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Short report

Phytochemical and antioxidant analysis of eight *Hypericum* taxa from Central Italy

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Abstract

Eight taxa of the *Hypericum* spp. growing in Central Italy (Appennino Umbro-Marchigiano) were analyzed by HPLC-DAD for constituents quantitation, for antioxidant and free radical scavenging activities. *H. perforatum* subsp. *veronense* was the richest in phenolic compounds and hyperforin was detected for the first time in *H. hircinum* subsp. *majus*. Significant values of antioxidant activity were found in the investigated *Hypericum* taxa.

Keywords: *Hypericum*; HPLC-DAD; Antioxidant activity

1. Plants

Aerial parts in blossom of eight *Hypericum* taxa were collected in June–July 2005 in various localities of the Appennino Umbro-Marchigiano, in the Central Italy (Table 1). Voucher specimens were authenticated and deposited in the Herbarium Camerinensis (Dept. of Environmental Sciences, Sect. of Botany and Ecology, University of Camerino, Italy), under accession codes CAME 7977–7987.

2. Uses in traditional medicine

Aerial parts of *H. perforatum* were used in central Italy against wounds and in case of burns, or, as infusion, against hypertension [1]. Leaves and stems of *H. hircinum* were used in southern Italy, as decotion, for treating cough and bronchitis [2].

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3. Previously isolated classes of constituents

Xanthones, flavonoids, napthodianthrones, phloroglucinols in *H. perforatum* [3–6].

4. New isolated constituents

No reports.

5. Tested material

Soxhlet extracts of stove-dried and milled aerial parts were obtained by a Soxhlet apparatus using a mixture MeOH–acetone (1:1) for 4 h (yield: 19.2%–34.6%). HPLC-DAD analyses were performed at 210 nm for phenolic compounds, 270 nm for hyperforin, 590 nm for hypericin. The mobile phase was similar to that previously reported [7], but EtOAc was used to clean up the column, according to Arnott [8]. The results were confirmed by use of HPLC-MS apparatus equipped with an ESI interface in negative ionization operating in the same chromatographic conditions. In addition, total phenolic content of extracts, expressed as gallic acid equivalents (GAE) in mg/g dry material, was measured by UV spectrophotometry at 750 nm, based on a colorimetric oxidation/reduction reaction using the Folin–Ciocalteu reagent [9].

6. Studied activity

Antioxidant activity was evaluated by Rancimat method, measuring the oxidation induction time with the use of A 743 Rancimat apparatus: a flow of air (20 l/h) was bubbled through the oil heated at 100 °C, and the volatile compounds were collected in cold water, increasing the water conductivity; each *Hypericum* sample was dispersed in 3 g of olive oil rich in linoleic acid (65% of fatty acids) at the concentration of 0.1%. Olive oil without added antioxidant as the control was run similarly. In addition, antioxidant activity was elucidated on heat-induced oxidation of aqueous emulsion system of β-carotene-linoleic acid [10]. After incubation at 50 °C, absorbance of each sample at 470 nm was monitored at time intervals of 15 min during 180 min. Antioxidant activities of *Hypericum* extracts were compared with those of BHT (butylated hydroxytoluene) at the same concentration and blank consisting of 0.2 ml of methanol. Tests were run in quadruplicate.

Free radical scavenging effects of the *Hypericum* extracts on DPPH (2,2-diphenyl-1-picrylhydrazyl) were measured at 517 nm by a UV-spectrophotometer, according to Sanchez-Moreno et al. [11] with some modification. The radical scavenging activity of the tested samples were compared with those of BHT at the same concentration and expressed as % of inhibition against DPPH. Free radical scavenging activity determination was repeated 4 times for each sample and the means are reported.

Statistical analysis was performed by one-way analysis of variance (ANOVA) and $P<0.05$ was accepted as statistically significant.

Table 1
Voucher specimen and collection locality of the studied entities of the *Hypericum* spp. growing in the Appennino Umbro-Marchigiano

Sample	Voucher specimen	Taxon	Collection locality	Height (m above sea level)
A	CAME 7982	*H. perforatum* subsp. *perforatum*	S. Maria Maddalena (Aquacanina)[a]	1280
B	CAME 7987	*H. perforatum* subsp. *perforatum*	Pian Grande (Norcia)	1300
C	CAME 7981	*H. perforatum* subsp. *veronense*	Paganico (Camerino)	670
D	CAME 7986	*H. perforatum* subsp. *veronense*	Nibbiano (Camerino)	640
E	CAME 7979	*H. montanum*	Capolapiaggia (Camerino)	560
F	CAME 7978	*H. montanum*	Montelago (Camerino)	870
G	CAME 7980	*H. hyssopifolium*	Pizzo di Meta (Sarnano)	1320
H	CAME 7977	*H. hirsutum*	Arcofiato (Camerino)	700
I	CAME 7984	*H. hircinum* subsp. *majus*	Botanical garden (Camerino)	630
L	CAME 7983	*H. tetrapterum*	Montelago (Sefro)	920

[a]Village closest to the collection locality in bracket.

7. Results

Concentrations of hyperforin, hypericin, chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin and quercetin in the *Hypericum* extracts are reported in Table 2, while the total phenolic compounds content is given in Table 3. Antioxidant and antiradical activity values are also reported in Table 3.

8. Conclusions

The present investigation constitutes the first screening for antioxidant/antiradical activity of some *Hypericum* spp. growing in central Italy. All the tested methanolic–acetone extracts contain pharmacologically important compounds that exhibited a mild to moderate antioxidant activity. *H. perforatum* s.l. (subsp. *veronense* in particular) was found to be the richest in hypericin, hyperforin and particularly in phenolic compounds, confirming its broad use both in the traditional medicine of central Italy and nowadays in many therapeutic applications. Moreover hyperforin was detected

Table 2
Concentrations (mg/g) of the constituents in investigated Hypericum taxa

Hypericum samples	Chlorogenic acid	Rutin	Hyperoside	Isoquercitrin	Quercitrin	Quercetin	Hyperforin	Hypericin
A	1.27	9.23	7.57	1.88	3.30	0.49	10.72	0.35
B	8.05	4.25	9.26	3.15	2.60	0.42	23.32	0.52
C	4.38	0.19	20.12	6.93	5.41	1.04	24.26	1.72
D	2.90	0.17	10.47	4.03	2.60	1.84	14.55	1.12
E	0.86	0.24	10.94	3.35	1.19	1.55	0.51	0.78
F	0.66	0.06	5.67	1.86	0.79	1.06	0.04	0.47
G	5.00	12.42	2.36	1.50	4.08	0.23	0.10	0.49
H	2.24	0.72	7.65	1.97	2.17	0.13	0.05	0.03
I	2.07	0.19	1.26	1.52	0.19	0.27	0.14	0.12
L	4.94	0.05	9.71	2.18	1.26	0.32	3.45	nd[a]
M	4.56	0.17	13.98	6.31	2.20	1.44	0.16	0.38

[a]nd = not detectable.

Table 3
Total phenolic content, antioxidant activities and scavenging effects of *Hypericum* extracts

Samples	Total phenolic content (in GA equivalent)[a]	Induction index (%)[b]	Antioxidant index[c]	DPPH[d]		
				3 μg/ml	6 μg/ml	12 μg/ml
A	7.78±0.05	1.32±0.001*	29.29±0.04*	2.72±0.12*	12.80±1.06*	33.56±0.52*
B	6.61±0.23	1.38±0.1*	38.49±0.10*	2.99±0.51*	8.21±1.89*	26.86±1.01*
C	11.24±0.05	1.1±0.07*	34.62±0.78*	6.45±0.31*	14.05±0.24*	36.31±0.85*
D	7.02±0.19	1.03±0.004*	43.82±0.40*	6.72±1.08*	12.71±0.56*	36.24±0.85*
E	3.92±0.06	1.71±0.22*	15.24±0.27*	2.24±1.13*	11.72±1.24*	23.66±1.24*
F	3.53±0.12	1.98±0.16*	25.00±0.61*	12.49±0.92	14.90±1.00	24.26±0.26*
G	4.67±0.07	1.57±0.02*	28.68±0.93*	4.72±0.73*	7.51±1.29*	21.56±1.09*
H	3.77±0.08	1.32±0.14*	35.00±0.83*	2.99±0.36*	8.27±0.61*	31.29±0.30*
I	4.26±0.16	1.13±0.04*	31.57±0.94*	2.79±0.65*	8.71±0.68*	29.43±0.71*
L	4.80±0.09	1.09±0.02*	48.68±0.12*	3.06±0.46*	8.33±0.33*	25.05±0.52*
BHT[e]		3.12±0.11	82.80±0.30	10.07±0.38	17.80±0.54	39.90±0.70

[a]mg GAE*/g extract.
[b]Induction index = induction time of olive oil + sample / induction time of olive oil.
[c]Percentage of inhibition estimated by means of β-carotene/linoleic acid system of extracts at 180 min.
[d]Scavenging effects at three different concentrations, expressed as % of inhibition against DPPH.
[e]Positive control, butylated hydroxytoluene.
*$P<0.05$ as compared with the control value.
Results are represented as means ± standard deviation.
Sample M has not been evaluated.

for the first time in *H. hircinum* subsp. *majus*. Significant values of phenolic compounds, detected also in other *Hypericum* taxa, justify antioxidant/antiradical properties of some extracts.

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References

[1] Guarrera PM. Fitoterapia 2005;76:1.
[2] Pieroni A, Quave CL, Santoro RF. J Ethnopharmacol 2004;95:373.
[3] Bystrov NS, Chernov BK, Dobrynin VN. Tetrahedron Lett 1975;32:2791.
[4] Gurevich AI, Dobrynin VN, Kolsov MN. Antibiotiki Moscow 1971;16:510.
[5] Brondz I, Greibrokk T. J Nat Prod 1983;46:940.
[6] Bombardelli E, Morazzoni P. Fitoterapia 1995;66:43.
[7] Brolis M, Gabetta B, Fuzzati N, Pace R, Panzeri F, Peterlongo F. J Chromatogr A 1998;825:9.
[8] Arnott C. Extraction and HPLC of hypericin; 2003. http://www.sbc.edu/honors/HJSpr03/Arnott.htm.
[9] Hoff JF, Singleton TJ. J Food Sci 1977;42:1956.
[10] Maure A, Cruz JM, Franco D, Dominquez JM, Sineiro J, Dominquez H, et al. Food Chem 2001;72:145.
[11] Sanchez-Moreno C, Larrauri JA, Saura-Calixto A. J Sci Food Agric 1998;76:270.