



# Isolation, characterization and production of a new recombinant lectin protein from leguminous plants

Ayyub Patel

Correspondence: [astricword@gmail.com](mailto:astricword@gmail.com)



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Department of Clinical Biochemistry, College of Medicine, King Khalid University, KSA, Saudi Arabia.

## Abstract

Lectins are present in microorganisms, plants and animals and have attracted great interest due to their varied physiological roles in cell agglutination, anti-tumour, immunomodulatory, antifungal and antiviral effects. Legume lectins are important to the pharmaceuticals but they are produced in low amounts in the plant seeds. Moreover, the genes controlling these proteins are conditionally active, i.e., they work under specific circumstances and not in regular manner. Looking into these limitations, we aimed this work to produce a recombinant lectin for pharmaceutical use especially for the treatment of cancer. Three different legume plant species were collected from Aseer region of Kingdom of Saudi Arabia viz., *Acacia seyal*, *Pisum sativum* (wild type) and *Pisum sativum* (Pea). The plant tissues were subjected to RNA extraction, the extracted RNA was used for lectin gene amplification using specific primers. Cloning, subcloning of the *Acacia* 400bp gene was carried out and *in vitro* transcription, combined with protein purification was undertaken. The cytotoxicity of the recombinant lectin was performed on two cell lines such as breast cancer MCF-7 and liver cancer HepG-2 cells. Our study resulted in the observation of two amplicones with all the three examined species, the amplicone molecular sizes were 800 and 400bp. The 400bp amplicone was excised from the agarose gel, purified and sequenced. The sequence analysis revealed that the nucleotide sequence belongs to lectin gene. The sequence analysis revealed that the lectin gene isolated from *Pisum sativum* (wild plant) is similar to the *Pisum sativum* lect1 with identity 90%, whereas, lectin isolated from *Pisum* (pea) showed similarity of 91% with the other lectins. On the other hand, *Acacia* lectin showed similarity with *Lotus japonicus* nod factor binding lectin gene with identity of 95%. Thus we conclude that new lectin protein of 17 and 15 kDa was produced that can be used by pharmaceutical industries.

**Keywords:** Legumes, acacia seyal, *Pisum sativum*, lectin, protein purification, cloning

## Introduction

*Acacia* is an important plant genus that is commonly used in a variety of infections. It is widely distributed in Asia, Australia and America and efficacy has been demonstrated in the treatment of gonorrhoea, leucorrhoea, diarrhea, dysentery and wounds [1]. Lectins are a well-known family of cationic antibacterial peptides (AMPs) isolated from fungi, plants, insects, mussels, birds and various mammals [25]. Lectins with different carbohydrate specificity have been isolated from forty nine different species, primarily from seeds. Lectins are the most abundant proteins (20-25% of total soluble proteins) in the park of several legume trees such as *Robinia pseudosacacia* and *Maackia amurensis*. Plant lectins have great potential as tools in the identification, purification, and stimulation of specific glycoconjugates. They also have been widely used to distinguish between cell types [5,10]. Lectins are also found in microorganisms, plants and animals and have attracted great interest due to their varied physiological roles in cell agglutination [13], anti-tumour [15,16], immunomodulatory [22], antifungal [12] and antiviral effects [29]. Furthermore, lectin-mediated drugs have been acquired to target specific cells and some lectins with anti-proliferative

properties were isolated and characterized from different parts of the plant, like; seeds [14], leaves [19] and roots [27,31].

It is reported that the total proteins from *pinellia pedatisecta* obviously inhibited ovarian cancer cell lines but showed no toxicity to human umbilical cord blood hematopoietic progenitor's *in vitro* [8,27,32]. It is also reported that the 30%  $(\text{NH}_4)_2\text{SO}_4$  deposition part of total proteins from *Pinellia ternata* rhizome could significantly inhibit human hepatocellular carcinoma cell line Bel-7402 growth and induce its apoptosis [8,24]. Succeed to isolate lectin like protein from *Acacia farnesiana* and they found that the isolated lectins protein sequence showed that AFAL has 68% and 63% sequence similarity with lectins of *Phaseolus vulgaris* and *Dolichos biflorus*, respectively.

Lectins with different carbohydrate specificity have been isolated from forty nine different species, primarily from seeds. Lectins are the most abundant proteins (20-25% of total soluble proteins) in the bark of several legume trees such as *Robinia pseudosacacia* and *Maackia amurensis*. Plant lectins have great potential as tools in the identification, purification, and stimulation of specific glycoconjugates. They also have been widely used to distinguish between cell types.

Recent and modern studies on these plants proved the occurrence of active principles in their different organs. Their pharmacological activity has been investigated. In view of their importance as a source of extracts and active constituents used in medicine, they were embodied in different pharmacopoeias. The collection of these plants from their natural habitats threatens them to a great measure. No means have been taken up till now to conserve these wild plants. In view of the rising demands for these plants and the limited amount of the wild supply, it is indispensable to undertake measures for their conservation for the sake of treatment of various diseases. A great deal of work has been conducted over the last half century to screen plant resources for unknown lectins and to characterize their carbohydrate specificities. Furthermore, as a result of recent developments in structural biology, much has been learned about the molecular basis of carbohydrate recognition by lectins. The general principles derived from studying the early structures have now been confirmed many times. It appears that legume lectins consist of a conserved scaffold composed of a basic set of essential and conserved residues, among which occur a limited number of variable residues that direct the specificity of the lectin.

Many reports have been published ascribing pharmacological roles/clinical effects to plant derived lectins. *Vescaum album* lectin had an inhibitory effect to tumor cells and reduced the number of tumor cells in lungs of mice injected with B16F10 cells; wheat germ lectins had potential as an adjuvant in the therapy of malignant neoplasia and other diseases caused by or following immune deficiency; mushroom lectins inhibited growth of sarcoma cells in the peritoneal cavity and exhibited clearly prolonged life-spans; mistletoe lectins were found to have anti cancer and immunomodulating effect in HIV- positive patients. Legume lectins are important to the pharmaceutical industry and in the treatment of many human ailments. Lowering of cost of production of

lectins and increasing their activity and clinical effectiveness would be very valuable to the pharmaceutical industry and, potentially, in cancer and HCV treatments. Novel lectins found in medicinal plants could hold great promise in that respect. Additionally, modern methods of biotechnology, genetic engineering could hold the key to improving the potency of these proteins. Work on cloning novel lectins from medicinal plants in arid regions and transfer cloned gene to *E. coli* to produce these lectins without altering their tertiary structure is also lacking. Production systems utilizing cell-suspension cultures could greatly facilitate cost-effective production of this bioactive protein for pharmaceutical interests. Finally, testing the effectiveness of recombinant protein (Lectin) in cancer treatment and inhibition of viral growth is a very promising aspect in its medicinal and Pharmaceutical applications.

The present work is an attempt to give a multidisciplinary study on these plants covering their taxonomy, ecology, phytochemistry, pharmacology...etc. and finally produced a recombinant organism able to produce high amounts of lectin compound in active form.

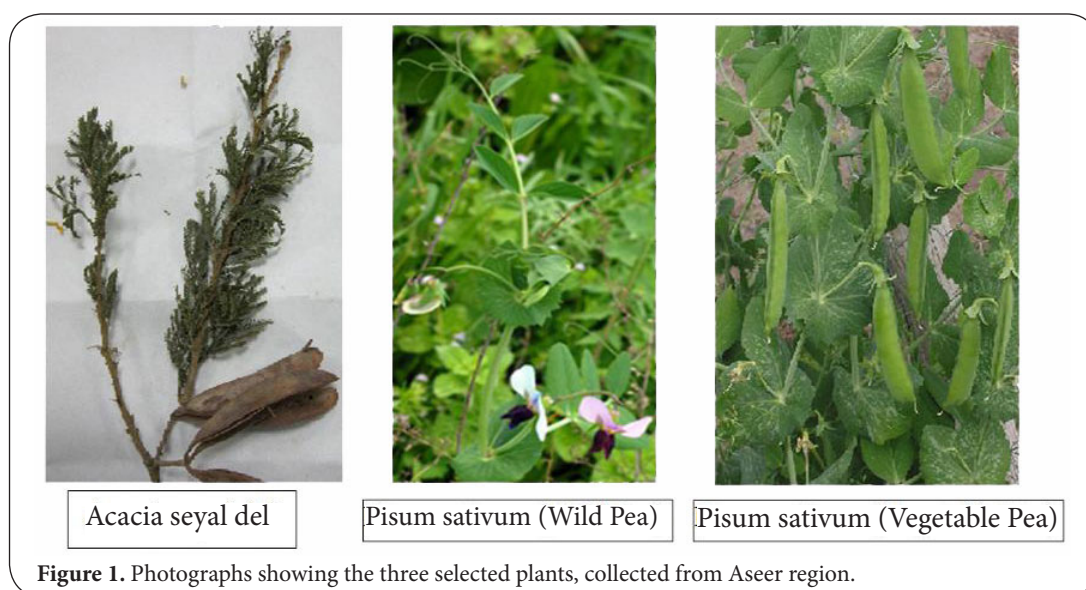
## Materials and methods

### Plant collection

Different legume plant tissues were collected from different areas in Aseer region of Saudi Arabia, but three different species were selected; *Acacia* (2 species), *Pisum* (Wild pea) and pea as cultivated plants. The collected young leaves and buds were washed with tap water, dried and then kept in aluminum foils and stored at -80°C until use. The *Acacia* tissues were used as a genetic pool, the tissues were ground as one sample (Figure 1).

### RNA isolation protocol

Total RNA was extracted from the plant tissues using Qiagene RNA extraction kit (Qiagene comp, Germany). There are two



species of *Acacia* found in Aseer, so genetic pool from these two species was made. 0.1 gm of the frozen tissues was minced in liquid nitrogen using mortar and pestle in the presence of buffer (100 mM Tris-HCl, 0.2% lysozyme and 0.1% glucose). The extraction process was performed according to the manufacturer procedure and the extracted RNA was re-suspended in 100  $\mu$ L of Diethyl pyrocarbonate (DEPC) water. In case of acacia the above mentioned methods did not give a high yield of RNA due to the high polysaccharides content in acacia tissues. So, we used CETAB (Cetyltrimethylammonium bromide) method to diminish the polysaccharide from the extract and then the extract was completed using Qiagene for RNA extraction kit (Qiagene, Germany).

**Reverse transcription of RNA:** Reverse transcription reactions were performed using oligo (dT) primer. Each 25  $\mu$ L reaction mixture containing 2.5  $\mu$ L (5x buffer with  $MgCl_2$ ), 2.5  $\mu$ L (2.5 mM) dNTPs, 1  $\mu$ L (10 pmol) primer, 2.5  $\mu$ L of plant extracted RNA and 0.2  $\mu$ L reverse transcriptase (MLVR, Fermentaz Company). PCR amplification was performed in a thermal cycler (Eppendorf) programmed at 95°C for 5 min, 42°C for 1 hr, 72°C for 10 min (Enzyme inactivation) and stored at 4°C [7].

#### **Lectin DNA amplification using specific PCR**

The synthesized cDNA of each plant was subjected to PCR amplification using the specific primers of lectin gene according to [17]. PCR amplification was carried out for each plant in separate manner in total volume of 25  $\mu$ L. The reaction conditions are showed as follows: 2  $\mu$ L of (100 ng cDNA), 0.2  $\mu$ L of 10  $\mu$ Mol each primer (forward 5'-TCAACGAAAACGAGTCTGGTG-3' and reverse 5'-GGTGGAGGCATCATAGGTAAT-3'), 2.5  $\mu$ L 10x PCR Buffer, 2.5  $\mu$ L of (2.5 mM) dNTPs, 0.3  $\mu$ L of 1U Taq DNA polymerase (Qiagene, Germany). The program was initiated for 5 min of denaturation, followed by 10 cycles of amplification with denaturation for 15 sec at 94°C, first annealing for 20 sec at 55°C and an extension at 72°C for 15 sec and followed by 40 cycles of amplification with denaturation for 1.5 min, second annealing for 1.5 min at 53°C, an extension at 72°C for 1 min and a final extension at 72°C for 5 min on Eppendorf thermal cycler.

#### **DNA sequencing and accession number**

The PCR product (400bp) was excised from the agarose gel using agarose DNA extraction kit, (Qiagene, Germany) and then the purified DNA was subjected to DNA sequencing (MACROGEN, Korea) using the designed forward primer in the sequence reaction. Sequence analysis using CLUSTAL W program were performed. The nucleotide sequences were analyzed with the BLAST database (www.ncbi.nlm.nih.gov) and the DNA sequence was submitted into the GenBank under accession numbers (JQ964105, JQ964106 and JQ964107).

#### **Cloning and subcloning lectin gene**

Cloning of amplified PCR products was done by T/A based cloning protocol by using TOPO TA Cloning<sup>®</sup> (with pCR<sup>®</sup> 2.1-TOPO<sup>®</sup> Cloning vector) and (a TOP 10 *E-coli* strain) (Invitrogen<sup>™</sup>,

USA). The recombinant bacteria were examined using Blue/White colony analysis. The well characterized clone were subjected to digestion using BamH1 restriction enzyme to release the gene from the pCR<sup>®</sup> 2.1-TOPO<sup>®</sup> vector, meanwhile the released fragment was purified by EzWay<sup>™</sup> Gel Extraction kit (Kombiotech comp, Korea) and ligated to the linearized prokaryotic expression pPROEX HT (life technologies, USA). The recombinant clones were examined by PCR for the extracted DNA plasmid and the recombinant clone was selected. The selected recombinant clones were grown on the LB medium containing ampicillin as antibiotic (100 $\mu$ g/ml). For gene induction IPTG was added to the bacterial culture after two hours from inoculation time. The culture was grown in incubator shaker over night at 37°C with shaking at 200 rpm and the cloning was done according to the protocols outlined by Life Technologies (Invitrogen Company, USA). For that reason the study aimed to design another primers to amplify the lectin full length gene and use it in this case with the 5 and 3 race RNA synthesis kit (SMARTer<sup>™</sup> RACE cDNA Amplification Kit, Clonetech Company, USA).

#### **Examination of gene function**

##### ***Lectin purification using 6x histidine affinity-tagged method***

Lectin purification was carried out by Ni-NTA resin matrix (QIAGEN Inc., USA). The induced bacterial cells was pelleted and re-suspended in 4 volumes of lysis buffer (50 mM Tris-HCl (pH 8.5 at 4°C), 5 mM 2-mercaptoethanol, 1 mM PMSF). The suspension was sonicated until 80% of the cells were lysed. The cell debris was removed by centrifugation; the supernatant was removed to a new tube (crude supernatant). Affinity purification was done according to the protocols outlined by Life Technologies, Invitrogen.

##### ***Solubilization and renaturation of lectin protein***

The inclusion pellets were solubilized in 8M urea buffer (pH 8) as per the procedures developed by [18]. The urea mixture was incubated at 25°C for 1 h before the insoluble molecules were removed by centrifugation. The urea solution was then diluted in a high pH buffer (pH 10.7) for renaturation of lectin. After solubilization in 8 M urea, the inclusion body solution was diluted with phosphate buffer pH 10.7, the solution was incubated at 25°C for 1 h, then pH adjusted to 8 and incubation was continued at 25°C for 1 h. The solution was transferred to dialyze against buffer (20 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM EDTA) at 4°C overnight.

##### ***Hybridization using the 400bp as a probe***

The small DNA fragment (400bp) was used as a probe to detect the lectin gene in the 800bp amplicones. The DIG System Nonradioactive and Highly Sensitive Detection of Nucleic Acids (Roche Applied Science, Germany) were used according to the manufacture procedures. The large fragment was purified from the agarose gel using agarose DNA extraction

kit (QIAGEN, Germany) and the purified DNA was subjected to PCR amplification using the same conditions above. The PCR product was cloned using T/A based cloning protocol by using TOPO TA Cloning<sup>®</sup> (with pCR<sup>®</sup> 2.1-TOPO<sup>®</sup> Cloning vector) and (a TOP 10 *E. coli* strain) (Invitrogen<sup>™</sup>, USA). The recombinant plasmid was screened using white/blue method and the plasmid DNA examined with the same primers (lectin primers).

### SDS-PAGE for the recombinant lectin

The purified lectin was separated on 12% polyacrylamide gel according to [23]. 10 µl of the purified protein was loaded and the gel was left for running at 80 Volt for two hours. Staining and destaining was performed and the gel was photographed.

## Results

### Hybridization using the 400bp as a probe

The results revealed that amplicons with 800bp were obtained as shown in (Figure 2). The gel indicated as (Figure 2) was transferred into Nylon membrane and then exposed to pre-hybridization solution, hybridization solution which contains the 400bp as probe and then the membrane was exposed to color reaction. The results showed that, negative reaction was obtained. Based on the obtained results, we can conclude that the large fragment was not lectin gene but may be another gene.

The results presented in Figure 3 revealed that two amplicons with different molecular sizes were amplified. The first amplicone was in molecular weight 400bp but the other one

was in molecular weight of about 800bp. Different annealing temperatures were examined to diminish the 400bp from the PCR reaction but nothing happened. For that reason we expected that the 400bp amplicone may be another copy from lectin gene.

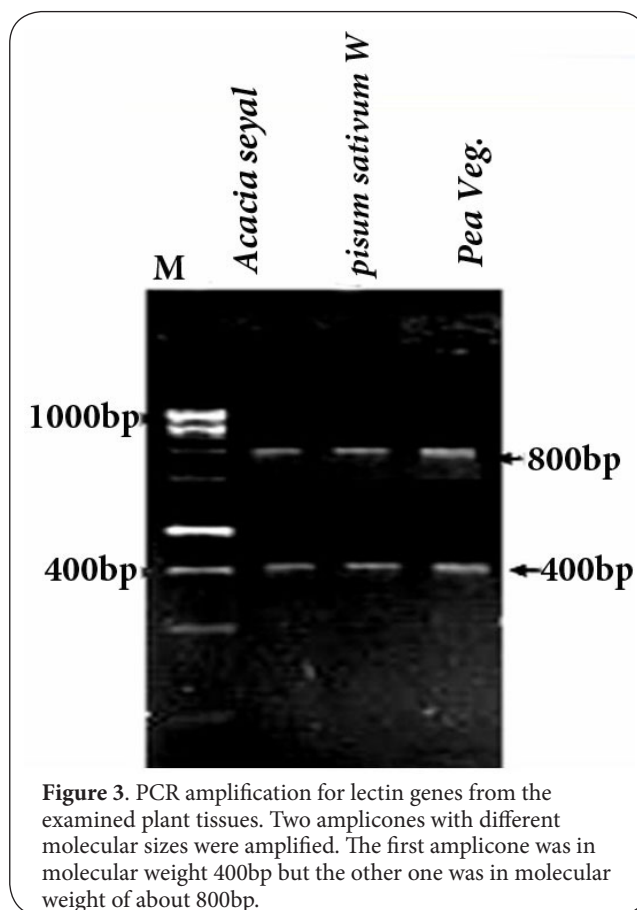


Figure 3. PCR amplification for lectin genes from the examined plant tissues. Two amplicons with different molecular sizes were amplified. The first amplicone was in molecular weight 400bp but the other one was in molecular weight of about 800bp.

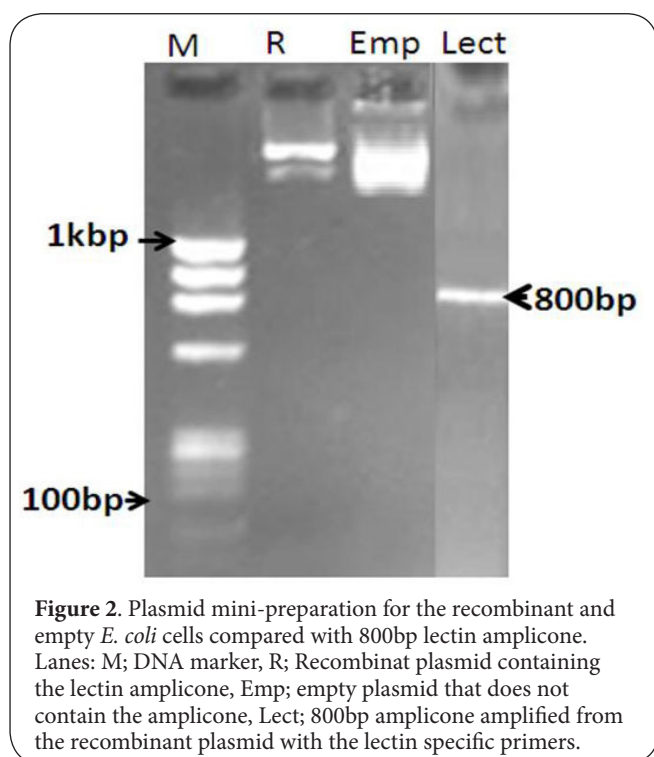


Figure 2. Plasmid mini-preparation for the recombinant and empty *E. coli* cells compared with 800bp lectin amplicone. Lanes: M; DNA marker, R; Recombinant plasmid containing the lectin amplicone, Emp; empty plasmid that does not contain the amplicone, Lect; 800bp amplicone amplified from the recombinant plasmid with the lectin specific primers.

### Sequence and sequence analysis

The sequence analysis using BLASTn revealed that the lectin gene isolated from *Pisum sativum* (wild plant) was similar to the *Pisum sativum* lect1 with an identity of 90%. However, the lectin isolated from *Pisum* (cultivated) showed a similarity of 91%. On the other hand, *Acacia* lectin showed similarity with *Lotus japonicus* nod factor binding lectin gene with identity of 95%. It is well known that, if the identity between two genes is not more than 97%, then the examined gene is a new gene. It could be concluded that the three obtained genes are new genes (Figure 4).

### Sequence alignment between the three obtained lectin genes

Results presented in (Figure 5) revealed that the similarity between the lectin gene of the *Pisum* wild plant and the cultivated *Pisum* was 95%. The similarity between the wild *Pisum* and *Acacia* was 50% and it was 52% with the cultivated

**Lectin sequence *Pisum sativum* wild type (Lectin PW)**

AAACCTAATCTTGCAGGAGCTGGCTACACGACAAAAGAGAAGCTGACACTGACCAAGG  
 CTGTAAGAACACTGTTGGCCGAGCTCTCTATTCTTACCAATCCCATATCTGGGATAG  
 CGAAACAGGCAACGTTGCTGATTTTACAACACCTTCATTTTGTGCATAGATGCACCCA  
 ACGGCTACAACGTTGCCGACGGGTTTACGTTCTCATCGCACCTGTAGATTCTAAAGCC  
 GCAAACCGCGGTTGGATATCTCGGAGTTTCAATGGCAAAGATTATCCCCGACAAAAC  
 GCTCAAACGTTGCTGTTGAGTTTGACACTTCTATAACGCTGCTGGGATCCAAGCAA  
 CGGAAAGAGACATATTGGAATCGGTGTGA

**Lectin *Pisum sativum* vegetable (Lectin PV)**

CCTTAACCTTAAGGCCAAGGAGTGGCTACACCACAAAAGAGAAGCTGACACTGACCAA  
 GGCTGTAAGAACACTGTTGGCCGAGCTCTCTATTCTTACCTATCCATATCTGGGATA  
 GCGAAACAGGCAACGTTGCTGATTTTACAACACCCCTTCATTTTGTCAAATAGATG  
 CACCAACGGCTACAACGTTGCCGACGGGTTTACGTTCTCATCGCACCTGTAGATACT  
 AAGCCGCAAAACCGCGGTTGGATATCTCGGAGTTTCAATGGCAAAGATTATGACAAAAC  
 TGCTCAAACGTTGCTGTTGAGTTTGACACTTCTATAACGCTGCTGGGATCCAAGCA  
 ACGGAAAGAGACATATTGGAATCGGTGTGA

**Lectin of *Acacia seyal* (Lectin AC)**

CCATGAACGATACGCTGCTAATCCTGAAGAAGCTGCAGAACTCTCTGATCCACTTCTAA  
 AAGAAGCAGAAAATGTGGTTCCTGTGACCCAGCAACCCACACACCCGTTAAGCTTGGG  
 GCAACTGCAGGTTAAGGCTTTTGGAGGGGAATGCTGCTGAAAATATATTGCAACGGGT  
 CGGGGATATGCTTTCAGCAACAGAAAAGTGCCCTTGATGCAGTATCTATTCTTGATGG  
 AACCAAGAAGGTTCTTATCTTTGGGTGACAATTAACATCCCTCTTGGGGAAGTTGG  
 GAAAAAGATTTACAAGACAGTGGGAGTAGTTGATCTAGGAGTGGGTGAGTCAAAATG  
 ACATATGCAGTCTCAAGGAACACAGCTAAAAATGCTCCTAAG

**Figure 4.** The DNA nucleotide sequence for the three amplified lectin genes from the three examined plants.

ones. On the other hand data presented in (Figures 5 and 6) revealed that when the alignment was performed based on the deduced amino acids, the same identity was obtained between the two *Pisum* (Wild & cultivated) plants but the similarity between the two *Pisum* and *Acacia* was decreased into 37 and 39% in successive manner.

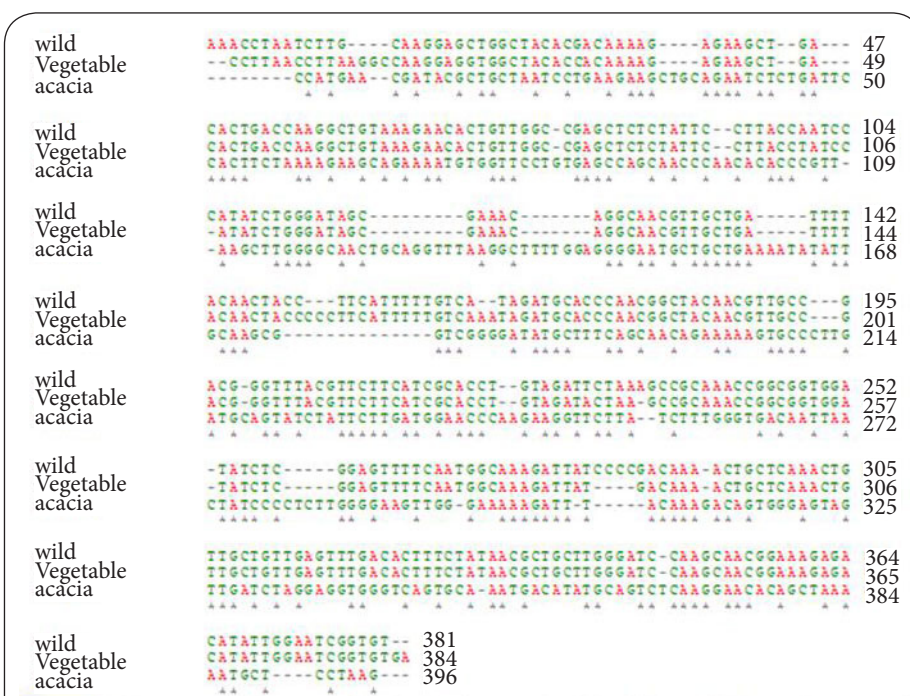
The phylogenetic tree for the three obtained lectin genes (Figures 6 and 7) revealed that, the three examined lectins were divided into two different groups but they have the same ancestor. Group 1 contains the two *Pisum* lectins but group two contains only the *Acacia* lectin. It could be concluded that the *Pisum* lectin was completely different from *Acacia* lectin. Moreover, the obtained DNA sequence is completely new. When the three obtained lectins were compared with the other lectins presented on the data base of the Gene Bank (Figure 7), it was observed that the two lectins of *Pisum* were closely related to other *Pisum* and *Vicia faba* lectins. However, the *Acacia* lectin was a new outer group for all the other lectins. For this reason this gene was chosen to be cloned and overexpressed.

**Recombinant lectin purification from the transformed *E.coli***

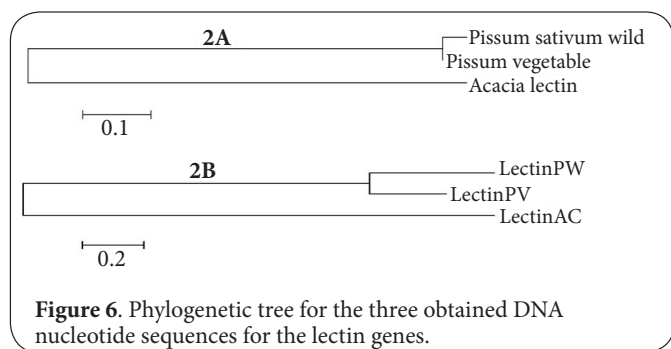
The purified lectin was separated on 12% polyacrylamide gel and the data presented in (Figure 8) revealed that, two proteins with molecular sizes of 17 and 15kDa were observed.

**Discussion**

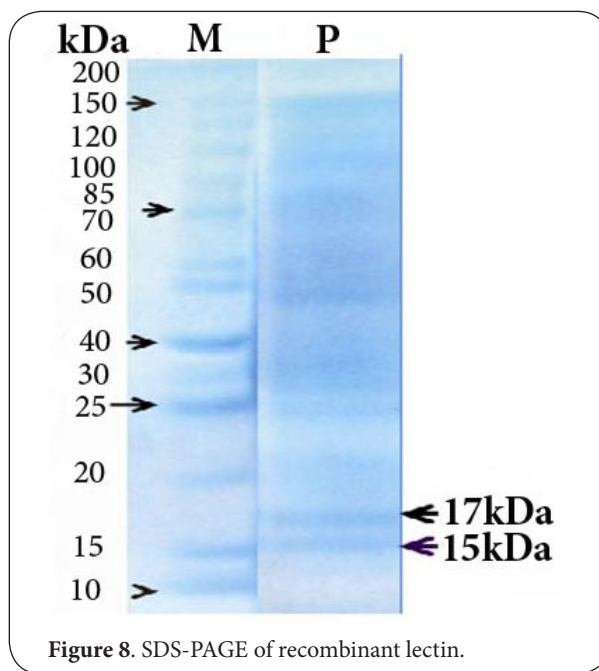
Carbohydrate-binding proteins which agglutinate erythro-



**Figure 5.** Sequence alignment for the lectin gene isolated from different legume wild plants. The sequence alignment based on the deduced amino acids using clustal W 1.4.



cytes and precipitate glycoconjugates are very common in plant tissues. These phytohaemagglutinins or lectins may be simple proteins or glycoproteins and they exhibit considerable binding specificity towards carbohydrates. The nature and properties of plant lectins have been reviewed by [4]. Leguminous seeds are a rich source of lectins, many of which possess homologous segments of amino acids and, hence, may have an evolutionary relationship [11]. Few examples of enzymes possessing lectin activity have been



reported but cumannosidase from *Phaseolus vulgaris* seeds [21] and  $\alpha$ -galactosidases obtained from several species of leguminous seeds [6] have been shown to be lectins. Lectin is very important for human health, so the studies aimed to purify and intensify the efficiency of new lectin specially derived from legumes. It is well known that several C-type lectins are present in serum in a soluble form (collectins, galectins, ficolins), where they function as agglutinins and opsonins, promoting phagocytosis by binding to microbial surface carbohydrates. Moreover, Membrane-bound C-type lectins are designed to capture pathogens for intracellular destruction, degradation and antigen loading of major histocompatibility complex molecules, whereas soluble lectins (such as the collectins) function by ligating and opsonizing microorganisms, or might have a role in antigen transport.

Lectins are generally considered to be non-enzymatic and non-immune proteins which selectively bind to a specific carbohydrate structure. The animal lectins are broadly classified into two major categories, the C type ( $\text{Ca}^{2+}$ -dependent) animal lectins which are structurally related to the asialoglycoprotein receptor while the S type (thiol-dependent) animal lectins form a distinct group which are generally  $\beta$ -galactoside binding. Embryonic and differentiating tissues of various vertebrates from teleosts, to amphibians, birds and mammals, contain a  $\beta$ -D-galactoside binding lectin that agglutinates trypsinized rabbit erythrocytes and is specifically inhibited by  $\beta$ -o-galactopyranosyl  $\beta$ -D-thiogalactopyranoside and lactose. The major  $\beta$ -galactoside binding lectin occurring in vertebrate tissues is a dimer with a subunit mass of about 14 kDa and requires thiol but not divalent metal ions for its haemagglutination activity, and exhibits considerable sequence homology among various species [20]. As in (Figure 8) the mass of lectin protein isolated are 15 and 17 kDa. Besides the 14 kDa lectin, other  $\beta$ -galactoside binding lectins have been found in mammalian tissues which include sheep, goat and buffalo liver having molecular weights of 18, 22 and 24 kDa, respectively [2]. Of these three  $\beta$ -galactoside binding hepatic lectins, only sheep lectin required 2-mercaptoethanol for its haemagglutination activity; in addition it requires  $\text{Ca}^{2+}$ . In contrast to major soluble  $\beta$ -galactoside specific lectins, goat and buffalo soluble hepatic lectins show haemagglutination activity against trypsinized rabbit red blood cells only in the presence of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  or  $\text{Sr}^{2+}$  [2] thus differing significantly in cofactor requirement from the known major mammalian  $\beta$ -galactoside specific lectins. We thus find it important to confirm the presence of a metal binding site of these lectins.

A new recombinant lectin, in a soluble form within *E. coli* cells has been recovered and purified as in (Figure 8) In contrast, the recombinant lectin from pea formed insoluble aggregates [26]. The sequence analysis using BLASTn revealed that the lectin gene isolated from *Pisum sativum* (wild plant) is similar to the *Pisum sativum* lect1 with identity 90%. While, the lectin isolated from *Pisum* (cultivated) showed similarity of 91%. On the other

hand, *Acacia* lectin showed similarity with *Lotus japonicus* nod factor binding lectin gene with identity of 95%. Results presented in (Figure 6) revealed that the similarity between the lectin gene of the *Pisum* wild plant and the cultivated *Pisum* was 95%. While, the similarity between the wild *Pisum* and *Acacia* was 50% but it was 52% with the cultivated ones. On the other hand data presented in (Figures 6 and 7) revealed that when the alignment was performed based on the deduced amino acids, the same identity was obtained between the two *Pisum* (Wild & cultivated) plants but the similarity between the two *Pisum* and *Acacia* was decreased into 37 and 39% in successive manner. Furthermore, the phylogenetic tree for the three obtained lectin genes (Figures 6 and 7) revealed that, the three examined lectins were divided into two different groups but they have the same ancestor (0.1 clade). Group 1 contains the two *Pisum* lectins but group two contains only the *Acacia* lectin. It could be concluded that the *Pisum* lectin was completely different from *Acacia* lectin.

Moreover, the obtained DNA sequence is completely new. When the three obtained lectins were compared with the other lectins presented on the data base of the Gene Bank (Figure 7), it was observed that the two lectins of *Pisum* were closely related to other *Pisum* and *Vicia faba* lectins. However, the *Acacia* lectin was a new outer group when compared with all the other lectins. For this reason this gene was chosen to be cloned and overexpressed. The recombinant clones were examined by PCR for the extracted DNA plasmid and the recombinant clone was selected. The Recombinant lectin purification from the transformed *E. coli* showed that the purified lectin is composed of two proteins with molecular sizes 17 and 15kDa as depicted in (Figure 8). The molecular weights are different from the other recorded before. These new recombinant lectin proteins encourage us to study their cytotoxicity as anticancer agent. Other legume lectins also exhibit antiproliferative activity towards cancer cell lines [30]. The cytotoxicity of the recombinant lectin was performed on breast cancer (MCF-7) cells, liver cancer (HepG-2) cells, and layrnx cancer (HEP-2) and colon cancer (HCT-116) cells. The cytotoxicity of the purified lectin was assayed by a cell viability assay, in the presence of different concentrations of the lectin compounds for 24 h (the two fractions). Under these experimental conditions, lectin exhibited a significant cytotoxic effect on all cell lines (Ayyub et al., unpublished work). Mitogenic lectins have a curative potential. They may provide protection and recovery from the immunosuppressive and mycelosuppressive effects of tumors and infections. They may also be used against malignancies [28]. Based on the IC50 for the 4 examined cell lines, both liver (HepG-2) and the Lung cancer (Hep-2) were treated with the IC50 of each and incubated at the previous conditions for 48 hours. Samples were taken after 24 and 48 hours in respective manner. The results revealed that the samples treated with lectin showed to be more effective as chemotherapeutic agents for cancer (Based upon further studies by the author and published elsewhere, [3].

Aseer region, in the southern region of Saudi Arabia, is at an altitude of about 2000 feet from the sea level and has its own confidential flora. With regard to the type I C-type lectins, there is evidence that macrophage mannose receptor is associated with a signal transduction pathway leading to tumour necrosis factor (TNF) and interleukin-12 production [9]. No such findings have yet been reported for the recombinant lectin from *Pisum sativum* (wild plant), *Pisum sativum* lect1 and *Acacia*. Hence, the author of this paper has carried out further studies with these recombinant lectins [3] and the results indicated that the expression of the investigated genes was increased in the cells treated with the Doxorubicin. But the expression was mostly the same in the hepatocarcinoma cells but lower in the breast cancer cells treated with lectin. This means that the expression of the TNF was inhibited/suppressed in the breast cancer cells rather than the HepG-2. The expression of the IL-12 was increased in the treated cells with two drugs and the expression was higher in case of hepatocarcinoma more than the breast cancer cell lines. This means that the immune system was induced when treated with the two examined drugs to resist the cancer effect. In case of the Bcl-2, the expression of this gene was increased with cells treated with doxorubicin compared with the non-treated ones. On the other hand this expression was lower in the cells treated with lectin. This means that presence of lectin makes more suppression for the Bcl-2 when compared with the doxorubicin. These results assure that the lectin is more effective than the Doxorubicin in the treatment of cancer.

## Conclusion

Lectins isolated from *Acacia* and *Pisum* had variance in their identity from the other identified ones in genbank. This study introduces new recombinant lectins (15 and 17kDa) in that can be used in treatment of cancer, as per our further studies using these recombinant lectins. The lectin is more effective than a widely used anticancer doxorubicin treating different kinds of cancer.

## Competing interests

The author declares that he has no competing interests.

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