Antimicrobial and Cytotoxic Activities of the Crude Extracts and Isolated Compounds of *Xylocarpus mollucensis*

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ABSTRACT: The fractionated crude extracts and three isolated pure compounds XM-1, XM-2 and XM-3 from stem bark of *Xylocarpus mollucensis* were screened for their antibacterial and antifungal activities and cytotoxicity against brine shrimp nauplii. Petroleum ether, ethyl acetate (EtOAc) and methanol (MeOH) extracts and the compounds isolated from EtOAc fractions were studied for their antimicrobial activities. Cytotoxic activities were conducted only with EtOAc extract and its selected fractions. The EtOAc extract showed promising antimicrobial activities against all the gram positive and gram negative bacteria whereas petroleum ether extract showed moderate activities and the MeOH extract did not show any antimicrobial activities. The isolated pure compounds XM-1, XM-2 and XM-3, whose structures were not elucidated, exhibited activities against most of the bacterial strains. The cytotoxicity towards brine shrimp nauplii of the crude EtOAC extract and its selected fractions were studied. The LC_{50} values of the EtoAc extract was 12.6 µg/ml and for the fractions 2, 5, 8 and 13 were 17.78, 13.34, 14.13 and 15.85 µg/ml, respectively.

Key words: Antimicrobial, Cytotoxic, *Xylocarpus,* Meliaceae.

INTRODUCTION

The genus *Xylocarpus mollucensis* (Meliaceae) comprises 16 species of trees, which are distributed in the Indian subcontinent, Malaysia and Australia.[1] Limonoids have been isolated from the species *Xylocarpus mollucensis*, *Amoora grandifoloia*, *A. rohituka* and *A. wallichii*.[2,3] Phytochemical investigation of the different species of Meliaceae resulted in the isolation of essential ester, terpenoids and steroids etc.[4] *Xylocarpus mollucensis* is a big tree that is widely distributed in the forest of Sundarban at Khulna district of Bangladesh. *Xylocarpus mollucensis* is said to cure dysentery, diarrhoea and abdominal troubles (Banglapedia).

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So far no detail phytochemical and biological studies have been carried out on this plant. In the present study, antimicrobial activities of the crude extracts and isolated compounds from EtOAc extract and cytotoxic activities of the EtOAc and selected fractions of the stem bark of *Xylocarpus mollucensis* have been discussed.

MATERIALS AND METHODS

The stem bark of *Xylocarpus mollucensis* was collected from the forest of Sundarban at Khulna district of Bangladesh. The sun-dried stem bark was ground mechanically and extracted in a soxhlet apparatus successively with petroleum ether, ethyl acetate and methanol. The extracts were then concentrated in *vacuo* using a Buchii rotavapor. The

EtOAc extract was then fractionated by vacuum liquid chromatography (VLC) over silica gel. Pure compounds were then isolated and purified from different fractions using different types of chromatographic techniques.

The *in vitro* antibacterial and antifungal activities of the crude extracts as well as the isolated pure compounds were determined by disc diffusion technique.[5] Sixteen bacterial strains, which included five gram positive and eleven gram negative organisms and eight fungi were collected from the Department of Microbology and Institute of Nutrition and Food Sciences, University of Dhaka. Nutrient agar media was used for the culture of bacteria and potato dextrose agar media was used for the culture of fungi. In brief, a measured amount of the test samples was dissolved in definite volumes of $CHCl_3$ to give solutions of known concentration (µg/ml). The sterile Matricel (BBL, cocksville USA) filter paper discs were impregnated with known amounts of the test substances and dried. Standard ampicillin disc (l0µg/ml) and disc on which $CHCl_3$ was adsorbed and dried (blank disc) were used as positive and negativre controls, respectively.

The disc was then placed in petridises (120 mm in diameter) containing Mueller- Hinton agar media seeded with the test organisms using sterile cotton swabs. The plates were then incubated at 37ºC for 24 hours. The antimicrobial activities were measured from zone of inhibition expressed in mm. All experiments were carried out in triplicate and the mean of the readings were recorded.[6] The cytotoxic activities were performed by Brine shrimp lethality test.[7]

RESULTS AND DISCUSSION

The antimicrobial and antifungal activities of the petroleum ether, EtOAc and methanol extracts of *Xylocarpus mollucensis* were determined against sixteen bacterial strains and eight fungi. The results were compared with those produced by the standard antibiotic, ampicillin trihydrate BP (10 µg/ml). The results of the sensitivity are summarized in Table 1. All strains showed sensitivity toward ethyl acetate

Table 1. Antibacterial activities of different extracts and compounds of *Xylocarpus mollucensis*

Bacteria		Zone of inhibition (mm ± SD)					
		Pet. Ether extract (3 mg/disc)	EtOAc extract (3 mg/disc)	XM-1 (l00µg/disc)	XM-2 (l00µg/disc)	XM-3 (l00µg/disc)	Ampicillin (l00 µg/disc)
Gram positive							
1	*Bacillus subtilis*	-	14 ± 0.5	12 ± 1	-	10 ± 0.5	22 ± 0.2
2	*B. megaterium*	14 ± 0.8	16 ± 0.5	14 ± 0.2	12 ± 0.4	-	20 ± 0.6
3	*B. cereus*	12 ± 0.2	12 ± 0.8	-	10 ± 0.3		
4	*Staphylococcus aureus*	12 ± 0.5	10 ± 0.4	-	-	12 ± 0.6	20 ± 0.3
5	*Sarcina lutea*	-	10 ± 0.5	11 ± 0.8	-	-	19 ± 0.2
Gram negative							
1	*Shigella sonii*	14 ± 0.4	18 ± 0.3	13 ± 0.5	12 ± 0.5	14 ± 0.6	20 ± 0.2
2	*Shigella boydii*	16 ± 0.2	17 ± 0.5	12 ± 0.8	-	14 ± 0.7	18 ± 0.3
3	*Shigella dysenteriae-type I*	12 ± 0.3	12 ± 0.2	-	12 ± 0.5	-	22 ± 0.6
4	*Shigella dysenteriae-type 2*	-	14 ± 0.5	-	-	10 ± 0.4	18 ± 0.3
5	*Escherichia coli*	-	14 ± 0.2	-	-	10 ± 0.5	20 ± 0.6
6	*Pseudomonas aeruginosa*	16 ± 0.4	18 ± 0.8	14 ± 0.7	12 ± 0.4	14 ± 0.6	22 ± 0.2
7	*Salmonella typhi*	15 ± 0.5	14 ± 0.2	12 ± 0.3	16 ± 0.5	16 ± 0.8	20 ± 0.5
8	*Salmonella paratyphi.A*	-	10 ± 0.5	-	-	-	16 ± 0.2
9	*Salmonella paratyphi B*	16 ± 0.5	16 ± 0.5	10 ± 0.2	14 ± 0.6	12 ± 0.5	19 ± 0.3
10	*Vibrio cholerae*	18 ± 0.2	16 ± 0.5	16 ± 0.4	14 ± 0.6	16 ± 0.2	22 ± 0.5
11	*V. mimicus*	14 ± 0.5	9 ± 0.8	12 ± 0.6	-	12 ± 0.4	20 ± 0.6

" - " = Indicates no zone of inhibition.

extract. Among the Gram-negative bacteria *Shigella sonii*, *Shigella boydii*, *Pseudomonas aeruginosa* and *Salmonella paratyphi B* and *Vibrio cholerae* showed promising sensitivity (16-18 mm) to ethyl acetate extract. *B. megaterium* is the Gram positive bacteria which showed promising sensitivity toward EtOAc extract. Other Gram positive and Gram-negative organism showed mild sensitivity. The petroleum ether extract demonstrated promising sensitivity against *Shigella boydii*, *Pseudomonas aeruginosa*, *Salmonella paratyphi B* and *Vibrio cholereae*; Mild activity against *B. megaterium*, *B. cereus*, *Staphylococcus aureus, Shigella sonii, Shigella dysenteriae-type I, Salmonella typhi* and *V. mimicus;* and no activity was observed against the other bacteria. Mild sensitivity was showed by *B. megaterium*, *B. cereus* and *Staphylococcus aureus* against petroleum ether extract. The methanol extract did not show any sensitivity (data not shown).

Table 2. Antifungal activities of the crude extracts and isolated compounds of *Xylocarpus mollucensis*

Name of Fungi		Zone of inhibition (mm ± SD) after 48 hr incubation					
		Pet. Ether extract (3 mg/l disc)	EtOAc extract (3 mg/l disc)	XM-1 (l00µg/disc)	XM-2 (l00µg/disc)	XM-3 (l00µg/disc)	Ampicillin (l00µg/disc)
1	*Aspergilus niger*	-	18 ± 0.6	14 ± 0.2	12 ± 0.8	13 ± 0.5	22 ± 0.4
2	*A. fumigatus*	-	16 ± 0.2	12 ± 0.5	-	12 ± 0.3	20 ± 0.5
3	*Candida albicans*	-	-	-	-	-	18 ± 0.6
4	*C. krusei*	-	-	-	-	-	20 ± 0.2
5	*Candida oryzae*	-	12 ± 0.5	12 ± 0.8	9 ± 0.8	-	22 ± 0.4
6	*Saccharromyces cerevisiae*	-	14 ± 0.6	10 ± 0.2	10 ± 0.6	-	22 ± 0.5
7	*Rhizopus oryzae*	-	12 ± 0.4	10 ± 0.6	-	10 ± 0.5	22 ± 0.8
8	*Trichoderma sp.*	-	15 ± 0.5	12 ± 0.6	12 ± 0.8	-	20 ± 0.2

"- " = Indicates no Zone of inhibition.

Table 3. Results of Brine shrimp lethality test of crude extract and selected fractions of *Xylocarpus mollucensis.*

Test samples	Group conc. (µg/ml)		Brine shrimp in each vial	Death in each vial		Average death	% mortality	Log conc.	LC_{50} (µg/ml)
Crude	A	25	15	12	8	10	67	1.4	12.6
	B	50	15	14	10	12	80	1.7	
	C	100	15	12	15	13.5	90	2.0	
	D	200	15	14	15	14.5	97	2.3	
	E	400	15	16	14	15	100	2.6	
F- 2	A	25	15	9	8	8.5	57	1.4	17.78
	B	50	15	10	10	10	67	1.7	
	C	100	15	12	11	11.5	77	2.0	
	D	200	15	13	16	14.5	97	2.3	
	E	400	15	16	14	15	100	2.6	
F – 5	A	25	15	10	11	10.5	70	1.4	13.34
	B	50	15	11	14	12.5	83	1.7	
	C	100	15	12	15	13.5	90	2.0	
	D	200	15	16	13	14.5	97	2.3	
	E	400	15	15	15	15	100	2.6	
F – 8	A	25	15	11	10	10.5	70	1.4	14.13
	B	50	15	13	11	12	80	1.7	
	C	100	15	12	14	13	87	2.0	
	D	200	15	15	12	13.5	90	2.3	
	E	400	15	14	16	15	100	2.6	
F - 13	A	25	15	9	10	9.5	63	1.4	15.85
	B	50	15	12	9	10.5	70	1.7	
	C	100	15	15	12	13.5	90	2.0	
	D	200	15	14	15	14.5	97	2.3	
	E	400	15	14	16	15	100	2.6	

Where, F = Fraction.

It was found that most of the bacterial strains exhibited moderate sensitivity (Table 1) against pure compounds XM-1, XM-2 and XM-3 except *Salmonella paratyphi-A, Shigella dysenteriae-type 2, Staphylococcus aureus* and *Sarcina lutea.* The ethyl acetate extract showed the prominent zone of inhibition against the fungus, *Aspergilus niger* and *A. fumigatus.* The petroleum ether extract did not show any antifungal activity. The isolated compounds XM-1, XM-2 and XM-3 for antifungal investigations showed moderate zone of inhibitions against *Aspergilus niger*; small zone of inhibition against *A. fumigatus, Candida oryzae, Saccharomyces cerevisiae, Rhizopus oryzae* and *Trichoderma sp.*; no zone of inhibition was observed against *Candida albicans* and *C. krusei* (Table 2).

It was found from the result of the brine shrimp lethality test in Table 3 that the crude EtOAc extract and the selected fractions of it exhibited toxicity towards brine shrimp. The crude extract was more potent than the selected fractions. Due to insufficiency of samples, the other fractions have not been included in this bioassay. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase of concentration of each sample. The percent of mortality of the brine shrimp nauplii was calculated for every concentration of each sample. A plot of log concentration of the sample versus percent of mortality showed an approximate linear correlation between them. The LC_{50} value of the crude ethyl acetate was 12.6 µg/ml and for the fractions 2, 5, 8 and 13 were 17.78, 13.34, 14.13 and 15.85 µg/ml respectively. This plant can be used as a good source of antimicrobial compounds for the treatment of different diseases.

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