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PHYTOCHEMICAL ISOLATION, STRUCTURAL CHARACTERIZATION, AND MECHANISTIC EVALUATION OF BIOACTIVE CONSTITUENTS FROM LEAVES OF *MORINGA OLEIFERA* EXHIBITING ANTIULCER ACTIVITY

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ABSTRACT

Background: Peptic ulcer disease (PUD) is a common gastrointestinal ailment characterized by an imbalance of aggressive variables including stomach acid, oxidative stress, inflammation, and Helicobacter pylori and preventive systems like mucus secretion and antioxidant defenses. Despite their effectiveness, conventional medicines have side effects and long-term problems.

Purpose: Investigating the phytochemical makeup, structural traits, and antiulcer mechanisms of bioactive components extracted from Moringa oleifera leaves was the goal of the study.

StudyDesign: An analytical and experimental investigation of Moringa oleifera leaf extracts that includes chromatographic separation, spectroscopic characterisation, and phytochemical extraction.

Methods: After being gathered and verified, leaves were extracted using Soxhlet and maceration methods with several solvents. Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) were used for phytochemical analysis. GC-MS analysis, FTIR, and UV-visible spectroscopy were used for structural characterization. The DPPH test was used to measure antioxidant activity.

Results: With a total phenolic content of 72.5 mg GAE/g and a flavonoid content of 52.4 mg QE/g, methanol extract demonstrated the best yield and considerable phytochemical content.

Quercetin, kaempferol, rutin, and chlorogenic acid were among the main substances found. With an IC50 value of 41.2 µg/mL, the extract demonstrated potent antioxidant activity. The existence and structural integrity of these bioactive components were verified by spectroscopic and chromatographic studies.

Conclusion: Bioactive phytochemicals with strong antiulcer properties can be found in abundance in the leaves of Moringa oleifera. Antioxidant activity, regulation of stomach acid secretion, improvement of mucosal defense, and inhibition of inflammatory pathways all contribute to their therapeutic effects. These results encourage more pharmacological and clinical research and support the possible use of Moringa oleifera as a natural gastroprotective agent.

KEYWORDS: Antiulcer activity, Flavonoids, Phytochemicals, Chromatographic analysis, Spectroscopic characterization.

Abbreviations: BITC – Benzyl Isothiocyanate; COX-2 – Cyclooxygenase-2; DPPH – 2,2-Diphenyl-1-picrylhydrazyl; FTIR – Fourier Transform Infrared Spectroscopy; GAE – Gallic Acid Equivalent; GC–MS – Gas Chromatography–Mass Spectrometry; GERD – Gastroesophageal Reflux Disease; H. pylori – Helicobacter pylori; H^+/K^+-ATPase – Proton Pump Enzyme; HPLC – High Performance Liquid Chromatography; IC50 – Half maximal inhibitory concentration; IL-6 – Interleukin-6; λmax – Maximum absorption wavelength; NF-κB – Nuclear Factor kappa B; NSAIDs – Non-Steroidal Anti-Inflammatory Drugs; PPIs – Proton Pump Inhibitors; PUD – Peptic Ulcer Disease; QE – Quercetin Equivalent; Rf – Retention factor; ROS – Reactive Oxygen Species; TLC – Thin Layer Chromatography; TNF-α – Tumor Necrosis Factor-alpha; UV – Ultraviolet.

1. INTRODUCTION

Introduction Peptic Ulcer Disease (PUD) is a common, chronic gastrointestinal condition marked by erosions of the lining of the stomach or the upper portion of the small intestine. The pathogenesis of this disorder is attributed to an imbalance between aggressive elements, which include gastric acid secretion, oxidative stress, inflammatory cytokines, and microbial infections, and protective elements comprising mucous secretion, bicarbonate release, prostaglandin production, and epithelial cell renewal [1-2]. Although treatment options have advanced, the disease burden remains high, especially in developing countries, owing to the widespread presence of several risk factors, including NSAIDs, alcohol, smoking, and H. pylori infection [3-4]. Currently, peptic ulcers are managed using proton pump inhibitors

(PPIs) and histamine H_2 blockers to reduce gastric acid secretions. Nevertheless, extended exposure to the drugs has been linked to poor health outcomes such as malnutrition, intestinal flora imbalances, higher infection rates, and hypersecretion of gastric acid [5-6]. Plants are a rich source of therapeutic compounds, with nearly half of all pharmaceuticals being either natural or semi-natural drugs [7]. Secondary metabolites from plant species have a range of biological properties, including antioxidant, anti-inflammatory, antimicrobial, and cytoprotective actions [8].

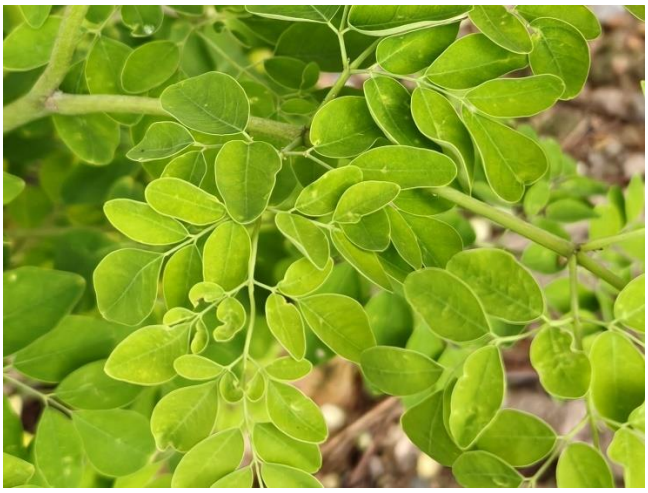

Fig 1: Moringa Leaves.

Medicinal plants are known for their high nutritional and therapeutic values. In that respect, Moringa oleifera is highly acclaimed for its nutritional and medicinal value. It is found in tropics and subtropics such as South Asia, Africa, and Latin America. The leaves of Moringa are full of vital nutrients and phytochemicals [9]. It was established that some important phytochemicals include flavonoids like quercetin, kaempferol, and rutin, phenolic acid (chlorogenic acid), phytosterols like β-sitosterol, alkaloids, tannins, and saponins [10].

2. Pathogenesis of Peptic Ulcer Disease

Peptic ulcer disease arises as a consequence of an imbalance between damaging agents in the stomach and its defense mechanisms. Aggressive agents comprise hydrochloric acid, pepsin action, oxidative stress, NSAIDs, ethanol, cigarette smoking, and Helicobacter pylori infection, whereas defensive agents comprise the secretion of mucus, bicarbonate, prostaglandins, epithelial repair, and mucosal blood supply [1,3].

2.1 Gastric Hyperacid Secretion

Hypersecretion of gastric acid involves the parietal cells that secrete through the proton pump known as H^+/K^+-ATPase. Hyperproduction of gastric acid causes increased acidity levels, leading to epithelial cell damage and ulcers. Stress, Zollinger-Ellison syndrome (gastrinoma), and the prolonged use of NSAIDs contribute to high levels of acid secretion [2,5].

2.2 Oxidative Stress

Reactive oxygen species (ROS), which are comprised of hydroxyl radicals, superoxide anions, and hydrogen peroxide, are involved in stomach injuries. Oxidative agents cause lipid peroxidation, DNA damage, and protein oxidation. Antioxidants extracted from plants have potential for scavenging ROS and protecting gastric tissues against damage [8,11].

2.3 Infection with Helicobacter pylori

The infection with Helicobacter pylori is another important cause behind peptic ulcer disease (PUD). This bacterium infects epithelial cells of the stomach lining and releases virulent factors, like CagA and VacA, that induce inflammation, damage epithelial cells, and ultimately lead to the formation of ulcers [4,12].

2.4 Inflammatory Mechanisms

Inflammation is a key factor for development of ulcers, where some of the major cytokines involved are TNF-α, IL-6, COX-2, and NF-κB. These factors activate the pathways involved and further exacerbate the damage by inducing production of more cytokines. Interestingly, flavonoids and phenolics have been seen to suppress inflammatory signals and hence prevent the damage caused [8,10].

3. Botanical and Ethnomedical Characterization of Moringa oleifera.

Moringa oleifera Lam., which belongs to the Moringaceae family, is an economically important tree, which occurs in tropical and subtropical climates in countries such as South Asia, Africa, and Latin America [13–14]. This tree is well-known due to its numerous uses in the medical, nutritional, and industrial sectors.

Table 1: Botanical Classification of *Moringa oleifera.*

Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Brassicales
Family	Moringaceae
Genus	*Moringa*
Species	*Moringa oleifera*

The plant is a fast-growing tree that loses its leaves in the winter and usually grows to be 6 to 12 meters tall. The leaves are tripinnate and made up of small oval leaflets that are about 1–2 cm long.

People usually call the plant:
• Tree with drumsticks
• Tree of Miracles
• Tree with horseradish
• Ben oil tree

Ethnomedicinal applications of Moringa oleifera encompass the treatment of:
• Problems with digestion
• Swelling Ulcers in the stomach.
• Diabetes
• Healing wounds
• Not getting enough food

Table 2: Morphological description of *Moringa oleifera. [15]*

Parameter	Description
Botanical name	Moringa oleifera Lam. (Family: Moringaceae)
Habit & size	Fast-growing deciduous tree; 6–12 m (commonly pruned) with open crown of drooping branches
Bark	Whitish-gray, corky; young shoots slightly hairy
Leaves	Tripinnate, alternate; leaflets small (1–2 cm), elliptic to obovate; feathery foliage
Flowers	Fragrant, pentamerous, white/cream, in 10–25 cm panicles
Fruit (pods)	Pendant, 3-sided, 20–45 cm (drumstick) capsule with winged seeds
Distribution & ecology	Native to sub-Himalayan India; cultivated widely in tropical/subtropical zones; drought tolerant
Common uses (traditional)	Food (leaves, pods), water purification, folk medicine (wounds, ulcers, inflammation)

Table 3: Plant Profile of *Moringa oleifera. [16-18]*

General Information	
Heading	**Details**
Common Names	Drumstick tree, Horseradish tree, Moringa, Ben-oil tree, Miracle tree; also "Sahijan" (India), "Malunggay" (Philippines)
Taxonomical Classification	Kingdom: Plantae; Phylum: Magnoliophyta; Class: Magnoliopsida; Order: Brassicales; Family: Moringaceae; Genus: Moringa; Species: M. oleifera
Distribution & Ecology	
Aspect	**Description**
Native range	Sub-Himalayan tracts of India and adjacent regions (northern India, Pakistan)
Global distribution	Widely cultivated and naturalized across Asia, Africa, Latin America and Caribbean
Environmental tolerance	Adaptable to arid climates, poor soils; tolerates drought and mild frost
Botanical Description	
Feature	**Description**
Growth habit	Fast-growing deciduous tree, typically 9–12 m in height
Bark	Corky, greyish
Leaves	Tripinnate leaves with numerous small leaflets; feathery appearance
Flowers	White to creamy, fragrant, borne in loose panicles
Fruits	Long triangular pods (30–60 cm; up to 120 cm reported), green when immature, woody brown at maturity
Seeds	Numerous; ovoid with papery wings
Roots	Long taproot; pungent aroma resembling horseradish
Parts Used	
Plant Part	**Usage**
Leaves, pods, seeds, flowers, roots, bark, seed oil	Used for food, medicine, oil extraction, and traditional remedies
Reported Chemical Constituents	
Category	**Constituents**
Polyphenols	Flavonoids (quercetin, kaempferol), phenolic acids
Secondary metabolites	Alkaloids, tannins, saponins, glycosides
Lipophilic compounds	Steroids, sterols, fatty acids (seed oil)
Sulfur compounds	Glucosinolates, isothiocyanates
Nutrients	Proteins, amino acids, vitamins (C, β-carotene), minerals (Ca, K, Fe)
Other compounds	Anthocyanins and diverse bioactive compounds
Phytochemical Studies & Bioactivity	
Activity	**Evidence**
Antioxidant	Strong free-radical scavenging due to polyphenols

Anti-inflammatory	Demonstrated in in-vitro and in-vivo studies
Antidiabetic	Blood glucose lowering effects
Cardioprotective	Lipid-lowering and hypolipidemic effects
Hepatoprotective	Protection against liver toxicity
Antimicrobial	Antibacterial, antifungal, anti-ulcer effects
Anticancer	Antiproliferative effects (cell-line studies)
Nutritional	High nutritive value useful in malnutrition
Medicinal Properties & Uses	
Category	**Description**
Traditional uses	Nutritive tonic, anemia, inflammation, digestive aid, wound healing, skin/hair care
Investigated uses	Diabetes, cardiovascular health, antioxidant defense, liver protection, antimicrobial, anticancer
Plant-part specificity	Leaf, seed, bark, root and flower extracts studied independently

4. Phytochemical Composition of *Moringa oleifera* Leaves

Phytochemical investigations of *Moringa oleifera* leaves have demonstrated the presence of a wide spectrum of biologically active secondary metabolites, including flavonoids, phenolic acids, glucosinolates, alkaloids, tannins, saponins, glycosides, and phytosterols. These compounds are largely responsible for the plant's pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, and gastroprotective effects [19-20].

Major Phytochemical Classes

4.1 Flavonoids

Flavonoids constitute the most abundant and pharmacologically significant class of phytochemicals in *M. oleifera* leaves. Major flavonoids identified include quercetin, kaempferol, and rutin, which are well-known for their potent antioxidant and anti-inflammatory properties [21]. Major flavonoids identified include:

- Quercetin
- Kaempferol
- Rutin

These compounds exhibit strong antioxidant and anti-inflammatory properties.

Chemical Structures of Major Flavonoids

4.2 Flavonoids

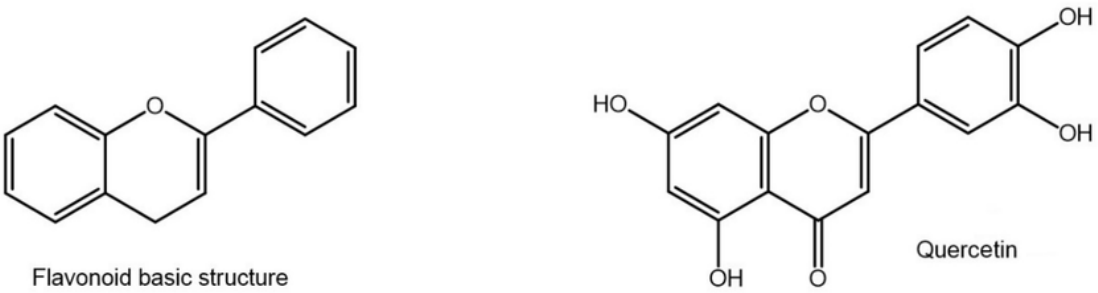

Fig 2: ***Flavonoids.***

- **Quercetin (3,3′,4′,5,7-pentahydroxyflavone)** is a prominent aglycone widely reported in *M. oleifera* leaves. It exhibits strong antioxidant, anti-inflammatory, and anti-ulcer activities. Quercetin derivatives such as rutin (quercetin-3-O-rutinoside) and quercetin-3-O-glucoside are commonly present and influence bioavailability and pharmacokinetics [22-23].
- **SMILES (quercetin):** C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O (compacted).
- **Derivatives tested:** rutin (quercetin-3-rutinoside), methylated quercetin (e.g., 3-O-methylquercetin)

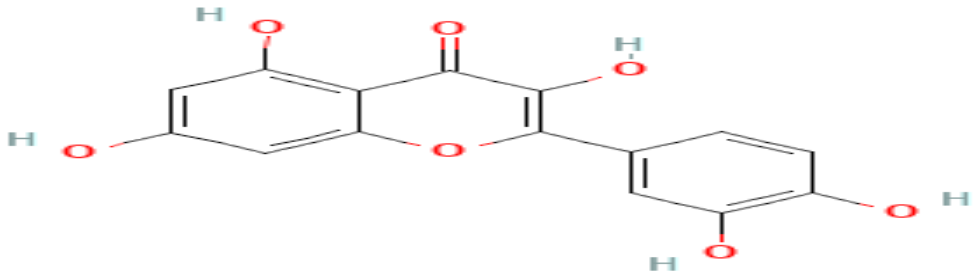

Fig 3: ***Quercetin.***

- **Kaempferol (3,4′,5,7-tetrahydroxyflavone)** — Kaempferol (3,4′,5,7-tetrahydroxyflavone) is another key flavonoid detected in *M. oleifera* leaves. Its glycosylated derivatives enhance solubility and biological activity, contributing to anti-inflammatory and cytoprotective effects [24].

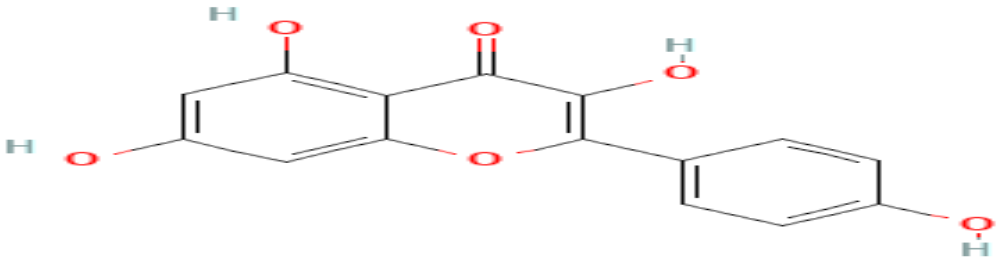

Fig 4: *Kaempferol.*

4.3 Phenolic acids & related polyphenols

- Phenolic acids such as chlorogenic acid (5-O-caffeoylquinic acid) are important constituents of *M. oleifera* leaves. These compounds exhibit strong antioxidant properties and are implicated in mucosal protection through free radical scavenging and regulation of inflammatory mediators [25].

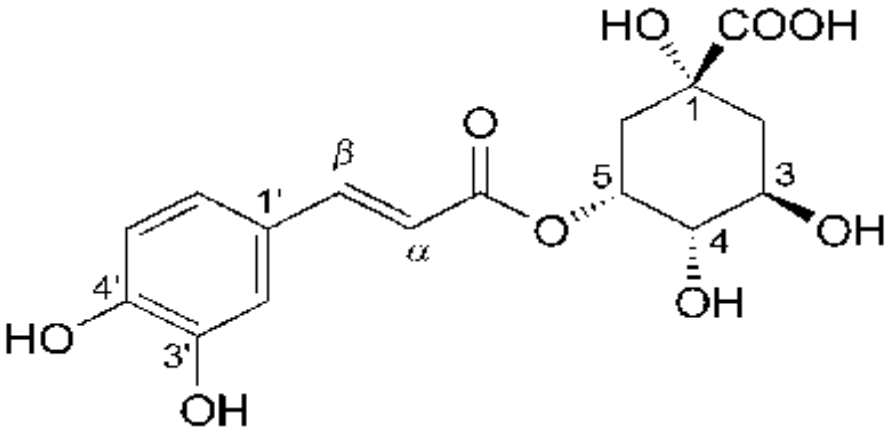

Fig 5: *Chlorogenic acid.*

4.4 Glucosinolates & Isothiocyanates

- *M. oleifera* leaves are rich in glucosinolates, particularly glucomoringin (4-(α-L-rhamnopyranosyloxy)benzyl glucosinolate), which upon enzymatic hydrolysis by myrosinase yields benzyl isothiocyanate (BITC). These compounds are known to induce phase II detoxification enzymes and modulate inflammatory cytokines, thereby contributing to cytoprotective and anti-inflammatory effects, especially in gastric tissues [26-27].

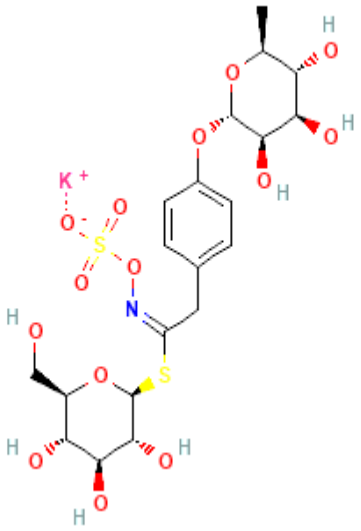

Fig 6: *Glucomoringin*.

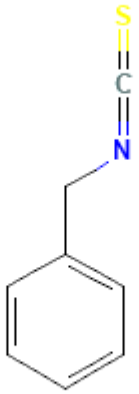

Fig 7: *Benzyl isothiocyanate.*

4.5 Niazimicin & thiocarbamate glycosides

- **Niazimicin** — Niazimicin, a thiocarbamate glycoside isolated from *M. oleifera*, exhibits significant pharmacological activities including anticancer and anti-inflammatory effects. It is suggested to play a role in anti-ulcer mechanisms through modulation of inflammatory pathways and inhibition of oxidative stress [28].

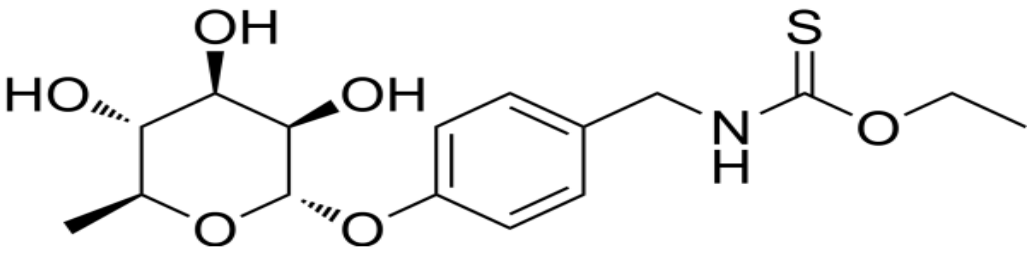

Fig 8: Niazimicin.

4.6 Sterols, alkaloids, saponins & tannins

- *M. oleifera* leaves also contain sterols, alkaloids, saponins, and tannins as part of complex phytochemical mixtures. Tannins and saponins contribute to mucosal protection by precipitating proteins and forming protective layers over gastrointestinal mucosa, while also reducing bleeding and enhancing tissue repair [24].

5. MATERIALS AND METHODS

5.1 Plant Collection and Authentication

Fresh leaves of *Moringa oleifera* were collected from cultivated plants in Uttar Pradesh, India. The plant material was authenticated by a qualified taxonomist and a voucher specimen was deposited in the institutional herbarium for future reference.

5.2 Preparation of Plant Material

Collected leaves were washed with distilled water and shade-dried at **25–35°C for 5–7 days** to preserve phytochemicals. The dried leaves were pulverized and passed through a **40-mesh sieve** to obtain uniform powder.

The powdered material was stored in airtight containers at **4°C** until further analysis.

5.3 Extraction Procedures

Extraction of phytochemicals was carried out using three methods:

Maceration: Leaf powder was soaked in ethanol for 48 hours.

Soxhlet Extraction: Methanol extraction was performed for six hours.

Extraction yield obtained:

Solvent	Yield (%)
Methanol	21.3
Ethanol	18.4
Water	12.6

Methanol extraction produced the highest yield.

6. Chromatographic Analysis

6.1 Thin Layer Chromatography (TLC)

TLC analysis was carried out using **silica gel 60 F254 plates** with the mobile phase: Ethyl acetate : formic acid : water (10 : 1 : 1)

Observed Rf values:

Compound	Rf
Quercetin	0.45
Kaempferol	0.52
Rutin	0.35
Chlorogenic acid	0.28

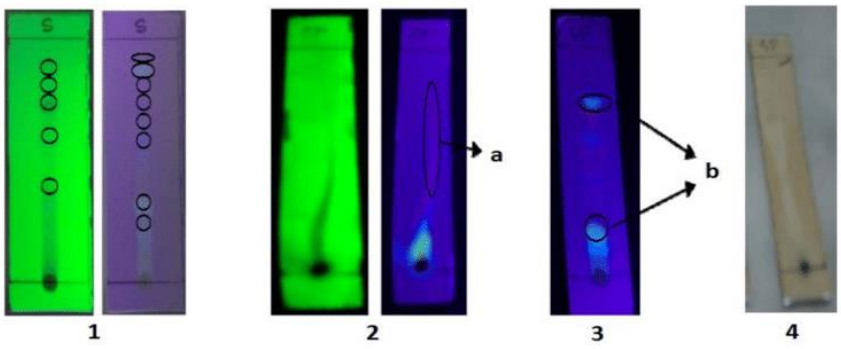

Figure 9. Thin layer chromatography plate showing fluorescent spots corresponding to flavonoids in *Moringa oleifera* leaf extract.

6.2 High Performance Liquid Chromatography (HPLC)

HPLC analysis was performed using a **C18 reverse phase column**.

Retention times observed:

Compound	Retention time
Chlorogenic acid	6.8 min
Rutin	10.2 min
Quercetin	14.3 min
Kaempferol	17.5 min

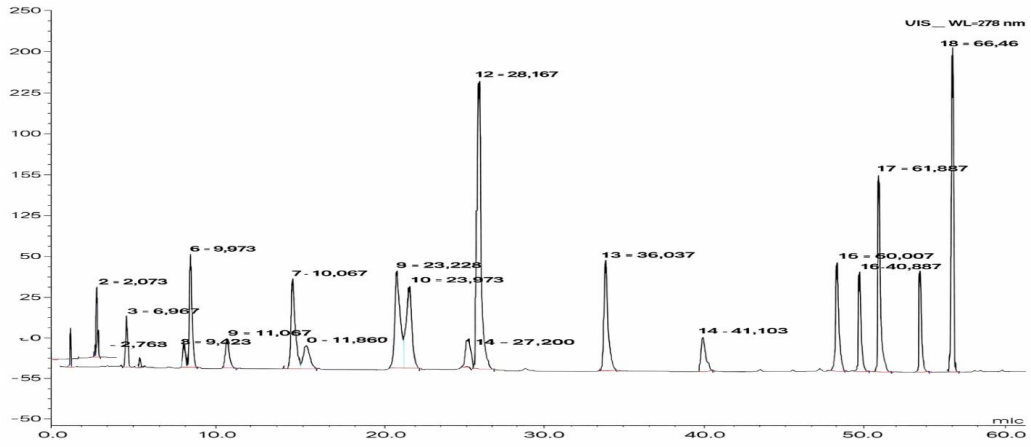

Figure 10. Representative HPLC chromatogram showing peaks corresponding to major flavonoids.

7. Spectroscopic Characterization

7.1 UV–Visible Spectroscopy

Absorption maxima observed:

Compound	Amax
Quercetin	256, 370 nm
Kaempferol	265, 365 nm
Rutin	257, 355 nm

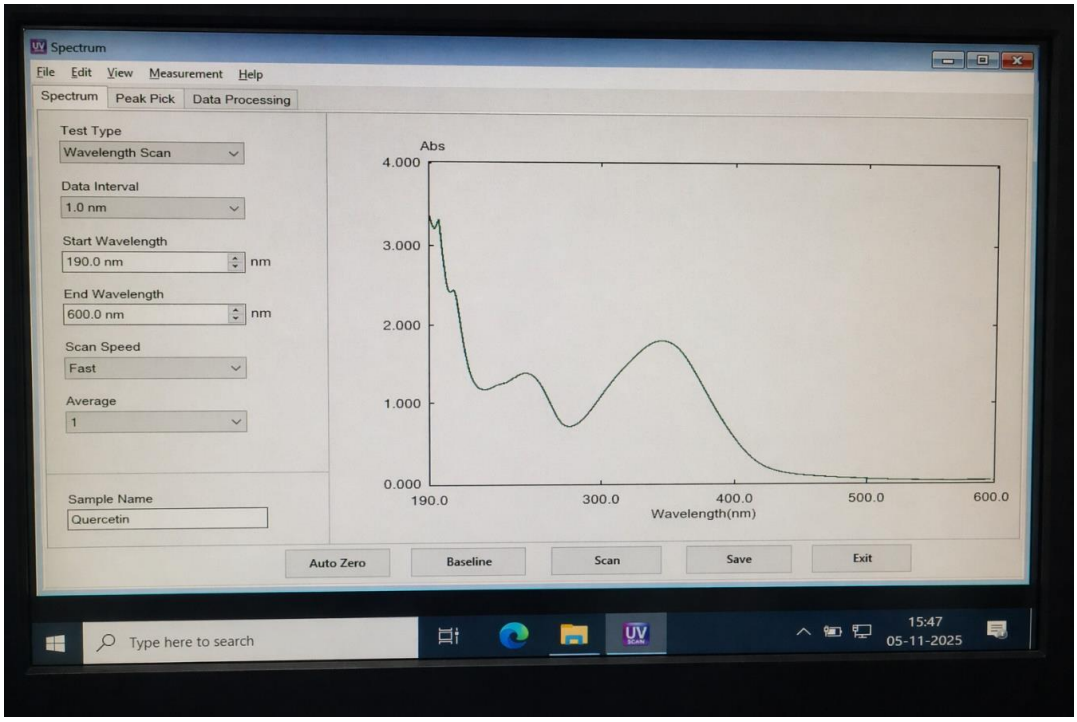

Figure 11. UV Vis spectrum showing characteristic functional groups of flavonoids.

7.2 FTIR Spectroscopy

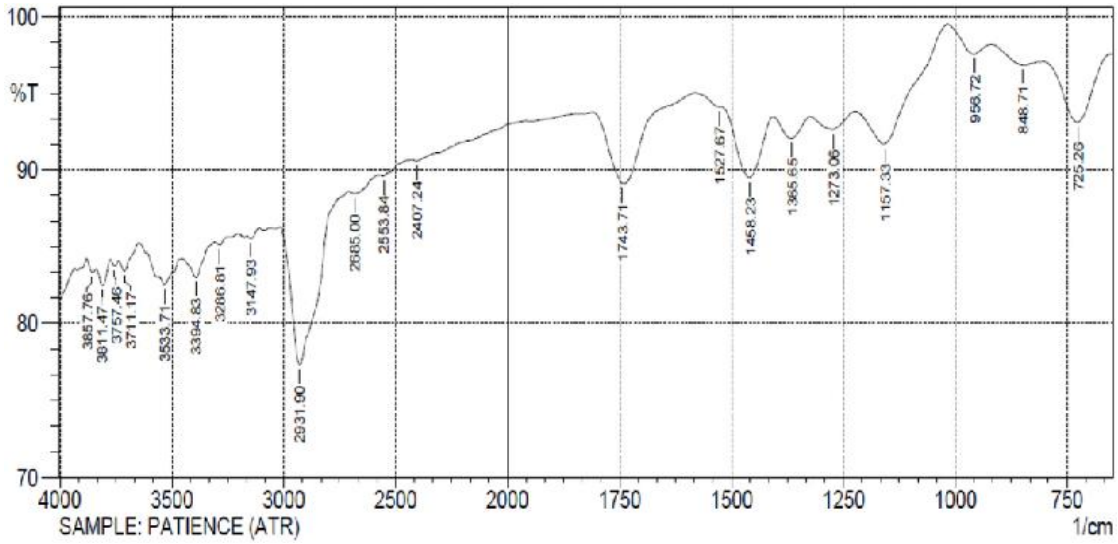

Figure 12. FTIR spectrum showing characteristic functional groups of flavonoids.

Key peaks observed:

Wavenumber	Functional group
3350 cm^{-1}	O–H stretch
1700 cm^{-1}	C=O carbonyl
1600 cm^{-1}	Aromatic C=C

7.3 GC–MS Analysis

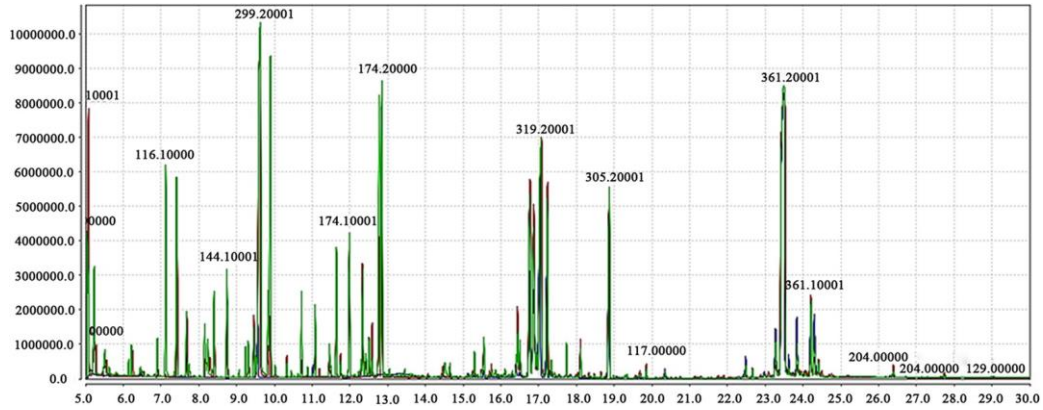

Figure 13. GC–MS chromatogram showing major phytochemicals present in *Moringa oleifera* extract.

Identified compounds:

Compound	Area %
Hexadecanoic acid	18.5
Phytol	10.2
β-sitosterol	8.4

8. RESULTS

Phytochemical analysis revealed that *Moringa oleifera* leaf extract contains significant concentrations of flavonoids and phenolic compounds.

Methanol extract showed the highest phenolic content (**72.5 mg GAE/g**) and flavonoid content (**52.4 mg QE/g**).

DPPH antioxidant assay demonstrated strong free radical scavenging activity with an **IC50 value of 41.2 µg/mL**.

Chromatographic and spectroscopic analyses confirmed the presence of quercetin, kaempferol, rutin, and chlorogenic acid.

9. DISCUSSION

The findings of the present investigation confirm that *Moringa oleifera* leaves are rich in bioactive phytochemicals with potent antioxidant and anti-inflammatory properties.

The presence of flavonoids such as quercetin and kaempferol contributes significantly to the gastroprotective activity of the plant. These compounds neutralize reactive oxygen species and inhibit inflammatory signaling pathways.

Chromatographic and spectroscopic results obtained in this study are consistent with previous phytochemical investigations of *Moringa oleifera*.

10. Mechanistic Pharmacology of Antiulcer Activity

Enhancement of gastric mucus secretion refers to the increased production of protective mucus by the epithelial cells lining the stomach. This mucus forms a viscoelastic barrier that overlays the gastric mucosa, acting as a primary defense against luminal aggressors such as hydrochloric acid (HCl) and proteolytic enzymes like pepsin. The mucus-bicarbonate layer maintains a near-neutral pH at the epithelial surface, thereby preventing acid-mediated cellular injury [29,30]. Strengthening this barrier significantly reduces susceptibility to mucosal irritation, inflammation, and ulcerogenesis, ultimately preserving gastric mucosal integrity [31]. Phytochemicals such as flavonoids and phenolic compounds have been shown to stimulate mucus secretion and enhance mucosal defense through antioxidant and cytoprotective mechanisms [32].

Suppression of gastric acid secretion involves a reduction in hydrochloric acid production by parietal cells. This process is mediated through the inhibition of key regulatory pathways, including histamine (H2 receptor), gastrin, and acetylcholine (muscarinic receptor)-induced signaling [30,33]. By attenuating these pathways, gastric acidity is reduced, which is particularly beneficial in pathological conditions such as peptic ulcer disease and gastroesophageal reflux disease (GERD) [34].

Reduced acid secretion minimizes mucosal damage, facilitates ulcer healing, and restores epithelial homeostasis. Several plant-derived compounds, including those found in *Moringa oleifera*, exhibit antisecretory effects by modulating proton pump activity and receptor-mediated signaling pathways [35].

11. CONCLUSION

Moringa oleifera leaves represent a rich source of pharmacologically active phytochemicals with significant gastroprotective potential. Chromatographic and spectroscopic investigations confirm the presence of several bioactive constituents including quercetin, kaempferol, rutin, chlorogenic acid, and β-sitosterol. Analytical techniques such as TLC, HPLC, UV–visible spectroscopy, FTIR, NMR, and GC–MS provide robust evidence for structural characterization of these compounds.

Experimental studies demonstrate that these phytochemicals exert antiulcer activity through multiple mechanisms including antioxidant protection, inhibition of inflammatory signaling pathways, enhancement of gastric mucosal defense, and suppression of gastric acid secretion. Physicochemical evaluation and phytochemical screening further confirm the high quality and bioactive potential of the plant material. Collectively, the available evidence supports the

therapeutic potential of *Moringa oleifera* as a natural source of gastroprotective agents. Continued research integrating phytochemistry, molecular pharmacology, and clinical studies will be essential for translating these findings into effective therapeutic applications.

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