# Evaluation of the anti-ulcer potential of ethyl acetate and 70% ethanolic extract of *Bryophyllum pinnatum* Lam. (Oken*)* leaves in rats

**Priyanka Yadav[1*], Arun K Mishra[2], Harpreet Singh[3]**

[1*]PhD Scholar, Faculty of Pharmacy, IFTM University, Moradabad, 244102, India.

[2] Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, 244102, India.

[3] School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University, Moradabad, 244102, India.

**[*]Corresponding author**

**Email: priyankagautamyadav@gmail.com**

**Phone: +91-91408 62725**

## Abstract

The present study evaluated the gastroprotective effects of ethanolic and ethyl acetate extracts of *Bryophyllum pinnatum* (200 and 400 mg/kg, p.o.) by using ethanol induced and stress-induced gastric ulcer model in rats. Ranitidine (30 mg/kg, p.o.) was used as standard drug. Parameters such as ulcer index, total acidity, acid volume, gastric pH were assessed in both ethanol-induced and stress induced ulcer model. Treatment with *Bryophyllum pinnatum* extracts showed a reduction in ulcer scores, index and in acidity. The extracts possessed significant protective action against the gastric lesions induced by stress and ethanol. The antiulcer activity may be due to the antisecretory and cytoprotective effects of the extract. This present study indicates that *Bryophyllum pinnatum* leaves extract have potential antiulcer activity and owned significant dose dependent action.

**Keywords**: Antiulcer activity, Ethanol induced, Stress induced, *Bryophyllum pinnatum*

## Introduction

Peptic ulcer disease includes both gastric and duodenal ulcers which posed a major threat to the world's population over the past two centuries with a high morbidity and mortality. (1) Peptic ulcer disease (PUD) is characterized by discontinuation in the inner lining of the gastrointestinal (GI) tract because of gastric acid secretion or pepsin. It extends into the muscularis propria layer of the gastric epithelium. It usually occurs in the stomach and proximal duodenum. It may involve the lower esophagus, distal duodenum, or jejunum. The estimated prevalence of peptic ulcer disease in the general population is 5–10%. (2) The management of peptic ulcer disease and its complications remain a challenge. The main therapeutic intervention for the gastric ulcer is the inhibition of aggressive factors combined with the stimulation of increased defensive factors (3). The existing therapeutic drugs for the treatment of this pathology are proton pump inhibitors (PPIs—lansoprazole, omeprazole) and H2-receptor antagonists (H2Ras—ranitidine, famotidine), in addition to antibiotics used to eradicate *H. pylori* (4). However, prolonged use of antisecretory drugs can cause many adverse effects (5) (6). For example, PPIs can cause abdominal pain, nausea, headache, diarrhea, osteoporosis, and fractures, in addition to pneumonia, insomnia, and kidney inflammation (7), and be associated with an increased gastric cancer risk (6). The long-term use of H2Ras can lead to the development of galactorrhea in women, gynecomastia in men, and alteration of the bacterial flora of the gastrointestinal tract (8). The development of new drugs from natural source is also encouraged because it is estimated that of the 300,000 plant species that exist in the world and only 15% have been evaluated to determine their pharmacological potential, moreover approximately 25% of the modern drugs are derived from natural products. (9) *B. pinnatum* belongs to the family Crassulaceae. It is widely distributed in tropical Africa, America, Hawaii, India, China, Australia, and Madagascar [6]. It has advantage to manage a variety of ailments such as conjunctivitis, edema, piles, wounds, eczema, chickenpox, and fever. (10) The leaves of *B. pinnatum* have been reported to possess hepatoprotective and antineoplastic [11, 12], anti-asthmatic and antitussives [12, 13], antidiabetic [13], antihypertensive [17], antimicrobial [15], anti-inflammatory and analgesic [16, 17], and antiulcer [18] activities. The phytochemical compounds present in the plants like sterols, terpenoids, flavones, tannins, and glycosides are responsible for the activities (19, 20, 21).

This study was aimed to evaluate the antiulcer activity of ethanolic and ethyl acetate leaves extract of *Bryophyllum pinnatum* Lam. (Oken) using different ulcer models in albino wistar rats.

## Materials and methods

### Drugs and chemicals

Ethanol, Ethyl acetate and Sodium hydroxide were purchased from S D Fine-Chem Limited (Mumbai, India).

### Plant materials

The plant *Bryophyllum pinnatum* Lam. (Oken) was collected during September-October 2020 from the ranch of Hari Singh in Lucknow. The plant was authenticated by Dr. Lal Babu Chaudhary, Senior Principal Scientist & Curator of Herbarium, Plant Diversity, Systematics and Herabarium Division, National Botanical Research Institute, Lucknow. A voucher specimen was deposited at the herbarium of the institute (No. LWG108140) for future reference. The weight of the leaves was around 2.7 kg in weight and has a dark green color.

### Preparation of ethyl acetate extract of *Bryophyllum pinnatum*

Fresh matured leaves of *Bryophyllum pinnatum* were washed thoroughly, air dried at room temperature under shade, and coarsely powdered using a mechanical grinder. The powder was kept in a tightly closed brown bottle until extraction. The plant material was then extracted successively with ethyl acetate using Soxhlet extraction apparatus. (22) The extract was concentrated using the Rota vacuum evaporator under reduced pressure until the extraction solvent was completely dried and stored in the refrigerator at 4 °C for further use. The dried extract was suspended in 0.5% Tween 80 and used for the experiments. The percentage yield was found to be 3.66%.

### Preparation of ethanolic extract of leaves of B.pinnatum

Extract of the leaves was prepared in accordance with the Soxhlet method described by Jensen, (2007). Fresh matured leaves of *Bryophyllum pinnatum* were washed thoroughly, air dried at room temperature under shade, and coarsely powdered using a mechanical grinder. The powder was kept in a tightly closed brown bottle until extraction. After ethyl acetate extraction then was further extracted successively with 70% ethanol using soxhlet extraction apparatus. (22) The ethanolic (70%) extract was concentrated using the Rota vacuum evaporator under reduced pressure until the extraction solvent was completely dried and stored in the refrigerator at 4 °C for further use. The dried extract was suspended in 0.5% Tween 80 and used for the experiments. The percentage yield was found to be 4.82%.

**Acute toxicity test**

Acute toxicity study was carried out using the limit test dose of 4000 mg/kg as described by OECD 423 guideline. Three female albino rats were fasted for 24 hours but allowed free access to water. A limit dose of 4000 mg/kg of BP was administered sequentially and animals were observed individually for behavioral profile (alertness, restlessness, irritability, and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response, and gait), physical states such as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea, and for morbidity or mortality, after dosing continuously for 4 hours, periodically during the first 72 hours (with special attention given during the first 4 hours) and daily thereafter, for a total of 15 days.

**Grouping and dosing of animals**

Albino Wistar rats of body weight 120-220gm (either sex) were utilized for the study. In this study, all experimental conditions and protocol were maintained in accordance with the Institutional Animal Ethics Committee received regulation (Reg. No-1088/PO/Re/S/07) under the rules 5(a) of the "Breeding of and experiments on animal control and supervision rules 1998".

Animals were randomly assigned to different groups each consisting of five rats. All treatments were given orally 1 hour before the experiment by oral gavage no.14. Doses were determined based on the acute toxicity studies as per OECD guidelines.

**Ethanol-induced ulcer model**

All treatments were given orally 1 hour before the experiment by oral gavage. Group-I: (negative control, treated with ethanol 2mL/kg), Group- II: (received Ranitidine 30 mg/kg), Group- III: (Test 1 received EE 200 mg/kg), Group- IV: (Test 2 received EE 400 mg/kg), Group- V: (Test 3 received EAE 200 mg/kg), Group- VI:(Test 4 received EAE 400 mg/kg).

The gastric ulcers were induced in rats by administrating absolute ethanol (90%) (1ml/200g) orally, after 60 minutes of test and standard drug treatment. All the animals were fasted for 36 hours before administration of ethanol. The Albino rats of either sex was divided into six groups, each consisting of five rats. They were kept in specially constructed cages to prevent coprophagia

during and after the experiment. The animals were anaesthetized 1h later with anesthetic ether and stomach was incised along the greater curvature and ulceration was scored. (23)

**Stress-Induced Gastric Ulcer Model**

All treatments were given orally 1 hour before the experiment by oral gavage. Group-I: (negative control, stress induced), Group- II: (received Ranitidine 30 mg/kg as standard group), Group- III: (Test 1 received EE 200 mg/kg), Group- IV: (Test 2 received EE 400 mg/kg), Group- V: (Test 3 received EAE 200 mg/kg), Group- VI:(Test 4 received EAE 400 mg/kg). The grouping and dosing for stress induced model were same as ethanol indued ulcer model. Immediately after the last dose administration, animals will be placed into plexiglass restraint cages without any movement possibility described by Gurbuz and Yesilada (24). Cages will be placed into the water at 19–21◦C so that the rats will be dipped until the xiphoid process. The animals will be kept in this stressful condition for 7 h and then sacrificed to collect their stomach. Then, each stomach will be dissected following the great curvature and ulceration will be scored (23)**.** The stomach was opened along the greater curvature, rinsed with saline and running water to remove gastric contents and blood clots and evaluated by a 10x magnifier hand lens. After the numbers of ulcerations would be counted, the scoring of the ulcer was made as follows based on the method designed by Kulkarni SK **(Kulkarni SK., 2005).** The parameters were also performed in the stress induced ulcer model as same was performed in ethanol induced ulcer model and tabulated in the following Table 5,6,7 and 8.

Ulcer scores were given as follows; 0.0 = normal colored stomach 0.5 = red coloration 1.0 = superficial ulcers 2= deep ulcers 3 = hemorrhagic streaks

Ulcer index was calculated using the equation

UI = UN + US +UP / 10

Where, UI = Ulcer Index

UN = Average number of ulcers per rats

US = Average of severity score

UP = Percentage of rats with ulcer.

Percentage inhibition was calculated using the following formula **(Sharma and Mishra, 2014).**

Percentage protection= Control U.I.—Test U.I X 100/ Control U.I

**Macroscopic evaluation of stomachs**

The Shay rat model described by Dashputre and Naikwade was followed with a slight modification. (23) The abdomen was opened, cardiac end of the stomach was dissected out, and the contents were drained into a glass tube. The volume of the gastric juice was measured after centrifugation (Eppendorf AG-5703DQ713856) at 2000 rpm for 10 minutes. From the supernatant, aliquots were taken for the determination of pH and total acidity.

**Determination of pH**

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water, and pH of the solution was measured using pH meter (Adwa AD8000). (23)

**Determination of total acidity and free acidity**

An aliquot of 1 ml of gastric juice was diluted with 1 ml of distilled water and was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color was observed. The volume of 0.01N NaOH consumed was noted and the volume of NaOH needed was taken as corresponding to the free acidity. The total acidity was expressed as mEq/L and calculated by the following formula (23)

Acidity = V NaOH x N x 100 mEq/ L / 0.1

where V is volume and N is normality.

**Statistical analysis**

Data were expressed as mean ± standard error of mean and statistically evaluated using one-way analysis of variance (ANOVA) using Graph Prism Pad, version 8.0.1; established statistical values of $P \leq 0.01$.

## Results and Discussion

### Acute toxicity test

With the acute toxicity test at the limit test dose of 4000 mg/kg, neither mortality nor changes related to behavioral, autonomic, neurologic, and physical profiles were observed within the first 24 hours and during the 14-day follow-up.

### Effects of the leaf extract on ethanol induced ulcer

Both the ulcer score and ulcer index were significantly reduced by BP200 ($P<0.01$), BP400 ($P<0.01$), and the standard drug Ranitidine ($P<0.01$). The effect of BP400 was comparable to Ranitidine regarding percent reduction in ulcer index (18.75% vs 8.65%) (Table 1, Figure 1).

This study was carried out to evaluate the anti-ulcer effect of ethyl acetate and 70% ethanol leaf extract of BP on ethanol-induced gastric ulcer and stress-induced gastric ulcer models. The extraction yield was found to be 3.66% and 4.82%w/w of ethyl acetate and ethanol extract respectively plant is similar with the 4% (25) reported in previous studies done in Nigeria, The hydroalcoholic solvent possesses a good extracting potential.

The acute toxicity study revealed that the plant extract was safe in rats at a limit dose of 4000 mg/kg. The finding supports the work done on rats in another study. (25) The acute toxicity test of ethanol leaf extract as shown in indicated that at a dose as high as 4000 mg/kg body weight, there was no mortality recorded in the animals and neither did they show any sign of toxicity. This is evidence that the leaves are relatively secure for human and animal consumptions.

In the present study, the ethanol-induced model was employed to confirm the gastric cytoprotective effect of the plant extract. Extract- and standard drug-treated groups showed a significant reduction (at least $P<0.01$) in both the ulcer score and ulcer index. Redness in ulcer which was caused by ethanol induced ulcer model and represented in figure 5. The extract effect increases with the dose and is comparable with that of the standard drug. This finding signifies that the extract possesses a gastroprotective effect, which is as good as the standard drug. The reduction in percentage of ulcer inhibition was (44.98% and 56.06%) for BP200 and BP400 respectively. The results of the present study showed that the BP leaf extract was capable of inhibiting gastric lesions formed by ethanol.

Absolute alcohol would extensively damage the gastric mucosa leading to increased infiltration of neutrophils, which are a major source of inflammatory mediators. Therefore, suppression of neutrophil infiltration during inflammation was found to enhance gastric ulcer healing. BP leaf extract has been shown to contain anti-inflammatory activity, and it is speculated that the gastroprotective effect exerted by this plant could be credited to its anti-inflammatory property. The extract of *B. pinnatum* might have probably worked via suppressing the release of inflammatory mediators to produce peripheral anti-inflammatory activity. (26,27,28,29) Compounds that act as antioxidants or activate the redox system are important for restoring gastric tissue. Therefore, strong ulcer healing effect of the extract in the ethanol-induced model might also be related to the antioxidant activity of the plant, which is well demonstrated in previous studies. (30) Extract- and standard drug-treated groups showed a significant reduction (at least $P<0.01$) in both the ulcer score and ulcer index. The extract effect increases with the dose and is comparable with that of the standard drug. Compounds that act as antioxidants or activate the redox system are important for restoring gastric tissue. Therefore, strong ulcer healing effect of the extract in the ethanol-induced model might also be related to the antioxidant activity of the plant, which is well demonstrated in previous studies. (30) The Test-2 (T2) group showed (18.75%) lessen number of ulcers, less acidity and high pH value (5.00) in comparison to other test groups of animals (Table 2,3 & 4, Figure 2,3 & 4).

**Table 1. Effects of B.pinnatum leaf extract on ulcer index in ethanol induced ulcer in rats**

| **Animals** | **Negative diseased Control** | **Standard (Std)** | **Test-1(T1) (EE; 200mg/kg)** | **Test-2(T2) (EE; 400mg/kg)** | **Test-3(T3) (EAE; 200mg/kg)** | **Test-4(T4) (EAE; 400mg/kg)** |
|---|---|---|---|---|---|---|
| Rat -1 | 35.375*** | 8.375*** | 18.625*** | 14.125*** | 23.375*** | 14.125*** |
| Rat -2 | 41.875*** | 8.375*** | 22.125*** | 19.375*** | 25.625*** | 23.125*** |
| Rat -3 | 43.125*** | 8*** | 25.625*** | 19*** | 26.625*** | 20.125*** |
| Rat -4 | 43.625*** | 9.625*** | 25*** | 20.5*** | 25.875*** | 21.625*** |
| Rat -5 | 49.375*** | 8.875*** | 26*** | 20.75*** | 28.125*** | 21.375*** |
| Mean | 42.68±4.998 | 8.65±0.627 | 23.48±3.109 | 18.75±2.688 | 25.93±1.727 | 20.08±3.493 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the diseased control group ***<0.01, ns= non-significant

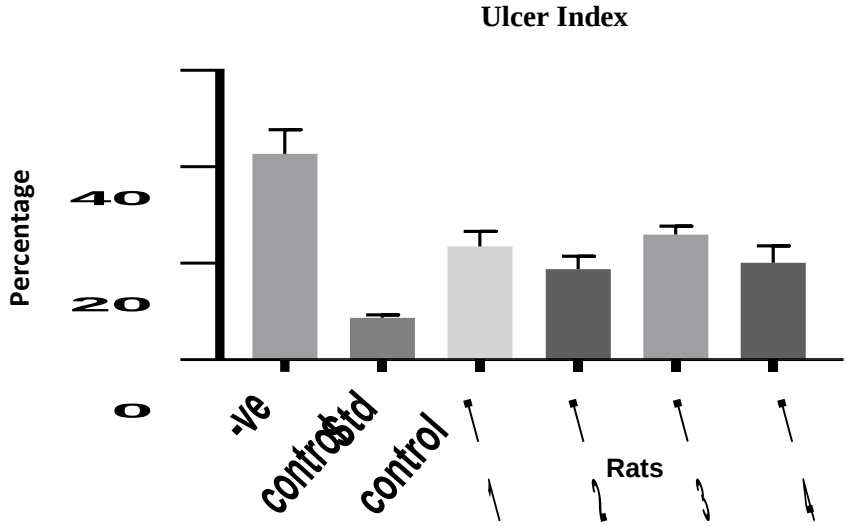


**Figure 1. Effects of B.pinnatum leaf extract on ulcer index in ethanol induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3(T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

**Table 2. Effects of B.pinnatum leaf extract on total acidity in ethanol induced ulcer in rats**

| Animals | Negative diseased Control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 96*** | 53*** | 75*** | 69*** | 85*** | 63*** |
| Rat -2 | 88*** | 58*** | 72*** | 69*** | 72*** | 68*** |
| Rat -3 | 84*** | 70*** | 76*** | 62*** | 80*** | 76*** |
| Rat -4 | 93*** | 60*** | 77*** | 65*** | 79*** | 79*** |
| Rat -5 | 92*** | 65*** | 75*** | 63*** | 81*** | 71*** |
| Mean | 90.60±4.669 | 61.20±6.535 | 75.00±1.871 | 65.60±3.286 | 79.40±4.722 | 71.40±6.348 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the Negative diseased control group ***<0.01, ns= non- significant

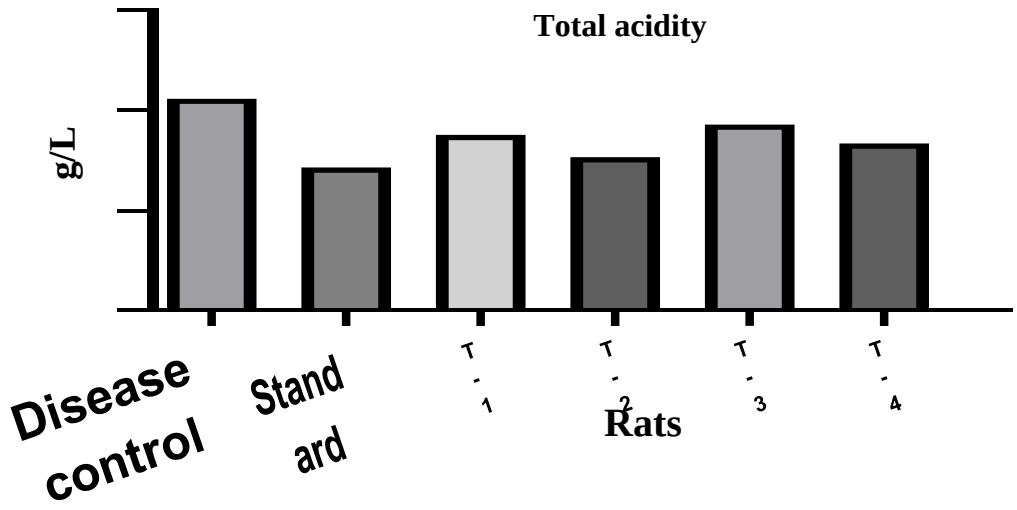


**Figure 2. Effects of B.pinnatum leaf extract on total acidity in ethanol induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3(T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

**Table 3. Effects of *B.pinnatum* leaf extract on Free acidity in ethanol induced ulcer in rats**

| Animals | Disease control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 73*** | 27*** | 52*** | 34*** | 41*** | 43*** |
| Rat -2 | 62*** | 30*** | 46*** | 31*** | 52*** | 49*** |
| Rat -3 | 60*** | 45*** | 46*** | 42*** | 59*** | 36*** |
| Rat -4 | 70*** | 32*** | 47*** | 46*** | 51*** | 39*** |
| Rat -5 | 59*** | 34*** | 45*** | 36*** | 56*** | 39*** |
| Mean | 64.80±6.301 | 33.60±6.877 | 47.20±2.775 | 37.80±6.099 | 51.80±6.834 | 41.20±5.020 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the Negative diseased control group ***<0.01, ns= non-significant

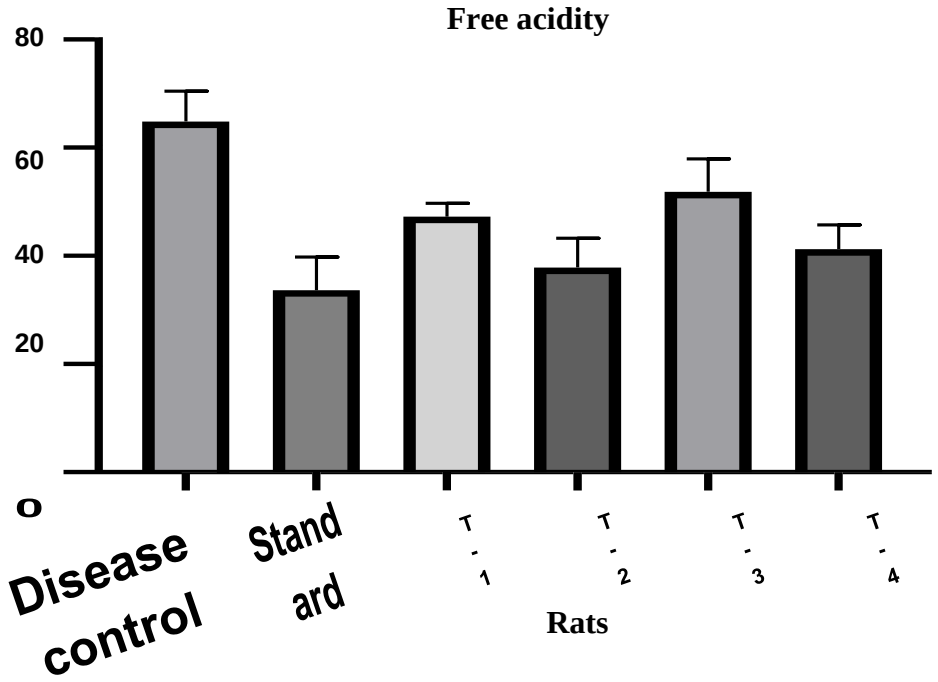


**Figure 3. Effects of *B.pinnatum* leaf extract on Free acidity in ethanol induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3(T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

**Table 4. Effects of B.pinnatum leaf extract on pH of gastric juice in ethanol induced ulcer in rats**

| Animals | Negative Disease control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 1*** | 6*** | 2*** | 6*** | 3*** | 4*** |
| Rat -2 | 2*** | 4*** | 4*** | 4*** | 3*** | 3*** |
| Rat -3 | 3*** | 6*** | 3*** | 6*** | 4*** | 4*** |
| Rat -4 | 3*** | 5*** | 3*** | 5*** | 4*** | 4*** |
| Rat -5 | 2*** | 5*** | 4*** | 4*** | 3*** | 3*** |
| Mean | 2.20±0.837 | 5.20±0.837 | 3.20±0.837 | 5.00±1.000 | 3.40±0.548 | 3.60±0.548 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the Negativediseased control group ***<0.01, ns= non-significant

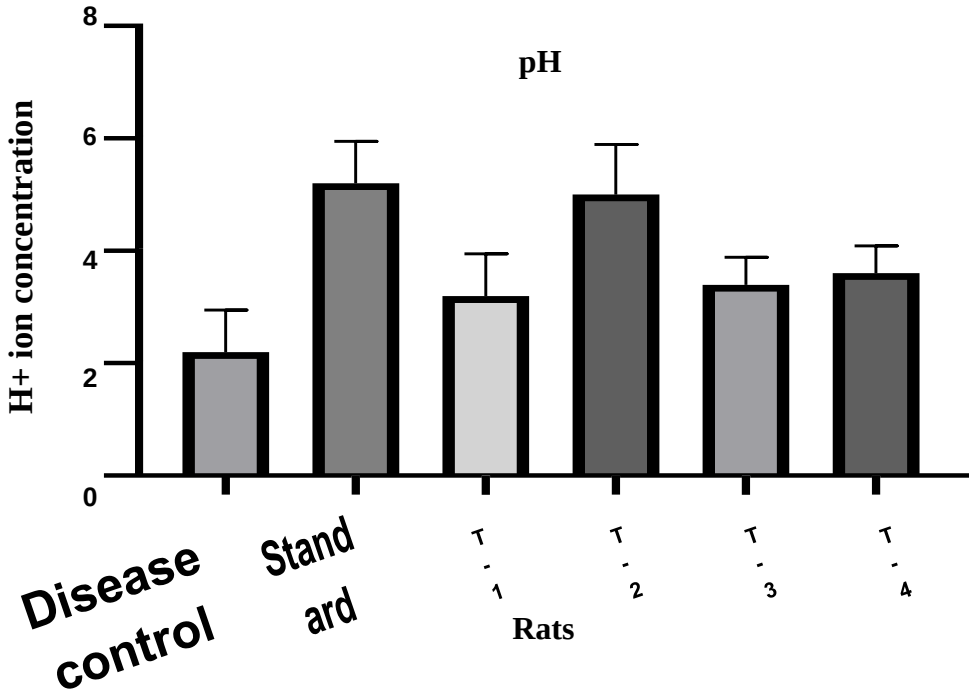


**Figure 4. Effects of *B.pinnatum* leaf extract on pH of gastric juice in ethanol induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3 (T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

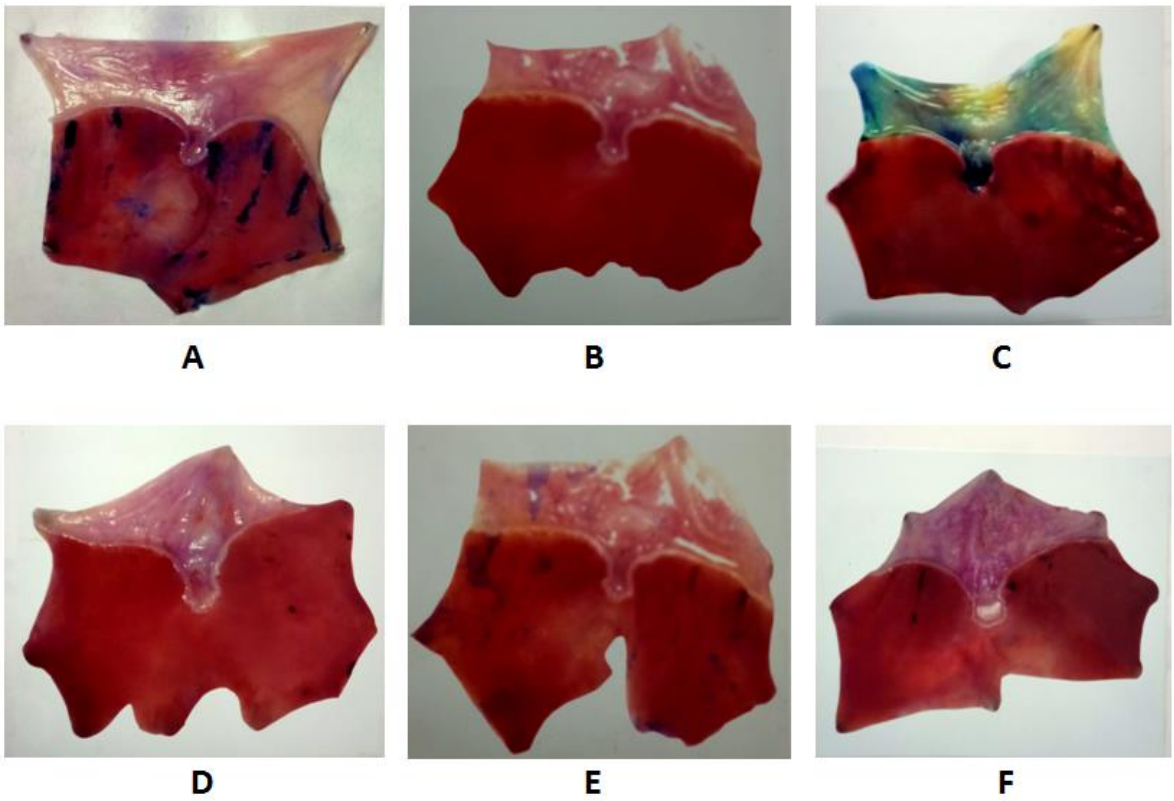


**Figure 5: Ethanol induced Ulcer A: Disease control; B: Standard; C: Test-1; D: Test-2; E: Test-3; F: Test-4**

**Effect of leaf Extract administration on Stress-Induced Ulcers**

The stress-induced gastric ulcer model showed lessen gastric ulcer index of both hydroethanolic leaves extract in comparison to control group, 17.82±1.594, 12.15±0.623 (***$p < 0.01$) respectively, while an opposite significant trend was observed for ranitidine, 11.42± 0.798(***$p < 0.01$) (Table 5, Figure 5). However, only the pretreatment with 400mg/kg significantly highly reduced both ulcer parameters to 12.15±0.623 (**$p < 0.01$), respectively. When compared with Ethyl acetate groups showed ulcer index 16.42±2.132, 13.92±1.396 and control group.

The Test-2 (T2) group showed (12.15%) lessen number of ulcers, less acidity 42.00 and 41.00 respectively, and high pH value (4.40) in comparison to other test groups of animals (Table 6,7 & 8, Figure 6,7 & 8). The ulcer score, redness was induced by stress model and represented in Figure 10.

The connection between the severe psychobiological and physiological stress and gastric damage has long been recognized (31). It has been widely accepted that stomach wall secretion and motility

alteration may manifest in stressful situations causing more or less severe ulcerations of gastrointestinal mucosa. This correlation was also observed in our animal model. Indeed, mucosa of stress-induced gastric ulcer rats showed a large number of ulcerative lesions, which disappeared when treated with ranitidine.

**Table 5. Effects of B.pinnatum leaf extract on ulcer index in stress induced ulcer in rats**

| Animal | Negative diseased Control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 25.62*** | 10.37*** | 18.62*** | 11.87*** | 15.37*** | 12.87*** |
| Rat -2 | 26.37*** | 11.87*** | 15.12*** | 11.37*** | 18.12*** | 12.37*** |
| Rat -3 | 21.62*** | 11.62*** | 17.87*** | 12*** | 18.62*** | 14.62*** |
| Rat -4 | 24.12*** | 10.87*** | 18.25*** | 12.5*** | 13.37*** | 13.87*** |
| Rat -5 | 23.62*** | 12.37*** | 19.25*** | 13*** | 16.62$^{ns}$ | 15.87*** |
| Mean | 24.27±1.851 | 11.42±0.798 | 17.82±1.594 | 12.15±0.623 | 16.42±2.132 | 13.92±1.396 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the diseasedcontrol group ***<0.01, ns= non-significant

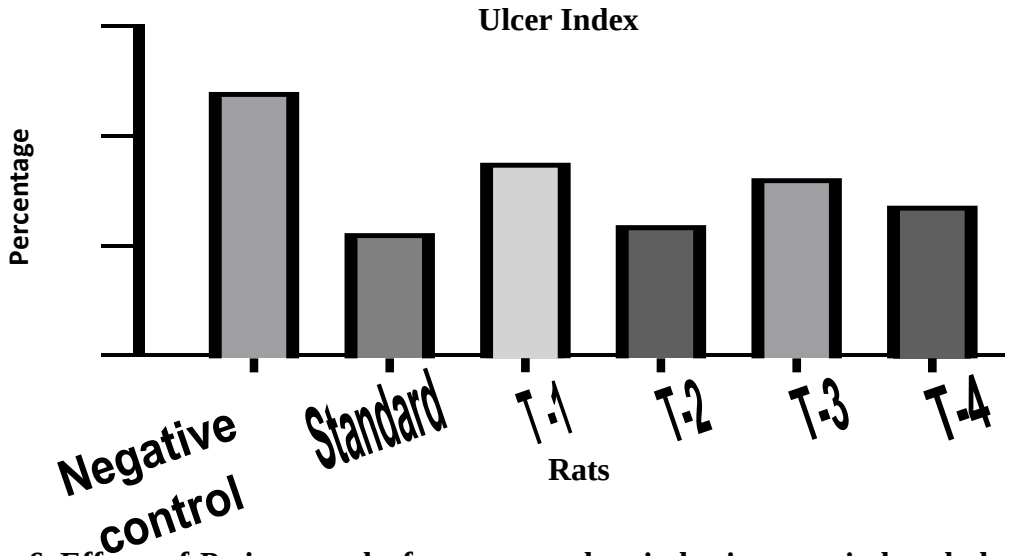


**Figure 6. Effects of *B.pinnatum* leaf extract on ulcer index in stress induced ulcer in rats**
**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3 (T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

In our experimental stress-induced gastric ulcer model, the *B.pinnatum* extracts were able to significantly protect the gastric mucosa from the stress damage, confirming the finding of Gazwi

and Mahmoud (32). In particular, all the extracts were able to decrease the severity and the number of stress ulcers.

**Table 6. Effects of *B.pinnatum* leaf extract on total acidity in stress induced ulcer in rats**

| Animal | Negative diseased Control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 62*** | 42*** | 50*** | 42*** | 60*** | 58*** |
| Rat -2 | 69*** | 36*** | 57*** | $37^{ns}$ | 71*** | 61*** |
| Rat -3 | 51*** | 31*** | 64*** | 52*** | 57*** | 48*** |
| Rat -4 | 81*** | 30*** | 59*** | 36*** | 73*** | 41*** |
| Rat -5 | 63*** | 43*** | 46*** | 45*** | 36*** | 39*** |
| Mean | 65.20±10.964 | 36.40±6.025 | 55.20±7.190 | 42.40±6.504 | 59.40±14.775 | 49.40±9.864 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the diseasedcontrol group ***<0.01, ns= non-significant

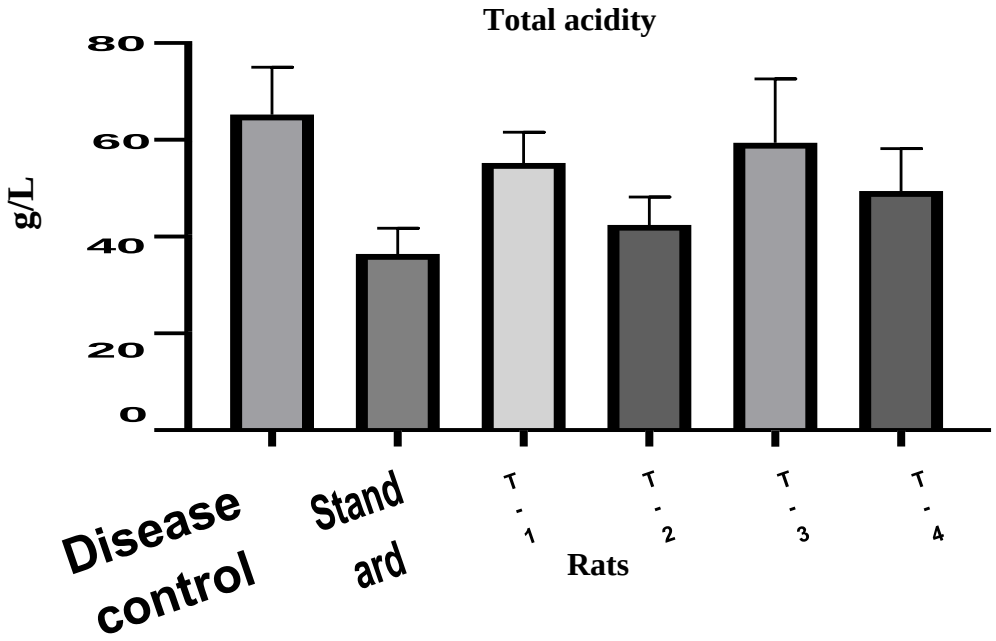


**Figure 7. Effects of B.pinnatum leaf extract on total acidity in stress induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3 (T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

**Table 7. Effects of *B.pinnatum* leaf extract on free acidity in stress induced ulcer in rats**

| Animals | Negative diseased Control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 53*** | 29*** | 39*** | 34*** | 61*** | 43*** |
| Rat -2 | 60*** | 35*** | 61*** | 49*** | 59*** | 49*** |
| Rat -3 | 43*** | 29*** | 57*** | 41*** | 50*** | 51*** |
| Rat -4 | 73*** | 36*** | 50*** | 45*** | 54*** | 40*** |
| Rat -5 | 58*** | 30*** | 53*** | 36*** | 40*** | 29$^{ns}$ |
| Mean | 57.40±10.922 | 31.80±3.421 | 52.00±8.367 | 41.00±6.205 | 52.80±8.349 | 42.40±8.706 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the diseasedcontrol group ***<0.01, ns= non-significant

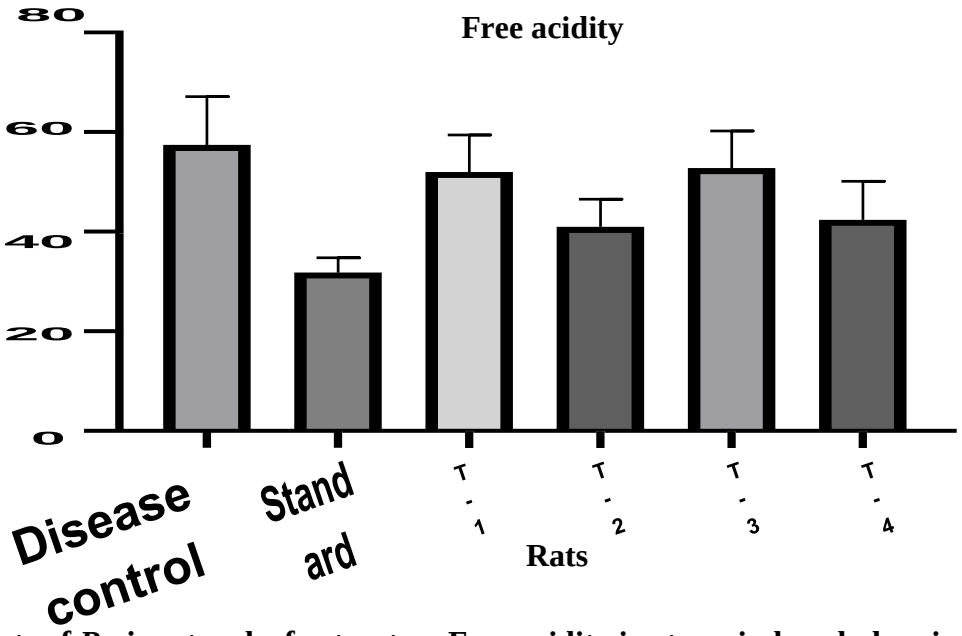


**Figure 8. Effects of *B.pinnatum* leaf extract on Free acidity in stress induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3(T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

**Table 8. Effects of B.pinnatum leaf extract on pH of gastric juice in stress induced ulcer in rats**

| Animals | Negative diseased Control | Standard (Std) | Test-1(T1) (EE; 200mg/kg) | Test-2(T2) (EE; 400mg/kg) | Test-3(T3) (EAE; 200mg/kg) | Test-4(T4) (EAE; 400mg/kg) |
|---|---|---|---|---|---|---|
| Rat -1 | 2*** | 6*** | 2*** | 4*** | 4*** | 4*** |
| Rat -2 | 3*** | 6*** | 4*** | 5*** | 2*** | 3*** |
| Rat -3 | 3*** | 5**** | 4*** | 6*** | 3*** | 5*** |
| Rat -4 | 4*** | 5*** | 3*** | 2*** | 4*** | 4*** |
| Rat -5 | 2*** | 6*** | 4*** | 5*** | 3*** | 3*** |
| Mean | 2.80±0.837 | 5.60±0.548 | 3.40±0.894 | 4.40±1.517 | 3.20±0.837 | 3.80±0.837 |

Values are mean ± SD of 05 rats in each group p value: <0.001 compare the respective group with the diseasedcontrol group ***<0.01, ns= non-significant

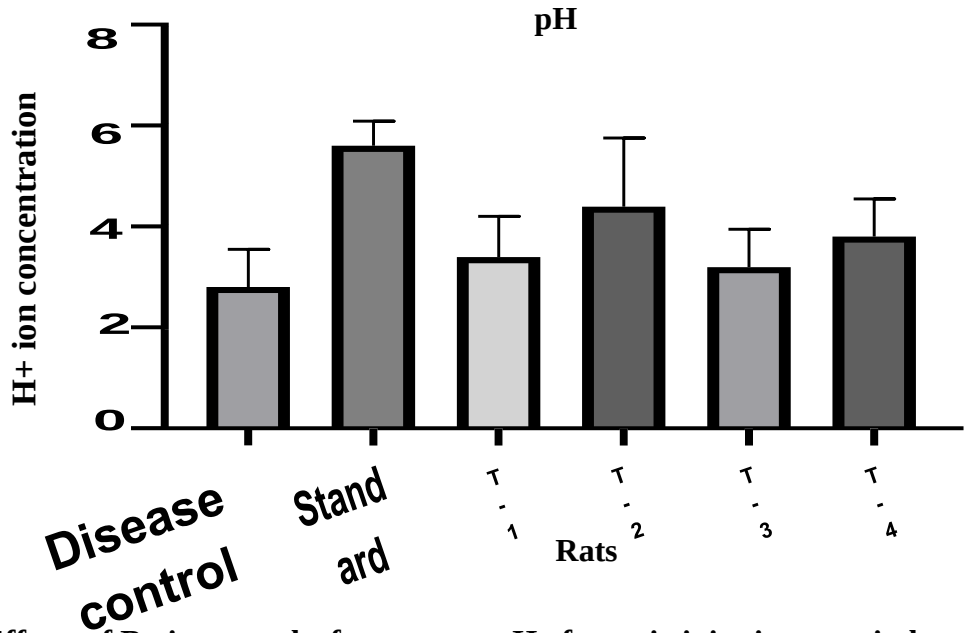


**Figure 9. Effects of B.pinnatum leaf extract on pH of gastric juice in stress induced ulcer in rats**

**[Test-1(T1) (EE;200mg/kg); Test-2(T2) (EE; 400mg/kg); Test-3(T3) (EAE;200mg/kg); Test-4(T4) (EAE;400mg/kg)]**

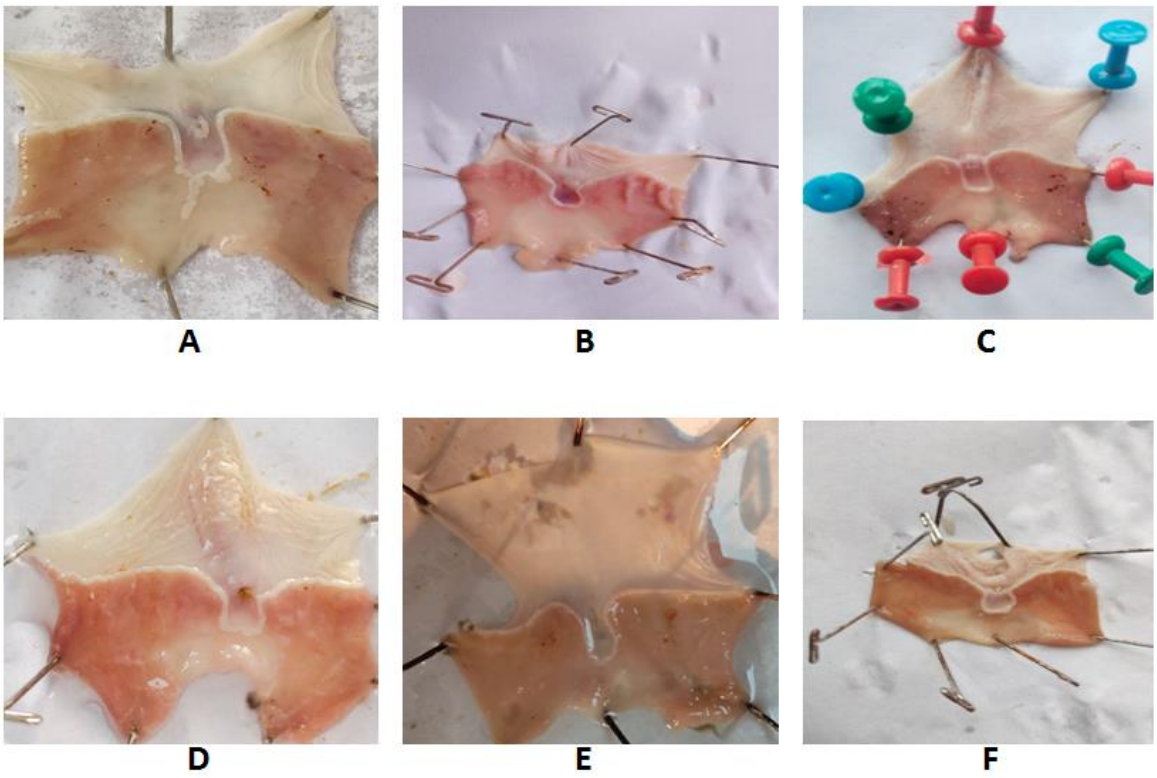


**Figure 10: Stress induced Ulcer A: Disease control; B:Standard; C: Tet-1; D: Test-2; E: Test-3; F:Test-4**

## Conclusion

From the present study, it can be concluded that ethanolic extract (70%) and ethyl acetate extract of *Bryophyllum pinnatum* Lam. (Oken) root have shown promising anti-ulcer activity on cold restraint stress, and ethanol- induced ulcer models, which upholds its folkloric use. The ethanolic extract (70%) was found to exhibit anti-ulcer activity more effectively than the ethyl acetate extract.

## References

1. An Overview of History, Pathogenesis and Treatment of Perforated Peptic Ulcer Disease with Evaluation of Prognostic Scoring in Adults, V Prabhu and A Shivani, Ann Med Health Sci Res. 2014 Jan-Feb; 4(1): 22–29.(1)
2. Lanas A,Chan FKL, Peptic ulcer disease. Lancet 2017 Aug 5;390 (10094):613-624.(2)

3. Wang, X. Y., Yin, J. Y., Zhao, M. M., Liu, S. Y., Nie, S. P., and Xie, M. Y. (2018). Gastroprotective Activity of Polysaccharide from Hericium erinaceus against Ethanol-Induced Gastric Mucosal Lesion and Pylorus Ligation-Induced Gastric Ulcer, and its Antioxidant Activities. Carbohydr. Polym. 186 (1), 100–109. doi:10.1016/j.carbpol.2018.01.004 (3)
4. Scally, B., Emberson, J. R., Spata, E., Reith, C., Davies, K., Halls, H., et al. (2018). Effects of Gastroprotectant Drugs for the Prevention and Treatment of Peptic Ulcer Disease and its Complications: a Meta-Analysis of Randomised Trials. Lancet Gastroenterol. Hepatol. 3 (4), 231–241. doi:10.1016/S2468-1253(18)30037-2 (4)
5. Kuna, L., Jakab, J., Smolic, R., Raguz-Lucic, N., Vcev, A., and Smolic, M. (2019). Peptic Ulcer Disease: a Brief Review of Conventional Therapy and Herbal Treatment Options. J. Clin. Med. 8 (2), 179. doi:10.3390/jcm8020179 (5)
6. Weltermann, T., Schulz, C., and Macke, L. (2021). Effect of Frequently Prescribed Drugs on Gastric Cancer Risk. Best Pract. Res. Clin. Gastroenterol. 50-51, 101741. doi:10.1016/j.bpg.2021.101741 (6)
7. Amang, A. P., Mezui, C., Siwe, G. T., Emakoua, J., Mbah, G., Nkwengoua, E. Z., et al. (2017). Healing and Antisecretory Effects of Aqueous Extract of Eremomastax Speciosa (Acanthaceae) on Unhealed Gastric Ulcers. Biomed. Res. Int. 2017, 1924320. doi:10.1155/2017/1924320 (7)
8. Youn, S. H., Lim, H., Ju, Y., Soh, J. S., Park, J. W., Kang, H. S., et al. (2018). Effect of Gastroprotective Agents on Upper Gastrointestinal Bleeding in Patients Receiving Direct Oral Anticoagulants. Scand. J. Gastroenterol. 53 (12), 1490–1495. doi:10.1080/00365521.2018.1541478 (8)
9. Exploring antiulcer and anti-inflammatory activities of methanolic leaves extract of an Indian mistletoe Helicantes elasticus (Desv.) Danser Jacob Jincy, Christudas Sunil, South African Journal of Botany, Volume 133, September 2020, Pages 10-16 (9)
10. Evaluation of Antidiarrheal Activity of Bryophyllum pinnatum Lam. (Oken) Leaves Extracts and Isolation of Active Principles Priyanka Yadav, Arun K Mishra and Harpreet Singh, ORIENTAL JOURNAL OF CHEMISTRY, 2022, Vol. 38, No.(5): Pg. 1110-1118 (10)
11. M. Afzal, I. Kazmi, and F. Anwar, “Antineoplastic potential of Bryophyllum pinnatum lam. on chemically induced hepatocarcinogenesis in rats,” Pharmacognosy Research, vol. 5, no. 4, pp. 247–253, 2013.(11)

12. R. I. Ozolua, C. J. Eboka, C. N. Duru, and D. O. Uwaya, “Effects of aqueous leaf extract of Bryophyllum pinnatum on guinea pig tracheal ring contractility,” Nigerian Journal of Physiological Sciences, vol. 25, no. 2, pp. 149–157, 2010.(12)
13. E. F. Aransiola, M. O. Daramola, E. O. Iwalewa, A. M. Seluwa, and O. O. Olufowobi, “Antidiabetic effect of Bryophyllum pinnatum leaves,” International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, vol. 8, pp. 89–93, 2014.(13)
14. S. Ghasi, C. Egwuibe, P. U. Achukwu, and J. C. Onyeanusi, “Assessment of the medical benefit in the folkloric use of Bryophyllum pinnatum leaf among the igbos of Nigeria for the treatment of hypertension,” African Journal of Pharmacy and Pharmacology, vol. 5, no. 1, pp. 83–92, 2011.(14)
15. S. Mudi and H. Ibrahim, “Activity of Bryophyllum pinnatum Kurz extracts on respiratory tract pathogenic bacteria,” Bayero Journal of Pure and Applied Sciences, vol. 1, pp. 43–48, 2008.(15)
16. L. A. Chibli, K. C. M. Rodrigues, C. M. Gasparetto et al., “Anti-inflammatory effects of Bryophyllum pinnatum (Lam.) Oken ethanol extract in acute and chronic cutaneous inflammation,” Journal of Ethnopharmacology, vol. 154, no. 2, pp. 330–338, 2014.(16)
17. M. Afzal, G. Gupta, I. Kazmi et al., “Anti-inflammatory and analgesic potential of a novel steroidal derivative from Bryophyllum pinnatum,” Fitoterapia, vol. 83, no. 5, pp. 853–858, 2012.(17)
18. S. Pal and A. K. N. Chaudhuri, “Studies on the anti-ulcer activity of a Bryophyllum pinnatum leaf extract in experimental animals,” Journal of Ethnopharmacology, vol. 33, no. 1-2, pp. 97–102, 1991.(18)
19. Pharmacological screening and evaluation of anti-peptic ulcer property of leaves of Artocarpus integrifolia. International Journal of Pharmacy and Pharmaceutical Sciences 6, 522–524. Sunil, K., Kopala, N., Maruthi, K.R., Alfarhan, A.H., Rajakrishnan, R., Thomas, J., 2013a. (19)
20. Hepatoprotective effect of Helicanthus elastica. Bangladesh Journal of Pharmacology 11, 45–49. Sunil, K., Kopala, N., Saraswathy, A., Amerjothy, S., 2013b. (20)
21. HPTLC fingerprinting of extracts of Mango Mistletoe Helicanthus elastica (Desr.) Danser with multiple markers. International Journal of Innovation of Science and Research 2, 864– 871. Sunil, K., Kopala, N., Shakila, R., Amerjothy, S., 2014. (21)

22. The Origin of the Soxhlet Extractor, J. Chem. Educ. 2007, 84, 12, 1913.
23. Evaluation of Anti-Ulcer Activity of Methanolic Extract of Abutilon indicum Linn Leaves in Experimental Rats, N. L. Dashputre, N. S. Naikwade, International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(2): 97-100.
24. Gurbuz I, Yesilada E. Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis by using various in vivo and biochemical techniques. J Ethnopharmacol. (2007) 112:284–91.
25. Antidiabetic potential of ethanol leaf extract of Bryophyllum pinnatum on alloxan-induced diabetic rats and their haematological profiles Casmir U. Ezeagu , Joshua Parker Elijah , Ukegbu Chimere Y, Eze Chuma S., Nwodo O. F. C.
26. Acute and Chronic Anti-inflammatory effect of Bryophyllum pinnatum Leaf Extract on Wistar Rats, Emmanuel Ikechukwu Onwubuya , Henrietta Aritetsoma Ogbunugafor , Chike Samuel Okafor, Afees Adebayo Oladejo, European Journal of Medicinal Plants 32(6): 6-14, 2021; Article no.EJMP.71303.
27. Al-Radahe S, Ahmed KA, Salama S, et al. Anti-ulcer activity of Swietenia mahagoni leaf extract in ethanol-induced gastric mucosal damage in rats. J Med Plants Res. 2013;7(16):988–997.
28. Abdulla MA, AL-Bayaty FH, Younis LT, Abu Hassa MI. Anti-ulcer activity of Centella asiatica leaf extract against ethanol-induced gastric mucosal injury in rats. J Med Plants Res. 2010;4(13):1253–1259.
29. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, et al. In vivo antioxidant and antiulcer activity of Parkia speciosa ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PLoS One. 2013;8(5):e64751.
30. Rached W, Benamar H, Bennaceur M, Marouf A. Screening of the antioxidant potential of some Algerian indigenous plants. J Biol Sci. 2010;10(4):316–324.
31. Gareau MG, Silva MA, Perdue MH. Pathophysiological mechanisms of stress-induced intestinal damage. Curr Mol Med. (2008) 8:274–81. doi: 10.2174/156652408784533760
32. Gazwi HSS, Mahmoud ME. Gastroprotective effect of Borago extracts against indomethacin-induced gastric ulcer in rats. J Food Dairy Sci. (2018) 2018:177–85. doi: 10.21608/jfds.2018.77776