Bot. Bull. Academia Sinica (1992) **33:** 185-190

Antimicrobial activity of legume seed proteins

Reda Helmy Sammour[1] and Abd-El-Raheem R. El-Shanshoury
Department of Botany, Faculty of Science, Tanta University, Tanta, Egypt

(*Received August 26, 1991; Accepted January 10, 1992*)

Abstract. The antimicrobial activity of ethanol extracts, total seed proteins, globulins, albumins, fractions of albumins (which eluted from Sephadex G-150 column), and lectins of *Dolichos lablab* L., *Trigonella foenum-graecum* L., *Trifolium alexandrium* L., *Bauhinia variegata* L., and *Delonix regia* (Boj.) Rat. seed meals were investigated. Ethanol extracts and globulins of the different species have no antimicrobial activity. The lectins and one of the albumin fractions of all species, except *Dolichos lablab*, showed antimicrobial activity. The lectins of these species agglutinate some strains, and this agglutination was attributed to variation in structural features of the bacterial cell envelope. It can be concluded that lectins of these species might be very helpful in studying the polysaccharide structure of the envelope of these strains.

Key words: Albumins; Agglutination; Antimicrobial activity; Globulins; Lectins; Total seed proteins.

Introduction

The antimicrobial activity of different plant organs has been reported by many authors (Allen *et al.*, 1980; Zaki and El-Gengeihy, 1981; Abou El-Souod *et al.*, 1983). A great deal of attention has focused on the agglutination nature of seed protein extracts of some plants, especially those belong to Leguminosae (Pereira *et al.*, 1974, 1976; Wood *et al* ., 1978; Allen *et al.*, 1980).

The legume seeds contain lectins and protease inhibitors that are reported to have antimicrobial activity (Mikola, 1983; Gatehouse *et al.*, 1980; Baker, 1978). Interactions with seed lectins have been used to obtain structural information about the cell envelope and cell wall polymers of several Gram-negative and Gram -positive microorganisms (Doyle and Birdsell, 1972; Hammarstrom *et al.*, 1972; Allen *et al.*, 1980).

In the present study, the seed extracts of a number of species belonging to the Leguminosae were tested for antimicrobial activity. In addition, the albumins of the seed proteins of the different species were separately eluted on gel filtration columns using Sephadex G -150, and the proteins eluted in the eluted peaks were tested for the antimicrobial activity. Lectins of all species were purified and their antimicrobial activity tested.

Materials and Methods

Microorganisms, Seeds and Chemicals

The test organisms (*Mycobacterium rhodochrous, Bacillus cercus* 1080, *Bacillus megaterium* 1057, *Bacillus sphaericus, Escherichia coli, Serratia marcescens, Corynebacterium xerosis* 1022, *Staphylococcus aureus* 1352) were obtained from the culture collection of the Microbial Resource Center, Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Seeds of *Dolichos lablab* L., *Trigonella foenum - graecum* L., *Trifolium alexandrinum* L., *Delonix regia* (Boj.) Rat. and *Bauhinia variegata* L. were collected from Egypt in Summer 1988.

Sephadex G-150 was obtained from LKB Instruments Ltd, South Croydon, Surrey, London W5 5SS, U. K. All other chemicals were purchased from BDH Chemicals Ltd, Poole Dotset, BH12 4NN, U. K. and

[1]Corresponding author.

were of "Analar" grade or the purest available.

Saponins Test

Ethanol extracts of seed meals were evaporated under vacuum to dryness. The dried samples were dissolved in 1 ml chilled chloroform plus 1 ml acetic anhydride. A few drops of concentrated sulfuric acid were then added. On standing, in the presence of saponin, a red or blue/green color is produced and sometimes a pink ring appears. Gypsophila root saponin was used as the positive control and bovine serum inhibitor as negative controls.

Alkaloids Detection

The presence of alkaloids in a 90 per cent ethanol extract was determined using Dragendorff,s Reagent.

Total Seed Protein Isolation

One hundred grams of the ground seed (meals) of *D. lablab, T. foenum-graecum, T. alexandrinum, D. regia*, and *B. variegata* were individually extracted with 0.135 M borate buffer at pH 8±0.02. The extracts were individually centrifuged at 23,000 g for 30 minutes. The supernatant of each extract was dialysed overnight against distilled water at 4°C, lyophilized, and freeze-dried.

Globulin and Albumin Preparations

One hundred grams of seed meal of *D. lablab, T. foenum-graecum, T. alexandrinum, D. regia* and *B. variegata* were mixed with 0.135 M borate buffer pH 8±0.02 for 24 h at 4°C. The extracts were separately centrifuged at 23,000 g for 30 minutes. The supernatant of each extract was dialysed against 33 mM sodium acetate buffer pH 5.2±0.02, at 4°C overnight. The precipitate of globulin proteins was collected by centrifugation at 23,000 g for 30 minutes, resuspended in distilled water, and lyophilized. The supernatant of albumins was dialysed against 5 mM sodium borate buffer pH 8 ± 0.02, at 4 °C overnight, centrifuged at 23,000 g for 30 minutes, resuspended in distilled water, and lyophilized.

Albumin Fractionation

Albumin (20 mg) of each sample was eluted on Sephadex G-150 columns equilibrated and eluted with 0.1 M tris buffer pH 8±0.02 adjusted to pH 8±0.02 with 1 N HCl containing 0.1% sodium azide. The fractions of each peak were pooled, dialysed and freeze-dried.

Lectin Isolation

After the elution of the albumin on Sephadex G-150, the column was washed with 0.5 M glucose (Croy *et al.*, 1984). The column was run at a rate of 21 ml/h and 7 ml fractions were collected. The fractions of the eluted peak, which contain lectin, were pooled, lyophilized, and then freeze dried.

Haemagglutination Assays

The freeze-dried lectins were tested for haemagglutination by a serial-dilution method. This method was carried out on microtitration plates with a 2% (v/v) suspension of untreated rabbit erythrocytes in phosphate-buffered saline (50 mM-sodium phosphate buffer (pH 7.5 + 0.02)/0.15M NaCl. Wheat germ agglutinin was used as standard haemagglutinins.

Agglutination

Two milligrams of freeze-dried sample of total seed proteins, globulins, albumins, the fractions of albumins (which eluted from Sephadex G-150 column), and lectins were individually dissolved in 0.1M Tris buffer, pH 7.6 ± 0.02, containing 0.15 M sodium chloride and 0.025% sodium azide. One hundred microliters of each extract was tested for agglutiation.

Ethanol extracts were dried under vacuum, and the remaining residues were dissolved in 0.1M Tris buffer, pH 7.6, containing 0.15 M sodium chloride and 0.025% sodium azide and purified by filtration. One hundred microliters of the clear filtrate was tested for antimicrobial activity.

Two milligrams of wheat germ agglutinin was dissolved in 0.1 M Tris buffer, pH 7.6±0.02, containing 0.15 M sodium chloride and 0.025% sodium azide. One hundred microliters was tested as control activity.

One hundred microliters of 0.1 M Tris buffer, pH 7.6 ± 0.02, containing 0.15 M sodium chloride, 0.025% sodium azide was tested as control.

Culture Medium

Nutrient broth medium composed of the following ingredients (g/L) was used in all preparations for bacterial cultures:

peptone	5.0 g
meat extract	3.0 g
tap water	0.1 L
pH	7±0.02

This medium was sterilized at 15 p. s. i., dispensed in test tubes and incubated at 37°C for 48 h with the test organisms.

Plate Medium

The same medium as mentioned above with the addition of 20 g/L agar for solidification was sterilized, transferred to sterile petri-dishes (20 ml/ plate), allowed to cool and solidify.

Antimicrobial Assay

The above plates were inoculated with the test organisms and left for 2 h, then the samples were tested by the method of Carlson *et al.* (1948). The plates were incubated at 37°C for 24-28 h, and the incubation zone was measured in millimeters.

Statistical Analysis

Agglutination data of each sample were combined and both the nean and analysis of variance (ONE - WAY ANOVA) were computed by the computerized program MICROSTAT.

Results and Discussion

Ethanol extracts of all species gave a positive result for both alkaloids and saponins. However when they were tested for antimicrobial activity, the extracts from all species did not agglutinate the bacterial species used in this study. This finding did not rule out that ethanol extracts of some legumes have antimicrobial activity against microorganisms (Zaki and El Gengeihy, 1981; Gatehouse *et al.*, 1980; Baker, 1978).

Table 1. shows that total protein extracts of all species, except *D. lablab*, inhibited the bacterial growth of some organisms. The variation between the antimicrobial activity of the total protein extracts and wheat germ agglutinin is highly significant. The albumins and globulins of all species were tested for antimicrobial activity and the results are shown in Table 2. Albumins of all species except *D. lablab* had some antimicrobial activity, while the globulins showed negative results. The data stated in Table 2 showed that the antibacterial proteins of legume seeds are extracted in the albumin fraction. The failure of *D. lablab* albumin to agglutinate the tested organisms might be because the cell envelope of these organisms is free of the polysaccharide ligands which can interact with *D. lablab* iectin combining sites. The albumin of *D. lablab* had no antimicrobial activity or the antimicrobial activity, if any, was present at low dose (Goldstein and Hayes, 1978). However absence of polysaccharide ligands which can interact with plant lectin combining sites was reported by Allen *et al.* (1980) to be the reason for the failure of 21 out of 31 strains of *Neisseria gonorrhoeae* to agglutinate with *D. biflorus* lectin. This indicated that the variation in structural features of the bacterial cell envelope is not only within species but also among the strains of the same species.

It is established that lectins, protease inhibitors, major albumin, and high molecular weight albumin are the major fractions of the albumin extract (Pusztai *et al.*, 1983; Croy *et al.*, 1984). The first two components were found to play a major role in the protection of

Table 1. *Antimicrobial activity of the total seed proteins*

Bacterial strains	Species					Analysis of variance One-Way ANOVA	
	Wheat germ agglutinin	*T. foenum*	*T. alex*	*D. regia*	*B. variegata*	F Ratio	Prob.
Mycobacterium rhodochrous	14.00	0.00	8.00	05.50	03.20	104.993	4.004E-08
Bacillus cercus 1080	17.00	6.00	5.30	14.60	09.30	080.829	1.422E-07
Bacillus megaterium 1057	13.00	0.00	8.20	00.00	07.40	159.260	5.222E-09
Bacillus sphaericus	15.00	8.90	7.30	10.20	06.30	031.877	1.146E-05
Escherichia coli	20.00	6.80	5.40	08.40	07.50	104.616	4.074E-08
Serratia marcescens	19.00	0.00	7.90	00.00	05.50	304.635	2.129E-10
Corynebacterium xerosis 1022	18.00	0.00	5.70	00.00	09.90	285.885	2.916E-10
Staphylococcus aureus 1352	15.00	0.00	8.60	00.00	09.00	209.260	1.363E-09

The figures are the mean of six readings.

Table 2. *Antimicrobial activity of albumins*

Bacterial strains	Wheat germ agglutinin	Species *T. foenum*	*T. alex*	*D. regia*	*B. variegata*	Analysis of variance One-Way ANOVA F Ratio	Prob.
Mycobacterium rhodochrous	14.00	00.00	10.00	07.50	04.00	108.938	3.346E-08
Bacillus cercus 1080	17.00	07.00	07.00	18.00	12.00	149.673	6.780E-12
Bacillus megaterium 1057	13.00	00.00	09.60	00.00	09.00	236.234	7.490E-10
Bacillus sphaericus	15.00	10.00	09.00	13.00	08.00	025.500	3.151E-05
Escherichia coli	20.00	00.00	07.00	08.00	10.00	192.000	2.082E-09
Serratia marcescens	19.00	00.00	09.00	00.00	07.00	307.028	2.048E-10
Corynebacterium xerosis 1022	18.00	00.00	10.00	00.00	13.00	321.000	1.643E-10
Staphylococcus aureus 1352	15.00	00.00	11.00	00.00	11.00	241.500	6.717E-10

The figures are the mean of six readings.

Table 3. *Antimicrobial activity of the proteins eluted in the last peaks of the elution profiles Fig. 1*

Bacterial strains	Wheat germ agglutinin	Species *T. foenum*	*T. alex*	*D. regia*	*B. variegata*	Analysis of variance One-Way ANOVA F Ratio	Prob.
Mycobacterium rhodochrous	14.00	09.00	09.20	13.00	10.00	015.924	2.448E-04
Bacillus cercus 1080	17.00	00.00	13.00	12.00	09.00	152.625	6.435E-09
Bacillus megaterium 1057	13.00	00.00	00.00	00.00	00.00		
Bacillus sphaericus	15.00	00.00	12.00	12.00	07.00	014.100	4.065E-04
Escherichia coli	20.00	10.00	11.00	10.00	10.00	057.600	7.214E-07
Serratia marcescens	19.00	09.00	08.00	00.00	12.00	021.443	4.612E-06
Corynebacterium xerosis 1022	18.00	12.00	17.00	00.00	10.00	194.250	1.966E-09
Staphylococcus aureus 1352	15.00	10.00	10.00	00.00	07.00	007.372	7.717E-03

The figures are the mean of six readings.

plants against microorganisms and insects (Baker, 1978; Mikola, 1983; Pusztai *et al.*, 1983). The other components did not inhibit trypsin inhibition assays nor show any haemagglutinating activity (Croy *et al.*, 1984). The albumins of all species were therefore individually fractionated on Sephadex G-150 column. Elution profiles of all samples showed two peaks (Fig.1). The proteins eluted in the last peak was found to have antimicrobial activity; however, its activity was lower than that of the corresponding albumin fraction (Table 3). This loss of activity might be attributed to the fact that lectin is bound within the column (Croy *et al.*, 1984), and the inhibitory activity might be due to protease inhibitors. This was reported by Mikola (1983) to have a minor role in protection of plants against microorganisms.

After elution of the albumin fraction on a Sephadex G-150 column, the column was washed with 0.5 M glucose to collect the lectins that adhered to the column. The fractions were pooled and then tested for antimicrobial activity (Table 4). The data indicates that lectins had the highest antimicrobial activity.

Total seed protein extracts and albumins of some of the investigated species had inhibitory activity against *B. megaterium*. The protein eluted in the last peaks of albumin elution profiles and lectins of all species had no effect. This antimicrobial activity might be due to some inhibitory factors other than lectins.

As shown in Table 3 and Table 4, the albumin fraction and lectin of *T. foenum-graecum* agglutinate *M. rhodochrous, E. coli, S. marcescens* 1247, *C. xerosis* 1022 and *S. aureus* 1352. However the total protein extract and albumin of this species did not inhibit the growth of these strains. This might be because the dose of lectin

Table 4. *Antimicrobial activity of the lectins eluted from Sephadex G-150*

Bacterial strains	Wheat germ agglutinin	Species *T. foenum*	*T. alex*	*D. regia*	*B. variegata*	Analysis of variance Two-Way ANOVA F Ratio	Prob.
Mycobacterium rhodochrous	14.00	16.00	17.00	20.00	17.00	014.100	4.065E-04
Bacillus cercus 1080	17.00	00.00	19.00	19.00	16.00	242.625	6.565E-10
Bacillus megaterium 1057	13.00	00.00	00.00	00.00	00.00		
Bacillus sphaericus	15.00	00.00	19.00	19.00	14.00	229.875	8.570E-10
Escherichia coli	20.00	17.00	18.00	17.00	17.00	005.100	0.0168
Serratia marcescens	19.00	16.00	15.00	00.00	19.00	277.150	3.400E-10
Corynebacterium xerosis 1022	18.00	18.00	25.00	00.00	17.00	323.625	1.578E-10
Staphylococcus aureus 1352	15.00	16.00	17.00	00.00	15.00	188.625	2.273E-09

The figures are the mean of six readings.

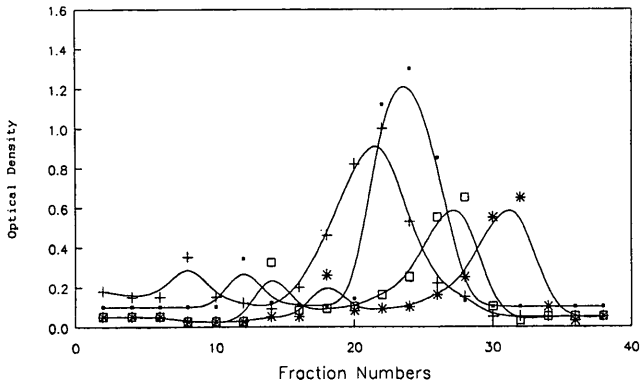

Fig. 1. Elution profiles of albumin seed proteins of *Trifolium alexandrinum*, *Delonix regia*, and *Bauhinia variegata*, and *Trigonella foenum-graecum* eluted with 0.1 M tris buffer pH 8±0.02 adjusted to pH 8±0.02 with 1 N HCl containing 0.1% sodium azide on gel filtration columns using Sephadex G-150. ■, *T. alexandrium*; +, *D. regia*; *, *B. variegata*; □, *T. foenum*.

in the total protein extract and albumin is very low (Goldstein and Hayes, 1978)

The variation between the antimicrobial activity of the total protein extracts, albumins, lectins and wheat germ agglutinin is highly significant, (Tables 1 -4). This indicated that antimicrobial activity is dependent on both the kind of bacterial strain and the kind of seed from which the extracts are taken.

In conclusion, the lectins of *Trigonella foenum - graecum*, *Trifolium alexandrium*, *Bauhinia variegata*, and *Delonix regia* agglutinate some of the tested microorganisms (which include both Gram positive and Gram negative bacteria). It was inferred that the cell envelope of *B. megaterium* 1057 has no polysaccharide ligands which can interact with *D. lablab* lectin combining sites. The agglutination reaction is proportional to the dose of the seed lectin. It can also be concluded that the lectins of *Trigonella foenum-graecum*, *Trifolium alexandrium*, *Bauhinia variegata*, and *Delonix regia* might be used very successfully to study the structural features of the tested organisms.

Literature Cited

Abou El-Souod, S. M., S. M. I. Moustafa, M. N. EL-Shourbagy, and I. M. El-Refae. 1983. Chemical and microbiological investigations of *Thymelaea hirsuta* Endl. (L.) growing in Egypt. Acad. J. Sci. **3**: 85-93.

Allen, P. Z., M. C. Connelly, and M. A. O. Apicella. 1980. Interaction of lectins with *Neisseria gonorrhea*. Can. J. Microbiol. **26**: 468-474.

Baker, A. M. R. 1978. Protease inhibitors and the biochemical basis of insect resistance in *Vigna unguiculata*. Ph. D. Thesis, Botany Department, Durham University.

Carlson, H. J., H. G. Douglas, and H. D. Bissel. 1948. Antibiotic substances separated from sumac. J. Bact. **55**: 607-612.

Croy, R. R. D., M. Houque, J. A. Gatehouse, and D. Boulter. 1984. The major albumin proteins from pea (*Pisum sativum* L.) Purification and some properties. Biochem. J. **218**: 795-803.

Doyle, R. J. and D. C. Birdsell. 1972. Interaction of concanavalian A with the cell wall of *Bacillus subtilis*. J. Bacteriol. **109**: 652 -658.

Gatehouse, A. M. R., J. A. Gatehouse, and D. Boulter. 1980. Isolation and characterization of trypsin inhibitors from cowpea (*Vigna unguiculata*). Phytochemistry **19**: 751-756.

Goldstein, I. J. and C. E. Hayes. 1978. The lectins: carbohydrate -binding proteins of plants and animals. Adv. Carbohydr. Chem. Biochem. **35**: 127-340.

Hammarstrom, S., A. A. Lindberg, and E. S. Robertson. 1972. Precipitation of lipopolysaccharides from rough mutants of

Salmonella typhimurium by an A-hemagglutinin from *Helix pomatia*. Eur. J. Biochem. **25**: 274-282.

Mikola, J. 1983. Proteinases, peptidases, and inhibitors of endogenous proteinases in germinating seeds. *In* J. Daussant, J. Mosse, and J. Vaughan (eds.), "Seed Proteins", Academic Press, New York, pp. 35-53.

Pereira, M. E. A., E. A. Kabat, and N. Sharon. 1974. Immunochemical study on the specificity of soybean agglutinin. Carbohydr. Res. **37**: 89-102.

Pereira, M. E. A., E. A. Kabat, R. Lotan, and N. Sharon. 1976. Immunochemical study of the specificity of the pea nut (*Arachis hypogaea*) agglutinin. Carbohydr. Res. **51**: 107-118.

Pusztai, A., R. R. D. Croy, G. Grant, and J. C. Stewart. 1983. Seed lectins : Distribution, location, and biological role. *In* J. Daussant, J. Mosse, and J. Vaughan, (eds.), "Seed Proteins", Academic Press, New York, pp. 53-82.

Wood, C. E., E. A. Kabat, S. Ebisu, and I. J. Goldstein. 1978. An immunochemical study of the combining sites of the second lectin isolated from *Bandeiraea simplicifolia* (BS II). Ann. Immunol. (Paris) **129**: 143-158.

Zaki, D. and S. El-Gengeihy. 1981. Antimicrobial activities of different extracts of Dovylis caffra. Egypt. J. Bot. **24**: 31-36.

豆科植物種子蛋白之抗菌活性

Reda Helmy Sammour and Abd-El-Raheem R. El-Shanshoury

Department of Botany, Faculty of Science
Tanta University, Tanta, Egypt

本報告以 *Dolichos lablab*, *Trigonella foenum-graecum*, *Trifolium alexandrium*, *Bauhinia variegata* 以及 *Delonix regia* 等豆科植物種子之酒精萃取物，種子總蛋白，球蛋白，白蛋白，經 Sephadex G-150 柱體劃分之白蛋白，以及凝集素研究抗菌活性。結果顯示，所有被測試品種之酒精萃取物及球蛋白，皆不具有抗菌活性。除了 *Dolichos lablab* 外，其他被測試品種之凝集素及白蛋白部分，都具有抗菌活性。這些品種之凝集素會凝集細菌。此凝集現象乃由於菌種外被膜之不同構造所致。所以，這些凝集素將可用來探討這些菌種外被膜多醣類之構造。