Journal of Applied Botany and Food Quality 88, 209 - 214 (2015), DOI:10.5073/JABFQ.2015.088.030

[1]Department of Analytics and Control of Medicines, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
[2]Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

Analysis of aucubin and catalpol content in different plant parts of four *Globularia* species

Miranda Sertić[1], Maja Crkvenčić[2]*, Ana Mornar[1], Kroata Hazler Pilepić[2], Biljana Nigović[1], Željan Maleš[2]

(Received May 7, 2015)

Summary

Iridoids are plant secondary metabolites that are gaining more scientific interest due to the wide range of their observed biological activities such as neuroprotective, anti-inflammatory, immunomodulatory, hepatoprotective and cardioprotective. The presence and content of aucubin and catalpol, two iridoid glucosides frequently present in iridoid-containing plants, was studied in methanolic extracts of leaves, flowers, woody stems and underground parts of four *Globularia* L. species, including the medicinal plant *Globularia alypum* L., using a specific and reliable HPLC-DAD-ESI/MS method. Aucubin was found in all four species, while catalpol was found only in *G. alypum* and *G. punctata*. Flowers contained the highest amounts of investigated iridoids, with catalpol content reaching 1.6% in *G. punctata* flowers. Comparing to the medicinal plant *G. alypum*, related species contained higher amounts of investigated iridoids, which makes them interesting candidates for further biological activity investigations.

Introduction

Medicinal plants produce various phytochemicals that are responsible for their preventive and/or healing properties. Along with different phenolic compounds, such as phenolic acids, flavonoids and tannins, which represent the largest part of plant secondary metabolites (Dai and Mumper, 2010), more attention considering biological activity and possible applications of plant extracts is recently given to iridoid-containing plants (Viljoen et al., 2012; West et al., 2014). Iridoids are a large group of monoterpenoid compounds (Andrzejewska-Golec, 1995), whose role in plants is mainly defence (Wink, 2003). They possess a wide range of biological activities, such as neuroprotective, anti-inflammatory, immunomodulatory, hepatoprotective, cardioprotective, anticancer, antioxidant, antimicrobial, hypoglycaemic, hypolipidemic, choleretic, antispasmodic and purgative (Tundis et al., 2008). However, the distribution of iridoids is with few exceptions limited to the plant orders Lamiales, Gentianales and Cornales (Jensen, 1991), which makes these compounds interesting as potential chemotaxonomic markers (Taskova et al., 2006).

Globularia L. is a small angiosperm genus recently included in the Plantaginaceae family (Albach et al., 2005). One of the members of this genus, namely *Globularia alypum* L., is a medicinal plant traditionally used in Mediterranean countries for some of the aforementioned activities attributed to iridoid compounds (Carrió and Vallès, 2012; Boudjelal et al., 2013; Bousta et al., 2014). Its hypoglycaemic, anti-ulcer and anti-inflammatory activity were confirmed by different studies (Zennaki et al., 2009; Fehri and Aiache, 2010; Amessis-Ouchemoukh et al., 2014b). Recent findings suggest that related species, such as *G. cordifolia* L. and *G. meridionalis* (Podp.) O. Schwarz, could also have potentially useful biological activities (Tundis et al., 2012a; Sipahi et al., 2014). The amount and type of iridoids present in these species might serve as an indicator of their potential health benefits, especially since these compounds were recognized as one of the main secondary metabolites of *Globularia* (Chaudhuri and Sticher, 1981; Kirmizibekmez et al., 2003; Tundis et al., 2012b).

Aucubin is the most widespread compound belonging to the group of iridoid glycosides (Andrzejewska-Golec, 1995). Aucubin is also a precursor of catalpol (Rønsted et al., 2000), one of the main secondary metabolites of *G. alypum* (Chaudhuri and Sticher, 1981), whose presence frequently accompanies the presence of aucubin in species of the Plantaginaceae (Taskova et al., 2006). Having in mind that medicinal use of different plant parts of *G. alypum* has been reported (Carrió and Vallès, 2012; Bousta et al., 2014), one of the aims of the present study was to investigate the content of aucubin and catalpol in leaves, flowers and woody stems of the medicinal plant to see if they could be connected to its recorded medicinal applications. Moreover, the study included three related species from the same genus, namely *G. cordifolia*, *G. meridionalis* and *G. punctata*, to try to predict their medicinal potential based on the comparison of the found amounts of investigated iridoids and known uses of the medicinal plant. Underground parts of the three less investigated species were also included in the analysis, while *G. alypum* was harvested without underground parts, due to its near threatened status in Croatia. The analysis was performed using a rapid method coupling high-performance liquid chromatography with diode array detector and electrospray ionization/mass spectrometry (HPLC-DAD-ESI/MS), which was optimized and validated in our laboratory.

Materials and methods

Chemicals

Aucubin was obtained from Fluka Chemie AG (Buchs, Switzerland) and catalpol from Sigma-Aldrich Co. (St Louis, MO, USA). HPLC-grade acetonitrile and formic acid were obtained from Merck (Darmstadt, Germany). Ultrapure water used in the mobile phase and for the preparation of sample and standard solutions was obtained by a Milli-Q water 140 purification system (Millipore, Bedford, USA). Methanol was obtained from T.T.T. d.o.o. (Sveta Nedelja, Croatia) and was of analytical grade.

Plant material

Aerial and underground plant parts of *G. cordifolia*, *G. meridionalis* and *G. punctata* were collected during the flowering period in May 2012 and *G. alypum* in March 2013 from natural populations growing in Croatia and Bosnia and Herzegovina. Geographical coordinates for localities of *G. alypum*, *G. cordifolia*, *G. meridionalis* and *G. punctata* were: 42° 30′ 50″ N/18° 19′ 07″ E, 43° 21′ 41″ N/17° 48′ 20″ E, 44° 31′ 41″ N/15° 08′ 38″ E and 45° 23′ 36″ N/13° 51′ 20″ E, respectively. Voucher specimens are deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia (Voucher No. 16 020, 16 030, 16 043, 16 051). The identity of each species was verified by Prof. Kroata Hazler Pilepić, Department of Pharma-

* Corresponding author

ceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

Preparation of sample and standard solutions

Dry powdered plant parts (leaves, flowers, woody stems and underground parts) (2.5 g) of each of the four *Globularia* species were extracted twice with methanol (25 mL) in a Bandelin SONOREX^{TM} SUPER ultrasonic extractor (30 min, room temperature) (Bandelin Electronic GmbH & Co. KG, Berlin, Germany), filtered and their volume was adjusted to 50 mL with methanol. Prior to analysis the extracts were diluted two times with water and filtered through an Acrodisc® GHP syringe filter (diameter 26 mm, pore size 0.20 μm) (Gelman, Ann Arbor, USA).

Stock solutions of aucubin and catalpol were prepared by dissolving each of the standards separately in water (0.2 mg/mL). All stock solutions were stored in a refrigerator at 4 °C for no longer than four weeks and were stable during that time. Working standard solutions used for method optimization and validation were prepared daily by serial dilutions with water just before measurements.

HPLC-DAD- ESI/MS analysis of aucubin and catalpol

Contents of aucubin and catalpol were analyzed by Agilent 1100 Series LC-MSD Trap system (Agilent Technologies, Waldbronn, Germany). Separation was performed on a Zorbax SB-C_{18} column (250 mm x 4.6 mm i.d.; 5 μm particle size) (Agilent Technologies, Waldbronn, Germany) under isocratic elution conditions using a mixture of ACN:H_2O:HCOOH (5:95:0.1, v:v:v) as the mobile phase, with a flow rate 0.5 mL/min, 25.0 °C column temperature and UV detection at λ 210 nm. Prior to use, the mobile phase was filtered through a cellulose nitrate filter (diameter 47 mm, pore size 0.45 μm) (Sartorius, Göttingen, Germany). Electrospray ionization (ESI) was performed in negative ionization mode. Nitrogen was used both as drying gas (5.0 L/min) and as nebulizer gas (15.0 psi). Electrospray source temperature was optimized at 325 °C and the capillary voltage was set at 3.5 kV. The UV detection was set at λ 210 nm. The full scan mass spectra were acquired over a range *m/z* 100 – 500. Data acquisition and processing were done using ChemStation for LC 3D and LC/MSD Trap v.5.2 software. All samples were injected in triplicate (10 μL, splitless mode). The contents of aucubin and catalpol were calculated from calibration curves obtained by the use of MS detector and the results were presented as mg/g dry plant material.

Method validation

The method was validated for linearity, precision, accuracy and limits of detection (LOD) and quantification (LOQ) according to guidelines of International Conference on Harmonization (ICH, 2005). Linearity was evaluated by least squares linear regression using seven different concentrations of standard solutions (10, 25, 50, 75, 100, 150 and 200 μg/mL). LOD and LOQ were evaluated by using the same dilutions of standard solutions as for linearity and were defined as the signal-to-noise ratio equal to 3:1 and 10:1, respectively. Accuracy of the method was determined using three different known concentrations (25, 50 and 100 μg/mL) of aucubin and catalpol, each analyzed individually three times. The intra- and inter-day precision of the method was validated with 100 μg/mL standard solution of aucubin and catalpol. For intra-day precision measurements were conducted six times within the same day, while for inter-day precision measurements were performed three times a day on three consecutive days. Relative standard deviation (RSD) for retention time and RSD for peak area, defined as the percentage ratio of standard deviation to the mean, were taken as measures of precision. All data were evaluated using Microsoft Office Excel 2007 (12.0.6683.5002).

Statistical analysis

The results are presented as means ± standard deviations of triplicate measurements. Exceptionally, reported retention times of aucubin and catalpol were based on six different analyses. A two-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test was carried out to determine significant differences between results. Significance level α was set at 0.05. The statistical analysis was carried out using GraphPad Prism 5.03 for Windows (GraphPad Software, San Diego, USA).

Results and discussion

In the present study, amounts of aucubin and catalpol in different plant parts of *G. alypum*, *G. cordifolia*, *G. meridionalis* and *G. punctata* were evaluated using a specific and reliable HPLC-DAD-ESI/MS method. The method provided good separation of analytes, with retention times being 7.56 ± 0.01 min for catalpol and 12.23 ± 0.01 min for aucubin (n = 6). The presence of aucubin and catalpol was determined by comparison of their retention times together with UV and mass spectra with those of standard compounds. Final quantification of analytes was based on the results obtained from the MS detector, which in comparison to a diode array detector provided better selectivity with obtained good sensitivity of the used method. After testing electrospray ionization in both positive and negative mode, much higher peaks were observed in negative ionization mode, similar as in some previous studies considering iridoid glycosides (Li et al., 2008; Deng et al., 2013). The observed fragmentation pattern of aucubin was similar to that of catalpol (Tab. 1). Deprotonated pseudo-molecular ions $[M - H]^-$ were present at *m/z* 345 for aucubin and at *m/z* 361 for catalpol. Also, characteristic fragment ions of $[M - H - 162]^-$ due to loss of a dehydroglucose residue were observed at *m/z* 183 for aucubin and *m/z* 199 for catalpol. A similar fragmentation pattern was observed previously (Kumar et al., 2013). In the mass spectrum of aucubin, an additional peak was present at *m/z* 137, most likely obtained by loss of glucose and CO $[M - H - 180 - 28]^-$. Peaks at *m/z* 381 and 383 observed for aucubin and at *m/z* 397 and 399 for catalpol, having a height ratio of about 3:1, indicated the presence of chlorine adducts (Zhu and Cole, 2000). The major ion observed for both compounds was a formic acid adduct of the pseudo-molecular ion $[M - H + 46]^-$. The formation of adducts with formic acid present in the mobile phase was reported previously for some other iridoid glycosides (Li et al., 2008; Deng et al., 2013). High peaks appearing at *m/z* 443 and 459 might be a result of adduct formation with phosphoric/sulfuric acid as possible residual contamination of the used system, leading to a rise of pseudo-molecular ion mass for approximately 98 Da (Tong et al., 1999).

Tab. 1: Peak identification for aucubin and catalpol mass spectra

m/z		
Auc	Cat	Peak identification
136.9	-	$[M - H - \text{glucose} - CO]^-$
182.8	198.8	$[M - H - \text{dehydroglucose}]^-$
344.9	360.9	$[M - H]^-$
381.0	396.9	$[M + Cl]^-$
391.0	406.9	$[M - H + HCOOH]^-$
442.9	458.9	$[M - H + H_3PO_4/H_2SO_4]^-$

Auc, aucubin; Cat, catalpol.

The developed method was validated for linearity, limits of detection (LOD) and quantification (LOQ), accuracy, intra- and inter-day precision. Validation parameters are presented in Tab. 2. The calibration curves for both analytes were linear over the concentration range used (10 – 200 μg/mL) (R > 0.99). The recovery values were between 98.11 % and 102.09 % indicating excellent accuracy of the method.

The results of the analysis showed that aucubin was present in all four investigated species, while catalpol was only found in *G. alypum* and *G. punctata* (Tab. 3). In a similar study done on different *Plantago* species, aucubin was also found to be more frequently present than catalpol (JURIŠIĆ et al., 2004), which could be connected with the fact that aucubin is a biosynthetic precursor of catalpol (RØNSTED et al., 2000).

Our results confirm the presence of aucubin in *G. alypum*, which was earlier reported by WIEFFERING (1966). This disproves the claims of CHAUDHURI and STICHER (1981), reporting its presence to be questionable in *G. alypum,* which were probably based on the fact that aucubin was never isolated from this species. Previous studies primarily reported aucubin and its derivatives as typical for *G. cordifolia* (KIRMIZIBEKMEZ et al., 2003), while for *G. alypum* mostly catalpol and catalpol derivatives were observed (CHAUDHURI and STICHER, 1981; AMESSIS-OUCHEMOUKH et al., 2014a).

When comparing plant parts of *G. punctata* it can be seen that flowers were richest in both aucubin and catalpol, followed by leaves ($p < 0.05$) (Fig. 1). The same iridoid distribution pattern was observed in all investigated species (Tab. 3). Recently, aerial parts of *G. meridionalis* were reported to contain higher amounts of iridoids than underground parts (TUNDIS et al., 2012a), as well. Similar observations were reported in other species belonging to the Plantaginaceae family, such as *Linaria dalmatica* (L.) P. Mill. (JAMIESON and BOWERS, 2010). Our findings and cited data are in accordance with the crucial ecological roles of secondary metabolites for plants, especially when having in mind that organs important for survival and reproduction usually have more potent compounds and higher amounts thereof (WINK, 2003).

Tab. 2: Validation parameters for the applied HPLC-DAD-ESI/MS method (linearity, limits of detection (LOD) and quantification (LOQ), accuracy, intra- and inter-day precision)

Validation parameters		Aucubin	Catalpol
Linearity range (μg/mL)		10-200	10-200
Regression equation		y = 23618x – 249491	y = 13700x – 25381
R		0.993	0.995
LOD (μg/mL)		1	2.5
LOQ (μg/mL)		3	7
Accuracy (%)	low	102.09	98.11
	medium	100.87	101.32
	high	101.21	99.48
Intra-day precision	RSD (%) for retention time	0.51	0.72
	RSD (%) for peak area	3.87	3.25
Inter-day precision	RSD (%) for retention time	0.54	0.87
	RSD (%) for peak area	4.92	4.85

LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation.

Tab. 3: Aucubin and catalpol contents in different plant parts of four *Globularia* species

Aucubin	Content/(mg/g dry plant material)			
	G. alypum	*G. cordifolia*	*G. meridionalis*	*G. punctata*
Leaves	0.58 ± 0.01	0.96 ± 0.03	1.53 ± 0.03	0.77 ± 0.01
Flowers	0.92 ± 0.00	1.65 ± 0.02	2.00 ± 0.01	1.29 ± 0.01
Woody stems	0.58 ± 0.01	0.59 ± 0.00	0.61 ± 0.01	0.66 ± 0.01
Underground parts	n.m.	0.81 ± 0.00	0.76 ± 0.01	0.57 ± 0.01
Catalpol	Content/(mg/g dry plant material)			
	G. alypum	*G. cordifolia*	*G. meridionalis*	*G. punctata*
Leaves	1.79 ± 0.03	ND	ND	2.52 ± 0.06
Flowers	12.58 ± 0.03	ND	ND	15.97 ± 0.63
Woody stems	1.47 ± 0.02	ND	ND	0.51 ± 0.01
Underground parts	n.m.	ND	ND	0.66 ± 0.01

Values are means ± SD, n = 3; n.m., not measured; ND, not detected.

In our study higher amounts of aucubin were found in leaves and flowers of the three related species comparing to those of *G. alypum* ($p < 0.05$). Catalpol concentrations found in *G. alypum* and *G. punctata* were generally higher than aucubin concentrations. They were especially high in flowers, with the content reaching almost 1.6% in *G. punctata*. A similar relationship between aucubin and catalpol was observed in *Rehmannia glutinosa* (Gaertn.) DC., one of the fundamental herbs used in traditional Chinese medicine (Won et al., 2010; Xu et al., 2012). Catalpol content was also seen to be higher than aucubin content in some *Plantago* species where both iridoids were present (Jurišić et al., 2004). From an ecological point of view these observations could be explained by the greater toxicity of catalpol to generalist herbivores (Nieminen et al., 2003).

According to different ethnobotanical studies flowers of *G. alypum* are usually used for treatment of diabetes, digestive disorders and eczema (Boudjelal et al., 2013; Bousta et al., 2014). The use of leaves and aerial parts of the same plant was reported for similar indications (Merzouki et al., 2000; Hammiche and Maiza, 2006), but also for some additional purposes, such as treatment of arterial hypertension (Carrió and Vallès, 2012; Sari et al., 2012). Based on data from previous studies considering biological activities of iridoids and the results presented in this study, it can be assumed that some of these activities could be attributed to aucubin and/or catalpol. However, it should be noted that this species contains a large number of compounds with medicinal potential (Amessis-Ouchemoukh et al., 2014a). Recently, the study of Merghache et al. (2013) confirmed hypoglycaemic and hypolipidemic activity of globularin, a cinnamoyl ester of catalpol isolated from *G. alypum*.

In *G. punctata* both investigated iridoids were present in higher amounts than in *G. alypum*, which makes it an interesting plant for further analysis, especially due to the fact that it is a well-distributed species of the genus (Tutin et al., 1972). However, one should bear in mind that besides genetic predisposition, other factors (internal and external) could have also contributed to the production of secondary metabolites in these plants (Jamieson and Bowers, 2010). For example, observed differences might be a consequence of different plant age (Andrzejewska-Golec, 1995) and/or reaction to damage caused by insect herbivores and pathogenic microorganism infections (Marak et al., 2002). It wasn't possible to eliminate all of these factors, due to the fact that harvesting was done from wild populations. However, all samples were collected in the same phenological period (flowering).

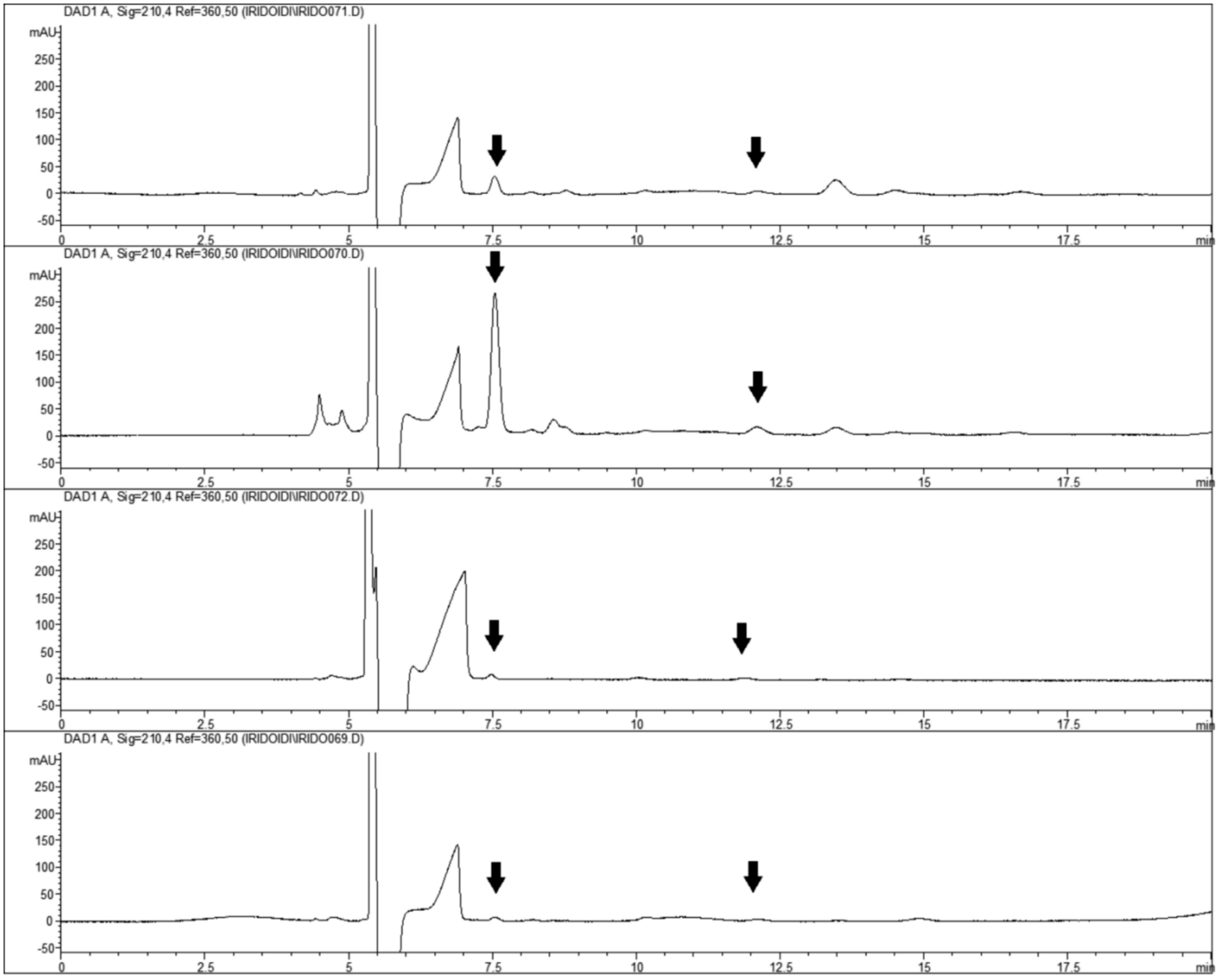

Fig. 1: Chromatograms of *G. punctata* leaves, flowers, woody stems and underground parts obtained by diode array detector (arrows indicating the presence of catalpol and aucubin, respectively).

Conclusions

Comparing aucubin and catalpol content of *G. cordifolia*, *G. meridionalis* and *G. punctata* to amounts of these iridoids found in the medicinal plant *G. alypum*, it can be assumed that the three species might have some potential for medicinal use. Certainly, possible biological activity investigations in the future should be accompanied by more detailed analyses of their chemical composition. These studies are currently in progress. So far the results show a greater similarity between the phytochemical composition of *G. punctata* and *G. alypum* than that between *G. cordifolia*/*G. meridionalis* and *G. alypum*. With regard to its wider distribution in Europe, *G. punctata* shows the highest potential for further investigations.

Acknowledgements

This research was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (project No. 006-0061117-1239 and 006-0061117-1240).

We would like to thank S. Maslo for his help in collecting plant material from Mostar.

References

Albach, D.C., Meudt, H.M., Oxelman, B., 2005: Piecing together the "new" Plantaginaceae. Am. J. Bot. 92, 297-315.

Amessis-Ouchemoukh, N., Abu-Reidah, I.M., Quirantes-Piné, R., Rodríguez-Pérez, C., Madani, K., Fernández-Gutiérrez, A., Segura-Carretero, A., 2014a: Tentative characterisation of iridoids, phenylethanoid glycosides and flavonoid derivatives from *Globularia alypum* L. (Globulariaceae) leaves by LC-ESI-QTOF-MS**.** Phytochem. Anal. 25, 389-398.

Amessis-Ouchemoukh, N., Madani, K., Falé, P.L.V., Serralheiro, M.L., Araújo, M.E.M., 2014b: Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Ind. Crop. Prod. 53, 6-15.

Andrzejewska-Golec, E., 1995: The occurrence of iridoids in plants. Acta Soc. Bot. Pol. 64, 181-186.

Boudjelal, A., Henchiri, C., Sari, M., Sarri, D., Hendel, N., Benkhaled, A., Ruberto, G., 2013: Herbalists and wild medicinal plants in M'Sila (North Algeria): An ethnopharmacology survey. J. Ethnopharmacol. 148, 395-402.

Bousta, D., Boukhira, S., Aafi, A., Ghanmi, M., El Mansouri, L., 2014: Ethnopharmacological study of anti-diabetic medicinal plants used in the Middle-Atlas region of Morocco (Sefrou region). Int. J. Pharma Res. Health Sci. 2, 75-79.

Carrió, E., Vallès, J., 2012: Ethnobotany of medicinal plants used in Eastern Mallorca (Balearic Islands, Mediterranean Sea). J. Ethnopharmacol. 141, 1021-1040.

Chaudhuri, R.K., Sticher, O., 1981: New iridoid glucosides and a lignan diglucoside from *Globularia alypum* L. Helv. Chim. Acta 64, 3-15.

Dai, J., Mumper, R.J., 2010: Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313-7352.

Deng, S., West, B.J., Jensen, C.J., 2013: UPLC-TPF-MS characterization and identification of bioactive iridoids in *Cornus mas* fruit. J. Anal. Methods Chem. 2013, 1-7.

Fehri, B., Aiache, J.M., 2010: Effects of *Globularia alypum* L. on the gastrointestinal tract. J. Nat. Prod. 3, 141-146.

Hammiche, V., Maiza, K., 2006: Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. J. Ethnopharmacol. 105, 358-367.

ICH, Validation of Analytical Procedures: Text and Methodology, 2005: ICH-Q2 (R1), International Conference on Harmonization: Geneva.

Jamieson, M.A., Bowers, M.D., 2010: Iridoid glycoside variation in the invasive plant Dalmatian toadflax, *Linaria dalmatica* (Plantaginaceae) and sequestration by the biological control agent, *Calophasia lunula*. J. Chem. Ecol. 36, 70-79.

Jensen, S.R., 1991: Plant iridoids, their biosynthesis and distribution in angiosperms. In: Harborne, J.B., Tomas-Barberan, F.A. (Eds.), Ecological chemistry and biochemistry of plant terpenoids, 133-158. Clarendon Press, Oxford.

Jurišić, R., Debeljak, Ž., Vladimir-Knežević, S., Vuković, J., 2004: Determination of aucubin and catalpol in *Plantago* species by micellar electrokinetic chromatography. Z. Naturforsch. C 59, 27-31.

Kirmizibekmez, H., Çalış, İ., Akbay, P., Sticher, O., 2003: Iridoid and bisiridoid glycosides from *Globularia cordifolia*. Z. Naturforsch. C 58, 337-341.

Kumar, V., Sood, H., Sharma, M., Chauhan, R.S., 2013: A proposed biosynthetic pathway of picrosides linked through the detection of biochemical intermediates in the endangered medicinal herb *Picrorhiza kurroa*. Phytochem. Anal. 24, 598-602.

Li, C.M., Zhang, X.L., Xue, X.Y., Zhang, F.F., Xu, Q., Liang, X.M., 2008: Structural characterization of iridoid glucosides by ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry. Rapid Commun. Mass Spectrom. 22, 1941-1954.

Marak, H.B., Biere, A., Van Damme, J.M.M., 2002: Systemic, genotype-specific induction of two herbivore-deterrent iridoid glycosides in *Plantago lanceolata* L. in response to fungal infection by *Diaporthe adunca* (Rob.) Niessel. J. Chem. Ecol. 28, 2429-2448.

Merghache, S., Zerriouh, M., Merghache, D., Tabti, B., Djaziri, R., Ghalem, S., 2013: Evaluation of hypoglycaemic and hypolipidemic activities of globularin isolated from *Globularia alypum* L. in normal and streptozotocin-induced diabetic rats. J. App. Pharm. Sci. 3, 1-7.

Merzouki, A., Ed-derfoufi, F., Molero Mesa, J., 2000: Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterapia 71, 278-307.

Nieminen, M., Suomi, J., Van Nouhuys, S., Sauri, P., Riekkola, M.L., 2003: Effect of iridoid glycoside content on oviposition host plant choice and parasitism in a specialist herbivore. J. Chem. Ecol. 29, 823-844.

Rønsted, N., Göbel, E., Franzyk, H., Jensen, S.R., Olsen, C.E., 2000: Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides. Phytochemistry 55, 337-348.

Sari, M., Sarri, D., Hendel, N., Boudjelal., A., 2012: Ethnobotanical study of therapeutic plants used to treat arterial hypertension in the Hodna region of Algeria. Global J. Res. Med. Plants & Indigen. Med. 1, 411-417.

Sipahi, H., Becker, K., Gostner, J.M., Charehsaz, M., Kirmizibekmez, H., Schennach, H., Aydin, A., Fuchs, D., 2014: Effects of globularifolin on cell survival, nuclear factor-κB activity, neopterin production, tryptophan breakdown and free radicals *in vitro*. Fitoterapia 92, 85-92.

Taskova, R.M., Gotfredsen, C.H., Jensen, S.R., 2006: Chemotaxonomy of Veroniceae and its allies in the Plantaginaceae. Phytochemistry 67, 286-301.

Tong, H., Bell, D., Tabei, K., Siegel, M.M., 1999: Automated data massaging, interpretation, and E-mailing modules for high throughput open access mass spectrometry. J. Am. Soc. Mass Spectrom. 10, 1174-1187.

Tundis, R., Loizzo, M.R., Menichini, F., Statti, G.A., Menichini, F., 2008: Biological and pharmacological activities of iridoids: recent developments. Mini Rev. Med. Chem. 8, 399-420.

Tundis, R., Bonesi, M., Menichini, F., Loizzo, M.R., Conforti, F., Statti, G., Pirisi, F.M., Menichini, F., 2012a: Antioxidant and anti-cholinesterase activity of *Globularia meridionalis* extracts and isolated constituents. Nat. Prod. Commun. 7, 1015-1020.

Tundis, R., Peruzzi, L., Colica, C., Menichini, F., 2012b: Iridoid and bisiridoid glycosides from *Globularia meridionalis* (Podp.) O. Schwarz aerial and underground parts. Biochem. Syst. Ecol. 40, 71-74.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A., 1972: Flora Europaea (Vol. 3). Cambridge University Press.

Viljoen, A., Mncwangi, N., Vermaak, I., 2012: Anti-inflammatory iridoids

of botanical origin. Curr. Med. Chem. 19, 2104-2127.

West, B.J., Uwaya, A., Isami, F., Deng, S., Nakajima, S., Jensen, C.J., 2014: Antiglycation activity of iridoids and their food sources. Int. J. Food Sci. Article ID 276950. http://dx.doi.org/10.1155/2014/276950.

Wieffering, J.H., 1966: Aucubinartige Glucoside (Pseudoindikane) und verwandte Heteroside als systematische Merkmale. Phytochemistry 5, 1053-1064.

Wink, M, 2003: Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64, 3-19.

Won, T.H., Ryu, S.K., Kang, S.S., Shin, J., 2010: Simultaneous analysis of bioactive metabolites from *Rehmannia glutinosa* by HPLC-DAD-MS/MS. Nat. Prod. Sci. 16, 116-122.

Xu, J., Wu, J., Zhu, L.Y., Shen, H., Xu, J.D., Jensen, S.R., Jia, X.B., Zhang, Q.W., Li, S.L., 2012: Simultaneous determination of iridoid glycosides, phenethylalcohol glycosides and furfural derivatives in Rehmanniae Radix by high performance liquid chromatography coupled with triple-quadrupole mass spectrometry. Food Chem. 135, 2277-2286.

Zennaki, S., Krouf, D., Taleb-Senouci, D., Bouchenak, M., 2009: *Globularia alypum* L. lyophilized methanolic extract decreases hyperglycemia and improves antioxidant status in various tissues of streptozotocin-induced diabetic rats. J. Complement Integr. Med. 6, Article 34.

Zhu, J., Cole, R.B., 2000: Formation and decompositions of chloride adduct ions, $[M + Cl]^-$, in negative ion electrospray ionization mass spectrometry. J. Am. Soc. Mass Spectrom. 11, 932-941.

Address of the corresponding author:
Maja Crkvenčić, Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Schrottova 39, HR-10000 Zagreb, Croatia.
E-mail: mcrkvencic@pharma.hr