Asian Journal of Green Chemistry 3 (2019) 518-524

Asian Journal of Green Chemistry

Journal homepage: www.ajgreenchem.com

Orginal Research Article

Identification of lectins from the seeds of Bangladeshi plants *Sesbania bispinosa* and *Senna occidentalis* by hemagglutination assay

Mst. Jesmin Sultana[a,*], Fazle Rabbi Shakil Ahmed[b], M. Taufiq Alam[c]

[a] *Department of Materials Science and Engineering, Faculty of Engineering, University of Rajshahi, Rajshahi, Bangladesh*

[b] *Department of Pharmacy, Khwaja Yunus Ali University, Sirajgonj, Bangladesh*

[c] *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering,University of Rajshahi, Rajshahi, Bangladesh*

ARTICLE INFORMATION

Received: 2 October 2018
Received in revised: 1 November 2018
Accepted: 11 November 2018
Available online: 2 February 2019

DOI: 10.33945/SAMI/AJGC/2019.4.8

KEYWORDS

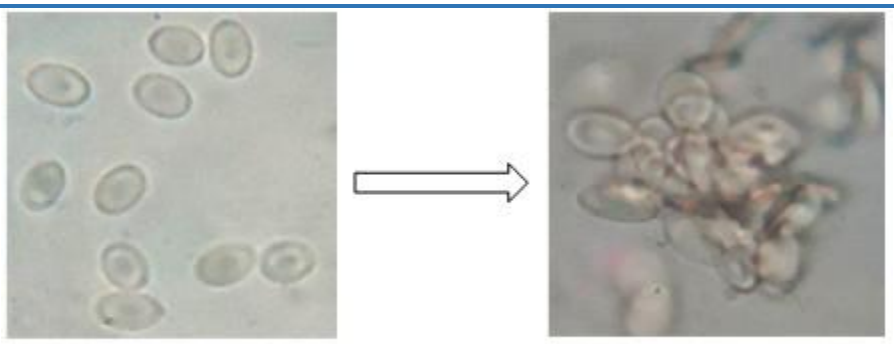
Hemagglutination
lectin
blood group
Sesbania bispinosa
Senna occidentalis

ABSTRACT

Hemagglutination assay is a simple and easy method to specify a lectin. An active lectin agglutinates erythrocytes by recognizing a carbohydrate on the cell surface and forming a cross-linked network in suspension. The assay is traditionally performed on a microtiter plate, where the lectin solution is serially diluted and the minimum concentration of a lectin causing agglutination is detected. The crude extract of Senna occidentalis and Sesbania bispinosa seeds contain considerable amount of protein. It was found that 10 gm of Sesbania bispinosa and Senna occidentalis seeds contain about 12.6 mg/mL and 9.8 mg/mL of protein, and minimum agglutination concentration was found to be 0.393 mg/mL for Sesbania bispinosa in each group of human erythrocytes (A, B, O and AB) and 0.613 mg/mL for Senna occidentalis in chicken erythrocytes. Finally, the hemagglutination assay confirmed that possible lectins present in the Sesbania bispinosa and Senna occidentalis plant sources.

Corresponding author, email: jssumi8@gmail.com, jesmin_mse@ru.ac.bd (M.J. Sultana)
Tel: +880 1818401313.

Graphical Abstract



Introduction

Lectins can be defined as a group of proteins found in all types of living organisms, either in soluble or in membrane-bound form that recognizes specific carbohydrate structures and thereby they may agglutinate cells by binding to cell-surface glycoproteins and glycoconjugates [1]. Till now, several plant lectins have been isolated from plant sources, and most of them have been arranged into the following categories; the legume lectins, monocot mannose-binding lectins, chitin-binding lectins composed of hevein domains, jacalin-related lectins, cucurbitaceae phloem lectins, ribosome-inactivating proteins, and amaranthine lectins [2]. The major sources of lectins are mature seeds and tubers. Small amount of lectins can be found in other tissues such as roots, barks, and leaves [3] that are preferred with great interest according to their diverse biological actions including, cell agglutination [4], anti-fungal [5], antiviral [6], and antiproliferative activities [7, 8].

Agglutination is the most easily method which basically involves the screening of lectin on erythrocytes. The ability to agglutinate cells distinguishes lectins from other sugar-binding macromolecules such as glycosidases and glycosyltransferases. Agglutination occurs when the bound lectin forms multiple cross bridges between the opposing cells [9].

In this research, *Sesbania bispinosa* and *Senna occidentalis* plant sources were used for lectins identification. *Sesbania bispinosa* local name is Dhaincha, Family: Fabaceae, 1-3 m tall, plant decoction as antacid and febrifuge. Bark and seed astringent for diarrhea; seed powder given to induce hunger. Leaf paste applied to small babies all over the body and also given bath against whooping cough. Flowers smoke as mosquito and insect repellent. And another plant *Senna occidentalis* local name is Kalkasunda. The plant (with the yellow flowers) grows wild on road side. Its fruit is pod brown with yellow margin and seed 20-25 in numbers with greenish brown. Decoction of the whole plant as flatulence and seeds are used in leprosy, erysipelas, pruritus, wounds, ulcers, cough, bronchitis,

hiccough, asthma, pharyngodynia, fever, and hydrophobia [10]. In this study, we have focused on confirming the lectins in the plant sources by hemagglutination assay.

Experimental

Collection of seeds

Initially, *Sesbania bispinosa* and *Senna occidentalis* seeds were collected from the local area and pulverized in a mortar and pastel to produce fine powder materials. The powder materials were screened by 50 mesh screen, and fat free powder of seeds were prepared with diethyl ether.

Extraction of lectin

At first, 25 mM of sodium phosphate buffer with the pH of 7.4 was added to the fat free powder of the tested sample A and B (50 mL for 10 gm) and was kept overnight at 4 °C with occasional stirring. The homogenate were filtered through double layer of silk cloth and centrifuged at 10,000 g for 10 min. The clear supernatants were used as crude extracts.

Protein precipitation by ammonium sulphate

The supernatants were adjusted to 90% saturation by adding solid ammonium sulphate and kept for 6-8 h at 4 °C. The precipitate was collected by centrifugation at 10,000 rpm for 10 min, then dissolved in 20 mM of Tris-HCl buffer containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$ and 1 mM $MgCl_2$ with the pH of 8. After centrifugation, the clear supernatants were dialyzed against same buffer solution and used as tested samples. The supernatants from *Sesbania bispinosa* and *Senna occidentalis* seeds were denoted as tested sample A and B, respectively.

Determination of the protein content by biuret method

Standard: Standard contains protein 80 gm/L

20 μL of crude extracts, tested samples (A and B), and standard protein sample were added to 1 mL of total protein reagent in different test tubes, the mixtures were shaken for few times, allowed to react for 10 min at room temperature. The absorbance of crude, tested samples and standard against the reagent blank were measured within 30 min at 546 nm using a UV-visible Spectrophotometer. The amount of protein contents were determined using the biuret method and the following equation.

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

Hemagglutination assay

Preparation of 2% NaCl solution

2 gm of NaCl was dissolved in 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.

Preparation of hemagglutination buffer

20 mM of Tris-HCl buffer with the pH of 7.8 containing 0.15 M of NaCl and 10 mM of $CaCl_2$ 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. Finally, 0.88 gm NaCl and 0.11 gm $CaCl_2$ were added and dissolved in that buffer.

Table 1. Total protein contain of tested sample.

Steps		Sources	Volume (mL)	Absorbance at 546 nm	Protein content (mg/mL)	Total protein (mg)
Standard		-		0.41	80	
Tested samples	A	*Sesbania bispinosa*	28	0.063	12.6	352.8
	B	*Senna occidentalis*	45	0.049	9.8	441

Table 2. A summary of hemagglutination assay during screening

Plant	Steps	Volume (mL)	Lectin activity (titer/mL)	Total activity (titer)	% Yield
Sesbania bispinosa	Crude extract	6	512	3072	29.21%
	Tested sample A	28	32	896	
Senna occidentalis	Crude extract	5	512	2560	28.12%
	Tested sample B	45	16	720	

Blood collection and preparation

Blood of human (Group A, B, O and AB), chicken, bovine and goat were used to test the blood group specificity. Samples of human blood were collected from four donors and other bloods were collected

from the respective species at their slaughter house. All the blood samples were collected in saline and centrifuged for 10 min. The erythrocyte pellet (Red blood cell, RBC) was washed thrice and re-suspended in the same saline to prepare a 2% suspension for hemagglutination assay.

Hemagglutination activity test

The hemagglutination assay was performed in 96 well microtiter *U*-bottomed plates in a final volume of 100 µL containing 50 µL serially diluted tested sample with equal amount of hemagglutination buffer (20 mM Tris-HCl buffer, pH of 7.8 containing 150 mM NaCl and 10 mM $CaCl_2$) and 50 µL of 2% suspension of erythrocytes previously washed with 150 mM NaCl. After a gentle shaking, the plate was kept at room temperature for 30 min. The visual agglutination titer of the maximum dilution giving the positive agglutination was recorded.

Results and Discussion

Determination of the protein content by biuret method

It was found that 10 gm of *Sesbania bispinosa* and *Senna occidentalis* seed contained 352.8 mg and 441 mg of total protein, respectively, as presented in Table 1.

Hemagglutination assay

Hemagglutination assay was performed using mice, chicken, bovine, goat, and human erythrocytes. Screened lectin from the tested sample A was powerfully agglutinated with human (A, B, O and AB) blood erythrocytes and there was no agglutinating activities in mice, chicken, bovine and goat erythrocytes. So, the sample A showed no human blood group specificity as it agglutinated A, B, O and AB all the blood groups tested. This behaviour was observed in other lectins, as well. For example, EspecL (*Erythrina speciosa* lectin) and BBL (*Belamyia bengalensis* lectin) can agglutinate all the human blood groups [11, 12]. A different result was also observed for HTTL (*Helianthus tuberosus* lectin), which could not agglutinate any human blood group (Table 2) [13].

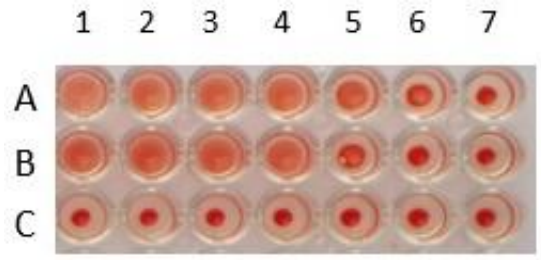
On the other hand, sample B was powerfully agglutinated with chicken erythrocytes and there were no agglutinating activities in mice, bovine, goat and human blood erythrocytes. Minimum agglutination activity were found to be 0.393 mg/mL in human (A, B, O and AB) blood erythrocytes and 0.613 mg/mL in chicken erythrocytes for tested sample A and B, respectively. The results of the hemagglutination activity are presented in Table 3, and the photograph of the hemagglutination activity is shown in Figure 1. The minimum concentration of other lectins from *D. batatas* [14] was found to be 2.7 and 3.9 µg/mL, respectively; whereas AJL (*Arisaema jacquemontii* lectin) from *A. jacquemontii* [15] required 11.5 µg/mL.

Table 3. Hemagglutination activity of various erythrocytes by tested sample A and B.

Erythrocytes	Group	Minimum plant concentration required for a visible agglutination (µg/mL)	
		Tested sample A	Tested sample B
Crude extract	-	49	38
Human	AB	393	NIL
	A	393	NIL
	B	393	NIL
	O	393	NIL
Chicken		NIL	613
Mice		NIL	NIL
Bovine		NIL	NIL
Goat		NIL	NIL

NIL, No hemagglutination activity

Figure 1. Hemagglutination activity test of screened lectin



A = *Sesbania bispinosa* =5 fold
B = *Senna occidentalis* = 4 fold
C = Control

A= Tested sample A in Tris-HCl buffer and human erythrocytes
B=Ttested sample B in Tris-HCl buffer and chicken erythrocytes
C= Tris-HCl buffer and erythrocytes(Control)

Conclusion

This study was performed to detect and characterize the possible presence of lectins from Bangladeshi plant sources by hemagglutination method. The results revealed that, the *Sesbania bispinosa* and *Senna occidentalis* plant sources contain considerable amount of lectin.

Acknowledgements

This research work has been partially funded by the Faculty of Engineering, University of Rajshahi and University Grants Commission of Bangladesh (sanction order no:A-438/5/52/UGC/Engg.-1/2017-2018).

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1]. Lis H., Sharon N. *Chem. Rev*., 1998, **98**:637

[2]. Van Damme E.J.M., Peumans W.J., Barre A., Rougé P. *Crit. Rev. Plant Sci*., 1998, **17**:575

[3]. Etzler M.E. Academic Press Inc Orlando., 1986, 371

[4]. Konkumnerd W., Karnchanatat A., Sangvanich P. *J. Sci. Food Agric*., 2010, **90**:1920

[5]. Sitohy M., Doheim M., Badr H. *Food Chem*., 2007, **104**:971

[6]. Tian Q., Wang W., Miao C., Peng H., Liu B., Leng F., Dai L., Chen F., Bao J. *Plant Sci*., 2008, **175**:877

[7]. Liu B., Bian H.K., Bao J.K. *Cancer Lett*., 2010, **287**:1

[8]. Kaur A., Singh J., Kamboj S.S., Sexana A.K., Pandita R.M., Shamnugavel M. *Phytochemistry*., 2005, **66**:1933

[9]. Goldstein I.J., Hughes R.C., Monsigny M., Osawa T., Sharon N. *Nature*, 1980, **285**:66

[10]. Warrier P.K., Nambiar V.P.K., Ramankutty C. *Orient Longman publication*, Madras, 1994, **2**:251

[11]. Konozy E.H.E., Bernardes E.S., Rosa C., Faca V., Greene L.J., Ward R.J. *Archiv. Biochem. Biophys.*, 2003, **410:**222

[12]. Banerjee S., Chaki S., Bhowal J., Chatterjee B.P. *Archiv. Biochem. Biophys*., 2004, **421**:125

[13]. Suseelan K.N., Mitra R., Pandey R., Sainis K.B., Krishna T.G. *Archiv. Biochem. Biophys*., 2002, **407:**241

[14]. Gaidamashvili M., Ohizumi Y., Iijima S., Takayama T., Ogawa T., Muramoto K. *J. Biol. Chem.,* 2004, **279**:26028

[15]. Kaur M., Singh K., Rup P.J., Kamboj S.S., Saxena A.K., Sharma M., Bhagat M., Sood S.K., Sing J. *J. Biochem. Mol. Biol.,* 2006, **39**:432

How to cite this manuscript: Mst. Jesmin Sultana*, Fazle Rabbi Shakil Ahmed. Identification of lectins from the seeds of Bangladeshi plants *Sesbania bispinosa* and *Senna occidentalis* by hemagglutination assay. *Asian Journal of Green Chemistry*, 3(4) 2019, 518-524. DOI: 10.33945/SAMI/AJGC/2019.4.8