Preliminary Antimicrobial Activity and Cytotoxicity of *Brunfelsia latifolia*

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The *n*-hexane and chloroform soluble portions of the methanolic extract of *Brunfelsia latifolia* were subjected to antimicrobial screening and brine shrimp lethality bioassay. The *n*-hexane soluble fraction exhibited moderate antibacterial activity, while the *n*-hexane and chloroform soluble materials demonstrated significant cytotoxicity with LC_{50} of 4.22 and 4.10 µg/ml, respectively.

The plant, *B. latifolia* (Synonym: *B. bonodora*, *B. australis;* Bengali name: Shusoma), is an evergreen shrub belonging to the Solanaceae family. The plant grows well in the tropical areas of the world and it is found all over Bangladesh.[1-3] The species of this genus are reported to have analgesic, anti-inflammatory and CNS depressant activities.[4,5] Fruits of *Brunfelsia* are toxic to canine and small children.[6] Previous phytochemical investigations with *Brunfelsia* led to the isolation of coumarins and alkaloids.[7]

The aerial parts of *B. latifolia* were collected from Bolda garden, Dhaka in January 2005. It was identified at the Department of Botany, University of Dhaka. The stem bark of the plant was separated from the stem and cut into small pieces, cleaned, dried and pulverized.

The powdered stem bark (200 g) of *B. latifolia* was soaked in 500 ml methanol, filtered and concentrated using a rotary evaporator at low temperature (36-40°C) and reduced pressure. An aliquot (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning protocol[8] into *n*-hexane, carbon tetrachloride and chloroform to afforded *n*- hexane (1.7 g), carbon tetrachloride (0.4 g), chloroform (1.3 g) and 1.4 mg aqueous soluble materials (1.4 g).

The preliminary antimicrobial activity of the extractives was determined at 400 µg/disc by the disc diffusion method[9] against a number of Gram positive and Gram negative bacteria and fungi (Table 1). The bacterial and fungal strains used in this experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Here standard Kanamycin (30 µg/disc) was used as the reference.

For *in vivo* cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia salina* for 24 hours.[10] For the experiment 4 mg of each of the plant extracts was dissolved in DMSO and by serial dilution technique, solutions of

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varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained. After 24 hours, the median lethal concentration, LC_{50}, of the test samples was obtained by a plot of percentage of shrimps killed against the logarithm of the sample concentration.

The *n*-hexane and chloroform soluble fractions of the methanolic extract of *B. latifolia* showed the average zone of inhibition of 10-15 mm and 7-14 mm, respectively at a concentration of 400 µg/disc. The *n*-hexane solubles of the bark strongly inhibited the growth of *B. cereus*, *S. bodii* and *C. albicans* having the zone of inhibition of 15 mm. The growth of *P. aureus* (14 mm), *A. niger* (14 mm) and *S. lutea* (13 mm) was also inhibited. It also showed mild inhibition of growth of *E. coli*, *S. dysenteriae* and *S. cerevacee*. On the other hand, the chloroform soluble material demonstrated mild activity against most of the organisms. In case of fungal strains, the *n*-hexane soluble materials showed strong inhibitory activity against *C. albican*s (15 mm) and *A. niger* (15 mm). However, the chloroform extracts revealed mild inhibitory activity against the fungal growth.

Table 1. Preliminary antimirobial activity of *B. latifolia* extractives.

Test bacteria and fungi	Diameter of zone of inhibition (mm)		
	HX (400 µg/disc)	CF (400 µg/disc)	KAN (30 µg/disc)
Gram positive bacteria			
Bacillus cereus	15	10	40
B. megaterium	--	14	50
B. subtilis	10	08	40
Sarcina lutea	13	07	24
Staphylococcus aereus	10	08	45
Gram negative bacteria			
Escherichia coli	12	10	26
Pseudomonas aeruginosa	14	08	23
Salmonella typhi	10	08	30
Salmonella paratyphi	10	07	50
Shigella boydii	15	08	23
Shigella dysenteriae	12	07	24
Vibro mimicus	10	07	45
V. parahemolyticus	10	08	30
Fungus			
Aspergillus niger	14	11	20
Candida albicans	15	08	25
Sacharomyces cerevaceae	12	10	23

HX: *n*-hexane soluble fraction of the methanol extract; CF: chloroform soluble fraction of the methanol extract extract; KAN: Kanamycin; "--": indicates no activity.

The *n*-hexane and chloroform soluble fractions of the methanol extract were subjected to brine shrimp lethality bioassay by using the method of Meyer.[10] In the brine shrimp lethality bioassay, the LC_{50} values exhibited by the *n*-hexane and chloroform soluble fractions were found to be 4.22 µg/ml and 4.10 µg/ml, respectively. The standard, vincristine sulphate, showed the LC_{50} value of 0.23 µg/ml. It is clearly evident from the experiment that the *n*-hexane and chloroform soluble fractions demonstrated strong cytotoxic activity. The antimicrobial activity and cytotoxicity revealed by the various extractives of *B. latifolia* substantiate the folk uses of this plant in various diseases.

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