IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
October 2022 Vol.:25, Issue:3

A Review on Therapeutic Potential of *Agles marmelos* Fruit Extract as an Anti-Ulcer

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Submitted: 23 September 2022
Accepted: 28 September 2022
Published: 30 October 2022

Keywords: Proton Pump Inhibitor, Antacid, DHPP, AOA, Radical Scavenging Activity (RSA)

ABSTRACT

The major goal of this study is to check the therapeutic potential of *Agles marmelos* fruit extract in anti-ulcer activity as well as consists of many pharmacological activities such as antioxidant, antidiabetic, dysentery, anticancer, antidandruff, Antileprotic, myalgia, antifungal, smallpox, antibacterial, ntiburn and radioprotective, antigenotoxic, anti inflammatory activity. Indian bael (Aegle marmelos) plant having fruit bark stem and leaves are showing above activities. Hence, we studied various activities and found the anti ulcer activity and the effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion.

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INTRODUCTION

An ulcer is a discontinuity or breaks in a bodily membrane that impedes the normal function of the affected organ. According to Robins's pathology, "ulcer is the breach of the continuity of skin, epithelium or mucous membrane caused by sloughing out of inflamed necrotic tissue." Common forms of ulcers recognized in medicine include:

Ulcer (dermatology), a discontinuity of the skin or a break in the skin. [2]

- Pressure ulcers, also known as bedsores
- Genital ulcer, an ulcer located on the genital area
- Ulcerative dermatitis, a skin disorder associated with bacterial growth often initiated by self-trauma
- Anal fissure, a.k.a. an ulcer or tear near the anus or within the rectum
- Diabetic foot ulcer, a major complication of the diabetic foot Corneal ulcer, an inflammatory or infective condition of the cornea Mouth ulcer, an open sore inside the mouth.
- Aphthous ulcer, a specific type of oral ulcer also known as a canker sore.
- Peptic ulcer, is a discontinuity of the gastrointestinal mucosa (stomach ulcer).
- Venous ulcer, a wound thought to occur due to improper functioning of valves in the veins.
- Stress ulcer, an ulcer located within the stomach and proximal duodenum
- Ulcerative sarcoidosis, a cutaneous condition affecting people with sarcoidosis
- Ulcerative lichen planus, a rare variant of lichen planus
- Ulcerative colitis is a form of inflammatory bowel disease (IBD).

Ulcerative disposition, a disorder or discomfort that causes severe abdominal distress, is often associated with chronic gastritis.

Mechanism of ulcer

Excessive gastric acid secretion is only one factor in the pathogenesis of peptic ulcer disease. Decreased mucosal defense against gastric acid is another cause. The integrity of the upper gastrointestinal tract is dependent upon the balance between "hostile" factors such as gastric acid,[3]

Complications

Left untreated, peptic ulcers can result in:

Internal bleeding. Bleeding can occur as slow blood loss that leads to anemia or as severe blood loss that may require hospitalization or a blood transfusion. Severe blood loss may cause black or bloody vomit or black or bloody stools. A hole (perforation) in your stomach wall. Peptic ulcers can eat a hole through (perforate) the wall of your stomach or small intestine, putting you at risk of serious infection of your abdominal cavity (peritonitis). Obstruction. Peptic ulcers can block the passage of food through the digestive tract, causing you to become full easily, to vomit, and to lose weight either through swelling from inflammation or scarring. Gastric cancer. Studies have shown that people infected with H. pylori have an increased risk of gastric cancer.

Causes of Ulcer Disease [4]

Common

- H. pylori infection
- NSAIDs
- Medications

Rare

- Zollinger-Ellison syndrome

- Malignancy (gastric/lung cancer, lymphomas)
- Stress (Acute illness, burns, head injury)
- Viral infection
- Vascular insufficiency
- Radiation therapy
- Crohn disease
- Chemotherapy

Anti-ulcer [3]

Antiulcer agents and medications for acid disease are commonly used drugs that rarely cause liver injury. Most agents act by inhibiting of gastric acid production, neutralization of acid or protection of the gastrointestinal mucosa from acid injury. These agents are used for both prevention and therapy of duodenal and gastric ulcer disease as well as to alleviate acid reflux, esophagitis and minor upper intestinal discomforts.

Proton pump inhibitor[5]

Histamine-2 receptor antagonists

Mechanism of action — Histamine-2 receptor antagonists (H2RAs) (eg, cimetidine, famotidine, and nizatidine) inhibit acid secretion by blocking H2 receptors on the parietal cell.

Mechanism of action

Proton pump inhibitors (PPIs) reduce the production of acid by blocking the enzyme in the wall of the stomach that produces acid. Acid is necessary for the formation of most ulcers in the esophagus, stomach, and duodenum, and the reduction of acid with PPIs prevents ulcers and allows any ulcers that exist in the esophagus, stomach, and duodenum to heal.[6,7]

Antacid

Antacids are used to neutralize stomach acid and reduce the symptoms of heartburn. There are many OTC medications available for this purpose, such as calcium carbonate, aluminum hydroxide, and magnesium hydroxide. Calcium carbonate is the prototype discussed as an example. Be sure to read drug-label information regarding antacids as you administer them because each type has its own specific side effects. Many antacids also contain simethicone, an antiflatulent used for gas relief. Simethicone is further described in the medication grid below[8].

Antibiotics

If your ulcer is caused by H. pylori bacteria, antibiotics can cure the ulcer. Usually, the doctor will prescribe triple or quadruple therapy, which combines several antibiotics with heartburn drugs. Eg penicillin

Other drugs include Sucralfate, Bismuth, Misoprostol, Potassium-competitive acid etc.[9]

Herbal treatment on Ulcer [10,11,12,13]

A type of medicine that uses roots, stems, leaves, flowers, or seeds of plants to improve health, prevent disease, and treat illness.

Common Name	Botanical Name	Parts Used	Effects
Tulsi	*Ocimum sanctum*	All parts	Anti-ulcer, Antibacterial
Tippani	*Allophylus serratus*	Leaves	Antiulcer
Shaparni	*Desmodium gangeticum*	Root Extract	Inflammation, typhoid, Anti-ulcer
Neem	*Azadirachta indica*	Dried Bark	GIT disorders
Indian Sarsaparilla	*Hemidesmus indicus*	Extract of Leaves	Antidiarrheal, Anti-ulcer
Satavari	*Asparagus racemosus*	Root extract	Anti-diarrheal, Anti-ulcer
Triphala	*Terminalia pallida*	Plant extract	Anti-ulcer
Aamla	*Embilica officinalis*	Fruit extract	Anti-ulcer
Gotu Kola	*Centella asiatica*	Fresh juice	Anti-ulcer
Brahmi	*Bacopa monniera*	Fresh juice	Anti-ulcer
Apple Bananas	*Musa sapientum*	Fruits	Anti-ulcer
Pappeta	*Carica papaya*	Seeds	Anti-amoebic, Anti-ulcer
Pausanto	*Kielmeyera coriacea*	Stem	Anxiolytic, Anti-ulcer
Brindle Berry	*Garcinia cambogia*	Fruit extract	Anti-ulcer
Winter melon	*Banincasa hispida*	Fruits	Anti-ulcer, epilepsy
Wild pipal	*Ficus arnottiana*	Fruits	Anti-ulcer, Demulcent
Indian devil tree	*Alstonia scholaris*	Whole plant	Anti-ulcer
Indian mulberry	*Morinda citrifolia*	Fruit	Anti-ulcer, Anti-diabetic
Indian borage	*Plectranthus amboinicus*	Whole plant	Diuretic, Anti-ulcer
Babul	*Acacia Arabica*	Leaves, Gums	Ulcer, Wound
Garlic	*Allium sativum*	Bulb juice	Antiulcer
Boabab	*Adansonia digitate*	Leaves and Bark	Antiulcer, syphilitic ulcer, irritable inflammatory ulcer disease
Bael tree	*Aegle marmelos*	Fruits	Antiulcer
Kattalai	*Aloe vera*	Leaves and powder	Anti-ulcer
Custard apple	*Annona sauamosal*	Leaves	Anti-ulcer
Satavari	*Asparagus racemosus*	Root extract	Anti-diarrheal, Anti-ulcer
Triphala	*Terminalia pallida*	Plant extract	Anti-ulcer
Aamla	*Embilica officinalis*	Fruit extract	Anti-ulcer
Gotu Kola	*Centella asiatica*	Fresh juice	Anti-ulcer
Brahmi	*Bacopa monniera*	Fresh juice	Anti-ulcer
Apple Bananas	*Musa sapientum*	Fruits	Anti-ulcer
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Bael tree	*Aegle marmelos*	Fruits	Antiulcer
Kattalai	*Aloe vera*	Leaves and powder	Anti-ulcer
Custard apple	*Annona squamosal*	Leaves	Anti-ulcer
Kanchanara/ Orchid tree	*Bauhinia variegate*	Bark and roots	Anti-ulcer
India or Nepal barberry	*Berberis aristata*	Root and wood	Anti-ulcer
Beetroot	*Beta vulgaris*	Roots	Anti-ulcer
Slow match tree	*Careya arborea*	Leaves, stem and bark	Anti-ulcer
Arasha-maram	*Ficus religiosa*	Bark and Leaves	Anti-ulcer

Significance

Safety concern is one of the most important aspects of drugs discovery and use.

Allopathic system of medicine involves very sophisticated process that involves very practical preclinical, clinical and post-clinical investigations that check not only the efficacy and effectiveness of medicines but also the safety and potential toxicity. It involves various quality control procedures that ensures the lack of toxicity of medicinal substances before approval and use and continues to monitor their usage while in the market. These extensive research and the strict rules and regulations ensure the safety of allopathic medicines.

Herbal medicine, however, is obtained from natural sources, so less side effects are expected. But few instances have been reported for the severe physical problems such as allergic reactions, liver or kidney breakdown, cancer, and even death caused by them. Most of the herbal products presently in use being marketed have not been brought to a drug-approval process to express their safety and efficiency. Some of them contain mercury, lead, arsenic, corticoids and poisonous unprocessed matter in injurious amounts. Hepatic dysfunction and even death after the ingestion of medication has been reported. Research has shown that 25% of the cases of childhood blindness in Nigeria and India is related with the employment of traditional eye medicines. While the adverse effects of some plants have been reported, perhaps the biggest problem in Nigeria concerning herbal medicine is the deficiency of standardization and few safety guidelines. The standardization of a medicine obtained from herbal sources that may include large number of chemical constituents, with minimal or absential evidence which might be accountable for the supposed or proven beneficial response, is not an easy matter. Because of these, public opinion of people is divided for and against the consumption of herbal medicine. Few of them agree with the safety and effectiveness, while others believe that the herbal medicines are dangerous for health and should be properly tested before consumption.

Pharmacological profile [14,15]

- Phytochemicals- It was shown that dry pulp of fruit contains mucilaginous mass. It was found that leaves, stem and root of this plant contains significant amount of tannins, alkaloids, coumarins and steroids.

1. Alkaloids: Leaves mainly contain rutacine, ysitosterol, aegelemine and aegeline, marmeline, fragrine, dictamen, cinnamate and different derivatives of cinnamide.

2. Coumarins: Marmin, marmesin, umbelliferine, umbeliferone, skimmianine, Scoporone, scopoletin, psoralen, marmelide, xanthotoxol and impertonin were identified from the bark leaves, fruit and root of plant.

3. Seed oil: seed oil is bitter and contains 15.6% palmitic acid, 8.3% stearic acid, 28.7% linoleic and 7.6% linolenic acid while seed residue contains about 70% protein.

4. Polysaccharide: reducing sugars such as galactose, arabinose and L-rhamnose are found in fruit.

- Carotenoids are present in fruit and responsible for the characteristics color of fruit. It was found that root of Aegle marmelos tree contain psoralin, xanthotoxin and scopolotein.

a. Wood: House building, cart construction, combs and different household's articles.

b. Leaf: Fodder, tooth brushes, chew sticks

c. Leaf juice: Added in bathing water to remove bad body smell

d. Flower: Infusion of flower is used in cooling drinks

e. Stem gum: Used for adhesive and bookbinding

f. Fruit: A yellow dye obtained from rind of unripe fruit is used in printing. Fruit pulp is used for making "Sharbat". Fruit pulp is added in water color paint to provide smoothness. Fruit pulps have detergent properties and so used in place of soap. Fruit pulp is pickled. Whole tree is used as wind barrier.

- **Pharmacological activity**

1. Diarrhoea and dysentery: Generally dried fruit pulp and its powder are used for the treatment of diarrhoea. It has been used for the treatment of amoebic dysentery.[16]

2. Antidiabetic activity: Leaf extract of the bael plant is generally known for their antidiabetic activity. It has been found that bael extract significantly reduces blood urea and cholesterol level in diabetic animals. It also decreases oxidative stress in diabetic animals. Leaf juice is directly employed in Unani system of medicine for antidiabetic activity. Various studies as detailed below have signified its use as an antidiabetic agent.[17]

3. Antispermatogenic activity: It has been found that leaf extract has significant antispermatogenic activity. It inhibits the process of spermatogenesis and also reduces sperm motility.(18)

4. Cardioprotective activity: Various studies have also reported the use of bael as a cardioprotective. The protective effect was estimated by the administration of leaf extract in isoprenaline-induced myocardial infarction in the experimental animal.(19)

5. Anticancer activity: Studies showed that Indian bael (Aegle marmelos) extract possess significant antiproliferative effect. It inhibits in vitro proliferation of human tumor cell lines including the leukemic K562 and Tlymphoid Jurhat.(24)

6. Leucoderma: Psoralen present in the pulp of Aegle marmelos, increases tolerance of sunlight which aids in the maintenance of normal skin color and thus, it is employed in the treatment of leukoderma.

7. Anti-asthmatic: Aegle marmelos leaf decoction is said to reduce phlegm in cold and alleviate asthma. In the traditional context of India, it is used in the management of asthma.(23)

8. Eye infections: The leaves of Aegle marmelos are considered an effective treatment for ophthalmia and various eye inflammations such as conjunctivitis. The decoction of its flowers is also used as eye lotion.

9. Snake bite antidote: The roots, leaves and barks of the plant are often used as an antidote in the treatment of snakebite.

10. Febrifuge: Different parts of the plants are used in treating various types of fevers.

11. Antiemetic: A decoction of the flowers and roots of Aegle marmelos is used as an antiemetic.

12. Haemostatic: The unripe fruit of the plant are used in the form of powder or paste as haemostatic.

13. Antipyretic and analgesic activity: In one study it was found that bael extract exhibits antipyretic, anti-inflammatory and analgesic activities in experimental animals [4, 7, 51].

14. Constipation: The riped bael fruit act as good laxative. It promotes peristaltic movements and thus helpful in the removal of fecal matter.

15. Peptic ulcer: Bael fruit and leaf infusion has been used for the treatment of peptic ulcer. One of the important advantages of this fruit is that it forms a mucilaginous layer on the gastric mucosa and thus prevents the interaction of acid with mucosal layer.

16. Antioxidant: estimated the antioxidant activity of methanolic extract of leaves, root and stem bark of plant Aegle marmelos. In this study free radical scavenging activity of the plant is estimated using DPPH (1, 1 -Diphenyl- 2 -picryl hydrazyl). Results showed that leaf extract have better antioxidant activity than stem bark and root extract.[20]

17. Anti-inflammatory: evaluated anti-inflammatory activity of leaves extract of Aegle marmelos using rat as experimental animal and aspirin as standard drug.

18. Antigenotoxic: Methanol and acetone extract of dried fruit were used to evaluate the antigenotoxic activity of Aegle marmelos in Human Blood Lymphocytes and E. coli PQ.

19. Insect infestation: Rajesh Kumar et al. extracted essential oil from the leaves of Aegle marmelos and studied the effect of this oil on the insect infestation of stored gram from.

20. Callosobruchus chinensis and wheat from Rhyzopertha dominica, Sitophilus oryzae and Tribolium castaneum. Results showed that extracted leaf oil significantly reduced the grain damage as well as weight loss in fumigated gram and wheat samples infested with all insects except T. castaneum.

21. Anti microfilariae:prepared methanolic extract of Aegle marmelos and further studied the antimicrofilariae effect using sterile plate. Results predicted the fact that methanolic extract of Aegle marmelos leaf have significant antimicrofilariae effect.

22. Hepatoprotective: Singanan et al. evaluated the hepatoprotective effect of bael leaves in alcohol induced liver injury in albino rats. Obtained results indicated that the bael leaves have excellent hepatoprotective effect.[20]

23. Antiburn and Radioprotective: Bael fruit powder mixed with mustard oil is used externally for the treatment of burn. It was found in various studies that due to free scavenging activity, bael fruits have radioprotective effect on experimental animal.

24. Ear Problems: The root of this tree possesses astringent activity and is used as a home remedy for curing ear problems. The juice of leaves processed in oil is also used as ear drops to treat ear infection.

25. Antiepileptic: The leaves of Aegle marmelos was found to have antiepileptic activity in combination with Cassia fistula, Lagerstroemia reginae, Premna mucronata, whole plants of Solanum xanthocarpum, Tribulus terrestris, Desmodium gangeticum, Desmodium pulchellum and branches and leaves of Solanum indicum.[25]

26. Antileprotic: The powdered and sundried leaves of Aegle marmelos when sprinkled on the wound after taking bath proved to be an effective remedy for the treatment of leprosy.

27. Myalgia: The paste of ground roots of Aegle marmelos with cold water taken early in morning in empty stomach helped in curing myalgia.

28. Smallpox: The paste of powdered leaves of Aegle marmelos is effectively used in the treatment of smallpox.

29. Antifungal: The unsaponifiable matter from the oil obtained from the seeds of Aegle marmelos was effective as antifungal agent [64-66].

30. Antibacterial: It was found that the fixed oil from seeds of Aegle marmelos and its unsaponifiable portion were found to be active against various strains of gram-positive and gram-negative bacteria.

31. Antidandruff: The rind of the fruit of bael is used in the treatment of dandruff.

32. Kidney problems: The ground leaves of Aegle marmelos can be used to treat kidney problems.

33. Insomnia: It was found that the paste of ground roots of bael in butter was effective in the treatment of insomnia. When applied on the soles of the feet, it can induce sleep.

34. Gynaecological problems: The bark of bael root is effective in various gynecological problems as proved by its famous ayurvedic formulation dashamoola (ten roots of medicinal plants).

35. Antiallergic: Since its pulp has a detergent property, it can be used as the herbal substitute of soaps, for allergic patients.

36. Antiarthritic: Raw bael fruit is used for treatment of arithritis and gout. Its pulp mixed with hot mustard oil can be applied on swollen joints for relief from these disorders.

37. Healthy mind and brain: The ripe fruit of Aegle marmelos keeps the body and mind cool, helps to sharpen intellect and concentration of mind.

38. Cure of Anaemia: Powdered pulp of bael consumed with boiled cow's milk helps to cure anaemia.

Need of work

The herbal preparation is devoid of allopathic side effects. As mention above *Aegle marmelos* consist of so many immersive activities the herbal preparation of these makes it free of side effects which makes the preparation more effective.

1. MATERIALS AND METHODS

Geographical source - *Aegle marmelos* (L.) Correa is a tree belongs to family Rutaceae is commonly known as bael; it is native to Northern India but widely found throughout Indian Peninsula and in a Ceylon, Burma, Bangladesh, Thailand and Indo-China (Rahman and Parvin, 2014).

The leaves of *Aegle marmelos* were harvested from November to December from local Herb garden and cleaned, air dried for 20-25 days , then pulverized for extraction.[23]

2. Collection and preparation

2.1 Collection and of plant material

The fresh, unripen fruits of Aegle marmelos were collected from healthy trees were growing at very hygiene and polluted-free area in the month of May, June, located at various regions of Jaipur, Rajasthan.

2.2 Preparation of Extract

Freshly collected seeds of Aegle marmelos were dried at 30 0C and at 18.9 % relative humidity conditions and milled with sieve to remove excess of mucilaginous hair. The plant extract was prepared using two different laboratory-grade solvents (double distilled water & methanol).

- Preparation of aqueous extract: The dried powdered plant part (1.0 kg of Aegle marmelos seeds) was extracted with 4.0 liters of double distilled water for 72 hours in a round bottom flask, by placing on water bath, attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (reddish brown) was obtained.

- Preparation of methanolic extract: The powdered plant material (1.0 kg of Aegle marmelos seeds) was extracted with 4.0 liters of analytical grade methanol for 72 hours in a round bottom flask, on water bath attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (yellow-reddish) was obtained.

The % yields of both the extracts (i.e. aqueous and methanol) were 19.71% and 10.84 %, respectively.

Ulcer healing potential

(Sharma et al.) Investigated anti-ulcer activity of methanolic and aqueous extract of A. marmelos seeds using indomethacin-induced ulceration, stressed induced ulceration and pylorus ligation induced ulcerations. Methanolic extract showed significant ($P<0.01$) ulcer protective action at the doses of 200 and 400 mg/kg body weight in all animal model. The

aqueous extract was also found to possess significant (P<0.05) ulcer healing property at the same doses as of methanolic extract. A significant reduction in volume of gastric juice, free acidity and total acidity, along with increase in pH was observed in pylorus ligated rats. The antiulcer property of both the extracts was attributed due to the presence of quercetin like (flavonoid) contents. Another study indicated that A. marmelos fruit pulp extract-treated albino rats show a significant decrease in mucosal thickness, superoxide dismutage, catalase activity and glutathione level. A significant increase in ulcer index, aspartate aminotransferase, alanine aminotransferase, lipid peroxidation activity was also observed.

These results suggest that gastro duodenal protective and antiulcerogenic properties of A. marmelos may also depend on antioxidant mechanism.

2.3 Assessment of antiulcer activity In vivo:

The male wistar rats were (180-200 g) were used for the study. They were housed in metal cages and were left for two days for acclimatization to animal room, was maintained under controlled condition of (12 hours light and dark cycle at 22 ± 3 0C), and were kept on standard pellet diet and water ad libitum. Before the study the animals were fasted overnight with the free access to water. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the purpose of control and supervision of experiments on experiments on animals).

Indomethacin-induced gastric ulcers:

A total of 36 rats were divided into 6 subgroups. Group I; received normal saline and served as control, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI; was treated with ranitidine (50 mg/kg b. w.) and served as reference group. All the treatments were made orally for 10 days, before gastric ulcer were induced in rats (except group-I) with indomethacin (20 mg / kg b.w.). The animals were killed under ether anesthesia after 6 hour of administration of indomethacin and the stomach was isolated and cut opened along the greater curvature. Screening of ulcer was done as; (0: normal colored stomach; 0.5: red coloration; 1.0: spot ulcers; 1.5: haemorrhagic streaks; 2.0= ulcers ≥ 3 mm but ≤ 5 mm; 3.0:

ulcers ≥ 5 mm). Ulcer index was calculated using formula;

UI = UN + US + UP X 10 -1

Where; (UI: ulcer index, UN: average no. of ulcer per animal, US: average of severity score and UP: percentage of animal with ulcer)

Stressed induced ulcers: The rats were divided into 6 groups, each containing 6 animals. Group I, received normal saline, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI; was treated with ranitidine (50 mg/kg b. w.) and served as standard. All the treatments were made orally 30 min prior to subjection of stress. The ulcer was induced by placing animals in a restraint cage maintained at temperature of 20 -40 C for 3 hours. The animals were sacrificed and scoring of ulcer was done as mentioned above.

Pylorus ligation-induced ulcers: The study was performed as method described by the Shay.

36 overnight fasted rats were divided in 6 subgroups; group I, received normal saline, group II, IV received 200 mg/kg, b.w and group III, V received 400 mg/kg, b.w of aqueous and methanolic extracts of Aegle marmelos seeds, sequentially. Group VI was treated with ranitidine (50 mg/kg b. w.) and served as standard. After 30 min. under ether anaesthesia, stomach was ligated and replaced carefully. Animals were deprived of both food and water during the post-operative period and were sacrificed at the end of 19-20 h, stomach was dissected out and contents were subjected to centrifugation (3000 rpm for 10 min) and then analyzed for pH, total volume of gastric secretion, free acidity, pH and total acidity.

The ulcer index was also calculated as described above. Acidity was expressed as:

$$\textbf{Total Acidity (m Eq/ L)} = \frac{\textbf{Vol. of NaOH X Normality}}{\textbf{0.1}} \textbf{ X 100}$$

Statistical Analysis: Data are expressed as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Dunnet test.

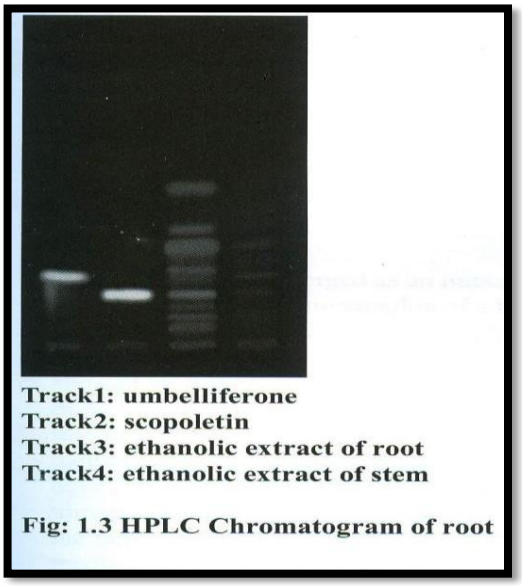
Chromatography

The plant materials (100g) were ground and then extracted with methanol for 24 h in a continuous extraction (Soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under a vacuum at a temperature of 45 IC to dryness. A part of this residue was dissolved in 2 N HCI and then filtered. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without Caffeine (Sahara, 2008).

Estimation of Total Tannins - The total tannins were estimated using Folin -Denis colorimetric method (Burns et al, 1974). Tannic acid was used to plot the calibration curve.

Thin layer chromatographic profile - Thin Layer Chromatography of aqueous extracts of both root and stem was carried out on a pre-coated silica gel 60F254 TLC plate (Merck India) using toluene, ethyl acetate and formic acid as mobile phase in the ratio of 5:2:1. The so far reported marker compounds umbelliferone and scopoletin were also spotted for their identification in the extracts. The plate was developed over a distance of 9 cm and visualized under UV-366 nm.

High-Pressure Liquid Chromatographic Analysis - 10 ml of the both extracts were filtered through 0.25 um membrane filters. The sample was then used for the HPLC profiling using a Shimadzu High-Performance Liquid Chromatographic system equipped with LC 10ATVP pump, SPD M10AVP Photo Diode Array Detector in combination with CLASS-VP 6.12 SP5 integration software. The mobile phase used for the separation was HPLC grade water(A) and acetonitrile (B) in a time programming 0-10 10% A, 10-20 30% A, 20-30 50%, 30-40.



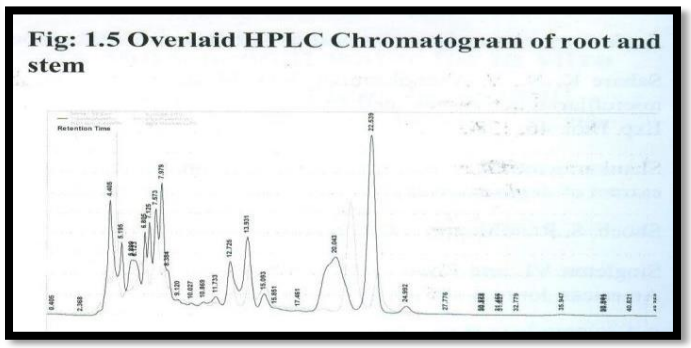


Figure: 1 **Figure: 2**

Evaluation and Characterization of Aeglo marmelous

DPPH assay: (2, 2-diphenyl-1-picrylhydrazyl)

The scavenging reaction between (DPPH) and an antioxidant (H_A) can be written as

(DPPH) + (H_A) →DPPH_H + (A)

(Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH_H and as consequence the absorbance was decreased from the DPPH radical to the DPPH_H form. The degree of discolouration indicates s the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Antioxidant activity by DPPH staining

An aliquot (3µL) of each sample and standard (Quercetin and Ascorbic acid) were carefully loaded onto a 10cm X 10cm Silica gel plate (silica gel 60 F254; Merck) and allowed to dry for 3 minutes. Drops of each sample were loaded in an order of decreasing concentration along the row. After 5 minutes, the TLC plate was sprayed with 0.2% DPPH in methanol. Discoloration of DPPH indicates scavenging potential of the compound tested.

Assay

The dried plant extract (200 µg) in 100 µl of the corresponding solvent was mixed with the solution (9.9 ml) mentioned above when necessary. BHT was used as a standard to evaluate the antioxidative activity of samples. The reacted solution obtained (1 ml) was used for TBA assay.

TBA assay

The degree of oxidation of oil was measured by the 2- thiobarbituric acid (TBA) assay (Ohkawa et al 1994). The reacted solution (1 ml) mentioned above was mixed with 0.2% (*w*/*v*) TBA (3 ml) and 0.05 M sulfuric acid (2.5 ml) and the mixture was heated for 30 min in a 95° water bath. After, the solution was then cooled in ice for 5 min; the colored substances were extracted by 4.0 ml of 1-butanol. The absorbance of the 1-butanol layer was measured at 532 nm. Antioxidant activity (AOA) was expressed as percentage inhibition of lipid peroxidation relative to the control using the following equation:

$$\text{AOA}\% \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance ofcontrol}} \times 100$$

Heat and pH stability

The extracts were heated in a boiling water bath for 15 min and the residual antioxidant activity was determined by radical scavenging activity using DPPH as described previously. For pH stability, antioxidant extracts were incubated for 24 h at pH 4, 7 & 9 and the residual antioxidant activity was determined during the incubation period at different time intervals (0 min, 30 min, and 24 h) as radical scavenging activity against DPPH (Arabshahi-Delouse et al. 2007).

Statistical analysis

Mean values of 4 determinations ($n = 4$) ($p < 0.05$) was subjected to one-way ANOVA and Tukey's multiple comparison tests using SPSS software (version 11).

Antioxidant component

The Aegle leaves were found to be a good source of several antioxidant components such as, β-carotene, glutathione, α-tocopherol, ascorbic acid and total polyphenols and flavonoids (Tables (Tables11 and and22).

Table 1

Antioxidant components, total polyphenols and extract yield of different solvent extracts from *Aegle* leaves

Antioxidant component	**Per 100 g**
α- Tocopherol (mg)[b]	27
β- carotene (μg) [b]	8600
Glutathione (m. moles) [b]	580
Ascorbic acid (mg) [b]	260
Total Flavonoids (mg)[a]	2.4
Total polyphenols (g) [b]	2.4
Polyphenol content[c]	

	Antioxidant component	**Per 100 g**	
	ME	9.9 (27.8)	
	EE	11.5 (12.3)	
	WE	8.9 (32.0)	

[an] mg/g extract, [b] per 100 g dry basis. [c] g of Gallic acid per 100 g of extract. Values in parenthesis indicate yield in g of extract per 100 g of dried leaves. *ME* methanol, *EE* ethanol, *WE* water extracts, $n = 4$ **Table 4**

Comparison of antioxidant properties and polyphenols of *Aegle* extracts and standards

Radical scavenging activity

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathogens such as cancer and cardiovascular diseases among others (Dorman et al. 2003a, b). The DPPH radical scavenging has been widely used to evaluate the free radical scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto et al 2000).

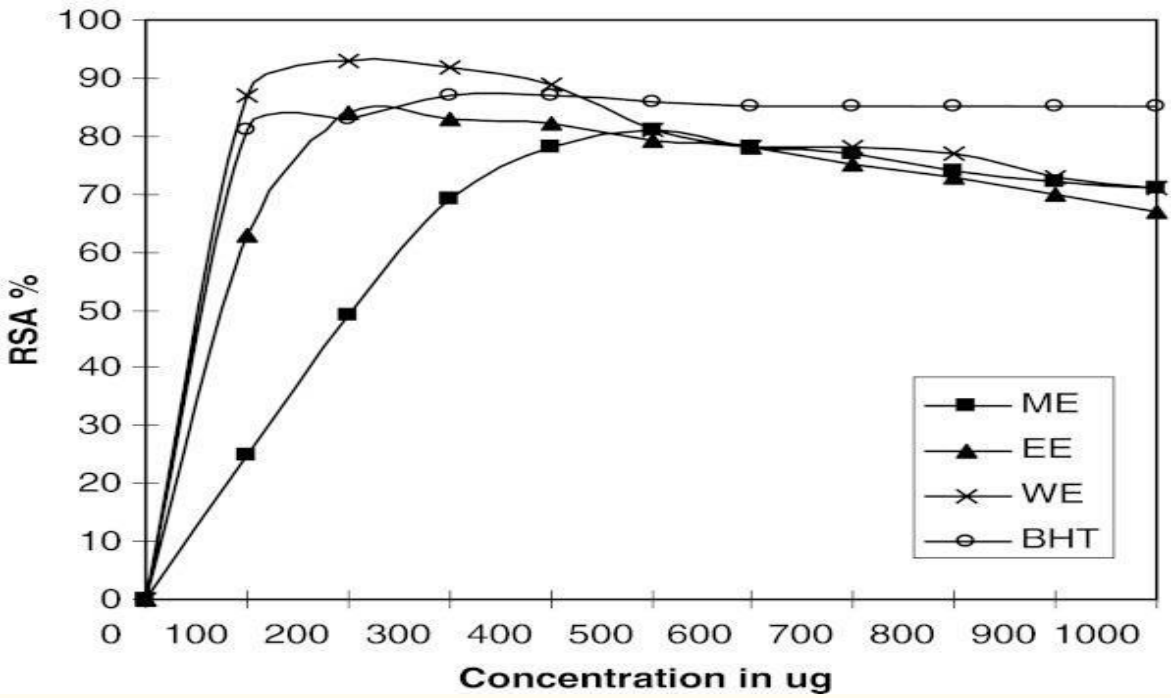
The radical scavenging activity (RSA) of the three solvent extracts (methanol, ethanol and water) as determined by DPPH assay at different concentrations is shown in Fig. 1. The scavenging ability of three extracts against DPPH was concentration dependent upto 300 µg., with increasing levels the RSA tended to decrease. This trend indicates that the scavenging potency of the extracts might reach a saturation point and at higher concentrations, it may act as a pro-oxidant. Similarly, at higher concentrations, phenolic compounds are reported to enhance $OH^{\cdot}$ formation and inhibit antioxidant enzymes (catalase and SOD) suggesting that the naturally occurring substances can have prooxidant effects under some reaction conditions (Sakihama et al 2002). At 200 µg concentration, WE showed maximum activity (92%) followed by EE (88%) and ME (78%) Table Table2.2. These percentages can be considered as a full absorption inhibition of DPPH because after completing the reaction in the final solution always possesses some yellowish color and therefore its absorption inhibition compared to colorless methanol solution cannot reach. 100% (Miliauskas et al.,

Solvent	Radical scavenging activity DPPH, %	Percent Inhibition of lipid oxidation (%)	Reducing power (absorbance at 700 nm)
ME	81 ± 2.064^{a}	23 ± 2.00^{a}	0.232 ± 0.003^{a}
EE	87 ± 1.00^{b}	46 ± 1.15^{b}	0.246 ± 0.004^{b}
WE	93 ± 0.57^{c}	28 ± 2.51^{a}	0.349 ± 0.003^{c}
Standard	87 ± 0.52^{ba}	80 ± 5.03^{cb}	0.973 ± 0.002^{dc}

[a]BHT, [b] α- Tocopherol, [c]Ascorbic acid, -standard conc. used 150 µg and extracts 200 µg;

ME-methanol, EE- ethanol, WE-water extracts, $n = 4$

2004).



DPPH radical scavenging activities (RSA) of methanol, ethanol and water extracts of *Aegle* leaves. BHT was used as positive control. % RSA relative to control. *ME* methanol, *EE* ethanol, *WE* water extracts

Figure 3

Reducing power assay

The reducing power (electron-donating capacity) of bioactive compounds is associated with antioxidant activity (Siddharaju et al 2002, Yen et al 1993). In the present study, the ability of extracts to reduce iron (III) to iron (II) was determined and compared with that of a standard (ascorbic acid). All three extracts showed some degree of electron-donating capacity (Table 3) in which EE extract showed a maximum reduction of iron (III) to iron (II) indicated by higher absorbance at 700 nm, but the capacities were inferior to that of ascorbic acid. It was interesting to note that despite containing the least amount of phenolics, WE were the most potent reducing agent.

Effect of pH and Temperature on the Antioxidant Activity of Methanol, Ethanol and Water Extracts of Aegle Leaves

Solvent extract	0 min	30 min.	24 hrs	Before heat treatment	After heat treatment
4ME	73 ± 1.52^{al}	71 ± 1.73^{abl}	70 ± 1.00^{al}	81 ± 2.064	57 ± 2.00
4EE	79 ± 1.15^{bl}	72 ± 0.57^{am}	$75 \pm .1.52^{bn}$	87 ± 1.00	71 ± 2.08
4WE	77 ± 1.52^{al}	68 ± 1.52^{bm}	72 ± 2.00^{abm}	93 ± 0.57	89 ± 1.2
7ME	67 ± 1.52^{al}	75 ± 1.52^{bl}	72 ± 2.51^{al}		
7EE	73 ± 2.64^{al}	68 ± 2.08^{al}	71 ± 2.64^{al}		
7WE	80 ± 1.00^{cl}	72 ± 2.08^{am}	72 ± 2.08^{am}		

Extracts conc. 200 µg; *ME* methanol, *EE* ethanol, *WE* water extracts, $n = 4$

Antioxidant activity (AOA)

The reducing power assay and radical scavenging activity assays are indicators of antioxidant activity. However, neither of these methods utilize a portion of food or biologically relevant oxidizable substrate, hence no direct information on the protective properties of the extracts can be determined (Dorman et al 2003a, b). In present study, the effect of various extracts on the % inhibition of lipid peroxidation in Linseed oil (rich in PUFA) was determined by TBA method. All the three extracts, were capable in preventing the formation of TBARS generated by Fenton's reagent (Table 3), however EE was the most effective in inhibiting lipid oxidation. A similar effect was not observed at higher concentrations of extracts (>500 µg) indicating a pro oxidant role at higher concentrations. Sakihama (2002) reported that, flavonoids and dihydroxycinnamic acids can nick DNA via the production of radicals in the presence of Cu and O_2. Phenoxyl radicals are reported to initiate lipid peroxidation while, Al, Zn, Ca, Mg and Cd have been found to stimulate phenoxyl radical-induced lipid peroxidation.

DISCUSSION

The peptic ulcer results from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms. To regain the balance, different therapeutic agents are used to inhibit gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal protection, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. The causes of gastric ulcer pyloric ligation are believed to be due to stress-induced increase in gastric hydrochloric acid secretion and or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating aci.

CONCLUSION

From the above information, we can conclude that *Aegle marmelos* consist of many pharmacological activities such as antioxidant, antidiabetic, dysentery, anticancer, antidandruff, Antileprotic, myalgia, antifungal, smallpox, antibacterial, ntiburn and radioprotective, antigenotoxic, anti-inflammatory activity and antiulcer. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The lesions produced by this method are located in the lumen region of the stomach. The Aegle marmelos leaves extract and Omeprazole significantly decreased the total acidity; this suggests that it having an antisecretory effect as well as markedly significant reduction in ulcer index. Non-steroidal anti-inflammatory agents, like Indomethacin induce gastric lesions in experimental animals by inhibition of gastric cyclooxygenase resulting in less formation of endogenous prostaglandin; Indomethacin also inhibits gastro duodenal bicarbonate secretion as well as gastric mucosal blood flow. The model shows drug's effect on cytoprotection through non prostaglandin-mediated mechanism. The extract shows protection against characteristic lesions produced by Indomethacin administration. This antiulcer effect of Aegle marmelos leaves extract may be due to both reductions in gastric acid secretion and gastric cytoprotection. Water immersion stress is one of the best models for stress-induced ulcer in animals. The model provides both emotional stress as well as physiological stress to the animal. The extract showed significant ulcer inhibition.

The anti-ulcer effect observed in the present study might be due to a possible relationship between protection of mucosal injury, inhibition of acid secretion and the antioxidant nature

of A. marmelos extract. Further studies are needed for their exact mechanism of action on gastric acid secretion and gastric cytoprotection.

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