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Short Communication

Biological Activity Study on a *Malvaceae* Plant: *Bombax ceiba*

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Abstract

The work described in this paper details the biological investigation on *Bombax ceiba*, species of *Malvaceae*. The methanol crude extract of *Bombax ceiba* was fractionated with kupchan method and *n*-hexane, carbon tetrachloride, chloroform fraction were made for screening the antimicrobial and antitumor potentials using disc diffusion method and brine shrimp lethality bioassay respectively. An established antibiotic (kanamycin, 30µg/disc) and cytotoxic agent (vincristine sulphate) were used to compare the results. From the graphs the concentration of methanolic crude extract give LC_{50} (50% mortality) value of 3.90µg/ml. LC_{90} was also determined from the graph to establish the therapeutic index and the value was found 150.0µg/ml. The four fractions were assayed for antimicrobial screening and the carbon tetrachloride fraction showed most prominent zone of inhibition against a number of bacterial and fungal strains.

Keywords: Bombax ceiba; Brine shrimp lethality bioassay; Disc diffusion method.

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1. Introduction

Bangladesh is a good storehouse of medicinal plants. *Malvaceae* is a family of flowering plants containing a number of genera, generally with five showy petals and five green sepals and numerous stamens that are fused at the base and form a tube around the pistil. Most species are herbs or shrubs but some species are trees. There are over 100 genera with close to 1,500 species in the family depending on the classification of some of the genera. In the broader APG circumscription, the *Malvaceae* are also a cladistically monophyletic group but take in a much larger number of genera, *Bombacaceae* is one of them that include a species named *Bombax ceiba*. They are native to tropical areas in

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western Africa, the Indian subcontinent, Southeast Asia. Common names for the genus include Silk Cotton Tree, Simal, Red Cotton Tree, Kapok [1-11].

Previous chemical studies revealed the isolation of 2-(2,4,5-trihydroxyphenyl)-3,5,7-trihydroxy-6-C- glucopyranosyloxy-4H-1-benzopyran-4-one [12], shamimicin (1''',1''''''-bis-2-(3, 4-dihydroxyphenyl)-3, 4-dihydro-3, 7-dihydroxy-5-O-xylopyranosyloxy-2H-1-benzopyran) [13], lupeol [14]. Antiangiogenic activity [14], antioxidant activity and cytotoxic activity [15] was detected from the methanolic extract of *B. Ceiba*. Furthermore, Inhibitory effects on tube-like formation of human umbilical venous cells [16], hypotensive and hypoglycemic activities were reported for this genus [17].

2. Plant materials

Root sample of *Bombax ceiba* was collected from an ayurbadic shop of Dhaka new market in September 2007. It was then air-dried and powdered with crushing machine. Then the powdered material was successively extracted with methanol by using cold extraction process [18]. The crude extract was then fractionated into *n*-hexane, carbon tetrachloride and chloroform by using kupchan partitioning method [19].

3. Methods for Biological Activity Study

3.1. *Method for cytotoxic study*

4.0 mg of each fraction (*n*-hexane, carbon tetrachloride, chloroform and methanol) of *Bombax ceiba* were taken and dissolved in 200µl of pure dimethyl sulfoxide (DMSO) in two vials to get stock solutions of 400µg/ml. A series of solutions of different concentrations were prepared from the stock solution by serial dilution method and the concentrations were as: 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml. Then the samples were subjected to brine shrimp lethality bioassay [20, 21] for cytotoxic studies. In each test tube, containing different concentrations of test sample, 10 brine shrimp nauplii (*Artemia salina*) were added.

Two control groups were used in cytotoxicity study, to validate the test method and results obtained due to the activity of the test agent. In the study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml and serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml and 0.0390 µg/ml of concentration. 30 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as negative control groups. After 24 hours, the test tubes were observed and the numbers of survived nauplii in each test tube were counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample.

3.2. *Method for antimicrobial assay*

Collected all fractions, i.e. *n*-hexane, carbon tetrachloride, chloroform and methanol extracts were tested for antimicrobial study by using standard disc diffusion method [22, 23]. In this study, 16 microorganisms were obtained from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka, Bangladesh. Standard Kanamycin (30 μg/disc) and blank sterile filter paper disc (diameter, 6 mm) were used as positive and negative controls, respectively. Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures. The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates, pre-inoculated with test bacteria. The discs were then incubated on the plate aerobically at 37ºC for 24 hours. The diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period.

4. Result and Discussion

4.1. *Cytotoxic study*

In the present bioactivity study, the crude methanolic extract showed positive results indicating that the test samples are biologically active. Plotting of log of concentration (log *C*) versus percent mortality (% mortality) for all test samples showed an approximate linear correlation (Fig. 1). From the graph, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined but in few cases due to higher mortality rates LC_{50} cannot be determined and for them LC_{90} values were determined.

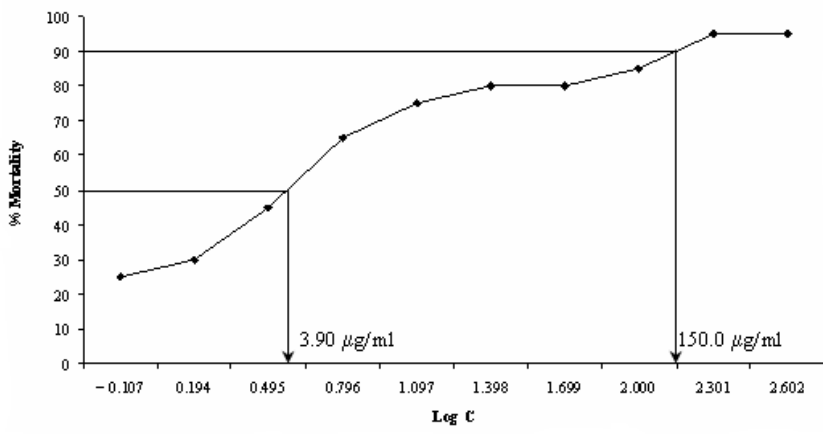

Fig. 1. Determination of LC_{50} and LC_{90} of methanolic extract of *Bombax ceiba.*

The crude extract of *Bombax ceiba* showed significant cytotoxic activity against brine shrimp nauplii and LC_{50} value was found to be 3.90μg/ml whereas the LC_{90} (90% mortality rate) was calculated 150.00μg/ml to get the therapeutic index (Table 1). For the conformity of the result, the test was done for two times. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC_{50} were calculated using Microsoft Excel 2000. All the values were compared with standard cytotoxic agent, vincristine sulphate for which LC_{50} was found to be 0.625 μg/ml.

Table 1. Brine shrimp lethality bioassay of methanolic extract of *Bombax ceiba.*

Conc. (μg/ml)	LogC	% Mortality (1)	% Mortality (2)	Mean	LC_{50} (μg/ml)	LC_{90} (μg/ml)
400	2.602	100	90	95	3.90	150.0
200	2.301	100	90	95		
100	2.000	90	80	85		
50	1.699	80	80	80		
25	1.398	80	80	80		
12.5	1.097	70	80	75		
6.25	0.796	60	70	65		
3.125	0.495	50	40	45		
1.563	0.194	20	40	30		
0.781	− 0.107	20	30	25		

4.2. *Antimicrobial study*

From the study, the zones of inhibition produced by the methanol, *n*-hexane, chloroform and carbon tetrachloride extract were found to be 10 − 14 mm, 09 − 15 mm and 13 − 20 mm respectively (Table 2) at a concentration of 200 μg/disc in case of 09 bacterial strain and 02 fungal strain but the bacterial strain of *B. subtilis, S. aureus, S. boydii, S. dysenteriae* and one fungal strain (*Saccharromyces cevevaceae*) showed no sensitivity. The significant activity was found by hexane extract against *Sarcina lutea* (13mm) and *Pseudomonas aeruginosa* (12mm). The chloroform extract showed prominent activity against *Vibrio mimicus* (15mm); significant activity against *Bacillus megaterium* (12mm) and *Vibrio parahemolyticus* (12mm). The carbon tetrachloride extract showed prominent (zone of inhibition >15mm) activity against almost all bacterial strain.

All the fractions of *Bombax ceiba* were also tested for antifungal activity against 03 fungi. The extracts had inhibitory effect against all the test pathogens in different degree. The chloroform extract and carbon tetra chloride extract showed profound activity against *Aspergillus niger* and *Candida albicans*, respectively.

Table 2. Antibacterial and antifungal activity of different extracts of *Bombax ceiba.*

Test organism	Diameter of zone of inhibition (mm)				
	Methanol extract (200 µg/disc)	Hexane extract (200 µg/disc)	$CHCl_3$ extract (200 µg/disc)	CCl_4 extract (200 µg/disc)	Kanamycin (30 µg/disc)
Gram positive bacteria					
Bacillus cereus (BTCC-19)	10	08	11	20	10
Bacillus megaterium (BTCC-18)	12	11	12	16	19
Bacillus subtilis	na	na	na	na	08
Staphylococcus aureus (BTCC-43)	na	na	na	na	10
Sarcina lutea (ATCC-9341)	11	13	09	15	17
Gram negative bacteria					
Escherichia coli (BTCC-172)	10	09	11	18	09
Pseudomonas aeruginosa (BTCC-1252)	13	12	09	18	15
Salmonella paratyphi	14	10	11	16	09
Salmonella typhi	12	08	12	17	15
Shigella boydii	na	na	na	na	11
Shigella dysenteriae	na	na	na	na	06
Vibrio mimicus	13	10	15	16	08
Vibrio parahemolyticus	11	07	12	16	12
Fungi					
Saccharromyces cevevaceae	na	na	na	na	07
Candida albicans	10	07	13	14	10
Aspergillus niger	12	13	14	13	09

na = no activity.

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References

1. M. D. Dassanayake and F. R. Fosberg, A revised handbook to the flora of Ceylon (Amerind Publishing Company, New Delhi, India, 1980).

2. A. J. C. Grierson, and D. J. Long, Flora of Bhutan including a record of plants from Sikkim (Royal Botanic Garden, Edinburgh & Royal Government of Bhutan, 1984).
3. H. Hara, A. O. Chater, and L. H. J. Williams, An enumeration of the flowering plants of Nepal (Springer, Netherlands, 1978-82).
4. R. J. Hnatiuk, Census of Australian vascular plants. Australian Flora and Fauna Series No. 11. (Australian Govt. Publication Service, 1990). ISSN: 0813-6726.
5. E. D. Merrill, An enumeration of Philippine flowering plants (New Day Publishers, Quezon City, Philippines, 1922-1926).
6. E. Nasir and S. I. Ali, Flora of [West] Pakistan (University of Karachi, Pakistan, 1970).
7. S. Rehm, Multilingual dictionary of agronomic plants (Kluwer Academic Publishers, Dordrecht-Boston-London, 1994).
8. A. Robyns, Taxon **10**, 156 (1961). doi:10.2307/1216003
9. A. Robyns, Bull. Jard. Bot. État **33**, 88 (1963).
10. B. D. Sharma, and M. Sanjappa, with assistannce from N. P. Balakrishnan (Ed.) Flora of India, Vol. 3 (BSI, Calcultta, Deep Printers, New Delhi, 1993).
11. R. E. Woodson and R. W. Schery, Flora of Panama (Missouri Botanical Garden Press, St. Louis, 1943-1980).
12. S. Faizi and M. Ali, Planta Med. **65** (4), 383 (1999). doi:10.1055/s-2006-960796
13. R. Saleem, S. I. Ahmad, M. Ahmed, Z. Faizi, S. Zikr-ur-Rehman, M. Ali, and S. Faizi, Biol Pharm Bull. **26** (1), 41 (2003). doi:10.1248/bpb.26.41
14. Y. J. You, N. H. Nam, Y. Kim, K. H. Bae, and B. Z. Ahn, Phytother Res. **17** (4), 341 (2003). doi:10.1002/ptr.1140
15. T. O. Vieira, A. Said, E. Aboutabl, M. Azzam, and T. B. Creczynski-Pasa, Redox Rep. **14** (1), 41 (2009). doi:10.1179/135100009X392485
16. N. H. Nam, H. M. Kim, K. H. Bae, and B. Z. Ahn, Phytother Res. **17** (2), 107 (2003). doi:10.1002/ptr.934
17. R. Saleem, M. Ahmad, S. A. Hussain, A. M. Qazi, S. I. Ahmad, M. H. Qazi, M. Ali, S. Faizi, S. Akhtar, and S. N. Husnain, Planta Med. **65** (4), 331 (1999). doi:10.1055/s-1999-14060
18. G. E. Trease and W. C. Evans. Trease and Evans' Pharmacognosy, 13th edition (Baillire Tindall, London, 1989).
19. B. C. Van Wagenen, R. Larsen, J. H. Cardellina II, D. Randazzo, Z. C. Lidert, and C. Swithenbank, J. Org. Chem. **58**, 335 (1993). doi:10.1021/jo00054a013
20. A. L. Goldstein and S. M. Kalkan, Principles of drug action, 2nd edition (Wiley Biochemical Health Publications, 1974) pp. 376-381.
21. B. B. Meyer, N. R. Ferringi, F. J. Futman, L. B. Jacobsen, D. E. Nichols, and J. L. Mclaughlin, Planta Medica **5**, 31 (1982). doi:10.1055/s-2007-971236
22. S. M. A. Zavala, G. S. Perez, and G. M. Perez, Phytotheraphy Res. **11**, 368 (1997). doi:10.1002/(SICI)1099-1573(199708)11:5<368::AID-PTR109>3.0.CO;2-6
23. P. R. Murray, E. J. Baron, M. A. Pfallar, F. C. Tenover, and R. H. Yolke, Manual of Clinical Microbiology, 6th edition (ASM, Washington DC, 1995) pp. 214-15.