

Discovery of Bioactive Triterpenoid Saponins and Antioxidant-Rich Tissues in the Endemic *Cyclamen adzharicum* from Adjara (Georgia)

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Abstract

To date, the diversity and distribution of ephemeroid plants in Adjara have not been comprehensively investigated. Field surveys conducted throughout the region documented 37 ephemeroid species representing 20 genera and 10 families. Of these, 18 species (45,9%) are endemic to different phytogeographical regions, including nine Caucasian endemics, four Colchic endemics, two Georgian endemics, two Adjara–Lazeti endemics. The most species-rich families were Amaryllidaceae (9 species), Ranunculaceae (8 species), Primulaceae (6 species), and Asparagaceae (5 species).. This high level of endemism underscores the exceptional conservation value of the region and confirms Adjara as one of the principal centers of plant diversity within the Caucasus biodiversity hotspot. Among the recorded taxa, the endemic species *Cyclamen adzharicum* (*Cyclamen coum* subsp. *caucasicum* (K.Koch) O.Schwarz), as a medicinal and CITES-listed species was selected for phytochemical investigation.

phytochemical analysis revealed that the leaves contained considerably higher levels of total phenolics, flavonoids, and catechins and exhibited stronger antioxidant activity than tubers, indicating tissue-specific accumulation of bioactive metabolites. Phytochemical analysis identified three major triterpenoid saponins—Myrabilin lactone, Cyclacoumin, and Desglucocyclamin These findings provide new information on the bioecology and phytochemical profile of *C. adzharicum* and highlight its potential value for conservation and future pharmacological research.

The results demonstrate the exceptional diversity, endemism, and ecological importance of Adjara's ephemeroid flora while highlighting increasing anthropogenic threats. The findings provide valuable baseline data for biodiversity conservation, sustainable utilization, and future ecological and phytochemical research on endemic geophytes of the Colchic region.

1. Introduction

The Floristic Region of Adjara is located in the southwestern part of Georgia, forming part of the Caucasus ecoregion. It belongs to the Colchic botanical-geographical province. The region is bordered on the north, east, and south by high mountain ranges—namely, the Arsiani, Adjara-Imereti, and Adjara-Trialeti ridges—while it opens to the west and is adjacent to the Black Sea (Nakhutsrishvili, 2013; UNESCO World Heritage Centre, 2021).

The mountainous surroundings protect Adjara from abrupt temperature fluctuations, and the Black Sea contributes to high levels of precipitation and humidity. These conditions have promoted the richness and diversity of the local flora. Due to such favorable climatic and geological factors, Adjara was largely unaffected by glaciations during the Tertiary and Quaternary periods, nor did it undergo sudden environmental shifts. As a result, many thermophilic (heat-loving) species survived in the region, making Adjara one of the prominent refugia in the Caucasus for relic thermophilic species (Nakhutsrishvili, 2013; Denk, Shatilova et al., 2022).

Modern-day Adjara serves as a significant sanctuary for relict species from the Tertiary period. Accordingly, the region has been included in the globally recognized Caucasus "hotspot" and is listed among the 200 ecoregions of global significance by the World-Wide Fund for Nature (WWF, 1997), characterized by extraordinary relic plant diversity, a high level of endemism, unique patterns of floristic evolution, and globally rare biomes.

Currently, the flora of Adjara comprises 1837 species, distributed across 159 families and 742 genera (Manvelidze et al., 2008). Among these, a notable group includes species that bloom in late winter to early spring or in autumn-winter, often forming conspicuous plant communities (aspects) that decorate the landscapes of the coastal slopes and foothills. These species play a dominant role in the formation of spring or autumn synusia. The majority of them are ephemeroïds.

Ephemeroïds represent a unique ecological group of shade-loving relict plants that undergo rapid vegetative growth, flowering, and fruiting before the forest canopy fully develops, after which they enter a dormant state. Their life cycle consists of three phases: a latent (dormant) phase, a pre-generative phase, and a generative phase (Shorina, Smirnova, 1985). The latent period lasts approximately 7–8 months, while the pre-generative and generative phases span 4–5 months.

The primary ecological factors influencing the life cycle of ephemeroïds—especially the timing of flowering—are meteorological conditions, particularly temperature, humidity and precipitation levels. Ephemeroïds include certain tuberous, tuber-bulbous, bulbous, and rhizomatous geophytes that have retained and repeat the developmental rhythm evolved during the Tertiary period. Among them are species with nectariferous, ornamental, medicinal, and edible value, though most are valued primarily for their ornamental qualities (Lapointe, 2001; Kleopov, 1941).

Currently, a high level of urbanization and increasing anthropogenic pressure—such as grazing, agriculture, construction, and commercial collection—pose a threat to the survival of these species in their natural habitats, pushing some toward extinction (Veatch et al., 2017).

As of today, three ephemeroïd species found in Adjara—*Leucojum aestivum* L., *Narcissus poeticus* L., and *Primula auriculata* Lam.—are included in the IUCN Red List. Two species, *Cyclamen adzharicum* and *Galanthus voronovii*, which are of commercial value, are listed in the CITES Appendix for endangered wild plant species under international trade regulations (<https://www.cites.org/>). Additionally, *Scilla winogradowii* and *Pulsatilla aurea* are listed in the Caucasus Red Data Book; *Galanthus alpinus* and *Leucojum aestivum* appear in the Turkish Red List; and *Galanthus krasnovii* and *Galanthus rizehensis* are included in the USSR Red Book.

1.1. Aim and Objectives

The aim of this study was to investigate the species diversity, taxonomic composition, endemism, and distribution patterns of ephemeroïd plants occurring in the floristic region of Adjara (Georgia), as well as

to carry out a phytochemical investigation of the leaves, flowers, and tubers of the endemic species *Cyclamen adzharicum* using gas–liquid chromatography (GLC).

The objectives of the present study were to:

- conduct a comprehensive survey of ephemeroïd plant diversity in Adjara and evaluate their taxonomic composition, distribution, endemism, and bioecological characteristics;
- identify an endemic medicinal species suitable for phytochemical investigation;
- characterize the triterpenoid saponin profile of *Cyclamen adzharicum* tubers using UPLC–PDA/MS;
- To evaluate the contents of essential oils, total phenolic compounds, flavonoids, catechins, and antioxidant activity in the leaves, flowers, and tubers of *Cyclamen adzharicum*. determine the contents of total phenolic compounds, flavonoids, catechins, and antioxidant activity in different plant organs;
- compare different extraction methods and storage conditions for their effects on the recovery of saponins

2. Materials and Methods

Field studies were conducted between 2015 and 2025 at altitudes ranging from 25 to 2025 meters above sea level, covering the Kakhaberi lowlands, adjacent coastal slopes, as well as oak-hornbeam forests of the lower forest belt, pine-oak forests of the middle belt, and fir-oak and fir-spruce forests of the upper belt. The route expedition method was employed for the field surveys.

Phytochemical analyses were performed at the Chromatographic Center of Western Georgia, affiliated with Shota Rustaveli State University of Batumi.

For species identification, the following floristic keys were used:

- *Key to the Plants of Adjara* (Dmitrieva, 1990. I. II),
- *Key to the Plants of Georgia* (Ketskhoveli, 1964, 1969),
- *Flora of Georgia* (Ketskhoveli et al., 2011).

Bioecological characteristics were studied using phenological observation methods (Serebryakov, 1964).

Latin names of species are cited according to the international nomenclatural index of the [www.World Flora Online](http://www.WorldFloraOnline.org) (2026).

For compound identification, the Ultra-High Performance Liquid Chromatography (UHPLC) method was used, specifically the Waters Acquity UPLC-PDA system.

2.1. Scientific Novelty

The scientific novelty of the present study lies in the first comprehensive investigation of the ephemeroid flora of Adjara combined with the bioecological and phytochemical characterization of the endemic species *Cyclamen adzharicum*. A total of 37 ephemeroid species belonging to 20 genera and 10 families were documented, and their taxonomic composition, endemism, ecological distribution, and practical significance were evaluated.

For the first time, the essential oil composition of *Cyclamen adzharicum* flowers was determined, and its major volatile constituents were identified by gas–liquid chromatography. In addition, the phytochemical profile of different plant organs was investigated, revealing the distribution of phenolic compounds, flavonoids, catechins, anthocyanins, and antioxidant activity.

Furthermore, UPLC–PDA/MS analysis enabled the identification of the principal triterpenoid saponins in the tubers of *Cyclamen adzharicum*, providing new information on the chemical composition of this endemic species. The obtained results expand current knowledge of the bioecology and phytochemistry of *C. adzharicum* and provide a scientific basis for its conservation and future pharmacological exploitation.

3. Result and discussion

According to our research, 37 ephemeroid plant species grow in the studied areas of Adjara. These species belong to 20 genera and 10 families. Among them, 14 species are bulbous, 12 are tuberous, 8 are rhizomatous, and 2 are tuber-tuberous. Out of the total, 18 species (45.9.%) are endemic, including 12 species endemic to the Caucasus, 4 to Colchis, 2 to Georgia, and 1 to the Adjara-Lazeti region. In terms of practical value: 20 species are ornamental; 8 species have both ornamental and medicinal value; 8 species are ornamental and edible; 4 species are used solely as food. In terms of vertical distribution: 10 species are found across all forest belts; 2 species occur in the lower and middle mountain belts; 3 species are distributed in the middle and upper belts; 3 species are present in the upper forest and subalpine belts; 4 species occur from the coastal lowlands to the middle belt; 14 species are found in subalpine meadows; and 4 species are distributed on coastal slopes (see Table 1).

Four species bloom in autumn: *Colchicum speciosum*, *Colchicum umbrosum*, *Crocus vallicola*, and *Crocus speciosus*. The remaining species bloom in late winter to early spring.

Table 1. Annotated List of Ephemeroide Species Distributed in Adjara

N	Family	Species	Geophyte	Distribution	Significance Endemism
1	Class: <i>Magnoliopsida</i> Family: <i>Brassicaceae</i>	<i>Cardamine</i> <i>quinquefolia</i> (M.Bieb.) Benth	Rhizomatous	Entire forest belt. Forest edges	Ornamental
2	Papaveraceae	<i>Corydalis caucasica</i> DC	Caucasian Tuberous	Forest edges up to the middle belt	Ornamental
3	Papaveraceae	<i>C. solida</i> (L.)Clav.	Tuberous	Beech and fir-spruce forests up to 1800 meters	Ornamental
4	<i>Ranunculaceae</i>	<i>Anemonastrum fasciculatum</i> (L.) Holub (<i>Anemone fasciculata</i> L. A)	Tuberous	Upper montane and subalpine belts, meadows, and shrublands	Ornamental
5	<i>Ranunculaceae</i>	<i>Anemonoides caucasica</i> (Rupr.) H. (<i>Anemone caucasica</i>)	Tuberous	Upper montane and subalpine belts, forest edges	Ornamental Caucasian
6	<i>Ranunculaceae</i>	<u><i>Anemonoides blanda</i> (Schott & Kotschy).</u> <u>Holub</u> (<i>Anemone blanda</i>)	Tuberous	From coastal lowlands to the middle montane belt, along forest edges	Ornamental
7	<i>Ranunculaceae</i>	<i>Ranunculus kochii</i> Lebed. (<i>Ficaria calthifolia</i> var. <i>adzharica</i>)	Tuberous	From coastal lowlands to the middle montane belt — forest edges, fields, and gardens	Ornamental Caucasian
8	<i>Ranunculaceae</i>	<i>Ranunculus cappadocicus</i> Willd. <i>Ranunculus ampelophyllus</i>	rooted	From coastal lowlands to the entire	Ornamental Colchian

				montane belt, in forest habitats	
9	<i>Ranunculaceae</i>	<i>Pulsatilla</i> <i>Paurea (N.Busch) Juz</i>	Tuberous	Upper montane and subalpine meadows and shrublands	Ornamental Colchian
10	<i>Ranunculaceae</i>	<i>P. albana</i> Bercht. & J. Presl	Tuberous	Subalpine cliffs	Ornamental Caucasian
11	<i>Ranunculaceae</i>	<i>P. violacea</i> Rupr	Tuberous	Subalpine meadows and cliffs	Ornamental Georgia
12	<i>Primulaceae</i>	<i>Cyclamen adzharicum</i> Pobed (<i>Cyclamen coum</i> subsp. <i>caucasicum</i> (K.Koch). O. Schwarz)	Tuberous	Coastal slopes and forests across the entire montane belt	Ornamental, medicinal Adjara
13	<i>Primulaceae</i>	<i>Primula auriculata</i> Lam.	rooted	Subalpine meadows	IUCN LC Ornamental
14	<i>Primulaceae</i>	<i>Primula algida</i> Adams	rooted	Subalpine meadows	Ornamental
15	<i>Primulaceae</i>	<i>P.pseudodenticulata</i> Pax	rooted	Subalpine meadows	Ornamental
16	<i>Primulaceae</i>	<i>P. amoena</i> M.Bieb.	rooted	Subalpine meadows	Ornamental
17	<i>Primulaceae</i>		rooted	Coastal slopes, upper forest zone valleys	Ornamental, medicinal
18	🌱🌱🌱 Liliopsida <i>Amaryllidaceae</i>	<i>Allium ursinum</i> L.,	Bulbous	Valleys of the lower and middle mountain belts	Ornamental, edible
19	<i>Amaryllidaceae</i>	<u><i>Allium pseudostrictum</i> Alb</u>	Bulbous	Subalpine meadows	Ornamental, edible

20	<i>Amaryllidaceae</i>	<i>Allium schoenoprasum</i> L.	Bulbous	Subalpine meadows and shrublands	Ornamental, edible
21	<i>Amaryllidaceae</i>	<i>Galanthus alpinus</i> Sosn.	Bulbous	Rare; of the mid and upper mountain zones.	Ornamental, medicinal Caucasian
22	<i>Amaryllidaceae</i>	G.krasnovii A.P. Khokhr.	Bulbous	Rare beech forest, within the 800-1000 m range.	Ornamental, medicinal Adjara-Lazeti
23	<i>Amaryllidaceae</i>	G.rizechensis Stern.	Bulbous	Rare coastal slopes	Ornamental, medicinal Adjara-Lazeti
24	<i>Amaryllidaceae</i>	G.woronowii Losink.	Bulbous	Rare From coastal slopes up to the upper forest zone.	Ornamental, medicinal Georgia
25	<i>Amaryllidaceae</i>	<i>Leucojum aestivum</i> L.,	Bulbous	Rare coastal lowlands and marshes.	IUCN LC. Ornamental
26	<i>Amaryllidaceae</i>	<i>Narcissus poeticus</i> L.	Bulbous	Coastal lowlands and slopes	IUCN LC. Ornamental
27	<i>Asparagaceae</i>	<i>Muscari armeniacum</i> H.J. Veitch	Bulbous	Rare; in a pine-oak forest in the Acharistskali valley, at 30-900 m.	Ornamental Colchian

28	<i>Asparagaceae</i>	<i>Convallaria majalis</i> L.	rooted	It is rare. Lower forest belt	Ornamental, medicinal Caucasuan
29	<i>Asparagaceae</i>	<i>Ornithogalum balansae</i> Boiss	Bulbous	Subalpine meadows	Ornamental Caucasian
30	<i>Asparagaceae</i>	<i>O.woronowii</i> Krasc	Bulbous	Coastal slopes and the lower mountain zone	Ornamental Caucasian
31	<i>Asparagaceae</i>	<i>Scilla monanthos</i> <i>C.koh</i> (<i>Scilla winogradowii</i>)	Bulbous	The entire forest belt	Ornamental Colchian
32	<i>Colchicaceae</i>	<i>Colchicum speciosum</i> Steven	Cormous Autumn-flowering	From the mid-forest zone up to the subalpine zone.	Ornamental, medicinal
33	<i>Colchicaceae</i>	<i>C. umbrosum</i> Steven.	Cormous Autumn-flowering	Mid-forest zone. Forest edges, shrublands.	Ornamental, medicinal
34	<i>Iridaceae</i>	<i>Crocus vallicola</i> Herb.	Cormous Autumn-flowering	Upper mountain and subalpine zones. Meadows.	Ornamental, edible
35	<i>Iridaceae</i>	<i>C.speciosus</i> M.Bieb.	Cormous Autumn-flowering	Mid and upper forest zones. Meadows.	Ornamental, edible
36	<i>Liliaceae</i>	<i>Gagea fragifera</i> (Vill.)E.Baier.	Bulbous	Subalpine meadows	Ornamental, edible
37	<i>Poaceae</i>	<i>Poa bulbosa</i>	rooted	Coastal lowlands	Ornamental, edible

Among the ephemeroids, a species of particular medicinal and decorative value is the tuberous, endemic, winter-early spring flowering plant *Cyclamen adzharicum* Pobed (*Cyclamen coum* subsp. *caucasicum*) shown in Figure 1 & 2 (Makaradze et al., 2019). This species is distributed at altitudes ranging from 20 to 900 meters above sea level. It grows on acidic soils (pH 4.9–5.0) within hornbeam-oak, hornbeam-chestnut, and chestnut-pine forests, as well as on forest edges, shrublands, moist slopes, and rocky outcrops. The vegetative period begins in late November, with flowering occurring in January–February. Fruiting occurs in March, and seed dispersal and senescence take place in April.

In folk medicine, juice extracted from the tubers of *Cyclamen adzharicum* is used by the local population to treat sinusitis, inflammations of the nasal mucosa, and chronic maxillary sinus infections (Zalmanovici, et al., 2018).

For the first time in Georgia, the essential oil composition of *Cyclamen adzharicum* (*Cyclamen coum* subsp. *caucasicum*) flowers was investigated. Essential oils were isolated by hydrodistillation, and their molecular composition was analyzed using gas–liquid chromatography (GLC). A total of eight saturated hydrocarbons, three organic acids, and two complex volatile compounds were identified in the essential oil. The saturated hydrocarbons included 3-methyleicosane ($C_{21}H_{44}$), heneicosane ($C_{21}H_{44}$), hexyldocosane ($C_{28}H_{58}$), heptacosane ($C_{27}H_{56}$), 2-methyltricosane ($C_{24}H_{50}$), hexacosane ($C_{26}H_{54}$), 3-ethyltetracosane ($C_{26}H_{54}$), and heptacosane ($C_{27}H_{56}$).

These long-chain hydrocarbons are widely used in cosmetic formulations as emollients and moisturizing agents. For example, hexyldocosane is commonly incorporated into hair-care products because it improves hair softness and enhances gloss. Three fatty acids were also identified: palmitic acid ($C_{16}H_{32}O_2$), linoleic acid ($C_{18}H_{32}O_2$), and stearic acid ($C_{18}H_{36}O_2$). These compounds are widely used in cosmetic and personal-care products owing to their emollient and skin-conditioning properties. In addition, two volatile compounds responsible for the characteristic fragrance of *Cyclamen adzharicum* essential oil were detected. One of these compounds, 3,7,7-trimethyl-1-(penta-1,3-dienyl)-2-oxabicyclo[3.2.0]hept-3-ene, as shown in Figure 3 possesses a complex bicyclic structure with terpene-like characteristics and is considered to contribute to the pleasant aroma of the essential oil. Compounds with similar structural features are commonly used in the fragrance and perfume industry.

Phytochemical analysis of *Cyclamen adzharicum* tubers resulted in the identification of the following triterpenoid saponins.

Compound 1 showed a protonated molecular ion at m/z 1083.30 $[M + H]^+$ with a retention time of 7.189 min. Based on comparison with the literature data, this compound was identified as **Desglucocyclamin I**. Positive FABMS analysis gave a molecular ion at m/z 1083 $[M + Na]$. Desglucocyclamin I was detected in all analyzed samples and represented the predominant saponin.

Compound 2 exhibited a molecular ion at m/z 1099.38 $[M + Na + H]^+$ with a retention time of 7.091 min. Comparison with published data allowed its identification as **Cyclacoumin**. Positive FABMS analysis

showed an ion at m/z 1099 [M + Na]. This compound was detected in the Ortabatumi and Tsoniarisi samples.

Compound 3 showed a molecular ion at m/z 1245.17 [M + Na + H]⁺ with a retention time of 7.223 min and a maximum UV absorption at 221 nm. According to the spectral characteristics and literature data, this compound was identified as **Myrabilin lactone**. Positive FABMS analysis gave an ion at m/z 1243 [M + Na]. Myrabilin lactone was detected only in tubers collected from Tsoniarisi and stored at low temperature.

Phytochemical investigations demonstrated that the leaves of *Cyclamen adzharicum* contain total phenolic compounds, flavonoids, catechins, and anthocyanins. Among the investigated populations, plants collected from Ortabatumi exhibited the highest antioxidant activity. The leaves contained the highest concentrations of phenolic compounds, flavonoids, and catechins, whereas the highest anthocyanin content was recorded in samples collected from Tskhmorisi.

Chromatographic analysis using a conductivity detector was performed to determine the qualitative and quantitative composition of mineral cations. Five cations were identified, with potassium (K⁺) being the predominant ion in all analyzed samples.

4. Conclusions

1. A comprehensive floristic survey conducted in Adjara documented 37 ephemeroid species belonging to 20 genera and 10 families, confirming the region as an important center of ephemeroid diversity within the Caucasus biodiversity hotspot. More than half of the recorded species are endemic, emphasizing their high conservation value.
2. The endemic species *Cyclamen adzharicum* exhibited distinct bioecological characteristics, including a well-defined phenological cycle and adaptation to acidic forest soils of the lower montane belt. Its restricted distribution and protected status further underline the necessity for conservation and sustainable management.
3. Phytochemical investigation of *Cyclamen adzharicum* demonstrated that the leaves are a rich source of phenolic compounds, flavonoids, catechins, and anthocyanins and possess pronounced antioxidant activity, indicating tissue-specific accumulation of bioactive metabolites.
4. UPLC–PDA/MS analysis of tuber extracts identified three major triterpenoid saponins—Desglucocyclamin I, Cyclacoumin, and Myrabilinlactone. Desglucocyclamin I was the predominant constituent and was detected in all analyzed samples, whereas Cyclacoumin and Myrabilinlactone showed population- and storage-dependent distribution patterns.
5. Essential oil analysis of *Cyclamen adzharicum* flowers revealed a diverse composition of saturated hydrocarbons, fatty acids, and volatile aroma compounds, suggesting potential applications in cosmetic, pharmaceutical, and fragrance industries.
6. The obtained results provide new information on the floristic diversity, bioecology, and phytochemical profile of *Cyclamen adzharicum*. These findings establish a scientific basis for the

conservation, sustainable utilization, and future pharmacological investigation of this endemic species and contribute to the understanding of the biodiversity of the Colchic region.

Declarations

Conflict of Interest

The authors declare that they have no known financial or personal conflicts of interest that could have appeared to influence the work reported in this paper.

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Author Contribution

N.V. conceived and designed the study, coordinated the research, conducted field investigations, interpreted the results, and wrote the main manuscript text. A.K. and M.V. performed the phytochemical and chromatographic analyses, contributed to data interpretation, and revised the manuscript. N.T., N.Z., K.D., I.D., and D.B. participated in field surveys, specimen collection, species identification, and data collection. E.J. contributed to the phytochemical investigation, interpretation of the analytical results, and manuscript revision. All authors reviewed, edited, and approved the final version of the manuscript.

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Figures



Figure 1

Cyclamen adzharicum Pobed.



Figure 2

Cyclamen adzharicum tubers

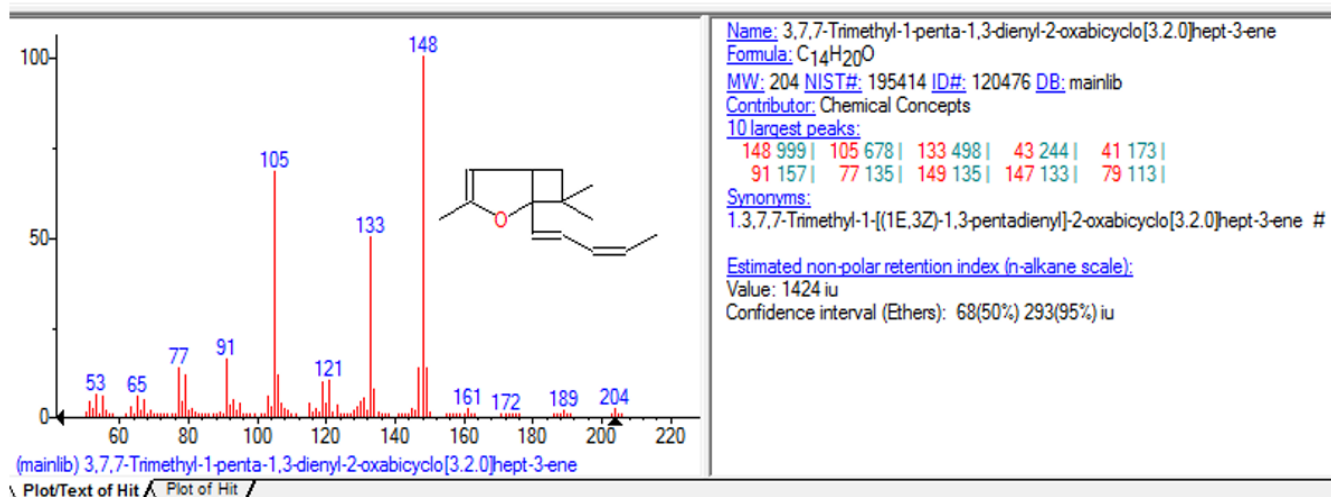


Figure 3

3,7,7-Trimethyl-1-(penta-1,3-dienyl)-2-oxabicyclo[3.2.0]hept-3-ene