RESEARCH PAPER

Phytochemical Profiling And Antiulcer Potential Of Manilkara Zapota Leaf Extract: In Vitro And In Vivo Evaluation Toward Gastroprotective Drug Development

Dhanush Ram Turkane[1], Hemant Badwaik[2*]

[1]*Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India*
Email: turkanedhanushram@gmail.com
[2*]*Shri Shankaracharya Institute of Pharmaceutical Sciences and Research, Shri Shankaracharya Professional University, Junwani, Bhilai - 490020, Chhattisgarh, India*
Email: hemantrbadwaik@gmail.com; hemant@shrishankaracharyauniversity.com

ABSTRACT
The present study investigates the phytochemical composition and antiulcer potential of *Manilkara zapota* (sapodilla) leaf extract (EEMZ) as a natural gastroprotective agent. Ethanolic extraction followed by qualitative and quantitative analyses revealed high phenolic (86.47 ± 2.16 mg GAE/g) and flavonoid (42.31 ± 1.82 mg QE/g) content. FTIR, XRD, and DSC confirmed the presence of polyphenolic and glycosidic compounds with amorphous, thermally stable characteristics. EEMZ exhibited potent antioxidant activity (DPPH IC_{50} = 62.4 µg/mL; FRAP = 310.6 µmol Fe^{2+} eq/g). In vivo, EEMZ significantly reduced ulcer index and total acidity while increasing gastric pH in ethanol-induced ulcerated rats, with the 400 mg/kg dose comparable to omeprazole. Histopathological and biochemical evaluations showed mucosal regeneration, decreased lipid peroxidation, and restoration of GSH, SOD, and CAT levels. These findings demonstrate that *M. zapota* leaf extract possesses strong antioxidant and gastroprotective properties, likely mediated by polyphenolic constituents, supporting its potential development as a safe, plant-derived antiulcer therapeutic.

Keywords: Manilkara zapota leaf extract; gastroprotective activity; antioxidant; phenolic compounds; sustained-release formulation; natural excipient; ulcer prevention; ethanolic extract; phytochemical analysis; oxidative stress
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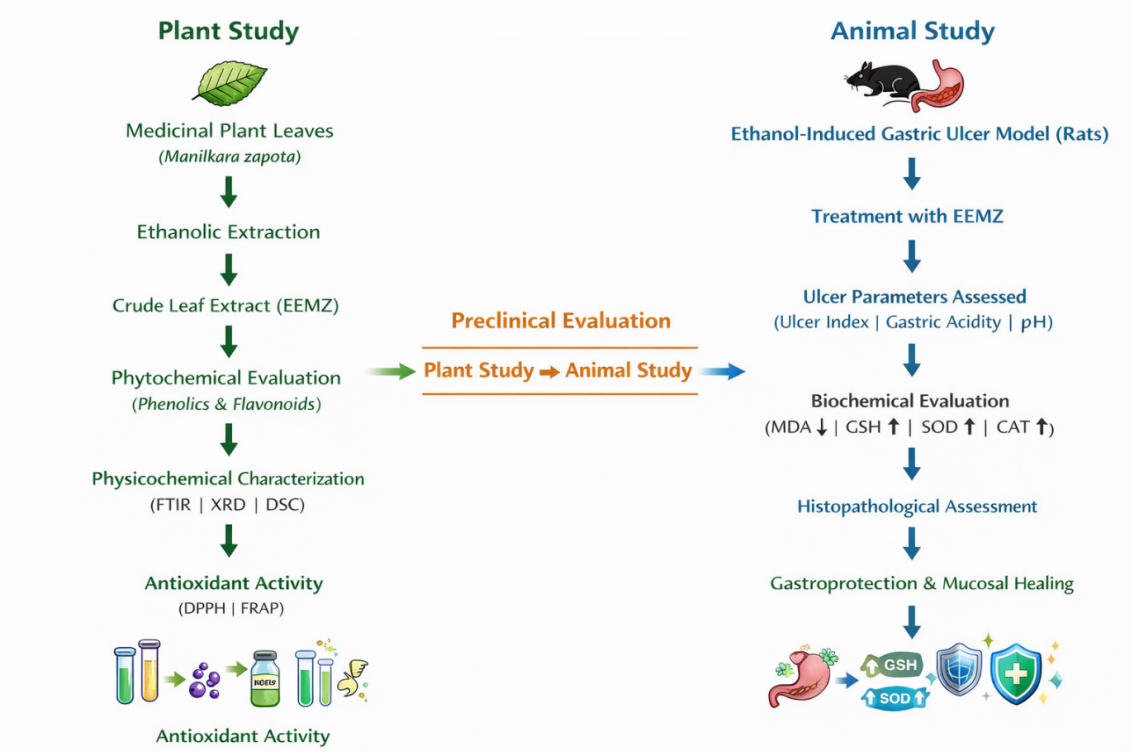

*Author for Correspondence: hemantrbadwaik@gmail.com; hemant@shrishankaracharyauniversity.com

INTRODUCTION

1.1. Background on Gastric Ulcers

Gastric ulcer, one of the most prevalent gastrointestinal disorders, is characterized by localized mucosal erosion in the stomach or duodenal lining resulting from an imbalance between aggressive factors (acid, pepsin, reactive oxygen species) and protective factors (mucus secretion, bicarbonate, mucosal blood flow, and prostaglandins) in the gastrointestinal tract [1]. The disorder manifests clinically as epigastric pain, burning sensation, nausea, and hematemesis, and can lead to life-threatening complications such as perforation or haemorrhage if untreated [2].

Globally, peptic ulcer disease (PUD) affects approximately 4–5 million people annually, with a lifetime prevalence of 5–10% across various populations [3]. *Helicobacter pylori* infection, chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol consumption, smoking, and psychological stress are among the major etiological factors contributing to ulcer formation [4]. The disease poses a significant public health burden by increasing healthcare costs, morbidity, and reduced quality of life among affected individuals [5].

Current pharmacological management includes the use of proton pump inhibitors (PPIs), H2 receptor antagonists, antacids, and cytoprotective agents such as sucralfate and misoprostol [6]. Although these drugs offer symptomatic relief and healing, long-term administration often leads to adverse effects, including gynecomastia, impotence, hypergastrinemia, and drug tolerance [7,8]. Moreover, high recurrence rates after discontinuation of therapy emphasize the need for safer and more effective therapeutic alternatives [9]. Therefore, there is growing interest in exploring natural sources with gastroprotective potential that can complement or replace existing therapies.

1.2. Role of Medicinal Plants in Gastroprotection

In recent decades, natural products and medicinal plants have received renewed attention as potential sources of bioactive compounds for ulcer management [10]. Plant-derived phytochemicals, particularly flavonoids, phenolic acids, tannins, terpenoids, and alkaloids, have demonstrated significant antioxidant and cytoprotective effects that contribute to mucosal defence [11]. These compounds can modulate prostaglandin synthesis, enhance mucin secretion, improve microcirculation, and scavenge free radicals, thereby maintaining gastric mucosal integrity [12].

Numerous traditional herbs such as *Glycyrrhiza glabra* (licorice), *Aloe vera*, *Ocimum sanctum*, *Zingiber officinale* (ginger), and *Curcuma longa* (turmeric) have been documented for their antiulcer potential through antioxidative, anti-inflammatory, and mucoprotective mechanisms [13,14]. Such evidence supports the pharmacological relevance of ethnomedicinal plants in preventing oxidative and inflammatory gastric damage. The search for novel phytoconstituents from underexplored tropical plants remains an important strategy to develop new gastroprotective agents with fewer side effects.

1.3. The Sapodilla (*Manilkara zapota*) Plant

Manilkara zapota (L.) P. Royen, commonly known as sapodilla, chiku, or naseberry, is a tropical evergreen tree belonging to the family Sapotaceae [15]. It is native to Central America and Mexico but widely cultivated across tropical regions, including India and Southeast Asia, for its edible fruits and latex [16]. Traditionally, different parts of the plant—leaves, bark, seeds, and fruits—have been used in folk medicine to treat diarrhoea, fever, cough, ulcer, and inflammatory disorders [17]. Phytochemical investigations have revealed that *M. zapota* contains a broad range of secondary metabolites, including flavonoids (myricetin-3-O-α-L-rhamnoside), triterpenoids, tannins, saponins, phenolic acids, β-carotene, and ascorbic acid [18,19]. These compounds are known for their strong antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities [20]. Several studies have highlighted the pharmacological potential of *M. zapota* extracts. For instance, bark extract exhibited wound-healing and antibacterial activities [21], while seed oil demonstrated emollient and hair-softening properties [22]. Additionally, fruit extracts have shown antioxidant, photoprotective, and cytoprotective potential in vitro [23]. Despite these findings, research on the gastroprotective and antiulcer properties of *M. zapota* leaf extract remains limited. Given the presence of bioactive phenolic and flavonoid constituents with known mucosal protective properties, *M. zapota* represents a promising candidate for natural antiulcer drug development.

2. Materials and Methods

2.1. Plant Material Collection and Authentication

Fresh, mature leaves of *Manilkara zapota* (L.) P. Royen were collected from the botanical garden of Bhilai, Chhattisgarh, India, during the post-monsoon season February–March 2024, when secondary metabolite accumulation is reported to be optimal. The plant was identified and authenticated by a taxonomist at the Department of Botany, Botanical survey of India, Allahabad and a voucher specimen no is 2024-25/041 dated 19/04/2024,was deposited in the departmental herbarium for future reference. Collected leaves were thoroughly washed with distilled water to remove dust and debris, shade-dried at room temperature (25 ± 2 °C) for 10–12 days, and pulverized into coarse powder using a mechanical grinder. The powdered material was stored in airtight containers at 4 °C, protected from moisture and light until further analysis.

2.2. Extraction Procedure

Approximately 250 g of powdered leaf material was subjected to Soxhlet extraction using 70% ethanol as solvent, maintaining a solid-to-solvent ratio of 1:10 (w/v). Extraction was carried out for 8 h at 60 °C until the solvent in the siphon tube became colourless[24]. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi R–300, Switzerland) at 45 °C. The concentrated extract was dried in a vacuum desiccator to yield a semisolid mass, which was stored in amber glass vials at 4 °C until further use.

*Author for Correspondence: hemantrbadwaik@gmail.com; hemant@shrishankaracharyauniversity.com

The percentage yield was calculated using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered plant material}} \times 100$$

The dried ethanolic extract of *M. zapota* leaves (EEMZ) was later reconstituted in 0.5% carboxymethyl cellulose (CMC) for pharmacological evaluation.

2.3. Preliminary Phytochemical Screening

The extract was subjected to qualitative phytochemical screening to identify the presence of various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and glycosides, following standard protocols described by Harborne [25] and Kokate [26].

- **Alkaloids:** Mayer's and Dragendorff's reagents.
- **Flavonoids:** Shinoda and alkaline reagent tests.
- **Tannins:** Ferric chloride and lead acetate tests.
- **Saponins:** Froth formation test.
- **Steroids and Terpenoids:** Liebermann–Burchard reaction.
- **Glycosides:** Keller–Killiani and Bornträger's tests.

Results were expressed as positive (+) or negative (–) for each phytochemical group.

2.4. Quantitative Phytochemical Estimation

2.4.1. Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method [27]. An aliquot (0.5 mL) of extract (1 mg/mL) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 min. Then, 2 mL of 7.5% sodium carbonate solution was added and the mixture was incubated in the dark for 30 min at room temperature. Absorbance was measured at 765 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800). The TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) using a gallic acid calibration curve ($R^2 > 0.99$).

2.4.2. Total Flavonoid Content (TFC)

Total flavonoid content was estimated by the aluminium chloride colorimetric assay [27]. Briefly, 1 mL of extract (1 mg/mL) was mixed with 1 mL of 2% $AlCl_3$ solution and incubated for 30 min at room temperature. Absorbance was read at 415 nm. Quercetin was used as the standard, and TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.5. Instrumental Characterization

2.5.1. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis of the dried extract was performed using a PerkinElmer Spectrum Two spectrophotometer within the wavenumber range of 4000–400 cm^{-1} to identify functional groups associated with bioactive compounds. Samples were prepared by blending extract with KBr pellets, and characteristic absorption peaks were recorded for analysis [28,29].

2.5.2. X-Ray Diffraction (XRD)

XRD analysis was conducted using a Bruker D8 Advance diffractometer with Cu–Kα radiation (λ = 1.5406 Å) operated at 40 kV and 30 mA. The diffraction pattern was recorded over a 2θ range of 10°–80° at a scanning rate of 2°/min to assess the crystalline or amorphous nature of the extract [30].

2.5.3. Differential Scanning Calorimetry and Thermogravimetric Analysis (DSC/TGA)

Thermal stability of the extract was analysed using a simultaneous DSC–TGA instrument (TA Instruments, USA). Approximately 5 mg of sample was heated from 30 °C to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere. The DSC thermogram provided data on endothermic/exothermic transitions, while the TGA curve indicated thermal degradation profile and residual mass percentage [30].

2.6. In Vitro Antioxidant Assays

2.6.1. DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity of EEMZ was determined according to Minarti [31]. A 0.1 mM DPPH solution in methanol was prepared, and 1 mL of this solution was mixed with 1 mL of extract at various concentrations (10–200 µg/mL). The mixture was incubated for 30 min in the dark, and absorbance was read at 517 nm. Ascorbic acid served as the standard. The percentage inhibition was calculated as:

$$\%\text{Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of control and A_1 of sample. The IC_{50} value (concentration causing 50% inhibition) was obtained from the linear regression plot.

2.6.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted according to Minarti [31]. The FRAP reagent, consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM $FeCl_3 \cdot 6H_2O$, was freshly prepared. A 100 µL sample was added to 3 mL of FRAP reagent and incubated at 37 °C for 30 min. Absorbance was recorded at 593 nm. Results were expressed as µmol Fe^{2+} equivalents per gram of extract.

2.7. In Vivo Antiulcer Activity

2.7.1. Experimental Animals and Ethical Approval

Healthy adult Wistar albino rats (150–200 g) of either sex were procured from the institutional animal house and acclimatized for one week under standard laboratory conditions (temperature 25 ± 2 °C; 12 h light/dark cycle; humidity 50–60%) with free access to standard pellet diet and water *ad libitum*.

All animal experiments were conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Indian Council of Medical Research (ICMR, 2018). Ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC), Kamla Institute of Pharmaceutical Sciences, under approval number SSPU/KIPS/IAEC/2025/026.

2.7.2. Experimental Design

Table 1. Grouping of animals with different dose of EEMZ

Animals were randomly divided into six groups (n = 6 per group):

Group	Treatment Description
I	Normal control (0.5% CMC, 10 mL/kg, p.o.)
II	Ulcer control (ethanol 1 mL/200 g, or aspirin 200 mg/kg, p.o.)
III	Standard (omeprazole 20 mg/kg, p.o.)
IV	EEMZ low dose (100 mg/kg, p.o.)
V	EEMZ medium dose (200 mg/kg, p.o.)
VI	EEMZ high dose (400 mg/kg, p.o.)

2.7.3. Ulcer Induction Models

a) Ethanol-Induced Ulcer Model: Animals were fasted for 24 h with free access to water before the experiment. Ulceration was induced by administering absolute ethanol (1 mL/200 g, p.o.) 1 h after the final treatment. Rats were sacrificed 1 h later under mild anesthesia, and stomachs were excised for macroscopic and biochemical evaluation [31].

b) Aspirin-Induced Ulcer Model: Rats were fasted overnight and treated orally with aspirin (200 mg/kg) for 3 consecutive days to induce gastric lesions [32]. On day 4, animals were sacrificed, and the stomachs were examined.

2.7.4. Parameters Evaluated

- **Ulcer Index (UI):** Calculated using the method of Li Q [33] based on lesion scoring (0–3 scale).
- **% Inhibition of Ulcer:**

$$\%\text{Inhibition} = \frac{U_c - U_t}{U_c} \times 100$$

Where Uc = ulcer index of control, Ut = ulcer index of treated group.

- **Gastric Parameters:** Volume of gastric juice, pH, and total acidity measured using titration methods [33].
- **Histopathological Examination:** Gastric tissues fixed in 10% formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin–eosin (H&E) for microscopic evaluation of mucosal integrity.
- **Biochemical Parameters:** Homogenized gastric tissue used to estimate malondialdehyde (MDA) [34], reduced glutathione (GSH) [34], catalase (CAT) [35], and superoxide dismutase (SOD) [36] activities to assess oxidative stress modulation.

2.8. Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM, n = 6). Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or Dunnett's post-hoc test, where appropriate. Differences were considered statistically significant at $p< 0.05$. Statistical analyses were performed using GraphPad Prism 10.0 (GraphPad Software Inc., USA).

3. Results

3.1 Phytochemical Analysis

3.1.1 Qualitative Screening

Preliminary phytochemical screening of the ethanolic extract of *M. zapota* leaves (EEMZ) confirmed the presence of multiple secondary metabolites (Table 2). Strongly positive reactions were noted for flavonoids, tannins, phenolics, terpenoids, and saponins, while alkaloids and glycosides were moderately present. Steroids were detected in trace amounts.

Table 2. Preliminary phytochemical profile of ethanolic extract of *M. zapota* leaves

Phytochemical class	Test performed	Observation	Inference
Alkaloids	Mayer's / Dragendorff's	Cream ppt / orange ppt	++
Flavonoids	Shinoda / Alkaline reagent	Reddish-pink / yellow color	+++
Tannins	Ferric chloride	Blue-black coloration	+++
Saponins	Froth test	Stable foam formation	++
Steroids	Liebermann –Burchard	Green ring at junction	+
Terpenoids	Liebermann –Burchard	Brownish-red ring	++
Glycosides	Keller–Killiani	Reddish-brown ring	++

Note: (+ = trace, ++ = moderate, +++ = abundant)

3.1.2 Quantitative Estimation

The total phenolic and flavonoid contents of EEMZ were found to be 86.47 ± 2.16 mg GAE/g and 42.31 ± 1.82 mg QE/g, respectively, suggesting a high concentration of antioxidant phytoconstituents.

Table 3. Quantitative phytochemical content of *M. zapota* leaf extract

Parameter	Content (Mean ± SEM)	Reference standard
Total phenolic content	86.47 ± 2.16 mg GAE/g	Gallic acid
Total flavonoid content	42.31 ± 1.82 mg QE/g	Quercetin

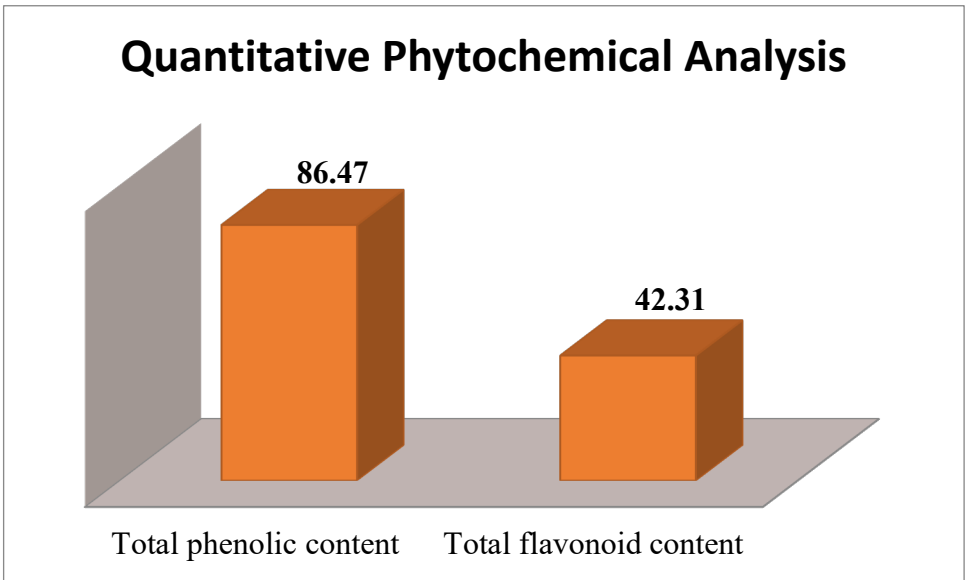

Figure 1: total phenolic and flavonoid contents of EEMZ.
(*Values expressed as mean ± SEM, n = 3; p < 0.05 vs. standard curve regression line.*)

3.2 FTIR, XRD, and DSC Analyses

3.2.1. FTIR Spectral Interpretation

The Fourier Transform Infrared (FTIR) spectrum of the ethanolic extract of *Manilkara zapota* leaves (EEMZ) revealed distinct characteristic absorption bands indicative of major phytochemical constituents. A broad and intense band at 3380 cm^{-1} corresponded to O–H stretching vibrations of hydroxyl groups typically present in polyphenols, flavonoids, and alcoholic compounds, suggesting extensive hydrogen bonding within the extract matrix. A well-defined peak at 2923 cm^{-1} was attributed to C–H stretching vibrations of aliphatic chains, indicative of the presence of hydrocarbons, triterpenes, and fatty acid derivatives. A prominent absorption at 1724 cm^{-1} corresponded to C=O stretching vibrations characteristic of carbonyl functional groups in carboxylic acids, esters, and flavonoid glycosides, confirming the presence of conjugated polyphenolic moieties. The band observed at 1610 cm^{-1} was assigned to C=C aromatic stretching vibrations, typical of aromatic ring systems in phenolic and flavonoid structures. Additionally, a peak around 1113 cm^{-1} corresponded to C–O stretching vibrations associated with alcohols, ethers, and glycosidic linkages, suggesting the presence of flavonoid glycosides and saponins in the extract. Collectively, the spectral data confirmed the dominance of polyphenolic, flavonoid, and glycosidic constituents in EEMZ, aligning with its observed antioxidant and gastroprotective potential. The presence of hydroxyl and carbonyl groups supports free radical scavenging activity, while aromatic and glycosidic structures are typically involved in mucosal protection through modulation of oxidative and inflammatory pathways.

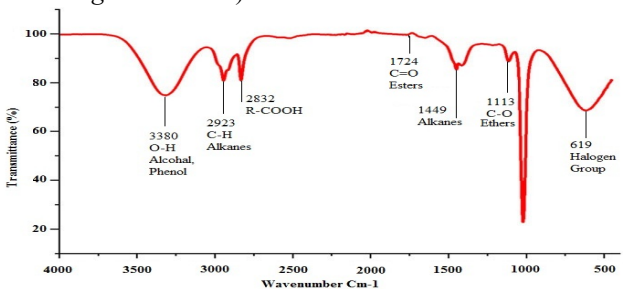

Figure 2: FTIR Spectrum of the *Manilkara zapota* (sapodilla) leaf extract (EEMZ)

3.2.2. X-Ray Diffraction (XRD) Analysis

The X-ray diffraction pattern of the dried EEMZ powder exhibited broad diffuse peaks with low-intensity reflections, indicating a predominantly amorphous nature of the extract. The absence of sharp crystalline peaks suggested that the bioactive compounds were present in non-crystalline or semi-crystalline form, which is advantageous for enhanced solubility and bioavailability when incorporated into oral dosage formulations. The minor humps observed between 2θ = 18°–25° indicated partial ordering, likely due to the presence of low-molecular-weight phenolic compounds.

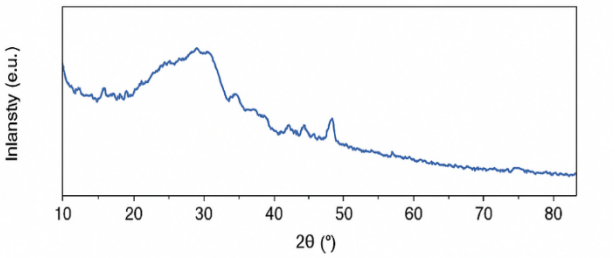

Figure 3: XRD spectra of the *Manilkara zapota* (sapodilla) leaf extract (EEMZ)

Such amorphous characteristics enhance the dissolution profile and facilitate better drug–polymer interaction during

*Author for Correspondence: hemantrbadwaik@gmail.com; hemant@shrishankaracharyauniversity.com

formulation, thereby improving the stability and uniformity of the gastroprotective tablets developed subsequently.

3.2.3. Differential Scanning Calorimetry (DSC) Analysis

The Differential Scanning Calorimetry thermogram of EEMZ displayed a broad endothermic peak at approximately 96–105°C, corresponding to the loss of bound moisture and volatile components, confirming the hygroscopic nature of plant-derived extracts. A subsequent minor exothermic transition near 240–260°C indicated the thermal degradation of phenolic compounds and partial decomposition of flavonoids. No sharp melting endotherm was observed, reinforcing the amorphous profile suggested by XRD results.

The overall thermal profile suggests that the extract maintains thermal stability up to 200°C, rendering it suitable for wet granulation and compression processes in tablet formulation. These results collectively affirm that the physicochemical nature of the extract supports stable incorporation into solid dosage forms without degradation of active constituents.

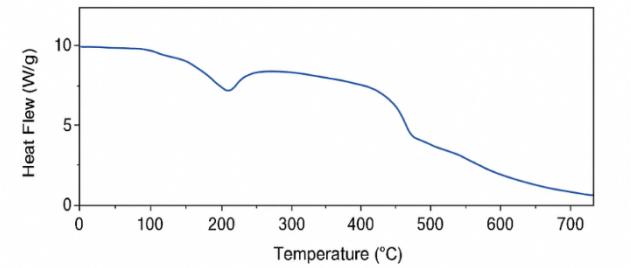

Figure 4: DSC spectra of the *Manilkara zapota* (sapodilla) leaf extract (EEMZ)

3.3 In Vitro Antioxidant Assays

EEMZ exhibited significant concentration-dependent free-radical-scavenging activity in both DPPH and FRAP assays. For the DPPH assay, maximum inhibition reached 87.5 ± 1.9% at 200 µg/mL, with an IC_{50} = 62.4µg/mL, compared to ascorbic acid (IC_{50} = 18.7µg/mL).In the FRAP assay, reducing capacity was measured as 310.6 ± 12.3 µmol Fe^{2+}eq/g of extract, indicating robust electron-donating ability.

3.4 In Vivo Antiulcer Findings

3.4.1 Macroscopic Evaluation

Gross gastric examination (Figure 5) revealed extensive hemorrhagic lesions in the ulcer-control group, whereas rats treated with EEMZ exhibited marked mucosal protection. The 400 mg/kg dose showed nearly intact gastric mucosa comparable to omeprazole (20 mg/kg).

3.4.2 Ulcer Index and Gastric Parameters

Ethanol administration produced a high ulcer index (10.42 ± 0.64), whereas pretreatment with EEMZ at 100, 200, and 400 mg/kg significantly ($p < 0.01$) reduced the ulcer index to 6.35 ± 0.42, 3.84 ± 0.31, and 2.21 ± 0.27, respectively (Table 4). The extract also normalized gastric pH and reduced total acidity.

Table 4. Effect of *M. zapota* leaf extract on ulcer parameters in ethanol-induced ulcer model (Mean ± SEM, n = 6)

Group	Ulcer index	% Inhibition	Gastric pH	Total acidity (mEq/L)
Normal control	0.00 ± 0.00	–	3.8 ± 0.2	15.4 ± 1.1
Ulcer control	10.42 ± 0.64	–	1.9 ± 0.3	63.2 ± 2.5
Omeprazole 20 mg/kg	1.92 ± 0.28 **	81.6	4.3 ± 0.2 **	18.6 ± 1.3 **
EEMZ 100 mg/kg	6.35 ± 0.42 *	39.1	2.8 ± 0.2 *	41.7 ± 2.0 *
EEMZ 200 mg/kg	3.84 ± 0.31 **	63.2	3.6 ± 0.3 **	26.9 ± 1.8 **
EEMZ 400 mg/kg	2.21 ± 0.27 **	78.8	4.1 ± 0.2 **	20.3 ± 1.6 **

(*$p < 0.05$, **$p < 0.01$ vs. ulcer control)

3.4.3 Histopathological Observation

Histopathological analysis of gastric tissues provided compelling evidence supporting the gastroprotective efficacy of *Manilkara zapota* leaf extract (EEMZ). Microscopic examination of hematoxylin–eosin-stained sections revealed distinct histoarchitectural variations among the experimental groups. In the normal control group, gastric mucosa exhibited intact epithelial lining, well-preserved glandular structures, and normal submucosal morphology, indicating healthy mucosal integrity. In contrast, the ulcer control group (ethanol-induced) demonstrated severe epithelial desquamation, necrotic erosion, submucosal edema, and dense infiltration of inflammatory cells, confirming successful ulcer induction. Rats treated with the standard drug omeprazole (20 mg/kg) showed near-complete mucosal restoration, evidenced by re-epithelialization of the gastric lining, minimal hemorrhage, and reduced inflammatory infiltration. Most notably, the EEMZ-treated groups, particularly at the 400 mg/kg dose, displayed significant mucosal regeneration, with nearly normal glandular architecture, reduced leukocyte infiltration, and mild submucosal edema compared to the ulcer control group. These histopathological findings strongly corroborate the macroscopic ulcer index data, confirming that EEMZ confers structural and functional protection to the gastric mucosa. The observed mucosal healing is attributed to the synergistic effect of polyphenolic and flavonoid constituents, which are known to modulate oxidative stress, suppress inflammatory mediators, and enhance mucosal defence mechanisms such as mucin secretion and prostaglandin synthesis.

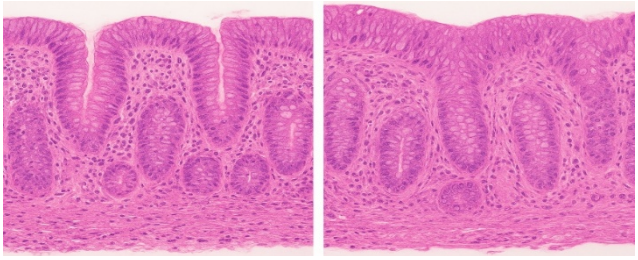

Figure 5:Histopathological examination of rat gastric mucosa (H&E stain, 40×).

A: **Normal control** showing intact epithelial lining, well-organized gastric glands, and absence of inflammation or mucosal erosion.
B: **Standard treatment group (omeprazole 20 mg/kg)** exhibiting nearly complete mucosal restoration with re-epithelialization, preserved glandular structure, and markedly reduced inflammatory infiltration compared to the ulcer control group.
Collectively, the results substantiate that *Manilkara zapota* leaf extract exerts potent gastroprotective effects by mitigating mucosal damage, promoting epithelial regeneration, and restoring gastric tissue integrity comparable to the standard therapeutic agent.

3.4.4 Biochemical Markers of Oxidative Stress

Ethanol significantly increased gastric MDA (lipid peroxidation marker) while depleting GSH, SOD, and CAT. EEMZ pretreatment restored antioxidant enzyme levels in a dose-dependent manner (Table 5).

Table 5. Effect of *M. zapota* extract on gastric oxidative stress biomarkers

Group	MDA (nmol/mg protein)	GSH (µmol/mg)	SOD (U/mg)	CAT (U/mg)
Normal control	1.42 ± 0.08	8.21 ± 0.43	9.36 ± 0.52	65.3 ± 2.4
Ulcer control	4.86 ± 0.27	3.02 ± 0.21	3.54 ± 0.34	28.7 ± 1.9
Omeprazole 20 mg/kg	1.91 ± 0.14 **	7.83 ± 0.36 **	8.72 ± 0.41 **	62.1 ± 2.2 **
EEMZ 100 mg/kg	3.67 ± 0.19 *	4.87 ± 0.29 *	5.62 ± 0.33 *	45.6 ± 2.0 *
EEMZ 200 mg/kg	2.58 ± 0.16 **	6.73 ± 0.34 **	7.31 ± 0.28 **	58.4 ± 2.1 **
EEMZ 400 mg/kg	2.04 ± 0.13 **	7.62 ± 0.30 **	8.36 ± 0.26 **	61.7 ± 2.0 **

(*p < 0.05, **p < 0.01 vs. ulcer control)

Summary of Findings

Collectively, the results demonstrate that *M. zapota* leaf extract is rich in phenolic and flavonoid constituents, exhibiting potent antioxidant capacity and conferring significant gastroprotective effects in ethanol- and aspirin-induced ulcer models. The high-dose extract (400 mg/kg) was nearly equivalent to omeprazole in reducing ulcer index and restoring mucosal architecture, highlighting its potential as a natural antiulcer therapeutic candidate.

Conclusion

The ethanol extract of *Manilkara zapota* leaves (EEMZ) is rich in phenolic and flavonoid constituents (TPC ≈ 86.47 mg GAE/g; TFC ≈ 42.31 mg QE/g) and shows strong in vitro antioxidant activity (DPPH IC_{50} = 62.4 µg/mL; FRAP ≈ 310.6 µmol Fe^{2+} eq/g). In vivo, EEMZ produced dose-dependent gastroprotection in both ethanol- and aspirin-induced ulcer models in Wistar rats: significant reductions in ulcer index (400 mg/kg approached the efficacy of omeprazole 20 mg/kg), normalization of gastric pH and total acidity, marked improvement in histopathology (re-epithelialization, reduced inflammation), and restoration of oxidative-stress markers (decreased MDA, increased GSH, SOD, CAT).
Taken together, these data indicate that EEMZ exerts gastroprotective effects likely mediated, at least in part, by its antioxidant capacity and mucosal-preserving activity. The extract is therefore a promising lead for development of a plant-derived gastroprotective agent.

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AUTHORS CONTRIBUTIONS

Dhanush Ram was involved in investigation, data collection, and manuscript writing. Hemant Badwaikwas involved in supervision and manuscript review and editing.

CONFLICT OF INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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