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

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## ABSTRACT

1  
2 The preventive effects of the American cranberry (*Vaccinium macrocarpon*)  
3 against urinary tract infections are supported by extensive studies which have  
4 primarily focused on its phenolic constituents. Herein, a phenolic-free  
5 carbohydrate fraction (designated cranf1b-F2) was purified from cranberry fruit  
6 using ion exchange and size exclusion chromatography. MALDI-TOF-MS  
7 analysis revealed that the cranf1b-F2 constituents are predominantly  
8 oligosaccharides possessing various degrees of polymerisation and further  
9 structural analysis (by GC-MS and NMR) revealed mainly xyloglucan and  
10 arabinan residues. In antimicrobial assays, cranf1b-F2 (at 1.25 mg/mL  
11 concentration) reduced biofilm production by the uropathogenic *Escherichia*  
12 *coli* CFT073 strain by over 50% but did not inhibit bacterial growth. Cranf1b-F2  
13 (ranging from 0.625 - 10 mg/mL) also inhibited biofilm formation of the non-  
14 pathogenic *E. coli* MG1655 strain up to 60% in a concentration-dependent  
15 manner. These results suggest that cranberry oligosaccharides, in addition to  
16 its phenolic constituents, may play a role in its preventive effects against urinary  
17 tract infections.

18

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

21

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 MgSO<sub>4</sub>, 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 H<sub>2</sub>O, 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 (Sephacryl 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 H<sub>2</sub>O 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 H<sub>2</sub>O 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 OD<sub>550</sub> was measured (Spectramax M2,  
101 Molecular devices, Sunnyvale, CA, USA). Percent biofilm formation was  
102 calculated as the average OD<sub>550</sub> of three replicate treatment wells divided by  
103 average OD<sub>550</sub> 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 (NaBD<sub>4</sub>) 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 NaBD<sub>4</sub>. 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 D<sub>2</sub>O shake and dissolved  
139 in D<sub>2</sub>O with addition of 1 µL of DMSO as internal reference. <sup>1</sup>H, <sup>13</sup>C, 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 H<sub>2</sub>O) 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 <sup>1</sup>H 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 <sup>1</sup>H 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<sub>3</sub>Pen<sub>4</sub> (5 hexoses and 4 pentoses), Hex<sub>4</sub>Pen<sub>3</sub>, Hex<sub>4</sub>Pen<sub>4</sub>,  
169 Hex<sub>5</sub>Pen<sub>3</sub>, Hex<sub>4</sub>Pen<sub>5</sub>, Hex<sub>5</sub>Pen<sub>4</sub>, Hex<sub>5</sub>Pen<sub>5</sub> and Hex<sub>6</sub>Pen<sub>4</sub>, 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- $\alpha$ -Arab, 3- $\alpha$ -Arab and 3,5- $\alpha$ -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.  $^1\text{H}$  and  $^{13}\text{C}$  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

### 267 **Acknowledgements**

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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  
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274 at a research facility supported in part by the National Science Foundation  
275 EPSCoR Cooperative Agreement #EPS-1004057.

276

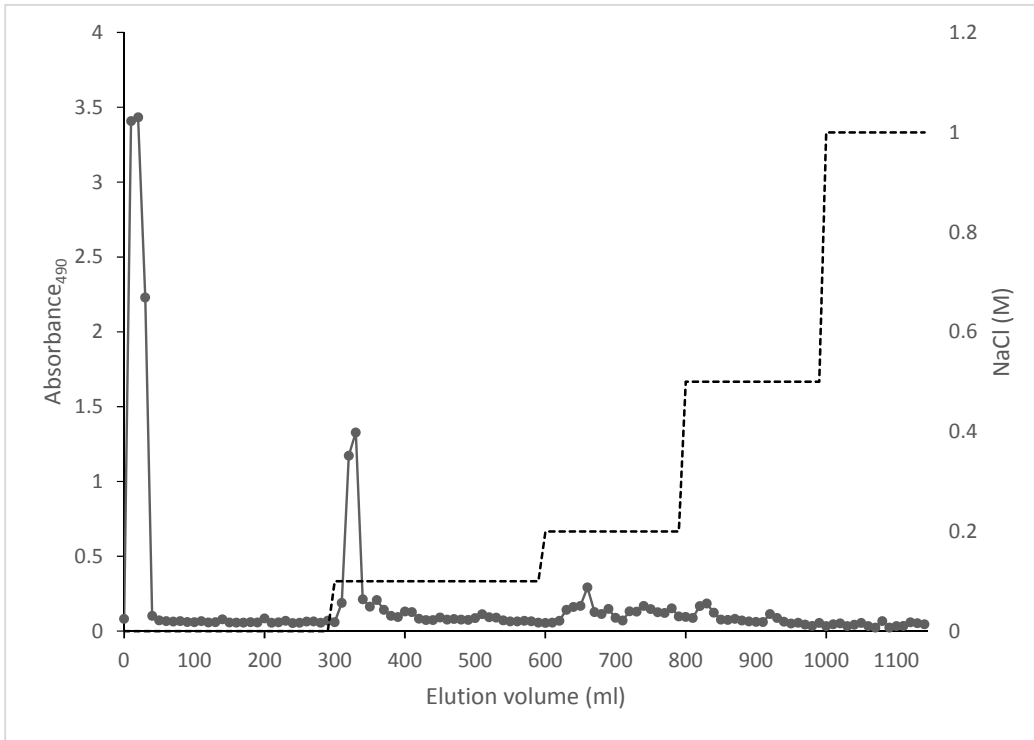
### 277 **Appendix A. Supplementary data**

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.

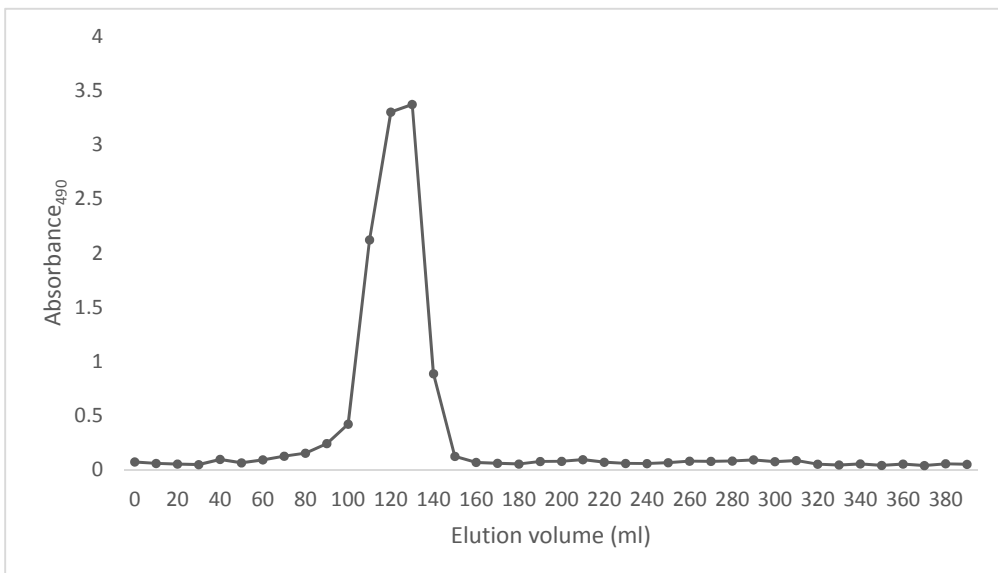
282



**Figure 1**

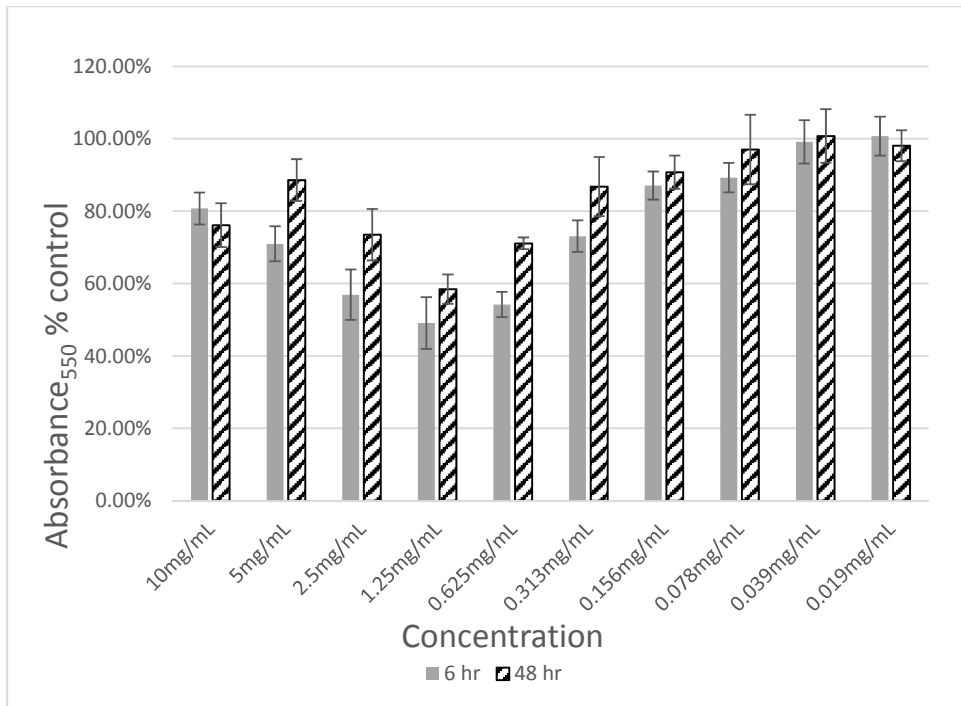


**Figure 1a. Elution profile of Cranf1b on Sepharose Q XL 16/10 column, eluted by stepwise gradient of NaCl (0-1 M) (total sugars, - - -).**

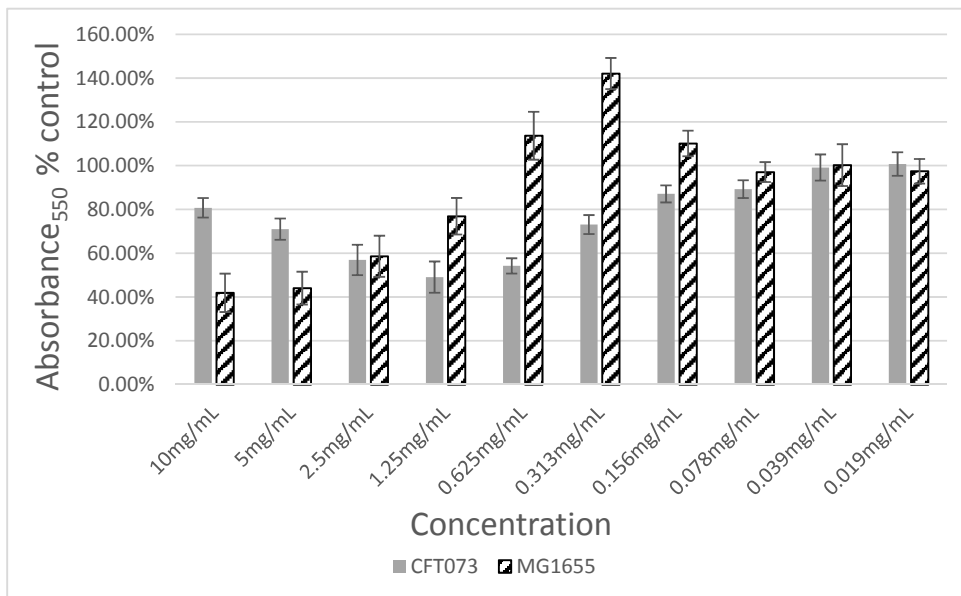


**Figure 1b. Elution profile of Cranf1b-F2 on Sephacryl S-100 HR 16/60 column, eluted by de-ionised water (total sugars, - - -).**

**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. <sup>13</sup>C NMR and <sup>1</sup>H 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%)	<u>G</u>	t-β-GlcP	105.24	-	-	-	-	-
			4.53	3.62	-	-	-	-
	<u>G</u>	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
	<u>G</u>	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 <sub>-</sub>	α-GlcP	92.40	71.85	-	-	-	-
			5.21	3.57	3.82	3.64	3.94	3.86
	G <sub>-</sub>	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

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