PERIODICUM BIOLOGORUM
VOL. 112, No 3, 307–310, 2010

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original scientific paper

Quantitative analysis of phenolic compounds of *Inula candida* (L.) Cass.

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Key words: *Inula candida* (L.) Cass. (*Asteraceae*), flavonoids, phenolic acids, total polyphenols, nontannin polyphenols, tannins, spectrophotometry, quantitative analysis

Received February 16, 2009.

Abstract

Background and Purpose: *Some species of the genus* Inula *L. are used in herbal medicine. Phenolic compounds contribute to many biological activities of the plants. No literature could be found in respect of the determination of the quantities of phenolic compounds of Inula candida. Quantitative analysis of phenolic compounds in different plant parts was therefore performed.*

Materials and Methods: *Plant material was collected from different locations in Croatia. The content of phenolic compounds (flavonoids, phenolic acids, total polyphenols, nontannin polyphenols and tannins) was determined by spectrophotometric methods.*

Results: *The quantity of flavonoids ranged from 0.008 to 0.079%, while the content of phenolic acids varied between 0.411 and 1.423%. The content of total polyphenols ranged from 1.53 to 3.57%. The quantity of polyphenols unadsorbed on hide powder (nontannin polyphenols) was between 0.56 and 1.99%, while the quantity of tannins ranged from 0.96 to 2.22%. The highest contents of all phenolic compounds were found in leaves, whereas stems contained the lowest quantities.*

Conclusion: *The content of all investigated compounds depended on the plant organ investigated, date of collection, and locality.*

INTRODUCTION

Inula L. is a large genus of about 90 species of flowering plants in the family *Asteraceae*, native to Europe, Asia and Africa. The species *Inula candida* (L.) Cass. is a perennial herb with widespread distribution in Mediterranean *(5)*.

Phenolic compounds (flavonoids, phenolic acids and tannins) are ubiquitous in plants. They are also found in many medicinal plants, and herbal medicines containing these compounds have often been used in pharmacy. Flavonoids and phenolic acids have analgesic, antiallergic, anticancer, antidiabetic, antihepatotoxic, antiinflammatory, antiosteoporotic, antioxidant, antispasmotic and antivascular effects (*1, 2, 4, 16*) while tannins have antidiarrhoeal, antioxidant and antiseptic properties (*15*).

This paper presents the investigation of the content of phenolic compounds (flavonoids, phenolic acids, total polyphenols, nontannin polyphenols and tannins) of *I. candida*, collected from separate geographic locations in Croatia.

MATERIALS AND METHODS

Plant material and chemicals

The leaves, flowers and stems of *I. candida* were collected in summer and autumn 2007 at three separated geographic localities in Croatia: Badija, Pakleni otoci and Klis.

Voucher specimens (No. 105000-105011) were deposited at the Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. Detailed information about the plant material is presented in Table 1.

All the solvents used were of analytical grade (Merck, Germany).

Quantitative analysis

The measurements were carried out using spectrophotometer Varian Cary 50 Bio (Varian Inc., USA).

The content of flavonoids calculated as quercetin in plant samples was determined by the method of Christ and Müller (*3*). After acid hydrolysis (with 25% hydrochloric acid in acetone for 30 minutes at 100 °C) the released aglycones were determined spectrometrically at 425 nm by forming a complex with $AlCl_3$ in a methanol-ethyl acetate-acetic acid medium (*6–9, 12*).

The total phenolic acid content was determined according to the European Pharmacopoeia spectrophotometric method (*13*). The absorbance of the complex formed between phenolic acids and sodium nitrite – sodium molybdate was measured at 505 nm.

The determination of total polyphenols, polyphenols unadsorbed on hide powder (nontannin polyphenols) and tannins was performed by the spectrophotometric method with phosphorous – volfram acid and hide powder (*7*).

TABLE 1

The investigated sources of the *Inula candida*.

Plant part	Abbreviation	Collection site	Collection date
Leaves	1 L	Badija	24.7.2007
Flowers	1 F		
Stems	1 S		
Leaves	2 L	Pakleni otoci	13.8.2007
Flowers	2 F		
Stems	2 S		
Leaves	3 L	Pakleni otoci	9.10.2007
Flowers	3 F		
Stems	3 S		
Leaves	4 L	Klis	7.10.2007
Flowers	4 F		
Stems	4 S		

Statistical analysis

The content of all investigated phenolic compounds was evaluated in five independent analyses and data were expressed as means ± SD. The significance of a between-group difference was determined by Student's *t*-test using SigmaStat(R) 3.5, Copyright (c) 2006 Systat Software, Inc. program (*14*).

RESULTS AND DISCUSSION

Table 2 shows results of the quantitative analysis of flavonoids and phenolic acids.

The leaves contained 0.061–0.079% flavonoids. The total flavonoid content in flowers was ranging from 0.031% to 0.047%, whereas the samples of stems contained 0.008–0.023% flavonoids. The highest flavonoid content (0.079%) was found in the sample of leaves collected in October at the locality Pakleni otoci. Significant differences in the flavonoid content in leaves were found only among the samples collected at Badija and Klis localities, whereas only the flowers collected at Pakleni otoci (August) and Klis localities showed significant difference in the flavonoid content. The flavonoid content in the stems collected at Pakleni otoci in August and October also showed the difference. Statistical analysis of the flavonoid content in the different plant parts collected at the same location showed significant differences for Pakleni otoci (August) and Klis localities among all plant parts, while significant difference among the samples collected at Badija was observed only between leaves and stems. There was no significant difference in the flavonoid content among plant parts collected at Pakleni otoci (October).

TABLE 2

Dried basis content of flavonoids and phenolic acids in the leaves (L), flowers (F) and stems (S) of *Inula candida*.

Sample	Flavonoids (%)	Phenolic acids (%)
1 L	$0.063 \pm 0.0015^{a,d}$	$1.268 \pm 0.0370^{k,l,r,v}$
1 F	0.041 ± 0.0027	$1.011 \pm 0.0290^{m,n,s,v}$
1 S	0.013 ± 0.0002^{d}	$0.516 \pm 0.2150^{r,s}$
2 L	$0.071 \pm 0.0020^{e,f}$	1.423 ± 0.2180^{q}
2 F	$0.031 \pm 0.0007^{b,e,i}$	$0.962 \pm 0.0516^{o,t}$
2 S	$0.008 \pm 0.0007^{e,f,i}$	$0.414 \pm 0.0817^{q,t}$
3 L	0.079 ± 0.0074	$1.075 \pm 0.0785^{k,w}$
3 F	0.041 ± 0.0055	$0.933 \pm 0.0418^{m,p,x}$
3 S	0.023 ± 0.0014^{e}	$0.437 \pm 0.0366^{w,x}$
4 L	$0.061 \pm 0.0016^{a,g,j}$	$1.031 \pm 0.0308^{l,u,z}$
4 F	$0.047 \pm 0.0018^{b,h,j}$	$0.640 \pm 0.0535^{n,o,p,u,y}$
4 S	$0.014 \pm 0.0004^{g,h}$	$0.411 \pm 0.0110^{y,z,}$

% = Mean ± SD, n = 5

Statistically significant difference between plant parts collected at different collection sites and plant parts collected at the same collection site at the significance level of: $^{a,v-y}$ $p < 0.02$, $^{b-h,k-q}$ $p < 0.05$, $^{i,j,r-u}$ $p < 0.01$, ^{z} $p < 0.001$.

The investigated samples contained higher quantities of phenolic acids in comparison with flavonoids. The highest quantity of phenolic acids was found in leaves (1.031–1.423%), while stems contained the lowest quantities (0.411–0.516%). The sample collected at Pakleni otoci in August was the richest in the content of phenolic acids (1.423%).

Significant differences in the content of phenolic acids for the samples collected at the same location were recorded for all plant parts collected at Badija and Klis localities, and also for all parts collected at Pakleni otoci (August and October) except for leaves and flowers. It is statistically evident that the content of phenolic acids in leaves showed significant differences among Badija/Pakleni otoci (October) and Badija/Klis samples, while differences in the content in flowers were observed among Badija/Pakleni otoci (October), Badija/Klis, Pakleni otoci (August)/Klis and Pakleni otoci (October)/Klis samples. There were no significant differences in the content of phenolic acids in stems among different collection sites.

The results of determination of the content of total polyphenols, nontannin polyphenols and tannins are presented in Tables 3-5.

Analogously to the content of flavonoids and phenolic acids in other plant parts, leaves contained the highest quantity of total polyphenols (2.98–3.57%), nontannin polyphenols (1.05–1.99%) and tannins (1.50–2.22%). Flowers were richer in the content of total polyphenols, nontannin polyphenols and tannins than stems. The highest quantity of total polyphenols (3.57%) and nontannin polyphenols (1.99%) was found in the leaves collected in October at Pakleni otoci, whereas the highest content of tannins (2.22%) was found in the leaves collected at the same locality in August.

Statistical analysis of the total polyphenol content showed significant differences for all samples except among leaves collected at Badija and Klis localities (Table 3.).

There were no significant differences in the content of nontannin polyphenols in stems among Pakleni otoci

TABLE 3

Dried basis content of total polyphenols in the leaves (L), flowers (F) and stems (S) of *Inula candida*.

Sample	Total polyphenols (%)
1 L	$2.98 \pm 0.010^{a,b,r,s}$
1 F	$2.27 \pm 0.001^{f,g,h,r,t}$
1 S	$1.53 \pm 0.016^{l,m,n,s,t}$
2 L	$3.27 \pm 0.019^{a,c,d,u,v}$
2 F	$2.60 \pm 0.006^{f,i,j,u,w}$
2 S	$1.73 \pm 0.010^{l,o,p,v,w}$
3 L	$3.57 \pm 0.009^{b,c,e,x,y}$
3 F	$2.83 \pm 0.007^{g,i,k,x,z}$
3 S	$1.74 \pm 0.015^{m,o,q,y,z}$
4 L	$3.00 \pm 0.002^{d,e,a',b'}$
4 F	$2.06 \pm 0.004^{h,j,k,a',c'}$
4 S	$1.81 \pm 0.013^{n,p,q,b',c'}$

% = Mean ± SD, n = 5
Statistically significant difference between plant parts collected at different collection sites and plant parts collected at the same collection site at the significance level of: $^{a,b,e-k,r-b'}$ $p < 0.001$, $^{c,d,l,n,p,q,c'}$ $p < 0.01$, ^{m} $p < 0.02$, ^{o} $p < 0.05$.

TABLE 4

Dried basis content of nontannin polyphenols in the leaves (L), flowers (F) and stems (S) of *Inula candida*.

Sample	Nontannin polyphenols (%)
1 L	$1.35 \pm 0.003^{a,b,c,p,q}$
1 F	$1.27 \pm 0.005^{g,h,i,p,r}$
1 S	$0.56 \pm 0.004^{m,n,o,q,r}$
2 L	$1.05 \pm 0.009^{a,d,e,s}$
2 F	$1.04 \pm 0.009^{g,j,k,t}$
2 S	$0.77 \pm 0.038^{m,s,t}$
3 L	$1.99 \pm 0.005^{b,d,f,u,v}$
3 F	$1.71 \pm 0.008^{h,j,l,u,w}$
3 S	$0.78 \pm 0.009^{n,v,w}$
4 L	$1.50 \pm 0.015^{c,e,f,x,y}$
4 F	$0.85 \pm 0.044^{i,k,l,x}$
4 S	$0.75 \pm 0.015^{o,y}$

% = Mean ± SD, n = 5
Statistically significant difference between plant parts collected at different collection sites and plant parts collected at the same collection site at significance level of: $^{a,b,d-j,l,n,q,r,v,w,y}$ $p < 0.001$, $^{c,o,p,s-u,x}$ $p < 0.01$, ^{k,m} $p < 0.02$.

TABLE 5

Dried basis content of tannins in the leaves (L), flowers (F) and stems (S) of *Inula candida*.

Sample	Tannins (%)
1 L	$1.63 \pm 0.007^{a,b,l,m}$
1 F	$1.00 \pm 0.004^{f,g,h,l}$
1 S	0.97 ± 0.012^{m}
2 L	$2.22 \pm 0.010^{a,c,d,n,o}$
2 F	$1.56 \pm 0.003^{f,i,j,n,p}$
2 S	$0.96 \pm 0.028^{o,p}$
3 L	$1.58 \pm 0.004^{c,e,q,r}$
3 F	$1.12 \pm 0.001^{g,i,q,s}$
3 S	$0.96 \pm 0.006^{k,r,s}$
4 L	$1.50 \pm 0.013^{b,d,e,t,u}$
4 F	$1.21 \pm 0.040^{h,j,t,v}$
4 S	$1.06 \pm 0.002^{k,u,v}$

% = Mean ± SD, n = 5
Statistically significant difference between plant parts collected at different collection sites and plant parts collected at the same collection site at the significance level of: $^{a,d,f,i,l-o,q,r,u}$ $p < 0.001$, ^{b,h,k,v} $p < 0.02$, ^{c,g,j,p,s,t} $p < 0.01$, ^{e} $p < 0.05$.

(August)/Pakleni otoci (October), Pakleni otoci (August)/Klis and Pakleni otoci (October)/Klis samples. The samples of leaves and flowers collected at Pakleni otoci (August) and flowers and stems collected at Klis showed no significant differences (Table 4).

There were no significant differences in the content of tannins among the leaves collected at Badija/Pakleni otoci (October) localities. The flowers and stems collected at Pakleni otoci (October)/Klis localities showed no significant differences. The samples of flowers and stems collected at Badija also showed no significant differences in tannin content (Table 5.).

The above results indicate high variability in the content of the investigated phenolic compounds. Significant differences in their content might be due to pedological and climatic factors.

The investigated samples of *I. candida* contained lower quannities of flavonoids, phenolic acids, total polyphenols, nontannin polyphenols and tannins in comparison with *I.* (*Limbarda*) *crithmoides* (L.) Dumort. (0.15–0.43% flavonoids) and *I. viscosa* (L.) Ait. (0.157–0.498% flavonoids, 1.71–5.92% phenolic acids, 4.56–11.30% total polyphenols, 2.65–7.37% nontannin polyphenols, 1.91–3.93% tannins) (*10*, *11*).

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

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