

# Determination of Biochemical Properties and Nutrient Element Contents in Cultivated *Allium* Species [*Allium schoenoprasum* L., *Allium vineale* L. and *Allium scorodoprasum* L. subsp. *rotundum* (L.) Stearn]

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**Abstract:** This study aimed to comparatively determine the biochemical characteristics, Dualex indices, and macro-micro nutrient element contents of three *Allium* species naturally distributed in Türkiye and cultivated under ex situ conditions. The plant material consisted of *Allium schoenoprasum* L., *Allium scorodoprasum* subsp. *rotundum* (L.) Stearn, and *Allium vineale* L., grown in the Medicinal and Aromatic Plants Garden of Van Yüzüncü Yıl University (Van, Türkiye). During the vegetation period, leaf samples were collected and analyzed for total phenolic content, total flavonoid content, total antioxidant activity, total ash, total dry matter, Dualex indices [nitrogen balance index (NBI), chlorophyll, flavonoid, and anthocyanin], and macro-micro nutrient elements using standard biochemical and instrumental methods. The results indicated that total ash ranged between 7.04-13.93% and total dry matter between 31.22-33.25%. Total flavonoid content varied from 16.66 to 17.60 mg QE 100 g<sup>-1</sup>, total phenolic content from 169.44 to 181.52 mg GAE g<sup>-1</sup>, and total antioxidant activity from 75.90 to 91.68 µmol TE g<sup>-1</sup>. Dualex measurements showed NBI values between 24.20-125.40 dx, chlorophyll 25.67-33.90 dx, flavonoid 0.27-1.69 dx, and anthocyanin 0.05-0.09 dx. When evaluated within each *Allium* species, it was determined that the concentrations of potassium, calcium, magnesium, zinc, and copper metals were higher in *Allium scorodoprasum* subsp. *rotundum* compared to the concentrations of other species. In biochemical content analyses, it was found that *Allium vineale* L. species had higher values. Overall, the findings demonstrate significant interspecific variation in nutritional and phytochemical traits, suggesting that *A. vineale* may be prioritized for phytochemical utilization and *A. scorodoprasum* subsp. *rotundum* for mineral nutrition studies and breeding programs.

**Keywords:** Antioxidant, geophyt, dualex index, onion, Van

## 1. Introduction

Türkiye hosts more than 12.000 plant taxa, 3.649 of which (a ratio of 3:1) are endemic (Şenkul and Kaya, 2017). In Türkiye, the *Allium* L. genus comprises 196 taxa, 60 of which are endemic species (Şelem et al., 2020). Ethnobotanical studies have revealed that wild *Allium* species have been collected from their natural habitats and consumed as food for generations (Pandey and Tripathi, 2017). Around thirty wild *Allium* species are used as vegetables, spices and ornamental plants, as well as for medicinal purposes. Ethnobotanical studies conducted in Türkiye have revealed that approximately 16 *Allium* taxa are used in the

production of herbed cheese. Various plants are utilised in herbed cheese production, with *Allium* species being preferred due to their taste, aroma, and natural growth (Tunçtürk and Tunçtürk, 2020). The leaves, stems, or bulbs of the plants are used in herbed cheese production.

People commonly use it for medical purposes such as earache relief, parasite treatment, hemorrhoids, deworming, expectorant, relief of rheumatic pains, diabetes, hypertension, infertility, and flu-like infections (Ekşi et al., 2020). Consumption of *Allium* species has also been found to reduce cancer risk compared to the control group (Valentina et al., 2016). *Allium* species generally

contain glucose, sucrose, saponins, cyanidin, quercetin (a flavonoid derivative), vitamins A, B, C and E, organic acids and sulphur-containing volatile compounds such as cysteine, propyl, isoalliin and alliin (Baytop, 1999). Considering their nutritional content, they offer local people an alternative food option in their natural habitats.

*Allium schoenoprasum* L., also known as the chive, is a perennial herbaceous plant found in eastern Anatolia. It grows at altitudes of 2.000-3.300 metres in alpine meadows, pastures, calcareous areas and along stream banks (Anonymous, 2024). It has been established that *A. schoenoprasum* (chives), a popular ingredient in herbed cheeses, exhibits various therapeutic properties (Shirshova et al., 2014; Deliorman et al., 2016; Zeng et al., 2017). It is rich in vitamins A and C, and contains small amounts of iron and sulphur. Due to its insect-repellent properties, it has the potential to be used as a biological warfare agent (Çelik et al., 2008).

*Allium scorodoprasum* L. subsp. *rotundum* (L.) Stearn is a perennial herbaceous plant found in northern and eastern Anatolia. It grows at altitudes of 0-1.400 metres in grassy areas, meadows and on calcareous and clayey slopes (Anonymous, 2024). The leaves and bulbs of *A. scorodoprasum* can be consumed raw or cooked and used as a spice. As well as being used as a food source, it is also employed in the prevention of diabetes. Studies have indicated that its bulb has antifungal, antiviral, antibacterial, and antioxidant properties. Pharmacological studies have utilised the antitumour, antihypertensive, antimicrobial, anti-obesity, diuretic and hepatoprotective properties of *A. scorodoprasum* (Tasci and Koca, 2015).

*Allium vineale* L. is a perennial herbaceous plant found in northwestern and central Anatolia. It grows at altitudes of 25-2.650 metres in alpine meadows, swamps and riverbeds (Anonymous, 2024). *Allium vineale* is widely used in traditional medicine and is one of the preferred species for cheese production thanks to its taste, aroma, and antibacterial properties (Firat, 2015; Firat and Aziret, 2016).

In this study, the biochemical contents, dual indices, and macro and micro-nutrient element contents of three commonly used *Allium* species (*A. schoenoprasum*, *A. vineale* and *A. scorodoprasum* subsp. *rotundum*) in herbed cheese production were aimed to be determined.

## 2. Materials and Methods

### 2.1. Plant material

The study material consists of *A. schoenoprasum*, *A. scorodoprasum* subsp. *rotundum* ve *A. vineale* species grown in the Medicinal and Aromatic Plants Garden of Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crops. This garden is located in 1680 m altitude. The coordinates are 38°33'46.21" N and 43°17'51.29" E. The soil properties of the area where the plant was cultivated were as follows: texture sandy-loamy, pH 7.65, total salt 8.8  $\mu\text{S cm}^{-1}$ , organic matter 0.94%. Leaves from each species were used for the analysis. Samples were taken from six plants of each species. Samples were taken in the first week of May in the second year after planting.

### 2.2. Determination of ash, dry matter, heavy metal, and nutrient contents

The total ash, dry matter, and mineral contents [including macro elements such as magnesium (Mg), calcium (Ca), and potassium (K), and micro elements such as manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe)] were analyzed. Dry matter content was determined by drying the samples in an oven at 105 °C for 24 hours. Total ash (inorganic matter) was measured using an electric muffle furnace operating at 550 °C. For mineral analysis, dried plant samples were incinerated and then treated with analytical-grade hydrochloric and nitric acids, following the Anonymous (1990) guidelines. The digested samples were diluted with distilled water to a final volume of 50 mL in a volumetric flask. All analyses were conducted in triplicate, and certified reference materials were used for accuracy in chemical analyses. Potassium, Ca, Mg, and Fe levels were determined using Atomic Absorption Spectrometry (AAS), while the concentrations of Mn, Zn, and Cu were measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

### 2.3. Total antioxidant, total phenolic and total flavonoids content

The total phenolic content was determined following the method described by Obanda and Owuor (1997). Antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay as outlined by Benzie and Strain (1996), with absorbance measured at 593 nm. Results were expressed as trolox equivalents (TE)

per milligram. The total flavonoid content was evaluated with slight modifications based on the procedure developed by Quettier-Deleu et al. (2000). Absorbance was recorded at 415 nm, and the flavonoid concentration was calculated as milligrams of quercetin equivalent (QE) per 100 grams of dry matter, using a calibration curve prepared with standard quercetin.

#### 2.4. Determination of nitrogen balance index, chlorophyll, anthocyanin and flavonoid

The nitrogen balance index (NBI), chlorophyll, flavonoid and anthocyanin content were measured on the leaf non-destructively using and in real time the Dualex scientific+ (FORCE-A, France) device before harvesting. The species were measured in the field using Dualex before the leaves were removed. These measurements were taken on the second leaf of each plant.

All analyses were conducted in triplicate, and standard deviations were calculated for the results. In the tables, values are given as mean  $\pm$  standard deviation (M $\pm$ SD). The obtained data were statistically analysed using COSTAT software. Duncan's multiple comparison test was used to evaluate the differences between the means.

### 3. Results

The total ash and dry matter ratio table is given in Table 1. The obtained data revealed that the total

ash and dry matter ratio in *A. schoenoprasum*, *A. scorodoprasum* subsp. *rotundum*, and *A. vineale* species were determined as 12.07 and 32.67%, 7.04 and 31.22%, and 13.93 and 33.25%, respectively (Table 1).

In terms of biochemical contents, *A. vineale* has exhibited the highest values across all parameters. While *A. scorodoprasum* subsp. *rotundum* has shown the lowest value in terms of total flavonoid content and total antioxidant activity, *A. schoenoprasum* has displayed the lowest value in terms of total phenolic content (Figure 1). The highest and lowest values were determined as follows: 16.66-17.08 mg QE 100 g<sup>-1</sup> for total flavonoid content, 169.44-181.52 mg GAE (Galic acid equivalent) g<sup>-1</sup> for total phenolic content, and 75.90-91.68  $\mu$ mol TE g<sup>-1</sup> for total antioxidant activity (Table 2, Figure 1).

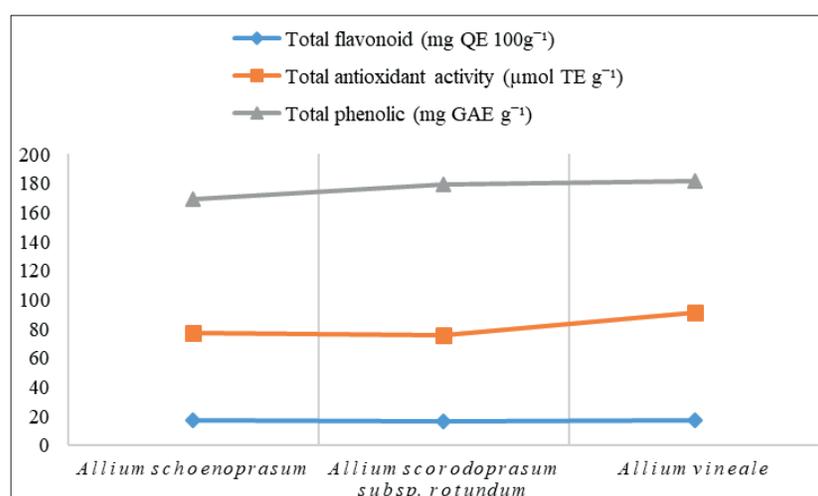
The dualex values, including NBI, chlorophyll, flavonoid, and anthocyanin, were determined to be 125.40 dx, 33.90 dx, 0.27 dx, and 0.09 dx, respectively, in *A. schoenoprasum*, 83.57 dx, 25.67 dx, 0.31 dx, and 0.07 dx, respectively, in *A. vineale*, 24.2 dx, 47.4 dx, 1.96 dx and 0.05 dx respectively in *A. scorodoprasum* subsp. *rotundum* (Table 2).

The amounts of macro-nutrient elements, including K, Ca, and Mg, were found to be highest in *A. scorodoprasum* subsp. *rotundum* species and lowest in *A. vineale* species. These ratios were determined within the ranges of 14.83-39.32 g kg<sup>-1</sup>

**Table 1.** The total ash and dry matter ratio of *Allium* species\*

	<i>A. schoenoprasum</i> (M $\pm$ SD)	<i>A. scorodoprasum</i> subsp. <i>rotundum</i> (M $\pm$ SD)	<i>A. vineale</i> (M $\pm$ SD)	CV	F value
Total ash (%)	12.07 $\pm$ 0.48 a	7.04 $\pm$ 0.66 b	13.93 $\pm$ 0.52 a	9.08	57.16**
Dry matter (%)	32.67 $\pm$ 0.39 a	31.22 $\pm$ 0.46 b	33.25 $\pm$ 0.48 a	1.79	1.07**

\*: Differences between means indicated by the same letter on the same line are not statistically significant, CV: Coefficient of variation, \*\*: p<0.01



**Figure 1.** Biochemical contents of *Allium* species

**Table 2.** The Dualex values of *Allium* species\*

Dualex values	<i>A. schoenoprasum</i> (M±SD)	<i>A. scorodoprasum</i> subsp. <i>rotundum</i> (M±SD)	<i>A. vineale</i> (M±SD)	CV	F value
Total flavonoid (mg QE 100g <sup>-1</sup> )	17.6±1.34	16.66±0.59	17.08±2.61	2.69	0.97
Total antioxidant (μmol TE g <sup>-1</sup> )	76.94±4.04 b	75.9±1.85 b	91.68±1.84 a	1.41	233.67**
Total phenolic (mg GAE g <sup>-1</sup> )	169.44±6.89 c	179.44±0.31 b	181.52±1.09 a	4.02	1.247**
NBI	125.40±19.17 a	24.20±9.10 c	83.57±5.85 b	3.10	683.72**
Chlorophyll	33.90±1.63 b	47.40±1.02 a	25.67±1.66 c	1.61	180.54**
Flavonoid	0.27±0.04 c	1.96±0.04 a	0.31±0.01 b	1.76	27901**
Anthocyanin	0.09±0.01 a	0.05±0.01 c	0.07±0.03 b	8.24	18.29**

\*: Differences between means indicated by the same letter on the same line are not statistically significant, CV: Coefficient of variation, \*\*: p<0.01

for K, 4.07-10.10 kg<sup>-1</sup> for Ca, and 1.94-3.88 kg<sup>-1</sup> for Mg (Table 3, Figure 2).

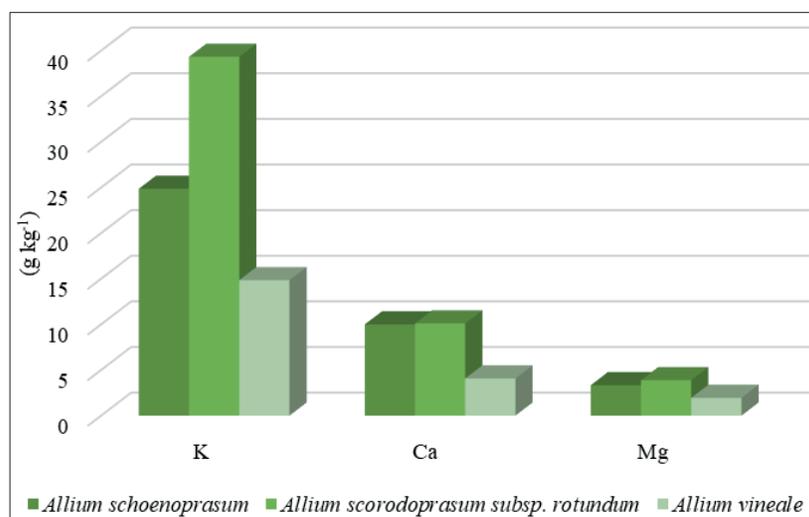
The micro-nutrient elements (Fe, Zn, Cu, and Mn) were determined as follows: for *A. schoenoprasum*, 377.80 mg kg<sup>-1</sup>, 29.22 mg kg<sup>-1</sup>,

6.95 mg kg<sup>-1</sup>, and 39.01 mg kg<sup>-1</sup> respectively; for *A. scorodoprasum* subsp. *rotundum*, 108.69 mg kg<sup>-1</sup>, 37.22 mg kg<sup>-1</sup>, 22.05 mg kg<sup>-1</sup>, and 33.75 mg kg<sup>-1</sup> respectively; and for *A. vineale*, 151.01 mg kg<sup>-1</sup>, 20.85 mg kg<sup>-1</sup>, 2.26 mg kg<sup>-1</sup>, and 16.61 mg kg<sup>-1</sup> respectively (Table 3, Figure 3).

**Table 3.** The amounts of macro and micro nutrient elements of *Allium* species\*

Macro-micro elements	<i>A. schoenoprasum</i> (M±SD)	<i>A. scorodoprasum</i> subsp. <i>rotundum</i> (M±SD)	<i>A. vineale</i> (M±SD)	CV	F value
K (g kg <sup>-1</sup> )	24.86±1.28 b	39.32±0.39 a	14.83±1.37 c	9.05	454.38**
Ca (g kg <sup>-1</sup> )	9.99±3.26 a	10.10±0.42 a	4.07±0.29 b	7.06	107.07**
Mg (g kg <sup>-1</sup> )	3.33±0.03 b	3.88±0.39 a	1.94±0.16 c	1.70	485.97**
Fe (mg kg <sup>-1</sup> )	377.80±14.83 a	108.69±1.27 c	151.01±8.09 b	2.44	1847.71**
Zn (mg kg <sup>-1</sup> )	29.22±0.40 b	37.22±0.17 a	20.85±0.02 c	1.87	596.84**
Cu (mg kg <sup>-1</sup> )	6.95±0.23 b	22.05±1.33 a	2.26±0.11 c	4.98	478.84**
Mn (mg kg <sup>-1</sup> )	39.01±2.51 a	33.75±1.47 b	16.61±0.33 c	1.93	205.80**

\*: Differences between means indicated by the same letter on the same line are not statistically significant, CV: Coefficient of variation, \*\*: p<0.01

**Figure 2.** The amounts of macro-nutrient elements of *Allium* species

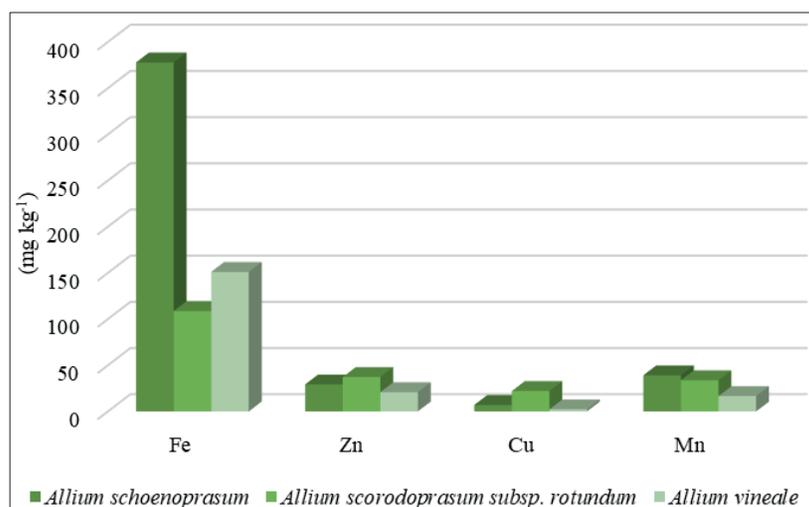


Figure 3. The amounts of micro-nutrient elements of *Allium* species

#### 4. Discussion and Conclusion

Significant differences in biochemical and mineral content were found among the three *Allium* species examined in this study. Specifically, *A. vineale* exhibited elevated levels of total phenolic content, flavonoid content, and antioxidant activity, suggesting that this variety is more prolific in the synthesis of secondary metabolites. Indeed, researchers have reported similar high phenolic and antioxidant values in *A. vineale* samples collected from the Van region (Cakmakci et al., 2022). Furthermore, it has been reported that there are differences in phenolic content between species and even between populations within species, which may be due to genetic factors as well as soil properties, microclimatic conditions.

The study found that *A. schoenoprasum* had a relatively high NBI value (125.40 dx), but low flavonoid values, compared to other species. This suggests that, despite high nitrogen use efficiency, secondary metabolite production is limited. The dual values for *Oenothera biennis* L. plants were determined by Nohutcu et al. (2024) to be 12.86 dx for NBI, 25.20 dx for chlorophyll, 1.97 dx for flavonol, and 0.03 dx for anthocyanin. Uçar et al. (2023) determined NBI, chlorophyll, flavonol and anthocyanin values in *Salvia officinalis* L. plants subjected to various bacterial treatments as 26.32-45.98 dx, 21.32-25.44 dx, 0.54-0.71 dx and 0.050-0.074 dx, respectively.

Analyses of different organs of *A. schoenoprasum* have demonstrated that the highest antioxidant activity is exhibited in the leaves, although other parts of the plant also display notable antioxidant capacity (Štajner et al., 2011). Similarly, another study reported that chive, wild mint, stinging nettle, shepherd's purse, and mint-

herbs frequently used in the preparation of herbed cheese-possess antioxidant properties to varying degrees and exhibit strong antimicrobial activity (Dağdelen, 2010).

Koca et al. (2015) reported that the shoots of *A. scorodoprasum* are rich in phenolic compounds, with flavonoid content measured at 14.95 mg RE g<sup>-1</sup>. Similarly, Mitic et al. (2014) compared the total phenolic and flavonoid contents of seed and bulb methanolic extracts, noting that the bulb exhibited higher levels of these compounds. Tasci et al. (2016) further emphasized that different organs of *A. scorodoprasum* possess distinct therapeutic properties, with flower extracts demonstrating strong antioxidant activity associated with high phenolic content (27.69 mg GAE g<sup>-1</sup>). In another study, Tasci and Koca (2015) determined that the flowers of *A. scorodoprasum* subsp. *rotundum* contained total phenolics ranging from 11.54 to 13.79 mg g<sup>-1</sup>, free radical scavenging activity of 47.23-54.86 μmol TE g<sup>-1</sup>, total anthocyanin content of 4.50-34.47 mg 100 g<sup>-1</sup>, and DPPH and FRAP values of 430.88-545.66 μmol Fe<sup>2+</sup> g<sup>-1</sup>.

In a related study, Tegin et al. (2019) reported that *A. vineale* exhibited a total phenolic content of 58.60% and DPPH radical scavenging activity of 12.00 μg mL<sup>-1</sup>. They also highlighted that elemental analysis revealed high levels of P, Fe, Na, K, and Mg-elements known to play a crucial role in the formation of pigments such as chlorophyll and carotenoids (Yakit and Tuna, 2006).

Ninfali et al. (2007) reported that the biologically active compound contents of leeks (mg 100 g<sup>-1</sup> FW) were 41.6 ± 4.0 for total phenols, 10.10 ± 1.02 for flavonoids, and 1.01 ± 0.09 for flavanols, while the mean oxygen radical absorbance capacity (ORAC) was 490 ± 43 μmol TE 100 g<sup>-1</sup>. Beşirli et

al. (2007) determined the dry matter content of onions (*A. cepa*) to be 12 g 100 g<sup>-1</sup>. In addition, the ash content was reported to range from 1.005 to 1.454 in Tunceli garlic (*A. tuncelianum*) and from 1.1021 to 1.5673 in Kastamonu garlic (*A. sativum*) (Takim, 2020). While there are quality criteria based on the ash content in foods, no study has been encountered regarding the determination of quality based on the ash content in garlic. It should be noted that a high ash content in foods is not always considered a positive result. For example, a high ash content in spices indicates the presence of additives (Takim, 2020). Considering the literature, it has been observed that the species are rich in ash and dry matter. Umaz et al. (2021) stated that many countries worldwide have determined acceptable maximum element concentration values differently in raw plant materials and foods. A general evaluation is made based on the values of the World Health Organization (Umaz et al., 2021). Considering the examined elements, they were found to be within the relevant range. The ash content obtained in the study ranged from 7.04% to 13.93%. These values are consistent with the ash content reported in other *Allium* species and are indicative of high mineral content. For example, a similar study on *A. cepa* and *A. sativum* reported ash contents of 5.58% and 3.71%, respectively (Abiola et al., 2017). In their study, Kefale et al. (2023) determined the total ash content to be 6.7% for red pepper, 3.37% for red onion, 6.33% for ginger, and 3.34% for garlic. The high ash contents were particularly pronounced in *A. vineale* and *A. schoenoprasum*, reflecting differences in the species' capacity to uptake minerals from the soil.

Umaz et al. (2021) determined the metal content of *A. pseudoampeloprasum* species as follows: K: 42190, Mg: 2667, Fe: 380, Cu: 3.60, Zn: 45.91, Mn: 43.54 mg kg<sup>-1</sup>, and the metal content of *A. shatakiense* species as: K: 35190, Mg: 2073, Fe: 214, Cu: 5.19, Zn: 59.90, Mn: 29.41 mg kg<sup>-1</sup>. In another study conducted on wild leek (*E. spectabilis*), the mineral element content of this plant was determined using an ICP-MS device. The mineral element contents of this plant were found to be: Ca: 76.00, Fe: 2.42, Mg: 15.23, P: 42.80, Zn: 0.36, K: 263, Na: 1.48, and Cu: 0.08 mg 100 g<sup>-1</sup> (Cinar et al., 2017). It has been reported that the metal contents of some geophyte species collected from different parts of Türkiye vary as follows: K: 19718-60066, Fe: 196-2285, Cu: 3.60-18.58, Mn: 29.41-91.89, Zn: 34.44-68.14, Mg: 1757-3506 mg kg<sup>-1</sup> (Umaz et al., 2021). The elemental data obtained from the conducted study were found to be within the relevant range. In the study, it was determined that *A. scorodoprasum* exhibited higher contents of K (14.83-39.32 g kg<sup>-1</sup>), Ca (4.07-10.10

g kg<sup>-1</sup>), and Mg (1.94-3.88 g kg<sup>-1</sup>) compared to the other species. This finding reveals that *A. scorodoprasum* is a mineral-rich species. The fact that *A. scorodoprasum* subsp. *rotundum* exhibited higher levels of K, Ca, Mg, Zn, and Cu compared to other species points to genetic differences such as mineral uptake capacity and root structure. The local consumption of this species, known as "sirmo," as a nutrient-rich food demonstrates the overlap between scientific evidence and traditional knowledge.

In the microelement analyses, the Fe content of *A. schoenoprasum* (377.80 mg kg<sup>-1</sup>) was found to be significantly higher than that of the other species. In the literature, it has been emphasized that the iron-rich nature of certain species increases their potential use as functional foods against anemia (Umoh et al., 2014). *Allium scorodoprasum* was distinguished particularly by its Zn (37.22 mg kg<sup>-1</sup>) and Cu (22.05 mg kg<sup>-1</sup>) levels. Zinc and copper act as cofactors in numerous enzymatic processes and play critical roles in antioxidant defense mechanisms (Abiola et al., 2017). Therefore, this species can be considered to have significant nutritional and medicinal potential. In contrast, *A. vineale* exhibited lower values for these microelements. However, previous studies have shown that *A. vineale* contains high levels of phenolic compounds and flavonoids (Demirtas et al., 2013). Thus, despite being relatively poor in minerals, this species stands out due to its biochemical composition. The biochemical and mineral diversity observed in *A.* species arises not only from genetic differences but also from environmental factors such as soil composition, pH, moisture, and microclimatic conditions.

The *Allium* species traditionally used in Van herby cheese are notable not only for their aromatic properties but also for their antimicrobial effects. For instance, in cream cheeses enriched with *A. roseum*, yeast and mold growth was inhibited, thereby extending shelf life (Gliquem et al., 2021). Similarly, the antimicrobial effects of different *Allium* species used in Van herby cheeses are known to enhance the product's durability.

The findings of the study indicate that *A. scorodoprasum* stands out with its mineral content, *A. schoenoprasum* with its richness in Fe, and *A. vineale* with its high levels of phenolic and flavonoid compounds. Thus, each species possesses distinct nutritional and functional advantages, establishing a strong link between traditional usage practices and scientific evidence.

Accordingly, the results demonstrate that the biochemical and mineral diversity observed in *Allium* species is primarily shaped by genetic

differences among the taxa, and that this diversity contributes to their functional properties and cultural significance within local food traditions. In particular, the selection and cultivation of *A. vineale* genotypes with high biochemical content may offer significant potential for both functional food production and the preservation of cultural heritage. The use of these species in the preparation of herby cheese in Anatolia, and especially in the Van region, illustrates how their biochemical richness is reflected in the local food culture in harmony with traditional knowledge. Studies on naturally growing *Allium* species have particularly focused on certain species. It is believed that conducting studies on other *Allium* species will enrich the literature.

### Ethical Statement

The authors declare that ethical approval is not required for this research.

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This research received no external funding.

### Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

### Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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