

Chemical Profile, Radical Scavenging and Cytotoxic Activity of Yellow Gentian Leaves (*Gentianeae luteae folium*) Grown in Northern Regions of Montenegro

Jasmina Balijagić^a, Teodora Janković^{b*}, Gordana Zdunić^b, Jelena Bošković^c, Katarina Šavikin^b, Dejan Godevac^d, Tatjana Stanojković^e, Miodrag Jovančević^a and Nebojša Menković^b

^aBiotechnical Faculty, Center for Continental Fruit-growing, Sandžadžka BB, 84000 Bijelo Polje, Montenegro

^bInstitute for Medicinal Plant Research, Tadeuša Koščuška 1, 11000 Belgrade, Serbia

^cFaculty of Biofarming, Megatrend University, Belgrade, Maršala Tita 34, 24300 Bačka Topola, Serbia

^dInstitute for Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia

^eInstitute of Oncology and Radiology of Serbia, University of Belgrade, Pasterova 14, 11000, Belgrade, Serbia

tjankovic@mocbilja.rs

Received: July 19th, 2012; Accepted: September 21st, 2012

LC-ESI-MS and HPLC were used for the identification of the constituents from *G. lutea* leaves collected at different localities, as well as for quantification of the main compounds. Seven secoiridoids, five C-glucoflavones and three xanthenes, were identified. Swertiamarin derivatives, namely eustomorusside (**2**), eustomoside (**3**) and septemfidoside (**5**), were detected in *G. lutea* for the first time. Concentrations of five constituents (swertiamarin, gentiopicrin, isovitexin, mangiferin and isogentisin) were determined. The relationship between concentrations of γ -pyrones and altitude was observed with statistically significant correlation ($r = 0.94$). The extracts were also evaluated for their content of total phenolics, and antiradical and cytotoxic activities. The total phenolics content ranged from 7.7 to 12.7 mg GAE/g, and the IC₅₀ values for DPPH radical scavenging activity varied between 0.45 to 2.02 mg/mL. The leaf extract exhibited moderate cytotoxic effects toward HeLa cells with an IC₅₀ value of 41.1 μ g/mL, while gentiopicrin, mangiferin and isogentisin exerted strong activity against HeLa cells, with IC₅₀ values ranging from 5.7 to 8.8 μ g/mL. The results confirm the traditional usage of *G. lutea* leaves and also suggest their possible utilization as hepatoprotective, hypoglycemic and anti-inflammatory agents.

Keywords: *Gentiana lutea* leaves, Secoiridoids, Flavonoids, Cytotoxicity.

Leaves of yellow gentian (*Gentiana lutea* L., Gentianaceae) are far less used in folk medicine than roots, which have a long tradition of usage as a bitter tonic in gastrointestinal ailments in Central and Southeast Europe, particularly among populations of the mountain regions [1]. There are reports that the leaves are used as a substitute for gentian root in Serbia and some other countries of the Central Balkans [1,2]. In folk medicine of Belarus and Uzbekistan the leaves are used as an appetite stimulant and against stomach and intestinal colic [3]. Leaf extracts also possess hepatoprotective properties, stimulating the liver and repairing its structure [4]. There are also reports that leaves are used in conditions connected with malignant diseases of the digestive tract [5].

Several studies report the phytochemical composition of the aerial parts of *G. lutea* [6,7], and one describes the simultaneous determination of secoiridoids, flavonoids and xanthenes by HPLC [8]. Iridoid glycosides (gentiopicrin and swertiamarin), flavonoids (isoorientin and isovitexin), and xanthenes (mangiferin, isogentisin, isogentisin-3-O-primeveroside) have been detected previously. In the present study, LC-ESI-MS and HPLC techniques were used for the comprehensive analysis of the leaves of *G. lutea* collected from different habitats in the mountains of Montenegro. Extracts were also evaluated for their antiradical and cytotoxic activities. Altogether, methanol extracts of fifteen *G. lutea* samples from various locations were analyzed. Identification of the compounds was based on chromatographic and mass spectral information, and the 15 identified compounds (Figure 1) are listed in Table 1.

Among the analyzed samples, two chromatographic patterns could be observed. Samples 1-9 and 12-15 had more complex chromatographic profiles, with 15 characteristic peaks, whereas samples 10 and 11 (mountain Sinjavina) showed 10 major peaks. The major difference between the samples was in the secoiridoid group. All analyzed samples contained loganic acid (**4**) and gentiopicrin (**9**). Samples 1-9 and 12-15 were characterized by the presence of eustomorusside (**2**), eustomoside (**3**), septemfidoside (**5**) and swertiamarin (**6**), whereas samples 10-11 contained only sweroside (**16**) in addition. According to the literature, swertiamarin (**6**) and gentiopicrin (**9**) have been previously reported in *G. lutea* leaves [8]. It should be noted that swertiamarin derivatives, namely eustomorusside (**2**), eustomoside (**3**) and septemfidoside (**5**), were detected in *G. lutea* for the first time. These compounds have limited distribution in the genus *Gentiana*, having been found only in *G. septemfida* and *G. olivieri* until now [9, 10]. Such a secoiridoid profile could support the traditional usage of *G. lutea* leaves as a substitute for its roots.

The polyphenol composition of *G. lutea* reported in our study is in accordance with previous reports [6,8,11]. The xanthenes mangiferin (**10**), gentioside (**14**) and isogentisine (**15**) were present in all analyzed samples, thus indicating the common xanthenes pattern. However, some variation in flavonoid profile was observed between the studied samples. Samples 1-9 and 12-15 contained isoorientin (**12**), isovitexin (**13**), and their O-heterosides isoorientin-4'-O-glucoside (**7**), isosaponarin (**8**) and isoorientin-2''-O-glucoside (**11**), while in samples 10 and 11, compounds **7** and **8** were not detected.

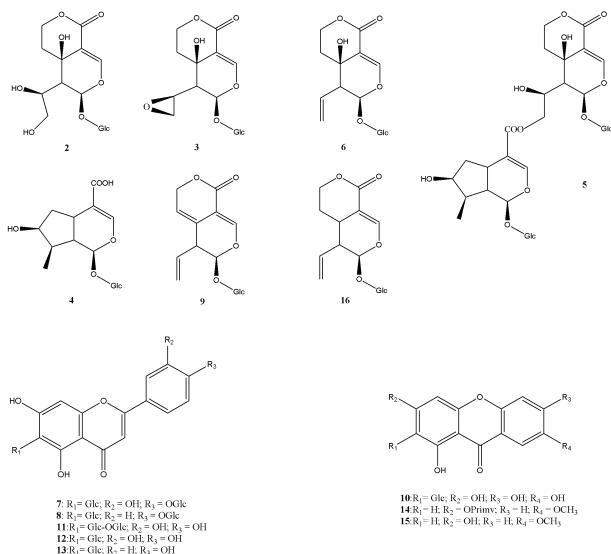


Figure 1: Chemical structures of the identified compounds (2-16) in *G. lutea* leaves.

Table 1: HPLC-DAD and LC-MS data of the constituents from *G. lutea* leaves.

Peak	t _R (min)	Compound	DAD		MS		
			λ _{max} (nm)		Species	Mass	Molecular formula
1	1.7	Unknown	228		M-H	342.1320	
2	2.9	Eustomorusside	240		M-H	408.1406	C ₁₆ H ₂₄ O ₁₂
3	6.1	Eustomoside	236		M-H	390.1202	C ₁₆ H ₂₂ O ₁₁
4	7.6	Loganic acid	238		M-H	376.1367	C ₁₆ H ₂₄ O ₁₀
5	9.8	Septemfidoside	240		M-H	826.2535	C ₃₂ H ₄₆ O ₂₁
6	10.2	Swertiamarin	238		M-H	374.1218	C ₁₆ H ₂₂ O ₁₀
7	11.2	Isoorientin-4'-O-glucoside	270, 338		M-H, 2M-H	610.1538	C ₂₇ H ₃₀ O ₁₆
8	11.5	Isosaponarin	270, 326		M-H, 2M-H	594.1589	C ₂₇ H ₃₀ O ₁₅
9	12.6	Gentiopictin	242, 274		M-H	356.1111	C ₁₆ H ₂₀ O ₉
10	12.8	Mangiferin	240, 258, 318, 366		M-H	422.0863	C ₁₉ H ₁₈ O ₁₁
11	19.6	Isoorientin-2"-O-glucoside	270, 350		M-H, 2M-H	610.1543	C ₂₇ H ₃₀ O ₁₆
12	20.8	Isoorientin	270, 350		M-H	448.1012	C ₂₁ H ₂₀ O ₁₁
13	23.5	Isovitexin	270, 336		M-H	432.1060	C ₂₁ H ₂₀ O ₁₀
14	26.9	Gentioside	236, 260, 302, 372		M-H	552.1483	C ₂₃ H ₂₈ O ₁₄
15	31.6	Isogentisin	234, 258, 310, 370		M-H	258.0531	C ₁₄ H ₁₀ O ₅
16	14.0	Sweroside	246		M-H	358.1269	C ₁₆ H ₂₂ O ₉

The results of the analysis of polyphenol compounds are of interest taking into account that flavonoids and xanthenes represent a group of secondary metabolites with various biological activities. The main C-glucoflavone, isoorientin, exhibited hepatoprotective, hypoglycemic, antihyperlipidemic, antiinflammatory and antioxidative effects [12-15]. The xanthone, mangiferin, the main constituent of some plants and extracts used in traditional medicine in many parts of the world, has been reported as a hepatoprotective, analgesic, anti-inflammatory and antioxidant agent [16-18]. Our previous studies reported antitubercular, antimicrobial and radioprotective activity of the aerial parts, as well as the polyphenol constituents (mangiferin and isogentisin) of *G. lutea* [19-21]. The main secoridoid, swertiamarin, has also been reported to possess hepatoprotective and antioxidant properties [22].

HPLC was used for the quantification of some constituents of *G. lutea* leaf extracts, and the results are summarized in Table 2. In all samples, swertiamarin (6) was present in higher concentration than gentiopictin (9). These compounds showed the broadest deviation among all the quantified compounds, differing from 1.3 to 34.7 mg/g (6), and 0.7 to 15.2 mg/g (9). As pointed out earlier, swertiamarin (6) was not detected in samples 10 and 11, which contained sweroside (16) instead. The xanthenes mangiferin (10)

Table 2: Contents of the secondary metabolites (mg/g DW) of *G. lutea* leaves.

Sample	6	9	10	13	15
1	9.9 ± 0.5	5.9 ± 0.3	3.4 ± 0.4	2.0 ± 0.09	0.5 ± 0.05
2	4.6 ± 0.8	4.0 ± 0.3	3.6 ± 0.3	0.6 ± 0.1	0.2 ± 0.08
3	2.7 ± 0.2	0.8 ± 0.09	3.2 ± 0.3	0.8 ± 0.1	0.3 ± 0.09
4	34.7 ± 2.9	3.3 ± 0.2	2.2 ± 0.2	0.7 ± 0.06	0.1 ± 0.02
5	11.1 ± 1.1	4.5 ± 0.4	7.3 ± 0.9	2.8 ± 0.2	0.5 ± 0.03
6	26.3 ± 0.09	0.7 ± 0.09	3.6 ± 0.4	0.8 ± 0.09	1.2 ± 0.01
7	1.3 ± 2.1	2.4 ± 0.04	4.0 ± 0.3	0.6 ± 0.07	0.2 ± 0.2
8	33.1 ± 0.1	7.1 ± 0.3	4.0 ± 0.2	2.4 ± 0.09	0.2 ± 0.05
9	10.1 ± 3.0	3.9 ± 0.6	2.5 ± 0.3	1.1 ± 0.2	0.6 ± 0.02
10	n.d.	15.2 ± 1.5	4.8 ± 0.5	2.8 ± 0.2	2.8 ± 0.2
11	n.d.	11.6 ± 1.2	4.4 ± 0.4	2.1 ± 0.2	6.1 ± 0.5
12	2.4 ± 1.2	0.9 ± 0.3	3.0 ± 0.3	0.3 ± 0.05	0.6 ± 0.03
13	11.9 ± 0.2	3.2 ± 0.08	2.3 ± 0.2	0.4 ± 0.02	0.4 ± 0.05
14	4.0 ± 0.4	3.5 ± 0.4	3.7 ± 0.3	0.5 ± 0.03	0.6 ± 0.07
15	7.0 ± 0.6	3.3 ± 0.3	3.4 ± 0.3	0.8 ± 0.8	0.1 ± 0.07

Legend: 6, swertiamarin; 9, gentiopictin; 10, mangiferin; 13, isovitexin; 15, isogentisin; n.d., not detected.

and isogentisin (15) were less variable in concentration, ranging from 2.2 – 7.3 mg/g and 0.1 to 6.1 mg/g, respectively. The amount of the C-glucoflavone isovitexin (13) was the most consistent (0.3 – 2.8 mg/g) among the samples. Our results show that the chemical profile of *G. lutea* leaves is very similar to that of the aerial parts of *G. olivieri* [10, 23], which are used in southeast Anatolia as a remedy for lowering blood glucose level in type-2 diabetes and as a component in a preparation for anaemia [24].

Table 3: The contents of UV-absorbing compounds in *G. lutea* leaves growing at different altitudes (L1-L8).

	L7	L1	L4	L8	L5	L6	L3
Altitude (m)	1450	1531	1550	1580	1620	1710	1775
10 (mg/g)	2.97	3.63	3.60	3.72	3.96	4.76	7.30
Σ 10+13+15 (mg/g)	3.87	5.83	5.61	4.79	6.61	10.38	10.55

It could be observed that the amount of mangiferin was larger in plants growing at higher altitudes (Table 3). A similar trend was noticed regarding γ-pyrone (xanthenes and flavonoids); their amount in plants collected from Strmenica (L3, sample 5, 1775 m) was 2.7-fold higher than that from Kobilja glava (L7, sample 12, 1450 m). Statistically significant correlation was observed between concentrations of γ-pyrone and mangiferin, and altitude ($r = 0.94$ and $r = 0.90$, respectively, at $p < 0.05$).

Our results are in accordance with previous findings that the contents of mangiferin and other UV-absorbing compounds are greater in plants growing at higher altitudes [25, 26].

The total phenolic content and DPPH radical-scavenging activity of leaves of *G. lutea* collected from different localities are shown in Table 4. The total phenolics content ranged from 7.7 to 12.7 mg GAE/g, and IC₅₀ for DPPH radical scavenging activity varied between 0.45 to 2.02 mg/mL. The relationship between total phenolics content and antiradical activity (expressed as reciprocal value of the calculated IC₅₀) of extracts was observed with statistically significant correlation ($r = 0.673$, $p < 0.05$). Positive correlation ($r = 0.696$, $p < 0.05$) between the sum of γ-pyrone determined by HPLC and radical scavenging activity was also noticed.

Table 4: Total phenolic content and DPPH radical-scavenging activity of leaves of *G. lutea*.

Sample	Total phenolics (mg GAE/g)	Σ 10+13+15 (mg/g)	DPPH radical scavenging activity IC ₅₀ (mg/mL)
1	11.5 ± 0.1	5.83 ± 0.18	0.58 ± 0.03
2	11.0 ± 0.1	4.39 ± 0.16	0.76 ± 0.02
3	10.3 ± 0.1	4.22 ± 0.17	0.90 ± 0.01
4	11.9 ± 0.1	3.02 ± 0.69	1.27 ± 0.04
5	11.7 ± 0.5	10.55 ± 0.37	0.45 ± 0.02
6	10.6 ± 0.1	5.61 ± 0.17	0.60 ± 0.03
7	8.4 ± 0.1	4.74 ± 0.21	1.21 ± 0.03
8	12.7 ± 0.1	6.61 ± 0.12	0.63 ± 0.03
9	10.6 ± 0.1	4.20 ± 0.18	0.78 ± 0.19
10	12.3 ± 0.1	10.38 ± 0.31	0.59 ± 0.02
11	11.2 ± 0.1	12.73 ± 0.38	0.69 ± 0.02
12	9.4 ± 0.1	3.87 ± 0.14	0.91 ± 0.03
13	7.7 ± 0.1	3.12 ± 0.10	2.02 ± 0.07
14	12.0 ± 0.01	4.79 ± 0.17	0.87 ± 0.03
15	8.5 ± 0.03	4.35 ± 0.09	1.15 ± 0.03

The cytotoxic activity of *G. lutea* leaf extracts which showed the highest antiradical activity, as well as gentiopicrin, mangiferin and isogentisin, were tested against human malignant cell lines (HeLa, MCF7, PC3 and LS174 cells). Cisplatin was used as a positive control and showed the highest growth inhibitory potency to all tested cells. The *G. lutea* extract exhibited moderate cytotoxic effects toward HeLa cells with an IC₅₀ value of 41.1 µg/mL (Table 5). As for the other cell lines, leaf extracts showed no cytotoxicity. All of the tested compounds exerted strong activity against HeLa cells, with IC₅₀ values ranging from 5.71 to 8.82 µg/mL. Isogentisin showed moderate activity against MCF7, PC3 and LS174 cells, with similar IC₅₀ values of 36.3, 36.2 and 39.6 µg/mL, respectively. Gentiopicrin and mangiferin exhibited no cytotoxicity against these cell lines, all IC₅₀ values being higher than 200 µg/mL.

Table 5: *In vitro* cytotoxic activity (IC₅₀) of *G. lutea* leaf extracts and selected compounds (µg/mL).

Sample	HeLa	MCF7	PC3	LS174
Leaves	41.1 ± 1.5	>200	>200	>200
Gentiopicrin	5.7 ± 0.4	>200	>200	>200
Isogentisin	8.8 ± 0.9	36.3 ± 4.4	36.2 ± 1.1	39.6 ± 4.4
Mangiferin	7.3 ± 1.2	>200	>200	>200
Cisplatin*	0.7 ± 0.14	1.1 ± 0.1	1.4 ± 0.2	2.4 ± 0.3

There are only a few reports regarding the cytotoxic activity of *Gentiana* spp. or the compounds that were tested in our study [27,28]. To our knowledge, this is the first report of the potent cytotoxic activity of gentiopicrin, mangiferin and isogentisin against the HeLa cell line.

The present study reports a detailed phytochemical investigation of *G. lutea* leaves. Our results show that leaves contain secoiridoids similar to those in the roots, which indicate their usage as a bitter tonic. Leaves are also rich in polyphenol compounds, especially mangiferin and isoorientin, which are reported to exhibit various biological activities. Based on the content of the main active principles, it is reasonable to believe that the examined species could be also be used as a hepatoprotective, hypoglycaemic and anti-inflammatory agent. The results from this study might also help the better utilization of *G. lutea* aerial parts for therapeutic purposes, which could have a protective role for this endangered plant species. Promotion of this part of the plant would certainly reduce the pressure on the root for exploitation, which would directly affect the conservation of natural resources of this species.

Experimental

Plant material: Leaves of *Gentiana lutea* L. were collected at 8 localities (L1-L8) on 7 mountains in northern Montenegro: Bihor (1531 m alt., L1, samples 1-3), Durmitor (1390 m alt., L2, sample 4), Bjelasica (1775 m alt., L3, sample 5; 1450 m alt., L7, samples

12 and 13), Prošćen (1550 m alt., L4, samples 6 and 7), Ljubišnja (1620 m alt., L5, samples 8 and 9), Sinjavina (1710 m alt., L6, samples 10 and 11) and Gutavica (1580 m alt., L8, samples 14 and 15), during the time of flowering (from June to September 2009). Voucher specimens have been deposited in the herbarium at the Natural Museum of Montenegro, Podgorica.

Sample preparation: Air-dried plant material was powdered, precisely weighed (1.0 g) and extracted twice with 10 mL methanol in an ultrasonic bath for 30 min. For HPLC and LC-MS analyses, extracts were filtered through a 0.45 PTFE filter prior to injection.

Determination of total phenolics: The total phenolic content was estimated by Folin-Ciocalteu method as described by Waterman and Mole [29], with slight modifications. The results were expressed as mg of gallic acid equivalents per g dry weight (mg GAE/g DW). Triplicate measurements were taken and mean values were calculated.

HPLC-DAD analysis: Analyses were carried out on an Agilent 1200 RR HPLC instrument, with diode array detector and a reverse phase Zorbax SB-C18 (Agilent) analytical column (150 mm × 4.6 mm i.d.; 5 µm particle size). The mobile phase consisted of solvent A (1%, v/v, solution of orthophosphoric acid in water) and solvent B (acetonitrile), using an elution gradient as follows: 0-5 min, 98-90% A; 5-15 min, 90% A; 15-20 min, 90-85% A; 20-25 min, 85-70% A; 25-30 min, 70-40% A; 30-34 min, 40-0% A. Detection wavelengths were set at 260 and 320 nm, and the flow rate was 1 mL/min. The injection volume was 5 µL and the column temperature was maintained at 25°C. The amounts of the compounds were calculated using calibration curves. The results are presented as mg per g dry weight.

LC-MS/MS analysis: LC-ESI-MS analyses were performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC, using a RR Zorbax Eclipse Plus C18 column (1.8 µm, 150 × 4.6 mm). The mobile phase A was H₂O containing 0.2% HCOOH and mobile phase B was MeCN. The injection volume was 5 µL, and elution at 0.995 mL/min with gradient program (0-3.02 min 10-20% B; 3.02-6.03 min 20% B; 6.03-12.06 min 20-30% B; 12.06-18.09 min 30-70% B; 18.09-24 min 70-38% B; 24-30 min 38-99% B; 30-33 min 99% B; 33-34 min 99-5% B). Mass spectra were acquired using an Agilent ESI-MSD TOF. Drying gas (N₂) flow was 12 L/min; nebulizer pressure was 45 psig; drying gas temperature was 350°C. For ESI analysis, the parameters were: capillary voltage, 4000 V; fragmentor, 140 V; skimmer, 60 V; Oct RF V 250 V, for negative modes. The mass range was from 100 to 2000 *m/z*. The data was processed with Molecular Feature Extractor and Mass Profiler software.

DPPH radical scavenging activity: The free radical scavenging activity was carried out according to the procedures described previously [30, 31]. All test analyses were run in triplicate. Trolox was used as a positive control exhibiting IC₅₀ value of 6.1 µg/mL.

Cytotoxic activity: Based on the total phenolic content and antiradical activity, sample 5 was selected for investigation of cytotoxic activity on 4 human cancer cell lines. Human cervix adenocarcinoma HeLa cells, human breast cancer MCF7, human prostate cancer PC3, and human colon carcinoma LS174 cell lines were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). Cancer cell lines were maintained in the recommended Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 100 g/L heat-inactivated (56°C) foetal bovine serum (FBS), 3 mmol/L, L-glutamine, 100 mg/mL

streptomycin, 100 IU/mL penicillin and 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and adjusted to pH 7.2 with bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air/5% CO₂ (v/v) at 37°C. Stock solutions (100 mg/mL) of extract and compounds, made in DMSO, were dissolved in corresponding medium to the required working concentrations. Neoplastic HeLa (2000 cells per well), MCF7 (3000 cells per well), PC3 cells (5000 cells per well) and LS174 cells (7000 cells per well) were seeded into 96-well microtitre plates; 24 h later, after cell adherence, investigated compounds were added to the wells, and the final concentrations applied to target cells were

200, 100, 50, 25 and 12.5 µg/mL. The cultures were incubated for 72 h.

The effect of extract and compounds on cancer cell survival was determined 72 h after the addition of extract/compounds by the microculture tetrazolium test (MTT) according to Mosmann [32]. All experiments were made in triplicate.

Acknowledgments - The authors acknowledge their gratitude to the Ministry of Education, Science and Technological Development of Serbia for financial support, project number 46013.

References

- [1] Tucakov J. (1996) *Phytotherapy*. Rad, Belgrade, 1-717.
- [2] Menković N, Šavikin K, Tasić S, Zdunić G, Stešević D, Milosavljević S, Vinček D. (2011) Ethnobotanical study on traditional uses of wild medicinal plants in Prokletije mountains (Montenegro). *Journal of Ethnopharmacology*, **133**, 97-107.
- [3] Sokolov PD. (1990) *Plant resources of the USSR*. Leningrad, 1-328.
- [4] Nikolaev SM, Shagzheeva GA, Dargaeva TD, Bakuridze AS. (1986) Effect of infusion of the aerial parts of *G. lutea* on the secretory and enzymatic functions of the stomach. *Rastitel'nye Resursy*, **22**, 401-404.
- [5] Hartwell JL. (1968) Plants used against cancer. A survey. *Lloydia*, **31**, 71-170.
- [6] Hostettmann K, Bellmann G, Tabacchi R, Jacot-Guillarmod A. (1973) Contribution à la phytochimie du genre *Gentiana* III. Etude des composés flavoniques et xanthoniques dans les feuilles de *Gentiana lutea* L. *Helvetica Chimica Acta*, **56**, 3050-3054.
- [7] Bakuridze AD, Tsagareishvili NT, Dargaeva TD, Patudin AV, Berashvili TD. (1991) Chromatospectrophotometric determination of γ -pyrone substances contents in the above-ground parts of *Gentiana lutea* L. *Rastitel'nye Resursy*, **27**, 115-119.
- [8] Menković N, Šavikin-Fodulović K, Savin K. (2000) Chemical composition and seasonal variations in the amount of secondary compounds in *Gentiana lutea* leaves and flowers. *Planta Medica*, **66**, 178-180.
- [9] Çaliş I, Ersöz T, Chulia A, Rüedi P. (1992) Septemfidoside: a new bis-iridoid diglucoside from *Gentiana septemfida*. *Journal of Natural Products*, **55**, 385-388.
- [10] Takeda Y, Masuda T, Honda G, Takaishi Y, Ito M, Ashurmetov O, Khodzhimatov O, Otsuka H. (1999) Secoroid glycosides from *Gentiana olivieri*. *Chemical and Pharmaceutical Bulletin*, **47**, 1338-1340.
- [11] Bellmann G, Jacot-Guillarmod A. (1973) Contribution à la phytochimie du genre *Gentiana* I. Etude des composés flavoniques et xanthoniques dans les feuilles de *Gentiana lutea* L. *Helvetica Chimica Acta*, **56**, 284-294.
- [12] Orhan DD, Aslan M, Aktay G, Ergun E, Yesilada E, Ergun F. (2003) Evaluation of hepatoprotective effect of *Gentiana olivieri* herbs on subacute administration and isolation of active principle. *Life Sciences*, **72**, 2273-2283.
- [13] Sezik E, Aslan M, Yesilada E, Ito S. (2005) Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. *Life Sciences*, **76**, 1223-1238.
- [14] Küpeli E, Aslan M, Gürbüz I, Yesilada E. (2004) Evaluation of *in vivo* biological activity profile of isoorientin. *Zeitschrift für Naturforschung*, **59c**, 787-790.
- [15] Budzianowski J, Pakulski G, Robak J. (1991) Studies on antioxidative activity of some C-glycosylflavones. *Polish Journal of Pharmacology Pharmacy*, **43**, 395-401.
- [16] Das J, Ghosh J, Roy A, Sil P. (2012) Mangiferin exerts hepatoprotective activity against D-galactosamine induced acute toxicity and oxidative/nitrosative stress via Nrf2-NFκB pathways. *Toxicology and Applied Pharmacology*, **260**, 35-47.
- [17] Dar A, Faizi S, Naqvi S, Roome T, Zikr-ur-Rehman S, Ali M, Firdous S, Moin ST. (2005) Analgesic and antioxidant activity of mangiferin and its derivatives: the structure activity relationship. *Biological and Pharmaceutical Bulletin*, **28**, 596-600.
- [18] Garrido G, González D, Delporte C, Backhouse N, Quintero G, Núñez-Sellés A, Morales M. (2001) Analgesic and anti-inflammatory effects of *Mangifera indica* L. extract (Vimang). *Phytotherapy Research*, **15**, 18-21.
- [19] Menković N, Šavikin-Fodulović K, Čebedžić R. (1999) Investigation of the activity of *Gentiana lutea* extracts against *Mycobacterium bovis*. *Pharmaceutical and Pharmacological Letters*, **9**, 74-75.
- [20] Šavikin K, Menković N, Zdunić G, Stević T, Radanović D, Janković T. (2009) Antimicrobial activity of *Gentiana lutea* L. extracts. *Zeitschrift für Naturforschung*, **64c**, 339-342.
- [21] Menković N, Juranić Z, Stanojković T, Raonić-Stevanović T, Šavikin K, Zdunić G, Borojević N. (2010) Radioprotective activity of *Gentiana lutea* extract and mangiferin. *Phytotherapy Research*, **24**, 1693-1696.
- [22] Jaishree V, Badami S. (2010) Antioxidant and hepatoprotective effect of swertiamarin from *Enicostemma axillare* against D-galactosamine induced acute liver damage in rats. *Journal of Ethnopharmacology*, **130**, 103-106.
- [23] Ersöz T, Çaliş I. (1991) C-glucoflavones from *Gentiana olivieri*. *Hacettepe University Journal of Pharmacy*, **11**, 29-38.
- [24] Başer KHC, Honda G, Miki W. (1986) Herb drugs and herbalists in Turkey. *Studia culturae islamicae*, Tokyo.
- [25] Yang H, Ding C, Duan Y, Liu J. (2005) Variation of active constituents of an important Tibet folk medicine *Swertia mussoitii* Franch. (Gentianaceae) between artificially cultivated and naturally distributed. *Journal of Ethnopharmacology*, **98**, 31-35.
- [26] Murai Y, Takemura S, Takeda K, Kitajima J, Iwashina T. (2009) Altitudinal variation of UV-absorbing compounds in *Plantago asiatica*. *Biochemical Systematics and Ecology*, **37**, 378-384.
- [27] Fan H, Zang Y, Zhang Y, Zhang HF, Zhao Z, Hu JF. (2010) Triterpenoids and iridoid glycosides from *Gentiana dahurica*. *Helvetica Chimica Acta*, **93**, 2439-2447.
- [28] Xu M, Zhang M, Wang D, Yang CR, Zhang YJ. (2011) Phenolic compounds from the whole plants of *Gentiana rhodantha* (Gentianaceae). *Chemistry and Biodiversity*, **8**, 1891-1900.
- [29] Waterman PG, Mole S. (1994) *Analysis of Phenolic Plant Metabolites*. Blackwell Scientific Publication, Oxford.
- [30] Silva BA, Ferreres F, Malva JO, Dias ACP. (2005) Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chemistry*, **90**, 157-167.
- [31] Bigović D, Šavikin K, Janković T, Menković N, Zdunić G, Stanojković T, Đurić Z. (2011) Antiradical and cytotoxic activity of different *Helichrysum plicatum* flower extracts. *Natural Product Communications*, **6**, 819-822.
- [32] Mosmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, **65**, 55-63.