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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF MANILKARA ZAPOTA ROOTS EXTRACTS FOR ANTI-ULCER ACTIVITY

[1]Miss. Ashwini D. Kale, [2]Dr. Karna B. More

[1]Student, [2]Research Guide,

Dr. Vedprakash Patil Pharmacy College Georai Tanda.

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***Corresponding Author**

Miss. Ashwini D. Kale

Student, Dr. Vedprakash Patil Pharmacy College Georai Tanda.

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ABSTRACT

The present study evaluated the pharmacognostic, phytochemical, antioxidant, and anti-ulcer potential of *Manilkara zapota* root extracts to validate its traditional medicinal use. The plant material was authenticated and subjected to physicochemical standardization, confirming its purity and quality. Successive extraction revealed higher extractive values in methanol (23%) and acetone (21%), indicating the presence of polar bioactive compounds. Preliminary phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, glycosides, and tannins. Quantitative analysis demonstrated higher total phenolic content in the acetone extract (42.50 ± 0.85 mg GAE/g) and higher flavonoid content in the methanol extract (46.25± 0.78 mg rutin/g). Antioxidant activity assessed by DPPH assay exhibited significant, concentration-dependent radical scavenging activity, comparable to standard antioxidants. The anti-ulcer activity was evaluated using an ethanol-induced gastric ulcer model in Wistar rats. The acetone extract showed significant gastroprotectiveactivity with a marked reduction in ulcer index, comparable to ranitidine, while the methanol extract showed moderate effects. Both extracts increased gastric pH and reduced total acidity, indicating antisecretory and cytoprotective mechanisms. In conclusion, *Manilkara zapota* root extracts, particularly the acetone extract, possess significant Anti-ulcer and antioxidant activity, supporting its potential as a natural therapeutic agent. Further studies are required to isolate active constituents and confirm clinical efficacy.

KEYWORD: *Manilkara Zapota*, Ethanol induced Model, Ranitidine Anti-ulcer activity.

1 INTRODUCTION

In this current era, the risk of developing serious diseases is rising due to unhealthy and contemporary lifestyle. Studies revealed that Gastric and Peptic Ulcer Disease (PUD) are the commonly developed acid-induced abrasions, generally in the stomach and proximal duodenum[1] The frequency rate of peptic ulcer and associated complications varies according to the time and region, with highest incidence of bleeding PUD was 80%, perforated PUD was 12% with a total of around 140 per 1,00,000 population[2] sually, after 15-45 mins of meal, epigastric pain occurs while the duodenal lesions develop after few hours of the meal due to excessive pepsin secretion.[3] Dyspepsia is a burning sensation in the stomach due to indigestion of food that possesses similar symptoms to epigastric ulcer such as bloating, discomfort, nausea which are difficult to diagnose[4] On the other hand, the risk of complications of peptic ulcer is increased four times in NSAID users, and two times in aspirin users.[11] The concomitant use of NSAIDs or aspirin with anticoagulants, corticosteroids, and selective serotonin reuptake inhibitors increase the risk of upper gastrointestinal bleeding.[12] Although many people who use NSAIDs or aspirin have concurrent H. pylori infection, their interaction in the pathogenesis of peptic ulcer disease remains controversial. A meta-analysis of observational studies resulted in a conclusion that NSAIDs, aspirin use, and H. pylori infection increase the risk of peptic ulcer disease independently.

The prevalence of peptic ulcers was 10-15% in the United States, with an increased frequency of more than 5, 00,000 cases every year.[1] The global estimates of peptic ulcers cover up to 5-10% population. However, ulcer incidence, mortality risks have been decreasing worldwide in the past few years.[5] Duodenal ulcers are four times more frequent in men than epigastric ulcers. Multicellular Organism have A system og organ in the gastrointestinal (GI) tract that enable them to ingest, digest, and Absorb nutrients from food, And eventually eliminate unwanted material.These essential Function, which for the most part occurs involuntarity, are Facilitated by the presence of a well organized nervous system. The GI tract also has the capacity to provide conscious awareness (sensation of pain) that alerts an organism of impending tissue damage which could hamper these vital physiological function. Normally, it involves interplay between immune, peripheral and central nervous syste.[6] Ulcer is an open sore on an external or internal surface of the body, caused by a break in the skin or mucous

membrane which fails to heal. Ulcers range from small, painfull sores in the mouth to bedsores and serious lesions of the stomach or intestine. Ulcer are most common on the skin of the lower extremities and in the gastrointestinal tract, althrought they may be encountered at almost any site. There Are many types of ulcer such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of these peptic ulcer is seen among many People, The Peptic ulcer Are erosion of lining of stomach or the duodenum. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer". The name refers to the site of ulceration. A person may have both gastric and duodenal at the same time. Gastric ulcer are located in the stomach, characterized by pain. Other symptoms may includes nausea, vomiting, And weight loss. In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain and cramps along with vomiting blood.**[7]**

The Pathophysiology of peptic ulcer disease involves imbalance between Aggressive factors (Acid, pepsin And Halicobacter pylori) And defensive Factors (musin, prostaglandins, biocarbonate, nitric acid and growth factors.). Peptic ulcer are once believed to be caused by spicy food and stress And other factors results from an imbalance between factors that can damage the gastroduodenal mucosal lining and defence mechanism that normally limit the injury. Aggressive factors include gastric juice (including hytrochloric Acid, pepsin, and bile salts refluxed from the duodenum), H pylori And NSAIDs (non steroids anti-inflammatory drugs). Diseases like Zollinger – Ellison syndrome, emotional stress, Alcohol abuse and smoking are the principle etiological factors associated with peptic ulcer. The gram negative bacterium Halicobacter pylori resides in the antrum but over time migrates towards the more proximal segments of the stomach.[8]

The pathophysiological factors associated with peptic ulcers are NSAIDS, Helicobacter pylori (H. pylori), tobacco consumption[6,7] Almost 90% of the ulcers are caused by H. pylori, which are distributed according to different ethnicities. So, H. pylori should be managed with various drugs as it causes the risk of gastric cancer development.[8] These gastroenterological bacteria infect 10% population in the rural and western countries, while affects more than 50% in the developing countries, but only a few develop clinical symptoms.[9] NSAIDs like diclofenac and aspirin secondary risk factors of gastric ulcer .Peptic ulcer disease vary according to the age groups: 22% in 0-5yrs, 55% in 6-10yrs and 86% in 11-20yrs.[10] Peptic ulcer disease is associated with several key pathophysiological factors, including the use of non-steroidal anti-inflammatory drugs (NSAIDs), *Helicobacter pylori* (*H. pylori*) infection, and tobacco

consumption. Approximately 90% of peptic ulcers are attributed to *H. pylori*, with its prevalence varying among different ethnic groups. Due to its strong association with an increased risk of gastric cancer, *H. pylori* infection requires appropriate management using combination drug therapy.

This bacterium infects about 10% of the population in rural and developed regions, whereas its prevalence exceeds 50% in developing countries; however, only a small proportion of infected individuals develop clinical symptoms. NSAIDs such as diclofenac and aspirin are considered important secondary risk factors, as they impair the gastric mucosal barrier and promote ulcer formation.

The incidence of peptic ulcer disease also varies with age, showing approximately 22% occurrence in children aged 0–5 years, 55% in those aged 6–10 years, and up to 86% in individuals aged 11–20 years.[10]

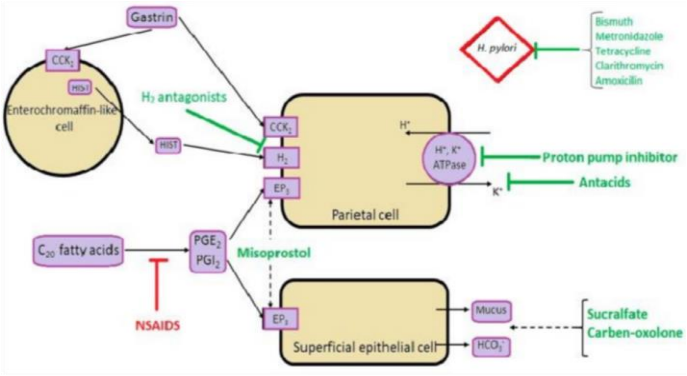


Figure 1: Schematic presentation of main pathophysiological mechanisms involved in the development of peptic ulcer disease, and the sites of action of the most commonly used pharmacological options in the treatment of peptic ulcer disease. CCK2 = Cholecystokinin Receptor; PGE2 = Prostaglandin E2; PGI2 = Prostaglandin I2 ; EP3 = Prostaglandin E receptor 3; HIST = Histamine.[14]

1.1 Type of ulcer: There are a few types of ulcers, including

1.1.1 Arterial Ulcers

Arterial ulcers, also known as ischemic ulcers, occur due to inadequate blood supply to the tissues, usually as a result of peripheral arterial disease. These ulcers are commonly found on the lower extremities, especially on the toes, heels, or pressure points of the feet. They are typically painful, have well-defined edges, and may appear pale or necrotic. Poor circulation delays healing, and the surrounding skin often appears cold, hairless, and shiny.

1.1.2. Venous Ulcers

Venous ulcers develop due to improper functioning of venous valves, leading to chronic venous insufficiency. These ulcers are most commonly located on the inner side of the lower leg, above the ankle. They are usually less painful compared to arterial ulcers and are characterized by irregular margins, edema, and surrounding skin discoloration (hyperpigmentation). The ulcer base is often moist with exudate, and healing may be slow without proper management of venous pressure.

1.1.3. MouthUlcers

Mouth ulcers, also known as aphthous ulcers, are small, painful lesions that occur on the soft tissues inside the oral cavity, such as the inner cheeks, lips, tongue, or gums. They are commonly caused by factors like stress, nutritional deficiencies (especially vitamin B12, iron, and folic acid), minor injuries, or immune responses. These ulcers are usually round or oval with a white or yellow center and a red border, and they typically heal spontaneously within 1–2 weeks.

1.1.4. GenitalUlcers

Genital ulcers are lesions that occur on the genital organs and are often associated with infections, particularly sexually transmitted infections such as herpes simplex virus or syphilis. They may present as painful or painless sores, depending on the underlying cause. In addition to infections, non-infectious conditions like trauma or inflammatory diseases can also lead to genital ulcers. Proper diagnosis and treatment are essential, as these ulcers may have significant implications for overall health and transmission risk.

1.2 General anatomy of stomach[52]

Stomach is a J-shaped cylindrical organ whose upper part is oesophagus and lower part is connected to duodenum. Stomach consists of convex curvature which extends to the liver and concave border which runs to the abdominal wall.

1.2.1 Stomach is divided into four part

Cardia: The esophagus is a muscular tube that transports food from the mouth to the stomach. It opens into the stomach at the cardia, which acts as the entry point. The cardia helps prevent the backflow of acidic gastric contents into the esophagus.

Fundus: The fundus is the upper dome-shaped portion of the stomach located above the cardia.

It primarily serves as a storage area for food and gases released during digestion.

Body of the Stomach: The body is the largest and central region of the stomach. It is the main site for digestion, where gastric glands secrete hydrochloric acid and digestive enzymes that convert food into a semi-liquid mass called chyme.

Curvatures of the Stomach: The stomach has two borders: the greater curvature (outer, convex surface) and the lesser curvature (inner, concave surface). These curvatures provide attachment points for blood vessels and supporting structures.

Muscle Layers: The stomach wall consists of three layers of smooth muscles: longitudinal (outer), circular (middle), and oblique (inner). These layers work together to churn, mix, and propel food, facilitating mechanical digestion.

Rugae: Rugae are folds present in the inner lining of the stomach. They allow expansion of the stomach when food enters and increase the surface area for efficient digestion.

Pyloric Region (Antrum and Canal): The pyloric region is the lower part of the stomach. The pyloric antrum helps in grinding food, while the pyloric canal serves as a passage leading to the pyloric sphincter.

Pyloric Sphincter and Duodenum: The pyloric sphincter is a thick muscular valve that regulates the passage of chyme from the stomach into the duodenum, the first part of the small intestine, ensuring controlled emptying**.**

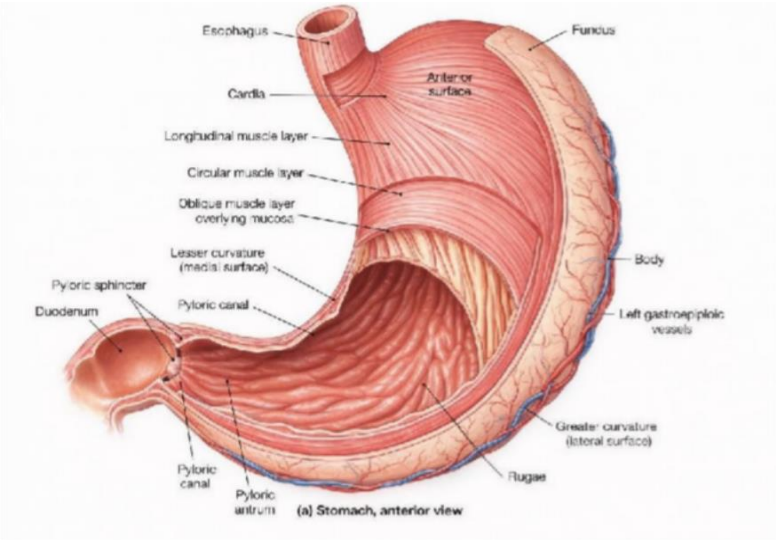


Figure 2: Anatomy of stomach.

1.2 Gastric Mucosal Defense Mechanisms

The gastric mucosa possesses a highly specialized defense system that protects it from continuous exposure to aggressive factors such as hydrochloric acid and pepsin. The primary components of mucosal defense include the mucus-bicarbonate barrier, epithelial cell integrity, prostaglandin synthesis, and mucosal blood flow. Mucus secreted by surface epithelial cells forms a gel-like layer that traps bicarbonate ions, maintaining a near-neutral pH at the epithelial surface despite the highly acidic gastric lumen. Prostaglandins, particularly PGE_2 and PGI_2, play a crucial role by stimulating mucus and bicarbonate secretion, enhancing mucosal blood flow, and promoting epithelial cell regeneration.

Additionally, tight junctions between epithelial cells prevent back diffusion of hydrogen ions. Rapid cell turnover and restitution mechanisms allow the gastric epithelium to repair superficial injuries within minutes. Any disruption in these protective mechanisms significantly increases susceptibility to ulcer formation.[61]

1.3 Role of Oxidative Stress in Peptic Ulcer

Oxidative stress is a critical factor in the pathogenesis of peptic ulcer disease. It results from an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defense system. ROS such as superoxide anion, hydroxyl radicals, and hydrogen peroxide cause lipid peroxidation, protein denaturation, and DNA damage in gastric mucosal cells. Studies have demonstrated increased levels of malondialdehyde (MDA), a marker of lipid peroxidation, in ulcerated tissues.

Endogenous antioxidants such as superoxide dismutase (SOD), catalase, and glutathione play protective roles. However, in ulcer conditions, these antioxidant defenses are often depleted. This oxidative damage weakens the mucosal barrier and exacerbates ulcer progression, highlighting the importance of antioxidant therapy in ulcer management.[61]

1.4 Inflammatory Mediators in Ulcer Development

Inflammation plays a central role in the development of peptic ulcers, particularly in infections caused by *Helicobacter pylori*. The bacterium stimulates the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which initiate and amplify the inflammatory response in gastric tissues. These mediators promote the recruitment of neutrophils, leading to the release of proteolytic enzymes and reactive oxygen species that damage the gastric mucosa. Persistent inflammation results in epithelial cell injury, impaired healing, and disruption of the

mucosal barrier. Additionally, inflammatory processes reduce mucosal blood flow and interfere with cellular regeneration, further aggravating tissue damage. The continuous activation of immune responses also contributes to chronic gastritis, which can progress to ulceration if untreated.

Furthermore, non-steroidal anti-inflammatory drugs (NSAIDs) exacerbate this condition by inhibiting *Cyclooxygenase (COX)*, particularly COX-1, which is essential for prostaglandin synthesis. Prostaglandins play a protective role by maintaining mucus secretion, bicarbonate production, and mucosal blood flow. Their inhibition weakens gastric defenses, making the mucosa more susceptible to injury. Thus, the combined effects of inflammation and reduced prostaglandin levels significantly accelerate the formation and progression of peptic ulcers.[61]

1.5 Pharmacological Targets in Peptic Ulcer Disease

Modern treatment strategies for peptic ulcer disease aim to reduce gastric acid secretion while strengthening mucosal defense mechanisms. Proton pump inhibitors (PPIs) act by irreversibly inhibiting the gastric proton pump (H^+/K^+-ATPase) in parietal cells, leading to profound and long-lasting suppression of acid secretion. H_2-receptor antagonists reduce acid production by blocking histamine-mediated stimulation of parietal cells, thereby decreasing basal as well as stimulated acid output.

Antacids provide rapid but short-term relief by neutralizing existing gastric acid, improving symptoms such as pain and discomfort. Cytoprotective agents like sucralfate form a protective barrier over the ulcer surface, while misoprostol, a prostaglandin analogue, enhances mucus and bicarbonate secretion and improves mucosal blood flow.

Eradication therapy for *Helicobacter pylori* typically involves a combination of antibiotics along with proton pump inhibitors, which not only suppress acid but also improve antibiotic efficacy. In addition, bismuth-containing regimens are sometimes used to enhance eradication rates.

However, prolonged use of these pharmacological agents may be associated with certain limitations. Long-term PPI therapy has been linked to nutrient malabsorption (such as vitamin B_{12}, calcium, and magnesium), increased risk of gastrointestinal infections, and possible alterations in gut microbiota. Similarly, inappropriate or excessive use of antibiotics may contribute to antimicrobial resistance. Recurrence of ulcers is also possible, particularly if

underlying risk factors such as NSAID use or persistent infection are not adequately managed.[62]

1.6 Role of Medicinal Plants in Ulcer Management:

Medicinal plants have gained significant attention in the management of peptic ulcer disease due to their multi-targeted mechanisms, favorable safety profile, and minimal side effects. Plants such as Butea monosperma, Azadirachta indica, and Glycyrrhiza glabra have demonstrated potent anti-ulcer activity in various experimental and clinical studies. The therapeutic effects of these plants are primarily attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, saponins, and alkaloids. These compounds exhibit strong antioxidant properties, helping to neutralize reactive oxygen species and reduce oxidative stress-induced mucosal damage. In addition, they enhance mucus and bicarbonate secretion, thereby strengthening the gastric mucosal barrier.

Many plant-derived compounds also inhibit gastric acid secretion by modulating histamine receptors or proton pump activity. Their anti-inflammatory action further contributes to ulcer healing by reducing the levels of pro-inflammatory cytokines and preventing tissue injury. Some phytochemicals also promote angiogenesis and epithelial regeneration, accelerating the healing process.

Experimental studies using various ulcer models have shown that these plant extracts significantly decrease ulcer index, gastric volume, and acidity, while reducing lipid peroxidation and increasing endogenous antioxidant enzyme levels such as superoxide dismutase and catalase. These findings highlight the potential of medicinal plants as effective and safer alternatives or adjuncts to conventional anti-ulcer therapy.[62]

1.2 EPIDERMIOLOGY

The lifetime risk for developing a peptic ulcer is approximately 5% to 10% with the rate of 0.1% to 0.3% per year. Peptic ulcers resulted in 301,000 deaths in 2013, down from 327,000 in 1990.[9] In Western countries, the percentage of people with H. pylori infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80, etc.). Prevalence is higher in third world countries, where it is estimated at 70% of the population, whereas developed countries show a maximum of a 40% ratio. Overall, H. pylori infections show a worldwide decrease, more so in developed countries. Transmission occurs via food, contaminated groundwater, or human saliva (such as from kissing or sharing food utensils).Peptic ulcer

disease had a tremendous effect on morbidity and mortality until the last decades of the 20th century when epidemiological trends started to point to an impressive fall in its incidence. The reason that the rates of peptic ulcer disease decreased is thought to be the development of new effective medication and acid suppressants and the rational use of nonsteroidal anti-inflammatory drugs (NSAIDs).[70,71]

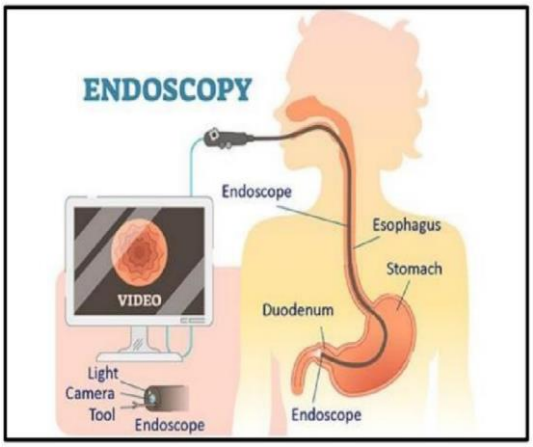


Figure 3: Upper GI endoscopy.

1.3 Pathogenesis of Peptic ulcer

Almost half of the world's population is colonized by H. pylori, which remains one of the most common causes of pepticulcer disease. The prevalence of H. pylori is higher in developing countries, especially in Africa, Central America, Central Asia, and Eastern Europe.

The organism is usually acquired in childhood in an environment of unsanitary conditions and crowding, mostly in countries with lower socioeconomic status. H. pylori causes epithelial cell degeneration and injury, which is usually more severe in the antrum, by the inflammatory response with neutrophils, lymphocytes, plasma cells, and macrophages. The mechanism by which H. pylori induces the development of different types of lesions in the gastroduodenal mucosa is not fully explained.[72]

H. pylori infection can result in either hypochlorhydria or hyperchlorhydria, thus determining the type of peptic ulcer. The main mediators of H. pylori infection are cytokines that inhibit parietal cell secretion, but H. pylori can directly affect the H+/K+ ATPase α-subunit, activate calcitonin gene-related peptide (CGRP) sensory neurons linked to somatostatin, or inhibit the production of gastrin.[73] Although the formation of gastric ulcers is associated with hyposecretion, 10-15% of patients with H. pylori infection have increased gastric secretion

caused by hypergastrinemia and reduced antral somatostatin content.

This leads to increased histamine secretion, and subsequently the increased secretion of acid or pepsin from parietal and gastric cells. Additionally, the eradication of H. pylori leads to a decrease in gastrin mRNA expression and an increase in somatostatin mRNA expression.[74] In the remaining majority of patients, gastric ulcers are associated with hypochlorhydria and mucosal atrophy. The main mechanism of NSAID-associated damage of the gastroduodenal mucosa is the systemic inhibition of constitutively expressed cyclooxygenase-1 (COX-1), which is responsible for prostaglandin synthesis, and is associated with decreased mucosal blood flow, low mucus and bicarbonate secretion, and the inhibition of cell proliferation.

NSAIDs inhibit the enzyme reversibly in a concentration-dependent manner. The co-administration of exogenous prostaglandins and cyclooxygenase-2 (COX-2)-selective NSAIDs use reduces mucosal damage and the risk of ulcers.[75] However, the different physicochemical properties of NSAIDs cause differences in their toxicity.[76] NSAIDs disrupt mucus phospholipids and lead to the uncoupling of mitochondrial oxidative phosphorylation, thus initiating mucosal damage.

When exposed to acidic gastric juice (pH 2), NSAIDs become protonated and cross lipid membranes to enter epithelial cells (pH 7.4), where they ionize and release H+. In that form, NSAIDs cannot cross the lipid membrane, and are trapped in epithelial cells, leading to the uncoupling of oxidative phosphorylation, decreased mitochondria energy production, increased cellular permeability, and reduced cellular integrity. Patients who have a history of peptic ulcers or haemorrhage, are over the age of 65, also use steroids or anticoagulants, and take high doses or combinations of NSAIDs are at the highest risk for acquiring NSAID-induced ulcers.[76]

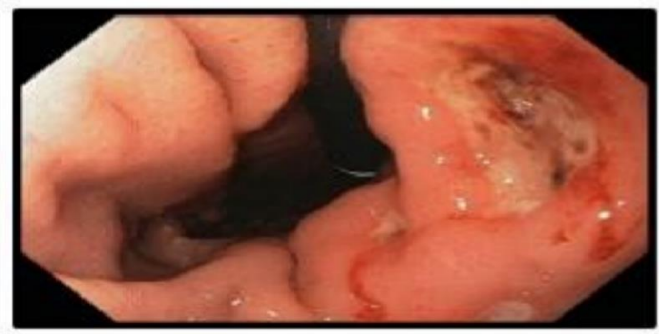


Figure. 4: Photograph of a peptic ulcer taken during an upper endoscopy.

1.3 Stages of Peptic ulcer

1. **Acute Stage:** Characteristic signs of acute peptic ulcer disease are symptoms that often appear suddenly, manifest clearly and progress in a short time. At this stage, if detected and treated properly, the disease can be completely cured. However, most patients often ignore the symptoms, subjectively do not go to the doctor, making the disease more complicated.[77]

2. **Chronic Stage**: Acute peptic ulcer disease, when left untreated, will cause inflammation and swelling for a long time, and after a while, it may turn into a chronic form.In the chronic stage, the lesions spread, the disease is more difficult to treat, and can even lead to dangerous complications such as atrophic inflammation, intestinal metaplasia, pyloric stenosis, hemorrhage, perforation, and gastric cance.[78]

1.4 Causes of peptic ulcer

1. Helicobacter pylori Infection

Infection with *Helicobacter pylori* represents the primary etiological factor in the development of gastric ulcers. Upon colonization of the stomach, the bacterium penetrates the protective mucous layer of the gastric epithelium and establishes itself within the mucosal lining. It produces cytotoxins and virulence factors that disrupt epithelial integrity, induce inflammatory responses, and impair mucosal defense mechanisms. This leads to progressive damage of the gastric mucosa, inhibition of protective factors such as mucus and bicarbonate secretion, and ultimately results in ulcer formation.[79]

2. Prolonged Use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Chronic administration of non-steroidal anti-inflammatory drugs, including Ibuprofen, Naproxen, and Diclofenac, is a well-established risk factor for peptic ulcer disease. These pharmacological agents exert their effects by inhibiting cyclooxygenase (COX) enzymes, leading to a reduction in prostaglandin synthesis. Since prostaglandins play a crucial role in maintaining gastric mucosal integrity by promoting mucus and bicarbonate secretion and regulating mucosal blood flow, their suppression compromises the protective barrier of the stomach, rendering it more susceptible to ulceration.[80]

3. Additional Contributing Factors

Hypersecretion of gastric acid is another significant contributor to mucosal injury and ulcerogenesis. This condition may arise from multiple factors, including genetic predisposition,

tobacco smoking, psychological stress, and dietary influences. Excessive acid production overwhelms mucosal defense mechanisms, leading to epithelial erosion and ulcer formation.

Furthermore, peptic ulcer disease may also be associated with rare pathological conditions such as Zollinger-Ellison syndrome. This syndrome is characterized by the development of gastrin-secreting tumors (gastrinomas) in the pancreas or duodenum, which stimulate excessive gastric acid secretion. The resulting hyperacidity can cause severe and recurrent ulcers, and the tumors may be either benign or malignant in nature.[81]

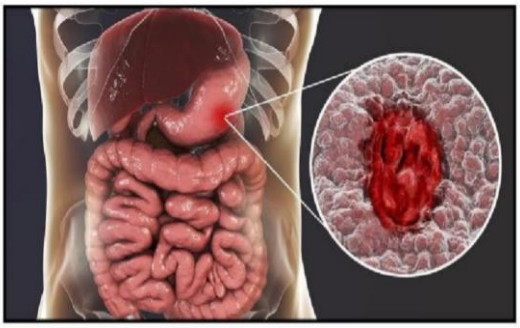


Figure 5: Peptic ulcer.

1.5 Symptoms

The most common peptic ulcer symptom is burning stomach pain. Stomach acid makes the pain worse, as does having an empty stomach. The pain can often be relieved by eating certain foods that buffer stomach acid or by taking an acid-reducing medication, but then it may come back. The pain may be worse between meals and at night. Many people with peptic ulcers don't even have symptoms. Less often, ulcers may cause severe signs or symptoms such as

- Vomiting or vomiting blood
- Nausea or vomiting
- Unexplained weight loss
- Appetite changes
- Feeling faint
- Trouble breathing
- Dark blood in stools, or stools that are black or tarry

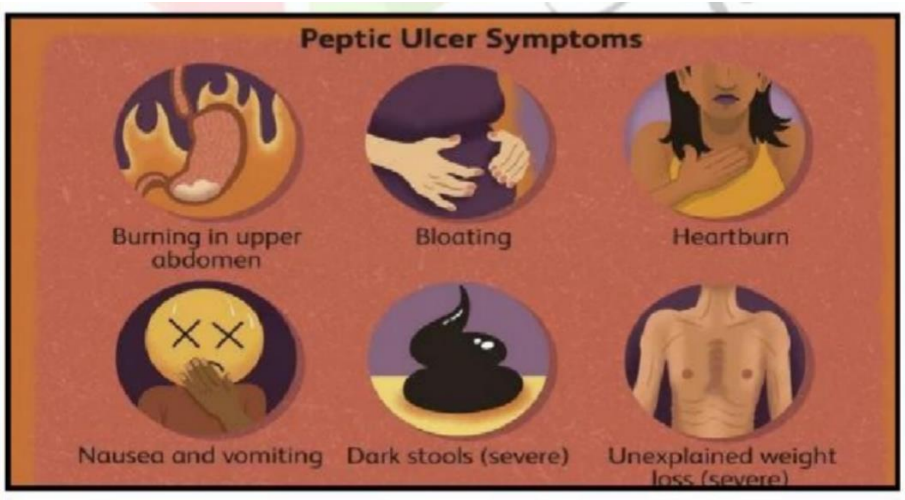


Figure 6: Peptic ulcer symptoms.

1.5 Treatment

The treatment of peptic ulcer disease primarily involves pharmacological agents aimed at reducing gastric acid secretion, enhancing mucosal defense, and eradicating *Helicobacter pylori* infection. Proton pump inhibitors (PPIs) such as omeprazole and pantoprazole are the most effective drugs, as they irreversibly inhibit gastric acid secretion by blocking the H^+/K^+-ATPase enzyme system. H_2-receptor antagonists like ranitidine and famotidine also reduce acid production, though they are less potent than PPIs. Antacids provide rapid symptomatic relief by neutralizing existing gastric acid. Cytoprotective agents such as sucralfate and misoprostol help in strengthening the mucosal barrier and promoting ulcer healing. In cases associated with *H. pylori*, combination therapy including antibiotics like amoxicillin, clarithromycin, or metronidazole along with PPIs is recommended for eradication. Additionally, discontinuation of non-steroidal anti-inflammatory drugs (NSAIDs) is crucial to prevent further mucosal damage. Overall, a combination of acid suppression, mucosal protection, and infection control forms the cornerstone of ulcer management.The management of *Amlapitta* in Ayurvedic therapeutics is based on a comprehensive approach involving both biopurificatory (Śodhana) and palliative (Śamana) interventions, aimed at correcting the underlying derangement of *Pitta dosha*. Śodhana procedures, including *Vamana* (therapeutic emesis), *Virechana* (purgation), *Basti* (medicated enema), and *Raktamokshana* (blood-letting), are employed to eliminate accumulated toxic metabolites (*Āma*) and restore systemic homeostasis. These interventions are particularly indicated in chronic and severe presentations to achieve sustained therapeutic outcomes. Śamana therapy encompasses the administration of single-drug preparations and polyherbal formulations,

frequently delivered in the form of medicated ghee (*Ghṛita*) and herbo-mineral compounds (*Bhasma*). These formulations exhibit multifaceted pharmacodynamic actions, including *Dīpana* (enhancement of digestive fire), *Pācana* (metabolic correction), and *Pitta-śamana* (pacification of aggravated Pitta), thereby contributing to the normalization of gastric secretory function and reinforcement of mucosal defense mechanisms.

The therapeutic management of *Parinamashoola*, which bears clinical resemblance to acid-peptic disorders, similarly incorporates Śodhana procedures alongside targeted herbal interventions and lipid-based formulations. These strategies aim to alleviate postprandial abdominal pain, regulate gastrointestinal motility, and restore digestive equilibrium.

Furthermore, strict adherence to dietary and lifestyle regulations (Pathya-Apathya) constitutes an essential component of Ayurvedic management. Patients are advised to avoid etiological factors that aggravate *Pitta*, including excessive intake of alcohol, saline, pungent, and sour foods, as well as black gram. Behavioral factors such as suppression of natural urges (*Vegadharana*), exposure to excessive heat, and psychological stressors particularly anger are also contraindicated. Such integrative management not only alleviates clinical symptoms but also plays a pivotal role in preventing recurrence and promoting long-term gastrointestinal health.[15,16]

Amlapitta is a well-described pathological entity in Ayurvedic literature, primarily characterized by the vitiation of *Pitta dosha* in association with impaired digestive fire (*Agni*) and accumulation of metabolic toxins (*Āma*). The etiopathogenesis (*Samprapti*) involves excessive intake of *Amla* (sour), *Lavana* (salty), *Katu* (pungent) foods, irregular dietary habits, psychological stress, and lifestyle disturbances, which collectively aggravate *Pitta* and disrupt gastrointestinal homeostasis.

From a modern biomedical perspective, Amlapitta shows strong clinical correlation with acid-peptic disorders, particularly **peptic ulcer disease (PUD)**, gastroesophageal reflux disease (GERD), and hyperacidity syndromes. The Ayurvedic concept of *Agnimandya* (hypofunction of digestive fire) parallels impaired gastric regulation, while *Pitta prakopa* reflects excessive gastric acid secretion and mucosal irritation.[63]

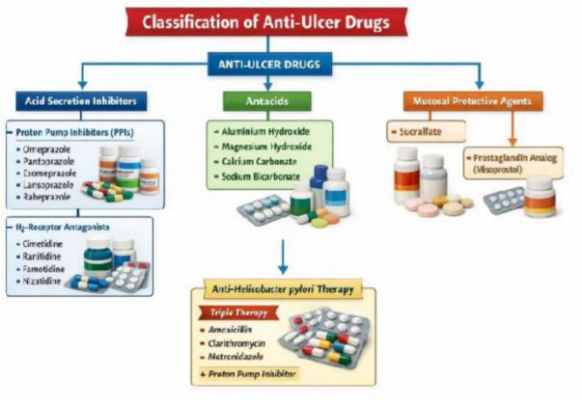


Figure 7: Drugs Classification.

2. **AIM: "Phytochemical and Pharmacological evaluation of *Manilkara Zapota* Roots extracts for Anti-Ulcer activity"**

OBJECTIVE

1. Phytochemical investigation and standardization of ***Manilkara Zapota Roots*** extracts.
2. *In-vitro* antioxidant activity of extracts.
3. Phytochemical screening for ***Manilkara Zapota Roots*** exract.
4. Preclinical Pharmacological evaluation of plants extracts for **Anti-Ulcer Activity.**

7. OBSERVATIONS AND RESULTS

7.1 Collection, identification and authentication of plant material.

The plant material used in the present study was collected from the Chhatrapati Sambhajinagar region, Maharashtra, India. The collected plant specimen was taxonomically identified and authenticated by a botanist from the Department of Botany, Rajarshi Shahu Arts, Commerce and Science College, Pathri. The plant was identified as *Manilkara zapota* belonging to the family *Sapotaceae*. An authentication letter was issued and the voucher specimen was deposited for future reference with accession number 17499. The authentication was carried out by **Dr. Bandewar S. T., Assistant Professor, Department of Botany, Rajarshi Shahu College, Pathri,** confirming that the plant is commonly found in the Marathwada region.

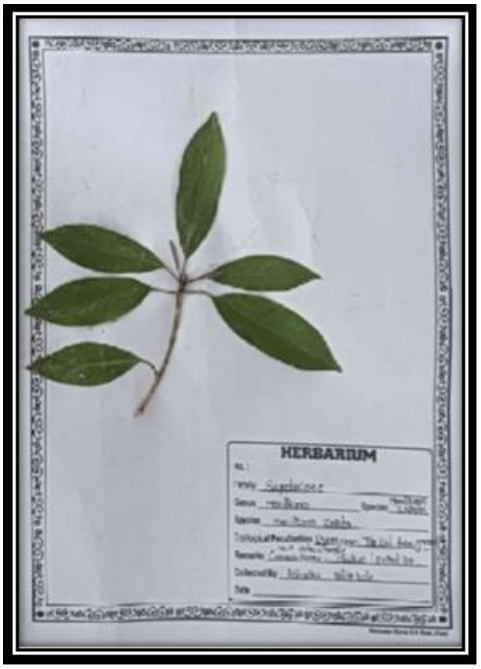


Fig. 13: Herbarium Sheet.

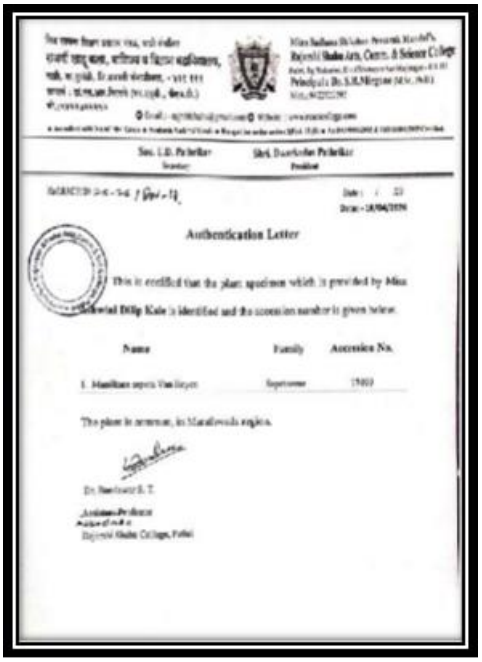


Fig. 14: Authantication Latter.

7.2. Pharmacognostic Evaluation

Table 03: Macroscopic Characteristics of *Manilkara zapota* Root.

Sr. No.	Parameter	Observation
1	Colour	Brown to dark brown
2	Odour	Slight characteristic
3	Taste	Slightly bitter and astringent
4	Shape	Cylindrical, irregularly branched
5	Size	Approximately 10–20 cm in length

7.3. Processing of crude drug

The collected plant material was washed thoroughly with water to remove soil and other foreign matter. The plant material was then shade dried at room temperature for several days until a constant weight was obtained. The dried material was pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was passed through a suitable sieve and stored in an airtight container for further experimental studies.

7.4. Physicochemical Standardization

Table 4: Physicochemical Standardization Parameters of -Plant Material.

Sr. No.	Standardization Parameters	Results(%w/w)
1	Ash value	**3 %**
2	Acid insoluble ash	**1 %**
3	Water soluble ash	**8.5 %**
4	Loss on Drying (LOD)	**6 %**

The standardization of the sample was carried out using physicochemical parameters such as ash value, acid insoluble ash, water soluble ash, and loss on drying. The total ash value was found to be 3 % w/w, which indicates the presence of inorganic components and mineral content in the sample. The acid insoluble ash was determined to be 1 % w/w, suggesting a low amount of siliceous matter such as sand and soil contamination.The water soluble ash value was observed to be 8.5 % w/w, indicating the presence of water soluble inorganic salts. This parameter helps in evaluating the purity and quality of the crude drug. The loss on drying (LOD) was found to be 6 % w/w, which represents the moisture content present in the sample. The low moisture content suggests better stability and reduced chances of microbial growth. Overall, these physicochemical parameters confirm that the sample complies with acceptable limits and indicate good quality and purity of the material.

7.5. Extractive value

Fig. 15: Extractive Values of *Manilkara zapota* Roots in Different Solvents.

Table 5: Extractive Values of *Manilkara zapota* Roots in Different Solvents.

Sr.No.	Solvent	Wt. of Empty Petri Dish (g)	Wt. of Petri Dish with Extract (g)	Wt. of Extract (g)	%Extractive Value (%)
1	Petroleum ether	91.95	92.08	0.13	6.5
2	Chloroform	92.46	92.60	0.14	7
3	Ethyl acetate	101.40	101.68	0.28	14
4	**Acetone**	**91.25**	**91.67**	**0.42**	**21**
5	**Methanol**	**83.86**	**84.32**	**0.46**	**23**
6	Ethanol	91.30	91.55	0.25	12.5
7	Water	91.53	91.70	0.17	8.5

Fig. 16: Soxhlet Assembly.

The extractive values of the sample were determined using solvents of increasing polarity including petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, and water. The extractive value indicates the amount of active constituents soluble in a particular solvent and helps in the evaluation of phytochemical constituents present in the crude drug.The petroleum ether extractive value was found to be 6.5 %, indicating the presence of small amounts of non-polar constituents such as fats and waxes. The chloroform extractive value was 7 %, suggesting the presence of moderately non-polar compounds. The ethyl acetate extractive value was 14 %, indicating the extraction of semi-polar phytoconstituents. The acetone extractive value was observed to be 21 %, showing a higher amount of soluble constituents. The methanol extractive value was highest at 23 %, indicating that the majority of phytochemicals present in the sample are polar in nature such as phenolics, flavonoids, and glycosides. The ethanol extractive value was 12.5 %, showing moderate extraction efficiency. The water extractive value was found to be 8.5 %, indicating the presence of water-soluble constituents such as sugars, tannins, and some glycosides. Overall, the results show that methanol and acetone yielded the highest extractive values, suggesting that polar solvents are more suitable for extraction of active constituents from the sample.

7.6. Extraction of plant material

The dried and coarsely powdered plant material was packed in a thimble and placed in a Soxhlet apparatus. Initially, the material was defatted with petroleum ether. After complete defatting, the marc was extracted successively with **Methanol** followed by **Acetone**. The extracts obtained were concentrated using a water bath and stored in airtight containers for further study.

7.7. Phytochemical screening

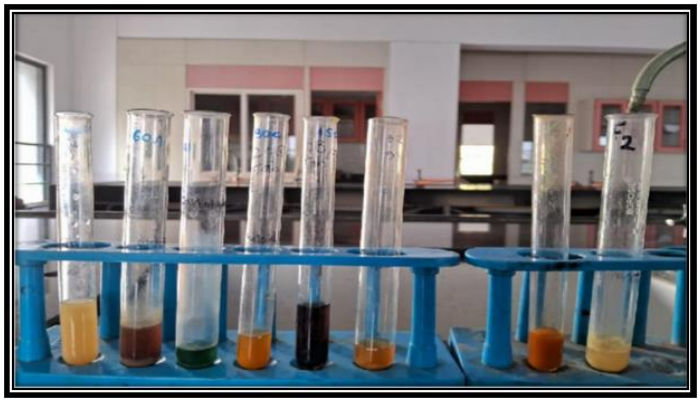


Fig. 17: Phytochemical Screening of Different Extracts.

Table 6: Preliminary Phytochemical Screening of Different Extracts.

(+) = Presence of phytoconstituent (−) = Absence of phytoconstituent

Sr. No.	Phytochemical Test	Pet. Ether	Methanol	Acetone
1	**Test for Alkaloids**			
a	Dragendorff's test	+	+	+
b	Mayer's test	–	–	–
c	Wagner's test	+	+	+
d	Hager's test	+	–	–
2	**Test for Flavonoids**			
a	Shinoda test	+	+	+
b	Sulphuric acid test	–	+	+
c	Lead acetate test	–	+	+
3	**Test for Glycosides**			
a	Keller–Killiani test	+	+	+
b	Legal test	–	+	–
c	Foam test	+	+	+
4	**Test for Steroids**			
a	Salkowski test	+	+	+
b	Liebermann–B urchard test	–	–	+
5	**Test for Proteins**			
a	Biuret test	+	+	+
b	Millon's test	–	–	–
6	**Test for Carbohydrates**			
a	Molisch test	+	+	+
b	Fehling's test	+	+	+
c	Benedict's test	–	+	+
d	Barfoed's test	–	–	–
7	**Test for Tannins & Phenolic Compounds**			
a	Lead acetate test	+	+	+
b	Dil. HNO_3 test	–	+	+
c	Dil. iodine solution	–	+	+

Preliminary phytochemical screening showed the presence of various bioactive compounds in petroleum ether, methanol, and acetone extracts. Alkaloids were present in all extracts (Dragendorff's and Wagner's positive), while flavonoids were more prominent in methanol and acetone extracts. Glycosides, proteins, and carbohydrates were detected in all extracts, whereas steroids were confirmed mainly in acetone extract. Tannins and phenolic compounds were also present, especially in methanol and acetone extracts. Overall, methanol and acetone extracts showed richer phytochemical composition compared to petroleum ether extract.

7.8. Quantitative analysis

7.8.1. Total Phenolic Content

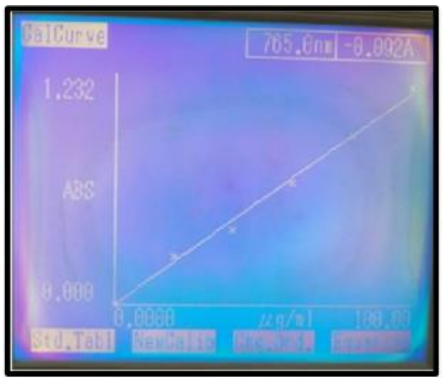


Fig. 18: Calibration curve of standard Gallic acid.

Table 7: Absorbance of Standard Gallic acid Solution (n = 3)

Values represent mean ± SEM (n=3)

Sr. No.	Concentration (µg/ml)	Absorbance (Mean ± SD)
1	20	0.271 ± 0.002
2	40	0.420 ± 0.002
3	60	0.691 ± 0.002
4	80	0.961 ± 0.003
5	100	1.231 ± 0.003

Table No. 8: Total phenolic Extract

Sr. No.	Concentration (µg/mL)	Extract	Absorbance (Mean ± SD)	Phenolic Content (mgGAE/g extract, Mean ± SD)
1	100	Methanol	0.890 ± 0.005	33.32 ± 0.72
2	100	Acetone	1.250 ± 0.006	42.50 ± 0.85

The total phenolic content was determined using different standard concentrations ranging from 20–100 µg/ml. The absorbance values increased progressively from 0.271 ± 0.002 to 1.231 ± 0.003, indicating a linear relationship between concentration and absorbance. This linearity confirms the suitability of the method for estimation of total phenolic content in the

extracts. The phenolic content of the extracts was calculated using the calibration curve. The methanol extract showed an absorbance of 0.890 ± 0.005, corresponding to a total phenolic content of 33.32 ± 0.72 mg GAE/g extract, indicating the presence of moderate amount of phenolic compounds. In contrast, the acetone extract exhibited higher absorbance of 1.250 ± 0.006 with total phenolic content of 42.50 ± 0.85 mg GAE/g extract, suggesting higher concentration of phenolic constituents.Phenolic compounds are known for their antioxidant and therapeutic properties. The higher phenolic content observed in the acetone extract indicates better extraction of phenolic constituents in acetone compared to methanol. Overall, the results suggest that both extracts contain significant amounts of phenolic compounds, with acetone extract showing comparatively higher total phenolic content.

7.8.2Total Flavonoid Content.

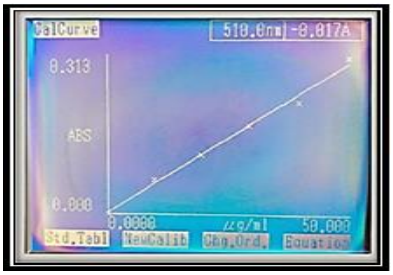


Fig. 19: Calibration curve of rutin.

Table 09: Absorbance of Standard Rutin Solution (n = 3).

Values represent mean ± SEM (n=3)

Sr. No.	Concentration (µg/mL)	Absorbance (Mean ± SD)
1	10	0.071 ± 0.002
2	20	0.123 ± 0.003
3	30	0.182 ± 0.002
4	40	0.228 ± 0.003
5	50	0.313 ± 0.004

Total flavonoid content

Table No. 10: Total phenolic Extract.

Values represent mean ± SEM (n=3).

Sr.No.	Extract	Concentration (µg/mL)	Absorbance (Mean ± SD)	TFC(mg Rutin/g extract, Mean ± SD)
1	Acetone	50	0.221 ± 0.003	39.02 ± 0.65
2	Methanol	50	0.274 ± 0.004	46.25 ± 0.78

The calibration curve for total flavonoid content was prepared using standard rutin solution at concentrations ranging from 10–50 µg/mL. The absorbance values increased gradually from 0.071 ± 0.002 to 0.313 ± 0.004, indicating a linear relationship between concentration and absorbance. This linearity confirms the reliability of the method for estimation of total flavonoid content in the extracts. The total flavonoid content of the extracts was calculated using the standard rutin calibration curve. The Acetone extract showed an absorbance of 0.221 ± 0.003 at 50 µg/mL, corresponding to 39.02 ± 0.65 mg rutin/g extract, indicating moderate flavonoid content. The methanol extract exhibited higher absorbance of 0.274 ± 0.004 with total flavonoid content of 46.25 ± 0.78 mg rutin/g extract, suggesting higher flavonoid concentration. Flavonoids are important phytoconstituents known for antioxidant and pharmacological activities. The higher flavonoid content observed in the methanol extract indicates better extraction efficiency of flavonoids in methanol compared to ethyl Acetone. Overall, both extracts contain appreciable amounts of flavonoids, with methanol extract showing comparatively higher total flavonoid content.

7.9. *In-vitro* antioxidant activity-DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical cavenging activity

Table 11: Antioxidant Activity.

Values represent mean ± SEM (n=3)

Conc (µg/ml)	Rutin (% inhibition)	Gallic acid (%inhibition)	Ascorbic acid (%inhibition)	Acetone Extract (%inhibitio)	Methanol Extract (%inhibition)
25	89.84 ± 0.42	80.61 ± 0.51	85.07 ± 0.46	86.92 ± 0.38	85.23 ± 0.41
50	92.76 ± 0.36	82.92 ± 0.44	86.30 ± 0.39	88.61 ± 0.35	87.54 ± 0.37
75	94.46 ± 0.31	85.69 ± 0.33	88.76 ± 0.34	89.38 ± 0.29	86.12 ± 0.42
100	96.30 ± 0.28	90.60 ± 0.27	90.00 ± 0.30	91.53 ± 0.25	90.54 ± 0.31
125	97.69 ± 0.22	94.76 ± 0.21	93.84 ± 0.24	93.38 ± 0.26	92.85 ± 0.28

The antioxidant activity of acetone and methanol extracts was evaluated using the DPPH radical scavenging assay and compared with standard compounds such as rutin, gallic acid, and ascorbic acid. The experiment was performed in triplicate, and results were expressed as mean ± SD.The percentage inhibition increased with increase in concentration (25–125 µg/ml) for both standards and extracts, indicating concentration-dependent antioxidant activity. Among the standards, rutin exhibited the highest scavenging activity (89.84 ± 0.42 to 97.69 ± 0.22%), followed by gallic acid (80.61 ± 0.51 to 94.76 ± 0.21%) and ascorbic acid (85.07 ± 0.46 to 93.84 ± 0.24%).Among the test extracts, acetone extract showed slightly higher antioxidant activity (86.92 ± 0.38 to 93.38 ± 0.26%) compared to methanol extract

(85.23 ± 0.41 to 92.85 ± 0.28%). The results suggest that both extracts possess significant free radical scavenging activity, which may be attributed to the presence of phenolic and flavonoid compounds.

7.10. Pharmacological Screening of *Manilkara zapota Root* extracts

7.10.1. IAEC Approval

Wistar rats of either sex weighing between 180–230 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in the animal house of Systemic Life Sciences & Research Pvt. Ltd., Telangana, approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were housed under standard environmental conditions with 12 h light/dark cycle, temperature (22 ± 2°C) and relative humidity 55 ± 5%, and were provided with standard pellet diet and water ad libitum. All animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The study titled *"Phytochemical and pharmacology Evaluation of Manilkara zapota Roots Extract for Anti-ulcer Activity in Rats"* was approved with IAEC approval number **13/IAEC-II/SLSRPL/2025**. All experimental procedures were carried out in accordance with CPCSEA guidelines for the care and use of laboratory animals.

7.10.2. Grouping of Animals

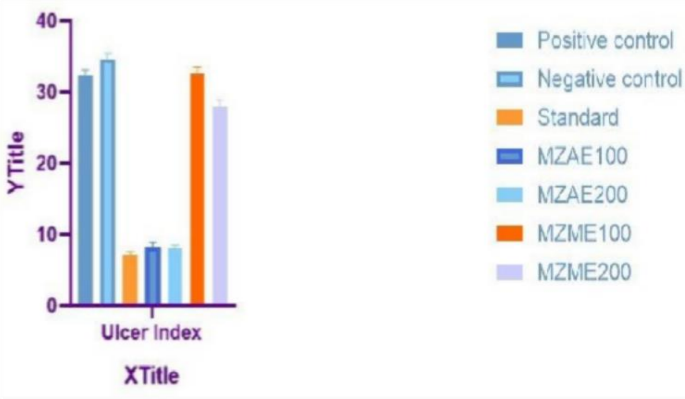
Table 12: Experimental grouping of animals for evaluation of anti-ulcer activity of *Manilkara zapota* Root extracts.

Group	Treatment	Dose	Route	Description
Group I	Normal control	—	Oral	Animals received normal saline only
Group II	Positive control	Ethanol(1ml/200g)	Oral	Animals received ethanol to induce gastric ulcer
Group III	Standard drug	20 mg/kg	Oral	Animals received Ranitidine before ethanol administration
Group IV	MZAE	100 mg/kg	Oral	Acetone extract of *Manilkara zapota* root (MZAE 100 g/kg)
Group V	MZAE	200 mg/kg	Oral	Acetone extract of *Manilkara zapota* root (MZAE 200 mg/kg)
Group VI	MZME	100 mg/kg	Oral	Methanolic extract of *Manilkara zapota* root (MZME 100 mg/kg)
Group VII	MZME	200 mg/kg	Oral	Methanolic extract of *Manilkara zapota* root (MZME 200 mg/kg)

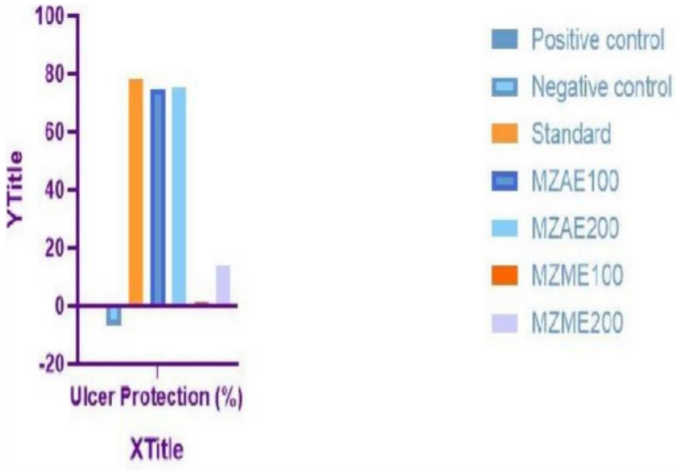
Table 13: Effect of *Manilkara zapota* Root Extract in Ethanol-Induced Gastric Ulcer.

Group	Ulcer Index	Ulcer Protection (%)
Positive Control	32.42 ± 0.66	0
Negative Control	34.62 ± 0.78	-6.79
Standard	7.1 ± 0.53	78.10**
MZAE100	8.2 ± 0.68	74.71**
MZAE200	8.1 ± 0.36	75.02**
MZME100	32.53 ± 0.98	-0.34
MZME200	27.98 ± 0.89	13.69*

*Values are expressed as mean ± standard deviation (SD) for six animals in each group (n = 6). Statistical analysis was performed by comparing treated groups with the negative control group. A value of *p < 0.05 was considered statistically significant, while **p < 0.01 was considered highly significant.*



Graph 1: Ulcer Index.



Graph 2: Ulcer Protection.

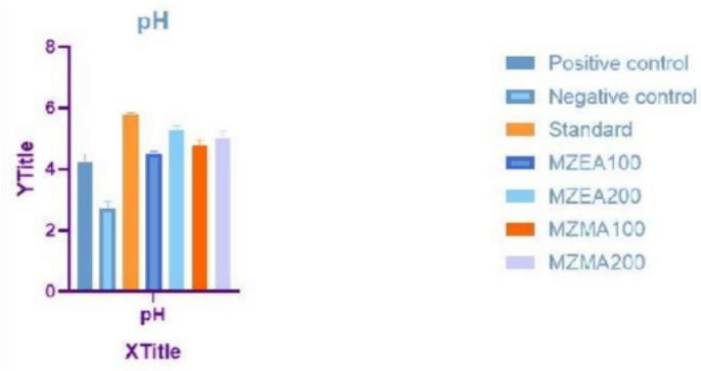
The anti-ulcer activity of *Manilkara zapota* root extracts was evaluated using ethanol-induced gastric ulcer model. The positive control group showed severe gastric mucosal damage with a

high ulcer index (32.42 ± 0.66). The negative control group also showed no protection and slightly increased ulcer index (34.62 ± 0.78), indicating absence of gastroprotective effect. The standard drug ranitidine (20 mg/kg) significantly reduced the ulcer index to 7.1 ± 0.53 with 78.10% ulcer protection, confirming the validity of the model. Among the test groups, Acetone extract exhibited marked gastroprotective activity. MZEA100 showed ulcer index of 8.2 ± 0.68 with 74.71% protection, while MZEA200 showed slightly better protection (75.02%) with ulcer index of 8.1 ± 0.36, indicating dose-dependent anti-ulcer activity. Methanolic extract showed comparatively lower protection. MZME100 did not produce significant protection (−0.34%), with ulcer index similar to control. However, MZME200 showed mild gastroprotective activity with ulcer index of 27.98 ± 0.89 and 13.69% protection. Overall, the results indicate that Acetone extract of *Manilkara zapota* roots exhibited significant anti-ulcer activity, whereas methanolic extract showed mild to moderate protection against ethanol-induced gastric ulcers.

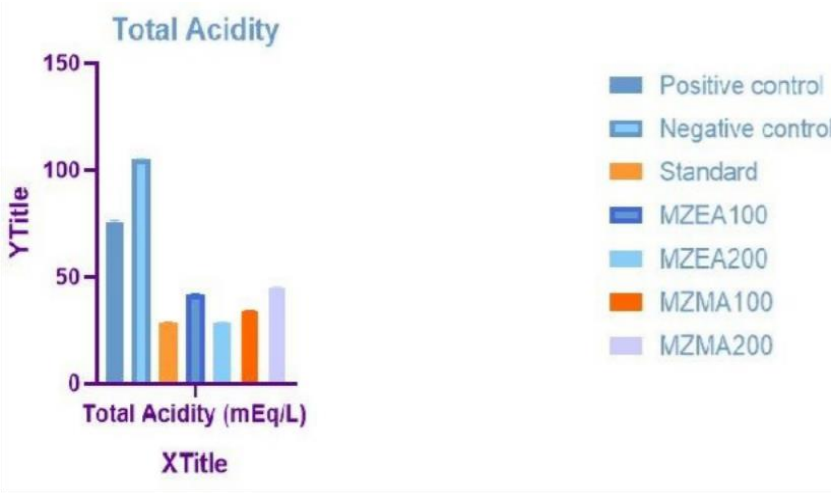
Table 14: Effect of *Manilkara zapota* Root Extract on Gastric pH and Total Acidity

Group	pH	Total Acidity (mEq/L)
Positive control	4.2 ± 0.30	75.5 ± 0.20
Negative control	2.7 ± 0.25	105.0 ± 0.50
Standard	5.8 ± 0.04	28.45 ± 0.40**
MZEA100	4.5 ± 0.08	41.45 ± 0.48**
MZEA200	5.3 ± 0.11	28.55 ± 0.10**
MZMA100	4.8 ± 0.16	34.12 ± 0.36**
MZMA200	5.0 ± 0.25	44.78 ± 0.46**

*Values are expressed as mean ± standard deviation (SD) for six animals in each group (n = 6). Statistical analysis was performed by comparing treated groups with the negative control group. A value of *p < 0.05 was considered statistically significant, while **p < 0.01 was considered highly significant.*



Graph 3: pH.



Graph 4: Total Acidity.

The effect of *Manilkara zapota* root extracts on gastric pH and total acidity was evaluated in ethanol-induced gastric ulcer model. The negative control group showed a marked decrease in gastric pH (2.7 ± 0.25) and a significant increase in total acidity (105.0 ± 0.50 mEq/L), indicating severe gastric acid secretion. In contrast, the positive control group showed moderate pH (4.2 ± 0.30) and reduced acidity (75.5 ± 0.20 mEq/L). Treatment with standard drug ranitidine significantly increased gastric pH (5.8 ± 0.04) and decreased total acidity (28.45 ± 0.40 mEq/L), demonstrating strong antisecretory activity. Among the test groups, ethyl acetate extract showed dose-dependent improvement. MZEA100 increased pH (4.5 ± 0.08) and reduced acidity (41.45 ± 0.48 mEq/L), while MZEA200 produced greater effect with higher pH (5.3 ± 0.11) and lower acidity (28.55 ± 0.10 mEq/L), comparable to standard. Methanolic extract also improved gastric parameters. MZMA100 showed pH of 4.8 ± 0.16 and total acidity of 34.12 ± 0.36 mEq/L. MZMA200 exhibited moderate antisecretory activity with pH 5.0 ± 0.25 and acidity 44.78 ± 0.46 mEq/L. Overall, the extracts increased gastric pH and decreased total acidity, suggesting significant gastroprotective and antisecretory activity of *Manilkara zapota* root extract.

7.10.3. Ethanol induced ulcer: Gross microscopic study of effect of *Manilkara zapota* Root extract on Gastric mucosal ulcer.

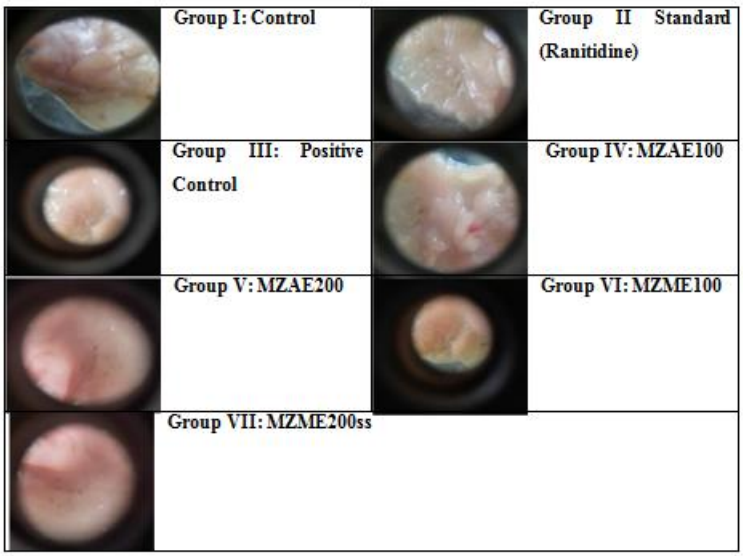


Fig. 20: Gross microscopic effect of *Manilkara zapota* Root extract on Gastric mucosal ulcer (gastroprotective) activity.

The ulcer protective activity of *Manilkara zapota* root extracts was evaluated by comparing gastric mucosal changes among different experimental groups. Group I (Control) showed normal gastric mucosa without any visible lesions. Group II (Standard - Ranitidine) exhibited marked protection with minimal ulceration and intact mucosal lining. Group III (Positive Control) showed severe gastric ulceration characterized by hemorrhagic streaks, mucosal erosion, and multiple ulcer lesions. Group IV (MZAE100) treated with *Manilkara zapota* acetone extract (100 mg/kg) showed mild ulceration with partial protection of gastric mucosa. Group V (MZAE200) demonstrated better protection with reduced number of ulcers and improved mucosal integrity, indicating dose-dependent activity of acetone extract. Group VI (MZME100) treated with methanol extract (100 mg/kg) showed moderate protection with fewer lesions compared to the positive control. Group VII (MZME200) exhibited significant gastroprotective effect with near normal gastric mucosa and minimal ulcer formation. Overall, both acetone and methanol extracts showed ulcer protective activity in a dose-dependent manner, with MZME200 showing maximum protection, comparable to the standard drug ranitidine.

3. Literature survey

1. **Jaiswal et al.2021:** Peptic ulcer disease (PUD) is a common gastrointestinal disorder characterized by the formation of lesions in the gastric or duodenal mucosa due to an

imbalance between aggressive and protective factors. Aggressive factors include gastric acid, pepsin, bile salts, and *Helicobacter pylori* infection, whereas protective factors involve mucus secretion, bicarbonate production, mucosal blood flow, and prostaglandins. When this balance is disrupted, mucosal damage occurs, leading to ulcer formation.[17]

2. **Vakial N et al. 2024:** Several studies have identified *Helicobacter pylori* infection and the use of non-steroidal anti-inflammatory drugs (NSAIDs) as the major etiological factors responsible for peptic ulcer disease. It has been reported that *H. pylori* contribute to approximately 42% of ulcer cases, while NSAID use accounts for about 36% of cases. These agents damage the mucosal barrier and increase gastric acid secretion, thereby promoting ulcer formation eptic ulcer disease is associated with increased hospitalization rates and mortality. Acid blocking with proton pump inhibitors, such as omeprazole or lansoprazole, is the primary treatment. Recurrence of ulcers can be prevented by eradicating H pylori if present and discontinuing aspirin or NSAIDs if applicable.[18]

3. **Saswat Swarup et al. 2021:** Epidemiologically, peptic ulcer disease affects approximately 5–10% of the global population, with a higher prevalence observed in older adults. The incidence has declined in recent years due to improved sanitation, better diagnosis, and effective eradication therapies for *H. pylori*. However, ulcer-related complications such as bleeding and perforation still pose significant health risks and contribute to morbidity and mortality worldwide. this review highlights the need for continued research efforts to address the challenges posed by peptic ulcer disease. By fostering awareness, promoting research, and encouraging the implementation of effective therapies, we can collectively strive towards reducing the burden of peptic ulcer disease and improving the health and well-being of individuals worldwide.[19]

4. **Singh et al.2025:** The pathophysiology of ulcer formation involves an imbalance between mucosal defensive mechanisms and damaging factors. Gastric acid and pepsin play a central role in mucosal injury, while oxidative stress and inflammatory mediators further aggravate tissue damage. Experimental studies have demonstrated that ethanol, stress, and NSAIDs induce ulcers by increasing reactive oxygen species (ROS) and reducing mucosal defense.[20]

5. **Vakial N et al. 2024:** Clinical manifestations of peptic ulcer disease include epigastric

pain, nausea, vomiting, and in severe cases, complications such as gastrointestinal bleeding, perforation, and gastric outlet obstruction. Among these, bleeding is the most common complication, occurring in approximately 73% of complicated cases, followed by perforation and obstruction. Peptic ulcer disease is associated with increased hospitalization rates and mortality. Acid blocking with proton pump inhibitors, such as omeprazole or lansoprazole, is the primary treatment. Recurrence of ulcers can be prevented by eradicating H pylori if present and discontinuing aspirin or NSAIDs if applicable. Eradication of H pylori decreases peptic ulcer recurrence rates from approximately 50% to 60% to 0% to 2%. Discontinuing NSAIDs heals 95% of ulcers identified on endoscopy and reduces recurrence from 40% to 9%. When discontinuing an NSAID is not desirable, changing the NSAID (eg, from ketorolac to ibuprofen), adding a proton pump inhibitor such as omeprazole or lansoprazole, and eradicating H pylori with treatment such as bismuth, metronidazole, and tetracycline combined with omeprazole can reduce recurrence rates.[21]

6. **Viana et al.2013:** Studied gastro-protective activity of ethanolic extract of leaves of Cenostigma macrophyllum Tul. Var. acuminata Teles Freire (Cm-FHA) on rats. Various ulcer inducing models such as Absolute ethanol induced gastric ulcer, HCl/ethanol-induced gastric ulcers, ischemia-reperfusion-induce gastric ulcers, cold restrain stress-induced gastric ulcers, indomethacin induced ulcer gastric ulcers were used to induce ulcers in rats. Extract was prepared using 4kg of dried leaves of CmFHA and subjected to extraction using 95% of ethanol. Concentrated ethanol extract was further subjected to methanol/water (1:2) and successively extracted with ethyl acetate, concentrated extract of which was further suspended with methanol/water (1:2) and was extracted with hexane. Cm-FHA when compared with standard drug cimetidine significantly exhibited gastroprotective effect in ethanol induced ulcer model at dose of 100 and 200mg/kg by showing lesion inhibition by 40%. In HCl /ethanol induced model lesion inhibition by 50 and 48% at a dose of 100 and 200mg/kg respectively. In ischemia reperfusion model lesion inhibition was 49% at 100mg/kg and 90% at 200mg/kg and in cold restrain stress induced model lesion inhibition was 63% at 100mg/kg and 76% at 200mg/kg. Study was concluded that results satisfactorily proved gastroprotective activity of Cenostigma macrophyllum which reinforces its traditional use in treatment of peptic ulcer as described in folk medicine.[22]

7. **Mota da silva et al 2015 :** Carried in vivo and in vitro studies to determine ulcer healing activity of hydro alcoholic extract of aerial plants of Mayenus robusta Reissek (HEMR) using acetic acid induced chronic ulcer model. Pylorus ligation induced ulcer induced model was used to study antisecretory property. In vitro antiulcer activity was determined by carrying out radical scavenging activity, cytoprotective effect and cell proliferation activity in fibroblast (L929 cells), with the assessment of antihelicobacter pylori activity. HEMR at oral dose of 10mg/kg caused reduction in gastric ulcer area by 53% in acetic acid induced chronic ulcer model. However in pylorus ligated ulcer induced model, HEMR 10mg/kg when administered by intraduodenal route did not show significant changes in volume, pH, total acidity and pepsin activity. In vitro studies of HEMR at the concentration of 1-1000µg/ml significantly scavenge free radical DPPH and also have shown cytoprotection in fibroblasts against hydrogen peroxide at concentration of 0.1-100 µg/ml.[23]

8. **De-Faria et al 2012:** Studied mechanism of action of antiulcer activity of Rhizophora mangle L. The bark of Rhizophora mangles L. was powdered and was extracted by maceration using acetone: water (7:3) to obtain crude extract (CE) which was later subjected to fractionation using different solvent to obtain aqueous fraction (Aq), ethyl acetate fraction (EtoAc) and butanolic fraction (BuOH). Gastic ulcer was induced in rats using ethanol induced and pylorus ligation induced ulcer model. Results showed that all the fractions of Rhizophora mangle L. showed a gastroprotective activity at all the tested doses. BuOH extract exhibited significant antiulcer activity so was further examined to determine the possible underlying mechanism of action. Involvement of Nitric oxide(NO), Sulfydril compounds(SH), mucus adhering to gastric wall and levels of PGE2 were determined along with the expression of COX-1,COX-2 and EGF. It was concluded that gastroprotective, ulcer healing and antisecretory effect of BuOH may be contributed by enhancement of PGE2 levels and upregulation of COX-2 and EGF.[24]

9. **Figueredo SM et al 2011:** Evaluated antiulcer activity of ethanolic extract of Malvastrum tricuspidatum using different ulcer inducing models such as ethanol induced, aspirin induced, cold restrain stress and pylorus ligation induced ulcer induced models. Whole plant of Malvastrum tricuspidatum was dried, powdered and defatted with petroleum ether and was extracted with ethanol. Ethanolic extract was evaluated for antiulcer activity using omeprazole as standard. Ethanolic extract of Malvastrum tricuspidatum at a dose of

500mg/kg showed a significant antiulcer activity in all the models[25]

10. **Santos RC et al 2012:** Evaluated Pluchea sagittalis (Lam) Cabrera for its antinociceptive and gastroprotective actions. Aerial plants of Pluchea sagittalis were dried, powdered and extracted with ethanol. Extract was studied for antinociceptive property in mice using different models such as abdominal constriction induced by acetic acid, nociception induced by glutamate, nociception induced by formalin and gastroprotective actions were investigated using ethanol induced ulcer model. Results showed that ethanolic extract of Pluchea sagittalis inhibition of acetic acid induced abdominal constriction with ID50 value of 624mg /kg and decrease in glutamate induced pain in mice with ID50 value of 368mg/kg. Extract also showed promising results by decreasing inflammatory phase induced by formalin at ID50 value of 411mg/kg. Ethanolic extract of Pluchea sagittalis also reduced gastric lesions produced by ethanol with ID50 value of 55 mg/kg.[26]

11. **Singh KD et al 2015:** Studied gastric and duodenal anti-ulcer activity of methanolic extract of leaves of Byrsonima intermdia A.Juss.(MBI). Antiulcer activity was determined by ethanol induced ulcer model in rats, NSAID-induced gastric ulcer, HCl/ethanol induced ulcer and pylorus ligation induced ulcer in mice. Ulcer protective effect of MBI was determined by evaluating following parameters such as gastric juice volume, pH, total acidity, mucus, NO, sulfhydryl compound, vanilloid receptor, glutathione level and myeloperoxide activity in gastric and duodenal mucosa. MBI was administered orally at the dose of 250, 500 and 1000mg/kg of body weight. Research work clearly demonstrates antiulcer activity of MBI by inhibiting gastric and duodenal ulcer by 69%. It is subsequently proved that gastroprotective action of MBI is mainly by participation of endogenous sulfhydryl compounds and increase in GSH level to provide gastric and duodenal protection.[27]

12. **Chouhan et al 2010:** studied antiulcer and in vitro antioxidant activity of Allium hookerii-an ethnomedicinal plant of Manipur. Leaves of Allium hookerii were collected, shade dried and powdered in mechanical grinder. Powder was defatted using petroleum ether and extracted with methanol. Methanolic extract of Allium hookerii was subjected to phytochemical evaluation to identify the presence of different phytoconstituents. MEAH was administered orally at the dose 200 and 400 mg/kg for seven days for acute ulcer protective study. Antiulcer activity was studied by pylorus ligation induced, ethanol induced and indomethacin induced gastric ulcer models. In vitro antioxidant activity was

evaluated by DPPH assay, reducing power assay, superoxide radical scavenging activity and hydrogen peroxide radical scavenging activity. Phytochemical studies revealed presence of flavonoids, carbohydrates, glycosides, steroids, saponins and phenolic compounds. Results showed that in pylorus ligated induced ulcer rats there was significant decrease total and free acidity 50% and 30.42% at dose of 200mg/kg and 55% and 41% at a dose of 400mg/kg respectively, with increase in pH i.e. 3.1 at 200mg/kg and 4.1 at 400mg/kg as compared to control 2.8, ulcer index showing 31.0% protection at 200mg/kg and 40.5% protection at 400mg/kg,total hexoses 20.4 and 28.6 at 200mg/kg and 400mg/kg respectively as compared to control 12.6, hexosamine 19.1 and 22.8 at 200mg/kg and 400mg/kg respectively as compared to control 11.6 which satisfactorily proved antisecretory activity of MEAH with in vitro antioxidant potential.[28]

13. **Balekar N, et al 2012:** studied Muktashukti Bhasma for antipeptic ulcer activity. Ash or paste of pearl oyster called as Muktashukti Bhasma (MSB) is used to treat various gastric disorders in ayurvedic system of medicine. In this study rats of either sex were divided into seven containing eight animals each. Group I was treated with distilled water and was kept as control, Group II, III and IV were treated with MSB with dose of 100mg/kg, 300mg/kg and 1000 mg/kg. Group V, VI and VII were treated subcutaneously with standard drug ranitidine with dose range of 0.5, 2 and 5 mg/kg of body weight. Assessment of peptic ulcer activity was carried out using pylorus ligated induced ulcer model. It was observed that MSB showed promising antiulcer activity with decrease in ulcer score and ulcer index in all administered doses. Ulcer score exhibited by MSB at dose of 100, 300, and 1000mg/kg of body weight was 1.66 ± 0.20, 0.66 ± 0.20 and 0.50 ± 0.22 as compared to control 2.00 ± 0.47. Ulcer index was 166, 44 and 25 as compared to control 200.0. At the same dose levels pH raised by MSB was 2.66, 3.66 and 6.0 as compared to control 2.0. It was concluded that MSB showed significant reduction in acid output and has anti-ulcer activity[29]

14. **Ingale AM et al 2016:** Evaluated anti-ulcer activity of grape (Vitis vinifera) seed extract using hydrochloric acid- ethanol induced ulcer model using wistar albino rats. Grape seed extract (GSE) was prepared by removing the seeds from grapes and were air dried. Seeds were then extracted with 95% ethanol. Rats of either sex were divided 4 groups containing 6 animals each. Group I served as control and were treated with 1ml distilled water, group II was treated with standard drug 100mg/kg of sucralfate, Group III and IV were treated with GSE at 100mg/kg and 200mg/kg respectively. All the treatment was administered orally. Ulcer was introduced 30 minutes after the treatment by administering 1ml of 0.3M

HCl and 60% ethanol. It was observed that though there was no significant change in the gastric volume, pH, total acidity and bound acidity, GSE significantly reduced ulcer number 30 + 3.23 and 27+ 2.97 as compared to control 41.66. Ulcer inhibition was 27.98% and 34.67% respectively[30]

15. **Pinheiro Silva L et al 2015:** collected leaves of Terminalia catappa (Combretaceae), shade dried and powdered and were extracted with ethanol. Aqueous fraction of Terminalia catappa (FrAq) was evaluated for antiulcer activity. Ulcers were induced in rats by ischemia reperfusion, pylorus ligation, ethanol induced ulcers and acetic acid induced gastric lesions. In vitro test was done to find out anti- helicobacter pylori activity. It was found that FrAq at 25mg/kg reduced ulcer lesions and also showed considerable activity against helicobacter pylori[31]

16. **Balekar N et al 2013:** evaluated antiulcer activity of bark extract of Albizzia lebbeck linn. Ulcers were induced using models such as ethanol induced ulcer, aspirin induced ulcer, cold stress restraint and pylorus ligation induced ulcer. Ethanolic extract of Albizzia lebbeck linn. at the dose of 500mg/kg showed substantial decrease in ulcer index number, gastric volume, free acidity and total acidity and substantial increase in mucous content as compared to control. This study reveals that Albizzia lebbeck Linn has good antiulcer potential due to its antisecretory activity.[32]

17. **Biondo TMA et al 2011:** Studied the antisecretory potential of aqueous extract of Baccharis trimera (Less.) DC and isolated active constituent to determine mechanism of action involved. In vivo antiulcer test were performed using cold restraint stress induced ulcer and pylorus ligated induced ulcer. Fractions were isolated using HPLC . 14C-aminopyrine [14C]-AP was used as measurement of gastric acid secretion. Fractions were also subjected to enzymatic assay of H+ K + ATPase. It was observed that aqueous extract Baccharis trimera (Aq) at dose of 25mg/kg reduced ulcer lesions. Fraction of aqueous extract (FrAq) signified that increase in mucus production, nitric oxide pathway and endogenous prostagalndin may be responsible for antiulcer efficacy of Baccharis trimera. Extract also showed considerable activity against Helicobacter pylori.[33]

18. **Heywood JB et al 1940:** Studied the occurrence and constitution of a sapogenin bassic acid in sapotaceous plants. It appeared th at bassic acid was characteristically present in the seeds of all except 2 of the sapotaceae plants examined. Seeds of M. hexandra, M. elengi,

M. djave and A. sapota were reported to contain 1.23%, 2.41%, 0.58 and 0 70% of bassic acid respectively.[34]

19. **Subramaniam SS et al 1973:** While studying distribution pattern of flavonoids in sapotaceae, observed presence of small am ounts of flavonoids chiefly quercitrin and quercetin in leaves of M. hexandra. Myricetin and quercetin aglycones along with myricetin 3-0-D-galactoside were shown to be present in leaves of M. elengi[35]

20. **Misra G et al 1974:** Reviewed the work carried out on the 3 well-known species of genus Mimusops, M. elengi, M. hexandra and M. m anilkara. They reported bassic acid as the only sapogemn of kernel. a-Spinosterol, p-D-glucoside of P-sitosterol and quercitol were reported as common com ponents m all the 3 plants. Quercitol, hentriacontane and taraxerol are common constituents of leaves. The seeds of all the 3 species were reported to contain identical compounds.[36]

21. **Bano M, Ahmed B et al 2014:** Examined fixed oil from seed. Seeds on extraction with P. ether (40-60°) yielded 13 6% of yellow colored oil. Purified crude oil upon saponification with KOH gave a and (3 sitosterols and fatty acids, capric acid 1.1%, launc acid 3. 38%, myristic acid 1.08%, palmitic acid 11.04%, stearic acid 11.35%, arachidic acid 0.55%, oleic acid 58.44% and linoleic acid 13 06% were separated from unsaponifiable m atter.[37]

22. **Orwa C et al 2009:** Related & objectives: Sesbania grandiflora has long been used in folk medicine in treatment of diarrhoea, snake bite, malaria, smallpox, fever, scabies; ulcer, and stomach disorders. Therefore, Present study was designed to investigate the antiulcer effect of ethanolic extract of leaves of S. grandiflora (EELSG) using different models of gastric ulceration in rats. EELSG at the dose of 400 mg/kg produced a significant reduction in the ulcer index. EELSG significantly inhibited gastric mucosal damage induced by aspirin, ethanol and indomethacin. In pylorus-ligated Shay rats, EELSG significantly reduced the basal gastric acid secretion. The antiulcer effect was further confirmed histologically. The anti-ulcer activity of EELSG was however, less than that of standard drugs. The present finding suggests that protective effect of EELSG might have been mediated by both anti-secretory and cytoprotective mechanisms. Moreover, further insight into the precise mechanism of action is essential to exploit the complete potency of EELSG and increase its usage in contemporary medicine.[54]

23. **Lanas A, Chan et al 2021:** Recent literature emphasizes the critical involvement of oxidative stress in the initiation and progression of peptic ulcer disease. Excessive generation of reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, leads to lipid peroxidation, protein oxidation, and DNA damage in gastric mucosal cells. Studies have reported elevated levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, in ulcerated gastric tissues. Endogenous antioxidant defense systems such as superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) play a protective role by neutralizing ROS. However, during ulcer conditions, these antioxidant systems are significantly depleted. Natural compounds derived from medicinal plants have demonstrated the ability to restore antioxidant enzyme levels and reduce oxidative stress, thereby preventing mucosal injury and promoting ulcer healing.[64]

24. **Sung JJY et al 2022:** Inflammatory mediators play a crucial role in the pathogenesis of gastric ulcers. Infection with *Helicobacter pylori* activates multiple signaling pathways, including nuclear factor-kappa B (NF-κB), leading to increased production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. These cytokines induce neutrophil infiltration, resulting in the release of reactive oxygen species and proteolytic enzymes that damage gastric tissues. Recent studies have highlighted the role of cyclooxygenase enzymes and nitric oxide pathways in ulcer development. NSAID-induced ulcers occur due to inhibition of prostaglandin synthesis, leading to decreased mucus production and impaired mucosal blood flow. Plant-derived compounds have shown potential in modulating these inflammatory pathways, thereby providing gastroprotective effects.[65]

25. **Kuipers EJ et al 2023:** In recent years, there has been growing interest in the development of plant-based anti-ulcer agents due to their safety and efficacy. Medicinal plants such as Butea monosperma, Azadirachta indica, and Glycyrrhiza glabra have been extensively studied for their anti-ulcer properties. Phytochemicals such as flavonoids and tannins exert protective effects by scavenging free radicals, enhancing mucus secretion, and inhibiting gastric acid secretion. Alkaloids and saponins also contribute to cytoprotective and anti-inflammatory activities. Recent pharmacological studies have demonstrated that these compounds can regulate multiple targets involved in ulcerogenesis, making them promising candidates for drug development.[66]

Plan of work

- ➢ Collection, Identification and authentication of plant material.
- ➢ Pharmacogenetic evaluation of plant material.
- ➢ Processing of crude drug.
- ➢ Extraction of plant material
- **Selection of extraction method.**
- **Selection of solvent.**
- **Phytochemical Screening**

❖ **Phytochemical screening Plant material for**

- **Extractive value and ash content.**
- **Acid soluble and water insoluble ash.**
- **Loss on drying.**

❖ **Phytochemical qualitative analysis of extract for**

- Carbohydrates, tannin, alkaloids, terpenoids, lipides, proteins, Phenolic acid etc.

❖ **Phytochemical quantitative analysis of extract for total phenolic & flavonoid content.**

- **Pharmacological Screening of plant extract for**
- *In-vitro* antioxidant activity.
- Anti-Ulcer activity.
- **Interpretation of data and its presentation.**

5. Plant Profile

5.1 Synonym: *Achras zapota* L. *Achras sapota* L. *Sapota zapotilla* (Jacq.) Coville, *Manilkara achras* (Mill.) Fosberg, *Achras mammosa* L and Chikuu**.**[37,38]

5.2 Plant parts: Roots of *Manilkara zapota*. were collected from district Chh. Sambhajinagar, Maharashtra. It was authenticated as. Ref.RSCP/2025-2026/Bot-14 from Associate professor Dr.Bandewar S.T. by Harbarium in the depatment of Botany Rajarshi Shahu clg Patri Tq. Phulambri Dist Chh. Sambhajinagar MH.

5.3 Manilkara Zapota: Classification

Kingdom : Plantae Subkingdom : Tracheobionta Class: Magnoliopsida

Family : Sapotaceae

Genus : Manilkara

Species: zapota L.

5.4 Botanical Description

Manilkara zapota. is a pyramidal evergreen tree. It typically grows to 15-30m tall and a diameter of about 1.5 m. Trees with sympodial branching system. Bark is long and cylindrical containing gummy latex called chicle. Leaves are green, simple, alternate, spirally arranged and clustered at the shoot tips, elliptic or oblong in shape, apex obtuse to acuminate and glabrous when mature.

The inflorescence is solitary axillary; cyathiform or campanulate, with a brown pubescent peduncle. Flowers small, bell-shaped, pinkish-white in colour and blooms in the month of April – June. Sepals are 6 in colour, (3 in outer whorl and 3 in inner whorl) with brown hairs. Corolla is subglobose; imbricate and 6 in number. Stamens are present opposite the corolla-lobes, and 6 in number. Ovary 10-12 celled; style bifid. Fruits are ovoid berry, rough brown skin, with sweet edible brown fleshy pulp. Seeds large ovoid, 3-5; black in colour.[39]

5.5 Microscopic and Anatomical Characteristics:

Microscopic evaluation of *Manilkara zapota* roots reveals a well-developed periderm comprising cork cells, cork cambium, and secondary cortex. The cortex consists of parenchymatous cells containing starch grains and tannins. The vascular tissue exhibits radial arrangement with exarch xylem. Secondary growth is prominent, characterized by lignified xylem vessels, fibers, and medullary rays. Powder microscopy shows diagnostic features such as lignified fibers, spiral xylem vessels, prismatic calcium oxalate crystals, starch granules (simple and compound), and tannin-containing cells. These characteristics are essential for pharmacognostic identification and quality control of the crude drug.

5.6 Chemical Constituents[40]

The phytochemical profile of *Manilkara zapota* reveals a diverse range of bioactive compounds distributed across various plant parts.The fruits are rich in sugars (glucose, fructose), dietary fiber, vitamins (A and C), and minerals such as calcium, phosphorus, and iron. Leaves and bark are abundant in polyphenolic compounds including flavonoids, tannins, and phenolic acids.

Triterpenoids such as saponins, along with compounds like sapotin and zapotin, have also been reported.Seeds contain fixed oils, alkaloids, and saponins, contributing to their pharmacological activity. The latex contains chicle, a natural gum of commercial importance The phytochemical profile of *Manilkara zapota* reveals the presence of a wide range of bioactive constituents distributed across different parts of the plant. The fruit is rich in sugars such as glucose and fructose, along with dietary fibers, vitamins (notably vitamin A and vitamin C), and minerals including calcium, phosphorus, and iron. The leaves and bark contain significant amounts of polyphenolic compounds such as flavonoids, tannins, and phenolic acids, which contribute to its antioxidant and medicinal properties. Triterpenoids like saponins and compounds such as sapotin and zapotin have also been reported. Additionally, latex obtained from the plant contains compounds like chicle, which is a natural gum. The seeds are known to contain fixed oils, saponins, and alkaloids, some of which exhibit pharmacological activities.[40]

Leaves: Contain hydrocarbons, sterols, lupeol-3- acetate, oleanolic acid, apigenin-7-O-α-L-rhamnoside, caffeic acid, myricetin-3-O-α-L-rhamnoside (5, 7, 3′, 4′, 5′-pentahydroxyflavon-3-O-α-L-rhamnoside), tannins, phlobatannins, saponins, cardiac glycosides, flavonoids, terpenoids, steroids, alkaloids, and other phenolic compounds.

Fruits: Contain cyanogenic glycosides, phenolic compounds, terpenoids, sapotin, saponins, achrassaponin, fixed oils, 3-O-acyl-L-rhamnose, Larabinose, 3-O-acetyl-D-methyl galacturonate, methyl 4-O-galloylchlorogenate, 4-O-galloylchlorogenic acid, methyl chlorogenate, dihydromyricetin, quercitrin, myricitrin, (+)-catechin, (–)-epicatechin, (+)-gallocatechin, and gallic acid.

Bark: Contains saponins, gums, reducing sugars, tannins, flavonoids, phenolic compounds, alkaloids, steroids, amino acids, proteins, anthraquinone glycosides, deoxy sugars, and terpenoids.

Seeds: Preliminary screening of various solvent extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, carbohydrates, proteins, fats, and oils**.**

(A) Leaves

(B) Stem

(C) Bark

(D) Fruits

Figure 08: ***Manilkara zapota.***

5.7 Geographic Range

Distribution: Manilkara zapota is widely distributed across tropical and subtropical regions of the world. It is extensively cultivated throughout the Caribbean islands and Central America, where it often grows into large forest trees. The plant is considered indigenous to northeastern Guatemala, northern Belize, and southern Mexico. Mexico remains one of the producers of sapodilla, while large-scale cultivation is also observed in tropical Asian countries such as India, Bangladesh, Pakistan, Thailand, Malaysia, Cambodia, Vietnam, and Indonesia. Its adaptability to diverse climatic conditions has contributed to its widespread cultivation and economic importance.[39]

Habitat: *Manilkara zapota* thrives optimally in tropical environments with moderate to full sunlight exposure and relatively low irrigation requirements. It prefers warm climatic conditions, although the reported temperature range of 1200–3600°C is likely a typographical error and should be interpreted as 12-36°C for optimal growth. The plant grows best in well-drained, slightly acidic to neutral soils (pH 6-8), particularly alluvial sandy loam. It demonstrates good adaptability to varied soil types but performs best under fertile conditions. Sowing is generally carried out during February-March and August-October to ensure

maximum yield. The application of organic manure such as farmyard manure (FYM), along with essential macronutrients like nitrogen, phosphorus, and potassium, significantly enhances growth and fruit production.[41]

Manilkara zapota grows best in warm, humid tropical climates with moderate rainfall ranging between 1250-2500 mm annually. It prefers temperatures between 12°C and 36°C, with optimal growth observed around 25-30°C. The species is sensitive to frost and prolonged cold conditions, which can adversely affect flowering and fruiting. Although relatively drought-tolerant once established, adequate moisture availability enhances vegetative growth and fruit yield. The plant thrives in well-drained soils, particularly alluvial sandy loam and lateritic soils, with a pH range of 6.0 to 8.0. It exhibits moderate tolerance to salinity and can grow in coastal regions, although excessive salinity may reduce productivity. One of the notable ecological traits of *M. zapota* is its ability to grow under partial shade, especially during the juvenile stage, making it suitable for mixed cropping and agroforestry systems. However, full sunlight exposure is essential for optimal flowering and fruiting in mature plants.

Nutrient availability plays a crucial role in its ecological performance. Organic manure such as farmyard manure (FYM), along with balanced fertilization using nitrogen, phosphorus, and potassium, significantly improves plant vigor and productivity. Seasonal planting is typically carried out during February–March and August–October to coincide with favorable climatic conditions.[98]

Growth Stages: The life cycle of *Manilkara zapota* involves several distinct developmental stages, including vegetative bud initiation, leaf emergence, shoot elongation, flowering (blooming), fruit set, fruit development, and eventual fruit maturation. These stages collectively determine the productivity and quality of the fruit, with environmental factors playing a critical role in regulating each phase.

The reproductive biology of *Manilkara zapota* plays a crucial role in its ecological success and genetic diversity. The species bears bisexual flowers, allowing both self- and cross-pollination. However, cross-pollination is more common due to the involvement of biotic pollinators, primarily insects such as bees. These pollinators are attracted by floral nectar and play a significant role in enhancing fruit set and yield. Open pollination in *M. zapota* results in considerable genetic variability among populations, which is advantageous for adaptation to diverse environmental conditions. The species also exhibits a degree of self-compatibility,

although cross-pollination generally leads to better fruit quality and higher yields. Seed dispersal in natural ecosystems is facilitated by animals and humans, contributing to the spread of the species. In cultivated systems, propagation is primarily carried out through vegetative methods such as grafting and air-layering to maintain desirable traits. Clonal propagation ensures uniformity in fruit quality and yield, which is essential for commercial cultivation.[98]

5.8 Biology and Ecology

Genetics: Genetic improvement programs for *Manilkara zapota* have been conducted since the 1950s, primarily focusing on hybridization techniques to enhance fruit yield, quality, and adaptability. Cytogenetic studies have established that the species possesses a diploid chromosome number of 2n = 26. These efforts have laid the foundation for future breeding strategies, although progress in developing superior cultivars has been relatively slow. *Manilkara zapota* interacts with various biotic and abiotic components of its ecosystem. It forms mutualistic relationships with pollinators such as bees, which are essential for reproduction. The plant also supports diverse microbial communities in the rhizosphere, which enhance nutrient uptake and soil fertility. Adaptations such as thick leaves, a deep root system, and latex production help the plant withstand environmental stress and herbivory. The latex acts as a defense mechanism against pests and pathogens. Additionally, the evergreen nature of the plant allows continuous carbon assimilation, contributing to its ecological stability. The species is also compatible with agroforestry systems, where it is grown alongside crops such as coconut, banana, and vegetables. This integration enhances biodiversity, improves soil health, and provides economic benefits to farmers.[41]

Reproductive Biology: The flowers of *Manilkara zapota* are bisexual, facilitating both self- and cross-pollination. However, the species predominantly exhibits open pollination, which contributes to high genetic variability within populations. Pollination is primarily mediated by insects, especially bees. Commercial propagation is usually achieved through clonal methods using selected superior seedlings. Breeding objectives include the development of varieties with larger fruit size, improved palatability, and reduced seed content. Although controlled hybridization efforts began in India during the 1950s, the successful release of improved cultivars has been limited[42]

5.7 Traditional uses[41,42]

Manilkara zapota (sapodilla) has long been utilized in traditional systems of medicine across tropical regions for its diverse therapeutic properties. Various parts of the plant, including

fruits, leaves, seeds, bark, and latex, are employed in indigenous remedies. The unripe fruits, rich in tannins, are commonly boiled to prepare a decoction used in the management of diarrhea and dysentery due to their astringent effect. Infusions made from young fruits and flowers are traditionally consumed to alleviate pulmonary ailments such as cough and bronchitis. Decoctions of aged or yellowed leaves are widely used for treating colds, coughs, and gastrointestinal disturbances. The seeds, when crushed, exhibit diuretic properties and are believed to aid in the expulsion of kidney and bladder stones. In some traditional practices, seed extracts are also used as sedatives and soporific agents. A mixture of sapodilla and chayote leaves is consumed to help regulate blood pressure. Topically, seed paste is applied to stings and bites from venomous organisms for its purported anti-inflammatory effects. The latex obtained from the plant has been used as a temporary dental filling in folk medicine. Additionally, preparations involving the fruit soaked in butter are believed to prevent biliousness and febrile conditions. Bark extracts are sometimes used for their antimicrobial and febrifuge properties. Overall, *Manilkara zapota* holds significant ethnomedicinal value due to its multifaceted therapeutic applications in traditional healthcare systems.

5.8 Pharmacological Study

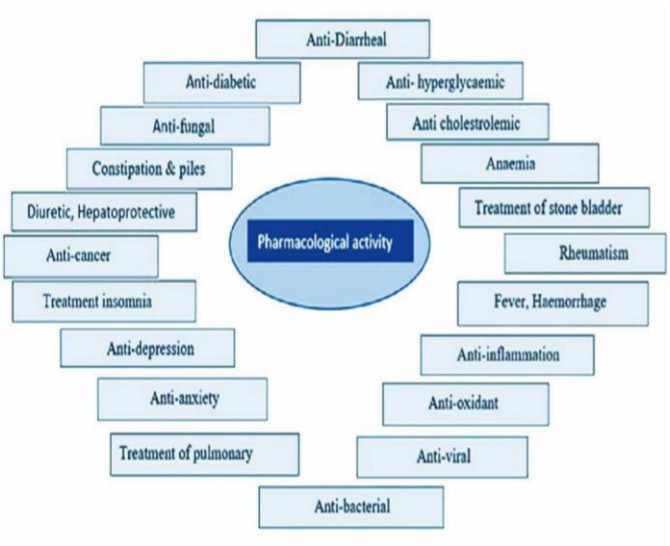


Fig. 9: Pharmacological Activity.

Anti-inflammatory and Anti-Pyretic Activities: The purpose of the study was to evaluate the anti-inflammatory and antipyretic potential of the ethanolic extract of *Manilkara zapota* leaves and its various solvent-soluble fractions using experimental albino Wistar rats. The anti-inflammatory activity was assessed using the carrageenan-induced paw edema model, which is a widely accepted method for evaluating inflammation. Additionally, the antipyretic activity

was evaluated using the yeast-induced pyrexia model in albino Wistar rats.[43]

Antidiarrheal activity: Scientific investigations on M. zapota have reclaimed its traditionally recognized efficacy in diarrhoea. In a study, the antidiarrheal activity of M. zapota bark ethanolic extract was demonstrated against castor oil induced diarrhoea in mice. Treatment with the bark extract reduced fecal output by 29.31% and 41.37%, at 250 mg/kg and 500 mg/kg doses, respectively.[44]

A pharmacological investigation of the ethanolic leaf extract of *Manilkara zapota* and its solvent fractions was carried out using Swiss albino mice to evaluate antinociceptive and antidiarrheal activities. The study employed both the acetic acid-induced writhing test and the radiant heat tail-flick method to assess peripheral and central analgesic effects, respectively. The extract and its fractions (petroleum ether and ethyl acetate) were administered orally at doses of 200 and 400 mg/kg body weight. Significant peripheral antinociceptive activity was observed at the higher dose (400 mg/kg), where the ethanolic extract, petroleum ether fraction, and ethyl acetate fraction exhibited writhing inhibition of 59.89%, 58.24%, and 46.70%, respectively, comparable to the standard drug diclofenac (59.34%). In the tail-flick assay, notable central analgesic activity was recorded at 90 minutes post-administration, with the ethanolic extract and petroleum ether fraction showing 74.15% and 82.15% prolongation of reaction time, respectively, approaching the effect of morphine (85.84%). Antidiarrheal activity was evaluated using the castor oil-induced diarrheal model, where the ethanolic extract significantly reduced defecation frequency by 53.57% and 60.71% at doses of 200 and 400 mg/kg, respectively, in comparison to the standard drug loperamide (71.42%). These findings indicate that *Manilkara zapota* leaves possess significant analgesic and antidiarrheal potential, supporting their traditional use in the management of pain and gastrointestinal disorders.[99]

Antibacterial activity: M. zapota has shown antibacterial activity against a range of clinically important Gram-positive and Gram-negative bacteria. Methanolic leaf extract of M. zapota exhibited moderate inhibitory effect against Staphylococcus aureus, Staphylococcus epidermidis Mucilaginibacter flavus, Pseudomonas pseudoalcaligenes, Enterobacter aerogenes, Morganella morganii, Alcaligenes fecalis and Klebsiella pneumonia, as determined by agar well diffusion assay The antibacterial activity of extracts made from Manilkara zapota seeds was evaluated using the broth dilution and disc diffusion techniques. It was discovered that Manilkara zapota seed acetone extract has antibacterial properties. When the quantity of P. oleovorans was enhanced by just 2 µg/mL (from 323 to 325 µg/mL), the acetone extract of

Manilkara zapota seeds induced an 18% greater inhibition (from 82 to 100%). Alkaloids, phenols, and flavonoids were detected in the acetone extract of Manilkara zapota seeds. More research should be done to determine the active ingredients in this extract. After being separated, active components may be studied structurally using appropriate methods including NMR and IR spectroscopy.[45]

Antioxidant Activities: Antioxidants are the chemical compounds that act on oxidation chain reactions by inhibiting or delaying the oxidation of other molecules. Antioxidants protect the human body from harmful effects of free radicals and ROS (Reactive Oxygen Species) Almost all the medicinal plants contain several antioxidants such as carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanins), polyphenols (ellagic acid, gallic acid, tannins), saponins, enzymes, vitamins (A, C, E, K) and minerals (copper, manganese, zinc, chromium, iodine, etc) Natural antioxidants are safer than synthesized antioxidants and they show anti-viral, anti-inflammatory, anti-cancer, anti-mutagenic, anti-tumour and hepatoprotective properties These natural antioxidants are produced in all or any part of plants but mostly leaves are considered as the main source for their synthesis he antioxidant potential of leaf extracts of *Manilkara zapota* has been extensively investigated using sequential solvent extraction techniques. The antioxidant activity was evaluated through established in vitro assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl), superoxide anion scavenging, and hydroxyl radical scavenging methods. Among the different solvent extracts, the acetone fraction demonstrated superior antioxidant activity, exhibiting stronger DPPH radical and superoxide scavenging effects compared to standard antioxidants such as ascorbic acid and gallic acid. The enhanced antioxidant capacity of the acetone extract may be attributed to the higher concentration of phenolic compounds and flavonoids, which are known to neutralize free radicals effectively. Furthermore, studies have reported a concentration-dependent increase in DPPH radical scavenging activity, indicating that the antioxidant potential of *Manilkara zapota* extracts improves with increasing extract concentration. These findings suggest that the plant possesses significant free radical scavenging ability and could serve as a natural source of antioxidants, with potential applications as a dietary supplement or functional food ingredient to protect against oxidative stress and related disorders.[46]

Anti-arthritic Activity: studied anti-arthritic effect of ethanolic extract of Manilkara zapota using in-vitro inhibition of protein denaturation model and found significant protection against denaturation of proteins suggesting the potential use of Manilkara as anti-arthritic agent.[47]

Antimicrobial Activity: The objective of the present investigation was to evaluate the antibacterial activity of *Manilkara zapota*. Ethyl acetate extracts obtained from the stem bark and leaves of *Manilkara zapota* were subjected to bioassays to determine their antimicrobial potential against a variety of pathogenic bacteria and fungi.

The present investigation aimed to evaluate the antibacterial and cytotoxic potential of *Manilkara zapota*. Ethyl acetate extracts of the leaves and stem bark were subjected to bioassays against a range of pathogenic microorganisms, including both bacterial and fungal strains. Phytochemical analysis using thin-layer chromatography (TLC) revealed the presence of bioactive constituents such as flavonoids, glycosides, and terpenoids, which are known to contribute to antimicrobial activity. The stem bark extract exhibited notable antibacterial effects, producing zones of inhibition ranging from 8 to 16 mm against organisms such as *Salmonella typhi*, *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, and *Escherichia coli*, as well as antifungal activity against *Aspergillus flavus*, *Fusarium* species, and *Vasianfactum* species. The leaf extract also demonstrated moderate inhibitory activity against the tested pathogens. The minimum inhibitory concentration (MIC) values of the extracts were found to range between 256 and 512 µg/mL, indicating moderate antimicrobial potency. In addition, cytotoxicity was evaluated using the brine shrimp lethality assay (*Artemia salina*), where the ethyl acetate leaf extract and stem bark extract exhibited LC_{50} values of 16.17 µg/mL and 50.26 µg/mL, respectively, compared to the standard drug ampicillin trihydrate (12.38 µg/mL). These findings suggest that *Manilkara zapota* possesses significant antimicrobial and cytotoxic properties, which may be attributed to its rich phytochemical composition and could be further explored for therapeutic applications.[98]

Anti-cancer activity: Silver nanoparticles synthesized from the aqueous leaf extract of *Manilkara zapota* demonstrated significant cytotoxic activity against various human cancer cell lines. The study revealed that these nanoparticles effectively inhibited the migratory potential of colorectal carcinoma cells. Cytotoxicity was evaluated using standard in vitro assays, including the trypan blue dye exclusion assay, MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay), and cell migration assays. The nanoparticles were tested against human colorectal carcinoma (HCT 116), cervical cancer (HeLa), and lung carcinoma (A549) cell lines. The IC_{50} values after 72 hours were found to be 8 ± 3 µg/mL for HCT 116, 16 ± 2 µg/mL for HeLa, and 29 ± 3 µg/mL for A549 cells. These results indicate that the silver nanoparticles exhibited higher cytotoxic potency against colorectal carcinoma cells

compared to HeLa and A549 cell lines, suggesting their potential as an effective anticancer agent.[48]

Hepato-protective

Aqueous extract of *M. zapota* fruit demonstrated CCl4-induced hepatic toxicity through analysis of biomarkers for liver function, serum lipid levels, malondialdehyde, nonprotein sulfhydryls, and histopathology. Hepatocyte population changes at 500 mg/kg lyophilized sapodilla extract were negligible. Proanthocyanins have a number of positive health effects, including hepatoprotection, which may be attributed to their ability to inhibit apoptosis in hepatic cells The anti-inflammatory and antipyretic potential of the ethanolic leaf extract of *Manilkara zapota* and its solvent fractions was evaluated using albino Wistar rats. Anti-inflammatory activity was assessed by the carrageenan-induced paw edema model, while antipyretic activity was determined using the yeast-induced pyrexia method. At a dose of 300 mg/kg (p.o.), both the crude ethanolic extract and the ethyl acetate fraction exhibited significant inhibition of paw edema, showing 91.98% and 92.41% inhibition, respectively, at the 4-hour mark, which was comparable to or even higher than the standard drug diclofenac (86.08%) ($P < 0.001$). In the antipyretic study, the ethanolic extract demonstrated a significant reduction in rectal temperature from 37.90°C to 37.41°C ($P < 0.01$) and 37.07°C ($P < 0.001$) at the third and fourth hours, respectively. Similarly, the ethyl acetate and petroleum ether fractions showed marked antipyretic effects ($P < 0.001$), with the petroleum ether fraction producing the most pronounced reduction in body temperature (36.86°C). These findings indicate that *Manilkara zapota* leaves possess potent anti-inflammatory and antipyretic activities, which may be attributed to the presence of bioactive phytoconstituents such as flavonoids, terpenoids, and glycosides, thereby supporting their potential therapeutic application in the management of inflammatory conditions and fever.[55]

Analgesic activity

Found that analgesic activity was assessed using acetic acid-induced writhing technique. At dosages of 200 mg/kg body weight, *M. zapota* leaf methanolic and petroleum ether extract indicated 96.42% and 94% pain inhibition. Because of presence of polyphenols and alkaloids, methanolic and petroleum ether extracts of *M. zapota* leaves showed high analgesic efficacy.[56]

Anti-arthritic activity

An ethanolic extract of *M. zapota* leaves displayed anti-arthritic action. Using a denaturation

inhibition technique, anti-arthritic efficacy is *in vitro* assessed. The ethanolic extract of *M. zapota* leaves 75.84% ± 2.31% suppression of protein denaturation at 250 g/mL suggests that *M. zapota* may be used as an anti-arthritic agent.[57]

Anti-tyrosinase activity

The bark extract of Manilkara zapota when screened for tyrosinase inhibitory activity shows the positive result. Sutthiduean Chunhakant and Chanya Chaicharoenpong utilised the EtOAc, MeOH, n-hexane and aqueous extracts of Manilkara zapota bark for the antityrosinase activity. According to their research ethanolic extract of Manilkara zapota identified to be having greater activity followed by n-hexane, MeOH and aqueous extract.[58]

CNS Depressant Activity

This study also demonstrated that Manilkara zapota leaves exhibit mild CNS depressant activity, as evidenced by the phenobarbitone-induced sleep test. The extract increased the duration of sleep in experimental animals, an effect that may be attributed to compounds capable of inducing sedation or hypnosis. This action is likely mediated through potentiation of GABAergic neurotransmission, enhancing postsynaptic inhibition via allosteric modulation of GABA receptors.[59]

Hepatoprotective and lipid lowering Activity

The tropical fruit sapodilla (Manilkara zapota syn. Achras zapota) is a nutrient-rich source of minerals and diverse bioactive phytochemicals, including flavonoids and catechins. Pharmacological studies have demonstrated its anti-bacterial, anti-parasitic, anti-fungal, antiglycative, hypocholesterolemic, and anti-cancer properties. However, its effects on hepatic tissue and serum lipids remain less explored To investigate this, an in vivo study was conducted using a carbon tetrachloride (CCl_4)-induced liver damage model in rats to assess the effects of lyophilized sapodilla extract (LSE). CCl_4 exposure elevated serum biomarkers of liver injury such as aspartate transaminase, alanine aminotransferase, γ-glutamyl transferase, and alkaline phosphatase as well as bilirubin, while disrupting serum lipid profiles (cholesterol and triglycerides). Treatment with LSE at doses of 250 and 500 mg/kg significantly and dosedependently reversed these changes.

Histological examination revealed that LSE reduced structural liver damage caused by CCl_4. Furthermore, assessment of oxidative stress markers demonstrated that LSE mitigated CCl_4-induced increases in malondialdehyde and preserved non-protein sulfhydryl levels. In vitro

assays, including DPPH and β-carotene-linoleic acid tests, confirmed the strong antioxidant activity of LSE. In conclusion, lyophilized sapodilla extract exhibits hepatoprotective and lipid-lowering effects against CCl_4- induced liver injury, effects that are at least partially mediated by its potent antioxidant propertie.[60]

Anti-aging activity: Skin aging is a complex process that results from both intrinsic and extrinsic factors. UV exposure is the most common extrinsic factor, whereas passage of time is the main intrinsic factor for aging. Collagen and elastin are the main proteins involved in maintaining the structural integrity of the skin. Collagenase is responsible for extracellular matrix (ECM) remodeling, including collagen breakdown. The combination of aging factors generates ROS, matrix metalloproteinases (MMPs), and elastase. Thus, aging is associated with oxidative damage. Depletion of collagen and elastin can generate aging signs and wrinkles on the skin. reported that 60 and 95% ethanolic fresh pulp extracts had a significant inhibitory activity against collagenase and elastase enzymes, both responsible for skin aging processes. It was revealed that both extracts, at a 140 µg/ml dose, showed a strong inhibition of collagenase enzyme, with 66.42 and 64.66%, respectively. However, they were lower compared to the 20 µg/mL standard dose of epigallocatechin gallate (EGCG), with 98.43%. In contrast, its inhibitory activity against elastase was examined. Of the 60 and 95% extracts, only the 95% showed a significant inhibitory effect. At 80 µg/ ml dose, the inhibition was 47.74%, higher than the standard 45.51%.[101]

6. MATERIALS AND METHODS

Table No. 1 List of Chemicals, Drugs and Kits.

Sr.no	**Drugs and Chemicals**	**Manufacturer**
1	Petroleum Ether	Fine Chem Industries, Mumbai
2	Chloroform	Fine Chem Industries, Mumbai
3	Methanol	Fine Chem Industries, Mumbai
4	Ethanol	Fine Chem Industries, Mumbai
5	Benzene	Fine Chem Industries, Mumbai
6	Ethyl Acetate	Fine Chem Industries, Mumbai
7	Sulphuric Acid	Hi Media Lab. Mumbai
8	Pholoroglucinol	Loba Chemie Pvt. Ltd. Mumbai
9	DPPH	Hi Media Lab. Mumbai
10	Ascorbic Acid	Hi Media Lab. Mumbai
11	Hytrogen peroxide	Hi Media Lab. Mumbai
12	Gallic Acid	Loba Chemie Pvt. Ltd. Mumbai
13	Indomethacin	Loba Chemie Pvt. Ltd. Mumbai
14	Hydrochloride Acid	Loba Chemie Pvt. Ltd. Mumbai

15	Silica gel	Hi Media Lab. Mumbai
16	Ferric chloride	Research Lab. Fine Chem. Industries, Mumbai
17	Ranitidine	Hi Media Lab. Mumbai
18	Sodium hydroxide	Loba Chemie Pvt. Ltd. Mumbai
19	Acetone	Fine Chem Industries, Mumbai

Table No. 2: List of Instruments.

Sr. No	Instrument	Manufacturer
1	Analytical weighing balance	Sony electronic scale, Mumbai.
2	Soxhlet Apparatus	Borosil, Mumbai
3	Laboratory Centrifuge	Remi Motors Ltd. Mumbai.
4	Auto analyzer	Orchid Scientifics, Mumbai.
5	UV-1800 Spectrophotometer	Shimadzu 1600, Japan.
6	Single pan balance	Dhona 200 Limited, Mumbai
7	Dessicator	Borosil, Mumbai
8	Microscope	Micron optics
9	Sonicator	PCI, Mumbai
10	Hot air oven	Metalab Scientific Industries, Mumbai

6.3 Collection, identification and authentication of plant material

The plant material used in the present study was collected from the Chhatrapati Sambhajinagar region, Maharashtra, India. The collected plant specimen was taxonomically identified and authenticated by a botanist from the Department of Botany, Rajarshi Shahu Arts, Commerce and Science College, Pathri. The plant was identified as *Manilkara zapota* belonging to the family *Sapotaceae*. An authentication letter was issued and the voucher specimen was deposited for future reference with accession number 17499. The authentication was carried out by **Dr. Bandewar S. T., Associate Professor, Department of Botany, Rajarshi Shahu College, Pathri,** confirming that the plant is commonly found in the Marathwada region.

Fig. 6: Herbarium Sheet.

Processing of Crude Drugs

The collected fresh Roots of *Manilkara Zapota* were subjected to shade drying and further crushed to powder, and then the powder is passed through the mesh No. 14, stored in air tight container and subjected to extraction by using Soxhlet extraction method.

6.5. Pharmacognostic evaluation of plant matrial

6.5.1 Morphological evaluation of Root

- **Colour:** Brown to dark brown
- **Taste:** Slightly bitter and astringent
- **Odour:** Slight characteristic
- **Shape:** Cylindrical, irregularly branched
- **Size:** Approximately 10–20 cm in length

6.6. Determination of physicochemical analysis of powdered plant material

Following physicochemical parameter were carried out.

- **Total Ash value**
- **Acid insoluble ash**
- **Water soluble ash**
- **Loss on drying**
- **Water soluble extractive**
- **Alcohol soluble extractive**

6.6.1. Total Ash value:

Principle: The residue remaining after incineration is the ash content of the drug.(inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium) is known as ash content. Ash value is a criterion to judge the identity OR purity of curd drug.

Procedure: Weigh and ignite flat, thin, porcelain dish or a tarred silica crucible. Weigh about 2 g of the powdered drug into dish/crucible. Support the dish on pipe-clay triangle placed on ring of retort stand. Heat with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost crease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off. Cool in desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug

6.6.2. Acid insoluble ash: *(K. R. Khandelwal, 2010)*

Acid insoluble ash is determined by dissolving ash in dilute hydrochloric acid (10%), the liquid filtered through an ashless filter paper and thoroughly washed with hot water. The filter paper is then ignited in the original dish, cooled and weighed.

Procedure: Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further using 25 ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into 100 ml beaker. Place mere gauze over a Bunsen burner and boil for five minutes. Filter through an 'ash less' filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter-paper and residue together into crucible; heat gently until vapours cease to be evolved and then more strongly until all carbon has been removed. Cool in desiccators. Weigh the residue and calculate acid–insoluble ash of the crude drug with reference to the air dried sample of the crude drug.

6.6.3 Water soluble ash: *(K. R. Khandelwal, 2010)*

When the total ash obtained from the above experiment is boiled in water, part of the ash dissolves (water-soluble ash) and part remains as insoluble ash.

Procedure: Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further-Using 25ml of water wash the ash from the dish used for total ash into 100 ml beaker. Place gauze over a burner and boil for five minutes. Filter through an 'ash less' filter paper, wash the residue twice with hot water. a crucible in the flame, cool and weigh. Put the filter paper and residue together into crucible; heat gently until vapours cease to be evolved and then more strongly until all carbon has been removed. Cool in a desiccator. Weigh the residue and calculate.

6.6.4 Loss of drying: *(K. R. Khandelwal, 2010)*

Principle: A method commonly used for moisture content determination is the loss-on-drying method, or LOD. It is used to specify many major quality specifications. This is based on the thermogravimetric principle, in which a substance is heated until no more weight is lost, that is, it is completely dry**.**

Procedure: Weigh about 2 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100◦c or 105◦c, until two consecutive weighing do not differ by more than 0.5 mg.Cool in desiccators and weigh. The loss in weight is usually recorded as moisture.

6.4.5. Extractive value: ***(K. R. Khandelwal, 2010)***

Principle: Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs.

Procedure: 2 gm of air-dried drug was macerated with 50 ml of different solvent in a closed flask for 24 hours; it was frequently shake during the first 6 hours and allowed to stand for 18 hours. After solvent evaporated. The percentage of solvent-soluble extractive value was calculated with reference to air dried drug.

6.4 Extraction of plant material: ***(K. R. Khandelwal, 2010)***

6.4.1. Selection of Solvent

On the basis of nature of phytochemical present in drug and extractive value solvents were selected for the extraction of the stem bark of *Manikkara zapota roots* use of solvent is petroleum ether, acetone and methanol.

6.4.2. Selection of Extraction method.

According to nature of phytochemical present in drug and literature review, extraction method was selected. The extraction method selected for extraction of stem bark of *Manilkara zapota Roots* was continuous hot extraction method using Soxhlet apparatus.

6.4.3. MATERIAL

Soxhlet apparatus, heating mental, powdered drug, petroleum ether, Acetone and Methanol.

6.5.3. Preparation of extracts

Three extracts of stem bark of *Manilkara Zapoat Roots* was prepared.

- Petroleum ether extract by continuous hot extraction method.
- Acetone extract by continuous hot extraction method.
- Methanol extract by continuous hot extraction method.

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\text{(\%) yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug.}} \times 100$$

6.5.3.1 Preparation of Petroleum ether extract (A)

Dried Roots powder was successively extracted with petroleum ether by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered Root power and 1000 ml petroleum ether was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container. Percentage yield of extract was calculated.

6.5.3.2 Preparation of Acetone extract (B)

Dried Roots powder was successively extracted with Acetone by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml Acetone was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container and Percentage yield of extract was calculated.

6.5.3.3. Preparation of Methanol extracts (C)

Dried Roots powder was successively extracted with Methanol by Soxhlet extractor apparatus according to the standard method till colourless solution was observed in siphon tube. 250 gm of the powdered plant and 1000 ml methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container. Percentage yield of extract was calculated.

6.6. Preliminary phytochemical evaluation: *(K. R. Khandelwal, 2010)*

Qualitative chemical tests were carried out for all three extracts to identify the presence of various chemical constituents All small quantity of Petroleum ether, Acetone and methanolic extracts are dissolved in few ml of water separately and following qualitative tests are carried out.

6.6.1. Test for carbohydrates

1. **Molisch's test:** To the extract, add few drops of alcoholic α-naphthol. Then add few drops of concentrated sulphuric acid through sides of test tube; purple to violet colour ring appears at the junction.

2. **Fehling's test:** 1ml of Fehling's A & Fehling's B solution were taken in test tube, boiled for one-minute equal volume of aqueous extract solution was added and heated in boiling water bath for 5-10 min formation of brick red ppt indicated the presence of carbohydrate**.**

3. **Benedict's test:** Equal volume of benedict's reagent and test solution was taken in test tube and heated in boiling water bath for 5 min formation of red coloured solution indicates the presence of carbohydrates.

6.6.2 Test for proteins

1. **Biuret test (general test):** To 3 ml of test solution, add 4 % sodium hydroxide add few drops of 1 % copper sulphate solution, violet or colour appears**.**

2. **Million's test (for proteins): -** Mix 3 ml of test solution with 5 ml Millon's reagent. White precipitate warm precipitate turns brick red or the precipitate dissolves giving red coloured solution.

6.6.3 Test for Amino acids

1 **Ninhydrin test:** Take 1 ml test solution in dry test tube and 1 ml distilled water in another tube as a control. Pour few drops of 2% ninhydrin in both the test tubes. Keep the test tubes in water bath for 5 minutes. Look for the development of blue or violet colour.

6.6.4. Test for Steroids

1. **Liebermann-Burchard test:** Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layer turns green which shows presence of steroids and formation of deep red color indicates presence of triterpenoids.

2. **Salkowski test:** Treat the extract with few drops of concentrated sulphuric acid red colour at lower layer indicates presence of steroids and formation of yellow coloured lower layer indicates presence of triterpenoids.

6.6.5. Test for alkaloids

1. **Hager's test:** Filtrates were treated with Hager's reagent to produce yellow precipitate indicates presence of alkaloids.

2. **Wagner's test:** Filtrates on treatment with Wagner's reagent give reddish brown precipitate indicates presence of alkaloids.

3. **Dragendroff's test:** To the extract, add Dragendroff's reagent, (Potassium Bismuth Iodide) reddish brown precipitate indicates presence of alkaloids.

4. **Mayer's test:** The extract on treatment with Mayer's reagent give cream coloured precipitate indicates presence of alkaloids.

6.6.6. Test for flavonoids

1. **Shinoda test:** Extract treated with few magnesium turning and few drops of concentrated hydrochloric acid gives pink scarlet, crimson red or occasionally green to blue color after few minutes**.**

2. **Lead acetate test**: In a test tube, 2 ml of the amino acid solution is taken. To this, 2 ml of NaOH is added, and the solution is boiled for a minute. Once the test tube cools down, a few drops of lead acetate are added to the solution. The test tube is then observed for the formation of a precipitate.

3. **Sulphuric acid test.** add dilute HNO3. This is to prevent precipitation of other insoluble barium compounds such as BaCO3 or BaSO3. Secondly, add Ba (NO3)2 (aq). If sulfuric acid is present a white precipitate will be immediately observed.

6.6.7. Test for glycosides

1. **Keller–Killiani test:** The Keller-Killiani test is a specific chemical test used to detect deoxysugars present in cardiac glycosides. Take a small quantity of the plant extract or drug sample. Add 2 ml of glacial acetic acid containing a trace of ferric chloride ($FeCl_3$). Carefully add 1 ml of concentrated sulfuric acid (H_2SO_4) along the side of the test tube (do not mix). A **brown or reddish-brown ring** appears at the junction of the two layers.

2. **Legal test:** A small quantity of the extract is dissolved in pyridine, then a few drops of sodium nitroprusside solution are added. The solution is made alkaline by adding sodium hydroxide, and the mixture is gently shaken. The appearance of a pink to red color indicates the presence of cardiac glycosides.

3. **Foam test:** A small quantity of the extract is diluted with water and shaken vigorously in a test tube. The formation of a stable and persistent froth (foam) that lasts for about 10–15 minutes indicates the presence of saponin glycosides.

6.6.7 Test for Tannins & Phenolic Compounds

1. **Lead Acetate Test:** A few drops of lead acetate solution are added to the extract. The formation of a white or yellow precipitate indicates the presence of tannins and phenolic

compounds.

2. **Shinoda Test (for Phenolics/Flavonoids):** To the extract, a small piece of magnesium and concentrated hydrochloric acid are added. The development of a pink or red color indicates the presence of phenolic compounds (flavonoids).

3. **Dil. HNO_3 test:** A small quantity of the extract is treated with dilute nitric acid and gently heated. The formation of a reddish to yellow coloration indicates the presence of tannins and phenolic compounds.

4. **Dil. iodine solution:** A small quantity of the extract is treated with dilute iodine solution. The appearance of a blue or blue-black coloration indicates the presence of starch, while a brownish coloration suggests the absence of starch.

6.7. Phytochemical quantitative analysis of extract for total phenolic & flavonoid content[49]

6.7.1. Total phenolic content

The total Phenolic content was determined by using the Folin-Ciocalteu assay. An aliquot of extract or standard solution of Gallic acid [10,20,30,40,50µg/ml] was added to 10 ml of volumetric flask. A blank reagent using distilled water was prepared. 0.5 ml of Folin- Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 20% NaHCO3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 60 minutes at room temperature, the absorbance against the reagent blank was determined at 637 nm with an UV- visible spectrophotometer.[49]

6.7.2. Total Flavonoid Content

Total flavonoid content was measured by the aluminum chloride colorimetric assay. Rutin was used as standard and flavonoid content was determined as Rutin equivalent. A calibration curve for Rutin was drawn for this purpose. An aliquot (1ml) of extracts or standard solutions of Rutin (10, 20, 30, 40 and 50µg/ml) was added to 10 ml volumetric flask containing of distilled water. To the flask was added 0.30 ml 5% NaNO2, after 5 minutes 0.3 ml 10 % AlCl3 was added. After 5 minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. Appearance of pink colour showed the presence of flavonoid content was expressed as mg Rutin equivalents (RuE)[50]

6.8 Pharmacological screening of plant extracts

6.8.1. *In-vitro* antioxidant activity-DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity.

In-vitro Anti-oxidant study of *Manilkara zapota roots* includes following antioxidant methods.

Principle

DPPH assay method is based on the reduction of Methanolic solution of coloured free radical DPPH by free radical scavenger. DPPH composed of unstable free radical molecules. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple colour. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration.

PROCEDURE

The reaction mixture consists of 2 ml of DPPH (0.8M) solution in methanol was mixed with 2 ml of extract solution of *Manilkara zapota Roots* and 1.0 ml of methanol. The various concentration of extract (25, 50, 75, 100 & 125 µg/ml) were prepared. A reaction mixture without test sample was served as control. After 30 min, the decrease in the absorbance of test mixture was measured at 510 nm and (%) inhibition was calculated against control.[51]**(%) Scavenging activity =** $\frac{\text{Control absorbance - Test absorbance}}{\text{Control absorbance}} \times 100$

6.8.2. Safe dose calculation

Acute oral toxicity study for plant extract carried out as per literature survey for referencing LD50 range of selected plant extracts and 1/10th of LD50 dose as par accordingly OECD Test Guideline 423 calculated and used as maximum experimental safe dose. The methanolic and acetone extract of *Manilkara zapota roots* was found to be safe up to 2000 mg/kg bodyweight after oral administration of the test compound.

6.8.3. Anti-ulcer activity

6.8.3.1 AEC Approval

Wistar rats of either sex weighing between 180–230 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in the animal house of Systemic Life Sciences & Research Pvt. Ltd., Telangana, approved by the Committee

for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were housed under standard environmental conditions with 12 h light/dark cycle, temperature (22 ± 2°C) and relative humidity 55 ± 5%, and were provided with standard pellet diet and water ad libitum. All animals were acclimatized to the laboratory conditions for at least one week before the commencement of the experiment. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The study titled *"Phytochemical and pharmacology Evaluation of Manilkara zapota Roots Extract for Anti-ulcer Activity in Rats"* was approved with IAEC approval number **13/IAEC-II/SLSRPL/2025**. All experimental procedures were carried out in accordance with CPCSEA guidelines for the care and use of laboratory animals.

6.8.3.2. Animal used

Systemic Life Sciences & Research Pvt. Ltd., Telangana,

For the study Wistar rats of either sex, of weight 180-230 gm were selected.

Housing Condition

Animals kept under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet and water regularly.

Experimental Protocol: On the day of the experiment, randomly 48 h fasted animals were given study medicines, standard drugs, and vehicles. One approach has been chosen as the anti-ulcer model for the current investigation. The animals were divided into Seven treatment groups. Each group contained six animals (n = 6).

6.8.3.3 Ethanol-induced model[53]

***Procedure*:** Male Wistar rats weighing 180-230 g were used for the study. The animals were fasted for 48 hours prior to the experiment, with free access to water. During the fasting period, they were housed in restraining cages to prevent coprophagy. The animals were divided into different groups and administered either the appropriate vehicle (control) or a cytoprotective drug (e.g., proteinoid) via the intra-arterial route for 30 minutes prior to ulcer induction. Ulceration was induced by administering 1 mL of absolute ethanol. Untreated animals served as control.

One hour after ethanol administration, the animals were euthanized using CO_2. The stomachs were immediately excised, opened along the greater curvature, and gently rinsed under running

tap water to remove gastric contents.

A circular, full-thickness section (approximately 13 mm in diameter) was obtained using a cork borer from each lobe of the fundus, just below the ridge separating the glandular and non-glandular regions of the stomach. The stomach tissues were then stretched and mounted on a foam board with the mucosal side facing upward. Gastric mucosal damage was assessed using a subjective scoring system, where lesions were graded from 0 (no damage) to 3 (severe damage), based on the extent of visible injury.

For uniform analysis, a Plexiglas template (19 × 14 × 0.3 cm), containing four rows of six holes (each 13 mm in diameter), was used. The template was polished on one side with emery cloth and fixed onto a clear glass sheet using photographic tape along its edges. Excised tissue samples from each stomach were carefully placed into the holes of the template. Tissue samples were analyzed in pairs to minimize sampling error. The template ispositioned on a rectangular central opening of anAristo Model T-16 cold cathode transilluminator(38 × 38 cm) containing a W-45 blue-white lamp.A camera is mounted on a copy stand directlyabove the template.Photographs were taken, thefilm was processed in a standard manner, and a contact sheet was made from the negatives.

1. Ulcer Index (% Ulceration)

$$\%\ Ulcer\ Index = \left(\frac{Ulcer\ Area}{Total\ Stomach\ Area}\right) \times 100$$

Where:

- *Ulcer Area* = Total area of gastric lesions
- *Total Stomach Area* = Total mucosal surface area examined

2. Percentage Inhibition of Ulcer

$$\%\ Inhibition = \left(\frac{UI_{control} - UI_{test}}{UI_{control}}\right) \times 100$$

Where

- *UIcontrol* = Ulcer index of control group
- *UItest* = Ulcer index of treated group

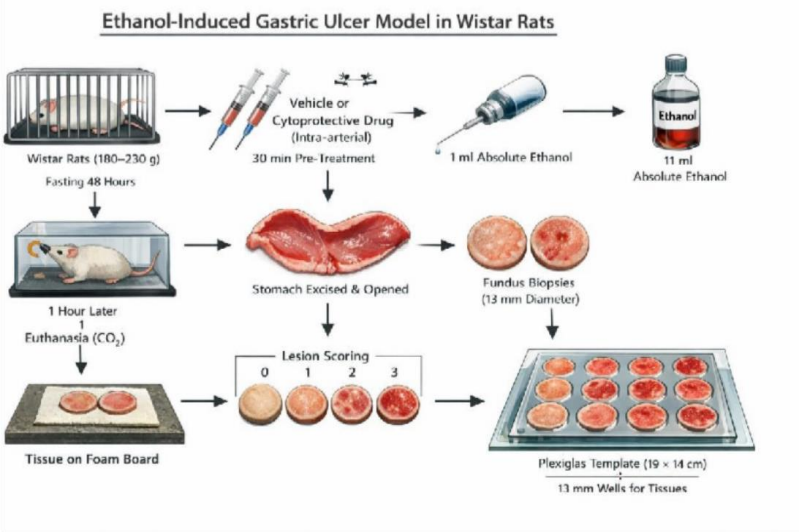


Fig. 08: Ethanol induced ulcer flow chart.

6.8.3.4 Determination of total Acidity[53]

The entire gastric contents of treated animals were carefully collected and transferred into centrifuge tubes for the measurement of gastric output. The samples were centrifuged at 1000 rpm for 5 minutes to separate the supernatant. An aliquot of 1 mL of the clear supernatant was diluted with 9 mL of distilled water. From this, 1 mL of the diluted gastric juice was taken into a 50 mL conical flask, and 1 mL of distilled water was added. Two drops of phenolphthalein indicator were introduced, and the solution was titrated against pre-standardized 0.1 N sodium hydroxide (NaOH) until a persistent pale pink color was observed, indicating the endpoint.

The volume of NaOH consumed during titration was recorded, and the total acidity was calculated.

Calculation of Total Acidity

$$Total\ Acidity\ (mEq/L) = \frac{V_{NaOH} \times N_{NaOH} \times 1000}{Volume\ of\ gastric\ juice\ (mL)}$$

Where:

- V_{NaOH}= Volume of NaOH consumed (mL)
- N_{NaOH}= Normality of NaOH (0.1 N)
- Volume of gastric juice = Volume of sample used for titration (mL)

6.8.3.6 Statistical Analysis

Each piece of data isrepresented as mean± S.E.M. A student t-test and aone-way analysis of

variance (ANOVA) were usedto evaluate the results. Additional multiplecomparisons were performed using Tukey's test as the post hoc test whenever the ANOVA was significant. The statistical analysis was carried out using GraphPad InStat version 5. P>0.05 wasdeemed non-significant (ns) when compared tothe control group, while p<0.05 to p<0.001 wasthe range of statistical significance. Non-significant(ns) was declared when p > 0.05 was compared to the control group.[53]

6.8.3.7. Ethanol induced ulcer

Grouping of animals:

Route of administration: Oral route administration.

Animal Grouping and drug administration:

- Group I : Animals were receive 0.5 % of DMSO/ Saline water orally.
- Group II : Positive Control Ethanol(1ml/200g) oral
- Group III : Animals were receive standard drug (Ranitidine 20mg/kg)
- Group IV : Animals were receive 100mg/kg dose of Acetone extract of *Manilkara zapota Roots Extract* (MZAE100mg/kg oral).
- Group V : Animal were receive 200 mg/kg dose of Acetone extract of *Manilkara Zapota Root extract* (MZAE200mg/kg oral).
- Group VI : Animals were receive 100mg/kg dose of Methanolic extract of
- *Manilkara Zapota Root Extract (*MZME100mg/kg oral)
- Group VII : Animals were receive 200mg/kg dose of Methanolic extract of *Manilkara Zapota Roots extract* (MZME 200mg/kg oral)

8. DISCUSSION

The present investigation was undertaken to evaluate the pharmacognostic, phytochemical, antioxidant, and anti-ulcer potential of *manilkara zapota* root extracts, thereby providing scientific validation for its traditional therapeutic use. The findings of this study collectively demonstrate that the plant possesses significant bioactive potential, particularly in the management of gastric ulcers.

Initially, pharmacognostic evaluation confirmed the identity and purity of the plant material. The macroscopic characteristics such as brown color, cylindrical shape, and astringent taste are consistent with standard descriptions of *manilkara zapota*. Physicochemical parameters including ash values, acid-insoluble ash, and loss on drying were within acceptable limits,

indicating minimal contamination and good stability of the crude drug. These parameters are essential for establishing quality control standards and ensuring reproducibility of herbal formulations.

The extractive value analysis revealed that methanol and acetone extracts exhibited the highest yields, suggesting that the majority of phytoconstituents present in the roots are polar or semi-polar in nature. This observation is consistent with the presence of phenolics, flavonoids, and glycosides, which are known to be efficiently extracted using polar solvents. The comparatively lower extractive values in non-polar solvents such as petroleum ether further support this inference.

Preliminary phytochemical screening demonstrated the presence of diverse bioactive compounds including alkaloids, flavonoids, glycosides, tannins, phenolics, carbohydrates, and proteins. Notably, methanol and acetone extracts showed a richer phytochemical profile compared to petroleum ether extract. The presence of phenolic and flavonoid compounds is particularly significant, as these constituents are well known for their antioxidant and cytoprotective properties, which play a crucial role in ulcer prevention and healing.

Quantitative estimation further supported these findings, where the acetone extract exhibited higher total phenolic content, whereas the methanol extract showed higher flavonoid content. This variation may be attributed to differential solubility of phytoconstituents in solvents of varying polarity. Phenolics and flavonoids are recognized for their ability to scavenge free radicals, inhibit lipid peroxidation, and enhance mucosal defense mechanisms, thereby contributing to gastroprotective effects.

The in-vitro antioxidant activity assessed using the DPPH radical scavenging assay demonstrated that both acetone and methanol extracts possess significant antioxidant potential in a concentration-dependent manner. Although the activity was slightly lower than standard antioxidants such as rutin and ascorbic acid, the extracts exhibited comparable efficacy. The antioxidant activity observed can be directly correlated with the high phenolic and flavonoid content, suggesting that these compounds play a key role in neutralizing oxidative stress, which is a major factor in the pathogenesis of gastric ulcers.

The anti-ulcer activity evaluated using the ethanol-induced gastric ulcer model in wistar rats provided compelling evidence of the gastroprotective potential of *manilkara zapota*. Ethanol

is known to cause gastric mucosal damage through oxidative stress, increased acid secretion, and disruption of the mucosal barrier. In this study, the acetone extract demonstrated significant reduction in ulcer index and increased ulcer protection in a dose-dependent manner, with effects comparable to the standard drug ranitidine. In contrast, the methanol extract showed comparatively lower protection, although higher doses exhibited moderate activity.

Furthermore, the extracts significantly modulated gastric parameters, as evidenced by increased gastric ph and reduced total acidity. These findings indicate that the extracts possess antisecretory as well as cytoprotective properties. The ability to reduce gastric acidity and enhance mucosal defense is a key mechanism in ulcer prevention. The gross morphological studies of gastric mucosa further corroborated these findings, where treated groups showed reduced ulceration and improved mucosal integrity.

The observed anti-ulcer activity can be attributed to multiple mechanisms, including antioxidant action, inhibition of gastric acid secretion, enhancement of mucosal defense, and possible modulation of inflammatory pathways. The presence of bioactive constituents such as flavonoids and tannins is likely responsible for these effects, as these compounds are known to promote mucus secretion, reduce oxidative damage, and stabilize gastric mucosal cells.

Overall, the study provides strong scientific evidence supporting the therapeutic potential of *manilkara zapota* roots as a natural anti-ulcer agent. Among the tested extracts, the acetone extract exhibited superior pharmacological activity, which may be due to higher extraction of phenolic compounds. The findings suggest that the plant could serve as a promising candidate for the development of herbal anti-ulcer formulations.

However, further studies involving isolation and characterization of active constituents, detailed mechanism-based investigations, and clinical evaluation are necessary to fully establish its efficacy and safety profile.

9. CONCLUSION

The present study was undertaken to systematically investigate the **pharmacognostic, phytochemical, antioxidant, and anti-ulcer potential of *Manilkara zapota* root extracts**, with the aim of providing scientific validation for its traditional medicinal use. The results obtained from the study clearly demonstrate that the plant possesses significant therapeutic potential, particularly in the management of gastric ulcers.

The **pharmacognostic and physicochemical evaluation** confirmed the identity, purity, and quality of the crude drug. Standard parameters such as ash values, extractive values, and loss on drying were found to be within acceptable limits, indicating that the plant material is suitable for further pharmacological investigations and can serve as a reliable source for herbal drug development.

The **phytochemical screening** revealed the presence of a wide range of bioactive constituents, including alkaloids, flavonoids, phenolic compounds, glycosides, tannins, and carbohydrates. Quantitative analysis demonstrated appreciable amounts of total phenolics and flavonoids in the extracts, which are well known for their antioxidant and therapeutic properties. The **acetone extract showed higher phenolic content**, whereas the **methanol extract exhibited higher flavonoid content**, indicating solvent-dependent extraction efficiency.

The **in-vitro antioxidant activity** confirmed that both acetone and methanol extracts possess significant free radical scavenging potential in a concentration-dependent manner. This antioxidant activity is likely attributed to the presence of phenolic and flavonoid compounds, which play an important role in reducing oxidative stress—a key factor in the pathogenesis of gastric ulcers.

The **anti-ulcer activity**, evaluated using the ethanol-induced gastric ulcer model in Wistar rats, demonstrated that the extracts possess significant gastroprotective effects. Among the tested extracts, the **acetone extract exhibited pronounced anti-ulcer activity**, showing a marked reduction in ulcer index and significant ulcer protection, comparable to the standard drug ranitidine. The extracts also significantly increased gastric pH and reduced total acidity, indicating both **antisecretory and cytoprotective mechanisms of action**. Gross morphological examination further supported these findings by showing improved gastric mucosal integrity in treated groups.

The overall findings suggest that the anti-ulcer activity of *Manilkara zapota* root extracts may be attributed to a combination of **antioxidant effects, inhibition of gastric acid secretion, enhancement of mucosal defense mechanisms, and possible anti-inflammatory actions**. The presence of bioactive phytoconstituents such as phenolics and flavonoids plays a crucial role in mediating these effects.

In conclusion, the study provides substantial scientific evidence supporting the **therapeutic**

potential of *Manilkara zapota* as a natural anti-ulcer agent. The acetone extract, in particular, demonstrated superior pharmacological activity and may serve as a promising candidate for the development of novel herbal formulations. However, further research is warranted to **isolate and characterize the active constituents, elucidate the precise mechanisms of action, and conduct clinical studies** to confirm its efficacy and safety in humans.

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