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

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Short title: berry juices against pneumococcal adherence

ABSTRACT

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

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

INTRODUCTION

Streptococcus pneumoniae is an important human pathogen and the most common cause for pneumonia, meningitis, and otitis media (Feldman and Anderson, 2009). It is estimated that up to 60 % of the population in the world carry the pneumococcus in the nasopharynx (Mitchell, 2003). Nasopharyngeal colonisation is mostly asymptomatic and it has a key role in pneumococcal disease and spread (Bogaert *et al.*, 2004). Pneumococcal infection is the cause of death for more than 1 million children in the world per year, most of them in developing countries (Kadioglu *et al.*, 2008). Risk factors for pneumococcal diseases include extremes of age, immunodeficiency (Bogaert *et al.*, 2004), as well as health habits and life style factors such as smoking and contact with pets (Almirall *et al.*, 2008). There is a vaccine available against *S. pneumoniae*. The pneumococcal vaccine is targeted against the capsular polysaccharide of *S. pneumoniae*. This 23-valent vaccine has a theoretical coverage of 90 % of disease causing serotypes. For young children a 7-valent capsular polysaccharide-protein conjugate vaccine is used due to weak response to polysaccharide vaccination (Bogaert *et al.*, 2004). High costs of the conjugate vaccine reduce its use in developing countries, where the need for prevention of pneumococcal diseases is most urgent (Kadioglu *et al.*, 2008). Antibiotics of different classes, e.g. β -lactams, macrolides, aminoglycosides, and fluoroquinolones, are used against pneumococcal infections (Woodhead *et al.*, 2005). However, antibiotic resistance to various classes of antibiotics has made the treatment of pneumococcal infections even more difficult (Feldman and Anderson, 2009).

Due to resistance and vaccination insufficiency it is important to find alternative means against pneumococcal diseases. Anti-adhesion therapy is a novel approach in preventing bacterial infections (Ofek *et al.*, 2003). In anti-adhesion therapy the important interaction between the pathogen and host cell is inhibited by soluble carbohydrates or their analogs (Ofek *et al.*, 2003; Zopf and Roth, 1996). Since anti-adhesion agents are aimed to interfere only with the bacterial attachment and they do not kill the pathogens, resistance towards these inhibitors is likely to evolve slowly (Joosten *et al.*, 2004). Results from animal experiments show that soluble carbohydrates can protect against experimental pneumococcal pneumonia (Idänpään-Heikkilä *et al.*, 1997).

Berries and their health effects have gained a lot of attention in recent years, and their anti-adhesion potential is an interesting field of research. Anti-adhesive properties of cultivated American cranberry (*Vaccinium macrocarpon* Ait.) have been studied widely and anti-adhesive activity has been found against different bacteria (Burger *et al.*, 2000; Howell *et al.*, 1998; Weiss *et al.*, 2002). We have discovered anti-adhesion activity of wild cranberry (*Vaccinium oxycoccos* L.), bilberry (*Vaccinium myrtillus* L.), lingonberry (*Vaccinium vitis-idaea* L.), and crowberry (*Empetrum nigrum* and *hermaphroditum* L.) juice fractions against the serious human pathogen *Neisseria meningitidis* (Toivanen *et al.*, 2009). In addition, our studies have demonstrated berry and berry juice binding activity of *Streptococcus agalactiae*, and hemagglutination inhibition activity against *Streptococcus suis* (Toivanen *et al.*, 2010). We have found as well that *S. pneumoniae* has binding activity to low molecular size fractions of bilberry (*Vaccinium myrtillus* L.) and cranberry (*Vaccinium oxycoccos* L.) juices in a microtiter well assay (Toivanen *et al.*, 2010). In the present study we investigated the anti-adhesion activity, or binding inhibition activity of low molecular size fractions and

subfractions from bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.), and crowberry (*Empetrum nigrum* and *hermaphroditum* L.) juices against *S. pneumoniae*. Adhesion and anti-adhesion experiments were conducted using Calu-3 cells (human bronchial epithelial cell line) as a model for adhesion. In addition, antimicrobial activity of the studied berry juice fractions was tested.

MATERIALS AND METHODS

Berry Juices. Bilberry (*Vaccinium myrtillus* L.), cranberry (*Vaccinium oxycoccos* L.), and crowberry (*Empetrum nigrum* and *Empetrum hermaphroditum* L.) (65 °Brix, content of soluble solids g/ 100 g of solution) juice concentrates were purchased from Kiantama Ltd (Suomussalmi, Finland). Juice concentrate of apple (mix of cultivars) (70 °Brix) was purchased from VIP-Juicemaker Ltd (Kuopio, Finland). Additive-free juice concentrates were stored at –20 °C.

Preparation of Berry Juice Molecular Size Fractions. Berry juice concentrates were diluted with water (1:4) and fractionated into three different molecular size fractions as described before (Toivanen *et al.*, 2009). Briefly, 15 mL of berry juice was loaded onto a 100 kDa cut-off Biomax Ultrafree-15 filter device (Millipore Corporation, MA, USA) and centrifuged (2000g at 4°C) until the volume was 1.5 mL. Top fraction (> 100 kDa) was collected and stored at -20°C. The filtrate was loaded onto a 10 kDa cut-off Biomax Ultrafree-15 centrifugal filter device (Millipore Corporation, MA, USA) and centrifuged as described above. Both the top fraction (10–100 kDa) and filtrate (< 10 kDa, referred here as FI) were stored at -20°C. The content of soluble solids in the FI fractions (mg/g) was analyzed by determining the °Brix values by using a refractometer (ATAGO NAR-IT, Tokyo, Japan). FI fractions of cranberry, bilberry, and crowberry were chosen for the present study according to our previous results, where

pneumococcal binding activity was detected to FI fractions of cranberry, bilberry, and crowberry (Toivanen *et al.*, 2010). These berry juice FI fractions composed mostly of sugars and small amounts of small size phenolics (Toivanen *et al.*, 2009; Toivanen *et al.*, 2010).

Solid-Phase Extraction (SPE) Subfractionation of Berry Juice FI Fractions.

In order to isolate phenolic compounds from the berry juice FI fractions for activity testing, the subfractionation method previously described was used (Toivanen *et al.*, 2009). Briefly, the FI fraction of berry juice (3 mL) was mixed with phosphate buffer (pH 7.0) (J. T. Baker, Deventer, The Netherlands) at 1:1 ratio (v/v), and passed through a C-18 SPE cartridge (Waters Corp., Milford, MA; cartridges of 3 cm³ capacity filled with 500 mg of adsorbent). The cartridge was conditioned with 15 mL of methanol (HPLC grade) (VWR International Ltd., Leuven, Belgium) and equilibrated with 5 mL of pH 7.0 phosphate buffer. After washing with 5 mL of diluted phosphate buffer (1:8, v/v, with water) and with 5 mL of water (purified on a Millipore Milli-Q apparatus, Molsheim, France) the cartridge was dried under vacuum for 30 sec. Then the elution with 5 mL of ethyl acetate (Laboratory-Scan, Dublin, Ireland) was performed. The cartridge was dried again under vacuum for 30 sec before the next elution with 3 mL of water. After that, the elution with 3 mL of 10 % aqueous methanol was discarded and the ones with 3 mL of 20 % and 3 mL of 60 % aqueous methanol were collected separately. Solvents in the subfractions were evaporated by a rotary evaporator at +30 °C. Finally, the solids were reconstituted with water to the concentration of 0.5 or 1.0 mg/mL. Subfractions eluted with water containing phenolic components according to

the methodology employed (Toivanen *et al.*, 2009; Sun *et al.*, 2006) were chosen for the experiments.

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

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

Isolation of *S. pneumoniae*. *S. pneumoniae* were harvested from ten plates and suspended in 40 mL of sterile PBS (Gibco, Paisley, U.K.) at 0 °C. The suspension was centrifuged at 2000g at 4 °C for 10 min and washed three times with cold sterile PBS.

The density of the bacterial suspension was standardized to absorbance value of 0.420 at A_{600} . After the last washing, bacteria were suspended to prewarmed (37 °C) Eagle's Minimum Essential Medium and diluted to the density of 10^8 /mL.

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

Inhibition of the Binding of *S. pneumoniae* to Calu-3 Cells. The assay was done as described above for binding, with the exception that 1.5 mL of berry juice FI fractions or berry juice subfractions diluted with culture medium (without antibiotics) were incubated over Calu-3 cells for one hour at 37 °C. After incubation with the berry juice fractions or subfractions, the bacterial suspension was added. Tested concentrations for the FI fractions and subfractions were 1.6–9.0 mg/g of soluble solids and 1–50 µg/mL, respectively. Colony forming units were counted next day and the binding inhibition activity was calculated as follows:

185
$$\frac{\text{CFU control} - \text{CFU sample}}{\text{CFU control}} \times 100 \%$$

188 **Antimicrobial Assay on Microtiter Plates.** Antimicrobial activities of the berry juice
189 FI fractions and subfractions were tested against *S. pneumoniae*. According to
190 literature, a microtiter broth microdilution method is most preferable for testing
191 antimicrobial activity of plant material (King *et al.*, 2008). In this study, a previously
192 described method (Amsterdam 2005) was used with modifications. Overnight plate-
193 cultured *S. pneumoniae* bacteria were suspended in cold sterile PBS to an optical
194 density of 0.420 at 600 nm. The corresponding colony forming unit was 10⁸. Diluted
195 bacterial suspension (50 µL) and different concentrations of diluted FI fractions and
196 subfractions (50 µL) were incubated in a microtiter plate (Falcon Flexible Plate, Becton
197 Dickinson Labware, NJ, USA) at 37 °C, in CO₂ atmosphere for one hour. As a control,
198 bacteria were incubated in the absence of berry juice samples or with Ampicillin (100
199 µg/mL). The antimicrobial activity of FI fractions and subfractions was analyzed by
200 plating the incubation mixtures in triplicate on sheep blood agar plates. The surviving
201 colony forming units were counted next day. Bacterial survival was calculated by
202 comparing the colony forming units (CFU) of bacteria–sample -mixture and control
203 bacteria:

204
$$\frac{\text{CFU sample}}{\text{CFU control}} \times 100 \%$$

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

inhibition; and differences between CFUs from pneumococcal control and pneumococcal-berry incubation mixtures. Significance was defined as a P of < 0.05 .

RESULTS AND DISCUSSION

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

In our previous studies we found that *S. pneumoniae* bound to cranberry and bilberry juice FI fractions in a microtiter well binding assay (Toivanen *et al.*, 2010). Slight adherence to crowberry juice FI fraction was also found. The concentrations of the tested fractions in the assay employed ranged between 160–180 mg/g. Due to differences in the methodologies, in the present cell culture study anti-adhesion activities were found even with 20–100 fold lower concentrations (1.6–9.0 mg/g). Our current results thus support the earlier findings on pneumococcal binding to berry juice FI fractions and gives further evidence on anti-adhesive properties of berry juices.

Anti-adhesion activity of cranberry (*Vaccinium macrocarpon* Ait.) has been studied widely *in vitro* against several bacteria (table 1). *In vivo* clinical trial has also been carried out (Kontiokari *et al.*, 2001). The anti-adhesive constituents in cranberry include fructose (Zafriri *et al.*, 1989), high molecular weight material (> 15 kDa) (Burger *et al.*, 2000), and proanthocyanidins (Foo *et al.*, 2000a; Foo *et al.*, 2000b). In this study we found adhesion inhibition against *S. pneumoniae* from low molecular size fractions of cranberry, bilberry and crowberry juices (< 10 kDa). We have shown that FI fractions contain only low-molecular-weight molecules, mainly sugar molecules and small amounts of lower molecular weight phenolic compounds (Toivanen *et al.* 2009; Toivanen *et al.* 2010). Carbohydrate receptors mediate the adhesion of *S. pneumoniae* to respiratory epithelial cells and pneumococcal adherence can be blocked by oligosaccharides (Bartehilson *et al.*, 1998; Andersson *et al.*, 1986) and by fractions of milk (Andersson *et al.*, 1986). In our previous microtiter well assay, pneumococcal adherence to cranberry and bilberry juice FI fractions was not due to sugar molecules (Toivanen *et al.*, 2010). It is known that proanthocyanidins from cranberry juice can prevent the adhesion of oligosaccharide α -Gal (1 \rightarrow 4) β -Gal recognizing adhesins in *E. coli* (Foo *et al.*, 2000a) and adhesion of *Neisseria meningitidis* pili can be blocked by milk oligosaccharides (Hakkarainen *et al.*, 2005) or by berry fractions containing phenolic components (Toivanen *et al.*, 2009). Here as well the phenolic compounds, such as proanthocyanidins and anthocyanins of the berry juice fractions (Toivanen *et al.*, 2009), could be the active molecules against the pneumococcal adherence.

Anti-adhesion activity of the water subfractions prepared from the berry juice FI fractions was also tested (Fig. 2). Adhesion inhibition achieved plateau in very low concentrations of the subfractions (1 μ g/mL of cranberry, 5 μ g/mL of bilberry, 15

259 $\mu\text{g/mL}$ of crowberry). The best activity was found with cranberry and bilberry.
260 Cranberry juice subfraction inhibited significantly the pneumococcal adhesion at the
261 highest concentration tested ($50 \mu\text{g/mL}$); the adhesion inhibition was found to be 38 %
262 (Fig. 2A). As a comparison, potent anti-adhesion activity against *E. coli* was found from
263 ethyl acetate extracts of Sephadex LH20-purified cranberry proanthocyanidins at a
264 concentration of $75 \mu\text{g/mL}$ (Foo *et al.*, 2000a). The adhesion inhibition activity of
265 bilberry with $50 \mu\text{g/mL}$ concentration was found to be 37 % and it was not significant
266 (Fig. 2B). Crowberry had the lowest activity; anti-adhesion activity was 19 % (50
267 $\mu\text{g/mL}$) (Fig. 2C).

268 Subfractions were prepared from juice FI fractions by SPE subfractionation in order to
269 extract the active phenolic compounds from FI fractions (Toivanen *et al.*, 2009; Sun *et*
270 *al.*, 2006). Adhesion inhibition activity of the cranberry subfraction was significant, but
271 it was lower than adhesion inhibition caused by the cranberry FI fraction. The detected
272 reduction in activity of the FI subfractions against the adhesion of *S. pneumoniae* may
273 result from loss of a combination of different phenolic molecules and/ or combination
274 with sugars or loss of a certain type of proanthocyanidins (Foo *et al.*, 2000b; Määttä-
275 Riihinen *et al.*, 2005) present in FI berry juice fractions and needed for the adhesion
276 inhibition. Detailed structural elucidation of the active phenolic molecules and the
277 possible combination of different molecules participating in the inhibition remains to be
278 carried out in future.

279 **Antimicrobial Activity of Berry Juice FI Fractions and Subfractions against *S.***
280 ***pneumoniae*.** Berries and especially their polyphenols are known to inhibit the growth
281 of many human pathogens (Cavanagh *et al.*, 2003; Matsushima *et al.*, 2008;
282 Puupponen-Pimiä *et al.*, 2005; Puupponen-Pimiä *et al* 2001). In the present study,

cranberry, bilberry, and crowberry juice FI fractions were found to possess high antimicrobial activity against *S. pneumoniae* (Fig. 3). The highest concentration used in antimicrobial tests (~86 mg/g) was extremely effective and the growth of *S. pneumoniae* was inhibited totally by all FI fractions. At the concentration of 3.5 mg/g cranberry juice fraction was still effective with nearly 100 % growth inhibition, but for bilberry and crowberry juice fractions the bacterial survival was 60 % and 39 %, respectively. At the lowest concentration tested (1.7 mg/g), the bacterial survival of all the berry juice FI fractions was found to be between 55–60 %. Subfractions did not have an effect on pneumococcal growth (data not shown). Bacterial survival was between 90–100 % for all subfraction concentrations, except for 50 µg/mL of bilberry juice subfraction (75 %). Both anti-adhesion and antimicrobial effects were minor after subfractionation when compared to anti-adhesion and antimicrobial effects of the F1 berry juice fractions.

Our findings showed that small molecular size FI fractions from cranberry, bilberry, and crowberry juices had significant effect on growth of *S. pneumoniae*. In addition, cranberry juice FI fraction inhibited significantly binding of *S. pneumoniae* to human bronchial cells, while the FI fractions from bilberry and crowberry juices had a lower anti-adhesion effect. Compared to significant anti-adhesion activity of cranberry juice FI fraction, the level of the anti-adhesion activity of the FI phenolic subfraction was substantially lower.

Here we examined adhesion inhibition in cell culture conditions by using excess amount of pneumococcal bacteria. The anti-adhesion activity was achieved with the cranberry juice FI fraction at the concentration of 8.7 mg/g of soluble solids, which contain small amount of polyphenols (Toivanen *et al.*, 2009; Toivanen *et al.*, 2010). The cranberry polyphenolic subfraction had inhibitory activity at the concentration of 5 mg/100ml.

307 The polyphenol concentration in cranberries is high, for example concentration of
308 proanthocyanidins is about 400mg/100g (Hellström *et al.*, 2009). Thus in the
309 physiological conditions anti-adhesion may be achieved with reasonable amount of
310 cranberry juice. However, without *in vivo* trials it is difficult to estimate the inhibitory
311 effect and the amount of cranberry juice needed for the anti-adhesion.

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

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Table 1. *In vitro* and *in vivo* anti-adhesion activity of cranberry (*Vaccinium 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

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

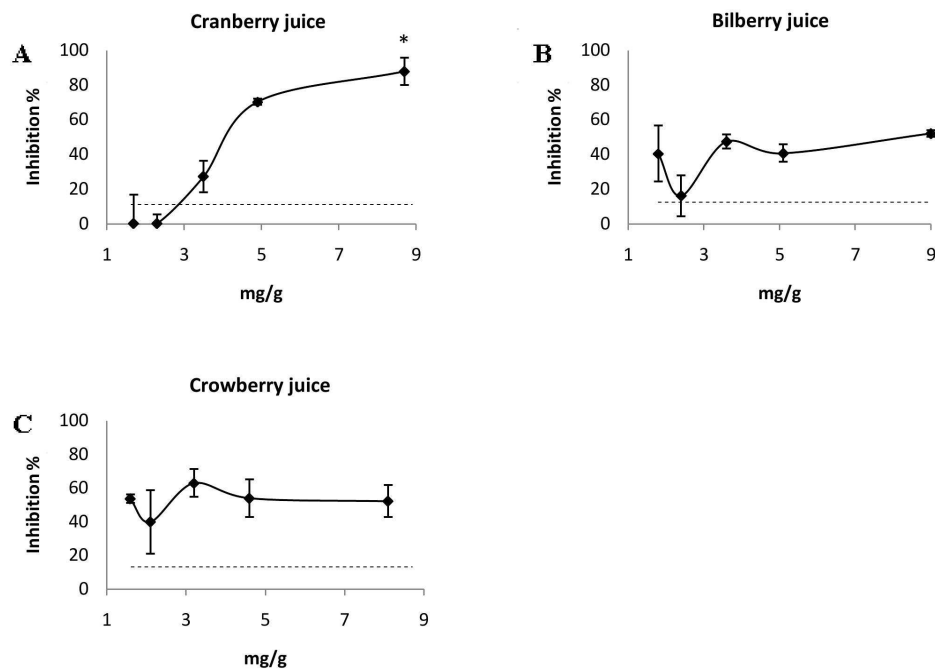
FIGURE LEGENDS

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

Figure 2. Inhibition of adhesion (mean \pm SEM, $n = 6$) of *S. pneumoniae* to Calu-3 human bronchial epithelial cell line by berry juice subfractions of **A)** cranberry juice, **B)** bilberry juice, and **C)** crowberry juice. Inhibition of adhesion expressed as the percentage of bacterial control. Apple juice FI fraction with constant concentration 11 mg/g was used as negative control (dotted line). * $P < 0.05$ against the bacterial control.

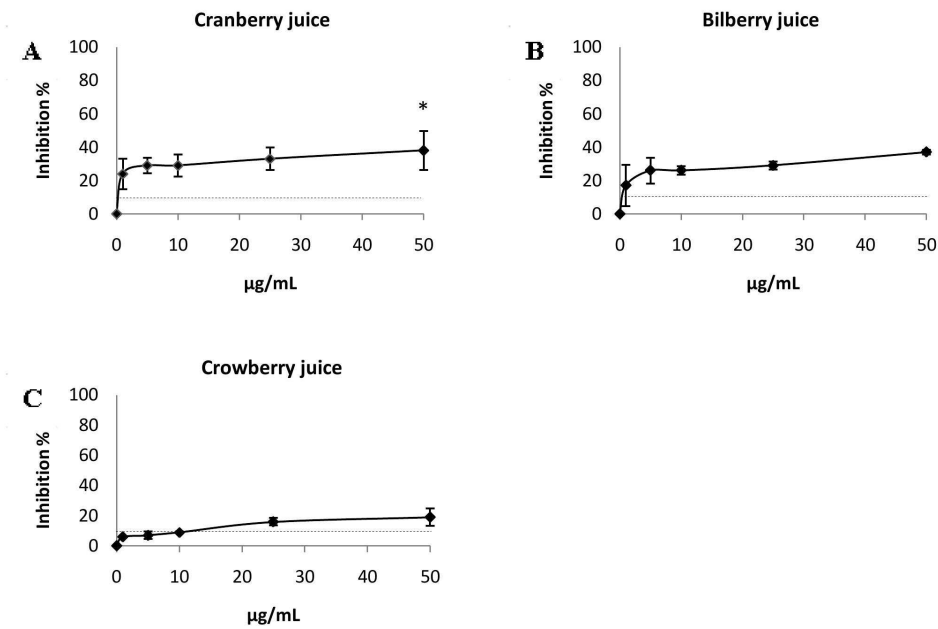
Figure 3. Antimicrobial activity of FI berry juice fractions (< 10 kDa) of cranberry, bilberry, and crowberry. Bacterial survival compared to control, mean \pm SEM of three experiments. Bacterial survival with Ampicillin (100 μ g/mL) was 5 %. * $P < 0.05$ against the bacterial control.

Huttunen S, Figure 1.



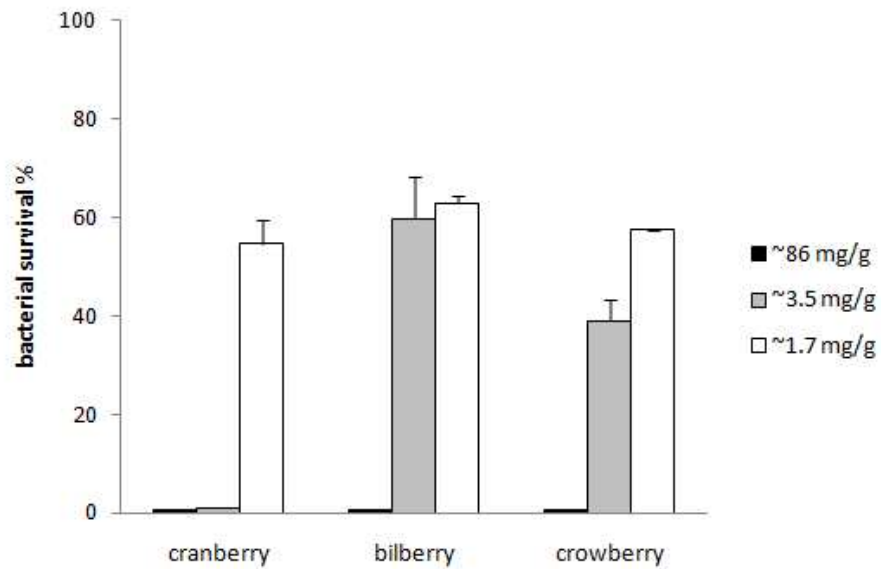
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Huttunen S, Figure 2.



180x143mm (300 x 300 DPI)

Huttunen S, Figure 3



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