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RESEARCH ARTICLE

Phytochemical characterization and antioxidant potential of *Allium ursinum* L. cultivated on different soil types- a preliminary study

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ABSTRACT

Wild garlic (*Allium ursinum* L.) has been used as nutrition and medicine for centuries. Although this plant species is a typical spring geophyte that grows spontaneously in moist, steep, shady beech forests, but information on phytochemical and antioxidant properties under various soil types are scarce. This study aimed to assess the phytochemical composition and antioxidant potential of the leaves of *A. ursinum* grown on different soil types, but under identical climatic conditions of South Banat, Serbia. For the purpose of reproduction, *A. ursinum* bulbs were collected from two different locations in Serbia and then planted on different types of soil, namely: Arenosol, Fluvisol, Cambisol and Chernozem. Fresh leaves of sprouted plants were sampled at the beginning of spring, morphologically analysed and stoma was counted. The leaf extract was prepared and its phytochemical composition and antioxidant potential were assessed. Regardless of the origin of the reproductive material (bulbs), the leaves of *A. ursinum* plants cultivated in Chernozem soil had the best morphological characteristics and the largest number of stomata. Phytochemical analyses revealed the following ranges for selected bioactive compounds (expressed on fresh weight, FW): chlorophyll content (289.9-642.4 μg/g for chlorophyll *a* i.e. 358.2-458.6 μg/g for chlorophyll *b*), total carotenoid content (TCC, 91.2-263.2 μg/g), total phenolic content (TPC, 1.43-1.98 mg/g GAE), total flavonoid content (TFC, 0.36-1.28 mg/g QE), and total dihydroxycinnamic acid derivative content (HCA, 0.53-0.59 mg/g CGAE). The highest values were obtained on Chernozem (chlorophyll *a*, chlorophyll *b* and TPC and HCA) and Cambisol (TCC and TFC). Chernozem appeared to be the best soil type during three applied standard antioxidant assays (CUPRAC, TAC and FRP) while DPPH radical quenching assay revealed no significant differences among all examined soil types. Based on the obtained results it could be assumed that Chernozem exhibited the most desirable physico-chemical properties for optimal development of *A. ursinum* (in particular its green parts) as a source of different antioxidants. Correlation analysis of phytochemical parameters has proved significant influence of total chlorophylls, phenolics, flavonoids and duhydroxycinnamic acid derivatives on antioxidant activity of *A. ursinum* leaves (unlike total carotenoid content) with the highest correlation between HCA and FRP assay ($r^2 = 1.00$). In addition, PCA analysis clearly determined Chernozem type of soil as the best choice for optimal leaf growth and development.

Keywords: Wild garlic; cultivation; morphology; leaves; phytochemicals

INTRODUCTION

Allium ursinum L., commonly known as wild garlic, is a perennial spring geophyte that grows in moist, shady habitats throughout Europe, Asia Minor and North Asia (Herault et al., 2005; Oborny et al., 2011). The period of its active growth and development lasts several months starting from February to June. The vegetation period begins with bulb elongation (4-6 cm). The three-limbed stem grows up to ca. 40 cm and it is surrounded by two or three elliptical leaves. At the top of the stem white flowers are formed, gathered in thyroid inflorescences that bloom from the beginning of April until the end of May (Sobolewska et al., 2015). The seeds are collected in a capsule and they are black-red in colour and round in shape (Błażewicz and Michowska, 2011). According to Ernest (1979), *A. ursinum* in natural habitats reproduces predominantly vegetatively by dividing the bulbs and only

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a small number of plants in the population reproduce by seeds. However, Eggert (1992) indicated that only the vegetative propagation ensures that adult plants reproduce themselves again, while recent research by Sobolewska et al. (2015), indicates that *A. ursinum* populations reproduce generatively (by seed).

A. ursinum appears to be of great value, both in nutrition and in modern and traditional medicine (Błażewicz and Michowska, 2011). It is a medicinal plant species that in its aboveground plant part, particularly in leaves, contains a wide range of bioactive compounds with pronounced pharmacological activities (Sendl et al., 1992). Its healing properties are associated with the content of sulphur, phenolic compounds (especially phenolic acids), saponins and carotenoids (Schmitt et al., 2005; Lachowicz et al., 2016; Marković et al., 2019). The fresh leaf of *A. ursinum* proved to have a high antioxidant potential due to the presence of phenolic compounds (Stajner et al., 2008). Li et al. (2020) showed that the production of secondary metabolites is generally affected by environmental factors, particularly climate and soil factors. Research of Djurdjevic et al. (2004), Trémolières et al. (2009) and Sobolewska et al. (2015), on wild *A. ursinum* showed that environmental factors (climate and soil), have a great impact on morphological, physiological, and chemical properties of the plant itself. Namely, research by Oguchi et al. (2018) indicated that the conditions of the external environment in *A. ursinum* directly affect the anatomical features of the leaf. Research by Hommel et al. (2014) explained that the regulation of H_2O and CO_2 through the stomatal apparatus in dry conditions in *A. ursinum* can directly affect the water conductivity through the leaf's mesophyll and the efficiency of water uptake from the soil, which can have different water capacities (Hansen et al. 2016). Recently, Bodó et al. (2021), revealed a great influence of soil type on the amount of nectar in *A. ursinum*. However, to the best of our knowledge, there were no studies on the influence of different soil types on the phytochemical properties of *A. ursinum*, cultivated under the same climatic conditions.

In that sense, current research aimed to reveal the most appropriate type of soil for wild garlic (*A. ursinum*) cultivation under agroecological conditions of South Banat (region of Serbia) as well as the soil type influences on several morpho-anatomical leaf parameters (weight, length, width of the leaf and number of stomata), content of selected phytochemicals (total phenolic content, total flavonoid content, total dihydroxycinnamic acid derivative content, total carotenoid content, content of chlorophyll *a* and chlorophyll *b*), and antioxidant properties of the edible part of wild garlic.

MATERIAL AND METHOD

Plant material

Planting material (bulbs) of *Allium ursinum* L. were collected from two different locations in the Republic of Serbia during mid-September 2020, where it grew spontaneously: 1) region of Fruška Gora (182 m above mean sea level (AMSL), 45°07'38.9" N 19°31'56.4 "E); 2) Mačva region (80 m AMSL 44°44'14.3"N 19°33'52.3"E) (Fig. 1). After collection, the bulbs were washed and sorted by size for their uniformity.

Experimental design

Bulbs with uniform size of wild *A. ursinum* were selected for further planting (Fig. 2); 16 vegetation pots (15 x 50 x 20 cm) per bulb origin (32 pots in total) were filled with four different soil types (Table 1). For further planting, 160 bulbs were selected from each location (2 location x 160), making a total of 320 uniform bulbs. All bulbs were sown in prepared pots with 10 bulbs per pot to a depth of 10 cm. Experimental design is presented on Fig. 2.

The planted pots were placed in a semi-shade, in natural conditions of the experimental field of Institute for Medicinal Plant Research 'Dr J. Pančić', Pančevo, South Banat, Serbia (77 m AMSL; 44°52'18.9"N 20°42'09.0"E). Vegetation pots were dug into the soil to their full height to avoid the bulbs freezing during winter. The experiment was set up according to the split plot system where the origin of the bulbs was the main plot (F1 = 2 locations)

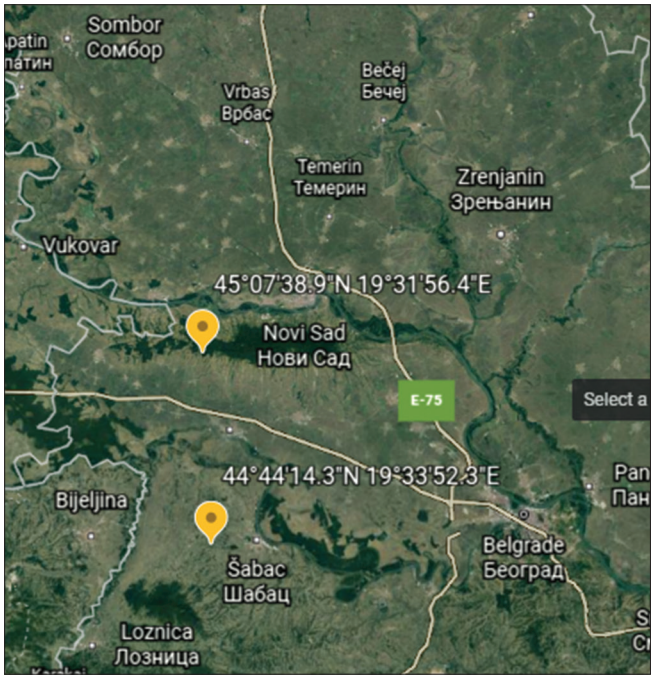

Fig 1. Localities where the reproductive material (bulbs) from native *A. ursinum* plants were sampled.

Table 1: General physico-chemical properties of tested soil types

Soil type	pH		$CaCO_3$ [%]	Humus [%]	N [%]	P_2O_2 [mg/100 g]	K_2O [mg/100 g]	Fine sand [%]	Coarse sand [%]	Powder [%]	Clay [%]
	in KCl	in H_2O						0.02-0.2 [mm]	0.2-2 [mm]	0.02-0.002 [mm]	<0.002 [mm]
Arenosol (1)	8.43	8.75	18.58	0.39	0.041	1.42	2.04	66.86	29.86	1.0	2.28
Fluvisol (2)	7.58	8.18	13.70	1.22	0.105	4.04	7.30	65.87	26.61	5.6	1.92
Cambisol (3)	3.73	4.85	0	1.85	0.12	2.80	13.40	30.26	1.74	79.92	18.08
Chernozem (4)	6.80	7.49	0.98	2.82	0.21	4.93	33.60	28.72	0.28	32.28	38.72

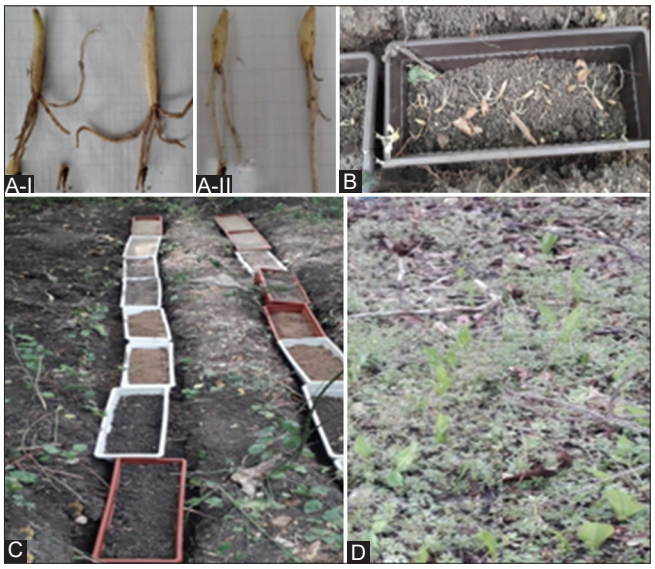

Fig 2. Vegetation pots with different soil types: AI, AII- the bulbs of *A. ursinum* from the original localities before planting; B, C- *A. ursinum* right after planting; D- six months later when the leaves appeared.

and the soil type (F2 = 4 soil types) was the sub-plot with four replications.

Meteorological data during the experiment were collected from an automatic meteorological station, 200 m away from the experiment site (Table 2).

In the second half of March 2021, the number of sprouted plants in all tested combinations was counted based on two factors previously defined as F1 and F2 (F1xF2). For morphological analyses, 10 leaves in their specific growth phase (70% of the final dimensions) were sampled. For further phytochemical analysis, the harvested leaves were first washed and the excess of water was removed at room temperature, then the leaves were immediately grounded and used for extract preparation.

Morpho-anatomical analysis of leaves

From each soil type treatment, 10 leaf sheets were randomly sampled from cultivated *A. ursinum* plants and all morpho-anatomical parameters (weight, length, width of the leaf and number of stomata) were determined according to Aslantaş and Karakurt (2009).

Table 2: Average monthly temperatures (°C/month) and precipitation (mm/month) during the vegetation period of *A. ursinum* L. (2020-2021)

Climatic parameters	2020.				2021.		
	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Temperature (°C)	19.5	12.3	6.5	4.9	3.9	5.3	6.1
Precipitation (mm)	33.3	81.6	10.2	32.7	89.1	19.8	37.8

Extraction procedure

A gram of fresh each leaf sample was soaked in 2x5 mL of 80% acetone and intensively shaken for 2 x 90 minutes in plastic cuvettes, protected from the light, at room temperature. The fresh sample to solvent ratio was 1:10. Separation of the precipitate from the supernatant was performed by filtering the extracts through a suitable filter paper. Afterwards, supernatants were stored at 4°C until they were analysed.

Determination of bioactive compounds

Total phenolic content (TPC)

Folin-Ciocalteu (FC) method was used for TPC determination as it is described in the literature (Ng et al., 2000). TPC quantification was done on the basis of the calibration curve prepared with ferulic acid (FA) as a standard because it is one of the predominant phenolics in *Allium* species (Simin et al., 2013; Asemani et al., 2019). Results were expressed as mg of ferulic acid equivalents (FAE) per g of fresh weight (FW).

Total flavonoid content (TFC)

The determination of TFC was evaluated using the spectrophotometric method as described by Kim et al. (2020). TFC was determined using a calibration curve with quercetin (Q) as a standard and the results were expressed as mg of quercetin equivalents (QE) per g of FW.

Total dihydroxycinnamic acid derivative content (HCA)

Total HCA content was estimated using the method described by Fraisse et al. (2011), with a small modification. Namely, 0.2 mL of undiluted plant extract was mixed with 0.4 mL of 0.5 M HCl, 0.4 mL of Arnow's reagent (obtained by dissolution of 10 g of $NaNO_2$ and 10 g of Na_2MoO_4 in 100 mL of distilled water), 0.4 mL of 2.215 M NaOH and 0.6 mL of distilled H_2O. The blank sample was prepared

by adding water instead of the plant extract to the reaction mixture. After a 20 minutes incubation of the mixture at room temperature, absorbance was recorded at 525 nm. Total HCA content in the extract was determined from the calibration curve with chlorogenic acid (CGA) as a standard. Results were expressed as mg of CGA equivalents (CGAE) per g of FW.

Total carotenoid content (TCC)

1 mL of properly diluted plant extract was used and then the absorbance of samples was read at 450 nm. The content of total carotenoids was determined as it was reported in literature (Gross, 1991) using the following equation:

$$\mu g \text{ carotenoid per } g = (A*V*106)/(E_{1cm}*100*m)$$

A- the absorbance of the sample at a wavelength of 450 nm; V - the total volume of extract; E_{1cm} - extinction coefficient for the used solvent (2500 for acetone); m - mass of sample. The obtained results were expressed as μg/g of FW.

Chlorophyll a and chlorophyll b content

Determination of chlorophyll *a* and *b* content was performed using a spectrophotometer (UV-1800, Shimadzu USA Manufacturing Inc., Canby, OR, USA) by reading the absorbance of samples at 646 nm and 663 nm in 2 mL of undiluted plant extract. The obtained values for the mentioned wavelengths were used to determine the chlorophyll *a* and *b* content using the following patterns suggested by Laware (2015):

$$\text{Chlorophyll } a \text{ } (\mu g/mL) = 12.21\ A_{663} - 2.81\ A_{646}$$

$$\text{Chlorophyll } b \text{ } (\mu g/mL) = 20.13\ A_{646} - 5.03\ A_{663}$$

A_{645} - the absorbance of the sample at a wavelength of 645 nm; A_{663} – the absorbance of the sample at a wavelength of 663 nm. The obtained results were expressed as μg/g of FW.

Determination of antioxidant activity

The antioxidant activity of the plant extracts was evaluated spectrophotometrically by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) scavenging assay, ferric reducing power assay (FRP), *in vitro* phosphomolybdenum total antioxidant capacity assay (TAC), and cupric ion reducing antioxidant capacity (CUPRAC) assay.

DPPH• assay

The determination of free radical scavenging activity of the extracts was done according to the method described by Gawron-Gzella et al. (2018). The percentage inhibition of DPPH• was calculated using the following formula:

$$\% \text{ of inhibition} = [A_b - A_s]/A_b *100$$

A_b -=the absorbance of blank sample; A_s = the absorbance of the sample extracts.

FRP assay

The antioxidant activity of *A. ursinum* extracts was determined by FRP assay according to the method previously described by Nibir et al. (2017). Ascorbic acid (AA) was used as a standard, and the obtained results were expressed as mg of ascorbic acid equivalents (AAE) per g of fresh weight (FW).

TAC assay

TAC assay was conducted by the method given by Prieto et al. (1999). The antioxidant capacity was calculated according to a calibration curve prepared with ascorbic acid as a standard. The results were expressed as mg of ascorbic acid equivalents (AAE) per g of FW.

CUPRAC assay

This assay was performed according to the procedure described by Yilar et al. (2020). A calibration curve was prepared using different concentrations of ascorbic acid as a standard and the results were expressed as mg of ascorbic acid equivalents (AAE) per g of FW. In each method, all samples were analysed in triplicates (n= 3).

Statistical analysis

Analysis of variance (ANOVA) was performed in order to find out if there are statistically significant differences between localities in different parameters. Normality was previously checked by Kolmogorov-Smirnov and Shapiro-Wilk tests. ANOVA was followed by Tukey's post-hoc test ($p< 0.05$). The correlation was done to see whether there is quantitative agreement (correlation relationship) between the variations of observed variables. The results of the chemical analysis are composed of 10 variables (methods) and 6 objects (localities). In order to get basic insights into similarities among the analysed samples, Principal Component Analysis was carried out. PCA represents a method for data reduction of possibly correlated variables into a smaller number of uncorrelated variables called principal components (PCs) (Miller and Miller, 1984). As a result, scatter and loading data are obtained. Data were analysed with SPSS 26.0 (SPSS, Inc., Chicago, IL) software.

RESULTS AND DISCUSSION

Morpho-anatomical of the leaves of cultivated *A. ursinum*

The results of morphological and anatomical study of the leaves of *A. ursinum* cultivated in South Banat climatic conditions in four different soil types are given in Table 3.

Table 3: Morphological parameters of *A. ursinum* leaves

Bulbs origin	Soil type	Leaf sheet	Leaf		Number of stomata per mm²		Ratio
		weight (g)	length (cm)	width (cm)	AB (abaxial side)	AD (adaxial side)	AB/AD
I	Arenosol (I_1)	0.40±0.03^{aA}	10.9±0.27^{aA}	2.0±0.14^{aA}	12.0±0.67^{aA}	38±2.16^{aA}	0.31±0.03^{aA}
	Fluvisol (I_2)	0.45±0.02^{aB}	12.0±0.50^{aB}	2.1±0.24^{aA}	13.0±1.83^{aA}	45.0±2.40^{aB}	0.28±0.03^{aA}
	Cambisol (I_3)	0.73±0.03^{aC}	12.1±0.38^{aB}	3.8±0.16^{aB}	20.0±1.56^{aB}	56.0±2.31^{aC}	0.35±0.03^{aB}
	Chernozem (I_4)	0.78±0.02^{aD}	14.5±0.84^{aC}	3.7±0.26^{aB}	28.0±1.33^{aC}	58.0±2.21^{aC}	0.48±0.03^{aC}
II	Arenosol (II_1)	0.45±0.03^{bA}	11.8±0.60^{bA}	1.9±0.17^{bA}	13.0±0.67^{bA}	41.0±2.00^{bA}	0.31±0.02^{aA}
	Fluvisol (II_2)	0.55±0.03^{bB}	12.9±0.67^{bB}	2.4±0.16^{bB}	15.0±1.89^{bB}	47.0±1.63^{bB}	0.31±0.04^{aA}
	Cambisol (II_3)	0.74±0.04^{aC}	12.6±0.23^{bB}	3.9±0.12^{aC}	21.0±1.33^{aC}	59.0±2.87^{aC}	0.35±0.0^{aB}
	Chernozem (II_4)	0.79±0.02^{aD}	14.9±0.55^{aC}	3.8±0.15^{aC}	29.0±1.41^{aD}	63.0±2.83^{bD}	0.46±0.04^{aC}

* I-locality: 45°07'38.9"N 19°31'56.4"E; II-locality: 44 ° 44'14.3 "N 19 ° 33'52.3" E; 1-Arenosol; 2-Fluvisol; 3-Cambisol; 4-Chernozem;
Means with the same small superscript letters within the same column between the same types of soil are not significantly different ($p < 0.05$)
Means with the same capital superscript letters between different soil types within the same locality are not significantly different ($p < 0.05$)

The final results show that statistically significantly higher values of leaf weight and length parameters were obtained in Fluvisol (II_2) and Chernozem (I_4 and II_4), whereas the highest parameters for leaf width were obtained in Cambisol (I_3 and II_3). However, significantly lower values for the same leaf parameters were observed in bulbs grown in Arenosol (I_1 and II_1). The highest number of stoma (Fig. 3) on the AB and AD side of the leaf was recorded with Cambisol (II_3) and Chernozem (I_4 and II_4), while statistically significantly lower values were recorded with Arenosol and Fluvisol. The presented results show that the soil type and the origin of *A. ursinum* bulbs (locality I and II), cause changes in all morphological parameters except AB/AD within Arenosol (I_1 and II_1) and Fluvisol (I_2 and II_2), (Table 3; Fig. 4).

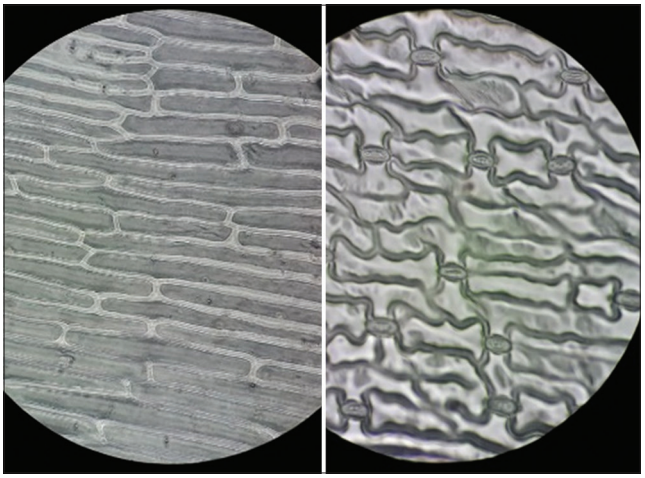

Fig 3. AB (abaxial, left) and AD (adaxial, right) side of leaf, treatment II4.

According to Tataranni et al. (2015) the morpho-anatomical structure of leaves is of great importance for the yield and photosynthetic activity of plants. Gönüz and Özörgücü (1999) further imply that photosynthetic activity depends on the illumination, which is directly correlated to the intensity of transpiration, while the intensity of transpiration largely depends on the anatomical structure of the leaf, particularly the number of stomata. Lawlor (2002) states that the number of stomata per leaf depends on the amount of water availability at its disposal during its physiological cycle, while Duursma et al. (2019) further explain that in case of less available water the plants use a smaller number of stomata per leaf to prevent losses. According to Kondryakov et al. (2009) the growth and development of plants in forest populations mostly depend on agro-ecological conditions, and for *A. ursinum* humidity and soil moisture seem to be particularly important. Regarding soil moisture, Hokkanen (2006) suggests that both, physical and chemical properties of soil are important. Based on results presented in the Table 1 textural properties of soils were quite different- Arenosol and Fluvisol contained fine sand fraction as predominant (65-87-66.86%), Cambisol was mostly powdered (79.92%) while Chernozem consisted of both powder (32.28%) and clay (38.72%) fractions. These differences were quite important since soils with significant share of clay materials are characterized with excellent water capacity and can provide enough water to plant preventing it from drought stress (McCauley et al., 2005). In this case, it can be assumed that Chernozem possessed the best textural properties.

Bearing in mind that during the vegetation period 2020-2021 examined *A. ursinum* plants were exposed to favourable climatic conditions (Table 2) and the given results support the statement of Lawlor (2002) that the morpho-anatomical structure of the plant leaves, particularly the number of stomata, depended on the amount of water availability at their disposal. Due to the favourable physicochemical properties of Chernozem (I_4, II_4), this soil type might be considered the most beneficial for the development of *A. ursinum* plants, particularly their leaves.

Phytochemical properties of *A. ursinum* leaf extracts

The phytochemical composition of *A. ursinum* leaves appear to be of great importance as constituents (chlorophylls, carotenoids, polyphenols, flavonoids, phenolic acids, etc.) proved to possess various beneficial effects (allelopathic, antioxidant, anticancer, antimutagenic, antibacterial, etc.)

and could be used in pharmaceutical and food industries (Štajner et al., 2003; Djurdjevic et al., 2013; Lachowicz et al., 2018; Kolašinac et al., 2021). In the current study, analysis of the contents of selected phytochemical compounds present in the leaves of cultivated *A. ursinum* revealed significant differences depending on the soil type (Table 4; Fig. 5).

According to Khanom et al. (2008) and Széles et al. (2012), the total chlorophyll content is greatly influenced by the soil type and by the amount of nutrients and irrigation. In the current study, the highest content of chlorophyll *a* and *b* was recorded in the leaves of *A. urisunum* cultivated on Chernozem – I_4 (642.39 μg/g FW; 458.57 μg/g FW, respectively) with values significantly different from all other soil types. According to Lachowicz et al. (2018) chlorophyll content and total carotenoid content (TCC) in the leaves of *A. ursinum* is mostly influenced by the time of leaf harvest, particularly the physiological phase of the plant from which it is collected, where the lowest content of chlorophyll and TCC was recorded in the leaves harvested in March and the highest ones in June. In this study, the results on the total chlorophyll content and TCC in the leaf were similar to those reported by Lupoae et al. (2010). Mutually different and the highest TCC content was achieved in the treatments II_3 and II_4 (263.24 μg/g and 248.66 μg/g, respectively).

The closest values but still significantly lower of TCCs was found at treatments I_1 and I_4 (212.55 μg/g and 212.74 μg/g, respectively), although they were also significantly higher than other treatments. Bearing in mind that the leaves from all treatments were harvested on the same day and that all plants were cultivated under the same climatic conditions it could be suggested that the type of soil (probably due to different water capacity and the content of nitrogen, phosphorus and potassium in the soils, Table 1), exhibited a dominant effect on the content of chlorophylls and TCC in the examined leaves of *A. ursinum*, which is in line with the previous studies (Dordas and Sioulas, 2008; Széles et al., 2012; Wang et al., 2015; Ahmad et al., 2019).

The total phenolic content (TPC) in leaf extracts varied from 1.42 mg/g FAE FW to 1.98 mg/g FAE FW, depending on the treatments. The highest TPC was detected in I_4 (1.98 mg/g FAE FW), followed by II_3 (1.80 mg/g FAE FW) while TPCs in other treatments were significantly lower. According to Mahmutovic et al. (2014), the leaf

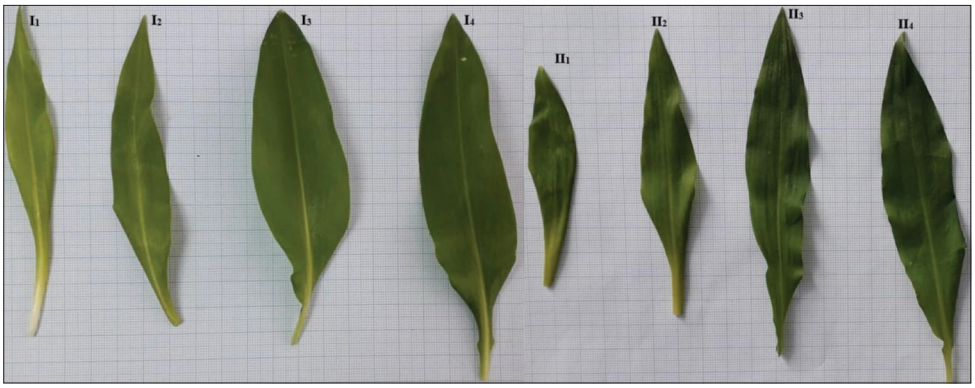

Fig 4. Morphological characteristics of A. ursinum leaves grown in different soil types.

Table 4: Content of selected phytochemicals in A. ursinum leaf extracts

Bulbs origin	Soil type	Chlorophyll *a* (μg/g FW**) ± SD	Chlorophyll *b* (μg/g FW) ± SD	TCC (μg/g FW) ± SD	TPC (mg/g FAE FW) ± SD	TFC (mg/g QE FW) ± SD	HCA (mg/g CGAE FW) ± SD
I*	Arenosol (I_1)	298.48±5.22a^{AB}	366.65±3.06^{aA}	212.55±3.82^{aC}	1.42±0.13^{aA}	0.66±0.02^{aA}	0.55±0.00^{aA}
	Fluvisol (I_2)	308.04±5.47^{aB}	416.66±5.76^{aB}	128.16±4.76^{aA}	1.44±0.11^{aA}	0.59±0.02^{aA}	0.59±0.01^{aB}
	Cambisol (I_3)	289.94±4.69^{aA}	423.49±4.11^{aB}	171.56±9.59^{aB}	1.78±0.12^{aB}	1.03±0.19^{aB}	0.54±0.02^{aA}
	Chernozem (I_4)	642.39±7.29^{aC}	458.57±14.06^{aC}	212.74±10.74^{aC}	1.98±0.10^{aB}	0.62±0.03^{aA}	0.56±0.01^{aAB}
II	Arenosol (II_1)	308.10±2.05^{bB}	358.15±5.26^{aA}	91.17±5.51^{bA}	1.44±0.12^{aA}	0.35±0.02^{bA}	0.52±0.01^{aA}
	Fluvisol (II_2)	302.18±2.89^{aB}	410.83±5.98^{aB}	120.88±5.34^{aB}	1.73±0.01^{bB}	1.00±0.06^{bB}	0.58±0.00^{aB}
	Cambisol (II_3)	302.68±4.43^{bB}	428.96±4.66^{aC}	263.24±5.21^{b}D	1.80±0.16^{bB}	1.28±0.14^{bC}	0.56±0.00^{aB}
	Chernozem (II_4)	289.75±0.78^{bA}	412.32±1.50^{bB}	248.66±5.18^{bC}	1.63±0.08^{bAB}	0.92±0.12^{bB}	0.53±0.01^{bA}

* I- the bulbs origin locality: 45°07'38.9"N 19°31'56.4"E; II- the bulbs origin locality: 44°44'14.3"N19°33'52.3"E;
** FW- fresh weight; TCC- total carotenoid content; TPC- total phenolic content; TFC- total flavonoid content; HCA- total dihydroxycinnamic acid derivative content; FAE- ferulic acid equivalents; QE- quercetin equivalents; CGAE- chlorogenic acid equivalents. Results are expressed as a mean value±SD (n=3). Means with the same small superscript letters within the same column between the same types of soil are not significantly different ($p < 0.05$). Means with the same capital superscript letters between different soil types within the same locality are not significantly different ($p < 0.05$).

sampled in May had a lower TPC (1.28 mg/g) than it was observed in all our treatments, which can be attributed to the differences in sample analysis and manipulation methodology as well as agroecological conditions.

Regarding the total flavonoid content (TFC), the highest values were observed in II_3 treatment (1.28 mg/g QE FW), followed by I_3 (1.03 mg/g QE FW) and II_2 (1.00 mg/g QE FW). For all soil types, obtained TFC values were significantly different. As previously mentioned, the plants from which the leaves were collected were in the same physiological phase while the origin of reproductive material (bulbs) differed (localities I and II) thus the observed differences in TFC could be attributed only to soil types. However, compared to our results, higher TFC values were recorded in some previous studies (Błażewicz-Woźniak and Michowska, 2011; Djurdjevic et al. 2004).

Djurdjevic et al. (2004) attributed the allelopathic effect of *A. ursinum* to phenolic acids and suggested dihydroxycinnamic acid as a very important constituent. In our study, the highest and significantly higher HCA was recorded in I_2 treatment (0.59 mg/g CGAE FW), followed by II_2 treatments (0.58 mg/g CGAE FW), and II_3 (0.56 mg/g CGAE FW) without statistically significant differences. Analogous to our results, Djurdjevic et al. (2013) also revealed HCA significantly lower than TPC.

Antioxidant properties of *A. urisinum* leaf extracts

Antioxidant potential is of great importance in determining the beneficial role of food products. As it is important to obtain complete information on the antioxidant potential of a product or plant raw material, several tests should be applied to achieve it (Rao et al., 2007). In the current study, four antioxidant parameters of leaf were analysed and the obtained results are presented in Table 5.

The CUPRAC test is used to determine the redox capacity of *A. ursinum* extracts and is based on determining the ability of a sample to reduce copper complex with neocuproine (Tirzitis et al., 2010). The obtained data following the CUPRAC test showed that the highest value was observed in I_4 treatment (2.75 mg/g AAE FW). In all other treatments significantly lower values were obtained ranging from 1.09 mg/g AAE (I_1) to 2.11 mg/g (I_2 and II_3).

Regarding the TAC test, that provides information on the total reducing power of the sample which according to Kampa et al. (2002) depends on the total content of phenolic and other present compounds, the highest and significantly higher value than in all other treatments in our study was again observed in I_4 (3.27 mg/g GAE FW). For other samples, the range for TAC values was from 1.93 (I_1) to 2.73 (II_3) mg/g AAE FW.

The DPPH• test is the most commonly used antioxidant test and provides information on the ability of a sample to prevent the formation of free radicals under adverse conditions. In current study, DPPH• assay revealed that there are no any statistically significant differences caused by soil type except for bulbs originated from Mačva region (location II) grown on Chernozem (II_4- 26.94%, Table 5).

The FRP test, which is used to reduce iron ions, showed the highest values of FRP was found in I_4 treatment (0.87 mg/g AAE FW) followed by II_2 (0.82 mg/g AAE FW), and II_3 (0.77 mg/g AAE FW) and I_3 (0.69 mg/g AAE FW) while the values recorded in all other treatments were lower. The outcomes of all antioxidant tests conducted in the current study revealed high antioxidant capacity of the leaf extracts of *A. ursinum*, which agrees with the results reported by Steiner et al. (2003). However, the obtained values differ from those reported by Stajner et al., (2008), Sapunjieva et al., (2012) and Mihaylova et al. (2014), probably due to the different method of the extract preparation and different sample origin.

The results presented in Tables 4 and 5 also indicate that the phytochemical composition and antioxidant potential

Table 5: Antioxidant properties of leaf extracts of *A. ursinum*

Bulbs origin	Soil type	CUPRAC**	TAC	DPPH•	FRP
		(mg/g AAE FW) ± SD	(mg/g GAE FW) ± SD	(% of inhibition) ± SD	(mg/g AAE FW) ± SD
I*	Arenosol (I_1)	1.09 ± 0.03^{aA}	1.93 ± 0.08^{aA}	27.72 ± 0.02^{aA}	0.61 ± 0.00^{aA}
	Fluvisol (I_2)	1.96 ± 0.05^{aC}	2.58 ± 0.00^{aB}	27.93 ± 0.78^{aA}	0.66 ± 0.04^{aAB}
	Cambisol (I_3)	1.57 ± 0.12^{aB}	2.60 ± 0.13^{aB}	27.72 ± 0.37^{aA}	0.69 ± 0.03^{aB}
	Chernozem (I_4)	2.75 ± 0.11^{aD}	3.27 ± 0.19^{aC}	27.69 ± 0.27^{aA}	0.87 ± 0.00^{aC}
II	Arenosol (II_1)	1.74 ± 0.17^{bA}	2.68 ± 0.23^{bB}	23.67 ± 2.18^{bA}	0.63 ± 0.01^{bA}
	Fluvisol (II_2)	1.40 ± 0.03^{bAB}	2.34 ± 0.04^{bA}	23.40 ± 1.22^{bA}	0.82 ± 0.02^{bB}
	Cambisol (II_3)	2.11 ± 0.09^{bC}	2.73 ± 0.08^{aB}	21.97 ± 2.38^{bA}	0.77 ± 0.02^{bB}
	Chernozem (II_4)	1.96 ± 0.18^{bBC}	2.11 ± 0.07^{bA}	26.94 ± 2.31^{aA}	0.65 ± 0.02^{bA}

* I- the bulbs origin locality: 45°07'38.9"N 19° 31'56.4"E; II- the bulbs origin locality: 44°44'14.3"N 19° 33'52.3"E

** CUPRAC- cupric ion reducing antioxidant capacity; AAE- ascorbic acid equivalents; FW- fresh weight; TAC- Total antioxidant capacity; DPPH- 2,2-diphenyl-1-picrylhydrazyl radical; FRP- ferric reducing power. Results are expressed as a mean value ± SD (n=3).

Means with the same small superscript letters within the same column between the same types of soil are not significantly different ($p < 0.05$).

Means with the same capital superscript letters between different soil types within the same locality are not significantly different ($p < 0.05$)

of studied leaves of *A. urisinum* are greatly influenced by the origin of the reproductive material (bulb). In short, it can be stated that the leaf from plants originating from different localities can be considered as leaves of different ecotypes. The best antioxidant potential and the highest content of phytochemical compounds were observed on Cambisol and Chernozem. Our results are in agreement with Bhattacharjee et al. (2013) who showed that the origin of reproductive material greatly influences not only growth and development of onion but also its chemical composition.

Statistical interpretation and correlation of results

The total content of phytochemical compounds in *A. ursinum* leaf extracts appears to greatly influence the total antioxidant capacity, as indicated by similar researches of Lachowicz et al. (2018). Namely, the authors observed same pattern and assumed that there is a correlation between phytochemical's content and antioxidant properties similar to the results in the current study. Correlation matrix of the content of phytochemical substances in *A. ursinum* leaf extracts and the achieved antioxidant potential is presented in Table 6.

According to the correlation matrix for phytochemical and antioxidative properties in wild garlic samples, for several parameters such as Chl. *a*-CUPRAC, Chl. *a*-TAC, Chl. *b*-TPC, Chl. *b*-CUPRAC, Chl. *b*-FRP, TPC-FRP, CUPRAC-TAC and TFC-HCA, high positive correlations were observed ($p < 0.05$) (Table 6). For TCC, there was no strong correlation with other examined parameters, which is not in agreement with the study of Lachowicz et al. (2018). However, this could imply that in this case phenolic compounds were more important antioxidants compared to carotenoids probably due to different growing conditions. In our study, the strongest correlation was observed between HCA and FRP ($r^2 = 1.000$). According to literature data different phenolic acids with two OH-groups in vicinal position (such as dihydroxicinnamic acid) had the strongest ability to participate in Fe^{3+} ions reduction process (Spiegel et al., 2020).

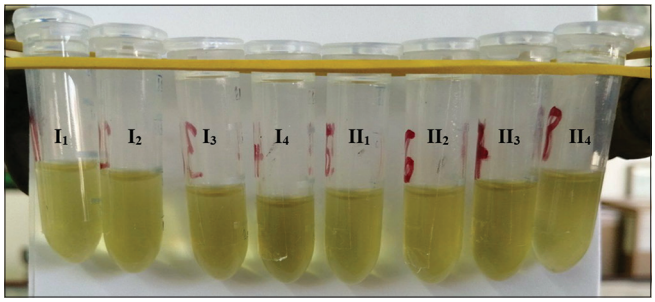

Fig 5. Leaf extracts of *A. ursinum* prepared for phytochemical analysis.

Principal component analysis (PCA)

Morphological parameters

According to eigenvalue values, two principal components were chosen, explaining 83.87 and 9.22 % of total variability (Fig. 6). Score plot showed two clusters (Chernozem and Cambisol) wheras Arenosol and Fluvisol were not clearly separated. The loading plot was used to present characteristic morphological parameters mostly contributing to the first two PCs. Accordingly, loading plot revealed AB as the most influential morphological parameter that discriminated Chernozem soil type. On the other hand, Cambisol was discriminated based on higher loading values for parameters leaf sheet weight, leaf width and AD (Fig. 6).

The discrepancies in morphological parameters in the leaves from plants cultivated on Cambisol and Chernozem have already been explained in the previous section. In short, the soil type had a dominant influence on leaf growth and development which is obvious from the Fig. 6.

Chemical parameters

Based on eigenvalue values, three principal components were chosen. The fourth principal component had

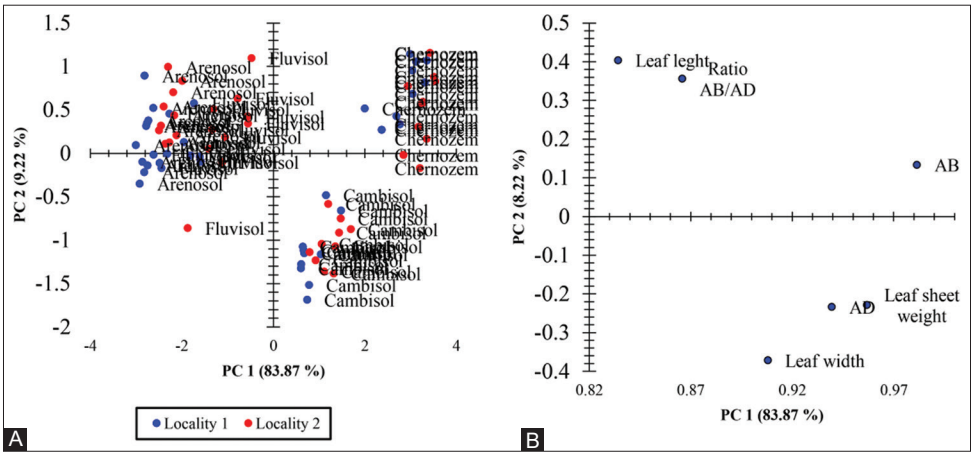

Fig 6. Principal component analysis (PCA) classification of soil types based on the morphological parameters: A-score plot, B-loading plot.

eigenvalue less than 1 and consequently it was excluded from further analysis. The first two and three principal components explained 75.81 and 88.50 %, respectively. Principal Component 1 (PC 1) and Principal Component 2 (PC 2) explained 47.71 and 28.10 % of total variability, respectively. Fig. 7 (A and D) reveals Chl. a, CUPRAC and TAC as the most influential parameters that discriminate I_4 locality. On the other hand, HCA and flavonoids are the most influential parameters that discriminate II_3 sample (Fig. 7A and 7D). Principal Component 3 shows the discrimination of locality I where DPPH$^{\bullet}$ had the highest positive value (Fig. 7C and 7F).

Conclusion and suggestion

Based on the obtained results, *A. ursinum* can be successfully grown in all four tested soil types in the agro-ecological conditions of South Banat (Serbia) related to the vegetative propagation. However, the best morphological and anatomical parameters could be achieved by growing *A. ursinum* on Chernozem. By analyzing the phytochemical composition of leaf extracts and their antioxidant potential, it can be suggested that both soil type and the origin of plant reproductive material (bulbs) had an influence on the achieved results, depending on monitored parameters. The highest content of Chlorophylls *a* and *b*, TCC, TPC, TFC,

Table 6: Correlation analysis for phytochemical parameters of the leaf extracts of *A. ursinum*

Variables	Chl. *a*	Chl. *b*	TCC	TPC	CUPRAC	TAC	TFC	DPPH·	HCA	FRP
Chl. *a*	1									
Chl. *b*	0.590	1								
TCC	0.170	0.416	1							
TPC	0.611	0.853*	0.418	1						
CUPRAC	0.752*	0.728*	0.305	0.596	1					
TAC	0.743*	0.618	-0.064	0.624	0.817*	1				
TFC	-0.273	0.465	0.557	0.537	-0.041	-0.110	1			
DPPH·	0.275	0.156	0.081	-0.101	0.049	-0.086	-0.361	1		
HCA	-0.273	0.465	0.557	0.537	-0.041	-0.110	1.000*	-0.361	1	
FRP	0.667	0.756*	0.144	0.856*	0.564	0.649	0.348	-0.274	0.348	1

Chl. *a*- Chlorophyll a; Chl. *b*- Chlorophyll *b*; TCC- total carotenoid content; TPC- total phenolic content; CUPRAC- cupric ion reducing antioxidant capacity; TFC- total flavonoid content; TAC- total antioxidant capacity; HCA- total dihydroxycinnamic acid derivative content; DPPH·- 2,2-diphenyl-1-picrylhydrazyl radical; FRP- ferric reducing power.
*Correlations are significant at $p < 0.05$

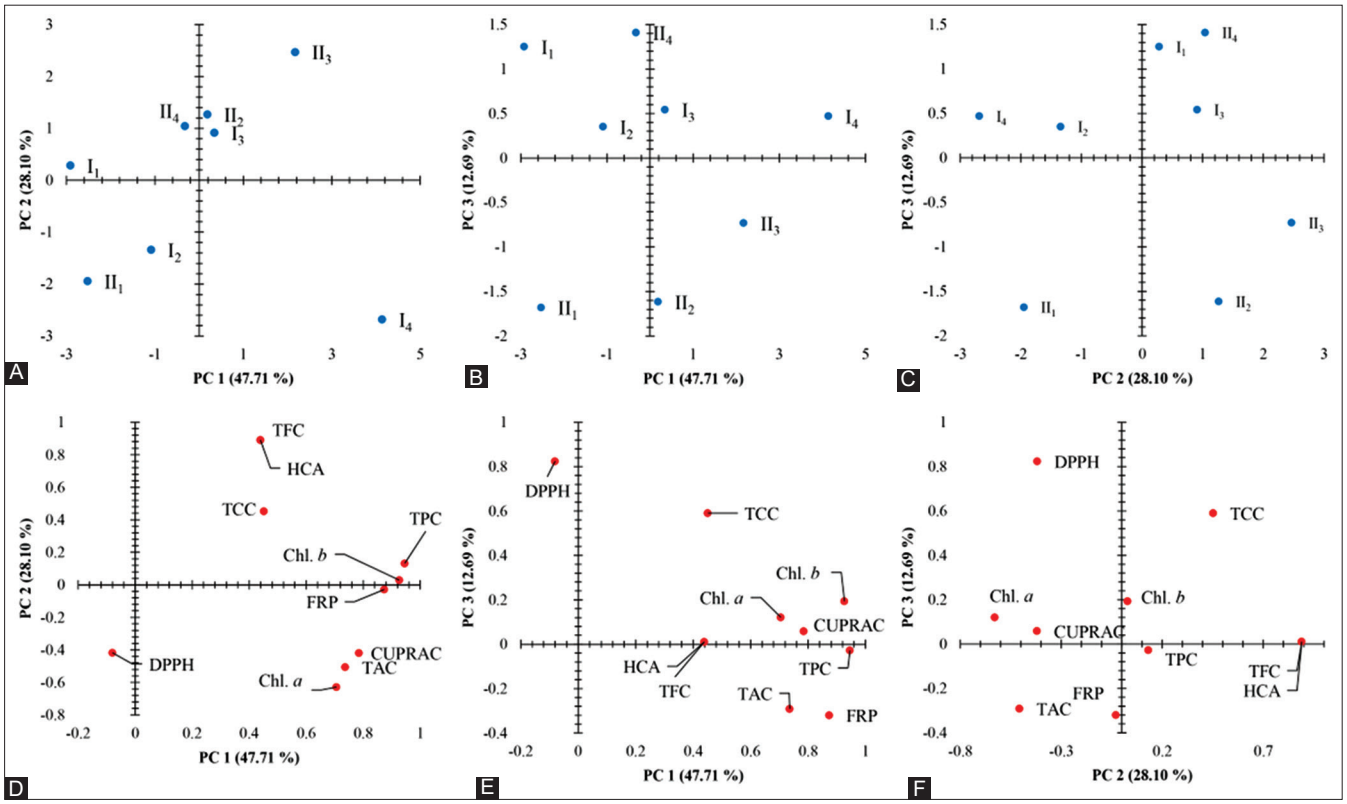

Fig 7. Principal component analysis (PCA) classification of soil types based on the chemical parameters: A, B, C, D-score plots; D, E, F, G-loading plots.

and HCA was achieved by growing *A. ursinum* on Cambisol and Chernozem. The best antioxidant potential using the CUPRAC and TAC tests was achieved with plant material originating from Cambisol and Chernozem, while the DPPH□ and FRP tests indicated that its highest antioxidant potential was achieved with plant material originating from Fluvisol and Chernozem. Correlation and PCA analyses confirmed an interdependence between the phytochemical composition and the strength of antioxidant capacity. Some further, more extensive studies are needed to confirm these preliminary findings.

Authors' contribution statement

S. Gordanić- conceptualized and performed field experiments and morphological analysis and wrote draft version of manuscript; D. Radanović- participated in field experiments and interpretation of results for morphological analysis; S. Vuković- performed chemical analyses and wrote part of draft version of manuscript; S. Kolašinac- performed statistical analyses, wrote part of draft version of manuscript and interpreted results; S. Kilibarda- performed chemical analyses; T. Marković- participated in writing of draft version of manuscript, interpreted results and supervised research; Đ. Moravčević- supervised research, critically reviewed manuscript and provided funding; A.Ž. Kostić; conceptualized chemical analyses; supervised research, critically reviewed manuscript and provided funding.

Conflicts of interest

There are no conflicts to declare.

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