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Exploring wild edible flowers as a source of bioactive compounds: New perspectives in horticulture

*Sonia Demasi, Matteo Caser, Dario Donno, Simone Ravetto Enri, Michele Lonati, Valentina Scariot**

Department of Agricultural, Forest and Food Sciences, University of Turin, Largo Paolo Braccini 2, 10095, Grugliasco (TO), Italy

ABSTRACT

The increasing interest in healthy and natural foods has raised the attention towards uncommon or unexplored ingredients, such as edible flowers. These products are proven to be a rich source of bioactive compounds, for example, vitamins or polyphenols that play an important role in health promotion and disease prevention. However, plant species with edible flowers are numerous and most of them still need to be studied with this aim. The high species richness of North-Western Italy provides interesting perspectives in the use of wild edible flowers, which are currently underutilized, but can be a valuable food source or food supplement for healthy diets. In this framework, the phytochemical composition of 22 wild edible flowers was analysed and compared with that of four cultivated species (*Borago officinalis* L., *Calendula officinalis* L., *Tagetes patula* L. and *Tropaeolum majus* L.) to evaluate their potentiality as sources of bioactive compounds. The total polyphenol content (TPC) and antioxidant activity of the fresh flowers were assessed, together with their phenolic profiles and vitamin C content, through spectrophotometric and chromatographic analyses. The evaluated parameters varied widely among species, with *Paeonia officinalis* L. and *Rosa pendulina* L. showing the highest values of polyphenols (1,930 mg gallic acid equivalents (GAE) · 100 g^{-1} and 1,774 mg GAE · 100 g^{-1}, respectively), followed by *Rosa canina* L. (1,397 mg GAE · 100 g^{-1}) and *Geranium sylvaticum* L. (1,268 mg GAE · 100 g^{-1}). The same species also showed the highest antioxidant activity, measured with three different assays [ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS)]. The phenolic profile differed among the studied species, with *Dianthus pavonius* Tausch and *R. pendulina* having the highest sum of detected polyphenols (2,522 mg · 100 g^{-1} and 2,366 mg · 100 g^{-1}, respectively). Vitamin C was identified in all but two flowers (*Allium ursinum* L. and *B. officinalis*) and *Primula veris* L. had the highest amount (45 mg · 100 g^{-1}). The study showed that wild edible flowers outperformed the cultivated species, except for *T. majus*, providing new insights for the use of wild edible flowers as sources of bioactive compounds.

Keywords: antioxidant activity, edible flowers, functional food, polyphenols, vitamin C

INTRODUCTION

The number of plant species considered edible in the world is about 30,000; however, very few of them are used to fulfil human food requirements (Shaheen et al., 2017). To this aim, the rich biodiversity and abundance of wild edible plants represent a precious resource still underutilized, and that can be used as food sources (Shaheen et al., 2017; Ceccanti et al., 2018; Brito et al., 2021). In this framework, there are numerous plant species with edible flowers and studies ongoing to explore their potential in the human diet as food, supplements or additives (Loizzo et al., 2016; Fernandes et al., 2017; Mulík and Ozuna, 2020). Eating flowers is a legacy of many cultures that have been using flowers in their food traditions during centuries, but nowadays

*Corresponding author.
e-mail: valentina.scariot@unito.it (Valentina Scariot).

edible flowers can also represent a source of nutrients and phytochemicals with health benefits. Despite this, only a small portion of species has been explored to date with this aim, such as *Centaurea cyanus* L., *Hibiscus rosa-sinensis* L., *Calendula officinalis* or *Rosa* spp. (Ceccanti et al., 2018; Pires et al., 2019; Takahashi et al., 2020). The legislation is currently lacking, as reported by Fernandes et al. (2017), since no international body (e.g. European Food Safety Authority – EFSA, Food and Drugs Administration – FDA, United Nations Food and Agriculture Organization – FAO) has released an official list of edible flowers to-date. Thus, the use, production and consumption must be carefully performed, especially when considering wild or underutilized plants, which need proper characterization of the species (Fernandes et al., 2017; Takahashi et al., 2020). Moreover, their eventual toxicity should be investigated, as well as the possibility that some flowers may be considered novel food according to legislation (e.g. European Regulation EU 2015/2283) (Egebjerg et al., 2018; Zhao et al., 2019).

The positive health effects of edible flowers are ascribed to their chemical composition, which are rich in phytochemicals with bioactive properties, such as vitamins (Fernandes et al., 2017; Scariot et al., 2018; Pires et al., 2019; Zhao et al., 2019; Mulík and Ozuna, 2020; Takahashi et al., 2020; Zheng et al., 2021). Vitamin C is a strong antioxidant that scavenges radicals, thus neutralizing oxidative stress and plays an important role in human metabolism, representing a fundamental supplement in the diet (Fascella et al., 2019; Caritá et al., 2020). Interesting results on the vitamin C content in flowers have been recorded in plants of Zingiberaceae (Rachkeeree et al., 2018), *Tropaeolum majus* L. (Lim, 2014a) and cultivars of *Paeonia lactiflora* Pall. (Weixing et al., 2017). An increased number of studies have focused on polyphenols in flower extracts (Chensom et al., 2019; Kalemba-Drożdż and Cierniak, 2019; Moliner et al., 2019; Pires et al., 2019; Demasi et al., 2020; Montoro et al., 2020), a wide group of non-nutritional plant secondary metabolites that possess several beneficial properties and exert a strong antioxidant activity, scavenging reactive oxygen species (Del Rio et al., 2013; Durazzo et al., 2019). Considering the health benefits provided by polyphenols and antioxidants, increasing the knowledge of their content in unconventional matrixes can be a new challenge (D'Angiolillo et al., 2018; Durazzo et al., 2019), despite the content of single compounds often being in traces or lower than 1 mg · 100 g^{-1} of fresh weight (FW), whatever the food considered (Pérez-Jiménez et al., 2010). This issue is of particular interest considering the edible flowers market, which is constantly increasing (Fernandes et al., 2020).

Polyphenols in foodstuffs are frequently evaluated as a whole group with colorimetric assays; however, the individual quantification of phenolic compounds is essential to understand the bioactivity potential and properties of food (Fernandes et al., 2017; Pérez-Jiménez et al., 2010; Skrajda-Brdak et al., 2020), especially when studying unexplored or underutilized edible flowers. So far, wide variability in terms of total polyphenols and antioxidant activity has been recorded in edible flowers from Asian countries, where flowers are commonly consumed as food or medicine, for example, *Bougainvillea glabra* Choisy, *Chrysanthemum* spp., *Hibiscus sabdariffa* L., *Nelumbo nucifera* Gaertn., *Osmanthus fragrans* Lour, *Paeonia* spp., *Rosa* spp., *Tagetes erecta* L. (Wong et al., 2006; Kaisoon et al., 2012; Li et al., 2014; Xiong et al., 2014; Zeng et al., 2014; Lu et al., 2016; Zheng et al., 2018). Similarly, interesting results derived from European studies, focused on *Borago officinalis* L., *C. officinalis* L., *Tagetes* spp., *Tropaeolum majus* L., *Rosa* spp. and related cultivars, the most studied and produced edible flowers, used as garnishment or ingredients in salads and other dishes (Rop et al., 2012; Fernandes et al., 2017, 2020; Pires et al., 2019).

The high species richness of European biogeographic regions gives interesting perspectives in the use of wild edible flowers as human foodstuff. Particularly, North-Western Italy, characterized by a wide variety of habitats and vegetation communities, harbours a total of 4,020 taxa (Bartolucci et al., 2018).

In this study, we explored flowers from wild plants that grow spontaneously in self-maintaining populations in semi-natural habitats of North-Western Italy. A total of 26 species (including 22 wild and four commonly cultivated species) were analysed to evaluate their potential as sources of bioactive compounds, through the assessment of total polyphenol content (TPC), antioxidant activity, phenolic profiles and vitamin C content.

MATERIALS AND METHODS

Plant material

An extended area in North-Western Italy was explored (including Aosta Valley and Piedmont administrative regions), collecting flowers from 22 wild species (Table 1). Wild species were selected to explore all altitudinal belts in the studied area, including plain, colline, montane and alpine belts, and to investigate many vegetation communities. Aiming at this, each species was associated with the corresponding phytosociological optimum (at class level, according to Aeschimann et al., 2004), which were then pooled in eight different vegetation communities characterized by homogeneous ecological features: (i) nutrient-rich grasslands (including *Molinio-Arrhenatheretea* phytosociological class), (ii) nutrient-poor grasslands (*Juncetea trifidi* class), (iii) dry grasslands (*Festuco-Brometea* class), (iv) edges (*Mulgedio-Aconitetea* and *Trifolio-Geranietea sanguinei* classes), (v) ruderal communities (*Stellarietea mediae* and *Artemisietea vulgaris* classes), (vi) shrublands (*Crataego-Prunetea* class), (vii) wetlands (*Phragmito-Magnocaricetea* class) and (viii) woodlands (*Carpino-Fagetea sylvaticae,*

Robinietea, and *Roso pendulinae-Pinetea mugo* classes). The month and site of sampling have been recorded for each species, as well as the soil and bedrock type of the sampling location. Besides, four commonly known and cultivated edible species were sampled in the nursery F.lli Gramaglia (45°05′22.4″N, 7°34′26.4″E, 302 m.a.s.l., Collegno - TO, Italy). An amount of circa 100 g of flowers were collected per species in spring and summer 2017 at the optimal phenological stage (i.e. at full flowering), placed in sealed polyethylene bags, immediately stored at 4°C in a portable refrigerator and transported to the laboratory for analyses. Species nomenclature followed Pignatti et al. (2017). The plant list was checked with the available literature to consider mostly species with documented use by human society, as either food or medical stuff (Table 1), and their eventual presence in the Novel food catalogue of the European Commission was checked (https://ec.europa.eu/food/safety/novel_food/catalogue_en).

Extract preparation

Fresh flower sample was grinded in a mortar using liquid nitrogen and then stored at −80°C until the preparation of the extracts that was performed with ultrasound-assisted extraction, a high reproducible, efficient, simple, time- and solvent-saving methodology. The solid–liquid extraction using organic solvents and water mixture is among the most common methodologies to extract polyphenols (Pires et al., 2019; Takahashi et al., 2020); thus flower powder (1 g) 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 (Demasi et al., 2020). First, the solution was filtered with one layer of filter paper (Whatman No. 1, Maidstone, UK) and afterwards using a 0.45 μm PVDF syringe filter (CPS Analitica, Milano, Italy). The extracts were stored at −20°C until the performance of colorimetric and chromatographic analyses.

TPC and antioxidant activity

The TPC in flower extracts and the evaluation of their antioxidant activity were performed using colorimetric methods, reading the absorbance with the spectrophotometer Cary 60 UV-Vis (Agilent, Santa Clara, CA, USA). In particular, the TPC was analysed using the Folin–Ciocalteu method (Slinkard and Singleton, 1977; Sánchez-Rangel et al., 2013; Demasi et al., 2020). An amount of 200 μL of flower extract were mixed with 1,000 μL of diluted (1:10) Folin–Ciocalteu reagent. The samples were left in the dark at room temperature for 10 min, then adding 800 μL of Na_2CO_3 (7.5%). After 30 min in the dark at room temperature, absorbance was read at 765 nm, expressing results as mg of gallic acid equivalents (GAE) per 100 g of FW (mg GAE · 100 g^{-1}). The antioxidant activity was evaluated through three different assays: the ferric reducing antioxidant power (FRAP) method (Benzie and Strain, 1998; Demasi et al., 2020), the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Wong et al., 2006) and the 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay (Tawaha et al., 2007; Dudonné et al., 2009). In the FRAP method, 30 μL of flower extract were mixed with 90 μL of deionized water and 900 μL of FRAP reagent. This was constituted of 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) and $FeCl_3 \cdot 6H_2O$ (20 mM). The samples were placed at 37°C for 30 min and absorbance was read at 595 nm. The results were expressed as mill moles of ferrous iron (Fe^{2+}) equivalents per kilogram of FW (mmol $Fe^{2+} \cdot kg^{-1}$). In the DPPH assay, 40 μL of flower extract was mixed with 3 mL of $DPPH^{\cdot}$ radical solution. The samples were left in the dark at room temperature for 30 min and absorbance was read at 515 nm. In the ABTS assays, 30 μL of flower extract was mixed with 2 mL of $ABTS^{\cdot}$ radical solution. The samples were left in the dark at room temperature for 10 min and absorbance was read at 734 nm. Both DPPH and ABTS results were expressed as micro moles of Trolox Equivalents (TE) per 1 g of FW (μmol TE · g^{-1}).

Phenolic profile and vitamin C

The bioactive compounds present in the extracts of edible flowers were determined using High-Performance Liquid Chromatography (HPLC) with Diode Array Detection (DAD) (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). The separation of compounds was obtained with a Kinetex C18 column (4.6 × 150 mm, 5 μm, Phenomenex, Torrance, CA, USA) and different mobile phases, according to previous validated methodology (Table 2; Caser et al., 2019; Donno et al., 2019). The identification of compounds was made by comparison with retention times and UV spectra of analytical standards and the quantification was achieved using calibration curves at the same chromatographic conditions. The following bioactive compounds were determined: phenolic acids (cinnamic acids: caffeic, chlorogenic, coumaric and ferulic acid; benzoic acids: ellagic and gallic acid); flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin); flavanols (catechin and epicatechin) and vitamin C. The results are expressed as mg · 100 g^{-1} of fresh flower.

Statistical analyses

Raw data of TPC, FRAP, DPPH, and ABTS, were transformed in standard scores and averaged to obtain the Relative Antioxidant Capacity Index (RACI) (Sun and Tanumihardjo, 2007). Then, mean differences between species concerning dry matter content, spectrophotometric data (TPC, FRAP, DPPH, ABTS, RACI) and chromatographic data (class of compounds and single compounds) were analysed using generalized linear models (GLMs) with Gaussian or gamma distribution according to the distribution of data. Tukey's post hoc-test with Bonferroni's adjustment was used to

Table 1. List of the 22 wild and 4 cultivated species collected and analysed in the study, with references on their botanical family, vegetation community, food and/or medicinal uses, month, site (WGS84/32N system) and soil (USDA soil taxonomy; the bedrock type is provided in brackets: C, calcareous; S, siliceous)* of sampling in 2017.

Species	Botanical family	Vegetation community	Reference of flower use		Flower sampling			Soil type
			Food	Medicinal	Month	Longitude	Latitude	
Allium ursinum L.	Amaryllidaceae	Woodlands	Lim (2014a)	Sobolewska et al. (2015)	April	7.878	45.490	Eutrudept (S)
Bellis perennis L.	Asteraceae	Nutrient rich grasslands	Lim (2014a)	Lim (2014a)	March	7.592	45.065	Hapludalf (S)
Centaurea cyanus L.**	Asteraceae	Ruderal communities	Lim (2014a)	Lim (2014a)	May	7.896	45.475	Eutrudept (S)
Cichorium intybus L.	Asteraceae	Ruderal communities	Lim (2014a)	Street et al. (2013)	June	7.843	45.192	Dystrudept (S)
Dianthus carthusianorum L.	Caryophyllaceae	Dry grasslands	-	Palma (1964)	June	7.219	45.301	Udifluvent (S)
Dianthus pavonius Tausch	Caryophyllaceae	Nutrient poor grasslands	-	-	July	7.122	44.391	Eutrocryept (C)
Erythronium dens-canis L.	Liliaceae	Woodlands	-	-	March	7.371	45.157	Dystrudept (C)
Geranium sylvaticum L.	Geraniaceae	Edges	-	-	June	7.186	45.300	Dystrudept (S)
Lavandula angustifolia Mill.**	Lamiaceae	Dry grasslands	Lim (2014b)	Lim (2014b)	June†	-†	-†	-†
Leucanthemum vulgare Lam.	Asteraceae	Nutrient rich grasslands	Lim (2014a)	Prinsloo et al. (2018)	April	7.592	45.065	Hapludalf (S)
Mentha aquatica L.	Lamiaceae	Wetlands	Lim (2014b)	Alvarado (2018)	September	7.485	45.120	Hapludalf (S)
Paeonia officinalis L.**	Paeoniaceae	Edges	Lim (2014b)	-	April	7.344	45.097	Udorthent (S)
Primula veris L.	Primulaceae	Woodlands	Lim (2014b)	Apel et al. (2017)	May	6.802	44.968	Eutrudept (C)
Primula vulgaris Huds.	Primulaceae	Woodlands	Lim (2014b)	Tuttolomondo et al. (2014)	March	7.379	45.145	Dystrudept (C)
Robinia pseudoacacia L.	Fabaceae	Woodlands	Lim (2014a)	Jarić et al. (2015)	May	7.593	45.065	Hapludalf (S)
Rosa canina L.**	Rosaceae	Shrublands	Lim (2014b)	Nemati et al. (2015)	May	7.677	45.715	Udorthent (S)
Rosa pendulina L.	Rosaceae	Woodlands	-	-	June	7.192	45.301	Dystrudept (S)
Salvia pratensis L.	Lamiaceae	Dry grasslands	Kucekova et al. (2013)	Kucekova et al. (2013)	May	7.603	45.036	Udifluvent (S)
Sambucus nigra L.	Adoxaceae	Shrublands	Lim (2014a)	Młynarczyk et al. (2018)	May	7.593	45.064	Hapludalf (S)
Taraxacum officinale Weber**	Asteraceae	Nutrient rich grasslands	Lim (2014a)	Lim (2014a)	March	7.593	45.064	Hapludalf (S)
Trifolium alpinum L.	Fabaceae	Nutrient poor grasslands	Abbet et al. (2014)	Agelet and Vallès (2001)	July	7.122	44.390	Eutrocryept (C)
Viola odorata L.	Violaceae	Ruderal communities	Lim (2014b)	Lim (2014b)	March	7.591	45.065	Hapludalf (S)
Borago officinalis L.	Boraginaceae	Cultivated	Lim (2014a)	Gupta and Singh (2010)	May	-§	-§	-§
Calendula officinalis L.	Asteraceae	Cultivated	Lim (2014a)	Lim (2014a)	May	-§	-§	-§
Tagetes patula L.	Asteraceae	Cultivated	Lim (2014a)	Lim (2014a)	July	-§	-§	-§
Tropaeolum majus L.	Tropaeolaceae	Cultivated	Lim (2014b)	Lim (2014b)	May	-§	-§	-§

*I.P.L.A., Regione Piemonte (2007); Regione Autonoma Valle d'Aosta (2018).
**Species already evaluated under the European Novel Food Regulation (Regulation EU 2015/2283), not considered novel food.
†Demasi et al. (2018).
§Flowers sampled from cultivated plants in the nursery F.lli Gramaglia.

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

Class of compounds	Mobile phase	Elution conditions	Wavelength (nm)
Cinnamic acids and 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.

identify homogeneous groups of means when $p < 0.05$ (R 3.6.2, R Foundation for Statistical Computing, Vienna, AT). Nonparametric Kruskal–Wallis test by stepwise comparison was performed on RACI data to avoid GLM misfunctioning due to the presence of non-positive values. Spearman's correlation analysis was used on TPC, FRAP, DPPH, ABTS, and phenolic profiles to evaluate the relationships between variables (SPSS, version 25.0, SPSS Inc., Chicago, Illinois, USA). Finally, the species were grouped according (i) to their TPC and antioxidant capacity and (ii) to their polyphenolic profiles and vitamin C content performing two hierarchical cluster analyses, respectively, using Euclidean distance measure and UPGMA linkage method (Past 3.11; Hammer et al., 2001).

RESULTS AND DISCUSSION

TPC and antioxidant activity

The flowers of the selected species showed highly significant differences in each of the recorded parameters (Table 3), including the content of dry matter, which ranged from 8.9% in *T. majus* to 31.2% in *L. angustifolia*. These results are in accordance with Fernandes et al. (2017) and Pires et al. (2019), who reported that water is the main constituent of edible flowers, accounting for 70–95% of the composition. Flowers were analysed fresh as they are mainly consumed fresh and since foods better retain their bioactive compounds when are minimally processed (Takahashi et al., 2020). Therefore, the results were expressed on an FW basis.

The highest amounts of TPC (Table 3) were detected in *P. officinalis* (1,930.5 mg GAE · 100 g^{-1}) and *R. pendulina* (1,773.7 mg GAE · 100 g^{-1}), with *R. canina* and *G. sylvaticum* also showing very high contents (1,396.6 and 1,267.8 mg GAE · 100 g^{-1}, respectively). The lowest TPC values were found in *T. officinale*, *B. officinalis*, *A. ursinum* and *C. officinalis* (159.4, 163.4, 184.4, 189.6 mg GAE · 100 g^{-1}, respectively). The TPC range recorded in this study is in line with those obtained from other reports on fresh edible flowers (Li et al., 2014; Petrova et al., 2016; Fernandes et al., 2017; Pires et al., 2019), whereas it is sensibly higher than values recorded in fresh rocket, basil, and Swiss chard microgreens (16–33 mg GAE · 100 g^{-1} of FW, Bulgari et al., 2017). Comparing the literature, our data on fresh flowers of *B. officinalis*, *C. cyanus* and *S. nigra* are lower than in previous studies (Rop et al., 2012; Grzeszczuk et al., 2016; Młynarczyk et al., 2018), while data on *C. officinalis*, *T. patula* and *T. majus* are comparable (Garzón and Wrolstad, 2009; Rop et al., 2012; Lim, 2014a, 2014b). Interestingly, *Rosa* spp. and *Paeonia* spp. have already been reported to own very high values of TPC among several edible flowers (Kumar et al., 2009; Fan et al., 2012; Li et al., 2014; Xiong et al., 2014), confirming our findings.

Despite showing slight differences in antioxidant activity ranking, depending on the assay used (Table 3), the results showed that *P. officinalis* had always the highest activity (303.8 mmol Fe^{2+} · kg^{-1}, 226.2 and 55.3 μmol TE · g^{-1} for FRAP, DPPH and ABTS, respectively), together with both roses and *G. sylvaticum*, whereas poor antioxidant activity was recorded in the flowers of *T. officinale*, *R. pseudoacacia* and *A. ursinum*. Generally, as well as for TPC, peony and rose showed high antioxidant activity also in a previous study, where these species outperformed other eight Chinese flowers (Xiong et al., 2014). In the case of FRAP analysis, our range of values recorded in 26 species is wider than that reported for 51 fresh edible flowers from China (Li et al., 2014), where nonetheless *Rosa* × *hybrida* had the highest activity (178 mmol Fe^{2+} · kg^{-1}), while the range of antioxidant activity measured with ABTS is consistent with our results. Comparing the data of single species, the antioxidant activity can be very variable according to the study. For example, our FRAP results on *C. officinalis* and *C. intybus* are much higher than previous reports on the same fresh flowers, while the results on *S. nigra* and *R. pseudoacacia* are similar (Butnariu and Coradini, 2012; Lim, 2014a; Loizzo et al., 2016). Concerning DPPH, our data on cultivated species *C. officinalis*, *T. patula* and *T. majus* are sensibly lower in comparison with the literature (Lim, 2014a; Petrova et al., 2016). Finally, Lim (2014b) reported values seven-fold higher than ours in fresh *T. majus* evaluating antioxidant activity with ABTS test, while 12 rose cultivars from Israel had minor values (2–36 μmol TE · g^{-1}) than our wild edible flowers of *R. canina* and *R. pendulina* (Friedman et al., 2010). It is thereby clear that even

Table 3. Dry matter, TPC and antioxidant activity (FRAP, DPPH and ABTS assays) in the 26 edible flowers.

Species	Dry matter (%)	TPC (mg GAE · 100 g^{-1})	Antioxidant activity		
			FRAP (mmol Fe^{2+} · kg^{-1})	DPPH (mmol TE · g^{-1})	ABTS (mmol TE · g^{-1})
Allium ursinum	10.2 jk	184.4 k	4.2 j	7.6 kl	0.7 m
Bellis perennis	16.9 ef	396.3 gi	81.6 cf	24.3 i	13.4 hj
Centaurea cyanus	26.4 b	378.5 hi	68.3 df	23.6 i	17.8 fh
Cichorium intybus	17.3 ef	618.4 df	138.4 ad	69.2 f	26.9 d
Dianthus carthusianorum	27.8 ab	936.3 bd	222.2 ab	81.1 ef	33.6 c
Dianthus pavonius	21.2 cd	752.8 ce	176.1 ac	106.5 c	24.3 de
Erythronium dens-canis	15.1 fh	364.3 hi	53.5 eg	20.4 ik	14.4 hj
Geranium sylvaticum	12.8 i	1,267.8 ab	267.0 ab	152.9 b	55.2 a
Lavandula angustifolia	31.2 a	396.0 gi	89.5 ce	14.8 il	14.0 hj
Leucanthemum vulgare	17.0 ef	448.8 fi	44.3 eh	20.9 ik	10.8 ij
Mentha aquatica	22.3 c	1,061.7 bc	256.0 ab	86.7 de	42.5 b
Paeonia officinalis	13.9 gi	1,930.5 a	303.8 a	226.2 a	55.3 a
Primula veris	18.8 de	1,044.9 bc	230.1 ab	97.1 cd	38.5 bc
Primula vulgaris	9.8 jk	602.9 dg	127.4 bd	41.7 gh	21.5 df
Robinia pseudoacacia	12.9 i	203.8 jk	15.7 i	4.5 l	2.4 m
Rosa canina	16.7 ef	1,396.6 ab	257.5 ab	146.2 b	55.6 a
Rosa pendulina	21.6 cd	1,773.7 a	253.8 ab	154.3 b	55.7 a
Salvia pratensis	17.7 ef	314.7 ij	38.9 fh	8.9 jl	9.0 jl
Sambucus nigra	16.8 ef	508.7 eh	78.8 cf	28.5 hi	18.3 fh
Taraxacum officinale	16.5 ef	159.4 k	13.0 i	7.7 kl	3.3 lm
Trifolium alpinum	10.0 jk	464.6 fi	91.5 ce	50.3 gh	20.3 eg
Viola odorata	13.0 hi	428.4 fi	66.1 dg	22.6 ij	15.6 gi
Borago officinalis§	15.3 fg	163.4 k	29.7 gi	22.8 i	3.7 km
Calendula officinalis§	13.7 gi	189.6 k	22.6 hi	3.6 l	9.2 jk
Tagetes patula§	10.7 j	470.8 fi	143.9 ad	44.1 gh	23.0 df
Tropaeolum majus§	8.9 k	355.8 hi	45.3 eh	14.8 il	12.8 hj
p	***	***	***	***	***

Data are expressed on a fresh-weight basis, except for dry matter. The level of statistical significance is given (***$p < 0.001$), different letters inside a column indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$).
ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents; TPC, total polyphenol content; TE, trolox equivalents.
§Flowers sampled from cultivated plants.

inside the same species, a wide range of results can be recorded on fresh flowers, possibly due to the growing conditions and the senescence of the plants (Fernandes et al., 2017; Piccolella et al., 2018). The production of secondary metabolites in plants is in fact regulated by various factors, triggered by both endogenous and exogenous signals. The quality and amount of plant secondary metabolites can be thus genetic-dependent as well as environment-dependent (Sangwan et al., 2001; Cutler et al., 2010; Akula and Ravishankar, 2011; Loreto et al., 2014; Ashraf et al., 2018; Caser et al., 2019; Najar et al., 2019).

Different analytical assays are necessary to explain the antioxidant potential of matrices, including TPC, FRAP, DPPH and ABTS, and comparison with other studies can be difficult due to differences in sample processing and extraction techniques (Santos-Buelga et al., 2012). To rank the flower species within our study according to their antioxidant potential, RACI was calculated, being a numerical scale that integrates different analytical methods (Sun and Tanumihardjo, 2007). The ranking of species antioxidant potential based on the calculated RACI is displayed in Figure 1, from the highest values of *P. officinalis* (2.32), *R. pendulina* (1.81), *R. canina* (1.59) and *G. sylvaticum* (1.58), to the lowest of *R. pseudoacacia* (−1.02), *T. officinale* (−1.02) and *A. ursinum* (−1.07).

The intake of polyphenols and antioxidants in the diet was associated with decreased inflammatory biomarkers (Maleki et al., 2019) and has been positively linked to a reduction of cardiovascular diseases and an improvement in microvascular function in hypertensive patients (Durazzo et al., 2019). High polyphenol intake has been also related to a reduced incidence of diabetes and a chemopreventive efficacy against experimental tumours, despite clinical results not providing univocal results (Li et al., 2013; Durazzo et al., 2019; Kumar and Goel, 2019; Lapuente et al., 2019). Phenolic compounds could also affect the gut microbiota composition, resulting in a greater abundance of beneficial microbes

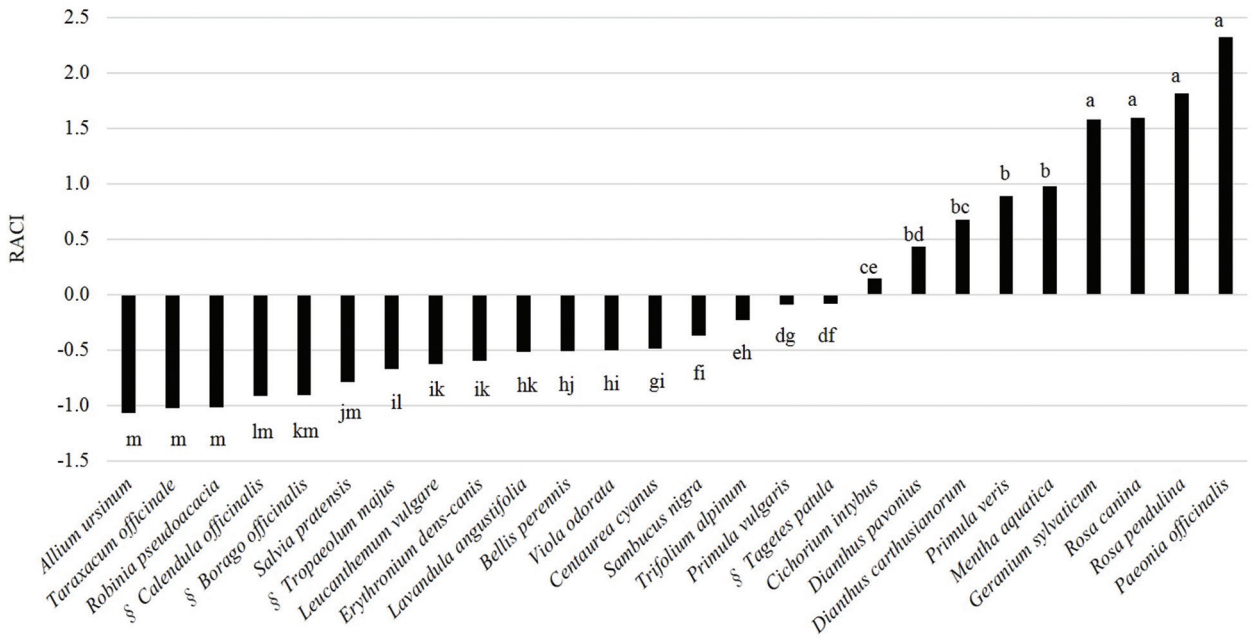

Figure 1. RACI calculated for the 26 studied flower species. Different lower case letters indicate significant differences between species according to Kruskal–Wallis' stepwise comparison ($p < 0.05$). RACI, relative antioxidant capacity index. §Flowers sampled from cultivated plants.

(Rinninella et al., 2019). Our screening of the TPC and antioxidant activity of 26 different flower species allowed to identify interesting wild plants with edible flowers, that is, *P. officinalis* and *G. sylvaticum*, together with more known species, namely roses, showing values always higher than cultivated flowers (*B. officinalis*, *C. officinalis*, *T. patula* and *T. majus*).

Phenolic profiles

Phenolics, with more than 8,000 compounds, are among the most numerous class of secondary metabolites, leading to a complex classification. However, they can be divided into flavonoids (including flavanols and flavonols, among the others) and non-flavonoid polyphenols (including phenolic acids) (Del Rio et al., 2013; Durazzo et al., 2019). HPLC analysis was performed to determine the phenolic compounds that mainly contributed to the antioxidant capacity of edible flowers, by evaluating the amount of six phenolic acids (four cinnamic and two benzoic acids), five flavonols and two flavanols (catechins), being among the most important compounds due to their biological and antioxidant activities (Durazzo et al., 2019; Takahashi et al., 2020). The results highlighted that each flower has a peculiar phenolic composition and the sum of detected polyphenols varied to a wide extent (Figure 2). *Dianthus pavonius* and *R. pendulina* had the highest content (2,522.1 and 2,365.7 mg · 100 g^{-1}, respectively), with values significantly higher than the species belonging to the same genus, that is, *D. carthusianorum* (772.7 mg · 100 g^{-1}) and *R. canina* (898.9 mg · 100 g^{-1}), respectively. The cultivated species, except for *T. majus*, had a lower content of phenolic compounds than the wild edible flowers analysed. The lowest quantity of polyphenols was indeed recorded in *C. officinalis* (17.3 mg · 100 g^{-1}).

The flowers with the highest RACI, that is, *P. officinalis*, *R. canina*, *R. pendulina* and *G. sylvaticum* had a statistically different amount of polyphenols detected with the chromatographic analysis, being 1,172, 899, 2,366 and 694 mg · 100 g^{-1}, respectively. Conversely, the high amounts of phenolics detected in *D. pavonius* and *T. majus* did not correspond to high RACI, indicating that further studies are needed to fully understand the phytochemical profile of each species and identify all the molecules that contribute to the antioxidant activity.

Considering each class of polyphenols, flavonols were on average 346 mg · 100 g^{-1}, cinnamic acids 183 mg · 100 g^{-1}, benzoic acid 133 mg · 100 g^{-1} and catechins 114 mg · 100 g^{-1}, confirming that flavonols are the main phenolics in edible flowers (Pires et al., 2019). Interestingly, analysing the Phenol-Explorer Database on 452 foods and beverages, Pérez-Jiménez et al. (2010) found a mean content of flavonols, benzoic acids and cinnamic acids equal to 11, 29, and 35 mg · 100 g^{-1} of FW, respectively, values considerably lower in comparison with edible flowers.

The detailed results on each class of polyphenols are reported in the following sections.

Flavonols

The evaluated flavonols (Figure 2 and Table 4; Figure A1 in Appendix) were present in 23 species, lacking in *B. perennis*, *B. officinalis* and *S. pratensis*.

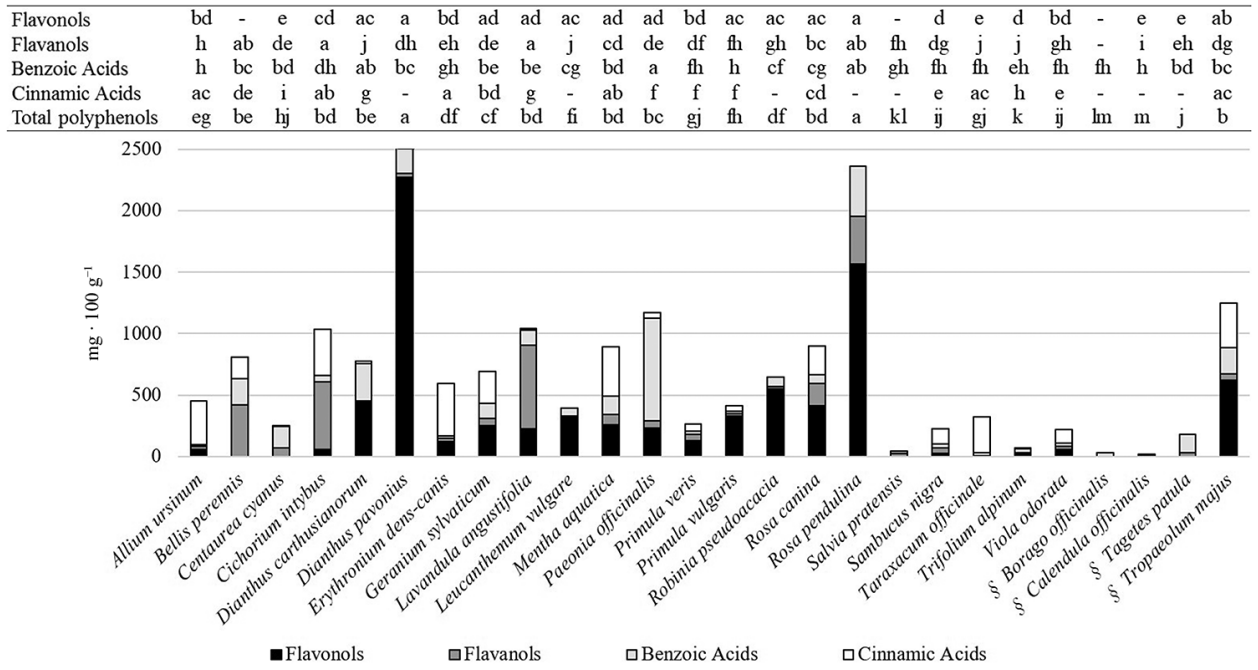

Figure 2. Total polyphenols and polyphenol classes (flavonols, flavanols, benzoic acids and cinnamic acids) content (mg · 100 g^{-1}) in the flowers of the 26 studied species. Different lower case letters in a row indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). -, compound not detected. §Flowers sampled from cultivated plants.

Where recorded, this class of flavonoids ranged from 0.5 mg · 100 g^{-1} (*T. patula*) and 2,269.6 mg · 100 g^{-1} (*D. pavonius*), always showing significant differences between species. Our range is particularly relevant, considering that the highest concentrations of flavonols in foods are 73–158 mg · 100 g^{-1} FW in onion and shallot, 119 mg · 100 g^{-1} FW in spinach, and 88 mg · 100 g^{-1} FW in black chokeberry (Pérez-Jiménez et al., 2010).

Considering single compounds (Table 4; Figure A1 in Appendix), hyperoside was detected in 13 out of 26 species, ranging from 0.6 mg · 100 g^{-1} (*G. sylvaticum* and *T. officinale*) to 262.4 mg · 100 g^{-1} (*D. carthusianorum*). Isoquercitrin was found in 11 species, ranging from 7.9 mg · 100 g^{-1} (*T. alpinum*) to 2,072.0 mg · 100 g^{-1} (*D. pavonius*). Quercetin was recorded in seven species, from 0.7 mg · 100 g^{-1} (*T. officinale*) to 328.1 mg · 100 g^{-1} (*L. vulgare*). Quercitrin was detected in 17 species, from 0.9 mg · 100 g^{-1} (*A. ursinum* and *C. cyanus*) to 1,353.4 mg · 100 g^{-1} (*R. pendulina*). Finally, rutin was found in 14 species, from 0.5 mg · 100 g^{-1} (*T. officinale*, *T. alpinum*, and *T. patula*) to 107.7 mg · 100 g^{-1} (*P. vulgaris*).

Considering each species, *A. ursinum* had very poor content of flavonols (59.4 mg · 100 g^{-1}), with hyperoside being the most abundant (Table 4). Exploring comparable bibliography, our findings on *B. perennis* were concordant with previous studies (Nazaruk and Gudej, 2001; Kucekova et al., 2013), since no amounts or very low amounts of quercetin and rutin were detected in the flower extract. In *C. cyanus*, only quercitrin is present, in extremely low amounts (0.9 mg · 100 g^{-1}). *Cichorium intybus* is very poor in flavonols, lacking in quercetin and quercitrin, concordant with a previous study (Kucekova et al., 2013), where also no amount of rutin was recorded; conversely, Loizzo et al. (2016) found very high concentrations of rutin (about 2,000 mg · 100 g^{-1} of dry extract) in *C. intybus*. *Dianthus* spp. were very rich but had diverse content of total flavonols, with *D. carthusianorum* having the highest concentration of hyperoside among the 26 studied species and *D. pavonius* the highest of isoquercitrin. *Erythronium dens-canis* was poor in flavonols with quercitrin as the highest (108.5 mg · 100 g^{-1}). The extract of *G. sylvaticum* was the only one to include all the five studied flavonols, containing about 250 mg · 100 g^{-1} of compounds. Similar concentrations were also recorded in *L. angustifolia*, *L. vulgare*, *M. aquatica*, and *P. officinalis*, where quercetin was the predominant compound. As for *Primula* spp., *P. vulgaris* flowers were slightly higher in flavonols than *P. veris*, also showing the highest concentration of rutin. Only one flavonol (quercitrin) was detected in *R. pseudoacacia*, as also occurring in *S. nigra*, *C. officinalis* and *T. majus*, with the first and the latter showing very high contents (547.3 and 619.6 mg · 100 g^{-1}). Contrasting results have been previously reported in *R. pseudoacacia* and *S. nigra*: Loizzo et al. (2016) found very high concentrations of rutin (about 2,000 mg · 100 g^{-1} of dry extract) in both species and of quercetin in *S. nigra*, while no traces of quercetin were found by Kucekova et al. (2013) in *S. nigra*, as our results. Concerning roses, *R. pendulina* was very rich in flavonols (1,566.2 mg · 100 g^{-1})

Table 4. Flavonols and flavanols content (mg · 100 g^{-1}) in the flowers of the 26 studied species.

Species	Flavonols					Flavanols	
	Hyperoside	Isoquercitrin	Quercetin	Quercitrin	Rutin	Catechin	Epicatechin
Allium ursinum	38.7 b	0.0 -	0.0 -	0.9 c	19.7 b	0.0 -	20.8 g
Bellis perennis	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.4 d	421.0 a
Centaurea cyanus	0.0 -	0.0 -	0.0 -	0.9 c	0.0 -	0.0 -	65.2 c
Cichorium intybus	23.5 bc	16.1 c	0.0 -	0.0 -	13.7 b	19.1 bc	533.3 a
Dianthus carthusianorum	262.4 a	163.6 b	0.0 -	8.0 c	17.8 b	0.0 -	0.1 h
Dianthus pavonius	0.0 -	2,072.0 a	0.0 -	163.6 c	34.1 b	11.7 c	26.3 dg
Erythronium dens-canis	9.0 cd	0.0 -	0.0 -	108.5 c	0.5 c	0.4 d	29.9 cg
Geranium sylvaticum	0.6 e	12.5 c	189.0 a	34.5 c	17.1 b	20.7 bc	37.7 cg
Lavandula angustifolia	17.3 bd	0.0 -	207.3 a	0.0 -	0.0 -	375.6 a	306.6 ab
Leucanthemum vulgare	0.0 -	0.0 -	328.1 a	0.0 -	0.0 -	0.0 -	0.1 h
Mentha aquatica	0.0 -	12.0 c	227.2 a	0.0 -	16.8 b	24.0 bc	59.2 cd
Paeonia officinalis	0.0 -	0.0 -	216.3 a	16.4 c	0.0 -	28.6 b	30.7 cg
Primula veris	14.7 bd	10.8 c	0.0 -	82.3 c	18.1 b	0.4 d	52.0 ce
Primula vulgaris	9.4 cd	100.1 b	0.0 -	109.6 c	107.7 a	0.0 -	23.9 eg
Robinia pseudoacacia	0.0 -	0.0 -	0.0 -	547.3 b	0.0 -	0.0 -	22.0 fg
Rosa canina	38.5 b	130.2 b	205.7 a	35.9 c	0.0 -	26.0 b	158.1 b
Rosa pendulina	15.3 bd	184.5 b	0.0 -	1,353.4 a	12.9 b	0.4 d	388.4 b
Salvia pratensis	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	25.0 eg
Sambucus nigra	0.0 -	0.0 -	0.0 -	23.4 c	0.0 -	0.0 -	48.3 cf
Taraxacum officinale	0.6 e	0.0 -	0.7 b	0.0 -	0.5 c	0.0 -	0.1 h
Trifolium alpinum	7.4 d	7.9 c	0.0 -	13.5 c	0.5 c	0.0 -	0.1 h
Viola odorata	16.3 bd	15.7 c	0.0 -	10.8 c	14.4 b	0.0 -	21.9 fg
Borago officinalis§	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Calendula officinalis§	0.0 -	0.0 -	0.0 -	1.7 c	0.0 -	0.4 d	0.1 h
Tagetes patula§	0.0 -	0.0 -	0.0 -	0.0 -	0.5 c	0.0 -	29.3 cg
Tropaeolum majus§	0.0 -	0.0 -	0.0 -	619.6 b	0.0 -	0.0 -	49.1 cf

Different lower case letters in a column indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates.
-, compound not detected.
§Flowers sampled from cultivated plants.

but lacked in quercetin and *R. canina* lacked in rutin. A previous report on roses (*R. damascena*, *R. bourboniana* and *R. brunonii*) instead identified both compounds, together with quercitrin (Kumar et al., 2009). Flowers of *T. officinale* had the lower content of flavonols (<1.7 mg · 100 g^{-1}), similar to *C. cyanus*, *C. officinalis* and *T. patula,* which had only traces of rutin. Also *T. alpinum* was poor in flavonols, with quercitrin as the most abundant compound (13.5 mg · 100 g^{-1}). The flowers of *V. odorata* contained a concentration of flavonols similar to *A. ursinum*, *E. dens-canis* and *P. veris*. Quercetin and rutin have been previously found in *Viola tricolor* L. and *Viola* × *wittrockiana* Gams., as well as rutin in *V. tricolor* (Vukics et al., 2008; Gamsjaeger et al., 2011; Gonçalves et al., 2012; Skowyra et al., 2014).

Flavonols seem to be the main phenolics exerting anti-cancer activity *in vitro* (Li et al., 2013) and inhibit *in vitro* oxidation of low-density lipoproteins, reducing thrombotic tendency (Del Rio et al., 2013). Among flavonols, quercetin represents an important molecule with wide therapeutic applications, owing to its anticancer and anti-inflammatory activity, together with cardiovascular disease and diabetes prevention (Durazzo et al., 2019). Thereof, *L. vulgare* and the species with a similar amount of quercetin (*G. sylvaticum, L. angustifolia, M. aquatica*, *P. officinalis*, and *R. canina*) are very interesting, as well as *R. pseudoacacia* and *T. majus*, for their amount of quercitrin, while *D. pavonius* and *R. pendulina* deserve attention for their impressive concentration of total flavonols.

Flavanols

Flavanols (Figure 2 and Table 4; Figure A2 in Appendix) were present in the flowers of all the studied species, except for *B. officinalis*, ranging from 0.1 mg · 100 g^{-1} (*D. carthusianorum, L. vulgare, T. officinale*, and *T. alpinum*) to 682.3 mg · 100 g^{-1} (*L. angustifolia*) with significant differences between species. At present, flavanols have been detected in 84 out of 452 foods (Pérez-Jiménez et al., 2010) and the richest sources are nuts (181–496 mg · 100 g^{-1} FW), strawberry (148 mg · 100 g^{-1} FW), and above all, berries, with content up to 659 mg · 100 g^{-1} FW, comparable with our highest values.

Catechin (Table 4; Figure A2 in Appendix) occurred in 12 species, from 0.4 mg · 100 g^{-1} (*B. perennis, E. dens-canis*, *P. veris, R. pendulina,* and *C. officinalis*)

to 375.6 mg · 100 g^{-1} (*L. angustifolia*). Epicatechin was instead more frequent, occurring in 25 species with a range of 0.1 mg · 100 g^{-1} (*D. carthusianorum, L. vulgare, T. officinale*, *T. alpinum,* and *C. officinalis*) and 533.3 mg · 100 g^{-1} (*C. intybus*).

The flavanols content was generally below 100 mg · 100 g^{-1} in most of the species, while interesting results are shown by five flowers, in which epicatechin always prevailed on catechin. *Lavandula angustifolia*, *C. intybus*, *B. perennis* and *R. pendulina* were around or above 400 mg · 100 g^{-1}, while *R. canina* had half of the content (184 mg · 100 g^{-1}). *Lavandula angustifolia* was the only species to contain a high concentration of catechin. Comparing bibliography, our findings in *B. perennis*, *S. nigra* and *T. officinale* are consistent with Kucekova et al. (2013), where no amounts of catechin were detected. The same authors found zero and 38 mg · 100 g^{-1} of dry weight of catechin in *C. intybus* and *S. pratensis,* respectively, and López-García et al. (2013) also found a small amount of catechin in *S. pratensis* (3.76 mg · 100 g^{-1} of dry weight). These results differ from our data since we recorded 19.1 mg · 100 g^{-1} in *C. intybus* and no detection in *S. pratensis*. Previous information on *S. nigra* (Młynarczyk et al., 2018) evidenced the presence of epicatechin in the flowers (25.43 mg · 100 g^{-1} FW) and of catechin 0.68 mg · 100 g^{-1} FW, similar to our results on the same species (48.3 mg · 100 g^{-1} of epicatechin and 0 mg · 100 g^{-1} of catechin).

Catechin and epicatechin belong to the subgroup of monomeric flavanols and are known to help in decreasing the body mass index and waist circumference (Durazzo et al., 2019); moreover, they help in preventing metabolic and cardiovascular diseases by improving the blood flow and exert antimicrobial, anti-inflammatory and antidiabetic properties (Ananingsih et al., 2013). Thus *R. canina, R. pendulina*, *B. perennis*, *C. intybus* and above all *L. angustifolia* are interesting genetic resources in this sense, whereas cultivated flowers (*B. officinalis* and *C. officinalis*) are of least interest.

Phenolic acids

Phenolic acids are commonly divided into benzoic and cinnamic acids, wide groups of polyphenols with at least 30 compounds reported in the past 10 years. Phenolic acids are recognized for their radical scavenging activity and their role in food preservation, as well as their therapeutic application, as reducing blood pressure and triglycerides (Kim et al., 2003; Ou and Kwok, 2004; Durazzo et al., 2019).

Benzoic acids

Benzoic acids (Figure 2 and Table 5; Figure A3 in Appendix) were present in every species ranging from 15.2 mg · 100 g^{-1} (*C. officinalis*) and 833.4 mg · 100 g^{-1} (*P. officinalis*), with significant differences among species. This compound content is interestingly elevated in comparison with foods (Pérez-Jiménez et al., 2010); apart from chestnut (1,215 mg · 100 g^{-1} FW), the other foods and beverages had a much lower amount of benzoic acids, that is, raspberry (121 mg · 100 g^{-1} FW), pomegranate juice (55 mg · 100 g^{-1} FW) and blackberry (50 mg · 100 g^{-1} FW).

Ellagic acid was detected in all the species (Table 5; Figure A3 in Appendix), ranging from 0.1 mg · 100 g^{-1} (*B. officinalis*) to 589.2 mg · 100 g^{-1} (*P. officinalis*), while gallic acid was found only in 9 species, with amounts of 0.1 mg · 100 g^{-1} (*A. ursinum*, *P. veris*, *R. canina*, *S. officinalis* and *S. nigra*) to 244.2 mg · 100 g^{-1} (*P. officinalis*).

Allium ursinum and other 11 species (*C. intybus*, *E. dens-canis*, *P. veris*, *P. vulgaris*, *S. pratensis*, *S. nigra*, *T. officinale*, *T. alpinum*, *V. odorata*, *B. officinalis* and *C. officinalis*) had similar concentrations of benzoic acids, up to 51.2 mg · 100 g^{-1}, with prevalence of ellagic acid, except in *C. intybus* and *B. officinalis*. A higher amount of benzoic acids was detected in the other species, containing only ellagic acid, except for *D. carthusianorum* and *R. canina* that had 27.5 mg · 100 g^{-1} and 0.1 mg · 100 g^{-1} of gallic acid, together with *P. officinalis* where the highest amount was measured. Gallic acid has been previously identified (18–458 mg · 100 g^{-1}) in *B. perennis*, *Rosa* spp., *S. pratensis*, *S. nigra*, *T. patula*, *T. officinale* and *T. majus* (Kumar et al., 2009; Kucekova et al., 2013; López-García et al., 2013; Lim, 2014a, 2014b), opposite to our study, where this compound is absent or present only in traces in the same species.

Gallic acid is mainly known for its antioxidant activity, while ellagic acid has anti-inflammatory properties and both exert anticancer and anti-HIV replication activities (Landete, 2011). Ellagic acid is also important in reducing the risk of cardiovascular diseases and obesity, since it decreases blood pressure and high blood cholesterol (Durazzo et al., 2019). Flowers of *P. officinalis* are therefore the most promising for these purposes.

Cinnamic acids

Cinnamic acids (Figure 2 and Table 5; Figure A4 in Appendix) were detected in the flowers of 18 species, ranging from 0.1 mg · 100 g^{-1} (*C. cyanus*) to 423.3 mg · 100 g^{-1} (*E. dens-canis*). Our highest values are double the foods and beverages with the highest concentrations, namely coffee (212 mg · 100 mL^{-1}), globe artichoke (202 mg · 100 g^{-1} FW), prune (192 mg · 100 g^{-1} FW) and red chicory (183 mg · 100 g^{-1} FW) (Pérez-Jiménez et al., 2010).

Caffeic acid (Table 5; Figure A4 in Appendix) was present in 13 species and ranged from 0.1 mg · 100 g^{-1} (*C. cyanus*) to 16.3 mg · 100 g^{-1} (*P. vulgaris*). Chlorogenic acid was detected in 8 species, from 0.2 mg · 100 g^{-1} (*L. angustifolia*) to 275.5 mg · 100 g^{-1} (*E. dens-canis*). Coumaric acid, found in 10 species, varied between 0.5 (*A. ursinum* and *T. alpinum*) and 158.5 mg · 100 g^{-1} (*B. perennis*). Finally, ferulic acid was detected in

Table 5. Benzoic acids and cinnamic acids content (mg · 100 g^{-1}) in 26 flower species.

Species	Benzoic acids		Cinnamic acids			
	Ellagic acid	Gallic acid	Caffeic acid	Chlorogenic acid	Coumaric acid	Ferulic acid
Allium ursinum	15.2 h	0.1 c	0.0 -	0.0 -	0.5 c	357.3 a
Bellis perennis	212.9 ad	0.0 -	15.6 ab	0.0 -	158.5 a	0.0 -
Centaurea cyanus	180.1 ad	0.0 -	0.1 d	0.0 -	0.0 -	0.0 -
Cichorium intybus	23.7 fh	27.5 b	0.0 -	230.0 b	148.5 a	0.0 -
Dianthus carthusianorum	278.5 ac	27.6 b	14.6 ab	0.0 -	0.0 -	0.0 -
Dianthus pavonius	214.4 ad	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Erythronium dens-canis	20.4 gh	0.0 -	16.2 a	275.5 a	110.7 a	20.9 bc
Geranium sylvaticum	121.4 be	0.0 -	0.0 -	244.1 b	16.4 b	0.0 -
Lavandula angustifolia	122.8 be	0.0 -	11.7 c	0.2 c	0.0 -	0.0 -
Leucanthemum vulgare	63.2 dg	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Mentha aquatica	153.8 bd	0.0 -	15.9 ab	270.6 a	113.8 a	0.0 -
Paeonia officinalis	589.2 a	244.2 a	13.9 b	0.0 -	0.0 -	32.6 bc
Primula veris	27.9 fh	0.1 c	15.2 ab	0.0 -	0.0 -	43.9 b
Primula vulgaris	16.3 h	0.0 -	16.3 a	0.0 -	0.0 -	29.3 bc
Robinia pseudoacacia	79.5 cf	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Rosa canina	72.5 dg	0.1 c	0.0 -	232.0 b	0.0 -	0.0 -
Rosa pendulina	410.7 ab	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Salvia pratensis	20.4 gh	0.1 c	0.0 -	0.0 -	0.0 -	0.0 -
Sambucus nigra	27.1 fh	0.1 c	15.3 ab	0.0 -	112.0 a	0.0 -
Taraxacum officinale	28.2 fh	0.0 -	15.7 ab	273.3 a	0.0 -	0.0 -
Trifolium alpinum	34.6 eh	0.0 -	0.0 -	0.0 -	0.5 c	0.2 c
Viola odorata	26.2 fh	0.0 -	1.9 d	0.0 -	113.4 a	0.2 c
Borago officinalis§	0.1 i	27.9 b	0.0 -	0.0 -	0.0 -	0.0 -
Calendula officinalis§	15.2 h	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Tagetes patula§	150.1 bd	0.0 -	0.0 -	0.0 -	0.0 -	0.0 -
Tropaeolum majus§	214.3 ad	0.0 -	14.1 b	241.2 b	109.6 a	0.0 -

Different lower-case letters in a column indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates.
-, compound not detected.
§Flowers sampled from cultivated plants.

seven species, from 0.2 mg · 100 g^{-1} (*T. alpinum* and *V. odorata*) to 357.3 mg · 100 g^{-1} (*A. ursinum*).

Table 5 shows that eight species lacked in cinnamic acids (*D. pavonius*, *L. vulgare*, *R. pseudoacacia*, *R. pendulina*, *S. pratensis*, *B. officinalis*, *C. officinalis* and *T. patula*), concordant with the results of Kucekova et al. (2013) on *S. pratensis*. Four species contained only traces (<15 mg · 100 g^{-1}, *C. cyanus*, *D. carthusianorum*, *L. angustifolia* and *T. alpinum*), with caffeic acid as the most present. *Paeonia officinalis*, *P. veris* and *P. vulgaris* had about 50 mg · 100 g^{-1} of cinnamic acids, containing only caffeic and ferulic acid, with the first one as the most abundant. With higher amounts, from 100 mg · 100 g^{-1} to 300 mg · 100 g^{-1}, *B. perennis*, *S. nigra* and *V. odorata* were characterized by the presence of coumaric acid, while *G. sylvaticum*, *R. canina* and *T. officinale* were characterized by chlorogenic acid. The five species containing the highest amounts of cinnamic acids had mainly chlorogenic and coumaric acids (*C. intybus*, *E. dens-canis*, *M. aquatica* and *T. majus*), except for *A. ursinum* that contained only ferulic acid and traces of coumaric acid. Contrasting results are reported by the study of Kucekova et al. (2013), where coumaric acid was absent in *B. perennis, C. intybus* and *S. nigra,* conversely to our study; caffeic acid was present in *B. perennis, C. intybus* and *S. nigra* but not in *T. officinale* and ferulic acid was present in *B. perennis* and *C. intybus* as we detected, but not in *S. nigra* and *T. officinale*.

Together with the other bioactive properties of phenolic acids, chlorogenic and ferulic acids are also characterized by working as antidiabetic agents (Kumar and Goel, 2019). Ferulic acid also counteracts the enzymes that catalyze the production of free radicals, while it enhances enzymes with free radical scavenging activity (Ou and Kwok, 2004). Our results showed the potentiality of 11 flowers (*A. ursinum, B. perennis*, *C. intybus*, *E. dens-canis*, *G. sylvaticum*, *M. aquatica*, *R. canina*, *S. nigra*, *T. officinale, T. majus* and *V. odorata*) with a very high amount of cinnamic acids that can be further evaluated for therapeutic application.

Vitamin C content

Vitamin C (Table 6) was detected in all the flowers, except for *A. ursinum* and *B. officinalis*, with values that ranged from 2.6 mg · 100 g^{-1} (*M. aquatica*) to 44.9 mg · 100 g^{-1} (*P. veris*).

Table 6. Vitamin C content (mg · 100 g^{-1}) in the flowers of the 26 studied species.

Species	Vitamin C
Allium ursinum	0.0 -
Bellis perennis	4.4 fi
Centaurea cyanus	3.3 gi
Cichorium intybus	4.0 gi
Dianthus carthusianorum	5.5 di
Dianthus pavonius	16.4 bc
Erythronium dens-canis	6.7 ch
Geranium sylvaticum	7.9 bg
Lavandula angustifolia	2.8 hi
Leucanthemum vulgare	5.9 di
Mentha aquatica	2.6 i
Paeonia officinalis	11.3 be
Primula veris	44.9 a
Primula vulgaris	3.8 gi
Robinia pseudoacacia	4.0 gi
Rosa canina	12.3 bd
Rosa pendulina	7.2 bg
Salvia pratensis	4.0 gi
Sambucus nigra	11.0 bf
Taraxacum officinale	3.5 gi
Trifolium alpinum	15.5 bc
Viola odorata	4.6 ei
Borago officinalis[§]	0.0 -
Calendula officinalis[§]	11.8 bd
Tagetes patula[§]	7.2 bg
Tropaeolum majus[§]	17.7 b

Different lower case letters in a column indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates.
-, compound not detected.
[§]Flowers sampled from cultivated plants.

Most of the flowers had a content of vitamin C up to 8 mg · 100 g^{-1}, whereas eight species were significantly higher (*D. pavonius*, *P. officinalis*, *P. veris*, *R. canina*, *S. nigra*. *T. alpinum*, *C. officinalis* and *T. majus*), with *P. veris* having at least a three-fold higher concentration. *Tropaeolum majus* has one of the highest values, indeed a previous report indicated that this species can contain high quantities of vitamin C, up to 71.5 mg · 100 g^{-1} (Lim, 2014b).

Vitamin C is one of the plant food components which contribute to lower the risk of cancer, chronic and cardiovascular diseases and premature mortality, together with antioxidants and other compounds (Barros et al., 2011; Aune, 2019). Moreover, vitamin C is essential as an enzymatic cofactor and in response to environmental stimuli. European Food Safety Authority established a Population Reference Intake of 95–110 mg per day for vitamin C (Fenech et al., 2019), easily satisfied by kiwifruit, which has an average content of vitamin C of 93 mg · 100 g^{-1} FW. In oranges the content is about 53 mg · 100 g^{-1} FW and in apple 5 mg · 100 g^{-1} FW (Cruz-Rus et al., 2012). Thus, most of the flowers have an interesting concentration of vitamin C, comparable to apples, and *P. veris* appears of particular interest as a supplement of vitamin C in the diet.

Correlation among variables and species clustering

The correlation analysis (Table 7) highlighted that the TPC of the 26 edible flowers was positively correlated with the antioxidant activity measured with the three assays (FRAP, DPPH and ABTS). These three methods of analysis also positively correlated with each other, confirming previous results on the positive link between TPC and antioxidant activity in edible flowers (Ji et al., 2012; Kaisoon et al., 2012; Xiong et al., 2014; Lu et al., 2016; Petrova et al., 2016). The abovementioned parameters also correlated with the content of flavonols, ellagic acid, both catechins and vitamin C, but they did not correlate with the content of the four cinnamic acids and ellagic acid, probably being the reason for the different ranking of the species evaluated through RACI and chromatographic analysis.

The hierarchical cluster analysis performed on TPC and antioxidant activity data identified five main groups (Figure 3), reflecting the ranking of the 26 species based on RACI (Figure 1). A first group (A) is composed of *P. officinalis* and *R. pendulina*, characterized by the highest values in all parameters (TPC, FRAP, DPPH, ABTS). Another group (B) consists of *D. carthusianorum*, *M. aquatica*, *P. veris*, *G. sylvaticum* and *R. canina*, with very high values except for DPPH. The third group (C) is characterized by low values for every analysis and includes 11 species, from *E. dens-canis* to *T. patula*. Then, in the fourth group (D), there are the species with the lowest values, namely *B. officinalis*, *T. officinale*, *C. officinalis*, *R. pseudoacacia* and *A. ursinum*. *Cichorium intybus*, *P. vulgaris* and *D. pavonius* belong to the fifth group (E) with intermediate values between groups (B) and (C).

The results of the cluster analysis performed on phenolic profiles and vitamin C (Figure 4) show that four species should be considered not related to the others due to their peculiar characteristics (*P. officinalis* – A, *L. angustifolia* – G, *R. pendulina* – H, and *D. pavonius* – I). Again, five groups formed, but the species differed from the previous cluster. The group with *R. pseudoacacia* and *T. majus* (B) has in common high values of quercitrin and ellagic acid, and a few other compounds were present. Species of the second group (C), from *B. officinalis* to *P. vulgaris*, shared low amounts of ellagic acid and epicatechin and have a few other compounds. Chlorogenic acid and quercetin are the major contributors of the third group (D) (from *E. dens-canis* to *R. canina*), while a miscellaneous few compounds are present in the fourth group (E) composed of *L. vulgare*, *A. ursinum* and *D. carthusianorum*. Finally, *B. perennis* and *C. intybus* belong to the fifth group (F), with high concentrations of epicatechin and coumaric acid.

A limited number of species resulted in the same groups in both dendrograms, namely: (i) *T. alpinum*,

Table 7. Spearman's correlation indexes between TPC, antioxidant activity (FRAP, DPPH and ABTS assays) and phenolic compounds recorded in the 26 edible flowers.

	FRAP	DPPH	ABTS	Caffeic acid	Chlorogenic acid	Coumaric acid	Ferulic acid	Hyperoside	Isoquercitrin	Quercetin	Quercitrin	Rutin	Ellagic Acid	Gallic Acid	Catechin	Epicatechin	Vitamin C
TPC	0.94**	0.93**	0.95**	0.07	0.06	0.05	0.09	0.26*	0.68**	0.43**	0.36**	0.43**	0.47**	0.18	0.50**	0.43**	0.44**
FRAP	1	0.93**	0.96**	0.09	0.09	0.06	0.04	0.23*	0.65**	0.40**	0.26*	0.40**	0.49**	0.21	0.56**	0.49**	0.37**
DPPH		1	0.93**	0.01	0.07	0.01	0.09	0.22	0.68**	0.33**	0.30**	0.42**	0.40**	0.31**	0.49**	0.37**	0.41**
ABTS			1	0.07	0.15	0.07	0.05	0.25*	0.67**	0.36**	0.29**	0.38**	0.47**	0.19	0.52**	0.47**	0.44**
Caffeic acid				1	0.30**	0.44**	0.35**	0.04	−0.01	−0.03	0.08	0.12	0.02	−0.03	−0.07	0.11	−0.05
Chlorogenic acid					1	0.49**	−0.07	−0.03	0.04	0.22*	0.06	0.01	−0.04	−0.06	0.35**	0.21	−0.01
Coumaric acid						1	−0.12	−0.08	−0.07	−0.04	0.02	0.05	−0.05	−0.06	0.09	0.40**	−0.02
Ferulic acid							1	0.30**	−0.03	−0.03	0.17	0.32**	−0.22*	0.08	−0.08	−0.05	0.00
Hyperoside								1	0.48**	−0.06	0.03	0.44**	−0.17	0.14	0.11	0.13	−0.05
Isoquercitrin									1	0.03	0.38**	0.69**	0.17	0.01	0.27*	0.14	0.30**
Quercetin										1	−0.09	−0.08	0.29*	0.06	0.70**	0.20	0.02
Quercitrin											1	0.23	0.20	−0.23*	0.01	0.26*	0.50**
Rutin												1	−0.04	0.04	0.14	0.07	−0.05
Ellagic acid													1	0.06	0.27*	0.34**	0.28*
Gallic acid														1	0.23*	−0.09	−0.12
Catechin															1	0.49**	0.05
Epicatechin																1	0.04

The level of statistical significance is given (**$p < 0.01$; *$p < 0.05$).
ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TPC, total polyphenol content.

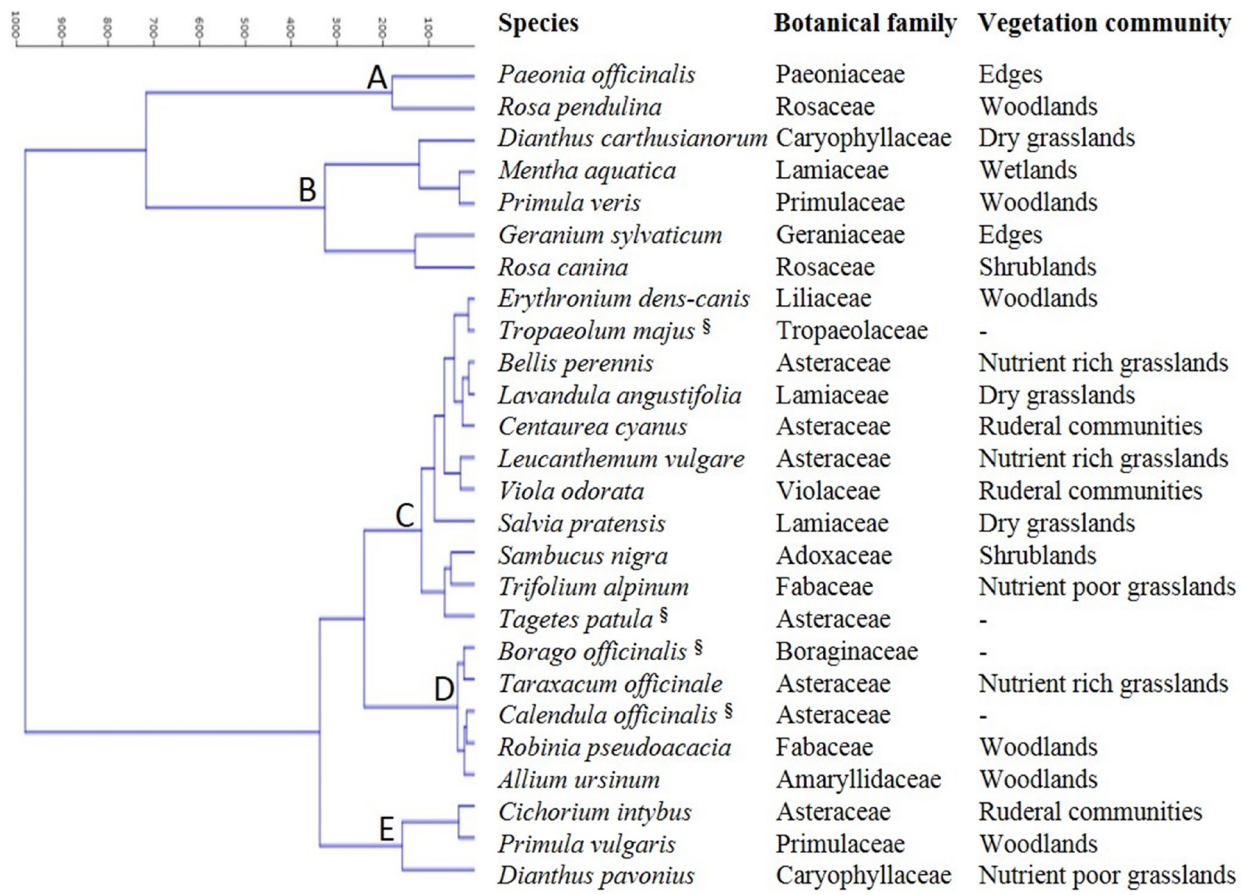

Figure 3. Hierarchical cluster analysis of the selected species according to their TPC and antioxidant activity (FRAP, DPPH and ABTS). The respective botanical family and vegetation community is provided for every species. §Flowers sampled from cultivated plants. ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; TPC, total polyphenol content.

S. pratensis, *S. nigra*, *V. odorata*, *C. cyanus* and *T. patula*; (ii) *B. officinalis* and *C. officinalis*; and (iii) *G. sylvaticum*, *M. aquatica* and *R. canina*. Interestingly, three couples of species belonging to the same genera showed significant differences both in their TPC and antioxidant activity and in their polyphenol profile and vitamin C content, as occurred in *Dianthus*, *Primula* and *Rosa*, therefore resulting separated in both dendrograms. The studied wild species derived from a wide variety of habitats and vegetation communities, namely semi-natural pastures and meadows, woodlands, shrublands, wetlands and agricultural fallows, resulting from the complex interactions among heterogeneous ecological, topographic and management conditions (Aeschimann et al., 2013; Mondino, 2007). However, generally there was no clear distinction among groups neither in terms of botanical family, vegetation community, soil type nor bedrock type. Thus, the chemical composition of the selected species appeared more species-dependent rather than taxonomic- or habitat-dependent.

CONCLUSIONS

This investigation on 22 wild edible flowers compared with four cultivated plants showed wide variability in their phenolic and vitamin C content, as well as in their antioxidant activity, disclosing valuable sources of bioactive compounds. Generally, these traits appeared more species-dependent rather than taxonomic- or habitat-dependent. However, it has to be considered that the phytochemical profile of flowers and their bioactive compounds content are susceptible to variation, depending also on environmental conditions and stresses. The results showed that flowers of *Dianthus pavonius* and *Rosa pendulina* had the highest concentrations of polyphenols, displaying also the highest antioxidant activity, together with *Geranium sylvaticum*, *Paeonia officinalis* and *Rosa canina*. Each studied species was characterized by a peculiar phenolic profile and in most of the flowers, vitamin C has been identified, deserving further investigations, for instance, for the development of new food supplements or additives. Wild edible flowers outperformed three of the cultivated species (*Borago officinalis*, *Calendula officinalis* and *Tagetes patula*) in most of the analysis, while *Tropaeolum majus* had comparable results. Environmental stresses during plant growth may contribute to the high accumulation of bioactive molecules. The use of wild plants may have a positive impact on the local economy, because the environmental and economic costs of emerging produce

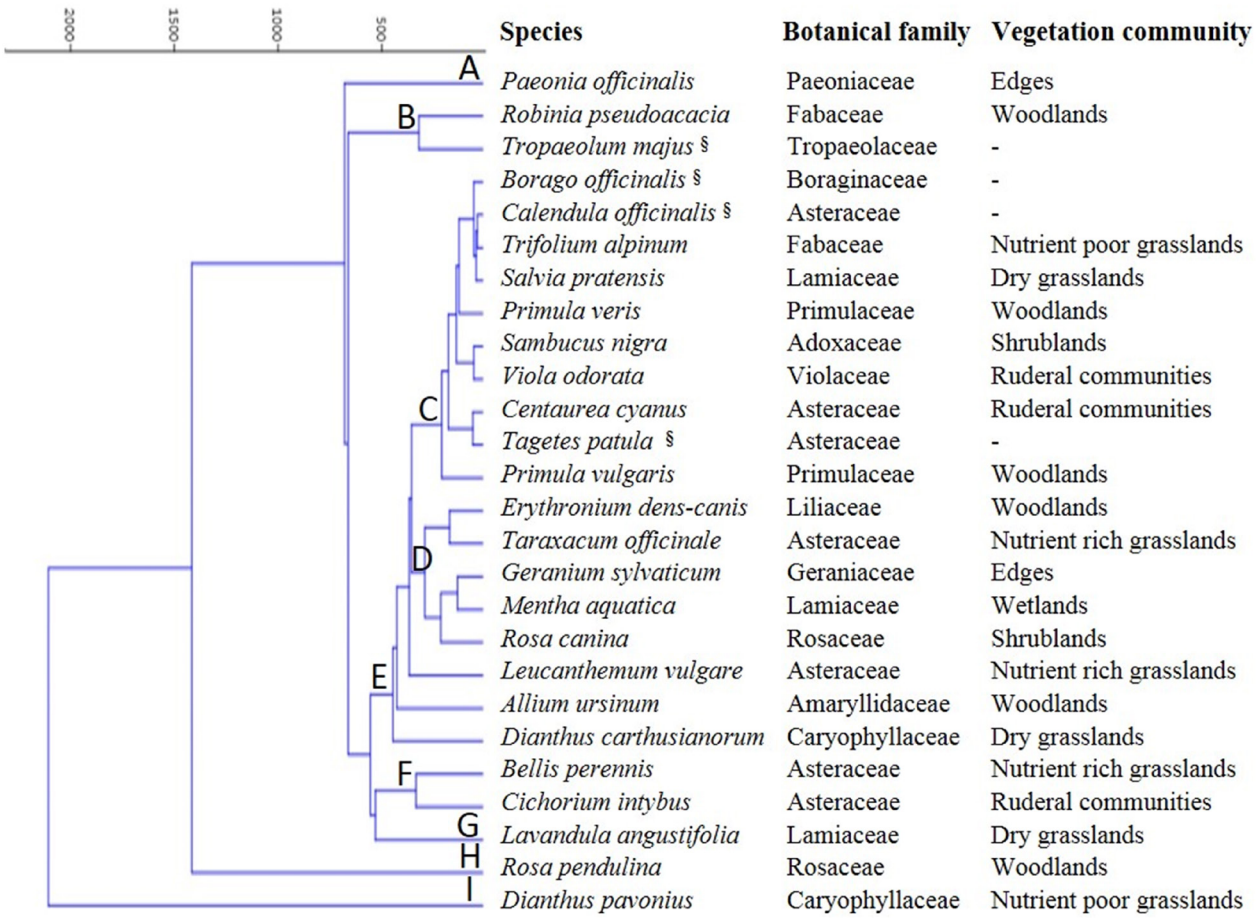

Figure 4. Hierarchical cluster analysis of the selected species according to their polyphenolic profile and vitamin C content. The respective botanical family and vegetation community is provided for every species. §Flowers sampled from cultivated plants.

with edible flowers (Falla et al., 2020; Fernandes et al., 2020) can be reduced, exploiting local resources. The valorization of quality and diversification of production can lead to higher revenue for growers, farmers or small enterprises (Takahashi et al., 2020). In order to support the consumption of edible flowers, it is of high importance in the future to evaluate and assess their sensory characteristics and postharvest performances. Furthermore, optimized cultivation protocols could standardize the produce. In this context, since lesser amount of flowers than vegetables and fruits is eaten, the application of intentional moderate stresses could foster the production of bioactive molecules (Caser et al., 2019). This will lead to increase and maintain across time the content of bioactive compounds, obtaining standard products that confer not only aesthetic value to the food, but also nutraceutical properties, to be accurately integrated into a healthy diet.

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AUTHOR CONTRIBUTIONS

VS and ML were responsible for conceptualization; SD and MC conducted the investigation. SD, MC and DD performed data curation. SD, MC, SRE, ML and DD took care of formal analysis. VS was in charge of supervision. SD and MC were responsible for writing the original draft. SD, MC, DD, SRE, ML and VS undertook writing of the review edition. All authors contributed to manuscript revision, and read and approved the submitted version.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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APPENDIX

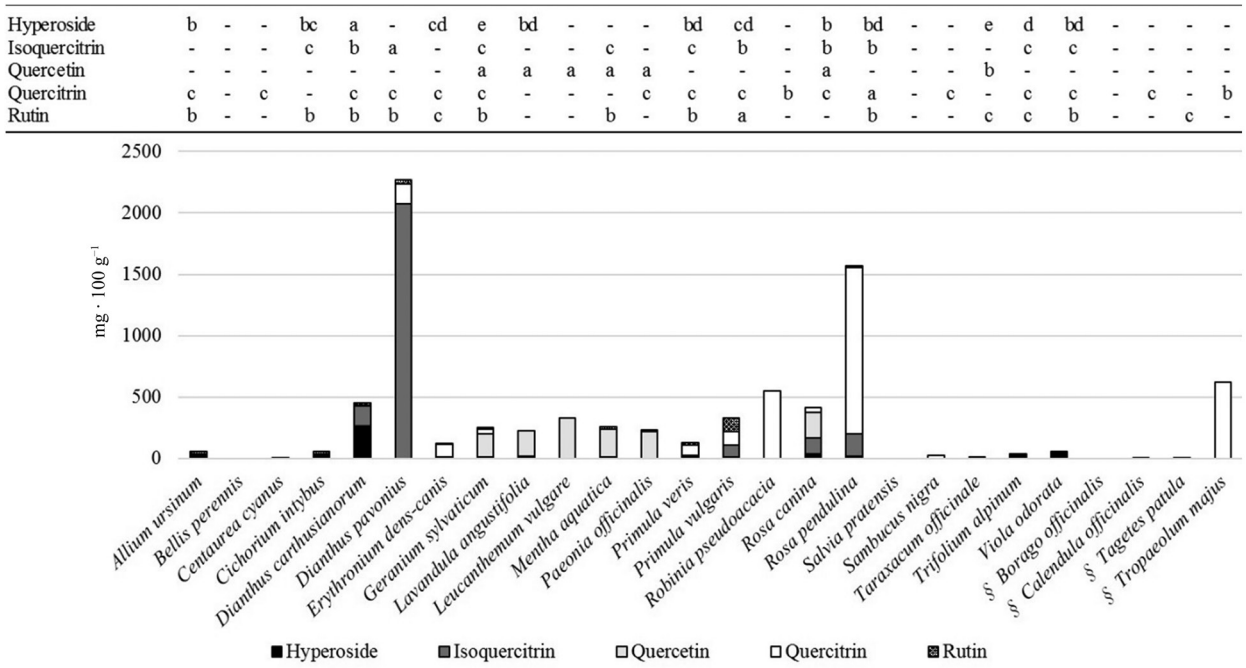

Figure A1. Flavonols (hyperoside, isoquercitrin, quercetin, quercitrin and rutin) content (mg · 100 g^{-1}) in the flowers of the 26 studied species. Different lower case letters in a row indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates. -, compound not detected. §Flowers sampled from cultivated plants.

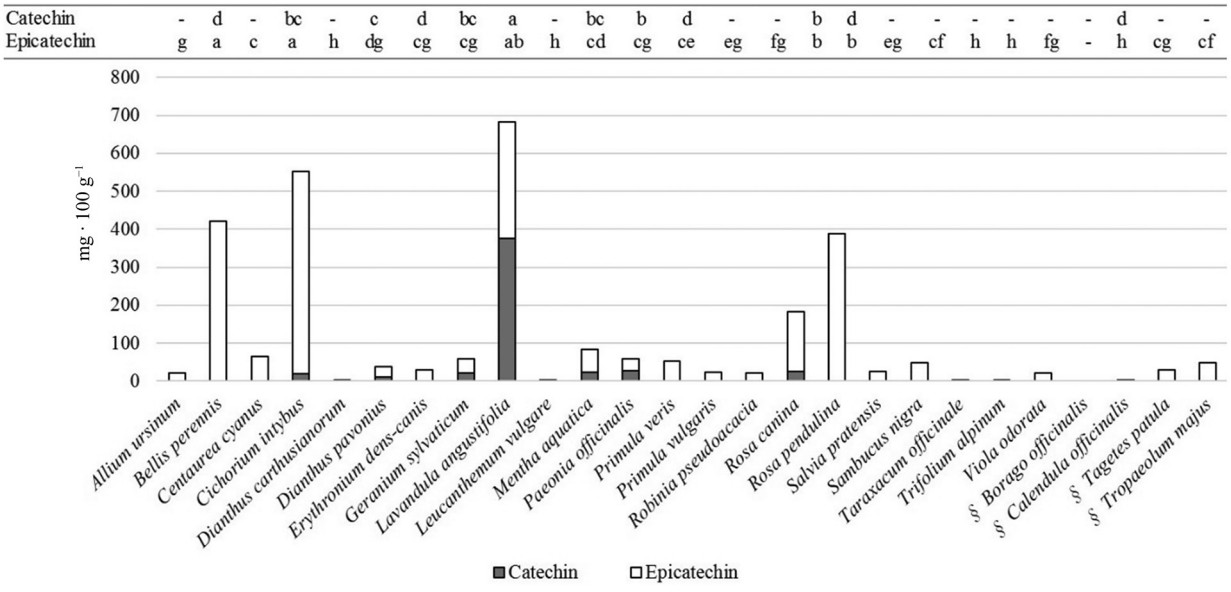

Figure A2. Flavanols (catechin and epicatechin) content (mg · 100 g^{-1}) in the flowers of the 26 studied species. Different lower case letters in a row indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates. -, compound not detected. §Flowers sampled from cultivated plants.

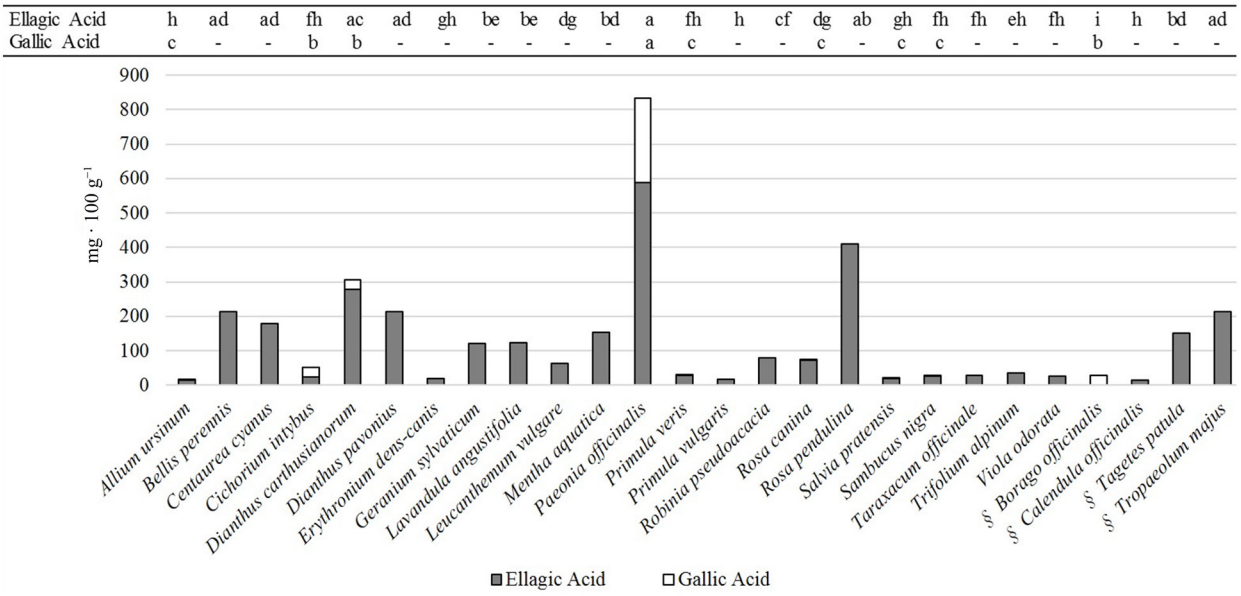

Figure A3. Benzoic acids (ellagic and gallic acid) content (mg · 100 g^{-1}) in the flowers of the 26 studied species. Different lower case letters in a row indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates. -, compound not detected. [§]Flowers sampled from cultivated plants.

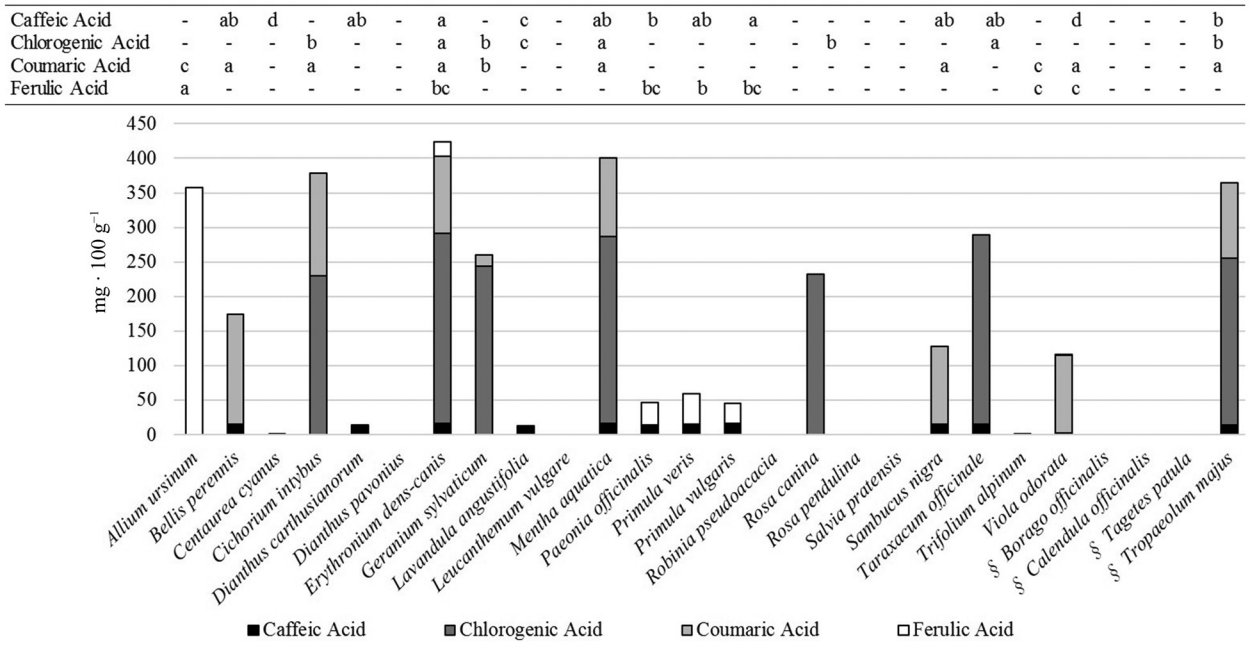

Figure A4. Cinnamic acids (caffeic, chlorogenic, coumaric and ferulic acid) content (mg · 100 g^{-1}) in the flowers of the 26 studied species. Different lower case letters in a row indicate significant differences between species according to Tukey's post-hoc test ($p < 0.05$). Data are means of three biological replicates. -, compound not detected. [§]Flowers sampled from cultivated plants.