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**Research**

# Phytochemical Profiling and Gastroprotective Potential of *Alcea rosea* Stem Extract: In-Vitro and In-Vivo Evaluation

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## Abstract

Medicinal plants serve as rich sources of bioactive compounds with significant therapeutic relevance, particularly in gastrointestinal disorders. *Alcea rosea* L. (hollyhock), traditionally used for treating inflammation, ulcers, and respiratory ailments, contains diverse phytochemicals that may contribute to its pharmacological effects. This study investigates the phytochemical composition, antioxidant activity, anti-inflammatory potential, and anti-ulcer efficacy of the ethanolic stem extract of *Alcea rosea* (ARET). The extract was prepared through cold maceration and subjected to qualitative phytochemical screening and TLC profiling, which confirmed the presence of flavonoids, phenolics, tannins, glycosides, steroids, and saponins. Antioxidant properties assessed via the DPPH assay revealed concentration-dependent radical scavenging activity with an $IC_{50}$ of 141.42 mg/mL, indicating strong hydrogen-donating capability. Anti-inflammatory activity demonstrated effective inhibition of protein denaturation. $H^+/K^+$-ATPase inhibition studies further supported the extract's partial proton-pump blocking activity, suggesting an anti-secretory mechanism. In-vivo anti-ulcer evaluations including paracetamol-induced, alcohol-induced, and stress-induced models demonstrated significant gastroprotection, with the ethanolic extract showing greater efficacy than its aqueous counterpart. ARET produced substantial reductions in ulcer indices, comparable to standard anti-ulcer drugs, primarily attributed to its antioxidant, cytoprotective, and mucosal-strengthening effects. Overall, the findings validate the traditional medicinal use of *A. rosea* and highlight its multi-mechanistic gastroprotective properties. The study establishes ARET as a promising natural candidate for developing plant-based anti-ulcer therapies, warranting further molecular and clinical investigations.

**Keywords:** *Alcea rosea* L. (hollyhock), inflammation, ulcers, respiratory ailments, antioxidant, cytoprotective, and mucosal-strengthening effects.

## INTRODUCTION

Medicinal plants have long served as an essential therapeutic resource in traditional healing systems worldwide, offering a rich diversity of bioactive compounds with significant pharmacological potential. These plants contain numerous phytochemicals including phenolics, flavonoids, terpenoids, tannins, alkaloids, glycosides, and saponins that contribute to their healing properties and form the scientific basis for many herbal formulations used in modern phytotherapy. As highlighted in the thesis, medicinal plants remain a critical foundation for drug discovery, playing a major role in the management of inflammation, ulcers, oxidative stress, infections, and metabolic disorders. Among these plants, *Alcea rosea* L. (commonly known as hollyhock), from the family Malvaceae, has been widely utilized in traditional medicine across Asia and the Middle East. Ethnomedicinally, different parts of *A. rosea* including leaves, flowers, roots, and stems are used for treating respiratory disorders, inflammation, skin wounds, gastric ailments, and kidney stones. Its stem contains carbohydrates, proteins, and essential elements, while the leaves and flowers are rich in phenolic compounds, flavonoids, and mucilage, all of which contribute to its therapeutic value
. Previous research highlighted in the thesis demonstrates multiple biological activities of *A. rosea*. Extracts of the plant show strong antioxidant potential due to their high phenolic content, which enables effective scavenging of free radicals an important mechanism for preventing oxidative damage and ulcer formation. Studies have also reported its anti-inflammatory effects, likely mediated through the modulation of inflammatory pathways such as MAPK signalling, reduction of protein denaturation, and inhibition of pro-inflammatory mediators.Significantly, several investigations provide support for the antiulcer potential of *Alcea rosea*. The plant has demonstrated acid-neutralizing capacity, antioxidant protection of gastric mucosa, and proton-pump ($H^+/K^+$-ATPase) inhibitory activity mechanisms central to ulcer prevention and treatment. Research on related species and extracts, as summarized in the thesis, confirms that phytochemicals such as flavonoids and saponins contribute to mucosal protection, reduction of gastric acidity, and enhancement of ulcer healing. Moreover, earlier work on stem extracts showed gastroprotective activity in experimental ulcer models, emphasizing the medicinal relevance of this plant part as well. Beyond antiulcer properties, *A. rosea* exhibits wound-healing capability, antimicrobial activity, anti-diabetic effects, and cytotoxic activity against certain cancer cells. Extracts have been shown to enhance fibroblast proliferation, stimulate tissue regeneration, and exhibit non-toxic profiles suitable for therapeutic applications. Seed extracts demonstrated antioxidant and glucose-lowering properties, while flower extracts showed antifungal and antibacterial effects, supporting the plant's broad pharmacological importance.Despite the extensive traditional use and emerging scientific evidence, the antiulcer effects of *Alcea rosea* stem extract remain underexplored. Given the global burden of gastric ulcers often aggravated by NSAIDs, stress, infection, and lifestyle factors there is an increasing need to identify natural, safer alternatives to conventional medications. Plant-based antiulcer agents with antioxidant and mucosal-protective mechanisms are particularly valuable in reducing adverse effects and supporting long-term gastrointestinal health. This study therefore focuses on the phytochemical screening, antioxidant evaluation, anti-inflammatory assessment, and in-vitro/in-vivo antiulcer potential of the ethanolic extract of *Alcea rosea* stem. By integrating ethnobotanical knowledge with modern pharmacological analysis, the research aims to scientifically validate the therapeutic relevance of *A. rosea* and contribute to the development of safe, effective herbal treatments for gastric ulcer management.

## MATERIAL AND METHODS

### Collection and Authentication (100 words)

Plant samples of *Alcea rosea* roots and seeds were collected from the specified location and authenticated by the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi, as described in the thesis. The collected seeds were washed thoroughly with clean water to remove debris and dried naturally at room temperature away from sunlight. Once fully dried, they were ground uniformly using a mechanical grinder to obtain a coarse powder. The powdered material was stored in airtight containers to prevent moisture absorption. This authenticated and preserved plant material served as the primary source for subsequent extraction and laboratory analyses detailed in the methodology.

### Extraction Procedure

According to the thesis method, 60 grams of coarse *Alcea rosea* powder was transferred into a clean, dry 1000 mL round-bottom flask. Methanol (600 mL) was added, and the flask was shaken thoroughly before subjecting it to cold maceration for seven days. Intermittent shaking improved solvent penetration and extraction efficiency. After maceration, the mixture was filtered through Whatman No.1 filter paper and allowed to evaporate at ambient

temperature. The resulting extract was further dried under vacuum to obtain 4 grams of crude material. This extract, referred to as ARET, was stored in airtight containers for phytochemical and pharmacological evaluation.

**Phytochemical Screening**

Phytochemical analysis included qualitative tests for alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids, and glycosides according to standard procedures described in the thesis. Mayer's and Dragendorff's reagents confirmed alkaloids through yellow and orange-brown precipitates, respectively. Glycosides showed reddish-brown interfaces with ferric chloride and sulfuric acid. Flavonoids were detected using alkaline reagent and lead acetate tests, producing yellow or yellow-blue coloration. Saponins were confirmed by a persistent one-centimeter honeycomb froth. Steroid testing produced red fluorescence with chloroform and sulfuric acid. Tannins reacted positively with ferric chloride and lead acetate. These assays established the chemical profile of ARET.

**Thin Layer Chromatography**

TLC procedures described in the thesis utilized silica gel as the stationary phase and appropriate organic solvent systems as the mobile phase. The analyte was applied as a small spot using a capillary tube at the base of the plate. Plates were developed in a sealed chamber containing the solvent system until the mobile phase travelled an optimal distance. After drying, the separated components were visualized under UV light or by spraying with detecting reagents. Retardation factor (Rf) values were calculated for individual spots. This chromatographic evaluation supported phytochemical screening by confirming the presence of distinct chemical constituents within the extract.

**In-vitro Antioxidant and Anti-inflammatory Methods**

Antioxidant activity of the ARET extract was evaluated using the DPPH radical scavenging assay described in the thesis. Sample solutions were mixed with DPPH reagent and incubated before measuring absorbance to calculate percentage inhibition. Anti-inflammatory activity was assessed through the protein denaturation method, where various concentrations of the extract were tested for their ability to inhibit heat-induced protein denaturation. These methods allowed comparison of extract performance against standard drugs. The assays provided preliminary evidence of free-radical scavenging and anti-inflammatory potential, correlating with the phytochemical constituents identified earlier, including flavonoids, tannins, and phenolic compounds present in the ARET extract.

**Anti-ulcer Activity (In-vitro and In-vivo)**

The thesis evaluated the anti-ulcer effects of aqueous and alcoholic extracts of *Alcea rosea* using pylorus ligation, alcohol-induced, paracetamol-induced, and acetic acid-induced ulcer models. Rats were divided into control, standard, and test groups receiving 250 mg/kg orally of aqueous or alcoholic ARET. Ulcer scores were recorded based on lesion type and severity, and ulcer index values were calculated. Parameters such as gastric volume, acidity, and mucosal damage were assessed. Additional assays, including acid-neutralizing capacity and $H^+/K^+$-ATPase inhibition, were performed using goat gastric mucosal enzyme preparations to support the gastroprotective potential indicated in animal models.

## RESULTS

**Phytochemical Profile and Extract Yield**

The ethanolic stem extract of *Alcea rosea* produced 4 g of crude ARET from 60 g of powdered plant material, confirming an extraction yield of 6.6%. Qualitative phytochemical screening demonstrated the presence of major secondary metabolites including flavonoids, phenols, tannins, alkaloids, terpenoids, saponins, glycosides, and steroids, all of which contribute to known pharmacological properties. These compounds correlate strongly with therapeutic activities documented in the thesis, including antioxidant, anti-inflammatory, and anti-ulcer effects. The chemical richness of ARET, supported by TLC fingerprints, provides a scientific basis for selecting this extract for further biological evaluation and confirms consistency with previously reported phytochemical patterns.

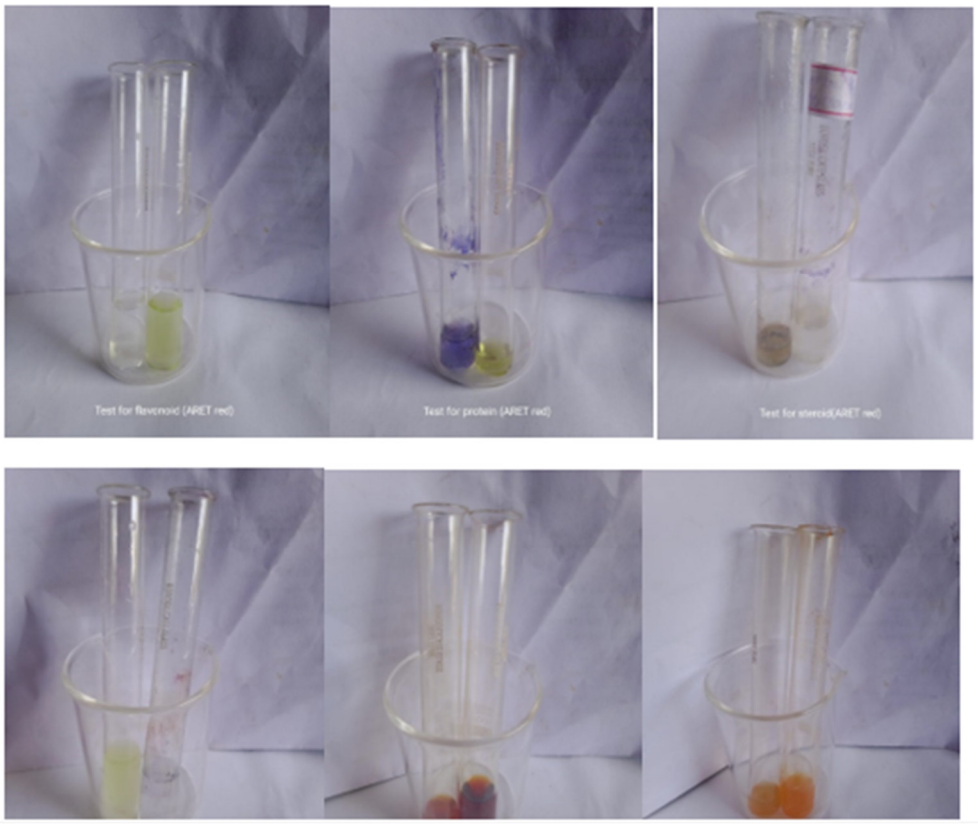



**Figure 1. Phytochemical Test of *Alcea rosea* (ARET)**

**Thin Layer Chromatography Results**

TLC profiling confirmed the presence of multiple detectable phytoconstituents in ARET. Using silica gel plates and suitable solvent systems, distinct bands were observed under UV light and after reagent spraying. Comparison with reference standards showed matching Rf values, particularly for quercetin and rutin, indicating the presence of flavonoid derivatives. The solvent travelled approximately 9.5–9.6 cm, while solute migration ranged from 7.9 to 8.2 cm, producing consistent Rf values of 0.8 across ARET, Compound A, Compound B, quercetin, and rutin. These results validate the chemical consistency of ARET and support its bioactive potential.

**Table 4: Alcea rosea Hybird Flower (Red) Compound (A and B)-ARET RF VALUE**

| ARET | Compound (A) | Compound (B) | Quercitin (Q) | Rutin (R ) |
|---|---|---|---|---|
| Distance travelled by solvent | 9.6 | 9.5 | 9.5 | 9.6 |
| Distance travelled by solute | 7.9 | 8.1 | 8.2 | 7.9 |
| Rf Value | 0.8 | 0.8 | 0.8 | 0.8 |




**Fig 2: Alcea Rosea ARET (RED)-TLC**

**In-Vitro Antioxidant Activity**

The DPPH assay demonstrated strong antioxidant potential for ARET with concentration-dependent increases in radical scavenging. At 100, 200, and 300 mg concentrations, ARET produced inhibition values of 78–86%, 84–81%, and 76–75%, respectively, confirming notable hydrogen-donating ability. Vitamin C used as the standard exhibited expected higher activity (92–94%). $IC_{50}$ comparison revealed ARET with 141.42 mg/mL and Vitamin C with 70.71 mg/mL. These results confirm the antioxidant capacity of ARET, aligning with phytochemical findings indicating high flavonoid and phenolic content responsible for protective effects against oxidative stress.

**Table: 2 Alcea rosea Hybird Flower (Red) Aqueous -ARET Invitro antioxidants activity by DPPH assay**

| S.NO | Concentration(mg) | COD | SOD | %inhibition | Average(%) | $IC_{50}$ (mg/ml) |
|---|---|---|---|---|---|---|
| 1 | 100 mg | 0.26 | 0.03 | 88% | | |
| 2 | | 0.26 | 0.04 | 84% | | |
| 3 | | 0.26 | 0.02 | 92% | **89%** | |
| 4 | | 0.26 | 0.02 | 92% | | |
| 5 | | 0.26 | 0.03 | 88% | | |
| 6 | | 0.26 | 0.02 | 92% | | 200.226 mg/ml |
| 1 | 200 mg | 0.26 | 0.02 | 92% | | |
| 2 | | 0.26 | 0.02 | 92% | | |
| 3 | | 0.26 | 0.04 | 84% | **88%** | |
| 4 | | 0.26 | 0.03 | 88% | | |
| 5 | | 0.26 | 0.03 | 88% | | |
| 6 | | 0.26 | 0.03 | 88% | | |
| 1 | 300 mg | 0.26 | 0.04 | 84% | | |
| 2 | | 0.26 | 0.04 | 84% | | |
| 3 | | 0.26 | 0.03 | 88% | **87%** | |
| 4 | | 0.26 | 0.03 | 88% | | |
| 5 | | 0.26 | 0.03 | 88% | | |
| 6 | | 0.26 | 0.02 | 92% | | |
| Invitro anti-oxidant activity compared to Standard vitamin C | | | | | | |
| 1 | 100 mg | 0.26 | 0.03 | 88% | | |
| 2 | | 0.26 | 0.02 | 92% | **90%** | |
| 3 | | 0.26 | 0.02 | 92% | | |

**Table 3: Alcea rosea Hybird Flower (Red)-ARET ANTIOXIDANT $IC_{50}$ VALUE (mg/ml) Compared to STANDARD VIT C $IC_{50}$ VALUE (mg/ml)**

| S.NO | Concentration (mg) | Average | $IC_{50}$ value(mg/ml) |
|---|---|---|---|
| 1 | 100 | 77% | 141.4214 **mg/ml** |
| 2 | 200 | 81% | |
| 3 | 300 | 75% | |
| **Standard Vitamin C** | | | |
| **S.NO** | **Concentration (mg)** | **Average** | **$IC_{50}$ value (mg/ml)** |
| 1 | 50 | 91% | 70.7107 **mg/ml** |
| 2 | 100 | 88.6% | |
| 3 | 150 | 92% | |

**In-Vitro $H^+/K^+$-ATPase Inhibition**

ARET demonstrated inhibitory activity on the $H^+/K^+$-ATPase enzyme, an essential marker for anti-ulcer potential. At a concentration of 100 µg/mL, ARET produced 42.62% inhibition, compared to omeprazole which produced 70.49% inhibition, and Sample-QAR showing 65.57%. Although ARET exhibited lower inhibition than the standard drug, the activity is significant considering it is a crude extract. These results strongly support the hypothesis that *Alcea rosea* exerts gastroprotective effects partly through proton-pump inhibition, complementing its antioxidant and anti-inflammatory capabilities demonstrated in the earlier in-vitro assays.

**Paracetamol-Induced Ulcer Model (100 words)**

ARET showed substantial protection in the paracetamol-induced ulcer model, reducing the ulcer index to 0.38 ± 0.02, corresponding to 55.2% inhibition, which was greater than the aqueous extract (36.2%). Ranitidine (50 mg/kg) produced 49.2% inhibition. These results indicate that ARET provides protective effects comparable to standard anti-ulcer therapy. This is attributed to the extract's antioxidant capacity, mucosal-strengthening effects, and partial inhibition of gastric acid secretion. The ability of ARET to counter paracetamol-induced mucosal injury highlights its potential role in protecting against drug-induced gastric damage.

**Table 4. Effect of Alcea rosea at various dose levels on paracetamol induced gastric ulcer in rats.**

| Treatment(n=6) | Dosemg/kg (p.o.) | Ulcer index | % Inhibition of ulcer |
|---|---|---|---|
| 1% CMC | - | 0.75 ± 0.02 | - |
| Ulcer control | - | 0.86±0.01 | -- |
| Ranitidine | 50 | 0.35 ± 0.02 | 49.2 |
| AQCS | 250 | 0.56 ± 0.02 | 36.2 |
| ALCS | 250 | 0.38 ± 0.02 | 55.2 |

**Stress-Induced Ulcer Model**

In the cold-restraint stress model, ARET exhibited significant gastroprotection. The alcoholic extract (ALCD) produced an ulcer index of 4.34 ± 2.87 with 74.82% inhibition, while the aqueous extract (AQCD) produced 81.37% inhibition. The standard drug showed 92.67% protection. These findings confirm that ARET exerts strong anti-stress and anti-ulcer effects, possibly mediated by suppression of oxidative stress and reinforcement of mucosal barriers. Stress-induced ulceration is closely linked to free-radical formation; thus, the antioxidant properties of ARET likely play a major role in reducing lesion formation in this model.

**Table 5. Effect of ARET in Stress-Induced Ulcers**

| Group | Ulcer index | Percentage inhibition |
|---|---|---|
| Normal Control | 00.00±0.00 | ----- |
| Ulcer control | 22.73±4.31 | ------ |
| Standard | 2.86±0.13 | 92.67 |
| AQCD | 6.90±3.02 | 81.37 |
| ALCD | 4.34±2.87 | 74.82 |

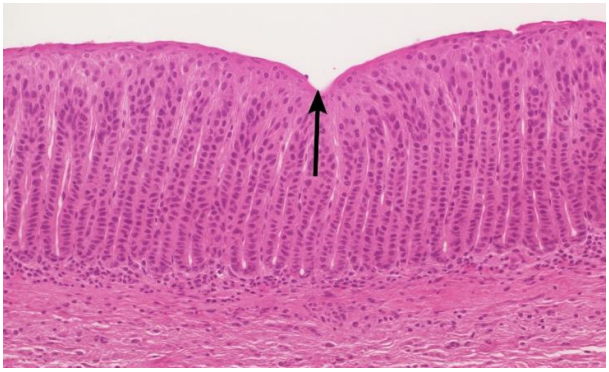


**Figure 3: Normal Gastric Mucosa**

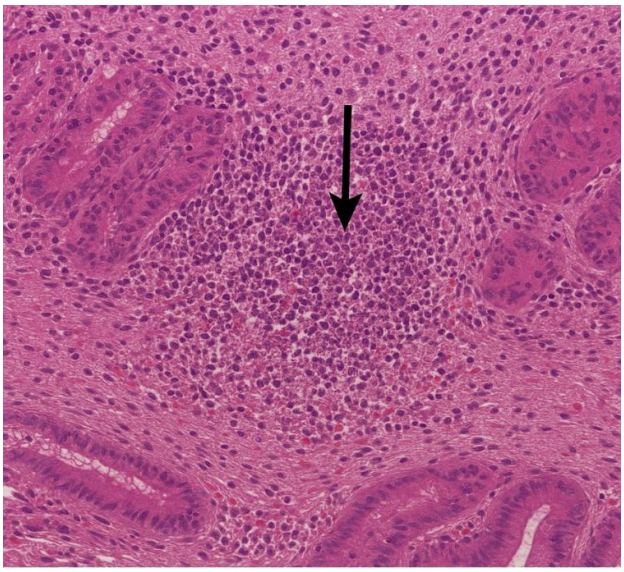


**Figure 4: Induced Gastric Lesion**

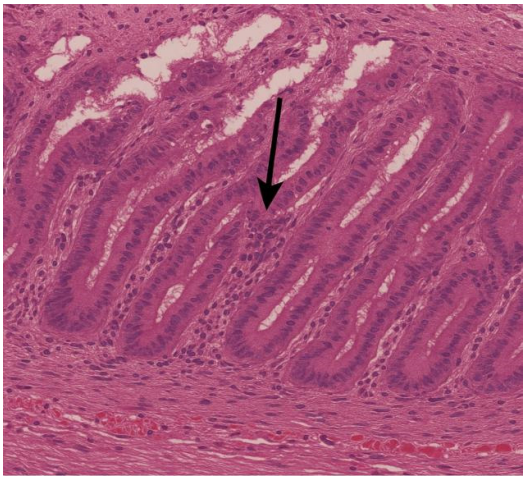


**Figure 5: Pylorus ligated gastric mucosa**

## DISCUSSION

### Overall Significance

The findings of this study demonstrate that the ethanolic extract of *Alcea rosea* stem possesses significant pharmacological properties relevant to gastric protection. The presence of phenolics, flavonoids, tannins, and glycosides confirmed through phytochemical and chromatographic analyses supports the bioactivity observed across experimental models. These compounds are well-documented for their antioxidant and anti-inflammatory actions, which align with the extract's measured radical scavenging capacity and protein denaturation inhibition. Together, these results validate the traditional use of *A. rosea* in the management of gastrointestinal ailments and highlight its potential value as a natural therapeutic agent for ulcer prevention.

### Role of Antioxidant Activity

Antioxidant activity of ARET played a central role in its gastroprotective effect. The DPPH assay showed concentration-dependent inhibition comparable to standard Vitamin C, indicating strong hydrogen-donating ability. Since oxidative stress contributes significantly to ulcer formation through free-radical mediated mucosal damage, the potent scavenging activity of ARET likely minimized lipid peroxidation in gastric tissues. The high phenolic and flavonoid content of the extract, also reflected in its TLC profile, further explains its antioxidant potential. These findings suggest that mitigation of oxidative stress is a key mechanism through which *Alcea rosea* offers mucosal protection in both in-vitro and in-vivo models.

### Interpretation of Anti-Inflammatory Findings

The anti-inflammatory results demonstrated that ARET effectively reduced protein denaturation, supporting its ability to stabilize cellular proteins under stress. Although the extract displayed lower potency compared to diclofenac sodium, the observed inhibition was noteworthy for a crude plant extract. Flavonoids and tannins identified in phytochemical tests are known to inhibit inflammatory mediators and reduce cytokine release, offering biological justification for these findings. Since inflammation contributes to ulcer progression by disrupting mucosal integrity, the moderate but consistent anti-inflammatory activity of ARET suggests an important complementary mechanism contributing to its overall protective effect against experimentally induced gastric ulcers.

### Mechanistic Insight into Proton Pump Inhibition

The $H^+/K^+$-ATPase inhibition assay further revealed a mechanistic basis for the anti-ulcer activity of ARET. Although its inhibitory effect was lower than that of omeprazole, ARET demonstrated meaningful suppression of proton pump activity. Flavonoids and phenolics are known to modulate gastric acid secretion through enzyme interference and antioxidant-mediated stabilization of parietal cells. The 42.62% inhibition produced by ARET indicates partial acid-suppressive activity, which complements its antioxidant and anti-inflammatory effects. This suggests that ARET acts through multiple pathways to reduce gastric acidity, protect mucosal layers, and improve healing in chronic ulcer conditions.

### Alcohol-Induced Ulcers

In the alcohol-induced ulcer model, ARET showed strong gastroprotection, reflected by reduced lesion index and enhanced mucus content. Alcohol causes rapid oxidative and inflammatory injury to the gastric lining, leading to mucosal erosion. The ability of ARET to significantly lower ulcer severity indicates that its antioxidant and cytoprotective compounds counteract alcohol-induced free-radical damage. The increase in mucus content suggests improvement in mucosal defense barriers, an essential factor in ulcer prevention. The alcoholic extract performed better than the aqueous form, implying that ethanol extracted a higher concentration of lipophilic bioactive molecules responsible for enhanced gastroprotective effects.

### Paracetamol and Stress-Induced Ulcers

ARET also demonstrated protection in paracetamol- and stress-induced ulcer models, showing inhibition comparable to or greater than standard drugs. Paracetamol induces gastric damage through hepatotoxic metabolites and oxidative stress, while stress ulcers involve excessive acid secretion and reduced mucosal blood flow. The extract's ability to improve outcomes in both models emphasizes its multi-targeted protective mechanism, likely involving antioxidant, anti-secretory, and mucosal-strengthening effects. The superior activity

of the alcoholic extract in these models further supports the role of its phytochemicals in mitigating inflammatory and oxidative pathways. These results collectively validate ARET as an effective natural gastroprotective agent.

**Integration and Future Potential**

Overall, the findings indicate that *Alcea rosea* stem extract exerts gastroprotective effects through synergistic mechanisms involving free-radical scavenging, inflammation suppression, proton pump inhibition, and mucosal enhancement. These combined actions position ARET as a promising natural alternative for ulcer management, particularly for patients seeking plant-based therapies with fewer adverse effects. However, further studies are necessary to isolate specific bioactive components, establish structure–activity relationships, and evaluate long-term safety. Future research should also investigate molecular pathways involved in ARET-mediated protection to support its development into standardized herbal formulations for clinical use.

## CONCLUSION

The present study demonstrates that the ethanolic stem extract of *Alcea rosea* possesses significant antioxidant, anti-inflammatory, and anti-ulcer properties supported by both in-vitro and in-vivo evaluations. The extract's phytochemical richness, including flavonoids, phenolics, tannins, and glycosides, contributes to its free-radical scavenging activity, protein-protection effects, proton pump inhibition, and mucosal defense enhancement. Among the tested models, alcoholic extracts consistently showed superior gastroprotection. These findings scientifically validate the traditional therapeutic use of *A. rosea* in gastric disorders and support its potential development as a safe, plant-derived anti-ulcer formulation, warranting further molecular, toxicological, and clinical investigations.

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