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Exploring the Anti-ulcer Potential of *Wodyetia bifurcata*: A Phytotherapeutic Approach

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Abstract

Background*: Wodyetia bifurcata*, commonly known as the foxtail palm, is an ornamental plant from the Arecaceae family that is increasingly being explored for its pharmacological potential due to its bioactive compounds. **Aim:** To evaluate the anti-ulcer potential of *W. bifurcata* seed pulp extract and its Phytotherapeutic approach. **Methods:** This study focused on identifying phytochemicals in the aqueous seed pulp extract of *W. bifurcata* (ASPeWB) using GC-MS mass spectrometry and evaluating its anti-ulcer activity through water immersion stress-induced ulcer models. **Results**: Preliminary phytochemical analysis confirmed the presence of phenols, tannins, steroids, and flavonoids. GC-MS profiling identified various metabolites, including organic acids, amino acids, fatty acids, sugars, polyols, and other constituents. Notably, compounds such as nitric oxide and N-(Trifluoroacetyl)-N,O,O',O''-tetrakis (trimethylsilyl) norepinephrine have been associated with anti-ulcer effects. *In vitro* experiments demonstrated that ASPeWB at a dose of 500 mg/kg significantly displayed acid neutralizing capacity and increased artificial gastric acid pH ($p<0.001$). *In vivo* assessments showed that ASPeWB (500 mg/kg) markedly modulated gastric parameters reducing gastric volume and total acidity and elevating pH ($p<0.001$) and decreased ulcer index and gastric acidity while increasing gastric pH and plasma corticosterone levels ($p<0.001$). Histopathological examination of gastric mucosa confirmed the anti-ulcer activity of ASPeWB, which was comparable to omeprazole (20 mg/kg). **Conclusion:** These findings suggest that *W. bifurcata* possesses significant potential as a natural anti-ulcer agent, warranting further clinical investigation to validate its efficacy and facilitate its integration into medical practice.

Major Findings: The decrease in gastric acid secretion, evidenced by reduced gastric volume and acidity, combined with the elevation of gastric pH, indicates a direct or indirect inhibitory effect on acid production. Decrease in plasma corticosterone levels suggests a systemic stress-modulating effect that may contribute to mucosal protection. Inhibit ulcer formation, promote mucosal healing, and restore gastric homeostasis, highlighting *W. bifurcata's* potential as a multifaceted natural anti-ulcer agent.

Key words: Anti-ulcer Activity, Omeprazole, *Wodyetia bifurcata* (ASPeWB)

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1. Introduction

Peptic Ulcer Disease (PUD) remains a significant global health issue, with a high prevalence in both developed and developing countries. It is primarily caused by an imbalance between aggressive factors like gastric acid, pepsin, *Helicobacter pylori* infection, and the protective factors of the gastric mucosa[1]. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), such as indomethacin, are also known to cause ulcers by inhibiting prostaglandin synthesis, thereby compromising mucosal defense mechanisms[2].

Although conventional treatment strategies involving proton pump inhibitors (PPIs), histamine H2-receptor antagonists, and antibiotics are effective, they are associated with significant adverse effects such

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as nutrient malabsorption, increased risk of infections, and drug resistance[3]. Therefore, there is a compelling need to explore safer and more sustainable alternatives. Natural products and plant-based medicines have long been valued for their therapeutic efficacy and safety profiles in the management of gastrointestinal disorders[4].

Wodyetia bifurcata (Family: *Arecaceae*), commonly known as the foxtail palm, is a palm species native to Australia, traditionally used in folk medicine for various ailments. Although primarily recognized for its ornamental value, preliminary studies suggest that *W. bifurcata* may contain phytochemicals with antioxidant and anti-inflammatory activities[5]. These pharmacological properties are particularly relevant to ulcer prevention and healing, as oxidative stress and inflammation play pivotal roles in mucosal damage[6,7].

This study aims to assess the anti-ulcer potential of aqueous extract of *W. bifurcata* seed pulp by evaluating its anti-ulcer effects using *in vitro* Acid Neutralising Capacity (ANC) and *in vivo* stress-induced ulcer models. Additionally, the physico-chemical properties and phytochemical profile of the aqueous extract of seed pulp of *Wodyetia bifurcata* were analyzed using GC-MS technology. The research seeks to validate the traditional use of *W. bifurcata* and explore its potential as a natural, plant-based therapeutic for ulcer treatment.

2. Materials and Methods

Evans blue, Pepsin, Omeprazole, Ketamine, Topfors reagent, Phenolphthalein, formalin were purchased from S D Fine-Chem Limited, India. Other chemicals of analytical grade were purchased from local vendors.

2.1 Collection of the *W. bifurcata* Seeds

The mature fruits of *W. bifurcata* (commonly known as Foxtail Palm) were collected from the Thoothukudi dist, Tamil Nadu, India and seeds were authenticated by Dr. V. Chelladuri, Retired Research Officer – Botany, Central Council for Research in Ayurveda and Siddha (C.C.R.A.S), Govt. of India. The voucher specimen with accession number XCH-40566 was deposited in departmental herbarium of the institute. After harvesting, the fruits were thoroughly washed with distilled water to remove surface impurities. The outer fibrous layer was carefully removed to isolate the seeds, and the pulp surrounding the seeds was manually separated using sterile tools. The collected pulp was then air-dried under shade at room temperature for several days until constant weight was achieved, ensuring minimal degradation of Phytoconstituents. The dried pulp was powdered using a mechanical grinder and stored in an airtight container at room temperature for further phytochemical and pharmacological evaluation.

2.2 Preparation of Crude Extracts of *W. bifurcata* Seed Pulp

Weigh a specific amount (100g) of the dried pulp and place it into a clean, heat-resistant container. Add distilled water in a typical ratio of 1:10 (w/v), meaning 1 part pulp to 10 parts water. Bring the mixture to a boil and let it simmer gently for approximately 30 to 60 minutes; this process facilitates the extraction of heat-stable, water-soluble phytochemicals. After simmering, allow the decoction to cool to room temperature. Then, filter the mixture using Whatman No. 1 filter paper to separate the liquid extract from the plant residue[8, 9]. The resulting aqueous extract of seed pulp of *W. bifurcata* (ASPeWB) can be used immediately for further applications or stored at 4°C for short-term use.

2.3 Preliminary Phytochemical Screening

The phytochemicals examination of *W. bifurcata* seed pulp was performed by using the standard methods[10].

2.4 GC-MS Analysis of *W. bifurcata* Seed Pulp

2.4.1 Extraction and Derivatization of Phytochemicals

Phytometabolites have been extracted and derivatized according to the previous method[11]. ASPeWB (200 mg) powder was mixed with 1 ml 80% cold aqueous methanol, vortex for 10 min followed by sonication for 10 minutes and centrifuged at 14000 rpm for 10 minutes at 4 °C. Experiments were performed in triplicates. An aliquot was lyophilized and dried the sample using O-methoxamine hydrochloride (60µL) solution in anhydrous pyridine. Sample was mixed briskly for 1 min, and incubated at 65°C for 30 min in a thermomixer. Subsequently, silylation of samples were performed using 100 µL of MSTFA, 1% TMCS which is

used as a catalyst and incubated at 60 °C for 60 min in a thermomixer (Eppendrof AG 5382, Germany).

2.4.2 GC-MS Instrumentation and Data Acquisition

Derivatized samples were mixed with n-hexane (500µL), vortexed. Metabolite profiling of ASPeWB using GC-MS was performed. Experimental conditions of GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30 m, ID: 0.25 mm, Film: 0.25 mm was used and the flow rate of the mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, the temperature program (oven temperature) was 40 C raised to 250 C at 5 C/min and the injection volume was 1 mL. Samples dissolved in chloroform were run fully at a range of 50e650 m/z and the results were obtained. The mass spectra were detected in 36 min.

2.4.3 Data Pre-processing

AMDIS software (version 2.0) was used to identify the phytochemicals in samples. GC-MS raw data file were exported into NetCDF format by means of Chemstation software. Peaks were compared by using Wiley Spectral library search program. Each peak of mass spectral data was deconvoluted by utilizing software developed and maintained by Wiley Spectral library search program. It is used to computationally separated the chromatographically over lapping peaks.

2.5 Evaluation of *In Vitro* Antiulcer Activity of ASPeWB

2.5.1 In Vitro Anti-ulcer Assays

2.5.1.1 Preparation of Artificial Gastric Juice

A solution of artificial gastric acid was prepared by dissolving 2 grams of NaCl and 3.2 mg of pepsin enzyme in 500 ml of water. To this mixture, 7.0 ml of HCL were added, and the volume was adjusted to 1000ml with distilled water, achieving a final pH of 1.2[12].

2.5.1.2 Determination of pH

About 90ml of the prepared different concentrations of ASPeWB (100, 500, 1000, and 1500 mg/mL) were used for the pH determination at temperatures ranging from 25 ºC-37 ºC. The pH values of the control solution were also determined[12] using Infra digi instrument pvt.ltd. Chennai, India. Omeprazole (20mg/mL) was taken as standard drug.

2.5.2 Acid Neutralizing Capacity (ANC) Method

The Fordtran's model is a well-established *in vitro* titration procedure[12] designed to simulate human gastric acid conditions. It evaluates how effectively a substance can neutralize hydrochloric acid (HCl), thereby mimicking the acid-buffering potential in the human stomach. Acid-neutralizing capacity of ASPeWB was evaluated at doses of 100mg, 500mg, 1000mg and 1500mg, with omeprazole (20 mg) serving as the standard reference. 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; the mixture was stirred 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. ANC of ASPeWB was calculated using following formula: ANC= Volume of HCL consumed (mL)× Normality of HCL×100/Volume of ASPeWB solution (mL). The result is expressed as milliequivalents of acid neutralized per gram of extract (meq/g).

2.6 Evaluation of *In Vivo* Antiulcer Activity of ASPeWB

2.6.1 Water Immersion Stress-induced Ulcer Model

Healthy adult Wistar Albino rats of either sex weighing between 150-200 g were used. Animals were housed in standard polypropylene cages and maintained under controlled temperature (23 ± 2 °C) in a 12 h light/dark cycle. All the experimental protocols were duly approved by Institutional Animal Ethics Committee (Protocol Approval No: SPCP/2024-25/COL/02; dated 26/09/2024). Rats (150–200 g) were randomized into four groups (n=6).

Group –I (Control): rats were fed with regular pellet food and water.

Group-II (Stress-induced ulcer group): Rats were subjected to cold restraint stress by submersion in water (22°C) for 16 hr[13].

Group-III (Stress+Omeprazole 20mg/Kg): rats were pre-treated with Omeprazole 20mg/Kg and followed by induction of cold restraint stress by submersion in water (22°C) for 16 hr.

Group –IV (Stress+ ASPeWB 500mg/Kg): rats were pre-treated with ASPeWB 500mg/Kg, once daily by oral

route) induction of cold restraint stress by submersion in water (22°C) for 16 hr.

Following this, Evans blue dye (30 mg/kg) was intravenously administered, and animals were euthanized after 10 minutes. The excised stomachs were fixed in 10% formalin, sectioned along the greater curvature, and lesion lengths were assessed under magnification. Total ulcer scores were calculated, and gastroprotective efficacy was expressed as percent inhibition of lesion formation relative to control.

2.6.2 Gastric Juice Parameters

Gastric juice parameters of rats were determined as per the previous method[13]. Total gastric content was into centrifuge tubes and centrifuged at 1000 rpm for 10 minutes. Dilute 1 ml of the supernatant to 10 ml of distilled water, titrate against 0.01N sodium hydroxide (NaOH) using Topfor's reagent and Phenolphthalein as indicator. The volume of NaOH consumed can be taken as a measure of the acid present. Total acid content was calculated using the following formula: Total acid content (mg) = Volume of NaOH (mL) × Normality of NaOH× Equivalent weight of NaOH/ Sample volume (mL). The total acid content was expressed typically in milligrams or milliequivalents per milliliter or per deciliter.

2.6.3 Ulcer Index Assessment

Ulcer index in experimental rats was determined using a previously reported method. Immediately after the completion of the study period, the rats were sacrificed, and the stomachs were dissected out. The stomachs were incised along the greater curvature, and the mucosa was rinsed with cold normal saline to remove any blood contaminants. The tissues were then kept in 10% formalin solution overnight. The next day, the stomachs were examined under a magnifying lens, and the ulcers were measured with the help of a Vernier caliper using the following arbitrary scale. Score 0 = No ulcers; Score 1 = Petechial haemorrhages; Score 2 = Ulcers < 2 mm; Score 3 = Ulcers > 2 < 4 mm; Score 4 = Ulcers > 4 mm]. Ulcer index=Total ulcer score/ number of animals ulcerated. Results are expressed as the number of ulcers, which refers to the count of lesions per stomach. Anti-ulcer activity of ASPeWB was calculated using; % Inhibition = [(Control-Standard (or) Test) / (Control)] ×100. Results were expressed as a percentage[13].

2.6.4 Estimation of Plasma Corticosterone (PC)

Estimation of plasma corticosterone was performed using reported method[14]. Blood was withdrawn from rats through supra orbital plexus. To 100 mL of plasma, add 300 microliters of isooctane. Mix thoroughly and then centrifuge the mixture; discard the supernatant. Add 600 microliters of chloroform to each tube containing the plasma, and after extraction, transfer 400 microliters of the chloroform layer into a new stoppered tube. To this, add 800 microliters of acid–alcohol solution (50%) in a 2:1 ratio. After allowing the mixture to stand for 1 hour, measure the fluorescence of the acid layer at an excitation wavelength of 462 nm and an emission wavelength appropriate for the fluorimeter. Results were expressed as micrograms per deciliter.

2.6.4.1 Statistical Analysis

Comparisons within the groups were done applying oneway ANOVA followed by post-test; Tukey's Multiple comparison test Statistical significance was set at $p<0.05$. Data analysis was performed by using GraphPad PRISM Version 5.01 (GraphPad Software Inc. California, USA).

3. Results and Discussion

3.1 Phytochemical Profiling of ASPeWB

Preliminary Phytochemical analysis of ASPeWB was performed by standard *in vitro* methods. Experiments were performed in triplicate. (+) indicates positive and (-) indicates negative. The percentage yield for aqueous extract was calculated as 25.45%, extract showed the presence of Phytoconstituents like Phenols,

Table 1. Preliminary phytochemical screening of ASPeWB

S. No	Test	Result
1	Phenol-Indole test	+
2	Saponins -Honey comb test	-
3	Tannins-Gelatine test	+
4	Steroids- Liberman test	+
5	Flavonoids- Lead acetate test	+
6	Glycosides- Killer killani test	+
7	Alkaloids - Mayers test	-
8	Carbohydrates – Molish test	-

Tannins, steroids, and flavonoids, as shown in Table 1. Phytochemical analysis of ASPeWB was performed by GC-MS mass spectrometry. Experiments were performed in triplicate.

Medicinal properties of plant extracts have been endorsed to the presence of different types of secondary metabolites[15]. Phytochemical analysis of ASPeWB was evaluated by using GC-MS based method (Figure 1). Selecting above 50% probability with Wiley Spectral library search program match of compounds, we could identify 10 phytochemicals by Wiley Spectral library. The identified metabolites belongs to organic acids, amino acids, fatty acids, sugars, polyols, and other constituents in Table 2. Compounds such as Nitric oxide[16] and N-(Trifluoroacetyl)-N,O,O',O"-tetrakis(trimethylsilyl) norepinephrine[17] are reported for its anti-ulcer activity. These findings support the therapeutic potential of ASPeWB as a natural source for anti-ulcer treatments.

3.2 Effect of ASPeWB on pH of Artificial Gastric Juice

The effect of ASPeWB on the pH of artificial gastric acid was evaluated. A statistically significant difference was observed compared to Omeprazole (20 mg/mL), with ***p < 0.001. One-way ANOVA followed by Tukey's multiple comparison test was used to analyze the data. Results are expressed as mean ± SD, with a sample size of n=3.

3.3 Acid Neutralizing Capacity (ANC) of ASPeWB

The Acid Neutralizing Capacity (ANC) of ASPeWB was assessed using artificial gastric acid. A significant difference was found compared to Omeprazole (20 mg/mL), with *p < 0.001. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Results are presented as mean ± SD, with a sample size of n=3.

3.4 Effect of ASPeWB on Acid Neutralisation Capacity

Determining pH and evaluating artificial acid neutralizing activity *in vitro* are crucial for understanding the efficacy and safety of antacids and other substances that aim to relieve acid-related conditions. These tests help assess how well a substance can raise pH and neutralize acid in a controlled environment, mimicking the stomach's acidic environment[18]. The *in-vitro* pH determination and acid neutralizing effects of ASPeWB in different concentrations (100 mg/mL, 500 mg/mL, 1000 mg/mL, and 1500 mg/mL) were compared with the standard antacid omeprazole 20 mg/ml. The results showed concentration dependent significant increase in pH of artificial gastric acid (p<0.001) (Figure 2) and reduction in acid neutralizing capacity per gram of antacid was found as 5.25±0.723, 18.25±0.025 16.75±0.034, 13.25±0.21, 12.25±0.19 respectively (p<0.001)

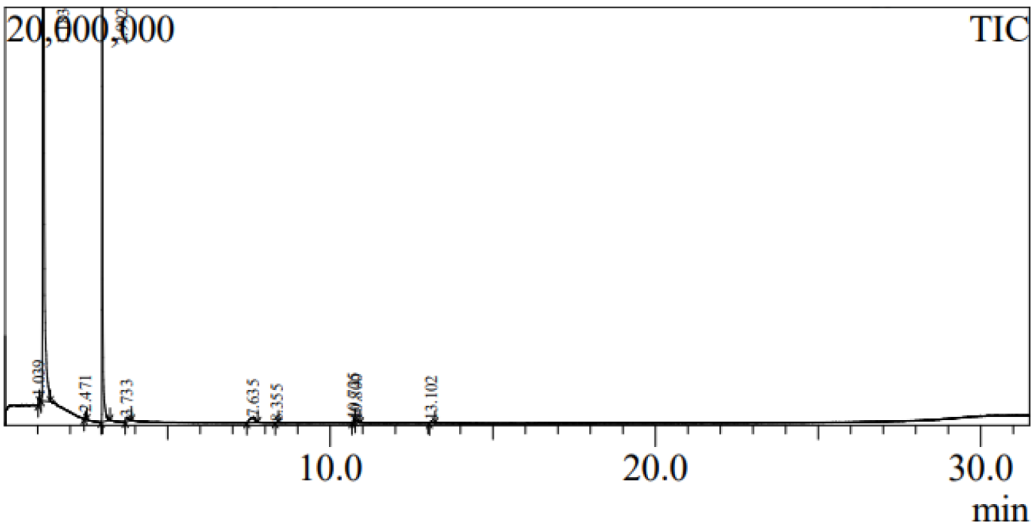


Figure 1. Total Ion Chromatogram (TIC) of ASPeWB using GC-MS analysis.

Table 2. Phytochemical profiling of ASPeWB

Peak	RT (min)	Area	% Area	Height	A/H	Mass m/z	Phytochemical names
1	1.039	178677	0.07	279491	0.64	44.10	Nitrous Oxide
2	1.183	216843842	84.30	96334818	2.25	57.85	Propane, 1, 2-dimethyl
3	2.471	430490	0.17	347260	1.24	43.15	2-Pentene
4	2.992	35259800	13.71	24352870	1.45	55.15	3-Penten-2one, 4-methyl-
5	3.733	1220059	0.47	210798	5.79	43.15	2-Pentanone, 4-hydroxy-4-methyl-
6	7.635	2481128	0.96	256760	9.66	73.15	N-(Trifluoroacetyl)-N,O,O′,O″-tetrakis(trimethylsilyl)norepinephrine
7	8.355	42078	0.02	30537	1.38	73.20	N-(Trifluoroacetyl)-N,O,O′,O″-tetrakis(trimethylsilyl)norepinephrine
8	10.725	334274	0.13	132354	2.53.	73.15	Cyclohexasiloxane,dodecamethyl-
9	10.800	173362	0.07	33774	5.13	73.20	Cyclohexasiloxane,dodecamethyl-
10	13.102	273598	0.11	81161	3.37	73.20	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasilo

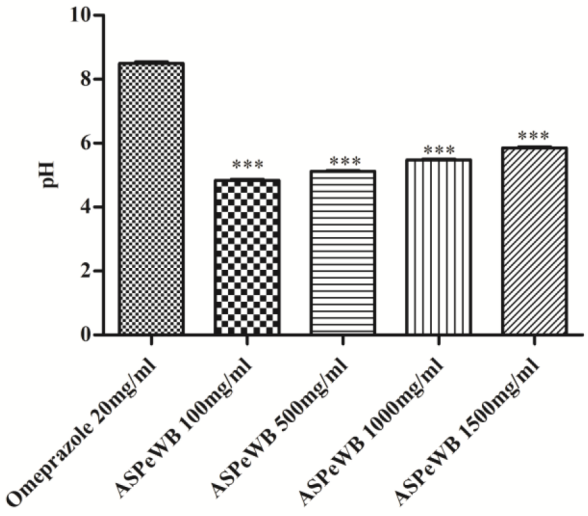


Figure 2. pH of ASPeWB and artificial gastric acid.

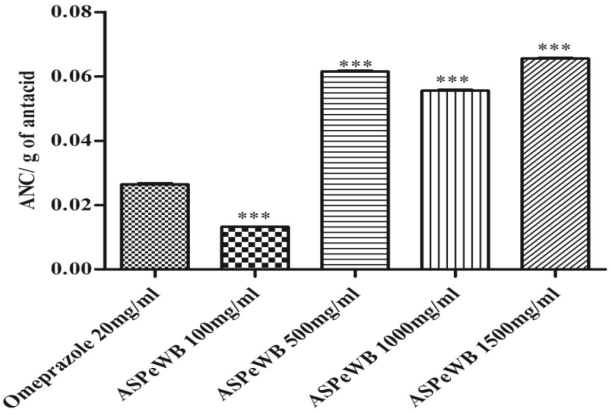


Figure 3. *In vitro* Acid Neutralizing Capacity (ANC) of ASPeWB.p.

(Figure 3). Similarly, Percentage of ANC value of the standard omeprazole (20 mg) and different doses of ASPeWB were found as 86.18, 51.80, 55.80, 65.03 67.67 respectively ($p<0.001$) (Figure 4). Whereas, ASPeWB 1000 and 1500mg/mL were found to neutralize acid more significantly as compared Omeprazole 20mg/kg ($p<0.001$) (Figure 4). Due to concerns regarding the toxicity of ASPeWB at higher doses, we selected 500 mg/kg as the optimal dose. This dose was used for all in *vivo* experiments. Together our findings confirm that ASPeWB has potential to tackle acidity.

The Acid Neutralizing Capacity (ANC) of ASPeWB was assessed using artificial gastric acid and Percentage of acid neutralization capacity was calculated. A significant difference was found compared to Omeprazole (20 mg/mL), with *$p < 0.001$. Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Results are presented as mean ± SD, with a sample size of n=3.

3.5 Effect of ASPeWB on Stress-induced Ulcer Rat Model

In stress-induced ulcers, gastric juice parameters often indicate an imbalance, with increased acidity and decreased mucosal blood flow. This can lead to erosions and ulcers in the stomach and duodenum19. The present study compared gastric juice parameters among four groups of rats (control, stress-induced, stress+omeprazole 20mg/Kg, and stress+ASPeWB 500mg/Kg). The data for gastric juice volume, total

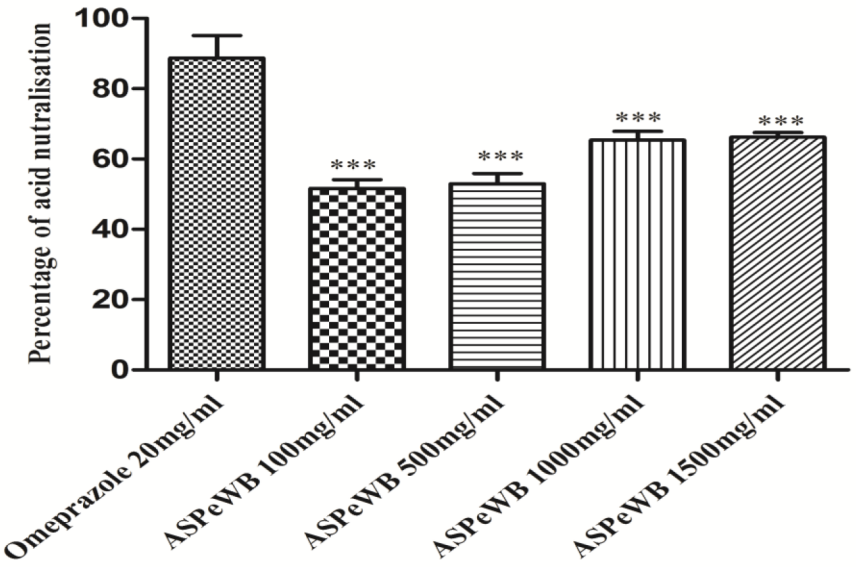


Figure 4. Percentage of acid neutralization capacity of ASPeWB.

acidity, and pH are summarized in Table 3. In rats with stress exposure notably increased gastric juice volume ($P < 0.001$; Table 3) and total acidity ($P < 0.001$; Table 3), while it led to an increase in gastric juice pH ($P<0.001$; Table 3). Similarly, in Omeprazole 20 mg/Kg and ASPeWB 500mg/Kg treated rats exhibited significantly lower gastric juice volume and total acidity compared to stress induced group ($P < 0.001$ for both; Table 3). Additionally, the gastric juice pH was significantly higher in treatment groups ($P<0.01$; table 3). These findings suggest that ASPeWB 500 mg/Kg exposure markedly reduces both the volume and acidity of gastric juice. Data indicates that ASPeWB can restore balance between gastric acidity and mucosal blood flow and is useful in the management of ulcers. It suggests that ASPeWB.

Gastric juice parameters including gastric volume, pH, total acidity, and free acidity were measured in treated groups (Stress + ASPeWB 500 mg/kg and Stress + Omeprazole 20 mg/kg) as well as in stress-induced groups. Statistical significance was indicated as ***$p < 0.001$ and **$p < 0.01$ compared to control, and ###$p < 0.001$ and ##$p < 0.01$ compared to the stress-induced group. Data are presented as mean ± SEM, with a sample size of n=6.

The Ulcer Index and percentage of ulcer inhibition were assessed in the groups: Stress + ASPeWB 500 mg/kg, Stress + Omeprazole 20 mg/kg, and stress-induced groups. Differences between groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Significance levels were indicated as ***$p < 0.001$ versus control, and ###$p < 0.001$ versus stress-induced group. Data are expressed as mean ± SEM, with a sample size of n=6.

Ulcer index and percentage of ulcer inhibition are key metrics used to assess the effectiveness of anti-ulcer treatments. The ulcer index quantifies the severity of ulcers, while the percentage of ulcer inhibition indicates the extent to which a treatment reduces ulcer formation. A significant ($p < 0.001$; Figure 5) increase in the ulcer index and percentage of ulcer was observed in the stress-induced ulceration group compared to the control group. Administration of ASPeWB 500mg/Kg significantly reduced the ulcer index and percentage of ulcer compared to stress induced group ($P < 0.001$; Figure 5). Additionally, the ulcer index was notably ($p < 0.001$) reduced in Omeprazole-induced ulcer models compared to the stress group. These findings suggest that ASPeWB has anti-ulcerogenic potential.

Plasma corticosterone levels were measured in the treated groups (Stress + ASPeWB 500 mg/kg and Stress + Omeprazole 20 mg/kg) as well as in stress-induced groups. Differences among groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. Significance levels were denoted as ***$p < 0.001$ versus control and ###$p < 0.001$ versus

Table 3. Measurements of gastric juice parameters

Group	Gastric Volume (ml/100g)	pH of Gastric Juice	Total Acidity (mEq/L)	Free Acidity (mEq/L)
Control	2.005 ± 0.29	2.57 ± 0.48	76.17 ± 4.09	41.9 ± 2.29
Stress- induced group	5.025 ± 0.46***	1.67 ± 0.14**	100.617 ± 6.09***	52.79± 3.74**
Stress+Omeprazole 20mg/kg	1.968 ± 0.22###	3.37 ± 0.315##	40.31 ± 3.78###	20.46 ± 2.13###
Stress+ASPeWB 500mg/kg	2.84 ± 0.87###	3.31± 0.23##	35.56 ± 3.322###	14.933 ± 2.45###

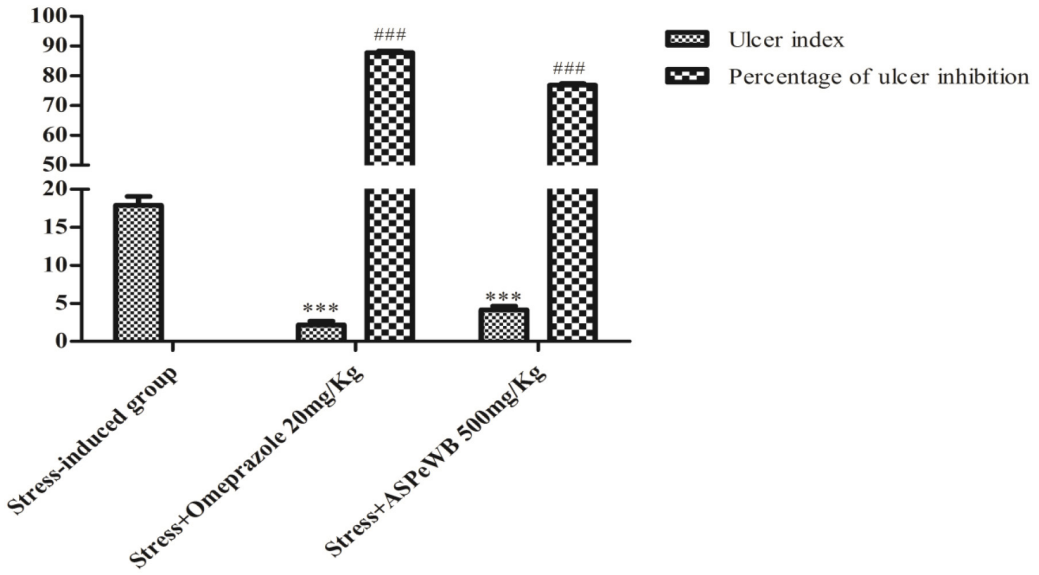


Figure 5. Ulcer Index and Percentage of ulcer inhibition in Wistar albino rats.

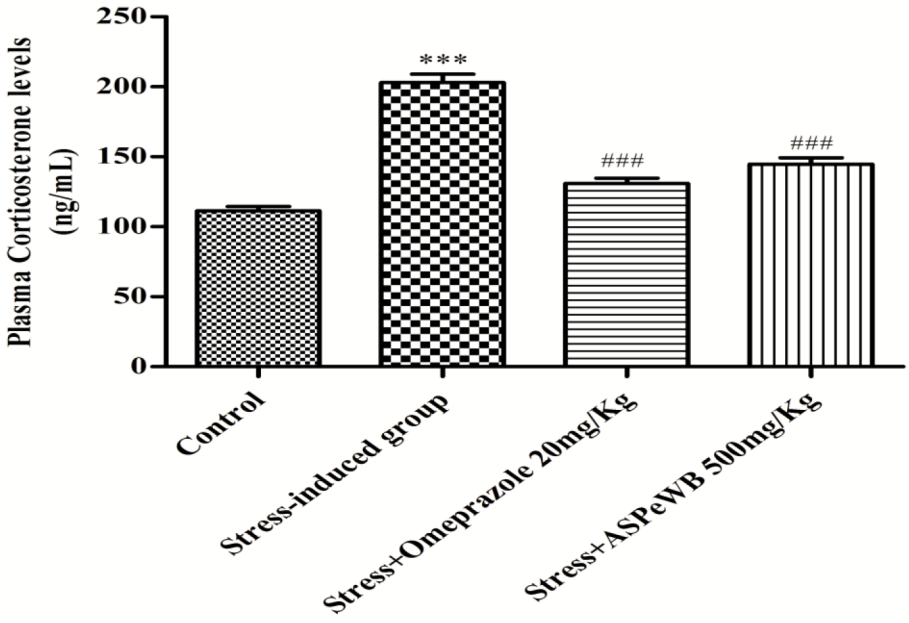


Figure 6. Plasma corticosterone levels determination.

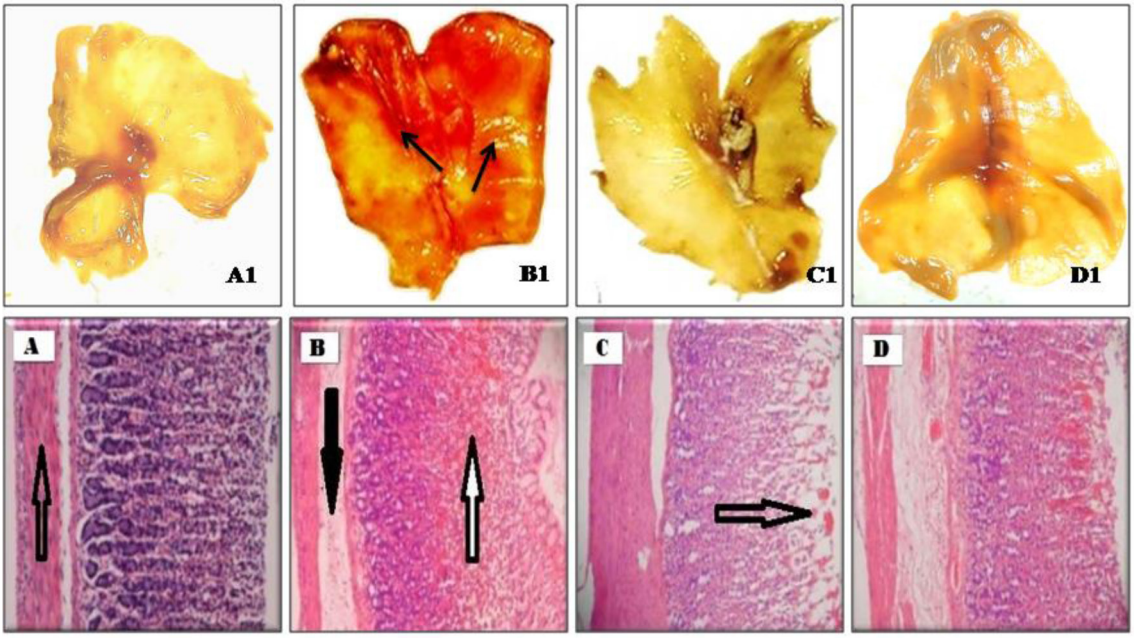


Figure 7. Effect of ASPeWB on Histological evaluation of stress-induced ulcer in rats.Whereas, Stomachs were collected from the following groups: A1 – control group; B1 – stress-induced ulcer group; C1 – stress + Omeprazole (20 mg/kg); D1 – stress + ASPeWB (500 mg/kg). Histological images were obtained for each group, with sections stained using Hematoxylin and Eosin (H&E) and observed at 20x magnification. Additionally, representative histological images include: A – control group; B – stress-induced ulcer group; C – stress + Omeprazole (20 mg/kg); D – stress + ASPeWB (500 mg/kg).

stress-induced group. Data are presented as mean ± SEM, with a sample size of n=6.

Corticosterone, a glucocorticoid hormone, plays a complex role in stress-induced ulcers, with evidence suggesting it can both protect against and exacerbate the condition14 (Filaretova, L., et al., 2016). Our results demonstrated that cold-restraint-produced significant increase in corticosterone levels ($p<0.001$; Figure 6). Whereas, ASPeWB and Omeprazole (500mg/Kg and 20mg/Kg respectively) treatments reduced corticosterone levels significantly ($p<0.001$ & $p<0.001$ respectively; Figure 6). These results provide further support for the hypothesis that ASPeWB can be used as anti-ulcer agent.

Stress ulcers arise from are a result of a complex interplay between reduced blood flow to the stomach, impaired mucosal defenses, and the exposure to aggressive factors like acid and pepsin. Which, ultimately results in the formation of superficial erosions in the upper GI tract20. Histological examination (Figure 7) of the gastric mucosa in rats pre-treated with ASPeWB at 500 mg/kg (D) or Omeprazole 20 mg/kg (C) revealed improved tissue architecture compared to the ulcer control rats (B). The ulcer control group exhibited extensive hemorrhagic necrosis of the gastric mucosa, along with edema and leukocyte infiltration in the submucosa. In the histological images, the black arrow indicates edema within the submucosa, while the white arrow points to disruption of the deep mucosal layer. The normal group (A) displayed whole gastric mucosa with normal histology.

4. Conclusion

Wodyetia bifurcata seed pulp extract (ASPeWB) exerts its anti-ulcer effects primarily through multiple interconnected pathways. The phytochemicals identified particularly phenols, tannins, flavonoids, and steroids contribute to its gastroprotective activity by scavenging free radicals, reducing oxidative stress, and strengthening the gastric mucosal barrier. The presence of bioactive compounds such as nitric oxide and norepinephrine derivatives suggests that ASPeWB may enhance mucosal defense by promoting vasodilation,

increasing mucosal blood flow, and stimulating mucus and bicarbonate secretion. The observed decrease in gastric acid secretion, evidenced by reduced gastric volume and acidity, combined with the elevation of gastric pH, indicates a direct or indirect inhibitory effect on acid production, possibly via modulation of proton pump activity or histamine pathways. Additionally, the decrease in plasma corticosterone levels suggests a systemic stress-modulating effect that may contribute to mucosal protection. Collectively, these mechanisms synergize to inhibit ulcer formation, promote mucosal healing, and restore gastric homeostasis, highlighting *W. bifurcate* potential as a multifaceted natural anti-ulcer agent. Further clinical studies are essential to confirm these effects and establish its safety and efficacy for potential incorporation into medical treatment strategies.

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Author Contribution

Dr. M. Kiranmai, Dr. K. Venu Madhav, and Dr. Komati Anusha contributed in experimental analysis, methodology, data generation, interpretations and preparation of manuscript drafts. Dr. Komati Anusha, Mrs. B. Suhasini and Mrs. E. Navya Pravala were involved in concept development, experiment design, data analysis, data interpretation, manuscript writing, editing, finalization and funding arrangements. All authors have read and agreed to the published version of the manuscript.