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ISOLATION AND CHARACTERIZATION OF ANTIFUNGAL LECTIN FROM SEEDS OF Cassia auriculata Linn.

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Abstract: *Cassia auriculata Linn., which is a shrub from Leguminosae family and widely distributed in India. The aim of this study is to isolate and characterize a lectin protein from seeds of Cassia auriculata and to evaluate for antifungal activity. The isolation and characterization of the lectins (SBL) from the seeds of Cassia auriculata have been reported. Affinity column chromatography along with Sephadex G-75 column used for the lactose binding lectins proteins purification from the Cassia auriculata seeds. To identify the fraction's molecular weight the affinity products were dialysed against PBS- ME and SDS-PAGE (10% w/v). Haemagglutination assay was performed for portion using AB, A, B and O type of blood group. Explorations of different techniques of lectin protein purification and describing their characteristics by performing the haemagglutination assay in present work. Lectin was isolated from Cassia auriculata seeds and purified by Sephadex G-75 column chromatography successfully with the maximum yield of 8 mg/ml in one fraction duly analyzed by UV-spectrophotometry. Molecular weight has been determined by SDS-PAGE. Haemagglutination and haemagglutination inhibition of purified lectin was found significant along with its carbohydrate binding specificity and Metal ions inhibition. The purified lectin was found stable at pH of 6-8 and at temperature up to 60°C for 30 minutes. Purified lectin inhibited the growth of Cassia albican with LIC of 0.1, 0.01 and 0.01 mg/ml, respectively which proved its antifungal potency. Lectin obtained from Cassia auriculata seeds is an important bioactive component, with potent antifungal activity, a new phyto-constituent orphan neglected diseases.*

Keywords: *Cassia auriculata, Lectin, Protein, Antifungal, Orphan disease.*

Introduction: Lectins are derived from a Latin term means I choose, due to their site-specific binding affinity. Lectins are naturally occurring non-immunogenic glycoprotein which selectively bind with carbohydrates[1, 2] and mediates several biological processes[3, 4]. Lectins are broadly available in the entire living system including plant, animals and microorganisms[5, 6], in plants lectins are mainly found in seeds, nuts, beans and grains in higher concentrations[7-9]. There are several biological functions in which lectins have the important role such as cell adhesion regulation, synthesis of glycoprotein, blood protein level regulation, etc. or we can say that it serves as a defensive mechanism[10]. Plant lectins attracted the researcher because of their high carbohydrate specificity and could characterize quickly without influencing sugar binding characteristics. Lectins have shown several biological activities such as antibacterial, antifungal, antiviral, anti-insect, anti-tumor, anti-proliferative, immune potentiating, etc.[11]. Lectins are found in human body parts like vascular endothelial linings (selectins) consecutively for blood cells to imprison microbes, as opsonins which cover foreign antigens and make prone for phagocytosis[12]. The antioxidants in food are much needed nutritional components which decrease the free radical induced oxidative damage to proteins, lipids and nucleic acids[13].

Cassia auriculata Linn., well-known as "avaram" in the Tamil which is a shrub from Leguminosae family and widely distributed in India and Sri Lanka. C. auriculata is used in the cure of orphan neglected disease[14-18].

Literature survey says that C. auriculata contains various phytochemicals such as

flavonoids, polysaccharides, tannins, and saponins glycosides, among other components. There are several phytoconstituents have been isolated and characterized from this plant, but still there is some scarcity regarding the chemical constituents like proteins from seeds. In the existing study we have isolated and characterized a lectin protein and evaluated for antifungal and antioxidant activity from seeds of C. auriculata.

Materials and Methods

Chemicals: Sodium hydroxide, Sodium carbonate, Glycine, Copper sulphate, Potassium sodium tartrate were purchased from SRL Pvt. Ltd., Mumbai. Acrylamide, Bisacrylamide, Ammonium persulphate, Sodium dodecyl sulphate, N,N,N‘, N‘-tetramethylenediamine, Bovine serum albumin, Tris were purchased from Sigma-Aldrich, USA. Folin-Ciocalteau phenol reagent, Potassium Dihydrogen Phosphate, Potassium hydrogen phosphate were obtained from S.D. fine chem. Ltd., Mumbai. Acetic acid, Bromophenol blue, agarose were purchased from Himedia, Mumbai. Glycerol was obtained from RANKEM Pvt. Ltd. Ethanol purchased from Trimurty Chemicals, India. Pre-stained molecular weight marker was purchased from Bio-Rad, India.

Lectin Source: *Cassia auriculata Linn.* seeds were obtained from traditional herbal plant supplier of MGR-Nagar, Chennai, India and authenticated by Plant Anatomy Research Centre (PARC), Chennai, India.

Blood Samples: Human blood (AB, A, B, and O group) cells ware collected from a healthy male donor (5mL) in EDTA anticoagulant blood storage vial, offered by Blood Bank, Madras Medical College, Chennai.

Extraction of Lectin from Cassia auriculata Seeds: *Cassia auriculata* seeds were grinded for removal of seed coats/shells. About 100 gm. of de-shelled Cassia auriculata seeds were homogenized in a mixer grinder and defatted thrice with 300 ml of distilled acetone. The defatted seed-meal was dried, mixed with 1 liter of 20 mM phosphate buffer saline (pH 7.4), containing 10 mM -mercaptoethanol (PBS-ME) and 0.15 M NaCl, and stirred at 48° C for 12 h. Then the suspension was faltered and the filtrate were separated by refrigerated centrifugation in Centrifuge R- C23, (REMI Elektrotechnik Ltd, India) at 6000 rpm for 30 min at 4° C and the suspended suspension was subjected to ammonium sulphate precipitation.

Lectin Purification from the Seeds of *Cassia auriculata*

Ammonium Sulphate Precipitation: The suspended supernatant was subjected to ammonium sulphate precipitation. The cells separation were done by centrifugation at 10,000 rpm for 15 minutes, after that supernatant was fractionated by precipitation with ammonium sulfate between 50% and 70% of saturation. All consequent stapes carried out at 4° C. Dialysis was performed with 14 KDa dialysis tubing. Lectin protein was re-suspended in 0.1 M Tris-HCl buffer (pH 7.8), and dialyzed (Memra-cel MD 44-14×100 CLR) using same buffer[19].

Gel Filtration Chromatography (Sephadex G-75): After the saturation of the protein pellet with ammonium sulphate 70% was dissolved in 0.1 M sodium phosphate buffer saline and loaded on to a preparative Sephadex G-75 column (2.2×60 cm) (Sigma-Aldrich, St. Louis, MO) equilibrated with phosphate buffer saline (pH 7.8). The flow rate of eluted column was 60 mL/h with 0.1 M sodium phosphate buffer containing 5mM and EDTA 10mM.From the elution profile, it was perceived that the lectin protein was shows as a well-resolved singlet peak of caseinase activity coinciding with a singlet protein peak. Fractions [19-23] with high lectin activities were pooled, dialyzed with the help of 14 kDa membrane and lyophilized by Lyophilizer, Vertisis, South Africa and go for further studies.

Analytical Methods

Protein Estimation of the Extract: The estimation of the protein content of the filtrate was done by using DYE binding method (Coomassie brilliant blue dye)[20].

Reagent: In 50 ml of ethanol reagent Coomassie Brilliant Blue G-250 (100 mg) was mixed properly. To this, 100 ml of 85% (v/v) phosphoric acid was added and the finale volume make up to one liter. The concentration in the reagent was 8.5% (v/v) phosphoric acid, 4.7% (v/v) ethanol and 0.01% (w/v) Coomassie Brilliant Blue G-250.

Procedure: In 1.0 ml of culture filtrate, 5 ml of CBB was added, mixed thoroughly and absorbance was observed in Double Beam UV-Vis Spectrophotometer LT-2800, (Labtronics, India) at wavelength of 595 nm. Sterile un-inoculated medium with the reagent served as a blank. Bovine Serum Albumin Fraction used as the referenced for the calculation of the protein amount.

Carbohydrate Estimation of the Extract: A convenient and sensitive process for the

estimation of reducing sugars, particularly when large numbers of samples are to be analyzed, was developed by Miller 1959 and involves the use of dinitro salicylic acid reagent[21].

Reagents

Dinitrosalicylic Acid (DNS) Reagent: Dissolve simultaneously, 1 gm. of dinitro salicylic acid and 50 mg of sodium sulfate, 200 mg of crystalline phenol placed in a beaker with 100 ml of 1% solution of NaOH by stirring. Store the reagent in a stoppered bottle at 4°C. The reagent deteriorates during storage due to atmospheric oxidation of the sulfate present. If required to be stored, prepare the reagent without sulphate and add it just before use.

40% Solution of Rochelle Salt (Sodium-Potassium Tartrate): Pipette 3 ml aliquot of the extract into a test tube and add 3 ml of DNS reagent. Heat the mixture for 5 min in a water bath. Colure has developed, add 1 ml of Rochelle salt (40%) when the contents of the tube still warm. Cool the tubes under running tap water. Absorbance was observed at wavelength of 575 nm. Calculate the amount of reducing sugar using standard sample prepared from glucose.

Haemagglutination and Haemagglutination Inhibition: Normal as well as trypsin-treated human AB, A, B and O erythrocyte were used to performed haemagglutination assays with 96-well ELISA plates. Serially diluted seed extract or the purified lectin protein solution 100 µl was placed in the first well. Haemagglutination titer was scored visually after the addition of the 100 µl of 4% erythrocyte suspension and incubating the plate for 1 h at 48° C. Following the procedure haemagglutination-inhibition assays was performed with the purified TCSL. PBS was added in the first well of the micro titer plate 50 µl of the sugar solution (400 or 100 mM). Then each well was added to 50 µl of the protein solution (25 mg/ml, which is 8 times the required concentration for 50% agglutination). 100 µl of 4% erythrocyte suspension was added after incubating this at 48° C for 1 h and the plate was further incubated for another one hour, the titer was visually scored[22].

Electrophoretic Studies

Electrophoresis Procedure: Separating gel polymerization was done on the glass plate. After inserting a comb stacking gel was polymerized over the separating gel. Buffer with was loaded in to wells and after that the power supply was attached with cathode in the bromophenol blue the known amount of protein sample mixed with sample upper tank and anode in the lower tank. Electrophoresis was carried out continuously with constant voltage and current supplies (20 mA) at 4° C for 2 h until the tracer dye reached 0.5 cm above the bottom end.

Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis (SDS-PAGE): SDS-PAGE gel electrophoresis was accomplished on slab gel with stacking and separating gels (10 and 5% w/v) according to the *Laemmli et.al. 1970*[23] as per (Table I a, I b and I c).

Table I a. Sample Preparation

Solution A	0.4% (w/v) SDS with 1.5 M Tris HCL buffer pH 8.8
Solution B	0.4% (w/v) SDS with 0.5 M Tris HCL buffer pH 8.8
Solution C	0.8% bisacrylamide with 30% (w/v) acrylamide
Solution D	Ersulphate
Solution E	1% SDS
Solution F	N,N,N,N, tetramethyl ethylene diamine (TEMED)

Table I b. Preparation of Gel

10% (w/v) Separating gel		5% (w/v) Stacking gel	
Solution A	0.75 ml	Solution B	0.38 ml
Solution C	2.0 ml	Solution C	0.5 ml
Solution D	0.3 ml	Solution D	0.15 ml
Solution E	0.6 ml	Solution E	0.3 ml
Distilled water	2.6 ml	Distilled water	1.98 ml
Solution F	0.005 ml	Solution F	0.005 ml

Table I c. Preparation of Buffer

Tank buffer pH 8.3		Sample buffer	
Tris	3.0 gm	Glycerol	2.0 ml
Glycine	14.4 gm	-mercaptoethanol	1.0 ml
SDS	1.0 gm	10% SDS (w/v)	4.0 ml
Distilled water	1.0 L	Solution B	1.7 ml
		Bromophenol blue (aqueous)	0.2 ml
		Distilled water	0.6 ml

Procedure: The equal volume of protein solution and sample buffer was mixed and boiled for 3 min. in a water bath, cooled and added to the walls then the power supply was attached to

anode in the lower tank and cathode in the upper tank. Electrophoresis was carried out continuously with constant voltage and current supplies (20 mA) at room temperature until the tracer dye reached 0.5 cm above the bottom end.

Staining of Separated Proteins: Staining was done at the end of the electrophoresis, the gel was removed and stained with silver staining method[24], and the gels were put in storage in 7% (v/v) acetic acid.

Determination of Molecular Mass: Purified lactin protein molecular mass was determined using SDS-PAGE. SDS-PAGE concurrent run with purified protein samples and standard protein markers consists of Bovine Serum Albumin (66.0), Phosphorylase b (97.4), Kda purchased from SRL difco Chemicals Ltd. The gels were stained with silver nitrate after the separation according to the Blum et al. 1987[24].

Thermal Inactivation: Incubated lectin samples (pH 7) at different temperatures (25 to 80°C at 5°C interval) for 30 min was used to determine the influence of temperature upon lectin hemagglutinating activity. At 4°C temperature the residual hemagglutinating activity was determined.

pH-dependence of Agglutination Activity of Purified Lectin: Influence of pH on lectin hemagglutinating activity was evaluated at different pH (1-12) and room temperature (25°C) for 60 min through incubation of lectin samples and then the mixture was adjusted to pH 7.0 and activity of residual hemagglutinating was evaluated.

Influence of EDTA, Ca^{2+} and Mn^{2+} on Purified Lectin: 1 mg/ml purified lectin sample, and 50 mM EDTA was incubated for 10 hours with constant shaking. Extensive dialysis of lectin sample was carried out in 150 mM NaCl medium, and assessment of hemagglutinating activity was done and also repeated after adding 50 mM Mn^{2+} and 50 mM Ca^{2+} ions.

Antifungal Effects of Purified Lectin: Antifungal activity was determiner using C. albican, on heavily seeded medium. Incubation was done for 48 hours at 37°C following the addition of the 10 µl of purified lectin in 0.05 M Sodium Phosphate buffer pH 7.0 in to the media. The clear zone of inhibition (inhibitory power) was determined by measuring the surrounding diameter[25].

Results

Purification of Antifungal Lectin from Seeds of Cassia auriculata: After the extraction with PSB buffer, cell free extract of *Cassia auriculata* was collected and precipitated proteins were salting out with Ammonium Sulphate (70%). The crude protein was further used for analysis after dialyzed and lophilization. The graphical representation for the purification of anti-fungal protein is presented in (Fig. 1). The anti-fungal lectin of *Cassia auriculata* was purified to homogeneity as given in the summarized procedure in (Table II).

Fig. 1.Flowchart for the partial purification of Lectin protein from the seeds of *Cassia auriculata*.

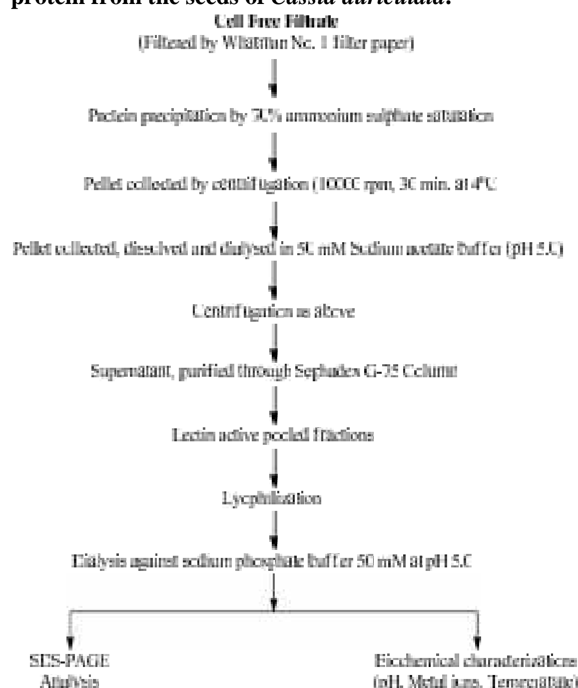


Table II. Purification of the Lectin from *Cassia auriculata*

Purification stage	Vol(ml)	Protein mg/ml	Total protein[a] mg/ml	Total lectin[b] (titer X ml)	Specific activity[c] (HU/mg)	Purification fold[d]	Yield %[e]
Crude extract	100	40	4000	12800	3.20	1	100
Dialysis	50	25	1250	6400	5.12	1.6	50
Sephadex G-75	30	8	240	2840	16	5	3.0

[a] Crude protein extract from 10 gm of cotyledons.

[b] Minimal concentration of protein able to cause visible agglutination of a 2-4% suspension of human erythrocytes.

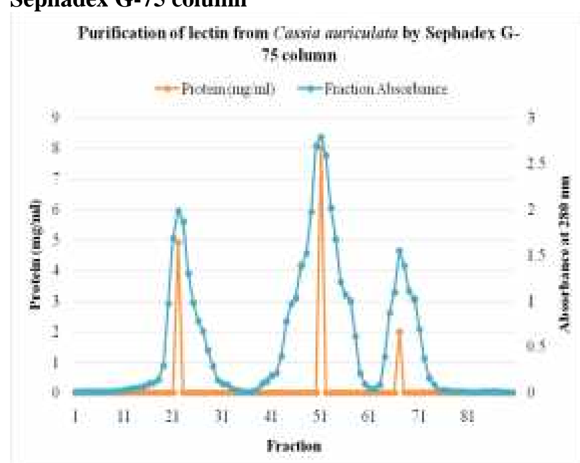
[c] Specific activity is defined as the hemagglutination unit (HU) divided by the protein concentration (mg/ml) of the assay solution. Human erythrocytes were used for the assay.

[d] Purification index was calculated as the ratio between the minimal concentration of the crude extract able to cause visible agglutination of the human erythrocytes and that of the protein fraction obtained at each purification step.

[e] The lectin was obtained by affinity chromatography on Saphadex G-75.

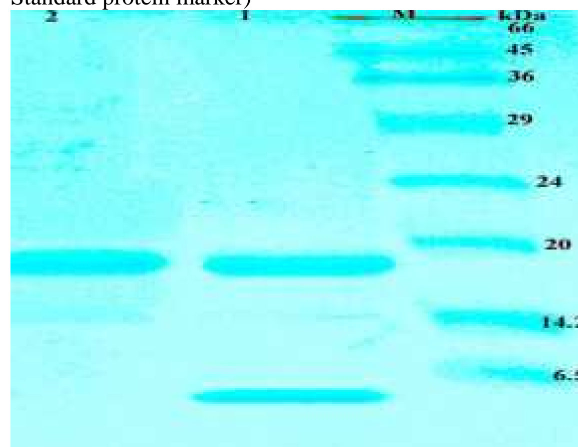
Sephadex G-75 Column Chromatography: After concentration of the protein (25 mg) was loaded on Sephadex G-75 column and each 2 ml of 80 protein fraction were collected. Their peaks of anti-fungal lectin were obtained: peak 1-fractions from 18 to 31, peak 2-fractions from 42 to 59 and peak 3-fraction from 63 to 74. The active protein fractions 22, 52 and 67 were isolated, concentrated, dialysed against sodium phosphate (10 mM; pH 7.0) and used for further purification (Fig. 2).

Fig. 2.Purification of lectin from *Cassia auriculata* by Sephadex G-75 column



Determination of Molecular Weight by SDS-PAGE: The purified anti-fungal protein (3.0 µg) was analyzed on 10% SDS-PAGE and stained with silver nitrate. Purified antifungal protein showed a single band on sodium dodecyl sulfate-poly acrylamide gel electrophoresis which was indicating that it was electrophoretically homogeneous. By comparing the relative mobility of the molecular mass of protein ladder as standard the molecular mass was determined as 20 kDa of the purified anti-fungal lectin (Fig. 3).

Fig. 3. Molecular mass determination of lectin from *Cassia auriculata* Linn. seeds by SDS-PAGE technique (Lane-1: Dialysis sample; Lane-2: Purified sample; Lane-M: Standard protein marker)



Haemagglutination and Haemagglutination Inhibition of Purified Lectin: The purified lectin from *Cassia auriculata* seeds showed altered haemagglutinating activities different blood group erythrocytes (Table III), and found relatively more potent for blood group "O".

Table III. Haemagglutinating activity of *Cassia auriculata* lectin against human erythrocytes

Source	Blood group	Agglutination (titer[a])
Human Blood	Type A	2^7
	Type B	2^7
	Type AB	2^7
	Type O	2^{10}

[a] Titer is the reciprocal of the end point dilution causing detectable agglutination of erythrocytes. The initial concentration of *purified* lectin was 500 µg used and diluted by ½ for the subsequent serial dilutions.

Haemagglutination on Carbohydrate Binding Specificity of the Lectin: The evaluation of carbohydrate binding specificity was carried out by hatching the lectin with a number of sugars in the haemagglutination assays of the tested carbohydrates D-Glucose and D-Mannose were fond the most potent inhibitors of haemagglutinating activity, reveled the absence of sulphur amino acid and affluent levels of hydroxyl amino acids (Table IV). In the haemagglutinating activity D-Mannose and D-Glucose were shows the IC_{50} value 5 mM and 7 mM, respectively which indicate that it was most potent inhibitors.

Table IV. Carbohydrate inhibition of the haemagglutinating activity of Cassia auriculata lectin

Sugar	IC_{50}[a] (mM)
D-Mannose	4
D-Glucose	8
D-Sucrose	9
D-Fructose	13
D-Galactose	22

[a]IC_{50}: Concentration required to inhibit haemagglutinating activity by 50%. 50 µg of CAL was used in these assays was 50 µg and diluted by ½ for the subsequent serial dilutions.

Influence of Purified Lectin from Seeds of Cassia auriculata on Metals: The *Cassia auriculata* purified lectin on haemagglutinating activity outstandingly declined after removing metal through continued dialysis against 50 mM EDTA, subsequently dialysis against NaCl (0.15 M).though, when Ca^{2+} and Mn^{2+} (50 mM) were readied to the assay medium, the CAL activity was fully restored (Table V).

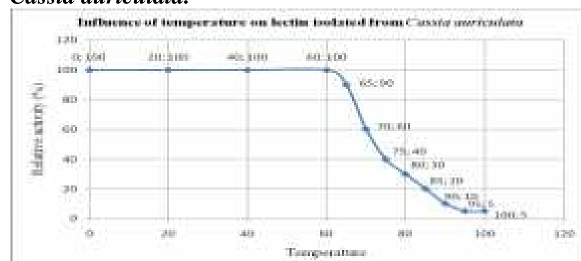
Table V. Metal ions inhibition of the haemagglutinating activity of *Cassia auriculata* lectin

Metal ions	Agglutination (titer[a])
Ca^{2+}	2^{10}
Mn^{2+}	2^{10}
K^{+}	2^{6}
EDTA	2^{7}
Fe^{2+}	2^{5}

[a] Titer is the reciprocal of the end point dilution causing noticeable erythrocyte agglutination. 500 µg of *Cassia auriculata* lectin was used in these assays and followed by 1/2 serial dilutions.

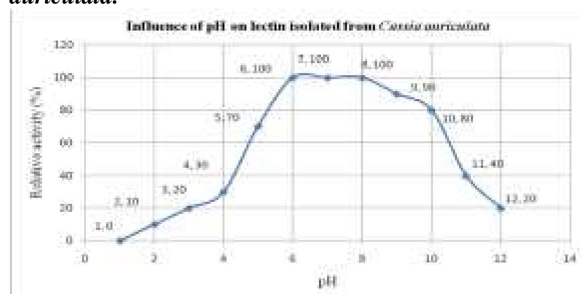
Thermal Inactivation of Purified Lectin from Seeds of Cassia auriculata: Generally, purified *Cassia auriculata* seed lectin is thermo-labile below to 60°C for 30 min, and maintains 100% potency. Temperature was increased beyond 60°C reduced the haemagglutinating activity gradually to 90, 60 and 20% while temperature was 75, 70 and 65°C, respectively, till the inactivation completely at 80°C for fewer than 15 min (Fig. 4).

Fig. 4.Influence of temperature on lectin isolated from *Cassia auriculata.*



Dependence on pH of Purified Lectin from Seeds of Cassia auriculata: Haemagglutination was clearly affected in acidic pH, while its activity was uninfluenced at pH range of 8-6. Lowering the pH to 4 and 5 simultaneously declining the potency by 70% and 30%, respectively, moreover the agglutination was fully stopped at pH below 4. While, rising the pH above 8, consistently lowers haemagglutination activity by 80%, 60%, 20% and 10% at the different range of pH 12, 11, 10and 9, respectively (Fig. 5).

Fig. 5.Influence of pH on lectin isolated from *Cassia auriculata.*



Anti-fungal Activity of Purified Lectin from Seeds of Cassia auriculata on Cassia albican: Growth inhibition of *Cassia albican* was observed in the presence of *Cassia auriculata* lectin at several serial dilution where the using concentration was 100 mg/ml. Growth of *Cassia albican* was inhibited by purified lectin with LIC of 0.1, 0.01 and 0.01 mg/ml, respectively (Fig. 6).

Fig. 6.Antifungal activity of purified sample of Lectin protein isolated from *Cassia auriculata* Linn.

*The influence on growth of hyphae for phyto-pathogenic fungi of the purified Psc-AFP.

(1) 20mM phosphate buffer pH 6.5
(2) 10 mM BSA in 20 mM phosphate buffer pH 6.5
(3) 5 mM Psc-AFP in 20 mM phosphate buffer pH 6.5
(4) 10 mM Psc-AFP in 20 mM phosphate buffer pH 6.

Discussion: The aim of this study is to isolate and characterize a lectin protein from seeds of *Cassia auriculata* and to evaluate this lectin for antifungal activity. With the help of affinity column chromatography on Sephadex G-75 lectin protein could be magnificently purified in a one-pot protocol. Purified extract loaded in to an affinity column chromatography followed by pounding out the unbound proteins then the specific activity led increments up to 320 titer/mg corresponding to 80% yield with the help of eluted bound lectin with 250 mM glucose. The final recovered lectin yield was 160 mg/100 gm dry seeds weight, which was exactly similar as reported by[26].

In the presence and absence of -mercaptoethanol SDS-electrophoretic patterns of purified *Cassia auriculata* lectin manifested two blends of 5.54 and 19.3 kDa which was

corresponding to and chains, bound non-covalently. Hence, the bands presence in SDS-PAGE most probable represented the subunits of a dimeric lectin; a common occurrence in plate lectins[27].

Cassia auriculata lectin did not show any specific aptitude to hemagglutinate human erythrocytes (AB, A, B, and O). The agglutinating activity of CAL was inhibited by mono and disaccharides, with D-Mannose shown highest potency while D-Galactose shown weakest potency refereeing to the highly selectively of *Cassia auriculata* lectin for mannose. EDTA (50 mM) was used to inhibit the hemagglutinating activity of the lectin, shown a divalent cations which was required for lectin potency, perhaps due to different protein structure.

Thermal denaturation result of *Cassia auriculata* lectin revealed that lectin remained significantly stable lower than 60°C for 30 min devoid of losing its hemagglutinating activity. The pH profile indicated that at pH 5.0-9.0 CAL was most stable. Dissociation of the lectin into it's a and b subunits at the acidic condition, complete abolishment of lectin hemagglutinating activity at pH lower than 4[28].

The examined anti-fungal activity of *Cassia auriculata* lectin against *Cassia albican* agrees with the result obtained from other plant legume lectins[29]. This activity was concluded to be related to the lectin carbohydrate binding property[30], that might endow lectin molecules with binding activity towards certain carbohydrates component in the fungal cell wall affecting its activity and viability as most lectins recognize either N-acetylneuraminic acid, N-acetylglucosamine, N- acetylgalactosamine, mannose, galactose or fructose in accordance with the conclusion of[3, 31].

Conclusion: Seeds of C. auriculata were homogenized in kitchen blander and defatted with distilled acetone (3×300 ml). The defatted and dried seed-meal was mixed with 11of 20 mM phosphate buffer (pH 7.4), containing 10 mM -mercaptoethanol and 0.15 M NaCl. The suspension was then filtered and the filtered centrifuged at 6000 rpm for 30 min at 4°C. The suspended supernatant was subjected to ammonium sulphate precipitation. The protein was loaded on to a Sephadex G-75 column. The affinity column was eluted and fractions with high lectin activities were pooled, dialyzed. Three peaks of anti-fungal lectin were obtained. The protein active fractions from were pooled, dialyzed against sodium phosphate buffer and lyophilized. According to Bardford and Dinitrosalicylic acid method protein content of the filtrate and carbohydrate content was estimated. The lectin from Cassia auriculata seed indicated variable hemagglutinating activities when tested against human erythrocytes (ABO system) referring to relatively greater potency against the O system of blood. The purified anti-fungal protein was analyzed on SDS-PAGE. By comparing the relative mobility of the molecular mass of protein ladder as standard the molecular mass was determined as 20 kDa of the purified anti-fungal lectin. The carbohydrate binding specificity of the lectin was studied, D-Mannose and D-Glucose were the most potent inhibitors of hemaggglutinating activity. Hemagglutination was markedly affected by acidic pH, while it motioned 100 of its activity at a pH range 6-8. Decreasing the pH to 5 and 4 lead to decreasing the activity and growth inhibition in the presence of *Cassia auriculata* lectin at serial dilutions.

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