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Extraction and HPLC Analysis of Phenolic Compounds in Leaves, Stalks, and Textile Fibers of *Urtica dioica* L.

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In the present study the phenolic composition of leaves, stalks, and textile fiber extracts from *Urtica dioica* L. is described. Taking into account the increasing demand for textile products made from natural fibers and the necessity to create sustainable "local" processing chains, an Italian project was funded to evaluate the cultivation of nettle fibers in the region of Tuscany. The leaves of two nettle samples, cultivated and wild (C and W), contain large amounts of chlorogenic and 2-*O*-caffeoylmalic acid, which represent 71.5 and 76.5% of total phenolics, respectively. Flavonoids are the main class in the stalks: 54.4% of total phenolics in C and 31.2% in W samples. Anthocyanins are second in quantitative importance and are present only in nettle stalks: 28.6% of total phenolics in C and 24.4% in W extracts. Characterization of phenolic compounds in nettle extracts is an important result with regard to the biological properties (antioxidant and antiradical) of these metabolites for their possible applications in various industrial activities, such as food/feed, cosmetics, phytomedicine, and textiles.

KEYWORDS: HPLC-DAD/MS; MS/MS spectra; *Urtica dioica* L. (nettle); flavonoids; anthocyanins; caffeoyl derivatives

INTRODUCTION

The interest of consumers in nonharmful natural fibers and textiles has greatly increased in recent years. Consumer demand for alternatives to the conventional, unsustainable textile chain and for information about all phases of the textile chain, from plant cultivation to fabric manufacturing (tracing), has also increased.

The present work is a part of the preliminary results from a large textile project relative to (i) technical−economic, environmental, and territorial evaluation for development of a new textile production in Tuscany; (ii) agroecological studies to evaluate production potentiality of fiber nettle plants in Tuscany; (iii) definition of sustainable methods for nettle fiber extraction and evaluation of the environmental impact of the process; and (iv) chemical characterization of different nettle plant parts to be used in different sectors, apart from textiles, according to the multifunctionality concept of agriculture.

Nettle (*Urtica dioica* L., Family: Urticaceae) is a dioecious plant with opposite, sharply toothed leaves, persistent stipules, and stinging trichomes. Nettle is a perennial, nitrophilous herb containing sclerenchymatic fibers in the bark, and it is widely distributed throughout the temperate regions of the world (*1*). In central Europe this plant was cultivated during the 19th century until the Second World War, and it has a long history as a fiber plant in Germany and Austria (*2*), where it was used, similarly to flax (*Linum usitatissimim* L.) and hemp (*Cannabis sativa* L.), for textiles before the introduction of cotton (*Gossypium* sp.). Recently, it has been reintroduced in Germany as a collection of nettle fabrics with the trade name Nettle World, confirming the evidence that the use of nettle for a sustainable and local fiber-producing industry, with low environmental impact, could be economically promising.

The fiber content of nettle clones ranges from 1.2 to 16% dry matter; this content is influenced mainly by genotype and somewhat by the cultivation method and environmental conditions (*3*). Fiber nettle cultivar 'Clone 13', selected by Bredemann (*2*) during the 1950s in Germany, is considered to be one of the most productive, and therefore it was also used in this project.

Various parts of the fiber nettle plant can also be used as food, fodder, and raw material for different purposes in cosmetics, medicine, industry, and biodynamic agriculture. In fact, this plant has been widely used for hundreds of years for its medicinal properties in the treatment of disease and disorders such as rheumatism, eczema, arthritis, gout, and anemia (*4, 5*).

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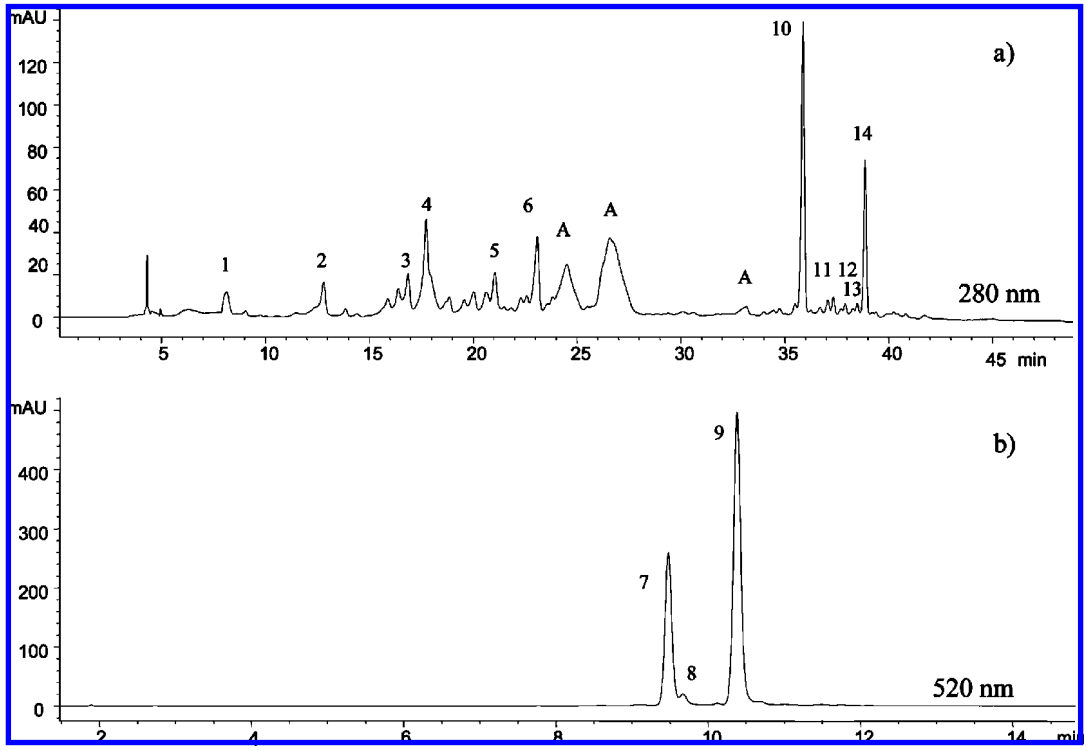

Figure 1. HPLC-DAD profiles acquired at two different wavelengths (280 and 520 nm) of hydroalcoholic extracts of nettle stalks. Peaks: 1, gallic acid (internal standard); 2, caffeic acid derivative; 3, *p*-coumaric acid; 4, chlorogenic acid; 5, caffeoylquinic acid; 6, 2-*O*-caffeoylmalic acid; A, anthocyanin compounds [detailed in **b**, as 7, peonidin 3-*O*-rutinoside; 8, peonidin 3-*O*-(6″-*O*-*p*-coumaroylglucoside); and 9, rosinidin 3-*O*-rutinoside]; 10, rutin; 11, quercetin *p*-coumaroyl glucoside; 12, kaempferol 3-*O*-glucoside; 13, kaempferol 3-*O*-rutinoside; 14, isorhamnetin 3-*O*-rutinoside.

Root tincture of stinging nettle may be an effective treatment for benign prostatic hyperplasia (BPH) (*6, 7*). In vitro and in vivo studies on nettle extracts have shown a mild anti-inflammatory effect, which may also be effective for treating certain individuals with allergic rhinitis (*8, 9*). In a previous work, the water extract of nettle was demonstrated to be a powerful antioxidant against various oxidative systems in vitro, due to phenolic compounds; moreover, this extract had antimicotic and antibacterial activity against *Staphylococcus aureus*, one of the most common Gram-positive bacteria causing food poisoning (*10*).

The biological activities of nettle leaves are assigned to the flavonoidic fraction: the methanolic extract of the aerial parts, containing quercetin and isorhamnetin glycosides, had an immunostimulatory activity on neutrophils, suggesting that it could be useful in treating patients suffering from neutrophil function deficiency and chronic granulomatous diseases (*11*); the aqueous methanolic extract of nettle roots has been used in clinics in Europe for the treatment of prostatic hyperplasia (*11*); the seeds and aqueous extract of the aerial parts of *U. dioica* L. have been occasionally used in Turkey as an herbal medicine by cancer patients (*11*). Furthermore, nettle leaves have demonstrated antiplatelet action, useful in the treatment and/or prevention of cardiovascular disease (*12*).

There are no comprehensive studies on the phenolic composition of leaves and stalks of *U. dioica* L., with previous work demonstrating the presence of caffeic acid, chlorogenic acid, 2-*O*-caffeoylmalic acid, and flavonoids in the aerial parts (*13*−*15*). To our knowledge very few data are available on the phenolic composition and anthocyanin content of the aerial parts (leaves and stalks) of the plant. Therefore, the aim of the present work was to completely characterize the content of phenolic metabolites in the raw material (i.e., fresh plant tissues, leaves, and stalks, these latter used to obtain a natural fiber by retting process), and to evaluate the competitiveness of the cultivated clone (Clone 13) on the textile market with respect to the spontaneous species.

MATERIALS AND METHODS

Nettle Cultivation. The experimental site is located in Prato (43° 53′ N, 011° 06′ E) in Tuscany. Because the high fiber content of a selected clone can be achieved only by vegetative propagation and planting of cuttings (*2, 16, 17*), the plants of nettle cultivar 'Clone 13' were multiplied in a greenhouse during the winter of 2005 and, after sufficient root development, they were transplanted into the field (May 8, 2006). Before transplanting, soil was prepared according to the methods used for the cultivation of herbs or vegetables, and a chemical fertilizer was applied (200 kg of N/ha). Plants were cultivated in rows 75 cm apart with a distance in the row of 50 cm, according to the method of ref *18*. Weed control, crucial in the first year of cultivation, was done mechanically (hoeing). Due to very small amounts of precipitation during summer 2006, two irrigations were carried out. From June to October, weekly measurements of stalk height (using a ruler) and diameter (using a caliper) were taken on 10 randomly selected plants. Even if the fiber of 1-year-old plants is not of the required quality for textile applications, fiber extractions were performed during the growing season to describe the increase of fiber content in the stalks. Fiber was also extracted from wild nettle plants collected in a field close to the nettle crop. Optimization of the analytical method to detect the permanence of antioxidants or antiradical substances, usually present in fresh leaves and stalks, was also carried out.

Extraction of Secondary Metabolites from Plant Material. Harvesting of plants was carried out on July 13, 2006 (67 days after transplanting), for the cultivated nettle ('Clone 13') and on July 1, 2006, for spontaneous plants. Leaves and stalks (1.5 g) of spontaneous and cultivated nettle were frozen in liquid nitrogen, comminuted into a powder, and then extracted with 3 × 25 mL of 70% EtOH adjusted to pH 3.2 by HCOOH at room temperature. The pH of the extract solution was 2.0 for the analysis of anthocyanins in the stalks. During the first extraction a quantity of 50 μL of internal standard (gallic acid 0.3 mg

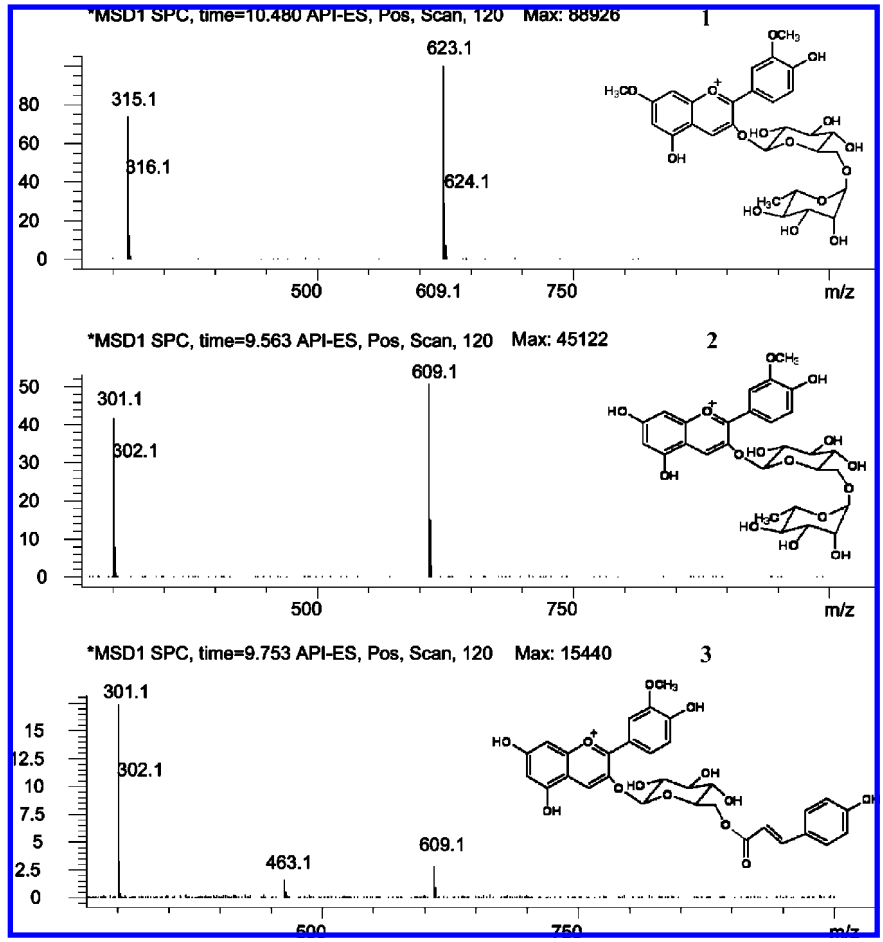

Figure 2. MS spectra of anthocyanins detected in nettle stalk extracts.

Table 1. HPLC-DAD Quali-quantitative Analysis of Phenolic Compounds in Nettle Extracts (C and W)[a]

	C		W	
	L	S	L	S
caffeic acid derivative	0.065 ± 0.023	0.207 ± 0.088	0.297 ± 0.284	0.129 ± 0.109
p-coumaric acid	nd	0.038 ± 0.003	0.052 ± 0.073	0.066 ± 0.042
caffeoylquinic acid	nd	0.148 ± 0.153	nd	0.066 ± 0.037
chlorogenic acid	1.925 ± 0.064	0.231 ± 0.145	0.589 ± 0.611	0.073 ± 0.039
2-*O*-caffeoylmalic acid	3.339 ± 1.118	nd	1.385 ± 1.010	nd
rutin	1.347 ± 0.480	1.359 ± 0.245	0.173 ± 0.140	0.168 ± 0.078
quercetin 3-*O*-glucoside	0.507 ± 0.151	0.095 ± 0.007	0.061 ± 0.060	0.024 ± 0.003
kaempferol 3-*O*-rutinoside	0.078 ± 0.018	0.041 ± 0.021	0.009 ± 0.002	0.008 ± 0.008
isorhamnetin 3-*O*-rutinoside	0.105 ± 0.039	0.503 ± 0.177	0,016 ± 0.015	0.034 ± 0.011
peonidin 3-*O*-rutinoside	nd	0.342 ± 0.119	nd	0.070 ± 0.035
peonidin 3- *O*-(6″-*O*-*p*-coumaroylglucoside	nd	0.034 ± 0.009	nd	nd
rosinidin 3-*O*-rutinoside	nd	0.675 ± 0.312	nd	0.113 ± 0.089
total phenolics	7.364 ± 0.421	3.670 ± 0.202	2.580 ± 2.048	0.750 ± 0.066

[a] Data are the mean ± SD of three determinations and are expressed as mg g^{-1} of fresh weight. L, leaves; S, stalks; nd, not detected.

Table 2. Stalk Density, Height, and Diameter and Fresh and Dry Biomass of Stalks and Leaves at Harvest (2007)[a]

			diameter (mm)			fresh biomass (g m^{-2})		dry biomass (g m^{-2})	
	stalk density (no. m^{-2})	height (cm)	bottom	middle	top	S	L	S	L
mean	131.7	170.8	8.1	5.2	2.7	4225.9	1542.1	1542.1	108.8
SD	16.8	25.7	1.6	1.0	0.7	625.2	86.0	287.6	27.2

[a] L, leaves; S, stalks.

mL^{-1}) was added. The raw hydroalcoholic extracts of leaves and stalks were then defatted with 2 × 30 mL of *n*-hexane, dried under vacuum (Rotavapor 144 R, Büchi, Switzerland), and rinsed with the extraction solvent to a final volume of 25 mL.

Table 3. Fibre Content (Percent of Stalk Dry Matter) of Different Stalk Portions of Cultivated Nettle during the 2007 Growing Season

DOY	stalk portion			mean yield (%)
	bottom	middle	top	
110	5.2 ± 0.5	4.8 ± 0.8	4.8 ± 2.2	4.9
130	5.3 ± 0.6	4.1 ± 0.4	3.4 ± 0.6	4.3
155	6.9 ± 1.8	11.3 ± 0.4	9.8 ± 1.1	9.3
169	9.9 ± 2.1	13.4 ± 1.3	6.5 ± 0.7	9.9
205	8.6 ± 1.7	13.2 ± 2.4	11.6 ± 2.0	11.1

Separation and identification of individual phenolic compounds were conducted on 20 μL aliquots using both HPLC-DAD (diode array detector) and HPLC-MS equipment. Authentic standards of quercetin 3-*O*-rutinoside (rutin), caffeic acid, and gallic acid were purchased from Fluka (Sigma-Aldrich, Milano, Italy); chlorogenic acid was from Roth (Karlsruhe, Germany); and kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-rutinoside, and peonidin 3-*O*-glucoside were purchased from Extrasynthèse S.A. (Lyon, Nord-Genay, France). All solvents were of HPLC grade and were obtained from Merck (Darmstadt, Germany).

HPLC-DAD Analysis. HPLC-DAD analyses were performed on an HP 1100 L liquid chromatograph equipped with a DAD detector and managed by an HP Chemstation (all from Agilent Technologies, Palo Alto, CA). Phenolic compounds were separated using a 4.6 × 250 mm Polaris E RP_{18} (5 μm) column (Varian, Germany) operating at 27 ± 0.5 °C. The eluent was H_2O (adjusted to pH 3.2 by HCOOH)/CH_3CN. A four-step linear gradient solvent system was used, starting from 100% H_2O to 100% CH_3CN during a 53-min period, at the flow rate of 0.8 mL min^{-1} (*19*). The anthocyanins were separated using an RP-80 C12 column (Phenomenex Synergi Max), 150 × 3 mm, 4 μm (Phenomenex), operating at 27 ± 0.5 °C. The eluent was H_2O (adjusted to pH 2.0 by HCOOH)/CH_3CN. A four-step linear gradient solvent system, at the flow rate of 0.4 mL min^{-1} for 28 min, was used (*20*). Determinations of phenolic contents were carried out in triplicate, and results are given as means ± standard deviation (SD).

HPLC-MS Analysis. HPLC-MS analyses were performed using the same analytical conditions of HPLC-DAD analysis. In detail, the HPLC-DAD was interfaced with an HP 1100 MSD API-electrospray (Agilent Technologies) operating in negative and positive ionization mode under the following conditions: nitrogen gas temperaturen 350 °C; nitrogen flow raten 10 L min^{-1}; nebulizer pressuren 30 psi; quadrupole temperaturen 30 °C; capillary voltagen 3500 V. The mass spectrometer operated at 120 eV of negative fragmentor for flavonoid and caffeic derivatives and at 120 eV of positive fragmentor for anthocyanins.

HPLC-MS/MS Analysis. HPLC-MS/MS analyses were performed using a liquid chromatograph HP 1100 L interfaced with an API3000 mass spectrometer with triple quadrupole (Applied Biosystem-Sciex, Toronto, Canada) equipped with a turbo ion spray (TIS). The mass spectrometer operated at −4500 V of energy potential, turbo gas flow 10 L min^{-1}, and T = 150 °C. Collision-activated dissociation (CAD) MS/MS was performed in a LINAC Q2 collision device, using N_2 at 10 mTorr as collision gas. Declustering potential (DP) and collision energy (CE) were automatically optimized for each molecule by the software Analyst 1.4. MS, and MS/MS spectra were registered in continuous flow, connecting the infusion pump directly at the turbo ion spray source. Data were processed by the software Analyst 1.4 with the "Explore" option for spectra identification.

Quantitative Analysis. Identification of individual phenolics was carried out using their retention times and both UV−vis, MS, and MS/MS spectra. Quantitation of the single phenolic compounds was directly performed by HPLC-DAD using a four-point regression curve built with the available standards. Curves with a correlation factor of $r^2 > 0.998$ were considered. In particular, caffeoyl acid amounts were calculated at 330 nm using chlorogenic as reference. Quercetin and isorhamnetin glycosides were calibrated at 350 nm using quercetin 3-*O*-rutinoside as reference. Kaempferol 3-*O*-rutinoside was calculated at 350 nm using the available standard. Finally, anthocyanin glycosides were calibrated at 520 nm using peonidin 3-*O*-glucoside as reference.

Fiber Measurements and Extraction. During both growing seasons (2006 and 2007), periodic measurements of stalk height and diameter (at 10 cm above the soil) were made on 10 randomly selected plants. At harvesting time in 2007, seven areas measuring 1 m^2 each were randomly chosen. Stalk number, fresh and dry biomass of stalks and leaves, and stalk diameter at three heights (bottom, middle, and top) were measured. A subsample of 10 stalks per replicate was collected and used for fiber extraction. Periodic fiber extractions were performed in both years during the growing season. Whereas in the first year fiber extraction was effected on entire stalks, in the second year extraction was carried out considering the different portions of the stalks: bottom, middle, and top. The length of portions was modified according to stem elongation. During the first year, fiber extraction was also carried out on wild nettle plants. Fiber extraction was performed by applying the physicochemical processing method, developed by Bredemann (*2*) for hemp, based on chemical separation in a 2% NaOH solution. This method allows determination of pure fiber content.

DPPH Test on Fiber. The free radical scavenging activity was evaluated with the DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl radical) assay directly on nettle fibers. The antiradical capacity of the samples was estimated according to the procedure reported by Brand-Williams (*21*) and slightly modified. A quantity of 500 mg of nettle fibers was plunged into 2 mL of an ethanol solution of DPPH$^{\bullet}$ (0.025 mg mL^{-1}) and kept at room temperature. After 20 min, the solution absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin-Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH$^{\bullet}$ solution was checked. The antiradical activity was calculated by the following ratio: $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorption of the DPPH$^{\bullet}$ solution and A_{sample} is the absorption of the DPPH$^{\bullet}$ solution after the addition of the sample (nettle fiber).

RESULTS AND DISCUSSION

In the fresh tissues of nettle (i.e., leaves and stalks), three classes of phenolics were characterized in the wild and cultivated samples: hydroxycinnamic acid derivatives (main compounds being chlorogenic acid and 2-*O*-caffeoyl-malic acid); flavonoids (rutin, quercetin *p*-coumaroyl-glucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, isorhamnetin 3-*O*-rutinoside); and anthocyanins [peonidin 3-*O*-rutinoside, rosinidin 3-*O*-rutinoside, peonidin 3-*O*-(6″-*O*-*p*-coumaroylglucoside)]. The anthocyanin glycosides were found only in stalk extracts, which were extracted with the hydroalcoholic solution at pH 2, by formic acid, to avoid anthocyanin degradation. As an example, **Figure 1** reports the chromatographic profiles registered at 280 and 520 nm of the hydroalcoholic stalk extracts. **Figure 1a** refers to the stalk phenolic composition, and **Figure 1b** is specific to the anthocyanin characterization. In particular, the phenolic compounds identified in stalk extracts of nettle are chlorogenic acid and another caffeoylquinic acid, 2-*O*-caffeoyl-malic acid, *p*-coumaric acid, and a caffeic acid derivative. This last molecule was hypothesized as sinapoyl alcohol esterified with caffeic acid by its UV and MS spectra. The MS spectrum shows the presence of a signal at *m*/*z* 371, corresponding to the quasi-molecular ion $[M - H]^-$, and the fragment at *m*/*z* 209, which correspond to the sinapoyl alcohol (data not shown). Concerning the flavonoid content, the stalk extract contains rutin, quercetin *p*-cumaroylglucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, and isorhamnetin 3-*O*-rutinoside.

The anthocyanin profile, composed of peonidin 3-*O*-rutinoside, peonidin *p*-coumaroylglucoside, and rosinidin 3-*O*-rutinoside, is reported here for the first time in the aerial parts of nettle, where only pelargonidin glycosydes have been previously detected by spectrophotometric method (*22*). The distribution of rosinidin glycosides is very limited in plants and reported only in the flowers of Primula (*23*).

In particular, the mass spectrum of peonidin 3-*O*-rutinoside (**1**) shows mass signals at *m*/*z* 609 and 301 (see **Figure 2**),

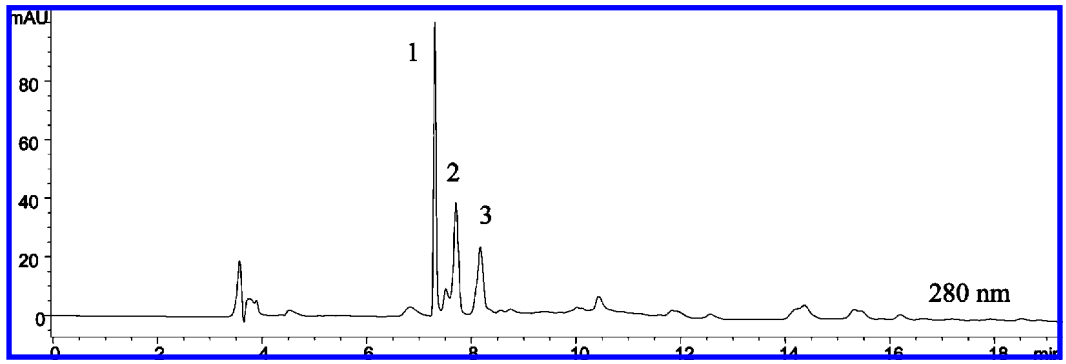

Figure 3. HPLC-DAD profile acquired at 280 nm of a hydrolyzed solution obtained from nettle fibers. Peaks: 1, dihydrosinapoyl alcohol; 2 and 3, hydroxycinnamic acid derivatives.

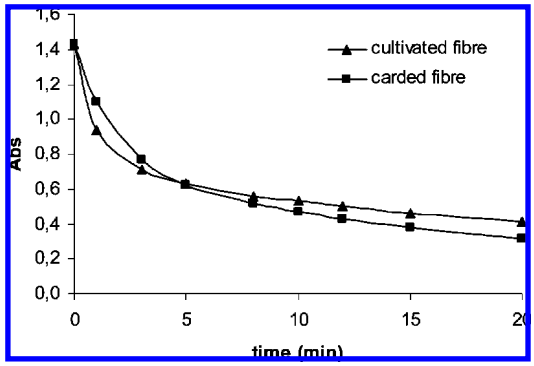

Figure 4. Antiradical activity of cultivated and carded fibers in DPPH test (radical concentration = 0.0025 g/100 mL).

corresponding to the quasi-molecular ion $[M]^+$ and to the fragment after the loss of rutinoside $[M - 308]^+$ that corresponds to the aglycon peonidin (*m/z* 301). The fragmentation pattern of rosinidin 3-*O*-rutinoside (**2**) shows two mass signals at *m/z* 623 and 315, corresponding to the quasi-molecular ion and to the fragment after the loss of rutinoside, that is, the aglycon rosinidin (*m/z* 315). The MS/MS analyses confirmed the previously reported fragmentation patterns. The rosinidin 3-*O*-rutinoside MS/MS spectrum shows the production of only one fragment corresponding to this rare aglycon, called rosinidin. The anthocyanin present in smaller amounts was hypothesized as peonidin 3-*O*-(*p*-coumaroylglucoside) (**3**) due to the following mass signals: *m/z* 609, corresponding to the quasi-molecular ion $[M]^+$, the fragment after the loss of *p*-coumaroyl moiety $[M - 146]^+$, and the fragment after the loss of *p*-coumaroylglucoside $[M - 308]^+$. The presence of the acylated moiety is confirmed by the UV−vis spectrum, which shows higher absorbance values in the range from 300 to 320 nm, with respect to the UV−vis spectrum of peonidin 3-*O*-rutinoside (data not shown).

The nettle extracts obtained from leaves, both cultivated and wild, differ in phenolic composition from a quantitative point of view. The extraction yield (95%) was controlled by adding gallic acid as internal standard. The choice of this molecule was based on its absence in our samples and on its retention time, which falls in an empty zone of the chromatogram (t_R = 8.21 min). The detection limits were calculated on the basis of a signal-to-noise ratio of 3:1 of the minor components. The detection limits evaluated for kaempferol 3-*O*-rutinoside and peonidin 3-*O*-rutinoside in all analyzed extracts are 15 and 80 $\mu g\ kg^{1-}$, respectively.

As reported in **Table 1**, the leaves of both cultivated and wild samples contain large amounts of caffeic acid derivatives, in particular, chlorogenic and 2-*O*-caffeoylmalic acid, which represent 71.5% in cultivated (C) and 76.5% in wild (W) of total phenolics. The flavonoids are represented by glycosides, in particular, quercetin 3-*O*-glucoside, and the 3-*O*-rutinosides of quercetin, kaempferol, and isorhamnetin. The main flavonol is rutin, which is present at 18.2% in C and at 6.7% in W leaves. Even if the polyphenol patterns are similar between C and W samples, there is a great difference from a quantitative point of view because C samples are on average 2.85 more concentrated than W ones.

Moreover, the phenolic composition of the leaves differs from that observed in the stalks (**Table 1**). The flavonoids are the main class in the stalks: 54.4% of total phenolics in C and 31.2% in W samples; furthermore, with respect to the leaves there is a large increase in isorhamnetin 3-*O*-rutinoside. The second class of quantitative importance is that of anthocyanins, present only in stalks: 28.6% of total phenolics in C and 24.4% in W stalks.

In the first year (2006), the stalks of cultivated nettle reached maximum height (about 75 cm) at 80−90 days after transplanting and maximum diameter (about 6 mm) at 60−70 days. The phase of intensive vegetative growth stopped when the flowering phase occurred. During the first year, all stalks were greatly branched, as already pointed out by other authors (*3*). In 2007, at the beginning of April, stalks were already 40 cm high, and they reached a mean height of 170 cm (**Table 2**) and a maximum diameter of 8 mm (at 10 cm from the soil). At harvest, a mean density of 132 stalks m^{-2} was found. Total fresh biomass was about 4600 g m^{-2}, and stalks represented >90%. Stalk mean dry matter was 1542 g m^{-2}, corresponding to a stalk production of 15.4 t ha^{-1} (**Table 2**).

In the first year, nettle stalks generally do not achieve the quality required for fiber processing (*2*); nevertheless, the fiber content trend during the growing season was determined. A maximum fiber yield of 10% was determined in cultivated nettle compared to a maximum fiber yield of about 5% for wild nettle (data not shown). As depicted in **Table 3**, in the second year, the highest fiber yields were always detected in the middle portion (maximum value of 13%). The maximum value of fiber yield considering the entire stalk was about 11%. Higher values, up to 15−16% of fiber yield, were previously found (*24*). To investigate the phenolic composition of nettle fibers, a basic hydrolysis was performed according to Fischbach et al. (*25*). The hydrolyzed solutions obtained from the fibers contain several components of the lignin, hypothesized as hydroxycinnamic derivatives, on the basis of their UV and MS spectra. The chromatographic profile of this hydrolyzed solution is reported in **Figure 3**. In detail, the mass spectrum of the main compounds (peak 1) shows a signal at *m/z* 211, hypothesized as dihydrosinapoyl alcohol in accordance with a previous paper (*26*); further investigations are in progress to completely

characterize the other compounds, which are always derivatives of this molecule from lignins. In light of these results, a method able to test the biological properties, in particular, the antiradical activity, directly on the fibers by means of a stable radical DPPH was optimized, in accordance with previous papers relative to standard, isolated phenolic compounds and standardized plant extracts (*27, 28*). The antiradical activity calculated after 20 min of contact with DPPH radical, as described under Materials and Methods, was 71% for raw fibers obtained from cultivated nettle and 78% for carded fibers. The radical scavenging activity of the fibers is reported in **Figure 4**.

The presence of phenolic compounds in nettle extracts, obtained from stalks, leaves, and fibers, too, is an important result for the biological property (antioxidants and antiradicals) of these metabolites and, thus, for their possible application in various industrial activities, such as food/feed, cosmetic, phytomedicine, and textiles. Moreover, further developments could be previewed about the evaluation of the antimicrobial and antimycotic effects of these secondary metabolites present in nettle fibers in order to create and test technological tissues of possible biomedical interest.

These initial results obtained in terms of biomass and fiber yield at harvest seem to positively support the feasibility of fiber nettle cultivation in central Italy, as production values are comparable to those found by German and Austrian research institutes. Nevertheless, further experimentation is essential to verify the suitability of these geographical areas, as well as the quality of the produced fiber.

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