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ABSTRACT

of the dissertation for the degree
of Doctor of Philosophy

PHARMACOGNOSTIC INVESTIGATION OF SOME SPECIES OF THE GENUS *GENTIANA* L. FROM THE FLORA OF AZERBAIJAN

Specialty: 3400.02 – Pharmaceutical chemistry, pharmacognosy

Field of science: Pharmaceutics

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GENERAL CHARACTERISTICS OF THE WORK

Relevance and Development of the Topic

The use of medicinal plants dates back to the earliest stages of human civilization. Historical and ethnographic evidence demonstrates that ancient societies employed local plants not only for their therapeutic properties but also for nutrition, rituals, and skincare[1]. Today, naturally derived products are of particular interest for the investigation of biologically active compounds and the development of novel pharmaceuticals, which has driven a growing demand for both wild and cultivated medicinal plants. In this regard, the study of wild plants native to the flora of Azerbaijan represents a timely and significant scientific endeavor.

Plants from diverse life forms contain a wide range of secondary metabolites, including flavonoids, iridoids, xanthones, and essential oils[2]. Flavonoids exhibit antioxidant activity through the scavenging of free radicals, alongside antibacterial, antifungal, and antiviral effects.

Iridoids possess appetite-stimulating, anti-inflammatory, hepatoprotective, antidiabetic, and antitumor activities[2,3]. Essential oils demonstrate antimicrobial, antiviral, diuretic, spasmolytic, and expectorant properties[2]. Xanthones, in addition to their cardiotonic, diuretic, and choleretic effects, also display antiviral, antioxidant, anti-inflammatory, and immunostimulatory activities[4].

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Gentiana L., commonly known as the gentian genus, is a plant group distributed in Azerbaijan that remains insufficiently studied from a pharmacognostic perspective. Of the 28 species present in the Caucasus region, nine occur in Azerbaijan[5], inhabiting the western and eastern Greater Caucasus, southern, central, and northern Lesser Caucasus, as well as the mountainous regions of Nakhchivan and Lankaran. These species are classified into four sections: Pneumonanthe (*G. asclepiadea*, *G. septemfida*, *G. lagodechiana*, *G. gelida*), Aptera (*G. cruciata*), Chondropylla (*G. pyrenaica*, *G. aquatica*), and Cyclostigma (*G. verna* subsp. *pontica*, *G. nivalis*)[5].

Phytochemical studies have revealed that gentian species are particularly rich in iridoids, xanthones, essential oils, flavonoids, and alkaloids[6]. These biologically active compounds are employed in the pharmaceutical industry for the preparation of medicinal products and in functional foods for disease prevention and therapy[6]. Traditionally, gentians have been used as appetite stimulants, choleretic agents, antipyretics, and hypotensive remedies[6,7]. In modern medicine, they exhibit antioxidant, hepatoprotective, appetite-stimulating, hypoglycemic, immunomodulatory, antimicrobial, antiviral, anti-inflammatory, and analgesic activities[6,8].

Overall, these findings highlight the considerable pharmacognostic potential of *Gentiana* L. species and justify further systematic investigation in Azerbaijan.

The object and subject of the research

The study focuses on *G. septemfida* Pall., *G. asclepiadea* L., *G. gelida* M. Bieb., and *G. cruciata* L. in Azerbaijan, addressing their distribution and raw material reserves, the isolation and identification

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of biologically active compounds, and the assessment of the biological activity of the resulting extracts and selected constituents. Additionally, the research examines the morphological and anatomical characteristics of *G. septemfida* and evaluates the potential use of these species in pharmaceutical applications.

The purpose and the tasks of the research

The study **aims** to identify promising *Gentiana* L. species in Azerbaijan through pharmacognostic investigations, assess their distribution and raw material reserves, characterize morphological and anatomical variations, isolate and identify biologically active compounds, evaluate the biological activity of the resulting extracts and selected constituents, develop and validate relevant regulatory documentation, and provide recommendations for their application in pharmaceutical practice.

To achieve the stated aim, the following **objectives** have been set:

1. To compile literature data on the systematics of *Gentiana* species, their distribution in Azerbaijan, their uses in traditional and modern medicine, phytochemical investigations, and the main groups of biologically active compounds present in these plants;
2. To determine the distribution areas and raw material reserves of *Gentiana* species occurring in Azerbaijan;
3. To study the morphological and anatomical structure of the most promising gentian species and to identify characteristic features of their raw material;
4. To investigate, using modern physicochemical methods, the biologically active compounds present in the selected gentian species, including iridoids, flavonoids, xanthones, triterpenoid saponins, essential oils, and polysaccharides;
5. To isolate and identify individual biologically active compounds belonging to flavonoids, xanthones, iridoids, and triterpenoid saponins from the studied species;
6. To evaluate the biological activities of the extracts and selected individual compounds obtained from the studied species, including antioxidant, antibacterial, antifungal effects, and inhibitory activity against digestive enzymes;

7. To develop and validate the relevant regulatory documentation for the raw material of the promising gentian species, facilitating its potential use in pharmaceutical practice.

Research methods

Metabolites from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were investigated using *HPLC-DAD-ESI-QQQ-MS*, and the quantitative determination of selected compounds was performed by *HPLC-DAD*. Individual compounds were isolated from *G. septemfida* and *G. asclepiadea* via column chromatography and reversed-phase liquid chromatography. Additionally, essential oils obtained from the aerial and underground parts of *G. septemfida*, *G. cruciata*, and *G. gelida*, and from the underground part of *G. asclepiadea* using the Clevenger method, were analyzed qualitatively and quantitatively by *GC* and *GC-MS*. The polysaccharide content of the aerial parts of the promising species was characterized using UV and IR spectroscopy, as well as *HPLC-MS-DAD*, and the antioxidant activity of the polysaccharide fractions against 2,2-diphenyl-1-picrylhydrazyl (*DPPH*) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (*ABTS*) radicals was evaluated.

The chemical structures of biologically active compounds isolated from *G. septemfida* and *G. asclepiadea* were elucidated using nuclear magnetic resonance (*NMR*) spectroscopy, including *^1H, ^{13}C, COSY, HMQC, and HMBC* experiments. Antioxidant activities of extracts and selected individual compounds from the promising species were assessed using *DPPH*, *NO*, superoxide (O_2^-), and lipid peroxidation assays, while their inhibitory effects on digestive enzymes, including α-amylase and α-glucosidase, were also examined. Furthermore, the antibacterial and antifungal activities of the extracts were evaluated using disk diffusion and serial dilution methods. Raw material reserves of the studied species were assessed using plot-based field surveys. Macroscopic and microscopic analyses were conducted for *G. septemfida* to characterize its morphological and anatomical features.

The main provisions of the defense:

- The results of resource studies on *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* contribute to the diversification of Azerbaijan's medicinal plant raw material base.
- The macroscopic and microscopic investigations of *G. septemfida* revealed distinctive diagnostic features that provide a reliable basis for the identification of the species.
- These species are rich in iridoids, flavonoids, xanthones, and triterpenoid saponins. In addition, *G. asclepiadea*, *G. cruciata*, and *G. septemfida* contain neutral and acidic polymers, as well as water-soluble polyphenol–polysaccharide complexes incorporating phenolic acids, which exhibit antioxidant activity. The essential oils of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were found to be rich in sesquiterpenes.
- Extracts and individual compounds isolated from *G. septemfida* and *G. asclepiadea* demonstrated strong antioxidant, antibacterial, antifungal, and digestive enzyme inhibitory activities, indicating their potential for further research aimed at developing new medicinal agents.
- Pharmacopoeial monographs prepared for the herb and root of *G. septemfida* were approved by the Pharmacological and Pharmacopoeial Expert Council of the Ministry of Health of the Republic of Azerbaijan, thereby contributing to the quality control of the plant's raw material.

The scientific novelty of the research

- ✓ For the first time, species of the family *Gentianaceae* distributed in the flora of Azerbaijan were studied from a pharmacognostic perspective using modern research methods, and *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were identified as promising species.
- ✓ The raw material resources of these species were determined for the first time, and diagnostic anatomical and morphological features of *G. septemfida* were established.
- ✓ Metabolites obtained from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were

analyzed for the first time using *HPLC-DAD-ESI-QQQ-MS*. In total, 126 compounds were identified, including 68 iridoid glycosides, 37 flavone C-, O-, and C, O-glycosides, 2 phenolic O-glycosides, 3 phenolic carboxylic acids, 7 xanthones, 6 triterpenoid saponin glycosides, and 3 carbohydrates. Quantitative determination of 6 iridoid glycosides, 9 glycosylflavones, and 1 xanthone was carried out by *HPLC-DAD*, and the method was validated.

- ✓ For the first time, the polysaccharide composition of the aerial parts of *G. asclepiadea*, *G. cruciata*, and *G. septemfida* was investigated and characterized. Neutral and acidic polymers, as well as water-soluble polyphenol–polysaccharide complexes containing phenolic acids, were identified, all of which exhibited antioxidant activity against *DPPH* and *ABTS* radicals.
- ✓ Essential oils obtained from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* by the Clevenger method were subjected to *GC* and *GC-MS* analysis, revealing the presence of monoterpenes such as limonene, linalool, and α-terpineol; sesquiterpenes such as spiroalol, β-caryophyllene, and α-bisabolol; and long-chain alkanes such as eicosane and pentacosane.
- ✓ For the first time, iridoids such as gentiopicroside and loganic acid, as well as ursane-type triterpenoid saponins: (2β,3β)-3,25-epidioxo-2,24-dihydroxyurs-12,20(30)-dien-28-oic acid and (2β,3β)-3,25-epidioxo-2,24-dihydroxyurs-12-en-28-oic acid—were isolated from the roots of *G. septemfida*. From the aerial parts of *G. asclepiadea* were isolated flavonoids isoorientin and isovitexin, as well as the xanthone glycoside mangiferin.
- ✓ Extracts, certain fractions, and individual compounds obtained from the aerial and underground parts of the studied species were evaluated for their radical scavenging activity using *DPPH*, *NO*, O_2^- and lipid peroxidation assays.
- ✓ Extracts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* demonstrated inhibitory activity against digestive enzymes such as α-amylase and α-glucosidase.

- ✓ The antibacterial and antifungal properties of extracts from the aerial and underground parts of *G. gelida*, *G. septemfida*, and *G. cruciata* were examined initially using the disk-diffusion method and subsequently by serial dilution techniques.
- ✓ For the first time, pharmacopoeial monographs for the herb and root raw materials of *G. septemfida* were prepared and approved by the Pharmacological and Pharmacopoeial Expert Council of the Ministry of Health of the Republic of Azerbaijan, thereby contributing to the implementation of quality control procedures during the collection, drying, and storage of the plant material.

Theoretical and practical significance of the study

- ✓ The research carried out has demonstrated that species of *Gentiana L.* (gentians) possess a sufficiently rich raw material base and are abundant in biologically active compounds. This highlights the potential of *G. septemfida Pall.*, *G. asclepiadea L.*, *G. cruciata L.*, and *G. gelida M. Bieb.* as promising species that can contribute to the diversification of natural medicinal plant resources of Azerbaijan
- ✓ Extracts and individual compounds isolated from these species have been proven to exhibit antioxidant, antifungal, and antibacterial effects, as well as inhibitory activity against digestive enzymes (α-amylase and α-glucosidase), thereby providing a scientific basis for the development of new medicinal preparations.
- ✓ Part of the phytochemical studies undertaken within the framework of this dissertation has been applied in quality control of certain herbal medicinal products manufactured by the pharmaceutical company "Azərfarm," with corresponding official documentation prepared.
- ✓ The results of the pharmacognostic investigation of promising gentian species have been integrated into the teaching process at Azerbaijan Medical University: the analysis of iridoids is incorporated into the undergraduate course "Pharmacognosy," while the analysis of xanthones is included in the graduate-level course "Phytochemistry."

- ✓ Based on the pharmacognostic studies conducted on the aerial and underground parts of *G. septemfida*, pharmacopoeial monographs were prepared and approved by the Pharmacological and Pharmacopoeial Expert Council of the Ministry of Health of the Republic of Azerbaijan. These normative documents provide a solid framework for ensuring quality control of the plant material at all stages, including collection, drying, and storage.

Personal involvement of the author

All scientific results presented and discussed in this dissertation were directly obtained and independently compiled by the author. Statistical processing of the numerical data was conducted by the author in collaboration with specialists in computer technology to ensure accuracy and reliability.

Approbation and application

The findings of the dissertation have been widely disseminated and applied in both scientific and practical contexts. Research outcomes were presented at national and international forums, including the 22nd Scientific Conference of Doctoral Students and Young Researchers (2018), the International Scientific-Practical Conference dedicated to the 100th anniversary of the Faculty of Medicine (2019), the 7th Scientific-Practical Conference of Residents of Azerbaijan Medical University (2019), the International Scientific-Practical Congress "*Current Issues of Medicine–2020*" (2020), the 5th International Scientific Congress "*Modern Problems of Pharmacy*" (2021), and the International Scientific-Practical Congress "*Current Issues of Medicine*" dedicated to the 100th anniversary of Heydar Aliyev (2023). Published in International conference dedicated to 105th anniversary of Baku State University 2024 – Green World Solidarity Year conference proceeding. A poster presentation was also delivered at the IV International Mediterranean Pharmacy Congress (Medphacon, 2019). The main results of the dissertation have been published in peer-reviewed journals, including *Metabolites* (2019), *Sağlamlıq* (2019), *Contemporary Achievements of Azerbaijani Medicine* (2019), *Medicine and Science* (2020), *Azerbaijan Pharmacy and Pharmacotherapy* (2020, 2024), *Chemistry of Natural Compounds* (2022), and *Azerbaijan Medical Journal* (2024).

The findings of this dissertation have been disseminated through a total of 19 scientific publications, comprising 9 research articles (including 2 published internationally, and 4 in journals indexed in recognized international databases), 8 conference proceedings and abstracts, and 2 pharmacopoeial monographs.

The work was discussed and approved at the Scientific Seminar of the BFD 4.18 One-Time Dissertation Council of Azerbaijan Medical University on 8th September, 2025 (Protocol № 1).

The approved pharmacopoeial monographs prepared for *G. septemfida* herb and root can be applied in quality control of plant raw materials during collection, processing, and storage.

Part of the phytochemical studies has been implemented in practice for quality control of herbal medicinal products produced by the pharmaceutical company "Azərfarm."

Moreover, the results of the pharmacognostic investigations have been integrated into the academic curriculum of Azerbaijan Medical University: the analysis of iridoids is included in the undergraduate course *Pharmacognosy*, while the analysis of xanthones is taught within the graduate-level course *Phytochemistry*.

Name of the organization where the dissertation work is performed

The dissertation was carried out at the Department of Pharmacognosy of Azerbaijan Medical University.

The scope and structure of the dissertation

The dissertation comprises 242 pages (237907 characters) and includes the following sections: Introduction (15921 characters), Literature Review (54180 characters), Materials and Methods (42208 characters), and four chapters presenting the author's original research (21854; 49597; 23529; and 15437 characters, respectively). The work also contains the Results (3892 characters), Practical Recommendations (820 characters), References, and Appendices. A total of 172 sources were cited in the dissertation. The dissertation is illustrated with 41 tables, 68 figures, and 2 schematics.

The State Registration Number of the dissertation was assigned by the Scientific Council on "Clinical, Regenerative, and Translational Medicine" under the Republican Coordination Council for Scientific Research on March 16, 2021 (Protocol № 10).

RESEARCH MATERIAL AND METHODS

The research objects consisted of aerial and underground parts of gentian species collected from various regions of the Republic of Azerbaijan: *G. septemfida* (seven-lobed gentian), *G. cruciata* (cross-shaped gentian), *G. asclepiadea* (slitted gentian), and *G. gelida* (cold-resistant gentian). Biologically active compounds were extracted from these materials by maceration and subsequently isolated using column chromatography and reversed-phase liquid chromatography. The antioxidant, antibacterial, antifungal activities, and inhibitory effects against digestive enzymes of the obtained extracts and individual compounds were evaluated.

Qualitative and quantitative analyses of plant metabolites were performed using *HPLC-DAD-ESI-QQQ-MS* and *HPLC-DAD*. Chromatographic separation was carried out on an *LC-MS-8050* system equipped with a diode-array detector and triple quadrupole electrospray ionization detector using a GLC Mastro C_{18} column at 35 °C. Solvent A was 0.5% aqueous formic acid, and solvent B was 0.5% formic acid in acetonitrile. The injection volume was 1 µL, with a flow rate of 100 µL/min. A gradient program of 0–6 min, 5–20% B; 6–12 min, 20–40% B; 12–16 min, 40–55% B; 16–21 min, 55–60% B; 21–30 min, 60–100% B; 30–35 min, 100–5% B was applied. Diode-array detection was performed in the 200–600 nm range. Mass spectra were recorded in both negative (−3 kV) and positive (+3 kV) ionization modes, with collision energies of 10–45 eV and an m/z range of 100–1900. ESI interface, desolvation line, and heat block temperatures were set to 3000 °C, 2500 °C, and 4000 °C, respectively. Nitrogen served as nebulizer gas (3 L/min), air as drying gas (10 L/min), and argon as collision gas (0.3 mL/min). Data processing was performed using the LabSolutions software with the internal LC-MS library.

Quantitative determination of iridoid glycosides, flavonoids, and xanthones was performed by *HPLC-DAD* using a ProntoSIL-120-5-C18 AQ column at 30 °C on an Econova MiLiChrom A-02 microcolumn chromatograph. Solvent A was 0.2 M $LiClO_4$ in 0.01 M $HClO_4$, and solvent B was 0.01 M $HClO_4$ in acetonitrile. Injection

volume was 1 µL, with a flow rate of 150 µL/min. The gradient program was 0–10 min, 12–35% B; 10–15 min, 35–70% B; 15–20 min, 70–12% B. Chromatograms were recorded at 210 nm.

GC analysis was conducted using an Agilent 6890N system with a flame ionization detector at 300 °C. *GC-MS* analysis was performed on an *Agilent 5975 GC-MSD* system using helium (0.8 mL/min) as the carrier gas and an Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness).

The polysaccharide content of the aerial parts of *G. asclepiadea*, *G. cruciata*, and *G. septemfida* was analyzed using a SF-2000 UV spectrophotometer and an FT-801 FTIR spectrometer. FTIR spectra were recorded in the 4000–600 cm^{-1} range on ZnSe substrates.

Antioxidant activities of extracts and individual compounds obtained from the studied species were evaluated using *DPPH*, *NO*, O_2^- and lipid peroxidation assays, while inhibitory effects on α-amylase and α-glucosidase enzymes were assessed spectrophotometrically. Antibacterial and antifungal activities of extracts from aerial and underground parts of *G. septemfida*, *G. gelida*, and *G. cruciata* were investigated using disk-diffusion and serial dilution methods.

RESEARCH RESULTS AND THEIR DISCUSSION

Metabolites from the aerial and underground parts of promising gentian species identified in Azerbaijan *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were analyzed using HPLC-DAD-ESI-QQQ-MS. A total of 126 compounds were identified in these species, including 68 iridoid glycosides (primarily loganic acid, loganin, sweroside, gentiopicroside, and sweroside derivatives), 37 flavone C-, O-, and C, O-glycosides (derivatives of luteolin, apigenin, chrysoeriol, and acacetin), 2 phenolic O-glycosides, 3 phenolic carboxylic acids, 7 xanthones, 6 triterpenoid saponin glycosides, and 3 carbohydrates. Quantitative determination of six iridoid glycosides, nine glycosylated flavones, and one xanthone was carried out using HPLC-DAD, and the method was validated for accuracy and reliability.

Table 1.

Quantitative determination of species-specific compounds in promising gentian species from Azerbaijan using HPLC-DAD

Compounds	Gentian species			
	GAS	GCR	GGE	GSE
Aerial parts				
Iridoids				
Loganic acid	11.83±0.21	3.40±0.06	1.97±0.04	4.07±0.08
Swertiamarin	1.53±0.03	tr.	9.04±0.16	9.47±0.18
Gelidoside	0.00	0.00	7.30±0.14	4.66±0.09
Gentiopicroside	91.74±1.85	14.77±0.29	4.41±0.08	5.08±0.10
Sweroside	tr.	tr.	tr.	tr.
Trifloroside	0.00	0.00	2.68±0.05	1.87±0.04
Total content of iridoids	105.10	18.17	25.40	25.15
Flavonoids				
Isovitexin	0.89 ± 0.02	2.27 ± 0.04	1.05 ± 0.02	3.59 ± 0.07
Isovitexin -2'-O-Glu	1.81 ± 0.04	2.40 ± 0.05	1.39 ± 0.03	7.03 ± 0.14
Saponarin	1.25 ± 0.03	1.42 ± 0.03	tr.	0.83 ± 0.02
Apigenin-7-O-Glu	2.52 ± 0.07	0.47 ± 0.01	0.67 ± 0.01	tr.
Isoorientin	18.85 ± 0.37	4.78 ± 0.09	33.59 ± 0.67	17.22 ± 0.34
Isoorientin-2'-O-Glu	40.62 ± 0.73	16.05 ± 0.32	15.16 ± 0.30	19.63 ± 0.39
Isoorientin-6'-O-Glu	0.00	0.00	3.22 ± 0.06	8.59 ± 0.17
Luteolin-7-O-Qlü	1.33 ± 0.02	tr.	0.78 ± 0.02	2.52 ± 0.05
Isoscoparin	0.00	0.00	1.14 ± 0.02	0.40 ± 0.01
Total content of flavonoids	**67.27**	**27.39**	**57.00**	**59.81**
Xanthones				
Mangiferin	17.48±0.33	5.75±0.11	0.00	0.00
Total content of xanthones	**17.48**	**5.75±0.11**	**0.00**	**0.00**
Total content of phenolic compounds	**84.75**	**33.14**	**57.00**	**59.81**

Table 1. Continued

Compounds	Gentian species			
	GAS	GCR	GGE	GSE
Total content of compounds	189.85	51.31	82.40	84.96
Underground parts				
Iridoids				
Loganic acid	11.75±0.23	17.31±0.34	6.14±0.12	8.43±0.16
Swertiamarin	5.87±0.11	2.63±0.05	3.47±0.07	3.83±0.07
Gelidoside	0.00	0.75±0.02	1.53±0.03	1.92±0.04
Gentiopicroside	64.71±1.29	57.51±1.15	61.37±1.22	75.90±1.51
Gentiopicroside-6-O-Glu	1.24±0.02	0.61±0.01	1.21±0.02	5.85±0.11
Sweroside	tr.	3.84±0.07	tr.	2.15±0.04
Trifloroside	0.00	0.54±0.01	7.07±0.14	5.60±0.11
Total content of iridoids	83.57	83.19	80.79	103.68
Flavonoids				
Isoorientin-2”-O-Glu	4.43±0.08	0.00	0.00	0.00
Total content of flavonoids	4.43.	0.00	0.00	0.00
Xanthones				
Gentioside	0.75±0.02	0.00	0.00	0.00
Total content of xanthones	**0.75**	**0.00**	**0.00**	**0.00**
Total content of phenolic compounds	**5.18.**	**0.00**	**0.00**	**0.00**
Total content of compounds	**88.75**	**83.19**	**80.79**	**103.68**

Essential oils obtained from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* using the Klevencer method were analyzed by GC and GC-MS. The oils were found to contain monoterpenes such as limonene, linalool, and α-

terpineol; sesquiterpenes including spiroanol, β-caryophyllene, and α-bisabolol; as well as long-chain alkanes such as eukosan and pentacosan.

The aerial parts of *G. asclepiadea*, *G. septemfida*, and *G. cruciata* were sequentially extracted with water at 20 °C and 90 °C, and with 0.5% oxalic acid at 50 °C. Following demineralization, deproteinization, and dialysis, the corresponding total polysaccharide fractions were obtained: WSPS20 and WSPS90 (water-soluble polysaccharides) and AcSPS50 (acid-soluble polysaccharides). Chemical analysis revealed that these polysaccharide fractions contained uronic acids (37.6–55.6%), starch (0.9–5.3%), protein (0.5–3.8%), arabinogalactan-protein complexes (AGP, 0.1–4.3%), and phenolic compounds (0.1–5.6%).

Extracts from the underground parts of *G. septemfida* and the aerial parts of *G. asclepiadea* were subjected to isolation procedures. Fractions were obtained using column chromatography with polyamide as the absorbent, followed by further purification of individual compounds using polyamide column chromatography (CC), silica gel CC, Sephadex CC, and reversed-phase liquid chromatography.

Based on the ^{1}H NMR spectrum of **compound G.s.k. 1**, signals corresponding to three vinyl protons (H-8, δ_H 5.72; H-10A, δ_H 5.22; H-10B, δ_H 5.18), one olefinic proton (–CH=CH–, H-3, δ_H 7.41), one hemiacetal proton (H-1, δ_H 5.59), one oxymethylene group (H-7a, δ_H 4.99; H-7b, δ_H 4.95), and one methine (=CH–) proton were observed, indicating that this compound is a mono-glycosidic secoiridoid heteroside. Furthermore, the weak-field signal of the H-6 proton (δ_H 5.64) confirms the presence of a double bond between C-5 and C-6. The ^{13}C NMR spectrum of G.s.k. 1 revealed signals for 16 carbon atoms, six of which were assigned to the β-glucopyranose residue. Interpretation of the spectral data, in accordance with literature reports, identified G.s.k. 1 as gentiopicroside.

For **compound G.s.k. 2A**, the ^{13}C NMR spectrum showed signals corresponding to 30 carbons, including four CH_3, eleven CH_2, seven CH, and eight quaternary carbons. In the low-field region of the ^{1}H

and ^{13}C NMR spectra, characteristic signals of a COOH group were observed at δ_C 177.39 and δ_H 11.96, respectively. Spectral interpretation, supported by literature data, identified G.s.k. 2_A as an ursane-type triterpenoid: (2β, 3β)-3,25-epidioxy-2,24-dihydroxyurs-12,20(30)-dien-28-oic acid.

For **compound G.s.k. 2_B**, the ^{13}C NMR spectrum exhibited signals for 30 carbons, including five CH_3, ten CH_2, eight CH, and seven quaternary carbons. Comparison with G.s.k. 2A revealed that the =CH- signals at δ_C 151.14 (C-20) and δ_C 106.51 (C-30) in G.s.k. 2_A were replaced by δ_C 31.81 (C-20) and δ_C 21.00 (C-30) in G.s.k. 2_B, indicating substitution of the =CH- group with a CH_3 group. Based on spectral interpretation and literature data, G.s.k. 2_B was identified as an ursane-type triterpenoid: (2β, 3β) - 3,25 - epidioxy - 2,24 - dihydroxyurs - 12 - en - 28 - oic acid.

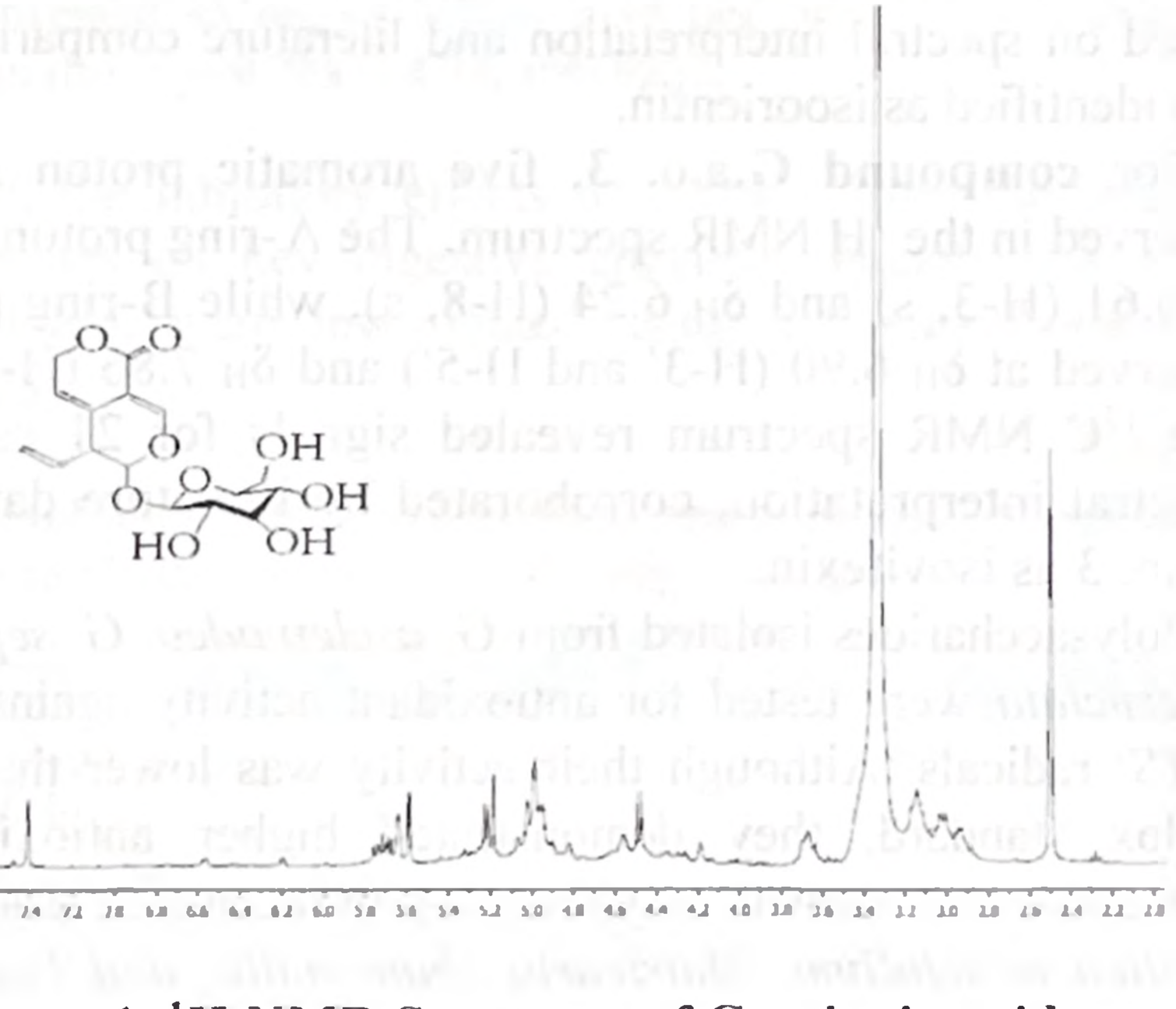

Figure 1. ^1H NMR Spectrum of Gentiopicroside

For **compound G.s.k. 3**, signals at δ_H 4.66 and δ_C 98.57 ppm in the ^1H and ^{13}C NMR spectra were assigned to the anomeric proton and carbon of a β-glucopyranose unit. In the ^1H NMR spectrum, three CH groups (H-5, δ_H 3.14; H-8, δ_H 1.90; H-9, δ_H 2.04) and one

CH_2 group (H2-6, δ_H 2.20, 1.76) corresponding to the cyclopentane moiety were observed. The ^{13}C NMR spectrum showed a total of 16 carbon signals. Spectral interpretation, in agreement with literature data, identified G.s.k. 3 as loganic acid.

For **compound G.a.o. 1**, the ^{1}H NMR spectrum exhibited a hydroxyl proton signal at δ_H 13.75, indicating a functional group attached to C-1. In the aromatic region, three proton signals were observed at δ_H 6.37, 6.86, and 7.38. Analysis of the sugar residue signals confirmed the presence of a β-glucopyranose unit. Spectral interpretation and comparison with literature data identified G.a.o. 1 as mangiferin.

For **compound G.a.o. 2**, five aromatic proton signals were observed in the ^{1}H NMR spectrum. The singlet at δ_H 6.40 ppm was assigned to H-8 in the A-ring of the flavone skeleton. Long-range correlations between H-8 and C-9 further supported this assignment. Based on spectral interpretation and literature comparison, G.a.o. 2 was identified as isoorientin.

For **compound G.a.o. 3**, five aromatic proton signals were observed in the ^{1}H NMR spectrum. The A-ring protons appeared at δ_H 6.61 (H-3, s) and δ_H 6.24 (H-8, s), while B-ring protons were observed at δ_H 6.90 (H-3′ and H-5′) and δ_H 7.86 (H-2′ and H-6′). The ^{13}C NMR spectrum revealed signals for 21 carbon atoms. Spectral interpretation, corroborated by literature data, identified G.a.o. 3 as isovitexin.

Polysaccharides isolated from *G. asclepiadea, G. septemfida, and G. cruciata* were tested for antioxidant activity against *DPPH* and *ABTS*· radicals. Although their activity was lower than that of the Trolox standard, they demonstrated higher antioxidant activity compared with known polyphenol–polysaccharide conjugates from *Achillea millefolium, Matricaria chamomilla, and Vaccinium vitis-idaea* subsp. minus.

The antioxidant properties of aerial and root extracts from *G. septemfida, G. asclepiadea, G. cruciata, and G. gelida* were further evaluated using DPPH, NO, O_2^-, and lipid peroxidation inhibition assays.

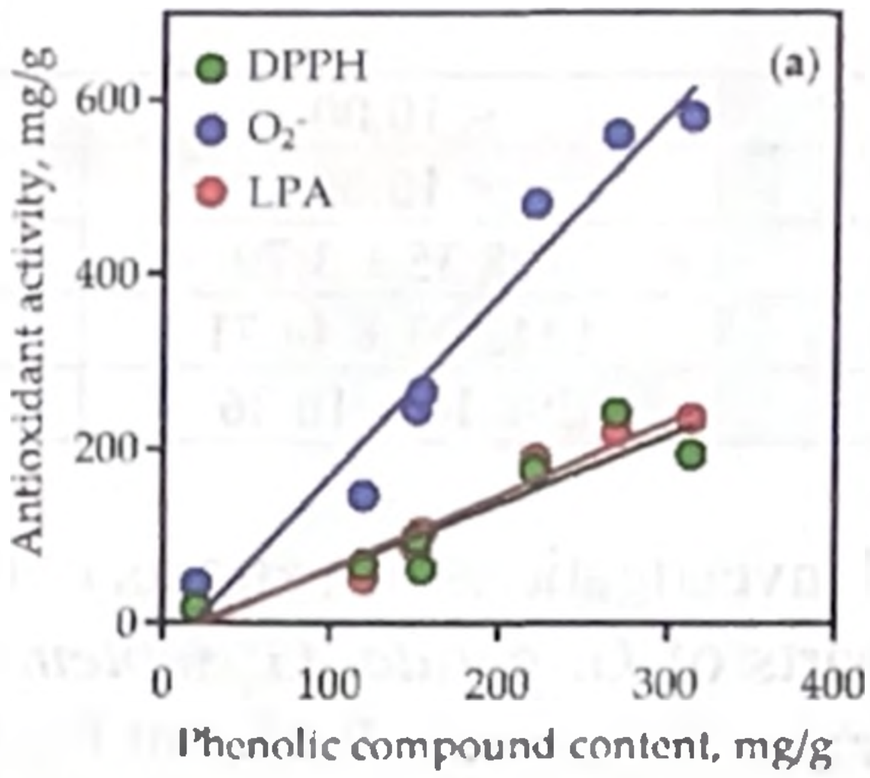

Figure 2. Correlation graphs between the total phenolic content (mg/g) in Gentiana extracts and their antioxidant activities. Activity against DPPH radicals, expressed as mg of Trolox per gram of dry extract; correlation equation: y=2.07x-38.48; r=0.9752. Activity against superoxide radicals, expressed as mg of quercetin per gram of dry extract; correlation equation: y=0.84x−20.21; r=0.9736. Lipid peroxidation inhibition, expressed as mg of caffeic acid per gram of dry extract; correlation equation: y=0.76x−14.74; r=0.9232.

In addition, the inhibitory effects of extracts from the selected promising species on key digestive enzymes, namely α-amylase and α-glucosidase, were investigated using a spectrophotometric microplate assay.

Table 2.

Results of the inhibitory effects of extracts and individual compounds from Gentiana species on the digestive enzymes α-amylase and α-glucosidase.

Ekstraktlar, maddələr	αAIP[r]	αGIP[r]
G. asclepiadea herb	530.11 ± 16.31	418.80 ± 12.98
G. cruciata herb	183.48 ± 6.62	194.90 ± 5.54
G. gelida herb	395.17 ± 14.02	311.48 ± 11.78
G. septemfida herb	480.20 ± 11.94	322.02 ± 11.69
G. asclepiadea root	38.87 ± 1.51	25.64 ± 0.97
G. cruciata root	< 10.00	< 10.00
G. gelida root	< 10.00	< 10.00
G. septemfida root	< 10.00	< 10.00
Loganic acid	< 10.00	< 10.00
Gentiopicroside	< 10.00	< 10.00

Table 2. Continued

Gelidoside	< 10.00	< 10.00
Trifloroside	< 10.00	< 10.00
Isovitexin	108.35 ± 3.79	52.63 ± 2.09
Isoorientin	1242.03 ± 44.71	811.10 ± 32.44
Mangiferin	296.14 ± 10.36	1562.84 ± 62.48

Microbiological investigations of extracts obtained from the aerial and underground parts of *G. gelida*, *G. septemfida*, and *G. cruciata* revealed that the extracts possess both antibacterial and antifungal activities. Notably, the root of *G. gelida* exhibited strong antibacterial activity against *Staphylococcus aureus*, the aerial parts of *G. septemfida* against *Escherichia coli*, the aerial and underground parts of *G. septemfida* against *Pseudomonas aeruginosa*, the aerial parts of *G. cruciata* against *Bacillus anthracoides*, the root of *G. gelida* against *Klebsiella pneumoniae*, and the root of *G. cruciata* against *Candida albicans*.

Anatomical studies of the root and leaf tissues of *G. septemfida* revealed distinct diagnostic features. The root's outer surface is covered by a single-layered epidermis, composed of thin-walled, fragmented, isodiametric cells, with strongly undulated cell walls. Microscopic examination of the thin lateral roots showed that the cortical cells are less layered and possess relatively large intercellular spaces. The cells of both epidermal layers are predominantly isodiametric and rectangular, exhibiting papillose structures. Such papillose epidermis is a characteristic anatomical feature of many Gentianaceae species. The stomata were primarily anomocytic, surrounded by 3–4 subsidiary cells, although some anizocytic stomata were also observed.

The *Gentiana* species in the flora of Azerbaijan are distributed across diverse geographic regions. Among them, *G. septemfida*, *G. cruciata*, *G. asclepiadea*, and *G. gelida* form extensive populations across various districts. Using the plot-based survey method, the distribution areas of these species were mapped, and the biological reserves of their aerial and underground parts were assessed. The studies further identified the exploitable reserves and estimated the annual harvestable quantities.

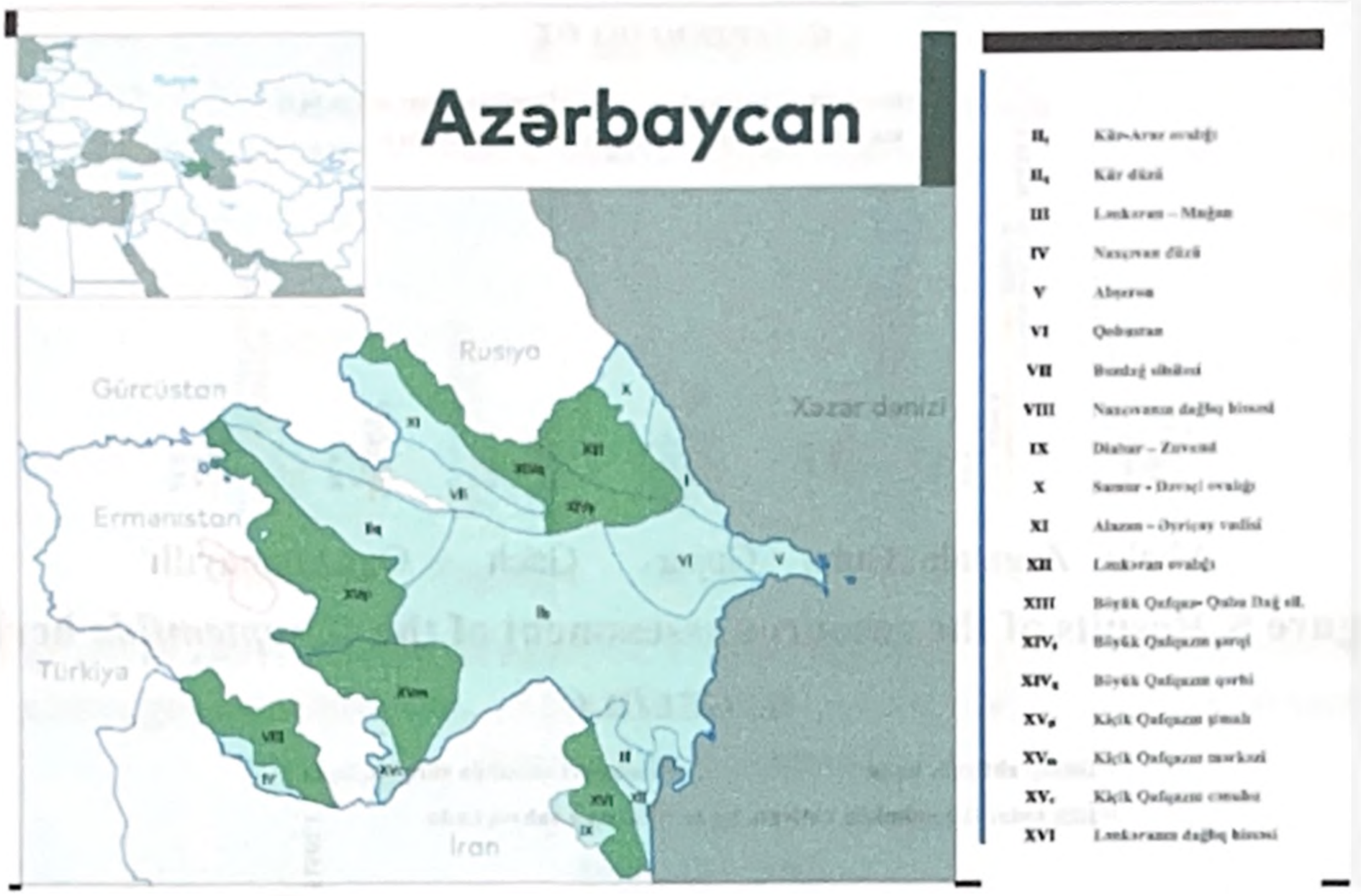

Figure 3. Distribution areas of *G. septemfida*, *G. cruciata*, *G. asclepiadea*, and *G. gelida* in the territory of Azerbaijan.

Based on the results of the conducted resource assessment studies, the distribution areas of *G. septemfida*, *G. cruciata*, *G. asclepiadea*, and *G. gelida* by region, as well as the exploitable and annual harvestable biomass of their aerial parts, are presented in Figures 4–8.

Figure 4. Results of the resource assessment of the *G. septemfida* roots.

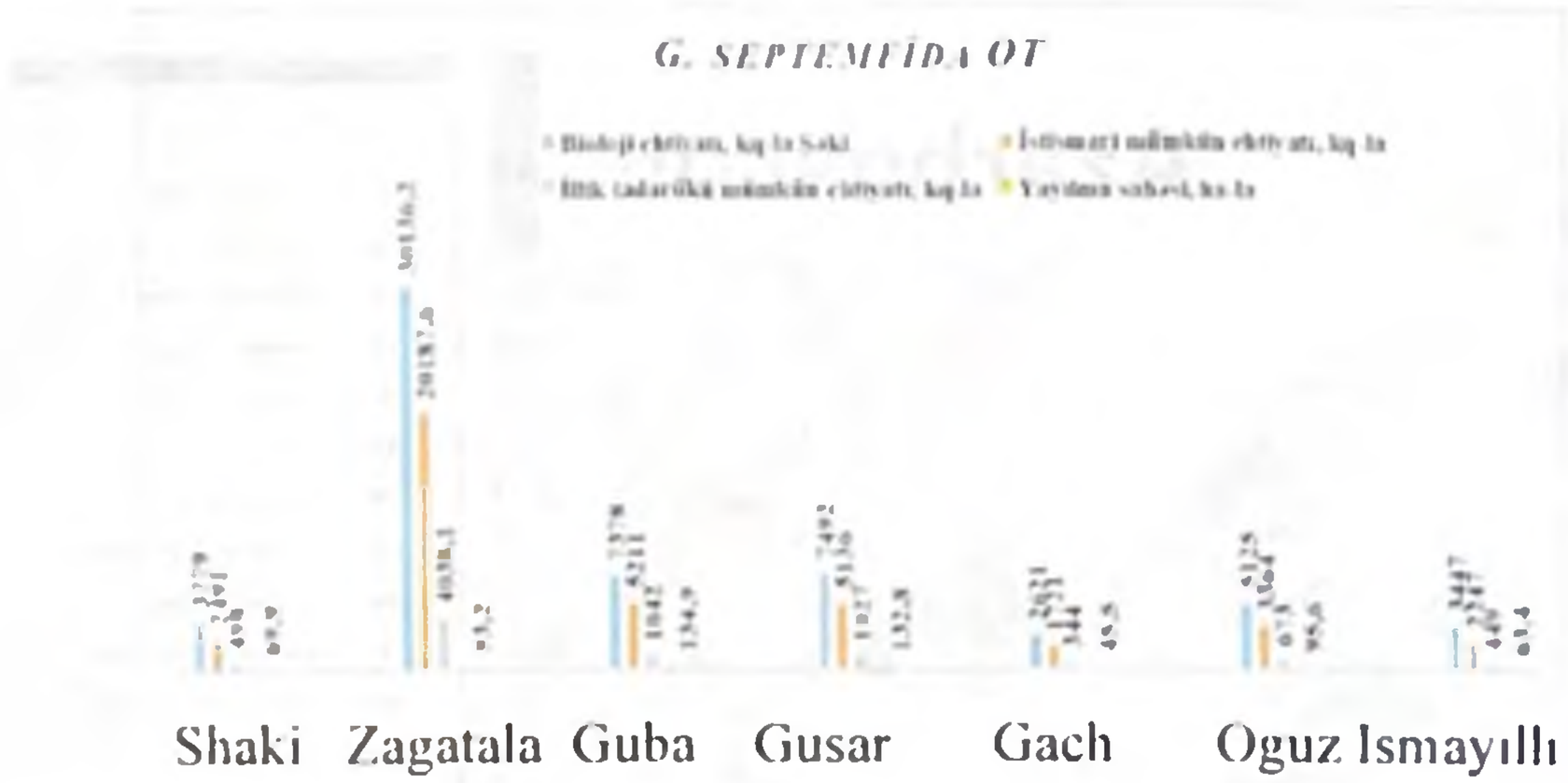

Figure 5. Results of the resource assessment of the *G. septemfida* herb.

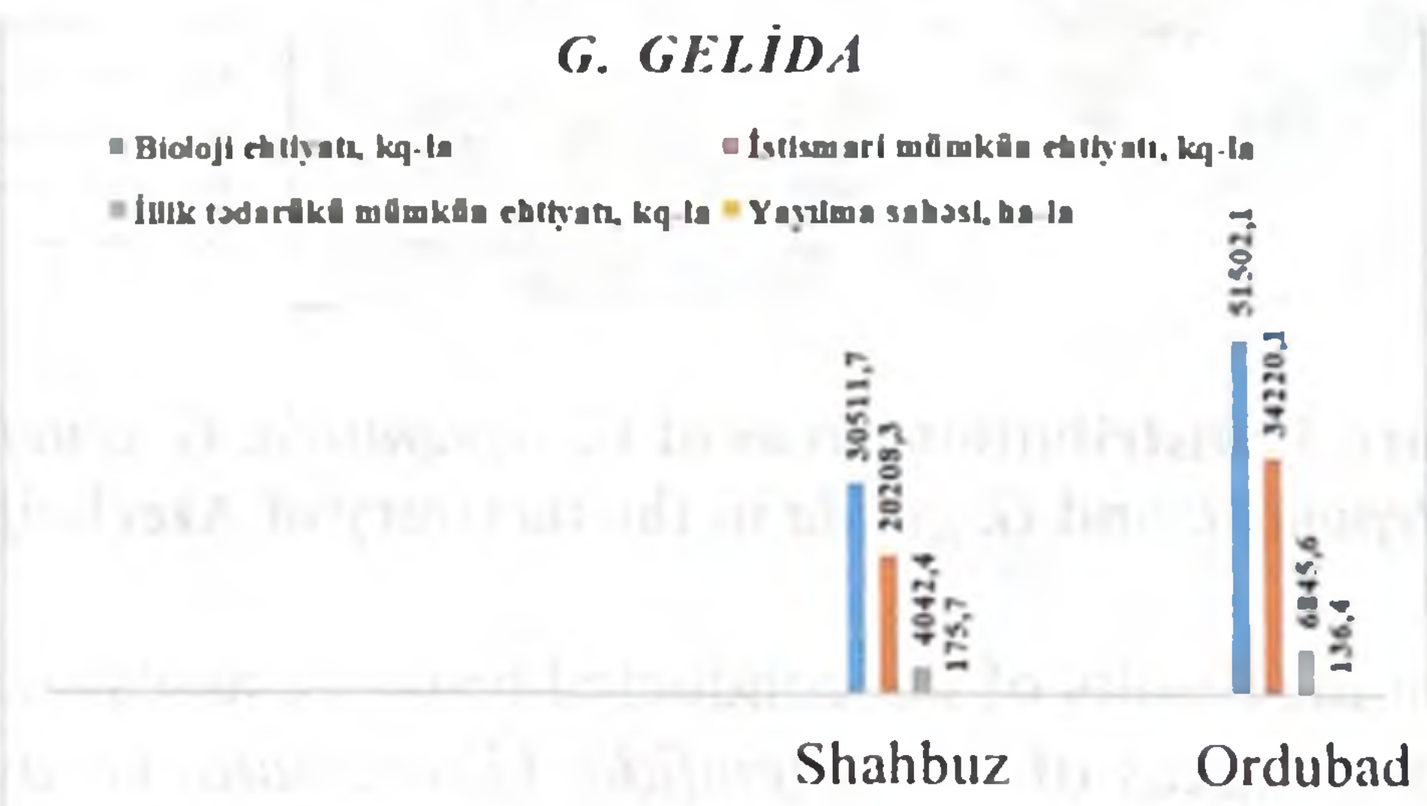

Figure 6. Distribution area of *G. gelida* by region, along with its aboveground biomass, exploitable resources, and potential annual harvestable reserves.

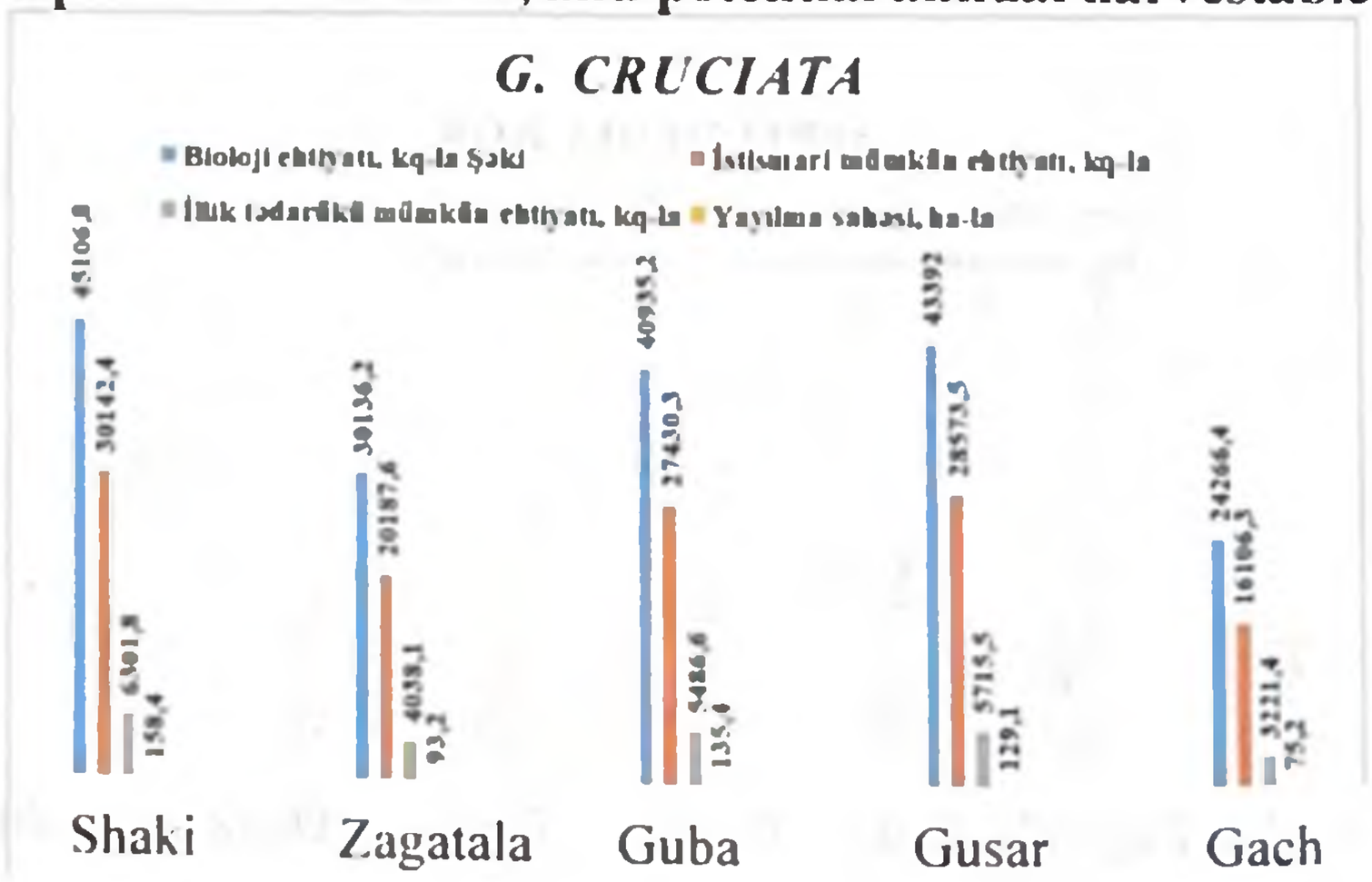

Figure 7. Distribution area of *G. cruciata* by region, along with its aboveground biomass, exploitable resources, and potential annual harvestable reserves.

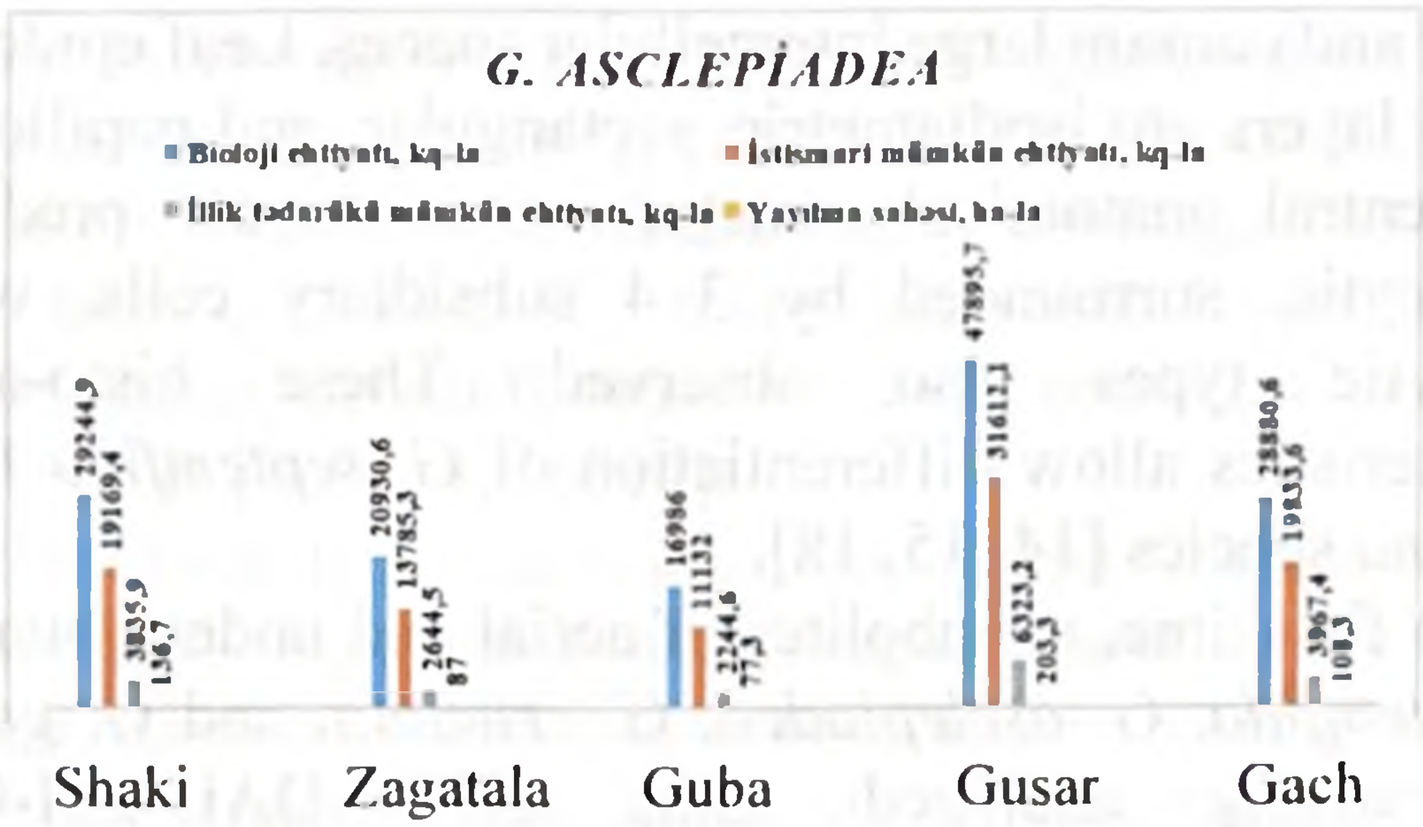

Figure 8. Distribution area of *G. asclepiadea* by region, along with its aboveground biomass, exploitable resources, and potential annual harvestable reserves.

RESULTS

1. Literature review revealed that iridoids, xanthones, flavonoids, and triterpenoid compounds are predominant in *Gentiana* species. Their raw materials have traditionally and scientifically been used as appetite stimulants, hepatoprotective agents, as well as antibacterial and antifungal remedies [3, 5, 17]. None of the nine *Gentiana* species found in Azerbaijan had been previously studied from a pharmacognostic perspective [1, 2, 4, 6, 10, 11].
2. For the first time, resource studies identified that *G. septemfida* occupies 542 ha of mass distribution in Azerbaijan, with annual harvestable reserves of 4.03 tons of aerial parts and 1.8 tons of roots. The mass distribution area of *G. cruciata* was 591.3 ha, with potential annual harvests of 24.8 tons of aerial parts and 6.6 tons of roots. *G. gelida* was found on 312.1 ha, with harvestable reserves of 6.8 tons of aerial parts and 1.5 tons of roots. For *G. asclepiadea*, the distribution area is 612.6 ha, with annual harvestable reserves of 19 tons of aerial parts and 4.4 tons of roots [8,18].
3. Histological studies revealed distinct anatomical features in the roots and leaves of *G. septemfida*. The root surface is covered by a single-layered epidermis. The cortex cells of lateral roots are

thinner and contain large intercellular spaces. Leaf epidermal cells in both layers are isodiametric, rectangular, and papillose, with a dorsoventral anatomical structure. Stomata are predominantly anomocytic, surrounded by 3–4 subsidiary cells, with some anisocytic types also observed. These histo-anatomical characteristics allow differentiation of *G. septemfida* from other *Gentiana* species [14, 15, 18].

4. For the first time, metabolites of aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were comparatively analyzed using HPLC-DAD-ESI-QQQ-MS, revealing 68 iridoid glycosides, 37 flavon C-, O-, and C, O-glycosides, 2 phenolic O-glycosides, 3 phenolic acids, 7 xanthones, 6 triterpenoid glycosides, and 3 carbohydrates, totaling 126 compounds. Quantitative determination of 7 iridoid glycosides, 9 glycosylated flavonols, and 2 xanthones was performed using HPLC-DAD. The aerial parts of *G. asclepiadea*, *G. septemfida*, and *G. cruciata* contained 83.1–98.9% total polysaccharide fractions, which included uronic acids, starch, protein, arabinogalactan-protein complexes, and phenolic compounds. Essential oils from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were obtained by the Klevencer method and analyzed using GC and GC-MS, revealing monoterpenes (limonene, linalool, α-terpineol) and sesquiterpenes (spirolanol, β-caryophyllene, α-bisabolol) [7, 13, 18, 19].
5. For the first time, iridoids such as gentiopicroside and loganic acid and ursane-type triterpenoids [(2β,3β)-3,25-epidioxy-2,24-dihydroxyurs-12,20(30)-dien-28-oic acid and (2β,3β)-3,25-epidioxy-2,24-dihydroxyurs-12-en-28-oic acid] were isolated from *G. septemfida* roots. From the aerial parts of *G. asclepiadea*, isoorientin, isovitexin flavonoids, and mangiferin xanthone glycoside were isolated. These compounds were identified using NMR (^{13}C, ^{1}H NMR, HMBC, HMQC, and COSY) spectroscopy [18].
6. Extracts from the aerial and underground parts of *G. septemfida*, *G. asclepiadea*, *G. cruciata*, and *G. gelida* were tested for

inhibitory activity against α-amylase and α-glucosidase, as well as for antioxidant activity using DPPH, NO, O_2^-, ABTS, and lipid peroxidation assays. Antibacterial and antifungal activities of extracts from *G. gelida*, *G. septemfida*, and *G. cruciata* were evaluated using disk/diffusion methods and further by serial dilution assays [7, 9, 14, 16, 18].

7. For the first time, pharmacopoeial monographs for the aerial parts and roots of *G. septemfida* were prepared and approved by the Pharmacological and Pharmacopoeial Expert Committee of the Ministry of Health of the Republic of Azerbaijan, providing regulatory standards for quality control of this plant [12, 14, 15].

PRACTICAL RECOMMENDATIONS

1. Determination of the raw material reserves of *Gentiana* species in the flora of Azerbaijan will enrich the country's wild medicinal plant resource base.
2. Results from microscopic studies of *G. septemfida* will allow this plant to be distinguished from related species during production and consumption processes.
3. Part of the phytochemical research conducted in this dissertation will support quality control of plant-based medicinal products manufactured by the "Azərfarm" company.
4. The pharmacognostic findings on promising *Gentiana* species will facilitate the teaching of modern research methods at Azerbaijan Medical University, including iridoid analysis in the undergraduate "Pharmacognosy" course and xanthone analysis in the graduate-level "Phytochemistry" course.

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LİST OF ABBREVIATIONS AND SYMBOLS

DPPH	–	2,2-diphenyl-1-picrylhydrazyl
ABTS	–	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
O_2^-	–	Superoxide
HPLC-DAD-ESI-QQQ-MS	–	High-Performance Liquid Chromatography Diode Array Detector Electrospray Ionization Triple Quadrupole Mass Spectrometry
HPLC-DAD	–	High-Performance Liquid Chromatography-Diode Array Detector
HMBC	–	Heteroniclear Multiple Bond Correlation
HMQC	–	Heteronuclear Multiple Quantum Coherence
COSY	–	Correlation spectrocopy
GC	–	Gas Chromatography
GC-MS	–	Gas Chromatography Mass Spectrometry
NMR	–	Nuclear Magnetic Resonance
WSPS20/90	–	water-soluble polysaccharides
AcSPS50	–	acid-soluble polysaccharides
G.s.k.	–	*Gentiana septemfida* root
G.s.o.	–	*Gentiana septemfida* herb
G.c.k.	–	*Gentiana cruciata* root
G.c.o.	–	*Gentiana cruciata* herb
G.g.k.	–	*Gentiana gelida* root
G.g.o.	–	*Gentiana gelida* herb
G.a.o.	–	*Gentiana asclepiadea* herb
G.a.k	–	*Gentiana asclepiadea* root

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