Journal of Pharma Research
Available online through
www.jprinfo.com
Vol. 15 Issues 04, 2026 ISSN: 2319-5622

Research Article

# EVALUATION OF ANTI-OXIDANT AND ANTI-ULCER ACTIVITY OF A POLYHERBAL FORMULATION IN NSAIDS-INDUCED ULCER MODEL

**Mandapati Apoorva[1]*, Saikrishna Nallajerla[2]**
*[1,2]Department of Pharmacology, AKRG College of Pharmacy, Nallajerla, East Godavari, Andhra Pradesh-534112*

**Received on: 04-06-2026** **Accepted & Published on: 24-07-2026**

## *ABSTRACT*

***Background:*** *Gastric ulcer is a common gastrointestinal disorder resulting from an imbalance between aggressive factors, such as gastric acid, non-steroidal anti-inflammatory drugs (NSAIDs), and oxidative stress, and protective mechanisms of the gastric mucosa. Although conventional anti-ulcer drugs are effective, their long-term use is associated with several adverse effects, prompting the search for safer plant-based alternatives.*

***Methods:*** *The selected medicinal plants were shade-dried, powdered, and formulated into a polyherbal preparation. Preliminary phytochemical screening was performed to identify major bioactive constituents. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with ascorbic acid as the reference standard. Anti-ulcer activity was assessed in aspirin-induced gastric ulcer models using Wistar rats. Gastric pH, ulcer index, gastric volume, and total protein content were determined. Acute oral toxicity was evaluated according to OECD Guideline 423.*

***Results:*** *Phytochemical analysis confirmed the presence of flavonoids, tannins, phenolic compounds, glycosides, and saponins. The formulation demonstrated concentration-dependent DPPH radical scavenging activity, indicating significant antioxidant potential. Acute toxicity studies revealed no mortality or observable toxic effects at 2000 mg/kg. In the aspirin-induced ulcer model, the polyherbal formulation significantly reduced ulcer index, increased gastric pH, restored total protein content, and exhibited dose-dependent gastroprotective activity comparable to omeprazole.*

***Conclusion:*** *The polyherbal formulation exhibited significant antioxidant and gastroprotective activities with an excellent safety profile. These findings support its potential as a safe, effective, and natural therapeutic alternative for the management of NSAID-induced gastric ulcers. Further mechanistic investigations and clinical studies are warranted to validate its therapeutic efficacy.*

***Keywords****: Gastric ulcer; Polyherbal formulation; Psidium guajava; Acacia catechu; Azadirachta indica; Glycyrrhiza glabra; Antioxidant activity.*

## INTRODUCTION

Peptic ulcer disease (PUD) remains one of the most prevalent gastrointestinal disorders worldwide, affecting millions of individuals and imposing a significant healthcare burden. It is characterized by the development of mucosal lesions in the stomach or proximal duodenum resulting from an imbalance between aggressive factors, including gastric acid, pepsin, Helicobacter pylori infection, non-steroidal anti-inflammatory drugs (NSAIDs), bile reflux, and reactive oxygen species (ROS), and protective mechanisms such as mucus-bicarbonate secretion, prostaglandin synthesis, mucosal blood flow, epithelial regeneration, and endogenous antioxidant defenses. Although the global prevalence of peptic ulcer disease has declined following the introduction of proton pump inhibitors (PPIs) and H. pylori eradication therapy, NSAID-induced gastric ulceration continues to represent a major clinical challenge, particularly

**Corresponding author:**
**Mandapati Apoorva**
Department of Pharmacology, AKRG College of Pharmacy, Nallajerla, East Godavari, Andhra Pradesh - 534112
**Email:** mandapatiapoorva@gmail.com
**DOI:** https://doi.org/10.5281/zenodo.21542835

among elderly patients and individuals receiving long-term anti-inflammatory therapy [1].

NSAIDs are among the most widely prescribed and self-administered medications for the management of pain, inflammation, and musculoskeletal disorders. However, their therapeutic benefits are frequently accompanied by gastrointestinal complications ranging from superficial mucosal erosions to severe gastric ulceration, bleeding, perforation, and gastric outlet obstruction. NSAID-induced gastric injury primarily results from cyclooxygenase (COX) inhibition, leading to reduced prostaglandin synthesis, impaired mucus and bicarbonate secretion, diminished mucosal blood flow, increased acid-mediated damage, and enhanced oxidative stress. Emerging evidence also suggests that inflammatory cytokines, mitochondrial dysfunction, and excessive production of reactive oxygen species significantly contribute to gastric mucosal injury and delayed ulcer healing. Oxidative stress has gained considerable attention as a central mechanism underlying gastric ulcer pathogenesis. Excessive generation of free radicals causes lipid peroxidation, protein oxidation, DNA damage, and apoptosis of gastric epithelial cells, thereby disrupting mucosal integrity. Consequently, antioxidants capable of scavenging reactive oxygen species and restoring endogenous antioxidant systems have emerged as promising therapeutic agents for ulcer prevention and treatment. Numerous experimental studies have demonstrated that natural phytochemicals, particularly flavonoids, phenolic acids, tannins, alkaloids, and saponins, possess potent antioxidant, anti-inflammatory, cytoprotective, and gastroprotective properties [2].

Current pharmacological management of gastric ulcers relies mainly on proton pump inhibitors, $H_2$-receptor antagonists, antacids, mucosal protective agents, and antibiotic regimens for H. pylori eradication. Although these therapies effectively promote ulcer healing, their prolonged use has been associated with several limitations, including recurrent disease, drug interactions, nutrient malabsorption, alteration of gut microbiota, increased susceptibility to infections, adverse drug reactions, and the emergence of antimicrobial resistance. These limitations have stimulated growing interest in identifying safer, more effective, and multi-targeted therapeutic alternatives. Medicinal plants have been utilized for centuries in traditional healthcare systems such as Ayurveda, Traditional Chinese Medicine, Siddha, and Unani for the treatment of gastrointestinal disorders. Scientific investigations over the past two decades have validated the anti-ulcer potential of numerous medicinal plants through diverse pharmacological mechanisms, including inhibition of gastric acid secretion, enhancement of mucus production, stimulation of prostaglandin synthesis, suppression of inflammatory mediators, antioxidant activity, and acceleration of mucosal regeneration. Among these, Psidium guajava, Azadirachta indica, Acacia catechu, and Glycyrrhiza glabra have attracted considerable scientific attention because of their rich phytochemical composition and experimentally proven gastroprotective properties. Their bioactive constituents—including flavonoids, catechins, glycyrrhizin, phenolic compounds, and tannins—collectively contribute to antioxidant, anti-inflammatory, antimicrobial, and mucosal healing activities [3].

Polyherbal therapy represents an advanced approach in herbal medicine based on the principle of phytochemical synergism, where multiple medicinal plants are combined to achieve enhanced therapeutic efficacy while minimizing toxicity. Unlike single-herb preparations or synthetic drugs targeting individual molecular pathways, polyherbal formulations simultaneously modulate multiple pathological mechanisms involved in gastric ulcer development. Experimental evidence indicates that polyherbal combinations can effectively suppress gastric acid secretion, improve antioxidant defense systems, inhibit inflammatory mediators, strengthen mucosal barriers, and accelerate tissue repair, thereby offering comprehensive gastroprotection [4].

In recent years, increasing numbers of experimental and clinical investigations have evaluated herbal and polyherbal formulations for the management of gastric ulcers. Despite encouraging findings, available evidence remains fragmented with respect to phytochemical composition, mechanisms of action, formulation strategies, pharmacological efficacy, safety, and translational potential. A comprehensive synthesis of current knowledge is therefore necessary to identify promising herbal candidates, highlight existing research gaps, and guide future therapeutic development [5].

Accordingly, this research aims to critically summarize the current evidence regarding the antioxidant and gastroprotective mechanisms of medicinal plants and polyherbal formulations used in gastric ulcer management, with particular emphasis on NSAID-induced gastric injury. Furthermore, the review discusses their phytochemical constituents, pharmacological mechanisms, preclinical and clinical evidence, formulation approaches, therapeutic advantages, current limitations, and future perspectives for developing safer and more effective anti-ulcer therapies.

## Materials and methods

### Materials

The polyherbal formulation was prepared using dried powders of Psidium guajava leaves, Acacia catechu heartwood, Azadirachta indica leaves, and Glycyrrhiza glabra roots as the principal herbal ingredients. Pharmaceutical-grade gum acacia, microcrystalline cellulose, sodium starch glycolate, magnesium stearate, and sodium benzoate were used as excipients for formulation development. Analytical-grade ethanol, methanol, and distilled water served as extraction solvents, while 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were employed for antioxidant evaluation. Pharmaceutical-grade aspirin and omeprazole were used to induce gastric ulcers and as the reference anti-ulcer drug, respectively. All chemicals and

 

reagents used in the study were of analytical or pharmaceutical grade and procured from certified commercial suppliers.

**Collection and Drying**

In the present work, the following plant materials were prepared in dried powder form: Acacia catechu (L.f.) Willd., Psidium guajava L. (guava leaves), Azadirachta indica A. Juss. (neem leaves), and Glycyrrhiza glabraL. (licorice stem). First, fresh plant materials were washed to remove dust and extraneous matter, then shade-dried at ambient temperature to preserve their bioactive principles.26Once adequately dried, the selected plant materials were pulverized using a mechanical grinder and subsequently sieved through a No. 80 mesh to achieve a uniform particle size distribution. The resulting fine powders were stored in airtight containers under cool and dry conditions to preserve their stability and phytochemical integrity until further use in Extract formulation. [6]

**Preparation of powder:**

The selected plant materials were air-dried thoroughly and pulverized to a fine consistency. The powdered samples were further passed through a No. 80 mesh sieve for uniform particle size. These sieved powders were then stored in air-tight containers in a controlled environment of low humidity and temperature in order to maintain the phytochemical integrity for further use in the formulation of polyherbal Extracts. [7]

**Phytochemical analysis of plants**

Phytochemical screenings are preliminary analytical techniques done on a plant powder to qualitatively show the presence of various bioactive compounds. A series of qualitative phytochemical screening tests were conducted to identify the presence of key bioactive constituents, including alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, steroids, proteins, carbohydrates, and amino acids. These assays provided preliminary insights into the chemical composition and therapeutic potential of the plant extracts. [8-10]

**Table 1: Composition of polyherbal Extract**

| Ingredients | Composition (mg) |
|---|---|
| **Neem** | 56 |
| **Acacia catechu** | 60 |
| **liquorice** | 60 |
| **Guava** | 59 |
| **Gum acacia** | 15 |
| **Theoretical Weight** | 250 |

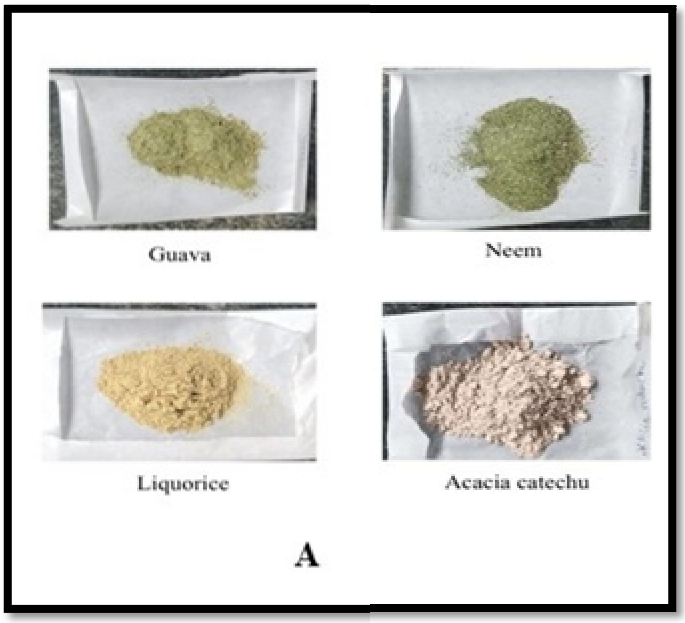


**Fig 1: A. Different herbs used in formulation B. Final poly herbal Extract formulation**

**In vitro antioxidant activity**

**Determination of in vitro Antioxidant activity**

**Free radical scavenging (DPPH) assay**

The free radical scavenging activity of MEPG was measured in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using the method of Blois (1958). About 0.3mM solution of DPPH in 100% ethanol was prepared and 1ml of this solution was added to 3ml of the formulation dissolved in ethanol at different concentrations (10–50 _g/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The IC50 value of the crude extract was compared with that of ascorbic acid, which was used as the standard. [10-12]

**Evaluation of Anti-Ulcer Activity:**

**Animal:**

Healthy adult Wistar rats of either sex, weighing between 180 and 200 grams, will be sourced and acclimatized under standardized laboratory conditions. Environmental parameters will be maintained at a controlled temperature of 22 ± 2°C, relative humidity of 55 ± 5%, and a regulated 12-hour light/dark photoperiod. Throughout the acclimatization period, animals will have unrestricted access to food and water (ad libitum) to ensure physiological stability prior to experimental procedures. The experiments will be performed in accordance with the guidelines set by CPCSEA, and prior approval will be sought from the Institutional Animal Ethics Committee of Sree Dattha Institute of Pharmacy, Hyderabad under approval number

 

:SDIP/IAEC/2026/11. Animals will be divided into five groups (n=6 per group) in a random manner to ensure statistical validity and to reduce any element of bias. [13-14]

**Experiment design:**

**Group 1:** Normal Control

**Group 2:** Disease Control Aspirin (300 mg/kg p.o) for 4 days

**Group 3:** Standard Control (Aspirin 300 mg/kg p.o) + Omeprazole 40 mg/kg p.o) for 4 days

**Group 4:** Test low dose (Aspirin 300 mg/kg p.o) + Test Drug 125 mg/kg p.o) for 4 days

**Group 5:** Test High Dose (Aspirin 300 mg/kg p.o) + Test Drug 250 mg/kg p.o) for 4 days

**Acute Oral Toxicity Study**

Acute oral toxicity assessment was conducted in accordance with OECD Guideline 423, which outlines the toxic class method for evaluating safety profiles. Based on prior data indicating minimal risk of mortality at the highest starting dose, three healthy female Wistar rats were selected for the study.35 The animals received the polyherbal formulation orally at a dose of 2000 mg/kg body weight. Post-administration, the rats were closely monitored for signs of toxicity, including changes in body weight, behavioural alterations, and mortality. Intensive observations were carried out during the initial four hours following dosing, followed by twice-daily evaluations over a 14-day period to detect any delayed adverse effects. [15-16]

**Weight Difference:**

In order to monitor systemic health and early signs of toxicity, each animal's body weight was recorded daily throughout the observation period of 14 days. Body weights were recorded at the same time each day using a calibrated digital balance. These data were used to assess weight gain or loss trends, which serve as indirect indications of metabolic disturbances, stress response, or organ dysfunction possibly induced by the test formulation. [17-18]

**Macroscopic Examination of the Stomach**

After euthanizing the animals, the stomachs were carefully opened along the greater curvature and rinsed with physiological saline to eliminate any remaining gastric contents and blood residues. The mucosal surfaces were then examined for ulcerative lesions using a 10× magnification lens. Ulcer severity was evaluated based on a standardized scoring system, which facilitated consistent assessment of lesion extent and intensity across samples. [19-20]

0 – Normal-colored stomach

0.5 - Red coloration

1 – Spot ulcers

1.5 – Hemorrhagic streaks

2 – 3 to 5 ulcers

3 – More than 5 ulcers

**Estimation of Ulcer Index**

The ulcer index for each animal was calculated by averaging the individual ulcer scores, providing a quantitative measure of the extent of gastric mucosal injury. [21-22]

**Determination of pH of Gastric Juice:** The pH of the centrifuged gastric fluid was determined using a properly calibrated digital pH meter to ensure accurate measurement.

**Determination of Total Protein Content by Dry Weight Method:** Total protein content was quantified using the dry weight method. A clean weighing bottle was pre-dried at 104–106 °C for 10 min, cooled in a desiccator, and weighed to obtain the tare weight. An aliquot (0.5–3 mL) of the protein sample, pre-treated to remove non-volatile interferents such as buffer salts, polysaccharides, pigments, or amino acid salts, was added to the container. The sample was dried at 104–106 °C for 4–6 h (overnight for volumes ≥5 mL) to eliminate water and volatile components. After cooling in a desiccator, the container was reweighed, and the protein content was calculated as the difference between dried and tare weights. The method provides a measurement accuracy of approximately 1–3% for samples containing 2–4 mg of protein. [23-25]

**Statistical Analysis**

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA) followed by a post hoc test to determine intergroup differences. Data were expressed as the mean ± standard error of the mean (SEM). A probability value of less than 0.001 ($p < 0.001$) was considered statistically significant, indicating a high level of confidence in the observed differences. [40, 41]

**Results:**

**Phytochemical analysis**

All four herbal extracts showed strong presence of flavonoids, tannins, and phenolic compounds—key constituents associated with antiulcer and antioxidant activity (Table 2).

 

**Table 2: Phytochemical Screening of Individual Herbal Extracts**

| Sr. No. | Phytochemicals | Chemical Test(s) Used | Guava Leaf | Acacia Catechu | Neem Leaf | Liquorice |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | Mayer's, Wagner's, Dragendorff's, Hager's | + | + | + | + |
| 2 | Flavonoids | Shinoda, Alkaline reagent, Zinc-HCl | + | + | + | + |
| 3 | Tannins | Gelatin, Ferric chloride, Phenazone | + | + | + | + |
| 4 | Saponins | Froth test, Foam test | ± | ± | + | + |
| 5 | Glycosides | Keller-Killiani, Legal, Baljet | ± | + | + | + |
| 6 | Phenolic compounds | Folin-Ciocalteu, Vanillin-HCl | + | + | + | + |
| 7 | Steroids | Liebermann-Burchard, Salkowski | ± | ± | + | + |
| 8 | Proteins | Biuret, Xanthoproteic, Heat test | ± | ± | ± | + |
| 9 | Carbohydrates | Molisch's, Fehling's, Benedict's | + | ± | ± | + |
| 10 | Amino acids | Ninhydrin, Millon's | ± | ± | ± | + |

**Legend:**

- + = Present
- ± = Slightly present or variable
- – = Absent

**In vitro anti-oxidant activity**

**DPPH Assay**

The in-vitro antioxidant activity of the polyherbal formulation was evaluated using the DPPH free radical scavenging assay, with ascorbic acid used as the standard. The results demonstrated a clear concentration-dependent increase in radical scavenging activity for both the formulation and the standard across the tested range of 10–50 µg/mL. The polyherbal formulation exhibited moderate to strong antioxidant activity, with percentage inhibition increasing from approximately 32% to 78%, whereas ascorbic acid showed higher activity ranging from about 48% to 92%. Although the formulation was less potent than the standard, its significant free radical scavenging ability can be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and phenolic compounds. These findings suggest that the formulation possesses appreciable antioxidant potential, which may contribute to its observed gastroprotective and anti-ulcer effects.

**Table 3: DPPH Radical Scavenging Activity**

| Concentration (µg/mL) | % Inhibition (Formulation) | % Inhibition (Ascorbic Acid) |
|---|---|---|
| **10** | 32.5 ± 1.2 | 48.6 ± 1.0 |
| **20** | 45.8 ± 1.5 | 62.4 ± 1.3 |
| **30** | 58.2 ± 1.1 | 74.8 ± 1.2 |
| **40** | 69.6 ± 1.3 | 85.2 ± 1.1 |
| **50** | 78.4 ± 1.4 | 92.6 ± 1.0 |

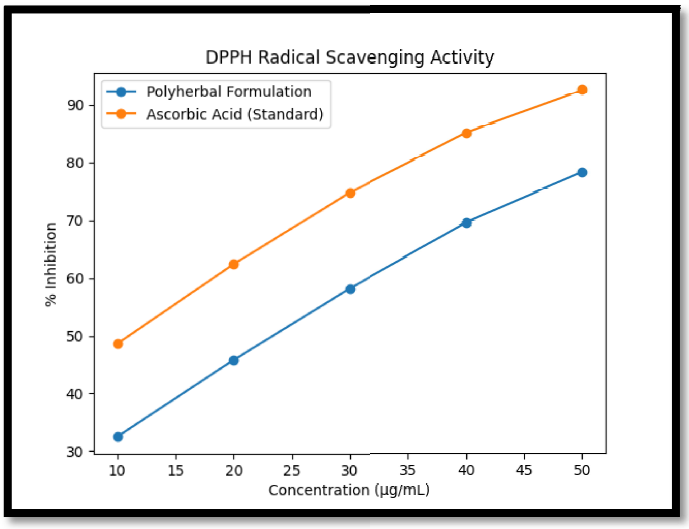


**Fig 2: DPPH assay of for formulation**

**Acue toxicity studies**

No mortality or treatment-related toxic effects were observed in female Wistar rats administered the polyherbal formulation at a limit dose of 2000 mg/kg. Throughout the 14-day observation period, all animals remained normal, exhibiting no behavioural abnormalities, clinical signs of toxicity, or changes in general appearance. Body weight of the treated rats increased gradually and followed the normal growth pattern, with no significant deviations from baseline values. No acute toxic manifestations were observed during the initial 4-hour intensive monitoring or during subsequent daily assessments. Based on these findings, the polyherbal formulation can be considered safe at the tested dose,

**Weight variation of rat**

Body weight patterns serve as sensitive indicators of metabolic disturbances or treatment-related toxicity. Across five days of monitoring, the rats maintained stable or slightly increased body weights within the expected physiological range (185–218 g). Minor fluctuations were observed but remained within normal biological variability attributed to feeding and hydration cycles. The absence of sharp weight decline reflects healthy metabolic status, indicating that the polyherbal formulation produced no adverse physiological effect even at high doses (Table 4).

**Table 4: Body Weight Variations During Acute Oral Toxicity Study**

| | R1 | R2 | R2 | R4 | R5 |
|---|---|---|---|---|---|
| **First Day** | 190gm | 189gm | 189gm | 189gm | 186gm |
| **Second Day** | 195gm | 192gm | 191gm | 189gm | 185gm |
| **Third day** | 210gm | 218gm | 218gm | 215gm | 215gm |
| **Fourth Day** | 185gm | 185gm | 188gm | 185gm | 182gm |
| **Fifth Day** | 200gm | 196gm | 196gm | 192gm | 192gm |

**Effect of Polyherbal Formulation on Gastric Parameters in Aspirin-Induced Ulcer Model**

Aspirin administration produced substantial gastric damage in the disease control group, which was reflected by a markedly elevated ulcer index (6.0 ± 0.22), a pronounced reduction in gastric pH (1.95 ± 0.65), and a significant decrease in total protein content (6.3 ± 0.12 mg/100 mg). These alterations collectively confirm the successful induction of acute gastric mucosal injury, indicating extensive acid-mediated erosion and compromised mucosal integrity. In contrast, the standard treatment group receiving omeprazole demonstrated strong gastroprotective activity. This group showed a sharp reduction in the ulcer index (0.5 ± 0.10), along with a notable increase in gastric pH (3.2 ± 0.42), indicating effective suppression of gastric acidity. Additionally, total protein content (14.3 ± 0.45 mg/100 mg) was restored toward normal levels, validating both the efficacy of omeprazole and the reliability of the experimental model.

The polyherbal formulation exhibited clear dose-dependent anti-ulcer effects. At the low dose, rats showed a moderate reduction in ulcer index (3.0 ± 0.85), an elevation in gastric pH (3.8 ± 0.31), and an improvement in total protein content (11.7 ± 0.42 mg/100 mg). These findings suggest partial mucosal protection and attenuation of aspirin-induced gastric injury (Figure 3).

At the high dose, the formulation produced marked gastroprotective effects. The ulcer index was reduced significantly to 1.5 ± 0.57, gastric pH increased to 3.1 ± 0.25, and total protein levels improved to 13.4 ± 0.25 mg/100 mg, indicating substantial restoration of mucosal structure and function. These improvements closely approached the efficacy observed with omeprazole (Table 7 & Figure 2).

Overall, the high-dose polyherbal formulation demonstrated therapeutic effects comparable to the standard drug, suggesting that the rich phytochemical constituents act synergistically to reduce gastric acidity, enhance mucus secretion, and preserve protein integrity essential for mucosal repair

**Table 5: Effect of Polyherbal Formulation on Gastric Parameters in Aspirin-Induced Ulcer Model**

| Group No | Treatment Dose (mg/kg) | Gastric Volume (ml) | pH | Mean Ulcer Index | Total Protein Content (Mg/100mg) |
|---|---|---|---|---|---|
| **I** | Normal Control | 8.05 ± 0.25 | 2.75 ± 0.50 | 0 | 15.5± 0.12 |
| **II** | Disease Control Aspirin | 5.69 ± 0.55 | 1.95 ± 0.65 | 6± 0.22 | 6.3± 0.12** |
| **III** | Group Standard Control (Aspirin + Omeprazole) | 6.98 ± 0.31 | 3.8 ± 0.42 | 0.5± 0.10 | 14.3± 0.45 |
| **IV** | Group Test low dose (Aspirin + Test Drug 125mg/kg) | 6.6 ± 0.55 | 3.1 ± 0.31 | 3± 0. 85 | 11.7± 0.42 |
| **V** | Group Test High Dose (Aspirin + Test Drug 200mg/kg) | 6.8 ± 0.25 | 3.60 ± 0.25 | 1.5± 0.57 | 13.4± 0.25*** |
| **I** | Normal Control | 8.05 ± 0.25 | 2.75 ± 0.50 | 0 | 15.5± 0.12 |

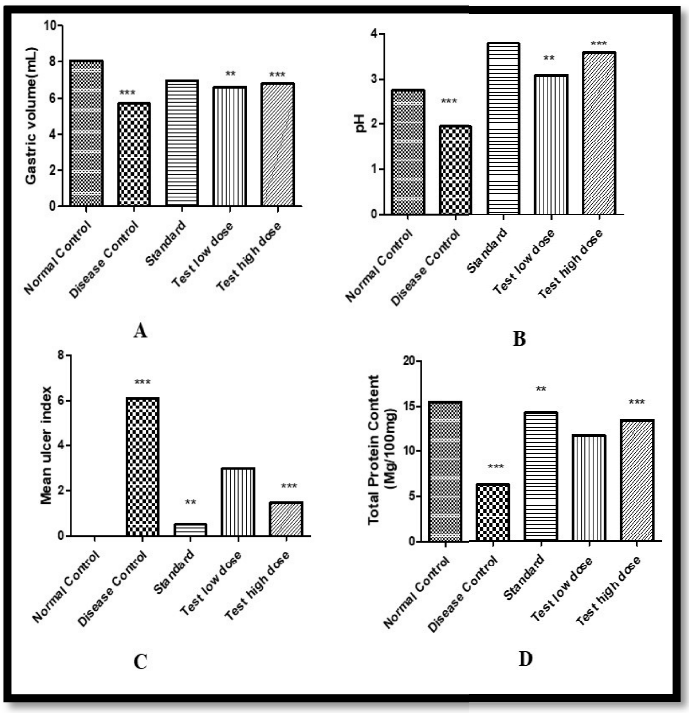


**Fig 3: Effect of Polyherbal Formulation on Gastric Parameters in Aspirin-Induced Ulcer Model A. Gastric volume B. pH C. Mean ulcer index D. Total protein content.**

Results were expressed as mean ± SEM (n=6); **p<0.01, and *** indicate p<0.001 respectively, all the groups were compared with Group-II

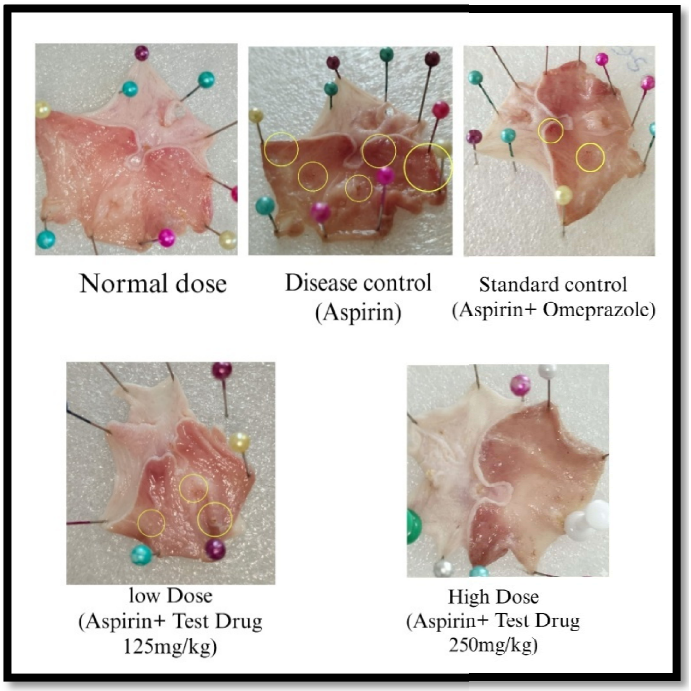


**Fig 4: Effect of Polyherbal Formulation on ulcer index in Aspirin-Induced Ulcer Model**

## DISCUSSION

The present study was undertaken to develop and evaluate a polyherbal Extract formulation containing *Psidium guajava, Acacia catechu, Azadirachta indica, and Glycyrrhiza glabra*, and to investigate its antioxidant and anti-ulcer potential. The findings of this investigation clearly demonstrate that the formulated polyherbal Extract possesses significant pharmacological activity supported by acceptable physicochemical properties, safety, and therapeutic efficacy.

Phytochemical screening revealed the presence of important bioactive constituents such as flavonoids, tannins, phenolic compounds, glycosides, and saponins in all selected plant materials. These phytoconstituents are well known for their antioxidant and cytoprotective effects. Flavonoids, in particular, are recognized for their ability to scavenge free radicals, inhibit lipid peroxidation, and enhance mucosal defense mechanisms. Tannins contribute by forming a protective layer over the gastric mucosa, thereby reducing irritation and preventing ulcer formation. The presence of these compounds provides a strong scientific basis for the observed biological activities of the formulation.

The in-vitro antioxidant activity evaluated by the DPPH assay demonstrated that the polyherbal formulation exhibits significant free radical scavenging activity in a concentration-dependent manner. The percentage inhibition increased progressively with increasing concentrations (10–50 µg/mL), indicating effective antioxidant potential. Although the activity was comparatively lower than the standard ascorbic acid, the formulation still showed substantial scavenging ability. This difference is expected, as ascorbic acid is a pure compound, whereas the polyherbal formulation is a complex mixture of phytoconstituents. The antioxidant activity observed can be attributed to the synergistic action of flavonoids, phenolic compounds, and tannins present in the formulation. These compounds neutralize reactive oxygen species (ROS), which play a crucial role in the pathogenesis of gastric ulcers.

The pre-compression parameters, including angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio, indicated good flowability and compressibility of the powder blend. These properties are essential for uniform die filling and successful Extract formation. Post-compression evaluation further confirmed that the Extracts possessed acceptable pharmaceutical characteristics such as uniform weight, adequate hardness, low friability, and rapid disintegration time. The low friability value (<1%) indicates strong mechanical resistance, while the disintegration time of less than 5 minutes ensures rapid release of active constituents. The near-neutral pH suggests that the formulation is non-irritant to the gastric mucosa. These findings confirm that the developed formulation is suitable for oral administration and meets pharmacopeial standards.

The acute oral toxicity study demonstrated that the polyherbal formulation is safe at a dose of 2000 mg/kg, with no mortality or observable toxic effects. The absence of behavioral changes and the maintenance of normal body weight indicate that the formulation does not produce any adverse systemic effects. This safety profile is an important advantage of herbal formulations compared to synthetic drugs, which often exhibit side effects upon prolonged use.

The anti-ulcer activity was evaluated using an aspirin-induced ulcer model, which is widely accepted for studying NSAID-induced gastric damage. Aspirin causes gastric injury by inhibiting cyclooxygenase (COX), leading to reduced prostaglandin synthesis, increased gastric acid secretion, decreased mucus production, and enhanced oxidative stress. In the present study, the disease control group showed a high ulcer index, low gastric pH, and reduced protein content, confirming severe mucosal damage.

Treatment with the polyherbal formulation resulted in a significant, dose-dependent reduction in ulcer index along with improvement in gastric pH and protein content. The high-dose group showed results comparable to the standard drug omeprazole, indicating strong gastroprotective activity. The increase in gastric pH suggests reduced acidity, while the restoration of protein content reflects improved mucosal integrity and healing. These findings indicate that the formulation not only prevents ulcer formation but also promotes healing of damaged gastric tissue.

The observed anti-ulcer activity can be attributed to multiple mechanisms. The antioxidant activity reduces oxidative stress, which is a major factor in ulcer formation. Flavonoids inhibit gastric acid secretion and enhance mucus production, thereby strengthening the mucosal barrier. Tannins provide a protective coating over the gastric lining, while saponins and glycosides contribute to mucosal regeneration and anti-inflammatory effects. Additionally, Glycyrrhiza glabra is known to increase prostaglandin levels and mucus secretion, further enhancing gastric protection. The combined action of these phytoconstituents results in a synergistic effect, making the formulation more effective than individual plant extracts.

Overall, the results of this study highlight the importance of polyherbal formulations in the management of gastric ulcers. Unlike synthetic drugs that target a single pathway, polyherbal formulations act through multiple mechanisms, providing a holistic approach to treatment. The comparable efficacy of the formulation with omeprazole, along with its excellent safety profile, suggests its potential as a natural alternative for ulcer management.

However, the study has certain limitations. The antioxidant activity was evaluated only by the DPPH method, and additional assays such as ABTS or FRAP could provide more comprehensive insights. Furthermore, detailed mechanistic studies and clinical trials are required to confirm the efficacy and safety of the formulation in humans.

In conclusion, the polyherbal Extract formulation demonstrated significant antioxidant and anti-ulcer activity, supported by strong phytochemical composition and favorable pharmaceutical properties. The formulation offers a promising, safe, and effective alternative to conventional anti-ulcer drugs, with potential for further development and clinical application.

## CONCLUSION

The present study successfully developed and evaluated a polyherbal Extract formulation containing Psidium guajava, Acacia catechu, Azadirachta indica, and Glycyrrhiza glabra, with the objective of assessing its antioxidant and anti-ulcer potential. The formulation process using the direct compression method yielded Extracts with satisfactory physicochemical properties, including uniform weight variation, adequate hardness, low friability, and rapid disintegration time. These characteristics confirm that the prepared Extracts meet pharmacopeial standards and are suitable for oral administration.

Phytochemical screening of the selected herbal ingredients revealed the presence of key bioactive compounds such as flavonoids, tannins, phenolic compounds, glycosides, and saponins. These constituents are well known for their antioxidant, anti-inflammatory, and cytoprotective activities, which play a crucial role in the prevention and healing of gastric ulcers. The in-vitro antioxidant study using the DPPH assay demonstrated that the polyherbal formulation possesses significant free radical scavenging activity in a concentration-dependent manner. Although the activity was lower than that of the standard ascorbic acid, the formulation exhibited substantial antioxidant potential, which can be attributed to the synergistic action of its phytoconstituents.

The acute oral toxicity study confirmed the safety of the formulation at a dose of 2000 mg/kg, with no signs of toxicity, mortality, or adverse behavioral changes observed in experimental animals. This finding highlights the safety and tolerability of the polyherbal formulation, making it a promising alternative to synthetic drugs that often produce undesirable side effects.

The anti-ulcer activity evaluated using the aspirin-induced ulcer model demonstrated significant gastroprotective effects of the formulation. The treatment resulted in a dose-dependent reduction in ulcer index, increase in gastric pH, and restoration of total protein content. Notably, the high-dose group exhibited effects comparable to the standard drug omeprazole, indicating strong anti-ulcer efficacy. The observed therapeutic effects can be attributed to multiple mechanisms, including antioxidant action, reduction of gastric acid secretion, enhancement of mucus production, and improvement of mucosal integrity.

Overall, the results of this study clearly indicate that the developed polyherbal Extract formulation possesses significant antioxidant and anti-ulcer activities with a high safety margin. The synergistic interaction of the selected medicinal plants provides a multi-targeted therapeutic effect, making the formulation more effective than single-herb preparations. This study supports the potential use of polyherbal formulations as safe, effective, and natural alternatives for the management of NSAID-induced gastric ulcers.

However, further studies, including detailed pharmacological investigations, long-term toxicity studies, and clinical trials in humans, are necessary to validate these findings and establish the formulation as a standardized therapeutic agent. With continued research and development, this polyherbal formulation holds strong potential for future clinical application in the treatment of gastric ulcers.

**Conflict of Interest**

All authors are declared no conflict of interest

**Acknowledgment:**

All authors are thankful to Principal and Management of AKRG College of Pharmacy,Nallajerla, East Godavari, Andhra Pradesh for providing facilities for conduct this work .

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**How to cite this article:**

**Mandapati Apoorva *, EVALUATION OF ANTI-OXIDANT AND ANTI-ULCER ACTIVITY OF A POLYHERBAL FORMULATION IN NSAIDS-INDUCED ULCER MODEL J Pharma Res, 2026; 15(04): 16-25. DOI: https://doi.org/10.5281/zenodo.21542835**

**Conflict of interest**: The authors have declared that no conflict of interest exists.

**Source of support:** Nil

 