J. bio-sci. 17: 89-94, 2009. ISSN 1023-8654
http://www.banglajol.info/index.php/JBS/index

ANTIBACTERIAL ACTIVITY AND CYTOTOXICITY OF THREE LECTINS PURIFIED FROM DRUMSTICK (*MORINGA OLEIFERA* LAM.) LEAVES

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Abstract

Context: Plant materials contain glycoproteins (phytolectins) that are toxic in nature may play a key role in the control of various normal and pathological processes in living organisms and have diverse biochemical and diagnostic applications.

Objectives: Screening of three lectins SLL-1, SLL-2 and SLL-3 purified from Drumstick (*Moringa oleifera* Lam.) leaves for their antibacterial activities and brine shrimp lethality bioassay.

Materials and Methods: Three bioactive lectins were purified from Drumstick leaves by conventional chromatographic methods. The lectins were tested for their antibacterial activities against three pathogenic bacteria– *Escherichia coli* (gram-negative) *Shigella dysenteriae* (gram-negative) and *Staphylococcus aureus* (gram-positive) using the standard disc-diffusion method. Mortality of the brine shrimp naupli was assessed by hality bioassay.

Results: All the lectins showed antibacterial activity against *E. coli*, S*h. dysenteriae* and *St. aureus.* They also showed cytotoxic effect in brine shrimp (*Artemia salina* L.) lethality bioassay. The LC_{50} values of SLL-1, SLL-2 and SLL-3 were found to be 15.8, 17.78 and 14.12 μg/ml respectively. The experimental results revealed that SLL-3 is more cytotoxic than other lectins. The lectin SLL-3 showed lowest activity whereas SLL-1 showed highest activity against the three bacteria.

Conclusion: Results suggest that the extracts from *M. oleifera* leaf can be a source of natural antimicrobials with potential applications in pharmaceutical industry to control coliform bacteria.

Key words: Drumstick, *Moringa oleifera*, lectins, antibacterial activity, brine shrimp, bioassay

Introduction

Lectins, are a group of glycoprotein's that binds sugar specifically and reversibly and possesses the unique ability to agglutinate erythrocytes and other types of cells (Boyd 1970). They are widely available in nature as they are found in animals, insects, plants and microorganisms (Lin *et al.*1981, Linear *et al*. 1986, Lis and Sharon1986, Sharon and Lis 1989). Lectins are widely distributed in the plant kingdom and are also referred to as phytohemagglutinin. The term phytolectin has been proposed in order to distinguish those lectins which are found in plants from those which are of animal or microbial origin. Both oncogenic and nononcogenic viral transformed cell possess increased susceptibility to agglutination by lectin that exhibit toxic substances even in very small amount to higher animal. Abrin and ricin possess such kinds of characteristics. They inhibit protein synthesis as well as DNA and also RNA in the cell culture.

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Moringa oleifera Lam. (Moringaceae) is a plant of subtropical West Indies, Indian origin, widely distributed in the Indo-Bangla subcontinent and cultivated throughout the tropical belt (Sastri 1962, Nadkarni 1976). A small genus of quick growing trees is distributed in Bangladesh, India, Arabia, Asia Minor, Africa, Egypt and Australia. It is widely cultivated in tropics for its edible fruits. The leaves contain vitamin A, vitamin C and are considered to be useful in scurvy and hay fever. In developing tropical countries, *M*. *oleifera* have been used to combat malnutrition, especially among infants and nursing mothers (Fuglie 1999). Its bark is regarded as an antiscorbic and it exudes a reddish gum sometimes used for diarrhea. The roots of *M*. *oleifera* are bitter, act as a tonic to the body and lungs, and an expectorant. The leaf tea is used to treat gastric ulcers and diarrhea while flower juice improves the quality and flow of mothers' milk when breast feeding and is also useful for urinary problems as it encourages urination. The pharmacological and biological activities of *M*. *oleifera* have been studied. Aqueous extract of *M*. *oleifera* has been found to have anti-nociceptive and anti-inflammatory activities (Sulaiman *et al*. 2008). Mahajan *et al*. (2007) reported that the seed extract of *M*. *oleifera* had an effect on toluene diisocyanate-induced immune-mediated inflammatory responses in rats. Almost all the parts of this plant exhibited various effects such as cardiovascular, gastrointestinal, hematological, and hepatorenal disorders inhibitory activities (Mahajan and Mehta 2007, Devaraj *et al*. 2007). Various publications have documented the antispasmodic, antitumor, antiulcer and hepatoprotective activities of *M*. *oleifera* (Ezeamuzie *et al*. 1996, Ali *et al*. 2004, Mahajan *et al*. 2007). It has also been reported to exhibit other diverse activities such as antiurolithiatic, antihypertensive, diuretic and cholesterol lowering activities (Anwar *et al*. 2007, Karadi *et al*. 2008).

The growing resistance of microorganisms to convectional antimicrobial agents is a source of concern to clinical microbiologists all over the world. As a result, efforts are being made to develop antimicrobial agents from local sources for better chemotherapeutic effects (Gills 1992). The demand for more natural antimicrobials has driven scientists to investigate the effectiveness of inhibitory compounds such as extracts from plants (Nasar-Abbas and Halkman 2004). Various publications have documented the antimicrobial activities of plant extracts (Mathabe *et al*. 2006, Ahmad and Aqil 2007). Thus plant extracts are promising natural antimicrobial agents with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. Plant materials contain mostly glycoproteins that are toxic in nature; they play a key role in the control of various normal and pathological processes in living organisms. So far more than hundred lectins have been purified and characterized but their antibacterial and toxicological studies against mortality of brine shrimp is scanty. So our attention was to carry out the antibacterial and toxicological studies on the lectins purified from drumstick (*M. oleifera*) leaves.

Materials and Methods

Materials: The mature leaves of *Moringa oleifera* L. were collected from Kazla, Rajshahi. Pure strains of *Escherichia coli* (gram-negative), *Shigella dysenteria* (gram-negative) and *Staphylococcus aureus* (gram-positive) were collected from the department of Pharmacy, University of Rajshahi, Bangladesh.

Isolation and purification of leaves: All the experimental procedure was carried out at 4 -10ºC. Drumstick leaves (155 g) were grinded in a mortar and pestle. A very small amount of polyvinylpyrolidine (PVP) was added (to avoid the phenolic compounds and other coloring substances) and mixed uniformly with pre-cooled distilled water containing 0.2M NaCl, pH-6.5 (6 ml/g of leaf) and kept overnight at 4ºC with occasional gentle shaking. The suspension was centrifuged (8,000 g) at 4ºC for 15 min. The clear supernatant was collected and adjusted to 100% saturation by adding solid ammonium sulfate. The resulting precipitate was collected by centrifugation, dissolved in minimum volume of pre-cooled distilled water and dialyzed against distilled water for 24 h with three changes and against 10 mM Tris-HCl buffer, pH-8.4 for 12 h at 4ºC. After centrifugation the clear supernatant was used as crude protein extract.

Three lectins SLL-1, SLL-2 and SLL-3 were extracted and purified from small sized leaves by Gel filtration of 100% ammonium sulfate saturated crude protein extract on Sephadex G-75 followed by ion-exchange chromatography on DEAE and affinity chromatography on Sepharose-4B. The purities of the three lectins were checked by polyacrylamide slab gel electrophoresis (Khatun *et al*. 2007).

Antibacterial screening: The purified lectins were screened for their antibacterial activities by the standard Disc-Diffusion Method (Barry 1980, Bauer *et al*. 1966) by measuring the diameter of the inhibitory zones in mm using 30μg/15μl of each of lectins in Tris-HCl buffer. The diameters of the zones of inhibitions of the samples were than compared with the diameter of the zone of inhibition produced by the standard antibiotic disc such as Doxycycline (DXT-30), erythromycin (E 15), ampicillin (Ap 10) and tetracycline (T 30). Blank discs were used as negative controls, which ensured that the residual solvents and the filter paper were not active themselves. Nutrients agar medium was used for determining antibacterial activity.

Lethality bioassay: Cytotoxicity study was done using Brine shrimp eggs placed in one side of a small tank divided by a net containing seawater (3.8% NaCl solution) for hatching. In the other side of the tank, a light source was placed in order to attract nauplii. All the eggs hatched within two days and in this period the nauplii were also sufficiently matured for lethality bioassay experiment (Persoone1980, Mayer *et al*.1982, Mclaughlin 1990).

From the stock solutions, specific volumes were transferred to the different vials containing 10 living shrimps and then seawater was added to make the volume up to 5 ml in each vial. The final concentration of the sample in the vials became 2, 4, 8, 16 and 32 μg/ml respectively. The experiments were carried out for the same concentration to get more accurate result and 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 survival in each vial was counted using a magnifying glass.

Results and Discussion

As shown in Table1, the three lectins obtained from showed antibacterial activity against most of the tested bacteria. The results were compared with those of doxycycline (DXT-30), erythromycin (E 15), ampicillin (Ap 10) and tetracycline (T 30) as standard antibiotic. Of the three lectins SLL-3 showed lowest antimicrobial activity whereas SLL-1 showed highest activity against the three bacteria. The lectins at 30μg/15μl concentration was reported to posses antibacterial activity against various gram positive and gram negative bacteria in particular against *Bacillus subtilis, Bacillus megaterium, Streptococcus β-haemolyticus, Streptococcus aureus, Sarcina lutea, Shigella sonnei, E. coli, Klebsiella species, Shigella shiga, Shigella boydii, Shigella flexneriae, Shigella dysenteriae, Salmonella typhi* and *Pseudomonas aeuginosa* (Ali *et al.* 2003).

The results of toxicity of experimental samples and standard Ampicillin trihydrate on brine shrimp are given in Table 2. All the three lectins and standard Ampicillin trihydrate exhibited significant cytotoxic activity in brine shrimp lethality bioassay. The mortality of the 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 50% mortality (LC_{50}) values of SLL-1, SLL-2 and SLL-3 were found to be 15.8, 17.78, and 14.12 μg/mL respectively.

It is evident from the results of brine shrimp lethality testing that the LC_{50} value of SLL-3 was the lowest (14.12 μg/ml) indicating its higher toxicity. On the other hand these LC_{50} values of SLL-1 and SLL-2 were

higher compared to SLL-3. The potency of the lectins was of the following order SLL-3 > SLL-1>SLL-2. This finding is very similar with that of mulberry seed (*Morus* spp) lectin (Hossain *et al*. 2002) and *Cassia fistula* L seed lectins (Ali *et. al*. 2003).

Table 1. Zone of inhibition exhibited by the lectins SLL-1, SLL-2 and SLL-3 purified from Drumstick (*Moringa oleifera* L.) leaves and standard antibiotics against different bacterial strains

Test Sample	Diameter of zone of inhibition (mm) against		
	E. coli	*Sh. dysenteriae*	*St. aureus*
SLL-1	13	17	31
SLL-2	11	14	24
SLL-3	10	13	20
Doxycycline (D-30)	33	28	32
Tetracycline (T 30)	-	17	16
Ampicillin (Ap 10)	-	20	14
Erythromycin (E 15)	-	29	32

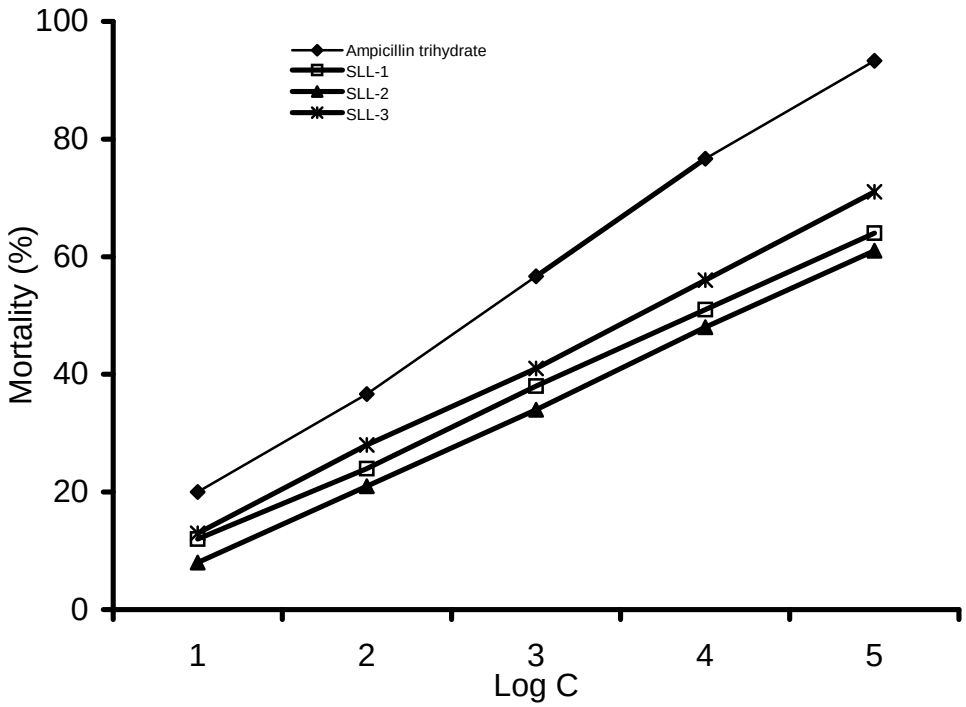

Fig. 1. Determination of LC_{50} of Drumstick leaves lectins.

Table 2. Effect of Drumstick leaves lectins and Ampicillin trihydrate on brine shrimp lethality bioassay

Test sample	Conc. (µg/ml)	Log conc. (LogC)	Mortality (%)	LC_{50} (µg/ml)
Control	0	0	0	
Ampicillin trihydrate	2	0.3010	20	6.31
	4	0.6020	36.66	
	8	0.9030	56.66	
	16	1.2041	76.66	
	32	1.5051	93.33	
SLL-1	2	0.3010	12	15.8
	4	0.6020	24	
	8	0.9030	38	
	16	1.2041	51	
	32	1.5051	64	
SLL-2	2	0.3010	8	17.78
	4	0.6020	21	
	8	0.9030	34	
	16	1.2041	48	
	32	1.5051	61	
SLL-3	2	0.3010	13	14.12
	4	0.6020	28	
	8	0.9030	41	
	16	1.2041	56	
	32	1.5051	71	

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