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Review

Bioactive compounds in cranberries and their role in prevention of urinary tract infections

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Cranberry (*Vaccinium macrocarpon* Ait.) ingestion has long been associated with prevention of urinary tract infections. The beneficial mechanism was historically thought to be due to the fruit acids causing a bacteriostatic effect in the urine. However, recently, a group of proanthocyanidins (PACs) with A-type linkages were isolated from cranberry which exhibit bacterial antiadhesion activity against both antibiotic susceptible and resistant strains of uropathogenic P-fimbriated *Escherichia coli* bacteria. The link between cranberry ingestion and maintenance of urinary tract health as well as the structural diversity, pharmacokinetics, quantification, and bacterial antiadhesion bioactivity of the A-linked cranberry PACs are reviewed.

Keywords: Antiadhesion / Cranberry / *Escherichia coli* / Proanthocyanidin / Urinary

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1 Incidence of urinary tract infection

Over 11 million women a year, in the United States alone, report having had a urinary tract infection (UTI), accounting for direct costs of over $1.6 billion [1]. Most women experience another UTI during their lifetime and 25% suffer recurrent infections [2]. The standard treatment for UTI is antibiotic therapy, however rising clinical failure rates of trimethoprim-sulfamethoxazole due to bacterial resistance has led physicians in some areas of the country to consider prescribing fluoroquinolones as first-line treatment [3]. Continual low-dose antibiotic regimes are often prescribed to prevent recurrent infections, resulting in further increases in bacterial immunity [4]. Pharmaceutical companies have been reluctant to fund the development of new antibiotics because many bacteria can begin to build up resistance within months. Alternative therapies for prevention of UTI are needed to slow the pace of antibiotic resistance development.

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Abbreviations: NIH, National Institutes of Health; **PAC,** proanthocyanidin, **UTI,** urinary tract infection

2 Cranberry consumption and prevention of UTI

Cranberry (*Vaccinium macrocarpon* Ait.) has been associated with prevention of UTI for nearly 100 years [5]. In recent years, more physicians have begun recommending daily cranberry consumption as a safe alternative for UTI prevention [1, 6–9]. The Cochrane Database Systematic Review [10] reported that there is positive clinical evidence that cranberry juice consumption can decrease the number of symptomatic UTIs over a 12-month period in women, citing two well-controlled randomized, double-blind, placebo-controlled studies [11, 12]; however, there is no evidence that cranberry can treat UTI once an infection is present. Additional studies, both clinical and mechanistic, are underway. The National Center for Complementary and Alternative Medicine, a branch of the US National Institutes of Health (NIH), is currently funding significant research projects (including several large-scale clinical trials) on the role of cranberry in promoting urinary tract health [13].

Cranberry juice, predominantly in the form of a juice cocktail drink with 27% cranberry, has been the traditional choice of most women seeking to prevent UTI. Single strength 100% cranberry juice is highly acidic and is therefore routinely formulated into diluted, sweetened highly palatable cocktail juice drinks. Most clinical trials have used sweetened cranberry juice, but one major study showed that the artificially sweetened cranberry juice cock-

tail was efficacious in UTI prevention [11]. Clinical research suggests that daily dosages of 240–300 mL of cranberry juice cocktail can prevent recurrence of UTI about 50% of the time and reduce bacteriuria and pyruria [11]. An *ex vivo* study examining human urine following cranberry juice cocktail consumption suggests that twice-daily dosing of cranberry might offer additional protection over a 24-h period [14]. Daily doses under 240 mL may not offer as much protection [15], but clinical confirmation is needed. NIH-funded dose–response clinical intervention trials are currently underway to determine and further clarify effective dosages of cranberry juice cocktail. Other forms of cranberry have demonstrated some benefits, including dried cranberry powder in the form of tablets or capsules [16–18], sweetened dried cranberries [19], and cranberry sauce [20], although further clinical research is needed on these products to determine dosage and efficacy.

3 Mode of action for UTI prevention

Research suggests that consumption of cranberry products may prevent UTI by preventing uropathogenic bacterial adhesion. Uropathogenic strains of *Escherichia coli* bacteria cause nearly 95% of UTIs [21]. Infection is initiated by bacterial adherence to the uroepithelium, followed by bacterial multiplication and colonization of the urinary tract [22]. Important virulence factors in the pathogenesis of UTI include adhesins (P fimbriae and type 1 fimbriae) that adhere to carbohydrate receptors on the surface of uroepithelial cells [22]. Nearly all uropathogenic strains of *E. coli* can express type 1 fimbriae that bind to mannose-like receptors [23]. P-fimbriated *E. coli* adhere to oligosaccharide receptor sequences (α-Gal(1 → 4)β-Gal) [24], and have been implicated in both cystitis and pyelonephritis [25].

Clinical researchers are attempting to employ techniques that inhibit bacterial adhesion in the prevention of UTI rather than prescribe low-dose antibiotic regimes, since the antiadhesion mechanism does not kill bacteria and leads to significant selection pressure to favor the survival of antibiotic resistant bacterial strains [26]. For this reason, there is particular interest in utilizing dietary inhibitors of adhesion, especially cranberry [26]. One study showed that antibiotic resistant P-fimbriated *E. coli* lost the ability to adhere to bladder cell receptors following incubation in urine of humans who had consumed 240 mL of cranberry juice cocktail [14]. This suggests that utilizing cranberry could potentially slow the pace of antibiotic resistance development by preventing UTI, thus reducing the need for antibiotic prophylaxis. This type of preventative strategy could become increasingly important as antibiotic resistance rates increase due to over-use of antibiotics [4].

The general antiadhesion properties of cranberry were discovered over 20 years ago [27–29], challenging the original belief that the acidity of fruit was responsible for the antibacterial effect. The majority of clinical research has all but dispelled the urinary acidification theory as the major mechanism of action in the prevention of UTI, demonstrating that a bacteriostatic pH is rarely achieved following normal serving sizes of cranberry [11, 18, 12, 30, 31]. In addition, recent studies have shown no effect on bacterial growth in urine following cranberry juice consumption [32, 33].

The details of the mechanism by which cranberry prevents bacterial adhesion are not fully understood, however new research offers some interesting insights. The binding of the proteinaceous bacterial fimbrial tips to mucosal surfaces on the uroepithelium occurs as a specific receptor–ligand association [34] favored by hydrophobic interactions [35]. One possible mechanism is that the cranberry compounds, acting as receptor analogs, competitively inhibit adhesion of *E. coli* to cells by binding to the fimbrial tips. Components in cranberry may also alter P-fimbriated uropathogenic bacteria in a number of ways, resulting in reduced adhesion capabilities. Cranberry may influence bacterial adhesion by altering cell surface properties of the bacteria and causing a shift in the distribution of zeta potentials (the electrical potential that exists across the interface of all solids and liquids) in a positive direction [36]. In a recent study, pH-neutralized cranberry juice induced conformational changes in the surface macromolecules of P-fimbriated *E. coli*, specifically reducing fimbrial length and density [37]. Evidence from other studies suggests that cranberry may be reducing fimbrial expression at the genetic level [37, 38]. Incubation in cranberry juice has also resulted in changes in the shape of bacteria from rods to spheres [37], and from rods to elongated rods [38], but it is unclear how these morphological changes may be influencing adhesion ability.

4 Active cranberry compounds that prevent bacterial adhesion

Cranberries contain two chemical compounds that have been implicated in prevention of bacterial adhesion, the monosaccharide fructose and a type of condensed tannin called proanthocyanidin (PAC). Fructose in cranberry juice inhibits adhesion of type 1 (mannose sensitive) fimbriae to uroepithelial cells, but this effect has only been demonstrated *in vitro* [28]. Dietary fructose has not been clinically proven to inhibit uropathogenic bacterial adhesion when administered orally. PACs are polyphenolic compounds found in cranberry that appear to be of primary importance for prevention of uropathogenic bacterial adhesion [37, 39, 40–43]. Cranberry PACs inhibit adhesion of P-fimbriated *E. coli* to uroepithelial cells, but not type 1 *E. coli* [39–41, 43]. PACs from cranberry and other plants are gaining attention from the medical and pharmaceutical communities for their wide array of potential health benefits [44].

PACs from different food sources account for the majority of flavonoids consumed in the Western diet [45]. PACs are defense compounds produced by the plant in response to environmental stress and microbial infection [46, 47]. The astringency of PACs protects the young fruit from animal and insect predators [48], and contributes to the unique taste of cranberry juice. One characteristic of PACs is their ability to bind proteins [49]. This characteristic further supports the theory that PACs may be binding the proteinaceous fimbriae on *E. coli*, inhibiting the specific receptor–ligand adhesion to uroepithelial cells.

5 Proanthocyanidin structures

The specific structure of PACs can influence their biological activity. The PACs are composed of oligomers and polymers of flavans, predominantly flavan-3,4-diols [50]. The B ring of the flavan monomer can be substituted with two or three *ortho* hydroxyl groups. The flavan-3-ol units (epicatechin or catechin) are most often linked through a single bond (B-type), which is present in common food sources of PAC, such as grapes and chocolate [51]. A less common structural feature of PACs is the A-type linkage, in which there is a second ether linkage between an A-ring of the lower unit and C-2 of the upper unit (O7 → C2). PACs isolated from cranberry fruit consist of predominantly epicatechin units with at least one A-type linkage [40, 41]. The A-type cranberry PACs prevent adhesion of P-fimbriated uropathogenic *E. coli* to uroepithelial cells *in vitro* [40, 41]. The structures of A-linked dimers and trimers were determined and found to be active in the antiadhesion assays; however, the oligomeric and polymeric A-linked PAC fractions (which were not structurally characterized) also possessed activity [40, 41]. Therefore, the A-linkage in cranberry PACs may represent an important structural feature for bacterial antiadhesion activity. To determine if the A-linkage is a prerequisite for antiadhesion activity, A-type PACs from cranberry juice cocktail and B-type PACs from other food sources (*i.e.*, commercial grape and apple juices, dark chocolate, and green tea) were isolated, quantified, and characterized for linkage type utilizing MALDI-TOF/MS and direct infusion (DI)/ESI-MS [52]. Single servings of each food (with standardized PAC content) were administered to human participants and urine samples were collected and tested for bacterial antiadhesion activity. Antiadhesion activity was detected only in the urine samples of participants who had consumed the cranberry juice cocktail, which contained the PACs with A-type linkages.

The structures of oligomeric and polymeric cranberry PACs are highly heterogeneous and have been difficult to elucidate, however, advances in spectroscopic techniques have allowed work to progress more rapidly in recent years. Cranberry PACs have been separated according to degrees of polymerization up to decamers using thiolytic degradation followed by normal-phase HPLC and MS/MS analysis [51] or normal-phase HPLC with MS fluorescent detection [53]. These techniques have shown that the A-linkage can occur at the terminal unit or between the extension units of the cranberry PAC molecule [51]. Recently, a group of cranberry PAC oligomers and polymers as large as 12 degrees of polymerization (DP) with as many as four A-type linkages were detected using MALDI-TOF MS [54]. The same technique was used to characterize the structural diversity of a series of anthocyanin-polyflavan-3-ol oligomers from cranberry [55]. These heterogeneous structures may arise naturally during fruit ripening or as a result of postharvest processing of the fruit [55]. Further research is needed to determine how molecular weight and structural complexity impact bacterial antiadhesion activity of the cranberry PACs.

6 Proanthocyanidin absorption and metabolism

The absorption and metabolism of cranberry PACs have not been well studied, due to the structural complexities of the molecules and the lack of commercial standards. Bioavailability studies on noncranberry PACs indicate that molecular weight can significantly impact metabolism and absorption [56]. PAC dimers and trimers are permeable through the Caco-2 human intestinal cell line, suggesting that they could be absorbed intact. PAC polymers may undergo degradation by colonic microflora and biotransformation into sulfate esters or glucoronide-conjugated metabolites [56]. PACs can be degraded by the microflora into phenolic acids which can be found in human urine following consumption of PAC-rich chocolate [57]. Oral delivery of ^{14}C-labeled grape PACs to rats resulted in 19% of the dose being excreted in the urine, and 45% in the feces [58]. A-type linkages may provide a degree of conformational rigidity to PACs [50], possibly impacting oxidation of the molecules. Further, the unusual heterogeneous structures of the A-linked cranberry PACs may be biotransformed to unique biologically active urinary metabolites or even reach the urine intact as dimers or trimers. Research has shown that bacterial antiadhesive compounds (either metabolites or intact low MW PACs) reach the urine of humans following consumption of cranberry products [14, 15, 17, 19, 27, 31, 43, 52] and the urine of mice fed isolated cranberry PACs [59]. The structures of the active urinary compounds are unknown; however, NIH-funded research is currently underway to identify and structurally characterize these molecules. Based on the metabolic fate of oligomeric and polymeric PACs from other plant sources, it is possible that cranberry PACs or metabolites could be active in the colon as well as the urinary tract [28]. They could bind to uropathogenic rectal *E. coli* isolates, thereby rendering them antiadherent prior to possible introduction into the urinary tract.

Or, they could alter the bacterial selection pressure in the colon to favor nonadherent strains. Further research is needed to determine site(s)-of-action of cranberry PACs and their metabolites.

7 Standardization of cranberry proanthocyanidins

Standardization of PAC content in cranberry products is needed to aid manufacturers in determining product stability and shelf-life, and to guide researchers in conducting dose–response research. Currently, there is no universally accepted standard method for quantification of cranberry PACs in products. Quantification of PACs is not straightforward and can lead to erroneous, irreproducible results [60]. The complexity of PACs in terms of molecular weight and linkage type makes it difficult to utilize a single quantification method for all products. This is especially true with cranberry, in that different processed products can have variable amounts of PACs with high levels of structural variation, due to oxidation or thermal degradation from particular processing techniques [61]. The A-linkages and heterogeneous structures of cranberry PAC make it difficult to apply standard PAC quantification methods to cranberry products [62]. Certain colorimetric assays, such as vanillin–HCl [50] are specific for flavan-3-ols and PACs, but lack of suitable standards and interferences from other sample components and extraction solvents can lead to inaccurate results. In addition, these methods can lead to an underestimation of larger polymers which tend not to respond as strongly to the vanillin–HCl reagent [63]. Colorimetric assays depend on reaction of chemical reagents with sites on the PAC molecule. The more complex the PAC, the more likely it is that the level of PAC will be underestimated, due to incomplete cleavage of the molecules by the reagent [63]. This normally results in an overestimation of lower MW PACs and an underestimation of higher MW PACs when compared to chromatographic techniques. Accuracy is improved if the PACs are isolated prior to analysis, but the amount of PAC that is isolated can vary depending on the extraction methodology employed. The DMAC method has been adapted for use in measuring PAC content of cranberry [62], but there are questions as to its accuracy for comparing different types of products, owing to the differences in PAC complexity among products. A major issue with all the colorimetric procedures is the lack of suitable reference standards. Many of the colorimeteric procedures tend to utilize catechin as a standard, which will have different reaction kinetics and a different molar absorptivity constant for the chromophores produced in the reaction [63] than the cranberry PACs. Gravimetric procedures based on fractionation and weighing of isolated compounds can provide accurate assessments of total PAC content on larger sample sizes, but, like colorimetric assays, they do not provide qualitative information on molecular weight composition [62]. Normal-phase HPLC methods work well for homogeneous PACs with B-type linkages, such as those in chocolate and grapes [64], but do not effectively resolve heterogeneous A-linked cranberry PAC peaks above tetramers [62]. The oligomers and polymers higher than tetramers, which may be important for urinary tract health, may not be accurately quantified. MS methods provide the greatest accuracy in quantifying and characterizing cranberry PACs [55, 62], but these methods require specialized expensive equipment and highly trained personnel.

The effect of cranberry PAC molecular weight and A-linkage location, type and number on bioactivity is not fully elucidated, thus it is critical to relate PAC quantification to bacterial antiadhesion activity. This is especially important because processing can alter PAC composition [61], which may positively or negatively impact bioactivity and shelf-life of cranberry products. A standard method for cranberry products that combines accurate PAC quantification with antiadhesion bioactivity level would provide the information that manufacturers need to formulate efficacious cranberry products for consumers.

8 Conclusion

There is intriguing evidence emerging on the antiadhesion bioactivity and structural complexity of the cranberry PACs. The unusual structural features of the cranberry PACs may help explain the positive clinical effects of cranberry on prevention of UTI. Further research is needed on dose–response, pharmacokinetics, and structure–activity relationships to fully substantiate the *in vivo* link between cranberry PACs and prevention of UTI.

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