


ORIGINAL CONTRIBUTION

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# Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*)

Samina Yesmin<sup>1,2†</sup>, Arkajyoti Paul<sup>3,4†</sup>, Tarannum Naz<sup>1\*</sup>, A. B. M. Atiqur Rahman<sup>1</sup>, Sarkar Farhana Akhter<sup>1</sup>, Mir Imam Ibne Wahed<sup>1</sup>, Talha Bin Emran<sup>3,4</sup> and Shafayet Ahmed Siddiqui<sup>5,6\*</sup> 

## Abstract

**Background:** This experiment is conducted to evaluate the anti-inflammatory effect of *Piper chaba* roots.

**Methods:** The in-vitro anti-inflammatory activity of *Piper chaba* was carried out by human red blood cell (HRBC) membrane stabilization method which includes heat-induced hemolysis and hypo tonicity- induced hemolysis and also by another method of egg albumin denaturation assay.

**Results:** Anti-inflammatory activity study of crude ethanolic extract was performed using heat induced membrane stabilization method, hypo-tonicity induced HRBC membrane stabilization method and egg albumin denaturation method. Crude ethanolic extracts of *P. chaba* showed promising in vitro anti-inflammatory activity in a concentration dependent manner. Using acetyl salicylic acid (ASA) as standard drug and was compared with ethanolic extract to determine anti-inflammatory activity. Heat induced anti-inflammatory test revealed that crude ethanolic extract of *P. chaba* (500 µg/ml) and positive control ASA(500 µg/ml) have 52.667% and 78% respectively, hypo tonicity induced anti-inflammatory test showed 35.67% and 59% inhibition of red blood cell (RBC) hemolysis. Egg albumin denaturation method also evaluated that crude ethanolic extract (1000 µg/ml) and ASA (1000 µg/ml) showed 60% and 97.12% inhibition of egg albumin denaturation.

**Conclusion:** The plant of *P. chaba* of the genus *Piper* possesses promising anti-inflammatory activities.

**Keywords:** *P. Chaba*, Anti-inflammatory, Denaturation, Hypo- tonicity induced hemolysis, Heat induced hemolysis

## Introduction

The use of natural products having therapeutic activities is an ancient practice for human civilization and plants, minerals and animal products (Epibatidine, African clawed frog) were the primary sources of drugs since ancient time [1]. Throughout the development of human culture using of natural products has had magical-religious significance-often been the only means to treat physical injuries and other diseases. During the last few decades in drug

discovery and drug development a secondary role was played by natural products [2].

Interest in using complementary medicine to alleviate and improve health conditions is increasing in developed countries [3]. Investigation of novel different medicinal plants from different regions of the world and their botanical utilization has increased all over the world including western world. We have a mechanistic information deficiency and a lack of possible differences amongst species from the same genus of a plant [4].

Due to harmful stimuli as pathogens, irritants and damaged cells, produce a vascular tissue response which we call inflammation [5]. Inflammation and oxidation are closely related: free radicals that damage cells lead to inflammation [6]. Inflammation, which is a preventive

\* Correspondence: [taranazbd@gmail.com](mailto:taranazbd@gmail.com); [shafayetahmed91.sa@gmail.com](mailto:shafayetahmed91.sa@gmail.com)

†Samina Yesmin and Arkajyoti Paul contributed equally to this work.

<sup>1</sup>Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh

<sup>5</sup>Department of Pharmacy, Noakhali Science and Technology University, Noakhali 3814, Bangladesh

Full list of author information is available at the end of the article

effort by organism for removing injurious stimuli alongside inflammation start a signal for healing process. The term anti-inflammatory refers to the attribute of a chemical substance alongside a treatment that can reduce inflammation. About half of analgesics are anti-inflammatory drugs, relieving pain by reducing inflammation as opposed to the mechanism of opioids, which affect our central nervous system (CNS) [7]. Using over-the-counter (OTC) with non-steroidal prescription medicaments are frequently commended in case of distinctive neurosurgical practice. It is very important processing both analgesic along with anti-inflammatory activities which decidedly can bring upon several novel prospects for inflammation related diseases. Natural compounds that are rectified of plants may suffice as very good templet for discovery and design of novel anti-inflammatory lead molecules and drugs with less toxicological status because gastrointestinal problems or liver cirrhosis associated with the use of synthetic anti-inflammatory drugs enduring dilemma of medical world [8]. The most substantial fallout profiles of NSAIDs and steroidal drugs is, an escalating concern for natural compounds among peoples, for instance herbal therapeutics and dietetic supplementations are expended for many centuries to scaled down inflammation and painful sensation [9]. There are confluence of naturally occurring bioactive compounds that too provide action in a similar mechanism as NSAIDs, which insert action by suppressing our inflammatory pathways. Furthermore, there are plies of naturally obtained chemical compounds that suppress nuclear factor- $\kappa\beta$  mediated inflammation pathways along with COX-pathway in our body. Reported anti-inflammatory herbal drugs with less toxicity are *Zingiber officinale* reduces subjective pain [10]. Anti-inflammatory activity has been observed in many other plants including *Piper* genus. Cloves, Rosemary, Turmeric has powerful anti-inflammatory punch [11]. *Piper betle* leaves extract (methanolic extract) has anti-inflammatory activity using carrageenan-induced hind paw edema model [12]. Piperine compound is isolated from *Piper nigrum* can cause acute changes in inflammatory processes. Another plant from *Piper* genus *Piper porphyrophyllum* (Piperaceae) a compound- 4',5-dihydroxy-3',7-dimethoxyflavone gave anti-inflammatory activity [13, 14].

*P. chaba* (Piperaceae) a glabrous climbing shrub popularly known as 'Choi' which is used in Bangladesh as a spice and also conceived to have medicinal property for a wide range of disease including bronchitis, asthma, piles and arthritis [15]. In Bangladesh and India *P. chaba* is popularly known as 'Choi', mostly used as a spice and used for a variety of disease for a belief to have medicinal activity [15]. Traditionally in different countries it is used as an anti-flatulent, gastro-protective, appetizing

property, as an expectorant, antitussive, anti-fungal agent. It also possesses cholesterol lowering properties and as a component of digestive and sleep-inducing preparation [16]. Previously reported that; chloroform extracts, extract of petroleum ether, ethyl acetate and methanolic extracts of *P. chaba* (Choi) roots showed antibacterial and antifungal properties compared with primary standard drugs Kanamycin and Nystatin, respectively and it contains compounds bornyl piperate, piperlonguminine, -1-undecylenyl-3,4-methylenedioxy benzene,  $\alpha$ - amyryrin,  $\beta$ - sitosterol, Chavicine, Cineol terpinan-4, 1- $\beta$ - caryophyllene, Fructose, Glucose, Guineesine, lignan, N-isobutyl-decatrans-2,trans-4-dienamide, Palmitic acid, Pellitorine, Piperidine, Piperocatalidine, Piperine, Piperlonguminirine, Piperonaline, Pipereicosalidine, Piplartine, Retrofractamide-D, Sesamin, Tetrahydropiperidic acid and Veneol etc. [17, 18].

We aimed in this study, to determine the in-vitro anti-inflammatory action of *P. chaba* root for ethanolic extract and further confirmation using molecular docking approach.

## Material and methods

### Plant collection and identification

The root of *P. chaba* about 5 kg were collected from the district of Bagerhat, Bangladesh, in the month of August 2015. The plant was authenticated by, Bangladesh National Herbarium (BNH) Mirpur, Dhaka, Bangladesh and a voucher specimen has been deposited in Bangladesh National Herbarium, voucher number-43198. The fresh roots were collected and preserved in Phytochemistry lab, Department of Pharmacy, University of Rajshahi, Bangladesh.

### Preparation of plant material

After washing the roots were cut into small parts. Parts were dried under the mild sun and finally dried in an oven at 40–45 °C for 36 h. After complete drying, the dried materials then fine-grained using a grinding machine to form a coarse powder (FFC-15, China) were stacked away for future use conserving in hermetically sealed container.

### Extraction with solvent

Extraction is the process in which the plant materials are treated with specific solvents whereby both medicinally active and inactive constituents are dissolved out. Powdered plant materials (roots) having a weight of about 700 g were taken in 3 different amber-colored reagent bottles separately and soaked in 700 ml of ethanol (98% pure) in each bottle. Then the plant extract was sealed for 5 days with episodic stirring. Firstly, filtering through cotton and then using Whatman No.1 filter papers the filtration process was meticulously done. The overall filtering process ran for consecutive three times

and then the solvent and extract mixture decocted using a rotary evaporator at 45 °C under reduced vapor pressure to yield crude plant extract.

#### Anti-inflammatory activity

The following standard methods were used for in vitro anti-inflammatory evaluation of crude ethanolic extract of *P. chaba* roots.

#### Human red blood cell (HRBC) membrane stabilization assay

To examine in vitro anti-inflammatory action of extract HRBC membrane stabilization technique is incorporated by following Gandhidasan et al. [19]. NSAIDs were used as standard and anti-inflammatory activity was expressed as the percentage of RBC lysis. HRBC membrane act similar as the lysosomal membrane [20]. If it stabilizes by using the extract it also stabilizes the lysosomal membrane. Spectrophotometer at 560 nm range was used for estimation of hemoglobin content in the suspension. Healthy human volunteer donated blood while any consumption of NSAIDs prior 2 weeks of the experiment are considered to be the prime exclusion criteria. Na-Oxalate was used to prevent clotting. All the blood samples were stored at 4 °C for 24 h before use. Centrifugation for 5 mins at 2500 rpm was used for supernatant removal. Sterile saline solution (0.9% w/v NaCl) was used for washing and centrifugation was done at 2500 rpm for 5 min. The process of clearing supernatant was done in three repeated times and the packed cell volume was measured. For cellular component reconstitution a 40% suspension (v/v) mixed with phosphate-buffered saline (10 mM, pH 7.4) comprised in 1 L of distilled water, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O- 0.26 g; Na<sub>2</sub>HPO<sub>4</sub>-1.15 g; NaCl - 9 g.

#### Heat-induced hemolysis

Portion of 5 ml of the isotonic buffer containing 50 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml of an ethanol solution of crude extract was put into two duplicate sets of centrifuge tubes. The same amount of vehicles was added up in another tube as control. 50 µl of RBC suspension was contributed to each tube and mingled gently by inverting the test tube. One pair of tubes were incubated at 54 °C temperature, 20 min in water bath. Other pair was preserved at temperature 0–5 °C in ice bath. Centrifugation of the mixture was done at 540 nm for 5 min at 5000 rpm and the absorbance was taken at 560 nm by using a spectrophotometer. Acetyl salicylic acid (ASA) 200 µg/ml was used as a reference standard. The percent inhibition of hemolysis was calculated according to the equation:

$$\% \text{inhibition of hemolysis} = 100 \times \left( 1 - \frac{\text{OD}_2 - \text{OD}_1}{\text{OD}_3 - \text{OD}_1} \right)$$

Where, OD<sub>1</sub> = Test Sample Unheated; OD<sub>2</sub> = Test Sample Heated and OD<sub>3</sub> = Control Sample Heated.

#### Hypotonicity-induced hemolysis

The isotonic solution was made by composing 154 mM NaCl in 10 mM sodium phosphste solution and the buffer of this solution was 7.4 p<sup>H</sup>. Stock RBC suspension 50 µl was mixed with 5 ml of the hypotonic solution containing the *P. chaba* ethanolic extract at concentrations of 50, 100, 200, 400 and 800 µg/ml, while the control sample was mixed with drug-free solution. After incubating for 10 min at room temperature the whole mixture was centrifuged 5000 rpm for 5 min and at 540 nm, the absorbance of the supernatant was assessed using UV-spectrophotometer. Acetyl salicylic acid (ASA) 200 µg/ml was used as a reference standard. The percent inhibition of hemolysis was calculated according to the equation:

$$\% \text{inhibition of hemolysis} = 100 \times \left( 1 - \frac{\text{OD}_2 - \text{OD}_1}{\text{OD}_3 - \text{OD}_1} \right)$$

Where, OD<sub>1</sub> = Test sample in isotonic solution; OD<sub>2</sub> = Test sample hypotonic solution and OD<sub>3</sub> = Control sample in hypotonic solution.

#### Egg albumin denaturation assay

Inflammatory and arthritic diseases are produced by denaturation of protein and a yield of autoantigen in several arthritic diseases might be for in vivo denaturation of proteins. So agents having the property of protein denaturation can be used for the anti-inflammatory drug development. In percent investigation, the in vivo anti-inflammatory effect of *P. chaba* was evaluated against denaturation of egg albumin. The reaction mixture (5 ml) consisted of the 0.2 ml of egg albumin with saline of 2.8 ml phosphate buffer (PBS, pH 6.4) and 2 ml of changing concentrations of *P. chaba* extract so that terminal concentrations become 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml. The similar volume of distilled water was used as control substance. Incubation was done for 15 min at 37.2 °C and after that it was heated for 5 min at 70 °C. After that absorbance was measured at 660 nm. For reference acetyl salicylic acid (ASA) at the final concentration of (200, 400, 600, 800 and 1000 µg/ml) was used at similar absorbance [21].

Following formula is used for calculating inhibition of protein denaturation:

$$\begin{aligned} &\% \text{inhibition of egg albumin denaturation} \\ &= 100 \times \left( 1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \right) \end{aligned}$$

Where, OD<sub>1</sub> = Test Sample Unheated; OD<sub>2</sub> = Test sample Heated and OD<sub>3</sub> = Control Sample Heated.

**Statistical analysis**

The results are expressed as mean ± SD using Graph Pad Prism (version 7). We used a one-way analysis of variance (ANOVA), followed by Scheffe’s post-hoc test or students paired or unpaired t-test where appropriate. The statistical method applied in each analysis was described in each figure. Results were considered to be significant when *p*-values were less than 0.05 (*p* < 0.05).

**In silico molecular docking**

**Protein preparation**

3-D crystal structure of the, catalytic domain of Cyclooxygenase-1 (PDB ID: 2OYE), Cyclooxygenase-2 (PDB ID: 6COX) and NF-κB (nuclear factor kappa light chain enhancer) (PDB ID: 5LDE) were downloaded using the Protein Data Bank database [22]. Following that, utilizing Protein Preparation Wizard of Schrödinger- Maestro v10.1 the protein structures were prepared and rectified. After that using Force Field OPLS\_2005, setting maximum heavy atom RMSD (root mean square deviation) at 0.30 Å; down-play of energy was carried out.

**Ligand preparation**

Employing PubChem database, six major representative compound structures i.e., bornyl piperate (CID: 274465980), piperine (CID: 638024) and piperlongumine (CID: 5320621) were regained. Then ligands to be docked were prepared with Lig-Prep tool ingrained in

Maestro 2015, followed by neutralization at pH 7.0 ± 2.0 using Epik and understated by force field OPLS-2005.

**Receptor grid generation**

For Glide ligand docking; receptor grids were calculated for the prepared receptors for searching suitable docking sites so that several ligands attach within the portended active site while molecular docking. In Glide, by maintaining the default parameters of Van der Waals scaling factor at 1.00 including charge cutoff at 0.25 subjugated to force field OPLS-2005 grids were generated. A cubic-like box of defined dimensions centered of the active site residues at the centroid (Reference ligand active site) was automatically generated for the receptor protein. Bounding box with a dimension of 14 Å × 14 Å × 14 Å was set for docking experiments.

**Glide standard precision (SP) ligand docking**

Using, Glide of Schrödinger-Maestro v 10.1 standard precision flexible ligand docking with receptor was carried out, where sanctions were applied for any non-cis/trans amide bonds. The scaling factor for Van der Waals and partial charge cutoff for ligand atoms were choose to be 0.80 and 0.15 respectively. Final scoring was performed on energy-minimized poses and displayed as Glide score. For each ligand, the best- docked pose (Figs. 1, 2 and 3) with lowest Glide score value was recorded [23, 24].

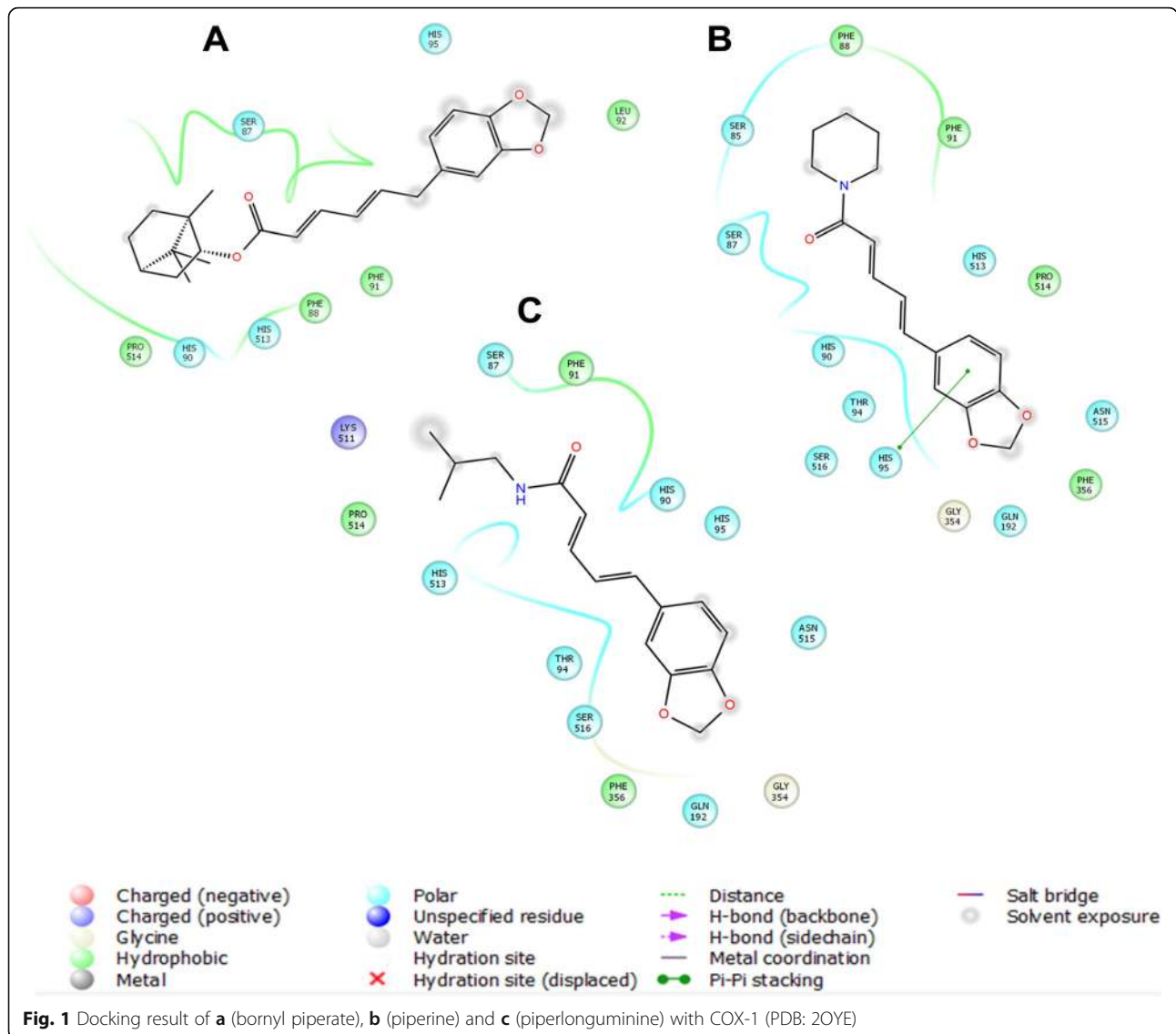
**Results**

**Effect of CEE (crude ethanolic extract) and ASA (acetyl salisylic acid) on heat-induced hemolysis of RBC membrane**

The recorded value of effect of CEE of *Piper chaba* roots and ASA on heat-induced hemolysis of RBC membrane is given in Table 1.

**Table 1** Effect of CEE and ASA on heat-induced hemolysis of RBC membrane

Sample	Conc. (µg/ml)	Absorbance			% of inhibition of RBC haemolysis			% Inhibition (Mean ± SD)
		a	b	c	a	b	c	
<b>ASA</b>	100	0.267	0.265	0.26	13	14	18	15 ± 2.646
	200	0.255	0.254	0.254	26	25	25	25.333 ± 0.577
	300	0.234	0.233	0.234	41	61	41	47.667 ± 11.547
	400	0.234	0.233	0.233	68	71	69	69.333 ± 1.528
	500	0.23	0.233	0.23	79	76	79	78 ± 1.7320
<b>CEE</b>	100	0.269	0.25	0.245	23	47	53	41 ± 15.875
	200	0.243	0.24	0.24	49	50	50	49.667 ± 0.577
	300	0.235	0.233	0.23	56	55	58	56.333 ± 1.527
	400	0.233	0.235	0.233	65	62	69	65.333 ± 3.512
	500	0.225	0.233	0.23	32	61	65	52.667 ± 18.009



**Effect of CEE (crude ethanolic extract) and ASA (acetyl salicylic acid) on hypotonicity-induced hemolysis of RBC membrane**

The recorded value of effect of CEE of *Piper chaba* roots and ASA on hypotonicity-induced hemolysis of RBC membrane is given in Table 2.

**Evaluation of anti-inflammatory activity by egg albumin denaturation**

In case of inflammatory arthritic diseases modification of tissue proteins occurs which is well-documented in several previous studies. In vivo denaturation of proteins and synthesis of auto antigen could be a hallmark marker of arthritic disease. Test results are shown in Table 3.

**In silico study: molecular docking for anti-inflammatory activity**

In this study among the three compounds which were isolated from *Piper chaba* root were performed molecular docking against Cyclooxygenase enzyme 1 (COX 1) (PDB ID: 2OYE) (Fig. 1), Cyclooxygenase enzyme 2 (COX 2) (PDB ID: 6COX) (Fig. 2) and NF-κB (nuclear factor kappa light chain enhancer) (PDB ID: 5LDE) (Fig. 3). From Tables 4 and 6, piperine showed best docking score against COX 1 and NF-κB. On the other hand, in Table 5, piperlonguminine showed best docking score against COX 2.

**Discussion**

The study involved using anti-inflammatory extracts as an important aspect. There are so many extracts or compounds which might show anti-inflammatory effects.



**Table 2** Effect of CEE and ASA on hypo tonicity induced hemolysis of RBC membrane

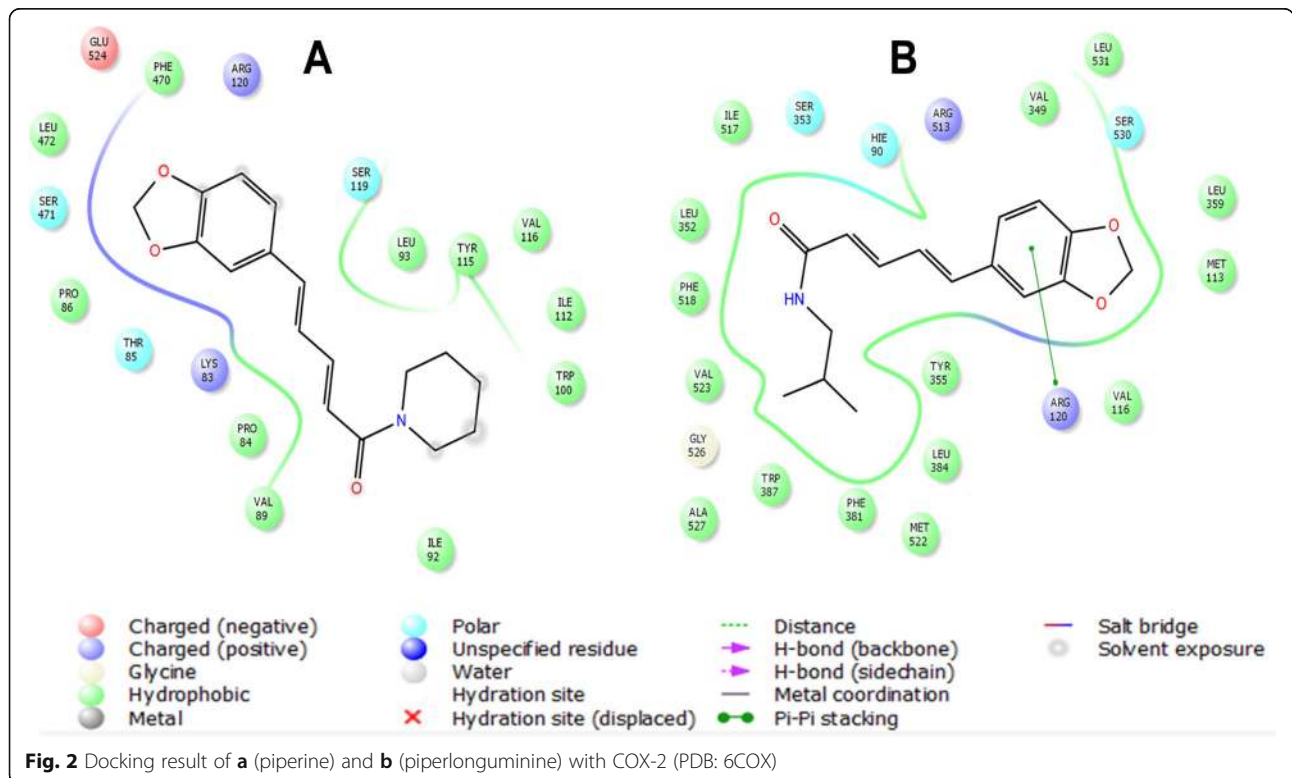
Sample	Conc. (µg/ml)	Absorbance			% of inhibition of RBC haemolysis			% Inhibition (Mean ± SD)
		a	b	c	a	b	c	
<b>ASA</b>	100	0.838	0.83	0.835	13	14	13	13.333 ± 0.577
	200	0.75	0.75	0.74	23	23	24	23.333 ± 0.577
	300	0.626	0.62	0.63	38	37.5	37	37.5 ± 0.5
	400	0.6	0.61	0.6	40	39	40	39.667 ± 0.577
	500	0.425	0.42	0.43	59	60	58	59 ± 1
<b>CEE</b>	100	0.842	0.84	0.844	13	13	12	12.667 ± 0.577
	200	0.711	0.71	0.715	28	28	27	27.667 ± 0.577
	300	0.7	0.71	0.7	28	27	28	27.667 ± 0.577
	400	0.68	0.68	0.675	30	30	31	30.333 ± 0.577
	500	0.65	0.65	0.6	34	34	39	35.667 ± 2.887

This effect can be demonstrated by heat-induced membrane stabilization method, hypotonicity induced HRBC membrane stabilization method and egg albumin anti-denaturation method. It is a concentration-dependent process and protection increased with increase in the concentration of the sample.

Recent study with *P. chaba* and *P. interruptum* ethanolic extract inhibited ear edema that was induced by ethyl phenylpiolate and edema of hind paws in carrageenan induced rat models [25]. Though having some

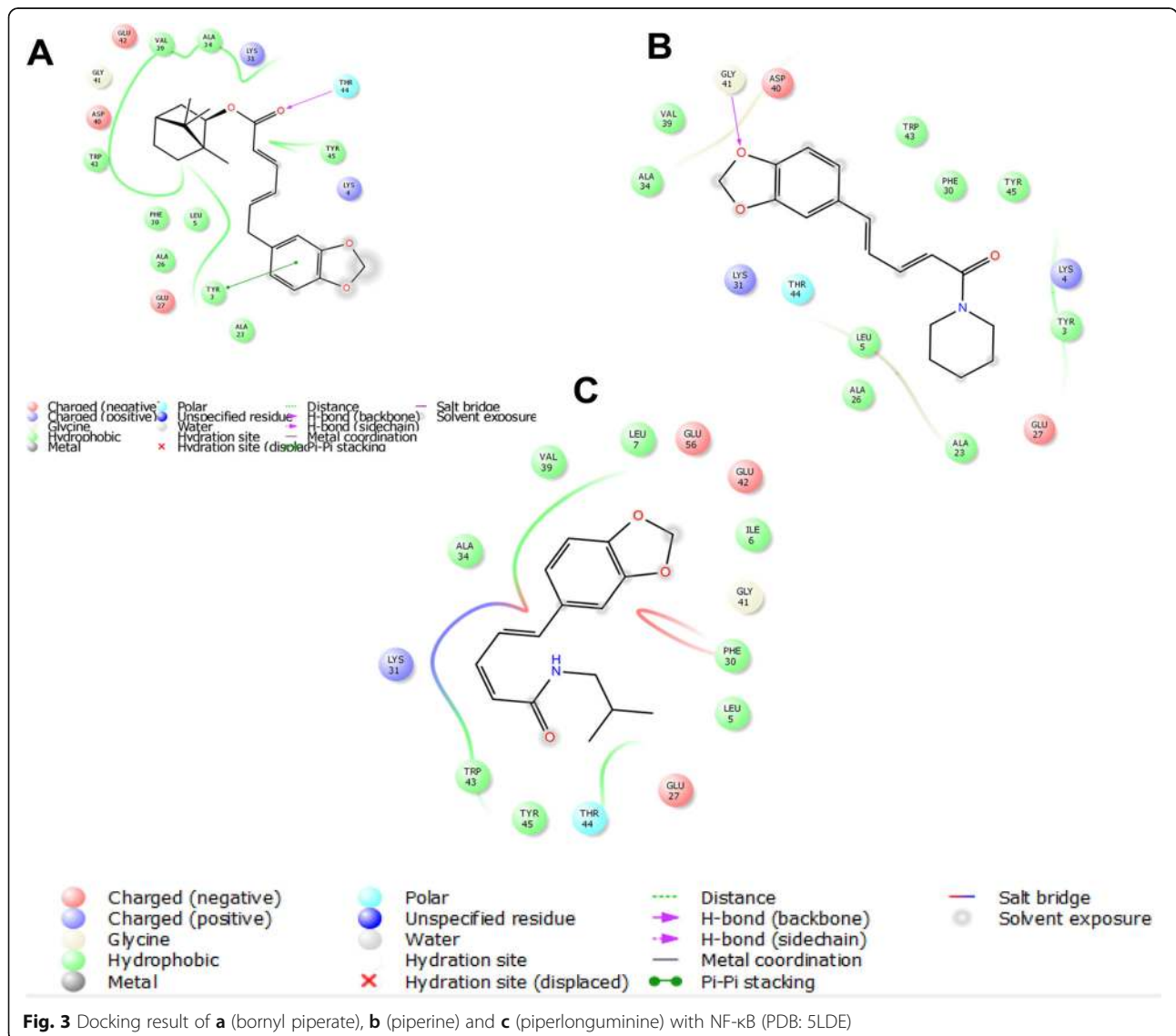
concern about procedure of the conducted study and for suspicious bias detection reports of those studies showed great promise for future study.

One of the major and well documented cause of inflammation is denaturation of proteins. The investigation is done on the mechanism of anti-inflammation activity. The further study was done on the ability of the extract to inhibit protein denaturation as the part of the investigation. For denaturation of albumin protein it seemed very efficient. The active compounds obtained from the plant part



**Table 3** Effect of CEE and ASA on egg albumin denaturation

Sample	Conc. (µg/ml)	Absorbance			% of inhibition of egg albumin denaturation			% Inhibition (Mean ± SD)
		a	b	c	a	b	c	
CEE	200	1.257	1.255	1.255	3	4	4	3.667 ± 0.577
	400	1.097	1.099	1.09	24	20	20	21.333 ± 2.309
	600	1.033	1.03	1.033	24	24	24	24 ± 0
	800	0.701	0.703	0.701	51	52	52	51.667 ± 0.577
	1000	0.513	0.515	0.513	64	64	52	60 ± 6.9282
ASA	200	0.319	0.32	0.32	77.44	77.36	77.43	77.41 ± 0.043
	400	0.277	0.277	0.275	80.48	80.48	80.7	80.553 ± 0.127
	600	0.198	0.195	0.198	86.6	86.7	86.6	86.633 ± 0.057
	800	0.086	0.088	0.086	95.3	97.3	95.39	95.997 ± 1.129
	1000	0.061	0.061	0.063	97	97.22	97.14	97.12 ± 0.111



**Fig. 3** Docking result of **a** (bornyl piperate), **b** (piperine) and **c** (piperlonguminine) with NF-κB (PDB: 5LDE)

**Table 4** Docking result of bornyl piperate, piperine and piperlonguminine with COX-1 (PDB: 2OYE)

Compound Name	Compound ID	Docking Score
bornyl piperate	274,465,980	-2.909
piperine	638,024	-5.648
piperlonguminine	5,320,621	-4.956

also had more or less anti-inflammatory activity. Acetyl salicylic acid a standard anti-inflammatory drug showed the maximum inhibition of 78% at the concentration of 500 µg/ml, whereas CEE of *P. chaba* showed 52.6% at that concentration. The extracts were effectively inhibiting the heat-induced hemolysis. An evidence regarding membrane stabilization is obtained from these results. The evidence proves that membrane stabilization is an additional mechanism of anti-inflammatory effect of the extract. Release of lysosomal contents from neutrophils might be inhibited at the inflammation sites for membrane stabilization [26].

The hypotonic solution has hemolytic effect. Hemolysis occurs when there is accumulation of excessive fluid into the cells resulting in rupture of the RBC membrane. When the red cell membrane gets injured, it will make the cell more susceptible to secondary damage. This damage is occurred by free radical-induced lipid peroxidation [27]. The leakage of serum protein and fluids into the tissue can be prevented by membrane stabilization. This process go on by inflammatory intermediators where there is an increase in permeability of membrane [28]. Ethanolic root-extract of *P. chaba* extract might be stabilize the membrane of RBC by precluding the discharge of lytic enzymes and other active inflammatory mediators. At lower dose 100 µg/ml the CEE of *P. chaba* showed almost similar activity compared to a reference standard.

For the evaluation of anti-inflammatory property of *P. chaba*, the anti-denaturation method of egg albumin was chosen. In the assay of anti-denaturation method, the egg albumin is denatured. This denaturation is induced by heat treatment. Some antigens are expressed by denatured proteins. These antigens are associated with hypersensitive reactions (type-III) which are associated with some diseases for example glomerulonephritis and serum sickness [29]. Heat denatured proteins can provoke delayed hypersensitivity. These proteins are as

effective as native proteins in such provoking [30]. Moreover, it has already been proved that conventional NSAID's like phenylbutazone and indomethacin don't only inhibit the endogenous prostaglandins production by blocking COX enzyme. In addition, they also prevent denaturation of proteins [31]. That's why for checking the anti-inflammatory activity, anti-denaturation assay is the convenient method. It can be observed from the present study that the extract has shown considerable anti-inflammatory activity. *P. chaba* is capable of controlling the production of autoantigen. Thus, it can inhibit the denaturation of proteins. This effect was compared with a standard drug. Aspirin was taken as standard drug for the comparison. In preliminary phytochemical screening, the secondary metabolites like phenolic compounds and alkaloids were found. These compounds might be responsible for such activity.

Nowadays molecular docking studies have been widely used to predict the ligand-target prediction and to obtain the biological activity of the natural products. Again, it gives us not only the possible mechanism of action of a protein or enzyme but also the binding moods inside the binding site of proteins or enzyme. So, we have also used the molecular docking of some compounds of *P. chaba* to make a collaboration between this compounds and different types of enzymes responsible for inflammation to illustrate the biochemical process of the anti-inflammatory activity [32–34]. These compounds were docked against three targets which were COX-1 (PDB ID: 2OYE), COX-2 (PDB ID: 6COX) and NF-κβ (PDB ID: 5LDE). From Tables 4 and 6, we can see that piperine give the best docking score against COX-1 and NF-κβ followed by bornyl piperate and piperlonguminine. It has been previously reported piperine have both analgesic and anticonvulsant effect [27]. But on the other hand, piperlonguminine showed highest docking score against COX-2 among of these three compounds in Table 5, which has been previously reported [35, 36]. In summary, the comprehensive analysis by using the complementary tool support the traditional use of this plant.

## Conclusions

The investigation indicated that the ethanolic root extract of *P. chaba* shows significant inhibition of hemolysis in vitro. Also, the inhibition effect confirmed

**Table 5** Docking result of bornyl piperate, piperine and piperlonguminine with COX-2 (PDB: 6COX)

Compound Name	Compound ID	Docking Score
bornyl piperate	274,465,980	-4.285
piperine	638,024	-4.285
piperlonguminine	5,320,621	-4.392

**Table 6** Docking result of bornyl piperate, piperine and piperlonguminine with NF-κB (PDB: 5LDE)

Compound Name	Compound ID	Docking Score
bornyl piperate	274,465,980	-4.33
piperine	638,024	-5.891
piperlonguminine	5,320,621	-4.733



by crude extracts of *P. chaba* was promising with that of standard drug acetylsalicylic acid. Moreover, from the in silico PASS prediction, the isolated phytoconstituents are significantly impacted in in vitro activities. This experimental evidence indicates *P. chaba* root extracts could have potential therapeutic efficacy in disease processes causing destabilization of biological membranes. The result of the present study assumed that this plant could play a dynamic role in anti-inflammatory activity.

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#### Declarations

All authors read the manuscript and approved it for submission. No portion or part of the manuscript has been published earlier, nor is any part of it under consideration for publication at another journal.

#### Authors' contributions

This work was carried out in coaction among all authors. All the authors have accepted responsibility for the whole content of this manuscript and approved for submission. Authors S.Y.; A. B. M. A. R and S. F. A collected the plant and prepared the extracts and fractions. S.Y.; A. B. M. A. R and S. F. A carried out the study design, performed the experiments, data collection, data interpretation, manuscript preparation, statistical analysis. Author A.P. performed the in silico docking study. S. Y. and S. A. S prepared the manuscript draft. T. N., M. I. I. W. and T. B. E designed and planned the studies, supervised the experiments and revised the manuscript for necessary changes in format, grammar and English standard and has thoroughly checked all authors read and approved the final version of the manuscript. All authors read and approved the final manuscript.

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#### Availability of data and materials

Not applicable.

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

#### Author details

<sup>1</sup>Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh.

<sup>2</sup>Department of Pharmacy, Atish Dipankar University of Science & Technology, Dhaka 1230, Bangladesh.

<sup>3</sup>Department of Pharmacy, BGC Trust University Bangladesh, Chittagong 4381, Bangladesh.

<sup>4</sup>Drug Discovery, GUSTO A Research Group, Chittagong 4000, Bangladesh.

<sup>5</sup>Department of Pharmacy, Noakhali Science and Technology University, Noakhali 3814, Bangladesh.

<sup>6</sup>Pratyasha Health Biomedical Research Center, Dhaka 1230, Bangladesh.

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