INVITRO EVALUATION OF ANTI-OXIDANT AND ANTI-ULCER ACTIVITY USING HERBAL EXTRACTS

Mrs. Sravani Batchu, Associate professor, Department of pharmacology, Mallareddy pharmacy college

Dr. G. Tuljarani, Professor & principal, Mallareddy pharmacy college

V. Sadhwika, M. Nikhil Reddy, O. Keerthana,

Student**,** Malla Reddy pharmacy college

ABSTRACT:

This study evaluated and compared the antioxidant and antiulcer activites of Moringa oleifera and Azadirachta indica leaf extracts prepared using solvent: methanol. A series of in vitro assays were performed, DPPH radical scavenging activity, hydrogen peroxide scavenging assay, nitric oxide scavenging assay, and acid neutralising capacity. Pharmacological studies have shown neem's ability to protect the gastric mucosa by scavenging hydroxyl radicals and inhibiting lipid peroxidation, thereby preventing oxidative damage linked to ulcer formation. In vitro experiments further support its protective role in maintaining gastric mucosal DNA integrity under oxidative stress. These findings validate the traditional use of Azadirachta indica in managing gastric ulcers and oxidative disorders, highlighting its potential as a natural therapeutic agent. M. oleifera leaves have been used in medicine to manage peptic ulcers, particularly in Indian communities. The flower buds are also utilized for their protective effects against gastric ulceration. Scientific investigations have confirmed the plant's significant antioxidant and anti-ulcer properties. Experimental studies revealed that treatment with M. oleifera leaf extract reduced malondialdehyde (MDA), a marker of oxidative stress, while enhancing the levels of key antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S-transferase (GST).

INTRODUCTION:

Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue [1]. There are many types of ulcers such as mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer. Of this peptic ulcer is seen common among many people. The peptic ulcers are erosion of lining of stomach or the duodenum. The two most common types of peptic ulcer are called "gastric ulcer" and "duodenal ulcer." [2]. In some cases, peptic ulcer can be life threatening with symptoms like bloody stool, severe abdominal pain, and cramps along with vomiting blood [3].

Different types of Ulcers:

1.Peptic ulcer

2.Gastric ulcer

3.Duodenal ulcer

4.Mouth ulcer

Gastric ulcers (GU) GU is particularly common in older age groups especially in females [4]. Although patients with GU have normal or even diminished acid production, ulcers rarely may occur even in complete absence of acid [5].

Duodenal ulcers (DU) DU occurs commonly in younger individuals [5] and predominantly affects males [6]. In the duodenum, there may appear ulcers on both the anterior and posterior walls called "kissing ulcers". Patients with DU produce more acids, particularly at night [7]

Mouth ulcer (MU) A painful open sore that develops inside the mouth, lips, or tongue often due to injury, infections, stress or vitamin deficiencies [7].

Signs and Symptoms:

- Abdominal pain
- Nausea and vomiting
- Loss of appetite
- Weight loss
- Bloating and discomfort
- Heartburn
- Fatigue
- Indigestion

Causes:

- Helicobacter pylori
- NSAIDS
- Stress
- Smoking
- Genetics
- Alcohol Consumption
- Diet
- Excessive acid production

Treatment:

Common anti ulcer drugs:

Proton pump inhibitors: Omeprazole, Pantoprazole

H2 Antihistamines: Cimetidine, Ranitidine

Systemic Antacids: Sodium bicarbonate, Sodium citrate

Non systemic Antacids: Magnesium hydroxide, Calcium carbonate

Ulcer protectives: Sucralfate, Colloidal bismuth subcitrate

Prostaglandin analogue: Misoprostol

Antioxidant constituents of plant materials act as radical scavengers, and convert the radicals to less reactive species. Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites [8]. Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists[9]. The most current research on antioxidant action focuses on phenolic compounds such as flavonoids. Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E and carotenoids, whose activities have been established in recent years[10]. Flavonoids, tannins and other phenolic constituents Present in food of plant origin are also potential antioxidants[9-10]. Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause[11]. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A and other substances (Sies, 1997). An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules[12]. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent[13]. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves[14].

1. Natural Antioxidants – Primary Enzymatic Antioxidants: Primary enzymatic antioxidants (also known as chain-breaking antioxidants) act as the first line of defence in preventing or suppressing the formation of radicals. They are essential in neutralising the threat of free radicals by converting them to a less reactive intermediate or block the transformation pathways that can lead to the formation of other radicals[15]. Key enzymes that belong under these groups are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Functions of SOD, catalase, and GPx intercept to remove the harmful reactive oxidative species from the body[16]. In a multistep process, SOD catalytically reduces superoxide radicals to form hydrogen peroxide with the help of metal ion cofactors such as copper, zinc, or manganese[17]. Hydrogen peroxide is then converted to water and oxygen by GPx and catalase. Glutathione peroxidase can also reduce and eliminate peroxidase by donating two electrons to ultimately form selenols[18]. These antioxidants are so crucial for the cells that they are extensively found in most aerobic cells and extracellular fluid[19].

2. Natural Antioxidants – Secondary Enzymatic Antioxidants: Secondary antioxidants also commonly known as hydroperoxide decomposers, can directly act on hydroperoxides and convert them to nonradical, nonreactive, and thermally stable products[20]. They work closely with the primary antioxidants to keep the peroxides level low whilst also supplying nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH) to the primary antioxidants. GSH plays a vital role as scavengers of oxygen and nitrogen reactive species. Glutathione is readily oxidised by ROS to form a dimer with a disulfide bond linking the two GSH molecules[21].

Azadirachta indica

Plant profile:

Fig no: 1 ***Azadirachta indica***

Systemic position of the plant :

- Common name - NEEM
- Botanical name - Azadirachta Indica
- Kingdom-Plantae
- Division-*Magnoliophyta*
- Class –*Magnoliopsida*
- Order-*Sapindales*
- Genus- *Azadirachta*
- Species-A.india
- Family-*Meliaceae*

PLANT INFORMATION :

Scientific name: *Azadirachta Indica*

Synonyms: :*Azadirachta indica*, Melia *Azadirachta*, arishth, margosa, neem tree, nim tree

Botanical Description

- **Type:** Evergreen tree
- **Height:** 15–30 meters (49–98 ft) tall
- **Leaves:** Pinnate with 20–31 medium to dark green leaflets; strongly aromatic
- **Flowers:** Small, white, fragrant; in drooping axillary panicles
- **Fruit:** Smooth, olive-like drupe; green when unripe, yellowish when ripe
- **Seeds:** Contain 1–3 kernels; used for neem oil extraction

Growing Conditions

- **Climate:** Tropical to subtropical; drought-tolerant
- **Sunlight:** Full sun
- **Soil:** Well-drained, sandy or loamy soil; tolerates poor and rocky soils
- **Water:** Low water requirement; does not tolerate waterlogging
- **Hardiness Zones:** USDA zones 10–12

Propagation

- **By seeds:** Fresh seeds germinate best
- **Cuttings and root suckers** can also be used, but less common.

Chemical constituents :

Category	Compound	Approximate % in Neem Leaf (Dry Weight)	Function/ Property
Triterpenoids	Azadirachtin	0.1% – 0.3%	Insecticidal, antifungal
	Nimbin	0.02% – 0.06%	Antiviral, anti-inflammatory
	Nimbolide	0.03% – 0.08%	Anticancer, antioxidant
	Gedunin	0.01% – 0.05%	Antimalarial, hepatoprotective
	Salannin	0.1% – 0.2%	Insect repellent
Flavonoids	Quercetin	0.05% – 0.2%	Antioxidant, anti-inflammatory
	Kaempferol	0.01% – 0.05%	Antioxidant, anticancer

Category	Compound	Approximate % in Neem Leaf (Dry Weight)	Function/ Property
	Myricetin	0.01% – 0.03%	Antioxidant, neuroprotective
Polyphenols	Gallic acid	0.05% – 0.1%	Antioxidant, antimicrobial
	Catechin	0.02% – 0.06%	Antioxidant
	Tannic acid	1% – 3%	Astringent, antimicrobial
Steroids	Beta-sitosterol	0.02% – 0.1%	Anti-inflammatory, cholesterol-lowering
Tannins	Tannins (Total)	1% – 3%	Antioxidant, astringent
Saponins	Saponins (Total)	0.5% – 1.5%	Immune-boosting, anti-inflammatory
Proteins	Total proteins	5% – 7%	Nutritional

Uses of Neem (*Azadirachta indica*)

1. Skin Disorders

- **Neem leaves and oil** are used to treat:
  - Acne, eczema, psoriasis, ringworm, and scabies
  - Skin ulcers and rashes
- Acts as an **antibacterial, antifungal, and anti-inflammatory** agent

2. Oral and Dental Care

- Neem twigs are traditionally used as **natural toothbrushes (chewing sticks)**
- Neem-based toothpaste and mouthwash help in:
  - Reducing plaque
  - Fighting gum disease and cavities
  - Maintaining overall oral hygiene

3. Anti-Parasitic & Insecticidal

- Neem oil is effective against **lice, ticks, fleas**, and **mosquitoes**
- Used topically to treat **head lice** and **scabies**

4. Antidiabetic Properties

- Neem leaf extract is known to help:
  - Regulate blood sugar levels
  - Improve insulin sensitivity

5. Liver and Gastrointestinal Health

- Neem bark and leaf extracts:
  - Act as hepatoprotective agents (protect the liver)
  - Aid in treating ulcers, indigestion, and intestinal worms

6. Immune System Booster

- Regular use of neem leaves (in controlled doses) can enhance immune function due to their **antioxidant** and **antiviral** properties

7. Fever and Malaria

- Traditional use of neem leaves and bark decoctions in managing **malaria symptoms**
- **Gedunin** (a compound in neem) has anti-malarial properties

8. Wound Healing

- Neem paste or oil promotes **faster healing of cuts, burns, and wounds**
- Helps prevent infection due to antimicrobial properties

9. Contraceptive & Antifertility

- Neem oil has been used as a **natural contraceptive**
- Research suggests potential **antifertility effects** in both males and females (needs medical supervision)

10. Hair Care

- Neem oil and neem-based shampoos are effective against:
  - Dandruff, hair fall, and scalp infections

Traditional uses:

Traditionally, neem has been used to treat ulcers due to its soothing and healing effects on the stomach lining. Neem leaf extracts help reduce gastric acid secretion and promote mucus production, offering protection against gastric and peptic ulcers. As an antioxidant, neem is rich in flavonoids and polyphenols that scavenge free radicals, thereby reducing oxidative stress and preventing cell damage. This makes it valuable in managing chronic diseases linked to oxidative damage.

Medicinal uses:

1. **Neem in Ulcer Treatment:** Medicinally, neem has shown significant gastroprotective effects in both animal and human studies. Neem leaf and bark extracts help reduce gastric acid secretion, increase the production of gastric mucus, and accelerate ulcer healing. Compounds like **nimbidin** and **nimbin** exhibit anti-inflammatory and anti-ulcer activity by protecting the stomach lining from damage caused by NSAIDs, alcohol, or

stress. Clinical studies have also reported neem's effectiveness in treating peptic ulcers without toxic side effects.

2. **Neem as an Antioxidant:** Neem contains a rich profile of antioxidants such as **quercetin**, **gallic acid**, **catechin**, and **vitamin C**, which neutralize free radicals and prevent oxidative stress-related damage to cells and tissues. These properties make neem beneficial in managing and preventing conditions like cardiovascular disease, neurodegenerative disorders, and cancer. Regular use of neem extract helps boost the body's natural defense system and supports overall cellular health.

Moringa oleifera

Plant profile :

Fig no:2 *Moringa Olifera*

- Common name – Drum stick, Moringa,Ben oil tree, Horseradish tree
- Botanical name – *Moringa olifera*
- Kingdom-Plantae
- Division-*Magnoliophyta*
- Class –*Magnoliopsida*
- Order-*Moringales*
- Genus- *Moringa*
- Species-*Moringa oleifera*
- Family-*Moringaceae*

PLANT INFORMATION :

Scientific name: *Moringa olifera*

Synonyms: Drumstick tree, Horseradish tree, Ben oil tree, Miracle tree, Munga / Moonga (in parts of India), Malunggay (in the Philippines),Sajina (in Bangladesh and Nepal)

Botanical Description

- **Type:** Deciduous tree or shrub
- **Height:** Typically grows 10–12 meters (33–39 feet), though can reach up to 15 meters (50 feet)
- **Leaves:** Small, oval, bright green, pinnate with three leaflets
- **Flowers:** Small, white or cream-colored, fragrant
- **Fruits:** Long, cylindrical, green pods (often referred to as "drumsticks")

Growing Conditions:

- **Sunlight:** Full sun
- **Soil:** Prefers well-draining, sandy or loamy soil; tolerates poor soil types
- **Watering:** Drought-tolerant once established, but best with moderate watering

- **Climate:** Grows well in tropical and subtropical climates (USDA zones 9–11)

Propagation:

- **Seeds:** Moringa is usually grown from seeds. The seeds should be soaked overnight before planting to help with germination. Plant the seeds about 1 inch deep in well-draining soil.
- **Cuttings:** Moringa can also be propagated by taking cuttings from mature trees, usually 1–2 feet long, and planting them directly in the soil.

Chemical constituents:

- **Flavonoids:** Quercetin, kaempferol, isoquercetin, and rutin are examples of flavonoids found in Moringa oleifera.
- **Phenolic Acids:** These include caffeic acid, ferulic acid, and p-coumaric acid.
- **Fatty Acids:** Moringa oil, particularly from the seeds, contains oleic acid and other unsaturated fatty acids.
- **Carotenoids:** Beta-carotene and lutein are important carotenoids present in the leaves.
- **Glucosinolates:** These are sulfur-containing compounds found in various parts of the plant, with the highest concentrations in the leaves and roots.
- **Alkaloids:** Moringine, moringinine, and other alkaloids are also present.

Uses of *moringa olifera* :

1. Health Benefits:

- Boosts immunity: Moringa leaves are rich in vitamins and help strengthen the immune system.
- Fights inflammation: It's used to reduce swelling and pain, especially for joint issues.
- Controls blood sugar: Moringa is used to help manage diabetes by lowering blood sugar levels.
- Improves digestion: It can treat digestive problems like constipation and indigestion.
- Supports skin and hair: Moringa oil is used to moisturize the skin and promote healthy hair.

2. Nutritional Uses:

- Rich in nutrients: Moringa leaves, seeds, and pods are packed with vitamins, minerals, and protein. The leaves are often eaten in salads, soups, or dried as a powder.

3. Cosmetic Uses:

- **Skin care:** Moringa oil is used to keep skin soft and reduce wrinkles.
- **Hair care**: It's used to strengthen hair and prevent hair loss.

Medicinal use:

1. Anti-Ulcer Medicinal Uses:

Moringa has been traditionally used for its ability to treat and prevent stomach ulcers. It works in the following ways:

- **Protects the stomach lining:** Moringa contains compounds like **flavonoids**, **phenols**, and **tannins**,

which help protect the stomach lining from harmful agents that can cause ulcers, such as excess gastric acid or bacterial infections.

- **Reduces inflammation:** The anti-inflammatory properties of moringa help reduce the swelling and irritation associated with ulcers, providing relief from pain and discomfort.
- **Antibacterial properties:** Moringa has natural antibacterial effects, especially against *Helicobacter pylori*, a bacteria known to cause stomach ulcers. By reducing bacterial load, moringa can support the healing of ulcers.
- **Regulates gastric acid secretion:** Moringa helps regulate the secretion of gastric acid, preventing excess acid that can worsen or cause ulcers.
- **Heals and repairs tissues:** The rich nutrients in moringa, such as vitamins (like Vitamin C) and amino acids, help promote tissue repair and healing of damaged stomach lining caused by ulcers.

Traditional Use:

- Moringa leaves and pods are boiled and consumed as a decoction to soothe the digestive system and promote healing.
- Moringa powder can be mixed with warm water and taken daily to help treat ulcers.

2. Antioxidant Medicinal Uses:

Moringa is packed with powerful antioxidants that contribute to its medicinal uses:

- **Rich in antioxidants:** Moringa is abundant in **vitamin C**, **beta-carotene**, **quercetin**, **chlorogenic acid**, and **zeatin**. These antioxidants protect the body from oxidative stress and the damage caused by free radicals.
- **Fights oxidative stress:** The antioxidant properties of moringa help neutralize harmful free radicals in the body, reducing the risk of chronic diseases, including heart disease, diabetes, and cancer.
- **Reduces inflammation:** Moringa's antioxidants also have anti-inflammatory effects, which can help reduce chronic inflammation that is linked to conditions like arthritis and heart disease.
- **Skin health and anti-aging:** Moringa is used for its anti-aging properties due to its ability to reduce oxidative damage to cells and tissues, promoting youthful and healthy skin.
- **Improves liver function:** The antioxidants in moringa support liver health by reducing oxidative stress and supporting detoxification processes.

Traditional Use:

- Moringa leaves and seeds are commonly consumed as a powder or in teas to increase the body's antioxidant intake.
- Moringa oil, rich in antioxidants, is also used topically to improve skin

health, reduce wrinkles, and promote overall skin rejuvenation.

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