RESEARCH PAPER

In Vitro Study of Anti-Ulcer Activity of Alpinia calcarata with Ethanol Extract

Revathy Leena Ravi[1*] and R Janet Rani[2]

[1]Research Scholar and [2]HOD & Assistant Professor, PG & Research Department of Microbiology, Sadakathullah Appa College (Autonomous), Affiliated to Manonmaniam Sundaranar University, Rahmath Nagar, Tirunelveli-627011, Tirunelveli, Tamil Nadu, India

***Corresponding author:** revathyravi3363@sadakath.ac.in*

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ABSTRACT

The peptic ulcer is a common gastrointestinal ailment that is caused by excessive gastric acid secretion or a weakened gastric mucosal barrier. Alpinia calcarata, a medicinal plant long used to treat gastrointestinal disorders, contains a variety of bioactive phytochemicals with potential gastroprotective properties. This study primarily examined the in vitro anti-ulcer activity of the ethanolic extract of Alpinia calcarata and assessed the in vitro anti-ulcer activity of ginger extract using three approaches: acid neutralization in simulated gastric fluid, inhibition of H^+/K^+-ATPase enzyme activity, and neutralizing capacity against artificial gastric acid. The extract's acid-neutralizing effect was evaluated by measuring pH changes after adding varying concentrations of the extract to artificial gastric fluid. Acid-neutralizing capacity was determined by titrating excess HCl with sodium hydroxide after treatment with various extract concentrations (100–1500 mg/mL), and the results were compared with those of standard antacids (magnesium and aluminum hydroxide). For proton pump inhibition, the H^+/K^+-ATPase enzyme was isolated from the gastric mucosa of a goat, and inhibition was determined spectrophotometrically by the Fiske-Subbarow method. Omeprazole was used as the standard. The ginger extract showed significant pH-neutralizing capacity, and its AN activity was concentration-dependent. At higher concentrations, the ginger extract was highly effective, similar to the standard antacids. The inhibition of the H^+/K^+-ATPase enzyme ranged from 37.32% to 75.20% at varying concentrations, and showed the highest inhibition close to Omeprazole (88.28%). The ginger extract has dominant in vitro anti-ulcer activity by both acid neutralization and proton pump inhibition, and all the results support its traditional use and suggest its potential as a natural anti-ulcer agent. Furthermore, in vivo and clinical studies are recommended to validate its safety and treatment effectiveness.

Keywords: Alpinia calcarata, Artificial Gastric Acid, Acid Neutralization, Anti-Ulcer activity, H^+/K^+-ATPase, Ethanolic Extract

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INTRODUCTION

Ulcer disorders significantly affect global health, affecting the stomach, large intestine, and oral mucosa. Conventional treatments have many disadvantages, including mucosal damage, decreased gastric acid secretion, and interactions with other medications. To address this health issue, alternative therapeutic approaches are required. Since ancient times, plants have been used for medicinal purposes because of their abundance of active metabolites. Because of their effectiveness, minimal side effects, and relative affordability, herbal medications are highly recommended, even when their physiologically active ingredients are unknown, and it is very essential, and regulations are required to guarantee the quality of products, especially in randomized studies to evaluate their safety and effectiveness in treating ulcer disorders. Numerous preclinical studies have reported the anti-ulcerative properties of chemical constituents derived from plants. Although most research has focused on pharmacological actions in animal models, many botanical products have been shown to have Anti-Ulcer (AU) activity [1].

Beyond its role as a spice, ginger (*Zingiber officinale* Roscoe) is among the top five foods rich in antioxidants and has long been used for medical purposes [2]. In addition to its culinary use in food and beverages, it has been used medicinally as an antipyretic, carminative, and treatment for bronchitis, rheumatism, and pain [3]. Various biological activities, including anti-bacterial, Anti-Inflammatory (AI), anti-angiogenic, and anti-tumor effects, have been investigated in its extracts [4-8]. In addition, it is utilized in the treatment of gastrointestinal disorders, such as gastric ulcerogenesis. This is done by eliminating the bacterium Helicobacter pylori, which produces ammonia in the stomach, leading to the development of many ulcers, especially in the duodenum, as well as other gastric problems such as gastritis [9-11].

Peptic ulcers are a very common gastrointestinal problem, often caused by excessive stomach acid secretion or a

**Author for Correspondence: revathyravi3363@sadakath.ac.in*

weakened gastric mucosal barrier, and it is noted that more than 10% of people worldwide suffer from Peptic Ulcer Disease (PUD), as per recent research. An outline of the preclinical and clinical research conducted by Prayoga et al. [1] in their review of medicinal plants. It focuses on how effectively they treat ailments, including PUD. To determine the mechanism by which each metabolite functions, more pharmacological activity assays are required. They added that these assays should examine which proteins, pathways, or genes are impacted by the plant extract or metabolite, and validating the possible application of these metabolites in the management of ulcers will require these investigations. In addition, they suggested the separation and synthesis of the active metabolites from plants in order to use them as targeted treatments with higher effectiveness and lower risk of side effects.

Because gastric ulcer therapy has limitations and the majority of currently available medications have significant side effects and less efficacy against gastric disorders, preventing or curing peptic ulcers is a challenging medical problem. Although very effective, Proton Pump Inhibitors (PPIs) have some side effects if taken for a long time. The PPIs and antacids are often prescribed for ulcers, but long-term use can have adverse effects and risk for drug interactions. Therefore, people are searching for safer natural remedies. PUD remains a major worldwide health issue, impacting millions of people and contributing to considerable morbidity [12]. The condition results from an imbalance between defensive systems, including mucus secretion, bicarbonate production, and aggressive forces such as pepsin, Helicobacter pylori infection, and nonsteroidal AI medicines (NSAIDs) [13]. Furthermore, ulcers develop because of erosion of the duodenal or stomach mucosa caused by prolonged exposure to these aggressive forces [14].

The PPIs, H_2-receptor antagonists, antacids, and antibiotic therapy for H. pylori eradication are conventional treatments for PUD [15]. Despite their effectiveness, prolonged utilization of PPIs and other synthetic medications resulted negatively, such as nutritional malabsorption, drug interactions, and relapse after discontinuation [16]. These limitations have prompted researchers to investigate various natural medicinal substances. Dharmani and Palit [17] reviewed the usage of herbal drugs and pointed out a few significant plants that have been shown in their study, as well as in recent studies, to have AU and ulcer-healing properties, and they suggested that separating, describing, and standardizing the active ingredients from herbal sources AU activity requires Ayurveda knowledge backed by contemporary science. The pathophysiology of peptic ulcers has been rapidly understood in recent years, and most research focuses on improved, advanced drug therapies. Nevertheless, the clinical assessment of these medications revealed the emergence of tolerance, the frequency of relapses, and adverse effects, rendering their effectiveness questionable. This has served as justification for developing novel AU medications, including herbal remedies. A valuable source of therapeutic agents for treating a variety of illnesses, including PUD, is medicinal plants and their derivatives.

In recent years, attention has turned to natural products and traditional medicinal plants as potential alternatives to conventional therapy[18]. *Alpinia calcarata* (*AC*) Roscoe, from the family *Zingiberaceae*, is commonly utilized to treat digestive disorders, inflammation, and gastric discomfort [19]. Phytochemical studies indicate that *AC* contains terpenoids, flavonoids, phenolic compounds, and essential oils with anti-inflammatory and antioxidant properties that may help preserve the stomach mucosa [20].

By targeting processes such as gastric acid neutralization and H^+/K^+-ATPase inhibition, in vitro studies provide a controlled and reliable approach for determining AU activity. This study is aimed to examine the in vitro AU activity of the ethanolic extract of *AC* by evaluating its neutralizing activity against artificial gastric juice, Acid-Neutralizing (AN) capacity, and inhibitory activity against H^+/K^+-ATPase. This study mainly focused on providing a mechanistic proof for the utilization of *AC* as a natural antacid and gastroprotectant [21-23].

MATERIALS AND METHODS

In-vitro AU Activity

Preparation of Artificial Gastric Acid (AGA)

Initially, the Artificial Gastric Juice (AGJ) is prepared based on United States Pharmacopeia (USP) guidelines, and a total of 2 g of sodium chloride and 3.2 mg of pepsin are dissolved in 7 mL of concentrated hydrochloric acid, and then diluted with deionized water to yield one liter of AGJ at pH 1.2. The AGJ is stored at 4˚C until utilization. After adding different concentrations of the extract to the AGJ, pH changes were measured to assess the extract's AN ability [21].

Neutralizing effect of the extract on AGA

Fifty milliliters (50 mL) of each test, water, and standard solution were mixed to 55 mL of AGJ at pH 1.2, and the resulting pH values were analyzed to determine the balancing effects on the AGJ, using the standard, Sodium bicarbonate (1 mg/mL). The pH was determined using a digital pen-type pH meter.

AN Capacity of the extract

The extract concentrations capable of AN were 100 mg/mL, 500 mg/mL, 1000 mg/mL, and 1500 mg/mL. Magnesium hydroxide and aluminum hydroxide (500 mg each) served as the standard. The volume was 70 mL after adding 5 mL of the sample, and the remaining 65 mL of water was added. The mixture was blended for one minute. 30 mL of 1.0 N HCl was added to the standard and test samples, and the samples were swirled for 15 minutes. Phenolphthalein was then added and mixed in. The excess HCl was titrated with 0.5 N sodium hydroxide until a pink color was achieved [22].

The Moles of Acid Neutralized (MAN) are evaluated by,

MAN = (vol. of HCl × Normality of HCl) - (vol. of NaOH × Normality of NaOH) (1)

AN capacity per gram of antacid = moles of HCl neutralized divided by Grams of Extract / Antacid (2)

H^+/K^+ - ATPase Inhibition activity of the extract

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

The H^+/K^+-ATPase enzyme sample was made using a fresh goat stomach that was bought from a butcher shop. The fundus' stomach mucosa was removed and exposed. The parietal cells were then extracted by scraping the stomach's inner layer. The stomach parietal cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100, then centrifuged at 6000 rpm for 10 minutes. The supernatant solution was then used to assess the H^+/K^+-ATPase activity.

The H^+ /K^+ ATPase inhibition

The sample was pre-incubated for 60 minutes at 37 °C with 0.1 mL of enzyme extract (300 g) and plant extract at different concentrations (20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, and 100µg/mL). The reaction was initiated by adding 200 mL of 2 mM $MgCl_2$ and 10 mL of KCl, as well as 2 mM ATP as the substrate. 4.5% ammonium molybdate was used to stop the reaction after 30 minutes at 37 ºC, and following the addition of 60% perchloric acid, the mixture was centrifuged for 10 minutes at 2000 rpm to extract the supernatant containing the released inorganic phosphate, which was subsequently detected using the Fiske-Subbarow [24] method at 660 nm. After 10 minutes at room temperature, 1 mL of supernatant, 4 mL of Millipore water, 1 mL of 2.5% ammonium molybdate, and 0.4 mL of ANSA were added. Absorbance at 660 nm was measured at various extract doses; enzyme activity (micromoles of Pi released per hour) was estimated using the absorbance of inorganic phosphate and compared to that of the well-known AU PPA inhibitor, omeprazole. The findings were presented as Mean ± SEM [23]. Percentage Enzyme Inhibition (PEI) is evaluated by:

PEI= [Activity (control) - Activity (test)/Activity (control)] × 100 (3)

RESULTS

Table 1 and **Figure 2** show the determination of the extract's neutralizing effect on AGA. When different concentrations of extract (100, 500, 1000, and 1500mg; each 90 mL) were added separately to 100 mL of AGJ (pH 1.2), the pH values of the extracts were 1.42, 1.51, 1.68, and 1.84, respectively. The pH values of water and Sodium bicarbonate solutions were 1.38 and 1.79, respectively. This finding suggests that the extract can neutralize acid in a dose-dependent manner.

Table 1. Determination of the neutralizing effect of the extract on AGA

Tested sample	pH Value
AGA	1.20
Water	1.38
100 mg/mL	1.42
500 mg/mL	1.51
1000 mg/mL	1.68
1500 mg/mL	1.84
Standard (Sodium bicarbonate) (1 mg/mL)	1.79

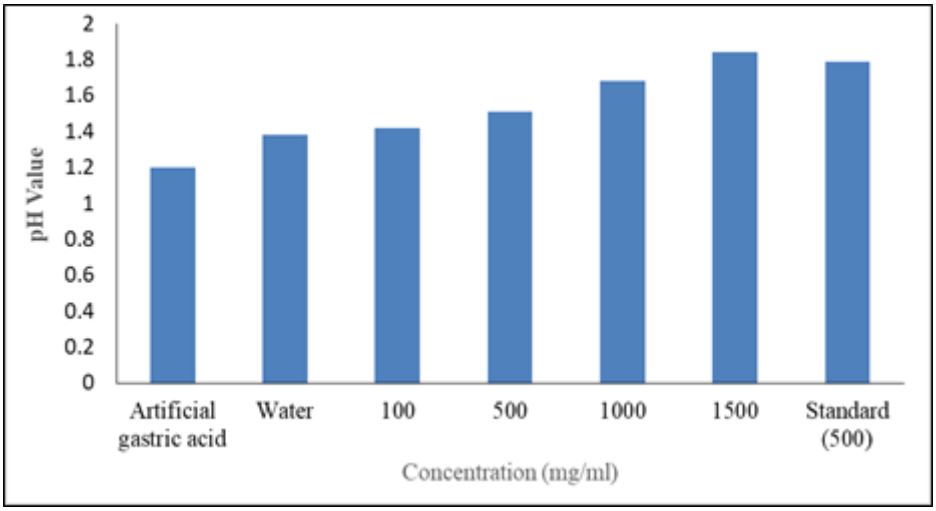

Figure 1. Neutralizing effect of the extract on AGA

The *in vitro* AN effects of the extract at various concentrations, 100 mg, 200 mg, 500 mg, 1000 mg, and 1500 mg/mL, are linked with the standard antacid AHMH (500 mg/mL) [25]. A concentration-dependent reduction

resulted in AN capacity per g of antacid: 88.5, 23.3, 9.8, and 5.6, respectively. Similarly, AHMH (500 mg), which has the AN capacity value of 13.50, has a very close concentration of the test drug. Whereas, extract concentrations 1000 and 1500mg were found to neutralize acid effectively in comparison with the standard. The results are presented in **Table 2** and **Figure 2.**

Table 2. The impact of the extract on AN capacity

Concentration (mg/mL)	Volume of NaOH consumed (mL)	mEq of Acid consumed	AN capacity per gram of acid
100	42.3	8.85	88.5
500	36.7	11.65	23.3
1000	40.4	9.80	9.8
1500	43.2	8.40	5.6
500 $Al(OH)_3$ + $Mg(OH)_2$	46.5	6.75	13.5

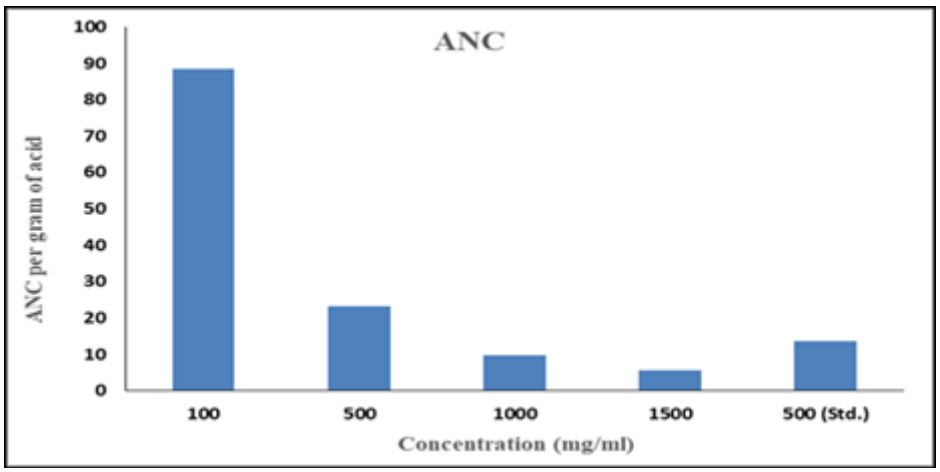

Figure 2. The impact of the extract on AN capacity

***In-vitro* H^+/K^+ - ATPase Inhibition Activity**

Various concentrations; 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, and 100µg/mL; of the extract are compared with those of the reference medicine Omeprazole; 20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL, and 100µg/mL. Inhibition of H+/K+-ATPase activity by both the test and standard drugs was concentration-dependent, with results ranging from 37.32 to 75.20% for the extract and from 21.25 to 88.28% for the standard drug at 20, 40, 60, 80, and 100 micrograms per milliliter. H+/K+ - ATPase activity was observed to be inhibited by the extract at concentrations 100µg/mL for 75%, and nearest to the standard Omeprazole (88.28%). The results are summarized in **Table 3** and **Figure 3.**

Table 3. The Impact of extract on H^+ /K^+ ATPase inhibition activity by *in vitro*

Concentration (µg/mL)	Ethanol extract	Standard (Omeprazole)
20	37.32	21.25
40	45.50	42.50
60	56.94	65.66
80	62.39	75.20
100	75.20	88.28

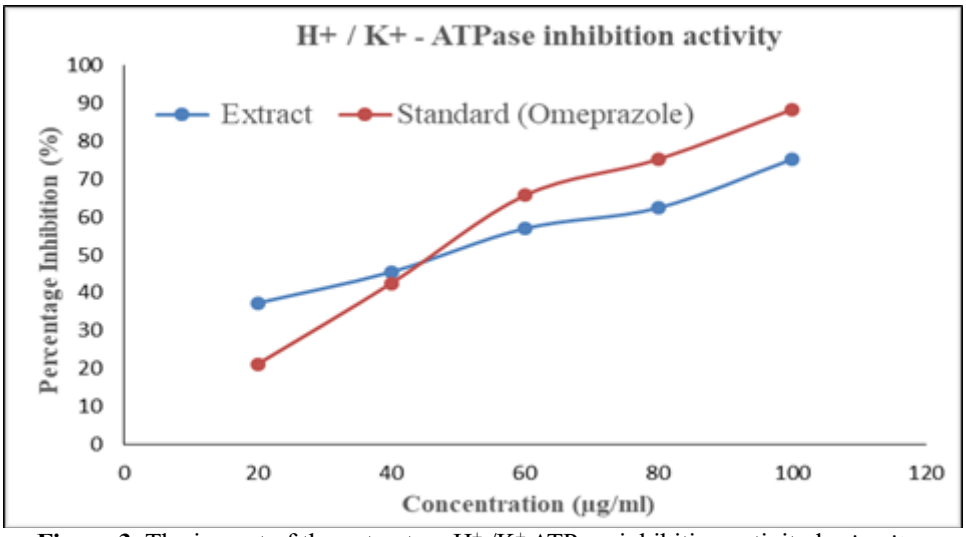

Figure 3. The impact of the extract on H^+ /K^+ ATPase inhibition activity by *in vitro*

CONCLUSION

Significant pH-neutralizing ability and concentration-dependent AN activity were demonstrated by the ginger extract. The ginger extract was just as effective at higher concentrations as conventional antacids. At different concentrations, the H^+/K^+-ATPase enzyme's inhibition varied from 37.32% to 75.20%, with Omeprazole (88.28%) exhibiting the highest inhibition. This study's findings pointed to ginger extract's potential as a natural AU agent and validated its traditional use. The extract exhibits dominant in vitro AU activity through both acid neutralization and proton pump inhibition. Based on the neutralizing effect of the extract, AN capacity, and H+/K+ ATPase inhibition activity, the ethanol extract is considered a source of novel AU and antacid drugs. The study findings indicated that the ethanol extract possesses an antacid and AU property, which might result from the existence of phyto-bioactive compounds in the mixture, and, to confirm its safety and efficacy as a treatment, in vivo and clinical research are advised.

FUTURE RESEARCH DIRECTION

Future studies should focus on in vivo evaluation of the AU and antacid activities of *AC* ethanolic extract using established animal ulcer models to confirm its therapeutic efficacy and safety. Furthermore, phytochemical isolation and characterization of the active bioactive compounds responsible for the observed effects can be carried out using advanced techniques, including HPLC, GC–MS, and LC–MS/MS. In addition, the molecular mechanisms of action can be further researched on proton pump inhibition, antioxidant activity, etc., and further investigations can be carried out for toxicological and pharmacokinetic studies to examine the dosage, bioavailability, and long-term safety. These studies will help develop standardized formulations and support the potential of *AC* as a novel, plant-based AU and antacid therapeutic agent.

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