Antimicrobial Activities of Lectins Extracted from Some Cultivars of *Phaseolus vulgaris* Seeds

Einas Hamed El-S¹, Magda Mahmoud Ibrahim El-A² and Mervat Mounir S*¹

¹Department of Microbiology, Ain-Shams University, Egypt
²Department of Botany, Ain-Shams University, Egypt

Abstract

Lectins of five cultivars of *Phaseolus vulgaris* seeds were isolated using ammonium sulfate precipitation followed by dialysis and their molecular characterization were determined using SDS-PAGE. The suggested regions of all isolated lectins ranged between 31 to 34 kDa. The isolated lectins demonstrated remarkable hemagglutination activity to all human blood groups (A, B, AB and O). The antimicrobial activity was studied using agar-well diffusion method and minimum inhibitory concentration (MIC) was determined. The results showed that the lectins of all tested *Phaseolus vulgaris* seeds had a potent antibacterial activity against all bacterial strains studied (*Staphylococcus aureus* ATCC 6538, and *Streptococcus mutants* ATCC 25175, *Pseudomonas aeruginosa* ATCC 10145 and *Klebsiella pneumonia*) with the exception of *Escherichia coli* 0157; H7 ATCC 51659 that was not affected. All tested lectins showed antifungal activity against *Candida albicans*. As far as we are aware, our work is the first approach that showed photographs of scanning electron microscope (SEM) before and after treatment of 90% saturated fraction of the lectins extracted from the Egyptian Shalatine cv. seeds against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, where a potential agglutination of bacterial cells was shown.

Keywords: Lectins; *Phaseolus vulgaris* seeds; Antimicrobial agents

Introduction

Lectins are natural substances that are ubiquitously present in nature (in animals, plants and micro-organisms). Plant lectins are classified into seven families based on their evolutionary and structural characteristics, one of them is a legume lectin family [1]. The most known lectins are found in legume seeds that compromising about 15% of the total proteins [2].

Lectins are proteins or glycoproteins that have at least one binding site without catalytic function or immunological characteristics. They bind reversibly to specific carbohydrates without any chemical modification [3]. Lectins differ from enzymes because they never change bound-carbohydrate properties. They are also unlike antibodies because they are not produced by immune system reference. In addition to specific binding to carbohydrates, lectins can cause cell agglutination and glycoprotein or carbohydrate precipitation. That is why the lectins are sometimes called "agglutinin" [3].

Lectins exhibit many interesting biological properties, such as specificity for human blood types, preferential agglutination of malignant cells and mitogenic stimulation of lymphocytes [4]. Lectins also showed biotechnological efficiency in diagnosis and therapeutic applications [5]. Among other functions, they are responsible for innate immunity and defense mechanisms in plants.

The lectin fractions from red kidney beans are known as erythroagglutinin (PHA-E) and leucoagglutinin (PHA-L). They are characterized by their amino acid sequences and their differential affinities for erythrocytes and leukocytes [6].

The binding capacity of lectins could be the cause of its biological activities against bacteria, fungi, viruses and tumor [7-10]. The interaction of lectin with teichoic and teichuronic acids, peptidoglycans and lipopolysaccharides present in bacterial cell walls are responsible for its antibacterial activity [11].

The continuous emergency of infections and the microorganisms developing resistance make the existing antibiotics less effective. Nowadays the natural products which are part of our daily diet serve as the best candidates for discovering new antibacterial drugs [12]. In this connection, lectins became one of the most focused interests for biologists in their research and applications in agriculture and medicine [13].

In this work, we separated lectins from five cultivars of *Phaseolus vulgaris* seeds. The study was devoted to detecting molecular weight, hemagglutination capacity and biological activity against some bacteria and fungi.

Materials and Methods

Plant materials

Seeds of five cultivars of *Phaseolus vulgaris* namely Bronco (White bean), Contender (Brown bean), 10YLH49 (Black turtle bean), Diacole (Red speckled kidney bean) and Shalatine (Pinto bean) were obtained from the Vegetable Breeding Research Department, Horticultural Research Institute, Agriculture Research Center, Egypt.

Protein marker

BLUeye prestained protein ladder (category number PM007-050 500 µl) with 12 prestained proteins covering a wide range molecular weight from 10 to 245 kDa were purchased from GeneDirex and used to monitor protein separation during SDS-PAGE technique.

*Corresponding author: Mervat Mounir S, Department of Microbiology, Ain-Shams University, Egypt. Tel: 00201141175244; 0020226673561; E-mail: mervatmounir84@yahoo.com

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Lectin standard

Lectin from red kidney bean (PHA-P) was purchased from Sigma-Aldrich (product number L-8754) and used as standard. PHA-P is a mixture of PHA-E (MW=128 kDa) and PHA-L (MW=126 kDa).

Dialysis tubing membrane

Dialysis tubing membrane was purchased from Spectra/Por. Its cut-off was 12-14 kDa, length 2 ml/cm and width 16 mm.

Blood group samples

Human blood group types A, B, AB and O was collected from healthy donors at Toplab medical laboratory, Cairo, Egypt.

Tested microorganisms

Tested bacteria: The Gram-negative bacteria Escherichia coli (ATCC 25922), Klebsiella pneumonia and Pseudomonas aeruginosa (ATCC 10145) and Gram-positive bacteria Staphylococcus aureus (ATCC 6538) and Streptococcus mutants (ATCC 25175) were obtained from the Department of Microbiology Ain Shams University.

Tested fungi: Candida albicans was purchased from the Regional Center for Mycology and Biotechnology, Al-Azhar University.

Methods

Lectin extraction: Extraction was carried out according to Zhang et al. in 2008 with slight modification. Phaseolus seeds (25 g) were ground to a powder and 250 ml of 50 mM phosphate buffer (pH 7.2) were added [14]. Then, the homogenized powder in buffer was left overnight for complete extraction at 4°C. After filtration, the filtrate was centrifuged at 6000 g for 20-25 min and the supernatant was fractionally precipitated with ammonium sulfate at 30%, 70% and 90% saturation, respectively. The pellets were collected in separate tubes each containing 15 ml phosphate buffers (pH 7.2).

Partial purification of lectins: The crude extracts of all tested seeds were dialysed extensively overnight in phosphate buffer (pH 7.2) at 4°C, using dialysis tubing membrane. The dialysed samples were considered as lectins.

Determination of protein concentration: The protein concentration of lectins was determined according to Bradford [15]. Bovine serum albumin (BSA) was used as standard and the absorbance was measured at 280 nm.

SDS-PAGE

SDS-PAGE was performed using 12% separating gel and 5% stacking gel according to Laemmli [16]. It was used to determine the molecular weight of the lectins. The red kidney bean lectin PHA-P was used as standard for the lectins. Monitoring for the lectins was carried out using BLUeye prestained protein ladder.

Hemagglutination activity test

The applied assay is based on the ability of lectins to agglutinate erythrocytes (so they are called phytohemagglutinin). The assay was carried out in 96 wells plate using two-fold serial dilutions of protein solution (50 μl) in 5 mM saline phosphate buffer (pH 7.2) which was added to 50 μl of 4% (v/v) RBCs, then the mixtures were incubated at 37°C for 1 h. A control containing 50 μl PBS instead of protein solution and 50 μl of 4% cell suspension were used as references [17]. The hemagglutination activity was expressed as the titer representing the reciprocal of the lowest concentration of protein at which visible agglutination could be observed.

Hemagglutination inhibition test

Serial two-fold dilutions of sugars (galactose, glucose, maltose, mannose, sucrose) were prepared in saline phosphate buffer. All sugar dilutions were mixed with an equal volume (50 μl) of the lectin solution showing positive hemagglutination activity. The mixtures were incubated for one hour at room temperature and then mixed with 50 μl of 4% human RBCs suspension. The negative control contained 50 μl protein solution and 50 μl of 4% RBCs. The inhibition of hemagglutination was observed and recorded [17].

Antibacterial and antifungal activity

The activity was measured using the agar-well diffusion method. Three replicates of nutrient agar plates were inoculated with bacterial culture, and three replicates of Sabouraud agar plates were inoculated with fungal culture, and three replicates of Sabouraud agar plates were inoculated with tested fungal growth (10⁵ CFU/ml) for 24 h. Wells of 8 mm were filled with 100 μl dialysed lectin solution (from all tested Phaseolus vulgaris seeds) obtained after ammonium sulfate precipitation (30%, 70% and 90%). 100 μl of 5 mM phosphate buffer solution (pH 7.2) was used as a control. The plates were kept in a refrigerator overnight for diffusion. After that, the plates were incubated for 24 h at 37°C. Average inhibition zone diameters around the wells were calculated [18].

Minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of the tested samples that inhibit the visible growth of bacteria after 24 h and fungi after 48 h. The MIC values were evaluated by the broth dilution test using inocula of 10⁵ CFU/ml for each tested micro-organism (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans) [19]. Serial two-fold dilutions (1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.8, 3.9, 1.95, 0.98, 0.49 and 0.24 μg/ml) of the dialysed lectin 90% ammonium sulfate saturation were prepared to 1 ml of brain heart infusion (BHI) medium. To each tube, 100 μl of the inoculum was added. Ampicillin, gentamycin and amphotericin B were used as standard drugs for Gram positive, Gram negative and antifungal activity, respectively.

Scanning electron microscopy

The samples of Staphylococcus aureus and Pseudomonas aeruginosa before and after treating with 100 μl fraction 90% of the lectin separated from Shalatine cv. Seeds were fixed using 2.5% glutaraldehyde and dehydrated by serial dilutions of ethanol using automatic tissue processor (Leica EMTP). Samples were dried using CO₂ critical point drier (Tousimis Autosamdri-815). The samples were then coated by gold sputter coater (SPI-Module). Finally, the samples were examined by scanning electron microscopy (JEOL-JSM-5500 LV) using high vacuum mode at the Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Statistical Analysis

Statistical analysis was done by one-way ANOVA test [20].

Results

Concentration of the extracted lectins

The crude extracts of lectins from the five cultivars of Phaseolus seeds (Bronco, Contender, 10YLHJ49, Diacole, and Shalatine) under study were fractionally precipitated and dialysed as described in the
methods. The concentrations of lectins were low in some cultivars and high in others (Figure 1). The highest significant value was recorded at 90% fraction with Shalatine cv. seeds followed by 10YLHJ49.

**SDS-PAGE**

SDS-PAGE was used to separate proteins according to their electrophoretic mobility as a function of molecular weight (Figure 2). Figure 2A shows that all the fractionated samples contained proteins at expected molecular weights for *Phaseolus vulgaris* lectins (31-34 kDa). It was clear that the 90% fraction of dialysed samples of all seeds contained highest concentrations of lectin protein. Moreover, Figure 2B shows SDS-PAGE of lectin from red kidney bean (PHA-P) that was used as a lectin standard.

**Hemagglutination activation and hemagglutination inhibition tests**

Lectins (fractions 70%, 90%) of the five *Phaseolus vulgaris* seeds were found to agglutinate all types of human blood groups (A, B, AB, O).
Antimicrobial activity

All the separated lectin fractions are significant and inhibited all tested fungi and bacteria except Escherichia coli. The highest significant inhibition zone was observed with fraction 90% of the lectins of cultivars. Shalatine, Diacole and Contender, against Pseudomonas aeruginosa (37, 34 and 29 mm, inhibiting zones, respectively).

Minimum inhibitory concentration (MIC)

The MIC of fraction 90% of lectins was tested and indicated that the tested microorganisms were inhibited by low concentrations of lectins ranging from 3.9 to 125 µg/ml of seeds of cultivars. Diacole (red kidney bean) followed by those of Contender (brown bean) showed the lowest MIC, compared to other Phaseolus cultivars under study (Table 4).

Scanning electron microscope (SEM) examination

In this work, scanning by electron microscope was used for the first time to examine the Gram-positive bacteria (Figures 3 and 4). Staphylococcus aureus (Figures 3B and 3C) and the Gram-negative bacteria Pseudomonas aeruginosa (Figures 4B and 4C), without and with lectin of Shalatine seeds (fraction 90%). The results proved that lectin solution had the ability to agglutinate and aggregate bacterial cells together which meant that it exhibited antibacterial activity.

Discussion

Phaseolus vulgaris is the most important leguminous seed and food crop in the world according to its importance in nutrition security and income generation [21,22]. The content of total protein in Phaseolus vulgaris seeds was reported to be between 17 to 23% of which 2.5-5% was lectins [8,23]. The extraction purification, characterization and biological applications of new plant lectins have been of concern [24].

In the present work, lectins were separated from the seeds of 5 cultivars of, namely: Bronco, Contender, 10YLHJ49, Diacole and Shalatine. Samples resulted with using different concentrations of ammonium sulfate showed that 90% saturation fraction contained the highest amount of lectin content recovered by the extraction in all the tested seeds (Figure 1). The highest significant value was recorded with Shalatine seeds (9.528 mg/g) followed by 10YLHJ49 (7.187 mg/g). The results proved that lectin solution had the ability to agglutinate and aggregate bacterial cells together which meant that it exhibited antibacterial activity.

Analysis of SDS-PAGE in this study clarified that molecular weights of lectins of the tested Phaseolus vulgaris seeds had molecular weights in the range of 31 to 34 kDa (Figure 2A). Similar results were obtained

Table 1: Hemagglutination activity of lectins extracted from five cultivars of Phaseolus seeds on human blood groups (A, B, AB and O).Highest activity (128) is shadowed.

<table>
<thead>
<tr>
<th>Phaseolus cultivars</th>
<th>Lectin fractions (% saturation)</th>
<th>Human blood groups</th>
<th>Hemagglutination activity (liter)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Bronco</td>
<td>70</td>
<td>32</td>
<td>128</td>
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<td></td>
<td>90</td>
<td>128</td>
<td>128</td>
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<tr>
<td>Contender</td>
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<td>32</td>
<td>32</td>
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<td></td>
<td>90</td>
<td>32</td>
<td>32</td>
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<tr>
<td>10YLHJ49</td>
<td>70</td>
<td>64</td>
<td>64</td>
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<td></td>
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<td>64</td>
<td>64</td>
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<td>Diacole</td>
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<td>16</td>
<td>16</td>
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<td>90</td>
<td>32</td>
<td>32</td>
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<tr>
<td>Shalatine</td>
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<td>32</td>
<td>32</td>
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<td></td>
<td>90</td>
<td>32</td>
<td>128</td>
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</tbody>
</table>

Titer=the reciprocal of the heights dilution of protein solution showing visible hemagglutination

Table 2: Hemagglutination inhibition of lectins extracted from five cultivars of Phaseolus seed using different sugars.

<table>
<thead>
<tr>
<th>Phaseolus cultivars</th>
<th>Lectin fractions (% saturation)</th>
<th>Sugars</th>
<th>Hemagglutination inhibition</th>
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<tr>
<td></td>
<td></td>
<td>galactose</td>
<td>glucose</td>
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<td></td>
<td>90</td>
<td>+ + +</td>
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<tr>
<td>Contender</td>
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<td>+</td>
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<td></td>
<td>90</td>
<td>+</td>
<td>-</td>
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<tr>
<td>10YLHJ49</td>
<td>70</td>
<td>+ + +</td>
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<td></td>
<td>90</td>
<td>+ + +</td>
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<tr>
<td>Diacole</td>
<td>70</td>
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<td>90</td>
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<td>Shalatine</td>
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<tr>
<td></td>
<td>90</td>
<td>+ + +</td>
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*: positive hemagglutination inhibition; -: negative hemagglutination inhibition. The number of + signs indicates the amplitude of relative hemagglutination inhibition
Table 3: Antimicrobial activity (measured as inhibition zones diameters of lectins extracted from five cultivars of Phaseolus seeds against tested microorganisms. The results are represented as mean of three replicates with ± standard deviation and p-value ≤ 0.05 and 0.01 considered statistically significant (95 and 99% confidence interval).

<table>
<thead>
<tr>
<th>Phaseolus cultivars</th>
<th>Lecin fractions (% saturation)</th>
<th>Inhibition zone diameters of the tested microorganisms (mm)</th>
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<tr>
<td></td>
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<td>Escherichia coli (-)</td>
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<tr>
<td>Bronco</td>
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<td>Contender</td>
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<td>10YLHJ49</td>
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<td>Diacole</td>
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p-value

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<th>P 0.01</th>
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<td>Gentamicin Ni</td>
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<td>Gentamicin 0.98</td>
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<td>Amphotericin B 1.95</td>
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</table>

by Chrispeels and Raikhal in 1991 [26] and Kovalchuk in 2006 [27] and who reported 31 kDa and 34 kDa bands, respectively. Moreover, phytohemagglutinin (PHA-P), is a lectin isolated from red kidney bean (Phaseolus vulgaris) consisting of four subunits with two different E and L type subunits. The configuration of this lectin results in five tetramer isolectins populations namely L, L, L, L, and L, [28]. In the present work, the molecular weights of subunits PHA-L and PHA-E were separated as bands 31 and 34 kDa, respectively using SDS-PAGE (Figure 2B).

Lectins have the ability to bind and agglutinate human and animal erythrocytes [29]. This made hemagglutination activity test the most convenient method of characterization and confirmation for presence of lectins. In this study, all lectins (fractions 70% and 90%) isolated from the five tested Phaseolus vulgaris seeds were able to agglutinate all types of human blood groups (A, B, AB and O). Similar results were reported by Zubcevic et al. in 2016 who indicated that lectin of Phaseolus vulgaris seeds had ability to agglutinate all human blood groups [30].

The agglutination activity of lectins could be inhibited by a specific simple monosaccharide, but for some lectins di-, tri- and even poly-saccharides are required. In this respect, lectins are classified into groups according to their sugar specificity, such as mannos, galactose, N-acetyl glucosamine, L-fucose and N-acetyl neuraminic acid; depend on the most effective monosaccharide that inhibited lectin mediated agglutination of erythrocytes [31]. Many lectins prefer binding to one sugar or two sugars [32,33]. In some cases, lectin activity can be inhibited by more than two sugars [34]. The results in this study showed that fractions 70% and 90% of the lectins separated from Bronco and Shalatine seeds were inhibited by two sugars (galactose and mannos), and fractions 70% and 90% of the lectins of Contender, Diacole and 10YLHJ49 seeds were inhibited by three sugars (galactose, maltose and mannos) (Table 2).
Figure 3: Effect of 100 µl of lectin isolated from Shalatine seeds. A shows the inhibition zones with the Gram-positive bacteria Staphylococcus aureus with lectin at 70% and 90% fractions. Scanning electron microscope images of Staphylococcus aureus are shown without B and with C 100 µl lectin fraction 90%.

Figure 4: Effect of 100 µl lectin of Shalatine seeds. A shows the inhibition zones with the Gram-negative bacteria Pseudomonas aeruginosa with lectin at 30%, 70% and 90% fractions. Scanning electron microscope images of Pseudomonas aeruginosa are shown without B and with C 100 µl lectin fraction 90%.
activity against the Gram-negative bacteria Pseudomonas aeruginosa and Klebsiella pneumoniae, and Gram-positive bacteria Staphylococcus aureus (Table 2). This could be further confirmed by the results that showed antibacterial activity of lectins obtained from Indigofera heterantha legume seeds and those extracted from Chenopodium quinoa seeds [37,38].

Chitin (N-acetyl glucosamine polymer) is the key component of fungal cell walls and lectin is considered as one of the chitin-binding proteins that exhibits antifungal properties [39]. In the present work, Table 3 shows that all the fractionated lectins of the five cultivars of Phaseolus vulgaris seeds under study had antifungal activity against Candida albicans. This conclusion agrees with that of Yan et al. in 2005 that proved that lectins from Astragalus mongholicus seeds had a potent effect on Candida albicans [40].

The minimum inhibitory concentration (MIC µg/ml) for each of the tested lectins against the microorganisms under study is shown in Table 4. The lowest MIC values consistently were displayed by the lectin from Diacole 3.9, 7.81, 15.63 and 31.25 µg/ml. These concentrations were being enough to mediate an effective antimicrobial activity against Klebsiella pneumonia and Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans, respectively. The previous results matched with those obtained by Oliveira et al. [7].

In our work, we applied a new approach to examine pathogenic bacteria treated with the lectins separated from Egyptian Phaseolus seeds of cv. Shalatine using scanning electro

References


antimicrobial activity of a lectin isolated and purified from Indigofera heterantha. Adv Biodi Biotec 4: 999-1006.

