Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 2, 2012

Full Proceeding Paper

ISOLATION AND IDENTIFICATION OF ADAPTOGENIC PROTEIN FROM *CICER ARIETINUM* LINN.

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Received: 16 March 2012, Revised and Accepted: 20 April 2012

ABSTRACT

In the present study, the fractions of crude protein from seeds of *C. arietinum* were assayed for its adaptogenic bioactivities. Plant lectin was isolated from the seeds of *Cicer arietinum* using a procedure that involved affinity chromatography. Lectin from *Cicer arietinum* showed one band with molecular weight of approx. 22 kDa as confirmed by SDS-PAGE. The crude protein sample from the *Cicer arietinum* L. was isolated by two step ammonium sulphate precipitation method. The dialysed sample was purified on cation exchange (CM-Sepharose) chromatography column. After isolating by column chromatography, identification of lectin was carried out by comparing with other plant lectins. The eluted fractions containing lectin were collected from the column and then used for hemagglutination activity study. The hemagglutination activity of various protein fractions were performed using peripheral human erythrocytes. Antioxidant activity of the lectin containing fraction was derived from free radical scavenging ability and the results indicated that adaptogenic activity of lectin from *Cicer arietinum* could be attributed to its antioxidant potential.

Keywords: *Cicer arietinum*, Protein precipitation, Ion exchange chromatography, Hemagglutination, Antioxidant, Adaptogen, Lectin.

INTRODUCTION

Stress may be defined as sum of all response to any external stimuli acting on biological system. Adaptability is probably the most distinct characteristic of life. Adaptogens are capable of acting against stress in all forms[3] and also they enhance resistance to stress, increase the performance and endurance during fatigue. Recently researchers are focusing towards the development of herbal adaptogens as they regulate and improve the stress response effectively. Plants are the richest and most convenient source of lectin. Plant lectin has been attracting much attention because of their ease of isolation and their usefulness as adaptogen. Lectins were identified by their agglutination ability of human and animal red blood cells. Lectins are particularly prevalent in fabaceous plants, where they are localized in the cotyledons of the seeds and roots. Besides fabaceous members are also proven adaptogens with adaptogenic proteins such a protein, CI-I from *Cajanus indicus* Spreng Arabinogalactan proteins from *Baptisia tinctoria* R.Br (AGPS) and agglutinin (PSA) from *Pisum sativum* L. In the present work *Cicer arietinum* L rich in lectins is selected for the identication of adaptogenic protein. This study reports the purification and characterization of lectin from *Cicer arietinum*.

MATERIALS AND METHODS

Protein Isolation and purification

Crude preparation and pre treatment

500gm of fresh pulse ground into small pieces and were defatted using ice cold Petroleum ether for atleast 2 hours. The above content was then filtered and the residue was smashed in a clean mortar pestle using chilled Phosphate buffer.

Protein fractionation using ammonium sulphate precipitation

The crushed material was then filtered again; to the filtrate 40% ammonium sulphate was added to precipitate the proteins as follows: equal volume of 40% ammonium sulphate saturated solution was added to the sample. After incubating for 2 hour and centrifugation at 14,000 g at 4°C for 25minutes, the supernatant was collected and precipitated again with 60% saturated ammonium sulphate. Further processing was done as per reported procedure[4].

Purification by desalting and ion exchange chromatography

This precipitated protein was then resuspended in approximately 3-5 ml of dialysis buffer (10 mM Ammonium bicarbonate, 1 mM DTT, 0.02% NaN_3) and pipetted into the dialysis tubing MWCO-10KDa. Dialyzed at 4°C for overnight against dialysis buffer, changed the dialysis buffer (1L) for the first 4 hours. Protein solution was collected from the dialysis tube. Dialysis tube was then washed with fresh 10 mM ammonium bicarbonate solution. Dialyzed protein solution was collected in a clean 2.0 ml micro centrifuge tube.

The dialyzed protein solution was then subjected to downstream processing and protein concentration estimated using Lowry's assay [5] and Lectin activity by hemagglutination activity[8].

SDS-PAGE

The molecular mass of purified lectin was estimated by SDS–PAGEusing known procedures[7].

Hemagglutination activity

This procedure was used as an indicator for the presence of sugar binding lectin (phyto-agglutinin)[8]. Lectins inactivate specific tumor cell types and precipitate certain glycoprotein and polysaccharides [9, 10]. Agglutination test for the detection of lectin is an established protocol [11, 12]and the activity was defined as the reciprocal of highest dilution with positive agglutination[13].

Free radical scavenging activity by DPPH assay

Determination of free radical-scavenging activity of the protein was done by following literature emthods[13, 14]. Various concentrations of herbal extract were added to methanol solution of 100μM DPPH and IC_{50} values derived from the data.

RESULTS AND DISCUSSION

Crude extracts from the seeds of *Cicer arietinum* were found to exhibit promissing agglutination activity. Peak 1 M of *Cicer arietinum* with 60% Ammonium sulphate precipitated fraction showed maximum lectin activity. The eluted protein sample after dialysis exhibited two protein peaks in CM-Sepharose chromatography (Fig. 1). SDS-PAGE of the purified lectin (peak 1) developed as a single protein band with a mass value of 22KDa (Fig. 2).

Hemagglutination activity

Lectins are a sugar-specific binding proteins and hemagglutination activity is considered as one of their key properties[15]. Lectins are used in the diagnostic and therapeutic purposes in cancer research [16]. The presence of Lectin protein from small to higher living organisms proved its diversity. In this study, lectin protein was isolated from *C. arietinium* by performing ammonium sulfate precipitation followed by dialysis.

To investigate hemagglutination activity, freshly collected human red blood cells were employed. Lectin isolated from *C. arietinium* showed a promising agglutination activity which is in agreement

with earlier work reported[17]. Agglutination assay was carried out using erythrocytes of 1% suspension in 20 mM Tris-HCl pH 7.4 containing 0.15M NaCl [18]. The agglutination strength was determined as the titre strength of lectin protein[19] (Table 1).

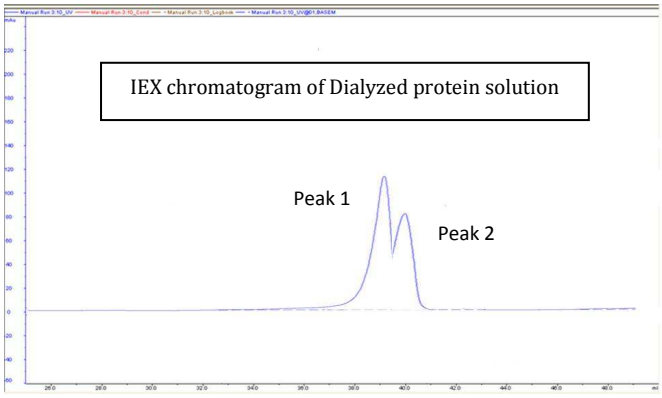


Fig. 1: CM-sepharose Ion Exchange chromatogram of dialyzed protein

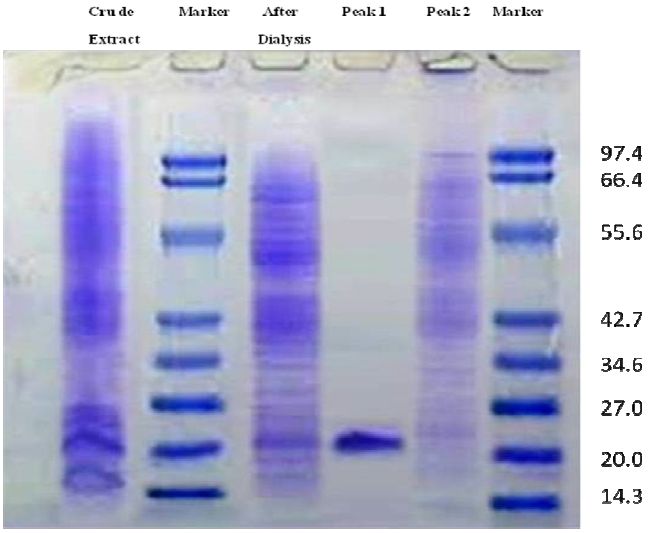
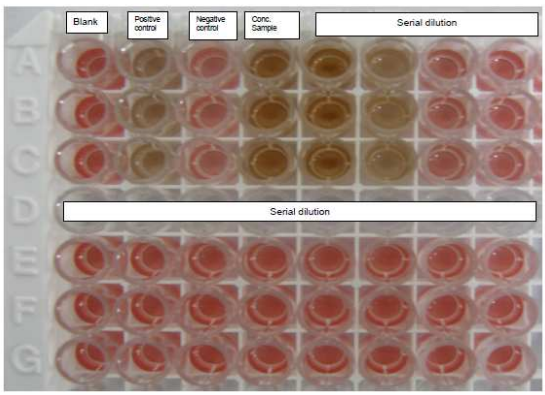


Fig. 2: Separation of a protein mixture by SDS-PAGE Electrophorsis. 5µg of Samples and 100ng of Protein Molecular weight standard were separated on a 12.5 % SDS-PAGE electrophoresis Gel at 150 volt untill complete. Protein were stained with Coomasie blue for 8 hours and destained overnight in 10 % Glacial acetic acid.

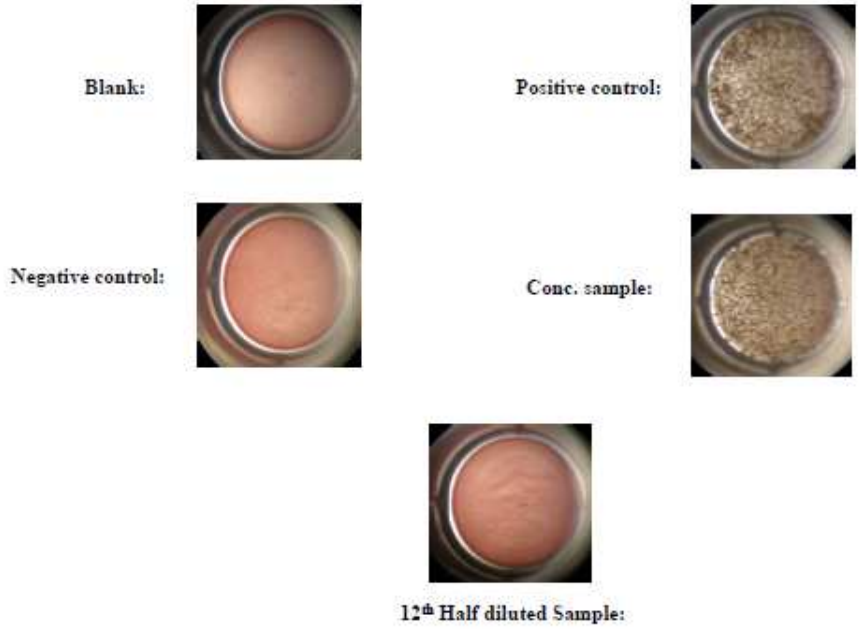
Table 1: Hemagglutination activity of isolated *Cicer arietinum* lectin.

Experiment	PBS alone (Negative control)	TCA (Positive control)	Crude Protein	Dialysis	IEX	
					Peak 1	Peak 2
Total Protein estimation	-	-	52.9 mg/ml	9.0	1.0	7.6
Hemeagglutination assay	-	++++	++	++	+++	+

- no agglutination, + strength of agglutination



A.



B.

Fig. 3: Hemagglutination assay performed in 96 well microtitre plates. A whole plate, B various controls and test wells.

Antioxidant Potential

The antioxidant potential of the isolated lectin protein was determined employing DPPH assay, which confirmed antioxidant activity: IC_{50} value of 0.170 mg/ml. It has been shown that the scavenging efficacy on the DPPH radical increases sharply with the increasing concentration of lectin.

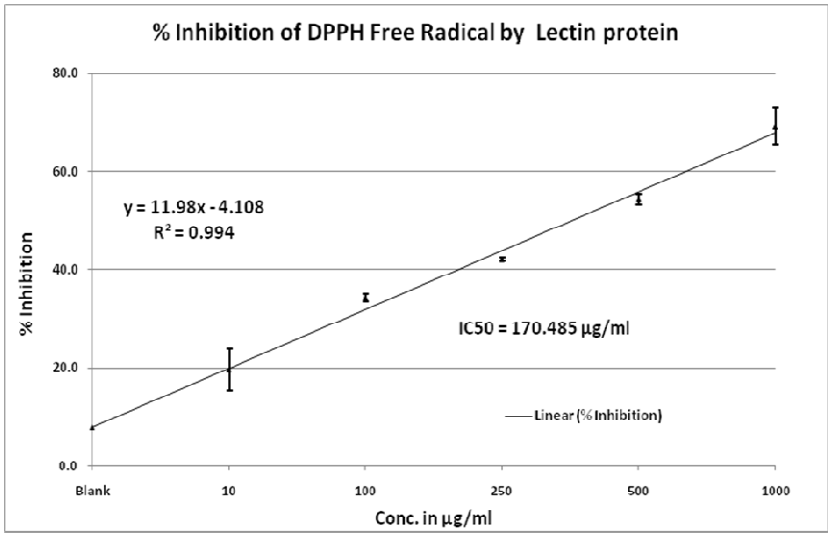


Fig. 4: The antioxidant activity of the crude protein extract was measured by the DPPH assay. n=3, mean ± SD.

Table 2: DPPH scavenging activity of isolated Lectin from *Cicer arietinum* Linn.

Lectin from *Cicer arietinum*	
Conc. (µg/ml)	**% Inhibition**
Blank	7.8 ± 0.01
10	19.5 ± 4.29
100	34.2 ± 0.77
250	42.0 ± 0.35
500	54.3 ± 1.14
1000	69.2 ± 3.67

(n=3, Mean ± SD)

CONCLUSION

Present work deals with isolation, characterization as well as agglutination and antioxidant potential of lectin protein from *Cicer arietinium L.* It is concluded from this study that crude protein of *Cicer arietinium Linn* possess good agglutination and antioxidant activity which may contribute towards providing adaptogenic property to this lectin containing fraction of *Cicer arietinium L.*

ACKNOWLEDGEMENTS

Support of the research through grant by Defence Research and Development Organization (DRDO), India is gratefully acknowledged. Authors also wish to acknowledge CARISM, SASTRA University, Thanjavur for providing necessary infrastructure facilities and support..

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