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Full Length Research Paper

Antibacterial and antioxidant activities of bilberry (*Vaccinium myrtillus* L.) *in vitro*

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Antibacterial and antioxidant activity, total phenolic and flavonoid concentrations of water, ethanol and ethyl acetate extract of fruits and leaves of *Vaccinium myrtillus* L. were studied. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been determined. Testing was performed on 30 clinical isolates, including strains of *Escherichia coli*, *Enterococcus faecalis* and *Proteus vulgaris*. The values for MIC were in the range from 5 to 40 mg/ml. The most sensitive bacterial strain was *Enterococcus faecalis* MF-Ef8 strain. The ethanol extract of fruits of *V. myrtillus* was found the most active. The total phenolic content was determined using Folin-Ciocalteu reagent and ranged between 31.44 to 119.17 mg GAE/g. The concentration of flavonoids in extracts was determined and the highest amount was in ethyl acetate extract of leaves of *V. myrtillus*. Antioxidant activity was monitored spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. The highest capacity to neutralize DPPH radicals (94%RSA) was found in the ethanol extract from fruits and in the water extract from leaves of *V. myrtillus*. The results of the total phenolic content determination of the examined extracts indicate that bilberry extracts are a rich source of phenolic compounds and also possess a significant antioxidant activity and moderate antibacterial activity.

Key words: Plant extracts, phenols, flavonoids, *Escherichia coli*, *Enterococcus faecalis*, *Proteus vulgaris.*

INTRODUCTION

Bilberry (*Vaccinium myrtillus* L.) is a deciduous shrub growing to 50 cm, with elliptical leaves. The flowers are single on short stems. The fruits are berries, globular, dark purple, juicy and sour (Kovačević, 2002). In many European countries, the bilberry is one of the most economically important wild berry species (Tomićević et al., 2011).

Different *Vaccinium* species (*V. myrtillus*, *V. vitis-idaea*, *V. macrocarpon*) are used in phytomedicine and pharmacy. Fruits of these species may have additional health benefits as they are rich in phytochemicals such as anthocyanins which are responsible for their red, purple and blue colours. Previous studies demonstrated that plants with high content of anthocyanins, had

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Abbreviations: MIC, minimum inhibitory concentration; **MBC,** minimum bactericidal concentration; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **DMSO,** dimethyl sulfoxide.

significant antibacterial effect (Hearst et al., 2010). *In vitro* studies indicate that anthocyanins and other polyphenols in berries could be substantial in the treatment of heart disease (Basu et al., 2010; Routray and Orsat, 2011), including antioxidant (Denev et al., 2010) and antiadhesion activity against bacteria (Huttunen et al., 2011). Compounds such as quercetin and phenolic acids could play an important role in the possible health effects of berries (Paredes-López et al., 2010). Bilberry fruits contain up to 10% tannins, anthocyanins, organic acids, and pectins. The leaves contain tannins, flavonoids, and a small amount of arbutin. In traditional medicine, fruits of *V*. *myrtillus* are much used as antidiarreal while leaves are used as astringent and diuretic (Sarić, 1989). In addition, *V*. *myrtillus* leaf infusions are traditionally used as a folk medicine treatment of diabetes, although recent studies show weak results (Helmstädter and Schuster, 2010). Urinary tract infections are among the most common bacterial infections acquired in the community and in hospitals. Treatment of these infections with antibiotics leads to a more rapid resolution of symptoms and is more likely to clear bacteriuria, but also selects for resistant uropathogens and commensal bacteria. So, it is advisable to seek alternative methods of prevention and treatment of urinary tract infections (Foxman, 2010).

Although there are papers on phytochemical analysis of leaves and fruits of this plant (Jaakola et al., 2002; Jaakola et al., 2004), the aim of this study was to determine and compare the antibacterial and antioxidant activity of different extracts of fruits and leaves of this plant collected on Borja Mountain (RS, Bosnia and Herzegovina, W. Balkans). The second aim of this paper was to determine the total phenol and flavonoid content in water, ethanol and ethyl acetate extracts using spectrophotometric methods.

MATERIALS AND METHODS

Plant material

In summer of 2009, ripe fruits and leaves of *V*. *myrtillus* were collected from natural populations on Borja mountain in the region of Teslić city in southeast Republic of Srpska, Bosnia and Herzegovina (position: 44°35′N, 17°35′E, altitude: 180.00 m, habitat: coniferous forest). Plants were identified and confirmed and voucher specimens were deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The collected plant material was air dried in darkness at ambient temperature (20°C). The dried plant material was cut up and stored in paper bags until needed.

Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from Zorka pharma" Šabac, Serbia. Gallic acid, rutin hydrate and aluminium chloride hexahydrate ($AlCl_3$) were purchased from Acros Organics, New Jersey, USA. Chlorogenic acid, Folin-Ciocalteu phenol reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co, St Louis, MO, USA.

Preparation of plant extracts

Prepared plant material (10g) was transferred to dark-coloured flasks with 200 ml of solvent (water, ethanol, ethyl acetate) and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40°C using Rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored at -20°C.

Determination of total phenolic contents in the plant extracts

The bilberry extracts were analyzed for total phenolics spectrophotometrically by the Folin-Ciocalteu procedure (Wootton-Beard et al., 2011). The reaction mixture was prepared by mixing 0.2 ml of methanolic solution of extract (1 mg/ml) and 1.5 ml of 1:10 Folin-Ciocalteu reagent dissolved in water. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml 6% $NaCO_3$ solution. After incubation for 90 min at room temperature in darkness, the absorbance of the mixture was read at 725 nm against a blank using spectrophotometer. The blank was prepared with methanol instead of extract solution. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for gallic acid which was used for calibration of standard curve. Total phenol content is reported as gallic acid equivalents by reference to linear equation of the standard curve ($y = 0.008x + 0.0077$, $R^2 = 0.998$). Then, the total phenolic content was expressed as gallic acid equivalents in miligrams per gram of extract (mg GAE/g of extract).

Determination of flavonoid concentrations in the plant extracts

The concentrations of flavonoids was determined using spectrophotometric method with aluminium chloride (Quettier-Deleu et al., 2000). The sample contained 1 ml of methanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The mixture was vigorously shaken, and after 10 min of incubation at room temperature, the absorbance *versus* a prepared blank was read at 430 nm using spectrophotometer. The samples were prepared in triplicate and the mean value of absorbance was obtained. Rutin was used as a standard for calibration of standard curve. The concentrations of flavonoids were calculated from the linear equation of standard curve ($y = 0.021x + 0.040$, $R^2 = 0.999$). Then, the concentrations of flavonoids were expressed as miligram of rutin equivalent per gram of extract (mg of RUE/g of extract).

Evaluation of DPPH scavenging activity

The ability of the plant extract to scavenge DPPH free radicals was assessed using the method described by Takao et al. (1994). The stock solution of the plant extract was prepared in methanol to achieve the concentration of 2000 µg/ml. Further, two-fold dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5 µg/ml. Diluted solutions of extract (2 ml each) were mixed with 2 ml of DPPH methanolic solution (80 µg/ml). After 30 min in darkness at room temperature, the absorbance was recorded in a spectrophotometer at 517 nm. The control samples contained 2 ml of methanol added to 2 ml of DPPH solution. Chlorogenic acid was used as a positive control. The experiment was performed in triplicate. Scavenging activity is expressed as the inhibition percentage calculated using the following equation:

Scavenging activity (%) = $100 \times [(A_{control} - A_{sample}) / A_{control})]$.

Table 1. Total phenolic contents and concentrations of flavonoids in fruits of *V. myrtillus* extracts.

Type of extract	Total phenolic content[1] (mg GAE/g of extract)	Flavonoid concentration[1] (mg RUE/g of extract)
Water	31.44 ± 0.17	5.20 ± 0.08
Ethanol	40.32 ± 0.24	10.06 ± 0.11
Ethyl acetate	99.34 ± 1.12	23.26 ± 0.21

[1]Values represent mean ± standard deviation.

Table 2. Total phenolic contents and concentrations of flavonoids from leaves of *V. myrtillus* extracts.

Type of extract	Total phenolic content[1] (mg GAE/g of extract)	Flavonoid concentration[1] (mg RUE/g extract)
Water	119.17 ± 0.52	43.08 ± 0.68
Ethanol	107.79 ± 1.23	81.98 ± 0.00
Ethyl acetate	66.76 ± 0.78	94.49 ± 3.61

[1]Values represent mean ± standard deviation

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

Test bacterial strains

Antibacterial activity of water, ethanol and ethyl acetate extract from dried fruits and leaves of *V. myrtillus* was tested against 30 strains of bacteria including ten strains of *Escherichia coli* (Mf-Ec1, Mf-Ec2, Mf-Ec3, Mf-Ec4, Mf-Ec5, Mf-Ec6, Mf-Ec7, Mf-Ec8, Mf-Ec9, Mf-Ec10), ten strains of *Enterococcus faecalis* (Mf-Ef1, Mf-Ef2, Mf-Ef3, Mf-Ef4, Mf-Ef5, Mf-Ef6, Mf-Ef7, Mf-Ef8, Mf-Ef9, Mf-Ef10) and ten strains of *Proteus vulgaris* (Mf-Pv1, Mf-Pv2, Mf-Pv3, Mf-Pv4, Mf-Pv5, Mf-Pv6, Mf-Pv7, Mf-Pv8, Mf-Pv9, Mf-Pv10). The *E. coli* strains and *P. vulgaris* strains represented Gram-negative bacteria. Bacterial strains of *E. faecalis* were Gram-positive. All clinical isolates were a generous gift from the Institute of Public Health, Banja Luka.

Suspension preparation

The original density of the bacterial suspension was 0.5 Mc Farland after which the additional dilution in saline at the proportion of 1:10 was made. The final concentration of the bacteria in the test tubes was 10^6 colony forming units (CFU)/ml.

Macrodilution method

The minimum inhibitory concentration (MIC) of the extracts was determined by the tube dilution method through the series of dilutions (NCCLS, 1997). In the test tubes filled with the Mueller Hinton broth, the solution of the extracts was added and the series of double dilutes was made. In each of the test tubes, the 100 µl of the suspension of the tested bacteria was added. The 24 h incubation at the temperature of 37°C was conducted. The minimum bactericidal concentration (MBC) is the lowest concentration of the tested substance which has the bactericidal effect. These values were collected by inoculation of the Mueller Hinton agar with the test tube content; it was the content from the test tubes in which the MIC was found and all the test tubes had more than the MIC found. Amoxicillin was used as a positive control. Whereas the extracts were dissolved in 10% dimethyl sulfoxide (DMSO), solvent control test was performed to study the effects of 10% DMSO on the growth of bacterial strains. It was observed that 10% DMSO did not inhibit the growth of bacteria.

Statistical analysis

SPSS program was applied only when the mean was calculated. Data are presented as means ± standard deviations.

RESULTS

Total phenolic content and flavonoid concentrations

The results of total phenolic content in the plant extracts are presented in Tables 1 and 2. The total phenolic content was expressed as gallic acid equivalents and ranged from 31.44 to 99.34 mg GAE/g in the extracts of fruits. The extracts obtained from leaves of *V. myrtillus* were richer in phenolic active compounds than the extracts of the fruit. The water leaves extract had the highest phenolic content with 119.17 mg of GAE/g of extract. The summary of quantities of flavonoids identified in the tested extracts is shown in Tables 1 and 2. The concentration of flavonoids in various extracts of *V. myrtillus* was determined using spectrophotometric met-hod with aluminium chloride. The content of flavonoids was expressed as rutin equivalent. Total flavonoid content

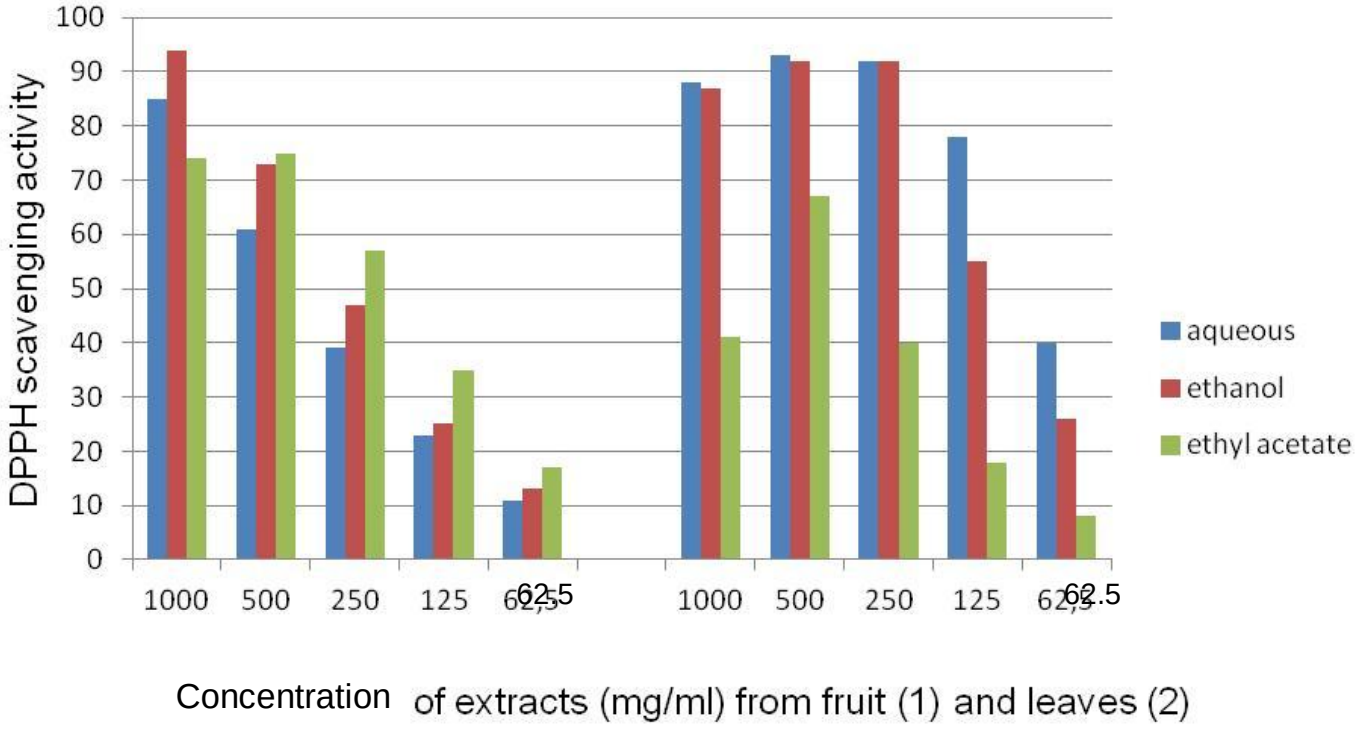

Figure 1. Antioxidant (DPPH scavenging, %RSA) activity of investigated fruit (1) and leaves (2) extracts of *V. myrtillus*

in plant extracts ranged between 5.20 to 94.49 mg RUE/g of extract. High concentrations of flavonoids were measured in ethyl acetate and ethanol extracts from leaves of *V. myrtillus*. Ethyl acetate is a low toxic solvent (Li at al., 2010).

Antioxidant activity

The antioxidant activity of six different extracts from *V. myrtillus* is expressed in Figure 1. The largest capacity in neutralization of DPPH radicals was measured in the ethanol extract from fruits of *V. myrtillus* and water extract from leaves of bilberry. In measuring total phenolic content, water extract showed the highest values. The extracts performing the highest antioxidant activity had the highest concentration of phenols.

Antibacterial activity

The results of *in vitro* antibacterial activities of water, ethanol and ethyl acetate extracts from fruits and leaves of *V. myrtillus* against 30 strains of Gram-positive and Gram-negative pathogenic bacteria are presented in Tables 3 and 4. Extracts from *V. myrtillus* inhibited several urinary pathogens extracted from urine samples. In general, the activity of extracts depended both on the species of bacteria and on the type and concentration of extract and varied between 10 and 40 mg/ml. The ethanol and the ethyl acetate extract of fruits showed better activity than water extract in relation to strains of *E. faecalis* and *P. vulgaris*. The all tested extract from leaves showed similar activity against strains of *E. coli, E. faecalis* and *P. vulgaris*. The most sensitive strain of tested bacteria towards ethanol extracts of fruits and leaves of *V. myrtillus* was strain of *E. faecalis* MFBL-Ef8. On the other hand, strains of *E. coli* were the most resistant. All tested extracts demonstrated approximately similar activity in relation to the tested different strains of same bacteria.

DISCUSSION

Based on the obtained values of the concentration of flavonoids in the examined extracts of *V. myrtillus*, it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005), different locations from which the plant came and plant growing conditions.

The ethanol extract from fruits and water extract from leaves of *V. myrtillus* had high concentration of total phenols, which is in correlation with the intense antioxidant activity of extracts.

Table 3. Antibacterial activities of water, ethanol and ethyl acetate extracts from fruits of *V. myrtillus* against tested strains of bacteria based on macrodilution method.

Species	Aqueous extract		Ethanol extract		Ethyl acetate extract		Amoxicillin	
	MIC[1]	MBC[2]	MIC	MBC	MIC	MBC	MIC	MBC
E. coli Mf-Ec1	20	40	40	>40	40	>40	1000	2000
E. coli Mf-Ec2	20	40	40	>40	40	>40	4000	8000
E. coli Mf-Ec3	20	40	40	>40	40	>40	4	8
E. coli Mf-Ec4	20	40	40	>40	40	>40	2	4
E. coli Mf-Ec5	20	40	40	>40	40	>40	2000	4000
E. coli Mf-Ec6	20	40	40	>40	40	>40	2000	4000
E. coli Mf-Ec7	20	40	40	>40	40	>40	4	4
E. coli Mf-Ec8	20	40	40	>40	40	>40	1000	2000
E. coli Mf-Ec9	20	40	40	>40	40	>40	4000	8000
E. coli Mf-Ec10	20	40	40	>40	40	>40	1000	2000
E. faecalis Mf-Ef1	20	>40	10	20	10	20	0.977	125
E. faecalis Mf-Ef2	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef3	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef4	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef5	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef6	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef7	20	>40	10	20	10	20	0.977	>125
E. faecalis Mf-Ef8	20	>40	5	10	10	20	0.488	125
E. faecalis Mf-Ef9	20	>40	10	20	10	20	0.488	>125
E. faecalis Mf-Ef10	20	>40	10	20	10	20	0.488	>125
P. vulgaris Mf-Ef1	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef2	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef3	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef4	20	40	10	20	10	40	0.977	7.812
P. vulgaris Mf-Ef5	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef6	20	40	10	20	10	40	0.977	7.812
P. vulgaris Mf-Ef7	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef8	20	40	10	20	10	40	2000	>4000
P. vulgaris Mf-Ef9	20	40	10	20	10	40	>4000	>4000
P. vulgaris Mf-Ef10	20	40	10	20	10	40	>4000	>4000

[1]Minimum inhibitory concentration (MIC) and [2]minimum bactericidal concentration (MBC) values are given as mg/ml for plant extracts and µg/ml for antibiotic.

Berry fruits are very rich sources of bioactive compounds as phenolics and organic acid. Comparison of the antibacterial effects of extracts from fruits and extracts from leaves of *V. myrtillus* showed that phenolic compounds were only partially responsible for the growth inhibition of bacterial strains and most of the antibacterial effects probably originated from other compounds such as organic acids.

Previous studies support our research data to a great extent (Puupponen-Pimia et al., 2005b; Badjakov et al., 2008). Differences in the results related to some strains of bacteria can also be explained by different sensitivity of tested species of bacteria, different methods of testing and the solvents used. In previous study, Puupponen-Pimia et al. (2005a) investigated the antimicrobial activity of extracts from fruits of bilberry on *Salmonella enterica* and *Staphylococcus aureus* and determined that *V. myrtillus* possess clear antimicrobial effects on these bacteria.

E. coli is a bacterium that is commonly found in the intestine. Most *E. coli* strains are commensals. However, some strains can cause severe disease (Brzuszkiewicz et al., 2011). Enterococci are Gram-positive commensals of the gastrointestinal tract of humans. *E. faecalis* is an important cause of infections in hospitalized, immunocompromized patients (Schaik et al., 2010). *Proteus* species have an important place in environmental pollution bioremediation. *Proteus* sp. from various environments are able to utilize and degrade many variety of toxic materials. Many researches have reported the potential biodegradation

Table 4. Antibacterial activities of water, ethanol and ethyl acetate extracts from leaves of *V. myrtillus* against tested strains of bacteria based on macrodilution method.

Species	Aqueous extract		Ethanol extract		Ethyl acetate extract		Amoxicillin	
	MIC[1]	MBC[2]	MIC	MBC	MIC	MBC	MIC	MBC
E. coli Mf-Ec1	40	>40	40	>40	20	40	1000	2000
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E. coli Mf-Ec3	40	>40	40	>40	20	40	4	8
E. coli Mf-Ec4	40	>40	40	>40	20	40	2	4
E. coli Mf-Ec5	40	>40	40	>40	20	40	2000	4000
E. coli Mf-Ec6	40	>40	40	>40	20	40	2000	4000
E. coli Mf-Ec7	40	>40	40	>40	20	40	4	4
E. coli Mf-Ec8	40	>40	40	>40	20	40	1000	2000
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E. faecalis Mf-Ef1	40	>40	20	40	40	>40	0.977	125
E. faecalis Mf-Ef2	40	>40	20	40	40	>40	0.488	>125
E. faecalis Mf-Ef3	40	>40	20	40	40	>40	0.488	>125
E. faecalis Mf-Ef4	20	>40	20	40	40	>40	0.488	>125
E. faecalis Mf-Ef5	40	>40	20	40	40	>40	0.488	>125
E. faecalis Mf-Ef6	40	>40	20	40	40	>40	0.488	>125
E. faecalis Mf-Ef7	20	>40	20	40	40	>40	0.977	>125
E. faecalis Mf-Ef8	20	>40	20	20	40	>40	0.488	125
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E. faecalis Mf-Ef10	20	>40	20	40	40	>40	0.488	>125
P. vulgaris Mf-Ef1	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef2	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef3	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef4	20	>40	20	40	40	>40	0.977	7.812
P. vulgaris Mf-Ef5	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef6	20	>40	20	40	40	>40	0.977	7.812
P. vulgaris Mf-Ef7	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef8	20	>40	20	40	40	>40	2000	>4000
P. vulgaris Mf-Ef9	20	>40	20	40	40	>40	>4000	>4000
P. vulgaris Mf-Ef10	20	>40	20	40	40	>40	>4000	>4000

[1]Minimum inhibitory concentration (MIC) and [2]minimum bactericidal concentration (MBC) values are given as mg/ml for plant ext racts and µg/ml for antibiotic.

of xenobiotics by the members of *Proteus* genus (Ceyhan, 2012).

The results of our research indicate good antibacterial activity of fruits of *V. myrtillus*. Thus, fruits and leaves of *V. myrtillus* should be considered a potential source of antibacterial substances. Anthocyanins and phenolic acid derivates were identified in previous investigations on *V. myrtillus*. Several studies demonstrated strong antio-xidant activity of these phenolic compounds (Nakajima et al., 2004; Viljanen et al., 2004; Ehala et al., 2005). Recent studies confirm these findings also for phenolic composition and antioxidant capacity of bilberry leaves (Martz et al., 2010).

Literature data indicate that the *V. myrtillus* is a medicinal plant in traditional medicine and it is applied as a source of active substances (Taruscio et al., 2004; Faria et al., 2005; Cooke et al., 2006). Leaves of *V. myrtillus* can prevent urinary tract infections. Fruits of bilberry have been used for the treatment of urinary tract infections.

Conclusions

The results of this research suggest that water, ethanol and ethyl acetate extracts of *V. myrtillus* inhibit the growth of human pathogens and can have significant effect on the prevention of the urinary tract infection. Antibacterial compounds from *V. myrtillus* may have important appli-cations as natural antibacterial agents. Therefore, the fruits and leaves of this plant are natural sources of anti-oxidant substances of high importance.

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REFERENCES

Badjakov I, Nikolova M, Gevrenova R, Kondakova V, Todorovska E, Atanassov A (2008). Bioactive compounds in small fruits and their influence on human health. Biotechnol. Biotechnol. Eq. 22:581-587.

Basu A, Rhone M, Lyons TJ (2010). Berries: emerging impact on cardiovascular health. Nutrition Rev. 68(3):168-177.

Brzuszkiewicz E, Thürmer A, Schuldes J, Leimbach A, Liesegang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R (2011). Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-aggregative-haemorrhagic *Escherichia coli* (EAHEC). Arch. Microbiol. 193(12):883-891.

Ceyhan N (2012). Biodegradation of pyrene by a newly isolated *Proteus vulgaris*. Sci. Res. Essays 7(1):66-77.

Cooke DN, Thomasset S, Boocock DJ, Schwarz M, Winterhalter P, Steward WP, Gescher AJ, Marczylo TH (2006). Development of analyses by high performance liquid chromatography and liquid chromatography/tandem mass spectrometry of bilberry (*Vaccinium myrtillus*) anthocyanins in human plasma and urine. J. Agric. Food Chem. 54:7009-7013.

Denev P, Ciz M, Ambrozova G, Lojek A, Yanakieva I, Kratchanova M (2010). Solid-phase extraction of berries' anthocyanins and evaluation of their antioxidative properties. Food Chem. 123(4):1055-1061.

Ehala S, Vaher M, Kaljurand M (2005). Characterization of phenolic profiles of Northern European berries by capillary electrophoresis and determination of their antioxidant activity. J. Agric. Food Chem. 53:6484-6490.

Faria A, Oliveira J, Neves P, Gameiro P, Santos B, Mateus N (2005). Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. J. Agric. Food Chem. 53:6896-6902.

Foxman B (2010). The epidemiology of urinary tract infection. Nat. Rev. Urol. 7:653-660.

Hearst C, Mc Collum G, Nelson D, Ballard ML , Millar BC , Goldsmith EC, Rooney JP, Loughrey A, Moore EJ, Rao RJ (2010). Antibacterial activity of elder (*Sambucus nigra* L.) flower or berry against hospital pathogens. J. Med. Plants Res. 4(17):1805-1809.

Helmstädter A, Schuster N (2010). *Vaccinium myrtillus* as an antidiabetic medicinal plant research through the ages. Pharmazie. 65(5):315-21.

Huttunen S, Toivanen M, Arkko S, Ruponen M, Tikkanen-Kaukanen C (2011). Inhibition activity of wild berry juice fractions against *Streptococcus pneumoniae* binding to human bronchial cells. Phytother. Res. 25(1):122-127.

Jaakola L, Määttä-Riihinen K, Kärenlampi S, Hohtola A (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. Planta 218:721-728.

Jaakola L, Määttä-Riihinen K, Pirttilä MA, Törrönen R, Kärenlampi S, Hohtola A (2002). Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development Plant Physiol. 130(2):729-739.

Kovačević N (2002). Basis of Pharmacognosy, Serbian schoolbook, Belgrade. pp. 162-162.

Li H , Hou Z, QiaoY, Feng B, Hu Y , Wang X , Zhao X (2010). Peroxopolyoxometalate-based room temperature ionic liquid as a self-separation catalyst for epoxidation of olefins. Catal. Commun. 11(5):470-475.

Martz F, Jaakola L, Julkunen-Tiitto R, Stark S (2010). Phenolic composition and antioxidant capacity of bilberry (*Vaccinium myrtillus*) leaves in Northern Europe following foliar development and along environmental gradients. J. Chem. Ecol. 36:1017-1028.

Min G, Chun-Zhao L (2005). Comparison of techniques for the extraction of flavonoids from cultured cells of *Saussurea medusa* Maxim. World J. Microb. Biot. 21:1461-1463.

Nakajima JI, Tanaka I, Seo S, Jamazaki M, Saito K (2004). LC/PDA/ESI-MS profiling and radical scavenging activity of antocyanins in various berries. J. Biomed. Biotechnol. 5:241-247.

NCCLS (National Committee for Clinical Laboratory Standards) (1997). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7-A4: Wayne PA.

Paredes-López O, Cervantes-Ceja ML, Vigna-Pérez M, Hernández-Pérez T (2010). Berries: improving human health and healthy aging, and promoting quality life, a review. Plant Foods Hum. Nutr. 65:299-308.

Puupponen-Pimia R, Nohynek L, Alakomi HL, Oksman KM (2005a). Bioactive berry compounds-novel tools against human pathogens. Appl. Microbiol. Biotechnol. 67:8-18.

Puupponen-Pimia R, Nohynek L, Hartmann-Schmidlin S, Kahkonen M, Heinonen M, Maatta-Riihinen K, Oksman-Caldentey KM (2005b). Berry phenolics selectively inhibit the growth of intestinal pathogens. J. Appl. Microbiol. 98:991-1000.

Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M,Cazin JC, Bailleul F, Trotin F (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol. 72:35-42.

Routray W, Orsat V (2011). Blueberries and their anthocyanins: Factors affecting biosynthesis and properties. Compr. Rev. Food Sci. F. 10(6):303-320.

Sarić M (1989). Medicinal plants of SR Serbia, Serbian academy of sciences and arts, Belgrade, pp. 567-568.

Schaik W, Top J, Riley DR, Boekhorst J, Vrijenhoek EP J, Schapendonk ME C, Hendrickx PA A, Nijman J I, Bonten JM M , Tettelin H, Willems JL R (2010). Pyrosequencing-based comparative genome analysis of the nosocomial pathogen *Enterococcus faecium* and identification of a large transferable pathogenicity island. BMC Genomics 11:239.

Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci. Biotechnol. Biochem. 58:1780-1783.

Taruscio TG, Barney DL, Exon J (2004). Content and profile of flavonoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. J. Agric. Food Chem. 52:3169-3176.

Tomićević J, Bjedov I, Obratov-Petković D, Milovanović M (2011). Exploring the Park-People Relation: Collection of *Vaccinium Myrtillus* L. by local people from Kopaonik National Park in Serbia. Environ. Manage. 48(4):835-846.

Viljanen K, Kylli P, Kivikari R, Heinonen M (2004). Inhibition of protein and lipid oxidation in liposomes by berry phenolics. J. Agric. Food Chem. 52:7419-7424.

Wootton-Beard PC, Moran A, Ryan L (2011). Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after in vitro digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. Food Res. Int. 44:217-224.