Chapter 14
Bioactive Secondary Metabolites in Several Genera of Gentianaceae Species from the Central Regions of the Balkan Peninsula

Katarina Šavikin, Ivana S. Aljančić, Vlatka E. Vajs, Slobodan M. Milosavljević, Milka Jadranin, Iris Đorđević and Nebojša R. Menković

Abstract The results are presented of phytochemical investigations, during the last decade, of some wild-growing species of the family Gentianaceae from Serbia and Montenegro. Some of the species investigated, members of the genera *Gentiana, Gentianella,* and *Swertia*, are endemic, and the emphasis in this report is on those exhibiting biological activities that could be regarded as a potential source of drugs. This review discusses more than fifty compounds, such as xanthones, secoiridoids, and C-glucoflavonoids.

14.1 Introduction

The process that leads from plants to bioactive pure constituents requires multi-disciplinary collaboration. This review summarizes the last two decades of results on Gentianaceae species of the rich Serbian and Montenegrin flora. In the search for

K. Šavikin · N.R. Menković (✉)
Institute for Medicinal Plant Research "Dr. Josif Pančić", Tadeuša Košćuška 1, Belgrade, Serbia
e-mail: nmenkovic@mocbilja.rs

I.S. Aljančić · V.E. Vajs · M. Jadranin
Center for Chemistry, Institute for Chemistry, Technology and Metallurgy, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia

S.M. Milosavljević
Faculty for Chemistry, University of Belgrade, Studentski trg 12–16, Belgrade, Serbia

I. Đorđević
Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja 18, Belgrade, Serbia

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biologically active and new compounds, targeted collection of wild-growing plants with special emphasis on endemics was based on chemotaxonomic and ethnomedicinal information of the corresponding genera. Combined efforts led to the isolation and structure elucidation of biologically active secondary metabolites, mostly belonging to three types of chemical structures, typical of the Gentianaceae, i.e., xanthones, flavone C-glycosides and secoiridoids (Šavikin-Fodulovic et al. 2002).

14.2 Genus *Gentiana*

Gentiana L. is a large cosmopolitan genus with about 400 species, occurring in alpine habitats of temperate regions of Asia, Europe, and the Americas. In the central regions of the Balkan Peninsula can be found 11 species of the genus *Gentiana*, such as *G. lutea*, *G. punctata*, *G. cruciata*, *G. asclepiadea*, *G. pneumonanthe*, *G. kochiana*, *G. dinarica*, *G. verna*, *G. tergestina*, *G. utriculosa*, and *G. nivalis* (Jovanović-Dunjić 1973). The best known is yellow gentian, *G. lutea* L., distributed in both Serbia and Montenegro.

14.2.1 Gentiana lutea *L. (Yellow Gentian)*

Gentiana lutea (Fig. 14.13a) is very popular as a stomachic as well as a component in preparations showing beneficial effects ingall bladder and liver diseases (Blumenthal 1998; Tasic et al. 2004). The roots and rhizome of *G. lutea* (*Gentianae radix*) are described in European Pharmacopoeia 7.0 (Ph. Eur. 7.0), (2011) as well as in many national pharmacopoeias (Pharmacopoea Yugoslavica 1984). The material is stabilized (unfermented), yellow colored, and very bitter. It is also used in traditional medicine, and in the course of his ethnomedicinal studies, the Serbian pharmacognosist Tucakov (1996) concluded that the yellow gentian root is among the most important remedies of the mountain inhabitants in Serbia. The fermented root, with a maroon-colored cross section, exhibiting a smell and taste like dried figs, is used for preparing alcoholic beverages (Tasic et al. 2004). Some studies showed that the root possesses cholagogue, anthelmintic, anti-inflammatory, and antimicrobial activity (Öztütk et al. 1998; Pontus et al. 2006; Menković et al. 1999; Šavikin et al. 2007). Underground organs of yellow gentian are characterized as having a diverse chemical composition, with secoiridoids as the main constituents. LC-ESI TOF MS chromatogram of *G. lutea* underground organs collected on Mountain Suvobor, Serbia, is presented in Fig. 14.1 with a tentative identification of the compounds in Table 14.1.

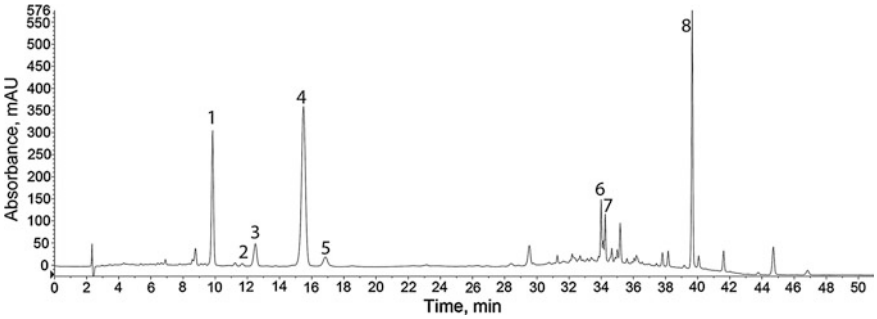
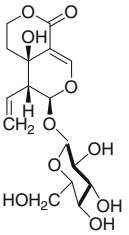


Fig. 14.1 LC-ESI TOF MS chromatogram of *G. lutea* underground organs

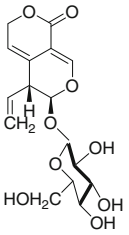
Table 14.1 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. lutea* underground organs

Peak no.	Retention time (min)	Compound
1	9.84	Loganic acid
2	11.69	Secologanosid
3	12.50	Swertiamarin
4	15.49	Gentiopicrin
5	16.85	Sweroside
6	34.00	Gentioside
7	34.25	Gentioside isomer
8	39.66	Isogentisin

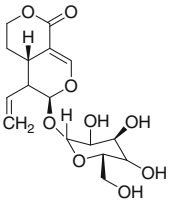
The bitter taste of the roots originates from secoiridoids. Among them, the most abundant are **1–3**.



1: Gentiopicrin

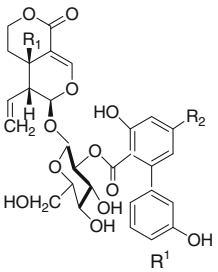


2: Swertiamarin



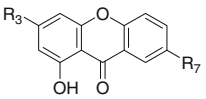
3: Sweroside

In addition, the roots contain biphenyl derivatives such amarogentin (**5**) located in bark of the roots, as well as the closely related amaropanin (**4**) and amaroswerin (**6**). Amarogentin is one of the most bitter natural compounds known (Wagner et al. 1983).



	R^1	R^2
4: Amaropanin	H	H
5: Amarogentin	H	OH
6: Amaroswerin	OH	OH

Another group of pharmacologically active constituents are xanthones **7–9**.



	R_3	R_7
7: Gentisin	OCH_3	OH
8: Isoogentisin	OH	OCH_3
9: Gentioside	$OGlc^6$-Xyl	OCH_3

The aerial parts of yellow gentian showed themselves to be very interesting and promising plant material (Menković et al. 2000). LC-ESI TOF MS chromatogram of *G. lutea* leaves collected from plants on the Mount Suvobor, Serbia, is presented in Fig. 14.2 with details of the compounds in Table 14.2.

The presence of **8** (Menković et al. 2000) and two flavonoid heterosides, **10** and **11**, was reported in the leaves of *G. lutea* by Hostettmann et al. (1973).

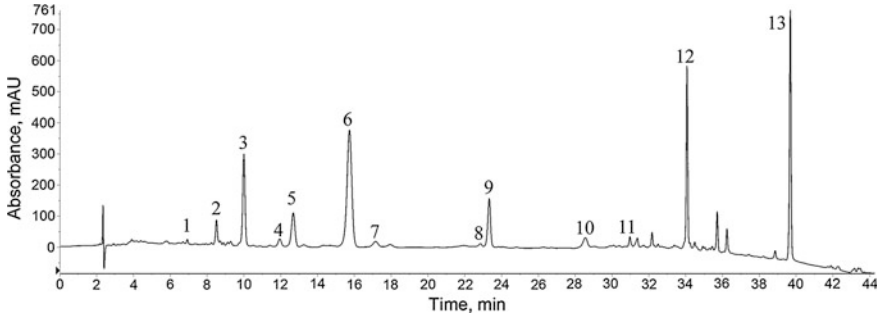
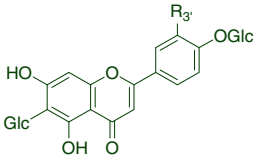


Fig. 14.2 LC-ESI TOF MS chromatogram of *G. lutea* leaves

Table 14.2 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. lutea* leaves

Peak no.	Retention time (min)	Compound
1	6.93	Eustomorussid
2	8.53	Secologanosid
3	10.00	Loganic acid
4	11.95	Septemfidosid
5	12.68	Swertiamarin
6	15.73	Gentiopicrin
7	17.17	Sweroside
8	22.84	Isosapoarin
9	23.33	Mangiferin
10	28.55	Isoorientin
11	30.97	Isovitexin
12	34.07	Gentioside
13	39.68	Isogentisin

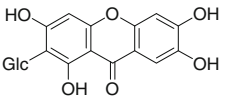


	$R_{3'}$
10: Isoorientin-4'-*O*-glucoside	OH
11: Isovitexin 4'-*O*-glucoside	H

From the aerial parts of *G. lutea*, three types of secondary metabolites, including xanthones, flavonoids, and secoiridoids were isolated in the laboratories of the authors of this chapter.

Xanthones

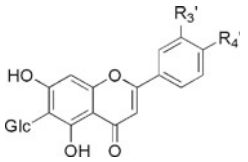
C-Glucoxanthone mangiferin (**12**) was accompanied by gentioside (**9**) and isogentisin (**8**).



12: Mangiferin

C-Glucoflavones

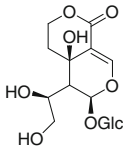
The aerial parts of *G. lutea* contained C-glucoflavones **13–15**:



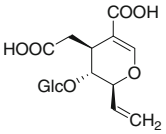
	$R_{3'}$	$R_{4'}$
13: Isoorientin	OH	OH
14: Isosaponarin	H	OGlc
15: Isovitexin	H	OH

Secoiridoids

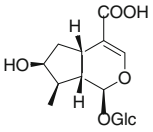
The aerial parts of *G. lutea* yielded seven secoiridoids, namely **1–3** and **16–19**:



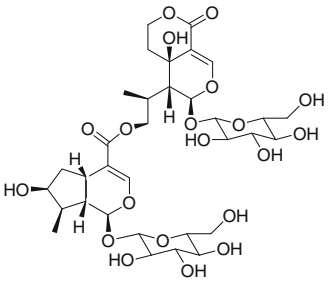
16: Eustomorussid



17: Secologanoside



18: Loganic acid



19: Septemfidoside

14.2.2 Gentiana dinarica *Beck*

Gentiana dinarica (Fig. 14.13b) is a rare, endemic, perennial species distributed in the Apennine and Balkan Peninsula. It grows on carbonate soils in subalpine and alpine regions at an altitude of 800–2300 m. According to authors' knowledge, it is not used in traditional medicine. In their investigations, plant materials (aerial parts and roots) were collected on the Mount Tara (1300 m), Serbia. A LC-ESI TOF MS chromatogram of *G. dinarica* leaves is presented in Fig. 14.3 with compounds identified in Table 14.3. The aerial parts of *G. dinarica* contained secoiridoids and C-glucoflavones, but xanthones were absent. Three C-glucoflavones, **13**, **15**, and **10**, were isolated from aerial parts.

A methanolic root extract contained three xanthones, namely **20–22** and two C-glucoflavones **13** and **10**. Xanthone aglycones were absent. Secoiridoid components isolated both from roots and from aerial parts were **1–3** and **5**. Considerable amount of compound **5** was found in the roots, which could be of interest.

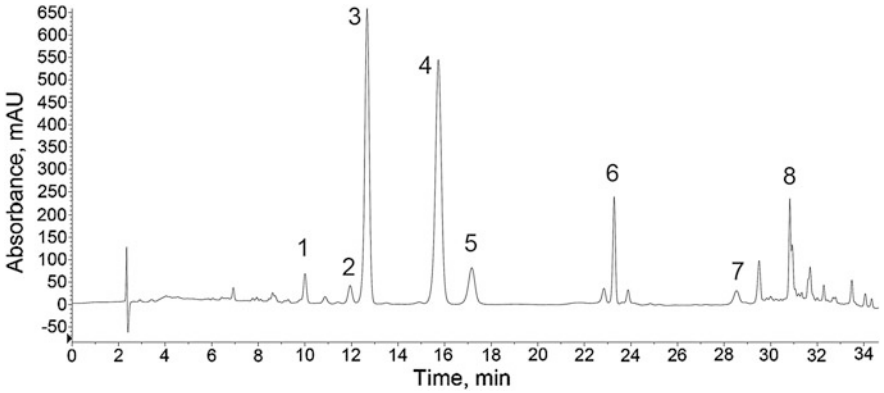
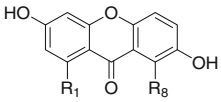


Fig. 14.3 LC-ESI TOF MS chromatogram of a methanolic extract of *Gentiana dinarica* leaves

Table 14.3 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. dinarica* leaves

Peak no.	Retention time (min)	Compound
1	10.01	Loganic acid
2	11.94	Secologanosid
3	12.67	Swertiamarin
4	15.73	Gentiopicrin
5	17.17	Sweroside
6	23.28	Isoorientin-3′-*O*-glucoside
7	28.53	Isoorientin
8	30.82	Isovitexin



	R_1	R_8	Plant part
20: Norswertianin-1-*O*-glucoside	OGlc	OH	Roots
21: Norswertianin-1-*O*-primeveroside	$OGlc^6$-Xyl	-OH	Roots
22: Norswertianin-8-*O*-primeveroside	-OH	$OGlc^6$-Xyl	Roots

14.2.3 Gentiana kochiana *E.P. Perrier and Songeon (*Syn. G. acaulis L.*)*

Gentiana kochina (Fig. 14.13c) is a small gentian native to Central and Southern Europe, from Spain east to the Balkans, growing especially in the mountainous regions, such as the Alps, Cevennes, and Pyrenees, at an elevation of 800–3000 m.

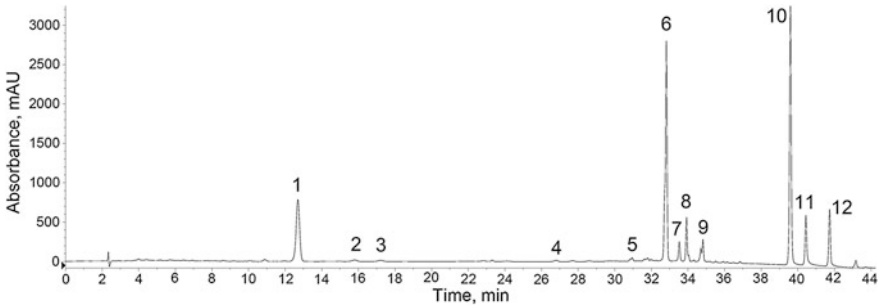


Fig. 14.4 LC-ESI TOF MS chromatogram of a methanolic extract of *G. kochiana* leaves

It grows in the mountain meadows with acidic silicate soil, inhabiting mostly south aspects. Extracts of this plant are used in traditional medicine in Tuscany (Italy) as an antihypertensive remedy (Manganelli et al. 2000).

The aerial parts and/or roots collected on the Mount Komovi, Montenegro, contained exclusively 1,3,7,8-tetraoxygenated xanthones, such as **23**, its 1-*O*-primeveroside (**24**), **25** and gentiacaulenin **26**, and their heterosides (**27–29**). Roots contain secoiridoids **1–3** and **18**. LC-ESI TOF MS chromatograms of *G. kochiana* leaves and roots are given in Figs. 14.4 and 14.5 and in Tables 14.4 and 14.5.

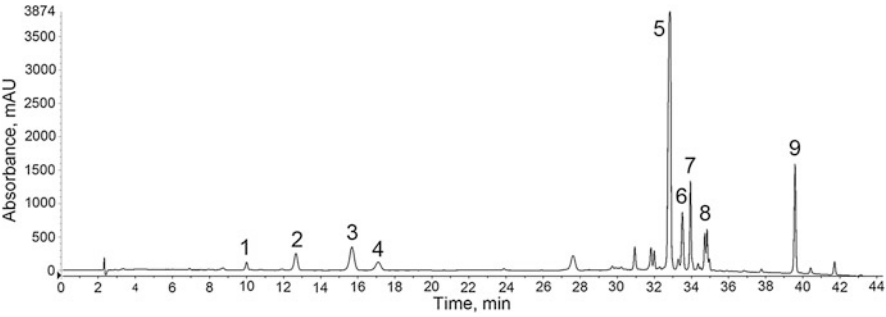


Fig. 14.5 LC-ESI TOF MS chromatogram of a methanolic extract of *G. kochiana* roots

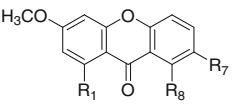
Table 14.4 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. kochiana* leaves

Peak no.	Retention time (min)	Compound
1	12.69	Swertiamarin
2	15.79	Gentiopicrin
3	17.25	Sweroside
4	26.79	Isoorientin-3′-*O*-glucoside
5	30.95	Isovitexin
6	32.82	Gentiacaulenin-1-*O*-primeveroside
7	33.55	Gentiacaulenin-1-*O*-glucoside
8	33.92	Decussatin-1-*O*-primeveroside
9	34.82	Gentiakochianin-7-*O*-primeveroside
10	39.60	Gentiacaulenin
11	40.44	Gentiakochianin
12	41.74	Decussatin

Table 14.5 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. kochiana* roots

Peak no.	Retention time (min)	Compound
1	10.00	Loganic acid
2	12.66	Swertiamarin
3	15.68	Gentiopicrin
4	17.10	Sweroside
5	32.84	Gentiakochianin-1-*O*-primeveroside
6	35.50	Gentiacaulenin-1-*O*-glucoside
7	33.94	Decussatin-1-*O*-primeveroside
8	34.84	Gentiakochianin-7-*O*-primeveroside
9	39.59	Gentiacaulenin

Xanthones



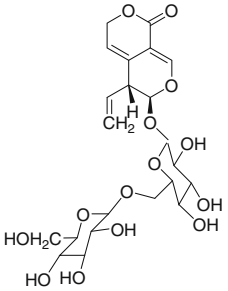
	R_1	R_7	R_8
23: Decussatin	OH	OCH_3	OCH_3
24: Decussatin-1-*O*-primeveroside	$OGlc^6$-Xyl	OCH_3	OCH_3
25: Gentiakochianin	OH	OH	OH
26: Gentiacaulein	OH	OH	OCH_3
27: Gentiokochianin-7-*O*-primeveroside	OH	$OGlc^6$-Xyl	OH
28: Gentiacaulein-1-*O*-primeveroside	$OGlc^6$-Xyl	OH	OCH_3
29: Gentiacaulein-1-*O*-primeveroside			

14.2.4 Gentiana asclepiadea *L. (Willow Gentian)*

Gentiana asclepiadea (Fig. 14.13d) is distributed in Central and Southern Europe from Schwarzwald to Olimp Mountain. It grows in high-mountain pastures and at the edges of forests to the subalpine tops of the mountains. It is also found in beech and spruce forests.

As the roots contain a similar chemical composition to yellow gentian, it is also used in traditional medicine as a remedy for poor appetite, for digestive problems, and for hepatitis A virus infections (Saric 1989; Mihailović et al. 2011). Although *G. asclepiadea* is very abundant in the flora of Serbia, comprehensive phytochemical and pharmacognostic investigations of plant material have not been carried out. From roots and rhizomes of *G. asclepiadea*, secoiridoid **1** was isolated together with its 6′-*O*-β-D-glucoside (**30**). Underground organs also contained sugars with gentianose being dominant. Unlike yellow gentian, the roots of willow gentian did not contain xanthone compounds. LC-ESI TOF MS chromatogram of *G. asclepiadea* roots is shown in Fig. 14.6 and in Table 14.6.

Secoiridoids



30: 6′-*O*-β-D-glucosylgentiopicroside

Aerial parts also contain sugars (fructose, glucose, gentiobiose, gentianose) similar to those in roots. The polyphenolic complex was diverse, being composed

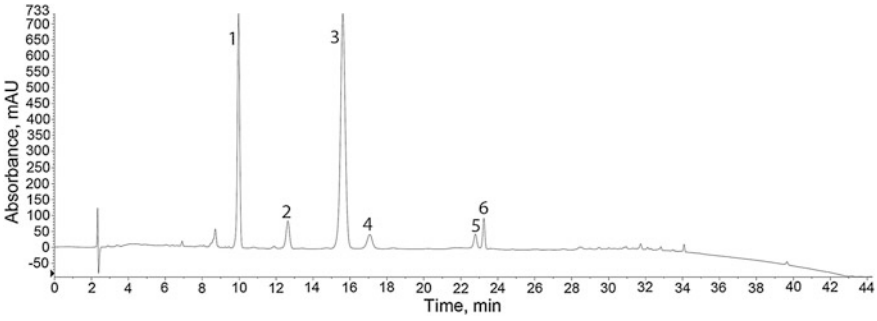


Fig. 14.6 LC-ESI TOF MS chromatogram of roots methanolic extract of *G. asclepiadea*

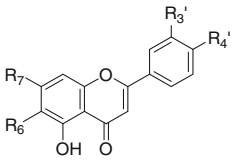
Table 14.6 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. asclepiadea* roots

Peak no.	Retention time (min)	Compound
1	9.97	Loganic acid
2	12.64	Swertiamarin
3	15.61	Gentiopicrin
4	17.07	Sweroside
5	22.79	Isosapoarin-*O*-glc
6	23.25	Isoorientin-3′-*O*-glc

of flavonoids and xanthones. LC-ESI TOF MS chromatogram of *G. asclepiadea* aerial parts is depicted in Fig. 14.7 and in Table 14.7.

C-Glucoflavones such as **11 and 13** and their glycosides **11**, **31**, **33**, and **10** were detected. The second group of γ-pyrone constituents were xanthone *C*-glucosides, with mangiferin (**12**) and its two *O*-glucosides **34** and **35** as the main constituents.

Flavonoids



	R_6	R_7	$R_{3'}$	$R_{4'}$
31: Isovitexin 2“-*O*-glucoside	Glc^2-glc	OH	H	OH
32: Isoorientin 2”-*O*-glucoside	Glc^2-glc	OH	OH	OH
33: Saponarin	Glc	OGlc	H	OH

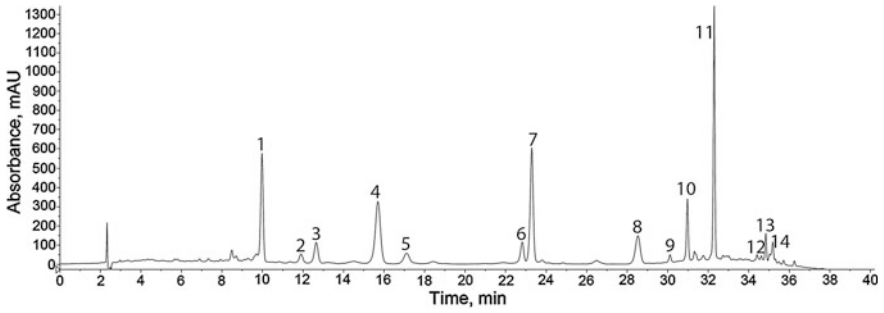
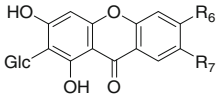


Fig. 14.7 LC-ESI TOF MS chromatogram of a methanolic extract of *G. asclepiadea* aerial parts

Table 14.7 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. asclepiadea* aerial parts

Peak no.	Retention time (min)	Compound
1	9.98	Loganic acid
2	11.91	Secologanosid
3	12.65	Swertiamarin
4	15.70	Gentiopicrin
5	17.11	Sweroside
6	22.72	Isosapoarin-*O*-glc
7	23.29	Mangiferin
8	28.53	Isoorientin
9	30.11	Isoscoparin
10	30.97	Isovitexin
11	32.29	Not identified
12	34.41	Gentiacauloside
13	34.81	Not identified
14	35.19	Not identified

Xanthones



	R_6	R_7
34: 6-*O*-β-D-glucosylmangiferin	OGlc	OH
35: 7-*O*-β-D-glucosylmangiferin	OH	OGlc

14.2.5 Gentiana utriculosa *L. (Bladder Gentian)*

Gentiana utriculosa (Fig. 14.13e) is an annual plant species found in Central Europe, mainly in the mountains of Italy and the Balkan Peninsula (Tutin 1972). According to our knowledge, it is not used in traditional medicine in the central regions of the Balkan Peninsula.

Analysis of the methanolic extract of the aerial parts showed the presence of secoiridoids, flavonoids, and xanthones. LC-ESI TOF MS chromatogram of *G. utriculosa* aerial parts is presented in Fig. 14.8 and in Table 14.8.

Previous phytochemical investigation of the *G. utriculosa* led to the isolation of xanthone aglycones **23** and **26**, as well as *C*-glycosides **12**, **13**, and **15** (Hostettmann and Jacot-Guillarmod 1977). Examination by the authors of *G. utriculosa* originating from the Mount Tara, West Serbia, revealed xanthone *O*-glycosides such as **24**, **36**, and **37**, and 4-C-glucoxanthones **12** and **38**, the latter being detected for the first time in the genus *Gentiana* (Janković et al. 2009).

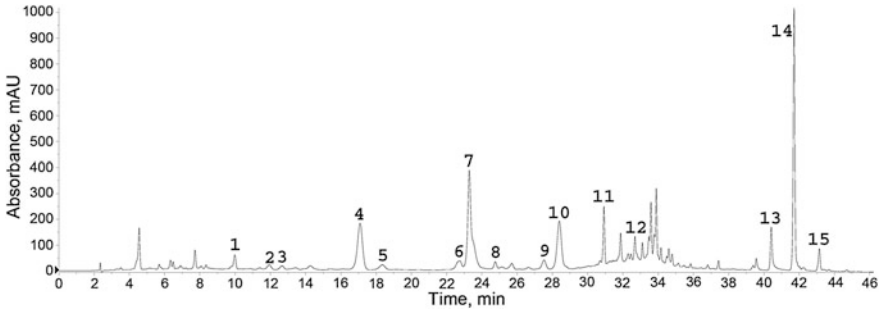
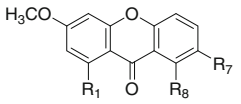
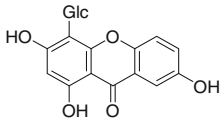


Fig. 14.8 LC-ESI TOF MS chromatogram of a methanolic extract of *G. utriculosa* aerial parts



	R_1	R_7	R_8
36: Isogentiakochianoside	$OGlc^6$-Xyl	OH	OH
37: Gentiakochianin-7-*O*-primeveroside	OH	$OGlc^6$-Xyl	OH

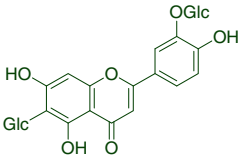


38: Lancerin

Table 14.8 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. utriculosa* aerial parts

Peak no.	Retention time (min)	Compound
1	9.98	Loganic acid
2	11.94	Secologanosid
3	12.67	Swertiamarin
4	17.09	Sweroside
5	18.36	Not identified
6	22.68	Isosapoarin
7	23.29	Mangiferin
8	24.76	Isoorientin-3′-*O*-glc
9	27.52	Lanceine
10	28.39	Isoorientin
11	30.92	Isovitexin
12	33.59	Gentopsid
13	33.89	Gentiakochianin
14	40.41	Gentiacaulenin
15	41.77	Decussatin

In addition, C-glucoflavones, **11** and **13** and **39**, were isolated from the same extracts.



39: Isoorientin-3'-*O*-glucoside

14.2.6 Gentiana punctata *L. (Spotted Gentian)*

Gentiana punctata (Fig. 14.13f) is a subalpine species of Southeast and Central Europe. The roots of spotted gentian are the officinal substitute for *G. lutea*, according to Yugoslav Pharmacopoeia IV (Ph. Yug. IV). As the roots and rhizomes contain bitter secoiridoids, they are also used in traditional medicine for the treatment of gastrointestinal tract diseases, as well as in the production of aperitifs (Tasić et al. 2004). *G. punctata* is an endangered species due to destructive harvesting (Šavikin-Fodulović et al. 2003).

Phytochemical investigations of *G. punctata* revealed significant differences in the chromatographic profiles of methanolic extracts of roots and aerial parts (Menković et al. 1998). In roots, a secoiridoid complex was dominant among secondary metabolites. Among them, **1** was the most abundant compound. However, flavonoids were not detected, but xanthone aglycon **7** was recorded. LC-ESI TOF MS chromatogram of *G. punctata* roots is presented in Fig. 14.9 and in Table 14.9.

The most abundant secoiridoid in the aerial parts was **17**, while **1** and **2** were also detected together with numerous flavonoid compounds. Xanthones were absent. LC-ESI TOF MS chromatogram of *G. punctata* aerial parts is presented in Fig. 14.10 and in Table 14.10.

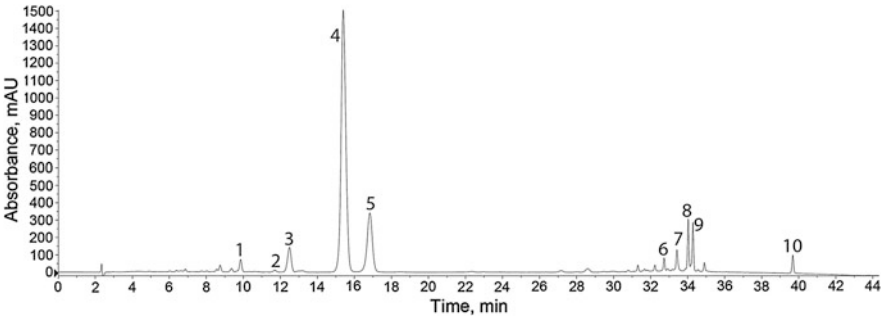


Fig. 14.9 LC-ESI TOF MS chromatogram of a methanolic extract of *G. punctata* roots

Table 14.9 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. punctata* roots

Peak no.	Retention time (min)	Compound
1	9.85	Loganic acid
2	11.70	Secologanosid
3	12.49	Swertiamarin
4	15.40	Gentiopicrin
5	16.83	Sweroside
6	32.73	Xanthone heteroside
7	33.42	Amarogentin
8	34.03	Gentioside
9	34.29	Gentioside isomer
10	39.68	Isogentisin

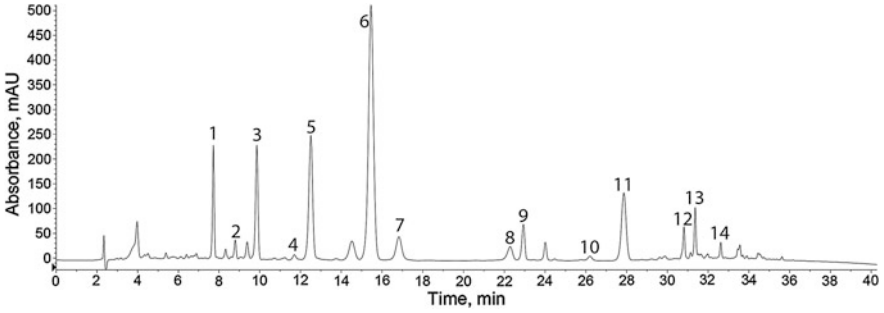


Fig. 14.10 LC-ESI TOF MS chromatogram of a methanolic extract of *G. punctata* aerial parts

Table 14.10 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *G. punctata* aerial parts

Peak no.	Retention time (min)	Compound
1	7.72	Eustomosid
2	8.33	Swertiamarin isomer
3	9.85	Loganic acid
4	11.70	Secologanosid
5	12.50	Swertiamarin
6	15.46	Gentiopicrin
7	16.83	Sweroside
8	22.29	Isosapoarin
9	22.94	Isoorientin-3′-*O*-glc
10	26.20	Isoorientin-3′-*O*-glc.-isomer
11	27.86	Isoorientin
12	30.81	Isovitexin
13	31.37	Isoscoparin
14	32.62	*Not identified*

14.3 Genus *Gentianella* Moench.

The genus contains about 250 species distributed over all continents. Six *Gentianella* species can be found in Serbia (Jovanović-Dunjić 1973). The main secondary metabolites of the genus are xanthones, flavone *C*-glucosides, and secoiridoids (Šavikin et al. 2010, Janković 2005). *Gentianella* species are not used in traditional medicine in the central regions of the Balkan Peninsula.

14.3.1 Gentianella austriaca *(A & J Kerner) Holub*

Gentianella austriaca is distributed in Southeast Europe and the Central Balkans. It grows on different bedrocks at altitudes of 950–2400 m in mountain and subalpine meadows and pastures, and rocky regions, even in the vegetation of glaciers (Jovanović-Dunjić 1973). The species, collected during flowering at the Mount Kopaonik in Serbia (at ca. 1750 m), was characterized by the presence of three classes of compounds typical for the Gentianaceae, such as secoiridoids, C-glucoflavones, and xanthones. The isolated and identified compounds in the aerial parts of *G. austriaca* were bellidifolin (**40**), demethylbellidifolin (**41**), corymbiferin (**42**), demethylbellidifolin-8-*O*-glucoside (**43**), bellidifolin-8-*O*-glucoside (swertianolin) (**44**), corymbiferin-1-*O*-glucoside (**45**), veratriloside (**46**), lanceoside (**47**), swertisin (**48**), campestroside (**49**), together with **1**, **12** and **13**. Compounds **46** and **47** have been found for the first time in the genus. The dominant compound isolated from the aerial parts was **40** and its 8-*O*-glycosyl derivatives. The roots also contained all the above-mentioned xanthones, except **49** and the flavonoids. LC-ESI TOF MS chromatogram of *G. austriaca* aerial parts is presented in Fig. 14.11 and in Table 14.11.

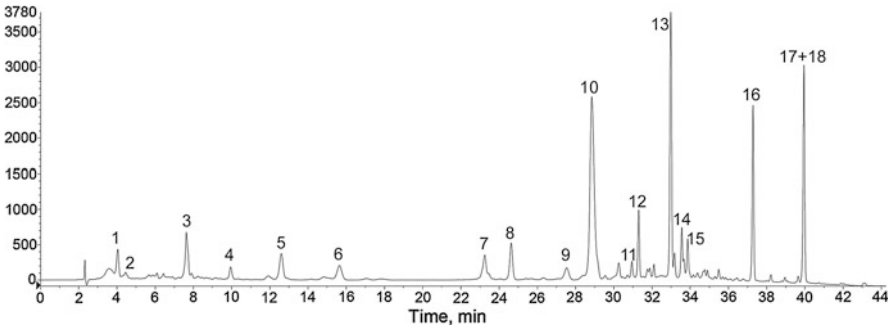
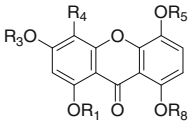


Fig. 14.11 LC-ESI TOF MS chromatogram of a methanolic extract of *Gentianella austriaca* aerial parts

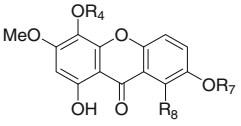
Table 14.11 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *Gentianella austriaca* aerial parts

Peak no.	Retention time (min)	Compound
1	4.04	Eustomorussid
2	4.45	Eustomosid
3	7.63	Campestroside isomer
4	9.95	Loganic acid
5	12.60	Swertiamarin
6	15.64	Gentiopicrin
7	23.25	Mangiferin
8	24.63	Campestroside
9	27.54	Lancerin
10	28.85	Demethylbellidifolin-8-*O*-glucoside
11	30.26	Isovitexin
12	31.30	Swertisin
13	32.98	Bellidifolin-8-*O*-glucoside
14	33.56	Corymbiferin-1-*O*-glucoside
15	33.90	Veratriloside
16	37.28	Demethylbellidifolin
17	39.90	Bellidifolin
18	39.95	Corymbiferin

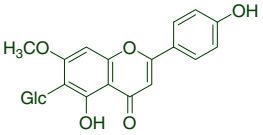
Xanthones



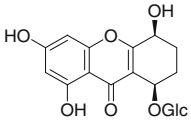
	R_1	R_3	R_4	R_5	R_8	Plant part
40: Bellidifolin	H	CH_3	H	H	H	Aerial
41: Demethylbellidifolin	H	H	H	H	H	Aerial
42: Corymbiferin	H	H	OMe	Me	H	Aerial
43: Demethylbellidifolin-8-*O*-glucoside	H	H	H	H	Glc	Aerial
44: Bellidifolin-8-*O*-glucoside	H	CH_3	H	H	Glc	Aerial
45 Corymbiferin-1-*O*-glucoside	Glc	H	OMe	Me	H	Aerial



	R_4	R_7	R_8	Plant part
46: Veratriloside	Me	Glc	H	Aerial
47: Lanceoside	Glc	Me	OH	Aerial



48: Swertisin



49: Campestroside

In the course of chemosystematic studies of the family Gentianaceae from Serbia and Montenegro, three additional *Gentianella* species were also collected at the time of flowering, namely *G. albanica* (Jav.) Holub and *G. crispata* (Vis.) Holub at the Mount Hajla, at ca. 1900 m, situated between Montenegro and Serbia (Kosovo), and *G. bulgarica* (Velen.) Holub on the slopes of the same mountain (at ca. 800 m). The HPLC/DAD (high-performance liquid chromatography with diode array detection) chromatograms of the MeOH (methanol) extracts of the aerial parts of these species indicated considerable similarity with *G. austriaca*, revealing almost the same constituents (Janković et al. 2005).

14.4 Genus *Swertia*

Due to the numerous pharmacological properties of its constituents, the genus *Swertia* has received considerable attention (Šavikin-Fodulovic et al. 2002; Šavikin et al. 2010). Some members of the genus, such as *S. japonica*, *S. chirata*, *S. hookeri*, *S. macrosperma*, *S. petiolata*, and *S. calycina*, have been used in traditional medicine in the Far East for many years. Among their active principles, xanthones (mostly 1,3,7,8- and 1,3,5,8-tetraoxygenated) have special significance because of their various biological activities, e.g., antidepressant, antileukemic, antitumor, antitubercular, choleretic, diuretic, antimicrobial, antifungal, anti-inflammatory, antiviral, cardiotonic, and hypoglycemic activities (Peres et al. 2000; Neerja et al. 2000).

Among the European *Swertia* species, only *S. perennis* (Fig. 14.13g) is recognized officially according to Flora Europaea, whereas a provisional status has been assigned to *S. punctata* Baumg (Tutin 1972). Tan and Vladimirov (2001) claimed that *S. punctata* is the precisely defined species. *S. punctata* is also described in flora of Serbia (Jovanović-Dunjić 1973), as the only species of the genus occurring in Serbia.

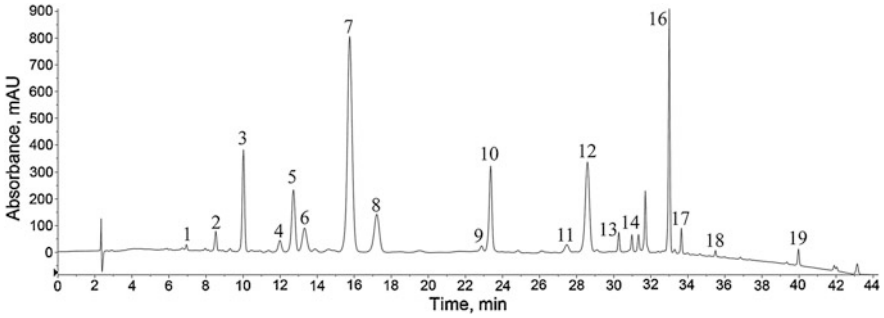


Fig. 14.12 LC-ESI TOF MS chromatogram of *S. punctata* leaves

14.4.1 Swertia Punctata *Baumg.*

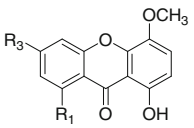
Swertia punctata Baumg. (Fig. 14.13h) is distributed in the Central Balkans. It grows in a zone of mountain wetland pastures, meadows, and turfs, mostly on silicate and serpentine substrates at altitude, between 1500 and 2000 m. Its population in Serbia is scarce and endangered, as reported recently in the Red Book of Serbian Flora (Jovanović 1999). Although several *Swertia* species are used extensively in traditional medicine, *S. punctata* is not mentioned in traditional medicine until the present times.

Methanol extracts have been analyzed of the aerial parts and roots of the *S. punctata* originating from Stara Planina, East Serbia (Menković et al. 2002). LC-ESI TOF MS chromatogram of *S. punctata* leaves is presented in Fig. 14.12 and in Table 14.12.

Apart from **12** and **13**, the compounds isolated from *S. punctata* are listed below:

1,3,5,8-Tetrasubstituted xanthones

In addition to **40** and **44**, the following 1,3,5,8-tetrasubstituted xanthones were identified:



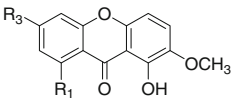
	R_1	R_3	Plant part
50: Isobellidifolin	OH	OH	Roots
51: Methylbellidifolin (swerchirin)	OH	OCH_3	Roots/aerial
52: Isobellidifolin-3-*O*-primeveroside	$OGlc^6$-Xyl	OH	Roots

Table 14.12 Tentatively identified components in the extract using LC-ESI TOF MS and UV data of *S. punctata* leaves

Peak no.	Retention time (min)	Compound
1	6.95	Eustomorussid
2	8.53	Secologanosid
3	10.02	Loganic acid
4	11.99	Septemfidosid
5	12.72	Swertiamarin
6	13.32	Homomangiferin
7	15.75	Gentiopicrin
8	7.22	Sweroside
9	22.87	Isosapoarin
10	23.36	Mangiferin
11	27.47	Isoorientin-2″-*O*-glucoside
12	28.58	Isoorientin
13	30.28	Swertisin
14	30.98	Isovitexin
15	31.71	Swertianol
16	33.00	Swerchirin
17	33.65	Not identified
18	35.49	Demethylbellidifolin
19	39.97	Bellidifolin

1,3,7,8-Tetrasubstituted xanthones

The roots of *S. punctata* afforded norswertianin-1-*O*-glucoside (**20**), as well as the following 1,3,7,8-tetrasubstituted xanthones (Menković et al. 2002):



	R_1	R_3	Plant part
53: Isoswertianin	OH	OH	Roots
54: Methylswertianin	OH	OCH_3	Roots
55: Methylswertianin-1-*O*-gentiobioside	$OGlc^6$-Glc	OCH_3	Roots

14.5 Biological Activity

Due to the diverse chemical composition and the presence of secoiridoids, xanthones, and C-glucoflavones, the numerous pharmacological activities have been demonstrated of Gentianaceae species (Jensen and Schripsema 2002; Neerja et al. 2000; Pinto et al. 2005; Šavikin et al. 2010).

Fig. 14.13 Flowers of some species of the family Gentianaceae (pictures taken from nature). **a** *Gentiana lutea*, **b** *G. dinarica*, **c** *G. kochiana*, **d** *G. asclepiadea*, **e** *G. urticulosa*, **f** *G. punctata*, **g** *Swertia perennis*, and **h** *S. punctata*

The bitter principles (secoiridoids), the usual constituents of the genus, stimulate secretion of gastric juices and bile, thus aiding appetites and digestion. *G. lutea*, as the officinal medicinal plant, was investigated extensively and activities, such as increasing salivation, appetite stimulation, and choleretic and immune-stimulating activity were reported (Öztütk et al. 1998, 2006). Gentiopicrin, a dominant compound in the secoiridoid complex, was indicated as the main active compound. Secoiridoids also exhibit several other biological activities. Swertiamarin and sweroside inhibited the growth of *Bacillus cereus, B. subtilis*, *Citrobacter freundii*

and *Escherichia coli*, swertiamarin was active against *Proteus mirabilis* and *Serratia marcescens*, while sweroside inhibited the growth of *Staphylococcus epidermidis* (Kumarasamy et al. 2003).

Methanolic extracts of flowers and leaves of *Gentiana lutea*, together with the isolated compounds mangiferin (**12**), isogentisin (**8**), and gentiopicrin (**1**), were used to investigate the antimicrobial activity. Both extracts and isolated compounds showed antimicrobial activity on a range of Gram-positive and Gram-negative bacteria as well as the yeast *Candida albicans* (Šavikin et al. 2009). The synergistic activity of the pure compounds may be responsible for the excellent antimicrobial effect of the extracts.

Gentiopicrin (**1**) has shown spasmolytic activity inhibiting, in a concentration-dependent manner, the spontaneous contractions of isolated guinea pig ileum. Contractions induced by histamine, acetylcholine, $BaCL_2$, and KCL on the ileum were also blocked significantly by this monoterpene glucoside, which suggests that this compound might be interfering with calcium influx into the smooth muscle cells (Rojas et al. 2000).

In a previous study, in vitro experiments showed that mangiferin (**12**) inhibited the cytotoxic action of ionizing irradiation (doses of 6 and 8 μGy) only on normal resting human PBMC (peripheral blood mononuclear cell), not stimulated for proliferation. Orally consumed *G. lutea* extract showed the potential to reduce the cytotoxic effect of irradiation on normal human immunocompetent cells PBMC of some healthy people, without changing the susceptibility of malignant cells to be destroyed by irradiation (Menković et al. 2010).

As reviewed by Neerja et al. (2000), bellidifolin (**40**), methylbellidifolin (**51**), methylswertianin (**54**), and mangiferin (**12**) isolated from the *Swertia* species exhibited various biological effects such as hypoglycemic, hepatoprotective, antituberculous, antioxidant, antimalarial, and anti-inflammatory activities. The xanthones, gentiacaulenin (**26**) and gentiakochianin (**25**), are responsible for antihypertensive activity, exerting a vasodilator action on in vitro aortic rings, probably linked to the blocking of the ryanodine-sensitive Ca++ channels (Chericioni et al. 2003).

Inhibition has been observed of type A and type B monoamine oxidases by a number of xanthones (Suzuki et al. 1980, 1981). Diethyl ether extracts of *G. kochiana* as well as gentiacaulenin (**26**) and gentiakochianin (**25**) were tested for CNS (central nervous system) pharmacological activity in rodents (Tomić et al. 2005). Extracts and **26** strongly inhibited rat microsomal MAO (monoamine oxidase) A. Examinations of behavior on mice showed that 10-day s.c. administration of the extract (20 mg/kg) decreased significantly immobility score in a forced swimming test and strongly inhibited ambulation and stereotypy in an open-field test. Studies suggest some antidepressant therapeutic potential of *G. kochiana* that is presumably connected to the action of **26**.

Xanthones **25** and **26** were also identified as the active principles responsible for in vitro antiglioma action of ether and methanolic extracts of *G. kochiana* (Isakovic et al. 2008). These compounds induced cell cycle arrest in G2/M and G0/G1 phases, respectively, in both C6 rat glioma and U251 human glioma cell lines. Both xanthones reduced mitochondrial membrane potential and increased the production

of reactive oxygen species in glioma cells, but only the effects of **25** were pronounced enough to cause caspase activation and subsequent apoptotic cell death. The assessment of structure-activity relationship showed that dihydroxylation at positions 7, 8 of the xanthonic nucleus is the key structural feature responsible for the ability of gentiakochianin to induce microtubule-associated G2/M cell blockage and apoptotic cell death in glioma cells.

Hudecová et al. (2012) showed that *G. asclepiadea* exerts antioxidant activity and enhances DNA repair of hydrogen peroxide- and silver nanoparticle-induced DNA damage. The same study also showed antioxidant, antigenotoxic, and biomodulatory effects of *G. asclepiadea* extracts on various cells (including lymphocytes and HEK 293 cells) exposed to different agents such as H_2O_2, Zeocin, and AgNPs (silver nanoparticles).

The aerial parts of *Gentianella austriaca* were evaluated for their antioxidative activity and protective properties on irradiated human peripheral blood lymphocytes in vitro (Leskovac et al. 2007). Aqueous ethanolic extracts showed protective effects, decreasing the incidence of radiation-induced micronuclei by 35.56 %, and significantly reduced lipid peroxidation for 30.88 %. The radioprotective effects of the water-soluble xanthones demethylbellidifolin (**41**), demethylbellidifolin-8-*O*-glucoside (**43**), bellidifolin-8-*O*-glucoside (**44**), and the flavonoid swertisin (**48**) were also reported (Jankovic et al. 2008). Among the compounds examined, the highest reduction by 27.92 % in the incidence of micronuclei was observed in irradiated lymphocytes treated with swertisin (**48**).

In addition, isovitexin (**15**), the co-occurring constituent of *G. lutea* leaves, exhibits considerable antioxidative and hypoglycemic effects. The potential antioxidative effect of the extract of the leaves of *G. lutea* could be assigned to xanthones and flavonoids, presumably mangiferin (**12**) and isoorientin **(13)**, respectively. It is well known (Rice-Evans et al. 1995) that the potential of flavonoids and xanthones to exert antioxidative effects is strongly dependent on their structure and the substituents of the heterocyclic and B rings. As far as the antiradical activity is concerned, the following structural features are essential:

(a) 3′,4′-Dihydroxy pattern (ring B)
(b) 2,3-double bond conjugated with keto group (ring A)
(c) 3,5-Dihydroxy substitution (ring A)
(d) Free 7-OH; it was established that glycosylation in positions 3, 5, and 7 reduces the activity.

The structure of isoorientin (**13**) fulfills most of the above conditions with *ortho*-3′,4′-OH groups, 5-OH and 7-OH, as well as a 2,3-double bond conjugated with the keto group. This could explain considerable antioxidative activity of **13**. 3′ and 4′-*O*-glucosides of **13**, lacking free *ortho*-3′,4′-OH groups that exhibit rather small inhibitory capacity (<1 %), (Burda and Oleszek 2001). In swertisin (**48**), free OH groups are only at C-4′ i C-5, whereas 7-OH is methylated, causing much lower activity in **48** (3 %) in comparison with that in **13** (66 %). At the same time, the considerable antioxidative activity of mangiferin (**12**) could be explained by the structural features similar to those in isoorientin (Krstić-Milošević 2008).

Based on the comparison of pharmacologic effects of the main components in the extracts of *G. lutea* leaves, one could expect antidiabetic, hepatoprotective, and anti-inflammatory effects. Thus, the continuation would be justified of the chemical investigation of this extract, aimed to discover a new source of drugs for use in phytotherapy. The use of yellow gentian leaves in the therapy instead of roots could have a protective effect on this species endangered by overexploitation. In addition, in the ethnomedicine of Turkey, as well as in the Indianmedicine Ayurveda, *Gentiana olivieri*, possessing a similar chemical profile to *G. lutea,* is used as tonic and bitter as well as for its antidiabetic, appetizer, and antipyretic effects (Sezik et al. 2005, Chopra et al. 2006, Mansoor and Malghani 1999).

Recently, Turkish authors have proved the hypoglycemic activity of the extracts of this species in which **13** was a dominant constituent, and at the same time, its hepatoprotective effect was evaluated (Orhan et al. 2003).

Similarly, in the Chinese province of Mongolia, the people use a plant "*gui-xincao*" in the form of a tea to cure colds, to clean blood by removing toxins and pathogens, as well as a diuretic (Min-Hui et al. 2010). This plant, identified as *Gentianella acuta* (Michaux.) Hulten, exhibits the chemical profile rather similar to that of *G. austriaca* with demethylbellidifolin, bellidifolin, and the corresponding heterosides being dominant.

Moreover, according to the chemical composition, *S. punctata* could be attractive as a source of medicinal raw material, but since its population is small, efforts are now concentrated on determining alternative ways for biomass production.

14.6 Experimental Design

14.6.1 Plant Material

For analysis, aerial parts and roots were collected from all species investigated. *G. lutea* was collected on the Mount Suvobor 750 m, Serbia; *G. dinarica* on the Mount Tara 1300 m, Serbia; *G. kochiana* on the Mount Komovi 2100 m, Montenegro; *G. asclepiadea* on the Mount Hajla 1900 m, Montenegro; and *G. utriculosa* on the Mount Tara (1100 m), Serbia. Four species of genus *Gentianella* were examined in the present studies. *G. albanica* and *G. crispata* were collected on the Mount Hajla at ca. 1900 m situated between Montenegro and Serbia (Kosovo), *G. bulgarica* on the slopes of the same mountain at ca. 800 m, and *G. austriaca* at Mount Kopaonik in Serbia at ca. 1750 m. *S. punctata* was collected on Stara mountain (1900 m), Serbia.

14.6.2 Chromatographic Techniques

A combination of different preparative chromatographic techniques was applied to isolate pure compounds. The analogous basic separation scheme, shown in

Fig. 14.14 for *Swertia punctata*, was applied to isolate pure compounds from all extracts examined.

The spectra were recorded with the following instruments: IR, Perkin-Elmer FT-IR spectrometer 1725 X; ^{1}H and ^{13}C NMR 1D and 2D NMR, Varian Gemini 2000 (200 MHz for ^{1}H), and Bruker AMX 500 (500 MHz for ^{1}H); UV, G113AA HP 8543 advanced UV–Vis spectrometer; DCIMS (150 eV, isobutane), Finnigan MAT mass spectrometer 8230, double focusing (BE geometry); ESIMS (a sample, dissolved in MeOH–H_2O, 1:1) Finnigan MAT 900, double focusing (EB geometry) equipped with a Finnigan MAT electrospray interface; optical rotations, Perkin-Elmer 141 MC polarimeter; melting points (not corrected), Boetius PHMK apparatus; analytical HPLC, Hewlett Packard HPLC model 1090, DAD detector (HP 1040), column, Lichrospher RP-18 (5 ì), 250 × 4 mm I.D. (Merck); preparative medium pressure chromatography (MPLC), Lobar column (silica gel Si 60, size A or B); dry-column flash chromatography (DCFC), silica gel Si 60; MN polyamide DC 6; TLC, 0.2 mm, silica gel 60 F 254 Merck, detection under UV or by heating after spraying with 50 % H_2SO_4; column chromatography (CC), polyamide-6-powder (polycaprolactam); Sephadex LH-20; elemental C,H-analysis, combustion method (Fig. 14.14).

14.6.2.1 LC-ESI TOF MS Analysis of MeOH Extracts

Air-dried plant material (0.5 g) was extracted with MeOH (10 ml) at room temperature for 48 h. The solvent was removed under vacuum at room temperature.

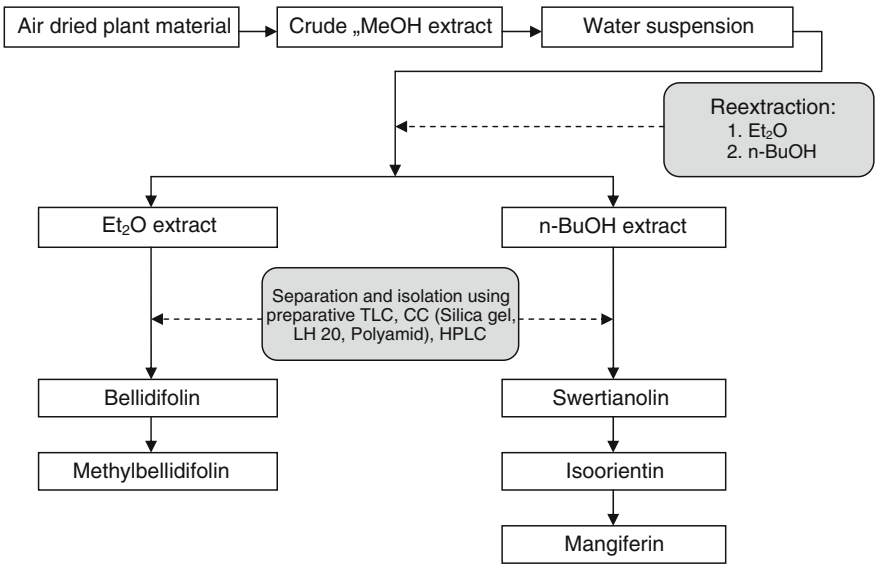


Fig. 14.14 Isolation of some compounds from *Swertia punctata* leaves

Before the analysis, the extract was dissolved in MeOH and the concentration was adjusted to ca. 10 mg/ml, followed by filtration through a Teflon Millipore filter HV, with a pore size 0.45 μm.

1. Injection volume: 5 μl.
2. Instrument: Agilent MSD ESI TOF coupled with Agilent 1200 series RR, liquid chromatography.
3. Column: LiChrospher 100 RP 18e, 150 × 4.0 mm i.d. 5.0 μm.
4. Mobile phase: A (water + 0.2 % HCOOH) + B (MeCN).
5. Elution: combination of gradient and isocratic modes according to following scheme:

Time (min)	Phase A (%)	Phase B (%)
0	98	2
5	90	10
18	90	10
20	85	15
25	85	15
30	70	30
40	30	70
50	30	70
51	98	2
55	98	2

6. Flow rate: 0.995 ml/min.
7. MSD conditions: drying gas (N_2) flow 12 l/min; nebulizer pressure 45 psig; drying gas temperature 350°; capillary voltage, 4000 V; fragmentor voltage, 140 V; skimmer, 60 V; Oct RF voltage 250 V; positive mode, mass range m/z 100–2500; 10,000 transients/scan.

14.7 Conclusions

The limited selection of bioactive plant constituents of some *Gentiana, Gentianella,* and *Swertia* species and the therapeutic applications described here give a general idea of the progress achieved over the last two decades. These achievements have been possible, in part, thanks to the development of techniques in separation and spectroscopy. LC DAD-ESI TOF MS chromatograms of plant extracts are presented and discussed.

Among numerous bioactive extracts, those of *G. lutea* leaves emerge as very promising since one could expect antidiabetic, hepatoprotective, and anti-inflammatory effects. Thus, the continuation is justified of the chemical investigation of this extract, aimed to discover a new source of drugs used in phytotherapy. The use

of yellow gentian leaves instead of roots in therapy could have a protective effect on this species endangered by overexploitation. Moreover, according to its chemical composition, *Swertia punctata* could be attractive as a source of medicinal raw material, but since its population is small, efforts should be focused in finding alternative ways for biomass production.

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