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PHYTOCHEMISTRY
Phytochemistry 67 (2006) 1271–1275
www.elsevier.com/locate/phytochem

Polysaccharide composition of the fruit juice of *Morinda citrifolia* (Noni)

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Received 13 March 2006; received in revised form 27 April 2006
Available online 14 June 2006

Abstract

An ethanol-insoluble, high molecular weight fraction was collected from the juice of *Morinda citrifolia* fruit grown in Viet Nam. The fraction is composed primarily of carbohydrate (67% (w/w)). The polysaccharide fraction consists predominantly of GalA*p* (53.6 mol%), Ara*f* (13.6 mol%), Gal*p* (17.9 mol%) and Rha*p* (9.5 mol%). Glycosyl linkage analysis suggests the polysaccharide fraction contains mostly the pectic polysaccharides, homogalacturonan (4-GalA*p*), rhamnogalacturonan I (4-GalA*p*, 2-Rha*p*, 2,4-Rha*p*), arabinan (5-Ara*f*, 3,5-Ara*f*, *t*-Ara*f*), type I arabinogalactan (4-Gal*p*, 3,4-Gal*p*, *t*-Ara*f*) and β-glucosyl Yariv-binding type II arabinogalactan (3,6-Gal*p*, *t*-Ara*f*). Low levels of xyloglucan (4-Glc*p*, 4,6-Glc*p*, *t*-Xyl*p*, *t*-Fuc*p*), heteroxylan (4-Xyl*p*) and heteromannan (4-Man*p*) are also present.

Keywords: *Morinda citrifolia*; Rubiaceae; Noni fruit juice; Polysaccharides

1. Introduction

The Noni plant, *Morinda citrifolia* L. Rubiaceae, is a medicinal plant that grows in tropical and temperate climates. In Viet Nam, *M. citrifolia* is abundant in the South with smaller populations in the North and centre of the country. The plant is a small evergreen tree with fruit that can grow up to 15 cm in size. The fruit, which has a foul taste and soapy smell when mature, was used as a food in times of famine. All parts of the plant, such as the bark, stem, root, leaf, and especially fruit, have also been used as herbal medicines by Polynesians for over 2000 years. According to traditional treatment and recent scientific research, the Noni plant has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumour, anthelmintic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Wang et al., 2002).

Despite the many therapeutic effects of Noni fruit, few reports on the identification of biologically active components are available. Low molecular weight compounds identified from the fruit include asperulosidic acid, caproic acid, caprylic acid (Levand and Larson, 1979) and various glycosides such as 6-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose, 6-*O*-(β-D-glucopyranosyl)-1-*O*-hexanoyl-β-D-glucopyranose and 3-methylbut-3-enyl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (Wang et al., 2000). The compounds 6-*O*-(β-D-glucopyranosyl)-1-*O*-octanoyl-β-D-glucopyranose and asperulosidic acid (designated NB10 and NB11, respectively) were shown to inhibit AP-1, an inducible eukaryotic transcription factor involved in cell proliferation, metastasis and metabolism (Liu et al., 2001).

In addition to the low molecular weight compounds, the fruit juice of Noni contains a polysaccharide-rich substance (Noni-ppt) with anti-tumour activity on Lewis lung carcinoma in mice (Hirazumi and Furusawa, 1999). A significant increase (119%) was observed in the life span of C57BL/6 mice implanted with Lewis lung carcinoma, with

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0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.04.023

41% of mice surviving for 50 days of treatment with a 15 mg per mouse dose. The polysaccharide-rich fraction extracted from Noni fruit juice also has antitumour activity on sarcoma 180 ascites tumour in mice (Furusawa et al., 2003). It was proposed that the Noni fruit juices suppressed growth of tumours by stimulating the immune system (Hirazumi and Furusawa, 1999).

The polysaccharides extracted from the Noni fruit juice have only been partially analysed by monosaccharide compositional analysis using cellulose thin-layer chromatography. According to this early analysis, they are composed of glucuronic acid (GlcA), galactose (Gal), arabinose (Ara) and rhamnose (Rha) (Hirazumi and Furusawa, 1999). In the current study the chemical structure of the polysaccharide fraction is further characterised by determining the glycosyl linkage composition and is shown to differ from this earlier study.

2. Results and discussion

The amount of Noni-ppt recovered from 100 g of fruit was 16 g, which is comparable to the yield recorded by Hirazumi and Furusawa (1999) (13% (w/w)). The compositional analysis accounted for approximately 83% of the mass. The colorimetric assays give an overall estimate of the composition of Noni-ppt. Noni-ppt is composed predominantly of carbohydrate (~67% (w/w)) of which uronic acids are a major component (62% of the sample). Low levels of protein are present (2% measured colorimetrically and 6% from nitrogen analysis), which is consistent with the findings of Hirazumi and Furusawa (1999) who detected 2% protein colorimetrically. Noni-ppt contains 5% (w/w) of AGP based on the specific binding of β-glucosyl Yariv reagent to arabinogalactan-proteins (AGPs) (Van Holst and Clarke, 1985). Total soluble phenolic assays estimated less than 1% (w/w) phenolics and moisture content of the sample is approximately 10%. The remaining mass (16%) is unaccounted for but some could arise from inorganic material (which was not measured), or as a consequence of the inaccuracies of the colorimetric assays.

Monosaccharide analysis by GC–MS of alditol acetates of the pre-reduced Noni-ppt (Table 1) shows that it is composed of Gal*A*p, 60% of which is methylesterified, Gal*p*, Ara*f*, Rha*p*, Glc*p*, Xyl*p*, GlcA*p*, Man*p* and Fuc*p*. Hirazumi and Furusawa (1999) partially characterised Noni-ppt by TLC of the sugars released after 1 M sulfuric acid hydrolysis. Apart from Gal*A*p that was not detected by Hirazumi and Furusawa (1999), the overall composition generally agrees with that found in this study. It is possible that the GalA was mistakenly identified as GlcA on the TLC since their R_Fs are very similar.

Glycosyl linkage analysis (Table 1) provides an insight into the polysaccharide composition of Noni-ppt. Consistent with the monosaccharide analysis, Gal*A*p is the most abundant sugar and it occurs primarily (52.1%) as 4-linked with 68% of the 4-Gal*A*p residues being methyl esterified at C(O)6. Gal*p* is mostly 4-linked and terminal, with lower amounts of 3-, 6-, 2,4-, 3,4- and 3,6-linkages. The next most abundant monosaccharide, Ara*f*, is mostly terminal, 5-linked and 3,5-linked, while Rha*p* occurs in a combination of 2- and 2,4-linkages along with some terminal residues. The minor monsaccharides are present as terminal-, 4- and 4,6-Glc*p*; terminal-, 2- and 4-Xyl*p*; terminal-GlcA*p*; 4-Man*p* and terminal-Fuc*p*.

Table 1
Monosaccharide linkage (based on permethylated alditol acetates) and monosaccharide composition (based alditol acetates) of Noni-ppt

Sugar	Deduced glycosidic linkage[a]	Linkage composition (mol%)[b]	Monosaccharide composition (mol%)
Rha*p*	Terminal	0.6	9.5
	2-	3.3	
	2,4-	2.1	
Fuc*f*	Terminal	0.3	0.3
Ara*f*	Terminal	7.7	13.6
	2-	tr	
	3-	0.1	
	5-	4.8	
	3,5-	2.1	
Xyl*p*	Terminal	0.6	1.2
	2-	0.3	
	4-	0.3	
Man*p*	4-	0.2	0.7
Glc*p*	Terminal	0.5	2.2
	4-	1.9	
	4,6-	0.3	
GlcA*p*	Terminal	0.9	1.1
Gal*p*	Terminal	4.3	17.9
	3-	0.5	
	4-	10.7	
	6-	1.1	
	2,4-	0.2	
	2,6-	tr	
	3,4-	0.6	
	3,6-	1.9	
Gal*A*p	Terminal	2.8 (10)	53.6 (60)
	4-	52.1 (68)	

[a] Terminal Ara*f* is deduced from 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, etc.
[b] Average of duplicate determination; ( ), % methyl esterification; tr, trace (<0.2%).

Based on neutral sugar analysis, Hirazumi and Furusawa (1999) suggested that Noni-ppt was composed of a "gum arabic heteropolysaccharide". Gum arabic is predominantly composed of arabinogalactan-proteins (AGPs) (Osman et al., 1993), which are classed as type II arabinogalactans where galactose is 3-, 6- and 3,6-linked (Bacic et al., 1988). While there is both linkage data (Table 1) and β-glucosyl Yariv binding data to confirm AGPs are present in Noni-ppt, at only 5% by weight, they are not the major constituent (Table 2). The glycosyl linkage composition of Noni-ppt (Table 1) indicates it is rich in pectic polysaccharides, with more than 80% of the linkages attributable to these polysaccharides (Ridley et al., 2001). Estimation of the overall poly-

Table 2
Deduced polysaccharide composition based on monosaccharide linkage data

Polysaccharide type	Component	Mol%[a]
Pectic polysaccharides	Homogalacturonan	49.5
	Rhamnogalacturonan I	10.8
	Arabinan	9
	Type I arabinogalactan	12.3
Type II arabinogalactan	Arabinogalactan-protein	5.5
Xyloglucan		1.5
Heteroxylan		0.3
Heteromannan		0.4
Undefined		10.7

[a] Based on sum of linkages as described by Sims and Bacic (1995).

saccharide composition of Noni-ppt by addition of the relevant linkages typical of plant polysaccharides confirms the abundance of pectic polysaccharides (Table 2). Pectic polysaccharides include components of homogalacturonan (HG), composed of linear (1,4)-GalA residues, some of which are carboxy methylesterified; rhamnogalacturonan I (RG-I), consisting of a backbone of alternating 4-GalA and 2-Rha with neutral (3- and 3,5-linked arabinan and 4- and 4,6-linked and/or 3 and 3,6-linked (arabino)galactans) and acidic oligosaccharides attached through C(O)4 of Rha; and rhamnogalacturonan II (RG-II). RGII has a 4-GalA backbone substituted with four different oligosaccharide side chains A (octasaccharide), B (nonasaccharide), C (Rha-(1,5)-Kdo disaccharide) and D (Ara-(1,5)-Dha disaccharide) (Ridley et al., 2001). All of the linkages typical of HG and RGI are present at high levels in Noni-ppt (Table 1). While there are many studies that show fruit juices including orange, apple, pear, guava, mango, papaya and strawberry contain pectins (Grassin and Fauquembergue, 1996), there have been few studies of the composition of juices or cell walls of the Rubiacae with the exception of coffee (*Coffea* spp.). Studies on coffee beans often report high levels of galactomannan and type II arabinogalactan or AGP (Navarini et al., 1999; Redgwell et al., 2002). In addition, pectin has been extracted from the mucilage (inner mesocarp) of *Coffea arabica* cherries in yields of 23–35% of the dry alcohol insoluble residue (AIR) (Avallone et al., 2000). Monosaccharide composition of the extracted pectin is similar to that of Noni-ppt (Table 1) in that it is composed of 60% uronic acid (60% of which is methyl esterified), with Ara and Gal the predominant neutral sugars (53 and 20 mol% of neutral sugars, respectively) (Avallone et al., 2000).

AGPs are known to stimulate the immune system (Pettolino et al., 2006). However, it is likely that the pectin component of Noni-ppt also contributes to its immunostimulatory activity as pectins have also been described as immunomodulators. For example, a pectic arabinogalactan from the Indian medicinal plant *Tinospora cordifolia* was isolated and shown to enhance the mitogenic activity of B-cells in vitro (Chintalwara et al., 1999); while pectic polysaccharide isolated from thyme (TV-3-IIIA-IIa; Chun et al., 2001), *Plantago major* (PMII; Samuelsen et al., 1996) and *Bupleurum falcatum* (BR-2Iib; Yamada et al., 1989), have complement activating abilities. In addition to the immunomodulatory effects of pectins, there is growing evidence that some pectins have direct anti-cancer activities, such as pH modified citrus pectin which presumably acts by inhibition of galectin-3-mediated processes (Nangia-Makker et al., 2002). Galectin-3 is a β-galactoside binding protein involved in cell growth, angiogenesis, apoptosis, signalling, differentiation, adhesion and RNA processing. Galectin-3 is expressed in normal cells, but has increased expression in cancer cells and may be involved in tumour progression and metastasis (Takenaka et al., 2004).

3. Concluding remarks

The glycosyl linkage composition of Noni-ppt has provided further insights into the potential biologically active components of Noni fruit juice. The high molecular weight fraction of Noni juice is predominantly composed of pectic polysaccharides including HG, RG and the neutral side chains of (arabino)galactan and arabinan. Low levels of AGP are also present along with trace amounts of xyloglucan, heteroxylan and heteromannan (Table 2). Pectins and AGPs have been implicated in the immunomodualatory effects of herbal medicines, and it is likely that both are responsible for the effects of Noni-ppt.

4. Experimental

4.1. Plant material and preparation of polysaccharide fraction

Yellowish-white (ripe) Noni fruits were collected from the South of Viet Nam, in December 2002. The fruit was washed and kept in a sterile covered glass jar for 1–3 days to allow the juice to seep out. The Noni juice was separated from the fruit by centrifugation. The addition of 4 volumes of 95% ethanol for 30 min at room temperature partitioned the juice into an ethanol-soluble fraction (Noni-sol) and ethanol-precipitated fraction (Noni-ppt). Noni-ppt was collected by centrifugation (2500 rpm for 10 min) and washed four times by repeatedly dissolving in water and precipitating with 95% ethanol. The final Noni-ppt was dissolved in water and the water-soluble fraction collected by centrifugation and freeze-dried.

4.2. Analytical methods

The colorimetric method for determination of sugar and related substances of Dubois et al. (1956) was used to estimate total carbohydrate. It was necessary to use a mixed standard to measure total carbohydrate due to the differential responses of hexoses, pentoses and uronic acids (Dubois et al., 1956). The standard was composed of a mixture of GalA, Gal, Ara and Rha in a ratio of 54:21:15:10, respectively. The ratio of sugars was determined from the

monosaccharide composition of the sample (Table 1). Uronic acid content of the polysaccharide was estimated using the method of Filisetti-Cozzi and Carpita (1991) with GalA as the standard. AGP content was determined by the method published by Van Holst and Clarke (1985). The reaction with β-glucosyl Yariv reagent, which specifically binds AGPs, was measured in radial diffusion gels. Gum arabic was used as a standard. The Bio-Rad Protein Assay was used to determine the presence of protein in Noni-ppt with bovine serum albumin (BSA) as standard according to manufacturer instructions. Total protein was also estimated from nitrogen analysis by the Kjedahl method. Nitrogen was analysed using a Carlos Erba NA 1500 Series 2 NCS Analyzer and AS-200 Autosampler (Fisons Instruments, Milan, Italy). Total soluble phenolics were measured using the Folin and Ciocalteu's reagent (Fry, 1988).

4.3. Polysaccharide analysis

The Noni-ppt was carboxyl reduced to detect uronic acids and to distinguish between methyl-esterified and free uronic acids (Sims and Bacic, 1995). Briefly, the polysaccharides were reduced with sodium borodeuteride to reduce methylesters, then free carboxylic acid groups were carbodiimide activated before a final reduction with sodium borohydride or sodium borodeuteride. The ratio of deuterated and non-deuterated ions corresponding to fragments of C(6) was used to determine the relative proportions of neutral, methylesterified and total uronic acids of the relevant hexoses. Glycosyl linkage composition of the carboxyl reduced polysaccharides was determined by GC–MS of the permethylated alditol acetates using a BPX70 column and conditions as described by Lau and Bacic (1993). Monosaccharide composition was determined by GC–MS of alditol acetates after carboxyl reduction and TFA hydrolysis of the polysaccharides using the same chromatographic conditions as above. Polysaccharide composition was estimated by addition of the relevant linkage types as described by Sims and Bacic (1995) and Shea et al. (1989).

Acknowledgements

This research was supported by funds from the Cooperative Research Centre for Bioproducts of Australia. Anh Kim Thi Bui wishes to acknowledge the assistance of The Ministry of Education and Training Vietnam in providing funds to complete this study in Australia.

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