

Analyses of Metals Impact on *Humulus lupulus* Strobili Antioxidant Capacity

DUŠAN Đ. PAUNOVIĆ¹, SNEŽANA S. MITIĆ² ([id https://orcid.org/0000-0003-3301-5981](https://orcid.org/0000-0003-3301-5981)),
IVANA D. RAŠIĆ MIŠIĆ^{2*} ([id https://orcid.org/0000-0003-4825-6980](https://orcid.org/0000-0003-4825-6980)),
MILAN N. MITIĆ² ([id https://orcid.org/0000-0002-1310-0540](https://orcid.org/0000-0002-1310-0540)),
ALEKSANDRA N. PAVLOVIĆ² ([id https://orcid.org/0000-0003-2053-3106](https://orcid.org/0000-0003-2053-3106)),
GORDANA M. KOCIĆ³ ([id https://orcid.org/0000-0003-2386-2466](https://orcid.org/0000-0003-2386-2466))

¹University of Union Nikola Tesla, Faculty of Applied Science, Department of Contemporary Food Technology, Niš, Serbia

²University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Serbia

³University of Niš, Faculty of Medicine Niš, Niš, Serbia

Abstract: This paper presents results of analyses of metal ions effects on hop strobili antioxidant characteristics. Determination of total phenols (TP), total flavonoids (TF) and fifteen phenolic compounds, as well as the antioxidant activity (DPPH, ABTS and FRAP) of hop extracts from eight samples was conducted using UV/Vis spectrophotometry and HPLC method. Contents of 24 elements in mineralized hop samples were determined by ICP-OES. Very strong negative relationship between TP, TF, antioxidant capacity results and Pb, Co, Cr, Sb and Na was determined applying principal component and cluster analyses. Namely, the higher concentrations of these metals were associated with lower contents of TP and TF and lower values of antioxidant tests. Impact of metal ions on phenolic and flavonoids content and antioxidant activity of hop strobili has not been previously published.

Keywords: hop; phenolic compounds; antioxidant capacity; metal ions; statistical analyses

1. Introduction

Hop (*Humulus lupulus* L) is a plant widely known for its usage in brewing industry, precisely in beer production. All parts of this plant are a source of polyphenolic compounds such as flavonoids, catechins, flavanones, phenolic acids, etc. Phenolic compounds or polyphenols are a wide group of plant metabolites and represent nonessential dietary components. Antioxidant action of plant extract is usually related to the presence of these compounds [1]. Large number of studies shows that among all phenolic compounds, flavonoids are of the greatest significance for antioxidant activity of plant. Hydroxyl groups, especially their position, play an important role for antioxidant activity of flavonoids, since they are responsible for donating hydrogen atom and thus scavenge free radicals [2, 3]. According to biological importance (antioxidant activity above all), phenolic compounds with two or three vicinal hydroxyl groups at C-atoms of benzene rings especially protrude as stated by Heim et al. [3] and Rice-Evans et al. [4, 5]. One of the most important properties of these compounds is that they get easily oxidized by molecular oxygen under physiological conditions. In these autooxidation reactions, semiquinones, quinones and reactive oxygen species (ROS) are produced.

Metals are omnipresent in various biological systems. The uptake of metals, both by roots and leaves of plants, increases with metal concentration in external medium. Shah et al. reported that Cd, Cr, Pb, As, Sb and Hg are toxic for plants [6]. They can inhibit electron transport, reduction of CO₂ fixation and affect plant growth through generation of free radicals and reactive oxygen species. However, plants use complex processes which activate the cells to adopt their metabolism to metal stress. Some heavy metals are essential for biological and physiological functions of plants, however, these metals become toxic depending on the nature and species of metal and plants. As stated by Lechno et al., Hernandez and Almansa, sodium ions can also be toxic for some plants [7, 8].

*email: ivana.rasic@pmf.edu.rs

The ability of metals to interact with polyhydroxy phenols during their transformation was investigated under different experimental conditions [9-13]. Recently, a few papers have been published that solely documented the metal content of hop and conveyed geographical characterization of hop origin based on their elemental plant and soil content [14, 15]. Lately, Liu et al. presented identification of geographical origin of this plant done by multi-metal elements fingerprinting along with the relationships with functional ingredients [16]. Few other studies focused exclusively on antioxidant activity of hop' extracts [17-24]. The impact of metal content (especially toxic metals) and antioxidant activity of hop strobili has not been analyzed by these authors.

Because of all above mentioned, the aim of this paper was to analyze metals content effect on antioxidant activity of hop strobili. In order to obtain this relationship, it was necessary to determine total phenols (TP) and total flavonoids (TF) in hop strobili, as well as their antioxidant activity (DPPH, ABTS and FRAP) and metal content. Various individual phenolic compounds were also quantified.

2. Materials and methods

2.1.Plant material

Strobili of eight hop samples were purchased at local markets. Each paper-aluminium bag contained 500 g of hop. Five bags of each sample were bought and mixed. In order to prepare samples for analysis, 2 grams from each mixed sample were taken in triplicate for each preparation method and further analyzed. Samples were stored in polyethylene bottles upon preparation.

2.2.Chemicals

The following certification standards were used: gallic acid (Carl Roth, Karlsruhe, Germany), quercetin-3-rutinoside, quercetin-glucoside, kaempferol-glucoside, p-coumaric acid, chlorogenic acid (Merck, Darmstadt, Germany) and quercetin-galactoside (Extrasynthese, Genay, France), 4-hydroxybenzoic acid, ferulic acid, (+)-catechin and (-)-epicatechin (Sigma Aldrich, Steineheim, Germany). Kaempferol-glucoside was used as the equivalent for kaempferol-rutinoside and quercetin-3-glucoside was used as the equivalent for quercetin-glycoside. The ICP multi-element standard solution of 20.00 ± 0.10 mg/L (Ultra - scientific (North Kingstown, RI, U.S.A.) was used as a stock solution for calibration. The following reagents were also used: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium peroxydisulfate, ethanol (96% by vol.), methanol (HPLC grade), acetic acid, formic acid and acetonitrile (HPLC grade) (J. T. Baker, Deventer, Netherlands). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) was purchased from Acros Organics, Geel, Belgium. Folin Ciocalteu's phenol reagent, iron(III) chloride, sodium hydroxide, sodium acetate, sodium nitrite, sodium carbonate, aluminium chloride hexahydrate, iron(II) sulfate heptahydrate, hydrochloric and nitric acid were purchased from Merck, Darmstadt, Germany, argon 5.0 (99.999% purity). All chemicals and solvents were analytical grade, unless indicated otherwise.

Polyethylene bottles had been previously cleaned using 20% nitric acid and washed with ultra-pure water ($0.05 \mu\text{S}/\text{cm}$ specific conductivity) in order to avoid contamination. All the glassware used was cleaned in the ethanol KOH solution, aqueous solution of HCl (1:1) and then thoroughly rinsed with tap, distilled and finally with ultra-pure water.

2.3.Instruments

The recording of spectra and absorbance readings were done using Agilent 8453 UV/Vis spectrophotometer, with 1 cm optical path quartz cuvettes. The pH measurements were carried out using Hanna Instruments pH-meter with glass electrode. The model 1200 Agilent Technologies, with analytical column C₁₈ (Zorbax Eclipse XDB-C18, 5 μm , 4.6×150 mm), was used for performing HPLC analysis. Muffle furnace VIMS Serbia equipped with microprocessor temperature program IVIGOS3123 (± 1 °C) was used for dry mineralization. Inductively coupled plasma optical emission spectrometer iCAP 6000 (Thermo Scientific Inc., Cambridge, UK) was applied for multielement

analysis. This instrument uses glass concentric nebulizer, an Echelle optical design (52.91 grooves/mm, 63.5° blaze angle) and a charge injection device solid-state detector (RACID86). High precision variable volume micropipettes (Lab Mate⁺) were used for pipetting the solutions. Ultra-pure water was obtained using MicroMed high purity water system, TKA Wasseraufbereitungssystem GmbH. Thermo-stating of refrigerated solutions and extracts was done in Julabo MP 5A Open Bath Circulations.

2.4. Sample preparation

Two methods were applied for the sample's preparation: extraction-maceration (for UV/vis and HPLC methods) and dry mineralization (for ICP-OES method).

Extraction of sample (2 g) was performed with 60 mL of solvent (ethanol/water, 30/70, v/v %) during 2 h at $t = 25^{\circ}\text{C}$. Suspension was filtered through Büchner funnel and Whatman No. 1 filter paper. Extracts were stored in polyethylene bottles and refrigerated till they were analyzed. The procedure was based upon methods reported by Iacopini et al. [25], Borowska et al. [26] and Katalinić et al. [27].

Dry mineralization of sample (2 g) was done in porcelain crucible in the electrical furnace at the increasing temperature interval from 50 to 450°C during the first 8 h, after it was held constant for the next 12 h. Cold ash-sample was dissolved in 2.5 mL HNO_3 (1:1, v/v), filtered (filter paper Whatman, 6 μm pore size) and filled with deionized water to 50 mL. The procedure was done according to the method by Radojević and Bashkin [28].

2.5. Spectrophotometric analysis

2.5.1. TP content determination

Sample extract volume of 0.1 mL was transferred into the 10 mL volumetric flask; after that it was added 2.5 mL of deionized (DI) water, 0.5 mL of Folin-Ciocalteu reagent solution, 2 mL of 20% Na_2CO_3 solution and filled with water to the mark. TP content was determined by measuring absorbance at $\lambda = 760\text{ nm}$ after 2 h against water as a blank [29, 30]. The measurements were compared to the calibration line obtained using gallic acid (GA) standard solution and the results of TP content were expressed as milligrams of gallic acid equivalents (GAE) per grams of hop ($\text{mg GAE/g} \pm \text{SD}$). The stated procedure was repeated in triplicates for each sample.

2.5.2. TF content determination

Reaction solution was prepared by mixing 0.5 mL of hop extract, 3 mL of DI water and 0.3 mL of 5% NaNO_2 according to the method by Zhishen et al. [31]. After incubation at room temperature for 5 min, 3 mL of 1% AlCl_3 was added. The mixture was left at room temperature for next 5 min, then 2 mL NaOH (1 mol/L) and water to the 10 mL were added. Absorbance was measured at $\lambda = 510\text{ nm}$ against water as a blank. According to the measured absorbance, the content of TF was determined using catechin calibration line and displayed as milligrams of catechin equivalent per g of hop ($\text{mg CE/g} \pm \text{SD}$). The stated procedure was repeated in triplicates for each sample.

2.6. Antioxidant activity determination

Antioxidant activity of hop extracts was evaluated applying three independent *in vitro* assays: DPPH, ABTS and FRAP.

The method by Brand-Williams et al., conducted with slight modifications, was used for DPPH assay [32]. The solution of DPPH radical ($1 \times 10^{-4}\text{ mol/L}$) was prepared in methanol. Initially, 5.0 mL of this solution and 0.1 mL of hop extract were mixed in 10 mL volumetric flask. Methanol was added to the mark. The discoloration of this radical was recorded by absorbance measurement at 520 nm, 30 min after the beginning of the reaction against methanol as a blank. Total antioxidant activity ($\mu\text{mol/L}$) was determined using Trolox calibration curve. It was plotted as a function of the absorbance decrease ($\Delta A = A_{\text{blank}} - A_{\text{sample}}$) of DPPH radical scavenging activity and the results were expressed as

micromoles of Trolox equivalent (TE) per g of hop ($\mu\text{mol TE/g} \pm \text{SD}$). The stated procedure was repeated in triplicates for each sample.

The assay of ABTS radical cation scavenging activity was performed according to the method described by Re et al. [33] and Arts et al. [34] with slight modifications [35]. ABTS was dissolved in methanol to the concentration of 7×10^{-3} mol/L. ABTS radical cation was produced by reacting ABTS stock solution with 2.4×10^{-3} mol/L $\text{K}_2\text{S}_2\text{O}_8$ and allowing the mixture to stand in the dark at room temperature 12–16 h prior use. ABTS radical cation solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.02 at 734 nm. An aliquot of each hop extract (0.1 mL) was mixed with 3.9 mL of diluted ABTS radical cation solution. The reduction in absorbance at 734 nm was measured after the reaction time of 6 min at room temperature. The Trolox calibration curve was plotted as a function of the decrease in absorbance ($\Delta A = A_{\text{blank}} - A_{\text{sample}}$) of ABTS radical cation scavenging activity. The results were expressed as micromoles of Trolox equivalents (TE) per gram of hop ($\mu\text{mol TE/g} \pm \text{SD}$). The stated procedure was repeated in triplicates for each sample.

FRAP working reagent was prepared daily by mixing following constituents: 25 mL of acetic buffer (0.3 mol/L, $\text{pH}=3.6$), 2.5 mL of TPTZ (10 mmol/L) in 40 mmol/L HCl and 2.5 mL of FeCl_3 (20 mmol/L). The reaction mixture was prepared in a 10 mL volumetric flask with 1 mL of hop extract, 2.1 mL of FRAP reagent and water to the mark. After the incubation time of 5 min at 37 °C, the absorbance at 595 nm was measured. The concentration ($\mu\text{mol/L}$) of Fe^{2+} equivalents was determined directly from the calibration curve. The final FRAP results were presented as micromoles of Fe^{2+} equivalents per g of hop ($\mu\text{mol Fe/g} \pm \text{SD}$). This assay was performed according to the method given by Benzie and Strain [35]. The stated procedure was repeated in triplicates for each sample.

2.7.HPLC analysis

Separation and identification of individual phenolic components are of importance in order to give information about real differences in phenolic profiles of studied hop samples. Chromatographic analyses of hop extracts was carried out using the gradient procedure with the mobile phase containing solvent A (5% formic acid in water) and solvent B (80% acetonitrile + 5% formic acid in water) as follows: 0–28 min, 0% B; 28–35 min, 25% B; 35–40 min, 50% B; 40–45 min, 80% B and for the last 10 min, 0% B. The temperature of column was set at 30 °C. The solvent flow rate was 0.8 mL/min and the injection volume was 5 μL . HPLC chromatograms were recorded at 360 and 320 nm, as well as using fluorescence detector ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 275/322$ nm). The identification of individual phenolic components was performed by comparing their retention times and spectral data with those of standards. Quantification of each phenolic compound was done using external standard method. Results were expressed as mg per g of hop.

2.8.ICP-OES analysis

The operating conditions for conducting ICP-OES analysis were: power of RF generator–1150 W, pump speed – 50 rpm, flush pump speed – 100 rpm, cooling gas flow – 12 L/min, nebulizer gas flow – 0.7 L/min, auxiliary gas flow – 0.5 L/min. The direction of plasma observation was dual.

2.9.Calibration parameters

The calibration line was plotted for each analyzed element. Linearity was considered satisfactory if the correlation coefficient (r) exceeded 0.9990, except for Al, Ca, Fe, K and Mg ($r < 0.9990$).

Selection of emission line in both axial and radial plasma viewing modes of each element was done based on the absence of spectral interferences and adequate sensitivity for determining low and high elements concentrations. Detection (LOD) and quantification (LOQ) limits were calculated using the following equations (1 and 2):

$$\text{LOD} = 3.3 \times \sigma / s \quad (1)$$

$$\text{LOQ} = 10 \times \sigma / s \quad (2)$$

were σ is residual standard deviation of the regression line and s is a slope of calibration curve [36, 37]. Both limits were expressed in $\mu\text{g/g}$. The selected wavelengths, correlation coefficients (r), detection limits (LOD) and limits of quantification (LOQ) were listed in Table 1.

Table 1. Emission wavelength, LOD, LOQ and correlation coefficient of the calibration curves for each element determination

Element	Parameter			
	λ (nm)	LOD ($\mu\text{g/g}$)	LOQ ($\mu\text{g/g}$)	Correlation coefficient
Al	308.215	0.1125	0.3713	0.9841
As	189.042	0.0650	0.2145	0.9997
Ba	455.403	0.0013	0.0043	0.9999
Ca	393.366	0.0033	0.0109	0.9901
Cd	228.802	0.0048	0.0158	0.9999
Co	238.892	0.0221	0.0729	0.9999
Cr	283.563	0.0556	0.1835	0.9997
Cu	324.754	0.0149	0.0492	1
Fe	259.940	0.0149	0.0492	0.9957
K	766.490	0.0438	0.1445	0.9979
Li	670.784	0.0015	0.0049	0.9998
Mg	279.553	0.0005	0.0017	0.9985
Mn	257.610	0.0026	0.0087	0.9999
Mo	202.030	0.0164	0.0541	0.9999
Na	588.995	0.0020	0.0660	1
Ni	231.406	0.0134	0.0442	0.9999
Pb	220.353	0.0629	0.2076	0.9999
Sb	217.581	0.0740	0.2442	1
Se	196.090	0.0707	0.2331	0.9993
Si	251.611	0.0133	0.0439	0.9993
Sn	189.989	0.0137	0.0452	0.9999
Sr	407.771	0.0005	0.0016	0.9995
V	309.311	0.0179	0.0591	1
Zn	213.856	0.0003	0.0010	0.9998

2.10. Statistical analysis

The aim of statistical analyses was to find correlations between the content of TP, TF, antioxidant capacity and mineral content in hop strobili samples. Principal component analysis was used to evaluate datasets and to establish the relationship among variables. Cluster analysis was performed in order to group samples based on their proximity. Analyses were performed using a statistical package Statistica 8.0. StatSoft, Inc., Tulsa, OK, USA. A probability of $p < 0.05$ was considered statistically significant [36].

3. Results and discussions

3.1. TP, TF content and antioxidant activity of hop extracts

The spectrophotometric results of TP, TF content and antioxidant activity of hop were presented in Table 2.

Table 2. TP and TF content and antioxidant activity of hop

Sample	Parameter					
	TP mg GAE/g	TF mg CE/g	TF/TP	DPPH $\mu\text{mol TE/g}$	ABTS $\mu\text{mol TE/g}$	FRAP $\mu\text{mol Fe/g}$
1	9.54 \pm 0.07	3.82 \pm 0.03	0.40	32.25 \pm 0.07	41.67 \pm 0.07	37 \pm 3
2	13.33 \pm 0.03	5.54 \pm 0.05	0.42	37.5 \pm 0.2	46.8 \pm 0.3	66 \pm 3
3	10.11 \pm 0.07	4.15 \pm 0.07	0.41	34.0 \pm 0.3	43.8 \pm 0.2	55 \pm 2
4	10.54 \pm 0.09	4.60 \pm 0.02	0.44	35.1 \pm 0.2	43.04 \pm 0.07	45 \pm 2
5	9.91 \pm 0.05	3.57 \pm 0.07	0.36	33.0 \pm 0.3	42.2 \pm 0.1	30 \pm 2
6	10.91 \pm 0.07	4.47 \pm 0.03	0.41	37.1 \pm 0.1	46.2 \pm 0.2	71 \pm 3
7	12.68 \pm 0.05	4.78 \pm 0.07	0.38	36.5 \pm 0.1	44.7 \pm 0.1	70.1 \pm 0.8
8	11.46 \pm 0.09	5.05 \pm 0.03	0.44	35.9 \pm 0.2	45.43 \pm 0.07	75 \pm 2

The content of TP in hop samples was in the range 9.54 mg GAE/g–13.33 mg GAE/g. The data obtained in this study are comparable to the results reported by Kirca and Arslan [24] and by Lermusieau et al. [38] while the results published by Proestos et al. [39], Mudura et al. [22] and Wojdylo et al. [19] were significantly lower. Higher TP content (23.1 mg/g and 151.42 mg/g) in hop extract was determined by Kahkonen et al. [20] and by Arsene et al. [18]. The content of TF in hop samples was in the range 3.57 mg CE/g–5.54 mg CE/g, which is 36%–44% from TP content.

Ethanol hop extracts showed relatively good radical-scavenging properties toward stable DPPH radical. The obtained results of DPPH radical scavenging activity ranged from 32.25 $\mu\text{mol TE/g}$ to 37.5 $\mu\text{mol TE/g}$, for ABTS from 41.67 $\mu\text{mol TE/g}$ to 46.8 $\mu\text{mol TE/g}$ and for FRAP assay from 30 $\mu\text{mol Fe/g}$ to 75 $\mu\text{mol Fe/g}$. The highest values of TP, TF, DPPH and ABTS were obtained for sample no. 2, while the highest values for FRAP were measured for sample 8. The lowest values of TP, DPPH, ABTS were determined for sample 1, while sample 5 has the lowest values for TF and FRAP.

3.2.HPLC analyses of individual phenolic compounds in hop strobili extract

In order to identify and quantify individual phenolic compounds, the content of flavonols, phenolic acids and flavan-3-ols was determined applying HPLC method. Seven phenolic compounds of quercetin and kaempferol were identified at 360 nm: quercetin-3-galactoside, quercetin-3-rutinoside, quercetin-3-glucoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, quercetin-glycoside and kaempferol-glycoside (Figure 1a). Four phenolic acids (4-hydroxybenzoic, chlorogenic, p-coumaric and ferulic) were identified at 320 nm (Figure 1b). The chromatographic profile for four flavan-3-ols (procyanidin B1, catechin, procyanidin B2 and epicatechin) identified using fluorescence detector at 275/322 nm is presented in Figure 1c.

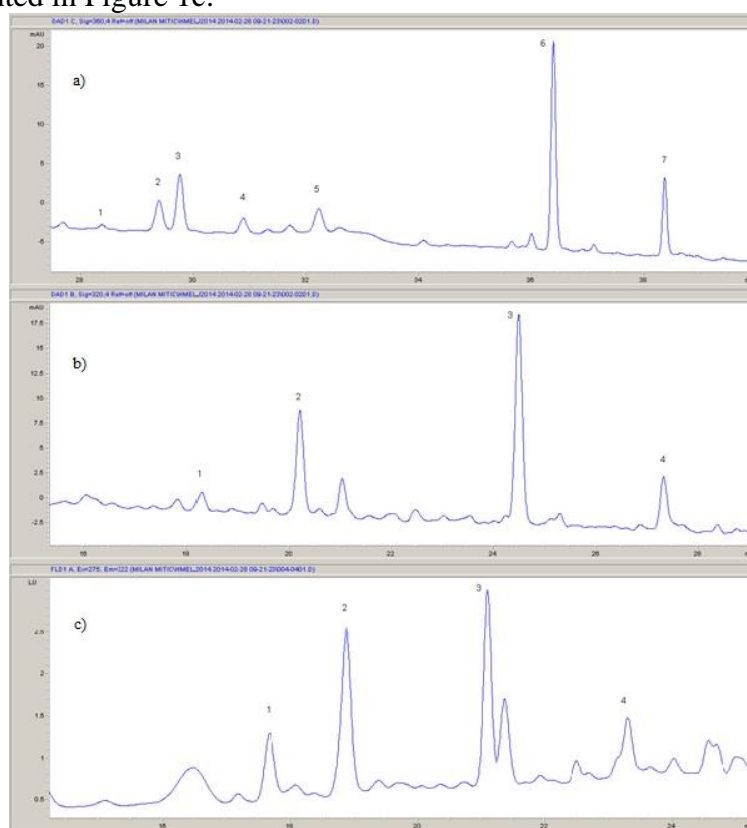


Figure 1. HPLC chromatograms of ethanol hop extract recorded at: a) 360 nm:

1) quercetin-3-galactoside; 2) quercetin-3-rutinoside; 3) quercetin-3-glucoside;

4) kaempferol-3-rutinoside; 5) kaempferol-3-glucoside; 6) quercetin-glycoside and 7) kaempferol-glycoside; b) 320 nm: 1) 4-hydroxybenzoic acid; 2) chlorogenic acid; 3) p-coumaric acid and 4) ferulic acid; c) 275/322 nm ($\lambda_{\text{ex}}/\lambda_{\text{em}}$): 1) procyanidin B1; 2) catechin; 3) procyanidin B2 and 4) epicatechin.

The results of flavonols, phenolic acids and flavan-3-ols contents in eight hop samples are listed in Table 3. The total content of flavonols was in the range 0.338 mg/g–1.427 mg/g. In all hop samples, quercetin-glycoside (0.158 mg/g–0.536 mg/g) is the major phenolic compound. The total content of quercetin-glycosides (0.290 mg/g–0.965 mg/g) was higher than the total content of kaempferol-glycosides (0.048 mg/g–0.546 mg/g). These results were in accordance with those given by Kovačova et al. [40], (0.165 mg/g and 0.126 mg/g), while results reported by McMurrough et al. [41] were 0.92 mg/g for quercetin-glycosides and 1.20 mg/g for kaempferol-glycosides.

Total phenolic acids content was in the interval 0.286 mg/g–0.752 mg/g. Chlorogenic acid with concentration interval 0.152 mg/g–0.349 mg/g is the major phenolic acid in all hop extracts. The phenolic acid found in the lowest concentration is 4-hydroxybenzoic acid (0.018 mg/g–0.124 mg/g). p-coumaric acid (0.019 mg/g–0.203 mg/g) and ferulic acid (0.039 mg/g–0.098 mg/g) are detected as well. Proestos et al. [39] published very similar results for 4-hydroxybenzoic acid (0.015 mg/g) and p-coumaric acid (0.038 mg/g), but ferulic acid was not detected. The higher contents of p-coumaric acid (0.228 mg/g) and ferulic acid (0.143 mg/g) in hop were reported by Wojdylo et al. [19].

Total quantity of flavan-3-ols was determined in the range 0.429 mg/g–0.996 mg/g. In all hop extracts, procyanidin B2 (0.132 mg/g–0.416 mg/g) was the major, while epicatechin (nd–0.159 mg/g) was the minor phenolic component. The similar content of catechin (0.238 mg/g), but significantly higher quantity of epicatechin (1.438 mg/g) was reported by Callemien et al. [42]. Magalhaes et al. confirmed presence of catechin, epicatechin and procyanidin in hop extracts [43]. Proestos et al. reported that catechin is the most common flavan-3-ol in plant materials [39].

Table 3. Flavonols, phenolic acids and flavan-3-ols content in the hop extracts (mg/g)

Compound	Sample							
	1	2	3	4	5	6	7	8
Flavonols								
Quercetin-3-galactoside	0.015	0.017	0.036	0.041	0.050	0.027	0.029	0.068
Quercetin-3-rutinoside	0.035	0.033	0.091	0.077	0.236	0.122	0.034	0.248
Quercetin-3-glucoside	0.082	0.135	0.319	0.178	0.177	0.236	0.108	0.187
Kaempferol-3-rutinoside	0.048	0.051	0.122	0.058	0.049	0.070	0.058	0.068
Kaempferol-3-glucoside	nd	nd	0.220	0.099	0.088	0.115	nd	0.086
Quercetin-glycoside	0.158	0.459	0.435	0.536	0.438	0.394	0.460	0.462
Kaempferol-glycoside	nd	nd	0.204	0.177	0.154	0.120	0.095	0.153
Σ Quercetin glycosides	0.290	0.644	0.881	0.832	0.901	0.779	0.631	0.965
Σ Kaempferol glycosides	0.048	0.051	0.546	0.334	0.291	0.305	0.153	0.307
Σ Flavonols	0.338	0.695	1.427	1.166	1.192	1.084	0.784	1.272
Phenolic acids								
4-hydroxybenzoic	0.018	0.020	0.124	0.111	0.047	0.019	0.018	0.118
Chlorogenic	0.152	0.316	0.349	0.304	0.257	0.294	0.273	0.245
p-coumaric	0.040	0.019	0.203	0.197	0.178	0.194	0.191	0.176
Ferulic	0.076	0.039	0.076	0.073	0.085	0.066	0.067	0.098
Σ Phenolic acids	0.286	0.394	0.752	0.685	0.567	0.573	0.549	0.637
Flavan-3-ols								
Procyanidin B1	0.131	0.165	0.143	0.123	0.089	0.117	0.148	0.079
Catechin	0.264	0.229	0.278	0.209	0.112	0.249	0.255	0.115
Procyanidin B2	0.311	0.339	0.416	0.310	0.260	0.331	0.325	0.132
Epicatechin	nd	nd	0.159	0.107	0.084	nd	0.159	0.103
Σ Flavan-3-ols	0.706	0.733	0.996	0.749	0.545	0.697	0.887	0.429

nd – not detected

3.3.ICP-OES analyses of hop strobili mineral content

Experimental values of hop mineral content were expressed as mean value (μg/g) ± SD for three replicate measurements and three groups of elements were established for obtained data (Tables 4, 5 and 6).

Table 4. The major elements content ($\mu\text{g/g}$) in hop strobili samples.

Sample	Element				
	K	Na	Ca	Mg	Na/K
1	10511 ± 5	89.3 ± 0.2	1253.6 ± 0.9	2056 ± 2	0.008
2	10603 ± 2	78.7 ± 0.2	1198.2 ± 0.3	2102.5 ± 0.7	0.007
3	9688 ± 3	82 ± 1	1224.2 ± 0.1	1945.6 ± 0.7	0.008
4	10124.5 ± 0.6	88.5 ± 0.1	1097 ± 1	1987.5 ± 0.8	0.009
5	10423 ± 1	89.67 ± 0.09	1255.4 ± 0.2	2176.4 ± 0.3	0.009
6	10734 ± 2	85.71 ± 0.04	1175.8 ± 0.8	1874.6 ± 0.5	0.008
7	10066 ± 3	80.1 ± 0.1	1167.5 ± 0.4	2067 ± 1	0.008
8	11007 ± 4	83.7 ± 0.3	1202.5 ± 0.6	2115.3 ± 0.6	0.008

Potassium concentration ranged from $9688 \mu\text{g/g}$ to $11007 \mu\text{g/g}$, while sodium was the metal present in the lowest concentrations ($78.7 \mu\text{g/g}$ – $89.67 \mu\text{g/g}$). Considering this, Na/K coefficients values were significantly low for all eight samples due to the high K content in all samples. The evaluated Ca content was in the interval of $1097 \mu\text{g/g}$ – $1255.4 \mu\text{g/g}$, while Mg concentrations were in the range of $1874.6 \mu\text{g/g}$ – $2176.4 \mu\text{g/g}$. Ghiselli et al. published elements concentrations in hop that are mostly in accordance with results of presented research, except for Ca that are 10 times higher (9380 mg/kg – 15515 mg/kg) [44]. Liu et al. quantified approximately the same content of Ca and Na as it was determined in this study, but two times higher concentration of Mg and 5 times smaller concentration of K [16]. Our research revealed the major elements content in hop samples decreased as following: $\text{K} > \text{Mg} > \text{Ca} > \text{Na}$.

The results of minor elements content in hop extracts are presented in Table 5.

Table 5. The minor elements content ($\mu\text{g/g}$) in hop strobili samples.

Sample	Element					
	Fe	Cu	Mn	Zn	Co	Cr
1	83.15 ± 0.05	8.92 ± 0.02	31.49 ± 0.04	39.18 ± 0.09	0.081 ± 0.005	0.781 ± 0.001
2	65.9 ± 0.1	9.54 ± 0.02	32.8 ± 0.1	31.7 ± 0.1	0.052 ± 0.006	0.456 ± 0.006
3	82.0 ± 0.3	9.776 ± 0.009	28.56 ± 0.01	42.74 ± 0.03	0.075 ± 0.001	0.678 ± 0.006
4	73 ± 1	8.93 ± 0.02	28.54 ± 0.04	37.7 ± 0.1	0.085 ± 0.003	0.871 ± 0.001
5	79.6 ± 0.2	8.113 ± 0.008	30.75 ± 0.05	36.8 ± 0.2	0.091 ± 0.005	0.61 ± 0.01
6	85.1 ± 0.2	7.47 ± 0.01	31.77 ± 0.04	36.43 ± 0.08	0.035 ± 0.001	0.688 ± 0.004
7	84.6 ± 0.1	8.354 ± 0.007	31.89 ± 0.09	39.8 ± 0.2	0.058 ± 0.009	0.487 ± 0.005
8	76.03 ± 0.09	7.881 ± 0.005	33.04 ± 0.05	35.75 ± 0.04	0.066 ± 0.002	0.556 ± 0.009
Sample	Se	Si	V	Mo	Ni	Sn
1	0.631 ± 0.003	12.02 ± 0.01	7.93 ± 0.06	1.23 ± 0.03	0.723 ± 0.001	0.681 ± 0.001
2	0.667 ± 0.006	11.56 ± 0.01	8.65 ± 0.02	2.056 ± 0.009	0.564 ± 0.009	0.792 ± 0.001
3	0.345 ± 0.006	12.67 ± 0.05	8.014 ± 0.009	1.89 ± 0.01	0.792 ± 0.002	0.626 ± 0.002
4	0.17 ± 0.01	9.6 ± 0.1	9.02 ± 0.08	1.346 ± 0.004	0.694 ± 0.002	0.46 ± 0.01
5	0.38 ± 0.01	11.74 ± 0.09	7.12 ± 0.06	1.68 ± 0.02	0.61 ± 0.01	0.667 ± 0.008
6	0.455 ± 0.008	9.45 ± 0.01	7.765 ± 0.008	1.125 ± 0.008	0.705 ± 0.007	0.551 ± 0.002
7	0.42 ± 0.01	8.98 ± 0.05	8.34 ± 0.02	1.87 ± 0.02	0.642 ± 0.006	0.611 ± 0.008
8	0.527 ± 0.009	10.64 ± 0.09	7.75 ± 0.09	1.254 ± 0.007	0.648 ± 0.002	0.712 ± 0.001

The minor elements content in hop samples decreased as following: $\text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Si} > \text{V} > \text{Mo} > \text{Ni} \approx \text{Cr} \approx \text{Sn} > \text{Se} > \text{Co}$. The highest content among these elements in all samples was determined for Fe ($65.9 \mu\text{g/g}$ – $85.1 \mu\text{g/g}$). Zinc was measured in concentration range $31.7 \mu\text{g/g}$ – $42.74 \mu\text{g/g}$, while manganese content in studied samples was about $30 \mu\text{g/g}$. Copper concentration range was $7.47 \mu\text{g/g}$ – $9.776 \mu\text{g/g}$, while cobalt was quantified in the interval of $0.035 \mu\text{g/g}$ – $0.091 \mu\text{g/g}$. The intervals for other elements are: $8.98 \mu\text{g/g}$ – $12.67 \mu\text{g/g}$ (Si), $7.12 \mu\text{g/g}$ – $9.02 \mu\text{g/g}$ (V), $1.125 \mu\text{g/g}$ – $2.056 \mu\text{g/g}$ (Mo), $0.564 \mu\text{g/g}$ – $0.792 \mu\text{g/g}$ (Ni), $0.456 \mu\text{g/g}$ – $0.871 \mu\text{g/g}$ (Cr), $0.46 \mu\text{g/g}$ – $0.792 \mu\text{g/g}$ (Sn) and $0.17 \mu\text{g/g}$ – $0.667 \mu\text{g/g}$ (Se). Ghiselli et al. presented partly similar or, for some of these elements, higher results (Si, V, Mo and Se were not examined in hop samples), while Cr and Ni content was higher, four times and ten times, respectively [44]. Liu et al. reported several times higher concentration of Fe, Mn, Cr and Co, but lower content for Zn compared to the quantified concentrations of these elements in this paper. In their research, the analysis of hop for Se, Si, Mo and Sn had not been done. Their samples

had up to 4 times higher content of Ni, but about 7 times lower content of V compared to the results reported in this study [16]. Melandrino et al. analyzed hop for the presence of Se and Sn but did not quantified these elements in samples they used [45]. A thorough search of the relevant literature yielded published articles that analyzed and quantified Sn and Se in hops.

The results of nonessential elements determination are shown in Table 6.

Table 6. Nonessential elements content ($\mu\text{g/g}$) in hop samples

Sample	Element			
	As	Pb	Cd	Al
1	0.35 ± 0.05	0.471 ± 0.009	0.098 ± 0.003	15.4 ± 0.1
2	0.11 ± 0.04	0.193 ± 0.005	0.066 ± 0.001	16.66 ± 0.09
3	0.36 ± 0.06	0.38 ± 0.01	0.078 ± 0.001	15.0 ± 0.2
4	0.21 ± 0.04	0.301 ± 0.009	0.092 ± 0.003	14.7 ± 0.1
5	0.30 ± 0.04	0.50 ± 0.02	0.055 ± 0.004	13.88 ± 0.02
6	0.28 ± 0.01	0.22 ± 0.02	0.082 ± 0.002	15.32 ± 0.08
7	0.24 ± 0.01	0.157 ± 0.005	0.098 ± 0.001	14.7 ± 0.2
8	0.38 ± 0.08	0.29 ± 0.03	0.059 ± 0.003	16.0 ± 0.1
Sample	Element			
	Ba	Li	Sr	Sb
1	4.22 ± 0.02	6.125 ± 0.008	9.234 ± 0.005	0.537 ± 0.001
2	4.002 ± 0.008	5.876 ± 0.009	7.653 ± 0.002	0.455 ± 0.002
3	3.453 ± 0.009	6.711 ± 0.002	6.98 ± 0.01	0.565 ± 0.008
4	3.769 ± 0.002	7.026 ± 0.009	7.112 ± 0.001	0.49 ± 0.01
5	4.319 ± 0.001	6.111 ± 0.003	7.656 ± 0.006	0.546 ± 0.005
6	4.07 ± 0.01	7.83 ± 0.01	8.62 ± 0.02	0.435 ± 0.002
7	2.871 ± 0.009	6.165 ± 0.003	8.142 ± 0.008	0.378 ± 0.005
8	3.886 ± 0.001	6.664 ± 0.009	9.189 ± 0.005	0.466 ± 0.001

The mean concentrations of third group elements were increasing in the following order: $\text{Cd} < \text{As} < \text{Pb} < \text{Sb} < \text{Ba} < \text{Li} < \text{Sr} < \text{Al}$. Among all nonessential elements, the highest contents of Al ($13.88 \mu\text{g/g}$ – $16.66 \mu\text{g/g}$) and Sr ($6.98 \mu\text{g/g}$ – $9.234 \mu\text{g/g}$) were determined. The metals such as Cd ($0.055 \mu\text{g/g}$ – $0.098 \mu\text{g/g}$), As ($0.11 \mu\text{g/g}$ – $0.38 \mu\text{g/g}$), Pb ($0.157 \mu\text{g/g}$ – $0.50 \mu\text{g/g}$), Sb ($0.378 \mu\text{g/g}$ – $0.565 \mu\text{g/g}$), Ba ($2.871 \mu\text{g/g}$ – $4.319 \mu\text{g/g}$) and Li ($5.876 \mu\text{g/g}$ – $7.83 \mu\text{g/g}$) were also quantified. Two times higher concentrations for Cd, twenty times for Ba and about five times for Sr, were reported by Ghiselli et al. [44]. Seven times higher content of Al, up to two times higher content of Cd, 35 times higher amount of Sr and approximately the same amount of As and Pb were presented by Li et al. [16]. A thorough search of the relevant literature yielded published articles that analyzed and quantified Sb in hops.

3.4. Statistical analyses of quantities of total phenols, total flavonoids, antioxidant capacity and mineral content of hop strobili

3.4.1. Principal component analysis (PCA)

PCA was applied to the entire data set and six principal components with eigenvalues higher than 1 were obtained, explaining 97.264% of variability. The first component explained 36.881%, the second 21.863%, the third 16.186%, the fourth 9.525%, the fifth 7.431% and the six component 5.378%. The PCA results are displayed in Table 7 and Figure 2. The first component explained the largest portion of the variance with significant loadings of following variables: DPPH, ABTS, FRAP, TP, TF, Na, Co, Cr, Pb, Sb (highest loadings), K, Ca, Fe, Mn, Zn, Se, Si, V, Ni, Sn, As and Al (moderate loadings) and Cu, Mg, Mo, Cd, Ba, Li and Sr (low loadings). On the first component Sb, Pb, Cr, Co, Na, Ca, Fe, Zn, Si, Ni and As have opposite influence compared to DPPH, ABTS, FRAP, TP and TF. This means that these elements are negatively correlated with antioxidant capacity tests and TP and TF content (*i.e.* if lower concentrations of Sb, Pb, Cr, Co, Na, Ca, Fe, Zn, Si, Ni and As are present, values of DPPH, ABTS, FRAP and TP and TF are higher). K, Mn, Se, V, Sn and Al are positively correlated with TP and TF content and antioxidant capacity tests. Variables with significant loadings for the second component are Ca, Mg and Sn.

Table 7. Factor coordinates of the variables, based on correlations

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
DPPH	-0.919	-0.323	0.085	-0.055	0.026	0.192
ABTS	-0.909	-0.105	0.126	0.086	0.205	0.306
FRAP	-0.822	-0.243	0.263	0.329	0.115	0.145
TP	-0.954	-0.034	-0.238	0.045	-0.157	-0.054
TF	-0.923	-0.118	-0.141	-0.117	0.181	-0.048
K	-0.407	0.512	0.674	-0.314	0.078	-0.040
Na	0.777	0.083	0.387	-0.464	-0.087	-0.105
Ca	0.328	0.809	0.031	0.442	0.053	0.072
Mg	-0.076	0.746	-0.264	-0.167	-0.502	-0.150
Fe	0.455	-0.243	0.485	0.623	-0.249	-0.036
Cu	0.097	-0.004	-0.859	0.096	0.462	-0.167
Mn	-0.684	0.541	0.412	0.116	-0.125	-0.196
Zn	0.655	-0.428	-0.102	0.593	-0.060	-0.068
Co	0.762	0.216	-0.384	-0.250	-0.195	-0.137
Cr	0.719	-0.412	0.181	-0.368	0.326	-0.191
Se	-0.441	0.693	0.160	0.258	0.284	-0.331
Si	0.424	0.629	-0.400	0.202	0.448	0.138
V	-0.376	-0.565	-0.461	-0.306	0.249	-0.400
Mo	-0.294	0.123	-0.851	0.283	-0.188	0.176
Ni	0.612	-0.475	0.127	0.408	0.455	0.007
Sn	-0.374	0.859	-0.180	0.267	0.124	-0.039
As	0.595	0.160	0.449	0.471	0.072	0.095
Pb	0.879	0.467	-0.022	-0.072	0.046	0.020
Cd	0.154	-0.639	0.019	0.195	0.039	-0.693
Al	-0.691	0.260	0.034	0.018	0.633	-0.181
Ba	0.268	0.597	0.333	-0.536	0.319	0.116
Li	0.065	-0.626	0.603	-0.112	0.281	0.383
Sr	-0.148	0.377	0.771	0.191	0.025	-0.433
Sb	0.796	0.351	-0.250	-0.060	0.367	0.195

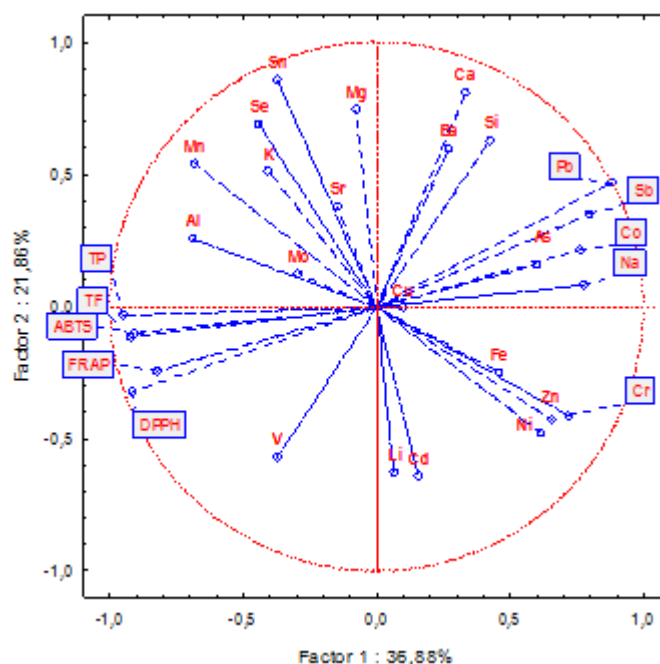


Figure 2. Projection of the variables on the factor plane (F1-F2)

According to the Table 7 and Figure 2 it can be observed that there is a strong positive relationship between DPPH, FRAP, ABTS, TP and TF. Group of elements (Pb, Sb, Co, Na and Cr) with highly positive factors is strongly negatively correlated with polyphenols and antioxidant tests.

3.4.2. Cluster analyses

Upon standardization of variables, Ward's method with squared Euclidian distance was used as a measure of proximity between the samples. Obtained dendrogram (Figure 3) presents clustering of hop strobili samples. Horizontal cluster analyses (HCA) showed that all the samples could be grouped into two main clusters. Cluster I was consisted of samples 7, 8, 6 and 2, while cluster II was formed by samples 4, 3, 5 and 1. The samples of cluster I are characterized by lower content of Pb, Sb, Co, Na and Cr and higher content of TP and TF and higher antioxidant capacity. The samples of cluster II are characterized by higher content of these metals and lower content of TP and TF and lower antioxidant capacity. Further analyses showed better similarity of samples by grouping them into the subclusters (S1 and S5 are similar to each other, and the nearest samples to them are S3 and S4, which are also similar to each other). The samples S8 and S6 are close to each other, while the sample S7 does have some similarities to either S8 or S6 (or both), but also with S2.

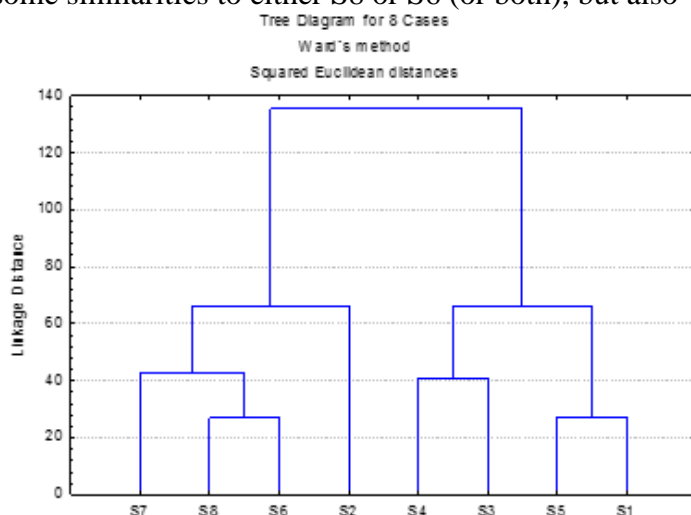


Figure 3. Dendrogram of cluster analyses of hop strobili samples (S) 1-8

4. Conclusions

The results of mineral content effect on TP and TF content, as well as on antioxidant activities of hop strobili are presented in this paper. The content of TP was determined in interval 9.54–13.33 mg GAE/g. TF were quantified in the range 3.57–5.54 mg CE/g. Hop extracts showed ability of neutralization DPPH (32.25–37.52 $\mu\text{mol TE/g}$) and ABTS (41.67–46.85 $\mu\text{mol TE/g}$) radicals, as well as high potential to reduce Fe^{3+} measured by FRAP assay (30.50–75.48 $\mu\text{mol Fe/g}$). Results obtained for these antioxidant assays have following trend $\text{FRAP} > \text{ABTS} > \text{DPPH}$. Hop extracts that do not follow this trend are sample 1 ($\text{ABTS} > \text{FRAP} > \text{DPPH}$) and sample 5 ($\text{ABTS} > \text{DPPH} > \text{FRAP}$). These two samples have the lowest content of TP and TF and the lowest antioxidant capacity, but have the highest total content of Pb, Sb, Co, Na and Cr. The best results considering TP, TF, DPPH and ABTS values were obtained for sample 2 due to the lowest total content of Pb, Sb, Co, Na and Cr. This study also included identifications and quantification of following phenolic compounds: seven flavonols (quercetin-3-galactoside, quercetin-3-rutinoside, quercetine-3-glucoside, quercetine-3-glycoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-glycoside), four phenolic acids (4-hydroxybenzoic acid, chlorogenic acid, p-coumaric acid and ferulic acid) and four flavan-3-ols (procyanidin B1, catechine, procyanidin B2 and epicatechine). The content of these compounds was 0.290–0.965 mg/g for total quercetin-glycosides, 0.048–0.546 mg/g for total kaempferol glycosides, 0.285–0.752 mg/g for total phenolic acids and 0.429–0.996 mg/g for total flavan-3-ols.

Furthermore, this is the first time the mineral content (24 elements) effect on TP and TF quantities and antioxidant capacity of hop strobili was analyzed. Statistical results showed strong positive

relationship between DPPH, FRAP, ABTS, TP and TF. Group of elements (Pb, Sb, Co, Na and Cr) with highly positive factors is strongly negatively correlated with polyphenols and antioxidant tests (*i.e.* higher concentrations of polyphenolic compounds and higher values for antioxidant capacity were associated with lower concentrations of these, for hop, potentially toxic metals).

Acknowledgements: This research was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (Agreement Numbers 451-03-68/2020-14-200124 and 451-03-68/2020-14/ 200113).

References

1. BRAVO, L., Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance, *Nutr. Rev.*, **56**(11), 1998, 317-333. <https://doi.org/10.1111/j.1753-4887.1998.tb01670.x>
2. COTELLE, N., Role of Flavonoids in Oxidative Stress, *Curr. Top. Med. Chem.*, **1**(6), 2001, 569-590. DOI : 10.2174/1568026013394750
3. HEIM, K.E., TAGLIAFERRO, A.R., BOBILYA, D.J., Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationships, *J. Nutr. Biochem.*, **13**(10), 2002, 572-584. [https://doi.org/10.1016/S0955-2863\(02\)00208-5](https://doi.org/10.1016/S0955-2863(02)00208-5)
4. RICE-EVANS, C.A., MILLER, N.J., PAGANGA, G., Structure-antioxidant Activity Relationships of Flavonoids and Phenolic Acids, *Free Radic. Biol. Med.*, **20**(7), 1996, 933-956. [https://doi.org/10.1016/0891-5849\(95\)02227-9](https://doi.org/10.1016/0891-5849(95)02227-9)
5. RICE-EVANS, C.A., MILLER, N.J., PAGANGA, G., Antioxidant Properties of Phenolic Compounds, *Trends Plant Sci.*, **2**(4), 1997, 152-159. [https://doi.org/10.1016/S1360-1385\(97\)01018-2](https://doi.org/10.1016/S1360-1385(97)01018-2)
6. ASHRAF M., OZTURK M., AHMAD M.S.A., *Plant Adaptation and Phytoremediation*, Springer Science+Business Media B.V., 2010, 71-97. DOI 10.1007/978-90-481-9370-7
7. LECHNO, S., ZAMSKI, E., TEL-OR, E., Salt Stress-Induced Responses in Cucumber Plants, *J. Plant. Physiol.*, **150**(1-2), 1997, 206-211. [https://doi.org/10.1016/S0176-1617\(97\)80204-0](https://doi.org/10.1016/S0176-1617(97)80204-0)
8. HERNANDEZ, J.A., ALMANSA, M.S., Short-term Effects of Salt Stress on Antioxidant Systems and Leaf Water Relations of Pea Leaves, *Physiol. Plant.*, **115** (2), 2002, 251-257. <https://doi.org/10.1034/j.1399-3054.2002.1150211.x>
9. ABRASH, H.I., SHIH, D., ELIAS, W., MALEKMEHR, F., A Kinetic Study of the Air Oxidation of Pyrogallol and Purpurogallin, *Int. J. Chem. Kinet.*, **21**(6), 1989, 465-476. <https://doi.org/10.1002/kin.550210609>
10. FRIEDMAN, M., JURGENS, H.S., Effect of pH on the Stability of Plant Phenolic Compounds, *J. Agric. Food Chem.*, **48**(6), 2000, 2101-2110. <https://doi.org/10.1021/jf990489j>
11. EATON, D.R., Complexing of Metal Ions with Semiquinones. An Electron Spin Resonance Study, *Inorg. Chem.*, **3**(9), 1964, 1268-1271. <https://doi.org/10.1021/ic50019a016>
12. TULYATHAN, V., BOULTON, R.B., SINGLETON, V.L., Oxygen Uptake by Gallic Acid as a Model for Similar Reactions in Wines, *J. Agric. Food Chem.*, **37**(4), 1989, 844-849. <https://doi.org/10.1021/jf00088a002>
13. MORAN, J.F., KLUCAS, R.V., GRAYER, R.J., ABIAN, J., BECANA, M., Complexes of Iron with Phenolic Compounds from Soybean Nodules and Other Legume Tissues: Prooxidant and Antioxidant Properties, *Free Radic. Biol. Med.*, **22**(5), 1997, 861-870. [https://doi.org/10.1016/S0891-5849\(96\)00426-1](https://doi.org/10.1016/S0891-5849(96)00426-1)
14. PEPI, S., CHICCA, M., TELLOLI, C., DI ROMA, A., GRISENTI, P., TESSARI, U., VACCARO, C., Discrimination of Geographical Origin of Hop (*Humulus lupulus* L.) Using Geochemical Elements Combined with Statistical Analysis, *Environ. Geochem. Health.*, **41**(3), 2019, 1559-1576. DOI: 10.1007/s10653-018-0232-7
15. OCVIRK, M., NEČEMER, M., KOŠIR, I.J., The Determination of the Geographic Origins of Hops (*Humulus lupulus* L.) by Multi-Elemental Fingerprinting, *Food Chem.*, **277**, 2019, 32-37. <https://doi.org/10.1016/j.foodchem.2018.10.070>

16. LIU, Z., WANG, Y., LIU, Y., Geographical Origins and Varieties Identification of Hops (*Humulus lupulus* L.) by Multi-Metal Elements Fingerprinting and the Relationships with Functional Ingredients, *Food Chem.*, **289**, 2019, 522-530. <https://doi.org/10.1016/j.foodchem.2019.03.099>
17. GORJANOVIĆ, S., PASTOR, F.T., VASIĆ, R., NOVAKOVIĆ, M., SIMONOVIĆ, M., MILIĆ, S., SUŽNJEVIĆ, D., Electrochemical Versus Spectrophotometric Assessment of Antioxidant Activity of Hop (*Humulus Lupulus* L.) Products and Individual Compounds, *J. Agric. Food Chem.*, **61**(38), 2013, 9089-9096. <https://doi.org/10.1021/jf401718z>
18. ARSENE, A.L., RODINO, S., BUTU, A., PETRACHE, P., IORDACHE, O., BUTU, M., Study on Antimicrobial and Antioxidant Activity and Phenolic Content of Ethanolic Extract of *Humulus Lupulus*, *Farmacia*, **63**(6), 2015, 851-857.
19. WOJDYLO, A., OSZMIANSKI, J., CZEMERY, R. Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs, *Food Chem.*, **105**(3), 2007, 940-949. <https://doi.org/10.1016/j.foodchem.2007.04.038>
20. KAHKONEN, M.P., HOPIA, A.I., VUORELA, H.J., RAUHA, J.P., PIHLAJA, K., KUJALA, T.S., HEINONEN, M., Antioxidant Activity of Plant Extracts Containing Phenolic Compounds, *J. Agric. Food Chem.*, **47**(10), 1999, 3954-3962. <https://doi.org/10.1021/jf990146l>
21. DI SOTTO, A., CHECCONI, P., CELESTINO, I., LOCATELLI, M., CARISSIMI, S., DE ANGELIS M., ROSSI, V., LIMONGI, D., TONIOLO, C., MARTINOLI, L., GIACOMO, S., PALAMARA, A.T., NENCIONI, L., Antiviral and Antioxidant Activity of a Hydroalcoholic Extract from *Humulus lupulus* L., *Oxid. Med. Cell. Longev.*, 2018, Article ID 5919237. <https://doi.org/10.1155/2018/5919237>
22. MUDURA, E., TOFANA, M., PAUCEAN, A., SOCACI, S., The Evaluation of Antioxidant Capacity of Romanian Hops, *J. Agroaliment. Proc. Technol.*, **16**(2), 2010, 262-264.
23. PREEDY, V. *Beer in Health and Disease Prevention*, 1st edn. Elsevier Inc. Academic Press, 2008, 467-474.
24. KIRCA, A., ARSLAN, E., Antioxidant Capacity and Total Phenolic Content of Selected Plants from Turkey, *Int. J. Food Sci. Tech.*, **43**(11), 2008, 2038-2046. <https://doi.org/10.1111/j.1365-2621.2008.01818.x>
25. IACOPINI, P., BALDI, M., STORCHI, P., SEBASTIANI, L., Catechin, Epicatechin, Quercetin, Rutin and Resveratrol in Red Grape: Content, *in vitro* antioxidant activity and interactions, *J. Food Compos. Anal.*, **21**(8), 2008, 589-598. <https://doi.org/10.1016/j.jfca.2008.03.011>
26. BOROWSKA, E.J., MAZUR, B., GADZAL, R., KOPCIUCH, V., BUSZEWSKI, B., Polyphenol, Anthocyanin and Resveratrol Mass Fractions and Antioxidant Properties of Cranberry Cultivars, *Food Technol. Biotechnol.*, **47**(1), 2009, 56-61. <https://hrcak.srce.hr/33053>
27. KATALINIĆ, V., MOŠINA, S.S., SKROZA, D., GENERALIĆ, I., ABRAMOVIĆ, H., MILOŠ, M., LJUBENKOV, I., PISKERNIK, S., PEZO, I., TERPINC, P., BOBAN, M., Polyphenolic Profile, Antioxidant Properties and Antimicrobial Activity of Grape Skin Extracts of 14 *Vitis vinifera* Varieties Grown in Dalmatia, *Food Chem.*, **119**(2), 2010, 715-723. <http://dx.doi.org/10.1016/j.foodchem.2009.07.019>
28. RADOJEVIĆ, M., BASHKIN, V., *Practical Environmental Analysis*. The Royal Society of Chemistry, Cambridge, UK, 1999, 378
29. SINGLETON, V. L., ROSSI, J. A., Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic.*, **16**, 1965, 144-158.
30. STRATIL, P., KLEJDUS, B., KUBAN, V., Determination of Phenolic Compounds and Their Antioxidant Activity in Fruits and Cereals. *Talanta*, **71**, 2007, 1741-1751. <https://doi.org/10.1016/j.talanta.2006.08.012>
31. ZHISHEN, J., MENGCHENG, T., JIANMING, W., The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals, *Food Chem.*, **64**(4), 1999, 555-559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

32. BRAND-WILLIAMS, W., CUVELIER, M.E., BERSET, C., Use of a Free Radical Method to Evaluate Antioxidant Activity, *LWT Food Sci. Technol.*, **28**(1), 1995, 25-30.
[https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
33. RE, R., PELLEGRIN, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay, *Free Radical Biol. Med.*, **26**(9-10), 1999, 1231-1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
34. ARTS, M.J., HAENEN, G.R., VOSS, H.P., BAST, A., Antioxidant Capacity of Reaction Products Limits the Applicability of the Trolox Equivalent Antioxidant Capacity (TEAC) Assay, *Food Chem Toxicol.*, **42**(1), 2004, 45-49. <https://doi.org/10.1016/j.fct.2003.08.004>
35. BENZIE, I.F., STRAIN, J.J., The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay, *Anal. Biochem.*, **239**(1), 1996, 70-76.
<https://doi.org/10.1006/abio.1996.0292>
36. MILLER, J.N., MILLER, J.C., *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education Limited, London, England, 2005, 15
37. Chandran, S., Singh, R.S., Comparison of Various International Guidelines for Analytical Method Validation, *Pharmazie*, **62**(1), 2007, 4-14. DOI: 10.1691/ph2007.1.5064
38. LERMUSIEAU, G., LIEGEOIS, C., COLLIN, S., Reducing Power of Hop Cultivars and Beer Ageing, *Food Chem.*, **72**(4), 2001, 413-418.
[https://doi.org/10.1016/S0308-8146\(00\)00247-8](https://doi.org/10.1016/S0308-8146(00)00247-8)
39. PROESTOS, C., BOZIARIS, I.S., NYCHAS, G.-J.E., KOMAITIS, M., Analysis of Flavonoids and Phenolic Acids in Greek Aromatic Plants: Investigation of Their Antioxidant Capacity and Antimicrobial Activity, *Food Chem.*, **95**(4), 2006, 664-671.
<https://doi.org/10.1016/j.foodchem.2005.01.049>
40. KOVAČOVA, J., LEHOTAY, J., URGEOVA, E., MOCAK, J., ČIŽMARIK, J., Determination of Selected Flavonoids in Hop Extract by HPLC, *J. Liq. Chromatogr. Relat. Technol.*, **34**(5), 2011, 329-340. <https://doi.org/10.1080/10826076.2011.551596>
41. MCMURROUGH, I., HENNIGAN, G.P., LOUGHREY, M.J., Quantitative Analysis of Hop Flavonols Using High-Performance Liquid Chromatography, *J. Agric. Food Chem.*, **30**(6), 1982, 1102-1106. <https://doi.org/10.1021/jf00114a024>
42. CALLEMIEN, D., JERKOVIĆ, V., ROZENBERG, R., COLLIN S., Hop as an Interesting Source of Resveratrol for Brewers: Optimization of the Extraction and Quantitative Study by Liquid chromatography/atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry, *J. Agric. Food Chem.*, **53**(2), 2005, 424-429. <https://doi.org/10.1021/jf040179n>
43. MAGALHAES, P.J., VIEIRA, J.S., GONCALVES, L.M., PACHECO, J.G., GUIDO, L.F., BARROS, A.A., Isolation of Phenolic Compounds From Hop Extracts using Polyvinyl-polypyrrolidone: Characterization by High-Performance Liquid Chromatography–Diode Array Detection–Electrospray Tandem Mass Spectrometry, *J. Chromatogr. A*, **1217**(19), 2010, 3258-3268. <https://doi.org/10.1016/j.chroma.2009.10.068>
44. GHISELLI, L., TALLARICO, R., ROMAGNOLI, S., DE ACUTIS, L., BENEDETTELLI, S., Antioxidant and Mineral Element Characterization in Spontaneous Hop (*Humulus lupulus* L.) in Central Italy, *Agrochimica*, **59**(4), 2015, 319-334.
DOI: 10.12871/0021857201544
45. MALANDRINO, M., GIACOMINO, A., ABOLLINO, O., ALLIO, A., TONIOLO R., COLOMBO, M.L., Determination of Major, Minor and Trace Elements in Glyceric Macerates and Mother Tinctures and in the Starting Plant Material, *J. Pharm. Biomed. Anal.*, **106**, 2015, 167-178. <https://doi.org/10.1016/j.jpba.2014.10.036>

