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Investigation Of In-Vitro Anti-Oxidant & Anti-Ulcer Activity Of Polyherbal Medicinal Plants

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ABSTRACT

Peptic ulcer disease (PUD) remains a significant global health burden, characterized by recurrent gastric lesions and complications. Although proton pump inhibitors (PPIs) like esomeprazole are the standard treatment, their adverse effects and high cost drive the search for safer, cost-effective alternatives. This study investigated the gastroprotective effects of *Rhodomyrtus tomentosa* and *Cynodon dactylon* leaf extracts in a cold-water immersion-induced ulcer model in rats, which replicates cold stress–mediated mucosal damage through increased permeability and vasoactive mediator release. The antioxidant and cytoprotective properties of these botanicals were evaluated by comparing their efficacy with esomeprazole in reducing gastric lesions and oxidative stress. Results demonstrated that both plant extracts significantly attenuated ulcer severity, with the 400 mg/kg dose showing near-comparable efficacy to esomeprazole (ulcer length: 3.60 ± 0.25 mm vs. 2.60 ± 0.85 mm). Histopathological analysis revealed dose-dependent mucosal protection, with the high-dose extract group exhibiting only mild lesions, in contrast to severe hemorrhagic necrosis in controls. Furthermore, the extracts normalized gastric pH (7.50 ± 0.36 vs. control 5.70 ± 0.82) and reduced gastric juice volume (2.00 ± 0.65 cm^3 vs. control 4.67 ± 0.54 cm^3), suggesting acid suppression and cytoprotective mechanisms.

Keywords: Peptic ulcer disease, *Rhodomyrtus tomentosa*, *Cynodon dactylon*, Gastroprotective activity, Cold-water immersion stress

1. INTRODUCTION

Peptic ulcer disease (PUD) is one of the most prevalent gastrointestinal disorders, affecting millions of people worldwide. It is characterized by recurrent episodes of mucosal erosion in the stomach or duodenum, leading to complications such as bleeding, perforation, and obstruction (Søreide et al., 2015). The pathogenesis of PUD involves an imbalance between aggressive factors (e.g., gastric acid secretion, Helicobacter pylori infection, and nonsteroidal anti-inflammatory drug [NSAID] use) and protective mechanisms (e.g., mucus secretion, bicarbonate production, and mucosal blood flow) (Malfertheiner et al., 2017). Despite advancements in conventional treatments, the high recurrence rate and adverse effects associated with long-term pharmacotherapy underscore the need for safer and more effective alternatives.

2. LIMITATIONS OF CONVENTIONAL ANTI-ULCER THERAPIES

Proton pump inhibitors (PPIs), such as esomeprazole, remain the gold standard for ulcer treatment due to their potent acid-suppressive effects (Scarpignato et al., 2016). However, prolonged PPI use has been linked to adverse effects, including nutrient malabsorption, increased risk of infections, and renal complications (Freedberg et al., 2017). Additionally, the high cost of these medications limits accessibility in low-resource settings. Similarly, while histamine H_2-receptor antagonists (e.g., ranitidine) and antacids provide symptomatic relief, they do not address underlying mucosal damage or oxidative stress, which plays a critical role in ulcer progression (Tarnawski et al., 2018)

3. THE ROLE OF OXIDATIVE STRESS IN ULCEROGENESIS

Oxidative stress is a key contributor to gastric mucosal injury. Reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide, exacerbate inflammation and impair tissue repair (Bhattacharyya et al., 2014). Cold-water immersion stress, an established experimental model, induces ulceration by triggering vasoconstriction, ischemia-reperfusion injury, and the release of pro-inflammatory cytokines (Konturek et al., 2011). This model mimics clinical scenarios where cold exposure (e.g., hypothermia or excessive cold beverage consumption) aggravates gastric lesions. The resultant mucosal permeability allows acid infiltration, further damaging epithelial cells and accelerating ulcer formation (Takeuchi et al., 2011).

4. NATURAL COMPOUNDS AS PROMISING ANTI-ULCER AGENTS

Given the limitations of synthetic drugs, there is growing interest in plant-derived compounds with gastroprotective and antioxidant properties (Ganguly et al., 2020). Medicinal plants like *Rhodomyrtus tomentosa* (commonly known as rose myrtle) and *Cynodon dactylon* (Bermuda grass) have demonstrated anti-inflammatory, antimicrobial, and free radical–scavenging activities in preclinical studies (Limsong et al., 2004; Singh et al., 2007). *R. tomentosa* is rich in flavonoids and tannins, which enhance mucus secretion and inhibit *H. pylori* growth (Srisawat et al., 2020), while *C. dactylon* contains polyphenols that attenuate oxidative damage and promote ulcer healing (Murali et al., 2015).

5. MATERIALS AND METHODS

Materials

Plant Extracts:

Leaves of *Rhodomyrtus tomentosa* (Myrtaceae family)

Leaves of *Cynodon dactylon* (Gramineae family)

Methods

The study utilized cultivated plant specimens. Leaves of *Rhodomyrtus tomentosa* and *Cynodon dactylon* were sourced from the Himalayan region of Dehradun, India.

Extraction Process

For *Cynodon dactylon*:

The leaves were mechanically dried, pulverized into powder, and approximately 600 grams of coarse powder was obtained. Sequential Soxhlet extraction was performed using solvents of varying polarity (chloroform, ethyl acetate, methanol, and petroleum ether) at 50–60°C. The resulting extracts were concentrated under reduced pressure (<40°C) and stored in a desiccator for further analysis.

For *Rhodomyrtus tomentosa*:

Leaves were shade-dried, milled into powder (~800 g), and subjected to Soxhlet extraction with different solvents (70% aqueous ethanol, chloroform, ethyl acetate, and water) at 50–60°C. The extracts were evaporated at <50°C under reduced pressure, yielding five distinct fractions stored in a desiccator.

Yield Calculation:

%Yield=Weight of extractWeight of crude drug×100%Yield=Weight of crude drug/ Weight of

extract×100

Phytochemical Screening

Carbohydrate Detection:

Molisch's Test: Reddish-violet ring formation confirmed carbohydrates.

Fehling's Test: Brick-red precipitate indicated reducing sugars.

Glycoside Detection:

Bornträger's Test: Reddish-brown interphase confirmed glycosides.

Keller-Killiani Test: Reddish-brown to blue-green transition under light indicated cardiac glycosides.

Saponin Detection:

Foam Test: Persistent foam (>1 cm) confirmed saponins.

Amino Acid & Protein Detection:

Millon's Reagent: Red precipitate indicated proteins.

- **Ninhydrin Test:** Purple color suggested amino acids.

Flavonoid Detection:

Shinoda Test: Pink-red color change confirmed flavonoids.

Ammonia Test: Color shift from white to orange on filter paper indicated flavonoids.

Total Phenolic Content (TPC) Analysis

Solvent Optimization: Water was selected over methanol due to minimal turbidity.

Standard Curve: Gallic acid (GAE, µg/mL) was used for calibration.

Procedure: Extracts (0.5–1 mg/mL) were mixed with Folin-Ciocalteu reagent and Na_2CO_3, incubated, and absorbance measured at 738 nm.

In Vitro Antioxidant Assays

DPPH Radical Scavenging:

Extracts (0.24–1000 µg/mL) were reacted with DPPH (purple → yellow).

Absorbance measured at 517 nm; IC_{50} calculated.

H_2O_2 Scavenging:

Extracts (0.24–500 µg/mL) were mixed with H_2O_2 in PBS.

Control: Methanol + PBS.

Nitric Oxide Scavenging:

Griess reagent detected nitrite formation (purple color).

Sulphanilic acid and NEDD reagents were used.

Animal Studies

Species: Wistar albino rats (200–250 g, 4–6 months, either sex, *n* = 48).

- **Antiulcer Activity**

Cold Water Immersion Model:

- **Groups:**

I: Control (normal saline, p.o.)

II: Standard (esomeprazole 25 mg/kg, i.p.)

III: *R. tomentosa* + *C. dactylon* (100 mg/kg each, p.o.)

IV: *R. tomentosa* + *C. dactylon* (200 mg/kg each, p.o.)

- **Procedure:**

Fasted rats were restrained in cold water (22°C, 16 hrs).

Evan's blue (30 mg/kg, i.v.) was injected before sacrifice.

Gastric lesions, pH, and volume of gastric juice were analyzed.\

6. HISTOPATHOLOGY

Stomach tissues were fixed in 10% formalin, sectioned, and stained with H&E for microscopic examination.

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's/Dunnett's tests (*p* < 0.05 significant) via GraphPad Prism v4.

Result & Discussion

Extraction and Yield of Plant Material

The percentage yields of successive leaf extracts from *Rhodomyrtus tomentosa* and *Cynodon dactylon* are detailed in Tables 1 and 2, respectively.

Phytochemical Screening of Polyherbal Formulation

Phytochemical Analysis of *Rhodomyrtus tomentosa*Preliminary phytochemical tests on successive leaf extracts revealed the

presence of steroids, terpenoids, tannins, alkaloids, saponins, phenolics, and flavonoids (Table 6.1).

Table 1: Physicochemical Properties and Phytochemical Composition of *Rhodomyrtus tomentosa* Leaf Extracts

Extract	Nature of Extract	Yield (% w/w)	Phytochemical Constituents
Petroleum ether	Greenish-brown solid	0.6	Steroids, terpenoids
Chloroform	Brown solid	1.4	Alkaloids, phenols, terpenoids
Ethyl acetate	Brownish solid	2.8	Flavonoids, phenols, tannins
Aqueous alcohol	Brownish semisolid	4.6	Flavonoids, phenols, saponins, terpenoids, tannins
Distilled water	Dark brown semisolid	2.5	Flavonoids, phenols, saponins, terpenoids, tannins

Table 2: Phytochemical Analysis and Extraction Yield of *Cynodon dactylon* Leaves

Extract	Nature of Extract	Yield (% w/w)	Phytochemical Constituents
Petroleum ether	Greenish-brown solid	1.4	Steroids, terpenoids
Chloroform	Brown solid	1.6	Alkaloids, phenols, terpenoids
Ethyl acetate	Brownish solid	2.2	Flavonoids, phenols, tannins
Aqueous alcohol	Brownish semisolid	6.0	Flavonoids, phenols, saponins, terpenoids, tannins
Distilled water	Dark brown semisolid	-	Flavonoids, phenols, saponins, terpenoids, tannins

Total Phenolic Content (TPC) Analysis

Phenolic Content in *Rhodomyrtus tomentos*The highest phenolic content was found in aqueous alcoholic extract (4.04 ± 0.22 mg GAE/g), followed by ethyl acetate (3.26 ± 0.24 mg GAE/g), chloroform (1.26 ± 0.24 mg GAE/g), and petroleum ether (0.48 ± 0.64 mg GAE/g) (Table 3).

Table 3: Phenolic Content of *Rhodomyrtus tomentosa* Leaf Extracts

Extract	Phenolic Content (mg GAE/g dry weight)
Petroleum ether	0.48 ± 0.64
Chloroform	1.26 ± 0.24
Ethyl acetate	3.26 ± 0.24
Aqueous alcohol (70%)	4.04 ± 0.22

Phenolic Content in *Cynodon dactylon*The methanolic extract exhibited the highest phenolic content (4.1 ± 0.18 mg GAE/g), followed by ethyl acetate (3.8 ± 0.48 mg GAE/g), chloroform (3.6 ± 0.40 mg GAE/g), and petroleum ether (1.4 ± 0.24 mg GAE/g) (Table 4).

Table 4: Phenolic Content of *Cynodon dactylon* Extracts

Extract	Phenolic Content (mg GAE/g dry weight)
Petroleum ether	1.4 ± 0.24
Chloroform	3.6 ± 0.40
Ethyl acetate	3.8 ± 0.48
Aqueous alcohol (70%)	4.1 ± 0.18

Antioxidant Activity of Plant Extracts

Antioxidant Capacity of *Rhodomyrtus tomentosa* Extracts

The antioxidant potential of *Rhodomyrtus tomentosa* extracts was evaluated using DPPH, H_2O_2, and nitric oxide (NO) radical scavenging assays (Figures 1– 4)

Fig: 1 Comparative Antioxidant Efficacy of *Rhodomyrtus tomentosa* Extracts in Nitric Oxide Scavenging

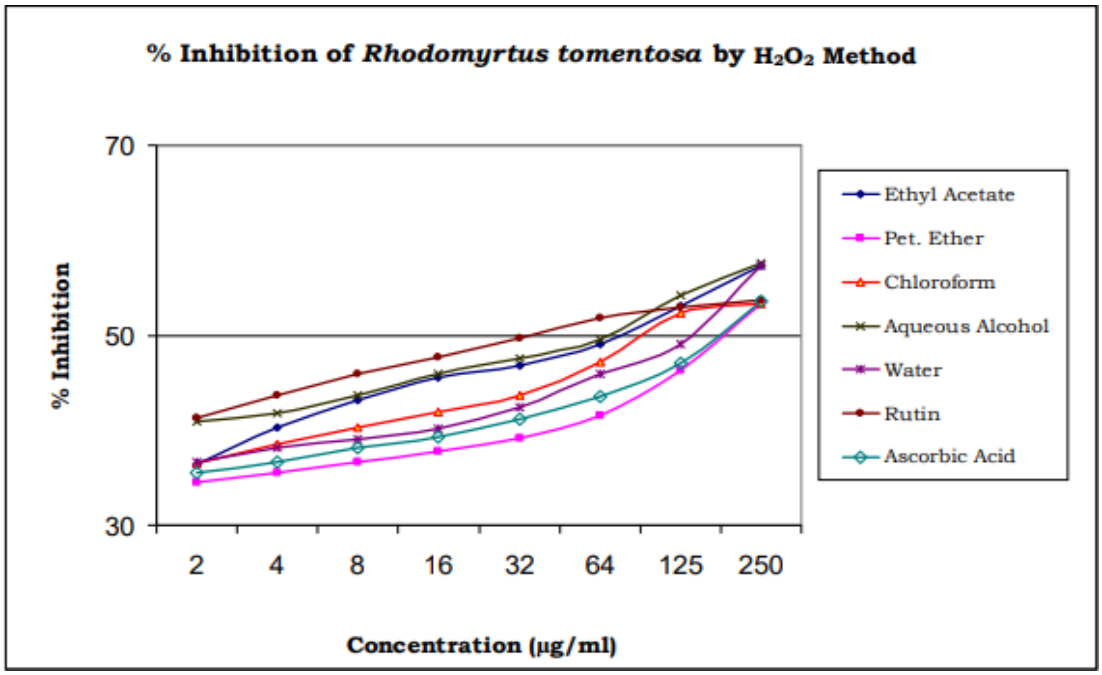


Fig: 2 Hydrogen Peroxide (H_2O_2) Scavenging Activity of R. tomentosa Extracts

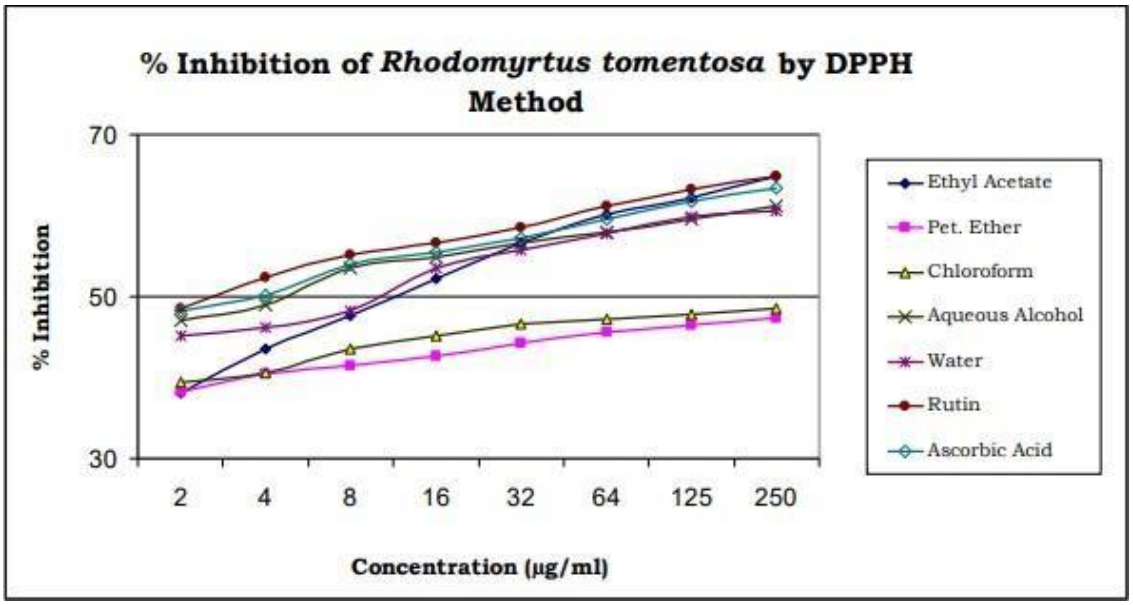


Fig: 3 DPPH Scavenging Activity of R. tomentosa Extracts

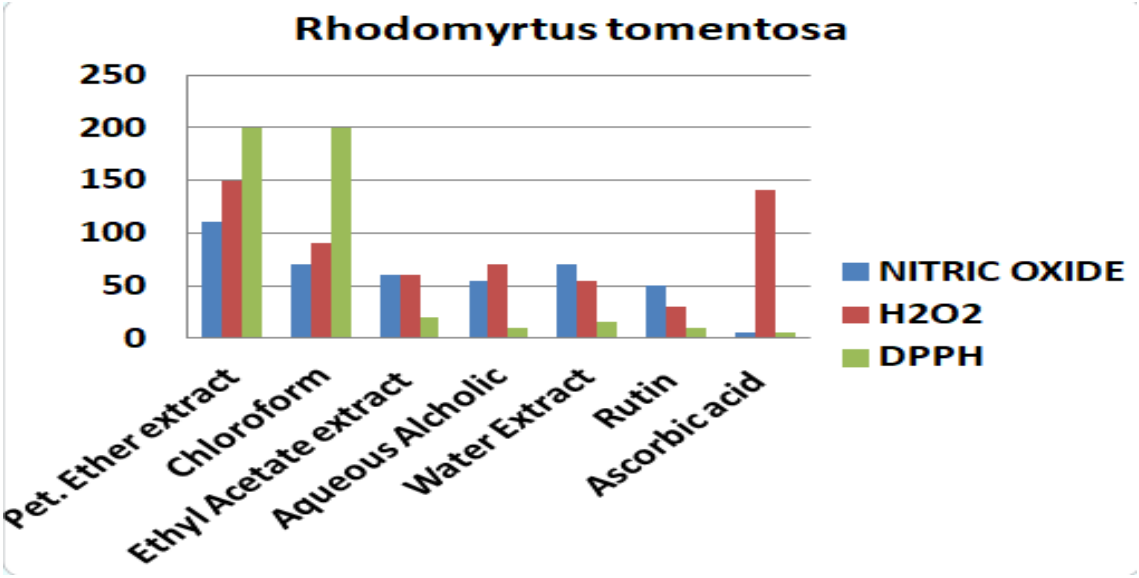


Fig: 4 In vitro evaluation of the anti oxidant potential of Rhodomyrtus tomentosa extracts

7. IN VITRO EVIDENCE OF THE ANTIOXIDANT CAPACITY OF *CYNODON DACTYLON* EXTRACTS

Figures 5, 6, and 7 illustrate the scavenging activity of *Cynodon dactylon* extracts and reference standards against nitric oxide (NO), hydrogen peroxide (H_2O_2), and DPPH radicals, respectively, at varying concentrations. The results demonstrate a dose-dependent increase in radical inhibition across all assays, with higher extract concentrations correlating to greater antioxidant activity.

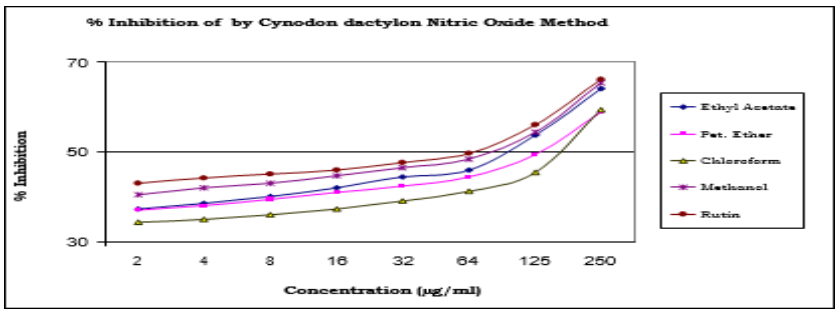


***Fig: 5* . The application of the nitric oxide method resulted in varying degrees of inhibition for the various Cynodon dactylon extract**

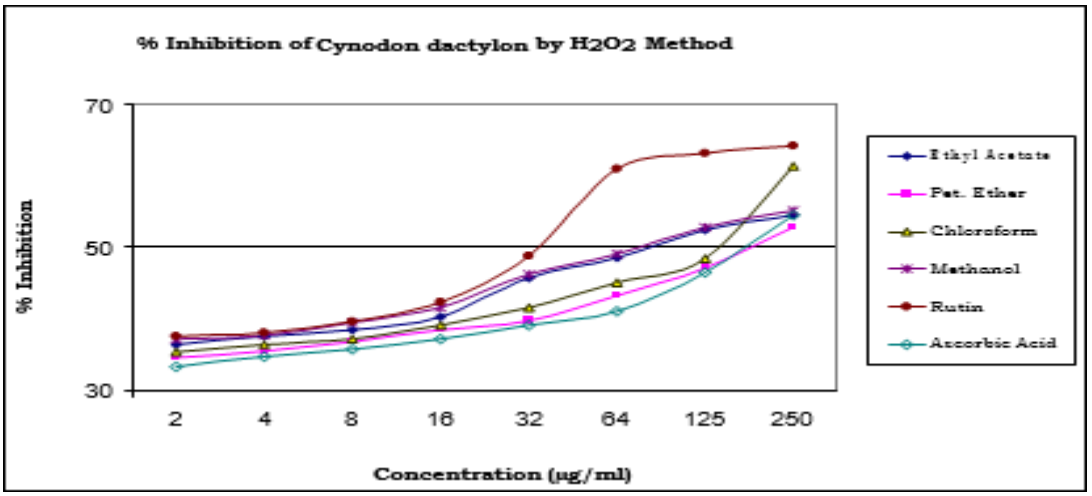


Fig: 6 Using Hydrogen Peroxide to Determine an Approximate Value for the Proportional Inhibition of Cynodon dactylon Extractives

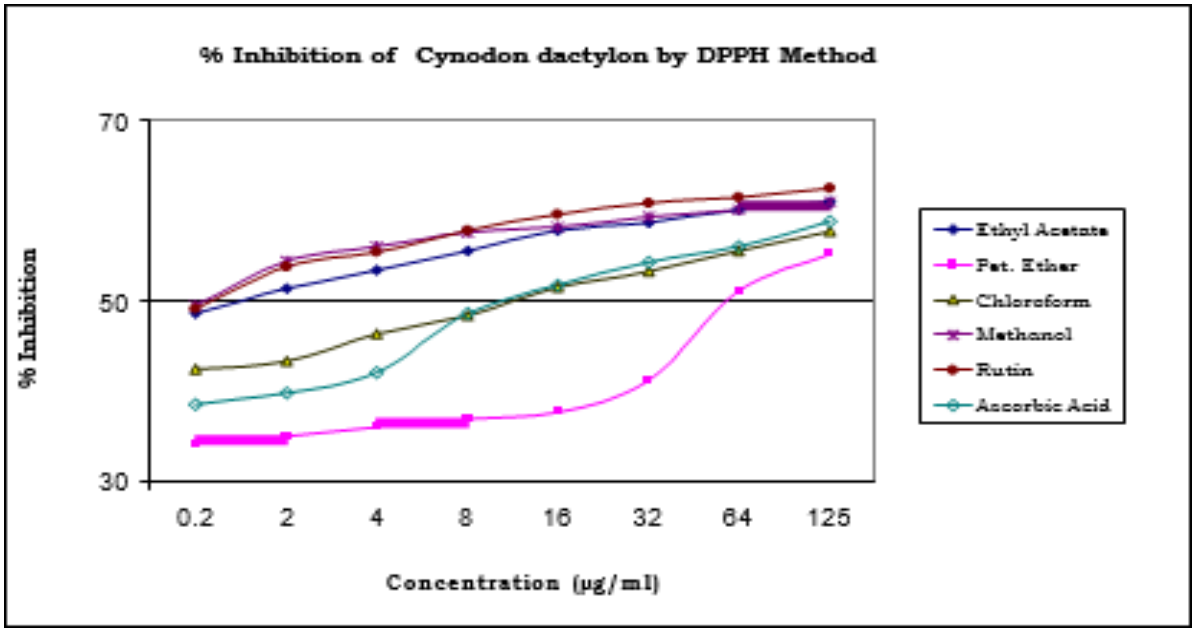


Fig: 7 The DPPH method for assessing the cancer-preventing potential of Cynodon dactylon samples

Cold Water Immersion Stress Ulcer Model

Exposure to cold water immersion (up to the xiphoid process for 18 hours) induced gastric lesions in rats by rapidly compromising the stomach lining. This study evaluated the anti-ulcer and antioxidant effects of a *Rhodomyrtus tomentosa* and *Cynodon dactylon* mixture compared to esomeprazole in this model.

Table 5 Effect of Cold-Water Immersion Stress on Gastric Ulcer Parameters

Groups	Number of Animals (n)	Ulcer Length (mm)	pH of Gastric Juice	Volume of Gastric Juice (cm^3)
Control group	6	7.90 ± 0.40*	5.70 ± 0.82	4.67 ± 0.54
Standard group (Esomeprazole)	6	2.60 ± 0.85	7.58 ± 0.54***	2.80 ± 0.18***
Rhodomyrtus tomentosa & Cynodon dactylon (200 mg/kg b.w.)	6	4.50 ± 0.16	6.60 ± 0.31**	4.30 ± 0.38
Rhodomyrtus tomentosa & Cynodon dactylon (400 mg/kg b.w.)	6	3.60 ± 0.25**	7.50 ± 0.36	2.00 ± 0.65**

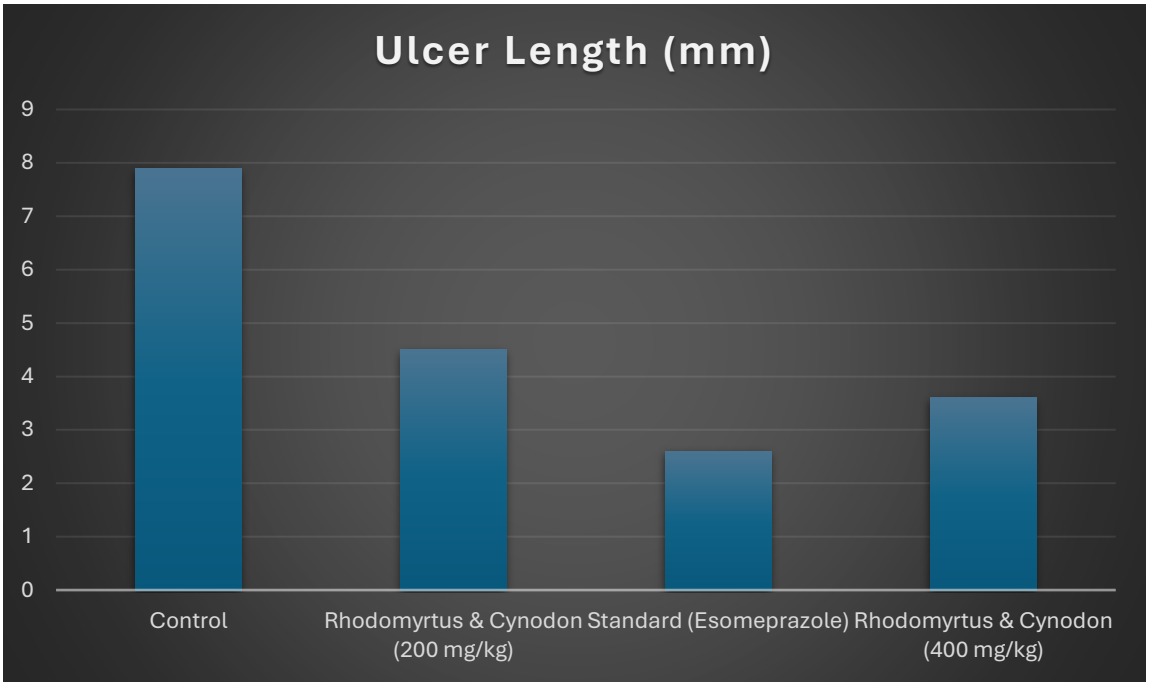


Fig: 8 : Effect of *Rhodomyrtus tomentosa* and Cynodon dactylon on gastric ulcer length in cold water immersion stress ulcer model

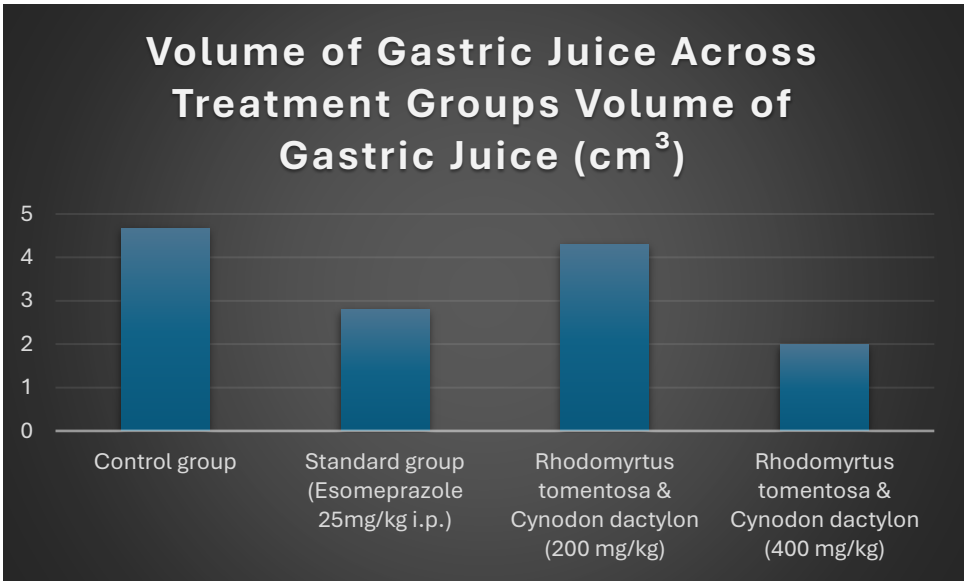
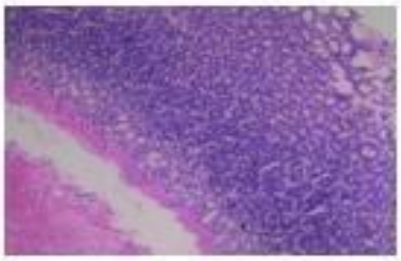
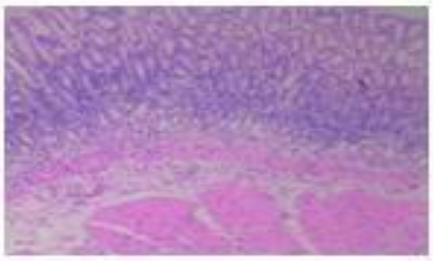


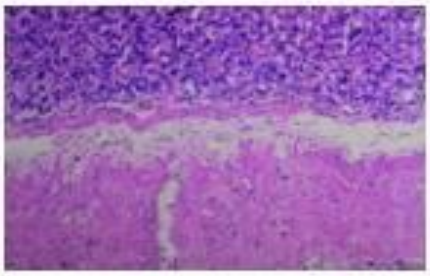
Fig: 9 Effect of *Rhodomyrtus tomentosa* and Cynodon dactylon powder in volume of gastric juice in cold water immersion stress ulcer model



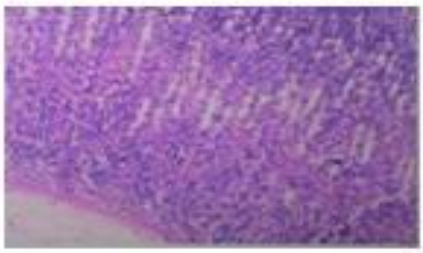
A. Control group (Normal saline)



B. Standard group (Esomeprazole) 25mg/kg



C *Rhodomyrtus tomentosa* and Cynodon dactylon powder 200mg/kg b.w p.o



D. *Rhodomyrtus tomentosa* and Cynodon dactylon powder 400mg/kg b.w p.o

Fig: 10 Photomicrographs showing Hematoxylin and Eosin-stained segments of rat stomach in cold water immersion stress ulcer model

Histopathological observation of stomach tissues after the treatment with *Rhodomyrtus tomentosa and Cynodon dactylon* powder has distinct effects on the severity of gastrointestinal lesions (gross inspection) in a cold-water immersion model: (A) Control group (Normal saline), severe lesions with extensive visible hemorrhagic necrosis of the gastric mucosa. (B) Standard group (Esomeprazole), architecture of mucosal lining is intact, no epithelial damage and ulcers were seen. (C) *Rhodomyrtus tomentosa and Cynodon dactylon* powder (200mg/kg b.w), mild lesions of the stomach mucosa when compared to the control group. (D) *Rhodomyrtus tomentosa and Cynodon dactylon* (400mg/kg b.w), abscess and very mild lesions

8. CONCLUSION

Peptic ulcer disease, a prevalent gastrointestinal disorder, is characterized by recurrent episodes and significant complications. While conventional treatments like proton pump inhibitors (PPIs, e.g., esomeprazole) are widely used, their high cost and adverse effects (e.g., tissue inflammation and oxidative damage) necessitate safer alternatives. This study evaluates the gastroprotective potential of *Rhodomyrtus tomentosa* and *Cynodon dactylon* leaf extracts against cold-water immersion-induced ulcers in rats—a model that mimics cold stress–induced mucosal permeability, vasoactive mediator release, and subsequent lesion formation. Recent research highlights natural compounds as promising anti-ulcer agents due to their antioxidant and cytoprotective properties. Here, we compare the efficacy of these botanicals with esomeprazole, focusing on their ability to mitigate gastric lesions and oxidative stress. Histopathological findings confirm that cold exposure rapidly compromises the gastric mucosa, validating the model's utility for testing protective interventions.

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