Panacea Journal of Pharmacy and Pharmaceutical Sciences
ISSN: 2349 7025

PJPPS
*Panacea Research Library*
http://internationaljournal.org.in/journal/index.php/pjpps

**Original Research Article** **Volume 10 Issue 3** **July-Sept 2022**

# STUDY OF PLANT SECONDARY METABOLITES AND ANTI ULCER ACTIVITY OF EXTRACT OF *PHYLLANTHUS URINARIA*

***Arun Kumar, Mr. Amit Dubey, Dr. Satkar Prasad, Dr. Sailesh Kumar Ghatuary**

RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)

*Corresponding Author's Email ID: arunmishra9520@gmail.com

**Abstract**

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *Phyllanthus urinaria* has several pharmacological properties including anti-inflammatory and anti-diarrhoeal. In spite of its uses in the traditional medicine against various ailments, this plant has so far not been screened for anti-ulcer activity. We report on the anti-ulcer activity of hydroalcoholic extract of *Phyllanthus urinaria.* The results of the present study have shown that hydroalcoholic extract of *Phyllanthus urinaria* possess gastro protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol. As flavonoids have been identified in the methanolic extract, we believe that the anti-ulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection already. Sub-acute toxicological studies have revealed that the methanolic extract of hydroalcoholic extract of *Phyllanthus urinaria* show slight CNS depression for a few hours after treatment at the dose of 2000 mg /kg. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe. Any substance that is not toxic at 2000 mg /kg is considered relatively safe.

**Key words:** *Phyllanthus urinaria*, hydroalcoholic extract, Anti Ulcer activity, Ethanol induced

## Introduction

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with a high morbidity and substantial mortality. Epidemiological data for this disease and its complications have shown striking geographical variations in incidence and prevalence. Development of ulcer disease and death from it has been associated with the birth of urbanisation and was interpreted as a birth-cohort event with the peak of disease in those born during the late 19th century.[1,2]

Our understanding of the disease changed greatly with the discovery of Campylobacter pyloridis (renamed Helicobacter pylori in 1989 because of a revised taxonomic classification) in 1982 by Warren and Marshall. [3,4]

This discovery switched the notion from an acid-driven disease to an infectious disease, opening a huge area for intensive research that resulted in the reconciliation of previously suggested mechanisms of pathogenesis. The fall of the acid dogma in peptic ulcer disease, which had found its undisputed acceptance during and after the introduction of histamine H2-receptor antagonists, led to the present therapeutic principle. Maintenance acid suppressive therapy for duodenal ulcer, which followed decades of dominance of surgical interventions (subtotal gastric resections, several forms of vagotomy), was replaced with a short-term antibiotic regimen targeting eradication of H pylori infection. [5,6]

H. pylori eradication as cure of peptic ulcer received its full recognition when the Nobel Prize for Medicine and Physiology was awarded to Warren and Marshall in 2005. This recognition has not, however, closed the chapter on peptic ulcers. The management of ulcer disease and its complications remains a clinical challenge. Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin are an increasingly important cause of ulcers and their complications even in H pylori-negative patients. Other rare causes of ulcer disease in the absence of H pylori, NSAIDs, and aspirin also exist.

The predominant symptom of uncomplicated peptic ulcer is epigastric pain, which can be accompanied by other dyspeptic symptoms such as fullness, bloating, early satiety, and nausea. In patients with duodenal ulcer, epigastric pain occurs typically during the

fasting state or even during the night and is usually relieved by food intake or acid-neutralising agents. Roughly a third of these patients also have heartburn, mostly without erosive oesophagitis. Chronic ulcers can be asymptomatic [7]

In particular, this absence of symptoms is seen in NSAID-induced ulcers, for which upper gastrointestinal bleeding or perforation might be the first clinical manifestation of disease. The most frequent and severe complication of peptic ulcers is bleeding, which is reported in 50–170 per 100 000, with the highest risk in people aged older than 60 years. Perforation is less frequent than is bleeding, with an incidence of around seven to ten per 100 000. Penetration of retroperitoneal organs is characterised by constant severe pain but fortunately is rare.15 Gastric outlet obstruction due to ulcer-induced fibrosis is also rare, and should raise suspicion of underlying malignant disease. [8]

The genus *Phyllanthus* (L.) belongs to a family of flowering plants Phyllanthaceae and consists of more than 1000 species widely distributed in various parts of the world. The species of this genus including trees, herbs and shrubs that is pharmacologically valuable as they contain various bioactive compounds. Previous scientific data indicate that more than 500 chemical compounds (phytochemicals) have been isolated from species of the genus *Phyllanthus*.

*Phyllanthus urinaria*, one of the herbal plants belonging to the genus *Phyllanthus* (Euphorbiaceae), is widely distributed in China, Southern India and South America. It has been used clinically in Asia to treat diarrhea, dysentery, hepatitis, edema, infantile malnutrition, acute conjunctivitis, aphthae and antibiotic resistant pyogenic infections. Aim of present work to evaluate antiulcer activity of the leaves of *Phyllanthus urinaria*.

## Material and Methods

### Collection of plant materials

The leaves of *Phyllanthus urinaria* were collected from local area of Bhopal in the month of January considering the seasonal conditions for obtaining maximum phytoconstituents.

## Extraction of plant material

Collected plant drugs namely *Phyllanthus urinaria* leaves were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (55.60 gm) and packed in (250 ml) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant drugs were subjected to extraction by ethanol and Water (ethanol: water; 70:30) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated [9].

## Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of leaves of *Phyllanthus urinaria*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids [10].

## Quantitative estimation of phenols and flavonoids

## Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [11].

## Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [12].

## Pharmacological activity

### Animals:

Wistar rats (180-200 g) and Swiss albino mice (males; 20–25 g) were used in the present study. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee.

### Acute toxicity study

Five groups (n = 5) of male albino mice were used in the acute toxicity study of *Phyllanthus Urinaria* Hydroalcoholic extract. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, and 2000 mg /kg) of the extract. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days.

### Experiment Design

Two treatment groups received 200 and 400 mg/kg plants extract orally via a stainless steel intubation needle. Two doses were given at 08:00 and 16:00 and a third dose was given on the second day 1.5 h before induction of gastric ulceration [64]. As the same time as case groups: a negative control group was given distilled water (10 mL/kg) and a positive control group was given Ranitidine at 50 mg/kg [13]. All animals were given

ethanol (Merck) 50 % (v/v) (in distilled water) at 10 mL/kg orally to induce gastric ulceration.

One hour after ethanol administration, all rats were killed by an overdose of chloroform and the stomachs were rapidly removed, opened along their greater curvature and gently rinsed under running tap water and spread on a paraffin plate. Lesions in the glandular part of the stomach were measured with a graticules under a stereomicroscope.

Long lesions were counted and measured along their greater length. Petechial lesions were counted. Each five petechial lesions were taken as 1mm of ulcer. The sum of the total length long ulcers and petechial lesions in each group of rats was divided by its number to calculate the ulcer index (mm). The macroscopic curative ratio was determined by the formula:

$$\text{Curative ratio} = \frac{\text{control ulcer index} - \text{test ulcer index}}{\text{control ulcer index}} \text{x100}$$

Immediately after macroscopic evaluation the stomachs were fixed in neutral buffered formalin (10%) then glandular parts were divided to four segments and routine histologic processing was carried out. 5-6µm sections were stained by H&E method and were evaluated microscopically. Microscopic ulcer index was obtained using published methods [66] by two pathologists, separately and a mean index was calculated.

Normal tissue = 0

Local damage to gastric pits cells = 1

Local damage to gastric glands = 2

Deep damage to gastric glands = 3

Microscopic ulcer index = (number of lesion 1) + (number of lesion 2) ×2+ (number of lesion3) ×3

**Grouping of animals:**

Group-I Control Received Distilled water 10 ml/kg, p.o

Group-II Received Ranitidine 50 mg/kg, p.o

Group-III Received HAPU 200 mg/kg, p.o

Group-IV Received HAPU 400 mg/kg, p.o

**Bio-Chemical estimation**

**Estimations in Gastric Juice**

The various biochemical parameters like carbohydrate content viz. fucose, hexosamine, total hexoses, and sialic acid and total carbohydrates were estimated. Gastric volume, pH, free and total acidity and total proteins were also evaluated [14].

**Results and Discussion**

Ulcer has long been recognized as one of the most important gastrointestinal problem. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer. *Phyllanthus urinaria* has several pharmacological properties including anti-inflammatory and anti-diarrhoeal. In spite of its uses in the traditional medicine against various ailments, this plant has so far not been screened for anti-ulcer activity. We report on the anti-ulcer activity of hydroalcoholic extract of *Phyllanthus urinaria.* The results of the present study have shown that hydroalcoholic extract of *Phyllanthus urinaria* possess gastro protective activity, as evidenced by its significant inhibition in the formation of ulcers induced by ethanol. The protective effect was confirmed by histological examination showing prevention of mucosal lesions and sub-mucosal edema. As flavonoids have been identified in the methanolic extract, we believe that the anti-ulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection already. Sub-acute toxicological studies have revealed that the methanolic extract of hydroalcoholic extract of *Phyllanthus urinaria* show slight CNS depression for a few hours after treatment at the dose of 2000 mg /kg. However, there was no sign of toxicity or mortality up to 14 days indicates that the extract is relatively safe. Any substance that is not toxic at 2000 mg /kg is considered relatively safe.

**Table 1: Extractive values obtained from *Phyllanthus urinaria* leaves using different solvents**

| S. No. | Solvent | % Yield |
|---|---|---|
| 1. | Petroleum ether | 13.58% |
| 2. | Methanol: Water | 19.44 % |

**Table 2: Preliminary phytochemical screening of *Phyllanthus urinaria* leaves**

| S.N. | Phytoconstituents | Test Name | Hydroalcoholic Extract |
|---|---|---|---|
| 1 | Alkaloids | Mayer's Test | **+(ve)** |
| | | Dragendorff's Test | **-(ve)** |
| 2 | Glycosides | Raymond's Test | **+(ve)** |
| | | Killer Killani Test | **+(ve)** |
| 3 | Carbohydrates | Molisch's Test | **-(ve)** |
| | | Fehling's Test | **-(ve)** |
| 4 | Tannins | Vanillin- HCl Test | **+(ve)** |
| | | Gelatin Test | **-(ve)** |
| 5 | Flavonoids | Lead acetate | **+(ve)** |
| | | Shinoda Test | **+(ve)** |
| 6 | Resins | Color detection with ferric chloride | **-(ve)** |
| | | Turbidity Test | **-(ve)** |
| 7 | Steroids | Libermann- Bur chard Test | **+(ve)** |
| | | Salkowski Reaction | **+(ve)** |
| 8 | Proteins & Amino acids | Biuret Test | **+(ve)** |
| | | Precipitation test | **-(ve)** |
| | | Ninhydrin Test | **+(ve)** |
| 9. | Phenols | Ellagic Acid Test | **+(ve)** |

**Table 3: Effect of Hydroalcoholic extract of *Phyllanthus urinaria* on ethanol-induced gastric ulcer**.

| **Groups** | **Groups Ulcer index ± SEM (Macroscopically)** | **Ulcer index ± SEM (Microscopically)** | **Macroscopic Curative ratio (%)** |
|---|---|---|---|
| Distilled water (10 mL/kg) | 12.51 ± 2.14 | 13.6 ± 1.22 | --------- |
| Ranitidine (50 mg/kg) | 5.19 ± 1.02 | 7.72 ± 0.87 | 58.51 |
| HAPU(200 mg/kg) | 2.99 ± 0.46* | 4.15 ± 0.78* | 76.09 |
| HAPU(400 mg/kg) | 2.47 ± 0.42* | 2.15 ± 0.42* | 80.25 |

*p < 0.01; n/group=6 HAPU: Hydroalcoholic extract of *Phyllanthus urinaria*

**Table 4: Effect of Hydroalcoholic extract of *Phyllanthus urinaria* on Antisecretory Parameters**

| **Treatment** | **Dose (mg/kg b.w)** | **pH** | **Free Acidity (mEq/l/ 100g)** | **Total Acidity (mEq/l/ 100g)** |
|---|---|---|---|---|
| Distilled water | 10 ml/kg | 1.84±0.12 | 61.01±3.1 | 77.84±3.8 |
| Ranitidine | 50 mg/kg | 5.19±2.55 | 21.13±3.4 | 31.25±3.5 |
| HAPU | 200 mg/kg | 3.11±0.22 | 41.32±0.05 | 39.16±2.66 |
| HAPU | 400 mg/kg | 3.81±0.44 | 34.47±2.19 | 33.47±2.46 |

**Values are mean ± S.E.M, n=6, NS-not significant, *p < 0.05 and **p < 0.01 Vs control, One way ANOVA followed by Dunnett's test**

**Table 5: Effect of Hydroalcoholic extract of *Phyllanthus urinaria* on Total Proteins and C/P**

| **Treatment** | **Dose (mg/kg b.w)** | Total proteins (µg/ml) | C/P |
|---|---|---|---|
| Distilled water | 10 ml/kg | 458.21±0.12 | 0.67 |
| Ranitidine | 50 mg/kg | 237.47±1.11** | 2.71 |
| HAPU | 200 mg/kg | 400.54±1.57** | 1.9 |
| HAPU | 400 mg/kg | 319.02±1.32** | 2.44 |

Values are expressed in terms of mean ± S.E.M, **p<0.01- One way ANOVA followed by Dunnett's test

**Table 6: Effect of Hydroalcoholic extract of *Phyllanthus urinaria* on Total Carbohydrates**

| **Treatment** | **Dose (mg/kg b.w)** | **Total Carbohydrates (µg/ml)** | | | |
|---|---|---|---|---|---|
| | | **Total Hexose** | **Hexosamine** | **Fucose** | **Sialic acid** |
| Distilled water | 10 ml/kg | 147.33±0.14 | 164.42±0.48 | 6.25±0.22 | 24.23±0.22 |
| Ranitidine | 50 mg/kg | 319.31±0.14** | 325.20±0.11** | 154.11±0.24** | 47.11±0.24** |
| HAPU | 200 mg/kg | 211.42±1.47** | 202.80±1.41** | 62.47±1.86** | 43.36±1.46** |
| HAPU | 400 mg/kg | 297.14±1.14** | 271.22±0.24** | 75.12±1.42** | 36.25±1.40** |

Values are expressed in terms of mean ± S.E.M, **p<0.01 Vs control- One way ANOVA followed by Dunnett's test

## Conclusion

From this study, it is clear that Hydroalcoholic extract of *Phyllanthus urinaria* have significant anti-ulcer activity in animal models. It has muco-protective activity and

gastric antisecretary when compared with that of reference drug omeprazole. The extract is non-toxic even at relatively high concentrations. The anti-ulcer activity is probably due to the presence of flavonoids. Further studies are being carried out to characterize and explore the biological activity of the compounds present in the extract.

## References

1. Susser M, Stein Z. Civilisation and peptic ulcer. Lancet 1962; 279: 116–19.
2. Sonnenberg A. Causes underlying the birth-cohort phenomenon of peptic ulcer: analysis of mortality data 1911–2000, England and Wales. Int J Epidemiol 2006; 35: 1090–97.
3. Warren JR, Marshall B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 321: 1273–75.
4. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 323: 1311–15.
5. NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in peptic ulcer disease. JAMA 1994; 272: 65–69.
6. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut 2007; 56: 772–81.
7. Dew MJ. Asymptomatic peptic ulcer disease. Br Med J (Clin Res Ed) 1987; 15: 401.
8. Gustavsson S. Peptic ulcer disease, complications. In: Gustavsson S, Kumar D, Graham DY, eds. The stomach. Edinburgh, London, Madrid, Melbourne, New York, Tokyo: Churchill Livingstone, 1992: 266–77.
9. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
10. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn., *Vallabh Prakashan*: 1994; 112:120.
11. Arpana Gaur Mishra, Richa Singh, Neha Patil, Geeta Parkhe. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *gloriosa superba* seed extract. Asian Journal of Pharmaceutical Education and Research. 2017, 6(2):12-17.

12. Alkofahi A, Atta AH. Pharmacological screening of antiulcerogenic effects of some Jordanian medicinal plants in rats. J Ethnopharmacol. 1999; 67:341-5.
13. Alvares A, Pomar F, Sevilla MA, Montero MJ. Gastric antisecretory and antiulcer activities of an ethanolic extract of Bidens pilosa L. var. radiate Schult. J Ethnopharmacol. 1999;67:333- 40.
14. Pandit S, Sur TK, Jana U, Bhattacharyya D, Debnath PK. Antiulcer effect of Shankha bhasma in rats: a preliminary study. Indian J Pharmacol. 2000;32:378-80