

Pharmacological Evaluation of the Antiulcer Activity of *Ricinus communis* L. Using *In vitro* Models

Rahul Nimba Patil*, Harshada Milind Jadhav, Ajay Jantar Pawara, Harish Sandesh Khairnar, Snehal Popat Bodke, Anushka Bajirao Rajole

Department of Pharmacology, MET's Institute of D. Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik, Maharashtra, INDIA.

ABSTRACT

Background: Peptic ulcer disease is a common gastrointestinal disorder resulting from an imbalance between aggressive factors such as gastric acid and defensive mechanisms of the gastric mucosa. Medicinal plants are widely explored as potential sources of safer antiulcer agents. The present study aimed to investigate the *in vitro* antiulcerogenic activity of *Ricinus communis* L. leaf extract. **Materials and Methods:** The antiulcer potential of the extract was evaluated using H⁺/K⁺-ATPase inhibition and Acid Neutralizing Capacity (ANC) assays. Different concentrations of the extract were tested and compared with standard drugs. The inhibitory effect on gastric proton pump activity and the ability to neutralize gastric acid were determined. **Results:** The extract exhibited concentration-dependent H⁺/K⁺-ATPase inhibitory activity, with percentage inhibition increasing from 26.15% to 61.54% over the tested concentration range. In the ANC assay, the extract demonstrated appreciable acid-neutralizing activity, indicating its ability to reduce gastric acidity. Although the activity was lower than that of the standard drugs, the extract showed significant antiulcer-related effects in both experimental models. **Conclusion:** The findings suggest that *Ricinus communis* L. leaf extract possesses promising *in vitro* antiulcerogenic properties through inhibition of gastric acid secretion and neutralization of excess acid. These results support its potential use as a natural therapeutic agent for the management of peptic ulcer disease and warrant further pharmacological investigations.

Keywords: Acid Neutralizing Capacity, Antiulcer activity, Gastric ulcer, H⁺/K⁺-ATPase Inhibition, *Ricinus communis* L.

Correspondence:

Rahul Nimba Patil

Department of Pharmacology, MET's
Institute of D. Pharmacy, Bhujbal
Knowledge City, Adgaon, Nashik,
Maharashtra-422003, INDIA.
Email: rahulp_iodp@bkc.met.edu
ORCID: 0009-0005-0558-8485

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INTRODUCTION

Ulcer is a circumscribed loss of epithelial tissue with necrosis extending into the submucosa or deeper layers, caused by an imbalance between tissue-damaging and protective factors, showing delayed healing (Tripathi, 2023; Vanderah, 2024). Peptic ulcer is defined by a disruption in the inner lining of the gastrointestinal tract due to the secretion of gastric acid or pepsin or an increase in *Helicobacter pylori*. It penetrates into the muscularis propria layer of the gastric epithelium. This condition typically occurs in the stomach and the proximal duodenum, but it can also affect the lower oesophagus, distal duodenum, or jejunum (Fatima *et al.*, 2018; Malik *et al.*, 2023).

Current treatment for peptic ulcer includes chemotherapy such as antibiotics, proton pump inhibitors, etc. Despite advancements in pharmacotherapy, peptic ulcer disease remains a recurrent condition due to limitations of synthetic drugs, including adverse

drug reactions, high treatment costs, antibiotic resistance to *Helicobacter pylori*, and long-term safety concerns associated with the use of non-steroidal anti-inflammatory drugs (Tripathi, 2023; Malfertheiner *et al.*, 2009). Herbal medicines have traditionally been used for the treatment of gastrointestinal disorders, including peptic ulcers. Natural products contain bioactive compounds such as flavonoids, alkaloids, and terpenoids that exhibit anti-ulcer, antioxidant, and anti-inflammatory properties. These compounds help reduce gastric acid secretion, enhance mucosal defence, promote ulcer healing, scavenge free radicals, and reduce *Helicobacter pylori* infection (Lanas and Chan, 2017).

One of the plants mentioned in Ayurveda and used since ancient times is *Ricinus communis* L., belonging to the family Euphorbiaceae and commonly referred to as the castor oil plant. It contains a variety of bioactive compounds that have been reported to possess antihistaminic, antimicrobial, anti-inflammatory, and hepatoprotective properties. The leaves of the plant contain flavonoids and phenolic compounds such as rutin, gallic acid, ellagic acid, and quercetin, as well as the alkaloid ricinine. The flavonoids rutin and quercetin present in the leaves of *Ricinus communis* L. possess strong anti-ulcer effects. Keeping this in mind, the present study was undertaken to evaluate the anti-ulcer effect of *Ricinus communis* L. Through *in vitro* assays (Teibo *et al.*, 2024; Jena and Gupta, 2012).



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MATERIALS AND METHODS

Plant Material Collection and Authentication

Ricinus communis L. (Euphorbiaceae) was collected from the premises of Bhujbal Knowledge City, Adgaon, Nashik. The plant was authenticated by experts from the Department of Pharmacognosy, MET's Institute of D. Pharmacy.

Drying and Grinding of Plant Material

Ricinus communis L. Leaves were shade-dried for 14 days and ground into a coarse powder using a mixer grinder. The coarse powder was further passed through a 4 mm sieve to obtain a uniform particle size.

Extraction of Plant Material

One hundred grams of powdered plant material was defatted by the maceration process using 1000 mL of petroleum ether to remove lipids, oils, and fatty acids. The obtained insoluble residue was dried and further extracted using the Soxhlet extraction process with 1000 mL of ethanol for 72 hr. After extraction, the obtained *Ricinus communis* L. leaf extract was concentrated using an electric water bath under controlled temperature and stored in a refrigerator at 2-4°C for further evaluation (Nour *et al.*, 2023; Khandelwal, 2010).

Physicochemical Properties and Phytochemical Study

The crude drug was analysed for quality and purity parameters such as total ash value, acid-insoluble ash value, sulphated ash, loss on drying, water-soluble extractive value, alcohol-soluble extractive value, petroleum ether-soluble extractive value, and foaming index. The obtained extract was further screened for the presence of alkaloids, carbohydrates, proteins, glycosides, flavonoids, terpenoids, saponins, steroids, and phenolic compounds (Nour *et al.*, 2023; Khandelwal, 2010; World Health Organization [WHO], 1998).

Thin Layer Chromatography

Standard methods were used to perform thin layer chromatography of the extracted plant material. Various solvent systems were tested and assessed. The appropriate solvent system was determined to be chloroform: ethyl acetate: methanol (6:2:2), and the spots were examined under UV light at 254 nm (Wagner and Bladt, 1996).

Pharmacological study

In vitro assessment of antiulcer activity.

- H⁺ K⁺ ATPase inhibition assay.
- Acid Neutralization Capacity Assay.

H⁺K⁺ ATPase Inhibition Assay

Procedure

Preparation of H⁺, K⁺-ATPase enzyme

Proton potassium ATPase was prepared from mucosal scrapings of goat stomach. Stomach from freshly slaughtered goats was washed gently with tap water. The mucosal layer of fundus was scrapped and homogenized in ice-cold phosphate buffer, pH 7.4. The homogenate was centrifuged for 20 min at 18,000 rpm. The supernatant so obtained was recentrifuged for 60 min at specified rpm. The pellet was resuspended in homogenisation buffer. Protein was determined by the method of Lowry *et al.* Different concentrations of the extract 200-1000 µg/mL were incubated in the reaction mixture (40 mM Tris- HCl buffer, pH 7.4, containing 2 mM MgCl₂ and 10 µg membrane protein) to make a volume of 1 mL. Then, 2 mM ATP Tris salt was utilized to start the reaction, this preparation was incubated for 20 min at 37°C. The reaction was terminated by adding 1 mL of ice-cold trichloroacetic acid (10% v/v). The H⁺ - K⁺ ATPase activity was assayed in the presence and the absence of different doses of the extract and omeprazole. The absorbance of inorganic Phosphate (Pi) released from the hydrolysis of ATP was recorded at 820 nm within 2 hr and expressed as µg Pi/mg prot/min.

Percentage of enzyme inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = \frac{[\text{Activity (control)} - \text{Activity (test)}]}{\text{Activity (control)}} \times 100$$

(Fiske and Subbarow, 1925; Gupta *et al.*, 2013).

Acid Neutralization Capacity Assay

Acid Neutralizing Capacity (ANC) Measurement

Objective

To determine the acid neutralizing capacity of a substance, typically used for evaluating buffers, antacids, or environmental samples such as soils and water.

Materials Required

- Sample (e.g., water, soil, or antacid).
- Standard Acid Solution (e.g., HCl, typically 0.1 M or 0.5 M).
- Standard Base Solution (e.g., NaOH, typically 0.1 M).
- pH Meter or pH Indicator.
- Burette and Pipette.
- Beaker (100 mL or 250 mL).
- Magnetic Stirrer (optional).
- Distilled Water.

Procedure

Sample Preparation

Water Sample: Use a measured volume (e.g., 50 mL) of the water sample. Soil Sample: Weigh a known amount (e.g., 5 g) of soil and suspend it in 50 mL of distilled water. Antacid Sample: Crush and dissolve a known mass (e.g. 1 mg/mL) in distilled water.

Acid Addition

Using a burette, titrate the sample with the standard acid (e.g., HCl) until the desired pH endpoint is reached. For environmental samples (water/soil): Titrate until pH 4.5 (or lower, based on requirements). For antacids: Titrate until pH 3.0 (simulating stomach conditions).

Titration and Neutralization

Record the volume of acid added. If back-titration is required (for strong neutralizers like antacids), add excess acid first, then titrate the unreacted acid with a base (NaOH) until the neutral pH endpoint is reached.

Calculation of ANC

ANC is expressed as milliequivalents per litre (meq/L) or per gram (meq/g) and is calculated using the following equations. The moles of acid neutralized are calculated as:

$$\text{Moles of acid neutralized} = (\text{Volume of HCl} \times \text{Normality of HCl}) - (\text{Volume of NaOH} \times \text{Normality of NaOH}).$$

Acid-Neutralizing Capacity (ANC) per gram of antacid/extract is calculated as:

$$\text{ANC} = \text{Moles of HCl neutralized} / \text{Grams of antacid or extract}$$

(College of Engineering and Applied Science, n.d.; Garad *et al.*, 2012).

Statistical analysis

All data were expressed as Mean $\pm$ SEM ($n=3$). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Physiochemical properties and Phytochemical screening

Phytochemical screening of *Ricinus communis* L. leaf extract revealed the presence of flavonoids, which are considered to be the major contributors to its antiulcer activity.

Thin Layer Chromatography

Thin-Layer Chromatography (TLC) analysis identified the flavonoids rutin and quercetin in the extract. The R_f value of

the extract was found to be 0.76, which corresponded with the R_f value of the standard compounds, thereby confirming the presence of rutin and quercetin.

Effect of RCLE on $H^+ K^+$ ATPase inhibition

The H^+/K^+ -ATPase enzyme, commonly known as the gastric proton pump, plays a crucial role in the secretion of gastric acid by parietal cells. Inhibition of this enzyme is an established therapeutic strategy for the management of gastric ulcers, Gastroesophageal Reflux Disease (GERD), and other acid-related disorders.

In the present study, the inhibitory effect of RCLE on H^+/K^+ -ATPase activity was evaluated and compared with the standard proton pump inhibitor, omeprazole. The results demonstrated a concentration-dependent inhibition of H^+/K^+ -ATPase by RCLE. The percentage inhibition increased progressively from 26.15% at 200 $\mu\text{g/mL}$ to 61.54% at 1000 $\mu\text{g/mL}$, indicating that the sample effectively reduced enzyme activity with increasing concentration.

The decrease in optical density values with increasing sample concentration further supports its inhibitory action against the proton pump enzyme. Omeprazole exhibited significantly higher inhibitory activity, producing 61.22% inhibition at 200 $\mu\text{g/mL}$ and reaching 86.22% inhibition at 1000 $\mu\text{g/mL}$. Although RCLE was less potent than the standard drug, it demonstrated substantial inhibitory activity at higher concentrations, suggesting the presence of bioactive constituents capable of suppressing gastric acid secretion through proton pump inhibition (Table 1 and Figure 1).

Effect of RCLE on Acid Neutralization Capacity

The Acid Neutralizing Capacity (ANC) assay was carried out to evaluate the antacid potential of RCLE in comparison with the standard antacid formulation containing aluminum hydroxide and magnesium hydroxide. The results demonstrated that both the standard and the test sample showed concentration dependent acid neutralizing activity. The standard formulation exhibited strong acid neutralization, with ANC values ranging from 58.0 to 33.8 mEq/g across the tested concentrations (Table 2 and Figure 2).

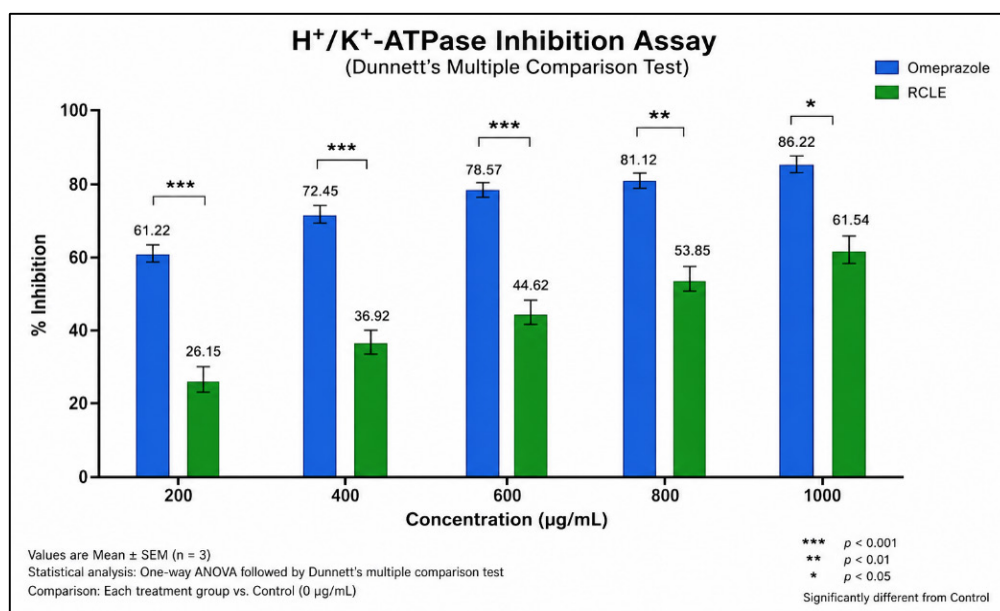
The higher acid consumed values and lower NaOH requirement indicated efficient neutralization of gastric acid by the standard antacid preparation. RCLE also showed appreciable acid neutralizing activity. As the concentration increased from 200 mg to 1000 mg, the amount of acid consumed increased from 8.2 to 26.5 mEq, indicating improved neutralization ability at higher concentrations. The ANC values of the sample ranged from 41.0 to 26.5 mEq/g. Although the activity was lower than that of the standard formulation, the sample demonstrated significant antacid potential.

Table 1: % Inhibition of H⁺ K⁺ ATPase enzyme.

Group	Conc. (µg/mL)	SEM±OD	% Inhibition
Control	-	0.653±0.023	-
Standard (Omeprazole)	200	0.253±0.009	61.22
	400	0.180±0.006	72.45
	600	0.140±0.006	78.57
	800	0.123±0.003	81.12
	1000	0.090±0.006	86.22
Test (RCLE)	200	0.480±0.006	26.15
	400	0.410±0.006	36.92
	600	0.360±0.006	44.62
	800	0.300±0.006	53.85
	1000	0.250±0.006	61.54

Table 2: Effect of RCLE on Acid Neutralization Capacity (ANC).

Group	Conc. (µg/mL)	Acid Consumed (mEq)	ANC (mEq/g)
Al(OH) ₃ + Mg(OH) ₂	200	11.6	58.0
	400	17.4	43.5
	600	22.8	38.0
	800	28.5	35.6
	1000	33.8	33.8
Test (RCLE)	200	8.2	41.0
	400	12.9	32.2
	600	17.6	29.3
	800	21.8	27.2
	1000	26.5	26.5

**Figure 1: % Inhibition of H⁺ K⁺ ATPase enzyme.**

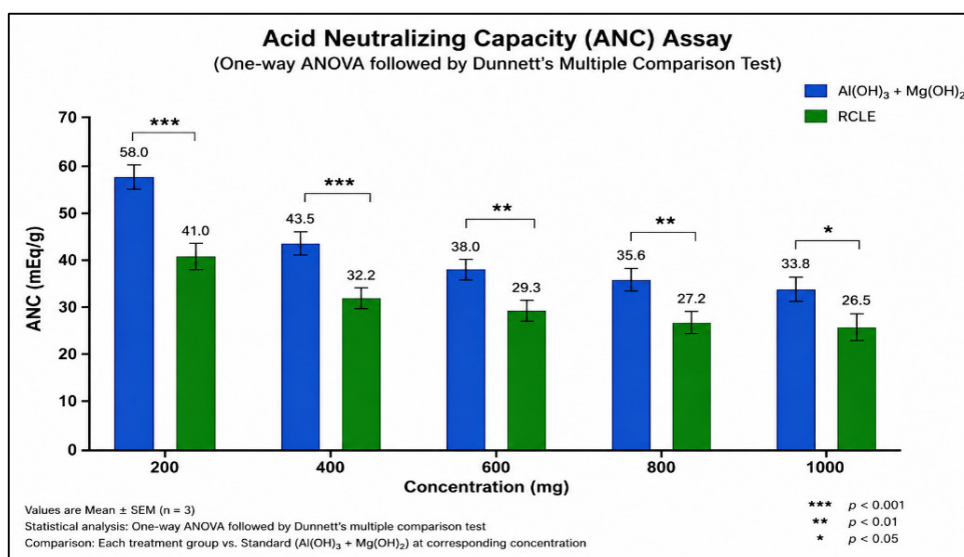


Figure 2: Effect of RCLE on Acid Neutralization Capacity (ANC).

CONCLUSION

Ricinus communis L. leaf extract exhibited significant and concentration-dependent inhibition of H^+/K^+ -ATPase activity, with percentage inhibition increasing from 26.15% at 200 $\mu\text{g}/\text{mL}$ to 61.54% at 1000 $\mu\text{g}/\text{mL}$. Although its inhibitory effect was lower than that of the standard drug omeprazole, the sample demonstrated appreciable proton pump inhibitory activity. These results indicate that extract possesses potential anti-ulcer and gastroprotective properties and may contribute to the reduction of gastric acid secretion.

The present study indicates that *Ricinus communis* L. leaf extract possesses considerable acid neutralizing capacity and exhibits dose-dependent antacid activity. However, its activity was comparatively lower than the standard $\text{Al}(\text{OH})_3 + \text{Mg}(\text{OH})_2$ formulation. These findings suggest that extract may serve as a potential natural antacid agent and could be further investigated for its gastroprotective and antiulcer properties.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding the publication of this work.

ABBREVIATIONS

TLC: Thin Layer Chromatography; **RCLE:** *Ricinus communis* leaves extract; **ANC:** Acid Neutralizing Capacity; **ATPase:** Adenosine Triphosphate.

SUMMARY

The present study investigated the antiulcer potential of *Ricinus communis* L. leaf extract through *in vitro* experimental models. The extract demonstrated notable inhibition of H^+/K^+ -ATPase activity along with appreciable acid-neutralizing capacity in a concentration-dependent manner. These findings indicate its potential role in reducing gastric acidity and supporting ulcer prevention. Further studies are required to validate its efficacy and elucidate the underlying mechanisms of action.

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