

Sempervivum rev 1

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Submission date: 13-Oct-2023 12:51PM (UTC+0200)

Submission ID: 2194498134

File name: ivum_Manuscript_King_Saud_University_Journal_rev_1_turnitin.docx (799.52K)

Word count: 5627

Character count: 30111

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Determination of the health-protective effect of different *Sempervivum* and *Jovibarba* species

Running title: Sempervivum species in human health

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Abstract

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This study aimed to examine the micro- and macroelements, as well as the total antioxidant, and polyphenol content of 22 different types of dried houseleek, and fresh plant decoctions. The measurement of antioxidant and polyphenol content was carried out with two drying methods- a drying cabinet and lyophilization. The plants, without exception, were provided by Berger Trio Kft. from Jánossomorja (Hungary), thus, the influencing effect of the environment is negligible in the case of the tested species. Based on the results, the type of houseleek has a clear influencing effect on the amount of the tested constituents. The investigated microelements were B, Cu, **21** Fe, Mn, and Zn, while Ca, K, Mg, Na, P, and S were examined among the macroelements. In terms of microelement content, Mn was present in outstanding amounts (20.67-148.00 mg/kg DW), while among macroelements Ca (44.43-95.27 g/kg DW) and Na (24.07-128.50 g/kg DW) was present in larger quantities in the samples. It can be said that in the case of samples with a high amount of antioxidants and polyphenols, the element content is low and the reverse is also true. During the examination of the dried houseleeks, outstanding values were obtained for the tested compounds. The antioxidant values of the dried leaves were between 2.22 and 317.74 mg AAE/g DW, while their polyphenol contents ranged from 5.52 to 144.16 mg GAE/g DW, however, the drying methods had some influencing effect. Regarding fresh plant decoctions, the polyphenol contents (0,02-1,11 mg GAE/g FW) were negligible, while the amount of antioxidants (2,38-4,61 mg AAe/g FW) were low. With other solvents (e.g. alcoholic extraction - tinctures), better results are likely to be achieved. Houseleek species (especially in dried form) are an excellent source of trace elements, antioxidants, and phenolic components so they can even be

used as additives to functional foods or consumed on their own in the form of encapsulated dietary supplements.

Keywords: antioxidants, elements, houseleek, polyphenols, spectrophotometry

Introduction

The houseleek comes in a wide variety of shapes, colors, and textures can be found in horticulture, and as a result, there are more than 4,000 varieties (Dégi et al. 2023) Very diverse plant, especially the rosette's diameter, width, and color. It ²⁶ can grow up to 15 cm high and 50 cm wide, with stemless 4-10 cm leaves, but when flowering it can be 20-25 cm. Some varieties has a narrower, more open rosette, and smooth or velvety leaves. Some plants are covered with spider web-like fibers or the tips of their leaves are covered with tiny hairs. Their colors are green, gray, and purple, they can vary in shades of yellow and red, and their tone changes according to the season. The houseleek is an evergreen, perennial, succulent plant. The houseleek rosette contracts in cooler weather and opens in hot weather. It can cling to the roofs of houses with its fibrous roots. In a moderate climate, and good soil, does not require additional nutrients (Fabritzek et al. 2018) It grows most actively in April and May. In summer, it develops from the center of the rosette leaves its inflorescence which is located on a long stem. (Brickell et al. 2008, Jankov et al. 2023). Some varieties bloom for much longer (Kelaidis et al. 2008). After flowering, the houseleek which bearing the flower, dies (Bernáth et al. 2013).

Many beneficial effects of houseleek on human health have been investigated in recent years. It is ⁷ an excellent source of antioxidants (Šentjurc et al. 2003; Knez Marevci et al. 2021), promotes wound healing (Cattaneo et al. 2019), and has an anti-inflammatory effect. It has a pain-relieving and detoxifying effect and helps the liver to regenerate (Blázovics ¹⁴ et al. 1993; Szentmihályi et al. 2004; Muselin et al. 2014; Stojković et al. 2015; Hegyi and Blázovics 2020). Its favorable physiological effects can be traced back to the phenolic ⁸ compounds that can be extracted from the leaves (Abram et al. 1999; Alberti et al. 2012).

Only one research is currently dealing with the elemental composition and effects of houseleek on health (Gentscheva et al. 2021a). However, no one has described the

relationship between trace elements and antioxidant compounds, especially in the case of the large number (22) species of houseleek described below. In order to reduce the influencing effects of the environment as much as possible, the plants included in the research, without exception, came from the same North-Western Hungarian horticulture (Jánossomorja, Hungary), where the same plant cultivation operations were carried out on them and they were grown in the same composition growing medium. Most of the peripheral region of Western Hungary belongs to the moderately warm, wet, mild winter climate zone. Another characteristic is the low-temperature fluctuation, as well as abundant precipitation at the national level. The region has more than 2,000 hours of sunshine per year. The average temperature in the region ranges between 9-10 °C.

Materials and Methods

Chemicals

For the ICP-OES analysis nitric acid (Iach: ner, Bratislava, Slovakia), and hydrogen peroxide (Molar Chemicals, Halásztelek, Hungary) were used for the destruction method. For calibration, mono-element standards for calcium, potassium, magnesium, and phosphorus, and multielement standards for microelements were purchased from CPAchem (Bogomilovo, Bulgaria). The argon gas required for the operation of the ICP-OES device was procured from Messer Hungarogáz Kft (Budapest, Hungary).

Chemicals for the determination of polyphenol, and antioxidant content were 97% ethanol (Reanal, Budapest, Hungary), anhydrous sodium carbonate (Riedel-de Haen, Seelze, Germany), Folin-Ciocalteu reagent (Merck), 2-4-6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, Budapest, Hungary), acetic acid (Reanal, Budapest, Hungary), anhydrous iron chloride (Merck, Budapest, Hungary), ascorbic acid (Sigma-Aldrich, Budapest, Hungary), and gallic acid (Sigma-Aldrich, Budapest, Hungary).

Houseleek samples

During the experiments, ¹ the total antioxidant and polyphenol content of 22 houseleek varieties were examined using spectrophotometric methods, and their elemental composition was also determined using ICP-OES equipment. Each variety of stone rose was in 3 densely overgrown pots with 10.5 cm diameter. The leaves of the same varieties were collected before drying and then divided into 3 equal parts to determine the dry matter content. All samples were subjected to two types of drying - oven drying (Heraeus T6, Germany) at 40°C, and lyophilization (Flexy-Dry MP, FTS Systems, USA)- until mass was constant. The dry matter ⁴ content of each sample was determined gravimetrically. The antioxidant and polyphenol contents were examined for both drying ²⁴ methods in order to determine the effect of the drying process and temperature on the compounds. The composition of micro- and macroelements is not affected by the drying method, so they were analyzed only in the case of drying cabinet samples. ⁴ The dried houseleek samples were chopped with a coffee grinder (Sencor, SCG 2050RD, FAST Hungary Kft., Hungary). The examined houseleek varieties were the following (Figure 1):

- | | |
|---|--|
| 1. ' <i>Sempervivum Tectorum</i> Var. <i>Pyreneum</i> ' | 12. ' <i>Sempervivum Celon</i> ' |
| 2. ' <i>Sempervivum Pacardian</i> ' | 13. ' <i>Sempervivum Mystic</i> ' |
| 3. ' <i>Sempervivum Orostachys Spinosa</i> ' | 14. ' <i>Sempervivum Elva</i> ' |
| 4. ' <i>Sempervivum Pilatus</i> ' | 15. ' <i>Sempervivum Grandiflorum</i> From Nufene
Pass' |
| 5. ' <i>Sempervivum Calcareum</i> ' | 16. ' <i>Jovibarbara Arenaria</i> ' |
| 6. ' <i>Sempervivum Arachnoideum Webbianum</i> | 17. ' <i>Jovibarbara Arenaria Ostirol</i> '
<i>Aureum</i> ' |
| 7. ' <i>Sempervivum Hey-Hey</i> ' | 18. ' <i>Sempervivum Koko Flanel</i> ' |
| 8. ' <i>Sempervivum Granat</i> ' | 19. ' <i>Sempervivum Noir</i> ' |

9. 'Sempervivum Reinhard'

20. 'Sempervivum Manuel'

10. 'Sempervivum For You'

21. 'Sempervivum Havendijker Teufel'

11. 'Sempervivum Ronsdorfer Hybride'

22. 'Sempervivum Gamma' voltak.

Sample destruction

From the dried, and chopped samples, 0.4 g were weighed into a 90 mL tetrafluoromethoxyl (TFM) digestive vessel. To the weighed samples was added 5 mL of 65% HNO₃, and 1 mL of 30% H₂O₂. The samples were digested in MARS 6 iWave (CEM Corporation, Matthews, NC, USA) microwave digestion system according to the following program: After a 15-minute heating phase, the destruction was performed at a temperature of 210 °C for 15 minutes under continuous temperature control. The reaction vessels were cooled, and then the resulting solutions were diluted to a volume of 25 mL with a 0.1 mol/L nitric acid solution. Parallel to the experimental samples, we also prepared blind samples. After digestion, samples were diluted to 25 mL and assayed with ICP-OES.

ICP-OES analysis

For the determination of the micro-and macroelement composition of the samples an Agilent (Santa Clara, CA, USA) 5110 ICP-OES type equipment was used. We measured the elements shown in Table 2 at the described wavelengths with the settings in Table 1. In the case of the examined macroelements, for the quantitative determination, analytical measuring solutions were prepared in the concentration range of 1-500 mg/L. In the case of microelements, we calibrated in a lower concentration range of 2.5-1000 µg/L.

Sample preparation for determination of total antioxidant and polyphenol content

To determine the amount of antioxidants and polyphenols in dried houseleek samples, the content of the active ingredient had to be extracted from the matrix by solvent extraction. For extraction, 0.5-0.5 g of samples were weighed into 100 mL Erlenmeyer flasks on an analytical balance and 10 mL of an extraction mixture containing ethanol and water (50:50 V/V%) was added. The extraction was performed at room temperature with a laboratory shaker (Elpan 358 S, Poland) for 2 hours at 120 rpm. The extracts were centrifuged at room temperature, 2500g, 20 min, and the filtrate was further analysed. We also examined the total polyphenol and, antioxidant content in the watery decoction (tea) of the plants. For this, 5 g of fresh houseleek leaves were scalded with 250 ml of boiling water. After it cooled down, it was filtered and the filtrate was examined.

FRAP assay

The FRAP assay procedure is based on the method described by Benzie and Strain (1996). 200 μ L of extracted sample or tea, 3mL of FRAP solution, and 100 μ L of water were pipetted into a test tube. The finished solutions were placed in a dark place for 5 min and then their absorbance was measured with a Spectroquant Pharo 100 spectrophotometer (Merck, Germany) at a wavelength of 593 nm against the blank. Ascorbic acid (40-500 mg/L) was used as a standard and the results were expressed as ascorbic acid equivalent (AAE)/ g dry matter.

Folin-Ciocalteu assay

Determination of total polyphenol content based on the Folin-Ciocalteu method described by Singleton et al. (1999) with some modifications (Barba et al. 2013). To 200 μ L of houseleek extract or tea, 1.5 mL of high-purity water was pipetted and the reagents were added. First 2.5 mL of 10 % Folin-Ciocalteu reagent, then 2 mL of 7,5 % Na_2CO_3 . The tubes containing the

mixture were placed in a dark place for 90 minutes, and then the absorbance was measured at 725 nm versus the blank. Gallic acid was used as a standard (25 - 1000 mg/L).

Data analysis

The total antioxidant, polyphenol, and micro-macroelement contents of houseleek samples and teas were determined in Microsoft Office Excel from the absorbance values measured for the samples using the equation of the second-order least squares analytical curve fitted to the measurement solutions using the nonlinear least-squares method. All the results are expressed as means (n=3) + / - standard deviation. All measurements were performed in triplicate, and the results are expressed as the mean standard deviation (SD). Analyses of variance (ANOVA) followed by the Tukey post hoc test were used to compare the significant differences in the data. Differences were considered statistically significant when $p < 0.05$. The statistical analyses were carried out with Microsoft Excel 2013 software.

Results and discussion

Dry matter content of houseleek samples

Table 3 shown the dry matter contents of the houseleek samples after drying to constant mass. In many cases, we detected significant differences in the dry matter content of the examined varieties. The lowest dry matter content had the '*Sempervivum orostachys spinosa*' variety with 3.02 m/m% value, in contrast, the '*Sempervivum granat*' species had the largest dry matter content (16.79 m/m%). The average moisture content of the plants was 91.21 m/m%. A dry matter content of 10.5 m/m% for *Sempervivum tectorum* leaves was determined by Mladenović et al. (2023), but in the case of succulent plants, this value also depends on how much moisture they can absorb from the soil and store in their leaves. The examined houseleek varieties were cared for with the same irrigation procedure, so it can be assumed

that the differences in the measured dry matter content resulted from morphological differences.

Microelement content of houseleek samples

¹³ Based on the results presented in Table 4, it can be said that the examined houseleek varieties have a high microelement content, which is influenced by the species. Among the microelements included in the study, the highest value is observed in manganese content. '*Sempervivum pilatus*' has the lowest manganese content (20.67 mg/kg), while '*Sempervivum orostachys spinosa*' contained the highest amount (140.00 mg/kg). Compared to the received concentrations, the recommended daily intake of manganese for an average, adult human body can be 5 mg (Kiss 2009). The smallest concentration of boron determined in the variety of '*Sempervivum havendijker teufel*' (10.19 mg/kg), while the '*Sempervivum calcareum*' species (31.58 mg/kg), contained the largest amount. The least amount of copper measured in the variety '*Sempervivum mystic*' (7.43 mg/kg), and the most in the '*Sempervivum arachnoideum webbianum aureum*' (30.75 mg/kg) species. Regarding zinc, '*Sempervivum mystic*' showed the least (16.03 mg/kg), and the variety of '*Sempervivum orostachys spinosa*' had the most zinc concentration (85.74 mg/kg). The smallest iron content was obtained in the '*Sempervivum mystic*' (13.57 mg/kg) variety, while the highest was in the species of '*Sempervivum havendijker teufel*' (67.04 mg/kg). The recommended daily intake is 10-15 mg for iron, 1.1 mg for copper, and 9 - 10mg for zinc (Forrai et al. 2020). Compared to the results of the studies presented by Szentmihályi et al. (2004),⁸ Turan et al. (2003), and Gentscheva et al. (2021a), we obtained similar results. The elements in the houseleek were better dissolved by the extractant with a higher water content, according to Gentscheva et al. (2021b), so it is assumed that these elements are water-soluble and easily accessed by living organisms.

Macroelement content of houseleek samples

Just like in the case of the microelement contents, the houseleek contains a large amount of macroelements (Table 5). The content of calcium and sodium should be emphasized. The smallest amount of calcium was detected in the case of '*Sempervivum havendijker teufel*' (44.43 g/kg), and the lowest sodium concentration was in the '*Sempervivum gamma*' (31.63 g/kg) species. The variety of '*Sempervivum orostachys spinosa*' has the most calcium, and sodium content, with 95.27 g/kg and 128.5 g/kg values. Regarding potassium the lowest concentration measured in the variety of '*Sempervivum granat*' (10.41 g/kg), and the highest in '*Sempervivum arachnoideum webbianum aureum*' (39.43 g/kg). The least magnesium was detected in the species of '*Jovibarba arenaria*' (5.82 g/kg), while the most were contained in the variety of '*Sempervivum orostachys spinosa*' (13.51 g/kg). Sulfur and phosphorus, samples showed lower amounts. For sulfur, the '*Sempervivum mystic*' (1.83 g/kg) variety contains the least, while '*Jovibarba arenaria ostirol*' (9.89 g/kg) variety the most amount. We measured the least concentration in the variety of '*Jovibarba arenaria*' (1.88 g/kg), while in the species of '*Sempervivum arachnoideum webbianum aureum*' (6.05 g/kg) the most phosphorus content. The recommended daily intake is 3.5 g for potassium, 800-1000mg for calcium, 2g for sodium, 620 mg for phosphorus, and 300-350 mg for magnesium (Fritz et al. 2019; Forrai et al. 2020). The measured concentrations compared to the result of Szentmihályi et al. (2004), Turan et al. (2003), Blázovics et al. (2003), and Gentscheva et al. (2021a), get almost similar quantities. The problem with using plants in the food industry is that they can accumulate toxic elements in addition to beneficial elements. Getcheva et al. (2021b) also examined the content of toxic elements in the houseleek extracts using the ICP-MS method, however, the content of titanium, arsenic, cadmium, and mercury was in all cases below the detection limit (0.02 mg/kg) and the highest measured lead concentration was 0.13 mg/kg. Comparing these values with WHO regulations, according to which the permissible limit value for cadmium in

plant samples is 0.3 mg/kg, while for lead is 10 mg/kg, it can be concluded that the stone rose does not accumulate large amounts of toxic elements harmful to health.

Antioxidant content of houseleek samples

¹³ Based on the results of Table 6, it can be said that overall better values were obtained using the lyophilization method, but this is not typical for all ²⁵ varieties. The lowest antioxidant content was measured in the species of '*Sempervivum arachnoideum webbianum aureum*' (2.22 mg AAE/g), this sample was dried in the drying cabinet. The most antioxidants were detected in '*Sempervivum manuel*' (305.91 mg AAE/g), in the case of all drying methods. For lyophilized samples, '*Sempervivum orostachys spinosa*' (41.45 mg AAE/g) contains the lowest concentration of antioxidant compounds, while the most was in '*Sempervivum manuel*' (317.74 mg AAE/g). Drawing a parallel between the houseleek varieties and the results, it can be concluded that the antioxidant content is influenced by the variety of plants and the used drying method. To our knowledge, there is no comprehensive publication in the literature that compares the antioxidant content of such a large number of houseleek varieties.

The used different drying processes resulted in distinct antioxidant values for the same houseleek species. However in terms of the tested compound groups, in most cases, lyophilization eventuated more favorable results, because it is a gentler drying method, thus caused less damaging to the compounds with heat-sensitive antioxidant effects. However, there were samples where on the contrary, oven drying resulted in higher polyphenol and antioxidant content. The reason for these differences could be that the different drying processes caused different plant cell walls' permeability, thus influencing the extraction efficiency, but the applied drying temperatures could also lead to the formation or decomposition of other compounds. Jankov et al. (2023) investigated the free ¹² radical scavenging capacity of the *Sempervivum tectorum* leaf extract and determined that the most

typical phenolic compounds with significant antioxidant effects in the houseleek leaves are ¹² kaempferol, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, caffeic acid, and gallic acid.

Polyphenol content of houseleek samples

The results of the total polyphenol content of the dried samples are shown in Table 7. Just as ⁶ in the case of antioxidant contents, clearly illustrates ¹⁵ that the amount of polyphenolic compounds is influenced by the type of plant, as well as in most cases these values are more favorable for samples dried with a lyophilizer. Mihai et al. (2019) investigated the phytochemical composition of '*Sempervivum ruthenicum*' and determined that the most typical polyphenolic acids in the plant are ²⁰ gallic acid, chlorogenic acid, caffeic acid, ferulic acid, cinnamic acid, p-coumaric acid, and ellagic acid. The sample with the least polyphenol content was '*Sempervivum arachnoideum webbianum aureum*' (5.63 mg GAE/g), which was dried with a drying cabinet. For this drying method, the highest concentration of polyphenols contained '*Sempervivum tectorum var. pyreneum*' (144.16 mg GAE/g). ³ In the case of lyophilized samples '*Sempervivum orostachys spinosa*' (19.75 mg GAE/g) ⁴ contained the least amount of polyphenolic compounds, on the other hand, '*Sempervivum ronsdorfer hybride*' contained in highest amount (126.45 mg GAE/g). Comparing the examined results with the article of Knez Marevci et al. (2021), similar polyphenol concentrations are seen.

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Antioxidant and polyphenol content of houseleek decoctions

Regarding ⁴ the total antioxidant and polyphenol content of teas made from different houseleek varieties, it can be clearly established that significantly fewer antioxidant and polyphenol compounds contain, like dried houseleek plants (Table 8). Plant decoctions containing antioxidant compounds in larger quantities, these amounts to each species are closely similar. The most antioxidants (4.61 mg GAE/g) were in the tea of '*Sempervivum arachnoideum webbianum aureum*' variety, and the least (2.38 mg GAE/g) was in the '*Sempervivum noir*' decoction. Polyphenolic compounds were only present in trace amounts in the teas, higher amounts were contained in the variety of '*Sempervivum tectorum*' var. ⁴ *pyreneum*' (1.11 mg GAE/g) and '*Sempervivum hey-hey*' (0.9 mg GAE/g). Although houseleek tea contains antioxidants, water is not the best extractant to extract these compounds, with other solvents, for example alcohols we could probably achieve better efficiency (Gentscheva et al. 2021b)

Conclusion

As a consequence of the research, it can be said that the dried and grounded houseleek contains a large amount of important trace elements for the human body, and accumulates toxic elements below the health risk limit. With the consumption of ground houseleek, we can ⁵ cover a significant part of the recommended daily intake of micro- and macroelements. After further processing, they can even be sold in the form of capsules as food supplements. Based on the results obtained, the houseleek (primarily in dried form) has physiologically favorable antioxidant and polyphenol content. ¹⁵ The amount of compounds is significantly influenced by the houseleek variety. However, we did not see a clear difference regarding the results of the drying procedures. In terms of polyphenol content, lyophilization was more favorable in most cases, we also experienced this with regard to the antioxidant content. Lyophilization is a

gentler drying method, thereby causing less damage to the heat-sensitive antioxidant compounds. However, there were results where, on the contrary, the drying cabinet resulted in more favorable values. These disparities may be due to different drying procedures. Each heat treatment made the plant cell walls permeable to varying degrees, thus also influencing the extraction efficiency, but the used temperatures also affect the formation of other compounds, and may lead to their decay. Furthermore, investigated houseleek varieties are likely to contain different amounts of individual antioxidants and phenolic compounds. Although the houseleek decoctions contain antioxidants, water is not the best solvent for the extraction of these compounds. Probably with other extractants, in the form of tinctures, we could achieve better efficiency. Also may be worthwhile to use dried houseleek additives for the production of functional foods. Overall, because of its high element content and excellent free radical scavenging capacity, houseleek may be appropriate for medicinal use. Napjainkban a szakirodalom középpontjában a növény különböző kivonatainak egészségügyi előnyei állnak, de a szárított levelek táplálkozással (kapszulák, élelmiszeripari adalékok) közvetlenül is bekerülhetnek az emberi szervezetbe. Due to its characteristic, slightly sour taste, it could be used mostly as a supplement to salads, pottages, and confectionery products with an intense taste.



Figure 1: Houseleek varieties in the experiment

Table 1 ICP-OES test program used for the determination of macro- and microelements

Parameters	Microelements	Macroelements
Number of elements	5	6
Read time	20	5
RF power (kW)	1.4	
Stabilization time (s)	15	
Viewing mode	axial	radial
Viewing height (mm)	-	8
Nebulizer flow (L/min)	0.75	
Plasma flow (L/min)	12	
Aux flow (L/min)	1	

Table 2 Wavelengths used to determine micro- and macroelements

Microelements	λ (nm)	Macroelements	λ (nm)
B	249.772	Ca	315.887
Cu	327.395	K	766.497
Fe	238.204	Mg	280.270
Mn	257.61	Na	589.592
Zn	202.548	P	213.618
		S	182.562

Table 3 ⁷ The dry matter content of the studied houseleek varieties, ³ different letters (a, b, c, d, e, f, g, h, i and j) denote significant differences ($p \leq 0.05$)

Houseleek species	Dry matter content (g / 100 g FW)
<i>Sempervivum tectorum var. pyreneum</i>	7.25 ± 0.36 ^a
<i>Sempervivum pacardian</i>	8.47 ± 0.35 ^b
<i>Sempervivum orostachys spinosa</i>	3.02 ± 0.41 ^c
<i>Sempervivum pilatus</i>	11.21 ± 1.11 ^d
<i>Sempervivum calcareum</i>	12.42 ± 0.75 ^e
<i>Sempervivum arachnoideum webbianum aureum</i>	11.03 ± 0.03 ^d
<i>Sempervivum hey-hey</i>	14.07 ± 0.79 ^f
<i>Sempervivum granat</i>	16.79 ± 0.08 ^g
<i>Sempervivum reinhard</i>	12.12 ± 0.23 ^e
<i>Sempervivum for you</i>	7.62 ± 0.37 ^a
<i>Sempervivum ronsdorfer hybride</i>	10.22 ± 0.56 ^d
<i>Sempervivum celon</i>	6.16 ± 0.35 ^h
<i>Sempervivum mystic</i>	7.10 ± 0.19 ^a
<i>Sempervivum elva</i>	5.74 ± 0.07 ^h
<i>Sempervivum grandiflorum from nufene pass</i>	5.37 ± 0.10 ⁱ
<i>Jovibarba arenaria</i>	6.08 ± 0.26 ^h
<i>Jovibarba arenaria ostriol</i>	9.72 ± 0.02 ^j
<i>Sempervivum koko flanel</i>	7.84 ± 0.15 ^a
<i>Sempervivum noir</i>	6.10 ± 0.30 ^h
<i>Sempervivum manuel</i>	8.92 ± 0.45 ^b

Sempervivum havendijker teufel

7.16 ± 0.15^a

Sempervivum gamma

8.41 ± 0.29^b

Table 4 The results of the microelement content of the examined houseleek varieties and their standard deviation values (mg/kg DW), different letters (a, b, c, d, e, f, g, h, i, j, k, l, m, n and o) denote significant differences ($p \leq 0.05$)

Houseleek species	B	Cu	Fe	Mn	Zn
<i>Sempervivum tectorum</i> var. <i>pyreneum</i>	24.90 ± 4.78 ^a	12.95 ± 1.87 ^a	24.35 ± 7.91 ^a	54.96 ± 0.10 ^a	23.31 ± 1.55 ^a
<i>Sempervivum pacardian</i>	18.63 ± 2.82 ^{a,c}	7.67 ± 2.88 ^b	17.80 ± 5.78 ^{a,b}	101.6 ± 1.55 ^b	26.77 ± 2.50 ^a
<i>Sempervivum orostachys spinosa</i>	31.38 ± 4.13 ^b	10.92 ± 1.21 ^b	52.45 ± 1.38 ^c	148.00 ± 2.39 ^c	85.74 ± 1.35 ^b
<i>Sempervivum pilatus</i>	21.17 ± 5.03 ^a	15.88 ± 1.46 ^c	35.22 ± 2.13 ^d	20.67 ± 12.97 ^d	31.09 ± 10.89 ^{a,c,d,e,h}
<i>Sempervivum calcareum</i>	31.58 ± 3.85 ^b	11.28 ± 5.52 ^{a,b,c}	36.39 ± 10.63 ^d	84.30 ± 2.81 ^e	42.86 ± 2.58 ^c
<i>Sempervivum arachnoideum webbianum aureum</i>	19.71 ± 1.33 ^a	30.75 ± 2.10 ^d	51.44 ± 4.72 ^c	80.63 ± 0.64 ^f	40.10 ± 0.31 ^c
<i>Sempervivum hey-hey</i>	17.64 ± 0.63 ^c	12.43 ± 5.55 ^{a,b,c}	25.47 ± 2.30 ^a	59.24 ± 2.32 ^g	23.19 ± 1.52 ^a
<i>Sempervivum granat</i>	20.49 ± 1.26 ^a	24.00 ± 0.93 ^e	34.31 ± 0.69 ^d	27.95 ± 0.49 ^d	34.14 ± 4.79 ^d
<i>Sempervivum reinhard</i>	15.75 ± 5.51 ^{a,c,d}	8.42 ± 6.60 ^{a,b,c}	21.64 ± 4.99 ^{a,b}	71.54 ± 0.28 ^h	30.57 ± 2.49 ^{d,e}
<i>Sempervivum for you</i>	20.16 ± 3.81 ^a	20.12 ± 2.58 ^f	40.05 ± 4.26 ^{d,e}	58.72 ± 3.31 ^g	32.77 ± 0.97 ^{d,e}
<i>Sempervivum ronsdorfer hybride</i>	22.56 ± 0.38 ^a	11.99 ± 0.76 ^{a,b}	24.07 ± 2.02 ^a	55.04 ± 6.27 ^{a,g}	36.13 ± 2.52 ^{a,g}
<i>Sempervivum celon</i>	16.06 ± 0.91 ^{a,d}	15.07 ± 1.62 ^c	19.75 ± 3.16 ^b	63.97 ± 2.20 ⁱ	29.24 ± 1.88 ^{d,e}
<i>Sempervivum mystic</i>	15.09 ± 0.53 ^d	7.43 ± 1.64 ^b	13.57 ± 6.20 ^b	91.44 ± 2.19 ^j	16.03 ± 0.94 ^f

<i>Sempervivum elva</i>	26.47 ± 2.32 ^a	12.24 ± 2.87 ^a	29.18 ± 0.77 ^a	36.57 ± 1.43 ^k	24.67 ± 2.57 ^a
<i>Sempervivum grandiflorum from nufene pass</i>	14.78 ± 0.90 ^{d,e}	17.78 ± 3.56 ^{c,f}	53.14 ± 3.77 ^c	67.86 ± 0.39 ^l	39.72 ± 0.98 ^h
<i>Jovibarba arenaria</i>	28.11 ± 2.65 ^{a,b,f}	14.67 ± 2.99 ^{a,c}	28.59 ± 1.82 ^a	26.97 ± 2.10 ^d	31.66 ± 2.84 ^{d,e}
<i>Jovibarba arenaria ostirol</i>	23.29 ± 0.76 ^a	19.77 ± 3.05 ^{c,f}	41.57 ± 5.51 ^{d,e}	31.28 ± 1.86 ^m	43.54 ± 1.67 ^c
<i>Sempervivum koko flanel</i>	18.00 ± 0.40 ^c	22.74 ± 3.54 ^{c,f}	58.82 ± 3.55 ^c	60.68 ± 2.86 ^{g,j}	33.13 ± 0.56 ^{d,e}
<i>Sempervivum noir</i>	16.34 ± 1.68 ^{c,d}	19.89 ± 4.83 ^{c,e,f}	33.23 ± 0.99 ^d	110.4 ± 0.38 ⁿ	37.90 ± 0.92 ^h
<i>Sempervivum manuel</i>	23.20 ± 0.71 ^a	8.57 ± 0.10 ^b	28.85 ± 2.67 ^c	81.95 ± 0.90 ^f	29.78 ± 0.31 ^{d,e}
<i>Sempervivum havendijker teufel</i>	10.19 ± 1.52 ^g	29.13 ± 3.27 ^d	67.04 ± 1.25 ^f	60.93 ± 0.59 ^g	38.71 ± 0.67 ^h
<i>Sempervivum gamma</i>	21.62 ± 1.27 ^a	10.53 ± 0.71 ^b	15.90 ± 3.99 ^b	94.77 ± 0.95 ^o	36.87 ± 0.96 ^{d,g}

Table 5 The results of the macroelement content of the examined houseleek varieties and their standard deviation values (g/kg DW), different letters (a, b, c, d, e, f, g, h, i, j, k and l) denote significant differences ($p \leq 0.05$)

Houseleek species	Ca	K	Mg	Na	S	P
<i>Sempervivum tectorum</i> var. <i>pyreneum</i>	71.43 ± 0.32 ^a	11.32 ± 0.32 ^a	8.96 ± 0.74 ^{ab}	73.96 ± 1.02 ^a	3.92 ± 1.06 ^a	3.39 ± 0.60 ^{a,c,f,g}
<i>Sempervivum pacardian</i>	71.13 ± 0.23 ^a	11.50 ± 4.06 ^{a,j}	9.25 ± 0.17 ^b	42.84 ± 5.97 ^b	3.08 ± 1.12 ^{a,d}	2.18 ± 0.02 ^{b,e}
<i>Sempervivum orostachys spinosa</i>	95.27 ± 1.84 ^b	21.29 ± 1.11 ^{b,c}	13.51 ± 2.69 ^{c,f}	128.5 ± 1.40 ^c	8.57 ± 1.37 ^b	5.37 ± 0.39 ^c
<i>Sempervivum pilatus</i>	45.50 ± 13.32 ^{c,e,g,i}	20.43 ± 2.41 ^b	7.05 ± 2.85 ^{a,e}	77.81 ± 9.10 ^{a,f}	2.47 ± 1.68 ^{a,d}	4.10 ± 0.73 ^a
<i>Sempervivum calcareum</i>	79.70 ± 3.71 ^d	19.36 ± 1.81 ^c	10.97 ± 4.93 ^{c,d,f}	120.6 ± 5.41 ^d	6.36 ± 1.38 ^{b,c,e}	3.92 ± 0.66 ^{a,c,f,g}
<i>Sempervivum arachnoideum webbianum aureum</i>	59.07 ± 0.55 ^e	39.43 ± 0.44 ^d	10.70 ± 1.04 ^{c,d}	93.18 ± 3.91 ^e	5.44 ± 1.46 ^c	6.05 ± 0.63 ^c
<i>Sempervivum hey-hey</i>	63.37 ± 1.37 ^f	16.27 ± 0.18 ^e	9.71 ± 1.48 ^{a,b}	82.21 ± 4.96 ^f	3.67 ± 1.71 ^{a,d}	2.52 ± 0.22 ^{d,e}
<i>Sempervivum granat</i>	49.60 ± 0.60 ^c	10.41 ± 1.27 ^{f,g}	8.24 ± 0.36 ^a	43.51 ± 0.30 ^b	4.48 ± 1.53 ^{a,c}	2.24 ± 0.49 ^{d,e}
<i>Sempervivum reinhard</i>	65.98 ± 6.14 ^f	12.68 ± 6.35 ^{a,c,g,h,i}	8.12 ± 0.29 ^a	53.10 ± 1.03 ^g	2.29 ± 0.26 ^d	2.88 ± 0.27 ^{d,e,f}
<i>Sempervivum for you</i>	55.07 ± 0.61 ^g	14.26 ± 3.98 ^{e,i}	7.53 ± 1.44 ^{a,e}	71.35 ± 6.72 ^{a,f}	3.85 ± 1.52 ^{a,d}	2.40 ± 0.92 ^{e,f}
<i>Sempervivum ronsdorfer hybride</i>	65.55 ± 7.38 ^f	14.41 ± 4.17 ^{e,j}	8.65 ± 4.39 ^{a,b,c,d,e}	24.07 ± 2.08 ^h	4.40 ± 2.30 ^{a,c,d}	2.57 ± 0.87 ^{e,f}
<i>Sempervivum celon</i>	75.99 ± 1.43 ^d	12.19 ± 0.64 ^a	9.67 ± 1.49 ^{a,b,c,d}	50.06 ± 5.68 ^{b,g,j}	3.36 ± 1.37 ^{a,d}	2.41 ± 0.57 ^{d,e,f}
<i>Sempervivum mystic</i>	84.88 ± 0.86 ^b	11.50 ± 2.91 ^a	12.47 ± 1.16 ^{c,d,f}	31.79 ± 3.39 ⁱ	1.83 ± 0.63 ^d	2.27 ± 0.25 ^{d,e}

<i>Sempervivum elva</i>	59.57 ± 2.84 ^e	17.47 ± 1.75 ^{e,h}	8.34 ± 1.68 ^{a,b}	49.23 ± 3.70 ^{b,g}	2.96 ± 0.67 ^{a,d}	2.95 ± 0.02 ^f
<i>Sempervivum grandiflorum from nufene pass</i>	45.94 ± 1.05 ⁱ	18.57 ± 1.60 ^h	8.12 ± 1.66 ^{a,b}	58.48 ± 3.96 ^j	4.61 ± 0.86 ^{a,c}	2.84 ± 0.40 ^f
<i>Jovibarba arenaria</i>	50.55 ± 1.23 ^c	12.83 ± 1.63 ^{a,i}	5.82 ± 1.31 ^e	45.65 ± 5.45 ^b	8.33 ± 1.18 ^b	1.88 ± 0.86 ^{d,e,f}
<i>Jovibarba arenaria ostirol</i>	49.01 ± 1.74 ^c	15.65 ± 0.93 ^e	6.25 ± 1.68 ^c	42.28 ± 3.02 ^b	9.89 ± 1.27 ^b	3.12 ± 0.24 ^g
<i>Sempervivum koko flanel</i>	48.62 ± 0.75 ^c	21.79 ± 2.86 ^b	6.99 ± 0.85 ^c	36.59 ± 1.52 ^{a,k}	5.04 ± 0.94 ^e	3.25 ± 0.25 ^g
<i>Sempervivum noir</i>	80.28 ± 0.26 ^d	14.22 ± 2.98 ^{e,i}	12.15 ± 0.65 ^f	57.62 ± 4.22 ^j	4.13 ± 1.22 ^a	2.82 ± 0.23 ^{d,e,f,g}
<i>Sempervivum manuel</i>	63.41 ± 0.06 ^f	13.44 ± 0.53 ⁱ	8.37 ± 0.11 ^a	38.25 ± 0.63 ^k	6.08 ± 0.08 ^e	3.60 ± 0.59 ^{a,e,f,g}
<i>Sempervivum havendijker teufel</i>	44.43 ± 1.10 ⁱ	25.53 ± 0.14 ^j	8.73 ± 0.96 ^{a,b}	61.59 ± 1.12 ^l	2.94 ± 0.21 ^{a,d}	3.62 ± 0.56 ^{a,e,f,g}
<i>Sempervivum gamma</i>	66.52 ± 0.36 ^f	11.39 ± 2.27 ^a	9.39 ± 0.91 ^{a,b,c,d}	31.63 ± 4.77 ⁱ	4.52 ± 0.98 ^{a,d}	2.46 ± 0.78 ^{d,e,f,g}

Table 6 The results of the antioxidant content (mg AAE / g DW) of the houseleek varieties and the associated standard deviation values, in the case of different drying methods, different letters (a, b, c, d, e, f, g, h, i, j, k, l and m) denote significant differences ($p \leq 0.05$)

Houseleek species	Total antioxidant content	
	of lyophilized samples (mg AAE/g DW)	of oven dried samples (mg AAE/g DW)
<i>Sempervivum tectorum</i> var. <i>pyreneum</i>	305.87 ± 1.99 ^a	294.55 ± 4.77 ^a
<i>Sempervivum pacardian</i>	122.52 ± 3.16 ^b	252.57 ± 8.10 ^b
<i>Sempervivum orostachys spinosa</i>	41.45 ± 1.53 ^c	7.42 ± 0.11 ^c
<i>Sempervivum pilatus</i>	259.34 ± 5.51 ^d	147.24 ± 7.02 ^d
<i>Sempervivum calcareum</i>	50.51 ± 2.11 ^e	6.30 ± 0.18 ^e
<i>Sempervivum arachnoideum webbianum aureum</i>	140.75 ± 15.94 ^f	2.22 ± 0.26 ^f
<i>Sempervivum hey-hey</i>	240.88 ± 9.47 ^g	178.01 ± 7.15 ^g
<i>Sempervivum granat</i>	295.04 ± 8.08 ^h	191.75 ± 9.39 ^g
<i>Sempervivum reinhard</i>	294.63 ± 27.08 ^h	202.15 ± 34.31 ^{g,h}
<i>Sempervivum for you</i>	279.86 ± 8.19 ^h	220.61 ± 5.64 ^h
<i>Sempervivum ronsdorfer hybride</i>	154.59 ± 1.70 ^f	298.35 ± 9.76 ^a
<i>Sempervivum celon</i>	267.82 ± 2.66 ⁱ	214.69 ± 38.46 ^{g,h}

<i>Sempervivum mystic</i>	291.24 ± 20.78 ^{h,j}	273.70 ± 4.41 ⁱ
<i>Sempervivum elva</i>	157.07 ± 4.26 ^f	224.95 ± 5.53 ^{h,i}
<i>Sempervivum grandiflorum from nufene pass</i>	213.19 ± 7.70 ^g	251.79 ± 12.28 ^{h,i}
<i>Jovibarba arenaria</i>	150.10 ± 9.70 ^f	92.71 ± 1.38 ^j
<i>Jovibarba arenaria ostriol</i>	56.71 ± 1.77 ^j	55.34 ± 3.15 ^k
<i>Sempervivum koko flanel</i>	120.75 ± 3.77 ^k	232.98 ± 30.49 ^h
<i>Sempervivum noir</i>	55.01 ± 6.22 ^j	187.96 ± 9.89 ^g
<i>Sempervivum manuel</i>	317.74 ± 7.88 ^l	305.91 ± 1.62 ^l
<i>Sempervivum havendijker teufel</i>	227.99 ± 5.80 ^m	147.10 ± 4.63 ^d
<i>Sempervivum gamma</i>	316.34 ± 6.84 ^l	283.62 ± 10.80 ⁱ

Table 7 The results of the polyphenol content (mg GAE / g DW) of the houseleek varieties and the associated standard deviation values, in the case of different drying methods, different letters (a, b, c, d, e, f, g, h, i, j, k, l, m, n and o) denote significant differences ($p \leq 0.05$)

Houseleek species	Total polyphenol content	
	of lyophilized samples (mg GAE/g DW)	of oven dried samples (mg GAE/g DW)
<i>Sempervivum tectorum</i> var. <i>pyreneum</i>	111.75 ± 3.87 ^a	144.16 ± 7.53 ^a
<i>Sempervivum pacardian</i>	112.27 ± 0.93 ^a	119.20 ± 3.86 ^b
<i>Sempervivum orostachys spinosa</i>	19.75 ± 1.80 ^b	6.07 ± 0.69 ^c
<i>Sempervivum pilatus</i>	81.66 ± 1.33 ^c	52.97 ± 1.70 ^d
<i>Sempervivum calcareum</i>	21.43 ± 0.59 ^b	5.52 ± 0.41 ^e
<i>Sempervivum arachnoideum webbianum aureum</i>	58.24 ± 1.51 ^d	5.63 ± 0.45 ^e
<i>Sempervivum hey-hey</i>	80.01 ± 4.48 ^c	66.16 ± 1.95 ^f
<i>Sempervivum granat</i>	90.07 ± 3.29 ^{e,j}	72.65 ± 8.37 ^{f,g}
<i>Sempervivum reinhard</i>	87.55 ± 1.58 ^c	81.55 ± 1.91 ^{g,j}
<i>Sempervivum for you</i>	89.95 ± 2.07 ^{e,j}	76.01 ± 3.48 ^g
<i>Sempervivum ronsdorfer hybride</i>	126.45 ± 3.66 ^f	97.78 ± 3.97 ^h

<i>Sempervivum celon</i>	78.36 ± 2.41 ^c	80.71 ± 2.45 ^s
<i>Sempervivum mystic</i>	101.17 ± 2.52 ^s	122.71 ± 5.85 ^b
<i>Sempervivum elva</i>	57.00 ± 1.64 ^d	83.66 ± 0.29 ^j
<i>Sempervivum grandiflorum from nufene pass</i>	71.63 ± 0.51 ^h	91.75 ± 1.82 ^j
<i>Jovibarba arenaria</i>	52.65 ± 4.90 ⁱ	36.30 ± 0.99 ^k
<i>Jovibarba arenaria ostriol</i>	24.07 ± 1.25 ^j	17.90 ± 0.90 ^l
<i>Sempervivum koko flanel</i>	46.68 ± 2.71 ^k	46.13 ± 0.96 ^m
<i>Sempervivum noir</i>	21.78 ± 0.56 ^b	31.23 ± 2.34 ⁿ
<i>Sempervivum manuel</i>	103.84 ± 5.36 ^s	107.23 ± 4.98 ^o
<i>Sempervivum havendijker teufel</i>	83.26 ± 4.39 ^c	66.33 ± 3.82 ^f
<i>Sempervivum gamma</i>	96.79 ± 5.00 ^l	112.97 ± 3.74 ^o

Table 8 Antioxidant (mg AAE/mL) and polyphenol (mg GAE/mL) content of plant decoctions made from houseleek species, with the corresponding standard deviation values, different letters (a, b, c, d, e, f, g, h, i, and j) denote significant differences ($p \leq 0.05$)

Houseleek species	Total antioxidant content of		Total Polyphenol content of	
	houseleek decoctions (mg AAE/mL)	houseleek decoctions (mg GAE/mL)	houseleek decoctions (mg AAE/mL)	houseleek decoctions (mg GAE/mL)
<i>Sempervivum tectorum</i> var. <i>pyreneum</i>	2.88 ± 0.02 ^a	1.11 ± 0.08 ^a		
<i>Sempervivum pacardian</i>	2.67 ± 0.06 ^b	0.20 ± 0.03 ^{b,d}		
<i>Sempervivum orostachys spinosa</i>	2.64 ± 0.04 ^b	0.16 ± 0.04 ^b		
<i>Sempervivum pilatus</i>	3.03 ± 0.06 ^c	0.02 ± 0.01 ^c		
<i>Sempervivum calcareum</i>	2.67 ± 0.18 ^{b,g}	0.26 ± 0.05 ^d		
<i>Sempervivum arachnoideum webbianum aureum</i>	4.61 ± 0.56 ^d	0.08 ± 0.02 ^e		
<i>Sempervivum hey-hey</i>	3.11 ± 0.03 ^e	0.90 ± 0.05 ^f		
<i>Sempervivum granat</i>	2.83 ± 0.01 ^b	0.88 ± 0.05 ^f		
<i>Sempervivum reinhard</i>	2.62 ± 0.01 ^f	0.05 ± 0.03 ^{e,e}		
<i>Sempervivum for you</i>	2.73 ± 0.05 ^g	0.11 ± 0.03 ^{b,e}		
<i>Sempervivum ronsdorfer hybride</i>	2.59 ± 0.02 ^h	0.05 ± 0.02 ^c		
<i>Sempervivum celon</i>	2.52 ± 0.03 ⁱ	0.02 ± 0.01 ^c		

<i>Sempervivum mystic</i>	2.93 ± 0.04 ^c	0.02 ± 0.01 ^c
<i>Sempervivum elva</i>	2.67 ± 0.03 ^b	0.03 ± 0.01 ^c
<i>Sempervivum grandiflorum from nufene pass</i>	2.81 ± 0.05 ^c	0.02 ± 0.01 ^c
<i>Jovibarba arenaria</i>	2.69 ± 0.11 ^b	0.08 ± 0.01 ^c
<i>Jovibarba arenaria ostriol</i>	2.72 ± 0.01 ^s	0.03 ± 0.01 ^c
<i>Sempervivum koko flanel</i>	3.00 ± 0.22 ^c	0.08 ± 0.02 ^c
<i>Sempervivum noir</i>	2.38 ± 0.03 ^j	0.04 ± 0.01 ^c
<i>Sempervivum manuel</i>	2.96 ± 0.18 ^c	0.13 ± 0.01 ^s
<i>Sempervivum havendijker teufel</i>	3.40 ± 0.52 ^c	0.45 ± 0.05 ^b
<i>Sempervivum gamma</i>	3.03 ± 0.05 ^c	0.85 ± 0.15 ^f

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