wastelands, hedges
Plant Type	Annual or perennial twining herb
Stem	Slender, green to purplish, glabrous or sparsely hairy
Leaves	Alternate, petiolate,

Gastric ulcers are a prevalent gastrointestinal disorder caused by an imbalance between aggressive factors such as gastric acid and pepsin and the protective mechanisms of the gastric mucosa, including mucus secretion, bicarbonate ions, prostaglandins, and adequate mucosal blood flow. Conventional management primarily focuses on suppressing gastric acid secretion through antacids, H_2 receptor antagonists, and proton pump inhibitors. Although these therapies are effective, their long-term use has been associated with several adverse effects, including nutrient malabsorption, increased susceptibility to infections, and drug interactions, which may limit prolonged treatment and patient compliance.

In recent years, increasing attention has been directed toward herbal and plant-based medicines due to their therapeutic potential and comparatively better safety profiles. *Ipomoea triloba*, a medicinal plant widely distributed in tropical and subtropical regions, has been traditionally used for various ailments, yet its antiulcer activity remains scientifically underexplored. Therefore, the present study aims to evaluate the pharmacological potential of *Ipomoea triloba* in experimentally induced peptic ulcer models and to compare its efficacy with standard antiulcer drugs. The findings of this study may contribute to the development of effective, safer, and natural antiulcer agents suitable for long-term use.

1. Plant Selection and Authentication

- *Ipomoea triloba* was selected based on its traditional medicinal use and easy availability in the local region.
- Fresh leaves (≈386 g) were collected from natural habitats.
- Leaves were washed thoroughly to remove soil and foreign matter.
- Shade drying was carried out for three days at room temperature to preserve bioactive constituents.
- Dried leaves were stored in airtight glass containers in a cool, dry place.
- The plant material was authenticated by a qualified taxonomist.
- A voucher specimen was prepared and deposited in a recognized herbarium for future reference.

2. Preparation and Extraction of Plant Material

- Dried leaves were powdered using a mechanical grinder.
- Soxhlet extraction was performed using continuous hot percolation.
- The powdered material was placed in a cellulose thimble.
- Sequential extraction was carried out using petroleum ether followed by methanol.
- Extraction temperature was maintained at approximately 60 °C.
- Extraction continued until the solvent became colorless.
- Extracts were concentrated under reduced pressure using a rotary vacuum evaporator at 40 °C.
- Percentage yield of the extract was calculated.
- Concentrated extracts were stored in airtight, labeled containers.

3. Preliminary Phytochemical Screening

- Qualitative phytochemical analysis was conducted using standard procedures.
- Tests were performed to detect:
  - Carbohydrates (Molisch's, Fehling's, Benedict's, Barfoed's tests)
  - Alkaloids (Dragendorff's, Wagner's, Mayer's, Hager's tests)
  - Saponins (Froth test)
  - Steroids and triterpenoids (Libermann–Burchard, Salkowski tests)
  - Tannins and phenolics (Ferric chloride, Gelatin, Lead acetate tests)
  - Flavonoids (Lead acetate, Alkaline reagent tests)
  - Glycosides (Borntrager's, Keller–Killiani tests)
  - Proteins and amino acids (Biuret, Ninhydrin tests)

4. Quantitative Phytochemical Analysis

- Total phenolic content (TPC) was estimated using the Folin–Ciocalteu method.

- Results were expressed as mg gallic acid equivalent (GAE)/g of extract.
- Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method.
- Results were expressed as mg rutin equivalent (RE)/g of extract.

Figure 3: Phytochemical investigation.

5. Evaluation of Antioxidant Activity

- DPPH free radical scavenging assay was used.
- Extract concentrations ranged from 20–100 µg/mL.
- Absorbance was measured at 517 nm using a UV spectrophotometer.
- Percentage inhibition was calculated to assess antioxidant potential.

6. Acute Toxicity Study

- Conducted according to OECD guideline 423.
- Male Wistar rats were administered extract doses of 5, 50, 300, and 2000 mg/kg orally.
- Animals were observed for 14 days for toxicity signs and mortality.
- Safe dose range and LD_{50} were determined.

7. Experimental Animals and Ethical Approval

- Male Wistar rats (150 ± 60 g) were used.
- Animals were housed under controlled temperature with standard feed and water.
- All experiments were approved by the Institutional Animal Ethics Committee (IAEC).

8. Induction of Gastric Ulcer

- Rats were fasted for 24 hours with free access to water.
- Gastric ulcers were induced using indomethacin (20 mg/kg, orally).
- Animals were divided into five groups: normal control, ulcer control, low-dose test, high-dose test, and standard treatment.

9. Treatment Protocol

- Test groups received *Ipomoea triloba* extract at 200 and 400 mg/kg.
- Standard group received ranitidine (20 mg/kg).
- After treatment, animals were sacrificed and stomachs were excised for analysis.

10. Assessment of Anti-ulcer Parameters

- Ulcer index was calculated using the Kulkarni scoring method.
- Gastric juice volume was measured after centrifugation.
- pH of gastric juice was determined using a pH meter.
- Free acidity was estimated by titration with 0.01 N NaOH.

11. Outcome

RESULTS AND DISCUSSION

- Data obtained were used to evaluate the antioxidant and anti-ulcer potential of *Ipomoea triloba* leaf extract compared with standard anti-ulcer therapy.
- Extraction yield analysis showed that the methanolic extract produced a higher percentage yield (2.24%) compared to the petroleum ether extract (0.49%), indicating better extraction efficiency of polar phytoconstituents using methanol.
- Preliminary phytochemical screening revealed that the methanolic extract was rich in bioactive compounds, including alkaloids, glycosides, carbohydrates, flavonoids, tannins, phenolic compounds, steroids, and triterpenoids, whereas proteins and amino acids were absent. The petroleum ether extract showed a comparatively limited phytochemical profile. These findings support the selection of the methanolic extract for further pharmacological evaluation.
- Quantitative phytochemical analysis demonstrated a substantial presence of phenolics and flavonoids in the methanolic extract. The total phenolic content was found to be 52.5 mg/g gallic acid equivalent, while the total flavonoid content was 27.5 mg/g rutin equivalent, indicating strong antioxidant-related constituents.
- In the DPPH free radical scavenging assay, the methanolic extract exhibited concentration-

dependent antioxidant activity. Although its scavenging potential (IC_{50} = 48.61 µg/mL) was lower than the standard ascorbic acid (IC_{50} = 21.54 µg/mL), the extract showed significant antioxidant capability, correlating with its high phenolic and flavonoid content.

- Acute oral toxicity studies conducted according to OECD-423 guidelines revealed no mortality or behavioral abnormalities even at a dose of 2000 mg/kg, indicating that the extract is safe and well tolerated.
- In the indomethacin-induced gastric ulcer model, *Ipomoea triloba* extract produced a dose-dependent gastroprotective effect. The ulcer control group showed severe ulceration, increased gastric volume, higher acidity, and altered pH. Treatment with the extract at 200 mg/kg significantly reduced ulcer index, gastric juice volume, and free acidity, while the 400 mg/kg dose showed more pronounced protection, approaching the efficacy of the standard drug ranitidine. The extract also improved gastric pH and reduced ulcer severity.
- Overall, the results demonstrate that *Ipomoea triloba* methanolic leaf extract possesses significant antioxidant and anti-ulcer activity, is non-toxic at therapeutic doses, and exhibits dose-dependent gastroprotective effects, supporting its potential as a natural anti-ulcer agent.

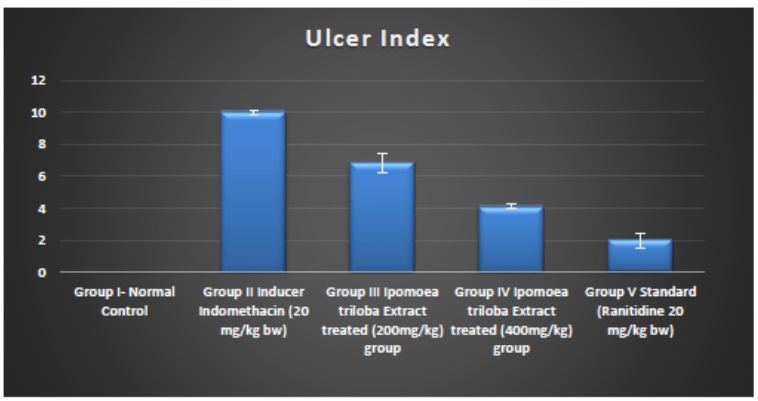
The results of the study clearly demonstrate the gastroprotective potential of *Ipomoea triloba* extract. Phytochemical analysis confirmed the presence of bioactive compounds such as phenolics, flavonoids, glycosides, tannins, and terpenoids. The methanolic extract showed high total phenolic (52.5 mg GAE/g) and flavonoid content (27.5 mg RE/g), indicating strong antioxidant capacity. This was supported by the DPPH assay, where the extract exhibited significant free radical scavenging activity (IC_{50} = 48.61 µg/mL), though less potent than ascorbic acid.

Acute toxicity studies revealed no signs of toxicity or mortality up to 2000 mg/kg, confirming a wide margin of safety. In the indomethacin-induced ulcer model, *Ipomoea triloba* produced dose-dependent gastroprotection. The 200 mg/kg dose showed moderate improvement, while the 400 mg/kg dose significantly reduced ulcer index, gastric juice volume, and free acidity, with improved gastric pH, approaching the effectiveness of ranitidine.

Macroscopic examination of gastric tissues further supported these findings, showing reduced mucosal damage and near-normal architecture at the higher dose. Overall, the study supports the traditional use of *Ipomoea triloba* and highlights its potential as a safe, natural anti-ulcer agent, warranting further mechanistic and clinical investigations.

Table 2: Observation of Ulcer Index.

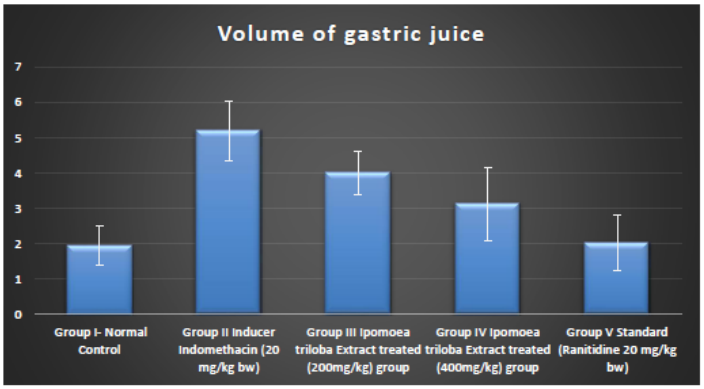
Groups	Ulcer Index
	Mean
Group I- Normal Control	0
Group II Inducer Indomethacin (20 mg/kg bw)	9.997±0.138
Group III *Ipomoea triloba* Extract treated (200mg/kg) group	6.841±0.602
Group IV *Ipomoea triloba* Extract treated (400mg/kg) group	4.113±0.132
Group V Standard (Ranitidine 20 mg/kg bw)	1.981±0.469



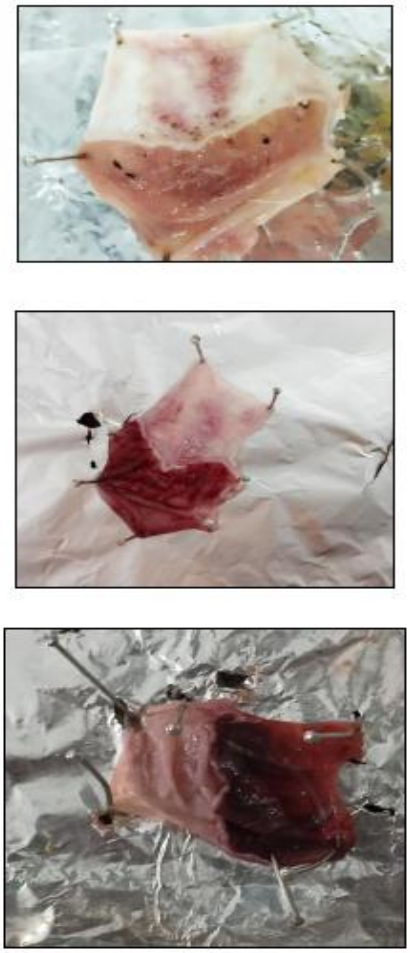
Graph 1: Bar chart represents ulcer index in Indomethacin induced ulcer in rats

Table 3: Observation of volume of gastric juice.

Treatment Group	Volume of gastric juice
Group I- Normal Control	1.955± 0.558
Group II Inducer Indomethacin (20 mg/kg bw)	5.199± 0.845
Group III *Ipomoea triloba* Extract treated (200mg/kg) group	4.012± 0.616
Group IV *Ipomoea triloba* Extract treated (400mg/kg) group	3.124± 1.034
Group V Standard (Ranitidine 20 mg/kg bw)	2.023± 0.79



Graph 2: Bar chart represents gastric volume in Indomethacin induced ulcer in rats



Group 1-3 Treated stomach with Standard drug and tested drug

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