Original Article

Detection of Optimum pH of *Momordica balsamina* Seeds Lectin

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ABSTRACT

Background: Lectins are carbohydrate binding proteins of non-immune origin that reversibly and non-enzymatically bind carbohydrates with high specificity for the chemical structure of the glycan array without changing their structure.

Objectives: The present study aimed to detect the optimum pH of *Momordica balsamina* seeds lectin (MbSL).

Materials and Methods:A season fresh of *Momordica balsamina*fruit seeds were brought from urban areas of Sudan (Gadarif and north Kurdofan states), then the lectin was isolated from saline extract by affinity chromatography on alpha agarose lactose matrix then the purified lectin activity was evaluated in different buffers to detect the optimum pH.

Results: The activity of the lectin remained stable in the pH range 2-12.

Conclusion: A lactose-binding lectin from seeds of *Momordica balsamina* medicinal plant shares a high degree of similarity with other Cucurbitaceae family lectins in term of their physicochemical features including sugar specificity, effect of pH on lectin stability.

Keywords: *Momordica balsamina;* lectin; seeds; plant lectin; pH; protein.

Lectins, multivalent cell-agglutinating proteins, by virtue of their exquisite sugar specificities are useful tools in widespread applications for monitoring the expression of cell-surface carbohydrates as well as for the purification and characterization of glycoconjugates[1]. Accordingly, lectin can be described as a substance which can agglutinate cells or precipitate glycoconjugates, with a structure resembling a carbohydrate binding protein or glycoprotein and is not of immune origin[2].

Lectins have been known since the turn of the 19th century. However, for a long time they attracted little attention, especially as it was assumed that they were confined to the plant kingdom and not present in humans or other animals[3]. The use of traditional medicine plants is now bringing attention of many scientists to examine the active components and to study toxicity for more safe valuable practice. Many of traditional medicinal plants have been studied for their protein content and lectin activity. However, only about 1% of these are proved through scientific studies to have real therapeutic value when used by humans[4].

Lectins have been classified according to their sugar-binding specificity to monospecific and polyspecific, the later can interact with more than one sugar[5].

Mature seeds are the main source of plant lectins, but they are also found in other vegetative tissues such as leaves, fruits but merely roots[6]. Plant lectins are secretary proteins that accumulate either in the vacuole or extracellular matrix. Most plants contain only one lectin, but in other cases, they contain two or more biologically different lectins[7,8].

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M. balsamina is a plant commonly known as Balsam apple, Dragon Flower in Arabic and Aeer locally in Sudan. Hutchinson,(1954) described the fruit as orange yellow, beaked, 21/2 inches in length bursting and exposing red brown seeds[9]. This species is closely related to *M. charantia* which occurs in areas of greater rainfall[10].

MATERIALS AND METHODS:

Plant materials *Momordica balsamina* fruit seeds were brought from urban area of Sudan (Gadarif and north Kurdofan states).

Erythrocytes:

Typed human blood cells (A, AB, B and O) were obtained from healthy donors, while animal blood cells were obtained from the animal house of Sudan University of Science and Technology, Khartoum, Sudan.

Chemicals:

Alpha agarose lactose affinity matrix was purchased from Sigma Chemicals. All other reagents were either of analytical grade or of highest quality available.

Preparation of seed extract:

The extraction was carried out as described by Konozy et al.[11]. Season fresh, mature, and good quality seeds of *M. balsamina* were ground to a fine flour in a coffee grinder and the meal (100 g) was defatted with petroleum ether in a ratio of (5mL/1g powder). Ether layer was removed by filtration system. Soluble protein was precipitated with equal amount of chilled acetone (added drop wise with continuous stirring). Extract was filtered through filtration system and further dehydrated extensively with chilled acetone to get acetone dried powder. Acetone dried powder was extracted for 4 hours with 0.145 mM NaCl at 4o C (in a ratio 5:1). The whole extract was filtered through filter paper and then centrifuged for 45 min at 6000rpm at 4^{o}C.

Purification of lectin on alpha agarose lactose matrix:

This was carried out essentially as described by Konozy et al.[12].In a syringe of 10mL capacity, 2mL of alpha agarose lactose were loaded; column was initially washed with 100mL of 100mM acetate buffer pH 5, equilibrated with 0.145M NaCl. Protein was loaded into the column; recycled for several times to ensure maximum retention of lectin on matrix. Unbounded proteins were washed off with equilibration saline till reading at OD280nm dropped to ≤0.2. Elution of bound lectins was done by 200 mM lactose; 3 ml fractions were collected at reduced flow rate of 3mL/min. Fractions were read for protein zZ content by spectrophotometer at 280 nm. Fractions that exhibited ODs above 0.06 were pooled, precipitated by ammonium sulphate 100%, then dialyzed against distilled water and tested for lectin activity.

Hemagglutination inhibition:

Hemagglutination test was conducted in a microtiter plates, in a final volume of 100µl. Each well contained 50µl of lectin solution and 50µl of 4% (v/v) suspension of either untrypsinized or trypsinized erythrocytes. Agglutination was assessed after incubation for 30 minutes at room temperature. Hemagglutinating activity was expressed titer, namely, the reciprocal of the highest dilution that gave a positive result[12]. The specific hemagglutinating activity was defined as titer (per mg lectin). Type O blood group was used throughout out this study.

Effect of pH on hemagglutinating activity:

This was performed as described by Konozy et al.[11], by incubating fixed concentration of the lectin 0.03 mg/ml with buffers of varying pH (between pH 2 to pH 13) at a total volume of 100 µl for 1 h at room temperature (25–27°C). The pH of lectin samples was adjusted to pH 7.0 by addition of 0.1 N NaOH or 0.1 N HCl before hemagglutinating activity was examined.

RESULTS:

Effect of pH on *Momordica balsamina* seeds lectin:

The lectin was stable over a wide range of pH values as shown in figures 1.

DISCUSSION:

The crude protein extracts of *Momordica balsamina* Seeds revealed high hemagglutinating activity when tested against human and animals RBCs and showed more

activity towards the O blood type (as shown in Table 1) which could be inhibited by many sugars mainly lactose. The purification was done in a single step by affinity chromatography on alpha agarose lactose matrix. The three dimensional structure of a protein is held together by non-covalent interactions viz. hydrogen bonds, ionic interactions, hydrophobic interactions, Vander Waals' forces and covalently by

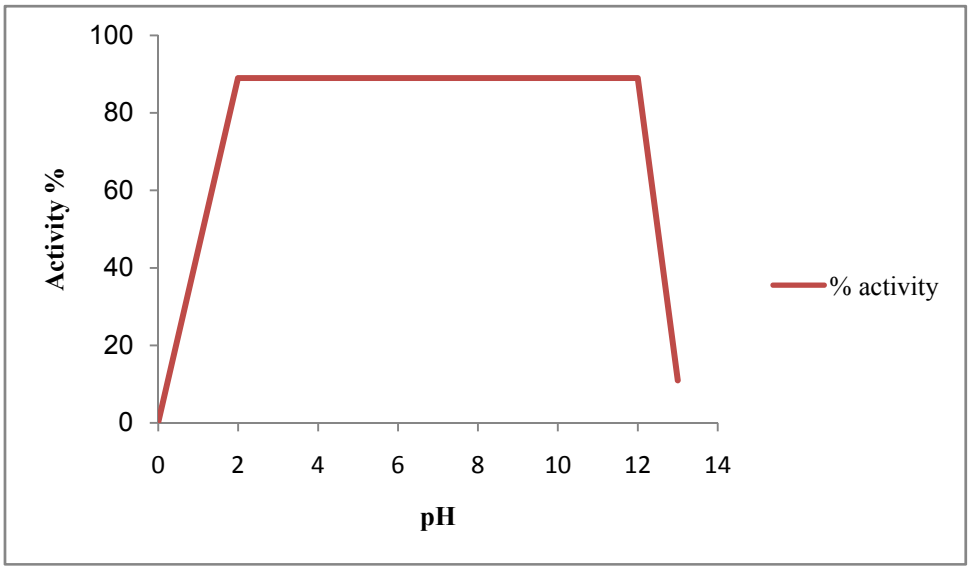

Figure (1): Effect of pH on the purified lectin (30ug of Lectin was incubated an hour at varing buffers ranging (2-13) at room temp. Lectin samples were neutralized either by 1% Hcl or 1% NaoH)

Table (1): Hemagglutinating activity of *Momordica Balsamina* seed lectin against different blood cells.

Erythrocytes type	Trypsin untreated (Specific activity)	Trypsin treated (Specific activity)
Human		
A	16	32
B	8	8
AB	4	130
O	16	130
Animal		
Mice	0.08	0.02
Donkey	NAD	NAD
Cow	0.8	NAD
Goat	NAD	NAD

NAD: No agglutination detected

disulfide linkages. Conditions, which disturb these stabilizing forces, affect the native conformation of the protein by changing its physical properties and biological activity[13]. Examination of purified MbSL toward different pH values showed that the activity of the lectin remained stable in the pH range 2-12. In comparison, the purified lectin from seeds of *Dolichos lablab* pH optimum found to be 7.4, *Trichosnthes dioica* lectin remains stable in pH range 6-12 was only partially stable at pH 4, while 60% activity was lost at pH 2[14].*Erythrinaspeciosa* seeds lectin was acidic pH sensitive and totally lost its activity when incubated with all pH values between pH 3 and pH 6. Above pH 6 and to pH 9.6 there was no effect on the lectin activity[12].

CONCLUSION:

MbSL shares a high degree of similarity with other *Cucurbitaceae* family lectins in sugar specificity and effect of pH on lectin stability.

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