Preliminary Phytochemical Screenings and Pharmacological Activities of *Blumea lacera* (Burn.f.) DC.

Md. Abul Khair[1], Mohammed Ibrahim[1], Qamrul Ahsan[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

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ABSTRACT: The methanol extract of the whole plant of *Blumea lacera* (Burn.f.) DC. (BLME) has been subjected to preliminary screenings for phytoconstituents and antipyretic, analgesic and anti-inflammatory activities. Antipyretic activity was assessed by the yeast-induced hyperthermia in mice. The analgesic property was evaluated by formalin-induced writhing test. Acetyl salicylic acid (ASA) was used as standard for *in-vitro* anti-inflammatory activity test. In yeast-induced pyrexia, the crude extract demonstrated a significant ($p=0.05$) reduction in body temperature of mice after elevation by the administration of yeast. These effects were pronounced at the 2nd and 3rd h of post-treatment with the extract. BLME exhibited a dose-dependent analgesic activity with 39.13% and 56.52% protection at 200-and 400-mg/kg, b.w., respectively as compared to 76.09% revealed by the standard diclofenac sodium. In the anti-inflammatory test, the crude extract at 400 µg/ml displayed 62.40% inhibition of protein denaturation whereas standard acetyl salicylic acid exhibited 76.74% inhibition. Results of the preliminary phytochemical screenings demonstrated the presence of alkaloids, flavonoids and triterpenoids in the extract.

Key words: *Blumea lacera*, phytochemical, antipyretic, analgesic, anti-inflammatory

INTRODUCTION

Plants are known to produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. The varieties of molecules in medicinal plants have proved to combat complicated diseases. Based on this, natural product scientists have always focused on the isolation of bioactive molecules from these precarious herbs and trees. Bangladesh being a subtropical country is a good repository of plants. The total medicinal plant market of Bangladesh is equivalent to US$ 14 billion each year.[1]

Correspondence to: Mohammad A. Rashid
Tel.: 880-2-9661900-73, Extn.- 8137, 8132; Fax: 880-2-8615583;
E-mail: rashidma@du.ac.bd

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 properties.[2] The plant also exhibited anti-leukemic, antiviral[3] and cytotoxic[4] activities against breast cancer cells. Phytochemical investigation of the plant has shown the presence of small amounts of acetylinic compounds, a thiophene derivative, a diester, campesterol, triterpenoid, prenylated phenol glycosides, monoterpene glycoside and flavonoids.[5-7] This plant has been reported to show depressant effect on central nervous system[12] along with insecticidal activities.[8-9]

As part of our continuing studies on medicinal plants of Bangladesh[10-12], we evaluated the phytochemical screenings and antipyretic, analgesic and anti-inflammatory activities of *B. lacera* as well as to find out the logical evidence for its folk uses.

MATERIALS AND METHODS

Plant material. 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.

Drying and grinding. After collection, the leaves were washed with running tap water. These clean leaves were first dried at a temperature not exceeding 40ºC in an oven. The dry materials were ground to a coarse powder with the help of a grinding machine and kept in an airtight container until extraction was commenced.

Hot extraction. Exactly 140 g of powdered leaf was extracted with 750 ml 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%).

Chemicals. All chemicals and solvents used in this study were of analytical grade and purchased from Merck, Germany. Standard drugs such as paracetamol, diclofenac sodium and acetyl salicylic acid were obtained from Square Pharmaceuticals Ltd as gift samples.

Experimental animals. For the experiment *Swiss albino* mice of either sex, 6-7 weeks of age, weighing between 25-30 g, 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 labratory conditions of temperature: (27.0 ± 1.0 °C), relative humidity: 55-65% and 12 h light/12 hr dark cycle and had free access to ICDDR,B formulated diet and water *ad libitum*. All protocols for animal experiment were followed[13] and 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.

Preliminary phytochemical investigations. For preliminary phytochemical investigation, the crude methanol extract of *B. lacera* was subjected to various tests to determine the chemical nature of the extract.[14-16]

Test for antipyretic activity. The antipyretic activity of *B. lacera* was evaluated on Swiss albino mice (25-30g) of either sex. The animals were divided into three groups, each group containing seven mice. The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by injecting 20% aqueous suspension of Brewer's yeast (10 ml/kg, s.c.).[17] All groups were fasted overnight but free accesses to drinking water was provided. After 24h rectal temperature of each mouse was recorded again. The induction of pyrexia was confirmed by rise in temperature of more than 32.9°F, while animals showing less than 32.9 °F rise of temperature were excluded from experiment. Group-I received saline (10 ml/kg, b.w.) as a negative control, group-II received paracetamol (150 mg/kg, b.w.) as a standard drug while the remaining group-III received 500 mg/kg body weight of the plant extract, respectively. Rectal temperature was recorded periodically at 1, 2 and 3 hr after drugs administration.

Test for analgesic activity. The analgesic activity of the crude extract was evaluated using formalin-induced writhing method in mice. Experimental animals (Swiss albino mice) were randomly selected and divided into three groups denoted as group-I, group-II, and group-III consisting of 7 mice in each group. The mice of each group received a particular treatment *i.e.* control, standard and the two doses of the extract. Test samples (about 200 and 400 mg/kg, b.w. of the plant extract), control and diclofenac sodium were given orally by means of a feeding needle. An interval of thirty minutes was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, formalin solution (5%) was administered intraperitoneally to each of the animals of all groups.

After an interval of 10 mins, which was given for absorption of formalin, number of squirms (writhing) was counted for 5 mins.[18]

Test for anti-inflammatory activity. To determine the anti-inflammatory activity of the methanol extract of *B. lacera*, 9 clean centrifuge tubes (three for standard acetyl salicylic acid, three for negative control methanol and three for crude extract) were used. 1.0 ml of 5% egg albumin solution was added to all test tubes. Later on, 1 ml of acetyl salicylic acid (0.1 mg), 1 ml of methanol and 1ml of crude extract (400 µg/ml) were added to the positive, negative control and test groups, respectively. The pH (5.6±0.2) of all the reaction mixtures was adjusted by 1N HCl, heated, cooled and after filtration, the absorbance was measured spectrophotometrically at 660 nm.[19]

Statistical analysis. Results are expressed as the mean ± SEM. 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.

RESULTS AND DISCUSSION

Phytochemical test. The crude extract of *B. lacera* was found to contain alkaloids, glycosides, flavonoids, reducing sugars and gums Table 1.

Table 1. Chemical analysis for phytoconstituents in the crude extract of *B. lacera.*

Examination	Test performed	Results
Alkaloids	Mayer's test	+
	Dragendorff's reagent	+
	Wagner's reagent	+
	Hager's reagent	+
	Tannic acid	-
Glycosides	Salkowski test	+
	Libermann-burchared test	+
Steroids	Salkowski test	-
	Libermann-burchared test	-
Tannins	Ferric chlorides	-
	Potassium dichromate	-
Flavonoids	Conc. HCl and alcoholic test	+
Saponins	Shake test (aq. solution)	-
Reducing sugars	Fehling's solution test	+
	Benedict's test	+
Gums	Molisch's reagent	+

(+) = present; (-) absent

Antipyretic activity. The experimental mice which showed at least an increase in rectal temperature to at least 34.27 ºF, 18 hrs after Brewer's yeast injection. The crude methanol extract (BLME) exhibited a significant (p=0.05) lowering of mices' body temperature which was elevated by the administration of yeast. These effects were pronounced at the 2nd and 3rd hr post-treatment with extract. The antipyretic effects of the extract were comparable to that of the standard paracetamol (Table 2).

Table 2. Effect of crude extract at 500 mg/kg, b.w i.p. in yeast-induced pyrexia.

Drug	Dose (mg/kg)	Rectal temperature in ºF at time (hr)			
		After administration of drug			
		Basal temperature	1 hr	2 hr	3hr
Control	150	98.77 ± 1.22	98.73 ± 1.19	98.77 ± 1.22	98.63 ± 1.19
BLME	500	99.03 ±0.15	98.17 ± 0.22	97. 3 ± 0.19*	96.06 ± 0.11*
Paracetamol	150	98.57 ± 0.88	92.5 ± 0.61*	91.2 ± 0.86*	92.87 ± 0.57*

Seven animals in each group; Values are mean ± SEM, * p=0.05 when compared to control, temperature after 18 hr of yeast injection and just after sample administration. BLME = *Blumea lacera* methanol extract

Analgesic activity. The results showed that the pain relief was achieved in a dose dependent manner, at all test doses (200 and 400 mg/kg i.p.) as shown in Table 3. The pain relieving dose 400 mg/kg, b.w. was found to be significant in comparison to the standard, diclofenac sodium. Total writhing were 28, 20 at the dose 200- and 400- mg/kg, b.w. respectively while the standard diclofenac sodium produced 11. The crude extract exhibited 39.13% and 56.52% protection at the dose of 200 and 400 mg/kg,

respectively as compared to 76.09% exhibited by standard diclofenac Na.

Anti-inflammatory activity. In the present study for *in-vitro* anti-inflammatory test, the crude extract at the dose of 400 µg/ml showed 62.40% inhibition of protein denaturation whereas standard acetyl salicylic acid (ASA) exhibited 76.74% (Table 4). The ability of this extract was found to be significant in inhibiting the heat-induced protein denaturation.

Table 3. Analgesic activity of crude methanol extract of *B. lacera* in formalin-induced test.

Animal group	Total writhing	% writhing	% protection
Control	46	100	0
Diclofenac sodium (25 mg/kg, b.w.)	11	23.91	76.09
BLME (200 mg/kg, b.w.)	28	60.86	39.13
BLME (400 mg/kg, b.w.)	20	43.47	56.52

Here, n = Number of animals= 07; BLME = *Blumea lacera* methanol extract

Table 4. *In vitro* anti-inflammatory activity of crude methanol extract of *B. lacera*.

Test groups	Total inhibition of protein denaturation
Control	00.00±0.0141
Standard ASA (100µg/ml)	76.74±1.141*
BLME (400 µg/ml)	62.40±0.56

SEM = Standard error of mean, n= 7; Total inhibition of protein denaturation = % MIPD ±SEM, *p= 0.05; BLME = *Blumea lacera* methanol extract

DISCUSSION

During our preliminary phytochemical screenings, important therapeutic principles like alkaloids, flavonoids, glycosides, reducing sugar, gums were detected in the crude extract of *B. lacera*. The results of the present research also suggested that the crude extract has considerable antipyretic, analgesic and anti-inflammatory effects. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plant materials as well as synthetic drugs for their antipyretic effect.[20,21] The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators are responsible for the antipyretic effect.[22] Subcutaneous administration of this plant extract significantly lowered the rectal temperature of yeast induced febrile mice. Thus, it can be postulated that *B. lacera* contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

In analgesic activity test, the formalin-induced pain as an experimental model of analgesia is useful for elucidating mechanism of pain and analgesia since it measures the response to a long-lasting nociceptive stimulus and, therefore, resembles clinical pain. Subcutaneous injection of dilute formalin into mice hind-paw produces biphasic nociceptive response namely: the first transient phase is caused by the direct effect of formalin on sensory C-fibers, and the second prolonged phase is associated with the development of the injury induced spinal sensitization, responsible for facilitated pain processing, a central sensitization of the dorsal horn neuron occurs during inflammatory pain.[23] Drugs that act centrally, such as the narcotics, inhibit both phases of formalin-induced pain, while peripherally acting drugs such as aspirin only inhibit the late phases.[24] Results of the present study showed that the crude extract of *B. lacera* inhibit both the early and late phases of formalin-induced pain, thus suggesting its central and peripheral anti-nociceptive actions.

Important therapeutic principles like alkaloids, flavonoids, glycosides, ctc. were detected during the preliminary phytochemical screenings of the crude extract. Therefore, the current findings can be attributed to all or some of these groups of chemical compounds. Further study is needed with *B. lacera* to find the exact mechanism of action for its antipyretic, analgesic and anti-inflammatory effects and to isolate the active molecules.

CONCLUSION

It can be concluded that the methanol extract of *B. lacera* has moderate antipyretic, analgesic and anti-inflammatory activities in various animal models and this strongly supports the ethno-pharmacological uses of this plant as antipyretic, analgesic and anti-inflammatory agent. The role of alkaloids, flavonoids and triterpenoids needs to be evaluated in future studies.

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