

## ORIGINAL ARTICLE

## IN VITRO ACTIVITY OF *VACCINIUM MACROCARPON* (CRANBERRY) ON URINARY TRACT PATHOGENS IN UNCOMPLICATED URINARY TRACT INFECTION

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**Background:** Urinary tract infection is the most common bacterial infection in the community, mainly caused by *Escherichia coli* (*E coli*). Due to its high incidence and recurrence, problems are faced in the treatment with antibiotics. Cranberry being herbal remedy have long been the focus of interest for their beneficial effects in preventing urinary tract infections. This study was conducted to analyse *in vitro* activity of cranberry (*Vaccinium macrocarpon*) on uropathogenic *E coli* in uncomplicated urinary tract infections. **Methods:** In this laboratory based single group experimental study, anti-bacterial activity of *Vaccinium macrocarpon* concentrate on urinary tract *E coli* was investigated, *in vitro*. Ninety-six culture positive cases of different uropathogens were identified. *Vaccinium macrocarpon* concentrate at different concentrations was prepared in distilled water and put in wells punched in nutrient agar. *E coli* isolates were inoculated on the plates and incubated at 37 °C for 24 hours. A citric acid solution of the same pH as that of *Vaccinium macrocarpon* was used and put in a well on the same plate to exclude the effect of pH. **Results:** A total of 35 isolates of *E coli* were identified out of 96 culture positive specimens of urine and found sensitive to *Vaccinium macrocarpon* ( $p < 0.000$ ). Results revealed that *Vaccinium macrocarpon* has antibacterial effect against *E coli*. Furthermore the antibacterial activity of *Vaccinium macrocarpon* has dose response relationship. Acidic nature of *Vaccinium macrocarpon* due to its pH is not contributory towards its antibacterial effect. **Conclusion:** *Vaccinium macrocarpon* concentrate may be used in urinary tract infection caused by *E coli*.

**Keywords:** *Vaccinium macrocarpon*, Urinary Tract Infection, *E coli*

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### INTRODUCTION

Urinary tract infection is one of the common bacterial infection in the general population with an estimated overall incidence of 18 per 1000 persons/year.<sup>1</sup> It is also an important source of morbidity in community.<sup>2</sup> It is a serious health problem and it has been estimated that about 6 million patients visit out-patients department and about 300,000 are treated in the wards every year for urinary tract infection worldwide.<sup>3</sup>

Urinary tract infection is defined as the invasion of some part of urinary tract by microorganisms causing disease.<sup>4</sup> It may be in the form of urethritis, cystitis, pyelonephritis or asymptomatic bacteriuria. The symptoms of urinary tract infection are frequently found in women with negative effects on their quality of life, especially on the medical, physical, social, psychological and economical aspects.<sup>5</sup> It may be in the form of asymptomatic bacteriuria (ASB) which is defined as: “the isolation of bacteria from the urine in significant quantities without symptoms”. Significant bacteriuria is regarded by the microbiologists as the presence of 10<sup>5</sup> colony forming units (CFU) per milliliter of urine.<sup>6</sup> Usually the patients attending the out-patients

clinics are diagnosed on their clinical presentation and urinalysis.<sup>7</sup> Depending upon the pathology urinary tract infection is divided into uncomplicated and complicated.<sup>8</sup> Uncomplicated urinary tract infection is community acquired and found in the normal urinary tract. It is mostly caused by *E coli* in more than 80% on the cases.<sup>9</sup> The estimated annual cost of community acquired urinary tract infection is approximately 1.6 billion dollars.<sup>10</sup> Keeping in view, the problems of urinary tract infections, its high incidence, drug resistance and a very high economic burden on the society worldwide,<sup>11</sup> it is now the time to explore the alternative strategies for the management of urinary tract infections. Analysis of clinical studies strongly suggest the use of cranberry (*Vaccinium macrocarpon*) in the prophylaxis of urinary tract infection.<sup>12–14</sup> *Vaccinium macrocarpon* has some active compounds called Proanthocyanidins (PACs). They adhere to the fimbriae of the pathogenic organisms, i.e., *E coli*, and prevent its attachment to the epithelium of the urinary tract so that they are washed away.<sup>15,16</sup>

The objective of the study was to determine the sensitivity of the urinary tract pathogens to *Vaccinium macrocarpon*.

## MATERIAL AND METHODS

It was a laboratory based single group experimental study conducted in the department of Pathology, Ayub Medical College and Ayub Teaching Hospital Abbottabad from 1st February to 2<sup>nd</sup> August 2014. Patients were selected through consecutive (non-probability) sampling technique. All the patients were enrolled from the Urology department of the hospital. After explaining the study protocol, informed consent was taken from all the symptomatic patients of the urinary tract infection. Clean catch technique was used to collect the samples in sterile containers.

For direct microscopy 50 ml of well mixed un-centrifuged urine was viewed under the high power objective. The presence of >5 pus cells/HPF was considered for culture purposes. The samples were inoculated on CLED media and incubated at 37°C for 24 hours. *E coli* was identified on the basis of cultural characteristics and colonial morphology, and API 20E standardized identification system.<sup>17,18</sup>

Cranberry concentrate (250 mg) was dissolved in 50 ml of distilled water to make stock solution. It was made fresh every time. The pH of stock solution was maintained at 3.9. Nutrient agar plates were inoculated with *E coli* isolates and 4 wells of 7 mm sizes each were punched in the media.<sup>19</sup> *Vaccinium macrocarpon* concentrate solution was put in 3 wells in different concentrations (25 µg, 50 µg and 100 µg) and the volume of *Vaccinium macrocarpon* solution was kept equal (25 µl) by adding distilled water.<sup>20</sup> A solution of citric acid of the same pH was prepared and put in 4<sup>th</sup> well on the same plate to exclude the effect of pH of *Vaccinium macrocarpon* on *E coli*. After this the plates were incubated for 24 hours at 37 °C. Sensitivity was noted by measuring the zone of inhibition around all the 4 wells. The sensitivity procedure was repeated 3 times in every case and their means were taken. A strain of *E coli* ATCC 25922 was used for control purposes.

The data was entered and analysed using SPSS 16.0. The antibacterial activity (zone of inhibition in mm) was expressed as mean±standard deviation. Difference between sensitivities was tested using Analysis of variance (ANOVA). Post hoc Tukey's test was applied to observe which group mean differs. The *p*-value was calculated to note the significance of the results. A *p*-value of ≤0.05 was considered as statistically significant.

## RESULTS

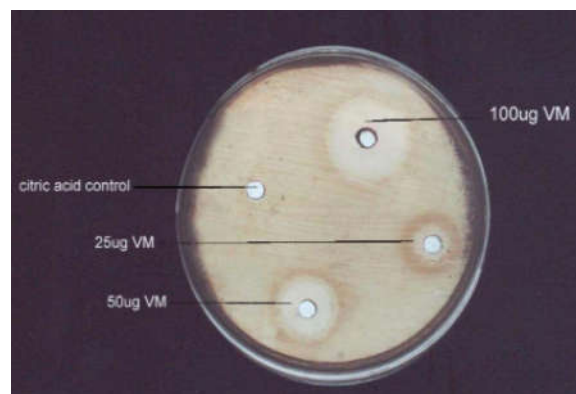
A total of 35 cases of *E coli* were isolated from 96 specimens. The remaining cases showed other uropathogens, i.e., *Staphylococcus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Enterobacter* and *Enterococcus*

*fecalis*. On CLED agar *E coli* fermented lactose, producing smooth yellow colonies.

Sensitivity test was performed using 3 concentrations of *Vaccinium macrocarpon* concentrate. Figure-1 shows the effect of these concentrations and citric acid control on growth of *E coli*. Zone of inhibition (mean±SD) was 9.77±2.10, 12.55±2.13 and 15.63±2.03 mm with 25µg, 50µg and 100 µg respectively Data represents mean±SD of 35 clinical isolates, each inoculated on three plates. Comparison of effect of three concentrations on *E coli* growth made by ANOVA, revealed that difference between three concentrations was significant (*p*<0.00)

Multiple comparisons of three concentrations revealed that zone of inhibition was significantly high with 100 µg when compared to 25 and 50 µg. (*p*<0.00).

Citric acid solution having the same pH as that of *Vaccinium macrocarpon* was used to exclude the effect of pH on the inhibition zone of *Vaccinium macrocarpon*.<sup>20</sup> It produced negligible zone of inhibition in all the cases.



**Figure-1: Effect of three concentrations of *Vaccinium macrocarpon* on *E coli* growth**

## DISCUSSION

Presently *Vaccinium macrocarpon* (cranberry) is widely used in the prevention of urinary tract infection.<sup>11-13</sup> It is available in the form of extract, capsules and juices for this purpose. Scientific work is required to better place the role of cranberry (*Vaccinium macrocarpon*) in the management of urinary tract infection.

*E coli* was the most common pathogen isolated in our study. A total of 35 cases of *E coli* were identified. This is in accordance with most of the studies showing *E coli* to be the most dominant uropathogen.<sup>21-24</sup>

Our study clearly indicated that *Vaccinium macrocarpon* had antibacterial activity against the most common uropathogen that is *E coli* and had a dose-response relationship. Sengupta *et al.* performed

a dose dependent clinical trial using whole cranberry powder in the subjects whose urine was culture positive with *E coli*.<sup>25</sup> There was a significant decrease ( $p<0.05$ ) in *E coli* culture positive subjects both in the low and high dose groups, i.e., 500mg and 1000mg cranberry consumption in a dose dependent manner. This study indicated the efficacy of cranberry in UTI with *E coli* and a dose response relationship is also produced as in our study.

In another study by Howell *et al.* dose response relation was seen after consumption of cranberry powder standardized for PACs.<sup>26</sup> There was significant reduction in the *E coli* adhesions and virulence. A significant dose dependent reduction in bacterial adherence of *E coli in vitro* and *in vivo* was noted after consumption of 108 and 36 mg of cranberry ( $p<0.001$ ) by Lavigne *et al.*<sup>14</sup>

Tempera *et al*<sup>27</sup> performed a study in 2 groups of female volunteers, having a negative and positive history for recurrent cystitis. Urine samples were taken before and after the treatment with cranberry extracts. Significant reduction of *E coli* bacterial adherence was observed in women who received cranberry extracts ( $p<0.001$ ). This study showed that the consumption of cranberry extract in suitable amounts can have an anti-adhesive activity on uropathogenic *E coli*. Gupta *et al.*<sup>28</sup> in their study also concluded reduction of *E coli* adherence to the bladder epithelial cells and this effect had a dose dependent relationship in a similar way as our findings.

In summary our study confirmed the inhibitory effects of *Vaccinium macrocarpon* against *E coli* with a clear dose dependent response.

## CONCLUSION

It is concluded that *Vaccinium macrocarpon* has antibacterial effect against uropathogenic *E coli* which is the most common cause of urinary tract infection in community. It is expected that the use of *Vaccinium macrocarpon* which is comparatively a cost effective, alternative, free from the hazards of antibiotics and resistance, will prove very beneficial for the community at large. It will also play important role in the management of UTI in pregnancy where the classical drugs are avoided.

## AUTHOR'S CONTRIBUTION

SM: Principle author, conceived the idea, data collection, literature review, write-up, SC, ST: Supervised the study, proof red the manuscript, MAA: Literature review, manuscript writing, MSW, MS: statistical analysis, bibliography.

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