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Short Communication

In vitro activity of cranberry extract against etiological agents of urinary tract infections

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Cranberries have long been the focus of interest for their beneficial effects in preventing urinary tract infections (UTIs). The objective of this study was to determine *in vitro* activity of cranberry extract on common etiologic agents of urinary tract infections isolated from patients. Filter sterilized methanol extract of cranberry was prepared and used in the present study. The minimum inhibitory concentration (MIC) was evaluated for active crude extract. The MIC value of methanol extract were 0.391 mg/ml for *Enterobacter aerogenes* and *Staphylococcus aureus* whereas the MIC of methanol extract of cranberry were 1.2500 and 0.0195 mg/ml for *Escherichia coli and Klebsiella pneumoniae* respectively. The lower MIC value of cranberry extract against *K. pneumoniae* in comparison to other three organisms suggests that *K. pneumoniae* showed greater sensitivity towards the extracts of the cranberry extract.

Key words: Antibacterial activity, cranberry, urinary tract infections.

INTRODUCTION

Urinary tract infections (UTIs) are common problem especially in women. One fourth to one fifth of women will have one at some time in their lives and the incidence increases with age (Harkins, 2000; Lynch, 2004). Antibiotics are used routinely for treatment of UTIs but these agents are not always effective and can cause side effects. Drinking of cranberry juice is one of the non-antibiotics strategies that have been advocated for the treatment and prevention of UTIs (Harkins, 2000; Griffiths, 2003; Abayomi, 1982). The scientific name for cranberry plant is *Vaccinium macrocarpon* (DerMarderosian et al., 2002). Cranberries fruits is native in some provinces of Iran and grow naturally. Commercial harvests occur in August and September. Cranberries contain 80% water and 10% carbohydrates (Lenter, 1991). Among other constituents are flavonoids, catechin, triterpenoids, organic acids and a small amount of ascorbic acid. The major organic acids are citric, malic and quinic acids, with small amounts of benzoic and glucuronic acids (Borukh et al., 1971). Anthocyanin pigments obtained from cranberry pulp are used for coloring applications Cranberries can be processed into fresh fruit, concentrate, sauce products and juice drinks. The single-strength juice is very acidic (pH = 2.5) and unpalatable. In 1930, cranberry juice cocktail, comprising a mixture of cranberry juice, sweetener, water and added vitamin C, was introduced. The leading brand of cocktail contains 33% pure cranberry juice. Dried cranberry powder formulated in capsules or tablets is also available (Raz et al., 2004).

Cranberries have long been the focus of interest for their beneficial effects in preventing urinary tract infections (Harkins, 2000; Vatem et al., 2005). Cranberries contain 2 compounds with anti-adherence properties that prevent fimbriated *Escherichia coli* from adhering to uroepithelial cells in the urinary tract.

Approximately one dozen clinical trials have been performed testing the effects of cranberries on the urinary tract infections. However, these trials suffer from a number of limitations. Most importantly, the trials have used a wide variety of cranberry products, such as cranberry juice concentrate, cranberry juice cocktail and cranberry capsules and they have used different dosing regimens. Further research is required to clarify unanswered questions regarding the role of cranberries in protecting

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Abbreviations: MIC, Minimum inhibitory concentration; **UTIs,** Urinary tract infections.

against UTI in general and in women with anatomical abnormalities in particular (Harkins, 2000). In spite of many studies in other country there was not any published data regarding to antibacterial activity of cranberry in our country .The aim of this study was to determine *in vitro* activity of cranberry juice on the common etiological agents of UTIs.

MATERIALS AND METHODS

Test microorganisms

Microorganisms used corresponded to *E. coli*, *Staphylococcus aureus, Enterobacter. aerogenes* and *Klebsiella pneumoniae* isolated from patients with urinary tract infections disease from the Imam Khomeini Hospital of Uremia. Urine specimen were cultured on blood and MacConkey agar. All plate were incubated for 24 h at 35°C. Colony count performed for positive cultures and significant isolates were identified. All patients had significant bacteriuria and UTI symptoms.

Conventional bacteriological methods such as colony morphology, gram stain and biochemical tests were used for identification of isolates (Mahon et al., 2006). Isolated microorganisms were kept on trypticase soy broth at -20°C. Before testing antibacterial activity stored microorganisms were subcultured on blood agar and used for further testing. We also used the strains *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC10031) *S. aureus* (ATCC 25923*) E. aerogenes* (ATCC13084) as quality control strains which provided from Iranian reference health laboratory.

Extraction of plant material

The cranberry fresh fruit

The fruits were rinsed under ruing tap water and dried in the oven at 40°C. The methanol extraction of the active ingredient of the cranberry were carried out using the method as described by solvent extraction 10 g of air dried powder was placed in 100 ml of organic solvent (methanol) in a conical flask, plugged with cotton and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 40 mg/0.1 ml. It was stored at 4°C in airtight bottles for further studies (Mbata et al., 2008; Nair et al., 2005).

Antibacterial test

The antibacterial activity of the plant extracts were carried out on the test isolates, using the agar-gel diffusion inhibition test. In the agar-gel diffusion inhibition test as described previously (Verporte et al., 1982) 0.2 ml of a 24 h broth culture containing 1×10^6 colony forming units/ml of organism was aseptically inoculated and evenly spread using bent sterile glass rod on the surface of gelled sterile Mueller-Hinton agar plates. Three wells of about 6.0 mm diameter were ascetically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 ml) of the extract were then introduced into the wells in the plates. The minimum inhibitory concentration (MIC) of the extracts was determined for tested organisms using the twofold serial microdiluation method with saline at a final concentration ranging from 5 to 0.0024 mg/ml. A control well also was in the center with 0.01 ml of the extracting solvent. The plates were allowed on the bench for 40 min for pre-diffusion of the extract to occur plates and then incubated at 37°C for 24 h (Mbata et al., 2008; Esimone et al., 1998).

RESULTS AND DISCUSSION

There are many studies regarding antibacterial activity and usage of cranberry extract in treatment of UTI. Cranbury is a native plant in many regions of Iran but there is not any information and published data about beneficial of this product. In the present study, the antibacterial activity of cranberry extract on *E. coli*, *E. aerogenes, K. pneumoniae* and *S. aureus* was studied. The results obtained from this study showed that the methanol extract of this plant inhibits the growth of the test isolates at varying concentrations.

Antibacterial activity of various concentrations of cranberry extract against different strains of urine isolates are shown in Table 1. The MIC value of methanol extract was the same for *E. aerogenes* and *S. aureus* (0.391 mg/ml) whereas the MIC of methanol extract were 1.2500 and 0.0195 mg/ml for *E. coli* and *E. aerogenes* respectively. The lower MIC value of cranberry extract against *K. pneumoniae* in comparison to other three organisms suggests that *K. pneumoniae* showed greater sensitivity towards the extracts of the cranberry extract.

These study suggests that concentrated cranberry juice has antibacterial activity especially on uropatogens Other study by Magaarinos et al showed that cranberry juice has inhibitory effects against pathogenic microorganisms including *E. coli, Salmonella* spp, *Listeria monocytogenes P. aeruginosa* and *S. aureus*. (Magariños et al., 2008) Other study also has shown that cranberry extract reduce biofilms formation on uroepthelial cells (Cimolai et al., 2007; Jass et al., 2009). Clinical trials have shown the effectiveness of cranberry juice in treatment of urinary tract infections. In a study trimethoprime had very limited advantages over cranberry extract in prevention of recurrent UTIs in older women (McMurdo et al., 2008; Pérez-López et al., 2009).

The explanation for the usefulness of cranberry juice was thought to be the excretion of hippauric acid in the urine. Hippauric acid is a strong bactriostatic agent. Hippuric acid has potential power to acidify urine (Harkins, 2000; Raz et al., 2004; Johnson et al., 2009). More recently attention has turned to the adherence of bacteria to the epithelial cells of the urinary tract, which is necessary if the bacteria cause infection. Sobota (1984) was the first to suggest that use of cranberry juice inhibit bacterial adherence to urinary tract epithelial cells. Other studies identified 2 compounds in cranberries that inhibit *E. coli* adhesions. One is fructose, which inhibit the mannose-sensitive fimbrial adhesions, the other one is a high molecular –weight compound that inhibits the mannose –resistant adherence of uropathogenic *E. coli*

Table 1. Antibacterial activity of cranberry extract against common uropathogenes.

Concentration	*E. coli* (n = 10)	*K. pneumoniae* (n =10)	*E. aerogenes* (n = 10)	*S. aureus* (n = 10)
5.0000	-	-	-	-
2.5000	-	-	-	-
1.2500	-	-	-	-
0.6250	+	-	-	-
0.3125	+	-	-	-
0.1563	+	-	-	-
0.0781	+	-	-	-
0.391	+	-	-	-
0.0195	+	-	+	+
0.0098	+	+	+	+
0.0049	+	+	+	+
0.0024	+	+	+	+

(Zafriri et al., 1989; Dugoua et al., 2008).

The anti-adhesive property of cranberries probably helps to prevent UTI in 2 ways: first it directly prevents *E. coli* from adhering to uroepithelial cells and second, it selects for less adherent bacterial strains in the stool. A recent study showed that regular consumption of cranberry juice was also effective in cases in patients with UTI caused by antibiotic resistant bacteria (Zafriri et al., 1989).

Conclusion

In conclusion the, extract of cranberry has antibacterial activity against uropathogens including *E. coli, S aureus*, *E. aerogenes* and *K. pneumoniae*. Therefore .the potential of cranberry product to act as a non-antibiotic alternative for preventing UTI, thereby reducing the total amount of antibiotics prescribed for treatment of UTIs and preventing drug resistance.

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