

## PHYTOCHEMICAL PROFILE AND BIOLOGICAL ACTIVITY OF SELECTED KIND OF MEDICINAL HERBS

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### ABSTRACT

Medicinal herbs are used due to their health benefits, a special aroma, taste and are considered as one of the richest sources of bioactive compounds. The present study aimed to determine antioxidant activity (DPPH and phosphomolybdenum method), a total polyphenol (using Folin-Ciocalteu reagent), flavonoid (aluminium chloride method), phenolic acid content (using Arnov reagent), antimicrobial activity (disc diffusion method) and chemical composition (ICP-OES instrument) of medicinal herbs (ginger, comfrey, valerian, chicory, horseradish, and ramsons) grown in Slovak republic. Antioxidant activity by DPPH method ranged from 0.61 (ramsons) to 3.62 (ginger) mg TEAC per g of dry matter (TEAC – Trolox equivalent antioxidant capacity); by phosphomolybdenum method from 66.67 (valerian) to 204.14 (ginger) mg TEAC per g of dry matter. Total polyphenol content ranged from 4.37 (comfrey) to 13.19 (ramsons) GAE per g of dry matter (GAE – gallic acid equivalent); total flavonoid content from 1.07 (chicory) to 47.55 (ramsons) QE per g of dry matter (QE – quercetin equivalent) and total phenolic acid content from 0.99 (horseradish) to 9.77 (ginger) CAE per g of dry matter (CAE – caffeic acid equivalent). In a sample of ginger was detected the highest antimicrobial activity against *Bacillus cereus* CCM 7934 (5 mm). Among the mineral compounds – in all observed samples were dominated ( $\text{mg}\cdot 100\text{g}^{-1}$ ) of potassium, phosphorus, magnesium, and calcium. The amount of cadmium, chrome, and lead in observed samples was detected only in a trace amount, so our results reveal that the medicinal herbs do not represent in this study a potential health risk regarding the content of toxic elements. The consumption and using of medicinal plants as a part of the food mode of consumers due to health benefits is recommended.

**Keywords:** mineral compounds; antioxidant activity; polyphenols; antimicrobial activity; plants

### INTRODUCTION

The use of herbal medicines is increasing steadily throughout the world as an alternative treatment for treating several health problems including civilisation diseases like cardiovascular problems, diabetes, high blood pressure and even certain types of cancer (Kaur, Kaur and Mahajan, 2013). Herbs have been recognized as potential drug candidates because they possess drug-like properties (Shakya, 2016). In Slovakia use of medicinal herbs is much more popular mostly in folk medicine. Unlike tablets and pills, herbal products are not regulated for purity and potency, so detection of benefits and risk is necessary.

Ginger (*Zingiber officinale* Rosco) which belongs to family *Zingiberaceae* is a plant containing a wide range of bioactive compounds especially gingerols, which are described by their pungency. The most abundant pungent component of ginger is 6-gingerol and is claimed to contain antioxidant and antimicrobial activity. Ginger has been widely used to treat nausea and vomiting; healing of wounds, cuts and has antipruritic, anticancer and anti-inflammatory activity (Danwilai et al., 2017; Mošovská, Nováková and Kaliňák, 2015).

The comfrey (*Symphytum officinale* L.) belongs to family *Boraginaceae*, is a plant that is used for therapeutic purposes mainly in traditional medicine. It is used externally for bruises, joint pains and rheumatic and also for the treatment of diseases of the gastrointestinal tract (Nossa González, Talero Pérez and Roza Núñez, 2016). The main bioactive compound of comfrey is allantoin responsible for cell division initiation and of growth of the conjunctive tissue, bones, cartilages and acceleration of wound healing (Neagu, Roman and Radu, 2010).

Valerian (*Valeriana officinalis* L.) which belongs to the family *Valerianaceae* has long been used in folk medicine for the treatment of insomnia all over the world, mainly in the United States and Europe. The dominant mechanisms for the pharmacological action of valerian have been described based on their agonistic effects via gamma-aminobutyric acid, adenosine, barbiturate, and benzodiazepine receptors. Indeed, the health properties of some components present in valerian are believed to be associated with their antioxidant activities (Sudati et al., 2009).

Chicory (*Cichorium intybus* L.) which belongs to family *Asteraceae* is widely distributed in Asia and Europe. All parts of this plant expose medicinal properties due to the presence of several bioactive compounds such as inulin, lactones, coumarins, vitamins, natural colorants, sterols, polyphenols, saponins and tannins (Abbas et al., 2015). Preparations from chicory exhibiting antioxidant, immunomodulating, anti-inflammatory activity due to the presence of cichoric acid as a specific active compound (Ahamad et al., 2015).

Horseradish (*Armoracia rusticana* Gaertn) which belongs to the family *Brassicaceae* is used freshly grated in gastronomy as an important ingredient for meat and fish products or into sauces and salads. The main bioactive substances of horseradish are glucosinolates that provide the characteristic flavour, aroma and are also responsible for health-promoting effects such as anti-cancer properties, antioxidant and antimicrobial activity (Calabrone et al., 2015).

Ramsons (*Allium ursinum* L.) which belongs to the family *Amaryllidaceae* has been used for centuries in alternative medicine. Mostly of studies based on its composition and biological activity are fairly recent and scarce. The main bioactive compounds of ramsons are sulfur-containing compounds that are responsible for distinct garlic-like scent and also for health benefits: digestive stimulant, antimicrobial agent, removing toxins from the body, and to prevent cardiovascular disorders (Sobolewska, Podolak and Makowska-Was, 2015).

### Scientific hypothesis

Medicinal herbs are a good source of bioactive compounds with antioxidant and antimicrobial activity. In some cases that can also be poisonous due to the heavy metals content. In our study we assumed a high biological activity and low level of hazard mineral compounds, providing the possibility of using these herbs in different kinds of industry.

## MATERIAL AND METHODOLOGY

### Biological material

The medicinal herbs were collected from nature and gardens in Slovakia (the Year 2018; locality Runina; 560 m.a.s.l.): *Zingiber officinale* Rosco – rhizome, *Symphytum officinalis* L. – root, *Valeriana officinalis* L. – root, *Cichorium intybus* L. – root, *Armoracia rusticana* Gaertn – root and *Allium ursinum* L. – leaves. Before the analysis samples were pulverized in the mortar.

### Sample preparation

The sample – 0.2 g was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 rpm (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). All analyses were realized in triplicate.

### Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

### Antioxidant activity

#### DPPH method – radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchez-Moreno, Larrauri and Saura-Calixto, 1998) with slight modification. The extracts (0.4 mL) were mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 1000 mg.L<sup>-1</sup>; R<sup>2</sup> = 0.998) was used as the standard and the results were expressed in mg.g<sup>-1</sup> Trolox equivalents.

#### Phosphomolybdenum method – reducing power

Reducing the power of samples was determined by the method of Prieto, Pineda and Aguilar (1999) with slight modification. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 1000 mg.L<sup>-1</sup>; R<sup>2</sup> = 0.998) was used as the standard and the results were expressed in mg.g<sup>-1</sup> Trolox equivalents.

#### Total polyphenol content

The total polyphenol content of samples was measured by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent. An amount of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 mg.L<sup>-1</sup>; R<sup>2</sup> = 0.998) was used as the standard and the results were expressed in mg.g<sup>-1</sup> gallic acid equivalents.

#### Total flavonoid content

The total flavonoid content of samples was determined using the modified method of Willett (2002). An amount of 0.5 mL of each sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5 – 20 mg.L<sup>-1</sup>; R<sup>2</sup> = 0.989) was used as the standard and the results were expressed in mg.g<sup>-1</sup> quercetin equivalents.

#### The total phenolic acid content

The total phenolic acid content of samples was determined using the method of Farmakopea Polska (1999). An amount of 0.5 mL of each sample was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnov reagent (10% NaNO<sub>2</sub> + 10% Na<sub>2</sub>MoO<sub>4</sub>), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg.L<sup>-1</sup>; R<sup>2</sup> = 0.999) was used as a standard and the results were expressed in mg.g<sup>-1</sup> caffeic acid equivalent.

### Microbial strains

Six strains of microorganisms were tested in this study. Gram-negative bacteria: *Escherichia coli* CCM 2024, *Citrobacter freundii* CCM 7187, *Yersinia enterocolitica* CCM 7204 and Gram-positive bacteria: *Bacillus cereus* CCM 7934, *Staphylococcus aureus* subsp. *aureus* CCM 2461, *Listeria monocytogenes* CCM 4699. All tested strains were collected from the Czech Collection of microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37 °C.

### Antimicrobial activity

The antimicrobial activity of each extract was determined by a disc diffusion method. Briefly, 100 µL of the test bacteria were grown in 10 mL of fresh media until they reached a count of approximately 10<sup>5</sup> cells per mL. Then 100 µL of the microbial suspension was spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 µL of distilled water were used as a negative control.

### Chemical (mineral compounds) composition

The amount of mineral elements was analysed by the ICP-OES method (ICP-OES spectrophotometer, Thermo iCAP Dual 6500, USA) – the samples (0.2 g) were subjected to mineralization under high pressure, in HNO<sub>3</sub> 65%, super pure. The samples were weighed and placed in Teflon vessels which were then filled with 8 mL of nitric acid and sealed tightly. For each group of nine samples, during the microwave dissolution process, the rotor of the digestion system was additionally filled with a blank sample comprising 8 mL of nitric acid alone. The samples were digested for one hour, with the applied algorithm of temperature increase as specified for biological samples, without exceeding 200 °C. This was carried out using Ethos One microwave digestion system from Milestone. The vessels were opened after the mineralization process had been completed and the samples with acid had been brought to room temperature. The samples were cooled down to room temperature and supplemented with water to the volume of 50 mL. The obtained detection threshold for each element was not lower than 0.01 mg.kg<sup>-1</sup> (with the assumed detection capacity of the measuring apparatus at a level exceeding 1 ppb). The measurements were performed with ICP-OES spectrometer, Thermo iCAP Dual 6500 with horizontal plasma, and the capacity of detection along and across plasma flame (Radial and Axial). Before measuring each batch of samples the method was calibrated with the use of certified Merck models. The measurement result for each element was compensated to account for the measurement of elements in the blank sample. In each case, a 3-point calibration curve was used for each element, with optics correction applying the method of internal models, in the form of yttrium and ytterbium ions, at the concentrations of 2 mg.L<sup>-1</sup> and 5 mg.L<sup>-1</sup>, respectively.

### Statistical analysis

All experiments were carried out in triplicate and the results reported are expressed as means with standard deviations. The experimental data were subjected to the analysis of variance (Duncan's test), at the confidence level of 0.05, using the SAS 2009 (USA) software.

## RESULTS AND DISCUSSION

### Antioxidant activity

Antioxidant activity (Table 1) of analysed samples tested by the DPPH method ranged from 0.61 to 3.62 mg TEAC.g<sup>-1</sup>. The highest value was determined in the sample of ginger, following by a sample of chicory and valerian. Antioxidant activity tested by the phosphomolybdenum method (Table 1) had a similar tendency – the highest value was measured in a sample of ginger (204.14 mg TEAC.g<sup>-1</sup>). Similar value determined in ginger by reducing power method **Akinola, Ahmad and Maziah (2014)** – 266.95 mg TEAC.g<sup>-1</sup>. The strong activity by DPPH, ABTS, and FRAP method of ginger was observed in the study of **Mustafa et al. (2019)**. This authors also reported that activity is influenced by the technological process – the best activity in FRAP and ABTS method was found in ginger sun-dried, while in the DPPH method the strongest activity was found in fresh ginger. Antioxidant activity of valerian was observed in the study of **Pilerood and Prakash (2014)**. These authors tested ethanol, methanol, acetone, 80% methanol, and 80% ethanolic extracts of valerian. The best activity determined by using 80% of ethanol – this extraction solution was also used in our study. In a sample of ramsons (Table 1) by the DPPH method was evaluated the lowest activity, whereas by the phosphomolybdenum method the activity was very strong. This result can be explained by the mechanism of action – in the DPPH method antioxidants from the sample act as scavengers of radical, whereas in phosphomolybdenum method antioxidants from the sample act as reductant agent. In the study of **Putnoky, Caunii and Butnariu (2013)** are described that the S-oxyde (+/-) of S-2-propenyl cysteine and the S-oxide of S-alkenyl cysteine, located in the cytoplasm and the enzyme alliinase located in the vacuole is the main bioactive compounds which bind reactive free radicals and act as reductant agent.

### Total polyphenol, flavonoid, and phenolic acid content

Total polyphenols (Table 1) in tested samples ranged from 4.37 to 13.19 mg GAE.g<sup>-1</sup>. The highest value was found in the sample of ramsons following by ginger and valerian. **Lachowicz et al. (2017)** determined in ramsons values ranged from 6.5 (bulbs) to 42.5 (leaves) mg GAE.100g<sup>-1</sup> fresh matter which is lower values compared to our study. **Pejatović, Samardžić and Krivokapić (2017)** determined values ranged from 13.05 to 18.33 mg GAE.g<sup>-1</sup>, which is comparable with our findings. **Qadir et al. (2017)** in ginger extract found total polyphenols in the amount of 98.37 mg GAE.100g<sup>-1</sup>, which is a lower level to compare with our results. These authors also published that 80% ethanol is discovered more effective for the recovery of antioxidants from herbal plants to compare with methanol and acetone.

**Table 1** Antioxidant activity, total polyphenol, flavonoid and phenolic acid content in tested samples.

Sample	DPPH (mg TEAC.g <sup>-1</sup> )	PM (mg TEAC.g <sup>-1</sup> )	TPC (mg GAE.g <sup>-1</sup> )	TFC (mg QE.g <sup>-1</sup> )	TPAC (mg CAE.g <sup>-1</sup> )
<b>Ginger</b> ( <i>Zingiber officinale</i> Rosco)	3.62 ±0.08a	204.14 ±2.99a	11.91 ±0.11a	8.62 ±1.11b	9.77 ±0.09a
<b>Horseradish</b> ( <i>Armoracia rusticana</i> Gaertn)	1.01 ±0.11e	73.94 ±3.08b	4.09 ±1.04b	1.96 ±0.07c	0.99 ±0.07f
<b>Comfrey</b> ( <i>Symphytum officinalis</i> L.)	1.16 ±0.03d	95.09 ±3.03c	4.37 ±0.06b	2.21 ±0.11c	3.31 ±0.09c
<b>Ramsons</b> ( <i>Allium ursinum</i> L.)	0.61 ±0.02f	169.18 ±2.44b	13.19 ±1.72a	47.55 ±2.31a	8.31 ±0.31b
<b>Cichory</b> ( <i>Cichorium intybus</i> L.)	2.79 ±0.04b	80.31 ±2.41d	5.39 ±0.83b	1.07 ±0.04c	1.79 ±0.01e
<b>Valerian</b> ( <i>Valeriana officinalis</i> L.)	2.52 ±0.07c	66.67 ±3.11f	5.53 ±0.14b	1.67 ±0.09c	2.61 ±0.07d

Note: DPPH – radical scavenging activity; PM – phosphomolybdenum method; TPC – total polyphenol content; TFC – total flavonoid content; TPAC – total phenolic acid content; TEAC – Trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; mean ±standard deviation; different letters in column denote mean values that statistically differ one from another.

**Table 2** Antimicrobial activity of tested samples determined by disc diffusion method.

Sample	<i>E.coli</i> CCM 2024 (mm)	<i>C. freundii</i> CCM 7187 (mm)	<i>Y. enterocolitica</i> CCM 7204 (mm)	<i>B. cereus</i> CCM 7934 (mm)	<i>S. aureus</i> CCM 2461 (mm)	<i>L. monocytogenes</i> CCM 4699 (mm)
<b>Ginger</b> ( <i>Zingiber officinale</i> Rosco)	-	-	1.00 ±0.07b	5.00 ±0.39a	-	1.00 ±0.02c
<b>Horseradish</b> ( <i>Armoracia rusticana</i> Gaertn)	-	-	-	1.33 ±0.11c	-	1.33 ±0.05c
<b>Comfrey</b> ( <i>Symphytum officinalis</i> L.)	1.00 ±0.01c	1.33 ±0.14b	1.00 ±0.06b	-	-	2.66 ±0.71a
<b>Ramsons</b> ( <i>Allium ursinum</i> L.)	2.00 ±0.11b	2 ±0.11a	2.33 ±0.01a	-	2.00 ±0.02a	1.00 ±0.11c
<b>Cichory</b> ( <i>Cichorium intybus</i> L.)	3.33 ±0.21a	-	1.00 ±0.01b	2.33 ±0.31b	2.00 ±0.03a	2.00 ±0.12b
<b>Valerian</b> ( <i>Valeriana officinalis</i> L.)	-	1.00 ±0.02c	1.00 ±0.02b	2.33 ±0.24b	2.00 ±0.01a	1.00 ±0.09c

Note: mm - millimetre; mean ±standard deviation; different letters in column denote mean values that statistically differ one from another.

**Table 3** Mineral compounds composition in tested samples.

Parameter (mg.100g <sup>-1</sup> )	<b>Ginger</b> ( <i>Zingiber officinale</i> Rosco)	<b>Horseradish</b> ( <i>Armoracia rusticana</i> Gaertn)	<b>Comfrey</b> ( <i>Symphytum officinalis</i> L.)	<b>Ramsons</b> ( <i>Allium ursinum</i> L.)	<b>Cichory</b> ( <i>Cichorium intybus</i> L.)	<b>Valerian</b> ( <i>Valeriana officinalis</i> L.)
<b>Aluminium (Al)</b>	11.50 ±2.04e	32.00 ±2.22d	55.00 ±1.42b	9.68 ±1.75e	41.91 ±1.12c	133 ±2.58a
<b>Cadmium (Cd)</b>	0.04 ±0.01a	0.03 ±0.01ab	0.02 ±0.01bc	0.01 ±0.01c	0.02 ±0.01bc	0.02 ±0.01bc
<b>Chrome (Cr)</b>	0.09 ±0.01b	0.19 ±0.05b	0.70 ±0.02b	0.35 ±0.02b	0.70 ±0.01b	2.85 ±1.17a
<b>Calcium (Ca)</b>	233 ±3.21f	365 ±3.45c	375 ±3.33b	702 ±2.59a	252 ±2.63e	321 ±2.41d
<b>Copper (Cu)</b>	0.76 ±0.13c	0.33 ±0.05d	1.31 ±0.12a	0.46 ±0.11d	0.90 ±0.01c	1.07 ±0.09b
<b>Iron (Fe)</b>	10.9 ±0.69e	22.9 ±1.14d	57.6 ±2.22b	12.3 ±1.24e	49.1 ±3.32c	189 ±1.52a
<b>Potassium (K)</b>	3772 ±2.45a	1961 ±18.80c	1778 ±3.58d	2655 ±10.81b	1234 ±3.14f	1698 ±4.48e
<b>Magnesium (Mg)</b>	390 ±1.79a	130 ±1.09e	118 ±4.21f	251 ±3.75c	182 ±1.97d	275 ±3.24b
<b>Manganese (Mn)</b>	182 ±1.24a	130 ±1.09b	4.02 ±0.11de	6.00 ±1.98d	2.72 ±0.58f	16.10 ±0.94c
<b>Sodium (Na)</b>	79.4 ±2.09c	6.23 ±1.96e	419 ±2.74b	3.24 ±1.11e	708 ±2.77a	20.3 ±1.12d
<b>Phosphorus (P)</b>	376 ±0.73b	196 ±2.80f	200 ±1.11e	391 ±2.13a	282 ±3.11d	343 ±1.14c
<b>Lead (Pb)</b>	0.09 ±0.02b	0.04 ±0.01c	0.08 ±0.01b	0.02 ±0.01c	0.08 ±0.02b	0.41 ±0.02a
<b>Sulphur (S)</b>	178 ±3.11c	615 ±3.56b	84.8 ±1.23f	932 ±3.21a	119 ±1.73e	0.41 ±1.11bc
<b>Strontium (Sr)</b>	1.93 ±0.09b	2.38 ±1.28b	1.53 ±0.09bc	0.30 ±0.10c	4.61 ±1.04a	1.44 ±0.09bc
<b>Zinc (Zn)</b>	5.06 ±1.79a	3.77 ±0.58ab	1.17 ±0.07c	1.34 ±0.08c	0.65 ±0.08c	2.99 ±0.07b

Note: mean ±standard deviation; different letters in column denote mean values that statistically differ one from another.

Total flavonoids (Table 1) in tested samples ranged from 1.07 to 47.55 mg QE.g<sup>-1</sup>. The best value was measured in a sample of ramsons following by a sample of ginger and comfrey. **Pejatović, Samardžić and Krivokapić (2017)** determined in ramsons leaves extract values ranged from 13.75 to 20.00 mg QE.g<sup>-1</sup>. **Ghasemzadeh, Jaafar and Rahmat (2010)** in a sample of ginger extract determined total flavonoids in amount ranged from 3.66 to 4.21 mg QE.g<sup>-1</sup>, which are lower values to compare with our results. These authors by HPLC method determined that in ginger rhizomes the most abundant flavonoid is quercetin. In valerian the most abundant flavonoids are 6-methylapigenin and hesperidin, which are responsible for the sedative and sleep-enhancing properties of valerian root (**Marder et al., 2003**).

Total phenolic acid content (Table 1) ranged from 0.99 to 9.77 mg CAE.g<sup>-1</sup>. The highest value was determined in a sample of ginger following by a sample of ramsons and comfrey. **Fahmi et al. (2019)** published that chlorogenic acid (63.85 ppm) and hesperidin (156.91 ppm) are among the major phenolic and flavonoid constituents in dry ginger. In comfrey **Sowa et al. (2017)** by HPLC-DAD method found rosmarinic, *p*-hydroxybenzoic, caffeic, chlorogenic, and *p*-coumaric acid.

#### Antimicrobial activity

In a sample of ginger (Table 2) was observed activity to inhibit *B. cereus* CCM 7934, *Y. enterocolitica* CCM 7204 and *L. monocytogenes* CCM 4699. In the study of **Nas, Ali and Ahmad (2018)** *E. coli* were found to be the highest susceptible organisms with an average zone of inhibition of 13.6 mm, followed by the *Shigella spp.* (13.3 mm), *Salmonella typhi* (12.7 mm), *Staphylococcus aureus* (12.5 mm), *Pseudomonas aeruginosa* (10.8 mm) while the least average zone of inhibition is shown by *Klebsiella pneumoniae* (9.2 mm).

In the sample of horseradish (Table 2) was detected low potential to inhibit growing of *B. cereus* CCM 7934 and *L. monocytogenes* CCM 4699. In comfrey extract (Table 2) was detected activity to inhibit *E. coli* CCM 2024, *C. freundii* CCM 7187, *Y. enterocolitica* CCM 7204 and *L. monocytogenes* CCM 4699. The antibacterial activity of the root extract of *Symphytum officinale* was tested by the disc diffusion method in the study of **Sumathi, Kumar and Bai (2011)** and it was found that methanol extract showed maximum inhibitory effect against the *Proteus vulgaris* and *Staphylococcus aureus*.

In ramsons sample (Table 2) was observed slight activity to inhibit *E. coli* CCM 2024, *C. freundii* CCM 7187, *Y. enterocolitica* CCM 7204, *S. aureus* subsp. *aureus* CCM 2461 and *L. monocytogenes* CCM 4699. **Krivokapić et al. (2018)** reported that the antimicrobial activity of the plant extract is influenced by the extraction solvent. A methanol extract of ramsons has been shown to exhibit antimicrobial activity against bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella enteritidis*. On the other hand, water extract was efficient only against *Bacillus subtilis*. Chicory extract in our study showed slight activity to inhibit *E. coli* CCM 2024, *B. cereus* CCM 7934, *Y. enterocolitica* CCM 7204, *S. aureus* subsp. *aureus* CCM 2461 and *L. monocytogenes* CCM 4699. In the study, **Liu et al. (2013)** were observed

several extracts of chicory which displayed activities against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Bacillus subtilis*, and *Salmonella typhi*. These authors also found that the best method for the chicory extract yield with antimicrobial activity was a combination of 70% ethanol v/v, 24-h impregnation time, 3 sonication rounds, and 300-W ultrasonic input power. In valerian extract (Table 2) was observed potential to inhibit the growth of *C. freundii* CCM 7187, *Y. enterocolitica* CCM 7204, *B. cereus* CCM 7934, *S. aureus* subsp. *aureus* CCM 2461 and *L. monocytogenes* CCM 4699.

#### Chemical composition

The results of mineral compounds composition are presented in Table 3. In a sample of ginger was determined potassium as the dominant compound following by magnesium and phosphorus. Very interesting was the amount of zinc which was the highest in this sample compared to other tested samples. According to **Chasapis et al. (2012)** zinc is one of the most important trace elements in the human body, with three major biological roles: catalyst, structural, and regulatory ion. Zinc has an important effect on homeostasis, immune function, oxidative stress, apoptosis, aging, and significant disorders of great public health interest are associated with zinc.

In samples of horseradish were dominant potassium, sulphur, calcium and phosphorus. The amount of sulphur was higher to compare with ginger, valerian, chicory, and comfrey. A higher amount of sulphur can be explained as the fact that horseradish is rich for glucosinolates. Glucosinolates are N-hydroxy-sulfates with a highly variable side chain and a sulfur-linked *beta*-D-glucopyranose. These compounds are the precursor molecules of the biologically active isothiocyanates components (**Bertóti et al., 2019**).

In a sample of comfrey dominant compounds was potassium, following by sodium, phosphorus, and calcium. This sample was determined the highest value of copper, which is an essential mineral for human health but at the same time can be toxic, depending upon the amounts of ingested. Copper is associated with bone health, immune function, and increased frequency of infections, cardiovascular risk, and alterations in cholesterol metabolism (**Araya, Manuel and Fernando, 2007**).

In the sample of ramsons was determined potassium as dominant compounds following by the sulphur, calcium, and magnesium. The amount of sulphur was the highest to compare with the other analysed samples. It is not surprising whereas the dominant bioactive compounds of this plant are based of sulphur (allicin).

In the sample of chicory, the main mineral compounds were potassium, sodium, magnesium and sulphur. The same tendency was observed in the study of **Mona, Wafaa and Elgindy (2009)** in which also authors determined higher content of iron in chicory root, which is comparable with our findings.

In the sample of valerian was determined potassium as dominant mineral compounds following by calcium and magnesium. In this sample was found the highest value of aluminium, this amount can be evaluated as a safe because according to Food Safety Authority (**Stahl et al., 2017**) the tolerable weekly intake of aluminium is 1 mg.kg<sup>-1</sup> body weight for all groups of people.

Valerian root is used mainly in folk medicine to prepare a decoction to treat psychological problems and usually is not consumed as a vegetable. Generally, the amount of heavy metals in observed samples was detected only in a trace amount, so our results reveal that the medicinal herbs do not represent in this study a potential health risk regarding the content of toxic elements.

## CONCLUSION

Medicinal herbs are a very important part of the industry, especially pharmacy and medicine. Nowadays these plants start to be very popular also in gastronomy and food technology. They are a good source of specific bioactive compounds, but their quality can be endangered due to the higher level of heavy metals. In our study, the highest antioxidant activity was determined in ginger (3.62 and 204.14 mg TEAC.g<sup>-1</sup>). In the sample of ramsons was detected the highest value of phenolics (13.19 mg GAE.g<sup>-1</sup>; 47.55 mg QE.g<sup>-1</sup>). The wide spectrum of mineral compounds was generally found in all observed samples, while the amount of heavy metals was detected only in a trace amount.

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