PERIODICUM BIOLOGORUM
VOL. 115, No 3, 459–462, 2013

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Short communication

Quantitative analysis of polyphenols in eighteen *Hypericum* taxa

KROATA HAZLER PILEPIĆ
ŽELJAN MALEŠ

University of Zagreb
Faculty of Pharmacy and Biochemistry
Department of Pharmaceutical Botany
Schrottova 39, 10000 Zagreb, Croatia

Correspondence:
Kroata Hazler Pilepić
University of Zagreb
Faculty of Pharmacy and Biochemistry
Department of Pharmaceutical Botany
Schrottova 39, 10000 Zagreb, Croatia
E-mail: khazler@pharma.hr

Key words: *Hypericum*, total polyphenols, nontannin polyphenols, tannins, spectrophotometry, quantitative analysis

Received May 15, 2013.

Abstract

Background and Purpose: *Phenolics are one of the most ubiquitous groups of plant secondary metabolites, which play an important role in plant stress tolerance, growth, reproduction, resistance to pathogens, pigmentation and etc. In this study we have analysed the content of polyphenols in the aerial parts of eighteen cultivated* Hypericum *taxa, collected over two consecutive seasons. No peer-reviewed literature could be found regarding determination of the quantities of polyphenols present in the majority of the analyzed taxa of the native or cultivated samples. Quantitative analysis of phenolic compounds was therefore investigated.*

Materials and Methods: *The content of phenolic compounds (total polyphenols, nontannin polyphenols and tannins) in the aerial parts of eighteen* Hypericum *taxa collected over two consecutive seasons was determined by spectrophotometric methods.*

Results: *The quantity of the total polyphenols of investigated taxa (Table 1), ranged from 3.96 to 16.88%. The highest content of total polyphenols was found in the samples of* H. kouytchense *(16.88%) collected in 2011 and* H. hookerianum *(14.75%) collected in 2012, while the samples of* H. richeri *subsp.* grisebachii *contained the lowest amount of total polyphenols (4.55 and 3.96%). The average content of tannins in the majority of investigated samples was found to be two times higher than the content of nontannin polyphenols, but in the rest of the samples, the most often ratio of tannins to nontannin polyphenols was 1:1.*

Conclusion: *The variable content of polyphenols examined here, probably reflects the plant adaptation to seasonal changes in its environment. The occurrence of these metabolites in the taxa explored, hopefully will contribute to future understanding of their pharmacological properties and activity.*

INTRODUCTION

Many species of *Hypericum* are known as healing herbs due to their various beneficial metabolites. The increasing interest in the chemistry of this genus has led to the isolation of many components with different biological activities. Methanolic extract from the aerial parts of *Hypericum* plants typically contains hypericins, hyperforins and phenolic compounds (*2*). Although hypericins were not found in the primitive taxa of the genus (*6*), these taxa also show a wide range of biochemical and pharmacological effects (for example antiproliferative effect *4, 9, 15*), obviously due to the presence of other constituents, especially phenolic compounds.

TABLE 1

Quantitative data analysis results of polyphenols compounds in 18 *Hypericum* taxa.

Taxon		Geographic distribution (13)	Year	Total polyphenols (%)*	Nontannin polyphenols (%)*	Tannins (%)*
1	*H. androsaemum* L.	Europe	2011.	5.88 ± 0.07	1.72 ± 0.03	4.16 ± 0.04
			2012.	6.26 ± 0.04	2.20 ± 0.10	4.06 ± 0.06
2	*H. balearicum* L.	Balearic Islands	2011.	11.79 ± 0.06	4.55 ± 0.02	7.24 ± 0.03
			2012.	11.35 ± 0.12	4.22 ± 0.06	7.13 ± 0.18
3	*H. delphicum* Boiss & Heldr	Greece	2011.	8.00 ± 0.08	1.95 ± 0.17[a]	6.05 ± 0.26[a]
			2012.	8.59 ± 0.23	3.78 ± 0.11[a]	4.81 ± 0.12[a]
4	*H. densiflorum* Pursh	N America	2011.	9.44 ± 0.24[a]	2.79 ± 0.23[a]	6.65 ± 0.19[a]
			2012.	13.16 ± 0.34[a]	3.34 ± 0.18[a]	9.82 ± 0.17[a]
5	*H. forrestii* N. Robson	China, Burma	2011.	8.11 ± 0.23	2.37 ± 0.01	5.74 ± 0.22
			2012.	8.23 ± 0.31	2.30 ± 0.15	5.93 ± 0.16
6	*H. hircinum* L.	C & E Europe	2011.	10.12 ± 0.36	5.36 ± 0.11[a]	4.76 ± 0.47[a]
			2012.	11.08 ± 0.11	3.86 ± 0.24[a]	7.22 ± 0.35[a]
7	*H. hookerianum* Wight & Arn.	E Asia	2011.	13.99 ± 0.08	7.04 ± 0.14[a]	6.95 ± 0.22[b]
			2012.	14.75 ± 0.05	8.56 ± 0.15[a]	6.19 ± 0.10[b]
8	*H. japonicum* Thunb. Ex Murray.	China, Japan	2011.	8.58 ± 0.12[a]	4.11 ± 0.13[a]	4.47 ± 0.25[a]
			2012.	13.65 ± 0.04[a]	5.91 ± 0.21[a]	7.74 ± 0.25[a]
9	*H. kalmianum* L.	N America	2011.	6.86 ± 0.03[b]	3.68 ± 0.06[a]	3.17 ± 0.09[b]
			2012.	5.48 ± 0.14[b]	1.52 ± 0.11[a]	3.96 ± 0.25[b]
10	*H. kouytchense* H.Lév.	China	2011.	16.88 ± 0.07[b]	7.35 ± 0.21[a]	9.53 ± 0.15[b]
			2012.	14.70 ± 0.10[b]	5.80 ± 0.03[a]	8.90 ± 0.07[b]
11	*H. linarifolium* Vahl	W Europe	2011.	5.68 ± 0.17[b]	3.66 ± 0.07[b]	2.02 ± 0.10
			2012.	4.61 ± 0.01[b]	2.90 ± 0.01[b]	1.71 ± 0.01
12	*H. oblongifolium* Choisy	India	2011.	12.93 ± 0.14[b]	5.29 ± 0.07[a]	7.64 ± 0.07[b]
			2012.	10.22 ± 0.09[b]	3.68 ± 0.06[a]	6.54 ± 0.03[b]
13	*H. olympicum* L.	SE Balkans	2011.	5.74 ± 0.26	3.20 ± 0.17	2.54 ± 0.10
			2012.	5.09 ± 0.02	2.89 ± 0.09	2.20 ± 0.08
14	*H. patulum* Thunb. Ex Murray	China, Taiwan, Japan	2011.	7.33 ± 0.01	2.26 ± 0.03	5.07 ± 0.04
			2012.	6.83 ± 0.05	2.65 ± 0.02	4.18 ± 0.03
15	*H. pseudohenryi* N. Robson	China	2011.	8.10 ± 0.02	2.57 ± 0.05	5.53 ± 0.04
			2012.	8.13 ± 0.05	2.19 ± 0.09	5.94 ± 0.04
16	*H. richeri* Vill subsp. *grisebachii* (Boiss.) Nyman	C & S Europe	2011.	4.55 ± 0.01	2.61 ± 0.00[a]	1.94 ± 0.00[b]
			2012.	3.96 ± 0.01	1.65 ± 0.01[a]	2.31 ± 0.02[b]
17	*H. triquetrifolium* Turra	SE Mediterranean	2011.	4.45 ± 0.02[b]	1.86 ± 0.06	2.59 ± 0.08[b]
			2012.	5.70 ± 0.05[b]	1.97 ± 0.03	3.73 ± 0.02[b]
18	*H. yezoënse* Maxim.	Asia	2011.	6.49 ± 0.07[b]	3.61 ± 0.10	2.88 ± 0.03[a]
			2012.	8.19 ± 0.14[b]	4.14 ± 0.02	4.05 ± 0.11[a]

*w/w, mean value ± SD, n = 3, difference between plant samples of the same taxa collected in different year at significance level of [a] $p < 0.05$ and [b] $p < 0.02$

Phenolics are one of the most ubiquitous groups of plant secondary metabolites, which play an important role in plant stress tolerance, growth, reproduction, resistance to pathogens, pigmentation and etc. (*5*). Moreover, some studies suggest that other phenolic compounds may be at least as important as flavonoids in UV protection (*14*). In this study we have analysed the content of phenolic compounds (total polyphenols, nontannin polyphenols and tannins) in the aerial parts of eighteen *Hypericum* taxa populations, cultivated under the same conditions and collected in the same period of vegetation through two consecutive years (Table 1). The main scope of this research is to check the annual fluctuations in the content of polyphenols, in the population which grows in the garden conditions influenced by slight meteorological changes during vegetation periods. Also, to the authors' knowledge, there are no published reports of total phenol content in the majority of the analyzed taxa in the native or in the cultivated samples. Up to now, data concerning phenol contents are available for only six *Hypericum* taxa from our research; *H. androsaemum* and *H. triquetrifolium* (*10*), *H. japonicum* and *H. patulum* (*12*), *H. hookerianum* (*11*) and *H. oblongifolium* (*1*).

MATERIALS AND METHODS

Plant material and chemicals

Research conducted involved 18 *Hypericum* taxa (Table 1). The plants were cultivated in the Pharmaceutical Botanical Garden »Fran Kušan« of the Faculty of Pharmacy and Biochemistry, University of Zagreb. Voucher specimens are deposited in the Herbarium of the Department of Pharmaceutical Botany. The shoots with full opened flowers were collected in June 2011 and 2012, and were properly dried at room temperature. The dried materials were assayed for total polyphenols, nontannin polyphenols and tannins. All solvents used were of analytical grade (Merck, Germany).

Quantitative analysis

The determination of total polyphenols, then polyphenols unadsorbed on hide powder (nontannin polyphenols) and tannins was performed by the spectrophotometric method with phosphorous – volframe acid and hide powder (*7*). The measurements were carried out using spectrophotometer Varian Cary 50 Bio (Varian Inc., USA). The content of total polyphenols, nontannin polyphenols and tannins was evaluated upon three independent analyses and data were expressed as means ± SD. The statistical analysis was performed using the Microsoft Office Excel 2003 (Microsoft Corporation) program.

RESULTS AND DISCUSSION

The quantity of total polyphenols of the investigated taxa (Table 1), ranged from 3.96 to 16.88%. The highest content of total polyphenols was found in the samples of *H. kouytchense* (16.88%), collected in 2011 and *H. hookerianum* (14.75%) collected in 2012, while the collected samples of *H. richeri* subsp. *grisebachii* contained the lowest amount of total polyphenols (4.55 and 3.96%). The average content of tannins in the majority of investigated samples was found to be twice or more times higher, than the content of nontannin polyphenols, while in other samples, the most often tannins to nontannin polyphenols ratio was found to be 1:1.

In half of the taxa already compared, significant difference was observed between the harvesting years. The total polyphenols content examined in 2012 was found to be significantly higher in four of the taxa samples, although in 4 taxa was calculated lower, whilst 10 taxa contained similar amounts of total polyphenols content in both harvesting years. Furthermore, significant differences were observed in the quantity of tannins and nontannin polyphenols measured in several samples among harvesting years. Total polyphenols values of *H. japonicum* and *H. patulum*, were found to be comparable with the results of Raghu Chandrashekhar *et al*. (*12*).

Polyphenol variations observed could be the consequence of the slight weather condition differences, which were noticed between two compared sampling seasons (*8*). Meteorological conditions for June 2011, Zagreb, were described as quite warm and the amount of rainfall was normal, while in June 2012, thermal conditions have been described as extremely warm and there was a plenty of rain. An increase in the content of phenols during extremely warm and wet period of the year, could be a concept for 4 taxa adaptation, that might be slightly more sensitive to changes in the weather conditions. Nevertheless, the majority of the taxa, although native in different regions of the world (Table 1), could give stabile phenolic compounds under the garden growth conditions. Such data are of great interest for cultivators of the medicinal *Hypericum* species, particularly because of its pharmacological activities mainly linked to the phenolic compounds (*2, 3*).

Acknowledgement: *This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (Project No. 006-0061117-1239).*

REFERENCES

1. ALI M, ARFAN M, AHMAD H, ZAMAN K, KHAN F, AMAROWICZ R 2011 Comparative antioxidant and antimicrobial activities of phenolic compounds extracted from five *Hypericum* species. *Food Technol Biotechnol 49*: 205–213
2. BARNES J, ANDERSON L A, PHILLIPSON J D 2001 St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol 53*: 583-600
3. BUTTERWECK V 2003 Mechanism of action of St John´s wort in depression: what is known? *CNS Drugs 17*: 539-562
4. DONGRE S H, BADAMI S, NATESAN S, CHANDRASHEKHAR R H 2007 Antitumor Activity of the Methanol Extract of *Hypericum hookerianum* Stem Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *J Pharmacol Sci 103*: 354-359
5. GHASEMZADEH A, GHASEMZADEH N 2011 Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *J Med Plants Res 5*: 6697-6703

6. KITANOV M K 2001 Hypericin and pseudohypericin in some *Hypericum* species. *Biochem Sys Ecol 29*: 171-178
7. MALEŠ Ž, BRANTNER A, SOVIĆ K, HAZLER PILEPIĆ K, PLAZIBAT M 2006 Comparative phytochemical and antimicrobial investigations of *Hypericum perforatum* L. subsp. *perforatum* and *H. perforatum* subsp. *angustifolium* (DC) Gaudin. *Acta Pharm 56*: 359-367
8. Meteorological and hydrological institute of Croatia 2013 http://meteo.hr
9. MUMTAZ A, ARFAN M, ZAMAN K, AHMED H, AKBAR N, ANIS I, SHAH M R 2011 Antiproliferative Activity and Chemical Constituents of *Hypericum oblongifolium*. *J Chem Soc Pakistan 33*: 772-777
10. OZEN T, AYAN A K, CIRAK C, KEVSEROGLU K 2005 Total phenol content of some *Hypericum* species growing in Turkey. *Chem Nat Comp 41*: 232-233
11. RAGHU CHANDRASHEKHAR H, VENKATESH P, PONNUSANKAR S, VIJAYAN P. 2009a Antioxidant activity of *Hypericum hookerianum* Wight and Arn. *Nat Prod Res 23*: 1240-1251
12. RAGHU CHANDRASHEKHAR H, VENKATESH P, ARUMUGAM M, VIJAYAN P 2009b Estimation of total phenols with special emphasis to antioxidant potentials of few *Hypericum* species *Pharmacologyonline 1*: 680-687
13. ROBSON N K B 1981 Studies in the genus *Hypericum* L. (Guttiferae). 2. Characters of the genus. *Bull Br Mus Nat Hist (Bot)* 8: 55-226
14. RYAN K G, SWINNY E E, WINEFIELD C, MARKHAM KR 2001 Flavonoids and UV photoprotection in *Arabidopsis* mutants. *Z Naturforsch 56c*: 745
15. VIJAYAN P, VINOD KUMAR S, DHANARAJ S A, MUKHERJEE P K, SURESH B 2003 In vitro cytotoxicity and antitumour properties of *Hypericum mysorense* and *Hypericum patulum*. *Phytother Res 17*: 952-956