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Isolation and characterization of water-soluble prebiotic compounds from Australian and New Zealand plants

J.K. Vidanarachchi [b], P.A. Iji [a,*], L.L. Mikkelsen [a], I. Sims [c], M. Choct [a]

[a] School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia
[b] Department of Animal Science, University of Peradeniya, Peradeniya 20400, Sri Lanka
[c] Industrial Research Limited, P.O. Box 31-310, Lower Hutt, New Zealand

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ABSTRACT

The water-soluble carbohydrates (WSCs) extracted from the underground parts (rhizome) of *Arthropodium cirratum* (Rengarenga lily extract); third order branches of *Cordyline australis* (Cabbage tree extract); a seaweed, *Undaria pinnatifida* (Undaria extract), and exudates from *Acacia pycnantha* (Acacia extract) were investigated. Extracts of Rengarenga lily, Cabbage tree, Undaria, and Acacia contained 576, 250, 275 and 794 g/kg DM WSCs, respectively. Constituent sugar analysis by gas–liquid chromatography (GLC) showed that extracts of Rengarenga lily and Cabbage tree contained predominantly fructose and glucose (82–95%). The analysis also revealed that Acacia extract contained mainly galactose (78%) and arabinose (22%) while Undaria extract, contained fucose (55%) and galactose (44%). Thin-layer chromatography (TLC) showed that, on the basis of R_F values, fructan composition of Rengarenga lily extract and Cabbage tree extract was different. Cabbage tree extract contained 45% (w/w) fructans while Rengarenga lily extract contained 65% (w/w) fructans. High performance size-exclusion chromatography coupled with multi-angle laser light scattering (SEC-MALLS) showed that the extracts had varying weight average molecular weight due to differences in the average chain length of the major carbohydrates. Data for the amino acid compositions differed considerably depending on the type of extract. Water-soluble carbohydrate extracts prepared from the four plant sources gave a wide range of WSC (250–794 g/kg DM) due to the different proportions of structural material in different species. It is not known how these differences will impact on animal production, if diets are supplemented with the extracts.

1. Introduction

The use of plants and their relevant bioactive compounds dates back thousands of years to the ancient Egyptians, Chinese, Indians and Greeks (Gill, 1999; Kamel, 2000). Chinese herbal medicines have been used for many centuries for treating various human and animal diseases (Li, 2000). Since the late 1990s and the birth of the prebiotic concept, scientists have begun to be interested in the health properties of prebiotic compounds, so today there are many scientific papers describing them in relation to human health. In recent years there has been an increased awareness of the potential that various natural plant bioactive compounds such as oligosaccharides and polysaccharides may act as phytobiotics or prebiotics in poultry feed and to exert growth-promoting effects as well as health-improving effects (Guo et al., 2004; Lan et al., 2004; Vidanarachchi, Mikkelsen, Sims, Iji, & Choct, 2005). Australia and New Zealand have a rich reservoir of bioactive compounds, and the health benefits of plant extracts have been empirically known for thousands of years by Aboriginal and Maori populations. The incidence of non-infectious diseases during pre-modern times was low in these populations, perhaps in part due to the presence of protective chemical constituents within the plants that were eaten (Cambie & Ferguson, 2003).

Many flowering plant species store fructans, which are polymers of fructose, as reserve carbohydrates. *Arthropodium cirratum* (Rengarenga lily) is a slender herb which has starchy edible rhizomes rich in fructans as storage carbohydrate (Harris, 1996). The occurrence of large amounts of fructans in Rengarenga lily rhizomes and readily hydrolysable glucofructofuranan in *Cordyline australis* (Cabbage tree) shoots, explains why the early settlers in Australia and New Zealand not only used the tubers and cooked stems of these species as food, but also used them in herbal medicine (Cambie & Ferguson, 2003; Fankhauser & Brasch, 1985; Harris, 1996).

Another abundant plant that has potential prebiotic properties is the wattle tree (*Acacia* spp.). Many *Acacia* species exude a complex arabinogalactan-type polysaccharide as sap. A report in the nineteenth century on Victorian Aborigines states that all their common ailments were effectively treated with wattle bark

* Corresponding author. Tel.: +61 2 6773 2082.
E-mail address: piji@une.edu.au (P.A. Iji).

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exudate (*Acacia* exudate), which is a slimy product from damaged portions of *Acacia* plants (Cribb & Cribb, 1981). Impressed with the "healing" powers of many wattle species, colonial doctors in Australia prescribed many of them for the treatment of dysentery and diarrhoea (Wickens & Pennacchio, 2002). American Indians also have traditionally consumed *Acacia* exudates for the prevention and treatment of gastro-intestinal disorders. Today, *Acacia* gum is widely used for its nutritional and surface properties by the human food industry.

Seaweeds are an abundant source of natural polysaccharides, many of which have commercial uses, particularly in the food, cosmetic and medical industries (Renn, 1997). Southern Australia is one of the richest areas in the world for seaweeds and more than 1000 species of macro-algae have been identified around Australia (Edgar, 1997). The health-promoting effects of extracts of certain seaweeds in human and animal models are well known (Sakai, Kimura, & Kato, 2002), but their influence on growth performance of farm animal species is less well-documented.

Prebiotic compounds from plants are obtained by one of three processes: direct extraction of natural carbohydrates (monosaccharides and oligosaccharides) from plants, controlled hydrolysis of natural plant polysaccharides and enzymatic synthesis, using hydrolases and/or glycosyl transferases (Grizard & Barthomeuf, 1999). Of the several methods which can be used for extracting bioactive compounds from plant sources, ethanol–water (warm or hot) extraction of water-soluble compounds is the most popular. Only a few polysaccharides are soluble in ethanolic extract, while all mono- and oligosaccharides are soluble (Carre, 2002). Research on biologically active carbohydrates from higher plants and related species in poultry nutrition is relatively new and in this study, water-soluble carbohydrates (WSCs) from underground parts (rhizome) of Rengarenga lily, third order branches of Cabbage tree and exudates of *Acacia*, and a seaweed *Undaria* were extracted and their chemical composition analysed in order to study their bio-activities *in vivo*.

2. Materials and methods

2.1. Isolation of water-soluble carbohydrates

2.1.1. Plant materials

The underground parts (rhizome) of *A. cirratum* (Rengarenga lily) and third order branches (stems) of *C. australis* (Cabbage tree) were collected in mid-winter of 2003 from a nursery in Wellington, New Zealand. Care was taken not to break the rhizomes, and they were thoroughly washed, to remove the soil. The exudates from *Acacia pycnantha* (Golden wattle) were obtained from trees grown in the Adelaide area of South Australia in December 2003. The *Undaria pinnatifida* (seaweed) samples were collected from Point Arthur, Wellington Harbour, New Zealand in June 2003 and the algae were washed thoroughly with seawater, followed by tap water, to remove soil particles and epiphytes. Several batches of plant extracts were prepared according to the following procedures in order to obtain sufficient materials for analyses and possible use in animal feeding. Material to solvent ratios was changed accordingly during the large-scale production of extracts.

2.1.2. Rengarenga lily and Cabbage-tree extracts

The cleaned rhizome samples of Rengarenga lily and third order branches of Cabbage-tree were air-dried, cut into small pieces and ground with a laboratory grinder (Mikro-Feinmuhle-Culatti MFC grinder, Janke and Kunkel GmbH and Co., Staufen, Germany). Powdered samples (1 g) from the two species were extracted in 50 mL of boiling 80% (v/v) ethanol for 10 min and WSCs were extracted twice with distilled water (50 mL/g, 70 °C, 60 min). The ethanol and two water extracts from each species were combined and concentrated *in vacuo* at 40 °C in a rotary evaporator (Buchi Rotavapor-R, Buchi Laboratories, Flawil, Switzerland). After freezing and thawing, the resulting insoluble materials were removed by centrifugation at 3500 *g* for 20 min at 20 °C using an MSE Mistral 2000R centrifuge (Sanyo Gallenkamp PLC, Leicestershire, UK), and the supernatants were passed through an ion exchange column consisting of both anion exchange resin (Amberlite IR 120, Na^+ form) and cation exchange resin (Amberlite IR 401, Cl^- form). The neutral elutes were immediately frozen in acetone cooled with dry-ice, and lyophilized for 72 h at −49 °C and 62×10^{-3} mbar (Eyela Freeze Dryer FD-1, Rikakikai Co., Tokyo, Japan). The freeze-dried powders of Rengarenga lily extract, (off-white) and Cabbage-tree extract (greenish-black) were analysed for chemical composition and stored for subsequent use in poultry diets.

2.1.3. Acacia extract

The *Acacia* exudate was dissolved in distilled water (50 g/L) and the solution was filtered through a 600 μm screen to remove particulate matter and then centrifuged at 12,000*g* for 10 min at 4 °C in a Beckman model J2-21M Induction Drive centrifuge with a JA-21 rotor (Beckman Instruments Inc., Palo Alto, CA, USA) in order to remove insoluble tannins. The supernatant was collected and dialysed (MWCO 12–14 kDa, Medicell International Ltd., London, UK) exhaustively against distilled water (48 h). The dialysate was collected and then lyophilized to yield a fine off-white powder (*Acacia* extract).

2.1.4. Seaweed extract

The seaweed samples (thalli) were air-dried and crushed into chips (less than 2.0 mm in diameter) using a laboratory grinder. The preparation of crude extracts of the samples was performed as described below. Unless indicated otherwise, all extraction steps were performed at 25 °C and all centrifugation steps were performed at 3500*g* for 20 min at 20 °C using an MSE Mistral 2000R centrifuge. Extraction of fraction I was performed by constant mechanical stirring (Heidolph MR 3001K stirrer, Heidolph Instruments GmbH and Co., Schwabach, Germany) of the algal powder in 150 mL of 1% (w/w) sulphuric acid, initially for 6 h, followed by overnight stirring with another 150 mL of 1% (w/w) sulphuric acid. After each stirring step, samples were centrifuged and supernatants were saved and finally pooled together (fraction I). The pooled fraction was neutralized with 1% (w/v) sodium hydroxide and dialysed exhaustively against distilled water (48 h). The dialysate was concentrated *in vacuo* at 40 °C in a rotary evaporator and immediately frozen in acetone cooled with dry-ice, and lyophilized.

The insoluble matter was resuspended in 150 mL of 1% (w/v) sodium carbonate and mechanically stirred (Heidolph Instruments GmbH and Co., Schwabach, Germany) three times (3 × 150 mL) for 4, 6 and 12 h, followed by centrifugation after each of the stirring steps. Supernatants were pooled together and neutralized with 10% (w/w) sulphuric acid and the pH was adjusted to 1.6 with the same sulphuric acid solution. A creamy white flocculate (alginate) which appeared at about pH 3.0 was removed by centrifugation and the resultant supernatant (fraction II) was neutralized with 10% (w/v) sodium hydroxide, followed by dialysis and lyophilization. The insoluble material was washed three times with distilled water (3 × 150 mL) and finally with 100 mL of acetone. Washed fractions were separated by centrifugation and pooled together (Fraction III), dialysed against distilled water (48 h) in a tubing with a normal 10–12 kDa molecular weight cut-off, and then lyophilised. Fractions I, II and III were combined (*Undaria* extract) for subsequent analysis.

2.2. Analytical techniques and measurements

2.2.1. Dry matter content

The DM content of extracts was determined gravimetrically according to the AOAC Official Method 934.01 (AOAC, 2002). Samples were accurately weighed (2–4 g) into preweighed silica crucibles and placed in a forced-air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd., Perth, Australia) which was preheated to 105 °C. Samples were held at this temperature overnight or until a constant mass was obtained. The DM content was calculated as percent ratio of the weight difference of samples before and after drying to that of the original material.

2.2.2. Crude protein

The nitrogen content of the extracts was determined according to the DUMAS combustion technique following the method described by Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyser (Leco Corp., St. Joseph, MI, USA). Nitrogen freed by combustion at high temperature in pure oxygen was measured by thermal conductivity detection and converted to equivalent protein by a numerical factor of 6.25. The furnace temperature was maintained at 1050 °C for pyrolysis of sample in ultra high purity oxygen. To interpret detector response as percentage nitrogen (w/w) calibration was done using pure primary standard of ethylenediaminetetra-acetic acid (EDTA).

2.2.3. Analyses of water-soluble carbohydrates

Total WSC were determined by the phenol–sulphuric acid method using glucose as a standard (Dubois et al., 1956). An equal volume (400 μL) of 5% (v/v) phenol was added to 400 μL plant extracts dissolved in distilled water (1 mg/mL) and gently mixed. Concentrated sulphuric acid (2 mL) was added and after 10 min incubation at room temperature the absorbance was read at 490 nm using a Hitachi 450-20 spectrophotometer (Hitachi Co., Japan).

2.2.4. Constituent sugar analysis

The reductive hydrolysis and acetylation method of Stevenson and Furneaux (1991) was used to convert the constituent sugars in WSC fractions into alditol acetate derivatives. This method utilises *in situ* reduction with *N*-methylmorpholineborane (MMB) during hydrolysis to prevent degradation of 3,6-anhydrogalactose units.

A fresh stock solution of aqueous MMB (80 mg/mL, 0.2 mL per sample) was made beforehand. The WSC fractions (1 mg) were placed in Kimax screw-cap tubes (13 × 100 mm) equipped with Teflon-lined caps and 50 μL aqueous MMB and 200 μL 3 M trifluoroacetic acid (TFA) which contained 2 mg/mL inositol (internal standard) were added and heated at 80 °C for 30 min. The tubes were cooled, a second portion of 50 μL MMB was added and reheated at 120 °C for 1 h. This step was repeated once more with the 100 μL aqueous MMB, and the solution was evaporated to dryness at 50 °C. Residual water was removed by adding acetonitrile (CH_3CN) twice (2 × 0.4 mL) and concentrating to dryness again. The resulting dry solid was acetylated by adding 100 μL of acetic anhydride (Ac_2O) and 100 μL of neat TFA, followed by heating at 50 °C for 30 min. Residual Ac_2O was decomposed by adding 1 mL of toluene and evaporating to dryness with a stream of dry air. The mixture was extracted with 2.5 mL of dichloromethane (CH_2Cl_2) and the organic phase was extracted first with 2.5 mL 0.5 M aqueous-sodium carbonate and then with 2.5 mL distilled water, discarding the upper aqueous layer each time. The phases were separated by brief, low speed (1000*g*, 5 min, 20 °C) centrifugation. The dichloromethane layer was then evaporated to near dryness and excess water was removed by adding 0.5 mL of CH_3CN and evaporating to dryness. The residue was dissolved in 350 μL of acetone and GLC was conducted on a Hewlett Packard 5890 Series II chromatograph (Global Medical Instrumentation Inc., Ramsey, MO, USA) fitted with a Supleco SP™ 2330 fused silica capillary column (15 m × 0.25 mm i.d., 0.25 μm film thickness). The sample was introduced by split injection using H_2 as the carrier gas with a split ratio of 65:1 (column flow, 1.5 mL/min). Injector and FID detector temperatures were 215 °C. Relative response factors were determined using synthesised alditol acetate standards and identification of components was by comparison of retention times with authentic standards.

2.3. High performance size-exclusion chromatography coupled with multi-angle laser light scattering (SEC-MALLS)

Ten milligrams (10 mg) of each of the water-soluble extracts were dissolved in 2 mL of 0.1 M lithium nitrate ($LiNO_3$) and heated at 95 °C for 15 min. Another 3 mL of 0.1 M $LiNO_3$ was added and left overnight. The SEC-MALLS system consisted of a Waters® 2690 XE Alliance separations module, a Waters® 490 E programmable multi-wavelength detector set at 280 nm, a DAWN-EOS multi-angle laser light-scattering detector (Laser Photometer) with a laser at 690 nm (Wyatt Technology Corp., Santa Barbara, CA, USA), and a Waters® 2410 refractive index monitor. Samples (0.2 mg/mL) were filtered (0.45 μm) before injection (100 μL) and eluted with 0.1 M $LiNO_3$ containing 0.02% NaN_3 (0.7 mL/min) from two columns (TSK-Gel G5000PWXL and G4000PWXL, 300 × 7.8 mm, Tosoh Co., Tokyo, Japan) connected in series. Data for molecular weight determination and conformation were analysed using ASTRA software (Wyatt Technology Corp., Santa Barbara, CA, USA) with a specific refractive index (*dn/dc*) of 0.145 mL/g (determined experimentally).

2.4. Determination of fructan contents and thin-layer chromatographic (TLC) analyses of fructans

Fructan content in Rengarenga lily extract and Cabbage-tree extract was determined using the full Megazyme fructan assay procedure (Megazyme, 2004). Structural composition of fructans was analysed by TLC using a method described by Sims (2003). Samples containing about 50 μg fructans were applied to the origin of a 20 × 20 cm silica gel TLC plate (Silica gel 60 F_{254}, Merck, Germany). Plates were developed three times in butan-1-ol/propan-2-ol/water (3:12:4, v/v/v) at room temperature. Compounds were visualised by spraying with the ketose-specific, urea-phosphoric stain.

2.5. Determination of amino acid composition of plant extracts

The amino acid composition of the plant extracts was determined using a gas chromatography–mass spectrometry (GC–MS) method described by Persson and Nasholm (2001). The extracts were hydrolysed with 6 M HCl containing 0.1% (w/v) phenol at 110 °C overnight before derivatization. The hydrolysed amino acids were derivatized to their *tert*-butyldimethylsilyl (tBDMS) form using *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluroacetamide (MTBSTFA). The derivitised amino acids were analysed by GC–MS using a Hewlett–Packard HP 6890 gas chromatograph (Global Medical Instrumentation Inc., Ramsey, MO, USA) on a CPSSil5 column (25 m × 0.3 mm, Chrompack, Bergen, The Netherlands) and detected by mass spectroscopy with a Hewlett–Packard 5973 mass selective detector. Quantitations were performed by the use of two internal standards, α-aminoisobutyric acid and hydroxyl-L-proline.

3. Results

The four plant extracts showed considerable variation in their WSC contents (Table 1). Rengarenga lily extract had 58% (dry

Table 1
Yield and chemical analyses (g/kg DM) of the plant extracts.

	Lily	Cabbage	*Undaria*	*Acacia*
Yield, water-soluble carbohydrates	576	250	275	794
Dry matter (g/kg)	938	880	885	930
Total sugar content	680	480	370	790
Crude protein	49	143	21	32
Molecular weight (Da)	~5000[a]	N.D	511,000	30,000
Polydispersity index[b] (M_w/M_n)	1.10	–	1.46	1.07
Fructan content (%, w/w)	65	45	–	–

N.D, molecular weight is too low for determination.
[a] Approximate molecular weight only; close to limit of method.
[b] Polydispersity index (M_w/M_n) = M_w (weight average molecular weight)/M_n (number–average molecular weight).

matter basis) WSC in the rhizomatous roots. The results also showed that Cabbage-tree extract and *Undaria* extract contained about one third (25–28%) of the WSC of *Acacia* extract (79%). The DM contents of lily extract and *Acacia* extract were similar (Table 1).

The DM contents of Cabbage-tree extract and *Undaria* extract were similar and lower than those of the lily and *Acacia* extracts. Water-soluble carbohydrates from lily extract contained the monosaccharides, fructose and glucose with similar retention times to those of a commercially available fructooligosaccharide (Frutafit) (Fig. 1). The results of the sugar composition analysis (Table 2 and Fig. 2) showed that *Acacia* extract contained mainly galactose and arabinose with a molar ratio of 7.8:2.2, while *Undaria* extract, contained fucose and galactose with a molar ratio of 5.5:4.4. Cabbage-tree extract contained mainly fructose and glucose with a molar ratio of 3.2:4.9.

The Megazyme fructan assay results indicated that lily extract and Cabbage-tree extract contained a high proportion of fructose and fructose-containing oligosaccharides. These sugars are highly acid-labile and are destroyed by the hydrolysing conditions in these analyses; as such values may have been underestimated in the composition analysis. Cabbage-tree extract contained 45% (w/w) fructans while lily extract contained 65% (w/w) fructans (Table 1). Thin-layer chromatography (TLC) using ketose-specific urea–phosphoric acid stain showed that lily extract fructans did not migrate from the origin at DP > 12, but that the Cabbage-tree extract fructans all moved from the origin (Fig. 3); therefore, Cabbage-tree extract fructans are much smaller fructans (oligofructans) than those of lily extract.

Of the four extracts, Cabbage-tree extract had the highest crude protein content (14.3%), about 3-, 4.5- and 7-fold those of lily extract, *Acacia* extract, and *Undaria* extract, respectively (Table 1). The amino acid analysis of plant extracts revealed that the lily extract and Cabbage-tree extract contained high proportions of glutamic acid which is similar to the higher levels of glutamic acid found in commercially available fructooligosaccharide (Frutafit®) (Table 3). As expected, Hyp and Ser were the main amino acids in *Acacia* extract and accounted for 23% (w/w) and 10% (w/w) of the total amino acids, respectively. *Undaria* extract had a comparatively high proportion (20% w/w) of Gly compared to other extracts. The concentrations of Met, Cys (sulphur-containing amino acids), and Tyr were lower in all plant extracts.

The analysis showed that the extracts had varying weight average molecular weights due to differences in the average chain length of major carbohydrates. The molecular weight of lily extract was ~5000 Da, meaning that the average chain length of the fructans in lily extract is about 30 (the number of sugar residues in the chain). *Undaria* extract and *Acacia* extract had weight-average molecular weights of 5.11×10^5 and 3.0×10^4 Da, respectively. The *Acacia* extract had a relatively low polydispersity index (M_w/M_n) of 1.07, whereas the value for *Undaria* extract was relatively high (1.46).

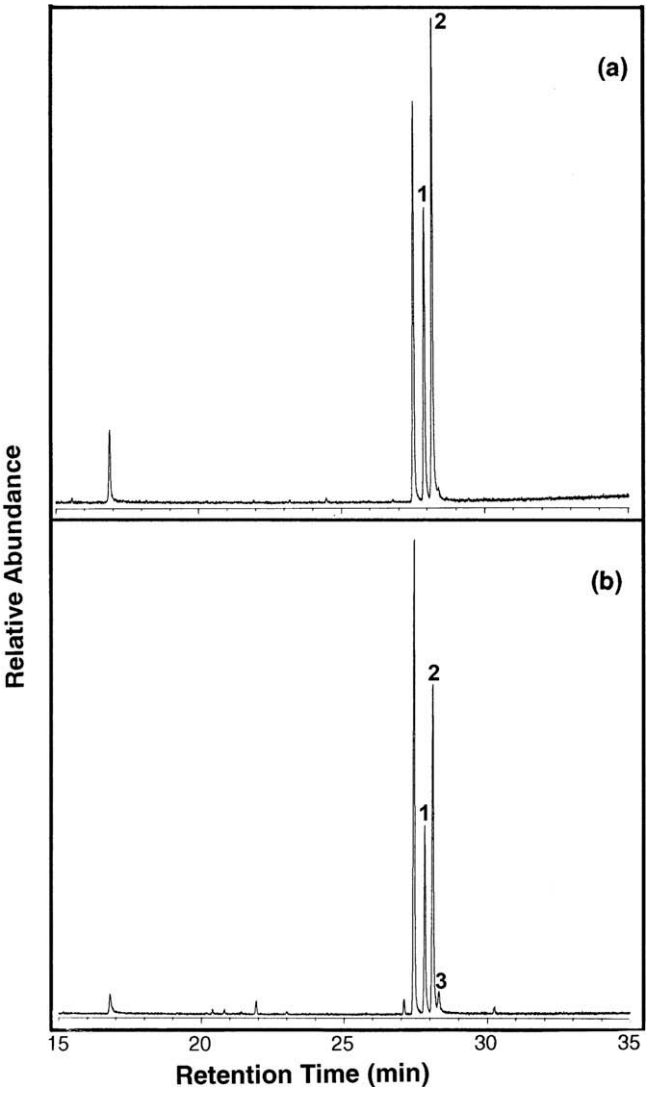

Fig. 1. Gas–liquid chromatograms of the alditol acetates of sugars from (a) Frutafit (fructooligosaccharide) and (b) Rengarenga lily extract obtained by reductive hydrolysis method. Peaks represent the sugars, mannose (1), fructose/glucose (2) and galactose (3).

Table 2
Comparison of the constituent sugar analyses of the plant extracts.[a]

Alditol acetate derivatives	Deduced sugar	Sugar composition (g/ 100 g)			
		Lily	Cabbage-tree	*Undaria*	*Acacia*
Rhamnitol	Rhamnose	N.D	4.7	N.D	0.5
Fucitol	Fucose	N.D	1.2	55.2	N.D
Arabinitol	Arabinose	N.D	5.8	N.D	21.6
Xylitol	Xylose	N.D	1.4	N.D	N.D
Mannitol	Mannose/ Fructose	34.4	32.1	1.0	N.D
Glucitol	Glucose/ Fructose	61.0	49.6	N.D	N.D
Galactitol	Galactose	4.6	5.1	43.9	77.9

N.D, not detected.
[a] Average of duplicate determinations.

4. Discussion

The extracts obtained from the dry powders of Rengarenga lily, Cabbage-tree, *Undaria* and exudate of *Acacia* were

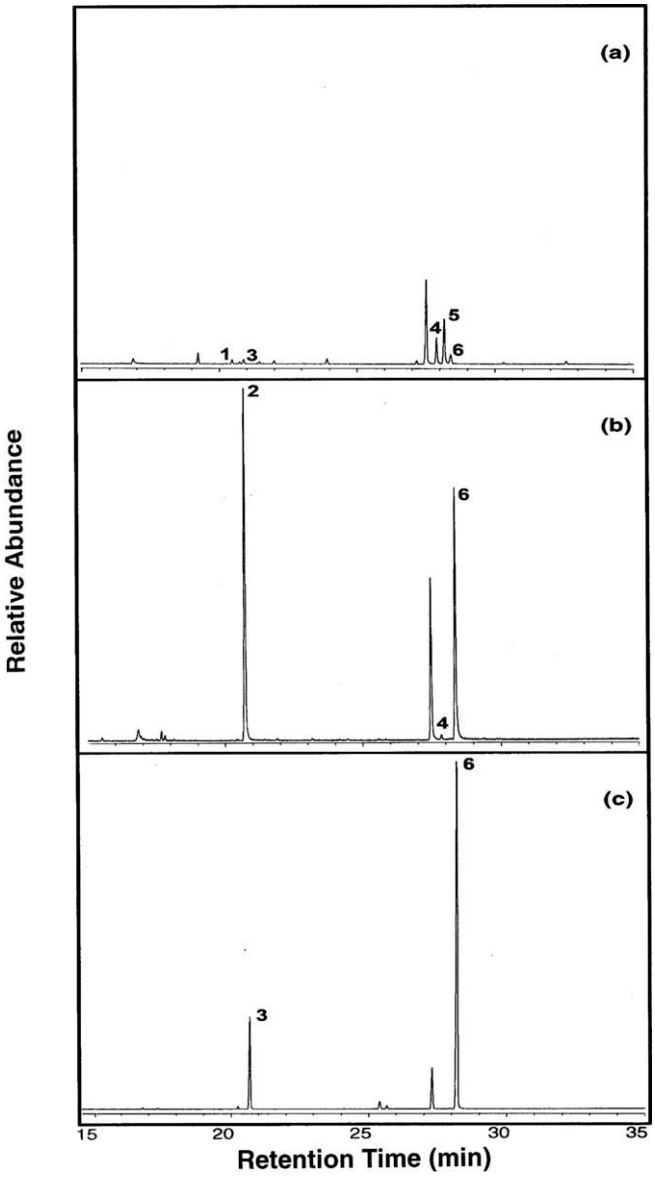

Fig. 2. Gas–liquid chromatograms of the alditol acetates of sugars from Cabbage tree extract (a), *Undaria* extract (b), and *Acacia* extract (c) obtained by reductive hydrolysis method. Peaks represent the sugars, rhamnose (1), fucose (2), arabinose (3), mannose (4), fructose/glucose (5) and galactose (6).

water-extractable carbohydrate compounds and part of the weight was likely to be water-extractable proteins and inorganic salts and other compounds such as phenolic compounds. The wide range of WSC (250–794 g/kg DM) is a reflection of the different proportions of structural material in different species.

The WSC content of the rhizomes of Rengarenga lily was lower than the WSC contents reported for the underground parts of chicory (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) (Schubert & Feuerle, 1997; van den Ende, Mintiens, Speleers, Onuoha, & van Laere, 1996; Wilson, Smith, & Yonts, 2004). Perhaps, the lower value of Rengarenga lily in this study was due to the limitations in the carbohydrate analysis as described below. Fructan contents can reach about 20% of the fresh weight (roughly 80% of the dry weight) in chicory taproots (Wilson et al., 2004). The fructan composition (mainly long chain oligosaccharides; DP > 12) of the lily extract in this study resembles that of the underground parts of chicory (Wilson et al., 2004). In contrast, Monti, Amaducci, Pritoni, and Venturi (2005) reported that chicory fructan contains a high proportion of short chain oligosaccharides (DP 2–10) instead of long chain fructans (DP > 11) and DP varies widely from 2 to more than 100. These variations could be due to differences in soil conditions, climate, biosynthetic factors, season (harvest time), cultivar and geographical locations (Monti et al., 2005; Wilson et al., 2004). Fructans from the Cabbage-tree extract contained greater amounts of monosaccharides (fructose and glucose) and sucrose, making them similar to fructans from adventitious root tubers of the *Caesia calliantha* (Incoll, Bonnett, & Gott, 1989). The amount of WSC extracted from Cabbage-tree extract (250 g/kg DM) in this study is comparable to the values reported by previous studies on the same species (211–388 g/kg DM) (Fankhauser & Brasch, 1985).The same authors reported that the polysaccharide precipitated from the water extracts of Cabbage-tree shoots is glucofructofuranan and contains more utilisable carbohydrate on a fresh weight basis than either sugar cane or sugar beet (Fankhauser & Brasch, 1985). The analytical data obtained for the sugar composition of *Acacia* extract are similar to those reported previously for *A. pycnantha* exudates (Annison, Trimble, & Topping, 1995). *Acacaia* extract had very high galactose content (78%) compared to similar types of arabinogalactans (18–49% galactose) extracted from Vulgares and Gummiferae series *Acacia* species in Africa (Al-Assaf, Phillips, & Williams, 2005b).

Fucose-containing carbohydrates (fucans) were extracted from *Undaria* seaweed with diluted sulphuric acid (1% (w/w)) during which alginate was extracted with 1% (w/v) Na_2CO_3 and discarded during the extraction process. Alginates form an insoluble precipitate at acidic pH (~3.0), but they are stable in solutions between pH 6 and 9. Total recovery corresponded to non-dialyzable compounds, as free minerals and low-molecular-weight substances were removed during exhaustive dialysis of the fractions. The sulphate (and its counter-ions) present in the galactofucan sulphate account for a significant proportion of the weight of the water-soluble carbohydrates in the *Undaria* extract. For the total carbohydrate content of the *Undaria* extract if every sugar residue has a sulphate attached then it could easily double the weight as being due to galactofucan sulphate. This means there is not a lot of weight left for other organic and inorganic compounds to make up the 885 g/kg dry matter.

In general, total carbohydrates determined in the plant extracts may have been underestimated in this study. The different sugars give different responses in the phenol–sulpuric acid total sugar assay. The response of fructose in the phenol–sulphuric acid assay was lower than glucose and therefore, the total sugar estimate in lily extract and Cabbage-tree extract was lower than expected. For instance, lily extract gave 680 g/kg carbohydrates using glucose as the standard. According to the Megazyme fructan assay lily extract contained 65% (w/w) fructans and because of the lower response of fructose in the phenol–sulphuric acid assay, total carbohydrate was underestimated. Out of the 209 g unaccounted for in analysis (938-680-49 = 209 g) (938 = DM, 49 = CP) it would not be surprising if 150 g or more of this was because of underestimation in the carbohydrate assay, and similarly for the other extracts.

It has long been established that the proteinaceous content of complex plant extracts may comprise a mixture of proteins, whose relative proportions vary for different plant species. It seems that proteins are extracted with the WSC, possibly due to the presence of water-soluble proteins in the intact materials or may be proteins are present in a carbohydrate–protein complex. For example, it is known that the polysaccharide fraction from *Acacia* tree exudates constitutes 95% of the dry weight of proteoglycans, which consist of highly branched galactan polymers, with galactose and/or arabinose side chains and 1–5% of covalently attached proteins rich in hydroxyproline and serine (Meance, 2004). This observation is typical for most of the type II arabinogalactans in many plant species

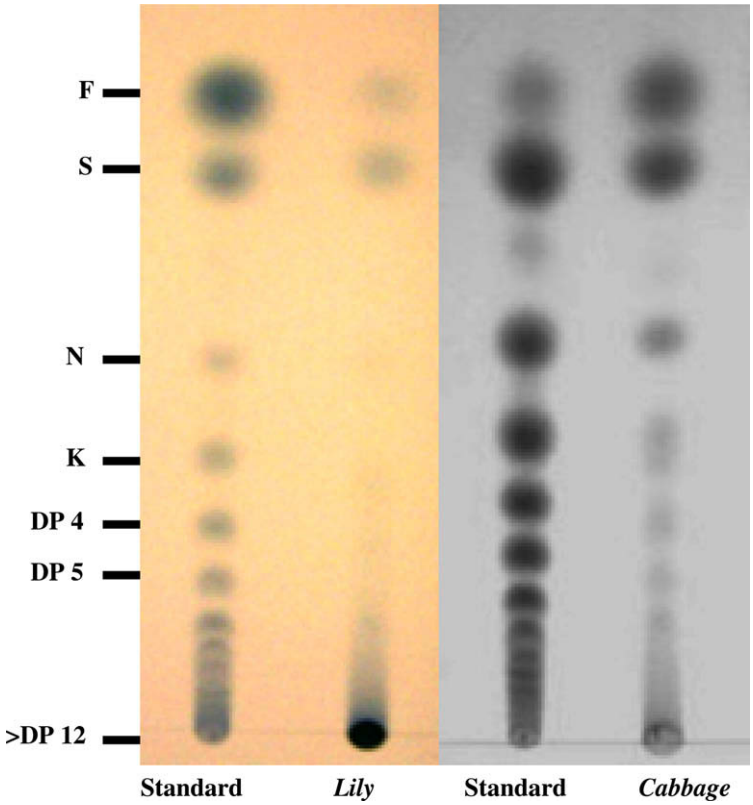

Fig. 3. Thin-layer chromatogram of fructans from Rengarenga lily extract (*A. cirratum*) and *Cabbage-tree extract* (*C. australis*). Markers represent the mobilities of fructose (F), sucrose (S), 6_G-kestotriose (N), 1-kestotriose (K), inulin tetrasaccharide (DP 4), and pentasaccharide (DP 5).

Table 3
The amino acid composition (weight percentage) of the plant extracts.[a]

	Amino acid composition (g/100 g protein)				
	Lily	Cabbage	*Undaria*	*Acacia*	Frutafit[b]
Alanine	11	10	10	5	6
Glycine	6	8	20	8	N.D
Valine	5	4	11	8	7
Leucine	6	6	11	11	7
Isoleucine	3	3	4	4	7
Proline	5	4	3	7	6
Methionine	1	Tr	2	N.D	N.D
Serine	4	4	9	10	N.D
Threonine	3	3	6	2	1
Phenylalanine	2	3	4	3	N.D
Aspartic acid	8	9	6	12	30
Hydroxyproline	2	3	6	23	N.D
Cystine	N.D	2	N.D	N.D	N.D
Glutamic acid	16	26	6	6	36
Lysine	7	4	N.D	N.D	N.D
Arginine	12	4	N.D	N.D	N.D
Histidine	8	4	N.D	N.D	N.D
Tyrosine	1	2	2	2	N.D

N.D, not detected.
Tr, trace amount (<1 wt%).
[a] Average of duplicate determinations.
[b] Commercially available fructooligosaccharide.

which possess immunomodulatory properties (Inngjerdingen et al., 2005; Paulsen, 2002). The Cabbage-tree extract was shown to contain a large (14% (w/w)) amount of protein compared to other extracts. This would also suggest that the considerable amount of water-soluble proteins in Cabbage-tree shoot powder may solubilise and fractionate with the carbohydrates during the extraction process.

To complete the analysis of the primary structure of carbohydrates, it is important to determine the mean number of constituent sugars or repeating units, that is, their weight average molecular weight (M_w), and the distribution of the molecules (polydispersity). The molecular weight of the *Acacia* extract (3.0×10^4 Da) is lower than the molecular weight of the arabinogalactans extracted from *Acacia senegal* (Al-Assaf, Phillips, & Williams, 2005a) and similar (3.0×10^4–3.9×10^4) to bioactive arabinogalactan polysaccharides extracted from *Glinus oppositifolius* (a medicinal plant) in Norway (Inngjerdingen et al., 2005). The weight average molecular weight of *Acacia* extract is one sixteenth that of *A. senegal* reported by Al-Assaf et al. (2005a) and low molecular weight means that its ability to become viscous is limited. The molecular weight of the Cabbage-tree extract was smaller than the lowest level that can be detected by the light-scattering system (SEC-MALLS). However, Brasch et al. (1988) reported that the number average molecular weight of the glucofructofuranan extracted from roots of the New Zealand Cabbage-tree is ~3000, which corresponds to a degree of polymerization of 19. The molecular weight of the seaweed fraction (5.11×10^5 Da) was similar to a value (6.8×10^5 Da) previously reported for high-molecular-weight fucans extracted from brown seaweeds (Nishino, Nishioka, Ura, & Nagumo, 1994; Patankar, Oehninger, Barnett, Williams, & Clark, 1993). Differences could be due to the extraction method or to seasonal or geographical variations. The high polydispersity index of *Undaria* extract may indicate that some degradation might have occurred during extraction of galactose-containing fucans from *Undaria* seaweed.

5. Conclusions

It can be concluded that WSC extracted from Rengarenga lily and Cabbage-tree contain mainly fructans and that their structures

are different. It appears that the Cabbage-tree extract contains mainly smaller fructans (oligofructans), whereas lily extract contains oligosaccharides with higher degree of polymerization (DP > 12). The *Acacia* extract contained around 80% (w/w) carbohydrate and only 3% (w/w) protein with a high level of hydroxyproline. The carbohydrates extracted from *Undaria* seaweed were composed mainly of fucose and galactose. Further studies, such as the fractionation of WSC compounds using gel-permeation chromatography and glycosyl linkage analysis using gas chromatography–mass spectrometry (GC–MS), are needed to elucidate and confirm the chemical structures in these plant extracts.

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