

## Isolation, Purify and Characterization of Lectin from the Seeds of *Artocarpus* Species

S. Krupa<sup>1\*</sup> and K. R. Siddalinga Murthy<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Bangalore University, Bangalore -56, India.

### Authors' contributions

This work was carried out in collaboration between both authors. Author SK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed the literature searches and author KRSM managed the analyses of the study. Both authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJBGMB/2021/v8i430199

#### Editor(s):

(1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India.

#### Reviewers:

(1) Nafiu Muhammad Ibrahim, Federal University Gusau, Nigeria.

(2) Abdul Munim Farooq, University of the Punjab, Pakistan.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/71809>

Received 24 May 2021

Accepted 28 July 2021

Published 31 July 2021

Original Research Article

### ABSTRACT

**Aim:** To isolate, partially purify and characterize lectin from the seeds of *Artocarpus* species - using ammonium sulphate, gel-filtration and ion exchange chromatography.

**Methodology:** ABO blood groups were screened for the assay of hemagglutinating property with seed lectins of both *heterophyllus* and *A. hirsutus*. The seed lectins were extracted using suitable buffer system pH 7.0 and partially purified by gel filtration and ion exchange chromatographic techniques.

**Results and Discussion:** Lectins were extracted from the defatted seed powders of *A. heterophyllus* and *A. hirsutus*. The extracts were used for optimization of assay buffer for hemagglutination activity and 50 mM Tris-HCl buffer, pH 8.8 containing 1 mM CaCl<sub>2</sub> and 1 mM MnCl<sub>2</sub> showed greater hemagglutinating property. Screening for optimum concentration of RBCs from different blood group (A, B, AB and O groups) indicated O group at 10 % concentration as ideal for the assay of hemagglutinating property with seed lectins of both *A. heterophyllus* and *A. hirsutus*.

**Conclusion:** In this study, lectin from the seeds of *Artocarpus heterophyllus* and *Artocarpus hirsutus* were isolated and partially purified. The isolated lectins were characterized for their hemagglutination activity. Among the human blood types (A<sup>+</sup>, B<sup>+</sup>, AB<sup>+</sup> and O<sup>+</sup>) used, all the blood groups showed agglutination while greater agglutination was observed with O<sup>+</sup> blood group.

\*Corresponding author: E-mail: [skrupa88@gmail.com](mailto:skrupa88@gmail.com);

**Keywords:** *Artocarpus*; DEAE-cellulose; hemeagglutination; lectin; Sephadex G- 200.

## 1. INTRODUCTION

Lectins are monovalent or divalent carbohydrate binding proteins which are non-immunoglobulin in nature with erythrocyte agglutinating properties [1]. The word lectin comes from the word 'legere' meaning 'to select' which was coined by William Boyd (1947). Lectin is a glycoprotein that contains a carbohydrate recognition domain (CRD) and do not modify the carbohydrates to which they bind. Lectins are used as probes for identifying and mapping sugars on cell surfaces. Lectins are important tools in cancer research - to differentiate between malignant cells from normal cells [2] and bone marrow transplantation [3]. Lectins are involved in nitrogen fixation where the root hairs of legume plants bind to *Rhizobium* [4]. Lectins are involved in providing innate immunity in animals. They are also involved in defense mechanism, lymphocytes homing and interactions of immunological cells. They find a prominent role in cell biology i.e. in cell-cell interactions, cell growth, apoptosis, cell division and cell cycle. Lectins are used in the isolation of saccharide containing biomolecules employing the technique of affinity chromatography. Lectins are also useful in blood typing, cell-separation, bacterial typing and bone-marrow transplantation [1].

Anti-nutritional factors can be natural or synthetic compounds that interfere with the absorption of nutrients and reduce the nutrient intake, digestion, and utilization. Antinutrients consumed in larger quantity can cause nausea, rashes, headaches, bloating, malnutrition etc [5]. However these compounds if consumed wisely with adequate food processing can be advantageous to humankind. Plants use anti-nutrients for their self-defense. Lectin is one of the anti-nutritional factor, which when consumed raw along with other nutrients reduces the nutrient bioavailability [6].

*Artocarpus* seeds are a very good source of lectins called Jacalin [4]. Jacalin and heterophyllin are the two important lectin studied in *A. heterophyllus* seeds [7]. Jacalin is of potential use in nano medicine. Jacalin is a tetrameric two chain lectin with a molecular mass of 65 kD comprising a heavy  $\alpha$ -chain of 133 amino acid residues and a light  $\beta$ -chain of 20 - 21 amino acid residues. Jacalin contributes to more than 50 % of the total protein present in the seed.

The objectives of this study is to isolate and partially purify lectins using ammonium sulphate, gel-filtration and ion exchange chromatography from the seeds of *A. heterophyllus* and *A. hirsutus* and to characterize for their hemagglutinating property using different ABO blood groups.

## 2. MATERIAL AND METHODS

### 2.1 Chemicals

Sodium dihydrogen orthophosphate ( $\text{NaH}_2\text{PO}_4$ ), disodium hydrogen orthophosphate ( $\text{Na}_2\text{HPO}_4$ ), hydrochloric acid (HCl), glacial acetic acid ( $\text{CH}_3\text{COOH}$ ), sodium hydroxide (NaOH), sodium chloride (NaCl), distilled water.

### 2.2 Collection of Seeds

*A. heterophyllus* and *A. hirsutus* seeds were collected from Kannur District in Kerala and Mangalore District in Karnataka respectively.

### 2.3 Preparation of Crude Extracts

The dried seed samples were dehulled manually and powdered using a grinder. The seed powders were defatted using n-hexane (1:5 w/v) in a Soxhlet extractor for 8 hours [8]. A 20 % extract of defatted powder was prepared using 33 mM phosphate buffer, pH 7.0 by continuous extraction in a shaker incubator for 16 hours at 4° C. The sample was then centrifuged at 7000 rpm for 15 minutes at 4° C. The residue was discarded and the supernatant was stored at 4° C for further use.

### 2.4 Preparation of Red Blood Cells

Blood of 15 ml from different blood groups (A, B, AB, and O) were collected (in the presence of EDTA to prevent coagulation) and red blood cells were obtained by centrifugation at 1000 rpm for 5 minutes at 4° C. The plasma was discarded. The residue (RBC) was washed with 33 mM Phosphate Buffer Saline (PBS) pH 7.0, and centrifuged at 1000 rpm for 5 minutes at 4° C. The supernatant was discarded and the dilution and centrifugation was repeated until the supernatant is clear. The cells were stored at 4° C for further use.

## 2.5 Optimization of Hemagglutination Assay Buffer

Crude seed extract (12.5  $\mu$ l) of *A. heterophyllus* and *A. hirsutus* were diluted with 12.5  $\mu$ l of different buffers (50 mM normal saline, 50 mM acetate buffer, pH 5.6, 33 mM phosphate buffer saline, pH 7.0, 50 mM Tris-HCl buffer saline, pH 7.0 and pH 8.8, 50 mM Tris-HCl buffer, pH 8.8 containing 1mM  $\text{CaCl}_2$  and 1mM  $\text{MnCl}_2$  and just water) each in U- shaped 96 well ELISA plates. Then 25  $\mu$ l of 10 % erythrocyte suspension were added to each buffers. After an incubation period of 30 minutes at room temperature, the plates were read at 595 nm using ELISA reader against a blank. The buffer showing maximum agglutination was used for assay of hemagglutination.

## 2.6 Screening for RBC Selection

Screening for RBC Selection of crude seed extract of 12.5  $\mu$ l from *A. heterophyllus* and *A. hirsutus* were diluted with 12.5  $\mu$ l of 50 mM Tris-HCl buffer, pH 8.8, containing 1 mM  $\text{CaCl}_2$  and 1 mM  $\text{MnCl}_2$ . 25  $\mu$ l erythrocyte suspension (A, B, AB and O) of 0.5 %, 1.0 %, 3.0 %, 5.0 % and 10 % dilutions were added. After an incubation period of 30 minutes at room temperature (30 $^{\circ}$ C), the plates were read at 595 nm using ELISA reader against a blank. The erythrocyte suspension showing maximum agglutination was recorded.

## 2.7 Ammonium Sulfate Precipitation

Addition of ammonium sulfate, standing and centrifugation procedures were performed at 4 $^{\circ}$ C to prevent protein denaturation. The crude extracts of *A. heterophyllus* and *A. hirsutus* were subjected to 90 % ammonium sulphate precipitation according to Cooper's nomogram, 1977. For 100 ml crude extract, 61.1 g of ammonium sulphate was added with continuous stirring for 30 minutes at 4 $^{\circ}$  C. The solution was allowed to stand for 30 minutes at 4 $^{\circ}$  C, then centrifuged at 7000 rpm for 15 minutes at 4 $^{\circ}$  C and the pellet was collected and dissolved in minimum amount of extraction buffer and stored at 0 $^{\circ}$  C for further use.

## 2.8 Dialysis

The ammonium sulfate used for the precipitation of proteins was removed by a method of dialysis. In this method the dialysis membrane was precreated by soaking in distilled water and washing it under running water. The dialysis

membrane with the sample was placed inside a beaker filled with 6 mM phosphate buffer, pH 7.0 and stirred for 24 hours at 4 $^{\circ}$  C with intermittent change of the buffer (a total of three times). The dialyzed sample was stored at 0 $^{\circ}$  C for further use.

## 2.9 Gel Filtration Chromatography

Dialyzed ammonium sulfate fraction (0 – 90 %) was subjected to gel filtration chromatography using Sephadex G - 200 column (50 cm x 1 cm) with a flow rate of 7 ml/hour. The column was equilibrated and eluted with 33 mM phosphate buffer, pH 7.0 containing 0.1M NaCl. Fractions of 1.0 ml were collected and checked for protein at 280 nm and assayed for lectin by hemagglutination assay. The peak fractions were pooled and subjected to ion exchange chromatography on DEAE-Cellulose.

## 2.10 DEAE-Cellulose Chromatography

The pooled sephadex G-200 fraction (2.0 ml) was subjected to DEAE-cellulose chromatography (10 cm x 2 cm). The column was equilibrated with 33 mM phosphate buffer, pH 7.0 containing 0.1 M NaCl. Elution was performed with an increasing linear gradient of 0.2 – 0.6 M NaCl in equilibration buffer at a flow rate of 30 ml/ h. Fractions of 2.0 ml were collected.

## 2.11 Lectin or Haemagglutination Assay

Fractions of 12.5  $\mu$ l from gel filtration and ion exchange chromatography were diluted with 12.5  $\mu$ l of 50 mM Tris-HCl buffer, pH 8.8, containing 1mM  $\text{CaCl}_2$  and 1 mM  $\text{MnCl}_2$ , 25  $\mu$ l of 10 % erythrocyte suspension of O blood group was added and incubated for 30 minutes at room temperature in U- shaped 96 well ELISA plates. The plates were read at 595 nm using ELISA reader against a blank (8).

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and Purification of Lectin

Lectins were extracted from the defatted seed powders of *A. heterophyllus* and *A. hirsutus*. The defatting of seed powders using hexane helped in the removal of fats and lipids which may interfere with agglutination. The extracts were used for optimization of assay buffer for hemagglutination activity and 50 mM Tris-HCl buffer, pH 8.8 containing 1 mM  $\text{CaCl}_2$  and 1 mM  $\text{MnCl}_2$  showed greater hemagglutinating property

with seed lectins of both *A. heterophyllum* and *A. hirsutus*.

Screening for optimum concentration of RBCs from different blood group (A, B, AB and O groups) indicated O group at 10 % concentration as ideal for the assay of hemagglutinating property with seed lectins of both *A. heterophyllum* and *A. hirsutus*.

Proteins were separated from other proteins by salting-out method. The proteins can be precipitated using either ammonium sulfate or magnesium sulphate. The salt solution decreases the solubility and thus precipitates the protein by absorbing the water of hydration and protecting the protein in the extract. In the present study, ammonium sulfate was chosen as precipitating agent due to its high solubility in water and produces high ionic strength. Increase in ionic strength decreases the protein solubility. The extracts were subjected to 90% ammonium sulphate saturation to confirm that most of the proteins are precipitated.

Desalting was carried out for the removal of ammonium sulfate as it may interfere with the agglutination and can give false results. This was achieved by dialysis against 6mM phosphate buffer, pH 7.0 for 24 hours at 4°C to prevent any protein denaturation.

### 3.2 Characterization of the Purified Lectin

Gel-filtration chromatography using Sephadex-G 200 resolve proteins of with molecular weights upto 600kD. Gel filtration chromatography of 90 % ammonium sulphate fraction on sephadex G-200 resolved into one minor peak (Seph-I) of high molecular weight and two major peaks (Seph-II and Seph-III) from the seeds of *A.*

*heterophyllum* (Fig 1). The peaks represents the greater haemagglutination activity of lectin. This suggested that Seph-II peak fractions (36 – 40) from the seeds of *A. heterophyllum* showed greater activity were pooled, lyophilized and subjected to ion exchange chromatography on DEAE-cellulose.

The hemagglutination activity was represented as one minor peak (Seph-I) of high molecular weight and three major peaks (Seph-II, Seph-III and Seph-IV) from the seeds of *A. hirsutus*, respectively (Fig 2). Seph-II peak fractions (31 – 34) from the seeds of *A. hirsutus* showing greater hemagglutination were pooled, lyophilized and subjected to ion exchange chromatography on DEAE-cellulose.

The fractions possessing hemagglutination activity from gel filtration chromatography subjected into DEAE-cellulose column was resolved into two peaks (DEAE-I and DEAE-II) in *A. heterophyllum* and three peaks (DEAE-I, DEAE-II and DEAE-III) in *A. hirsutus* (Fig. 3 and 4).

Lectin, was isolated from the seeds of *A. heterophyllum* and *A. hirsutus*. The isolated lectin was subjected to ammonium sulfate precipitation. Ammonium sulfate is a precipitating agent, due to its high solubility in water and produces high ionic strength. Increase in ionic strength decreases the protein solubility. Lectin was then subjected to gel-filtration and ion exchange chromatography for partial purification and characterization. All the fractions of the partially purified protein obtained from the column chromatographic techniques was then assayed for hemagglutinating property on different ABO blood groups.

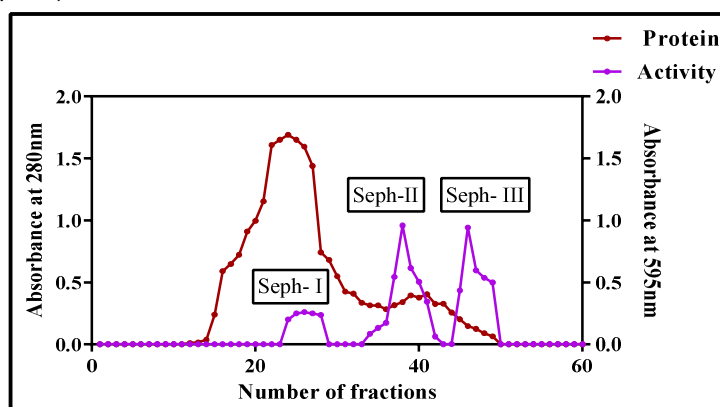


Fig. 1. Sephadex G-200 gel filtration elution profile of lectin from the seeds of *A. heterophyllum*

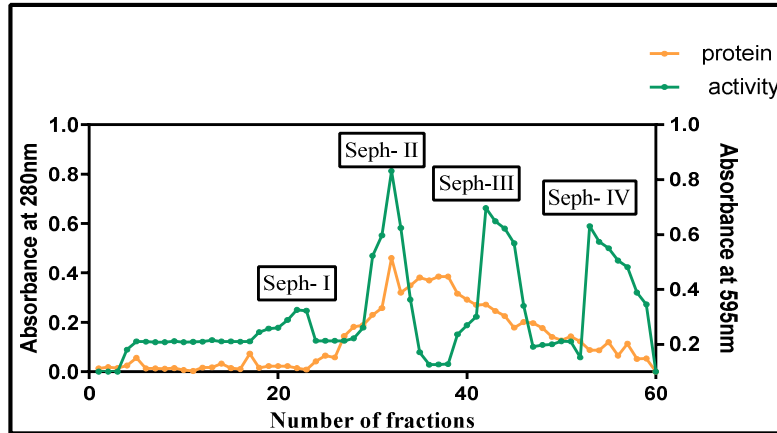


Fig. 2. Sephadex G-200 gel filtration elution profile of lectin from the seeds of *A. hirsutus*

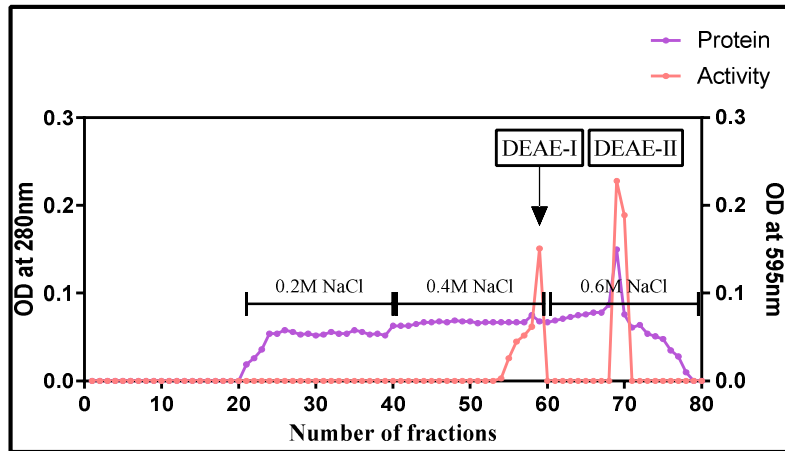


Fig. 3. DEAE-cellulose stepwise elution profile of lectin from the seeds of *A. heterophyllus*

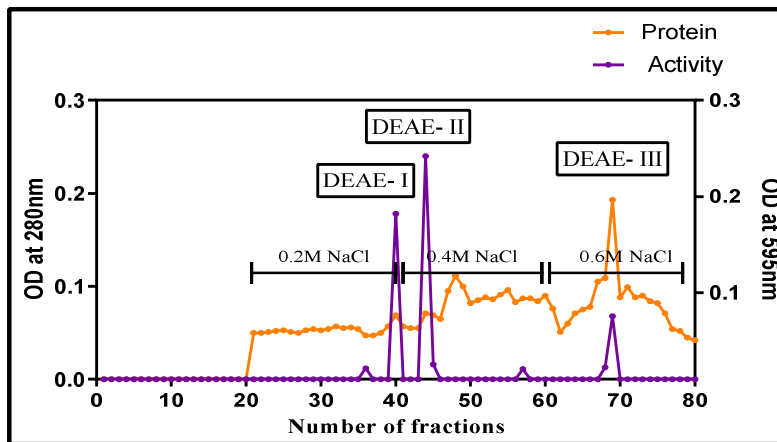


Fig. 4. DEAE-cellulose stepwise elution profile of lectin from the seeds of *A. hirsutus*

#### 4. CONCLUSION

In this study, lectin from the seeds of *Artocarpus heterophyllus* and *Artocarpus hirsutus* were isolated and partially purified. The isolated lectins were categorized for their hemagglutination activity. Among the human blood types (A<sup>+</sup>, B<sup>+</sup>, AB<sup>+</sup> and O<sup>+</sup>) used, all the blood groups showed agglutination. This explains that the lectin present is a non- blood type while greater agglutination was observed with O<sup>+</sup> blood group.

#### ACKNOWLEDGEMENTS

Department of Biochemistry, Bangalore University and DST-INSPIRE Fellowship Programme are kindly acknowledged.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Eikenella corrodens. Hiroyuki Azakami, Hiromu Nakashima, Hiromi Akimichi, Yuichiro Noiri, Shigeyuki Ebisu, and Akio Kato. Involvement of n-acetyl-D-galactosamine-specific lectin in biofilm formation by the periodontopathogenic bacterium. Biosci. Biotechnol. Biochem. 2006;70(2):441–446.
2. Smetana K Jr., Dvorankova B, Chovanec M, Boucek J, Klima J. Nuclear presence of adhesion-growth-regulatory galectins in normal/ malignant cells of squamous epithelial origin. Histochem. Cell Biol. 2006;125:171-182.
3. Lis H and Sharon N. Lectins as molecules and as tools. Annu. Rev. Biochem. 1986;55:35-67.
4. Barondes SH. Lectins: their multiple endogenous cellular functions. Ann. Rev. Biochem. 1981; 50:207-231.
5. Essack H, Odhav B, Mellem JJ. Screening of traditional South African leafy vegetables for selected anti-nutrient factors before and after processing. Food Sci Technol. 2017;3:462-1.
6. Sarita ES, Singh E. Potential of millets: nutrients composition and health benefits. Journal of Scientific and Innovative Research. 2016;5(2):46–50.
7. Gaikwad SM, Khan MI. Binding of T-antigen disaccharides to *Artocarpus hirsute* lectin and jacalin are energetically different. Photochem. Photobiol. 2006; 82:1315-1318.
8. Irving v. Occena, Elmer- Rico E. Mojica, Florinia E. Merca. Isolation and partial characterization of a lectin from the seeds of *Artocarpus camansi* Blanco. Asian Journal of Plant Sciences. 2007; 6(5):757-764.

© 2021 Krupa and Murthy; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<https://www.sdiarticle4.com/review-history/71809>