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Anthelmintic and analgesic activities of different solvent fractions of crude methanolic extract of *Baccaurea ramiflora* (Lour.) fruit peels and seeds with molecular docking analysis

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ABSTRACT

Introduction: *Baccaurea ramiflora* Lour, locally known as Latkan, is a popular fruit in Bangladesh used for its anthelmintic and analgesic activities. This study aimed to compare the anthelmintic and analgesic activities of different solvent fractions of crude extract from *B. ramiflora* fruit seed and peel.

Methods: Cold extraction followed by the Kupchan scheme was employed to obtain various solvent fractions. *In vivo* anthelmintic activity was assessed using *Pheretima posthuman*, while analgesic activity was evaluated via writhing and tail immersion tests on Swiss albino mice. Molecular docking studies of four reported phytochemicals from *B. ramiflora* on analgesic and anti-parasitic protein targets were conducted using the PyRx.

Results: Results revealed significant anthelmintic activity in seed petroleum ether, chloroform, ethyl acetate, and acetone solvent fractions in a dose-dependent manner, with acetone extract exhibiting the highest activity. Writhing was significantly reduced in all seed and peel extracts, with the highest inhibition observed at 200 mg/kg dose. Moreover, seed chloroform and peel ethyl acetate extracts displayed the longest pain reaction times in the tail immersion model (6.924 ± 0.264 seconds and 6.562 ± 0.157 seconds, respectively). Molecular docking revealed strong binding interactions of Baccariminose C and D (≥8.2 kcal/mol) with all studied protein targets.

Conclusion: This study confirms the anthelmintic and analgesic activities of *B. ramiflora* fruit peel and seed extracts. These results contribute to the understanding of the medicinal properties of *B. ramiflora* and its potential applications in pharmaceuticals.

Implication for health policy/practice/research/medical education:
Different solvent fractions of crude extracts of *Baccaurea ramiflora* peel and seed showed significant analgesic and anthelmintic potentials through *in vivo* and *in silico* studies, which may imply the use of the fruit's peel as herbal medicine.

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Introduction

Plants are considered one of the most prominent sources of important secondary metabolites with medicinal benefits (1,2). Various phytochemicals, derived as secondary metabolites from plants, substantially contribute to identifying new pharmacological compounds, exhibiting a broad spectrum of pharmacological activities (3). Fruits, in particular, are considered the most common source of essential macro- and micronutrients such as β-carotene (4), thiamine, riboflavin, cyanocobalamin, ascorbic acid, and tocopherol (5) among others. Over the past four decades, researchers and drug scientists have been searching for new drugs of natural origin more than synthetic molecules, probably because currently available

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drugs are either too expensive or tend to produce higher side effects (6).

Helminthiasis is an acute and/or chronic pathological condition caused by infestation with intestinal parasitic worms – such as roundworm (*Ascaris lumbricoides*), hookworm (*Ancylostoma caninum*), and whipworm (*Trichuris trichuris*). According to WHO, it affects the health of a large fraction of humans and livestock, mostly in developing countries. Numbers of pathogenic nematodes and schistosomes have already developed long-term resistance against the available drugs (7). Moreover, the analgesic medicines currently available for the treatment of pain (central or peripheral) are also losing patient compliance majorly due to triggering adverse effects e.g. non-steroidal anti-inflammatory drugs (NSAIDs) cause gastric lesions, opiates develop tolerance and dependence, etc. So, the assessment of analgesic potential is attracting the development of new analgesic generics without adverse effects (8).

Baccaurea ramiflora Lour. (Family: Euphorbiaceae), locally known as "Latkan" in Bengali, is an underutilized fruit crop found in Bangladesh, India, Nepal, Myanmar, Thailand, China, and Malaysia (9). In Chinese Dai medicine, it is used as an anodyne against cellulitis, abscesses, rheumatoid arthritis, injuries, protozoal infections, and diarrhea (10). Scientific studies have documented the traditional and ethnomedicinal uses of different parts of *B. ramiflora,* as well as its isolated chemical constituents. The stem of *B. ramiflora* contains bioactive polyphenols such as 4'-O-(6-O-vanilloyl)-β-D-glucopyranosyl tachioside D, 6'-O-vanilloylpicra-quassioside D, 6'-O-vanilloylicariside B5 (11). The leaves have been shown to contain polyphenols, flavonoids, proanthocyanidin, rosmarinic acid, 6'-O-vanilloylisotachioside, and 6'-O-vanilloyltachioside (12,13). Additionally, the roots, pericarp, fruit pulp, peel, and seeds contain a number of phytochemicals (14–17). With such a diverse range of phytochemicals, the extracts of different solvents of the whole plant of *B. ramiflora* possess a wide variety of biological activities, including antioxidant and anti-inflammatory, antibacterial, CNS depressing and sedative activities, insecticidal and anti-cancer activities of leaves and stem as well as the anthelmintic, hypoglycemic, and hypolipidemic activities of leaves and bark (15-18). The fruit of *B. ramiflora* has been reported to have a high nutritional composition of vitamin C, protein, calcium, potassium, and phosphorus. Also, it is rich in plant metabolites like flavonoids, tannins, alkaloids, saponins, and phenolic compounds (19-22).

A number of studies have reported that tannins, phenolic compounds, alkaloids, flavonoids, and saponins, particularly those extracted from plant parts like fruit peel and seed, possess antioxidant, anthelmintic, and analgesic efficacy (23-25). Therefore, the primary objective of the study was to investigate the anthelmintic and both central and peripheral analgesic activities of petroleum ether, chloroform, ethyl acetate, and acetone fractions from the crude methanolic extract of *B. ramiflora* seed and peel. To our knowledge, no such combined study has been performed yet with such different fractions of either seed or peel parts of *B. ramiflora* as used in the study, thus providing the rationale for conducting this research.

Materials and Methods

Drugs and chemicals used

The reagents and chemicals used in the study included Petroleum ether (60-80°C), Chloroform, Ethyl acetate, Acetone (Merck India ltd.); Methanol (Sigma Aldrich Ltd., Japan); 2,2-diphenyl-1-picrylhydrazyl (DPPH), Sodium chloride, Carboxymethylcellulose, and Tween-80. Analytical grade reagents were used and procured from the local chemical supplier company (Active Fine Chemicals Ltd, Bangladesh).

The drugs that were used as standard in this study were Albendazole oral suspension (Alben®, Eskayef Pharmaceuticals Ltd., Bangladesh) for the anthelmintic study and indomethacin capsule (Indomet®, Opsonin Pharma Bangladesh) for the analgesic activity assessment. Both drugs were purchased from a local pharmacy in Cumilla.

Collection and preparation of plant material

The fresh fruits of *B. ramiflora* Lour. were collected during the fruiting season from Narshingdi district, Bangladesh. The sample was identified and authenticated by the chief scientific officer, National Herbarium Bangladesh, Mirpur, Dhaka, and was retained at both the National Herbarium, Bangladesh, and the Department of Pharmacy, East West University, Bangladesh, under accession number DACB-46500 for future reference.

The seeds and peels were separated from the collected fruits, thoroughly washed, sun-dried for a couple of days, and finally in a hot air oven for 1 hour at 40 °C. The plant materials were milled and ground into a fine powder, yielding approximately 200 g of each type. After that, the seed and peel powders were separately subjected to maceration process for 15 days in methanol with random stirring and shaking. The resulting mixtures were filtered first through coarse filtration material (pieces of clean and white cotton cloth), then with Whatman® filter paper 1 (Merck, Germany). Solvent-solvent partitioning was carried out according to polarity order following the method instituted by Kupchan and developed by Van Wagnen and his team (26). Four types of solvents (Petroleum ether, chloroform, ethyl acetate, and acetone) of different polarities were chosen. Each partitioning was conducted with as many repetitions as required to ensure proper partitioning was achieved. The eluent was pooled and dried using a rotary evaporator. The dry weight of each extract was recorded as total yield. The fractionated

B. ramiflora seed extract of petroleum ether (BRSPE), chloroform (BRSCH), ethyl acetate (BRSEA), and acetone (BRSAC), and peel extract of petroleum ether (BRPPE), chloroform (BRPCH), ethyl acetate (BRPEA) and acetone (BRPAC) were kept at -4 °C in the refrigerator for further studies.

Animals used

For the anthelmintic experiment, the healthy adults of Indian earthworm, *Pheretima posthuma* (Nematoda), 3-5 cm long with a width of 0.2 cm, were used for its resemblance (both anatomical and physiological) with the intestinal worm-type parasites of human. From moist soil the earthworms were collected, and washed with water and saline solution to remove fecal matter.

For performing analgesic activity, 5 weeks old healthy Swiss albino mice (weighing ~16-23 g) were purchased from the Animal House facility of the Department of Pharmacy of the State University of Bangladesh and were transferred to the Laboratory Animal House facility of the Department of Pharmacy of Comilla University, Cumilla. The mice were kept in standard polycarbonate cages with autoclaved paddy husk as the bedding and stainless-steel wire as the covering. They were fed regular pellet food (Icddr,b, Bangladesh) with unlimited accessible water prior to and after the experimentation. The temperature and humidity were maintained at 25 ± 1 °C and 60 ± 5% respectively, with a 12-hour dark/light cycle. Before initiating, the *in vivo* assay protocols were approved by the Animal Ethics Committee of the State University of Bangladesh, Dhaka (2023-01-04/SUB/A-ERC/005). After completion of the experiments, the mice were sacrificed as humanely as possible with the intra-peritoneal administration of 150 mg/kg dose of Pentobarbital. The study was carried out ensuring strict maintenance of "The Guide for the Care and Use of Laboratory Animals" published by the "National Institute of Health (NIH publication 86-23, revised in 1985)" and in accordance with the guidelines of ARRIVE (Animal Research Reporting In Vivo Experiments). Additionally, specific institutional and national laws were followed where applicable throughout the experimentation, to meet the requirement of the Department of Pharmacy, Comilla University, Bangladesh.

Experiments

Anthelmintic activity

The peel and seed extracts were dissolved in water with 1% tween-80 and tested in various doses (25, 50, 100 mg/mL). Normal saline water with tween-80 was used as a control. Albendazole was used as the standard drug for the anthelmintic study at 10 mg/mL suspension dissolved in water.

Pheretima posthuma of uniform size were selected randomly and divided into nine groups (5 each). Prior to the experimentation, solutions of all the test compounds, along with the standard drug-albendazole, were freshly prepared. The earthworms were placed on petri dishes for every extract at room temperature. Time taken for paralysis of the earthworm was observed and noted, mainly when no movement was observed in the worms without physical stimulation. The times of death of worms were recorded after ensuring that worms did not move even when shaken vigorously. The outcomes of this assay were expressed as mean ± standard error of mean (SEM) values.

Analgesic activity

After a week of acclimatization, the acetic acid-induced writhing test and tail immersion test were performed on the healthy mice to observe both peripheral and central mechanisms of pain. The mice were randomly divided into groups (n = 5 in each group) and were fasted for 12 hours. To evaluate the activity, the animals of the test groups received test samples at the doses of 100 mg/kg and 200 mg/kg b.w (body weight), respectively. The standard group received indomethacin (10 mg/kg b.w.), and the control group was treated with 0.5% CMC (Carboxymethyl cellulose) solution PO in both tests.

Acetic acid-induced writhing test

The extracts, control, and standard drug were administered orally 30 minutes before intra-peritoneal administration of 0.1% acetic acid. After an interval of 5 minutes, the mice were observed for writhing, such as characteristic stretching or bending of the body, for the next 20 minutes. Dose-dependent inhibition was also measured. The percent inhibition (% analgesic activity) was calculated as Inhibition (%) = {(A–B)/A} × 100; where, A=Average number of writhing of the control group; B=Average number of writhing of the test group.

Tail immersion test

The test was conducted using the method developed by Olaleye and his colleagues (27). The tail of the mice was submerged to a constant level (3-4 cm) in a hot water bath maintained at 55 ± 0.5 °C temperature. The actual tail flicking responses of mice, i.e., the time taken in seconds to withdraw the tail from a hot water source (response time), were calculated, and the results were compared with the control and standard groups. The test termination time was set as 28 seconds to prevent thermal injury or tissue damage. The reaction time was measured at 0, 30, 60, and 90 minutes after treatment, respectively. A significant delay in the response time compared to the control group animals was considered a positive analgesic response.

Molecular docking analysis

Molecular docking analysis was performed for four trinoditerpenoids, which are baccaramiones-A, B, C and

D, isolated from *Baccaurea ramiflora* and reported by Chen and his team (28). These compounds were docked against cyclooxygenase-2 (cox-2) and tubulin. The crystal structures of cox-2 (PDB id: 5kir) (29) and tubulin (PDB id: 7pje) (30) were obtained from the protein data bank (RCSB-PDB) (31). Preparation of the target proteins and their energy minimization were done using PyMol (32), and Swiss PDB viewer (33), respectively. 3D structures of the standard drugs were downloaded from PubChem (34), and structures of target compounds were drawn using ChemDraw ultra (version 12.0.2). Energy minimization of ligands was done using Avogadro (35). AutoDock Vina (36) was used to study the protein-ligand interactions, and the visualization of interactions was done using BIOVIA Discovery Studio (37). Docking was done using a maximized search space to include the whole protein surface to compare the binding sites of standard and test compounds.

Statistical analysis

All the results were demonstrated as mean ± SEM and were analyzed by ANOVA, followed by Dunnett's multiple comparison tests with a single pooled variance. The ANOVA test was performed using GraphPad Prism version 7.0. The P value< 0.05 was considered to be statistically significant.

Results

Anthelminthic study

Different extracts of *B. ramiflora* seeds and peels showed significant anthelmintic activities in a dose-dependent manner compared to the control and similar to the reference drug Albendazole (Table 1), especially the seed fractions. Among different seed fractions, acetone extract (BRASAC) had recorded highest anthelmintic properties both in terms of paralysis (22.5 ± 1.32) and death (24.25 ± 1.93) at 100 mg/mL dose, which was an even more effective response than the standard drug, Albendazole (39.5 ± 1.527).

Different fractions of *B. ramiflora* peel showed lesser anthelmintic activity than those of *B. ramiflora* seed fractions. Significant effects were not observed in petroleum ether, chloroform, and ethyl acetate fractions compared to the standard drug, but ethyl acetate and acetone fractions exhibited good potency, especially peel acetone extract at 100 mg/mL concentration, which

Table 1. Anthelmintic activity of different solvent fractions of *Baccaurea ramiflora* seed and peel at variable doses

Sample	Fraction type	Dose (mg/mL)	Response against treatment	
			Time taken for paralysis (min)	Time taken for death (min)
	Control (Saline)	-	-	-
	Standard (Albendazole)	10	28.25 ± 1.54	39.5 ± 1.527
Seed fractions of *Baccaurea ramiflora*	Petroleum ether (BRSPE)	25	39.25 ± 1.75***	96 ± 2.94****
		50	28.75 ± 2.81	89 ± 5.26****
		100	23.25 ± 1.11	71 ± 2.198****
	Chloroform (BRSCH)	25	42.5 ± 2.90****	72.75 ± 2.01****
		50	28.25 ± 2.80	59.25 ± 5.26****
		100	24.5 ± 1.04	42.5 ± 1.55
	Ethyl acetate (BRSEA)	25	66.75 ± 1.03****	86.75 ± 1.****
		50	36.25 ± 1.37**	56.75 ± 1.37***
		100	25.5 ± 0.86	37.00 ± 1.08
	Acetone (BRSAC)	25	38.25 ± 0.85**	53.75 ± 3.70**
		50	29.75 ± 0.85**	43 ± 1.29
		100	22.5 ± 1.32****	24.25 ± 1.93***
Peel fractions of *Baccaurea ramiflora*	Petroleum ether (BRSPE)	25	173.5 ± 2.10****	247 ± 4.14****
		50	161.5 ± 3.23****	233.75 ± 1.75****
		100	152.25 ± 3.30****	223.75 ± 1.93****
	Chloroform (BRSCH)	25	88 ± 3.135****	163.75 ± 3.30****
		50	64 ± 2.857****	136.5 ± 1.55****
		100	52.75 ± 2.689****	119.25 ± 1.88****
	Ethyl acetate (BRSEA)	25	102.25 ± 1.38****	133.5 ± 1.55****
		50	36.75 ± 1.702	73.25 ± 1.75****
		100	25.5 ± 1.707	52.25 ± 3.22 **
	Acetone (BRSAC)	25	51 ± 1.29****	92.5 ± 1.93****
		50	34.5 ± 1.55	54.75 ± 1.25***
		100	14.5 ± 1.04**	30.25 ± 1.49

Values are mean ± SEM (n = 5 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to only saline treated group.

showed the lowest death time (30.25 minutes), closest to the death time of the group treated with Albendazole (39.5 minutes).

Overall, *B. ramiflora* seeds demonstrated comparatively higher anthelmintic activities than peel extracts, and acetone fraction of *B. ramiflora* seed showed the highest efficacy among all the samples.

Analgesic activity

Acetic acid induced writhing test

All fractions except ethyl acetate of *B. ramiflora* seed had comparable levels of inhibition in acetic acid-induced writhing test. Acetone, chloroform, and petroleum ether showed almost a similar percentage of inhibition (88.94%, 87.55%, and 87.5%, respectively) as the standard at 200 mg/kg (Table 2). The highest level of inhibition was observed in the acetone fraction (88.94%) as compared to the standard group.

Among peel extracts, ethyl acetate and acetone (200 mg/kg) showed notable inhibition (86.05% and 86.78%, respectively). The analgesic activity of *B. ramiflora* seed was comparatively greater than the peel extracts in a dose-dependent fashion.

Tail immersion test

The tail immersion test is designed to evaluate the central analgesic activity of test sample where sensation of pain is induced by heat. In the present study, responses observed at different doses and at different times are respectively shown in Figure 1 and Figure 2.

The maximum inhibition of thermal stimulus was all observed upon 60 and 90 minutes of extract administration, with the highest inhibition observed at 200 mg/kg dose in BRSCH (6.924 ± 0.264) and BRSEA (6.276 ± 0.157) as shown in Figure 1, and BRPEA (6.562 ± 0.157) in Figure 2 as compared to the standard group (7.278 ± 0.177). All the other fractions of the *B. ramiflora* peel and seed exhibited increased but comparable analgesic activity.

Molecular docking analysis

Molecular docking analysis was performed using PyRx among the four novel phytochemicals reported by Chen and colleagues (28) in maximized mode following a protocol documented for PyRx operation. The binding sites for standard and test compounds were compared. The structures of albendazole, indomethacin, and baccaramiones A, B, C, and D are shown in Figure 3.

The docking interactions of albendazole and baccaramiones-A, B, C, and D against tubulin (PDB ID-7PJE) are shown in Figure 4. Binding energy and binding interactions are summarized in Table 3.

From the binding interactions, it was found that albendazole and baccaramione A bind at the same chain (Chain A) of 7PJE whereas baccaramione B binds with chain C and baccaramiones C and D bind to chains C and D of 7PJE. Albendazole had the lowest binding affinity for the target protein as target for helminthiasis whereas

Table 2. Evaluation of peripheral analgesic activity of different fractions of *Baccaurea ramiflora* seed and peel extracts at variable doses

Treatment	Fraction	Dose (mg/kg)	Number of writhing	Inhibition (%)
Negative control (CMC)	-	-	41.6 ± 1.24899	0
Positive control (Indomethacin)	-	10	4.5± 0.41833	89.18
Seed fractions of *Baccaurea ramiflora*	Petroleum Ether (BRSPE)	100	9.4 ± 0.57879****	77.40
		200	5.2 ± 0.78421****	87.50
	Chloroform (BRSCH)	100	9.7 ± 0.75166****	76.68
		200	5.18 ± 0.56780*	87.55
	Ethyl acetate (BRSEA)	100	10.8 ± 0.64420****	74.04
		200	8.9 ± 0.29154***	78.60
	Acetone (BRSAC)	100	8.5 ± 1.22474**	79.57
		200	4.6 ± 0.53385	88.94
Peel fractions of *Baccaurea ramiflora*	Petroleum Ether (BRSPE)	100	9.7 ± 0.51478****	76.68
		200	7.7 ± 0.46368*	81.49
	Chloroform (BRSCH)	100	10.1 ± 0.50990****	75.72
		200	8.9 ± 0.67823***	78.60
	Ethyl acetate (BRSEA)	100	9.5 ± 0.65192****	77.16
		200	5.8 ± 0.75166	86.05
	Acetone (BRSAC)	100	10.4 ± 0.43011****	75.0
		200	5.5 ± 0.57008	86.78

Values are mean ± SEM (n = 5 in each group). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 compared to only saline treated group. CMC: Carboxymethyl cellulose.

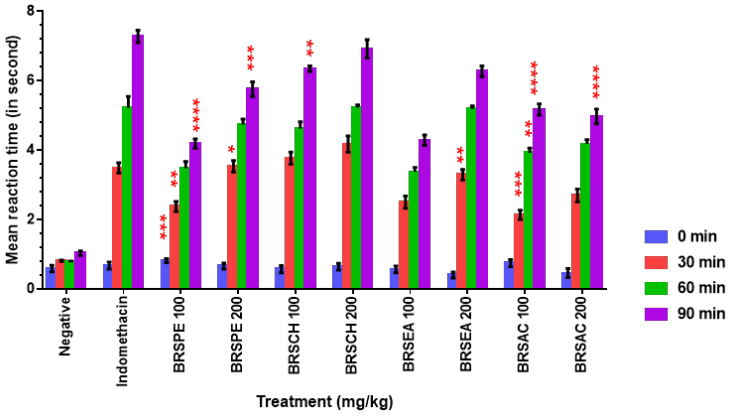

Figure 1. Analgesic effects of different solvent fractions of the *Baccaurea ramiflora* seed extract in the rat tail immersion test. Each value point represents the mean ± SEM of the reaction time (n = 5); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ significantly different from only vehicle (Carboxymethyl cellulose; CMC)-treated group. Negative control group received vehicle (CMC solution), positive control received Indomethacin 10 mg/kg body weight, tested animals were treated with 100 and 200 mg/kg body weight (p.o.) dose of *B. ramiflora* seed extract of petroleum ether (BRSPE), chloroform (BRSCH), ethyl acetate (BRSEA) and acetone (BRSAC).

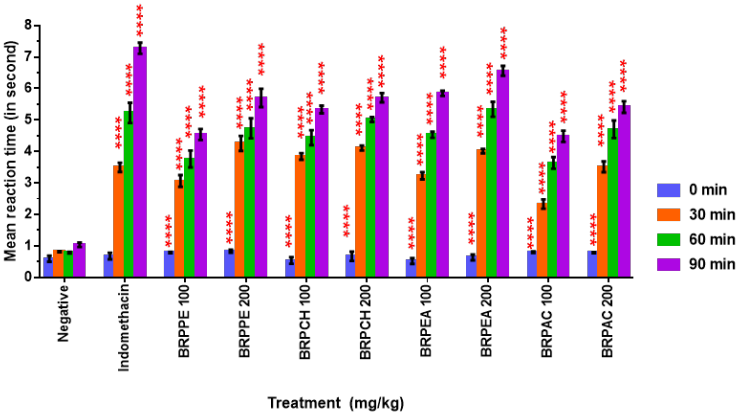

Figure 2. Analgesic effects of different solvent fractions of the *Baccaurea ramiflora* peel extract on rats in tail immersion test. Each value point represents the mean ± SEM of the reaction time (n = 5); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ significantly different from only vehicle (CMC solution) treated group. Negative control group received vehicle (Carboxymethyl cellulose; CMC)), positive control received Indomethacin 10 mg/kg body weight, tested animals were treated with 100 and 200 mg/kg body weight (p.o.) dose of *B. ramiflora* peel extract of petroleum ether (BRPPE), chloroform (BRPCH), ethyl acetate (BRPEA) and acetone (BRPAC).

baccaramione D showed maximum binding affinity. All four selected ligands form an H-bond with 7PJE but there was absence of an H-bond between albendazole and 7PJE.

The docking interactions of indomethacin and baccaramiones-A, B, C, and D with cyclooxygenase 2 enzyme crystallographic structure (PDB ID-5KIR) are shown in Figure 5 and relevant binding energy and binding interactions are summarized in Table 4.

All the ligands and indomethacin showed similar binding affinity towards 5KIR even though there were variations at the binding sites. Baccaramiones C and D were found to be binding at the same site as indomethacin and baccaramiones A and B bind to different sites of 5KIR. Except for baccaramiones A, all the ligands and the standard drug indomethacin were found to be forming hydrogen bond with the cyclooxygenase 2 protein in the molecular docking analysis.

Discussion

The present study evaluated the anthelmintic activity based on a previous investigation by Uddin et al, which revealed the qualitative presence of tannins in the seeds and peel of *B. ramiflora* fruit (38). Our experimentation revealed comparable anthelmintic efficacy of the different seed extracts to the standard drug, among which BRSAC showed efficacy higher than the standard drug. Moreover, the seed fractions demonstrated greater activity than peel fractions, establishing their superiority as potential anthelmintic development. Further *in vitro* and *in vivo* studies are essential to elucidate the phytochemical bioactive contents and their application as preventive/curative medicine and in new drug development.

Different classes of NSAID drugs are available for the treatment of central and peripheral analgesia, working by inhibiting pain mediators initiated by bradykinin,

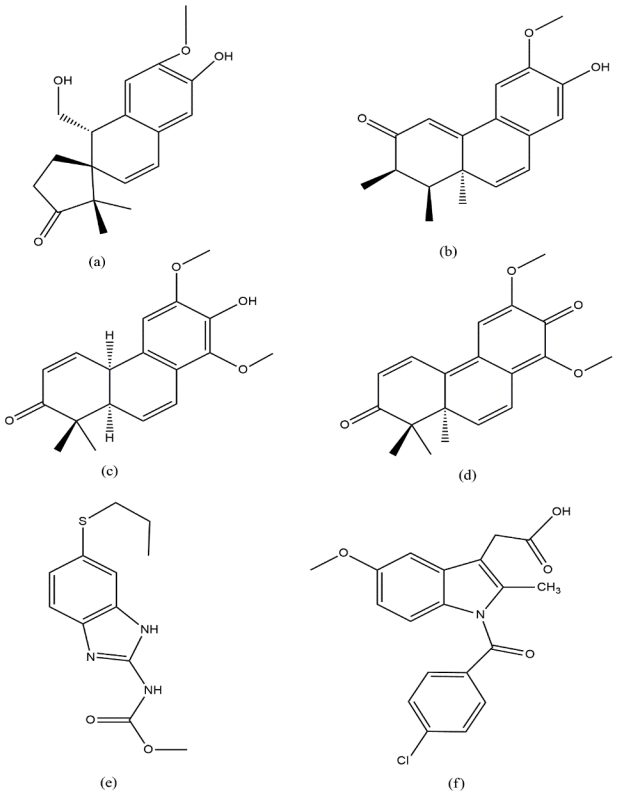

Figure 3. Chemical structures of a) baccaramione A, b) baccaramione B, c) baccaramione C, d) baccaramione D, e) albendazole, and f) indomethacin

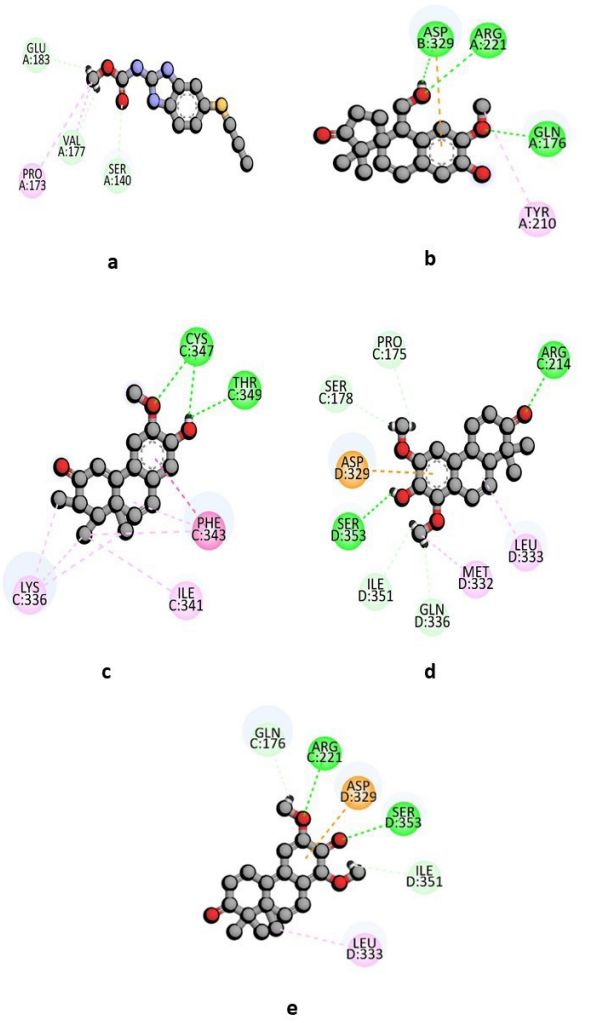

Figure 4. Interaction of tubulin with a) albendazole, b) baccaramione A c) baccaramione B, d) baccaramione C, and e) baccaramione D. Green: conventional hydrogen bond; pink-violet: hydrophobic; orange: pi-cation/ pi-anion; cyan: carbon-hydrogen bond

prostaglandin, leukotriene, histamine, TNF-α, etc. Several studies concluded that flavonoids, polyphenols, phenolic derivatives, and phenolic acids might provide analgesic and anti-inflammatory activities (39,40). The fruit juice of *B. ramiflora* has long been used as a painkiller in South Asia (41). Moreover, several investigations have revealed analgesic activities in different parts of the plant and fruit of *B. ramiflora* (9,38,42). Based on these studies, we performed an acetic acid-induced writhing test (chemical model) for peripheral analgesic activity and a tail immersion test (thermal model) for central analgesic activity compared to the standard drug. Indomethacin/*B. ramiflora* significantly reduced abdominal contractions in a dose-dependent manner. Various seed fractions displayed a greater level of inhibition than the peel fractions at 200 mg/kg. However, the acetone extract proved to be effective in both peel and seed samples (86.78% and 88.94%, respectively). Compounds with more than 70% inhibition are considered to have good analgesic activity (43). Thus, it can be concluded that the acetone extracts of seed and peel sample possess promising analgesic activities as compared to standard. In the tail immersion test, we observed a significant reduction of thermally induced hyperalgesia by all extracts in a dose-dependent manner. The highest activity, comparable to the standard drug, was observed in seed chloroform, ethyl acetate, and peel ethyl acetate extracts.

Overall, different seed extracts exhibited more potency in terms of anthelmintic and analgesic activity. The seed acetone extract showed promising activity against helminthiasis and peripheral neuropathy, whereas the seed chloroform extract was the most effective as a central analgesic. However, more elaborative studies should be designed to identify and isolate the precise active components and discover the accurate mechanism

Table 3. Summary of binding affinity and binding interaction of tubulin (PDB ID: 7PJE) with selected ligands and albendazole

Ligand	Binding affinity (kcal/mol)	Residues involved in hydrogen bond
Indomethacin	-6.7	-
Baccaramione A	-7.4	GLN176A, ARG221A, ASP329B
Baccaramione B	-7.5	CYS347C, THR349C
Baccaramione C	-8.1	ARG214C, SER353D
Baccaramione D	-8.2	ARG221C, SER353D

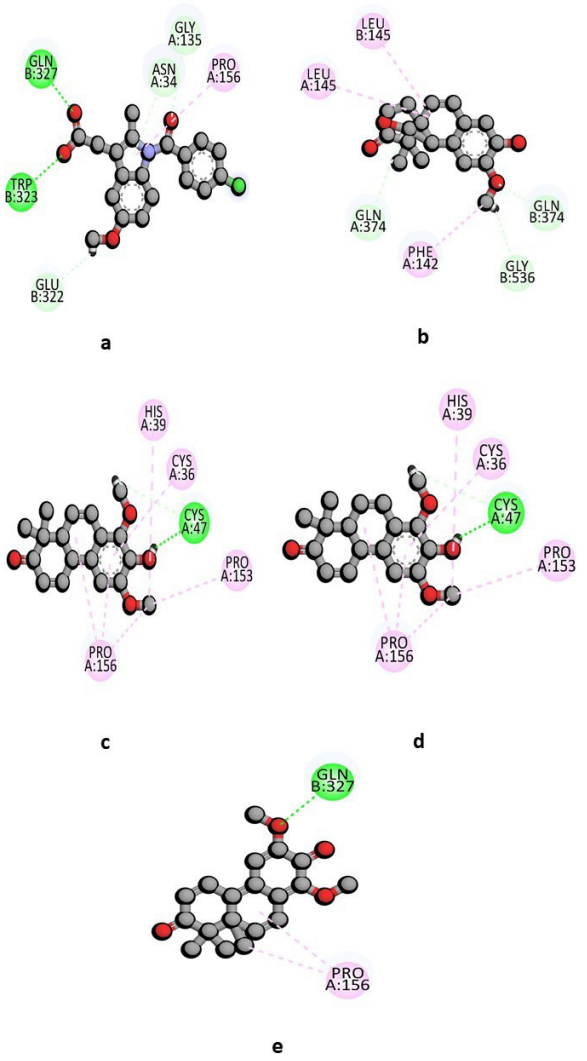

Figure 5. Interaction between cyclooxygenase-2 with a) indomethacin, b) baccaramione A, c) baccaramione B, d) baccaramione C and e) baccaramione D. Green: conventional hydrogen bond; pink-violet: hydrophobic; cyan: carbon-hydrogen bond.

of peripheral and central analgesic action of the sample fractions of this study.

Later, to validate the findings of anti-inflammatory and anthelmintic assay and to develop an understanding of the underlying molecular mechanism of the given activities, molecular docking analysis was performed against selected targets, i.e., cyclooxygenase 2 or COX-2 enzyme (PDB ID: 5KIR) and tubulin (PDB ID: 7PJE). COX-2 and tubulin were selected to evaluate the anti-inflammatory and anthelmintic potentials of selected ligands, respectively (44,45). The focus of the molecular docking analysis was to determine whether the standard and selected ligands bind to the same site on the target proteins. Hence, the molecular docking was performed using maximized mode in PyRx (46). From the 2D target protein-ligand interactions, it was found that in the case of tubulin, baccaramione A and albendazole bind at the same chain but at different sites. However, the former has a higher binding affinity (Figure 4). Baccaramione A forms three hydrogen bonds with tubulin involving Gln176A, Arg221A and Asp329B residues. No hydrogen bond was seen in the case of interaction between albendazole and tubulin, which explains the higher binding affinity values for baccaramione A than albendazole towards tubulin. Among all the baccaramiones, baccaramione D showed the highest binding affinity towards tubulin with a binding affinity value of -8.4 kcal/mol; the binding affinity values for all four baccaramiones were higher than albendazole itself. Previously, an *in silico* study was carried out using different medicinal plants, which were known to possess anthelmintic activity against tubulin. From that study, it was found that compounds limonoids, which have known anthelmintic activities, showed a binding affinity of -8.9 kcal/mol. The ligands analyzed in this study, baccaramiones C and D showed binding affinities close to that of limonoids, which indicated the potential anthelmintic activity of *B. ramiflora* (47). Analgesic activity of *B. ramiflora* was well documented in a previous study, though the findings were not validated through molecular docking studies to estimate the tentative pain-reliving mechanism (9). Thus, the current work aimed at investigating the binding patterns of the isolated compounds to understand the molecular basis of analgesic activity the different solvent fractions of *B. ramiflora* seed and peel. In the case of COX-2 (Figure 5), baccaramiones C and D bind to the same place as indomethacin. Indomethacin and baccaramiones A, B, C and D exhibited almost equal binding affinities *in silico*, ranging between -8.1 kcal/mol and -8.4 kcal/mol. Baccaramione D had the highest binding affinity towards COX-2 (-8.4 kcal/mol). both indomethacin and baccaramione D formed a hydrogen bond with Gln327B residue. Other residues that participated in the interaction between indomethacin, baccaramiones C and D involved Trp323B, Gly135A, Asn34A, Pro156A, His39A, Cys36A, Cys47A, and Pro153A. On the other hand, baccaramiones A and B bind to the same site of COX-2, which is different from the binding site of indomethacin. Here, the actively involved residues included Gln374B, Gly536B, Phe142A, Gln374A, Leu145A, and Leu145B for baccaramiones

Table 4. Summary of binding affinity and binding interaction of cyclooxygenase-2 enzyme (PDB ID:5KIR) with selected ligands and Indomethacin

Ligand	Binding affinity (kcal/mol)	H-bond
Indomethacin	-8.3	TRP323B, GLN327B
Baccaramione A	-8.1	-
Baccaramione B	-8.2	GLY225B, HIS226B, ARG376A
Baccaramione C	-8.3	CYS47A
Baccaramione D	-8.4	GLN327B

A and Arg376A, Gly225B, His226B, Val538B, Phe142A, Gly356B, Leu145, Leu145B, and Asn375B for baccaramione B. In the case of tubulin, albendazole did not form any H-bond with tubulin, but all four ligands formed H-bonds with tubulin, which may be responsible for their higher binding affinities (48). From the binding affinities, it can be concluded that all four ligands have comparable affinities for the respective target proteins, and in both cases, some ligands bound to the same or at least somewhat at a near site to the binding site of the standards. Although *in silico* studies shed some light on the probable mechanism of the obtained activities, further research is needed to develop a proper understanding of the molecular mechanism underlying the analgesic and anthelmintic activity of *B. ramiflora* (Lour.).

Conclusion

The outcomes of this study clearly demonstrated the significant potency and efficacy of different fractions of *B. ramiflora* fruit seed and peel as anthelmintic and analgesic means. Moreover, this study provided an ethnomedicinal and pharmacological rationale for the traditional use of different parts of this plant as natural remedy of various pathological conditions. The extracts are expected to have high content of phytochemical(s) that might contribute for the isolation of bioactive compound and novel lead compound for drug development. Hence, we strongly suggest for further investigations on the isolation and evaluation of isolated bioactive chemical constituents through *in vitro*, *in vivo,* and *in silico* approach.

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Conflict of interests

The authors declare no conflict of interest

Ethical considerations

Animal Ethics Committee of State University of Bangladesh approved the protocols for *in vivo* experiments performed on laboratory animals (2023-01-04/SUB/A-ERC/005). This research did not include any human subjects.

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