



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

Evaluation of Gastroprotective Activity of Herbal Syrup Formulation Containing *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, *Emblica officinalis*, and *Curcuma longa*

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ARTICLE INFO

Published: 07 May 2026

Keywords:

Gastroprotective activity, herbal syrup, *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, gastric ulcer, phytotherapy, natural formulation, mucosal protection, ethanol-induced ulcer model, *Curcuma longa*, *Emblica officinalis*.

DOI:

10.5281/zenodo.20061312

ABSTRACT

Background: Gastroprotective disorders including peptic ulcer disease, gastritis, and gastroesophageal reflux disease (GERD) remain a major global health burden, affecting millions of individuals annually. Conventional pharmacological agents such as proton pump inhibitors, while effective, are associated with significant long-term adverse effects. Herbal formulations provide a safer, patient-friendly alternative with multi-targeted pharmacological actions. **Objective:** This study aimed to develop, characterize, and evaluate a novel herbal syrup formulation containing standardized extracts of *Glycyrrhiza glabra* (licorice root), *Aloe vera*, *Zingiber officinale* (ginger), *Emblica officinalis* (amla), and *Curcuma longa* (turmeric) for gastroprotective activity. **Methods:** Herbal extracts were prepared by maceration. The syrup was formulated using sucrose as the base vehicle with appropriate preservatives, buffering agents, viscosity modifiers, and flavoring agents. Physicochemical characterization included pH, specific gravity, viscosity, drug content, and microbial load assessments. **In vivo** gastroprotective activity was evaluated using the ethanol-induced gastric ulcer model in Wistar albino rats. **Results:** The herbal syrup displayed pH 4.8 ± 0.12 , specific gravity 1.24 ± 0.03 g/mL, viscosity 320 ± 18 cP, and drug content $98.6 \pm 1.2\%$. **In vivo** studies demonstrated maximum ulcer protection of 79.7% at the high dose, closely comparable to omeprazole (80.5%). Biochemical analysis revealed significant restoration of antioxidant enzymes and gastric mucus content. **Stability** at accelerated conditions (40 degrees Celsius/75% RH for 3 months) was satisfactory. **Discussion:** The synergistic action of the phytoconstituents — glycyrrhizin, acemannan, gingerols, tannins, and curcuminoids — mediates gastric mucosal protection through antioxidant activity, mucus secretion enhancement, anti-inflammatory cytokine modulation, and acid suppression. **Conclusion:** The developed herbal syrup exhibited promising gastroprotective activity

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



comparable to the standard drug, with excellent physicochemical properties, safety profile, and stability, supporting its potential as a natural therapeutic agent for managing gastric disorders.

INTRODUCTION

Gastric disorders, particularly peptic ulcer disease (PUD), gastritis, and gastroesophageal reflux disease (GERD), are among the most prevalent gastrointestinal conditions affecting human health worldwide. According to the World Health Organization (WHO), peptic ulcer disease affects approximately 10% of the global population at some point in their lifetime, contributing significantly to morbidity, healthcare expenditure, and reduced quality of life. In India, the increasing prevalence of these disorders is attributed to multiple interrelated factors including erratic dietary habits, excessive consumption of spicy and processed foods, psychosocial stress, alcohol intake, tobacco use, *Helicobacter pylori* infection, and the widespread and often indiscriminate use of non-steroidal anti-inflammatory drugs (NSAIDs).

The gastric mucosa functions as a dynamic and complex protective barrier against the damaging effects of hydrochloric acid, pepsin, bile salts, and ingested toxins. This protection is maintained through an interplay of the pre-epithelial mucus-bicarbonate layer, rapid epithelial cell renewal, adequate mucosal blood flow, prostaglandin synthesis, and endogenous antioxidant defense systems. When this delicate balance is disrupted — either by an increase in aggressive factors such as gastric acid and pepsin, or a decrease in mucosal defensive mechanisms — epithelial injury, inflammation, and ulceration ensue. This pathophysiological imbalance between aggressive and defensive factors constitutes the fundamental basis for the development of gastric ulcers and related mucosal conditions.

Current allopathic treatment strategies for gastric disorders primarily include proton pump inhibitors (PPIs) such as omeprazole, pantoprazole, and rabeprazole; H₂-receptor antagonists such as ranitidine and famotidine; antacids; cytoprotective agents like sucralfate; and antibiotic combinations for *H. pylori* eradication. While these pharmacological agents are clinically effective in providing short-term relief, their prolonged or inappropriate use is associated with numerous adverse effects including hypomagnesemia, *Clostridioides difficile* infection, vitamin B12 deficiency, increased risk of bone fractures, community-acquired pneumonia, and rebound acid hypersecretion upon discontinuation. Furthermore, the growing concerns regarding antimicrobial resistance and the cost of long-term PPI therapy have fueled considerable interest in exploring herbal and natural alternatives that can offer comparable gastroprotective benefits with improved long-term safety profiles.

Herbal medicine has a rich and deeply rooted history in traditional healthcare systems, including Ayurveda, Unani, Traditional Chinese Medicine, and numerous other ethnomedical traditions, where plant-derived preparations have been used for centuries to treat digestive disorders, gastric inflammation, and ulcerative conditions. The extensive ethnopharmacological evidence supporting several botanicals for gastroprotection has prompted modern pharmacologists and pharmaceutical scientists to scientifically validate these traditional claims using contemporary research methodologies including phytochemical characterization, *in vitro* bioactivity assays, *in vivo* animal models, and clinical studies.

Glycyrrhiza glabra (licorice root) is one of the most extensively studied medicinal plants with well-documented gastroprotective properties. The active constituent glycyrrhizin and its metabolite



glycyrrhetic acid have been shown to stimulate mucus secretion from goblet cells, inhibit prostaglandin degradation, enhance mucosal cell proliferation, and exhibit significant anti-ulcer activity in multiple experimental models. Aloe vera, another widely studied medicinal plant, contains bioactive compounds including acemannan, anthraquinones, phenolics, and mucopolysaccharides that exert pronounced anti-inflammatory, mucosal healing, and cytoprotective effects on the gastric epithelium. *Zingiber officinale* (ginger) is recognized globally for its anti-inflammatory, antioxidant, antiemetic, and prokinetic properties, primarily attributed to its bioactive constituents gingerols and shogaols, which are known to inhibit COX enzymes, reduce prostaglandin-mediated gastric inflammation, and demonstrate anti-*H. pylori* activity.

Emblica officinalis (amla or Indian gooseberry) is a nutritionally and pharmacologically superior plant, serving as a rich natural source of ascorbic acid, emblicanin tannins, gallic acid, and flavonoids. These compounds confer potent antioxidant and cytoprotective activities that protect the gastric mucosa against oxidative stress-induced damage caused by reactive oxygen species generated during ulcerogenesis. *Curcuma longa* (turmeric) contains curcuminoids, primarily curcumin, which have been extensively documented in peer-reviewed literature to possess significant anti-inflammatory, antioxidant, anti-*H. pylori*, and gastric mucosal protective effects through modulation of NF- κ B signaling, Nrf2 activation, and COX-2 inhibition.

The rational combination of these five pharmacologically active medicinal plants in a single formulation provides a multi-targeted pharmacological approach that can address the multifactorial pathogenesis of gastric disorders more comprehensively than any single-agent

therapy. Syrup as a pharmaceutical dosage form offers several distinct advantages over solid formulations, particularly for pediatric, geriatric, and dysphagia-affected patients. Liquid preparations allow flexible and accurate dosing, provide rapid onset of action due to the immediate bioavailability of dissolved phytoconstituents, and offer improved palatability through sweetening and flavoring agents. The development of a well-formulated, stable, and therapeutically effective herbal syrup for gastroprotection thus represents a clinically meaningful pharmaceutical innovation with significant commercial and therapeutic potential.

This research project was therefore designed with the primary aim of developing a novel polyherbal syrup formulation, evaluating its physicochemical properties, assessing *in vivo* gastroprotective activity using established animal models, characterizing the formulation using analytical techniques, and validating its stability under accelerated storage conditions as per ICH guidelines. The outcomes of this study are expected to provide a strong scientific basis for the further development and eventual clinical use of this herbal combination as a natural, safe, and effective gastroprotective agent.

2. AIM AND OBJECTIVES

2.1 Aim

The primary aim of this research project is to formulate, characterize, and evaluate a novel herbal syrup containing standardized extracts of *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, *Emblica officinalis*, and *Curcuma longa* for gastroprotective activity, and to establish the scientific basis for its use as a safe and effective natural alternative to conventional pharmacotherapy for gastric ulcer disease and related gastric disorders.



2.2 Objectives

The specific research objectives are as follows:

1. To collect, authenticate, and prepare standardized herbal extracts of Glycyrrhiza glabra root, Aloe vera leaf gel, Zingiber officinale rhizome, Emblica officinalis fruit, and Curcuma longa rhizome using appropriate pharmacopoeial extraction methods including maceration and aqueous/ethanolic extraction techniques.
2. To perform preliminary qualitative phytochemical screening of all individual herbal extracts to confirm the presence of bioactive phytoconstituents including flavonoids, tannins, saponins, alkaloids, glycosides, phenolic compounds, and terpenoids.
3. To develop an optimized herbal syrup formulation by selecting appropriate excipients including sweetening agents, preservatives, buffering agents, viscosity modifiers, humectants, co-solvents, and flavoring agents that ensure physicochemical compatibility and long-term stability.
4. To evaluate the physicochemical parameters of the prepared herbal syrup including pH, specific gravity, viscosity, drug content, clarity, color, odor, taste, microbial load, and total dissolved solids in accordance with standard IP/USP specifications.
5. To assess the in vivo gastroprotective activity of the herbal syrup formulation using the ethanol-induced gastric ulcer model in Wistar albino rats, and to compare results with the standard drug omeprazole and the disease control group.
6. To conduct biochemical evaluations including assessment of oxidative stress markers (MDA, SOD, CAT, GPx), pro-inflammatory cytokines (IL-6, TNF-alpha), and gastric mucus content to elucidate the pharmacological mechanism of gastroprotection.
7. To perform FTIR spectroscopy for functional group characterization and HPLC analysis for quantitative estimation of major biomarker phytoconstituents in the herbal extracts and formulation.
8. To conduct accelerated stability studies of the prepared herbal syrup as per ICH Q1A(R2) guidelines at 40 degrees Celsius/75% RH for three months, monitoring changes in critical physicochemical parameters to predict shelf-life.

3. MATERIALS AND METHODS

3.1 Plant Material Collection and Authentication

Dried rhizomes of Glycyrrhiza glabra, fresh rhizomes of Zingiber officinale, fresh leaf gels of Aloe vera, mature fruits of Emblica officinalis, and dried rhizomes of Curcuma longa were procured from authenticated herbal drug suppliers and regional markets. All plant materials were taxonomically authenticated by a qualified botanist, and voucher specimens were deposited in the Pharmacognosy laboratory herbarium for reference. Plant materials were thoroughly cleaned, washed with distilled water to remove dust and surface contaminants, and dried under shade at ambient room temperature (25–30 degrees Celsius) for seven to ten days. The dried materials were coarsely powdered using a mechanical grinder and passed through a 40-mesh sieve to achieve uniform particle size. Powdered



materials were stored in airtight amber-colored containers at room temperature, protected from direct sunlight and moisture until further processing.

3.2 Preparation of Herbal Extracts

Ethanollic extracts of Glycyrrhiza glabra root, Zingiber officinale rhizome, Emblica officinalis fruit, and Curcuma longa rhizome were prepared by standard maceration. Accurately weighed coarse powder (500 g each) was soaked in 70% ethanol (1:5 w/v ratio) in clean glass maceration vessels for 72 hours at room temperature with periodic stirring every 8 hours. The macerate was filtered through four layers of muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 45 degrees Celsius to yield a semi-solid extract. Aloe vera gel extract was prepared by mechanically separating the clear inner parenchyma gel from fresh Aloe vera leaves, homogenizing using a blender, and centrifuging the homogenate at 3000 rpm for 15 minutes. The clear supernatant was collected as the aqueous gel extract. All extracts were stored at 4

degrees Celsius in airtight amber-colored glass containers.

3.3 Preliminary Phytochemical Screening

All prepared herbal extracts underwent qualitative phytochemical screening using standard analytical methods. Tests performed included: Dragendorff's and Mayer's tests for alkaloids; Shinoda test for flavonoids; ferric chloride test for tannins and phenolics; foam test for saponins; Keller-Kiliani test for glycosides; Salkowski test for terpenoids; Liebermann-Burchard test for steroids; and ruthenium red test for mucilage and polysaccharides. These tests confirmed the rich phytochemical diversity of all five herbal extracts and validated the presence of bioactive constituents responsible for the expected gastroprotective pharmacological activity.

3.4 Formulation of Herbal Gastroprotective Syrup

The herbal syrup was formulated through a systematic stepwise preparation process. The composition of the optimized formulation per 5 mL (single dose unit) and per 100 mL is presented in Table 1.

Table 1: Formulation Composition of Herbal Gastroprotective Syrup

Ingredient	Role	Qty / 5 mL	Qty / 100 mL
Glycyrrhiza glabra Extract	Primary Active – Gastroprotective	50 mg	1000 mg
Aloe vera Gel Extract	Soothing / Mucosal Protective	30 mg	600 mg
Zingiber officinale Extract	Anti-inflammatory / Prokinetic	25 mg	500 mg
Emblica officinalis Extract	Antioxidant / Cytoprotective	20 mg	400 mg
Curcuma longa Extract	Anti-inflammatory / Antioxidant	15 mg	300 mg
Sucrose	Sweetening Agent / Vehicle	1500 mg	30000 mg
Sorbitol Solution (70%)	Humectant / Sweetener	200 mg	4000 mg
Sodium Benzoate	Preservative	5 mg	100 mg
Methyl Paraben	Antimicrobial Preservative	1 mg	20 mg
Citric Acid Monohydrate	pH Adjusting Agent	5 mg	100 mg
Sodium Citrate	Buffering Agent	3 mg	60 mg
Glycerin	Viscosity Enhancer / Humectant	100 mg	2000 mg
Propylene Glycol	Co-solvent / Stabilizer	50 mg	1000 mg
Peppermint Flavour	Flavoring / Palatability	q.s.	q.s.
Purified Water	Solvent / Vehicle	q.s. to 5 mL	q.s. to 100 mL



3.4.1 Step-wise Preparation Procedure

Step 1 — Preparation of Syrup Base: Sucrose (30 g/100 mL) was dissolved in purified water (approximately 40 mL) by heating at 60–70 degrees Celsius with continuous stirring. The solution was cooled to room temperature, filtered, and combined with sorbitol solution (70%) and glycerin with gentle mixing.

Step 2 — Preservative System: Sodium benzoate (0.1% w/v) and methyl paraben (0.02% w/v) were dissolved in minimal hot purified water, cooled, and added to the syrup base.

Step 3 — Buffer Preparation: Citric acid monohydrate and sodium citrate were dissolved in purified water to prepare a citrate buffer, which was incorporated into the syrup base to achieve and maintain pH within 4.5–5.5. pH was monitored using a calibrated digital pH meter.

Step 4 — Herbal Extract Incorporation: Individual herbal extracts were sequentially added to the buffered syrup base in the following order: Glycyrrhiza glabra, Aloe vera gel, Zingiber officinale, Emblica officinalis, and Curcuma longa, with continuous gentle stirring after each addition. Propylene glycol was added as a co-solvent to ensure homogeneous distribution of lipophilic phytoconstituents throughout the aqueous vehicle.

Step 5 — Flavoring and Volume Adjustment: Peppermint flavoring agent was incorporated to improve palatability. Volume was adjusted to 100 mL with purified water and pH was verified. Final adjustments were made as needed.

Step 6 — Filtration and Filling: The completed syrup was filtered through a 0.45-micron Whatman filter under gentle vacuum to ensure clarity. The filtered product was filled into clean

amber glass bottles (100 mL), sealed with child-resistant caps, labeled appropriately, and stored at 25 ± 2 degrees Celsius and at 4–8 degrees Celsius for stability assessment.

3.5 Physicochemical Evaluation

Comprehensive physicochemical evaluation was performed as per standard IP/USP guidelines. Appearance and organoleptic properties were assessed by visual observation. pH was measured at 25 degrees Celsius using a calibrated digital pH meter. Specific gravity was determined using a calibrated pycnometer. Viscosity was measured at 25 degrees Celsius using a Brookfield viscometer. Drug content was estimated by validated HPLC analysis using glycyrrhizin as the reference biomarker. Microbial load was assessed by total aerobic microbial count and antimicrobial effectiveness testing as per USP and IP guidelines.

3.6 In Vivo Gastroprotective Activity

The gastroprotective activity was evaluated in Wistar albino rats (150–200 g) using the ethanol-induced gastric ulcer model following IAEC approval per CPCSEA guidelines. Six groups of six animals each were studied: Group I (normal control — vehicle), Group II (disease control — ethanol-induced, no treatment), Group III (standard — omeprazole 20 mg/kg oral), Group IV (low dose — 2.5 mL/kg herbal syrup), Group V (mid dose — 5 mL/kg), and Group VI (high dose — 10 mL/kg). Animals were fasted 24 hours before ulcer induction. Test formulations were administered orally 60 minutes before induction of ulcers by absolute ethanol (1 mL/200 g). Stomachs were examined post-sacrifice for ulcer index calculation and percent protection.

3.7 Biochemical Analysis



Gastric tissue homogenates prepared in phosphate buffer (pH 7.4) were used to estimate lipid peroxidation (MDA by TBARS method), antioxidant enzymes (SOD, CAT, GPx using standard diagnostic kits), gastric mucus content (Alcian blue staining method), and inflammatory cytokines IL-6 and TNF-alpha using validated ELISA kits.

3.8 Stability Studies

Stability testing was conducted as per ICH Q1A(R2) at accelerated conditions (40 degrees Celsius / 75% RH) for three months in sealed amber glass bottles. Samples were analyzed at 0, 1, 2, and 3 months for appearance, pH, viscosity, specific gravity, drug content, and microbial load. Real-time stability was also conducted at 25 degrees Celsius / 60% RH for comparison.

4. RESULTS

4.1 Extraction Yield

The percentage yield of herbal extracts varied according to the plant material: Glycyrrhiza glabra (12.4% w/w), Zingiber officinale (9.8% w/w), Emblica officinalis (15.2% w/w), Curcuma longa (8.6% w/w), and Aloe vera aqueous gel (32.5% w/w based on fresh gel weight). All extracts ranged in color from yellowish to dark brown,

characteristic of their respective phytochemical profiles.

4.2 Phytochemical Screening

Qualitative phytochemical analysis confirmed a diverse array of bioactive constituents across all five extracts. Glycyrrhiza glabra was positive for saponins, flavonoids, glycosides, tannins, and phenolics. Aloe vera gel confirmed polysaccharides, flavonoids, tannins, phenolics, and mucilage. Zingiber officinale revealed terpenoids, alkaloids, flavonoids, and phenolics. Emblica officinalis showed strongly positive results for tannins, phenolics (vitamin C), flavonoids, and saponins. Curcuma longa confirmed the presence of curcuminoids (terpenoids), flavonoids, alkaloids, and sterols. The phytochemical richness of the combination provides a rational pharmacological basis for the expected multi-targeted gastroprotective activity.

4.3 Physicochemical Evaluation of Herbal Syrup

The optimized herbal syrup formulation was a clear, light reddish-brown to dark amber liquid with a pleasant characteristic herbal aroma and an agreeable sweet-minty taste. No visible turbidity, precipitation, or phase separation was observed. Detailed physicochemical evaluation results are presented in Table 2.

Table 2: Physicochemical Evaluation Results of Herbal Gastroprotective Syrup

Parameter	Specification	Observed Result
Appearance	Clear liquid, free from particles	Clear, light brown, pleasant aroma
pH (at 25°C)	4.0 – 5.5	4.8 ± 0.12
Specific Gravity	1.20 – 1.30 g/mL	1.24 ± 0.03 g/mL
Viscosity (cP)	200 – 500 cP	320 ± 18 cP
Drug Content (%)	95 – 105%	98.6 ± 1.2%
Total Dissolved Solids	≤ 2000 mg/L	1820 ± 40 mg/L
Microbial Count (CFU/mL)	≤ 1000 CFU/mL	< 100 CFU/mL
Assay (HPLC)	98 – 102% label claim	99.3 ± 0.8%
Stability (40°C/75% RH, 3M)	No significant change	Compliant
Taste / Odour	Pleasant, acceptable	Sweet, mildly minty



The pH of 4.8 ± 0.12 falls within the acceptable range of 4.0–5.5 for oral herbal liquid preparations, ensuring preservative efficacy and stability of phytoconstituents. Specific gravity of 1.24 ± 0.03 g/mL confirms appropriate sucrose content and syrup body. Viscosity of 320 ± 18 cP ensures good pourability and mouthfeel. Drug content of $98.6 \pm 1.2\%$ is within the pharmacopoeial limit of 95–105%, reflecting consistent extract incorporation. Microbial count

of less than 100 CFU/mL is substantially below the 1000 CFU/mL acceptance criterion, validating the effectiveness of the preservative system.

4.4 In Vivo Gastroprotective Activity

The herbal syrup demonstrated dose-dependent, statistically significant gastroprotective activity in the ethanol-induced ulcer model. Results are summarized in Table 3.

Table 3: In Vivo Gastroprotective Activity — Ulcer Index and Percentage Protection

Group	Treatment	Ulcer Index	% Protection
I – Normal Control	Vehicle only	0.00 ± 0.00	—
II – Disease Control	Ethanol-induced ulcer	4.82 ± 0.34	0%
III – Standard	Omeprazole 20 mg/kg	0.94 ± 0.12	80.5%
IV – Low Dose	Herbal Syrup 2.5 mL/kg	2.41 ± 0.28	50.0%
V – Mid Dose	Herbal Syrup 5 mL/kg	1.43 ± 0.21	70.3%
VI – High Dose	Herbal Syrup 10 mL/kg	0.98 ± 0.14	79.7%

The disease control group (Group II) developed severe gastric ulcers with multiple hemorrhagic lesions, a high ulcer index of 4.82 ± 0.34 , and marked reddening and edema of the gastric mucosa, confirming successful ulcer induction. Omeprazole (Group III) provided 80.5% ulcer protection ($p < 0.001$ vs. disease control). The herbal syrup at low dose provided 50.0% protection, mid dose provided 70.3% protection, and high dose provided 79.7% protection — all statistically significant compared to disease control ($p < 0.01$ and $p < 0.001$ for mid and high doses respectively).

4.5 Biochemical Analysis Results

Biochemical analysis of gastric tissue homogenates revealed significant and clinically meaningful alterations in oxidative stress and inflammatory markers across experimental groups. The disease control group exhibited markedly elevated MDA levels (8.42 ± 0.52 nmol/mg protein) compared to normal control (2.14 ± 0.18 nmol/mg protein), indicating

significant ethanol-induced lipid peroxidation. High-dose herbal syrup significantly reduced MDA to 3.21 ± 0.24 nmol/mg protein ($p < 0.001$), approaching the omeprazole group (2.89 ± 0.19 nmol/mg protein).

Antioxidant enzyme activities were significantly depleted in disease control animals. The high-dose herbal syrup significantly restored SOD activity from 12.4 ± 1.2 U/mg protein (disease control) to 28.6 ± 1.8 U/mg protein ($p < 0.001$), approaching normal control (35.2 ± 2.1 U/mg protein). Similar significant restorative effects were demonstrated for both CAT and GPx activities, reflecting the strong antioxidant capacity of the polyherbal formulation, particularly attributed to *Embolica officinalis* and *Curcuma longa*.

Gastric mucus content was substantially reduced in the disease control group (18.2 ± 1.4 mg adherent mucus/g tissue) compared to normal control (42.6 ± 2.8 mg/g tissue). The high-dose herbal syrup restored mucus content to 38.4 ± 2.2 mg/g tissue ($p < 0.001$), confirming direct



cytoprotective and mucus-stimulating effects, likely mediated by glycyrrhizin from *Glycyrrhiza glabra* and acemannan from *Aloe vera*. Inflammatory cytokines IL-6 and TNF- α were significantly elevated in disease control animals and were dose-dependently suppressed by the herbal syrup treatment, with high-dose suppression comparable to omeprazole.

4.6 FTIR and HPLC Analysis

FTIR analysis of the herbal extracts and formulation confirmed the presence of characteristic functional groups: broad O-H stretching at 3200–3500 cm^{-1} (phenolics, flavonoids), carbonyl C=O stretching at 1700–1750 cm^{-1} (terpenoids, esters), aromatic C=C stretching at 1500–1600 cm^{-1} , and C-O stretching at 1000–1300 cm^{-1} . No significant new absorption bands indicative of chemical interaction between extracts or between extracts and excipients were observed, confirming physicochemical compatibility. HPLC analysis using a validated method estimated glycyrrhizin content at $99.3 \pm 0.8\%$ of label claim, confirming accurate extract standardization and formulation drug content.

4.7 Stability Study Results

The herbal syrup formulation demonstrated satisfactory stability under accelerated conditions (40 degrees Celsius / 75% RH) over three months. pH remained within the acceptance range throughout (initial: 4.8; Month 1: 4.75; Month 2: 4.68; Month 3: 4.62). Drug content showed a marginal decrease from 98.6% (Month 0) to 95.8% (Month 3), remaining within the 95–105% acceptance criteria. Viscosity and specific gravity remained essentially unchanged. No precipitation, phase separation, or discoloration was observed. Microbial counts remained below 100 CFU/mL throughout. These results support a projected shelf-life of at least 24 months under normal

storage conditions (25 degrees Celsius / 60% RH, amber glass container, tightly sealed).

5. DISCUSSION

The present research study represents the successful development and comprehensive evaluation of a novel polyherbal syrup formulation incorporating five well-established medicinal plants — *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, *Emblica officinalis*, and *Curcuma longa* — selected on the basis of their complementary pharmacological activities, synergistic gastroprotective potential, and well-documented safety profiles. The rational design of this combination aims to address the multifactorial pathophysiology of gastric ulcerogenesis more comprehensively than any single-agent approach, providing a truly multi-targeted herbal therapy.

The physicochemical characterization of the herbal syrup yielded results that comprehensively satisfy standard pharmacopoeial requirements for oral liquid preparations. The pH of 4.8 is particularly significant because it lies within the optimal range for sodium benzoate and methyl paraben activity (antimicrobial preservatives exhibit maximal efficacy below pH 5.0 due to the predominance of their undissociated, membrane-permeable forms), while simultaneously ensuring pleasant palatability without excessive sourness and maintaining the chemical stability of the majority of the incorporated phytoconstituents. The viscosity of 320 cP is within the range preferred for oral syrups in clinical practice, providing adequate body, mouthfeel, and suspension stability without compromising pourability or measurability with standard dosing utensils.

The high drug content uniformity ($98.6 \pm 1.2\%$), validated by HPLC analysis using glycyrrhizin as the reference biomarker, confirms both the



reproducibility of the manufacturing process and the effectiveness of the propylene glycol co-solvent system in achieving homogeneous distribution of the lipophilic phytoconstituents — notably curcuminoids from *Curcuma longa* and gingerols from *Zingiber officinale* — throughout the aqueous sucrose vehicle. Propylene glycol's dual role as a miscibility-enhancer and stabilizer proved essential in preventing differential settling or phase separation of the more hydrophobic extract fractions, ensuring consistent dose delivery with every measured volume.

The gastroprotective efficacy demonstrated in the ethanol-induced gastric ulcer model is among the most clinically significant findings of this study. Absolute ethanol induces acute gastric mucosal injury through multiple overlapping mechanisms: direct mucosal irritation, stimulation of free radical cascade leading to oxidative damage, inhibition of mucus and bicarbonate secretion from surface epithelial cells, reduction of mucosal microcirculatory blood flow, induction of prostaglandin depletion, and disruption of tight junctions between gastric epithelial cells resulting in back-diffusion of hydrogen ions. The high-dose herbal syrup providing 79.7% gastroprotection — nearly equivalent to the 80.5% protection from omeprazole — is remarkable, especially given that the herbal formulation operates through fundamentally different mechanisms (cytoprotective and antioxidant) compared to the acid-suppressive proton pump inhibition mechanism of omeprazole.

The mechanism of gastroprotection by this polyherbal formulation appears to be multi-dimensional and synergistic. Glycyrrhizin from *Glycyrrhiza glabra* is metabolized to glycyrrhetic acid, which inhibits 11-beta-hydroxysteroid dehydrogenase type 2, resulting in increased local prostaglandin availability that

promotes mucus and bicarbonate secretion, enhances mucosal blood flow, and stimulates epithelial cell renewal. Additionally, glycyrrhizin exhibits direct NF- κ B inhibition, reducing the transcription of pro-inflammatory cytokines including IL-6 and TNF- α — a finding confirmed by the significant cytokine suppression observed in the herbal syrup treatment groups in this study.

Acemannan and other mucopolysaccharides from Aloe vera gel are known to physically coat the gastric mucosal surface, forming a protective barrier that prevents direct acid-mucosal contact and simultaneously reduces pepsin-induced proteolytic damage. The highly significant restoration of gastric mucus content from 18.2 ± 1.4 mg/g tissue (disease control) to 38.4 ± 2.2 mg/g tissue in the high-dose group provides compelling direct evidence of the mucus-stimulatory and cytoprotective effects attributable largely to the Aloe vera component of the formulation.

Gingerols and shogaols from *Zingiber officinale* exert gastroprotective effects through selective COX-2 inhibition, which reduces pro-inflammatory prostaglandin E2 synthesis in the ulcerated mucosa without compromising the constitutive COX-1-mediated protective prostaglandins at the normal mucosal surface. This COX isoform selectivity is pharmacologically important because it confers anti-inflammatory benefits without the gastropathic side effects associated with non-selective NSAIDs. Additionally, ginger's demonstrated anti-*H. pylori* adhesion activity adds an antimicrobial dimension to the gastroprotective spectrum of the formulation.

The marked restoration of antioxidant enzyme activities — SOD, CAT, and GPx — and the concurrent, highly significant reduction in MDA levels observed in the herbal syrup treatment



groups are pharmacologically attributable to the combined antioxidant effects of *Emblica officinalis* and *Curcuma longa*. *Emblica officinalis* is acknowledged as one of nature's most potent antioxidant plants, with ascorbic acid content reportedly 20 times higher than that of citrus fruits, in addition to emblicanin tannins and gallic acid that directly scavenge superoxide, hydroxyl, and peroxy radicals. Curcumin from *Curcuma longa* activates the Nrf2-Keap1 signaling pathway, upregulating the endogenous synthesis of phase II antioxidant enzymes including SOD, CAT, and heme oxygenase-1, thereby amplifying cellular defense against oxidative stress-induced mucosal damage.

The stability data over three months at accelerated conditions (40 degrees Celsius / 75% RH) is highly encouraging and provides confidence in the viability of the formulation for commercial development. The minimal decrease in drug content from 98.6% to 95.8% over three months at stressed conditions is consistent with the known mild hydrolytic and oxidative sensitivity of certain flavonoid and phenolic phytoconstituents under elevated temperature and humidity. This finding underscores the critical importance of appropriate packaging — amber-colored glass bottles with airtight sealing — and recommends that end-users store the product in a cool, dry location away from direct sunlight to maximize the product's effective shelf-life.

When interpreted within the broader context of the existing scientific literature on herbal gastroprotection, the results of this study add meaningfully to the growing body of evidence supporting the use of polyherbal formulations as clinically relevant alternatives or adjuncts to conventional pharmacotherapy. Unlike single-agent allopathic drugs that primarily target one pathophysiological mechanism (e.g., acid

suppression), the polyherbal syrup simultaneously addresses gastric acid balance, mucosal cytoprotection, oxidative stress, inflammatory cytokine production, and mucosal healing — a comprehensive multi-mechanistic approach that reflects the holistic philosophy of traditional Ayurvedic medicine and represents a significant conceptual advantage in managing the complex, multifactorial pathophysiology of gastric ulcer disease.

6. CONCLUSION

This research project successfully achieved the development, comprehensive physicochemical characterization, in vivo pharmacological evaluation, and accelerated stability assessment of a novel polyherbal syrup formulation designed specifically for gastroprotective activity. The rational combination of *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, *Emblica officinalis*, and *Curcuma longa* in a well-designed syrup vehicle represents a scientifically rationalized, multi-targeted pharmacotherapeutic approach to managing gastric ulcers and mucosal inflammatory disorders that are among the most prevalent gastrointestinal conditions globally.

The physicochemical parameters of the herbal syrup — including pH (4.8 ± 0.12), specific gravity (1.24 ± 0.03 g/mL), viscosity (320 ± 18 cP), and drug content ($98.6 \pm 1.2\%$) — satisfactorily conform to standard pharmacopoeial specifications for oral liquid preparations, confirming the quality, safety, and acceptability of the formulation. The organoleptic properties including appearance, color, taste, and odor were found to be pleasant, clear, and acceptable, indicating favorable patient compliance potential particularly among pediatric and geriatric populations.



The *in vivo* gastroprotective activity assessment using the ethanol-induced gastric ulcer model demonstrated dose-dependent, statistically significant ulcer protection across all treatment groups. The high-dose group (10 mL/kg) achieved 79.7% ulcer protection, closely and meaningfully comparable to the standard drug omeprazole (80.5%), providing robust preclinical pharmacological evidence for the therapeutic efficacy of the formulation. This equivalence in gastroprotective activity is particularly significant because the herbal formulation achieved it through cytoprotective, antioxidant, and anti-inflammatory mechanisms rather than through acid suppression — suggesting that it may complement rather than merely duplicate conventional pharmacotherapy.

Biochemical analysis further validated and mechanistically elucidated the gastroprotective activity through demonstrated restoration of gastric antioxidant enzyme activities (SOD, CAT, GPx), significant reduction of lipid peroxidation markers (MDA), normalization of gastric mucus content, and effective suppression of pro-inflammatory cytokines (IL-6, TNF- α). These multi-dimensional biochemical findings confirm that the polyherbal formulation exerts a comprehensive mucosal protective effect through simultaneous antioxidant, anti-inflammatory, and mucus-stimulatory mechanisms attributable to the synergistic interaction of the phytoconstituents present in the five component medicinal plants.

Accelerated stability studies confirmed that the formulation maintains all its critical quality attributes — pH, drug content, viscosity, microbial safety, and appearance — within pharmacopoeial acceptance criteria over three months at stressed conditions, supporting a projected shelf-life of at least 24 months under appropriate normal storage conditions. These stability results validate the pharmaceutical formulation design and confirm

the adequacy of the preservative system and packaging strategy.

In conclusion, the developed herbal gastroprotective syrup formulation demonstrates excellent physicochemical properties, clinically meaningful gastroprotective efficacy comparable to the standard drug, a mechanistically sound and pharmacologically rational biochemical profile, and satisfactory long-term stability. It represents a promising, safe, cost-effective, and patient-friendly alternative to conventional allopathic gastroprotective agents. Future research directions should encompass well-designed randomized controlled clinical trials in human patients with confirmed gastric ulcer disease, bioavailability studies, optimization of the formulation using novel drug delivery platforms such as nanoemulsification or self-microemulsifying systems to further enhance phytoconstituent bioavailability, and detailed long-term toxicological assessment of the polyherbal combination.

ACKNOWLEDGEMENT

We extend our deepest respect and gratitude to our project guide, Prof. Arif sheikh, Anuradha College of Pharmacy, for his invaluable guidance, continuous support, and scholarly mentorship throughout this research project. His expertise in herbal pharmacology and pharmaceutical formulation provided us with the direction and motivation needed to navigate the complexities of this study. His patient guidance during every stage of formulation, evaluation, and documentation was indispensable.

.We acknowledge Sant Gadge Baba Amravati University, Amravati, for providing the academic framework and guidelines that shaped this project. We are grateful to all researchers and authors whose published works provided the scientific



foundation and inspiration for this study. Their contributions to the field of herbal pharmacology made this research possible.

Finally, we express our deepest gratitude to our parents, family members, and friends for their unconditional love, moral support, and unwavering encouragement throughout this entire academic journey. This work is dedicated to them.

7. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest — financial, professional, or personal — associated with this research project and the results, interpretations, and conclusions presented herein. This work was conducted solely for academic and scientific purposes as part of the B. Pharm 8th Semester curriculum at Anuradha College of Pharmacy, Chikhali. No external organization, pharmaceutical company, commercial entity, or funding body had any involvement in the study design, data collection, data analysis, data interpretation, or the decision to publish the results of this research. All data reported in this manuscript are original, authentic, independently generated, and have not been submitted or published elsewhere.

8. FUNDING STATEMENT

This research project was entirely self-funded by the student investigators. No external funding, grants, or financial support of any kind was received from any government agency, non-governmental organization, private institution, pharmaceutical company, or any other funding body for any aspect of this research work. All expenses related to plant material procurement, extract preparation, formulation development, laboratory reagents, consumables, evaluation instruments, analytical standards, and manuscript preparation were borne solely and personally by

the student authors. This ensures complete financial independence and freedom from any potential conflict of interest that could influence the objectivity of the research findings.

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HOW TO CITE: Vaibhav Jadhav, Rahul Jaybhaye, Ram Wadekar, Roshan Daberao, Arif Sheikh, Dr. R. H. Kale, Dr. K. R. Biyani, Evaluation of Gastroprotective Activity of Herbal Syrup Formulation Containing *Glycyrrhiza glabra*, *Aloe vera*, *Zingiber officinale*, *Emblica officinalis*, and *Curcuma longa*, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 5, 1265-1278. <https://doi.org/10.5281/zenodo.20061312>

