Antioxidant and Anticancer Properties of Leaf and Fruit Extracts of the Wild Raspberry (*Rubus idaeus* L.)

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Abstract

In this study the leaves and fruits of wild raspberry (*Rubus idaeus* L.) populations from the central Balkan region were examined to determine the level of secondary metabolites and related antioxidant activity, as well as biological activity, upon existing ethnobotanical evidence, primarily linked to gastrointestinal disorders. The values obtained for total phenols ranged from 59.68 to 96.83 mg GA g⁻¹ and 24.29 to 38.71 mg GA g⁻¹ in leaf and fruit extracts, respectively. The highest values of tannins and anthocyanins were determined for leaf extracts from a population of east Serbia at a level of 1.27 mg mL⁻¹ and 9.00 mg mL⁻¹. Antioxidant activity was evaluated by measuring the scavenging capacity of the extracts on DPPH. Higher antioxidant activity was detected in the leaf extracts than in the fruit extracts. Leaf and fruit extract were the most effective against *Escherichia coli* (ATCC 8739). Anticancer activity was studied on a human colorectal cancer cell line HCT-116. Leaf extracts exhibited anticancer activity with IC₅₀/24 h 162.38 μg mL⁻¹ and IC₅₀/48 h 95.69 μg mL⁻¹. Wild raspberry leaf and fruit extracts contain numerous secondary metabolites providing marked antioxidant, antimicrobial and anticancer activity.

Keywords: biological activity; methanolic extracts; phytochemical analysis; secondary metabolites

Introduction

Plant secondary metabolites, mainly phenolic compounds, are characterized by strong biological activity. There are four main classes of polyphenols: flavonoids, phenolic acids, lignans, and tannins (Rao and Snyder, 2010), presented in fruits and vegetables, which are linked to particular nutritional and health benefits (Wang and Lin, 2000; Seeram, 2008). These compounds possess a capacity for scavenging of reactive oxygen species (ROS), thus exhibiting an antioxidant activity (Cao et al., 1997; Trivedi et al., 2016). Biological activity of various fruit berries, including raspberries, is attributed to the high content of anthocyanins (e.g. cyanidin 3-glucoside, cyanidin 3-rutinoside, pelargonidin 3-glucoside), flavonoids (quercetin, kaempferol), tannins, phenolic acids (e.g. ellagic acid), and lignans (Seeram et al., 2006; Gramza-Michalowska et al., 2017). Composition and concentration of phenolic compounds in berries and related antioxidant activity and other biological effects, are highly dependent on fruit maturity, agro-environmental conditions and post-harvest technologies (Bobinaite et al., 2012).

The genus *Rubus* is one of the largest in the family Rosaceae with about 700 species, being divided into 12 subgenera of which are only a few species cultivated. *Ideobatus* subgenus includes raspberries, which are widespread in Asia, Europe, Africa and North America. This subgenus is comprised of approximately 200 species (Marulanda et al., 2007). Modern raspberry cultivars are derived from the European red raspberry (*R. idaeus* L.), North American red raspberry (*R. strigosus* Michx.), the black raspberry (*R. occidentalis* L.) and the purple raspberries (*R. neglectus* Peck) that are hybrids between red and black raspberries (Castillo, 2006). Most of commercial varieties are hybrids developed by breeding of the wild raspberries (Çekiç and Özen, 2010).

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The wild *R. idaeus* in region of Balkans and Serbia grows on altitudes above 1000 m a.s.l., mainly on the edge of beech forests, as well as in vineyards, extensive orchards, hedges, pastures and in abandoned meadows and other under-managed habitats (Dujmović Purgar et al., 2012).

Biological activity of the raspberry fruit, such as antimicrobial (Rauha et al., 2000), antioxidant (Babji et al., 2017), antioxidant (Kong et al., 2003), as well as prevention of cardiovascular diseases and type II diabetes (Rao and Snyder, 2010), is linked to various phenolic compounds, such as hydroxyacinnamic acid, gallic acid, galloyl esters, ellagic acid conjugates, flavonols, and anthocyanins (Määttä-Riihinen et al., 2004). Because of rich phenolic profile and high concentration of vitamin C (Benvenuti et al., 2010), the raspberry belongs to a group of fruit of high antioxidant capacity (Beckwilder et al., 2005; Jakobek et al., 2009).

Leaf is less studied than fruit, and most of reports mainly refers to phytochemical composition (Gudej and Tomczyk, 2004), while the biological effects are most related to fruit, probably because of wide use of fruit in human nutrition (Rauha et al., 2000).

The leaves of wild and cultivated raspberries are listed in traditional Balkan and Southeast European medicine, with evidence of use for relief of morning sickness during pregnancy, preventing miscarriage and easing labour pains (Parel et al., 2004), in addition to ethnomedical notes on antimicrobial and anticancer effects of it fruit (Rauha et al., 2000; Seeram et al., 2006). Study of Çekiç and Özgen (2010) pointed out the better antioxidant properties of the wild raspberry in comparison of the standard (cultivated) varieties, which was related to higher content of the total phenolic compounds and organic acids. However, there are still missing data on biological activity and medicinal uses of the raspberry (Holst et al., 2004), especially referring to wild populations.

The present study aims to determine the variability of main groups of secondary metabolites in different wild raspberry populations, collected from the central Balkan region, as well as to assess any related biological effects of its fruits and leaves, including antioxidant, antibacterial (studied on the bacterial strains of gut microflora differing in pathological potential) and anticancer effects using human colorectal cancer cell line HCT-116 model.

## Materials and Methods

### Plant material

Plant material (leaves and fruits) of wild raspberry (*R. idaeus*) was collected during field research in the period June - August 2016. The material was collected in the mountainous region of Serbia from seven different sites: Golija (P1), Kopaonik (P2), Goč (P3), Studena Mountain (P4), Željin (P5), Ozren (P6) and Stara Mountain (P7) (Table 1). Leaf and fruit material was collected as bulk sample of at least 20 individuals of each population.

### Chemicals

All the reagents used in this study were analytical grade and were obtained from Sigma Chemical Company (St. Louis, USA) and Merck (Darmstadt, Germany).

### Preparation of methanolic fruit and leaf extracts

Plant material (10 g of fresh leaves and fruits) was transferred to dark bottles to which 200 mL of methanol was added and the sample was stored at room temperature. After 24 hours, the content was filtered through Watman filter paper and the residue was then added to the same solvent volume. After 48 hours the procedure was repeated. Process of extraction was performed in a dark room. The combined supernatant was then evaporated using a vacuum evaporator at 40°C. The samples were then stored in a refrigerator until use for biochemical analyses at a temperature of 4°C.

### Phytochemical analyses of plant extracts and antioxidant activity

#### Determination of total phenolic content

Spectrophotometric method was used to determine the overall phenol concentration (Wootton-Beard et al., 2011). The reaction mixture was prepared by mixing 0.5 mL of the prepared methanolic solution of the extract (1 mg mL⁻¹), 2.5 mL of 10% water-soluble Folin-Ciocalteu reagent and 2.5 mL 7.5% NaHCO₃. The samples were then incubated at 45°C for 15 minutes. Blank was prepared in the same way, only methanol was added instead of the extract. The absorbance of the samples and the blank (methanol instead of extract solution) was measured on the spectrophotometer λₘₐₓ = 765 nm (Jenway 6105; Bibby Scientific Limited, Staffordshire, UK). The same procedure was repeated for gallic acid (GA) to calculate the equivalent concentration of total phenols (mg GA g⁻¹ of extract).

#### Determination of flavonoid concentrations

A spectrophotometric method was used to determine the flavonoid concentration (Quettier-Deleu et al., 2000). The samples were prepared by mixing 1 mL of the methanolic solution of the extract (1 mg mL⁻¹) and 1 mL of 2% AlCl₃ dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was measured at λₘₐₓ = 415 nm. The same procedure was repeated for rutin (Ru) to calculate the equivalent concentration of flavonoids (mg Ru g⁻¹ of extract).

### Table 1. Geographical position of the localities

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P1 – Golija; P2 – Kopaonik; P3 – Goč; P4 – Studena Mountain; P5 – Željin; P6 – Ozren; P7 – Stara Mountain

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**Determination of tannins concentration**

The total amount of tannins was determined by spectrometry measurement (Hosu et al., 2014). The samples were prepared by mixing 2 mL of the extract, 3 mL of concentrated HCl and 1 mL of distilled water. The content of the first sample was incubated for 30 min at 100 °C whereas 0.5 mL of ethanol was added to the second sample.

The absorbance of the samples was determined at wavelengths $\lambda_{\text{max}} = 470, 520$ and 570 nm. The differences ($\Delta A$) between the samples values obtained were determined at the same wavelengths ($\Delta A_{470}, \Delta A_{520}, \Delta A_{570}$). The values for wavelengths $\Delta A_{470}, \Delta A_{520}$ and $\Delta A_{570}$ were calculated as follows: TTC (g/L) = $15.7 \times$ minimum ($\Delta A_{520}$).

**Determination of anthocyanins concentration**

Samples containing 0.5 mL of extract, 0.5 mL of 0.1% ethanolic HCl solution and 10 mL of 2% aqueous HCl solution were used to determine the total amount of anthocyanins. The procedure was as follows: 4.4 mL of distilled water was added to the first sample while 4.4 mL of 13% sodium bisulphate was added to the second sample and diluted at a ratio of 1:1.

The absorbance of the samples was determined at a wavelength of $\lambda_{\text{max}} = 520$ nm using a starting solution made up of 4.9 mL distilled water, 0.5 mL of 0.1% ethanolic HCl solution and 10 mL of 2% aqueous HCl solution. The values obtained ($\Delta A$) were multiplied by the coefficient 875. The total amount of anthocyanins in a sample is expressed in μg mL$^{-1}$ of the extract (Hosu et al., 2014).

**Evaluation of DPPH scavenging activity**

Antioxidant activity (AOA) was measured using DPPH method according to Takao et al. (1994). Process of analysis was performed in a dark room. The procedure was as follows: for this method, a solution of 2.2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (80 μg mL$^{-1}$) was used. The test sample solution (extract) in methanol was at a concentration of 1000 μg mL$^{-1}$, as were the solutions of positive control (vitamin C and butyl hydroxytoluol-BHT). The concentrations tested were 1000 μg mL$^{-1}$, 500 μg mL$^{-1}$, 250 μg mL$^{-1}$, 125 μg mL$^{-1}$, 62.5 μg mL$^{-1}$, 31.25 μg mL$^{-1}$ and 15.625 μg mL$^{-1}$. In 6 tubes, 2 mL of methanol was measured, then 2 mL of the initial concentration extract (1000 μg mL$^{-1}$) was added to the first tube and a series of double dilutions was prepared in the other tubes.

The same preparation was carried out for the positive controls. Then, 2 mL of DPPH solution was added to each tube and they were incubated for 30 min/RT/ in a dark place. The reading of the absorbance was carried out at $\lambda = 725$ nm. For each sample, three absorbencies were measured and the mean value was calculated. A control (blank) was prepared by measuring 2 mL of methanol in one tube, adding 2 mL of DPPH solution and then incubating it for min/RT/ in a dark place. The reading of the absorbance was at $\lambda = 725$ nm.

For each sample, three absorbencies were measured and the mean value calculated. On the basis of the results, the IC$_{50}$ concentration was determined.

**Biological activity**

**Screening of antimicrobial activity for selected gut microbiota**

**Test microorganisms**

The antimicrobial activity of the methanolic extracts of the leaves and fruits of wild raspberry, were tested against a panel of ATCC (American Type Culture Collection) microorganisms, including Gram-positive *Sarcina lutea* (ATCC 9431) and *Bacillus subtilis* (ATCC 6633) and Gram-negative *Escherichia coli* (ATCC 8739). Three selected microorganisms are parts of normal human gut microbiota, differing among each other in term of effects on gastro-intestinal functionality. The *B. subtilis* is known as non-pathogenic bacteria, often used in probiotics. The *S. lutea* was selected as possibly pathological gastric bacteria known to be frequent in foodborne infections and intoxications, related to gastric obstruction and gastric disorders (Lam-Himlin et al., 2012), while for selected pathogenic strains of *E. coli*, including the one which we used in the current study it is known the role in gastric disorders (Rowe, 1979). There are some recent reports on linkage between gut microbiome and colorectal cancer (Sun and Kato, 2016), including the role of *E. coli* (Khan et al., 2017).

**Microdilution method**

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the microdilution method (Sarker et al., 2007). Stock solutions of the extracts were prepared in pure dimethyl sulfoxide (DMSO). The concentration of the stem of the herbal extracts tested was 40 mg/mL. A double dilution of the plant extracts was made in 96-well plates containing Mueller-Hinton broth for bacteria. The tests concentrations ranged from 20 mg mL$^{-1}$ to 0.16 mg mL$^{-1}$. The final concentration was 5 X 105 CFU mL$^{-1}$. Bacterial growth was monitored by the addition of resusurin, a bacterial growth indicator. As positive control doxycycline was used. The plates were incubated for 24 hours at 37 °C. The MIC is defined as the lowest concentration of the test substance that prevents the resusurin to change color from blue to pink. The minimum bactericidal activity was determined by transferring 10 μL of plaque samples whereby no color change was observed on the nutrient agar. At the end of incubation, the lowest concentration without bacterial growth is defined as the minimum bactericidal concentration. All tests were performed in triplicate.

**Anticancer activity**

**Cell cultures and treatment**

For testing the anticancer activity of the plant extracts we used the human colorectal cancer cell line HCT-116, purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in DMEM (Dulbecco’s Modified Eagle Medium) (Gibco, USA) and supplemented with 10% fetal calf serum, penicillin (100 IU mL$^{-1}$) and streptomycin (100 μg mL$^{-1}$) at 37 °C in a 5% CO$_2$ humidified atmosphere.
The cell viability and proliferation were determined at 80% to 85% confluence using the MTT assay. For the MTT assay, the cells were seeded in a 96-well plate (10^4 cells per well) and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO₂. After 24 hours, the medium was replaced with 100 μL solutions of various concentrations of the plants extracts. Stock solutions of the extracts were made in dimethyl sulfoxide (DMSO) at a concentration of 200 μg mL⁻¹. The final concentration of DMSO in the stock solutions was 1% (v/v). The stock solutions were serially diluted 2-fold, in concentrations ranging from 200 μg mL⁻¹ to 6.33 μg mL⁻¹ in the DMEM medium. Untreated cells were used as controls. Cells with the treatment were incubated at 37 °C and 5% CO₂ in a humidified atmosphere for 24 h and 48 h. The control and each of the extracts were tested in triplicate in three independent tests.

**Cell viability and proliferation assay (MTT assay)**

After 24 h and 48 h of treatment, the cell viability and proliferation were determined using the MTT assay (Mosmann, 1983). The MTT assay is based on the reduction of yellow tetrazolium salt MTT [3-(4,5-diethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] by the mitochondrial dehydrogenase of living cells to purple formazan that is soluble. After the incubation period, the medium with the treatments was removed and 100 μL of the MTT solution at a final concentration 5 mg mL⁻¹ was added to each well and incubated in a 5% CO₂ humidified atmosphere at 37 °C for 3 h. After incubation, the MTT solution was discarded and the colored crystals of formazan that were produced were dissolved in 150 μL of DMSO.

The absorbance values were measured on a microplate reader at 550 nm. The cell viability was calculated as the ratio of absorbance of the group treated divided by the absorbance of control group and multiplied by 100 to give the percentage of viability.

The data were expressed as the means ± standard errors (S.E.). The effects of the plant extracts were expressed by IC₅₀ (the half-maximal growth inhibitory concentration). The IC₅₀ values were calculated from the dose curves by a computer program (Calcusyn).

**Statistical analysis**

Statistical analyses were performed with the software packages SPSS for Windows (version 10.0). All measurements were carried out in triplicate, and the results are presented as mean ± S.E.M. Statistical analysis was performed via linear correlation coefficient and Pearson’s correlation coefficient.

**Results and Discussion**

**Phytochemical analyses**

Results of phytochemical analyses are presented in Table 2. Total phenolic compounds of leaf methanolic extracts ranged from 59.68 to 96.83 mg GA g⁻¹. The lowest values were recorded in the samples from the P1 study site, while the highest values were found for samples of populations assigned as P4 and P7. Sample of P7 population had a slightly lower concentration than sample from P4 (95.50 mg GA g⁻¹). The concentration of phenolic compounds of wild raspberry fruits ranged from 24.29 mg GA g⁻¹ (P5 population) to 38.71 mg GA g⁻¹ (P7). The lowest values of total phenolic content were recorded in samples taken from shadowy sites (P5 and P3), while the highest values had samples from sunny localities and higher altitudes. Previous studies ascertained significant correlation between production of secondary metabolites in plants and enhancement of plant protective mechanisms. Namely, plants produce larger quantities of secondary metabolites so as to efficiently protect protein synthesis from adverse effects of UV radiation that is particularly intense at higher altitudes (Duval et al., 1999). Taking into account that environmental factors influence plant metabolism reactions, Mikulić-Petkovšek et al. (2012) suggested that a higher content of phenols in the wild berries comparing with the cultivated ones, could be a result of adaptive mechanism, allowing growth of wild berry in their harsh natural environment.

The flavonoid concentration in studied wild raspberry leaf samples ranged from 7.02 mg Ru g⁻¹ (P7 population), to 7.53 mg Ru g⁻¹ (P1). Concentration of flavonoid values in the leaf extracts was fairly uniform. The content of flavonoids in leaves of the wild and cultivated raspberry was reported by Gudej and Tomczyk (2004), who concluded that raspberries are a rich source of flavonoids, ellagic acid and tannins. In the fruit extracts of the wild raspberry, the flavonoid concentration ranged from 4.39 mg Ru g⁻¹, as recorded in sample of P3 population, up to 4.97 mg Ru g⁻¹, recorded for P2 population. Similar values of flavonoids (6.0 mg g⁻¹ fresh weight) in raspberry fruits were recorded in study of Rauha et al. (2000). The highest concentrations of the flavonoids in the raspberry fruit were found in one wild ecotype among different samples of domesticated and wild grown raspberries in Turkey (Gülçin et al., 2011).

The concentration of total tannins in the methanol extracts of wild raspberry leaves ranged from 0.73 mg mL⁻¹, as recorded for the samples from P1, up to 1.27 mg mL⁻¹, in population P7. Results on tannin leaf concentration are similar to those reported by Durgo et al. (2012). In the fruit extracts, tannin concentration ranged from 0.20 mg mL⁻¹ (P5) to 0.45 mg mL⁻¹ (P3). In colored fruits, which are rich in anthocyanins, tannins stabilize the anthocyanins by linking them to form copolymers (Lopes da Silva et al., 2007). Berries rich in anthocyanins or their derived extracts exhibit numerous protective effects with potential benefits for human and animal health (Konić-Ristić et al., 2011). The red fruits have a higher content of cyanide-based glycosides, while the dark colored fruits contained more delphinidine glycosides. There are no significant differences in the anthocyanin composition between the cultivated and wild-growing fruits of individual species (Veberić et al., 2015). The concentration of anthocyanins in the methanol leaf extracts ranged from 4.43 to 9.00 μg mL⁻¹. The highest concentration was recorded in the of P6 population, while the lowest was recorded in P5 population. The highest concentration of anthocyanins in the methanol extracts of the fruit was observed in samples from P1 site (4.73 μg mL⁻¹), while the lowest was observed for P5 population (0.31 μg mL⁻¹). Wang and Lin (2000) have shown that the concentration of the total anthocyanins increases with the degree of maturity of the fruits, and their results are in line with values determined in our study for mature fruits which were evaluated.
Measurements of antioxidant activity (AOA) were performed using the free radical (DPPH) assay. The results are expressed as an IC50 concentration, and AOA is inversely proportional to the numerical value of the IC50 values. The results of the AOA extract of the leaves ranged from 110.17 to 199.18 μg mL⁻¹. The highest AOA was detected in the leaf extract from P7 population. The methanol extracts from P3 and P4 site were also characterized by high values of AOA. The similar data were obtained by Durgo et al. (2012) indicating the high antioxidative potential of raspberry leaf extracts. Therefore, raspberries can be added to tea blends to increase the antioxidant capacity of the beverages for a better effect on human health. The AOA methanol extract of the fruit ranged from 246.09 to 489.60 μg mL⁻¹. The highest AOA was shown for methanol extracts of wild raspberry fruit from P7 site, while the lowest values were from the samples of the P5 population. Upon obtained results, it seems that fruits of wild raspberry from the higher and sunny mountains are better source of natural antioxidants (P7 and P4). Our results are in a line with some previous studies which showed that the extracts of the samples collected at higher altitudes had greater antioxidant capacity than the extracts of the samples taken at lower altitudes (Ghasemi et al., 2011). Tosun et al. (2009) showed that wild raspberry genotypes, compared with cultivated varieties, had a higher content of total phenols, a higher concentration of ascorbic acid and better AOA. By comparing the AOA of leaves and fruits, it can be seen that the leaves had a higher level of AOA. Based on this, it can be concluded that the antioxidant capacity is specific to the type of tissue due to different phytochemical content of bioactive antioxidant compounds (Wang and Lin, 2000). The antioxidant activity was significantly higher in the leaves than in fruits of the wild raspberry populations, which is correlated with total phenolics content, including flavonoids, tannins, and anthocyanins, similarly to results obtained for some berry fruits (Vyas et al., 2013). Leaf extracts from P1, P2 and P6 populations had the higher content of flavonoids while the other populations had more total phenols. In the case of fruit extracts, the P1 population with the high content of secondary metabolites is separated (high content of the total phenols and anthocyanins, but also a high content of tannins and flavonoids). Populations P2 and P4 exhibited a high content of flavonoids while population P3 had the highest tannins content.

Among the values of phenolic components and AOA obtained it is possible to examine the statistical dependence of the method of linear regression. The correlation coefficient (R), as well as the coefficient of determination (R²), which show how much of the variation of the dependent variables explains the model for AOA related to the total phenols, flavonoids, tannins and anthocyanins (Table 3). As reported previously, all studied compounds, primarily phenols are highly related to express AOA (Stankovic et al., 2015). A relatively strong and weak link between content of total phenolics and flavonoids respectively implies the possibility that other classes of phenolic secondary metabolites, aside from flavonoids, contribute to antioxidant activity in raspberry. Former studies on major secondary metabolites in fruits and leaves of the R. idaeus revealed the presence of elagic, p-coumaric, ferulic acid and quercetin (Jacobek et al., 2009). Significant biological activity of these phenolic acids and flavonoids has already been confirmed for antioxidant activity of these compounds (Kadoma and Fujisawa, 2008). Also, tannins and flavonoid content showed strong correlation coefficient (0.847 and 0.838) with AOA. Based on these statistical data, it can be seen that phenolic compounds are the dominant components contributing to AOA, which is in agreement with range of previous reports.

### Biological activity

The test results of antimicrobial activity are presented in the Table 4. The methanolic extract samples of the leaves from all of the populations showed a mean inhibitory activity between 2.5 and 20.00 mg mL⁻¹. The E. coli exhibited the highest sensitivity to the studied extracts, in range from 2.50 to 10.00 mg mL⁻¹, while the most resistant was Gram-positive bacteria B. subtilis with MIC from 10.00 to 20.00 mg mL⁻¹. The methanolic extracts of the fruits also showed a mean inhibitory activity of 5.00 to 20.00 mg mL⁻¹,
with the most susceptible Gram-positive bacteria *S. lutea*, while the most resistant was *B. subtilis* with a MIC of 20.00 μg mL⁻¹ for all samples. Rauha et al. (2000) demonstrated the moderate antimicrobial activity of the methanol extracts of raspberry fruits in relation to *B. subtilis*, while the methanolic extract of fruits exhibits mild antimicrobial activity in relation to some bacteria, including *S. lutea* and *E. coli*, similarly to our findings. The most effective antibacterial effect was determined for the leaf extract of P1 population, which was rich in flavonoids and anthocyanins, so the raspberry leaf can be recommended in form of a hot water infusion and decoct, i.e. herbal tea in the treatments of infections. Since methanolic leaf extract did not exhibit any significant activity against Gram positive *B. subtilis* - widely used as probiotic (Liu et al., 2012), simultaneously affecting pathogenic bacteria *S. lutea* and *E. coli*, the ethnobotanical data on use of herbal tea from raspberry leaf for stabilization of the normal gut flora and diarrhea (Menković et al., 2014) treatment might be confirmed. All extracts exhibited much lower antimicrobial activities than the tested antibiotic (Table 4). During the study, the ability of methanol extracts from the leaves and fruits to inhibit the growth of human colorectal cancer cell line HCT-116 were tested. The IC₅₀ values of leaf extracts after 24 h and 48 h are presented in Fig. 1. Methanolic extracts of raspberry leaves from P1 showed the strongest anticancer activity with IC₅₀/24 h 162.38 and IC₅₀/48 h 95.69 μg mL⁻¹. On the other hand, leaf extracts from P4 did not show anticancer activity, while the leaf extracts from P7 had a mild anticancer activity after 48 h. It is important to note that the extracts of leaves from P7 and P4 had the highest AOA, leading to the conclusion that anticancer and AOA are not positively, but negatively correlated (Table 5). Boivin et al. (2007) reached similar conclusions, showing that strawberry juice was a very weak (15% at 50 μl mL⁻¹) inhibitor of cancer cell proliferation, but exhibited the second highest ORAC value (22.6 μmol TE mL⁻¹). Strawberries were also found to have a relatively high antioxidant capacity (14.6 μmol TE mL⁻¹). The methanolic extracts of wild raspberry fruit have shown very low anticancer activity (Fig. 2.). Bowen-Forbes et al. (2012) examined the anticancer activity of several extracts of raspberry fruits, yielding results that indicate that only the ethanol extract shows a mild anticancer activity, while the methanolic extract has no anticancer activity, which coincides with our findings. On the other hand, this finding contradicts that of Seeram et al. (2006) according to whom increasing the concentration of extracts increases the inhibition of carcinogenic growth.

The statistical dependence between the phytochemical parameters tested in the methanol extracts of the leaves and fruits and their anticancer activity was determined on the basis of Pearson’s correlation coefficient (Table 5). There are statistically significant correlations between those tested values in which Sig. P < 0.05. Positive correlations exist between MTT 24 h and total phenols (P-value = 0.001 < P-critical = 0.05), confirming notes on an important role of natural polyphenols in cancer prevention (Huang et al., 2008).

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<th>Population</th>
<th>MIC</th>
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</tr>
<tr>
<td>Doseycycline</td>
<td>&lt;0.50</td>
<td>3.80</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Pearson’s correlation coefficient

<table>
<thead>
<tr>
<th>MTT 24 h</th>
<th>MTT 48 h</th>
<th>Leaf AOA (DPPH)</th>
<th>Leaf flavonoids</th>
<th>Leaf total phenol content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.882</td>
<td>-0.787</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.882</td>
<td>0.876</td>
<td>-0.973</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.787</td>
<td>0.876</td>
<td>-0.857</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.950</td>
<td>-0.885</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).
Fig. 1. Anticancer activity of methanolic leaf extracts of the wild Rubus idaeus populations: (A) IC$_{50}$/24 h; (B) IC$_{50}$/48 h (µg mL$^{-1}$)

Fig. 2. Anticancer activity of methanolic fruit extracts of the wild Rubus idaeus populations: (A) IC$_{50}$/24 h; (B) IC$_{50}$/48 h (µg mL$^{-1}$)

Positive correlations also exist between AOA and flavonoids (P-value = 0.010 < P-critical = 0.05). Negative correlations exist between MTT 24 h and AOA (P-value = 0.009 < P-critical = 0.05), MTT 24 h and flavonoids (P-value = 0.036 < P-critical = 0.05), AOA and total phenols (P-value = 0.000 < P-critical = 0.05) and between flavonoid and phenol (P-value = 0.014 < P-critical = 0.05). By analysing the Pearson coefficient of correlation of the anticancer activity of the methanolic fruit extracts and the tested phytochemical parameters, it was found that there is no correlation.

In general, the most expressed biological activity (antimicrobial and anticancer) exhibited leaf extracts of the population with the highest content of tannins and anthocyanins (P1). It has been already reported on anticancer (Kong et al., 2003) and antimicrobial activity (Burdulis et al., 2009) of anthocyanins.

Conclusions

Based on the results presented in this research, wild raspberry from the central Balkan region – the territory of Serbia, can be recommended as a rich source of natural antioxidants, especially phenolic compounds. Also, wild raspberry leaves can be recommended as a supplement to tea blends in the treatment of various infections (especially for gastrointestinal disorders), due to their antimicrobial activity against E. coli. Raspberry leaf extracts were found to have mild anticancer activity, while the fruits did not show any anticancer activity. It is noticeable that the samples of plant populations from the high-altitude locality have more secondary metabolites and better biological activity. In addition to this fact, genetic material from the Serbian wild raspberry can be used as a basis for creating new varieties with improved trait characteristics.

Acknowledgements

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References


Boivin D, Blanchette M, Barrette S, Moghrabi A, Beliveau R (2007). Inhibition of cancer cell proliferation and suppression of TNF-induced


