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Carbohydrate Polymers 56 (2004) 471–481
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Water-soluble polysaccharides with pharmaceutical importance from Durian rinds (*Durio zibethinus* Murr.): isolation, fractionation, characterisation and bioactivity

Sanya Hokputsa[a,*], Waraporn Gerddit[b], Sunanta Pongsamart[b], Kari Inngjerdingen[c], Thomas Heinze[d], Andreas Koschella[d], Stephen E. Harding[a], Berit S. Paulsen[c]

[a]*The NCMH Physical Biochemistry Laboratory, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, UK*
[b]*Department of Biochemistry, Faculty of Pharmaceutical sciences, Chulalongkorn University, Bangkok, 10330, Thailand*
[c]*Institute of Pharmacy, Department of Pharmacognosy, P.O.Box 1068, Blindern, N-0316 Oslo, Norway*
[d]*Kompetenzzentrum für Polysaccharidforschung an der Friedrich-Schiller-Universität Jena, Lessingstrasse 8, D-07743, Germany*

Received 3 December 2003; revised 1 March 2004; accepted 25 March 2004
Available online 28 May 2004

Abstract

Crude, pharmaceutically useful water-soluble polysaccharides have been isolated from durian rinds (*Durio zibethinus*) by hot water extraction followed by ethanol precipitation. The polysaccharides were fractionated by anion exchange chromatography and size exclusion chromatography. Characterisation of the sub-fractions by methanolysis, methylation analysis and NMR spectroscopy revealed that the principal components were pectic polysaccharides with starch as a contaminant. Physical features namely molecular weight (M_w) and intrinsic viscosity of the main fractions were investigated by size exclusion chromatography coupled to multi angle laser light scattering (SEC/MALLS) and capillary viscometer, respectively. The main fractions were subjected to the complement-fixation assay and the relationship of the chemical features of the polysaccharide fractions with their activity was also considered.

Keywords: *Durio zibethinus*; Durian; Pectic polysaccharide

1. Introduction

Durian (*Durio zibethinus* Murr.), a tropical fruit native to Southeast Asia, is one of the most highly valued and desired fruits among Southeast Asians due to its distinct flavour and unique taste. The fruit is ovoid or ovoid-oblong to nearly round shaped with an average size weighing between 2 and 4.5 kg depending on their varieties. The rind which usually weighs more than half of the total fruit weight, is green to yellowish brown, thick and semi-woody with sharply pointed pyramidal thorns.

Thailand is one of the primary producers and a world exporter of fresh and frozen durian. Beginning in May and extending through August, the durian seasonally announces its presence in Thai market and massive amounts of the rind is disposed of as waste which could lead to environmental problems.

In the interest of the environment, attempts have recently been made to use this agricultural waste as a source of valuable materials of commercial importance; for example, Khedari and colleagues developed a new particleboard as a component of construction panels for energy conservation in building from durian rinds (Khedari, Charoenvai, & Hirunlabh, 2003). Previous studies dedicated by Pongsamart and co-workers have isolated the water-insoluble and water-soluble polysaccharides from the rinds, which have been found potential as pharmaceutical excipients. Their applications such as a tablet binder, tablet disintegrator and gelling agent have been well reported (Pongsamart & Panmaung, 1998; Umprayn, Chanpaparp, & Pongsamart, 1990a,b).

Further investigations have also shown that the crude water-soluble polysaccharides have antibacterial activities

* Corresponding author. Present address: The Government Pharmaceutical Organization, R&D Institute, Phytochemical Research Laboratory, 75/1 Rama VI Rd. Rajthevi, Bangkok, 10400, Thailand. Tel.: +66-22038121; fax: +66-22462134.
E-mail address: sbxsh1@nottingham.ac.uk (S. Hokputsa).

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doi:10.1016/j.carbpol.2004.03.018

against certain strains of gram positive and negative bacteria (Lipipun, Nantawanit, & Pongsamart, 2002). Dressing film can be prepared from the water-soluble polysaccharides and in vivo study demonstrated that the dressing films enhanced wound healing in pig skin.

Because the water-soluble polysaccharides isolated from the rinds of durian have a variety of pharmaceutical benefits, it would be of some interest to investigate the chemical and hydrodynamic properties of the polysaccharides since these properties are of fundamental importance for further research. The detailed structure of the water-soluble polysaccharides present in the crude polysaccharide has not yet been elucidated.

In the present study after a detailed analysis of the chemical composition we report on its hydrodynamic properties and in relation to the complement-fixing activity of these polysaccharides.

2. Materials and methods

2.1. Extraction of polysaccharides

Ground fresh fruit rinds were dried in hot-air oven at 60 °C, which gave 19.6 ± 1.4% w/w of dried material. The dried sample was extracted with water at 90–100 °C adjusted to pH 4.5 with citric acid and purified as described previously (Pongsamart & Panmaung, 1998) as illustrated in Fig. 1. Briefly, the water extract was concentrated under reduced pressure and the gel-like precipitate was obtained by addition of acidified aqueous ethanol. The precipitate was collected by filtration, dried and ground. The dried precipitate was re-dissolved with water and afterwards concentrated. To the concentrated solution, aqueous ethanol was added and the precipitate was recovered. The precipitate was washed twice with 75% ethanol and then 95% ethanol, then dried, ground and sieved (designated as PG).

2.2. Fractionation

Anion-exchange chromatography: PG (3.7 g) was fractionated by anion exchange chromatography on a column (22 cm × 5 cm) of DEAE-Sepharose Fast-flow (Pharmacia) previously regenerated with 1 M NaCl and equilibrated with distilled water. The sample was dissolved in distilled water and left overnight. The solution was filtered through a 0.8 μm membrane filter and applied onto the column coupled to a P-3 peristaltic pump (Pharmacia) at a flow rate of 0.5 ml/min. The column was first eluted with distilled water at a speed of 1.0 ml/min followed by NaCl solution gradient (0 → 2 M). Fractions of 10 ml were collected using a Pharmacia LKB-Suprafrac fraction collector and monitored for the presence of carbohydrate using the phenol–sulphuric acid assay (Chaplin, 1994). Fractions containing carbohydrate from the elution step were pooled, dialysed against distilled water in a Spectrapor dialysis tubing (molecular weight cut off = 3500 Da) and lyophilised. PG was thus separated into two main fractions (PG1 and PG2) as shown in Fig. 2.

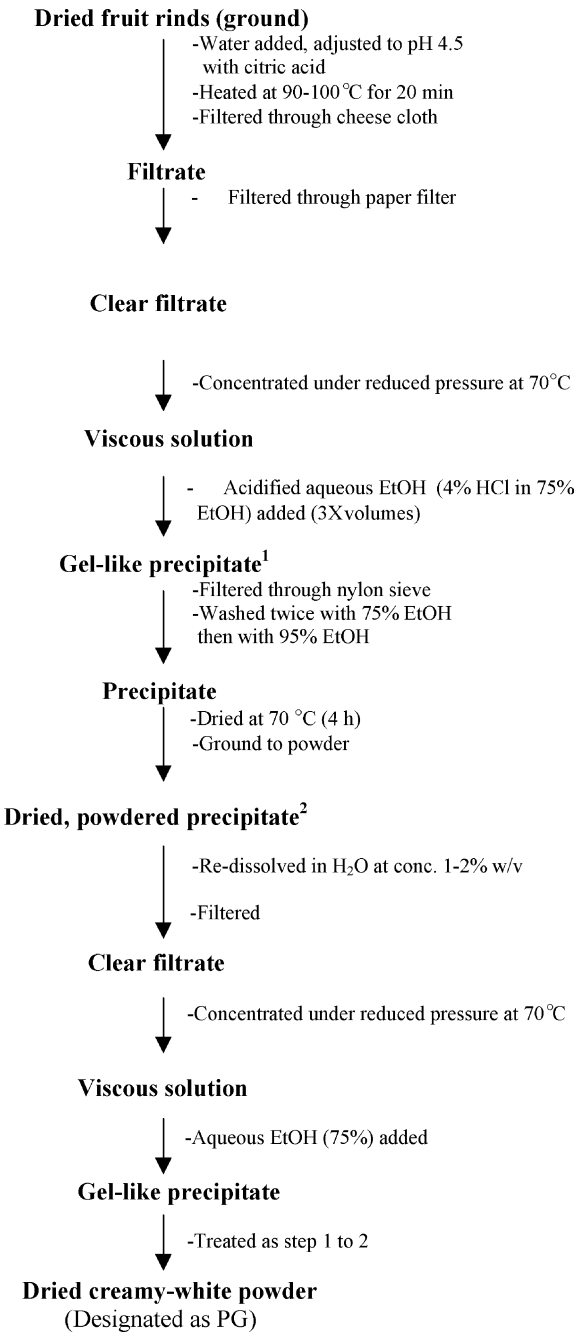

Fig. 1. Schematic presentation of isolation of water-soluble polysaccharides (PG) from *Durio zibethinus* Murr. Rinds.

Size exclusion chromatography: PG1 and PG2 were further fractionated on a column (80 cm × 3 cm) of Sephacryl 400, eluted with 10 mM NaCl. Fractions containing carbohydrate were pooled, dialysed and lyophilised. The SEC elution profiles for PG1 and PG2 are as Figs. 3 and 4, respectively.

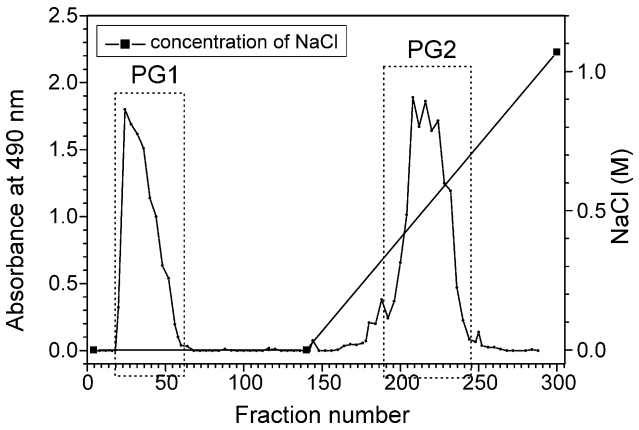

Fig. 2. Fractionation profile of PG on DEAE-Sepharose Fast-flow.

2.3. Quantitative determination of protein content

The protein content of the samples was determined by Micro BCA™ Protein Assay Reagent Kit (US Patent No. 4,839,295; Pierce, Rockford, IL) using albumin as protein standard.

2.4. Determination of the monosaccharide composition of polysaccharides

The sugar composition analysis was determined by methanolysis and gas chromatography (GC). Briefly, the polysaccharide samples were subjected to methanolysis with 4 M HCl in anhydrous methanol for 24 h at 80 °C. Mannitol was added as an internal standard followed by trimethylsilylation. The trimethylsilylated samples were subjected to gas chromatographic analysis (Samuelsen et al., 1995).

2.5. Determination of the glycosidic linkage composition of polysaccharides

Prior to methylation, PG1 and PG2 were reduced to the corresponding neutral sugars on the polymer level (Sims & Bacic, 1995). Methylation of the polysaccharides were carried out by the following procedure. The free hydroxyl groups in the polysaccharides were deprotonated by a mixture of sodium hydroxide and dimethyl sulphoxide and subsequently methylated with methyl iodide. The fully methylated polysaccharides were then hydrolysed with 2.5 M trifluoroacetic acid, followed by reduction of the partially methylated sugars to the corresponding partly methylated alditols using sodium borodeuteride. Those originally involved in a linkage are finally acetylated to give partly acetylated, partly methylated alditols (Kim & Carpita, 1992) followed by gas chromatography-mass spectroscopy (GC-MS) analysis of the derived partially methylated alditol acetates (Barsett, Paulsen, & Habte, 1992;Samuelsen et al., 1995).

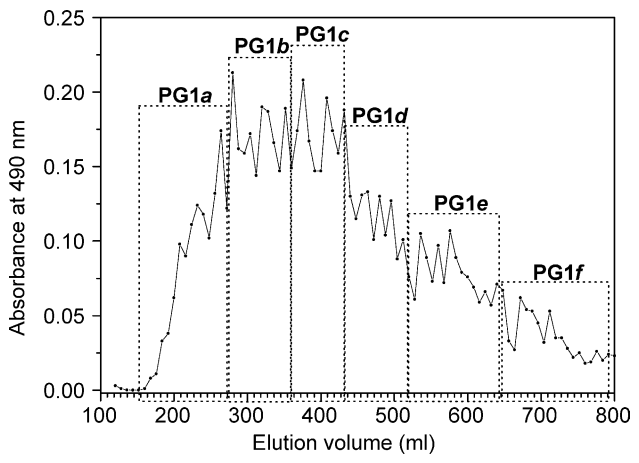

Fig. 3. Fractionation profile of PG1 on Sephacryl 400.

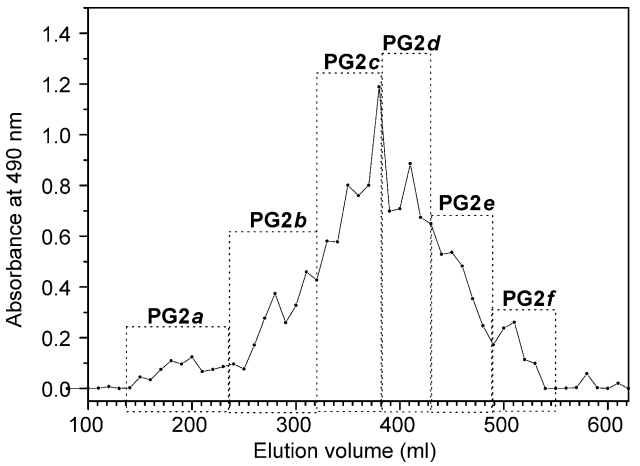

Fig. 4. Fractionation profile of PG2 on Sephacryl 400.

2.6. ^{1}H and ^{13}C-NMR

Samples PG1 and PG2 were dissolved in D_2O at concentrations of approximately 30 mg/ml. ^{1}H and ^{13}C-NMR spectroscopy were performed with a Bruker DRX-400 spectrometer at 400.2 and 100.6 MHz, respectively, at 70 °C. The chemical shifts were expressed in ppm relative to the resonance of the internal standard 3-trimethylsilyl-1-propanesulfonic acid (sodium salt).

2.7. Homogeneity and molecular weight determination

Homogeneity and molecular weights of the polysaccharide fractions were determined by size exclusion chromatography coupled to multi-angle laser light scattering (SEC-MALLS). The chromatography consisted of a HPLC pump (Model PU-1580, Jasco corp, Tokyo, Japan), a Rheodyne injection valve (Model 7125, Rheodyne, St Louise, MS) fitted with a 100 μl loop and the following column system: Phenomenex guard column, TSK G6000PW and TSK G4000PW connected in series. Samples were dissolved at a concentration of 1 mg/ml for PG1 subfractions and 5 mg/ml for other samples in phosphate

buffered saline, pH 7.0, $I = 0.1$ buffer (Green, 1933). Sample solutions (injection volume 100 μl) were injected onto the column system at room temperature. An eluent identical to the solvent used to dissolve the samples was pumped at flow rate of 0.8 ml/min and monitored using a Dawn DSP multi-angle laser light scattering photometer and an Optilab 903 interferometric refractometer (both instruments from Wyatt Technology, Santa Barbara, CA). Signals from the light scattering photometer and the refractometer were captured and analysed on a PC using the dedicated ASTRA™ software supplied by the manufacturer.

2.8. Intrinsic viscosity

Measurements on all samples ranging from 0.5 to 5 mg/ml were performed using an Automated Viscosity Measuring Unit (AVS 310, Schott Geräte, Hofheim, Germany) at a temperature of (20.00 ± 0.01) °C.

The relative, η_{rel}, and reduced viscosities, η_{red} were calculated from

$$\eta_{rel} = \left(\frac{t}{t_0}\right)\left(\frac{\rho}{\rho_0}\right) \approx \frac{t}{t_0}$$

where t, and ρ refer to the flow time and density for polysaccharide samples, respectively, and t_0 and ρ_0 to solvent flow time and density. At the low concentrations used, the density correction is not required and η_{rel} can be calculated from t/t_0 (Harding, 1997). The reduced specific viscosities were found from

$$\eta_{red} = (\eta_{rel} - 1)/c$$

where c is the concentration of polysaccharide (g/ml). The value of η_{red} when extrapolated to zero concentration is defined as intrinsic viscosity $[\eta]$ (ml/g).

2.9. Complement fixing activity of the polysaccharides

Samples were subjected to a complement-fixation assay (Method A) (Michaelsen, Gilje, Samuelsen, Hogasen, & Paulsen, 2000) using polysaccharide fraction PM II from *Plantago major* as a positive control (Samuelsen et al., 1995).

3. Results and discussion

3.1. Isolation and fractionation

Extraction of crude water-soluble polysaccharides (PG) yielded approximately 10% w/w of dried durian fruit rinds as creamy white powder according to the procedure as shown in Fig. 1. PG was further fractionated by ion exchange column chromatography (IEC) on a DEAE-Sepharose Fast-flow column eluted with water and NaCl (0 → 2 M) gradient. PG was separated into two main fractions, PG1 and PG2. PG1 was eluted with water whereas PG2 was eluted with NaCl solution ranging from 0.3 to 0.7 M (see Fig. 2). Appearance, yield, protein content and sugar composition of PG and its fractions obtained after anion exchange chromatography are given in Table 1. All samples had low protein content (less than 3%) but the previous elemental analysis showed that nitrogen was not detected in PG (Pongsamart & Panmaung, 1998). The protein assay used is based on the reduction of copper by proteins; there might be reducing agents/sugars present in the samples which also reduce copper. Hence the cumulative effect could give an exaggerated protein content. However, the protein content in samples seemed negligible which is not taken into further consideration.

Table 1
Appearance, yield, protein content and sugar composition (mol%) of the crude water-soluble polysaccharides (PG) and its polysaccharide fractions (PG1 and PG2) obtained after separation on the DEAE-Sepharose Fast-flow column

	PG	PG1	PG2
Appearance	creamy white powder	slightly cream, fluffy	white, fluffy
Yield[a]	10.0	22.0	68.6
Protein content (w/w)	2.6	1.4	2.4
Sugar composition[b]			
Ara	1.2	2.2	0.7
Rha	4.8	10.7	2.1
Xyl	0.4	0.8	-
Gal	4.9	8.1	4.9
Glc	20.9	43.3	6.0
GalA	67.9	34.9	86.2

[a] Calculated as weight% of applied material.
[b] Mol% of total carbohydrate content.

3.2. Structural features

A previous study showed the presence of fructose in acid hydrolyzate of PG by thin layer chromatography and high performance liquid chromatography, however, the amount of fructose has not yet determined (Pongsamart & Panmaung, 1998). Hence, the amount of fructose which can not be determined by methanolysis, was quantitatively determined by a separate assay, the phenol-boric-sulfuric acid assay for ketose (Chaplin, 1994) and it was found that the amount of fructose in PG, PG1 and PG2 were 3.7, 6.0 and 1.9%, respectively.

The glycosidic linkage present in PG1 and PG2 determined by methylation and GC-MS are given in Table 2. Due to the presence of galacturonic acid and glucose as the major components in comparable amounts in PG1, we would like to suggest that PG1 is most likely comprised of a mixture of polysaccharides, mainly pectic polysaccharides and a glucan. A high amount of (1 → 4) linked galacturonic acid confirms the presence of pectic polysaccharide in PG1. Rhamnose residues were most likely incorporated in the pectin due to the presence of (1 → 2)

Table 2
Linkage composition (mol% of total carbohydrate content) in the polysaccharides fractions isolated from *D. zibethinus*

Sugar and linkage type	PG1 (neutral fraction)	PG2 (acidic fraction)
Ara T*f*	0.8	0.2
1 → 2	1.3	0.3
1 → 3	–	0.2
1 → 5	0.1	trace
1 → 3,5	–	0.1
Rha T	3.8	1.4
1 → 2	3.1	–
1 → 2,4	3.8	0.7
Xyl T	trace	–
Gal T	2.8	1.1
1 → 4	2.8	–
1 → 6	0.5	0.3
1 → 3	1.2	1.3
1 → 3,6	0.7	2.3
Fru T	n.d	trace
2 → 1	n.d	trace
Glc T	9.2	1.6
1 → 4	25.3	4.0
1 → 4,6	8.5	0.5
GalA T	1.7	9.9
1 → 4	31.8	71.0
1 → 3,4	1.6	2.8

n.d, not determined.

linked rhamnose residues which are normally inserted in the galacturonan backbone giving rise to a rhamnogalacturonan backbone, a typical feature of pectic polysaccharide. Galactose and arabinose residues were probably attached to the rhamnogalacturonan backbone as neutral sugar side chains in pectic polysaccharide because these sugars are commonly found in the pectin and usually linked to *O*-4 of the rhamnose residues, which matched with result from methylation analysis where rhamnose residues with (1 → 2,4) linkage were found. Glucose units in PG1 were mostly (1 → 4)-linked and had a branch point at C-6 and strong positive test with iodine-KI for PG1 was observed suggesting that most of the glucan in PG1 is starch. It was found from literatures that starch is commonly extracted along with pectin, especially when pectin is isolated at high temperature (Iagher, Reicher, & Ganter, 2002; Kravtchenko, Voragen, & Pilnik, 1992; Mukhiddinov, Khalikov, Abdusamiev, & Avloev, 2000). From methylation analysis, we came to know that fructose is present as a terminal non-reducing sugar and also as (2 → 1)-linked. However, the amount of each linkage was not determined due to the peaks corresponding to the fructose units were not resolved (data not shown). But this suggests that PG1 may contain fructans which are the most abundant non-structural polysaccharides after starch found naturally in a wide variety of plants (Franck & De Leenheer, 2002) as a minor component (approx 6% w/w according to the phenol-boric-sulfuric acid assay as mentioned earlier).

PG2 was composed principally of galacturonic acid (80% of total carbohydrate content) and most of which is (1 → 4)-linked. Due to the dominant feature of PG2 consisting mainly of a linear chain of (1 → 4)-linked galacturonic acid units (polygalacturonic acids), it is also a pectic polysaccharide. Rhamnose, galactose and arabinose residues in PG2 were encompassed in the polygalacturonic acids or smooth region of the pectin and the reasons for these are similar to those given for PG1. Owing to small amounts of glucose found in PG2 compared to PG1, glucose residues in PG2 were either included in the pectin molecules as glucose can also be found in the molecular chain of pectin (Rolin, Neilsen, & Glahn, 1998) or being separate glucan in small quantity which will be considered later.

In comparison between PG1 and PG2, pectin in PG1 had a higher neutral sugar content—more specifically rhamnose and galactose—which normally are present in areas rich in neutral sugars, the so-called 'hairy regions'. This infers that the pectin in PG1 were richer in the neutral sugar side chains than PG2.

The chemical structure of PG1 and PG2 were also studied by ^{1}H and ^{13}C-NMR spectroscopy. The ^{13}C-NMR spectra for PG1 and PG2 are shown in Figs. 5 and 6, respectively. In the ^{13}C-NMR spectrum of PG2 (Fig. 6), the signals from galacturonic acid units which are the principal sugar in this fraction are dominating. Assignments were

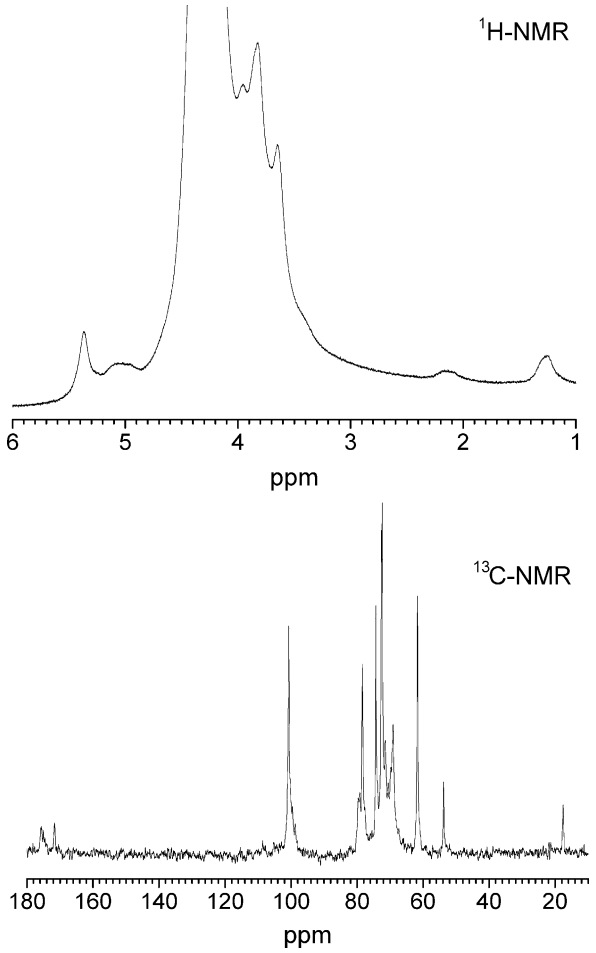

Fig. 5. ^{1}H and ^{13}C-NMR spectra for PG1.

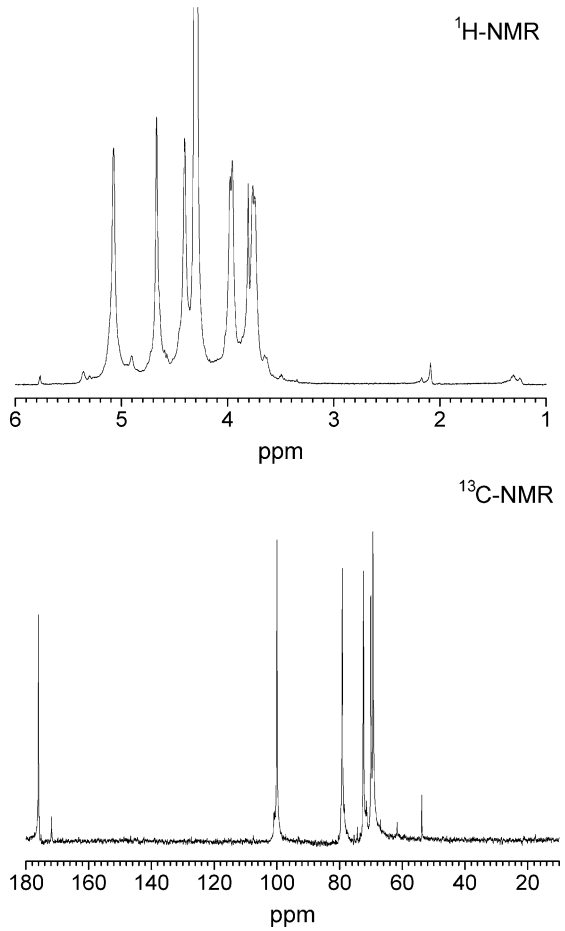

Fig. 6. ^{1}H and ^{13}C-NMR spectra for PG2.

made in reference to previous works (Golovchenko, Ovodova, Shashkov, & Ovodov, 2002; Iagher et al., 2002; Mukhiddinov et al., 2000; Sinitsya, Copikova, & Pavlikova, 1998). Predominant signals at 100.0, 69.4, 70.1, 79.1 and 72.4 ppm were assigned to C-1 to C-5 of (1 → 4) α-galacturonic acid residues, respectively. The signals in the region for the resonances of carbonyl groups (carboxyl and ester carbonyls) at 176.0 and 171.8 ppm correspond to the C-6 of unesterified (*C*OOH) and (methyl) esterified (*C*OOCH$_3$) galacturonic acid units, respectively. The signal at 53.8 ppm represents methyl carbons of the methyl ester (COO*C*H$_3$) of galacturonic acid units. In the ^{1}H-NMR spectrum of PG2, signals at 5.1, 3.8, 4.0, 4.4 and 4.7 ppm were assigned to H-1 to H-5 of (1 → 4) α-galacturonic acid residues, respectively. Signals at 1.3 and 2.1 ppm possibly relate to C-6 in rhamnose residues and acetyl protons, respectively. However, no signals around 17 and 21 ppm which correspond to C-6 rhamnose residues and methyl carbons of acetyl groups, respectively, were detected in the ^{13}C-NMR spectrum. These were probably due to the presence of rhamnose in PG2 as minor component and galacturonic acid residues are acetylated in negligible amounts together with the lower sensitivity of ^{13}C-NMR compared to ^{1}H-NMR.

In the ^{13}C-NMR spectrum of PG1 (Fig. 5), the signals from the main sugar components i.e. glucose, galacturonic acid and rhamnose were the detectable resonances. The signals at 100.7 ppm region probably belonged to the C-1 of the main sugars and their signals from C-2 to C-5 carbons appeared in the region 65–80 ppm. The signal at 17.6 ppm belonged to methyl carbon of rhamnose residues. Similar to the assignment for carbonyl groups in PG2, signals at 175.5 and 171.6 ppm were assigned to the C-6 of unesterified and (methyl) esterified galacturonic acid units, respectively. The signal at 53.8 ppm was assigned to methyl carbons of the methyl ester. Signals assigned above agree very well with previous results for the residues of pectins from various sources. Signals at 74.3, 78.4 ppm and strong intense signal at 61.7 ppm were tentatively assigned to the C-3, C-4 and C-6 of the glucose units, respectively, which probably related to starch. ^{1}H-NMR of PG1 failed to provide significant evidence for significant information on the structure of the polysaccharides as a high viscous solution was formed leading to line broadening. The signal at 1.3 ppm represented the methyl group at position 6 of rhamnose. The signal at 2.1 ppm from acetyl protons was found, but the signal around 21 ppm from ^{13}C-NMR appeared as traces suggesting that minute amounts of galacturonic acid units are acetylated.

According to the NMR spectra for both PG1 and PG2, it can be concluded that the pectin in PG1 and PG2 was vastly different in terms of the degree of esterification (DE) as clearly seen from the ratio of signal intensities of esterified galacturonic acid units and unesterified galacturonic acid units. The pectin part of PG2 had much lower DE as compared with PG1. This coincides with the fact that buffers or salt solutions with higher concentrations are required for the desorption of the less methylated pectins on DEAE column (Mukhiddinov et al., 2000) from which PG1 was eluted with water, whereas PG2 was eluted in the range 0.3–0.7 M NaCl.

PG1 were further separated by SEC as shown in Fig. 3. Polysaccharides in PG1 were not well separated, coalescing in a wide peak according to the system chosen. This was probably due to the polydisperse nature of both the glucan and pectin. However, PG1 was separated into six fractions (Fig. 3) and their sugar compositions are shown in Table 3.

Table 3
Yield and sugar composition of PG1 sub-fractions obtained after fractionation on Sephacryl 400

	PG1	PG1a	PG1b	PG1c	PG1d	PG1e	PG1f
Yield[a]	–	15.7	13.9	13.2	8.9	11.2	13.6
Sugar composition[b]							
Ara	2.2	2.3	2.1	1.8	1.7	1.8	2.4
Rha	10.7	12.4	9.8	7.4	7.6	7.6	14.0
Xyl	0.8	–	–	trace	trace	0.8	0.7
Gal	8.1	7.5-	6.8	6.5	7.1	6.8	8.2
Glc	43.3	34.6	44.7	41.6	47.8	48.9	28.0
GalA	34.9	43.2	36.6	42.2	35.9	34.1	46.7

[a] Calculated as weight% of applied material.
[b] Mol% of total carbohydrate content.

Table 4
Yield and sugar composition of PG2 sub-fractions obtained after fractionation on Sephacryl 400

	PG2	PG2a	PG2b	PG2c	PG2d	PG2e	PG2f
Yield[a]	–	5.6	12.0	23.0	20.6	23.5	8.2
Sugar composition[b]							
Ara	0.7	–	–	–	–	–	–
Rha	2.1	11.5	4.1	2.0	1.8	2.0	2.0
Xyl	–	–	–	–	–	–	–
Gal	4.9	7.8	4.9	4.9	4.3	4.7	4.3
Glc	6.0	31.3	15.6	2.4	1.8	2.8	2.0
GalA	86.2	49.3	75.4	90.6	92.2	90.6	91.7

[a] Calculated as weight% of applied material.
[b] Mol% of total carbohydrate content.

As can be seen in Table 3, glucose and galacturonic acid were found in considerable amounts in all PG1 sub-fractions indicating all PG1sub-fractions contain a mixture of starch and pectin. Rhamnose, galactose and arabinose which belong to pectin molecules were found in all sub-fractions in comparable amounts to those found in the original sample (PG1). Fractions PG1a which eluted earliest and PG1f which eluted latest from Sephacryl 400 column seem to contain pectin in higher amounts when compared to the others as suggested by the higher amounts of galacturonic acid and rhamnose found in these two sub-fractions. PG2, the fraction containing principally pectin type polysaccharide was also further separated by SEC and it was separated into six fractions as shown in Fig. 4. Sugar compositions of PG2 sub-fractions are given in Table 4.

Galacturonic acid is the principal sugar for all PG2 sub-fractions. The presence of glucose in significant amounts in PG2a and PG2b compared to the others suggests that these two fractions contained significant amounts of starch which can be non-convalently bound with pectin. In other words, starch is more likely to loosely bind with high molecular weight pectin. The presence of starch in PG2a and PG2b was confirmed by a positive test with KI/I_2 test, whereas other PG2 sub-fractions showed negative results. The fractions PG2c, PG2d, PG2e and PG2f contained principally galacturonic acid along with rhamnose, galactose, and glucose in similar proportions indicating that these four are pure pectin. It can be seen that the total amount of neutral sugars from PG2a to PG2f (PG2c-PG2f) decrease with increasing elution volume. This finding agrees with our previous observation with the fractionation by SEC of pectic polysaccharide from *Acanthus ebracteatus* (Hokputsa et al., 2004).

3.3. Homogeneity and molecular weight of polysaccharides

SEC coupled to refractive index (RI) and multi-angle laser light scattering (MALLS) detectors was used to characterise the polysaccharide fractions. This technique can provide absolute molecular weight and its distribution (Wyatt, 1992). The RI gives a signal proportional to concentration and the light scattering signal from MALLS depends on both concentration and molecular weight. Fig. 7a shows light scattering (at 90°) and RI chromatograms of PG. The RI chromatogram shows a bi-modal peak suggesting the sample contains more than one species with the very high molecular weight species present as minor component indicated by comparatively smaller RI peak at lower elution

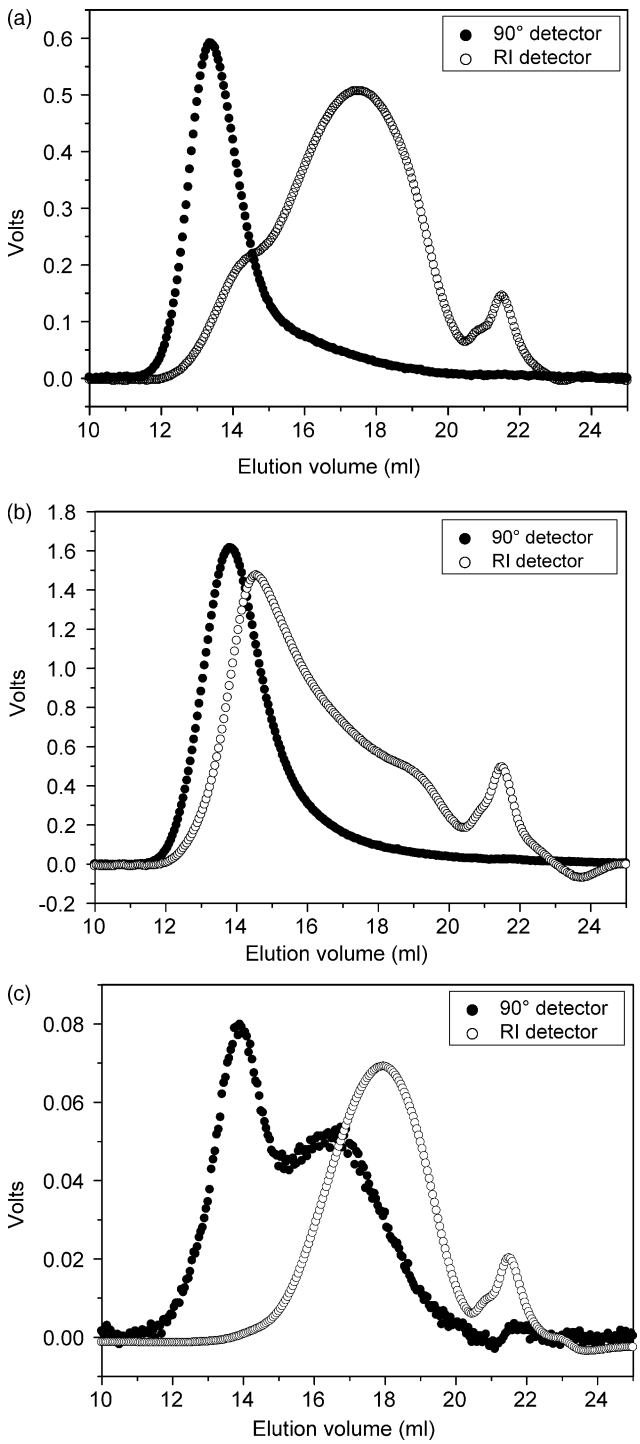

Fig. 7. Light scattering and refractive index profiles from SEC/MALLS experiments on the water-soluble polysaccharides from *Durio zibethinus* Murr: (a) PG; (b) PG1; (c) PG2.

volume and there is a large light scattering peak associated with this region. The majority of the sample is medium molecular weight polysaccharide as indicated by large RI peak which corresponds to a peak elution volume of 17.7 ml. The corresponding light scattering trace appeared comparatively lower due to the dominating LS peak of the high molecular weight species. Fig. 7b shows light scattering and RI chromatograms of PG1. The RI profile has broad elution volume with a shoulder on the low molecular weight side. The main component of PG1 probably corresponds to the comparatively smaller RI peak of PG.

Fig. 7c shows the light scattering and RI chromatogram of PG2. The RI chromatogram shows one large, relatively symmetrical peak, but the light scattering chromatogram shows a bi-modal peak. However, there is very little RI signal associated with the earlier light scattering peak eluting at exclusion volume which indicates that there is a very high molecular weight component present in a very small amount probably arising from the presence of pectin aggregates. The aggregation of pectin molecules in solution is highly practicable, especially in the case for LM pectins (Sawayama, Kawabata, Nakahara, & Kamata, 1988). PG2 is considered as low esterified pectin compared to PG1 as discussed earlier.

The differential molecular weight distributions as a function of molecular weight obtained from SEC/MALLS for PG, PG1 and PG2 are shown in Fig. 8. It can be seen that the high molecular weight polysaccharide is confined to PG1 whereas the lower molecular weight polysaccharide is considered to be the principal component in PG2 and also a slight decrease in molecular weight obtained compared to the original sample, PG.

SEC/MALLS experiments for PG1 and PG2 sub-fractions were also performed, the overlay of RI profiles of PG1 and PG2 sub-fractions are shown in Fig. 9. As can be seen in Fig. 9a, all PG1 sub-fractions had broad elution volume especially PG1c, PG1d, PG1e and PG1f appeared

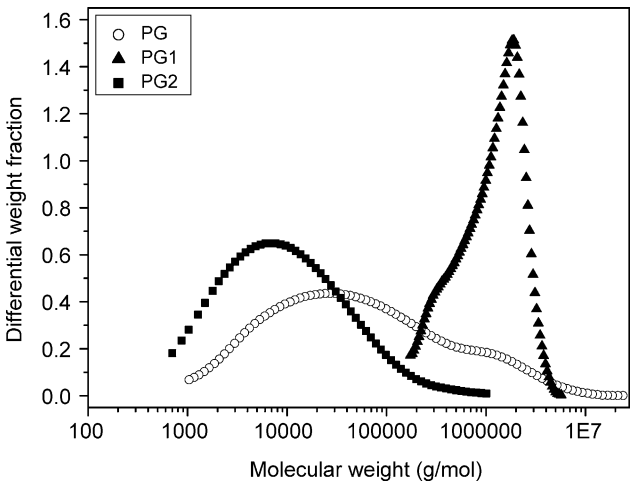

Fig. 8. Differential molecular weight distribution obtained from SEC/MALLS of polysaccharide fractions from *Durio zibethinus* Murr.

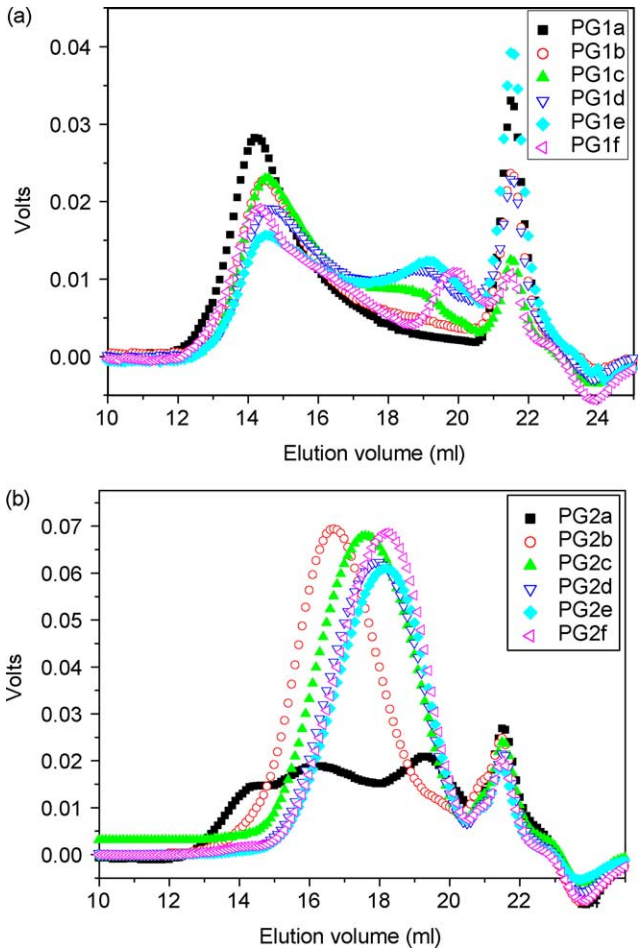

Fig. 9. The overlay of RI profiles from SEC/MALLS experiments: (a) PG1 and its sub-fractions; (b) PG2 and its sub-fractions.

as bi-modal peaks, probably arising from the samples containing a mixture of polysaccharides in accordance with the speculation from their sugar composition results that glucose and galacturonic acid were found in considerable amounts in all sub-fractions. Regardless of the heterogeneity of the samples, the number and weight average molecular weight obtained from SEC/MALLS for all sub-fractions are given in Table 5.

As for PG2 sub-fractions (Fig. 9b), the RI profiles for all excluding PG2a and PG2b appeared as symmetrical single peak, suggesting homogeneous polysaccharides (pectins) as speculated from sugar composition analysis. PG2a appeared as multi-modal peak indicating a mixture of polysaccharides which is in keeping with the results from the sugar composition analysis. The RI profile of PG2b had a relatively small shoulder at the low molecular weight side; this shoulder probably belongs to glucan (probably starch), as glucose was also present in PG2b in significant amounts. The shoulder at low molecular weight side in the RI profile of PG2b had the same elution range as did the last peak in the RI profile of PG2a and these probably belong to a separate glucan. This is likely because in theory, if the glucan is present in the original sample, it should be

Table 5
Average molecular weight and intrinsic viscosity of polysaccharide fractions from *D. zibethinus*

Sample	[η], ml/g	Molecular weight		Polydispersity (M_w/M_n)
		M_n	M_w	
PG	310 ± 30	–	–	–
PG1	230 ± 10	562,000	1,090,000	1.9
PG1a		744,000	1,260,000	1.7
PG1b		535,000	833,000	1.6
PG1c		298,000	604,000	2.0
PG1d		207,000	478,000	2.3
PG1e		78,000	459,000	5.9
PG1f		78,000	475,000	6.1
PG2	83 ± 1	44,000	78,000	1.8
PG2a		219,000	480,000	2.2
PG2b		46,000	103,000	2.2
PG2c		38,000	55,000	1.4
PG2d		19,000	34,000	1.8
PG2e		18,000	32,000	1.8
PG2f		7000	21,000	3.0

eluted with water, not with NaCl solution applied to the DEAE column because it may interact with pectin. Generally (1 → 4) linked glucan is water-insoluble. If soluble, it should be the low molecular weight glucan which corresponds to the RI profile at high elution volume.

3.4. Intrinsic viscosity

Intrinsic viscosity [η] is influenced by the so-called hydrodynamic volume of a macromolecule in solution. It reflects of size or physico-chemical properties of the molecule that depends primarily on the molecular weight, chain rigidity and solvent quality. The value for [η] can be obtained from η_{red} when extrapolated to zero concentration as is demonstrated by the Huggins plots for PG, PG1 and PG2 as shown in Fig. 10 and the intrinsic viscosity values are given in Table 5. PG1 has higher intrinsic viscosity than PG2 which agrees with the results from SEC/MALLS study; PG1 was eluted at lower elution volume provided there is no non-size exclusion mechanism. Neutral polysaccharides have low hydrodynamic volume and neutral sugar side chains do not contribute significantly to the intrinsic viscosity. According to the Mark-Houwink relation, this would mean that the pectin in PG1 appears to be made of much higher molecular weight molecules. This also agrees with molecular weights obtained from SEC/MALLS. Intrinsic viscosities of both PG1 and PG2 were lower than PG, possibly due to the depolymerisation of polysaccharide chains during fractionation. The intrinsic viscosity of PG is comparable with previously reported values for commercial citrus pectin (345 ml/g) determined under the same solvent (Morris, Foster, & Harding, 2000). Intrinsic viscosities for pectins typically lie in the range 100-600 ml/g (Rolin et al., 1998).

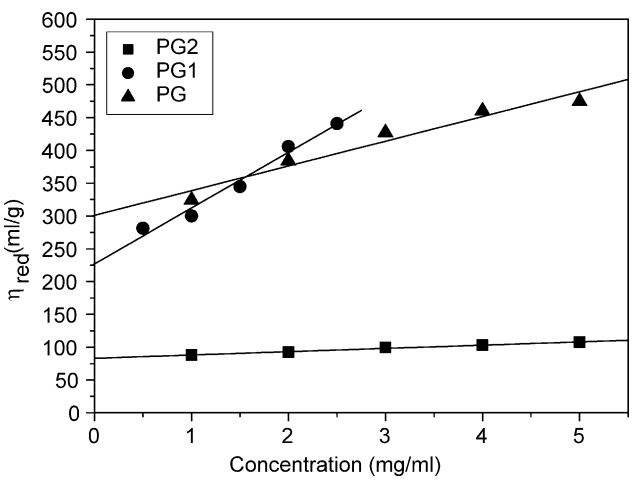

Fig. 10. Plots of reduced viscosity (η_{red}) against concentration (Huggins plot) of polysaccharides PG, PG1 and PG2.

3.5. Effect on the complement system

Various polysaccharides from different plants were reported to be responsible for the effects associated with the healing of wounds. Some of these have an influence on the immune system and are often called immunomodulators when the complement system is involved (Diallo, Paulsen, Liljeback, & Michaelsen, 2001). A previous study has shown that the crude water-soluble polysaccharides, PG possesses wound-healing properties as demonstrated in pig skin. The crude polysaccharides (PG) and its main fractions (PG1 and PG2) were therefore evaluated for the effect on the complement system using the widely used haemolytic complement assay. The effect of the polysaccharides on the complement system expressed as % inhibition of haemolysis by the polysaccharides was compared to that of the pectic polysaccharide PM II which is known to possess this activity (Michaelsen et al., 2000). The results from the complement-fixation assay are shown in Fig. 11. Essentially, PG2 had very low or negligible activity at

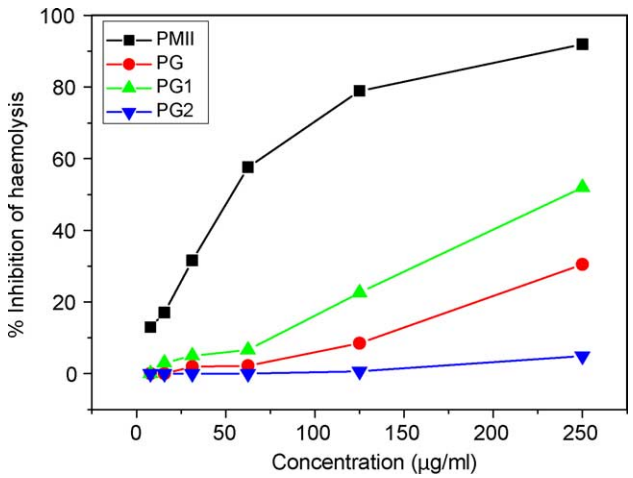

Fig. 11. Concentration and activity (inhibition of haemolysis) profiles of polysaccharides from *Durio zibethinus* Murr: PG, PG1 and PG2 compared to pectic polysaccharide from *Plantago major*, PM II.

the range of concentrations studied whereas PG1 exhibited the activity at the higher concentrations studied and its activity is concentration dependent. However, the activity of PG1 was much lower than that of PM II. The activity of PG lies between PG1 and PG2 probably as a result of the combined effects of PG1 and PG2.

Many pectic polysaccharides isolated from medicinal plants have shown biological activity. Information on structure activity relationship is available for pectic type polysaccharide, but little is known on the biological activity of pectins isolated from fruit and vegetables. However, not all pectins have the effect on the complement system, it has been summarised that, in general, the bioactive pectins (with the effect on the complement system) are the pectins rich in neutral sugar side chains of (arabino)galactans containing β-(1 → 6) linkage. Though the pectins from *Durio zibethinus* contain arabinogalactans side chains containing β-(1 → 6) linkage, the very low activity of the isolated pectin (PG2) is due to very low content in neutral sugar side chains (as indicated by the amounts of arabinose and galactose). Pectic polysaccharide PM II contains 9% arabinose and 8% galactose (Samuelsen, 2000). Another pectic polysaccharide (A1002) possessing the activity from *A. ebracteatus* which has been studied by our research group, contains 6.1% arabinose and 11% galactose (Hokputsa et al., 2004). It is clearly seen that arabinose and galactose content of PG2 is much lower than that of PM II and A1002. Our result reinforces the previous finding stating that pectins having the effect on the complement system are classified as rhamnogalacturonan with several neutral sugar side chains (Yamada & Kiyohara, 1999). However, for the time being, the minimum or optimum contents of arabinose and galactose required for activity have not yet been elucidated.

Recalling the sugar composition of PG1, provided that the starch content in the PG1 arbitrarily accounts for all the glucose present (or the pectin molecules in PG1 contained insignificant amounts of glucose) all other sugars except for glucose would therefore belong to the pectin, hence sugar composition of pectin in PG1 (mol%) are as follows: Ara (3.9%), Rha (18.9%), Xyl (1.4%), Gal (14.3%) and GalA (61.6%). PG1 had higher activity as compared to PG2, this may be explained by its higher amounts of neutral sugar side chains of pectin. In spite of the difference in fine structure of the pectins, it is interesting to note that the activity of PG1 is lower than PM II possibly due to the lower concentration of the pectin in PG1 which contains considerable amounts of starch as mentioned earlier. If the non-pectic polysaccharide in PG1 is removed, the activity of PG1 may be higher.

Furthermore, any neutral sugar side chains in PG might be cleaved leading to lower amount of neutral sugars in side chains, especially arabinose and galactose, than those present in nature because highly acidic condition was involved in the isolation of PG. The condition used to isolate PG is similar to those for the isolation of commercial pectins which normally contain low neutral sugar side chains, this probably explains why commercial pectins have rarely been reported to have the effect on the complement system.

According to the result, the wound healing properties of film made from PG might not be associated with the complement system, but probably due to the antibacterial activity as previously reported. Pectins isolated from other sources have been reported to have antibacterial activity (el-Nakeeb & Yousef, 1970; Takenaka, Muto, Yatsunami, & Echigo, 1994; Zaporozhets, Besednova, Lyamkin, Loenko, & Popov, 1991). Films made from natural products are of increasing scientific and commercial interest, they are not only biodegradable but also acceptable for pharmaceutical applications. A recent study has also revealed that films made from pectin isolated from passion fruit-maracuya (*Passiflora edulis*) enhances wound healing as demonstrated in mice model, but the mechanism of the healing properties has not yet investigated (Segura-Ceniceros et al., 2003).

4. Concluding remarks

Crude, pharmaceutically useful polysaccharides (PG) extracted from the rinds of *Durio zibethinus*, are composed of pectin as the principal component and starch as a contaminant, which is often found in commercial pectins and referred as a ballast compound. The crude polysaccharides demonstrated a high viscosity and may therefore be used as an alternative viscosity enhancer instead of commercial pectins. However, the lengthy purification of PG may diminish this potential as demonstrated by the lower intrinsic viscosity of PG1 and PG2 compared to the original sample, PG. It has been known that physicochemical properties of pectin varies with the conditions used during isolation and purification. Durian rind could be a profitable agricultural waste as a potential source of the pectin if the optimum condition for isolation of pectin from the durian rind is studied in order to obtain the pectin with desired properties especially the rheological properties which is in our interest for future research.

Acknowledgements

The authors would like to thank Dr Wolfgang Günther at the Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich-Schiller-University of Jena for performing the NMR experiments.

References

Barsett, H., Paulsen, B. S., & Habte, Y. (1992). Further Characterization of polysaccharides in seeds from Ulmus-Glabra huds. *Carbohydrate Polymers*, *18*(2), 125–130.

Chaplin, M. F. (1994). Monosaccharides. In M. F. Chaplin, & J. F. Kennedy (Eds.), *Carbohydrate analysis. A practical approach* (pp. 1–42). Oxford: Oxford University Press.

Diallo, D., Paulsen, B. S., Liljeback, T. H. A., & Michaelsen, T. E. (2001). Polysaccharides from the roots of Entada africana Guill. et Perr, Mimosaceae, with complement fixing activity. *Journal of Ethnopharmacology*, *74*(2), 159–171.

Franck, A., & De Leenheer, L. (2002). Inulin. In A. Steinbüchel (Ed.), *Biopolymers* (pp. 439–479). Weinheim: Wiley-VCH.

Golovchenko, V. V., Ovodova, R. G., Shashkov, A. S., & Ovodov, Y. S. (2002). Structural studies of the pectic polysaccharide from duckweed Lemna minor L. *Phytochemistry*, *60*(1), 89–97.

Green, A. A. (1933). The preparation of acetate and phosphate buffer solutions of known pH and ionic strength. *Journal of the American Chemical Society*, *55*, 2331.

Harding, S. E. (1997). The intrinsic viscosity of biological macromolecules. Progress in measurement, interpretation and application to structure in dilute solution. *Progress in Biophysics and Molecular Biology*, *68*(2-3), 207–262.

Hokputsa, S., Harding, S. E., Inngjerdingen, K., Jumel, K., Michaelsen, T. E., Heinze, T., Koschella, A., & Paulsen, B. S. (2004). Bioactive polysaccaharides from the stems of the Thai medicinal plant *Acanthus ebracteatus*: thier chemical and physical features. *Carbohydrate Research*, *339*(4), 753–762.

Iagher, F., Reicher, F., & Ganter, J. L. M. S. (2002). Structural and rheological properties of polysaccharides from mango (*Mangifera indica* L.) pulp. *International Journal of Biological Macromolecules*, *31*(1–3), 9–17.

Khedari, J., Charoenvai, S., & Hirunlabh, J. (2003). New insulating particleboards from durian peel and coconut coir. *Building and Environment*, *38*(3), 435–441.

Kim, J. B., & Carpita, N. C. (1992). Changes in esterification of the uronic-acid groups of cell- wall polysaccharides during elongation of maize coleoptiles. *Plant Physiology*, *98*(2), 646–653.

Kravtchenko, T. P., Voragen, A. G. J., & Pilnik, W. (1992). Analytical comparison of 3 industrial pectin preparations. *Carbohydrate Polymers*, *18*(1), 17–25.

Lipipun, V., Nantawanit, N., & Pongsamart, S. (2002). Antimicrobial activity (in vitro) of polysaccharide gel from durian fruit-hulls. *Songklanakarin Journal of Science and Technology*, *24*(1), 31–38.

Michaelsen, T. E., Gilje, A., Samuelsen, A. B., Hogasen, K., & Paulsen, B. S. (2000). Interaction between human complement and a pectin type polysaccharide fraction, PMII, from the leaves of Plantago major L. *Scandinavian Journal of Immunology*, *52*(5), 483–490.

Morris, G. A., Foster, T. J., & Harding, S. E. (2000). The effect of the degree of esterification on the hydrodynamic properties of citrus pectin. *Food Hydrocolloids*, *14*(3), 227–235.

Mukhiddinov, Z. K., Khalikov, D. K., Abdusamiev, F. T., & Avloev, C. C. (2000). Isolation and structural characterization of a pectin homo and ramnogalacturonan. *Talanta*, *53*(1), 171–176.

el-Nakeeb, M. A., & Yousef, R. T. (1970). Study of antimicrobial action of pectin. I. Antibacterial and antifungal activities of pectin. *Planta Medica*, *18*(3), 201–209.

Pongsamart, S., & Panmaung, T. (1998). Isolation of polysaccharides from fruit-hulls of durian (*Durio Zibethinus* L.). *Songklanakarin Journal of Science and Technology*, *20*(3), 323–332.

Rolin, C., Neilsen, B. U., & Glahn, P. E. (1998). Pectin. In S. Dumitriu (Ed.), *Polysaccharides: structural diversity and functional versatility* (pp. 377–431). New York: Marcel Dekker.

Samuelsen, A. B. (2000). Structure features of biologically acitive polysaccharides fractions from the leaves and seeds of Plantago major. In B. S. Paulsen (Ed.), *Bioactive carbohydrate polymers* (pp. 37–46). Dordrecht: Kluwer Academic Publishers.

Samuelsen, A. B., Paulsen, B. S., Wold, J. K., Otsuka, H., Yamada, H., & Espevik, T. (1995). Isolation and partial characterization of biologically-active polysaccharides from Plantago-major L. *Phytotherapy Research*, *9*(3), 211–218.

Sawayama, S., Kawabata, A., Nakahara, H., & Kamata, T. (1988). A light scattering study on the effects of pH on pectin aggregation in aqueous solution. *Food Hydrocolloids*, *2*(1), 31–37.

Segura-Ceniceros, E. P., Ilyina, A., Contreras-Esquivel, J. C., Rodriguez-Menchaca, D., Flores-Espinoza, J. C., & Montes-Rodriguez, O. E. (2003). Entrapment of enzymes in natural polymer extracted from residue of food industry: preparation methods, partial characterisation and possible application. *Moscow University Chemistry Bulletin*, *44*(1), 84–87.

Sims, I. M., & Bacic, A. (1995). Extracellular polysaccharides from suspension-cultures of Nicotiana-Plumbaginifolia. *Phytochemistry*, *38*(6), 1397–1405.

Sinitsya, A., Copikova, J., & Pavlikova, H. (1998). C-13 CP/MAS NMR spectroscopy in the analysis of pectins. *Journal of Carbohydrate Chemistry*, *17*(2), 279–292.

Takenaka, T., Muto, O., Yatsunami, K., & Echigo, T. (1994). Antibacterial activity of pectin hydrolyzates. *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi*, *41*(11), 785–792.

Umprayn, K., Chanpaparp, K., & Pongsamart, S. (1990a). The studies of durian rind extracts as an aqueous binder I: Evaluation of granule properties. *Thai Journal of Pharmaceutical Sciences*, *15*(2), 95–115.

Umprayn, K., Chanpaparp, K., & Pongsamart, S. (1990b). The studies of durian rind extracts as an aqueous binder II: evaluation of tablet properties. *Thai Journal of Pharmaceutical Sciences*, *15*(3), 173–186.

Wyatt, P. J. (1992). Combined differential light scattering with various liquid chromatography separation techniques. In S. E. Harding, D. B. Sattelle, & V. A. Bloomfield (Eds.), *Laser light scattering in biochemistry* (pp. 35–58). Cambridge: The Royal Society of Chemistry.

Yamada, H., & Kiyohara, H. (1999). Complement-activating polysaccharides from medicinal herbs. In H. Wagner (Ed.), *Immunomodulatory agents from plants* (pp. 161–202). Basel: Birkhäuser.

Zaporozhets, T. S., Besednova, N. N., Lyamkin, G. P., Loenko, Y. N., & Popov, A. A. (1991). Antibacterial and therapeutic efficacy of pectin from Zostera, a sea grass. *Antibiotiki I Khimioterapiya*, *36*(4), 24–26.