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CRANBERRY, A POTENTIAL ALTERNATIVE TREATMENT FOR URINARY TRACT INFECTIONS

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Abstract

Vaccinium macrocarpon is perennial plant traditionally used as an herbal medicine in treatment and prevention of UTIs. Although the mechanisms of action are not jet fully understood it is presumed that they involve interferation with bacterial adhesion and changes in bacterial morphology mainly attributed to the plants proanthocyanidins.

Cranberry extracts (CE) standardized for different concentrations of proanthocyanidinnes (PACs), CE in combination with antibiotics (norfloxacin and vancomycin) and antibiotics alone (only antibiotics) were investigated for their effect on different strains of uropathogenic *E.coli, S. saprophyticus* and *E. faecalis*.

As a source of CE we used commercial herbal supplements containing only Vaccinium macrocarpon extract (37.5 mg PACs) or CE in combination with D-manoza (25, 3 mg PACs). We used bacterial strains isolated from outpatients with UTIs reffered for routine urine examination at the Institute of microbiology and parasitology. Sensitivity of the pathogens to CE (as monoagent or combined in herbal mix) was evaluated with disc diffusion method.

Our results showed stronger effect of CE on the growth of *E.coli* compared to Gram-positive strains. *S. saprophyticus* strains were more susceptible to the extract/herbal mixes compared to the enterococci which predominantly presented as recalcitrant to the inhibitory activity of cranberry/herbal mixes.

The sample size of this study was small to draw definite conclusions but our results illuminate avenues for future research of the potential of cranberry as an alternative treatment in patients with UTIs.

***Keywords*:** cranberry, UTIs, *E.coli*, antimicrobial resistance

Introduction

Urinary Tract Infections (UTIs) represent one of the most frequent disorders of the urinary system in both genders particularly in women with prevalence of more than 50% of the women at least once during their lifespan [1].

Patients with an episode of acute UTI might develop recurrent infection, defined as two episodes of UTI in a period of six months or three or more episodes of UTI during a twelve month period caused by the same (relaps) or different (re-infection) pathogens as that involved in the original UTI [2, 3] which represents a substantial burden to the healthcare system.

Uropathogenic *Escherichia coli* is the leading Gram negative isolate associated with urinary tract infections both in ambulatory and hospitalized patients being represented as a causative agent of over 85% of the UTIs, while most prevalent Gram positive etiological agens include *Staphylococcus saprophyticus* and *Enterococcus faecalis* [4].

Taking into consideration that the number of multiple drug resistant bacteria has raised in the last years, herbal medicine research on antimicrobial effects of natural plant substances with potential to affect the microorganism without having negative impact on the host are an emerging trend [5, 6].

Currently, various plant derived active substances with antimicrobial effect have been in the centre of scientific interest because they can mediate in prevention or/and treatment of infective diseases with minor or no negative adverse effects on the human organism [5].

Vaccinium macrocarpon (cranberry) is an edible perennial plant that has been used in humans nutrition for centuries. Essential oils and extracts derived from different parts of the plant have been a subject to extensive research since the whole plant, in particularly the fruits of the species are rich source of multiple active ingredients including phenolics, organic acids and mostly significant, proanthocyanins – a special class of phenols recognized for their various bioactive properties, particularly their effect as adjuvant in relieving the symptoms of lower urinary tract infection.

Data from one systematic review of ten randomized trials (RCTs) of Cranberry (juice and tablets) controlled with placebo/control (juice or water), conducted in 1049 volunteers indicate that cranberry supplements may improve the condition in patients with UTIs.

Results from this review showed significant decrease of the incidence of UTIs after a 12 months period compared to placebo/control [7].

Some studies have shown that mechanisms behind this effect of the cranberry products involve interaction with the mannose binding domain of type-1 fimbriae, resulting in direct inhibition of attachment of *Escherichia coli* to the epithelium of the urethra which is a crucial step in the pathogenesis of the infection [7, 8].

It was also discovered that cranberry derived proanthocyanidins (PACs) have important role in this process presenting as potent inhibitors of adhesion of P-fimbriated *E. coli in vitro* [9,10, 11].

It another study it was demonstrated that the reduced adhesion may be attributed to decrease in P-fimbrial expression associated with exposure to PACs [12].

Studies have shown that cranberry beneficial effects in patients with UTIs are attributed to indirect effects as well. Namely, in some studies admistration of cranberry extracts led to induction of Tamm-Horsfall protein production from the distal part of the loop of Henle and suppression of the inflammatory cascade that occurs as a normal immune response to pathogen invasion [13, 14].

Study objectives

The objective of the study was to determine the antimicrobial potential of cranberry extract (Vaccinium macrocarpon-VmE) and to investigate whether fermented cranberry juice and commercial antibiotics can be used in combination as an alternative treatment to bacterial infections, by pinpointing the decrease of the minimum inhibitory concentration of the uropathogens with simultaneous consumption of cranberry juice.

Materials and methods

- *Samples and bacterial strains*

This study comprised urine specimens from 32 outpatients, with suspected infection of the urinary system referred for microbiological urine analysis at the Institute of Microbiology and parasitology, Medical Faculty, Skopje. A total number of 20 bacterial isolates [(10 Gram-negative isolates (E. coli strains) and 10 Gram-positive isolates (5 S. saprophyticus strains and 5 Enterococcus spp. strains) from 20 culture-positive urine samples were examined in the antimicrobial assay. The remaining 12 urine specimens (out of 32 included in the study) were with mixed or no bacterial growth and did not undergo further investigation.

The usual culturing methods (quantitative urine culture on blood sheep agar or chromogenic agar) following standard laboratory procedures were used for isolation and identification of the bacterial strains. Gram staining and standard biochemical analysis were applied where needed.

- *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing for vancomycin and norfloxacine, antibiotics which are commonly active against staphylococci./enterococci and E.coli, accordingly, was performed with EUCAST's (European Society of Clinical Microbiology and Infectious Diseases) disc diffusion method using commercial antibiotic discs (Oxoid, England) of vancomycin (VAN; 10 μg) and norfloxacine (NOR; 5 μg)(15).

Antibacterial activity of 2 herbal supplements (commercial lyophilized Vaccinium macrocarpon extracts) including 100% cranberry extract (CE)(standardized for 37.5 mg PACs) and herbal mix containing 70% CE + D-mannose (standardized for 25.3 mg PACs) and the activity of CE+antibiotic

(vancomycin for staphylococci./enterococci; norfloxacine for E.coli) combination was measured by standard Kirby-Bauer disk diffusion method (16).
To determine the sensitivity to CE and CE + antibiotic we used sterile filter paper discs and suitable antibiotic discs to which 10 μL suspension of the lyophilized cranberry extract in 200 μL saline was added. Three types of discs were formed containing as follows: type 1 100% CE, type 2 was saturated with 70% CE, and type 3 was vancomycin/or norfloxacine disc infused with 100% CE. For the antibacterial assay, an inoculum of 106 CFU/ml of each bacterial strain was grown for 24 h at 37°C on Muller Hinton Agar (Oxoid, England) on which the paper discs (6 mm in diameter) were placed.
After an incubation period of 48 h at 37°C, antimicrobial activity against the test strains was noted by zones of inhibition of growth around the discs, and the diameters of the clear growth-free zones around each disk were measured.

Results

Our results showed stronger effect of cranberry extract on the growth of E.coli compared to Gram-positive strains which might be associated with the sensitive phenotype of E.coli isolates included in the study. Forty percents (4/10) E.coli isolates had equal, 30% (3/10) larger and 30% (3/10) provided smaller growth inhibition zones for VmE and VmE+NOR compared to the inhibitory effect of norfloxacin alone (Table 1).

Table 1: Inhibition zones for cranberry extract (standardized for 37.5 mg PACs), herbal mix (cranberry extract in combination with D-manoza; standardized for 25, 3 mg PACs), Norfloxacine and cranberry extract in combination with Norfloxacine (5 μg/disk) against *E.coli*

E.coli isolates	**VmE 37.5 mg PACs ZOI (mm)**	**VmE 25.3 mg PACs ZOI (mm)**	**NOR ZOI (mm)**	**NOR+ VmE ZOI (mm)**
1	16	16	6	16
2	15	15	15	20
3	18	18	18	21
4	16	16	16	24
5	6	6	6	6
6	10	10	24	25
7	16	16	6	16
8	22	22	20	22
9	18	18	22	18
10	24	24	26	25

All of the *S. saprophyticus* strains and 80% (4/5) were susceptible to VmE and the the combination VAN+ VmE, accordingly. This represents notable difference from the other investigated genus of Gram-positive uropathogens, *Enterococcus spp*., which were mainly resistant (60%; 3/5 isolates) to the inhibitory activity of both VmE and VmE in herbal mix and to the VAN+ VmE combination.

Table 2: Inhibition zones for Vaccinium macrocarpon extract (standardized for 37.5 mg PACs), herbal mix (Vaccinium macrocarpon extract in combination with D-mannose; standardized for 25, 3 mg PACs), Vancomycin and Vaccinium macrocarpon extract in combination with Vancomycin (5 μg/disk) against *S. saprophyticus*

***S. saprophyticus* isolates**	**VmE 37.5 mg PACs ZOI (mm)**	**VmE 25.3 mg PACs ZOI (mm)**	**VAN ZOI (mm)**	**VAN+ VmE ZOI (mm)**
1	10	10	12	16
2	10	10	10	17
3	9	9	11	15

4	7.5	7	9.5	6
5	10	10	10	6

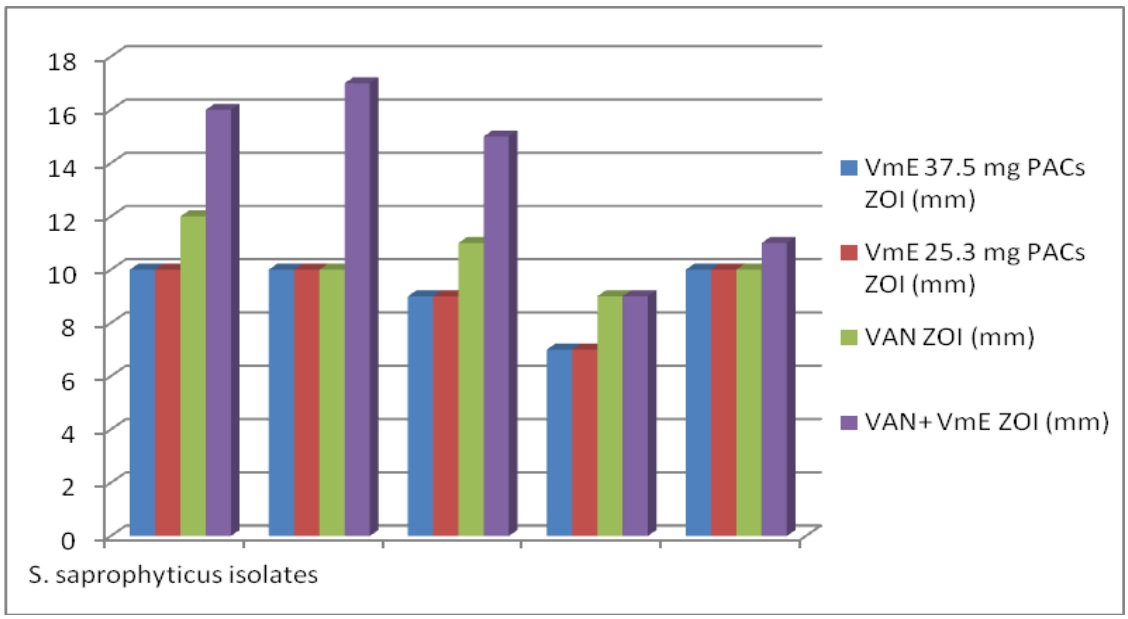

Figure 1: Comparison of the effects of VmE, VmE in herbal mix, VAN and VmE +VAN on each *Staphylococcus saprophyticus* isolate

Enterococcus spp. strains were the most resistant, even when tested with vanocmycin and cranberry extract. Ssixty percents (3/5) of the isolates were resistant to VmE, VmE in herbal mix and VAN+ VmE. One of the remaining two strains displayed higher values of inhibition zones for VmE and VmE in herbal mix reaching diameter of 19 mm compared to ZOI of vancomycin and vancomycin + VmE, while the other *Enterococcus spp*. isolate exhibited largest ZOI with all VmE combinations (23 mm) versus relatively small inhibition zone for vancomycin alone (11 mm) indicating highest susceptibility of this strain to cranberry extract.

Table 3: Inhibition zones for Vaccinium macrocarpon extract (standardized for 37.5 mg PACs), herbal mix (Vaccinium macrocarpon extract in combination with D-manoza; standardized for 25, 3 mg PACs), Vancomycin and Vaccinium macrocarpon extract in combination with Vancomycin (5 µg/disk) against *Enterococcus spp.*

***Enterococcus spp* isolates**	**VmE 37.5 mg PACs ZOI (mm)**	**VmE 25.3 mg PACs ZOI (mm)**	**VAN ZOI (mm)**	**VAN+ VmE ZOI (mm)**
1	19	19	14	15
2	6	6	15	6
3	6	6	10	6
4	6	6	8	6
5	23	23	11	23

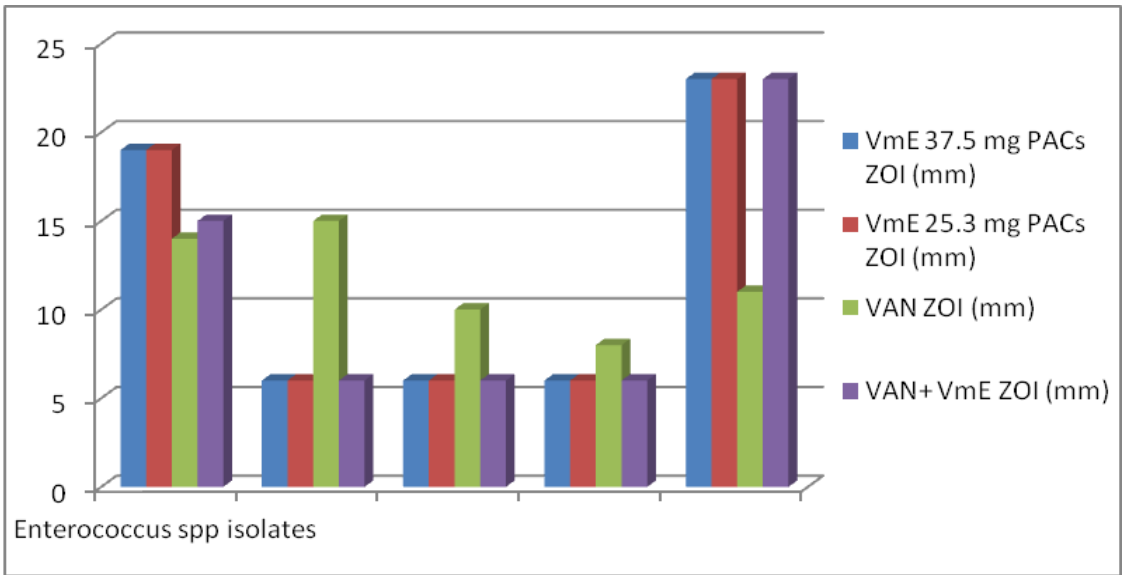

Figure 2: Comparison of the effects of VmE, VmE in herbal mix, VAN and VmE +VAN on each *Enterococcus spp* isolate

Discussion

Up to date, numerous studies, in vitro and in vivo, have been conducted on the application of cranberry and its bioactive constituents in prophylaxis of recurrent UTIs as well as their mechanisms of action (17].

Although the utilization of Vaccinium macrocarpon has been related with a reduced incidence of UTIs and alleviation of the symptoms of active UTI compared to placebo/control, in most of the studies [18-23] some varying results can be found throughout the literature [7, 23], possibly due to vague definition of the dosage, concentration and formulation of proanthocianidins and variability in

PAC-A concentration between cranberry formulations in the different studies [24].

In this study we used dietary supplements containing lyophilized Vaccinium macrocarpon extracts and two antibiotics commonly effective against Gram-negative and Gram-positive uropathogens.

Our results indicate that CE alone or in combination with the antibiotic have more potent inhibitory effect on the bacterial growth compare to the antibiotic alone, especially in regard to the growth of *E.coli* and *S. saprophyticus*. Similar outcomes were achieved from Lian et al. who investigated the effects of cranberry and four other berry fruit extracts on the growth of *S. aureus*.

Results of their study showed significant antimicrobial against S. aureus and the most potent among the berries studied. By comparison of this data with the effect on NaOH-neutralized samples they also concluded that antimicrobial effect is not ascribable to the acidity of the berries rather it's entirely related to the polyphenol classes detected in the extracts [25].

In a similar study performed by LaPlante et al. three proprietary PAC-standardized cranberry extracts were assessed for their effects on the inhibition of bacterial growth and biofilm production against different clinically significant pathogens: *Staphylococcus epidermidis, Staphylococcus aureus*, methicillin-resistant *S. aureus, Staphylococcus saprophyticus* and *Escherichia coli*.

Their efficacy outcome corresponded to the results from our study in terms of the inhibitory effect of CE against staphylococci but showed contrary effect i.e. insignificant influence on the growth of the Gram-negative species (*E. coli*) [26].

Unlike previously mentioned study, Mantzourani et. al in their evaluation of the antimicrobial activity of unfermented and fermented cranberry juice combined with vancomycin or tigecycline obtained larger ZOI and lower minimum inhibitory concentrations in *Enterococcus spp*. strains in favor of the combination vancomycin/ tigecycline compared to the respective ZOI with vancomycin and unfermented juice [27].

Conclusion

It is well known that cranberry is advocated for treatment of urinary tract infection and plays certain role in symptom alleviation; however in the absence of data from well-designed and controlled studies the application of cranberry for this purpose remains controversial.

The sample size of this study was small to draw definite conclusions but our results illuminate avenues for future research of the potential of cranberry as an alternative treatment in patients with urinary tract infections.

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