



Exploring the potential of wild leafy vegetables widespread in European Alps as functional food

Simone Ravetto Enri^a, Nicole Mélanie Falla^a, Sonia Demasi^{a,b}, Daniela Manila Bianchi^c, Stefania Squadrone^c, Giampiero Lombardi^a, Valentina Scariot^{a,*}

^a Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Italy

^b Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125, Torino, Italy

^c Istituto Zooprofilattico Sperimentale Del Piemonte Liguria e Valle D'Aosta - Experimental Zooprophyllactic Institute of Piedmont, Liguria and the Aosta Valley, Via Bologna 148, 10154, Torino, Italy

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ABSTRACT

Wild leafy vegetables, historically vital for Alpine biodiversity and nutrition during hardship, are regaining importance due to the demand for healthy and sustainable food. This study evaluated mineral and phytochemical compositions of eight species: *Achillea millefolium*, *Alchemilla xanthochlora*, *Bistorta officinalis*, *Blitum bonus-henricus*, *Phyteuma betonicifolium*, *Plantago lanceolata*, *Silene vulgaris*, *Taraxacum* sect. *Taraxacum*. Total trace element concentrations ranged from 26 mg kg⁻¹ (*T. officinalis*) to 92 mg kg⁻¹ (*B. officinalis*), and potential toxic elements were below legal limits. The sum of the phenolic compounds analysed ranged from 155.30 mg GAE 100 g⁻¹ FW (*A. millefolium*) to 1200.38 mg GAE 100 g⁻¹ FW (*A. xanthochlora*). Flavanols were the most abundant phenolic class, but cinnamic acids, flavonols, benzoic acids and vitamin C also showed high values in the analysed species, contributing to high antioxidant activity (from 0.59 μmol TE g⁻¹ FW in *A. millefolium* to 149.39 μmol TE g⁻¹ FW in *A. xanthochlora* for DPPH). Overall, *A. xanthochlora* stood out for its richness in phenolic compounds. These findings underscore wild vegetables potential as functional foods, offering bioactive compounds comparable to or exceeding cultivated plants. Emphasizing preservation of traditional diets and rural innovation, this research supports the integration of wild leafy vegetables into contemporary nutrition strategies.

1. Introduction

Functional foods are increasingly recognized for their role in preventing nutrition-related diseases and enhancing human well-being [1]. They are rich in bioactive compounds such as phytochemical compounds with antioxidant activity. These compounds can be provided by many fruits, vegetables, whole grains, nuts, seeds, herbs, and spices, both domesticated and wild [2].

Wild edible plants (WEPs) are non-domesticated plant species which are usually gathered from the wild (forests, bushlands, grasslands, etc.) for human nutrition. They can be eaten as whole or only some of their parts, such as fruits, flowers, leaves, roots [3,4].

In the past centuries, WEPs, and among them wild leafy vegetables (WLVs), were important to human society, since they were a key source of nutrients during periods of famine and war, integrating agricultural food [5–8], especially in the Alps [9–12]. However, in developed

countries, their importance in the human diet declined over the years, due to first agricultural expansion and then the industrial revolution, which brought changes in agriculture, nutrition, economy, and society [13]. Therefore, WLVs are currently underutilised in developed countries, and almost only older people still preserve their knowledge and culinary tradition [7,8,14]. In recent years, this limited use of WLVs has been regarded as a wasted opportunity for integrative food for the local economy and well-being of people in various regions of the world. Several studies on WLVs asserted their contribution to food sovereignty (especially in non-industrialized countries), their central role in extensive farming systems and subsistence economy, and their function in maintaining rural identities by recovering rural economies and preserving biodiversity [8,15]. Furthermore, holistic policies and sustainable practices appear required to recognize the intricate interplay between livestock farming, community well-being, and environmental conservation, ensuring a balanced and resilient future for these interconnected

* Corresponding author.

E-mail addresses: simone.ravettoenri@unito.it (S. Ravetto Enri), nicolemelanie.falla@unito.it (N.M. Falla), sonia.demasi@unito.it (S. Demasi), manila.bianchi@izsto.it (D.M. Bianchi), stefania.squadrone@izsto.it (S. Squadrone), giampiero.lombardi@unito.it (G. Lombardi), valentina.scariot@unito.it (V. Scariot).

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systems. Such strategies should address collaborative efforts between stakeholders, governments, and conservation organisations to ensure the preservation of these invaluable ecological and cultural resources for generations to come.

Recently, the increasing knowledge about compositional properties of WLVs, along with the growing demand for healthy and sustainable food, has caused a renewed interest towards them [6,16]. Based on the amount and caloric contribution of proteins, carbohydrates, fat, and dietary fibre, WLVs can be regarded as appropriate food for low-calorie diets [8]. In addition, WLVs are a source of nutrients and phytochemicals which may provide health benefits (immune system enhancement, prevention of age-related diseases), such as phenolic compounds, vitamins, and minerals, often found in higher amounts than in cultivated species, leading to considering several of them as functional food [4, 16–19]. Moreover, WLVs could be used in low-input sustainable food production systems, eventually increasing the diversification and productivity of agro-ecosystems, since they are adapted to stressful environmental conditions [20].

However, attention should be paid before consuming wild plants, since they may also produce or accumulate potential anti-nutritional and toxic compounds, such as phytates, oxalates and saponins, and trace elements like lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd), which could be harmful to human health if they exceed some defined limits [8,21].

Therefore, agro-ecological and nutritional studies are very important to explore the revival and commercialisation of the resources provided by such a rich biodiversity of WLVs [3,8].

In this framework, this study focused on eight species which grow spontaneously in self-maintaining populations in semi-natural grasslands of the European Alpine region. The WLVs were selected among the most representative wild food plants with acknowledged functional benefits, as reported in literature [12,22–24]. Investigations on their mineral and phytochemical (pigments, antioxidant, phenolic, and bioactive compounds) composition were performed aiming to

encourage their use to preserve traditional food habits as cultural heritage, to contribute to local sustainable development with innovative options for rural areas, such as the possible applications of WLVs as functional foods, and thus to promote a healthier diet.

2. Materials and methods

2.1. Plant material

Eight plant species (Fig. 1) were selected among the most widespread in the European Alpine region [25] and traditionally consumed by local people: *Achillea millefolium* L., *Alchemilla xanthochlora* Rothm., *Bistorta officinalis* Delarbre, *Blitum bonus-henricus* (L.) Rchb., *Phyteuma betonicifolium* Vill., *Plantago lanceolata* L., *Silene vulgaris* (Moench) Garcke, *Taraxacum* F.H.Wigg. sect. *Taraxacum* (hereafter *Taraxacum officinale*).

Species selection considered their ease of harvesting, size of populations, phenology, and aimed to explore a wide ecological gradient, in terms of soil nutrient availability and moisture, suitable temperature, tolerance to defoliation and typical growing habitat, across different botanical families (Table 1). Collection sites were semi-natural grasslands traditionally exploited through extensive cattle grazing and/or haymaking in summer time. Collection sites were characterised by shallow soils with acidic parent rock material and gentle slopes, in a temperate sub-oceanic climate (average annual valley conditions: temperature, 10.1 °C; precipitation, 1355 mm). Therefore, three different collection sites, characterised by different ecological conditions, were identified in Valchiusella (North Western Italian Alps; Fig. 2).

Plant material was manually harvested in the wild from May to June 2021, before the first annual grazing or haymaking. *Achillea millefolium*, *A. xanthochlora*, *B. officinalis*, *P. betonicifolium*, *P. lanceolata*, and *T. officinale* were harvested at Piane (830 m a.s.l.), *B. bonus-henricus* in Palit (1640 m a.s.l.), and *S. vulgaris* in Colla (1250 m a.s.l.). About 500 g of fresh plant material per species were collected, preserved in plastic bags in a portable refrigerator and processed in the University

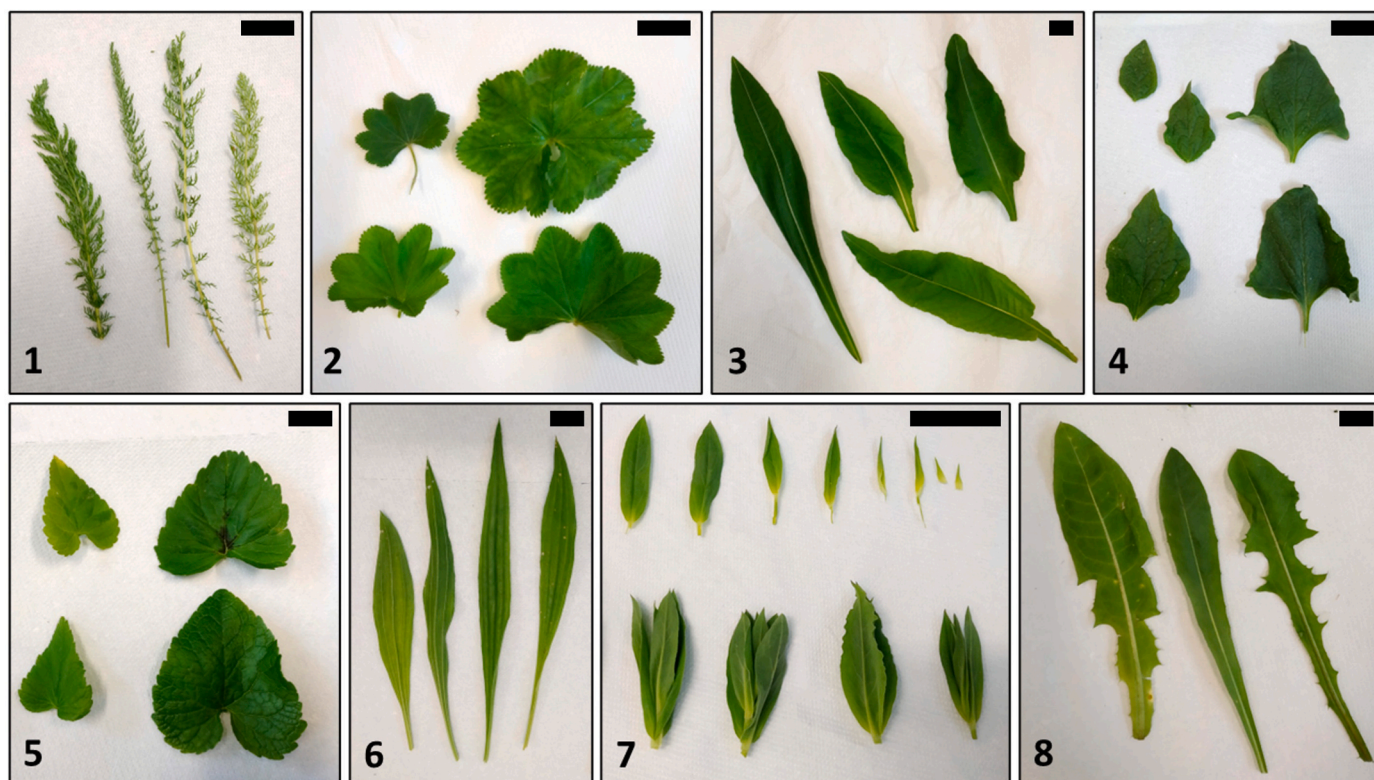


Fig. 1. Leaves of the eight wild leafy vegetables species: Ach.mil (1); Alc.xan (2); Bis.off (3); Bli.bon (4); Phy.bet (5); Pla.lan (6); Sil.vul (7); Tar.off (8). Black rectangles are 2 cm scales. For plant codes see Table 1.

Table 1

Ecological characteristics of the eight wild edible plants. Landolt's indicator values refer to Landolt et al. [66] while habitats (ecological unit and optimal phytosociological class) are provided according to Aeschimann et al. [17].

Plant code	Species	Vernacular name	Family	Landolt's indicator values				Habitat
				Moisture	Temperature	Nutrients	Defoliation tolerance	
Ach.mil	<i>Achillea millefolium</i>	Common yarrow	Asteraceae	2	3	3	3	Nutrient-rich grasslands (Molinio-Arrhenatheretea)
Alc.xan	<i>Alchemilla xanthochlora</i>	Intermediate lady's mantle	Rosaceae	3.5	3	4	4	Nutrient-rich grasslands (Molinio-Arrhenatheretea)
Bis.off	<i>Bistorta officinalis</i>	Common bistort	Polygonaceae	4	2.5	4	3	Nutrient-rich grasslands (Molinio-Arrhenatheretea)
Bli.bon	<i>Blitum bonus-henricus</i>	Good-King-Henry	Amaranthaceae	2	2.5	5	3	Nitrophilous ruderal communities (Artemisietea vulgaris)
Phy.bet	<i>Phyteuma betonicifolium</i>	Betony-leaved rampion	Campanulaceae	3	2	2	2	Alpine belt grasslands (Juncetea trifidi)
Pla.lan	<i>Plantago lanceolata</i>	Ribwort plantain	Plantaginaceae	2.5	3	3	4	Nutrient-rich grasslands (Molinio-Arrhenatheretea)
Sil.vul	<i>Silene vulgaris</i>	Bladder campion	Caryophyllaceae	2.5	3	2	2	Nutrient-rich grasslands (Molinio-Arrhenatheretea)
Tar.off	<i>Taraxacum officinale</i>	Dandelion	Asteraceae	4	3	4	3	Nutrient-rich grasslands (Molinio-Arrhenatheretea)

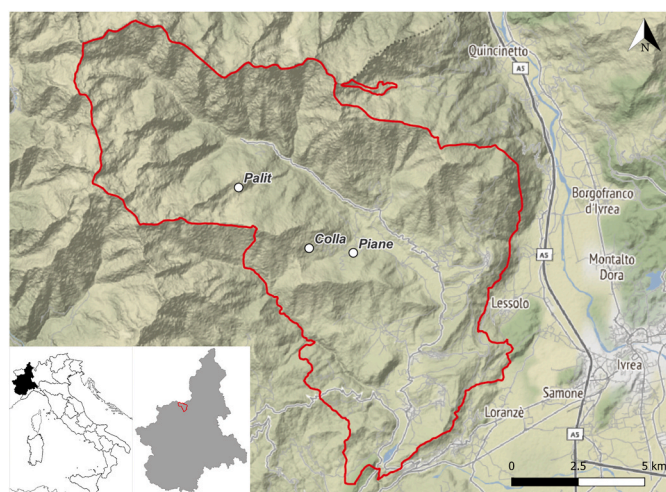


Fig. 2. Map of the three sites where the plant material was collected. Red borders contour the Valchiusella in the Piedmont region (TO, NW-Italy). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

laboratory (Department of Agricultural, Forest and Food Sciences - Grugliasco (TO). Long. 7.589, Lat. 45.066), on the same day of harvest. Each sample was divided into two parts: (i) stored at $-20\text{ }^{\circ}\text{C}$ for trace elements analyses; (ii) stored at $-80\text{ }^{\circ}\text{C}$ for phytochemical analyses.

2.2. Mineral composition

Twenty grams of fresh leaves of each species were analysed for mineral composition. Samples stored at $-20\text{ }^{\circ}\text{C}$ were homogenised and subjected to mineralization with acids and oxidants (HNO_3 and H_2O_2) using a microwave digestion lab station (Ethos 1, Milestone, Shelton, CT, USA), equipped with a 10-positions rotor for high pressures PTFE (polytetrafluoroethylene) digestion tubes.

Eleven trace elements were analysed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS Agilent 7800, USA): five essential elements, namely chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and six nonessential elements, namely arsenic (As), cadmium (Cd), lead (Pb), nickel (Ni), tin (Sn), and vanadium (V).

Particularly, metals and metalloids have been recognized as potentially hazardous to men and biota; among them, arsenic and cadmium

were classified as the Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) while organic mercury, nickel and lead were classified as probable human carcinogens (group 2B).

Vanadium in the environment originates from anthropogenic activities and may pose toxic hazards to plants, since many industrial processes may release high amounts of V into the soil. Tin is used in the plastic and canning industry and for pest eradication in agriculture, where it penetrates the soil and consequently the plants. Essential elements, i.e., chromium, copper, iron, manganese, zinc, which participate in several biological pathways as constituents or cofactors of enzymes, can also exert toxic effects if assumed above recommended levels [8,21].

The instrument is equipped with a collision/reaction cell optimised to significantly improve the performance of helium (He) collision mode for the removal of polyatomic interferences.

Direct Mercury Analyser (DMAEvo, Milestone, Shelton, CT, USA), which performs thermal decomposition, catalytic reduction, amalgamation, desorption, and atomic absorption spectroscopy without sample pre-treatment, was used for total mercury (Hg) determination.

In each analytical session, the accuracy of the analytical procedure was verified by analysing Certified Reference Materials (Tea leaves INCT TL-1 from the Institute of Nuclear Chemistry and Technology). Recoveries were reported in Table S1. Reagent blanks were also determined in each series of the analysis.

The limit of detection (LODs) and the limits of quantification (LOQ) were calculated according to Commission Regulation (EU) 333/2007 laying down the methods of sampling and analysis for the official control of the levels of Pb, Cd, Hg, inorganic Sn, 3-MCPD and benzo(a)pyrene in foodstuffs. LODs and LOQs for the analyte elements were calculated using the standard deviation (σ) of 20 independent measurements of a blank solution, with 3σ for LODs, and 10σ for LOQs. The repeatability was calculated with the formula HORRATr, where observed RSDr (relative standard deviation) values were divided by the RSDr values estimated from the modified Horwitz equation; all values must be less than 2. The LOQs of the analysed elements range between 0.001 and 0.010 mg kg^{-1} (Table S1). The LOQ for each element was set at 0.010 mg kg^{-1} to facilitate comparison. All values were expressed on a wet weight basis.

The analytical methods were validated according to UNI CEI EN ISO/IEC 17025 (General Requirements for the Competence of Testing and Calibration Laboratories).

2.3. Phytochemical analysis

2.3.1. Pigment content

Chlorophylls (a+b) and carotenoids were determined using the method described by Lichtenthaler [26] with slight modifications. The analysis was performed as follows: 50 mg of frozen leaves were ground using a Tissue Ruptor homogenizer (Qiagen, Switzerland) and then added to 5 mL of a 90 % aqueous MeOH (methanol) solution. The samples were then left in the dark at 4 °C for 24 h. The obtained phytoextract was centrifuged (Eppendorf Centrifuge 5417 R, Germany, Hamburg) for 10 min at 3000 rpm, at 4 °C. After collection of the supernatant, absorbance was read at 470, 652.4, and 665.2 nm for each sample by means of a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA). The results were expressed as μg of chlorophylls a+b or carotenoids per mg of fresh weight ($\mu\text{g mg}^{-1}$ FW).

2.3.2. Extract preparation

Phytoextracts of 70 % aqueous MeOH were obtained as follows: 500 mg of frozen leaves (-80 °C) were ground in 5 mL of a MeOH 70 % solution by means of a Tissue Ruptor homogenizer (Qiagen, Switzerland). Samples were left in the dark at 4 °C for 24 h. The obtained phytoextract was then centrifuged for 15 min at 3000 rpm, at 4 °C. The extracts were filtered using a 0.45 mm PVDF syringe filter (CPS Analytica, Milano, Italy).

Extracts were then used to analyse the nitrates, the antioxidant activity, the total phenolic content (TPC), the phenolic profile and the vitamin C. Each sample was processed and analysed in three replicates.

2.3.3. Nitrates

Nitrate concentration was evaluated directly in the phytoextracts by semi-quantitatively measurement, using test strips (MQuant® Nitrate test, Merck KGaA, Darmstadt, Germany), based on Parks et al. [27]. The evaluation was performed by comparing the colour of the strip reaction zone to a red-violet colour scale (0-10-25-50-100-250-500 $\text{mg L}^{-1} \text{NO}_3^-$). Results were then expressed as $\text{mg NO}_3^- \text{kg}^{-1}$ FW and data were compared to the nitrate limits for lettuce and spinach provided by European Commission Regulation (EC) No. 915/2023.

2.3.4. Antioxidant activity

The antioxidant activity was assessed through three different assays as described by Demasi et al. [3] and briefly reported hereafter.

In the FRAP assay, 30 μL of phytoextract were mixed with 90 μL of deionized water and 900 μL of FRAP reagent. After the incubation time, the absorbance of the solutions was read at 595 nm and the results expressed as $\text{mmol Fe}^{2+} \text{kg}^{-1}$ FW.

In the DPPH assay, 20 μL phytoextract was mixed with 1.5 mL of DPPH radical solution, then samples were left in the dark at room temperature for 30 min. The absorbance was read at a wavelength of 515 nm.

In the ABTS assay, 30 μL phytoextract was mixed with 2 mL of ABTS radical solution, then samples were left in the dark at room temperature for 10 min. The absorbance was read at a wavelength of 734 nm.

For both DPPH and ABTS assays, the values were plotted against a Trolox calibration curve ($0.008\text{--}1 \mu\text{g TE } \mu\text{L}^{-1}$, $R^2 = 0.9626$ for DPPH, and $0.002\text{--}0.5 \mu\text{g TE } \mu\text{L}^{-1}$, $R^2 = 0.9989$ for ABTS), to quantify the antioxidant activity of the samples analysed; results were expressed as $\mu\text{mol TE g}^{-1}$ FW.

The absorbances were measured through the spectrophotometer Cary 60 UV-Vis (Agilent Technologies, Santa Clara, CA, USA).

Raw data from FRAP, DPPH, and ABTS of each sample were transformed into standard (T) scores and averaged in order to obtain the Global Antioxidant Score (GAS [20]). The T-scores were calculated for each of the three variables using the following equation: $T\text{-score} = (X - \text{min}) / (\text{max} - \text{min})$, where min and max represent the smallest and the largest values of variable X among the investigated extracts. The obtained values of GAS ranged from 0 to 1 and allowed a direct and easy

comparison among species with different antioxidant capacities.

2.3.5. Total phenolic content

The TPC was determined through the Folin-Ciocalteu method [28].

Two hundred μL of phytoextract (or extracting solution for the control sample) were mixed with 1000 μL of diluted (1:10) Folin-Ciocalteu reagent (consisting of phosphomolybdenum and phosphotungsten acids). The samples were left in the dark at room temperature for 10 min, then 800 μL of Na_2CO_3 (7.5 %) were added. Samples were left in the dark at room temperature for 30 min. Absorbance was then read at 765 nm, through the spectrophotometer Cary 60 UV-Vis (Agilent Technologies, Santa Clara, CA, USA).

The results were compared with a calibration curve constructed using gallic acid as a standard ($1.95\text{--}250 \text{ mg L}^{-1}$; $R^2 = 0.9982$), and expressed as mg GAE g^{-1} FW.

2.3.6. Phenolic compounds and vitamin C

The bioactive compounds were determined by means of HPLC-DAD (Agilent 1200, Agilent Technologies, Santa Clara, CA, USA). Phytochemical separation was obtained with a Kinetex C18 column ($4.6 \times 150 \text{ mm}$, 5 mm, Phenomenex, Torrance, CA, USA) and different mobile phases, according to previous validated methodology [3]. Identification of compounds was performed by comparison with retention times and UV spectra of analytical standards, and quantification was obtained from calibration curves under the same chromatographic conditions. The following bioactive compounds were screened: 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 (ascorbic and dehydroascorbic acids). Results were expressed as $\text{mg } 100 \text{ g}^{-1}$ of fresh leaves ($\text{mg } 100 \text{ g}^{-1}$ FW).

2.4. Statistical analysis

Differences among species in terms of pigment content, antioxidant activity (i.e. GAS), TPC, and bioactive compounds were tested through generalised linear models (GLM). Being the analysed values continuous response variables, each of them was modelled with both Gaussian and Gamma distributions and then the best fitting model (i.e., that one showing the lowest Akaike Information Criterion [29]) was retained. Where significant effects were found ($p < 0.05$), the differences were explored through Tukey's post-hoc tests.

Two principal component analyses (PCA) were performed to assess the relationships among (i) the main trace elements and (ii) the main phenolic compounds recorded in the analysed species. Pearson correlation was used to calculate the distance matrices. The relationship among the main phytochemical compounds and the antioxidant activity was also explored through a multivariate constrained analysis. First, a detrended correspondence analysis (DCA) was run and, being the length of gradients less than 3, a redundancy analysis (RDA) was selected [29] and furtherly performed with 499 permutations under reduced model. A matrix of FRAP, DPPH, ABTS was used as set of dependent variables, while pigment (chlorophylls and carotenoids), and TPC as well as the scores of the first three axes of the PCA carried out on bioactive compounds were selected as explanatory variable matrix. The first two axes of the PCA carried out on mineral composition were furtherly added to the multivariate plot as passive variables.

Univariate analyses were carried out with R software [30]. The GLM were run with the 'glmmTMB' package [31] and Tukey's post-hoc tests were computed with the 'emmeans' package [32]. Multivariate analyses were performed using CANOCO 4.5 software (Ithaca, NY, USA).

3. Results

3.1. Mineral composition

3.1.1. Trace elements

Total trace elements concentrations ranged from 92 mg kg⁻¹ (*B. officinalis*) to 26 mg kg⁻¹ (*T. officinale*) following the decreasing trend *B. officinalis* > *P. betonicifolium* > *A. millefolium* > *A. xanthochlora* > *S. vulgaris* > *B. bonus-henricus* > *P. lanceolata* > *T. officinale* (Fig. 3).

Generally, Fe, Mn, Zn, and Cu were the most abundant micro-minerals in all species, while As, Sn, and V were found in traces. Iron showed a mean value of 14.7 mg kg⁻¹, being highest in *P. betonicifolium* (24.1 mg kg⁻¹) and lowest in *P. lanceolata* (7.8 mg kg⁻¹). Manganese showed a mean value of 30.6 mg kg⁻¹, being highest in *B. officinalis* (57.8 mg kg⁻¹) and lowest in *T. officinale* (7.9 mg kg⁻¹). Zinc showed a mean value of 13.0 mg kg⁻¹, being highest in *P. betonicifolium* (22.8 mg kg⁻¹) and lowest in *T. officinale* (7.0 mg kg⁻¹). Copper showed a mean value of 1.4 mg kg⁻¹, being highest in *A. millefolium* (2.5 mg kg⁻¹) and lowest in *S. vulgaris* (0.9 mg kg⁻¹).

Cultivated green leafy vegetables generally showed similar or lower trace elements values than WLVs, i.e., 7.05–18.9 mg Fe kg⁻¹ FW, 1.07–1.48 mg Mn kg⁻¹ FW, 2.37–4.17 mg Zn kg⁻¹ FW, and 0.24–0.46 mg Cu kg⁻¹ FW in lettuce [33]; 2.4 mg Fe kg⁻¹ FW, 1 mg Mn kg⁻¹ FW, 1.6 mg Zn kg⁻¹ FW, and 0.51 mg Cu kg⁻¹ FW in chicory [34].

The observed values of metal concentrations were below the maximum limits set by EU Regulation 915/2023 for certain contaminants in food, i.e. Cd (0.20 mg kg⁻¹) and Pb (0.30 mg kg⁻¹ in leaf vegetables excluding fresh herbs and edible flowers), and by EU Regulation 73/2018 on maximum residue levels for Hg compounds in or on certain products (0.01 mg kg⁻¹). Indeed, Cd showed a mean value of 0.054 mg kg⁻¹, being highest in *T. officinale* (0.103 mg kg⁻¹) and lowest in *B. bonus-henricus* (0.003 mg kg⁻¹). Lead showed a mean value of 0.026 mg kg⁻¹, being highest in *A. millefolium* (0.070 mg kg⁻¹) and lowest in *B. bonus-henricus* (0.009 mg kg⁻¹) and in *S. vulgaris* (0.009 mg kg⁻¹). Mercury was found at 0.001 mg kg⁻¹ in all samples (results not reported in Fig. 3).

Regarding the other nonessential elements, nickel (Ni) showed a mean value of 0.44 mg kg⁻¹, being highest in *B. officinalis* (1.25 mg kg⁻¹) and lowest in *B. bonus-henricus* (0.049 mg kg⁻¹). Arsenic (As) showed its highest value in *A. millefolium* (0.163 mg kg⁻¹), its lowest value in *T. officinale* (0.005 mg kg⁻¹), and a mean value of 0.031 mg kg⁻¹. Conversely, chromium (Cr) showed the highest value in *B. officinalis* (0.074 mg kg⁻¹) and its lowest value in *S. vulgaris* (0.005 mg kg⁻¹), with a mean value of 0.034 mg kg⁻¹. Tin (Sn) showed its highest value in *P. betonicifolium* (0.023 mg kg⁻¹) and its lowest in *T. officinale* (0.002 mg kg⁻¹) and *P. lanceolata* (0.003 mg kg⁻¹), and a mean value of 0.009 mg kg⁻¹. Vanadium (V) showed its highest value in *P. betonicifolium* (0.015 mg kg⁻¹) and its lowest values were found in *A. millefolium* (0.002 mg kg⁻¹) and *B. officinalis* (0.003 mg kg⁻¹), with a mean value of 0.007 mg kg⁻¹.

These results are confirmed by the PCA plot (Fig. 4), which explained a total variance of 71.6 % on the first two axes. The multivariate analysis clearly separated the eight WLVs from each other based on their mineral compositions. Particularly, it highlighted higher concentrations of nonessential micronutrients (especially As, Pb, Cd, and Ni) on the first axis and, consequently, in *A. millefolium*, and a strong connection of *P. betonicifolium* with the essential metal contents Fe, Zn, and V on the second axis. Other species like *P. lanceolata*, *T. officinale*, and *S. vulgaris* confirmed to have the lowest concentrations of the analysed trace elements, being arranged opposite most of the mineral arrows in the plot. Therefore, the first axis highlighted an increasing concentration of potentially toxic elements in the assessed species, while the second axis showed a gradient of micronutrients required for human dietary integration.

3.1.2. Nitrates

The presence of nitrates was observed only in *B. bonus-henricus* (100–250 mg kg⁻¹) and in *B. officinalis* (<100 mg kg⁻¹), being far below the legal limits determined by European Commission Regulation for certain contaminants in foodstuffs (EC No. 915/2023). Indeed, the maximum admitted levels for the presence of nitrates in lettuce and spinach, either fresh or frozen, are 2000–4500 mg kg⁻¹ FW, according to the product and harvest period.

3.2. Pigment status

Chlorophyll and carotenoid contents varied among species (Fig. 5), showing higher values in *B. bonus-henricus* (2.05 and 0.47 µg mg⁻¹ FW for chlorophylls and carotenoids, respectively) and *P. betonicifolium* (2.11 and 0.52 µg mg⁻¹ FW) and lower values in *P. lanceolata* (0.84 and 0.21 µg mg⁻¹ FW).

3.3. Phenolic composition and vitamin C

The TPC showed its highest value in *A. xanthochlora* (14.42 mg GAE g⁻¹ FW) and its lowest value in *A. millefolium* (1.22 mg GAE g⁻¹ FW) (Fig. 6).

The sum of the phenolic compounds analysed was the highest in *A. xanthochlora* (1200.38 mg GAE 100 g⁻¹ FW) and the lowest in *A. millefolium* (155.30 mg GAE 100 g⁻¹ FW (Fig. 7). Overall, flavanols were the most abundant phenolic class, followed by benzoic acids, while cinnamic acids and flavonols were detected in a lower amount (Fig. 7).

Alchemilla xanthochlora also presented the highest content of flavanols (954.581 mg 100 g⁻¹) and high content of benzoic acids (102.72 mg 100 g⁻¹) and flavonols (121.01 mg 100 g⁻¹), while it showed low values of cinnamic acids (22.07 mg 100 g⁻¹).

Conversely, *A. millefolium* was poor in all phenolic classes.

Bistorta officinalis and *B. bonus-henricus* showed both high phenolic contents; they showed the highest content of flavanols (468.49 and 418.00 mg 100 g⁻¹ respectively), and *B. officinalis* resulted the richest in cinnamic acids (240.34 mg 100 g⁻¹), while *B. bonus-henricus* resulted the richest in benzoic acids (235.87 mg 100 g⁻¹), together with *P. betonicifolium* and *S. vulgaris* (227.22 and 372.86 mg 100 g⁻¹ respectively). *Silene vulgaris* showed the highest content of flavonols (223.67 mg 100 g⁻¹).

Phyteuma betonicifolium was also the richest in vitamin C (144.41 mg 100 g⁻¹), together with *A. xanthochlora* (116.65 mg 100 g⁻¹); this latter was not different from *B. officinalis* (88.66 mg 100 g⁻¹). The other five species, namely *A. millefolium*, *B. bonus-henricus*, *P. lanceolata*, *S. vulgaris*, and *T. officinale* showed very low or null content (Fig. 7).

The contribution of single phenolic and vitamin C to the phytochemical profiles of the different species is shown in the PCA reported in Fig. 8. The multivariate analysis explained the 67.9 % of the variance on the first three axes. The first axis highlighted a gradient of the main cinnamic acids (caffeic, chlorogenic, and ferulic acids) together with hyperoside flavonol acid, with *B. officinalis* showing the highest concentration of these compound group. The second axis less clearly segregated classes of compounds; quercetin and ellagic (in the bottom part) acids were the most relevant ones, these latter being associated with *S. vulgaris*. The third axis highlighted an increasing abundance of most flavonols and vitamin C, with *A. xanthochlora* being mainly associated with high concentrations of these compounds.

3.4. Antioxidant activity

GAS was highest in *A. xanthochlora* leaves (almost five times higher than the other species), and lowest in *A. millefolium* and *S. vulgaris* (Fig. 9).

Alchemilla xanthochlora showed the highest values for FRAP (340.728 mmol Fe²⁺ kg⁻¹ FW), DPPH 149.39 µmol TE g⁻¹ FW) and ABTS (182.23 µmol TE g⁻¹ FW).

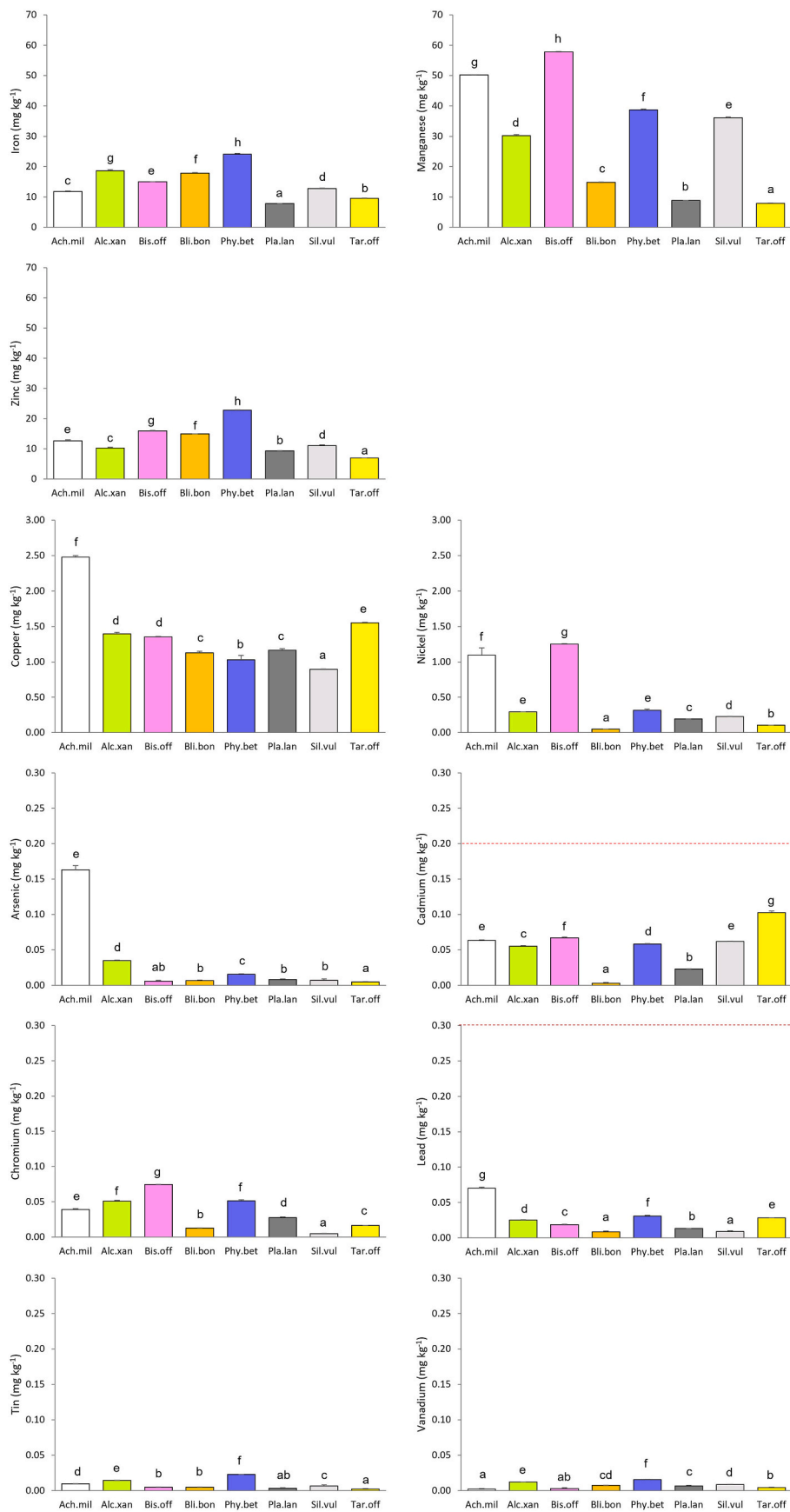


Fig. 3. Differences among WLVs species in terms of trace elements. Error bars represent the standard deviation of the means, while different letters indicate significant differences ($p < 0.05$) among species. Red lines indicate the maximum concentrations defined by the Regulation (CE) No. 915/2023 for Cd and Pb. For plant codes see Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

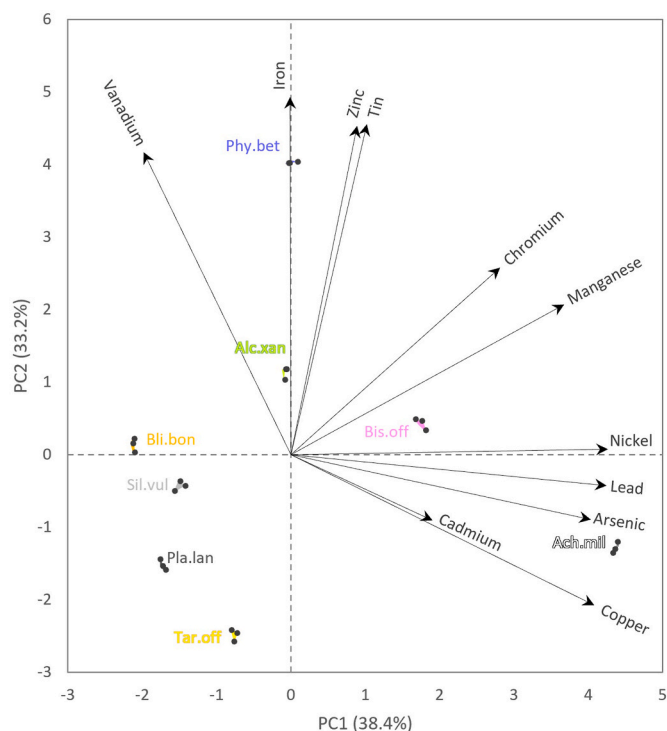


Fig. 4. PCA plot showing the relationships among the concentrations of the main trace elements of the eight analysed WLVs. The percentage variance explained by each axis is reported in brackets. For plant codes see Table 1.

Conversely, *A. millefolium*, *S. vulgaris*, and *T. officinale* showed the lowest values for FRAP (28.052, 31.518, and 32.158 mmol Fe²⁺ kg⁻¹ FW respectively). *Achillea millefolium* and *S. vulgaris* also showed the lowest DPPH values (0.59 and 1.51 μmol TE g⁻¹ FW respectively). The ABTS assay showed low similar values for all species, except for *A. xanthochlora*.

The relationships among antioxidant activity (in terms of ABTS, DPPH, and FRAP) and the main phytochemical variables obtained by the previous analyses are reported in the RDA (Fig. 10). The adjusted R² of the multivariate analysis was 0.975, with 98.1 % of variance explained by the first two axes. As noticeable on the first axis, the antioxidant activity (ABTS, DPPH, FRAP) was mainly influenced by TPC and by the third component of bioactive compound PCA (Bioactive_PC3, related to main flavonols and vitamin C). These drivers were the most relevant in segregating the species and confirmed their strong relationship with *A. xanthochlora*.

4. Discussion

The nutritional value is a key aspect of food product quality assessment [35]. This study assessed the phytochemical profiles of eight WLV species, to enhance traditional food habits and to investigate the possibility of using them along with cultivated vegetables, such as lettuce and spinach, and as functional food products, similarly to microgreens [36]. The major determinants of the nutritional quality of leafy vegetables are: (i) the mineral composition [37]; (ii) the pigments status, namely chlorophylls and carotenoids [16,38,39]; and (iii) the phenolic and vitamin content [35,39].

4.1. Mineral composition

4.1.1. Trace elements

Green leafy vegetables contain numerous minerals which are essential for the correct functioning of the human body and are gaining attention in the prevention of various diseases [8,21].

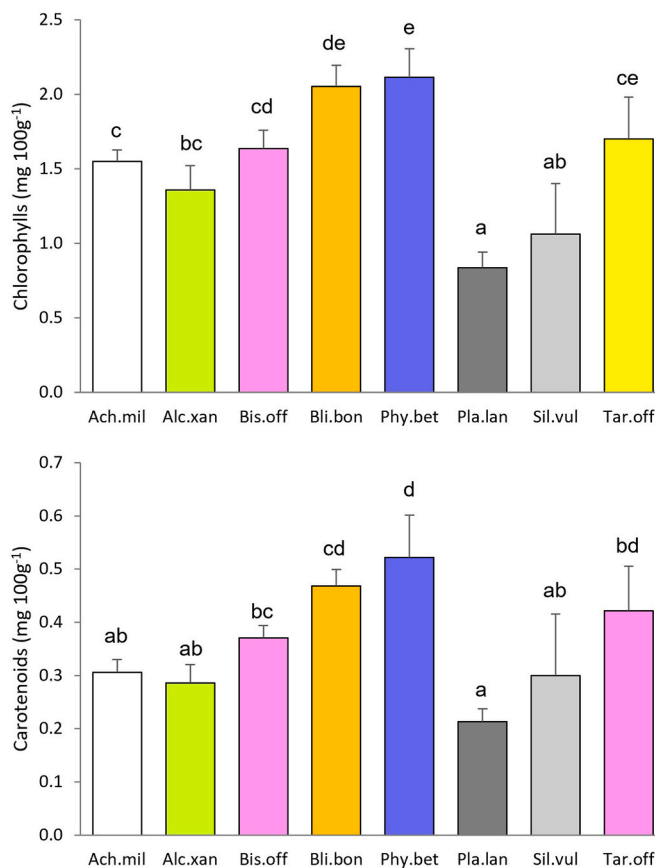


Fig. 5. Differences among WLVs species in terms of pigment (chlorophylls and carotenoids). Error bars represent the standard deviation of the means, while different letters indicate significant differences ($p < 0.05$) among species. For plant codes see Table 1.

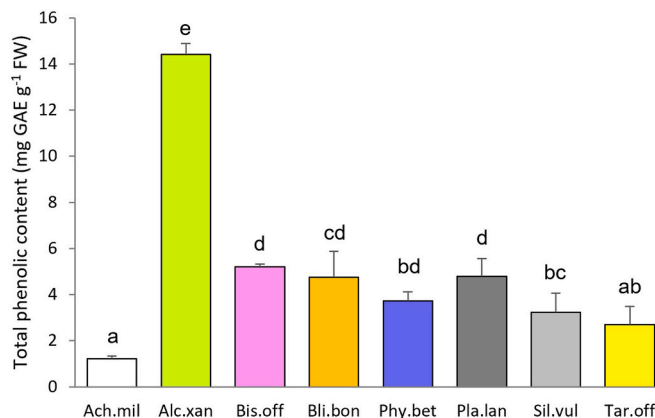


Fig. 6. Differences among WLVs species in terms of total phenolic content (TPC). Error bars represent the standard deviation of the means, while different letters indicate significant differences ($p < 0.05$) among species. For plant codes see Table 1.

Several WLVs already showed higher mineral contents than cultivated green leafy vegetables, such as lettuce, chicory, spinach, or cabbage [18,34].

Compared to lettuce (*Lactuca sativa* L.), all the eight WLVs analysed showed a higher Cu and Zn content (0.27–0.42 mg Cu kg⁻¹ FW; 4,53–5,55 mg Zn kg⁻¹ FW [33]). *Alchemilla xanthochlora*, *B. bonus-henricus*, *B. officinalis* and *P. betonicifolium* also showed a higher Fe content, while *A. millefolium*, *P. lanceolata*, *S. vulgaris* and *T. officinale*

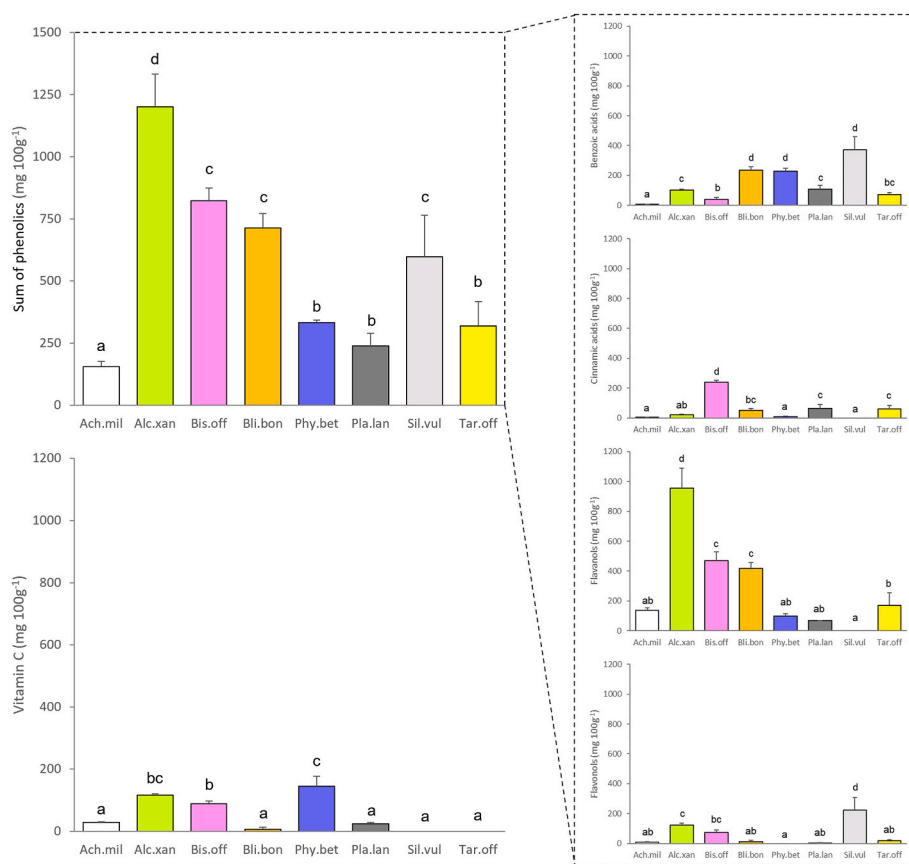


Fig. 7. Differences among WLVs in terms of phenolic profile and vitamin C content (sum of ascorbic acid and dehydroascorbic acid). Phenolic profile is presented as both singular class (i.e. benzoic acids, cinnamic acids, flavanols, and flavonols) concentration and sum of phenolics. Error bars represent the standard deviation of the means, while different letters indicate significant differences ($p < 0.05$) among species. For plant codes see [Table 1](#).

showed similar Fe values (6.34–11.7 mg Fe kg⁻¹ FW [33]). Regarding Mn, *A. millefolium*, *A. xanthochlora*, *P. betonicifolium*, *B. officinalis* and *S. vulgaris* showed higher values, while *B. bonus-henricus*, *P. lanceolata* and *T. officinale* showed similar values (8,11–13,1 mg Mn kg⁻¹ FW [33]).

The eight WLVs analysed showed a higher Cu, Zn, Fe and Mn content than chicory (*C. intybus*) (0.51 mg Cu kg⁻¹ FW; 1.6 mg Zn kg⁻¹ FW; 2.4 mg Fe kg⁻¹ FW; 1 mg Mn kg⁻¹ FW [34]).

Therefore, including WLVs in the diet can contribute to the Recommended Daily Allowance of trace elements, i.e., the average level of daily intake of elements in the diet, sufficient to meet the needs of almost all healthy individuals (men and women aged from 31 to 50) [8].

Compared to other wild vegetables, the eight WLVs analysed contained 0.9–2.5 mg Cu kg⁻¹, values similar to or lower than those found in wild *Rumex acetosa* L. (2.7 mg kg⁻¹), *Picris hieracioides* L. (4.7 mg kg⁻¹), *Cichorium intybus* L. (2.9 mg kg⁻¹), and *Plantago coronopus* L. (2.7 mg kg⁻¹) [40], and in wild *Rumex pulcher* L. (1 mg kg⁻¹) leaves [6]. Particularly, the high *A. millefolium* Cu content (2.5 mg kg⁻¹) suggests that 100 g of it may contribute to almost 30 % of the Cu Recommended Daily Allowance of 0.9 mg day⁻¹ [8].

The eight WLVs contained 7.8–24.1 mg Fe kg⁻¹, values similar to those found in *R. acetosa* (22.73 mg kg⁻¹), *C. intybus* (24.62 mg kg⁻¹), and *P. coronopus* (28.55 mg kg⁻¹) [40] and in *R. pulcher* (12.6 mg kg⁻¹) [6], but they were lower than the Fe content found in *P. hieracioides* (124.86 mg kg⁻¹) [40]. *Phyteuma betonicifolium* Fe content (24.1 mg Fe kg⁻¹) suggests that 100 g of it may contribute to 30 % of the Fe Recommended Daily Allowance (8 mg day⁻¹) for male adults, and to 13 % (18 mg day⁻¹) for female adults [8].

The eight WLVs contained 7.9–57.8 mg Mn kg⁻¹, values similar to or higher than those found in *R. acetosa* (15 mg kg⁻¹), *P. hieracioides* (11.6

mg kg⁻¹), *C. intybus* (7.1 mg kg⁻¹), and *P. coronopus* (4.8 mg kg⁻¹) [40], and higher than those found in *R. pulcher* (2.2 mg kg⁻¹) [6]. *Polygonum bistorta* Mn content (57.8 mg kg⁻¹) suggests that 100 g of it may contribute to more than the whole Mn Recommended Daily Allowance for male (2.3 mg day⁻¹) and female (1.8 mg day⁻¹) adults [19].

The eight WLVs contained 7–22.8 mg Zn kg⁻¹, values similar to or higher than those found in *R. acetosa* (6.9 mg kg⁻¹), *P. hieracioides* (10.3 mg kg⁻¹), *C. intybus* (6.4 mg kg⁻¹), and *P. coronopus* (4.5 mg kg⁻¹) [40], and in *R. pulcher* (3.9 mg kg⁻¹) [6]. *Phyteuma betonicifolium* Zn content (22.8 mg kg⁻¹), suggests that 100 g of it may contribute to 20 % of the Zn Recommended Daily Allowance (11 mg day⁻¹) for male adults, and to more than 28 % (8 mg day⁻¹) for female adults [8].

The multivariate analysis highlighted a strong connection of *P. betonicifolium* with the essential metal contents Fe, Zn, and V, confirming its high essential element content and the benefits of its use as a human diet supplement.

Although trace elements are important in the human diet (e.g. nickel is a cofactor of various enzymes [41]) and their scarcity may cause various diseases, attention must be paid to toxic minerals, such as Pb, Hg, As, and Cd, which can accumulate in plants due to anthropogenic activities, sometimes beyond limits, making them unsuitable for human consumption [21,41]. For instance, Pb and Cd can become harmful to human health affecting nerve, gastrointestinal, reproductive, or biochemical activities [21].

The values of toxic elements in the eight analysed WLVs were below legal limits, therefore they resulted suitable for human consumption. However, the multivariate analysis confirmed high concentrations of As, Pb, Cd, and Ni in *A. millefolium*, suggesting that this species should be consumed with caution, and not in excessive quantities.

The eight WLVs showed 0.004–0.163 mg As kg⁻¹ and 0.003–0.103

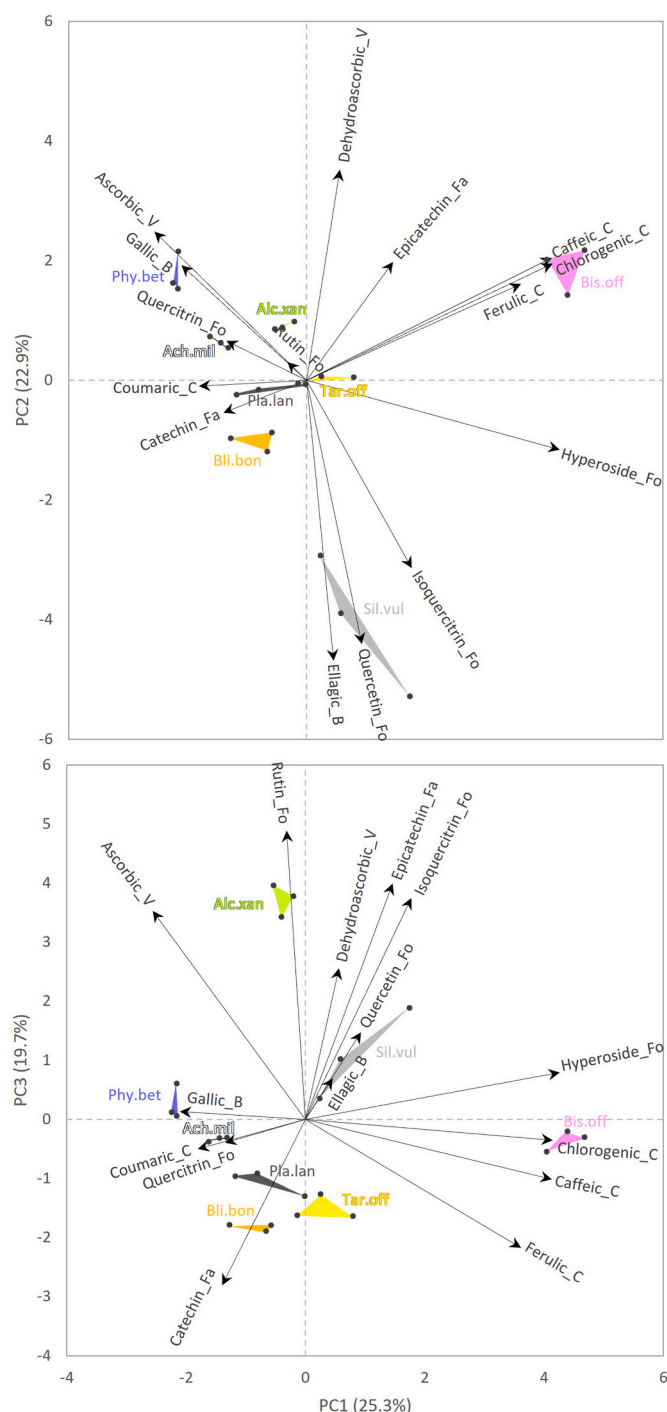


Fig. 8. PCA plots (first-second and first-third axes are displayed) highlighting the relationships among the main bioactive compounds found in the eight analysed species. The percentage variance explained by each axis is reported in brackets. Suffixes indicate the main compound class: B, benzoic acids; C, cinnamic acids; Fa, flavanols; Fo, flavonols; V, vitamin C. For plant codes see Table 1.

mg Cd kg⁻¹, values similar to or higher than three microgreen and baby green species, i.e., *Sanguisorba minor* Scop. (0.024 mg As kg⁻¹ and 0.005 mg Cd kg⁻¹), *Sinapis arvensis* L. (0.01 mg As kg⁻¹ and 0.004 mg Cd kg⁻¹), *T. officinale* (0.041 mg As kg⁻¹ and 0.006 mg Cd kg⁻¹), grown in a hydroponic system [36]; conversely, the eight WLVs showed 0.005–0.074 mg Cr kg⁻¹, values lower than the range found by Lenzi and colleagues (0.593–2.673 mg kg⁻¹ FW) [36]. Also Pb values (0.009–0.07 mg kg⁻¹) were similar to those found in *S. arvensis* (0.018 mg kg⁻¹) and

T. officinale (0.046 mg kg⁻¹) [36], similarly to Ni values (0.049–1.25 mg kg⁻¹), which were similar to those found in *S. minor* (1.385 mg kg⁻¹) and *T. officinale* (1.398 mg kg⁻¹) [36].

The eight WLVs showed 0.002–0.023 mg Sn kg⁻¹, values like those found in *Cannabis sativa* L. microgreens, in the ‘Antal’ variety (0.002 mg kg⁻¹ FW) [42].

Vanadium showed low values, not exceeding the recommended dietary intake [43].

4.1.2. Nitrates

Nitrates themselves are not toxic, but their metabolites produced within the human body can be extremely harmful [20,40]. Therefore, a European Commission Regulation [44] established the highest nitrate limit allowed to be present in certain leafy vegetables (spinach and lettuce), which are known to accumulate high amounts of these compounds. Depending on the species and the type of product (either fresh, frozen, or deep-frozen), the limits range between 2000 and 4500 mg kg⁻¹ nitrate.

The only nitrates presence was found in leaves of *B. bonus-henricus* (100–250 mg kg⁻¹) and *B. officinalis* (<100 mg kg⁻¹), similarly to the value obtained by Ceccanti and colleagues [40] in the leaves of *P. hieracioides*, *C. intybus*, and *P. coronopus* (130, 250, and 110 mg g⁻¹ FW respectively) collected in the wild. However, these values were far below the legal limits.

4.2. Pigment status

Chlorophylls and carotenoids have antioxidant effects and can be useful in the production of natural food colorants [38,45]. Chlorophylls and carotenoids are important in the metabolism of light energy and in catalyzing the formation of carbohydrates; they can also be useful in the production of natural food colorants. Moreover, they have anti-cancer, anti-mutagenic and antioxidant effects [38,45].

The chlorophylls and carotenoids ranges of values were similar to those present in the five WLVs analysed by Ceccanti and colleagues [16], i.e., *R. acetosa*, *C. intybus*, *P. hieracioides*, *S. minor*, and *P. coronopus*. The chlorophylls values were also similar to the four WEPs (*Urtica dioica* L., *Aegopodium podagraria* L., *T. officinale*, *Stellaria media* L.) studied by Ozola and colleagues [38], that showed however higher carotenoid content (from 0.74 to 1.62 µg mg⁻¹ FW) than ours. Conversely, *B. bonus-henricus* and *S. vulgaris* showed a higher carotenoid content than the same species analysed by Milani and colleagues (0.10 µg mg⁻¹ FW and 0.12 µg mg⁻¹ FW respectively; [12]).

All eight WLVs showed higher chlorophyll values than *L. sativa* (0.5–0.6 µg mg⁻¹ FW [33]). Six WLVs showed higher chlorophyll values than cabbage (*B. oleracea* var. *capitata*) leaves (1.27 µg mg⁻¹ FW [46]), while *P. lanceolata* and *S. vulgaris* showed a lower value. All eight WLVs analysed showed higher carotenoids values than *B. oleracea* var. *capitata* leaves (0.011 µg mg⁻¹ FW [46]). Comparing our values with other wild species, four WLVs analysed showed similar chlorophyll values to *S. minor*, *S. arvensis*, and *T. officinale* microgreens and baby leaves (1.44–1.55 µg mg⁻¹ FW [36]), with the exception of *P. lanceolata* and *S. vulgaris*, which showed lower values, and *B. bonus-henricus* and *P. betonicifolium*, which showed higher values.

Therefore, WLVs have good chlorophyll and carotenoid contents compared to other WLVs, as well as to normal vegetables.

4.3. Phenolic composition and vitamin C

Phenolic compounds can have an important influence over appearance, flavour and health-promoting properties, therefore affecting the quality of food products [8]. Moreover, they have antioxidant, anti-inflammatory, antitumor and antimicrobial properties, among other effects, and their inclusion in a regular diet has beneficial health effects [8,47].

Recently, several studies have highlighted how WLVs microgreens

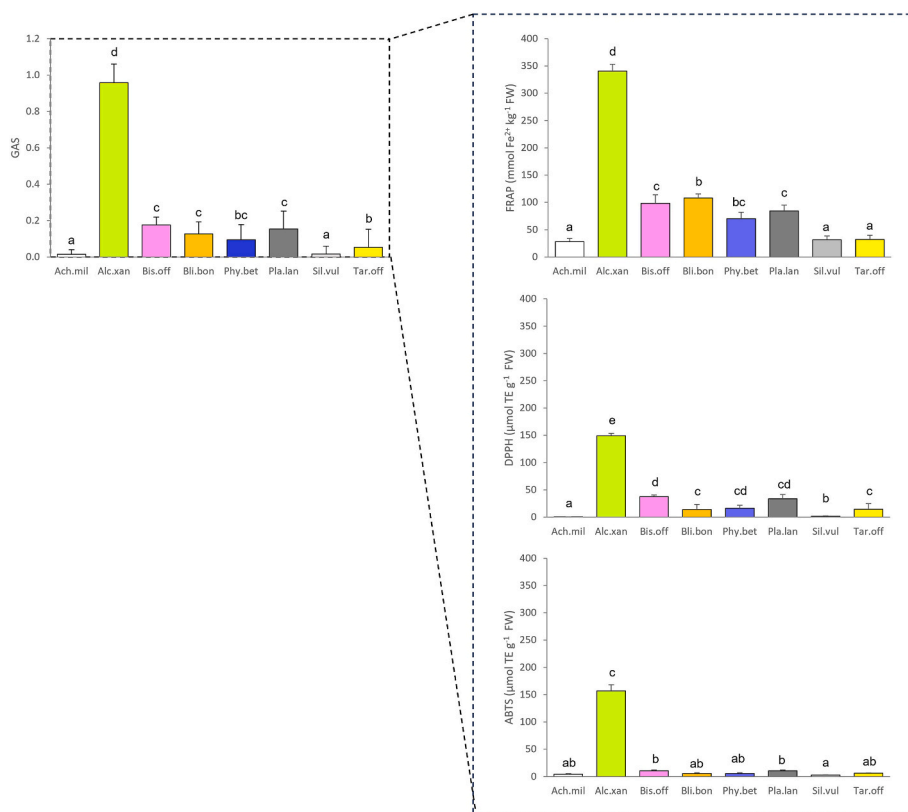


Fig. 9. Differences among WLVs according to their antioxidant activity in terms of FRAP, DPPH, ABTS, and GAS. Error bars represent the standard deviation of the means, while different letters indicate significant differences ($p < 0.05$) among species. For plant codes see Table 1.

and baby greens are rich in antioxidant and health-promoting compounds [36]. The WLVs analysed showed higher TPC values than *L. sativa* ($2.69 \text{ mg GAE g}^{-1} \text{ FW}$) [48], with the exception of *A. millefolium*, which showed a lower value; they also showed higher TPC values than white cabbage (*B. oleracea* var. *capitata*) ($0.21\text{--}0.3 \text{ mg GAE g}^{-1} \text{ FW}$) and savoy cabbage (*B. oleracea* var. *sabauda*) ($0.48\text{--}0.54 \text{ mg GAE g}^{-1} \text{ FW}$) [49]. Compared to other WLVs, the analysed species showed overall lower TPC values than *R. acetosa*, *C. intybus*, *S. minor* and *P. coronopus*, except for *A. xanthochlora* which showed similar results to wild-collected *P. hieracioides* [16]. Conversely, *B. bonus henricus* showed a higher TPC than the same species analysed by Milani and colleagues ($2.95 \text{ mg GAE g}^{-1} \text{ FW}$), whereas *S. vulgaris* and *Taraxacum officinale* showed a similar TPC than the same species (3.75 and $2.64 \text{ mg GAE g}^{-1} \text{ FW}$ respectively) [12].

Cinnamic acids are a wide group of polyphenols with antioxidant activity and they are effective in reducing blood pressure and triglycerides [50]. *Bistorta officinalis* showed a chlorogenic acid content similar to *Fagopyrum tataricum* ($129 \text{ mg } 100 \text{ g}^{-1}$ [51]) and *Ocimum basilicum* L. ($14.83 \text{ mg } 100 \text{ g}^{-1}$ green basil and $13.97 \text{ mg } 100 \text{ g}^{-1}$ purple basil [52]) microgreens. *Blitum bonus-henricus* and *P. lanceolata* showed coumaric acid values higher than in some microgreens, i.e., *Corchorus olitorius* L., *Brassica oleracea* L. var. *gongylodes*, *Brassica rapa* L. subsp. *chinensis* ranging, *O. basilicum*, *Raphanus sativus* L., *Beta vulgaris* L. subsp. *vulgaris*, and *Brassica rapa* L. subsp. *narinosa*, which ranged from 0.51 to $2.59 \text{ mg } 100 \text{ g}^{-1}$ [52]. *Blitum bonus-henricus*, *P. lanceolata*, *B. officinalis* and *T. officinale* showed a higher ferulic acid content than Kaur and colleagues' [53] results for mung bean (*Vigna radiata* L.) microgreens ($0.36 \text{ mg } 100 \text{ g}^{-1}$) grown in soil. The multivariate analysis confirmed the high content of cinnamic acids in *B. officinalis*, suggesting that its use may be helpful in the counteraction of reactive oxygen species.

Flavonols show in vitro anti-tumor activity and reduction of thrombotic tendency [54]. *Achillea millefolium*, *A. xanthochlora*, and *S. vulgaris* showed a rutin content similar to cress, green basil, mibuna,

mustard and purple basil microgreens (1.83 , 1.95 , 3.71 , 8.56 , and $1.90 \text{ mg } 100 \text{ g}^{-1}$ respectively), but lower than coriander microgreens ($239.22 \text{ mg } 100 \text{ g}^{-1}$; [52]). *Silene vulgaris* showed a quercetin amount higher than *F. tataricum* microgreens ($129 \text{ mg } 100 \text{ g}^{-1}$; [51]). The multivariate analysis confirmed its high quercetin content, suggesting that its use may be helpful against inflammatory and cardiovascular diseases, and in tumour prevention. A 33 g portion of these leaves may cover the estimated mean quercetin intake of 29.4 mg day^{-1} [50].

Benzoic acids are another large group of polyphenols with effects similar to those of cinnamic acids [50]. The highest gallic acid content of *P. betonicifolium* was higher than the range of values obtained by Kaur and colleagues [53] in black gram (*Vigna mungo* L.), mung bean (*Vigna radiata* L.) and chickpea (*Cicer arietinum* L.) microgreens (12.55 , 12.33 , and $8.55 \text{ mg } 100 \text{ g}^{-1}$ respectively). Ellagic acid, as highlighted by the multivariate analysis, showed its highest concentration in *S. vulgaris*, making this species useful against inflammatory diseases and in reducing blood pressure and cholesterol levels [50].

Flavonols are related to beneficial health effects reducing the occurrence of chronic cardiovascular diseases and cancer, as well as having anti-obesity potential, since they help decrease the body mass index and waist circumference [50]. The highest value of catechin in *B. bonus-henricus* was higher than the values found by Kaur and colleagues [53] in the three soil-grown microgreens ($66.32\text{--}77.58 \text{ mg } 100 \text{ g}^{-1}$); similarly, epicatechin values were higher than in mung bean grown in soil ($1.06 \text{ mg } 100 \text{ g}^{-1}$) [53]. *Blitum bonus-henricus* consumption may therefore be useful in the prevention of cardiovascular diseases and cancer [50].

Vitamin C is associated to beneficial health effects as the prevention of ageing effects, cardiovascular and cancer diseases [55,56]. Total vitamin C was not detected in *S. vulgaris* and *T. officinale*. The other six WLVs showed similar or higher vitamin C content than *L. sativa* ($10.3\text{--}19.4 \text{ mg } 100 \text{ g}^{-1}$; [57]) and savoy cabbage (*B. oleracea* var. *sabauda*: $61.9 \text{ mg } 100 \text{ g}^{-1} \text{ FW}$; [58]), with the exception of

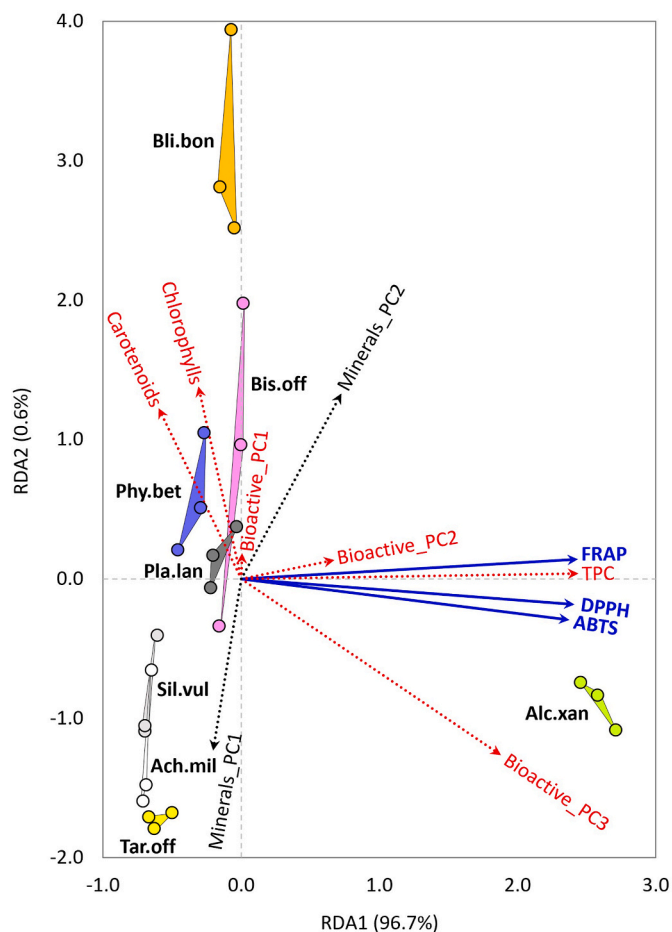


Fig. 10. Redundancy analysis showing the relationships among the antioxidant activity (ABTS, DPPH, FRAP, blue solid arrows) and the main phytochemical variables (red dashed arrows) obtained by the PCA on mineral composition. PC, principal component; TPC, total phenolic content. For plant codes see Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

B. bonus-henricus, which was lower. Compared to white cabbage (*B. oleracea* var. *capitata*: 36.60 mg 100 g⁻¹ FW [59]; 34.70 mg 100 g⁻¹ FW; [60]), *A. millefolium*, *B. bonus-henricus*, and *P. lanceolata* showed a lower vitamin C content, while *A. xanthochlora*, *P. betonicifolium*, and *B. officinalis* showed higher values.

Phenolic compounds are bioactive non-nutrients, for which there is no specific recommended dietary intake, as in Europe it varies depending on country and dietary pattern, from about 600 to 1700 mg day⁻¹ [61]. They are very important for their antioxidant activity, which enables the scavenging of stress-induced reactive oxygen species, and for their anti-inflammatory, antitumor and antimicrobial properties [8]; therefore WLVs rich in phenolic compounds are important for human dietary implementation.

Total vitamin C was similar to or higher than that of six raw WLVs (*Rumex pulcher* L., *Silene vulgaris* (Moench) Garcke., *Asparagus acutifolius* L., *Bryonia dioica* L., *Humulus lupulus* L., *Tamus communis* L.) (25.1–60.9 mg 100 g⁻¹) analysed by García-Herrera and colleagues [6]. Particularly, the high *P. betonicifolium* vitamin C content (144.41 mg 100 g⁻¹ FW) suggests that 100 g of it may cover the vitamin C Recommended Daily Allowance of 90 mg day⁻¹ [8]. *Alchemilla xanthochlora* was mainly associated with high concentrations of flavonols and vitamin C, however, due to its correlation with potential toxic trace elements, it is advisable to consume it with moderation.

4.4. Antioxidant activity

The antioxidant activity values depend on the extraction method, reaction times and standards used for normalisation; for these reasons, data obtained by different researches are sometimes difficult to compare [62]. GAS values may be used as an important ranking tool for plant material evaluation. Examples reported in the literature concern red wine [63], lavender [64] and rosmarinus [65]. Freshly harvested and unprocessed *A. xanthochlora* leaves showed the highest antioxidant activity among the eight species as also shown by its highest GAS value.

In details, compared to other cultivated green leafy vegetables, five WLVs, namely *A. millefolium*, *B. bonus-henricus*, *P. betonicifolium*, *S. vulgaris*, and *T. officinale*, showed DPPH values lower than *L. sativa* (40.26 μmol TE g⁻¹ FW; [48]), two WLVs, namely *P. lanceolata* and *B. officinalis*, showed a similar value, while *A. xanthochlora* showed a higher value.

All eight WLVs showed a higher antioxidant activity than savory cabbage (FRAP: 2.6 mmol Fe²⁺ kg⁻¹ FW) and white cabbage (FRAP: 2.0 mmol Fe²⁺ kg⁻¹ FW; ABTS: 2.44–3.26 μmol TE g⁻¹ FW), with the exception of *S. vulgaris*, which showed similar ABTS values [49,60].

Compared to other WLVs, the analysed species showed higher FRAP values than those found in other edible wild green species, i.e., *Asparagus acutifolius* L., *Borago officinalis* L., *C. intybus*, *Diplotaxis erucoides* L., *Sinapis incana* L., and *Sinapis nigra* L. (from 10.33 to 19.03 mmol Fe²⁺ kg⁻¹ of wet weight; [66]), and *P. lanceolata* and *S. vulgaris* (43.8 and 27.4 mmol Fe²⁺ kg⁻¹ FW respectively; [67]), but it must be noted that, in the first study samples were boiled, while in the second extractions occurred in an ethanol/water solution (85 : 15, v/v).

Concerning ABTS assay, seven WLVs analysed showed values similar to or lower than *Allium cepa* L., *Brassica juncea* L., *Daucus carota* L., *Foeniculum vulgare* Mill., *Helianthus annuus* L., *Hibiscus sabdariffa* L., *O. basilicum*, *Raphanus sativus* L., *Spinacia oleracea* L., and *Trigonella foenum-graecum* L. microgreens (from 10.9 to 22.8 μmol TE g⁻¹ FW; [68]), with the exception of *A. xanthochlora*, which was much higher than their results. Conversely, all eight WLVs showed higher values than *A. acutifolius*, *B. officinalis*, *C. intybus*, *D. erucoides*, *S. incana*, and *S. nigra* (from 2.99 to 4.59 μmol TE g⁻¹ of wet weight; [66]).

The RDA analysis highlighted how the antioxidant activity was mainly influenced by TPC, flavonols and vitamin C, also confirming the high antioxidant activity of *A. xanthochlora*.

4.5. Overall quality assessment of WLVs

The quality assessment of the selected WLVs was challenging due to the lack of literature on the same species and, even when present, the use of different extraction and analytical methods, which made data comparison difficult or not possible.

Overall, the selected species showed interesting characteristics, making them suitable as potential functional foods.

Achillea millefolium was found to be rich in minerals, especially in Cu, Mn, and Zn, and in flavanols, and to contain vitamin C. However, attention must be paid to its excessive consumption, since it can contain oxalate, a potentially toxic compound that may cause the formation of kidney stones or the reduction of the intestinal absorption of calcium [5].

Alchemilla xanthochlora showed a high mineral content, especially in Fe and Cu. It also showed very high total phenolic content and antioxidant activity. This species contained vitamin C and flavanols, particularly epicatechin, which probably contributes to the bitter flavour of this species [69].

Bistorta officinalis was found to be rich in minerals of Mn, Cu, Fe and Zn; moreover, it showed high chlorophylls, carotenoids and total phenolic content, and it contained vitamin C. Particularly, *B. officinalis* showed a high cinnamic acid content, and specifically in three of the four acids evaluated, namely caffeic acid, ferulic acid (responsible for bitterness), and chlorogenic acid (responsible for astringency) [70,71]. This species also showed a high epicatechin content.

Blitum bonus-henricus was found to be rich in the minerals Cu, Fe, and Zn. It also showed high chlorophylls, carotenoids and total phenolic content. Particularly, this species was rich in benzoic acids, specifically in ellagic acid, and in flavanols, especially in catechins, which are responsible for astringency and bitterness [70]. Caution should be taken with its overconsumption, however, as it can have high oxalate contents in the fresh leaves, from 610.5 (less mature leaves) to 867.4 mg 100 g⁻¹ (large mature leaves), which significantly decreased after boiling [72].

Phyteuma betonicifolium showed high Fe, Zn, Mn, and Cu contents, and chlorophylls and carotenoids. Compared with the other species, it showed lower phenolic content and antioxidant activity, but had a high content of benzoic acids, specifically in gallic acid (responsible for astringency - [70]), and the highest vitamin C content.

Plantago lanceolata showed high Zn and Cu contents, even if they were lower than in the other species. It had a high phenolic content, particularly benzoic and cinnamic acids, as well as high antioxidant activity, and contained vitamin C.

Silene vulgaris showed high Mn, Zn, and Cu contents, but the lowest antioxidant activity together with *A. millefolium*. However, it showed a high content of phenolic acids, among which it showed the highest values of benzoic acids, specifically of ellagic acid, and the highest values of flavonols, specifically of isoquercitrin and quercetin (the latter responsible for astringency - [35]). Caution should be taken with its overconsumption, though, as the leaves can contain calcium oxalate [20] and phytates, which decreases the bioavailability of mineral elements absorbed through the diet [73].

Finally, *T. officinale* showed high Cu content, as well as high chlorophylls and carotenoids contents. Conversely, it was poor in total phenolic content and showed a low antioxidant activity. According to Huang and colleagues [74], *T. officinale* showed a very low soluble oxalate content.

4.6. Environmental sustainability

The selected species develop in highly biodiverse secondary habitats, maintained by traditional silvo-pastoral activities in the Alpine region. These extensive silvo-pastoral systems ensure high environmental sustainability [9] and provide many ecosystem services [75], including healthy food provisioning. In these habitats, WLVs are more resistant to biotic and abiotic disturbances (such as heatwaves, diseases) than domesticated crops, ensuring their availability even under extreme environmental conditions. Indeed, the selected WLVs showed moderate plasticity to mountain ecological conditions, as almost all of them displayed intermediate preference values for moisture and temperature, which are the most common ecological conditions found in the Alps. The only exception was *B. officinalis* regarding soil moisture, as it typically thrives in very moist soils. Concerning the influence of agro-pastoral management, some of the selected species appeared more prone to harvesting, especially *A. xanthochlora*, *P. lanceolata*, and *T. officinale*. Additionally, certain species (namely, *A. xanthochlora*, *B. officinalis*, and *T. officinale*) were strictly dependent on balanced agro-pastoral activities (fertile value of 'nutrients' indicator), and a cessation of traditional extensive grassland management would directly impact on their presence and performance. This is confirmed by their optimal habitat: nutrient-rich grasslands are open habitats that require active management practices to maintain the presence of such grassland specialists species. Conversely, although *B. bonus-henricus* can form wide population patches, it is only found in over-rich conditions of soil nutrients, which occur sporadically in Alpine areas. The complex mosaic of contrasting ecological and management conditions, which results in the wide variety of vegetation communities of Alpine pastures including such small patches of low value for agro-pastoral purposes, can represent a huge source of WLVs with high nutraceutical value for human nutrition. Agri-environmental schemes promoting extensive silvo-pastoral practices appear therefore advisable to enhance the presence of these functional WLVs in the context of sustainable food

system and biodiversity conservation.

5. Conclusions

According to our results, the eight assessed WLVs hosted by semi-natural Alpine habitats can play a pivotal role in the implementation of a healthy human diet, being a source of minerals, vitamins and antioxidant compounds. Nowadays, given their low productivity, their integration into modern diets can only be achieved in the context of traditional local preparations, while representing a potential integration for local labour and low impact tourism in the Alpine communities.

To avoid the eventual overexploitation of biodiversity that would result from uncontrolled harvesting, and which could threaten the species and their related ecosystems, future research could be aimed at domestication and cultivation of the WLVs as an alternative to wild harvesting. Additionally, it is well-known that phytochemical and mineral contents may widely vary in plants, due to climate and soil characteristics. Therefore, cultivation processes may be useful to standardize their nutritional status, and to reduce the presence of anti-nutritional or toxic compounds such as nitrates, oxalates, saponins or phytates.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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Disclosure statement

The authors report there are no competing interests to declare.

CRediT authorship contribution statement

Simone Ravetto Enri: Writing – original draft, Investigation, Data curation. **Nicole Mélanie Falla:** Writing – original draft, Formal analysis. **Sonia Demasi:** Writing – review & editing, Methodology, Formal analysis. **Daniela Manila Bianchi:** Investigation. **Stefania Squadrone:** Writing – original draft, Investigation, Formal analysis. **Giampiero Lombardi:** Project administration, Funding acquisition. **Valentina Scariot:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Giampiero Lombardi reports financial support was provided by Piedmont Region. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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