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Partial Characterization of the Lectin of Runner Beans (*Phaseolus coccieneus*) Var. Alubia

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Abstract: We extracted and partial characterized lectin from runner beans (*Phaseolus coccineus* L.). This lectin shows a great affinity to fetuin-agarose column like others lectins and the electrophoretic gels point one band of ≈ 45 kDa. In addition to the previous assays, we detected the presence of lectins by agglutination assays. We know that lectins are non-enzymatic proteins or glycoproteins that bind carbohydrates. The biological function of plant lectins is not fully understood, but they are hypothesized to be involved in a number of intrinsic processes. Many of those processes include hemagglutination. We believe that the *P. coocineus* lectin will be an important tool for know the properties of many lectins, included their capacity to detected and quantify tumor markers.

Key words: *P. coccineus*, lectin, fetuin-agarose column

INTRODUCTION

Lectins are non-enzymatic proteins or glycoproteins that bind carbohydrates (Houlès Astoul *et al*., 2002; Luac *et al*., 2002; Bourne *et al*., 2002, 2004; Van Damme *et al*. 2007; Fouquaert *et al*., 2008). Although, they were first identified in plants over a century ago, they are now known to exist throughout nature, including both eukaryotic and prokaryotic organisms. While all lectins have a Carbohydrate Recognition Domain (CRD) that determines their specificity, the classification of lectins depends on the source, i.e., plant, animal, microbial, etc (Skelton and Wong, 1990; Benerjee *et al*., 1994; Bourne *et al*., 2002, 2004).

Two major problems hamper the discovery of the remaining sugar-binding motifs in plants. First, unless homologous lectins have been identified in other organisms, no relevant information is provided by genome/proteome analyses. Second, evidence is accumulating that the expression level of lectins with a specific endogenous role is so low that they escape detection by the currently available activity assays. Though at present virtually all abundant plant lectins can be classified into well-defined protein families, there are still a few exceptions for which sufficient sequence information is not available. One of these orphan lectins is the Euonymus Europaeus Agglutinin (EEA) (Fouquaert *et al*., 2008).

The animal lectins are classified based on their amino acid sequence homology and evolutionary relatedness, while the plant lectins are grouped according to the plant family. Lectins found in microorganisms tend to be classified according to the function, e.g., adhesions or hemagglutinins and toxins (Wong *et al*., 1985, 1986; O'Sullivan *et al*., 1990; Hynes *et al*., 1999; Bourne *et al*., 2002, 2004; Fouquaert *et al*., 2008). Lectins are proteins with binding specificities for certain carbohydrate moieties and have been used previously for typing bacteria, including other *Campylobacters* and the closely related *Helicobacter pylori*. As with other gram-negative bacteria, lipopolysaccharide (LPS) contributes to the pathogenesis of Campylobacters (Aabenhus *et al*., 2002).

Lectins found in plants of the monocotyledonous group are distinct from those of the dicotyledons and exhibit several unique properties. Snowdrop lectin (*Galanthus nivalis* agglutinin) was first isolated from bulbs of the snowdrop *G. nivalis* L. and characterized as a tetrameric protein of 50 kDa composed of four identical subunits (Longstaff *et al*., 1998). Lectins are found in both soluble and cell-associated forms and exhibit a wide range of functions. Animal lectins are involved in a variety of cellular processes, including enzyme trafficking, tissue

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homing and immune function. Microbial carbohydrate binding proteins largely function in host cell attachment, tissue colonization and invasion. The biological function of plant lectins is not fully understood, but they are hypothesized to be involved in a number of intrinsic processes. A number of plant lectins, including snowdrop lectin, have been shown to exhibit antimetabolic or insecticidal effects on insects when fed in artificial diets, suggesting that such compounds, if they could be transferred into plants normally lacking lectins, may have significance as plant protectants (Longstaff *et al*., 1998).

By other hand, other properties include deposition of storage proteins, maintenance of seed dormancy, defense against pathogens (Longstaff *et al*., 1998; Bell *et al*., 1999; Shukla *et al*., 2005) and animal predators, symbiosis, transport of carbohydrates, mitogenic stimulation of embryonic plant cells, elongation of cell walls and recognition of pollen. Despite the lack of information regarding the biological function of plant lectins, there is an enormous interest in these molecules and, thus, they have been well-defined with regard to their carbohydrate specificities (Sharon and Lis, 1990; Barbieri *et al*., 1993; Barre *et al*., 1996; Van Damme *et al*., 2007; Fouquaert *et al*., 2008). They are found in multiple parts of the plant, including the seeds, bark, stems and leaves. They are relatively easy to extract and have become invaluable research tools in many disciplines, including glycobiology, immunology and cell biology. Preliminary studies indicated that the potent insecticidal lectin, Gleheda, from the leaves of *Glechoma hederacea* (ground ivy) preferentially agglutinates human erythrocytes carrying the Tn (GalNAcα1-Ser/Thr) antigen (Singh *et al*., 2006).

MATERIALS AND METHODS

Lectin was extracted from 20 g of seed (*P. coccineus*) used to a blender with glass and in cooling (4°C), using 200 mL of phosphates buffer (PBS) in 2 h. Then the extract was centrifuged to 5000 x g by 20 min at 4°C. The pH of supernatant was adjustment to 4 with glacial acetic acid and shook by 2 h and was left stand all night. The supernatant was centrifuged to 12000 x g by 20 min at 4°C. The supernatant was towards with ammonium sulphate to 80%. Then was centrifuged to 40000 x g by 30 min at 4°C. Finally, the pill was resuspended in PBS and was dialyzed against PBS for future analysis. Then, the sample with the lectin of *Ph. coccineus* was resuspended at 1.5 mL of PBS and added onto fetuin-agarose column with flew of 0.02 mL sec^{-1}. Then, washed the column with 20 mL of PBS (0.02 M PO_4/0.9% NaCl/0.02% Na Azide). Finally, the lectin was eluted with 30 mL of 0.2 M of acetic acid at pH 2.5. The electrophoretic separation of proteins was performed in 10% (w/v) polyacrylamide gels (SDS-PAGE) following the method described by Schägger and Von Jagow (1987). Electrophoreses were run at room temperature. After electrophoresis, gels were fixed in 50% methanol and 10% acetic acid for 30 min and stained with Coomassie blue (Serva blue G, SERVA Electrophoresis GmbH, Heidelberg, Germany) (Schägger and Von Jagow, 1987). Protein concentration was determined by a modification of Stoscheck (1990) method, using Bovine Serum Albumin (BSA) as standard.

RESULTS AND DISCUSSION

As shown in Fig. 1, we had two peaks of protein. One, the larger peak (dotted arrow) eluted with PBS and

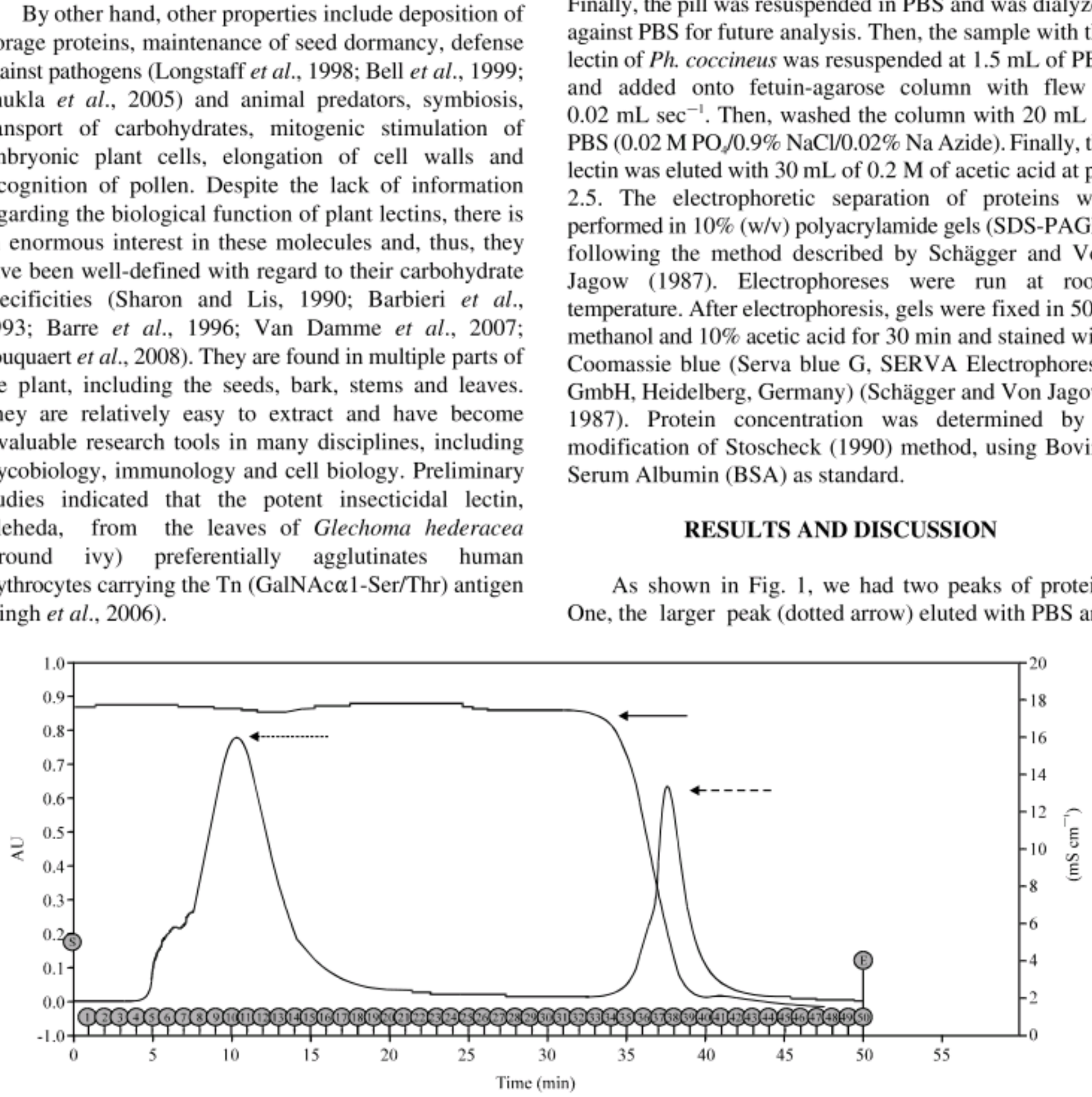

Fig. 1: Partial purification of lectin from runner beans (*Ph. coocineus*). Elution profile of a Fetuin-Agarose column loaded with a lectin extract of runner beans seed. One milliliter fraction were collected. Solid arrow point the conductivity curve. Dotted arrow point the protein eluted with PBS. Broken arrow point the lectin of *Ph. coocineus*, eluted with 0.2 M acetic acid pH 2.5

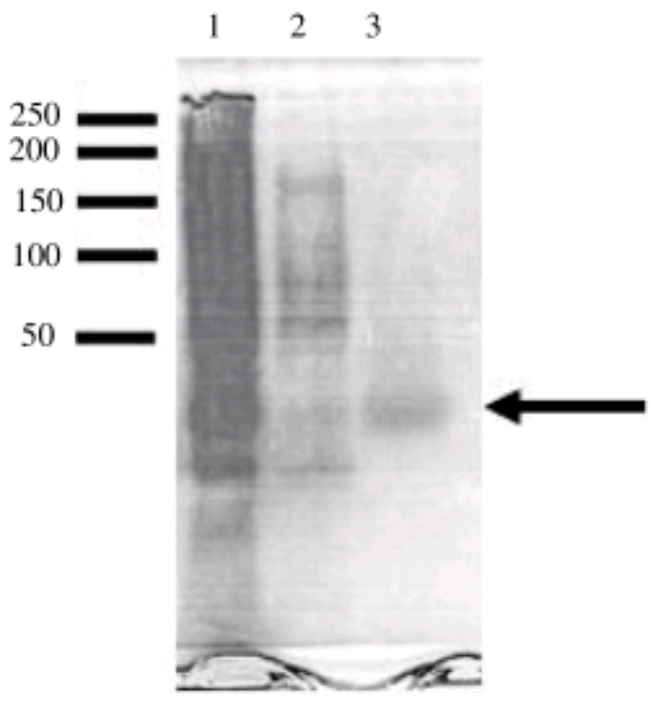

Fig. 2: Protein patterns of fractions obtained from runner beans purification lectin. Protein were separated by SDS-PAGE 10% and the gels were stained with Coomassie blue. Line 1: Curd extract of lectin. Line 2: Fraction eluted with PBS. Line 3: Lectin eluted with 0.2 M acetic acid pH 2.5. Solid arrow pint the lectin o *Ph. coccineus*

not shown affinity with the fetuin-agarose column. By other hand, the minor peak (broken arrow) eluted with 2 M of acetic acid at pH 2.5, showed affinity with the fetuin-agarose column, conductivity value of 5.25 and yield of 36% (data not shown). The electrophoretic gel (Fig. 2, line 3) show the fraction eluted with 2 M of acetic acid at pH 2.5. This line only had one band of protein of ≈ 45 kDa, suggested that is the *P. coccineus* lectin.

Those results show the presence of one protein like a lectin, the affinity to fetuin-agarose column, ensure this asseveration (Fig. 1, 2). But more studies we have to do for complete its characterization.

REFERENCES

Aabenhus, R., S.O. Hynes, H. Permin, A.P. Moran and L.P. Andersen, 2002. Lectin typing of *Campylobacter concisus.* J. Clinical Microbiol., 40: 715-717.

Barbieri, L., M.G. Battelli and F. Stirpe, 1993. Ribosome-inactivating proteins from plants. Biochim. Biophys. Acta, 1154: 237-282.

Barre, A., E.J. Van Damme, W.P. Peumans and P. Rougé, 1996. Structure-function relationship of monocot mannose-binding lectins. Plant Physiol., 112: 1531-1540.

Bell, H.A., E.C. Fitches, R.E. Down, G.C. Marris, J.P. Edwards, J.A. Gatehouse and A.M. Gatehouse, 1999. The effect of snowdrop lectin (GNA) delivered via artificial diet and transgenic plants on *Eulophus pennicornis* (Hymenoptera: Eulophidae), a parasitoid of the tomato moth *Lacanobia oleracea* (Lepidoptera: Noctuidae). J. Insect. Physiol., 45: 983-991.

Benerjee, R., S.C. Mande, V. Ganesh, K. Das and V. Dhanaraj, 1994. Crystal structure of peanut lectin, a protein with an unusual quaternary structure. Proc. Natl. Acad. Sci. USA., 91: 227-231.

Bourne, Y., C.H. Astoul, V. Zamboni, W.J. Peumans, L. Menu-Bouaouiche and E.J. Van Damme, 2002. Structural basis for the unusual carbohydrate-binding specificity of jacalin towards galactose and mannose. Biochem. J., 364: 173-180.

Bourne, Y., V. Roig-Zamboni A. Barre, W.J. Peumans, C.H. Astoul and E.J. Van Damme *et al*., 2004. The crystal structure of the *Calystegia sepium* agglutinin reveals a novel quaternary arrangement of lectin subunits with a β-Prism fold. J. Biol. Chem., 279: 527-533.

Fouquaert, E., W.J. Peumans D.F. Smith, P. Proost, S.N. Savvides and E.J. Van Damme, 2008. The old *Euonymus europaeus* agglutinin represents a novel family of ubiquitous plant proteins. Plant Physiol., 147: 1316-1324.

Houlès Astoul, C., W.J. Peumans, E.J. Van Damme, A. Barre, Y. Bourne and P. Rougé, 2002. The size, shape and specificity of the sugar-binding site of the jacalin-related lectins is profoundly affected by the proteolytic cleavage of the subunits. Biochem. J., 367: 817-824.

Hynes, S.O., S. Hirmo, T, Wadström and A.P. Moran, 1999. Differentiation of *Helicobacter pylori* isolates based on lectin binding of cell extracts in an agglutination assay. J. Clin. Microbiol., 37: 1994-1998.

Longstaff, M., K.S. Powell, J.A. Gatehouse, R. Raemaekers, C.A. Newwell and W.D. Hamilton, 1998. Production and purification of active snowdrop lectin in *Escherichia coli*. Eur. J. Biochem., 252: 59-65.

Luac, G., J. Durmic, S. Šupraha and M. Flögel, 2002. Lectins labeled with digoxin as a novel tool to study glycoconjugates. Food Technol. Biotechnol., 40: 289-292.

O' Sullivan, N., J. Benjamin and M.B. Skirrow, 1990. Lectin typing of *Campylobacter* isolates. J. Clin. Pathol., 43: 957-960.

Schägger, H. and G. Von Jagow, 1987. Tricine-sodium dodecyl sulfatepolyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem., 166: 368-379.

Sharon, N. and H. Lis, 1990. Legume lectins-a large family of homologous proteins. FASEB J., 4: 3198-3208.

Shukla, S., R. Arora and H.C. Sharma, 2005. Biological activity of soybean trypsin inhibitor and plant lectins against cotton bollworm/legume pod borer, *Helicoverpa armigera*. Plant Biotechnol., 22: 1-6.

Singh, T., J.H. Wu, W.J. Peumans, P. Rougè, E.J. Van Damme and R.A. Alvarez *et al*., 2006. Carbohydrate specificity of an insecticidal lectin isolated from the leaves of *Glechoma hederacea* (ground ivy) towards mammalian glycoconjugates. Biochem. J., 393: 331-341.

Skelton, S.K. and K.H. Wong, 1990. Simple, efficient purification of filamentous hemagglutinin and pertussis toxin from *Bordetella pertussis* by hydrophobic and affinity interaction. J. Clin. Microbiol., 28: 1062-1065.

Stoscheck, C.M., 1990. Increased uniformity in the response of the Coomassie blue G protein assay to different proteins. Anal Biochem., 184: 111-116.

Van Damme, E.J., S. Nakamura-Tsuruta, D.F. Smith, M. Ongenaert and H.C. Winter *et al*., 2007. Phylogenetic and specificity studies of two-domain GNA-related lectins: Generation of multispecificity through domain duplication and divergent evolution. Biochem. J., 404: 51-61.

Wong, K.H., S.K. Skelton and J.C. Feeley, 1985. Interaction of *Campylobacter jejuni* and *Campylobacter coli* with lectins and blood group antibodies. J. Clin. Microbiol., 22: 134-135.

Wong, K.H., S.K. Skelton and J.C. Feeley, 1986. Strain characterization and grouping of *Campylobacter jejuni* and *Campylobacter coli* by interaction with lectins. J. Clin. Microbiol., 23: 407-410.