

SCREENING OF LECTIN IN TUBERS OF *ARACEAE* AND PARTIAL CHARACTERIZATION OF *XANTHOSOMA VIOLACEUM* LECTIN (XVL)

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ABSTRACT

Present study describes the screening of lectin from different edible tubers belongs to *Araceae* family. Among the different tubers screened *Xanthosoma Violaceum* showed higher lectin activity and chosen for further studies. Crude lectin was extracted from *Xanthosoma Violaceum* tuber using various buffers like acetate buffer saline (pH-4.3), Phosphate buffer saline (pH 7.2) and Tris buffer saline (pH-8.5). Hemagglutination activity was performed for the crude extract and partially purified lectin sample. XVL showed agglutination towards rabbit, goat, sheep, horse, cow, and pig erythrocytes but not towards human erythrocytes. Protein profiling of crude and ammonium sulphate fraction at different pH was done using SDS-PAGE. More number of proteins and large amount of lectin was extracted in both in PBS and TBS. Partially purified XVL was used for the determination of its carbohydrate specificity by hemagglutination inhibition assay.

XVL activity was inhibited by simple sugars like galactose, mannose, glucosamine and also by complex glycoproteins such as thyroglobulin, transferrin, fetuin, asialofetuin, mucin, asialomucin, and ovalbumin. Partially purified lectin was stable over a wide range of pH 3.0 –8.5 and retains its activity up to 70⁰C. The present investigation increases the knowledge about the occurrence of lectins and their contribution towards the medicinal properties of edible tubers of *Araceae* family.

KEYWORDS: *Xanthosoma violaceum*, *Araceae*, Mannose, Lectins, Glycoproteins.

1. INTRODUCTION

Lectins are heterogeneous group of (glyco) proteins of nonimmune origin that agglutinate cells and/or precipitate complex glycoconjugates.^[1] Because of their unique ability to recognize and bind reversibly to specific carbohydrate ligands either free in solution or on cell surfaces without chemically altering them that distinguishes lectins from other carbohydrate-binding proteins and enzymes and which make them invaluable tools in biomedical and glycoconjugates research. Lectins are ubiquitous in all living forms and found in viruses, bacteria, fungi, plants, and animals.^[2] In the beginning, lectin research mainly focused on lectins in seeds due to their abundance and rapid purification. Now large number of lectins have been reported in other tissues of the plants such as leaves, bark, bulbs, tubers, rhizomes, etc. The physiological functions and mechanisms of various animal lectins have been studied precisely.^[3-6] However, those of plant lectins have not been well understood. There are still numerous plant lectins yet to be identified and studied. Plant storage organs are rich in proteins and are one of the best targets for identification and isolation new lectins, which help to unravel their biological potentials (e.g. Anti-tumor, anti insecticidal activities).

In general, plant tubers are rich in starch and indeed they often considered solely as a source of carbohydrate for diets and industrial uses. However, they do contain protein which varies in amount from about 1-10 (D. Wt.). Tubers offer numerous desirable nutritional and health benefits such as antioxidant, hypoglycemic, hypocholesterolemic, antimicrobial and immunomodulatory activities.^[7-11] These observed bioactivities are due to the presence of large number of bioactive constituents such as phenolic compounds, saponins, bioactive proteins, glycoalkaloids, and phytic acids.^[12] Also, plant tubers are known to possess proteins such as chitinase and lectins^[13] that are responsible for various defense related functions. During the last two decades accumulated evidence reveals that several new lectins with interesting properties have been purified and Characterized from monocotyledons plant species belonging to *Alliaceae*, *Amaryllidaceae*, *Araceae*, *Lilliaceae* and *Orchidaceae*.^[14-17] However, a very few lectins are characterized from *Araceae* species as compared to several lectins reported from other monocot families.^[18-20,15] Plant tubers are widely consumed as a staple food in various parts of Karnataka especially Western Ghat region. Some of these possess a good medicinal value. In this direction, efforts are being made to search for new lectins having novel (unique) sugar specificity and potential applications. Therefore a total of seven plants species belonging to *Araceae* family were screened for the lectin activity and their novel sugar binding property. Tubers of *Xanthosoma violaceum*, an aroid found in

Western Ghats of South India showed very high lectin activity and hence this lectin has been chosen for studies. The present investigation focused on tracing for lectin activity in tubers of different aroids and partial purification and characterization of *Xanthosoma violaceum* lectin (XVL).

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

Fresh tubers of *Colocasia esculenta* var. *esculenta*, *Colocasia esculenta* var. *antiquorum*, *Colocasia esculenta* var. *fontanesii*, *Xanthosoma sagittifolium* (L.) Schott, *Xanthosoma violaceum*, *Alocasia macrorhiza* Schott and *Remusatia vivipara* (Roxb) were collected from Madikere district, Karnataka, India, during the month of November-December and stored at -20°C. The collected tubers were identified by Dr. M. Sivadasan, Professor, Department of Botany & Microbiology, College of Science, King Saud University, P. B. No. 2455, Riyadh – 11451, Kingdom of Saudi Arabia and also with the help of Dr. V. Krishna, Department of Biotechnology, Kuvempu University, Shankarghatta, Karnataka, India.

2.2. Preparation of tuber extracts

Fresh Tubers were peeled, diced and homogenized in 50mM phosphate buffered saline (PBS), pH 7.2 (w/v 1:10) at 4°C overnight. The homogenate was filtered through cheesecloth followed by centrifugation at 10,000 rpm for 20 min at 4°C. The clear supernatant (crude extract) was collected and used for screening.

2.3. Screening for lectin activity

The extracts from different tubers were screened for lectin activity by hemagglutination assay using both animal and human erythrocytes. Tuber extract showing highest activity was selected for further studies.

2.4. Extraction of lectin from *Xanthosoma violaceum* (XVL) tuber in different pH buffers

Three buffers such as acetate buffer saline (ABS) pH 4.3, Phosphate buffer saline (PBS) pH 7.2 and Tris buffer saline (TBS) pH 8.5 were employed for the extraction of lectin from the tuber. These three extracts were tested for lectin activity using both trypsinized and untrypsinized rabbit erythrocytes.

2.5. Erythrocyte preparation

Both human erythrocytes of A, B, AB and O blood groups and rabbit erythrocytes were collected in Alsever's solution (Dextrose, trisodium citrate, sodium chloride and citric acid pH (7.2) washed 4-5 times with PBS, pH 7.2. A 3% (v/v) suspension of the erythrocytes in the above buffer was prepared and treated with trypsin (0.05% w/v) at 37⁰C for 1 h, subsequently washed three times with same buffer.

2.6. Heamagglutination assay

Heamagglutination assay was routinely performed in standard microtitre plates by 2-fold serial dilution method of,^[21] A 50 μ l aliquot of the erythrocytes suspension was mixed with 50 μ l of serially diluted lectin and agglutination was examined visually after incubation for 1 h. The unit of heamagglutination activity (U) was expressed as the reciprocal of the highest dilution (titer) of the lectin that showed complete agglutination.

2.7. Determination of Protein concentration

Protein concentration was determined both in crude and partially purified lectin preparations by the method of Bradford^[22] using bovine serum albumin as a standard.

2.8. Partial purification of XVL

The crude extract of three different buffers was subjected to partial purification of lectin by Ammonium sulphate precipitation. Briefly, Ammonium sulphate was added to 60% saturation with constant stirring and allow it stand for overnight. The solution was centrifuged at 10,000 rpm for 20 min at 4⁰C. The precipitate was dissolved in respective buffers and dialyzed against several changes with the same buffer for 48 hours. Hemagglutinating activity was examined both in supernatant and precipitate. The supernatant was discarded as there was no agglutination activity. The dialyzed samples were used for characterization studies.

2.9. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein profiling of both crude and partially purified lectin was analyzed by 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in accordance with the procedure of Laemmli.^[23] The protein bands were visualized after staining with Coomassive Brilliant Blue G-250.

2.10. Hapten inhibition assay (carbohydrate binding specificity)

To ascertain the carbohydrate binding specificity of the partially purified lectin, sugar / hapten inhibition assay was performed in a manner analogous to the hemagglutination test^[21] except that serial dilutions of the sugar solutions (25 μ l) were pre-incubated for 1 h at 37⁰C with 25 μ l of the lectin (titer adjusted to 4 or 8 U). Erythrocyte suspension (50 μ l) was added, mixed and the plates were visually observed after 1 h. Lack of hemagglutination is the indication of specific binding and inhibitory property of the sugar (or a glycoprotein). For this purpose a battery of 25 sugars and their derivatives were used which included 3 pentoses: Xylose, D-Arabinose and ribose; 10 hexoses and their derivatives: glucose, mannose, galactose, L-fucose, L-rhamnose, glucosamine, N-acetylgalactosamine, N-acetylglucosamine, N-acetylmannosamine and mannopyranoside, 3 disaccharides: maltose lactose, and Melibiose; Complex glycoproteins like transferrin, thyroglobulin, fetuin, asialofetuin, mucin, asialomucin, ovalbumin were also used. The various sugars and their derivatives were tested at a concentration of 200mM while glycoproteins at a concentration of 1mg/ml. The lowest concentration of the sugar/glycoprotein, which inhibited the agglutination, was taken as the minimal inhibitory concentration (MIC).

2.11. pH and thermal stability

Effect of pH on agglutinating activity was performed by incubating aliquots of partially purified lectin for 1 h with buffers of different pH values ranging from 3.0 to 12.

The heat stability of hemagglutinating activity of partially purified lectin was determined by incubation of lectin sample at different temperatures (30, 40, 50, 60, 70, 80, and 90⁰C) for 1 hr. At each temperature an aliquot of 50 μ l of sample was taken, cooled to room temperature and agglutinating activity was examined.

3. RESULTS AND DISCUSSION

3.1. Screening of plant tubers for lectin activity

Among the seven plant species examined for lectin activity, *Colocasia esculenta* var. fontanesii, *Xanthosoma violaceum* and *Remusatia vivipara* (Roxb.) showed lectin activity (Table1). But characterization and biological activity of lectin has been well documented in the literature for *Colocasia esculenta* var. fontanesii and *Remusatia vivipara* (Roxb.)^[24,15] Despite the report of lectin activity in large number of tubers from Araceae species, to the best of our knowledge no information is available in the literature regarding the lectin activity in *Xanthosoma violaceum* (XVL). Also the tubers of *Xanthosoma violaceum* are widely used

in food preparation and folk medicine for treatment of various ailments. We have therefore partially purified the lectin and studied its properties. Like other reported monocot tuber lectins XVL exclusively agglutinates both untrypsinized and trypsinized rabbit erythrocytes but not human ABO erythrocytes.

Table 1: Screening of tubers of Araceae for lectin activity.

Sl. No	Plant	Hemagglutinating activity
1	<i>Colocasia esculenta</i> var. <i>esculenta</i>	-
2	<i>Colocasia esculenta</i> var. <i>antiquorum</i> *	+
3	<i>Colocasia esculenta</i> var. <i>fontanesii</i>	-
4	<i>Xanthosoma sagittifolium</i> (L.) Schott	-
5	<i>Xanthosoma violaceum</i>	+
6	<i>Alocasia macrorrhiza</i> Schott	-
7	<i>Remusatia vivipara</i> (Roxb.)*	+

Note: +, hemagglutinating activity; -, no hemagglutinating activity; *Already reported

3.2. Extraction of XVL in various buffers

Crude extracts of *X. violaceum* were prepared in various buffers of different pH. Among the various buffers used, less amount of lectin was extracted in ABS compared to that of PBS and TBS as indicated by highest hemagglutination activity. Within the erythrocytes, XVL agglutinates significantly towards trypsinized rabbit erythrocytes compared with untrypsinized rabbit erythrocytes. This is due to exposure of glycans of glycoproteins / lipids upon trypsinization which could be available for interaction with the lectin. Figure 1 showing the hemagglutination activity of XVL towards human ABO and rabbit erythrocytes. Sedimentation of erythrocytes in 1st, 2nd, 3rd and 4th rows indicating the no agglutination towards human ABO erythrocytes where as formation of mat like texture in the last row represents agglutination towards rabbit erythrocytes.

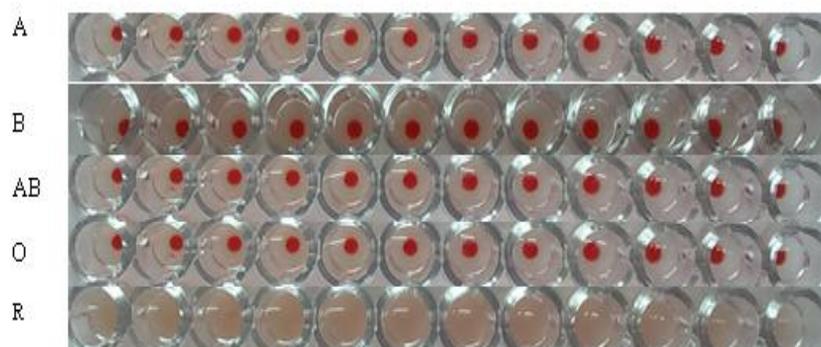


Fig 1: Hemagglutination assay where rows A, B, AB, O indicates Human erythrocytes and R indicates Rabbit erythrocytes.

3.3. SDS-PAGE of crude extract of XVL in different buffers

In order to determine the suitable extraction buffer for maximum extraction of lectin in tubers of XVL and to know the protein profiling, total Proteins of both crude extract and ammonium sulphate precipitation were subjected to SDS-PAGE (15%). The electrophoretic analysis reveal that maximum number of proteins extracted in PBS and TBS with few intense bands near 10, 12 and 18 kDa (Lane 3, 4, 5, & 6). Less intense protein bands near 10, 12 and 18 kDa in lane 1 and 2 corresponding to ABS indicates not much protein were extracted and found lower lectin activity. Figure 2 below depicts the protein profiling of both crude and partially purified XVL form different pH.

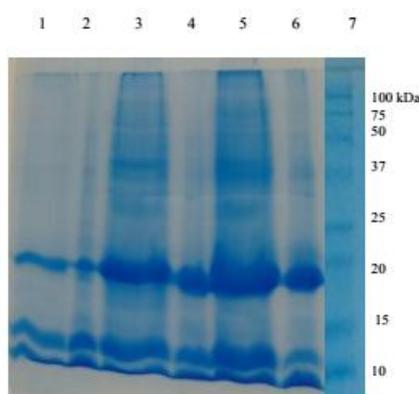


Fig 2: Protein profiling of crude and ammonium sulphate fractions by SDS-PAGE in 15% gel.

Lane 1 & 2: Crude & 60% ammonium sulphate precipitate from ABS; 3 & 4: crude & 60% ammonium sulphate precipitate from PBS; 5 & 6: crude & 60% ammonium sulphate precipitate from TBS; 7: Molecular weight Marker (10-100kDa).

3.4. Hapten inhibition assay (carbohydrate – binding specificity)

Among the carbohydrates tested, the lectin-induced hemagglutination was inhibited by some simple sugars as well as by complex glycoproteins (**Table.2**). Among different glycoproteins tested it showed strong inhibition towards thyroglobulin, fetuin and transferrin indicating its complex sugar specificity similar to earlier reported monocot lectins *Colocacia esculanta*, *Xanthosoma sagittifolium*, *Arum maculatum*, *Typhonium divaricatum* and *Remusatia vivipara*.^[24-27,16] Inhibition towards galactose is not reported in any of the lectins from *Araceae* family. These proteins are characterized by the ability to bind specifically to mannose, and are currently referred to as *Galanthus nivalis* agglutinin (GNA)-related

lectins.^[14] They have attracted increasing attention in recent years because of their novel sugar specificity and remarkable biological properties, Valuable tools for biotechnological applications.^[28]

Table 2: Carbohydrate binding specificity of partially purified XVL.

Sl. No	Sugar	Minimum Inhibitory Concentration (MIC)
1	Galactose	3.12mM
2	Glucosamine	3.12mM
3	Mannose	1.56mM
4	Thyroglobulin	0.39 μ g
5	Fetuin	0.39 μ g
6	Asialofetuin	0.39 μ g
7	Transferrin	0.39 μ g
8	Ovalbumin	0.39 μ g
9	Mucin	1.56 μ g
10	Asialomucin	1.56 μ g

3.5. pH and thermal stability

Effect of temperature on heamagglutination activity of partially purified XVL has shown in fig 3. Lectin was found to be stable up to 70⁰C and above which there is a gradual fall in the lectin activity due to denaturation of the lectin.

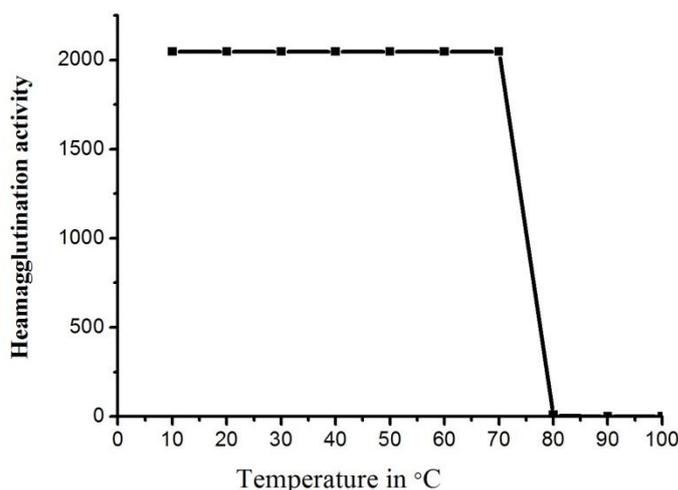


Fig 3: Effect of temperature on heamagglutination activity of lectin.

The partially purified lectin was found to be stable over a pH range of 3-12 as shown in fig 4. The results showed that lectin was stable in the pH range 3-8.5. Partially purified XVL showed stability at broad pH range but its activity decreases below pH 3 and above pH 8.5.

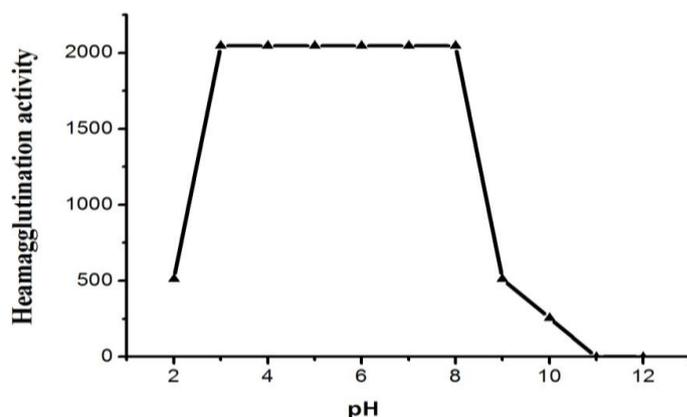


Fig 4: Effect of pH on heamagglutination activity of lectin.

4. CONCLUSION

Among the different tubers from *Araceae* family were screened *Xanthosoma violaceum* showed higher activity. Because of its high lectin activity and limited information available in the literature XVL was chosen for further biochemical characterization. Lectin was more specifically agglutinating rabbit erythrocytes but not human erythrocytes which is characteristic of many of the lectins reported from *Araceae*.^[24,16] Like other monocot mannose binding lectins (MBL), XVL is specific to mannose, galactose and complex glycoproteins.

Because of its novel sugars/glycoproteins Specificity,, XVL has got potential application in cancer biology, particularly, in understanding aberrant glycosylation changes occurred during metastasis. Further purification, Characterisation and its Biological studies are under progress.

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