



Nutraceutical compounds of edible wild plants collected in Central Italy

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Abstract

Edible wild plants (EWP_s) are a part of the ethnobotanical heritage of a territory. Several species are used in the local traditional food in central Italy, and unfortunately, the risk of losing this knowledge is currently present. The objective of this work is to evaluate the nutritional value, some active biological components, and total antioxidant capacity (ORAC) of some EWPs present in central Italy: *Allium neapolitanum* Cirillo, *Allium triquetrum* L., *Foeniculum vulgare* Mill. *Melissa officinalis* L., *Thymus serpyllum* L. *Sonchus oleraceus* L., *Reichardia picroides* L. Roth, *Rhagadiolus stellatus* L. Gaertn and *Plantago coronopus* L. The analysis determines the proximate composition, some minerals (Ca, K, Mg, Na, Fe, and P) and antioxidant vitamins (A, β -carotene, E, and C) of major nutritional interest, total polyphenols, and ORAC. The EWPs showed an interesting qualitative/quantitative composition due to the interesting fiber values, antioxidant vitamins, total polyphenols, and great values of total antioxidant capacity (3.670-20.071 μ mol TE / 100g). These results suggest an undoubted nutritional and dietary interest for the four EWPs. Their valorization and adequate consumption/intake, due to the presence of numerous nutrients and biologically active components, could contribute to significant beneficial implications for our health.

Keywords: Edible wild plants; nutraceutical activity; antioxidant capacity; ethnobotanical species

Introduction

Since time immemorial, edible wild plants (EWP_s) have constituted a large part of daily food intake for many populations, and today, particularly in rural areas, they represent an essential part of daily food consumption. EWP_s represent a rich store of food diversity that continues to be exploited in various rural areas and, in many contexts, still lies at the basis of the Mediterranean diet. Some authors maintain that diet is partly responsible for the relatively long life of Mediterranean peoples. (Pérez-López, Chedraui, Haya, & Cuadros, 2009), and indeed, the Mediterranean Basin is a hotspot of EWP biodiversity (Ceccanti, Landi, Benvenuti, Pardossi, & Guidi, 2018), whose therapeutic and culinary uses have long been known and documented. Numerous studies have shown how widespread knowledge of these species is, and the vast number of uses made them, for example, in Bulgaria (Nedelcheva, 2013), Croatia (Dolina & Łuczaj, 2014; Łuczaj, Zovko Končić, Miličević, Dolina, & Pandža, 2013), Greece (Psaroudaki, Nikoloudakis, Skaracis, & Katsiotis, 2015) and Spain (Menendez-Baceta, Aceituno-Mata, Tardío, Reyes-García, & Pardo-de-Santayana, 2012), where they have been used for centuries in cooking, medicine, cosmetics, and as fuel. In Italy, around 828 species from 98 botanical families are defined as edible species and are a part of the ethnobotanical heritage. The interest in these plant species is based on the positive health benefits associated with their chemical composition, such as the availability of numerous nutrients and bioactive compounds (Maurizi et al., 2015; Romojaro, Botella, Obón, & Pretel, 2013). Various authors have studied their nutraceutical properties as they are readily available and rich in essential nutrients such as proteins, vitamins, minerals, fats, and carbohydrates (Ranfa & Bodesmo, 2017; Ranfa et al., 2015) as well as having antioxidant activity and a notable polyphenol content, which is of interest, although susceptible to variations during the year (Piccolella, Crescente, Pacifico, & Pacifico, 2018). Oxidant stress is known to be responsible for some forms of cancer (Cohen, Kristal, & Stanford, 2000), as well as for degenerative pathologies such as those affecting the cardio-circulatory system, hypertension, atherosclerosis, heart attack, and stroke (Polidori et al., 1998; Yang, Devaraj, & Jialal, 2001) and also the autoimmune system (Iborra, Palacio, & Martínez, 2005).

The nutraceutical potential contained in edible wild plants comes from the biological activity of their secondary metabolites, which is why they are widely used in health food preparation, pharmaceuticals, and cosmetics EWP valuation, however, is more essential than ever since there is a perception of the loss of this ethnobotanical knowledge, which is generally transmitted from generation to generation (Nebel et al., 2006). For example, before the development of penicillin,

garlic (*Allium sativum* L.) was one of the most widely used remedies against infectious diseases, in use until the Second World War (Petrovska & Cekovska, 2010). Today, garlic is known in popular phytotherapy as a hypotensive, due to the presence of allicin, and is also used to treat hypercholesterolaemia. This folk knowledge has been completely absorbed into European culture and in the Mediterranean basin.(Leonti & Verpoorte, 2017).

Moreover, in modern civilization, where industrial ones have replaced traditional activities, the risk of loss is high (Salerno, Maria Guarrera, Caneva, & Nazionale Arti Tradizioni, 2005), and so for the local traditions linked to EWP consumption.

This study aims to evaluate the chemical and nutraceutical properties of nine edible wild plant species belonging to the Italian ethnobotanical heritage in order to demonstrate their potential health benefits with their consumption.: *Reichardia picroides* L. Roth, *Rhagadiolus stellatus* L. Gaertn., *Sonchus oleraceus* L. and *Plantago coronopus* L., *Allium neapolitanum* Cirillo, *Allium triquetrum* L., *Melissa officinalis* L., *Thymus serpyllum* L., *Foeniculum vulgare* Mill., which were collected in the Central Italy. The first three species belong to the *Asteraceae* family, the fourth species to Plantaginaceae one, the fifth and sixth species belong to the *Alliaceae* family, the seventh and eighth species to Labiateae, and finally, the last one to the *Apiaceae* family. In particular, the first two species are more frequently collected and found on local street markets, resulting in the most commonly known species by the Italian population (Ranfa & Bodesmo, 2017).

Material and methods

Collection and preparation of plant species

The specimens of the nine species examined: *R. picroides* L. Roth, *R. stellatus* L. Gaertn., *S. oleraceus* L., *P. coronopus* L., *A. neapolitanum* Cirillo, *A. triquetrum* L., *M. officinalis* L., *T. serpyllum* L., *F. vulgare* Mill., were collected in the wild between February and October 2018 on Subasio Mount near Assisi and Spello, Central Italy; the two harvested area are located at an altitude of 200 to 500 meters (Fig. 1). The plant material was immediately transferred to the laboratories after the harvest, washed gently with deionized water, and the non-edible parts of the plants were removed before following analysis. The plant classification was verified by using the stereomicroscope SX45, following the Italian Flora Checklist (Pignatti et al., 2018), and by using the *exiccata* plant species of the University of Perugia's Erbario PERU. The survey of plant species according to the Braun-Blanquet (Braun-Blanquet, 1964) method and the collection of samples was carried out in some

areas near Spello and Assisi and in the central and upper parts of Mt. Subasio. The sample of each EWP species was a composite sample using 10-15 plants per sample.

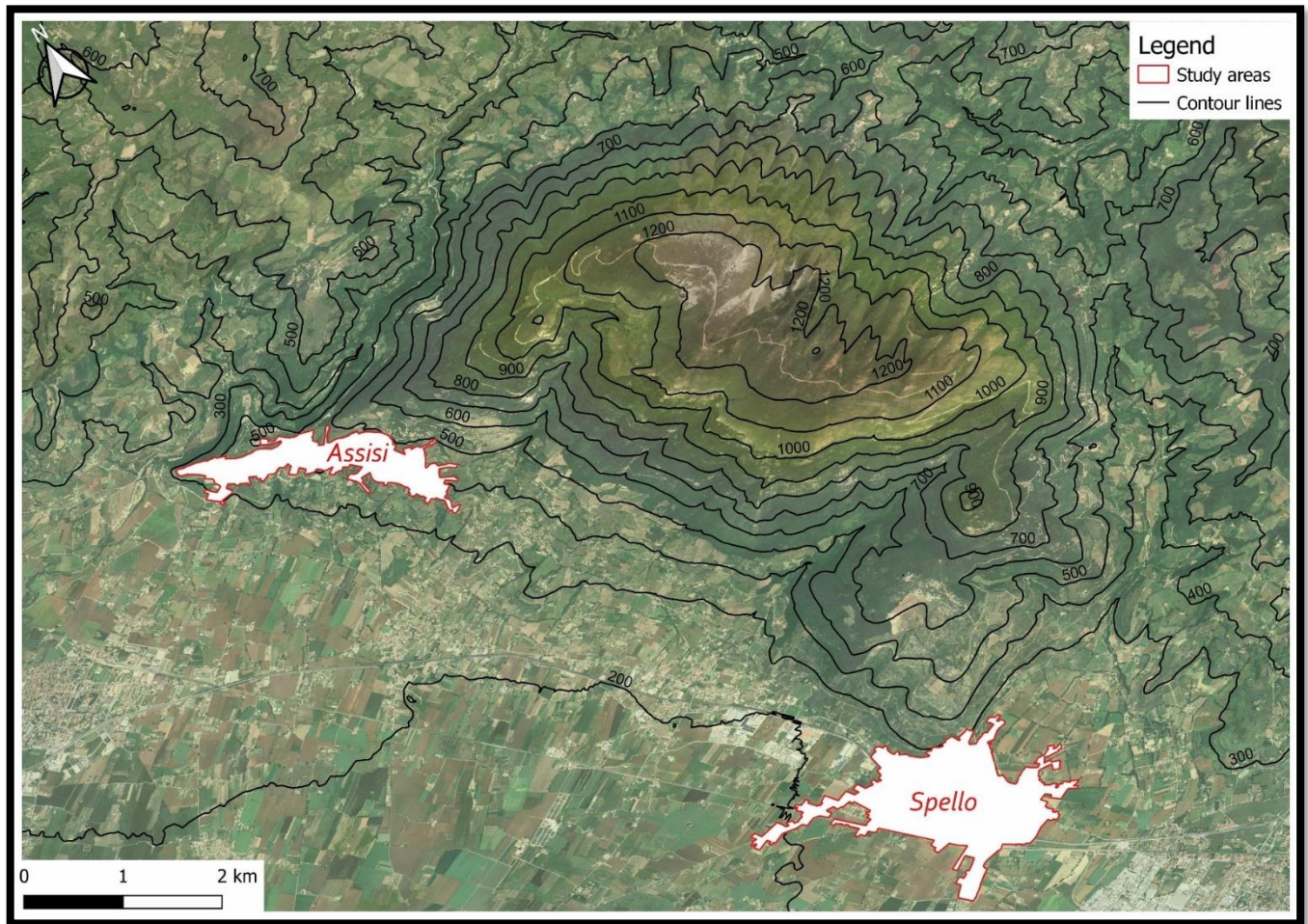


Figure 1. Geographic area with altitude values (Subasio Mount, Central Italy), where plants were collected

Chemical composition

The moisture content of the nine raw edible wild plants was determined by drying the leaves in an oven at 100 °C until a constant weight, according to the Association of Official Analytical Chemists AOAC method (AOAC 1990), was obtained. Crude protein content was calculated using the conversion factor 6.25 ($N \times 6.25$) from the nitrogen content determined by the Kjeldahl method (AOAC 1990). Total lipid content was evaluated using chloroform/methanol extraction based on the Bligh and Dyer procedure as described in the AOAC method (AOAC 1990). Ash content was determined according to the AOAC method (AOAC 1990) by incineration in a muffle furnace at 500° C for six h. The enzymatic-gravimetric method determined Total dietary fiber according to the AOAC method (AOAC 1995). Carbohydrates were calculated by difference.

Calcium, magnesium, and iron determination, according to the AOAC method (AOAC, 2006) conducted by AA-6800 Model flame (air-acetylene) atomic absorption spectrophotometer (AAS) (Shimadzu, Kyoto, Japan) on the ashes which were obtained from 4 g of each lyophilized sample dissolved in 10 mL of 6 N of HCl, then transferred into a 50 mL volumetric flask and the volume adjusted with distilled water. Stock standard solutions, 1000 ppm in HNO₃ are used. Potassium and sodium were determined using a PFP7 Flame Photometer (Bibby Scientific, Jenway, Techne Inc., UK) on the same solution. Certified stock industrial standard solutions were purchased from Jenway (Jenway, Essex, UK). Analysis accuracy was confirmed for all the metals studied using certified standard reference material (NIST-SRM-1573rd, tomato leaves). Phosphorus content was colorimetrically determined on 0.2 g of each lyophilised sample by reading the absorbance at 650 nm using ammonium molybdate, hydroquinone and sodium sulphide solutions according to the AOAC method (AOAC, 1990). Total polyphenols were determined on 250 mg of each lyophilised sample by the Folin-Ciocalteu method with the measurement at 760 nm with a spectrophotometer (Varian UV/Vis 50 Cary Bio model, Palo Alto, CA, USA), and the results were expressed as mg of gallic acid equivalent (GAE)/ 100 g fresh weight (Burini, 2007; Singleton & Rossi, 1965).

Simultaneous determination by normal phase high performance liquid chromatograph (NP-HPLC) of tocopherols and β -carotenes (vitamin E, provitamin A)

Tocopherols (vitamin E) and β -Carotene (provitamin A) were simultaneously carried out by a modified NP-HPLC operating with a Spectra Physics SP8800 Model ternary pump (Mountain View, CA, USA), and a Rheodyne 7125 (Cotati, CA, USA) sample-injection valve with a 10 μ L injection loop. The two detectors used in series were a Jasco FP-920 Model, Tokyo, Japan fluorometric detector and a UV/Vis detector (Shimadzu SPD-10 A VP Model, Kyoto, Japan), for the determination in a single run of tocopherols (α -, β -, γ - and δ -tocopherol) and β -carotene (Maurizi et al., 2015; Ranfa & Bodesmo, 2017; Ranfa, Maurizi, Romano, & Bodesmo, 2014a; Ranfa et al., 2015). Vitamin A, expressed as μ g of retinol equivalents (RE) per 100 grams, is calculated considering a 6:1 ratio of μ g of β -carotene and μ g RE per 100 grams (National Research Council - US).

Total L-ascorbic acid determination

Total L-ascorbic acid (vitamin C) determination was carried out according to the Burini method (Burini, 2007), based on radical oxidation of L-ascorbic acid (AA) to obtain dehydro-L-ascorbic

acid (DHAA) using a peroxy radical generated *in situ* by thermal decomposition of an azo-compound, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as already described in previous studies (Maurizi et al., 2015; Ranfa, Maurizi, Romano, & Bodesmo, 2014b).

Total antioxidant capacity determination by Hydrophilic-Oxygen Radical Absorbance Capacity (H-ORAC).

The analysis of total antioxidant capacity by ORAC assay (Cao, Alessio, & Cutler, 1993; Ou, Hampsch-Woodill, & Prior, 2001) was carried out in triple, after treatment of the sample in the following way: 0,5 g of each fresh leaf was added with 20 mL of a CH₃COCH₃:H₂O:CH₃COOH (70:29,5:0,5 v/v/v) mixture in a 50 mL polypropylene graduate conical tube (Falcon 2070 Blue Max, Boston Dickinson Labware, NJ, USA). After homogenization (IKA TI 18 Ultra Turrax, Milan, Italy) to obtain a refined suspension and centrifugation at 4000 rpm for 5 min (Eppendorf centrifuge 5810 R Model, Eppendorf, Milan, Italy), the supernatant was diluted 1:50 with phosphate buffer, and the resulting solution was used for ORAC evaluation (Ranfa & Bodesmo, 2017; Ranfa et al., 2015).

Results

The results of proximate composition, total polyphenols, minerals, antioxidant vitamins content, and total antioxidant capacity of the nine EWP_s are reported in Table 1, expressed as the mean and standard deviation (SD) and referred to on 100 g of fresh weight (fw). Regarding the proximate profile, the results are almost similar between all EWP_s. In particular, *A. neapolitanum* records the highest value (95%) for moisture content, while *M. officinalis* is the lowest (86.5%). Carbohydrates, which are an important energy source and which can balance food intake, represent on average the greatest fraction, although with a wide range of variability among the species, from 2.5% in *R. picroides* to 14.4% in *M. officinalis*. Protein content was low in *A. neapolitanum* and *A. triquetrum* but decidedly higher in *F. vulgare* and *M. officinalis*. Lipids were consistently lower than 1% in all cases, with slight differences among the nine species. The total crude fiber is the most abundant component, of which concentration varies between 1.2 and 6.3 g/100g fw, where the two highest values are recorded for *R. stellatus* (6.3 g/100g) and *R. picroides* (6.0 g/100g). Total polyphenol components have various properties (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; Kähkönen et al., 1999; Ozcan, Akpınar-Bayazit, Yilmaz-

Ersan, & Delikanli, 2014), showed extremely interesting values, with very high concentrations in *T. serpyllum* and *M.officinalis*.

Variations in composition among the nine species determined differences in energy content, which remained very limited, ranging from 16 to 68 kcal/100g per edible part (Table 1).

The concentrations of several minerals, which are important from a nutritional point of view, were also determined, with very interesting results (Table 2). In particular, high concentrations of K in *Rhagadiolus stellatus*, Fe and P in *M. officinalis* and Ca e Fe in *T. serpyllum* were of interest, while Na was very low, particularly in *T. serpyllum*. It is necessary to note that the low bio-availability of iron is due to the presence of some components that combine with it to form compounds that are not easily soluble or absorbable, such as polyphenols, phytic acid, phosphates, carbonates, and oxalic acid.

Table 1. Chemical composition (*g/100g edible weight*) and energy content of nine edible wild plants (EWPs)

Component	<i>Allium triquetrum</i>	<i>Allium neapolitanum</i>	<i>Tymus serpyllum</i>	<i>Foeniculum vulgare</i>	<i>Melissa officinalis</i>	<i>Sonchus oleraceus</i>	<i>Reichardia picroides</i>	<i>Rhagadiolus stellatus</i>	<i>Plantago coronopus</i>
Water	93.3±1.28	95.0±0.76	86.0±0.71	86.1±0.81	79.2±0.65	88.8±0.72	88.1±0.80	86.5±0.66	89.0±0.91
Protein	0.8±0.01	0.7±0.08	1.3±0,10	3.3±0.06	3.0±0.09	1.9±0.06	1.7±0.08	2.0±0.10	1.2±0.06
Lipid	0.2±0.01	0.2±0.04	0.2±0.03	0.2±0,03	0.3±0.02	0.4±0.03	0.3±0.04	0.3±0.03	0.2±0.03
Carbohydrates	4.1±0.12	3.0±0.28	10.7±0.24	7.8±0,28	14.4±0,28	3.0±0.23	2.5±0.28	2.6±0.24	3.8±028
Ash	1.0±0.02	1.0±0.11	1.2±0.09	2.3±0.12	2.5±0.13	1.7±0.10	1.2±0.11	2.1±0.09	1.4±0.12
Crude fiber	1.3±0.06	1.2±0.35	3.7±0.34	1.8±0.27	2.2±0.31	4.0±0.29	6.0±0.35	6.3±0.34	4.2±0.27
Energy (<i>kcal/100g</i>)	20	16	47	44	68	22	19	21	21
Energy (<i>kJ/100g</i>)	85	66	199	184	287	94	79	87	89

Table 2. Mineral content of edible wild plants (EWPs) (*mg/100g edible weight*).

Element	<i>Allium triquetrum</i>	<i>Allium neapolitanum</i>	<i>Thymus serpyllum</i>	<i>Foeniculum vulgare</i>	<i>Melissa officinalis</i>	<i>Sonchus oleraceus</i>	<i>Reichardia picroides</i>	<i>Rhagadiolus stellatus</i>	<i>Plantago coronopus</i>
K	324±32	355±35	523±51	424±41	644±62	497±34	418±29	690±30	123±12
Na	37±3	30±3	6±2	157±12	4±1	48±4	98±3	85±4	96±4
Ca	143±12	135±11	425±35	249±20	352±29	143±12	102±9	186±9	124±10
Mg	13±2	5±2	60±7	33±4	97±11	11±0.9	9±0.9	16±0.8	17±1.0
Fe	1.1±0.2	1.8±0.4	7.6±1.5	1.5±0.3	6.4±1.3	1.2±0.09	0.8±0.09	0.8±0.10	1.1±0.10
P	34±3	20±2	59±5	71±6	103±8	52±3	32±3	58±4	24±2

Regarding antioxidant content, the main hydrosoluble and liposoluble vitamins and total polyphenolic substances were determined. The results obtained (Table 3) showed excellent β -carotene (provitamin A) values (from 1,334 to 4,601 $\mu\text{g}/100\text{ g fw}$) as well as exciting values for ascorbic acid (vitamin C) in *F. vulgare*, *R. stellatus*, *P. coronopus* and *R. picroides* and α -tocopherol (vitamin E) also showed interesting values (from 0.83 to 4.0 $\text{mg}/100\text{g fw}$). It must be pointed out that by consuming as little as 100 g of these plants, the excellent β -carotene content will contribute as a precursor up to as much as 80% of daily vitamin A intake (Table 3).

Total Antioxidant Capacity dosage was determined by Hydrophilic Oxygen Radical Absorbance Capacity (H-ORAC) (Romojaro et al., 2013). The importance of this type of determination, which has been included in the USDA database for several years now, is that numerous studies have shown an inverse association between vegetable consumption and death from degenerative diseases deriving, so it seems, from the protective function attributed to the 'antioxidant' hypothesis (Hung et al., 2004). Table 4 shows the Total Antioxidant Capacity values of nine samples of EWPs obtained by the ORAC method and expressed in μmol of Trolox Equivalent/100 g ($\mu\text{mol TE}/100\text{ g}$). Among the analyzed species, *M. officinalis* showed the highest ORAC value of 22,071 $\mu\text{mol TE}/100\text{ g}$, followed by *R. picroides* and *S. oleraceus* 10,491 and 8,053 $\mu\text{mol TE}/100\text{ g}$, respectively.

Table 3. Antioxidant vitamin and total polyphenol content of edible wild plants (EWPs)

Component	<i>Allium triquetrum</i>	<i>Allium neapolitanum</i>	<i>Thymus serpyllum</i>	<i>Foeniculum vulgare</i>	<i>Melissa officinalis</i>	<i>Sonchus oleraceus</i>	<i>Reichardia picroides</i>	<i>Rhagadiolus stellatus</i>	<i>Plantago coronopus</i>
α -Tocopherol (mg/100 g)	0.83±0.016	2.10±0.053	4.00±0.065	1.20±0.028	1.10±0.037	2.00±0.01	1.80±0.03	1.10±0.09	1.70±0.12
β -Carotene (μ g/100 g)	1,546±24	2,186±17	3,297±68	2,297±70	1,334±20	4,601±60	3,505±164	3,052±45	2,060±35
Vitamin A (μ gRet.Eq./100g)	258.00±4	364±3	550±11	383±12	222±3	767±10	584±27	509±8	343±6
Ascorbic acid (mg/100 g)	5.00±0.24	4.0±0.16	23.7±0.52	41.2±1.10	13.0±0.43	31±1.3	36±1.7	39±2.0	37±1.6
Total polyphenols (mg/100g)	82.00±3	92±3	2,771±49	541±22	3,473±62	98±9	215±12	154±11	284±15

Table 4. ORAC values of edible wild plants EWP_s.

	<i>Allium triquetrum</i>	<i>Allium neapolitanum</i>	<i>Thymus serpyllum</i>	<i>Foeniculu m vulgare</i>	<i>Melissa officinalis</i>	<i>Sonchus oleraceus</i>	<i>Reichardia picroides</i>	<i>Rhagadiol us stellatus</i>	<i>Plantago coronopus</i>
ORAC (μ mol TE/100g)	6,593±220	4,436±163	4,065±397	5,035±219	22,071±325	8,053±328	10,491±194	7,696±310	3,670±202

Discussion

The scientific interest is turning to plants not commonly known, such as the local species with several important genetic characteristics. In some case, they call attention to the pharmaceutical industries also for the presence of phytochemicals with a nutraceutical potential (Berni et al., 2018).

The same consideration could also be turned to the wild edible species, which are promising species, also in consideration that they usually have several folk medicinal uses (Ranfa & Bodesmo, 2017). In particular, the results on the studied EWP, suggested the presence of phytochemicals in high concentrations, such as bioactive compounds (vitamins and polyphenols) which have positive health properties and nutraceutical potentials. The high ORAC values also enhance this interesting composition. The Total Antioxidant Content results are all very interesting as they show high values, particularly for *M. officinalis*, all of which are superior than those found in other widely consumed vegetables such as lettuce, kale, tomatoes, rocket, radicchio, spinach, etc. (Ninfali, Mea, Giorgini, Rocchi, & Bacchiocca, 2005; Sánchez-Mata et al., 2012). Therefore, if even modest quantities of these species were consumed, it would be possible to satisfy the average daily antioxidant quota (5000 units ORAC/die) for a standard diet, which the United States Department of Agriculture (USDA, 2010) deems capable of countering alterations caused by oxidative processes. Regarding the β -carotene, the highest value is detected on *S. oleraceus* (4,601 $\mu\text{g}/100\text{ g}$), which is similar to those recorded for *Carpobrotus edulis* L., *Salicornia persica* Akhani and *Sarcocornia fruticosa* (L.) A.J. Scott, three halophytic plants with a high tolerance of salinity (Rocha et al., 2017; Ventura & Sagi, 2013). Moreover, also the α -tocopherol values agree with those found in some species of *Asteraceae* family, with a maximum value of 1.70 mg/100 g fw, found in *S. oleraceus*, but the highest was found in *T. serpyllum* (*Lamiaceae*) 4.00 mg/100g (Morales et al., 2014). Furthermore, the total ascorbic acid values are much higher than the data reported by (Sánchez-Mata et al., 2012), in particular, the level of vitamin C found in this study for *F. vulgare*, 41.20 mg/100g, is much higher than that reported in the 2012 study.

The determined polyphenolic compound content values agree with those reported by Maurizi et al. (2015). Since all plants, except *P. coronopus*, can be consumed fresh, the impact of preparation methods and cooking on EWP composition is not considered in this study. However, some of them are often consumed after cooking, and some probable modifications can occur, such as reported for the fennel (*Foeniculum vulgare* Mill.), for which the boiling effect at 100°C per 30 minutes decreases polyphenolic concentration both for principally the thermal processing and also for an effect of leaching in the water (Rawson, Hossain, Patras, Tuohy, & Brunton, 2013).

The variability of the total polyphenol components was due to the different characteristics of each EWP. However, within each species, the stage of development when the plants were collected (phenological stages) and the daily harvest time also influenced the composition of the polyphenol content. (Gori, Nascimento, Ferrini, Centritto, & Brunetti, 2020). Moreover, the considered EWP_s are characterized by other fundamental ecosystem benefits. These species are characterized by high physical resistance (to drought, wind, ice, and so on) derived from genetic features established and fixed over the centuries. These plants showed natural rhythms of growth linked exclusively to the seasonal climate and environmental potential of the growing areas, where their presence enriched the biodiversity rate.

Conclusion

This study demonstrates how EWP_s can play a very important role in daily food consumption by indicating interesting and appreciable nutritional characteristics such as significant potassium, magnesium, calcium, iron, and sodium content, numerous biologically active components (polyphenols, vitamin A, ascorbic acid, vitamin E), crude fiber, low lipid content, absence of cholesterol, and low energy. It is precisely the presence of the components with antioxidant action that is of considerable interest given that currently, oxidative stress (i.e., the lack of balance in the organism between the formation of oxygen and nitrogen radical species and endogenous and exogenous antioxidants) is believed to be responsible for the development of numerous degenerative diseases.

Therefore, the diet itself may be protective in preventing major chronic diseases if these EWP_s are habitually consumed, though in the modest quantities indicated by the Mediterranean culinary tradition. So, it is not by chance that these species are present as staples in the Mediterranean Diet, which is included in the UNESCO Intangible Cultural Heritage (UNESCO, 2010) thanks to its rationality and preventive capacity (Pitsavos et al., 2005). So, EWPs constitute not only a real botanical treasure but also a precious culinary, nutritional, and cultural heritage. Future studies could also target EWPs collected at different times of the year to identify the best harvesting moments according to their most suitable nutraceutical properties.

Finally, with an exposition field trip, the limitation of losing all knowledge linked to these EWP will be reached between the population, thanks to the promotion of integrative and training education events. So, in this direction, the consumer will be aware that EWP is “good for health” and that they have an added value linked to the Umbrian territory, defining local and highly sustainable food.

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