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Evaluation of toxicological, anti-allergic and neuropharmacological activities of *Bridelia stipularis*: *In-vivo* and *in-silico* studies

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Abstract
Bridelia stipularis locally known as 'Harinhara' native in hill tract area. The plant is traditionally used in cough, asthma and catarrh by the Chakma ethnic people of Bangladesh. In this study, we investigated the toxicological, anti-allergic, neuropharmacological potential of leaves extract and profiling of its bioactive polyphenols by HPLC for *in-silico* molecular docking. Phytochemical test confirmed the existence of phytochemicals including tannin, flavonoid, saponin, alkaloid, glycoside etc. In toxicity study, the extract was safe up to 3 g/kg oral dose in mice. The studied showed no organ specific toxicity at 500 mg/kg oral dose. Anti-allergic activity performed by using TDI-induced allergic mice model. The extract significantly reduced the TDI-induced allergy like symptoms such as scratching, sneezing, swelling ($P < 0.05$). Additionally, the extract also ameliorated the leukocytes count in blood and bronchoalveolar lavage (BAL) fluid. The neuropharmacological activity test was conducted by following the open field method in mice model, at both doses of extract at 300 and 500 mg/kg showed mild CNS depressant effect. HPLC profiling identified the presence of seven phenolic compounds, among these rutin hydrate demonstrated the best docking score of -7.4 kcal/mol same as anti-allergic drug against (H_1R). On the other hand exhibited rosmarinic acid and myricetin -7.9 and -7.8 kcal/mol against $GABA_A$ whereas standard CNS drug showed -7.7 kcal/mol. In conclusion, *B. stipularis* is safe and rich in different polyphenolic compounds which were found effective against diseases.

Keywords: *Bridelia stipularis*, allergy, histamine, molecular docking

Introduction
Bangladesh has a rich and inheritance heritage of herbal plants in the worlds. Large number of people in Bangladesh and mainly in tribal communities depends on traditional medicine for cure of their diseases [1]. Traditional records and ecological diversity indicates that hill tract plants can be an exciting resource for possible leads for the development of better drugs against different life threatening diseases [2]. About 25% of all modern medicines are active or passively isolated from plants [3]. Lots of bioactive compounds of pharmaceutical importance have been reported from the hill tract ecosystem by this time. Chittagong hill tracts area of Bangladesh is renowned as a great source of medicinal and herbal plants [4]. Hill tract plants are popular in folk medicine for curing several different diseases like asthma, rheumatism, diabetes, small pox, diarrhea etc.

Bridelia stipularis commonly known as harinhara belonging Euphorbiaceae family, is traditionally used to treat cough, asthma and catarrh by the Chakma ethnic community of Chittagong hill tract area [5]. *B. stipularis* is used in pleurisy and exudation. Warm leaf poultice and leaf powder are used to white spots in skin for the treatment of allergies in children [6]. *B. stipularis* is used in amoebic dysentery, constipation, chest pain, leucoderma, diarrhoea, and strangury decoction [7].

Histamine, a prominent contributor to allergic diseases is produced during decarboxylation of histidine in the enzyme presence of L-histidine decarboxylase [8]. Histamine H_1 receptor antagonists, such as, cetirizine, loratadine, and epinastine are popularly used to suppress the histamine H_1 receptor (H_1R) activity, and thus exert anti-allergic activity [9]. However, most of the anti-allergic drugs altered CNS function causes drowsiness and impaired psychomotor performance is often a consequence of traditional antihistamine administration [10]. Though many antihistamines are now in clinical use, few research studies on new anti-allergic agents have found their path to success [11]. Toluene 2,4 diisocyanate (TDI) is a respiratory sensitizer and known to cause occupational allergies that is used in laboratory induction of allergy [12]. TDI is responsible for causing varied allergic conditions such as allergic rhinitis, scratching, sneezing and nasal score [13]. It is estimated that about (2–15)% chronically TDI exposed workers suffer from asthma [14]. The existing anti-allergic drugs in the market are trashed with the side effects of headache, somnolence, body weight gain, cardiac arrhythmia, appetite simulation etc. [15]. This inconvenience has encouraged new research and discovery of newer anti-allergic drugs.

The objective of the neuropharmacological study is to reveal whether these plant have CNS depressant effect or not like the existing anti-allergic drugs in the market e.g., cetirizine, loratadine etc. As the existing anti-allergic drugs are already known for their CNS depressing effect. The neuropharmacological test measured the excitability of CNS and the reduce in locomotor activity is nearly related to sedation [16].

HPLC-DAD profiling of bioactive phytochemicals present in the extract followed by *in-silico* molecular docking study to demonstrate possible interaction of the found compounds with the specific receptors H_1R and $GABA_A$. The main objective of the research was to investigate the anti-allergic and neuropharmacological activities of popular *Bridelia stipularis* hill tract species.

Plant collection and extraction

Bridelia stipularis leaves were picked up from Rangamati region, Chattagram, Bangladesh during at day time and identified by Bangladesh National Herbarium (Accession No. 46537 DACB). The collected leaves were separated from unnecessary materials and dried for 4 weeks. The leaves were grinded with the help of a suitable grinder. About 500 gm of each powder was soaked in 2000 ml of 96% ethanol in containers. After 14 days, the mixture was filtered through whatman filter paper. Then evaporated by rotary evaporator. The yield value (%) of ethanolic extract of *Bridelia stipularis* is 5.1%.

Chemicals and Reagents

Standard drug cetirizine, diazepam was purchased from square pharmaceuticals Ltd., Dhaka, Bangladesh. All other chemicals were of HPLC grade such as methanol, ethanol, chloroform, ascorbic acid, gallic acid etc. and purchased from Loba and Sigma. TDI, biochemical parameters measurements kits such as SGOT, SGPT, ALP, bilirubin, creatinine, urea kits were purchased from Germany.

Experimental Animal

5-6 weeks aged swiss albino mice about 22-27 gm weights were used. Mice were kept in separated cages at laboratory conditions for 1 week (temperature 24–27 °C, humidity 55±5%) in the pharmacology for better adaptation. Animal Ethics Committee, Khulna University approved the procedures of all animal experiments and the reference number is: KUAEC-2021/06/10.

Phytochemical screening

Presence of different phytochemicals and secondary metabolites previously reported to have anti-allergic potentials was tested using standard testing protocols [17].

Toxicologcal Study

Acue toxicity test

Acute toxicity test was conducted by OECD guideline-425 with some modification [18]. Experimental animal were separated into four groups, containing six mice. First group denoted as control which received 2% tween-80 water solution orally. Three groups given *B. stipulais* at the dose of 1000, 2000 and 3000 mg/kg b. wt. Before any treatment, each mice and test samples were weighed accurately [19].

Sub-acute toxicity test

The sub-acute toxicity test of *B. stipulais* extract was done on established procedure by McConnell *et al.*, 1978 with slight changes [20]. Mice were selected and seperated into three groups onecontrol group and two test groups congaing six mice each. Test-I group received 500 mg/kg *B. stipulais* extract regularly for 14 days and vehicle only given the control group. All experimental animal were observed regularly for any abnormal signs or mortality. For biochemical parameters analysis SGPT, SGOT, ALP, urea, bilirubin, cholesterol, triglyceride and creatinine were measured [21].

Anti-allergic activity evaluation

TDI sensitizations and provocation

TDI (Toluene 2, 4-diisocyanate) allergen was used for allergy produced in mice that established by Dev S. *et al.*, 2009 with slight modification [22]. Mice were arbitrarily chosen and separeted in five groups: Control, TDI-control, Standard and two test groups (*B. stipulais* 300 and 500 mg/kg). 5%, 10 µL of TDI solution was applied bilaterally on nasal vestibule in mouse except for the control mice who only received the vehicle ethyl acetate (10 µL). All the groups received respective doses of treatments orally. The TDI provocation and the whole experimental method are described in (Figure 1).

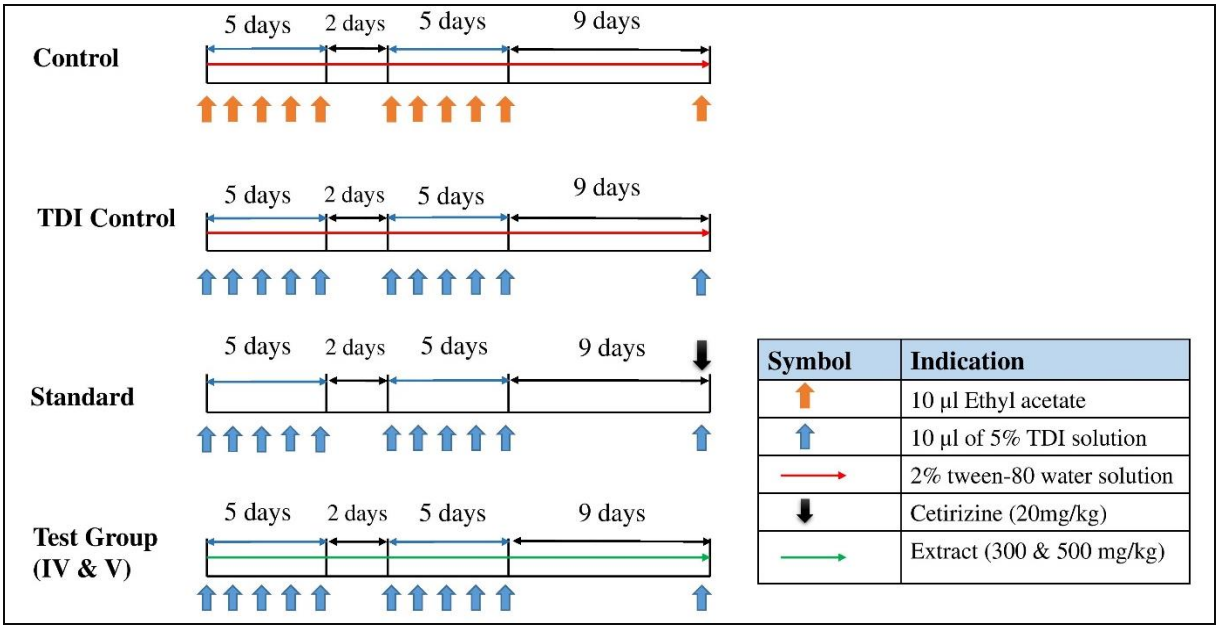


Fig 1: Experimental protocol at a glance

Assessment of allergy-like symptom

On the 21st day, nasal allergic symptoms were recorded just after TDI provocation and assessed carefully for 10 min by accommodating the animal into different cages [23].

Differential analysis in blood

On the 21st day, allergy like symptoms were measured. After 24 h. of provocation with TDI, mice were anesthetized by chloroform and blood samples were collected from vein by Chakrabarty *et al*., 2022 method [24]. Total WBC count was done on an automated cell counter. For differential WBC count, prepared slides and stained with Leichman stain.

Differential analysis in BAL (*Bronchoalveolar lavage*) fluid

In order to collect BAL fluid, 0.9% sterile saline was introduced into lung via cannulated tracheal tube as per the process described by Sozmen, S.C. *et al*., 2016 with some modifications [36]. BAL fluid was collected from each mouse separately and centrifuged at 3500 rpm for 15 min. BAL fluid slides were prepared same as blood cell slides preparation.

Neuropharmacological activity

The test was performed as per the method established by Gupta *et al.*, 1971 [26]. Experimental animal were selected randomly and separated into six groups, containing six mice. Control, standard and test sample solution were given orally. After treatment, mice were placed individually and number of square visited by mouse was counted for 3 min on 0, 30, 60, 90 and 120 min during the experiment in a sound attenuated room.

HPLC analysis and quantification of polyphenols

Detection of selected polyphenolic compound in *B. stipularis* extract was measured by HPLC-DAD analysis as followed by Dev *et al.*, 2021 methods [27]. HPLC analysis was performed using LC-20A (Shimadzu Japan) and the detector was set at 270 (Figure 2, and 3).

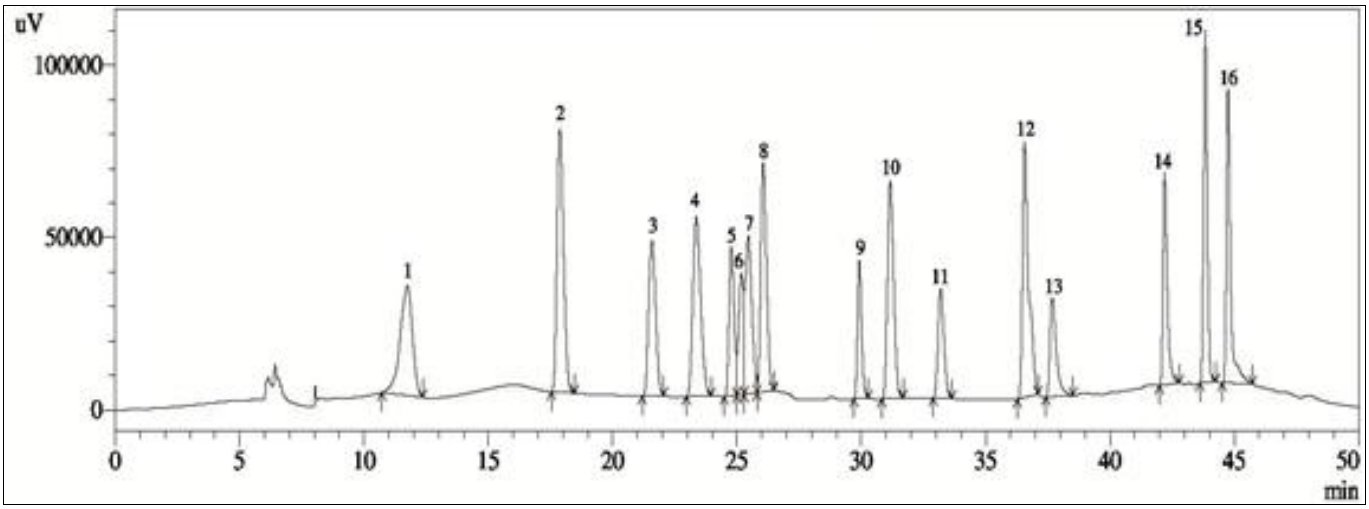


Fig 2: Standard mixture of sixteen polyphenolic compounds HPLC chromatogram.

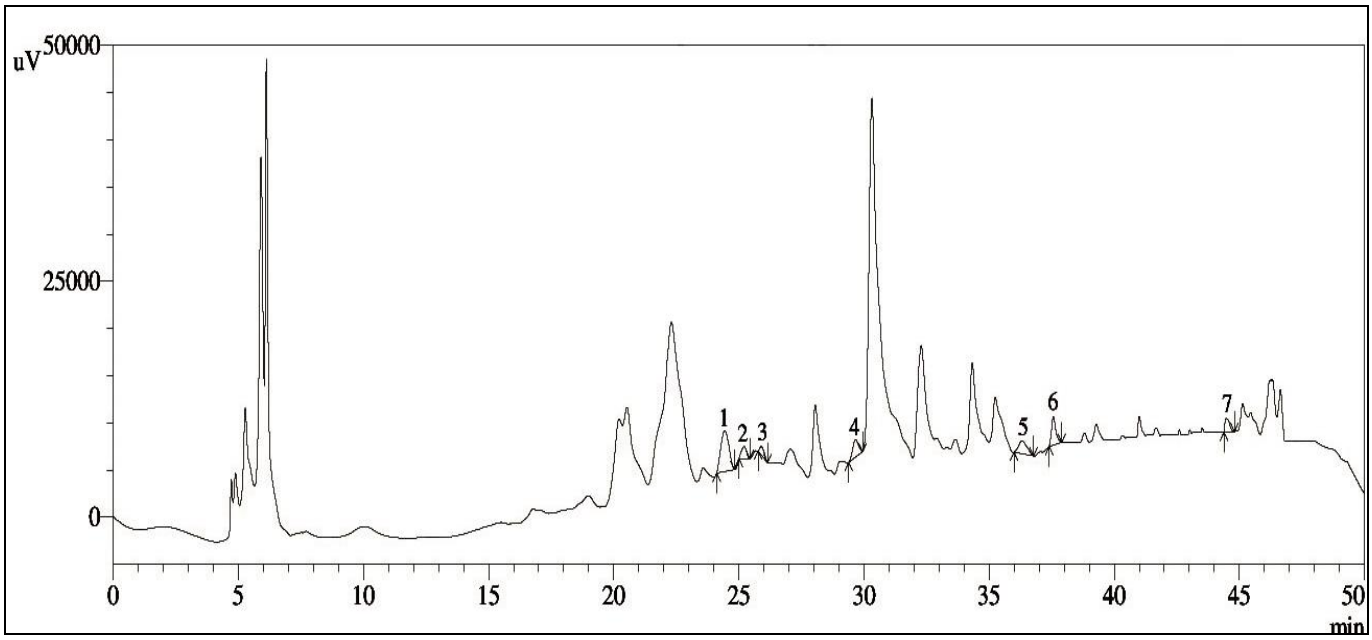


Fig 3: *B. stipularis* leaves HPLC chromatogram. Peaks: 1. (-) Epicatechin; 2. Caffeic acid; 3. Syringic acid; 4. Rutin hydrate; 5. Rosmarinic acid; 6. Myricetin; 7. Kaempferol.

In-silico molecular docking study for anti-allergy activity

Ligand Preparation

All nine ligands along with the standards, i.e. (-) Epicatechin (CID: 72276); Caffeic acid (CID: 689043); Syringic acid (CID: 10742); Rutin hydrate (CID:45479757); Rosmarinic acid (CID: 5281792); Myricetin (CID: 5281672); Kaempfero (CID: 5280863); Standar drugs Cetirizine (CID: 2678) and Diagepam (Pubchem CID: 3016) were downloaded from PubChem [28]. All the ligands were optimized via Avogadro (Avogadro: advanced molecular builder andvisualizer; Version 1.2.0 http://avogadro.cc/) where the UFF force field was applied during the optimization process. Finally, all the ligands were saved as .pdb (Protein Data Bank) format.

Preparation of the receptor protein

The histamine H1 receptor (PDB ID: 3RZE), and $GABA_A$ (PDB ID: 6X3V) were downloaded from 'Protein Data Bank' [29]. The downloaded proteins were then cleaned with PyMOL and optimized using Swiss-PdbViewer.

Molecular Docking and Visualization

Molecular docking analysis between the receptors and the ligands (separately) were conducted using the 'Vina Wizard' program in PyRx – Python Prescription 0.8 [30]. Firstly, the ligands (separately) and the receptors were loaded into the PyRx program with appropriate declaration of the compound, i.e. macromolecule, or ligand. Two separate maximised cube sized boxes of specific dimensions (69.29 Å × 59.35 Å × 94.71 Å for H_1R and 78.26 Å × 57.29 Å × 98.52 Å for $GABA_A$) aligned to the core active site residues of the receptors. Afterwards, the docked ligands (separately) and the optimized receptor was combined by PyMOL. The combined structures were viewed with Discovery Studio for proper visualization. The ligand interactions with amino acids were observed and images were saved at the best poses.

Statistical analysis

Results were presented as Mean ± Standard Error of Mean (SEM). For analyzing all the data using student's t test. $p<0.05$ was considered as statistically significant.

Results and Discussion

Phytochemical screening

Phytochemicals tests confirmed the presence of alkaloid, tannin, flavonoid, reducing sugar, glycoside, terpenoid and acidic compounds and the absence of gums and protein in *B. stipularis* (Table 1). Plants stored antioxidant possess anti-allergic potential [31].

Table 1: Phytochemical screening of *Bridelia stipularis*

Phytochemical group	*Bridelia stipularis*
Reducing sugar	+
Tannins	+
Flavonoid	+
Saponins	-
Gum	-
Steroid	+
Alkaloid	+
Glycoside	+
Protein	-
Acidic Compound	+

'+' presence and '–' absence of phytochemical group.

Acute toxicity study

B. stipularis extract showed its non-toxic nature in toxicological screening. The extract on tested mice was found safe and it was evidenced by no behavioral changes. There was no toxic reaction or lethality found at any doses (Table 2).

Table 2: Effects of *B. stipulais* leaves body weight

Group	No. of dead mice	1st day average weight in gm.	14th day average weight in gm.
Control group	0	23.40±0.51	31.0±0.55
B. stipulais (1 g/kg)	0	21.5±0.43	29.67±0.49
B. stipulais (2 g/kg)	0	22.83±0.48	30.33±0.42
B. stipulais (3 g/kg)	0	23.5±0.43	32±0.36

Mean ± Standard Error of Mean (SEM). (n = 6), *$p<$ 0.05 vs. control, **$p<0.001$ vs. control.

Sub-acute toxicity study

In sub-acute toxicity, animals were given at the dose 500 mg/kg of extract for 14 days and measurement of several

biochemical parameters. None of the biochemical contents showed any significant sign of side effect at 500 mg/kg dose (Table 3).

Table 3: Effect of *B. stipulais* leaves on biochemical parameters.

Parameter	Control	Test- I (*B. stipulais* 500 mg/kg)
SGPT (U/L)	47.6±3.187	57±3.10
SGOT (U/L)	41.4±2.158	49±2.43
ALP (U/L)	141.8±4.223	131±2.76
Bilirubin (mg/dL)	1.04±0.092	1.09±0.21
Creatinine (mg/dL)	1.02±0.115	1.34±0.43
Urea (mg/dL)	35.6±3.90	45.8±5.43
Total Cholesterol (mg/dL)	197±4.049	188.8±9.482
Triglyceride (mg/dL)	153.6±6.71	174.1±9.40

Mean ± Standard Error of Mean (SEM). (n = 6), *p<0.05 vs. control, **p<0.001 vs. control.

Anti-allergic activity

Assessment of allergic symptoms: TDI produced allergic symptoms like sneezing, scratching, nasal scores and redness. But administration of *B. stipulais* significantly reduced these symptoms. The efficacy of extract at a dose of 500 mg/kg was compared with the standard allergic drug (Table 4). The current therapies primarily focus on control the symptom and prevention of the inflammation [32]. Anti-allergic agents act by reducing the action on target cell [33].

Table 4: Assessment of allergic symptoms in mice.

Group	No. of sneezes	No. of Scratch	Nasal score
Negative control	10.5±1.07	64.2±3.78	0.4±0.0
Positive control (TDI-control)	30.2±1.03#	211.8±4.04#	2.4±0.37#
Standard (Cetrizine 20 mg/kg)	14.5±1.49***	128.8±8.35***	1.3±0.24***
B. stipularis (300 mg/kg)	14.80±1.59**	155.60±8.72**	1.80±0.37
B. stipularis (500 mg/kg)	11.80±0.97***	129.20±8.15***	0.8±0.37**

Mean ± Standard Error of Mean (SEM). (n = 6), #p<0.05 vs. control, *p<0.05 vs. TDI-control; **p<0.001 vs. TDI-control; ***p<0.0001 vs. TDI-control.

Effect on WBC count

In blood analysis found that administration of extract decreased total WBC count in blood and the amount of lymphocyte, neutrophil and eosinophil in dose dependent. The efficacy of extracts were comparable with the anti-allergic drug at both doses of 300 and 500 mg/kg extract (Table 5). The amount of leukocytes upsurge considered as allergic symptoms [34]. But *B. stapularis* leaves at 300 and 500 mg/kg decreased the WBC cells.

Table 5: Assessment of WBC cells in blood.

Group	TC (WBC)	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophils
Negative Control	5670±163.34	3370±109.59	1850±61.92	270±30	125±25	55±18.93
Positive control (TDI-control)	12380±200.45#	7870.4±114.55#	3485±108.54#	710±45.83#	215±19.79	130±30
Standard (Cetrizine 20 mg.kg)	6340±149.96***	3650±142.40***	2010±56.67***	495±27.34**	130±13.34**	45±15.73*
B. stipularis (300 mg/kg)	6000±216.79***	3945.8±141.78**	1323.8±157.47*	693.4±104.14	94.8±23.49	72.2±12.634
B. stipularis (500 mg/kg)	4900±306.59***	3332.2±214.47***	1188.6±46.99**	280±45.27**	70.6±15.78*	28.6±11.70

Mean ± Standard Error of Mean (SEM). (n = 6), #p<0.05 vs. control, *p<0.05 vs. TDI-control; **p<0.001 vs. TDI-control; ***p<0.0001 vs. TDI-control.

Effect on BAL (Bronchoalveolar lavage) fluid

The analysis of BAL fluid showed that, administration of extract decreased the WBC count in BAL fluid and the number of lymphocyte, neutrophil and eosinophil in dose dependent way. The efficacy of the extract was comparable with standard drug at the dose of 500 mg/kg (Table 6).

Table 6: Assessment of WBC cells in BAL Fluid.

Group	TC (WBC)	Lymphocytes	Neutrophils	Eosinophils	Monocytes	Basophil
Negative control	5970±150.59	3320±112.35	2060±101.32	395±35.32	125±38.91	70±20
Positive control (TDI-control)	13680±230.84#	8240±155.78#	4255±120.52#	920±55.38#	165±38.04	110±19.44
Standard (Cetrizine 20 mg/kg)	6880±112.35 ***	3850±113.78***	2420±78.60***	495±56.49 ***	55±18.93*	40±14.53*
B. stipularis (300 mg/kg)	6225±309.23 **	1324.5±79.40**	4171±210*	573.75±54.12 *	1088.75±16.44 *	47±15.96
B. stipularis (500 mg/kg)	5700±267.7 ***	1255±93.57**	3955.25±148**	430.75±61.85 *	70.25±12.14 *	28.75±16.75

Mean ± Standard Error of Mean (SEM). (n = 6), #p<0.05 vs. control, *p<0.05 vs. TDI-control; **p<0.001 vs. TDI-control; ***p<0.0001 vs. TDI-control.

Neuropharmacological activity

The objective of the neuropharmacological study was to reveal whether this plant has CNS depressant effect or not like the existing anti-allergic drugs in the market e.g., cetirizine, loratadine etc. The neuropharmacological test measured the level of excitability of CNS [35]. *B. stipularis* at both doses (300 and 500 mg/kg) did not show any significant CNS depression activity in comparison with the negative control group (Table 7). Therefore, it is to signify that extract have significant anti-allergic potential without having the side effect of CNS depression.

Table 7: Effect of extract on the neurological behavior of mice.

Group	No. of squares crossed by mice				
	At 0 min	At 30 min	At 60 min	At 90 min	At 120 min
Control	124.6 ± 7.03	95.2±1.98	85.2 ± 2.44	75.6 ± 2.84	70.2 ± 4.66
Standard Diazepum (1 mg/kg)	105.6 ± 1.81	47±4.69**	37.8 ± 1.07**	29.8 ± 2.71**	14.8 ± 4.79**
B. stipularis (300 mg/kg)	110.20±9.44	107±4.32	93.60±3.28	103.20±6.10	96±7.95
B. stipularis (500 mg/kg)	109±1.87	107± 2.97	91.80± 4.58	97.80± 6.87*	95.40± 5.90*

Mean ± Standard Error of Mean (SEM). n=6, *p< 0.05 vs. control and **p<0.001 vs. control.

HPLC Analysis

HPLC analysis demonstrated that *P. pubinerve* and *B. stipularis* contains multiple polyphenolic compound that includes (-) Epicatechin; Caffeic acid; Syringic acid; Rutin hydrate; Rosmarinic acid; Myricetin and Kaempferol.

Table 8: Amount of polyphenolic compound in the ethanolic extract of *B. stipularis* leaves.

Polyphenolic Compounds	Contain in (mg/100 g dry extract)
	B. stipularis
(-) Epicatechin	41.99±0.15
Caffeic acid	3.43±0.17
Syringic acid	1.01±0.01
Rutin hydrate	6.08±0.12
Rosmarinic acid	7.20±0.16
Myricetin	5.64±0.01
Kaempferol	1.56±0.22

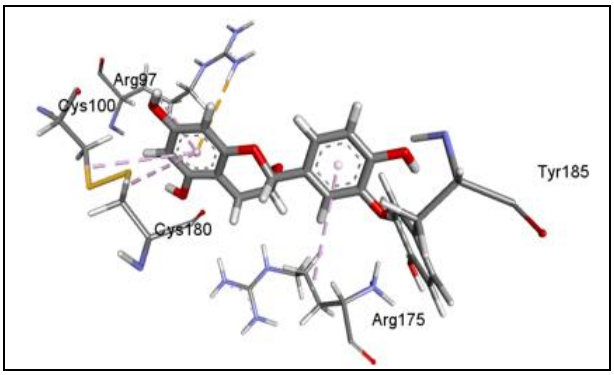
Mean ± Standard Error of Mean (SEM). (n=3)

***In-silico* molecular docking study for anti-allergic activity**

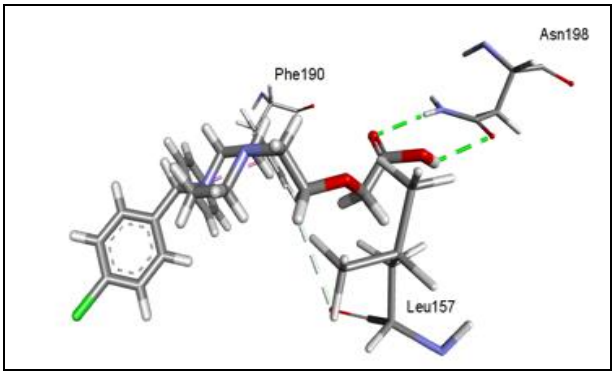
In histamine signaling pathway Histamine H_1 receptor (H_1R) mainly involved in allergic symptoms [8]. During the *in-silico* molecular docking study, among the nine polyphenoic compounds found after HPLC analysis with the H_1R protein, we observed the docking score. Among the nine found compounds rutin hydrate (-7.4 kcal/mol), trans ferulic acid and kameferol (-7.2 kcal/mol), rosmarinic acid and myricetin (-7.1 kcal/mol) demonstrated satisfactory docking score in comparison with the docking score of the standard ligand cetirizine (-7.4 kcal/mol) same as rutin hydrate with the H_1R (Table 9). It is evident from various studies that rutin hydrate, and rosmarinic acid have significant anti-allergic activity, which are present in extracts. Therefore, it is resultant, anti-allergic potential of this plant might have been exhibited because of the presence of these polyphenolic compounds (Fig. 4 and 5).

Table 9: Docking score for anti-allergic activity along with interacting amino acids.

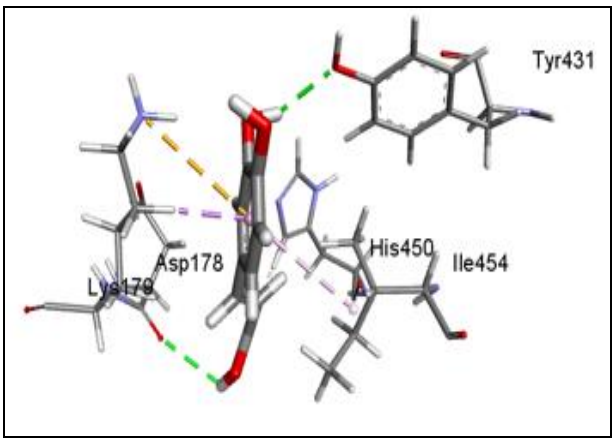
Ligand Name	Docking score (kcal/mol)	Interacting amino acids
Cetirizine (Standard drug)	-7.4	ASN198, LEU157, PHE190
(-) Epicatechin	-6.6	TYR185, ARG175, ARG97, CYS100, CYS180
Caffeic acid	-6.5	TYR431, HIS450, ILE454, ASP178, LYS179
Syringic acid	-5.4	TRP189, HIS167, TRP165, ASP183, VAL187, PHE190, PHE184, PRO161
Rutin hydrate	-7.4	THR1157, ASP1159, ARG411, LYS412, HIS220, LYS415, ALA414, TYR210
Rosmarinic acid	-7.1	ASN198, HIS167, PHE190, GLY164, PRO161, TRP158
Myricetin	-7.1	ARG409, ARG139, LYS57, ASN474, ASN472, ALA413, GLU410, SER128, ARG125
Kaemferol	-7.2	ARG139, THR60, ASN474, ARG409, ALA413, ARG125, ASN63, ASN472



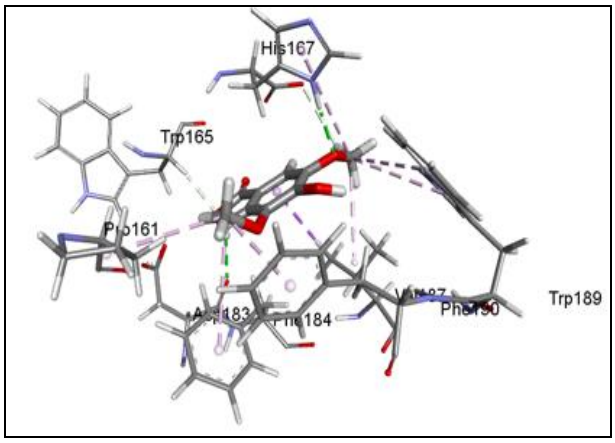
Cetirizine



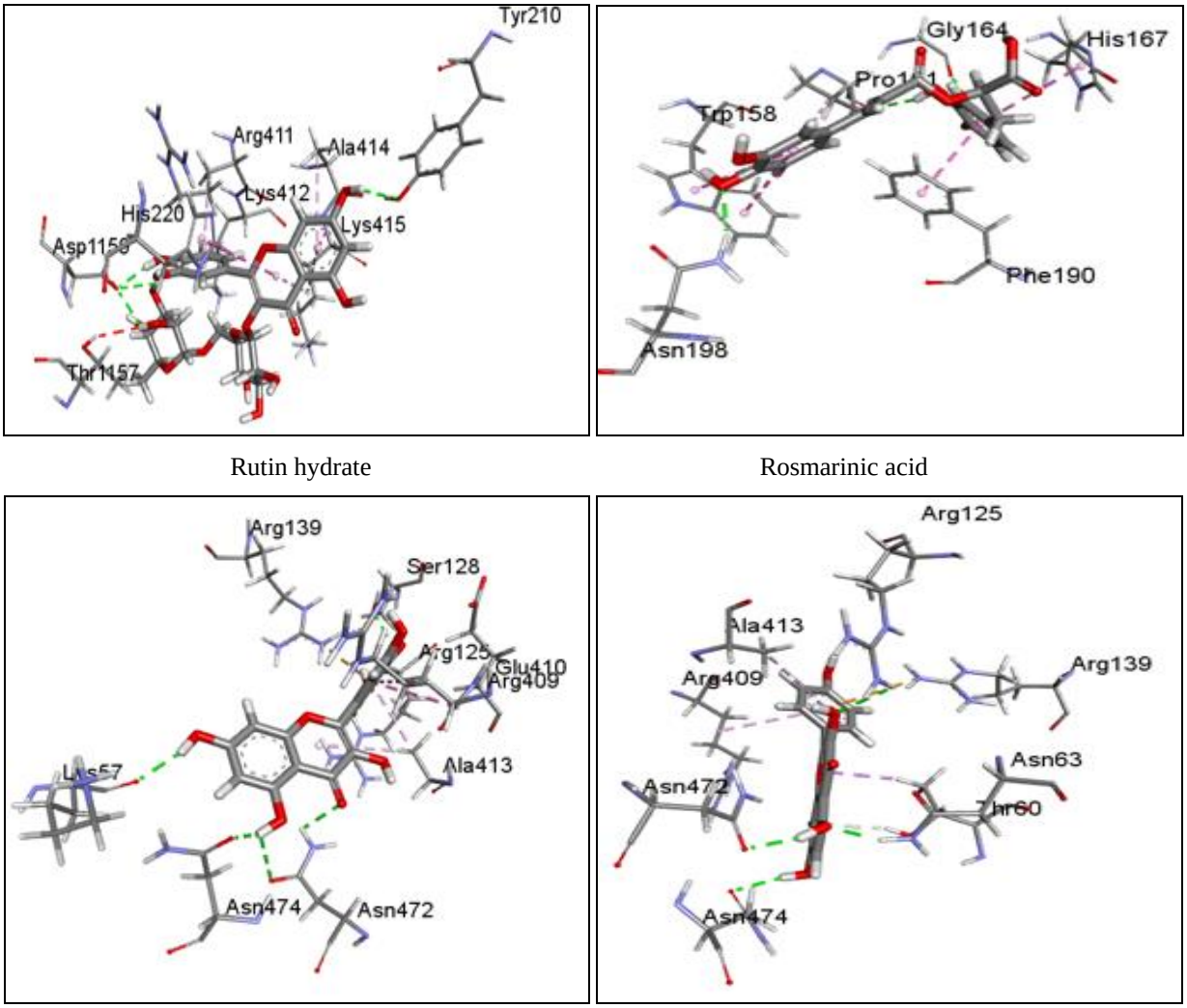
(-) Epicatechin



Caffeic acid



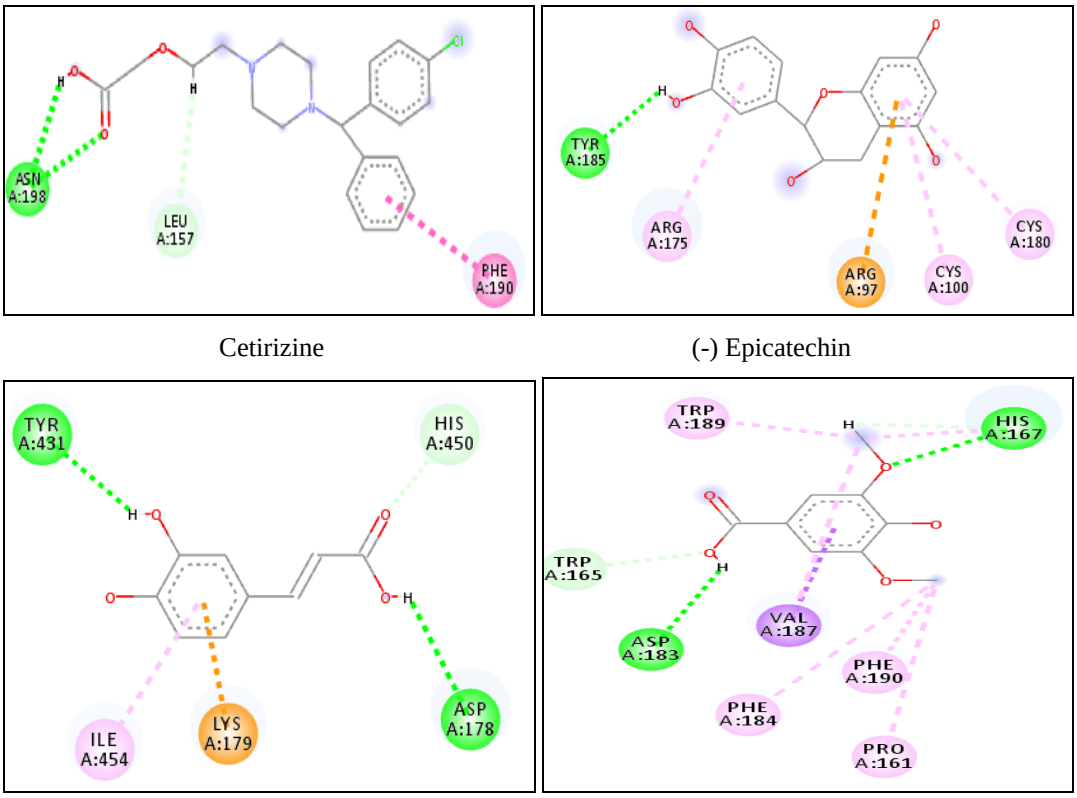
Syringic acid



Rutin hydrate | Rosmarinic acid

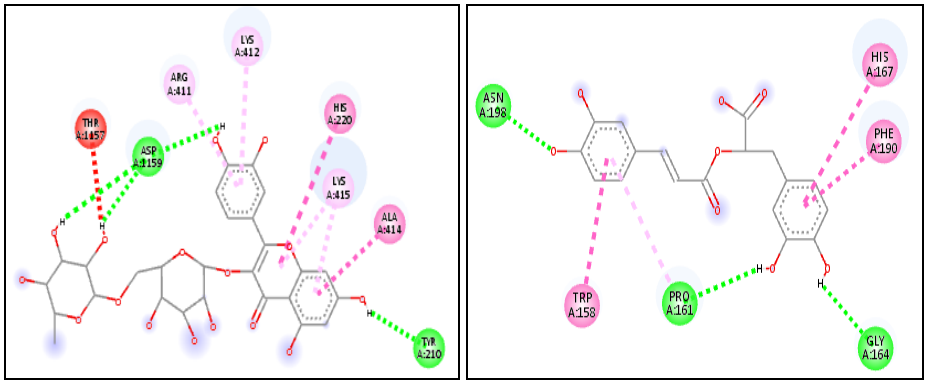
Myricetin | Kaemferol

Fig 4: 3D interactions between H_1R (PDB ID: 3RZE) and ligands according to the best ranked pose of standard drug cetirizine and seven found legends.

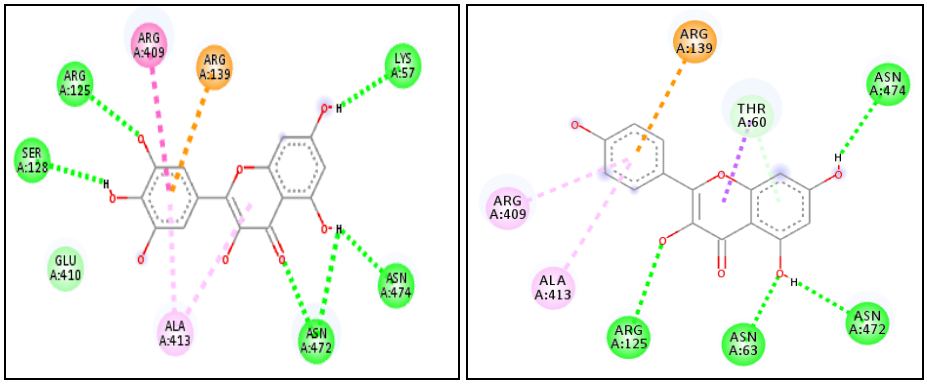


Cetirizine | (-) Epicatechin

Caffeic acid | Syringic acid



Rutin hydrate Rosmarinic acid

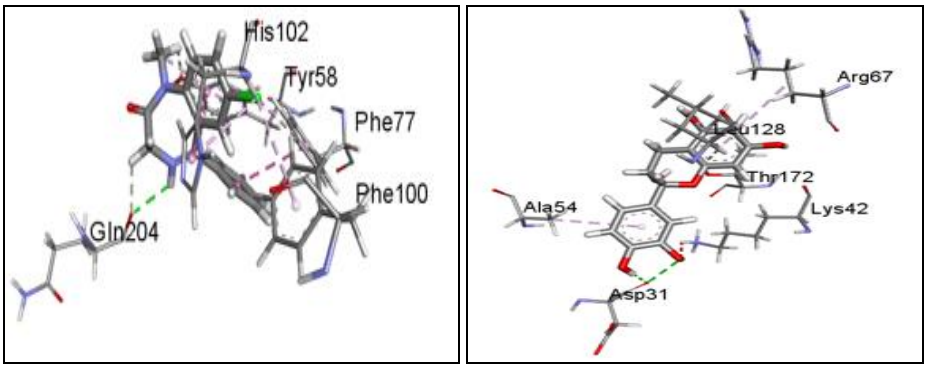


Myricetin Kaemferol

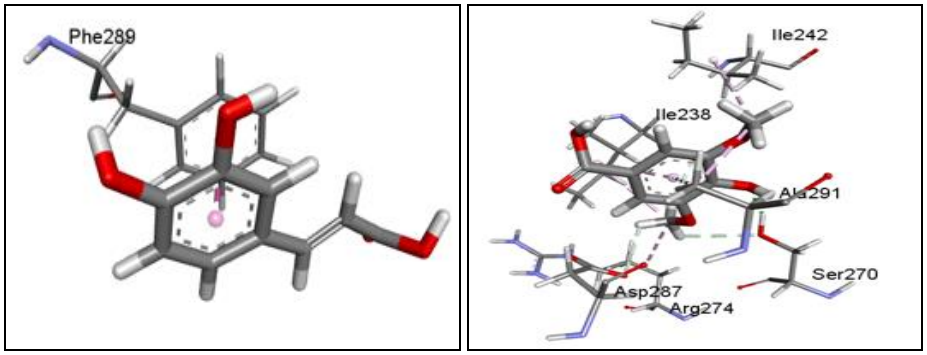
Fig 5: 2D interactions between H_1R (PDB ID: 3RZE) and ligands according to the best ranked pose of ligands. Here, colors indicate the residue or species type of bond interactions; green: conventional H-bond, light green: carbon-hydrogen bond, red: unfavorable acceptor/donor, orange: pi-cation, pink: pi-pi T-shaped, light pink: pi-alkyl. Interactions between ligand atoms and protein residues are marked with lines

***In-silico* molecular docking study for neuro-pharmacological activity**

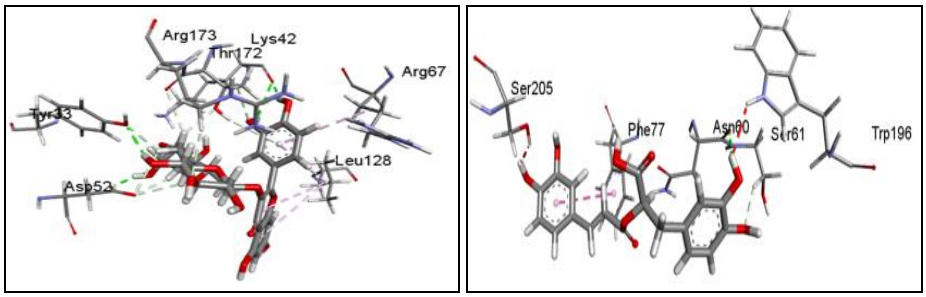
During the *in-silico* molecular docking study, interactions with the $GABA_A$ the docking score of the standard ligand diagepam -7.7 kcal/mol followed by rosmarinic acid (-7.9 kcal/mol), and myricetin (-7.8 kcal/mol) (Table 10) suggesting the efficacy of extracts against CNS depressant *in-silico* studies whereas, *in-vivo* mild support its CNS effects, these anomalies demand further investigations (Figure 6 and 7).



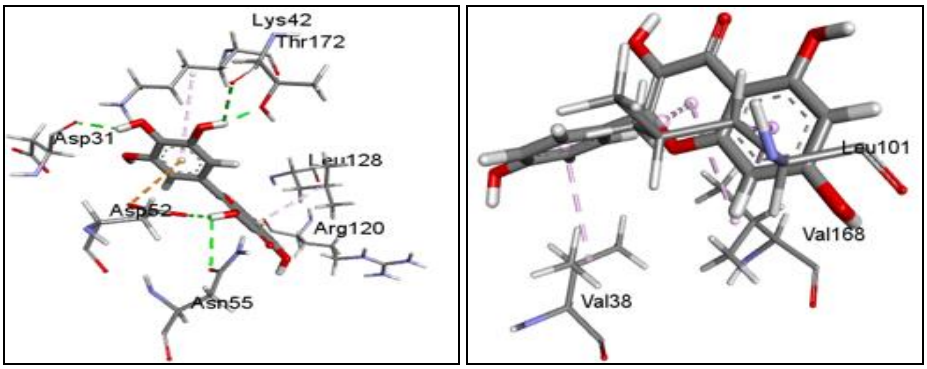
Diazepam (-) Epicatechin



Caffeic acid Syringic acid

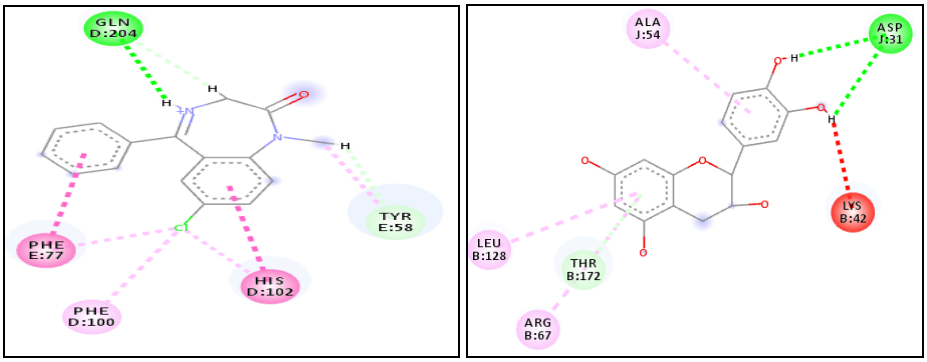


Rutin hydrate Rosmarinic acid

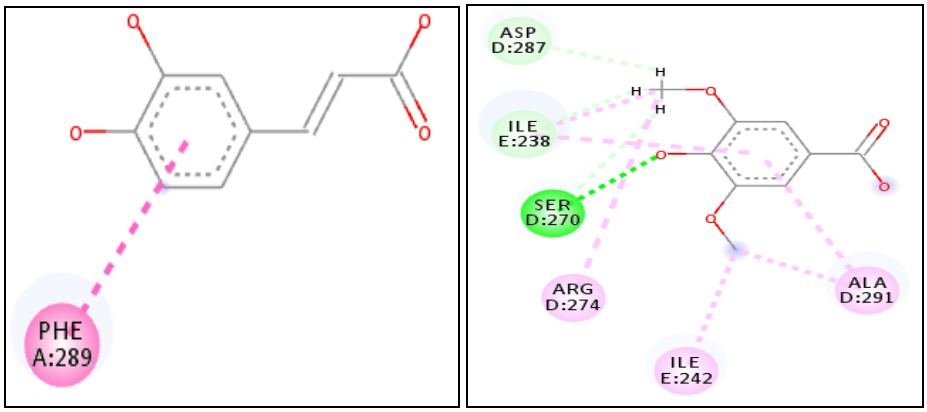


Myricetin Kaemferol

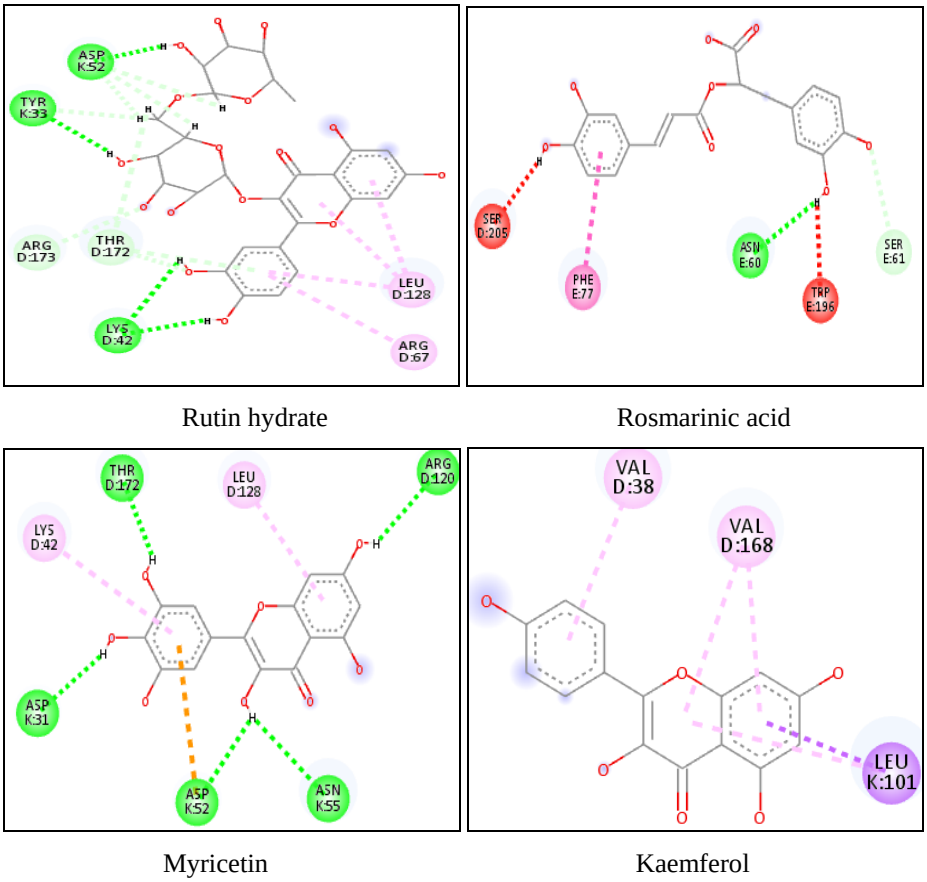
Fig 6: 3D interactions between $GABA_A$ (PDB ID: 6X3v) and ligands according to the best ranked pose of stanadard drug cetirizine and seven found legands.



Diazepam (-) Epicatechin



Caffeic acid Syringic acid



Rutin hydrate Rosmarinic acid

Myricetin Kaemferol

Fig 7: 2D interactions between GABA$_A$ (PDB ID: 6X3V) and ligands according to the best ranked pose of ligands. Here, colors indicate the residue or species type of bond interactions; green: conventional H-bond, light green: carbon-hydrogen bond, red: unfavorable acceptor/donor, orange: pi-cation, pink: pi-pi T-shaped, light pink: pi-alkyl. Interactions between ligand atoms and protein residues are marked with lines

Table 10: Docking score for neuro pharmacological activity along with interacting amino acids.

Ligand Name	Docking score (kcal/mol)	Interacting amino acids
Diazepam (Standard drug)	-7.7	GLN204, PHE77, PHE100, HIS102, TYR58
(-) Epicatechin	-5.7	ALA54, ASP31, LYS42, LEU128, THR172, ARG67
Caffeic acid	-7	PHE289
Syringic acid	-5.2	ASP287, ILE238, SER270, ARG274, ILE242, ALA291
Rutin hydrate	-6.6	ASP52, TYR33, ARG173, THR172, LYS42, ARG67, LEU128
Rosmarinic acid	-7.9	SER205, PHE77, ASN60, TRP196, SER61
Myricetin	-7.8	ARG120, LEU128, THR172, LYS42, ASP31, ASP52, ASN55
Kaemferol	-5.8	VAL38, VAL168, LEU101

Conclusions

The present study reveals the anti-allergic and Neuro-pharmacological potentials of ethanolic crude extracts. Polyphenolic compounds like myricetin, kaemferol, rutin hydrate, rosmarinic acid, and syringic acid might be behind the therapeutic activities which support its folkloric uses. This study also conforms the safety of these plants. Further investigations for characterization of the novel compounds could be helpful for development of better therapies targeting the allergy and CNS diseases.

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Authors' Declaration

The authors declared that they have no conflict of interest.

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