ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 19, Issue 2, 2026

Online - 2455-3891
Print - 0974-2441

Research Article

COMPREHENSIVE PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL SCREENING OF ETHNOMEDICINAL PLANT EXTRACTS TO INVESTIGATE THEIR ANTI-INFLAMMATORY, ANTIOXIDANT POTENTIAL AND ANTIULCER EFFICACY

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Received: 12 November 2025, Revised and Accepted: 30 December 2025

ABSTRACT

Objectives: The objective of the study was to evaluate the phytochemical composition and antioxidant, anti-inflammatory, and anti-ulcer activities of selected ethnomedicinal plant stem bark extracts.

Methods: Stem bark extracts were prepared using standard solvent extraction methods and subjected to phytochemical screening and chromatographic profiling. Antioxidant activity was assessed by superoxide radical scavenging and reducing power assays. Anti-inflammatory effects were evaluated using the carrageenan-induced paw edema model, while anti-ulcer activity was determined using the pylorus ligation–induced gastric ulcer model. Gastric secretion parameters and histopathological analysis were performed to confirm gastroprotection.

Results: The extracts exhibited a high content of phenolics and flavonoids, correlating with strong, dose-dependent antioxidant activity. Significant reductions in ulcer index, gastric volume, and total acidity were observed, with effects comparable to standard drugs. Anti-inflammatory studies showed marked inhibition of paw edema. Histopathological findings confirmed preservation of gastric mucosal integrity and reduced lesion severity in treated groups.

Conclusion: The results validate the traditional use of the investigated ethnomedicinal plants and demonstrate that acetone and ethanol extracts possess potent antioxidant, anti-inflammatory, and anti-ulcer activities, supporting their potential development as natural gastroprotective agents.

Keywords: *Psidium guajava*, Phytochemical screening, Antioxidant activity, Anti-inflammatory activity, Anti-ulcer activity, Pylorus ligation model.

 DOI: http://dx.doi.org/10.22159/ajpcr.2026v19i2.57471. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

This article aims to comprehensively summarize and critically analyze research studies related to herbal drugs, with particular emphasis on their therapeutic applications. Relevant and recent publications were systematically retrieved from databases including PubMed, ResearchGate, and Google Scholar. Studies were selected based on predefined criteria such as relevance, scientific reliability, year of publication, journal quality, applicability of the findings, and accessibility of the full text. In addition to experimental investigations, studies focusing on the pharmacological evaluation relevance of herbal drugs and plant-derived phytoconstituents were also included [1]. According to the World Health Organization, nearly 80% of the population in developing countries relies on plant-based traditional medicines for primary health care. The high cost of treatment and the associated adverse effects of synthetic drugs have increased interest in herbal therapies. The therapeutic efficacy of medicinal plants is attributed to the presence of multiple bioactive constituents that act synergistically, which explains why whole plant extracts are often preferred over isolated compounds [2]. Medicinal plants are often regarded as chemical goldmines due to their wide acceptability by both human and animal systems. The World Health Organization estimates that nearly 80% of the population in developing countries depends on traditional medicine, predominantly plant-based drugs, to meet their primary health care needs. This reliance is largely attributed to the diverse pharmacological properties of medicinal plants, including antioxidant, antibacterial, antifungal, and antiviral activities. Traditional indigenous systems of medicine, such as Ayurveda, Siddha, and Unani, extensively utilize a wide range of plant species for the prevention and treatment of various ailments [3].

Psidium guajava L., commonly known as guava, is an important medicinal and nutritional plant belonging to the family Myrtaceae. Native to tropical and subtropical regions of Central America, it is now widely cultivated across Asia, Africa, and South America due to its strong adaptability and significant economic value. Guava has long been recognized in traditional medicine systems, including Ayurveda, Unani, and folk practices, for its wide range of therapeutic benefits [4,5].

The plant is rich in bioactive phytoconstituents, particularly flavonoids (quercetin, kaempferol), tannins, phenolic acids, triterpenoids, saponins, carotenoids, and essential oils. These constituents contribute to its diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiarrheal, antidiabetic, hepatoprotective, cardioprotective, and gastroprotective properties. The leaves, in particular, are extensively used for treating gastrointestinal disorders such as diarrhea, dysentery, ulcers, and abdominal pain [6,7].

Modern scientific investigations have validated many of its traditional uses. The strong antioxidant potential of *P. guajava* is attributed to its high polyphenolic content, which helps in neutralizing free radicals and reducing oxidative stress – a key factor involved in the pathogenesis of ulcers, inflammation, metabolic disorders, and chronic diseases. In addition, its antiulcer activity is linked to its ability to enhance mucosal defense, reduce gastric acidity, inhibit oxidative damage, and promote tissue healing [8].

Due to its nutritional richness – particularly Vitamin C, dietary fiber, and important micronutrients – *P. guajava* is considered a functional food with promising health benefits. Its low toxicity, wide availability,

and strong therapeutic potential make it an attractive candidate for pharmaceutical and nutraceutical development [8].

Given its ethnomedicinal relevance, phytochemical richness, and diverse biological activities, *P. guajava* continues to draw significant research interest for developing safe, natural, and effective therapeutic agents, especially for gastrointestinal and oxidative stress-related disorders.

METHODS

Collection, identification, and authentication of plant material

Fresh stem samples were collected from the local areas of Nanded City. The plant material was initially identified through its characteristic morphological features with assistance from a qualified taxonomist. Formal authentication was carried out by Dr. Shrirang S. Bodke, Associate Professor and Head, Department of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded. The reference number H-01 confirming the plant as *P. guajava* L. belonging to the family Myrtaceae.

Preparation of plant extracts

Coarsely powdered stem material of *P. guajava* L. (300 g) was first defatted thoroughly with petroleum ether (60–80°C) using a Soxhlet apparatus. The marc obtained after defatting was then successively extracted with acetone, followed by ethanol. Each extract was filtered through Whatman filter paper, concentrated to dryness, and stored in an airtight desiccator.

The percentage yield of each extract was subsequently calculated and given as per the following:

S. No.	Drug taken (g)	Solvent (ml)	Consistency	Color of extract	Yield (g)	% Yield
1	300 g	Pet-Ether (1000 ml)	Sticky	Dark Brown	0.5	0.16
2	297 g	Acetone (1000 ml)	Sticky	Dark Green	7	2.3
3	292 g	Ethanol (1000 ml)	Sticky	Dark Brown	9	3

The percentage yield of the acetone and ethanol extracts was calculated with respect to the initial weight of dried plant material. The acetone extract yielded 2.3%, whereas the ethanol extract yielded 3%.

Phytochemical screening of plant extracts

Preliminary phytochemical screening of the extract was carried out using standard qualitative tests.

Alkaloids were detected by adding Mayer's or Dragendorff's reagent to the extract, producing a characteristic precipitate.

Flavonoids were confirmed by the Shinoda test, where the addition of magnesium ribbon and concentrated hydrochloric acid yielded a pink or red color. Phenolic compounds were identified through the ferric chloride test, which produced a blue or green coloration.

Tannins also responded to ferric chloride by forming a blue-black or green-black color. Saponins were tested using the foam test, where vigorous shaking with water resulted in persistent froth.

Glycosides were detected by the Keller–Kiliani test, showing a brown ring at the interface.

Carbohydrates were identified by Molisch's test through the formation of a violet ring after adding α-naphthol and concentrated sulphuric acid.

Proteins were confirmed using the Biuret test, producing a violet color.

Steroids and terpenoids were detected using the Salkowski and Liebermann–Burchard tests, respectively, which produced characteristic reddish-brown or greenish-blue color changes.

In vitro anti-oxidant activity

Principle

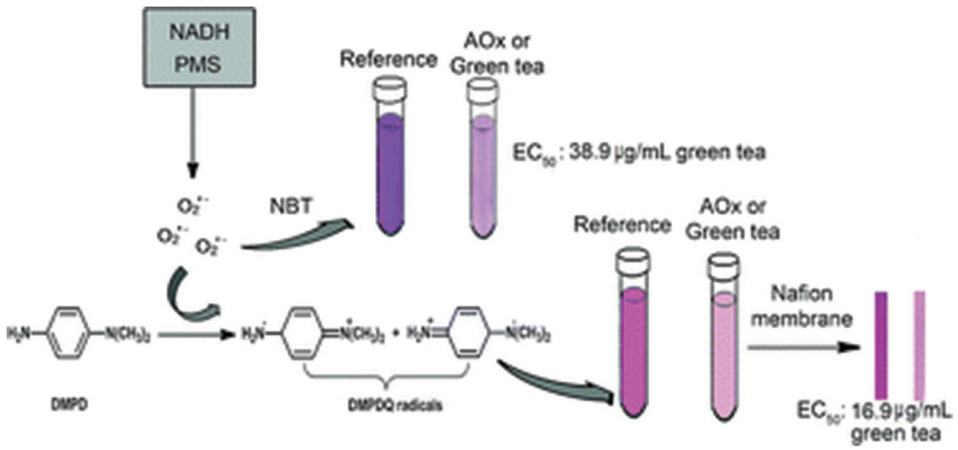
Superoxide anions are generated in a phenazine methosulfate–nicotinamide adenine dinucleotide system and reduce nitro blue tetrazolium (NBT) to a blue-colored formazan, which is measured at 560 nm. Antioxidants scavenge the superoxide anions and inhibit formazan formation.

The superoxide anion scavenging activity of the extracts was evaluated using the NBT reduction method. The reaction mixture contained phosphate buffer, NBT, riboflavin, and different concentrations of the test extracts. The tubes were exposed to light to initiate the photochemical generation of superoxide radicals, which reduce NBT to a blue-colored formazan. The decrease in absorbance at 560 nm in the presence of the extracts indicated their ability to scavenge superoxide radicals. A control was prepared without the extract, and ascorbic acid was used as the standard. The percentage inhibition was calculated to determine the antioxidant potential of each extract [9].

$$\% \text{ Inhibition} = (\text{Control value} - \text{Test value}/\text{Control value}) \times 100$$

Identification of targeted constituents by liquid chromatography–mass spectrometry (LC-MS/MS)

Following preliminary phytochemical screening, total phenolic content, total flavonoid content, and antioxidant activity studies, the selected Acetone and ethanolic extracts of *P. guajava* were subjected to LC-MS/MS for targeted analysis.



The analysis was performed using an Agilent 6460 Triple Quadrupole LC-MS/MS system (Agilent Technologies, USA) equipped with an Agilent 1260 Infinity II LC system. The mass spectrometric detection was conducted using a heated electrospray ionization source operated in both positive and negative ionization modes with unit mass resolution. The mass scan range was set from 100 to 800 atomic mass units (amu). High-purity nitrogen was employed as both the nebulizing and collision gas.

Fig. 1: *Psidium guajava* plant Photograph taken by the authors during field collection (Nanded, Maharashtra, India). Source: Author's own photograph (no permission required)

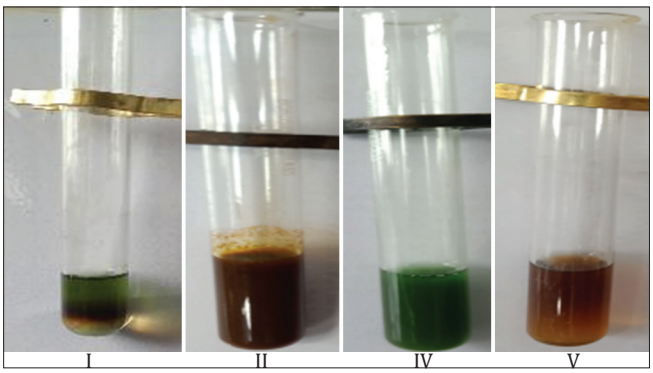


Fig. 2: Chemical tests of plant extracts

Instrument parameters were optimized as follows: Capillary voltage at 3500 V, gas temperature at 250°C, gas flow at 10 L/min, nebulizer pressure at 55 psi, sheath gas temperature at 350°C, sheath gas flow at 11 L/min, and nozzle voltage at 500 V. Separation was achieved using a Syncronis™ C18 silica column (100 mm × 4.6 mm, 5 µm; Agilent Technologies) under a stepwise gradient elution method. The mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B), as detailed in Table 2. The flow rate was maintained at 0.8 mL/min, with a total run time of 15 min and an injection volume of 5 µL. The column temperature was set at 25°C.

Mass spectral data were acquired in MS^2 scan mode, and molecular ion peaks were identified using MassHunter Workstation software (Agilent Technologies, USA) for qualitative analysis. Sample preparation involved vortexing using a Labtek multi-tube vortexer (Labtek Instruments, India) and centrifugation with an Eppendorf 5424 R centrifuge (Eppendorf, India).

Drugs and chemicals

The standard omeprazole at 20 mg per kg was purchased from Nitin Lifesciences Ltd. The test extracts, *P. guajava Acetone* extract (PGAE) and *P. guajava* ethanolic extract (PGEE) were made in the departmental laboratory at 100 mg/kg and 200 mg/kg, respectively. Common analytical chemicals such as petroleum ether, ethyl acetate, and methanol were acquired from Fine Chem Industries in Mumbai.

Experimental animals

The study used Wistar rats that weighed between 200 and 250 grams, with six rats in each group. The rats were obtained from the certified breeding centre, Biotox, in Nashik. The animals were cared for properly according to the guidelines set by the Committee for Control and Supervision of Experiments on Animals. The Institutional Animal Ethics Committee (IAEC) approved the study plan with Biotox/IAEC/03/2024/RP-23 approval.

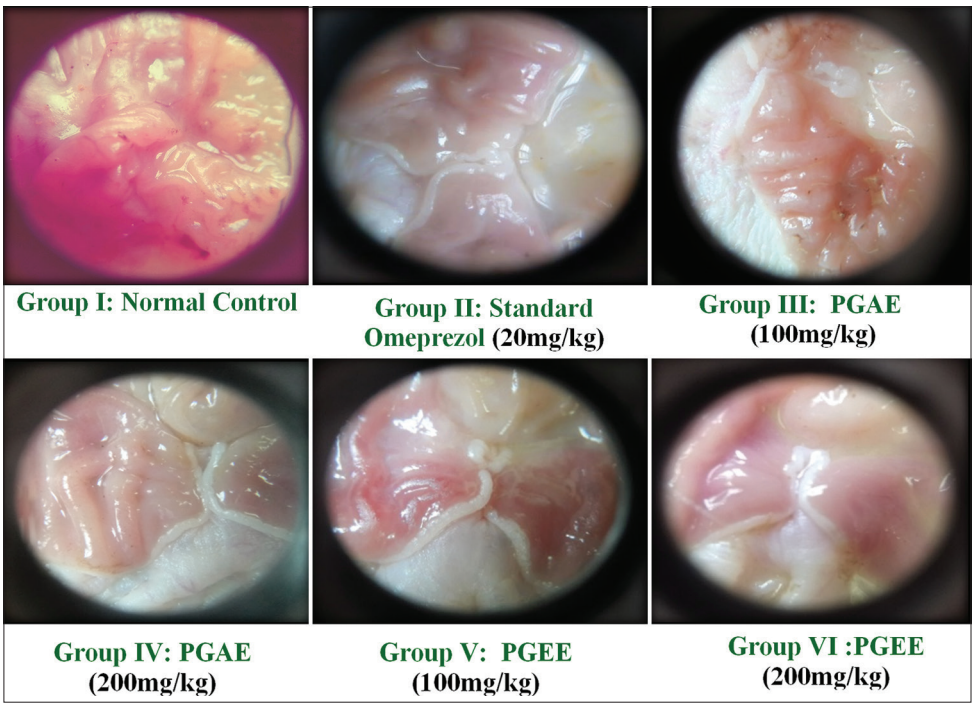


Fig. 3: Gross morphological appearance of the gastric mucosa in the pylorus ligation-induced ulcer model in rats. Panel I: Normal control group showing intact gastric mucosa; Panel II: Omeprazole-treated group showing marked protection against gastric lesions; Panel III: *Psidium guajava* acetone extract (PGAE)-treated group showing moderate protection; Panel IV: PGAE-treated group showing Good protection; Panel V: *P. guajava* ethanolic extract (PGEE)-treated group showing notable gastroprotective effect; Panel VI: PGEE-treated group showing Good gastroprotective effect

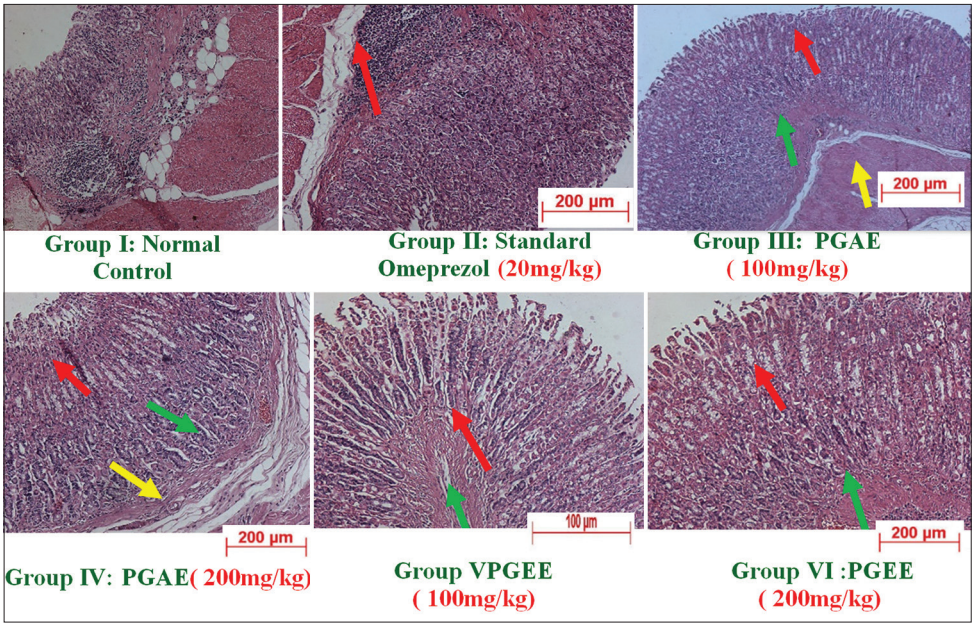


Fig. 4: Histological sections of gastric tissue (the staining method hematoxylin and eosin [H&E], stain, 100×) from different treatment groups showing varying degrees of mucosal damage and protection. Histopathological examination of gastric tissue in rats following treatment. Tissue sections were stained with H&E and observed under a light microscope at 100× magnification. Scale bar.=100–200 µm. Panel I: Normal control; Panel II: Standard drug-treated group; Panel III: PPAE test extract (100 mg/kg); Panel IV: PPAE Test extract (200 mg/kg); Panel V: PPEE test extract (100 mg/kg); Panel VI: PPEE test extract (200 mg/kg)

Table 1: Experimental design for carrageenan-induced rat paw edema

S. No.	Groups	Treatment
1	Group I	Animals were administered DMSO 0.5% dissolve in water.
2	Group II	Animals were administered 0.1 mL of 1% carrageenan rat to the sub-plantar region
2	Group III (Standard Group)	Standard group (carrageenan+indomethacin 10 mg/kg b. w. p. o.)
3	Group IV (Test Group)	PGAE (100 mg/kg); Animals were received 100 mg/kg of Acetone extract.
4	Group V (Test Group)	PGAE (200 mg/kg); Animals were received 200 mg/kg of Acetone extract
5	Group VI (Test Group)	PGETH (100 mg/kg); Animals were received 100 mg/kg Ethanolic extract
6	Group VII (Test Group)	PGETH (200 mg/kg); Animals were received 200 mg/kg Ethanolic extract

b.w.p.o.=By weight, per os (oral administration) *b.w.p.o.* indicates drug/extract dose expressed as milligrams per kilogram of body weight and administered orally

Table 2: Experimental grouping of animals and treatment protocol

S. No.	Group	Treatment description
1	Group I (Control Group)	Animals received saline water orally, followed by pylorus ligation.
2	Group II (Standard Group)	Animals received Omeprazole 20 mg/kg orally, followed by pylorus ligation.
3	Group III (Test Group)	PGAE 100 mg/kg: Animals received 100 mg/kg of acetone extract followed by pylorus ligation.
4	Group IV (Test Group)	PGAE 200 mg/kg: Animals received 200 mg/kg of acetone extract followed by pylorus ligation.
5	Group V (Test Group)	PGETH 100 mg/kg: Animals received 100 mg/kg of ethanolic extract followed by pylorus ligation.
6	Group VI (Test Group)	PGETH 200 mg/kg: Animals received 200 mg/kg of ethanolic extract followed by pylorus ligation.

PGAE=*Psidium guajava* acetone extract; PGEE=*Psidium guajava* ethanol extract. Each experimental group consisted of six animals (n=6)

Carrageenan-induced paw edema for anti-inflammatory activity

The anti-inflammatory activity of the acetone and ethanol extracts of *P. guajava* stem bark was evaluated using the carrageenan-induced rat paw edema model. Wistar rats (150–200 g) were divided into groups and administered the test extracts orally at doses of 100 and 200 mg/kg, while indomethacin (10 mg/kg) served as the standard drug and 0.5% CMC as the control. Treatment groups and dosing schedule in carrageenan-induced rat paw edema model given in table 1. One hour after treatment, acute inflammation was induced by injecting 0.1 mL of 1% carrageenan into the subplantar region of the right hind paw. Paw volume was measured before induction and again at 1, 2, 3, and 4 h after carrageenan injection using a plethysmometer. The increase in paw volume was calculated, and the percentage inhibition of edema was determined by comparing the treated groups with the control group. This model evaluates both early and late phases of inflammation, primarily mediated by histamine, serotonin, and prostaglandins [10-12].

Grouping of animals for above test was carried out as follows

The animals were divided into six experimental groups (n = 6 per group) as follows: Group I (Control) received saline orally followed by pylorus ligation; Group II (Standard) received omeprazole (20 mg/kg, p.o.) followed by pylorus ligation; Group III (Test) received acetone extract of *Psidium guajava* stem bark (PGAE, 100 mg/kg, p.o.) followed by pylorus ligation; Group IV (Test) received PGAE (200 mg/kg, p.o.) followed by pylorus ligation; Group V (Test) received ethanolic extract of *Psidium guajava* stem bark (PGETH, 100 mg/kg, p.o.) followed by pylorus ligation; and Group VI (Test) received PGETH (200 mg/kg, p.o.)

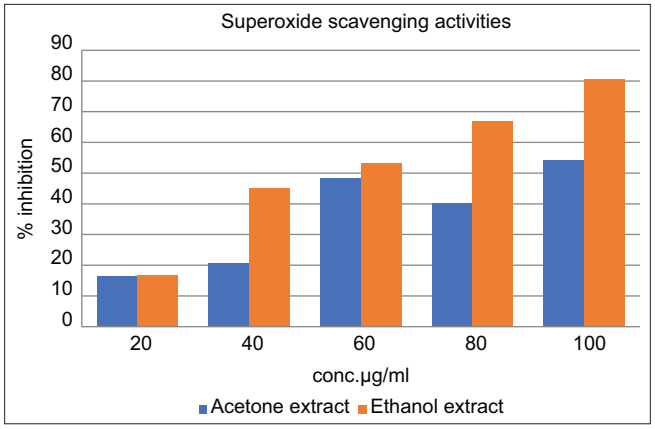


Chart 1: Effect of plant extract on antioxidant activity. X-axis: Concentration (µg/mL). Y-axis: Percentage antioxidant activity (%). Values are expressed as mean±standard error of the mean (n=6)

Table 3: Qualitative phytochemical screening of *Psidium guajava* stem bark extracts

S. No.	Phytochemical test	Petroleum ether extract	Acetone extract	Ethanol extract
1	Alkaloids	–	+	+
2	Carbohydrates	–	+	+
3	Proteins	–	+	+
4	Glycosides	–	+	+
5	Saponins	–	+	+
6	Amino acids	–	–	+
7	Steroids	–	+	+
8	Phenolic compounds	–	+	+
9	Flavonoids	–	+	+

Phytochemical screening was performed on *P. guajava* stem bark extracts. The symbols '+' and '–' indicate the presence and absence of the respective phytochemical constituents, respectively

followed by pylorus ligation.

Evaluation parameter of anti-inflammatory activity

Parameter to evaluate anti-inflammatory activity of the extract is the difference occurs in paw volume of rat.

Experimental design

The study used male and female Wistar rats weighing between 200 and 250 g. These animals were kept in a lab under standard conditions, which meant they had a 12-h cycle of light and darkness, and the temperature and humidity were controlled. The rats were given regular lab food and water for 7 days before the experiments started to help them get used to the environment. The study tested how effective the stem bark of *P. guajava* was in preventing ulcers. They used two methods to create ulcers: one by tying off the pylorus. The test groups were given plant extracts in Acetone, Ethanol, and ranitidine was used as the standard drug for comparison.

Pylorus ligation ulceration in rat

Scientists utilize pylorus ligation as a recognized experimental technique, which causes stomach ulcers in rats because it allows gastric secretions to build up and create erosion, followed by ulcer formation. The research involved Wistar albino rats, which weighed between 150 and 200 g. Before the procedure, the animals received 24 h of fasting. Animals were fasted for 24 h before sample collection to minimize variability in metabolic and biochemical parameters, such as serum glucose and lipid levels, which are influenced by recent food intake and can affect the interpretation of antioxidant and biochemical assays. Overnight fasting is a commonly used procedure in preclinical studies to establish a consistent baseline metabolic state before analysis [13]. While they could drink water freely [14].

Animals were divided into seven groups containing six members each (n=6).

The rats in Group I functioned as the negative control because they received 0.5% DMSO through oral administration.

Table 4: Superoxide radical scavenging activity of acetone and ethanol extracts of *Psidium guajava* L. stem bark at different concentrations

S. No.	Conc. (µg/mL)	Absorbance		% Inhibition	
		Acetone extract	Ethanol extract	Acetone extract	Ethanol extract
1.	20	0.644±0.002	0.607±0.001	16.55	16.82
2.	40	0.576±0.0021	0.411±0.003	20.70	45.19
3.	60	0.480±0.004	0.331±0.002	48.18	53.25
4.	80	0.340±0.006	0.260±0.004	40.18	66.90
5.	100	0.221±0.004	0.197±0.007	54.23	80.54

Values are expressed as mean±SEM. Conc.=Concentration expressed in micrograms per milliliter (µg/mL). A standard antioxidant was included as a reference control for comparative evaluation

Table 5: LCMS profiling of bioactive compounds identified in *Psidium guajava* stem bark extract

Compound	Compound class	Retention time (min)	Mode	Concentration (ng/mL)
Gallic acid	Phenolic acid	2.15	Positive	1846.78
Catechin	Flavonoid	3.48	Positive	2157.92
Quercetin	Flavonoid	4.12	Positive	1674.90
Quercetin-3-O-glucoside	Flavonoid glycoside	4.85	Positive	2076.67
Kaempferol-3-O-glucoside	Flavonoid glycoside	5.43	Positive	2559.74
Rutin	Flavonoid glycoside	6.22	Positive	2835.38
Corilagin	Hydrolyzable tannin	7.04	Positive	2122.58
Ellagic acid	Polyphenolic compound	8.26	Negative	914.11
Procyanidin B2	Condensed tannin	9.58	Negative	899.98
Guavin B (ellagitannin)	Ellagitannin	10.22	Negative	984.67
Myrecetine	Flavonoid	8.26	Negative	5122.58
Epigenin	Flavonoid	9.58	Negative	3081.73
Beta sitosterol	phytosterols	10.22	Negative	2359.74

All concentrations listed in the LCMS table are expressed as nanograms per milliliter (ng/mL) of the extract solution

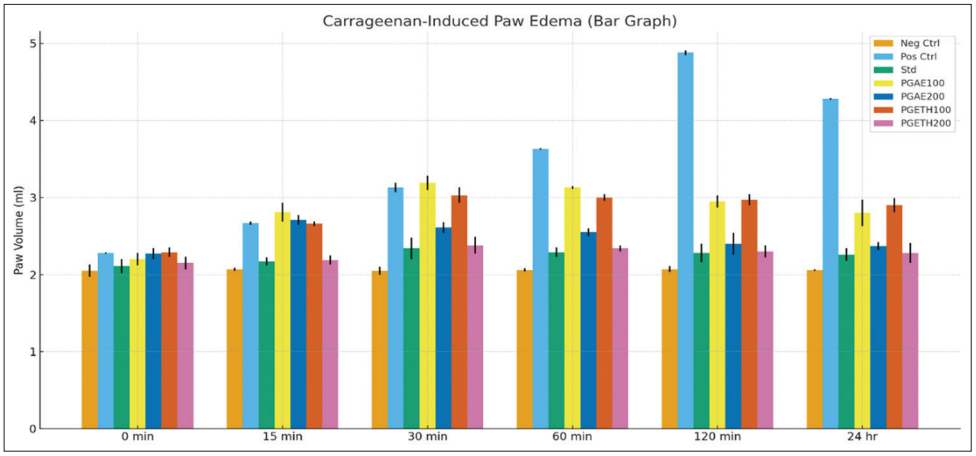


Chart 2: Anti-inflammatory activity of extract in carrageenan-induced paw edema model. X-axis: Time (h). Y-axis: Paw volume (mL). Values are mean±standard error of the mean (n=6)

Table 6: Anti-inflammatory activity of *Psidium guajava* stem bark extracts evaluated using the carrageenan-induced paw edema model

Experimental group	Mean paw volume (ml)					
	0 min	15 min	30 min	60 min	120 min	24 h
Negative control	2.05±0.08	2.07±0.02	2.05±0.05	2.06±0.02	2.07±0.04	2.06±0.01
Positive control (0.1 mL of 1% carrageenan)	2.28±0.01	2.67±0.02	3.13±0.06	3.63±0.01	4.88±0.03	4.28±0.01
Standard (indomethacin 10 mg/kg)	2.11±0.09	2.17±0.05*	2.34±0.14**	2.29±0.06**	2.28±0.12**	2.26±0.08**
PGAE (100 mg/kg p.o)	2.20±0.08	2.81±0.12*	3.19±0.09*	3.13±0.02*	2.95±0.08*	2.80±0.17*
PGAE (200 mg/kg p.o)	2.27±0.07	2.71±0.06**	2.61±0.07**#	2.55±0.05**	2.40±0.14**	2.37±0.05**
PGETH (100 mg/kg p.o)	2.29±0.06	2.66±0.03*	3.03±0.10*	3.00±0.04*	2.97±0.07*	2.90±0.09*
PGETH (200 mg/kg p.o)	2.15±0.08	2.19±0.06**	2.38±0.11**	2.34±0.04**#	2.30±0.08**#	2.28±0.13**#

Values are expressed as mean±SEM (n=6), *Significant difference (p<0.05 or less) and **Highly significant difference (p<0.001) when compared with control, (One way ANOVA followed by Tukey's test.) #p>0.05 non-significant difference when compared with standard

Table 7: Anti-ulcer activity of *Psidium guajava* stem bark extracts evaluated using the pylorus ligation–induced gastric ulcer model

Group	Treatment and dose mg/kg	Gastric volume (ml)	P^H	Total acidity	Ulcer index (In%)	% Protection
I	Control	1.13±0.003	1.32±0.003	6.21±0.001	96.22+0.614	-
II	Omeprezol (20 mg/kg p.o)	2.03±0.004**	5.12±0.003**	9.11±0.005**	23.81±1.092**	96.01**
III	PGAE (100 mg/kg p.o)	2.21±0.005**	4.17±0.011**	10.56±0.042**	26.98±1.486**	63.98**
IV	PGAE (200 mg/kg p.o)	2.30±0.019**	4.20±0.012**	9.18±0.033**	29.68±1.213**	70.58**
V	PGETH (100 mg/kg p.o)	2.40±0.008**	4.10±0.010**	10.45±0.033**	24.06±1.548**	82.51**
VI	PGETH (200 mg/kg p.o)	2.48±0.010**#	5.06±0.005**#	10.12±0.016**#	25.73±1.215**#	90.41**#

Data are expressed as mean±SEM (n=6). *p<0.05 and **p<0.01 compared with the normal control group; #p<0.05 compared with the disease control group. The values are represented as mean±SEM (n=6) for all groups and statistically significance between treated and control groups was analyzed using One-way ANOVA, Followed by Tukey's test. *p<0.05-Significant difference when compared with control, **p<0.001 Highly Significant difference when compared with control, #p>0.05-Non-Significant difference with Standard. Pg: *Psidium guajava,* AE: Acetone extract, ETH: Ethanol extract

Table 8: Histopathological evaluation of gastric tissues in pylorus ligation–induced ulcerated rats treated with *Psidium guajava* L. stem bark extracts

Group I	Control (Vehicle-saline water)	Microscopically shows necrosis of gastric mucosa, sub-mucosal edema, and hemorrhage.
Group II	Standard drug (Omeprazole 20 mg/kg)	Microscopically shows stomach fairly protected.
Group III	PGAE (100 mg/kg)	Microscopically show congestion of sub-mucosal blood vessels associated with edema.
Group IV	PGAE (200 mg/kg)	Microscopically shows normal gastric histology.
Group V	PGETH (100 mg/kg)	Microscopically show inflammation of sub-mucosal layer.
Group VI	PGETH (200 mg/kg)	Microscopically show normal gastric histology.

The rats in Group II functioned as the standard because they obtained omeprazole through intraperitoneal administration at 20 mg/kg [15]. The rats in Groups III and IV were given *P. guajava* stem bark extract (PGAE) by mouth at 100 and 200 mg/kg of body weight. The rats in Groups V and VI were orally given *P. guajava* ethanolic extract (PGEE).

Before the pylorus ligation procedure, all treatments took place 60 min in advance while using light ether anesthesia. Each animal was kept in a separate environment to prevent them from eating each other and consuming their feces. About one inch long, a small midline incision was made just below the xiphoid process. The pyloric end of the stomach was gently uncovered and handled carefully to avoid damage. It was then tied off to keep its blood supply safe. The stomach was put back into the abdomen, and the cut was sealed with stitches. The animals were put to sleep 4 h later, and their stomach tissues were removed. The collected stomach samples were put into marked centrifuge tubes for measurement [16,17].

Then the gastric juice pH was recorded, followed by a centrifugation process to separate the supernatant. The research team used 0.1 N

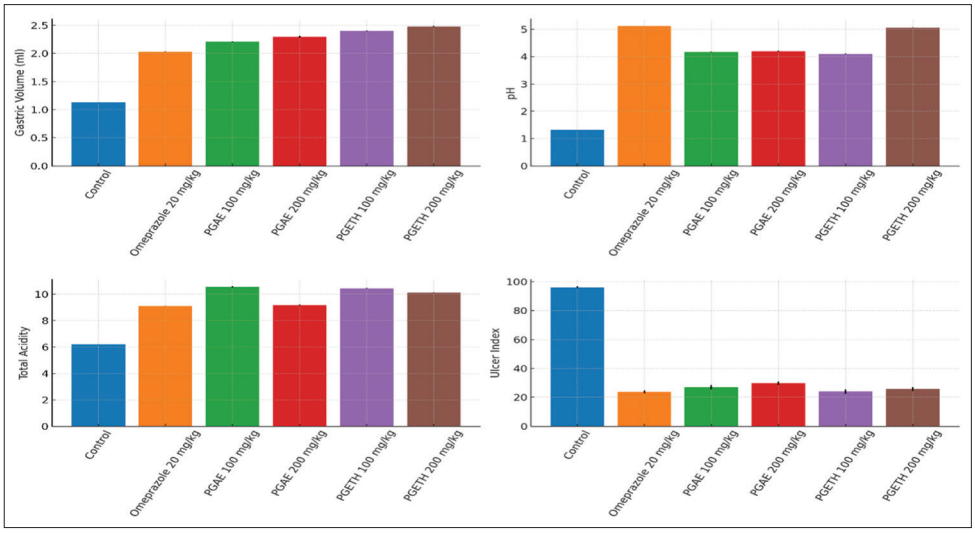


Chart 3: Effect of treatments on gastric volume, pH, total acidity, ulcer index, and % protection in pylorus-ligated rats

NaOH for titration to determine free and total acidity levels. Afterwards, they opened the stomachs through their greater curvature before securing them on cork boards to perform ulcer evaluation under a binocular microscope [18,19].

The ulcer index measurement occurred through a visual assessment of the pinned stomach tissue.

Determination of ulcer index

After the sacrifice, the stomachs were removed and cut along the greater curvature. The stomach contents were carefully washed with tap water to remove solid particles. Each stomach was placed on a glass slide and examined under 10 times magnification to check the depth of ulcers and whether they were present. The average ulcer score was determined using standard methods and presented as an index. The level of ulcer protection was also measured by comparing the results to those of the control group [20,21].

Ulcer score system [22]

Vogel's ulcer scoring system (widely used in pylorus ligation models).

Many anti-ulcer studies adopt the scoring criteria where the stomach is examined macroscopically, and lesions are graded (0–3 scale), and an ulcer index is calculated. A representative example and reference is:

Scoring of ulcer was performed based on severity and number of lesions (0=Normal stomach, 0.5=Red coloration, 1=Spot ulcer, 1.5=Hemorrhagic streaks, 2=Ulcers, 3=Perforation), and ulcer index was calculated. Normal stomach: 0, Red color: 0.

5, Spot ulcers: 1, Hemorrhagic streaks: 1. 5, Ulcers: 2, Perforation: 3

Calculation of ulcer index: $U1=UN+US+UP\times10^{-1}$

Percentage of protection=(Control mean ulcer index−Test mean ulcer index)×100/Control mean ulcer index

Where:
UN=Average number of ulcers per animal;
US=Average severity score;
UP=Percentage of animals with ulcers.

The ulcer index refers to the average ulcer score per animal.

Calculation of percentage protection [23].

To calculate the percentage of protection, the following formula was used:

Percentage protection=(C−T/C)×100

Where:
C is the ulcer index of the control group
T is the ulcer index of the treated group.

Determination of gastric pH

Once the stomach was taken out, the contents inside were poured out through a small cut on the larger curve of the stomach into a graduated centrifuge tube. The mixture was then spun in a centrifuge at 1000 revolutions/min for 10 min. After spinning, the liquid on top was collected and tested for acidity level using a digital pH meter that had been properly calibrated [24].

Determination of total acidity

To determine the total acidity, 1 mL of previously spun gastric juice was mixed with 9 mL of distilled water in a 50 mL flask. Two drops of phenolphthalein were added, and the mixture was titrated with 0.1 N sodium hydroxide (NaOH) until a lasting pink color appeared. The amount of NaOH used was noted [25]. The volume of NaOH used was recorded, and acidity was calculated as:

$$\text{Acidity} = \frac{\text{V NaOH} \times \text{N} \times 100 \text{ mEq / L}}{0.1}$$

Where,
V=Volume of NaOH used (in mL)
N=Normality of NaOH.

Statistical analysis

All experimental results are expressed as mean±standard error of the mean (SEM). Statistical evaluation was carried out using one-way analysis of variance (ANOVA). When significant differences were observed by ANOVA, group comparisons were further analyzed using Tukey's *post hoc* multiple comparison test. Data analysis was performed using GraphPad InStat software (version 5). Differences were considered statistically significant at $p<0.05$, with higher levels of significance noted up to $p<0.001$, while $p>0.05$ was regarded as statistically non-significant when compared with the control group.

RESULTS

Phytochemical screening of plant extracts

The phytochemical screening given in table 2 shows that the ethanol extract contains the maximum number of phytoconstituents, followed by the acetone extract, while the petroleum ether extract shows the least presence of phytochemicals.

Alkaloids, carbohydrates, proteins, glycosides, saponins, steroids, phenolic compounds, and flavonoids are present in both acetone and ethanol extracts. Amino acids are detected only in the ethanol extract.

***In vitro* anti-oxidant activity**

Both acetone and ethanol extracts show a concentration-dependent increase in % inhibition. However, the ethanol extract exhibits significantly higher inhibitory activity than the acetone extract at all tested concentrations, with a maximum inhibition of 80.54% at 100 µg/mL (Table 4). This suggests that the ethanol extract possesses stronger bioactive potential compared to the acetone extract (Chart 1).

Identification of constituents by LC-MS technique

After considering preliminary phytochemical evaluation, total phenolic content, total flavonoid content, and antioxidant study, the selected methanolic and ethanolic extracts were subjected for HRLC-MS study.

The phytochemical profiling of the extract revealed a rich composition of bioactive compounds, predominantly belonging to the flavonoid, phenolic acid, tannin, and phytosterol classes (Table 5). Several compounds were detected in high concentrations, particularly Myrecetine (5122.58 ng/mL), Rutin (2835.38 ng/mL), Kaempferol-3-O-glucoside (2559.74 ng/mL), Epigenin (3081.73 ng/mL), Beta-sitosterol (2359.74 ng/mL), Catechin (2157.92 ng/mL), Corilagin (2122.58 ng/mL), and Quercetin-3-O-glucoside (2076.67 ng/mL). The dominance of flavonoids (such as Myrecetine, Epigenin, Rutin, Catechin, Quercetin derivatives) and tannins (Corilagin, Procyanidin B2, Guavin B) indicates that these are the major phytochemical constituents of the extract. These compounds are well known for their strong antioxidant, anti-inflammatory, anti-ulcer, and cytoprotective properties. The presence of phenolic acids (Gallic acid, Ellagic acid) and phytosterols (Beta-sitosterol) further enhances the therapeutic potential of the extract. Overall, the phytochemical profile suggests that the extract possesses significant pharmacological value, largely attributable to its high flavonoid and tannin content.

Pylorus ligation ulceration in rat

Treatment with PGAE and PGETH significantly improved gastric parameters compared to the control group. Both extracts increased gastric pH and volume, reduced total acidity and ulcer index, and showed dose-dependent gastroprotective activity given in tables 7 and 8 and also illustrated in chart 3. The ethanol extract (PGETH) demonstrated greater ulcer protection than the acetone extract (PGAE), with PGETH 200 mg/kg showing 90.41% protection, comparable to the standard drug omeprazole (96.01%). This indicates strong anti-ulcer potential, particularly for the ethanol extract.

DISCUSSION

The present study provides compelling evidence that the stem bark of *P. guajava* is a rich source of bioactive secondary metabolites with pronounced antioxidant, anti-inflammatory, and gastroprotective activities. Phytochemical screening revealed a predominance of phenolics and flavonoids, alongside alkaloids, glycosides, and tannins, all of which are widely recognized for their pharmacological relevance. Previous investigations on *P. guajava* leaves and bark have emphasized the central role of phenolic-rich fractions in mediating antioxidant and anti-inflammatory effects. The current findings not only corroborate these reports but also extend them by highlighting the stem bark as a particularly potent reservoir of therapeutically significant phytoconstituents.

The antioxidant activity, assessed through superoxide radical scavenging, demonstrated a clear and concentration-dependent response, with the ethanol extract exhibiting markedly superior efficacy compared to the acetone extract. The high level of inhibition (80.54% at 100 µg/mL) observed for the ethanolic extract underscores the effectiveness of polar solvents in extracting antioxidant-active compounds. This observation aligns with earlier studies on *P. guajava* and other members of the Myrtaceae family, wherein ethanolic extracts consistently exhibited enhanced radical-scavenging potential. Importantly, HRLC-MS profiling provided molecular-level validation of this activity by confirming the presence of major flavonoids such as myricetin, apigenin, rutin, and kaempferol-3-O-glucoside, as well as tannins including corilagin. These compounds are well documented to regulate oxidative stress through free radical neutralization, modulation of endogenous antioxidant defenses, and suppression of redox-sensitive inflammatory signaling pathways, thereby substantiating the robust antioxidant profile of the extracts.

The anti-inflammatory efficacy demonstrated in the carrageenan-induced paw edema model further reinforces the pharmacological relevance of *P. guajava* stem bark (Table 6). Both extracts significantly attenuated acute inflammation, particularly at the 200 mg/kg dose, with effects comparable to the non-steroidal anti-inflammatory drug indomethacin. Given that carrageenan-induced inflammation is mediated by a biphasic release of histamine, serotonin, prostaglandins, and proinflammatory cytokines, the observed inhibition suggests interference with multiple inflammatory mediators. Flavonoids such as quercetin derivatives, rutin, and myricetin – identified in the extracts – are known inhibitors of cyclooxygenase and lipoxygenase pathways and suppressors of cytokines such as tumor necrosis factor alpha and interleukin-1 beta. Similar mechanisms have been reported for *P. guajava* leaf and bark extracts, indicating a conserved anti-inflammatory pharmacodynamic profile across different plant parts (Chart 2).

The gastroprotective activity evaluated using the pylorus ligation-induced ulcer model revealed a significant reduction in ulcer index and gastric acidity, accompanied by a pronounced increase in percentage protection. Notably, the ethanol extract at 200 mg/kg achieved 90.41% protection, approaching the efficacy of the standard proton pump inhibitor omeprazole. This substantial gastroprotection may be attributed to the combined antioxidant and mucosal-protective effects of flavonoids and tannins, which are known to enhance gastric mucus secretion, stabilize epithelial integrity, and mitigate oxidative injury induced by excessive acid secretion. Earlier reports on *P. guajava* and related species have similarly linked anti-ulcer activity to phenolic-mediated attenuation of oxidative stress and inflammation, lending strong support to the mechanistic basis of the present findings.

Collectively, the integration of phytochemical characterization, HRLC-MS profiling, and *in vivo* pharmacological evaluation provides a coherent and mechanistically plausible framework supporting the therapeutic potential of *P. guajava* stem bark. The convergence of antioxidant, anti-inflammatory, and anti-ulcer activities underscores the multifaceted pharmacological relevance of this plant part and positions it as a promising candidate for further translational and drug development studies.

CONCLUSION

The study demonstrates that *P. guajava* L. stem bark extracts possess significant phytochemical richness and strong therapeutic potential. Both acetone and ethanol extracts showed potent antioxidant, anti-inflammatory, and anti-ulcer activities. LCMS analysis confirmed the presence of major bioactive compounds, especially and tannins which likely contribute to the observed pharmacological effects. The ethanol extract, particularly at 200 mg/kg, exhibited superior efficacy in antioxidant scavenging, inflammation reduction, and ulcer protection, comparable to standard drugs. These findings support the traditional medicinal use of *P. guajava* stem bark and suggest its potential application in developing natural therapeutic agents for oxidative stress, inflammation, and gastric ulceration.

AUTHORS' CONTRIBUTIONS

Shagufta A. Farooqui Conceptualization, writing original draft, data curation, formal analysis, visualization, and writing of both the original draft and the review. S. S. Patil. Supervision and validation were provided.

FUNDING SOURCES

No funding was available for this work

CONFLICTS OF INTEREST

Nil.

ACKNOWLEDGMENT

I would like to express my gratitude to the Department of Science and Technology for providing the research facility at the School of Pharmacy under DST-FIST, which I utilized for my project.

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