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Research article

Edible flowers of wild allium species: Bioactive compounds, functional activity and future prospect as biopreservatives

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Abstract: Edible flowers are rich in phenolic compounds with antioxidant and antimicrobial properties. Fresh flower extracts of four wild underutilized allium species (i.e., *Allium narcissiflorum* Vill., *Allium schoenoprasum* L., *Allium sphaerocephalon* L., and *Allium ursinum* L.) were analyzed to assess their potential applications in the food industry. *Allium narcissiflorum* exhibited the highest total phenolic content, together with *A. schoenoprasum*, the highest anthocyanin content, and the highest antioxidant activity. Conversely, *A. ursinum* generally showed the lowest values. The analysis of specific phenolic compounds, including cinnamic acids, benzoic acids, flavonols, and flavanols, and vitamin C further differentiated the allium species. *Allium narcissiflorum* showed high content of coumaric and ferulic acid. *Allium schoenoprasum* was the richest in quercetrin and epicatechin, and showed a high content of quercetin and coumaric acid. *Allium sphaerocephalon* was the richest in ellagic acid and showed a high content of quercetin. *Allium ursinum* was the richest in hyperoside and rutin and showed a high content of ferulic acid; moreover, this species was the only one in which gallic acid was detected. Antibacterial properties were tested against two ubiquitous bacteria strains associated with food spoilage (i.e., *Bacillus cereus* and *Bacillus subtilis)*. While *A. sphaerocephalon* was totally ineffective, the other three species showed a similar antibacterial activity against both strains. Allium flower extracts inhibited *B. cereus* less than the synthetic antibiotic chloramphenicol, and *A. narcissiflorum* and *A. ursinum* were effective against *B. subtilis* as much as chloramphenicol. The results revealed the potential of fresh edible allium flowers to be used as functional food and biopreservatives.

Keywords: *Allium ursinum*; *Allium sphaerocephalon*; *Allium narcissiflorum*; *Allium schoenoprasum*; antioxidant activity; antibacterial activity

1. Introduction

Wild edible plants are non-cultivated species that are typically collected from natural environments (e.g., forests, scrubland, and grassland) for human consumption, particularly in times of conflict or famine, and have recently enjoyed a resurgence in popularity [1]. These plants are rich in nutrients and phytochemicals that offer health benefits, including phenolic compounds, vitamins, and minerals, which have led to their renewed use as functional foods [2]. For their antioxidant and antimicrobial properties, phenolic-rich foods are increasingly valued in dietary planning [3]. Different parts of the plants, such as fruits, flowers, leaves, and roots, can be edible [4]. Particularly, the flowers, as the reproductive organs of the plant, can contain a different spectrum of bioactive compounds compared to leaves, bulbs, or other vegetative tissues. Flowers often produce specialized secondary metabolites that play key roles in pollinator attraction and reproductive success. These include a wide variety of volatile organic compounds responsible for floral scent, as well as pigments, such as anthocyanins, flavonoids, and carotenoids, that contribute to coloration [5]. Unlike leaves, which typically accumulate higher levels of chlorophyll and photosynthetic-related compounds, flowers may have lower chlorophyll content but higher levels of specific phenolic compounds, nectar sugars, and essential oils [6]. Furthermore, flowers can also accumulate defensive compounds to protect reproductive tissues from herbivores and pathogens [7]. These differences in chemical composition not only influence the organoleptic properties (taste and aroma) of edible flowers but also their potential nutritional and medicinal properties [8]. In addition, flower extracts have been shown to have antimicrobial effects, likely due to inhibitory substances targeting specific microorganisms [8]. This characteristic is particular interesting as the use of natural compounds in food production is becoming popular as alternatives to combat multidrug resistance, aligning with consumer demand for "green label foods" [9]. Plant extracts, considered generally recognized as safe (GRAS), offer a natural alternative to synthetic antimicrobials with broad-spectrum antimicrobial properties, cultural significance, low toxicity, and high availability, making them suitable as biopreservatives [10]. For example, *Hibiscus rosa-sinensis* L. flowers exhibit antibacterial effects on several Gram-positive and Gram-negative foodborne pathogens, possibly due to polyphenols, flavonoids, and tannins [11].

The genus *Allium* (Amaryllidaceae family) is one of the largest among flowering plants, comprising over 800 perennial rhizomatous or bulbous species, mainly distributed in temperate or boreal regions of the Northern Hemisphere [12,13]. Allium species have long been used for their health-promoting properties due to their high content of bioactive compounds, such as flavonoids, phenolic acids, anthocyanins, saponins, phytosterols, and organosulfur compounds [14,15]. Only a few species are commonly used in cooking, including garlic (*Allium sativum* L.), onion (*Allium cepa* L.), leeks (*Allium porrum* L.), and shallots (*Allium ascalonicum* L.), primarily for their bulbs and leaves, which are known for their bioactive and antimicrobial properties due to polyphenols and organosulfur compounds like allicin [12,16].

The edible flowers of certain allium species also contain significant concentrations of phytochemicals, including flavonoids, carotenoids, chlorophylls, and vitamin E [5]. These species have shown various bioactivities, such as antibacterial (*A. sativum*, *A. ampeloprasum* L., *A.*

ascalonicum, *A. cepa*, *A. roseum* L.), fungicidal (*A. ascalonicum*, *A. sativum*), anticarcinogenic (*A. sativum*, *A. schoenoprasum* L., *A. ampeloprasum*), and anti-inflammatory (*A. cepa*), as well as the ability to reduce cholesterol and support cardiovascular health [17–21]. However, only a few allium flowers, such as *A. schoenoprasum* and *A. ursinum* L., are traditionally consumed in Europe [17].

Allium schoenoprasum (chives) is a circumboreal species common in the Alps, mainly in marshes, peatlands, and wet meadows, from 600 to 2600 m above sea level [22,23]. Its flowers bloom from May to July, depending on local climate conditions and altitude. The individual flowers are small, campanulate, and measure approximately 5–7 mm in diameter, with tepals about 7–15 mm long. The inflorescence forms a compact, spherical umbel that can reach a diameter of 1.5–5 cm. A review on edible flowers [24] mentions their flowers having zesty onion taste. Although primarily the leaves are used in cooking, research has identified phenolic compounds like gallic, coumaric, and ferulic acids in the flowers, with ferulic acid contributing to the species' anti-proliferative properties, which may aid in tumour prevention [25].

Allium ursinum (ramsons) is widespread in Eurasian broadleaf forests, thriving in shady, moist, humus-rich soils from 0 to 800 m above sea level [22,23]. Known for its antioxidant effects, primarily due to its high phenolic content [5,26], ramsons can help neutralize reactive oxygen species. *Allium ursinum* flowers in spring, typically from April to June, sending up leafless stems of 15–40 cm topped with loose umbels of 13–20 (up to 30) star-shaped white flowers 1.9–5.4 cm across. Each tepal measures about 0.8–1 cm in length. Demasi et al. [6] described the taste of its flowers, noting that the intensity of their smell and garlic aroma was very strong, and that the flowers were easy to chew. The leaves, with a garlicky aroma, are consumed raw or cooked. This species has traditionally been used to help prevent gastrointestinal, cardiovascular, and respiratory conditions [16].

Other minor allium species in Europe may also have phytochemically rich flowers, such as *A. narcissiflorum* Vill. and *Allium sphaerocephalon* L. *Allium narcissiflorum* (Piedmontese garlic), located in limited areas of the Western Alps, is a subendemic species that can be found in a limited area of the Western Alps, including the provinces of Imperia, Cuneo, Turin and Aosta in Italy, and in the Isère, Drôme, Hautes-Alpes, Alpes-de-Haute-Provence, Vaucluse, and Alpes-Maritimes in France. It is a perennial bulbous geophyte species, 15 to 40 cm tall. It is found mainly in rocky environments with an altitudinal distribution of mountain, subalpine and alpine type, from 800 to 2600 m a.s.l. [22,23]. It produced compact umbels in late spring to early summer, with flowers roughly 8–12 mm in diameter. It is known for containing saponins, phenols, and coumarins in its above-ground parts [27]. *Allium sphaerocephalon* (round-headed leek) is a steno-mediterranean and paleotemperate species, widespread throughout the Italian territory. It is a perennial bulbous geophyte species, 20-100 cm tall that blooms in late spring to early summer (May–July), producing slender scapes 50–90 cm tall, topped with dense, egg- to spherical umbels approximately 2.5 to 3.8 cm wide composed of numerous tiny florets. It is commonly found on rocky slopes, arid soils, fallow land, and vineyards with an altitudinal distribution of hilly to subalpine type, from 0 to 1900 m a.s.l. [22,23]. and is noted for its antioxidant and mild bactericidal properties [28].

Given the well-known health-promoting properties of the genus *Allium* and the scarcity of information about allium edible flowers, this study deepened the knowledge of four allium species investigating their flower's antioxidant and antibacterial properties. Together with two species traditionally consumed, i.e., *A. schoenoprasum* and *A. ursinum*, analyses involved two other neglected species (i.e, *A. narcissiflorum* and *A. sphaerocephalon)*. Information could be helpful to assess their potential as functional food and biopreservatives.

2. Materials and methods

2.1. Plant material

The fresh edible flowers of four wild allium species, namely *A. narcissiflorum*, *A. schoenoprasum*, *A. sphaerocephalon*, and *A. ursinum*, were harvested in the Piedmont region (north-west Italy) (Figure 1). For each species, three samplings of about 35 g each of fresh flowers at full flowering stage were performed in the collection sites reported in (Table 1). The flowers were placed in sealed polyethylene bags, immediately stored at 4 ℃ in a portable refrigerator and transported to the laboratory for analysis.

Figure 1. Flowers of *A. narcissiflorum* (A), *A. schoenoprasum* (B), *A. sphaerocephalon* (C), and *A. ursinum* (D).

Table 1. Characteristics of the collection sites of the four wild allium species.

Species	Collection site (Italy)	Latitude (WGS84/32N)	Longitude (WGS84/32N)	Elevation a.s.l.	Sampling period
A. narcissiflorum Vill.	Colle Fauniera, Demonte (CN)	4916388.555	350379.309	2473	July
A. schoenoprasum L.	Chiappi, Castelmagno (CN)	4917348.024	350854.385	2293	July
A. sphaerocephalon L.	Pian della Mussa, Balme (TO)	5018116.863	357840.662	1749	June
A. ursinum L.	Terre Ballerine, Montalto Dora (TO)	5038206.091	412439.786	310	April

2.2. Extract preparation

Fresh flowers of each species were grinded with mortar and pestle and liquid nitrogen. For each species, three replicate samples were taken from the same batch of fresh flowers. The obtained flower

powders were stored at −80 °C until ultrasound extraction. One gram of flower powder was extracted with 50 mL of a water:methanol solution (1:1), at room temperature with an ultrasound extractor (Sarl Reus, Drap, France) at 23 kHz for 15 min [5]. The obtained phytoextract was filtered through one layer of filter paper (Whatman No. 1, Maidstone, UK), then with a 0.45 mm PVDF syringe filter (CPS Analitica, Milano, Italy), and then maintained at −20 °C until analyses.

2.3. Bioactive compounds and antioxidant activity

2.3.1. Total phenolic content, total anthocyanin content, and antioxidant activity

Allium phytoextracts were evaluated via colorimetric methods as described by Demasi et al. [5], using a Cary 60 UV-Vis spectrophotometer (Agilent, Santa Clara, CA, USA).

Total phenolic content (TPC) was analysed following the Folin–Ciocalteu method. Briefly, an amount of 1000 µL of diluted (1:10) Folin reagent (Scharlab, Lodi (LO), Italy) was mixed with 200 µL of allium extract. Following a 10-minute incubation in the dark at room temperature, 800 µL of Na_2CO_3 (7.5%) were added to each sample. Samples were then kept in the dark at room temperature for an additional 30 minutes. Absorbance readings were taken at 765 nm. The results were compared with a calibration curve constructed using gallic acid as a standard (1.95–250 mg/L) and expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg GAE/100 g FW).

Total anthocyanin content (TAC) was estimated by the pH differential method. Briefly, each sample was diluted in two 10 mL volumetric flasks (1 mL of sample for each flask), each containing a different buffer solution: one adjusted to pH 1.0 (KCl and HCl – 25 mM) and the other to pH 4.5 ($C_2H_3NaO_2$ and $C_2H_4O_2$ – 0.4 M). Absorbance readings were taken at both 510 nm and 700 nm. Absorbance (A) was calculated as follows:

$$A = (A510\ nm - A700\ nm)\ pH\ 1.0 - (A510\ nm - A700\ nm)\ pH\ 4.5 \quad (1)$$

Then, the TAC of each extract was as follows:

$$TAC = [A \times MW \times DF \times 1000] \times 1/\varepsilon \times 1 \quad (2)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-Oglucoside (449.2 D), DF is the dilution factor (25), ε is the molar extinction coefficient of cyanidin-3-glucoside (26.900), and results were presented in milligrams of cyanidin-3-O-glucoside per 100 g (mg C3G/100 g FW).

Antioxidant activity (AOA) was analysed using three assays: the ferric reducing antioxidant power (FRAP) method, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay.

The FRAP solution was prepared by mixing a buffer solution at pH 3.6 ($C_2H_3NaO_2$ + $C_2H_4O_2$ in water), 2,4,6-tripyridyltriazine (TPTZ, 10 mM in HCl 40 mM; Sial, Darmstadt, Germany), and $FeCl_3 \cdot 6H_2O$ (20 mM). To assess the antioxidant activity, 30 µL of allium extract were mixed with 90 µL of deionized water and 900 µL of FRAP reagent. The samples were then incubated at 37 °C for 30 minutes, after which absorbance readings were taken at 595 nm. The antioxidant activity was plotted against a $FeSO_4 \cdot 7H_2O$ calibration curve (100–1000 µmol/L), and values were expressed as millimoles of ferrous iron equivalents per kilogram of fresh weight (mmol Fe^{2+}/kg FW).

The working solution of DPPH radical cations ($DPPH^{\cdot}$, 100 µM; Aldrich, Darmstadt, Germany) was prepared by dissolving 2 mg of DPPH in 50 mL of Methanol (MeOH). The absorbance at 515 nm

of the DPPH$^{\cdot}$ solution was adjusted to a value of 1000 (±0.005) by adding MeOH. Then, 20 µL of allium extract (or extraction solvent for the control) were mixed with 1.5 mL of the DPPH$^{\cdot}$ radical solution. The samples were then incubated in the dark at room temperature for 30 minutes, after which absorbance readings were taken at 515 nm. The radical scavenging activity was calculated as follows:

$$[(Abs_0 - Abs_1)/Abs_0] \times 100 \quad (3)$$

where Abs_0 is the absorbance of the control sample and Abs_1 is the absorbance of the sample containing the allium extract. The values were plotted against a Trolox calibration curve (0.0078–1 µg/µL) and values were expressed as micro moles of Trolox Equivalents per 1 g of fresh weight (µmol TE/g FW).

The ABTS radical cation ($ABTS^+$; Aldrich, Darmstadt, Germany) solution was obtained by the reaction of 2.45 mM $K_2S_2O_8$ and 7.0 mM ABTS; incubation for 12–16 h in the dark at room temperature followed. The resulting $ABTS^+$ was then diluted with deionised water to achieve an absorbance of 0.70 (± 0.02) at 734 nm. For the assay, 2 mL of diluted $ABTS^+$ were mixed with 30 µL of the allium extract. The absorbance readings were taken at 734 nm after 10 min in the dark at room temperature. The radical scavenging activity was calculated as described for the DPPH assay. The values were plotted against a Trolox calibration curve (0.002–1 µg/µL) and values were expressed as micro moles of Trolox Equivalents per 1 g of fresh weight (µmol TE/g FW).

2.3.2. Phenolic profile and vitamin C

The presence of specific bioactive compounds in allium flowers' phytoextracts was investigated by means of high-performance liquid chromatography (HPLC) with diode array detection (DAD) (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). Compound separation was performed through a Kinetex C18 column (4.6 × 150 mm, 5 mm, Phenomenex, Torrance, CA, USA) with different mobile phases, according to previously validated protocols (Table 2) [5]. Compounds were identified through the comparison of their retention times and UV spectra with those of the analytical standards with the same chromatographic conditions. Fifteen compounds were investigated, namely phenolic acids (cinnamic acids: caffeic, chlorogenic, coumaric, and ferulic acids; benzoic acids: ellagic and gallic acids); flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin); flavanols (catechin and epicatechin); and vitamin C (ascorbic and dehydroascorbic acids). Results were expressed as mg/100 g FW. Analytical HPLC grade solvents (acetonitrile, methanol, and formic acid), reagents for HPLC buffer (potassium dihydrogen phosphate and phosphoric acid) were purchased from Fluka Biochemika (Buchs, Switzerland) and Sigma–Aldrich (St Louis, MO, USA). All polyphenolic standards with HPLC purity (caffeic acid ≥98%, chlorogenic acid ≥95%, coumaric acid ≥98%, ferulic acid ≥99%, hyperoside ≥97%, isoquercitrin ≥98%, quercetin ≥95%, quercitrin ≥98%, rutin ≥95%, ellagic acid ≥95%, gallic acid ≥97%, catechin ≥97% and epicatechin ≥98%) were purchased from Sigma–Aldrich. Milli-Q ultrapure water was produced by Sartorius Stedim Biotech mod. Arium (Sartorius, Göettingen, Germany). Stock solutions of cinnamic acids and flavonols with a concentration of 1.0 mg mL^{-1} were prepared in methanol. From these solutions, four calibration standards (1000 ppm, 50 ppm, 250 ppm, 125 ppm) were prepared by dilution with methanol; stock solutions of benzoic acids, tannins, and catechins with a concentration of 1.0 mg mL^{-1} were prepared in a solution of 95% methanol and 5% water. From these solutions, four calibration standards were prepared by dilution with 50% methanol-water.

Table 2. Mobile phases, elution conditions and wavelength used to detect the five classes of compounds with HPLC analysis.

Classes of compounds	Mobile Phase	Elution conditions	Wavelenght (nm)
Cinnamic acids Flavonols	A: 10 mM KH_2PO_4/H_3PO_4, pH = 2.8 B: CH_3CN	5%B to 21%B in 17 min + 21%B in 3 min (2 min conditioning time); flow: 1.5 mL min^{-1}	330
Benzoic acids and Flavanols	A: $H_2O/CH_3OH/HCOOH$ (5:95:0.1 *v/v/v*), pH = 2.5 B: $CH_3OH/HCOOH$ (100:0.1 *v/v*)	3%B to 85%B in 22 min + 85%B in 1 min (2 min conditioning time); flow: 0.6 mL min^{-1}	280
Vitamin C	A: 5 mM $C_{16}H_{33}N(CH_3)_3Br$/50 mM KH_2PO_4, pH = 2.5 B: CH_3OH	Isocratic, ratio of phase A and B: 95:5 in 10 min (5 min conditioning time); flow: 0.9 mL min^{-1}	261, 348

HPLC, High-Performance Liquid Chromatography.

2.4. Antimicrobial activity

2.4.1. Bacterial strains

The antimicrobial activity was tested using the following bacterial strains: *Bacillus cereus* (ATCC 11778) and *Bacillus subtilis* (ATCC 6051), purchased from Microbiologics (St. Cloud, Minnesota, US). These bacteria were selected as they are ubiquitous and associated with food spoilage. Strains were cultured following manufacturer instructions, inoculated in Brian Hearth Infusion Broth (BHI-Brain Heart Infusion -VWR International - Leuven Belgium) and incubated for 24 h at 37 °C. After growth, all strains were cryobanked in glycerol solution and then diluted until the concentration of 7 Log (CFU)/mL used for the preparation of the BHA plates. Each plate was inoculated with 9 mL of melted culture media (52 °C) and 1 mL of bacterial culture, reaching a final concentration of 6 Log (CFU)/mL.

2.4.2. Antibacterial activity assay

The agar diffusion method was used as an evaluation method. For each plate, three wells of 0.8 cm were cut in agar. One well was filled with 20 µL of sterilized water as negative control, one was filled with 20 µL of chloramphenicol as positive control, and the latter was filled with 20 µL of flower extract (at a concentration of 8 g per 25 mL of deionized water). Plates were then incubated at 37 °C and inhibition zone (cm) of each well were measured after 48h. Each inhibition zone was measured in 3 directions. In addition, each combination of allium species and bacterial strain was tested in triplicate.

2.5. Statistical analysis

All data were subjected to statistical analysis of the normality and homoscedasticity through a Shapiro–Wilk test and Levene test, respectively. Species comparisons were computed using the one-way ANOVA, using the REGWF's post-hoc test by means of SPSS 28 software (version 28.0; SPSS Inc., Chicago, IL, USA). A nonparametric Kruskal–Wallis test with stepwise comparison was

performed on HPLC data where the variances were not homogeneous after data transformation, with the exception of quercitrin and ellagic acid, as per the Levene test. A principal component analysis (PCA) biplot was performed for the antioxidant assays, single phenolic compounds and antimicrobial activity by means of PAST 4.03 software. The same software was used to compute correlations among the bioactive compounds and the antioxidant activity assays, as well as among the bioactive compounds and antibacterial activities by Pearson's correlation coefficient test. Raw data from FRAP, DPPH, and ABTS of the studied allium flowers were transformed into standard scores and averaged to obtain the global antioxidant score (GAS) as described by Caser et al. [29].

3. Results and discussion

3.1. Total anthocyanin content, total phenolic content, and antioxidant activity

The four allium species showed differences in TAC, TPC, and FRAP, DPPH and ABTS assays, generally exhibiting the same pattern for each parameter (Table 3).

Table 3. Total anthocyanin content (TAC; mg C3G/100 g FW), total phenolic content (TPC; mg GAE/100 g FW), and antioxidant activity (FRAP; mmol Fe^{2+}/kg FW, DPPH; µmol TE/g FW and ABTS; µmol TE/g FW) in *A. narcissiflorum*, *A. schoenoprasum*, *A. sphaerocephalon*, and *A. ursinum* flowers. Data are expressed on a fresh weight basis (FW) and are presented as the mean value ± standard deviation.

Species	TAC	TPC	FRAP	DPPH	ABTS
A. narcissiflorum	26.63 ± 7.38a	260.20 ± 58.79a	42.62 ± 15.94a	19.29 ± 1.25a	8.07 ± 1.52a
A. schoenoprasum	7.46 ± 3.69b	232.62 ± 48.39a	26.65 ± 5.42a	9.30 ± 1.20b	9.14 ± 0.52a
A. sphaerocephalon	9.59 ± 5.54b	147.77 ± 7.89b	25.83 ± 7.57a	6.37 ± 0.23b	6.76 ± 1.08a
A. ursinum	0.00 ± 0.00c	184.44 ± 7.37ab	4.19 ± 2.76b	7.64 ± 1.69b	0.75 ± 0.52b
p	**	*	**	***	***

The statistical relevance is provided (*** = $p < 0.001$; ** $p < 0.01$; * $p < 0.05$). Different letters in the columns indicate significant differences among the subspecies according to REGWF's post-hoc test ($p < 0.05$).

Allium narcissiflorum always showed the highest values, *A. ursinum* the lowest, while the other two species presented mid properties. Both *A. schoenoprasum* and *A. sphaerocephalon* showed intermediate TAC, low DPPH, similarly to *A. ursinum*, and high FRAP and ABTS, similarly to *A. narcissiflorum*. For TPC, a trend was highlighted, with *A. schoenoprasum* like *A. narcissiflorum* and *A. sphaerocephalon* similar to *A. ursinum*.

More in detail, TAC values ranged from 0.00 to 26.63 mg C3G/100 g FW. *Allium ursinum* showed the lowest TAC value, coherently with its white flowers. In the other species, with pink to purple flowers, *A. narcissiflorum* exhibited significantly higher TAC compared to *A. schoenoprasum* and *A. sphaerocephalon*. However, it should be noted that the analytical method used does not detect all the anthocyanin compounds, including polymeric anthocyanins [30], such as cyanidin-3-laminaribioside that was found in different allium species (i.e., *A. schoenoprasum*, *A. sativum*, *A. porrum*, *A. cepa*, and *A. ursinum* [18,31]) and 3-acetylglucoside, 3-glucoside, 3-(6-malonylglucoside) and 3-(3,6-dimalonylglucoside) of cyanidin that where specifically identified in *A. schoenoprasum* flowers [32]. *Allium narcissiflorum*'s TAC resulted comparable to other edible flower species such as *Paeonia*

officinalis or *Centaurea cyanus* L. (25.96 and 34.67 mg C3C/100 g FW, respectively) [6].

For TPC (range: 147.77 - 260.20 mg GAE/100 g FW), *A. narcissiflorum* and *A. schoenoprasum* showed the highest values, *A. sphaerocephalon* the lowest, while *A. ursinum* showed intermediate values. Results of *A. ursinum* were higher than those found in a previous study by Demasi et al. [6], i.e. 99.26 mg GAE/100 g FW, while results of *A. schoenoprasum* were lower than those found by Grzeszczuk et al. [33], i.e. 375.76 mg GAE/100 g FW. The influence of the harvesting environment on the phytochemical composition of the flowers is probably responsible for these differences. In general, allium flowers showed TPC values comparable to other specie's flowers, such as *Borago officinalis* L. (163.4 mg GAE/100 g FW), *Calendula officinalis* L (189.6 mg GAE/100 g FW), *Leucanthemum vulgare* (Vaill.) Lam. (230.8 mg GAE/100 g FW) and *Robinia pseudoacacia* (203.8 mg GAE/100 g FW), but lower than very rich TPC flowers such as *Rosa pendulina* L., *Rosa canina* L., *Primula veris* L., *Dianthus carthusianorum* L., or *Paeonia officinalis* L. (1181.9, 884.44, 609.16, 470.5, 1270.7 mg GAE/100 g FW respectively) [5].

Regarding the antioxidant activity (AOA) assays, FRAP ranged from 4.19 to 42.62 mmol Fe^{2+}/kg FW, DPPH ranged from 6.37 to 19.29 µmol TE/g FW and ABTS ranged from 0.75 to 9.14 µmol TE/g FW. *Allium narcissiflorum* showed always high values across all assays (FRAP, DPPH and ABTS), being similar to other species' flowers, such as *L. vulgare* (44.3 mmol Fe^{2+}/kg FW, 20.9 µmol TE/g FW and 10.8 µmol TE/g FW, respectively) and *T. majus* (45.3 mmol Fe^{2+}/kg FW, 14.8 µmol TE/g FW and 12.8 µmol TE/g FW, respectively), but lower than *P. officinalis* (303.8 mmol Fe^{2+}/kg FW, 226.2 µmol TE/g FW and 55.2 µmol TE/g FW, respectively) and *R. canina* (257.5 mmol Fe^{2+}/kg FW, 146.2 µmol TE/g FW and 55.6 µmol TE/g FW, respectively) [5].

GAS index, integrating the different analytical methods, ranked the allium according to this gradient from high to low: *A. narcissiflorum* (0.821) > *A. schoenoprasum* (0.533) > *A. sphaerocephalon* (0.373) > *A. ursinum* (0.066) (Figure 2).

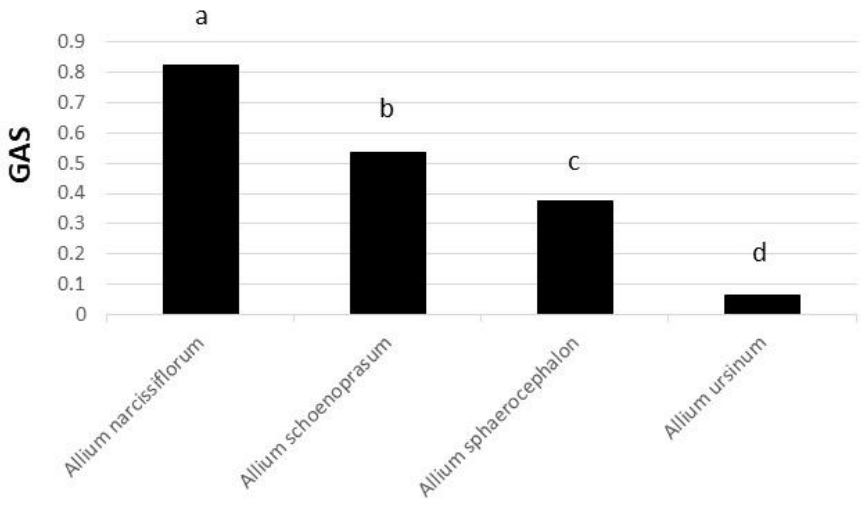

Figure 2. Global Antioxidant Score (GAS) in *A. narcissiflorum*, *A. schoenoprasum*, *A. sphaerocephalon* and *A. ursinum*. Small case letters indicate significant differences among species, according to the REGWF post hoc test ($p < 0.05$).

GAS values could represent a ranking tool of high-quality plant material useful to select plants with potential applications as functional foods. In literature, data about GAS values are available only for a few beverages (e.g., red wines; [34]) and herbs (e.g., lavenders; [29]). In a previous study, Demasi et al. [5] calculated the GAS for several edible flowers. Compared to this, allium flowers ranked low, especially compared to flowers of *P. officinalis*, *R. pendulina*, and *R. canina* (data not shown). *Allium ursinum* resulted as the species with the least antioxidant properties. While the other three allium species presented values like different wild species (i.e., *S. pratensis*, *Viola odorata* L., and *T. officinale*) and commonly cultivated food species, such as *Calendula officinalis* L., *B. officinalis,* and *T. majus*.

3.2. Bioactive compounds

All classes of the phenolic compounds investigated were found to be present (flavonols, benzoic acids, cinnamic acids, and catechins), as well as vitamin C, in varying amounts according to the species; four compounds were not detected in any of the four allium species (i.e., isoquercitrin, caffeic acid, chlorogenic acid and catechin; Table 4). Four out of five flavonols were detected (i.e., hyperoside, quercetin, quercitrin, and rutin), making them the most abundant class in the four allium species, followed by cinnamic acids (two out of four compounds detected, i.e., coumaric and ferulic acids), benzoic acids (ellagic and gallic acids), vitamin C (ascorbic and dehydroascorbic acids), and catechins (one of two compounds detected, i.e. epicatechin).

Table 4. Bioactive compounds in *A. narcissiflorum*, *A. schoenoprasum*, *A. sphaerocephalon*, and *A. ursinum* flowers. The values are given in mg/100 g of fresh weight (FW). Data are presented as mean ± standard deviation.

	Flavonols				Benzoic acid	
Species	Hyperoside	Quercetin	Quercetrin	Rutin	Ellagic acid	Gallic acid
A. narcissiflorum	8.98 ± 2.66b	0.00 ± 0.00b	5.62 ± 2.78c	0.00 ± 0.00c	10.38 ± 3.82bc	0.00±0.00b
A. schoenoprasum	0.00 ± 0.00c	156.38 ± 9.19a	104.62 ± 2.52a	6.13 ± 0.90b	5.96 ± 3.52c	0.00±0.00b
A. sphaerocephalon	0.00 ± 0.00c	170.74 ± 33.71a	77.64 ± 5.45b	0.00 ± 0.00c	25.25 ± 0.62a	0.00±0.00b
A. ursinum	38.72 ± 8.15a	0.00 ± 0.00b	0.91 ± 0.00c	19.75 ± 2.41a	15.24 ± 2.40b	0.07±0.00a
p	*	*	***	*	***	*
	Cinnamic acids		Catechins	Vitamin C		
Species	Coumaric acid	Ferulic acid	Epicatechin	Ascorbic acid	Dehydroascorbic acid	
A. narcissiflorum	99.72 ± 3.33a	362.91 ± 60.64a	84.24 ± 22.35b	3.72 ± 3.22	6.06 ± 0.34	
A. schoenoprasum	70.15 ± 36.33a	0.00 ± 0.00b	276.92 ± 107.95a	3.93 ± 3.40	5.68 ± 1.05	
A. sphaerocephalon	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00d	1.83 ± 3.16	4.86 ± 2.26	
A. ursinum	0.49 ± 0.00b	357.33 ± 31.94a	20.82 ± 0.57c	0.00 ± 0.00	0.00 ± 0.00	
p	*	*	*	ns	ns	

The statistical relevance is provided (*** = $p < 0.001$; * $p < 0.05$; ns = not significant). Different letters in the columns indicate significant differences among the subspecies according to REGWF's post-hoc test ($p < 0.05$).

Of the 11 compounds detected, eight were found in *A. narcissiflorum*, *A. schoenoprasum*, and *A. ursinum*, while five were found in *A. sphaerocephalon*, although each species displayed a unique combination of these compounds. In general, *A. narcissiflorum* showed a high level of coumaric

acid (99.7 mg/100 g FW) and ferulic acid (362.9 mg/100 g FW), along with intermediate levels of hyperoside, ellagic acid, and epicatechin, but lacked quercetin, rutin, and gallic acid. *Allium ursinum* exhibited high levels of hyperoside (38.7 mg/100 g FW), rutin (19.7 mg/100 g FW), and ferulic acid (357.3 mg/100 g FW), as well as intermediate levels of ellagic acid and traces of quercetrin, gallic acid, and coumaric acid. Quercetin was absent in its flowers. In contrast, *A. sphaerocephalon* was characterized by the highest levels of quercetin (170.7 mg/100 g FW) and ellagic acid (25.2 mg/100 g FW), intermediate levels of quercetrin, and no detectable amounts of other compounds. Meanwhile, *A. schoenoprasum* displayed elevated levels of quercitrin (104.6 mg/100 g FW), epicatechin (276.9 mg/100 g FW), quercetin (156.3 mg/100 g FW) and coumaric acid (70.1 mg/100 g FW), and low levels of rutin and ellagic acid, but other compounds were absent. In a previous study, the phenolic profile of several edible flower species showed similar or lower levels of individual compounds [5]. For example, *A. ursinum* exhibited hyperoside levels comparable to or exceeding those in *Erythronium dens-canis* and *Rosa canina* (9.0 and 38.5 mg/100 g FW, respectively), while its rutin content was superior to that of *Cichorium intybus*, *Dianthus carthusianorum*, *Geranium sylvaticum*, and *Primula veris* (13.7–18.1 mg/100 g FW). Similarly, *A. sphaerocephalon* had quercetin levels slightly lower than *Geranium sylvaticum* (189.0 mg/100 g FW). The quercetin content of *A. schoenoprasum* exceeded that of *Centaurea cyanus* (0.9 mg/100 g FW), *Dianthus carthusianorum* (8.0 mg/100 g FW), and *Calendula officinalis* (1.7 mg/100 g FW) and was comparable to *Erythronium dens-canis* (108.5 mg/100 g FW), *Primula veris* (82.3 mg/100 g FW), and *Primula vulgaris* (109.6 mg/100 g FW). In contrast to Demasi et al. [5], this study identified coumaric acid and ferulic acid, particularly present in *A. narcissiflorum*, and ellagic acid, gallic acid, and vitamin C in other allium species. Kucekova et al. [25] also analysed phenolic compounds in *A. schoenoprasum* flowers, using a 90:10 methanol:water solution, and reported higher gallic acid (20.1 mg/100 g FW) but lower coumaric acid (20.7 mg/100 g FW), ferulic acid (88.7 mg/100 g FW), and rutin (2.02 mg/100 g FW) compared to this study. In agreement with Kucekova et al. [25], our results also identified ferulic acid as the most abundant phenolic compound in the allium species. Regarding vitamin C, no significant differences were observed among the four allium species, although it was only detected in *A. narcissiflorum*, *A. schoenoprasum*, and *A. sphaerocephalon*.

3.3. Principal component analyses

PCAs were conducted to better visualize the diversity among the allium species investigated. The PCA shown in Figure 3 grouped the flowers of allium species according to their antioxidant profile based on TPC, TAC, FRAP, DPPH, ABTS, ascorbic acid, and dehydroascorbic acid content. PC1 explained almost the total variation (99.9 %) and differentiated allium species according mainly to TPC; PC2 explained 0.1% according to FRAP value. *Allium narcissiflorum* and *A. schoenoprasum* mainly separated from *A. ursinum* and *A. sphaerocephalon* by PC1, having higher TPC content.

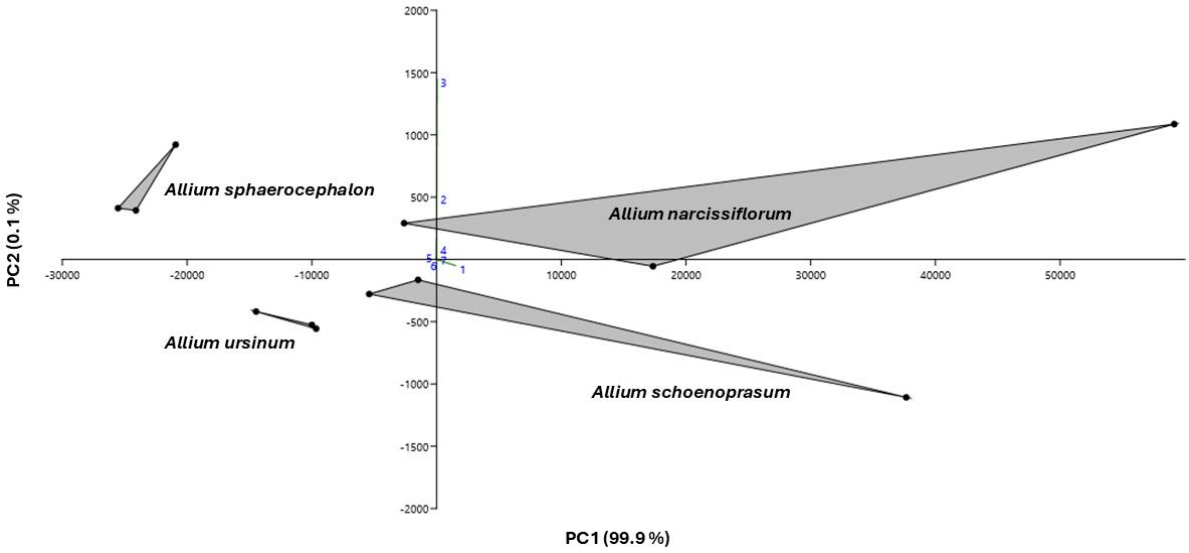

Figure 3. Principal component analysis (PCA)-biplot performed for TPC (1), TAC (2), FRAP (3) DPPH (4), ABTS (5), ascorbic acid (6), and dehydroascorbic acid (7) values of *A. narcissiflorum*, *A. schoenoprasum*, *A. ursinum*, and *A. sphaerocephalon.*

The PCA shown in Figure 4 grouped the flowers of the allium species according to the phenolic profile, based on the first two principal components (PC1 explaining 77.3 % and PC2 explaining 22.7 % of the variation). Using factor loading analysis, the best markers that separated the groups were ferulic acid, quercetin and quercitrin for PC1, and coumaric acid and epicatechin for PC2. *Allium schoenoprasum* and *A. sphaerocephalon* were clearly distinct from *A. narcissiflorum* and *A. ursinum*, having higher ferulic acid and similar traits.

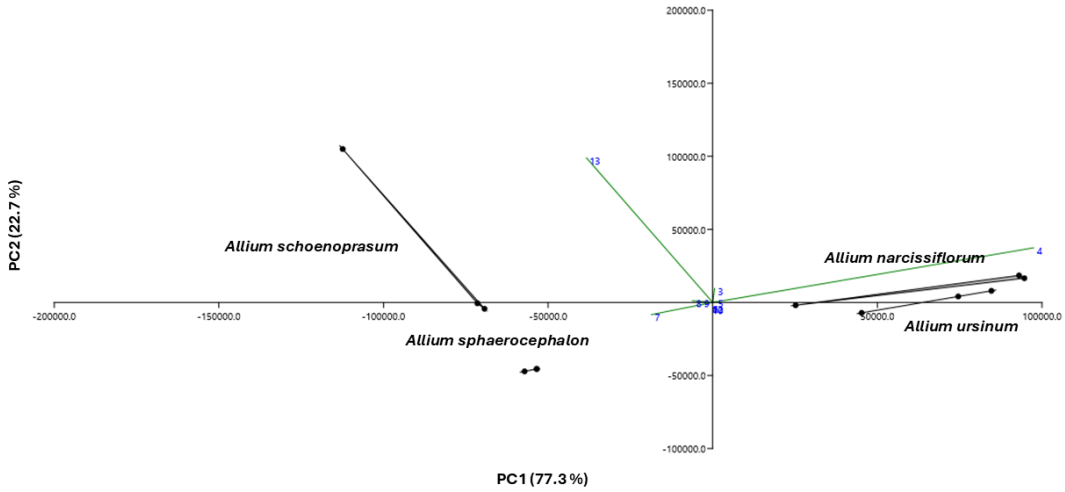

Figure 4. Principal component analysis (PCA)-biplot performed for phenolic compounds of *A. narcissiflorum*, *A. schoenoprasum*, *A. ursinum*, and *A. sphaerocephalon*, calculated and drawn using PAST 4.03 software. 1. Caffeic acid; 2. Chlorogenic acid; 3. Coumaric acid; 4. Ferulic acid; 5. Hyperoside; 6. Isoquercitrin; 7. Quercetin; 8. Quercitrin; 9. Rutin; 10. Ellagic acid; 11. Gallic acid; 12. Catechin; 13. Epicatechin.

3.4. Antimicrobial activity

All allium flower extracts showed antibacterial activity (ABA) against both bacteria tested, except for *A. sphaerocephalon* (Table 5), which was ineffective. Concerning *B. cereus*, allium flowers of *A. narcissiflorum*, *A. schoenoprasum*, and *A. ursinum* showed similar antibacterial activity, but all were less effective than the positive control (Chloramphenicol). Concerning *B. subtilis*, interestingly *A. narcissiflorum* and *A. ursinum* showed an antibacterial activity as effective as the positive control, whereas *A. schoenoprasum* lower.

Table 5. Inhibition zones (cm) of *A. narcissiflorum*, *A. schoenoprasum*, *A. sphaerocephalon*, and *A. ursinum* flower extracts on *Bacillus cereus* and *Bacillus subtilis* strains after 48h. Data are presented as the mean value ± standard deviation.

	Bacillus cereus	*Bacillus subtilis*
Species	Inhibition zone	Inhibition zone
A. narcissiflorum	0.37 ± 0.21b	0.93 ± 0.54ab
A. schoenoprasum	0.50 ± 0.29b	0.53 ± 0.31b
A. sphaerocephalon	0.00 ± 0.00c	0.00 ± 0.00c
A. ursinum	0.73 ± 0.42b	0.97 ± 0.56ab
Chloramphenicol	1.14 ± 0.57a	1.24 ± 0.62a
p	***	***

The statistical relevance is provided (*** = $p < 0.001$). Different letters in the columns indicate significant differences according to REGWF's post-hoc test ($p < 0.05$).

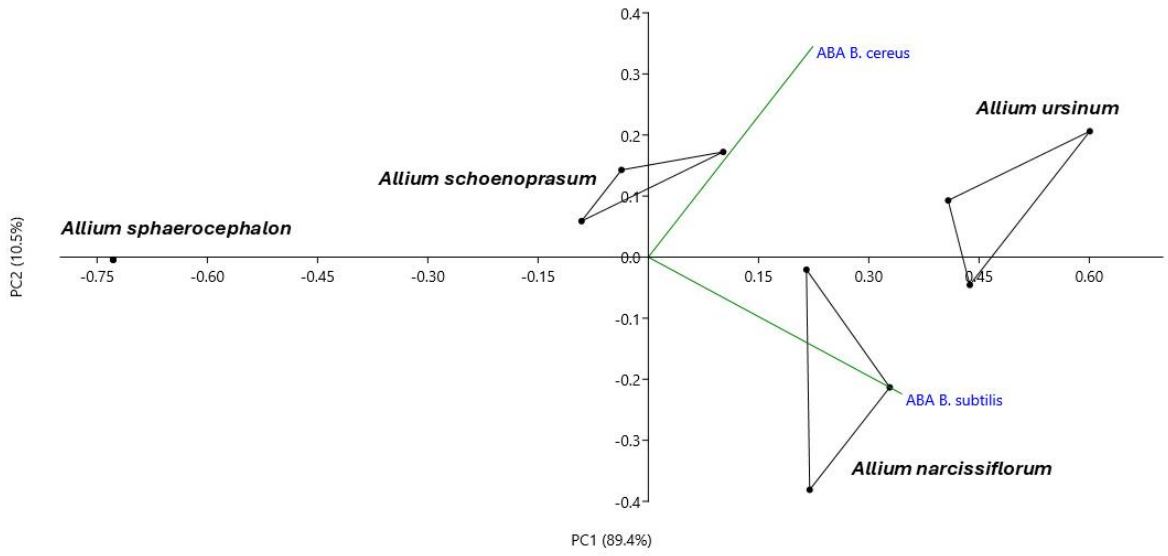

Figure 5. Principal component analysis (PCA)-biplot performed for antibacterial activity (ABA) of *A. narcissiflorum*, *A. schoenoprasum*, *A. ursinum*, and *A. sphaerocephalon* extracts on *B. cereus* and *B. subtilis*.

Figure 5 reported the PCA computed on the ABA of the flower extracts studied. PC1 explained 89.4% and PC2 10.5% of the variation, giving a total of 99.9%. *Allium narcissiflorum* and *A. ursinum*

were clearly separated from the other species for positive value of PC1. Using factor loading analysis, the best markers that separated species were both ABA *vs B. cereus* and *B. subtilis*.

The ABA *vs B. subtilis* resulted positively correlated ($p < 0.05$) with the ferulic acid content (data not shown). While no correlation was found for ABA vs *B. cereus.* Ferulic acid is reported to have a stronger antimicrobial activity against Gram-positive bacteria than Gram-negative [35]. However, Lemos and colleagues [36] observed how it had a low to moderate effect in inhibiting *B. cereus* formation on biofilm. In literature other phenolics were reported to have antibacterial activities, such as benzoic and cinnamic acids [3], ellagic acid [37], *p*-coumaric acid [38], flavonols, and catechins [39–41], but no correlations were found for them in the present work.

4. Conclusions

Based on the analyses conducted in this study, wild allium fresh flowers are rich in bioactive compounds with significant variations in phenolic content, antioxidant activity, and antibacterial properties observed among species. Notably, *A. narcissiflorum* exhibited the highest total phenolic and anthocyanin content, alongside remarkable antioxidant capacity, resulting a strong candidate for applications requiring antioxidant-rich food. Furthermore, both *A. narcissiflorum* and *A. ursinum* flowers demonstrated notable antibacterial activity, particularly against *B. subtilis*. This could be ascribed to their content in ferulic acid. Overall, *A. narcissiflorum* emerges as the best species due to its unique phytochemical profile and high content of bioactive compounds. However, its natural habitat at high altitudes poses significant challenges for its exploitation. To overcome this limitation, domestication and cultivation of this species should be addressed. This approach could not only facilitate practical applications by ensuring availability but also standardize product quality while preserving biodiversity. Interestingly, *A. ursinum* also stands out for its high antibacterial activity. Being more accessible and widespread at lower altitudes than *A. narcissiflorum*, *A. ursinum* offers a viable alternative for large-scale applications.

In conclusion, this study provided new information about antioxidant and antimicrobial properties of underutilized and neglected species, focusing on flower's polyphenolic composition. In order to better assess flower's bioactivity, future research should investigate also organosulfur compounds like allicin, which is mainly responsible for antimicrobial properties of allium bulbs and leaves, together with polyphenols. A more comprehensive phytochemical characterization could strengthen their potential applications as functional food and biopreservatives.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Matteo Caser: Data curation, Formal analysis, Writing—original draft. Nicole Mélanie Falla: Data curation, Formal analysis, Writing—original draft. Sonia Demasi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—review and editing. Daniele Nucera: Methodology, Writing—review and editing. Valentina Scariot: Conceptualization, Funding acquisition, Supervision, Writing—review and editing.

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