INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

**ISSN: 2454-132X**
**Impact factor: 6.078**
***(Volume 6, Issue 1)***
Available online at: www.ijariit.com

# Isolation, partial purification and characterization of Lectin protein from soybean seeds (Glycine Max) and its cytotoxicity against Hela Cells

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## ABSTRACT

***Plant lectin proteins have a wide range of biological importance. In this research work, the isolation, partial purification and characterization of the lectin proteins from the Soybean seeds (Glycine max) have been reported. The lectin proteins were purified with the help of ion exchange chromatography and further gel filtration chromatography on DEAE Cellulose column and Sephadex G-100 column respectively. Human blood group A was used for the agglutination assay. Lectin Protein concentration was determined by the Lowry method and further analysis was done by SDS- PAGE. Samples were dialysed against phosphate buffer saline (PBS) and SDS-PAGE was done to identify the molecular weight of fractions. Further, haemagglutination assay was performed for a sample of crude, 20% cut, 60% cut, 90% cut, dialysed samples, 60% ion exchange chromatography portion using A type blood group. The highest titer value was obtained from agglutination assay the sample from gel filtration chromatography i.e.512HU and the specific activity is 6400 HU/mg. The partially purified lectin protein gave a single protein peak on ion exchange chromatography. On the SDS-PAGE electrophoretogram, many bands were observed, out of which one band was of 30 kDa which is supposed to be the band of lectin. The partially purified lectin shows cytotoxic activity against HeLa cell line. The highest inhibition of HeLa cells i.e. 63.8% was observed at the concentration of 250µg/ml. Thus, the IC50 of partially purified lectin protein was found to be 99.2µl/ml.***

***Keywords***— *Soybean Lectin, SBL, Ion Exchange Chromatography, HeLa Cells, SDS-PAGE, Haemagglutination Assay*

## 1. INTRODUCTION

The soybean or soya bean (*Glycine max*) is a species of legume native to East Asia, widely grown for its edible bean, which has numerous uses. Soybean, (*Glycine max*), also called soja bean or soya bean, annual legume of the pea family (Fabaceae) and its edible seed. The soybean is economically the most important bean in the world, providing vegetable protein for millions of people and ingredients for hundreds of chemical products. The soybean is one of the richest and cheapest sources of protein and is a staple in the diets of people and animals in numerous parts of the world. The seed contains 17 percent oil and 63 percent meal, 50 percent of which is protein. Because soybeans contain no starch, they are a good source of protein for diabetics. The lectins purified from soybean showed equal agglutination with all blood groups of human beings i.e. A, B, AB, and O. Soybean has haemag lutinating activity and contains a protein which reacted with antibodies directed against soybean seed lectin is the same as root lectin. The word 'Lectin' has been derived from the latin word which means 'I Choose'. Because lectins are very specific to the site to which it binds Lectins are carbohydrate-binding proteins that bind to glycoproteins, glycolipids, and also polysaccharides (Goldstein and Hayes, 1978) which mediates various kind of biological processes by binding to the different sugar moiety. Lectins are highly diverse in structure, molecular weight, composition, and number of sugar binding sites present per molecule (Laija, et al.2010) Lectins are widely distributed in nature and found in all forms of life including plant products such as fruits, vegetables but nuts, grains, beans, and seeds contains high lectin amount (Lis and Sharon, 1986). Researchers have great interest and lectins have been studied and isolated from various sources including plants, animals, fungi, lichens, and bacteria(Liener1976; Hapner and Robins 1979; Damjanov 1987; Sharon and Lis 1989).

The lectin activity was determined by measuring agglutination assay. The plant lectins are stable proteins that can be characterized without affecting their sugar binding properties. The banana lectin has been isolated from *Musa paradisiacal* and characterized in 1990 (Koshte et al., 1990). The dimeric structure of lectin was shown to be mannose-specific (Singh, 2004). Lectins have also been isolated from vegetative tissues of plants like leaves, stems, barks, and roots (Callow 1975; Goldstein and Haye 1978; Quinn

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and Etzler1987). Lectins are also suited for analysis and isolation of animals and human glycocojugates. The mature seed contains about 3% of the weight of it (Laija et al., 2010). The snail *Helix pomatia* contains large amounts of lectins which agglutinate with human type A erythrocytes (Uhlenbruck&Prokop, 1966). It has been reported (Vasta, 1992) that this lectin, which aggregates in the snail albumin gland (Prokop et al., 1968), plays a major role in the protection of eggs as well as developing embryos against bacterial and fungal infections. The lectins from legume and cereal has altered the microflora present in the gut (Liener, 1986; Pusztai et al. 1993b), causes inflammation (Liener, 1986; Pusztai et al., 1993b) and increases the intestinal permeability (Greer et al., 1985) which also helps in the translocation of gut pathogens to the periphery. Pusztai et al (1991) reported that beans having a higher content of lectins cause most serious damages to the luminal surface of the intestine in rats compared to those having lesser lectin contents. An inhibitory effect of lectins on the activity of peptidase as well as disaccharidase of enterocytes has also been reported (Oliveira, 1989; Kim et al., 1976). Lectins isolated from bean species have influenced the intestinal structure and function negatively (Liener, 1986) leading to diseased situations Distinction between a malignant cell and a normal cell has been done by using lectins (Sharon and Lis 1989, 2004). Lectins have the property which modifies the cell cycle by inducing non-apoptotic G1-phase accumulation mechanisms, which arrests the G2/M phase cell cycle and does apoptosis and also has the ability to activate the caspase cascade. Plant lectins have the capacity in cell separation and bone marrow transplantation (Reisner et al., 1983). Introduction of plant lectins in the form of microarrays as a unique means for high throughput analysis of protein glycosylation (Rosenfeld et al., 2007) and profiling the global changes in the mammalian and bacterial (Hsu et al., 2006) cell surface glycomes. Lectins can be used as probes for the characterization and isolation of simple and complex sugars (Rudiger and Gabius, 2001). Lectins can be used in immunological studies as a tool (Moreira et al., 1991). Dietary lectins can cross the gastrointestinal barrier and enters the circulation intact (Pusztai et al.1989), and also be able to interact with synovial tissues directlyVarious plant lectinshas the tendency to bind with the intestinal mucosa which disturbs the functions of intestine and may cause enlargement of pancreas (Pusztai and Bardocz, 1996). Plant lectins play a vital role in defense itself from the predators is not new. Lectins are used in the activation of lymphocytes and for the induction of proteins like enzymes, interleukins or cytokines (Kilpatrick, 1991). To determine the 3-dimensional structure of the carbohydrate binding sites of lectins NMR and XRD are used.

## 2. OBJECTIVES

(a) Isolation of proteins from soybeans (Glycine max)
(b) Protein Estimation by Lowry Method.
(c) Purification of lectin
(d) Estimation of the concentration of lectins by Haemagglutination Assay
(e) Determination of lectins by SDS-PAGE
(f) Cytotoxicity of lectins against the HeLa cell line.

## 3. MATERIALS AND METHODS

### 3.1 Chemicals

Sodium hydroxide (NaOH), Sodium carbonate (Na2CO3), glycine, Cuppersulphate (CuSO4) Potassium sodium tartarate (KNaC4H4O6) Acrylamide, Bisacrylamide, Ammonium persulphate (APS), Sodium dodecyl sulphate (SDS), N, N, N‘, N‘-tetramethylenediamine (TEMED), Bovine serum albumin (BSA), Tris, Folin-Ciocalteau phenol reagent, Potassium Dihydrogen Phosphate (KH2PO4), Potassium hydrogen phosphate (K2HPO4) Acetic acid, Bromophenol blue, agarose Glycerol. Ethanol, etc.

### 3.2 Sample collection

The Soybean (Glycine max) seeds were collected for isolation and purification of lectins from a local store of the market, Ahmednagar.

### 3.3 Seed coat removal

Soybean seeds were taken and soaked in PBS (50gm soybean seeds) and seed coat was removed. Then, the seeds were grinded with the minimum volume of PBS and the paste was collected in centrifuge tubes and was centrifuged at 7500 rpm, at 40C for 20 min. The supernatant was taken and subjected to the salting out process.

### 3.4 Salting Out/Ammonium Sulphate Precipitation

The ionic strength of a protein solution is increased by adding ammonium salt, the solubility decreases, and protein gets precipitates. The salt molecules compete with the protein molecules in binding with water. The concentration of salt requires for precipitation of the protein out of the solution is varies greatly in different proteins. It is also used to concentrate dilute solutions of proteins. Ammonium sulphate salt was used in the salting out process. Crude sample was taken for 20% cut off in the salting out process. According to the salt chart, 5.35 gm for 100 ml, ammonium sulphate was added to the crude by pinch by pinch and continue stirring was done by a magnetic stirrer. Then the sample was stored at 4°C overnight. On the next day, the sample was taken for centrifugation and then the supernatant was collected for further 60% ammonium sulphate precipitation. Crude sample was taken for 60% cut off in the salting out process. According to the salt chart, 12.2 gm for 50 ml ammonium sulphate was added to the crude by pinch by pinch and continuous stirring was done by a magnetic stirrer. Then the sample was stored at 4°C temperature overnight. On the next day, the stored sample was taken and centrifugation was done at 4°C temperature for 30 min at 10,000 rpm and the pellet was dissolved in PBS for further process. The supernatant was taken for 90% cut off in the salting out process. According to the salt chart, 10.2gm ammonium sulphate was added to the stored sample by pinch by pinch and continuous stirring was done by a magnetic stirrer. Then the sample stored at 4°C temperature overnight.

### 3.5 Dialysis

A dialysis bag was used for this purification. The pore size of the dialysis bag was 10-100 A0. The dialysis was done against PBS at 4°C of 20% cut off, 60% cut off, 90% cut off samples. Every 2 hrs after starting the dialysis PBS buffer was changed.

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**3.6 Ion Exchange Chromatography**

DEAE (3 gm) was weighed and soaked in 30 ml 1N NaOH for 30 min. After 30 min, excess NaOH was removed carefully and distilled water was added to remove excess NaOH. Then, 30 ml 1N HCL was added to keep for 20 min. Excess HCL was removed and washed with distilled water. DEAE was washed with distilled water until it gets neutral. After treatment, DEAE was used for column packing. Glass wool was placed at the bottom of the syringe and then the small volume of buffer was added to avoid trapping of air bubbles. Immediately, the syringe was filled with gently stirring DEAE carefully from the trap of syringe. The column was washed with 0.1 M acetate buffer (pH 4.6) several times. Aliquote of 2ml of 60% dialysed sample was gently poured on column and flow was plugged off for 5 min. For elution of sample, acetate buffer was used as the mobile phase. Elution buffers of 0.1M, 0.2M, 0.3M, 0.4M, 0.5M NaCl concentration was used. Elution buffers (acetate) gently added on column and 1ml of first elution collected in the first vial. Likewise, other elutes were collected in separate vials. These samples were assayed and protein concentration was determined by the Lowry method and its O.D was determined at 280nm.

**3.7 Gel Filtration Chromatography**

Sephadex G-100 column was prepared in 1N NaOH and fixed on a stand. The column was equilibrating with a 4 ml gel filtration buffer. The gel filtration buffer was drained out completely. Aliquote of 0.2 ml of the sample loaded on to the column, along the sides of the column. The sample was allowed to sink completely and then 0.2 ml of gel filtration buffer was added. The gel filtration buffer allowed flowing out completely. Keep toping the column with gel filtration buffer, till all the protein has eluted out. Fractions were collected in different tubes. Then, the lower cap and then the upper cap was fixed to stop the flow of buffer.

**3.8 Determination of Concentration of Protein**

The concentration of crude, 20% cut, 60% cut, 90% cut, and 60% ion exchange chromatography and 60% Gel filtration chromatography were measured by the Lowry method using Bovine Serum Albumin (BSA) as the standard protein (Lowry et al., 1951). Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) in the test tube. The final volume in each of the test tubes was 5 ml. The BSA range is 0.05 to 1 mg/ ml. From these different dilutions, a 0.2 ml protein solution was pipetted out to different test tubes and 2 ml of alkaline copper sulphate reagent (analytical reagent) was added. The solutions were mixed well. The test tubes were incubated at room temperature for 10 mins. and 0.2 ml of reagent Folin Ciocalteau solution was added to each tube and incubated for 30 min. The optical density was measured at 660 nm. The graph was plotted as absorbance against protein concentration to get a standard calibration curve. Finally, the absorbance of the unknown sample was determined and the concentration of the unknown sample was analyzed using the standard graph.

**3.9 Preparation of Human Erythrocyte**

Healthy human venous blood (4-6 ml) was collected in a 15 ml tube to which the anticoagulant EDTA was previously added.

**3.10 Haemagglutination Assay**

A blood sample of 1 ml was centrifuged in a 2ml micro tube at 1000 rpm for 5min at room temperature. Then the pellet was collected and was added 10ml of PBS. The mixture of blood and PBS was centrifuged at 1000 rpm for 5min at room temperature. After centrifuge, the pellets were collected and 100µl of the pellet was added to10ml of PBS solution (pH 7.2). The haemagglutination activity of Soybean lectin was detected when blood erythrocytes were added to it. The assay was carried out in a 96 well round bottom microtitre plate. The first well of each row was served as a positive control to which 100µl of normalized sample and 100µl of blood was added and the last well served as a negative control since it contains 100µl of blood and 100µl of PBS solution. Between the positive and negative control, each well contains blood, PBS and lectins. First of all, 100µl PBS was added to all the wells. Then 10µl of normalized crude was poured to the first well and it was serially diluted till the negative control. A similar procedure was followed for the other samples. Finally, 100µl of the processed blood sample was poured to each well. After that, the plate was placed on a plane surface without disturbing it. After two hours the haemagglutination assay, the result was observed.

**3.11 SDS-PAGE**

The electrophoresis apparatus assembly was clamped and sealed with the help of a 1% agarose gel. The assembly was checked for leakage by filling water between plates. The separating gel mixture was poured between plate immediately after preparation and gel mixture was allowed to polymerize and water was removed after confirmation using filter paper. Distilled water was added to this gel to confirm the polymerization using filter paper. The stacking gel was prepared and immediately poured on to the separating gel. Then the comb was inserted into the gel approximately 1 cm. After the stacking gel had set, the comb was removed carefully and wells were washed with distilled water to remove non polymerized acrylamide. The plates were then fixed to the apparatus and with the notch plate, facing the top reservoir. The top and bottom reservoirs were filled with running buffer. The 10 µl protein sample namely standard, crude protein extract, precipitated protein, dialysis protein and protein purified by chromatography were loaded into wells, sequentially along with 10µl gel loading buffer. The power supply was turned on and the protein sample was electrophoresed for 1-2 hrs. After electrophoresis, the glass plates were removed from the apparatus and separated by holding under running tap water. The gel was removed from plates into the petri plate and stained with CBB stain. The gel was then washed with a distaining solution and the banding pattern of protein on the gel was observed.

**3.12 Cytotoxicity of partially purified lectins against HeLa cell line**

In this assay, the cancer cells were seeded in complete medium in a 96-well plate at a density of 1x105 cells/ml. After reaching confluence, the cells were incubated with different concentrations of the sample (µl) for 48 hr. DMSO was used as controls. The medium was then discarded and the adherent cells were washed twice with phosphate buffer solution (PBS), then 20 µl of MTT stock solution (5 mg/ml in PBS) was added to each well and the plates were further incubated overnight at 37°C. 100 µl of DMSO was added to each well to solubilize the formazan crystals produced by viable cells. After the complete dissolving of formazan

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blue, the absorbance was measured at 570 and 690 nm, as reference wavelength, using a microplate reader (Thermo). The percentage of cell viability was calculated according to the equation described in Moongkarndi et al. (2004). % of cell viability = (OD of treated cells / OD of control cells) × 100 The concentrations required for inhibition of 50% of cell viability (IC50) were calculated.

## 4. RESULTS AND DISCUSSION

### 4.1 Sample collection and identification of the variety

The Soybean (Glycine max) seeds were collected for isolation and purification of lectins from a local store of the market, Ahmednagar. The soybean variety was identified by the Agriculture specialist as Mahabeej JS 335.

### 4.2 Ammonium Sulphate Precipitation

Ammonium Sulphate was taken for salting out process. Salting out process was started from 20% cut off to 90% cut off. At each step, the sample was centrifuged and the supernatant was taken for successive cut off and the pellet was dissolved in PBS and used for the further purification step. The highest protein concentration was observed in 60% cut off a sample. The protein concentration of a 60% cut off sample is 3.64 mg/ml.

### 4.3 Dialysis

In this purification step, all the samples came from the salting out process get dialyzed against PBS at 4°C and concentration was measured with the help of Lowry method of measurement of the concentration of proteins. The highest concentration was observed in 60% dialyzed sample and the protein concentration was found to be 0.52 mg/ml.

### 4.4 Purification of protein by ion exchange chromatography and gel filtration chromatography

Soybean lectin was purified by Ion Exchange Chromatography by DEAE Cellulose column and Gel Filtration Chromatography by Sephadex G-100 column. Soybean lectin was purified by Ion Exchange Chromatography by the DEAE Cellulose column. The chromatogram shows the highest peak observed in 16 numbered fractions therefore the partially purified protein is present in this fraction. In Gel Filtration Chromatography, the sample came from ion exchange chromatography was poured on to the Sephadex G-100 column. In this chromatogram, the highest peak showed the presence of protein. The fractions collected during ion exchange and gel filtration chromatography were subjected to the Lowry method to determine the total protein concentration. The result was shown in fig no.8 and 9. The highest peak was observed in fraction no.12.and fraction no.21 respectively.

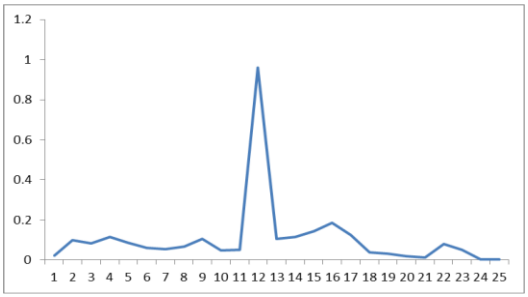


**Fig. 1: Ion exchange chromatography**

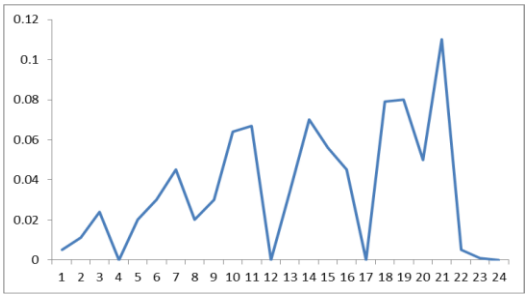


**Fig. 2: Gel filtration chromatography (12th fraction) (60% dialyzed sample.)**

### 4.5 Determination of total protein concentration

The total protein concentration of crude, ammonium salt precipitated, dialyzed and purified protein sample by chromatography was measured by the Lowry method using Bovine Serum Albumin (BSA) as the standard protein (Lowry et al., 1951). The total protein concentration was decreased from crude to gel filtration chromatography sample as the purification level goes on increasing. The total protein concentration of the crude sample was 112.8 mg/ml whereas the protein concentration from ion exchange chromatography and gel filtration chromatography were 0.04 and 0.08 mg/ml respectively. The results were shown in the table.

**Table 1: Concentration of Proteins**

| Sample | Volume (ml) | Concentration (µg/ml) | Total Concentration (mg/ml) |
|---|---|---|---|
| Crude | 47 | 2400 | 112.800 |
| 20% cut | 42 | 1720 | 72.240 |
| 60% cut | 33 | 3640 | 120.120 |
| 90% cut | 29 | 2760 | 80.040 |
| 20% Dialysis | 9 | 1000 | 9.000 |
| 60% Dialysis | 8 | 520 | 4.160 |
| 90% Dialysis | 6 | 3480 | 20.880 |
| 60% Ion exchange chromatography | 1 | 40 | 0.04 |
| 60% gel filtration chromatography | 1 | 80 | 0.08 |

### 4.6 Haemagglutination Assay

To characterize the desired protein, a haemagglutination assay was performed by using human erythrocyte suspension of 'type A 'blood group. The assay was carried out in vials by serially diluting the sample and allowing it to incubate for 2 hours. Then, it

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was found that there was an agglutination reaction in each sample. In the present investigations, lectins extracted from soybean seeds were used for erythrocytes agglutination assay. Hemagglutination activity performed in V shaped vials with 1% Human blood in PBS (pH7.2) all the samples give agglutination activity. This is evident by the formation of the carpet layer on the bottom of a vial. The reciprocal of dilution is calculated as titer value, which reflects lectin activity. Higher the titer value, the higher is the lectin activity. On the other hand, the absence of lectin marked a distinctive red colour on the bottom of the vials. Protein content varied lowest of 0.04 mg/ml in the sample of ion exchange chromatography to highest of 3.64 mg/ml in a 60% dialysed sample. In soybean seeds, the highest hemagglutination and specific activity were observed in the gel filtration chromatography sample (512 HU) having a specific activity of 6400 HU/mg.

**Table 2: Erythrocytes agglutination by soybean seed extracts**

| Sr. No. | Samples | Protein Conc. (mg/ml) | Hemagglutination activity/unit (HU) | Specific activity (HU/mg) |
|---|---|---|---|---|
| 1. | Crude | 2.4 | 512 | 213.33 |
| 2. | 20% cut | 1.72 | 385 | 233.83 |
| 3. | 60%cut | 3.64 | 256 | 70.32 |
| 4. | 90%cut | 2.76 | 16 | 5.797 |
| 5. | 60%dialysis | 0.52 | 64 | 123.07 |
| 6. | 60% ion exchange chromatography | 0.04 | 128 | 3200 |
| 7. | 60% gel filtration chromatography | 0.08 | 512 | 6400 |

### 4.7 SDS- PAGE

The SDS-PAGE was performed for determination of molecular weight using polyacrylamide as the resolving gel (12%) and polyacrylamide as the stacking gel (4%) and the protein bands were further stained by the staining solution. Then, the bands were visualized by the gel documentation system and then the molecular weight of the desired protein was found to be 30 kD.

### 4.8 Cytotoxicity of lectins against HeLa cell line

Cytotoxicity of partially purified lectin purified after gel filtration chromatography was evaluated against the HeLa cell line. The graph shows the 0 concentration of lectins there was no inhibition. As the concentration of lectins increases the inhibition was observed. The highest inhibition of HeLa cells i.e. 63.8% was observed at the concentration of 250µg/ml. Thus, the IC50 of partially purified lectins was found to be 99.2µl/ml.

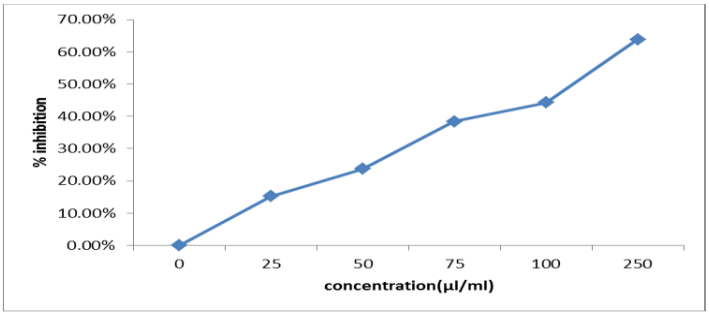


**Fig. 3: cytotoxicity of partially purified lectin isolated from soybean seeds**

## 5. CONCLUSION

In summary, this research work concluded that the Soybean Lectin (SBL) was isolated successfully and purified partially from the seeds of Soybean (Glycine max) by ammonium sulphate precipitation (ASP), dialysis, ion-exchange chromatography, and further gel filtration chromatography. The haemagglutination assay was done for the characterization of the Soybean Lectin (SBL) which showed that these lectin proteins could agglutinate with the human red blood cells(RBS's) due to the carbohydrate-binding site present on the red blood cells. The Soybean Lectin (SBL) agglutinates with the red blood cells of A-type blood group. This research work explored a variety of techniques of protein (lectin) purification and describing their characteristics by doing the haemagglutination assay. The highest titer value was obtained from agglutination assay the sample from gel filtration chromatography i.e.512HU and the specific activity is 6400 HU/mg. The partially purified lectin proteins gave a single peak on the spectrophotometric analysis of a fraction of ion-exchange chromatography. On the SDS-PAGE electrophoretogram, many bands were observed, out of which one band was of 30 kDa which is supposed to be the band of lectin. The partially purified lectin shows cytotoxic activity against the HeLa cells. The highest inhibition of HeLa cells i.e. 63.8% was observed at the concentration of 250µg/ml. Thus, the IC50 of partially purified lectins was found to be 99.2µl/ml

## 6. REFERENCES

[1] Albertazzi P., Pansini F., Bonaccori G., Zanotti L., Forini E., De Aloysio D., (1998). The Effect of dietary soybean supplementation on hot flushes. Obstetrics & Gynecology 91: 6-11.

[2] Baintner K., Farningham DA., Bruce LA, MacRae JC, Pusztai A., (1993). The fate of the antinutritive proteins of soybean in the ovine gut. Zentralbl Veterinarmed A, 40: 427−431.

[3] Bashir H., Khan T., A. Masood and Hamid R., (2010). Isolation, Purification, and Characterization of a Lectin from a Local Kashmiri Variety of Soybean (Glycine max). Asian Journal of Biochemistry, 5: 145-153.

[4] Bohlool, B. B. and Schmidt, E. L., (1974). Lectins: a possible basis for specificity in the Rhizobium—legume root nodule symbiosis, ‖ Science, vol. 185: 269–271.

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[5] Brooks-Kaiser JC, Bourque LA, Hoskin DW. (1993). Heterogeneity of splenic natural suppressor cells induced in mice by treatment with cyclophosphamide. Immunopharmacology, 25: 117−129.
[6] Damjanov, I. (1987). Biology of disease: lectin cytochemistry and histochemistry. Lab Inv 57: 5-20.
[7] De Mejia EG, Bradford T, Hasler C. (2003). The anticarcinogenic potential of soybean lectin and lunasin.Nutr Rev, 61: 239−246.
[8] Goldstein, I.J., and Hayes, C.E. (1978). The lectins: Carbohydrate-binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. 35: 127-340.
[9] Grant G., Oliviera J.T.A, Dorward P.M., Annand M.G., Waldorn M., Pusztai A. (1987). Metabolic and hormonal changes in rats resulting from the consumption of kidney beans or soybeans. Nutrition Reports International, 36:763-772.
[10] Grant, G., Bardocz, S., Brown, D. S., Watt, W, B., Stewart, J. C. and Pusztai, A.(1990). Greer F and Pusztai A (1985). Toxicity of kidney bean (Phaseolus vulgaris) in rats: changes in intestinal permeability. Digestion32:42–46.
[11] Hoskin DW, Bowser DA, Brooks-Kaiser JC. (1992). Soybean agglutinin positive natural suppressor cells in mouse bone marrow inhibit interleukin 2 production and utilization in mixed lymphocyte reactions. J LeukocBiol, 51: 649−656.
[12] Julieta Pérez-Giménez, Elías J. Mongiardini, M. Julia Althabegoiti, Julieta Covelli, J. Ignacio Quelas, Silvina L. López-García, and Aníbal R. Lodeiro, (2009). Kennedy AR (1995). The evidence for soybean products as cancer preventive agents. J. Nutr. 125: 733S-743S.
[13] Kilpatrick, D.C Kilpatrick (1991). Lectin interactions with human leukocytes: mitogenicity, cell separation, clinical applications, in D.C Kilpatrick, E Van Driessche, T.C Bog-Hansen (Eds.), Sigma Chemical Company, St Louis, USA.Lectin Reviews, vol. 1: 69–80.
[14] Kim, Y. S., Brophy, E. J. & Nicholson, J. A. (1976). Rat intestinal brush border membrane peptidase. H. Enzymatic properties, immunochemistry, and interactions with lectins of two different forms of the enzyme. J. Biol. Chem. 251: 3206-3212. Kocourek, J. & Horejsi, V. (1981). Nature (London), 290:188.

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