Research Article

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EXTRACTION AND PURIFICATION OF LECTIN FROM PLANT SEEDS AN EMPIRICAL STUDY ON *ARTOCARPUS* SP

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ABSTRACT

Present study was aimed to isolate the jacalin from the jack fruit which is humans consumes more frequently in their daily lives. Secondly, to purify the lectin by agglutinin method preferred PBS with a combination of sulfate precipitate was used to perform agglutination. But, agglutinating activity levels of erythrocytes will enhance when they undergone trypsinization. The purified lectin is an entire lectin because it is agglutinated from erythrocytes without trypsin remedy and as well as without addition of metal ions. Heam-agglutination was performed and assayed for the crude extract, dialyzed sample and purified lectin samples. Incubated for one hour the resultant plates were formed a layer of erythrocytes on the surface of wells and it was confirmed the indication of agglutination. The extracted lectin was used for agglutination inhibition assay and specificity in the direction of the distinct sugars used in the present study. The present study revealed that lectin proteins have the abilities to differentiate determinants of carbohydrates in mammalian blood cells. Our ancient's have been used plant lectins for detecting the blood groups. An enormous plant lectins and some kind of glycan compounds have the ability to bind with high affinity and appropriate haptens for blocking of interactions. Due to these fusion and duplication combination of defense genes may leads evolutionary advantage and to generate new diversified protein specificities.

Keywords: Lectin, Jacalin, Artocarpus sps, Heamagglutination and micro well plate.

INTRODUCTION

Plant lectins are divalent or multivalent sugar binding proteins which can agglutinates cells of different organs having multiple saccharide and as well as sufficient complementary sites for binding the lectins. Lectins are also glycol-proteins which may agglutinate mammalian erythrocytes, polysaccharides and carbohydrate complex. Lectins are the first discovered phytohemagglutinins in vegetation and later termed as agglutinins or hemagglutinins. Most of the recent researches were confirmed that the lectins may also be present in various sources of kingdom of plants, animals and even lower forms of some microorganisms. The maximum abundant lectins are determined from the leguminous seeds, however may also be obtained from various parts of plants and as well as hole plant material^{1, 2, 3}.

Surprisingly, lectins are even unique for the sugar moieties of binding proteins and act as central dogma in biological and biochemical phenomena of cells and proteins. As an instance, some viruses will also be able to utilize and attach themselves to the point of host infected organism's cells. Lectins are also can be disabled by specific mono and oligo-saccharides which bind to them and prevent their bonding with the membranes of infected cells. However, now research scientist are recognized that the lectins to be gift of nature and time- honored in microbial world, wherein they have a tendency to be called by means of other names, which includes hemagglutinins and adhesions³.

At present synthetic lectins are commercially available and widely using as biological tools in glycobiology come from flora

and widely scattered within the plant kingdom, despite of that legume plants are the most widely using plants to extract natural lectins. These magnificent characteristics have additionally been appreciably exploited in the wide variety of fields in biochemical and biomedicine fields¹ and are divalent or multivalent proteins which can agglutinates the cells and other saccharide substances^{2, 3, 4}.

The magnanimous properties of lectins will be purely depending on their efficacy to influencing ability with carbohydrates combine with glycol-components on its mobile surface, furtherly it may lead biological building blocks of important proteins⁵. To the greatest extent immense lectins were discovered from the seeds of leguminous plant as a source life but they also can be prevalent from different wide variety of plant parts like leaves, roots and stems. Lectins are capable of tensely bind to and specific polysaccharides and glycoproteins precipitation, why because of polyvalent characteristic range of lectins, however, it depends on molecular length, composition of an amino acid metallic ion requirements and architecture of specific lectin⁶

Lectins are typically detected in, and sooner or later isolated from, dried seeds due to their convenience of acquiring and easy and flexibility of working with seeds. Indeed, lectins were discovered in numerous other tissues, including potato (*Solanum tuberosum*) tubers, soya-bean (*Glycine max*) roots, fruit skins of cucumber own family. Boyd and Reguera stated that tomato (*Lycopersicon esculentum*) seeds possessed no agglutinating activity toward human A-, B- or O- type erythrocytes. However, located agglutinating interest closer to glutaraldehyde-fixed human erythrocytes were observed in the extracts all parts from

a tomato plant including the seeds of tomato plant. This obvious inconsistency may be because of absolutely replicative difference among clean and glued cells of tomato in assay⁶. The production of Jackfruit in Thailand is 392 tons and used to prepare a canned sugary syrup and frequently exporting to America and Europe countries⁷.

The Jackfruit tissue is starchy fibrous flavor compatible with other fruits like pineapple, mango and apple and banana respectively^{7, 8}. Most of the Asian countries culture, Artocarpus used as staple food in boiled form⁸. Especially in Thailand boiled young Artocarpus fruits are used to prepare a Thai salad namely tam kanun. jackfruit consist bulbs which made up of easily digestible flesh and having rich energy sources 100 gm of fruit will provide approximately 93 calories. It is a rich source of Antioxidants, 13 gm of Vitamin-C and other essential minerals^{7, 8, 9}.

MATERIALS AND METHODS

Artocarpus Heterophyllus was selected for this study and prepared a herbarium (DND-JF-012016) deposited in Pharmacology Department at St. Theresa International College. Dry seeds were collected from local area of Ongkharak, Nakhon Nayok, Thailand and de-hulled manually. Powder was extracted from the seeds by grinding with mortar and pestle furtherly de-fatted with n hexane (1:5 ratios w/v) for fifteen minutes and was repeated twice to ensure until the hexane was clear from the fat. Furtherly PBS (0.02 M, pH 7.2) was added to the above sample at the ratio of 1:5 (v/w) with continuous stirring by using magnetic stirrer for 16 hours, followed by immediate cheese filtration. The obtained solution was centrifuged at 10000 rpm/15 min at 4°C, the residue of precipitation was discarded and the resultant solution was stored at 0°C for further use.

Precipitation of Ammonium Sulfate

Was carried out by using 90% Ammonium sulfate as precipitant and, incubated for 1 hour the mixture was centrifuged at 10000 rpm for ten minutes. Precipitated pellet was collected dissolved in adequate amount of buffer and stored for further use.

Desalting

Membrane dialysis tubing was done to remove ammonium sulfate from the above solution with subjecting it in to running water and tubes were sealed at one end ammonium sulfate was poured with the help of pipette to avoid bubble formation then sealed the other end stirred for 6 hours at 4°C. finally desalted sample was collected and centrifuged. Supernatant was collected and stored at 0°C.

Red blood cells preparation

A quantity of 200 µL A, B, AB and O were mixed with 10 mL of PBS and the mixture was centrifuged at 10000 rpm for 5 minutes. Supernatant solution was discarded pellet of compacted RBC were diluted with out to 10 mL mark same repeated until obtaining the clear solution. The same repeated to erythrocytes and was incubated trypsinized made a 1.5 percent RBC suspension

Heamagglutination assay

A quantity of 50 µL Crude extract, ammonium sulfate and gel filtration fractions are weighed and serially diluted with PBS in multi well microtiter plates and same quantity of 2% erythrocyte suspension were added to each well after one hour visually observed.

Agglutination of inhibition

Glucose, xylose and maltose were used to examine agglutination reaction. 1000 500 mM of standard sugars, a 50 µL of the extracted lectin was placed in microtiter which contains 50 µL of the above said sugars. Furtherly 50 µL of erythrocyte suspension was added and observed for the inhibition of agglutination and formation of button shaped distinct cells at the bottom.

Determination of protein

The Bradford method was used to determine the crude protein from the extract, ammonium sulfate and Sephadex G – 100 shows activity levels of lectin. Here as a standard BSA was used and 1.5 mL of Coomassie blue was added to the 0.5 mL of the sample mixed thoroughly after 5 minutes the absorbance were recorded at 595 nm¹⁰.

Estimation of protein

We can measure the protein content in a solution quantitatively by different kind methods. Amongst Bradford is easy and simple and expensive as well. Assay based on the capability of proteins to bind Coomassie brilliant blue G250 and forms a complex coefficient was much more than that of the other free dyes.

MATERIALS AND REAGENTS

Preparation of Dye Concentrate^{10, 11}

100 mg of Coomassie brilliant blue G-250 dissolved in 50 ml of ethanol 95% furtherly 100 ml of concentrated orthophosphoric acid added followed by distilled water to a final volume of 200 ml and store in amber colored bottle. Later whenever we need to use mix 1:4 volume of ratio concentrated dye solution in distilled water. This was subjected to filter with whatmann no one paper.

Preparation of Protein¹¹

Stock 50 mg of BSA was weighed and dissolved in distilled water and make up to 50 ml in standard flask^{11, 12}

Preparation of working standard^{10, 11}

Solution 10 mg of stock solution diluted in 50 ml of distilled water in standard flask of 200 µg.

Steps Involved in Procedure^{10, 11}

0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard was pipette out in to a series of tube and labeled. Add 5 ml of diluted dye binding solution mix well incubate for 5 minutes to develop blue color when it binds with proteins and absorbance was recorded at 595 nm.

RESULTS AND DISCUSSION

Pre weighed de hulled seeds were ground in a mortar and pestle resulting powder extracted with hexane –n to omit the fat contents or lipids which may interferes in agglutination. 1 gm of seed powder was mixed with 5 ml of n hexane the same was repeated twice to get defatted powder and was stirred for 16 hours and centrifuged supernatant was used as crude source of lectin for 10 gm of extracted powder dissolved in 150 ml of PBS solution (Figure 1-4 and Table 1 and 2).

Proteins were separated by precipitation with ammonium sulfate the resultant was the desalted substance solution declines the solubility and leads to protein precipitation. Ammonium sulfate was choosing as a precipitating agent due its high solubility and magnesium sulfate absorbs the water from the dehydrated surroundings and protects the protein extract to coagulate (fig: 1,

2, 3, 4 and table: 1 and 2). Hence, increased levels of ionic strengths decline the solubility of protein. From zero to ninety percent of ammonium sulfate saturation was confirmed that the most of the proteins gets precipitated.

Extensive dialysis was carried out by Desalting with distilled water at 4°C to prevent denaturation of protein. The membrane which was used in the present study has a 12 KDa of molecular cut off and it allows the proteins with more than 12 KDa of molecular weight to sustain in dialysis sac for further investigation by PAGE.

Partial characterization of lectins from *Artocarpus* sp by using assay of heamagglutination

Heam-agglutination was performed and assayed for the crude extract, dialyzed sample and purified lectin samples. Incubated for one hour the resultant plates were formed a layer of erythrocytes on the surface of wells and it was confirmed the indication of agglutination.

Figure 1: The below picture showing the Agglutination reaction of crude extract, dialyzed sample and purified form of a lectin after incubation over a period of one hour

- 1: two wells contains crude extract showing agglutination
- 2: two wells contains dialyzed sample showing agglutination
- 3: Shows agglutination in two wells which contains purified lectin

Assay of Heamagglutination inhibition

Inhibition of agglutination was performed for the crude extract, dialyzed and for purified lectins which was assayed by using sugars and it was observed that no inhibition for all four different sugars use in the present study. It indicates that the lectins contain enormous number of binding sites that can be compatible with wide ranges of receptors of monosaccharides. Accordingly, if the sugar lectin interaction is more abundant than the erythromycin and lectin interventions, hence there would be a possibility of occurring of inhibition at bottom of wells.

Figure 2: Crude extract Inhibition with Lactose (L: 1, 0.5), Xylose (X: 1, 0.5), Glucose (G: 1, 0.5) and Maltose (M: 1, 0.5).

Figure 3: Inhibition assay of dialyzed sample with Xylose (X: 1, 0.5), Lactose (L: 1, 0.5), Glucose (G: 1, 0.5) and Maltose (M: 1, 0.5).

Figure 4: Inhibition assay of purified lectin with Lactose (L: 1, 0.5), Xylose (X: 1, 0.5), Glucose (G: 1, 0.5) and Maltose (M: 1, 0.5).

Protein concentration determination by Bradford's method

Table 1: Extracellular protein content was calculated by Bradford's estimation. It is useful when the amount of the unknown protein is limited

S. No	Standard solution volume in ml	Standard solution concentration in µg	Volume of distilled water ml	Volume of Bradford's reagent ml	OD values 595 nm
1.	Blank	0	1	5	0.00
2.	0.2	40	0.8	5	0.09
3.	0.4	80	0.6	5	0.16
4.	0.6	120	0.4	5	0.24
5.	0.8	160	0.2	5	0.30
6.	1	200	-	5	0.33

Table 2: Protein concentration for crude extract, dialyzed sample and purified lectin is determined by Bradford's assay

S. No	Name of Lectin sample	Sample volume µg	Volume of distilled water	Bradford's reagent volume	OD at 595 nm
1.	Crude extract	400	600	5	0.31
2.	Dialyzed sample	400	600	5	0.28
3.	Purified Lectin	400	600	5	0.16

According to the OD values of the samples the concentrations of lectins in dialyzed sample 170 µg, Crude extract 150 µg and purified lectin 100 µg respectively.

Furthermore, it is possible that Yeoman and co-employees were detected that a non-lectin agglutinin occur in tomato extracts. One of the most abundant famous lectins have been isolated from the jackfruit seeds and till now it is the one which was maximum studied lectin^{13, 12}. Another lectin namely well known as heterophyllin changed into additionally extracted from the seeds of *Artocarpus heterophyllus*¹¹⁻¹⁴. Moraceae own family are the other lectins isolated included seeds, *A. lakoocha*¹⁵, *A. hirsuta*¹⁶, *A. integer*¹⁷, *A. incisa*¹⁸, *A. lignanensis*¹⁹, *A. integrifolia*²⁰ and these days species from Vietnam particularly *A. champeden* and *A. masticata*²¹.

Furtherly Lectins were using as probes for identifying and mapping sugars on cellular surfaces of particular reagent for the isolation of saccharide-containing biomolecules and its agglutinating assets and the levels of adherence potentiality towards glycol-conjugates by means of affinity chromatography. These properties are also for the usage of lectins for blood typing, cell separation, bacterial typing and transplantation of bone marrow and as a device in most cancers and biomedical research due to its potentiality to distinguish normal cell from the malignant or converted cells²².

Despite of these abilities of lectins now a days using as a biotechnological tool in specific research-studies^{23, 24, 25, 26, 27}.

SUMMARY AND CONCLUSION

Although, many researchers identified that the natural functions of plant lectins and many other researchers across the globe still trying to identify more properties. Most profound novelty and wide variety of functional properties of lectins are now being understood and different lectin abilities are recognized to differentiate determinants of carbohydrates in mammalian blood cells. Our ancient's have been used plant lectins for detecting the blood groups. An enormous plant lectins and some kind of glycan compounds have the ability to bind with high affinity and appropriate haptens for blocking of interactions.

Additionally, many cereal plant lectins with tandem repeats arose through duplication of lectin domains. Most of all such kind of lectin proteins have the natural biological defense ability

against to pathogens and predators which contains chitin. Due to these fusion and duplication combination of defense genes may leads evolutionary advantage to generate new diversified protein specificities. The authors are trying to characterization by simple PAGE method to know more about jacalin lectins.

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