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[1]Department of Horticulture, Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina
[2]Institute of Genetic Resources, University of Banja Luka, Banja Luka, Bosnia and Herzegovina
[3]Department of Plant Physiology, Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Morphological characteristics and antioxidant properties of *Allium ursinum* L. wild growing in the northwestern part of the Republic of Srpska (Bosnia and Herzegovina)

Vida Todorović[1], Nikolina Đekić[1], Marina Antić[2], Borut Bosančić[1], Jelena Davidović Gidas[1], Senad Murtić[3*]

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Summary

Allium ursinum L. (ramson) has been used for centuries as a food and medicinal herb. Generally, the phenology, morphology, as well as health benefits of *Allium ursinum* plants have been scientifically validated; however, the knowledge about geographic variation in morphological characteristics and antioxidant properties of *Allium ursinum* are fairly scarce. The aim of this study, therefore, was to reveal the habitat preferences of *Allium ursinum* in different geographical regions of the Republic of Srpska (Bosnia and Herzegovina) and to evaluate its morphological characteristics of stems, leaves and bulbs and its antioxidant properties. Morphological characteristics as well as antioxidant properties of *Allium ursinum* plants including total phenolics and flavonoids contents, and ferric reducing antioxidant power (FRAP) were determined. In this study, the high abundance of *Allium ursinum* plants was recorded at five different locations: Laktaši, Kozara, Prnjavor, Kneževo and Drinić. The results of this study revealed that *Allium ursinum* prefers forest habitats and that their morphological characteristics and antioxidant properties are strongly dependent on both geographical location and habitat conditions. We hereby suggest that *Allium ursinum* can be considered a valuable source of phenolic compounds with relevant antioxidant activity.

Keywords: climate, habitats, natural antioxidants, plant growth

Introduction

Allium ursinum L. (ramson), also known as wild garlic or bear's garlic, is a bulb-forming perennial plant native to Europe and Northern Asia. It can be found in forest habitats from the Mediterranean region to Scandinavia and Siberia, especially in moist deciduous forests, under bushes, along forest roads and streams, from the plains up to 1.900 m above sea level. This plant grows most successfully in wet, slightly acidic, well-drained and nutrient-rich soils, in full shade and semi-shade habitats. In such places, *Allium ursinum* often forms dense populations covering large areas, especially in horn-beam-oak and beech forests (BODÓ et al., 2015).

As far as morphological and anatomical characteristics are concerned, *Allium ursinum* is a typical representative of the *Allium* genus. This plant is a vernal geophyte, with triquetrous, erect, flower stem, and 2-3 smooth, elliptic-lanceolate, dark green leaves, which are shorter than the stem. In favorable conditions, the stem reaches a height of 50 cm. Atop the stem is an umbel-like inflorescence consisting of 15-20 starry, snowy-white flowers that bloom from mid-spring to early summer. *Allium ursinum* produces small bulbs at the base. Its bulbs are narrow, elongated, about 1.5-6 cm in size. However, in contrast to many other *Allium* species, *Allium ursinum* regenerates prevalently by seeds.

Two subspecies of *Allium ursinum* are recognized: *Allium ursinum* ssp. *ursinum* and *Allium ursinum* ssp. *ucrainicum* Kleopow et Oxner. The subdivision is based on pedicel morphology (KARPAVICIENE, 2006). The pedicels of *Allium ursinum* ssp. *ursinum* are rough with numerous papillae, while *Allium ursinum* ssp. *ucrainicum* Kleopow et Oxner has smooth pedicels without papillae (FARKAS, 2012). There is also a significant difference in the distribution area of these two subspecies. The former is widely distributed in western and central Europe, whereas the latter in eastern and southeastern Europe. It is important to note, however, that *Allium ursinum* ssp. *ucrainicum* Kleopow et Oxner has been cultivated in home gardens across Europe for medicinal and other purposes since ancient times, and this fact perhaps explains its rather surprising occurrence in some localities in western and central Europe (ROLA, 2012).

Both *Allium ursinum* subspecies have acquired a reputation as a medicinal plants as well as a spice due to their powerful biological activity and unique flavor and aroma (SCHMITT et al., 2005). Moreover, *Allium ursinum* has been recognized as a great source of pharmacologically active compounds that provide beneficial effects on human health and cardiovascular and respiratory disease risk reduction. In addition, this plant is used for both insulin and cholesterol level normalization, and as an anti-microbial and anti-inflammatory agent efficient in the treatment of cold, fever, chronic gastritis and gastric ulcers (AMAGOVA et al., 2022). *Allium ursinum* is also a plant characterized by very high antioxidant properties. It is very rich in bioactive compounds such as phenolic compounds and carotenoids that scavenge free radicals and lower the oxidative stress. Interestingly, all parts of *Allium ursinum* plants are edible and have culinary uses, so they can be also classified as a functional food (VOĆA et al., 2022).

Up to our knowledge, there are no studies that have evaluated morphological characteristics, antioxidant properties and distribution of *Allium ursinum* populations in the territory of the Republic of Srpska (Bosnia and Herzegovina). The aim of this study, therefore, was to reveal the distribution and habitat preferences of *Allium ursinum* in the northwestern part of the Republic of Srpska and to estimate its morphological characteristics and antioxidant properties.

Materials and methods

The study was conducted in the nortwestern region of the Republic Srpska. According to the Köppen and Geiger classification (KOTTEK et al., 2006), this region has a a temperate oceanic climate with hot summers and cold, snowy winters. These climate characteristics are influenced by the Adriatic Sea as well as the Dinarides Mountains, which separate the continental Balkan Peninsula from the Adriatic Sea. The monthly average temperatures range from -6.0 °C to 6.2 °C in the winter months (November to February) and from 9.8 °C to 24.7 °C in the warmer months (May to September). Rainfall in this region is largely constant throughout the year.

* Corresponding author

Across the study region, we selected five locations: Laktaši, Kozara, Prnjavor, Kneževo and Drinić, characterized by high presence of *Allium ursinum* Geographical illustration of sampling locations are presented in Fig. 1.

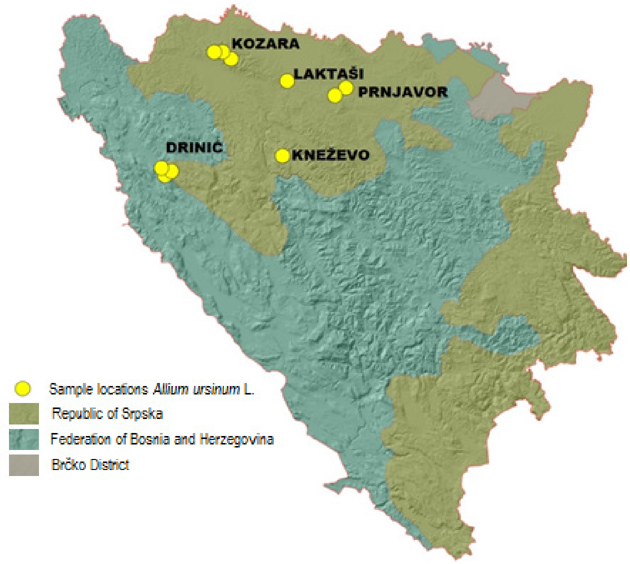

Fig. 1: Map of the study area with geographical illustration of sampling locations.

All selected locations, with elevations ranging from 118 m to 836 m above sea level, are primarily covered by natural beech forests, while the aboveground layer is largely dominated by *Allium ursinum* in spring. The geographical features and habitat data that were collected in the studied sites are summarized in Tab. 1.

Plant material collection

Plant material collection was conducted during the period from March 2021 to May 2021. The investigated areas were located at different altitudes ranging from 118 m to 836 m above sea level. Consequently, the start and timing of *Allium ursinum* phenophases were not the same in different localities. As the aim was to examine the morphological characteristics and antioxidant properties of *Allium ursinum* in the same phenophase of plant‘s development, the collection period was that long. Since the *Allium ursinum* plants were not equally distributed across the study locations, only the areas where the *Allium ursinum* plants grew in high density were the subject of research. The surface area of experimental plot in each study location was approximately 50 m^2. Field work also included the measurement of plant density within the vegetation survey (quadrat) area. Each experimental plot had three quadrats (each 1 m × 1 m). Plant density was determined by counting the number of individual plants of *Allium ursinum* and dividing it by the quadrat's area. A quadrat was also used to sample *Allium ursinum* plants. The *Allium ursinum* plants, including both aerial and underground parts (10 individuals per 1 quadrat; 30 individuals per location), were sampled before the flowering phase when they have reached their maximum height. Plants were sampled very carefully, then placed in paper bags and transported to the laboratory of the Faculty of Agriculture, University of Banja Luka.

Morphological evaluation

The morphological analysis of *Allium ursinum* plants were processed as soon as they arrived at the laboratory. A total of 30 *Allium ursinum* plants from each study location were collected to study the morphological traits: number of coarse roots, length of the leaves (cm), width of the leaves (cm), length of the petiole (cm), width of the petiole (cm), length of the bulb (cm), width of the bulb (cm) and mass of the whole plant (g). Morphological evaluation was performed according to descriptors for *Allium* species (IPGRI, 2001). All measurements were done using a calibrated analytical balance and micrometer caliper.

Extraction procedure for phenolic compounds

Before extraction, *Allium ursinum* plants were washed with distilled water, separately dried and grinded, and then stored in paper bags. The extraction of phenolic compounds was performed using 30% aqueous ethanol solution as follows: 1 g of dried plant sample was steeped with 30 ml of aqueous ethanol solution (water/ethanol 50/50 v/v) at room temperature for 24 h. Then, the obtained extracts were filtered through Whatman filter paper (11 µm pore size) into a 50 mL flask and diluted to the mark with extract solution. The extracts thus obtained were used for the quantification of total phenolics, total flavonoids and total antioxidant activity.

Determination of total phenolics

Total phenolics were determined by the Folin-Ciocalteu method (OUGH and AMERINE, 1988) as follows: Sample extract solution (0.1 mL) was mixed with distilled water (6 mL) and Folin-Ciocalteu reagent (500 µL) was added (Folin-Ciocalteu reagent was diluted with distilled water in the ratio 1:2 before use). After 3 min, 20% Na_2CO_3 (1.5 mL) was added, and the total volume was adjusted to 10 mL with distilled water. The samples were heated at 40 °C for 30 min in a water bath. After cooling to room temperature, the absorbance of the resulting solutions was measured at 765 nm against the reagent blank. Total phenolics quantification was based on a standard curve of gallic acid (0-500 mg/L), and the results were expressed as mg gallic acid equivalents per gram dry matter of sample (mg GAE/g DM).

Determination of total flavonoids

Total flavonoids were determined by the aluminium chloride colorimetric assay (ZHISHEN et al., 1999) as follows: Sample extract solution (1 mL) was mixed with distilled water (4 mL) and 5% $NaNO_2$ (0.3 mL) was added. After 5 min, 10 % $AlCl_3$ (0.3 mL) was added. Then after 1 min, 1M NaOH (2 mL) was added, and the total volume

Tab. 1: The geographical features and habitat type in the selected study localities.

Locality	North	East	Altitude	Habitat type	Date of collection
Drinić	44° 28‘ 22“	16º 31‘ 25“	836 m	*Picea abies* L. and *Fagus sylvatica* L.	April 30, 2021
Kneževo	64° 41‘ 014“	49º 35‘ 094“	790 m	*Fagus sylvatica* L. and *Carpinus betulus* L.	April 18, 2021
Kozara	64° 144‘ 78“	49º 88‘ 101“	492 m	*Picea abies* L. and *Fagus sylvatica* L.	April 2, 2021
Laktaši	44° 55‘ 13“	17º 19‘ 18‘	118 m	*Fagus sylvatica* L. and *Carpinus betulus* L.	March 3, 2021
Prnjavor	64° 72‘ 914“	49º 68‘ 772“	184 m	*Quercus cerris* L. and *Fagus sylvatica* L.	April 4, 2021

was adjusted to 10 mL with distilled water. The mixture was incubated at room temperature for 1 h, and, thereafter, the absorbance was measured at 510 nm against the reagent blank. Total flavonoids quantification was based on a standard curve of catechin (0-100 mg/L), and the results were expressed as mg catechin equivalents per gram dry matter of sample (mg CE/g DM).

Ferric reducing antioxidant power assay

Total antioxidant capacity (TAC) was determined by the Ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) as follows: 240 µL distilled water, 80 µL sample extract solution, and 2080 µL FRAP reagent was added into a 10 mL flask and heated in water bath at 37 °C for 5 min. FRAP reagent was prepared immediately before use by mixing acetate buffer (300 mM, pH=3.6), 10 mM TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in a volume ratio of 10:1:1. Samples were incubated at 37 °C for 15 min in a water bath and, thereafter, the absorbance was measured at 595 nm against the reagent blank. Ferric reducing antioxidant power quantification was based on a standard curve of $FeSO_4 \times 7\ H_2O$ (0-2 mmol/L), and the results were expressed as mmol Fe^{2+} per gram dry matter of sample (mmol Fe^{2+}/g DM). Amersham ultrospec 2100 pro spectrophotometer (Biochrom, USA) was used for all spectrophotometric determinations.

Statistical analysis

All assays were performed in triplicates. Statistical analysis was performed using the SPSS 22.0 software package (IBM, SPSS Inc., Chicago, IL, USA) and the results were expressed as means ± standard deviation. Significant differences were determined by one-way analysis of variance (ANOVA) and least significant difference (LSD). The correlation between total phenolic/flavonoids and total antioxidant capacity was presented by Pearson correlation coefficient.

Results and discussion

The distribution of *Allium ursinum* in the studied area

The occurrence of *Allium ursinum* in the North-western area of Republic Srpska (Bosnia and Herzegovina) was already recognized, but without providing any respective localities. This study has confirmed its occurrence in five different localities: Laktaši, Kozara, Prnjavor, Kneževo and Drinić. In each of the above-mentioned localities, a high presence and high percent cover of *Allium ursinum* was recorded (Fig. 2).

The maximum density was 512 individuals/m^2 in Laktaši, while minimum density was observed in Drinić (148.7 individuals/m^2) areas (Tab. 2). In general, the density of *Alium ursinum* tends to be negatively correlated with increasing elevation. This is expected, considering the fact that low temperature at high altitudes limits plant growth and consequently its abundance.

This study also revealed that *Allium ursinum* occurring in the studied area mostly forms dense populations in forest habitats, especially in beech forests, as a continuous underwood layer. Sobolewska et al. (2015) reported the formation of *Allium ursinum* dense carpet in beech forests is associated with adaptation mechanisms that help *Allium ursinum* plants to survive in its habitats. *Allium ursinum* plants start with active growth in early spring, before the growth of beech tree leaves, thus providing enough light for their initial growth and development. Beech leaves grow with the flowers (they stand together in inflorescences) from April to May, protecting the *Allium ursinum* adult plants from direct sunlight. At the same time, beech crown provides appropriate air-humidity needed for optimal *Allium ursinum* growth and development. As a consequence, *Allium ursinum* plants strongly prefers forest habitats; it can rarely be found outside of a forest. The present study confirms this trend.

Interestingly, *Allium ursinum* was found only in dense stands in which the other plant species are either rarely present or absent. The basic reason for this lies in the fact that *Allium ursinum* can spread invasively by bulbs and self-seeding, which makes it difficult for other plants to exist in those habitats. Another basic reason for this is the fact that *Allium ursinum* produces allelochemicals, such as p-coumaric and ferulic acid, which negatively affect the growth, development and productivity of other plant species (Đurđević et al., 2004).

The morphological characteristics of *Allium ursinum*

Morphological evaluation performed in this study have indicated that all individuals of *Allium ursinum* belong to the subspecies *ursinum*.

Fig. 2: The distribution of *Allium ursinum* in the studied area.

All observed plants are characterized by rough pedicels with numerous papillae, and that is the basic criteria for their classification to subspecies *ursinum* (Fig. 3). Conversely, the individuals that belong to the subspecies *ucrainicum* have smooth pedicels without papillae; however, they were not found in the studied areas.

Fig. 3: Morphological characteristics of *Allium ursinum* plants from studied area.

The morphological analysis also revealed that there was no significant difference in above-ground plant biomass among *Allium ursinum* populations from different localities. On the other hand, analysis of the leaf size (leaf length and width) as well as the petiole length and width indicated a statistically significant difference among studied *Allium ursinum* populations. Accordingly, the *Allium ursinum* populations occurring in Kneževo had significantly higher leaf length and width than those grown in other studied localities, whereas the *Allium ursinum* populations occurring in Prnjavor and Laktaši had the highest petiole length. Interestingly, in this study, *Allium ursinum* plants from Kozara area had the highest petiole width, while other *Allium ursinum* populations had lower petiole width without significant difference among them (Tab. 2).
Overall, considerable variations in leaf morphological traits among *Allium ursinum* plants were observed in this study and the variations in all evaluated traits within populations were lower than those among populations. These data strongly suggest that the environmental factors, including among others geographic location and climate that changes rapidly along elevational gradients, play a key role in the morphological variation in *Allium ursinum* plants.
The most pronounced change associated with increasing elevation is a decrease in temperature, making locations at higher elevations colder (Wessely et al., 2022). In general, the leaf size tends to be smaller in plants that thrive in cold environments (Li et al., 2020); however, the present study failed to confirm it for *Allium ursinum* populations. For example, *Allium ursinum* plants occurring in Kneževo (740 meters above sea level) had higher average leaf size (length and width) than those grown at lower altitudes. These results are not entirely unexpected, however, since the growth and distribution pattern of *Allium ursinum* plants is very specific and therefore cannot be considered as a generalist. In this relation, it is important to note that the *Allium ursinum* plants can live up to 10 years; in the first two years of life the plant growth is slow, rapid growth starts in the third year and lasts until the fifth year, and then the growth slows down again. The years above-mentioned are rough estimates because large individual differences are possible between stages of a life cycle in *Allium ursinum* plants depending on local conditions. To get a better understanding of the life cycle of *Allium ursinum* it is also important to note that the length and width of leaves in *Allium ursinum* plants over 3-year-old are typically much larger than in individuals of 1 to 2 year old. In addition, the distribution of individuals according to age in an *Allium ursinum* population can vary significantly within and across localities. With this in mind, it is quite clear that the growth, development and distribution pattern of *Allium ursinum* is unique to each locality (Oborny et al., 2011).
The results of this study also showed that there were significant differences in the number of coarse roots between studied *Allium ursinum* populations. *Allium ursinum* plants occurring in the Kozara area had the significantly highest number of coarse roots, while the *Allium ursinum* plants from Laktaši and Drinić had the lowest (Tab. 3).

Tab. 3: Morphological characteristics of underground parts of *Allium ursinum* plants.

Locality	Coarse roots (no.)	Bulb length (cm)	Bulb width (cm)
Drinić	8.76 ± 3.09c	2.31 ± 0.43	0.28 ± 0.09^{b}
Kneževo	10.77 ± 3.57^{b}	2.10 ± 0.53	0.28 ± 0.10^{b}
Kozara	13.33 ± 4.32^{a}	2.10 ± 0.56	0.55 ± 0.21^{a}
Laktaši	8.70 ± 3.05^{c}	2.27 ± 0.48	0.28 ± 0.09^{b}
Prnjavor	11.43 ± 3.46^{b}	2.14 ± 0.58	0.31 ± 0.21^{b}
$LSD_{0.05}$	1.81	-	0.08*

Means followed by different letters indicate significant differences at $p < 0.05$. A total of 30 *Allium ursiunum* plants from each location were evaluated for each trait.

Eggert (1992) reported that the number of coarse roots (contractile roots) is an important indicator of the stage of the *Allium ursinum* plant. In contrast to *Arisarum vulgare* O. Targ. Tozz., *Hemerocallis fulva* (L.) L. and other plants in which contractile roots develop from an early stage of their life (Pütz, 1998), development of contractile roots in *Allium ursinum* starts approximately from the age of three years. According to results from the analysis of coarse roots, it can be assumed that examined plots in the Kozara area had more adult individuals compared to other studied localities. However, it is almost impossible to determine the age of the *Allium ursinum* plants based only on visual observation due to several reasons. The two main reasons are: (1) the flowering stage of *Allium ursinum* plants starts quite late in their life, mostly from the fifth year of its life,

Tab. 2: The number of plants per m^2, above-ground mass and morphological characteristics of *Allium ursinum* leaves.

Locality	Plants per m^2 (no.)	Above-ground mass (g)	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Petiole width (cm)
Drinić	148.67 ± 40.80	4.02 ± 0.96	10.42 ± 2.47^{b}	3.54 ± 1.07^{c}	4.89 ± 1.27^{a}	0.15 ± 0.05^{b}
Kneževo	303.67 ± 68.37^{b}	3.99 ± 1.56	14.42 ± 3.63^{a}	5.42 ± 2.03^{a}	3.59 ± 0.51^{c}	0.16 ± 0.06^{b}
Kozara	206.33 ± 63.32^{b}	4.56 ± 2.04	10.54 ± 2.91^{b}	4.31 ± 1.06^{b}	4.21 ± 1.71^{b}	0.20 ± 0.07^{a}
Laktaši	512.01 ± 15.03^{a}	4.04 ± 0.95	10.45 ± 2.43^{b}	3.53 ± 1.05^{c}	4.93 ± 1.27^{a}	0.15 ± 0.05^{b}
Prnjavor	274.33 ± 86.95^{b}	3.65 ± 1.58	8.62 ± 1.65^{c}	3.10 ± 0.81^{c}	5.28 ± 1.33^{a}	0.17 ± 0.08^{b}
$LSD_{0.05}$	109.56	-	1.38	0.63	0.65	0.32*

Means followed by different letters indicate significant differences at $p < 0.05$. A total of 30 *Allium ursiunum* plants from each location were evaluated for each trait.

and therefore, the fluctuations in environmental conditions greatly influence the timing of flowering; (2) *Allium ursinum* plants possess the capacity for both sexual reproduction through seeds and asexual reproduction through bulbs, however, *Allium ursinum* plants that regenerate by bulbs reach maturity faster than those produced from seeds, i.e. they can develop an inflorescence already in the second year. As a consequence, the habitus of individuals of *Allium ursinum* plants of the same age can differ significantly from each other even in the same locality (ERNST, 1979).

The results of this study also showed that *Allium ursinum* plants occurring in Kozara had the highest bulb width, while other *Allium ursinum* populations had lower bulb width without significant difference among them (Fig. 4). Assuming that bulb length is positively correlated with plant age, the results of this analysis also support the hypothesis that examined plots on the Kozara area have more adult individuals compared to the other localities in this study.

The antioxidant properties of *Allium ursinum*

It is well known that *Allium ursinum* plants as well as many other plants belonging to the genus *Allium* are rich sources of bioactive compounds (STANISAVLJEVIĆ et al., 2020), however, studies on its composition and biological properties are still fairly scarce. Phenolics are commonly known as the largest group of plant-derived compounds that have biological properties including antioxidant, antimicrobial and anti-inflammatory activities. The health benefits of *Allium* vegetable consumption have been related, at least in part, to their phenolic compounds content (KOTHARI et al., 2020). The results of this study showed that the concentration of total phenolics in *Allium ursinum* plants ranged from 12.93 to 20.15 mg GAE/g DM (dry mass) and total flavonoids ranged from 5.20 to 7.55 mg CE/g DM (Tab. 3).

Tab. 3: Total phenolics, total flavonoids, and total antioxidant capacity of *Allium ursinum* plants.

Locality	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Total antioxidant capacity (mmol Fe^{2+}/g)
Drinić	18.62 ± 2.57^{a}	6.93 ± 0.63^{b}	0.144 ± 0.010^{ab}
Kneževo	18.30 ± 2.11^{a}	6.83 ± 0.45^{bc}	0.145 ± 0.014^{ab}
Kozara	12.93 ± 1.88^{c}	5.20 ± 0.41^{d}	0.129 ± 0.014^{c}
Laktaši	20.15 ± 6.80^{a}	7.55 ± 1.43^{a}	0.153 ± 0.051^{a}
Prnjavor	16.66 ± 2.51^{b}	6.30 ± 0.56^{c}	0.139 ± 0.014^{b}
$LSD_{0.05}$	3.48	0.59	0.009^{*}

Means followed by different letters indicate significant differences at $p < 0.05$ (n=10).

Literature references describe a variety of ranges for total phenolic content of *Allium ursinum* plants from different geographical origins, depending on both plant parts used for extraction and solvent used. In the study of SAPUNJIEVA et al. (2012) total phenolic content of *Allium ursinum* leaves showed a range from 16.84 to 28.11 mg GAE/g DM with the highest content in 70% ethanol extract and the lowest in the water extract of dry leaves. PEJATOVIĆ et al. (2017) extracted phenolic compounds from *Allium ursinum* leaves in the mixture of 70% ethanol/80% methanol (50/50, v/v) and obtained results in the range from 10.3 to 21.1 mg GAE/g DM for *Allium ursinum* plants collected from Donje Lipovo (Montenegro) and Čemerno (Bosnia and Herzegovina). These results are similar to our findings. On the other hand, more than three times higher amounts of total phenolics were noticed in the leaves of *Allium ursinum* plants from three different sites in Poland (BŁAŻEWICZ-WOŹNIAK and MICHOWSKA, 2011).

The above-mentioned studies support the argued hypothesis that the

Fig. 4: Bulb width and length of *Allium ursinum* plants from studied area.

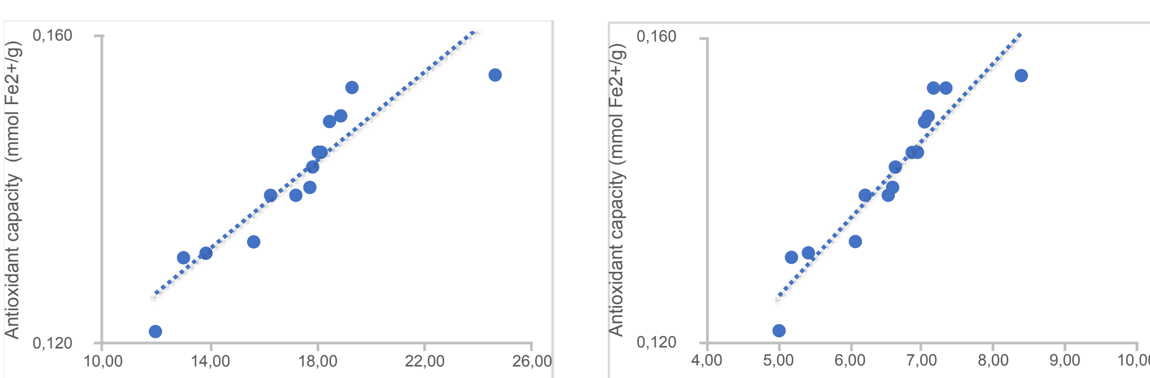

Fig. 5: Relationship between antioxidant capacity (FRAP) and total phenolics/flavonoids in *Allium ursinum*.

phenolic contents in plants are greatly influenced by both geographical location and habitat conditions (Vilkickyte and Raudone, 2021; Chalbi et al., 2023). The findings of this study also support this hypothesis. Namely, significant differences in the total phenolics were found among the studied *Allium ursinum* populations in this study; the *Allium ursinum* plants occurring in Laktaši had significantly higher levels of total phenolics than those grown in other studied localities, whereas the *Allium ursinum* plants occurring in the Kozara had the lowest total phenolic content. The total flavonoid contents also showed the same trend as that of the values recorded for total phenolics. This is to be expected, since flavonoids are the most frequent phenolic compounds (Mutha et al., 2021).

Many studies also provide evidence that geographical location and habitat conditions significantly affect the chemical composition of plants including among others total phenolic and flavonoid contents (Zargoosh et al., 2019; Elsharkawy et al., 2020). A large number of researchers have reported that plants synthesize and accumulate phenolic compounds in higher amounts when faced with stress conditions (Sharma et al., 2019; Al-Hmadi et al., 2021). These findings lead to the assumption that *Allium ursinum* plants occurring in Kozara area were exposed to less severe stress conditions than those grown in other studied localities. However, further studies are needed to confirm this hypothesis.

In this study, a highly positive linear relationship was observed between total phenolics and antioxidant capacity as weel between total flavonoids and antioxidant capacity in *Allium ursinum* plants (Fig. 5). Numerous studies have also reported positive relationship between total phenolics/flavonoids and antioxidant activity in plants (Khan et al., 2020; Kiani et al., 2021; Chalbi et al., 2023). These findings suggest that phenolic compounds, in general, represent an important source of natural antioxidants in plants.

Conclusion

As a conclusion, the results obtained in this study demonstrate that environmental factors play a key role in the variation of morphological characteristics and antioxidant properties of *Allium ursinum* among studied localities. The study also confirmed that *Allium ursinum* strongly prefers forest habitats and that it is an important source of phenolics with relevant antioxidant activity.

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Conflict of interest

No potential conflict of interest was reported by the author.

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ORCHID:
Vida Todorović https://orcid.org/0000-0002-5018-2899
Nikolina Đekić https://orcid.org/0000-0001-5468-2949
Marina Antić https://orcid.org/0000-0002-5792-9916
Borut Bosančić https://orcid.org/0000-0001-8475-400X
Jelena Davidović Gidas https://orcid.org/0000-0003-0144-2583
Senad Murtić https://orcid.org/0000-0003-0887-1970

Address of the corresponding author:
Dr. Senad Murtić, Department of Plant Physiology, Faculty of Agriculture and Food Sciences University of Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
E-mail: murticsenad@hotmail.com, s.murtic@ppf.unsa.ba

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