RESEARCH PAPER

# Phytochemical Characterization and Assessment of Anti-Ulcer Activity of *Psidium Guajava* Extract

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## ABSTRACT

*Psidium guajava* is a medicinal plant widely used in traditional medicine for gastrointestinal disorders. The present study aims to characterize the phytochemical constituents and evaluate the anti-ulcer activity of the ethanolic extract of Psidium guajava. The extract was prepared using Soxhlet extraction and subjected to preliminary phytochemical screening, revealing the presence of flavonoids, tannins, phenolics, saponins, and alkaloids. The anti-ulcer activity was evaluated using experimental models such as ethanol-induced and pylorus ligation-induced ulcer models. The extract exhibited significant gastroprotective activity, as evidenced by reduced ulcer index, decreased gastric acidity, and increased mucus production. The observed activity may be attributed to the presence of polyphenolic compounds and flavonoids. These findings suggest that Psidium guajava possesses promising anti-ulcer potential and could serve as a natural therapeutic agent.

**Keywords:** *Psidium guajava, Anti-ulcer activity, Gastroprotective effect, Ethanolic extract, Phytochemical screening, Flavonoids, Tannins, Phenolic compounds, Soxhlet extraction, Gastric ulcer models.*

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## INTRODUCTION

Medicinal plants have played a crucial role in traditional healthcare systems for centuries and remain invaluable sources for discovering new therapeutic agents. As chronic diseases become more common and synthetic drugs face challenges like side effects and drug resistance, there's a growing enthusiasm for plant-based medicines. These natural products are packed with a variety of phytoconstituents that offer a wide array of pharmacological benefits, including antioxidant, anti-inflammatory, antimicrobial, and gastroprotective properties. (1,2)

eptic ulcer disease stands as one of the most prevalent gastrointestinal issues, impacting a significant number of people worldwide. This condition is marked by the presence of lesions in the gastric or duodenal lining, resulting from a disruption between harmful elements like gastric acid secretion, pepsin activity, oxidative stress, and infection from Helicobacter pylori, and the body's protective measures, such as mucus production, bicarbonate generation, and the synthesis of prostaglandins. (3) Lifestyle elements like stress, alcohol use, smoking, and the extended use of non-steroidal anti-inflammatory drugs (NSAIDs) can significantly increase the risk of developing ulcers. While traditional anti-ulcer medications, including proton pump inhibitors and $H_2$-receptor antagonists, can be effective, relying on them for a long time may lead to various side effects, such as relapses, tolerance, and gastrointestinal issues. (4) This has sparked a growing curiosity about discovering safer and more effective alternatives derived from natural sources.

In recent years, there has been a growing interest in medicinal plants and their promising role in managing gastric ulcers. This is largely due to their remarkable ability to boost mucosal defense mechanisms and lower gastric acid secretion. One standout among these plants is Psidium guajava, or guava, a tropical gem from the Myrtaceae family that thrives in India and other tropical areas. Traditionally, various parts of this versatile plant, particularly its leaves, have been employed to tackle a range of health issues, including diarrhea, dysentery, inflammation, diabetes, and gastrointestinal disorders. (5,6)

xploring the phytochemicals in Psidium guajava has unveiled a treasure trove of bioactive compounds, such as flavonoids like quercetin and guaijaverin, along with tannins, phenolic acids, triterpenoids, and essential oils. These remarkable compounds are celebrated for their powerful antioxidant and anti-inflammatory effects, which are vital in safeguarding the gastric mucosa against damage from free radicals and inflammatory agents. (7) Flavonoids are known for their remarkable ability to reduce gastric acid secretion, boost mucus production, and enhance blood flow in the mucosal lining. These actions come together to create a protective effect for the stomach, showcasing the incredible benefits of these compounds.

Oxidative stress plays a significant role in the development of gastric ulcers. When there's an overproduction of reactive oxygen species (ROS), it can cause lipid peroxidation, damage to proteins, and compromise the integrity of the gastric mucosa. Fortunately, antioxidants from medicinal plants can help neutralize these harmful free radicals, protecting tissues from damage. Moreover, their anti-inflammatory properties can reduce the levels of inflammatory mediators like prostaglandins and cytokines, ultimately supporting the healing of ulcers. [8]

Even though Psidium guajava has a long history of traditional use and is believed to have various pharmacological benefits, it's essential to conduct a thorough scientific evaluation of its anti-ulcer properties. This study is set out to identify the phytochemical components and examine the anti-ulcer effects of the ethanolic extract of Psidium guajava through experimental models. The goal of this research is to offer scientific support for its traditional applications and to delve into its potential as a natural treatment option for managing peptic ulcer disease.

## MATERIALS AND METHODS

### 1. Plant Material Collection and Authentication

Fresh leaves of Psidium guajava were collected and authenticated by a qualified botanist. The leaves were washed, shade-dried, and powdered using a mechanical grinder. The powdered material was stored in an airtight container for further use. [9]

**Fig. 1. *Psidium guaja va (Leaf) (*Myrtaceae*)***

### 2. Preparation of Extract

The dried powdered material was extracted using ethanol in a Soxhlet apparatus for 6–8 hours. The extract was concentrated under reduced pressure using a rotary evaporator and dried to obtain a semisolid mass. The percentage yield was calculated and the extract was stored at 4°C until further use. [10]

**Fig.2. Soxhlet Extraction**

**Preliminary Phytochemical Screening**

A preliminary phytochemical screening was carried out on the ethanolic extract of Psidium guajava in order to identify the presence of important secondary metabolites responsible for various biological activities. Standard qualitative tests were performed for the detection of alkaloids (Mayer's and Dragendorff's tests), flavonoids (Shinoda test), tannins and phenolic compounds (Ferric chloride test), saponins (Foam test), glycosides (Keller–Killiani test), carbohydrates (Molisch's test), and proteins (Biuret test). These phytochemical investigations provide preliminary evidence for the presence of bioactive constituents that may contribute to antioxidant, anti-inflammatory, and gastroprotective activities. [11,12]

**Isolation of Flavonoid Fraction**

Column chromatography was employed for the isolation of the flavonoid-rich fraction from the ethanolic extract of Psidium guajava. Silica gel was used as the stationary phase, while toluene was initially used for column packing and sample loading. Approximately 20 g of ethanolic extract adsorbed on silica gel was carefully loaded onto the column. Elution was carried out using different ratios of toluene and ethyl acetate with gradually increasing polarity. The separated fractions were collected and monitored by thin-layer chromatography (TLC). Fractions showing similar TLC profiles were pooled together and further subjected to characterization and evaluation studies (13,14)

**Characterization of Isolated Fraction**

**Thin-Layer Chromatography (TLC)**

Thin-layer chromatography was carried out for the isolated flavonoid fraction obtained from the ethanolic extract of Psidium guajava using silica gel 60 F254 precoated TLC plates. The mobile phase consisted of toluene:ethyl acetate:formic acid (5:4:1). The developed TLC plates were visualized under UV light at 254 nm and 366 nm. Distinct fluorescent spots with characteristic Rf values confirmed the presence of flavonoid constituents in the isolated fraction. [15]

**UV–Visible Spectroscopy Characterization of Isolated Fraction**

The isolated flavonoid fraction of Psidium guajava was analyzed using a UV–visible spectrophotometer in the wavelength range of 200–800 nm. The spectrum exhibited characteristic absorption maxima around 245 nm and 275 nm, indicating the presence of flavonoid phytoconstituents. These absorption bands are mainly associated with aromatic benzoyl and cinnamoyl systems present in flavonoid compounds. [16]

**Fourier Transform Infrared Spectroscopy (FTIR)**

FTIR spectroscopy was performed to identify the functional groups present in the isolated flavonoid fraction of Psidium guajava within the scanning range of 4000–400 $cm^{-1}$. The obtained spectra showed characteristic absorption peaks corresponding to hydroxyl (–OH), carbonyl (C=O), aromatic C=C, and C–O stretching vibrations, confirming the presence of flavonoid-related functional groups in the isolated fraction. [17]

**Nuclear Magnetic Resonance (NMR) Spectroscopy**

The purified flavonoid fraction isolated from *Psidium guajava* was subjected to ^1H NMR and ^13C NMR analysis after dissolution in a suitable deuterated solvent. The obtained spectra revealed characteristic aromatic proton signals, hydroxyl proton peaks, and carbon resonances corresponding to flavonoid skeletal structures, thereby confirming the flavonoid nature of the isolated compound. [18]

**Mass Spectrometry**

Mass spectrometric analysis of the isolated fraction from the ethanolic extract of Psidium guajava was carried out using Electrospray Ionization Mass Spectrometry (ESI-MS). The molecular ion peak and fragmentation pattern obtained from the spectra confirmed the presence of flavonoid constituents in the isolated fraction and supported the structural characterization of the phytocompounds. [19]

**Anti-Ulcer Activity**

**1. Acid Neutralizing Capacity (ANC) Assay**

**Principle**

The Acid Neutralizing Capacity assay evaluates the ability of a test sample to neutralize gastric acid (HCl). It is commonly used to assess antacid-like activity of plant extracts. (19,20)

**Procedure**

- Accurately weigh the test extract (e.g., 500 mg).
- Add 25 mL of 0.1 N HCl.
- Stir the mixture for 15 minutes.
- Add 2–3 drops of phenolphthalein indicator.
- Titrate the excess acid with 0.1 N NaOH until a faint pink endpoint is obtained.
- Perform the same procedure for a blank (without sample). (20)

**Calculation**

$$ANC = (V_{blank} - V_{sample}) \times N$$

Where:

- $V_{blank}$= Volume of NaOH for blank (mL)
- $V_{sample}$= Volume of NaOH for sample (mL)
- $N$= Normality of NaO

**2. $H^+/K^+$-ATPase Inhibition Assay**

**Principle**

The $H^+/K^+$-ATPase enzyme (proton pump) is responsible for gastric acid secretion. Inhibition of this enzyme reduces acid production, similar to drugs like omeprazole. (21)

**Procedure**

- Prepare gastric mucosal homogenate (source of $H^+/K^+$-ATPase enzyme).

- Incubate enzyme with:
    - Test extract (different concentrations)
    - Standard drug (Omeprazole)
- Add ATP solution to initiate reaction.
- Incubate at 37°C for 20–30 minutes.
- Stop reaction using trichloroacetic acid (TCA).
- Measure inorganic phosphate (Pi) released using spectrophotometric method (usually at 400–660 nm depending on reagent). (21,22)

**Calculation of Enzyme Activity**

$$\text{Enzyme Activity} = \frac{\text{Amount of Pi released}}{\text{Time} \times \text{Protein concentration}}$$

Percentage Inhibition

$$\% \, Inhibition = \frac{Control - Sample}{Control} \times 10$$

**RESULTS AND DISCUSSION**

**Phytochemical Screening of Guava**

Preliminary phytochemical screening of the ethanolic extract of *Psidium guajava* leaves revealed the presence of sev1eral bioactive constituents. The results are summarized below:ochemical Screening (23,24)

**Table 1: Phytochemical Screening of *Psidium guajava* (Guava) Leaf Extract**

| Sr. No. | Phytoconstituents | Test Performed | Observation | Result |
|---|---|---|---|---|
| | | | | |
| 1 | Alkaloids | Mayer's / Dragendorff's Test | Creamy / Orange precipitate | +++ |
| 2 | Flavonoids | Shinoda Test | Pink / Red coloration | +++ |
| 3 | Tannins | Ferric Chloride Test | Blue-black / Green color | +++ |
| 4 | Phenolic Compounds | Ferric Chloride Test | Deep bluish-black color | ++ |
| 5 | Saponins | Foam Test | Persistent froth | + |
| 6 | Glycosides | Keller–Killiani Test | Brown ring formation | + |
| 7 | Carbohydrates | Molisch's Test | Violet ring | + |
| 8 | Proteins | Biuret Test | No violet coloration | – |
| 9 | Steroids | Salkowski Test | No reddish-brown ring | – |

Key
- (+++) = Abundantly present
- (++) = Moderately present
- (+) = Present
- (–) = Absent


**Fig.3.Phytochemical Screening**

**Chromatographic Column Separation**

The ethanolic extract of Psidium guajava was subjected to column chromatography using silica gel as the stationary phase and ethyl acetate:toluene:formic acid (5:4:1) as the mobile phase. During elution, the appearance of a distinct yellow-colored band indicated the successful separation of flavonoid-rich constituents based on differences in polarity. The collected eluted fractions were further analyzed by thin-layer chromatography (TLC), which resulted in the identification of two major fractions, namely F1 and F2. Each isolated fraction exhibited characteristic flavonoid profiles and was subsequently subjected to further characterization using UV–Visible spectroscopy, FTIR, NMR, and mass spectrometry techniques for confirmation of phytoconstituents. [25,26]

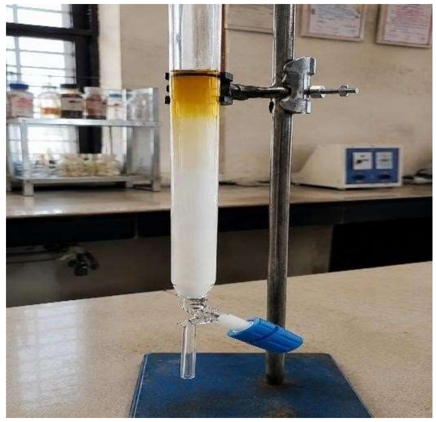


**Fig.4 Flavonoid-rich fraction extracted from *Psidium guajava* ethanolic extract using column chromatography**

**Thin-Layer Chromatography (TLC) [27,28]**

The separated fraction obtained from the ethanolic extract of Psidium guajava was subjected to thin-layer chromatography (TLC) using silica gel as the stationary phase and Ethyl acetate:Toluene:Formic acid (5:4:1) as the mobile phase. After chromatographic development, the TLC plate was observed under UV light, where a distinct fluorescent spot corresponding to the flavonoid-rich fraction was detected.

The isolated sample exhibited an Rf value of 0.54, which closely matched the standard flavonoid reference value, indicating the presence of flavonoid constituents in the extract. The similarity in Rf values between the standard and the test sample confirmed the successful isolation of flavonoid-rich phytoconstituents from the ethanolic extract of Psidium guajava. The TLC profile demonstrated effective separation and purification of the bioactive flavonoid fraction using the selected solvent system of Ethyl acetate:Toluene:Formic acid (5:4:1).

**Table 2: TLC Observation of Isolated Fraction**

| Sr. No. | Constituent | Mobile Phase | Ratio | Standard | Standard Rf Value | Sample Rf Value | Observation | Inference |
|---|---|---|---|---|---|---|---|---|
| 1 | Flavonoid | Toluene:Ethyl acetate:Formic acid | 5:4:1 | Quercetin | 0.54 | 0.53 | Yellow fluorescent spot observed under UV light | Flavonoids present |

**UV–Visible Spectroscopy [29,30]**

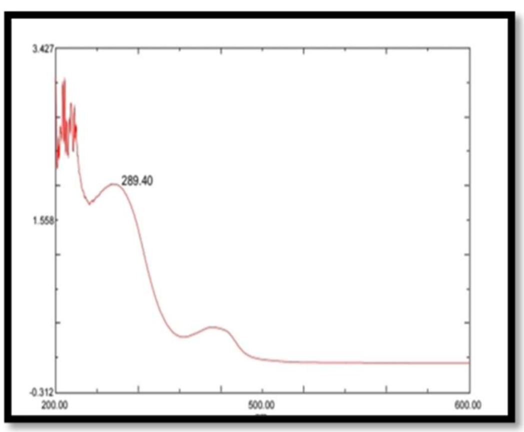



**Fig 5 Flavonoid Fraction UV-Visible Spectral Analysis**

**Table 3 UV-Visible Spectral Analysis**

| Sr, No. | Wavelength (nm) | Absorbance |
|---|---|---|
| 1 | 289.00 | 1 0.889 |

The isolated fraction from Psidium guajava exhibited characteristic absorption peaks at 255 and 275 nm during UV–visible spectroscopy analysis, indicating the presence of flavonoid and polyphenolic phytoconstituents. These absorption maxima are associated with π→π* electronic transitions of conjugated aromatic systems commonly observed in flavonoid compounds such as quercetin and guaijaverin present in guava leaves. The obtained spectral pattern confirmed that the isolated fraction contained polyphenolic compounds with significant antioxidant potential.

**FTIR Spectroscopy [31]**

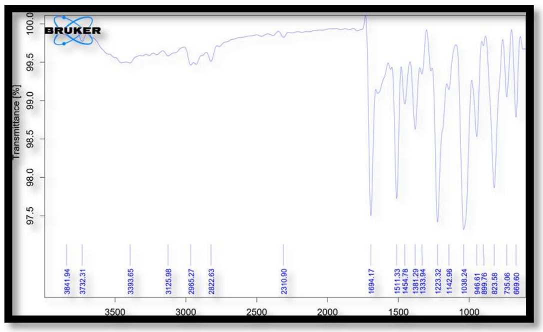



**Fig 6. FTIR spectra of isolated fraction of Guava extract**

**Table 4. FTIR Spectral Analysis of Fractions**

| Peak ($cm^{-1}$) | Functional Group |
|---|---|
| 3393.65 | O–H / N–H stretching |
| 2965.27 | C–H stretching |
| 1694.17 | C=O stretching |
| 1511.33 | Aromatic C=C stretching |
| 1381.29 | C–N stretching |

The FTIR spectral analysis of the isolated extract from Psidium guajava confirmed the presence of important functional groups associated with flavonoids and polyphenolic compounds. Broad absorption bands observed around 3400–3455 $cm^{-1}$ indicated O–H stretching vibrations corresponding to hydroxyl groups of phenolic and flavonoid compounds. Prominent peaks appearing in the region of 1650–1735 $cm^{-1}$ represented carbonyl (C=O) stretching vibrations, suggesting the presence of conjugated ketones and flavonoid structures. Peaks around 1510–1600 $cm^{-1}$ were attributed to aromatic C=C stretching vibrations, confirming aromatic ring systems characteristic of flavonoids present in guava leaves. Furthermore, peaks between 1000 and 1300 $cm^{-1}$ corresponded to C–O and C–O–C stretching vibrations of phenolic, alcoholic, and ether functional groups. The observed FTIR spectral pattern confirmed that the isolated extract was rich in flavonoid and polyphenolic phytoconstituents. (32,33)

**NMR Spectroscopy [34,35]**

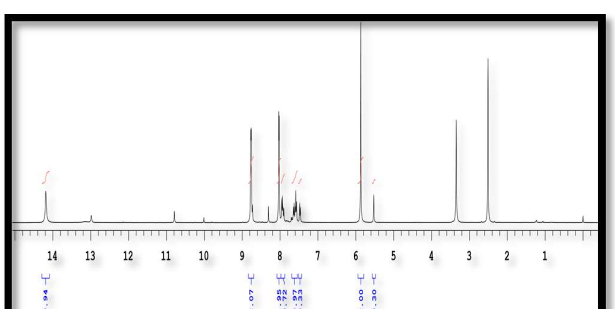



**Fig.7.1H NMR spectra of fractions**

**Table 5. Interpretation of 1H NMR spectral peaks of fractions**

| Chemical Shift (δ ppm) | Assignment |
|---|---|
| 6.8 – 8.2 | Aromatic protons |
| 5.2 – 5.5 | –OH proton |
| 2.5 – 3.5 | $–OCH_3$ proton |

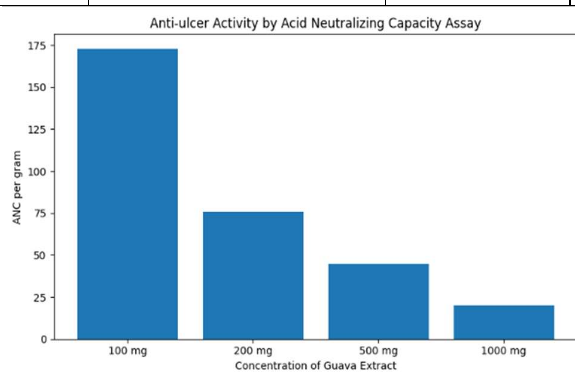
**ANTI-ULCER ACTIVITY**

- **The anti-ulcer activity of** the ethanolic extract was evaluated using **Acid Neutralizing Capacity (ANC)** and **$H^+/K^+$-ATPase inhibition assays**, which are widely used to assess the ability of plant extracts to neutralize gastric acid and inhibit gastric acid secretion.
- In the **Acid Neutralizing Capacity assay**, the extract was treated with excess hydrochloric acid (0.1 N HCl) followed by back titration with 0.1 N NaOH using phenolphthalein indicator. The amount of acid neutralized indicates antacid potential.
- In the **$H^+/K^+$-ATPase inhibition assay**, the enzyme (proton pump) responsible for gastric acid secretion was incubated with different concentrations of the extract (50, 100, and 200 µg/mL). The released inorganic phosphate was measured spectrophotometrically, and % inhibition was calculated.
- **Omeprazole** was used as the standard drug. Increased acid neutralization and higher enzyme inhibition indicate significant anti-ulcer activity. [36,37]

**Acid Neutralizing Capacity (ANC) Assay**

**Table 6. Acid Neutralizing Capacity**

| Sr. No. | Concentration of Guava Extract | NaOH Volume Consumed (mL) | Acid Consumed | ANC per gram | % Acid per gram |
|---|---|---|---|---|---|
| 1 | 100 mg Guava Extract | 34.6 | 17.30 | 173.0 | 57.66% |
| 2 | 200 mg Guava Extract | 30.4 | 15.20 | 76.0 | 50.66% |
| 3 | 500 mg Guava Extract | 44.8 | 22.40 | 44.8 | 74.66% |
| 4 | 1000 mg Guava Extract | 38.5 | 19.25 | 19.25 | 64.16% |



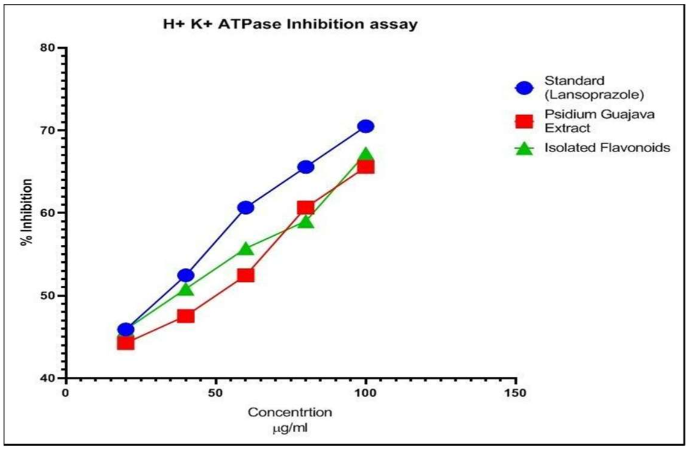

**Graph: Acid Neutralizing Capacity of Guava Fraction**

**$H^+/K^+$-ATPase Inhibition Assay**

**Table 7. $H^+/K^+$-ATPase Inhibition Assay of Guava Fraction**

| Sr. No | Sample Code | Concentration (µg/ml) | Absorbance | Percent Inhibition (%) |
|---|---|---|---|---|
| 1 | Control | | 0.61 | |
| 2 | Standard (Lansoprazole) | 20 | 0.33 | 45.90 |
| | | 40 | 0.29 | 52.45 |
| | | 60 | 0.24 | 60.65 |
| | | 80 | 0.21 | 65.57 |
| | | 100 | 0.18 | 70.49 |
| 3 | Psidium guajava extract | 20 | 0.34 | 44.26 |

| | | | | |
|---|---|---|---|---|
| | | 40 | 0.32 | 47.54 |
| | | 60 | 0.29 | 52.45 |
| | | 80 | 0.24 | 60.65 |
| | | 100 | 0.21 | 65.57 |
| 4 | Isolated flavonoids of Psidium guajava | 20 | 0.33 | 45.90 |
| | | 40 | 0.30 | 50.81 |
| | | 60 | 0.27 | 55.73 |
| | | 80 | 0.25 | 59.01 |
| | | 100 | 0.20 | 67.21 |




**Graph: $H^+/K^+$-ATPase Inhibition Assay**

**DISCUSSION:**
The present study demonstrates that the ethanolic extract of *Psidium guajava* possesses significant anti-ulcer activity, as evidenced by its performance in both **Acid Neutralizing Capacity (ANC)** and **$H^+/K^+$-ATPase inhibition assays**. These findings indicate that the extract acts through **dual mechanisms**, namely **neutralization of gastric acid** and **inhibition of gastric acid secretion**, both of which are essential in ulcer management.

The **ANC assay** results revealed that the extract effectively neutralizes hydrochloric acid in a **dose-dependent manner**, suggesting its potential as a natural antacid. This activity may provide immediate relief from gastric irritation and acidity. Similar mechanisms of acid neutralization have been reported for several plant-based anti-ulcer agents and are considered an important first line of defense against ulcer formation [38,39,40]

The **$H^+/K^+$-ATPase inhibition assay** further confirmed the anti-secretory activity of the extract. The proton pump enzyme plays a crucial role in gastric acid secretion, and its inhibition leads to reduced acid production. The extract showed a concentration-dependent increase in percentage inhibition, indicating effective suppression of proton pump activity. This mechanism is comparable to standard proton pump inhibitors like omeprazole, which are widely used in clinical practice [41,42]

The observed anti-ulcer activity of *Psidium guajava* can be attributed to its rich phytochemical composition. The presence of **flavonoids and phenolic compounds** contributes to strong antioxidant activity, which helps in scavenging free radicals and preventing lipid peroxidation. These compounds also enhance prostaglandin synthesis, thereby improving mucosal defense mechanisms [43,44]

**Tannins**, identified in high amounts, exert a protective effect by forming a layer over the gastric mucosa, reducing irritation and preventing further damage. This barrier effect is crucial in promoting ulcer healing. Additionally, **saponins** are known to stimulate mucus secretion, enhancing the protective lining of the stomach [45]

The anti-ulcer activity of *Psidium guajava* is also supported by previous studies, which reported its ability to reduce gastric lesions and decrease acid secretion. These findings validate the traditional use of guava leaves in the treatment of gastrointestinal disorders [46]

**CONCLUSION:**
The present study demonstrates that the ethanolic extract of *Psidium guajava* leaves possesses significant antioxidant and anti-ulcer activities due to the presence of bioactive phytoconstituents such as flavonoids, tannins, phenolic compounds, alkaloids, and saponins. The extract exhibited a concentration dependent free radical scavenging effect in the DPPH assay, indicating strong antioxidant potential, which plays a key role in protecting gastric mucosa from oxidative damage. Furthermore, the anti-ulcer activity assessed through Acid Neutralizing Capacity (ANC) and

$H^+/K^+$-ATPase inhibition assays confirmed that the extract acts through both antacid and anti-secretory mechanisms by neutralizing gastric acid and inhibiting proton pump activity. The overall findings suggest that *Psidium guajava* exerts a multi-mechanistic gastroprotective effect involving acid neutralization, reduction of acid secretion, antioxidant action, and enhancement of mucosal defense, thereby supporting its traditional use in the management of gastric ulcers and highlighting its potential as a natural therapeutic agent for further pharmaceutical development.

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