



***In-vitro* Anti-Ulcer Activity of Ethanolic Extract of Tecoma Stans Flower**

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Keywords

Tecoma stans; acid neutralization capacity assay and H⁺/K⁺-ATPase inhibition method; phytochemicals; antiulcer activity.

Abstract

Aims: The present in-vitro investigation assessed the anti-ulcer potential of Tecoma stans flower extract through acid neutralization capacity assay and Proton pump inhibitor (H⁺/K⁺-ATPase) assay and preliminary phytochemical analysis. Study design: Experimental laboratory based in-vitro study. **Methods:** Flower extract of Tecoma stans was screened for phytochemical constituents. Anti-ulcer property was assessed by using acid neutralization capacity assay and Proton pump inhibitor (H⁺/K⁺-ATPase) inhibition method. The percentage inhibition, the optical density at 660nm and IC₅₀ values of H⁺/K⁺-ATPase inhibition method were determined. **Results:** The preliminary phytochemical constituents of the extract was identified as alkaloids, flavonoids, phenolic compounds, saponins and tannins indicating that the bioactive constituents are capable of neutralize stomach acid. The acid neutralizing capacity assay revealed that the dose -dependent increase with the extract closely comparable to the standard dose and the H⁺/K⁺-ATPase inhibition assay demonstrated concentration-dependent enzyme inhibition with an IC₅₀ value confirming moderate proton pump inhibitory activity. **Conclusion:** These findings suggest that Tecoma stans contains potent bioactive phytochemicals capable of modulating ulcer pathways through acid neutralization capacity assay and proton pump inhibitor (H⁺/K⁺-ATPase) inhibition method.

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1. Introduction

An ulcer is a wound or erosion that occur in the protective layer of an organ, skin or digestive tract. According to the gastrointestinal health, the common type of ulcers are stomach ulcers, which form in the inner most layer of stomach or inner most layer of the duodenum [1]. A stomach ulcer is an acid produced lesion in the gastrointestinal tract, often in the stomach or the superior part of the duodenum. The beginning of severe injury to the GI layer, mucosa, and first segment of small intestine that spreads through the mucosal tissue is known as gastric ulcer. The main aspect of gastric ulcer is induced with gram negative *Helicobacter pylori* bacteria with excess secretion of hydrochloric acid, deficient epithelial defence protect form gastric acidity, medications are muscarinic agonist [2]. There are two main types of gastro-duodenal ulcer. The acute gastric ulcer does not spread when it enter to the muscle layer of mucosa unlike to the submucosa layer. It also developed due to neurological damage, burns induced stress which leads to ulcer. In chronic gastric ulcer, the muscular layer has an entire-

thickness, and the base is in serosal layer, involving extra gut tissues which includes the both stomach and peptic ulcer of duodenum [3].

Gastric erosion develops, that the harmful agents damage the stomach layer or when it gets damaged. The internal body damaging factors in the stomach are hydrochloric acid, pepsin, bile reflux, lipid breakdown. Even though the acid reducing agents are available, the agents have many adverse effects; therefore, we have to research for anti-ulcer agents having less adverse effect or not any side effects [4]. However, the antiulcer drugs produce adverse effect or some side effects by the using of drugs for chronic conditions and it may involve the changing of some physiological process of our body. So that the herbal medications are now used to control the adverse or side effects when used in chronic conditions. Therefore, many herbal medicines have proved that it has antiulcer activity to reduce the erosion of the protective lining layer by their potential effective factors [5].

Over past few decades, natural products have emerged as a rich reservoir of bioactive compounds with diverse pharmacological activities. Medicinal plants, in particular, offer multi-targeted therapeutic potential and often present fewer side effects compared to synthetic drugs [6]. *Tecoma stans* is usually called as trumpet flower and it is a yellow-coloured flower and the *tecaoma stans* flower belongs to the family Bignoniaceae and it is native to the Americas. The common name of the *Tecoma stans*

are yellow trumpet brush or it is also called as yellow bells. The family of *Tecoma stans* consists of small tree species. The *Tecoma stans* is a decorative plant and it is fast growing plant in India. It is a widespread species throughout tropical and subtropical areas. It is also native to the various countries like America, West Indies, Mexico, South America. The leaf of *Tecoma stans* extract has been given orally for the treatment of Diabetes mellitus and Abdomen pain [7].



Figure 1: Tecoma Stans

2. Materials and Methods

2.1 Plant Material and Extraction

Raw flowers of *Tecoma stans* were collected, shadow-dried and the flowers were powdered. Then the powder plant material was extracted by using ethanol, the extract was stored and used for further experimental analysis [8], [9].

2.2 Preliminary Phytochemical Analysis

Different phytochemical screening analysis were conducted on the plant extract to identify the presence of major secondary metabolites. Thus, phytochemical test is conducted to detect the presence of Alkaloids, Flavonoids, Resins, glycosides, phenolic and sulfur-containing compounds in *Tecoma stans* flower extract [10]. The Phytochemical constituents are the fingerprint of the plant materials, and that suggests the biological and pharmacological activity of the plant material. The therapeutic properties of the *Tecoma stans* depend upon the secondary metabolites and the percentage yield of the extract [11].

2.3 Proton Pump ($H^+ / K^+ - ATPase$) inhibition method

Preparation of $H^+ / K^+ - ATPase$ Enzyme

Raw goat stomachs were obtained from the local meat market, from that fresh stomach inner lining, the parietal cell lining was collected. Thus, parietal cells were homogenised in a 16Mm tris buffer solution that has pH of 7.4, containing 10% triton x-

100. And then centrifuge for 10 mints at 6000rpm. From that centrifugation process, the supernatant separated and then used for proton pump ($H^+ / K^+ - ATPase$) inhibition study. By using Boadford's method, the protein content was measured by using BSA as a standard [12].

Assessment of Proton Pump ($H^+ / K^+ - ATPase$) inhibition

The reaction mixture of the sample having 0.1ml of enzyme extract (300 μ g) and the *Tecoma stans* extract of various concentration (500,250, 100, 50 and 10 μ g/ml) is pre-heated for 60 minutes at 37°C. This reaction was started by adding 10mMKCL (200 μ L) and 2mMMgCl₂ (200 μ L). After 30 minutes of pre heated at 37°C, by the addition of 4.5% ammonium molybdate and 60% perchloric acid, the reaction was stopped and centrifuge at 2000rpm for 10 minutes. After 10 minutes the inorganic phosphate has released and at 660nm it was measured. The components of 1ml of supernatant, 4ml of Millipore water and 1ml of 2.5% ammonium molybdate and 0.4ml of ANSA has added at room temperature. An inorganic phosphate, the absorbance of various dose of the extract were measured at 660nm and the enzyme activity was calculated by micromoles of Pi which is released per hour. The final results were compared with the standard antiulcer therapeutic agents of proton pump inhibitor of Omeprazole (100 μ g/ml) and the Mean expression as $\pm$ SEM 16% and the percentage inhibition was calculated by the

formula [13].

$$\text{Percentage inhibition} = \frac{[\text{Activity (control)} - \text{Activity (test)}] / \text{Activity (control)}}{1} \times 100.$$

2.4 Acid Neutralization Capacity

The acid neutralization capacity of the extract of *Tecoma stans* of various concentration were compared with the standard acid neutralizer Aluminium hydroxide + Magnesium hydroxide (50 mg/ml). The water was added to the 5ml quantity of this mixture to make up the volume of 70ml and mixed for one minute. And add 30ml of 0.1 N HCL to the standard and test solution and mixed it for 15 minutes., and add drops of phenolphthalein. the excess amount of hydrochloric acid was titrated with 0.5N sodium hydroxide by adding drops till pink colour is obtained [14].

The moles of acid neutralization capacity was calculated by,

$$\text{Moles of acid neutralization} = (\text{volume of HCL} \times \text{Normality of HCL}) - (\text{volume of NaOH} \times \text{Normality of NaOH})$$

$$\text{Acid neutralizing capacity (ANC) per gram of antacid} = \frac{\text{moles of HCL neutralized}}{\text{Grams of Antacid or extract}}$$

3. Results

3.1 Preliminary phytochemical analysis

The phytochemical investigation of the plant extract *Tecoma stans* revealed the secondary metabolites. From the investigation results Alkaloids, Flavonoids, Phenolic compounds, Saponins, and Tannins were identified. The bioactive compounds of *Tecoma Stans* may act by reacting with the hydrogen ions (H+) in the gastric fluid to form neutral molecules, thus increasing the pH of the stomach contents and reducing acidity.

3.2 H⁺/K⁺ - ATPase Inhibition Activity

The percentage inhibition of the H⁺/K⁺ - ATPase enzyme of various concentrations of the test sample is shown in Table 1. The results exhibit 55.94% inhibition of H⁺/K⁺ - ATPase enzyme at maximum concentration of 500 mg/ml and 41.91% inhibition of H⁺/K⁺ - ATPase enzyme at the minimum concentration of 10 mg/ml. From the obtained results, our extract possesses the concentration dependent inhibitory effect. where increasing concentrations of the sample led to higher percentages of enzyme inhibition. The IC₅₀ value of our plant extract was 106.1 µg/ml, suggests that our plant extract have moderate acid neutralizing capacity against standard acid neutralizing agent omeprazole.

Table 1: Percentage of Inhibition of H⁺/K⁺-ATPase Enzyme.

S. No.	Test sample concentration(µg/ml)	Percentage Inhibition in triplicates			Mean Value (%)
1.	Omeprazole(100 µg/ml)	89.2586	89.3008	89.5535	89.371
2.	500 µg /ml	55.9393	57.3294	56.7818	56.6835
3.	250 µg /ml	51.8534	52.4853	52.9486	52.4291
4.	100 µg /ml	50.9688	49.5788	51.2637	50.6038
5.	50 µg /ml	48.5678	46.2511	46.2932	47.0373
6.	10 µg /ml	41.9124	44.6925	44.9452	43.85

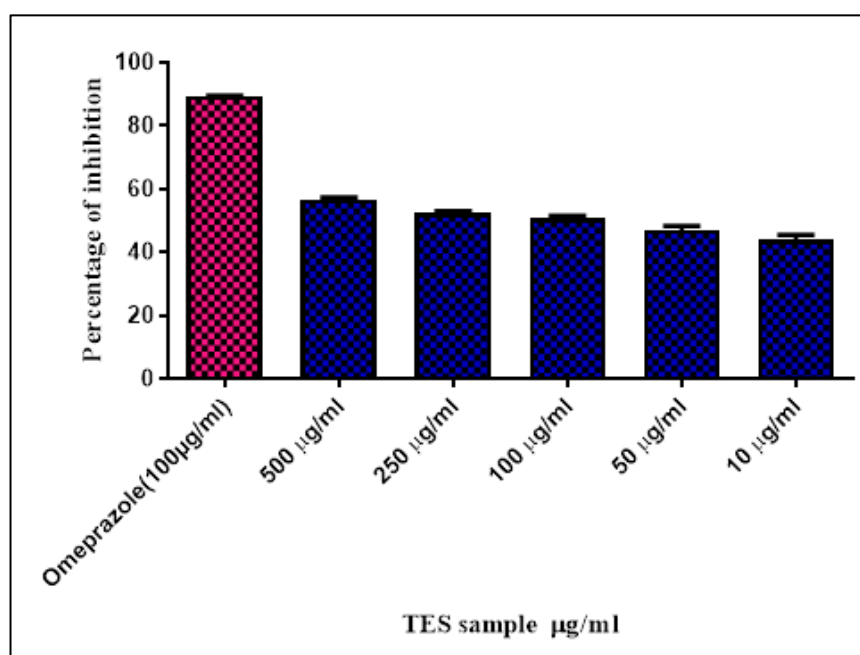


Figure 2: Percentage Inhibition of H⁺/K⁺ - ATPase Enzyme.

3.3 Acid Neutralization Capacity

The acid neutralization capacity (ANC) of *Tecoma Stans* sample (TES) was determined by comparing the acid-neutralizing ability of different concentrations against standard antacid mixture of Aluminium hydroxide + Magnesium hydroxide (50 mg/ml). This assay measures samples capacity to neutralize hydrochloric acid (HCl) in presence of phenolphthalein as an indicator. The results indicate the higher concentrations of extract exhibit greater ANC values. The ANC values of different concentrations of *Tecoma Stans* extract are shown in the table 2:

the ANC values are highest for 500 µg/ml and 250 µg/ml concentrations, both are exhibiting a value of 45 g of antacid per gram of sample. The ANC decreases slightly at lower concentrations, for 100 µg/ml and 50 µg/ml concentrations of values of 42 g and the 10 µg/ml concentration shows value of 41 g. The control (Aluminium hydroxide + Magnesium hydroxide) exhibits the highest ANC value of 54 g, confirming its well-established efficacy as a standard antacid.

Table 2: Acid neutralizing capacity.

S. No.	Name of the Sample	Concentration (µg/ml)	Reading on Burette (mL)	Moles of Acid neutralization	Acid neutralization capacity (ANC) (g)
1.	TES	500 µg/ml	1.5	2.25	45
2.	TES	250 µg/ml	1.5	2.25	45
3.	TES	100 µg/ml	1.8	2.1	42
4.	TES	50 µg/ml	1.8	2.1	42
5.	TES	10 µg/ml	1.9	2.05	41
6.	Control (Aluminium hydroxide + Magnesium hydroxide)	50 mg/ml	0.6	2.7	54

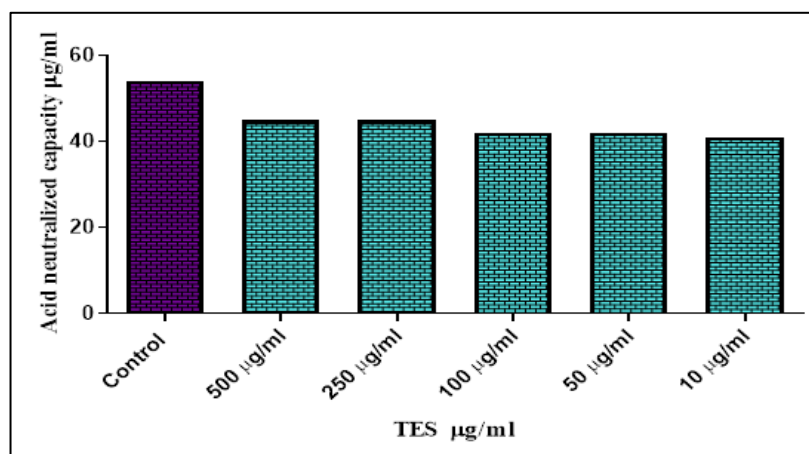


Figure 3: Acid neutralizing capacity.

4. Discussion

The antiulcer property of *Tecoma stans* is evaluated in the present study by qualitative phytochemical screening method and acid neutralization capacity assay and proton pump (H^+/K^+ - ATPase) inhibition method. The phytochemical constituents of the plant extract show the presence of secondary metabolites such as Alkaloids, Flavonoids, Phenolic compounds, Saponins and Tannins. Thus, secondary metabolites perform to produce antiulcer activity because of their neutralization capability. The antacids that they act by neutralizing gastric acid and also it reduces the gastric pH [15], [16], [17].

of acid that it can neutralize and it is measured by the process called as back titration. In acid neutralization capacity, the ethanolic extract of *Tecoma stans* of 500µg/ml concentration shows a reduction in acid neutralization capacity value. The proton pump H^+/K^+ - ATPase is an enzyme that it has the inducing capacity and it located on the apical secretory membrane of parietal cells. The proton pump H^+/K^+ - ATPase inhibition method, the higher concentration of the extract given the maximum percentage inhibition. The data of the plant sample indicate it possess an antiulcer activity, also proved by IC₅₀ value results. The mechanism of action and the active principles included in the antiulcer activity are to be established in the further studies [18], [19], [20].

The acid neutralization capacity (ANC) is the amount

Conclusion

As per the results, that the ethanolic extract of *Tecoma stans* may determine that it be consider as an antiulcer agent. However, the However, a elaborate study on the isolation of the active constituents involved in the plant of *Tecoma stans* and the mode of action which is responsible for its antiulcer activity is to be studied in future.

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Author Contributions

HP: Experiment design and execution of research work; **RS:** Data analysis and interpretation; **SK:**

Manuscript preparation and **BS:** Supervision and final approval.

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Not applicable

Conflict of Interest

There are no conflicts of interest.

Declaration

The author declares that the use of AI-powered language tools (ChatGPT and Grammarly) to enhance clarity and readability of the manuscript. The generated content is reviewed and edited by the author to ensure the alignment, validation, and accuracy of the content for the intended meaning of the research.

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