- Short communication

Antimicrobial and Cytotoxic Activities of the Crude Extracts of *Hopea scaphula*

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The extractives of *Hopea scaphula* (Dipterocarpaceae) were subjected to antimicrobial screening and brine shrimp lethality bioassay. In case of antimicrobial screening, petroleum ether and ethyl acetate extracts exhibited moderate antibacterial activity, while the petroleum ether extract demonstrated highest cytotoxicity with LC_{50} of 2.00 µg/ml in the brine shrimp lethality bioassay.

Hopea scaphula (Syn: *Anisoptera scaphula, Vatica scaphula, Anisopteris glabra* and *Scaphula glabra*; Bengali name - Boilsur, Boilam or Sada Boilam; Family- Dipterocarpaceae) is a very tall tree which grows in Chittagong and the neighboring Hill-Tracts areas of Bangladesh.[1] The species of this genus are reported to have anititumor,[2] cytotoxic, anti-HIV[3], astringent, CNS depressant, hypotensive and antifungal[4] properties.

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Previous phytochemical investigations with *Hopea* species led to the isolation of hopeanolin,[5] (-)-hopeaphenol,[6] balanocaprol, dibalanocaprol,[3] cycloartane triterpenoid,[7] hopeaphenol A, isohopeaphenol A[8] and vaticanol C.[2]

The stem bark of *H. scaphula* was collected from Mirpur, Dhaka in the month of August 2004. A voucher specimen (DACB - 32064) representing this collection has been deposited in Bangladesh National Herbarium, Dhaka.

The air-dried and powdered stem bark (200.5 g) was successively extracted with petroleum ether (7 days), ethyl acetate (7 days) and methanol (7 days) at room temperature with occasional shaking and stirring. The extractives were filtered through fresh cotton plug and followed by Whatman No.1 filter paper. The filtrates were then concentrated by a Buchii rotavapor at low temperature and pressure and afforded pet-ether extract (PE, 1.7 g), ethyl acetate extract (EA, 1.5 g) and methanol extract (ME, 1.0 g).

The antimicrobial activity of the crude extracts was determined by the disc diffusion method,[9] against the microbial strains listed in Table 1. These

were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Here, Kanamycin (30 µg/disc) was used as the standard. The pet-ether and ethyl acetate extracts were dissolved separately in chloroform and applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent.

For cytotoxicity screening, DMSO solutions of the petroleum ether, ethyl acetate and methanol extracts were applied against *Artemia salina*[10] in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the pet-ether, ethyl acetate and methanol extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml) were obtained by serial dilution technique for each extract.

Table 1. Antimicrobial activity of *H. scaphula* extractives (400 µg/disc) and Kanamycin (30 µg/disc)

Test microorganisms	Diameter of zone of inhibition (mm)		
	PE	EA	KAN
Gram Positive			
Bacillus cereus	14.20 ± 0.10	9.36 ± 0.09	34.77 ± 0.25
B. megaterium	-	-	34.60 ± 0.40
B. subtilis	11.43 ± 0.40	11.30 ± 0.15	37.00 ± 0.20
Staphylococcus aureus	10.07 ± 0.12	11.30 ± 0.19	35.10 ± 0.36
Sarcina lutea	10.37 ± 0.32	10.11 ± 0.11	31.83 ± 0.21
Gram Negative			
Escherichia coli	10.18 ± 0.16	11.33 ± 0.17	35.57 ± 0.40
Pseudomonas aeruginosa	11.27± 0.25	12.10 ± 0.09	35.17 ± 0.16
Salmonella paratyphi	10.11 ± 0.10	12.30 ± 0.17	37.70 ± 0.26
S. typhi	12.27 ± 0.25	13.14 ± 0.10	34.97 ± 0.15
Shigella boydii	11.12 ± 0.13	12.21 ± 0.12	32.14 ± 0.12
S. dysenteriae	11.17 ± 0.15	11.32 ± 0.16	34.93 ± 0.21
Vibrio mimicus	9.13 ± 0.12	-	34.80 ± 0.22
V. parahemolyticus	10.17 ± 0.15	10.25 ± 0.16	36.71 ± 0.42
Fungi			
Candida albicans	12.21 ± 0.18	10.30 ± 0.16	24.90 ± 0.10
Aspergillus niger	12.33 ± 0.15	10.35 ± 0.19	22.98 ± 0.23
Sacharomyces cerevacae	11.07 ± 0.12	11.35 ± 0.18	22.88 ± 0.29

The diameter of zone of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; PE: pet. ether extract; EA: ethyl acetate extract; KAN: kanamycin.

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 2. LC_{50} data of *H. scaphula* extracts and vincristine sulfate.

Samples	LC_{50} (µg/ml)
Vincristine sulphate	0.212 ± 0.10
PE	2.00 ± 0.23
EA	4.47 ± 1.77
ME	4.90 ± 1.33

The values of LC_{50} are expressed as mean ± SD (n=3). VS: vincristine sulphate (Std.); PE: pet-ether extract; EA: ethyl acetate extract; ME: methanol extract.

The crude pet-ether and ethyl acetate extracts showed moderate antibacterial activity with the average zone of inhibition of 9 - 14 mm and 9 - 13 mm, respectively, at 400 µg/disc. The pet-ether extract showed the highest activity against the growth of *B. cereus* having the zone of inhibition of 14 mm. Besides this, the growth of *S. typhi* (12.27 mm), *B. subtilis* (11.43 mm), *P. aeruginosa* (11.27 mm), *S. boydii* (11.12 mm) and *S. dysenteriae* (11.17 mm) were moderately inhibited. In case of fungi, the average zone of inhibition was found to be 11-12 mm. At the same time, the ethyl acetate extract also inhibited the growth of *S. typhi* (13.14 mm), *P. aeruginosa* (12.10 mm), S. *paratyphi* (12.30 mm), *S.*

boydii (12.21 mm), *B. subtilis* (11.30 mm), *S. aureus* (11.30 mm), *E. coli* (11.33 mm) and *S. dysenteriae* (11.32 mm) moderately. The same extract also exhibited mild to moderate inhibitory activity against the growth of fungal strains.

Following the procedure of Meyer[10], the lethality of the pet-ether (PE), ethyl acetate (EA) and methanol (ME) extracts to brine shrimp were evaluated on *A. salina* after 24 hours[10] of exposure the samples and the positive control, vincristine sulphate (VS). The LC_{50} were found to be 2.00, 4.47, 4.90 and 0.21 µg/ml for PE, EA, ME extracts and VS, respectively. The cytotoxicity exhibited by the crude extracts was promising and this clearly indicates the presence of potent bioactive compounds.[10]

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