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Anti-Allergic Potentials of *Actinodaphne angustifolia*: Integrated In Vivo Evaluation and In Silico Analysis of Bioactive Compounds

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ABSTRACT

Actinodaphne angustifolia (Lauraceae), traditionally used for treating inflammatory and allergic disorders was investigated for its anti-allergic potential using integrated in vivo and in silico approaches. Safety evaluation in accordance with OECD guidelines confirmed the non-toxic nature of the *ethanolic* leaf extract. In a toluene 2,4-diisocyanate (TDI)-induced allergic model, the extract reduced allergic symptoms, including sneezing, nasal scratching, and nasal scores, also normalizing elevated white blood cell counts in blood and bronchoalveolar lavage fluid. A marked reduction in serum IgE levels further indicated suppression of hypersensitivity responses, with effects comparable to cetirizine. HPLC analysis revealed a rich polyphenolic profile dominated by (–) epicatechin and rutin hydrate, while GC–MS identified 15 additional bioactive constituents. Molecular docking demonstrated strong binding affinities of these compounds toward key allergic targets, namely histamine H1 receptor, interleukin-4, and phospholipase A2, supporting their mechanistic roles in anti-allergic activity. The findings validate the ethnomedicinal relevance of *A. angustifolia* and highlight its potential as a source of plant-derived anti-allergic agents.

1 | Introduction

Medicinal plants have been among the most ancient and reliable sources of therapeutic agents, with nearly 80% of the global population still relying on traditional remedies for primary healthcare [1]. The chemical diversity and structural complexity of plant-derived metabolites make them invaluable resources for modern drug discovery, especially for chronic diseases where conventional therapies often exhibit limited efficacy and adverse

effects [2, 3]. In Bangladesh, a rich heritage of medicinal plants exists, many of which play a central role in traditional healing systems. A large proportion of the rural and tribal populations relies on these herbal remedies to treat diverse ailments due to limited access to modern healthcare [4, 5]. Among these communities, the hill tract regions represent an especially valuable repository of ethnomedicinal knowledge, where plants are routinely used to address life-threatening diseases, such as diabetes, asthma, rheumatism, and allergic disorders. This ecological

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diversity, coupled with centuries of empirical use, highlights the therapeutic potential of hill-tract flora as promising sources of novel bioactive compounds for modern drug development [6, 7]. Indeed, several pharmaceutical industries have already explored plant-derived metabolites from such ecosystems for incorporation into commercial therapies.

Allergic disorders remain a major global health concern, affecting hundreds of millions of individuals worldwide. Allergy, or hypersensitivity, is defined as a triggered immune response to foreign substances present in the environment. The consequences of allergies range from minor symptoms, such as atopic dermatitis, itching, sneezing, and rhinitis, to severe manifestations, such as asthma and anaphylaxis [8, 9]. Sometimes, the severity of allergic syndromes is such that it can even result in death. These allergic reactions are mediated by a biomolecule named histamine. Histamine, a biogenic amine formed by the decarboxylation of histidine, plays a central role in mediating allergic responses through activation of histamine H1 receptors (H1R), leading to symptoms, such as sneezing, nasal irritation, and airway inflammation [10, 11]. In addition to histamine signaling, other mediators such as interleukins and phospholipase A2 also contribute significantly to the allergic cascade. Antihistaminic agents such as cetirizine and loratadine are routinely used to alleviate allergic symptoms, particularly sneezing, itching, and rhinitis. [12, 13], despite their clinical effectiveness, prolonged use may be accompanied by undesirable effects, including drowsiness, central nervous system disturbances, and, in certain circumstances, cardiovascular-related effects. [14]. Experimental models using toluene diisocyanate (TDI), a well-known respiratory sensitizer, further highlight the complexity of allergic mechanisms, as exposure can induce allergic rhinitis and asthma-like symptoms [15, 16]. Notably, approximately 15% of individuals with chronic occupational exposure to TDI develop asthma, emphasizing the urgent need for safer and more effective therapeutic alternatives [17]. Hence, exploring and validating plant-based alternatives holds great promise in addressing these challenges.

Actinodaphne angustifolia (Lauraceae), locally known as Modon mosta, is traditionally used by the indigenous community in Southeast Asia for the management of inflammatory and allergic conditions [18]. Phytochemical studies of *Actinodaphne* species have revealed a structurally diverse range of secondary metabolites, including isoquinoline alkaloids, such as aporphines and oxoaporphines, lactones, lignans, phenolic amides, flavonoids, sterols, and terpenoid constituents [19]. These findings indicate that *A. angustifolia* is a rich source of pharmacologically relevant secondary metabolites. Several of these metabolite classes are associated with biological activities, such as antioxidant, antibacterial, antifungal, anti-inflammatory, and cytotoxic effects. In *A. angustifolia*, previously reported constituents include β -sitosterol, quercetin-3-O-rhamnoside, vitexin, friedelin, and various hydrocarbon derivatives [20]. The presence of these chemically diverse constituents suggests that the biological effects of the species may arise from the combined actions of multiple phytochemicals rather than from a single constituent. The anti-allergic potential associated with its constituents has not been investigated with molecular targets involved in allergic responses. Consequently, the traditional use of this species for allergic disorders has remained without experimental validation. Considering

its ethnomedicinal significance and promising phytochemical composition, *A. angustifolia* was selected for a comprehensive investigation to evaluate its anti-allergic activity and to identify the bioactive constituents that may contribute to this effect. Here, we investigated the anti-allergic potential of the *ethanolic* leaf extract of *A. angustifolia* using an integrated in vivo and in silico approach. Anti-allergic activity was evaluated in a toluene diisocyanate (TDI)-induced mouse model by assessing clinical symptoms, including sneezing, nasal scratching, and nasal scores, together with hematological parameters, such as white blood cell (WBC) counts in blood and bronchoalveolar lavage (BAL) fluid and serum IgE levels. HPLC–DAD and GC–MS analyses were employed to characterize the phytochemical composition of the extract. The pharmacokinetic and drug-likeness properties of selected constituents were further examined through ADME analysis. Molecular docking was performed to explore the binding interactions of the identified phytoconstituents with key allergy-related targets, including the histamine H1 receptor (PDB ID: 3RZE), interleukin-4 (PDB ID: 3BPN), and phospholipase A2 (PDB ID: 1DB4), while molecular dynamics simulations were used to further assess the stability of selected protein–ligand complexes. Collectively, the experimental and computational findings provide scientific support for the traditional use of *A. angustifolia* in allergic conditions and offer insights into the phytoconstituents and molecular targets potentially associated with its anti-allergic activity.

2 | Results and Discussion

2.1 | Phytochemical Screening

Preliminary phytochemical evaluation of the *ethanolic* extract of *A. angustifolia* revealed the presence of diverse classes of secondary metabolites, including alkaloids, tannins, flavonoids, glycosides, terpenoids, and reducing sugars, while gums and xanthoproteins compounds were absent (Table S1). These phytoconstituents are widely associated with significant pharmacological activities. In particular, flavonoids and phenolic compounds are well known for their antioxidant and anti-allergic effects, as they scavenge free radicals and modulate immune responses. The presence of bioactive compounds provides a rational basis for *A. angustifolia*'s observed bioactivities.

2.2 | Safety Study (Acute and Sub-Acute Toxicity) of the Extract

The acute toxicity study demonstrated that oral administration of the extract up to 3000 mg/kg produced no mortality, behavioral changes, or visible signs of toxicity during the 14-day observation period (Table S2). Body weight remained stable compared to the control group, indicating the absence of systemic toxicity (Figure S1). Similarly, subacute toxicity assessment (500 mg/kg) showed no significant alterations in physiological behavior or biochemical parameters. Hepatic enzymes (SGPT, SGOT, ALP) and renal function markers (creatinine, urea, bilirubin) remained within normal physiological limits (Figure 1). These findings classify the extract as practically non-toxic under OECD Guideline 425, indicating a wide safety margin for further pharmacological use.

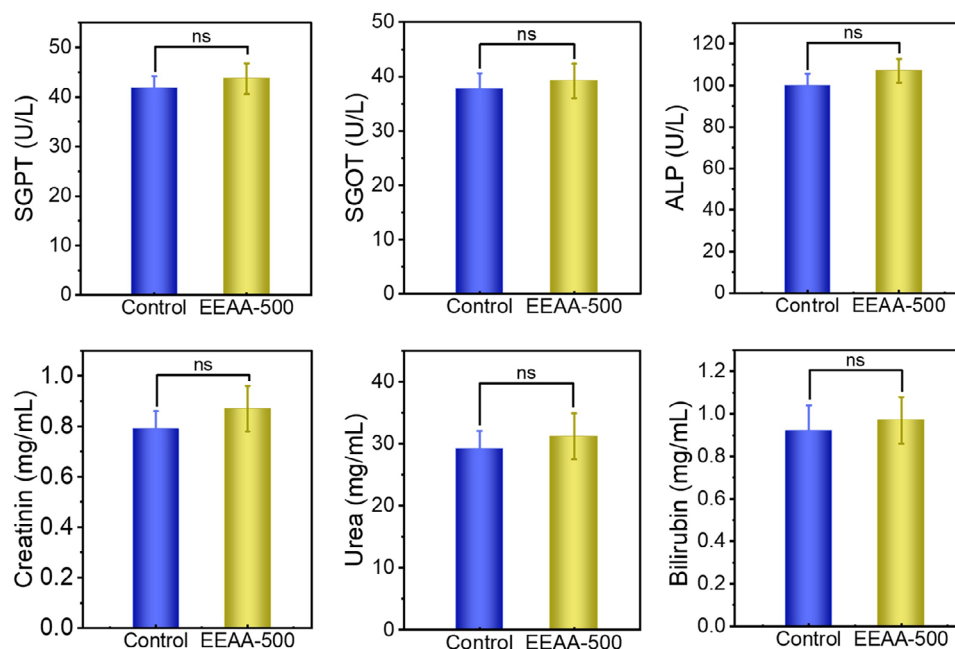


FIGURE 1 | Safety evaluation of the *ethanolic* extract of *A. angustifolia* (EEAA) based on serum biochemical parameters. Effects of EEAA on liver function markers: (a) Serum glutamate pyruvate transaminase (SGPT), (b) serum glutamate oxaloacetate transaminase (SGOT), (c) alkaline phosphatase (ALP), and (d) serum bilirubin; and kidney function markers: (e) Serum creatinine, and (f) serum urea. Data are presented as mean $\pm$ standard error of the mean (SEM) ($n = 6$). ns: not significant.

2.3 | Evaluation of Anti-Allergic Activity

TDI sensitization successfully induced nasal allergy-like symptoms in mice, including sneezing, scratching, rhinorrhea, and nasal score. The TDI control group exhibited significantly elevated sneezing frequency, scratching behavior, and nasal scores of 41.2 ± 4.25 , 234 ± 19.9 , and 2.8 ± 0.20 , respectively (Figure 2). Oral administration of the *ethanolic* extract of *A. angustifolia* (EEAA) produced a marked reduction in all allergy-associated symptoms. At 500 mg/kg, the extract markedly decreased sneezing, scratching, and nasal scores to levels comparable to those of the standard antihistamine cetirizine. Notably, lessened the allergic symptoms, such as sneezing, scratching, and nasal scores with the value of (EEAA-500 vs Cetirizine 11.8 ± 2.97 vs 11.2 ± 3.07), scratches (EEAA 500 vs Cetirizine vs 111.2 ± 18.5), and nasal scores (EEAA-500 vs Cetirizine 0.8 ± 0.37 vs 1.3 ± 0.24). These findings indicate that *A. angustifolia* possesses substantial anti-allergic potential, possibly through modulation of histamine-mediated pathways. Serum IgE levels, a key biomarker of allergic response, were significantly elevated in TDI-treated mice. EEAA administration (500 mg/kg) markedly reduced IgE levels, approaching the effect of cetirizine (Figure 2d). This suggests that the extract effectively modulates IgE-mediated hypersensitivity responses, possibly through regulation of cytokine signaling pathways such as IL-4.

TDI exposure significantly increased total and differential WBC counts, including lymphocytes, neutrophils, eosinophils, basophils, and monocytes. EEAA treatment effectively normalized these elevated counts (Figure 3), suggesting suppression of immune cell activation and infiltration. The extract at 500 mg/kg demonstrated a comparable efficacy to cetirizine, indicating

its capacity to regulate immune cell infiltration and mitigate hypersensitivity responses.

Consistent results were observed in bronchoalveolar lavage (BAL) fluid, where TDI-induced elevation of inflammatory cells was significantly reduced following extract treatment (Figure 4). This demonstrates that the extract exerts both systemic and localized anti-inflammatory effects within the respiratory tract.

2.4 | HPLC Standardization and Quantification of Polyphenols

HPLC-DAD analysis was performed using a mixed standard containing 16 authentic polyphenolic reference compounds under identical chromatographic conditions (Figure 5). The number of polyphenolic compounds identified and quantified was based on their retention times (Figures S2, S3). The identified compounds included catechol (5.69 mg/100 g), (-)epicatechin (106.55 mg/100 g), rutin hydrate (8.08 mg/100 g), rosmarinic acid (17.92 mg/100 g), myricetin (26.46 mg/100 g), and kaempferol (10.21 mg/100 g). Among these, (-)epicatechin was the most abundant, indicating its possible role as a dominant bioactive component (Table S3). These polyphenols are well documented for their antioxidant and immunomodulatory effects. Polyphenols such as catechol, (-)epicatechin, rutin hydrate, myricetin, and kaempferol are well-documented for their immunomodulatory and antihistaminic effects, acting through suppression of mast cell degranulation, inhibition of histamine release, and modulation of inflammatory mediators [21]. Their combined presence indicates a synergistic mechanism

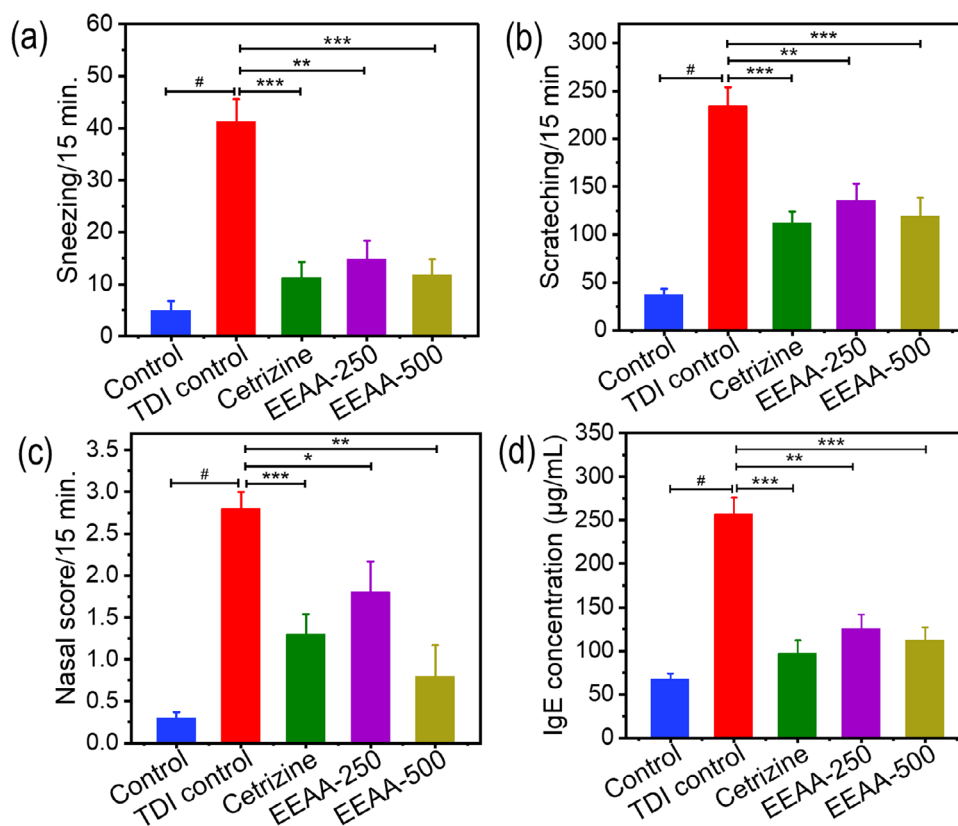


FIGURE 2 | Evaluation of the anti-allergic effects of the *ethanolic* extract of *A. angustifolia* (EEAA) in mice. The assessed parameters include (A) sneezing frequency, (B) scratching behavior, (C) nasal symptom scores, and (D) serum IgE levels. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 6$). Statistical significance was determined using Dunnett's test: # $p < 0.05$ versus the control group; * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.0001$ versus the TDI control group.

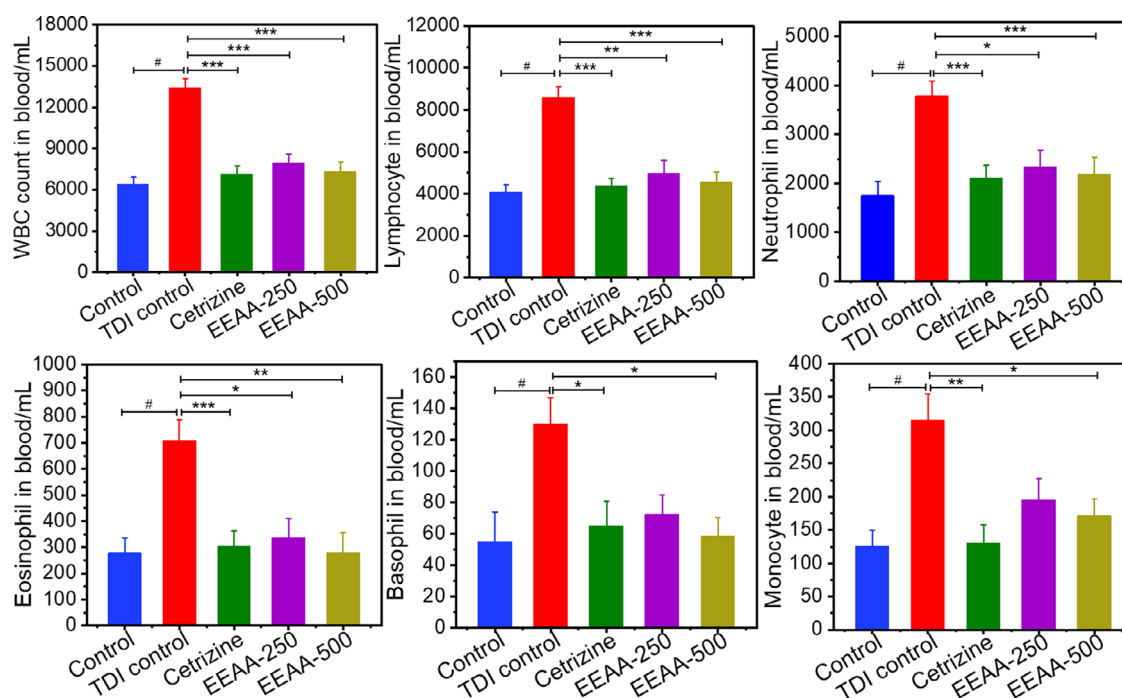


FIGURE 3 | Effect of the *ethanolic* extract of *A. angustifolia* (EEAA) on total and differential white blood cell (WBC) counts in mice. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 6$). Statistical significance was performed using Dunnett's test: # $P < 0.05$ versus control group, * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.0001$ versus TDI control group.

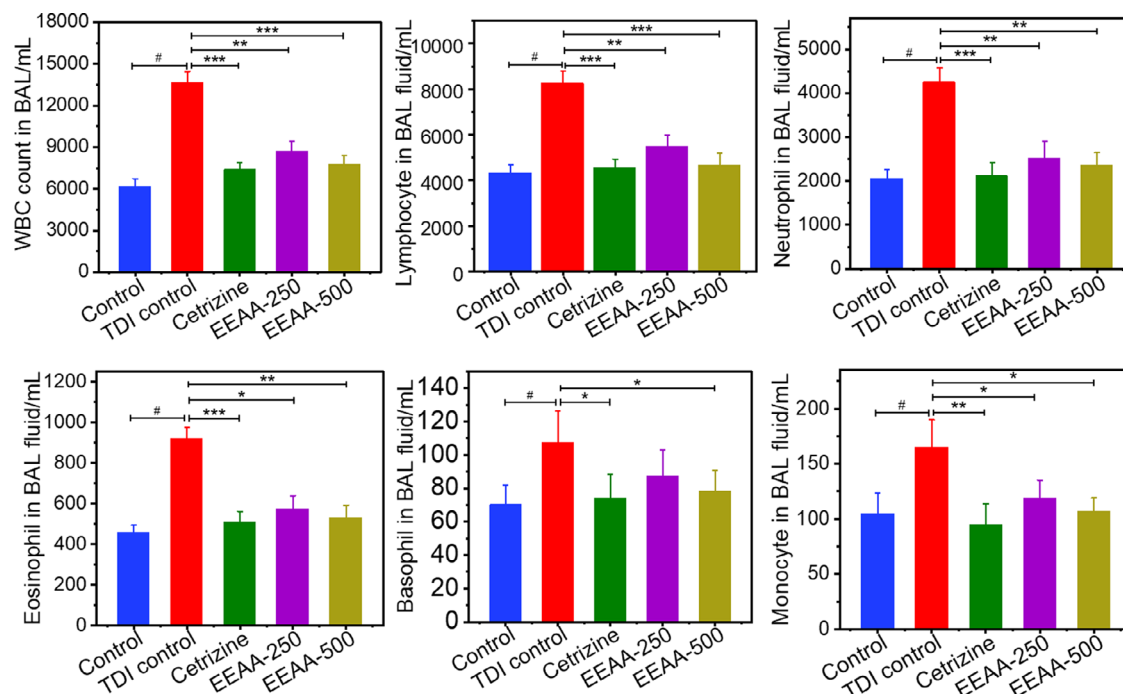


FIGURE 4 | Effect of *ethanolic* extract of *A. angustifolia* (EEAA) total and differential white blood cell (WBC) counts in bronchoalveolar lavage (BAL) fluid of mice. Data are presented as mean $\pm$ standard error of the mean (SEM) ($n = 6$). Statistical significance was performed using Dunnett's test: # $P < 0.05$ versus control group, * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.0001$ versus TDI control group.

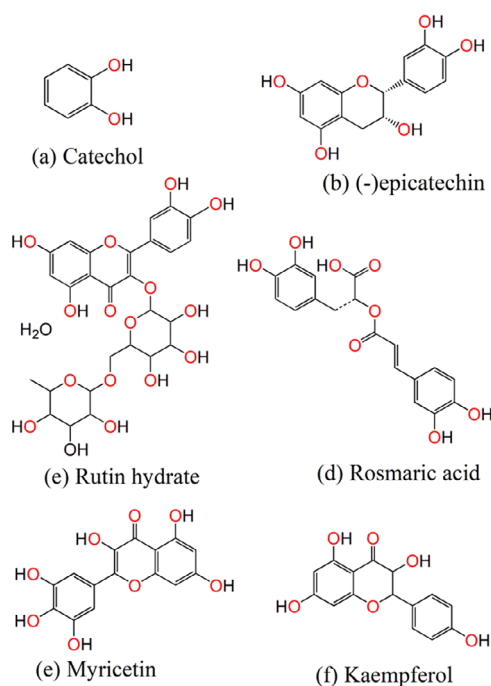


FIGURE 5 | Chemical structures and quantification of major polyphenolic compounds identified in the *ethanolic* extract of *A. angustifolia* using HPLC analysis. The detected compounds include (a) catechol, (b) (-)-epicatechin, (c) rutin hydrate, (d) rosmarinic acid, (e) myricetin, and (f) kaempferol.

underlying the observed anti-allergic activity. GC-MS and FT-IR analyses were additionally performed to provide complementary information on the broader chemical composition of the extract.

2.5 | GC-MS Analysis of Bioactive Phytoconstituents

GC-MS analysis tentatively identified fifteen volatile and semi-volatile constituents in the 95% *ethanolic* leaf extract of *A. angustifolia* based on their mass spectral fragmentation patterns and comparison with the NIST library. The identified constituents comprised aliphatic hydrocarbons, terpenoid derivatives, esters, and other volatile or semi-volatile metabolites. (Figure S4 and Table S4). Among them (1S,15S)-bicyclohexadecan-2-one, (2R)-2-[(1R)-4-methylcyclohex-3-en-1-yl] propan-1-ol, 5-methylhept-3-yne, and dibutyl benzene-1,2-dicarboxylate—were found to be dominant (Figure 6). These compounds have been associated with diverse pharmacological activities, including antioxidant and anti-inflammatory properties, suggesting a multifactorial mechanism of action for the extract. These compounds are associated with diverse pharmacological activities, including antioxidant and anti-inflammatory effects. Notably, (1S,15S)-bicyclohexadecan-2-one, a hydrophobic bioactive molecule, may play a complementary role by interacting with non-polar regions of biological targets, thereby enhancing membrane permeability and ligand stability [22]. The coexistence of volatile (GC-MS identified) and non-volatile (HPLC identified) phytoconstituents provides a comprehensive biochemical basis for the biological activities of the extract.

2.6 | FT-IR Analysis

FT-IR analysis was additionally performed to provide complementary information on the major functional groups present in the *ethanolic* leaf extract of *A. angustifolia* (Figure S5). Seven characteristic absorption bands were observed at 847, 1037, 1177,

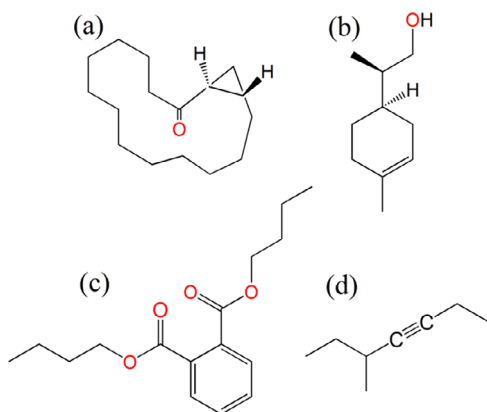


FIGURE 6 | GC-MS-based identification and structural characterization of bioactive constituents present in the *ethanolic* extract of *A. angustifolia*. A total of 15 compounds were identified, among which four major bioactive compounds are highlighted: (a) (1S,15S)-bicyclohexadecan-2-one, (b) (2R)-2-[(1R)-4-methylcyclohex-3-en-1-yl] propan-1-ol, (c) dibutyl benzene-1,2-dicarboxylate, and (d) 5-methylhept-3-yne, demonstrating the chemical complexity and potential pharmacological relevance of the extract.

1437, 1557, 2328, and 2917 cm^{-1} . These bands were tentatively associated with aromatic C–H out-of-plane bending (847 cm^{-1}), C–O stretching (1037 and 1177 cm^{-1}), C–H bending (1437 cm^{-1}), aromatic C=C stretching (1557 cm^{-1}), atmospheric C=O related absorption (2328 cm^{-1}), and aliphatic C–H stretching (2917 cm^{-1}). The FT-IR spectrum therefore provides complementary evidence for the presence of diverse oxygenated, aromatic, and aliphatic functional groups in the extract. However, the FT-IR findings are considered supportive evidence of the functional groups present in the extract and does not by themselves, confirm the identity of individual phytochemical compounds.

2.7 | In Silico Molecular Docking Study

To elucidate the molecular basis of the observed anti-allergic activity, molecular docking studies were performed against key allergic targets: Histamine H1 receptor, Interleukin-4 (IL-4), and Phospholipase A2 (PLA2). The docking performance of the polyphenolic compounds was compared with the standard antihistamine drug cetirizine (Table 1).

Among the HPLC-identified compounds, (-) epicatechin exhibited the strongest binding affinity toward the Histamine H1 receptor, with a docking score of -8.8 kcal/mol (Figure 7), which is significantly better than that of cetirizine (-7.1 kcal/mol) (Figure S6). This compound formed multiple stabilizing interactions with key amino acid residues, including TRP158, TYR431, PHE432, TYR458, ASP107, and TYR108, indicating strong receptor binding and potential antagonistic activity. Rutin hydrate (-7.9 kcal/mol) and myricetin (-7.8 kcal/mol) also demonstrated notable binding affinities, forming hydrogen bonds and electrostatic interactions with residues, such as SER128, GLU410, ASN63, and ARG409. These residues are known to be crucial for ligand binding within the active pocket of the receptor. In contrast, catechol showed comparatively

weaker binding (-5.8 kcal/mol), suggesting a contribution to antihistaminic activity. The stronger binding interactions of phenolic compared to cetirizine suggest that these compounds may act as natural H1 receptor antagonists, potentially inhibiting histamine-mediated allergic responses [23]. The superior binding affinity compared to cetirizine highlights its potential to modulate cytokine-mediated immune responses, which may lead to IL-4-driven IgE production.

Docking analysis against Interleukin-4 revealed that rutin hydrate exhibited the highest binding affinity (-9.0 kcal/mol) (Figure 8), among the identified polyphenols and cetirizine (-7.4 kcal/mol). The interaction profile included multiple hydrogen bonds with residues such as ASP66, GLU45, LYS84, TYR56, and ARG88, indicating strong stabilization within the binding site. Similarly, (-) epicatechin (-7.5 kcal/mol), rosmarinic acid (-7.9 kcal/mol), and myricetin (-7.9 kcal/mol) also showed favorable interactions with key residues involved in cytokine signaling (Figures S7–S10). These interactions suggest a potential inhibitory effect on IL-4-mediated responses, which play a central role in IgE allergic responses [24].

A similar binding pattern was observed in the docking analysis with Phospholipase A2. Rutin hydrate again demonstrated the highest binding affinity (-9.0 kcal/mol) than cetirizine (-7.4 kcal/mol), followed by rosmarinic acid, myricetin, and kaempferol, all of which showed stronger binding. The ligands interacted with critical residues, such as ASP66, GLU45, LYS84, TYR56, and ARG88, which are associated with enzymatic activity and inflammatory mediator release. These findings suggest that the phytoconstituents may inhibit PLA2 activity, thereby reducing the production of pro-inflammatory lipid mediators involved in allergic responses [25].

Notably, the GC-MS-identified bioactive phytochemicals (1S,15S)-bicyclohexadecan-2-one also demonstrated appreciable binding interactions with the selected targets (Figure 9). Other potential compounds include (2R)-2-[(1R)-4-methylcyclohex-3-en-1-yl] propan-1-ol, 5-methylhept-3-yne, and dibutyl benzene-1,2-dicarboxylate also show the potential binding affinity (Table S5). Although its binding affinity was comparatively moderate relative to polyphenols, its hydrophobic scaffold enabled favorable interactions within non-polar regions of the receptor binding pockets (Figures S11–S13). Such hydrophobic interactions are crucial for ligand stabilization and membrane permeability, suggesting that this compound may complement the activity of polar compounds by enhancing overall binding diversity and pharmacokinetic behavior.

2.8 | In Silico Drug-Likeness and Pharmacokinetic (ADME) Analysis

The drug-likeness of the selected compounds was evaluated using Lipinski's rule of five (RO5), which is commonly applied to estimate the oral drug-development potential of small molecules (Table 2). According to this guideline, compounds generally show favorable drug-like properties when their molecular weight is below 500 Da, logP is ≤ 5 , hydrogen-bond acceptors (HBA) are ≤ 10 , and hydrogen-bond donors (HBD) are ≤ 5 , with no more than one criterion being exceeded. Based on these parameters,

TABLE 1 | Interacting amino acid residues of the identified bioactive compounds by HPLC and standard drug (cetirizine) with key antiallergic target proteins— Histamine H1 receptor (PDB ID: 4RZE), Interleukin-4 (PDB ID: 3BPN), and Phospholipase A2 (PDB ID: 1DB4) —As determined from molecular docking studies.

Ligands	Histamine H1 Receptor (3RZE)	
	Docking score	Interacted amino acid
Catechol	−5.8	TRP428, PHE432, SER111, ILE115, ASN198, THR112
(-) Epicatechin	−8.8	TRP158, ALA195, TYR431, PHE432, TYR 458, ILE454, ASP107, TYR108
Rutin hydrate	−7.9	SER128, GLU410, ASN63, ARG125, ASN472, THR60, LYS57, ARG409
Rosmarinic acid	−7.1	ARG409, LYS412, ASN472, GLU410, SER128, THR60, ALA413
Myricetin	−7.8	GLU447, ASN443, ARG176, LYS442, ILE438, ASP186
Kaempferol	−7.1	ASN63, ARG125, ALA413, ARG409, ARG139, THR60, LYS 57
Cetirizine	−7.1	ARG409, LYS412, ARG139, SER128, GLN131, ARG127, ALA413
Interleukin 4 (PDB ID: 3BPN)		
Catechol	−5	ILE C176, PHE C183
(-) Epicatechin	−7.5	GLN A106, GLU A103, LEU A27, ASN C105, THR C104, LYS C109, ARG A64, THR A28, VAL A29
Rutin hydrate	−9	ASP B66, GLU B45, LEU B39, LYS A84, TYR A56, ARG A88, ASP A87, ARG A81
Rosmarinic acid	−7.9	ASP B67, ASP B66, PHE B41, ARG A88, TYR A56, LYS A84
Myricetin	−7.9	TYR B74, ASP B67, ASP B66, LYS A84, SER B44
Kaempferol	−7.9	HIS B131, LEU C319, GLN C278, ASP C324, LYS C318, GLU A19, LYS A12
Cetirizine	−7.4	LEU C319, LYS A12, THR A13, ASP B125, ARG A85, LYS C318
Phospholipase A2 (PDB ID: 1DB4)		
Catechol	−7.5	ILE C176, PHE C183
(-) Epicatechin	−7.5	GLN A106, GLU A103, LEU A27, ASN C105, THR C104, LYS C109, ARG A64, THR A28, VAL A29
Rutin hydrate	−7.9	ASP B66, GLU B45, LEU B39, LYS A84, TYR A56, ARG A88, ASP A87, ARG A81
Rosmarinic acid	−8.2	ASP B67, ASP B66, PHE B41, ARG A88, TYR A56, LYS A84
Myricetin	−4.6	TYR B74, ASP B67, ASP B66, LYS A84, SER B44
Kaempferol	−7.8	HIS B131, LEU C319, GLN C278, ASP C324, LYS C318, GLU A19, LYS A12
Cetirizine	−7.3	LEU C319, LYS A12, THR A13, ASP B125, ARG A85, LYS C318

all examined compounds satisfied the RO5 criteria. This minor deviation may still be acceptable because several naturally derived therapeutic compounds possess relatively high hydrogen-bond donor counts. The predicted pharmacokinetic profiles further indicated that three of the four compounds exhibited high gastrointestinal (GI) absorption and favorable molar refractivity. Their oral drug-likeness was additionally assessed using Veber's criteria, which consider ≤ 10 rotatable bonds and a topological polar surface area (TPSA) $\leq 140 \text{ \AA}^2$ as favorable characteristics for oral bioavailability. Rotatable bonds provide an indication of molecular flexibility, whereas TPSA is associated with the ability of a compound to interact with and cross biological membranes

[26]. Rosmarinic acid showed a slightly higher TPSA than the recommended range, which may account for its comparatively lower predicted GI absorption. The other three compounds met the Veber criteria, suggesting favorable physicochemical and pharmacokinetic characteristics and supporting their potential for further investigation as drug candidates.

2.9 | Molecular Dynamics (MD) Simulation

Molecular dynamics (MD) simulations were performed to further assess the stability of the predicted (−) epicatechin–Histamine

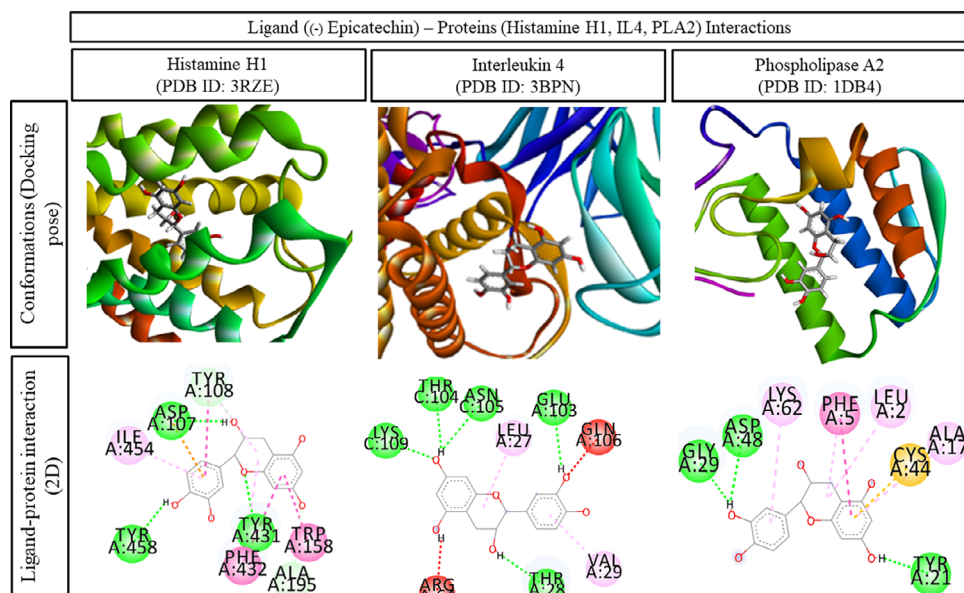


FIGURE 7 | In silico molecular docking interactions of (–) epicatechin (PubChem ID: 107957) with key receptor proteins associated with antiallergic activity. The interactions of (–)-epicatechin with (a) Histamine H1 receptor (PDB ID: 4RZE), (b) Interleukin-4 (PDB ID: 3BPN), and (c) Phospholipase A2 (PDB ID: 1DB4) are presented. The upper panel depicts the three-dimensional (3D) docking conformations of the ligand–protein complexes, whereas the lower panel presents the corresponding two-dimensional (2D) interaction diagrams. Interaction types are color-coded as follows: Green, conventional hydrogen bonds; light green, carbon–hydrogen bonds; red, unfavorable donor–donor or acceptor–acceptor interactions; pink, π – π T-shaped interactions; orange, π –cation interactions, and light pink, π –alkyl interactions.

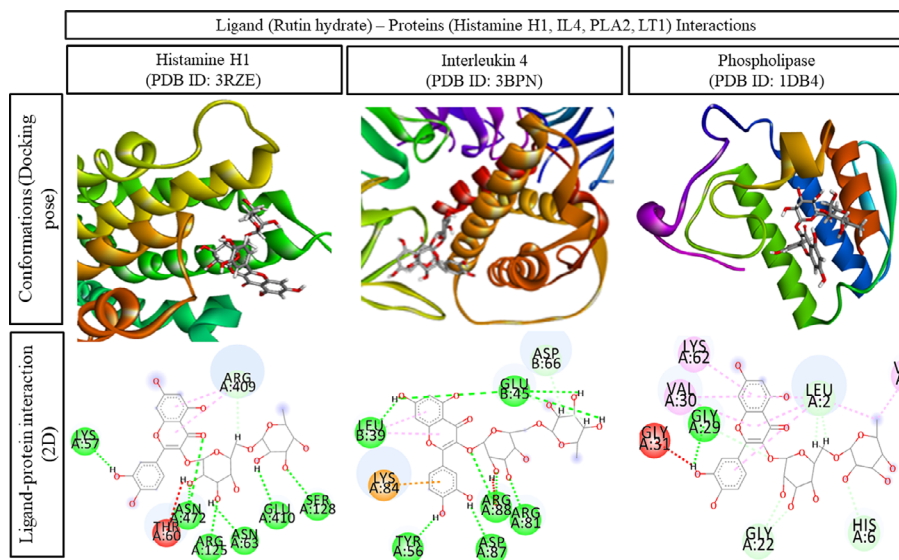


FIGURE 8 | In silico molecular docking interactions of Rutin hydrate (PubChem ID: 107957) with key receptor proteins associated with antiallergic activity. The figure illustrates the binding interactions of Rutin hydrate with (a) Histamine H1 (PDB ID: 4RZE), (b) Interleukin 4 (PDB ID: 3BPN), and (c) Phospholipase A2 (PDB ID: 1DB4). The upper panel presents the three-dimensional (3D) ligand–protein docking complexes, whereas the lower panel shows the corresponding two-dimensional (2D) interaction maps. Color codes represent the interaction types: Green, conventional hydrogen bond; light green, carbon–hydrogen bond; red, unfavorable acceptor/donor; pink, π – π T-shaped interaction; orange, π –cation interaction; and light pink, π –alkyl interaction.

H1 receptor complex, using the cetirizine–Histamine H1 receptor complex as a reference. The dynamic behavior of both systems was assessed using RMSD, RMSF, radius of gyration (R_g), and solvent-accessible surface area (SASA). As shown in

Figure 10a, both complexes exhibited an initial adjustment followed by fluctuations around relatively stable RMSD values. The (–) epicatechin–Histamine H1 complex maintained an average RMSD of approximately 3.4 Å, compared with 3.7 Å for the

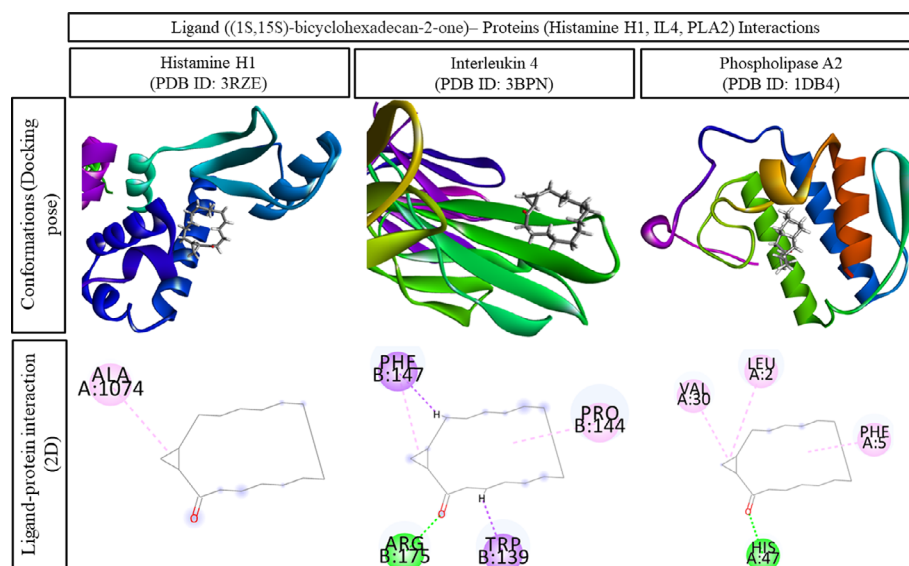


FIGURE 9 | In silico molecular docking interactions of (1S,15S)-bicyclohexadecan-2-one (PubChem ID: 107957) with key receptor proteins associated with antiallergic activity. The interactions of (1S,15S)-bicyclohexadecan-2-one with (a) Histamine H1 receptor (PDB ID: 4RZE), (b) Interleukin-4 (PDB ID: 3BPN), and (c) Phospholipase A2 (PDB ID: 1DB4) are presented. The upper panel depicts the three-dimensional (3D) docking conformations of the ligand–protein complexes, whereas the lower panel presents the corresponding two-dimensional (2D) interaction diagrams. Interaction types are color-coded as follows: Green, conventional hydrogen bonds; light green, carbon–hydrogen bonds; red, unfavorable donor–donor or acceptor–acceptor interactions; pink, π – π T-shaped interactions; orange, π –cation interactions, and light pink, π –alkyl interactions.

TABLE 2 | ADME analysis of selected compounds.

Compounds	GI absorption	MR	Lipinski criteria				Violation	Veber criteria	
			M. Weight	HBA	HBD	Log P		RB	TPSA
	Range/limit	40–130	≤500	≤10	≤5	≤5	≤1	≤10	≤140 Å ²
Catechol	High	79.03	110.12	7	6	0.53	1	1	112.61
(–) Epicatechin	High	87.30	290.27	7	5	0.99	0		
Rutin hydrate	High	68.03	610.52	7	5	1.22	0	1	131.36
Rosmarinic acid	Low	91.40	360.31	8	5	1.52	0	7	144.52
Myricetin	High	72.06	318.23	6	5	0.77	0	1	121.3
Kaempferol	High	59.33	286.23	7	6	0.91	0	5	127.5

cetirizine–Histamine H1 complex. The absence of a continuous increase in RMSD during the simulation suggests that the overall receptor structure remained stable in the presence of both ligands. The RMSF profile (Figure 10b) showed different degrees of flexibility among individual receptor residues. The (–) epicatechin–Histamine H1 complex displayed slightly greater fluctuations than the cetirizine complex, with most residue movements occurring within approximately 1–5.5 Å. These variations may reflect local flexibility of the receptor rather than disruption of the ligand-bound structure [27]. The R_g values (Figure 10c) remained within a relatively narrow range for both complexes, indicating that the receptor retained its overall compact structure throughout the simulation. Similarly, the SASA profile (Figure 10d) showed no substantial or progressive change in solvent exposure, further suggesting that the global structure of the receptor was maintained. The RMSD, RMSF, R_g , and SASA results indicate that (–) epicatechin remained stably associated with the Histamine H1 receptor during the simulation. Its overall

dynamic profile was comparable to that of cetirizine, supporting the docking prediction and suggesting that the interaction of (–) epicatechin with the H1 receptor is structurally favorable. These computational findings provide additional evidence for the potential role of (–) epicatechin as a bioactive compound targeting the Histamine H1 receptor.

These findings strongly support the experimental in vivo results and provide mechanistic insight into the anti-allergic activity of *A. angustifolia*. These findings highlight the therapeutic potential of its bioactive constituents as promising candidates for the development of safer, plant-derived anti-allergic agents.

3 | Conclusion

The present study provides experimental and computational evidence supporting the anti-allergic potential of the *ethanolic*

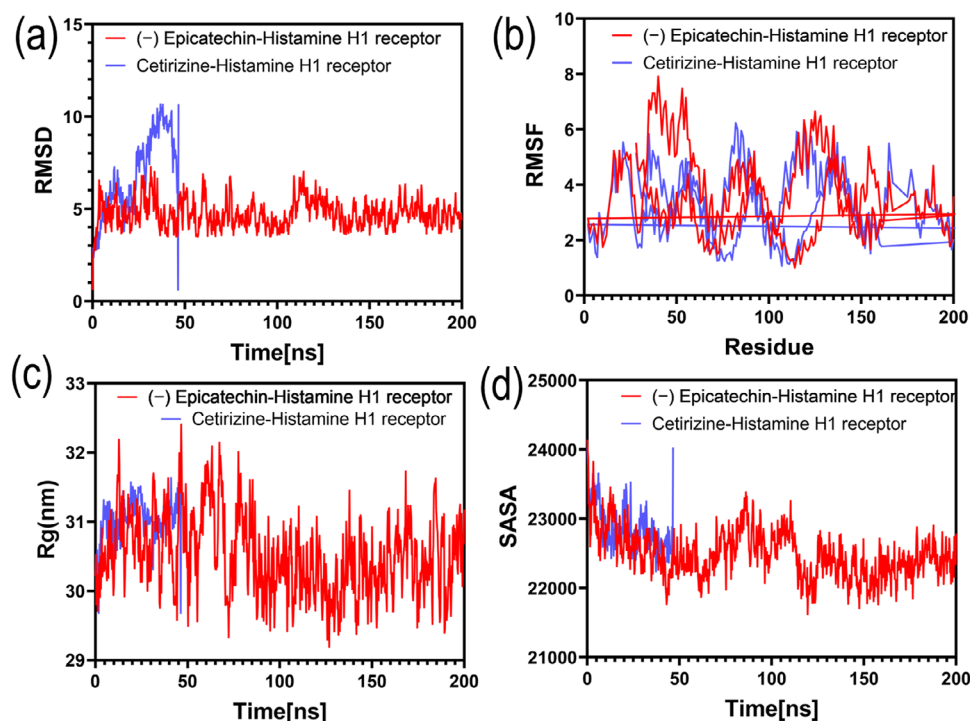


FIGURE 10 | (a) Root-mean-square deviation (RMSD) of the C α atoms of the Histamine H1 receptor in complexes with (-) epicatechin and cetirizine at 310 K. (b) Root-mean-square fluctuation (RMSF) profiles of the Histamine H1 receptor residues in the (-) epicatechin and cetirizine complexes. (c) Radius of gyration (Rg) profiles of the (-) epicatechin-Histamine H1 and cetirizine-Histamine H1 complexes during the MD simulations at 310 K. (d) Solvent-accessible surface area (SASA) profiles of the (-) epicatechin-Histamine H1 and cetirizine-Histamine H1 complexes.

leaf extract of *A. angustifolia*. The extract showed beneficial effects in the TDI-induced allergic mouse model, as reflected by reductions in allergic symptoms, WBC counts, BAL inflammatory responses, and serum IgE levels. HPLC-DAD and GC-MS analyses revealed several phytoconstituents, while FT-IR analysis provided complementary evidence for the major functional groups present in the extract. The identified compounds also showed generally favorable drug-likeness and ADME profiles. In silico molecular docking further supported these findings, revealing strong binding affinities of major phytoconstituents toward key allergic targets, including Histamine H1 receptor, Interleukin-4, and Phospholipase A2. Notably, (-) epicatechin and rutin hydrate exhibited superior binding interactions compared to the standard drug cetirizine, supporting their potential role as natural antihistamine agents. MD simulations further indicated that the (-) epicatechin-Histamine H1 receptor complex maintained stable structural behavior comparable to the reference cetirizine-H1 complex, supporting the persistence of the predicted interaction under simulated physiological conditions. The findings indicate that *A. angustifolia* exerts its anti-allergic effects by suppressing histamine. These results suggest that *A. angustifolia* extract has potential for traditional use for allergic disorders and serves as a promising source for isolation of bioactive compounds.

4 | Experimental Section

4.1 | Plant Sample and Extraction Process

Leaves of *A. angustifolia* were collected from the Chittagong Hill Tracts region of Bangladesh. The plant material was taxonomi-

cally identified by experts at the Bangladesh National Herbarium, Mirpur, Dhaka-1216, where a voucher specimen (**DACB 65236**) was deposited for future reference. The leaves were thoroughly cleaned, shade-dried, and pulverized into coarse powder. The powdered leaves were extracted with ethanol using a maceration technique at room temperature (sample-to-solvent ratio 1:5, w/v) in a sealed jar. The crude extract was filtered, and the solvent was removed under reduced pressure at 40 °C using a rotary evaporator (Büchi Rotavapor, Switzerland). Ethanol was selected because of its relatively low toxicity, suitability for biological studies, and ability to recover a broad range of moderately polar phytochemicals. Although a single solvent cannot extract all phytochemical constituents present in the plant material, the ethanolic extract provides a broad range of bioactive compounds suitable for evaluating the anti-allergic potential of *A. angustifolia*. The dried extract was stored at 2 °C in airtight vials until further phytochemical and pharmacological investigations.

4.2 | Chemicals and Reagents

All experiments were conducted using analytical-grade reagents. Toluene-2,4-diisocyanate (TDI) was obtained from Wako Chemicals (Tokyo, Japan). The standard antihistamine drug cetirizine was procured from Square Pharmaceuticals Ltd. (Dhaka, Bangladesh). Commercial diagnostic kits for estimating biochemical parameters, including alkaline phosphatase (ALP), bilirubin, creatinine, and urea, were obtained from certified suppliers in Germany. All solvents, such as methanol, ethanol, and diethyl ether, were of HPLC grade and purchased from Loba Chemie (India).

4.3 | Experimental Animals

Swiss albino mice (5–6 weeks old, body weight 22–28 g) were used for in vivo experiments. Animals were housed under controlled environmental conditions (24–27 °C, relative humidity 55 ± 5%, 12 h light/dark cycle) with ad libitum access to standard pellet diet and water. Before the experiments, the animals (mice) were acclimatized for one week in the Pharmacology Laboratory, Discipline of Pharmacy, Khulna University, 9208, Bangladesh. All experimental protocols were approved by the Animal Ethics Committee of Khulna University (**Approval No.: KUAEC-2025-03-04**) and conducted in accordance with institutional and national guidelines for the care and use of laboratory animals.

4.4 | Phytochemical Screening

Preliminary phytochemical analysis of the *ethanolic* extract of *A. angustifolia* was performed following standard qualitative protocols [28]. The presence of major secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids, was investigated. A detailed description of test procedures is provided in the [Supporting Information](#).

4.5 | Safety Evaluation of the *Actinodaphne angustifolia* Extract

4.5.1 | Acute Toxicity Test

The acute oral toxicity of the *ethanolic* extract of *A. angustifolia* (EEAA) was evaluated in accordance with OECD Guideline 425, with minor modifications [29]. Healthy Swiss albino mice were randomly divided into six groups ($n = 6$ per group). The control (Group I) received distilled water, whereas the test groups (EEAA-250, EEAA-500, EEAA-1000, EEAA-2000, and EEAA-3000) were administered EEAA at graded doses of 250, 500, 1000, 2000, and 3000 mg/kg body weight via oral gavage. Before dosing, all animals were individually weighed to ensure accurate dose calculations. Following administration by oral gavage, the animals were carefully monitored for the first 30 min, observed periodically over the subsequent 24 h, and assessed daily for 14 consecutive days to detect any behavioral changes, physical abnormalities, or signs of mortality.

4.5.2 | Sub-Acute Toxicity Test

Sub-acute toxicity was assessed following a previously reported protocol with minor modifications [30]. 12 Swiss albino mice were randomly divided into two groups: Group I (control) and a test group (EEAA-500) ($n = 6$ per group). The control group received water, while the test group was administered the extract orally at a dose of 500 mg/kg body weight once daily for 14 consecutive days. Throughout the experimental period, all animals were closely observed for signs of behavioral abnormalities, physiological changes, and mortality. At the end of the treatment period, the animals' body weight was measured. The animals were then anesthetized, and blood samples were collected in heparinized tubes from the cervical vein. The collected blood samples were centrifuged at 3000 rpm for 10 min to obtain serum. Biochemical parameters, including hepatic function markers (SGPT, SGOT,

and ALP) and renal function markers (creatinine, urea, and bilirubin), were analyzed in triplicate, and the mean values were used for statistical analysis.

4.6 | Evaluation of Anti-Allergic Activity

4.6.1 | TDI Sensitization and Provocation Protocol

Allergic responses were induced using toluene-2,4-diisocyanate (TDI) following a previously established method with minor modifications to optimize experimental conditions [31, 32]. A total of thirty mice were randomly divided into five groups ($n = 6$ per group): Group I (control), Group II (TDI control), Group III (standard, cetirizine 20 mg/kg), and Groups IV and V (EEAA-treated groups at 250 and 500 mg/kg, respectively). The extract and standard drug were administered orally once daily for three weeks. Sensitization was carried out by applying 10 µL of 5% TDI solution (in ethyl acetate) to the nasal vestibule of mice (Groups II–V) 1 h after treatment, for five consecutive days. Following a two day interval, the sensitization cycle was repeated for another five days. After a nine days rest period, final provocation was performed using the same TDI dose. The negative control group received ethyl acetate alone following the same schedule.

4.6.2 | Assessment of Allergy-Like Symptoms

Allergic symptoms were evaluated immediately after TDI provocation and observed for 15 min. Parameters included sneezing frequency, nasal scratching (rubbing), and the nasal score. Symptoms were scored using a standardized scale ranging from 0 to 3 (0 = no symptoms, 3 = severe symptoms) [33].

4.6.3 | White Blood Cell (WBC) Analysis

On day 21 after the final provocation, allergy-like symptoms were recorded. After 24 h, mice were anesthetized to reduce the pain, and blood samples were collected from the cervical vein. Blood samples were diluted (1:10) with 1% acetic acid, and total and differential WBC counts were determined using an automated hematology analyzer (DS-500i, Nihon Kohden, Japan). Each sample was analyzed in triplicate, and the mean values were used for statistical evaluation.

4.6.4 | Brochoalveolar Lavage Fluid (BAL) Analysis

BAL fluid was collected by intratracheal cannulation with sterile saline (0.9%), following standard protocol [34]. BAL fluid samples were centrifuged at 3000 rpm for 10 min, and the resulting cell pellets were used to prepare Leishman-stained smears for differential leukocyte assessment. Each sample was analyzed in triplicate, and the average values were used for statistical analysis.

4.7 | HPLC Standardization and Quantification of Polyphenols

Polyphenolic compounds in the *ethanolic* extract of *A. angustifolia* were analyzed by high-performance liquid chromatography

(HPLC) using a Shimadzu LC-20A system (Shimadzu, Japan) equipped with dual solvent delivery pumps, an autosampler, and a photodiode array detector, all controlled by LabSolution software. Separation was performed on a Luna C18 column (250 × 4.6 mm, 5 µm; Phenomenex, USA) maintained at 33 °C. The mobile phase consisted of solvent A (1% acetic acid in acetonitrile) and solvent B (1% acetic acid in water), with gradient elution programmed as follows: 0–20 min, 5%–25% A; 20–30 min, 25%–40% A; 30–35 min, 40%–60% A; 35–40 min, 60%–30% A; 40–45 min, 30%–5% A; and 45–50 min, 5% A. The flow rate was 0.5 mL/min, the injection volume was 20 µL, and detection was carried out at 270 nm. Polyphenols were quantified against calibration curves prepared from authentic standards, and the method was validated for linearity, precision, and reproducibility. The identity of detected compounds was confirmed by comparing their retention times and UV spectra with those of reference standards, and by quantifying major bioactive constituents [35].

4.8 | GC–MS Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis was performed using a Clarus 690 gas chromatograph (PerkinElmer, CA, USA) equipped with an Elite-35 column (30 m × 0.25 mm, 0.25 µm film thickness; PerkinElmer, CA, USA) and coupled to a Clarus SQ 8C mass spectrophotometer (PerkinElmer, CA, USA). 1 µL sample was injected in splitless mode, with high-purity helium (99.999%) serving as the carrier gas at a constant flow rate of 1 mL/min for a total run time of 40 min. The ionization was carried out in electron ionization (EI) mode at 70 eV. The inlet temperature was maintained at 280 °C, while the column oven was programmed as follows: initial temperature 60 °C (0 min hold), increased at 5 °C/min to 240 °C, and maintained for 4 min. The mass spectral data were acquired over an *m/z* range of 50–600 with a scan time of 1 s. Compounds were identified by comparing their fragmentation patterns with the National Institute of Standards and Technology (NIST) library database [36].

4.9 | FT-IR Analysis

Fourier-transform infrared (FT-IR) spectroscopy was performed using an ATR-FTIR spectrometer (Nexus 670, Tokyo, Japan) to characterize the major functional groups present in the *ethanolic* leaf extract of *A. angustifolia*. A small amount of the dried extract was directly placed on the ATR crystal, and the spectrum was recorded over the appropriate mid-infrared range. The spectrum was collected after background correction, and the characteristic absorption bands were analyzed to assign the major functional groups present in the extract.

4.10 | In Silico Molecular Docking Study

4.10.1 | Ligand Preparation

Phytochemical constituents identified through HPLC and GC–MS, along with the reference drug cetirizine, were retrieved

from the PubChem database [37]. The compounds included catechol (CID: 289), (–)-epicatechin (CID: 72276), rutin hydrate (CID: 45479757), rosmarinic acid (CID: 5281792), myricetin (CID: 5281672), kaempferol (CID: 5280863), (1S,15S)-bicyclo[13.1.0]hexadecan-2-one (CID: 13760785), (2R)-2-[(1R)-4-methylcyclohex-3-en-1-yl]propan-1-ol (CID: 12571838), 5-methylhept-3-yne (CID: 521962), dibutyl benzene-1,2-dicarboxylate (CID: 3026), and cetirizine (CID: 2678). The chemical structures were subjected to energy minimization and geometry optimization using Avogadro (v1.2.0) with the Universal Force Field (UFF). The optimized ligands were subsequently saved in Protein Data Bank (.pdb) format for further docking analysis.

4.10.2 | Protein Preparation

The three-dimensional crystal structures of the allergy-related target proteins- Histamine H1 receptor (PDB ID: 4RZE), Interleukin-4 (PDB ID: 3BPN), and Phospholipase A2 (PDB ID: 1DB4) were obtained from the Protein Data Bank [26]. Protein structures were prepared by removing co-crystallized ligands, water molecules, and other non-essential components using PyMOL v2.0 (Schrödinger, LLC). Energy minimization was then performed using Swiss-PdbViewer (v4.1) to ensure structural stability before docking.

4.10.3 | Molecular Docking and Interaction Analysis

Molecular docking simulations were carried out using AutoDock Vina implemented in PyRx 0.8 [27]. Both ligands and receptors were imported into PyRx and defined appropriately as “ligand” or “macromolecule.” Grid boxes were defined to encompass the active binding sites of the selected target proteins. The grid box dimensions were: Histamine H1 receptor (PDB ID: 3RZE), 69.29 × 59.35 × 94.71 Å; for Interleukin-4 (PDB ID: 3BPN), 97.63 × 64.98 × 121.08 Å; and for Phospholipase A2 (PDB ID: 1DB4), 27.23 × 29.52 × 31.71 Å. The default AutoDock Vina settings were applied for all docking simulations. The best docking poses were selected based on binding affinity (lowest binding energy scores). The docked ligand–receptor complexes were further visualized and analyzed in Discovery Studio Visualizer v4.5 (BIOVIA, Dassault Systèmes) to identify hydrogen bonding, hydrophobic, and electrostatic interactions with amino acid residues.

4.10.4 | Drug-Likeness and ADME Analysis

Drug-likeness assessment was performed to estimate the suitability of the selected compounds as potential orally active drug candidates. The compounds were evaluated using the SwissADME web server based on their physicochemical and pharmacokinetic properties, including absorption, distribution, metabolism, and excretion (ADME). Their drug-like characteristics were further assessed according to Lipinski’s rule of five, which provides commonly used criteria for evaluating oral bioavailability and drug-development potential.

4.10.5 | Molecular Dynamics (MD) Simulation

The Protein–ligand Complexes showing favorable docking interactions were subsequently subjected to molecular dynamics (MD) simulations to examine their structural stability and conformational behavior under conditions approximating a physiological environment. Simulations were performed using the AMBER14 force field implemented in YASARA Structure v. 24.4.10. Each system was placed in a cubic simulation cell extending 20 Å beyond the complex and solvated with water to achieve a density of 0.997 g cm⁻³. The systems were maintained at 310 K, 1 Atm, and pH 7.4, with 0.9% NaCl to approximate physiological ionic conditions. periodic boundary conditions were applied throughout the simulations. long-range electrostatic interactions were calculated using the Particle-mesh Ewald (PME) method, while an 8 Å cutoff was applied for short-range van der Waals and Coulombic interactions. A 2.5 fs integration time step was used, and trajectory frames were recorded at regular intervals during the 00 ns simulation. The resulting trajectories were analyzed using root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (R_g), solvent-accessible surface area (SASA), molecular surface area (MolSA), hydrogen-bond interactions, and secondary-structure analysis to assess the stability, flexibility, compactness, and interaction behavior of the protein–ligand complexes.

4.11 | Statistical Analysis

All experimental results were expressed as mean $\pm$ standard error of mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for group-wise comparisons. A *p*-value of < 0.05 was considered statistically significant.

Author Contributions

Rabindra Nath Acharyya: conceptualization, data curation, investigation, and writing – original draft. **Md. Abid Muktadir Risha:** data curation and software. **Novi Dwi Widya Rini:** formal analysis. **Sarita Manandhar:** investigation and methodology. **Sabina Shahi:** formal analysis and methodology. **Biswa Nath Bhadra:** validation and editing. **Asish Kumar Das:** investigation, validation, review, and editing. **Katsuhiko Ariga:** review and editing. **Shrabanti Dev:** conceptualization, funding acquisition, and review and editing. **Lok Kumar Shrestha:** review and editing. The authors declare that the works presented in this article are original.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. R. Latif and T. Nawaz, “Medicinal Plants and Human Health: A Comprehensive Review of Bioactive Compounds, Therapeutic Effects, and Applications,” *Phytochemistry Reviews* 25 (2025): 2299–2342, <https://doi.org/10.1007/s11101-025-10194-7>.
2. X. Lin, H. Li, T. Wang, X. Xiao, and B. Huang, “The Traditional Uses, Nutrition, Phytochemistry, and Pharmacology of the Medicinal Abrus Species: A Comprehensive Review,” *Chemistry & Biodiversity* 22 (2025): e00182, <https://doi.org/10.1002/cbdv.202500182>.
3. N. Nasim, I. S. Sandeep, and S. Mohanty, “Plant-Derived Natural Products for Drug Discovery: Current Approaches and Prospects,” *Nucleus* 65 (2022): 399–411, <https://doi.org/10.1007/s13237-022-00405-3>.
4. S. Meshram, P. Itankar, S. Prasad, et al., “Plant Metabolomics in Natural Product Drug Discovery: Emerging Approaches, Challenges, and Translational Potential,” *Current Pharmacology Reports* 12 (2026): 9, <https://doi.org/10.1007/s40495-026-00452-3>.
5. A. Beyaoui, M. Kaplan, I. Saidi, et al., “Phenolic Profile, Bioactivities and in Silico Analysis of the Trunk Bark of Acacia Cyanophylla Lindl,” *Chemistry & Biodiversity* 21, no. 8 (2024): e202401061, <https://doi.org/10.1002/cbdv.202401061>.
6. M. Halder and S. Jha, “Medicinal Plants and Bioactive Phytochemical Diversity: A Fountainhead of Potential Drugs Against Human Diseases,” *Medicinal Plants: Biodiversity, Biotechnology and Conservation* 33 (2023): 39–93, https://doi.org/10.1007/978-981-19-9936-9_2.
7. K. Chakma, M. M. Alam, D. Chakma, and R. H. Bhuiyan, “Traditional Uses of Ethno-Medicinal Plants in Chittagong Hill Tracts (CHTs), Bangladesh: A Review,” *Journal of Pharmaceutical Research International* 33 (2021): 70–79, <https://doi.org/10.9734/jpri/2021/v33i30B31642>.
8. A. Dey, S. Rani, R. N. Acharyya, et al., “Anti-Allergic Potentials of *Ceriops decandra* Leaves in TDI-Induced Allergic Mice: Comprehensive in Vivo and in Silico Studies,” *Phytomedicine Plus* 5 (2025): 100670, <https://doi.org/10.1016/j.phyplu.2024.100670>.
9. M. Chandra Shill, H. A. S. El-Nashar, P. Prova Mollick, et al., “Longevity Spinach (*Gynura procumbens*) Ameliorated Oxidative Stress and Inflammatory Mediators in Cisplatin-Induced Organ Dysfunction in Rats: Comprehensive in Vivo and in Silico Studies,” *Chemistry & Biodiversity* 21 (2024): e202301719, <https://doi.org/10.1002/cbdv.202301719>.
10. T. Moriguchi and J. Takai, “Histamine and Histidine Decarboxylase: Immunomodulatory Functions and Regulatory Mechanisms,” *Genes to Cells* 25 (2020): 443–449, <https://doi.org/10.1111/gtc.12774>.
11. I. Mahmud, R. N. Acharyya, A. Paul, et al., “Antiallergic Activity of *Amoora cucullata* Roxb Bark Extract and Profiling of Its Polyphenolic Compounds: In Vivo and in Silico Studies,” *Pharmaceutical Sciences Asia* 50 (2023): 317–330, <https://doi.org/10.29090/psa.2023.04.23.347>.
12. S. Linton, L. Hossenbaccus, and A. K. Ellis, “Evidence-Based Use of Antihistamines for Treatment of Allergic Conditions,” *Annals of Allergy, Asthma & Immunology* 131 (2023): 412–420, <https://doi.org/10.1016/j.anai.2023.07.019>.
13. R. N. Acharyya, K. Mitra, M. A. M. Risha, et al., “Evaluation of Toxicological, Anti-Allergic and Neuropharmacological Activities of *Bridelia stipularis*: In Vivo and in Silico Studies,” *Journal of Medicinal Plants Studies* 10 (2022): 113–123, <https://doi.org/10.22271/plants.2022.v10.i4b.1442>.
14. A. G. Corsico, S. Leonardi, A. Licari, et al., “Focus on the Cetirizine Use in Clinical Practice: A Reappraisal 30 Years Later,” *Multidisciplinary Respiratory Medicine* 14 (2019): 40, <https://doi.org/10.1186/s40248-019-0203-6>.

15. M. Ban, G. Morel, I. Langonné, N. Huguet, E. Pépin, and S. Binet, "TDI Can Induce Respiratory Allergy With Th2-Dominated Response in Mice," *Toxicology* 218 (2006): 39–47, <https://doi.org/10.1016/j.tox.2005.09.013>.
16. P. K. Sardar, S. Dev, M. A. Al Bari, et al., "Antiallergic, Anthelmintic and Cytotoxic Potentials of Dried Aerial Parts of *Acanthus ilicifolius* L.," *Clinical Phytoscience* 4 (2018): 34, <https://doi.org/10.1186/s40816-018-0094-7>.
17. R. N. Acharyya, S. Mithila, S. Rani, et al., "Anti-Allergic and Anti-Hyperglycemic Potentials of *Lumnitzera racemosa* Leaves: In Vivo and in Silico Studies," *Proceedings of National Academy of Science India B-Biological Science* 93 (2022): 147–158, <https://doi.org/10.1007/s40011-022-01399-3>.
18. M. Sharma, "Comparative Wood Anatomy of *Actinodaphne* Species," *International Journal of Plant, Animal and Environmental Sciences* 4 (2014): 165–169.
19. M. N. Uddin, T. Alam, and M. A. Islam, "Evaluation of Carbon Tetrachloride Fraction of *Actinodaphne angustifolia* Nees (Lauraceae) Leaf Extract for Antioxidant, Cytotoxic, Thrombolytic and Antidiarrheal Properties," *Bioscience Reports* 40 (2020), BSR20201110, <https://doi.org/10.1042/BSR20201110>.
20. A. S. Putri, F. F. Purba, I. W. Kusuma, and H. Kuspradini, "Chemical Compositions and Antimicrobial Potential of *Actinodaphne macrophylla* Leaves Oils From East Kalimantan," *IOP Conference Series: Earth and Environmental Science* 144 (2028): 012021, <https://doi.org/10.1088/1755-1315/144/1/012021>.
21. K. Gairola, S. Gururani, and S. K. Dubey, "Polyphenols and Its Effect on the Immune System," *Nutraceuticals and Functional Foods in Immunomodulators* (2022): 121–140, https://doi.org/10.1007/978-981-19-2507-8_5.
22. A. Falanga, R. Bellavita, S. Braccia, and S. Galdiero, "Hydrophobicity: The Door to Drug Delivery," *Journal of Peptide Science* 30 (2024): e3558, <https://doi.org/10.1002/psc.3558>.
23. D. Ciupei, A. Colișar, L. Leopold, A. Stănilă, and Z. M. Diaconeasa, "Polyphenols: From Classification to Therapeutic Potential and Bioavailability," *Foods* 13 (2024): 4131, <https://doi.org/10.3390/foods13244131>.
24. A. Shankar, J. W. McAlees, and I. P. Lewkowich, "Modulation of IL-4/IL-13 Cytokine Signaling in the Context of Allergic Disease," *Journal of Allergy and Clinical Immunology* 150 (2022): 266–276, <https://doi.org/10.1016/j.jaci.2022.06.012>.
25. K. C. Torres Costa, V. Santana Vieira Santos, E. Rezende Vaz, et al., "A Novel Peptide Able to Reduce PLA2 Activity and Modulate Inflammatory Cytokine Production," *Toxicon* 231 (2023): 107207, <https://doi.org/10.1016/j.toxicon.2023.107207>.
26. D. Wang, Q. Guo, Z. Wu, et al., "Molecular Mechanism of Anti-histamines Recognition and Regulation of the Histamine H1 Receptor," *Nature Communications* 15 (2024): 84, <https://doi.org/10.1038/s41467-023-44477-4>.
27. O. Trott and A. J. Olson, "AutoDock Vina: Improving the Speed and Accuracy of Docking With a New Scoring Function, Efficient Optimization, and Multithreading," *Journal of Computational Chemistry* 31 (2010): 455–461, <https://doi.org/10.1002/jcc.21334>.
28. A. Mazumder, R. N. Acharyya, N. Kundu, M. K. Kundu, M. A. Islam, and M. M. Rahman, "Phytochemical Screening and Evaluation of Pharmacological Potential of *Baliospermum solanifolium* Leaf Extract," *Clinical Complementary Medicine and Pharmacology* 4 (2024): 100123, <https://doi.org/10.1016/j.ccmp.2023.100123>.
29. B. Chakrabarty, R. N. Acharyya, N. Kundu, et al., "Evaluation of Anti-Diabetic and Anti-Allergic Activities of *Brownlowia tersa* (L.) Kosterm Leaves Extract and Determination of Its Phenolic Compounds by HPLC/DAD," *Tropical Journal of Natural Products Research* 4 (2020): 326–333, <https://doi.org/10.26538/tjnpr/v4i8.1>.
30. R. N. Acharyya, M. D. A. M. Risha, S. Dev, K. Ariga, A. K. Das, and L. K. Shrestha, "Multi-Target Antidiabetic Potentials of *Xylocarpus mekongensis*: In Vivo Efficacy, Enzyme Inhibition, and Molecular Docking," *Journal of Oleo Science* 75 (2026): 427–442, <https://doi.org/10.5650/jos.ess25270>.
31. M. A. Islam, M. S. Huq Atanu, M. A. Siraj, et al., "Supplementation of Syringic Acid-Rich *Phrynium pubinerve* Leaves Imparts Protection Against Allergic Inflammatory Responses by Downregulating iNOS, COX-2, and NF- κ B Expressions," *Heliyon* 9 (2023): e13343, <https://doi.org/10.1016/j.heliyon.2023.e13343>.
32. Y. Sugawara, Y. Okamoto, T. Sawahata, and K. Tanaka, "An Asthma Model Developed in the Guinea Pig by Intranasal Application of 2, 4-Toluene Diisocyanate," *International Archives of Allergy and Immunology* 101 (1993): 95–101, <https://doi.org/10.1159/000236504>.
33. S. Dev, R. N. Acharyya, S. Akter, et al., "Toxicological Screening and Evaluation of Anti-Allergic and Anti-Hyperglycemic Potential of *Sonneratia caseolaris* (L.) Engl. Fruits," *Clinical Phytoscience* 7 (2021): 69, <https://doi.org/10.1186/s40816-021-00301-4>.
34. S. G. Mahajan, R. G. Mali, and A. A. Mehta, "Effect of *Moringa Oleifera* Lam. Seed Extract on Toluene Diisocyanate-Induced Immune-Mediated Inflammatory Responses in Rats," *Journal of Immunotoxicology* 4 (2007): 85–96, <https://doi.org/10.1080/15476910701337472>.
35. S. Dev, R. N. Acharyya, M. C. Shill, et al., "Bioactivities of Colocasia Affinis Schott and Profiling of its Bioactive Polyphenols by HPLC-DAD," *Bangladesh Pharmaceutical Journal* 24 (2021): 1–10, <https://doi.org/10.3329/bpj.v24i1.51629>.
36. X. Wei, I. Koo, S. Kim, and X. Zhang, "Compound Identification in GC-MS by Simultaneously Evaluating the Mass Spectrum and Retention Index," *Analyst* 139 (2014): 2507–2514, <https://doi.org/10.1039/C3AN02171H>.
37. J. A. Cabezas, M. L. Arias, N. Ferllini, et al., "Design, Synthesis, Biological Evaluation, and in Silico Analysis of Novel Antifungal Agents Targeting Trichophyton Species," *Chemistry & Biodiversity* 23 (2026): e01477, <https://doi.org/10.1002/cbdv.202501477>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: cbdv71727-sup-0001-SuppMat.pdf.