Mediterranean non-cultivated vegetables as dietary sources of compounds with antioxidant and biological activity

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Abstract

Non-cultivated vegetables whose basal leaves have been traditionally consumed in Spain were evaluated for their potential in human nutrition, considering vitamin C, organic acids, tocopherols, phenolics and flavonoids, and antioxidant activity. As far as we know, this is the first report on organic acids and vitamin C of *Anchusa azurea* and *Apium nodiflorum*, on tocopherols of *Anchusa azurea*, *Beta vulgaris, Chondrilla juncea, Rumex papillaris, Rumex pulcher, Silybum marianum and Taraxacum obovatum,* as well as on the antioxidant capacity of most of them. Data revealed that the mentioned non-cultivated vegetables are good sources of bioactive compounds. *Rumex pulcher, R. papillaris* and *Papaver rhoeas* are rich in vitamin C, *Sonchus oleraceus* and *Rumex papillaris* in tocopherols. *Rumex pulcher, Papaver rhoeas* and *Anchusa azurea* showed promising antioxidant properties, which are related to their high levels of phenolic and flavonoids. Some species presented high levels of oxalic acid. Therefore, people with a trend of developing kidney calculus should avoid eating these greens (especially *Silybum marianum*, *Sonchus oleraceus* and *Beta maritima)* and choose species with low oxalic acid content such as *Taraxacum obovatum* and *Cichorium intybus.* The traditional consumption of these species after boiling and rejecting the water may decrease the amount of oxalic acid.

Keywords: non-cultivated leafy vegetables; vitamin C; organic acids; tocopherols; antioxidant properties

1. Introduction

There is an increasing public awareness that nutrition and dietary components have a very relevant contribution to personal well-being and health (Biesalski et al. 2011). In the case of non-cultivated traditional vegetables, despite its intermingled nutritional and medicinal role has been widely documented (Etkin, 1996; Guarrera, 2003; Heinrich, Leonti, Nebel, & Peschel, 2005; Pardo-de-Santayana, Tardío, & Morales, 2005; Carvalho & Morales, 2010), there are regions where they are mainly seen as an inferior, low-calorie part of the diet (e.g., Poland, Łuczaj, 2010). On the contrary there are *herbophilous* regions, where people appreciate non-cultivated greens and even perceive their health benefits (Ishtiaq et al., 2007). This is the case of many Mediterranean countries, where these vegetables have been an important part of the Mediterranean diet (Pieroni, 2001; Parada, Carrió, & Vallès, 2011).

For instance, non-cultivated vegetables have been traditionally consumed in the Iberian Peninsula and have played a nutritional role in complementing agricultural foodstuff, especially during times of shortage (Tardío, Pardo de Santayana, & Morales, 2006; Carvalho & Morales, 2010). However, there is general trend of decline in the use of non-cultivated vegetables due to social and food habit changes (Carpenter et al. 2009) and only a few species are still widely consumed (Pardo-de-Santayana, Pieroni, & Puri, 2010). Specifically, there is an increasing scientific interest on studying the health benefits of these greens, since their nutritional properties and their richness in bioactive components such as antioxidants, have demonstrated health-promoting properties (Burton & Traber, 1990; Scalbert & Williamson, 2000).

Epidemiological evidences indicate a correlation between the intake of food rich in antioxidants and the reduction of certain chronic diseases. Furthermore, non-cultivated vegetables are rich in micronutrients and have been reported to address micronutrient

malnutrition (Kennedy, Nantel, & Shetty, 2003). Vegetables (including non-cultivated plant foods) have interest for their micronutrient content, especially vitamins and minerals, but also for other phytochemical compounds with antioxidant properties (Scalbert & Williamson, 2000). These micronutrients are important in the prevention of various pathologies including degenerative, cardiovascular and neurological diseases (Shah & Channon, 2004), several kinds of cancer, as well as embryonic development (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006)

Vitamin C, both in the form of ascorbic acid (AA) and its oxidized one, dehydroascorbic acid (DHAA), is nutritionally important. It is an essential vitamin and has a high redox potential, alone or/and coupled to other antioxidants, either in food or in human body (Phillips et. al 2010). Recent research in the role of dietary antioxidants in general, and of specific food components have shown the need for accurate food composition data to facilitate epidemiological studies related to the intake of vitamin C, which is often underestimated by the measurement of exclusively AA form.

Another antioxidant of great interest is vitamin E (formed by tocopherols and tocotrienols), being an essential component of the human diet (Trumbo, 2002). Tocopherols are naturally occurring antioxidant nutrients that play important roles in health by inactivating free radicals produced through normal cellular activity and from various stressors. They act as antioxidants by donating a hydrogen atom to peroxyl radicals of unsaturated lipid molecules, forming a hydroperoxide and a tocopheroxyl radical, which reacts with other peroxyl or tocopheroxyl radicals forming more stable adducts (Traber, 2007). It has been reported that γ-tocopherol and its physiological metabolite 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), inhibit COX-2-catalyzed formation of PGE2, inducing anti-inflammatory properties (Barreira, Alves, Casal, & Ferreira, 2009). Moreover, it is also believed that tocopherols and

tocotrienols protect against degenerative processes, such as cancer and cardiovascular diseases (Burton & Traber, 1990; Kamal-Eldin & Appelqvist, 1996).

Other important nutritional compounds are organic acids. However, there is a lack of data about their profile in non-cultivated vegetables. These compounds are photosynthetic intermediates, mainly produced in mitochondria through the tricarboxylic acid or Krebs cycle and to a lesser extent in the glyoxysome as part of the glyoxylate cycle. Some organic acids may have biological activity, and thus a significant impact on human health. Tartaric, malic, citric or succinic acids have shown positive health benefits as antioxidants due to their ability to chelate metals (Lopez-Bucio, Nieto-Jacobo, Ramírez-Rodríguez, & Herrera-Estrella, 2000; Seabra et al. 2006).

As the available data about nutrients and bioactive compounds composition of many non-cultivated edible vegetables are still scarce, and with the aim of improving the knowledge on their nutritional value, the present study provides new data about the content of some compounds with biological activity, such as vitamin C (ascorbic and dehidroascorbic acid), organic acids, vitamin E (tocopherols), phenolics, as well as the *in vitro* measurement of the overall antioxidant capacity of eleven non-cultivated leafy species traditionally used in Spain.

2. Material and methods

2.1. Sampling of plant material

According to their high cultural relevance shown in a previous ethnobotanical review (Tardío et al., 2006), eleven different species of non-cultivated vegetables traditionally used in Spain were chosen (**Table 1**): *Anchusa azurea* Mill., *Beta maritima* L., *Cichorium intybus* L., *Chondrilla juncea* L., *Papaver rhoeas* L., *Rumex papillaris* Boiss. & Reut., *Rumex pulcher* L., *Scolymus hispanicus* L., *Silybum marianum* (L.)

Gaertn., *Sonchus oleraceus* L. and *Taraxacum obovatum* (Willd.) DC. Their basal leaves were harvested and their non-edible portion was eliminated. All these species are usually gathered before flowering (sterile specimens), when they look similar to other species. Therefore a deep knowledge about their vegetative stages and morphological features is needed for a correct identification of the desired species. Fertile material was collected at later date and a herbarium voucher of flowered plants from each studied species and each population was mounted to make easier and to confirm the identification.

The whole leaves were used in most of the species, except for the two thistles (*Scolymus hispanicus* and *Silybum marianum*), whose leaves were peeled removing their spines and leaving only the fleshy midribs. The samples were prepared with at least twenty five specimens of each species randomly chosen, gathered in springtime from the middle of March to late May. From each sample, a minimum of 500 g of edible portion was gathered, cleaned by removing soil particles and damaged parts, packed in plastic bags and carried to the laboratory in a cold system within the day. All the samples of the selected species presented a healthy external appearance. Vitamin C and organic acid contents were measured in fresh samples (immediately after collection and transport to the laboratory), whereas tocopherols and antioxidant assays were performed in freeze-dried samples (stored at -20ºC until analysis, less than two years). Moreover, moisture was analyzed in all samples according to Method 925.09 (AOAC, 2005).

In order to have representative samples that take into account the geographical and environmental variability, each studied species was collected from two different wild populations of Central Spain and during at least two years from 2007 to 2009 (three years in some cases). Therefore, the number of surveyed samples for each species was 4 or 6, depending on a two year or three year period of gathering.

Physicochemical, vitamin C and organic acids analyses were performed in all the independent samples and three replicates were used. So the number of measures varied between 12 and 18 according to two or three periods of gathering (e.g. 4 or 6 samples for each species). For the other analyses, a global sample was prepared by mixing the independent samples of each species. Therefore the determinations were carried out in the mixed sample and three replicates were also used (e.g a total of 1×3 measures).

2.2. Standards and reagents

The eluents *n*-hexane 95% and ethyl acetate 99.98% were of HPLC grade from Lab-Scan (Lisbon, Portugal). Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). Tocopherol standards (α, β, γ and δ), trolox and gallic acid, L (+)-ascorbic acid, oxalic acid, malic acid, citric acid and succinic acid were purchased from Sigma (St. Louis, MO, USA). Glutamic acid and L-Cystein were purchased from Merck (Darmstadt, Germany). Racemic tocol, 50 mg/ml, was purchased from Matreya (Pleasant Gap, PA, USA). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Analysis of vitamin C and organic acids composition

Vitamin C (ascorbic acid) and individual organic acids (oxalic, glutamic, malic, citric and fumaric) were determined based on procedures optimized and described by Sánchez-Mata et al. (2011). An aliquot of the extracts were also subjected to reduction with L-cysteine (4 g/100 mL) to transform the DHAA in AA and analyse the total vitamin C content. DHAA was estimated by difference between total vitamin C and AA

contents.

The HPLC equipment was a liquid chromatographer equipped with an isocratic pump (model PU-II, Micron Analítica, Madrid, Spain), an AS-1555 automatic injector (Jasco, Tokyo, Japan), a Sphereclone ODS (2) 250 × 4.60, 5 μm Phenomenex column (Torrance, CA, USA), a UV-visible detector (Thermo Separation Specta Series UV100, San Jose, CA, USA). The mobile phase was 1.8 mmol/L H_2SO_4 (pH= 2.6). For AA analysis a flow-rate of 0.9 mL/min and UV detection at 245 nm was used. Conditions for organic acids were 215 nm UV detection and 0.4 mL/min flow rate. Data were analysed using Biocrom 2000 3.0 software (Madrid, Spain). Identification was performed comparing retention times with those obtained from commercial pure standards. Quantification was based on the UV signal response, and the resultant peak areas in the chromatograms were plotted against concentrations obtained from standards. Vitamin C and organic acids contents in non-cultivated greens are expressed in mg/100 g of fresh weight (fw).

2.4. Analysis of tocopherols composition

Tocopherols content was determined after the extraction procedure previously described by Barros, Heleno, Carvalho, & Ferreira (2010), using tocol as internal standard (IS). The HPLC equipment consisted of an integrated system with a Smartline pump 1000 (Knauer, Berlin, Germany), a degasser system Smartline manager 5000, an AS-2057 auto-sampler and a 2500 UV detector at 295 nm (Knauer, Berlin, Germany) connected in series with a FP-2020 fluorescence detector (Jasco, Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm. Data were analysed using Clarity 2.4 Software (DataApex, Prague, The Czech Republic). The chromatographic separation was achieved with a Polyamide II (250 × 4.6 mm) normal phase column from YMC

Waters (Dinslaken, Germany). The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min, and the injection volume was 10 μL. The compounds were identified by chromatographic comparisons with authentic standards. Identification was performed comparing retention times with those obtained from commercial pure standards. Quantification was based on the fluorescence signal response, using the internal standard method and peak areas in the chromatograms were plotted against concentrations obtained from each standard. Tocopherol contents in non-cultivated greens are expressed in mg/100 g of fresh weight (fw).

2.5. Evaluation of antioxidant activity

2.5.1. Extracts preparation

A fine dried powder (1 g) was extracted by stirring with 40 ml of methanol at 25 °C for 1 h and filtered through Whatman No. 4 filter paper. The residue was then extracted with one additional 40 mL portion of methanol. The combined methanolic extracts were evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), re-dissolved in methanol at a concentration of 5 mg/mL, and stored at 4 °C for further use.

2.5.2. Total phenolics and total flavonoids

Total phenolics were estimated based on procedures described by Wolfe, Wu, & Liu (2003) with some modifications. An aliquot of the extract solution (0.5 mL) was mixed with Folin–Ciocalteu reagent (2.5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/L 2 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany). Gallic acid was used to

calculate the standard curve (0.05–0.8 mmol/L), and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Flavonoids content was determined using the method of Jia, Tang, & Wu (1999), with some modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5 g/100 mL, 0.15 mL). After 6 min, $AlCl_3$ solution (10 g/100 mL, 0.15 mL) was added and allowed to stand further 6 min. Thereafter, NaOH solution (4 g/100 mL, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510nm. (+)-Catechin was used to calculate the standard curve (0.0156–1.0 mmol/L) and the results were expressed as mg of (+)-Catechin equivalents (CEs) per g of extract.

2.5.3. DPPH radical-scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), according to Barros et al. (2010). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μL) and aqueous methanolic solution (80:20 v/v, 270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorbance at 515 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of

RSA percentage against extract concentration. Trolox was used as standard.

2.5.4. Reducing power

Different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1 g/100 mL, 0.5 mL). The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10 g/100 mL, 0.5 mL) was added. The mixture (0.8 mL), deionised water (0.8 mL) and ferric chloride (0.1 g/100 mL, 0.16 ml) were poured in the 48-wells and the absorbance was measured at 690 nm in the Microplate Reader described above (Barros et al., 2010). The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration. Trolox was used as standard.

2.5.5. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath (Barros et al., 2010). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2 h of assay/initial β-carotene content) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration. Trolox was used

as standard.

2.5.6. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances

Brains were obtained from pig (*Sus scrofa*), dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mmol/L, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate, which was centrifuged at 3000g for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different concentrations of the extracts (0.2 mL) in the presence of $FeSO_4$ (10 μmol/L; 0.1 mL) and ascorbic acid (0.1 mmol/L; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28 g/100 mL, 0.5 mL), followed by thiobarbituric acid (TBA, 2 g/100 mL, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000g for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured by its absorbance at 532 nm (Barros et al., 2010). The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(AxB)/ A] x 100%, where A and B were the absorbance of the control and the extract solution, respectively. The extract concentration providing 50% lipid peroxidation inhibition (EC_{50}) was calculated from the graph of TBARS inhibition percentage against extract concentration. Trolox was used as standard.

2.6. Statistical analysis

Each sample was analysed in triplicate. The results were expressed as means and standard deviations: $12 \leq n \leq 18$ (analytical triplicate of 4 or 6 samples) in the case of physicochemical, vitamin C and organic acids analyses, and n=3 (analytical triplicate of 1sample) for the other analyses. Analysis of variance (ANOVA), followed by Duncan`s test, was conducted using Statgraphics Plus 5.1 software to analyze data at the 95%

confidence level.

3. Results and discussion

3.1. Vitamin C and organic acids composition

In general, the leafy vegetables with the highest moisture content (between 80 and 90 g/100g; **Table 2**) are tender and succulent. *Silybum marianum* stands out with the highest moisture value (93.4 g/100g), whereas *Chondrilla juncea* and *Rumex pulcher* presented the lowest moisture contents.

Vitamin C content of the species analysed was quite variable. As shown in **Table 2**, *Rumex pulcher* presented the highest value (35.07 mg/100 g), followed by *Papaver rhoeas* (31.03 mg/100 g). Ascorbic acid was the main form found in *Rumex* species, which presented 55 to 57% of total vitamin C, whereas both chemical forms were found in equal percentages in *Papaver rhoeas* and *Beta maritima* (**Table 2**). On the other hand, DHAA was the predominant form found in *Anchusa azurea* and in the samples from the Asteraceae family (*Silybum marianum, Scolymus hispanicus, Sonchus oleraceus* and *Taraxacum obovatum*).

The species that presented the lowest vitamin C (3.30 mg/100 g and 2.74 mg/100 g, respectively) and AA content (1.11 mg/100 g and 0.36 mg/100 g) were those whose fleshy midribs (peeled basal leaves) were traditionally consumed, i.e. *Scolymus hispanicus* and *Silybum marianum*. This may indicate that vitamin C in plants is often located at higher levels in photosynthetic tissues, as previously reported by Loewus (1999) and Sánchez-Mata et al. (2012).

Vardavas, Majchrzak, Wagner, Elmadfa, & Kafatos (2006) reported vitamin C content for many Cretan non-cultivated vegetables, some of them being the same species of our study. The species from the Asteraceae family, such as *Scolymus hispanicus, Sonchus*

oleraceus and *Taraxacum* spp., presented higher values compared to those analysed in our study, except for chicory (*Cichorium. intybus*) which showed a similar content (23 mg/100 g). On the contrary, *Papaver rhoeas* from Creta presented lower contents (around 40-50 %) than the Spanish sample. Our samples of wild sea beet (*Beta maritima*) presented lower vitamin C content than those of Cretan cultivated *Beta vulgaris* (21.36 and 39 mg/100 g, respectively). Comparing our wild chicory data with those of Souci, Fachmann, & Kraut (2008) for the cultivated chicory, our samples presented around double content of vitamin C (19.80 and 8.7 mg/100 g, respectively).

Besides ascorbic acid, six different organic acids were characterized in the samples analysed: oxalic acid, glutamic acid, malic acid, citric acid and fumaric acid. Their profile and total content in plants depends upon the species, age and tissue type, therefore organic acids accumulation modulates plant adaptation to the environment conditions (López-Bucio et al., 2000).

As also found by Guil, Torija, Giménez, Rodríguez-García, & Giménez (1996), oxalic acid was the major organic acid in the basal leaves of all the analyzed non-cultivated species (**Table 2**), except in *Taraxacum obovatum* and *Cichorium intybus* that revealed malic acid as the main organic acid with values around 106.20 and 92.51 mg/100 g, respectively. Other authors previously reported high oxalic acid accumulation in the leafy tissues of some species from the families Asteraceae, Euphorbiaceae, Chenopodiaceae, Portulacaceae or Liliaceae, which has been attributed to the way of incorporation of HCO_3^- and CO_2 in photosynthesis mechanisms, for organic acid biosynthesis pathways. Moreover, the biosynthesis of these compounds is specially induced in tender leaves (Coll, Rodrígo, García, & Tamés, 1995; Lea & Leegood, 1997).

The fleshy midribs of *Silybum marianum,* and to a lesser extend *Scolymus hispanicus*, were the samples with a high oxalic acid levels (1030 mg/100 g and 486 mg/100 g, respectively). This may be related to the localization of this organic acid, which is biosynthesised in the leaf mesophyll and stored in the leaf stalks, the edible part of this species (Coll et al., 1995). *Beta maritima* presented also a high oxalic acid content (581 mg/100 g), but lower than its cultivated relative (*Beta vulgaris*) (1145 mg/100 g) as previously reported by Chai & Liebman (2005).

Oxalic acid is a major compound in many leafy vegetables and although it has a quite low toxicity (with 5 g as the minimal lethal dose for an adult), this compound may cause some negative effects on animal or human health through the reduction of dietary calcium bioavailability (by the formation of an insoluble complex), or the formation of calcium oxalate kidney calculus. For those reasons although oxalic acid has a quite low toxicity (with 5 g as the minimal lethal dose for an adult), the ingestion of high levels of this organic acid is not desirable. Some authors (Guil et al., 1996) recommended an oxalic acid/Ca ratio not higher than 2.5 in the foods to avoid this toxic effect.

These results show that even species with highest amounts of oxalic acid, such as *Silybum marianum*, would never provide more than 2 g of this undesirable compound in a standard 100 g portion. Very high amounts of about 250 g that are hardly eaten by humans would be necessary to reach toxic doses. However, people who easily form kidney oxalate calculus, should avoid the ingestion of oxalic acid rich species, and choose other species such as *Chondrilla juncea*, *Cichorium intybus* or *Taraxacum obovatum* leaves, which provide less than 80 mg per 100 g portion, and thus its oxalic acid content does not represent a potential risk.

Regarding other organic acids, glutamic acid was detected in six of the total samples analyzed, with values around 43.69 and 253.67 mg/100 g for *Sonchus oleraceus* and

Rumex papillaris, respectively (**Table 2**). The highest citric acid content was detected in *Papaver rhoeas* (149.04 mg/100 g). Fumaric acid was found in all the samples with the exception of *Rumex* species, being *Silybum marianum* the one with the highest value (123.09 mg/100 g).

3.2. Tocopherols composition

The four tocopherol chemical forms (α, β, γ and δ- tocopherol) were identified. Alfa-tocopherol, the chemical form with the highest vitamin E activity (Caretto, Nisi, Paradiso, & DeGara, 2009), was the most abundant one in all the studied samples (**Table 3**), except for *Silybum marianum* whose predominant form was δ-tocopherol (0.10 mg/100 g). The predominance of α-tocopherol is in agreement with the results previously reported by other authors (Vardavas et al., 2006; Barros et al., 2010; Morales et al., 2011). This form is considered the most active in humans, due to a preferential absorption and distribution in the human body. It has also been described as inhibiting the radical chain propagation in the lipid peroxidation process, by its own conversion into oxidized products (Tucker & Townsend, 2005; Caretto, Nisi, Paradiso, & DeGara, 2009).

As shown in **Table 3**, *Cichorium intybus* presented the highest total tocopherols content (2.98 mg/100 g), followed by *Sonchus oleraceus* (2.22 mg/100 g), while *Scolymus hispanicus* and *Silybum marianum* again stood out for their lowest contents (0.05 mg/100 g and 0.15 mg/100 g, respectively), which may be related to the fact that these samples are peeled leaves, only containing midrib tissues.

The highest levels of α-tocopherol were found in *Sonchus oleraceus* (1.70 mg/100 g) and *Rumex papillaris* (1.29 mg/100 g), while the lowest content was found in *Scolymus hispanicus* (0.02 mg/100 g). The highest content in β-tocopherol was found in *Papaver*

rhoeas (0.66 mg/100 g). Delta-tocopherol was found in all the samples, except in the *Rumex* species (**Table 3**).

As far as we know, this is the first report on tocopherols composition for most of the species studied. In the case of *Taraxacum obovatum*, there is information about its cultivated relative *Taraxacum officinale* (Souci et al., 2008), with a value of total tocopherols of 2.5 mg/100 g, which was higher than the value found in the non-cultivated species (0.60 mg/100 g). Alfa-tocopherol was also the main chemical form (2.5 mg/100 g), which is in agreement with the results obtained in the present study (0.51 mg/100 g).

Vardavas et al. (2006) reported α- and γ-tocopherols contents in some wild Cretan species, such as in *Cichorium intybus*, *Beta maritima*, *Papaver rhoeas*, *Scolymus hispanicus*, *Sonchus oleraceus* and *Taraxacum* sp., but they analysed the whole aerial part, being these data not comparable to those presented in this work.

3.3. Phenolics and flavonoids

Total phenolics and flavonoids contents for the analyzed samples are shown in **Table 4**. *Anchusa azurea* presented the highest phenolics content (148.62 mg GAE/g extract) followed by *Rumex papillaris* (104.18 mg GAE/g extract) while the highest flavonoids content was found in *Anchusa azurea* (84.81 mg CE/ g extract).

Italian samples (Conforti et al., 2009) of *Cichorium intybus, Papaver rhoeas* and *Sonchus oleraceus* revealed lower phenolic contents (0.190 mg GAE/100 g, 0.072 mg GAE/g and 0.061 mg GAE/g, respectively) than the Spanish samples studied herein. Furthermore, *Cichorium intybus* and *Papaver rhoeas* basal leaves analyzed in this study presented higher flavonoids content (31.35 mg CE/g extract and 12.00 mg CE/g extract, respectively) than the Italian samples (10 mg CE/g extract and 4.68 mg CE/g extract)

studied by Conforti et al. (2009). This variability could be due to the different climatic conditions, soil fertility, postharvest and environmental conditions (Prohens et al., 2005), as well as to genetic differences or particular.

3.4. Antioxidant properties of non-cultivated greens

The antioxidant activity of the studied vegetables is shown in **Table 4**. *Anchusa azurea*, the species with the highest phenolics and flavonoids content, also presented the highest antioxidant activity measured by DPPH scavenging activity (EC_{50} 0.02 mg/mL), reducing power (EC_{50} 0.01 mg/mL), and inhibition of β-carotene bleaching (EC_{50} 0.02 mg/ml) methods. *Chondrilla juncea, Papaver rhoeas, Rumex pulcher and Silybum marianum* presented the highest TBARS inhibition capacity (EC_{50} 0.02 mg/mL). Comparing with other authors, Conforti et al. (2009) reported higher DPPH scavenging activity for *Cichorium intybus, Papaver rhoeas* and *Sonchus oleraceus* (EC_{50} 0.026, 0.049 and 0.164 mg/mL, respectively) but lower TBARS inhibition (0.127, 0.283 and 0.435 mg/mL, respectively).

The correlation coefficient (r) and p-values between the analysed compounds and the antioxidant capacity of the studied vegetables are given in Table 5. The results of the reducing power and DPPH assays (EC_{50}) were significantly correlated with most of the antioxidant compounds content, such as ascorbic acid, phenolics, flavonoids, α, γ and total tocopherols, which, in turn, is strongly and positive correlated with α-tocopherol ($r = 0.928$; $p = 0.000$). Nevertheless, the results of β-carotene bleaching inhibition assay were correlated to phenolics, flavonoids and γ and total tocopherol, whereas TBARS assay only was negatively correlated with α and δ-tocopherol.

As can be seen in **Table 5**, the highest correlation coefficients were found for DPPH, reducing power and β-carotene bleaching inhibition EC_{50} results and phenolics and

flavonoids content, suggesting that these compounds are major contributors to the antioxidant capacity of these vegetables. A strong correlation was also found for total tocopherol content and β-carotene bleaching inhibition assay (r=-0.552, p = 0.000),

The β-carotene undergoes a rapid discolouration in the absence of an antioxidant since the free linoleic acid radical attacks the β-carotene molecule, which loses the double bonds and, consequently, loses its characteristic orange colour (Gutierrez et al., 2006). Classical antioxidants (especially tocopherols) can donate hydrogen atoms to quench radicals and prevent decolourization of carotenoids; tocopherols (TOH) act as antioxidants by their capacity to scavenge lipid peroxyl radicals of unsaturated lipid molecules, preventing propagation of lipid peroxidation, mainly in polyunsaturated fatty acids (PUFAs) (Palozza et al., 1992):

β-carotene—H (orange) + $ROO^{\bullet}$ → β-carotene$^{\bullet}$ (bleached) + ROOH

β-carotene—H (orange) + $ROO^{\bullet}$ + TOH → β-carotene—H (orange) + ROOH + $TO^{\bullet}$

This highly correlations between tocopherols and β-carotene bleaching inhibition assay was also observed by the authors in other food matrix as is the case of wild fruits (*Arbutus unedo*, *Crataegus monogyna*, *Prunus spinosa*, and *Rubus ulmifolius*) (Morales et al., 2013), which may confirm the relation between the lipid mechanism of this assay, which mostly depends on lipid bioactive compounds and lipidic environment (linoleate and other free radicals; $ROO^{\bullet}$) and tocopherols.

In relation to these results, DPPH showed a strong correlation with reducing power assay (r = 0.978, p = 0.000), and TBARS assay only correlated to β-carotene bleaching inhibition assay, which may be related to the fact that both methods evaluate the antioxidant action against all the lipid fractions.

4. Conclusions

This study shows that the non-cultivated vegetables analyzed are good sources of bioactive compounds, such as vitamin C (AA + ADHA) in the case of *Rumex pulcher, R. papillaris* and *Papaver rhoeas*, and tocopherols (*Sonchus oleraceus* and *Rumex papillaris*). *Rumex pulcher, Papaver rhoeas* and *Anchusa azurea* showed promising antioxidant properties, which are related to their high levels of phenolics and flavonoids.

The basal leaves of some of the species studied presented very high levels of oxalic acid. As the ingestion of high levels of this antinutrient is not desirable for people who suffer from kidney calculus problems, they should avoid or reduce the ingestion of leafy vegetables, such as *Silybum marianum*, and choose other species with low oxalic acid content, such as *Taraxacum obovatum* or *Cichorium intybus.* Boiling may be a good option, since it decreases the amount of oxalic acid, and its toxic effects, due to the loss of oxalic acid by solubilization of it in the cooking liquid

Considering that Spain already exports huge amounts of cultivated vegetables, the incorporation of spontaneous or semi-cultivated products may open new commercial opportunities for rural areas. Their nutritional potential could make them especially attractive given the public's increasing awareness that what is eaten is essential for personal well-being and health.

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Table 1. Edible and medicinal uses of the non-cultivated vegetables surveyed, according to several ethnobotanical studies carried out in the Iberian Peninsula (Benítez, González-Tejero, & Molero-Mesa, 2008; Carvalho & Morales, 2010; Carvalho, 2010; Tardío, 2010).

Species	Edible use	Medicinal uses
Anchusa azurea	Cooked	Gastralgia, cold, kidney stones, pain, skin problems and so on
Beta maritima	Cooked	Digestive disorders, burns and throat pains and anaemia
Papaver rhoeas	Raw and cooked	Nervousness, insomnia, digestive and respiratory disorders, baldness, eye infection, as well as for measles treatment
Rumex papillaris	Raw in salads	-
Rumex pulcher	Cooked	-
Chondrilla juncea	Raw in salads	-
Cichorium intybus	Raw and cooked	Digestive disorders such as laxative, diuretic and invigorative, hypoglycaemic, depurative, disinfectant of urinary tract, hepatoprotective, and in skin diseases
Sonchus oleraceus	Raw and cooked	As depurative and diuretic, in contusions and burns, and also to prevent haemorrhoids
Taraxacum obovatum	Raw in salads	Hepatoprotective, in kidney diseases, malfunction, and asthenia
Scolymus hispanicus	Boiled and fried in olive oil with garlic; raw in salads	Digestive disorders as gastralgias, in Malta fever and in eye infection
Silybum marianum	Boiled and fried in olive oil with garlic; raw in salads.	Liver diseases, gall-blander infection and haemorrhoids

Table 2. Moisture contents (g/100g of fresh weight), vitamin C and organic acids composition (mg/100 g of fresh weight) of the edible basal leaves of different non-cultivated vegetables.

Species	Moisture	Ascorbic acid	Dehidroascorbic acid	Total Vitamin C	Oxalic acid	Glutamic acid	Malic acid	Citric acid	Fumaric acid
Anchusa azurea	90.32 ^{cd}	0.67 ^{a} (0.14)	11.74 ^{def} (6.41)	12.09 ^{bcd} (6.02)	378 ^{bc} (266.39)	191.29 ^{c}	42.90 ^{b} (18.16)	8.02 ^{ab}	71.81 ^{c} (45.89)
Beta maritima	84.51 ^{ab}	9.99 ^{cd} (1.62)	10.63 ^{cde} (2.51)	21.36 ^{cde} (3.12)	581 ^{bcd} (428.64)	nd	51.36 ^{bc} (7.41)	126.24 ^{d} (11.37)	7.14 ^{b} (0.60)
Chondrilla juncea	82.54 ^{abc}	1.76 ^{a} (1.58)	15.22 ^{de} (3.75)	16.51 ^{cd} (4.96)	93 ^{ab} (11.71)	nd	92.65 ^{bc} (80.43)	45.65 ^{b}	0.78 ^{a} (0.58)
Cichorium intybus	89.10 ^{bcd}	4.78 ^{ab} (2.82)	15.04 ^{de} (3.89)	19.80 ^{cde} (2.26)	40 ^{a} (36.06)	70.22 ^{ab}	92.51 ^{bc} (57.35)	30.32 ^{b}	2.40 ^{a} (0.98)
Papaver rhoeas	85.36 ^{abc}	14.11 ^{d} (2.25)	16.86 ^{ef} (10.06)	31.03 ^{fg} (12.29)	446 ^{bcd} (321.56)	95.73 ^{ab}	147.19 ^{bc} (92.49)	149.04 ^{d}	4.40 ^{ab} (3.86)
Rumex	86.40 ^{abc}	14.28 ^{cd} (8.24)	10.96 ^{de} (5.34)	25.81 ^{ef} (6.48)	251 ^{abc} (20.55)	253.67 ^{cd}	117.53 ^{bc} (19.65)	30.35 ^{b}	nd
Rumex pulcher	79.11 ^{bcd}	20.22 ^{f} (3.56)	15.79 ^{def} (5.43)	35.07 ^{fg} (5.24)	317 ^{bc} (259.33)	nd	31.07 ^{b} (3.24)	79.52 ^{bc}	nd
Sonchus oleraceus	88.45 ^{bcd}	2.80 ^{ab} (1.24)	10.00 ^{cd} (1.64)	11.92 ^{bc} (1.81)	539 ^{bcd} (359.43)	43.69 ^{a} (2.43)	318.00 ^{d} (170.31)	13.16 ^{b}	1.14 ^{a} (0.74)
Taraxacum	83.89 ^{ab}	1.99 ^{ab} (0.84)	14.48 ^{de} (4.82)	16.72 ^{cd} (5.40)	20 ^{a} (16.65)	62.95^{ab} (42.83)	106.20 ^{bc} (55.35)	27.61 ^{b}	3.28 ^{ab} (0.50)
Scolymus hispanicu	84.15 ^{abc}	1.11 ^{a} (0.18)	2.73 ^{ab} (0.51)	3.30 ^{a} (1.12)	485 ^{bcd} (470.45)	nd	79.89 ^{c} (13.46)	59.51 ^{cd}	1.29 ^{a} (1.04)
Silybum	93.44 ^{d}	0.36 ^{a} (0.00)	2.39 ^{a} (0.59)	2.74 ^{a} (0.58)	1030 ^{g} (859.46)	nd	4.16 ^{a} (0.65)	1.16 ^{a}	123.09 ^{d} (83.30)

Values are expressed as mean (standard deviation, n-1), $12 \leq n \leq 18$. In each column, different letters mean statistically significant differences ($p < 0.05$)

nd = not detected

* Only peeled basal leaves (fleshy midribs) were analyzed.

Table 3. Tocopherols composition (mg/100 g of fresh weight) of the basal leaves of different non-cultivated vegetables.

Species	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	Total
Anchusa azurea	0.36 ^{abc} (0.08)	0.05 ^{a} (0.00)	0.11 ^{a} (0.02)	0.01 ^{a} (0.00)	0.48 ^{b} (0.09)
Beta maritima	0.51 ^{abc} (0.02)	0.01 ^{a} (0.00)	0.14 ^{a} (0.00)	tr	0.63 ^{b} (0.15)
Chondrilla juncea	0.57 ^{bc} (0.18)	0.01 ^{a} (0.01)	0.12 ^{a} (0.01)	0.05 ^{c} (0.01)	0.74 ^{b} (0.21)
Cichorium intybus	0.99 ^{c} (0.11)	0.04 ^{a} (0.01)	1.88 ^{c} (0.09)	0.08 ^{c} (0.00)	2.98 ^{e} (0.19)
Papaver rhoeas	1.13 ^{c} (0.06)	0.66 ^{b} (0.13)	0.05 ^{a} (0.00)	0.02 ^{a} (0.01)	1.87 ^{cd} (0.19)
Rumex papillaris	1.29 ^{d} (0.44)	0.05 ^{a} (0.02)	0.34 ^{b} (0.13)	nd	1.67 ^{cd} (0.58)
Rumex pulcher	0.44 ^{abc} (0.02)	0.03 ^{a} (0.01)	0.07 ^{a} (0.00)	nd	0.54 ^{b} (0.02)
Sonchus oleraceus	1.70 ^{d} (0.05)	0.04 ^{a} (0.01)	0.47 ^{b} (0.03)	0.01 ^{a} (0.00)	2.22 ^{d} (0.06)
Taraxacum obovatum	0.51 ^{abc} (0.02)	0.01 ^{a} (0.00)	0.05 ^{a} (0.00)	0.03 ^{b} (0.01)	0.60 ^{b} (0.01)
Scolymus hispanicus *	0.02 ^{a} (0.00)	0.02 ^{a} (0.00)	0.01 ^{a} (0.00)	tr	0.05 ^{a} (0.00)
Silybum marianum *	0.04 ^{ab} (0.00)	tr	0.01 ^{a} (0.00)	0.10 ^{d} (0.00)	0.15 ^{a} (0.00)

Values are expressed as mean (standard deviation, n-1), n=3. In each column, different letters mean statistically significant differences ($p < 0.05$)

nd = not detected

tr = traces; tr ≤ 0.005 mg/100 g

* Only peeled basal leaves (fleshy midribs) were analyzed

Table 4. Extraction yields (η, g/100g) and antioxidant capacity EC_{50} values (mg/mL) of the edible basal leaves of different non-cultivated vegetables.

Species	η	Total phenolics (mg GAE/g extract)	Total flavonoids (mg CE/g extract)	EC_{50} values (mg/mL)			
				DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS inhibition
Anchusa azurea	14.04	148,62 ^{h} (2,00)	84.81 ^{i} (4,03)	0.02^{a} (0.00)	0.01 ^{a} (0.00)	0.02 ^{a} (0.00)	0.03 ^{b} (0.00)
Beta maritima	37.34	61.91 ^{e} (7,51)	21.55 ^{e} (0,87)	1.35 ^{d} (0.03)	0.47 ^{d} (0.00)	0.38 ^{cd} (0.00)	0.05 ^{d} (0.00)
Chondrilla juncea	24.28	37.66 ^{c} (2,40)	7.43 ^{b} (0,28)	1.64 ^{e} (0.15)	0.34 ^{b} (0.01)	0.38 ^{cd} (0.02)	0.12 ^{f} (0.00)
Cichorium intybus	19.34	73.68 ^{f} (0,66)	31.35 ^{g} (1,00)	1.11 ^{c} (0.05)	0.57 ^{e} (0.01)	0.45 ^{f} (0.01)	0.02 ^{a} (0.00)
Papaver rhoeas	20.95	25.86 ^{i} (3,52)	12.00 ^{c} (0,46)	1.28 ^{f} (0.03)	0.40 ^{c} (0.00)	0.56 ^{g} (0.11)	0.02 ^{a} (0.00)
Rumex papillaris	27.95	104.18 ^{g} (4,17)	39.49 ^{h} (3,26)	2.45 ^{d} (0.09)	0.60 ^{e} (0.01)	0.30 ^{b} (0.01)	0.03 ^{b} (0.00)
Rumex pulcher	28.83	73.44 ^{j} (5,32)	26.14 ^{f} (0,87)	3.31 ^{g} (0.10)	0.84 ^{f} (0.01)	0.34 ^{bc} (0.00)	0.02 ^{a} (0.00)
Sonchus oleraceus	27.68	51.33 ^{d} (1,75)	14.83 ^{d} (0,98)	1.36 ^{d} (0.02)	0.89 ^{g} (0.05)	0.03 ^{a} (0.00)	0.05 ^{d} (0.00)
Taraxacum obovatum	26.20	58.26 ^{e} (0,90)	30.03 ^{g} (0,66)	0.79 ^{b} (0.10)	0.48 ^{d} (0.01)	0.37 ^{cd} (0.00)	0.07 ^{e} (0.00)
Scolymus hispanicus	21.69	21.51 ^{b} (1,51)	8.39 ^{b} (1,12)	4.97 ^{h} (0.08)	5.97 ^{h} (0.04)	0.65 ^{h} (0.01)	0.04 ^{c} (0.00)
Silybum marianum *	47.34	3.72 ^{a} (0,36)	1.13 ^{a} (0,27)	13.09 ^{i} (0.04)	1.82 ^{i} (0.01)	0.44 ^{df} (0.03)	0.02 ^{a} (0.00)

Values are expressed as mean (standard deviation, n-1), n=3. In each column, different letters mean statistically significant differences ($p < 0.05$).

GAE = Galic acid equivalents; CE = Cathequin acid equivalents

nd = not detected

* Only peeled basal leaves (fleshy midribs) were analyzed

Table 5. Correlations coefficients and (*P*-values) between the antioxidant compounds content and the antioxidant capacity of the studied non-cultivated vegetables.

	AA	Vit C	Phen	Flav	α-T	β-T	γ-T	δ-T	T. T	DPPH	RP	β_Car
Vit C	**0.596**											
	(0.000)											
Phen	0.121	**0.282**										
	(0.146)	**(0.000)**										
Flav	-0.033	**0.186**	**0.945**									
	(0.674)	**(0.025)**	**(0.000)**									
α-T	0.114	0.000	**0.229**	0.046								
	(0.170)	(0.013)	**(0.005)**	(0.577)								
β-T	**0.327**	**0.341**	**-0.237**	**-0.187**	**0.181**							
	(0.000)	**(0.000)**	**(0.004)**	**(0.024)**	**(0.029)**							
γ-T	-0.081	0.126	**0.237**	0.124	**0.450**	-0.138						
	(0.335)	(0.130)	**(0.004)**	(0.138)	**(0.000)**	(0.098)						
δ-T	**-0.359**	**-0.411**	**-0.504**	**-0.350**	**-0.319**	-0.130	-0.055					
	(0.000)	**(0.000)**	**(0.000)**	**(0.000)**	**(0.000)**	(0.118)	(0.506)					
T. T	0.082	**0.232**	**0.193**	0.031	**0.928**	**0.234**	**0.715**	**-0.214**				
	(0.326)	**(0.005)**	**(0.020)**	(0.707)	**(0.000)**	**(0.004)**	**(0.000)**	**(0.009)**				
DPPH	**-0.252**	**-0.507**	**-0.675**	**-0.525**	**-0.368**	0.011	**-0.286**	**0.877**	**-0.329**			
	(0.002)	**(0.000)**	**(0.000)**	**(0.000)**	**(0.000)**	(0.892)	**(0.000)**	**(0.000)**	**(0.000)**			
RP	**-0.270**	**0.000**	**-0.626**	**-0.471**	**-0.343**	-0.086	**-0.229**	**0.901**	**-0.302**	**0.978**		
	(0.001)	**(0.000)**	**(0.000)**	**(0.000)**	**(0.000)**	(0.300)	**(0.005)**	**(0.000)**	**(0.000)**	**(0.000)**		
β-Car	0.153	**0.091**	**-0.561**	**-0.591**	**-0.550**	-0.044	**-0.365**	0.141	**-0.552**	**0.381**	**0.267**	
	(0.065)	**(0.010)**	**(0.000)**	**(0.000)**	**(0.000)**	(0.598)	**(0.000)**	(0.091)	**(0.000)**	**(0.000)**	**(0.001)**	
TBARS	0.129	0.693	**0.177**	0.089	**-0.214**	**-0.144**	-0.006	-0.033	0.128	0.061	0.024	**0.230**
	(0.123)	(0.246)	**(0.033)**	(0.288)	**(0.009)**	**(0.083)**	(0.939)	(0.693)	(0.124)	(0.468)	(0.767)	**(0.005)**

Statistically significant correlations are shown in bold letter ($p<0.05$). AA: ascorbic acid; Vit C: Vitamin C; Phen: Phenolics; Flav: Flavonoids; DPPH: EC_{50} DPPH assay; RP: EC_{50} Reducing Power; β -car: EC_{50} β -carotene bleaching inhibition; TBARS: EC_{50} TBARS assay; α -T: α -tocopherol; β -T: β -tocopherol; γ -T: γ -tocopherol; δ -T: δ -tocopherol