



ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *BUNIAS ORIENTALIS* L. AND *SCORZONERA HISPANICA* L. ETHANOL EXTRACTS

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The current study was aimed to evaluate an accumulation in plant raw material of *Bunias orientalis* L. and *Scorzonera hispanica* L. the total content of phenolic compounds, phenolic acids, flavonoids, antioxidant activity (by DPPH-method) and reducing power of alcoholic extracts. Raw of investigated plants collected from experimental collections of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv) and dried for next investigation. Obtained results showed that total content of polyphenols in the above-ground parts of *B. orientalis* extracts was 52.88 mg g⁻¹ GAE (gallic acid equivalent), phenolic acids – 11.29 mg g⁻¹ CAE (caffeic acid equivalent), flavonoids – 39.91 mg g⁻¹ QE (quercetin equivalent), antioxidant activity – 8.94 mg g⁻¹ TE (Trolox equivalent) and reducing power of extracts – 184.59 mg g⁻¹ TE (Trolox equivalent). Total content of polyphenols in the above-ground parts of *S. hispanica* plants was 60.16 mg g⁻¹ GAE, phenolic acids – 20.71 mg g⁻¹ CAE, flavonoids – 36.24 mg g⁻¹ QE, antioxidant activity – 5.35 mg g⁻¹ TE and reducing power of extracts – 125.40 mg g⁻¹ TE. Also, total content of polyphenols in the roots of *B. orientalis* extracts was 9.75 mg g⁻¹ GAE, phenolic acids – 1.73 mg g⁻¹ CAE and reducing power of extracts – 138.70 mg g⁻¹ TE. Root extracts of *S. hispanica* had total content of polyphenols of 10.87 mg g⁻¹ GAE, phenolic acids – 2.62 mg g⁻¹ CAE and reducing power of extracts – 110.96 mg g⁻¹ TE. Flavonoids and antioxidant activity in the root extracts of both species weren't determined. The antimicrobial activity of alcoholic extracts of two investigated species was tested against 8 microorganisms by disc diffusion method. It was found that higher activity against microbial strains had alcoholic extracts of *B. orientalis* roots.

Keywords: *Bunias orientalis*, *Scorzonera hispanica*, antioxidant activity, polyphenols, flavonoids, phenolic acids, antimicrobial activity

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Introduction

Plants are potential sources of natural antioxidants with therapeutic properties that can be used in traditional and folk medicine (Chandra et al., 2014; Ivanišova et al., 2017). Representatives of Asteraceae Bercht. & J. Presl and Brassicaceae Burnett also are the good source of antioxidant compounds of different nature (Martinez-Sanchez et al., 2007; Vijayalakshmi et al., 2009; Sikora and Bodziarczyk, 2012). Cruciferous vegetables act as a good source of natural antioxidants due to the high levels of carotenoids, tocopherols and ascorbic acid (Ateya et al., 2016). *Bunias orientalis* L. (Turkish cabbage) is biennial or perennial hemicryptophyte that belongs to Brassicaceae. A recent study of *B. orientalis* connected with a high possibility to root because of powerful taproot leading to massive sprawl (Oliver et al., 2015). The complex of characteristics (biochemical toxicity, allelopathic effect, powerful taproot etc.) explained this plant as invasive (Dietz and Winterhalter, 1996; Dietz et al., 1999; Patamsitè et al., 2013). Also, it was studied nectar production and carbohydrate composition of this species (Denisow et al., 2016). Nevertheless, the accumulation of some biochemical compounds in different organs causes the useful properties of *B. orientalis* (Vinogradova and Kuklina, 2018). In the M.M. Gryshko Botanical Garden of the NAS of Ukraine have been studying these plants as forage and energetic culture and has been conducted selection work. Raw of this species is rich on protein, lipids, ash, ascorbic acid, carotene etc. (Uteush and Lobas, 1996).

Another species from Asteraceae family belongs to the genus of *Scorzonera* L. Some species of this genus have been used as traditional medicines with analgesic, antirheumatic, anthelmintic, stomachic and diuretic effects. These plants can be used for the treatment of the wound, hypertension, infertility, lung oedema, diarrhoea etc. (Sari et al., 2009). As reported Acikara Bahadir et al. (2013a), the plants of *Scorzonera* species used as medicinal and vegetable cultures possess promising antioxidant activity. One of the most effective compounds from antioxidants was found a chlorogenic acid. Extracts from some species of *Scorzonera* showed the activity on the wound healing that can be connected with the combined effect of the constituents (Küpeli et al., 2011). Useful properties of *Scorzonera* species for the food industry because of valuable biochemistry content were described (Mahjoub et al., 2009; Çitoğlu et al., 2010; Bashta et al., 2015). Some studies reported that raw of these plants contains flavonoids (apigenin, luteolin, kaempferol, rutin etc.) and triterpenoids (lupeol, daucosterol etc.) (Küpeli et al., 2011; Acikara et al., 2013; Benabdelaziz et al., 2014). Çetin et al. (2018) reported about seventeen triterpenoids in *S. veratrifolia*. However, the information about *Scorzonera* species is considerably limited in the existing literature (Erden and Kirbağ, 2015).

The aim of this study was to assess an antioxidant and an antimicrobial potential of *Bunias orientalis* L. and *Scorzonera hispanica* L. in the conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methodology

The plants were grown in 2017 at the experimental fields of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) in the Kiev city (50° 24' 55" N, 30° 33' 45" E).

Biological material

Observation on plants was conducted in the experimental collection of forage plants (*Bunias orientalis* L.) and vegetables crops (*Scorzonera hispanica* L.) of Cultural Flora Department of NBG. Plant raw material of investigated plants was collected in the stages of spring vegetation. In this study used above-ground part and roots of *B. orientalis* and above-ground part of *S. hispanica*.

Biochemical analysis

The biochemical analysis was done in the Slovak University of Agriculture in Nitra (Slovak Republic). For planned analyses, 0.2 g of milling fraction was extracted with 20 ml of 80% ethanol for 24 hours. After centrifugation at 4000 g with Rotofix 32 A (Hettich, Germany) for 20 min, the supernatant was used for measurement of the total content of polyphenols.

Radical scavenging assay

The radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sanchez-Moreno et al., 1998). The extracts (0.5 mL) were mixed with 3.6 mL of radical solution (0.025 g of 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) (10–100 mg.L⁻¹; $R^2 = 0.988$) was used as the standard and the results were expressed in mg g⁻¹ Trolox equivalents.

Reducing power

Reducing power of extracts was determined by the phosphomolybdenum method of Prieto et al. (1999) with slight modifications. The mixture of 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) 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 (10–1000 mg.L⁻¹; $R^2 = 0.998$) was used as the standard and the results were expressed in mg g⁻¹ Trolox equivalents.

Total polyphenol content

Total polyphenol content extracts was measured by the method of Singleton and Rossi (1965) using Folin-Chiocalteu reagent. 0.1 mL of each sample extract was mixed with 0.1 mL of the Folin-Chiocalteu 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 (25–250 mg.L⁻¹; $R^2 = 0.996$) was used as the standard and the results were expressed in mg g⁻¹ gallic acid equivalents.

Total flavonoid content

Determination of total flavonoids content was conducted according to procedure which was described by Shafii et al. (2017). 0.5 mL of sample extract was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M sodium 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 (0.01–0.5 mg.L⁻¹; $R^2 = 0.997$) was used as the standard and the results were expressed in $\mu\text{g g}^{-1}$ quercetin equivalents.

Total phenolic acid content

Determination total phenolic acids content of extracts was carried out using method of Farmakopea Polska (1999). 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1–200 mg.L⁻¹, $R^2 = 0.999$) was used as a standard and the results were expressed in mg g⁻¹ caffeic acid equivalents.

Preparation of plant extracts for determination of antimicrobial activity

Plant raw material was dried, crushed and weighed out to 2 g and soaked in 20 mL of ethanol p.a. (Sigma, Germany) during two weeks at room temperature. After this ethanol plant extracts were filtered through the Whatman No. 1 filter paper. The obtained extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the ethanol (Stuart RE300DB rotary evaporator, Bibby Scientific Limited, UK, and vacuum pump KNFN838.1.2KT.45.18, KNF, Germany). For the antimicrobial assays, the crude plant extracts were dissolved in dimethylsulfoxide (Penta, Czech Republic) to 102.4 mg mL⁻¹.

Microbial strains

Eight strains of microorganisms were tested in this study, including *Bacillus cereus* CCM 869, *Candida albicans* CCM 8215, *Candida glabrata* CCM 8270, *Candida tropicalis* CCM 8264, *Clostridium perfringens* CCM 4435T, *Haemophilus influenza* CCM 4456, *Klebsiella pneumoniae* subsp. *pneumoniae* CCM 4415, *Salmonella enterica* subsp. *enterica* CCM 7189. All tested strains collected from the Czech Collection of microorganisms. The bacterial suspensions were cultured in the nutrient broth at 37 °C.

Disk diffusion method of determination of antibacterial activity

Antibacterial activity of ethanol extracts of *B. orientalis* and *S. hispanica* were 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 approximately 10^5 cells.mL⁻¹. 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 millimetres. All antimicrobial assays were performed in at least triplicate. Negative control was a filter disc impregnated with 10 μL of distilled water.

Statistical analysis

The statistically treated data are given in table as the arithmetical mean values and their standard errors.

Results and discussion

Phenolic compounds are a large group of the secondary metabolites widespread in plants. These compounds possess different biological activities, but the most important is antioxidant activity (Soobrattee et al., 2005; Podsędek, 2007). Study of Mahboubi et al. (2013) expected that ethanolic or ethyl acetate extracts with higher level of total phenolic content would exhibit higher antioxidant activity. However, for example, Kähkönen et al. (1999) reported that antioxidant activity does not necessarily correlates with high amounts of phenolics.

Our previous data concerning Brassicaceae showed that total antioxidant activity of selected species was 16.94–36.91% in methanol extracts and 26.53–65.66% in water extracts (Vergun and Rakhmetov, 2018). As shown in Tables 1 and 2, total content of polyphenols, phenolic acids and flavonoids was higher in the above-ground part of plants of *B. orientalis* than in roots. The considering of obtained results in details showed that content of polyphenols in above-ground part was 5.4 times more than in roots, content of phenolic acids – 6.5 times respectively. Similar results obtained for *S. hispanica* extracts.

The most widespread and diverse group of the polyphenols are the flavonoids. They have been suggested to play a preventive role in the development of some disease. They are highly effective scavengers of most oxidizing molecules (Baba and Malik, 2015; Hudz et al., 2017). The total content of flavonoids and antioxidant activity by DPPH-method weren't determined in the roots of the investigated plants.

Table 1 The content of phenolic compounds and antioxidant activity in above-ground parts of *Bunias orientalis* L. and *Scorzonera hispanica* L. in the period of spring vegetation

Parameter	<i>Bunias orientalis</i>	<i>Scorzonera hispanica</i>
Total content of polyphenols (mg g ⁻¹ GAE)	52.88 ±1.84	60.16 ±1.01
Total content of phenolic acids (mg g ⁻¹ CAE)	11.29 ±1.00	20.71 ±2.55
Total content of flavonoids (mg g ⁻¹ QE)	39.91 ±0.43	36.24 ±2.14
Antioxidant activity (mg g ⁻¹ TE)	8.94 ±0.07	5.35 ±0.12
Reducing power of extract (mg g ⁻¹ TE)	184.59 ±0.37	125.40 ±1.00

Notes: Means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of three independent experiments (\pm SD)

As reported Acikara Bahadir et al. (2013b), the chlorogenic acid is one of the major constituent of *Scorzonera* species, which probably responsible for the biological activities of these plants. As described by Erden and Kirbağ (2015), the total phenolic content of soil surface parts of different species of *Scorzonera* was from 14.92 to 40.19 mg g⁻¹ GAE and flavonoids – from 30.47 to 110.08 mg g⁻¹ QE. Granica et al. (2015) revealed that both subaerial and aerial parts of *S. hispanica* contain caffeoylquinic acid derivatives, which are the major phenolics in this plant. Also, in this study showed that presence of flavonoids was confirmed in aerial part of *S. hispanica*.

Table 2 The content of phenolic compounds and antioxidant activity in the roots of *Bunias orientalis* L. and *Scorzonera hispanica* L. in the period of spring vegetation

Parameter	<i>Bunias orientalis</i>	<i>Scorzonera hispanica</i>
Total content of polyphenols (mg g ⁻¹ GAE)	9.75 ±0.54	10.87 ±0.29
Total content of phenolic acids (mg g ⁻¹ CAE)	1.73 ±0.05	2.62 ±0.26
Total content of flavonoids (mg g ⁻¹ QE)	ND	ND
Antioxidant activity (mg g ⁻¹ TE)	ND	ND
Reducing power of extract (mg g ⁻¹ TE)	138.70 ±1.41	110.96 ±6.88

Notes: Means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of three independent experiments (\pm SD); ND – non determined.

The interest of natural plants products as antimicrobials is supported by the necessity to reduce the use of conventional antibiotics in food preservation (Ateya et al., 2016). Study of Prasad (2014) showed that different extracts of selected plants of Brassicaceae exhibited significant antimicrobial activity.

Investigation of the antimicrobial activity of root extracts of *B. orientalis* showed in the Figure 1. Our results of antibacterial testing with disc diffusion method demonstrate that *Candida albicans* and *Salmonella enterica* subsp. *enterica* were the most sensitive to *B. orientalis* root extract (5.00 and 4.33 mm respectively). Among tested bacteria, *Bacillus cereus* was the least sensitive (1.00 mm).

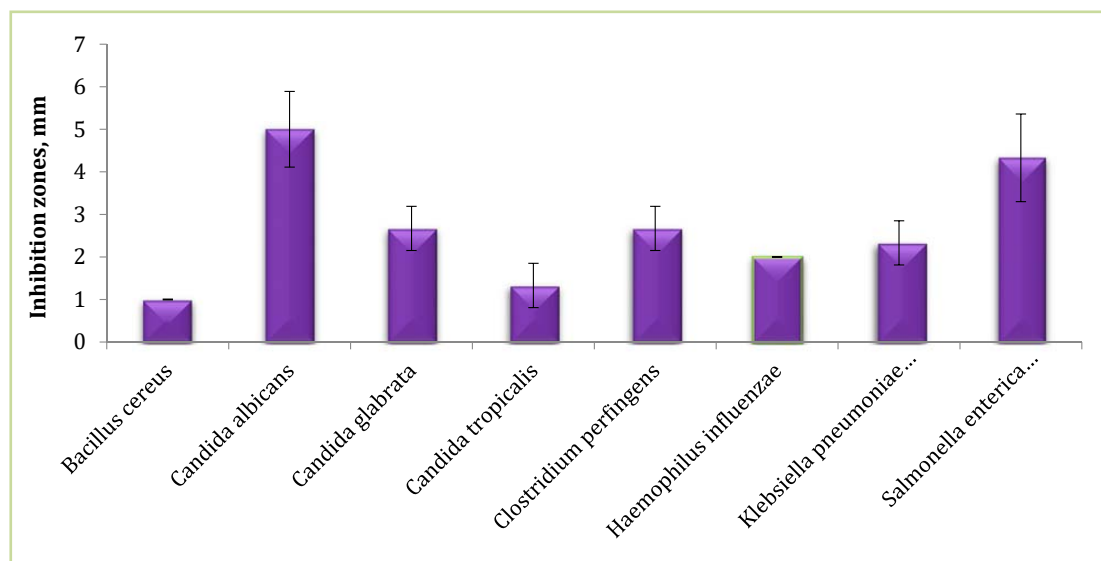


Figure 1 Antimicrobial activity of root plant extracts of *Bunias orientalis* L. (Means in columns followed by different letters are different at $p = 0.05$; each value represents the mean of six independent experiments (\pm SD))

Our study showed also, that antimicrobial activity of above-ground extracts of *B. orientalis* wasn't determined. Ethanol root extracts weren't effective against all investigated microorganisms.

In the current research plant extracts (above-ground part) of *S. hispanica* showed antimicrobial activity against *Salmonella enterica* only. Inhibition zone in this case was 1.33 ± 0.52 mm. Root extracts weren't effective against all microorganisms. Results of Sari et al. (2009) showed that *S. latifolia* and *S. veratrifolia* ethanol extracts were able to inhibit the growth of *Staphylococcus aureus*, *S. epidermidis*, *Shigella flexneri*, *Candida albicans*. Better results were found for petroleum ether fraction. Another study of *S. humilis* L. reported that no antibacterial activity against *Bacillus subtilis* and nor antifungal activity against *Candida albicans* wasn't found (Zidorn et al., 2000; Zidorn et al., 2002). As reported Erden and Kirbağ (2015), three species of genus of *Scorzonera* showed antimicrobial effect on *Escherichia coli* (17–27 mm of inhibition zone) and none of the plant extracts showed any effect against *Klebsiella pneumoniae*. Two species showed the antimicrobial activity against *Staphylococcus aureus* (8 mm of inhibition zone).

Conclusions

Thus, results of current study showed that ethanol extracts of *B. orientalis* and *S. hispanica* demonstrated a higher antioxidant activity of the above-ground parts of plants then of roots (content of polyphenols and phenolic acids). The content of flavonoids was observed in the above-ground parts of plants; however, in roots it wasn't determined. Reducing power was higher in the extracts of above-ground parts. Alcoholic extracts from the roots of *B. orientalis* demonstrated antimicrobial effect against all tested microorganisms. The highest antimicrobial activity was found against *Candida albicans* and *Salmonella enterica* subsp. *enterica*. Root extracts of *S. hispanica* weren't effective against all tested microbial strains but extracts of above-ground part was non-significant effective against *Salmonella enterica*. Authors considered that alcoholic extracts of investigated plants could be beneficial for new microbiological and pharmacological study.

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