Ethnobotany and antibacterial potentiality of *Blumea lacera* L. from Sundarban Mangrove forest of Bangladesh

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Abstract

Indigenous knowledge on the utilization of *Blumea lacera* L. by the interim people as well as ethnic communities living at the vicinity of Sundarban mangrove forest of Bangladesh were documented through survey, interview, collection and identification. Native people of Sundarban use the herb in bleeding piles. Leaf of the plant was found to be the most useful part for ethnomedicinal use. Generally, leaves of *B. lacera* have been used to treat most kinds of colds. Warm leaf paste used as diuretic whereas leaf paste with black pepper powder was found to apply in dog bite. Furthermore, *B. lacera* collected from Sundarban mangrove habitat as well as from natural plain land habitat (JU campus) were subjected to a comparative analysis of antibacterial activity. Antibacterial study from the present experiment suggested that the ethyl acetate fraction of *B. lacera*, irrespective of habitat choice, was active against the gram-positive bacteria *Bacillus cereus*. The methanol fraction of Sundarban sample showed significant dose dependent increase in antibacterial activity against the gram-negative bacteria *Serratia sp., Proteus mirabilis, Salmonella typhi* and *Vibrio cholera* while the JU sample did not produce any inhibition zone. The *n*-hexane fractions of both type plant samples were entirely non-responsive to the bacterial growth.

Key words: Ethnobotany, antibacterial activity, *Blumea lacera*, Sundarban mangrove forest.

INTRODUCTION

Primary tropical forest is generally considered to be the most likely habitat to discover new pharmaceuticals due to high biodiversity and endemism. However, many indigenous groups rely on forested and non-forested environments for medicinal plants. An ethnobotanical approach to drug discovery suggests that extreme environments may also harbor potentially useful medicinal compounds (Gentry, 1993; Macilwain, 1998). During the last ten years pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically (Akinpelu *et al.*, 2009). According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Systematic screening of folk medicines and plants may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002).

Blumea lacera L. belonging to the Asteraceae family is a common plain land weed of Bangladesh. This plant species also grows in Sundarban area and known as Kukursunga in that locality (Uddin *et al.*, 2011). B. lacera has an enormous medicinal value and been widely used in the traditional medicinal system of Bangladesh for a long time. It is used

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as antipyretic, stimulant, astringent, anthelmintic, febrifuge, diuretic and in hemorrhoids (Pandit *et al.*, 1996) bronchitis (Jha & Verma, 1996) as antimicrobial for healing cuts (Joshi, 1980) in urinary troubles (Singh, 1988) in irritation, as haemostatic (Kakrani & Saluja, 1994) and anti-inflammatory (Pal, 1972). There are scanty reports on ethnobotany and antimicrobial activity of *B. lacera* that are found in mesophytic habitat (Tiwari *et al.*, 2012; Singh *et al.*, 2010) but yet now there is no aforesaid information of this plant grows in mangrove or halophytic habitat. Considering the above fact, present study was aimed to document the indigenous knowledge on the utilization of *B. lacera* by the ethnic communities living in the vicinity of Sundarban mangrove forest of Bangladesh followed by comparative analysis of antibacterial activity of the aerial parts of the plant collected from Sundarban as well as from Jahangirnagar University campus.

MATERIALS AND METHODS

Ethnobotanical study: The survey was conducted among the interim fisherman, forest guard, and honey collector of Kalagachia, Dublar Char and Kotka sites of Sundarban mangrove forest of Bangladesh. Data were collected in the field note book on the plant parts used, preparation of the drug, its dosage and administration etc. as given by the informants. Interviews were conducted with the help of a semi-structured questionnaire and the guided field-walk method of Martin (1995) and Maundu (1995). Interviews were conducted in the Bengali language. Notably, the Kavirajes practiced among the mainstream Bengali-speaking population.

Collection and Identification: Plant samples were collected from two different habitats namely Jahangirnagar University campus and Sundarban and were identified and authenticated by the Bangladesh National Herbarium (DACB), Mirpur, Dhaka, Bangladesh. The voucher specimens (accession no. of JU sample: DACB 38210 and Sundarbans sample: DACB 32624) have been deposited in DACB for further reference.

Preparation of crude extract and fraction: The collected aerial parts of both the samples were sun-dried followed by drying in a hot air oven (Gallenkamp) at reduced temperature (<50°C). About 200 g powder was digested with 1000 ml of ethanol for three days accompanying with occasional shaking and stirring. The whole mixtures was filtrated by a piece of clean, white cotton material. The extract was concentrated at 45°C under reduced pressure using a rotary evaporator.

Partition of the crude methanolic extract: Solvent-solvent partitioning was done by using the protocol designed by Kupchan & Tsou (1973) and modified version of Wagenen *et al.* (1993). The crude ethanolic extract of the sample was first partitioned with *n*-hexane and methanol at ratio 1:1 using separating funnel and these two fractions were evaporated separately to dryness by rotary evaporator at 40°C. Then the methanol fraction was further partitioned to ethyl acetate and methanol fractions and evaporated to dryness. Fraction samples were then marked and stored in dark, cold and dry place.

Determination of antibacterial activity: Antibacterial potentiality of the fractions (*n*-hexane, ethyl acetate and methanol fractions) were determined by disc diffusion method following Saad *et al.* (2012). As per availability, two Gram positive bacteria *Bacillus cereus* and *B. subtilis* and eight Gram negative bacteria *Erwinia* sp., *Proteus mirabilis*, *Pseudomonas* sp., *Salmonella* sp., *Salmonella typhi*, *Serratia* sp., *Shigella flexneri* and *Vibrio cholera* were used to determine antibacterial activities. The sterile filter paper disc (8 mm diameter) containing three different concentration of the dry extracts (40 μg/ml, 80 μg/ml and 120 μg/ml), standard antibiotic disc (5 μg/ml Streptomycine) and negative control (blank disc soaked with methanol solvent only) were used in the experiment and the results recoded as the mean values of three replications. The bacterial strains used for the experiment were collected as pure cultures from Microbiology laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka.

RESULTS AND DISCUSSION

Traditional medicinal practitioners tend to keep the treatment methods within the family, where the knowledge is passed on from generation to generation. As a result over the centuries, traditional medicine practitioners have developed a familiarity with plant species found in their region or habitat and the applicability of those plant species in treatment of various types of diseases. In the present study, interim peoples of the three sites namely Dublar char, Kalagachia and Kotka were surveyed to collect the information on medicinal use of *B. lacera*. About 25 people were asked to collect the information on *B. lacera* growing in Sundarbans. The plant was found non familiar to the local people as most of them could not recognize it. Restricted use and application was another sign of its less popularity in traditional medicinal system of Sundarban area. However, five of the respondent, all of them were illiterate, including kavirajes, fishermen and forest guard took the interviewers in areas from where they collected the plant, pointed out the plant, and mentioned their uses. They learned this knowledge from their forefathers. So it was difficult to gather much information in shortened period field works.

Kavirajes and folk medicine practitioners of our country have been using *B. lacera* and its relatives in their medicinal system for a long time (Islam *et al.*, 2012). *B. lacera* grow in Sundarban area locally known as Bonpalang, Bontamak etc. It had strong pungent smell when squashed. Among the visited three sites, Dublar Char had the highest salinity (about 20 ppt). The salinity in Kalagachia and Kotka ranged between 13 and 14 ppt (Hossain, 2003). Among the five informants, two form Dublar Char, another two from Kalagachia and one from Kota were interviewed (Table 1).

Rahman (2013) reported the use of *B. lacera* as fuels and juice of the leaves as an anthelmintic, febrifuge, astringent, and diuretic in Rajshahi district of Bangladesh. He also mentioned use of the herb in bleeding piles. Mollik *et al.* (2010) reported the ethnomedicinal use of *B. lacera* for gastrointestinal disorder and as an insect repellent in Bagerhat Sadar and Rampal area of Bagerhat district of Bangladesh.

Table 1. Ethnobotanical information on *B. lacera* at different sites of Sundarban mangrove forest

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Site	GPS coordinates and forest type*	Name and age of the informant	Profession	Uses			
Dublar char		Aziz (37) Local practition		 Warm leaf paste is applied to lower abdomen as diuretic. Leaves juice (approx. 1 tea spoonful) with honey orally taken to treat most kinds of colds. 			
	N21°43'47" E89°36'15.2" (Gewa-Kewra type forest)	Mrinmoy Nandi (63)	Kaviraj	1. Root and stem decoction is taken to treat blood dysentery. 2. Leaf paste (2½ tea spoonful) with black pepper powder (1 tea spoon) is applied in dog bite. 3. Leaf juice (1 cup) mixed with a tea spoonful of mustard seed is orally taken to relieve piles.			
Kalagachia	N22°13.124' E89°14.329' (Gewa-Goran- Poshur type forest)	Shafiuddin (51)	Fisherman	 Leaf paste is applied on forehead for the treatment of fever and to the joints for gout. Pellet of leaf and seed powder is taken in leucorrhoea. 			
		Malek (45) Forest Guard		Native people use the herb in the treatment of bleeding piles.			
Kotka	N21°51.263" E89°47.257' (Sundari-Gewa- Kewra type forest)	Idris miah (39)	Forest Guard	Warm decoction of the plant is applied to hasten the expulsion of placenta after childbirth.			

^{*} Source: Hossain, 2003.

Kumar & Bhagat (2012) reported that roots and leaves of *B. lacera* are used as antipyretic and root decoction of *Blumea hieracifolia* for gastric trouble in Nepal. Leaf juice used as an astringent, febrifuge, diuretic and anti-helminthic whilst leaf paste along with a pinch of common salt is applied externally to cure eczema and ring worm (Satapathy *et al.*, 2012).

9.50c

10.33c

10.67b

Fractions	Sample	Dose (µl)	Bacillus subtilis	Bacillus cereus	Vibrio cholerae	Pseudom- onas sp.	Serratia sp.	Erwinia sp.	Protius mirabilis	Shigella flexneri	Salmone- lla sp.	Salmone- lla typhi
		40	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
	JU	80	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
havana		120	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
<i>n</i> -hexane	Sundar- ban	40	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
		80	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
		120	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
		40	8a	9.33a	8b	8a	8a	8a	8a	8a	8a	8a
	JU	80	8a	11a	9.17b	8a	8a	8a	8a	8a	8a	8a
Ethyl		120	8a	14a	10.33b	8a	9.50b	8a	8a	8a	8a	8a
acetate	Sundar- ban	40	8a	10.67a	8a	8a	8a	8a	8a	8a	8a	8a
		80	8a	13.83a	8a	8a	8a	8a	8a	8a	8a	8a
		120	8a	14.50a	8a	8a	8a	8a	8a	8a	8a	8a
		40	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
	JU	80	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
Methanol		120	8a	8a	8a	8a	8a	8a	8a	8a	8a	8a
ivieulanoi		40	0.02 1	0	0.50	0	10.50	0.501	1 1 221	0 1	0	0.50

Table 2. Antibacterial activities of different fractions of *B. lacera* collected from JU campus and Sundarban mangrove forest

NB: In a column means followed by common letters are not significantly different at 5% level by DMRT. *Disc diameter = 8 mm

12.50a 8.50de

13.17a 8.83de

10.33b

11.33b

12.17b

9cd

9.83cd

11.17b

8a

8a

8a

8a

10.33b

40 8.83cde

9.33cd

80

Sundar-

8a

8a

10.17b

9.50c

10.17c

11.33b

The use of plant extracts or phytochemicals with known antimicrobial properties can be of great significance for therapeutic treatments (Jaimini *et al.*, 2011). Plants have been found *in vitro* to have antimicrobial property as they are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids (Cowan, 1999). In this study, the antibacterial property of methanolic extracts of the aerial parts have been tested against ten bacterial strains to evaluate the broad-spectrum antimicrobial properties of *B. lacera* (Table 2).

Two gram positive bacteria namely *Bacillus subtilis* and *B. cereus* and eight gram negative bacteria namely *Vibrio cholerae*, *Pseudomonas* sp., *Serratia* sp., *Salmonella typhi*, *Salmonella* sp., *Proteus mirabilis*, *Erwinia* sp., and *Shigella flexneri* were used in the present experiment. *n*-hexane fractions of JU and Sundarban samples showed no antibacterial activity whereas ethyl acetate fraction of both the samples showed inhibitory affect against all the tested bacteria under investigation (Fig. 1). Maximum zone of inhibition produced by ethyl acetate fraction was 14 mm for 120 μ g/ml against *Bacillus cereus* followed by 13.83 mm for 80 μ g /ml and 10.67 mm for 40 μ g/ml respectively. Thus the finding from the present experiment suggest that the ethyl acetate fraction of *B. lacera*, irrespective of habitat choice was active against the gram positive bacteria *Bacillus cereus* following dose dependency (Fig. 2).

The methanol fraction of JU sample did not produce any inhibition zone whereas Sundarban sample showed broad spectrum inhibition against both gram positive and gram negative bacteria except *Salmonella* sp. The methanol fraction of Sundarban sample

showed significant dose dependent increase in antibacterial activity against the gram negative bacteria namely, *Serratia* sp., *Proteus mirabilis*, *Salmonella typhi* and *Vibrio cholerae*. The highest inhibition zone of this fraction recorded as 14 mm and 13 mm against *Serratia* sp. and *Proteus mirabilis*, respectively whereas *Bacillus cereus* showed the lowest inhibition zone (10.17 mm) (Table 2). These observations were more likely to be the fact that an outer membrane in gram negative bacteria, which acts as a barrier to many environmental substances including antibiotic (Panda *et al.*, 2012).

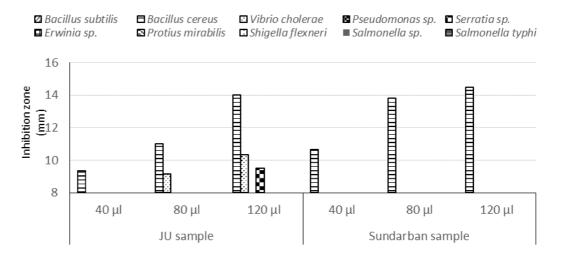


Fig. 1. Antibacterial activity of ethyl acetate fractions of *B. lacera* collected from JU campus and Sundarban mangrove forest

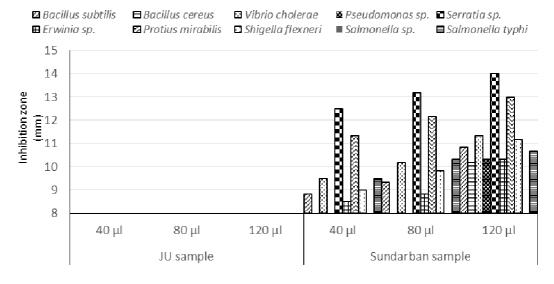


Fig. 2. Antibacterial activity of methanol fractions of *B. lacera* collected from JU campus and Sundarban mangrove forest

A number of review and research article provide the information of the antibacterial activities in *B. lacera* and the members of Asteraceae family. Singh *et al.* (2010) reported that the methanol extract showed good inhibitory activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Serratia marcescens*, *Staphylococcus aureus and Candida albicans* which corroborates with findings of Sundarbans sample of the present study. In contrast, Tiwari *et al.* (2012) reported 13mm (100µg/ml) inhibition zone against *Bacillus subtilis* and *Escherichia coli* respectively with the chloroform fraction of *B. lacera*. However, Islam *et al.* (2008) reported that *B. lacera* showed most promising antibacterial properties indicating the potential for discovery of new antibacterial drugs. The present study suggests that the methanol fraction of the aerial parts of *B. lacera* collected from Sundarban could be relatively potential source of antibacterial agent than the other fractions. Thus further extensive work on *B. lacera* growing in Sundarban may lead to the isolation of interesting therapeutic compound and future work in this regard is needed.

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