WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 120-130. Research Article ISSN 2277– 7105

PHYTOCHEMICAL INVESTIGATION AND ASSESSMENT OF *IN VIVO* AND *IN VITRO* PHARMACOLOGICAL ACTIVITIES OF *BLUMEA LACERA* (BURM.F.) DC.

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Article Received on
19 Dec 2014,

Revised on 13 Jan 2015,
Accepted on 07 Feb 2015

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ABSTRACT

The present study was designed to investigate different *in vivo* & *in vitro* biological activities and phytoconstituents of methanolic leaf extract of *Blumea lacera* (Burm.f.) DC. The leaves of *Blumea lacera* are used ethnomedically across various regions of India, Asia Minor, Philippines, West Africa as well as some rural areas of Bangladesh without scientific basis or safety concerns. Determination of its phytochemical constituents, *in vivo* and *in vitro* pharmacological properties will provide supportive scientific evidence in favor of its continuous usage. Methanolic leaves extract of *Blumea lacera* was assessed with the Swiss albino mice to evaluate *in vivo* antidiarrheal activity. Tubifex worms (*Panagrellus redevises*) were used to evaluate *in vitro* anthelmintic effect. It has been evaluated as antidiarrheal agent compared to Loperamide, as a standard drug. The 400mg/kg concentration in both case of testing showed closely percentage (40.275%) to Loperamide (62.068%). The extract showed remarkable anthelmintic properties compared to Levamisole, as a standard drug. These findings demonstrate that methanolic leaves extract of *Blumea lacera* have considerable antidiarrheal and anthelmintic property. There were remarkable amount of pharmacologically active constituents e.g. alkaloids, terpenoids, flavonoid, steroid, carbohydrate, cardiac glycoside and resin. Thus, which compounds is responsible for the present pharmacological actions and to

know their mechanism of action, extensive pharmacological and phytochemical experiments are essential.

KEYWORDS: *Blumea lacera*, antidiarrheal activity, anthelmintic activity, phytochemical investigation, tubifex worms.

INODUCTION

Medicinal plants may be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents and are used for medicinal purposes.[1] There is a continuous and urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and re-emerging diseases. Plants constitute the important sources of active natural products which differ widely in terms of structures, biological properties and mechanisms of actions. Around 25% of the prescribed medications in the world are of plant origin[2] and at about 80% people rely on traditional plant-based medications for their primary health care needs in most developing countries.[3] Various phytochemical components, especially poly phenols (such as flavonoids, phyenyl propanoids, phenolic acids, tannins etc.) are known to be responsible for the key activities of any plant. The screening of plant extract has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases. Plant derived agents are being used for the treatment of cancer. Several anticancer agents including taxol, vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, and etoposide derived from epipodophyllotoxin are in clinical use all over the world.[4-7]

Blumea lacera (Burm.f.) DC. (Bengali name: Kukursunga; Family-*Asteraceae*) is an erect, villous, foetid herb. *Blumea* consists of at around 80 species.[8] Literature review revealed that the leaves of *Blumea lacera* are used ethnomedically across various regions of Asia Minor, India, West Africa, Philippines, Western Australia (n.)[9] as well as some rural (e.g. hilly) areas of Bangladesh. In Ayurveda, it is considered bitter, astringent, acrid, thermogenic, errhine, anti-inflammatory, styptic, ophthalmic, digestive, anthelmintic, tonic, expectorant, diuretic, deobstruent and stimulant.[10-11] Juice from crushed leaves is used to apply topically over burnt area.[12] In the Philippines, a decoction of fresh flowers is given before meals for bronchitis - 30 gm in 1 liter of water, boiled to half of its volume.[13] Study also isolated two new glycosides, the triterpenoid glycoside 19α-hydroxyurs-12-ene-24,28-dioate and the phenol glycoside 2-isoprenyl-5-isopropylphenol from the whole plant of *B. lacera*. Essential

oil of leaves yielded thymoquinol dimethyl ether as main constituent; together with β-caryophyllene, α-humulene and E-β-farnesene[14] and this essential oil from leaves have analgesic and tranquillizing activities.[1] The plant also showed anti-leukemic, antiviral[15] and cytotoxic[16] activities against breast cancer cells. The plant belonging to family Compositae had been reported for its anthelmintic use in ancient literature, but it was not scientifically proved.[17-19] So, through this study we tried to evaluate scientifically either it has really anthelmintic property or not. Therefore, the goals of the study were to determine the phytochemical constituents and evaluation of various pharmacological effects e.g. antidiarrheal, anthelmintic properties of the methanolic leaves extract of *Blumea lacera* in comparison of commercial standard.

MATERIALS AND METHODS

Plant authentication and extraction

The leaves of *Blumea lacera* were collected from Vatiary, Chittagong beside of Chittagong Cantonment, Bangladesh and identities of the plants were confirmed by Dr. Shaikh Bokhtear Uddin, Associate Professor and Taxonomist, Department of Botany, University of Chittagong. The solvent (methanol) extraction procedure was used to prepare extracts. The extracts were filtered and concentrated using rotary vapor at a temperature of 40°C-50°C to get solid extracts.

Chemicals

All chemicals and solvents used in this investigation were of analytical grade and purchased from Merck, Germany. Standard drug such as Loperamide was purchased from Square Pharmaceuticals Limited, Bangladesh and Levamisole was purchased from ACI Limited, Bangladesh. Castor oil was purchased from WELL's Health Care, Spain.

Experimental animals and organisms

Swiss albino mice of either sex having age of 6-7 weeks and weight of 30-40gm were obtained from the animal house of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR,B).The animals were housed under the standard laboratory conditions (relative humidity 55-65%, room temperature 24.0 ± 1.0^{o}C and 12h light: dark cycle) for one week to acclimate and fed with ICDDR,B formulated standard diet and water ad libitum. Appropriate measures were taken to minimize the discomfort of animals and all protocols for animal experiment were followed by the institutional animal ethical committee.[20] The free living nematode *Panagrellus redivivus* (sour paste nematode) is

known to many aquarium enthusiasts and fish keepers as the micro worm or black worm were collected from Aquarium fish center, Chittagong, Bangladesh which is used as fish food.

Preliminary phytochemical investigation

The methanolic extract of *Blumera lacera* was screened for the presence of various bioactive phytochemical compounds. Specific qualitative tests were performed to identify bioactive compounds of pharmacological importance through standard methods. 1 gm of the methanol leave extract of *Blumea lacera* was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents.[21-25]

***In vivo* anti-diarrheal activity test**

Acute toxicity test

Acute toxicity test for the extract of *Blumea lacera* was carried out following the method of Lorke to evaluate any possible toxicity.[26] Different doses of methanolic leaves extract were injected intraperitonealy into groups of 12 Swiss albino mice. The injected maximum dose was 600 mg/kg. At the same time, the control group only received distilled water. The number of deaths of experimental mice was counted at 48 h after treatment.

Methodology

The method which was followed by Galvez et al. for antidiarrheal activity test was modified to suit the experimental needs.[27-28] Experimental albino mice were fasted 24 h before the test with free access to water and divided into 4 groups of 4 animals each. Diarrhoea was induced by administering 0.5ml of castor oil orally. Group A treated as control (received distilled water 0.5ml, p.o.), Group B received standard drug (Loperamide 5mg/ kg body weight, p.o.) and Group C and Group D received methanolic leaves extract of *Blumea lacera* (200mg/kg and 400 mg/kg respectively). 30 min later castor oil was administrated (0.5ml) to the mice. Each animal was placed in individual cage, where the floor of cage was lined by blotting paper. The floor lining was changed every hour interval. The consistency of the faecal matter and the number of both the wet and the dry diarrhoeal droppings were counted every hour up to 4 hours. During an observation period of 4 hours, the total number of faeces which were excreted by the animals was recorded. The total number of diarrheal faeces of the control group was considered 100%. Percent inhibition (PI) in defecation was calculated using the following formula-

PI= Mean defecation (Control group - Treated group) ×100/ Mean defecation of control group.

***In vitro* anthelminthic activity test**

Preparation of extract solution

100mg extract (*Blumea lacera*) of each was suspended in 10ml distilled water and the suspension was shaken vigorously on a vortex mixture. The suspension was kept overnight at room temperature to solubilize the water soluble part of the extract in aqueous medium and sediment the water insoluble water. After that supernatant aqueous part was separated through a paper filter (Whatman No.1). This extract solution was ready to use for *in vitro* anti anthelmintic activity study. Different concentration of each extract solutions (Mentioned amount in result) were prepared for the experimental analysis.

Application of extract solution to the black worms (tubifex)

In each test tube approximately 10-12 *Panagrellus redivivus* (tubifex worms) taken and approximately 2ml of extract solution of different concentration were given in each test tube. Then the starting time, time for paralysis and death time of the worm were noted carefully. Two control groups were used in this study to validate the test method and results obtained due to the activity of the test agent. In case of negative control test, only distilled water was added in Petridis containing 10-12 *Panagrellus redivivus*. No extract was added to prepare control solution. In case of positive control test, Levamisole syrup was used at different concentration mentioned at Table 04 and careful observation was made to see the paralyzing time and death time of *Panagrellus redivivus*. The time was noted for calculating results.

RESULTS

Preliminary phytochemical investigation

In case of phytochemical investigation, the final outcomes showed in Table 01 respectively.

Table 01: Phytochemical investigation of methanolic extracts of *Blumea lacera*

Sl No.	Phytochemical Tests	Methanolic Extracts of *Blumea lacera*
1	Alkaloids	+
2	Carbohydrate	+
3	Cardiac Glycoside	+
4	Phenol	-
5	Flavonoid	+
6	Resin	+

7	Tannin	-
8	Cholesterol	-
9	Saponins	-
10	Terpenoids	+
11	Steroids	+
12	Quinone	-
** *(+) = Present and (-) = Absent*		

Castor oil induced *in vivo* antidiarrheal activity test

In case of acute toxicity test, the assay did not show any toxic effects as none of death of the experimental animal found after 48 h. The results for *in vivo* antidiarrheal activity test are mentioned to the Table 02 and Figure 01.

Table 02: Effect of *Blumea lacera* in antidiarrheal activity test on Swiss albino mice

Groups	Treatment	Dose	No. of faeces in 4 h	% inhibition in defecation
Group A	Distilled Water	0.5 ml/mice	14.5 ± 1.0	00
Group B	Loperamide	5 mg/kg	5.5 ± 0.57	62.068
Group C	BLME	200 mg/kg	10.66 ± 1.15	26.482
Group D	BLME	400 mg/kg	8.66 ± 0.57	40.275

Values are presented as mean ± SD, (n=4); one-way ANOVA followed by Dunnett's t-test where P<0.05 was considered as statistically significant.
BLME= Blumea lacera methanol extract.
SD= Standard Deviation.

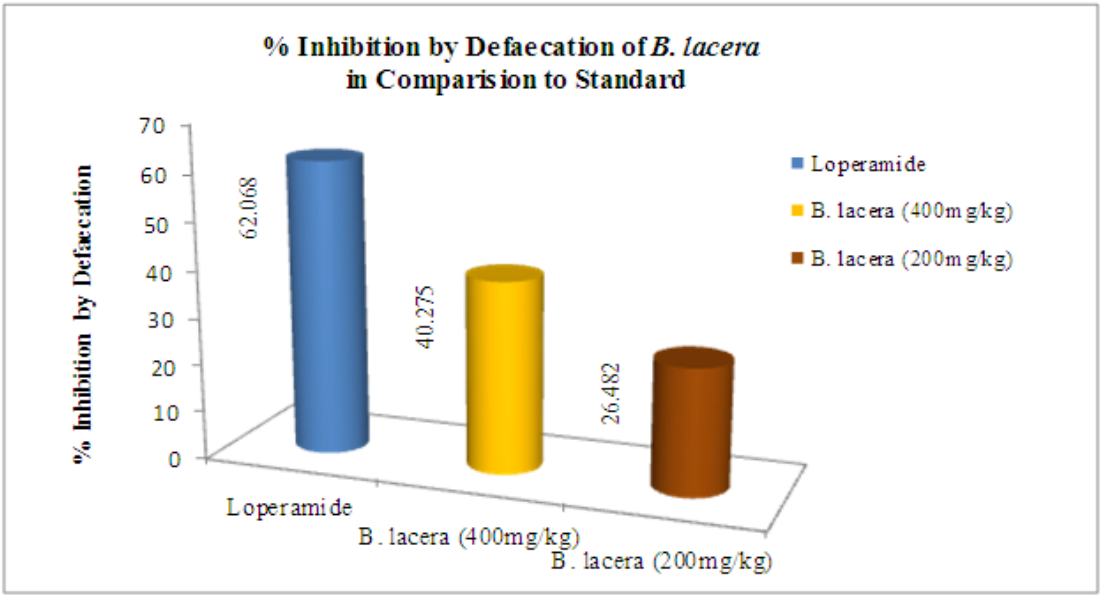

Figure 01: % inhibition defecation in case of castor oil induced diarrhea test

***In vitro* anthelminthic activity test**

The findings of *in vitro* anthelmintic activity test of *Blumea lacera* are given below tables (Table 03, Table 04, Table 05 and Table 06 respectively)

Table 03: Table for anthelmintic activity test (*Blumea lacera*)

Concentration (mg/ml) *(Blumea lacera)*	Paralysed time (h:min:sec)	Death time (h:min:sec)
2	00:27:18	01:06:30
2.5	00:04:07	00:45:04
3	00:03:28	00:26:58

Table 04: Table for anthelmintic activity test (positive control)

Concentration (mg/ml) (Levamisole)	Paralysed time (h:min:sec)	Death time (h:min:sec)
0.5	00:14:41	02:01:32
0.8	00:06:26	00:12:21
1	00:03:32	00:06:57

Table 05: Table for anthelmintic activity test (negative control)

No. of observation for distilled water	Paralysed time (h:min:sec)	Death time (h:min:sec)
1	None	None
2	None	None
3	None	None

Table 06: Table for anthelmintic activity of *Blumea lacera* in comparison with positive and negative control

Sample	Concentration mg/ml	Paralyzed time (h:min:sec)	Death time (h:min:sec)
Blumea lacera	2	00:27:18	01:06:30
	2.5	00:04:07	00:45:04
	3	00:03:28	00:26:58
Positive control (Levamisole)	0.5	00:14:41	02:01:32
	0.8	00:06:26	00:12:21
	1	00:03:32	00:06:57
Negative control	Distilled Water	None	None

DISCUSSION

It has been estimated that there are approximately 500,000 species of plants available on earth[29] and among them only a few percentage (1% to 10%) are used as food supplement by humans and other animal species together. In addition, just more than 10% of plants are used for medicinal purposes.[30] The methanolic extract of *Blumea lacera* was screened for the presence of various phytoconstituents (Table 01). Specific qualitative tests were performed to

detect bioactive compounds of pharmacological importance through standard methods. The analysis revealed the presence of alkaloids, terpenoids, flavonoid, steroid, carbohydrate, cardiac glycoside and resin.

The anti-diarrheal activity of *Blumea lacera* has also good result (Table 02 and Figure 01). The 400mg/kg concentration shows closely percentage (40.275%) to standard drug Loperamide (62.068%). Therefore, the anti-diarrheal efficacy of the selected plant extract can also be a sign of good hope.

In anthelmintic activity study using tubifex worms (*Panagrellus redivivus)* the methanolic extract of *Blumea lacera* leaves showed significant effect in comparison with the positive control Levamisole. The worms found to be paralyzed within 3.28 min & death time found 26.58 min taking concentration of 3mg/ml for extract where the worms found to be paralyzed within 3.32 min & death time found 06.57 min taking concentration of 1mg/ml for standard drug, Levamisole (Table 06). In case of negative control (distilled water), the worms were alive even after one day (24 h). During this study, the paralyzed time was noted by observing the reduced movement and the death time was noted while the movement of worms totally stopped. There was no movement observed even after vigorous shaking of mentioned time. So, it can be assumed that extract is pharmacologically active and may have good anthelmintic activity.

CONCLUSION

Herbal medicines can gain the confidence of orthodox health practitioners when there are scientifically established proofs of their claimed efficacies. The result of the present study showed that the extract of *Blumea lacera* which contain remarkable amount of Phytoconstituents. On the basis of the overall results from our investigations, the use of *Blumea lacera* in the treatment of diarrhoea is justified. As the results of this experiment leaves extracts possess pharmacologically active ingredients with antidiarrheal properties, the active constituents responsible for antidiarrheal activity remain to be identified; further studies are required to understand the pharmacological action of its antidiarrheal activity.

In anthelmintic activity study using tubifex worms (*Panagrellus redivivus*) the extract showed positive effect in comparison with the positive control Levamisole. However, this is only a preliminary investigation and to make final comment the extract should thoroughly

investigated both phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

ACKNOWLEDGEMENT

We would like to express our deep sense of gratitude and heartfelt appreciation to the authority of Department of Pharmacy, International Islamic University Chittagong, Bangladesh for providing us the facilities of all kinds to conduct the research.

CONFLICT OF INTERSET

Authors declare that there is no conflict of interests.

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