Nutritional composition and bioactive properties of commonly consumed wild greens: potential sources for new trends in modern diets

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ABSTRACT

Beyond the composition of the usual macronutrients and micronutrients, it is important to provide information on the composition of bioactive compounds and antioxidant capacity of foods, particularly of wild species to regain them for nowadays dietary habits. Many greens are known as excellent sources of natural antioxidants, and consumption of fresh plants in the diet may contribute to the daily antioxidant intake. In the present study five leafy wild greens traditionally consumed (*Borago officinalis, Montia fontana, Rorippa nasturtium-aquaticum, Rumex acetosella, Rumex induratus*) were studied in order to document macronutrients, micronutrients and non-nutrients composition. *R. induratus* revealed the highest levels of sugars, ascorbic acid, tocopherols, lycopene, chlorophylls, flavonoids, and one of the highest antioxidant activity expressed as DPPH scavenging activity, β-carotene bleaching inhibition, and TBARS formation inhibition. *R. nasturdium-aquaticum* showed the healthier PUFA/SFA and n-6/n-3 ratios, and *B. officinalis* proved to be a source of γ-linolenic acid and other fatty acids from n-6 series that are precursors of mediators of the inflammatory response. The nutritional characteristics and antioxidant potential of these wild greens require reconsideration of their role in traditional as well as in contemporary diets. Furthermore, their extracts might find applications in the prevention of free radical-related diseases, as functional food formulations.

Keywords: Wild greens; Macronutrients; Micronutrients; Non-nutrients; Antioxidants.

1. Introduction

Consumers demand more detailed information about the nutritional value and ingredient composition of foods. Moreover, they request new formulations consistent with current health recommendations. In this way, beyond the composition of the usual macronutrients and micronutrients, it seems important to also provide information on the composition of bioactive compounds and the antioxidant capacity of foods (Hassimoto, Genovese, & Lajolo, 2009).

Macronutrients (such as carbohydrates, proteins and fats) constitute the majority of an individual's diet, supplying energy, and the essential nutrients that are needed for growth, maintenance, and activity. Micronutrients (such as vitamins or trace minerals) are required in very small amounts to orchestrate a whole range of physiological functions, but which the organism itself cannot produce. Active non-nutrients (chemical compounds found in certain foods that are nonessential for the body's operation but enhance health by playing an active or protective role) alongside nutrients in human diet are also very important. Antioxidant phytochemicals such as phenolic compounds are a good example of healthy non-nutrients (Ferreira, Barros, & Abreu, 2009).

Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants increases the antioxidant capacity of the plasma and reduces the risk of some cancers, heart diseases, and stroke (Justesen & Knuthsen, 2001; Hassimoto et al., 2009). These properties are attributed to a variety of constituents, including vitamins and numerous phytochemicals, mainly phenolic compounds, such as flavonoids (Justesen & Knuthsen, 2001).

Vegetables are known as excellent sources of natural antioxidants, and consumption of fresh plants in the diet may therefore contribute to the daily antioxidant intake.

Traditional rural diets used to include interesting amounts of leafy greens, i.e. plant leaves sometimes attached to tender petioles and shoots, which were eaten raw in salads, stir-fried, stewed or steamed and made in tortillas. Several greens are claimed to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients (Heinrich et al., 2005; Pardo de Santayana, Tardío, & Morales, 2005; Carvalho & Morales, 2010). Herein, five wild edible species widely consumed as leafy greens in many rural communities in the Mediterranean (Tardío, Pardo de Santayana, & Morales, 2006; Hadjichambis et al., 2008; Carvalho & Morales, 2010) were studied in order to document macronutrients, micronutrients and non-nutrients composition and to provide new insights into local foods and traditional cuisine which can be useful for modern diet and nutrition.

2. Materials and methods

2.1. Samples

Five of the most frequently cited leafy greens according to several ethnobotanical surveys (Tardio et al., 2006; Hadjichambis et al., 2008; Carvalho, 2010) were selected for analysis, i.e., *Borago officinalis* L. (borage; pt: borragem), *Montia fontana* L. (water-blinks; pt: merujas), *Rorippa nasturtium-aquaticum* (L.) Hayek (water-cress; pt: agrião), *Rumex acetosella* L. (sheep sorrel; pt: azedinhas), *Rumex induratus* Boiss. & Reut. (sorrel; pt: azedas).

Plant material was gathered in 2010 early spring in Trás-os-Montes, Portugal considering local consumers' sites, criteria and preferences (Carvalho, 2010; Carvalho & Morales, 2010) and samples were prepared accordingly. Young basal leaves of

borage and fully expanded leaves of sorrel were gathered in human disturbed habitats, the shoots of sheep sorrel in meadows and aerial parts of water-blinks and water-cress were collected in streams. All the species are claimed to have nutritional and healing properties when gathered in the proper season and stage of growth. They are widely eaten raw or cooked, except borage that is mainly consumed boiled, because of some harmful effects as reported by several informants (Carvalho, 2010).Voucher specimens are deposited in the Escola Superior Agrária de Bragança herbarium (BRESA). The samples were lyophilized (Ly-8-FM-ULE, Snijders, Holland) and kept in the best conditions (- 80 ºC and protected from light) for subsequent use.

2.2. Standards and Reagents

Acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, L-ascorbic acid, sugars (D(-)-fructose, D(+)-glucose anhydrous, D(+)-melezitose hydrate, D(+)-raffinose pentahydrate, D(+)-sucrose, and D(+)-trehalose) standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid, malvidin 3-glucoside, quercetin dehydrate, and (+)-catechin. Racemic tocol, 50 mg/ml and tocopherols (α-, β-, γ-, and δ-isoforms) were purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Macronutrients

2.3.1. Nutritional value. The samples were analysed for chemical composition (moisture, proteins, fats, carbohydrates and ash) using the AOAC procedures (AOAC, 1995). The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations: Energy (kcal) = 4 × (g proteins +g carbohydrates) + 9 × (g fats).

2.3.2. Sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by Barros, Carvalho, Morais, & Ferreira (2010a), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

2.3.3. Fatty Acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Barros et al., 2010a). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30ºC/min ramp to 125 ºC, 5ºC/min ramp to 160 ºC, 20ºC/min ramp to 180 ºC, 3ºC/min ramp to 200 ºC, 20ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 ml/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

2.4. Micronutrients

2.4.1. Tocopherols. Tocopherols content was determined following a procedure previously optimized and described by Barros, Heleno, Carvalho, & Ferreira (2010b), using tocol as IS. The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30ºC. 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. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response,

using the internal standard method, and tocopherols contents were further expressed in mg per 100 g of dry weight (dw).

2.4.2. Vitamin C. Ascorbic acid was determined following a procedure previously described by the authors (Barros et al., 2010b) with 2,6-dichloroindophenol, and measuring the absorbance at 515 nm (spectrophotometer AnalytikJena). Content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (6×10^{-3}-0.1 mg/ml), and the results were expressed as mg of ascorbic acid per 100 g of dry weight (dw).

2.4.3. Pigments. β-carotene, lycopene, chlorophylls a and b were determined following a procedure previously described by the authors (Barros et al., 2010a), measuring the absorbance at 453, 505, 645, and 663 nm. Contents were calculated according to the following equations: β-carotene (mg/100 ml) = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Lycopene (mg/100 ml) = $- 0.0458 \times A_{663} + 0.204 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$; Chlorophyll a (mg/100 ml) = $0.999 \times A_{663} - 0.0989 \times A_{645}$; Chlorophyll b (mg/100 ml) = $- 0.328 \times A_{663} + 1.77 \times A_{645}$, and further expressed in mg per 100 g of dry weight (dw).

2.5. Non-nutrients and in vitro antioxidant properties

2.5.1. Extraction procedure. A fine dried powder (20 mesh; ~1g) stirring with 50 ml of methanol at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 50 ml portion of methanol. The combined methanolic extracts were evaporated at 35ºC under reduced pressure (rotary

evaporator Büchi R-210), re-dissolved in methanol at 5 mg/ml, and stored at 4 ºC for further use.

2.5.2. Phenolics.

For total phenolics quantification, the extract sample concentrated at 2.5 mg/ml (1 ml) was mixed with *Folin-Ciocalteu* reagent (5 ml, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/l, 4 ml). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development (Wolfe, Wu, & Liu, 2003). Absorbance was then measured at 765 nm. Gallic acid was used to calculate the standard curve (0.050-0.80 mM), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

For flavonoids quantification, the extract sample concentrated at 2.5 mg/ml (0.5 ml) was mixed with distilled water (2 ml) and $NaNO_2$ solution (5%, 0.15 ml). After 6 min, $AlCl_3$ solution (10%, 0.15 ml) was added and allowed to stand further 6 min. NaOH solution (4%, 2 ml) was added to the mixture, followed by distilled water until a final volume of 5 ml. The mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm (Jia, Tang, & Wu, 1999). (+)-Catechin was used to calculate the standard curve (1.5×10^{-2}-1.0 mM) and the results were expressed as mg of (+)-catechin equivalents (CE) per g of extract.

For Flavonols and Anthocyanins quantification, the extract sample concentrated at 2.5 mg/ml (250 µl) was mixed with HCl 0.1% in 95% etanol (250 µl) and HCl 2% (4550 µl). After 15 min the absorbance was measured at 360 and 520 nm. $A_{360\ nm}$ was used to estimate flavonols; quercetin was used to calculate the standard curve (0.2-3.2 mM) and the results were expressed as mg of quercetin equivalents (QE) per g of extract.$A_{520\ nm}$

was used to estimate anthocyanins; malvidin 3-glucoside was used to calculate the standard curve (0.1-2.3 mM) and the results were expressed as mg of malvidin 3-glucoside equivalents (ME) per g of extract (Mazza, Fukumoto, Delaquis, Girard, & Ewert, 1999).

2.5.3. DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek). 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 absorption 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 (Barros et al., 2010). 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. This methodology was performed using the Microplate Reader described above. The 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% w/v, 0.5 ml). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 ml) was added. The mixture (0.8 ml) was poured in the 48-wells, as also deionised water (0.8 ml) and ferric chloride (0.1% w/v, 0.16 ml), and the absorbance

was measured at 690 nm. 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 40ºC 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 50ºC in a water bath. 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 2h 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 (TBARS). Brains were obtained from pig (*Sus scrofa*) of body weight ~150 Kg, dissected and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (w/v) brain tissue homogenate which was centrifuged at 3000*g* (Centorion K24OR refrigerated centrifuge) 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 μM; 0.1 ml) and ascorbic acid (0.1 mM; 0.1 ml) at 37ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 0.5 ml), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 ml), and the mixture was then heated at 80 ºC 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. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] x 100%, where A and B were the absorbance of the control and the compound 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

For each one of the species three samples were used and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 16.0 program.

3. Results and discussion

3.1. Macronutrients

The results of the macronutrients composition and energetic value (expressed on dry weight basis) obtained for the studied wild edible greens are shown in **Table 1**. *M. fontana* revealed the highest moisture content (95 g/100 g). Carbohydrates, calculated

discounting protein and fat levels, were abundant macronutrients and the highest levels were found in *R. acetosella* (79 g/100 g), which may be due to the morphological type of plant material consumed, i.e. fibrous steams and highly veined leaves. *B. officinalis* and *R. nasturtium-aquaticum* revealed the highest ash contents (~17 to 18 g/100g), while *M. fontana*, *R. nasturtium-aquaticum* and *R. induratus* gave the highest levels of protein (~13 to 14 g/100 g) without significant statistical differences (p<0.05). The highest levels of fat were found in *R. induratus* (4 g/100g), contributing to its higher energy value (376g/100g). A sample of *Rumex vesicarius* obtained in Saudi Arabia, a species from the same genus of *R. induratus* and with similar uses, gave higher levels of protein (19 g/100 g) and ash (17 g/100 g), but slightly lower fat concentration (3 g/100 g) (Alfawaz, 2006).

The studied wild greens showed high nutritional value with a low energetic contribution of 356 kcal/100 g, on average. As far as we know this is the first report on nutritional value of those greens, with the exception of *B. officinalis* (Medrano, Masoud, & Martinez, 1992) that is largely studied due to its increasing agricultural interest because of the potential market for γ-linolenic acid (GLA) and antioxidant potential (Mhamdi, Wannes, Sriti, Ksouri, & Marzouk, 2010).

The five wild edible greens presented fructose, glucose, sucrose, trehalose and raffinose as main sugars (**Table 1**). *R. induratus* revealed the highest total sugars content (5 g/100 g), with the highest levels of fructose (2 g/100g) and glucose (1 g/100g). Sugars are only a small part of carbohydrates that also include polysaccharides such as amide and cellulose.

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and the ratios of PUFA/SFA and n-6/n-3 of the studied wild edible greens are shown in **Table 2**. *M. fontana* and *Rorippa nasturtium-aquaticum* revealed a similar profile with α-linolenic acid (C18:3n3) as the main fatty acid, followed by palmitic acid (C16:0) and linoleic acid (C18:2n6). The same was observed for *Rumex* species, but with an inversion in palmitic and linoleic acids contents. The present study adds detailed information about fatty acids composition to the work described by Taveira, Pinho, Gonçalves, Andrade, & Valentão (2009) in *Rumex induratus* leaves.

The four mentioned species revealed a clear prevalence of PUFA (>72%). Otherwise, *Borago officinalis* leaves revealed a quite different fatty acids composition (**Figure 1**), with prevalence of SFA due to the contribution of palmitic, arachidic (C20:0), and behenic (C22:0) acids (12% each SSFA). The main PUFA were linoleic (10%); γ-linolenic (C18:3n6; GLA; 11%), α-linolenic (12%); and *cis*-11,14-eicosadienoic acid (C20:2c; 13%).

The family Boraginaceae Juss. is one of the best known sources of GLA, an unusual fatty acid in plants, highly appreciated because of its nutritional and medical benefits. The concentrations of linoleic, α-linolenic, GLA, and erucic acid (22:1n9) are of special chemotaxonomic importance within this family (Velasco & Goffman, 1999). Most of the studies available were focused on *B. officinalis* seeds oil (Velasco & Goffman, 1999; Wettasinghe & Shahidi, 1999). Herein, we could found the mentioned four fatty acids also in *B. officinalis* leaves.

For "good nutritional quality", including health beneficial effects, PUFA/SFA ratios should be higher than 0.45, while n-6/n-3 fatty acids ratios should be lower than 4.0

(Guil, Torija, Giménez, & Rodriguez, 1996), as it is observed in the present study (**Table 2**). The best values were registered in *R. nasturtium-aquaticum* (4.9 and 0.2, respectively).

3.2. Micronutrients and pigments

Micronutrients such as vitamins contents were determined and the results are given in **Table 3**. Ascorbic acid was the most abundant vitamin in *B. officinalis* and in the two *Rumex* species. *R. induratus* revealed the highest concentration (1064 mg/100 g). Ascorbic acid (or vitamin C) is an indicator commonly used to evaluate frozen vegetables quality. In spite of its importance for human health, it is generally observed that if this vitamin is well preserved, the other nutrients are also well retained (Gonçalves, Cruz, Abreu, Brandão, & Silva, 2009).

The four vitamers of tocopherols were detected, being α-tocopherol the major compound in all the species. *Rumex* species presented the highest content of tocopherols (84 to 99 mg/100 g) without significant statistical differences (p<0.05), with the highest levels of all the isoforms. The tocopherols profile of *R. acetosella* is shown in **Figure 2**. *R. induratus* gave higher vitamins levels than a sample of *Rumex vesicarius* from Saudi Arabia (253 and 4.7 mg/100 g of ascorbic acid and tocopherols, respectively) (Alfawaz, 2006).

Pigments such as chlorophylls a and b were found in all the studied greens being the first pigment higher in *Rumex* species without significant statistical differences (p<0.05), and the second one in *R. induratus* (14 mg/100 g; **Table 3**). β-carotene was not detected in *R. nasturtium-aquaticum* and *Rumex* species. Nevertheless, this pigment was found in a cultivated sample of *R. nasturtium-aquaticum* obtained in the UK; 4.7

mg/100g fresh weight) using a HPLC method (Hart & Scott, 1995). Lycopene was not detected in *B. officinalis* and *M. fontana*, and the highest levels were found once more in *R. induratus* (5 mg/100 g).

The analyses performed allowed the first characterization of these wild greens in vitamins (ascorbic acid and tocopherols) and pigments (carotenoids and chlorophylls) composition, since the available reports in literature describe results in *B. officinalis* seeds (Velasco & Goffman, 1999) and cultivated *Nasturtium officinale* (Gonçalves et al., 2009).

3.3. Bioactive non- nutrients

The composition in non-nutrients and antioxidant activity of the studied wild greens is shown in **Table 4**. *Rumex* species gave the results in DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition, which is in agreement to its highest levels of phenolics (117 or 142 mg GAE/g extract), flavonoids (90 or 68 mg CE/g extract), and flavonols (57 mg QE/g extract). Particularly, *Rumex induratus* was already reported as a source of flavonoids, including glycosylated flavones (Ferreres, Gil-Izquierdo, Andrade, Valentão, & Tomás-Barberán, 2007).

B. officinalis also showed a high β-carotene bleaching inhibition with the highest anthocyanins levels (52 mg ME/g extract), and flavonoids concentration without statistical differences ($p<0.05$) of *R. induratus* (88 and 90 mg CE/g extract, respectively). Seeds methanolic extracts of *B. officinalis* from Tunisia revealed much lower phenolics content and DPPH scavenging activity than leaves (Mhamdi et al., 2010). The antioxidant properties of *B. officinalis* seeds were attributed to their phenolic constituents including phenolic acids such as rosmarinic, syringic and sinapic acids

(Wettasinghea, Shahidia, Amarowicz, & Abou-Zaid, 2001). Ethanolic extracts of leaves from Italy revealed lower phenolics and flavonoids contents (97 and 9 mg chlorogenic acid equivalents/g extract, respectively), but slightly higher DPPH scavenging activity (0.06 mg/ml) (Conforti et al., 2008). It seems that its antioxidant properties are related to other antioxidants rather than phenolic compounds.

The studied methanolic extract of *R. nasturtium-aquaticum* showed similar phenolics levels, higher flavonoids concentration and TBARS inhibition, and lower DPPH scavenging activity than ethanolic:water extract of a sample from Iran: 96-97 mg GAE/g extract, 62-63 mg CE/g extract, 0.27 mg/ml and 0.12 mg/ml, respectively) (Yazdanparast, Bahramikia, & Ardestani, 2008; Bahramikia & Yazdanparast, 2010). There are also available reports on antioxidant properties (Hassimotto et al., 2009) and flavonoids composition (Justesen & Knuthsen, 2001) of cultivated samples.

Significantly linear correlations (**Table 5**) were established between the non-nutrients contents, and antioxidant activity EC_{50} values. This proves that the wild green with the highest bioactive non-nutrients contents is the most efficient in antioxidant activity (with the lowest EC_{50} values). The correlations were more significant for reducing power assay. DPPH scavenging activity correlates better with flavonols, reducing power with phenolics and flavonols, β-carotene bleaching inhibition with phenolics and TBARS inhibition also with phenolics.

In conclusion, the studied wild greens are nutritionally well-balanced vegetables, and based on the antioxidant potential of their extracts, they might find applications in the prevention of free radical-related diseases, as functional food formulations. Particularly *R. induratus* revealed the highest levels of total sugars, fructose, glucose, ascorbic acid,

tocopherols, lycopene, chlorophylls, flavonoids and also presented one of the highest antioxidant activity expressed as DPPH scavenging activity, β-carotene bleaching inhibition, and TBARS formation inhibition. *R. nasturdium-aquaticum* showed the healthier PUFA/SFA and n-6/n-3 ratios, and *B. officinalis* proved to be a source of GLA, and other fatty acids from n-6 series that are precursors of mediators of the inflammatory response.

The culinary and organoleptic characteristics of these wild greens and their high content in antioxidants require reconsideration of their role in the traditional as well as in the contemporary diet.

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Table 1. Macronutrients composition and energetic value of the wild edible greens.

	Borago officinalis	*Montia fontana*	*Rorippa nasturtium-aquaticum*	*Rumex acetosella*	*Rumex induratus*
Moisture (g/100 g fw)	86.85 ± 0.40 e	95.22 ± 0.74 a	93.23 ± 0.99 b	89.09 ± 1.01 d	90.29 ± 0.53 c
Ash (g/100 g dw)	17.88 ± 0.86 a	15.07 ± 0.10 b	16.81 ± 0.08 a	10.93 ± 1.06 c	11.07 ± 0.30 c
Proteins (g/100 g dw)	8.93 ± 1.58 b	13.38 ± 0.01 a	13.58 ± 0.38 a	7.85 ± 1.86 b	13.54 ± 0.28 a
Fat (g/100 g dw)	1.25 ± 0.23 d	3.09 ± 0.47 b	2.97 ± 0.47 b	2.35 ± 0.28 c	3.97 ± 0.14 a
Carbohydrates (g/100 g dw)	71.94 ± 1.70 b	68.46 ± 0.29 c	66.64 ± 0.31 c	78.87 ± 1.50 a	71.42 ± 0.28 b
Energy (kcal/100 g dw)	334.73 ± 3.26 e	355.18 ± 0.84 c	347.61 ± 1.24 d	368.03 ± 3.98 b	375.55 ± 0.36 a
Fructose (g/100 g dw)	0.14 ± 0.03 d	0.76 ± 0.17 cb	0.89 ± 0.02 b	0.60 ± 0.00 c	1.71 ± 0.09 a
Glucose (g/100 g dw)	0.58 ± 0.06 c	1.00 ± 0.02 ba	0.82 ± 0.08 bc	0.73 ± 0.01 c	1.26 ± 0.20 a
Sucrose (g/100 g dw)	1.52 ± 0.13 a	0.44 ± 0.05 c	0.99 ± 0.17 b	0.21 ± 0.07 c	1.25 ± 0.31 ba
Trehalose (g/100 g dw)	0.22 ± 0.06 c	0.38 ± 0.06 bc	0.33 ± 0.05 bc	0.63 ± 0.14 a	0.55 ± 0.12 ba
Raffinose (g/100 g dw)	nd	0.28 ± 0.07 a	0.07 ± 0.01 c	0.12 ± 0.03 bc	0.20 ± 0.02 ba
Total Sugars (g/100 g dw)	2.46 ± 0.16 bc	2.86 ± 0.32 bc	3.10 ± 0.27 b	2.29 ± 0.19 c	4.97 ± 0.31 a

In each row, different letters mean significant differences ($p<0.05$).

Table 2. Fatty acids (percentage) composition of the wild edible greens.

	Borago Officinalis	*Montia fontana*	*Rorippa nasturtium-aquaticum*	*Rumex acetosella*	*Rumex induratus*
C6:0	0.70 ± 0.09 a	0.04 ± 0.01 b	0.03 ± 0.00 b	0.10 ± 0.01 b	0.09 ± 0.01 b
C8:0	0.31 ± 0.03 a	0.03 ± 0.00 b	0.06 ± 0.01 b	0.06 ± 0.00 b	0.03 ± 0.00 b
C10:0	0.35 ± 0.06 a	0.05 ± 0.01 b	0.05 ± 0.01 b	0.08 ± 0.02 b	0.09 ± 0.02 b
C12:0	0.92 ± 0.19 a	0.17 ± 0.01 c	0.28 ± 0.06 cb	0.54 ± 0.02 b	0.93 ± 0.20 a
C14:0	2.80 ± 0.69 a	0.43 ± 0.01 c	0.64 ± 0.06 c	1.69 ± 0.13 b	2.66 ± 0.34 a
C14:1	0.23 ± 0.03 d	0.94 ± 0.01 c	0.55 ± 0.05 d	3.55 ± 0.27 b	4.25 ± 0.34 a
C15:0	0.28 ± 0.01 a	0.04 ± 0.00 c	0.20 ± 0.01 b	0.20 ± 0.08 b	0.25 ± 0.04 ba
C16:0	12.03 ± 0.70 cb	17.22 ± 1.06 a	13.20 ± 0.31 b	11.23 ± 0.73 c	9.36 ± 0.71 d
C16:1	0.13 ± 0.01 d	0.33 ± 0.06 c	0.49 ± 0.01 b	0.71 ± 0.15 a	0.72 ± 0.00 a
C17:0	0.17 ± 0.01 a	0.11 ± 0.02 b	0.15 ± 0.01 a	0.16 ± 0.00 a	0.16 ± 0.00 a
C18:0	2.28 ± 0.04 a	0.10 ± 0.03 c	0.92 ± 0.02 b	0.95 ± 0.07 b	2.34 ± 0.09 a
C18:1n9	2.08 ± 0.20 b	2.37 ± 0.38 b	0.70 ± 0.03 c	3.43 ± 0.32 a	2.20 ± 0.05 b
C18:2n6	9.50 ± 1.25 e	18.71 ± 0.45 b	11.84 ± 0.18 d	20.18 ± 0.48 a	13.76 ± 0.01 c
C18:3n6	10.86 ± 0.92 a	0.04 ± 0.00 b	0.08 ± 0.00 b	nd	nd
C18:3n3	12.26 ± 1.90 e	55.57 ± 0.80 c	68.42 ± 0.24 a	51.34 ± 1.41 d	58.84 ± 1.03 b
C20:0	11.91 ± 0.30 a	1.90 ± 0.26 b	0.14 ± 0.02 c	0.86 ± 0.01 cb	1.48 ± 0.15 b
C20:1	0.26 ± 0.05 a	0.19 ± 0.00 a	0.01 ± 0.00 a	0.18 ± 0.00 a	0.07 ± 0.00 a
C20:2	12.56 ± 2.01 a	0.28 ± 0.04 b	0.04 ± 0.00 b	0.67 ± 0.17 b	0.13 ± 0.04 b
C20:3n6	nd	0.27 ± 0.02 a	0.04 ± 0.00 b	0.14 ± 0.02 ba	nd
C20:4n6	nd	nd	0.04 ± 0.00 a	nd	nd
C20:3n3+C21:0	0.21 ± 0.01 a	0.19 ± 0.01 ba	0.15 ± 0.05 c	0.16 ± 0.02 bc	0.15 ± 0.03 c
C20:5n3	nd	0.05 ± 0.01 b	0.60 ± 0.03 a	0.02 ± 0.00 b	nd
C22:0	12.00 ± 1.06 a	0.50 ± 0.04 c	0.22 ± 0.00 c	1.44 ± 0.09 b	0.85 ± 0.09 cb
C22:1n9	0.44 ± 0.06 a	0.17 ± 0.06 b	nd	0.07 ± 0.01 b	nd
C22:2	nd	0.04 ± 0.00 c	nd	0.08 ± 0.03 b	0.13 ± 0.02 a
C23:0	3.02 ± 0.05 a	nd	0.01 ± 0.00 c	0.49 ± 0.03 b	0.56 ± 0.12 b
C24:0	4.55 ± 0.43 a	0.24 ± 0.06 d	0.80 ± 0.02 c	1.67 ± 0.13 b	0.94 ± 0.16 c
C24:1	0.17 ± 0.02 b	0.01 ± 0.00 c	0.36 ± 0.04 a	0.02 ± 0.00 c	nd
Total SFA	**51.32 ± 0.93 a**	**20.83 ± 0.14 b**	**16.68 ± 0.51 c**	**19.45 ± 1.12 b**	**19.76 ± 0.73 b**
Total MUFA	**3.30 ± 0.49 b**	**4.02 ± 0.50 b**	**2.11 ± 0.00 c**	**7.96 ± 0.06 a**	**7.24 ± 0.39 a**
Total PUFA	**45.38 ± 1.92 d**	**75.15 ± 0.36 b**	**81.20 ± 0.50 a**	**72.59 ± 1.06 c**	**73.01 ± 1.12 cb**
PUFA/SFA	0.88 ± 0.05 c	3.61 ± 0.01 b	4.87 ± 0.18 a	3.74 ± 0.27 b	3.70 ± 0.19 b
n-6/n-3	1.64 ± 0.08 a	0.34 ± 0.01 b	0.17 ± 0.00 c	0.39 ± 0.02 b	0.23 ± 0.00 c

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); γ-Linolenic acid (C18:3n6); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); Dihomo-γ-linolenic acid (C20:3n6); Arachidonic acid (C20:4n6); *cis*-11,14,17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); *cis*-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3); Behenic acid (C22:0); Erucic acid (C22:1n9); Docosandienoic acid (C22:2); Tricosanoic acid (C23:0); Lignoceric acid (C24:0); Nervonic acid (C24:1).
The results are expressed in percentage. In each row different letters mean significant differences ($p<0.05$).

Table 3. Micronutrients and pigments composition of the wild edible greens.

	Borago officinalis	*Montia fontana*	*Rorippa nasturtium-aquaticum*	*Rumex acetosella*	*Rumex induratus*
α-tocopherol (mg/100 g dw)	8.75 ± 0.57 d	45.23 ± 5.65 b	22.30 ± 0.16 c	52.24 ± 9.18 ba	62.55 ± 1.61 a
β-tocopherol (mg/100 g dw)	0.31 ± 0.02 c	1.76 ± 0.35 b	0.66 ± 0.02 cb	4.83 ± 0.93 a	0.50 ± 0.02 c
γ-tocopherol (mg/100 g dw)	2.30 ± 0.15 c	8.63 ± 1.37 cb	2.09 ± 0.05 c	24.91 ± 5.68 a	15.65 ± 0.41 b
δ-tocopherol (mg/100 g dw)	0.13 ± 0.01 c	0.98 ± 0.04 c	1.37 ± 0.24 c	16.71 ± 1.08 a	5.58 ± 0.56 b
Total tocopherols (mg/100 g dw)	11.49 ± 0.70 c	56.60 ± 7.40 b	26.42 ± 0.43 c	98.69 ± 16.86 a	84.28 ± 0.62 a
Vitamin C (mg/100 g dw)	19.09 ± 1.73 d	39.48 ± 7.23 c	17.34 ± 3.22 d	180.48 ± 4.38 b	1064.51 ± 2.56 a
β-carotene (mg/100 g dw)	2.35 ± 0.04 b	3.80 ± 0.32 a	nd	nd	nd
Lycopene (mg/100 g dw)	nd	nd	1.42 ± 0.04 c	3.42 ± 0.06 b	4.80 ± 0.06 a
Chlorophyll a (mg/100 g dw)	2.40 ± 0.05 d	8.80 ± 0.64 c	10.26 ± 0.04 b	11.18 ± 0.05 a	10.86 ± 0.09 a
Chlorophyll b (mg/100 g dw)	0.54 ± 0.03 e	2.70 ± 0.33 d	10.87 ± 0.10 b	7.25 ± 0.22 c	13.62 ± 0.22 a

nd- not detected. In each row different letters mean significant differences ($p<0.05$).

Table 4. Non-nutrients composition and antioxidant properties (EC_{50} values) of the wild edible greens.

	Borago officinalis	*Montia fontana*	*Rorippa nasturtium-aquaticum*	*Rumex acetosella*	*Rumex induratus*
Phenolics (mg GAE/g extract)	113.58 ± 0.92 c	47.47 ± 1.62 d	50.42 ± 2.77 d	141.58 ± 3.67 a	117.08 ± 2.54 b
Flavonoids (mg CE/g extract)	88.17 ± 2.73 a	25.88 ± 1.01 d	35.17 ± 3.36 c	67.91 ± 3.02 b	89.78 ± 2.81 a
Flavanols (mg QE/g extract)	51.71 ± 0.53 b	24.40 ± 1.23 d	32.76 ± 0.67 c	56.95 ± 0.40 a	57.17 ± 1.17 a
Anthocyanins (mg ME/g extract)	51.50 ± 1.29 a	14.02 ± 0.32 d	14.81 ± 0.42 d	35.49 ± 0.10 c	42.94 ± 1.58 b
DPPH scavenging activity (mg/ml)	0.07 ± 0.00 c	0.22 ± 0.01 a	0.13 ± 0.03 b	0.03 ± 0.00 d	0.03 ± 0.00 d
Reducing power (mg/ml)	0.23 ± 0.01 c	0.84 ± 0.02 a	0.74 ± 0.02 b	0.16 ± 0.01 d	0.22 ± 0.01 c
β-carotene bleaching inhibition (mg/ml)	0.13 ± 0.02 c	0.46 ± 0.04 b	0.85 ± 0.16 a	0.12 ± 0.01 c	0.19 ± 0.03 c
TBARS inhibition (mg/ml)	0.14 ± 0.00 c	0.25 ± 0.01 b	0.38 ± 0.06 a	0.11 ± 0.02 cd	0.10 ± 0.01 d

In each row different letters mean significant differences ($p<0.05$).

Table 5. Correlations established between phenolics, flavonoids, flavonols, anthocyanins, tartaric esters and antioxidant activity EC_{50} values of the wild edible greens.

	DPPH scavenging activity (mg/ml)		Reducing power (mg/ml)		β-carotene bleaching inhibition (mg/ml)		TBARS inhibition (mg/ml)	
	Linear equation	R^2	Linear equation	R^2	Linear equation	R^2	Linear equation	R^2
Phenolics (mg ClAE/g extract)	y=-452.14x+136.65	0.7995	y=-127.83x+149.96	0.9580	y=-113.77x+134.26	0.7319	y=-303.97x+153.78	0.7713
Flavonoids (mg CE/g extract)	y=-0.0024x+0.2423	0.7256	y=-0.0101x+1.0603	0.8564	y=-0.008x+0.8458	0.5547	y=-0.0033x+0.3977	0.6272
Flavonols (mg QE/g extract)	y=-0.0054x+0.3330	0.9189	y=-0.0213x+1.3883	0.9726	y=-0.0158x+1.0569	0.5518	y=-0.0066x+0.4907	0.6538
Anthocyanins (mg ME/g extract)	y=-0.0039x+0.2180	0.6052	y=-0.0176x+0.9963	0.8241	y=-0.0152x+0.8377	0.6413	y=-0.0058x+0.3812	0.6318

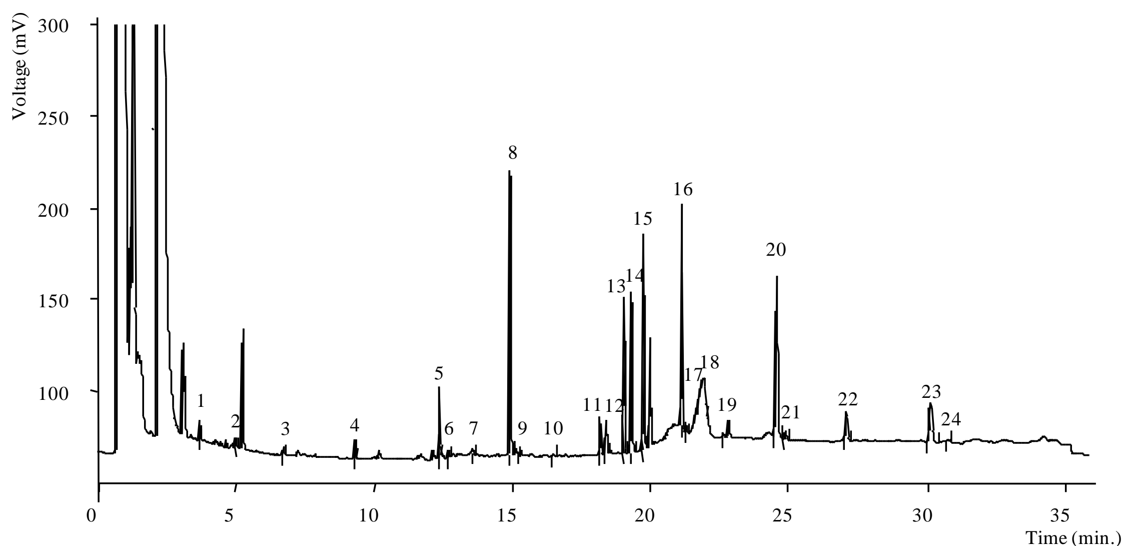

Figure 1. Individual fatty acids chromatograms of *Borago officinalis*.

1- C6:0; 2- C8:0; 3- C10:0; 4- C12:0; 5- C14:0; 6-C14:1; 7- C15:0; 8- C16:0; 9- C16:1; 10- C17:0; 11- C18:0; 12- C18:1n9; 13 C18:2n6; 14- C18:3n6; 15-C18:3n3; 16- C20:0; 17- C20:1; 18- C20:2c; 19- C20:3n3+C21:0; 20- C22:0; 21- C22:1n9; 22- C23:0; 23- C24:0; 24- C24:1.

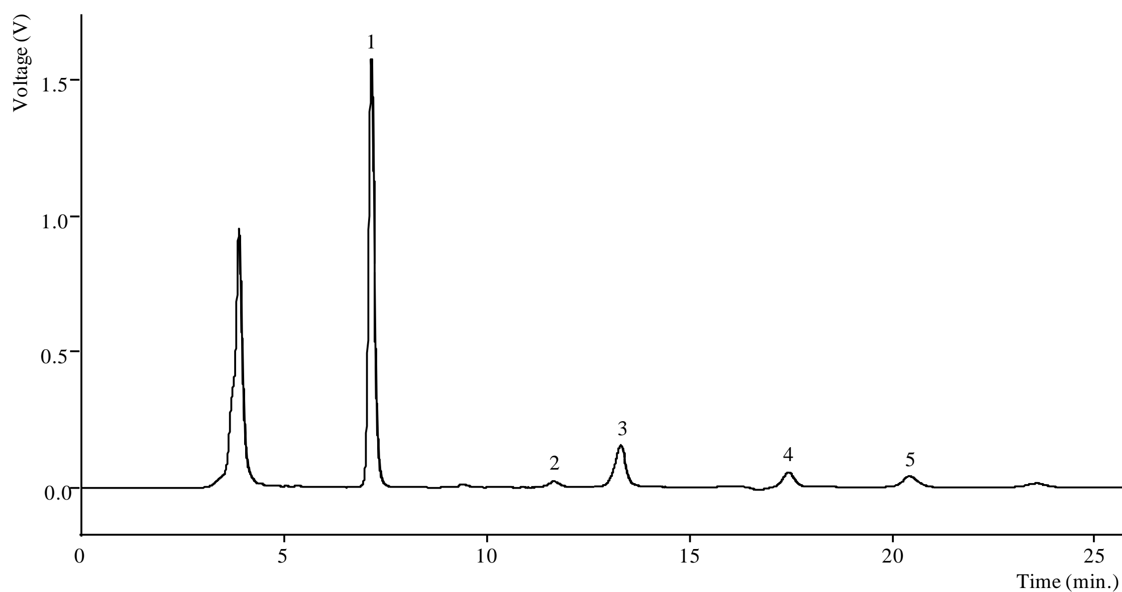

Figure 2. Individual tocopherols chromatogram of *Rumex acetosella*: 1-α-tocopherol; 2-β-tocopherol; 3- γ-tocopherol; 4- δ-tocopherol; 5-tocol (IS).