Therapeutic Potential of *Spondias pinnata* for Asthma: Evidence from *In vitro, In vivo* and Phytochemical Studies

Swathi S, Lakshman K

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, PES University, Bengaluru, Karnataka, India. E-mail: swathi.sridharagatta@gmail.com

Address for Correspondence

Swathi S
Department of Pharmacognosy,
Faculty of Pharmaceutical Sciences,
PES University, Bengaluru,
Karnataka, India.
E-mail: swathi.sridharagatta@gmail.com

Abstract

Introduction: This study investigated the anti-asthmatic effects of *Spondias pinnata* using isolated guinea pig ileum and performed molecular docking analysis to identify potential asthma-related molecular targets.

Methodology: The extract of *S. pinnata* underwent phytochemical analysis and isolated β-sitosterol, Epicatechin using column chromatography. The anti-asthmatic activity of the extract was assessed using isolated guinea pig ileum, measuring its inhibitory effect on histamine-induced contractions, mast cell degranulation inhibition, and acute toxicity studies were conducted. The extract's impact on bronchospasm inhibition was measured, including its effect on pre-convulsive time. Molecular docking analysis assessed interactions between β-sitosterol, Epicatechin, IL-13, and endothelial nitric oxide synthase.

Results and Discussion: The phytochemical analysis confirmed the presence of pharmacologically active compounds. The extract demonstrated dose-dependent inhibition of

histamine-induced contractions in guinea pig ileum, indicating its anti-asthmatic potential. Mast cell degranulation inhibition showed dose-dependent efficacy. Acute toxicity studies indicated the extract's safety at medium doses. The extract also exhibited inhibitory effects on clonidine-induced mast cell degranulation and histamine-induced bronchospasm, with a higher dose increasing pre-convulsive time, indicating protection against convulsions. Molecular docking analysis supported potential interactions between β-sitosterol, Epicatechin, and IL-13, endothelial nitric oxide synthase, suggesting their involvement in the anti-asthmatic properties of *S. pinnata*.

Conclusion: This study highlights the anti-asthmatic potential of *S. pinnata* extract, demonstrated by its inhibition of histamine-induced contractions, mast cell degranulation, and bronchospasm. Epicatechin in S. pinnata may contribute to its anti-asthmatic properties through interactions with asthma-related molecular targets.

Keywords: *Spondias pinnata*, Anti-asthmatic, guinea pigs, Wistar rats, Molecular docking

1. Introduction:

Asthma is a chronic inflammatory disease of the airways with various etiologies such as environmental allergens, viral infections, tobacco smoke, stress, and genetic predisposition that can trigger asthma. The inflammatory process leads to bronchoconstriction, increased mucus secretion, and airway hyperresponsiveness, resulting in wheezing, coughing, and shortness of breath.[1] Treatment options include inhaled corticosteroids, long-acting beta-agonists, leukotriene modifiers, and immunomodulatory therapies. Research on the pathophysiology of asthma has led to improved management of acute exacerbations, but the underlying mechanisms of the condition are still not fully understood. [2]

Plants have been used in traditional medicine to treat asthma, and modern scientific research has investigated their potential as alternative or complementary therapies. Plants such as Ginger, Garlic, Ginkgo, Turmeric, Eucalyptus, Boswellia, and Liquorice have been found to have anti-inflammatory and bronchodilation effects, which can improve respiratory function and reduce asthma symptoms. [3] Scientific studies have shown that extracts from different plant parts, such as the bark, leaves, and fruits, possess anti-inflammatory and bronchodilation properties to help improve asthma symptoms. [4]

Spondias pinnata is a plant known for its medicinal properties, and its phytochemical constituents have been studied extensively. Gas chromatography-mass spectrometry (GC-MS) analysis revealed compounds such as fatty acids, sterols, terpenoids, and flavonoids in *S. pinnata* leaves. [5] Few researchers identified and isolated phenolic compounds such as gallic acid, chlorogenic acid, and quercetin from *S. pinnata* leaves using high-performance liquid chromatography (HPLC) and mass spectrometry.[6] HPLC analysis identified various flavonoids, including quercetin, kaempferol, and luteolin, in the methanol extract of *S. pinnata* leaves. Tannins, such as punicalagin and ellagic acid, were isolated and identified from *S. pinnata* leaves using HPLC. The bark of *S. pinnata* has also been studied, isolating, and identifying various flavonoids and phenolic compounds, including gallic acid, ellagic acid, and quercetin, from the ethanolic extract of the bark using HPLC. Triterpenoids were isolated from the methanol extract of *S. pinnata* bark using column chromatography and spectroscopic techniques. Alkaloids, β-sitosterol, and stigmasterol were identified from the ethanolic extract of *S. pinnata* bark using GC-MS.[7] *S. pinnata* has been extensively studied for its pharmacological properties. It exhibited significant antioxidant activity, anti-inflammatory activity, antidiabetic activity, antimicrobial

activity against various microorganisms, hepatoprotective activity, and anti-cancer activity in vitro against various cancer cell lines.[8]

S. pinnata possesses potent anti-inflammatory and immunomodulatory properties, which could make it a promising natural remedy for asthma. However, despite the promising findings from previous studies, further research is still needed to investigate the safety and effectiveness of *S. pinnata* as a treatment option. This study aims to address this gap by providing more robust evidence for the therapeutic potential of *S. pinnata* for asthma through *in vitro* and *in vivo* studies for the development of new and effective treatments for asthma.

2. Materials and methods

2.1. Preliminary phytochemical analysis

Various phytochemicals in the *S. pinnata* ethanolic extract were qualitatively identified by standard protocols.[9]

2.2. Total flavonoid content

The total flavonoid content of the ethanolic extract of *S. pinnata* was determined using the Dowd colorimetric method with the $AlCl_3$ method. 1 mL of the extract or quercetin was combined with 0.2 mL of a 10% $AlCl_3$ solution in ethanol, 0.2 mL of a 1 M potassium acetate solution, and 5.6 mL of distilled water. The mixture was incubated for 30 minutes at room temperature, followed by measuring the absorbance at 415 nm against a blank. Using a calibration curve, the total flavonoid content was expressed as micrograms of quercetin equivalents per milligram of dry matter. The experiments were conducted in triplicate, and the mean values and standard deviations were calculated.[10]

2.3. Isolation of phytochemicals from *S. pinnata*:

Two compounds (SP-1 & SP-2) were isolated from the ethanolic extracts of *S. pinnata* by using the column chromatography method. In this study, silica gel (60-120 mesh) was used as the stationary phase, and different solvents (n-Hexane and Ethyl acetate) were used as the mobile phase, with increasing order of polarity. SP-1 was isolated in a ratio of 90:10 as a colourless solid with a yield of 28 mg, while SP-2 was isolated in a ratio of 70:30 with a yield of 7 mg. To confirm the identity of these compounds, various analytical techniques were used, including thin-layer chromatography (TLC), Fourier-transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR), and mass spectroscopy. TLC analysis was performed using a mobile phase of 7:3 n-Hexane, Ethyl acetate, and Anisaldehyde-Sulphuric acid as a Spraying agent. The Rf value of SP-1 was found to be 0.64, while that of SP-2 was 0.42. FT-IR was used to determine the functional groups in the compounds, while ^{1}H NMR and ^{13}C NMR were used to determine the carbon and hydrogen atoms' positions in the molecules. Mass spectroscopy was used to determine the molecular weight of the compounds.[11]

2.4. *In silico* docking studies

The cDOCKER method based on CHARMM force filed in receptor-ligand interaction protocol of Discovery Studio v3.5 software was used to dock Epicatechin into the active site of 1IJZ, 3NOS, 3LB6, 1N26, and 2AZ5, where the protein cavity was kept rigid, and the ligand molecules were flexible to move freely inside the binding cavity, to determine the potential binding conformations, analyse the interaction patterns, and correlate with reported activity. The scoring function was based on -cDOCKER interaction energy; the higher -cDOCKER interaction energy signifies greater favourable binding between the protein and the ligand.[12-14]

2.5. *In vitro* anti-asthmatic activity

Guinea pigs of either sex weighing between 400 and 450 g were starved overnight with free access to water. After sacrifice, their intestines were dissected, and the distal portion was sectioned into pieces 2-3 cm long. These tissue pieces were perfused with Tyrode solution until the effluent was clear and then placed in a 25 mL organ bath containing Tyrode physiological solution at 37°C with a constant tension of 1 g and aeration. After an equilibration period, increasing concentrations of histamine and extracts were added. The concentration of histamine that elicited a submaximal response was determined and used for subsequent testing. Test samples viz., standard drug (Mepyramine 0.04 µg/mL), extracts (2, 4, and 8 mg/ml) were added individually after five minutes to evaluate the effect on histamine-mediated contraction. The contraction of the tissue was recorded using a micro dynamometer.[15]

2.6. Mesenteric Mast Cell Count

The adult Wistar rats to be studied are anesthetized and euthanized by cervical dislocation. The mesentery is carefully dissected from the abdominal cavity and placed in a petri dish containing a 10% neutral buffered formalin fixative solution. After fixation, staining was done by embedding in paraffin and sectioning into thin slices of size 5-6 microns. The stained sections are examined under a microscope using 40x and 60x resolution to identify and count the number of mast cells per unit area of the mesentery. The mast cell count is expressed as the average number of mast cells per unit area of the mesentery. The data obtained is analysed statistically to determine significant differences between experimental groups.[16]

2.7. Acute toxicity studies

The acute toxicity was studied by following OECD guidelines using the 'up and down' method with a limit test at 2000 mg/kg body weight and a progression factor of 1.3, starting with a dose of 500 mg/kg. Non-pregnant female Wistar rats were fasted overnight and weighed before administering test extracts. The animals were dosed one at a time at 24-hour intervals and observed for a minimum of 24 hours for symptoms of a moribund state, such as shallow, laboured, or irregular respiration, muscular weakness or tremors, absence of voluntary response to external stimuli, cyanosis, and coma. The animals were monitored for 14 days, and the number of deaths within this period was recorded.[17]

2.8. Histamine-induced bronchospasm in guinea pigs

Bronchospasm was induced experimentally in guinea pigs by exposing them to histamine aerosol according to the method described by Taur et al., 2017. Briefly, guinea pigs of either sex were selected and grouped as follows (n = 5): Group I - Vehicle control, Group II - Standard Ketotifen (10 µg/ml), and Group III and IV - Extracts (Low and High concentrations). Animals showed progressive dyspnea when challenged with 1% w/v histamine aerosol at 40 mmHg pressure from a nebulizer of the histamine chamber (M/s Inco Ambala, India). The onset of pre-convulsive dyspnea (PCD) was recorded as pre-convulsive time (PCT), and the moment the PCD occurred, animals were immediately relocated to the fresh air. Guinea pigs with more than 120 s pre-convulsion dyspnea were considered insensitive and discarded. On the 5^{th} day, the PCT was recorded 2 h after the last dose of extracts.[18]

The percentage increase in PCT was estimated using the equation: $\% PCT = (1 – T1/T2) \times 100$

Where,

T1 = time for PCD onset on day 0

T2 = time for the PCD onset on day 5

2.9. The Clonidine-induced mast cell degranulation in rats

This experiment can help understand the plant extracts' effect on the degranulation of mast cells, which is an important step in allergic reactions, especially in asthma. The effect of extracts on rat mast cell suspensions and the extent of degranulation can be studied. Wistar rats were divided into four groups of five rats each. Group I received vehicle 5 ml/kg orally. Group II received sodium cromoglycate 50 mg/kg intraperitoneally. Group III and IV received low and high doses of the *S. pinnata* extract orally, respectively. The treatment continued for seven days. On day 7, two hours after the assigned treatment, mast cells were collected from the peritoneal cavity.

Ten millilitres of normal saline solution were injected into the peritoneal cavity, and the abdomen was gently massaged for 90 sec. The peritoneal cavity was carefully opened, and the fluid containing mast cells was aspirated and collected in a siliconized test tube containing 7–10 ml of RPMI-1640 Medium (pH 7.2–7.4). The mast cells were washed thrice by centrifugation at low speed (400–500 r.p.m.), and the pellet of mast cells was taken in the medium. The mast cell suspension (approximately 1×10^6 cells/ml) was challenged with 0.5 µg/ml of clonidine solution and stained with 1% toluidine blue, and observed under a high-power microscope field. Cells (100) were counted from different visual areas, and the number of intact and degranulated cells was counted.[19]

3. Results

3.1. Preliminary phytochemical analysis

The standard protocols were followed to conduct the preliminary phytochemical analysis of *S. pinnata*. The analysis revealed the presence of various compounds such as Alkaloids,

Glycosides, Flavonoids, Terpenoids, Steroids, Tannins, Proteins, Carbohydrates, Amino acids, and Saponins.

3.2. Total flavonoid content

The plant extract's total flavonoid content was determined using the colorimetric method with $AlCl_3$. A calibration curve ($y = 7.074x + 0.0757$, $R^2 = 0.9956$) was generated by utilizing varying concentrations of standard quercetin (0–100 µg/mL) and was represented as quercetin equivalents (QE) per gram. The total flavonoid content of the extract was calculated and represented as milligrams of quercetin equivalents (QE) per gram of dry weight sample (mg/g). In this representation, y represents the absorbance at 415 nm, and x represents the total flavonoid content in the extracts.

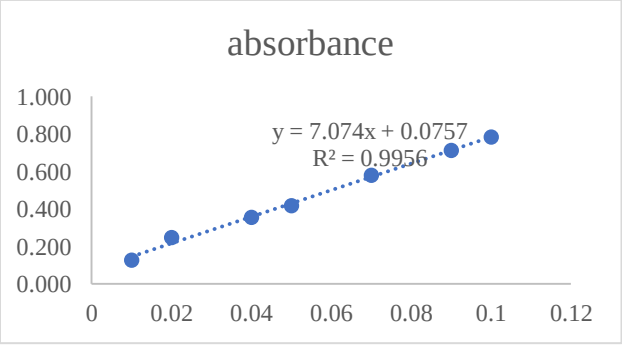


Figure 1. Standard curve of quercetin

The ethanolic extract of *S. pinnata* has a flavonoid content (73.7±0.67mgQE/g) at the given conditions and procedures.

3.3. Isolation of Phytochemical from *S. pinnata*

3.3.1. SP-1: (β – sitosterol)

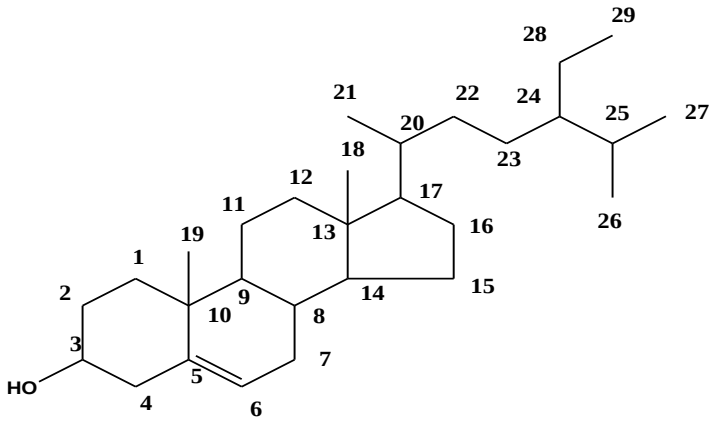


Figure 2. Structure of β-sitosterol

^{1}H NMR ($CDCl_3$, 400 MHz); The ^{1}H NMR spectrum of the compound (ppm) showed the presence of six methyl signals that appeared as two methyl singlets at δ 0.70 and 1.03, which corresponds to the angular methyl singlets. Three methyl doublets appeared at δ 0.95, 0.84, and 0.85, and a methyl triplet at δ 0.87correspounding to the methyl protons. The ^{1}H NMR spectrum of this substance also showed one olefinic proton at δ 5.37. Its ^{1}H NMR spectrum showed a proton corresponding to the hydroxyl group, which appeared as a multiplet at δ 3.55ppm. The signals at 140.9 and 121.9 ppm have been assigned to C5 and C6 double bonds, respectively, while the value at 19.2 ppm corresponds to the angular carbon atom (C19). Mass m/z value of 397 and 414 indicates the characteristic fragment peak of β – sitosterol (mol. wt 414)

3.3.2. SP-2 (Epicatechin):

The ^{1}H NMR spectrum showed M.P. ranged from175-180 ^{0}C.^{1}H NMR spectrum showed δ 2.68 (1H, d, C-2), 4.76 (1H, m, C-3), 6.42 (1H, d, C-6), 6.45 (1H, d, C-8), 6.65 (1H, d, C-2'), 6.60 (1H,d C-5'), 6.64 (1H, d, C-6'), two doublets at 2.68 & 3.18 (1H, dd, dd, C-4)confirms the absence of carbonyl functional group on fourth position and Hydroxyl peaks at 1.17 (C-3), 3.45 (C-3')and 3.57 (C-4') represents the polyhydroxy nature of the molecule. The peaks at the range

of 6ppm represent the aromatic ring system in the molecule. Similarly, the ^{13}C NMR spectrum of the compound indicated 15 carbon signals with no free methyl groups and methoxy groups with δ values 81.35(C-2), 68.64(C-3), 29.44(C-4), 154.80(C-5), 94.67(C-6), 157.74(C-7),94.66(C-8), 155.04(C-9), 99.94(C-10), 131.07(C-1'),115.65(C-2'),145.02(C-3'),145.06(C-4'),116.07(C-5'),120.06(C-6'). The quaternary carbon (C-9) linked to the oxygen atom and the carbons that bear hydroxyl groups exhibit higher ppm values in the ^{13}C NMR spectrum. A mass m/z value of 291 indicates the characteristic fragment peak of Epicatechin (mol.wt-290.08)

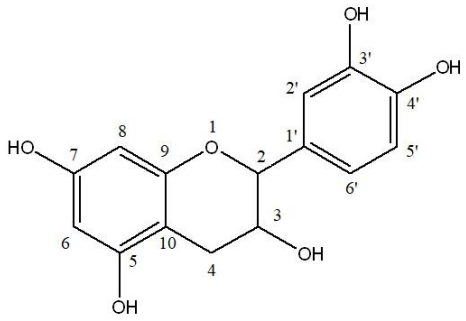
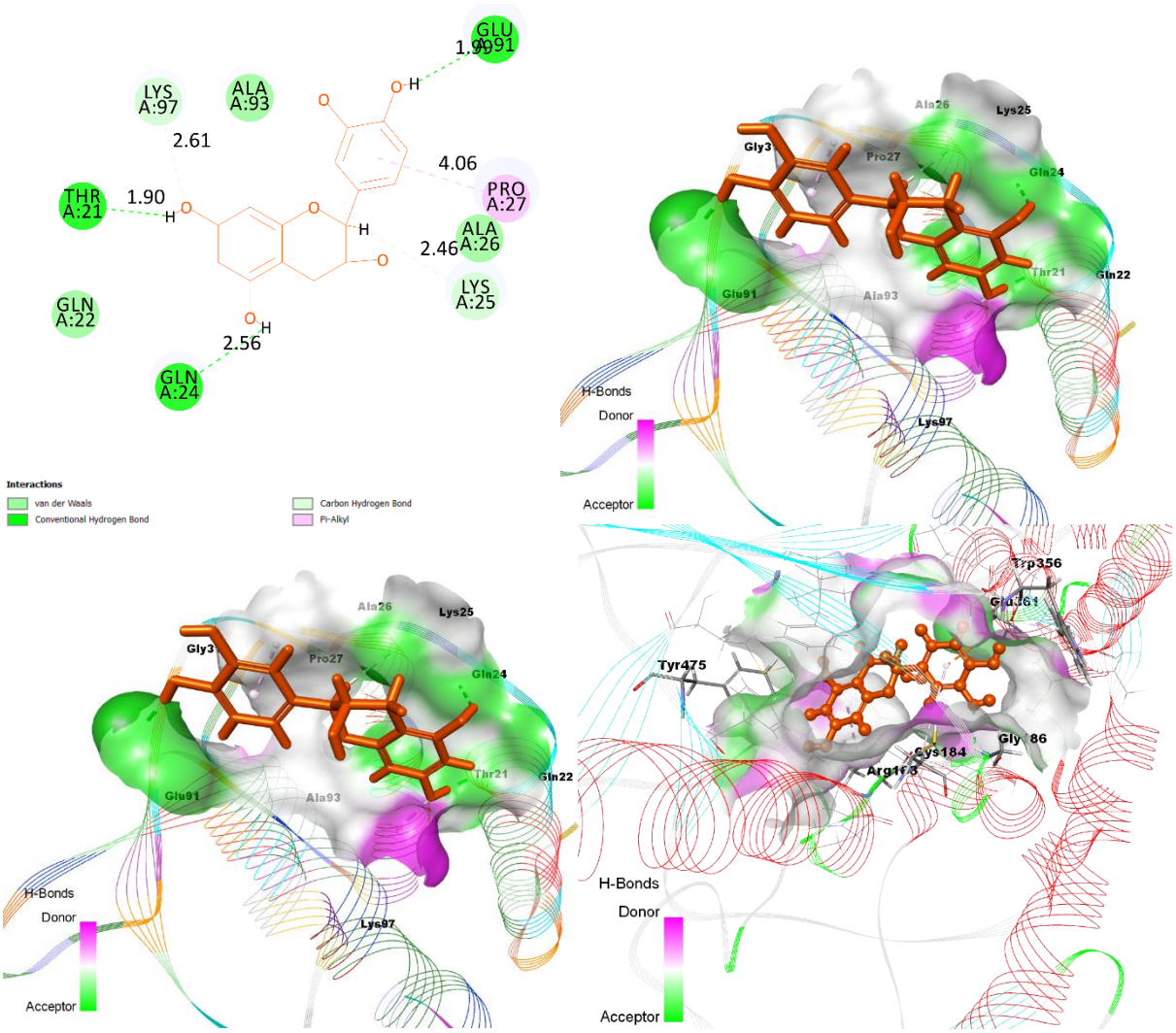


Figure 3. Structure of Epicatechin

3.4. *In silico* docking studies

The docking results were demonstrated in Table 1. Epicatechin and β-sitosterol can easily be accommodated into the studied targets' binding sites (Figure 4&5). The reasonable docking poses yielded docking scores with 1IJZ, 3NOS, 3LB6, 1N26, and 2AZ5. Epicatechin with PDB: 1IJZ showed three hydrogen bonds with GLU91, THR21, and GLN24 amino acids. Epicatechin with PDB: 3NOS (GLY186, TRP356, and TYR475) and 3LB6 (ARG111 and ASN113) showed three and two hydrogen bonds, respectively. PDB: 1N26 (GLN68) and 2AZ5 (TYR151) displayed one hydrogen interaction each with Epicatechin. The β-sitosterol binding score was comparatively insignificant, indicating the non-specificity of the molecule with the selected targets.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LYS A:97
ALA A:93
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1.99
4.06
PRO A:27
THR A:21
1.90
ALA A:26
2.46
LYS A:25
GLN A:22
2.56
GLN A:24
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Alkyl
Ala26
Lys25
Gly3
Pro27
Gln24
Thr21
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Acceptor
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Cys184
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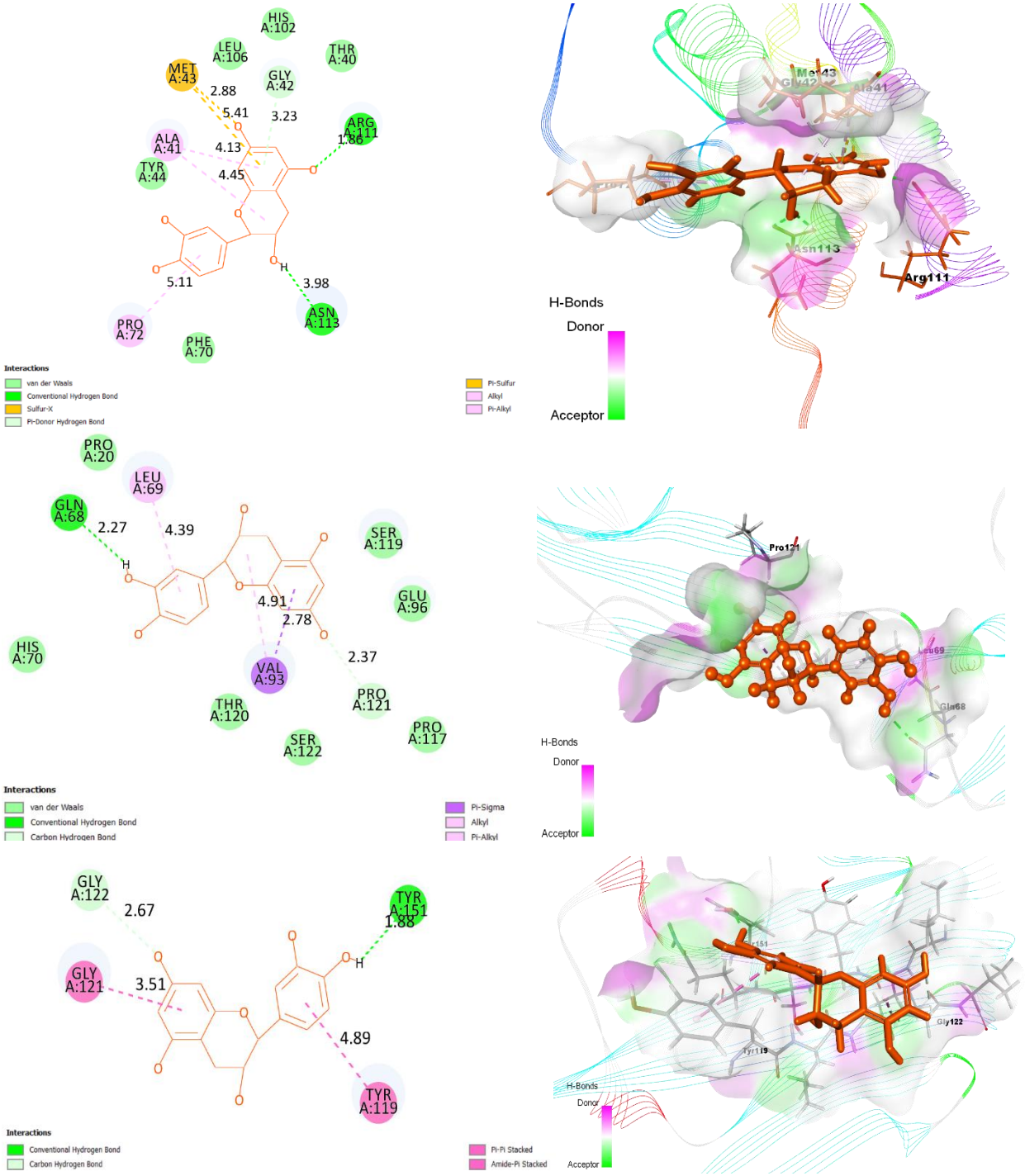
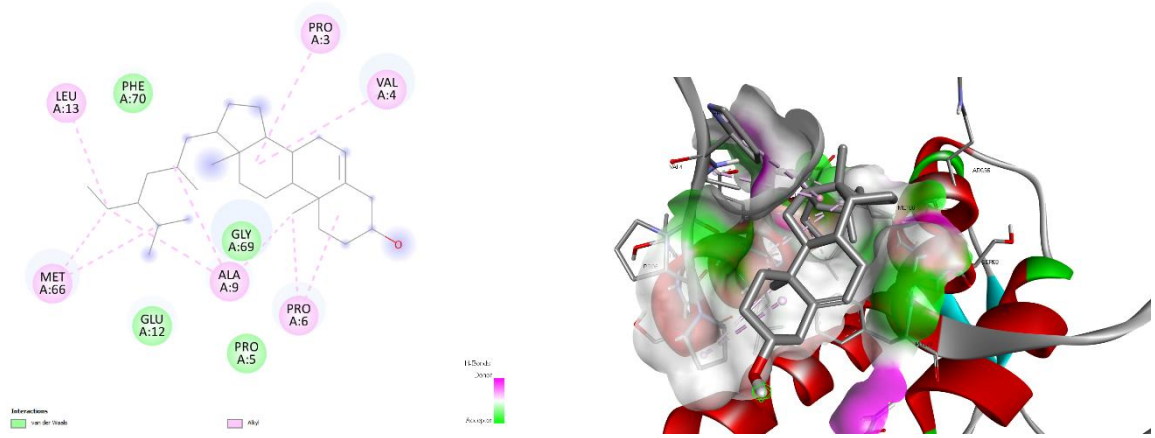


Figure 4. Docking score of Epicatechin with multiple targets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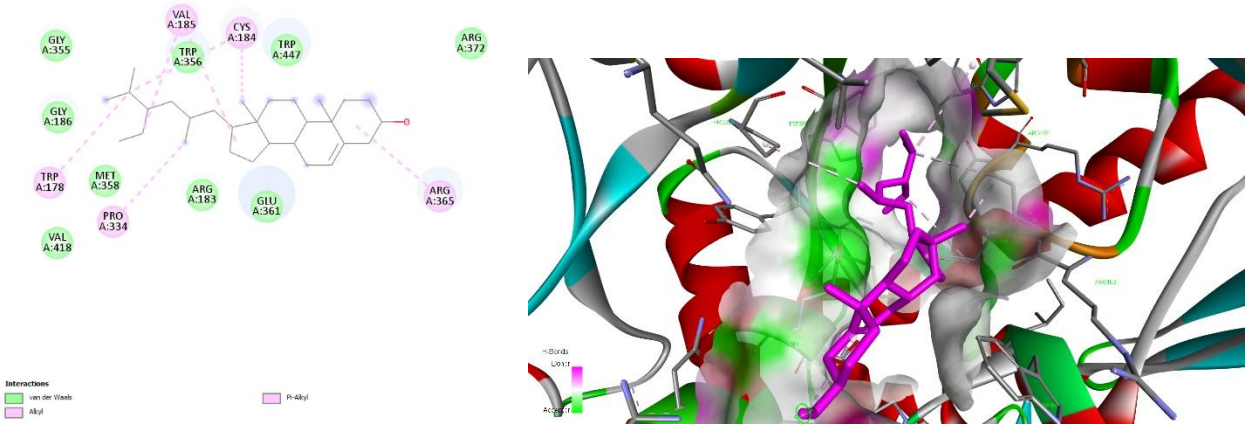
PRO A:5
Interactions
van der Waals
Alkyl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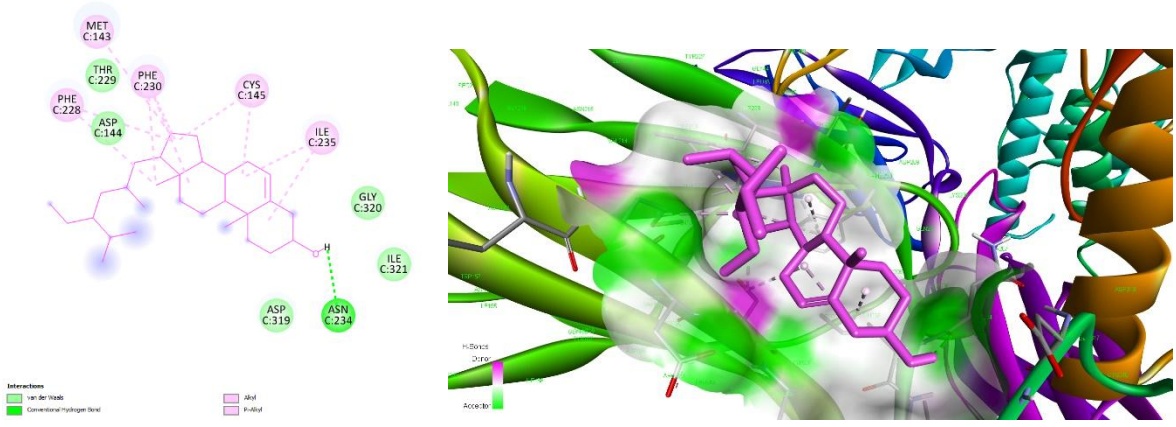
Pi-Alkyl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ASP C:144
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van der Waals
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Pi-Alkyl

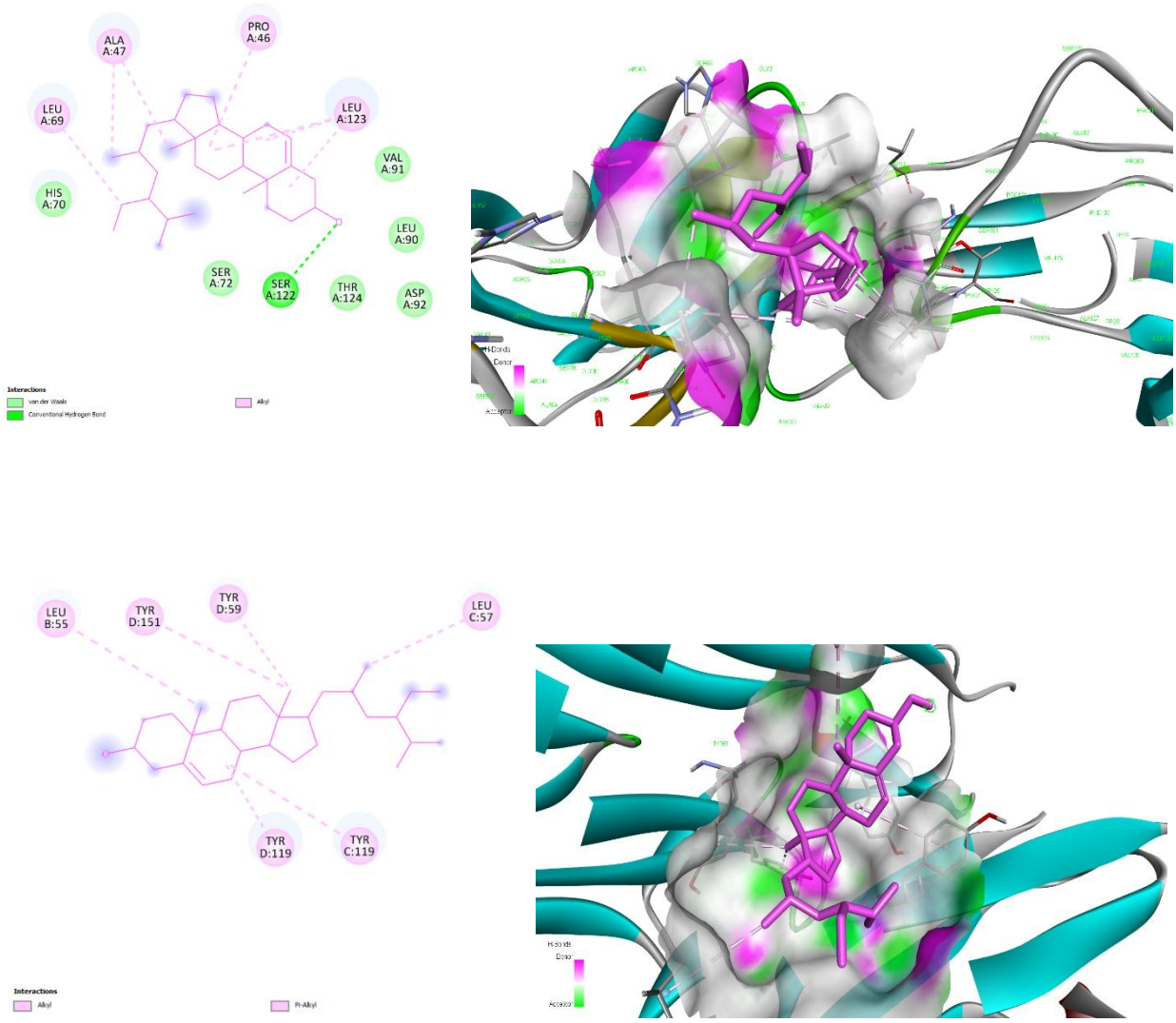

Figure 5. Docking score of β-sitosterol with multiple targets

Table 1. Docking score of Epicatechin with multiple targets

S. No.	PDB	Epicatechin	β-sitosterol
		cDOCKER (Kcal/mol)	
1.	1IJZ (Human IL-13)	-39.23	-6.35
2.	3NOS (Human endothelial nitric oxide synthase)	-39.54	-7.33
3.	3LB6 (IL-13)	-38.21	-5.48
4.	1N26 (Human Interleukin-6)	-32.44	-4.9
5.	2AZ5 (TNF-alpha)	-27.70	-7.82

Based on the identified compound epicatechin, which exhibited strong docking scores with human IL-13 and human endothelial nitric oxide synthase, there is a scientific rationale to propose a potential correlation between the in silico docking results and the observed in vitro and in vivo data. The favorable docking scores indicate a strong binding affinity of Epicatechin towards these specific protein targets, suggesting its capability to interact with them and potentially modulate their activity.

The anti-asthmatic effects demonstrated by *S. pinnata*, including the inhibition of histamine-induced contractions and mast cell degranulation, provide experimental evidence that supports the hypothesis of the involvement of Epicatechin in the observed outcomes. Histamine-induced contractions and mast cell degranulation are closely associated with IL-13 and nitric oxide synthesis, processes known to be implicated in asthma.

The convergence of the docking scores indicating a potential interaction between Epicatechin and the specific protein targets, along with the observed anti-asthmatic effects of *S. pinnata* on processes related to IL-13 and nitric oxide, provides scientific support for the notion that Epicatechin may contribute to the anti-asthmatic activity of *S. pinnata*.

3.5. *In vitro* anti-asthmatic activity

At a 1.6µg/ml volume of histamine, the control group showed a mean contraction of 91.25±1.27 units. The standard group showed a mean contraction of 1.48±1.25 units, indicating effective inhibition of histamine-induced contractions. Treatment with *S. pinnata* at a dose of 200mg/kg resulted in a mean contraction of 61.73±2.97 units, which was significantly lower than the control group, and treatment with a higher dose of 400mg/kg resulted in a mean contraction of 36.82±3.93 units, which was significantly lower than both the control group and the lower dose

group. The data (Table 2) show that *S. pinnata* can significantly inhibit histamine-induced contractions in isolated guinea pig ileum, and the inhibitory effect is dose-dependent. The higher dose of 400mg/kg of *S. pinnata* was more effective in inhibiting histamine-induced contractions than the lower dose of 200mg/kg, indicating that the extract has the potential as an anti-asthmatic agent. The data suggest that *S. pinnata* could have potential therapeutic value in treating asthmatic conditions.

Table 2. Effect on histamine-induced contraction in isolated guinea pig ileum

Volume of Histamine (25µg/ml)	**Control group**	**Standard Group**	***S. Pinnata* (200mg/kg)**	***S. Pinnata* (400mg/kg)**
0.1	20.36±0.93	0.23±0.49**	9.63±1.72*	5.32±0.97*
0.2	35.24±0.84	0.26±1.15**	25.64±3.19*	19.71±2.67**
0.4	60.71±0.38	1.31±1.27**	34.82±1.64*	26.62±2.18**
0.8	89.34±0.85	1.53±1.34**	51.37±3.45**	31.45±3.21**
1.6	91.25±1.27	1.48±1.25**	61.73±2.97**	36.82±3.93**

3.6. Mesenteric Mast Cell Count

The presented data show different treatments' percentage inhibition of mast cell degranulation. Inhibition of mast cell degranulation is an important target in treating inflammatory conditions. The data shows that ketotifen, a known antiallergic agent, has the highest inhibitory effect on mast cell degranulation, with a percentage inhibition of 82.61±1.18. The two doses of *S. pinnata* tested, 200mg/kg and 400mg/kg, showed significant inhibition of mast cell degranulation

compared to the control group, with a percentage inhibition of 56.82±2.94 for 200mg/kg and 71.56±1.18 for 400mg/kg respectively. These results (Table 3) suggest that *S. pinnata* has shown a significant inhibitory effect on mast cell degranulation. The inhibitory effect was also dose-dependent, with the higher dose of 400mg/kg showing a more significant than the lower dose of 200mg/kg.

Table 3. Effect of ethanolic extracts on mast cell degranulation

Treatment	**% inhibition of degranulation**
Ketotifen	82.61±1.18
S. pinnata (200mg/kg)	56.82±2.94**
S. pinnata (400mg/kg)	71.56±1.18**

3.7. Acute toxicity studies

Acute toxicity studies performed according to OECD guidelines 425 showed that *S. pinnata* does not show any toxicity symptoms in rats at a dose of 2000 mg/kg body weight orally, and it is considered safe to use the extract at medium doses. The working dose was calculated as $1/5^{th}$ and $1/10^{th}$ of the highest safe dose, i.e., 400 and 200mg/kg body weight, respectively.

3.8. The Clonidine-induced mast cell degranulation in rats

The data (Table 4) shows the percentage of disrupted mast cells in rats treated with different doses of *S. pinnata* and sodium cromoglycate, compared to a control group, in the presence of clonidine, an α_2-adrenergic receptor agonist that induces mast cell degranulation.

The control group showed 80.36±1.15% disrupted mast cells, indicating significant mast cell degranulation in the presence of clonidine. Treatment with sodium cromoglycate showed a significant inhibitory effect on mast cell degranulation, with only 25.16±0.38% disrupted mast cells. *S. pinnata* tested at 200mg/kg and 400mg/kg doses also showed a significant inhibitory effect on mast cell degranulation, with 52.54±2.09% and 40.92±2.17% disrupted mast cells, respectively. The inhibitory effect of *S. pinnata* was dose-dependent, with the higher dose showing a more significant inhibitory effect. These results suggest that *S. pinnata* has the potential to inhibit mast cell degranulation induced by clonidine. The inhibitory effect was comparable to sodium cromoglycate, indicating that *S. pinnata* could be an alternative or complementary treatment for asthmatic conditions.

Table 4. Effect of *S. pinnata* on Clonidine-induced mast cell degranulation in rats

Treatment	**% of disrupted mast cells**
Control	80.36±1.15
Sodium cromoglycate	25.16±0.38
S. pinnata (200mg/kg)	52.54±2.09**
S. pinnata (400mg/kg)	40.92±2.17**

3.9. Histamine-induced bronchospasm in Guinea pigs

The effect of different treatments on pre-convulsive time in guinea pigs over 5 days was measured (Table 5). Pre-convulsive time is the time taken for the onset of convulsions after administering histamine. The control group showed relatively stable pre-convulsive times on Day 0 and Day 5, with mean values of 93.55±6.38 and 94.48±6.45, respectively. Ketotifen, a known antiallergic drug, showed a significant increase in pre-convulsive time on Day 5, with a mean value of 146.55±10, compared to Day 0 (101.77±6.94), indicating a protective effect

against convulsions. *S. pinnata* tested at 200mg/kg and 400mg/kg showed a slight increase in pre-convulsive time on Day 5 at 104.70±7.14 and 122.13±8.33, respectively. The results suggest that *S. pinnata* has a mild protective effect against convulsions induced by the tested agent compared with ketotifen.

Table 5. Effect of extracts on pre-convulsive time in Guinea pigs

Treatment	**Day 0**	**Day 5**
Control	93.55±6.38	94.48±6.45
Ketotifen (10 µg/ml)	101.77±6.94	146.55±10
S. pinnata (200mg/kg)	99.72±6.8	104.70±7.14
S. pinnata (400mg/kg)	95.60±6.52	122.13±8.33

4. Discussion

The histamine-induced contraction in isolated guinea pig ileum model is an experimental model used in pharmacology and physiology research to study the effects of drugs and compounds on smooth muscle contraction in the guinea pig ileum. This model has significant importance as it allows researchers to evaluate the efficacy of potential drugs for treating gastrointestinal disorders and to investigate the mechanisms of action of neurotransmitters on smooth muscle contraction. The model has also been used to test the effects of various anti-asthmatic, antiallergic agents to evaluate their safety and efficacy. *S. pinnata* has a significant inhibitory effect on histamine-induced contractions in isolated guinea pig ileum. The study found that the higher dose of 400mg/kg of the extract was more effective in inhibiting contractions, suggesting

that the extract could be used as an anti-asthmatic agent. Thus, the data support the potential therapeutic value of *S. pinnata* in treating asthmatic conditions.

Mast cells are an essential component of the immune system that play a crucial role in initiating and maintaining allergic and inflammatory reactions. Mast cell degranulation, which releases histamine and other inflammatory mediators from mast cells, is critical in developing allergic and inflammatory diseases. Inhibition of mast cell degranulation is an important target for treating inflammatory conditions. The study found that *S. pinnata* showed significant inhibition of mast cell degranulation. The two doses of *S. pinnata* tested, 200mg/kg and 400mg/kg, showed percentage inhibitions of 56.82±2.94 and 71.56±1.18, respectively, compared to the control group. These results suggest that *S. pinnata* could potentially treat inflammatory conditions at higher doses by inhibiting mast cell degranulation.

The effect of *S. pinnata* on clonidine-induced mast cell degranulation in rats is a crucial event in the pathogenesis of asthma. The findings revealed that the control group had a high percentage of disrupted mast cells, indicating significant mast cell degranulation. However, sodium cromoglycate and *S. pinnata* treatment showed a significant inhibitory effect on mast cell degranulation, indicating their potential in asthma treatment. The study found that sodium cromoglycate had the highest inhibitory effect, with only 25.16% disrupted mast cells. Interestingly, *S. pinnata*, tested at 200mg/kg and 400mg/kg doses, also exhibited a significant inhibitory effect on mast cell degranulation, with 52.54% and 40.92% disrupted mast cells, respectively.

The effect of different treatments on pre-convulsive time in guinea pigs was measured. The control group showed stable pre-convulsive times on Day 0 and Day 5, indicating that histamine consistently induced convulsions in the guinea pigs. However, when treated with ketotifen, a

known antiallergic drug, there was a significant increase in pre-convulsive time on Day 5 compared to Day 0, indicating a protective effect against convulsions. *S. pinnata* was also tested at doses of 200mg/kg and 400mg/kg, and it showed a significant increase in pre-convulsive time on Day 5 compared to Day 0, suggesting its mild protective effect against convulsions. These findings support the potential of *S. pinnata* as a treatment for bronchospasm.

Column chromatography was employed to isolate and characterize two compounds, SP-1 and SP-2, from the ethanolic extracts of *S. pinnata*. This analysis aimed to gain insight into the bioactive compounds present in the plant. After a comprehensive spectral analysis, the isolated compounds were identified as β-sitosterol and Epicatechin. Interestingly, Epicatechin is the first report identified in this plant species. The phytochemical analysis and total flavonoid content support that the plant is rich in flavonoids and can contribute to the observed pharmacological activity. Considering the identified compound epicatechin, which showed strong docking scores with human IL-13 and human endothelial nitric oxide synthase, it is reasonable to propose a potential correlation between the in silico and in vitro/in vivo data. The docking scores suggest that compared to β-sitosterol, Epicatechin may interact with these specific targets, possibly influencing their activity and associated asthma-related processes. This aligns with the observed anti-asthmatic effects of *S. pinnata* extract in inhibiting histamine-induced contractions and mast cell degranulation, as these processes are closely linked to IL-13 and nitric oxide synthesis. However, more studies are required to fully comprehend the mechanisms behind these compounds' activities.

5. Summary and Conclusion

The preliminary phytochemical analysis of *S. pinnata* revealed the presence of various compounds such as Alkaloids, Glycosides, Flavonoids, Terpenoids, Steroids, Tannins, Proteins,

Carbohydrates, Amino acids, and Saponins. *In vitro* anti-asthmatic activity results showed that *S. pinnata* could significantly inhibit histamine-induced contractions in isolated guinea pig ileum, and the inhibitory effect is dose-dependent. The higher dose of 400mg/kg of *S. pinnata* was more effective in inhibiting histamine-induced contractions than the lower dose of 200mg/kg, indicating that the extract has the potential as an anti-asthmatic agent. Mast cell count inhibition results indicated that *S. pinnata* has the potential as an anti-asthmatic agent, significantly inhibiting mast cell degranulation. Acute toxicity studies showed that *S. pinnata* is safe to use at medium doses of 200 and 400mg/kg body weight. The Clonidine-induced mast cell degranulation in rats demonstrated that *S. pinnata* has the potential as an anti-asthmatic agent by inhibiting clonidine-induced mast cell degranulation. Histamine-induced bronchospasm in guinea pigs showed that *S. pinnata* slightly increased pre-convulsive time on Day 5, indicating a protective effect against convulsions.

Isolation and characterization of compounds SP-1 and SP-2 from the ethanolic extracts of *S. pinnata* using column chromatography were carried out to understand the bioactive compounds in the plant further. From the detailed spectral analysis, the isolated compounds SP-1 and SP-2 were identified as β-sitosterol and Epicatechin, which is reported for the first time from this species. Further studies are required to fully understand the mechanisms behind these activities and the potential use of these compounds in developing new drugs for asthmatic conditions. The study suggests that *S. pinnata* could be a promising natural alternative or complementary treatment for asthmatic conditions.

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Conflict of interest

The authors declare no conflict of interest

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