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Pharmacological Activities of *Blumea lacera* (Burm. f) DC: A Medicinal Plant of Bangladesh

Abul Khair[1], Mohammed Ibrahim[1], Qamrul Ahsan[1], Zilly Homa[1], Md. Ruhul Kuddus[2], Ridwan Bin Rashid[3] and Mohammad Abdur Rashid[2*]

[1]Department of Pharmacy, Southern University Bangladesh, Chittagong, Bangladesh.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[3]Department of Microbiology, University of Dhaka, Dhaka-1000, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MI and QA designed the study, performed the statistical analysis. Authors MRK and MAR wrote the protocol and wrote the first draft of the manuscript. Authors AK and MI managed the analyses of the study. Author RBR managed the literature searches. Author MAR revised manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The crude methanol extract of whole plant of *Blumea lacera* (Burn.f.) DC. has been investigated for anti-diarrheal, antimicrobial, anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities.
Place and Duration of Study: The study was carried out in 2013 in the Department of Pharmacy, Southern University Bangladesh, Chittagong, Bangladesh.
Methodology: Test for anti-diarrheal activity was carried out by castor oil-induced diarrhea in mice. The preliminary antimicrobial activity was determined by the agar disc diffusion method. The anxiolytic activity was examined in mice by using the hole board test and open field test (OFT). The anti-atherothrombosis activity was evaluated using standard streptokinase. The membrane stabilizing activity was assessed by using hypotonic solution induced hemolysis of human erythrocyte. The plant extract was also assessed for anti-diabetic ability using *In vitro* α-amylase inhibitory potential. The α-amylase inhibitory activity of *B. lacera* was measured using the starch-iodine method.

**Corresponding author: Email: rashidma@du.ac.bd;*

Results: The crude extract of *B. lacera* showed anti-diarrheal activity in dose-dependent manner. In antimicrobial assay, this extract showed better activity against the tested fungi compared to the bacteria used in the screening. Significant anxiolytic activity was found for this plant extract. In the *In vitro* anti-atherothrombosis test, the extract exhibited 46.17% clot lysis as compared to the standard, streptokinase (81.53%). In membrane stabilizing activity test, the plant extract at 1.0mg/ml inhibited the heat-induced hemolysis of RBCs by 52.27% whereas the standard acetyl salicylic acid (ASA) demonstrated 81.72% inhibition of hemolysis. Our results revealed that the extract had dose dependent prevention of digestion of carbohydrates by inhibiting α-amylase. The ability of *B. lacera* to inhibit thermal-and hypotonic-enzyme activity was found to be statistically significant (p=0.05).
Conclusion: These results demonstrated that *B. lacera* may be used in pharmaceutical applications because of its effective pharmacological properties.

Keywords: Blumea lacera; anti-diarrheal; antimicrobial; anxiolytic; anti-atherothrombosis; membrane stabilizing; alpha-amylase.

1. INTRODUCTION

The use of natural products or natural product-based medicine is increasing all over the world especially in the developing countries such as Bangladesh, India, China, and the Middle East. About 25% of the prescribed drugs in the world are of plant origin [1]. Approximately 80% people rely on traditional plant-based drugs for their primary health care needs in the developing countries [2]. Since ancient times, different parts of medicinal plants have been used for ailments caused by microorganisms. There is wide range of medicinal plant parts possessing a variety of pharmacological activities; such are used as powerful raw drug. Recent widespread interest in plant-derived drugs reflect its recognition of the validity of many traditional claims regarding the values of natural products in health care [3]. For quality control of traditional medicines, phytochemical screenings are mainly applied. Now a days, secondary plant metabolites with previously unknown pharmacological activities have been extensively investigated as source of medicinal agents. Thus, it is anticipated that phytochemicals with enough antibacterial efficacy will be used for the treatment of bacterial infections [4]. According to WHO, medicinal plants are the best sources to obtain a variety of new herbal drugs. Therefore, in order to determine the potential use of herbal medicine, it is important to emphasize the study of medicinal plants that found in folklore [5].

Blumea lacera (Burn.f.) DC. (Bengali name: Kukursunga; Family-Asteraceae) is an erect herb which grows as a weed in uncultivated lands all over Bangladesh. The alcoholic extract of the herb exhibited marked anti-inflammatory activity against carrageenin- and bradykinin-induced inflammation in rats. Essential oil from leaves have analgesic, hypothermic and tranquillizing activities [6]. The plant also exhibited anti-leukemic, antiviral [7] and cytotoxic [8] activities against breast cancer cells.

As part of our ongoing efforts to study medicinal plants of Bangladesh [9-11], we evaluated the anti-diarrheal, antimicrobial, anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities of *B. lacera* as well as to find out the logical evidence for its folk uses of this plant as described in Ayurveda. Although in an earlier experiment, the ethanol extract of root of *B. lacrera* has been reported to exhibit the anti-diarrheal activity

[12], but the present study was designed to evaluate the pharmacological activities of crude methanol extract of whole plant of this plant.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Leaves of *B. lacera* were collected from Chittagong Pahartoli, Bangladesh in June 2012 and were identified at the Forest Research Institute, Chittagong, Bangladesh where a voucher specimen has been maintained for future reference.

2.1.1 Drying and grinding

After collection, the leaves were washed with running tap water. These clean leaves were dried at a temperature not exceeding 50ºC. The dry materials were ground to a coarse powder with the help of a grinder and kept in an airtight container. The container was then stored in a cool and dark place until extraction was commenced.

2.1.2 Hot extraction by soxhlet extractor

Exactly 140gm of powdered leaf was extracted with 750ml of methanol (99.98%) with a Soxhlet apparatus (Quickfit, England). The extract was concentrated with a rotary evaporator (Heidolph, Germany) under reduced temperature and pressure to provide a gummy residue (yield 18.70%).

2.1.3 Chemicals

All chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as loperamide, ciprofloxacin, fluconazole, diazepam, acetyl salicylic acid and acarbose were obtained from Square Pharmaceuticals Ltd as gift samples.

2.1.4 Experimental animals

For the experiment *Swiss albino* mice of either sex, 6-7 weeks of age, weighing between 25-30g, were collected from the Animal Resources Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). The mice were maintained under standard environmental conditions of temperature: (27.0±1.0ºC), relative humidity: 55-65% and 12h light/12hr dark cycle and had free access to ICDDR,B formulated diet and water *ad libitum*. Appropriate measures were taken to minimize the pain or discomfort of animals and the mice were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee [13].

2.2 Test for Anti-diarrheal Activity

Anti-diarrheal activity of crude methanol extract of *B. lacera* was assessed by castor oil-induced diarrhea in mice [14]. The animals were divided into control, positive control and two test groups containing seven mice in each. Control group received 1% Tween-80 (10ml/kg, p.o). The positive control group received loperamide (25mg/kg, p.o.) while the test groups

received the methanol extract (100 and 200mg/kg b.w.) orally. Acute diarrhea was produced by oral administration of 0.4ml of castor oil in each mouse. The latency period and diarrheic secretion were counted for 4 hours.

2.3 Test for Antimicrobial Activity

The preliminary antimicrobial activity of the extractive was determined at 500µg/disc by the agar disc diffusion method [15] against a number of Gram-positive and Gram-negative bacteria and fungi. The bacterial and fungal strains used in this experiment were collected from the Microbiology Lab., Chittagong University, Chittagong, Bangladesh. Standard ciprofloxacin (30µg) and fluconazole (50µg) disc were used as reference.

2.4 Test for Anxiolytic Activity

2.4.1 Treatment schedule

The anxiolytic activity of *B. lacera* was examined by using the hole board test and open field test (OFT). The animals were divided in to four groups, with each group consisting of seven mice. First group received normal saline, second group received diazepam (1mg/kg b.w., p.o.), while the third and fourth groups received plant extract at 200 and 400mg/kg b.w. respectively.

2.4.2 Hole board test

The hole board is a white painted wooden board (30cm×20cm×14cm) with 16 holes (each of diameter 3cm) evenly distributed on the base of box. The test groups orally received crude extract of *B. lacera* at the dose of 200 and 400mg/kg b.w. respectively. The control group received saline and positive control received diazepam (2mg/kg-i.p.) respectively. The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 5min at 30min after oral administration of both doses of the test drug [16].

2.4.3 Open field test

The open field test is used to observe general motor activity, exploratory behavior and measures of anxiety. The open field area was made of plain wood and consisted of a square area (45cm×45cm×20cm). The floor had a square sheet of wood (45cm×45cm) with the surface divided into sixteen small squares. Mice were divided into four groups of 7 mice and treated similarly as described in hole cross test. About 30min after treatment, mice of both the control and treated groups were placed individually at the center of the open field and behavioral activities were recorded for 5min. Subsequently, hand operated counters and stopwatches were used to score the following behavioral parameters for a period of 5min: (1) the number of entries and time spent in the centre, (2) periphery and corners of the field, (3) the number of crossings (number of square floor units entered) as a measure of distance traveled, (4) rearing (number of times the animal stood on hind legs) and (5) assisted rearing (forepaws touching the walls of the apparatus) [16].

2.5 Test for Anti-atherothrombosis Activity

The anti-atherothrombosis activity of the crude extract was evaluated using streptokinase as standard [17]. For this study, 4ml venous blood was drawn from healthy volunteers and distributed in three (for extract, reference standard and for negative control) pre-weighed sterile micro-centrifuge tubes (0.5ml/tube). The tubes were incubated at 37ºC for 45min. After clot formation, serum was completely removed without disturbing the clot and each tube was weighed again to determine the weight of clot (clot weight=weight of clot containing tube–weight of tube alone). Then, 100µl of methanol extract at a dose of 5µg/µl, 100µl of streptokinase and 100µl of methanol were separately added to the pre-marked tubes containing the clot. The tubes were then incubated at 37ºC for 90min and observed for clot lysis. Afterwards, the fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated for three times in different days with fresh blood samples collected from 10 healthy volunteers (male and female) having no history of contraceptives and anticoagulants.

2.6 Test for Membrane Stabilizing Activity

For this experiment, three clean centrifuge tubes were taken for positive control (acetyl salicylic acid), three for negative control (99.8% methanol) and six for crude methanol extract. One milliliter of 10% RBC suspension was added to each tube. Then 1.0ml methanol and 1.0ml acetyl salicylic acid were added to the negative control and positive control tubes, respectively. On the other hand, for the test group, 1.0ml of methanol extract (1000mg/kg) was added. The pH (7.4±0.2) of the reaction mixtures was adjusted by phosphate buffer. The tubes were incubated in water bath and after cooling, these were centrifuged at 2500rpm for 5min. After filtration the absorbance of the supernatants were measured at 556nm. The total inhibition of hemolysis was then calculated by determining the % inhibition of hemolysis [18].

2.7 Test For Alpha-amylase Inhibitory Activity

The α-amylase inhibitory activity of *B. lacera* was measured using the starch-iodine method [19]. Twenty microliter of α-amylase solution (0.030mg/ml) was mixed with 1.3ml of Tris-HCl buffer (0.01M containing 0.006M NaCl, pH 6.8) and the crude extract (80, 160, 320µl). After incubation at 37ºC for 20min, 100µl of the starch solution (0.1%) was added, and the mixture re-incubated for 20min, after which 2ml of 0.01% acidic iodine solution was added. The absorbance of the sample was measured at 565 nm and percentage inhibition was calculated as % Inhibition of enzyme activity =(A-C)×100/ (B-C) where, A=absorbance of the sample, B=absorbance of blank (no extract), and C=absorbance of control (no starch).

2.8 Statistical Analysis

Results are expressed as the mean±SEM (SEM=Standard Error of Mean). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group; p=0.05 was considered as statistical significant.

3. RESULTS

3.1 Anti-diarrheal activity

In the castor oil-induced diarrheal experiment in mice, the crude methanol extract of *B. lacera* at 100 and 200mg/kg b.w. significantly reduced the total number of episodes of defecation as well as delayed the onset of diarrhea in a dose dependent manner Table 1. These results were shown to be statistically significant (p=0.05).

3.2 Antimicrobial Activity

In the anti-bacterial screening by disc diffusion method, the zones of inhibition were found within the range of 9.0 to 14.0mm. The highest zone of inhibition (14.0mm) was observed against *Shigella dysenteriae* Table 2. But the plant extract also showed mild antibacterial activity against *Salmonella* Typhi*, Bacillus cereus, Staphylococcus aureus*. During the anti-fungal test, the zones of inhibition were found within the range of 11.0 to 18.17mm with the highest zone (18.17mm) observed for *Blastomyces dermatitidis* Table 2.

3.3 Anxiolytic Activity

3.3.1 Hole board test

The number of line crossings was found to increase significantly in case of diazepam treated animals as compared to control animals. The plant extracts at the 200 and 400mg/kg b.w. (p.o) dose showed significant increase in the number of line crossing as compared to control animals as shown in Table 3.

3.3.2 Open field test

Significant anxiolytic activity was observed for diazepam as well as plant extracts when compared to control. In the open field test, administration of plant extract in mice showed significant increase in the number of squares crossed during 5min intervals of test as compared with control as show in Table 3.

3.4 Anti-atherothrombosis Activity

In the anti-atherothrombosis activity test, streptokinase, a positive control (30,000IU) showed 81.53% clot lysis. On the other hand, clots when treated with 100µl methanol (negative control) showed only negligible lysis (2.87%). In the same time, by treating clots with 500µl (µg/ml) of the extract, 46.17% clot lysis was obtained. Statistical representation of the effective clot lysis percentage has been shown in Table 4.

3.5 Membrane Stabilizing Activity

The extract at 500, 1000µg/ml inhibited the heat induced hemolysis of RBCs by 35.90, 52.27%, respectively whereas standard acetyl salicylic acid showed 71.36% inhibition of hemolysis Table 4. The stabilization of cell membrane for crude methanol extract was found to be moderate. Although the precise mechanism of this membrane stabilization is yet to be elucidated, it is thought that the plant might inhibit the release of lysosomal content of neutrophils at the site of inflammation.

Table 1. Effect of crude methanol extract of *B. lacera* on castor oil-induced diarrhea in mice

Test groups	Mean latent period (min)	% defecation	% Inhibition of defecation	TNF (240min)
Control	27.67	100	0	68±1.78
Loperamide (3mg/kg)	73.67	19.12	80.88	13±0.41
BLME (200mg/kg)	58.26	47.06	52.94	46.98±0.81
BLME (100mg/kg)	44.0	69.12	30.88	31.98±0.41

*TNF=Total number of feces; Values are mean±SEM (n=7); **p=0.05 by Dunnett's T test for values between the sample and vehicle treated group; SEM=Standard Error of Mean; BLME=B. lacera methanol extract*

Table 2. Antimicrobial activity of crude methanol extract of *B. lacera* at 500µg/disc

Microorganisms	Zone of inhibition (MZI±SD) mm	
	BLME (500µg/disc)	Standard (30µg/disc)
Gram positive bacteria ciprofloxacin		
Bacillus cereus	11.66±0.68	15.33±0.41
B. megaterium	10.66±0.40	20.67±0.41
B. subtilis	11.33±0.82	15.00±0.71
Staphylococcus aureus	11.67±0.41	18.00±1.41
Gram negative bacteria		
Escherichia coli	9.0±0.70	16.67±0.41
Pseudomonas aeruginosa	9.66±0.40	17.00±1.22
Salmonella Typhi	13.66±1.08	15.67±0.41
Shigella dysenteriae	14.0±0.71	15.33±0.82
Fungi fluconazole		
Aspergillus niger	12.62±0.41	16.5±0.35
Blastomyces dermatitidis	18.17±1.74	19.67±0.54
Cryptococcus neoformans	15.16±1.816	18.5±1.06
Protozoa fluconazole		
Plasmodium ovale	11.00±0.70	15.33±0.74

MZI: Mean zone of inhibition (mm); zone of inhibition under 7mm were considered as less active and were discarded. Values are expressed as mean±SD (n=3). Means with different letters are significantly different (p=0.05). BLME=B. lacera methanol extract

Table 3. Anxiolytic effect of crude methanol extract of *B. lacera* in mice by hole cross test and open field test

Treatment	Dose (mg/kg)	Hole cross test		Open field test	
		Number of hole crossing	% Inhibition of hole cross	Number of square crossed	% Inhibition of square cross
Saline	1ml	25.67±1.08	0.0	251.67±2.94	0.0
Diazepam	1	3.33±0.71	87.01	39.67±2.54	84.24
BLME	200	8.67±0.41	66.23	118.33±1.42	32.67
BLME	400	6.0±0.41	76.62	67.31±1.78	52.99

All values are mean±SEM (n=7). SEM=Standard Error of Mean; BLME=B. lacera methanol extract

3.6 Alpha-amylase Inhibitory Activity

The extract of *B. lacera* displayed concentration dependent inhibitory effect on the starch breakdown *In vitro* as shown in Table 5. In the present study, the crude extract showed 29.82%, 46.49%, 63.15% inhibition of α-amylase enzyme activity at 80, 180 and 320µg/ml, respectively whereas the standard acarbose (50µg/ml) produced 79.80% inhibition. The ability of *B. lacera* to inhibit and hypotonic and thermal enzyme activity was found to be statistically significant (p=0.05).

Table 4. Anti-atherothrombosis and membrane stabilization activity of crude methanol extract of *B. lacera*

Test groups	Anti-atherothrombosis activity	Membrane stabilizing activity
	% Clot lysis	Total inhibition of hemolysis
Control	2.87±0.94	00.00±0.018
Streptokinase (positive control)	81.53±3.7	ND
Positive control (ASA, 0.1mg/ml)	ND	71.36±0.021*
BLME (1.0mg/ml)	ND	52.27±0.0212*
BLME (0.5mg/ml)	46.17±3.7	35.90±0.01414*

*Values are mean±SEM,*p= 0.05; ND=Not determined; SEM=Standard Error of Mean; BLME=B. lacera methanol extract*

Table 5. Inhibition (%) of amylase activities of crude methanol extract of *B. lacera*

Test group	Total inhibition of α-amylase activity
Control (DW)	0
Positive Control (50µg/ml)	79.80±0.00141*
BLME (320µg/ml)	63.15±0.0014*
BLME (160µg/ml)	46.49±0.00147*
BLME (80µg/ml)	29.82±0.00147*

*DW=Distilled water, Values are mean±SEM,*p=0.05; SEM=Standard Error of Mean; BLME=B. lacera methanol extract*

4. DISCUSSION

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, which is accompanied by an excess loss of fluid in the faeces. In some types of diarrhea, the secretory component predominates, while other types of diarrhea are characterized by hyper motility. Castor oil causes diarrhea due to its active metabolite, ricilonic acid [20], which stimulates the peristaltic activity in the small intestine, leading to the changes in the electrolyte permeability of the intestinal mucosa. Its action stimulates the release of endogenous prostaglandins [21]. The results of the present study show that there has been a statistically significant reduction in the incident and severity of diarrhea with the crude extract of *B. lacera* in experimental animals. The plant extract at 100, 200mg/kg b.w. doses significantly lowered several typical parameters of diarrhea. Further studies are required to confirm the underlying mechanism of the observed activity of the plants.

Results obtained from the present study revealed that the extract exhibited a relatively high inhibitory activity against the tested fungal strains, with *Blastomyces dermatitidis* being the most sensitive fungus. Amongst Gram-positive bacteria, *B. cereus* and *Staphylococcus aureus* were the most sensitive (widest inhibition zones), whereas *Shigella dysenteriae* was the most sensitive Gram-negative bacteria. The antibacterial activity may be due to the presence of flavone type compounds in this plant [22].

The adrenergic and dopaminergic system have been shown to play a role in anxiety. Benzodiazepine have been extensively, used for the last 40 years to treat several forms of anxiety, but due to their unwanted side effects, alternative treatment strategies were sought with favorable side effect profiles. Medicinal plants are a good source to find new remedies for these disorders. Despite the wide spread traditional use of *B. lacera* for treating various disorders, there are no reports of scientific evaluation of its anxiolytic activity. The present work demonstrates that *B. lacera* extract had anxiolytic activity in mice as evident by open field and hole cross models [23].

The red blood cell stability test is based on the result that a number of non-steroidal anti-inflammatory agents inhibit heat-induced rupture of erythrocytes, most probably by stabilizing the membrane of the cell. The erythrocyte membrane may be considered as a model of the lysosomal membrane. Agents that can prevent the rupture of the latter, and thereby prevent damage to the tissue caused by the release of the hydrolytic enzymes contained within the lysosome maybe expected to improve some symptoms of inflammation. It has been demonstrated that certain herbal preparations were capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity [24].

The anti-diabetic activity of medicinal plants could be evaluated using several methods; *in vitro* α-amylase inhibitory assay is one of such techniques. The extract of *B. lacera* inhibited α-amylase but its activity was significantly less than that of positive control acarbose ($p=0.05$). The inhibitory effect of methanol extract was comparable to than that of the positive control, indicating that the α-amylase inhibitory ability resides in this extract. Alpha-amylase is an enzyme responsible for breaking down of α-1,4-glycosidic bonds in starch. Therefore, the enzyme increases the availability of glucose in the blood. *B. lacera* extract could be useful in post-prandial hyperglycemia by reducing the hydrolysis of carbohydrates. The observed activity may be due to the presence of chemical constituents such as phenolic compounds (tannins and flavonoids) and terpenoids in the extract [25,26]. Phenolics have been reported to inhibit α-amylase activities. They also have anti-hyperglycemic activity and inhibit the development of diabetes [27,28].

5. CONCLUSION

These primary findings suggest the presence of bioactive secondary metabolites in this plant extract that are responsible for anti-diarrheal, antimicrobial, anxiolytic, anti-atherothrombosis, membrane stabilizing and alpha-amylase inhibitory activities.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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