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Antimicrobial and Cytotoxic Activities of the Crude Methanolic Extracts of *Xylia dolabriformis*

Fataha Nur Robel[1], Md. Shaikh Ahsan Ullah[1], Choudhury M. Hasan[2], A.M. Sarwaruddin Chowdhury[1*] and Md. Abul Kaiser[2]

[1]*Department of Applied Chemistry and Chemical Engineering, Dhaka University, Dhaka-1000, Bangladesh.*

[2]*Department of Pharmaceutical Chemistry, Dhaka University, Dhaka-1000, Bangladesh.*

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Abstract

The partition fractions of crude methanolic extracts such as chloroform, n-hexane, carbon tetrachloride and aqueous soluble fractions of *Xylia dolabriformis* (Family- Fabaceae) were subjected to antimicrobial screening and brine shrimp lethality bioassay. In case of antimicrobial screening only the chloroform soluble fraction showed mild to moderate antimicrobial activity. From the results of brine shrimp lethality bioassay it can be safely predicted that carbon tetrachloride & chloroform soluble fractions were highly cytotoxic with $\mathbf{LC_{50}}$ of 3.89 and 3.80 µg/ml respectively and n-hexane and aqueous phase were moderately cytotoxic with $\mathbf{LC_{50}}$ of 7.08 and 5.01 respectively.

Keywords: Antimicrobial activity, Cytotoxic activity, Brine shrimp lethality bioassay.

I. Introduction

Xylia dolabriformis is a large deciduous tree. Its bark is grey or reddish-brown with short cracks irregularly distributed. Its leaves are tripinnate. Their flowers are pale yellow, in globose long-pedunculate heads. Seeds 6-10, compressed, testa brown, shining. Geogrphicaclly they are distributed in Africa and Asia [1].

II. Materials and Methods

Plant collection

The stem of the plant *Xylia dolabriformis* was collected from Bangladesh National Botanical Garden in the month of May 2009 and was identified by a taxonomist. A voucher specimen that contained the identification characteristics of the plant was submitted to the Bangladesh National Herbarium; Dhaka for future reference. The specimen bears **DACB Accession no. 32761.**

Extraction

The stem bark of the plant was collected in fresh condition. Then it was washed with water to remove dust and other foreign particles. It was then chopped into small pieces and sun-dried for few days and then, dried in an oven at reduced temperature (not exceeding 50^0C) to make it suitable for grinding purpose. The stem barks were ground into coarse powder using a grinding machine.

400 g of the powder materials was extracted with methanol in an air tight bottle for 7 days with occasional shaking and stirring. The extract was then filtered off through a cotton plug and finally with Whatman no. 1 filters papers. The volume of the filtrate was concentrated with a rotary film evaporator at a low temperature and pressure. The concentrated methanol extract was subjected to solvent-solvent partitioning by using different types of solvent as n-hexane, carbon tetrachloride and chloroform respectively. Thus n-hexane, carbon tetrachloride, chloroform as well as aqueous phase were collected separately and evaporated to dryness.

Antimicrobial Activity

The antimicrobial activity of the partition fractions was determined by the disc diffusion method [2,3] 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 standard. The partition fractions were dissolved separately in chloroform and then applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent. 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 and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

Cytotoxic Activity

Brine shrimp lethality bioassay [4,5,6] technique was applied for the determination of cytotoxic propety of the plant extractives. Vincristine sulphate and DMSO were used as positive and negative control, respectively. 4 mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for eachconcentration. The median lethal concentration (LC50) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

* Corresponding Author: E-mail: profdrsarwar@yahoo.com

III. Result and Discussion

Antimicrobial activities of four partition fractions, viz, chloroform, n-hexane, carbon tetrachloride and aqueous soluble fractions of *Xylia dolabriformis* and kanamycin (30 µg/disc) were investigated.

Only chloroform fraction significantly exhibited antibacterial and antifungal activity. Other fractions did not show any activity.

The zone of inhibition produced by the chloroform fraction was found to be 9-10 mm at a concentration of 400 µg/disc.

The chloroform fraction showed notable activity against *Bacillus cereus* (9 mm), *Bacillus megaterium* (10 mm), *Staphylococcus aureus* (10 mm), *Sarcina lutea* (10 mm), *Escherichia coli* (1o mm), *Pseudomonas aeruginosa* (10 mm), *Salmonella paratyphi* (10 mm), *Salmonella typhi* (9 mm), *Shigella boydii* (10 mm), *Shigella dysenteriae* (10 mm), *Vibrio mimicus* (10 mm), *Vibrio parahemolyticus* (10 mm), *Candida albicans* (9 mm), *Aspergillus niger* (10mm) & *Sacharomyces cerevacae* (9mm). The results are shown in Table-1.

It is concluded from the above data that none of the fractions but chloroform fraction had significant activity against the pathogenic bacteria.

Partition fractions such as n-hexane, carbon tetrachloride, chloroform and aqueous soluble extracts and positive control vincristine sulphate were screened by brine shrimp lethality bioassay for probable cytotoxic activity.

The LC_{50} value for n-hexane, chloroform, carbon tetrachloride, aqueous soluble fractions and vincristine sulphate were found to be 7.08, 3.80, 3.89, 5.01 and 0.33 µg/ml, respectively (listed in Table-2). This indicated that carbon tetrachloride & chloroform soluble fractions were highly cytotoxic. And n-hexane and aqueous soluble fractions had shown moderate cytotoxicity. The results have been summarized in Table-2.

Comparing with positive control, vincristine sulphate signifies that the cytotoxicity exhibited by the partition fractions is optimistic and they might have antitumor or pesticidal compounds. A thorough chemical study is required to isolate the molecules that are responsible for the activities. [4, 5, 6]

Table.1. Antimicrobial activity of four partition fractions (chloroform, n-hexane, carbon tetrachloride and aqueous soluble fractions) of *Xylia dolabriformis* and kanamycin (30 µg/disc).

Test bacteria and fungi	Diameter of zone of inhibition (mm)				
	Chloroform	n-hexane	Carbon tetrachloride	Aqueous phase	Kanamycin
Gram positive					
Bacillus cereus	9	7	-	-	34
Bacillus megaterium	10	-	7	-	34
Bacillus subtilis	10	-	-	-	34
Staphylococcus aureus	10	-	-	-	34
Sarcina lutea	10	-	-	-	34
Gram Negative					
Escherichia coli	10	-	-	-	34
Pseudomonas aeruginosa	10	7	-	-	34
Salmonella paratyphi	10	-	7	-	34
Salmonella typhi	9	-	-	-	34
Shigella boydii	10	-	-	-	34
Shigella dysenteriae	10	7	-	-	34
Vibrio mimicus	10	-	7	-	34
Vibrio parahemolyticus	10				34
Fungi					
Candida albicans	9	-	7	-	34
Aspergillus niger	10	7	-	-	34
Sacharomyces cerevacae	9	-	-	-	34

Table.2. LC_{50} data of partition fractions of *Xylia dolabriformis* and vincristine sulphate.

Fraction/ sample	LC_{50} (µg/ml)
Vincristine	0.33
n-Hexane	7.08
Chloroform	3.80
Carbon tetrachloride	3.89
Aqueous Phase	5.01

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