Metabolite profiling of flavonols and *in vitro* antioxidant activity of wild shoots of *Humulus lupulus* L. (hop) of different origin

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Abbreviations: reactive oxygen species, ROS; formic acid, HCOOH; methanol, MeOH; acetonitrile, ACN; water, H_2O; limit of detection (LOD); limit of quantification (LOQ); photochemiluminescence (PCL).

35 **Abstract**

36

37 *Humulus lupulus* L., commonly named hop, is well-known for its sedative and estrogenic activity.
38 While hop cones are widely characterized, only few works have been carried out on the young
39 shoots of this plant.
40 In the light of this, the aim of this work was to identify for the first time the flavonoids present in
41 hop young shoots and to compare their composition with the antioxidant activity of samples
42 harvested from different locations in Northern Italy. The samples were extracted by means of
43 dynamic maceration with methanol as the extraction solvent. The HPLC-UV/DAD, HPLC-ESI-MS
44 and MS^2 analysis were carried out by using an Ascentis C_{18} column (250 × 4.6 mm I.D., 5 μm),
45 with a mobile phase composed of 0.1 M formic acid in both water and acetonitrile, under gradient
46 elution. Quercetin and kaempferol glycosides were the main compounds identified and quantified in
47 hop shoot extracts by HPLC-UV/DAD, HPLC-ESI-MS and MS^2. Total flavonol compounds ranged
48 from 2698.1 ± 185.4 to 517.3 ± 47.5 μg/g (fresh weight).
49 The antioxidant activity was determined by using a photochemiluscence assay with a Photochem®
50 apparatus and varied from 1.067 ± 0.083 to 0.683 ± 0.044 μg Trolox equvalents/g. The results
51 showed that hop shoots represent a potential source of flavonols; therefore, they should be
52 considered as a potential new source of bioactive compounds to be used in the nutraceutical ambit.

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67 **Keywords:** *Humulus lupulus*; hop shoots; flavonols; HPLC; MS; antioxidant activity.

68 **1. Introduction**

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70 The harvesting and consumption of edible wild plants is an ancient custom for many people.
71 Although in the last century their use has been limited, the interest for their healthy properties has
72 never lessened. Today, the increase of cardiovascular, cancer and neurodegenerative diseases in
73 industrialized countries has stimulated new interest in edible wild plants. Indeed, edible wild plants
74 have a high nutritional value and are a rich source of bioactive compounds [1], such as vitamins,
75 carotenoids and polyphenols, which have been found to possess a great variety of biological
76 activities, including the antioxidant activity. Several scientific studies have shown free radicals and,
77 in particular, reactive oxygen species (ROS) as the main cause of aging and tissues damage. The
78 overproduction of ROS, most frequently either by excessive stimulation of NAD(P)H by
79 cytokines or by the mitochondrial electron transport chain and xanthine oxidase, results in oxidative
80 stress. Oxidative stress is a deleterious process that can be an important mediator of damage to cell
81 structures and, consequently, cardiovascular disease, cancer, neurological disorders, including
82 Alzheimer and Parkinson, and ageing [2]. Dietary antioxidants and other nutrients play an
83 important role in preventing cells from radical-induced cytotoxicity [3].
84 *Humulus lupulus* L., commonly named hop, is a dioecious perennial plant belonging to the
85 *Cannabaceae* family. Although hop is natural from central Europe, today it is widely cultivated in
86 all temperate regions. Hop female flowers are used in the brewing process of beer, providing
87 bitterness, flavour and aroma. The characteristic bitter grade and aroma that define a particular beer
88 are influenced by many factors, including the hop cultivars employed.
89 In ancient times hop was used for its sedative action effect. The sedation, pre-anesthetic and anti-
90 anxiety properties of hop extracts have been recently demonstrated in rats [4]. The frequent
91 menstrual disturbances observed in female hop-pickers has suggested a potential hormonal activity
92 of hop extracts. The estrogenic effects of hop has been attributed to 8-prenylnaringenin [5]. Other
93 hop prenylflavonoids, including xanthohumol and other prenylchalcones, do not have any
94 estrogenic activity, but they are considered to be possible cancer chemopreventive compounds
95 [5,6].
96 Hop cones are widely characterized for their phenolics [7], stilbenes [8], prenylflavonoids and
97 prenylphloroglucinols (bitter acids) [9,10]. Hop young shoots have been widely used as vegetables
98 by the Romans. Today, hop young shoots, together with those from *Asparagus acutifolius* L. (wild
99 asparagus), *Bryonia dioica* Jacq. (white or red bryony) and *Tamus communis* L. (black bryony), are
100 hand-picked in fresh and fertile lands at the edge of the woods and ditches. They have been

101 traditionally consumed boiled as wild asparagus or to cook risotto and omelettes. In Italy, the
102 harvest of hop young shoots is typical in the North and Central regions and it is made on the banks
103 of rivers or in hill areas, where there is a high humidity. As regards the composition, only few
104 studies have been carried out on hop young shoots for their content of carotenoids [11], organic
105 acids [12] and tocopherol [13].
106 In the light of all the above, the aim of this study was to identify for the first time the flavonoid
107 composition of hop young shoots by means of a new method based on HPLC-UV/DAD, HPLC-
108 ESI-MS and MS^2 and to compare these data with the antioxidant activity of the plant material
109 harvested from four different points in Northern Italy.
110
111 ## 2. Material and methods
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113 ### *2.1. Chemicals and solvents*
114
115 Quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside were purchased
116 from Extrasynthese (Genay, France). Kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside
117 were purchased from Sigma-Aldrich (Milan, Italy). All reference compounds were of
118 chromatographic grade. Formic acid (HCOOH), HPLC-grade methanol (MeOH) and acetonitrile
119 (ACN) were from Sigma-Aldrich (Milan, Italy). Water (H_2O) was purified using a Milli-Q Plus185
120 system from Millipore (Milford, MA, USA).
121
122 ### *2.2. Plant material*
123
124 Hop young shoots were hand-picked in April-May 2014 in three different locations of the Po river
125 bancks, including Castelmassa (Rovigo), Cologna (Rovigo) and Santa Maria in Punta (Ferrara), and
126 in a hill area of Tuscany, named Vicchio (Florence). Each sample was packed in plastic bags,
127 frozen at ─20 °C on the same day and preserved until analysis.
128
129 ### *2.3. Extraction of secondary metabolites from hop shoots*
130
131 Dynamic maceration was chosen for the extraction of the secondary metabolites present in hop
132 shoots and MeOH was used as the extraction solvent. In particular, the extraction procedure was
133 performed on 2.0 g of fresh sample with 10 mL of solvent at room temperature for 30 min under

134 magnetic stirring. The mixture obtained from the extraction was then centrifuged at 4000 rpm for 5
135 min and the supernatant solution was filtered under vacuum into a volumetric flask. The residue of
136 the first extraction was re-extracted as previously described. Finally, the filtrates of the two
137 extractions were combined and brought to 25 mL in a volumetric flask. An aliquot of 5 mL of the
138 extract was concentrated under vacuum at 35°C and then brought to the final volume of 1 mL with
139 MeOH in a volumetric flask. The concentrated extract was subsequently filtered by using a 0.45 µm
140 PTFE filter into a HPLC vial prior to the injection into the HPLC system.
141 The extraction procedure was carried out in duplicate for each sample.
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143 *2.4.HPLC-UV/DAD analysis*
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145 HPLC-UV/DAD analyses were performed on an Agilent Technologies (Waldbronn, Germany)
146 modular model 1100 system, consisting of a vacuum degasser, a quaternary pump, an autosampler,
147 a thermostatted column compartment and a diode array detector (DAD). The chromatograms were
148 recorded by using an Agilent Chemstation for LC and LC-MS systems (Rev. B.01.03).
149 The HPLC analyses were carried out on an Ascentis C_{18} column (250 × 4.6 mm I.D., 5 µm,
150 Supelco, Bellefonte, PA, USA). The mobile phase was composed of (A) 0.1 M HCOOH in H_2O and
151 (B) ACN. The gradient elution was modified as follows: 0-15 min from 10% to 20% B, 15-35 min
152 from 20% to 30% B, 35-40 min from 30% to 40% B, 40-45 min from 40% to 50% B. The post-
153 running time was 5 min. The flow rate was 1.0 mL/min. The column temperature was set at 25 °C.
154 The sample injection volume was 10 µL. The UV/DAD acquisitions were carried out in the range
155 190-550 nm and chromatograms were integrated at 352 nm. Three injections were performed for
156 each sample.
157
158 *2.5. HPLC-ESI-MS and MS^2 analysis*
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160 HPLC-ESI-MS and MS^2 analyses were carried out by using an Agilent Technologies modular 1200
161 system, equipped with a vacuum degasser, a binary pump, a thermostatted autosampler, a
162 thermostatted column compartment and a 6310A ion trap mass analyzer with an ESI ion source.
163 The HPLC column and the applied chromatographic conditions were the same as reported for the
164 HPLC-UV/DAD system. The flow rate was split 5:1 before the ESI source.
165 The HPLC-ESI-MS system was operated both in the positive and in the negative ion modes. For the
166 positive ion mode, the experimental parameters were set as follows: the capillary voltage was 3.5

kV, the nebulizer (N_2) pressure was 32 psi, the drying gas temperature was 350 °C, the drying gas flow was 10 L/min and the skimmer voltage was 40 V. For the negative ion mode, the conditions were set as follows: the capillary voltage was 4.0 kV, the nebulizer (N_2) pressure was 35 psi, the drying gas temperature was 350 °C, the drying gas flow was 11 L/min and the skimmer voltage was 40 V.

Data were acquired by Agilent 6300 Series Ion Trap LC/MS system software (version 6.2). The mass spectrometer was operated in the full-scan mode in the *m/z* range 100-1000. MS^2 spectra were automatically performed with helium as the collision gas in the *m/z* range 50-1000 with the SmartFrag function.

2.6. HPLC-UV/DAD method validation

The validation of the HPLC-UV/DAD method was performed in agreement with the international guidelines for analytical techniques for the quality control of pharmaceuticals (ICH guidelines) [14].

The stock standard solution of each compound (quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside) was prepared as follows: an accurately weighed amount of pure compound (2.1-5.0 mg) was placed into a 10 mL volumetric flask; then, MeOH was added and the solution was diluted to volume with the same solvent. The external standard calibration curve was generated by using six data points, covering the concentration ranges: 7.8-313.0 µg/mL for quercetin-3-*O*-rutinoside (**3**); 5.3-213.0 µg/mL for quercetin-3-*O*-galactoside (**5**); 7.8-312.0 µg/mL for kaempferol-3-*O*-rutinoside (**7**); 6.3-505.0 µg/mL for kaempferol-3-*O*-glucoside (**10**). Ten µL aliquots of each standard solution were used for HPLC analysis. Injections were performed in triplicate for each concentration level. The calibration curve was obtained by plotting the peak area of the compound at each level versus the concentration of the sample. The quantification of compounds **3**, **5**, **7** and **10** was performed by using their calibration curves. The amount of the other flavonols found in hop shoots was carried out by using the calibration curves of the reference compounds with the same chromophore. In particular, the calibration curve of quercetin-3-*O*-rutinoside was used for compound **1**, that of quercetin-3-*O*-galactoside for compound **6**, that of kaempferol-3-*O*-glucoside for compounds **11** and **12** and that of kaempferol-3-*O*-rutinoside for compounds **2**, **4**, and **9**.

For reference compounds, the limit of detection (LOD) and the limit of quantification (LOQ) were experimentally determined by HPLC analysis of serial dilutions of a standard solution to reach a signal-to-noise (S/N) ratio of 3 and 10, respectively.
The accuracy of the analytical method was evaluated by means of the recovery test. This involved the addition of a known quantity of standard compound to half the sample weight of grounded sample (Vicchio) to reach 100% of the test concentration. The fortified samples were then extracted and analysed with the proposed method.
The precision of the extraction technique was validated by repeating the extraction procedure on the inflorescence of the same hemp sample (Vicchio) six times. An aliquot of each extract was then injected and quantified. The precision of the chromatographic system was tested by performing intra- and inter-day multiple injections of one extract (from sample Vicchio) and then checking the %RSD of retention times and peak areas. Six injections were performed each day for three consecutive days.

2.7. Determination of antioxidant capacity by photochemiluminescence (PCL) method (Photochem®)

The photochemiluminescence (PCL) assay, based on the methodology of Popov *et al.* [15], was used to measure the antioxidant activity of hop young shoot extracts with a Photochem apparatus (Analytic Jena, Jena, Germany) against superoxide anion radicals generated from luminol.
The antioxidant activity of the extracts was measured by means of the ACL kits (Analytic Jena, Jena, Germany). For the ACL assay, 2.3 mL of reagent 1 (solvent and dilution reagent, MeOH), 0.2 mL of reagent 2 (buffer solution), 25 μL of reagent 3 (photosensitizer, luminol 1 mmol/L) and 10 μL of standard or sample solution were mixed and measured. Luminol is used as a photosensitiser when exposed to UV light at λ_{max} of 351 nm, and as a detecting substance for free radicals. Trolox was employed for the standard calibration curve from 0.25 to 2 nM.
In the PCL-ACL assay, the photochemical generation of free radicals is combined with a sensitive detection obtained by using chemiluminescence. In ACL studies, the kinetic light emission curve was monitored for 3 min and expressed as μg/g Trolox equivalents. The areas under the curves were calculated by using the PCL soft control and analysis software. The presence of Trolox (used as the standard for the calibration curve) or any antioxidants from the samples reduces the magnitude of the PCL signal, and hence, the area calculated from the integral. The observed inhibition of the signal was plotted against the concentration of Trolox added to the assay medium. The

231 concentration of the added sample was such that the generated luminescence during the 3-min
232 sampling interval fell within the limits of the standard curve.
233
234 *2.8. Statistical analysis*
235 All statistical analyses were performed by using Excel software Office 2013. Concentrations,
236 recovery, means and standard deviation were calculated with Excel. Statistica 6.1 (Statsoft) was
237 used for the analysis of variances (ANOVA) and a Tukey's adjustment for multiple comparisons to
238 test for significant differences between the means. *P*-values under the significance level (α) of 0.05
239 were considered statistically significant.
240

241 ## 3. Results and Discussion
242

243 3.1. *Method development and identification of flavonols in hop shoot extracts*
244
245 In this study, the identification of flavonols in hop shoot extracts was carried out for the first time
246 on the basis of their UV/Vis spectra, together with MS and MS^2 data, which were compared with
247 those of reference standards, when commercially available. The flavonols identified in the
248 methanolic extracts of hop shoots are shown in Table 1.
249 Table 1
250 Since there are no studies focused on the phenolic composition of hop shoots, the characterization
251 of this plant material represents an interesting topic to be properly investigated. Firstly, the research
252 was focused on the identification of the characteristic bioactive compounds present in well-known
253 hop cone extracts, including prenylflavonoids and prenylphloroglucinols [10,16,17]. To do this, the
254 extraction and HPLC analysis of hop shoot samples was initially performed under the same
255 conditions previously described in the literature [10]. However, the chromatograms recorded did not
256 reveal the presence of both prenylflavonoids and bitter acids in the hop shoot extracts analysed in
257 this study. This is probably due to the fact that these samples are composed of embryonal tissues,
258 where the biosynthesis of both prenylflavonoids and bitter acids does not take place.
259 In the light of all the above, the extraction and analytical conditions were completely modified and
260 optimized, in order to have a good recovery of bioactive compounds from hop shoots and a
261 satisfactory separation of the peaks observed in the HPLC chromatograms. The chromatographic
262 peaks were preliminary assigned to a chemical class according to their UV/Vis spectra. Indeed, all
263 polyphenols have a peculiar UV/Vis spectrum with different λ_{max}: in particular, flavonoids exhibit a

264 first maximum in the 240-285 nm range (band II) and a second one in the 300-550 nm range (band
265 I) [18]. By combining this information with those obtained from MS experiments, a preliminary
266 identification was carried out, and then it was confirmed by the HPLC analysis of the reference
267 standards commercially available under the same chromatographic conditions.
268 As regards HPLC-ESI-MS and MS^2 analyses, both positive and negative ion modes were applied
269 for the structural characterization of hop shoot constituents [18,19]. On the basis of this approach, a
270 total of 12 flavonol glycosides were identified (Table 1).
271 In particular, quercetin and kaempferol glycosides were firstly distinguished due to their different
272 UV/Vis behavior and λ_{max} in the band I range: in fact, quercetin glycosides showed a λ_{max} in the
273 355-360 nm range, while kaempferol glycosides in the 348-352 nm range.
274 As regards mass spectrometry, in the MS^2 spectra of these compounds recorded in the positive ion
275 mode, the cleavage of the glycosidic bond led to the elimination of the sugar residue, resulting in a
276 strong fragment at *m/z* 303 and 287, corresponding to the aglycones, quercetin and kaempferol,
277 respectively. In the negative ion mode, most of the identified constituents generated the
278 corresponding aglycone at *m/z* 300 (homolytic cleavage) and *m/z* 301 (heterolytic cleavage) for
279 quercetin glycosides and at *m/z* 284 (homolytic cleavage) and *m/z* 285 (heterolytic cleavage) for
280 kaempferol glycosides, suggesting that the glycosylation site was probably located at the 3 position
281 [20,21].
282 As regards the sugar moiety identification, MS methods can be used to obtain information on the
283 carbohydrate type and sequence. Even if glucose is the most common monosaccharide in flavonoid
284 glycosides, galactose along with rhamnose, xylose and arabinose are not uncommon [21].
285 Disaccharides are also often found in glycosylated flavonoids, the more common ones being
286 rutinose (rhamnosyl-(α1→6)-glucose) and neohesperidose (rhamnosyl-(α1→2)-glucose) [21]. The
287 cleavage at the glycosidic *O*-linkages with a concomitant H-rearrangement leads to the elimination
288 of monosaccaride residues, such as the loss of 162 u (hexose), 146 u (deoxyhexose), 132 u
289 (pentose) or 176 u (uronic acid), thus allowing the determination of the carbohydrate sequence [21].
290 In addition, the analysis of the product ion spectra of $[M + Na]^+$ adduct ions can provide additional
291 information on the size and pattern of glycoside substitutions on flavonols [22].
292 The MS^2 experiments carried out on the $[M + Na]^+$ adduct ion of compound **1** generated a base peak
293 at *m/z* 477, corresponding to $[\text{rhamnose-glucose-rhamnose+Na}]^+$, thus indicating the presence of a
294 trisaccharidic moiety [22]. This compound, showing a $[M + H]^+$ ion at *m/z* 757, according to its UV
295 spectrum and MS^2 fragmentation in the positive ion mode with the subsequent loss of a rhamnose
296 (*m/z* 611) and a rhamnose-glucose disaccharide unit (*m/z* 303), was identified as quercetin-3-*O*-(2-

297 rhamnosyl)-rutinoside [23]. The presence of the rutinoside moiety was deduced by the absence of
298 the $[M - H - 120]^-$ fragment in the negative ion mode, which is typically due to the presence of a
299 neohesperidoside disaccharide [23]. The fragmentation pattern of this constituent in the negative ion
300 mode was also found to be in agreement with other previous studies [24,25]. By following the same
301 strategy, compound **2** was identified as kaempferol-3-*O*-(2-rhamnosyl)-rutinoside on the basis of
302 the good agreement of its MS and MS^2 data with the literature [24,25].
303 Flavonol glycosides having a rhamnosyl-glucose as the sugar moiety (such as compounds **3, 4** and
304 **7**) showed a base peak at *m/z* 331 $[\text{rhamnose-glucose-+Na}]^+$, suggesting that these compounds
305 possess a disaccharide unit and not two sugars linked at different positions. Compounds **3** and **7**
306 were confirmed as rutinosides by the analysis of reference standards. As regards compound **4** it was
307 identified as kaempferol-3-*O*-neohesperidoside, due to the product ion $[M - H - 120]^-$ at *m/z* 473
308 in the negative ion mode, which revealed the presence of a (1→2) interglycosidic linkage between
309 the two monosaccharides [23]. In addition, the two product ions at *m/z* 447 and 285, which
310 correspond to the loss of rhamnose (−146 u) and rhamnosyl-glucose (−308 u), respectively, have
311 relative abundances strikingly different, with a 1→2 linkage between the monosaccharides which
312 favours the elimination of the disaccharide residue to yield a deprotonated aglycone ion [26].
313 Finally, the MS^2 data of compound **4** were found to be in good agreement with the literature
314 [24,25,27].
315 The fragmentation pathway observed for the $[M + H]^+$ ions of compounds **8**, **9**, **11** and **12** was based
316 on the release of the malonyl-glucose moiety (– 248 u) [21]. As regards the negative ion mode, the
317 major product ion in the MS^2 spectra of these compounds corresponds to $[M - H - CO_2]^-$,
318 originating from the decarboxylation of a malonic acid moiety [22]. The exact location of the
319 malonyl group on the glycosidic part is difficult to be defined on the basis of MS data, but it is
320 known to be predominantly located at the 6-position of a hexose moiety [21]. On the basis of the
321 good agreement of the MS and MS^2 data of compounds **8**, **11** and **12** in both the positive [26] and
322 the negative ion mode [28,29], they were identified as quercetin-3-*O*-(6''-*O*-malonyl)-hexoside (**8**)
323 and kaempferol-3-*O*-(6''-*O*-malonyl)-hexoside (**11** and **12**); indeed, both glucose and galactose are
324 possible as the sugar moiety for these compounds [28]. Compound **9** was identified as kaempferol-
325 3-*O*-(6''-*O*-malonyl)-neohesperidoside, due to the match of its MS and MS^2 data with the literature
326 [27]. In particular, the $[M + H]^+$ ion of **9** was 86 mass units larger than that of compound **4**,
327 indicating that compound **9** contains a malonyl group; this was further supported by the presence of
328 the product ion at *m/z* 593 in the MS^2 spectrum acquired in the negative ion mode, attributable to
329 $[M - H - \text{malonyl}]^-$. The specific biosynthetic pathway for this natural compound has been

330 described by Kogawa *et al*. [30], as a malonylated derivative of its precursor kaempferol-3-*O*-
331 neohesperidoside (**4**).
332
333 *3.2. Method validation*
334
335 Over the concentration range tested, the method showed good linearity ($r^2 \geq 0.9995$) for the
336 reference standards chosen in this study.
337 The LOD values had a range from 1.8 to 2.5 μg/mL, while the LOQ range was from 5.3 to 7.8
338 μg/mL , which indicate that the method is sensitive.
339 The accuracy of the analytical procedure was evaluated by using the recovery test. The percentage
340 recovery values, obtained by comparing the results from samples and fortified samples, were found
341 to be higher than 80% and they can be considered satisfactory.
342 The low intra- and inter-day %RSD for retention times (≤ 0.1) and peak area (≤ 3.0) relative to the
343 target compounds and their low intra- and inter-day SD (≤ 76 μg/g) values for content indicate the
344 high precision of both the chromatographic system and the extraction procedure.
345 By taking into account all the information described above, it can be concluded that this method is a
346 reliable tool for the analysis of flavonols in hop shoots, conforming to the ICH guidelines.
347
348 3.3. *Quantitative analysis of flavonols in hop shoot extracts*
349
350 Figure 1 shows a representative HPLC-UV/DAD chromatogram of a hop shoot extract obtained
351 with the method developed in this study (sample Vicchio).
352 Figure 1
353 The HPLC-UV/DAD method was applied to the quantitative analysis of flavonol glycosides in hop
354 shoot samples. Quantitative data, expressed as μg/g fresh weight, are shown in Table 2.
355 Table 2
356 A noteworthy difference in the content of total flavonol compounds was observed between hop
357 shoots of different origin, with the sample Vicchio being the richest one (2698.1 ± 185.4 μg/g) and
358 the sample Castelmassa the poorest (517.3 ± 47.5μg/g). In general, the most abundant compounds
359 found in hop shoot samples were kaempferol derivatives, such as kaempferol-3-*O*-(6"-*O*-malonyl)-
360 glucoside (or galactoside) (769.5-226.0 μg/g), kaempferol-3-*O*-glucoside (490.7-65.2 μg/g) and
361 kaempferol-3-*O*-(6"-*O*-malonyl)-neohesperidoside (401.4-53.7 μg/g).

362 Since the present work is the first one focused on the quali- and quantitative analysis of hop shoot
363 flavonoids, there are no comparative data in the literature. For this reason, the quantitative data
364 obtained in this study have to be considered as preliminary; additional samples collected from
365 different areas are necessary to assess also the influence of abiotic and biotic factors on the
366 bioactive constituents of this plant material.
367
368 *3.4. Antioxidant activity of hop shoot extracts*
369
370 The antioxidant activity of hop young shoot extracts is shown in Figure 2 and the results are
371 expressed as μg of Trolox equivalents for g of shoots (fresh weight).
372 Figure 2
373 Vicchio hop shoot sample had the greatest antioxidant activity (1.067 ± 0.083 μg/g), followed by
374 Santa Maria in Punta and Cologna; Castelmassa presented the lowest antioxidant activity (0.683 ±
375 0.044 μg/g).
376 It is interesting to note that the antioxidant activity data have a good correlation with the total
377 flavonol contents, with an r^2 value of 0.9577.
378 As regards the influence of the environmental conditions, it should be pointed out that the Vicchio
379 sample came from hill of Tuscany at 203 m a.s.l., while Cologna, Santa Maria in Punta and
380 Castelmassa samples, which had similar antioxidant activity, came from different points of Po river
381 bancks.
382 In literature are reported as the antioxidant activity of vegetal and the phenolic profile show
383 quantitative differences as a function of the cultivars, environmental conditions (temperature,
384 humidity, UV irradiation) of growing, and different growing stages. In particular the high salt
385 concentration induce an increment of antioxidative activity and polyphenol concentration [31]. The
386 Cologne and Santa Maria in Punta samples, locations near the Po river mouth, presented a
387 significantly higher antioxidant activity.
388
389 **4. Conclusions**
390 The flavonol composition of hop young shoots was studied in this work for the first time by means
391 of a validated method based on HPLC-UV/DAD, HPLC-ESI-MS and MS^2. Quercetin and
392 kaempferol glycosides were the main compounds in the extracts obtained from this plant material.
393 These components were quantified and their content was found to be related to the antioxidant
394 activity of the extracts, determined *in vitro* by means of a PCL assay.

395 These results highlight the importance of hop shoots as a potential source of flavonols, which can
396 be useful for their role against biological radicals, such as superoxide radical $O_2^{\bullet-}$. Therefore, hop
397 young shoots should be considered as a new source of bioactive compounds to be used in the
398 nutraceutical field. The differences in the antioxidant activity among samples of different origin
399 suggests an influence of the environmental conditions on the biosynthesis of flavonol compounds.
400

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Figure captions

Figure 1: Representative chromatogram obtained by HPLC-UV/DAD of an extract of hop shoots (sample Vicchio) at 352 nm.

Figure 2: Antioxidant activity (PCL-ACL) of hop young shoot extracts of different origin. Data are expressed as µg of Trolox equivalents for g of shoots. Values with no small letters in common are significantly different ($P < 0.05$) (mean ± SD, $n = 6$).