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Some New Findings Regarding the Antiadhesive Activity of Cranberry Phenolic Compounds and Their Microbial-Derived Metabolites against Uropathogenic Bacteria

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ABSTRACT: Findings concerning the antiadhesive activity of cranberry phenolic compounds and their microbial-derived metabolites against Gram-negative (*Escherichia coli* ATCC 53503 and DSM 10791) and Gram-positive (*Enterococcus faecalis* 04-1) bacteria in T24 cells are reported. A-Type procyanidins (A2 and cinnamtannin B-1) exhibited antiadhesive activity (at concentrations ≥250 μM), a feature that was not observed for B-type procyanidins (B2). The metabolites hippuric acid and α-hydroxyhippuric acid also showed effective results at concentrations ≥250 μM. With regard to conjugated metabolites, sulfation seemed to increase the antiadhesive activity of cranberry-derived metabolites as 3-(3,4-dihydroxyphenyl)propionic acid 3-*O*-sulfate presented active results, unlike its corresponding nonsulfated form. In contrast, methylation decreased antiadhesive activity as 3,4-dihydroxyphenylacetic acid was found to be active but not its corresponding methylated form (4-hydroxy-3-methoxyphenylacetic acid). As a whole, this work sustains the antiadhesive activity of cranberry-derived metabolites as one of the mechanisms involved in the beneficial effects of cranberries against urinary tract infections.

KEYWORDS: *cranberry, microbial-derived phenolic metabolites, urinary tract infections (UTI), uropathogens, bacteria adherence, bladder cells*

INTRODUCTION

Urinary tract infections (UTI) are the most pervasive of bacterial infections and represent a large economic and medical burden worldwide. Antibiotics are generally prescribed as treatment against UTI, although they can have adverse side effects, including the development of antimicrobial resistance and reduction of beneficial intestinal bacteria.[1] As a natural alternative, cranberry (*Vaccinium macrocarpum*) has been widely recommended in the prevention and treatment of UTI prophylaxis.[2,3] Among other components, cranberry is rich in polyphenols, particularly proanthocyanidins, anthocyanidins, and flavonols, together with phenolic acids and benzoates.[4] Although many epidemiological and intervention studies have proved the efficacy of cranberry products in UTI prophylaxis,[2,3] others have shown mixed results.[5,6] One of the last meta-analyses concerning this topic[7] reported a large interindividual variability in cranberry efficiency against UTI; it also concluded that patients at some risk of these infections were more susceptible to the beneficial effects of cranberry consumption.[7]

Among other possible mechanisms behind the protective effects of cranberry against UTI is the capacity of cranberry polyphenols to act as antiadhesive agents in preventing/inhibiting the adherence of pathogens to uroepithelial cell receptors,[8] which appears to be a major step in the pathogenesis of these infections.[9] One of the very first studies about this topic reported the in vitro inhibition of the adherence of uropathogenic P-fimbriated *Escherichia coli* by procyanidin A2 and other cranberry A-type procyanidins.[10] Numerous studies have further proven the antiadhesive activity of different cranberry phenolic compounds/fractions/extracts against strains of uropathogenic *E. coli* (UPEC) using cell culture methodologies.[11−13] Experiments on cell cultures have also evidenced antiadhesive activity against UPEC of urine collected after the consumption of cranberry products.[5,14−16] But it is unlikely that the compounds responsible for the antiadhesive activity of urine collected after cranberry consumption would be the original forms present in the original food. It is known that polyphenols, and especially proanthocyanidins, are poorly absorbed in the small intestine, and reach the colon where they are catabolized by the gut microbiota to give rise to a great battery of phenolic metabolites that can be absorbed and further secreted in urine.[17] In fact, in a recent study, Peron and co-workers[18] demonstrated that the antiadhesive properties of urine after cranberry consumption could not be ascribable to the direct effect of A-type proanthocyanidins because their levels in urine were in the low-concentration range (<nM).[18] Therefore, the question that remains to be answered is what is the compound(s) present in urine after cranberry consumption that prevents/inhibits effective adherence of uropathogens to uroepithelial cells. Just recently, Feliciano and co-workers published valuable data regarding the absorption, metabolism, and excretion of cranberry polyphenols[19,20] that could help in the resolution of this cranberry antiadhesive activity puzzle.

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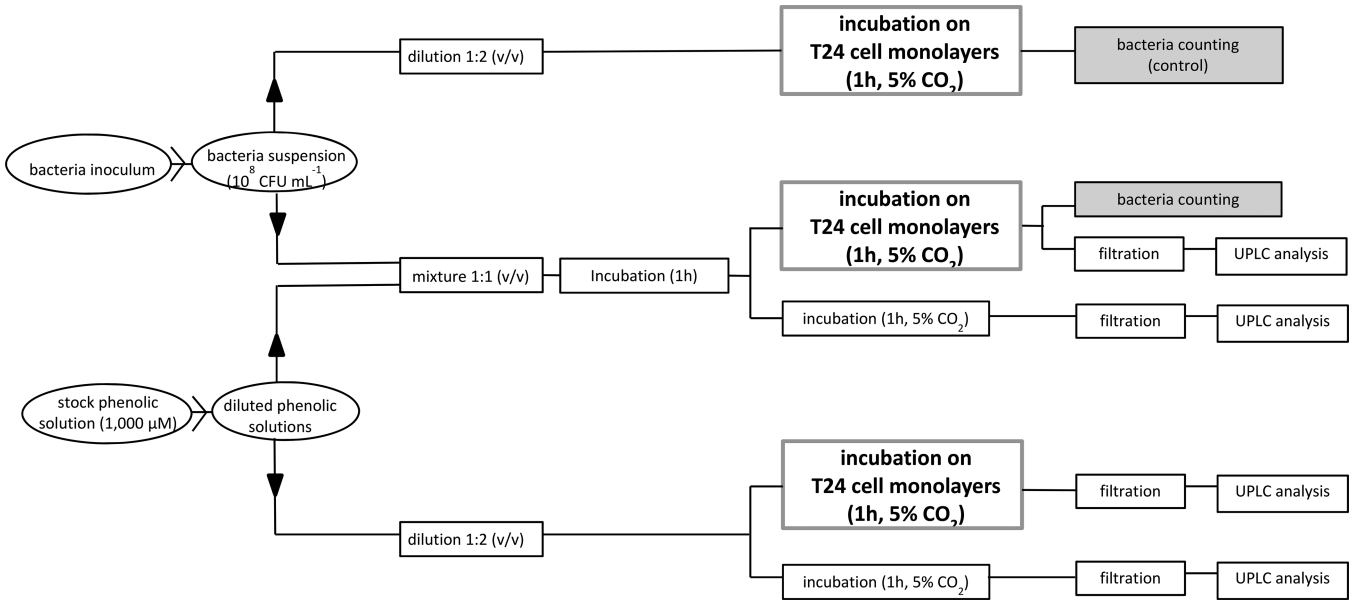

Figure 1. Schematic representation of the in vitro antiadherence assays carried out in this study.

After cranberry juice consumption by healthy men, a total of 60 phenolic-derived metabolites were quantified in plasma and urine, including hippuric acids, pyrogallol sulfates, valerolactone, benzoic acids, phenylacetic acids, and flavonol glucuronides, as well as cinnamic acid sulfates and glucuronides.[19] Although some of these compounds were tested for antiadhesive activity such as phenolic acids[21] and valerolactones,[22] no data are available for the majority of them, particularly for compounds found in high concentrations in human fluids, such as hippuric and α-hydroxyhippuric acids. On the other hand, the majority of the in vitro studies about antiadhesive activity against uropathogens have focused on P-fimbriated *E. coli* strains. However, Gram-positive bacteria, including some staphylococcal and enterococcal species, also seem to be implicated in the etiopathogenesis of UTIs,[23] particularly among the elderly population, pregnant women, and people with any other risk factor for UTIs.[24] Therefore, the goal of this paper has been to assess the effect of cranberry phenolic compounds and their metabolites on bacteria adherence to bladder uroepithelial cells, not only for Gram-negative (*E. coli* ATCC 53503 and DSM 10791) but also for Gram-positive (*Enterococcus faecalis* 04-1) species. Among the compounds tested, we have included cranberry flavan-3-ols (both A-type and B-type proanthocyanidins) and phenolic metabolites exhibiting a high abundance in urine after cranberry consumption (i.e., hippuric and α-hydroxyhippuric acids) as well as other conjugated metabolites such as sulfate and methyl derivatives [i.e., 3-(3,4-dihydroxyphenyl)propionic acid 3-*O*-sulfate and 4-hydroxy-3-methoxyphenylacetic acid]. Additionally, and to ensure that the compounds tested were the forms responsible for the observed effects in the cells, their stability after bacteria adherence assays was evaluated through ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) analysis.

MATERIALS AND METHODS

Phenolic Compounds. A total of nine phenolic compounds were used in this study. Procyanidin A2 [epicatechin-(4β→8, 2β→*O*→7)-epicatechin], procyanidin B2 [epicatechin-(4β→8)-epicatechin], and cinnamtannin B-1[epicatechin-(4β→8, 2β→*O*→7)-epicatechin-(4α→8)-epicatechin] were purchased from Extrasynthèse (Genay, France); hippuric acid (benzoylaminoacetic acid), α-hydroxyhippuric acid [(benzoylamino)hydroxyacetic acid), homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid), and 3,4-dihydroxyphenylacetic acid were obtained from Sigma-Aldrich Chemical Co. (St. Louis); and 3-(3,4-dihydroxyphenyl)propionic acid 3-*O*-sulfate sodium and its free acid [3-(3,4-dihydroxyphenyl)propionic acid or hydrocaffeic acid] were acquired from Toronto Research Chemicals, Inc. (Toronto, Canada).

Stock standard solutions were prepared in Dulbecco's phosphate-buffered saline solution (DPBS, Lonza Walkersville, Inc., USA) by weighing individual phenolic compounds to achieve a starting concentration of 1000 μM, and assayed solutions were prepared via serial dilutions.

Uropathogenic Strains and Growing Conditions. Two Gram-negative bacteria (*E. coli* ATCC 53503 and *E. coli* DSM 10791) and a Gram-positive bacteria (*E. faecalis* 04-1) were selected for the study. The UPEC ATCC53503 strain that proved to give rise to P fimbriae was grown in Tryptic Soy broth (TSB; Scharlau, Barcelona, Spain). The *E. coli* 10791 DSM 10791 strain that harbors the fimH, papGII/III, focG, sfaS, and hlyA genes[25] was grown in Luria broth (LB; Laboratorios Conda, Madrid). The *E. faecalis* 04-1 strain was a clinical isolate kindly donated by Prof. Juan Miguel Rodriguez (Complutense University of Madrid, Madrid) and was also grown in TSB. These strains were kept frozen at −70 °C in a sterilized mixture of glycerol in a culture medium (20% v/v). The contents of thawed cryovials were added to the medium and grown overnight at 37 °C. Overnight cultures were harvested by centrifugation (10 000g, 10 min, 4 °C) and resuspended in DPBS at concentrations of about 10^6 and 10^8 CFU mL^{-1} for antibacterial and adherence assays, respectively.

Antibacterial Activity Assays. The antibacterial assays were performed using the method of Garcia-Ruiz et al.[26] Inhibition of the growth of *E. coli* ATCC 53503, *E. coli* DSM 10791, and *E. faecalis* 04-1 by the tested phenolic compounds was determined by the microtiter dilution method, using serial double dilutions of the compounds and initial bacteria inocula (10^6 CFU/mL). Bacteria growth was determined by reading the absorbance at 600 nm. Growth inhibitory activity was expressed as a mean percentage (%) of growth inhibition with respect to a control without the phenolic compounds. The assays were conducted in triplicate.

Cell Culture. We used T24 cells (ATCC HTB4) as they have been shown to be similar to primary human bladder epithelial cells.[27] T24 bladder cells were grown and maintained in McCoy's 5A medium (Sigma-Aldrich, St. Louis, USA) supplemented with 10% (v/v) fetal bovine serum at 37 °C in an atmosphere of 5% CO_2/95% air at

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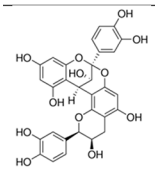
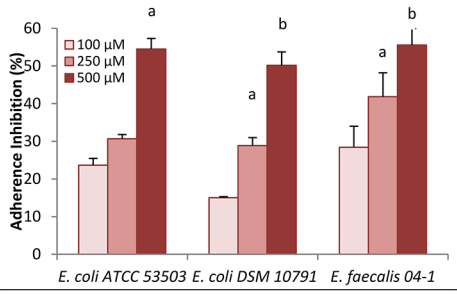
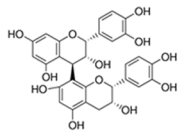
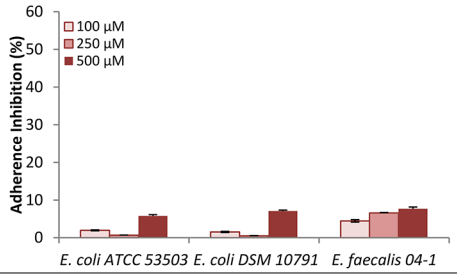
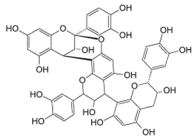
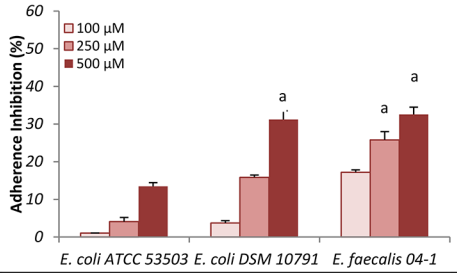
Compound	C (μM)	% of Bacteria Adherence: *E. coli* ATCC® 53503™ Mean±SD	*P* value	*E.coli* DSM 10791 Mean±SD	*P* value	*E. faecalis* 04-1 Mean±SD	*P* value	% of Adherence Inhibition
Procyanidin A2								
	100	8.02±0.60	0.450	7.90±0.15	0.201	6.60±1.30	0.188	
	250	7.29±0.18	0.485	6.60±0.48	0.159	5.36±0.81	**0.042**	
	500	4.79±0.25	**0.041**	4.64±0.33	**0.016**	4.09±0.32	**0.012**	
Procyanidin B2								
	100	9.22±0.55	0.813	9.61±0.96	0.644	8.76±0.53	0.492	
	250	9.34±0.49	0.344	9.41±0.80	0.780	8.57±0.41	0.304	
	500	8.86±0.52	0.275	8.76±0.16	0.429	8.47±0.12	0.059	
Cinnamtannin B1								
	100	9.02±0.41	0.479	8.87±1.49	0.729	7.81±0.17	0.080	
	250	8.55±0.63	0.347	7.72±0.30	0.063	7.00±0.43	**0.017**	
	500	7.73±0.57	0.288	6.32±0.46	**0.008**	6.36±0.88	**0.038**	

Figure 2. Antiadhesive activity (% of bacteria adherence and % of adherence inhibition) of procyanidin A2, procyanidin B2, and cinnamtannin B-1 against *E. coli* ATCC 53503, *E. coli* DSM 10791, and *E. faecalis* 04-1 in T24 cells. For the % of bacteria adherence, *P* values express differences from the control (no compound present) for the corresponding uropathogenic bacteria strain. For the % of adherence inhibition, values with different letters (a, b) indicate statistically significant differences ($P < 0.01$) among compound concentrations that were found to significantly decrease the % of bacteria adherence.

constant humidity. For the experiments, cells were seeded in 24-well tissue plates and grown for approximately 24 h to enable cell attachment and to obtain confluent cell monolayers.

Cytotoxicity Assay/Cell Viability. The cytotoxicity of the tested phenolic compounds against T24 cells was evaluated using the colorimetric 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay,[28] which is based on the reduction of the dye MTT to formazan, an insoluble intracellular blue product, by cellular dehydrogenases. Briefly, T24 cells seeded in a 96-well plate the previous day (5×10^5 cells/well) were washed with DPBS solution to eliminate antibiotics, overlaid with 0.5 mL of phenolic solution, and incubated for 24 h at 37 °C under 5% CO_2 atmosphere. Then the supernatant was removed, the monolayer was washed with DPBS, and MTT was added to each well (0.5 mg/mL) and the cell culture incubated for 3 h at 37 °C. Absorbance was measured at 570 nm with a plate reader (Multiskan FC Thermo Fisher Scientific, New Hampshire, USA). The absorbance ratio between the cell culture treated with the phenolic compound and the untreated control × 100 represented cell viability (percentage of control). Assays were conducted in triplicate.

Adherence Assay. The in vitro antiadherence assays of uropathogens by phenolics are schematically shown in Figure 1. Uropathogenic bacteria overnight cultures (10^8 CFU/mL) were incubated with the same volume of the phenolic solution (200, 500, and 1000 μM) for 1 h, at 37 °C, in agitation (180 rpm) (bacteria + phenolic compound mixtures). Therefore, the final concentration of phenolics in these mixtures were 100, 250, and 500 μM. The selection of these concentration values was based on previous studies with other phenolic compounds[21,22] in which we found significant antiadhesive activity ($p < 0.01$) at concentrations higher than 100 μM. Then confluent T24 cell monolayers (5×10^5 cells/well) were washed with DPBS solution and overlaid with 0.5 mL of the uropathogenic bacteria preincubated with each phenolic compound or with DPBS (control) (Figure 1). Supernatants were collected after 1 h of incubation at 37 °C under 5% CO_2 atm, and an aliquot was immediately frozen (−80 °C) for further UHPLC MS/MS analysis. Cells and adhered bacteria were then detached using a 0.05% trypsin-ethylenediaminetetraacetic acid solution and sonicated in an ultrasonic sonication bath (3 pulses, 10 s on, 3 s off) at 40 kHz to recover bacteria associated with cells. Bacteria counts (CFU/mL) were carried out using the serial dilution plate method on TSA plates for *E. coli* strains and *Enterococcus* Selective Agar plates (Laboratorios Conda) for enterococci, as previously described.[21] The adherence percentage (%) was calculated as the number of adhered bacteria (CFU/mL) relative to the total number of bacteria added initially × 100. The percentage of inhibition by a phenolic compound/extract was calculated as $[1 - (\%\ \text{Adherence}_{\text{sample}}/\%\ \text{Adherence}_{\text{control}})] \times 100$. The bacteria + phenolic compound mixtures were subjected to

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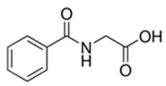
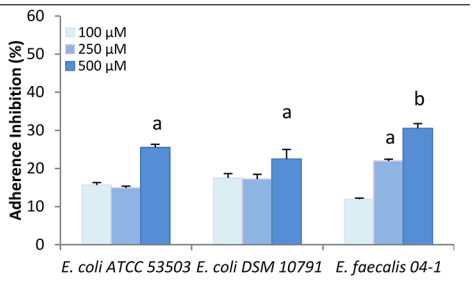
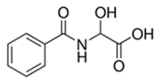
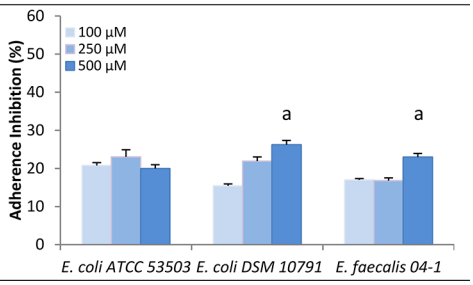
Compound	C (μM)	% of Bacteria Adherence: *E. coli* ATCC® 53503™ Mean±SD	*P* value	*E.coli* DSM 10791 Mean±SD	*P* value	*E. faecalis* 04-1 Mean±SD	*P* value	% of Adherence Inhibition
				Hippuric acid				
	100	7.44±0.32	0.067	7.90±0.53	0.133	8.96±0.09	0.054	
	250	7.51±0.24	0.054	7.93±0.59	0.150	7.94±0.18	**0.037**	
	500	6.57±0.21	**0.020**	7.42±0.84	**0.045**	7.07±0.20	**0.032**	
				α-Hydroxyhippuric acid				
	100	6.71±0.24	0.056	7.85±0.25	0.068	9.25±0.20	0.054	
	250	6.52±0.54	0.119	7.25±0.37	0.076	9.27±0. 40	0.133	
	500	6.78±0.32	0.074	6.85±0.29	**0.048**	8.58±0.36	**0.048**	

Figure 3. Antiadhesive activity (% of bacteria adherence and % of adherence inhibition) of hippuric acid and α-hydroxyhippuric acid against *E. coli* ATCC 53503, *E. coli* DSM 10791, and *E. faecalis* 04-1 in T24 cells. For the % of bacteria adherence, *P* values express differences from the control (no compound present) for the corresponding uropathogenic bacteria strain. For the % of adherence inhibition, values with different letters (a, b) indicate statistically significant differences ($P < 0.01$) among compound concentrations that were found to significantly decrease the % of bacteria adherence.

the same incubation but in the absence of T24 cells (Figure 1), and supernatants were collected and immediately frozen (−80 °C) for further UHPLC MS/MS analysis.

In parallel, the diluted phenolic solutions were incubated in the absence and presence of T24 cells as indicated above (Figure 1), and supernatants were collected and immediately frozen (−80 °C) for further UHPLC MS/MS analysis. All assays were conducted in triplicate.

UHPLC-DAD-ESI-TQ MS Targeted Analysis of Phenolic Metabolites. Supernatants from incubations of cell monolayers with phenolic compounds previously incubated or not with uropathogens as well as incubations of phenolic compounds in the absence and presence of uropathogens (Figure 1) were assayed for phenolic metabolites. Prior to their analysis, supernatants were filtered (0.22 μM, Symta, Spain) and 200 μL of sample was mixed with 50 μL of internal standard (4-hydroxybenzoic-2,3,5,6-tetradeutered acid) (IS) prepared in acetonitrile and 0.1% formic acid at a final concentration of 0.25 mg/mL. Then 2.0 μL of sample was injected into the chromatographic system. Each analysis was performed in duplicate.

Phenolic metabolites were analyzed using UHPLC-ESI-MS/MS following a method previously published.[29] A total of 54 phenolic compounds [3-(hydroxyphenyl)-propionic, hydroxyphenylacetic, hydroxycinnamic, hydroxybenzoic, and hydroxymandelic acids] were targeted.[29] All metabolites were quantified using the calibration curves of their corresponding standards, and the results were presented as the percentage (%) of loss in relation to the phenolic solution incubated in the absence of T24 cells and/or pathogens.

Statistical Analysis. A paired sample *t* test was used to evaluate whether the inhibition of adherence of uropathogenic strains to T24 uroepithelial cells in the presence of a phenolic compound was different ($P \leqslant 0.01$) from pathogen adherence in its absence (control). Also, one-way analysis of variance (ANOVA) post hoc comparison using Tukey's HSD test ($P \leqslant 0.01$) was used for comparing the values of inhibition (%) among those phenolic compounds that significantly decreased the percentage of adherence with respect to the control. The IBM SPSS program (v.22) for Windows was used for data processing.

RESULTS

Effects of Cranberry Phenolic Compounds on the Adherence of Gram-Negative and Gram-Positive Uropathogens to Bladder Epithelial Cells. First, any antimicrobial or cytotoxic activity of the tested compounds at the concentrations used (100, 250, and 500 μM) was discarded (data not shown). Initial assays also showed that the capacity of *E. coli* ATCC 53503 (9.31 ± 0.63% of total bacteria), *E. coli* DSM 10791 (9.34 ± 0.31%), and *E. faecalis* 04-1 (10.3 ± 0.75%) to adhere to T24 cells was similar to that previously reported (9.82%, 9.01%, and 11.3%, respectively).[30]

The effect of the three flavan-3-ols present in cranberry (procyanidins A2 and B2 and cinnamtannin B-1) on the adherence of both Gram-negative (*E. coli* ATCC 53503, *E. coli* DSM 1079) and Gram-positive (*E. faecalis* 04-1) bacteria is reported in Figure 2. Significant differences ($P \leqslant 0.01$) in the percentage (%) of bacteria adherence from the control (no phenolic compound added) were indicated for each tested concentration of phenolic compound (100, 250, and 500 μM). Only A-type procyanidins (A2 and cinnamtannin B-1) produced significant decreases in the percentage of bacteria adhered to uroepithelial cells, particularly at 250 μM for procyanidin A2 against *E. coli* DSM 1079 and *E. faecalis* 04-1 and for cinnamtannin B-1 against *E. faecalis* 04-1, and at 500 μM for both compounds against the three strains tested, except for cinnamtannin B-1 against *E. coli* ATCC 53503 (Figure 2). The percentage (%) of adherence inhibition rose to 28.4%, 41.9%, and 55.8% for procyanidin A2 and to 17.2%, 25.8%, and 32.65% for cinnamtannin B-1 respectively for *E. coli* ATCC

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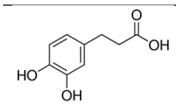
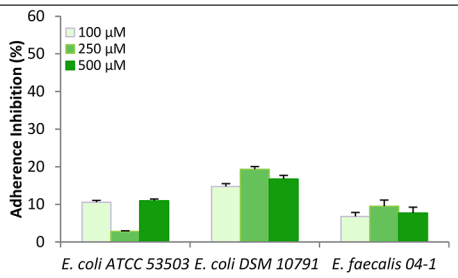
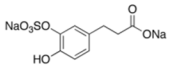
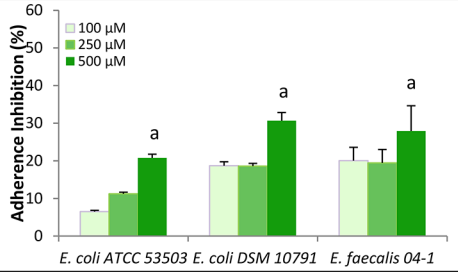
Compound	C (μM)	% of Bacteria Adherence: *E. coli* ATCC® 53503™ Mean±SD	*P* value	*E. coli* DSM 10791 Mean±SD	*P* value	*E. faecalis* 04-1 Mean±SD	*P* value	% of Adherence Inhibition
				3-(3,4-dihydroxyphenyl)propionic acid				
	100	8.19±0.37	0.062	7.91±0.41	0.112	9.65±1.51	0.596	
	250	8.93±0.49	0.117	7.49±0.29	0.074	9.38±1.64	0.556	
	500	8.18±0.35	0.105	7.74±0.45	0.097	9.56±1.93	0.699	
				3-(3,4-dihydroxyphenyl)propionic acid 3-O-sulfate sodium salt				
	100	8.52±0.43	0.112	7.40±0.28	0.103	8.83±0.58	0.082	
	250	8.09±0.31	0.082	7.41±0.37	0.117	8.97±1.32	0.065	
	500	7.22±0.34	**0.034**	6.32±0.19	**0.026**	8.03±1.98	**0.012**	

Figure 4. Antiadhesive activity (% of bacteria adherence and % of adherence inhibition) of 3-(3,4-dihydroxyphenyl)propionic acid (dihydrocaffeic acid) and 3-(3,4-dihydroxyphenyl)propionic acid 3-*O*-sulfate sodium salt against *E. coli* ATCC 53503, *E. coli* DSM 10791, and *E. faecalis* 04-1 in T24 cells. For the % of bacteria adherence, *P* values express differences from the control (no compound present) for the corresponding uropathogenic bacteria strain. For the % of adherence inhibition, values with different letters (a,b) indicate statistically significant differences ($P < 0.01$) among compound concentrations that were found to significantly decrease the % of bacteria adherence.

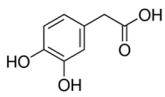
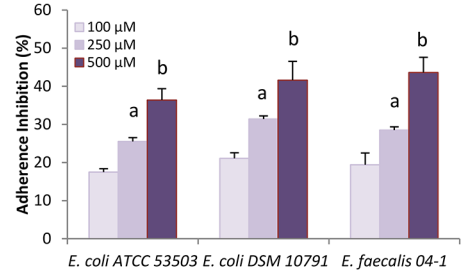
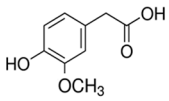
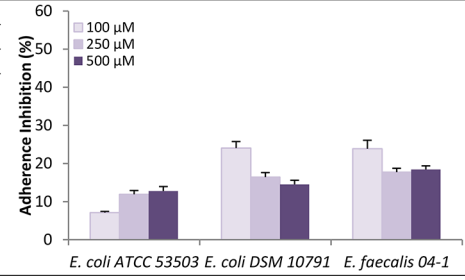
Compound	C (μM)	% of Bacteria Adherence: *E. coli* ATCC® 53503™ Mean±SD	*P* value	*E.coli* DSM 10791 Mean±SD	*P* value	*E. faecalis* 04-1 Mean±SD	*P* value	% of Adherence Inhibition
				3,4-Dihydroxyphenylacetic acid				
	100	7.63±0.38	0.091	7.02±0.49	0.099	8.48±1.35	0.174	
	250	6.89±0.27	**0.043**	6.11±0.16	**0.013**	7.50±0.23	**0.049**	
	500	5.89±0.48	**0.021**	5.21±0.62	**0.047**	5.90±0.54	**0.038**	
				4-Hydroxy-3-methoxyphenylacetic acid				
	100	9.35±0.40	0.375	7.59±0.54	0.058	7.68±0.72	0.116	
	250	8.86±0.74	0.318	8.36±0.60	0.096	8.30±0.47	0.094	
	500	8.78±0.81	0.372	8.55±0.60	0.109	8.23±0.43	0.082	

Figure 5. Antiadhesive activity (% of bacteria adherence and % of adherence inhibition) of 3,4-dihydroxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid) against *E. coli* ATCC 53503, *E. coli* DSM 10791, and *E. faecalis* 04-1 in T24 cells. For the % of bacteria adherence, *P* values express differences from the control (no compound present) for the corresponding uropathogenic bacteria strain. For the % of adherence inhibition, values with different letters (a, b) indicate statistically significant differences ($P < 0.01$) among compound concentrations that were found to significantly decrease the % of bacteria adherence.

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J. Agric. Food Chem. 2019, 67, 2166−2174

53503, *E. coli* DSM 1079, and *E. faecalis* 04-1, at the concentration of 500 μM (Figure 2).

Effects of Cranberry-Derived Metabolites on the Adherence of Gram-Negative and Gram-Positive Uropathogens to Bladder Epithelial Cells. As seen for procyanidin A2 and cinnamtannin B-1, an inhibitory concentration-dependent effect on the adherence of *E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04-1 to T24 cells was observed for hippuric and α-hydroxyhippuric acids (Figure 3). Hippuric acid resulted in being significantly effective ($P \leqslant 0.01$) against the three uropathogenic strains at the concentration of 500 μM (inhibition % of 25.5, 22.5, and 30.5 for *E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04-1, respectively), whereas only the adherence of *E. faecalis* 04-1 was significantly reduced by this compound at the concentration of 250 μM (inhibition % of 22.0) (Figure 3). α-Hydroxyhippuric reduced significantly only the adherence of *E. coli* DSM 1079 (inhibition % of 26.2%) and *E. faecalis* 04-1 (inhibition % of 23.0%) at the concentration of 500 μM (Figure 3).

The sulfate form of 3-(3,4-dihydroxyphenyl)propionic acid [i.e., 3-(3,4-dihydroxyphenyl)propionic acid 3-*O*-sulfate sodium] also showed antiadhesive activity against the three uropathogenic strains tested (Figure 4). At the concentration of 500 μM, the percentages (%) of adherence inhibition rose to 20.8%, 30.7%, and 27.9% for *E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04-1, respectively (Figure 4). In contrast, the free acid form [3-(3,4-dihydroxyphenyl)propionic acid] did not lead to significant effects ($P > 0.01$) on the adherence of uropathogenic strains, at least at concentrations of up to 500 μM (Figure 4).

The microbial-derived metabolite of flavan-3-ols, 3,4-dihydroxyphenylacetic acid, showed a clear concentration-dependent effect on the adherence of the three uropathogenic strains tested, with significant decreases ($P \leqslant 0.01$) being observed at the concentrations of 250 and 500 μM (Figure 5). At the concentration of 500 μM, the percentage of adherence inhibition rose to 36.4%, 41.6%, and 43.6% for *E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04-1, respectively (Figure 5). However, its methyl derivative, homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid), showed no significant antiadhesive activity, at least at concentrations of up to 500 μM (Figure 5).

In parallel to the bacteria adherence evaluation, the stability of the compounds during the assays was determined through UPLC-MS analysis of the supernatants after incubation of the compounds in the presence and absence of pathogenic bacteria and bladder epithelial cells (Figure 1). Among the 54 phenolic metabolites targeted, only the initial compounds used were detected after their incubation with pathogenic bacteria and/or bladder epithelial cells (see Table 1 for chromatographic and spectrometric data of the compounds). In other words, there seemed to be no sign of bacterial and/or cellular metabolites derived from the phenolic compounds tested after their incubation with bacteria and/or cells. Moreover, the concentration of the compounds after their incubation with the cells was not significantly different from that of the compounds incubated on their own (% of loss of between −18.7 and 19.2), thereby confirming the stability of the compounds during their incubation with cells. However, the concentration of the compounds in the supernatants after their incubation with the pathogenic bacteria (following or not incubation with the cells) was significantly lower than that of the compounds incubated on their own (perecentage of loss of up to 57.6). To investigate this fact, the residues retained in the filter (0.22 μM) after filtration of the bacteria + phenolic mixtures (Figure 1) were redissolved and analyzed by UHPLC-MS. Some traces of the phenolic compound were found, indicating that there could be certain retention of the compound inside the bacteria mass during filtration, which could explain these latter results.

Table 1. Phenolic Metabolites Detected in the Supernatants from the In Vitro Adherence Assays of *E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04-1 on Bladder Epithelial Cells

metabolite	retention time (min)	MRM transition (m/z)	cone voltage (V)	collision energy (V)
hippuric acid	5.26	178 > 134	30	10
α-hydroxyhippuric acid	3.92	194 > 73	25	10
3-(3,4-dihydroxyphenyl) propionic acid 3-*O*-sulfate	5.06	261 > 181	30	15
3-(3,4-dihydroxyphenyl) propionic acid	5.25	181 > 137	32	12
4-hydroxy-3-methoxyphenylacetic acid	6.04	181 > 137	27	8
3,4-dihydroxyphenylacetic acid	4.18	167 > 123	20	12

DISCUSSION

Consumption of cranberry (*Vaccinium macrocarpon*) has been widely recommended for prophylaxis against urinary tract infections (UTI),[2,3] although the mechanisms behind these effects have not been fully revealed.[8] This study points out some new features regarding the antiadhesive activity of cranberry phenolic compounds and their microbial-derived metabolites against uropathogens. First, this paper has demonstrated that the adherence of enterococcal strains (Gram-positive) such as *E. faecalis* to bladder uroepithelial cells was also inhibited after incubation of the bacteria with certain phenolic compounds. In fact, among the three strains used, sensitivity against phenolic compounds was in the order *E. faecalis* 04-1> *E. coli* DSM 10791 > *E. coli* ATCC 53503. For example, cinnamtannin B-1 and hippuric acid were active against *E. faecalis* 04-1 at 250 μM, but not against *E. coli* DSM 10791. Although most studies about the antiadhesive activity of cranberry phenolic compounds have subscribed to P-fimbriated *E. coli* strains, some studies have also proven in vitro antiadhesive activity against other Gram-negative bacteria such as *Proteus mirabilis*, a species that has been associated with infections in catheterized patients or those having structural anomalies of the urinary tract.[31] Recently, *E. faecalis* species have received much attention because of their resistance to aminoglycosides and vancomycin.[32] In the same vein, Wojnicz and co-workers[33] explored the effects of a cranberry extract on the growth, virulence factors, and biofilm formation of *E. faecalis* urine isolates. As far as we know, this paper reports, for the first time, data concerning the activity of cranberry phenolic compounds on the adherence of *E. faecalis* to uroepithelial cells. Together, the results suggest a wide activity spectrum against uropathogenic bacteria by cranberry phenolic compounds and their microbial-derived metabolites, at least in in vitro conditions.

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Another new finding reported in this paper concerns the antiadhesive activity of flavan-3-ols, particularly of trimer cinnamtannin B-1 [epicatechin-(4β→8, 2β→O→7)-epicatechin-(4α→8)-epicatechin]. To the best of our knowledge, this is the first time that an A-type procyanidin molecule different from A2 [epicatechin-(4β→8, 2β→O→7)-epicatechin] has been tested for in vitro antiadhesive activity against uropathogens. Both A2 and cinnamtannin B-1 significantly inhibited the adherence of three strains of bacteria tested (*E. coli* ATCC 53503, *E. coli* DSM 1079, and *E. faecalis* 04−1) at concentrations ≥250 μM, a feature that was not observed for procyanidin B2 [(−)-epicatechin-(4β-8)-(−)-epicatechin]] in line with previous reports.[11,21] Therefore, it could be suggested that a certain structure−activity relationship for A-type procyanidins as dimers (i.e., A2) led to higher inhibition percentages (%) than trimers (i.e., cinnamtannin B-1) for the uropathogenic stains tested. The lower number of A-type linkages and/or the greater molecular volume might be the chemical structural features that explained the inverse relationship between the polymerization degree and the antiadherence activity of A-type procyanidins. But in any case, the antiadherence activity of A-type procyanidins is unlikely to be relevant in vivo as their concentration in urine is null or very low as a consequence of their extensive microbial metabolism in the gut.[18,19]

Following this reasoning, another achievement of this paper relates to the phenolic compounds known to be present in urine after cranberry consumption, such as hippuric acid (benzoylaminoacetic acid) and α-hydroxyhippuric acid [(benzoylamino)hydroxyacetic acid). Our results demonstrated that both acids exhibit antiadhesive activity in a dose-dependent manner (100−500 μM) against the three strains of bacteria, with hippuric acid being more active than α-hydroxyhippuric acid. Hippuric acid is known to be generated from protein and amino acid metabolism,[34] but it is also a common metabolite of many flavonoids after glycination of benzoic acid in the liver.[35] In fact, hippuric acid has been validated as a biomarker of overall flavonoid intake in specific populations including healthy adolescents.[36] With regard to cranberry consumption, hippuric and α-hydroxyhippuric acids have shown maximum excretion in 24 h urine amounts (69 717 nmol and 74 538 nmol, respectively) after the intake of cranberry juice (787 mg of polyphenols).[19] Therefore, our results suggested that the relatively high concentration of hippuric acids might be responsible, at least partially, for the antiadhesive activity of the urine samples collected after consumption of cranberry products.[5,14−16]

In regard to other minor metabolites present in urine after cranberry consumption, 3,4-dihydroxyphenylacetic acid and dihydrocaffeic acid 3-O-sulfate also proved to be active ($C \geq$ 250 μM) against bacteria adherence to uroepithelial cells for the three uropathogens studied. Their total amount excreted in urine after 24 h after the intake of cranberry juice (787 mg of polyphenols) were 1597 and 417 nmol respectively for 3,4-dihydroxyphenylacetic acid and dihydrocaffeic acid 3-O-sulfate.[19] In comparison to the nonconjugated forms, sulfation seemed to induce/increase antiadhesive activity as 3-(3,4-dihydroxyphenyl)propionic acid showed no significant anti-adhesive activity. This was in accordance with a previous study[22] that assayed the antiadhesive activity of 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and differently sulfated derivatives against UPEC to uroepithelial cells; all the compounds tested were able to significantly inhibit the adherence of UPEC at concentrations of 100 μM, but only 5-(3′-hydroxyphenyl)-γ-valerolactone-4′-O-sulfate inhibited significantly the adherence of UPEC to T24 cells when incubated at 50 μM.[22] In contrast, methylation impeded/decreased metabolite antiadhesive activity as 4-hydroxy-3-methoxyphenylacetic acid did not lead to significant effects on the adherence of uropathogenic strains, at least at the concentrations used in this study. As this is very preliminary data tested only with one compound of each class, more work is needed to confirm that sulfation may increase antiadhesion activities and that methylation may decrease it. Moreover, further studies in this subject should also consider the physiological concentration of the phenolic metabolites as well as the possible additive (and even synergistic) effects among the cranberry-derived phenolic metabolites and other metabolites present in the urine matrix.

The wide interindividual differences observed in the human urinary/plasmatic phenolic metabolite profile have led to the introduction of the concept of polyphenol "metabotypes" or polyphenol metabolizing phenotypes,[37] which groups subjects with similar capacity to metabolize dietary polyphenols. Some studies have shown subject clusterization according to the capacity to metabolize polyphenols from soy,[38] from pomegranate,[39] or from wine,[40] among other foods. It remains to be proven if the intake of cranberry polyphenols would also cluster subjects according to their phenolic metabolite profile in physiological fluids (urine, plasma, and feces). However, recent studies reporting a preliminary elucidation of urinary metabotypes concerning the metabolism of flavan-3-ols,[41] one of the main classes of polyphenols present in cranberry, make us hypothesize that there could be different phenolic metabotypes related to the production of gut microbial metabolites from cranberry polyphenols, and that these hypothetical metabotypes could be one of the factors putatively associated with the great variability observed in the efficacy of cranberry products.

Finally, in relation to the in vitro conditions used to evaluate the antiadhesive activity against uropathogens, the UHPLC-MS analysis carried out in this study ensured stability of the phenolic metabolites tested under the cell culture conditions and UHPLC-MS parameters used in this study. Moreover, there was no apparent cellular and/or bacterial metabolism of the phenolic compounds tested as no new compounds were detected. In a previous paper, the possible transformations of phenyl-γ-valerolactones in bladder epithelial cells infected or not with UPEC were evaluated.[22] While 5-(3,4-dihydroxyphenyl)-γ-valerolactone did not undergo any metabolic transformation, its sulfate derivatives underwent a certain metabolism by T24 cells that comprised the opening of the lactone ring and/or desulfation, the latter only to a very small extent (0.5−2%).[22] Compound stability and cell metabolism during cell culture assays is indeed a matter about which great care should be taken in studying bioactivity in vitro.[42] Although the results of this study reinforced the utility of the T24 cell model for the study of the adherence of UTI-related bacteria, we should bear in mind that adherence of pathogens to uroepithelial cell receptors is a complex process involving multidimensional interactions between commensal microbiota, uropathogens, immune cells, and epithelial cells.[30] More evolved cell models considering multispecies microbial biofilms and also evaluating other fimbriae-type UPEC strains such as type 1fimbriae ones will be of interest for future experiments.

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In summary, the results of this paper demostrate that A-type procyanidins (A2 and cinnamtannin B-1) but not B-type procyanidins (B2) are able to inhibit not only the adherence of uropathogenic *E. coli* but also of Gram-positive uropathogenic bacteria to bladder cells in in vitro conditions, although it is unlikely that procyanidins were present in urine at relevant physiological concentrations. It is also reported for the first time that cranberry-derived phenolic compounds detected in urine after consumption of cranberries and/or cranberry products such as hippuric acid, α-hydroxyhippuric acid, 3,4-dihydroxyphenylacetic acid, and dihydrocaffeic acid 3-*O*-sulfate are also able to inhibit the adherence of the uropathogenic bacteria tested. Additive (and even synergistic) effects among all the cranberry-derived phenolic metabolites present in urine are expected in vivo, which could explain, at least partly, the preventive and/or curative effects of the consumption of cranberry against urinary tract infections. Although a lack of standardization in terms of composition and dosage for cranberry products has been considered one of the main reasons for the large differences in the efficacy of cranberry consumption observed among the many epidemiological and intervention studies that have been carried out, interindividual variability in the capacity to metabolize cranberry polyphenols may have a lot to do with the beneficial effects associated with cranberry. New investigations into the gut microbial metabolism of cranberry polyphenols and their further conjugation, mainly in the liver, will give more clues in moving toward a resolution of the cranberry UTI-preventing puzzle.

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Notes

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REFERENCES

(1) Montorsi, F.; Gandaglia, G.; Salonia, A.; Briganti, A.; Mirone, V. Effectiveness of a combination of cranberries, *Lactobacillus rhamnosus*, and vitamin C for the management of recurrent urinary tract infections in women: results of a pilot study. *Eur. Urol.* **2016**, *70*, 912−915.

(2) Occhipinti, A.; Germano, A.; Maffei, M. E. Prevention of urinary tract infection with Oximacro®, a cranberry extract with a high content of a-type anthocyanidins: a pre-clinical double-blind controlled study. *Urol. J.* **2016**, *13*, 2640−2649.

(3) Maki, K. C.; Kaspar, K. L.; Khoo, C.; Derrig, L. H.; Schild, A. L.; Gupta, K. Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. *Am. J. Clin. Nutr.* **2016**, *103*, 1434−42.

(4) Pappas, E.; Schaich, K. M. Phytochemicals of cranberries and cranberry products: characterization, potential health effects and processing stability. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 741−781.

(5) Stapleton, A. E.; Dziura, J.; Hooton, T. M.; Cox, M. E.; Yarova-Yarovaya, Y.; Chen, S.; Gupta, K. Recurrent urinary tract infection and urinary *Escherichia coli* in women ingesting cranberry juice daily: a randomized controlled trial. *Mayo Clin. Proc.* **2012**, *87*, 143−150.

(6) Juthani-Mehta, M.; Van Ness, P. H.; Bianco, L.; Rink, A.; Rubeck, S.; Ginter, S.; Argraves, S.; Charpentier, P.; Acampora, D.; Trentalange, M.; Quagliarello, V.; Peduzzi, P. Effect of cranberry capsules on bacteriuria plus pyuriaamong older women in nursing homes: a randomized clinical trial. *JAMA* **2016**, *316*, 1879−1887.

(7) Luís, Â.; Domingues, F.; Pereira, L. Can cranberries contribute to reduce the incidence of urinary tract infections? A systematic review with meta-analysis and trial sequential analysis of clinical trials. *J. Urol.* **2017**, *198*, 614−621.

(8) Blumberg, J. B.; Camesano, T. A.; Cassidy, A.; Kris-Etherton, P.; Howell, A.; Manach, C.; Ostertag, L. M.; Sies, H.; Skulas-Ray, A.; Vita, J. A. Cranberries and their bioactive constituents in human health. *Adv. Nutr.* **2013**, *4*, 618−632.

(9) Sun, X.; Wu, J. Food derived anti-adhesive components against bacterial adhesion: Current progresses and future perspectives. *Trends Food Sci. Technol.* **2017**, *69*, 148−156.

(10) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli in vitro*. *Phytochemistry* **2000**, *54*, 173−181.

(11) Howell, A. B.; Reed, J. D.; Krueger, C. G.; Winterbottom, R.; Cunningham, D. G.; Leahy, M. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. *Phytochemistry* **2005**, *66*, 2281−2291.

(12) Gupta, A.; Dwivedi, M.; Mahdi, A. A.; Nagana Gowda, G. A.; Khetrapal, C. L.; Bhandari, M. Inhibition of adherence of multi-drug resistant *E. coli* by proanthocyanidin. *Urol. Res.* **2012**, *40*, 143−150.

(13) Ermel, G.; Georgeault, S.; Inisan, C.; Besnard, M. Inhibition of adhesion of uropathogenic *Escherichia coli* bacteria to uroepithelial cells by extracts from cranberry. *J. Med. Food* **2012**, *15*, 126−134.

(14) Di Martino, P.; Agniel, R.; David, K.; Templer, C.; Gaillard, J. L.; Denys, P.; Botto, H. Reduction of *Escherichia coli* adherence to uroepithelial bladder cells after consumption of cranberry juice; a double-Blind randomized placebo-controlled cross-over trial. *World J. Urol.* **2006**, *24*, 21−27.

(15) Lavigne, J. P.; Bourg, G.; Combescure, C.; Botto, H.; Sotto, A. *In vitro* and *in vivo* evidence of dose-dependent decrease of uropathogenic *Escherichia coli* virulence after consumption of commercial *Vaccinium macrocarpon* (cranberry) capsules. *Clin. Microbiol. Infect.* **2008**, *14*, 350−355.

(16) Howell, A. B.; Botto, H.; Combescure, C.; Blanc-Potard, A. B.; Gausa, L.; Matsumoto, T.; Tenke, P.; Sotto, A.; Lavigne, J. P. Dosage effect on uropathogenic *Escherichia coli* anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. *BMC Infect. Dis.* **2010**, *10*, 94.

(17) Monagas, M.; Urpi-Sarda, M.; Sánchez-Patán, F.; Llorach, R.; Garrido, I.; Gómez-Cordovés, C.; Andrés-Lacueva, C.; Bartolomé, B. Insights into the metabolism and microbial transformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food Funct.* **2010**, *1*, 233−253.

(18) Peron, G.; Sut, S.; Pellizzaro, A.; Brun, P.; Voinovich, D.; Castagliuolo, I.; Dall'Acqua, S. The antiadhesive activity of cranberry phytocomplex studied by metabolomics: intestinal PAC-A metabolites but not intact PAC-A are identified as markers in active urines against uropathogenic *Escherichia coli*. *Fitoterapia* **2017**, *122*, 67−75.

(19) Feliciano, R. P.; Boeres, A.; Massacessi, L.; Istas, G.; Ventura, M. R.; Nunes dos Santos, C.; Heiss, C.; Rodriguez-Mateos, A. Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols. *Arch. Biochem. Biophys.* **2016**, *599*, 31−41.

DOI: 10.1021/acs.jafc.8b05625
J. Agric. Food Chem. 2019, 67, 2166−2174

(20) Feliciano, R. P.; Mills, C. E.; Istas, G.; Heiss, C.; Rodriguez-Mateos, A. Absorption, metabolism and excretion of cranberry (poly) phenols in humans: a dose response study and assessment of inter-individual variability. *Nutrients* **2017**, *9*, 268.
(21) González de Llano, D.; Esteban-Fernández, A.; Sánchez-Patán, F.; Martín-Alvarez, P.; Moreno-Arribas, M. V.; Bartolomé, B. Antiadhesive activity of phenolic compounds and their microbial-derived metabolites against uropathogenic *Escherichia coli* in bladder epithelial cell cultures. *Int. J. Mol. Sci.* **2015**, *16*, 12119−12130.
(22) Mena, P.; González de Llano, D.; Brindani, N.; Esteban-Fernández, A.; Curti, C.; Moreno-Arribas, M. V.; Del Rio, D.; Bartolomé, B. 5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone and its sulphate conjugates, representative circulating metabolites of flavan-3-ols, exhibit anti-adhesive activity against uropathogenic *Escherichia coli* in bladder epithelial cells. *J. Funct. Foods* **2017**, *29*, 275−80.
(23) Flores-Mireles, A. L.; Walker, J. N.; Caparon, M.; Hultgren, S. J. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.* **2015**, *13*, 269−84.
(24) Kline, K. A.; Lewis, A. L. Gram-Positive uropathogens, polymicrobial urinary tract infection, and the emerging microbiota of the urinary tract. *Microbiol. Spectrum* **2016**, *4*, UTI-0012-2012.
(25) Rafsanjany, N.; Lechtenberg, M.; Petereit, F.; Hensel, A. Antiadhesion as a functional concept for protection against uropathogenic *Escherichia coli: In vitro* studies with traditionally used plants with antiadhesive activity against uropathognic *Escherichia coli*. *J. Ethnopharmacol.* **2013**, *145*, 591−597.
(26) García-Ruiz, A.; Moreno-Arribas, M. V.; Martín-Álvarez, P. J.; Bartolomé, B. Comparative study of the inhibitory effects of wine polyphenols on the growth of enological lactic acid bacteria. *Int. J. Food Microbiol.* **2011**, *145*, 426−431.
(27) Hilbert, D. W.; Pascal, K. E.; Libby, E. K.; Mordechai, E.; Adelson, M. E.; Trama, J. P. Uropathogenic *Escherichia coli* dominantly suppress the innate immune response of bladder epithelial cells by a lipopolysaccharide- and toll-Like receptor 4-independent pathway. *Microbes Infect.* **2008**, *10*, 114−121.
(28) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and citotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.
(29) Sánchez-Patán, F.; Monagas, M.; Moreno-Arribas, M. V.; Bartolomé, B. Determination of microbial phenolic acids in human faeces by UPLC-ESI-TQ MS. *J. Agric. Food Chem.* **2011**, *59*, 2241−2247.
(30) González de Llano, D.; Arroyo, A.; C Ardenas, N.; Rodríguez, J. M.; Moreno-Arribas, M. V.; Bartolomé, B. Strain-specific inhibition of the adherence of uropathogenic bacteria to bladder cells by probiotic *Lactobacillus* spp. *Pathog. Dis.* **2017**, *75* (4), ftx043.
(31) Nicolosi, D.; Tempera, G.; Genovese, C.; Furneri, P. M. Anti-adhesion activity of A2-type proanthocyanidins (a cranberry major component) on uropathogenic *E. coli* and *P. mirabilis* strains. *Antibiotics* **2014**, *3*, 143−154.
(32) European Center for Diseases Prevention and Control (ECDC). *Surveillance of antimicrobial resistance in Europe 2016*, pp 55−57. Available from: https://www.ecdc.europa.eu/en/publications-data/summary-latest-data-antibiotic-resistance-european-union (accessed February 2018).
(33) Wojnicz, D.; Tichaczek-Goska, D.; Korzekwa, K.; Kicia, M.; Hendrich, A. Study of the impact of cranberry extract on the virulence factors and biofilm formation by *Enterococcus faecalis* strains isolated from urinary tract infections. *Int. J. Food Sci. Nutr.* **2016**, *67*, 1005−1016.
(34) Pero, R. W. Health consequences of catabolic synthesis of hippuric acid in humans. *Curr. Clin. Pharmacol.* **2010**, *5*, 67−73.
(35) de Ferrars, R. M.; Czank, C.; Zhang, Q.; Botting, N. P.; Kroon, P. A.; Cassidy, A.; Kay, C. D. The pharmacokinetics of anthocyanins and their metabolites in humans. *Br. J. Pharmacol.* **2014**, *171*, 3268−3282.
(36) Penczynski, K. J.; Krupp, D.; Bring, A.; Bolzenius, K.; Remer, T.; Buyken, A. E. Relative validation of 24-h urinary hippuric acid excretion as a biomarker for dietary flavonoid intake from fruit and vegetables in healthy adolescents. *Eur. J. Nutr.* **2017**, *56*, 757−766.
(37) Bolca, S.; van de Wiele, T.; Possemiers, S. Gut metabotypes govern health effects of dietary polyphenols. *Curr. Opin. Biotechnol.* **2013**, *24*, 220−225.
(38) Kwak, H. S.; Park, S. Y.; Kim, M. G.; Yim, C. H.; Yoon, H. K.; Han, K. O. Marked individual variation in isoflavone metabolism after a soy challenge can modulate the skeletal effect of isoflavones in premenopausal women. *J. Korean Med. Sci.* **2009**, *24*, 867−873.
(39) Gónzalez-Sarrias, A.; Garcia-Villalba, R.; Romo-Vaquero, M.; Alasalvar, C.; Orem, A.; Zafrilla, P.; Tomas-Barberan, F. A.; Selma, M. V.; Espin, J. C. Clustering according to urolithin metabotype explains the interindividual variability in the improvement of cardiovascular risk biomarkers in overweight-obese individuals consuming pomegranate: A randomized clinical trial. *Mol. Nutr. Food Res.* **2017**, *61* (5), 1600830.
(40) Muñoz-Gonzalez, I.; Jiménez-Girón, A.; Martin-Alvarez, P. J.; Bartolome, B.; Moreno-Arribas, M. V. Profiling of Microbial-Derived Phenolic Metabolites in Human Feces after Moderate Red Wine Intake. *J. Agric. Food Chem.* **2013**, *61*, 9470−9479.
(41) Mena, P.; Ludwig, I. A.; Tomatis, V. B.; Acharjee, A.; Calani, L.; Rosi, A.; Brighenti, F.; Ray, S.; Griffin, J. L.; Bluck, L. J.; Del Rio, D. Inter-individual variability in the production of flavan-3-ol colonic metabolites: preliminary elucidation of urinary metabotypes. *Eur. J. Nutr.* **2018**, DOI: 10.1007/s00394-018-1683-4.
(42) Aragones, G.; Danesi, F.; Del Rio, D.; Mena, P. The importance of studying cell metabolism when testing the bioactivity of phenolic compounds. *Trends Food Sci. Technol.* **2017**, *69*, 230−242.

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