- Short communication

Antimicrobial and Cytotoxic Activities of *Bryophyllum daigremontianum*

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The *n*-hexane, carbon tetrachloride and chloroform soluble fractions of a crude methanol extract of the whole plant of *Bryophyllum daigremontianum* were subjected to antimicrobial activity and brine shrimp lethality bioassay. The carbon tetrachloride soluble partitionate of the methanolic extract exhibited significant antimicrobial activity and strongest cytotoxicity having LC_{50} of 0.78 µg/ml.

B. daigremontianum (Bengali name- Pathorkuchi, Family- Crassulaceae) is a perennial, glabrous, succulent herb with simple, opposite, succulent, oblong-lanceolate, serrate, obtuse, purple blotched beneath, petiole 2-5 cm long leaves. It is native to Madagascar and naturalized in many parts of tropical and subtropical Africa, Asia (Indian Ocean islands), North America and South Africa and also found in Bangladesh. *Bryophyllum* is reported for various ethnomedical uses such as antitumor[1], antinociceptive, anti-inflammatory and antidiabetic[2] and antimicrobial activities.[3] Previous phytochemical studies with *Bryophyllum* species revealed the occurrences of bryophollenone, bryophollone, cholestane-3,6,14-triol, 3,3',4',5,5',7-hexahydroxyflavan, 3-hydroxy-12,20-ursadien-11-one, 2-(9-decenyl) phenanthrene, bryophyllin-A,[4] bryophyllin B,[5] bryotoxin B, bryotoxin C and 3,5,11,14-tetrahydroxy-12, 19-dioxobufa-20, 22-dienolide.[6]

The aerial part of *B. daigremontianum* was collected from Savar, Dhaka in January 2004. A voucher specimen has been deposited in the Department of Botany, University of Dhaka.

About 533 gm of the powdered material was soaked in 1.5 liter of methanol in a large flask and was kept for 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. A portion (5.0 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[7] which afforded of *n*-hexane (0.35 g), carbon tetrachloride (0.25 g), chloroform (0.15 g) and aqueous soluble (2.29 g) materials.

The antimicrobial activity of the crude extract as well as *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions was determined by the disc diffusion method.[8] The bacterial and fungal strains used for the experiment were collected as pure

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cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. The extractives were dissolved separately in chloroform and methanol as required and applied to sterile filter paper discs at 300 µg/disc and carefully dried to evaporate the residual solvent. Standard kanamycin (30 µg/disc) discs were used as positive control.

For cytotoxicity screening the *n*-hexane (HF), carbon tetrachloride (CTF), chloroform soluble materials (CF) and the crude methanol extract (ME) were separately dissolved in DMSO. The test samples were then applied against *Artemia salina* in a 1-day in *vitro* assay.[9, 10] Four mg of each of the extractives (HF, CTF, CF and ME) was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/ml were obtained by serial dilution technique. Vincristine sulphate and DMSO were used as the positive and negative control, respectively. Table 1 shows the results of the brine shrimp lethality bioassay after 24 hr exposure of the shrimps to all the samples and the positive control, vincristine sulfate.

Both the bioassays were performed in triplicate. The zone of inhibition and LC_{50} were calculated as mean ± SD (n=3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

Table 1. Brine shrimp lethality bioassay of *B. daigremontianum* extractives

Sample	LC_{50} (µg/ml)	95% Confidence Limit	Regression equation	K^2	
				Calculated	Tabular
VS	0.44	0.20-0.98	Y=0.5805X +1.502	1.125	15.507
HF	70.71	28.64-174.57	Y=0.5841X +0.6107	0.503	15.507
CTF	0.78	-	Y=0.2818X+ 4.534	1.634	15.507
CF	4.42	2.37-8.23	Y=0.3955X+ 3.636	1.559	15.507
ME	59.46	16.09-219.77	Y=0.7503X -1.2367	-	15.507

The values of LC_{50} are expressed as mean ± SD (n=3). VS: vincristine sulphate (Std.); HF: *n*-hexane soluble partitionate; CTF: carbon tetrachloride soluble partitionate; CF: chloroform soluble partitionate; ME: methanolic extract.

Table 2. Antimicrobial activity of *B. daigremontianum* extractives

Test microorganisms	Diameter of zone of inhibition (mm)				
	HF	CTF	CF	ME	KAN
Gram positive bact.					
Bacillus cereus	-	10.23 ± 0.21	-	10.2 ± 0.17	15 ± 0.17
B. megaterium	12.27 ± 0.23	10.20 ± 0.2	-	-	18 ± 0.2
B. subtilis	-	12.27 ± 0.25	-	-	17 ± 0.17
Staphylococcus aureus	-	13 ± 0.2	-	-	15 ± 0.2
Sarcina lutea	-	12.23 ± 0.21	10.20 ± 0.2	-	15 ± 0.21
Gram negative bact.					
Escherichia coli	-	14.23 ± 0.21	-	-	16 ± .0.25
Pseudomonas aeruginosa	-	8.17 ± 0.15	8.27 ± 0.25	-	12 ± 0.26
Salmonella paratyphi	-	10.30 ± 0.26	-	-	16 ± 0.15
S. typhi	11.23 ± 0.25	10.20 ± 0.2	10.30 ± 0.26	10.2 ± 0.25	15 ± 0.23
Shigella boydii	-	10.23 ± 0.21	9.20 ± 0.2	-	15 ± 0.2
S. dysenteriae	-	8.30 ± 0.26	-	-	16 ± 0.17
Vibrio mimicus	-	10.23 ± 0.25	-	-	16 ± 0.26
V. parahemolyticus	-	9 ± 0.2	-	-	15 ± 0.2
Fungi					
Candida albicans	-	12.20 ± 0.2	8.10 ± 0.17	-	15 ± 0.17
Aspergillus niger	8.23 ± 0.21	8.30 ± 0.26	8.23 ± 0.25	-	15 ± 0.21
Saccharomyces cerevacae	8.23 ± 0.25	-	8.30 ± 0.26	-	10 ± 0.23

The diameter of zone of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; HF: *n*-hexane soluble partitionate; CTF: carbon tetrachloride soluble partitionate; CF: chloroform soluble partitionate; ME: methanolic extract; KAN: kanamycin.

The carbon tetrachloride soluble partitionate showed prominent activity against the entire range of test microorganisms (Table 2). The growth of *E. coli* was strongly inhibited with the zone of inhibition 14 mm, while it showed moderate inhibitory activity against *S. aureus* (13 mm), *S. lutea* (12 mm) and *B. subtilis* (12 mm). Mild inhibitory activity was noticed against *B. cereus* (10 mm), *B. megaterium* (10 mm), *V. mimicus* (10 mm) and *S. paratyphi* (10 mm), *S. typhi* (10 mm), *S. boydii* (10 mm). In case of fungi, the growth of *C. albicans* was strongly inhibited (12 mm). The crude methanolic extract of the whole plant showed mild inhibitory activity against the growth of *B. cereus* and *S. typhi,* each having zone of inhibition of 10 mm. The rest of the microorganisms were almost insensitive to it. At the same time, the *n*-hexane soluble partitionate of methanolic extract showed moderate inhibitory activity against *B. megaterium* (12 mm). The growth of *S. typhi* was moderately inhibited having zone of inhibition 11 mm. On the other hand, the chloroform soluble fraction of the methanolic extract exhibited mild inhibitory activity against *S. typhi* (10 mm) and *S. lutea* (10 mm). In case of fungi the average zone of inhibition was 08 mm.

The LC_{50} values of *n*-hexane, carbon tetrachloride, chloroform soluble fraction and methanol extract were found to be 70.71μg/ml, 0.78 μg/ml, 4.42 μg/ml and 59.46 μg/ml, respectively. From the results of the brine shrimp lethality bioassay it can be well predicted that the crude extract and Kupchan fractions have considerable cytotoxic potency. It has been found from the above discussion that the crude methanolic extract along with *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions of *B. daigremontianum* have significant antimicrobial and cytotoxic activities, which supports the traditional use of this plant in various infectious diseases.

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