Wild Mediterranean Plants as Traditional Food: A Valuable Source of Antioxidants

Paola Vanzani, Monica Rossetto, Veronica De Marco, Linda E. Sacchetti, Maurizio G. Paoletti, and Adelio Rigo

Abstract: Some wild Mediterranean plants used as traditional food are an extraordinary source of antioxidants. We tested some properties of 10 of these herbaceous plants, used in Liguria (Northwest Italy) to prepare a traditional dish known as “prebuggiun.” A total of 9 of them were found to have a polyphenol content and antioxidant properties similar or better than those of red chicory and blueberry, which are, in the case of vegetables and fruits, among the richest of antioxidants.

Keywords: antioxidant activity, edible herbs, lipid peroxidation, polyphenols, radical scavenging activity

Practical Application: In this article, we reported a study on wild plants growing in the Mediterranean area. These herbs have been neglected and this study aimed to revalue these plants because they are an extraordinary source of antioxidants. The increasing demand for natural antioxidants (additives in the food industry too) justifies the search for new sources of natural antioxidants. The revaluation of these plants will be interesting for: (1) consumer health, rediscovering a vegetable source of high antioxidant power; (2) possibility of producing new commercial products, such as food supplements of high quality and low cost; (3) pharmacological applications.

Introduction

Epidemiological data indicate that cardiovascular diseases are low in the Mediterranean area where plant foods rich in antioxidants make up a considerable portion of the diet (Willett and others 1995; La Vecchia 2009). These observations are supported by recent field studies. For instance, significantly higher values of total antioxidant activity and of fibrinogen were measured in plasma of elderly women living in rural area of Crete and were correlated with high wild plant consumption (Manios and others 2005). To this regard, in the Mediterranean basin, wild plants rich of antioxidants are harvested and are eaten seasonally; however, it is important to know more about their contribution to healthy diets. Furthermore the increasing demand for natural antioxidants justifies the search for new sources of these compounds (Herrero and others 2006; Mohamed and others 2007; Gruenwald 2009; Huber and Rupasinghe 2009; Mustafa and others 2010).

In this regard, attention has recently been paid to the possible health benefits of foods characterized by high free-radical trapping activity since some of these radicals are involved in human diseases and in particular in cardiovascular and neurological pathologies (Lee and others 2004; Rossetto and others 2008; Tosetti and others 2009). Among these foods, blueberries and chicories (wild Cichorium intybus, and cultivated, that is, red chicories) rank at the top for their antioxidant activity (Rossetto and others 2005; Salvatore and others 2005). Therefore, the radical trapping properties of the Mediterranean flora, comprising more than 10000 plant species with various properties (antinflammatory, diuretic, and so on), deserve further investigation. To this respect, we considered a traditional food from Liguria region (Northwest Italy, especially in the province of Genova) called “prebuggiun” or “prebuggion,” which consists of dozens of wild herbs. The new leaves and shoots of these herbs are usually harvested from early spring to summer and are used raw, or boiled for a few minutes, as key ingredients for soups, stuffing for pies, and vegetable raviolis (the typical pansotti) or simply as “a side-dish” (Bisio and Minuto 1999). In particular, prebuggiun is a “mixture” of wild or semidomesticated herbs collected in farmed or uncultivated land. These herbs belong to 15 families, more than half of which from the Compositae family (Bisio and Minuto 1999).

According to some researchers a variety of phenolics such as hydroxycinnamic acids, benzoic acids, flavonols, flavones, anthocyanins beyond cumarins, terpenes, alkaloids, and carotenoids, are present in some of prebuggiun plants (Paoletti and others 1995; Mimaki and others 2001; el-Mousallamy 2002; Vlcek and others 2002; Ayoub 2003; Zeghichi and others 2003; Parejo and others 2004; Galvez and others 2005; Heinrich and others 2005; Adam and others 2009; Conforti and others 2009). However, despite the richness of phenolic compounds of some of these plants, homogeneous and quantitative data on the antioxidant activity and phenolic composition have not been reported yet.

We examined 10 of these herbs for polyphenol content, their antioxidant properties, including iron reducing power and their capacity and efficiency in trapping peroxyl radicals (LOO·), and found that almost all of these plants show antioxidant features similar or better than those characterizing red chicories and blueberries, which are at the top among vegetables and fruits for antioxidant activity (Rossetto and others 2005).
Wild plants as source of antioxidants

Materials and Methods

Chemicals
Rac1-lauroylglycerol and AlCl3 hexahydrate was obtained from Sigma-Aldrich (Milano, Italy). Linoleic acid, deoxycholic acid sodium salt monohydrate, sodium acetate, FeSO4, FeCl3, and 2,4,6-Tri(2-pyridyl)-5-triazine were purchased from Fluka (Buchs, Switzerland). 2,2’-azobis(2-[2-imidazolin-2-yl]propene) dihydrochloride (ABIP) was a kind gift of Wako Chemicals (Neuss, Germany). Folin–Ciocalteu phenol reagent was obtained from Sigma-Aldrich. Chlorogenic acid, gallic acid, cyanidin-3-glucoside, and quercetin were purchased from Extrasynthèse (Genay Cedex, France).

Quartz distilled water was used to prepare all the aqueous solutions. Buffers were equilibrated in batch with Chelex-100 (Bio-Rad, Richmond, Calif., U.S.A.) to minimize the concentration of heavy metal ions.

Harvesting and sample preparation
Each of the wild herbaceous plant was harvested in 4 different areas of the seaside hills east of Portofino (Genova, Italy) in spring, when they are particularly good for eating. The plants were processed within 48 h from the harvesting.

Verona red chicory, wild chicory (Cichorium intybus), and blueberry (Vaccinium myrtillus), used as comparison, were obtained from local markets in Padova.

The extracts of the plants were prepared using about 100 g of edible part (leaves) of this plant from each area. The raw herbs, randomly sampled, 400 g, were homogenized in 1 L of ethanol/water solution (85 : 15, v/v) containing 0.12 M HCl (Rossetto and others 2005). The homogenates were centrifuged and the supernatants were stored at −80 °C until measurements.

Spectrophotometric characterization and quantification of various families of antioxidant
Plant extracts (5 and 180 mg fresh weight per milliliter in ethanol/water solution, 85 : 15, v/v, containing 0.12 M HCl) were analyzed spectrophotometrically in the 250 to 800 nm UV-Vis range.

Total hydroxycinnamic acid content. Hydroxycinnamic acid content was determined measuring the absorbance of the complex of hydroxycinnamic acids with aluminum (III) (Porozhets and others 2003). Appropriately diluted extracts were mixed with a solution of AlCl3 in sodium acetate buffer, (50 mM Al[III] final concentration). Optical density was measured at 365 nm. Chlorogenic acid was used as a standard, and the results were expressed as millimoles of chlorogenic acid per kilogram of vegetable fresh weight.

Total anthocyanin content. Anthocyanin content was determined according a pH-differential method (Yang and Zhai 2010). Briefly, absorbances of the hydro-alcoholic extract (0.1 M HCl), and of the extract buffered at pH 4.5 (with sodium acetate) were measured at 530 and 700 nm, respectively.

The absorbance due to anthocyanins was calculated as Abs = (Abs530 − Abs700) pH 1 − (Abs530 − Abs700) pH 4.5 and the anthocyanin concentration was calculated using cyanidin 3-glucoside as standard compound. Anthocyanin concentration was express as millimoles of cyanidin 3-glucoside per kilogram of vegetable fresh weight.

Total phenol content
The total phenol content (TP) was measured according to the Folin–Ciocalteu method (Lee and others 2003). The absorbance measurements were carried out by a Varian Cary 50 spectrophotometer at 770 nm after incubation of the reaction mixture for 60 min at room temperature. The TP of extracts was expressed as millimoles of gallic acid per kilogram of plant fresh weight.

The ferric reducing antioxidant power (FRAP)
The ferric reducing antioxidant power (FRAP assay) of the ethanolic extracts was estimated according to the procedure described by Benzie and Strain (1996). This method is based on the electron transfer between the antioxidant molecules and Fe3+ ions. The change of absorbance of the FRAP reagent (prewarmed at 37 °C) was monitored at 596 nm after 10 min of reaction. A Varian Cary 50 UV-Vis spectrophotometer was used. Solutions of FeSO4 of known concentration were used for calibration.

Measurement of the peroxyl radical scavenging activity
The peroxyl radical scavenging activity of various ethanolic extracts was calculated by measuring their ability to inhibit the peroxidation of linoleic acid in a micellar system (Zennaro and others 2007). The oxygraphic method that we have used on the basis of a rigorous kinetic model permits obtaining the peroxyl radical trapping capacity (PRTC) and the equivalent peroxyl radical trapping efficiency (ePRTE) of the extracts. PRTC is expressed as millimoles of LOO• trapped by 1 kg of plant fresh weight and ePRTE is the efficiency of food in trapping LOO• expressed as millimoles of Trolox in 1 kg of plant fresh weight. The rate of peroxidation of linoleic acid was measured from the rate of O2 disappearance by a Metrohm 663 VA stand equipped with a Yellow Spring Oxygen electrode, inserted into a thermostated oxygraphic cell. The current study was recorded by a personal computer equipped with a data acquisition board (DAQ PCI-6221, M series, Natl. Instruments, Austin, Tex., U.S.A.). The working electrode was poised at −800 mV against Ag/AgCl. The experimental oxygraphic traces were automatically processed by means of a computational procedure to obtain oxygen consumption rates from which the PRTC and PRTE values were calculated (Zennaro and others 2007).

The reaction mixture was prepared by evaporating the solvent from a solution of Rac1-lauroylglycerol in dichloromethane and by dissolving the resulting film in 20 mM phosphate buffer, pH 7.4, containing 5 mM deoxycholic acid sodium salt, to obtain 0.5 mM final concentration of Rac1-lauroylglycerol. Linoleic acid (2 mM final concentration) was then added and the solution vigorously vortexed. The micelle containing solution was equilibrated with atmospheric oxygen in the oxygraphic cell thermostated at 37 ± 0.1 °C. After thermal equilbrium, ABIP, 4 mM final concentration, was added as a constant source of LOO•, which start lipid peroxidation and the plant extracts were injected (at a final concentration 0.1 to 0.01 g/mL) 1 min after the ABIP addition.

Statistical analysis
The measurements were usually carried out in quadruplicate and values are expressed as means ± standard deviations (SD). The Student’s t-test was used to compare data of raw and boiled herbs. Analysis of variance (ANOVA) was performed and differences between means were considered statistically significant at P ≤ 0.05 according to Fisher’s least significant difference (LSD test). Multiple linear regressions were carried out to correlate important phenolic constituents to antioxidant activity tests.

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Wild plants as source of antioxidants...

All statistical analyses were performed using SigmaStat 3.1, Advisory Statistics for Scientists.

Results and Discussion

Wild herbs used as food

The wild Mediterranean plants examined in this study are listed in Table 1 together with their traditional properties. All these plants may be eaten raw or boiled for a few minutes and, according to popular belief, most of them present a variety of properties such as depurative, diuretic, antiinflammatory, analgesic, digestive, cicatrizing, and so on (Trichopoulou 2001; Guarrera 2003; Herold and others 2003; García-Lafuente and others 2009).

Spectrophotometric characterization of polyphenols present in the plant extracts

Figure 1A and B show the UV-Vis spectra in the region 250 to 500 nm of the acid hydroalcoholic extracts obtained from the wild herbs. For sake of comparison in Figure 1A, the spectra of blueberry (l) and of wild (f) and red chicory (h) extracts together with that of chlorogenic acid (i), a hydroxycinnamic acid, are also reported.

Most of these spectra are characterized by the presence of a peak at 335 nm and of a shoulder at about 300 nm, which are spectroscopic features of hydroxycinnamic acids. In Figure 1C we have reported the spectra of the concentrated extracts of some prebuggiun herbs together with that of cyanidin-3-glucoside in the 450 to 650 nm region, where the anthocyanins absorb. In all the extracts, with the exception of those of Sanguisorba minor L. and Foeniculum vulgare, the presence of a peak around 535 nm was found which could be attributed to anthocyanins.

The confirmation of the presence of these important families of antioxidants was carried out according to validate and selective colorimetric methods using specific reagents (see Materials and Methods) and the results obtained are reported in Table 2. According to these methods, the large presence of hydroxycinnamic acids in the prebuggiun plants was confirmed with the exclusion of Rumex crispus and of Sanguisorba minor where this family of phenols was found in negligible amount. However, only in the case of these 2 plants, a significant amount of flavonoids was found according to the flavonoid test of Tabart and others (2010).

Furthermore anthocyanins, which are present in a large amount in blueberry and Verona red Chicory came out to be present in a very small amount (<1/100 of the total amount of polyphenols) in the prebuggiun plants we have examined, see Table 2, last column.

Table 1–Characteristics of some Mediterranean herbaceous plants (prebuggiun) used as food in Liguria region.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Family</th>
<th>Preparations</th>
<th>Traditional properties</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hyoseris radiata</em> L.</td>
<td>Wild chicory</td>
<td>Compositae</td>
<td>leaves boiled and/or eaten raw in salads</td>
<td>blood depurative, diuretic, depurative, analgesic against toothache</td>
</tr>
<tr>
<td><em>Reichardia picroides</em> (L.) Roth</td>
<td>French Scorzonera</td>
<td>Compositae</td>
<td>leaves boiled and/or eaten raw in salads</td>
<td>antiinflammatory, cicatrizing action, lenitive</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em> L.</td>
<td>Ribwort Plantain</td>
<td>Plantaginaceae</td>
<td>leaves boiled. Taste: slightly bitter</td>
<td>astringent, tonic, laxative, cholagogue</td>
</tr>
<tr>
<td><em>Sonchus oleraceus</em> L.</td>
<td>Sow Thistle</td>
<td>Compositae</td>
<td>leaves boiled and/or eaten raw in salads.</td>
<td>astringent, digestive, cough-relieving</td>
</tr>
<tr>
<td><em>Foeniculum vulgare</em> Miller</td>
<td>Wild fennel</td>
<td>Apiaceae</td>
<td>leaves and stem boiled and/or eaten raw in</td>
<td>aperitive, digestive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>salads</td>
<td></td>
</tr>
<tr>
<td><em>Sanguisorba minor</em> L.</td>
<td>Salad Burnet</td>
<td>Rosaceae</td>
<td>leaves boiled and/or eaten raw in salads.</td>
<td>astringent, digestive, cough-relieving</td>
</tr>
<tr>
<td><em>Centranthus ruber</em> (L.) DC.</td>
<td>Red Valerian</td>
<td>Valerianaceae</td>
<td>leaves boiled; young leaves are eaten raw</td>
<td>nerve, antispasmodic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>in salads</td>
<td></td>
</tr>
<tr>
<td><em>Rumex crispus</em> L.</td>
<td>Curled Dock</td>
<td>Polygonaceae</td>
<td>boiled leaves</td>
<td>antinflammatory, cicatrizing action, tonic, ristorative, antiseptic, astringent revulsive and cicatrizing action, against haemorrhoids remineralizing</td>
</tr>
<tr>
<td><em>Silene vulgaris</em> (Moench) Garcke</td>
<td>Bladder Campion</td>
<td>Caryophyllaceae</td>
<td>boiled leaves and/or eaten raw in salads</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1–UV-Vis spectra of the acid hydroalcoholic extracts. A and B: 5 mg of fresh weight per milliliter of a, Reichardia picroides (L.) Roth; b, Sonchus oleraceus L.; c, Plantago lanceolata L.; d, Hyoseris radiata L.; e, Foeniculum vulgare Miller; f, wild Cichorium intybus; g, Centranthus ruber (L.) DC.; h, 40 μM chlorogenic acid; i, Verona Red Chicory; j, blueberry; and m, Sanguisorba minor L.; n, Rumex crispus L.; o, Silene vulgaris (Moench) Garcke; p, Ranunculus ficaria L. C: 0.18 g of fresh weight per milliliter, of some Prebuggiun herbs; q, 5 μM cyanidin 3-glucoside.
Wild plants as source of antioxidants...

Antioxidant properties

Plant extracts were examined for the TP, for the electron exchange properties (FRAP) and for the capacity (PRTC), and efficiency (ePRTE) in trapping LOO⁻, measured according to the oxygraphic method previously described (Zennaro and others 2007).

PRTC represents the amount of peroxyl radical trapped by a given amount of food, while ePRTE represents the reactivity by which LOO⁻ is trapped and is correlated with the kinetic rate constant of the inhibition reaction. The obtained values are reported in Table 2. Although the red chicory and blueberry are characterized by a high content of polyphenols (some millimoles of gallic acid per kilogram fresh weight) in the case of the prebuggin herbs that we have examined, the TP content is similar or higher. The amount of polyphenols present in Sanguisorba minor L. is particularly high. In fact this content is many times higher than the polyphenol content in wild blueberry. In column 4 of Table 2, the results of ferric iron reducing antioxidant power measured according to the FRAP assay on the herb extracts are also reported. The FRAP values of prebuggin herbs are only in 2 cases (Sanguisorba minor L. and Rumex crispus L.) remarkable higher than those of reference plants (wild and cultivated chicory and blueberry), while in the other cases the FRAP values are similar to the reference value.

It is interesting to observe the linear relationship between the TP and FRAP values of the prebuggin herbs (r > 0.97) if the Sanguisorba minor L. value is excluded. For these herbs a ratio FRAP/TP = 1.5 ± 0.3 was calculated, while this ratio in the case of Sanguisorba minor L. and of red chicory and blueberry was 2.8 ± 0.3 (see Table 3).

Regarding the ePRTE and PRTC, the values of these parameters are in most of the herbs we have examined similar or higher than those we have found for blueberries and red chichories, see Table 2. In particular, the PRTC values of all the herbs, with the exception of Ranunculus ficaria L., are significantly higher than the PRTC of blueberry. Low linear correlation was found between the ePRTE and PRTC values (r = 0.47), as expected for kinetic and stoichiometric parameters. Also, the linear relationship

Table 2—Antioxidant properties of some wild plants (prebuggin) and comparison to reference plants.

<table>
<thead>
<tr>
<th>Prebuggin plants</th>
<th>ePRTEb (mmol/kg)</th>
<th>PRTCc (mmolAAO/100 g)</th>
<th>FRAPd (mmol Fe2+/kg)</th>
<th>TPd (mmol/kg)</th>
<th>Hydroxy-cinnamic acids (mmol/kg)</th>
<th>Anthocyanins E (mmol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyoseris radiata L. (L.) Roth</td>
<td>105 ± 2.0a</td>
<td>171 ± 10c</td>
<td>31.1 ± 2.0e</td>
<td>19.8 ± 1.1de</td>
<td>17.5</td>
<td>0.027</td>
</tr>
<tr>
<td>Reichardia procida (L.) Roth</td>
<td>103 ± 4.8a</td>
<td>153 ± 9.4d</td>
<td>38.7 ± 1.6d</td>
<td>22.9 ± 1.5d</td>
<td>19.3</td>
<td>0.025</td>
</tr>
<tr>
<td>Plantago lanceolata L.</td>
<td>91.1 ± 5.2b</td>
<td>223 ± 11a</td>
<td>43.8 ± 1.4d</td>
<td>27.6 ± 1.9c</td>
<td>14.4</td>
<td>0.012</td>
</tr>
<tr>
<td>Sonchus oleraceus L.</td>
<td>88.3 ± 5.2b</td>
<td>129 ± 5.5e</td>
<td>31.8 ± 2.2e</td>
<td>18.6 ± 1.7e</td>
<td>13.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Foraminium vulgare Müller</td>
<td>72.3 ± 3.6c</td>
<td>105 ± 4.1f</td>
<td>27.9 ± 0.7e</td>
<td>17.8 ± 1.1f</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Sanguisorba minor L.</td>
<td>69.9 ± 1.6c</td>
<td>212 ± 10b</td>
<td>257 ± 12a</td>
<td>98.2 ± 4.6a</td>
<td>&lt; 0.5</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Centaurea ruber (L.) DC.</td>
<td>43.1 ± 2.0d</td>
<td>99.2 ± 7.3f</td>
<td>26.2 ± 0.5e</td>
<td>20.6 ± 1.6d</td>
<td>12.0</td>
<td>0.012</td>
</tr>
<tr>
<td>Rumex crispus L.</td>
<td>35.4 ± 6.8e</td>
<td>N.M.</td>
<td>131 ± 5.8b</td>
<td>61.8 ± 2.0b</td>
<td>&lt; 0.5</td>
<td>0.051</td>
</tr>
<tr>
<td>Ranunculus ficaria L. (Verona Red)</td>
<td>7.7 ± 0.5f</td>
<td>33.2 ± 1.7l</td>
<td>12.8 ± 1.0f</td>
<td>11.0 ± 0.4g</td>
<td>5.1</td>
<td>0.019</td>
</tr>
<tr>
<td>Silene vulgaris (Moench) Garcke</td>
<td>5.7 ± 1.1f</td>
<td>N.M.</td>
<td>27.4 ± 0.6e</td>
<td>26.8 ± 1.6c</td>
<td>6.5</td>
<td>0.025</td>
</tr>
</tbody>
</table>

Reference plants

| Chichonius intybus L.                  | 92.7 ± 7.0b      | 74.3 ± 2.0b            | 38.1 ± 1.3d          | 13.5 ± 0.6g  | 7.9                             | 1.9                      |
| Chichorium (Verona Red)                |                  |                       |                     |              |                                 |                          |
| Chichorium myrtilus (wild blueberry)   | 102.4 ± 4.8a     | 62.3 ± 5.0a            | 64.5 ± 4.5c         | 21.0 ± 2.4d  | < 1                             | 7.6                      |
| Chichorium intybus L. (wild chicory)   | 37.8 ± 2.8d      | 87.3 ± 5.0g            | 16.9 ± 0.83f        | 12.6 ± 0.6g  | 6.8                             | 0.013                    |

All the values are referred to the fresh weight of plants.

bEPRTE: equivalent Peroxyl Radical Trapping Efficiency. Values are expressed in terms of millimoles of Trolox equivalent present in 1 Kg of food.

cPRTC: Peroxyl Radical Trapping Capacity. Values are expressed in terms of millimoles of peroxyl radicals (LOO⁻) trapped by 1 Kg of food.

dFRAP = ferric reducing antioxidant power. Values are expressed in terms of millimoles of iron reduced by 1 Kg of food.

ePRTC/TP = Total Phenols content from Folin–Ciochot method. Values are expressed as millimoles of gallic acid equivalent per kilogram of food.

*Quantified as chlorogenic acid.

Statistical difference between plants were analyzed by ANOVA (LSD, P ≤ 0.05). Means with the same letter within a column were not significantly different (a to l).

Table 3—Comparison of antioxidant properties of prebuggin plants.

<table>
<thead>
<tr>
<th>Prebuggin plants</th>
<th>ePRTE/TP</th>
<th>PRTC/TP</th>
<th>ePRTE/PRTC</th>
<th>FRAP/TP</th>
<th>FRAP/ePRTE</th>
<th>FRAP/PRTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyoseris radiata L. (L.) Roth</td>
<td>5.3b</td>
<td>8.6a</td>
<td>0.61d</td>
<td>1.66d</td>
<td>0.30d</td>
<td>0.186</td>
</tr>
<tr>
<td>Reichardia procida (L.) Roth</td>
<td>4.5bc</td>
<td>6.7b</td>
<td>0.67c</td>
<td>1.7d</td>
<td>0.39d</td>
<td>0.25ef</td>
</tr>
<tr>
<td>Plantago lanceolata L.</td>
<td>3.3d</td>
<td>8.1a</td>
<td>0.41e</td>
<td>1.6d</td>
<td>0.48d</td>
<td>0.206gh</td>
</tr>
<tr>
<td>Sonchus oleraceus L.</td>
<td>4.7b</td>
<td>6.9b</td>
<td>0.68c</td>
<td>1.7d</td>
<td>0.36d</td>
<td>0.25eg</td>
</tr>
<tr>
<td>Foraminium vulgare Müller</td>
<td>4.1c</td>
<td>5.9c</td>
<td>0.69c</td>
<td>1.6d</td>
<td>0.39d</td>
<td>0.27e</td>
</tr>
<tr>
<td>Sanguisorba minor L.</td>
<td>0.7f</td>
<td>2.2f</td>
<td>0.33f</td>
<td>2.6b</td>
<td>3.68b</td>
<td>1.21a</td>
</tr>
<tr>
<td>Centaurea ruber (L.) DC.</td>
<td>2.1e</td>
<td>4.8d</td>
<td>0.43e</td>
<td>1.3f</td>
<td>0.61d</td>
<td>0.27e</td>
</tr>
<tr>
<td>Rumex crispus L.</td>
<td>0.66g</td>
<td>–</td>
<td>–</td>
<td>2.1c</td>
<td>3.70b</td>
<td>–</td>
</tr>
<tr>
<td>Ranunculus ficaria L.</td>
<td>0.76g</td>
<td>3.0e</td>
<td>0.23g</td>
<td>1.25g</td>
<td>1.66c</td>
<td>0.39d</td>
</tr>
<tr>
<td>Silene vulgaris (Moench) Garcke</td>
<td>0.2g</td>
<td>–</td>
<td>–</td>
<td>1.3g</td>
<td>4.83a</td>
<td>–</td>
</tr>
<tr>
<td>Chichorium intybus L. (Verona Red Chicory)</td>
<td>6.9a</td>
<td>5.5c</td>
<td>1.25b</td>
<td>2.8b</td>
<td>0.41d</td>
<td>0.51c</td>
</tr>
<tr>
<td>Chichorium myrtilus (wild blueberry)</td>
<td>4.9b</td>
<td>3.0e</td>
<td>1.64a</td>
<td>3.1a</td>
<td>0.63d</td>
<td>1.04b</td>
</tr>
<tr>
<td>Chichorium intybus L. (wild chicory)</td>
<td>3.0d</td>
<td>6.9b</td>
<td>0.43e</td>
<td>1.3ef</td>
<td>0.45d</td>
<td>0.19gh</td>
</tr>
</tbody>
</table>

Different letters within a column (a to h) indicate significant difference, analyzed by LSD test, P ≤ 0.05.
Wild plants as source of antioxidants...

between the PRTC and FRAP values, which reflects the relationship between the H and the electron exchange ability of the plants we have examined, is poor ($r = 0.51$). To this regard, the strong electron donating ability of *Sanguisorba minor* L. appears to be not paralleled to it by H-donation ability. In fact, the value of the ratio FRAP/PRTC is the highest among those of the plants we have examined.

The large variations of the ratios ePRTE/TP and of PRTC/TP, see Table 3 columns 2 and 3, are an indication of the different quality of the polyphenols present in the wild herbs we have studied.

Multiple linear regression test indicate that the antioxidant properties of the plants we have reported in Table 2 can be predicted from a linear combination of important phenolic constituents (TP, hydroxycinnamic acids, anthocyanins, which were considered as independent variables and are expressed as millimoles per kilogram of fresh weight).

The obtained multiple linear regression equations are the following:

$$e_{PRTE} = -29.1 + 0.93 \times TP + 6.23 \times \text{hydroxycinnamic acids} + 14.9 \times \text{anthocyanins}; \ r = 0.89.$$  

In this case, all the independent variables appear to contribute to the prediction of ePRTE being $P = 0.008$, $<0.001$, $<0.001$ for TP, hydroxycinnamic acids, and anthocyanins, respectively.

$$PRTC = -16.9 + 2.35 \times TP + 7.52 \times \text{hydroxycinnamic acids} + 2.77 \times \text{anthocyanins}; \ r = 0.90.$$  

In this case, the independent variables TP and hydroxycinnamic acids appear to account for the ability to predict PRTC being $P < 0.001$, 0.89, 0.038 for TP, hydroxycinnamic acids, and anthocyanins, respectively. In particular, from this analysis it appears that the important role of hydroxycinnamic acids and of anthocyanins is to determine the ePRTE of TP and of hydroxycinnamic acids to determine the PRTC and of TP to determine FRAP values.

It is worth underlining that the radical trapping activities (measured by ePRTE and PRTC values) of the prebuggiun herbs were assessed in a system that mimics the conditions occurring in the upper small intestine, where peroxidation of the poly-unsaturated fatty acids present in foods may easily occur and where the antioxidants present in these plants provide a real protection, since there are no bioavailability problems.

It is interesting to compare the antioxidant characteristics of boiled prebuggiun (a mixture containing 20 g of each wild herbs of Table 1) with those of some vegetables which are usually eaten boiled. In general, according to Figure 2, it appears that about 70% or more of the original activity of these vegetables is present in the cooking water, while from 30% to 44% of this activity is retained by boiled prebuggiun, and in much lower amount (in some case practically disappeared) by boiled spinach (*Spinacea oleracea* L.) and

$\text{FRAP} = -25.7 + 2.75 \times TP + 0.11 \times \text{hydroxycinnamic acids} + 4.81 \times \text{anthocyanins}; \ r = 0.99.$  

In this case, the independent variables TP and anthocyanins appear to account for the ability to predict FRAP being $P < 0.001$, 0.89, 0.038 for TP, hydroxycinnamic acids, and anthocyanins, respectively. In particular, from this analysis it appears that the important role of hydroxycinnamic acids and of anthocyanins is to determine the ePRTE of TP and of hydroxycinnamic acids to determine the PRTC and of TP to determine FRAP values.
Wild plants as source of antioxidants...

catalogna (Cichorium intybus L. var. foliosum). As a consequence, the antioxidant properties of the boiled prebuggian are significantly higher than those of boiled spinach and of boiled Cichorium intybus L. var. foliosum (P < 0.001). Furthermore, 2 more consideration can be done: (1) the antioxidant properties of boiled prebuggian are higher than that of fresh spinach and of fresh Cichorium intybus L. var. foliosum (P < 0.007); (2) in the case of these latter vegetables the ePRTE, PRTC, and FRAP values after boiling (boiled vegetable + cooking water) are below to that of fresh vegetables (P < 0.05), while in the case of prebuggian after boiling (boiled vegetable + cooking water) ePRTE (P = 0.017) and TP (P = 0.001) values appear significantly higher than those of fresh vegetables. This behavior may indicate that, during boiling, hydrolytic processes may occur increasing the antioxidant activity.

The valuable antioxidant activity of some plants of prebuggian is very high with respect of other plants of Mediterranean area. In fact Salvatore and others (2005) examined wild plants of Sicily very rich of antioxidant and found that Cichorium intybus (wild chicory), which has been used from immemorial time as vegetable in the Mediterranean area, has a significant high total antioxidant activity value with respect to other wild green herbs frequently eaten of this region, such as Sinapis incana, Sinapis nigra, Diplotaxis endiviae, Asparagus acutifolius, and Borage officinalis. In the case of prebuggian, herbs it appears that 7 or more of these plants present antioxidant parameters higher to that of wild Cichorium intybus that we have examined.

Conclusions

For the first time, the antioxidant properties of prebuggian were studied. These properties appear very relevant with respect to those of other wild plants of the Mediterranean flora and to those of red chicory and blueberry, which are, in the case of vegetables and fruits, among the richest of antioxidants. Therefore these properties, and in particular those related to capacity and efficiency in trapping LOO• characterizing some wild herbs present in prebuggian, appear important given the extremely limited number of plants used in modern diets, which may be improved if more antioxidant-rich plants are considered as possible food.

References


