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ISOLATION AND CHARACTERIZATION OF LECTIN FROM *Carica papaya* AND *Manilkara zapota*

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ABSTRACT

Lectins are sugar binding proteins or glycoproteins of non-immune origin which bind to specific mono or oligosaccharides without altering the bound ligand and having the ability to agglutinate cells, which are extensively found in plants, vertebrates, and invertebrates. The aim of this study was to comparatively analyze the physicochemical properties, haemagglutination pattern and sugar specificity of both the lectins from Carica papaya and Manilkara zapota respectively. The haemagglutination pattern studies showed that lectin from ripen papaya and papaya seeds have affinity towards A+ve human blood group whereas the raw papaya lectin showed affinity towards O+ve human blood group unlikesapota lectin. In the present study the papaya lectin and sapota lectin have shown affinity towards different sugar binding. Electrophoretic separation of raw papaya extract and sapota extract showed single bands in 10% non-denaturing gel (Native PAGE). The same lectins showed three bands (subunits) of molecular weight ~39 kDa, ~80 kDa and ~101 kDa for papaya and a single band of ~33 kDa in case of sapota respectively.

KEY WORDS

Lectins, Carica papaya, Manilkarazapota,haemagglutination, haemagglutination inhibition assay, Electrophoretic separation.

INTRODUCTION

Lectins are sugar binding proteins or glycoproteins of non-immune origin which bind to specific mono or oligosaccharides without altering the bound ligand and having the ability to agglutinate cells, which are extensively found in plants, vertebrates, and invertebrates. They are most abundant in the plant kingdom, and are found in seeds, leaves, barks, tubers, rhizomes, roots, bulbs, depending on the plant species [1]. Lectins are currently used as tools in elucidating membrane structure and cell transformation (Lis and Sharon, 1981). Lectins are also used for determining the oligosaccharide structure glycoconjugates. [2].

These are proteins/glycoproteins, which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharides or oligosaccharides (Peumans and Van –Damme, 1995).

These lectins can be employed in a range of biomedical studies, including cancer and immunological research, isolation and characterization of glycoconjugates, and blood typing. In glycoproteomics, studies are facilitated by the large number of natural lectins that recognize and bind to carbohydrates.

When immobilized on inert matrices, these lectins are used in affinity chromatography, assisting in the purification and separation of glycoproteins for analytical testing. [3]

Legume lectins are implicated in recognition of specific nodulating rhizobia [4]. It was shown that a lectin from a particular legume binds only to the corresponding rhizobial species and not to rhizobia infecting other legumes.

Several studies have confirmed lectins as being insecticidal and transgenic crops expressing lectin genes have been introduced in many economically important crops [5].

Lectins were first described in 1888 by Stillmark, who observed that crude extracts of castor beans (*Ricinuscommunis*) contained a toxic substance

named ricin that agglutinated human and some animal red blood cells. Ehrlich which is considered to be Father of Immunology has done the research showing that the small amount of lectin containing seeds when fed to rabbits cause partial immunity to the toxicity demonstrating lectins are also antigenic (induce Ag-Ab reaction).

These are dynamic contributors to tumor cell recognition (surface markers), cell adhesion and localization, signal transduction across membranes, mitogenic stimulation, and augmentation of host immune defense, cytotoxicity and apoptosis[6].

Lectins play a major role in nitrogen fixation in leguminous and non-leguminous plants. It has been suggested that root lectin recognized by bacterial receptor molecules is an important determinant of host plant specificity in *Rhizobium* legume symbiosis[7]. These have been implicated in direct first-line defense against pathogens, cell trafficking, immune regulation and prevention of auto-immunity[8].

MATERIALS AND METHODS

(i) Extraction of agglutinin:

Carica papaya and *Manilkarazapota* were obtained from the local market sold as food and cleaned with water to remove dirt. 50% homogenate was prepared by crushing the fleshy part of *Carica papaya* with PBS in mortar-pestle and with TBS in case of *Manilkarazapota*. Further papaya sample was homogenized in Down's homogenizer and both papaya and sapota extracts were filtered through muslin cloth. Samples were quickly centrifuged at maximum rcf (8000g) and supernatant from each sample was used as agglutinin.

Preparation of erythrocyte suspension:

The human blood was obtained from the volunteers in sodium citrate. 2ml blood was taken in the centrifuge tube and 8ml of 0.9% NaCl was added to make the volume 10ml. The blood was properly resuspended with the help of Pasteur pipette. It was centrifuged in cooling centrifuge at 10,000 rpm for 10 min at 4°c. The supernatant was discarded and pellet was again resuspended in 0.9% NaCl to make up the volume upto 10ml. 5ml of blood was transferred into another tube and 5ml of 0.9% NaCl was added to each tube to make it 10ml.

(ii) Haemagglutination assay:

The haemagglutination assay was performed in 96 well polystyrene U bottomed microtitre plate.[9] The series of decreasing dilutions of agglutinin was made with 100µl of PBS for papaya sample and of TBS in case of sapota sample (0.1 M, pH-7.2). A control was prepared by using only PBS for papaya sample and by using TBS for sapota sample. 100µl of 2.5% erythrocyte suspension was added to every well and mixed by resuspending the mixture. The plate was kept for incubation at 37°c for 1 hour. Haemagglutination was examined visually and reciprocal of maximum dilution of the agglutinin solution showing haemagglutination was recorded as titer.

(iii) Haemagglutination inhibition assay:

Haemagglutination inhibition assay was performed by carrying out haemagglutination assay of agglutinin, along with equal volume of inhibitors (dextrose, lactose and maltose) and keeping PBS and TBS provided with respective concentration of inhibitor as control. The degree of haemagglutination was examined and maximum dilution of agglutinin showing inhibition was recorded.

(iv) Physiochemical properties:

pH stability:

The pH stability of lectin was found out by using 0.1 M PBS (pH- 7.2) of different pH ranging from pH 1 to pH 14 in case of papaya agglutinin and by using 0.1 M TBS (pH- 7.2) in case of sapota agglutinin, in which the agglutinin is serially diluted. The haemagglutination assay was performed as described above.

Temperature sensitivity:

The agglutinin from both the samples in 0.1M PBS (pH-7.2) and 0.1 M TBS (pH- 7.2) were incubated in water bath at temperature ranging from 0°c-100°c for 15 minutes. The aliquots (100µl) were withdrawn, cooled and haemagglutination assay was performed as described above.

Protein estimation:

The protein concentrations in all the agglutinin fractions were determined by the Lowry et.al (1951) method[10]. To 100µl of the sample solution 900µl of

distilled water was added to make the volume up to 1 ml. To this 5ml of Alkaline copper reagent and followed by 5 minutes incubation at room temperature. 0.5ml of FC reagent was added to this mixture. Tube was kept in dark for 30 minutes and the absorbance was measured at 660 nm. BSA (200µg/ml) was used as the standard and a blank was prepared with water. The amount of protein was determined from the standard curve obtained from the absorbance of BSA.

Polyacrylamide slab gel electrophoresis:

(v) 10 % Non-Denaturing gel:

The stacking gel consists of 500µl of 30% Acrylamide-0.8% bisacrylamide solution, 1.25ml of 0.5M Tris base (pH 6.8), 50µl of 10% Ammonium persulfate,1.5ml of Distilled water and 4µL of TEMED. The separating gel consisted of 1.4ml of 1.5M Tris (pH 8.9), 1.86ml of 30%Acrylamide-0.8%Bisacrylamide solution, and 50µl of 10% Ammonium persulfate, 2.46ml of Distilled water and 4µl of TEMED. The running buffer contained 1.5g of Tris (pH 8.9), 7.2g of Glycine and final volume was made up to 500ml using distilled water.

(vi) 10% Denaturing gel:

The stacking gel consists of 350µl of 30%Acrylamide-0.8%bisacrylamide solution, 625µl of 0.5M Tris base(pH 6.8),20µl of 10% Ammonium persulfate,1.54ml of Distilled water, 4µl of TEMED and 50 µl of 10% SDS. The separating gel consisted of 1.25ml of 1.5M Tris (pH 8.9), 1.65ml of 30%Acrylamide-0.8%Bisacrylamide solution, 50µl of 10% Ammonium persulfate, 2.05ml of Distilled water, 4µl of TEMED and 75µl of 10% SDS. The running buffer contained 1.5g of Tris (pH 8.9), 7.2g of Glycine, 5g of SDS and final volume was made upto 500ml using distilled water.

RESULTS AND DISCUSSION

The papaya and sapota lectin strongly agglutinate only untreated human erythrocytes, that to also papaya lectin exhibit a preference for O + ve and A + ve as compared to B and AB blood cells. On the other hand sapota lectin shows high level of agglutination of A + ve blood as compared to B, AB and O blood groups. [Table I, II]

In the present study the haemagglutination activity of papaya seed is found to be low as it contains weak agglutinin which was in reference with the earlier work done on *Carica papaya.* [11]. [Table III]

The sapota lectin was found to be stable at a pH range of 9-13 as that of papaya lectin which was found to be stable at the pH range of 7-13.The sapota lectin shows enhanced haemagglutination activity with the addition of divalent metal ions while papaya lectin doesn't require any metal ion for its activity [Table V].Both the lectins were found to get inactivated above 40^{o}C. [Table VI].

Papaya seed lectin 'Caricin' have high specificity for monosaccharides [12], which is similar to the inhibition test performed with ripen and raw papaya extracts in the present study. The papaya lectin has shown inhibition of haemagglutination activity at sugar concentration above 0.4 mg/ml-0.6 mg/ml.

In the present study the sugar specificity of papaya and sapota lectin was studied by using different monosaccharides viz. lactose, maltose and dextrose. The papaya lectin showed high binding affinity for the lactose sugar followed with maltose and dextrose. The minimum concentration of lactose for the inhibition of haemagglutination activity of papaya lectin is noted to be 0.25 mg/ml and above 0.5 mg/ml, the haemagglutination activity gets inhibited completely. Dextrose, on the other hand have shown less affinity and gives positive haemagglutination results at concentrations 0.1 mg/ml- 0.4 mg/ml. Maltose have shown moderate haemagglutination as well as inhibition activity which is relatively similar to that of lactose such that at concentration 0.1 mg/ml – 0.4 mg/ml, it shows positive haemagglutination while inhibits the lectin activity above 0.5 mg/ml. In case of sapota lectin, lactose and maltose have showed high binding affinity followed by dextrose. The minimum concentration of lactose and maltose for the inhibition of haemagglutination activity of sapota lectin is noted to be 0.25 mg/ml and above 0.5 mg/ml, the Haemagglutination activity gets inhibited completely. Dextrose on the other hand have shown less affinity and gives positive haemagglutination result at concentrations 0.1 mg/ml -0.4 mg/ml. [Table VII] [Fig.1]

Table I: Pattern of Haemagglutination shown by ripen papaya extract prepared in PBS

SAMPLE	PREPARED IN PBS			
	RIPEN PAPAYA			
BLOOD GROUP DILUTION	A+ ve	O + ve	B + ve	AB + ve
0:0 (CONTROL)	-	-	-	-
1:2	+ +	-	-	-
1:4	+	-	-	-
1:8	-	-	-	-
1:16	-	-	-	-
1:32	-	-	-	-
1:64	-	-	-	-

PBS: Phosphate buffer saline

Table II Pattern of Haemagglutination shown by sapota extract prepared in TBS

SAMPLE	PREPARED IN TBS			
	SAPOTA			
BLOOD GROUP DILUTION	A+ ve	O + ve	B + ve	AB + ve
0:0 (CONTROL)	-	-	-	-
1:2	++	-	-	-
1:4	+	-	-	-
1:8	+	-	-	-
1:16	+	-	-	-
1:32	+	-	-	-
1:64	+	-	-	-
1:128	-	-	-	-
1:256	-	-	-	-
1:512	-	-	-	-

TBS: Tris buffer solution

Table III Pattern of Haemagglutination shown by raw papaya extract and papaya seeds

SAMPLES	RAW PAPAYA				PAPAYA SEEDS			
BLOOD GROUP DILUTION	A+ ve	O + ve	B + ve	AB + ve	A+ ve	O + ve	B + ve	AB + ve
0:0 (CONTROL)	-	-	-	-	-	-	-	-
1:2	-	+ +	-	-	+ +	-	-	-
1:4	-	+	-	-	+	-	-	-
1:8	-	-	-	-	-	-	-	-
1:16	-	-	-	-	-	-	-	-
1:32	-	-	-	-	-	-	-	-
1:64	-	-	-	-	-	-	-	-

Table IV Effect of pH on haemagglutination activity of papaya and sapota extract

DILUTION pH VALUE	0:0 (CONTROL)	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
pH 8	-	+	+	-	-	-	-	-	-	-
pH 9	-	+	+	+	-	-	-	-	-	-
pH 10	-	+	+	+	+	+	+	-	-	-
pH 11	-	+	+	+	+	+	-	-	-	-
pH 12	-	+	+	+	+	-	-	-	-	-
pH 13	-	+	+	+	-	-	-	-	-	-
pH 14	-	-	-	-	-	-	-	-	-	-

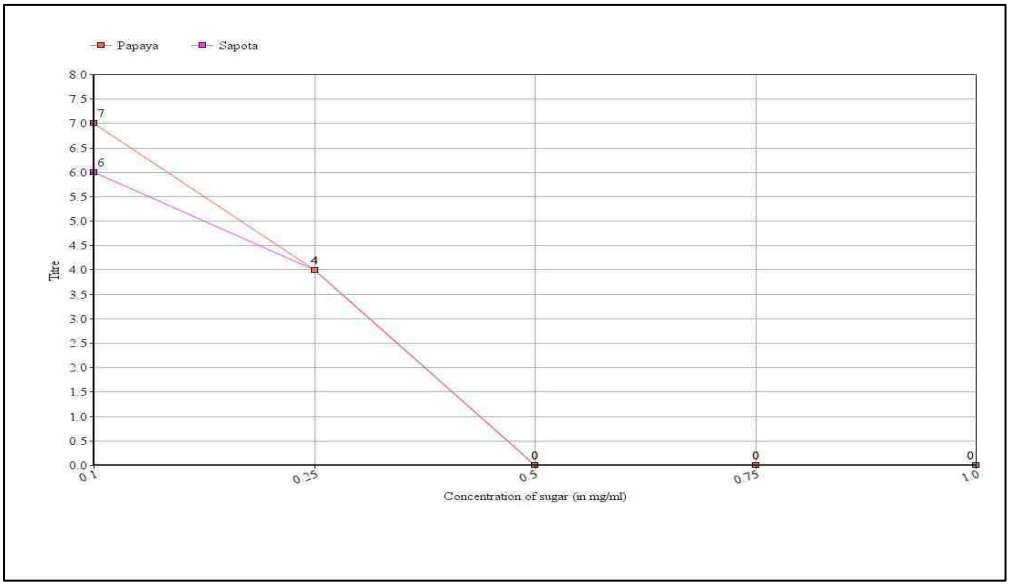
Table V Effect of pH and calcium ions on haemagglutination activity of sapota extract

SAMPLES	PAPAYA SAMPLE						SAPOTA SAMPLE					
TEMPERATURE DILUTION	**0° C**	**20°C**	**40°C**	**60°C**	**80°C**	**100° C**	**0° C**	**20°C**	**40°C**	**60°C**	**80°C**	**100° C**
0:0 (CONTROL)	-	-	-	-	-	-	-	-	-	-	-	-
1:2	+	+	+	-	-	-	+	+	+	-	-	-
1:4	+	+	+	-	-	-	+	+	+	-	-	-
1:8	-	+	+	-	-	-	+	+	+	-	-	-
1:16	-	-	+	-	-	-	+	+	+	-	-	-
1:32	-	-	-	-	-	-	-	+	+	-	-	-
1:64	-	-	-	-	-	-	-	-	+	-	-	-
1:128	-	-	-	-	-	-	-	-	-	-	-	-
1:256	-	-	-	-	-	-	-	-	-	-	-	-
1:512	-	-	-	-	-	-	-	-	-	-	-	-

Table VI Effect of temperature on haemagglutination activity of papaya and sapota sample

SAMPLES	PAPAYA SAMPLE												SAPOTA SAMPLE									
DILUTION CONCENTRATION	SUGARS	0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512		0:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
0.1mg/ml	LACTOSE	-	+	+	-	-	-	-	-	-	-		-	+	+	-	-	-	-	-	-	-
	MALTOSE	-	+	+	-	-	-	-	-	-	-		-	+	+	-	-	-	-	-	-	-
	DEXTROSE	-	+	+	+	-	-	-	-	-	-		-	+	+	-	-	-	-	-	-	-
0.25mg/ml	LACTOSE	-	+	-	-	-	-	-	-	-	-		-	+	-	-	-	-	-	-	-	-
	MALTOSE	-	+	-	-	-	-	-	-	-	-		-	+	-	-	-	-	-	-	-	-
	DEXTROSE	-	+	+	-	-	-	-	-	-	-		-	+	+	-	-	-	-	-	-	-
0.5mg/ml	LACTOSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
	MALTOSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
	DEXTROSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
0.75mg/ml	LACTOSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
	MALTOSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-
	DEXTROSE	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-

Table VII Inhibitory effect of sugars on haemagglutination activity of papaya and sapota lectin



The protein concentration estimated by Lowry's method was found to be 1.16 mg/ml for papaya lectin and about 0.68 mg/ml for sapota lectin.

Electrophoretic separation of raw papaya extract and sapota extract have shown single bands in 10% non-denaturing gel (Native PAGE). The same papaya and sapota lectin have shown to get denatured by SDS and β- Mercaptoethanol which have resulted in the appearance of three bands (subunits) with the molecular weight ~ 39 kDa, ~ 80 kDa and ~ 101 kDa respectively in case of papaya lectin and single band with molecular weight ~33 kDa in case of sapota lectin calculated by $R_f$value and plotting graph of molecular weight of standard protein marker against calculated $R_f$values.

Fig. II Separation on 10% Non- denaturing gel (Native PAGE)

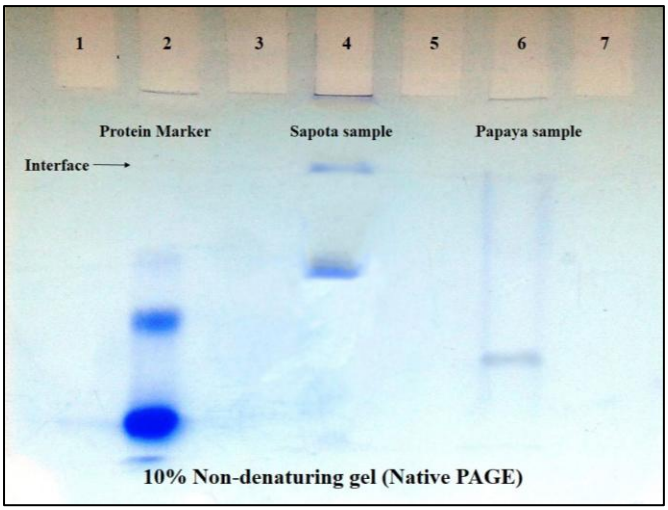
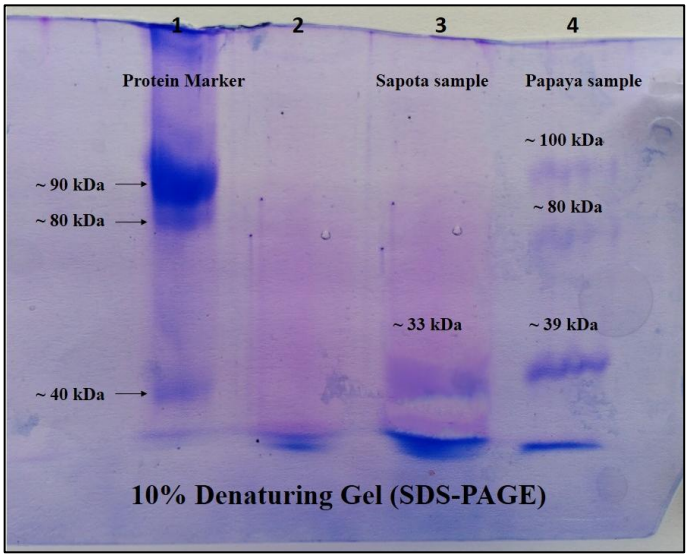


Fig. III Separation on 10% Denaturing gel (SDS PAGE)



CONCLUSION

In the present study it has been found that the papaya lectin is having the potential to agglutinate the human erythrocytes especially RBCs with O and A antigen. Also it has been experimented that the haemagglutination activity of papaya lectin vary with respect to variation in physicochemical parameters such as temperature, pH of the buffer etc. In the sugar inhibition test it has been observed that lectin from papaya has greater affinity towards the monosaccharides. So this explains its interaction with cell surface receptors and this interaction may be responsible for the immunomodulatory activity of this lectin.

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Conflict of Interest: NONE

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