

# Screening and Partial Purification of Lectin from Various Bangladeshi Plant Seeds

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**Abstract** Lectins are group of proteins or glycoprotein of non immunological origins, which can recognize specific carbohydrate structure. In this research work hemagglutination was used as technique for screening Bangladeshi vegetables for lectin. Five varieties of legume plant seeds *Canavalia gladiata*(Sword bean),*Lens culinaris* (Moshordal), *Peasum sativam* (Motorsuti), *Vigna unguiculata* subsp. *sesquipedalis* (Borboti), *Cajanus cajan* (Arhordal), one Amaranthaceae *Amaranthus caudate* (katoadata) and one fruit *Citrullus lanatus* (Watermelon) species from Bangladesh were examined for lectins with chicken and human erythrocytes. Crude extracts from all the species showed agglutinating activity against the erythrocytes used. The lowest protein concentration required to produce erythrocytes agglutination varied remarkable ranging from 0.7 µg/ml to 8080 µg/ml. The strongest activities were shown in the agglutination of human blood erythrocytes by partial purification of lectin from Moshordal and chicken blood erythrocyte from Katoadata and Sword bean. Inhibition assays performed with mono and disaccharides showed that agglutinating of erythrocytes by Sword bean, Moshordal, Motorsuti and Arhordal extracts were completely inhibited where others three were not inhibited by the sugars. Amonium sulphate at 90% saturation followed by dialysis. Sword bean, Arhordal, Watermelon, Borboti, Motorsuti, Moshordal and Katoadata respectively which correspond to the 144.21 mg, 202.5 mg, 135 mg, 179.4 mg, 6 mg, 288 mg, and 72.8 mg are the total protein content.

**Keywords:** agglutinating activity, partial purification, lectins, protein content, inhibition assays

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## 1. Introduction

Bangladesh is a riverine country. It is rich in water resources. It is very much potential for production of various plants. Sword bean, Moshordal, Motorsuti, Borboti, Arhordal , Katoadata ,and Watermelon plants are

those of them. The common name of the *Canavalia gladiata* is the "Sword Bean", *Lens culinaris* is the "Moshordal" *Peasum sativam* is the Motorsuti, *Vigna unguiculata* subsp. *sesquipedalis* is the Borboti, *Cajanus cajan* is the Arhordal, *Amaranthus caudate* is the katoadata, and *Citrullus lanatus* is the Watermelon.

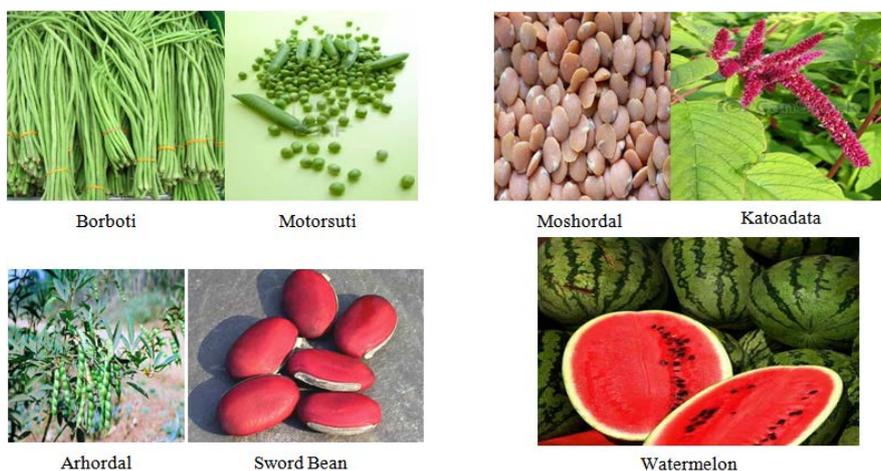


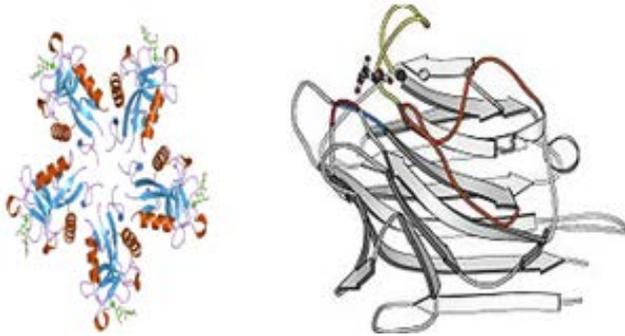
Figure 1: Photograph of Borboti, Motorsuti, Moshordal , Katoadata, Arhordal , Sword bean and Watermelon

They contain considerable amount of lectin/protein. Lectins are sugar-binding proteins which are highly specific for their sugar moieties. They typically play a role in biological recognition phenomena involving cells and proteins. For example, some viruses use lectins to attach themselves to the cells of the host organism during infection. These are the source of an enzyme, unease, which has been exploited commercially. Another definition is, Lectins are a class of proteins that bind sugar specifically and reversibly and that agglutinate cells, are widely distributed in nature, being found in animals, insects, plants and microorganisms [1]. All lectins are glycoproteins, but all glycoproteins are not lectins. In the modern classification, there are 3 main types of lectins: - (1) C-type (2) S-type and (3) legume

(1) C- type lectins: - C-type plants lectins are characterized by a calcium dependent carbohydrate recognition domain. There are two main types of C-type lectins. (a) Mannose-binding protein and (b) Selectins.

(2) S-type lectins:- S-type lectins (S for soluble) are small, soluble proteins with calcium independent affinity for lactosamine and  $\beta$ - galactoside. There are 3 main S-type lectins: (a) S-Lectins (b) Galactose binding and (c) Galectin.

(3) Legume lectins:- They have both calcium and manganese binding site.



**Figure 2.** A typical legume lectin and Pentameric structure of rattlesnake venom lectin

But lectins are usually classified on the basis of source. They are of various types: plant lectin, vertebrate lectin, invertebrate lectin, lectin of slime mold, lectin of protozoa, viral lectin and bacterial lectin according to their respective sources.

**Table 1. Vertebrate Soluble  $\beta$ -Galactoside-binding Lectins**

Designation	Major source
Calf $\beta$ -galactoside lectin	Heart, Spleen, Muscle
Chicken-lactose-lectin-I (CLL-I)	Adult liver
Chicken-lactose-lectin-II (CLL-II)	Intestinal mucosa
Electro lectin	Eel electric organ
Erythroid developmental agglutinin	Rabbit bone marrow
Human $\beta$ -galactoside lectin	Lung
Rat $\beta$ -galactoside lectin (RL-14.5)	Lung
Rat lectin (RL-18)	Lung
Rat lectin (RL-29)	Lung
Thrombo lectin	Snake venom
Xenopus skin lectin (XL-16)	Skin
Xenopus oocyte lectin (XL-43)	Oocytes, eggs
Xenopus serum lectin (XL-69)	Serum

Vertebrate lectins can be classified into two groups- soluble lectins that may play a role in the secretion or organisation of extracellular glycoconjugates [2] and membrane lectins that are integrated into membranes and are apparently involved in translocation of glycoconjugates in cells [3]. Soluble tissue lectins: Aqueous extracts of many vertebrate tissues contain materials that agglutinate appropriate test erythrocytes. Since this hemagglutination activity can be inhibited by simple sugars or complex glyco-conjugates these materials have been classified as lectins. Like plant lectins, some have also been shown to be mitogens [4,5]. The first vertebrate purified lectin was from electric organ of the electric eel *Electrophorus electricus* [6] and from calf heart and lung [7] by affinity chromatography.

### 1.1. Invertebrate Lectins

These lectins are found impractically all of the approximately 30 phyla and the various classes and subclasses of invertebrates [9,12] mainly in the hemolymph and sexual organs, e. g. albumin glands and eggs [10,11]. They are also present in the membranes of hemocytes cells that function as primitive and rather unspecific immunological protectors [13].

### 1.2. Biological Properties of Lectin

Since virtually all cells come in a sugar coating, it is not surprising that Lectins binds readily to cells. Such binding may result in a variety of biological effects.

### 1.3. Agglutination

Agglutination is the most easily detectable manifestation of the interaction of a Lectin with cells, and to this very day is used to reveal the presence of a Lectin in a biological source. The ability to agglutinate cells distinguishes Lectins from other sugar-binding macromolecules, such as glycosidases and glycosyltransferases, and is therefore included in the definition of Lectins according to [8].

For agglutination to occur the bound Lectin must form multiple cross bridges between apposing cells. There is, however, no simple relation between the amount of Lectin bound and agglutination. Cases are even known where considerable amounts of a Lectin are bound to cells without causing agglutination. This is because agglutination is affected by many factors such as the molecular properties of the Lectin (e.g. number of saccharide binding sites, molecular size), cell-surface properties (for example, number and accessibility of receptor sites, membrane fluidity), and metabolic state of the cells [14]. In addition, agglutination is affected by external conditions of assay such as Temperature, cell concentration, mixing and so on. Agglutination is inhibited by appropriate sugar.

## 2. Materials and Methods

### 2.1. Collection and Delipidation of Seeds

Borboti, Motorsuti, Moshordal, Katoadata, Arhordal, Sword Bean, Watermelon seeds were purchased from local seed market. After removing the seed coat, seeds

were pulverized in a mortar and pestle to produce fine powder materials. The meal of seed thus obtained were delipidated by refluxing with diethyl ether. The delipidated seed meal was air dried at room temperature to remove last traces of diethyl ether and used as a starting material for lectin purification.

## 2.2. Crude Extraction of Lectin

Delipidated seed meal (10 gm) was homogenized with 50 ml of 0.02 M Tris-HCl buffer pH 7.4 for 12 hours at 10-15°C. The suspension was filtered through double layer of silk cloth. The filtrate was collected and centrifuged at 10,000 rpm for 10 minutes to remove any solid material. The clear supernatant (48 ml) was used as crude extract.

**Partial Purification:** 90% NH<sub>4</sub>SO<sub>4</sub> in gm was added into the crude extract of the seeds by the following formula.

$$\frac{\text{Crude vol.} \times 66.2}{100}$$

And kept it for 6-8 hours at 10-15°C. The suspension was centrifuged at 10,000 rpm for 10 minutes and the clear supernatant was removed. After removing the clear supernatant the precipitation was collected and dissolved in minimum amount (2-3 ml) of buffer solution. Centrifuged at 10,000 rpm for 10 minutes to remove solid material. The clear supernatant (2-3 ml) was used as partial pure solution of lectin.

## 2.3. Determination of the Protein Content by Biuret Method

**Reagent preparation:** The reagent was prepared by mixing the following solution.

Sodium hydroxide	200 mM
Potassium sodium tartrate	32 mM
Copper sulfate	12 mM
Potassium iodide	30 mM

**Standard:** Standard contains protein 80 gm/l and sodium azide 0.095%.

20 µl protein sample or 20 µl standard protein sample were added to 1.0 ml of total protein reagent in different test tube, the mixture was shaken for few times, allowed to react for 10 min at room temperature. The absorbance of sample and standard against the reagent blank were measured within 30 min at 546 nm using a UV-Vis Spectrophotometer. The amount of protein content was determined using the following equation.

$$C = 80 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \text{ [mg/ml]}$$

## 2.4. Dialysis

The partial purified lectin was poured into activated dialysis bag of selectively permeable membrane (e.g. cellophane), immersed in a large volume of buffer. After some hours of stirring, the buffer was replaced by fresh buffer. This was done for several times. After dialysis the purified lectin was collected in a stoppered bottle.

## 2.5. Hemagglutination Studies

i) **Preparation of 1% NaCl solution:** 1 gm of NaCl was dissolved in about 80 ml of deionized distilled water in a 100 ml volumetric flask. The final volume was made up to the mark by adding deionized distilled water.

ii) **Preparation of hemagglutination buffer (20 mM Tris-HCl buffer pH 7.8 containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub>):** 20 mM Tris-HCl was prepared by dissolving 0.24 gm of Tris base in about 90 ml of deionized distilled water. After adjusting the pH to 7.8 with concentrated HCl, the final volume was made up to 100 ml with deionized distilled water. 0.88 gm NaCl and 0.11 gm CaCl<sub>2</sub> were added and dissolved in that buffer.

## 2.6. Blood Collection and Preparation

Just before experiment, blood from human and chicken was collected in a centrifuge tube containing sufficient amount of 1% NaCl solution. The blood sample was immediately centrifuged at 3000 rpm for 3 minutes. The supernatant was discarded and the cells were washed similarly for three times with the above solution. Finally a 1% suspension (w/v) of RBC was prepared with hemagglutination buffer.

## 2.7. Hemagglutination Test

The hemagglutination was performed in U-bottom polystyrene microtiter plate as follows: 50 µl of 20 mM Tris buffer pH 7.8 containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub> were poured into A1 through H12 of the titer plate. 50 µl of all protein solutions in Tris-HCl buffer were added in A1, B1, C1, D1, E1, F1, G1 of Borboti, Watermelon, Sword bean, Motorsuti, Moshordal, Katoadata and Arhordal, respectively of the titer plate. The protein and buffer were mixed well. Two-fold serial dilution was carried out from 1:1 dilutions in wells A1 to G1 down to 1:2048 dilutions in wells A12, to G12 control containing 50 µl of 20 mM Tris buffer pH 7.8 containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub>, instead of protein solution and 50 µl cell suspensions were used in H1 to H10. The mixture in the titer plate was mixed well by gentle shaking with vortex mixture. The mixture was incubated at 37°C for an hour. After 1 hour of incubation, the sediment erythrocytes were gently mixed with the supernatant and one drop of this suspension was examined under microscope. (Figure 4) The agglutinating activity was expressed as the titer, the reciprocal of the greatest dilution at which visible agglutination could be detected. The specific activity was expressed as titer/mg of protein

## 3. Result Discussion

### 3.1. Determination of the Protein Content by Biuret Method

20 µl protein samples or 20 µl standard protein samples were added to 1.0 ml of total protein reagent in different test tube, the mixture was shaken for few times, allowed to react for 10 min at room temperature. The absorbance of samples and standard against the reagent blank were measured within 30 min at 546 nm using a UV-Vis Spectrophotometer. And the absorbance of standard for all

samples is 0.410. The amount of protein content was determined using the following equation.

$$C = 80 \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} [\text{mg/ml}]$$

It was found that 10 gm seed contains about 144.21mg, 202.5mg, 135mg, 179.4mg, 6mg, 288mg; 72.8mg are the total protein content for Sword bean, Arhordal, Watermelon, Borboti, Motorsuti, Moshordal and Katoadata respectively

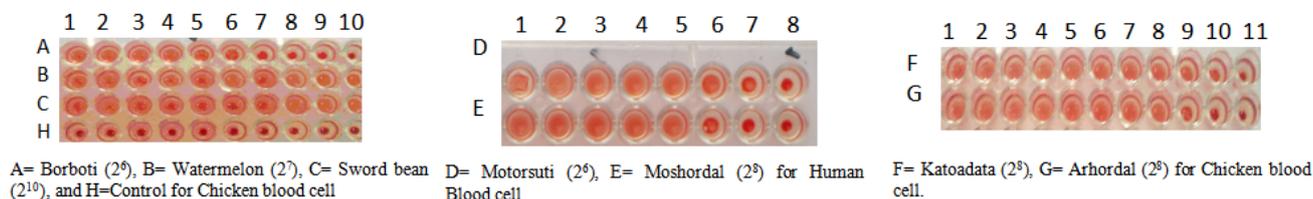
**Table 2. A summary of partial purification of lectins produced by vegetables seeds**

Name of Crude Extract	Crude volume (ml)	90% NH <sub>4</sub> SO <sub>4</sub> (gm) Crude vol.×66.2 100	Partial pure solution (ml)	Absorbance at 546 nm	Protein contain mg/m	Total protein (mg)
Standard				0.41	80	
Sword bean(C)	11	7.28	1.5	0.067	13.11	144.21
Arhordal (G)	15	9.93	2	0.069	13.5	202.5
Watermelon(B)	10	6.62	1.1	0.069	13.5	135
Borboti (A)	13	8.6	1.2	0.071	13.8	179.4
Motorsuti(D)	10	6.62	3	0.003	0.6	6
Moshordal(E)	15	9.93	5	0.098	19.2	288
Katoadata(F)	13	8.6	4	0.029	5.6	72.8

### 3.2. Hemagglutination Test

Hemagglutination assay has been widely used for the measurement of lectin activity. In the hemagglutination test, 50 µl of 20 mM Tris buffer pH 7.8 containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub> were poured into A. to H. 50µl of protein solutions in Tris-HCl buffer were added in A to G of the titer plate. The protein and buffer were mixed well. Two-fold serial dilution was carried out from 1:1 dilutions in wells A1, B1, C1, D1, E1, F1, and G1 down to 1:2048

dilutions in wells A12, to G12. The control containing 50 µl of 20 mM Tris buffer pH 7.8 containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub>, instead of protein solution and 50 µl cell suspensions were used in H1 to H12. The mixture in the titer plate was mixed well by gentle shaking with vortex mixture. The mixture was incubated at 30°C for an hour. The RBC in the control wells have settled. The end-point was taken as the dilution in the last pair of wells that showed complete (100 percent end point) hemagglutination.

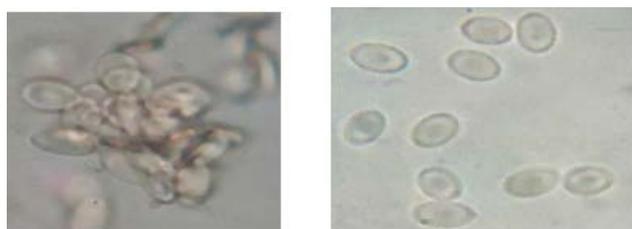


**Figure 3.** Photograph of hemagglutination tests in a microtiter plate

### 3.3. Hemagglutination Activity

Hemagglutination assay was performed using chicken and human blood erythrocytes. Sword bean lectin powerfully agglutinated Chicken and Human blood erythrocytes in order of hemagglutinating activity. Arhordal, Watermelon, Borboti and Katoadata lectin were agglutinated Chicken erythrocytes. Motorsuti and Moshordal agglutinated by human blood erythrocytes.

The result of hemagglutination activity was presented in Table 3.



**Figure 4.** Photograph of hemagglutination tests under microscope (a) lectin agglutinate red blood cells (b) Not agglutination

### 3.4. Haemagglutination Inhibition Assay

Haemagglutination inhibition assay was performed by mixing 25 µl of appropriately diluted lectin (twice the lowest concentration capable of visible agglutination) with 25 µl of 200 mM stock solution of sugar in microtiter plates. After one hour of incubation at room temperature, 50 µl of 1% erythrocyte suspension was added to each well and plates were further incubated for 30 min at 37°C temperature. A control was also run containing 25 µl of PBS, instead of sugar solution. The plates were stabilized at room temperature for 2-3 hrs. The formation of button in the presence of sugar indicated the inhibition of lectin activity i.e. a positive reaction, while formation of mat indicated non-specific sugars. The agglutination inhibition of the crude extract lectins from different vegetables and fruit seeds were tested with glucose and maltose. Lectins from sword bean, arhordal, Motorsuti and Moshordal showed an almost similar sugar inhibition profile and all were found to be specific for glucose and maltose. However, glucose and maltose could not inhibit the agglutinating activity of borboti and watermelon seed

lectins. And Katoadata were completely inhibited by the sugars.

**Table 3. Hemagglutination activity of lectin**

	1	2	3	4	5	6	7	8	9	10	11	12
	Dilution (Sample: Buffer)											
	2	4	8	16	32	64	128	256	512	1024	2048	4096
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
C	a	+	+	+	+	+	+	+	+	+	-	-
	b	+	+	+	+	+	+	+	-	-	-	-
G	a	+	+	+	+	+	+	+	-	-	-	-
	b	-	-	-	-	-	-	-	-	-	-	-
B	a	+	+	+	+	+	+	-	-	-	-	-
	b	-	-	-	-	-	-	-	-	-	-	-
A	a	+	+	+	+	+	-	-	-	-	-	-
	b	-	-	-	-	-	-	-	-	-	-	-
D	a	-	-	-	-	-	-	-	-	-	-	-
	b	+	+	+	+	+	-	-	-	-	-	-
E	a	-	-	-	-	-	-	-	-	-	-	-
	b	+	+	+	+	+	+	+	-	-	-	-
F	a	+	+	+	+	+	+	+	-	-	-	-
	b	-	-	-	-	-	-	-	-	-	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

a= Chicken Blood Cell, b = Human Blood Cell

+, Hemagglutination activity; -, Control.

C = Sword bean, G= Arhordal, B= Watermelon, A = Borboti, D = Motorsuti, E = Moshordal,

F = Katoadat, H = Control

**Table 4. A summary of hemagglutination assay during partial purification of lectin from vegetables seeds**

Name of Crude Extract		Volume (ml)	Lectin activity Titer/ml	Total activity (Titer)	Total protein (mg)	Specific activity (Titer/mg)	Fold purification
Sword bean(A)	Crude	11	512	5632	144.21	39.05	1
	Partial pure	1.5	2048	3072	48.81	62.94	1.61
Arhordal(B)	Crude	15	128	1920	202.5	9.48	1
	Partial pure	2	1024	2048	65.00	31.51	3.32
Watermelon(C)	Crude	10	64	640	135	4.74	1
	Partial pure	1.1	512	563.2	38.61	14.59	3.08
Borboti(D)	Crude	13	32	416	179.4	2.32	1
	Partial pure	1.2	256	307.2	38.40	8	3.45
Motorsuti(E)	Crude	10	32	320	6	53.33	1
	Partial pure	3	256	768	3.5	219	4.12
Moshordal(F)	Crude	15	128	1920	288	6.66	1
	Partial pure	5	1024	5120	92.90	55.11	8.27
Katoadata(G)	Crude	13	128	1664	72.8	22.86	1
	Partial pure	4	1024	4096	24.27	168.77	7.38

## 4. Conclusion

We have identified and develop a protocol for Lectin extracted and partially purified from Borboti Motorsuti, Moshordal, Katoadata Sword bean, Arhordal and Watermelon by using a alternative source. The lectin was shown the heamagglutination activity and the agglutination inhibition. By using this alternative source we can easily purify the lectin Because lectin has anticancer activities. Thus it helps to improve country economic condition.

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