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**Inhibition activity of wild berry juice fractions against
Streptococcus pneumoniae binding to human bronchial cells**

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4 1 **Inhibition activity of wild berry juice fractions against *Streptococcus***
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7 2 ***pneumoniae* binding to human bronchial cells**
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59 20 **Short title:** berry juices against pneumococcal adherence
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21 **ABSTRACT**

22 Bacterial adhesion to the cell surface is a crucial step before infection can take place.
23 Inhibition of bacterial binding offers a novel preventive approach against infections.
24 Cranberry (*Vaccinium macrocarpon* Ait.) juice has been found to have anti-adhesive
25 activity against different bacteria. *Streptococcus pneumoniae* is an important pathogen
26 and the most common cause for pneumonia, meningitis, and otitis media. In this study
27 the inhibitory activity of cranberry (*Vaccinium oxycoccos* L.), bilberry (*Vaccinium*
28 *myrtillus* L.), and crowberry (*Empetrum nigrum* and *Empetrum hermaphroditum* L.)
29 juice fractions against pneumococcal binding was tested using human bronchial cells
30 (Calu-3) as an adhesion model. In addition, antimicrobial activity of the berry juice
31 fractions was tested. It was found that the studied berry juice fractions had anti-adhesion
32 activity and cranberry juice was the most active. The adhesion inhibition activity of
33 cranberry juice was nearly 90 % at the concentration of 8.7 mg/g of soluble solids.
34 Antimicrobial activity of the studied berry juice fractions was found to be remarkable;
35 pneumococcal growth was inhibited totally at the concentration of ~86 mg/g. Both anti-
36 adhesion and antimicrobial activities were reduced after solid-phase extraction of the
37 berry juices, which may suggest molecular synergistic effects of the berry juice
38 molecules against *S. pneumoniae*. The findings indicate that cranberry, bilberry, and
39 crowberry juices have potential against pneumococcal infections.

40 **Keywords:** *Streptococcus pneumoniae*, berries, anti-adhesion, antimicrobial, Calu-3

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43 INTRODUCTION

44 *Streptococcus pneumoniae* is an important human pathogen and the most common
45 cause for pneumonia, meningitis, and otitis media (Feldman and Anderson, 2009). It
46 is estimated that up to 60 % of the population in the world carry the pneumococcus
47 in the nasopharynx (Mitchell, 2003). Nasopharyngeal colonisation is mostly
48 asymptomatic and it has a key role in pneumococcal disease and spread (Bogaert *et*
49 *al.*, 2004). Pneumococcal infection is the cause of death for more than 1 million
50 children in the world per year, most of them in developing countries (Kadioglu *et al.*,
51 2008). Risk factors for pneumococcal diseases include extremes of age,
52 immunodeficiency (Bogaert *et al.*, 2004), as well as health habits and life style
53 factors such as smoking and contact with pets (Almirall *et al.*, 2008).

54 There is a vaccine available against *S. pneumoniae*. The pneumococcal vaccine is
55 targeted against the capsular polysaccharide of *S. pneumoniae*. This 23-valent
56 vaccine has a theoretical coverage of 90 % of disease causing serotypes. For young
57 children a 7-valent capsular polysaccharide-protein conjugate vaccine is used due to
58 weak response to polysaccharide vaccination (Bogaert *et al.*, 2004). High costs of the
59 conjugate vaccine reduce its use in developing countries, where the need for
60 prevention of pneumococcal diseases is most urgent (Kadioglu *et al.*, 2008).
61 Antibiotics of different classes, e.g. β -lactams, macrolides, aminoglycosides, and
62 fluoroquinolones, are used against pneumococcal infections (Woodhead *et al.*, 2005).
63 However, antibiotic resistance to various classes of antibiotics has made the
64 treatment of pneumococcal infections even more difficult (Feldman and Anderson,
65 2009).

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5 66 Due to resistance and vaccination insufficiency it is important to find alternative means
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7 67 against pneumococcal diseases. Anti-adhesion therapy is a novel approach in preventing
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9 68 bacterial infections (Ofek *et al.*, 2003). In anti-adhesion therapy the important
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11 69 interaction between the pathogen and host cell is inhibited by soluble carbohydrates or
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13 70 their analogs (Ofek *et al.*, 2003; Zopf and Roth, 1996). Since anti-adhesion agents are
14
15 71 aimed to interfere only with the bacterial attachment and they do not kill the pathogens,
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17 72 resistance towards these inhibitors is likely to evolve slowly (Joosten *et al.*, 2004).
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19 73 Results from animal experiments show that soluble carbohydrates can protect against
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21 74 experimental pneumococcal pneumonia (Idänpään-Heikkilä *et al.*, 1997).
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26 75 Berries and their health effects have gained a lot of attention in recent years, and their
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28 76 anti-adhesion potential is an interesting field of research. Anti-adhesive properties of
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30 77 cultivated American cranberry (*Vaccinium macrocarpon* Ait.) have been studied widely
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32 78 and anti-adhesive activity has been found against different bacteria (Burger *et al.*, 2000;
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34 79 Howell *et al.*, 1998; Weiss *et al.*, 2002). We have discovered anti-adhesion activity of
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36 80 wild cranberry (*Vaccinium oxycoccos* L.), bilberry (*Vaccinium myrtillus* L.),
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38 81 lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* and
39
40 82 *hermaphroditum* L.) juice fractions against the serious human pathogen *Neisseria*
41
42 83 *meningitidis* (Toivanen *et al.*, 2009). In addition, our studies have demonstrated berry
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44 84 and berry juice binding activity of *Streptococcus agalactiae*, and hemagglutination
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46 85 inhibition activity against *Streptococcus suis* (Toivanen *et al.*, 2010). We have found as
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48 86 well that *S. pneumoniae* has binding activity to low molecular size fractions of bilberry
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50 87 (*Vaccinium myrtillus* L.) and cranberry (*Vaccinium oxycoccos* L.) juices in a microtiter
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52 88 well assay (Toivanen *et al.*, 2010). In the present study we investigated the anti-
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54 89 adhesion activity, or binding inhibition activity of low molecular size fractions and
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4 90 subfractions from bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos*
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7 91 L.), and crowberry (*Empetrum nigrum* and *hermaphroditum* L.) juices against *S.*
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9 92 *pneumoniae*. Adhesion and anti-adhesion experiments were conducted using Calu-3
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11 93 cells (human bronchial epithelial cell line) as a model for adhesion. In addition,
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13 94 antimicrobial activity of the studied berry juice fractions was tested.

17 95 **MATERIALS AND METHODS**

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19 96 **Berry Juices.** Bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.),
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21 97 and crowberry (*Empetrum nigrum* and *Empetrum hermaphroditum* L.) (65 °Brix,
22
23 98 content of soluble solids g/ 100 g of solution) juice concentrates were purchased from
24
25 99 Kiantama Ltd (Suomussalmi, Finland). Juice concentrate of apple (mix of cultivars) (70
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27 100 °Brix) was purchased from VIP-Juicemaker Ltd (Kuopio, Finland). Additive-free juice
28
29 101 concentrates were stored at -20 °C.

30
31 102 **Preparation of Berry Juice Molecular Size Fractions.** Berry juice concentrates were
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33 103 diluted with water (1:4) and fractionated into three different molecular size fractions as
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35 104 described before (Toivanen *et al.*, 2009). Briefly, 15 mL of berry juice was loaded onto
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37 105 a 100 kDa cut-off Biomax Ultrafree-15 filter device (Millipore Corporation, MA, USA)
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39 106 and centrifuged (2000g at 4°C) until the volume was 1.5 mL. Top fraction (> 100 kDa)
40
41 107 was collected and stored at -20°C. The filtrate was loaded onto a 10 kDa cut-off Biomax
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43 108 Ultrafree-15 centrifugal filter device (Millipore Corporation, MA, USA) and
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45 109 centrifuged as described above. Both the top fraction (10–100 kDa) and filtrate (< 10
46
47 110 kDa, referred here as FI) were stored at -20°C. The content of soluble solids in the FI
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49 111 fractions (mg/g) was analyzed by determining the °Brix values by using a refractometer
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51 112 (ATAGO NAR-IT, Tokyo, Japan). FI fractions of cranberry, bilberry, and crowberry
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53 113 were chosen for the present study according to our previous results, where
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5 114 pneumococcal binding activity was detected to FI fractions of cranberry, bilberry, and
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7 115 crowberry (Toivanen *et al.*, 2010). These berry juice FI fractions composed mostly of
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9 116 sugars and small amounts of small size phenolics (Toivanen *et al.*, 2009; Toivanen *et*
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11 117 *al.*, 2010).
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16 119 **Solid-Phase Extraction (SPE) Subfractionation of Berry Juice FI Fractions.** In
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18 120 order to isolate phenolic compounds from the berry juice FI fractions for activity
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20 121 testing, the subfractionation method previously described was used (Toivanen *et al.*,
21
22 122 2009). Briefly, the FI fraction of berry juice (3 mL) was mixed with phosphate buffer
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24 123 (pH 7.0) (J. T. Baker, Deventer, The Netherlands) at 1:1 ratio (v/v), and passed through
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26 124 a C-18 SPE cartridge (Waters Corp., Milford, MA; cartridges of 3 cm³ capacity filled
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28 125 with 500 mg of adsorbent). The cartridge was conditioned with 15 mL of methanol
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30 126 (HPLC grade) (VWR International Ltd., Leuven, Belgium) and equilibrated with 5 mL
31
32 127 of pH 7.0 phosphate buffer. After washing with 5 mL of diluted phosphate buffer (1:8,
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34 128 v/v, with water) and with 5 mL of water (purified on a Millipore Milli-Q apparatus,
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36 129 Molsheim, France) the cartridge was dried under vacuum for 30 sec. Then the elution
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38 130 with 5 mL of ethyl acetate (Laboratory-Scan, Dublin, Ireland) was performed. The
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40 131 cartridge was dried again under vacuum for 30 sec before the next elution with 3 mL of
41
42 132 water. After that, the elution with 3 mL of 10 % aqueous methanol was discarded and
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44 133 the ones with 3 mL of 20 % and 3 mL of 60 % aqueous methanol were collected
45
46 134 separately. Solvents in the subfractions were evaporated by a rotary evaporator at +30
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48 135 °C. Finally, the solids were reconstituted with water to the concentration of 0.5 or 1.0
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50 136 mg/mL. Subfractions eluted with water containing phenolic components according to
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5 137 the methodology employed (Toivanen *et al.*, 2009; Sun *et al.*, 2006) were chosen for the
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7 138 experiments.

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10 139 **Cell Culture.** The human bronchial epithelial cell line (Calu-3) was a kind gift from
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12 140 Professor Jouni Hirvonen (University of Helsinki, Finland), and it was used between
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14 141 passages 28 and 45. Cells were cultured in Eagle's Minimum Essential Medium
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16 142 (BioWhittaker, Cambrex, Verviers, Belgium) (10 % v/v) supplemented with heat
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18 143 inactivated fetal bovine serum (Gibco, Paisley, U.K) (10–20 % v/v), L-glutamine 100x
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20 144 (Euroclone, Pero, Italy) (1 % v/v), non-essential amino acid solution 100x (Euroclone,
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22 145 Pero, Italy) (1 % v/v), sodium pyruvate (BioWhittaker, Cambrex, Verviers, Belgium) (1
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24 146 % v/v), penicillin-streptomycin 100x (Euroclone, Pero, Italy) (1 % v/v) and NaHCO₃
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26 147 (Merck, Darmstadt, Germany) (0.15 % w/v). Cells were cultivated in 75 cm² flasks
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28 148 containing 10–20 mL medium and maintained in a humidified 5 % CO₂-95 %
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30 149 atmospheric air incubator at 37 °C. The culture medium was changed every 2–3 days
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32 150 and cells were passaged once or twice per week at a 1:2–1:5 split ratio using trypsin-
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34 151 EDTA solution (Gibco, Paisley, UK).

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36 152 **Bacterial Strain and Culture Conditions.** *Streptococcus pneumoniae* clinical strain
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38 153 SB 53845 (isolated from lung) was received from Sauli Haataja (University of Turku,
39
40 154 Finland). Bacteria were cultivated at 37 °C for 18 h on sheep blood agar plates
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42 155 (Microbiological Laboratory, Kuopio University Hospital, Finland or Labema Inc.,
43
44 156 Kerava, Finland). An atmosphere of increased CO₂ was provided for optimal growth by
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46 157 using a candle-extinction jar (Weiser *et al.*, 1994).

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48 158 **Isolation of *S. pneumoniae*.** *S. pneumoniae* were harvested from ten plates and
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50 159 suspended in 40 mL of sterile PBS (Gibco, Paisley, U.K.) at 0 °C. The suspension was
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52 160 centrifuged at 2000g at 4 °C for 10 min and washed three times with cold sterile PBS.
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5 161 The density of the bacterial suspension was standardized to absorbance value of 0.420 at
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7 162 A_{600} . After the last washing, bacteria were suspended to prewarmed (37 °C) Eagle's
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9 163 Minimum Essential Medium and diluted to the density of 10^8 /mL.

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11 164 **Binding of *S. pneumoniae* to Calu-3 Cells.** A previously described method (Rytönen
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13 *et al.*, 2004; Todoriki *et al.*, 2001) was used with modifications. Calu-3 cells were
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15 166 seeded on 6-well plates with the cell density of 10^5 per well 20–24 hours before the
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17 167 experiment, and were let grown into confluence. A culture medium without antibiotics
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19 168 was used. Diluted bacterial suspension (1.5 mL) prepared as described above was
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21 169 incubated over the cells for one hour at 37 °C. Nonadherent bacteria were removed by
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23 170 three washings with sterile PBS and the infected cells were detached with 0.5 mL of
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25 171 trypsin-EDTA solution. Infected cells were diluted and appropriate dilutions were
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27 172 plated (100 µl) on the sheep blood agar plates and bacteria were cultivated overnight at
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29 173 37 °C in a CO₂ atmosphere. Attachment of bacteria to Calu-3 cells was determined from
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31 174 duplicate cultures on sheep blood agar plates. Colony forming units (CFU) were
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33 175 counted next day.

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35 176 **Inhibition of the Binding of *S. pneumoniae* to Calu-3 Cells.** The assay was done as
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37 177 described above for binding, with the exception that 1.5 mL of berry juice FI fractions
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39 178 or berry juice subfractions diluted with culture medium (without antibiotics) were
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41 179 incubated over Calu-3 cells for one hour at 37 °C. After incubation with the berry juice
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43 180 fractions or subfractions, the bacterial suspension was added. Tested concentrations for
44
45 181 the FI fractions and subfractions were 1.6–9.0 mg/g of soluble solids and 1–50 µg/mL,
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47 182 respectively. Colony forming units were counted next day and the binding inhibition
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49 183 activity was calculated as follows:
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$$\frac{\text{CFU control} - \text{CFU sample}}{\text{CFU control}} \times 100 \%$$

Antimicrobial Assay on Microtiter Plates. Antimicrobial activities of the berry juice F1 fractions and subfractions were tested against *S. pneumoniae*. According to literature, a microtiter broth microdilution method is most preferable for testing antimicrobial activity of plant material (King *et al.*, 2008). In this study, a previously described method (Amsterdam 2005) was used with modifications. Overnight plate-cultured *S. pneumoniae* bacteria were suspended in cold sterile PBS to an optical density of 0.420 at 600 nm. The corresponding colony forming unit was 10^8 . Diluted bacterial suspension (50 μL) and different concentrations of diluted FI fractions and subfractions (50 μL) were incubated in a microtiter plate (Falcon Flexible Plate, Becton Dickinson Labware, NJ, USA) at 37 °C, in CO₂ atmosphere for one hour. As a control, bacteria were incubated in the absence of berry juice samples or with Ampicillin (100 $\mu\text{g}/\text{mL}$). The antimicrobial activity of FI fractions and subfractions was analyzed by plating the incubation mixtures in triplicate on sheep blood agar plates. The surviving colony forming units were counted next day. Bacterial survival was calculated by comparing the colony forming units (CFU) of bacteria–sample -mixture and control bacteria:

$$\frac{\text{CFU sample}}{\text{CFU control}} \times 100 \%$$

Statistical Analysis. Results for adhesion inhibition and antimicrobial tests were reported as means \pm standard error of the mean (SEM). Two-tailed, unpaired Student's t-test (Microsoft Excel 2007) was used to calculate the significance of the differences between CFUs from pneumococcal adhesion (control) and pneumococcal adhesion

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4 211 inhibition; and differences between CFUs from pneumococcal control and
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6 212 pneumococcal-berry incubation mixtures. Significance was defined as a P of < 0.05 .
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10 213 **RESULTS AND DISCUSSION**

11 214 **Inhibition of the Binding of *S. pneumoniae* to Calu-3 Cells.** Carbohydrate receptors
12 215 in human airway mediate the adhesion of *S. pneumoniae* to respiratory epithelial cells
13 216 (Andersson *et al.*, 1983). In order to test the anti-adhesion activity of selected wild berry
14 217 juice fractions and their subfractions against *S. pneumoniae*, human bronchial cells
15 218 (Calu-3) were chosen for the cell culture model (Elm *et al.*, 2004). Cranberry juice FI
16 219 fraction inhibited the adhesion of *S. pneumoniae* to Calu-3 cells in a dose dependent
17 220 manner (Fig. 1A). The adhesion inhibition activity of cranberry juice was significant
18 221 and reached nearly 90 % at the concentration of 8.7 mg/g ($P < 0.05$). Activity decreased
19 222 towards lower concentrations and the lowest concentrations used (2.3 mg/g and 1.7
20 223 mg/g) did not have an effect on pneumococcal adherence. Both bilberry and crowberry
21 224 juice FI (Fig. 1B, C) fractions possessed lower anti-adhesion activity (no dose-
22 225 dependent) compared to cranberry. Adhesion inhibition of both bilberry (9 mg/g) and
23 226 crowberry (8.1 mg/g) juice fractions was 52 %.

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36 227 In our previous studies we found that *S. pneumoniae* bound to cranberry and bilberry
37 228 juice FI fractions in a microtiter well binding assay (Toivanen *et al.*, 2010). Slight
38 229 adherence to crowberry juice FI fraction was also found. The concentrations of the
39 230 tested fractions in the assay employed ranged between 160–180 mg/g. Due to
40 231 differences in the methodologies, in the present cell culture study anti-adhesion
41 232 activities were found even with 20–100 fold lower concentrations (1.6–9.0 mg/g). Our
42 233 current results thus support the earlier findings on pneumococcal binding to berry juice
43 234 FI fractions and gives further evidence on anti-adhesive properties of berry juices.
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4 235 Anti-adhesion activity of cranberry (*Vaccinium macrocarpon* Ait.) has been studied
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6 236 widely *in vitro* against several bacteria (table 1). *In vivo* clinical trial has also been
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8 237 carried out (Kontiokari *et al.*, 2001). The anti-adhesive constituents in cranberry include
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10 238 fructose (Zafriri *et al.*, 1989), high molecular weight material (> 15 kDa) (Burger *et al.*,
11
12 239 2000), and proanthocyanidins (Foo *et al.*, 2000a; Foo *et al.*, 2000b). In this study we
13
14 240 found adhesion inhibition against *S. pneumoniae* from low molecular size fractions of
15
16 241 cranberry, bilberry and crowberry juices (< 10 kDa). We have shown that FI fractions
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18 242 contain only low-molecular-weight molecules, mainly sugar molecules and small
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20 243 amounts of lower molecular weight phenolic compounds (Toivanen *et al* 2009;
21
22 244 Toivanen *et al* 2010). Carbohydrate receptors mediate the adhesion of *S. pneumoniae* to
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24 245 respiratory epithelial cells and pneumococcal adherence can be blocked by
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26 246 oligosaccharides (Bartehlson *et al.*, 1998; Andersson *et al.*, 1986) and by fractions of
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28 247 milk (Andersson *et al.*, 1986). In our previous microtiter well assay, pneumococcal
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30 248 adherence to cranberry and bilberry juice FI fractions was not due to sugar molecules
31
32 249 (Toivanen *et al.*, 2010). It is known that proanthocyanidins from cranberry juice can
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34 250 prevent the adhesion of oligosaccharide α -Gal (1 \rightarrow 4) β -Gal recognizing adhesins in *E.*
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36 251 *coli* (Foo *et al.*,2000a) and adhesion of *Neisseria meningitidis* pili can be blocked by
37
38 252 milk oligosaccharides (Hakkarainen *et al.*, 2005) or by berry fractions containing
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40 253 phenolic components (Toivanen *et al.*, 2009). Here as well the phenolic compounds,
41
42 254 such as proanthocyanidins and anthocyanins of the berry juice fractions (Toivanen *et*
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44 255 *al.*, 2009), could be the active molecules against the pneumococcal adherence.
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55 256 Anti-adhesion activity of the water subfractions prepared from the berry juice FI
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57 257 fractions was also tested (Fig. 2). Adhesion inhibition achieved plateau in very low
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59 258 concentrations of the subfractions (1 μ g/mL of cranberry, 5 μ g/mL of bilberry, 15
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5 259 $\mu\text{g/mL}$ of crowberry). The best activity was found with cranberry and bilberry.
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7 260 Cranberry juice subfraction inhibited significantly the pneumococcal adhesion at the
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9 261 highest concentration tested ($50 \mu\text{g/mL}$); the adhesion inhibition was found to be 38 %
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11 262 (Fig. 2A). As a comparison, potent anti-adhesion activity against *E. coli* was found from
12
13 263 ethyl acetate extracts of Sephadex LH20-purified cranberry proanthocyanidins at a
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15 264 concentration of $75 \mu\text{g/mL}$ (Foo *et al.*, 2000a). The adhesion inhibition activity of
16
17 265 bilberry with $50 \mu\text{g/mL}$ concentration was found to be 37 % and it was not significant
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19 266 (Fig. 2B). Crowberry had the lowest activity; anti-adhesion activity was 19 % (50
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21 267 $\mu\text{g/mL}$) (Fig. 2C).
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27 268 Subfractions were prepared from juice FI fractions by SPE subfractionation in order to
28
29 269 extract the active phenolic compounds from FI fractions (Toivanen *et al.*, 2009; Sun *et*
30
31 270 *al.*, 2006). Adhesion inhibition activity of the cranberry subfraction was significant, but
32
33 271 it was lower than adhesion inhibition caused by the cranberry FI fraction. The detected
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35 272 reduction in activity of the F1 subfractions against the adhesion of *S. pneumoniae* may
36
37 273 result from loss of a combination of different phenolic molecules and/ or combination
38
39 274 with sugars or loss of a certain type of proanthocyanidins (Foo *et al.*, 2000b; Määttä-
40
41 275 Riihinen *et al.*, 2005) present in FI berry juice fractions and needed for the adhesion
42
43 276 inhibition. Detailed structural elucidation of the active phenolic molecules and the
44
45 277 possible combination of different molecules participating in the inhibition remains to be
46
47 278 carried out in future.
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53 279 **Antimicrobial Activity of Berry Juice FI Fractions and Subfractions against *S.***
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55 280 ***pneumoniae*.** Berries and especially their polyphenols are known to inhibit the growth
56
57 281 of many human pathogens (Cavanagh *et al.*, 2003; Matsushima *et al.*, 2008;
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59 282 Puupponen-Pimiä *et al.*, 2005; Puupponen-Pimiä *et al.* 2001). In the present study,
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5 283 cranberry, bilberry, and crowberry juice FI fractions were found to possess high
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7 284 antimicrobial activity against *S. pneumoniae* (Fig. 3). The highest concentration used in
8
9 285 antimicrobial tests (~86 mg/g) was extremely effective and the growth of *S. pneumoniae*
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11 286 was inhibited totally by all FI fractions. At the concentration of 3.5 mg/g cranberry
12
13 287 juice fraction was still effective with nearly 100 % growth inhibition, but for bilberry
14
15 288 and crowberry juice fractions the bacterial survival was 60 % and 39 %, respectively. At
16
17 289 the lowest concentration tested (1.7 mg/g), the bacterial survival of all the berry juice FI
18
19 290 fractions was found to be between 55–60 %. Subfractions did not have an effect on
20
21 291 pneumococcal growth (data not shown). Bacterial survival was between 90–100 % for
22
23 292 all subfraction concentrations, except for 50 µg/mL of bilberry juice subfraction (75 %).
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25 293 Both anti-adhesion and antimicrobial effects were minor after subfractionation when
26
27 294 compared to anti-adhesion and antimicrobial effects of the FI berry juice fractions.
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33 295 Our findings showed that small molecular size FI fractions from cranberry, bilberry, and
34
35 296 crowberry juices had significant effect on growth of *S. pneumoniae*. In addition,
36
37 297 cranberry juice FI fraction inhibited significantly binding of *S. pneumoniae* to human
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39 298 bronchial cells, while the FI fractions from bilberry and crowberry juices had a lower
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41 299 anti-adhesion effect. Compared to significant anti-adhesion activity of cranberry juice
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43 300 FI fraction, the level of the anti-adhesion activity of the FI phenolic subfraction was
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45 301 substantially lower.
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51 302 Here we examined adhesion inhibition in cell culture conditions by using excess amount
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53 303 of pneumococcal bacteria. The anti-adhesion activity was achieved with the cranberry
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55 304 juice FI fraction at the concentration of 8.7 mg/g of soluble solids, which contain small
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57 305 amount of polyphenols (Toivanen *et al.*, 2009; Toivanen *et al.*, 2010). The cranberry
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59 306 polyphenolic subfraction had inhibitory activity at the concentration of 5 mg/100ml.
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4 307 The polyphenol concentration in cranberries is high, for example concentration of
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7 308 proanthocyanidins is about 400mg/100g (Hellström *et al.*, 2009). Thus in the
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9 309 physiological conditions anti-adhesion may be achieved with reasonable amount of
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11 310 cranberry juice. However, without *in vivo* trials it is difficult to estimate the inhibitory
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13 311 effect and the amount of cranberry juice needed for the anti-adhesion.

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17 312 In summary, these results indicate bilberry, crowberry, and especially cranberry juices
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19 313 as novel sources for both anti-adhesive and antimicrobial agents against pneumococci.
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21 314 Studies on molecular characterization of the active phenolic molecules remain to be
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23 315 carried out in future. The studied berry juice FI fractions were solvent-free and water
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25 316 soluble, thus their possible future utilization in food industry would be easy.
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31 318 **ACKNOWLEDGMENTS**

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425 **Table 1.** *In vitro* and *in vivo* anti-adhesion activity of cranberry (*Vaccinium*
 426 *macrocarpon* Ait.)

	Cranberry material	Bacteria	Examples of diseases in humans	References
<i>In vitro</i>	A-type proanthocyanidin trimers of cranberry ^a ; Purified proanthocyanidin extracts of cranberry ^b ; Cranberry juice ^c	<i>Escherichia coli</i>	UTI	Foo <i>et al.</i> , 2000b ^a ; Howell <i>et al.</i> , 1998 ^b ; Zafriri <i>et al.</i> , 1989 ^c
	High molecular weight material of cranberry juice	<i>Helicobacter pylori</i>	gastritis	Burger <i>et al.</i> , 2000
	Polyphenolic fractions of cranberry* juice	<i>Neisseria meningitidis</i>	meningitis, septicemia	Toivanen <i>et al.</i> , 2009
	High molecular weight material of cranberry juice	Oral bacteria	dental caries	Weiss <i>et al.</i> , 2002
<i>In vivo</i>	Cranberry juice	<i>Escherichia coli</i>	UTI	Kontiokari <i>et al.</i> , 2001

427 * *Vaccinium oxycoccos* L.; UTI, urinary tract infection

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4 428 **FIGURE LEGENDS**

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6 429 **Figure 1.** Inhibition of adhesion (mean \pm SEM, $n = 6$) of *S. pneumoniae* to Calu-3
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8 430 human bronchial epithelial cell line by fraction F1 (< 10 kDa) of **A)** cranberry juice, **B)**
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10 431 bilberry juice, and **C)** crowberry juice. Inhibition of adhesion expressed as the
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12 432 percentage of bacterial control. Apple juice fraction F1 with constant concentration 11
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14 433 mg/g was used as negative control (dotted line). * $P < 0.05$ against the bacterial control.
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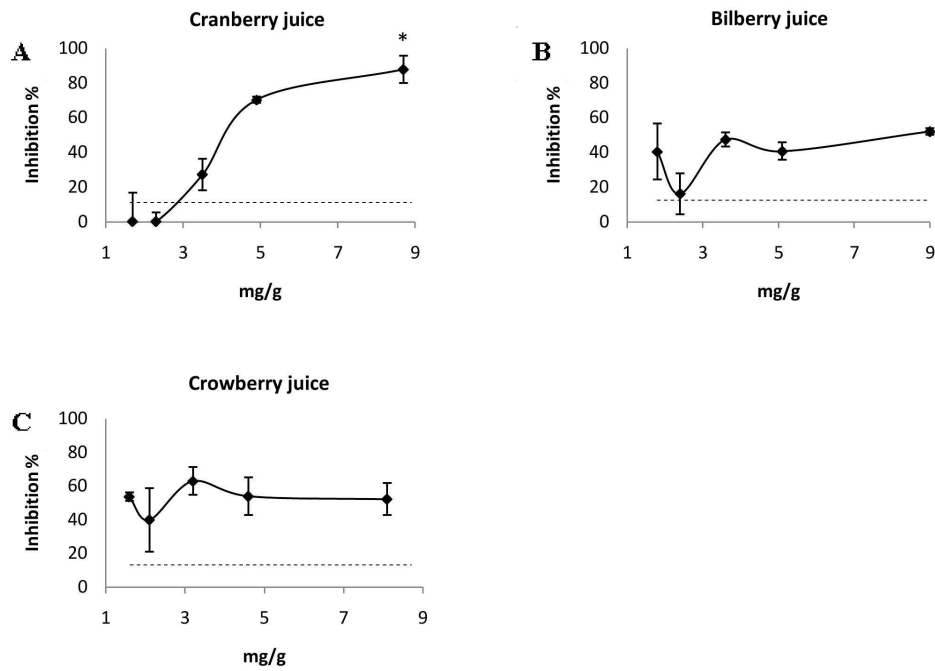
19 434

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22 435 **Figure 2.** Inhibition of adhesion (mean \pm SEM, $n = 6$) of *S. pneumoniae* to Calu-3
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24 436 human bronchial epithelial cell line by berry juice subfractions of **A)** cranberry juice, **B)**
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26 437 bilberry juice, and **C)** crowberry juice. Inhibition of adhesion expressed as the
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28 438 percentage of bacterial control. Apple juice FI fraction with constant concentration 11
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30 439 mg/g was used as negative control (dotted line). * $P < 0.05$ against the bacterial control.
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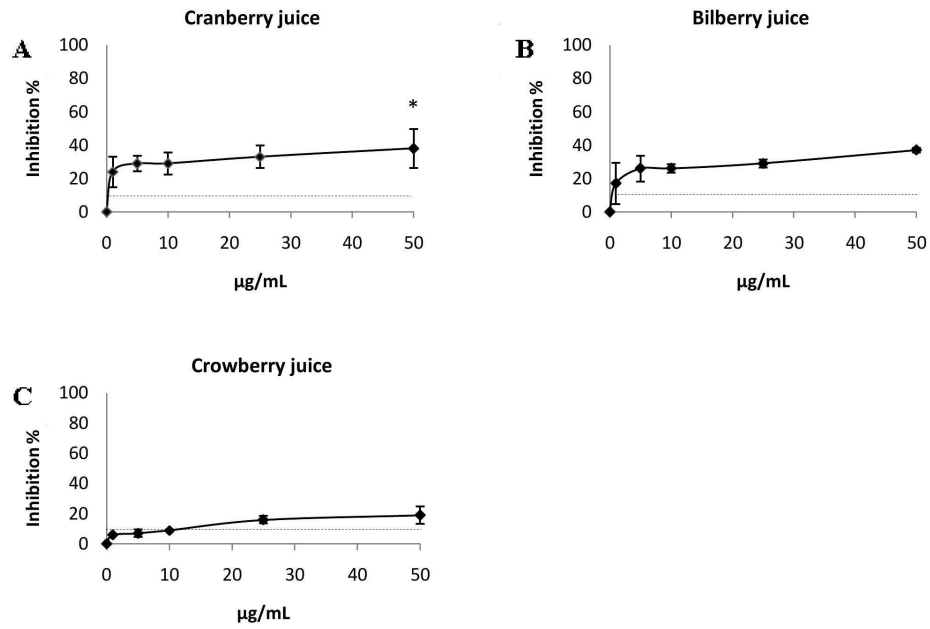
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38 441 **Figure 3.** Antimicrobial activity of FI berry juice fractions (< 10 kDa) of cranberry,
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40 442 bilberry, and crowberry. Bacterial survival compared to control, mean \pm SEM of three
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42 443 experiments. Bacterial survival with Ampicillin (100 μ g/mL) was 5 %. * $P < 0.05$
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44 444 against the bacterial control.
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Huttunen S, Figure 1.



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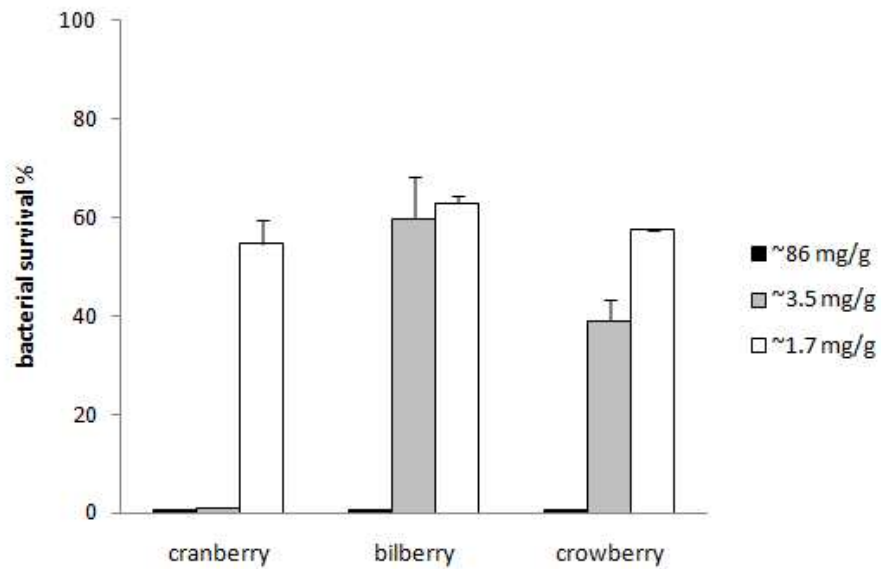
Huttunen S, Figure 2.



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view

Huttunen S, Figure 3



203x140mm (72 x 72 DPI)

Review