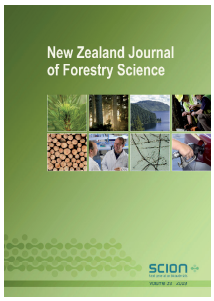
3rd New Zealand and German CELL WALL SYMPOSIUM

SCION Next generation biomaterials

New Zealand Journal of Forestry Science

39 (2009) 217-223

http://nzjfs.scionresearch.com



Biologically Active Polysaccharides in Medicinal Plants†

Yves S.Y. Hsieh[1,2,*], Shih-Fen Liao[2], Wen-Bin Yang[2]

[1]*School of Biological Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

[2]*Genomic Research Center, Academia Sinica, Taipei 115, Taiwan*

(Submitted for publication 10 February 2009; accepted in revised form 25 September 2009)

Corresponding author: yves@gate.sinica.edu.tw

Abstract

Biologically active polysaccharides from medicinal plants have been studied for many years. In addition to immuno-modulating effects, human clinical studies have shown that some polysaccharides have certain benefits for human health. Most studies have focused on the side chains of pectic rhamnogalacturonan I (RG-I), particularly the Type II arabinogalactans, which have been isolated and shown to have anti-complementary and other immuno-activities. These studies have provided valuable information about how carbohydrate moieties induce certain biological events.

Keywords: cell wall polysaccharides; glucomannans; heteroxylans; medicinal plant; pectins, rhamnogalacturonan I; xyloglucans.

† Based on a paper presented at the 3rd Joint New Zealand-German Symposium on Plant Cell Walls, 13-15 February 2008, Auckland, New Zealand

Introduction

Polysaccharides are major components of plant cell walls and some of the most abundant biomaterials on earth. Cell wall polysaccharides are vital to human nutrition and health, and are sold commercially as dietary fibres and as health supplements (Paulsen, 2001; Tungland & Meyer, 2002; Harris & Smith, 2006). Many polysaccharides extracted from medicinal plants are recognised as having therapeutic potential as anti-cancer, anti-inflammatory, anti-bacterial, anti-viral, and immunodulatory agents (Darshan & Doreswamy, 2004). Some polysaccharides have been subjected to clinical trails and significant improvements in certain disease states have been reported (Guess et al., 2003). However, how polysaccharides induce complex cellular events in the human body remains largely unknown. Nevertheless, it is likely that they act by regulating receptor mechanisms. If this is so, then it is essential to know the structures of specific polysaccharides and how they interact with the different receptors that lead to their biological effects. Such studies form the basis of glycobiology.

To determine the detailed structures of complex polysaccharides is a tedious task which often requires multiple strategies and requires enormous effort. Polysaccharides range in their structures from homopolymers to complex heteropolymers with different monomers, ring sizes, anomeric configurations, glycosidic linkages, and non-carbohydrate substituents. Their structures vary

ISSN 0048 - 0134

depending on their botanical origin and on the organ and cell type from which they were extracted, making it hard to evaluate which part of the structure contributes to its pharmaceutical activity. This review provides an overview of the structures of biologically active cell wall polysaccharides and related polysaccharides in medicinal plants.

Biologically active cell wall polysaccharides

Pectins

Pectins are the major non-cellulosic components in the primary cell walls of medicinal plants belonging to eudicotyledons and non-commelinid monocotyledons (Harris, 2005). Examples of sources for commercial preparations are the dried peels of lemons (*Citrus limon* (L.) Burm.f.) and limes (*Citrus aurantiifolia* (Christm.) Swingle) (Harris & Smith, 2006). Pectins have complex structures and are classified according to their domain structures, namely homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) (Harris, 2005). Homogalacturonan (HG) is an acidic domain composed solely of galacturonic acid (α-D-Gal*p*A) residues with no side chains. The residues are usually methyl-esterified or acetylated to different extents depending on the botanical origin of the HG (Willats et al., 2001). Rhamnogalacturonan I has a backbone structure of alternating α-D-Gal*p*A and rhamnopyranosyl (α-L-Rha*p*) residues, with some of the α-L-Rha*p* residues bearing neutral polysaccharide side chains: arabinan, galactan, arabinogalactan Type I, or arabinogalactan Type II (see Figure 1). Rhamnogalacturonan II is a very complex domain. It has an HG backbone with four structurally different side chains designated A, B, C, and D. These side chains contain rare sugars including β-D-apiose, α-L-galactose, 2-*O*-methyl-α-L-fucose, 2-*O* methyl-α-D-xylose, and 3-deoxy-D-manno-octulosonic acid (Kdo) (see Figure 1) (Glushka et al., 2003).

Studies on medically related biological activities of pectins have focused mostly on the RG I domain. This domain has been shown to be associated with modulating the immune system including anti-complementary activity, B-cell proliferation and up-regulation of immune cytokines (Kiyohara et al., 1988; Samuelsen et al., 1996; Sakurai et al., 1999; Inngjerdingen et al., 2007).

The complement system plays an important role in host defence by mediating the lysis of erythrocytes and invading bacteria, the inflammatory response, the opsonisation of antigens and viral neutralisation. Overstimulation of the complement system can have negative effects such the development of aschronic hemolytic anemia caused by the excess lysis of host erythrocytes. Interestingly, a preparation containing RG-I and HG isolated from *Angelica acutiloba* (Siebold & Zucc.) Kitag. has been shown to induce anti-complementary activity (Kiyohara et al., 1988). This preparation was composed of 90% α-D-Gal*p*A residues with some α-L-Rha*p* residues, and neutral polysaccharide side chains attached to O-4 of the α-L-Rha*p* residues. Treatment of this anti-complementary pectin with *endo*-α-(1→4)-polygalacturonanase hydrolysed the HG domains, but the RG-1 domains, rich in neutral polysaccharide side chains, were enzyme resistant. Although the oligogalacturonides derived from enzyme-sensitive HG had weak or neglible activity, the RG-I domains had potent anti-complementary activity (Kiyohara et al., 1988).

Similar studies have also been carried out on the neutral polysaccharide side chains of RG I from leaves of *Panax ginseng* C.A.Mey (Gao et al., 1988; Gao et al., 1990), roots of *A. acutiloba* (Yamada et al., 1985a) and leaves of *Plantago major* L. (Samuelsen et al., 1996). An arabinogalactan Type II with a β-(1→3)-galactan backbone and α-L-arabinofuranosyl (α-L-Ara*f*) residues linked at O-6 was shown to have the greatest effect on the complement system by neutralising the immune complement; it, therefore, has potent anti-complementary activity (Samulsen et al., 1996). Some of the analyses of these active fractions showed a high proportion of (1→6)-linked β-D-Gal*p* residues. The α-(1→5)-arabinans from fruits of *Zizyphus jujube* Lam. and roots of *Bupleurum falcatum* L. have also been shown to activate the complement system through both the alternative pathway and classical pathway, initiated by antibody and formation of antigen-antibody complexes, respectively (Yamada et al., 1985b; Yamada et al., 1988).

Immunomodulatory activities have also been reported for pectins with RG-I domains bearing arabinogalactan Type I and II side chains. For example, the GOA2-I fraction of the Malian medicinal plant (*Glinus oppositifolius* (L.) Aug. DC.) was found to induce B-cell proliferation and upregulate the expression of the gene IL-1β and IFN-γ (Inngjerdingen et al., 2007). Clinical studies showed that oral administration of modified citrus pectin lengthened the doubling time of prostate specific antigen (PSA) in prostate cancer patients, thus slower cancer progression (Guess et al., 2003). In addition, Jackson et al. (2007) reported that citrus pectin that had been treated by heating to 123 °C induced apoptosis in human prostate cancer cells. However, it remains unclear what structural or physical changes occurred on heating the pectin and it appears that not all citrus pectins are effective against prostate cancer cells.

Several studies have also shown that oral administration of pectins can relieve gastric related symptoms, cause detoxification and lower serum-cholesterol concentration (Durrington et al., 1976; Brown & Juhl, 1979; Holt et al., 1979; Leed et al., 1981; Kiyohara et al., 1994).

 ISSN 0048 - 0134

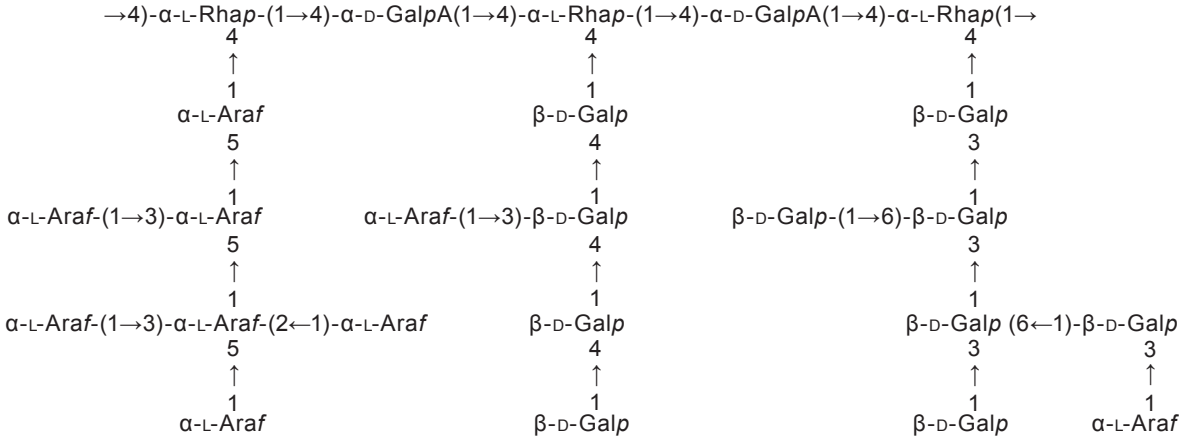
FIGURE 1: Structures of pectins from plant cell walls (modified from Harris, 2005 and Harris & Stone, 2008).

Pectins

Homogalacturonan

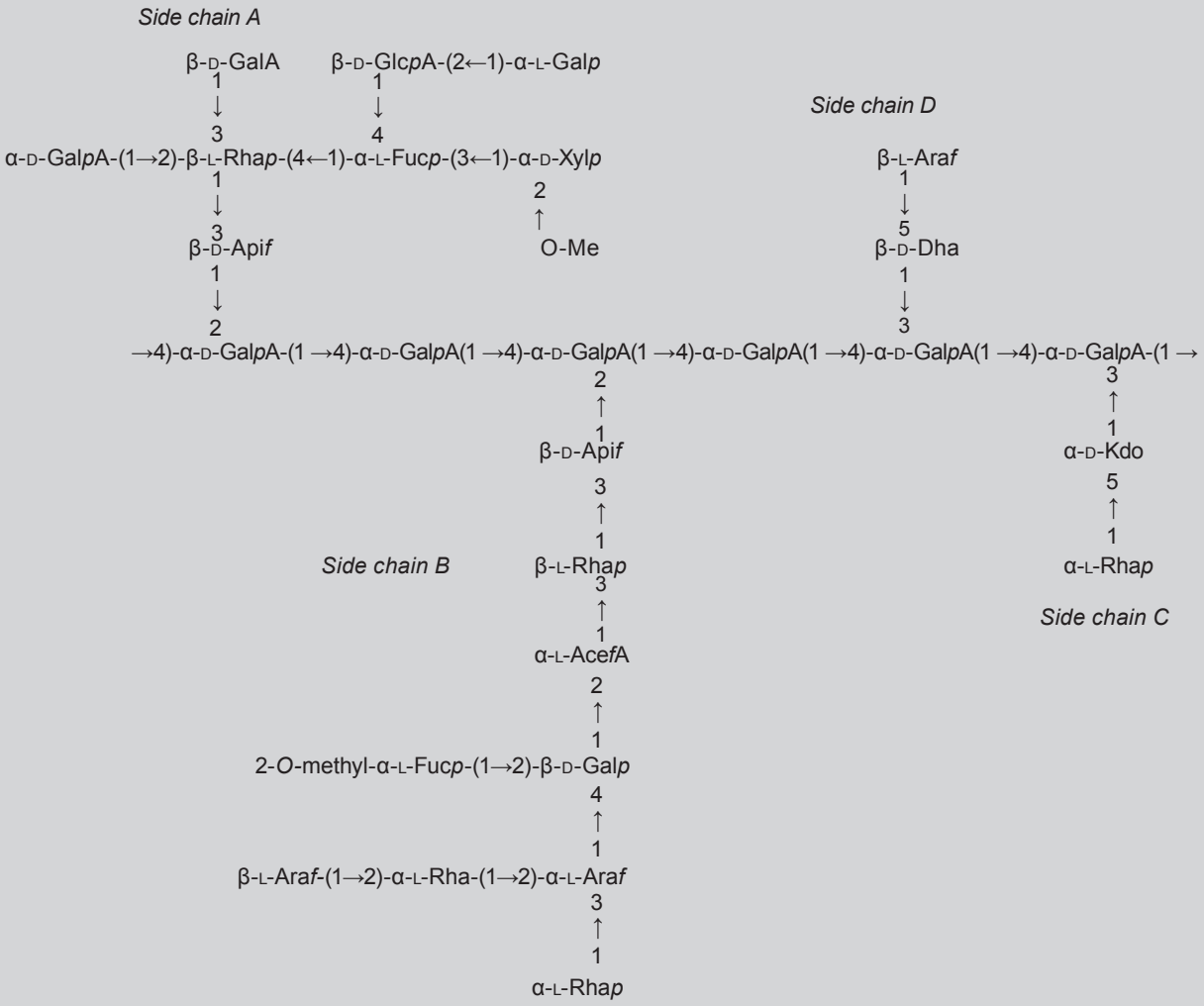
→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1

Rhamnogalacturonan I



Arabinan *Arabinogalactan Type I* *Arabinogalactan Type II*

Rhamnogalacturonan II

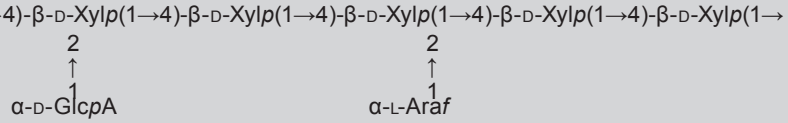


 ISSN 0048 - 0134

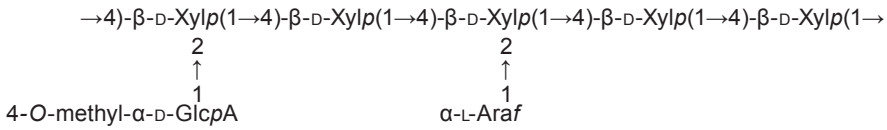
FIGURE 2: Structures of heteroxylans, xyloglucans, and heteromannns from plant cell walls (modified from Harris, 2005 and Harris & Stone, 2008).

Heteroxylans

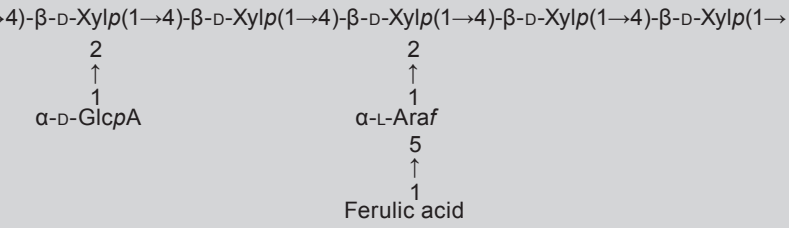
Glucuronoarabinoxylan (GAX)



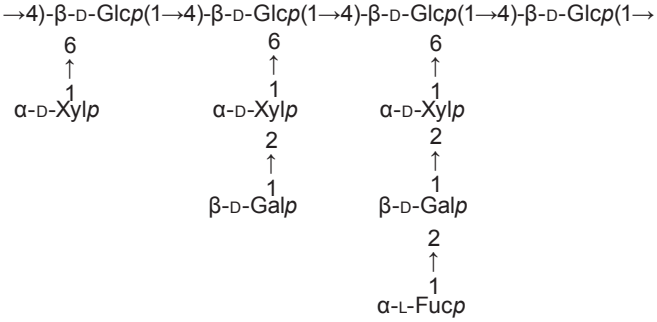
4-O-methyl glucuronoarabinoxylans



Feruloylated glucuronoarabinoxylans



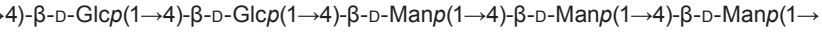
Xyloglucans



Heteromannans

Mannan

Glucomannan



 ISSN 0048 - 0134

Heteroxylans

There are only a few reports on the biological activities of heteroxylans because it is challenging to extract these polysaccharides from cell wall preparations. To gain quantitative yields, strong alkalis, such as 4 M potassium hydroxide or 6 M sodium hydroxide, are required to break hydrogen-bonds that link these molecules to cellulose microfibrils. Heteroxylans have linear backbones of β-D-Xyl*p* residues, some of which are substituted with single α-D-glucuronopyranosyluronic acid (or its 4-*O*-methyl derivative) residues at O-2 of β-D-Xyl*p*, and/or with single α-L-Ara*f* residues at O-2 or O-3 of β-D-Xyl*p,* see Figure 2 (Whistler et al., 1954; Hoffmann et al., 1992). Oligosaccharide side chains have also been reported in the glucuronoarabinoxylans of cereals. For example, L-Gal*p*-(1→4)-D-Xyl*p*-(1→2)-L-Ara*f* was found attached at O-2 of the backbone β-D-Xyl*p* residues of glucuronoarabinoxylans from maize hulls (Whistler & Corbett, 1955). The sugar L-Gal*p* rarely occurs in cell wall polysaccharides, but has also been found in the side chains of rhamnogalacturonan II (O'Neill et al., 2004).

Glucuronoarabinoxylans occur in the primary cell walls of a group of plants known as commelinid monocotyledons and have ester-linked ferulic acid attached, see Figure 2 (Harris, 2005). These glucuronoarabinoxylans have been extracted from wheat (*Triticum aestivum* L.) cell walls and were found to have potent antioxidant activity due to the ferulic acid (Ferguson et al. 2003; Yuan et al., 2005). The biological activity of ferulic acid has been studied in cultured human HT-29 cells, including its activity against DNA and chromosomal breakage. It was suggested that free ferulic acid released from cell walls by esterases in the human colon may play an important role in protection against colorectal cancer (Ferguson et al., 2001; Ferguson et al., 2005).

Extracts containing 4-*O*-methyl glucuronoarabinoxylans from the roots of *Echinacea purpurea* Moench were tested on granulocytes *in vitro* and were shown to enhance phagocytotic activities by up to 23% at extract concentrations of 1-100 µg/mL (Proksch & Wagner, 1987).

Xyloglucans

Xyloglucans also have to be extracted from cell walls using strong alkali. They have backbones composed of (1→4)-linear β-D-glucopyranosyl (β-D-Glc*p*) residues substituted at O-6 with α-D-Xyl*p* residues, Figure 2. Kato et al. (2001) reported that fucogalactoxyloglucans, the most commonly occurring type, induced cytotoxicity in the model colon cancer cell line COLO 205; no activities was shown in xyloglucans without α-L-Fuc*p* or β-D-Gal*p* residues. This provided the first evidence that these monosaccharides in the side chains of xyloglucans play an important role in the cytotoxicity (Kato et al., 2001).

Heteromannans

Glucomannans are composed of both β-D-Glc*p* and β-D-Man*p* residues joined by (1→4) linkages in a linear chain (Figure 2). They are often present in large proportions in the viscous hydrogel of several medicinal orchids. Acemannan (acetylated glucomannan) extracted from the succulent leaves of *Aloe* spp. has shown significant clinical activity in wound healing (Mandal & Das, 1980; Paulsen, 2001). *In vitro* experiments have indicated that acemannan stimulated the production of mouse macrophage cytokine IL-6 and TNF-α (Zhang & Tizard, 1996). Heteromannans have also been shown to be effective adjuvants in avian vaccination against avain viral and tumoural diseases *in vivo* (Djeraba & Quere, 2000).

The detailed structures of the glucomannans extracted from the stems of the medicinal orchid *Dendrobium huoshanense* Z.Z.Tang & S.J.Cheng have been studied. The ratio of β-D-Man*p* to β-D-Glc*p* was found to be 5 : 1 and acetyl groups were found attached at O-2 of the β-D-Man*p* residues (Hsieh et al., 2008). The effects of *D. huoshanense* acetyl glucomannan on immune cytokines were studied and compared with the glucomannans from konjac (*Amorphophallus konjac* K.Koch) and a pure mannan, the (1→4)-β-D-mannan from ivory nut (*Phytelephas macrocarpa* Ruiz. & Pav.). Cytokine profiling indicated a significant increase in the expression of IFN-γ, IL-10, IL-6, and IL-1α. Significant amounts of hematipoietic growth factors GM-CSG and G-CSF were also induced in a dose-dependent manner, whereas konjac glucomannan and mannan from ivory nut failed to induce cytokine expression.

Conclusions

For decades, polysaccharides have been studied mostly for their structural and energy-storage roles. More recently, some polysaccharides have been shown to modulate immuno-activities. However, there is much still to be learnt. In particular, we need to understand the relationship between polysaccharide structure and biological recognition and activities. Certain fragments isolated from cell wall polysaccharides are valuable as they can lead to specific biological events, for example the anti-complement activity induced by Type II arabinogalactans of pectins and the expression of several cytokines induced by 2-*O*-acetyl glucomannans. The use of cell wall polysaccharides in carbohydrate-based drug discovery is promising.

Acknowledgements

Hsieh Y.S.Y would like to thank University of Auckland Doctoral Scholarship and The Genomic Research Center, Academia Sinica for supporting this project. We would like to thank Professor P. J. Harris for critically reading the manuscript.

 ISSN 0048 - 0134

References

Brown, D. D., & Juhl, R. P. (1976). Decreased bioavailability of digoxin due to antacids and kaolin-pectin. *The New England Journal of Medicine, 295*, 1034-1037.

Darshan, S., & Doreswamy, R. (2004). Patented anti-inflammatory plant drug development from traditional medicine. *Phytotherapy Research, 18*, 343-357.

Djeraba. A., & Quere, P. (2000). *In vivo* macrophage activation in chickens with acemannan, a complex carbohydrate extracted from *Aloe vera*. *International Journal of Immunopharmacology, 22*, 365-372.

Durrington, P. M., Manning, A. P., Bolton, C. H., & Hartog, M. (1976). Effect of pectin in serum lipids and lipoproteins, whole-gut transit-time, and stool weight. *The Lancet, 21*, 394-396.

Ferguson, L. R., Chaven, R. R., & Harris, P. J. (2001). Changing concepts of dietary fiber: implications for carcinogenesis. *Nutrition and Cancer, 39*, 155-169.

Ferguson, L. R., Lim, I. F., Pearson, A. E., Ralph, J., & Harris, P. J. (2003). Bacterial anitmutagenesis by hydroxycinnamic acids from plant cell walls. *Mutation Research, 542*, 49-58.

Ferguson, L. R., Zhu, S. T., & Harris, P. J. (2005). Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells. *Molecular Nutrition & Food Research, 49*, 585-593.

Gao, Q. P., Kiyohara, H., Cyong, J. C., & Yamada, H. (1988). Characterisation of anti-complementary acidic heteroglycans from the leaves of *Panax ginseng* C.A.Meyer. *Carbohydrate Research, 181*, 175-187.

Gao, Q. P., Kiyohara, H., & Yamada, H. (1990). Further structural studies of anti-complementary acidic heteroglycans from the leaves of *Panax ginseng* C.A.Meyer. *Carbohydrate Research, 196*, 111-125

Glushka, J. N., Terrell, M., York, W. S., O'Neill, M. A., Gucwa, A., Darvill, A. G., Albersheim, P., & Prestegard, J.H. (2003). Primary structure of the 2-*O*-methyl-α-L-fucose-containing side chain of the pectic polysaccharide, rhamnogalacturonan II. *Carbohydrate Research, 338*, 341-352.

Guess, B. W., Scholz, M. C., Strom, S. B., Lam, R. Y., Johnson, H. J. & Jennrich, R.I. (2003). Modified citrus pectin (MCP) increases the prostate-specific antigen doubling time in men with prostate cancer: a phase II pilot study. *Prostate Cancer and Prostatic Diseases, 6,* 301-304

Harris, P. J. (2005). Diversity in plant cell walls. In R. J. Henry (Ed.), *Plant diversity and evolution: genotypic and phenotypic variation in higher plants* (pp. 201-227). Wallingford, Oxon, UK: CAB International Publishing.

Harris, P. J., & Smith, B. G. (2006). Plant cell walls and cell-wall polysaccharides: structures, properties and uses in food products. *International Journal of Food Science and Technology, 41*, 129-143.

Harris, P. J., & Stone, B. A. (2008). Chemistry and molecular organization of plant cell walls. In M. E. Himmel (Ed.), *Biomass recalcitrance: deconstructing the plant cell wall for bioenergy* (pp. 61-93). Oxford: Blackwell Publishing.

Hoffmann, R. A., Wijk, J. V., Leeflang, B. R., Kamering, C., Altona, C., & Vliegethart, J. F. G. (1992). Conformational analysis of the α-L-arabinofuranosides present in wheat arabinoxylans from proton-proton coupling constants. *Journal of the American Chemical Society, 114*, 3710-3714.

Hsieh, Y. S. Y., Chein, C., Lia, S. K. S., Liao, S. F., Hung, W. T., Yang, W. B., Lin, C. C., Chen, T. J., Chang, C. C., Fang, J M., & Wong. C. H. (2008). Structure and bioactivity of polysaccharides in medicinal plant *Dendrobium huoshanense*. *Bioorganic & Medicinal Chemistry, 16*, 6054-6068.

Holt, S., Heading, R. C., Carter, D. C., Prescott, L .F., Laurence, F., & Tothill, P. (1979). Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol. *The Lancet, 313*, 636-639.

Inngjerdingen, K. T., Patel, T., Xinyong, C., Lennart, K., Stephanie, A., Morris, G. A., Harding, S. E., Matsumoto, T., Diallo, D., Yamada, H., Michaelsen, T. E., & Ingjerdingen, M. (2007). Immunological and structural properties of a pectic polymer from *Glinus oppositifolius*. *Glycobiology, 17*, 1299-1310.

Jackson, C. L., Dreaden, T. M., Theobald, L. K., Tran, N. M., Beal, T. L., Eid, M., Gao, M. Y., Shirley, R. B., Stoffel, M. T., Kumar, M. V., & Mohnen, D. (2007). Pectins induces apoptosis in human prostate cancer cells: correlation of apoptotic function with pectin structure. *Glycobiology, 17*, 805-819.

Kato, Y., Uchida, J., Ito, S., & Mitshishi, Y. (2001). Structural analysis of the oligosaccharide units of xyloglucan and their effects on growth

 ISSN 0048 - 0134

of COLO 201 human tumor cells. In M. Endo, S. Harata, Y. Saito, A. Munukata, M. Sasaki, & S. Tsuchida (Eds), *New developments in glycomedicine* (pp. 161-164). Amsterdam: Elsevier Science.

Kiyohara, H., Cyong, J. C., & Yamada, H. (1988). Structure and anti-complementary activity of pectic polysaccharides isolated from the root of *Angelica acutiloba* Kitagawa. *Carbohydrate Research, 182*, 259-275.

Kiyohara, H., Hirano, M., Wen, X. G., Matsumoto, T., Sun, X. B., & Yamada, H., (1994). Characterisastion of an anti-ulcer pectic polysaccharide from leaves of *Panax ginseng* C.A.Meyer. *Carbohydrate Research, 263*, 89-101.

Leed, A. R., Ralphs, D. N., Ebied, F., Metz, G., & Dilawari, J. B. (1981). Pectin in the dumpling syndrome: reduction of symptoms and plasma volume changes. *The Lancet, 16*, 1075-1078.

Mandal, G., & Das, A. (1980). Structure of the glucomannan isolated from the leaves of *Aloe barbadensis* Miller. *Carbohydrate Research, 87*, 249-256.

O'Neill, M. A., Ishii, T., Albersheim, A., & Darvill, A. G. (2004). Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review of Plant Biology, 55*, 109-139.

Paulsen, B. S. (2001). Plant polysaccharides with immunostimulatory activities. *Current Organic Chemistry, 5*, 939-950.

Prosksch, A., & Wagner, H. (1987). Structural analysis of a 4-*O*-methyl glucuronoarabinoxylans with immune-stimulating activity from *Echinacea purpurea*. *Phytochemistry, 26*, 1989-1993.

Sakurai, M. H., Matsumoto, T., Kiyohara, H., & Yamada, H. (1999). B-cell proliferation activity of pectic polysaccharide from a medicinal herb, the roots of *Bupleurum falcatum* L. and its structural requirement. *Immunology, 97*, 540-547.

Samuelsen, A. B., Paulsen, B. S., Wold, J. K, Otsuka, H., Kiyohara, H., Yamada, H., & Knutsen, S. H. (1996). Characterization of a biologically active pectin from *Plantago major* L. *Carbohydrate Polymers, 30*, 37-44.

Tungland, B. C., & Meyer, D. (2002). Nondigestible oligo- and polysaccharides (dietary fiber): their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety, 1*, 73-92.

Whistler, R. L., Conrad, E., & Hough, L., (1954). 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose from hemicellulose-B of corn cob. *Journal of the American Chemical Society, 76*, 1668-1670.

Whistler, R. L., & Corbett, W. M. (1955). Oligosaccharides from partial acid hydrolysis of corn fiber hemicellulose. *Journal of the American Chemical Society, 77*, 6328-6330.

Williats, W. G. T., McCartney, L., Mackie, W. & Knox, J. P. (2001). Pectin: cell biology and prospects for functional analysis. *Plant Molecular Biology, 47*, 9-27.

Yamada, H., Nagai, T., Cyong, J. C., & Otsuka, Y. (1985a). Studies on polysaccharides from *Angelica acutiloba*-IV. Characterization of an anti-complementary arabinogalactan from the roots of *Angelica acutiloba* Kitagawa. *Molecular Immunology, 22*, 295-304.

Yamada, H., Nagai, T., Cyong, J. C., Otsuka, Y., Tomoda, M., Shimizu, N., & Shimada,K. (1985b). Relationship between chemical structure and anti-complementary activity of plant polysaccharides. *Carbohydrate Research, 144*, 101-111.

Yamada, H., Ra, K. S., Kiyohara, H., Cyong, J. C., Yang, H. C., & Otsuka, Y. (1988). Characterization of anti-complementary neutral polysaccharides. *Phytochemistry, 27*, 3163-3168.

Yuan, X., Wang, J., & Yao, H. (2005). Antioxidant activity of feruloylated oligosaccharides from wheat bran. *Food Chemistry, 90*, 759-764.

Zhang, L., & Tizard, I. R. (1996). Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. *Immunopharmacology, 35*, 119-128

 ISSN 0048 - 0134