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Exploring the therapeutic potential of *Psidium guajava* and *Beta vulgaris* extracts for peptic ulcers: A natural alternative to conventional medications

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Abstract
Peptic ulcers pose a significant challenge in modern medicine, necessitating the exploration of alternative treatments to conventional medications. This study investigates the potential of *Psidium guajava* and *Beta vulgaris* extracts as alternative treatments for peptic ulcers. Physicochemical analysis reveals that *Psidium guajava* extract is rich in flavonoids, while *Beta vulgaris* extract contains saponins. Fractionation followed by GC-MS analysis identifies beta-bisabolene and caffeic acid in the *Psidium guajava* extract, compounds known for their anti-inflammatory and antioxidant properties. Comparative chromatography confirms the presence of caffeic acid in *Beta vulgaris* extract, which is recognized for its high antioxidant properties. Using a rat model of pylorus ligation-induced ulcers, the combined extract of *Psidium guajava* and *Beta vulgaris* effectively reduces gastric acid secretion, inhibits ulcers, and provides Cytoprotective effects. Histopathological analysis confirms the effectiveness of *Psidium guajava*'s flavonoids and Beta vulgarise Saponins in treating peptic ulcers. These findings highlight the potential of *Psidium guajava* and *Beta vulgaris* extracts as promising alternatives for peptic ulcer management, offering a safer and more efficient approach to ulcer therapy.

Keywords: Peptic ulcers, *Psidium guajava*, *Beta vulgaris*, alternative treatment, flavonoids, saponins, gastric acid secretion, cytoprotective, ulcer inhibition, natural remedies, GC-MS study

Introduction
A peptic ulcer is a common gastrointestinal disorder characterized by the breakdown of the mucosal epithelium in the stomach and/or duodenum [1]. Factors contributing to its development include excessive acid or pepsin production and impaired mucosal resistance [15]. Acid secretion in the stomach involves the release of hydrogen ions (H+) and chloride ions (Cl-) by parietal cells, along with bicarbonate ions (HCO3-) into the gastric venous circulation [3]. Gastric acid secretion is regulated by various chemical, neural, and hormonal factors, with gastrin release inhibited at low pH levels. Pepsin, a proteolytic enzyme, can also contribute to gastric acid hypersecretion [1]. Mucosal defence against acid and pepsin involves several factors that are not fully understood. Peptic ulcers can be caused by various factors, including infection with Helicobacter pylori [4], ethanol consumption, bile salts, and non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin [5]. Helicobacter pylori infection, in particular, has been identified as a significant factor in peptic ulcer disease [6]. The management of peptic ulcers typically involves the use of synthetic drugs, but there is ongoing research on natural anti-ulcer plant products. Medicinal plants and herbal formulations, such as *Psidium guajava* (guava) and *Beta vulgaris* (beetroot), have shown efficacy in ulcer treatment, potentially reducing costs and side effects associated with synthetic drugs. *Psidium guajava* and *Beta vulgaris* have been studied for their anti-ulcer properties [7] *Psidium guajava* contains active components like pentacyclic triterpenoid acid and beta-sitosterols, while *Beta vulgaris* contains betalain pigments such as betacyanins and betaxanthins. These plants exhibit antioxidant, anti-inflammatory, antimicrobial, and wound-healing activities [8]. Traditional use of *Psidium guajava* and *Beta vulgaris* for treating ulcers and other ailments is well-documented in various cultures.
Scientific research supports the medicinal properties of *Psidium guajava* and *Beta vulgaris*, and their extracts have been evaluated for anti-ulcer activity using different models and techniques [9]. Considering the significant prevalence of peptic ulcer disease worldwide, the

search for natural anti-ulcer plant products continues. Traditional herbal remedies and plant-based medicines offer potential alternative treatments for peptic ulcers, aiming for pain relief, ulcer healing, and prevention of recurrence.

Material and Methods

The leaves of *Psidium guajava* were obtained from CSA Garden in Prayagraj, and *Beta vulgaris* leaves were obtained from the drugs market in Prayagraj, Uttar Pradesh, India, in the month of October. The collected leaves were dried in the shade until they produced a cracked sound when broken. The leaves of *Psidium guajava* and *Beta vulgaris* were authenticated by a Taxonomist from the Botanical Survey of India in Allahabad. Voucher specimens of the leaves were submitted to the Botanical Survey of India in Prayagraj.

Preparation of Plant Extract

The leaves of *Psidium guajava* and *Beta vulgaris* were finely powdered after collection and drying and then milled using an electric blender. Both drugs were defatted with petroleum ether for 2 days. 100 g of the fine leaf powder of *Psidium guajava* was homogenized in 500 ml of methanol in a conical flask for 3 days, and *Beta vulgaris* leaves were homogenized in 500 ml of ethanol in a conical flask for 2 days. The mixture was then filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and concentrated under reduced pressure using an Eyela rotary evaporator (Sigma-Aldrich, USA). The extracts were stored at -20 °C [9].

Evaluation of Physicochemical Parameters

The physicochemical parameters were performed on *Beta vulgaris* and *Psidium guajava* leaves powder according to the references of the World Health Organization (2002) and the Indian Pharmacopoeia (2007) [14].

Foreign Matter

A total of 100 g of plant material was measured and spread in a thin layer. The foreign matter was visually investigated using a magnifying lens (6x or 10x) or sorted using an appropriate sieve based on the specific plant material requirements. The remaining sample was sifted through a No. 250 sieve, and the dust was regarded as a mineral admixture. The sorted foreign matter was weighed, and the percentage w/w was calculated.

Determination of Moisture Content (Loss on Drying)

1.0 g of the powdered drug was placed into a weighed flat and thin porcelain dish. The sample was dried in an oven at 100-105°C until two consecutive weighings did not vary by more than 0.5 mg. The dish was cooled in a desiccator and weighed. The loss in weight was recorded as moisture. The results were expressed as a percentage.

Formula: Moisture Content % = (X - Y) / W x 100

Determination of Extractive Value [10]

Alcohol Soluble Extractive Value - Cold Maceration

5 g of powdered dried material was weighed and macerated with 95% ethanol by shaking consistently and frequently. The mixture was allowed to stand for 24 hours, filtered, and transferred to a tarred flat-bottom dish. It was further evaporated to dryness in a water bath. The dried extract was cooled in a desiccator and weighed. The extractable matter in mg per g of air-dried material was calculated. The result was expressed as a percentage.

Water soluble extractive value - cold maceration

5 g of the powdered sample material was weighed and macerated with chloroform water (preservative) in a 250 ml conical flask. The flask was corked and left. The flask was corked and left undisturbed for 24 hours. The mixture was then filtered, and the filtrate was transferred to a tared flat-bottom dish. It was evaporated to dryness in a water bath, and the dried extract was cooled in a desiccator and weighed. The extractable matter in mg per g of air-dried material was calculated, and the result was expressed as a percentage.

Total Ash Value

2-5 g of the powdered material was accurately weighed and placed in a previously ignited and tarred crucible. The crucible was heated gradually until the carbon was completely burnt, and the residue was white or nearly white. It was then cooled in a desiccator and weighed. The percentage of total ash with respect to the air-dried drug was calculated.

Acid-Insoluble Ash Value

The total ash obtained above was boiled with 25 ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on an ash less filter paper and washed with hot water until the washings were neutral. The filter paper along with the insoluble matter was transferred to the crucible previously used, ignited, cooled, and weighed. The percentage of acid-insoluble ash with respect to the air-dried drug was calculated.

Determination of pH

A 10% w/v aqueous suspension of the powdered material was prepared, and the pH of the suspension was measured using a digital pH meter.

Determination of Loss on Drying

1-2 g of the powdered material was accurately weighed and placed in a previously weighed and tarred Petri dish. The dish with the sample was dried in an oven at 105 °C for 5 hours. It was then cooled in a desiccator and weighed. The loss in weight was recorded as the percentage of loss on drying.

Determination of Heavy Metals

The heavy metal content in the plant extracts was determined using standard procedures such as atomic absorption spectroscopy (AAS) or inductively coupled plasma-mass spectrometry (ICP-MS).

Separation and Identification of active fractions using Gas Chromatography-Mass spectroscopy

The GC-MS analysis of the aqueous extract of *Psidium guajava* leaf was conducted following a laboratory protocol [11]. The analysis was performed using an Agilent Technologies 6890 Series GC system coupled with an Agilent 5973 mass selective detector, controlled by Agilent compatible software. An HP-5MS capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm was used. For the analysis, ultra-pure helium gas was used as the carrier gas at a flow rate of 1.0 mL/min, resulting in a linear velocity of 37 cm/sec. The injector temperature was set at 250 °C. The initial oven temperature was 60 ◦C and was then programmed to increase to 280 ◦C at a rate of 10 ◦C/min, with a hold time of 4 min at each increment. Sample injections of 2 µL were made in the splitless mode, with a split ratio of 20:1. The mass spectrometer parameters for the

analysis were as follows: Electron ionization mode with an electron multiplier voltage set at 70 eV and 1859 V, respectively; ion source temperature at 230 °C; quadrupole temperature at 150 °C; solvent delay set at 4 min; and can range from 50 to 700 am. To identify the compounds, the retention times and mass spectral data were compared with those available in the Central Drug Research Institute (CDRI) library.

Results

Physicochemical parameters

Physicochemical parameters like loss on drying, percentage moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive, and pH were determined and depicted in Table. The results of the fluorescence analysis of the powdered drug are mentioned in Table 1.

Table 1: Physicochemical characters of *Psidium guajava* and *Beta vulgaris* leaves powder

S. No.	Parameters	Value of *Psidium guajava*	Value of *Beta vulgaris*
1.	Loss on drying at 105 ºC (%w/w)	6.01	5.33
2.	Total ash value (%w/w)	6.22	6.56
3.	Acid insoluble ash value (%w/w)	5.87	6.11
4.	Water soluble extractive value (%w/w)	20.56	21.3
5.	Alcohol soluble extractive value (%w/w)	12.8	11.44
6.	pH (Filter of 10% w/v aqueous solution)	4.22	4.98
7.	Foreign organic matter (%w/w)	0.2	0.6

Fluorescence analysis Fluorescence analysis was performed on the powder of *Psidium guajava* and *Beta vulgaris* and the result is mentioned in Tables 2 & 3.

Table 2: Fluorescence analysis of *Psidium guajava* leaves powder

S. No.	Reagents	UV Light (254 nm)	UV Light (366 nm)	Visible light
1.	As such powder	Green	Yellowish green	Pale green
2.	Picric acid	Dark green	Greenish black	Yellow
3.	H_2SO_4	Dark brown	Green	Dark brown
4.	$FeCl_3$	Green	Greenish black	Pale green
5.	HCl	Light green	Green	Brown
6.	Acetic acid	Green	Green	Brown
7.	HNO_3	Brown	Brownish black	Orange
8.	NaOH	Dark green	Green	Dark green
9.	H_2O	Green	Green	Green

Table 3: Fluorescence analysis of *Beta vulgaris* leaves powder

S. No.	Reagents	UV Light (254 nm)	UV Light (366nm)	Visible light
1.	Powder as such	Green	Dark green	Dark green
2.	NAOH	Light green	Black	Green
3.	H_2SO_4	Dark brown	Brown	Light brown
4.	HCL	Light blue	Blue	Reddish purple
5.	Acetone	Dark red	Light green	Light green
6.	Chloroform	Red	Dark green	Greenish yellow
7.	HNO_3	Green	Bluish black	Pale yellow
8.	$FeCl_3$	Light blue	Dark blue	Black
9.	KOH	Light blue	Dark blue	Reddish yellow
10.	Acetic acid	Black	Black	Light red

Preliminary phytochemical screening

Preliminary phytochemical screening was performed on methanolic extract of leaves part of *Psidium gaujava* and ethanolic extract of leaves part of *Beta vulgaris* and results were shown in Table 4.

Table 4: Phytochemical screening of methanolic and ethanolic extract of leaves part of *Psidium gaujava* and *Beta vulgaris*

S. No.	Test	Methanolic extract of *Psidium guajava*	Ethanolic extract of Beta vulgaris
1	**Alkaloids**		
	Mayer's reagent	−	−
	Dragendorff's reagent	++	−
	Wagner's reagent	−	−
	Hager's reagent	+	−
2	**Saponins**		
	Froth test	−	+
3	**Steroids**		

	Salkowasld test	++	++
	Leibermann's reagent	++	+
4	**Carbohydrates**		
	Molisch's test	++	++
	Fehling's test	+	+
5	**Anthraquinone Glycosides**		
	Borntrager's test	−	+
6	Cardiac Glycosides		
	Legal test	−	++
	Keller killiani test	−	++
7	**Tannins**		
	Lead acetate solution	+++	+
	Ferric chloride solution	++	++
8	**Proteins**		
	Xanthoprotein test	−	+
	Biuret test	−	++
9	**Flavonoids test**		
	Aq. Sodium hydroxide solution	++	++
	Conc. Sulphuric acid test	++	+
	Shinoda's test	++	++

+ = present, - = absent

Antiulcer activity

The effect of the oral administration of separate plant extracts and a combination of plant extracts gives an appreciable response to ulcer index reduction. The administration of crude methanolic extract of *Psidium guajava* in doses: 500 mg/kg body weight, ethanolic extract of *Beta vulgaris* in doses: 250 mg/kg body wt. and a combination of both drugs in doses: (1) 250 mg/kgBV and 250 mg/kg PG (1:1) and (2) 250 mg/kg body wt. BV and 500 mg/kg of PV (1:2) causes a decrease in the ulcer index of ethanolic induce ulceration in the stomach of Wistar rats (table 6.5). Ethanol-induced gastric ulcer was employed to study the ulcer protective effect of guava leaves extracts and Beta vulgaris leaves extract. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. The effect of the standard drug ranitidine (50 mg/kg) reduction in ulcer levels of different groups is significant (p<0.0001).

Table 5: Effect of *Beta vulgaris* ethanolic extract (BVEE) in pylorus ligation-induced ulcer in rats

Groups	Ulcer index	Gastric volume	Free acidity	Total acidity	Ph
Normal control	1.008±0.09	0.138±0.09	12.47±0.5	21.74±0.2	3.69±0.3
Control	14.45±0.03	2.64±0.2	245.6±0.03	693.6±0.1	1.9±0.1
Ran 50	4.67±0.02***	0.903±0.038**	24.9±0.2**	42.65±0.1	4.45±0.1**
BVEE 250mg/kg	8.63±0.01**	1.47±0.18*	61.98±0.1*	159.01±0.2	2.46±0.2*

The values are the mean SEM of the six animals in each group. Statistical analysis was performed using one-way ANOVA followed by Tukeye's Kramer multiple comparison test. **p<0.0001 compared with control.

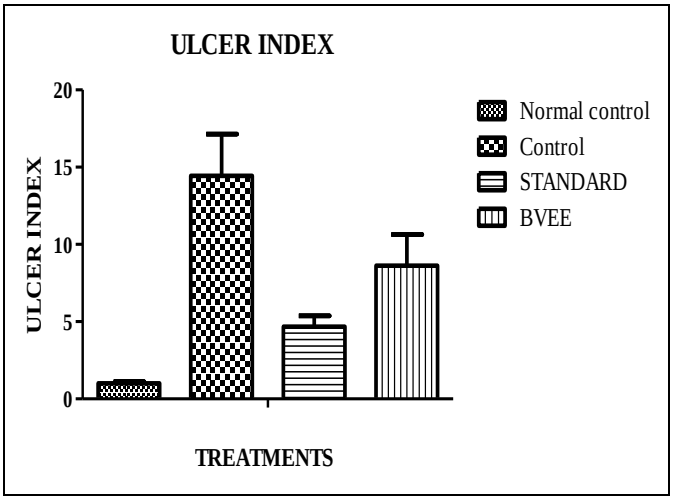


Fig 1: Effect of BVEE on ulcer index correlation between ulcer index and treatments

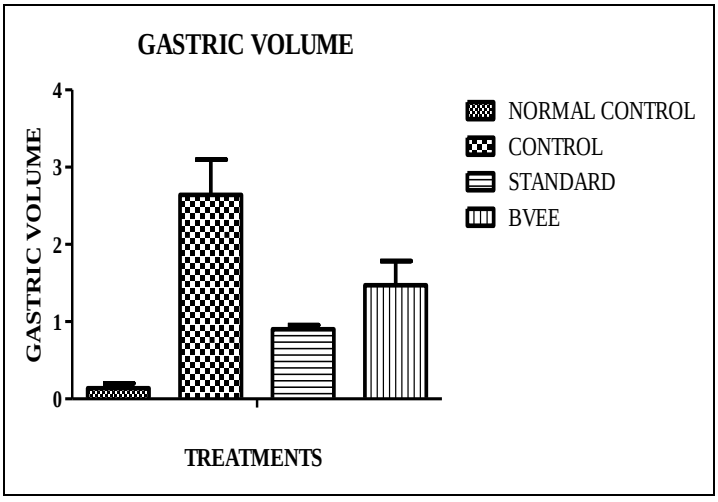


Fig 2: Effect of BVEE on gastric volume correlation between gastric volume and treatments

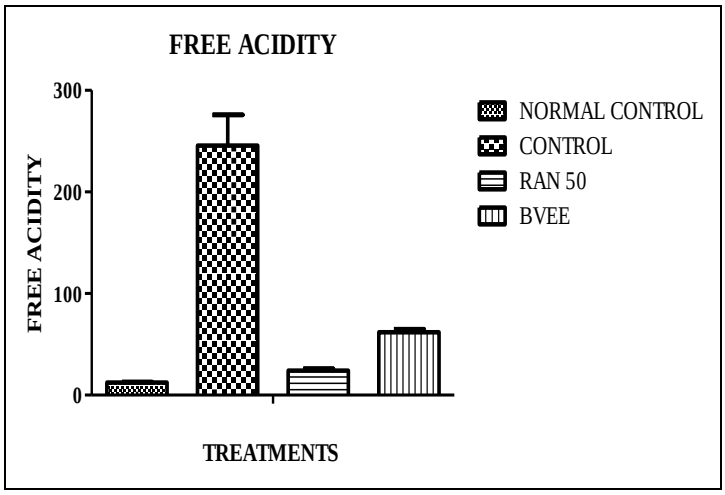


Fig 3: Effect of BVEE on free acidity correlation between free acidity and treatments

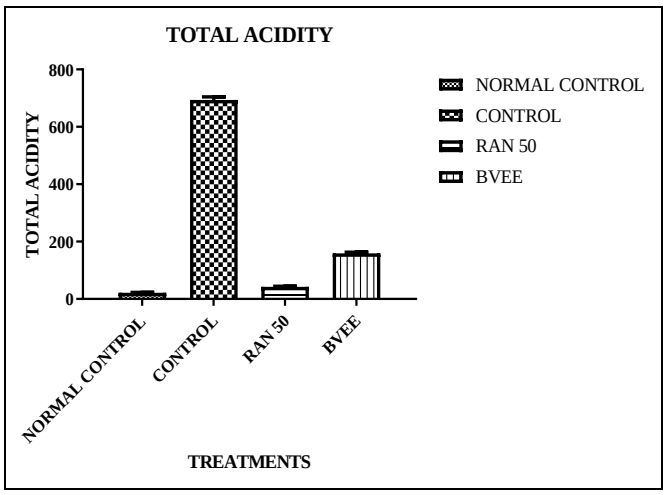


Fig 4: Effect of BVEE on total acidity correlation between total acidity and treatments

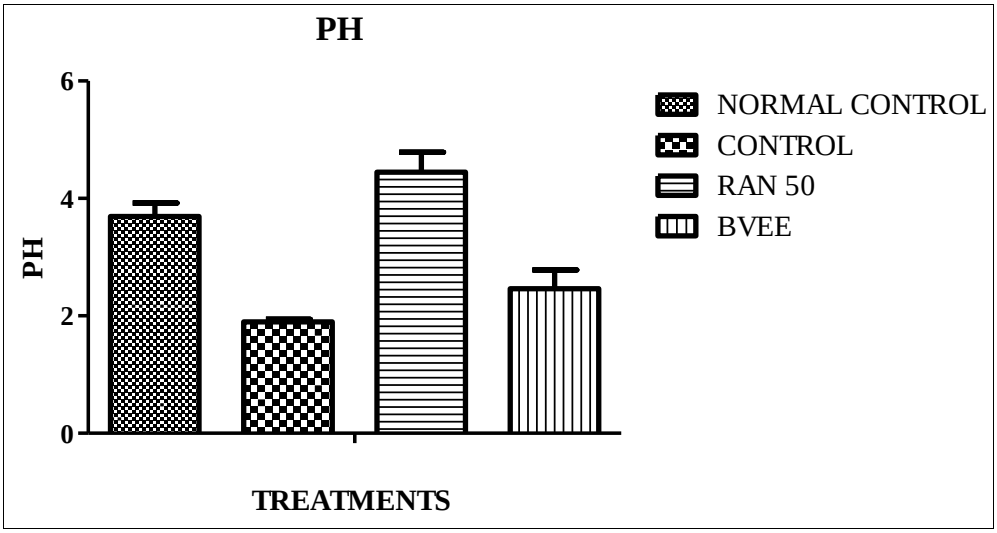


Fig 5: Effect of BVEE on pH and correlation between pH and treatments

Table 6: Effect of *Psidium guajava* methanolic extract (PGME) in pylorus ligation-induced ulcer in rats

Groups	Ulcer index	Gastric volume	Free acidity	Total acidity	Ph
Normal control	1.008±0.09	0.138±0.09	12.47±0.5	21.74±0.002	3.69±0.3
Control	14.45±0.3	2.64±0.2	245.6±0.03	693.6±0.1	1.9±0.1
Standard	4.67±0.2***	0.903±0.03**	24.9±0.2**	42.65±0.01**	4.45±0.1**
PGME500mg/kg	7.42±0.1***	1.13±0.1*	47.66±0.1*	96.93±0.2*	2.86±0.2*

The values are the mean SEM of the six animals in each group. Statistical analysis was performed using one-way ANOVA followed by Tukeye's Kramer multiple comparison test. ** p<0.0001 compared with control.

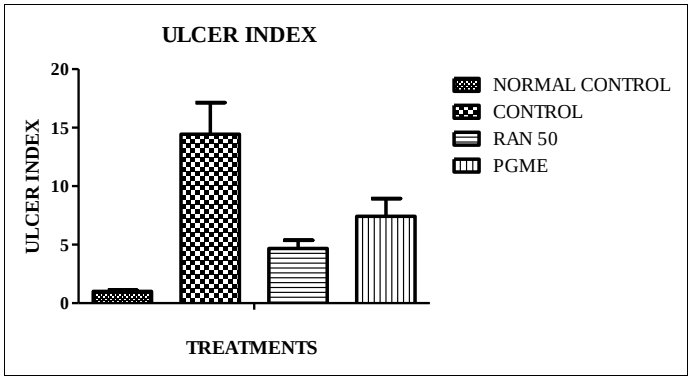


Fig 6: Effect of PGME on ulcer index correlation between ulcer index and treatments

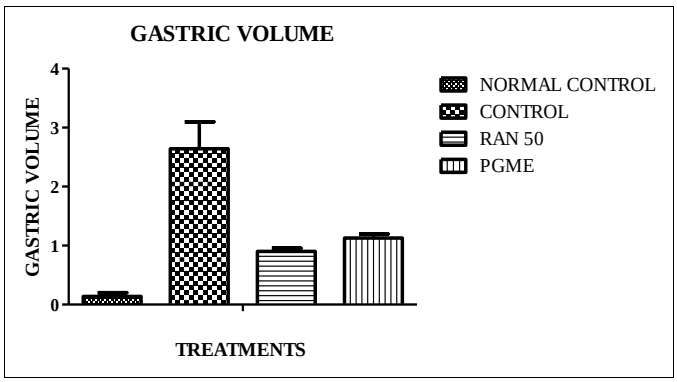


Fig 7: Effect of PGME on gastric volume correlation between gastric volume and treatments

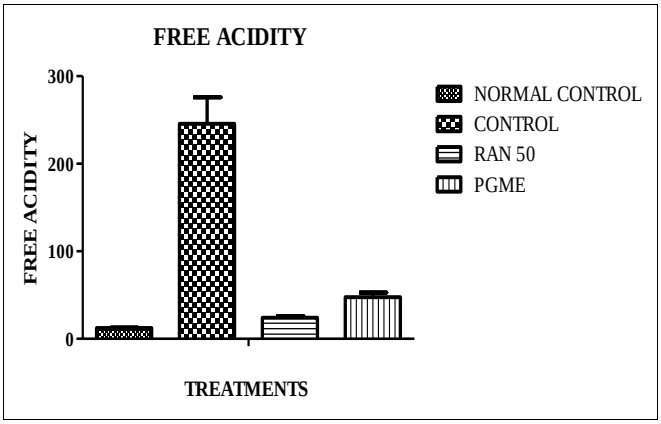


Fig 8: Effect of PGME on free acidity correlation between free acidity and treatments

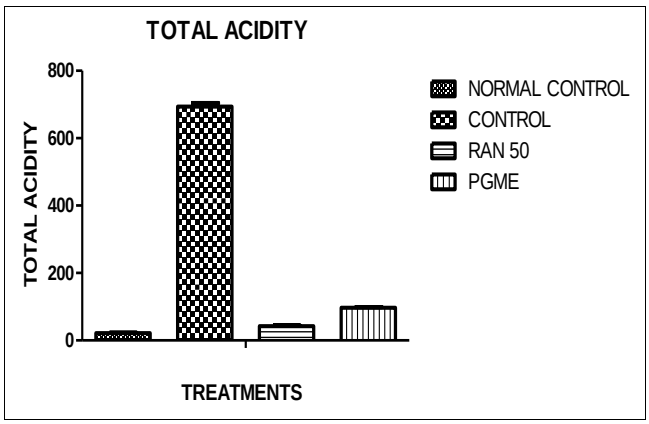


Fig 9: Effect of PGME on total acidity and correlation between total acidity and treatments

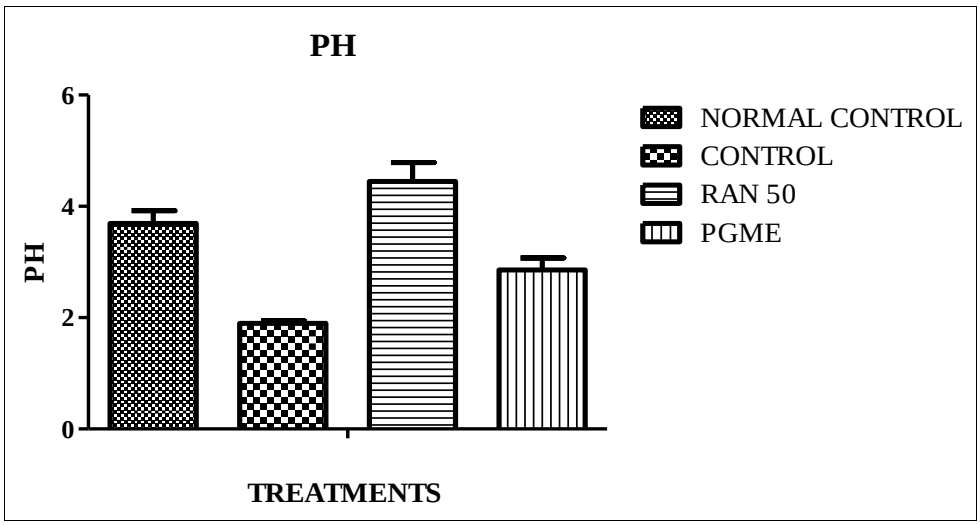


Fig 10: Effect of PGME on pH correlation between pH and treatments

Table 7: Effect of Combination of drug extract in pylorus ligation induced Ulcer in rats

Groups	Ulcer index	Gastric volume	Free acidity	Total acidity	Ph
Normal control	1.008±0.09	0.138±0.09	12.47±0.5	21.74±0.2	3.69±0.3
Control	14.45±0.3	2.64±0.2	245.6±0.3	693.6±0.1	1.9±0.1
Standard	4.67±0.2***	0.903±0.03**	24.9±0.2**	42.65±0.1**	4.45±0.1**
COM 1 BV250 PG 250	5.43±0.1**	1.6±0.3*	25.55±0.3*	53.53±0.2*	4.13±0.1*
COM 2 BV250 PG 500	4.44±0.2***	0.981±0.01**	24.05±0.7**	52.18±0.9**	3.566±0.02**

The values are the mean SEM of the six animals in each group. Statistical analysis was performed using one way ANOVA followed by Tukeye's Kramer multiple comparison test. ***p*<0.0001 compared with control.

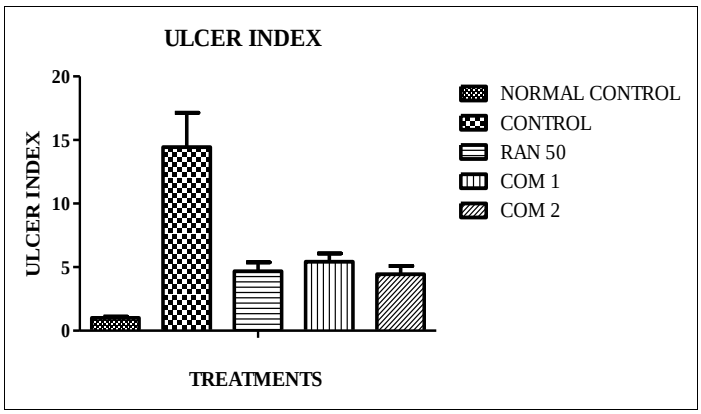


Fig 11: Effect of combination (COM1 and COM2) on ulcer index correlation between ulcer index and treatments

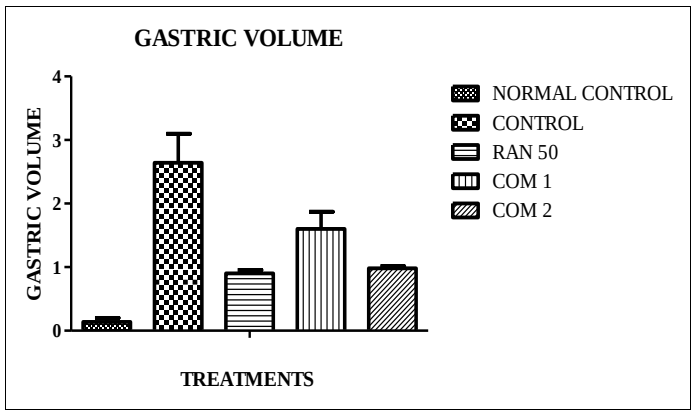


Fig 12: Effect of combination (COM1 and COM2) on gastric volume correlation between gastric volume and treatments

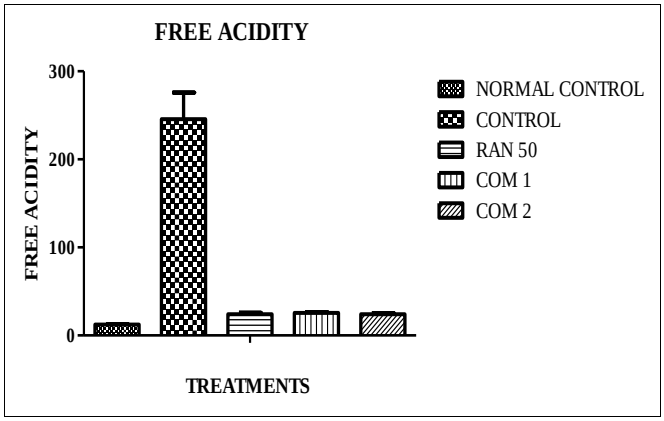


Fig 13: Effect of combination (COM 1 and COM 2) on free acidity correlation between free acidity and treatments

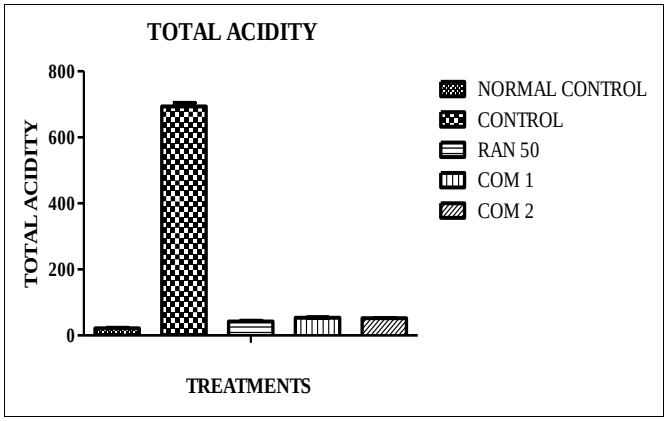


Fig 14: Effect of combination (COM 1 and COM 2) on total acidity correlation between total acidity and treatments

The histopathological examination of the stomach sections from normal control rats revealed no signs of ulceration, indicating a healthy gastric condition. The transverse section of the stomach displayed a consistent and uniform arrangement of cells, as depicted in Fig 6.16.

In contrast, the control group of rats exhibited significant distortion of cells, indicating the presence of ulcers. The arrangement of cells in this group was highly disordered, indicating the severity of gastric damage. This observation is supported by Fig 6.17.

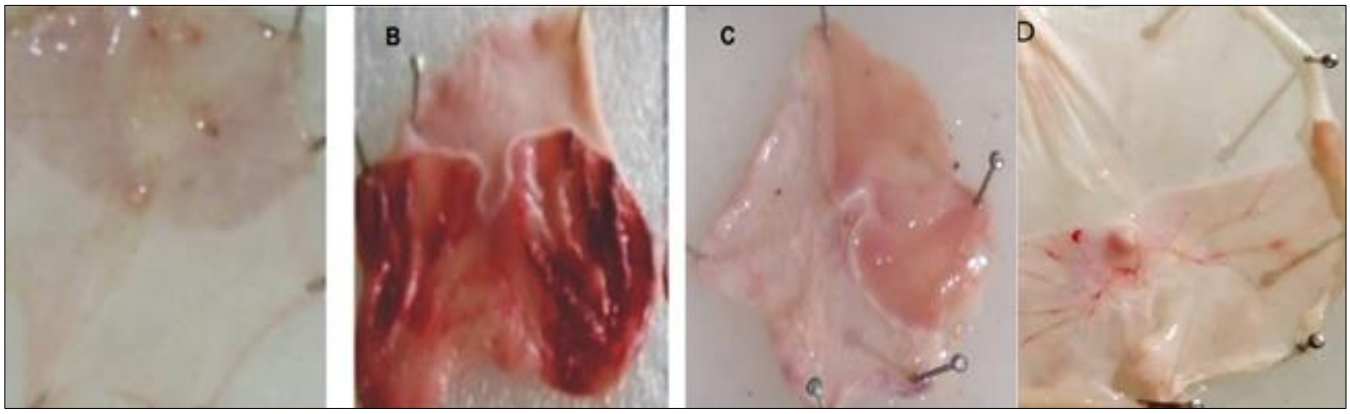
However, when treated with the standard drug Ranitidine, the rats displayed a marked improvement in cell arrangement. The cells appeared to be more organized, suggesting a therapeutic effect against ulcer formation (Fig 6.17).

Upon treatment with the ethanolic extract of *Beta vulgaris*, the rats exhibited fewer ulcers compared to the control group. However, the extract was not as effective as the standard drug or the combination treatment. Figures 6.16 and 6.17 illustrate this finding.

Similarly, the administration of the *Psidium guajava* extract resulted in a reduced formation of ulcers in the treated rats, although some ulcers were still present. This is demonstrated in Figures 6.18 and 6.19.

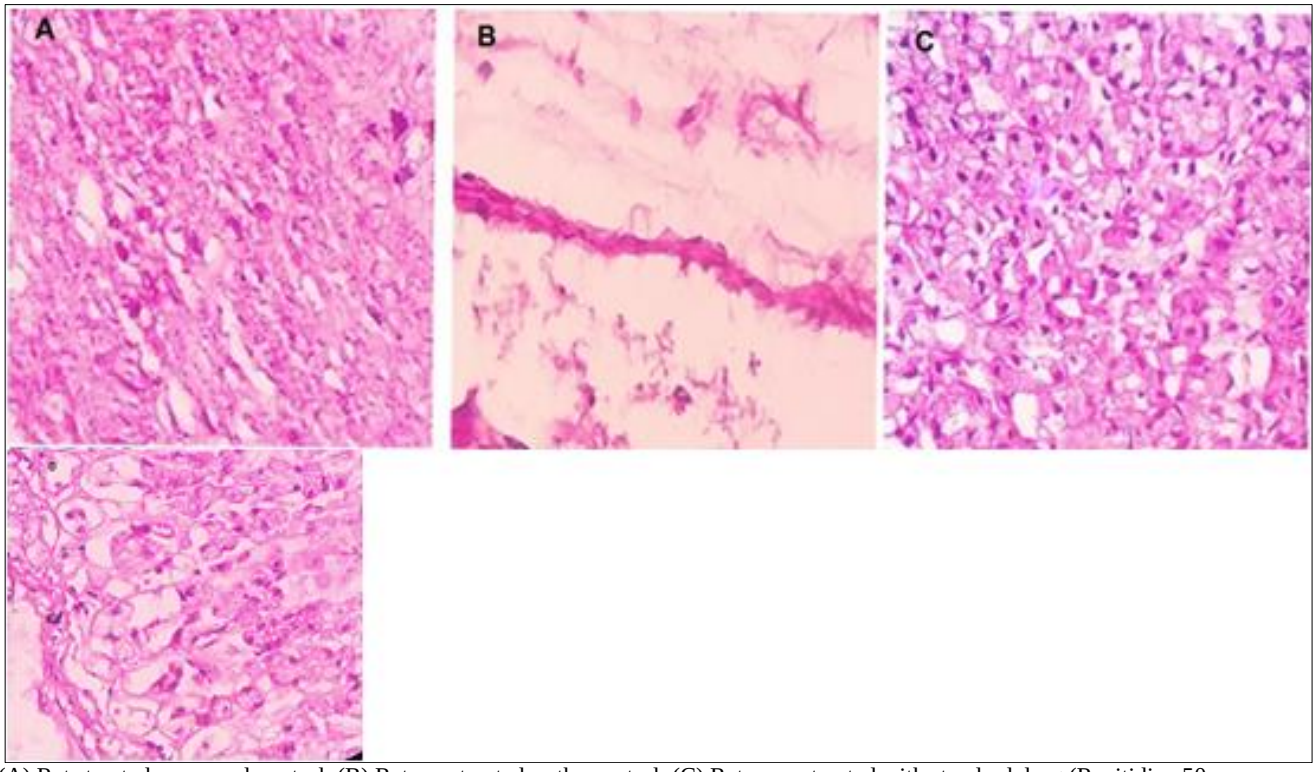
Remarkably, the histopathological examination of the stomach sections from rats treated with the combination dose extract (COM 1 and COM 2) revealed a significant reduction in ulceration or complete absence of ulcers when compared to the *Psidium guajava* extract, *Beta vulgaris* extract, and the standard drug. This favourable outcome is represented in Figures 6.20 and 6.21.

Overall, the histopathological analysis indicates that the combination treatment exhibited superior efficacy in reducing ulcer formation compared to the individual plant extracts or the standard drug. These findings suggest the potential of the combination treatment as a promising therapeutic approach for managing gastric ulcers.



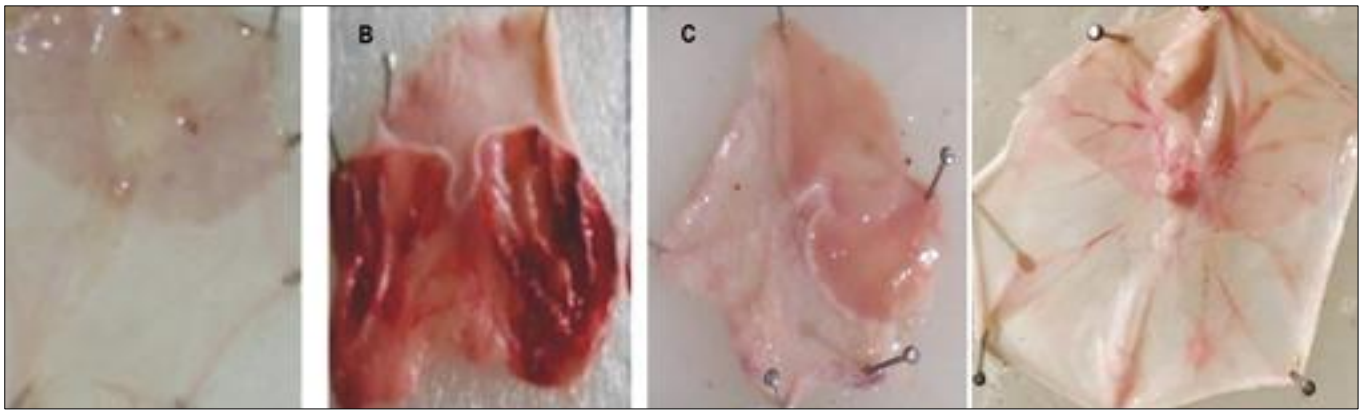
(A) Rats treated as normal control. (B) Rats are treated as the control. (C) Rats were treated with a standard drug (Ranitidine 50 mg/kg). (D) Rats treated with test extract (*Beta vulgaris* 250 mg/kg).

Fig 16: Gastric mucosa of the pylorus Iigated rat showing ulcers mostly in the rumen of the stomach and the stomach was treated with *Beta vulgaris* extract



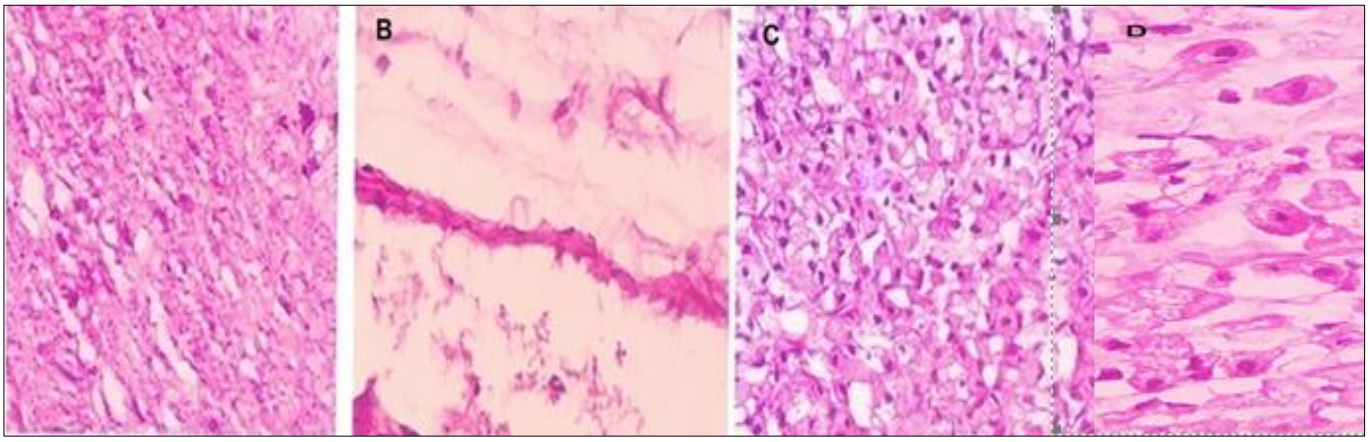
(A) Rats treated as normal control. (B) Rats are treated as the control. (C) Rats were treated with standard drug (Ranitidine 50 mg/kg). (D) Rats treated with test extract (Beta *vulgaris* 250 mg/kg).

Fig 17: Histological sections of the stomach from rat



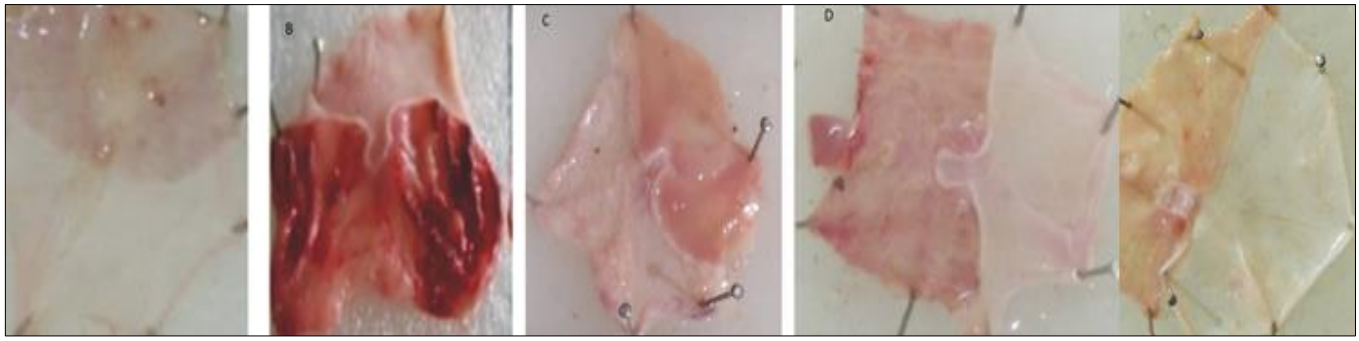
(A) Rats treated as normal control. (B) Rats are treated as the control. (C) Rats treated with standard drug (Ranitidine 50 mg/kg). (D) Rats treated with test extract (*Psidium guajava* 250 mg/kg).

Fig 18: Gastric mucosa of the pylorus Iigated rat showing ulcers mostly in the rumen of the stomach and the stomach was treated with *Psidium guajavas* extract.



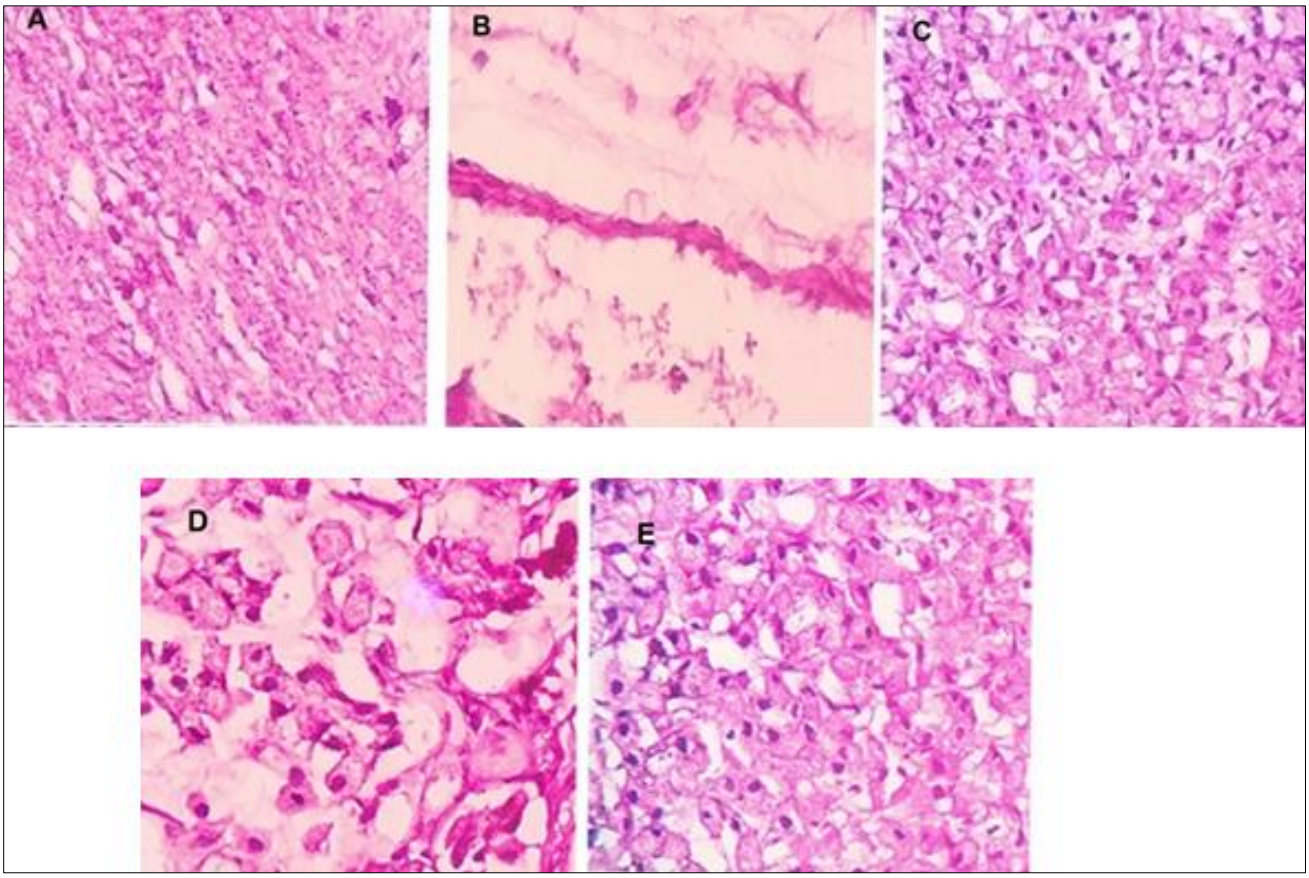
(A) Rats treated as normal control. (B) Rats are treated as the control. (C) Rats treated with standard drug (Ranitidine 50 mg/kg). (D) Rats treated with test extract (*Psidium guajava* 250 mg/kg).

Fig 19: Histological sections of the stomach from rat.



(A) Rats treated as normal control. (B) Rats are treated as the control. (C) Rats treated with standard drug (Ranitidine 50 mg/kg). (D) Rats treated with the combination dose 1 (*Beta vulgaris* (1): (1) *Psidium guajava*). (E) Rats treated with the combination dose 2 (*Beta vulgaris* (1): (2) *Psidium guajava*).

Fig 20: Gastric mucosa of the pylorus Iigated rat showing ulcers mostly in the rumen of the stomach and the stomach was treated with *Psidium guajavas* extract



(A) Rats treated as normal control. (B) Rats treated as the control. (C) Rats treated with standard drug (Ranitidine 50 mg/kg). (D) Rats treated with the combination dose 1 (*Beta vulgaris* (1): (1) *Psidium guajava*). (E) Rats treated with the combination dose 2 (*Beta vulgaris* (1): (2) *Psidium guajava*).

Fig 21: Histological sections of the stomach from rat

Result

Fractionation followed by GC-MS was conducted to determine the bioactive compounds responsible for the observed antiulcer effect. Through comparison of their MS spectra with standard spectra from the CDRI library, two compounds, beta-disabling and caffeic acid, were identified in the *Psidium guajava* extract. These findings suggest that the extract contains phytochemicals with the potential to inhibit the accumulation of free radicals, which are known to cause cell damage and ulceration. Comparative chromatography confirmed the presence of caffeic acid in the extract of *Beta vulgaris* leaves, which is known for its high antioxidant properties [12, 13]. However, it is not yet clear whether these compounds contribute to the antiulcer properties of guava leaves and beetroot. In summary, the results of this study demonstrate that the combined extract of *Psidium guajava* leaves exhibits an antiulcer effect in pylorus ligation-induced ulcers.

Discussion

The physicochemical analysis of the drugs provided valuable insights into their properties. The total ash content was found to be 6.22% in *Psidium guajava* leaves and 6.56% in *Beta vulgaris* leaves. The presence of foreign organic matter was negligible, measuring less than 0.6%. Both plants exhibited water-soluble extractives values exceeding 20%. The pH values of the extracts were measured as 4.2 for *Psidium guajava* and 4.9 for *Beta vulgaris*, indicating their acidic nature. Additionally, fluorescence analysis confirmed the quality and purity of both drugs. Preliminary phytochemical screening of the plant materials revealed the presence of various phytochemicals. The methanolic leaf extract of

Psidium guajava showed a lower number of alkaloids but a higher amount of flavonoids, which are known for their antiulcer activity. Conversely, *Beta vulgaris*, which is rich in saponins, demonstrated antiulcer properties attributed to this particular phytochemical. To induce ulcers in the study, pylorus ligation was performed for 6 hours using Shay's method. This method was chosen due to its ability to produce severe ulcers in the stomach, with the added advantage of easily collecting gastric juice. The results confirmed that the pylorus ligation technique predominantly caused circular and inner lesions in the rumen of the stomach, which is lined by squamous epithelium lacking glandular structures and secretions.

The involvement of the rumen in ulceration during pylorus ligation can be attributed to increased stress-induced gastric hydrochloric acid secretion from the glandular portion into the rumen. The presence of acid in the stomach and stasis of secreted acid are important factors in ulcer formation. The study also suggested the potential involvement of free radicals in the pathogenesis of pylorus ligation-induced ulcers in rats.

Peptic ulcers are recurrent diseases resulting from an imbalance between defensive (Cytoprotective) and offensive factors (gastric acid), often associated with Helicobacter pylori infection and the use of non-steroidal anti-inflammatory drugs. In this study, a combination of both plant extracts significantly reduced basal gastric secretion and inhibited ulcers, indicating a Cytoprotective mechanism of action on the gastric mucosa. This effect may be attributed to the extracts' ability to reduce gastric acid secretion, possibly through various mechanisms. Fractionation followed by GC-MS analysis identified beta-bisabolene and caffeic acid in the *Psidium guajava* extract, compounds known for their anti-inflammatory and antioxidant properties. Comparative chromatography confirmed the presence of caffeic acid in Beta vulgaris extract, which is recognized for its high antioxidant properties.

The combination of the methanolic extract of *Psidium guajava* and the ethanolic extract of *Beta vulgaris* demonstrated anticholinergic and vagolytic activities, contributing to the inhibition of aggressive factors and irritant receptors. The protective coating provided by the extracts reinforced the resistance of the mucosal barrier, leading to decreased acidity levels and increased pH in gastric fluid. Moreover, the extracts showed a decrease in pepsin levels (an aggressive factor) and an increase in cumin (a defensive factor), further supporting their antiulcer effects.

Histopathological analysis confirmed that the antiulcer effects of the *Psidium guajava* and *Beta vulgaris* extracts were attributed to the presence of flavonoids and Saponins, respectively. The flavonoids in the *Psidium guajava* methanolic leaf extract and the Saponins in the Beta vulgaris ethanolic extract exhibited significant efficacy in treating peptic ulcers. However, it is not yet clear whether the identified compounds, beta-bisabolene and caffeic acid, contribute to the observed antiulcer properties of guava leaves and beetroot.

In summary, the physicochemical properties, preliminary phytochemical screening, experimental findings, and the GC-MS study demonstrated the potential of the combination treatment using *Psidium guajava* and *Beta vulgaris* extracts for their antiulcer activity. These extracts exhibited anti-secretory, Cytoprotective, anticholinergic, and vagolytic effects, leading to a reduction in aggressive factors and an enhancement of defensive factors. The histopathological analysis, along with the identification of flavonoids and Saponins through the GC-MS study, further supported the effectiveness of these plant extracts in treating peptic ulcers.

Cconclusion

Based on the discussed findings, it can be concluded that the combination treatment using *Psidium guajava* and *Beta vulgaris* extracts holds significant promise as an alternative approach for the management of peptic ulcers. The physicochemical analysis of the extracts revealed their acidic nature and the absence of foreign organic matter, ensuring their quality and purity.

Preliminary phytochemical screening demonstrated the presence of various bioactive compounds in *Psidium guajava* and *Beta vulgaris* extracts. The GC-MS study identified beta-bisabolene and caffeic acid in the *Psidium guajava* extract, known for their anti-inflammatory and antioxidant properties, respectively. Comparative chromatography confirmed the presence of caffeic acid in *Beta vulgaris* extract, recognized for its high antioxidant properties.

In experimental studies, the combination of *Psidium guajava* and *Beta vulgaris* extracts showed notable antiulcer effects. It effectively reduced basal gastric secretion, inhibited the formation of ulcers, and exhibited Cytoprotective properties. The extracts exhibited anticholinergic and vagolytic activities, contributing to the inhibition of aggressive factors and irritant receptors. They also enhanced the resistance of the mucosal barrier by decreasing acidity levels, increasing pH in gastric fluid, and modulating pepsin and mucin levels.

The histopathological analysis further supported the efficacy of *Psidium guajava* and *Beta vulgaris* extracts in treating peptic ulcers. The presence of flavonoids in *Psidium guajava* and Saponins in *Beta vulgaris* extracts played a significant role in their antiulcer effects.

Overall, these findings highlight the potential of the combined extract of *Psidium guajava* and *Beta vulgaris* as a promising alternative for peptic ulcer management. By targeting multiple factors involved in ulcer formation, including gastric acid secretion, aggressive factors, and defensive factors, this combination treatment offers a safer and more efficient approach to ulcer therapy.

Further research and clinical studies are warranted to explore the specific mechanisms of action, optimal dosages, and potential synergistic effects of these extracts. Nevertheless, the results of this study provide valuable insights into the therapeutic potential of *Psidium guajava* and *Beta vulgaris* extracts, opening avenues for the development of novel and effective antiulcer therapies.

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