



## Production and identification of intricate bioactive oligosaccharides from *Nyctanthes arbor-tristis* leaves by a combination of enzymatic, HPAEC and MALDI-TOF-MS techniques

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Non-digestible oligosaccharides are associated with positive health outcomes; however, hemicellulosic oligosaccharides of *N. arbor-tristis* have not been studied. Herein, we report production of hemicellulosic oligosaccharides from its leaves and their identification. A series of complex neutral oligosaccharides (**1-16**), produced by *endo*-(1,4)- $\beta$ -D-glucanase digestion of the alkali extracted xyloglucan, have been identified by chemical, HPAE chromatographic and MALDI-mass spectrometric techniques. Additionally, xylan enriched hemicellulose-A fraction upon *endo*-(1,4)- $\beta$ -D-xylanase digestion yield several acidic and neutral xylo-oligosaccharides (**17-24**), each containing a backbone of  $\beta$ -(1,4)-D-Xylp units substituted at position 2 with a single stub of D-Xylp or 4-*O*-Me-D-GlcpA residues. The structural diversity and high water solubility of these enzyme-generated oligosaccharides suggested promising health implications.

**Keywords:** *N. arbor-tristis* leaves, hemicelluloses, oligosaccharides, HPAEC, MALDI-TOF-MS

Recently, purification of a pectic polysaccharide from the water extract of *N. arbor-tristis* leaves was reported<sup>1</sup>. This polysaccharide (75 kDa) was stated to be highly branched and possessed esterified phenolic acid. Generation of several pectin derived oligosaccharides epitomizing macromolecular structure by using chemical as well as enzymatic protocols, and their structural features have also been described. Moreover, this polymer was reported to have dose dependent antioxidant activity analogous to standard antioxidants, and ester-linked phenolic acids were related to the activity. Additionally, this bioactive polymer was able to form a water-soluble complex with bovine serum albumin in the range of pH 4.0–7.4.

Hemicelluloses that comprise of a complex group of polysaccharides, represent one of the major sources of renewable organic matter in nature<sup>2</sup>. Some of these hemicelluloses act as connecting macromolecules between cellulose fibrils, imparting flexibility to plant fibres. This property of the hemicellulosic polysaccharides has been exploited to regulate the barrier characteristics of cellulosic materials. Moreover, hemicelluloses have also been used as food additives<sup>3</sup>, and packaging films<sup>4,5</sup>.

Prebiotics are dietary ingredients that cannot be digested by human-secreted digestive enzymes, yet

provide a health benefit to the host by selectively stimulating the growth and/or activity of one or a limited number of host gut microbiota<sup>6,7</sup>. A number of oligosaccharides were reported to have pronounced prebiotic activity<sup>8-10</sup>. For instance, xylo-oligosaccharides augmented the growth of bifidobacteria and, hence, are labelled as prebiotics<sup>11</sup>. As a consequence of anti-cancer property, xylitol is used in food applications such as in chewing gum and tooth paste<sup>12</sup>. Additionally, some of oligosaccharides themselves regulate plant growth, organogenesis, and defence against pathogens<sup>10</sup>. They also exhibited antimicrobial activity against pathogenic bacteria or fungi<sup>13</sup> and, consequently, might be beneficial to replace synthetic additives<sup>14</sup> used in food preservations. Hence, oligosaccharides are targets of new investigations. Incidentally, the prebiotic properties of oligosaccharides depend on their chemical profile including monosaccharide composition, glycosidic linkage pattern, and degree of polymerization. Since *N. arbor-tristis* is a medicinal plant, and as oligomers have considerable potential for application as prebiotics, studies on hemicellulose derived oligosaccharides will be of relevance from scientific as well as industrial point of view.

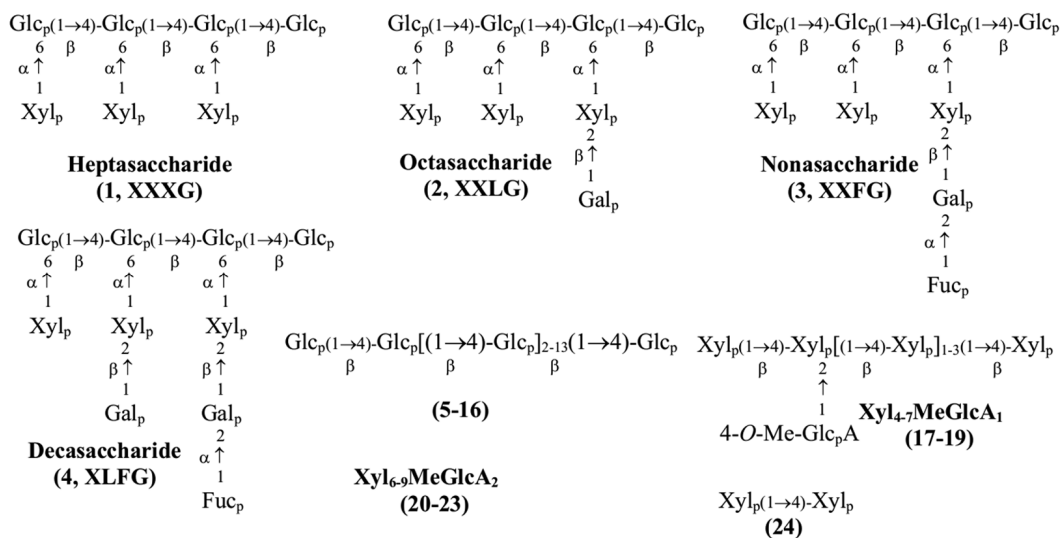
Herein, we report information on oligosaccharides produced from the hemicellulosic polysaccharides

(A1OH and B1OH) of *N. arbor-tristis* leaves. First, generation of oligosaccharides by the digestion of a xyloglucan enriched fraction (B1OH-N) with *endo*-(1,4)- $\beta$ -D-cellulase and their characterization is demonstrated. Incidentally, purification of B1OH fraction through anion exchange chromatography yielded a neutral fraction designated as B1OH-N. Then, production of xylo-oligosaccharides from the xylan enriched fraction (A1OH) by *endo*-(1,4)- $\beta$ -D-xylanase digestion and their structural characterization is also reported. Investigation on resulting oligosaccharides (Scheme I) was performed by amalgamating chemical, chromatographic and mass spectrometric techniques.

### Results and Discussion

Alkali extraction produces xyloglucan and xylan as major hemicelluloses. Extraction of the water-insoluble residues<sup>1</sup> of *N. arbor-tristis* leaves using aqueous 4% solution of sodium hydroxide yielded the anticipated

hemicelluloses. In reality, this alkali extract gets precipitated during dialysis and the mixture upon centrifugation separated into two fractions: the hemicellulose-A fraction (named as A1OH) comprising of the precipitate and the hemicellulose-B fraction (marked as B1OH) containing the water-soluble polymer. Saccharide analysis of the water-soluble B1OH fraction showed the existence of Glc, Xyl, Gal and Fuc residues (Table I) indicative of xyloglucan (XG) type polymer. The Rha, GalA, Ara and a portion of Gal residues probably originates from pectic polymer co-extracted with hemicelluloses. Contrarywise, the hemicellulose-A fraction consists mainly of Xyl residue indicative of xylan. As composition analysis could generate vague information<sup>15</sup>, the water-soluble B1OH fraction was further purified by anion exchange chromatography (AEC) using DEAE-Sepharose FF column. The yield from the anion exchanger was 91% based on total



Scheme I

Table I — Glycosyl makeup (mol %) of hemicellulosic fractions (B1OH, A1OH and B1OH-N) of *N. arbor tristis* leaves and of oligosaccharides containing fractions produced by digestion with *endo*-glucanase (fraction XG<sub>OSSES</sub>) and *endo*-xylanase (fraction X<sub>OSSES</sub>)

Oligosaccharide	B1OH	A1OH	B1OH-N	XG <sub>OSSES</sub>	X <sub>OSSES</sub>
Rha	1	2	Tr <sup>a</sup>	Nd <sup>b</sup>	1
Fuc	3	Tr	4	5	Nd
Ara	7	9	2	1	Nd
Xyl	32	80	31	30	97
Man	2	Tr	1	Nd	1
Gal	18	4	16	12	1
Glc	31	2	45	52	Nd
GalA	6	3	1	Tr	Nd

<sup>a</sup> Tr: trace

<sup>b</sup> Nd: not detected

saccharide recovered. What is more, 42% of the recovered saccharide was not-retained (designated as B1OH-N) in the exchanger; whereas the rest was eluted from using salt gradient. Compositional analysis of B1OH-N demonstrated the presence of Glc, Xyl, Gal and Fuc as the major neutral saccharide typical of XG type polysaccharide (Table I). Based on standard dextran, the average molecular mass of this XG is 43 kDa as estimated by size exclusion chromatography. This xyloglucan (XG) had a specific rotation of  $[\alpha]_D^{25} +8.1^\circ$  (*c* 1.1, H<sub>2</sub>O) as well. The assignments of the D- and/or L-configuration to the saccharide residues are based on results from previous hemicellulosic polysaccharide<sup>16</sup>. The IR spectrum of B1OH-N fraction, as shown in Figure 1, displayed absorption bands suggestive of hemicellulosic polysaccharides<sup>17</sup>. For instance, bands characteristic of C–H stretching vibrations of methylene or methyl groups (2860–2920 cm<sup>-1</sup>), and the O–H stretching vibrations of hydrogen bonded hydroxyl groups (3350 cm<sup>-1</sup>) were present. The low intensity band at 895 cm<sup>-1</sup> indicative of β-glycosidic linkages connecting the monosaccharide residues<sup>18</sup> as well as bands between 1150 and 1000 cm<sup>-1</sup> characteristic of C–O stretching absorption of polysaccharide were also detected.

#### **endo-Glucanase digestion of B1OH-N produces several xyloglucan oligosaccharides (XG<sub>OSSES</sub>)**

Generally, *endo*-glucanase hydrolyses (1,4)-β-D-glycosidic linkage of XG adjacent to a non-substituted Glc unit while retaining the side chains intact<sup>15</sup>. In reality, the B1OH-N fraction was submitted to a depolymerisation reaction with an *endo*-glucanase, and the enzyme-resistant fraction was removed from the digest by precipitation with EtOH. The *endo*-

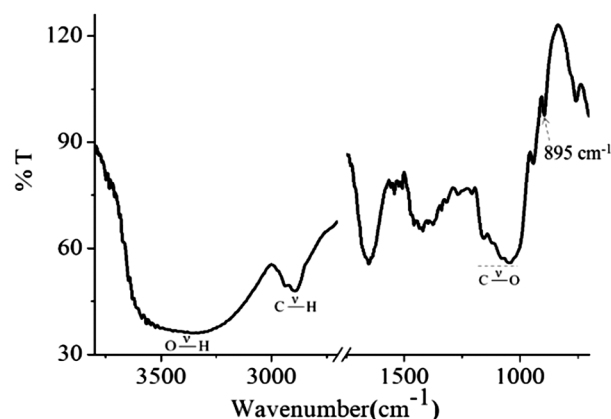


Figure 1 — Infrared spectrum of xyloglucan enriched hemicellulosic fraction (B1OH-N) isolated from *N. arbor-tristis* leaves.

glucanase generated xyloglucan oligosaccharides namely, XG<sub>OSSES</sub>, recovered from the supernatant of the digest, contained Xyl (*ca.* 30%), Glc (*ca.* 52%), Gal (*ca.* 12%), and Fuc (*ca.* 5%) units as major saccharides (Table I). The sugar composition of this water-soluble fraction is thus consistent with the presence of xyloglucan. However, the ratio of Glc to Xyl for this fraction is 1.77. Therefore, it is likely that the glucan present in B1OH-N was also hydrolysed.

#### **Glycosidic linkage pattern of xyloglucan oligosaccharides (XG<sub>OSSES</sub>)**

Complete methylation of XG<sub>OSSES</sub> generated a product that was converted into methylated glycoses by acid hydrolysis. Subsequently these glycoses were transformed into their corresponding partially methylated alditol acetates (PMAAs), and investigated by GC and GC-MS. The results as illustrated in Table II revealed the existence of (1,6) and (1,4,6)-linked Glcp residues as expected from oligosaccharides of XG. Terminal-Fuc, Gal and Xyl (all), as well as (1,2)-linked Xyl and (1,2)-linked Gal were present too. Collectively, these results validate the existence of XG thus corroborating sugar compositional data. In contrast, T-Glc and (1,4)-linked Glcp units are possibly the end result from the methylation analysis of glucan. This outcome is in agreement with the sugar compositional analysis data wherein the existence of glucan has been deduced.

#### **Matrix-Assisted Laser Desorption Ionization-Time of Flight- mass spectrometry (MALDI-TOF -MS)**

As highlighted in Figure 2, MALDI-TOF mass spectrum of XG<sub>OSSES</sub> showed the existence of two series of oligosaccharides. Based on molecular mass, ions generated from the first series of oligosaccharides at *m/z* 1085, 1247, 1393, and 1555, respectively, are consistent with  $[M + Na]^+$  of Hex<sub>4</sub>Pent<sub>3</sub>, Hex<sub>5</sub>Pent<sub>3</sub>, Hex<sub>5</sub>Pent<sub>3</sub>dHex<sub>1</sub>, and Hex<sub>5</sub>Pent<sub>3</sub>dHex<sub>1</sub>. Considering the mode of action of *endo*-(1,4)-β-D-glucanase, saccharide composition and glycosyl linkage pattern data, and the molecular masses of established xyloglucan oligosaccharides<sup>19–25</sup>, tentative structures for oligomers of 1<sup>st</sup> series present in XG<sub>OSSES</sub> fraction, are proposed as hepta(1)-, octa(2)-, nona(3)- and deca(4)-saccharides (Scheme I). In addition, a 2<sup>nd</sup> series of ions at *m/z* from 689 to 2693 with a mass difference of 162 Da corresponding to cello-oligosaccharides has also been spotted in this spectrum, with degrees of polymerization ranging from four to sixteen. Grounded on glycosyl make-up,

Table II — Partially *O*-methylated alditol acetates obtained by methylation analysis of oligosaccharides containing fractions derived by *endo*-glucanase (fraction XG<sub>OSES</sub>) and *endo*-xylanase (fraction X<sub>OSES</sub>) of hemicelluloses of *N. arbor tristis* leaves

O-methyl alditol acetates <sup>a</sup>	Deduced linkages	<i>m/z</i> values of ions generated by EI-MS of alditol acetates	Peak area <sup>b</sup>	
			XG <sub>OSES</sub>	X <sub>OSES</sub>
2,3,4,6-Me <sub>4</sub> -Gal	T-Galp	43, 45, 102, 118, 145, 161, 162 and 205	14	Nd
3,4,6-Me <sub>3</sub> -Gal	1,2-Galp	43, 45, 101, 130, 190, 161 and 205	1	Nd
2,3,4-Me <sub>3</sub> -Fuc	T-Fucp	43, 72, 102, 118, 131, 162 and 175	1	Nd
2,3,4-Me <sub>3</sub> -Xyl	T-Xylp	43, 101, 102, 117, 118, 161 and 162	16	5
2,3-Me <sub>2</sub> -Xyl	1,4-Xylp	43, 87, 102, 118, 129, 189 and 233	16	76
3,4-Me <sub>2</sub> -Xyl	1,2-Xylp	43, 88, 101, 117, 130 and 190	1	Nd
3-Me-Xyl	1,2,4-Xylp	43, 129, 130, 189 and 190	Nd	19
2,3,6-Me <sub>3</sub> -Glc	1,4-Glcp	43, 45, 102, 118, 129, 162, 173 and 233	10	Nd
2,3,4-Me <sub>3</sub> -Glc	1,6-Glcp	43, 45, 118, 130, 145, 161, 190 and 205	12	Nd
2,3-Me <sub>2</sub> -Glc	1,4,6-Glcp	43, 102, 118, 127, 162, 201, 261 and 305	22	Nd

<sup>a</sup> 2,3,4,6-Me<sub>4</sub>-Gal denotes 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, etc.

<sup>b</sup> Percentage of total area of the identified peaks.

<sup>c</sup> Nd: not detected.

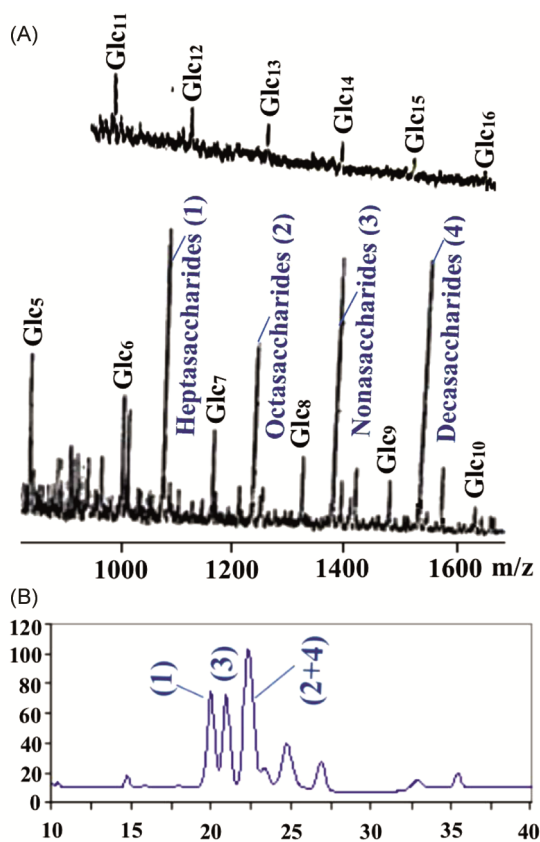


Figure 2 — (A) MALDI-TOF mass spectrum (Inset: Expansion of *m/z* 1790–2650 region) and (B) HPAE chromatographic elution profile of oligosaccharides generated from the xyloglucan of *N. arbor-tristis* leaves by *endo*-β-(1,4)-D-glucanase digestion. Scheme I provide structures of the identified oligosaccharides.

linkage pattern of the constituent saccharide and the molecular mass, the structure of oligomers corresponding to these  $[M+Na]^+$  ions can be assigned as  $Glcp(1,4)-Glcp[(1,4)-Glcp]_{2-13}(1,4)-Glcp$  (5-16).

### High Performance Anion Exchange-Pulse Amperometric Detection (HPAE-PAD) chromatography

HPAE chromatographic investigation of fraction XG<sub>OSES</sub> validates the outcome of MALDI-TOF mass spectrometry. In reality, elution profile (Figure 1) of this fraction demonstrates the presence of three major peaks possessing different intensities. Retention times of these peaks resembled xyloglucan oligosaccharides XXXG (1, heptasaccharide), XXFG (3, nonasaccharide), and XXLG (2, octasaccharide) + XLFG (4, decasaccharide), respectively, produced by the action of *endo*-glucanase on the xyloglucan of *Arabidopsis thaliana*<sup>20</sup>, *Argania spinosa*<sup>19,21</sup>, *Benincasa hispida*<sup>23</sup>, *Brassica campestris*<sup>24</sup>, *Linum usitatissimum*<sup>25</sup> and *Sesamum indicum*<sup>22</sup>. These oligosaccharides (1-4) have been labeled according to Fry *et al.*, 1993 (Ref 26). Xyloglucans, based upon the types of oligosaccharides released after hydrolysis, are classified as XXXG and XXGG type<sup>27</sup>. Since the side chain in *endo*-glucanase generated oligomers is substituted with Xyl-Gal-Fuc residues, the xyloglucan of present study is of XXXG-type<sup>27</sup> characteristic of many dicots<sup>27,28</sup>. The second types of XG namely XXGG type is the one in which side chain contains T-Gal residues as in tobacco and tomato cells<sup>27,28</sup>.

### Glycosyl linkage pattern of xylan

Complete methylation of A1OH generated an orange compound possessing a specific rotation of  $[\alpha]_D^{25} -31.8^\circ$  (*c*1.09, CHCl<sub>3</sub>) characteristic of β-D-glycosidic linkages. The methylated polysaccharide hydrolyzed, and the resulting methylated glycoses were transformed into their PMAAs and analyzed by

GC and GC-MS. Glycosidic linkage analysis (Table II) showed that A1OH is a branched polysaccharide, containing non-reducing end-unit of Xylp (2,3,4-Me<sub>3</sub>-Xyl). The other Xylp units were mainly, (1,2)- and (1,2,4)-linked in accord with 3,4-Me<sub>2</sub>-Xyl, and 3-Me-Xyl derivatives, respectively.

### *endo*-(1,4)- $\beta$ -D-xylanase produces acidic and neutral xylo-oligosaccharides (X<sub>OSES</sub>)

Additional information on xylan structure was acquired by digesting A1OH using *endo*-(1,4)- $\beta$ -D-xylanase, an enzyme specific for  $\beta$ -D-xylan. Saccharide analysis of the generated xylo-oligosaccharides (X<sub>OSES</sub>) indicated the presence of Xyl units (97 mol%) accompanied by minute quantities of Rha, GalA and GlcA residues (Table I). HPAE chromatographic analysis of X<sub>OSES</sub> fraction indicated the presence of xylose and xylobiose as well as peaks eluted with high concentration of NaOAc arising from acidic xylo-oligosaccharides.

MALDI mass spectrum (Figure 2) of fraction X<sub>OSES</sub> exhibited one main peak at  $m/z$  759 consistent with an oligosaccharide containing one 4-*O*-methyl-D-glucopyranuronosyl (4-*O*-MeGlcA) residue joined to four pentose units **17** (Scheme I). In view of the fact that Xyl is the exclusive pentose identified in X<sub>OSES</sub> (Table I), this ion was assigned to Xyl<sub>4</sub>-4-*O*-MeGlcA. By the same token, [M+Na]<sup>+</sup> ions at  $m/z$  = 891 and 1023 were assigned to Xyl<sub>5</sub>-4-*O*-MeGlcA<sub>1</sub> (**18**) and Xyl<sub>6</sub>-4-*O*-MeGlcA<sub>1</sub> (**19**), respectively. An additional group of ions at  $m/z$  1213, 1345, 1477 and 1609 was assigned to oligosaccharides (**20-23**) containing two 4-*O*-MeGlcA units linked to six, seven, eight and nine Xyl residues, respectively. Additionally, [M+Na]<sup>+</sup> ion at  $m/z$  305 corresponding to Xyl<sub>2</sub> (**24**) has also been detected (not shown). Consequently, a number of acidic as well as neutral oligosaccharides were produced from A1OH fraction using *endo*-(1,4)- $\beta$ -D-xylanase (Figure 3). Taken together, it may be concluded that the studied xylan consists of a backbone of (1,4)-linked  $\beta$ -D-Xylp units substituted at position 2 with a single D-Xylp, 4-*O*-Me-D-GlcA residues in proportions of one branch to every five units of Xyl.

### Experimental Section

Chemicals used were analytical grade or best available. All determinations were done at least in duplicate. Solvent evaporations were performed <50°C (Eyela N-1100 rotary evaporator) and concentrated sample solutions were lyophilized (Cool Safe 55-F

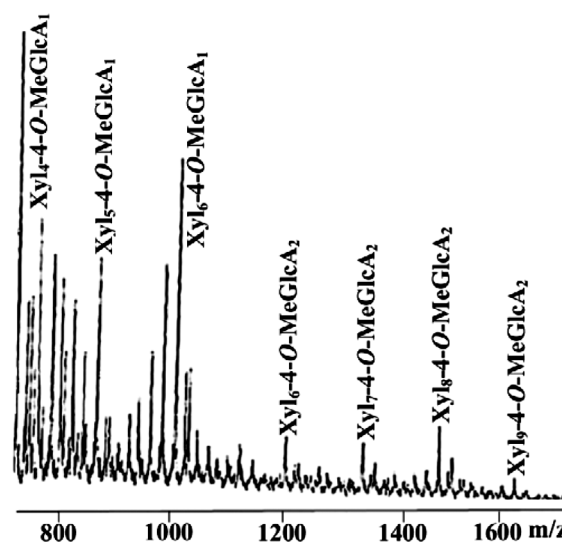


Figure 3 — MALDI-TOF mass spectrum of oligosaccharides produced from xylan enriched fraction A1OH of *N. arbor-tristis* leaves using *endo*- $\beta$ -(1,4)-D-xylanase. See Scheme I for the structure of oligosaccharides.

freeze drier, Scanvac, Lyngø, Denmark). Dialysis was performed using a 12 kDa molecular weight cut-off tube (Sigma Aldrich, Steinheim, Germany). Gas chromatographic (GC; Shimadzu GC-17A, Shimadzu, Kyoto, Japan) and gas chromatography mass spectrometric (GCMS; Shimadzu QP 5050 A, Shimadzu) analysis were performed using conditions as described<sup>25</sup>. The infrared (IR) spectrum was recorded by using KBr discs containing finely ground sample (Perkin Elmer FTIR-spectrum RX 1 spectrophotometer). UV-Vis spectrometric analyses were performed on a Shimadzu UV-2450 spectrophotometer.

### Isolation of polysaccharides

The residue (18 g), produced after aqueous extraction of *Nyctanthes arbor-tristis* leaves by using method as described previously<sup>1</sup>, was extracted twice with 500 mL of 1M NaOH solution containing 20mM NaBH<sub>4</sub> for 9 h. Combined extracts were acidified with AcOH to pH 5.5 and dialysed extensively against water. The precipitate was removed by centrifugation to yield A1OH (420 mg) fraction. The soluble fraction from 1M NaOH extract has been designated as B1OH (557 mg).

### Anion-exchange chromatography

A solution (25 mL) of fraction B1OH (200 mg) in 50 mM NaOAc (pH 5.5) was injected to a column (20 cm  $\times$  1.6 cm) of DEAE-Sepharose FF (AcO<sup>-</sup>; Amersham Biosciences AB, Uppsala, Sweden)

equilibrated previously with same buffer. Elution was carried out initially with the same buffer at a flow rate of 55 mL h<sup>-1</sup> to yield, after dialysis and lyophilization, the non-retained fraction B1OH-N (106 mg). Polysaccharides bound to the column matrix were eluted using salt gradient. Fractions (10 mL) were collected and analysed for total saccharide content by the phenol-sulfuric acid<sup>29</sup> assay using glucose as standard.

### Size exclusion chromatography (SEC)

The molecular mass of B1OH-N fraction was determined by Size Exclusion Chromatography on a Sephacryl S-100 FF (Amersham Biosciences AB, Uppsala, Sweden) column (90 cm × 2.6 cm). The column, subsequent to loading with a solution of B1OH-N in 0.5 M NaOAc buffer (pH 5.5), was eluted in a descending manner with the same buffer (20 mL h<sup>-1</sup>) at 25–35°C. Fractions were collected using a Pharmacia Biotech RediFrac fraction collector and analyzed for total sugar content. A set of standard dextrans (10–500 kDa) was used to calibrate the column.

### Preparation of oligosaccharides

Fraction B1OH-N (10 mg) was dissolved in 4 mL of NaOAc buffer (50 mM, pH 5.5) and the mixtures incubated with 30 units of *endo*-(1→4)-β-D-glucanase (Megazyme International, Ireland) for 24 h at 35–40°C. The glucanase resistant polymer was then precipitated in 80% ethanol (v/v) and removed by centrifugation. The soluble fraction containing the xyloglucan oligosaccharides was concentrated, desalted on a column of Sephadex G-25 and finally lyophilized to yield the xyloglucan oligosaccharides (XG<sub>oses</sub>).

Similarly, xylan containing fraction (A1OH, 9 mg) was hydrolyzed with 40 units of *endo*-(1→4)-β-D-xylanase (Megazyme International Ireland, xylanase M6) in 3 mL of NaOAc (50 mM, pH 5.5) at 35–40°C for 24 h. For removing xylanase resistant polymeric material, the hydrolysate was diluted with 12 mL chilled EtOH. Afterward the precipitate was removed by centrifugation and the xylo-oligosaccharides present in the supernatant was concentrated and lyophilized to yield a fraction named as X<sub>oses</sub>. The enzyme-resistant material in the pellet was isolated by lyophilization.

### Sugar analysis

Neutral sugars released by hydrolysis with 2 M CF<sub>3</sub>CO<sub>2</sub>H (3 h, 100°C) were converted into their alditol acetates<sup>30</sup>, and analyzed by GC as well as GC-MS. Alternatively, per-*O*-trimethyl-silylated-derivatives of methyl glycosides generated by the method of

York *et al.*, 1985 (Ref 31) were analyzed by GC using conditions as described previously<sup>32</sup>.

### Glycosidic linkage analysis

The pool of xyloglucan-oligosaccharides (XG<sub>oses</sub>) was permethylated according to Blakeney<sup>33</sup>. Permethylated material purified by solvent extraction was hydrolysed, and the liberated methylated glycoses converted into their partially methylated alditol acetates (PMAA). Finally, PMAAs were analysed by GC and GC-MS using conditions as described<sup>25</sup>.

### High performance anion exchange (HPAE) chromatography

Enzyme generated oligosaccharides (XG<sub>oses</sub> and X<sub>oses</sub>) were analysed on a Dionex DX 500 system equipped with a GP 50 gradient pump, an eluent degas module, a CarboPac PA-1 column and a pulse amperometric detector (PAD). Samples (10–100 μL) were injected and eluted (1 mL min<sup>-1</sup>) with NaOAc gradient in 100 mM NaOH as described<sup>25</sup>.

### Matrix-assisted laser desorption ionization-time of-flight-mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS analysis of oligosaccharides was done using a Bruker Daltonics flex Analysis MALDI-TOF mass spectrometer. The mass spectra were recorded in reflectron mode using 2,5-dihydroxybenzoic acid (10 mg mL<sup>-1</sup>) as matrix.

### Conclusions

The findings of this study highlight several significant aspects of *N. arbor-tristis* leaves derived oligosaccharides. First, a number of neutral xyloglucan derived oligosaccharides (**1-4**) along with twelve cello-oligosaccharides (**5-16**) were produced and identified. Moreover, several acidic in addition to neutral xylo-oligosaccharides (**17-24**) were also generated and characterised. Thus, enzymatic hydrolysis presents an attractive option for selective modification of polysaccharides, especially for the production of complex oligosaccharides. Apart from GC-MS, HPAEC amalgamated with MALDI-MS provides the most powerful analytical method for structural characterization of oligosaccharides. The twenty-four oligosaccharides produced not only stand for fine structures of hemicellulosic polysaccharides, but also can serve as tools for studying their biology. Seeing that oligosaccharides exhibit highly specific biological functions, the oligosaccharides generated herein could be effective in designing innovative functional foods.

### Conflicts of interest

The authors confirm that this article content has no conflicts of interest.

### Acknowledgements

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