



Evaluation of *in-vitro* Antiulcer Activity and Phytochemical analysis in *Alcea rosea* (Hollyhock)

Shoab Mohammad Syed^{1,2}, B. Sabitha^{1*}, T. Sowmya³

¹School of Pharmacy, Shri Venkateshwara University, Gajraula, Amroha, UP, India

²Department of Pharmaceutics, Dayanand College of Pharmacy Latur MS, India

³Department of Pharmaceutics, Smt. Sarojini Ramulamma College of Pharmacy, Palamuru University, Mahabubnagar, Telangana, India

Corresponding Author: B. Sabitha

Address: 8-139/3, Near Pochamma Temple, Marlu, Mahabubnagar-509001, Telangana, Ph. 8179703690

Mail.id: sabithabandaru320@gmail.com

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ABSTRACT

Alcea rosea, a member of the Malvaceae family, generally recognized as Hollyhock, is widely grown in gardens and parks across Asia and Southern Europe. Several medicinal investigations have revealed that this plant has pain-reducing, antibacterial, and anti-inflammatory qualities. The present study, *In-vitro* Antiulcer Activity of *Alcea rosea*, incorporated H⁺, K⁺-ATPase and Acid neutralizing capacity (ANC). At a concentration of 250 mg, sample QAR displayed the highest ANC per gram (98.0 meq/g), surpassing the given standard antacid (97.2 meq/g), and implying significant acid neutralizing capability. Although slightly less than QAR, sample ARET as well exhibited considerable activity (82.4 meq/gat250mg). The acid neutralizing capacity (ANC) investigation demonstrated that both the herbal samples tested and standard antacids had considerable acid neutralizing features. Sample-QAR and ARET were examined for their capacity to suppress H⁺, K⁺-ATPase, using omeprazole as the reference standard. Sample-QAR and omeprazole displayed inhibition rates of 70.49% and 65.57%, respectively, at a concentration of 100µg/ml. In the meantime, Sample-ARET displayed a 42.62% inhibition rate, showing that it has a reasonable amount of activity.

Keywords: *Alcea rosea* (ARET), Antiulcer Activity, Acid neutralizing capacity (ANC) and H⁺, K⁺-ATPase.

INTRODUCTION

Herbal Medicine

Integrative medicine is an emerging field within alternative and complementary medicine that is increasingly being recognized worldwide and may soon be integrated into conventional medical practices. This approach is embraced by diverse populations across various demographics, encompassing different genders, socioeconomic backgrounds, and age groups. As the popularity of holistic medicine continues to rise globally, its market share expands, with annual sales approaching \$562 billion. Elements driving this increase in marketing and consumption encompass the cost-effectiveness of these products, their recognition as natural solutions, and their standing as low-toxicity options known for effectively addressing specific challenging conditions. Additionally, their adaptability in production, accessibility, and usage also play a significant role. Certain approaches in herbal medicine may include ingredients like fungi, bee products, insect ash, shells, insects, and animal parts to develop natural products that demonstrate biological activity. These products are used for promoting health and treating a range of illnesses. The advantages of herbal medicine are plentiful, with minimal downsides. Secondary metabolites are associated with both the pharmacological effects and the majority of the toxic effects of herbal medicines. These substances have been used appropriately, misused, and misunderstood in various contexts. To ensure the effective use of herbal medicines as a healthcare option, it is essential to have accurate and comprehensive knowledge and experience.

Benefits of herbal medicine

Herbal medicines (HM) are characterized as any substance utilized for diagnosing, treating, or preventing diseases, where the active components originate from any part of a plant, other plant materials, or a combination of these elements (Dhalwal K. et al 2009). In numerous remote regions of Africa and Asia, herbal medicine remnants play a vital role in fundamental healthcare. Additionally, they are integral to the cultural practices surrounding various diets worldwide. There exists a rich history of communal use and reported health benefits linked to numerous plants and herbal formulations. Scientific research emphasizes the pharmacological effects both beneficial and adverse of the intricate chemical constituents present in herbal medicines (Bent 2008). These remedies have been employed for both proactive and reactive health maintenance, addressing a wide spectrum of ailments, from minor issues to severe health conditions (Mosihuzzaman M. 2012; Coleman, 1995; Akhter S. 2008; Parasuraman U. 2014).

Taxonomical Classification

Kingdom	:	Plantae
Division	:	Mangoliophyta
Order	:	Malvales
Family	:	Malvaceae
Genus	:	<i>Alcea</i>
Species	:	<i>Alcea rosea</i>
Common Name	:	Hollyhock



Synonyms

Althaea rosea (L.) Cav.

Habitat

Anthropogenic (man-made or disturbed habitats), meadows and fields

Description

Alcea rosea, commonly known as hollyhocks, have long been cherished in gardens. These flowers grow on strong, tall spikes that typically reach heights of 5 to 8 feet and generally do not need support. The leaves are large and heart-shaped, with 3, 5, or 7 lobes, becoming smaller as they ascend the spike. They bloom for an extended period from June to August and are sometimes found in nursery catalogs under the name *Althaea*. The genus name derives from the Latin, which comes from the Greek word "alkaia," referring to a type of mallow. The specific epithet translates to pink. This group of plants produces large, outward-facing, single flowers measuring 4 to 5 inches in diameter, available in a broad spectrum of colours, including reds, pinks, whites, and pale yellows.

Distribution

Hollyhock, likely originating from western Asia, is a classic garden ornamental that is extensively cultivated and boasts numerous cultivars. Many of these have naturalised globally, including in North America, where they are frequently found, although they do not tend to be overly invasive.

Phytoconstitution

Phytochemicals including flavonoids, tannins, terpenoids, saponins, and cardiac glycosides, as well as high-molecular-weight acidic polysaccharides referred to as mucilages.

Alcea rosea

Alcea rosea, commonly known as Hollyhock and part of the Malvaceae family, is frequently cultivated in gardens and parks throughout Southern Europe and Asia. Numerous pharmacological studies have shown that this plant possesses anti-inflammatory, antibacterial, and pain-relieving effects (Dar, P. A., et al 2017; Mert, T., et al 2010). In traditional Iranian medicine, the roots of *Alcea rosea* have been used to treat various health conditions, such as bronchitis, diarrhea, constipation, inflammation, severe coughs, and angina (Ahmadi, M., et al 2012). Research into the ethnobotanical and ethnopharmacological aspects of different *Alcea rosea* species indicates their potential for addressing a wide array of health issues. Hollyhock refers to the common name for *Alcea rosea*, a species that is prevalent from the Mediterranean region to Central Asia. This plant originates from China and Greece and is cultivated in gardens throughout India.

Alcea rosea is a perennial herb that can grow between 100 to 200 cm tall, and occasionally even up to 300 cm. The timing of its flowering depends on the region where it is grown. It features vibrant, bowl-shaped blooms in various colors, from white to deep violet, along with large, rounded, serrated, and textured leaves (Fahamiya, N., et al 2016). In addition to its aesthetic appeal, the hollyhock yields edible flowers that can be brewed into tea, which has a slightly bitter flavor (Newman, S. E.; O'connor, A. S. 2009). Additionally, this plant has a long-standing tradition of medicinal use in folklore throughout the Eastern Mediterranean. Different parts of the hollyhock are employed for their health advantages, which include properties that are antioxidant, antimicrobial, antiviral, and protective for the liver, among others (Abdel-Salam, A. A. et al 2018; Azab, A. 2016; Kalemba-Drożdż, M. and Cierniak, A. 2019).

The third strategy involves the utilization of medicinal plants, where all parts of the plant possess valuable secondary metabolites. One instance of this method is Hollyhock (*Alcea rosea* L.), a perennial plant known for its medicinal and aromatic properties. The roots, seeds, shoots, and flowers of this plant produce secondary metabolites that provide health benefits and have been used traditionally to treat a range of health concerns in the Middle East and nearby areas (Rehman, R., et al 2019). Furthermore, the seeds of this species contain mucilage, which can aid in seed germination in arid and semi-arid environments. Since 6000 years ago, people have utilized *A. rosea* for medicinal purposes (Cowan M.M. 1999).

Recent studies have shown that various parts of the plant, including its flowers, roots, and seeds, possess antibacterial, anti-inflammatory, cytotoxic, sedative, analgesic, anti-cough, immunomodulatory, and antibiotic effects (Choi ES, et al 2012). The plant is particularly significant due to its wealth of valuable phytochemical compounds, such as anthocyanins, pectin, starch, monosaccharides, disaccharides, mucilage, and flavonoids (including hypolaetin-8-glucoside, isoquercitrin, kaempferol, caffeic acid, and p-coumaric acid). Additionally, it contains coumarins, scopolamine, phytosterols, tannins, asparagine, and certain amino acids, highlighting the importance of *Alcea rosea* as a rich source of secondary metabolites (Chopra RN, et al 1958; Pullaiah T. 2006).

Alcea rosea, also known as *Althaea rosea* or 'Garden hollyhock' (locally called Gul-e-Khera), belongs to the mallow family (Malvaceae) (Munir M, et al 2012). This biennial plant, native to the southwestern regions of China, has been grown since the 15th century. It is often used for ornamental purposes and can grow as tall as 8 feet, flourishing in various soil types, especially those that are moist and well-drained (Shaheen N, et al 2010). Its distribution spans temperate regions globally. The flowers display a range of colors such as red, purple, white, pink, yellow, and black-purple, blooming individually on straight stems (Lim TK (2014).

Flavonoids and anthocyanins have been isolated from *Alcea rosea*, contributing to the production of natural medications known for their anti-microbial and anti-inflammatory properties, particularly concerning the gastrointestinal system (Munir M, et al 2012). *Althaea rosea* commonly referred to as garden Hollyhock or Gulekhera in local terminology is a plant species valued for both its ornamental and medicinal properties, classified within the mallow family (Malvaceae). This species is grown in various colors such as white, pink, and red, and it originates from China. It flourishes in soils that are moderately fertile, moist, and well-drained (Still, M.S. 1994) as well as clay soils (Abraham, D.K. 1999). Notably, this plant exhibits a remarkable ability to tolerate and accumulate significant levels of cadmium (Liu, J.N., et al 2008; Liu, J.N., et al 2009).

Furthermore, Hollyhock is recognized for its use in the production of paper for wrapping and creating paper bags (Matsumae, R., et al 1956). Hollyhock, scientifically known as *Alcea rosea*, is a self-seeding member of the Malvaceae family and is classified as an ornamental dicot flower. This plant has been utilized for various purposes, including traditional medicine and culinary applications, with its leaves, roots, and seeds often being used (S.B. Aziz et al. 2019; M.F. Majnis et al.2022).

Ulcers

An ulcer is a painful lesion that can take a significant amount of time to heal and may come back after treatment. These sores are quite prevalent. The underlying cause and the location of the ulcer on your body influence its appearance and the associated symptoms. Ulcers can develop in various areas, including the lining of the stomach and the outer layer of the skin. While some ulcers may resolve without intervention, others require medical care to prevent serious complications. Ulcers, which are lesions found on the skin or mucous membranes, are defined by tissue that is inflamed on the surface and has undergone necrosis (Chan FKL and Graham DY 2004). Peptic ulcers are the most common gastrointestinal disorder (Chan FKL and Graham DY 2004; Goyal RK 2008), affecting approximately 10% of the global population (Shimoyama AT, et al 2013). Research indicates that these ulcers arise from an imbalance between the stomach's protective mechanisms and its aggressive factors (Malfertheiner P, et al 2009; Shimoyama AT, et al 2013; Rao CV, et al 2000).

Factors that can contribute to peptic ulcers include the consumption of alcohol and non-steroidal anti-inflammatory drugs (NSAIDs), poor dietary choices, the presence of free oxygen radicals, heightened production of pepsin and hydrochloric acid (HCl), stress, and infection with *Helicobacter pylori*. Conversely, protective elements include growth factors, mucus, nitric oxide, prostaglandins, bicarbonates, and sufficient blood flow to the stomach (Sowndhararajan K, Kang SC. 2013; Lemos M, et al 2015). To treat peptic ulcers, healthcare providers typically prescribe medications such as proton pump inhibitors, antacids, histamine H2 receptor antagonists, and anticholinergics (Chan FK, leung WK. 2002).

On the other hand, thrombocytopenia, nephrotoxicity, hepatotoxicity, and impotence are among the serious side effects that might arise from long-term usage of these medications (Sheen FK, Triadafilopoulos G. 2011).

In vitro Antiulcer Activity

(i) Acid Neutralizing Capacity

Acid-neutralizing capacity (ANC) and anti-ulcer effects are connected because ANC shows how well an antacid can neutralize stomach acid, which plays a crucial role in both the formation and healing of ulcers. Simply put, a higher ANC means a stronger ability to lower stomach acidity, which could help in treating ulcers. ANC is the measure of how much acid a substance can neutralize, typically expressed in milli equivalents (mEq) for each gram or milli liter of the antacid. This ability plays an important role in figuring out how well antacids work to lower stomach acidity.

Ulcers are painful spots that can form on the inner surface of the stomach or small intestine, usually caused by too much acid or a lack of protective factors. Anti-ulcer medications are compounds that can help lower stomach acid, shield the stomach and intestinal lining, or aid in the healing process of ulcers.

Activity

Antacids contain basic compounds that react with the hydrochloric acid (HCl) present in the stomach, aiding in its neutralization and raising the pH level. This action helps to reduce the acidity and discomfort commonly associated with heartburn and ulcers. A higher Acid Neutralising Capacity (ANC) means that the antacid can neutralize more acid, potentially leading to more effective relief (Divya, J.O. and Rasheed, F.M., 2021).

Ulcers and ANC

Peptic ulcers, a specific kind of ulcer, may arise from excessive acid production or harm to the stomach lining. Antacids can help control these ulcers by neutralizing the acid responsible for their formation (Yafout, M., et al. 2022). Furthermore, certain antacids contain components such as sucralfate, which adheres to the ulcers and forms a protective barrier over the stomach lining.

Role of ANC in Choosing Antacids

Antacids with higher Acid Neutralizing Capacity (ANC) values are generally considered more effective in neutralizing stomach acid (Divya, J.O. and Rasheed, F.M., 2021). When choosing an antacid, it is important to consider the individual's specific requirements, such as the severity of their symptoms and any existing health conditions (Yafout, M., et al. 2022). Some research highlighted in the International Journal of Herbal Medicine suggests that low-dose antacids may be sufficient for healing peptic ulcers; however, there are concerns about "acid rebound" with certain types, where the body increases acid production after their use (Hade, J.E. and Spiro, H.M., 1992). Furthermore, the World Journal of Biology Pharmacy and Health Sciences reports that Emilia sonchifolia extract showed a decrease in acid neutralizing capacity that was dependent on the concentration per gram of antacid (Faseela V A., et al. 2023).

A lot of research looks at how well anti-ulcer treatments work by checking ANC and other factors, such as the inhibition of H⁺/K⁺-ATPase, which is another way that medications can lower acid production.

(ii) H⁺/K⁺ - ATPase Inhibition Activity

Blocking H⁺/K⁺-ATPase is a key way to help with ulcers. This enzyme, known as the proton pump, is found in the stomach and is responsible for producing gastric acid. When this pump is inhibited, it leads to less acid being made, which is important because too much acid can cause ulcers. Medications called proton pump

inhibitors (PPIs), such as omeprazole, lansoprazole, and esomeprazole, work by stopping this pump from functioning.

The H⁺/K⁺-ATPase enzyme is situated in the parietal cells of the stomach lining. It pumps hydrogen ions (H⁺) into the stomach while taking in potassium ions (K⁺) into the cells. This action helps to acidify the stomach contents, which is necessary for digestion, but if there's too much acid, it can result in ulcers. Inhibiting H⁺/K⁺-ATPase is a common treatment for issues like peptic ulcers, gastroesophageal reflux disease (GERD), and dyspepsia, where too much acid is a significant concern. Lowering the amount of gastric acid by blocking H⁺/K⁺-ATPase helps safeguard the stomach lining and supports ulcer healing by lessening the damaging impact of acid.

H⁺/K⁺-ATPase Inhibition Works

(i) Lowered Acidity

Inhibition of the H⁺/K⁺-ATPase enzyme plays a vital role in decreasing gastric acid (HCl) secretion, leading to a marked reduction in stomach acidity (Laloo, D., et al 2021).

(ii) Restoration and Protection

In cases such as peptic ulcers and GERD, an overproduction of acid can harm the stomach lining, resulting in ulcers. Inhibiting H⁺/K⁺-ATPase can lower acidity, facilitating the healing of current ulcers and aiding in the prevention of new ones (Adinortey, M.B., et al 2018).

(i) Relief from Symptoms

Ulcers often cause pain and discomfort, including abdominal pain and heartburn, primarily due to excessive acid production. Inhibiting H⁺/K⁺-ATPase can alleviate these symptoms by lowering acidity, offering relief to ulcer patients (Mei, D. and Zou, C., 2025).

Medications that block H⁺/K⁺-ATPase

(i) Proton Pump Inhibitors (PPIs)

The most frequently utilized medications for inhibiting H⁺/K⁺-ATPase are omeprazole, lansoprazole, and pantoprazole. These proton pump inhibitors (PPIs) function by attaching to the active site of the H⁺/K⁺-ATPase enzyme, thereby blocking its ability to transport H⁺ ions.

(ii) Potassium-Competitive Acid Blockers (PCABs)

This is an advanced category of medications that inhibit the H⁺/K⁺-ATPase. They function by attaching to the K⁺ active site of the enzyme, thereby effectively preventing its activity (Adinortey, M.B., et al 2018).

Clinical Uses

Proton pump inhibitors (PPIs) are frequently prescribed for various medical issues

(i) Peptic Ulcers: They promote the healing of ulcers by reducing the damaging impact of stomach acid.

(ii) Gastroesophageal Reflux Disease (GERD): They alleviate symptoms associated with acid reflux, such as heartburn, by decreasing acid production.

(iii) Zollinger-Ellison Syndrome: In this rare disorder, the stomach produces an excessive amount of acid. PPIs help manage this overproduction of acid (Fujii, T., et al 2021).

In vitro Antiulcer Activity

S.NO	PLANT NAME	EXTRACT	IN-VITRO MODELS	AUTHORS
1.	<i>Hedranthera barteri</i>	-	H ⁺ /K ⁺ -ATPase Inhibition Activity	Onasanwo, S.A., et al 2010
2.	<i>Acalypha wilkesiana</i>	Leaf Extract	H ⁺ /K ⁺ -ATPase Inhibition Activity	Gupta, R.K.P. and Hanumanthapp, M., 2013
3.	<i>Cassia tora</i> <i>Pithecellobium dulce</i> <i>Butea monosperma</i> <i>Pongamia pinnata</i> <i>Tephrosia purpurea</i> <i>Mucuna pruriens</i>	Methanolic, Chloroform, Butanolic, Water Extracts	H ⁺ /K ⁺ -ATPase Inhibition Activity	Gupta, A., et al
4.	<i>Emilia sonchifolia</i>	Whole Plant Extract	Acid Neutralizing Capacity	Faseela V A., et al. 2023
5.	<i>Dissotis rotundifolia</i>	Whole Plant Extract	H ⁺ /K ⁺ -ATPase Inhibition Activity	Adinortey, M.B. et al 2018
6.	<i>Carissa carandas</i>	Methanol Extract	H ⁺ /K ⁺ -ATPase Inhibition Activity	Shukla, A., et al 2016

METHODOLOGY

Collection and Authentication of Plants

In this study, samples of *Alcea rosea* root were collected from Place. The seeds were sourced and confirmed by the Department of Botany at Ayya Nadar Janaki Ammal College in Sivakasi. After collection, the seeds

were washed with water and allowed to dry naturally at room temperature, out of direct sunlight. Once they were dry, they were ground evenly with a mechanical grinder to produce a coarse powder. This powdered substance was then kept in an airtight container.

Procedure for preparing the sample

A 1000 millilitre round-bottom flask was selected for preparing the sample and was cleaned with methanol, ensuring it dried thoroughly. Then, 60 grams of coarse *Alcea rosea* powder was added to the flask, along with 600 millilitres of methanol. The flask was thoroughly shaken and left for a cold maceration process lasting 7 days. During which the flask was shaken periodically to enhance extraction. Subsequently, the blend was passed through Whatman Filter Paper No. 1 and allowed to evaporate naturally at ambient temperature.

Please note that while I have rephrased the original text. It is essential to make certain that the paraphrased material accurately reflects the meaning and intent of the original text, while employing your own vocabulary and sentence construction, and dried under vacuum. 4 gram of crude extract was obtained from the 60 gram of the powdered seeds of *Alcea rosea*. Crude Extract will term as ARET.

PHYTOCHEMICAL ANALYSIS TEST

Phytochemical screening is the process used to identify various compounds found in plant extracts. Plants contain numerous chemical constituents that can elicit different physiological responses and offer therapeutic advantages. Consequently, it is standard practice to evaluate plants for the existence of biologically active and medicinally significant phytochemicals. Constituents responsible for a particular biological activity. Some of the examples of phytoconstituents include alkaloids, steroids, carbohydrates, saponins, tannins, flavonoids etc (Farnsworth NR. et al 1966 & Haseen, A., et al 2024).

In vitro Evaluation of Antiulcer Activity

(i) **Acid Neutralizing Capacity** (Garad MC, et al 2012; Sumia Fatima, et al 2018)

Objective

To determine the acid neutralizing capacity of a substance, typically used for evaluating buffers, antacids, or environmental samples such as soils and water.

Materials Required

- Sample (e.g., water, soil, or antacid)
- Standard Acid Solution (e.g., HCl, typically 0.1M or 0.5 M)
- Standard Base Solution (e.g., NaOH, typically 0.1M)
- pH Meter or pH Indicator
- Burette and Pipette
- Beaker (100 mL or 250 mL)
- Magnetic Stirrer (optional)
- Distilled Water

Procedure

1. Sample Preparation

- Water Sample: Use a measured volume (e.g., 50 mL) of the water sample.
- Soil Sample: Weigh a known amount (e.g., 5g) of soil and suspend it in 50 mL of distilled water.
- Antacid Sample: Crush and dissolve a known mass (e.g. 1mg/ml) in distilled water.

2. Acid Addition

- Using a burette, titrate the sample with the standard acid (e.g., HCl) until the desired pH endpoint is reached.
 - For environmental samples (water/soil): Titrate until pH 4.5 (or lower, based on requirements).
 - For antacids: Titrate until pH 3.0 (simulating stomach conditions).

3. Titration & Neutralization

- Record the volume of acid added.
- If back-titration is required (for strong neutralizers like antacids), add excess acid first, then titrate the unreacted acid with a base (NaOH) until the neutral pH endpoint is reached.

4. Calculation of ANC

ANC is expressed as milli equivalents per liter (meq/L) or per gram (meq/g) and is calculated using:

The mole of acid neutralized is calculated by, Moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. Of NaOH × Normality of NaOH)

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

(ii) **H⁺/K⁺ - ATPase Inhibition Activity** (Gupta, R.K.P. and Hanumanthappa, M., 2013; Fiske CH and Subbarow Y 1925)

Preparation of H⁺/K⁺ - ATPase Enzyme:

Proton potassium ATPase was prepared from mucosal scrapings of goat stomach. Stomach from freshly slaughtered goats was washed gently with tap water. The mucosal layer offundus was scrapped and homogenized in ice-cold phosphate buffer, pH 7.4. The homogenate was centrifuged for 20 min at 18,000 rpm. The supernatant so obtained was re centrifuged for 60 min at specified rpm. The pellet was re suspended in homogenization buffer. Protein was determined by the method of Lowry *et al.* Different concentrations of the extract 20-100 $\mu\text{g/ml}$ were incubated in the reaction mixture (40 mM Tris-HCl buffer, pH7.4, containing 2m MMgCl_2 and 10 μg membrane protein) to make a volume of 1ml. Then, 2 mM ATP Tris salt was utilized to start the reaction; this preparation was incubated for 20 min for 37°C. There action was terminated by adding 1ml of ice-cold trichloro acetic acid (10%v/v). The H⁺- K⁺ ATPase activity was assayed in the presence and the absence of different doses of the extract and omeprazole. The absorbance of inorganic phosphate (Pi) released from the hydrolysis of ATP was recorded at 820 nm within two hours and expressed as $\mu\text{g Pi/mg prot/min}$.

Percentage of enzyme inhibition was calculated by using the formula;

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

RESULTS**Percentage of Yield (%)**

S.NO	Name of the Plant	Percentage of inhibition (%)
1	<i>Alcea rosea</i>	1.5%w\w

Preparation of ethanolic extract of *Alcea rosea* by cold macretion method, that extract gives 1.5% w/w.

(i) PHYTOCHEMICAL ANALYSIS

Table 1: *Alcea rosea* -ARET Phytochemical Test
[- absence; + presence]

S.No	Phytochemical Test	Ethyl acetate Extract (Sample)	Ethyl acetate Extract (Aqueous)
1.	Alkaloids	-	-
2.	Flavonoids	+	-
3.	Tannins	+	+
4.	Phenols	-	-
5	Carbohydrates	+	+
6.	Proteins	-	+
7.	Steroids	+	-
8.	Saponins	-	-

Table2: *Alcea rosea* Compound (A and B)-ARET Phytochemical Study
[- absence; + presence]

S.No	Phytochemical Test	Compound A	Compound B
1.	Flavonoids	+	+

(ii) IN VITRO EVALUATION OF ANTIULCER ACTIVITY**1. Acid Neutralizing Capacity**

Table3: Effect of samples - Von Acid Neutralizing Capacity

S. no	Sample	Conc. (mg)	NaOH Used (mL)	Acid Consumed (mEq)	ANC (meq/g)
1	Control	-	35.6	4.4	-
2	Al(OH) ₃ +Mg(OH) ₂	250	15.7	24.3	97.2
		500	9.3	30.7	61.4
		1000	5.4	34.6	34.6
3	Sample-QAR	250	15.5	24.5	98.0
		500	12.2	27.8	55.6
		1000	10.4	29.6	29.6
4	Sample- ARET	250	19.4	20.6	82.4
		500	17.6	22.4	44.8

	1000	15.4	24.6	24.6
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The acid neutralizing capacity (ANC) study revealed that both standard antacids and the tested herbal samples exhibited significant acid neutralizing properties. Among the tested formulations:

- Sample QAR demonstrated the highest ANC per gram (98.0 meq/g) at 250 mg concentration, exceed in given the standard antacid (97.2meq/g), suggesting potent acid neutralizing potential.
- Sample ARET also showed substantial activity (82.4meq/gat250mg), though slightly lower than QAR.

2. H⁺/K⁺ - ATPase Inhibition Activity

Table 4: Effect of sample A on *In Vitro* H⁺/K⁺-ATPase Inhibition Activity

S. No	Sample Code	Concentration (µg/ml)	ODat820nm	Percent Inhibition (%)
1	Control		0.61	
2	Standard omeprazole	20	0.33	45.90
		40	0.29	52.45
		60	0.24	60.65
		80	0.21	65.57
		100	0.18	70.49
3	Sample QAR	20	0.34	44.26
		40	0.32	47.54
		60	0.29	52.45
		80	0.24	60.65
		100	0.21	65.57
4	Sample-ARET	20	0.49	19.67
		40	0.44	26.22
		60	0.41	32.78
		80	0.39	36.06
		100	0.35	42.62

Sample-QAR and ARET were tested for their ability to inhibit H⁺, K⁺-ATPase, with omeprazole serving as the reference standard. At a concentration of 100µg/ml, Sample-QAR and omeprazole demonstrated inhibition rates of 65.57% and 70.49%, respectively. Meanwhile, Sample-ARET exhibited a 42.62% inhibition rate, indicating that it has moderate activity.

Fig 1: Alcea Rosea ARET - Acid Neutralizing Capacity (ANC)



Fig 2: Alcea Rosea - ARET Phytochemical Test (Sample)

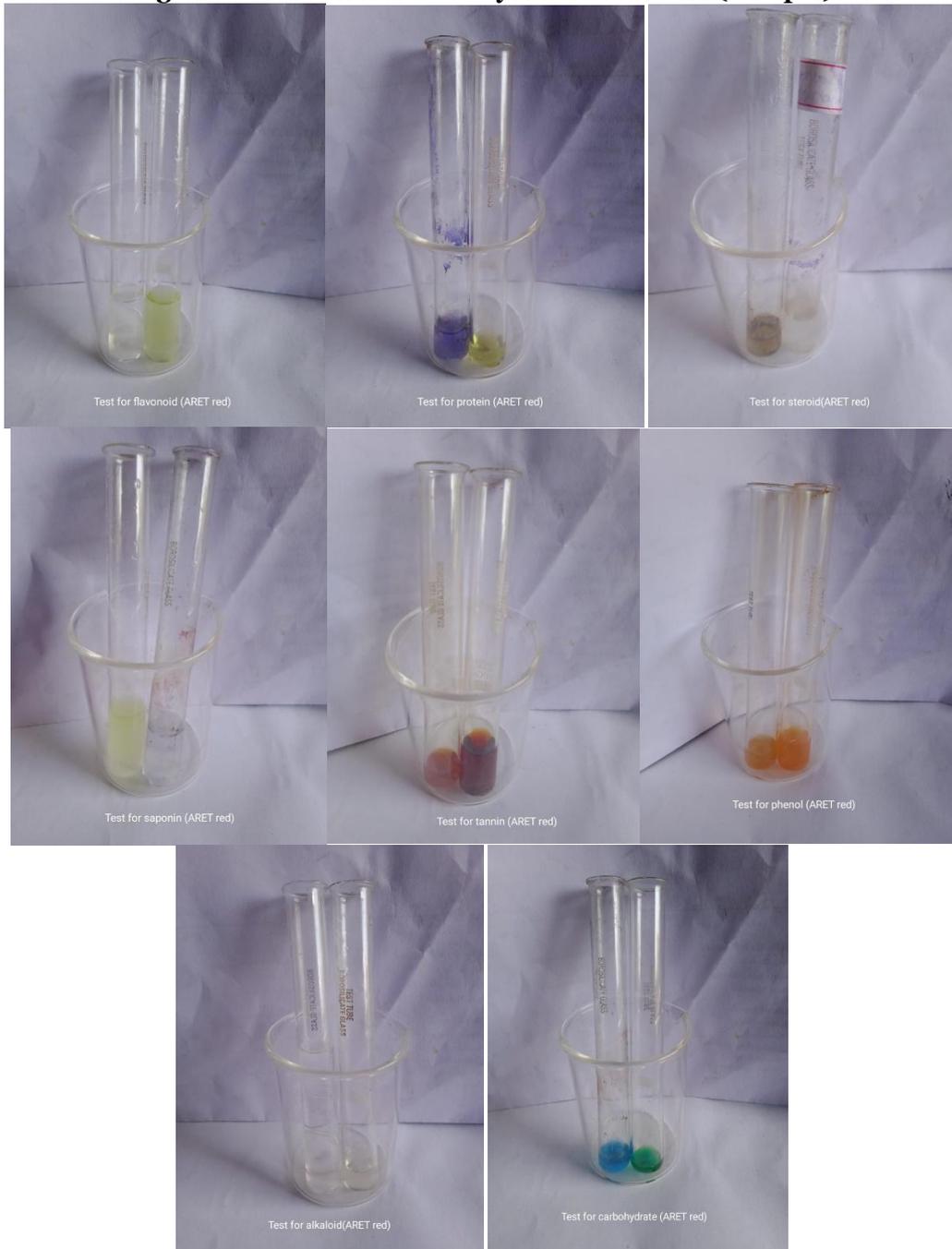
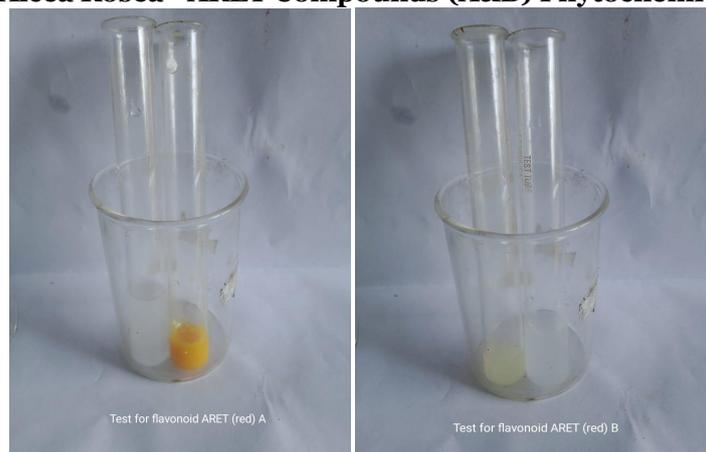


Fig 3: Alcea Rosea Aqueous - ARET Phytochemical Test





Fig 4: Alcea Rosea - ARET Compounds (A&B) Phytochemical Test



DISCUSSION

The exact cause of peptic ulcers is often unclear, but it is generally thought to result from a disruption between harmful factors and the body's natural defence mechanisms that protect the mucosal lining (Prasad K, et al. 2014). Acidity is a common gastrointestinal problem associated with a functional abnormality that can arise from various factors. Overproduction of stomach acid, also known as gastric acid (HCl), causes ulceration by inflaming the stomach lining (Houshia OJ et al 2012). Antacids reduce the stomach's pH by neutralising gastric acid (MI Thabrew and LDAM Arawwawala 2016). The capacity of an antacid to neutralise acid is referred to as its acid neutralising capacity (ANC), which is measured using a method known as back titration. Although they don't cause ulcers, stress and spicy meals can exacerbate them. *Helicobacter pylori*, a spiral-shaped bacterium that flourishes in the acidic conditions of the stomach, is associated with 70–90% of ulcer cases. Additionally, medications like aspirin and other NSAIDs can induce or exacerbate ulcers. The antiulcer action of several substances has been extensively studied pharmacologically. This study investigates the anti-peptic and gastroprotective effects of the most commonly utilised herbal remedies, along with the overall active compounds that have been documented. In this research, we have reviewed the literature on various medicinal plants known for their anti-ulcer properties (Maury, P.K., et al 2012). Relieving pain, healing the ulcer, and preventing recurrence are the ideal goals of peptic ulcer disease treatment. This article seeks to present details about various medicinal plants that can be used in both contemporary science and ayurveda for the prevention or management of peptic ulcers (Vimala, G. and Gricilda Shoba, F., 2014). In contrast, natural products rich in active metabolites have demonstrated the ability to enhance the quality of life for individuals suffering from ulcer disease by protecting the mucosa, mitigating ulcer impacts, inhibiting

pro-inflammatory factors, and reducing bacterial load. Because they lessen discomfort and lesions, natural extracts have shown promise in treating ulcers and other health issues. This study provides a summary of both preclinical and clinical research regarding medicinal plants, focusing on their effectiveness in treating conditions such as aphthous ulcers, ulcerative colitis, and peptic ulcers (Prayoga DK, et al 2024). Peptic Ulcer Disease (PUD), which encompasses both duodenal and gastric ulcers, is the most prevalent gastrointestinal disorder. The pathophysiology of PUD involves an imbalance between protective substances such as nitric oxide and growth factors, and harmful elements like acid and pepsin. The clinical assessment of antiulcer medications revealed adverse effects, tolerance, and a relapse rate that cast doubt on their effectiveness. It is evident that ulcers are often lessened when *Musa sapientum* aqueous extract is administered at the dosage employed in this investigation. More research should be done to examine its use in indigenous medicine from a scientific standpoint (Prabha P. et al., 2011).

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