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Phytochemical composition, antioxidant activity and cytotoxicity of *Blumea lacera* Linn. from two different habitats

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Abstract

Blumea lacera collected from JU campus and the Sundarbans showed strong presence of carbohydrates, flavonoids, trace amount of alkaloids, weak presence of some glucosides, terpenoids and steroids following preliminary phytochemical screening. DPPH free radical scavenging activity increased with the increase of concentration of methanol, ethyl acetate and *n*-hexane fractions of both the samples. Methanol and ethyl acetate fractions of JU sample showed strong scavenging activity of DPPH free radical. Concentration dependent increment in percent mortality of brine shrimp nauplii was observed in all the fractions of both the samples. Ethyl acetate and methanol fractions of JU sample showed less cytotoxicity than that of Sundarbans samples. Therefore, methanol and ethyl acetate fractions of JU sample should be the choice to identify new bioactive compound(s) in the future.

Key words: Phytochemicals, antioxidant activity, cytotoxicity, *Blumea lacera*, mangrove habitat

INTRODUCTION

Habitat is the natural environment in which an organism lives or the physical environment that surrounds a species population (Dickinson, 1963; Abercrombie *et al.*, 1966). Plants growing in different habitat or ecological condition may evolve through stable genetic polymorphisms and produce diverse secondary metabolites (Binns, 2002). *Blumea lacera* Linn. is one of the most common weeds of Bangladesh. It is a small size annual herb belongs to the family Asteraceae commonly known as Janglimulli, Kakaronda, Siyalmutra and Susksampatra. The plant has an enormous medicinal value and been widely used in the traditional medicinal system of Bangladesh for a long time. The plant is used as antipyretic, stimulant, astringent, anthelmintic, febrifuge, diuretic and to treat hemorrhoids (Pandit *et al*., 1996), bonchitis (Jha and Verma, 1996), sores and wound healing (Ahluwalia, 1968). The root is used as blood purifier while leaves are used to rectify urinary complaints (Varandani, 1969). Seeds of this plant have been reported to exhibit antibacterial activity (Gayake, 2012).

As one of the geographically-widespread species, *B. lacera* has adapted naturally to different habitats in Bangladesh including mangroves. Many workers reported on the presence and isolation of bioactive compounds from *B. lacera* that are found in mesophytic habitat (Agarwal *et al*., 1995; Rao *et al*., 1977). But there is no information

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on the phytochemical composition and bioactivity of this plant grows in mangrove habitat. Thus the present investigation aims to a comparative study on phytoconstituents, DPPH free radical scavenging activity and cytotoxicity of *B. lacera* growing in two different ecological conditions namely, meshophytic and mangrove habitats.

MATERIALS AND METHODS

Plant material: The aerial parts of the plants were collected from JU campus and from the yard Kalagachia Range office, The Sundarbans, Khulna, Bangladesh and The plant was finally identified as *Blumea lacera* by the Bangladesh National Herbarium (BNH), Mirpur, Dhaka, Bangladesh. The voucher specimens (accession no. of JU sample: DACB38210 and for Sundarbans sample: DACB32624) have been deposited in the Bangladesh National Herbarium (BNH), Mirpur, Dhaka, Bangladesh for further reference.

Preparation of crude extract and fraction: The collected plant parts (aerial parts) were sun-dried followed by drying in a hot air oven (Gallenkamp) at reduced temperature (<50ºC) for 4 days to make suitable for grinding purpose. About 200 g powder was digested with 1000 ml of 100% methanol for three days accompanying with occasional shaking and stirring. The whole mixtures then underwent a coarse filtration by a piece of clean, white cotton material followed by filtration using Whatman filter paper. The extract was concentrated at 45ºC under reduced pressure using a rotary evaporator and resultant residue was stored under refrigerated conditions until further studies.

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 methanolic extract of the sample was first partitioned with *n*- hexane and mthanol at ratio 1:1 using separating funnel and these two fractions were evaporated separately to dryness by rotary evaporator at 40ºC. Then the mthanol 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.

Phytochemical screening: The crude methanolic extract and the fractions were subjected to different qualitative tests to find out the presence of chemical constituents using standard procedure (Evans, 1989; Sofowara, 1993; Ghani, 1998 and Dev, 2002). Molisch's and Fehling's reagents were used to investigate the presence of carbohydrates and reducing sugar, respectively. Hagger's reagent, Wagner's reagent, Mayer's reagent and Dragendroff's reagents were used to test the presence of alkaloids while $FeCl_3$ test and Keller Killiani's test were carried out for glycosides and cardenolides, respectively. Borntrager's test was conducted to test the presence of anthraquinone glycosides; Lead acetate, Alkali, $FeCl_3$ and Conc. H_2SO_4 were used for flavonoids. $FeCl_3$, ammonia and lead acetate were used to test the presence of phenolic compounds. Concentrated H_2SO_4 was used to detect terpenoids whereas acetic anhydride was used to check the presence of triterpene. The presence of phytosterols/steroids was indicated by the Salkowski's test while the presence of saponins was confirmed by foam test.

Determination of antioxidant activity: The antioxidant activities of the extracts were measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical following the method described by Blois, (1958) and Aoshima *et al*., 2004. 150 µl DPPH solution was added to 3ml methanol and absorbance was taken immediately at 517 nm for control reading. 50 µl of various concentrations of different fractions as well as standard compound (ascorbic acid) were taken and the volume was made uniformly to 150 µl using methanol. Each of the samples was then further diluted with methanol up to 3ml and to each 150 ml DPPH was added. Finally, absorbance at 517 nm was determined after 30 min. and the percent inhibition activity was calculated as

$$\text{DPPH free radical scavenging activity (\%)} = \frac{\text{control absorption} - \text{corrected sample absorption}}{\text{control absorption}} \times 100$$

IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated following the method of Brand-Williams *et al*. (1995).

Brine shrimp lethality bioassay: Brine shrimps (*Artemia salina*) lethality bioassay was followed by Meyer *et al*. (1982).

RESULTS AND DISCUSSION

Blumea lacera is a common weed found throughout the Bangladesh. Traditional use of this plant inspired to investigate the phytochemical composition along with some selected bioactivity of the aerial parts of *B. lacera*. The crude methanolic extract of both the sample collected from JU campus and the Sundarbans were first partitioned into *n*-hexane and methanol, the methanol fraction was further partitioned into ethyl acetate and water. Then all fractions were marked and stored in dark, cold and dry place. JU sample and the sundarbans samples yielded 51.43% and 56% methanolic crude extract, respectively. The results of fraction amount obtained from methanolic crude extract have been presented in Table 1. The yield percentage was higher in JU sample compared to Sundarbans sample for *n*-hexane and ethyl acetate fractions. After partitioning the yield of *n*-hexane fractions were 47.14% (JU sample) and 38% (Sundarbans sample). Whereas the yield of ethyl acetate fractions were 41.12% and 35.71% for JU and Sundarbans samples, respectively (Table 1).

Table 1. Yield of fractions of *B. lacera* collected from JU campus and the Sundarbans

Fractions		JU sample (% yield)	Sundarbans sample (% yield)
Methanolic crude extract		51.43	56
Partition of methanolic crude extract	*n*-Hexane fraction	47.14	38
	Methanol fraction	50.27	60.09
Further partition of methanol fraction	Ethyl acetate fraction	41.12	35.71
	Water fraction	55	62

The data of qualitative chemical examination of *Blumea lacera* collected from JU campus and the Sundarbans have been presented in Table 2. Phytochemical analysis of the aerial parts showed the presence of various phytochemicals of therapeutic significance. The qualitative chemical examination of fractions obtained by successive solvent extraction of the aerial parts were found to contain carbohydrates, flavonoids, trace amount of alkaloids, some glucosides, terpenoids and steroids in the polar fractions collected from JU campus and Sundarbans (Table 2). These phytoconstituents present in the extracts may account for their various pharmacological activities (Ghani, 2003). Pattewar *et al.* (2012) reported that *B. lacera* contains tannins, alkaloids, saponins, anthraquinone glycosides, steroids, flavonoids, phenolics and terpenoids from the aqueous extract of *B. lacera*. Tiwari *et al.* (2012) also reported the presence of carbohydrates, alkaloids, glycosides, phytosterols, phenolic compounds and flavonoids from this plant. Whereas, Pratap & Parthasarathy (2012) reported that *B. lacera* contains steroids, terpenoids, alkaloids, saponin and absence of tannins and phenolic compounds.

Natural products are beneficial to physiological health (Hossain *et al*., 2008). It has long been recognized that naturally occurring substances in higher plants have antioxidant activity and increasing attention has been focused on medicinal plants. DPPH free radical scavenging is a popular and reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of the plant extracts (Espin *et al*., 2000). The DPPH free radical scavenging of antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy *et al*., 2007).

In the present investigation, the highest value (96.33%) of DPPH free radical scavenging activity at 200 µg/ml concentration was observed in the methanol fraction of JU sample. The ethyl acetate fraction of the Sundarbans sample showed 93.47% scavenging activity at the concentration of 200 µg/ml, followed by methanol (79.07%) and *n*-hexane (66.16%) fractions, respectively. On the other hand the *n*-hexane fraction of JU sample of *B. lacera* showed the lowest value (60.32%) for free radical scavenging activity (Table 3). In both the samples free radical scavenging activity was found to increase with concentration of the fractions. But the increasing trends varied depending on fractions. Bhaumik *et al.* (2008) reported that the methanol fraction of *B. lanceolaria* exhibited significant DPPH free radical scavenging activity with an IC_{50} value of 45.79 µg/ml whereas the IC_{50} value of the standard drug L-ascorbic acid was found to be 13.76 µg/ml. Roy *et al.*, (2012) reported that the ethyl acetate and aqueous soluble fraction of *B. membranacea* showed mild to moderate free radical scavenging activity. Prajakta *et al.* (2013) reported that the essential oil of *B. eriantha* showed DPPH free radical scavenging with IC_{50} value of 437.92 µg/ml.

Brine Shrimp Cytotoxicity Test (BSCT) is a useful tool to screen a wide range of chemical compounds for their various bioactivities. It has been well utilized to screen and fractionation of physiologically active plant extracts as well. Table 4 showed the lethality of the three fractions of the aerial parts of *B. lacera* collected from JU campus and the Sundarban to the Brine Shrimp nauplii. Concentration dependent increment in percent

mortality of Brine Shrimp nauplii was observed in all the fractions of both the samples. Ethyl acetate and methanol fractions of the Sundarbans samples showed the higher cytotoxicity compared to JU sample. JU sample showed relatively increased toxicity than the Sundarbans sample only for *n*-hexane fraction. LC_{50} value 115.38 µg/ml of *n*-hexane fraction indicates the presence of cytotoxic compounds in the extract. LC_{50} values of *n*-hexane fraction of the Sundarbans sample was 181.53 µg/ml. LC_{50} values of methanol fraction of JU and the Sundarbans samples were 183.80 µg/ml and 133.41µg/ml, respectively whereas that was for ethyl acetate fraction of JU and the Sundarbans samples were 245.43 µg/ml and 159.82 µg/ml, respectively. In comparison with vincristine sulphate (LC_{50}=0.069 µg/ml) the results were very poor. Preliminary phytochemical screening revealed the presence of steroids and trace amount of alkaloids. So the observed cytotoxic action may be due to the presence of such compounds.

Roy *et al*. (2012) reported that in Brine shrimp lethality bioassay, the petroleum ether soluble fraction of *B. membranacea* showed the maximum toxicity towards the shrimp with LC_{50} value of 0.841 µg/ml whereas the standard drug vincristine sulphated showed the LC_{50} value of 0.544 µg/ml. Zhu & Tian (2011) reported that the essential oil of *B. martiniana* showed the significant larvicidal activity and it also showed a dose dependent effect on mortality. Moshi *et al.,* (2010) reported that the ethanol fraction of *B. auriculata* showed significant mildy cytotoxic activity with the LC_{50} value of 41.80 µg/ml. Uddin *et al.*(2011) reported that the methanol fraction of *B. lacera* showed the maximum cytotoxicity with LC_{50} values of 0.01 to 0.08 mg/ml against healthy mouse fibroblast and three human cancer cell lines.

Table 2. Qualitative chemical examination of aerial parts of *Blumea lacera* collected from two different habitats

Name of the test		Specific test	*n*-Hexane fraction		Ethyl acetate fraction		Methanol fraction	
			JU sample	Sundarbans sample	JU sample	Sundarbans sample	JU sample	Sundarbans sample
Carbohydrates test	a.	Molicsh's	-	-	+	+	+	+
	b.	Fehling's	+	+	+	+	+	++
Alkaloids test	a.	Hager's	-	-	+	+	+	+
	b.	Wagner's	-	-	-	+	+	+
	c.	Mayer's	-	-	-	-	-	-
	d.	Tannic acid (10%)	+	+	+	+	+	+
Flavonoids test	a.	Ferric chloride	-	-	-	-	-	-
	b.	Lead acetate	-	-	++	+	++	+
	c.	Alkali	-	-	++	+	+	-
	d.	Con. H_2SO_4	-	-	-	-	-	-
Glycosides test Common test	a.	Ferric chloride	-	-	+	+	-	-
Anthraquinon glycosides	b.	Borntrager's	+	+	-	-	+	+
Cardiac glycosides	c.	Keller killiani	-	-	-	-	-	-
Test for Tannins and Phenolic compounds	a.	Ferric chloride	-	-	-	-	-	-
	b.	Lead acetate	-	-	-	-	++	+
	c.	Amonia (NH_3)	-	-	-	-	-	-
Saponin test	a.	Foam	-	-	-	-	-	++
Terpenoids test Common test	a.	Con. H_2SO_4	-	-	-	-	-	-
Triterpenoids test	b.	Acetic anhydride	-	-	-	-	-	-
Phytosterol/Steroid test	a.	Salkowski	+	+	+	+	++	++

NB: +++ =Strong, ++ = moderate, + = week, - = Negative

Table 3. DPPH free radical scavenging activity of *B. lacera* collected from JU campus and the Sundarbans

Concentration (µg/ml)	Activity (%)					
	n-Hexane		Ethyl acetate		Methanol	
	JU sample	Sundarbans sample	JU sample	Sundarbans sample	JU sample	Sundarbans sample
5	2.12(f) ± 0.56	5.12(f)±0.10	57.51(e)±0.41	12.76(e)±1.12	65.33(d)±0.34	6.87(f)±0.81
10	5.02(e) ± 0.42	8.30(d)±1.05	58.84(d)±0.57	15.85(e)±0.62	73.55(c)±0.40	9.33(e)±0.16
25	11.54(d) ± 0.28	12.65(df)±0.31	88.15(c)±0.39	22.79(d)±0.29	82.25(b)±0.32	18.87(d)±0.56
50	21.06(c) ± 0.89	22.04(c)±0.76	90.66(b)±0.11	37.62(c)±1.12	91.34(a) ±1.15	36.36(c)±0.11
100	40.41(b) ± 0.47	42.18(b) ± 0.48	91.65(b)±0.27	68.66(b)±0.80	93.88(a) ±0.24	53.93(b)±0.06
200	60.32(a) ± 0.19	66.16(a)±1.36	93.94(a)±0.12	93.47(a)±1.78	96.33 (a) ±1.08	79.07(a)±0.37

NB: In a column means followed by common letters in parenthesis are not significantly different at 5% level by DMRT. Data are mean ± SE values of three replications.

Table 4. Cytotoxicity of different fractions of *B. lacera* collected from JU campus and the Sundarbans

Sample concentration (µg/ml)		*n*-Hexane fraction							Ethyl acetate fraction							Methanol fraction							Vincristine sulphate (VS)						
		10	20	40	80	160	200	400	10	20	40	80	160	200	400	10	20	40	80	160	200	400	0.06	0.125	0.25	0.5	1	5	10
JU sample	Mortality (%)	5	20	35	50	75	90	100	0	0	5	25	40	55	70	5	15	25	30	45	70	85	10	20	30	40	50	90	100
	LC_{50} (µg/ml)	115.38							245.43							183.8													
Sundarbans sample	Mortality (%)	0	10	25	35	50	70	85	0	15	25	40	60	75	90	10	25	35	40	55	80	100	0.0699						
	LC_{50} (µg/ml)	181.53							159.82							133.41													

Note: Vincristine sulphate used as a positive control.

Phytochemical study of *Blumea lacera* showed the presence of carbohydrates, flavonoids, trace amount of alkaloids, some glucosides, terpenoids and steroids in the polar fraction of *B. lacera* collected from JU campus and the Sundarbans. Ethyl acetate and methanol fractions of JU sample had strong scavenging of DPPH free radical with low ctytotoxicity than that of the sundarbans sample. Thus it is important to carry out extensive phytochemical and pharmacological studies on the ethyl acetate and methanol fractions of JU sample to eventually find out new bioactive compound.

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