

# EVALUATION OF THE PHENOLIC PROFILE OF BEAR'S GARLIC (*ALLIUM URSINUM* L.) LEAVES

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*European wild garlic (Allium ursinum L.) syn. wood garlic, ramsons or bear's garlic is a perennial plant that is common in Europe and Asia, but rare in the Mediterranean region. Bear's garlic is included in the Latvian Red Data Book. The plant species has high commercial value and a high amount of biologically active compounds. Because of the conservation status of wild populations, raw material should be obtained by cultivation. For the experiment, leaves of six accessions of bear's garlic from the ex situ collection of medicinal and aromatic plants at the Latvia University of Life Sciences and Technologies, were used. The aim of this study was to identify the individual phenolic compounds in bear's garlic leaves. The composition of the phenolic profile was determined using a high-performance liquid chromatograph (HPLC) Shimadzu Nexera LC-40 with diode-array detector (DAD). Overall, 18 phenolic compounds (gallic acid, 3,5-diOHbenzoic acid, catechin, chlorogenic acid, 4-hydroxybenzoic acid, epicatechin, homovanillic acid, caffeic acid, vanillic acid, syringic acid, vanillin, p-coumaric acid, rutin, sinapic acid, ferulic acid, 2-OHcinnamic acid, quercetin, luteolin, and kaempferol) were detected in the samples. The gallic acid was the predominant phenolic compound, with a range in concentration from 75.8 mg·100 g<sup>-1</sup> to 322.5 mg·100 g<sup>-1</sup> in bear's garlic.*

**Keywords:** wild garlic, polyphenols, HPLC.

## INTRODUCTION

*Allium ursinum* L. (wild garlic syn. wood garlic, ramsons or bear's garlic) is a perennial plant found in swamps and riparian forests of Europe and Asia, but it is rare in the Mediterranean region (Pârva *et al.*, 2014; Pavlović *et al.*, 2017; Krivokapic *et al.*, 2018). It is widely distributed in the Northern Hemisphere: North America, North Africa, Europe and Asia (Pejatović *et al.*, 2017), including Ukraine, Russia, and Caucasus (Błażewicz-Woźniak and Michowska, 2011). The species is also common in the Czech Republic and Germany (Kęsik *et al.*, 2011). Bear's garlic is a plant of the family *Amaryllidaceae* (subfamily *Allioideae*) and in Latvia it is a protected species included in the Latvian Red Data Book. The health-promoting properties of *A. ursinum* were already known by our ancestors. The first information about this species, along with a botanical description, was

mentioned in the herbarium of Hieronymus Bock in 1539 (Tomaszewska-Sowa *et al.*, 2015). Bear's garlic is characterised by its composition, which is similar to that of common garlic (*Allium sativum* L.) and recognised as an important source of biologically active compounds, with the long history of application in traditional medicine as a natural remedy for gastrointestinal, cardiovascular, and respiratory disorders (Błażewicz-Woźniak and Michowska, 2011; Sobolewska *et al.*, 2015; Stanisavljević *et al.*, 2020). Extracts of bear's garlic have high antibacterial and antioxidant activity (Radusin *et al.*, 2019). *A. ursinum* possesses antiplatelet, cardioprotective and blood pressure-lowering activity, antimicrobial, anti-inflammatory and antioxidant properties (Pavlović *et al.*, 2017; Batiha *et al.*, 2020). The major biologically active substances *A. ursinum*, which are responsible for the effects on maintaining good health, are phenolic compounds (especially flavonoids), vitamins, min-

eral substances, lectins, polysaccharides, fatty acids (palmitic, linoleic, oleic, palmitoleic, stearic,  $\alpha$ -linolenic, and myristic acid), many amino acids (asparagine, glutamine, aspartic acid, glutamic acid, arginine, alanine, glycine, threonine), alliins (in particular methylalliin, i.e. methiin, and allylalliin, i.e. alliin) and other sulphur-containing molecules, for example allyl methyl disulphide and allicin pigments (Vlase *et al.*, 2013; Lenková *et al.*, 2016; Pavlović *et al.*, 2017; Pejatović *et al.*, 2017; Krivokapić *et al.*, 2018). Sulfur-containing compounds of *A. ursinum* have traditional use in culinary as well as medicine (Pärvi *et al.*, 2014). The plant has an excellent taste and all over the world it is widely used as a spice and as an aromatic addition to salads (Tomaszewska-Sowa *et al.*, 2015; Batiha *et al.*, 2020). Bear's garlic is well-known in food preparation, especially in dried foods for storage and some types of soup, and it can be utilised in both fresh and dehydrated states (Batiha *et al.*, 2020). Nowadays, there are more and more products containing bear garlic on the market — it is sold as raw, pickled, or salted (Kęsik *et al.*, 2011). Pollen of bear's garlic is also very valuable for honey-bee and wild pollinating insects due to its high content of protein (Tomaszewska-Sowa *et al.*, 2015). Despite the fact, that *A. ursinum* has been used for centuries in traditional medicine, there is a lack of studies about its composition and pharmacological activity (Sobolewska *et al.*, 2015).

Bear's garlic has high commercial value. Because of the conservation status of wild populations, raw material needs to be obtained by cultivation. In the scientific literature, there is little information about the quality of *A. ursinum*, and therefore more research on biochemical compounds is needed. The aim of this study was to identify the individual phenolic compounds of bear's garlic leaves.

## MATERIALS AND METHODS

**Plant material and soil agrochemical characteristics.** On 19 May 2021, leaves were collected from six accessions of *A. ursinum* from the *ex situ* collection of genetic resources of aromatic and medicinal plants (N 56°39'47"; E 23°45'13") of the Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Institute of Soil and Plant Sciences, Laboratory of Horticulture and Apiology (Jelgava, Strazdu iela 1). Historically, the plant material had been collected from the wild in 2001–2006, from different locations in Latvia: accessions No. 1, 2, and 3 from Talsi Municipality, including the territory of Slītere National Park; accessions No. 4 and 5 from Aizkraukle Municipality; and accession No. 6 – from Tukums Municipality (territory of Ķemeri National Park). In the collection, every 5–6 years plants are propagated by cloning. The soil at the trial site is strongly altered by cultivation loam, with organic matter content 2.7 g·kg<sup>-1</sup>, pH<sub>KCl</sub> 6.3, P<sub>2</sub>O<sub>5</sub> content 102 g·kg<sup>-1</sup> and K<sub>2</sub>O content 207 g·kg<sup>-1</sup>.

**Meteorological conditions.** According to data of the Latvian Environment, Geology and Meteorology Centre, the

winter of 2020/2021 was one of the coldest since 2012/2013. The average air temperature was –2.8 °C. December was warm, which continued until mid-January, when –31.0 °C was recorded on 17 January. The frost lasted until the last week of February. The continuous snow cover was from the beginning of January to the end of February. In active vegetation period of bear's garlic (March – May), the average air temperature was +5.8 °C (0.2 °C above long-term average observations), with a range was from –21.8 °C on 9 March to +28.1 °C on 12 May. The end of April to beginning of May, when active growth of *A. ursinum* occurs, was in a cold period that was a few degrees below the norm. The total amount of precipitation was 152.7 mm, which was 24% above the seasonal norm. Both March and April were drier than normal in spring, but May was the wettest since 1924.

**Extraction of phenolic compounds from bear's garlic leaves.** Fresh ground leaves of bear's garlic (moisture 85%), 1.0 g per sample, was extracted on a magnetic stirrer with 20 ml of methanol (HPLC grade CHROMASOLV®) for 60 min. The extract was centrifuged at 15 000 rpm 5 min. Then the supernatant was filtered through a 0.45 µm pore size membrane filter and kept at temperature –18 °C until HPLC analysis.

**HPLC method for the analysis of phenolic compounds.** Experiments were performed at the Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

**Preparation of calibration solution.** To prepare the calibration solution, the following components were added to a 100 ml narrow neck volumetric flask: 6.8 ± 0.1 mg gallic acid, 7.4 ± 0.1 mg 3,5-dihydroxybenzoic acid, 12.0 ± 0.1 mg catechin, 12.8 ± 0.1 mg 4-hydroxybenzoic acid, 13.1 ± 0.1 mg chlorogenic acid, 12.1 ± 0.1 mg homovanillic acid, 14.5 ± 0.1 mg vanillic acid, 13.8 ± 0.1 mg caffeic acid, 16.0 ± 0.1 mg epicatechin, 18.8 ± 0.1 mg syringic acid, 9.8 ± 0.1 mg vanillin, 12.1 ± 0.1 mg p-coumaric acid, 88.1 ± 0.1 mg sinapic acid, 9.2 ± 0.1 mg ferulic acid, 6.1 ± 0.1 mg rutin, 11.2 ± 0.1 mg 2-hydroxycinnamic acid, 4.3 ± 0.1 mg quercetin, 9.1 ± 0.1 mg luteolin and 9.6 ± 0.1 mg kaempferol. The flask was then filled with HPLC grade CHROMASOLV® methanol till mark and mixed.

**Parameters of chromatography.** Content of individual phenolic compounds was determined with a high-performance liquid chromatograph (HPLC) Shimadzu Nexera LC-40 with diode-array detector (DAD). An analytical column YMC C18, 4.6 mm · 250 mm, 5 µm and temperature +30 °C was used for separation of polyphenols at wavelength 278 nm. Injection volume of the sample was 50 µl. The mobile phase consisted of: A (deionized water), B (HPLC grade CHROMASOLV® methanol) and C (acetic acid solution for HPLC). Gradient conditions were used according to the procedure described by Cinkmanis *et al.* (2018). Start flow rate was 1.0 ml·min<sup>-1</sup>.

**Determination of moisture.** Moisture content of bear's garlic leaves was determined by ISO 6496:1999 using a KERN balance (Germany) with precision  $\pm 0.001$  g.

**Statistical analysis.** The results are presented as means and standard deviation for three replicates. Two-factor analysis was used to determine significant differences. The Fisher's criterion was applied to estimate the effects of production systems and cultivars. Significance was defined at  $p < 0.05$ .

## RESULTS

The following individual phenolic compounds were isolated from bear's garlic leaves using methanol extract: gallic acid, 3,5-dihydroxybenzoic acid, catechin, 4-hydroxybenzoic acid, chlorogenic acid, chlorogenic acid, homovanillic acid, vanillic acid, caffeic acid, epicatechin, syringic acid, vanillin, p-coumaric acid, sinapic acid, ferulic acid, rutin, quercetin, luteolin and kaempferol (Figs. 1, 2, and 3). The concentration of individual phenolic compounds was variable, depending on sampling location in Latvia. Gallic acid, ferulic acid, caffeic acid, chlorogenic acid, and sinapic acid were the main phenolic acids present in all analysed bear's garlic leaf samples. The highest concentration was found for gallic acid in all bear's garlic leaves (Fig. 1). The amount of this acid ranged from 75.8 to 322.5 mg·100 g<sup>-1</sup>. The highest concentration of gallic acid was found in sample No. 5, and the lowest concentration found in sample No. 3. Ferulic acid

also was detected in the analysed samples. The range of ferulic acid was from 8.3 till 47.8 mg·100 g<sup>-1</sup>. The highest concentration of ferulic acid was found in sample No. 5 and the lowest in sample No. 1. Vanillic acid also was found in the analysed bear's garlic samples (Fig. 2). The concentration of vanillic acid was higher than the concentration of homovanillic acid, 2-OHcinnamic acid and 3,5-diOHbenzoic acids. The concentration of vanillic acid in the analysed bear's garlic's samples ranged from 13.5 to 0.9 mg·100 g<sup>-1</sup>, sample No. 2 had the highest concentration of vanillic acid. Concentration of flavonoid compounds in *A. ursinum* accessions varied within a large range (Fig. 3). Among the flavonoid compounds quantified in the leaf samples, the highest concentration of rutin reached 29.8 mg·100 g<sup>-1</sup>. A relatively high concentration of catechin hydrate and epicatechins was also observed in the samples (Fig. 3). Chromatographic analysis was not able to quantify amounts of luteolin, quercetin, and kaempferol in the bear's garlic leaves.

## DISCUSSION

There are many steps to obtain phytochemicals from plants, such as milling, grinding, homogenisation, and extraction. Among these steps, extraction is the main step for recovering and isolating phytochemicals from plant materials. Extraction efficiency is affected by the chemical nature of phytochemicals, the extraction method used, sample particle

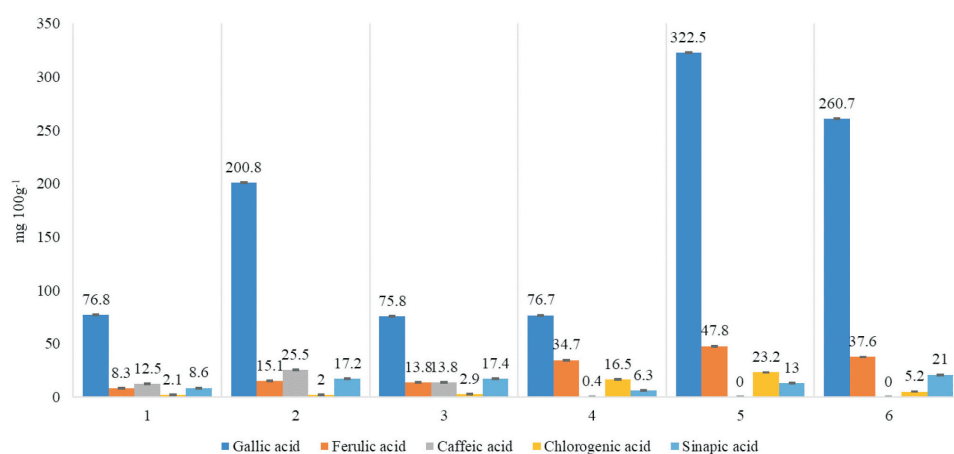


Fig. 1. Concentration of predominant phenolic acids in bear's garlic accessions, mg·100 g<sup>-1</sup>.

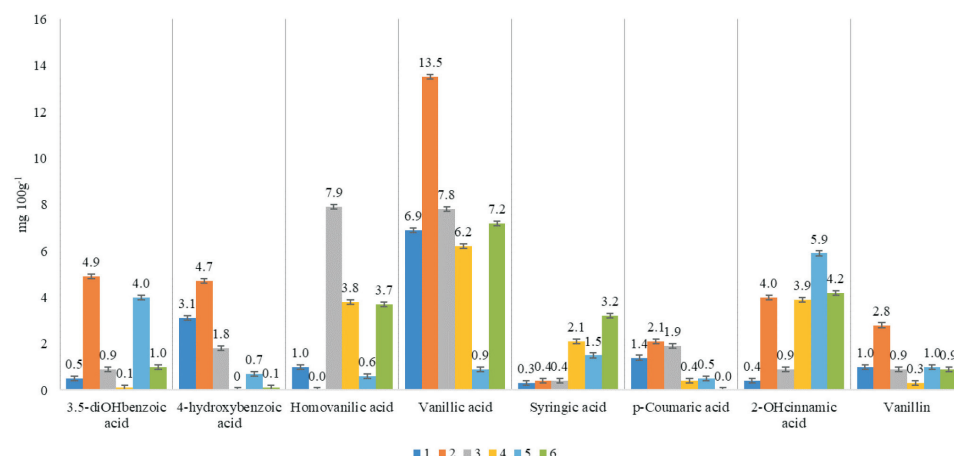


Fig. 2. Concentration of minority phenolic acids in bear's garlic accessions, mg·100 g<sup>-1</sup>.

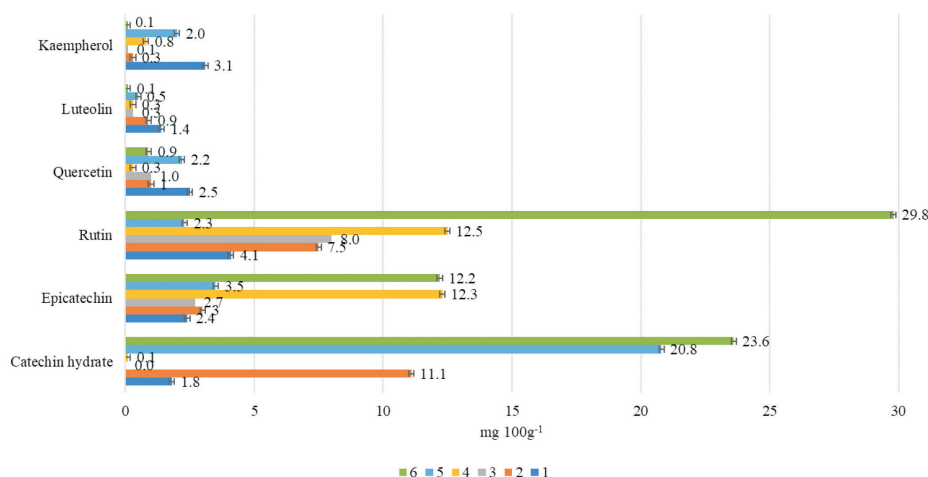


Fig. 3. Concentration of flavonoid compounds in bear's garlic accessions.

size, the solvent used, as well as the presence of interfering substances (Stalikas, 2007). Extraction of bear's garlic leaves depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. For extracting phenolic compounds from plant material, the typically used solvents are methanol, ethanol, acetone, and ethyl acetate (Złotek *et al.*, 2016). In the present study, 100% methanol was used for extracting phenolic compounds from bear's garlic leaf extracts. According to Lachowicz *et al.* (2016), it is the phenol fraction in plants depends mainly on the species, cultivars and the agronomic and climatic conditions during the growing season.

Medicinal plants rich in polyphenols can retard the oxidative degradation of lipids and improve the quality and nutritional value of food (Vlase *et al.*, 2013). As study from Romania on *Allium ursinum* (bear's garlic) leaf extracts reported that methanol extracts (96%, 80%) had a gallic acid concentration ranging between 0.0011–0.0576 mg·ml<sup>-1</sup> extract, while ethanol extracts (96%, 60%) had a gallic acid concentration of 0.0010–0.0182 mg·ml<sup>-1</sup> (Condrat *et al.*, 2010). It has been confirmed that the yield of phenolic compounds depends on extraction method, extraction solvent and part of the plant used for extraction (Krivokapic *et al.*, 2018). The possible discrepancy in the amount of phenolics found between different authors may be the consequence of different methods used for quantification (Krivokapic *et al.*, 2018). Flavonoids are natural products widely found in the plant kingdom and are currently consumed in large amounts in the daily diet (Augšpole *et al.*, 2018). Rutin has a wide range of bioactivity including antioxidant and anti-inflammatory, and therefore researchers are searching for its potential sources (Camponogara *et al.*, 2020; Kherbache *et al.*, 2020). The results of the current study confirm that leaves of bear's garlic can be a potential source of rutin. Several previous studies have shown that the main polyphenols present in leaves of *A. ursinum* are flavonoids, among which kaempferol derivatives were predominant (Stanislavljević *et al.*, 2020). Another study found that kaempferol was present only in hydrolysed extracts of leaves (252.76 µg·100 g<sup>-1</sup>) and flowers (1839.33 µg·100 g<sup>-1</sup>) of *A. ursinum* (Vlase *et al.*, 2013).

## CONCLUSIONS

1. The obtained results of this study showed that bear's garlic leaves are a natural source of phenolic compounds. The concentrations and presence of phenolic compounds varied among bear's garlic leaf samples.
2. Among phenolic acids, a higher content of gallic acid (75.8 to 322.5 mg·100 g<sup>-1</sup>) and ferulic acid (8.3 to 47.8 mg·100 g<sup>-1</sup>) was observed in all six accessions of *A. ursinum* from the *ex situ* collection of genetic resources of aromatic and medicinal plants.
3. The lowest concentration of phenolic acids was observed for p-Coumaric acid (0.4 to 2.1 mg·100 g<sup>-1</sup>) and vanillin (0.3 to 2.8 mg·100 g<sup>-1</sup>).
4. The dominant flavonoid compounds were rutin (2.3 to 29.8 mg·100 g<sup>-1</sup>), catechin hydrate (0.1 to 23.6 mg·100 g<sup>-1</sup>) and epicatechin (2.4 to 12.3 mg·100 g<sup>-1</sup>).

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## FENOLU SAVIENOJUMU NOVĒRTĒJUMS LAKŠU (*ALLIUM URSINUM* L.) LAPĀS

Lakši vai meža loki jeb mežloks (*Allium ursinum*) ir daudzgadīgi amariļļu dzimtas lakstaugi. Lakši ir iekļauti Latvijas Sarkanajā grāmatā. Lakšiem ir augsta komerciāla un bioloģiski aktīvu savienojumu vērtība. Eksperimentam tika izmantotas Latvijas Lauksaimniecības universitātes ārstniecības un aromātisko augu ģenētisko resursu *ex situ* kolekcijas seši lakšu paraugi. Pētījuma mērķis bija identificēt lakšu paraugu lapās atsevišķus fenola savienojumus. Fenolu saturu noteica, izmantojot augstas izšķirtspējas šķidrums hromatogrāfu (HPLC) Shimadzu Nexera LC-40 ar diožu matricas detektoru (DAD). Kopumā noteica 18 fenola savienojumus (galluskābi, 3,5-diOHbenzoscābi, katehinu, hlorogēnskābi, 4-hidroksibenzoscābi, epikatehinu, homovanilskābi, kafijskābi, vanilīnskābi, ceriņskābi, vanilīnu, p-kumarskābi, rutīnu, sinapīnskābi, ferulskābi, 2-OHkanēliskābi, kvercetinū, luteolīnu un kempferolu). Pētījumā tika konstatēts, ka dominējošais fenola savienojums bija galluskābe. Lakšos galluskābe bija no 75.8 mg·100 g<sup>-1</sup> līdz 322.5 mg·100 g<sup>-1</sup>.