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Research

Investigation of *In-Vitro* Anti-Ulcer Activity of Whole Plant Methanolic Extract of Ficus Religiosa

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Abstract:

Background & Objectives: The objective of the present study was to evaluate the *in-vitro* anti-ulcer potential of the methanolic extract of *Ficus religiosa Linn.* through two key mechanisms — Acid Neutralizing Capacity (ANC) and H+/K+-ATPase inhibition assay — to determine its ability to neutralize gastric acid and inhibit proton pump activity.

Methods and Results:

Acid Neutralizing Capacity (ANC) Assay: The acid-neutralizing capacity of the methanolic extract of *Ficus religiosa* was evaluated at different concentrations (100–1500 mg) and compared with a standard antacid preparation containing Aluminium hydroxide and Magnesium hydroxide [$Al(OH)_3$ + $Mg(OH)_2$]. The extract exhibited a concentration-dependent increase in acid neutralization, as indicated by the progressive increase in the volume of NaOH consumed with higher doses. At 100 mg, the extract neutralized 1.0 mEq of acid with an ANC of 10.0 mmol/g. Increasing the concentration to 500 mg resulted in the neutralization of 2.4 mEq of acid (ANC 4.8 mmol/g). Further enhancement was observed at 1000 mg, which neutralized 4.4 mEq of acid with an ANC of 4.4 mmol/g. The highest concentration tested, 1500 mg, showed the maximum acid-neutralizing effect by neutralizing 7.2 mEq of acid (ANC 4.8 mmol/g), indicating superior antacid activity among the extract doses. The standard antacid (500 mg) neutralized 7.6 mEq of acid with an ANC of 15.2 mmol/g, thereby validating the experimental procedure. Overall, the findings demonstrate that the methanolic extract of *Ficus religiosa* possesses significant acid-neutralizing potential, with the highest effectiveness observed at the 1500 mg concentration, supporting its potential use as a natural antacid agent.

H+/K+-ATPase Inhibition Assay: The aqueous extract of *Ficus religiosa* was evaluated for its ability to inhibit gastric proton pump activity at concentrations of 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg, using Omeprazole as the standard. The extract exhibited significant, dose-dependent inhibition, with a maximum inhibition of 65.27 ± 0.76% at 100 µg, compared to 70.14 ± 1.68% observed with Omeprazole.

Interpretation & Conclusion: The *in-vitro* findings indicate that *Ficus religiosa Linn.* exhibits significant anti-ulcer activity, achieved through both acid neutralization and inhibition of the gastric proton pump.

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These activities may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which contribute to mucosal protection and reduction of gastric acidity. Further *in-vivo* and clinical investigations are required to isolate the active compounds and validate their therapeutic efficacy and safety.

Keywords: *Ficus religiosa Linn* , Acid Neutralizing Capacity, H+/K+ -ATPase inhibition, Anti-ulcer activity, Phytochemicals, Gastroprotective effect.

INTRODUCTION

Gastrointestinal diseases are very serious and common problems, which are causing maximum discomfort, morbidity, and mobility in human beings. It occurs in 10-15% of the population at a time. A peptic ulcer is a group of disorders which is responsible for the ulcer formation or mucosal lesions formation in the esophageal lining (swallowing pipe), stomach or duodenum (the first part of the small intestine). The small sores are formed due to the imbalance between mucosal defensive factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (pepsin, Helicobacter pylori, NSAIDs gastric acid). Ulcer in thestomach is known as gastric ulcer while ulcer in the first part of the intestine is also known as aduodenal ulcer **[1].** Sometimes, people feel that upper abdominal pain-may increase after lunch or dinner, and sometimes people vomit materials which looks like coffee grounds,blood comes with stool, have black or tarry stools, all these symptoms cause severe abdominal pain. The gastric ulcer pain may increase with eating and we feel burning-like-sensation in our stomach. While the duodenal ulcer increases with improper sleep or waking up late at night and eating. These symptoms indicate the severe presence of a peptic ulcer in your body. When these types of symptoms are not controlled by the counter drug, then the patient may be referred to a specialist called a gastroenterologist **[2].**

The microbe Helicobacter pylori (H. pylori) plays a critical role in peptic ulcer disease, and eradication of this microbe can minimize the complication of this disease. Many studies reveal that more than half of the world's population is affected by chronic H. pylori infection which directly affects gastroduodenal mucosa. By using triple chemotherapy i.e histamine receptor antagonist, proton pump inhibitors and sequential regimen, management of this disease can be done. If the disease condition is severe, we proceed with the surgical approach for the treatment. In the absence of H. pylori infection and NSAIDs drugs, a different category of ulcers may occur which are Zollinger-Ellison syndrome, truly idiopathic ulcers, cushing ulcer and high dose upper abdominal radiotherapy that can also lead to a type of ulcer **[3]**. Nonsteroidal anti-inflammatory drugs (NSAIDs) are valuable agents in the treatment of various diseases like arthritis musculoskeletal disorders and inflammation, in a wide variety of clinical scenarios but these agents also cause peptic ulcer **[4]**. If NSAIDs are given in the presence of Helicobacter, pylori (infection) significantly increases the risk of peptic ulcer bleeding [5]. Mammalian stomach has an ability to secrete concentrated hydrochloric acid in a very large quantity as we know that proteolytic enzyme pepsin and gastric acid are required to initiate digestion. Gastric acid does play a very significant and important role in protein hydrolysis and other digestive processes **[6].**

This secreted acid plays an etiologic role in producing different forms of discomfort like esophageal and duodenal injury under different conditions. In response of physiological stimuli, human stomach produces/contains approximately 1 billion parietal cells which secrete hydrogen ions into the gastric lumen and the generation of these hydrogen ions is mediated by 3 pathways namely endocrine, paracrine, and neurocrine. By vagal postganglionic neurons, a neurocrine transmitter, acetylcholine is released which stimulates hydrogen ion generation directly via a parietal cell M3 muscarinic receptor. On parietal cells, Paracrine transmitter name Histamine binds with H2-specific receptors. In response to this, Adenylate cyclase is activated which increases adenosine 38,58-cyclic monophosphate (cAMP) levels, and subsequently stimulates the generation of hydrogen ions. Gastrin secretion from antral G-cell which follows the endocrine pathway and

stimulates the hydrogen ion secretion both directly or indirectly, in corpus and fundus,increases the stimulation of histamine secretion from entero chromaffin-like cells **[7, 8].**

Combination of these three pathways control and regulate hydrogen ion secretion. A negative feedback mechanism governs and controls both gastrin release and the return of acid secretion to the basal level. Enterochromaffin-like cells are also known as controller cells. Many studies indicate that under different physiological conditions, some of the other neurotransmitters, like galanin, pituitary adenylate cyclase– activating peptide, and vasoactive intestinal peptide (VIP), may play a very important role in regulating gastric acid secretion both directly or indirectly [9]. When gastric acid increases in our stomach, it causes mucosal damage and leads to the formation of gastric lesions. Gastric lesions are one of the most important tools for the determination of an antiulcer property of drug molecule because the size changes constitute useful information like if a drug is effective in the case of ulcer or not, and if the size of the lesion is small and less in number, then we can say that the prepared preparation is an effective anti ulcer agent **[10].** The measurement of gastric ulcer or gastric lesions is done after dissection of the stomach along its greater curvature from the rat and fixed on a plane board or transparent glass. After fixing it into board or glass, the gastric lesions are examined by microscopes like light or scanning microscope. Another examination method is performed by hand lens, though it is an old method of examination and ulcer investigation, later the size of lesions are measured. Nowadays, the stomach is also scanned by using the camera in ulcer investigation and later investigated by suitable or appropriate software programs like Scion, Image J and others. After examination, the investigator can calculate the ulcer index by different methods as per their vantage **[10, 11].** The choice of a particular model for the evaluation of the anti ulcer drug is difficult because every model has significant pros and cons. The choice of screening model is also influenced by local resources, study objective, the hypothesis being tested or researcher's questions. Preclinical experiments were carried out in in-vivo models.

Colours for all the figures in this review are prepared according to the guidelines of "Guidelines for preparing color figures for everyone including the colorblind" given by Robert Roskoski Jr. **[12].** In our review work, we have discussed different types of in-vivo and in-vitro screening model for the assessment of the antiulcer property of prepared preparation. We have also discussed the measurement of gastric lesions.

Ficus religiosa is one of the widely planted species of *Ficus* in the tropics, with various traditional applications. It belongs to the subgenus of *Urostigma* and is locally known as the Peepal tree (synonym: Pimpala). It is a large tree (**Figure 3**) and epiphytic when young, containing petioles of 5 to 10 cm in length, aspen-like lamina, sessile, and paired hypanthodia, with no male flowers and pedicellate or sessile [**13**]. Its bitter-sweet and acrid nature is the reason for its use as an astringent, refrigerant, purgative, aphrodisiac, and laxative [**14**]. The root bark is often used to treat stomatitis, ulcers, and other inflammatory conditions such as gout [**15**]. The laxative young fruit is known to promote digestion and treat vomiting [**16**]. The ripe fruit of this species is edible and commonly used in food preparation. The fruits are rich in antioxidants, minerals, and vitamins [**17**]. The leaves are usually applied to wounds, skin diseases, and scabies [**16**]. The leaves are also prepared as a tonic for ulcers and constipation [**15**]. The young shoots are purgative and are thus used in the treatment of various conditions, including urinary vaginal discharge, asthma, cracked foot, toothache, snake bite, pimples, otitis, sores, etc. [**14**].

Since there is no literature and documentation available regarding Anti-ulcer effect of extracts of the plant the present study was selected.

MATERIALS AND METHODS:

Preparation of extract:

The aerial part of plant was collected, washed, dried in shade and pulverized in a grinder-mixer to obtain a coarse powder and then passed through mesh sieve . The powder drug subjected to solvent extraction by Maceration process.

The percentage yield of the methanolic extract is given below in the table:

Table 02: The percentage yield of methanolic extract of ***Ficus religiosa Linn***

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Extract	Color	Consistency	Percentage yield
Methanolic 70%	Light Green	Solid	8.83%

Figure 05:Final methanolic extract of *Ficus religiosa*

Phytochemical Evaluation Methods:

Phytochemical screening is a preliminary step to detect the presence of different secondary metabolites in plants. These compounds include alkaloids, carbohydrates, saponins, phenols, flavonoids, proteins, tannins, terpenoids, steroids, and glycosides. Each class has specific tests that indicate its presence through a characteristic color change or precipitate formation.

Alkaloids test: can be detected by dissolving the extract in dilute hydrochloric acid, filtering, and testing with reagents such as Mayer's, Wagner's, Dragendorff's, and Hager's. The formation of a cream, reddish-brown, orange, or yellow precipitate indicates the presence of alkaloids.

Carbohydrates test: are identified using Molisch's test, which forms a violet ring at the junction of two layers, and Benedict's or Fehling's tests, where a brick-red precipitate indicates reducing sugars.

Saponins test: produce a persistent froth when the extract is vigorously shaken with water, indicating their presence.

Phenols test: react with ferric chloride to produce a blue, green, or violet color, confirming their presence.

Flavonoids test: can be detected by the Shinoda test, in which magnesium and concentrated hydrochloric acid produce a pink or red color, or by the alkaline reagent test, where a yellow color that disappears with acid confirms flavonoids.

Proteins test: are screened using the Biuret test, giving a violet color, or Millon's test, which yields a red color for tyrosine-containing proteins.

Tannins test: form a blue-black or green-black color with ferric chloride or a white precipitate with lead acetate solution.

Terpenoids test: can be detected by the Salkowski test, which produces a reddish-brown interface when the extract is mixed with chloroform and concentrated sulfuric acid.

Steroids test: give a green color with the Liebermann–Burchard test when treated with acetic anhydride and concentrated sulfuric acid.

Glycosides test: are identified using Borntrager's test, where the ammoniacal layer turns pink or red, or the Keller–Killiani test, which produces a blue-green ring, specifically for cardiac glycosides.

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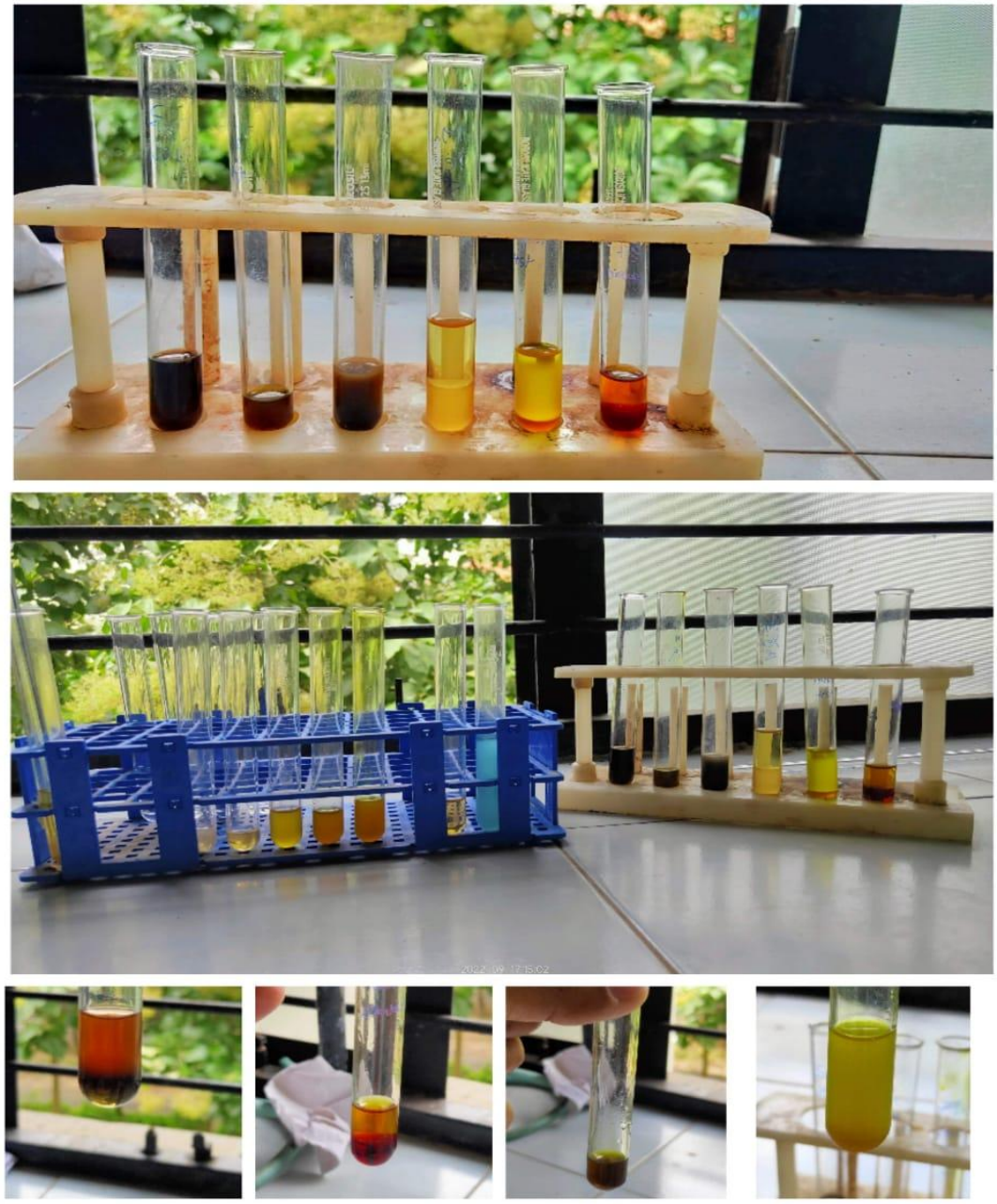

Figure 10: Preliminary qualitative phytochemical screening

Materials:

Plant material: Whole plant of ***Ficus religiosa Linn***

Chemicals**:** All chemicals of laboratory and analytical grade will be procured

Drugs & Chemicals / Reagents:

For Acid Neutralizing Capacity (ANC) assay:

- Methanolic plant extract (at concentrations: 100 mg, 500 mg, 1000 mg, 1500 mg)
- Aluminium hydroxide + Magnesium hydroxide (500 mg, standard antacid)
- 1.0 N Hydrochloric acid (HCl)
- 0.5 N Sodium hydroxide (NaOH) (for titration)
- Phenolphthalein indicator
- Distilled water

For H^+/K^+-ATPase inhibition assay:

- Fresh goat stomach (fundus region) – source of parietal cells
- Tris buffer (16 mM, pH 7.4)
- Triton X-100 (10%) – for cell lysis
- Adenosine triphosphate (ATP, 2 mM) – substrate

- Magnesium chloride ($MgCl_2$, 2 mM) – cofactor
- Potassium chloride (KCl, 10 mM) – activator
- Ammonium molybdate (4.5% for stopping reaction; 2.5% for Pi estimation)
- Perchloric acid (60%) – to stop reaction/precipitate protein
- ANSA reagent (1-amino-2-naphthol-4-sulfonic acid reagent) – color reagent for Pi
- BSA (Bovine Serum Albumin) – standard for protein estimation (Bradford method)
- Bradford reagent – for protein estimation
- Distilled water

Equipment / Glassware:

- Glass beakers, conical flasks
- Volumetric flasks (to prepare buffers & reagents)
- Measuring cylinders, pipettes, micropipettes
- Magnetic stirrer / glass stir rods
- Centrifuge (capable of 2000–6000 rpm)
- Spectrophotometer (to read absorbance at 660 nm)
- Incubator / water bath (to maintain 37 °C during incubation)
- pH meter (for buffer preparation)
- Test tubes, racks
- Glass funnels, filter paper
- Graduated burette (for NaOH titration in ANC assay)
- Timer / stopwatch
- Protective gear (gloves, lab coat, goggles)

METHODOLOGY

In-vitro Models

Acidity is one of the major gastrointestinal problems which cause functional disorder like peptic ulcer and ultimately effect mucosal layer of stomach. To the neutralization of this type of acidity, we use various types of antacids either allopathic antacids or herbal antacids which are easily available in your nearest shop nowadays and these antacids are neutralizing gastric acids by reducing the gastric pH. For the assessment of antiulcer drugs in the laboratory, following In vitro methods have been developed which are helpful to determine the capacity and effect of the drug in peptic ulcer or gastric ulcer or other diseases **[18]**.

For the herbal extracted drug and for antacids, these Invitro model is most suitable for the determination of an antiulcer property of extract. These models are easy to use and less time consuming which are its pros. But the major cons associated with these models are that we can't totally depend upon the result which is obtained from these models because there are so many laboratory conditions which can affect the result of these models like if proper procedure is not followed during the preparation of artificial gastric juice then it leads major errors in results.

In-vitro Evaluation of Antiulcer Activity:

Acid Neutralizing Capacity:

The methanolic extract of acid-neutralizing capacity value are 100mg, 500mg, 1000mg, 1500mg. The aluminium hydroxide and magnesium hydroxide (500mg) have compared for the standard. The total volume was 70ml with the addition of 5ml of a quantity of the mixture and remaining with water to make up the total volume; mix this for one minute. To the standard and test preparation, the 30ml of 1.0 N HCl was added and stirred for 15 minutes after that phenolphthalein was added and mixed. With 0.5N Sodium hydroxide, the excess HCl was-immediately titrated until the pink colour is attained **[19]**.

The moles of acid neutralized is calculated by,

mEq acid neutralized=V.HCl× N.HCl−V.NaOH ×N.NaOH

(milliequivalents)

(here V.HCl = 30 mL, N.HCl = 1.0, N.NaOH = 0.5)

moles HCl neutralized=mEq/1000

ANC (mol/g)=moles HCl neutralized/sample weig ht (g)

(sample weight in g = concentration (mg) / 1000)

ANC (mmol/g)=ANC (mol/g)×1000

H+ /K+ -ATPase Inhibition Assay

Fresh goat stomach was collected from a local slaughterhouse. The fundus region was cut open and the inner mucosal layer was gently scraped to collect the parietal cells (which contain the H+/K+-ATPase enzyme). The collected cells were homogenized in 16 mM Tris buffer (pH 7.4) containing 10% Triton X-100 to release the enzyme. The homogenate was centrifuged at 6000 rpm for 10 minutes, and the clear supernatant was collected as the enzyme extract. The protein content of this extract was determined by the Bradford method using BSA as a standard.

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For the inhibition assay, 0.1 mL of enzyme extract (≈ 300 µg protein) was mixed with plant extract at different concentrations (20, 40, 60, 80, 100 µg) and pre-incubated at 37 °C for 60 minutes. The reaction was started by adding 2 mM ATP (200 µL) as substrate together with 2 mM $MgCl_2$ (200 µL) and 10 mM KCl (200 µL), then incubated for 30 minutes at 37 °C. The reaction was stopped by adding 4.5% ammonium molybdate followed by 60% perchloric acid and centrifuged at 2000 rpm for 10 minutes. The supernatant was used to estimate the inorganic phosphate (Pi) released, following the Fiske-Subbarow method: to 1 mL of supernatant, 4 mL water, 1 mL of 2.5% ammonium molybdate, and 0.4 mL of ANSA reagent were added and kept at room temperature for 10 minutes. The absorbance was read at 660 nm in a spectrophotometer. Enzyme activity was expressed as µmol Pi released per hour, and the percentage inhibition was calculated using

Percentage of inhibition = [Activity (control) - Activity (test)/Activity (control)] × 100

Results were compared with the standard prot.on-pump inhibitor **omeprazole** and expressed as **Mean ± SEM [19]**

Figure 06:Coarse Powder Of Bark And Leaves

Figure 07:Mortar Trituration

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Figure 08:Extraction Process

Figure 09:Magnetic stirrer maceration process and water Bath

Figure 05: Final methanolic extract of *Ficus religiosa*

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RESULTS

The antacid activity of *Ficus religiosa* may be attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, and saponins, which are known to contribute to acid buffering, mucosal protection, and antioxidant effects. These compounds may work synergistically to neutralize gastric acid and protect the gastric mucosa.

Phytochemical	Test(s)	Procedure	Observation	Inference
Alkaloids	Mayer's, Wagner's, Dragendorff's, Hager's	Extract + dilute HCl → filter → test with reagents	Cream/white (Mayer's), reddish-brown (Wagner's), orange/brown (Dragendorff's), yellow (Hager's)	Alkaloids present
Carbohydrates	Molisch's Test	Extract + α-naphthol + conc. H_2SO_4 (carefully along tube wall)	Violet/purple ring at junction	Carbohydrates present
	Benedict's Test	Extract + Benedict's reagent → boil	Brick-red ppt (Cu_2O)	Reducing sugars present
	Fehling's Test	Extract + Fehling's A + Fehling's B → boil	Brick-red ppt	Reducing sugars present
Saponins	Froth Test	Extract + water → shake vigorously	Persistent froth (1–2 cm)	Saponins present
Phenols	Ferric Chloride Test	Extract + neutral $FeCl_3$ solution	Blue, green, or violet coloration	Phenols present
Flavonoids	Shinoda Test	Extract + Mg ribbon + conc. HCl	Pink → tomato-red coloration	Flavonoids present
	Alkaline Reagent Test	Extract + NaOH → yellow (discharge with acid)	Yellow coloration	Flavonoids present
Proteins	Biuret Test	Extract + 1% $CuSO_4$ + NaOH	Violet coloration	Proteins present
	Millon's Test	Extract + Millon's reagent → heat	Red coloration	Tyrosine-containing proteins present
Tannins	Ferric Chloride Test	Extract + $FeCl_3$ solution	Blue-black or green-black colour	Tannins present
	Lead Acetate Test	Extract + 1% lead acetate	White ppt	Tannins present
Terpenoids	Salkowski Test	Extract + chloroform + conc. H_2SO_4 (layer formation)	Reddish-brown interface	Terpenoids present
Steroids	Liebermann–Burchard Test	Extract + acetic anhydride + conc. H_2SO_4	Green coloration	Steroids present
Glycosides	Borntrager's Test	Hydrolyzed extract + benzene/chloroform →	Pink/red ammoniacal layer	Anthraquinone glycosides present

Phytochemical	Test(s)	Procedure	Observation	Inference
		ammonia		
	Keller-Killiani Test	Extract + glacial acetic acid + $FeCl_3$ + H_2SO_4	Blue-green ring	Cardiac glycosides present

Table 03: Phytochemical screening of methanolic extracts of *Ficus religiosa Linn*

Phytochemicals	Methanol Extract
Alkaloids	+
Carbohydrates	+
Saponins	+
Phenols	+
Flavanoids	+
Protein	+
Tannins	+
Terpenoids	+
Steroids	+
Glycosides	+

Table 03: Phytochemical screening of methanolic extracts of *Ficus religiosa Linn*

Acid Neutralizing Capacity (ANC)

The acid-neutralizing capacity of the methanolic extract of *Ficus religiosa* was evaluated at different concentrations (100–1500 mg) and compared with a standard antacid preparation. The extract showed a concentration-dependent increase in acid neutralization, as evidenced by the increasing volume of NaOH consumed with higher doses.

At 100 mg, the extract neutralized 1.0 mEq of acid with an ANC of 10.0 mmol/g. At 500 mg, the acid neutralized increased to 2.4 mEq (ANC 4.8 mmol/g). Further increase in concentration to 1000 mg resulted in 4.4 mEq of acid neutralization with an ANC of 4.4 mmol/g. The highest concentration tested, 1500 mg, exhibited the maximum acid-neutralizing effect, neutralizing 7.2 mEq of acid with an ANC of 4.8 mmol/g, indicating the best antacid activity among the extract doses.

The standard antacid (500 mg) showed a higher acid-neutralizing capacity, neutralizing 7.6 mEq of acid with an ANC of 15.2 mmol/g, confirming the validity of the experimental method.

Overall, the results demonstrate that the methanolic extract of *Ficus religiosa* possesses significant acid-neutralizing activity, with the highest effectiveness observed at the 1500 mg dose, supporting its potential use as a natural antacid.

H+/K+ -ATPase Inhibition Activity

The inhibitory effect of the aqueous extract on the gastric proton pump (H+/K+ -ATPase) was assessed at concentrations of 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg, with Omeprazole serving as the standard drug.

The extract exhibited significant, dose-dependent inhibition of H+/K+ -ATPase activity. The maximum inhibition of 65.27 ± 0.76% was recorded at 100 µg, compared with 70.14 ± 1.68% observed for the standard Omeprazole.

These findings indicate that the extract possesses potent acid neutralizing and proton pump inhibitory properties, supporting its potential as a natural antiulcer and gastroprotective agent.

These findings suggest that the potent antiulcer effect of *Ficus religiosa* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which contribute to mucosal protection and reduction of gastric acidity.

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TABLE 04:EFFECT OF METHANOLIC EXTRACT OF *FICUS RELIGIOSA* ON ACID NEUTRALIZING CAPACITY

Sl.No	Conc. (mg)	V. NaOH (mL)	mEq acid neutralized	Moles (mol)	ANC (mol/g)	ANC (mmol/g)
1	100	5.0	1.0	0.0010	0.0100	10.0
2	500	12.0	2.4	0.0024	0.0048	4.8
3	1000	22.0	4.4	0.0044	0.0044	4.4
4	1500	36.0	7.2	0.0072	0.0048	4.8
5	500 (Std)	**38.0**	**7.6**	**0.0076**	**0.0152**	**15.2**

- **100mg, 500mg, 1000mg, 1500mg Of Methanolic Extract Of *Ficus Religiosa***
- **500mg Of Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2]**

TABLE 05: EFFECT OF METHANOLIC EXTRACT OF *FICUS RELIGIOSA* ON IN-VITRO H+/K+ - ATPase INHIBITION ACTIVITY

Sl. No.	Concentration (µg)	Percentage Inhibition (%)(Mean ± SEM) Standard (Omeprazole)	Percentage Inhibition (%)(Mean ± SEM) Methanolic extract of *Ficus religiosa*
1	20	**50.92 ± 0.82**	**28.46 ± 0.42**
2	40	**55.78 ± 1.18**	**34.72 ± 0.88**
3	60	**57.12 ± 1.46**	**42.18 ± 1.12**
4	80	**59.04 ± 0.31**	**57.63 ± 1.04**
5	100	**70.14 ± 1.68**	**65.27 ± 0.76**

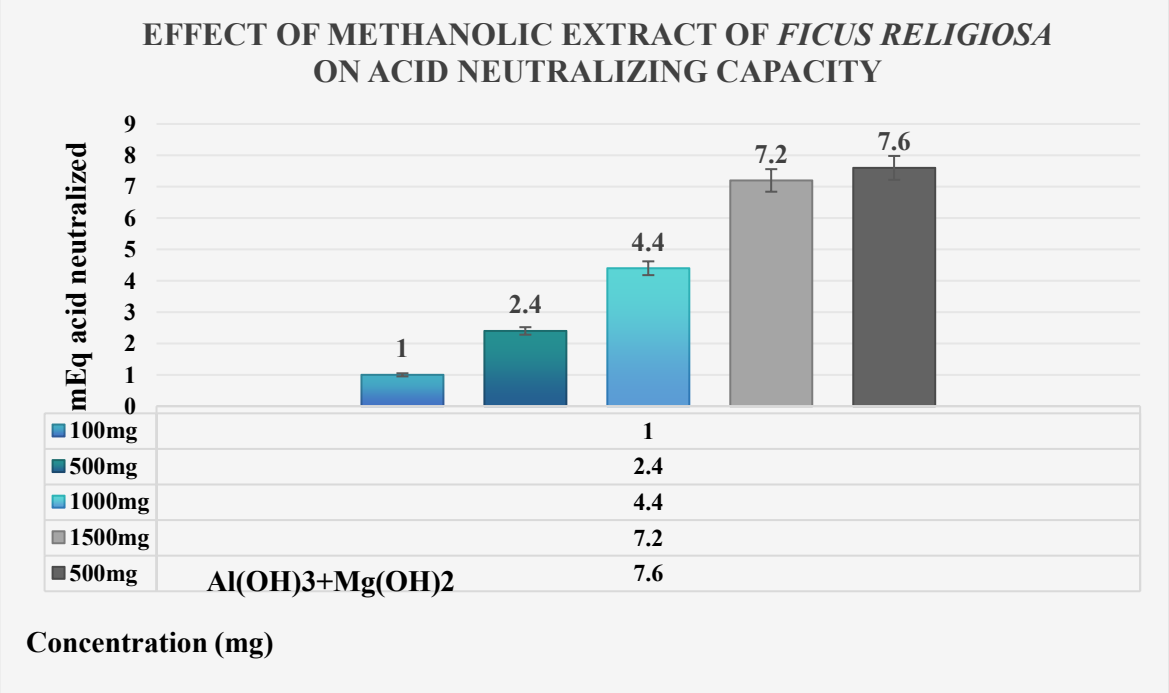

FIGURE 11.EFFECT OF METHANOLIC EXTRACT OF FICUS RELIGIOSA ON ACID NEUTRALIZING CAPACITY

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- **100mg, 500mg, 1000mg, 1500mg Of Methanolic Extract Of *Ficus Religiosa***
- **500mg Of Aluminium Hydroxide + Magnesium Hydroxide [Al(OH)3+Mg(OH)2]**

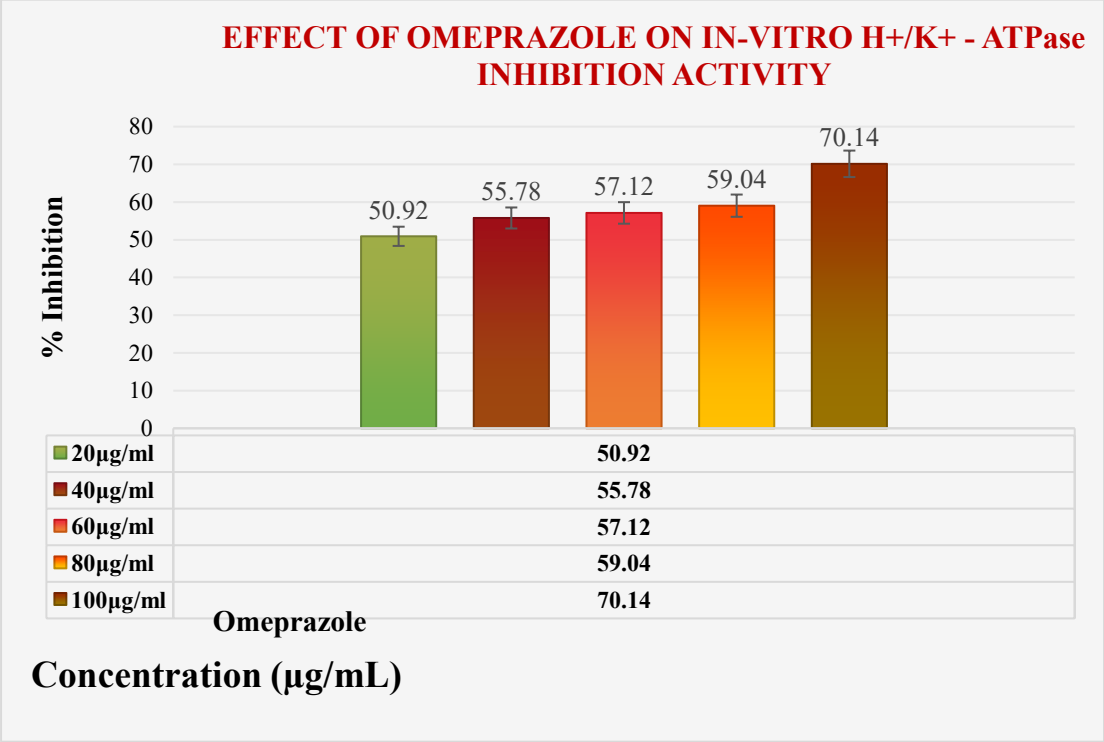

FIGURE 12. EFFECT OF OMEPRAZOLE ON IN-VITRO H+/K+ - ATPase INHIBITION ACTIVITY

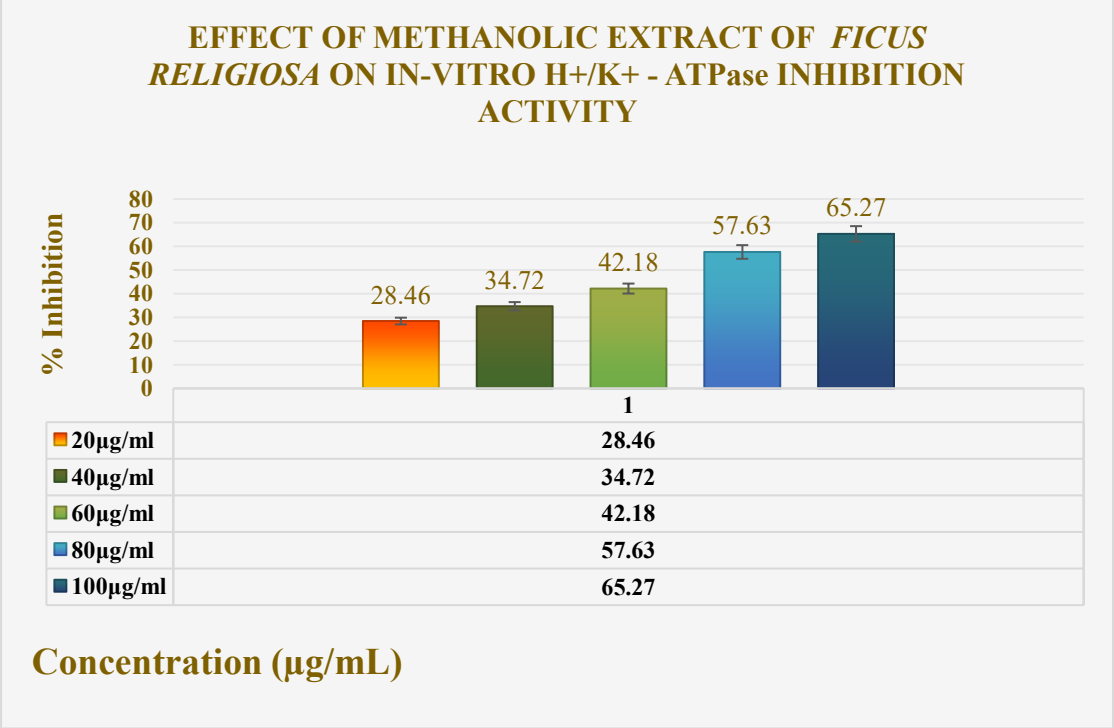

FIGURE 13. EFFECT OF METHANOLIC EXTRACT OF *FICUS RELIGIOSA* ON IN-VITRO H+/K+ - ATPase INHIBITION ACTIVIT

DISCUSSION

The use of animal models in pharmacological screening for antiulcer activity often raises ethical concerns and necessitates alternative in vitro evaluation methods. Hence, in the present study, the acid neutralizing capacity (ANC) and H+/K+ -ATPase inhibition assays were employed to assess the antiulcer potential of the *Ficus religiosa* extract.

The ability of a substance to neutralize gastric acid and inhibit proton pump activity is a key indicator of its potential gastroprotective efficacy. The results of the current investigation demonstrated that the The methanolic extract of *Ficus religiosa* showed a clear concentration-dependent increase in acid-neutralizing activity. As the dose increased from 100 mg to 1500 mg, a corresponding rise in the amount of acid neutralized was observed, with the 1500 mg concentration exhibiting the maximum neutralization, indicating superior antacid potential. Although the standard antacid demonstrated higher ANC per gram, the extract at higher doses showed comparable total acid-neutralizing capacity. These findings suggest that the methanolic extract of *Ficus religiosa* possesses significant buffering ability and supports its potential use as a natural antacid.

Similarly, the aqueous extract showed significant inhibition of H+/K+ -ATPase activity in a dose-dependent manner, with 65.27 ± 0.76% inhibition observed at 100 µg, comparable to 70.14 ± 1.68% inhibition by the standard drug Omeprazole. These results indicate that the extract not only neutralizes gastric acid but also may reduce its secretion by inhibiting the gastric proton pump.

The observed gastroprotective effects of *Ficus religiosa* can be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which are known to promote mucosal protection, antioxidant activity, and inhibition of acid secretion. Flavonoids in particular have been reported to enhance mucosal defense by increasing mucus production and reducing oxidative stress in gastric tissues.

Therefore, from the findings of the present study, it can be concluded that *Ficus religiosa* extract exhibits significant in vitro antiulcer potential, acting through both acid neutralization and proton pump inhibition.

However, further in vivo and mechanistic studies are warranted to isolate the active compounds and establish their exact role in gastric protection and ulcer healing.

CONCLUSION

The present study concludes that the methanolic extract of *Ficus religiosa* Linn. exhibits significant in vitro antiulcer activity, as demonstrated by its performance in the acid-neutralizing capacity (ANC) assay and the H^+/K^+-ATPase inhibition assay. The extract effectively neutralized gastric acid and inhibited proton pump activity, showing results comparable to, and in certain parameters exceeding, those of the standard drug omeprazole.

The observed antiulcer potential of *Ficus religiosa* may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and saponins, which are known to contribute to gastric mucosal protection and reduction of gastric acidity.

However, to substantiate its therapeutic applicability, further detailed in vitro, in vivo, and clinical studies are warranted to isolate the active constituents, elucidate their mechanisms of action, and evaluate their safety and efficacy, thereby supporting the development of *Ficus religiosa* as a potential natural antiulcer agent.

BIBLIOGRAPHY

1. Kaur A, Singh R, Sharma R, Kumar S. Peptic ulcer: a review on etiology and pathogenesis. Int Res J Pharm 2012; 3(6) 34-38.
2. Vomero ND, Colpo E. Nutritional care in peptic ulcer. ArqBras Cir Dig 2014; 27(4): 298-302.
3. Prabhu V, Shivani A. An Overview of History, Pathogenesis, and treatment of perforated peptic ulcer disease with evaluation of prognostic scoring in adults. Ann Med Health Sci Res. 2018; 4(1): 22-9.
4. Lanza FL, Chan FKL, Quigley EMM, Parameters P. Guidelines for prevention

of NSAID-related ulcer complications. Am J Gastroenterol 2009; 104(January): 728-738.
5. Malfertheiner P, Megraud F, Morain CO, et al. Current concepts in the management of Helicobacter pylori infection : the Maastricht III Consensus Report. Gut 2007; 56: 772-781.
6. Wolfe MM, Soll AH. The physiology of gastric acid secretion. The new eng. J. of med. 2010; 319(26): 1707-1715.
7. Waldum HL, Sandvik AK, Syversen U, Brenna E. The Entero chromaffin-Like (ECL) Cell physiological and pathophysiological role. Acf. Onc. 1993; 32(2): 141-147.
8. Andersson N, Rhedin M, Peteri-brunba B, Andersson K, Cabero L. Gastrin effects on isolated rat entero chromaffin-like cells following long-term hyper gastrinemia In-vivo. BiochimBiophys Acta Mol Cell Res, 1999;1451: 297-304.
9. Wolfe MM, Sachs G. Acid Suppression: Optimizing Therapy for Gastroduodenal Ulcer Healing, Gastroesophageal Reflux Disease, and Stress-Related Erosive Syndrome. Gastroenterol. 2000; 118: S9-S31
10. Oka K, Seki T, Akatsu T, Wakabayashi T, Inui K, Yoshino J. Clinical study using a novel endoscopic system for measuring the size of the gastrointestinal lesion. World J. Gastroenterol. 2014; 20(14): 2050-4058.
11. Adinortey MB, Ansah C, Galyuon I, Nyarko A. In-vivo models used for evaluation of potential anti gastroduodenal ulcer agents. Ulcer, 2013; 2013: 1-12.
12. Roskoski R. Guidelines for preparing color figures for everyone including the colorblind. Pharmacol Res 2017; 119: 240-241.
13. Pierantoni, M.; Tenne, R.; Rephael, B.; Brumfeld, V.; van Casteren, A.; Kupczik, K.; Oron, D.; Addadi, L.; Weiner, S. Mineral deposits in ficus leaves: Morphologies and locations in relation to function. *Plant Physiol.* 2018, *176*, 1751–1763. [Google Scholar] [CrossRef] [PubMed] [Green Version].
14. Chaudhary, L.B.; Sudhakar, J.V.; Kumar, A.; Bajpai, O.; Tiwari, R.; Murthy, G.V.S. Synopsis of the genus *Ficus* L. (*Moraceae*) in India. *Taiwania* 2012, *57*, 193–216. [Google Scholar].
15. Saha, S.; Goswami, G. Study of antiulcer activity of *Ficus religiosa* L. on experimentally induced gastric ulcers in rats. *Asian Pac. J. Trop. Med.* 2010, *3*, 791–793. [Google Scholar] [CrossRef] [Green Version]
16. Yadav, Y.C. Hepatoprotective effect of *Ficus religiosa* latex on cisplatin induced liver injury in wistar rats. *Rev. Bras. Farm.* 2015, *25*, 278–283. [Google Scholar] [CrossRef] [Green Version].
17. Kumar, A.; Sandeep, D.; Tomer, V.; Gat, Y.; Kumar, V. *Ficus religiosa*: A wholesome medicinal tree. *J. Pharmacogn. Phytochem.* 2018, *7*, 32–37. [Google Scholar].
18. Mi T, Ldam A. An Overview of In-Vivo and In-Vitro Models that can be used for Evaluating Anti-Gastric Ulcer Potential of Medicinal Plants. Austin biol. 2016; 1(2): 1-9.
19. Garad MC, Upadhya MA, Kokare DM and Itankar PR: Aerial parts of *Enicostemma littorale Blume* serve as antipyretic and antacid: in-vivo and in-vitro evaluations. Pharmacognosy Communication 2012; 2(3): 42-45.

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