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# "Evaluation of anti-ulcer activity of *sphagenticola trilobata* plant extract in alcohol induced gastric ulcer in rats"

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## ABSTRACT

The present study investigates the anti-ulcer activity of *Sphagneticola trilobata* plant extract in an alcohol-induced gastric ulcer model in rats. Gastric ulcers, a common gastrointestinal disorder, are often exacerbated by alcohol consumption. This research aims to evaluate the protective effects and potential mechanisms of *Sphagneticola trilobata*, a plant known for its medicinal properties, against gastric mucosal damage. Rats were administered alcohol to induce gastric ulcers and subsequently treated with *Sphagneticola trilobata* extract. Gastric lesions were assessed macroscopically to determine ulcer index, and other relevant parameters. Results indicated a significant reduction in ulcer formation and improvement in gastric mucosal integrity in treated groups compared to controls. These findings suggest that *Sphagneticola trilobata* extract possesses considerable anti-ulcerogenic properties, making it a promising candidate for further pharmacological studies.

**Keywords:** Anti-ulcer activity, *Sphagneticola trilobata*, Gastric ulcer, Alcohol-induced, Rats.

## 1. INTRODUCTION

Gastric ulcers, a prevalent gastrointestinal disorder, represent a significant health concern worldwide due to their impact on quality of life and the potential for serious complications, such as bleeding and perforation. The etiology of gastric ulcers is multifaceted, encompassing factors such as Helicobacter pylori infection, prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs), and lifestyle factors, including alcohol consumption and stress. In particular, alcohol is known to induce gastric ulcers by disrupting the mucosal barrier, increasing acid secretion, and generating free radicals, which collectively contribute to mucosal damage and ulceration[1,2].

The search for effective treatments for gastric ulcers has historically focused on synthetic drugs, such as proton pump inhibitors (PPIs), H2 receptor antagonists, and antacids[3]. While these treatments can be effective, they are often associated with side effects and potential drug interactions. As a result, there is growing interest in the exploration of natural remedies that can provide safer alternatives for the management of gastric ulcers.

One such promising natural remedy is *Sphagneticola trilobata*, a perennial herb belonging to the Asteraceae family, which has been traditionally used in various regions for its medicinal properties. Also known as *Wedelia trilobata*, this plant is native to Central America and has been widely introduced to other tropical and subtropical regions around the world. The leaves and aerial parts of *Sphagneticola trilobata* are rich in bioactive compounds, including flavonoids, terpenoids, and saponins, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties[4].

Recent pharmacological studies have highlighted the potential of *Sphagneticola trilobata* in the treatment of various ailments, including inflammation, liver disorders, and diabetes[5]. However, its potential anti-ulcer activity, particularly against alcohol-induced gastric ulcers, remains underexplored. The existing literature suggests that the plant's rich phytochemical profile may provide protective effects against gastric mucosal damage through several mechanisms. These include scavenging of free radicals, inhibition of gastric acid secretion, and enhancement of mucosal defense mechanisms[6-8].

The current study aims to evaluate the anti-ulcer activity of *Sphagneticola trilobata* plant extract in an experimental model of alcohol-induced gastric ulcer in rats. Alcohol-induced ulcers serve as a relevant experimental model due to their similarity to human ulcers in terms of pathophysiology and response to treatment[9]. This model involves the administration of ethanol, which induces gastric mucosal lesions by mechanisms such as increased gastric acid secretion, and oxidative stress. By assessing the effects of *Sphagneticola trilobata* extract in this model, the study seeks to provide insights into its potential efficacy and mechanisms of action in ulcer prevention and treatment.

The findings of this study are expected to contribute to the growing body of evidence supporting the use of natural products in the management of gastric ulcers and may pave the way for the development of novel, plant-based therapeutic agents. Such agents could offer a valuable alternative to conventional pharmacotherapy, with potentially fewer side effects and improved patient compliance.

## 2. MATERIAL AND METHOD

### 2.1 Plant collection

The medicinal plant Sphagneticola trilobata, collected in 300 gm, was dried under shade and oven at 45°C, stored in air-tight containers, and authenticated by a plant taxonomist to confirm its identity and purity.

### 2.2 Extraction

The study used a Soxhlet device to extract plant material from *Sphagneticola trilobata* powder. Soxhlation was performed at 60°C with petroleum ether as the non-polar solvent. The material was dried and re-extracted with methanol solvent. The extraction was confirmed by the absence of residual solvent when evaporated. The extracts were evaporated at 40°C.The dried extract was weighed, and each extract's % yield was calculated using the following formula:

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

Prepared extracts was observed for organoleptic characters (percentage yield, colour and odour) and was packed in air tight container and labelled till further use[10].

### 2.3 Phytochemical investigation

Experiment was performed to identify presence or absence of different phytoconstituents by detailed qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were used[11].

### 2.4 Quantitative Phytochemical Estimation

#### 2.4.1 TPC (Total Phenolic Content)

The Folin-Ciocalteu Assay was used to determine the total phenolic content of *Sphagneticola trilobata* extract. The extracts were mixed with Folin-Ciocalteu Reagent and sodium carbonate, diluted, and then spectrophotometrically measured at 760 nm. Calibration curves were created using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. The reagent produces a blue color upon reaction, which was measured spectrophotometrically[12].

#### 2.4.2 TFC (Total Flavonoid Content)

The flavonoid content of *Sphagneticola trilobata* extract was determined using the Aluminium chloride method. The extract was mixed with distilled water, sodium nitrite, aluminium chloride, sodium hydroxide, and a UV spectrophotometer. The absorbance was estimated at 510 nm using a UV spectrophotometer. Calibration curves were created using standard solutions of Rutin Equivalent (RE) mg/gm, with concentrations of 20, 40, 60, 80, and 100 µg/mL. The total flavonoid content was determined from the calibration curve, and results were expressed as mg Rutin equivalent per gram dry extract weight[13].

### 2.5 DPPH (2,2-Diphenyl-1-picrylhydrazyl)

The antioxidant activity of *Sphagneticola trilobata* extract was assessed using the DPPH free radical scavenging assay. A methanol solution of extracts/standards was prepared from a stock solution with 0.1mM DPPH solution. The mixture was vortexed, incubated for 30 minutes, and analyzed using a UV spectrophotometer. A control was prepared with 3ml of 0.1mM DPPH solution and incubated for 30 minutes. The absorbance of the control was measured against methanol[14]. Percentage antioxidant activity of sample/standard was calculated by using formula:

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

### 2.6 Acute Toxicity Study

The guideline outlines a step-by-step acute toxic class approach, using three animals of the same sex in each phase. Depending on the mortality and moribund stage of the animals, 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 a specified dose, and the absence or presence of compound-related mortality in one stage determines the next phase. Dosing three further animals with the same dose and dosing three other animals at the next higher or lower dose level is done in each step[15].

### 2.7 Experimental work

### 2.7.1 Animals

All animal experiments were approved by Institutional Animal Ethics Committee (IAEC). Wistar rats of either sex or approx. 200-250g in body weight were housed in a group of six in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly.

### 2.7.2 Induction of ulcer in rats:

Wistar rats weighing 200±50 were fasted for 24 hr with free access to water and rats randomly divided into five groups. The control group received a vehicle (distilled water, 5 ml/kg, through oral route) and Second group is inducer group which was treated with Ethanol 20 mg/kg through oral route. And treatment groups III and IV were given Ethanol 20 mg/kg and test sample (*Sphagneticola trilobata* extract- 100, 200 mg/kg body weight) and Standard group (V) was treated with the standard antiulcer drug (Ranitidine 20 mg/kg through oral route). The rats were sacrificed after one hours of Ethanol administration and the stomach was removed and opened along the greater curvature[16].

### 2.7.3 Parameters assessed for anti-ulcer activity

- Determination of Ulcer Index
- Determination of pH and Volume of gastric juice
- Free acidity determination

### 2.8 Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. The stomachs were then dissected along the larger curvature, cleaned with normal saline to remove gastric contents, and examined with a 10x magnifying lens to determine the presence of ulcers. Ulcers were counted and evaluated using the Kulkarni technique (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = hemorrhagic streaks, 3 = ulcers > 3 but < 5, and 5 = ulcers > 5). The ulcer Index and percentage of ulcer inhibition were determined as follows:

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

Where,

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

### 2.9 Volume and pH of gastric juice

The volume of gastric juice of each animal was measured after centrifugation with 1000 rpm for 10 minutes and analyzed. The volume of the centrifuged sample was expressed as ml/ 100g body weight. A pH meter is used for determining pH after diluting 1 ml of gastric juice aliquot with 1 ml of distilled water.

### 2.10 Determination of free acidity

1 ml of distilled water was used to dilute 1 ml of gastric juice aliquot and then transferred to a conical flask (50 ml) with the addition of 2 drops of phenolphthalein indicator. 0.01 N NaOH was used for titration until a permanent pink color was resulted; its consumed volume was determined**.** The free acidity was calculated by the formula:

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

## 3. RESULTS

### 3.1. Percentage Yield

**Table 1: Percentage Yield of crude extracts of *Sphagneticola trilobata* extract**

| S. No | Plant name | Solvent | Theoretical weight | Yield(gm) | % yield |
|---|---|---|---|---|---|
| 1 | *Sphagneticola trilobata* | Pet ether | 284 | 1.61 | 0.56% |
| 2 | | Methanol | 298.98 | 6.10 | 2.04% |

### 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** | | |
| 1.1 | Dragendroff's test | Present | Absent |
| 1.2 | Mayer's reagent test | Present | Absent |
| 1.3 | Wagner's reagent test | Present | Absent |
| 1.3 | Hager's reagent test | Present | Absent |
| **2.** | **Glycoside** | | |
| 2.1 | Borntrager test | Absent | Present |
| 2.2 | Legal's test | Absent | Present |
| 2.3 | Killer-Killiani test | Absent | Present |
| **3.** | **Carbohydrates** | | |
| 3.1 | Molish's test | Absent | Present |
| 3.2 | Fehling's test | Absent | Present |
| 3.3 | Benedict's test | Absent | Present |
| 3.4 | Barfoed's test | Absent | Present |
| **4.** | **Proteins and Amino Acids** | | |
| 4.1 | Biuret test | Absent | Absent |
| **5.** | **Flavonoids** | | |
| 5.1 | Alkaline reagent test | Absent | Present |
| 5.2 | Lead Acetate test | Absent | Present |
| **6.** | **Tannin and Phenolic Compounds** | | |
| 6.1 | Ferric Chloride test | Absent | Present |
| **7.** | **Saponin** | | |
| 7.1 | Foam test | Present | Present |
| **8.** | **Test for Triterpenoids and Steroids** | | |
| 8.1 | Salkowski's test | Absent | Absent |
| 8.2 | Libbermann-Burchard's test | Absent | Absent |

### 3.3 Quantitative Analysis

#### 3.3.1 Total Phenolic content (TPC) and Total Flavonoid Content (TFC) estimation

**Table 3 Standard table for Gallic acid**

| 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 |

**Table 4 Standard table for Rutin**

| S. No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1. | 20 | 0.174 |
| 2. | 40 | 0.202 |
| 3. | 60 | 0.276 |
| 4. | 80 | 0.315 |
| 5. | 100 | 0.330 |

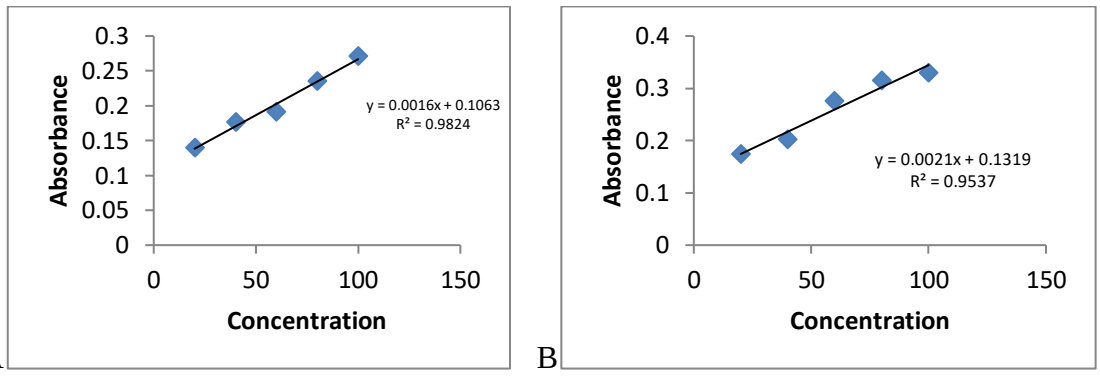



**Figure 1: Represent standard curve of Gallic acid (A) and Rutin (B)**

**Table 5: Total Phenolic Content of extract *Sphagneticola trilobata***

| Extracts | Total Phenolic content (mg/gm equivalent of Gallic acid) |
|---|---|
| **Methanol** | 62 |

**Table 6: Total Flavonoid Content of extract *Sphagneticola trilobata***

| Extracts | Total Flavonoid content (mg/gm equivalent of rutin) |
|---|---|
| **Methanol** | 17 |

### 3.4 *In vitro* Antioxidant Assays

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

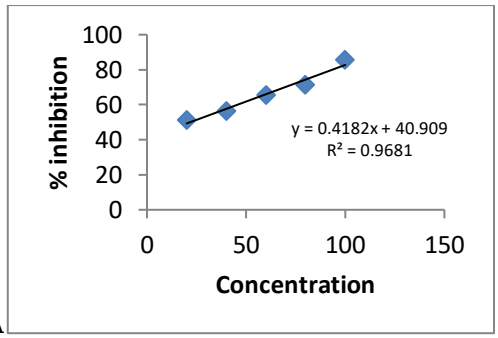
**Table 7: DPPH radical scavenging activity of Std. Ascorbic acid**

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 20 | 0.482 | 51.313 |
| 40 | 0.433 | 56.262 |
| 60 | 0.342 | 65.454 |
| 80 | 0.283 | 71.414 |

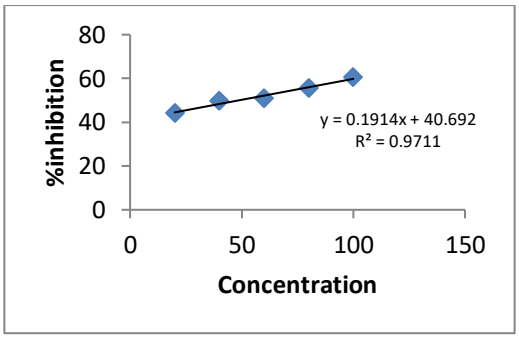
| | | |
|---|---|---|
| 100 | 0.143 | 85.555 |
| **Control** | | 0.990 |
| **IC50** | | **21.77** |

**Table 8: DPPH radical scavenging activity of methanol extract of *Sphagneticola trilobata***

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| 20 | 0.518 | 44.108 |
| 40 | 0.464 | 49.837 |
| 60 | 0.453 | 51.027 |
| 80 | 0.412 | 55.459 |
| 100 | 0.366 | 60.432 |
| Control | 0.925 | |
| IC50 | | 48.74 |




A




B

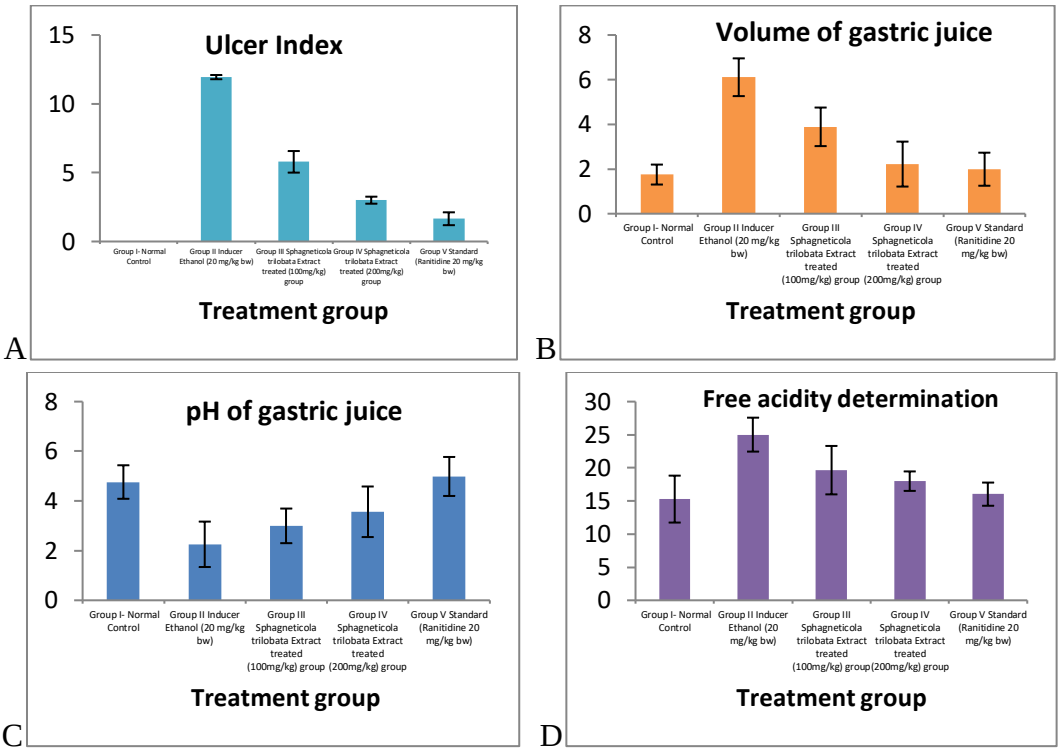
**Figure 2: DPPH radical scavenging activity of Std. Ascorbic acid (A) and extract of *Sphagneticola trilobata* (B)**

### 3.5 Determination of Ulcer Index, volume, pH and free acidity of gastric juice

**Table 9: Observation of Ulcer index, volume, pH and free acidity of gastric juice**

| Treatment Group | Ulcer Index Mean | Volume of gastric juice | pH of gastric juice | Free acidity determination (mE/L) |
|---|---|---|---|---|
| Group I- Normal Control | 0 | 1.752± 0.447 | 4.762±0.675 | 15.280±3.543 |
| Group II Inducer Ethanol (20 mg/kg bw) | 11.942±0.153 | 6.110± 0.845 | 2.254±0.914 | 25.012±2.561 |
| Group III *Sphagneticola trilobata* Extract treated (100mg/kg) group | 5.789±0.784 | 3.890± 0.863 | 2.997±0.697 | 19.648±3.655 |
| Group IV *Sphagneticola* | 3.001±0.255 | 2.221± 1.006 | 3.564±1.018 | 17.986±1.470 |

| | | | | |
|---|---|---|---|---|
| *trilobata* Extract treated (200mg/kg) group | | | | |
| Group V Standard (Ranitidine 20 mg/kg bw) | 1.654±0.467 | 1.992± 0.74 | 4.986±0.785 | 16.023±1.750 |




**Graph 1**: **Bar chart represents ulcer index (A), gastric volume (B), pH (C) and free acidity (D) in Ethanol induced ulcer in rats**

### 3.6 Images


**Figure 3: 1. Normal Stomach, 2. Ulcers in stomach and 3. Treated stomach**

## 4. DISCUSSION

The phytochemical examination of *Sphagneticola trilobata's* methanolic extract revealed the presence of alkaloids, phenolics, flavonoids, saponins, glycosides, saponin, steroids, tannin, and phenolic. TPC and TFC were calculated as part of a quantitative phytochemical experiment. The TPC was calculated with gallic acid (standard), while the TFC was calculated with rutin as the standard. *Sphagneticola trilobata* extract inhibited DPPH radicals by 60.43%, with an IC 50 value of 48.74µg/mL. Ascorbic acid was utilized as a reference molecule,

exhibiting 85.55% inhibition and an IC 50 value of 21.77µg/ml. In the acute toxicity study, no signs of toxicity were found upto the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 100 mg/kg and 200 mg/kg have been fixed for study. Five groups of adult albino wistar rats were taken for the study. Rats were divided into five groups each containing 6 animals. Group first is normal group received the saline for 7 days. Group second is Ethanol inducer group (20 mg/kg bw). Group third is *Sphagneticola trilobata extract* (100 mg/kg bw). Fourth group is *Sphagneticola trilobata extract* (200mg/kg bw) and Group fifth is standard (Ranitidine 20 mg/kg bw). The extract of *Sphagneticola trilobata* was evaluated by using ethanol induced peptic ulcer model. Ulcer produced in this model was seen as red sores. The stomachs of rats in the ethanol induced peptic ulcer showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs. There was a significant decrease in the measured gastric ulcer index in the stomach of *Sphagneticola trilobata* (200 mg/kg bw) treated animals when compared with the *Sphagneticola trilobata* (100 mg/kg bw) treated. The volume of gastric juice was observed as 2.221 ml of *Sphagneticola trilobata* (200 mg/kg bw) in decreased level as compared to *Sphagneticola trilobata* (100 mg/kg bw) treated group showed gastric volume of 3.890. The pH of gastric juice was observed as 3.564 of *Sphagneticola trilobata* (200 mg/kg bw) treated group and it showed the reduction in acidic pH as compared to *Sphagneticola trilobata* (100 mg/kg bw) showed 2.997 pH. The free acidity was observed as mE/L of *Sphagneticola trilobata* (200 mg/kg bw) treated group 17.986 and it showed the reduction in acidity as compared to *Sphagneticola trilobata* (100 mg/kg bw) showed 19.648 mE/L.

## 5. CONCLUSION

Thus, from the present study it was concluded that the treatment of *Sphagneticola trilobata* flower extract maintains the normal range of acidity and also maintain the pH level of stomach. Present study supports the use of *Sphagneticola trilobata* extract by local healers as traditional medicine in treatment of ulcer. This effect can be attributed to presence of various bioactive components present on extract and also be due to protective potential of extract confirms the mechanism of anti-ulcer activity against ethanol induced peptic ulcer.

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