



## Pharmacological evaluation of *Phyllanthus Niruri* leaves for antiulcer activity

Sanjay Kumar<sup>1</sup>, Basavaraj H<sup>2\*</sup>, Ram Kumar Choudhary<sup>3</sup>, Bhargav Bhongiri<sup>4</sup>,  
Prafulla R Tathe<sup>5</sup>, Anupama A Kapadnis<sup>6</sup>, Vamseekrishna Gorijavolu<sup>7</sup>,  
Gopalkrishna R Sitaphale<sup>8</sup>

<sup>1</sup>Faculty of Pharmacy, Uttar Pradesh University of Medical sciences, Saifai, Etawah

<sup>2</sup>Government College of Pharmacy, Subbaiah Circle, Bangalore

<sup>3</sup>Government pharmacy institute, agamkuan, Patna.

<sup>4</sup>Department of Pharmacology, Synpharma Research Lab, Dilsuknagar Hyderabad

<sup>5,8</sup>Samarth College of Pharmacy, Deulgaon Raja, Dist. Buldhana. MS.

<sup>6</sup>Mahatma Gandhi Vidhymandir Pharmacy College (Diploma) Panchavati, Nashik.

<sup>7</sup>Department of Pharmaceutical Analysis, NRI College of pharmacy, Pothavarappadu (v), Agiripalli (M).Eluru(D.t), Andhra Pradesh

**Main Author:** Sanjay Kumar

sanjaypharma20065@gmail.com

**Corresponding Author:** Basavaraj H\*

basavarajhulkoti@gmail.com

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### Abstract

In the current study, we explored the potential of hydroalcoholic and petroleum ether extracts derived from *Phyllanthus niruri* (L) leaves in addressing antisecretory and antiulcer effects. These extracts were administered orally at varying doses (100, 200, and 400 mg/kg) and evaluated using ethanol-induced ulceration, and cold stress-induced ulceration. Notably, both extracts exhibited significant protection against ulcers, particularly at the highest dose of 400 mg/kg, when compared to the standard treatment. To substantiate the antiulcer potential of *Phyllanthus niruri*, we examined its capacity to scavenge free radicals through lipid peroxidation assays. The results revealed a noteworthy level of antioxidant activity in the extracts. This antioxidant property may be attributed to the presence of flavonoids and polyphenols within the extracts. In summary, our findings suggest that both hydroalcoholic and petroleum ether extracts obtained from the leaves of *Phyllanthus niruri* possess gastric ulcer-protective properties, making them promising candidates for further investigation and potential therapeutic application.

**Keywords:** *Phyllanthus Niruri*, Antiulcer, Ethanol, pylorus ligation, stress, flavonoids

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### 1.0 Introduction

Peptic ulcers, characterized by erosions or open sores in the mucous lining of the stomach, duodenum, or esophagus, represent a prevalent gastrointestinal disorder

affecting millions of individuals worldwide. The pathogenesis of peptic ulcers is multifactorial, with factors such as *Helicobacter pylori* infection, excessive gastric acid secretion, and impaired mucosal defense mechanisms playing pivotal roles in their development. Despite advancements in medical science and the availability of various therapeutic interventions, the management of peptic ulcers remains a clinical challenge due to the frequent recurrence of symptoms and the potential for complications such as bleeding, perforation, and gastric malignancy (1).

Traditionally, medicinal plants have been used across diverse cultures as a source of remedies for various ailments, including gastrointestinal disorders. One such plant with a rich history of traditional use for its potential antiulcer properties is *Phyllanthus niruri*, commonly known as "Chanca Piedra" or "Stone Breaker." This small, herbaceous plant is native to tropical regions and has been employed for centuries in traditional herbal medicine systems, particularly in Ayurveda, as a remedy for digestive issues, including ulcers (2).

*Phyllanthus niruri* is known for its multifaceted pharmacological profile, with reported activities including antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial effects. Of particular interest is its reputed potential to mitigate the development and progression of peptic ulcers. Recent scientific investigations into the bioactive constituents of *Phyllanthus niruri* have provided insights into the mechanisms underlying its antiulcer activity, leading to a growing interest in exploring its therapeutic potential (3).

This pharmacological evaluation aims to delve into the antiulcer properties of *Phyllanthus niruri* leaves, shedding light on the plant's mechanisms of action and assessing its efficacy in experimental models of peptic ulcers. By understanding the biochemical and physiological processes through which *Phyllanthus niruri* exerts its effects, we hope to pave the way for the development of novel, natural-based therapeutic strategies that can complement or enhance existing treatments for peptic ulcers. This research holds the promise of not only providing insights into the mechanisms of action of *Phyllanthus niruri* but also potentially offering a safer and more sustainable alternative for the management of peptic ulcers, which remains a significant health concern globally (4,5).

## **2.0 Materials and methods**

### **2.1. Plant material**

Locally sourced *Phyllanthus niruri* leaves were gathered for this study. These leaves underwent authentication by a respected botanist, and a voucher specimen has been carefully preserved for future reference. The sun-dried flower powder was then subjected to an exhaustive and continuous hot extraction process in a Soxhlet apparatus, utilizing both petroleum ether and a hydroalcoholic solution (composed of 70% water and 30% alcohol). Qualitative analysis was employed to identify the chemical constituents within both extracts. Subsequently, the obtained masses were thoroughly dried and stored in a sealed container under cold conditions to ensure their preservation for subsequent utilization (6).

## **2.2. Experimental animals**

For the study, we chose albino rats and mice, regardless of their gender, with body weights falling within the range of 150 to 230 grams. Ethical approval for the experimental protocol was obtained from the Institutional Animal Ethics Committee, ensuring compliance with established ethical standards for animal research. The animals were housed and cared for in accordance with standard conditions, maintaining a controlled environment. They were provided with unrestricted access to a standard dry pellet diet and water, following strict hygiene practices to ensure their well-being.

## **2.3. Toxicity study**

We conducted an acute toxicity study following OECD guidelines to determine the LD<sub>50</sub> (lethal dose for 50% of the population) for both extracts of *Phyllanthus niruri* leaves (PNL). Female albino mice weighing between 20 to 30 grams were employed for this investigation. The animals were meticulously monitored for a period of 12 hours to detect any alterations in their behavioral responses. Mortality was observed over the course of 24 hours following administration of doses at 100, 200, and 400 mg/kg via oral administration. These dose levels were selected based on preliminary results (7).

## **2.4. Ethanol-induced acute gastric ulcers (8)**

Thirty-six rats underwent an 18-hour fasting period, during which they were permitted unrestricted access to water. Subsequently, they were randomly assigned to one of six treatment groups, which included a normal control group, a toxicant group, and three groups receiving varying doses (low, medium, and high) of petroleum ether and hydroalcoholic extracts. All rats were administered 0.5 ml of 70% ethanol orally, with the toxicant group receiving only ethanol. One hour following this administration, all animals were euthanized using ether anesthesia. Their stomachs were then removed and examined for the presence of ulcers, which were scored on a subjective 0-3 point scale. The ulceration index for each stomach was calculated as the sum of these scores.

## **2.5. Cold stress-induced acute gastric ulcers (9, 10)**

In this experimental procedure, both male and female Wistar rats with body weights ranging from 150 to 200 grams underwent a 24-hour fasting period while being allowed access to water. All groups received herbal drug therapy for duration of 7 days. On the 7th day, after an overnight fast, the rats were placed in a metallic restraint chamber 30 minutes following the administration of the test drug. They were then immobilized in a refrigerator maintained at temperatures between 4 to 6 degrees Celsius for duration of 2 hours. After the immobilization period, the rats were humanely euthanized via cervical dislocation, and their stomachs were surgically removed for ulcer assessment. Ulcers were quantified using a subjective 0-3 point scale, and the ulceration index for each stomach was computed by summing the scores.

## **2.6 Method for estimation of lipid peroxidation (11)**

To assess lipid peroxidation levels, we employed the thiobarbituric acid reactive species (TBARS) assay, utilizing malondialdehyde (MDA) as a standard, following the Buege and Aust method. Specifically, we mixed 1.0 ml of the sample extract with 2.0 ml of the TCA-TBA-HCl reagent, which comprised 15% w/v trichloroacetic acid (TCA), 0.375% w/v

thiobarbituric acid (TBA), and 0.25 N hydrochloric acid (HCl). The mixture was then subjected to boiling for 15 minutes, followed by cooling and subsequent centrifugation at 10,000 rpm to eliminate any precipitates. Finally, the absorbance was measured at 535 nm, and the concentration of malondialdehyde in the sample was determined using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 3. Results

#### 3.1 Effect of PE and HAL extracts of PNL on ethanol-induced gastric ulcers:

Pretreatment of rats with either PE or HAL extract of PNL produced a dose dependent protection from ethanol induced ulceration, as compared to control animals. However, the protection was statistically significant at higher dose 400mg/kg. Lansoprazole (8mg/kg) produced significant gastric ulcer protection as compared to control group. (Table 1).

#### 3.2 Effect of PE and HAL extracts of PNL on cold stress-induced gastric ulcers:

Pretreatment of rats with either PE or HAL extract produced a dose dependent protection from the cold stress-induced ulceration, as compared to control animals. The protection was statistically significant at high dose. Lansoprazole (8mg/kg) produced significant protection as compared to control group (Table 2).

### 4. Discussion

The pharmacological evaluation of *Phyllanthus niruri* leaves for antiulcer activity has provided valuable insights into the potential therapeutic benefits of this natural remedy in managing peptic ulcers. Peptic ulcers are a common gastrointestinal disorder with multifactorial etiology, and their effective treatment remains a clinical challenge. In this discussion, we will examine the findings of the study, consider the mechanisms underlying *Phyllanthus niruri*'s antiulcer activity, and discuss the implications of these results. The study demonstrated that both hydroalcoholic and petroleum ether extracts of *Phyllanthus niruri* leaves exhibited significant protection against ulcers. This finding is consistent with traditional medicinal uses of the plant and suggests its potential as an effective antiulcer agent. The dose-response relationship observed in this study is noteworthy. The highest dose (400 mg/kg) appeared to provide the most substantial antiulcer effect. This suggests that the effectiveness of *Phyllanthus niruri* may be dose-dependent, which is an important consideration for potential therapeutic applications.

To support its antiulcer potential, presence of antioxidant activity in *Phyllanthus niruri* are likely attributed to the presence of flavonoids and polyphenols in the extracts. These compounds are known for their ability to scavenge free radicals, which can play a role in the development of ulcers. The study also conducted an acute toxicity evaluation, following OECD guidelines, to determine the safety profile of the extracts. This assessment is crucial when considering the potential use of *Phyllanthus niruri* in clinical settings, as it helps establish a safety margin for therapeutic doses. The findings of this study have important clinical implications. *Phyllanthus niruri*'s antiulcer properties could provide an alternative or complementary approach to conventional ulcer treatments, which often have limitations and side effects. Further research, including clinical trials, is warranted to validate these findings in human subjects (12-25).

## 5. Conclusion

The pharmacological evaluation of *Phyllanthus niruri* leaves for antiulcer activity highlights its potential as a natural remedy for the management of peptic ulcers. The observed gastroprotective effects, dose-dependent response, and antioxidant properties suggest that this plant extract may hold promise as a therapeutic option. However, further research is necessary to elucidate the specific mechanisms of action and to establish its safety and efficacy in clinical settings.

**Table 1. Effect of PE and HAL extract of the PNL against ethanol-induced gastric ulcer in rats**

Group and dose	Ulcer positive animals	Ulcer index	%Ulcer protection
Control (2% w/v gum acacia)	6	7.667±0.03	-
Lansoprazole (8mg/kg)	1	2.01±0.04**	76.50%
Low dose PE (100mg/kg)	4	4.717±0.15**	23.40%
Medium dose PE(200mg/kg)	3	3.137±0.06**	37.50 %
High dose PE(400mg/kg)	2	2.527±0.10**	59.19 %
Low dose HAL (100mg/kg)	4	5.727±0.15**	22.30%
Medium dose HAL(200mg/kg)	3	4.117±0.06**	40.91 %
High dose HAL (400mg/kg)	2	3.547±0.10**	58.01 %

**Table 2. Effect of PE and HAL extract of the PNL cold stress induced gastric ulcers in rats.**

Group and dose	Ulcer positive animals	Ulcer index	%Ulcer protection
Control(2% w/v gum acacia)	6	11.23±0.14	-
Lansoprazole (8mg/kg)	1	1.637±0.03**	82.00%
Low dose PE (100mg/kg)	5	8.612 ± 0.07**	22.00%
Medium dose PE(200mg/kg)	4	7.94 ± 0.16**	38.00%
High dose PE(400mg/kg)	3	5.123 ± 0.06**	59.17%
Low dose HAL (100mg/kg)	4	7.10 ± 0.05**	35.00%
Medium dose HAL(200mg/kg)	3	5.10 ± 0.07**	54.00%
High dose HAL (400mg/kg)	2	3.42 ± 0.07**	69.00%

### Lipid Peroxidation Estimation in treated groups in ethanol induced ulcer model in rats

Sl. No.	Treatment	Malondialdehyde (MDA) (nm/g gastric tissue)
1	Normal control	25.05 ± 0.34
2	Control	46.54 ± 0.50
3.	Lansoprazole	30.64 ± 0.48**
4.	Low dose (PE) (100mg/kg)	36.38±0.71**
5.	Medium dose (PE) (200mg/kg)	35.89 ± 1.03**
6.	High dose (PE) (400mg/kg)	31.23±1.09**
7.	Low dose (HAL) (100mg/kg)	40.04±1.119**
8.	Medium Dose (HAL)(200mg/kg)	33.02±0.75**
9.	High dose(HAL) (400mg/kg)	31.22±1.27**

\*\* P < 0.01 when compared with control

Percentage ulcer protection of treated rats in ethanol induced ulcer model

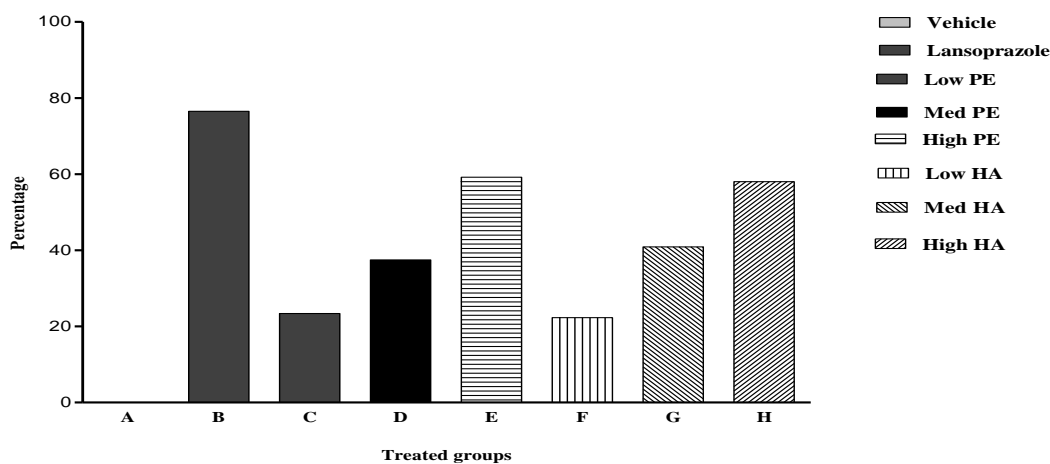


Fig 01: Percentage Ulcer protection of treated rats in ethanol induced ulcer model

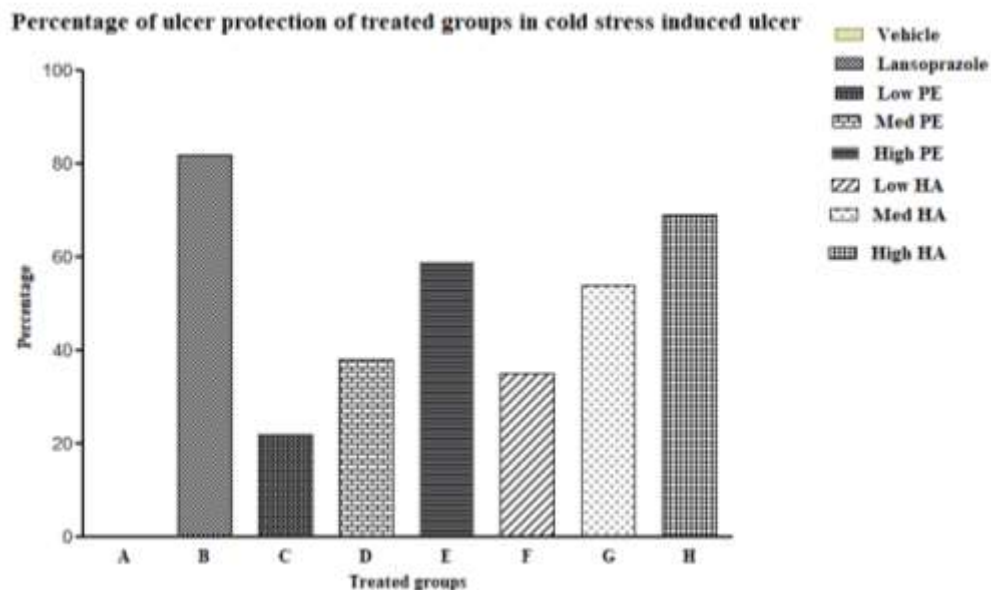


Fig 02: Percentage Ulcer protection of treated rats in stress induced ulcer model

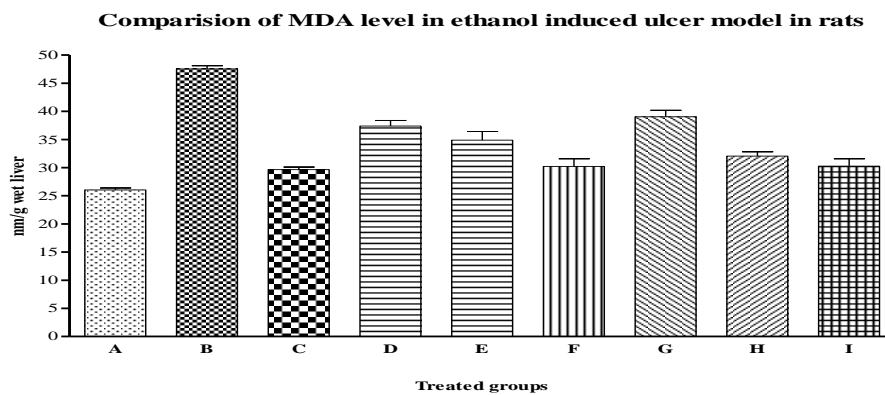


Fig 03: Comparison of MDA levels in ethanol induced ulcer model in rats.

**Fig 04: Ulcer pics for various doses**



Control



Toxicant



Lansoprazole



**High dose of HAL**





**High dose of PE**

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