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Partial Characterization of Lectin from *Artocatpus* sps.

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ABSTRACT

Plants are the most convenient and richest source of lectins due to their ease in isolation and availability. The present study was aimed to characterize the plant lectin isolated from the Artocarpus sps. After extraction of lectin from the jackfruit was subjected to SDS-PAGE which shows diverse biological active properties and also possible to examine their biological activity levels to elute and separate the bands of subunits of lectin from gel. In present study, it was found that all most all subunits are active and retains their natural biological activity of lectin. The present study revealed that smaller subunits of lectins has more agglutinating activity, it may be due to the more concentrated amount of the lectins subunits as the whole lectin. Native PAGE for the dialysed sample of *Artocarpus sp.* with different concentrations (25µl, 35µl, 45µl and 55µl) was shown that Purified lectin sample of *Artocarpus sp.* corresponds to 72 kDa with standard protein marker. According to the results of the present study lectin units are trimeric and have similar in lectin activity.
Keywords Artocarpus sps, Lectin, SDS-PAGE, Biological activity and characterisation.

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INTRODUCTION

Plant lectins were firstly discovered by researchers due to their ability to precipitate and agglutinate the erythrocytes. Determination of jackfruit Lectin activity remains agglutination of human and animal red blood cells or other cells. In leguminous plants lectins are prevalent, where they are centralized in seeds and roots of cotyledons. Matured seeds of plant may contain lectins up to 3% of its weight and have precisely specific abundant binding activity to a specific carbohydrate irrespective of their structural arrangement. Surprisingly, Simple sugars and also oligosaccharides can block activity of a lectin. Since their magnanimous specificity towards particular structures of carbohydrates. In light of the available research data, lectins can be able to distinguish and separate oligosaccharides with in their identical sugar composition according to the perceptions of other researchers.

Plants are the most convenient and richest source of lectins due to their ease in isolation and availability. In recent years plant lectins becomes attracting and much more attention because of their usefulness as active biological reagents for glycol-conjugates in a solution and surfaces of the cells. Plant lectins are structurally diverse and carbohydrate binding proteins of a non-immune origins. Most of the plant lectins will be able to precipitate the complex conjugates of carbohydrates with multiple binding sites. Lectins have been isolated and purified from the different sources which includes plants, animals and microorganisms like bacteria and some viruses. In the year 1985 Namjunatra et al reported that jack fruit lectin has the agglutinating activity towards the human and rat sperm [1, 2].

Most importantly, the actual structure recognized by the binding site of lectin. It will be revealed when lectins binding with its natural ligands with larger and compacted than that of single monosaccharides. Lectins containing similar specificity to monosaccharides and may differ in the affinity towards the particular di, oligosaccharides or with the affinity of glycopeptides. In recent years, in immunological studies plant lectins are reliably acting as one of the most powerful and useful tools [3]. Hence, now a days, lectins have become predominant and engrossing because of their exceptional usage as a probe in characterization isolation of simple and complex sugars [4, 5].

Despite of all other uses lectins are innumerable in the nature. In considering the ease which they could be prepare in purified form, uncountable availability to chemical manipulations and in fact inhibition by sugars make them pippin as an exceptional biological tool in biological and pharmacological researches. Although, plant lectins are ubiquitously exhibiting in seeds of most of the plant species, even though they may differ in their architecture. Specific activities of lectins depends on from where they originated and according to the species of plant. Consequently the lectins isolation from the plant seeds purification and characterization of lectins are attracting the glycobiologists in the field of glycobiology. The present study summarizes and reports that the extraction, purification and characterization of lectin from the seeds of jackfruit.

Based on recent reports Lectins may exhibits multiple charges, due to this property they may able to agglutinate red blood cell, but as of now very few such attempts have been made for lectin which isolated from the seeds of Artocarpus sps. Yet to do a lot of investigative research on plant seed lectins activity, whether it depends on any one of the subunits or on the whole unit of lectin. The present study was undertaken to investigate on characterization of isolated lectin from seeds of jackfruit by using simple technique like SDS-PAGE.

MATERIALS AND METHODS

Electrophoresis

Sodium Dodecyl Sulfate - polyacrylamide gel electrophoresis was subjected and carried out according [6]. Samples were dialyzed to remove excess of salt content against distilled water. Later subjected to lyophilized and re-dissolved in phosphate buffer solution to activate and protein determination. Determination of protein was done by re-electrophoresis of lectin samples on SDS-PAGE.

Gel permeation chromatography was carried out by purification of the ammonium sulfate dialyzed with Sephadex G 100 column, then it was equilibrated with PBS and to elute the column. Absorbance was red

at 280 nm fractions with peak values were pooled and freeze-dried for further usage. The crude extract, dialyzed sample and the column purified sample were subjected to hem-agglutination, inhibition assay and protein determination.

Extraction and purification of lectin

Seeds were collected from the ripened Artocarpus sps and about 50 gms of seeds were peeled off the outer layer. Deskinned seeds were subjected to ground by using mechanical mortar and pestle. Flour was furtherly defatted by soaking in ether for 24 hours at 35^0c. Defatted powder was stirred for period of overnight on a magnetic stirrer with PBS at 4^0c. The extract was filtered through the whatmann No.1 filter and spun at 10000g for period of 30 minutes. Ammonium sulphate was added to the supernatant with proper stirring and kept it overnight. Resultant precipitants were removed from by centrifugation at 20000 g for 30 min. then clear supernatant of the above resultant solution was adjusted to 60% saturation with ammonium sulfate with constant stirring kept overnight. Further the precipitate was collected centrifugation process as the same as above and subjected dialyzed rapidly against three different PBS concentration. Clear solution was collected and resultant is lectin and was then purified by chromatography.

Soluble polysaccharide was cross linked with epichlohydrin to prepare insoluble sugar-gum. Further it was kept in z hot air oven for a period of 8 hours at 70°C once it is solidified it was soaked in water. It was then rinsed and several times repeated the washing with distilled water until p^H become neutral. The same was transferred in to 20 mL of PBS and then homogenized in blender with particle size of about 300 mm and repeatedly washed with PBS. Obtained sugar gum was packed in 2.5 cm diameter glass column attached and equipped with flow adapter. The column was kept at 4°C was run at a rate of 100 mL/h, first with PBS until the absorbency of the effluent at 280nm became equal to that of PBS. Then on the top of column isolated lectin was applied. Then the column was extensively washed with PBS until the absorbance of the effluent becomes neutral or less than 0.05. Lectin was then eluted using a solution of N-acetyl D-galactosamine. Finally 2 mL aliquots were collected and the absorbance was read at 280 nm for each fraction and maximum absorbance were pooled. Dialyzed against distilled water then jackfruit lectin was lyophilized and kept it in dessicator.

RESULTS AND DISCUSSION

After, lectin Extraction and purification (table: 1) from seeds of Artocarpus sps, it was subjected to SDS-PAGE. Eluted jacalin lectin shows two specific different bands with KDa 12 and 15 respectively (fig: 1 and 2) with 12.0 KDa band was stained more intensively. So it is suggesting that lectins are retains their activity irrespective of their heterogeneity and are trimeric in structures.

To achieve the main objective of the present investigation further continued to investigate the activity levels of isolated lectin subunits separated and elution was confirmed through re-electrophoresis of the SDS-PAGE (Fig. 1 and 2). The study prevails that the eluted lectin agglutinating activity is more in smaller it may be due to more number of subunits than the larger units. The results of the SDS-PAGE were also suggests that each trimeric units may have the similarities in sugar binding sites. Similarly, the Plant latex has various different health benefits to mammalians and our ancients had been used in folk medicine. Biological activity and benefits of latex of *Artocarpus heterophyllus* on human blood coagulation have been reported [7].

In this study Gel filtration chromatography was carried by using Sephadex G-100 and it resolved proteins with molecular weights from 4,000 to 1, 50,000 Da. The elution profile of the dialyzed 0-90% ammonium sulphate fraction at a flow rate of 1 mL/minute. While reading the absorbance it was shows that the two peaks of lectin elution were visible at 280nm in UV Visible Spectrophotometer. Whereas 20 fractions were collected at 3 mL per well but agglutination was shown only 4-6 fractions, which falls on the first peak. This also may suggested that it is an indication of lectin is larger with high molecular weight because it was eluted first. Similarly aberman [8] was isolated the lectins from the seeds of Artocarpus fruit by using affinity chromatography on an egg white prepared absorbent. Whereas galactomannan molecules which can soluble in sugar gum with approximate average weight of 220 kDa structurally arranges in a linear β 1-4 mannan liked chains and substituted with mannose unit at every single galactose in α 1-6 linkages [9].

Denser distributed galactose terminal links facilitates the single step purification of a specific lectin on sugar gums. Similarly jacalin lectin was purified from Artocarpus integrifolia by preparative anion exchange high performance liquid chromatography in a single step [10]. The content of protein was more in jackfruit and seeds of jackfruit lectins are rich in protein as well as starch. This assumed that the cellular and sub cellular localization of paranchyma cells off seeds are comparable with pea and jack bean seeds. To understand the molecular evolution of jacalin lectins it is very important that the cytoplasm and vacuoles plays an important role in localization of plant lectins [11-13].

The seeds of different Artocarpus species plants was exhibited unusual carbo hydrate binding specificity towards galactose and mannose [14]. The large size of the carbohydrate-binding site of jacalin lectin isolated from jackfruit seeds will be able accommodate monosaccharides due to its hydroxyl confirmations. The molecular weight of the lectin has been reported as 39500 Da with 10 kDa of identical subunits [15] and other authors have been reported that the lectins isolated from seeds of jackfruit with the weight of 62, 65 and 56 kDa [16-18].

In the present study the results of the gel filtration results of lectin used in the present study shows that it contains 72 kDa protein (fig. 3), which yielded a single band in native gel electrophoresis. The two dissimilar bands of molecular weight 17 and 11 kDa, similarly have reported that two tetrameric lectins, jacalin and artocarpin for *Artocarpus integrifolia* [18]. In another research reported that on jacalin lectin molecular weight of 46,000 consisting with two subunits 12,000 and 15,000 [19]. Molecular weight with 11500 and 15000 kDa were reported for *Artocarpus integrifolia* by [20-22] reported a molecular weight of 39,500 for Artocarpus *integrifolia lectin* with a single polypeptide of 10,500 Dalton. Lectin agglutinated human type A, B and O erythrocytes almost equally [23].

From Japanese jack bean lectin was purified through affinity chromatography [24] and similarly by using sephadex G-50 by [25]. In the present study Sephadex G-100 column was used for the isolation of lectin from the seed sample. Under SDS PAGE lectin band was found that (72 kDa). Purified lectin sample of *Artocarpus sp.* corresponds to 72 kDa with standard protein marker (fig: 1, 2, and 3) indicating that they are not linked by disulfide bonding. Similarly a lectin of Japanese jack bean was shown the similar results with molecular weight of 30000 of protein subunit [25, 26] whereas, in the present study results also similar in accordance with exhibiting high molecular weight of 110000 isolated from c. ensiform with five bands of molecular weights 78, 74, 54, 32 and 30 kDa on SDS PAGE [26, 27].

Table: 1 shows elution profile of plant lectins by Gel filtration column chromatography Sephadex G-100

S.No	Elutions	OD at 280 nm
1.	ELUTION 1	0.122
2.	ELUTION 2	0.184
3.	ELUTION 3	0.190
4.	ELUTION 4	0.263
5.	ELUTION 5	0.282
6.	ELUTION 6	0.279
7.	ELUTION 7	0.141
8.	ELUTION 8	0.113
9.	ELUTION 9	0.081
10.	ELUTION 10	0.053
11.	ELUTION 11	0.055
12.	ELUTION 12	0.054
13.	ELUTION 13	0.048
14.	ELUTION 14	0.057
15.	ELUTION 15	0.043
16.	ELUTION 16	0.045
17.	ELUTION 17	0.038
18.	ELUTION 18	0.035
19	ELUTION 19	0.033
20.	ELUTION 20	0.029

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Figure: 1 the crude extract, dialysed sample and eluted lectin from the gel filtration chromatography was subjected to native PAGE.

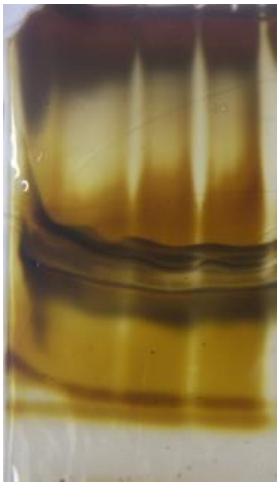


Figure: 2 Native PAGE for the crude extract from *Artocarpus sp.* with different concentrations (20µl, 30µl, 40µl and 50µl)

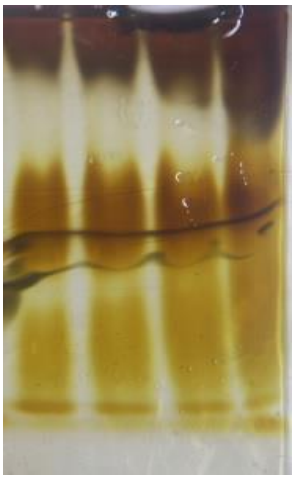
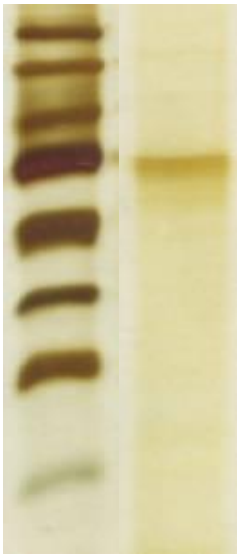


Figure: 3 Native PAGE for the dialysed sample of *Artocarpus sp.* with different concentrations (25µl, 35µl, 45µl and 55µl)



Lane 1: standard protein marker (Range: 170, 130, 100, 72, 55, 40, 33 and 24 kDa)
Lane 2: Purified lectin sample of *Artocarpus sp.* corresponds to 72 kDa with standard protein marker

SUMMARY AND CONCLUSIONS

Lectins differ in size, in composition, molecular weight, number of sugar binding sites and structure of subunits per molecule. Most of the research reports on lectins are confined only to different species in legume family. By virtue of most of the lectins abundant with legume plants only few studies have been made for their biological application.

The defining feature of lectins is binding to a carbohydrate especially mono and disaccharides and it is fundamental base for the classification of many methods in plant lectins. Lectins utilized in purification, sequencing of poly saccharides characterization of polypeptides and glycoproteins due to saccharide binding special feature. Most important advantage of the plant lectins are mono-specificity and readily available. The development of parallel monoclonal antibodies will be costliest affair and time consuming for detection of biological differences in cells. Lectins are potential to utilize as diagnostic markers due their ready availability, ease in purification and their uniqueness in attachment with moieties of the carbohydrates.

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