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Research Article

EVALUATION OF ANTIULCER ACTIVITY OF *PLUMERIA PUDICA* FLOWERS ON WISTAR ALBINO RATS**Hithesh C M^{1*}, Ahalyadevi K H², Suresha B S³, T. Balasubramanian⁴**¹2nd year M Pharma, student of Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.

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²Principal and Professor, Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.³Professor and Head, Department of Pharmacology, Bharathi College of Pharmacy, Bharathinagara, Mandya, Karnataka, India – 571422.**Abstract:**

The present study evaluated the antioxidant, acid-neutralizing, and gastroprotective activities of the hydroethanolic flower extract of *Plumeria pudica*. The extract exhibited concentration-dependent DPPH radical scavenging activity, increasing from 18.83% at 20 µg/mL to 66.92% at 100 µg/mL, with an IC₅₀ value of 70.36 µg/mL. HEPP also demonstrated antioxidant activity in the phosphomolybdate and ferric reducing assays, with ascorbic-acid-equivalent activities of 10.44 and 14.35 µg/mL, respectively. The extract showed acid-neutralizing capacity ranging from 261.51 to 27.15 mEq/g at the tested concentrations. In the ethanol-induced gastric ulcer model, HEPP at 250 and 500 mg/kg reduced the ulcer index to 2.08 ± 0.20 and 1.75 ± 0.11, respectively, compared with 2.67 ± 0.21 in the ulcer-control group. Overall, the findings demonstrate that the hydroethanolic flower extract of *P. pudica* possesses antioxidant, acid-neutralizing, and gastroprotective properties.

Keywords: *Plumeria pudica*, hydroethanolic extract, anti-ulcer activity, gastric ulcer, antioxidant activity, DPPH, acid-neutralizing capacity, Wistar albino rats.

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INTRODUCTION:

Gastric hyperacidity and ulcer are common gastrointestinal disorders that contribute much to human suffering. Ulcers result from imbalance between aggressive factors in the gastric lumen and protective mechanisms of gastroduodenal mucosa ¹

The gastric mucosa is normally protected by a dynamic balance between mucosal defensive mechanisms and aggressive factors, particularly gastric acid and pepsin. Disruption of this equilibrium, either through increased aggressive factors or impaired mucosal defenses, can lead to mucosal injury and ulceration. The pathophysiology of gastric ulcers is multifactorial, involving several interacting factors ². Sloughing of inflammatory dead tissue is a characteristic of ulcers, which are open sores of the skin or mucous membrane ³. Lesions on the skin's or a mucous membrane's surface that exhibit a superficial loss of tissue are called ulcers. Although they can appear anywhere, ulcers are most frequently found on the skin of the lower limbs and in the digestive system. There are numerous kinds of ulcers, including vaginal, peptic, esophageal, and oral ulcers. Peptic ulcers are common among these individuals. ⁴

Peptic ulcer disease (PUD) is an important gastrointestinal disorder characterized by a mucosal defect in the stomach or duodenum that extends through the muscularis mucosae into the submucosa or deeper layers. Unlike superficial erosions, peptic ulcers represent deeper mucosal lesions. The development of PUD is associated with an imbalance between factors that protect the gastric and duodenal mucosa and those that cause mucosal injury.⁵ The term "peptic" reflects the involvement of gastric acid and pepsin in the development of these lesions.⁶ Gastric ulcers commonly occur along the lesser curvature of the stomach, particularly in the antrum and at the angularis junction, whereas duodenal ulcers predominantly occur in the first portion of the duodenum.⁷

Plumeria pudica is native to Colombia, Panama, and Venezuela and has been widely introduced and naturalized throughout tropical regions, including South and Southeast Asia.⁸ In India, it is cultivated in gardens, temple premises, and roadsides, particularly in tropical and subtropical regions.⁹ Although traditional medicinal uses of *P. pudica* have been reported, systematic experimental evaluation of its flower extract for gastroprotective activity remains limited. Therefore, the present study was undertaken to evaluate the anti-ulcer potential of *P. pudica* flower extract using an ethanol-induced gastric ulcer model in Wistar albino rats.

MATERIALS AND METHODS:

2.1 Plant material

Fresh flowers of the plant *Plumeria pudica* were collected from Bharathinagara, Maddur taluk,

Mandya district, Karnataka. The flowers were identified, confirmed and authenticated by Dr. Thejesh Kumar M.P., M.Sc., Ph.D., Coordinator, Department of Botany (PG), Bharathi College (PG & RC), Bharathinagara. The collected flowers were washed, shade dried, subjected to size reduction into a fine powder, and stored in an airtight container at room temperature for further use.

2.2 Preparation of extract

The dried powdered flowers of *P. pudica* were extracted using a hydroethanolic solvent system (70:30) for approximately 72 h by Soxhlet extraction. The solvent was removed using a rotary evaporator, and the concentrated extract was stored for subsequent pharmacological and phytochemical investigations.

2.3 Preliminary phytochemical screening ¹⁰

The hydroethanolic flower extract was subjected to preliminary qualitative phytochemical screening using standard methods for the detection of alkaloids, flavonoids, carbohydrates, steroids, saponins, glycosides, proteins, phenolic compounds, and tannins by phytochemical screening using standard methods.

2.4 In vitro antioxidant activity

The antioxidant potential of the *P. pudica* flower extract was evaluated using DPPH radical scavenging, phosphomolybdate, and ferric reducing power assays. Ascorbic acid was used as the reference standard.

2.4.1 DPPH radical scavenging assay ¹¹

The extract and ascorbic acid were prepared as stock solutions at a concentration of 1 mg/mL using DMSO. Different concentrations ranging from 20 to 120 µg/mL were prepared. Each concentration was mixed with 1 mL of methanolic DPPH solution and incubated in the dark at room temperature for 20 min. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. The percentage of radical-scavenging activity was calculated using the following equation:

$$\% \text{ DPPH radical scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extract or standard.

2.4.2 Phosphomolybdate assay ¹²

The phosphomolybdate reagent was prepared using sulfuric acid, sodium phosphate, and ammonium molybdate. Appropriate concentrations of the extract and ascorbic acid were mixed with 3 mL of the freshly prepared reagent and incubated at 95°C for 90 min in a water bath. After cooling, the absorbance was measured at 695 nm using a UV-visible spectrophotometer

2.4.3 Ferric reducing power assay ¹³

Appropriate concentrations of the extract and ascorbic acid were mixed with phosphate buffer (pH 6.6) and 1% potassium ferricyanide and incubated at 50°C for 20 min. After cooling, 10% trichloroacetic acid was added and the mixture was centrifuged at

3000 rpm for 10 min. The upper layer was mixed with distilled water and freshly prepared ferric chloride solution, and the absorbance was measured at 700 nm.

2.5 *In vitro* Acid-neutralizing capacity¹⁴

Acid-neutralizing capacity (ANC) is the ability of a substance to neutralize hydrochloric acid (HCl). A known quantity of test substance was reacted with HCl, and the remaining acid was determined by back-titration with standard sodium hydroxide (NaOH). ANC was expressed as mEq of HCl neutralized per gram or dose. Hydroethanolic extract (100, 200, 500 and 1000 mg) was tested, with magnesium hydroxide (500 mg) and aluminium hydroxide as standards. The preparations were mixed with water, followed by 30 mL of 1.0 N HCl and swirling for 15 minutes. Phenolphthalein was added, and the remaining HCl was titrated with 0.5 N NaOH until a pink colour appeared.

The milliequivalents of acid neutralized were calculated as:

$$\text{mEq} = (\text{vol. of HCl} \times \text{Normality of HCl}) - (\text{vol. of NaOH} \times \text{Normality of NaOH})$$

Acid neutralizing capacity (ANC) per gram of antacid = mEq of HCl neutralized / Grams of Antacid or Extract

2.6 Experimental animals¹⁵

Healthy Wistar albino rats weighing 150–200 g were used for the evaluation of anti-ulcer activity. The animals were procured from Vertebrates, Magadi Road, Bengaluru, and acclimatized in the animal house facility of Bharathi College of Pharmacy, Bharathinagara. The animals were housed in polypropylene cages containing bedding material under standard husbandry conditions with a 12 h light/dark cycle. They were provided commercial pelleted rat chow and water *ad libitum*. The experimental protocol was conducted in accordance with CCSEA guidelines after obtaining approval from the Institutional Animal Ethical Committee (IAEC No. BCP/IAEC/2025/05, dated 12/07/2025).

2.7 Experimental design

A total of 30 Wistar albino rats were randomly divided into five groups, consisting of six animals per group. The groups were as follows:

- **Group I:** Normal control
- **Group II:** Ulcer control
- **Group III:** Ulcer control + Omeprazole 20 mg/kg
- **Group IV:** Ulcer control + Extract 250 mg/kg
- **Group V:** Ulcer control + Extract 500 mg/kg

Omeprazole was used as the standard anti-ulcer drug. The extract doses of 250 and 500 mg/kg were selected based on the reported LD₅₀ value of 5000 mg/kg, corresponding to one-twentieth and one-tenth of the reported LD₅₀, respectively.

2.8 Ethanol-induced gastric ulcer model:

The ethanol-induced gastric ulcer model was employed to evaluate the gastroprotective activity of the *P. pudica* flower extract. The animals were weighed and acclimatized under standard laboratory conditions. The animals, except those in the normal control group, were fasted for 24 h with free access to water. The respective treatments were administered orally by gavage. After 1 h, absolute ethanol was administered orally at a dose of 8 mL/kg to induce gastric mucosal injury. One hour after ethanol administration, the animals were sacrificed and the stomachs were carefully removed and opened along the greater curvature. The stomachs were gently rinsed with normal saline and examined macroscopically for gastric lesions. The extent of ulceration was recorded for determination of the ulcer index and percentage of gastric protection.

2.9 Ulcer index¹⁶

The gastric ulcer index (UI) is calculated by examining the gastric lesions and evaluating their size and severity. A protective effect against ethanol-induced stomach mucosal damage is indicated by a reduced ulcer index in the treatment group when compared to the ethanol-treated ulcer control group.

An ulcer index UI is calculated:

$$\text{UI} = \text{UN} + \text{US} + (\text{UP} \times 10^{-1})$$

UN = average of numbers of ulcers per animal

US = average of severity score

UP = percentage of animals with ulcer.

2.9 Statistical analysis

Statistical analysis was carried out using GraphPad Prism software. Results were expressed as mean ± SEM and compared using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Statistical significance was considered at $p < 0.05$.

3. Results

3.1 *In vitro* antioxidant activity

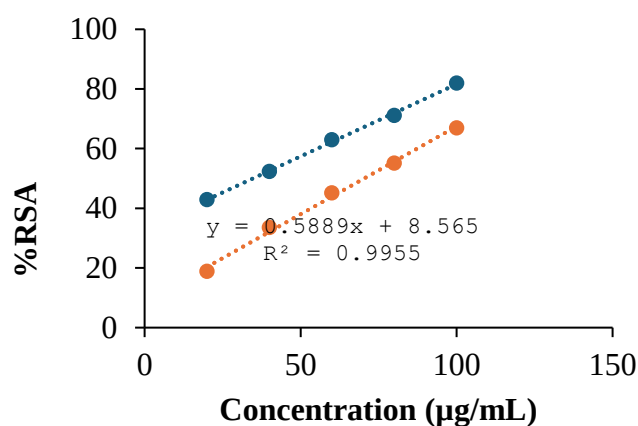
DPPH assay:

The DPPH assay, both ascorbic acid and hydroethanolic extract of HEPP demonstrated concentration-dependent increases in percentage inhibition. The percentage radical scavenging activity (RSA) produced by HEPP increased from 18.83% at 20 µg/mL to 66.92% at 100 µg/mL, while ascorbic acid increased from 42.88% to 81.92% over the same concentration range. The calculated IC₅₀ values were 70.36 µg/mL for HEPP and 34.73 µg/mL for ascorbic acid. Thus, the DPPH findings indicate the antioxidant potential of the hydroethanolic flower extract of *P. pudica*. The DPPH radical scavenging potential of ascorbic acid and *P. pudica* flower extract is shown in Table 1 and Figure 1.

Table 1: Concentration and % RSA, IC₅₀ Value of standard ascorbic acid and HEPP.

Sample	Concentration (µg/mL)	%RSA	IC ₅₀ Value (µg/mL)
Standard (Ascorbic acid)	20	42.88	34.73
	40	52.32	
	60	62.98	
	80	71.08	
	100	81.92	
Sample (HEPP)	20	18.83	70.36
	40	33.51	
	60	45.12	
	80	55.1	
	100	66.92	

%RSA= Percentage radical scavenging activity, IC₅₀= Half-maximal inhibitory concentration.

Figure 1: DPPH radical scavenging potential of ascorbic acid and HEPP.

Phosphomolybdate assay

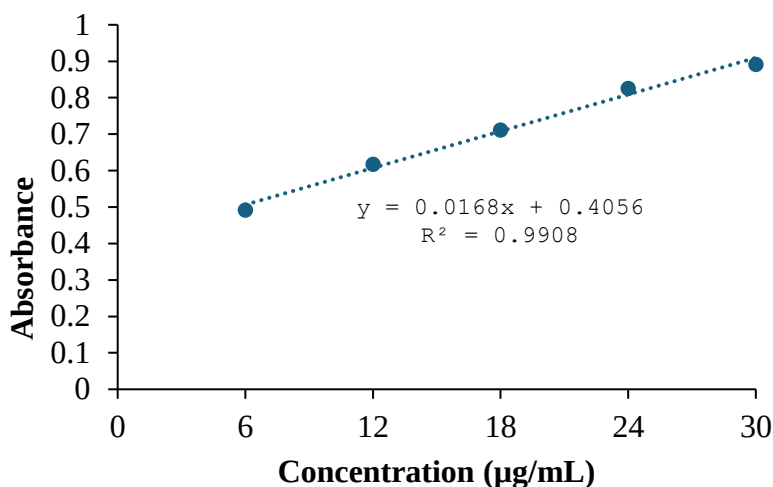
The phosphomolybdate assay demonstrated antioxidant activity in both ascorbic acid and the hydroethanolic extract of HEPP. Based on the standard calibration curve, the antioxidant activity of HEPP was equivalent to 10.44 µg/mL of ascorbic acid. The extract exhibited an absorbance of 0.581 at

a concentration of 100 µg/mL, whereas ascorbic acid showed a concentration-dependent increase in absorbance. These findings indicate the antioxidant potential of the hydroethanolic flower extract of *P. pudica*. The phosphomolybdate antioxidant activity of ascorbic acid and *P. pudica* flower extract is presented in Table 2 and Figure 2.

Table 2: Concentration and absorbance values of standard ascorbic acid and HEPP.

Sample	Concentration (µg/mL)	Absorbance (765 nm)
Standard (Ascorbic acid)	6	0.492
	12	0.618
	18	0.712
	24	0.826
	30	0.892
Sample (HEPP)	100	0.581

Figure 2: Phosphomolybdenum assay of standard ascorbic acid.

**Ferric reducing power assay**

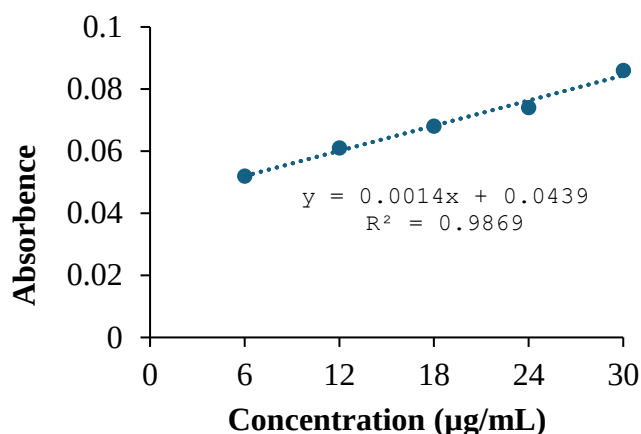
The ferric reducing power assay revealed antioxidant reducing activity for both ascorbic acid and the hydroethanolic extract of *P. pudica* (HEPP). The reducing activity of HEPP was equivalent to 14.35 µg/mL of ascorbic acid based on the standard calibration curve. At a concentration of 60 µg/mL, HEPP exhibited an absorbance of 0.064 at 700 nm,

while ascorbic acid showed an increase in absorbance with increasing concentration. The observed reducing activity further supports the antioxidant potential of the hydroethanolic flower extract of *P. pudica*. The ferric reducing activity of ascorbic acid and *P. pudica* flower extract is presented in Table 3 and Figure 3.

Table 3: Concentration and absorbance values of standard ascorbic acid and HEPP.

Sample	Concentration (µg/mL)	Absorbance (700nm)
Standard (Ascorbic acid)	6	0.052
	12	0.061
	18	0.068
	24	0.074
	30	0.090
Sample (HEPP)	60	0.064

Figure 3: Ferric reducing assay of standard ascorbic acid.



3.2 *In vitro* Acid-neutralizing capacity

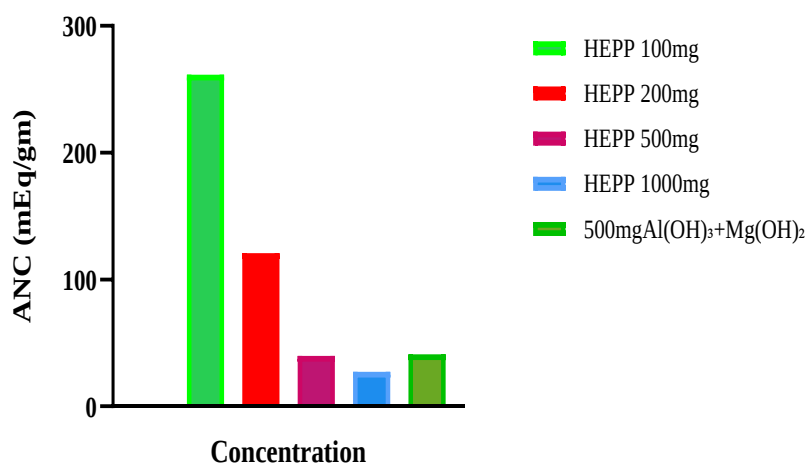
The hydroethanolic extract of *P. pudica* (HEPP) exhibited acid-neutralizing activity at all the tested concentrations. The acid-neutralizing capacity of HEPP was found to be 261.51, 120.75, 39.76 and 27.15 mEq/g at concentrations of 100, 200, 500 and 1000 mg, respectively. The standard preparation

containing aluminium hydroxide and magnesium hydroxide showed an acid-neutralizing capacity of 41.12 mEq/g. These findings demonstrate the acid-neutralizing potential of the hydroethanolic flower extract of *P. pudica*. The acid-neutralizing capacity of HEPP at different concentrations and the standard preparation is presented in Table 4 and Figure 4.

Table 4: Effect of Hydroalcoholic extract of *Plumeria pudica* on ANC

Sl No	Concentration	Volume of NaOH consumed (mL)	mEq of Acid consumed	ANC (mEq/g extract)
1.	HEPP 100mg	7.7	26.15	261.51
2.	HEPP 200mg	11.7	24.15	120.75
3.	HEPP 500mg	20.3	19.85	39.76
4.	HEPP 1000mg	5.7	27.15	27.15
5.	Aluminum hydroxide + Magnesium hydroxide (500mg)	18.9	20.55	41.12

Figure 4: Acid neutralizing capacity of Hydroalcoholic extract of flowers of *Plumeria pudica* *invitro*.



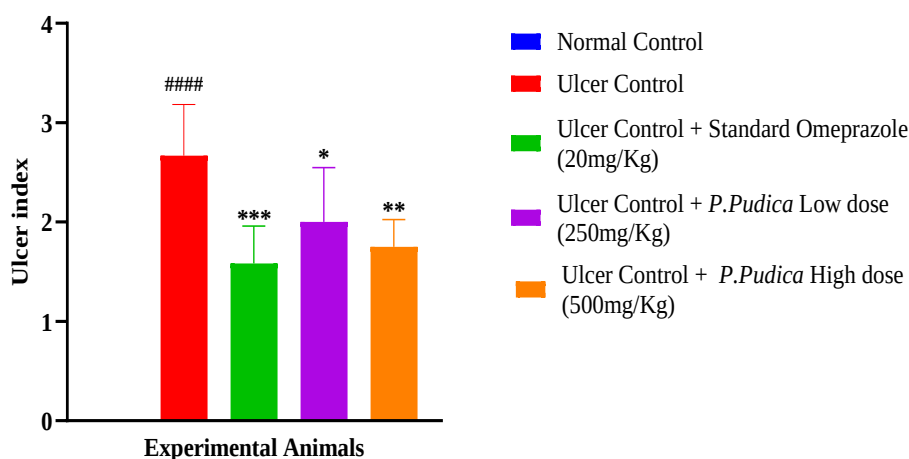
3.3 Ethanol-induced gastric ulcer model

The hydroethanolic extract of *P. pudica* (HEPP) showed gastroprotective activity against ethanol-induced gastric ulceration. The ulcer index was 2.67 ± 0.21 in the ulcer-control group, which was reduced to 1.50 ± 0.15 by omeprazole (20 mg/kg). HEPP at 250 and 500 mg/kg reduced the ulcer

index to 2.08 ± 0.20 and 1.75 ± 0.11 , respectively. The reductions were significant at 250 mg/kg ($p < 0.05$) and 500 mg/kg ($p < 0.01$), while omeprazole showed a highly significant effect ($p < 0.001$). The anti-ulcer activity and ulcer index of HEPP are presented in Table 5 and Figure 5.

Table 5: Anti-ulcer activity of Hydroethanolic extract of flowers of *Plumeria pudica* on Ulcer index of ethanol-induced gastric ulcer model.

Sl no	Treatment	Dose	Ulcer index
1	Normal control	-	0.00±0.00
2	Ulcer control	Absolute alcohol (8 mL/kg)	2.67 ± 0.21####
3	Ulcer + Standard	20 mg/kg	1.50 ± 0.15***
4	Ulcer + HEPP (LD)	250 mg/kg	2.08± 0.20*
5	Ulcer + HEPP (HD)	500 mg/kg	1.75 ± 0.11**

Figure 5: Anti-ulcer activity of Hydroalcoholic extract of flowers of *Plumeria pudica* on Ulcer index of Ethanol induced model.**CONCLUSION:**

The present study demonstrated that the hydroethanolic flower extract of *Plumeria pudica* (HEPP) possesses antioxidant, acid-neutralizing, and gastroprotective properties. HEPP showed concentration-dependent antioxidant activity in the DPPH assay, along with activity in the phosphomolybdate and ferric reducing assays. The extract also exhibited acid-neutralizing capacity at the tested concentrations. In the ethanol-induced gastric ulcer model, HEPP at 250 and 500 mg/kg reduced the ulcer index and provided gastric protection, with the higher dose showing greater protection than the lower dose. Further studies are necessary to establish its precise mechanism of action and therapeutic potential.

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