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Original Article

Integrative Pharmacological Profiling of Leaf-Derived Steroids from *Belosynapsis vivipara* for Ulcer Protection: *in silico* and *in vivo* Approaches

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Abstract

This investigation focused on evaluating the anti-ulcer properties of a steroidal compound extracted from the ethanol-based leaf extract of *Belosynapsis vivipara*. A combination of polar and nonpolar solvents was used to facilitate the isolation process, and the resulting fractions were characterized using thin-layer chromatography, Fourier-transform infrared spectroscopy, and high-performance liquid chromatography. The extract was administered orally to Wistar albino rats at doses of 250 mg/kg and 500 mg/kg, using ethanol-induced gastric lesions as the experimental model. Ranitidine was used as the reference drug for comparison. To support the biological findings, molecular docking and ADMET profiling were conducted against human carbonic anhydrase isoforms I and II. *β*-sitosterol was identified as a major constituent through column chromatography. The higher dose of the extract significantly improved ulcer-related parameters, including ulcer index, gastric pH, and acidity levels, suggesting a dose-dependent therapeutic effect. Docking analysis revealed notable interactions between *β*-sitosterol and the target protein (PDB ID: 6G3V), including hydrogen bonding with ALA132 and hydrophobic contacts with ALA135 and LEU131. The compound also met Lipinski's criteria for drug-likeness. These findings indicate that the ethanol extract of *B. vivipara* leaves, particularly due to *β*-sitosterol, holds promise as a natural agent for gastric ulcer management through mucosal protection and acid regulation.

***Keywords**: Antiulcer Activity; Belosynapsis vivipara; Beta-Sitosterol; Docking Studies; Isolation*

Introduction

Peptic ulcer disease primarily manifests as gastric or duodenal ulcers and affects a significant portion of the global population. These ulcers result from compromised mucosal defenses, leading to tissue erosion within the gastrointestinal tract. Protective factors such as prostaglandins, mucus secretion, adequate blood flow, and growth regulators play a vital role in maintaining mucosal integrity (Rashid *et al*., 2025; Hamed *et al*., 2022). However, various contributing factors—including poor nutrition, bacterial infections, psychological stress, tobacco use, excessive gastric acid secretion, and prolonged intake of nonsteroidal anti-inflammatory drugs—can disrupt this balance. Although conventional treatments such as proton pump inhibitors, H_2-receptor blockers, and antacids are widely prescribed, they are often associated with adverse effects, including cardiac irregularities, allergic reactions, and hematological disturbances (Sharma *et al*., 2025). In response to these

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limitations, medicinal plants have emerged as promising sources of therapeutic agents, particularly because of their rich content of secondary metabolites known for their protective health benefits.

In these plants, *Belosynapsis vivipara*, an epiphytic herb from the Commelinaceae family first documented in China in 1871, has attracted interest because of its unique ecological niche and limited distribution across the Western Ghats (Kohale *et al*., 2025). It typically grows on moss-laden branches of native trees such as *Syzygium cuminii* and *Memecylon bellatum* in riparian forest zones. Despite its rarity, the pharmacological potential of *B. vivipara* remains largely unexplored. Preliminary studies have reported its microscopic characterization (Das, 2025), anti-inflammatory (Kavade *et al*., 2012; Sasane *et al*., 2021), antibacterial (Das & Singirikonda, 2023; Kalyani *et al*., 2023), and antioxidant properties (Bylappa & Handral, 2025), yet comprehensive investigations into its bioactive profile are still lacking. Recent efforts have focused on identifying the key phytoconstituents responsible for its therapeutic effects, with particular attention to its capacity to counteract ulcer formation, marking a significant step toward validating its role in gastroprotective interventions.

Materials and Methods

Plant Collection and Identification

The plant specimen of *Belosynapsis vivipara* was collected from the forested region of Wayanad in Kerala, India. Its botanical identity was verified and authenticated by Dr. P. E. Rajshekharan, Principal Scientist at the Indian Institute of Horticultural Research, Bengaluru. This expert validation ensured accurate species identification, providing a reliable foundation for the subsequent phytochemical and pharmacological investigations.

Preparation of Plant Extract

After the roots were separated from the leaves, the leaf material was thoroughly rinsed with distilled water to remove surface impurities and then dried under shade conditions for approximately two weeks. Once dried, the leaves were ground into a coarse powder using a mortar and pestle to facilitate efficient extraction. The powdered sample was subjected to Soxhlet extraction using 80% ethanol as the solvent and maintained over an eight-hour cycle to ensure optimal recovery of phytoconstituents. The resulting extract was passed through Whatman No. 1 filter paper to remove particulate matter, and the filtrate was concentrated by evaporating the solvent at 45 °C using a rotary flash evaporator (Das, 2013), yielding a semi-solid crude extract suitable for further analysis.

Phytochemical Screening of Plant Extract

The ethanol extract of *Belosynapsis vivipara* leaves was subjected to preliminary phytochemical screening to identify the presence of bioactive constituents. Specific chemical assays, including Salkowski's and Liebermann-Burchard tests, were used to confirm the presence of steroidal compounds and phytosterols in the extract. In addition to these targeted evaluations, a series of standard phytochemical tests was conducted to detect other classes of secondary metabolites, such as flavonoids, alkaloids, tannins, saponins, and glycosides (Rathod *et al*., 2025). This comprehensive profiling provided insight into the diverse chemical composition of the extract, laying the groundwork for further pharmacological investigations.

Isolation and Characterization of Plant Extract

A total of 5 grams of the ethanol-based leaf extract of *Belosynapsis vivipara* was loaded onto a chromatographic column measuring 40 × 500 mm, packed with silica gel of 200–400 mesh size as the stationary phase. Fractionation was carried out using a gradient of solvents with increasing polarity, with each solvent applied in 5 mL volumes (Das *et al*., 2018). The solvent system included *n*-hexane, diethyl ether, ethyl acetate, ethanol, methanol, and water (Dali *et al*., 2024). The elution sequence began with *n*-hexane gradually enriched with diethyl ether (100:0 to 0:100 v/v), followed by diethyl ether with ethyl acetate, ethyl acetate with ethanol, ethanol with methanol, and finally methanol with water, each using similar gradient ratios. Thin-layer chromatography was performed on silica gel plates using a solvent mixture of *n*-hexane, ethyl acetate, and acetic acid to monitor compound

separation. Each collected fraction was analyzed using Fourier-transform infrared spectroscopy to identify functional groups. For further profiling, high-performance liquid chromatography was conducted using a Shimadzu Lab Solution system equipped with a reversed-phase C8 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of buffer A, 0.05% orthophosphoric acid, and mobile phase B, acetonitrile, with a flow rate of 1.5 mL/min, detection at 202 nm, and an injection volume of 20 µL. Methanol served as the diluent throughout the analysis (Ruba *et al*., 2024).

Docking and ADME Studies

Molecular docking studies were carried out using AutoDock version 4.2.6 to evaluate the interaction between *β*-sitosterol and the target proteins (Yuenyong *et al*., 2024). The two-dimensional structure of *β*-sitosterol was initially sketched using ChemSketch and saved in MOL format. It was then converted into a three-dimensional structure using PyMOL and exported in PDB format (Das *et al*., 2023). Protein structures corresponding to human carbonic anhydrase isoforms I (PDB ID: 6G3V) and II (PDB ID: 6G3Q), both complexed with famotidine, were retrieved from the Protein Data Bank. This was performed as an exploratory analysis to evaluate potential secondary interactions of *β*-sitosterol, since CA isoforms have been reported to play a minor role in modulating gastric physiology. The inclusion of CA docking was intended to broaden the pharmacological profile assessment rather than replace established models of gastric acid regulation. Prior to docking, the receptor models were refined by removing water molecules and co-crystallized ligands using PyMOL to ensure accurate binding-site accessibility. Protein-ligand interactions were visualized using Discovery Studio Visualizer, which provided detailed insights into binding conformations. Additionally, *β*-sitosterol was evaluated using SwissADME to assess its pharmacokinetic properties and compliance with Lipinski's rule of five, confirming its potential as a drug-like candidate (Vani *et al*., 2024).

Pharmacological Studies

The experimental protocol for assessing anti-ulcer activity was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Krupanidhi College of Pharmacy under approval number KCP/IAEC/PCOL/PCHEM/115/2023. Wistar albino rats of both sexes, weighing between 160 and 200 g, were selected for the study. The animals were maintained under controlled environmental conditions, with the ambient temperature regulated at approximately 23 ± 2 °C and relative humidity kept consistent. A 12-hour light-dark cycle was followed, with illumination scheduled from 7:00 a.m. to 7:00 p.m. The rats were housed in wire-mesh cages and provided with standard pellet feed and clean drinking water ad libitum throughout the experimental period (Kurian, 2024). In this study, the animals were pretreated for 21 consecutive days prior to the ethanol challenge. Although the ethanol-induced gastric ulcer model is classically acute, the extended regimen was deliberately chosen to evaluate both the prophylactic efficacy and the tolerability of *β*-sitosterol under repeated administration. This design allowed the simulation of sustained exposure, bridging acute gastroprotection with a preliminary chronic safety assessment.

Acute oral Toxicity Studies

The OECD 423 recommendations were followed for the acute oral toxicity test. For the toxicity experiments, nine adult's healthy Wister rats were experimented (Kumar *et al*., 2025).

Evaluation of Antiulcer Activity

The animals were pretreated with either the test extract or the standard reference drug for 21 days. Following this regimen, Groups II through V, each comprising six Wistar rats, were administered 1 mL of absolute ethanol to induce gastric ulceration, with Group II serving as the positive control without any drug intervention. One hour after ethanol exposure, the animals were euthanized using a carbon dioxide overdose. The stomachs were carefully exercised, opened along with the greater curvature, and rinsed with chilled phosphate-buffered saline to remove residual blood and debris (Li *et al*., 2025). Gross examination of the gastric mucosa was performed to assess visible lesions. Gastric contents were collected for analysis of pH, total acidity, and free acidity. Ulcer index values and percentage inhibition were calculated to determine the extent of mucosal protection. The experimental design

included four groups: Group I received only the vehicle and served as the normal control; Group II served as the ulcer control; Group III was treated with a low dose of the plant extract; and Group IV received a higher dose, allowing for comparative evaluation of dose-dependent therapeutic effects.

Total Acidity Estimation

To determine the total and free acidity of the gastric contents, equal volumes of gastric juice and distilled water were mixed in a conical flask. Two drops of phenolphthalein were added to the mixture to assess total acidity, while Toffer's reagent was used to estimate free acidity. The solution was then titrated with 0.01 N sodium hydroxide until a stable pink coloration appeared, indicating the endpoint. The volume of sodium hydroxide consumed during titration was recorded. The acidity values, expressed in milliequivalents per liter (mEq/L), were calculated using the following formula: Acidity = Volume of NaOH × Normality × 100. This method provided quantitative insight into the acid levels present in the gastric fluid (Patidar *et al*., 2021).

Statistical Analysis

Experimental results were expressed as mean values accompanied by their respective standard deviations (mean ± SD) for each treatment group. Statistical evaluation of intergroup differences was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify specific pairwise comparisons. A significance threshold of $p < 0.001$ was applied to determine the reliability of observed differences, indicating a high level of statistical confidence in the outcomes. This analytical approach ensured rigorous assessment of the treatment effects across all experimental groups.

Results

From 250 g of coarsely powdered *Belosynapsis vivipara* leaves, 25.2 g of ethanol extract was obtained following Soxhlet extraction. Initial phytochemical screening of the extract, as summarized in Table 1, revealed the presence of several bioactive groups. The tests showed positive results for steroids, phytosterols, alkaloids, flavonoids, glycosides, tannins, saponins, proteins, terpenoids, and phenolic compounds. However, the extract showed negative responses for resins and gum, suggesting their absence in the leaf material. This diverse phytochemical profile highlights the therapeutic potential of the plant and supports its further pharmacological evaluation.

Table 1: *Phytochemical Test of Ethanolic Extract*

Sl No.	Chemical Test	Ethanolic Extract
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Saponins	+
5	Steroidal compounds	+
6	Tannins	+
7	Phytosterols	+
8	Phenolics	+
9	Gum	_
10	Proteins	+
11	Terpenoids	+
12	Resins	--

+ = Present -- = Absent

Column chromatography of the ethanol extract from *Belosynapsis vivipara* leaves produced multiple fractions separated according to solvent polarity gradients (Figure 1). The initial eluent, comprising *n*-hexane and diethyl ether, produced six distinct fractions. Subsequent elution with diethyl ether and ethyl acetate yielded five fractions, followed by five additional fractions from the ethyl acetate and ethanol combination. The ethanol and methanol mixture generated six fractions, whereas the final methanol and water eluent did not yield any detectable fractions. Each collected fraction was analyzed using thin-layer chromatography (TLC) and Fourier-transform infrared spectroscopy (FTIR).

For TLC analysis, *β*-sitosterol was used as the reference compound. Both the sample and standard were spotted on a 5 × 5 cm precoated silica gel plate and developed in a solvent system consisting of *n*-hexane, ethyl acetate, and acetic acid. The solvent front was allowed to rise to a height of 4 cm. After visualization in an iodine chamber, the sample showed three distinct spots at migration distances of 0.9 cm, 1.4 cm, and 3.7 cm (Figure 2), while the standard *β*-sitosterol spot appeared at 3.7 cm. The corresponding retention factor (Rf) for the standard was calculated as 0.57, indicating a match with one of the sample components.

Infrared spectral analysis of the isolated compound revealed distinct absorption bands corresponding to various functional groups. A prominent peak at 3332.4 cm^{-1} indicated the presence of O–H stretching vibrations, suggesting hydroxyl functionality. The spectrum also displayed characteristic bands at 2973.4 cm^{-1} and 2925.3 cm^{-1}, attributed to asymmetrical stretching of methyl and methylene groups, respectively, while a symmetrical CH_2 stretch was observed at 2894.9 cm^{-1}. A strong absorption band at 1044.6 cm^{-1} corresponded to C–O stretching vibrations, indicating alcohol or ester linkages. Additional bending vibrations were noted at 1380.3 cm^{-1} for CH_3 groups and at 1451.8 cm^{-1} for CH_2 groups. These spectral features were recorded from a fraction eluted using a solvent mixture of ethyl acetate and ethanol in a 10:90 v/v ratio, confirming the presence of steroidal and phytosterol-like structures within the extract (Figure 3).

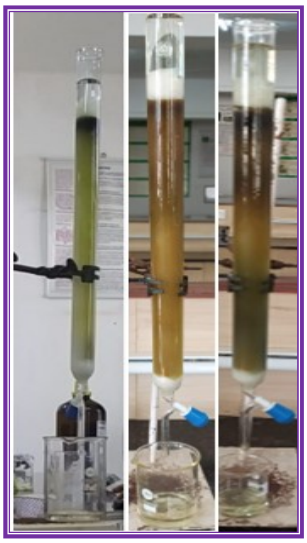


***Figure1**: Isolation of ß-sitosterol*

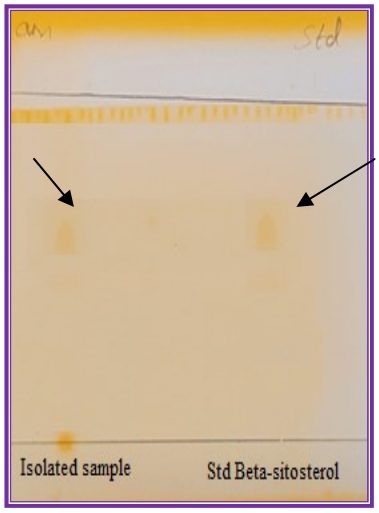


***Figure 2:** TLC of Isolated Sample*

Column chromatography profile of the phytosterol fraction, demonstrating separation and enrichment of *β*-sitosterol. The elution pattern illustrates the predominance of *β*-sitosterol with minor co-elution of stigmasterol.

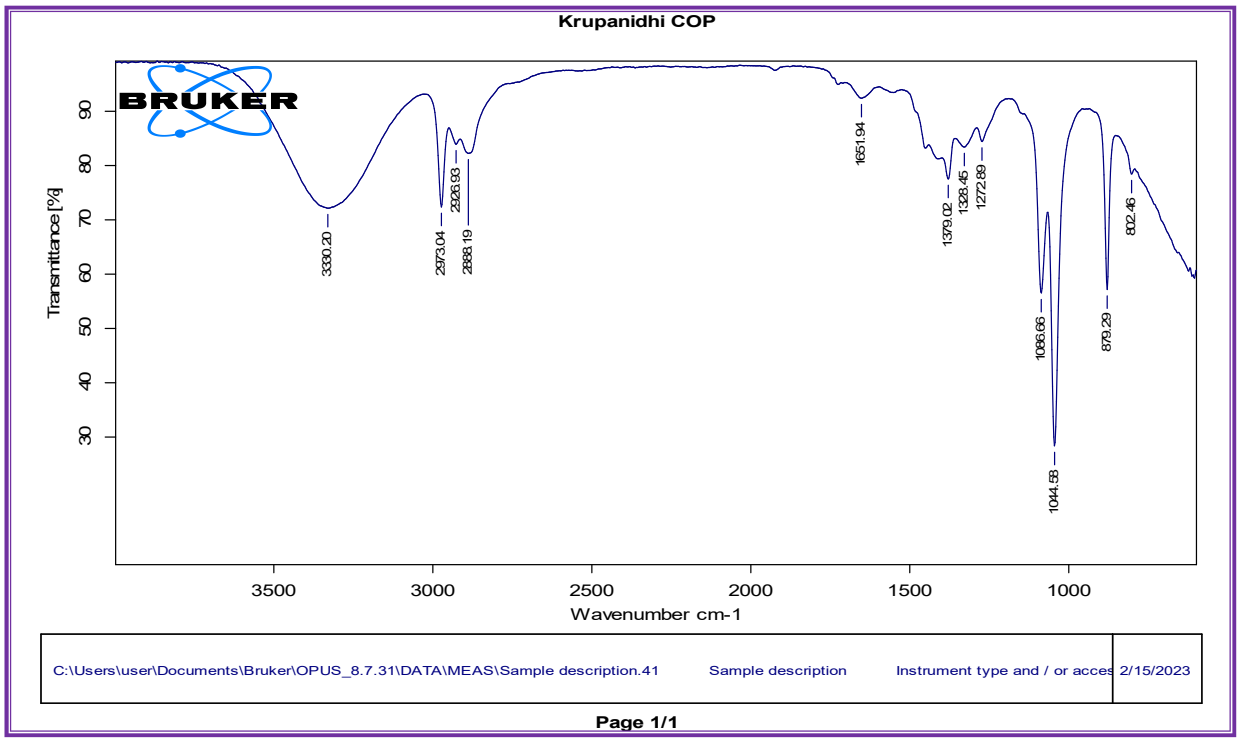


Figure 3: *FTIR Spectrum of the Isolated Compound, Highlighting Characteristic Absorption Bands Consistent With B-Sitosterol. The Spectrum Supports Structural Identification and Complements Chromatographic Findings*

HPLC column C_8 peak time of *β*-sitosterol was found at Rt 7.2 in 202nm detector and stigmasterol peak was found at Rt of 4.1 (Figure-4a and b). The amount of beta-sitosterol present in BV leaves extract was 0.035%.

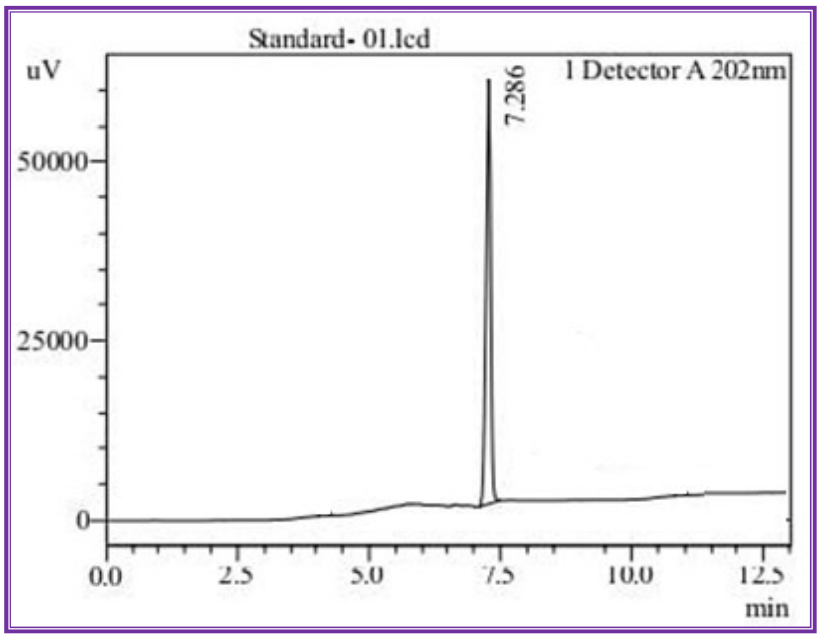


Figure 4a: *HPLC of Standard Beta-Sitosterol*

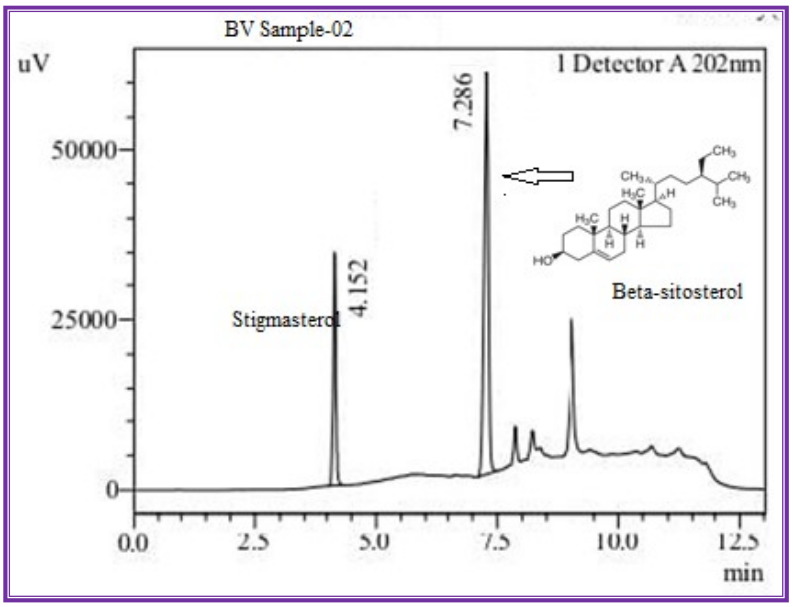


Figure 4b: *HPLC Chromatogram of The Isolated Phytosterol Fraction Showing Distinct Peaks for Stigmasterol (Rt 4.1) And B-Sitosterol (Rt 7.2). The Chromatogram Confirms the Presence of a Phytosterol Mixture Enriched In B-Sitosterol*

HPLC analysis of the isolated fraction revealed two distinct peaks: stigmasterol at a retention time (Rt) of 4.1 and *β*-sitosterol at an Rt of 7.2. Column chromatography predominantly yielded *β*-sitosterol, which was confirmed through FTIR characterization. However, the chromatographic profile indicated the presence of stigmasterol in a minor proportion, suggesting that the isolated material represented a *β*-sitosterol-enriched mixture containing traces of stigmasterol.

In silico Study

β-Sitosterol satisfies the criteria outlined in Lipinski's Rule of Five, a widely accepted guideline for evaluating the oral bioavailability of drug-like compounds. Its molecular weight remains below the 500-Dalton threshold, and its calculated partition coefficient (log P) is less than 5, indicating favorable lipophilicity. Additionally, the molecule has fewer than five hydrogen bond donors and fewer than ten hydrogen bond acceptors, supporting its potential for membrane permeability. The number of rotatable bonds is also below ten (Table 2).

Table 2: *Lipinski's Rule of Five for β-sitosterol*

Sl. No	Compound Name	Molecular Formula	Molecular Weight (G/Mol)	Rotatable Bonds	Hydrogen Bond Donors	Hydrogen Bond Acceptors	Log *P*
1	*β*-sitosterol	$C_{29}H_{50}O$	414.71	6	1	1	4.79

Docking was performed using AutoDock version 4.2.6. The docking score was found to be -11.05 kcal/mol for protein 6G3V and -10.16 kcal/mol for protein 6G3Q, indicating good binding affinity of both proteins for the ligand *β*-sitosterol. The amino acid interactions observed for protein 6G3V included a conventional hydrogen bond with ALA132 and a Pi-alkyl bond with ALA135. For protein 6G3Q, the interactions included a conventional hydrogen bond with LEU240 and a Pi-alkyl bond with PHE231 (Figure 5).

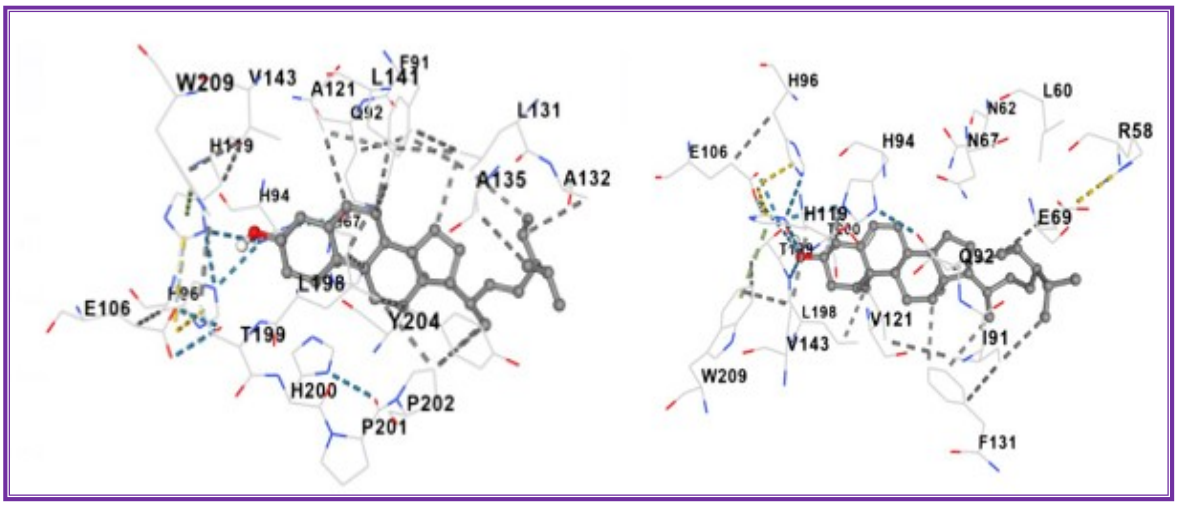


***Figure 5**: Protein and Ligand Docking of 2D View of 6g3v and 6g3q*

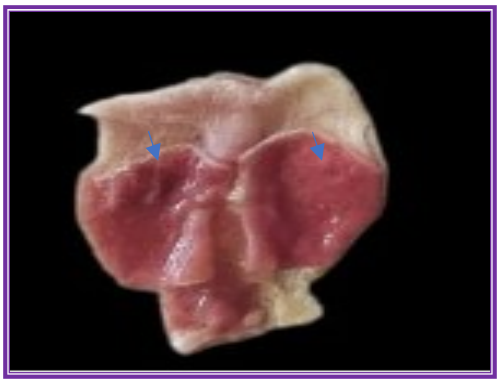
Molecular docking interaction of *β*-sitosterol with human carbonic anhydrase isoform I (PDB ID: 6g3v and 6g3q). The figure depicts hydrogen bonding and hydrophobic interactions within the active site, suggesting potential secondary binding affinity. The binding pose illustrates supplementary interactions, reinforcing exploratory evaluation of off-target potential.

β-Sitosterol demonstrated measurable binding affinity toward CA isoforms I and II, although these interactions were weaker compared to standard ligands. The docking outcomes suggest possible secondary binding potential, complementing the primary mechanisms of gastric protection mediated through H2 receptor and proton pump pathways.

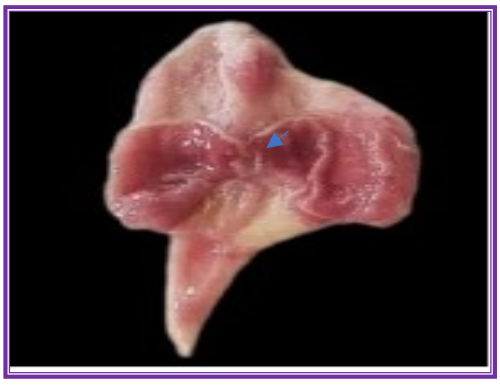
Antiulcer Activity

Acute Toxicity Analysis

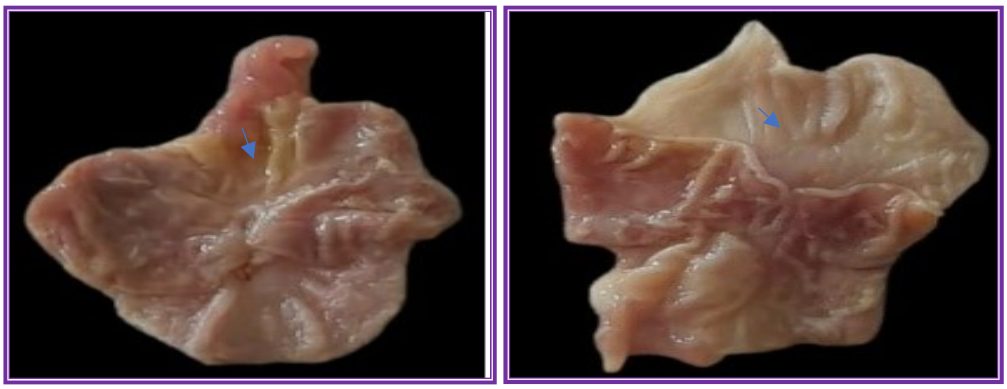
Acute oral toxicity assessment of BV leaf extract was conducted in accordance with OECD guideline 423, using a limit dose of 2000 mg/kg. No signs of toxicity, behavioral alterations, neurological disturbances, autonomic irregularities, or mortality were observed during the initial 24-hour period or throughout the 14-day observation period. Based on these findings, experimental doses were established at 250 mg/kg (low dose) and 500 mg/kg (high dose) for subsequent studies. Following treatment, gastric tissues were carefully excised along the greater curvature, rinsed with saline to remove residual blood and contents, and examined under 10× magnification for mucosal damage (Figure 6). Ulceration was quantified by counting the lesions and calculating the ulcer index, which incorporated the total number of ulcers, severity scores, and percentage of affected animals, multiplied by a factor of ten (Table 3). Comparative analysis revealed that the high dose of BV extract conferred superior gastroprotective effects against ethanol-induced ulcers, as evidenced by a reduced ulcer index relative to the low-dose group.



(a)



(b)



(c) (d)

Figure 6: *Macroscopic Evaluation of Wistar Albino Rats A) Ethanol Induced, B) Low Dose of Extract, C) High Dose of Extract, D) Standard Drug Ranitidine Induced*

Histopathological section of the gastric mucosa in ethanol-induced ulcer control rats, showing severe epithelial disruption and hemorrhagic lesions. This image represents classical acute ulcer pathology. Histopathological section of the gastric mucosa in *β*-sitosterol-treated rats, demonstrating preserved epithelial integrity and reduced ulceration after 21 days of pretreatment. The figure highlights the gastroprotective effect of prolonged administration.

Continuous administration of *β*-sitosterol for 21 days resulted in significant protection against ethanol-induced gastric lesions. The prolonged pretreatment not only reduced ulcer indices but also maintained mucosal integrity throughout the study period. These findings suggest that *β*-sitosterol exhibits durable gastroprotective effects under repeated dosing conditions.

Table 3: *Results on Ulcer Index, Ph, Free and Total Acidity of BV Extract*

Sl no	Treatment	Ulcer index	pH	Free acidity mEq/L/100g	Total acidity mEq/L/100g
1	Vehicle control	0	3.0 ± 0.01	5.0 ± 0.6	10.5 ± 1.6
2	Ethanol induced	13.4 ±0.48$^{\#}$	1.4 ± 0.4$^{\#}$	8.4 ± 0.6$^{\#}$	28.1 ± 1.4$^{\#,\#}$
3	Low dose of BV [250mg/kg]	6.2 ± 0.43**	1.6 ± 1.5*	6.3 ± 0.3*	17 ± 0.1^{ns}
4	High dose ofBV[500mg/kg]	3.2 ± 0.41***	1.9 ± 1.1***	5.4 ± 0.3**	13 ± 1.3**
5	Ranitidine [21.6mg/kg]	3.0 ± 0.66****	2.1 ± 0.11****	4.5 ± 0.5**	11 ± 0.8***

All the values are significantly represented Mean±SD and analyzed by ANOVA followed by Turkey's test. Statistical significance of the results was tested by comparing ethanol induced with vehicle control group (# *P*<0.05, ##*P*<0.01, ###*P*<0.001, ####*P*<0.0001). Low dose, high dose and ranitidine treated group is compared with ethanol inducer (*P*<0.05) ns: no significance, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

Gastric pH measurements were conducted using a calibrated pH meter, with equal volumes of gastric juice and distilled water mixed for analysis. Rats administered low and high doses of BV extract exhibited pH values within the physiological range of 1.5 to 3.0, in contrast to the ethanol-induced group, which showed a markedly acidic pH of 1.4, as detailed in Table 3. Subsequent graphical representations showed that vehicle-treated animals displayed no ulcer index, while both the ranitidine and high-dose BV extract groups demonstrated significantly reduced ulcer indices (*P*< 0.001), as shown in Figure 7. In Figure 8, the ethanol group maintained a highly acidic gastric environment (1.4 ± 0.4), whereas the high-dose BV extract elevated the pH to 1.9 ± 1.1 (*P*< 0.001), closely approximating the effect of ranitidine. Figures 9 and 10 further confirmed that the high-dose

BV extract significantly lowered both free and total gastric acidity ($P< 0.01$), underscoring its potential anti-ulcer efficacy comparable to that of the standard reference drug.

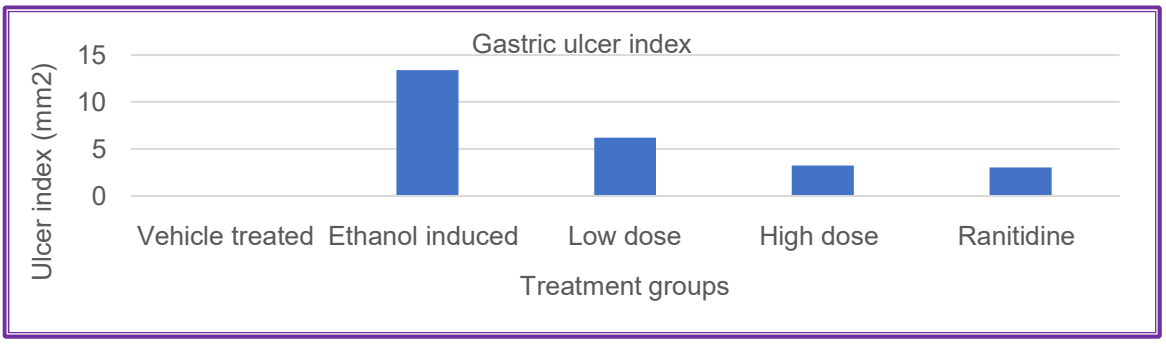


Figure 7: *Effect of BV Leaves Extract and Ranitidine on Gastric Ulcer Index with Ethanol Induced Antiulcer Activity in Wistar Albino Rats*

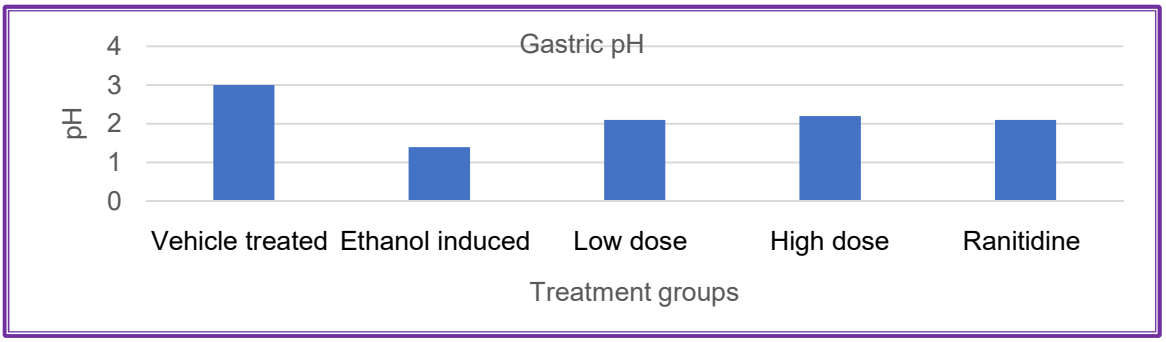


Figure 8: Effect of BV *Leaves Extract and Ranitidine on Gastric Ph with Ethanol Induced Antiulcer Activity in Wistar Albino Rats*

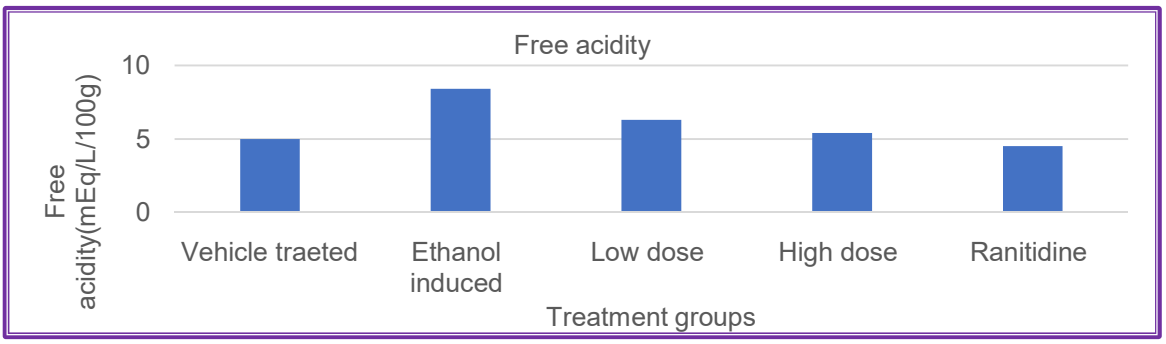


Figure 9: *Effect of BV Leaves Extract and Ranitidine on Gastric Free Acidity with Ethanol Induced Antiulcer Activity in Wistar Albino Rats*

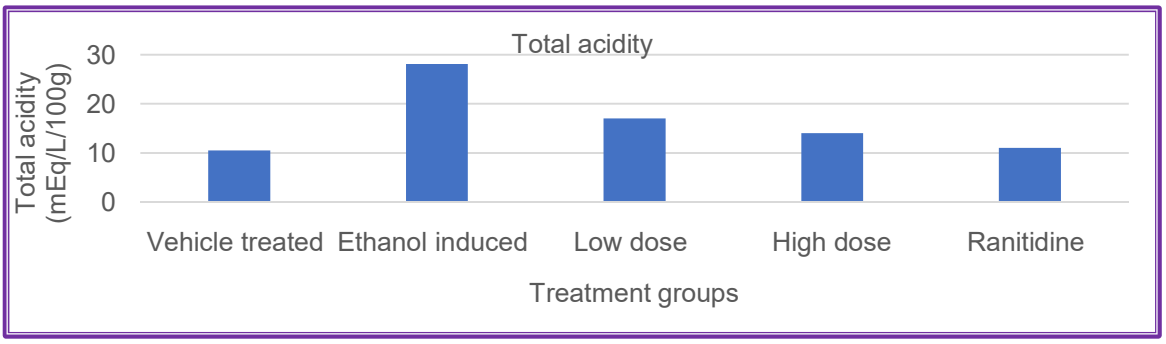


Figure 10: *Effect of BV Leaves Extract and Ranitidine on Gastric Total Acidity with Ethanol Induced Antiulcer Activity in Wistar Albino Rats*

Discussion

The present investigation demonstrated that the ethanol leaf extract of *Belosynapsis vivipara* possesses significant anti-ulcer activity, with *β*-sitosterol identified as a major bioactive constituent. Sequential phytochemical screening confirmed the presence of diverse secondary metabolites, including steroids, phytosterols, flavonoids, alkaloids, tannins, saponins, and terpenoids. This broad chemical profile supports the traditional use of medicinal plants as reservoirs of therapeutic agents and provides a rationale for further pharmacological exploration.

Furthermore, column chromatography and spectral characterization established *β*-sitosterol as a key compound, as corroborated by TLC, FTIR, and HPLC analyses. The identification of *β*-sitosterol aligns with earlier reports on phytosterols as gastroprotective agents, suggesting that its structural features contribute to mucosal defense. The FTIR spectrum revealed hydroxyl and methyl group vibrations, while HPLC confirmed its retention time and concentration, strengthening the evidence for its isolation and purity. The detection of stigmasterol alongside *β*-sitosterol highlights the natural tendency of phytosterols to coexist and frequently co-crystallize in plant extracts. Although *β*-sitosterol was the major constituent isolated and characterized, the minor presence of stigmasterol reflects the complexity of phytosterol mixtures in botanical sources. This finding underscores the importance of reporting both major and minor phytosterols to ensure consistency across analytical and isolation data. The revised manuscript clarifies that the isolated fraction was enriched in *β*-sitosterol but contained stigmasterol, thereby aligning the HPLC results with the isolation and FTIR discussion.

The presence of phytosterols represents a novel phytochemical finding, as such compounds have not previously been documented in this species. Plant-derived steroidal metabolites are known to exhibit a broad spectrum of pharmacological properties, including anti-inflammatory, lipid-lowering, hepatoprotective, and antimicrobial effects (Okokon *et al*., 2022; Hieu *et al*., 2024; Yigit *et al*., 2025). *β*-Sitosterol has been recognized for its therapeutic potential in the management of gastric ulcers (Miszczuk *et al*., 2024). The experimental outcomes of the current study align with these reports, demonstrating notable anti-ulcer activity (Jazeela *et al*., 2022; Gupta *et al*., 2022). Computational docking analyses further supported these findings, revealing strong binding affinities of *β*-sitosterol toward ulcer-associated proteins 6G3V and 6G3Q, with docking scores of –11.05 kcal/mol and –10.5 kcal/mol, respectively, indicating robust interactions (Loosli *et al*., 2024; Mesileya *et al*., 2025). The number of rotatable bonds in phytosterols was less than ten, suggesting structural rigidity conducive to oral absorption. Collectively, these physicochemical properties support the suitability of *β*-sitosterol for oral administration in pharmacological formulations.Docking of *β*-sitosterol against CA isoforms was included to highlight potential off-target interactions, recognizing that carbonic anhydrase contributes only indirectly to gastric acid regulation. While the standard therapeutic relevance of famotidine and related agents lies in H_2 receptor antagonism and proton pump inhibition, the observed CA binding provides supplementary insight into the broader pharmacological spectrum of *β*-sitosterol. This clarification ensures consistency with established gastric protection models while acknowledging the exploratory findings. According to Lipinski's criteria, *β*-sitosterol is orally bioavailable and does not penetrate the blood-brain barrier, enhancing its suitability as a gastrointestinal agent.

Specifically, ethanol-induced gastric lesions were markedly attenuated in the extract-treated groups. The ulcer index decreased from 13.4 in the ulcer control group to 6.2 and 3.2 in the low- and high-dose groups, respectively. Gastric pH values improved significantly, increasing from 1.4 in the ulcer control group to 1.9 in the high-dose group, approaching the ranitidine-treated value of 2.1. Similarly, both free and total acidity levels were reduced, highlighting the extract's ability to restore gastric homeostasis. Collectively, these results demonstrate that *β*-sitosterol-rich fractions of *B. vivipara* exert protective effects by enhancing mucosal integrity and regulating acid secretion.Ethanol, a known ulcerogenic compound, exacerbates oxidative stress and gastric acid secretion. However, pretreatment with BV extract significantly mitigated these effects, with the high dose showing superior gastroprotective efficacy compared with both the low dose and the standard drug ranitidine (Ghareeb *et al*., 2024). The use of a 21-day pretreatment protocol in an acute ethanol model was intended to extend the evaluation window beyond conventional short-term designs. This approach provided valuable insight into the sustained protective potential and preliminary safety profile of *β*-sitosterol. Although acute models typically employ shorter pretreatment durations, the findings demonstrate that prolonged administration can reveal consistent gastroprotection and tolerability, thereby strengthening translational relevance. The revised manuscript clarifies this rationale to ensure coherence between the study design and pharmacological interpretation. Statistical analysis confirmed the significance of these findings ($P < 0.001$), reinforcing the therapeutic promise of BV extract in ulcer management.

The findings are consistent with previous reports on plant-derived phytosterols and flavonoids that exhibit anti-ulcer activity through antioxidant, anti-inflammatory, and cytoprotective mechanisms. The dose-dependent efficacy observed in this study underscores the importance of phytochemical concentration in achieving therapeutic outcomes. Moreover, the convergence of silico and in vivo data strengthens the translational potential of *B. vivipara* as a candidate for natural anti-ulcer drug development.

Limitations

The present study was limited to in vitro assays and computational analyses, without validation in animal models or clinical settings. The phytochemical profiling focused primarily on alkaloids, phytosterols, and flavonoids, leaving other potential bioactive constituents unexplored. Additionally, the study did not assess the long-term safety, pharmacokinetics, or bioavailability of the BV extract, which are essential for translational applications.

Future Scope

Future investigations should focus on expanding the study to larger and more diverse populations to enhance reproducibility and clinical relevance. Incorporating advanced analytical approaches such as omics and network pharmacology could provide deeper mechanistic insights. Translational studies and formulation optimization may ultimately support regulatory approval and therapeutic application.

Conclusion

Natural products have long served as foundational elements in both traditional and modern healthcare systems, with medicinal plants offering a rich reservoir of therapeutic agents. In this context, the current investigation highlights *Belosynapsis vivipara* as a promising source of bioactive constituents, notably *β*-sitosterol, which emerged as a key compound contributing to its anti-ulcer potential. Experimental findings demonstrated that administration of the leaf extract effectively mitigated gastric ulceration, supporting its role in gastrointestinal protection. The identification of bioactive molecules was facilitated by their prolonged retention times during analysis, indicating their stability and pharmacological relevance. The dose-dependent efficacy observed in this study underscores the importance of phytochemical concentration in achieving therapeutic outcomes. Moreover, the convergence of silico and in vivo data strengthens the translational potential of *B. vivipara* as a candidate for natural anti-ulcer drug development. Collectively, these results underscore the therapeutic promise of BV, suggesting its utility in the formulation of future plant-derived anti-ulcer agents.

Conflict of Interest

The authors declare that they have no competing interests.

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