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Effect of *Vaccinium vitis-idaea* tea and *Arctostaphylos uva-ursi* tea on growth of causative agents of urinary tract infections

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Abstract - **Urinary tract infections pose a serious problem to people, both in the hospital environment and outside world. They are characterized by high mortality and ability to cause health problems in areas of the human body other than the urinary tract. It has been long clinical practice to treat these infections with antibiotics, a tactic made very ineffective with the advent of antibiotic-resistant microbial strains. The research has turned to alternative modes of treatment, such as use of herbal remedies to combat urinary tract infections. Effect of two types of herbal teas was observed through use of broth microdilution assay, to test varying concentrations of teas on the growth of selected microorganisms. Results were verified by assessment of colony growth on Mueller Hinton Agar plates. Tested microorganisms exhibited very dense colony growth. Similarity of conditions between urinary retention and conditions under which microorganisms were cultured in 96-well plates possible reason for density of growth. Methods with higher degree of confidence in treatment of urinary tract infections could likely be the combination of antibiotics with herbal teas.**

Keywords: **antibiotic resistance, *Arctostaphylos uva-ursi*, broth microdilution assay, urinary tract infections, *Vaccinium vitis-idaea***

1. Introduction

Urinary tract infections (UTIs) are, by definition, categorized as diseases according to clinical symptoms, laboratory indicators and microbiological findings, and are most often caused by various bacterial species. They are designated as cystitis or as infections affecting lower urinary tract, or as prostatitis. Based on clinical factors, they are classified in different groups: acute uncomplicated cystitis, urinary tract infections caused by indwelling catheters, recurrent cystitis in young women, urinary tract infections in men, complicated urinary tract infections and asymptomatic bacteriuria [1,2]. In the cases of often repeated and inadequately treated urinary tract infections, these infections may become permanent or chronic diseases [3], which can lead to development of other types of diseases, and further increase the already high mortality rate exhibited by urinary tract infections [4].

Antibiotics are the most common method of treatment for urinary tract infections. Some of the antibiotics have been highly effective in treatment of UTIs, while others had little to no effect. However, the misuse of antibiotics has led to development of bacterial resistance, where increasing numbers of antibiotics have no effect in treatment of urinary tract infections [5,6]. This has led to discovery of alternative methods of treatment for UTIs, such as use of herbal products to combat these infections. Certain herbs have exhibited

ability to prevent bacterial invasion into the urinary tract (*Agropyron repens*), to impede adhesion of bacteria to bladder walls (*Urtica* spp., *Betula* spp.), and to inhibit formation of bacterial colonies [7,8]. Bioactive compounds of *Vaccinium vitis-idaea* (lingonberry, mountain cranberry) have demonstrated antimicrobial, anti-inflammatory and antioxidative activity [9,10], while *Arctostaphylos uva-ursi* (bearberry) was also noted to have anti-inflammatory effect in lower urinary tract [11].

The objective of this experiment was to determine the effect of *Vaccinium vitis-idaea* tea and *Artctostaphylos uva-ursi* tea on growth of primarily bacteria which are responsible for the development of various urinary tract infections.

2. Materials and methods

Microorganisms used in this experiment were *Escherichia coli* ATCC 14169, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 12493, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 and *Pseudomonas aeruginosa* ATCC 27853. They were cultured and kept in Tryptic Soy Broth (TSB) containing 50% glycerol, since it was necessary to store them at -80˚C.

The herbal teas chosen for this experiment were the *Vaccinium vitis-idaea* tea and *Arctostaphylos uva-ursi* tea, both of which were acquired from herbal pharmacy ″Faveda″ in Sarajevo. Teas were made and tested in two different concentrations: in concentration recommended on tea packaging and in concentration two times stronger than the recommended one.

Since the objective of this experiment was to study the effect of teas on bacterial growth, it was necessary to detect minimal inhibitory concentration (MIC) for both teas. Broth microdilution assay was used for detection of minimal inhibitory concentrations for each of the tested microorganisms. Tested microorganisms were first grown in the medium Tryptic Soy Broth until desired growth phase. In each well of microtiter plate a 100µl of medium was pipetted. Tea, also made in TSB medium, was added in column 3 (100µl) and mixed using micropipette to suck the liquid up and down a few times. Then, a 100µl of dilution was transferred from column 3 to column 4 and mixed using micropipette. The procedure was repeated until the last column, so the concentration of tea was lessened by half in each subsequent well. Following that, a 10 µl of microorganism was added (each tested microorganism to an individual plate) in columns 2 to 12. Column 1 did not contain microbes, as it represented negative control. Plates were incubated overnight at 37˚C.

After incubation, microbes from wells containing different concentrations were inoculated onto Mueller Hinton Agar plates and incubated overnight at 37˚C. Bacterial growth was observed from the plates and it was visually assessed and numerically classified by the density of formed colonies, as shown in Table 1.

3. Results

Bacterial growth was assessed from Mueller Hinton Agar plates. The objective was to find minimal inhibitory concentration, so microorganisms were inoculated only from certain wells of 96-well plates (where the probability of finding minimal inhibitory concentrations seemed the highest). Starting concentrations of tea were the recommended concentration (RC) on the tea packaging (Table 2 and 3),

adjusted for the volume of the well, and the second concentration that was twice as strong as the recommended one (Tables 4 and 5). Using broth microdilution assay as described in the previous section, the concentration of tea was lowered by half in each subsequent well. Recommended concentration on the tea packaging was one coffee spoon (10 g) per 200 ml of liquid (TSB, in this case).

Table 1. Evaluation of microbial growth

Evaluation	**Area of plate covered by microbial growth**
10^7	100%
10^6	85%-90%
10^5	70%-75%
10^4	50%
10^3	35%
10^2	15%
10^1	<15%
Sterile	0%

Table 2. Effect of recommended concentration of *Vaccinium vitis-idaea* tea on bacterial growth

	0,5*RC	**0,25*RC**	**0,125*RC**	**0,0625*RC**
***E. faecalis* 29212**	10^7	10^7	/	/
***C. albicans* 10231**	10^7	10^7	/	/
***P. aeruginosa* 27853**	10^7	10^7	/	/
***E. coli* 25922**	10^7	10^7	/	/
***E. coli* 14169**	10^7	10^7	10^7	10^7
***S. aureus* 25923**	10^7	10^7	/	/
***S. aureus* 12693**	10^7	10^7	/	/
***S. aureus* 6538**	10^7	10^7	/	/

Table 3. Effect of recommended concentration of *Arctostaphylos uva-ursi* tea on bacterial growth

	0,5*RC	**0,25*RC**	**0,125*RC**	**0,0625*RC**
***E. faecalis* 29212**	10^7	10^6	/	/
***C. albicans* 10231**	10^6	10^7	10^7	10^7

***P. aeruginosa* 27853**	10^6	10^7	10^7	/
***E. coli* 25922**	10^7	10^7	/	/
***E. coli* 14169**	10^7	10^7	/	/
***S. aureus* 25923**	10^7	10^7	/	/
***S. aureus* 12693**	10^6	10^6	10^6	10^6
***S. aureus* 6538**	/	10^7	10^7	10^7

Table 4. Effect of second concentration of *Vaccinium vitis-idaea* tea on bacterial growth

	RC	**0,5*RC**	**0,25*RC**
***E. faecalis* 29212**	10^7	10^7	10^7
***C. albicans* 10231**	10^7	/	/
***P. aeruginosa* 27853**	10^7	10^7	/
***E. coli* 25922**	10^7	/	/
***E. coli* 14169**	10^7	10^7	/
***S. aureus* 25923**	10^7	10^7	/
***S. aureus* 12693**	10^7	10^7	/
***S. aureus* 6538**	10^7	10^7	/

Table 5. Effect of second concentration of *Arctostaphylos uva-ursi* tea on bacterial growth

	RC	**0,5*RC**	**0,25*RC**	**0,125*RC**
***E. faecalis* 29212**	/	10^7	10^7	10^7
***C. albicans* 10231**	10^7	10^7	/	/
***P. aeruginosa* 27853**	10^7	10^7	/	/
***E. coli* 25922**	10^7	10^7	/	/
***E. coli* 14169**	10^6	10^7	10^7	/
***S. aureus* 25923**	10^7	10^7	/	/
***S. aureus* 12693**	10^7	10^7	10^7	/

***S. aureus* 6538**	10^7	10^7	/	/

As observed from results presented in tables above, microorganisms have demonstrated formation of highly dense bacterial colonies, covering the entirety of inoculated surface of agar plates, both in case of recommended tea concentrations and the second tested concentration.

4. Discussion

Urinary tract infections present a great issue, since they are capable of causing a multitude of other health complications, as well as possessing a high mortality rate, which is 3% in women and 1% in men [4]. A fact which further exacerbates this problem is the ability of microorganisms to form structures such as biofilm and to produce antibiotic-negating enzymes, resulting in bacteria being capable of withstanding and surviving antibiotic activity, which is a characteristic termed antibiotic resistance. Microbial strains resistant to a variety of different antibiotics, designated as multidrug-resistant strains, have also emerged [6]. It is likewise highly concerning that the discovery of new types of antibiotics capable of successfully combating UTIs has substantially decreased, which has led researchers to turn their investigations to alternative methods of treatment for UTIs. One of these alternative methods is the use of herbal products, since some of their compounds are capable of mitigating or eliminating the symptoms of UTIs.

In this experiment, we observed the growth of microorganisms under influence of herbal tea. One of the objectives was to determine whether tea alone has the ability to impede the growth of bacteria. Broth microdilution assay was used to test the effect of different concentrations of tea on microbial growth, because it provides quantitative data and it is possible to use this method in any laboratory. Inoculation onto Mueller Hinton Agar plates was used to verify the results. After overnight incubation at 37˚C, it was observed that tested microorganisms exhibited highly dense growth, under both normal and doubled concentrations. Conclusion is that this might have happened because the conditions in 96-well plates, during the performance of broth microdilution assay, were similar to those occurring in urinary retention. Urinary retention is an inability to completely remove urine from the bladder, which can lead to kidney and bladder damage, and development of urinary tract infections. Bacteria within urine, which are normally mostly harmless since they are removed with urine in healthy people, in case of urinary retention are able to accumulate and cause the development of various urinary tract infections [12]. Increased liquid intake was reported to have beneficial effects for the patients affected by UTIs, since it causes dilution of metabolic waste products which serve as nutrients for microorganisms [13]. In addition, diuresis that results from increased liquid intake yields the benefit of so-called mechanical ″flushing″, meaning that bacteria are physically removed from the urinary tract along with urine, denying them the time necessary to accumulate and cause further issues. As noted in introduction, some herbs possess compounds which exhibit the ability to prevent bacterial adhesion or inhibit bacterial growth [7,8]. ″Flushing″ the urinary tract with herbal tea used for alleviating the symptoms of UTIs can possibly exhibit higher effectiveness in treatment of these infections.

Herbal teas used in this experiment consisted of dried leaves (in case of *A. uva-ursi*) and dried fruits or *baccae* (in case of *V. vitis-idaea*). This might have contributed to their low effect on microbial growth.

Extracts of fresh leaves and fruits could be more effective in treatment of urinary tract infections. Despite not being able to inhibit the growth of microbes on their own, both *V. vitis-idaea* and *A. uva-ursi* have properties which make them useful in treatment of UTIs, such as the anti-inflammatory activity and antioxidative activity, as well as other properties. Their use in treatment of UTIs is a subject of many studies [9-11,14-17].

Antibiotic resistance of microorganisms responsible for development of UTIs extends to some of the most commonly used antibiotics in treatment of UTIs. However, there are several antibiotics which still demonstrate significant effect in inhibiting the growth of UTI causative agents. There is a high possibility that the combined effect of antibiotics and herbal teas could result in greater effectiveness of treatment of urinary tract infections. Further research into combined use of antibiotics and herbal remedies should be done, with aim of reduction of unnecessary antibiotic use, which leads to the development of antibiotic resistance.

5. References

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