World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Pharmacological evaluation, anti-oxidant and ant-ulcer activity of *Uncaria tomentosa* extract in experimental rats

Brajlata Prajapati [1,*], Pradeep Kumar Mohanty [2] and Akhlesh Kumar Singhai [3]

[1] *Research Scholar, School of Pharmacy, LNCT University, Bhopal (M.P), India.*
[2] *HOD, department of Pharmacology, School of Pharmacy, LNCT University, Bhopal (M.P), India.*
[3] *Director, School of Pharmacy, LNCT University, Bhopal (M.P), India.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 22(03), 211-221

Publication history: Received on 01 April 2025; revised on 01June 2025; accepted on 04June 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.3.0497

Abstract

The present study aimed to evaluate the pharmacological, antioxidant, and anti-ulcer activities of *Uncaria tomentosa* extracts using both *in vitro* and *in vivo* experimental models. The plant material was extracted successively using petroleum ether and methanol via Soxhlet extraction. The methanolic extract exhibited a higher percentage yield (2.23%) compared to the petroleum ether extract (0.55%). Phytochemical screening indicated the presence of alkaloids, glycosides, flavonoids, tannins, and carbohydrates predominantly in the methanolic extract. Quantitative analysis showed significant levels of total phenolic content (61.7 mg GAE/g) and total flavonoid content (16.25 mg RE/g). Antioxidant activity was confirmed through DPPH radical scavenging assay, with the methanolic extract demonstrating an IC_{50} of 48.68 µg/mL, compared to 22.04 µg/mL for standard ascorbic acid.*In vivo* anti-ulcer studies in ethanol-induced ulcer models in rats revealed that *Uncaria tomentosa* extract at 400 mg/kg significantly reduced the ulcer index (3.001 ± 0.255), gastric juice volume (2.221 ± 1.006 mL), and free acidity (17.986 ± 1.470 mEq/L), while mildly increasing gastric pH (3.008 ± 0.650). These effects were comparable to the reference drug ranitidine. The findings validate the traditional use of *Uncaria tomentosa* and highlight its potential as a natural source for antioxidant and anti-ulcer therapies.

Keywords: *Uncaria tomentosa*; Antioxidant activity; Anti-ulcer; Phytochemical screening; DPPH assay; Ethanol-induced ulcers; Gastric protection; Medicinal plants

1. Introduction

Medicinal plants have been utilized for therapeutic purposes since ancient times and continue to be a significant source for drug discovery. A substantial portion of the population in developing regions of Asia and Africa relies on plant-based traditional medicines for primary healthcare. The widespread use of these plants is primarily due to their easy accessibility and affordability. Herbal therapies typically involve parts of plants or unrefined herbal extracts containing a variety of phytochemicals, which are believed to act synergistically. These complex mixtures can serve as lead compounds for the development of numerous drugs currently used in the treatment of various diseases.[1]

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of these peptic ulcers is seen among many people. The peptic ulcers are erosion of lining of stomach or the duodenum. [2] Antiulcer medications are crucial for managing different forms of ulcers, notably peptic ulcers, which can cause serious

*Corresponding author: Brajlata Prajapati

pain and perhaps serious consequences if left untreated. Here are some crucial details emphasising the significance of antiulcer medications: Pain and discomfort relief Antiulcer medications aid in reducing ulcer-related symptoms such heartburn, indigestion, and burning belly discomfort. These medications ease discomfort and raise the standard of living for ulcer sufferers by lowering stomach acid production and/or preserving the stomach lining. [3]

The therapeutic potential of *Uncaria tomentosa* (cat's claw) as an anti-ulcer agent was supported by both traditional use and modern pharmacological research. Its diverse chemical constituents, including alkaloids and proanthocyanidins, demonstrated promising biological activities contributing to its effectiveness in managing ulcerative conditions, particularly peptic ulcers. With increasing demand for natural and affordable treatments, *U. tomentosa* served as a valuable source for the development of novel plant-based anti-ulcer therapies. However, further clinical investigations were required to fully understand its mechanisms of action and therapeutic efficacy. [4]

2. Material and methods

2.1. Chemicals

All chemicals and reagents used in the experimental procedures were of analytical grade and sourced from reputable suppliers to ensure reliability and accuracy of results. Glacial acetic acid, nitroprusside, sodium hydroxide, and ammonia were procured from Merck, known for its high-quality laboratory reagents. Petroleum ether was obtained from Research lab, and ethanol was supplied by Molychem, both recognized for their consistent reagent-grade products. Concentrated sulfuric acid was sourced from Fizmerck, while 95% alcohol, concentrated hydrochloric acid, and chloroform were provided by Clorofiltind, a well-established supplier of laboratory chemicals. Magnesium, necessary for various qualitative tests, was acquired from Himedia, a trusted name in microbiological and chemical supplies. The use of these reagents supported the precision and reproducibility of the experimental analyses carried out in this study.

2.2. Plant collection

Uncaria tomentosa , a medicinal plant, was collected around 350gm in the localised area of Bhopal. After cleaning, the plant was dried under shade at room temperature for three days before being oven dried at 45°C until completely dry. To avoid contamination and deterioration, dried plant parts (leaves) were stored in airtight glass containers in a dry, cool location.

2.3. Extraction

In the present study, the extraction of plant material was carried out using the continuous hot percolation technique with a Soxhlet apparatus. Finely powdered *Uncaria tomentosa* was loaded into the thimble of the Soxhlet extractor. The extraction process was conducted at a controlled temperature of 60°C, employing petroleum ether and methanol as non-polar solvents. The Soxhlation was continued separately for each solvent until the siphon tube exhibited no further change in color, indicating exhaustive extraction. To ensure complete removal of the solvents, the extracts were concentrated under reduced pressure using a rotary vacuum evaporator (Buchi type) at 40°C. The dried residues were weighed, and the percentage yield of each extract was determined using the following formula: [5]

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

2.4. Phytochemical investigation

An experiment was carried out to determine the presence or absence of several phytoconstituents using thorough qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were used. **[6]**

2.5. Quantitative Phytochemical Estimation

2.5.1. TPC

The total phenolic content of *Uncaria tomentosa* extract was estimated using the Folin-Ciocalteu assay. A 0.2 mL aliquot of the extract was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate, then diluted with distilled water to a final volume of 7 mL. The mixture was incubated at room temperature for 2 hours. Absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Gallic acid was used as the standard, and a calibration curve

was prepared with concentrations ranging from 20 to 100 µg/mL. The assay is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds, producing a blue chromophore measurable spectrophotometrically. [7]

2.5.2. TFC

The flavonoid content was measured using the aluminium chloride technique. 0.5 mL of *Uncaria tomentosa* extract solution was mixed with 2 mL of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and stirred thoroughly. After that, wait 6 minutes before adding 0.15 mL of 10% aluminium chloride and allowing standing for 6 minutes. Then, 2 millilitres of 4% sodium hydroxide were added. The mixture was shaken and properly combined. The absorbance of the combination was measured at 510 nm using a UV spectrophotometer. Calibration curves were created with standard solutions of Rutin Equivalent (RE) mg/gm. Rutin was concentrated to 20, 40, 60, 80, and 100 µg/mL. The calibration curve was used to determine the total flavonoid concentration, which was expressed as mg Rutin equivalent per gram of dry extract weight. [8]

2.6. DPPH

Uncaria tomentosa extract's antioxidant activity was assessed utilizing the DPPH free radical scavenging test. A methanol solution containing 1 mg/ml extracts/standard was produced. *Uncaria tomentosa* extracts/standards (20-100µg/ml) were produced from a 1mg/mL stock solution with 2mL of 0.1mM DPPH solution added. The resulting mixture was vortexed, incubated for 30 minutes at room temperature in a relatively dark environment, and measured at 517 nm with a UV spectrophotometer. For the control, add 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. The absorbance of the control was measured against methanol (as a blank) at 517 nm. **[9]**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

2.7. Acute Toxicity Study

The acute toxic class approach outlined in the guideline is a stepwise procedure that uses three animals of the same sex per phase. Depending on the animals' mortality and/or moribund stage, 2-4 steps may be required to determine the acute toxicity of the test chemical. The drug is given orally to a group of experimental animals at one of the prescribed dosages. The chemical is evaluated in stages, with each step including three animals of the same sex. The absence or presence of compound-related mortality in animals dosed at one stage determines the next phase, i.e., no further testing is required, dosing of three further animals with the same dose, and dosing of three other animals at the next higher or lower dose level. Each phase requires three animals. The beginning dose is set from one of four fixed levels: 5, 50, 300, and 2000 mg/kg body weight of *Uncaria tomentosa* . [10]

2.8. Experimental work

2.8.1. Animals Protocol

- **IAEC Approval** All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).
- **Animal used**
- **Weight** 270±20 gm
- **Strain** Wistar rat
- **Sex:** Wistar Male

Housing Condition- The animals were kept in six separate cages at a controlled temperature of 22 ± 2°C. All animals were fed a conventional food (golden feed, New Delhi) and provided with water on a regular basis.

2.8.2. Induction of ulcer in rats:

Male Wistar rats weighing 270±20 g was fasted for 24 hours with free access to water and randomly divided into 5 groups. The control group was given a vehicle (5 ml of distilled water orally), and the inducer group was given 20 mg/kg of ethanol orally. Treatment groups III and IV were given ethanol 20 mg/kg and a test sample (*Uncaria tomentosa* extract- 200, 400 mg/kg), whereas the standard group (V) was given the standard antiulcer medicine (Ranitidine 20 mg/kg via oral route). The rats were slaughtered after an hour, and the stomach was removed and opened along the larger curvature. [11]

2.8.3. Experimental design

- Group 1- Normal control
- Group 2- Inducer group Ethanol 20 mg/kg orally
- Group 3- Treated with *Uncaria tomentosa* extract 200 mg/kg orally
- Group 4- Treated with *Uncaria tomentosa* extract 400 mg/kg orally
- Group 5- Treated with standard drug (Ranitidine) 20 mg/kg orally

2.9. Parameters assessed for anti-ulcer activity

2.9.1. Ulcer index

The incidence and severity of the lesion were graded using the arbitrary scoring method listed below. The stomachs were then dissected along their greater curvature, cleaned with normal saline to remove gastric contents, and examined under a 10x magnifying lens for ulcer formation. Ulcers were counted and evaluated using the Kulkarni technique (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = haemorrhagic streaks, 3 = ulcers > 3 but < 5, and 5 = ulcers > 5).

The ulcer Index and percentage of ulcer inhibition were determined as follows:

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

Where,
UN = Average number of ulcers per animal,
US = Average of severity score,
UP = Percentage of animals with ulcers

2.9.2. Volume of gastric juice

Each animal's gastric juice volume was determined after centrifugation at 1000 rpm for 10 minutes and evaluated. The volume of the centrifuged sample was calculated as mL per 100g body weight.

2.9.3. pH of gastric juice

To determine pH, dilute 1 mL of gastric juice with 1 mL of distilled water and use a pH meter.

2.9.4. Determination of free acidity

Dilute 1 ml of gastric juice with distilled water and transfer to a 50 ml conical flask. Add 2 drops of phenolphthalein indicator. 0.01 N NaOH was used for titration until a permanent pink color was obtained; the consumed volume was calculated. The free acidity was estimated with the following formula:

$$\boldsymbol{Acidity} = \frac{\boldsymbol{VolumeofNaOH \times NormalityofNaOH \times 100}}{\boldsymbol{0.1}}$$

3. Results

3.1. Percentage Yield

Table 1 Percentage Yield of crude extracts of *Uncaria tomentosa* extract

S.no	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Uncaria tomentosa*	Pet ether	350	1.67	0.55%
2		Methanol	295.23	6.59	2.23%

3.2. Preliminary Phytochemical study

Table 2 Phytochemical testing of extract

S. No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	**Methanolic extract**
1.	Alkaloids		
	Dragendroff's test	Present	Present
	Mayer's reagent test	Present	Present
	Wagner's reagent test	Present	Present
	Hager's reagent test	Present	Present
2.	Glycoside		
	Borntrager test	Absent	Present
	Legal's test	Absent	Present
	Killer-Killiani test	Absent	Present
3.	Carbohydrates		
	Molish's test	Present	Present
	Fehling's test	Present	Present
	Benedict's test	Present	Present
	Barfoed's test	Present	Present
4.	Proteins and Amino Acids		
	Biuret test	Absent	Absent
	Ninhydrin test	Absent	Absent
5.	Flavonoids		
	Alkaline reagent test	Present	Present
	Lead Acetate test	Present	Present
6.	Tannin and Phenolic Compounds		
	Ferric Chloride test	Present	Present
	Lead Acetate test	Present	Present
	Gelatin test	Present	Present
7.	Saponin		
	Foam test	Present	Absent
8.	Test for Triterpenoids and Steroids		
	Salkowski's test	Absent	Absent
	Libbermann-Burchard's test	Absent	Absent

3.3. Quantitative Analysis

3.3.1. Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

Table 3 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.174
2.	40	0.202
3.	60	0.278
4.	80	0.313
5.	100	0.330

Table 4 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.140
2.	40	0.176
3.	60	0.191
4.	80	0.235
5.	100	0.271

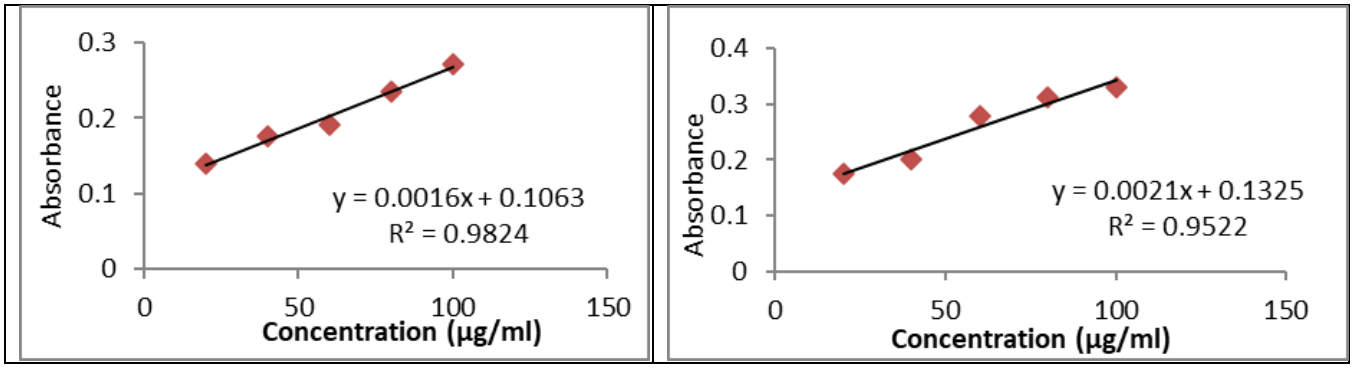

Figure 1 Represent standard curve of Gallic acid and Rutin

Total Phenolic Content and Total Flavonoid Content in extract

Table 5 Total Phenolic Content in extract

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	61.7 mg/gm

Table 6 Total Flavonoid Content in extract

Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Methanol	16.25 mg/gm

3.4. *In vitro* Antioxidant Assays

3.4.1. DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 7 DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.489	50.854
40	0.430	56.783
60	0.346	65.226
80	0.286	71.256
100	0.143	85.628
Control	0.995	
IC50 22.04		

Table 8 DPPH radical scavenging activity of methanol extract of *Uncaria tomentosa*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.518	44.120
40	0.466	49.730
60	0.453	51.132
80	0.414	55.339
100	0.365	60.625
Control 0.927		
IC50 48.68		

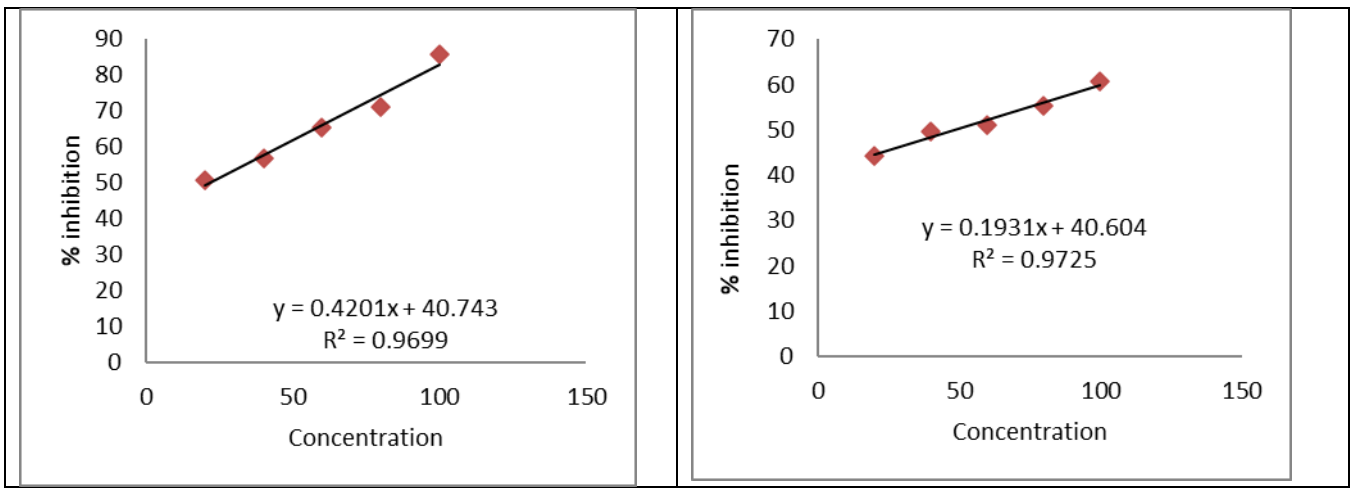

Figure 2 DPPH radical scavenging activity of Std. Ascorbic acid and extract of *Uncaria tomentosa*

3.5. Analysis of general parameters

3.5.1. Determination of Ulcer Index

Table 9 Observation of Ulcer Index

Groups	**Ulcer Index**
	Mean
Group I- Normal Control	0
Group II Inducer Ethanol (20 mg/kg bw)	10.789±0.784
Group III *Uncaria tomentosa* Extract treated (200mg/kg) group	9.942±0.153
Group IV *Uncaria tomentosa* Extract treated (400mg/kg) group	3.001±0.255
Group V Standard (Ranitidine 20 mg/kg bw)	1.654±0.467

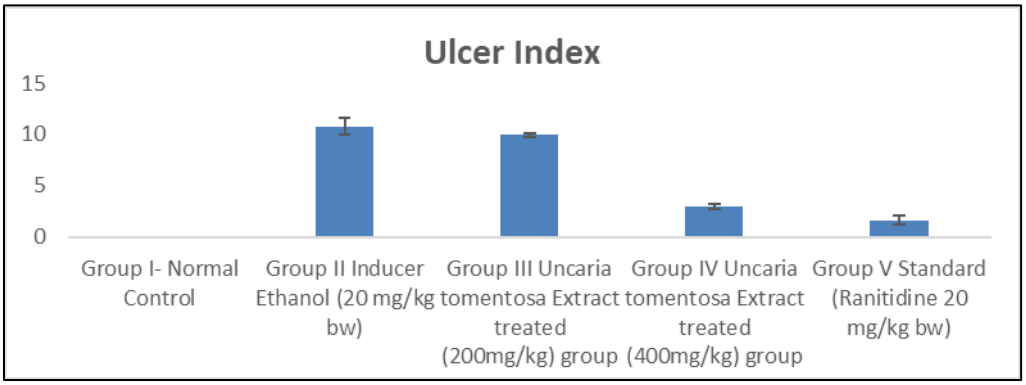

Figure 3 Bar chart represents ulcer index in Ethanol induced ulcer in rats

3.5.2. Determination of Volume of gastric juice

Table 10 Observation of volume of gastric juice

Treatment Group	**Volume of gastric juice**
Group I- Normal Control	1.752±0.447
Group II Inducer Ethanol (20 mg/kg bw)	6.890±0.863
Group III *Uncaria tomentosa* Extract treated (200mg/kg) group	5.110±0.845
Group IV *Uncaria tomentosa* Extract treated (400mg/kg) group	2.221±1.006
Group V Standard (Ranitidine 20 mg/kg bw)	1.992±0.741

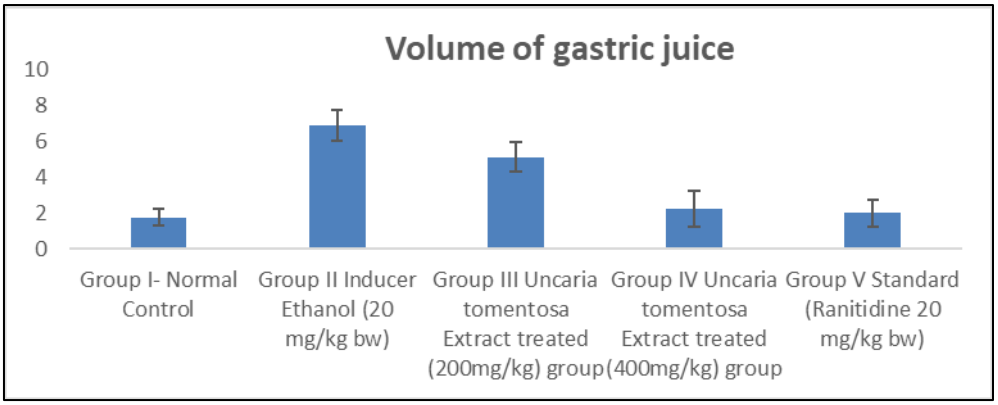

Figure 4 Bar chart represents gastric volume in Ethanol induced ulcer in rats

3.5.3. Determination of pH of gastric juice:

Table 11 Observation of pH of gastric juice

Treatment Group	pH of gastric juice
Group I- Normal Control	2.117±0.73
Group II Inducer Ethanol (20 mg/kg bw)	3.950±0.580
Group III *Uncaria tomentosa* Extract treated (200mg/kg) group	2.966±0.560
Group IV *Uncaria tomentosa* Extract treated (400mg/kg) group	3.008±0.650
Group V Standard (Ranitidine 20 mg/kg bw)	2.876±0.850

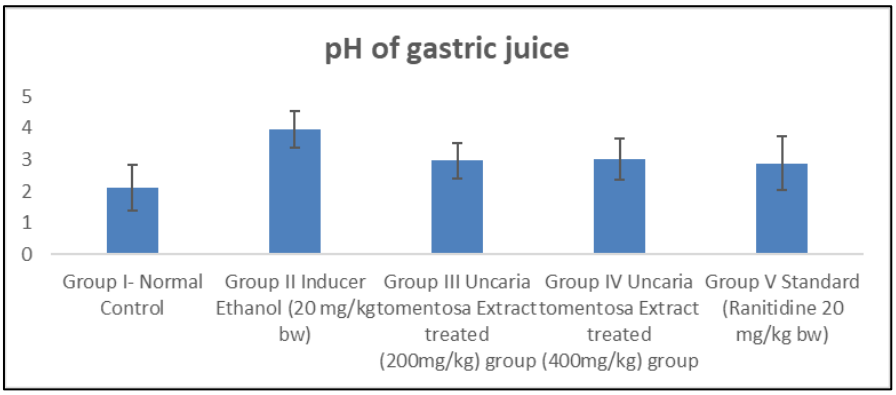

Figure 5 Bar chart represents pH in Ethanol induced ulcer in rats

3.5.4. Free acidity determination:

Table 12 Observation of free acidity in Ethanol induced peptic ulcer in rats

Treatment Group	Free acidity determination (mE/L)
Group I- Normal Control	15.280±3.543
Group II Inducer Ethanol (20 mg/kg bw)	26.648±3.640
Group III *Uncaria tomentosa* Extract treated (200mg/kg) group	25.012±2.561
Group IV *Uncaria tomentosa* Extract treated (400mg/kg) group	17.986±1.470
Group V Standard (Ranitidine 20 mg/kg bw)	16.023±1.750

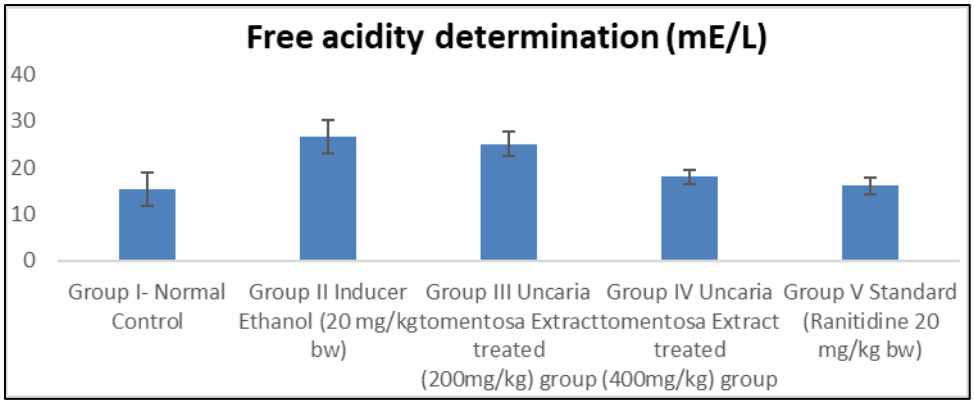

Figure 6 Bar chart represents free acidity determination in Ethanol induced ulcer in rat

The methanolic extract of *Uncaria tomentosa* yielded a higher percentage (2.23%) compared to the petroleum ether extract (0.55%), indicating better solubility of phytoconstituents in polar solvents. Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, and phenolic compounds, particularly in the methanolic extract, supporting its traditional medicinal use. Quantitative analysis showed a notable total phenolic content (61.7 mg GAE/g) and flavonoid content (16.25 mg Rutin/g), which correlate with strong antioxidant activity demonstrated by the DPPH assay (IC_{50} = 48.68 µg/mL). In vivo studies showed significant anti-ulcer activity of the extract, with a dose-dependent reduction in ulcer index, gastric juice volume, and free acidity, especially at 400 mg/kg, comparable to the standard drug Ranitidine. These findings support the gastroprotective potential of *Uncaria tomentosa* and highlight its value in developing plant-based anti-ulcer agents.

4. Conclusion

In conclusion, the study highlights the significant pharmacological potential of *Uncaria tomentosa* as both an antioxidant and an anti-ulcer agent. The extract exhibited strong antioxidant activity, which is crucial in reducing oxidative stress that contributes to various gastrointestinal disorders. Moreover, the anti-ulcer activity demonstrated in the ethanol-induced ulcer model further supports the therapeutic efficacy of *Uncaria tomentosa* in managing gastric ulcers. Given its phytochemical profile, especially its high phenolic and flavonoid content, the extract shows promise as a natural alternative for the treatment and prevention of ulcers, warranting further research for its clinical applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology advances. 2015 Dec 1;33(8):1582-614.

[2] Jaiswal F, Rai AK, Wal P, Wal A, Singh SP. Peptic ulcer: a review on etiology, pathogenesis and treatment. Asian Journal of Pharmaceutical Education and Research. 2021;10(4):1.

[3] Lal N, Singh rv. an overview: formulation and evaluation of herbal formulation for its antiulcer activity.

[4] Batiha GE, MagdyBeshbishy A, Wasef L, Elewa YH, Abd El-Hack ME, Taha AE, Al-Sagheer AA, Devkota HP, Tufarelli V. *Uncaria tomentosa* (Willd. ex Schult.) DC.: A review on chemical constituents and biological activities. Applied Sciences. 2020 Apr 13;10(8):2668.

[5] Baidya Rai M, Bhattarai S, Feitosa CM, editors. Ethnopharmacology of wild plants. Boca Raton: CRC Press; 2021 Feb 14.

[6] Egbuna C, Ifemeje JC, Maduako MC, Tijjani H, Udedi SC, Nwaka AC, Ifemeje MO. Phytochemical test methods: qualitative, quantitative and proximate analysis. In Phytochemistry 2018 Dec 12 (pp. 381-426). Apple Academic Press.

[7] Kaur C, Kapoor HC. Anti-oxidant activity and total phenolic content of some Asian vegetables. International journal of food science and technology. 2002 Feb;37(2):153-61.\

[8] Parthasarathy S, Azizi JB, Ramanathan S, Ismail S, Sasidharan S, Said MI, Mansor SM. Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from Mitragynaspeciosa (Rubiaceae family) leaves. Molecules. 2009 Oct 9;14(10):3964-74.

[9] Athavale A, Jirankalgikar N, Nariya P, De S. Evaluation of in-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. International journal of pharmaceutical sciences and research. 2012 Aug 1;3(8):2543.

[10] Gribaldo L, Gennari A, Blackburn K, Clemedson C, Degeneracy A, Meneguz A, Pfaller W, Ruhdel I. 3.1. Acute Toxicity. Alternatives to laboratory animals. 2005 Jul;33(1_suppl):27-34.

[11] Abdulla MA, Ahmed KA, Al-Bayaty FH, Masood Y. Gastroprotective effect of Phyllanthusniruri leaf extract against ethanol-induced gastric mucosal injury in rats. African Journal of Pharmacy and Pharmacology. 2010 May 31;4(5):226-30.