Bulgarian Chemical Communications, Volume 49, Special IssueD (pp. 176 – 180) 2017

Phenolic constituents and antioxidant capacity of *Inula oculus-christi* from Bulgaria

A. Trendafilova[1]*, M. Todorova[1], V. Ivanova[1], I. Aneva[2]

[1] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 9, 1113 Sofia, Bulgaria*
[2] *Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., 1113 Sofia, Bulgaria*

Received May 01, 2017; Revised May 30, 2017

Dedicated to Acad. Ivan Juchnovski on the occasion of his 80th birthday

The aim of this study was to evaluate the total phenolic and flavonoid contents and antioxidant capacity of different *Inula oculus-christi* extracts and to isolate the potential antioxidant compounds. The methanol extract from flowers showed the highest total phenolic (68.34±0.66 mg GAE/g DE) and flavonoid (42.63±0.75 mg CE/g DE) contents, and the highest antioxidant capacity compared to other extracts through DPPH and ABTS assays (1.167±0.007 and 0.582±0.010 mM TE/ g DE, resp.). Six flavones (apigenin, luteolin, hispidulin, nepetin, scutellarein-4'-methyl ether and jaceosidin), two flavone glucosides (nepetrin and hispidulin-7-O-glucoside), chlorogenic acid and 3,5-dicaffeoylquinic acid were isolated from the most active extract. Their structures were established by spectroscopic methods. With exception of hispidulin, all identified compounds were found for the first time in the studied species.

Key words: *Inula oculus-christi;* Asteraceae, phenolics; flavonoids; DPPH; ABTS assays

INTRODUCTION

Free radicals and reactive oxygen species are known to be the major reason for various chronic and degenerative diseases associated with oxidative stress, such as diabetes mellitus, inflammation, cancer, hypertension, atherosclerosis, cardiovascular and neurodegenerative diseases [1]. Recently, plants and plant-derived antioxidants (vitamins, flavonoids and phenolic acids) have received growing attention, since they play an important role as preventive agents against damage caused due to oxidative stress with long-term physiological benefits without any harmful side effects [2]. The genus *Inula* (Asteraceae) comprises about 100 species widespread in temperate regions of Europe, Africa and Asia. Many *Inula* species are frequently used in traditional medicines throughout the world and reports on their ethnopharmacological applications have been recently reviewed [3]. Plants belonging to this genus have shown to possess various biological activities - antitumor, antiinflammatory, antibacterial, antiproliferative, antitussive, antidiabetic and hepatoprotective, etc., which were attributed to the abundance of bioactive components mainly sesquiterpene lactones, phenolic acids, and flavonoids [3,4]. *I. oculus-christi* is native to Iran, the Caucasus, Turkey, eastern Central Europe, Austria and the Balkan Peninsula [5]. Literature survey revealed several articles dealing mainly with the content of sesquiterpene lactones and their cytotoxic, antitumor and acetylcholinesterase activities [6-9]. A little is known about antioxidant properties of this species and the content of phenolic compounds. To the best of our knowledge, only hispidulin in *I. oculus-christi* from Serbia [6] and a DPPH scavenging activity of aqueous extracts of the species from Turkey [10] have been reported.

Continuing our research on *Inula* species, growing in Bulgaria we have focused our attention on the phenolic constituents and antioxidant capacity of *I. oculus-christi*.

EXPERIMENTAL

Plant material

Wild growing *I. oculus-christi* was collected in full flowering stage in July 2016 from western Rhodope Mts in Bulgaria. The plant was identified by Dr. Ina Aneva (Institute of Biodiversity and Ecosystem Research, BAS, Sofia). A voucher specimen (SOM 1360) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, BAS, Sofia, Bulgaria.

* To whom all correspondence should be sent:
E-mail: *trendaf@orgchm.bas.bg*

Extraction and isolation

Air-dried and powdered flowers (180 g) from *I. oculus-christi* were sequentially extracted with chloroform (2 x 2L) and methanol (2 x 1L) at room temperature for 24 hrs each. After filtration, the solvent from the combined extracts was evaporated under vacuum to give corresponding chloroform (7.4 g) and methanol (10.6 g) extracts. A portion of methanol extract (2.5 g) was dissolved in CH_3OH (15 ml) and centrifuged at 5800 rpm in order to remove insoluble parts. Clear methanolic solution was concentrated up to 5 ml and subjected to a Sephadex LH-20 column (equilibrated with CH_3OH) to give two main fractions A (1.5 g) and B (0.3 g). Fraction B was further applied to MPLC on LiChroprep RP-18 and eluted with increasing concentrations of CH_3OH in H_2O (20 to 80%). Further purification of selected fractions by MPLC (LiChroprep RP-18, CH_3OH/H_2O, 50:50) and/or prep. TLC (Silica gel, $CHCl_3/CH_3OH$, 10:1) yielded individual compounds: **1** (2.8 mg), **2** (5.3 mg), **3** (2.3 mg), **4** (12.3 mg), **5** (2.1 mg), **6** (2.2 mg), **7** (5.1 mg), **8** (4.6 mg), **9** (10.2 mg), and **10** (16.3 mg).

Chloroform and methanol extracts (0.41 and 0.49 g, respectively) from leaves of *I. oculus-christi* were obtained from 10 g of dry plant material using the same procedure. TLC comparison of the methanol extracts obtained from leaves and flowers was performed on Silica gel using Toluene/Dioxane/CH_3COOH (90:25:4) and EtOAc/ $HCOOH/CH_3COOH/H_2O$ (100:11:11:26) followed by spraying with NP/PEG reagent and UV visualization at 366 nm [11].

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was measured using Folin–Ciocalteu method [12]. Gallic acid was used as a standard compound and TPC was expressed as mg gallic acid equivalents (GAE) per 1 g of dry extract.

Determination of total flavonoid content (TFC)

Total flavonoid content was measured using a colorimetric assay developed previously [13]. (+)-Catechin was used as a standard compound and TFC was expressed as mg catechin equivalents per 1 g of dry extract.

Determination of antioxidant capacity

DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis (3)-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activities were determined according to the previously described methods [14]. Results were expressed as Trolox equivalent antioxidant capacity (mM Trolox equivalents per gram dry extract, mM TE/g DE), using calibration curve (absorption vs. concentration) of Trolox dissolved in methanol at different concentrations.

Statistical analysis

All data were reported as means ± standard deviation (SD) using at three independent measurements. Analysis of variance with a confidence interval of 95% was performed using MS Excel software.

RESULTS AND DISCUSSION

Phenolic compounds and flavonoids contribute to the overall antioxidant potential of plants, so that the chloroform and methanol extracts of the leaves and flowers of *I. oculus-christi* were analyzed for their total phenolic and flavonoid contents (Table 1). Total phenol content was expressed as mg GAE/g DE. The highest amount of phenolics was detected in methanol extracts. Leaves and flowers contained almost equal amounts of phenolics (68.34±0.66 and 66.95±1.13 mg GAE/g DE, respectively). Flavonoid content was expressed as mg catechin equivalents per gram of dry extract (mg CE/g DE) and its values varied widely for both solvents used and in the different plant parts. The methanol extract from flowers was the richest of in flavonoids (42.63±0.75 mg CE/g DE).

Antioxidant capacity of plants is commonly evaluated using more than one method to measure various oxidation products [15]. In this study, DPPH and $ABTS^{\bullet+}$ assays were used to estimate free radical scavenging properties of the studied extracts and the obtained results were expressed as mM Trolox equivalents per gram of dry extracts (mM TE/g DE (Table 1). Antioxidant capacity of the studied extracts measured by the DPPH method ranged from 0.023 to 1.167 mM TE/g DE. As shown in Table 1, methanol extract from flowers showed the highest radical scavenging activity, followed by methanol extract from leaves, while chloroform extract from leaves was almost inactive. Lower antioxidant capacities were determined by ABTS•+ assay and the values varied between 0.056 and 0.582 mM TE/g DE. The different antioxidant activity levels obtained from the assays probably due to the difference in the ability of antioxidant

compounds in the extracts to quench ABTS and DPPH free radicals in *in vitro* systems. A good correlation was observed between antioxidant capacity assessed with the DPPH and ABTS tests and phenolic content in studied extracts (R^2 = 0.9726 and 0.9684, respectively).

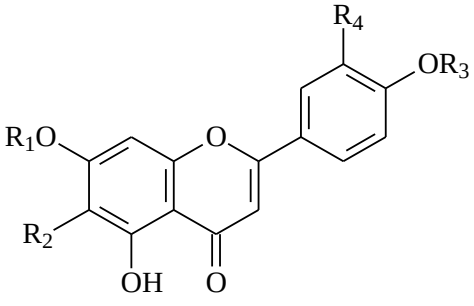
The most active methanol extract obtained from flowers was worked up for isolation of the Sephadex LH-20 and further purification afforded apigenin (**1)** [16, 17], luteolin (**2**) [16-19], scutellarein-4'-methyl ether (**3**) [20], nepetin (**4**) individual compounds. Column chromatography on [18, 19], jaceosidin (**5**) [21], hispidulin (**6**) [19, 22], nepetrin (**7**) [22], hispidulin-7-glucoside (**8**) [23], chlorogenic acid (9) [24] and 3,5-dicaffeoylquinic acid (10) [24] (Fig. 1). The isolated compounds were identified using spectral data (UV, ^{1}H NMR and MS) compared with those published in the literature. TLC comparison of the methanol extracts obtained from leaves and flowers of *I. oculus-christi* in the presence of the isolated compounds did not show significant qualitative differences.

The results described above showed that *I. oculus-christi* was characterized by the presence of flavones (**1-8**) and caffeoylqunic acid derivatives (**9** and **10**). Compounds **3-8** were substituted at C-6 with hydroxyl or methoxyl group. With exception of jaceosidin (**5**), all isolated flavonoids have been previously found in species of genus *Inula* such as *I. britannica, I. japonica, I. helenium, I. salsoides, I. helenium, I. viscosa, I. germanica*, etc. [3, 4, 25, 26]. Mono- and dicaffeoylquinic acid derivatives are widespread in the plant kingdom including *Inula* species. Phenolic acids **9** and **10** have been detected previously in *I. viscosa, I. britannica, I. helenium, I. cappa,* etc. [3, 4]. It is worth also to

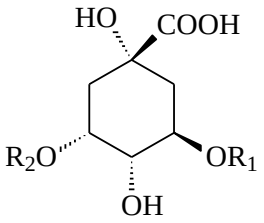
Table 1. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (ABTS and DPPH assay) of different *I. oculus-christi* extracts**.**

Sample	Extract	TPC	TFC	Antioxidant capacity [mM TE/g DE]	
		[mg GAE/g DE]	[mg CE/g DE]	ABTS	DPPH
leaves	$CHCl_3$	5.48±0.62^a	4.90±0.16^a	0.056±0.003^a	0.023±0.001^a
flowers	$CHCl_3$	18.31±1.63^b	16.08±1.10^b	0.105±0.003^b	0.076±0.002^b
leaves	MeOH	66.95±1.13^c	19.12±1.34^c	0.470±0.009^c	0.981±0.003^c
flowers	MeOH	68.34±0.66^c	42.63±0.75^d	0.582±0.010^d	1.167±0.007^d

Values are means ± SD. Different letters in same columns are significantly different at $p < 0.05$



	R_1	R_2	R_3	R_4
1	H	H	H	H
2	H	H	H	OH
3	H	OH	CH_3	H
4	H	OCH_3	H	OH
5	H	OCH_3	H	OCH_3
6	H	OCH_3	H	H
7	Glu	OCH_3	H	OH
8	Glu	OCH_3	H	H



9 R_1 = H, R_2 = Caffeoyl
10 R_1 = R_2 = Caffeoyl

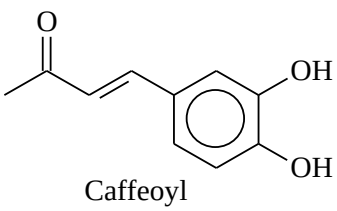


Fig. 1. Structures of the isolated compounds

mention that besides hispidulin (**7**) [6] all other isolated compounds were registered for the first time in *I. oculus-christ*.

CONCLUSION

The highest antioxidant capacity of the methanol extract obtained from flowers of *I. oculus-christi* could be attributed to the high content of phenolic components such as caffeoylquinic acid derivatives and flavonoids. Moreover, the domination of C-6 substituted flavonoids in *I. oculus-christi* is in accordance with the known flavonoid patterns of *Inula* species, i.e. they could be assumed as chemotaxonomic markers.

Acknowledgements: This work was supported by the National Science Fund, Ministry of Education and Science, Bulgaria, Project DN 09/11.

REFERENCES

1. J. M. McCord, *Am. J. Med.* **108**, 652 (2000).
2. S. Alok, S.K. Jain, A. Verma, M. Kumar, A. Mahor, M. Sabharwal, *Asian Pac. J. Trop. Biomed.*, **4**, 78 (2014).
3. A. M. L. Seca, A. Grigore, D. C. G. A. Pinto, A. M. S. Silva, *J. Ethnopharmacol*., **154**, 286 (2014).
4. A. M. L. Seca, D. C. G. A Pinto, A. M. S. Silva, *Chemistry & Biodiversity*, **12**, 859 (2015).
5. http://en.hortipedia.com/wiki/Inula_oculus-christi
6. V. Vajs, M. Nevešćanin, S. Macura, N. Juranić, N. Menković, S. Milosavljević, *Fitoterapia*, **74**, 508 (2003).
7. M. Mosaddegh, M. H. Moghadam, S. Ghafari, F. Naghibi, S.N. Ostad, R.W. Read, *Nat. Prod. Commun.*, **5,** 511 (2010).
8. M. H. Moghadam, F. Naghibi, A. Atoofi, M. A. Rezaie, M. Irani, M. Mosaddegh, *Z. Naturforsch.*, **68c**, 108 (2013).
9. H. Hajimehdipoor, M. Mosaddegh, F. Naghibi, A. Haeri, M. Hamzeloo-Moghadam, *An. Acad. Bras. Cien*., **86**, 801 (2014).
10. S. Berk, B. Tepe, S. Arslan, *Afr. J. Pharm. Pharmacol*., **5**, 1695 (2011).
11. H. Wagner, S. Bladt, Plant Drug Analysis. A Thin Layer Chromatography Atlas. Second Edition, Springer-Verlag, Brooklyn, New York, 1996.
12. K. M. Yoo, Ch. H. Lee, H. Lee, B. K. Moon, Ch. Y. Lee, *Food Chem*., **106**, 929 (2008).
13. J. Zhishen, T. Mengcheng, W. Jianming, *Food Chem*., **64**, 555 (1999).
14. A. Marchev, I. Ivanov, P. Denev, M. Nikolova, V.Gochev, A. Stoyanova, A. Pavlov, V. Georgiev, *J. BioSci. Biotechnol.*, **4**, 219 (2015).
15. K. Schlesier, M. Harwat, V. Böhm, R. Bitsch, *Free Radic Res*., **36**, 177 (2002).
16. T. Fossen, Ø. M. Andersen, in: Flavonoids – Chemistry, Biochemistry and Applications, Ø. M. Andersen, K. R. Markham (eds.), Taylor & Francis, USA (2005).
17. K. R. Markham, H. Geiger, in: The Flavonoids - Advances in Research 1986-1990, J. B. Harborne (ed.), Chapman and Hall, London (1993).
18. 18. N. Bai , Z. Zhou , N. Zhu , L. Zhang , Z. Quan, K. He, Q. Y. Zheng, C.-T. Ho, *J. Food Lipids*, **12,** 141 (2005).
19. 19. G. Flamini, E. Antognoli, I. Morelli, *Phytochemistry*, **57**, 559 (2001).
20. 20. H. Sabina, R. Aliya, *Pak. J. Bot*., **41**, 1927 (2009).
21. 21. L. Hammoud, R. Seghiri, S. Benayache, P. Mosset, A. Lobstein, M. Chaabi, F. León, I. Brouard, J. Bermejo, F. Benayache, *Nat Prod Res*., **26,** 203 (2012).
22. S. Shi, Y. Ma, Y. Zhang, L. Liu, Q. Liu, M. Peng, X. Xiong, *Sep. Puri. Technol*., **89**, 225 (2012).
23. I. Fernandez, B. Garcia, J. R. Pedro, A. Varea, *Phytochemistry*, **30**, 1030 (1991).
24. H. Morishita, H. Iwahashi, N. Osaka, R. Kido, *J. Chromatography A*, **315**, 253 (1984).
25. E. Wollenweber, M. Dörr, H. Fritz, K. M. Valant-Vetschera, *Z. Naturforsch*., **52c,** 137 (1997).
26. E. Wollenweber, M. Christ, R. H. Dunstan, J. N. Roitman, J. F. Stevens, *Z. Naturforsch*., **60c**, 671 (2005).

ФЕНОЛНИ СЪЕДИНЕНИЯ И АНТИОКСИДАНТЕН КАПАЦИТЕТ НА *INULA OCULUS-CHRISTI* ОТ БЪЛГАРИЯ

А. Трендафилова[1]*, М. Тодорова[1], В. Иванова[1], И. Анева[2]

[1] *Институт по органична химия с Център по фитохимия, Българска академия на науките, ул. "Акад. Г. Бончев", бл. 9, 1113 София, България*
[2] *Институт по биоразнообразие и екосистемни изследвания, Българска академия на науките, ул. "Гагарин" 2, 1113 София, България*

Постъпила на 1 май 2017 г.; Коригирана на 30 май 2017 г.

(Резюме)

Целта на това изследване е да се определи тоталното фенолно и флавоноидно съдържание и антиоксидантния капацитет на различни екстракти от *Inula oculus-christi*, както и да се изолират потенциалните антиоксидантни съединения. Метанолният екстракт получен от цветовете на растението показа най-високо тотално съдържание на фенолни съединения (68.34±0.66 mg GAE/g DE) и флавоноиди (42.63±0.75 mg CE/g DE) и най-висок антиоксидантен капацитет спрямо DPPH and ABTS радикали (1.167±0.007 and 0.582±0.010 mM TE/ g DE, съответно). Шест флавона (апигенин, лутеолин, хиспидулин, непетин, скутелареин-4'-метил етер и яйцеозидин), два флавонови глюкозида (непетрин и хиспидулин-7-О-глюкозид), хлорогенова и 3,5-дикафеоилхинова киселини бяха изолирани от най-активния екстракт. Тяхната структура е определена с помощта на спектрални методи. С изключение на хиспидулин, всички идентифицирани съединения се откриват за първи път в изследваното растение.