Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Purification of a lectin from *Eugenia uniflora* L. seeds and its potential antibacterial activity

M.D.L. Oliveira[1], C.A.S. Andrade[1], N.S. Santos-Magalhães[1,2], L.C.B.B. Coelho[1], J.A. Teixeira[3], M.G. Carneiro-da-Cunha[1,2] and M.T.S. Correia[1]

1 Departamento de Bioquímica – Laboratório de Glicoproteínas, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil
2 Laboratório de Imunopatologia Keizo-Asami (LIKA), Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil
3 IBB-Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Braga, Portugal

Keywords
antibacterial lectin, bacteria, *Eugenia uniflora*, *Eugenia uniflora* seed, lectin, lectin purification.

Correspondence
Profa. Dra. Maria Tereza Santos Correia, Departamento de Bioquímica, Universidade Federal de Pernambuco – UFPE, 50670-901, Recife, PE, Brazil.
E-mail: mtscorreia@gmail.com

2007/0869: received 4 June 2007, revised and accepted 9 December 2007

doi:10.1111/j.1472-765X.2007.02319.x

Abstract

Aims: The aim of this work was to analyse the antimicrobial properties of a purified lectin from *Eugenia uniflora* L. seeds.
Methods and Results: The *E. uniflora* lectin (EuniSL) was isolated from the seed extract and purified by ion-exchange chromatography in DEAE-Sephadex with a purification factor of 11·68. The purified lectin showed a single band on denaturing electrophoresis, with a molecular mass of 67 kDa. EuniSL agglutinated rabbit and human erythrocytes with a higher specificity for rabbit erythrocytes. The haemagglutination was not inhibited by the tested carbohydrates but glycoproteins exerted a strong inhibitory action. The lectin proved to be thermo resistant with the highest stability at pH 6·5 and divalent ions did not affect its activity. EuniSL demonstrated a remarkable nonselective antibacterial activity. EuniSL strongly inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella* sp. with a minimum inhibitory concentration (MIC) of 1·5 μg ml^{-1}, and moderately inhibited the growth of *Bacillus subtilis, Streptococcus* sp. and *Escherichia coli* with a MIC of 16·5 μg ml^{-1}.
Conclusions: EuniSL was found to be effective against bacteria.
Significance and Impact of the Study: The strong antibacterial activity of the studied lectin indicates a high potential for clinical microbiology and therapeutic applications.

Introduction

Lectins are proteins which have the ability to bind specifically and reversibly monosaccharides and glycoproteins (Sharon and Lis 1995). Lectins are widely distributed in the nature occurring in animals, micro-organisms and plants. In the plant kingdom, these proteins are abundant in seeds, roots, fruits, flowers and leaves. They are mainly obtained from mature seeds of leguminous and possess one or multiple molecular forms (Sharon and Lis 1990) and its chemical characteristics, physical-chemistry structure and biological properties have been extensively investigated.

Eugenia uniflora L., a Myrtaceae plant distributed in South of Africa, is commonly used in the treatment of hypercholesterolemia, gout and hypertension (Arai *et al.* 1999). *Eugenia uniflora* extracts have the capacity to reduce blood pressure in hypertension patients (Consolini *et al.* 1999) as well as to inhibit DNA polymerase of Epstein–Barr virus (Lee *et al.* 2000).

Structural characterization of plant lectins revealed the presence of specific binding sites which react with carbohydrates exposed on the surface of micro-organisms, making possible the identification of pathogenic bacteria based on the selective agglutination between lectins and bacteria (Calderon *et al.* 1997; Munoz-Crego *et al.* 1999). Lectins can be used to analyse carbohydrates present in the cell walls of Gram-positive and Gram-negative bacteria (Doyle 1994) as specificity to a wide variety of complex carbohydrates, such as teichoic acid, peptidoglycan

and lipopolysaccharides has been demonstrated. Although interactions between plant lectins and human pathogenic bacteria present have been reported (Slifkin and Doyle 1990), little attention has been paid to the interaction between lectins and phytopathogens.

The main goal of the present work was to purify of a lectin from *E. uniflora* seeds, a common indigenous fruit in Brazil and to analyse its antimicrobial activity against Gram-negative and Gram-positive bacteria.

Materials and methods

Lectin purification

Seeds of *E. uniflora* were powdered and homogenized (10% w/v) by gentle shaking for 4 h at room temperature in 0·01 mol l^{-1} sodium-phosphate buffer, pH 7·0 containing 0·15 mol l^{-1} sodium chloride (PBS). The homogenate was filtrated through cheesecloth and the filtrate centrifuged at 12 000 ***g*** for 15 min. From the centrifugate, a clarified extract (crude extract, CE) was obtained by applying three successive dialysis steps with PBS. The CE (10 ml) containing 11·3 mg ml^{-1} of protein was applied directly in a DEAE-Sephadex ion-exchange column (10 ml) previously equilibrated with PBS. The column eluate was collected at a flow rate of 20 ml h^{-1}, and the elution profile was monitored at 280 nm. Non-adsorbed protein fractions were collected with the equilibrium buffer, while adsorbed protein materials were eluted by the addition of PBS adjusted to pH 2·0. The nonadsorbed fractions were not considered for further analysis as, in chromatographic processes, the adsorbed proteins are expected to have a higher degree of purification. The largest absorbance fractions obtained by elution of the adsorbed proteins were pooled (this pool was designated EuniSL, purified lectin from *E. uniflora* seed lectin) and SDS-PAGE was performed for denatured and reduced samples and evaluated by the method of Laemmli (1970).

Haemagglutinating activity and inhibition assays

The *E. uniflora* lectin haemagglutinating activity (HA) and HA inhibition were determined with glutaraldehyde-treated erythrocytes according to Correia and Coelho (1995). The HA (inverse of the titre) was defined as the lowest sample dilution showing full haemagglutination. Specific HA (SHA) was determined dividing HA by protein concentration. Rabbit erythrocytes were chosen for subsequent assays. HA inhibition in the presence of several glycoproteins (bovine serum fetal, fetuin, tyroglobulin, asialofetuin, casein and rabbit serum) and sugars [D(+)-arabinose, D(+)-galactose, D(+)-raffinose, methyl-β-D-galactopyranoside, methyl-α-D-mannopyranoside, *N*-acetyl-D-galactosamione, D(+)-lactose, D(+)-mannose, D(+)-glucose, *N*-acetyl-D-glucosamine, D-glucuronic acid, L(+)-rhaminose, trehalose, D(+)-cellobiose, D(−)-fucose, L-fucose, D(−)-ribose, D(−)-fructose, D(+)-xylose, sucrose and D(+)-maltose] was used to determine the lectin carbohydrate binding specificity.

Effect of pH, temperature and metal ions on haemagglutinating activity

The effect of pH, temperature and metal ions on HA was evaluated by incubating 1 ml of EuniSL samples (136 μg ml^{-1}) at 30, 40, 50, 60, 70, 80, 90 and 100°C for 30 min or at different pH values for 1 h at room temperature (25°C) in 1 ml of selected buffers (0·01 mol l^{-1} Tris–hydrochloric acid buffer at pH values 7·5, 8·0, 8·5, and 9·0 and 0·01 mol l^{-1} citrate phosphate buffer at pH values 4·5, 5·0, 5·5, 6·0, 6·5 and 7·0).

The same procedure was used to evaluate the effect of Mg^{2+} and Ca^{2+}. In this case, incubation time was 15 min and metal solutions tested were 5, 10 and 0·02 mol l^{-1} $CaCl_2$ and $MgCl_2$ solutions in 0·15 mol l^{-1} NaCl aqueous solution.

In all experiments, an aliquot (50 μl) of the mixture was distributed in a microtitre plate well and the HA assay was performed.

Radial diffusion assay

The interactions of EuniSL with leave or seed extracts from *E. uniflora* or with Cramoll 1,4, a glucose/mannose lectin from *Cratyllia mollis* seeds, a native plant of north-east of Brazil (Correia and Coelho 1995), were analysed by a radial diffusion test on agar gel (Ashford *et al.* 1982). Radial diffusion was carried out to evaluate the presence of saccharide residues and the binding specificity to glycoproteins.

Tested micro-organisms

All micro-organisms were isolated from medical material and provided by Departamento de Antibióticos (DA), Universidade Federal de Pernambuco (UFPE), and Laboratório de Bacterioses (LB), Universidade Federal Rural de Pernambuco (UFRPE). Gram-positive bacterial strains were – *Staphylococcus aureus* (UFPEDA-01), *Streptococcus* sp. (UFPEDA-295) and *Bacillus subtilis* (UFPEDA-16) and Gram-negative bacterial strains were – *Klebsiella* sp. (LBUFRP-01), *Pseudomonas aeruginosa* (LBUFRPE-02), *Corynebacterium bovis* (LBUFRPE-03) and *Escherichia coli* (LBUFRPE-04). All bacteria were maintained in Difco™ Nutrient Agar (NA) and stored at 4°C. For agglutination

studies and evaluation of antimicrobial activity of EmaL, bacteria growth was undertaken in shake flasks (250 ml), containing Difco™ Nutrient Broth (NB) incubated overnight in an orbital shaker at 37°C. Biomass concentration was determined by measuring suspension turbidity at 600 nm and converted to colony forming units (CFU) ml^{-1} using appropriate calibration curves. All the experiments were carried out with a biomass concentration of 10^5–10^6 (CFU) ml^{-1}.

Antibacterial activity assay

Antibacterial activity of CE and the purified EuniSL was investigated by the disc diffusion method (Cole 1994). The microbial strains were obtained from stock cultures in nutrient agar (0·7%). One-hundred millilitres of warm NA (43°C) and 0·5 ml of bacteria suspension (10^5–10^6 CFU ml^{-1}) were mixed and 10 ml volumes were distributed in sterile Petri plates (90 × 15 mm) and allowed to solidify. Sterile blank paper discs (6 mm diameter) impregnated with 20 μl of sterile solution of CE (2·0 mg ml^{-1}), EuniSL (2·0 and 1·0 mg ml^{-1}), positive control (amoxicillin, 2·5 mg ml^{-1}) and negative control 0·15 mol l^{-1} NaCl, were added on the agar plates. Plates were incubated at 37°C for 24 h. A transparent ring around the paper disc revealed antimicrobial activity. Zones of growth inhibition around discs were measured in millimetres.

Determination of the minimum inhibitory concentration and the minimum bactericidal concentration

Minimum inhibitory concentration corresponded to the minimum lectin concentration that inhibited visible bacterial growth. MIC was determined by the dilution tube test (Courvalin *et al.* 1985). Serial dilutions of EuniSL in PBS were prepared and added to the bacteria cultures (in NB) containing 10^7 cells ml^{-1}in the exponential growth phase (turbidity equivalent to 0·5 in the McFarland scale). The samples were incubated for 24 h at room temperature (25°C). Afterwards, cultures were seeded onto NA and incubated for 24 h at room temperature (25°C). The minimum bactericidal concentration (MBC) corresponded to the minimum concentration of the lectin that reduced the number of CFU to 0·1% of the initial concentration.

Bacterial agglutination test

For quantitative determination of the agglutinating activity, the minimum agglutinating concentration (MAC) corresponding to the minimum concentration of lectin that promoted bacterial agglutination was recorded. Bacterial cultures, which were grown for 16 h, were diluted at a ratio of 1 : 100 with NB. Agglutination assay was performed in 96-well microtitre plates with twofold serial dilutions of lectin in 0·15 mol l^{-1} NaCl. To each well, 100 μl of diluted bacterial suspension was added to a final volume of 200 μl. MAC was determined by visual agglutination after 16 h incubation of plates at 37°C.

The glycoprotein inhibition of EuniSL induced bacteria agglutination was performed in microplates. The lectin (50 μl) solution was mixed with an equal volume of diluted bovine fetal serum (0·5 mg ml^{-1}). After incubation at room temperature (25°C) for 30 min, 50 μl of bacterial suspension was added and the mixture was left at rest for 30 min and MAC in the presence of bovine fetal serum was determined.

Results

The HA of the CE was 8192. A purified EuniSL lectin was obtained when the CE was applied in an anion-exchange column (Fig. 1a). This one-step process was highly reproducible as the same result was obtained for more than 20 assays. The overall purification yield of the lectin is summarized in Table 1. SDS-PAGE with disulfide-reducing agent of the purified EuniSL besides confirming that a pure fraction has been obtained, showed that the molecular mass of EuniSL lectin was 67 kDa (Fig. 1b). EuniSL agglutinated human and rabbit erythrocytes and does not bind specifically to simple carbohydrates (Fig. 2a). In addition, EuniSL HA was inhibited by glycoproteins present in bovine fetal serum, rabbit serum, tyroglobulin, casein and fetuin but not by asialofetuin (Fig. 2b). The HA of EuniSL was maintained at 100°C for 60 min was pH-dependent [the highest value was obtained at pH 6·5 (Fig. 2c)] and was not dependent on metal ions, such as Ca^{2+} and Mg^{2+}.

Radial diffusion assays showed that EuniSL recognized glycoproteins present in *E. uniflora* seed and leaf extracts, differently from Cramoll 1,4 that only recognized glycoproteins present in *E. uniflora* seed extract. Furthermore, EuniSL was not recognized by Cramoll 1,4.

In vitro antibacterial assays demonstrated that the purified lectin exhibited antibacterial activity against the tested pathogenic bacteria (*Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus* sp., *Staphylococcus aureus, Corinebacterium bovis, Escherichia coli* and *Klebsiella* sp.) being the diameter of the corresponding inhibition halos shown in Table 2.

The obtained values for MIC, MBC and MAC are presented in Table 2 as well as results on HA inhibition of EuniSL by bovine fetal serum.

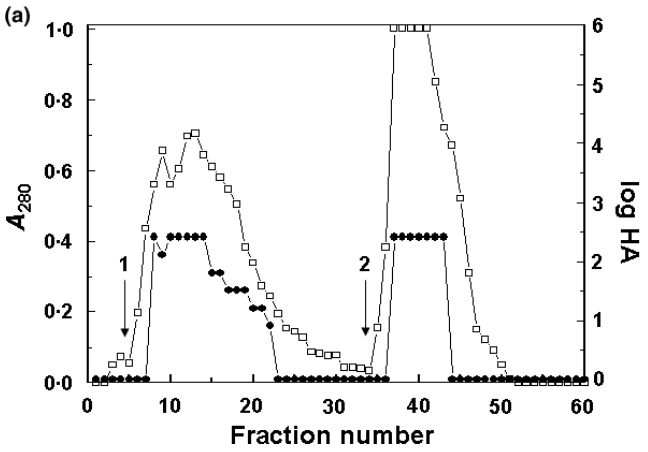

Figure 1 Isolation and characterization of EuniSL. (a) Chromatography of EuniSL on DEAE-Sephadex G50. A sample (10 ml) containing 11·3 mg ml^{-1} of protein was applied at flow rate of 20 ml h^{-1} and 2 ml fractions were collected. Arrows indicate elution with (1) PBS, followed by (2) PBS, pH 2·0 (○ absorbance at 280 nm; ● log of HA). (b) SDS-PAGE of EuniSL. Standard marker proteins: bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20·1 kDa) and α-lactalbumin (14·4 kDa); (2) EuniSL.

Table 1 Yields of EuniSL obtained during the purification of lectin from *Eugenia uniflora* seeds

Samples	Volume (ml)	Total protein (mg)	Specific HA (titre $protein^{-1}$)	Purification (folds)
CE	200	14 000	7·3	1·00
(EuniSL)	73	129	85·3	11·68

CE, crude extract of *E. uniflora* seeds.
Haemagglutinating activity (HA) was performed with 2·5% (v/v) suspension of glutaraldehyde-treated erythrocytes. Specific HA (SHA) = titre (mg $ml^{-1})^{-1}$ of protein.

Discussion

The isolation, purification, characterization and biological applications of new plant lectins have been of interest (Latha *et al.* 2006; Wong *et al.* 2006). EuniSL is a new lectin obtained from *E. uniflora* seeds as lectins from Eugenia species have not been described. As other lectins, EuniSL bind unspecifically to human erythrocytes and it is not inhibited by monosaccharides. EuniSL was inhibited by glycoproteins as isolectins from *Acacia constricta* and *Phaseolus vulgaris* that were only inhibited by complex carbohydrates, which are present in fetuin and tyroglobulin (Guzmán-Partida *et al.* 2004). Our finding suggests that EuniSL belongs to the same complex class of the isolectins from *A. constricta* and *P. vulgaris*.

EuniSL showed to be a heat-stable lectin with a similar behaviour to the protein reported by Ngai and Ng (2004) that maintained its HA at 100°C for 60 min.

Simple radial diffusion assay is a method used for the qualitative detection of lectin interactions with saccharide compounds being used to determine the saccharide residues present in glycoproteins (Lima *et al.* 1997). EuniSL displayed the same behaviour of *Peanut agglutinin* and *Agaricus bisporus agglutinin* lectins as precipitation bands with samples containing glycopeptides were observed

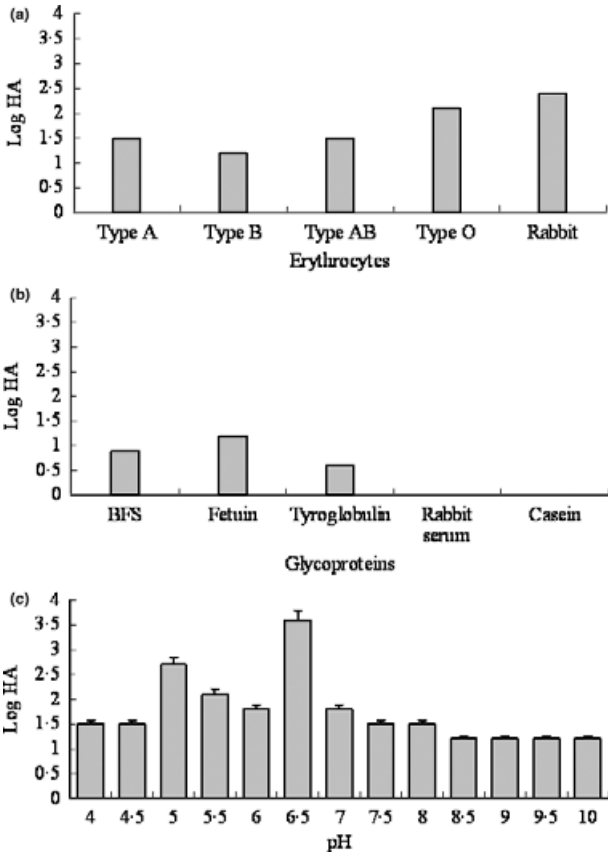

Figure 2 Log HA of EuniSL in the presence of erythrocytes (a), glycoproteins (b) and under different pH values (c). The Log of EuniSL samples (136 μg ml^{-1}) in PBS with rabbit erythrocytes was 2·4. Each data position of (a) and (b) is the given value of three experiments, exactly, with the same figure. Each data position of (c) is an average of three experiments and error bars show the standard deviation.

(Zeng *et al.* 2000). On the other hand, the simple radial diffusion assay demonstrated that no saccharide residues (glucose, mannose or its derivates) are present in the

Table 2 Antimicrobial activity, MIC, MBC and MAC of EuniSL* against tested bacteria

Bacteria	Clearing zone Diameter (mm)†	MIC (μg ml^{-1})	MBC (μg ml^{-1})	MAC (μg ml^{-1})	Inhibition (titre^{-1})‡
Staphylococcus aureus (+)	20·0 ± 0·57	1·5	16·5	0·250	0
Streptococcus sp. (+)	17·3 ± 0·57	16·5	180·0	0·250	8
Bacillus subtilis (+)	12·0 ± 0·00	16·5	180·0	NT	NT
Klebsiella sp. (–)	19·6 ± 3·30	1·5	16·5	0·063	0
Pseudomonas aeruginosa (–)	18·6 ± 0·60	1·5	16·5	0·250	4
Corinebacterium bovis (–)	14·0 ± 0·10	16·5	180·0	NT	NT
Escherichia coli (–)	12·0 ± 1·00	16·5	180·0	NT	NT

NT, not determined.
Gram-positive (+) and Gram-negative (–) bacteria.
MIC, minimal inhibitory concentration which corresponds to the minimum lectin concentration capable to inhibit the visible growth of the micro-organism.
MBC, minimal bactericide concentration which corresponds to the minimum concentration of the lectin capable to reduce the number of CFU for 0·1% of the initial inoculum.
MAC, minimal agglutination concentration which corresponds to the minimum lectin concentration which promotes bacterial agglutination.
*Initial concentration of lectin = 1·0 mg ml^{-1}.
†Diameter of paper disc.
‡Numbers represent the titre^{-1} of agglutination activity inhibition by bovine fetal serum (0·5 mg ml^{-1}). Titre^{-1} agglutination was 64.

EuniSL structure as no precipitation band was observed between EuniSL and Cramoll 1,4. As Cramoll 1,4 is not a glycoprotein, it can be used to verify the presence of glucose, mannose or its derivates residues in glycoproteins (Lima *et al.* 1997).

The studied lectin showed a remarkable antibacterial activity against all the tested bacteria, while an irrelevant antibacterial activity was detected with the seed CE. Proteins from seed extracts of some plant species showed higher antibacterial activity than the other parts of the plant (Basile *et al.* 1997) and have an important role in the protection of seeds against microbial invaders. These results demonstrate that EuniSL is the responsible for the antibacterial action observed in the seed extract. An outstanding feature of the antibacterial activity of the isolated lectin is its nonselective activity against bacteria and its activity on several species of human pathogenic bacteria.

The results describing the effect of EuniSL on the agglutination of several bacterial strains confirm the important interaction between the lectin and all the strains under consideration. From the tested strains, *Klebsiella* sp. seemed to be the most sensitive to the presence of EuniSL.

It is known that lectins do not bind to sugars of glycoprotein reduced terminals only, but may also react with internal components of the carbohydrate chains or with noncarbohydrates (Goldstein and Poretz 1986). Inhibition of bacteria agglutination by EuniSL using bovine fetal serum showed that lectin binding to the tested bacteria occurs by interaction with bacterial surface carbohydrates. Similar to EuniSL, the lectin of *Morus alba* exhibited antibacterial activity against *Pseudomonas syringae* pv. *mori* and the bacteria agglutination was also inhibited by the glycoproteins, fetuin and tyroglobulin (Ratanapo *et al.* 2001).

Although the mechanism of action of the peptides has not yet been elucidated in detail, the presented data confirm the *in vitro* antibacterial activity of EuniSL against pathogenic bacteria. It has been proposed that the proteins with antibacterial action form a channel on cell membrane and the cell dies as a result of the out flowing of cellular contents, being this mechanism different from that of antibiotics (Talas-Ogras *et al.* 2005).

The purification of a new lectin (EuniSL) by ion-exchange chromatography is described. Denaturing electrophoresis shows that this lectin has a major subunit with an apparent molecular mass of 67 kDa. This lectin has a high antibacterial activity as demonstrated by the growth inhibition (*in vitro*) of important pathogenic bacteria. These results point out that future findings of lectin applications obtained from medicinal plants can be of great importance for clinical microbiology and possible therapeutic applications.

Acknowledgements

The authors are deeply grateful for the technical assistance of Maria Barbosa Reis da Silva, João Antonio Virgínio, VALNATURA (AML/B7-311/97/0666/II-0440-FA) of ALFA Programme and the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq for

financial support (Proc. 472529/2006-4/MCT/CNPq 02/2006-Universal).

References

Arai, I., Amagaya, S., Komatsu, Y., Okada, M., Hayashi, T., Kasai, M., Arisawa, M. and Momose, Y. (1999) Improving effects of the extracts from *Eugenia uniflora* on hyperglycemia and hypertriglyceridemia in mice. *J Ethnopharmacol* **68**, 307–314.

Ashford, D., Allen, K. and Neuberger, A. (1982) The production and properties of an antiserum to potato (*Solamum tuberosum*) lectin. *Biochem J* **201**, 641–645.

Basile, A., Vuotto, M.L., Violante, U., Sorbo, S., Martone, G. and Castaldo-Cobianchi, R. (1997) Antibacterial activity in *Actinidia chinensis*, *Feijoa sellowiana* and *Aberia caffra*. *Int J Antimicrob Agents* **8**, 199–203.

Calderon, A.M., Buck, G. and Doyle, R.J. (1997) Lectin-microrganism complexes. In *Lectins, Biology, Biochemistry, Clinical Biochemistry* Vol. 12 ed. van Driessche, E., Beeckmans, S. and Bøg-Hansen, T.C. Lemchesvej. Hellerup, Denmark: TEXTOP. http://plab.ku.dk/tcbh/Lectins12/Calderon/paper.htm.

Cole, M.D. (1994) Key antifungal, antibacterial and anti-insect assays – a critical review. *Biochem Syst Ecol* **22**, 837–856.

Consolini, A.E., Baldini, O.A.N. and Amat, A.G. (1999) Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. *J Ethnopharmacol* **66**, 33–39.

Correia, M.T.S. and Coelho, L.C.B.B. (1995) Purification of a glucose/mannose specific lectin, isoform 1, from seeds of *Cratylia mollis* Mart. (Camaratu bean). *Appl Biochem Biotechnol* **55**, 261–273.

Courvalin, P., Goldstein, F., Philippon, A. and Sirot, J. (1985) *L'antibiogramme*. Paris, Bruxelles: MPC-Videocom.

Doyle, R.J. (1994) Introduction to lectins and their interactions with microorganisms. In *Lectin-microorganism Interactions* ed. Doyle, R.J. and Slifkin, M.V. pp. 1–65. New York: Marcel Dekker, Inc.

Goldstein, I.J. and Poretz, R.D. (1986) Isolation, physicochemical characterization and carbohydrate-binding specificity of lectins. In *The Lectins: Properties, Functions and Applications in Biology and Medicine* ed. Liener, I.E., Sharon, N. and Goldstein, I.J.V. pp. 33–247. Orlando, FL, USA: Academic Press.

Guzmán-Partida, A.M., Robles-Burgueño, M.R., Ortega-Nieblas, M. and Vázquez-Moreno, I. (2004) Purification and characterization of complex carbohydrate specific isolectins from wild legume seeds: *Acacia constricta* is (vinorama) highly homologous to *Phaseolus vulgaris* lectins. *Biochimie* **86**, 335–342.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Latha, V.L., Rao, R.N. and Nadimpalli, S.K. (2006) Affinity purification, physicochemical and immunological characterization of a galactose-specific lectin from the seeds of *Dolichos lablab* (Indian lablab beans). *Protein Expr Purif* **45**, 296–306.

Lee, M.H., Chiou, J.F., Yen, K.Y. and Yang, L.L. (2000) EBV DNA polymerase inhibition of tannins from *Eugenia uniflora*. *Cancer Lett* **154**, 131–136.

Lima, V.L.M., Correia, M.T.S., Cechinel, Y.M.N., Sampaio, C.A., Ownen, J.S. and Coelho, L.C.B.B. (1997) Immobilized *Cratylia mollis* lectin as a potential matrix to isolate plasma glycoproteins including lecithin-cholesterol acyltransferase. *Carbohydr Polym* **33**, 27–32.

Munoz-Crego, A., Alvarez, O., Alonso, B., Rogers, D.J. and Lvovo, J. (1999). Lectin as diagnostic probes in clinical bacteriology-an overview. In *Lectins, Biology, Biochemistry, Clinical Biochemistry* Vol. 13 ed. van Driessche, E., Beeckmans, S. and Bøg-Hansen, T.C. Lemchesvej. Hellerup, Denmark: TEXTOP. http://plab.ku.dk/tcbh/Lectins12/Calderon/paper.htm.

Ngai, P.H.K. and Ng, T.B. (2004) A mushroom (*Ganoderma capense*) lectin with spectacular thermostability, potent mitogenic activity toward tumor cells. *Biochem Biophys Res Commun* **314**, 988–993.

Ratanapo, S., Ngamjunyaporn, W. and Chulavatnatol, M. (2001) Interaction of a mulberry leaf lectin with a phytopathogenic bacterium, *P. syringae* pv *mori*. *Plant Sci* **160**, 739–744.

Sharon, N. and Lis, H. (1990) Legum lectins, a large family of homologous proteins. *FASEB J* **4**, 3198–3208.

Sharon, N. and Lis, H. (1995) Lectins with a sweet tooth: functions in cell recognition. *Essays Biochem* **30**, 59–75.

Slifkin, M. and Doyle, R.J. (1990) Lectins and their application in clinical microbiology. *Clin Microbiol Rev* **3**, 197–218.

Talas-Ogras, T., Ipekci, Z., Bajrovic, K. and Gozukirmizi, N. (2005) Antibacterial activity of seed proteins of *Robinia pseudoacacia*. *Fitoterapia* **76**, 67–72.

Wong, J.H., Wong, C.C.T. and Ng, T.B. (2006) Purification and characterization of a galactose-specific lectin with mitogenic activity from pinto beans. *Biochim Biophys Acta* **1760**, 808–813.

Zeng, X., Nakaaki, Y., Murata, T. and Usui, T. (2000) Chemoenzymatic synthesis of glycopeptides carrying α-Neu5Ac-(2 → 3)-α-D-GalNAc, and related compounds and analysis of their specific interactions with lectins. *Arch Biochem Biophys* **383**, 28–37.