

**EVALUATION OF SYNERGISTIC ANTIULCER POTENTIAL OF ETHANOLIC EXTRACT OF *BETA VULGARIS* (TAPROOT) & *FICUS RELIGIOSA* (BARK) IN WISTAR RATS**\*Rahul Arora<sup>1</sup> and Neelanchal Trivedi<sup>2</sup><sup>1,2</sup>Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India.

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**ABSTRACT**

**Objective:** To authenticate the synergistic anti-ulcer potential of the ethanolic extract of *Ficus religiosa* bark and *Beta vulgaris* root aligned with *in vivo* pylorus ligation assay. **Methods:** The root and bark of the plants were extracted by ethanol. The ethanolic extract was subjected to evaluate its phytochemical profiles. Gastric ulcers induced in male wistar rats via ligating the pylorus part of stomach. The antiulcer activity was checked by formative and comparing the ulcer index in the experimental drug groups with that of the vehicle control and standards. Omeprazole was taken as standard drug. Dose was kept as 20 mg/kg as standard and 250 & 500 mg/kg for the plant extracts. The parameters taken to evaluate antiulcer activity were gastric juice volume, free acidity, total acidity and ulcer index. **Result:** The obtained results were: Percent Protection (71.02% & 68.45%), Mean Ulcer Index ( $0.31 \pm 0.13$ ,  $0.674 \pm 0.279$  and  $0.982 \pm 0.415$ ), Gastric volume ( $2.13 \pm 0.139$ ,  $2.86 \pm 0.101$  and  $3.55 \pm 0.194$ ), Gastric pH ( $4.25 \pm 0.140$ ,  $3.80 \pm 0.201$  and  $3.67 \pm 0.098$ ), Free Acidity ( $29.99 \pm 2.904$ ,  $69.01 \pm 2.03$  and  $63.21 \pm 2.994$ ), Total Acidity ( $1.72 \pm 1.69$ ,  $35.98 \pm 0.44$  and  $72.87 \pm 0.244$ ) for 20 mg/kg standard drug and 250 & 500 mg/kg of ethanolic extract of EEBV+EEFR. **Conclusion:** The Ethanolic extract represented a noteworthy decrease in gastric pH, free acidity and total acidity as while compared with ulcer control using Pylorus Ligation Model.

**KEYWORDS:** *Beta vulgaris*, *Ficus religiosa*, Ethanolic Extract, Omeprazole, Pylorus Ligation.**INTRODUCTION**

Indian herbal plants and their derivatives are an important source in stabilizing human wellbeing & amending the superiority of life for many years and have satisfied human being as precious mechanism of medicines, eatables, seasonings, beverages, cosmetics and dyes. More than the last decade there has been an increasing attention in drugs of plant source and these drugs possessed a significant role for disease management.<sup>[1]</sup>

Peptic ulcer is the main gastro-intestinal disorders. Peptic ulcer happens because of a discrepancy among the destructive factors (acid, pepsin, *Helicobacter pylori*) and protective factors (bicarbonate secretion, Prostaglandins, gastric mucus, native confrontation of the mucosal cell factors).<sup>[9]</sup> It is the ulceration of mucous covering piercing during the mucosa uncovered to most harmful agents like acid, pepsin, bile acids, food ingredients, bacterial products, certain drugs and pathological condition such as Zollinger –Ellison Syndrome, in stomach and duodenum. If the ulceration occurs in Stomach, it is called Gastric ulcer and when it occurs in duodenum, it is called duodenal Ulcer. Peptic ulcer is a widespread medical crisis with yearly frequency of about

100/100,000 adults and overall mortality of 10 to 15% according to latest studies. In the beginning of 20th century peptic ulcers were considered to be caused by poignant stress and spicy foods.<sup>[12]</sup> Peptic ulcer occurs frequently in men than in women. It is now well established that peptic ulcer can be prevented by amplification the self-protective mechanisms of gastric and duodenal mucosa rather than diminishing factors of assault causing ulceration.<sup>[2]</sup>

There is a lot expectation of judgment energetic anti-ulcer compound from endogenous plants as these are still used in beneficial in spite of the development through conservative chemistry and pharmacology for manufacturing helpful drugs. As there are various information's of plants regarding antiulcer activity, we have selected two plants having different medicinal values to identify the antiulcer effect.<sup>[3]</sup>

## Beet Root



**Fig 1: Beet root**

**Phytochemical Constituents:** Initial phytochemical screening of *Beta vulgaris* showed the existence of Carbohydrates, Amino acids, Betain, Volatile constituents like pyridine, geosmin, Protein, lipids and Vitamin A, B, C and minarls.<sup>[5]</sup>

## Peepal Tree



**Fig 2: Peepal Tree**

**Phytochemical Constituents:** Initial phytochemical screening of *F. religiosa* barks, resulted the existence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides.

Therefore this study was conducted to compare the synergistic antiulcer effect of ethanolic extract of *Beta vulgaris* root and *Ficus religiosa* bark aligned *in vivo* pylorus ligation assay, with the standard drug Omeprazole.<sup>[4]</sup>

## MATERIALS AND METHODS

**Plant collection:** The root of *Beta vulgaris* and the bark of *Ficus religiosa* bark were assembled from local market of Moradabad (U.P.) in the month of October 2015. The specimens of plant material were identified by the Department of Botany, Hindu College, Moradabad (Ref no. HC/Bot/028).

**Plant Extraction:** Fresh plants were collected, cleaned under running tap water, the roots and barks were removed from the fresh stems and desiccated on filter paper in dark place at room temperature, then were

powdered and stored in airtight container till further processing.<sup>[6]</sup>

The dried bark of *Ficus religiosa* and the root of *Beta vulgaris* were powdered using mixer grinder. The powdered bark and root were packed in a paper bag and stored in air tight container for further use. The powder first taken in soxhlet assembly for extraction with petroleum ether to remove the fatty compounds, the defatted extract was then extracted further with Ethanol and water respectively. Each extract was concentrated by distilling off the solvent under reduced pressure below 50°C. Extracted material was dried in hot-air oven below 50°C. The extracts were used for pharmacological evaluations.<sup>[7]</sup>

**Selection of Animals:** Healthy albino rats of each sex (weighing 100-150g) were taken in the experiment. All animal experiment was conducted in accordance with CPCSEA guidelines.

**Selection of Standard Drug:** For antiulcer activity Omeprazole was chosen as standard drug.

## Pharmacological Screening of Plant Extract

### Pylorus ligation induced gastric mucosal ulcer

First of all the animal were kept on overnight fasting for 24 hours, then they were administered thiopental sodium (10 mg/kg, i.p.) to anesthetized, and later the stomach of animal was dissected and the pylorus is ligated. The rats were separated into four groups, each group containing 6 rats. First group treated as control and received distilled water by oral route. Second group was administered Omeprazole (20 mg/kg) as standard drug for ulcer protective studies. Third & Fourth Groups received combination of ethanolic extracts of *Ficus religiosa* and *Beta vulgaris* respectively, four hours later animals were sacrificed; opened the stomach carefully and removed all the gastric content, composed and centrifuged at 3000 revolution/minute for 10-12min. The amount of gastric acid level (ml) and the pH values were ascertained. Total acid secretion in gastric juice was determined by titration of the supernatant volume.<sup>[8,13]</sup>

### Measurement of various parameters

The mean values were calculated by the subsequent scoring system

- (i) Normal coloured stomach– 0
- (ii) Red coloured streaks - 0.5
- (iii) Ulcer spots – 1
- (iv) Haemorrhagic bands – 1.5
- (v) Ulcers – 2
- (vi) Perforated stomach - 3.

Percent inhibition will be calculated by applying the following formula

$$\% \text{ Inhibition} = \frac{(U I_{\text{ulcer control}} - U I_{\text{treated}}) \times 100}{U I_{\text{ulcer control}}}$$

### Estimation of gastric pH

The gastric content, collected from stomach of rat will be transferred into centrifuge tubes to estimate the gastric volume and pH. The tubes will be centrifuged at 1000 revolutions/min for 10 min and the gastric volume is observed from the graduation on the tubes. The supernatant will be collected and pH was determined by using a digital pH meter.<sup>[14]</sup>

### Estimation of total acidity

The multiples of 1 ml gastric juice was diluted with 9 ml of distilled water then it will take into a 100 ml flask and few drops of phenolphthalein indicator will be added to it and titrated with 0.01N Sodium hydroxide till a stable pink colour occurs. The volume of 0.01N Sodium hydroxide consumed is noted. The total acidity is expressed as mEq/L by the following formula:

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}$$

### Estimation of free acidity

Topfer's reagent was used for the estimation of free acidity. Equally divisible amount of gastric juice were titrated with 0.01N Sodium hydroxide solution until orange colour was persisted. The volume of 0.01N Sodium hydroxide consumed was distinguished. The free acidity was intended by the same formula as for determining of total acidity.<sup>[10,11]</sup>

**Statistical Analysis:** All results were expressed as Mean  $\pm$  SEM for 6 rats. The difference among means been analyzed by unpaired student's t-test.

## RESULTS AND DISCUSSION

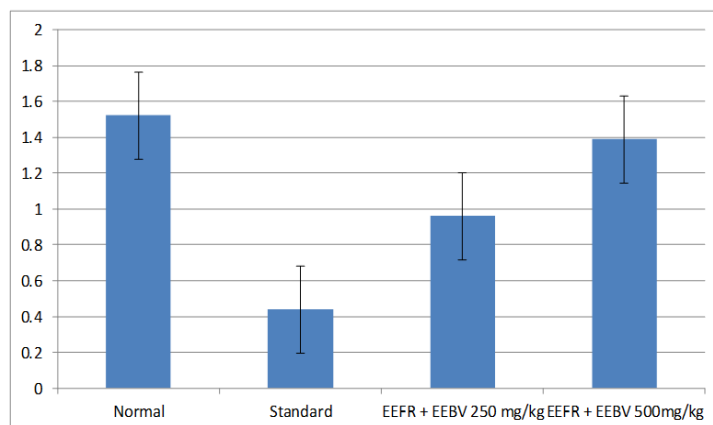
The Ethanolic extract of bark and root of *Ficus religiosa* and *Beta vulgaris* represented a noteworthy decrease in gastric pH, free acidity and total acidity as while compared with ulcer control ( $p < 0.05$ ). The two behaviors fashioned important consequence on ulcer index ( $p < 0.05$ ). The EEFR + EEBV experienced 71.02 (250mg/kg) & 68.45% (500mg/kg) ulcer fortification.

The results obtained with ethanolic extracts of combination of *Ficus religiosa* and *Beta vulgaris* at the dose of 250mg/kg & 500 mg/kg (Table 1) was set up near to the standard drug and the consequence showed that the ethanolic extract of *Ficus religiosa* and *Beta vulgaris* was found to be more effective than the ethanolic extract of individual plant. The antiulcer effects of *Ficus religiosa* and *Beta vulgaris* can be due to the occurrence of flavanoids, tannins and antioxidant activity in the extracts.

The ethanolic extracts increased the gastric pH, reduced the total acidity, ulcer index & free acidity while compared to the control group (Table 1, Figure 3-7). The histopathological examination signified minimal damage to the gastric mucosa while treated with ethanolic extracts of *Ficus religiosa* and *Beta vulgaris* (Figure 9). The current experiment recommended that the synergistic antiulcer activity of EEFR & EEBV by pylorus ligation induced gastric ulcer is because of their anti secretory activity while the coordination of defense in ethanol induced gastric ulcer of EEFR & EEBV may be recognized to cyto protective and free radical search or antioxidant properties.

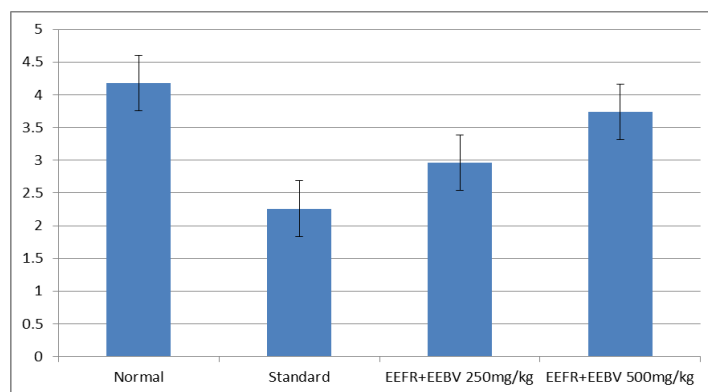
**Table 1: Effect of EEFR + EEBV on Pylorus Ligation- Induced Ulcer Model**

Sr. No.	Group	Dose	Mean Ulcer Index $\pm$ SEM	% Protection	Gastric Volume (ml)	Gastric pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)
1	Normal	-	1.214 $\pm$ 0.398	-	4.0 $\pm$ 0.176	2.57 $\pm$ 0.170	87.49 $\pm$ 0.15	76.01 $\pm$ 0.24
2	Standard (Omeprazole)	20mg/kg	0.31 $\pm$ 0.13	79.24	2.13 $\pm$ 0.139	4.25 $\pm$ 0.140	29.99 $\pm$ 2.90	17.2 $\pm$ 1.69
3	EEFR + EEBV	250mg/kg	0.674 $\pm$ 0.279	71.02	2.86 $\pm$ 0.101	3.80 $\pm$ 0.201	69.01 $\pm$ 2.03	35.98 $\pm$ 0.44
4	EEFR + EEBV	500mg/kg	0.982 $\pm$ 0.415	68.45	3.55 $\pm$ 0.194	3.67 $\pm$ 0.098	63.21 $\pm$ 2.99	72.87 $\pm$ 0.24

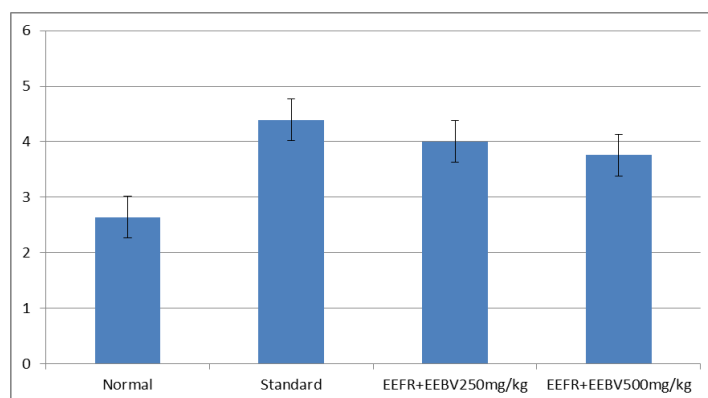


**Figure 3: Mean Ulcer Index**

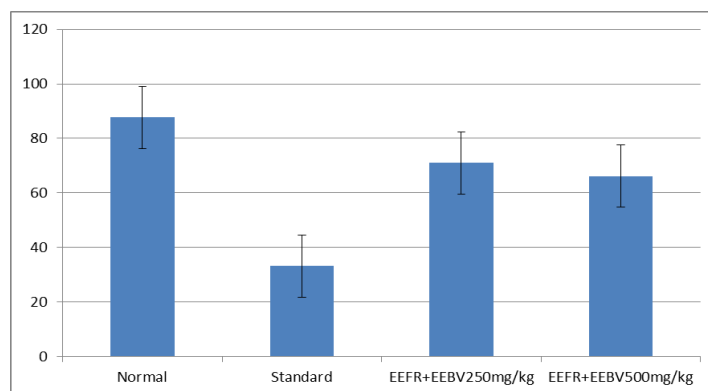
(Values are expressed as (Mean  $\pm$  SEM) (n=6), significant at  $p < 0.05$  as compared with normal.)

**Figure 4: Gastric Volume**

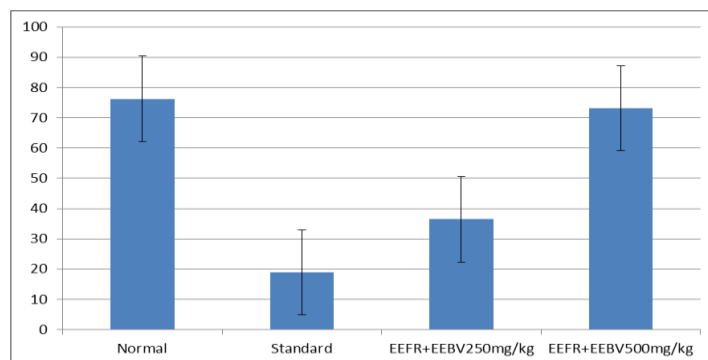
(Values are expressed as (Mean ± SEM) (n=6), significant at  $p < 0.05$  as compared with normal.)

**Figure 5: Gastric pH**

(Values are expressed as (Mean ± SEM) (n=6), significant at  $p < 0.05$  as compared with normal.)

**Figure 6: Free Acidity**

(Values are expressed as (Mean ± SEM) (n=6), significant at  $p < 0.05$  as compared with normal.)

**Figure 7: Total Acidity**

(Values are expressed as (Mean ± SEM) (n=6), significant at  $p < 0.05$  as compared with normal.)

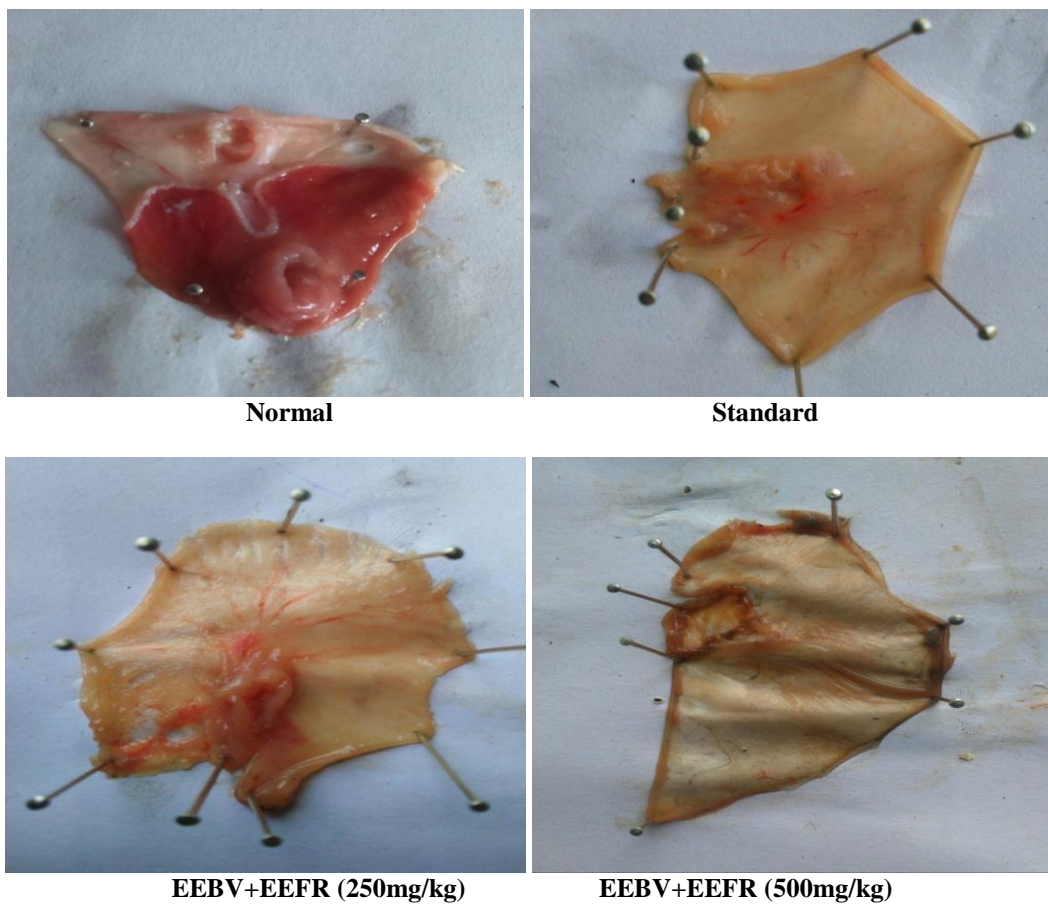


Figure 8: Macroscopical Pictures of Rat's Stomach using Pylorus Ligation Induced Ulcer Model.

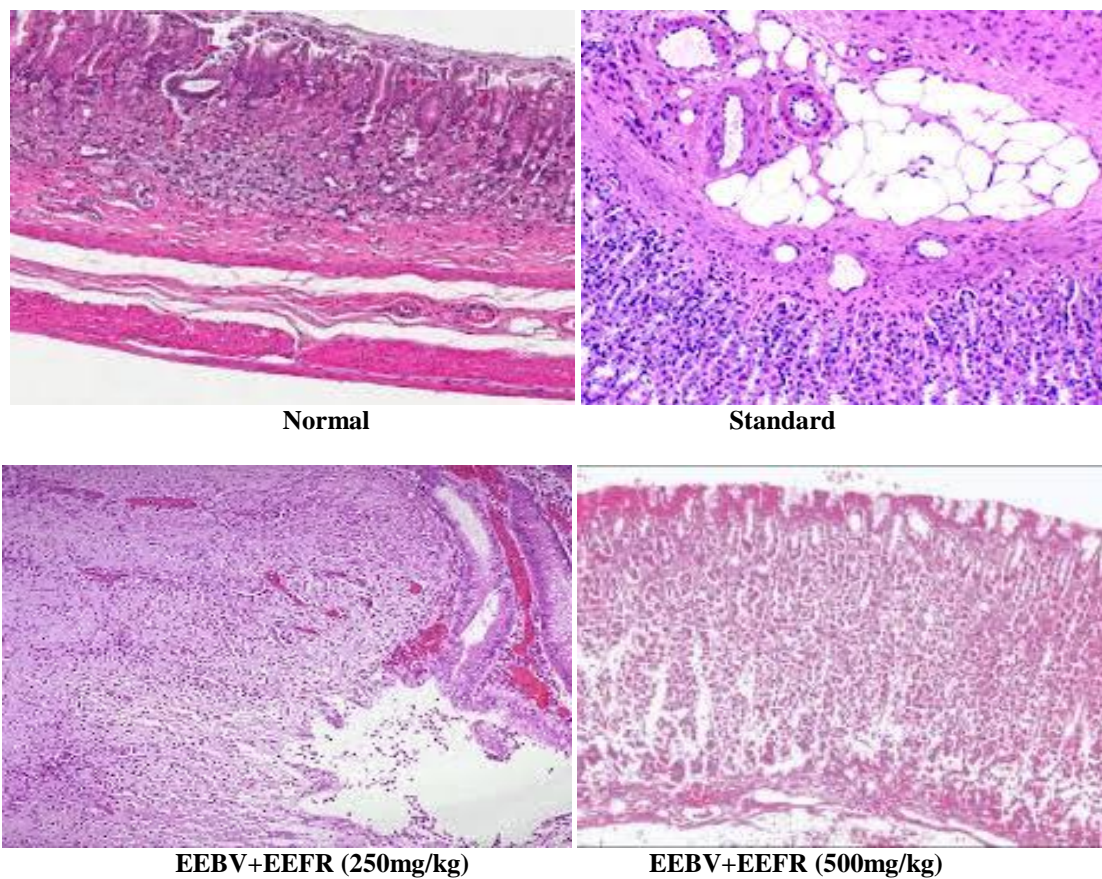


Figure 9: Histopathological Pictures of stomach tissue of Wistar Rat.

**CONCLUSION**

The consequences of our experiment showed that ethanolic extracts of *Ficus religiosa* and *Beta vulgaris* overcome considerable antiulcer property and supports the conventional use of *Ficus religiosa* and *Beta vulgaris* in management of gastrointestinal disorders. The dried plants were subjected to phytochemical work. The preliminary phytochemical investigation showed the presence of glycoside, steroids, flavonoids, alkaloids, carbohydrate and tannins in the ethanolic extract, these extracts showed significant anti-ulcer activity.

The results lead to the conclusion that the ethanolic extracts of *Ficus religiosa* and *Beta vulgaris* showed a noteworthy anti-ulcer activity in experimental animals. The combination of *Ficus religiosa* and *Beta vulgaris* ethanolic extract exhibited comparatively improved anti-ulcer activity than individual plant extract. Pylorus ligation induced and ethanol induced ulceration models were used for assessing anti-ulcer activity and different parameters like gastric pH, free acidity, anti-oxidant study, gastric volume, total acidity, mucin estimation, protein estimation ulcer index and histopathological examinations were resolute.

Thus, significant anti-ulcer activity of ethanol extract of combination of *Ficus religiosa* and *Beta vulgaris* is because of the presence of flavonoids, tannins and anti-oxidant activity.

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**REFERENCES**

1. Vyawahare N. S., Deshmukh V. V. et al.; Plants with anti ulcer activity; Pharmacognosy Review, 2009; 21(3): 108-15.
2. Hoogerwerf W. A., Pasricha P. J. ;Agents used for control of Gastric Acidity and treatment of Peptic ulcer and Gastro Esophageal Reflux Disease; McGraw Hill;10<sup>th</sup> edition, 2011; 1005-19.
3. Kumar R; A review on Medicinal Plants for peptic ulcer; Scholor Research Library; Der Pharmacia Lettre, 2011; 3: 414-20.
4. <http://en.wikipedia.org/wiki/Ficus religiosa>.
5. <http://en.wikipedia.org/wiki/Beta vulgaris>.
6. Gregory Marslin, Divya B. et al; Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extract, Asian Pacific Journal of Tropical Biomedicine, 2013; 555-559.
7. Sowjanya Akula et al.; Evaluation of antiulcer activity of *Beta vulgaris* in rats, International Journal of Pharmacy and Technology, 2015; 9264-7.
8. Divya B., Palanivel V. and Senthil Kumar KL.; Study of anti ulcer activity of *Ficus religiosa* leaf extract on experimentally induced gastric ulcers in rats, international journal of pharmaceutical and chemical sciences, 2012; 1(3): 1062-5.
9. Kumar Hemant, Srivastava Shrish Kumar, Chandana V. Rao, Yadav Sanjay, anti-ulcer activity of *Ficus religiosa* l. leaves on experimental animals, International Journal of Pharmaceutical Sciences Review and Research, 2011; 11(2): 83-6.
10. Khan M S A, Hussain Syed Ahmed et al, Anti-ulcer activity of *Ficus religiosa* stem bark ethanolic extract in rats, Journal of Medicinal Plants Research, 2011; 5(3): 350-4.
11. Kulkarni, S.K.; "Hand Book of Experimental Pharmacology", Vallabh Prakashan, New Delhi, 2000; 149-150.
12. Emanuel Rubin, M.D. John, Farber M.D. "The Text Book of Pathology", J.B. Lippincott Company, Philadelphia: 2nd edition, 1994; 638-41.
13. Whiteley, P.E., Dalrymple, S.A. "Current Protocols in Pharmacology", New York, John Wiley & Sons, 1998; 10.2.1-4.
14. Nair, V., Arjuman, A., Gopalakrishna, H.N., Dorababu, P., Mirshad, P.V., Bhargavan, D.; Evaluation of the Anti-Ulcer Activity of A Polyherbal Formulation in Aspirin & Pyloric Ligation Induced Gastric Ulcers in Albino Rats, Indian Journal of Medicine and Research, 2010; 132: 218-23.