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Antibacterial Activity and Cytotoxicity of Three Lectins Purified from *Cassia fistula* Linn. Seeds

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Three lectins CSL-1, CSL-2 and CSL-3, purified from the *Cassia fistula* seeds were tested for their antibacterial activities against 14 pathogenic bacteria using 30 µg/disc. The lectin CSL-3 was found to be active against all of the bacterial strains and showed strong activity against *Bacillus megaterium*, *Streptococcus β-haemolyticus* and *Shigella boydii*. The lectin CSL-2 showed poor activity against most of the bacterial strains and has strong activity against only *Streptococcus β-haemolyticus*. But the lectin CSL-1 was found to be inactive against all the bacterial strains except *Streptococcus β-haemolyticus* and *Sarcina lutea*. All the lectins affect significantly the mortality rate of brine shrimp. Among them CSL-2 was found to be highly toxic (6.68 µg ml^{-1}) followed by CSL-1 (10.47 µg ml^{-1}) and then CSL-3 (13.33 µg ml^{-1}).

Key words: Antibacterial activity, *Cassia fistula*, brine shrimp lethality bioassay, toxicity

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Introduction

Cassia fistula L., a semi-wild Indian laburnum, is frequently planted on city roads and avenues in almost all the districts of Bangladesh as a handsome ornamental tree for its beautiful bunches of yellow flowers and also used in traditional medicine for several indications like fever, heart disease, gout, rheumatism, ringworm, facial paralysis, thoracic obstructions etc (Blatter and Millard, 1954). from the time immemorial. The seed of *Cassia fistula* has been attracted much attention since it contains about 24% protein as reported by Roskoski *et al.* (1980) which is higher than that contained in most of the plant sources and may be considered as an important source of lectins. Lectins are sugar-binding proteins that agglutinate cells and/or precipitate glycoconjugate molecules with a carbohydrate portion like polysaccharide, glycoproteins, glycolipids and other. Lectins play a key role in the control of various normal and pathological processes in living organisms. Research in the field of lectins has been going on in many research laboratories of the world. So far more than hundred lectins have been purified and characterized but their antibacterial and toxicological studies against mortality of brine shrimp have not yet been carried out extensively. So our attention was concentrated to carry out research work on antibacterial and toxicological studies on the lectins purified from *Cassia fistula* seeds.

Materials and Methods

Extraction and purification of lectins

The ripe fruits of *Cassia fistula* commonly known as Bandarlati in Bangladesh were collected from Rajshahi University campus, Rajshahi, Bangladesh. Seeds were separated from the fruits, washed with water, dried under sunlight and used for experimental purposes. Distilled water containing 0.2% NaCl, pH 6.5 was used as extracting solvent for preparation of crude protein extract from fat free dry powder, the highest ratio of absorbance at 280 and 260 nm found in this solvent system. Three lectins were extracted and purified from *Cassia fistula* seeds in biologically active form by gel filtration of 100% ammonium sulfate saturated crude protein extract on Sephadex G-50 followed by ion-exchange chromatography on DEAE cellulose and then affinity chromatography on Sepharose 4B (personally contact for purification process). The lectins were found to be homogeneous justified by polyacrylamide disc gel electrophoresis which was conducted at room temperature, pH 8.4 on 7.5% gels as described by Ornstein (1964).

Antibacterial screening

Three lectins CSL-1, CSL-2 and CSL-3, purified from the seeds were screened for their antibacterial activities against 14 pathogenic bacteria by the Standard Disc-Diffusion Method (Barry, 1980; Bauer *et al.*, 1966) by measuring the diameter of the inhibitory zones in mm using 30 μg/15 μl of each of lectins in Tris-HCl buffer. The diameters of the zones of inhibitions of the samples were then compared with the diameter of the zone of inhibition produced by the standard antibiotic disc (kanamycin, 30 μg/disc) used. Blank discs were used as negative controls, which ensure that the residual solvents and the filter paper were not active themselves. Nutrient agar medium was used for determining antibacterial activity.

Brine shrimp lethality

Cytotoxicity was studied using Brine shrimp eggs. Shrimp eggs were placed in one side of a small tank divided by a net containing sea water (3.8% NaCl solution) for hatching. In the other side of the tank, a light source was placed in order to attract the nauplii. Two days were allowed to hatch all the eggs and in this period the nauplii were also sufficiently matured for experiment (Meyer ***et al.***,1982; Mclaughlin, 1990; Persoone, 1980).

From the stock solutions of the protein samples, specific volumes were transferred to the different vials containing 10 living shrimps and then sea water was added to make the volume upto 5 ml in each vial. The final concentration of the sample in the vials became 2, 4, 8, 16 and 32 μg ml^{-1} respectively. Three experiments were carried out for the same concentration to get more accurate result and a control experiment was performed similarly taking 10 living shrimps in 5 ml seawater. The same assay procedure was performed for the standard Ampicillin trihydrate.

After 24 h incubation, the vials were observed and the number of deaths in each vial was counted using a magnifying glass. From this data, the mean percentage of mortality of the nauplii was calculated at each concentration.

Results and Discussion

As shown in Table 1, all the three lectins obtained from ***Cassia fistula*** seeds showed mild to severe activities against most of the tested bacteria. The results were compared with those of kanamycin as a standard antibiotic. Of the three lectins CSL-1 was found to be inactive against

Table 1: Zone of inhibition exhibited by the lectins CSL-1, CSL-2 and CSL-3 purified from *Cassia fistula* seeds and standard antibiotic Kanamycin against different bacterial strains

	Diameter of the zone of inhibition in mm Standard			antibiotic
Name of bacteria	CSL-1 (30 μg/disc)	CSL-2 (30 μg/disc)	CSL-3 (30 μg/disc)	Kanamycin (30μg/disc)
Gram-positive				
Bacillus subtilis	-	-	07	20
Bacillus megaterium	-	08	20	14
Streptococcusβ-haemolyticus	08	22	20	14
Streptococcus aureus	-	07	10	35
Sarcina lutea	07	07	13	20
Gram-negative				
Shigella sonnei	-	08	12	13
Escherichia coli	-	08	08	30
Klebsiella species	-	07	08	16
Shigella shiga	-	07	08	20
Shigella boydii	-	-	17	35
Shigella flexneriae	-	07	12	15
Shigella dysenteriae	-	07	15	30
Salmonella typhi	-	07	10	19
Pseudomonas aeruginosa	-	-	12	15

- = Inactive against the organisms

Table 2: Effect of *Cassia fistula* seed lectins and Ampicillin trihydrate on brine shrimp lethality bioassay

Test samples	Conc. (µg ml^{-1})	Log conc. (Log C)	No. of Shrimp taken	No. of shrimp died			Average No. of death	Mortality (%)	LC_{50} (µg ml^{-1})
				Vial 1	Vial 2	Vial 3			
Nil (Control)	0	0	10	0	0	0	0	0	
Ampicillin	2	0.3010	10	2	2	2	2.000	20.00	6.31
trihydrate	4	0.6020	10	4	3	4	3.666	36.66	
	8	0.9030	10	5	6	6	5.666	56.66	
	16	1.2041	10	7	8	8	7.666	76.66	
	32	1.5051	10	10	9	9	9.3.33	93.33	
CSL-1	2	0.3010	10	1	2	1	1.333	13.33	10.47
	4	0.6020	10	3	3	3	3.000	30.00	
	8	0.9030	10	4	4	5	4.333	43.33	
	16	1.2041	10	6	6	6	6.000	60.00	
	32	1.5051	10	8	8	7	7.666	76.66	
CSL-2	2	0.3010	10	2	2	2	2.000	20.00	6.68
	4	0.6020	10	3	4	4	3.666	36.66	
	8	0.9030	10	5	5	6	5.333	53.33	
	16	1.2041	10	7	7	8	7.333	73.33	
	32	1.5051	10	9	9	9	9.000	90.00	
CSL-3	2	0.3010	10	2	1	1	1.333	13.33	13.33
	4	0.6020	10	3	2	3	2.666	26.66	
	8	0.9030	10	4	4	4	4.000	40.00	
	16	1.2041	10	5	5	6	5.333	53.33	
	32	1.5051	10	7	7	7	7.000	70.00	

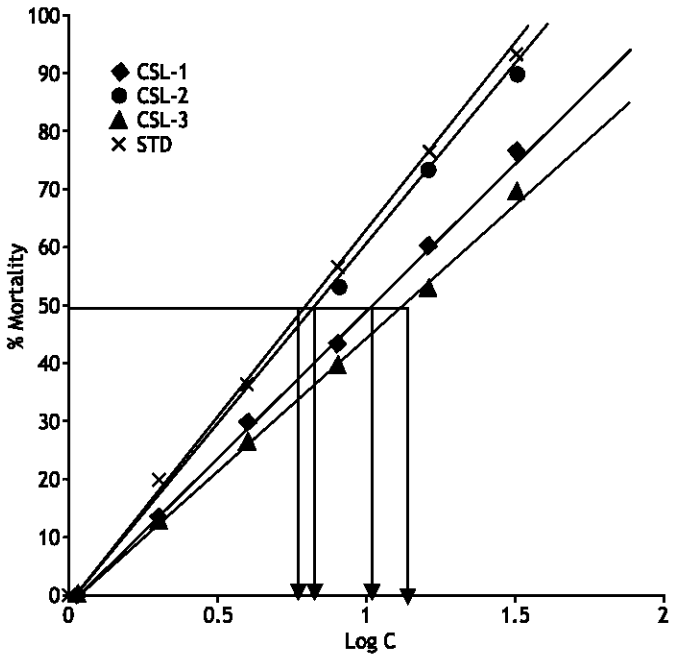

Fig 1: Determination of LC_{50} of *Cassia fistula* seed lectins (CSL-1, CSL-2 and CSL-3) and standard Ampicillin trihydrate (STD) against brine shrimp nauplii

all the bacterial strains except gram-positive ***Streptococcus β-haemolyticus*** and ***Sarcina lutea***. The lectin CSL-2 did not show any activity against gram-positive ***Bacillus subtilis*** as well as gram-negative ***Shigella boydii*** and ***Pseudomonas aeruginosa*** but it displayed strong activity against only gram-positive ***Streptococcus β-haemolyticus***. On the other hand, the lectins, CSL-3 was found to be active against all the tested bacteria and exhibited severe activity against gram-positives ***Bacillus megaterium*** and ***Streptococcus β-haemolyticus*** and gram-negative ***Shigella boydii*** and ***Shigella dysenteriae***.

The results of toxicity of experimental samples and standard Ampicillin trihydrate on brine shrimp are depicted in Table 2. All the three lectins and standard Ampicillin trihydrate exhibited significant toxic effect on brine shrimp lethality bioassay. The mortality rate of brine shrimp nauplii was found to increase with the increase in concentration of the samples and a plot of log of concentration vs. percent of mortality gave almost linear correlation (Fig. 1).

From the graph, the LC_{50} (concentration at which 50% mortality of the nauplii occurs) as estimated (Goldstein ***et al.***, 1974) by the extrapolation was found to be 10.47 μg ml^{-1} for CSL-1, 6.68 μg ml^{-1} for CSL-2, 13.33 μg ml^{-1} for CSL-3 (Table 2) and 6.31 μg ml^{-1} for Ampicillin trihydrate. From these results it might be concluded that the lectin CSL-2 is highly toxic than the other two lectins.

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