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Cranberry (*Vaccinium macrocarpon*) oligosaccharides decrease biofilm formation by uropathogenic *Escherichia coli*

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ABSTRACT

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The preventive effects of the American cranberry (*Vaccinium macrocarpon*) against urinary tract infections are supported by extensive studies which have primarily focused on its phenolic constituents. Herein, a phenolic-free carbohydrate fraction (designated cranf1b-F2) was purified from cranberry fruit using ion exchange and size exclusion chromatography. MALDI-TOF-MS analysis revealed that the cranf1b-F2 constituents are predominantly oligosaccharides possessing various degrees of polymerisation and further structural analysis (by GC-MS and NMR) revealed mainly xyloglucan and arabinan residues. In antimicrobial assays, cranf1b-F2 (at 1.25 mg/mL concentration) reduced biofilm production by the uropathogenic *Escherichia coli* CFT073 strain by over 50% but did not inhibit bacterial growth. Cranf1b-F2 (ranging from 0.625 - 10 mg/mL) also inhibited biofilm formation of the non-pathogenic *E. coli* MG1655 strain up to 60% in a concentration-dependent manner. These results suggest that cranberry oligosaccharides, in addition to its phenolic constituents, may play a role in its preventive effects against urinary tract infections.

Keywords: American cranberry, *Vaccinium macrocarpon*, phenolic, oligosaccharide, biofilm, *Escherichia coli*

22 **1. Introduction**

23 Urinary tract infections (UTI) commonly occur anywhere from the kidney in the
24 upper urinary tract to the bladder in the lower urinary tract. Although UTIs are
25 generally easy to treat with antibiotics, acute infections can be dangerous for
26 elderly, infant and immunocompromised patients (Jepson, Williams, & Craig,
27 2012). Some UTI patients can experience frequent recurrent infections and
28 increased susceptibility to drug resistant uropathogens (Jepson et al., 2012;
29 Reid et al., 2001). Over 80% of UTIs are associated with *Escherichia coli*, which
30 may be transmitted from the bowel to urethra. Biofilms that form on the
31 bladder wall help prevent the bacteria from being eradicated by the immune
32 system and antibiotics (Anderson et al., 2003; Moreno et al., 2008). Evidence
33 suggests that consumption of the American cranberry (*Vaccinium macrocarpon*
34 Aiton) juice can inhibit the presence of bacteria in urine and reduce UTI
35 symptoms associated with bacteriuria and pyuria (Avorn et al., 1994; Reid et
36 al., 2001). Our group (LaPlante, Sarkisian, Woodmansee, Rowley, & Seeram,
37 2012), and others (Côté et al., 2011; Iswaldi et al., 2012; Lian, Maseko, Rhee,
38 & Ng, 2012) have studied the antimicrobial effects of the phenolic constituents
39 of cranberries. Some studies (Foo, Lu, Howell, & Vorsa, 2000a, 2000b; Gupta
40 et al., 2012; Howell et al., 2005) have shown that cranberry proanthocyanidins
41 (commonly known as PACs), with at least one A-type linkage, inhibit the
42 adherence of type p-fimbriated *E. coli* to uroepithelial cells and human red blood
43 cells. The chemistry of cranberry PACs (Lee, 2013) and their absorption and

44 metabolism have been studied (Ou & Gu, 2014). However, the non-phenolic
45 constituents in cranberry have been less investigated (Hotchkiss, Nunez, Khoo,
46 & Strahan, 2013). Herein, we provide the first report describing the structural
47 characterization of a phenolic-free carbohydrate fraction purified from cranberry
48 and its evaluation for inhibition of biofilm formation by both uropathogenic (*E.*
49 *coli* CFT073) and non-pathogenic (*E. coli* MG1655) strains of *E. coli*.

50

51 **2. Materials and methods**

52 **2.1. Bacterial strains and media**

53 *E. coli* strains CFT073 and MG1655 were gifts from Dr. Paul Cohen (University
54 of Rhode Island). Luria Bertani (LB) medium (BD, NJ, USA) was
55 supplemented with 5 g/L dextrose. M63 medium (Bioworld, OH, USA) was
56 supplemented with 1 mM MgSO₄, 2 g/L dextrose and 5 g/L casamino acid.

57

58 **2.2. Fractionation of cranberry materials**

59 **2.2.1. Purification of crude cranberry hull extract (Cranf1)**

60 Scheme S1 (see Supplementary data) shows the fractionation flow chart of
61 cranberry materials with yields and their total phenolic contents. Briefly, a
62 pectinase (Klerzyme 150, DSM Food Specialties, South Bend, IN, USA)
63 degraded cranberry hull extract (Cranf1) was fractionated using an Agilent 971-
64 FP flash purification system (Agilent Technologies, Santa Clara, CA, USA) with
65 Biotage SNAP KP-C18-HS 120g cartridges (Biotage, Charlotte, NC, USA).

66 mL of Cranf1 aqueous solution (100 mg/mL) was loaded onto the pre-
67 conditioned C18 column cartridge and eluted sequentially with 500 mL of de-
68 ionised H₂O, 500 mL of 15% methanol/water, and finally 500 mL of MeOH at 35
69 mL/min. Fractions eluted with 100% water were pooled as Cranf1W with a yield
70 of 38.1% (w/w), fractions eluted with 15% methanol were pooled as Cranf1b
71 with a yield of 23.8%, and fractions eluted with 100% methanol were pooled as
72 Cranf1M with a yield of 28.1% (see Scheme S1, Supplementary data).

73

74 **2.2.2. Purification of oligosaccharide enriched fraction Cranf1b**

75 Cranf1b was introduced onto an anion exchange column (Sephacrose Q XL
76 16/10, GE Healthcare Life Sciences, Pittsburgh, PA, USA) and eluted with step-
77 wise gradient of NaCl aqueous solution (0-1 M) at 5 mL/min on a ÄKTA fast
78 protein liquid chromatography (FPLC) system (GE Healthcare Life Sciences).
79 Ten mL fractions were collected and assayed for total carbohydrate content
80 assay.(Masuko et al., 2005) The pooled carbohydrate-containing fractions were
81 freeze-dried and desalted (10×300 mm Bio-gel P2 column; BIO-RAD, Hercules,
82 CA, USA). The constituents that eluted with 100% de-ionised H₂O and 0.1 M
83 NaCl were combined and further purified by gel filtration (Sephacryl S-100 HR
84 16/60, GE Healthcare Life Sciences; elution with de-ionised H₂O at 0.25
85 mL/min), yielding two fractions designated as cranf1b-F1 and cranf1b-F2.

86

87 **2.3. Biofilm assay**

88 The antibiofilm property of the cranberry materials was measured against *E.*
89 *coli* CFT073 and MG1655 using a modified crystal violet staining method in
90 round bottom 96-well microtiter plates (George, 2011; Naves et al., 2008; Niu
91 & Gilbert, 2004). Bacteria colonies from TSA plates were inoculated into LB
92 broth and incubated at 37 °C with 175 rpm shaking for 24 h. The cultures were
93 then diluted 100-fold in M63 medium, distributed in microtiter wells, and treated
94 with a series of two-fold dilutions of test samples (10 - 0.019 mg/mL). The
95 plates were incubated at 37 °C for 6 h or 48 h, gently washed with de-ionised
96 water, and stained with 125 µL of 0.1% crystal violet solution for 15 min. The
97 solution was removed and the wells were again gently washed with de-ionised
98 water and dried for 1 h. 125 µL of 30% acetic acid solution was added to each
99 well and incubated for 15 min. 100 µL from each well was transferred to a flat
100 bottom microtiter plate and the OD₅₅₀ was measured (Spectramax M2,
101 Molecular devices, Sunnyvale, CA, USA). Percent biofilm formation was
102 calculated as the average OD₅₅₀ of three replicate treatment wells divided by
103 average OD₅₅₀ of replicate control wells (30 wells/plate). Each experiment was
104 conducted in duplicate.

105

106 **2.4. High Performance Size Exclusion Chromatography (HPSEC)**

107 HPSEC was carried out at 40 °C on a TSKgel G3000PW column [7.5 × 300 mm
108 column, Tosoh Bioscience LLC, King of Prussia, PA, USA; Hitachi LaChrom
109 Elite HPLC, Tokyo, Japan; 0.6 mL/min de-ionised water, refractive index (RI)

110 detection]. The molecular weights of compounds were determined by
111 comparison of retention times to a standard curve (Supplementary Fig. S1)
112 generated with standard dextrans of molecular weights ranging from 1000 to
113 50000 Daltons.

114

115 **2.5. Glycosyl composition analysis**

116 Sugar composition was determined by GC-MS analysis of monosaccharides
117 (York, Darvill, McNeil, Stevenson, & Albersheim, 1986). Briefly, 100 µg of
118 sample was hydrolysed with 2M TFA for 2 h at 121 °C. The hydrolyte was
119 reduced with sodium borodeuteride (NaBD₄) at room temperature for 1.5 h. The
120 reduced monosaccharides were O-acetylated with acetic anhydride at 50 °C for
121 20 min. The resulting product was extracted with dichloromethane and
122 analysed by GC-MS (DB-1 column, GC Model 6890/MS Model 5973, Agilent
123 Technologies, Santa Clara, CA, USA). The monosaccharide composition was
124 determined by comparison with a GC-MS profile of monosaccharide standards.

125

126 **2.6. Glycosyl linkage analysis**

127 Partially methylated acetate alditols (PMAAs) of cranf1b-F2 were analysed by
128 GC-MS (Ciucanu & Kerek, 1984; York et al., 1986). Briefly, 600 µg of sample
129 was permethylated with iodomethane and concentrated sodium hydroxide in
130 DMSO. The permethylated oligosaccharide was hydrolysed with 2M TFA and
131 reduced with NaBD₄. The sample was then acetylated with acetic anhydride

132 and extracted with dichloromethane. GC-MS analysis was conducted using a
133 Supelco SP2331 column (Sigma-Aldrich, St. Louis, MO, USA). The GC-MS
134 profile was analysed by comparison of retention time and electron-impact
135 fragmentation spectra with PMAA standards.

136

137 **2.7. NMR analysis**

138 The cranf1b-F2 was deuterium exchanged twice by D₂O shake and dissolved
139 in D₂O with addition of 1 µL of DMSO as internal reference. ¹H, ¹³C, 2D COSY,
140 TOCSY, NOESY, HSQC and HMBC spectra were obtained on a 500 MHz NMR
141 spectrometer (Varian VNMRS 500MHz, Agilent Technologies) at 25 °C.

142

143 **2.8. MALDI mass spectrometry**

144 Cranf1b-F2 (1 mg/mL in H₂O) was mixed with 2,3-dihydrobenzoic acid (DHB)
145 matrix solution (v/v=1:1). Two µL of the mixture was analysed by MALDI-TOF-
146 MS (Axima Performance, Shimadzu, Kyoto, Japan) in positive reflectron mode
147 with power set at 80kV. 500 profiles were collected for each experiment.

148

149 **3. Results and discussion**

150 In this study, we investigated a carbohydrate fraction extracted from cranberry
151 and evaluated its inhibitory effect on biofilm formation of two strains of *E. coli*.
152 The ¹H NMR spectra of the original cranberry starting material (Cranf1) and its
153 three major purified fractions namely, Cranf1W, Cranf1b and Cranf1M were

154 obtained (see Supplementary data). The ^1H NMR spectrum of Cranf1b showed
155 only trace resonances above 7.0 ppm, indicating that phenolics were mostly
156 removed by C18 column chromatography. The crude cranberry extract cranf1b
157 was purified by anion exchange chromatography and four fractions, cranf1b-F1
158 (64.0%), cranf1b-F2 (17.5%), cranf1b-F3 (2.5%) and cranf1b-F4 (<1%), were
159 collected (Figure 1a). Due to the limited quantities of the latter fractions, only
160 cranf1b-F1 and cranf1b-F2 were further studied. Cranf1b-F1 and cranf1b-F2
161 were next purified by gel filtration, resulting in only one peak for each sample
162 (Figure 1b). The homogeneity of cranf1b-F2 was further confirmed by HPSEC
163 profile (Supplementary Fig. S6) and the average molecular size was predicted
164 to be 1370 Da. However, MALDI-TOF MS spectrometry of cranf1b-F2 produced
165 a series of oligosaccharide sodium adduct ions (Supplementary Fig. S7),
166 revealing it to be a mixture of oligomers within a close molecular weight range.
167 The ions at approximately 1055, 1085, 1217, 1247, 1349, 1379, 1511, 1541 can
168 be attributed to Hex_3Pen_4 (5 hexoses and 4 pentoses), Hex_4Pen_3 , Hex_4Pen_4 ,
169 Hex_5Pen_3 , Hex_4Pen_5 , Hex_5Pen_4 , Hex_5Pen_5 and Hex_6Pen_4 , respectively.
170 Clusters of less abundant ions were observed above 1700 representing
171 oligosaccharides with degrees of polymerisation (DP) larger than 11.

172

173 The GC-MS profile (Supplementary Fig. S8a) of the monosaccharide acetate
174 alditols (Table 1) indicated that the cranf1b-F2 was primarily composed of
175 arabinose (46%), glucose (40%), xylose (12%) and trace quantities of galactose

176 (2%). The predominance of glucose, xylose and arabinose suggests that
177 cranf1b-F2 is likely a xyloglucan (FRY, 1989; McNeil, Darvill, Fry, & Albersheim,
178 1984).

179

180 Glycosyl linkages of each monosaccharide are listed in Table 1 (GC-MS profile
181 see Supplementary Fig. S8b). In addition to the common glycosyl linkages
182 known for xyloglucan (Fry et al., 1993) 5- α -Arab, 3- α -Arab and 3,5- α -Arab were
183 also found in cranf1b-F2. These additional linkages are consistent with
184 arabinan side chains that are commonly present in cell-wall pectic substances
185 (Caffall & Mohnen, 2009). In xyloglucan nomenclature for side chain subunits
186 (Fry et al., 1993) cranf1b-F2 glycosyl linkages belong to side chain subunits S,
187 L, X and G. ^1H and ^{13}C NMR chemical shifts were assigned for the identified
188 cranf1b-F2 subunits (Table 1) based on the recorded 1D NMR and 2D NMR
189 spectra (see Supplementary data) and in consideration of previous reports
190 (Busato et al., 2005; Hoffman et al., 2005; Jia, Cash, Darvill, & York, 2005;
191 Shakhmatov, Toukach, Michailowa, & Makarova, 2014; Watt, Brasch, Larsen,
192 & Melton, 1999).

193

194 Although commonly found as separate polymer components of plant cell walls,
195 a portion of xyloglucan and pectic polysaccharides are proposed to be
196 covalently bound (Femenia, Rigby, Selvendran, & Waldron, 1999; Popper & Fry,
197 2005, 2008; Thompson & Fry, 2000; Vidal, Williams, Doco, Moutounet, &

198 Pellerin, 2003). The putative xyloglucan-pectin complex model was first
199 introduced by Albersheim and coworkers in 1973 (Keegstra, Talmadge, Bauer,
200 & Albersheim, 1973). Thompson and Fry (Thompson & Fry, 2000) observed
201 xyloglucan that co-eluted with anionic pectin during anion exchange
202 chromatography and remained part of the complex after treatment with 8M urea,
203 6M NaOH and proteinase. Treatment with arabinanase and/or galactanase
204 converted a great portion of the complex into neutral compounds, suggesting
205 that covalent bonding occurs between xyloglucan and the Ara/Gal-rich pectic
206 domain, likely on the arabinan and/or arabinogalactan side chains of a
207 Rhamnogalacturan I region (Abdel-Massih, Baydoun, & Brett, 2003; Popper &
208 Fry, 2005; Thompson & Fry, 2000). However, no NMR spectroscopic evidence
209 for a covalent linkage has yet been reported. In our study, co-elution of the
210 xyloglucan and arabinan components of cranf1b-F2 in every chromatography
211 step, coupled with its slight acidity, (Thompson & Fry, 2000) suggests the
212 existence of a covalent linkage.

213

214 The original cranberry material (Cranf1) and its three major purified fractions,
215 namely, Cranf1W, Cranf1b and Cranf1M were tested for the prevention of
216 biofilm formation against *E. coli* MG1655, a non-uropathogenic strain, and *E.*
217 *coli* CFT073, a well-studied uropathogenic strain (Welch et al., 2002) (see Table
218 S1, Supplementary data). At equivalent concentrations (1.25 mg/mL), Cranf1b
219 showed the most reduction in biofilm formation against the uropathogenic *E.*

220 *coli* CFT073 strain, therefore its sub-fractions, Cranf1b-F1 and cranf1b-F2 were
221 further tested against this strain. Although no activity was observed for cranf1b-
222 F1, cranf1b-F2 reduced biofilm formation of *E. coli* CFT-073 by as much as 50 %
223 at 1.25 mg/mL after 6 h of incubation (Figure 2a). The reductive effect on
224 biofilm formation was maintained for at least 48 h (Figure 2a) with no growth
225 inhibition, demonstrating that the reduced biofilm after 6 h is not merely due to
226 a delay in the initiation of biofilm production. Interestingly, the highest
227 inhibitory effect was not achieved at the highest concentration tested. While the
228 reason for the declining prevention at higher concentration is not yet known, we
229 hypothesise that aggregation of the cranf1b-F2 sample may be partially
230 responsible. HPSEC analysis showed that large particles (>100,000 Da)
231 formed at the higher concentration (Supplementary Fig. S6). Aggregation of
232 oligosaccharides would lead to less concentration of active molecules in
233 solution, hence having a potential impact on the overall activity. Biofilm
234 formation by *E. coli* MG1655 was also sensitive to the effects of cranf1b-F2
235 (Figure 2b), but not to cranf1b-F1. A concentration-dependent reduction in
236 biofilm formation was observed between 10 and 0.625 mg/mL; however, an
237 increase in biofilm formation was consistently observed between 0.625 and
238 0.156 mg/mL of cranf1b-F2. The distinct dose-response patterns between
239 CFT073 and MG1655 may derive from their different abilities to form and
240 sustain biofilms. MG1655 naturally produces much lighter biofilm than CFT073,
241 which likely makes it more vulnerable to biofilm modifying agents.

242

243 As previously discussed, the role of the polyphenols (including PACs) present
244 in cranberries in its preventive effects against urinary tract infections has been
245 extensively studied by several groups (LaPlante, Sarkisian, Woodmansee,
246 Rowley, & Seeram, 2012; Gupta et al., 2012; Howell et al., 2005). Thus, it is
247 possible that the multiple constituents, including polyphenols and
248 oligosaccharides, present in the cranberry whole fruit act additively,
249 complementarily, and/or synergistically in its overall biological effects.
250 Interestingly, in the current study, we did not observe any growth inhibitory and
251 anti-biofilm effects of the Cranf1M fraction (which was enriched in polyphenol
252 constituents) on both of the *E. coli* strains which was in agreement with our
253 previous report (LaPlante, Sarkisian, Woodmansee, Rowley, & Seeram, 2012).
254 Therefore, while it appears that the phenolic constituents did not contribute to
255 the inhibition of biofilm formation by the uropathogenic *E. coli* CFT073 strain
256 (based on our bioassays), their overall contribution to the prevention of urinary
257 tract infections by the whole cranberry fruit should not be discounted.

258

259 **4. Conclusion**

260 In conclusion, our study demonstrates that a phenolic-free, oligosaccharide
261 component of cranberry modifies the biofilm formation of *E. coli* strains CFT073
262 and MG1655. Thus, in addition to PACs and other polyphenols, certain
263 carbohydrate components in cranberry may also contribute to its overall anti-

264 infective properties. Further investigation to clarify the structure-activity
265 relationships of these oligosaccharides is currently being pursued by our group.

266

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269 Middleboro, MA, USA). Bacterial strain *E. coli* CFT073 was a gift from Dr. Paul
270 Cohen (University of Rhode Island). Instruments used for the various chemical
271 analyses were supported by an Institutional Development Award (IDeA) from
272 the National Institute of General Medical Sciences of the National Institutes of
273 Health (grant number 2 P20 GM103430). NMR and GC-MS data were acquired
274 at a research facility supported in part by the National Science Foundation
275 EPSCoR Cooperative Agreement #EPS-1004057.

276

277 **Appendix A. Supplementary data**

278 Scheme of fractionation of cranberry materials. Detailed structural analysis data
279 of cranberry materials including Cranf1, Cranf1W, Cranf1b, Cranf1M and
280 cranf1b-F2. Complementary biofilm assay results of Cranf1, Cranf1W, Cranf1b
281 and Cranf1M.

282

Figure 1

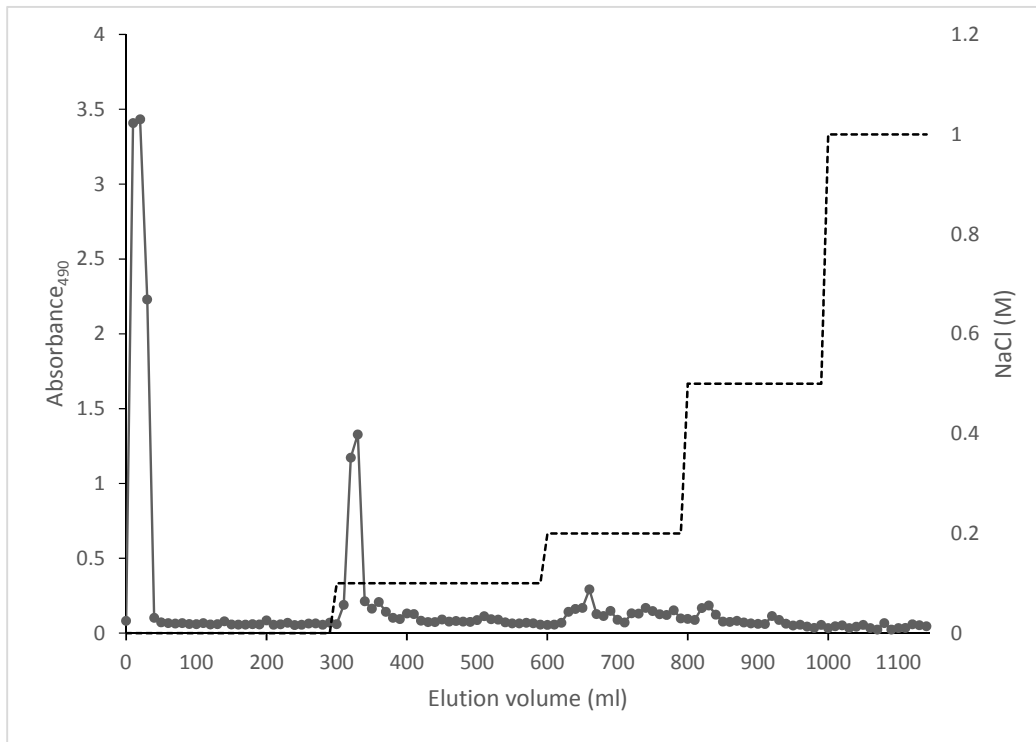


Figure 1a. Elution profile of Cranf1b on Sepharose Q XL 16/10 column, eluted by stepwise gradient of NaCl (0-1 M) (total sugars, - -).

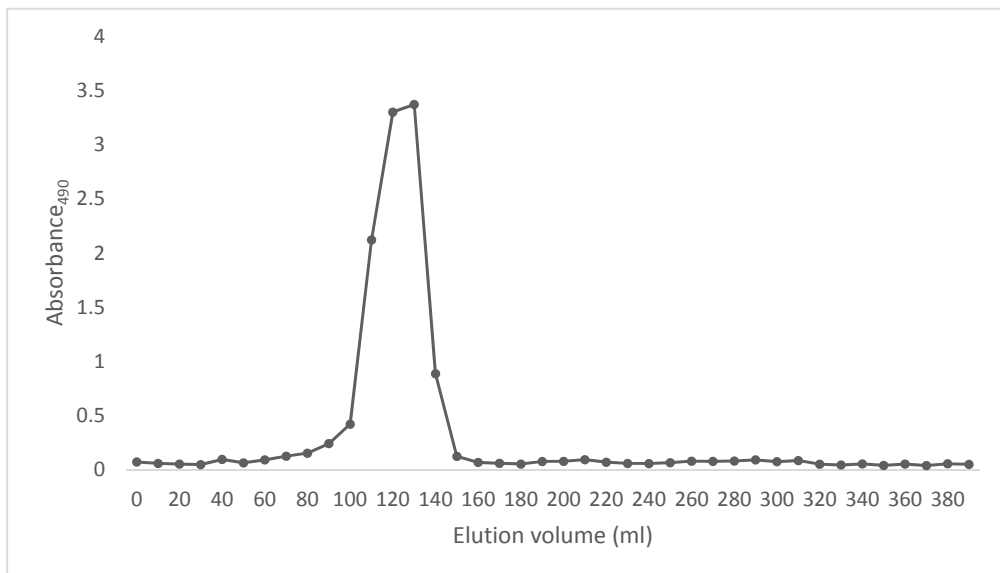


Figure 1b. Elution profile of Cranf1b-F2 on Sephacryl S-100 HR 16/60 column, eluted by de-ionised water (total sugars, - -).

Figure 2

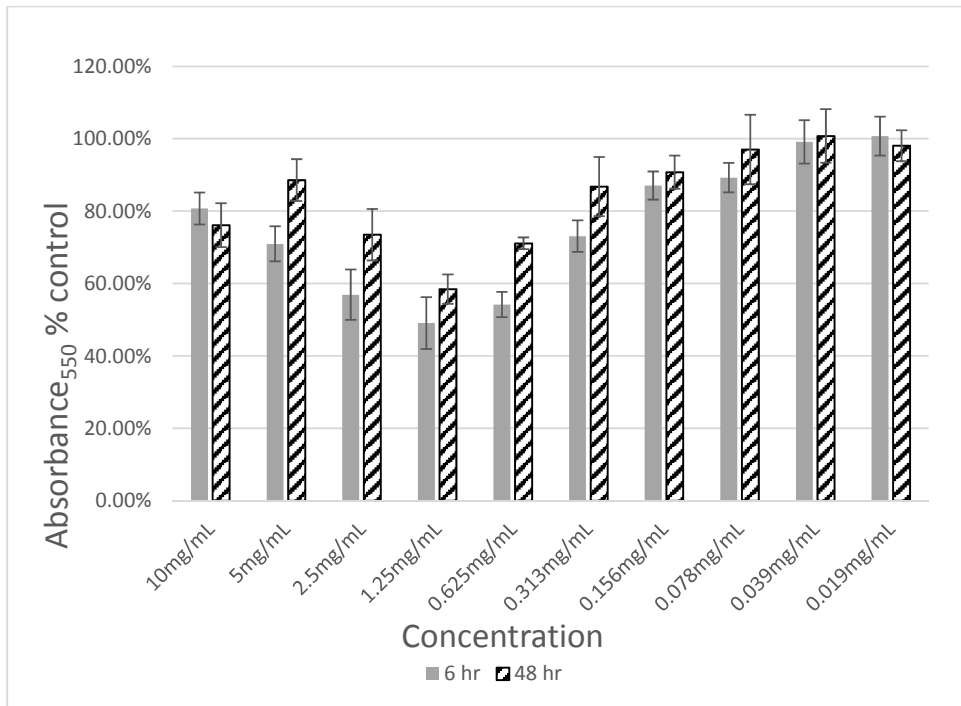


Figure 2a. Inhibition of *E. coli* CFT073 biofilm formation by Cranf1b-F2 at concentration from 0.019 mg/mL to 10 mg/mL.

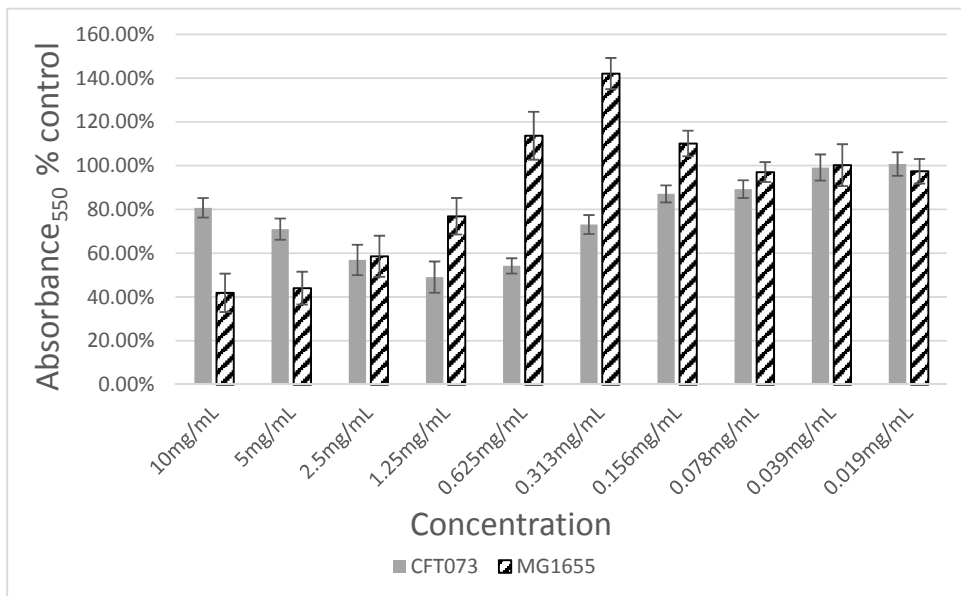


Figure 2b. Inhibition of *E. coli* MG1655 biofilm formation by Cranf1b-F2 at concentration from 0.019 mg/mL to 10 mg/mL.

Table 1. ¹³C NMR and ¹H NMR chemical shifts (δ in ppm) for cranf1b-F2.

Residue (Mol %)	Subunits	Linkages	C1/H1	C2/H 2	C3/H 3	C4/H 4	C5/H5	C6/H6
Araf (56%)	Arabina n	t-α-Araf	107.69	81.56	77.17	84.58	61.75	-
			5.14	4.13	3.95	4.03	3.84	-
	Arabina n	3,5-α-Araf	108.11	79.83	82.90	82.11	67.14	-
			5.11	4.28	4.09	4.30	3.83/3.9 3	-
	Arabina n	5-α-Araf	108.15	81.53	77.25	83.02	66.89	-
			5.08	4.12	4.02	4.21	3.88/3.7 9	-
	Arabina n	3-α-Araf	107.72	80.26	84.33	83.09	-	-
			5.18	4.36	3.95	4.14	-	-
	S	t-α-Araf	109.87	81.66	77.05	84.44	61.81	-
			5.15	4.19	3.93	4.06	3.71	-
Xylp (14%)	S	2-α-Xylp	99.20	79.47	72.47	70.03	61.87	-
			5.08	3.56	3.85	3.65	3.55	-
	L	2-α-Xylp	98.98	81.14	-	-	-	-
			5.14	3.6	-	-	-	-
	X	t-α-Xylp	99.48	72.06	73.67	70.14	-	-
			4.94	3.54	3.71	3.61	-	-
Galp (2%)	L	t-β-Galp	105.10	-	-	-	-	-
			4.60- 3.73	-	-	-	-	-
GlcP (27%)	_G	t-β-GlcP	105.24	-	-	-	-	-
			4.53	3.62	-	-	-	-
	G	4,6-β- GlcP	103.35	73.5	74.77	79.74	74.32	67.47
			4.53	3.38	3.66	3.67	3.82	3.87/3.8 0
	G	4,6-β- GlcP	103.20	73.5	74.77	79.64	74.32	67.04
			4.52	3.37	3.66	3.69	3.82	3.93/3.8 2
	G	4-β-GlcP	103.10	73.79	76.11	79.55	-	-
			4.51	3.3	3.49	3.54	-	-
	G_	α-GlcP	92.40	71.85	-	-	-	-
			5.21	3.57	3.82	3.64	3.94	3.86
	G_	β-GlcP	96.34	74.44	75.32	81.15	75.41	60.52
			4.65	3.28	3.63	3.62	3.59	3.80/3.9 4

G = -4)-β-D-GlcP-(1-

S = α-L-AraF(1-2)-α-D-XylP-(1-6)-β-D-GlcP-(1-

L = β-D-GalP-(1-2)-α-D-XylP-(1-6)-β-D-GlcP-(1-

X = α-D-XylP-(1-6)-β-D-GlcP-(1-

G: -4)-β-D-GlcP-(1-

G_: Reducing end glucose

_G: Non-reducing end glucose

References

- Abdel-Massih, R. M., Baydoun, E. A., & Brett, C. T. (2003). In vitro biosynthesis of 1,4-beta-galactan attached to a pectin-xyloglucan complex in pea. *Planta*, 216(3), 502-511.
- Anderson, G. G., Palermo, J. J., Schilling, J. D., Roth, R., Heuser, J., & Hultgren, S. J. (2003). Intracellular bacterial biofilm-like pods in urinary tract infections. *Science*, 301(5629), 105-107.
- Avorn, J., Monane, M., Gurwitz, J. H., Glynn, R. J., Choodnovskiy, I., & Lipsitz, L. A. (1994). Reduction of bacteriuria and pyuria after ingestion of cranberry juice. *Journal of the American Medical Association*, 271(10), 751-754.
- Busato, A. P., Vargas-Rechia, C. G., Gorin, P. A., Petkowicz, C. L., Tischer, C. A., Bochicchio, R., & Reicher, F. (2005). New 4-O-substituted xylosyl units in the xyloglucan from leaves of *Hymenaea courbaril*. *International Journal of Biological Macromolecules*, 35(5), 277-282.
- Caffall, K. H., & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, 344(14), 1879-1900.
- Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research*, 131(2), 209-217.
- Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. F., & Lacroix, M. (2011). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22(8), 1413-1418.
- Femenia, A., Rigby, N., Selvendran, R., & Waldron, K. (1999). Investigation of the occurrence of pectic-xylan-xyloglucan complexes in the cell walls of cauliflower stem tissues. *Carbohydrate Polymers*, 39(2), 151-164.
- Foo, L., Lu, Y., Howell, A. B., & Vorsa, N. (2000a). The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. *Phytochemistry*, 54(2), 173-181.
- Foo, L., Lu, Y., Howell, A. B., & Vorsa, N. (2000b). A-Type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. *Journal of Natural Products*, 63(9), 1225-1228.
- FRY, S. C. (1989). The structure and functions of xyloglucan. *Journal of Experimental Botany*, 40(1), 1-11.
- Fry, S. C., York, W. S., Albersheim, P., Darvill, A., Hayashi, T., Joseleau, J. P., Kato, Y., Lorences, E. P., Maclachlan, G. A., & McNeil, M. (1993). An unambiguous nomenclature for xyloglucan-derived oligosaccharides. *Physiologia Plantarum*, 89(1), 1-3.
- George, A. (2011). Microtiter dish biofilm formation assay. *Journal of Visualized Experiments*, (47).

- Gupta, A., Dwivedi, M., Mahdi, A. A., Nagana Gowda, G. A., Khetrapal, C. L., & Bhandari, M. (2012). Inhibition of adherence of multi-drug resistant *E. coli* by proanthocyanidin. *Urological Research*, *40*(2), 143-150.
- Hoffman, M., Jia, Z., Pena, M. J., Cash, M., Harper, A., Blackburn, A. R., 2nd, Darvill, A., & York, W. S. (2005). Structural analysis of xyloglucans in the primary cell walls of plants in the subclass *Asteridae*. *Carbohydrate Research*, *340*(11), 1826-1840.
- Hotchkiss, A. T., Nunez, A., Khoo, C., & Strahan, G. D. (2013). Cranberry xyloglucan oligosaccharide composition. Patent Application US20130316025 A1.
- Howell, A. B., Reed, J. D., Krueger, C. G., Winterbottom, R., Cunningham, D. G., & Leahy, M. (2005). A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry*, *66*(18), 2281-2291.
- Iswaldi, I., Gomez-Caravaca, A. M., Arraez-Roman, D., Uberos, J., Lardon, M., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2012). Characterization by high-performance liquid chromatography with diode-array detection coupled to time-of-flight mass spectrometry of the phenolic fraction in a cranberry syrup used to prevent urinary tract diseases, together with a study of its antibacterial activity. *Journal of Pharmaceutical and Biomedical Analysis*, *58*, 34-41.
- Jepson, R. G., Williams, G., & Craig, J. C. (2012). Cranberries for preventing urinary tract infections. *Cochrane Database of Systematic Reviews*, *10*, CD001321.
- Jia, Z., Cash, M., Darvill, A. G., & York, W. S. (2005). NMR characterization of endogenously O-acetylated oligosaccharides isolated from tomato (*Lycopersicon esculentum*) xyloglucan. *Carbohydrate Research*, *340*(11), 1818-1825.
- Keegstra, K., Talmadge, K. W., Bauer, W., & Albersheim, P. (1973). The structure of plant cell walls III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiology*, *51*(1), 188-197.
- LaPlante, K. L., Sarkisian, S. A., Woodmansee, S., Rowley, D. C., & Seeram, N. P. (2012). Effects of cranberry extracts on growth and biofilm production of *Escherichia coli* and *Staphylococcus* species. *Phytotherapy Research*, *26*(9), 1371-1374.
- Lee, J. (2013). Proanthocyanidin A2 purification and quantification of American cranberry (*Vaccinium macrocarpon* Ait.) products. *Journal of Functional Foods*, *5*(1), 144-153.
- Lian, P. Y., Maseko, T., Rhee, M., & Ng, K. (2012). The antimicrobial effects of cranberry against *Staphylococcus aureus*. *Food Science and Technology International*, *18*(2), 179-186.
- Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S.-I., & Lee, Y. C.

- (2005). Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical Biochemistry*, 339(1), 69-72.
- McNeil, M., Darvill, A. G., Fry, S. C., & Albersheim, P. (1984). Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry*, 53(1), 625-663.
- Moreno, E., Andreu, A., Pigrau, C., Kuskowski, M. A., Johnson, J. R., & Prats, G. (2008). Relationship between *Escherichia coli* strains causing acute cystitis in women and the fecal E. coli population of the host. *Journal of Clinical Microbiology*, 46(8), 2529-2534.
- Naves, P., Del Prado, G., Huelves, L., Gracia, M., Ruiz, V., Blanco, J., Rodríguez-Cerrato, V., Ponte, M., & Soriano, F. (2008). Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent. *Journal of Applied Microbiology*, 105(2), 585-590.
- Niu, C., & Gilbert, E. (2004). Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure. *Applied and Environmental Microbiology*, 70(12), 6951-6956.
- Ou, K., & Gu, L. (2014). Absorption and metabolism of proanthocyanidins. *Journal of Functional Foods*, 7, 43-53.
- Popper, Z. A., & Fry, S. C. (2005). Widespread occurrence of a covalent linkage between xyloglucan and acidic polysaccharides in suspension-cultured angiosperm cells. *Annals Botany*, 96(1), 91-99.
- Popper, Z. A., & Fry, S. C. (2008). Xyloglucan-pectin linkages are formed intraprotoplasmically, contribute to wall-assembly, and remain stable in the cell wall. *Planta*, 227(4), 781-794.
- Reid, G., Hsiehl, J., Potter, P., Mighton, J., Lam, D., Warren, D., & Stephenson, J. (2001). Cranberry juice consumption may reduce biofilms on uroepithelial cells: pilot study in spinal cord injured patients. *Spinal Cord*, 39(1), 26-30.
- Shakhmatov, E. G., Toukach, P. V., Michailowa, E. A., & Makarova, E. N. (2014). Structural studies of arabinan-rich pectic polysaccharides from *Abies sibirica* L. Biological activity of pectins of *A. sibirica*. *Carbohydrate Polymers*, 113, 515-524.
- Thompson, J. E., & Fry, S. C. (2000). Evidence for covalent linkage between xyloglucan and acidic pectins in suspension-cultured rose cells. *Planta*, 211(2), 275-286.
- Vidal, S., Williams, P., Doco, T., Moutounet, M., & Pellerin, P. (2003). The polysaccharides of red wine: total fractionation and characterization. *Carbohydrate Polymers*, 54(4), 439-447.
- Watt, D., Brasch, D., Larsen, D., & Melton, L. (1999). Isolation, characterisation, and NMR study of xyloglucan from enzymatically depectinised and non-depectinised apple pomace. *Carbohydrate Polymers*, 39(2), 165-180.
- Welch, R., Burland, V., Plunkett, G., Redford, P., Roesch, P., Rasko, D., Buckles, E., Liou, S.-R., Boutin, A., & Hackett, J. (2002). Extensive mosaic

structure revealed by the complete genome sequence of uropathogenic Escherichia coli. *Proceedings of the National Academy of Sciences*, 99(26), 17020-17024.

York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T., & Albersheim, P. (1986). Isolation and characterization of plant cell walls and cell wall components. *Methods in Enzymology*, 118, 3-40.