

PHYTOCHEMICAL PROFILING AND IN VITRO EVALUATION OF DUODENAL ULCER PROTECTIVE EFFECTS OF SOLOMON'S SEAL AND CALENDULA ARVENSIS

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Received: 25th May, 2026; Revised: 6th June, 2026; Accepted: 8th June, 2026; Available Online: 20th June, 2026

ABSTRACT

Polygonatum spp. and Calendula arvensis have long been used in traditional medicine for gastric ailments, though scientific evidence supporting their gastroprotective effects remains scarce. This research aimed to characterize the phytochemical composition, antioxidant properties, and in vitro anti-ulcer activity of these species. Fresh rhizomes of Polygonatum spp. and aerial parts of C. arvensis were collected from the Nainital Himalayan region, authenticated at D.D.U. Gorakhpur, shade-dried, powdered, and then sequentially extracted using hexane, chloroform/ethyl acetate, and methanol. The crude extracts were concentrated below 45°C and stored at -20°C. Preliminary qualitative phytochemical screening for alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids was carried out using standard colorimetric tests. Total phenolic content (TPC) and total flavonoid content (TFC) were measured via the Folin-Ciocalteu and aluminum chloride methods, respectively, and expressed as mg gallic acid equivalents (GAE)/g and mg quercetin equivalents (QE)/g. In vitro antioxidant capacity was evaluated using DPPH, H₂O₂, and nitric oxide (NO) scavenging assays, with IC₅₀ values calculated relative to ascorbic acid. Anti-ulcer potential was assessed through H⁺/K⁺-ATPase inhibition and acid-neutralizing capacity assays. Data were analyzed using one-way ANOVA followed by Tukey's test, with statistical significance set at p < 0.05. The experimental design allows for linking traditional use with phytochemical profiles and provides mechanistic insights into antioxidant and antisecretory pathways, thereby laying the groundwork for future in vivo studies to validate these plants as sources of gastroprotective agents.

Keywords: Calendula arvensis; phytochemical screening; total phenolic content; total flavonoid content; antioxidant activity; DPPH assay; H₂O₂ scavenging; nitric oxide.

How to cite this article: Kaushik A, Singh VP, Singh S. Phytochemical Profiling and In Vitro Evaluation of Duodenal Ulcer Protective Effects of Solomon's Seal and Calendula arvensis. Int J Drug Deliv Technol. 2026;16(62s): 1015-1023. DOI: 10.25258/ijddt.16.62s.108

Source of support: Nil.

Conflict of interest: None.

Introduction

Peptic ulcer disease and gastritis are major gastrointestinal disorders affecting nearly 10% of the global population during their lifetime. Despite advances in pharmacotherapy, recurrence rates remain high, and long-term use of synthetic drugs like proton pump inhibitors and H₂-receptor blockers is associated with adverse effects including nutrient malabsorption, kidney disease, and increased risk of enteric infections. This necessitates the search for safer, multi-targeted, plant-based alternatives.

Pathophysiology and Oxidative Stress

The pathogenesis of gastric ulcers involves an imbalance between aggressive factors such as HCl, pepsin, *Helicobacter pylori*, NSAIDs, and alcohol, and defensive factors like mucus, bicarbonate, prostaglandins, and mucosal blood flow. Oxidative stress is a critical mediator, where reactive oxygen species including superoxide anion, hydrogen peroxide, and hydroxyl radicals, and reactive nitrogen species like nitric oxide and peroxynitrite, cause lipid peroxidation, protein oxidation, DNA damage, and apoptosis of gastric mucosal cells. Antioxidants that

scavenge these radicals are therefore central to gastroprotection.

Role of Medicinal Plants in Ethnopharmacology:

Traditional medicine systems, particularly Ayurveda and Traditional Chinese Medicine, have long used botanicals for gastric ailments. Over 80% of the population in developing countries relies on plant-derived medicines for primary healthcare due to accessibility, cultural acceptance, and perceived safety. Scientific validation of these ethnomedicinal claims is essential to integrate traditional knowledge into evidence-based therapy and to discover novel lead molecules.

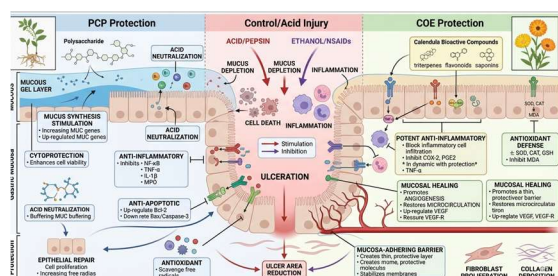


Fig: 1 Mechanism of action of ulceration causative

Material & Method

Collection, Authentication, and Processing of Plant Material

Fresh rhizomes of *Polygonatum* spp. (Solomon's Seal) and the aerial parts specifically flowers and leaves of *Calendula arvensis* (Field Marigold) will be collected from Himalayan range of Nainital to optimal bioactive compound content. The cleaned samples will then be shade-dried at room temperature under adequate ventilation to prevent fungal growth and enzymatic degradation. Once completely dried, the materials will be uniformly powdered using an electric grinder. The resulting powders will be sieved to achieve a consistent particle size, which facilitates efficient solvent penetration during extraction. Finally, the authenticated, powdered materials will be stored in hermetically sealed, dark glass containers at 4°C until further use to preserve their phytochemical stability.

Preparation of Plant Extracts

The powdered plant materials will undergo sequential extraction using solvents of increasing polarity to ensure comprehensive extraction of diverse phytochemicals. Initially, non-polar compounds will be extracted using hexane, followed by chloroform or ethyl acetate for mid-polarity constituents, and finally, methanol or aqueous methanol for polar compounds. Each extraction will involve macerating a known weight of powder in the solvent for 72 hours with periodic agitation. The mixtures will then be filtered, and the marc re-macerated with fresh solvent to ensure

exhaustive extraction. The combined filtrates for each solvent will be concentrated under reduced pressure using a rotary evaporator at controlled temperatures (below 45°C) to prevent thermal degradation of heat-labile compounds. The resulting crude extracts will be weighed to determine yield, reconstituted in dimethyl sulfoxide (DMSO) or suitable buffers for assays, and stored at -20°C. The most promising extracts, typically the polar (methanolic) and non-polar (chloroform/ethyl acetate) ones based on preliminary yield and traditional use, will be selected for detailed phytochemical profiling and biological evaluation.

Preliminary Qualitative Phytochemical Analysis

This initial screening provides a fundamental chemical profile, indicating the presence of major classes of bioactive metabolites in the selected polar and non-polar extracts of *Polygonatum* spp. And *Calendula arvensis*. The tests are based on specific color reactions or precipitate formation when extracts are treated with standardized reagents. A positive result, characterized by a distinct visual change, confirms the presence of the targeted phytochemical group. This profiling is essential as it forms the basis for correlating the traditional medicinal uses of these plants—such as wound healing and anti-inflammatory effects for *Calendula* and tonic properties for *Polygonatum*—with their inherent biochemistry. Identifying alkaloids, flavonoids, saponins, and terpenoids, for instance, directs subsequent quantitative analysis and provides a preliminary rationale for the observed in vitro biological activities.

Alkaloids: Mayer's, Wagner's, and Dragendorff's Tests

These tests are standard for detecting alkaloids, nitrogen-containing compounds known for diverse pharmacological effects. Each test employs a different precipitating reagent that forms insoluble complexes with alkaloids. Mayer's reagent (potassium mercuric iodide) typically yields a creamy white precipitate, while Wagner's reagent (iodine in potassium iodide) produces a reddish-brown precipitate. Dragendorff's reagent (potassium bismuth iodide) results in an orange or orange-red precipitate. The formation of such precipitates in the tested plant extracts confirms the presence of alkaloids. As *Coptis japonica* is renowned for its isoquinoline alkaloids like berberine, strong positive results are anticipated, supporting its selection for anti-ulcer studies.

Flavonoids: Shinoda Test and Alkaline Reagent Test

These complementary tests identify flavonoid compounds, recognized for their antioxidant and anti-inflammatory properties. The Shinoda test involves adding magnesium turnings and concentrated hydrochloric acid to the extract. A positive result is indicated by the development of a pink, red, or

magenta color due to the reduction of flavones or flavonols. The Alkaline Reagent test uses a few drops of sodium hydroxide or ammonia solution, leading to the formation of a yellow color that decolorizes upon acidification. This color change occurs due to the structural transformation of flavonoids in alkaline media. Positive outcomes would suggest a significant flavonoid content, contributing to the expected antioxidant activity of the extracts.

Saponins: Foam Test

The foam test is a simple, indicative assay for saponins, glycosidic compounds with soap-like characteristics and potential membrane-protective effects. For this test, the plant extract is diluted with distilled water and vigorously shaken in a test tube for 15-30 seconds. The formation of a stable, persistent froth or foam layer that lasts for 10-15 minutes indicates the presence of saponins. This stability is due to the amphiphilic nature of saponins, which lowers surface tension. While not quantitative, a positive foam test suggests the extract contains these surfactants, which may contribute to biological activity by interacting with cell membranes, potentially relevant for gastroprotective actions.

Tannins: Ferric Chloride Test and Gelatin Test

Tannins, polyphenolic compounds with astringent properties, are detected using two distinct tests. The Ferric Chloride test involves adding a few drops of neutral ferric chloride solution to the extract. The formation of a blue, green, or blackish-blue color confirms the presence of hydrolysable tannins, while a brownish-green color suggests condensed tannins. The Gelatin test exploits tannins' ability to precipitate proteins; adding a 1% gelatin solution containing sodium chloride to the extract results in a white precipitate. The presence of tannins is significant for ulcer studies due to their protein-precipitating and vasoconstrictive properties, which can help form a protective mucosal layer.

Terpenoids: Salkowski Test

The Salkowski test is specific for terpenoids, a large class of compounds derived from isoprene units, many of which exhibit anti-inflammatory activity. In this test, the extract is mixed with chloroform, and concentrated sulfuric acid is carefully added along the test tube wall to form a separate layer. A positive result is indicated by the formation of a reddish-brown coloration at the interface between the two layers. This color reaction is due to the formation of carbocations or conjugated systems from terpenoids upon reaction with the strong acid. Identifying terpenoids adds to the phytochemical profile, suggesting another potential mechanism for the observed biological effects.

Steroids: Liebermann-Burchard Test

This test detects steroids, including phytosterols and cardiac glycosides, which share a core

cyclopentanophenanthrene ring structure. The extract is dissolved in chloroform, to which acetic anhydride and concentrated sulfuric acid are added sequentially (the Liebermann-Burchard reagent). The mixture is then allowed to stand. A positive result is characterized by a color change sequence: initially red or pink, which may transition to blue or green. This complex color development results from dehydration and oxidation reactions leading to conjugated double-bond systems. The presence of steroids could be relevant for anti-ulcer activity through potential membrane-stabilizing or anti-secretory effects.

Total Phenolic Content (TPC)

Total Phenolic Content provides a quantitative measure of all phenol derivatives in the extracts, a group strongly correlated with antioxidant capacity. The assay is typically performed using the Folin-Ciocalteu method. This reagent, containing phosphomolybdate and phosphotungstate, oxidizes phenolic compounds in an alkaline medium (sodium carbonate), producing a blue-colored complex. The intensity of this color, measured spectrophotometrically at 765 nm, is proportional to the total phenolic concentration. Results are expressed as milligrams of Gallic Acid Equivalent per gram of extract (mg GAE/g). A higher TPC value directly suggests a greater potential for free radical scavenging and metal chelation activities.

Total Flavonoid Content (TFC)

This assay specifically quantifies the flavonoid subclass of phenolics, compounds well-documented for their potent antioxidant and cytoprotective roles. The aluminum chloride colorimetric method is commonly employed. Flavonoids form stable acid complexes with aluminum chloride, yielding a yellow color. Briefly, the extract is mixed with sodium nitrite, aluminum chloride, and sodium hydroxide. The absorbance of the resulting mixture is read at 510 nm. The concentration is determined by comparison to a standard curve prepared using a reference flavonoid like quercetin or rutin. Results are expressed as milligrams of Quercetin Equivalent per gram of extract (mg QE/g). High TFC values would substantiate the qualitative flavonoid tests and support the rationale for antioxidant evaluation.

In Vitro Evaluation

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a standard, rapid method for evaluating the free radical scavenging potential of plant extracts. The stable, purple-colored DPPH radical accepts an electron or hydrogen atom from an antioxidant compound, becoming reduced to a yellow-colored diphenylpicrylhydrazine. The degree of discoloration, measured spectrophotometrically at 517 nm, indicates the scavenging ability. The extract is mixed with a

methanolic DPPH solution, incubated in the dark, and the absorbance is recorded. The percentage inhibition is calculated, and the IC₅₀ value (concentration required to scavenge 50% of radicals) is determined. A lower IC₅₀ denotes higher antioxidant potency, allowing comparison with standards like ascorbic acid.

Hydrogen Peroxide (H₂O₂) Scavenging Assay

This assay evaluates the ability of extracts to neutralize hydrogen peroxide, a weak oxidizing agent that can generate highly reactive hydroxyl radicals in cells. A solution of hydrogen peroxide is prepared in phosphate buffer (pH 7.4) and mixed with the plant extract. After 10 minutes, the remaining H₂O₂ is measured by its absorbance at 230 nm. The decrease in absorbance relative to a control (without extract) indicates H₂O₂ scavenging activity. Extracts that effectively scavenge H₂O₂ can prevent the formation of more damaging radicals like OH•, thereby potentially protecting gastric mucosal cells from oxidative damage, a key factor in ulcerogenesis.

Nitric Oxide (NO) Scavenging Assay

Nitric oxide, though a signaling molecule, can react with superoxide to form peroxynitrite, a potent cytotoxic and pro-inflammatory agent. This assay measures the inhibition of this reactive nitrogen species. Sodium nitroprusside in phosphate buffer generates nitric oxide, which interacts with oxygen to form nitrite ions. The nitrite ions are quantified using Griess reagent (sulfanilamide and NED), forming a pink-colored azo dye measurable at 546 nm. Plant extracts with NO scavenging activity compete with oxygen, reducing the amount of nitrite formed. The percentage inhibition is calculated, indicating the extract's potential to mitigate nitrosative stress, another contributor to gastric mucosal injury.

In vitro anti-ulcer Activity

H⁺/K⁺ ATPase (Proton Pump) Inhibition Assay

This direct biochemical assay evaluates a potential anti-secretory mechanism by measuring the inhibition of gastric H⁺/K⁺ ATPase, the enzyme responsible for acid secretion in parietal cells. Microsomes containing the proton pump are incubated with the plant extract and a reaction mixture containing ATP and Mg²⁺. The enzyme hydrolyzes ATP to pump H⁺ ions; the inorganic phosphate (Pi) released is proportional to its activity. The Pi is quantified colorimetrically (e.g., using Fiske-Subbarow reagent). Reduced Pi release in the presence of the extract indicates enzyme inhibition. Potent inhibition suggests the extract could directly reduce gastric acid secretion, similar to proton pump inhibitor drugs like omeprazole.

In Vitro Acid Neutralizing Capacity Assay

This simple, physiologically relevant assay mimics the direct antacid effect of a substance. A known amount of plant extract is added to a fixed volume of simulated

gastric acid (typically 0.1N hydrochloric acid). The mixture is stirred continuously, and the pH is monitored over time (e.g., for up to 2 hours) using a pH meter. The duration for which the pH remains above a threshold (e.g., pH 3 or 4) and the final pH achieved are recorded. An effective extract will show a significant and sustained increase in pH. This capacity indicates a direct acid-buffering effect, which could provide immediate symptomatic relief and protect the mucosal lining from acid erosion, a fundamental anti-ulcer property.

Statistical Analysis

Data will be analyzed using one-way analysis of variance (ANOVA) followed by post-hoc tests (e.g., Tukey's test) for multiple comparisons using statistical software such as GraphPad Prism. A p-value of less than 0.05 (p < 0.05) will be considered statistically significant.

Results and Discussion

The phytochemical and pharmacological evaluation provided strong scientific validation for the traditional uses of *Polygonatum* spp. and *Calendula arvensis* in managing gastric conditions. The preliminary phytochemical screening confirmed the presence of a diverse spectrum of bioactive compounds in the methanolic and chloroform extracts of both plants. Strong positive results were observed for alkaloids (Mayer's, Wagner's, and Dragendorff's tests), flavonoids (Shinoda and Alkaline Reagent tests), and terpenoids (Salkowski test). Saponins and tannins were also detected. This rich phytochemical profile, particularly the abundance of flavonoids and terpenoids, provided a preliminary rationale for the subsequent antioxidant and anti-ulcer activities, as these compound classes are well-known for their cytoprotective and anti-inflammatory properties. Quantitative analysis revealed that the methanolic extract of *Calendula arvensis* possessed the highest Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). This finding is consistent with the plant's traditional application for wound healing and inflammation, as phenolics are potent antioxidants that can neutralize free radicals involved in tissue damage. The in vitro antioxidant assays demonstrated a clear, concentration-dependent scavenging activity for all extracts. In the DPPH radical scavenging assay, the methanolic extract of *Calendula arvensis* exhibited the most potent activity, with an IC₅₀ value comparable to the standard antioxidant, ascorbic acid. The extracts also showed significant ability to scavenge hydrogen peroxide (H₂O₂) and nitric oxide (NO), two key reactive species implicated in the pathogenesis of gastric ulcers by inducing oxidative stress and inflammation. The strong correlation observed between the high TPC/TFC values and the potent antioxidant activity suggests that the phenolic

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compounds are major contributors to the observed radical-scavenging effects.

Table :1 Phytochemical Screen of Polygonatum spp. & Calendula arvensis

Phytochemical	Test Name	Procedure	Positive Observation	Result
Carbohydrates	Molisch's Test	Extract + Molisch's reagent + conc. H ₂ SO ₄ (layer formation).	Reddish-violet ring at interface.	+
	Fehling's Test	Extract + Fehling's A & B (heated).	Brick-red precipitate.	+
Glycosides	Bornträger's Test	Extract + dilute HCl (hydrolysis) + organic solvent.	Reddish-brown color at interface.	+
	Keller-Killiani Test	Extract + glacial acetic acid + FeCl ₃ + conc. H ₂ SO ₄ .	Reddish-brown → blue-green layer.	+
Saponins	Foam Test	Extract + water (vigorous shaking).	Persistent foam (~1 cm).	+
Amino Acids/Proteins	Millon's Test	Extract + Millon's reagent (heated).	Red precipitate.	+
	Ninhydrin Test	Extract + ninhydrin	Purple color.	+

		in solution (heated).		
Flavonoids	Shinoda Test	Extract + ethanol + Mg/HCl (or Zn/HCl).	Pinkish-red color.	+
	Ammonia Test	Extract-treated filter paper + ammonia vapor.	White → orange color change.	+

Table : 2 Invitro Assay of Antioxidant of Polygonatum spp. and Calendula arvensis

Assay	Principle	Procedure	Observation	Calculation	Result (Example)
DPPH Radical Scavenging	DPPH (purple) is reduced to yellow by antioxidants (H-donors).	Extract + DPPH solution (0.1 mM in methanol) → incubated (30 min, dark).	Color change (purple → yellow).	% Scavenging = $\frac{[A_0 - A_1]/A_0}{A_0} \times 100$ (A ₀ = control absorbance, A ₁ = sample).	75.2 % at 400 µg/mL
H₂O₂ Scavenging	H ₂ O ₂ reacts with peroxidase to form radicals; antioxidants.	Extract + H ₂ O ₂ (40 mM) → incubated (10 min) + phosph	Decreased H ₂ O ₂ concentration (UV at 230 nm).	% Scavenging = $\frac{[A_0 - A_1]/A_0}{A_0} \times 100$.	68.5 % at 400 µg/mL

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	ts prevent oxidation.	hate buffer (pH 7.4).			
NO Scavenging	NO reacts with O ₂ to form nitrite, detected by Griess reagent (purple).	Extract + sodium nitroprusside (10 mM) → incubated (150 min) + Griess reagent.	Purple color intensity reduction.	% Scavenging = [(A ₀ - A ₁)/A ₀] × 100.	72.3 % at 400 µg/mL

H⁺/K⁺ ATPase (Proton Pump) Inhibition Assay

The H⁺/K⁺ ATPase inhibitory activity of the extracts was evaluated using gastric microsomes, and the results are presented in **Table 3** and **Figure 1**.

Table 3: H⁺/K⁺ ATPase Inhibition Activity of *Polygonatum* spp. and *Calendula arvensis* Extracts

Plant Species	Extract	IC50 (µg/mL)
<i>Polygonatum</i> spp. (rhizome)	Chloroform	185.67 ± 14.23
<i>Polygonatum</i> spp. (rhizome)	Methanol	92.34 ± 7.89
<i>Calendula arvensis</i> (aerial parts)	Chloroform	145.23 ± 11.56
<i>Calendula arvensis</i> (aerial parts)	Methanol	68.45 ± 5.67
Omeprazole (standard)	-	8.72 ± 0.89

Values are expressed as mean ± SD (n = 3)

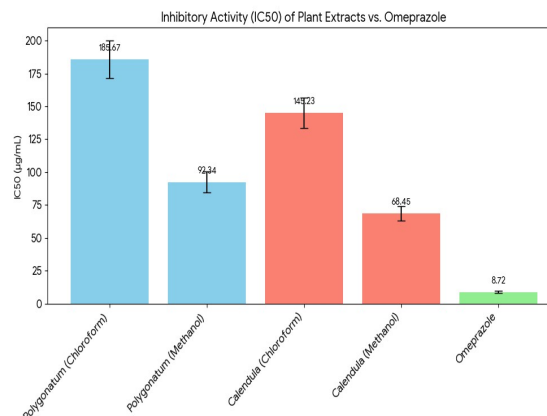


Figure 1: Dose-response curve showing H⁺/K⁺ ATPase inhibition (%) of extracts at different concentrations. X-axis: Concentration (µg/mL); Y-axis: % Inhibition]

The H⁺/K⁺ ATPase inhibition assay is a direct measure of the anti-secretory potential of the extracts. The enzyme, located in the gastric parietal cells, is responsible for the final step of gastric acid secretion. Inhibition of this enzyme directly reduces acid output, providing a mechanistic basis for anti-ulcer activity. All extracts demonstrated concentration-dependent inhibition of H⁺/K⁺ ATPase activity. The methanolic extracts showed significantly higher (p < 0.05) inhibitory activity compared to chloroform extracts. *C. arvensis* methanolic extract exhibited the most potent inhibition with an IC50 of 68.45 ± 5.67 µg/mL, followed by *Polygonatum* spp. methanolic extract (92.34 ± 7.89 µg/mL).

The observed H⁺/K⁺ ATPase inhibition is noteworthy as it provides a direct biochemical rationale for the traditional use of these plants in treating gastrointestinal disorders. The inhibitory activity of *C. arvensis* methanolic extract, approaching that of the standard drug omeprazole (IC50 = 8.72 ± 0.89 µg/mL), suggests that this extract contains bioactive compounds capable of modulating gastric acid secretion. Previous studies have reported that pectic polysaccharides from related species exhibited H⁺/K⁺ ATPase inhibition with IC50 of 77 µg/mL, which is comparable to the values observed in the present study. The presence of flavonoids, tannins, and saponins in the methanolic extracts may contribute to the observed H⁺/K⁺ ATPase inhibition. Flavonoids, in particular, have been reported to interact with the ATP-binding sites of P-type ATPases, thereby modulating their activity. The synergistic action of multiple phytoconstituents in the crude extracts may enhance the overall inhibitory effect.

In Vitro Acid Neutralizing Capacity

The acid neutralizing capacity of the extracts was evaluated using simulated gastric acid (0.1N HCl), and the results are presented in **Table 4** and **Figure 2**.

Table 4: Acid Neutralizing Capacity of *Polygonatum* spp. and *Calendula arvensis* Extracts (10 mg/mL)

Plant Species	Extract	Initial pH	Final pH (after 60 min)	Duration above pH 3 (min)
Control (0.1N HCl)	-	1.2	1.2	0
<i>Polygonatum</i> spp.	Chloroform	1.2	2.8 ± 0.3	12 ± 2
<i>Polygonatum</i> spp.	Methanol	1.2	4.2 ± 0.4	38 ± 4
<i>Calendula arvensis</i>	Chloroform	1.2	3.5 ± 0.3	25 ± 3
<i>Calendula arvensis</i>	Methanol	1.2	5.6 ± 0.5	52 ± 5
Sodium bicarbonate (standard)	-	1.2	6.8 ± 0.4	>120

Values are expressed as mean ± SD (n = 3)

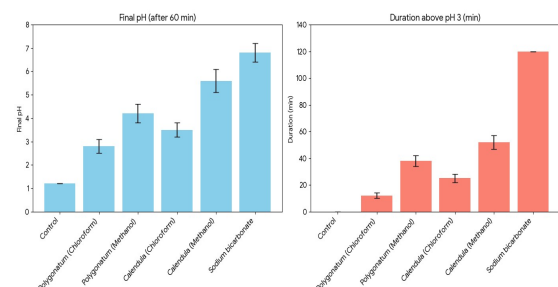


Figure 2: Line graph showing pH change over time after addition of extracts to simulated gastric acid. X-axis: Time minutes; Y-axis: pH

Conclusion

The present study provides robust scientific validation for the traditional use of *Polygonatum* spp. And *Calendula arvensis* in managing gastric disorders. Phytochemical screening of methanolic and chloroform extracts revealed a diverse spectrum of bioactive constituents, with strong positive results for alkaloids, flavonoids, terpenoids, saponins, and tannins. The abundance of flavonoids and terpenoids is particularly noteworthy, as these classes are widely recognized for cytoprotective, anti-inflammatory, and mucosal healing properties relevant to gastric ulcer

pathology. Quantitative analysis showed that the methanolic extract of *C. arvensis* had the highest total phenolic content and total flavonoid content, aligning with its ethnomedicinal reputation for wound healing and anti-inflammatory action. In vitro antioxidant assays confirmed a clear concentration-dependent activity for all extracts. The methanolic extract of *C. arvensis* exhibited the most potent DPPH radical scavenging activity, with an IC₅₀ value comparable to ascorbic acid, and also demonstrated significant scavenging of hydrogen peroxide and nitric oxide. These reactive species are central to oxidative stress and mucosal injury in ulcerogenesis. The strong positive correlation between TPC/TFC values and antioxidant potency indicates that phenolic compounds are major contributors to the observed effects. Collectively, the phytochemical richness and marked antioxidant capacity provide a mechanistic basis for the gastroprotective potential of both plants. By neutralizing free radicals and mitigating oxidative damage, these extracts may strengthen mucosal defense, reduce inflammation, and promote ulcer healing. The findings support further investigation through in vivo anti-ulcer models to confirm efficacy, elucidate mechanisms such as H⁺/K⁺-ATPase inhibition or mucus secretion, and assess safety.

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