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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ZIZIPHUS RUGOSA LEAVES FOR GASTROPROTECTIVE BENEFITS

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ABSTRACT

This study investigates the phytochemical composition and gastroprotective potential of Ziziphus rugosa leaves, emphasizing its antioxidant and antiulcer activities. Ethanolic extraction yielded the highest recovery of bioactive compounds (8.0%), rich in flavonoids (93.36 mg QE/g) and phenolics (493.42 mg GAE/g). Phytochemical screening confirmed the presence of tannins, saponins, alkaloids, glycosides, and steroids. Antioxidant assays (DPPH, ABTS) demonstrated strong radical inhibition, particularly at higher concentrations. In vitro antiulcer screening showed significant acid-neutralizing capacity and potent inhibition of H^+/K^+-ATPase activity. The ethanolic extract displayed dose-dependent proton pump inhibition with a maximum of 62.18% at 100 µg/mL, closely comparable to omeprazole (69.56%). Acid-neutralization assays further supported the extract's antacid-like properties. These effects are likely mediated through antioxidant mechanisms and direct modulation of proton pump Activity. The findings strongly support the traditional use of Z. rugosa in managing gastric disorders. The combined antioxidant, acid-neutralizing, and H^+/K^+-ATPase inhibitory effects of its phytoconstituents suggest its promising potential as a natural gastroprotective agent. Further investigations into its mechanism of action and bioactive constituents are warranted to develop safe, plant-based antiulcer therapies.

KEYWORDS: Ziziphus rugosa, gastroprotection, H^+/K^+-ATPase inhibition, antiulcer activity etc.

INTRODUCTION

Humans and animals have relied on plant, animal and mineral sources for medicine. Medicinal plants play a dominant role in large portion of world's population. Ancient manuscripts of Indian, Unani, Papyrus from Egyptian and Chinese herbal book writings represented the utilization of herbs as curative agents. The use of medicinal plants was also described in Rig-Veda as the main source of medications used to treat a variety of illnesses.[1] Nearly 80% of people worldwide use herbal medicines, especially in underdeveloped countries where they constitute an important part of primary healthcare. Western nations have shown an increasing interest in plant-based therapeutic products in recent years. Growing knowledge of the possible long-term health hazards connected to synthetic medications is a major factor driving this trend.[2] India is rich in medicinal plant resources, many of which are commonly used by local communities as traditional remedies. These plants also serve as vital components in various indigenous medical systems, either in their natural form or as part of processed formulations for modern treatments. Globally, around 422,000 species of flowering plants have been documented, with over 50,000 of them recognized for their medicinal value.[3]

From the various literature surveys, the medicinal plants are proven to be very effective in treating many diseases but yet failed to show effective antiulcer activity. Though there are many medicinal plants exist, Ziziphus rugosa have more importance than that of other plants due to its wide range of ethno medical action and pharmacological action. It is found from the literature survey that this plant was not screened for its gastroprotective mainly anti-ulcer activity. Hence, we selected this plant for present research work.

Plant profile

The genus Ziziphus, belonging to the family Rhamnaceae, comprises around 100 species of deciduous and evergreen shrubs or small to medium-sized trees. Around the world, these species are frequently found in tropical and subtropical areas. Notable species within this genus include Ziziphus jujuba, Ziziphus mauritiana, Ziziphus rugosa, and Ziziphus oenoplia. Members of this genus can be erect or sprawling, and many exhibit climbing behaviour. They may be either evergreen or deciduous and are often characterized by the presence of spines. The leaves are alternate and stalked, with a distinctive pattern of three prominent veins. Stipules are typically modified into one or two spines, either upright or curved. The small, yellowish-green flowers are bisexual and arranged in axillary or terminal inflorescences such

as racemes or thyrses. The fruit is a drupe with a fleshy or corky middle layer (mesocarp), and a woody or cartilaginous stone (endocarp), typically containing a single seed.[4]

Taxonomical details of Ziziphus rugosa

Kingdom: Plantae

Class: Dicotyledonae

Sub-Class: Polypetalous

Series: Disiflorae

Order: Rosales

Family: Rhamnaceae

Genus: Ziziphus (Zizyphus)

Species: rugosa Lam.

Figure 1: Whole Fresh Plant of Ziziphus rugosa.

Local and Common names of Ziziphus rugosa Plant

Hindi: Ber, khat-bor. Kothamullu.

Telugu: Pindiparimi, Pinduparighamu.

Kannada: Bela hudukee gida, Belahadu kina, Bommaralu, Bommarlu, Chotte,

Tamil: Avicuppam, Avucucikacceti, Avucucikam, Kattilantai.[5]

Botanical Overview

Ziziphus rugosa is a moderately sized tree that typically reaches a height of about 10 to 15 meters.[6]

Geographical Distribution

Ziziphus rugosa is commonly found throughout tropical and subtropical regions of Asia. It grows best in moist, well-drained soil and is typically seen along riverbanks, in forest openings, and at the edges of tropical woodlands. This plant thrives in warm environments and is capable of growing from lowland regions to altitudes reaching around 1,500 meters[7] Traditionally, the fruit has been used for managing tumours and is believed to possess sedative, hepatoprotective, blood-purifying, and cardiotonic properties in regions like Sylhet. In India, a bark decoction is commonly applied to wounds and used to treat diarrhoea. Additionally, a mixture of the plant's Menorrhagia can be managed using leaves and flowers. Ziziphus rugosa has also traditionally been used to treat a range of diseases, including skin illnesses, it has been traditionally used to address various health conditions such as mouth sores, water retention, skin boils, digestive issues like diarrhea and flatulence, rapid heartbeat, syphilis, miscarriage, hysteria, and also functions as a natural astringent.[8]

Phytochemical Constituents

Extensive research on the phytochemical composition of Ziziphus rugosa has revealed a diverse array of bioactive compounds, including the plant is rich in several bioactive compounds, including flavonoids, tannins, glycosides, terpenes, steroids, saponins, and cyclopeptide alkaloids The existence of particular cyclopeptide alkaloids such as sativanine-H, rogosanine-A, rugosanine-B, and nummularine-P was established through root bark analysis. The plant is a rich source of pentacyclic triterpenoids, including compounds such as betulinic acid, betulinaldehyde, oleanolic acid, alphitolic acid, 2-α-hydroxyursolic acid, and lupeol. It also contains various flavonoids, such as kaempferol, quercetin, myricetin-3-O-rhamnoside, apigenin, and its glycosylated form, apigenin-7-O-glucoside, and vanillic acid (a dihydroxybenzoic acid). Additionally, compounds such as rugoside, zizyphoside, β-sitosterol, and β-sitosterol glycoside have been identified in the root bark.[9,10]

Antioxidants

Free radicals are highly reactive, short-lived molecules generated during oxidation processes. These unstable molecules can trigger chain reactions that damage cellular structures. Oxygen, being a highly reactive element, often contributes significantly to the development of these

dangerous species. Free radicals stabilize themselves by capturing electrons from surrounding molecules, which can disrupt the integrity and function of healthy cells. This oxidative damage is believed to be a major contributor to aging and the progression of several degenerative disorders, such as cancer and heart-related diseases, visual disorders, immune system decline, and potential impairments in brain function.[11]

Ulcers

Ulcers are open lesions that form on the surface of the skin or mucous membranes, typically involving the loss of the epithelial layer. In the digestive system, ulcers most commonly form in the top section of the small intestine (duodenal ulcers) or the stomach (gastric ulcers). These are collectively referred to as peptic ulcers. Common symptoms include mild to severe upper abdominal pain, often accompanied by a burning sensation. Other associated signs may include acid reflux (heartburn), a feeling of fullness or bloating, as well as more serious complications like gastrointestinal bleeding, obstruction, or perforation.[12] In 2012, the United States reported approximately 435,000 hospital 796,000 ED visits and hospitalizations pertaining to the gastrointestinal tract injuries, representing about 1% of all gastrointestinal emergency cases recorded that year.[13] Ulcers usually form due to a disruption in the natural balance between aggressive factors and the protective mechanisms of the gastrointestinal lining. This imbalance may stem from increased harmful agents or a weakened mucosal defense. Currently, peptic ulcer treatment focuses on two main approaches: reducing gastric acid production and enhancing the protection of the stomach lining against damaging factors. Serious side effects, including internal bleeding, perforation, tissue spread, and even an elevated risk of cancer, can result from poorly treated ulcers.[14,15]

REVIEW OF LITERATURE

1. **Mabuza M. et al., 2025** assessed the in vitro antiplasmodial activity and cytotoxicity of Z. mucronata, Z. rivularis, and Z. zeyheriana. It further identified antiplasmodial constituents using ^{1}H NMR-based metabolomics and GC-MS analyses. The study confirmed the significant antiplasmodial activity of Z. mucronata, and for the first time, it reported the antiplasmodial activity of Z. rivularis and Z. zeyheriana. It further demonstrated that the tested samples have no apparent cytotoxicity.[16]

2. **Agrawalet P. al., 2024** examine the anxiolytic properties of the ethanolic extract of Ziziphus mauritiana (EEZM) in several behavioral paradigms using Swiss albino mice as experimental subjects. The findings of this study indicate that the administration of Z.

mauritiana has an anxiolytic effect on mice. Its properties as a novel therapeutic option for the management of anxiety in humans deserve analysis.[17]

3. **Yu C. et al 2022** explored the chemical constitutes, gastroprotective effects, and the active fraction of C. fimbriata, as well as elucidating the underlying mechanisms. Firstly, four in vitro antioxidant tests were applied to determine the oxidation resistance of C. fimbriata methanol extract and its fractions. The results indicated that CfEF is a promising source of gastroprotective agents. The antioxidant activity of this herb, as well as prevention of gastrin secretion and inhibition of H+K+ -ATPase, was found to be the underlying mechanism of action.[18]

4. **Figueiredo F. et al., 2022** investigate the F. chica hydroethanolic extract of leaves (HEFc) preventative and curative antiulcer gastrointestinal efficacy as well as the mechanisms of action using in vivo rodent models. These outcomes confirmed the advantages of Fridericia chica leaves for the treatment of stomach ulcers, which are well-known.[19]

5. **Qiang F. et al., 2017** isolated two new C-glucosyl flavonoids 6″-O-feruloylspinosin and 6″-O-feruloyl-6‴-p-hydroxybenzoylspinosin, together with five known compounds, from the seeds of Ziziphus jujube (Rhamnaceae family). Their structures were elucidated on the basis of chemical and spectroscopic evidences. Compounds 1–7 showed moderate inhibitory effects against COX-1 and COX-2 enzymes.[20]

6. **Prashith K. et al., 2011** determined antibacterial, insecticidal and free radical scavenging activity of methanol extract of Ziziphus rugosa Lam. fruit pericarp. The phytochemical analysis of extract showed the presence of alkaloids, saponins, flavonoids and glycosides. The extract, in suitable form, may be used to control bacterial diseases, free radical damage and arboviral diseases. The phytoconstituents present in the extract may be responsible for the tested biological efficacies of extract. Further studies on isolation of active constituents from the extract and their biological activity are under investigation.[21]

MATERIALS AND METHODS

Collection and Confirmation of Plant Material Identity

Ziziphus rugosa, the botanical specimen utilized in this research, was chosen because of its documented pharmacological properties and traditional medicinal uses. Twigs were gathered from forested areas in the Sindhudurg district of Maharashtra, India. The Dep. Of Botany at

Anandibai Raorane College of Arts, Commerce, and Science, Vaibhavwadi, verified the plants identify. For documentation and future use, a reference specimen has been kept in the college herbarium.

Chemicals and Reagents

The study employed chemicals and solvents of analytical grade standard. The sources of methanol, butanol, and ethyl acetate were approved chemical suppliers. Additional reagents needed for phytochemical testing were purchased from HI Media Laboratories Pvt., including bovine serum albumin, aluminum chloride, DPPH, Folin–Ciocalteu reagent, and DNSA reagent.

Extraction Procedures

A sequential Soxhlet extraction using solvents of varying polarity is a reliable approach for isolating a broad spectrum of phytoconstituents from Ziziphus rugosa leaves. This gradient extraction technique ensures effective isolation of compounds ranging from non-polar to highly polar in nature. Initially, healthy and mature leaves of Ziziphus rugosa are collected, thoroughly rinsed with water, and dried under shade for approximately 7 to 10 days to preserve heat-sensitive constituents. The fully dried leaves are mechanically crushed into coarse powder and stored in sealed containers to protect against moisture. For extraction, roughly 50 grams of the ground plant material is packed in filter paper, placed in an extraction thimble, and positioned in the Soxhlet unit's central compartment. The extraction is then carried out in a stepwise manner using solvents with increasing polarity to ensure a comprehensive recovery of bioactive phytochemicals.[22]

Phytochemical Screening

Table no. 1: Chemical test used for phytochemical screening.

Metabolites	Test	Procedure
Alkaloids	Dragendorff's Test	To 2 ml of extract add few drops of Dragendorff's reagent. Alkaloids give orange brown precipitates.
Glycosides	Borntrager's Test	Add 5-10 ml of hydrochloric acid to 1 gm of the crude drug and let it sit for 2-3 minutes. Filter the mixture and cool the filtrate. Add an equal volume of benzene or carbon tetrachloride into the filtrate and shake it well. Keep this mixture aside until the benzene layer separates. Remove the benzene layer and add 10% amount of ammonium solution to the filtrate. Shake the mixture well and allow it to have the ammoniacal layer to separate and settle at the bottom. The ammonical layer

		turns pink or red indicating the presence of anthroquinone glycoside in the crude drug.
Flavonoids	Shinoda Test	Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow color appeared but it gradually became colorless by adding few drops of dilute HCL, indicating that flavonoids were present.
Tannins and Phenolics	FeCl3 Test	Add 5% FeCl3 solution to the extract. Development of deep blue-black color shows presence of tannins and phenolics.
Coumarins	Fluorescence Test	The extract when made alkaline shows blue or green fluorescence if Coumarins are present.
Saponins	Foam Test	Shake the extract vigorously with water. Persistent foam indicates presence of saponins
Amino Acids	Ninhydrin Test	To 3 ml of test solution add 3 drops of 5% ninhydrin solution and heat in a boiling water bath for 10 min. appearance of purple or bluish color shows presence of amino acids.
Protein	Biuret Test	To 3 ml of aq. extract add 4% NaOH solution and few drops of 1% CuSO4 solution. Appearance of violet or pink color indicates presence of protein.
Carbohydrates	Molisch's Test	To 2-3 ml of aq. extract add few drops of alpha naphthol solution in alcohol, shake and add conc. H_2SO_4 from sides of the test tube. A violet ring formed at the junction of two liquids indicates presence of carbohydrates

Calculating The Total Phenolic and Flavonoid

Total flavonoid content (TFC) was assessed using a colorimetric assay with aluminum chloride ($AlCl_3$), employing quercetin as the calibration standard. Meanwhile, total phenolic content (TPC) was estimated using the Folin–Ciocalteu method, with gallic acid used as the standard reference compound. Absorbance readings were recorded with a UV-visible spectrophotometer at 415 nm for flavonoids and 765 nm for phenolic compounds.[23]

Antioxidant Activity

1. DPPH Free Radical Scavenging Assay

The antioxidant activity of the ethanol extract of Ziziphus rugosa (ZREE) was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. In this procedure, 1 mL of a 0.1 mM DPPH solution in methanol was mixed with 1 mL of the extract at concentrations ranging from 25 to 125 µg/mL. The mixture was incubated in the dark at room temperature for 30 minutes to allow the reaction to proceed. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, with ascorbic acid serving as the standard. The percentage of radical scavenging activity was calculated using a suitable formula.

% Scavenging = $[(A_0 - A_1) / A_0] \times 100$

"In this formula, A_0 represents the absorbance of the control solution, while A_1 denotes the absorbance measured for the sample containing the plant extract."

2. Assay for ABTS Radical Cation Decolorization

The $ABTS^+$ radical cation was produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate, followed by incubation in the dark at room temperature for approximately 12 to 16 hours. The resulting solution was then diluted with ethanol until its absorbance at 734 nm reached 0.70 ± 0.02. Subsequently, 1 mL of the $ABTS^+$ solution was mixed with varying concentrations (25–500 µg/mL) of the ethanol extract of Ziziphus rugosa.Following a 6-minute incubation period, absorbance was measured at 734 nm using a UV-Vis spectrophotometer. Trolox was used as the reference antioxidant for comparative analysis.[24]

Assay for Acid Neutralizing Capacity (ANC)

The ability to neutralize acid (ANC) of the ethanolic extract of Ziziphus rugosa bark (ZREE) was evaluated using a titrimetric method adapted from the Indian Pharmacopoeia with slight modifications. This method simulates gastric conditions to assess the extract's ability to neutralize hydrochloric acid. For the test, 1 gram of ZREE are accurately A precise amount of the sample was weighed and combined with 100 mL of distilled water. Then, 30 mL of 0.1 N hydrochloric acid was added, and the mixture was stirred continuously for 1 hour at 37 ± 1°C using a magnetic stirrer. Following incubation, the residual free acid was the solution was then titrated with 0.1 N sodium hydroxide using phenolphthalein as the indicator. The endpoint was identified by the development of a faint pink hue that persisted for a minimum of 30 seconds. A blank titration was conducted under identical conditions without the extract. A standard Antacids were utilized as positive controls, with distilled water serving as negative controls. The acid neutralizing capability was then estimated using the following formula:

$$\mathrm{ANC} = (V_b - V_s) \times N \times 1000$$

Where:

- V_b = volume (in mL) of sodium hydroxide used in the blank titration
- V_s = volume (in mL) of sodium hydroxide consumed during the sample titration)

- N = normality of the sodium hydroxide solution
- ANC is expressed in terms of mEq of HCl neutralized per gram of extract.[25]

RESULTS AND DISCUSSION

Plant Material Selection, Authentication & Procurement

The plant material was collected from Maharashtra's Sindhudurg district and authenticated by a renowned botanist and Associate Professor at A.R. College of Vaibhavwadi.

Phytochemical analysis from previous literature indicates that Ziziphus rugosa includes bioactive substances such sterols, flavonoids, tannins, and saponins. These compounds are recognized for their roles in wound healing and gastrointestinal protection, primarily due to their antioxidant and antiulcer effects. Given these attributes, the twigs of Ziziphus rugosa were selected for further investigation into their antioxidant and antiulcer potential.

Plant Extracts preparation

In the current study, sequential extraction of Z. rugosa leaf powder (50 grams) was performed using solvents in order of increasing polarity: water, ethanol, petroleum ether, and chloroform. Each extract's yield % was determined. And is presented in the following Table. The ethanol extract yielded the highest at 9.55%, followed by petroleum ether (6.84%), chloroform (4.37%), and water (3.70%). The higher yield obtained with ethanol suggests a richer presence of polyphenolic compounds This solvent extract contains flavonoids, phenols, and tannins.

Table no. 2 physical nature of the extracts.

Sr. No	Solvent	Physical nature	Color	Yield (%)
1.	Petroleum Ether	Sticky	Yellowish	0.5
2.	Chloroform	Partially sticky	Yellowish green	1.2
3.	Ethanol	Hygroscopic Powder	Dark brown	8.0
4.	Aqueous	Sticky	Brown	2.5

Qualitative Phytochemical Evaluation

The qualitative phytochemical analysis of various Ziziphus rugosa leaf extracts revealed that the ethanolic extract tested positive for all the phytoconstituents examined. In contrast, the chloroform extract lacked both tannins and steroids. The aqueous extract indicated the presence of several key Compounds include phenols The presence of compounds such as alkaloids, glycosides, flavonoids, tannins, sugars, and saponins was confirmed. A comprehensive summary of the results is presented in Table no.3

Table no. 3: Phytochemical test results of the various extracts of Ziziphus rugosa leaves.

Phytochemicals	Petroleum ether extract	Chloroform extract	Ethanol extract	Water extract
Alkaloids	++	++	++	++
Glycosides	+	+	+	++
Flavonoids	+	+	++	+
Tannins	-	-	++	+
Carbohydrates	+	+	++	++
Saponins	+	+	++	++
Terpenoids	+	+	++	-
Steroids	+	+	++	-
Phenols	-	-	++	++

Where, + shows presence, ++ strongly confirms and– shows the absence of phytochemicals.

Total flavonoid content

Flavonoid levels in the leaf extracts of Ziziphus rugosa were assessed using different solvents and expressed in terms of quercetin equivalents (mg QE/g). Among the extracts, the ethanol-based sample exhibited the highest flavonoid content at 91.24 ± 4.62 mg QE/g, followed by the chloroform extract with 81.8 ± 3.32 mg QE/g, and the aqueous extract with 76.37 ± 1.45 mg QE/g. A detailed summary of these results is shown in Table no 4.

Total phenolic content

The extract of Ziziphus rugosa leaves in various solvents were estimated. for phenolic content and reported. Ethanolic extract of Ziziphus rugosa leaves showed 493.42± 8.23 mgGAEg−1 phenolic content, The aqueous extract showed a total phenolic content of 92.75 ± 8.03 mg GAE/g, whereas the chloroform extract exhibited comparatively lower value 24.7± 2.60 mgGAEg−1, as indicated in Table no. 4.

Table no. 4 Total flavonoid and phenolic contents of the extracts of Ziziphus rugosa leaves.

Extracts	Total Flavonoid Content (mgQE/gram)	Total Phenolic Content (mgGAE/gram)
Ether	80.77	30.22±1.44
Chloroform	79.6±3.25	21.7±1.42
Ethanol	93.36±2.13	52.43±4.63
Water	74.62±1.93	93.54±6.04

In-vitro Antioxidant activity

A Leaf extract of Ziziphus rugosa prepared using ethanol (ZREE) was tested for antioxidant capability utilizing DPPH, ABTS, hydrogen peroxide (H_2O_2), and nitric oxide (NO) radical

scavenging tests. All of the methods evaluated showed that the extract had considerable free radical scavenging properties. ZREE inhibited DPPH radicals by 55.84% at 125 µg/mL and scavenged nitric oxides at 500 µg/mL. The extract demonstrated dose-dependent antioxidant activity in ABTS and H_2O_2 tests, as shown in Figure no. 2

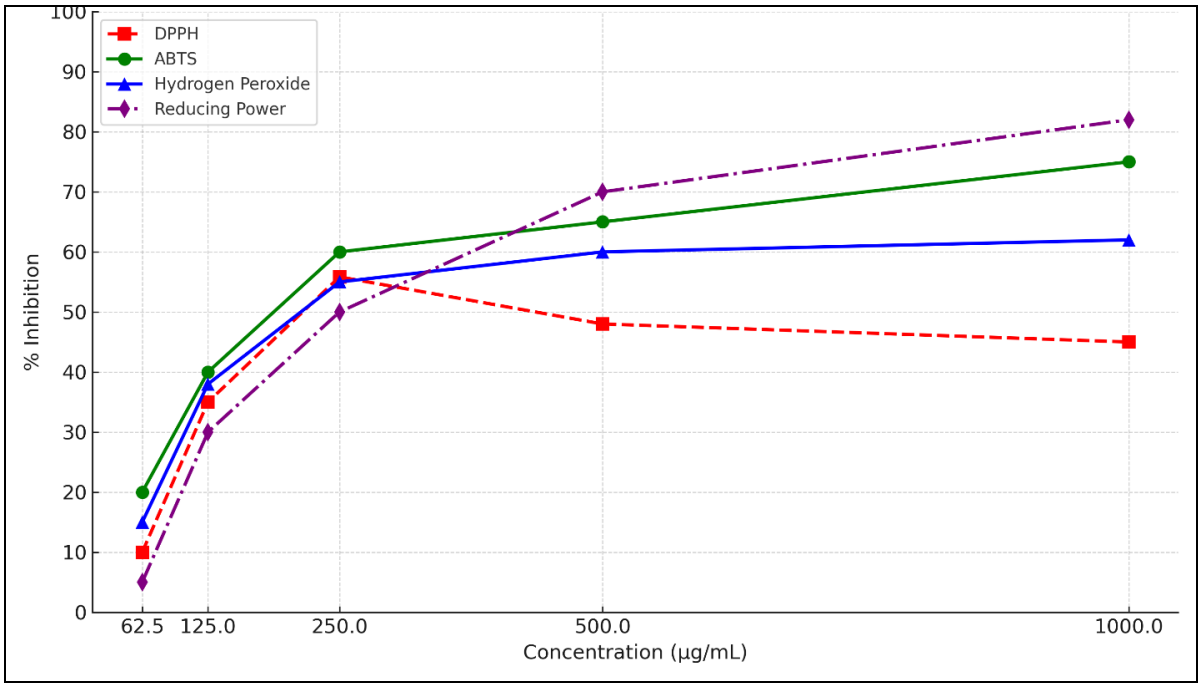

Figure 2: Antioxidant activity of ZREE.

In-vitro Evaluation of Antiulcer Activity

Acid Neutralizing Capacity

To assess the acid-neutralizing potential of the ethanolic extract, varying doses (100 mg, 500 mg, 1000 mg, and 1500 mg) were evaluated. For comparison, a standard antacid formulation containing 500 mg of aluminum and magnesium hydroxides was used. Each test and control sample were brought to a total volume of 70 mL, with 5 mL of the sample extract and the remaining volume adjusted using distilled water. The solution was gently swirled for one minute before further analysis.

Next, 30 mL of 1.0 N hydrochloric acid (HCl) was added to both the test sample and standard solutions, and the mixtures were stirred thoroughly for 15 minutes. A few drops of phenolphthalein were then introduced as an indicator, and the solutions were immediately titrated with 0.5 N sodium hydroxide (NaOH) until a consistent pale pink color signified the endpoint. To determine the number of moles of hydrochloric acid neutralized, subtract the

product of the volume and normality of sodium hydroxide used from the product of the volume and normality of hydrochloric acid added.

Table no. 5: Effect of Ethanolic Extract of on Acid Neutralizing Capacity.

Sr. No.	Concentration (mg)	Volume of NaOH Consumed (ml)	mEq of Acid Consumed	ANC per gram of Antacid
1	100	36.8	12.05	120.50
2	500	28.4	17.56	35.12
3	1000	38.6	8.68	8.68
4	1500	43.0	11.55	7.70
5	500 mg $Al(OH)_3$ + $Mg(OH)_2$	47.3	6.78	13.56

H+/K+ - ATPase Inhibition Activity

The inhibitory potential of the ethanolic extract on H^+/K^+-ATPase activity was evaluated across varying concentrations of 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg and compared with Omeprazole, a standard proton pump inhibitor. The extract demonstrated significant dose-dependent inhibition of enzyme activity. The highest inhibitory effect was recorded at 100 µg, showing 62.18 ± 0.54% inhibition, while Omeprazole exhibited 69.56 ± 1.72% inhibition at the same concentration. These findings are presented in Table no. 6.

Table no. 6 Effect of Ethanolic Extract of on In-Vitro H+/K+ - ATPase Inhibition Activity.

S. No.	Concentration (µg)	Standard Omeprazole (%)	Ethanolic Extract (%)
1	20	-51.25 ± 0.78	-30.12 ± 0.26
2	40	-56.32 ± 1.24	-18.48 ± 1.86
3	60	36.58 ± 1.58	31.64 ± 0.68
4	80	58.62 ± 0.24	55.36 ± 1.54
5	100	69.56 ± 1.72	62.18 ± 0.54

CONCLUSION

The objective of this study was to investigate the potential into the phytochemical composition and evaluate the in-vitro antioxidant and antiulcer potential of Ziziphus rugosa leaves, with a particular emphasis on the ethanolic and aqueous extracts. The findings provide strong scientific support for the traditional use of this plant in managing gastrointestinal issues and conditions linked to oxidative stress.

Preliminary phytochemical analysis confirmed that Ziziphus rugosa leaves are rich in biologically active constituents, including flavonoids, phenols, tannins, alkaloids, glycosides, and saponins—all of which are associated with potent antioxidant and gastroprotective

effects. Of all the extracts evaluated, the ethanolic extract demonstrated the greatest yield and the richest phytochemical profile. It contained a total flavonoid concentration of 93.36 ± 2.13 mg QE/g and a phenolic content of 52.43 ± 4.63 mg GAE/g.

The ethanolic extract was tested in four in-vitro antioxidant assays: DPPH, ABTS, and it shown considerable dose-dependent activity in all techniques. At 125 µg/mL, the extract showed 55.84% DPPH scavenging activity, with the maximum nitric oxide inhibition at 500 µg/mL. These effects can be related to the abundance of phenolics and flavonoids, particularly quercetin, which are recognized for their ability to neutralize free radicals.

Additionally, the aqueous extract was tested for its antiulcer potential Using assays for both acid-neutralizing capacity (ANC), the extract's potential for reducing gastric acidity was evaluated. The ANC evaluation indicated a dose-dependent neutralization of hydrochloric acid, with a value of 7.70 mEq/g at 1500 mg concentration. Although this was slightly lower than the standard antacid formulation (aluminium and magnesium hydroxides), it still reflects notable antacid potential, suggesting that the extract can help buffer gastric acidity and protect the stomach lining.

These promising in-vitro findings clearly indicate that Ziziphus rugosa leaves possesses strong antioxidant and antiulcer properties.

In conclusion, the research confirms that Ziziphus rugosa leaves is a potent natural source of antioxidants and exhibits significant antiulcer activity through both acid neutralization and proton pump inhibition. These findings lend strong scientific This lends credibility to the plant's traditional usage in folk medicine to cure ulcers and oxidative damage. With additional research and confirmation, this plant has the potential to become a safe and effective natural treatment agent for oxidative stress-related stomach illnesses.

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