Antihistamine Potential of Red Alga *Acanthophora spicifera* Through Combination *In Vivo*, *In Vitro*, and *In Silico* Study

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Abstract: *Red alga* Acanthophora spicifera *is a marine species with potential as a source of natural antihistamines. This study evaluated the bioactivity of its secondary metabolites through* in vivo, in vitro, *and* in silico *approaches. Extracts were obtained by successive maceration using* n*-hexane, ethyl acetate, and methanol.* In vivo *testing on BALB/c mice showed that extract administration did not cause significant weight loss. However, its effect on allergic symptoms was significant* ($p < 0.05$), *with the methanol extract identified as the most effective by the Mann–Whitney test.* In vitro *assays revealed a significant reduction in histamine levels* ($p < 0.05$) *during sensitization, treatment, and post-challenge phases, particularly in the methanol extract group.* In silico *docking demonstrated that two compounds from the methanol extract exhibited strong binding affinity to the H1 receptor (PDB ID: 3RZE), with binding energies of −6.27 and −5.06 kcal/mol. These findings suggest that* A. spicifera, *particularly its methanol extract, is a promising source of natural antihistamines with potential applications in the development of safer allergy treatments.*

Keywords: Acanthophora spicifera*; antihistamine;* in vivo*;* in vitro*;* in silico

■ INTRODUCTION

Allergy is an immunological disorder with a rising global prevalence, representing a significant public health concern, including in Indonesia. According to the World Allergy Organization, over 20% of the global population is affected by one or more forms of allergic disease, a number that continues to increase due to lifestyle changes and growing environmental exposure [1]. Histamine, the primary mediator in allergic reactions, is released by mast cells and basophils and is responsible for symptoms such as pruritus, edema, and nasal congestion through the activation of H1 histamine receptors [2]. H1-antihistamines of both first- and second-generation have been widely used in managing allergic symptoms. However, side effects such as sedation, dry mouth, and impaired concentration limit their widespread use, especially among patients requiring long-term treatment [3]. Therefore, the search for natural product-based antihistamines has gained growing interest, particularly those offering improved safety and efficacy profiles. Some naturally derived compounds show potential antihistamine activity, including quercetin [4], polyphenols [5], terpenoids [6-7], polypeptides [8], indole-3-carbaldehyde [9], and viridicatol [10].

The red alga *Acanthophora spicifera*, widely distributed in tropical waters including Indonesia, is known to be rich in secondary metabolites such as flavonoids, alkaloids, saponins, phenolics, and terpenoids [11]. These compounds have been associated with a range of biological activities, including anticancer [12], anti-inflammatory, and antibacterial effects [13]. Previous studies have reported that *A. spicifera* contain apigenin with anti-inflammatory properties [14], and quercetagetin flavonols that show significant activity

against both Gram-positive and Gram-negative bacteria [15]. Although its biological potential has been explored in various contexts, no studies to date have specifically investigated the potential of *A. spicifera* as an antihistamine agent. This study aims to evaluate the antihistamine activity of *A. spicifera* extracts and their bioactive compounds using an integrated approach involving *in vivo*, *in vitro*, and *in silico* methods to assess both biological efficacy and binding affinity to the H1 histamine receptor. The findings are expected to contribute to the development of allergy therapies based on marine bioresources from Indonesia.

■ EXPERIMENTAL SECTION

Materials

The solvents used for maceration were *n*-hexane, ethyl acetate, and technical-grade methanol, which was purified prior to use. Additional materials included thin-layer chromatography (TLC) plates, distilled water (Onemed Waterone), aluminum foil, ovalbumin (from broiler chicken egg white), cetirizine HCl 10 mg (generic), 70% ethanol (Onemed), physiological saline (0.9% NaCl infusion, OEM), sulfuric acid p.a., aluminum hydroxide powder (Chalco), hydrochloric acid p.a., 10% $FeCl_3$, magnesium powder, sodium carboxymethyl cellulose (NaCMC/Tylose) powder, Mayer's reagent (Merck), Dragendorff's reagent (Merck), Wagner's reagent (Merck), glacial acetic acid (Merck), standard laboratory feed (ADII), chloroform (Merck), and ELISA reagents from Bioassay Technology Laboratory (catalog no. E0248MO). The use of BALB/c mice in this study was conducted in accordance with the approved animal ethics protocol from the Research Ethics Committee, Faculty of Pharmacy, Universitas Hasanuddin (Approval No. 500/UN4.17/KP.06.05/2025).

Instrumentation

The equipment used in this study included standard laboratory glassware, Büchner funnel, animal hair clipper (Kimei), syringes (Onemed, 3 and 5 mL), cannulas (Onemed), EDTA blood collection tubes (Onemed Vaculab, 3 mL), digital balance (Ohaus), rotary vacuum evaporator (Hahn Shin HS-2000NS), centrifuge (PLC Series), microtiter plates, micropipettes, ELISA reader (Thermo Scientific Multiskan FC), incubator (Heidolph Unimax 1010), gas chromatography–mass spectrometry (GC-MS) (Shimadzu QP2010 Ultra), pulverizer (KME), and a computer for molecular docking analysis.

Procedure

Sample preparation

Specimens of the marine alga *A. spicifera* were collected from Barrang Lompo Island, South Sulawesi, Indonesia, at the coordinates 5°02'58"S, 119°19'16"E. The collected algae were thoroughly rinsed and air-dried at room temperature in a shaded area, protected from direct sunlight. The dried material was then ground using a pulverizer with a 200-mesh size. Extraction was conducted through sequential maceration with *n*-hexane, ethyl acetate, and methanol, followed by concentration of the extracts using a rotary evaporator to obtain semi-solid masses. This procedure was adopted from preliminary trials and previous studies as a standard method to recover a broad spectrum of metabolites.

Extract identification

The chemical constituents of the extracts were identified using colorimetric methods with specific chemical reagents. Phytochemical screening was performed for alkaloids, steroids/terpenoids, flavonoids, phenolics, and saponins using established protocols described by Harborne [16]. The secondary metabolites present in the extract that exhibited the highest antihistaminic bioactivity, as determined by both *in vivo* and *in vitro* assays, were further analyzed using GC-MS.

In vivo *antihistamine activity assay*

The *in vivo* method employed in this study was adapted from the protocol by Mine et al. [17], with specific modifications made to suit the experimental parameters of this research, as illustrated in Fig. 1. The allergen inducer used was ovalbumin, a protein found in egg white [18]. Physiological parameters were evaluated using the method described by Mine et al. [19].

In vitro *antihistamine activity assay*

Fresh blood was collected from the experimental mice and centrifuged at 1500 rpm for 15 min to obtain the plasma. The ELISA assay was conducted according

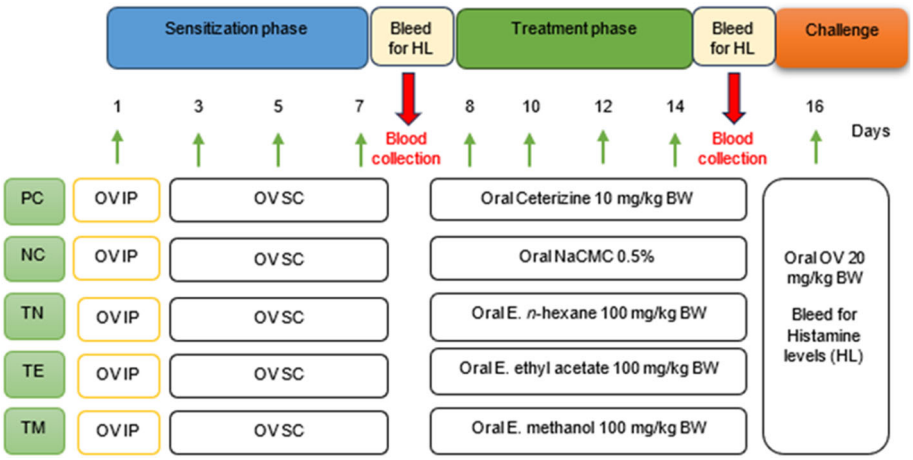

Fig 1. Experimental design. Five groups. (PC) positive control, (NC) negative control, (TN) treatment with *n*-hexane extract, (TE) treatment with ethyl acetate extract, and (TM) treatment with methanol extract. (n = 5/group) using BALB/c mice. (OV IP) ovalbumin with Intraperitoneal injection and (SC) subcutaneous injection

to the standard operating procedures provided by BT LAB (Bioassay Technology Laboratory) [20].

Data analysis

Statistical analyses for the in vivo and in vitro experiments were performed using SPSS version 25. Parametric data were analyzed using repeated measures analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test [17]. For non-parametric data, the Friedman test was employed, followed by the Wilcoxon signed-rank test. Additionally, the Kruskal–Wallis test was conducted, followed by post hoc analysis using the Mann–Whitney U test.

In silico *antihistamine activity assay*

The drug-likeness of the identified phytochemicals was assessed using Lipinski's Rule of Five (Ro5) via the SwissADME web tool (http://www.swissadme.ch) [21]. Phytochemicals that fulfilled these criteria were selected as test ligands for molecular docking analysis. Three-dimensional structures of the major compounds identified in the methanolic extract were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Ligand optimization was performed using Chimera 1.17.3 software with the AMBER ff14SB force field, and the ligands were saved in .pdb format. The same preparation procedure was applied to the standard ligand, cetirizine. The 3D structure of the H1 histamine receptor protein was obtained from the Protein Data Bank (PDB ID: 3RZE) [22]. Using Chimera 1.17.3, all non-essential residues were removed from the protein structure. The native ligand of the protein complex was isolated and prepared using the same protocol as the receptor. Both the protein and ligands were saved in .pdb format [23]. Molecular docking was conducted using AutoDock4, with preparation and analysis performed using AutoDockTools version 1.5.7 [24]. The active site was defined with grid box coordinates centered at x = 27.017, y = 27.640, and z = 45.951, using a grid box size of 40 × 40 × 40 Å. The ligands were converted to .pdbqt format. Molecular docking was performed using the Lamarckian Genetic Algorithm with the following parameters: 10 runs, a population size of 150, and a maximum of 2.500.000 evaluations. Validation of the docking protocol was performed by redocking the native ligand (doxepin), yielding an RMSD less than 2.0 Å, consistent with benchmarks reported by Shamsian et al. [25] and Aziz et al. [26]. The generated non-covalent interactions between ligand and receptor were visualized using Discovery Studio Visualizer.

■ RESULTS AND DISCUSSION

Extraction Yield and Phytochemical Screening

Based on the study, the extraction yields obtained are presented in Table 1. The results indicate that methanol yielded the highest extract recovery at 5.46%.

Table 1. Extraction yield of red algae *A. spicifera*

Extract	% Yield
n-Hexane	0.37
Ethyl acetate	0.37
Methanol	5.46

Table 2. Phytochemical screening results of *A. spicifera* extracts

Test	*n*-Hexane	Ethyl acetate	Methanol
Alkaloids	+	+	+
Flavonoids	-	+	+
Steroids	+	-	-
Terpenoids	-	+	+
Saponins	-	-	+
Phenolics	-	-	+

The correlation between the high yield and the chemical composition is further supported by the phytochemical screening results presented in Table 2, which reveal that the methanolic extract contains a greater diversity of bioactive compound classes compared to the ethyl acetate and *n*-hexane extracts. It highlights the efficiency of methanol, a polar solvent, in extracting bioactive compounds from macroalgal tissues. These findings align with previous studies suggesting that polar solvents, such as methanol and ethanol, are generally more efficient than non-polar solvents in extracting a broad spectrum of biologically active compounds from marine algae, particularly phenolic and flavonoid constituents [27].

Effect of Extract on Body Weight in the *In Vivo* Method

Data presented in Table 3 and the profile diagram in Fig. 2 show a general trend of increasing body weight across all treatment groups. Repeated measures ANOVA

Table 3. Effect of *A. spicifera* extract on the mice's body weight

Treatment groups	Body weight (g)		
	Sensitization	Treatment	Challenge
Positive control	30.3 ±3.130	33.2 ±3.271	34.0 ±3.606
Negative control	24.2 ±4.50	25.2 ±4.606	25.4 ±4.50
n-Hexane extract	28.4 ±1.817	33.2 ±3.421	34.0 ±3.317
Ethyl acetate extract	32.4 ±4.775	34.4 ±5.771	34.6 ±5.367
Methanol extract	29.0 ±4.062	30.8 ±5.675	30.6 ±5.413

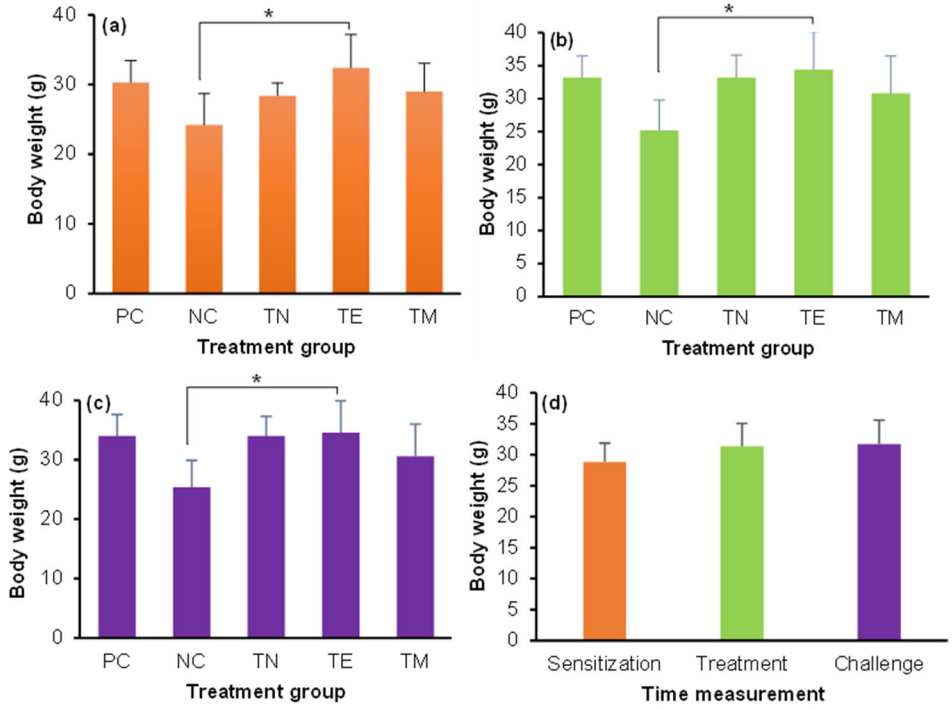

Fig 2. The effect of *A. spicifera* extract on the body weight of BALB/c mice varied by (a) sensitization phase, (b) treatment phase, (c) challenge test, and (d) time-based measurement effect. (*) indicate statistically significant differences (*$p < 0.05$)

with Greenhouse–Geisser correction showed a significant effect of time across the sensitization, treatment, and post-challenge phases on mice's body weight ($F(1.384, 27.674) = 77.696$, $p < 0.001$, $\eta^2 = 0.795$). The analysis also revealed a significant main effect of treatment group on body weight ($F(4, 20) = 3.205$, $p = 0.035$, $\eta^2 = 0.391$), as well as a significant interaction between time and treatment group ($F(5.353, 27.674) = 6.491$, $p < 0.001$, $\eta^2 = 0.565$). These results indicate that both the measurement time points and the type of treatment significantly influenced the dynamics of body weight. However, the observed significance does not indicate a trend of weight loss. Instead, it shows a differential pattern of weight gain among treatment groups over time. The stability of body weight in mice during the observation period suggests that *A. spicifera* extract is relatively safe and does not disrupt the animals' metabolic balance. This finding is consistent with the study by Bello et al. [28], which stated that one of the key indicators of the safety of herbal preparations in *in vivo* studies is the absence of significant body weight loss during the observation.

Effect of Extract on Allergic Symptoms

The effect of *A. spicifera* extract on allergic symptoms across treatment groups is presented in Table 4. The analysis began with the Friedman test, which showed a statistically significant difference in median scores across observation times (Chi-Square = 153.620; df = 8; Asymp. Sig. = 0.000). Subsequent Wilcoxon signed-rank tests were conducted for pairwise comparisons between time points. The results indicated no significant differences ($p > 0.05$) within the first 15 min. However, beyond the initial 15-min interval, all remaining time-point pairs demonstrated significant differences ($p < 0.05$), confirming that observable changes in allergic symptoms began to occur after this time point.

The Kruskal-Wallis test results, as shown in Fig. 3, revealed statistically significant differences among treatment groups at 120 min ($p < 0.042$), 180 min ($p < 0.050$), 240 min ($p < 0.035$), and 300 min ($p < 0.002$). These findings indicate significant intergroup variation in allergic symptoms during those time intervals, with the strongest difference observed at 300 min, as indicated by by the lowest *p*-value ($p < 0.002$). Overall, the results suggest that treatment effects became more pronounced over time, with significant differences emerging after 120 min.

Further analysis using the Mann–Whitney U test demonstrated that administration of methanol, ethyl acetate, and *n*-hexane extracts led to statistically significant differences in allergic symptoms compared to the negative control group ($p < 0.05$). Mean rank analysis revealed that the methanol extract group had the lowest average symptom score among the extract-treated and control-negative groups, though still higher than that of the positive control group (cetirizine). This suggests that while the methanol extract effectively reduced allergic symptoms, its efficacy did not match that of the pharmacological standard (cetirizine) [29].

The observed efficacy of the methanol extract is likely attributed to its content of flavonoids, phenolics, and other polar compounds known for their anti-inflammatory and immunosuppressive activities [30]. These compounds function by inhibiting the release of inflammatory mediators, such as histamine and leukotrienes, from mast cells, key drivers of allergic pathogenesis. In contrast, the ethyl acetate and *n*-hexane extracts, which are more suitable for extracting semi-polar to non-polar compounds, exhibited comparatively lower antiallergic activity. This supports the hypothesis that the bioactivity of each extract is strongly influenced

Table 4. Effect of *A. spicifera* extract on allergic symptoms in BALB/c mice

Treatment groups	Allergy symptoms score			
	120 min	180 min	240 min	300 min
Positive control	1.0 ± 0	1.2 ± 0.447	1.8 ± 0.447	2.0 ± 0
Negative control	1.8 ± 0.894	2.4 ± 0.548	2.6 ± 0.894	3.6 ± 0.548
n-Hexane extract	1.2 ± 0.447	1.6 ± 0.548	2.0 ± 0	2.0 ± 0
Ethyl acetate extract	1.0 ± 0	1.4 ± 0.548	2.2 ± 0.447	2.4 ± 0.894
Methanol extract	1.0 ± 0	1.4 ± 0.548	1.4 ± 0.548	1.6 ± 0.548

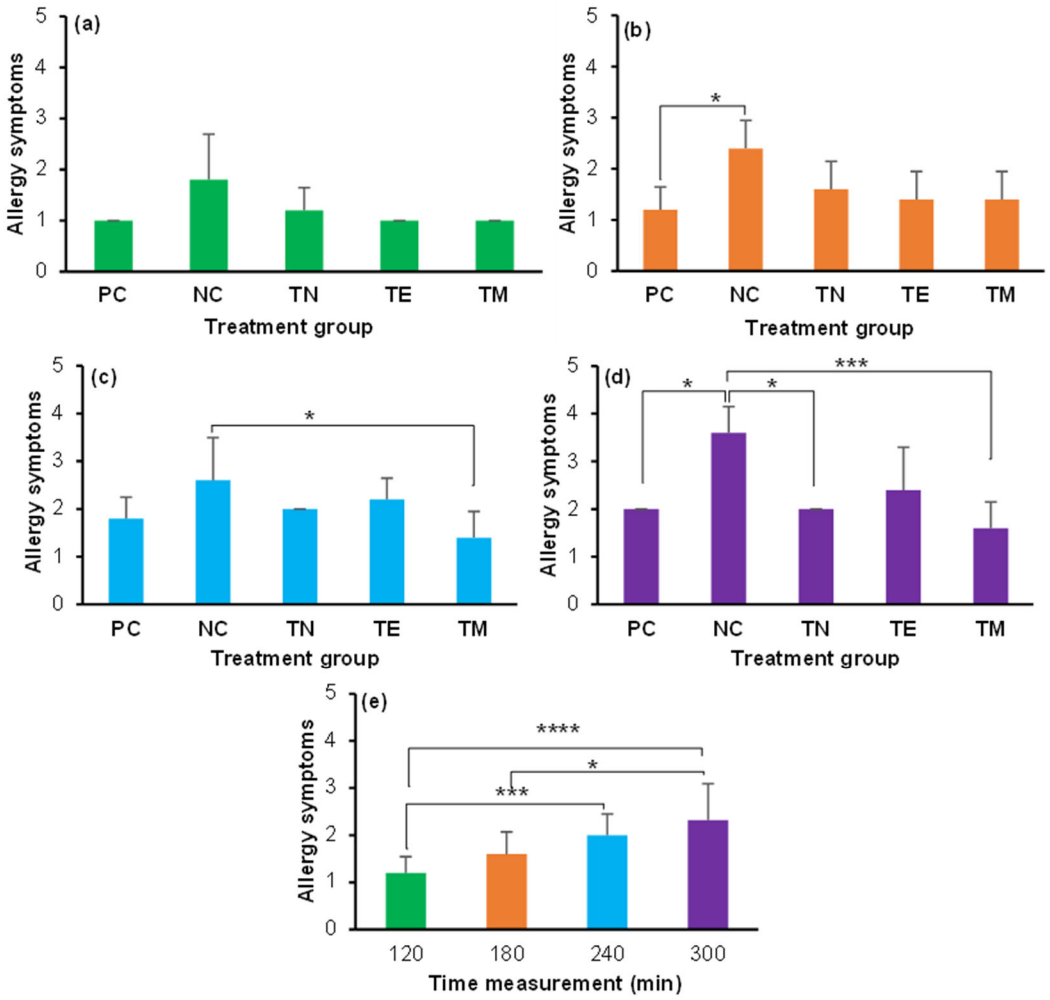

Fig 3. Effect of *A. spicifera* extract on allergic symptoms in BALB/c mice at various time points: (a) 120 min, (b) 180 min, (c) 240 min, (d) 300 min, and (e) overall effect across time points. (*, ***, ****) indicate statistically significant differences (*$p < 0.05$, ***$p < 0.01$, ****$p < 0.001$)

by solvent polarity [31]. Differences in extract efficacy may also be affected by factors such as the bioavailability of active compounds, metabolic stability, and synergistic interactions among chemical constituents within the extracts [32].

Effect of *A. spicifera* Extract on Histamine Levels: *In Vitro* Method

The *in vitro* assay was conducted to evaluate the ability of *A. spicifera* extract to inhibit histamine release from sensitized mast cells. The effects of the extract on histamine levels are presented in Table 5. Normality testing confirmed that all data were normally distributed ($p > 0.05$). Repeated-measures ANOVA revealed a significant main effect of time on histamine levels ($F(2, 40) = 55.264$, $p < 0.001$, $\eta^2 = 0.734$). The type of treatment also had a significant effect ($F(4, 20) = 3.420$, $p = 0.028$, $\eta^2 = 0.406$). Moreover, a significant interaction was found between time and treatment group ($F(8, 40) = 15.624$, $p = 0.001$, $\eta^2 = 0.758$), indicating that the pattern of histamine level reduction was strongly influenced by the specific treatment administered.

Fig. 4, which shows the methanol extract, exhibited the most significant reduction in histamine levels throughout the observation period, from the sensitization phase through treatment and culminating in the challenge phase. However, its effect did not surpass that of the positive control group (cetirizine). In contrast,

Table 5. Effect of *A. spicifera* extract on histamine levels in BALB/c mice

Treatment groups	Histamine levels (ng/mL)		
	Sensitization	Treatment	Challenge
Positive control	40.22 ± 8.618	36.78 ± 5.760	3.49 ± 0.229
Negative control	35.09 ± 8.407	31.70 ± 2.664	33.39 ± 4.253
n-Hexane extract	33.37 ± 4.662	33.67 ± 2.914	31.66 ± 3.525
Ethyl acetate extract	41.39 ± 7.687	37.64 ± 6.398	34.10 ± 6.398
Methanol extract	43.30 ± 15.312	23.80 ± 10.930	9.82 ± 9.950

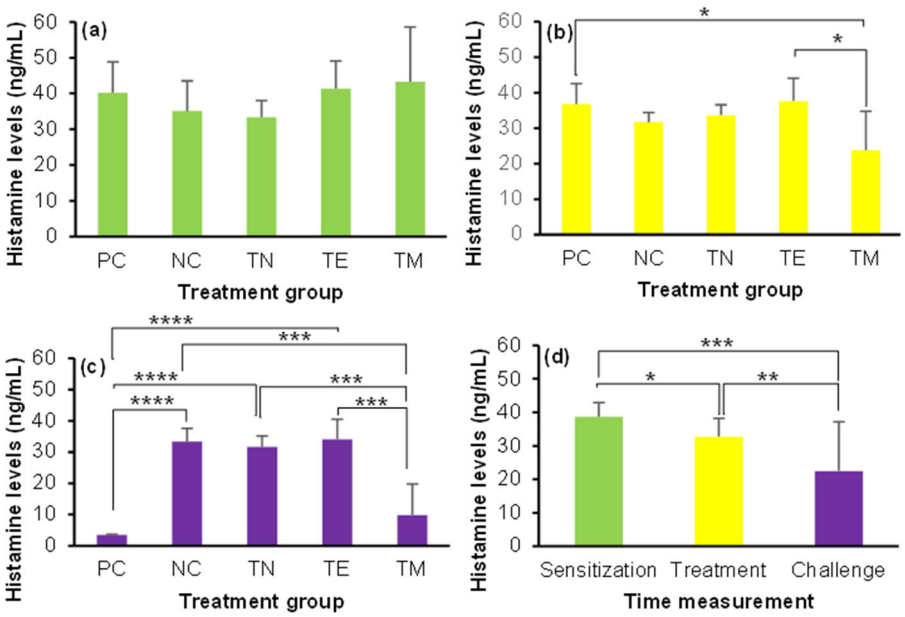

Fig 4. Effect of *A. spicifera* extract on histamine levels in BALB/c mice during different experimental phases varied by (a) sensitization phase, (b) treatment phase, (c) challenge phase, and (d) time-dependent measurement effect. (*, **, ***, ****) indicate statistically significant differences (*$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; ****$p < 0.001$)

the *n*-hexane extract and negative control group showed minimal changes in histamine concentration, indicating that non-polar fractions were less effective in inhibiting histamine release. Post hoc analysis using Tukey's test confirmed that the reduction in histamine levels in the methanol extract group was statistically significant compared to the ethyl acetate extract group ($p = 0.035$). No significant differences were observed between the other treatment groups. These findings support the hypothesis that polar compounds in the methanol extract, such as flavonoids, phenolics, terpenoids, and alkaloids, actively contribute to the inhibition of mast cell degranulation [33]. Therefore, the methanol extract of *A. spicifera* demonstrates promising potential as a natural antihistamine agent.

GC-MS Analysis

GC-MS analysis of the methanol extract of *A. spicifera* successfully identified various chemical constituents with potential biological activities. The chromatographic analysis revealed 166 peaks. The GC-MS chromatogram of the methanolic extract is presented in Fig. 5. From these results, the five major compounds with the highest peak area percentages were selected for further *in silico* molecular docking analysis. These compounds are listed in Table 6.

Several compounds identified as minor constituents in the methanol extract, such as undecanal, methyl dodecanoate, heptadecane, and methyl 12-methyl-tridecanoate, have also been reported [11], indicating that these compounds are commonly present

Table 6. Phytochemical composition of the methanol extract of *A. spicifera*

RT	%Area	Compounds	Bioactivity
18.038	14.32	Methyl 14-methylpentadecanoate	Antifungal [34]
21.468	7.08	2-Ethyldodecan-1-ol	-
19.198	8.17	Octadecanoic acid	Anti-inflammatory [35]
36.595	7.18	(1*R*,4a*R*,5*S*)-5-[(*E*)-5-Hydroxy-3-methylpent-3-enyl]-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2*H*-naphthalene-1-carbaldehyde	Antimicrobial, anti-inflammatory [36]
15.076	7.03	3,7,11,15-Tetramethylhexadecyl acetate	Antioxidant [37]

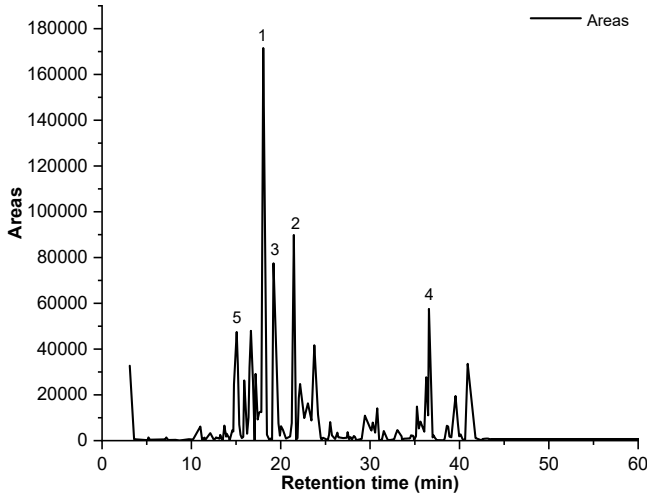

Fig 5. Chromatogram of methanol extract *A. spicifera*

in the secondary metabolite profile of *A. spicifera*, although their concentrations may vary. The composition of identified compounds in natural extracts can differ significantly due to environmental factors, extraction methods, and the sensitivity or spectral matching accuracy of the GC-MS instrumentation and compound libraries used. Therefore, the absence of identical dominant compounds across different studies is a typical and expected outcome in metabolomic investigations of natural products**.** One limitation of the present study is that only GC-MS analysis was employed, which primarily detects volatile and semi-volatile compounds. Consequently, non-volatile metabolites, which may also contribute to the biological activity of *A. spicifera*, were not characterized. Future studies incorporating LC-MS or complementary analytical techniques are therefore recommended to obtain a more comprehensive metabolite profile.

Lipinski's Rules of Five and Molecular Docking Analysis

The evaluation of the compounds 1–5 shown in Table 7 revealed that they complied with Lipinski's Ro5, indicating that they possess molecular characteristics conducive to oral absorption and membrane permeability [38]. Molecular docking analysis presented in Fig. 6 revealed that compound 4 exhibited the lowest binding energy against the histamine H1 receptor, with a binding affinity of −6.27 kcal/mol. The interactions involved numerous van der Waals bonds with several protein residues, as well as conventional hydrogen bonds formed between the ligand's carbonyl group and residues Tyr210 and Asn408. Additionally, carbon–hydrogen bond interactions were observed between the ligand's carbonyl group and residues Lys415 and Gly418. These two types of hydrogen bonds are believed to contribute to the stabilization of the ligand–receptor complex [39].

Table 7. Lipinski's Rule of Five parameters and molecular docking analysis

Compounds	Lipinski rules					Binding energy (kcal mol^{-1})
	MW (< 500 g mol^{-1})	HBA(< 10)	HBD (< 5)	M log p (≤ 4.15)	Lipinski's violations (≤ 1)	
1	270.45	2	0	4.44	1	−2.93
2	214.39	1	1	3.95	0	−3.79
3	284.48	2	1	4.67	1	−3.82
4	304.47	2	1	3.73	0	−6.27
5	340.58	2	0	5.58	1	−5.06
Cetirizine	388.89	5	1	2.35	0	−4.09

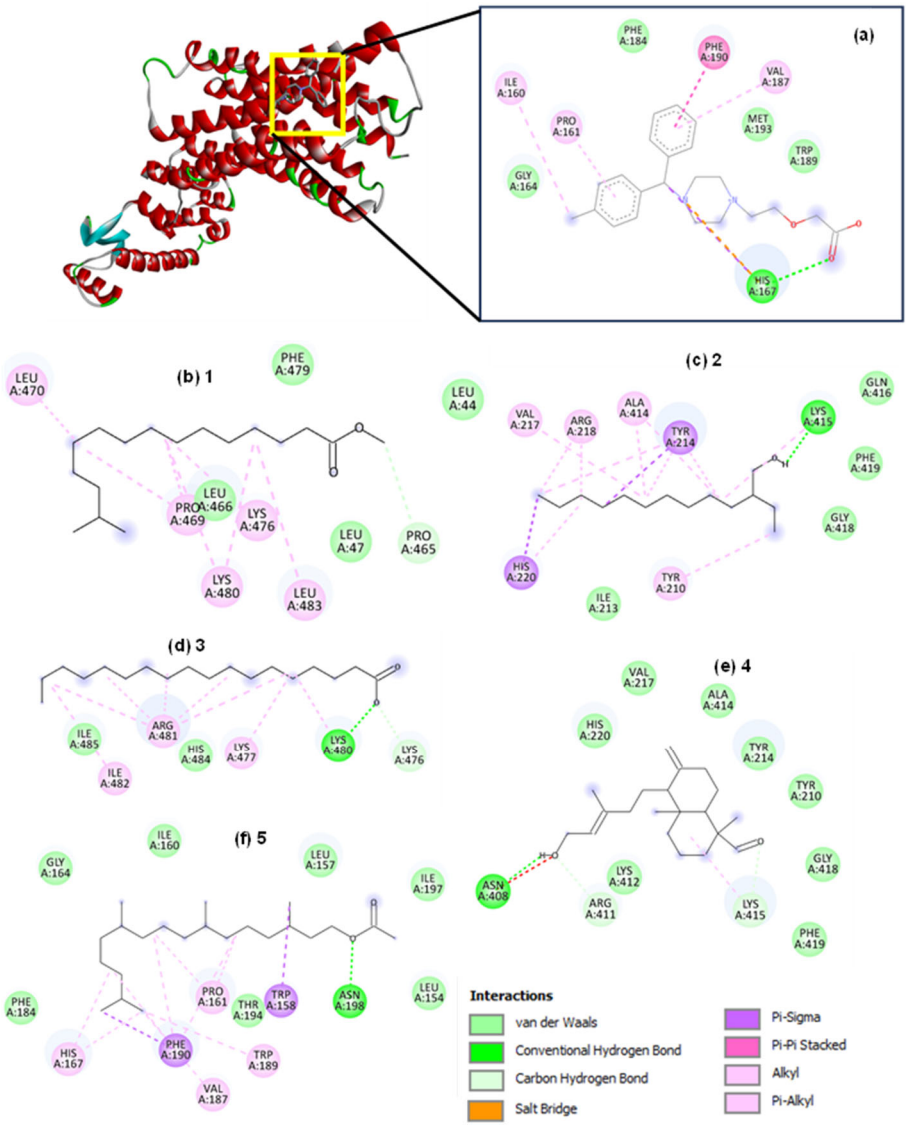

Fig 6. Binding site and interaction of ligands with the histamine H1 receptor. (a) Cetirizine, (b) methyl 14-methylpentadecanoate, (c) 2-ethyldodecan-1-ol, (d) octadecanoic acid, (e) (1*R*,4a*R*,5*S*)-5-[(*E*)-5-hydroxy-3-methylpent-3-enyl]-1,4a-dimethyl-6-methylene-3,4,5,7,8,8a-hexahydro-2*H*-naphthalene-1-carbaldehyde, and (f) 3,7,11,15-tetramethylhexadecyl acetate

Compound 5, with a binding affinity of −5.06 kcal/mol, exhibited interaction types similar to those of compound 4, although no carbon–hydrogen bonds were detected. Instead, π-alkyl interactions were observed with Pro161, His167, and Val187 along with π-sigma interactions involving Trp158 and Phe190. For comparison, cetirizine, used as the positive control, showed a binding energy of −4.09 kcal/mol, indicating a lower binding affinity than both tested compounds. Although neither compound interacted directly with His167, as observed for cetirizine, these alternative interactions contributed to the formation of stable ligand–receptor complexes. Previous studies have also reported that structural differences and interaction types strongly influence variations in binding affinity [40-41]. Thus, compounds 4 and 5 remain promising candidates with favorable binding profiles despite differences in binding modes.

■ CONCLUSION

This study revealed the potential of *A. spicifera* as a

source of secondary metabolites with antihistamine activity, utilizing an integrated *in vivo*, *in vitro*, and *in silico* approach. The highest yield was from the methanol extract (5.46%), containing flavonoids, terpenoids, steroids, phenolics, alkaloids, and saponins in the extract. *In vivo* analysis of body weight in treated BALB/c mice demonstrated that *A. spicifera* extract is safe and does not interfere with the animals' metabolic balance. The clinical allergic symptom evaluation further revealed that the methanol extract was more effective in suppressing allergic responses compared to other extracts, although it did not surpass the positive control. *In vitro* results showed a significant reduction in histamine levels in the methanol extract group throughout the observation period, consistent with its richer profile of polar bioactive compounds, including alkaloids, flavonoids, terpenoids, phenolics, and tannins. GC-MS analysis of the methanol extract identified five major compounds, with (1*R*,4a*R*,5*S*)-5-[(*E*)-5-hydroxy-3-methylpent-3-enyl]-1,4a-dimethyl-6-methylene-3,4,5,7,8,8a-hexahydro-2*H*-naphthalene-1-carbaldehyde and 3,7,11,15-tetramethylhexadecyl acetate showing the strongest binding affinities in *in silico* docking studies (−6.27 and −5.06 kcal/mol, respectively) against the histamine H1 receptor. These findings suggest potential antagonist activity at the H1 receptor site. Furthermore, favorable ADMET profiles supported both the efficacy and pharmacological safety of these compounds. Altogether, the methanol extract of *A. spicifera* shows promising potential as a natural antihistamine candidate acting through multiple mechanisms and warrants further exploration in preclinical and clinical studies.

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■ CONFLICT OF INTEREST

The authors have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Nunuk Hariani Soekamto: Supervision, Conceptualization. Ilmi Wahyuni: Data Curation, Investigation, Writing - Preparation of the Original Draft. Herlina Rasyid: Supervision, Validation. Nurul Annisa, Risda Adriana, Alifiah Alfaniah Al Putri: Research Assistants, Djabal Nur Basir, Ahyar Ahmad, St. Fauziah: Supervisors. Rizal Irfandi: Editing and Publication. All authors agreed to the final version of this manuscript.

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