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In-Vitro Evaluation of Antiulcer Activity of Ethanol Extract of *Saponaria Officinalis*

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Abstract

For the treatment and prevention of illnesses and disorders, medicinal plants are recognized to contain conventional therapeutic substances. This study examined the potential of ethanol extracts of *Saponaria officinalis* to block H⁺/K⁺ ATPase and neutralize acids in order to determine whether they may be utilized to cure ulcers. In traditional medicine, the *Saponaria officinalis* is used to treat gastrointestinal disorders and inflammation. The acidity of the extract at a 1500 mg/ml concentration was much lower than that of conventional aluminium hydroxide + magnesium hydroxide (500mg), falling to 5.33 from 12.7. The ethanol extract of *Saponaria officinalis* at a concentration of 100µg, showed maximum percentage of inhibition of 67.17±1.96% as compared to omeprazole 71.47±0.52%, demonstrated the highest percentage of inhibition of the H⁺/K⁺ - ATPase. The results of this study suggest that *Saponaria officinalis* ethanol extract contains substances that can block enzymes and neutralize acids, making it a potential alternative treatment for digestive issues.

Keywords: Anti-ulcer, Acid, H⁺ / K⁺ - ATPase, *Saponaria officinalis*, HCL.

Introduction

An ulcer is one of the frequent reasons for hospital visits, and its incidence is rising globally [1]. Peptic ulcers are a non-fatal condition that are primarily characterized by recurrent sensations of epigastric pain, which are frequently eased by food or alkali, in addition to giving patients great discomfort, upsetting their daily schedules, and also inflicting mental anguish [2]. Stress, long-term use of anti-inflammatory medications, and other causes can cause ulcers in people. Though the cause of ulcers is mostly unknown, it is widely acknowledged that they are the result of an imbalance between aggressive factors and the endogenous defense mechanism's ability to maintain the integrity of the mucosa [3]. In order to effectively treat peptic ulcers, a medicine must either decrease the aggravating factors on the gastroduodenal mucosa or increase the mucosal

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resistance to them [4]. The digestive tract's acid peptic damage, which causes mucosal breaks that extend to the submucosal epithelium, is the cause of this condition [5]. Ulcers are open sores on transparent skin or mucous membranes. They can also occur internally in the digestive tracts, where they are surrounded by irritated dead tissue. Peptic ulcers are an erosion of the stomach or duodenum's lining [6]. Organ bleeding and perforation are caused by the ulcers, which range from superficial epithelium injury to deeper abrasions [7]. Saponarin and Saponaretin, two flavonoids found in plants, appear to play a significant role in increasing mucus secretion for the treatment and prevention of peptic ulcers [8]. Additionally, saponarin has been shown in in-vitro tests to limit the growth of the *H. pylori* bacterium. Because saponarin prevents the production of histamine in the gastric mucosa, it has a protective effect [9]. There is an urgent need for the safest, most effective anti-ulcer medications that also aim to reduce pain and postpone ulcer recurrence. Due to their natural constituents and lack of adverse effects, herbal medicines are therefore seen as safer choices. In the current investigation, acid neutralizing ability and H^+/K^+ - ATPase inhibitory activity were used to assess the antiulcer effectiveness of an ethanol extract of *Saponaria officinalis*. As a result, an effort was made to support its traditional claim as an anti-ulcer agent using these chosen approaches.

Material and Methods

Plant Material

The plant material was harvested, shade-dried at room temperature, coarsely ground, and then exposed to a solvent extraction process utilizing ethanol for 7-8 hours to finish the extraction. The solvent was ultimately evaporated, producing green extract, which was then refrigerated for later use.

In-Vitro Evaluation of Antiulcer Activity

Acid neutralizing capacity

The amount of aqueous extract that can neutralize acid is 100 mg, 500 mg, 1000 mg, and 1500 mg. Magnesium hydroxide (500 mg) and aluminum hydroxide have been compared for the standard. After adding 5ml of the mixture and adding the remaining 70ml of water, the total volume was 70ml. This was blended for one minute. The 30ml of 1.0 N HCl was added to the standard and test preparation and swirled for 15 minutes. Then, phenolphthalein was added and combined. The surplus HCl was promptly titrated until the pink color was achieved using 0.5N sodium hydroxide [10].

The moles of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl \times Normality of HCl) - (vol. Of NaOH \times Normality of NaOH)

Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract.

H^+/K^+ - ATPase Inhibition Activity

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

The gastric mucosa of the fundus was cut off and opened, and the inner layer of the stomach was scraped out for the parietal cell in order to prepare the fresh goat stomach that had been obtained from the neighborhood butcher. The stomach parietal cell was homogenized in 16 mM Tris buffer with a pH of 7.4, 10% Triton X-100, and centrifuged at 6000 rpm for 10 minutes. The supernatant solution was then used to inhibit H^+/K^+ ATPase. Bradford's technique is used to determine protein content, and BSA is used as a reference.

Assessment of H^+/K^+ ATPase inhibition

The reaction mixture of the sample containing 0.1 ml of enzyme extract (300 g) and plant extract at various concentrations (20 g, 40 g, 60 g, 80 g, and 100 g) was pre-incubated for 60 min at 37 °C. 2 mM ATP was added as the substrate, along with 200 mL each of 2 mM $MgCl_2$ and 10 mL each of KCl, to start the reaction. After 30 minutes at 37 degrees Celsius, the reaction was halted with 4.5% ammonium molybdate. Then, 60% perchloric acid was added, and the mixture was spun at 2000 rpm for 10 minutes to liberate the inorganic phosphate, which was then detected at 660 nm using the Fiske-Subbarow technique. In a nutshell,

1ml of supernatant, 4ml of Millipore water, 1ml of 2.5% ammonium molybdate, and 0.4ml of ANSA were added after 10 min at room temperature. At different extract dosages, absorbance at 660 nm inorganic phosphate has been measured; the enzyme activity has been estimated as micromoles of Pi released per hour was compared to the well-known anti-ulcer PPA inhibitor Omeprazole. Results were expressed as Mean \pm SEM [11] % enzyme inhibition has calculated using the formula:

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

Result and Discussion

The neutralizing effect of the ethanolic extract was examined at four concentrations (100, 500, 1000, and 1500 mg) and standard Aluminium hydroxide + Magnesium hydroxide [Al(OH)₃ + Mg(OH)₂] (500 mg). The findings showed that, in comparison to the industry standard of 12.7 mg/mg, the extract's ability to neutralize acid dramatically decreased at doses of 100, 500, 1000, and 1500 mg, or by 100.5, 28.5, 9.25, and 5.33 mg/mg, respectively. It has been found that the extract considerably improves the capacity of acid to be neutralized when diluted to a concentration of 1500 mg. The results are tabulated in Table no:1 and Figure no:1

Table -1: Effect of ethanol extract of *Saponaria officinalis* on acid neutralizing capacity

Sl. No	Concentration (mg)	Volume of NaOH consumed (ml)	mEq of Acid consumed	ANC per gram of acid
1	100	39.9	10.05	100.5
2	500	31.5	14.25	28.5
3	1000	41.5	9.25	9.25
4	1500	44	8.0	5.33
5	500 Al(OH) ₃ + Mg(OH) ₂	47.3	6.35	12.7

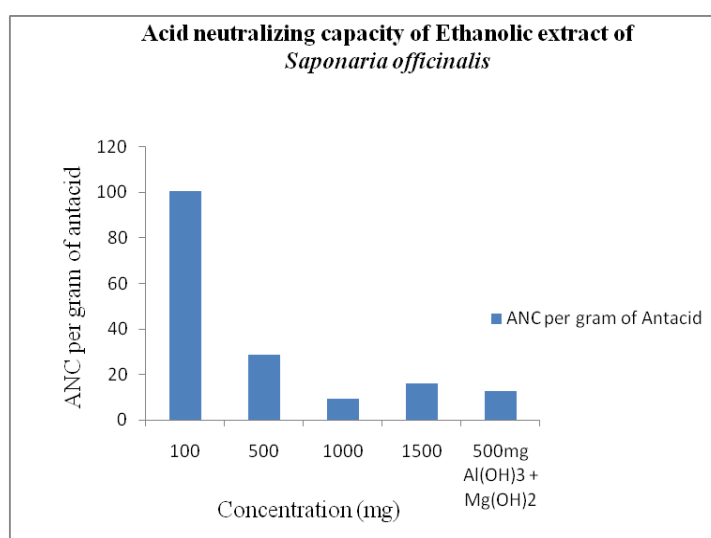


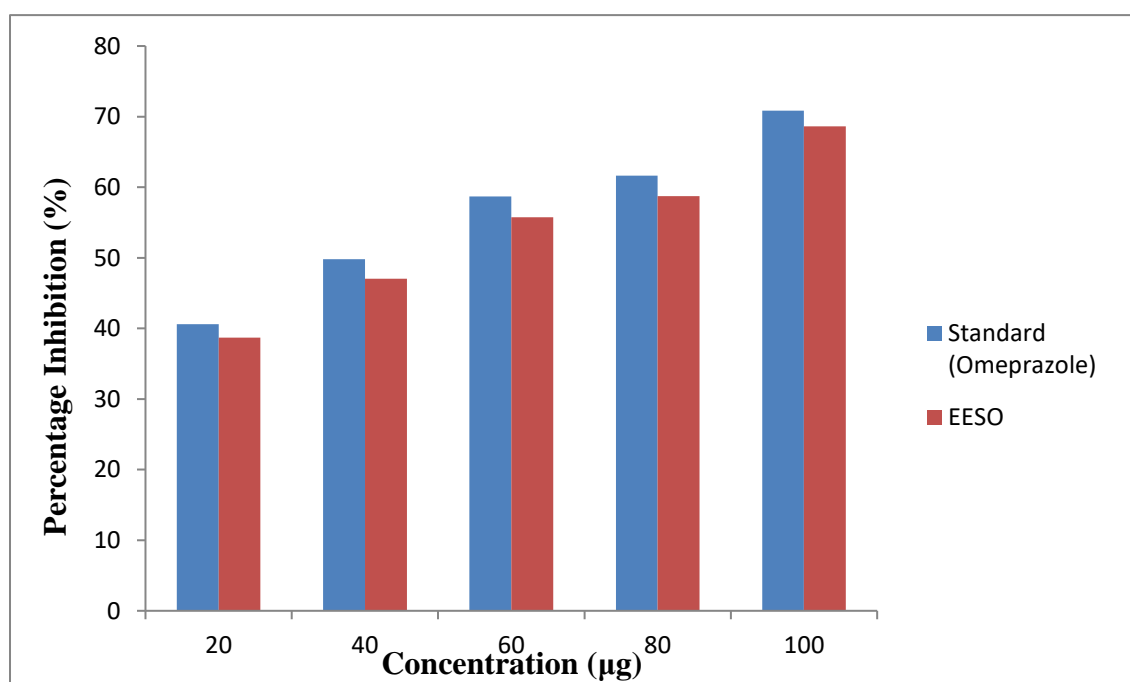
Figure -1: Effect of ethanol extract of *Saponaria officinalis* on Acid neutralizing capacity

H⁺/K⁺ - ATPase Inhibition Activity

The H⁺/K⁺ -ATPase inhibitory activity of *Saponaria officinalis* ethanol extract at different concentrations of 20, 40, 60, 80, and 100µg was compared with omeprazole as the reference drug. Significant action in a dose-dependent manner was demonstrated by the extract. At a concentration of 100µg, extract showed the highest percentage inhibition at 68.62±1.03%, whereas conventional omeprazole showed the highest percentage inhibition at 70.83±0.31%. The outcome is shown in Table 2 and Figure 2. IC₅₀ value was found to be 49.46µg/ml.

Table -2: Effect of EESO on In-vitro H⁺/K⁺ - ATPase inhibition activity

Sl.no	Concentration (µg/ml)	Percentage Inhibition (%) (Mean±SEM)	
		Standard (Omeprazole)	Ethanol extract of <i>Saponaria officinalis</i>
1	20	40.60±1.07	38.70±1.21
2	40	49.80±1.36	47.05±1.45
3	60	58.70±0.89	55.73±1.41
4	80	61.63±0.52	58.73±1.39
5	100	70.83±0.31	68.62±1.03

**Figure-2:** Effect of EESO on In-vitro H⁺/K⁺ - ATPase inhibition activity

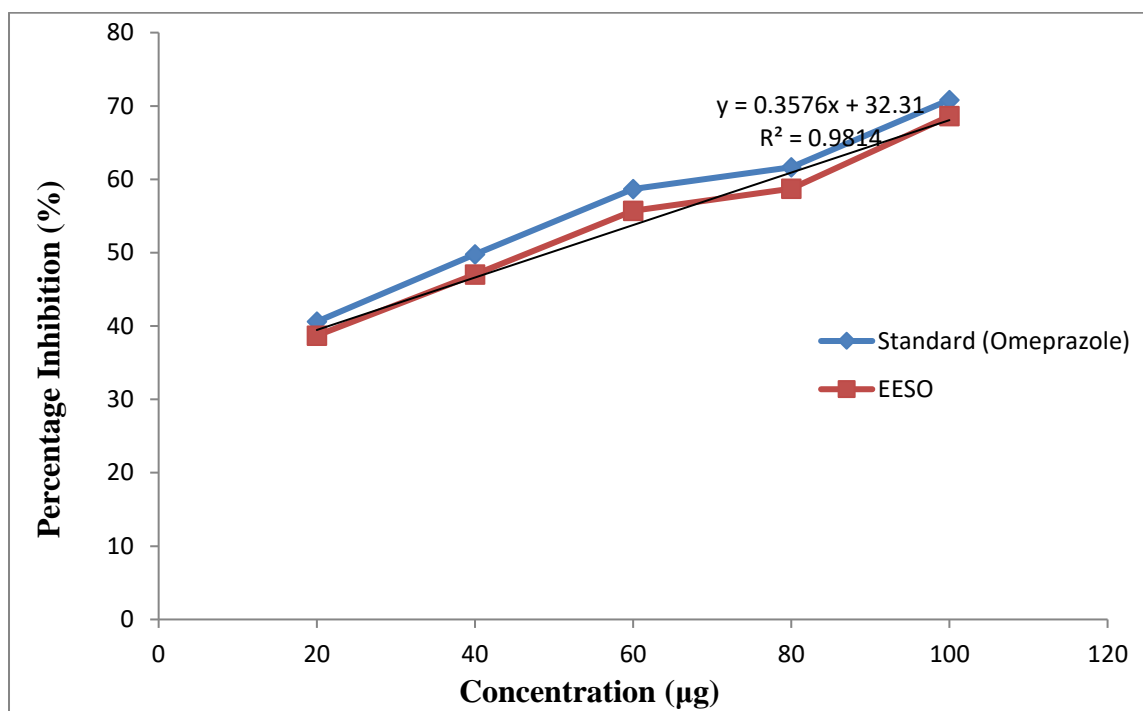


Figure-3: Graph showing IC₅₀ value of EESO on In-vitro H⁺ / K⁺ - ATPase inhibition activity

Discussion

Most of the time, the cause of peptic ulcers is unknown, but it is widely acknowledged that it is caused by an imbalance between aggressive factors and the endogenous defense mechanism's ability to maintain mucosal integrity [12]. Acidity is a frequent digestive issue that is linked to a functional condition that may develop for a number of different reasons [13]. Inflammation and ulceration of the stomach lining result from the overproduction of stomach acid, also known as gastric acid or HCl [14]. Antacids work by neutralizing stomach acid and lowering the stomach's pH [12]. The amount of acid that an antacid can neutralize is known as its acid-neutralizing capacity (ANC), and it has been evaluated using a technique called back titration. The aqueous extract significantly decreased ANC by 5.33 at a dosage of 1500 mg. Using the proton pump, the parietal cells of the stomach mucosa secrete excessive amounts of hydrochloric acid, which is what is known as hyperchlorhydria. An important enzyme for producing acidity is H⁺/K⁺ - ATPase, which is found in the apical secretory membrane of parietal cells [15]. At a concentration of 100µg, the extract exhibited a maximum percentage inhibition of 68.62±1.03% in H⁺/K⁺ - ATPase activity.

Hence, the EESO reduced ATP hydrolysis by gastric ATPse (IC₅₀ of 49.46µg/ml). According to the results presented here, the ethanol extract may have antacid, antisecretory, and antiulcer properties that may be brought on by the presence of certain chemicals in the mixture. However, more research is needed to determine its precise mechanism of action and the key ingredients responsible for its antiulcer efficacy.

Conclusion

On the basis of the results, we may conclude that the ethanol extract of the species may be considered as a sole source of novel antiulcer drugs. However, a detailed study on the isolation of active constituents from this species and its underlying mechanism of action responsible for its antiulcer effect is to be studied in the future.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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