

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHARMACOGNOSY

**ISOLATION OF SECONDARY METABOLITES
FROM *GENTIANA ASCLEPIADEA* AND *G.
CRUCIATA* AND EVALUATION OF THEIR ANTI-
INFLAMMATORY AND ANALGESIC ACTIVITIES**

DOCTOR OF PHILOSOPHY THESIS

RİMA KONYA, M.Sc. Pharm.

İstanbul-2023

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RİMA KONYA, M.Sc. Pharm.

SUPERVISOR
Prof. Dr. Hasan KIRMIZIBEKMEZ

İstanbul-2023

THESIS APPROVAL

Institute : Yeditepe University Institute of Health Sciences
Programme : Pharmacognosy
Title of the Thesis : Isolation of Secondary Metabolites from *Gentiana asclepiadea* and *G. cruciata* and Evaluation of Their Anti-inflammatory and Analgesic Activities
Owner of the Thesis : Rima Konya
Examination Date : 19.06.2023

This study has been approved as a Doctorate Thesis in regard to content and quality by the Jury.

	Title, Name-Surname (Institution)	Signature
Chair of the Jury:	Prof. Dr. Erdem Yeşilada Yeditepe University	
Supervisor:	Prof. Dr. Hasan Kırmızıbekmez Yeditepe University	
Member/Examiner:	Prof. Dr. Leyla Bitiş Marmara University	
Member/Examiner:	Assoc. Prof. Dr. Muhammed Hamitoğlu Yeditepe University	
Member/Examiner:	Asst. Prof. Dr. Dicle Çevik Trakya University	

APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated and numbered

Director of Institute of Health Sciences

Prof. Dr. Bayram YILMAZ

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

19/06/2023

Rima Konya



DEDICATION

*I dedicate this thesis to my lovely family,
for their endless support, encouragement and guidance.*

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LIST OF SYMBOLS AND ABBREVIATIONS

1D	1-Dimensional
2D	2-Dimensional
Ac	Acetyl
ANOVA	Analysis of Variance
br	Broad
BuOH	Butanol
Bz	Benzoyl
Calcd	Calculated
CC	Column Chromatography
CD ₃ OD	Deuterated Methanol
CH ₂ Cl ₂	Dichloromethane
CHCl ₃	Chloroform
COSY	Correlation Spectroscopy
COX	Cyclooxygenase
d	Doublet
DMEM	Dulbecco's Modified Eagle's Medium
EtOAc	Ethyl Acetate
Fr/Frs	Fraction/Fractions
GABA	Gamma-Aminobutyric Acid
Gen	Gentiobiose
Glc	Glucose
H ₂ SO ₄	Sulfuric acid
HMBC	Heteronuclear Multi-Bond Correlation
HR-ESI-MS	High Resolution-Electrospray Ionisation- Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
IC ₅₀	The Half Maximal Inhibitory Concentration
IL	Interleukin
iNOS	Inducible Nitric Oxide Synthase
IR	Infrared Spectroscopy
LPS	Lipopolysaccharide
m	Multiplet

MeOH	Methanol
MPLC	Medium Pressure Liquid Chromatography
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
MW	Molecular Weight
NF- κ B	Nuclear Factor kappa B
ni	No Inhibition
NMR	Nuclear Magnetic Resonance
NO	Nitric Oxide
NOESY	Nuclear Overhauser Enhancement Spectroscopy
PG	Prostaglandin
Prim	Primveroside
rH ₂ O	Remaining H ₂ O
ROS	Reactive Oxygen Species
Rut	Rutinoside
s	Singlet
SiO ₂	Silicon dioxide (silica gel)
TLC	Thin Layer Chromatography
TNF	Tumor Necrosis Factor
UV	Ultraviolet
Vis	Visible
WHO	World Health Organisation
Xyl	Xylose

ABSTRACT

Konya, R. (2023). Isolation of Secondary Metabolites from *Gentiana asclepiadea* and *G. cruciata* and Evaluation of Their Anti-inflammatory and Analgesic Activities. Yeditepe University, Institute of Health Sciences, Department of Pharmacognosy, Ph.D. Thesis, İstanbul.

Chronic inflammatory diseases account for nearly half of all deaths worldwide and the potential adverse effects of current treatment protocols necessitate to develop safer alternatives. Over recent years, plant extracts and plant-derived drugs have gained more attention as natural therapeutic agents. Members of the genus *Gentiana* have long been used for the prevention and treatment of a wide variety of diseases. Extracts obtained from this genus, as well as the isolates, were reported to possess anti-inflammatory and analgesic activities according to the latest findings. The aim of this thesis was to purify the compounds from the MeOH extracts prepared from the aerial parts of *Gentiana asclepiadea* L. and *G. cruciata* L. and to evaluate their *in vitro* anti-inflammatory and analgesic activities. A new secoiridoid glycoside, cruciatoside (**1**), along with ten known compounds, eustomoside (**2**), eustomorusside (**3**), gentiopicroside (**5**), 6'-*O*- β -D-glucosyl gentiopicroside (**6**), loganic acid (**7**), isoorientin (**8**), isovitexin (**9**), isovitexin 2''-(*E*)-ferulate (**10**), mangiferin (**11**) and 2-methyl inositol (**13**) were isolated from *G. cruciata*, while depressine (**4**), compounds **5**, **7-9**, **11** and adenosine (**12**) were obtained from *G. asclepiadea*. The structures were elucidated mainly by HR-MS, 1D and 2D NMR experiments. The anti-inflammatory activities of the isolated compounds were evaluated through nitrite and IL-6 levels in RAW 264.7 cells activated by LPS. Among all the tested compounds, **10** exerted the most potent nitrite inhibition with 39.5% rate at 200 μ M, comparable to positive control indomethacin. Also, compounds **4**, **5**, and **8** exhibited a moderate nitrite inhibition over 20%. On the other hand, **4** showed the highest decrease in IL-6 levels among all the tested compounds, which was followed by compounds **5**, **8** and **1**, respectively. Compounds **4** and **5** displayed the most potent *in vitro* analgesic activity by decreasing PGE₂ secretion from LPS-activated RAW264.7 cells, comparable to the positive control, indomethacin. Consequently, compounds **1**, **4**, **5**, **8**, and **10** were determined as potent anti-inflammatory and analgesic agents, which deserve further *in vitro* and *in vivo* investigations.

Key Words: *Gentiana cruciata*, *G. asclepiadea*, secoiridoid, flavonoid, anti-inflammatory and analgesic activities

ÖZET

Konya, R. (2023). *Gentiana asclepiadea* ve *G. cruciata*'dan Sekonder Metabolitlerin İzolasyonu, Antienflamatuvar ve Analjezik Aktivitelerinin Araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Farmakognozi Anabilim Dalı, Doktora Tezi, İstanbul.

Kronik enflamatuvar hastalıklar dünya çapındaki tüm ölümlerin yaklaşık yarısını oluşturmaktadır ve güncel tedavi protokollerinin advers etkileri daha güvenli alternatifler geliştirilmesini gerekli kılmıştır. Son yıllarda bitki ekstraları ve bitkisel kaynaklı ilaçlar doğal terapötik ajanlar olarak dikkat çekmektedir. *Gentiana* türleri birçok hastalığın tedavisinde ve önlenmesinde uzun zamandır kullanılmaktadır. Son bulgulara göre, bu cinsten elde edilen ekstraların ve bileşiklerin antienflamatuvar ve analjezik özelliklere sahip olduğu bildirilmiştir. Bu tez çalışmasının amacı *Gentiana asclepiadea* L. ve *G. cruciata* L. türlerinin toprak üstü kısımlarından hazırlanan MeOH ekstralarından bileşiklerin saflaştırılması, *in vitro* antienflamatuvar ve analjezik aktivitelerinin araştırılmasıdır. *G. cruciata* türünden yeni bir sekoiridoit glikoziti olan krusiyatozit (1) ve bilinen 10 bileşik, östomozit (2), östomoruzit (3), gentiopikrozit (5), 6'-O- β -D-glukozil gentiopikrozit (6), loganik asit (7), izoorientin (8), izoviteksin (9), izoviteksin 2''-(E)-ferulat (10), mangiferin (11) ve 2-metil inozitol (13) izole edilmişken, *G. asclepiadea* türünden depressin (4), bileşikler 5, 7-9, 11 ve adenosin (12) elde edilmiştir. Bileşiklerin yapıları başlıca HR-MS, 1D ve 2D NMR teknikleri ile aydınlatılmıştır. İzole edilen bileşiklerin antienflamatuvar aktiviteleri, LPS tarafından indüklenmiş RAW 264.7 hücrelerinde nitrit ve IL-6 seviyeleri aracılığıyla incelenmiştir. Test edilen bileşiklerden 10, 200 μ M konsantrasyonda %39.5 nitrit inhibisyonuyla, pozitif kontrol olan indometazin ile mukayese edilebilecek şekilde en güçlü aktiviteyi göstermiştir. Ayrıca, bileşikler 4, 5 ve 8 de %20'nin üzerinde bir nitrit inhibisyonu yapmıştır. Diğer taraftan, tüm bileşikler arasından bileşik 4, IL-6 seviyesinde en fazla düşüşe neden olmuş ve bunu sırasıyla 5, 8 ve 1 izlemiştir. Bileşikler 4 ve 5 LPS ile indüklenmiş RAW264.7 hücrelerinde PGE₂ salınımını pozitif kontrol olan indometazin ile kıyaslanabilir şekilde en fazla azaltarak en güçlü *in vitro* analjezik aktiviteyi göstermiştir. Sonuç olarak, 1, 4, 5, 8 ve 10 güçlü antienflamatuvar ve analjezik etkili bileşikler olarak daha ileri *in vitro* ve *in vivo* araştırmaları hak etmektedir.

Anahtar kelimeler: *Gentiana cruciata*, *G. asclepiadea*, sekoiridoit, flavonoit, antienflamatuvar ve analjezik aktivite

1. INTRODUCTION AND AIM

Inflammation is a protective response of the body to injurious stimuli, including pathogens, toxicants, or irradiation. It can be considered as a complex defense process that aims to remove harmful stimuli and initiate tissue recovery (1). Understanding the role of inflammation in developing major diseases is crucial in combatting present-day morbidity and mortality. Globally, around 50% of deaths are thought to be inflammatory diseases, including cardiovascular diseases, cancer, kidney diseases, and many autoimmune and neurodegenerative disorders (2). Due to the duration of the response, inflammation can be classified as acute or chronic. Acute inflammatory response can be caused by infections or tissue injuries. It is the initial response to the stimuli that lead to the recognition of infection and the generation of a variety of inflammatory mediators. Chronic inflammation is generally associated with the dysfunction of tissues and homeostatic imbalance. Nevertheless, unresolved acute inflammation may also become chronic over time (3).

Pain is a distressing sensation due to harmful stimuli and also a cardinal sign of inflammation. Inflammatory responses in the peripheral and central nervous systems play a crucial role in the pathophysiology of pain (4)). Current treatment strategies for inflammatory diseases are limited and sometimes ineffective; they are usually used to relieve symptoms instead of treating underlying reasons. Non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids are commonly prescribed as treatment options for inflammatory diseases. However, their potentially dangerous side effects and interactions with conventional treatments necessitate to discovery and development of safer alternatives (5).

Natural products have long been consumed for their medicinal benefits from antiquity and plant remedies are invaluable medical resources due to their chemical diversity and therapeutic potential (6). Numerous plant-derived compounds were shown to possess anti-inflammatory and anti-nociceptive properties. Amongst, several secondary metabolite groups, such as polyphenolic compounds (i.e. flavonoids, hydroxycinnamic acid derivatives, and stilbenoids), terpenoids, and alkaloids were found effective against inflammation (7).

The genus *Gentiana* L. (Gentianaceae) is a large genus containing around 400 species, mainly distributed in Southeast Asia, Europe, and North America (8). Previous

phytochemical and biological studies on different extracts of the genus *Gentiana*, as well as the isolated compounds indicated their antioxidant, antiatherosclerotic, anti-inflammatory, antityrosinase, cardioprotective, gastroprotective, hepatoprotective, and immunomodulatory activities (9). Moreover, the roots of *G. lutea* (*Gentiana radix*) are described as an official drug and listed in many pharmacopoeias, including European Pharmacopoeia, British Pharmacopoeia, and Japanese Pharmacopoeia (10). Over 500 secondary metabolites from *Gentiana* species were isolated, mainly belonging to iridoids, xanthenes, flavonoids, triterpenoids, and alkaloids (8).

The genus *Gentiana* is represented by 12 species in the flora of Türkiye (11). Among these species, *G. cruciata* L. and *G. asclepiadea* L. are traditionally used as tonic and appetizer, also against fever and kidney pain (12,13). Recent studies on these two species revealed their antioxidant, antibacterial, and hepatoprotective activities (14–18). However, there are limited studies on the purification of the potential bioactive secondary metabolites from both species. Therefore, this thesis aimed to purify and establish the chemical structures of secondary metabolites from the above-ground parts of *G. asclepiadea* and *G. cruciata* growing wild in Türkiye and to evaluate their *in vitro* anti-inflammatory and analgesic effects. For this reason, aerial parts of both species were extracted with MeOH separately. The crude extracts were dispersed in H₂O and partitioned against CHCl₃, EtOAc, and *n*-BuOH, respectively to yield subextracts. EtOAc and *n*-BuOH subextracts of both species were submitted to multiple chromatographic separations to isolate bioactive compounds. The chemical structures of the isolates were determined with the help of 1D and 2D NMR experiments, as well as HR-MS. Moreover, *in vitro* anti-inflammatory and analgesic activities of the isolated compounds were assessed in LPS-induced RAW 264.7 murine macrophage cells.

2. GENERAL DESCRIPTION

2.1. General Information on the Genus *Gentiana* L.

2.1.1. Botanical Information

2.1.1.1. Gentianaceae

Gentianaceae plants are annual, biennial, and perennial herbs. Leaves are mostly glabrous, entire, basal usually forming a rosette, cauline usually opposite and sessile, often connate at the base. The flowers of the members are 4-8-merous and actinomorphic. Calyx $\pm$ deeply divided and corolla lobes are contorted in the bud, usually glabrous. Stamens are inserted on corolla tube whereas anthers are slightly cordate at the base, basifixed or rarely versatile. Ovary is superior, unilocular, with 2 parietal placentae, and ovules numerous. Fruits are capsule dehiscing by 2 valves (11).

2.1.1.2. *Gentiana* L.

Members belonging to the *Gentiana* genus are mostly perennial (rarely annual) herbs. Habit various: sometimes forming $\pm$ branched creeping mats, sometimes with 1-several shoots from a fleshy rootstock. Flowers are 4-5(-9)-merous, calyx $\pm$ deeply divided and lobes are partly joined by an inner membrane. Corolla is hypocrateriform to infundibular, pale-, deep- or violet-blue, yellow or cream (rarely white), usually with smaller subsidiary lobes (plicae) alternating with main lobes; throat not fringed inside. Anthers are basifixed, ovary is tapering above to a short style, or style absent; stigmas 2, persistent. Nectaries are present at the base of ovary. Capsule is ellipsoid to oblong, stipitate or sessile (11).

The family Gentianaceae has more than 1700 species belonging to 90 genera with a wide variety of habitats, morphology, and ecology. The genus *Gentiana* is the largest genus of the Gentianaceae family containing over 400 species (19). There are 12 species growing wild in the flora of Türkiye (11).

The dichotomous key for *Gentiana* species growing in Türkiye:

1. Basal leaves very large (to 40 x 25 cm); corolla divided to more than 1/2, bright yellow

1. *lutea*

1. Basal leaves to 15x3 cm (often much smaller); corolla divided to less than 1/2, white, pale yellow, blue, or violet

2. Stems tall, usually more than 30 cm, with axillary flowers

3. Leaves acute; flowers 5-merous; anthers connate

2. *asclepiadea*

3. Leaves obtuse; flowers 4-merous; anthers free

3. *cruciata*

2. Stems shorter, usually less than 30 cm; flowers terminal or terminal and lateral

4. Annuals

5. Corolla pale blue, lobes obtuse; capsule stipitate **5. *aquatica***

5. Corolla bright blue, lobes acute; capsule sessile **12. *nivalis***

4. Perennials

6. Rootstock sheathed with fibrous collar at apex; stem erect, 10-40 cm **4. *olivieri***

6. Rootstock not sheathed with fibrous collar at apex; stems usually procumbent or less than 10 cm

7. Corolla pale yellow **9. *gelida***

7. Corolla blue (rarely white)

8. Flowers 3 or more in a terminal head; stems simple **7. *septemfida***

8. Flowers usually solitary, terminal; stems simple or branched

9. Flowering stems with 11 or more internodes; stems simple, prostrate **8. *boissieri***

9. Flowering stems with fewer than 11 internodes; stems usually much branched at base, forming a mat

10. Subsidiary corolla lobes $\pm$ equalling main lobes, corolla appearing 10-merous; internodes $\pm$ equal **6. *pyrenaica***

10. Subsidiary lobes small, less than 1/2 length of main lobes; internodes elongating after flowering

11. Longest rosette leaves much longer than cauline leaves; Corolla (20-)28 mm or more **10. *verna***

11. Rosette leaves $\pm$ equalling cauline leaves; corolla less than 20 mm

11. *brachyphylla*

2.1.1.3. *Gentiana asclepiadea* L.

Robust herbs that can grow up to 100 cm. Stems are several and simple, of 14-20 internodes; flowers are in upper leaf axils and forming a $\pm$ elongated head. Leaves are ovate-lanceolate to lanceolate, narrower, more crowded above, and usually are 5-nerved, green. Flowers are usually 5-merous, and sessile. Calyx is 12-20 mm, divided into c. 1/2 lobes narrow, acute and spreading. Corolla is mid to deep blue, rarely white, 30-50 mm, divided to c. 1/6 lobes triangular, acute; subsidiary lobes are shorter and $\pm$ irregular. Capsule is ellipsoid, shortly stipitate (11).

Flowering season: August-September

Habitat: Grassy places and clearings in woodland

Altitude: 300-2000 m.

Distribution in Türkiye: Northern Türkiye, Southern Anatolia: Kırklareli (Mahyadağ), Kocaeli (Suadiye to Keltepe), Bolu (Bolu Mountain), Kastamonu (İlgaz Mountain), Amasya (Akdağ Mountain), Ordu (Ünye to Karakuş), Giresun (Yavuzkema), Rize (Rize to Karadere), Çoruh, Balıkesir (Çeyizderesi), Hatay (Amanus).

Worldwide distribution: Central & Southern Europe, Northern Iran, Caucasia, Europe-Siberia (11).



Figure 1. *Gentiana asclepiadea* L.

2.1.1.4. *Gentiana cruciata* L.

Robust herbs which can reach to 50 cm. Stems are simple, stout, often several from a basal rosette, of 6-10(-12) internodes; flowers forming a terminal head and also in axils of upper leaves. Leaves are 30-100 x 10-30 mm, ovate-lanceolate, subacute, 3-nerved, $\pm$ connate at base and, especially lower ones, forming a sheath. Flowers are 4-merous, sessile. Calyx is 8-15 mm, divided to c. 1/2 lobes triangular and corolla is blue, often purplish and green-marked outside, 15-30 mm; intermediate lobes irregular, short. Capsule is narrow, sessile or subsessile (11).

Flowering season: July-September

Habitat: Woodland margins and grassland

Altitude: 800-2500 m.

Distribution in Türkiye: North & Inner Anatolia. Bolu (Lake Abant), Zonguldak (Keltepe), Karabük, Sinop (Ayancik, Çangal), Ordu: (Ünye to Karakuş), Giresun (Tamdere to Yavuzkema), Gümüşhane, Kars to Ardahan, Tunceli (Pülümür), Muş, Bingöl, Van (Kavuşşahap Mountain).

Worldwide distribution: Mainly Central & Southern Europe, Northern, Central & Southern Russia, Caucasia, Northern & Western Iran (rare), Western Siberia, Europe-Siberia (11).



Figure 2. *Gentiana cruciata* L.

2.1.1.5. Local Names of *Gentiana* sp.

Gentianaceae family was named after Gentius, the last king of ancient Illyria (181-168 BC), who may have discovered the healing properties of the roots of gentian (*Gentiana lutea*) against malaria (20).

In Turkish: *Gentiana lutea* (Güşadotu, Centiyan, Sarı gentiyan, Defneyezit, Sivri kökü), *G. asclepiadea* (Mavi centiyan, Sütü güşadotu), *G. olivieri* (Afat) (12).

In Other Languages: Enzian (German), Gentiane (French). *G. asclepiadea* (willow gentian), *G. lutea* (big yellow gentian), *G. nivalis* (snow gentian), *G. macrophylla* (bigleaf gentian), *G. septemfida* (crested gentian), *G. cruciata* (cross gentian).

2.1.1.6. Traditional Use of *Gentiana* sp.

Some of the *Gentiana* sp. have long been utilized for centuries to treat a broad range of ailments. Shennong's Materia Medica Classic (25-220 AD), a Chinese book on agriculture and medicinal plants, is one of the oldest records that define the utilization of *Gentiana* species as a part of Traditional Chinese Medicine (TCM). According to this book, two herbal medicines that contain different *Gentiana* species were consumed for their liver purging and pain-relieving properties (9). These two traditional remedies are still utilized as a part of TCM. *Gentianae Radix et Rhizoma* (Longdan) contains dried roots and rhizomes of *G. manshurica*, *G. rigescens*, *G. triflora*, and *G. scabra* whereas *Gentianae Macrophyllae Radix* (Qin-Jiao) consists of *G. crassicaulis*, *G. dahurica*, *G. macrophylla*, and *G. straminea* (21). Honghualongdan, another TCM remedy, made from *G. rhodantha* was listed in Chinese pharmacopoeia and consumed as a cough reliever (9). *G. algida* is consumed to treat athlete's foot and pneumonia (22). *G. davidii* var. *formosana* is distributed widely in Taiwan and has been utilized for the treatment of gastrointestinal tract disorders, as a part of TCM (23). Leaves and roots of *G. macrophylla* and *G. scabra* have been consumed as a tonic for the digestive system (24).

Gentiana species have been also used in other Asian countries. For instance, *G. scabra* roots are commonly-used folk remedy in Korean traditional medicine to combat diabetes (25). As a part of Indian traditional medicine, the root of *G. kurroo* is frequently utilized to relieve stomach aches and urinary infections, and also employed as an astringent, expectorant, anti-inflammatory, anthelmintic, antibacterial, antipsychotic, and sedative agent (26). *G. straminea* and *G. urnula* have been used for common cold, bacterial and fungal infections, fever, hepatitis, constipation, rheumatism, pain, poisoning, and hypertension as a part of Tibetan folk traditional medicine (27,28). *G. veitchiorum* is used in Tibetan traditional medicine against liver jaundice, headache, and chronic pharyngitis (29). *Gentianae Scabrae Radix*, a Japanese crude drug, prepared from *G. scabra* var. *buergeri* has been consumed as a stomachic and appetite stimulant (30). *G. algida*, *G. barbata*, and *G. decumbens* have long been utilized as a part of Mongolian traditional medicine for wound healing, against fever and cough, and to treat lung, liver, and bile disorders (31). One of the species native to the Himalaya Region, *G. floribunda* is used against gastrointestinal, respiratory, hepato-biliary, and neurological problems (32). *G. olivieri* has been consumed for a wide variety of conditions, such as common cold, diarrhea, stomachache, and wound healing in Uzbekistan Republic (33).

In Europe, *Gentiana* species are commonly preferred as a part of traditional medicine throughout the Balkan Peninsula. In Montenegro, roots and aerial parts of *G. lutea*, *G. asclepiadea*, *G. kochina*, *G. cruciata*, and *G. punctata* have been utilized as an appetizer, stomachic and against gall and liver disorders (34). In Bosnia-Herzegovina, *G. asclepiadea*, *G. lutea*, and *G. symphandra*, *G. punctata* have been used against flatulence, appetite loss, and influenza infections, and also for cardiovascular, liver, and gall disorders (35). In Serbia, *G. lutea*, *G. asclepiadea*, *G. cruciata*, and have been consumed against various health issues such as high cholesterol, diabetes, diarrhea, and stomach ulcers and to improve the immune system (36–39). In Norway, roots of *G. purpurea* were utilized to treat diarrhea, colic, loss of appetite, bronchitis, and cough (40). In Italy, *G. kochina* roots have been employed for loss of appetite, gastric atony, and flatulence whereas roots of *G. lutea* have been preferred in the case of fever, hypertension, and intestinal colic, and *G. asclepiadea* has been utilized against hypertension (41).

Flowering herbs of *G. cruciata* and *G. asclepiadea* have been consumed as an appetite stimulant and against fever in various regions of Anatolia (12). In the northern region of Anatolia, flowering aerial part infusion of *G. cruciata* has been consumed to relieve kidney pain whereas its whole plant decoction has been utilized as tonic (13). *G. lutea* has been widely used as folk medicine. Powder and infusion of its dried roots have been used for gastric stimulation, as an appetizer, and antipyretic agent while its decoction has been used for wound healing (12). Flowering herbs of *G. olivieri* has been prepared as an infusion (2-3%) or macerate in different regions of Anatolia. These folk remedies have been consumed as a bitter tonic, stomachic, appetizer, antipyretic agent, and also to lower blood glucose and treat some mental disorders (12,42).

In addition, leaves and flowers of *G. neurotheca* are employed in the case of arthritis and infectious hepatitis in Nigeria (43), while *G. spathaceae* is consumed for gastrointestinal disorders by Otomi Indians in Mexico (44).

2.1.2. Biological Activity Studies on *Gentiana* sp.

2.1.2.1. Antioxidant Activity

Mihailovic and colleagues investigated the effect of methanolic extracts of *G. asclepiadea* and *G. cruciata* and published a series of studies. In their first study in 2013, they aimed to assess the antioxidant and antigenotoxic properties of the methanolic root extract of *G. asclepiadea* through its chloroform, ethyl acetate, and *n*-butanol fractions. DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging activity, total antioxidant capacity, lipid peroxidation inhibition activity, and measurement of reducing power

techniques were applied to evaluate *in vitro* antioxidant activity whereas sex-linked recessive lethal (SLRL) test was applied on *Drosophila melanogaster* to evaluate *in vivo* antigenotoxic activity. The EtOAc fraction displayed more potent antioxidant and radical-scavenging activities than other fractions and the activity was attributed to its phenolic content (total phenolics, flavonoids, flavonols, and gallotannins). Fractions also showed antigenotoxic effects on DNA damage induced by alkylating agent EMS (ethyl methane sulfonate) (45). Two years later, Mihailovic et al. published a study focused on the antioxidant potential of the methanolic extracts obtained from roots and aerial parts of *G. cruciata*. For this purpose, the extracts were subjected to various pH, heating, and an *in vitro* digestion experiments. Additionally, the protective potential of the extracts was evaluated via comet assay. Both extracts showed a protective effect against CCl₄-induced genotoxicity. According to the results, the aerial parts showed stronger antioxidant potential than the roots and it was ascribed to the higher phenolic content. Furthermore, both two extracts displayed thermal and pH stability. Besides, a remarkable increase in the concentration of phenolic compounds was measured in both extracts during the duodenal phase of the digestion model. Additionally, the genotoxicity potentials of the extracts were evaluated against CCl₄-induced DNA damage via comet assay. The roots extract displayed no genotoxicity however, the aerial part extract demonstrated a slight genotoxicity (46).

Azman et al. searched the antioxidant potential of *G. lutea* root extracts (of water or water with ethanol 50:50 v/v) used as a food additive via total phenolic content (The Folin–Ciocalteu method), oxygen radical absorbance capacity (ORAC value) and ferric reducing antioxidant power (FRAP) assessments. Additional assays such as color, pH, and resistance to lipid oxidation (via TBARS) were also performed. Water with ethanol extract showed higher total phenolic content, also higher ORAC and FRAP activities than the aqueous extract. Furthermore, the TBARS assay was applied to evaluate the extent of lipid oxidation of food over the storage period. According to the results, 2 g kg⁻¹ lyophilized *G. lutea* significantly prevented the deterioration of the food product. Furthermore, adding 0.5 g kg⁻¹ ascorbic acid to the lyophilizate led to better results such as delayed oxidation rates and improved color of the food product via a synergistic effect (47).

Kusakari and colleagues isolated three iridoid glucosides (gentiournoside A, E, and depressoside) from the flowers of *G. urnula* and evaluated their antioxidant activities through DPPH radical scavenging and SOD (superoxide dismutase)-like

activity assays. All the isolates displayed DPPH radical scavenging activities approximately within the same range as those of standard antioxidants, Trolox, and L-ascorbic acid. On the other hand, depressoside exhibited the most potent SOD-like activity with $IC_{50} = 45.5 \mu\text{mol L}^{-1}$, more than ten times higher than that of Trolox (28).

Hudecova et al. examined the antioxidant potency and potential ability to enhance DNA repair of aqueous or methanolic extracts of *G. asclepiadea* flower and haulm. The antioxidant potencies in ABTS post-column derivatization, potential ability to enhance DNA repair in human peripheral blood mononuclear cells after hydrogen peroxide (H_2O_2) treatment and DNA repair in human kidney HEK 293 cells after 20 nm silver nanoparticles (AgNPs) were evaluated. Residual DNA damage was measured with the comet assay to assess DNA recovery in the absence or presence of *Gentiana* extracts. Based on the represented results, haulm extracts displayed slightly stronger antioxidant potencies, in comparison to flower extracts. Nevertheless, all four extracts exerted a remarkable ability to enhance DNA repair in both cell types after treatment with H_2O_2 and AgNP (48).

In another study based on aerial part and root extracts of *G. asclepiadea*, total phenol and flavonoid content were assessed and DPPH radical-scavenging capacity was measured. The highest total phenolic and flavonoid content was seen with diethyl ether extract among the root extracts. Within the aerial part extracts, diethyl ether and ethyl acetate extracts displayed the highest content of flavonoids, whereas the ethanol extract demonstrated the highest total phenol content. The aerial part extracts exhibited (181.3 to 614.3 $\mu\text{g/mL}$) remarkably higher flavonoid concentrations in comparison to root extracts (426.6 to $>1000 \mu\text{g/mL}$), and also showed significantly higher antioxidant activity according to the DPPH values. Based on these results, the EtOH aerial part extract (181.3 $\mu\text{g/mL}$) and the diethyl ether root extract (426.6 $\mu\text{g/mL}$) demonstrated the highest activity (18).

Wani et al. revealed the antioxidant properties of the methanolic root extract of *G. kurroo* via hydroxyl radical, DPPH, protective oxidative DNA damage, and lipid peroxidation assays. The results were compared with those of standard antioxidants α -tocopherol, catechin, and BHT. Methanolic extract of *G. kurroo* demonstrated radical scavenging activity (at 100 $\mu\text{g/mL}$: 25.60% and at 700 $\mu\text{g/mL}$: 69.90% of DPPH scavenging effect) and hydroxyl radical scavenging activity (at 100 $\mu\text{g/mL}$: $53.93 \pm 1.53\%$) in a concentration-dependent manner. Microsomal lipid peroxidation was measured through malonaldehyde (MDA) and the extract inhibited MDA formation,

which was also concentration-dependent. The protective effect on DNA oxidative damage was also evaluated and the extract exhibited a protective effect between 10–100 µg /ml. These antioxidant properties were attributed to the extract's high phenolic and flavonoid content (49).

In another study based on the antioxidant activity of *G. kurroo*, MeOH extracts were prepared from leaves and roots and were evaluated through DPPH, FRAP, and NBT (nitroblue tetrazolium test) assessments. The root extract displayed higher antioxidant activity than the leaf extract and these results were ascribed to its higher total phenolic and flavonoid content (For leaf extract TPC: 34 ± 1.8 , TFC: 20 ± 1.5 , for root extract TPC: 68 ± 2.4 , TFC: 41 ± 2.2) (50).

Karaliya and colleagues published a study on the biological activities of various *G. lutea* extracts (petrol ether, ethanol, water, and chloroform extracts of flowers, stems, and leaves). The antioxidant capacity of *G. lutea* extracts was assessed with the help of the DPPH assay. According to the results, petrol ether stem and flower extracts exhibited the highest antioxidant potential with the lowest IC₅₀ values (105.4 ± 10.5 µg/mL to 94.5 ± 9.4 µg/mL) (51).

In a study performed by Dou et al., total flavonoid extraction was applied to the *G. veitchiorum* flowers with water, 70% methanol, or 95 % methanol (50 ml for each). The extract of 70% methanol showed the highest amount of flavonoid content (4.52 ± 0.14 mg/g) and apigenin was defined as the most abundant flavonoid in the extract. Apigenin was then isolated for further analyses and its antioxidant properties were determined with the help of FRAP, ABTS, and DPPH assays. Results revealed the increased radical scavenging rates (8.26 ± 1.23 to 80.31 ± 6.96) and total antioxidative capacity (19.85 ± 2.02 to 89.24 ± 9.85) with elevated concentrations of apigenin. In addition, higher antioxidant activity was observed (11.29 ± 1.02 to 133.26 ± 9.17) by the reducing power assay due to the higher apigenin concentrations (52).

In a study conducted by Wang et al., two polysaccharide fractions (GSP I-a and GSP I-b) obtained from roots of *G. scabra*, comprising mannose, fructose, rhamnose, glucose, galacturonic acid, fucose, and galactose were evaluated through hydroxyl radical scavenging, superoxide radical scavenging, DPPH scavenging, and reducing power assays. Both fractions exhibited relatively low scavenging and reducing power activities against hydroxyl radicals and superoxide anions (53).

Ko et al. isolated isoorientin-6"-O-glucoside from *G. arisanensis* to evaluate its antioxidant properties via superoxide scavenging, free radical scavenging, hydrogen

peroxide scavenging, peroxy radical reactions in an aqueous solution and in hexane, copper and iron chelation assays. Results were compared with other antioxidant standards (Trolox, Probutol, BHT, α -tocopherol) and confirmed the remarkable antioxidant activity of the isolate, isoorientin-6"-*O*-glucoside (54).

The antioxidant properties of *G. decumbens* aqueous extract was investigated by Myagmar and Aniya. The extract demonstrated strong antioxidant properties through superoxide, hydroxyl radical scavenging, and DPPH radical scavenging assessments. Moreover, mild to moderate activity was recorded through measurements of chemiluminescence and inhibitory action on xanthine oxidase (55).

Seal investigated the antioxidant potential of aqueous methanol and acetone extracts of *G. pedicellata* leaves. Aqueous methanol extract of the species exhibited more potent activity through DPPH and reducing power tests (0.23 ± 0.0007 ; 13.07 ± 0.04) compared to acetone extract (1.22 ± 0.02 ; 5.79 ± 0.10). These properties were ascribed to the higher phenolic, flavonoid, and flavonol content of the aqueous methanol extract (23.46 ± 0.32 , 34.79 ± 0.013 , 23.12 ± 0.006) (56).

Wu et al. purified six compounds from the methanolic whole plant extract of *G. piasezkii* and evaluated their antioxidant properties through a DPPH assay. Compounds 6'-*O*-vanilloylarbutin, 7-*O*-feruloylorientin, isoorientin, and luteolin displayed free radical scavenging activity (49.66 ± 2.30 , 5.20 ± 0.10 , 78.64 ± 5.42 , 6.64 ± 0.39) (57).

2.1.2.2. Hepatoprotective and Choleretic Activities

Mihailovic and colleagues examined the hepatoprotective effect of the methanolic extracts of roots and aerial parts of *G. asclepiadea* against carbon tetrachloride-induced liver injury in rats. Extracts were orally administrated to Wistar rats at 100, 200, and 400 mg/kg b.w. doses once a day for a week, prior to carbon tetrachloride treatment, and the activity was compared with the reference drug silymarin. Methanolic extracts significantly reduced serum transaminases, total bilirubin levels, and alkaline phosphatase, whereas elevated the level of total protein in CCl₄-treated animals. Also, a remarkable increase in the levels of superoxide dismutase, catalase, and reduced glutathione, with a significant reduction in MDA levels was observed with the extracts, compared to the CCl₄-treated group. Moreover, histopathological examinations support the hepatoprotective effect of the extracts. The aerial part extract displayed no genotoxicity by comet assay, however, the root part extract exhibited slight DNA damage via an increase in tail length (16). In 2014, Mihailovic et al. published a study aimed to

assess the effects of the methanol extracts of the roots and aerial parts of *G. cruciata* against CCl₄-induced liver injury. Pretreatment with the extracts resulted in dose-dependently and remarkably reduced alkaline phosphatase, serum transaminases, and total bilirubin levels, while elevated total protein levels in comparison with the CCl₄-treated group. Furthermore, oral administration of extracts remarkably raised antioxidant enzyme activities (catalase and superoxide dismutase) and also increased the content of glutathione. With the help of microscopic evaluations of the liver, CCl₄-induced lesions and related toxic formations were observed minimum in the rat livers pretreated with *G. cruciata* extracts (400 mg per kg b.w.) (17).

Zhang et al. conducted a study to assess the hepatoprotective effect of methanol extract of *G. veitchiorum* (oral administration: doses between 100-400 mg/kg⁻¹) against CCl₄-induced oxidative stress and liver injury in mice. Antioxidant enzyme activities including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in liver tissue and hepatic enzyme levels including aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in serum were measured and also some additional experiments (histopathological examinations and Western blot) were conducted. 200 and 400 mg/kg⁻¹ of doses of *G. veitchiorum* extracts remarkably inhibited the increased serum and liver tissue levels of ALT, AST, ALP, SOD, CAT, and GPX whereas the 400 mg/kg⁻¹ of the extract exhibited similar effect with the positive control silymarin. Histological examinations ended up with similar results; 400 mg/kg⁻¹ of the extract ameliorated liver injury comparable to that of silymarin. Western blot analysis of the high-dose extract (400 mg/kg⁻¹) on the TNF- α and HO-1 expressions also resulted in identical results (58).

Orhan and colleagues performed a study on the hepatoprotective activity of the ethanolic extract of *G. olivieri* flowering herbs against CCl₄-induced hepatic injury. Hepatic and plasma tissue MDA formation, plasma transaminase enzyme levels (ALT and AST), liver tissue GSH level, and histopathological examinations were evaluated. According to the results, 125 mg/kg dose of the extract demonstrated high anti-lipid peroxidation activity on MDA (tissue MDA: 39.4%, plasma MDA: 30.6%), GSH (tissue GSH 71.4%) and plasma transaminase enzyme levels (AST 44.3% and ALT 53.4%). The plasma AST level of the extract showed (at 125 mg/kg: 53.4% inhibition) potent activity comparable to the standard *C. scolymus* bracts, which are widely used for antihepatotoxic purposes. Furthermore, bioassay-guided fractionation was performed and

hepatoprotective properties of the active subfractions were attributed to their high isoorientin content (59).

Öztürk et al. conducted an *in vivo* study based on the EtOH extract of *G. lutea* ssp. *symphyandra* roots. A bile flow experiment was performed to assess bile production and then rat livers were removed for the histological examination. According to the results, multiple-dose treatment with the ethanol extract ameliorated the impaired bile flow (60).

In Handoussa and colleagues' study, the aqueous ethanolic extract of *G. spicata* aerial parts exhibited a hepatoprotective effect against CCl₄-induced hepatic injury in rats. Pre-treatment with the aqueous ethanolic extract prevented the increase in serum ALT and AST levels. Biochemical assays (TBARS, GSH) of liver homogenate and histopathological examinations also confirmed alleviation of the hepatotoxic effects and amelioration in liver tissue (61).

In a study performed by Wang et al., the methanolic extract of *G. manshurica* roots and rhizomes was evaluated against acetaminophen (APAP)-induced liver injury. Pretreatment with the extract improved serum ALT, AST, and MDA levels, and also hepatic antioxidant status (GSH content, GSH-px, and SOD activity) (62). In another study, methanol extract of *G. manshurica* was assessed on acute alcohol-induced fatty liver. *G. manshurica* extract administration helped to diminish elevated serum ALT, AST, and hepatic triglyceride (TG) levels, supported CAT, SOD and glutathione peroxidase activities, restored GSH levels and suppressed the increase in MDA levels. Histopathological studies also confirmed its efficacy against alcohol-induced hepatic steatosis and necrosis. These hepatoprotective properties were attributed to two possible mechanisms, which are and SREBP-1-regulated fatty acid synthesis and blocking CYP2E1-mediated free radical scavenging effects (63).

Aktay et al. investigated the hepatoprotective properties of plants used in Turkish folk medicine, including *G. olivieri* against CCl₄-induced hepatotoxicity. Ethanolic extract of the *G. olivieri* flowering herbs showed a remarkable reduction in elevated plasma ALT (86.39%) and AST (69.57%) levels, and a marked inhibition of lipid peroxidation with reduced plasma (70.45%) and liver homogenate (32.31%) MDA levels. Histological examinations also confirmed the results of biochemical assays (64).

Cao et al. conducted a study based on the effects of *G. dahurica* ethanolic extract on alcoholic liver disease (ALD). The extract ameliorated steatosis and liver

damage and the mechanism was attributed to the inhibition of elevated transaminase activities in the lipid and serum levels (65).

Various studies have been performed to evaluate the hepatoprotective and choleretic effects of gentiopicroside. In a study performed by Han et al., gentiopicroside was found effective in cholestasis induced by alpha-naphthylisothiocyanate (ANIT), which was ascribed to its ability to regulate bile acid, fatty acid, and lipid metabolisms (66). In another study gentiopicroside was evaluated against ANIT-induced cholestatic liver injury, concentrations of bile acids in serum, urine, feces, and liver were measured. Results revealed that pretreatment with gentiopicroside alleviated bile acid metabolism and liver injury induced by ANIT (67). Yang et al. investigated gentiopicroside isolated from *G. manshurica* and its effect on the progression of alcoholic hepatic steatosis to fibrosis induced by chronic ethanol intake. Gentiopicroside inhibited the advancing of hepatic steatosis via alleviating imbalanced lipid metabolism and promoting lipid oxidation (68). Li et al. investigated the *in vitro* and *in vivo* activity of gentiopicroside on P2X7 receptor-mediated alcoholic hepatosteatosis. Gentiopicroside reduced serum aminotransferases and triglyceride accumulation in both acute and chronic alcohol-induced hepatosteatosis mice models, and also reduced lipogenesis and promoted lipid oxidation in ethanol-exposed HepG2 cells. These features were attributed to its effect on liver kinase B1/AMP-activated protein kinase (LKB1/AMPK) mediated regulation (69).

2.1.2.3. Antidiabetic Activity

Sezik et al. investigated *in vivo* hypoglycaemic effect of aqueous and methanolic extracts of *G. olivieri* flowering herbs on normal, glucose-hyperglycemic, or streptozotocin-induced diabetic rats. According to the results, the aqueous extract of did not exhibit any significant activity in normal or glucose-loaded nor in diabetic rats, whereas the methanolic extract displayed a significant hypoglycemic effect on glucose-hyperglycaemic rats. As a part of further experiments, bioassay-guided fractionation was performed on methanolic extract. Chloroform, ethyl acetate, *n*-butanol, and H₂O subfractions were obtained and all these were studied on both normal and glucose-hyperglycaemic models. EtOAc, *n*-BuOH, and H₂O fractions demonstrated significant activity in glucose-hyperglycaemic models and tolbutamide had been chosen as a reference drug to compare with subfractions. Since tolbutamide didn't display a desirable and safe hypoglycaemic profile, (due to the hypoglycaemic shock risk) EtOAc extract was considered as a safer and better alternative. Isoorientin was isolated from EtOAc

subfraction and its subacute administration to streptozocin (STZ)-induced diabetic rats produced significant hypoglycemic and antihyperlipidemic effects (70).

In a study conducted by Ghazanfar et al., the antidiabetic properties of methanolic and hydroethanolic extracts of *G. kurroo* were compared to the reference drug glibenclamide on STZ-induced diabetic rats. Both extracts displayed antihyperglycemic effect, but the methanolic extract showed higher efficacy in decreasing the fasting glucose compared to the reference drug. Both methanolic and hydroethanolic extracts were found efficient on elevated biochemical parameters (LDL, triglycerides, creatinine, cholesterol, ALT, ALP, AST levels) in diabetic rats. Polyphagia, polydipsia, and polyuria, also known as '3Ps' are cardinal signs of diabetes. Hydroethanolic and methanolic extracts (at the dose of 500 mg/kg b.w.) showed similar effects with glibenclamide and resulted in a reduction in food and water consumption and the amount of urine excretion. Additional experiments such as histopathological examinations and oral glucose tolerance test (OGTT) also supported the represented results (71).

Shin et al. performed a study on methylene chloride, *n*-hexane, *n*-butanol, and ethyl acetate fractions of *G. scabra* aqueous extracts and their effect on glucagon-like peptide-1 (GLP-1) secretion, which is believed to regulate insulin secretion. *In vitro* experiments on enteroendocrine NCI-H716 cells revealed that *G. scabra* extracts induce GLP-1 secretion through the GPCR signaling pathway. Ethyl acetate showed the highest efficiency on NCI-H716 cells among all the fractions (72).

Suh et al. investigated the hypoglycaemic effect of aqueous ethanol extract of *G. scabra* roots. The *db/db* mice were used as diabetic models and several parameters such as glucagon-like peptide-1 (GLP-1), blood glucose, and insulin have been monitored. Plant extract exhibited a blood glucose-lowering effect due to GLP-1 secretion in enteroendocrine cells. According to HPLC-MS results, bitter iridoid glycosides were found as active constituents, and the hypoglycaemic effect of loganic acid has been additionally evaluated. Loganic acid also exhibited a GLP-1 secreting effect similar to the extract (25). Another study was conducted on the antidiabetic potency of loganic acid in STZ-induced rats by Dzydzan and colleagues. Loganic acid didn't exhibit any remarkable activity on blood glucose levels however improved antioxidant status in leukocytes by decreasing GSH and activities of CAT, GPx, and glutathione reductase (GR) along with reducing levels of intracellular reactive oxygen species. It also inhibited the formation and accumulation of advanced glycation end products (AGEs), advanced oxidation protein products (AOPPs), and MDA derivatives (73).

In a study conducted by Akileshwari et al., inhibitory activities of water, ethanol, methanol, and ether extracts of *G. lutea* roots were evaluated on aldose reductase (ALR), which leads to diabetic complications due to sorbitol accumulation. Ether and methanol extracts demonstrated the highest ALR2 inhibitory activity among all extracts and the activity was attributed to amarogentin, a secoiridoid glycoside (74).

2.1.2.4. Cardioprotective Activity

Huang and colleagues extracted *G. macrophylla* roots with 80% methanol and investigated its activity against cholesterol-aggravated cardiac abnormalities associated with systemic lupus erythematosus (SLE). Extract demonstrated anti-apoptotic effects in NZB/WF1 mice fed with a high-cholesterol diet according to results of microscopic examination, immunoblotting, and terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assays. *G. macrophylla* root extract successfully suppressed intrinsic and extrinsic apoptotic pathways and also enhanced the cardiac insulin-like growth factors (IGF)-1 survival signaling (75).

Kesavan et al. conducted a study on the anti-atherosclerotic effect of *G. lutea* aqueous root extract and isovitexin on human umbilical vein endothelial cells (HUVECs), rat aortic smooth muscle cells (RASMCs) and STZ-induced diabetic rats. Both extract and compound displayed protective activity on endothelial inflammation and smooth muscle cell migration and also inhibited ERK1/2 activation, iNOS expression, adhesion of leukocytes, and generation of reactive oxygen species (ROS) (76).

In a study conducted by Khan et al., the hypotensive activity of aqueous methanolic extract of *G. floribunda* whole plant was evaluated in anesthetized rats and isolated thoracic aorta tissues. The plant extract demonstrated blood pressure lowering and endothelial-independent vasodilatory effects via Ca^{++} antagonism (32).

Lin et al. performed a study on *G. scabra* aqueous extract and assessed its anti-atherosclerotic activity via oxidized LDL (OxLDL) uptake and cell cytotoxicity assay. The plant extract successfully inhibited oxidized LDL uptake and expression of scavenger receptor- A (SR-A) (77).

Manganelli et al. investigated the methanolic extract of *G. kochiana* roots and its antihypertensive properties were tested on the aortic rings of Wistar rats. *G. kochiana* extract displayed a dose-dependent vasorelaxant effect (41). Another study revealed the mechanism of action. Methanolic extract of *G. kochiana* roots displayed an endothelium-independent vasodilator activity in pre-contracted aortic rings and caused a marked depression of the contracturant effect induced by KCl and caffeine. The possible

mechanism was attributed to the inhibition of ryanodine receptors, which are responsible for the release of Ca^{++} from the sarco/endoplasmic reticulum (78).

In Mansoor and colleagues' study, aqueous and ethanolic extracts of *G. olivieri* were prepared and gentianine alkaloid was isolated to evaluate their cardioprotective properties in normotensive rats. Both extracts and pure compound reduced systolic, diastolic, and mean arterial blood pressure (fall in BP percentages: 47.5 ± 1.24 , 46.8 ± 1.50 , 50.2 ± 0.85), respectively. Gentianine demonstrated the highest hypotensive activity in a dose-dependent manner (19.6 ± 1.3 at 1 mg kg^{-1} , 35.2 ± 1.1 at 3 mg kg^{-1} , 50.3 ± 0.88 at 10 mg kg^{-1}) (79).

2.1.2.5. Anticoagulant and Thrombotic Activity

Cai et al. performed a study on polysaccharide fractions (GSP 1-3) of *G. scabra* roots and assessed their *in vitro* anticoagulant activity via activated partial thromboplastin time (APTT), thrombin time (TT), and prothrombin time (PT) assays. All three fractions exhibited anticoagulant effects via prolonging APTT and TT. GSP-3, which consisted of rhamnose, arabinose, galactose, and galacturonic acid demonstrated the highest activity among all fractions (80).

Bakuridze et al prepared dry extract from the aerial parts of *G. lutea* and evaluated its effect on hemostasis. Oral administration of the extract to the mice resulted in a prolonged time of the formation of active thromboplastin and shortened time of thrombin and fibrinous cluster formation (81).

Lin and colleagues isolated flavonoids from *G. arisanensis* and assessed their antiplatelet and vasorelaxant properties. Amongst, luteolin exhibited a potent antiplatelet activity via arachidonic acid (AA)-induced platelet aggregation, and also inhibited Ca^{++} -induced contraction in a high- K^{+} medium and noradrenaline-induced phasic and tonic contraction in a dose-dependent manner (82). In a study conducted by Huh et al., MeOH extract of *G. scabra* was tested against anti-platelet activating factor (PAF) induced anaphylactic shock. The extract demonstrated potent antagonistic activity and was subjected to further partitions. The EtOAc fraction exhibited the highest activity and prevented PAF-induced systemic anaphylaxis successfully. This fraction was then subjected to chromatographic separations and yielded 2-hydroxy-3-methoxybenzoic acid glucose ester. The compound also displayed potent PAF-antagonistic activity at low doses (0.3 mg/mouse) and was defined as active component of the fraction (83).

Yen and colleagues investigated amarogentin, an active compound from *G. lutea*, and its activity on platelet activation. The compound inhibited platelet activation

through the inhibition of the phospholipase-protein kinase C (PLC γ 2-PKC) cascade and mitogen-activated protein kinases (MAPK) pathway (84).

2.1.2.6. Neuroprotective Activities

Şenol et al. prepared DCM, EtOAc, and MeOH extracts from the leaves, roots, and flowers of *G. asclepiadea*, *G. cruciata*, *G. olivieri*, *G. septemfida*, and *G. verna* to evaluate their inhibitory effects against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes as well as their *in vitro* antioxidant properties. The highest AChE inhibitory effect was observed with DCM extract of *G. verna* flowers ($53.65 \pm 1.03\%$), while the highest BChE inhibitory effect was measured with EtOAc extract of *G. cruciata* roots ($50.72 \pm 0.75\%$). The antioxidant activity has been assessed via various assays such as DPPH radical scavenging, metal-chelation capacity, and ferric-reducing antioxidant power. *G. verna* extracts exhibited the highest antioxidant activity among all five species (85).

Xu et al. conducted a study on the AChE inhibitory effect and cytotoxicity of *G. rhodantha* chemical components. The MeOH extract of the whole plant was subjected to several column chromatography methods and 19 compounds were isolated including lancerin, mangiferin, neomangiferin, apigenin 7-*O*- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranoside, and rhodanthenones A-D, which were tested for their biological activities. Among the compounds, rhodanthenone D and mangiferin demonstrated 18.4 and 13.4% of AChE inhibitory effects, while eight (mentioned above) of these nineteen compounds exhibited no cytotoxicity at the concentration of 40 μ M (86).

In a study performed by Urbain et al., the MeOH extract of *G. campestris* leaves was assessed for AChE inhibitory activity. Bioactivity-guided fractionation was applied to the extract to isolate its active principles. Four xanthenes, bellidin, bellidifolin, swertianolin, and norswertianolin were isolated and tested for AChE inhibition. Bellidifolin displayed similar activity (0.01 μ g minimum inhibitory quantity) to the reference compound galanthamine (87).

Li et al. investigated the neurogenesis and neuroprotective effect of the mixture of benzoates (gentisides A-K) from *G. rigescens* roots. Nerve growth factor (NGF) mimicking activity was assessed in PC12 cells and the neurogenesis effect was tested in ICR mice via 5-bromo-2-deoxyuridine (BrdU) and neuronal nuclei (NeuN) double immunostaining assays while the neuroprotective effect on memory was evaluated through scopolamine (SCO)-induced mice behavior tests such as Y-maze, novel object

reorganization (NOR), Morris Walter Maze (MWM) tests. According to the results, the benzoate fraction showed *in vivo* neurogenesis and NGF mimicking effects as well as beneficial effects on impaired memory through inhibition of AChE and oxidative stress (SOD activity and MDA levels were also calculated). Regulation of the IGF-1R/ERK/CREB signaling pathway was also linked to neuroprotective activity (88). Gao et al. published two other studies in 2010, based on gentisides from *G. rigescens* and their neuritogenic activities. In their first study, the isolated compounds gentiside A and B demonstrated a significant neuritogenic activity in PC12 cells at 30 μ M, which was comparable to the activity of nerve growth factor (NGF) at 40 ng/mL. In their second study, gentisides (C-K) were isolated and again tested in neurite outgrowth on PC12 cells. Gentiside C showed the highest neuritogenic activity among all the isolated compounds and this effect was attributed to its structural properties such as (shortest) alkyl chain length. The isolate exhibited a potent neuritogenic effect which was similar to that seen with the positive NGF control at 40 ng/mL. According to structural comparisons of gentisides A–K, alkyl chain length affects activity regardless of the structural diversity of the alkyl chain (89,90).

Cheng et al. conducted another study on *G. rigescens* and its activity on nerve growth factor (NGF)-mimicking and NGF-enhancing activities. Active principle amarogentin was isolated from the roots of the plant and its activity was tested in the PC12 cell line and primary cortical neuron cells. Amarogentin-induced neuritogenic effect was evaluated through various signaling pathways (Ras/Raf/MEK/ERK, INSR/PI3K/AKT, GR/PLC/PKC) and target protein identification methods such as cellular thermal shift assay (CETSA) and small-interfering RNA (siRNA). Results revealed that amarogentin demonstrated neurogenesis by induction of phosphorylation of the insulin receptor (INSR) and regulating the PI3K/AKT/Ras/Raf/ERK and GR/PLC/PKC signaling pathways. INSR was also determined as the potential target protein of amarogentin (91). In a study conducted by Luo et al., structure-activity relationships of gentiside derivatives were illuminated. Several gentiside derivatives with different alkyl chain lengths such as 2,3-dihydroxybenzoates, tetradecylbenzoates, tetradecyl-benzamides, phenyl ketones 2,3-dihydroxy phenyl tetradecanoate and 3-(tetradecyloxy)benzene-1,2-diol derivatives were synthesized to evaluate the activity and determine the optimum length of the alkyl chain. Tetradecyl 2,3-dihydroxybenzoate, tetradecyl 3,4-dihydroxybenzoate, and tetradecyl 3,4,5-trihydroxybenzoate exhibited the highest neuritogenic activities on PC12 cells at 1 or 3 μ M. Moreover, tetradecyl 2,3-

dihydroxybenzoate demonstrated the highest neurotogenic activity at 1 μM as well as inducing remarkable neurite outgrowth via activation of the ERK signaling pathway (92).

The neuropharmacological properties of *G. kochiana* were investigated by Tomic and colleagues. Diethyl ether extract of the aerial parts and two isolated xanthenes, gentiacaulein and gentiakochianin were evaluated through *in vivo* and *in vitro* assays. Both compounds and the extract exhibited inhibitory activity on MAO enzymes. Gentiacaulein demonstrated strong MAO A inhibition ($\text{IC}_{50}=0.49 \mu\text{M}$), whereas gentiakochianin acted as a weak MAO A ($\text{IC}_{50}=164 \mu\text{M}$) inhibitor. Both compounds exhibited moderate MAO B inhibitory activity. Behavioral examinations revealed that diethyl ether extract (sc, 10 days, 20 mg/kg) affected both force swim test (FST) and open-field tests similar to those induced by 10 mg/kg imipramine (93). Another study was conducted on *G. kochiana* extract and xanthenes. Diethyl ether extract of the aerial parts and two isolated xanthenes were assessed to evaluate *in vivo* and *in vitro* anxiolytic-like activity. The extract was found effective through behavioral tests such as elevated plus maze (EPM) and staircase tests, which were comparable to the effects of the reference drug diazepam. However, *in vitro* assays concluded that neither the extract nor the xanthenes displayed interaction with $\alpha 1$ -adrenergic receptors, GABAergic receptors, and acetylcholine esterase (94).

Kaya et al. performed a study on three benzophenone glucosides (2,3'-dihydroxy-4-methoxy-benzophenone-6-*O*- β -glucopyranoside, 2,4,3',4'-tetrahydroxy-benzophenone-6-*O*- β -glucopyranoside and 2,4,3'-trihydroxy-benzophenone-6-*O*- β -glucopyranoside) isolated from the methanolic extract of *G. verna* and their MAO A inhibitory activity. The isolates exhibited MAO A inhibition with IC_{50} values $31.3 \pm 4 \mu\text{M}$, $41 \pm 4.7 \mu\text{M}$, and $>250 \mu\text{M}$, respectively (95).

In a study performed by Öztürk et al., the methanolic extract of *G. lutea* ssp. *symphyandra* roots (at 250 and 500 mg/kg doses) were evaluated on the central nervous system (CNS) of mice. The extract increased the swimming time (4.40 ± 1.91 and 2.80 ± 1.79) and swimming performance (2.80 ± 0.73 and 4.00 ± 0.84) in the swimming endurance test. Moreover, slight analgesic activity was reported (96). Mustafa et al. investigated the neurotogenic activity of the methanolic extract of *G. lutea* roots on the PC12 cell line. Differentiation assay by real-time cell analysis system (RTCA DP), neurite outgrowth image analysis, and immunofluorescence staining was performed to evaluate activity. According to the results, *G. lutea* extracts (at 12.5 and 25 $\mu\text{g/ml}$ doses)

exhibited a significant stimulation of neuritogenesis with or without NGF, which was assessed through enhanced lengths and the number of neurite-positive cells (97).

Yang et al. extracted *G. delavayi* flowers with chloroform to assess anti-BACE1 (β -secretase 1) action and amyloid precursor protein (APP) processing, which plays an important role in β -amyloid peptide accumulation and Alzheimer's Disease. Results revealed that the chloroform extract inhibited BACE1 action, declined A β 40 and A β 42 peptide levels, and also supported A β oligomer degradation in Chinese hamster ovary (CHO) cells (98). In another study based on Alzheimer's Disease, *G. kurroo* methanolic extracts were tested *in vivo* through an EPM test. Orally administered extract (100 mg/kg and 200 mg/kg) attenuated scopolamine-induced cognitive impairment in mice (99).

Aslan et al. extracted *G. olivieri* flowering herbs with 80% EtOH and evaluated their *in vivo* anticonvulsant effects through different experimental models (pentylenetetrazole, picrotoxin, and maximal electroshock). The rotarod test was also performed to assess neurotoxicity. The extract displayed a protective effect against maximal electroshock (MES)-induced seizures at doses 200, 750, and 1000 mg/kg, and also prolonged the onset of picrotoxin-induced (PIC) seizures at 1000 mg/kg and pentylenetetrazole (PTZ)-induced seizures at 200 and 750 mg/kg. In the rotarod performance test, intraperitoneal administration of 200 mg/kg extract led to a decrease in the time of permanence of the mice in the rotating bar (100).

2.1.2.7. Gastroprotective Activities

Niiho et al. investigated the gastroprotective effects of active components from the methanolic extracts of *G. lutea* root and *Swertia japonica* herb. The methanolic extracts were then partitioned with ether, ethyl acetate, and *n*-butanol. Bitter principles amarogentin, gentiopicroside, amaroswerin and swertiamarin were isolated through multiple chromatographic steps. The methanolic extract, EtOAc, and *n*-BuOH fractions of *G. lutea* roots reduced gastric acid secretion and total acid output dose-dependently and also displayed protective activity against gastric ulcers induced by aspirin plus pyloric ligation. Moreover, the EtOAc fraction showed a stronger inhibitory effect (91.2%) on ulcer induction than that of the reference drug, cimetidine (78.9%). Oral administration of EtOAc and *n*-BuOH as well as amarogentin, gentiopicroside, amaroswerin, and swertiamarin demonstrated a gastroprotective effect against gastric ulcers induced by immersion stress. Amarogentin and amaroswerin exhibited strong protective activities against stress-induced ulcer lesions (ID_{50} = 5.76 and 2.58 mg/kg). Oral administration of

EtOAc fraction, amarogentin, amaroswerin, and swertiamarin suppressed ethanol-induced gastric lesions remarkably (101).

In a study conducted by Rojas and colleagues, *in vivo* spasmolytic activity of some Mexican traditional remedies was investigated including *G. spathacea*. CHCl₃-MeOH (1:1) extract of the plant exerted a potent activity (IC₅₀= 4.58 ± 1.10) in isolated rat ileum (44). The following year, Rojas et al. performed bioassay-guided fractionation to detect major spasmolytic principles in *G. spathacea*. Then the CHCl₃-MeOH (1:1) extract of *G. spathacea* aerial parts was subjected to several partition steps to isolate compounds. Isolated compound gentiopicroside (IC₅₀= 2.80 ± 0.71) inhibited the spontaneous contractions in guinea pig ileum dose-dependently. Furthermore, cholinergic, histaminergic, and calcium-induced contractions were evaluated. The extract reduced KCl (32.2% of inh.) and BaCl₂-induced (52.4% of inh.) contractions significantly whereas cholinergic (19% of inh.) and histaminergic (22.6% of inh.) contractions moderately (102).

Yang et al. isolated gentiopicroside from *G. macrophylla* to evaluate *in vivo* gastroprotective activity against ethanol-induced gastric mucosal injury. MDA, GSH, TNF- α , IL-6, heat shock protein 70 (HSP-70), epidermal growth factor (EGF), vascular endothelial (VEGF) levels as well as SOD and myeloperoxidase (MPO) activities were assessed. Moreover, histopathological examinations were performed. Gentiopicroside pretreatment (at doses of 20, 40, and 80 mg/kg) significantly reduced gastric mucosal injury, decreased TNF- α , IL-6, MDA, HSP-70 levels and MPO activity, increased GSH level and SOD activity and normalized EGF and VEGF levels. Gentiopicroside demonstrated amendatory effects on oxidative stress markers and pro-inflammatory cytokines as well as gastric mucosal defensive factors. It enhanced HSP-70 levels that contributed to gastric mucosa protection while normalized EGF and VEGF levels exhibited a supportive effect on angiogenesis in gastric mucosa and ameliorated alcohol-induced gastric erosions (103). In another study, gentiopicroside from *G. macrophylla* was assessed against stress-induced gastrointestinal motility disorder. Gastric peptide hormone levels and receptor expressions were evaluated. Gentiopicroside (at doses 40, 80 and 160 mg/kg) successfully alleviated gastrointestinal peristalsis via modulation of somatostatin (SST) and gastrin (GAS) levels as well as increasing MTL receptor (MLTR) and decreasing vasoactive intestinal peptide VIP receptor 2 (VIPR₂) expressions (104).

2.1.2.8. Antimicrobial Activity

In a study conducted by Yin and colleagues, the methanolic extract of *G. macrophylla* roots was tested on bacterial strains isolated from wound burns. In addition, phytochemical screening and antioxidant activity (via NBT and DPPH assays) experiments were performed. Antibacterial activity was determined with the help of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The methanolic extract displayed significant antioxidant activity via NBT and DPPH assays. The highest antibacterial effect was measured against *Staphylococcus epidermidis* (MIC= 60 µg/mL) whereas *Escherichia coli* was shown as the less sensitive bacterium (MIC= 140 µg/mL). Phytochemical analysis showed the presence of chlorogenic acid, rutin, sweroside, isogentisin, and gentisin, which potentially made a major contribution to the biological activity (105).

Baba et al. investigated the *in vitro* antibacterial effect of methanolic extracts of *G. kurroo* leaves and roots on both Gram-positive and negative bacteria strains. The root extract (MIC value ranged from 0.15 ± 0.04 to 0.75 ± 0.05 mg/mL) exhibited higher antibacterial activity than the leaf extract (MIC value ranged from 0.22 ± 0.08 to 0.90 ± 0.02 mg/mL). The highest antibacterial activity of the root extract was measured against *Micrococcus luteus* (0.15 mg/ml) whereas the lowest was observed against *Salmonella enteritidis* (0.75 ± 0.05). The activity was attributed to the high phenolic and flavonoid content (50).

Buza et al. extracted *G. asclepiadea* roots with ethanol to evaluate *in vitro* antibacterial activity through the standard well diffusion and broth microdilution assays. According to the zone of inhibition (ranged from 10.00 to 17.33 mm) and MIC index values, *G. asclepiadea* inhibited both Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*) and negative (*Salmonella enteritidis*, *Salmonella typhimurium*, *Escherichia coli*) bacterial strains. Biological activity was attributed to the major phytochemical components, which were identified as amarogentin, apigenin, luteolin-7-*O*-glucoside, naringenin, and rutoside (14). Stefanovic et al. investigated *in vitro* antimicrobial and antibiofilm activities of aqueous, ethanol, ethyl acetate, acetone, and diethyl ether extracts of *G. asclepiadea* aerial parts and roots on both Gram-positive and negative bacteria (*Bacillus*, *Enterococcus*, *Staphylococcus*, *Escherichia*, *Proteus*, *Pseudomonas*, *Klebsiella* and *Salmonella* sp.) as well as fungi (*Candida*, *Penicillium* and *Aspergillus* sp.) strains. The antibacterial and antifungal activities were determined by MIC values whereas antibiofilm activity was determined by biofilm inhibition

concentration (BIC₅₀) calculations. Antibacterial activity of the extracts was found more effective on Gram-positive than Gram-negative bacteria. Especially, the ethanol, acetone, and ethyl acetate extracts displayed remarkable activity on *Bacillus* species (*B. subtilis*, *B. cereus*, *B. subtilis* ATCC 6633) with MICs ranging from 0.16 to 5 mg/mL. Contrarily, Gram-negative bacteria demonstrated low sensitivity to the extracts as well as low to moderate activity was observed on fungi strains. The highest antifungal activity was measured with ethanol extract of *G. asclepiadea* on *Candida* species. The antibiofilm activity was tested on various *Staphylococcus*, *Pseudomonas*, and *Proteus* strains. The extracts were found effective in biofilm formation, with BIC₅₀ values ranging from 2.12 to 37.04 mg/mL. The highest antibiofilm activity was observed with aqueous and ethanolic extracts on *Staphylococcus* species and acetone extract on *Pseudomonas* species (18). Milutinovic and colleagues prepared various plant extracts to obtain the highest polyphenol content. According to an optimized procedure, one of these plants, *G. asclepiadea* was extracted with 25% EtOH. Antimicrobial activity was tested on both Gram-positive and Gram-negative bacteria, yeast, and probiotic microorganism strains and determined by MIC values. The prebiotic activity of the extract was also assessed through probiotic microorganisms and measuring their growth stimulation potential. The extract inhibited the growth of *S. aureus*, *L. monocytogenes*, *E. coli*, and *C. albicans* strains (with MIC values ranging from 5 to 20 mg/mL). The extract also supported *S. boulardii* growth when added to the growth medium in the final concentration of 0.5 or 0.25 mg/mL. The prebiotic activity of the extract has been associated with secoiridoid content instead of polyphenols since gentiopicroside has been reported to be metabolized by some of the *Lactobacillus* species by their β -glucosidase action (106,107). Mihailovic et al. investigated the chemical composition and antimicrobial activity of the methanol and *n*-butanol extracts of *G. asclepiadea* as well as its essential oil. Essential oil from the underground parts (roots and rhizomes) consisted of caryophyllene oxide, β -damascenone, and β -ionone, whereas the main contents of the essential from the aerial parts were identified as toluene, tetradecanoic acid, linalool, and caryophyllene oxide. Antimicrobial activity was tested on various bacteria and fungi strains. Essential oil from the underground parts exhibited significant activity on *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* strains with MIC values of 0.62, 2.5, 2.5, 2.5, and 5 μ L/mL. *Micrococcus lysodeikticus* was found resistant to essential oil from the underground parts (>5) while the same bacteria strains exhibited a sensitive profile to the essential oil from aerial parts with a MIC value of 2.5

$\mu\text{g/ml}$. The antimicrobial effect of the essential oils was attributed to the high sesquiterpene content and their derivatives. The methanol extract demonstrated antimicrobial activities on all tested bacteria and fungi strains with MIC values ranging from 100 to 1600 $\mu\text{g/mL}$ whereas *n*-butanol extract was found to be less effective on bacteria and fungi strains with MIC values ranging from 312.5 to 2500 $\mu\text{g/mL}$. The methanolic extract displayed the highest antibacterial activity on *S. aureus* (ATCC 25923), *E. faecalis*, and *M. lysodeikticus* strains whereas *n*-butanol extract was found also most effective on *S. aureus* strain. The methanolic extract exhibited the highest antifungal activity on *Penicillium* species (*P. verrucosum*, *P. chrysogenum*, *P. cyclopium*, *P. canescens*) while *P. canescens* demonstrated the highest susceptibility to the *n*-butanol extract (108). Jovanovic et al. extracted *G. asclepiadea* underground parts with 70% aqueous ethanol and partitioned them with petroleum ether, ethyl acetate, and *n*-butanol. All these fractions were evaluated for their *in vitro* antimicrobial activity against various bacteria and fungi strains with the help of MIC and minimum bactericidal activity (MBC) values. In addition, molecular binding simulation of several secondary metabolites (gentiopicroside, swertiamarin, sweroside, isovitexin, isoorientin, and isogentisin) was performed on the *Enterococcus faecalis* lipote-protein ligase A (lplA-1). MIC values of Gram-positive strains were measured between 0.78-25 mg/ml whereas Gram-negative strains were calculated slightly higher (3.13 to 50 mg/ml). Moreover, MBC values supported the previous data (ranging from 0.78 - 50 mg/ml compared to 3.13 - > 200 mg/ml for Gram-positive and negative strains). The lowest MIC values were observed with petroleum ether and ethyl acetate fractions, against *Bacillus cereus* and *Staphylococcus aureus* strains (MIC of 0.78 mg/ml) while the highest bactericidal effect was observed with ethyl acetate fraction, against *Enterococcus faecalis* (MBC of 0.78 mg/ml). Isoorientin and isovitexin exhibited the best binding affinity according to the molecular binding simulation (109).

Karalija et al. performed a study on petrol ether, chloroform, ethanol, and aqueous extracts of *G. lutea* flowers, leaves, and stems to determine their phytochemical constituents and biological activities. Ethanol extracts from leaves and stems exhibited a potent antimicrobial activity (with 29.33 ± 1.15 and 27.50 ± 1.78 mm inhibitory zone) on *C. albicans* compared to reference drug nystatin (with 20.00 mm inhibitory zone). Moreover, ethanol extracts were shown to possess higher activity. According to the results of the activity of single compounds, rosmarinic acid exhibited activity on the

widest spectrum whereas salicylic acid and naringenin showed the highest activities on *C. albicans* strains, with 25.33 ± 0.58 and 22.33 ± 1.52 mm inhibitory zones (51).

2.1.2.9. Radioprotective Activity

Menkovic and colleagues investigated the *in vitro* and *ex vivo* radioprotective properties of the aqueous EtOH extract of *G. lutea* roots and its active component mangiferin. The radioprotective activity of mangiferin was assessed in human peripheral blood mononuclear cells (PMBC) and phytohemagglutinin-activated PMBC. Mangiferin prevented PMBC from the cytotoxic action of ionizing irradiation at doses 6 and 8 Gy. The *ex vivo* radioprotective activity was evaluated in PMBC cells from healthy volunteers and cell survival of malignant cells was measured using the human cervical cancer (HeLa) cell line. Results revealed that oral consumption of the extract (15 g of *G. lutea* 30% EtOH extract) provided radioprotective effect against the cytotoxic effect of irradiation on human PMBC while not modulating the susceptibility of malignant cells (110).

Petrovic et al. isolated polyphenols including tetraoxygenated xanthenes and their glycosides, C-glucoflavones, and secoiridoids from *G. dinarica* root, and investigated their *in vitro* radioactive effects. Among the tested compounds, gentiacaulein, gentiakochianin, norswertianin-1-*O*-primveroside, norswertianin-8-*O*-primveroside, isoorientin, and isoorientin-4-*O*-glycoside were tested in human peripheral blood lymphocytes exposed to 2 Gy of gamma radiation and were evaluated through lipid peroxidation, micronucleus, thiobarbituric acid, and apoptosis assays. Norswertianin 8-*O*-primveroside (26.92%), isoorientin 4'-*O*-glycoside (25.90%), norswertianin-1-*O*-primveroside (21.66%), gentiakochianin (21.55%) and isoorientin (4.97%) significantly reduced radiation-induced micronuclei. Isoorientin 4'-*O*-glycoside (39.75%), gentiakochianin (35.56%), norswertianin 1-*O*-primveroside (33.07%), and plant infusion (11.49%) significantly reduced MDA levels whereas norswertianin 8-*O*-primveroside and gentiacaulein led to an increase (20.24 and 7.45%). The whole plant infusion (249.50%), norswertianin 1-*O*-primveroside (85.49%), isoorientin 4'-*O*-glycoside (41.29%), norswertianin 8-*O*-primveroside (33.07%) and isoorientin (8.97%) elevated percentage of apoptotic cells while gentiakochianin reduced the percentage by 37.87%. Norswertianin 8-*O*-primveroside (18.06%), isoorientin 4'-*O*-glycoside (16.13%), norswertianin-1-*O*-primveroside (10.97%) and gentiakochianin (9.68%) decreased cell proliferation in irradiated human lymphocytes remarkably (111).

2.1.2.10. Antigenotoxic, Cytotoxic, and Antitumor Activities

Hudecova et al. performed a study on methanolic and aqueous extracts of *G. asclepiadea* flower and haulm to evaluate their DNA repair ability in human PMBC after H₂O₂ treatment and in human kidney HEK 293 cells after silver nanoparticles (AGNP) exposure. DNA damage of untreated cells and cells treated with extracts (0.25 mg/mL) was assessed by single-cell gel electrophoresis. PMBC treated with H₂O₂ and incubated without extracts had rejoined 40% of strand breaks (SBs) whereas cells incubated with aqueous haulm extract had rejoined 90% of SBs, with methanolic flower extract 86% of SBs, with methanolic haulm extract 81% of SBs and with aqueous flower extract 72% of SBs after 8 minutes of recovery. On the other hand, cells incubated with extracts displayed faster recovery than those incubated without extracts. The highest DNA recovery following AgNP exposure was observed between 5 and 10 mins with an increase in SB rejoining and a decrease in DNA damage. The methanolic haulm, aqueous haulm, and methanolic flower extracts reduced the tail DNA damage from 26.6% to 13% whereas the aqueous flower extract led to a decrease to 11%. Haulm extracts exhibited a significant recovery between 30 and 60 mins. DNA damage reduced from 13.7% to 5.9% with the methanolic haulm extract whereas decreased from 12.4% to 6.7% with the aqueous haulm extract (15). Hudecova and colleagues published another study on aqueous and methanol extracts of *G. asclepiadea* haulms and flowers and their DNA repair ability in human kidney HEK 293 cells after silver nanoparticles (AgNP) exposure. Comet assay was performed, and DNA strand breaks and oxidized base lesions (8-oxoG) were quantified. The methanolic haulm extract led to the highest increase in DNA SBs. According to the results of the comet assay, the methanolic haulm extract was found most potent against the 8-oxoguanine (8-oxoG) formation (112). Mihailovic et al. extracted *G. asclepiadea* roots with methanol and partitioned with the solvents chloroform, ethyl acetate and *n*-butanol. The antigenotoxic activity of the fractions was evaluated SLRL mutations in ethyl methanesulfonate (EMS)-treated *D. melanogaster* males. Post-treatment with all the root fractions decreased the frequency of mutations and genotoxicity significantly in germinative cell lines (45). Mihailovic and colleagues performed another study on antigenotoxic activity. The methanolic extracts of *G. cruciata* aerial parts and roots were evaluated through the alkaline comet assay. According to the results, both extracts exhibited antigenotoxic effects against CCl₄-induced DNA damage. These properties were attributed to the high polyphenolic content of the extracts and their potential to protect from ROS (46).

Rodrigues et al. prepared aqueous and ethanolic extracts of *G. lutea* roots and tested their *in vitro* cytotoxic activities against HeLa and breast carcinoma (MCF-7) cell lines at different concentrations (31.25 to 500 µg/mL). The highest growth inhibition (25%) was detected at 500 µg/mL with the ethanolic extract on the MCF-7 cell line whereas the aqueous extract supported cell growth (15–20%) at low doses and demonstrated growth inhibition (15-25%) at the highest extract concentration. These critical differences in activity were ascribed to the different secondary metabolite content of the extracts (113).

Sobot et al. extracted *G. lutea* roots with water to evaluate their cytotoxic and genotoxic effects in human peripheral blood mononuclear cells. Oxidative stress parameters were assessed by pro/antioxidant balance (PAB) and lipid peroxidation (LP). 1 and 2 mg/mL doses of the extract slightly decreased the PAB and LP levels. Increased chromosome aberrations were observed after 48 h of treatment with the extract however, the percentage of chromosomal breaks decreased after 72 h of treatment for concentrations of 1 and 2 mg/mL (0.93 and 5.1%). Cell viability was reduced with the increasing doses (0.5, 1, and 2 mg/mL) and treatment duration. The results revealed concentration- and time-dependent cytotoxic and genotoxic effects of the extract. However, the lower frequency of chromosomal breaks after 72 h of treatment with 1 and 2 mg/mL doses was ascribed to the activation of DNA repair mechanisms in the cells with DNA lesions as well as the removal of the cells with irreparable damage (114).

In a study conducted by Isakovic et al., aerial parts of *G. kochiana* were extracted with methanol and then partitioned with ether, ethyl acetate, and *n*-butanol, respectively to yield subfractions. Gentiakochianin, gentiacaulein, decussatin, isogentiakochianoside, gentiacaulein- 1-*O*-glucoside, and gentiabavaroside were obtained from the fractions after multiple chromatographic steps. All the extracts and isolates were tested in C6 rat glioma and U251 human glioma cell lines. Methanolic and ether extracts exhibited *in vitro* anti-glioma activity and xanthones gentiakochianin (IC₅₀ = 27.3 µM and 31.4 µM for U251 and C6 cells) and gentiacaulein (IC₅₀ = 53.2 µM and 56.6 µM for U251 and C6 cells) were determined as their active principles. Isolated compounds gentiakochianin and gentiacaulein induced cell cycle arrest in G₂/M and G₀/G₁ phases and decreased mitochondrial membrane potential in glioma cells. Gentiakochianin exhibited a stronger antiproliferative effect through its ability to induce microtubule stabilization, induction of caspase action, and subsequent apoptotic cell death. Furthermore, combinations of gentiakochianin-gentiacaulein and gentiacaulein-

decussatin exhibited synergistic activity. Additionally, structure-activity relationship (SAR) analyses were performed and activity was attributed to the dihydroxylation at positions C7 and C8 of the xanthonic nucleus (115).

Wani et al. investigated the antiproliferative activity of the methanolic extract of *G. kurroo* roots (at 10-100 µg/mL conc.) through sulphorhodamine B (SRB) assay against various cancer lines such as human colon cancer cell line (HCT-116), lung carcinoma cell line (A-549), pancreatic cancer cell line (Miapaca-2), lung cancer cell line (HOP-62) and acute monocytic leukemia cell line (THP-1). The methanolic extract exhibited a concentration-dependent anti-proliferative effect on all five cancer cell lines. The most potent activity was measured at the highest (100 µg/mL) concentration against THP-1 and HCT-116 cell lines (with 87 and 74 % of cell growth inhibitions). The protective effect of the methanolic extract on oxidative damage was assessed in calf thymus DNA. According to the results, *G. kurroo* root extract prevented oxidative damage induced by FeSO₄ and H₂O₂ in a concentration-dependent manner. Cell cycle progression and mitochondrial membrane potential were assessed by flow cytometry analyses. The methanolic extract induced cell cycle arrest in G₀/G₁ phase whereas the population of apoptotic cells changed (from 11.4% in the case of control to 49.6% at the highest concentration). The extract also displayed a significant effect on mitochondrial membrane potential loss in a concentration-dependent manner (49).

Matsukawa et al. extracted *G. triflora* var. *japonica* roots with boiling water to evaluate *in vitro* and *in vivo* antiproliferative properties. The aqueous extract inhibited (by 30-40% compared to the control) the growth of implanted solid tumors (murine renal cell carcinoma, Renca cells) in BALB/c mice by daily oral administration. This result confirmed the *in vivo* antiproliferative effect of the extract on solid tumors. The plant extract induced apoptosis of cultured human Daudi cell line (Burkitt's lymphoma) and the mechanism of the *in vitro* cell death was investigated. Morphological changes were observed in extract-treated cells due to apoptotic cell death, such as shrank, fragmented, condensed cells and nuclei appearance as well as DNA degradation (116). Ogata and colleagues performed another study on *G. triflora* var. *japonica* aqueous extract and its *in vitro* antiproliferative effect using various cell lines including rat myeloma (Y3-Ag1.2.3) and HeLa cells. Inhibition of caspase activity was assessed via pan-caspase inhibitor Z-VAD-fmk whereas the number of viable cells was calculated with WST (water-soluble tetrazolium salt)-1 assay or trypan blue exclusion. The plant extract induced apoptosis via caspase-independent pathways. Y3-Ag1.2.3 cell lines exhibited

higher susceptibility to the extract compared to HeLa cells with a higher rate of mitochondrial respiration. (117).

2.1.2.11. Anti-Inflammatory and Analgesic Activities

Wang et al. investigated the anti-inflammatory activity of the compounds isolated from *G. dahurica* roots, which is one of the four plants that consisting “Qin-Jiao”. Iridoid glycosides, ecdysteroids, lignans, triterpenes, a lignan glycoside, a phenylpropanoid, and a triterpenoid were isolated from *G. dahurica* after a series of chromatographic steps. In addition, iridoid glycosides obtained from other Qin Jiao plants (*G. crassicaulis*, *G. straminea*, and *G. rigescens*) from prior studies were also tested. Inhibition of NO release (in macrophage RAW264.7 cells) and COXs-2/1 (on zebrafish model) as well as cytotoxicity (by MTT, in myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549 cells, MCF-7, and colon cancer SW480 cell lines) assays were conducted. Roburic acid exhibited a moderate inhibitory effect on NO release (IC_{50} = 16.85 μ M) and weak cytotoxicity on HL-60 cells (IC_{50} = 16.43 μ M). Twelve of the isolated compounds (gentiopicroside, 6'-*O*- β -D-glucopyranosyl gentiopicroside, olivieroside C, epi-kingiside, kingiside, loganic acid, 20-hydroxyecdysone 3-acetate, liriodendrin, roburic acid *G. dahurica*, qingjiaoside C, 4'-*O*- β -D-glucosylgentiopicroside from *G. crassicaulis* and 11-*O*- β -D-glucopyranosylester from *G. straminea*) displayed inhibitory effects on COXs-2/1 (at conc. of 30 μ M) more than the positive control, indomethacin (56%, at conc. of 0.9 μ M). Among these compounds, roburic and (79%) liriodendrin (73%) exhibited the strongest inhibitory activities (118).

Jia et al. extracted *G. macrophylla* and *G. straminea* with 75% EtOH to evaluate their *in vivo* and *in vitro* biological activities. As a part of Chinese, Tibetan, and Mongolian traditional medicine, these two species have long been utilized for pain and inflammation. Experimental inflammation and pain models (carrageenan-induced paw edema, xylene-induced ear edema, writhing test, and hot plate test in mice), as well as macrophage RAW 264.7 and TL4-expressing HEK 293 cells were used in the assays. Inhibition of nuclear factor kappa B (NF- κ B) transcriptional activity and nuclear translocation of NF- κ B (p50 and p65) was examined by using the luciferase-based assay and immunoblotting. According to the results, both extracts demonstrated remarkable and identical anti-inflammatory and analgesic effects on all four experimental models in a concentration-dependent manner. Results also revealed that pretreatment with the extracts

significantly suppressed LPS-induced NF- κ B transactivation and inhibited LPS-induced p65 and p50 nuclear translocation. These biological effects of the extracts were attributed to the secoiridoid glycosides, flavonoids, and triterpenes which were detected by HPLC-ESI-MS analysis (119). Shi and colleagues performed a study on polysaccharides from *G. macrophylla* roots to assess their anti-inflammatory activities. 3D NH₂-graphene oxide hydrogel was used for separation for selective adsorption with a high decoloration ratio (96.4%). Structural elucidations were determined by high-performance gel permeation chromatography (HPGPC) according to the molecular weight of the compounds. Anti-inflammatory activity was evaluated through TNF- α , IL-1 β , IL-6, and IL-10 levels, using cultured RAW264.7 cells. *G. straminea* decolored polysaccharide fraction reduced TNF- α , IL-6, and IL-1 β levels concentration-dependently (120).

Zhao et al. isolated gentiopicroside from *G. macrophylla* to assess its cytotoxicity and anti-inflammatory properties on rat chondrocytes (50, 500, and 1500 μ g/mL concentrations). According to the MTT and Hoechst staining assays, no significant toxicity or morphological feature of apoptosis-necrosis was observed with gentiopicroside exposure. The isolate diminished PGE₂ (induced by 10 ng/mL IL-1 β) levels, down-regulated COX-2 mRNA, caused a reduction in MMP-1, MMP-3, and MMP-13 gene expression levels, and upregulated type II collagen reduction (induced by 10ng/ml IL-1 β). The isolate was also evaluated for its inhibitory activity on MAPK signaling pathways (induced by 10 ng/mL IL-1 β) and reduced P38, JNK, and ERK activation (121). Zhang et al. conducted another study on the anti-inflammatory activity of gentiopicroside. The compound was isolated from the aqueous extract of *G. officinalis* and its activity was evaluated by both *in vivo* and *in vitro* assays. LPS-induced RAW 264.7 cells were pretreated with different concentrations of gentiopicroside (25, 50, 100 μ g/mL). According to the results, production of the NO, PGE₂, and IL-6 was strongly inhibited by gentiopicroside treatment. The *in vivo* anti-inflammatory activity was assessed using the xylene-induced ear edema assay and gentiopicroside displayed a potent activity with an inhibition rate of 34.2%. The molecular docking experiment also confirmed its iNOS and COX-2 inhibitory interactions (122).

In a study performed by Cao and colleagues, EtOAc extract of *G. striata* was tested (at 100 and 200 mg kg⁻¹ conc.) on rheumatoid arthritis-induced inflammation. The extract decreased rat serum NO levels, with a higher inhibition rate compared to positive control prednisone while reducing PGE₂ level with a similar extent as that produced by

the control. The anti-inflammatory properties of the extract were attributed to its components such as loganin, sweroside, apigenin, luteolin, quercetin, and rutin (123).

Zhang et al. conducted a study on hot water extract of *G. purpurea* roots and the anti-inflammatory potential of its active principles. Among the isolated compounds, gentiopicroin, amarogentin, erythrocentaurin, and gentiogenol inhibited TNF- α secretion dose-dependently (40).

Wani et al. extracted *G. kurroo* roots with MeOH to assess *in vivo* analgesic activity through Eddy's hot plate method and acetic acid-induced writhing test in mice. Oral administration of the extract (250 and 500 mg/kg) showed a dose-dependent analgesic effect. The extract exerted 63.4% and 73.7% inhibition of the writhing response and increased reaction time in Eddy's hot plate method (124). Mubashir and colleagues conducted another study on the MeOH extract of *G. kurroo* to assess anti-inflammatory and immunomodulatory properties by using *in vivo* and *in vitro* assays. Haemagglutination antibody titre (HAT) and delayed-type hypersensitivity (DTH) assays were used to evaluate *in vivo* activity. The extract (200 mg/kg) exerted 66.2 and 55.3% inhibition of primary and secondary humoral response and 70.3 and 75.7% inhibition of cellular response after 24 and 48 h of paw thickness, with a higher inhibition rate compared to control cyclophosphamide. The methanolic extract reduced NO, TNF- α , and IL-6 levels and NF- κ B expression in LPS-induced peritoneal macrophages while suppressing T and B-cell proliferation in splenocytes in a dose-dependent manner (125).

2.1.3. Compounds Isolated from *Gentiana* sp.

The phytochemical studies conducted on roots and aerial parts of different *Gentiana* species up to date indicated that the main components isolated from this genus include secoiridoids, iridoids, flavonoids, and xanthenes. The secondary metabolites isolated from *Gentiana* species were presented in the following tables particularly focusing on the recent articles (Tables 1-80).

2.1.3.1. Secoiridoids

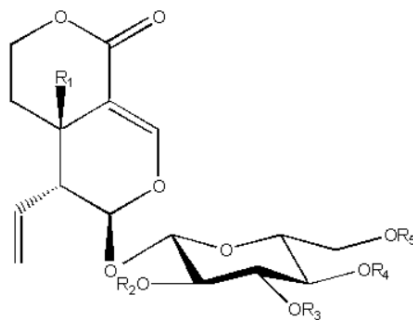


Table 1. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
Sweroside	H	H	H	H	H	<i>G. algida</i>	(126,127)
						<i>G. asclepiadea</i>	(15,16), (45)
							(128–131)
						<i>G. boissieri</i>	(132)
						<i>G. crassicaulis</i>	(133–135)
						<i>G. cruciata</i>	(17), (46)
							(136–138)
						<i>G. dahurica</i>	(133), (139,140)
						<i>G. dinarica</i>	(141)
						<i>G. formosana</i>	(142)
						<i>G. gelida</i>	(131)
						<i>G. loureirii</i>	(143)
						<i>G. lutea</i>	(144–147)
						<i>G. lutea</i> ssp.	(148)
						<i>symphyandra</i>	
						<i>G. macrophylla</i>	(119), (133)
							(149–155)
						<i>G. manshurica</i>	(156), (157)
						<i>G. officinalis</i>	(133)
						<i>G. olivieri</i>	(33), (158)
						<i>G. paradoxa</i>	(131)
						<i>G. pneumonanthe</i>	(138), (159)

Table 1. Secoiridoids obtained from *Gentiana* sp. (continue)

Sweroside	H	H	H	H	H	<i>G. purpurea</i> <i>G. rhodantha</i> <i>G. rigescens</i> <i>G. scabra</i> <i>G. septemfida</i> <i>G. siphonantha</i> <i>G. straminea</i> <i>G. striata</i> <i>G. tibetica</i> <i>G. triflora</i>	(40) (160) (156), (161–165) (156), (166) (131), (167) (133), (168) (133), (169–173) (123) (174) (156)
Swertiamarin	OH	H	H	H	H	<i>G. apiata</i> <i>G. asclepiadea</i> <i>G. campestris</i> <i>G. crassicaulis</i> <i>G. cruciata</i> <i>G. dahurica</i> <i>G. dinarica</i> <i>G. gelida</i> <i>G. kurroo</i> <i>G. lutea</i> <i>G. lutea</i> ssp. <i>symphyandra</i> <i>G. macrophylla</i> <i>G. manshurica</i>	(175) (14–16), (45), (130), (131) (176) (133–135), (177,178) (17), (46) (136–138) (133), (179) (139,140) (141), (180) (131), 181 (71) (138), (145), (182–184) (148) (119), (133), (149–154) (156,157)

Table 1. Secoiridoids obtained from *Gentiana* sp. (continue)

Swertiamarin	OH	H	H	H	H	<i>G. officinalis</i> <i>G. ottonis</i> <i>G. paradoxa</i> <i>G. pneumonanthe</i> <i>G. punctata</i> <i>G. rhodantha</i> <i>G. rigescens</i> <i>G. septemfida</i> <i>G. scabra</i> <i>G. straminea</i> <i>G. triflora</i>	(133) (185), (186) (131) (138), (159) (187) (160), (188) (153), (163– 165), (184,185) (167), (131) (156), (166), (188–190) (119), (133), (171,172), (191) (156)
Swertiapunimarin	H	H	H	H	Glc	<i>G. macrophylla</i> <i>G. olivieri</i>	(155) (33)
3'-acetylsweroside	H	H	Ac	H	H	<i>G. formosana</i>	(142)
6'-acetylsweroside	H	H	H	H	Ac	<i>G. manshurica</i>	(157)
6'-O-(3-(β-D-glucopyranosyloxy)-2-hydroxybenzoyl)-sweroside	H	H	H	H	A	<i>G. manshurica</i> <i>G. straminea</i>	(157) (171–173)
6'-O-acetyl-3'-O-(3-(β-D-glucopyranosyloxy)-2-hydroxybenzoyl)-sweroside	H	H	A	H	Ac	<i>G. manshurica</i>	(157)
6'-(2,3-dihydroxybenzoyl)-sweroside	H	H	H	H	B	<i>G. algida</i>	(192)
6'-(2,3-dihydroxybenzoyl)-swertiamarin	OH	H	H	H	B	<i>G. algida</i>	(192)
Olivieroside B	H	B	H	H	H	<i>G. olivieri</i>	(33), (193)

Table 1. Secoiridoids obtained from *Gentiana* sp. (continue)

Gentistraminoside A	H	H	H	Ac	C	<i>G. straminea</i>	(191)
Gentistraminoside B	OH	H	Ac	Ac	C	<i>G. straminea</i>	(191)
2'-(2,3-dihydroxy benzoyl)-sweroside	H	C	H	H	H	<i>G. algida</i> <i>G. rigescens</i> <i>G. scabra</i> <i>G. straminea</i> <i>G. tibetica</i>	(126) (156), (194) (166) (172), (173) (174)
2'-(2,3-dihydroxy benzoyl)-swertiamarin	OH	C	H	H	H	<i>G. rigescens</i>	(194)
Trifloroside	H	Ac	Ac	A	Ac	<i>G. algida</i> <i>G. cruciata</i> <i>G. gelida</i> <i>G. linearis</i> <i>G. lutea</i> <i>G. manshurica</i> <i>G. olivieri</i> <i>G. paradoxa</i> <i>G. pneumonanthe</i> <i>G. scabra</i> <i>G. scabra</i> var. <i>buergeri</i> <i>G. septemfida</i> <i>G. siphonantha</i> <i>G. straminea</i> <i>G. tibetica</i> <i>G. triflora</i>	(126) (131) (131), (181) (195) (144) (157) (158) (131) (129) (190), (196) (30), (197) (131) (168) (179) (174) (198)
Scabraside	H	Bz	Ac	A	Ac	<i>G. manshurica</i> <i>G. scabra</i> <i>G. scabra</i> var. <i>buergeri</i>	(157) (199) (30)

Ac: acetyl Bz: benzoyl Glc: glucosyl A: 3-(β -D-glucopyranosyloxy)-2-hydroxybenzoyl)

B: 2,3-dihydroxybenzoyl C: 2-hydroxy-3- (1- β -D-glucopyranosyloxy)-benzoyl

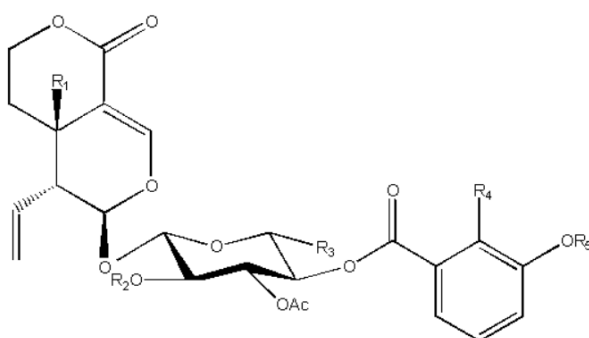


Table 2. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
Deglucosyltrifloroside	H	Ac	CH ₂ OH	OH	H	<i>G. scabra</i> <i>G. triflora</i>	(200) (196,201)
Deglucoscabroside	H	Bz	CH ₂ OH	OH	H	<i>G. triflora</i>	(201)
2''-dehydroxy trifloroside	H	Ac	CH ₂ OH	H	Glc	<i>G. scabra</i>	(200)
4'''-O-β-D-glucopyranosyltrifloroside	H	Ac	CH ₂ OH	OH	A	<i>G. triflora</i>	(199)
4'''-O-β-D-glucopyranosylscabroside	H	Bz	CH ₂ OH	OH	A	<i>G. triflora</i>	(199)
6'''-O-β-D-glucosyl trifloroside	H	Ac	CH ₂ OH	OH	B	<i>G. linearis</i>	(195)
Macrophylloside A	H	C	CH ₂ OH	OH	Glc	<i>G. macrophylla</i> <i>G. straminea</i> <i>G. tibetica</i>	(155) (170), (191) (174)
Macrophylloside B	OH	C	CH ₂ OH	OH	Glc	<i>G. macrophylla</i>	(155)
Isomacrophylloside	H	C	CH ₂ OH	OGlc	H	<i>G. tibetica</i>	(174)
Gelidoside (Rindoside)	OH	Ac	CH ₂ OH	OH	Glc	<i>G. algida</i> <i>G. gelida</i> <i>G. macrophylla</i> <i>G. scabra</i> var. <i>buergeri</i> <i>G. septemfida</i>	(126) (181) (152) (197) (167)

Table 2. Secoiridoids obtained from *Gentiana* sp. (continue)

						<i>G. siphonantha</i>	(166)
						<i>G. tibetica</i>	(174)
Deglucogelidoside	OH	H	CH ₂ OH	H	H	<i>G. triflora</i>	(201)
2-gentiosylgelidoside	OH	D	CH ₂ OH	OH	Glc	<i>G. siphonantha</i>	(168)

Ac: acetyl Bz: benzoyl A: (4'''-*O*- β -D-glucopyranosyl)- β -D-glucopyranosyl B: (6'''-*O*- β -D-glucopyranosyl)- β -D-glucopyranosyl C: 2,3-dihydroxybenzoyl D: 2,5-dihydroxybenzoyl (gentiosyl)

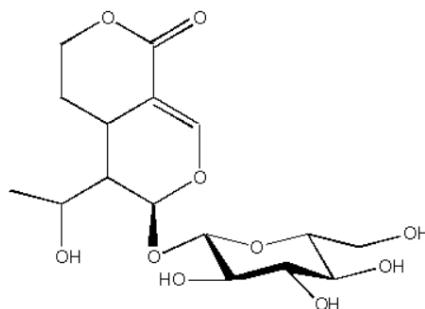


Table 3. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
8-hydroxy-10-sweroside	<i>G. macrophylla</i>	(155)
	<i>G. scabra</i>	(190)
	<i>G. tibetica</i>	(174)

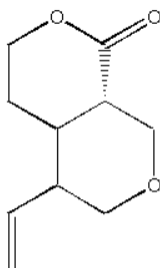


Table 4. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Olivierigenin	<i>G. olivieri</i>	(193)

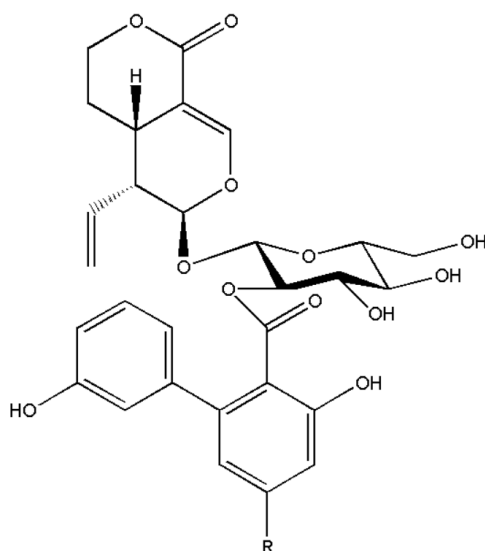


Table 5. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Amaropandin	H	<i>G. burseri</i>	(202)
		<i>G. pannonica</i>	(203)
Amarogentin	OH	<i>G. asclepiadea</i>	(138)
		<i>G. burseri</i>	(202)
		<i>G. dinarica</i>	(141)
		<i>G. gelida</i>	(131)
		<i>G. lutea</i>	(138), (145), (204–206)
		<i>G. lutea</i> ssp. <i>symphyandra</i>	(207)
		<i>G. pannonica</i>	(203)
		<i>G. pneumonanthe</i>	(138)

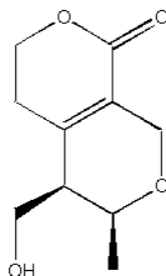


Table 6. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Swerpalactone	<i>G. macrophylla</i>	(208,209)

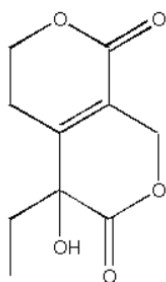


Table 7. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Gentiolacton	<i>G. purpurea</i>	(210)
	<i>G. siphonantha</i>	(168)
	<i>G. triflora</i>	(211)

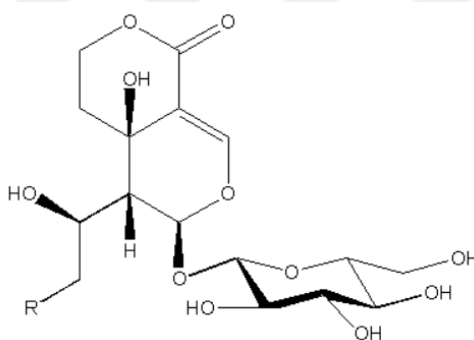


Table 8. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Eustoside	Cl	<i>G. campestris</i>	(176)
		<i>G. septemfida</i>	(167)
Eustomorusside	OH	<i>G. campestris</i>	(176)
		<i>G. gelida</i>	(181)
		<i>G. lutea</i>	(184)
		<i>G. olivieri</i>	(33)
		<i>G. septemfida</i>	(167)

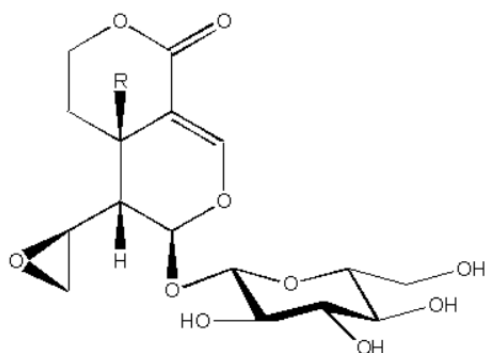


Table 9. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Desoxyeustomoside	H	<i>G. campestris</i>	(176)
Eustomoside	OH	<i>G. campestris</i> <i>G. gelida</i> <i>G. lutea</i> <i>G. olivieri</i> <i>G. septemfida</i>	(176) (181) (184) (33) (167)

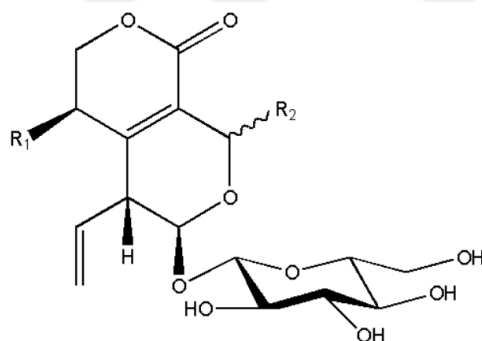


Table 10. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Swertiajaposide A	H	β -OCH ₃	<i>G. scabra</i>	(200), (212)
6 β -hydroxy swertiajaposide A	OH	β -OCH ₃	<i>G. crassicaulis</i> <i>G. scabra</i>	(134) (212)
6 β -hydroxy-3-epi-swertiajaposide A	OH	α -OCH ₃	<i>G. scabra</i>	(200)

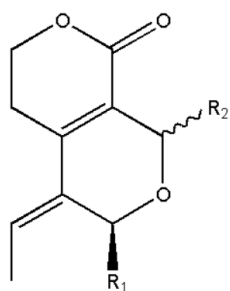


Table 11. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
3-epi-swertiajaposide C	—OGLc	OCH ₃	<i>G. apiata</i>	(175)
(Z)-5-ethyliden-3,4,5,6-tetrahydro- <i>cis</i> -6,8-dimetoksi-1 <i>H</i> ,8 <i>H</i> -pyrano (3,4- <i>c</i>) pyran-1-one	—OCH ₃	—OCH ₃	<i>G. macrophylla</i>	(209)
Swerimilegenin E	CH ₃	—OCH ₃	<i>G. macrophylla</i>	(213)
Swerimilegenin H	—OH	H	<i>G. crassicaulis</i> <i>G. macrophylla</i>	(200) (154)
Dehydroxy swerimilegenin H	H	H	<i>G. veitchiorum</i>	(214)
Swerimilegenin I	—OCH ₃	H	<i>G. crassicaulis</i> <i>G. macrophylla</i>	(200) (154)

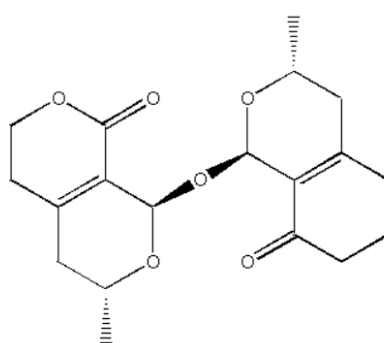


Table 12. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Swerimilegenin G	<i>G. macrophylla</i>	(213)

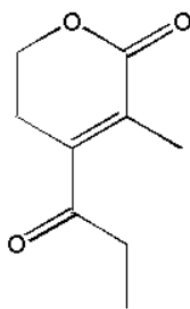


Table 13. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Swermilegenin C	<i>G. veitchiorum</i>	(213)

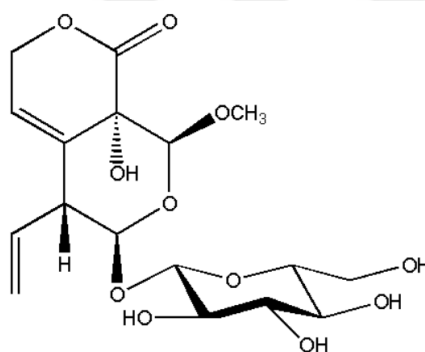


Table 14. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Gentiascabraside A	<i>G. scabra</i>	(212)

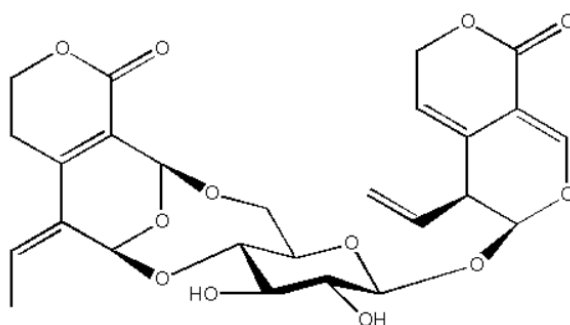


Table 15. Secoiridoid dimer obtained from *Gentiana* sp.

Compound	Species	References
Gentimacroside	<i>G. macrophylla</i>	(209)

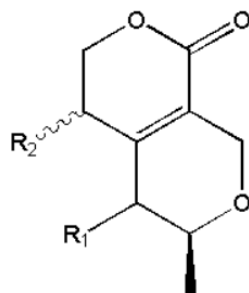


Table 16. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Swerimuslactone A	H	H	<i>G. veitchiorum</i>	(213)
9-oxoswerimuslactone A	=O	H	<i>G. veitchiorum</i>	(213)
6-hydroxy swerimuslactone A	H	OH	<i>G. macrophylla</i>	(154)

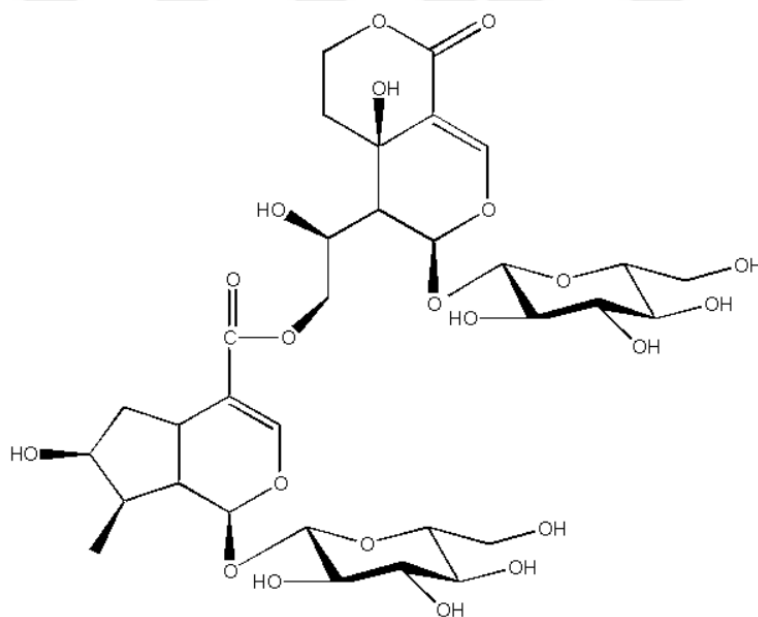


Table 17. Iridoid-secoiridoid dimer obtained from *Gentiana* sp.

Compound	Species	References
Septemfidside	<i>G. septemfida</i>	(167)

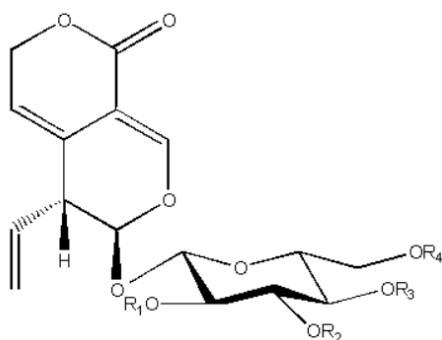


Table 18. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
Gentiopicroside	H	H	H	H	<i>G. algida</i>	(126,127)
					<i>G. apiata</i>	(175)
					<i>G. asclepiadea</i>	(15,16), (138), (128–131), (215)
					<i>G. boisseri</i>	(132)
					<i>G. burseri</i>	(202)
					<i>G. crassicaulis</i>	(133–135), (177,178)
					<i>G. cruciata</i>	(17), (136–138)
					<i>G. dahurica</i>	(133), (139,140), (216)
					<i>G. dinarica</i>	(141)
					<i>G. gelida</i>	(181)
					<i>G. linearis</i>	(195)
					<i>G. lutea</i>	(144–147), (10,217)
					<i>G. lutea ssp. symphyandra</i>	(148), (207)
					<i>G. macrophylla</i>	(103), (119), (133), (150), (152), (154,155) (209)
					<i>G. manshurica</i>	(62), (157), (209)
					<i>G. officinalis</i>	(133)
					<i>G. olivieri</i>	(33), (193)
					<i>G. paradoxa</i>	(131)
					<i>G. pneumonanthe</i>	(131), (138)

Table 18. Secoiridoids obtained from *Gentiana* sp. (continue)

Gentiopicroside	H	H	H	H	<i>G. rhodantha</i> <i>G. rigescens</i> <i>G. septemfida</i> <i>G. scabra</i> <i>G. siphonantha</i> <i>G. spathacea</i> <i>G. straminea</i> <i>G. tibetica</i> <i>G. triflora</i> <i>G. veitchiorum</i>	(165), (188) (156), (161–165) (218,219) (131), (167) (156), (166), (189), (199) (219,220) (133), (168) (102) (119), (133) (171–173), (191) (174), (221) (156), (201), (211) (222)
3'-acetyl-gentiopicroside	H	Ac	H	H	<i>G. dahurica</i>	(216)
6'-acetyl-gentiopicroside	H	H	H	Ac	<i>G. dahurica</i> <i>G. straminea</i>	(216) (172,173)
3'-O-β-D-glucosyl-gentiopicroside (olivieroside C)	H	Glc	H	H	<i>G. crassicaulis</i> <i>G. dahurica</i> <i>G. macrophylla</i> <i>G. olieri</i>	(177,178) (140) (154) (33)
4'-O-β-D-glucosyl-gentiopicroside	H	H	Glc	H	<i>G. crassicaulis</i> <i>G. scabra</i>	(178) (223)
6'-O-β-D-glucosyl-gentiopicroside	H	H	H	Ac	<i>G. apiata</i> <i>G. asclepiadea</i> <i>G. crassicaulis</i> <i>G. cruciata</i> <i>G. dahurica</i> <i>G. manshurica</i> <i>G. macrophylla</i>	(175) (224) (134), (178) (131) (216) (156) (119), (153–155)

Table 18. Secoiridoids obtained from *Gentiana* sp. (continue)

6'-O-β-D-glucosyl-gentiopicroside	H	H	H	Ac	<i>G. olivieri</i> <i>G. rigescens</i> <i>G. scabra</i> <i>G. straminea</i> <i>G. triflora</i>	(33) (156) (156), (223) (119), (171,172), (191) (156)
6'-O-β-D-xylopyranosyl gentiopicroside	H	H	H	Xyl	<i>G. crassicaulis</i>	(134)
Olivieroside	H	Glc	H	H	<i>G. scabra</i>	(223)
Olivieroside A	A	H	H	H	<i>G. olivieri</i>	(33), (193)
Scabran G₃	H	H	H	B	<i>G. crassicaulis</i> <i>G. macrophylla</i> <i>G. scabra</i>	(134) (154) (212)
Scabran G₄	H	H	H	C	<i>G. crassicaulis</i> <i>G. scabra</i>	(134) (212)
Scabran G₅	H	H	H	D	<i>G. scabra</i>	(212)

Ac: acetyl Bz: benzoyl A: *p*-coumaryl B: β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside C: β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside D: 2x β -D-glucopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside Xyl: Xylulose

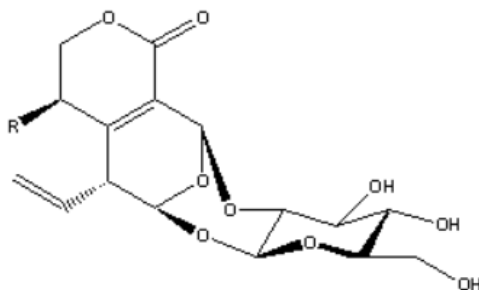


Table 19. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Gentiananoside A	OH	<i>G. crassicaulis</i>	(134)
Gentiananoside B	H	<i>G. crassicaulis</i>	(134)

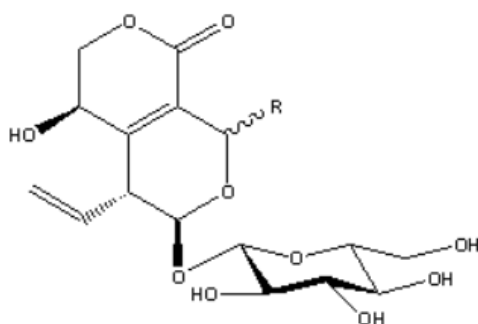


Table 20. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Gentiananoside C	α -EtO	<i>G. crassicaulis</i>	(134)
Gentiananoside D	β -EtO	<i>G. crassicaulis</i>	(134)

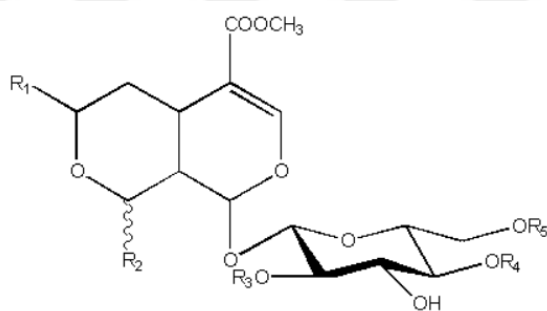


Table 21. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
Kingiside	=O	β -CH ₃	H	H	H	<i>G. dahurica</i> <i>G. pyrenica</i> <i>G. rhodantha</i>	(140) (225) (226)
2'-O-(3''-hydroxy benzoyl)-kingiside	=O	β -CH ₃	A	H	H	<i>G. rhodantha</i>	(226)
8-epikingiside	=O	α -CH ₃	H	H	H	<i>G. dahurica</i> <i>G. pyrenica</i> <i>G. rhodantha</i>	(140) (227) (226)
2'-O-(3''-hydroxy benzoyl)-8-epi kingiside	=O	α -CH ₃	A	H	H	<i>G. rhodantha</i>	(226)

Table 21. Secoiridoids obtained from *Gentiana* sp. (continue)

6'-<i>O</i>-<i>p</i>-coumaroyl-8-epikingiside	=O	$\alpha\text{-CH}_3$	H	H	B	<i>G. rhodantha</i>	(226)
6'-gentisoyl-8-epikingiside	=O	$\alpha\text{-CH}_3$	H	H	C	<i>G. siphonantha</i>	(228)
6'-vanilloyl-8-epikingiside	=O	$\alpha\text{-CH}_3$	H	H	Van	<i>G. pyrenica</i>	(225,227)
Morroniside	OH	$\beta\text{-CH}_3$	H	H	H	<i>G. pyrenica</i>	(229)
4'-<i>O</i>-<i>p</i>-coumaroyl-8-morroniside	OH	$\beta\text{-CH}_3$	H	B	H	<i>G. pyrenica</i>	(229)
7(<i>S</i>)-<i>n</i>-butyl morroniside		$\beta\text{-CH}_3$	H	H	H	<i>G. straminea</i>	(172,173)
7(<i>R</i>)-<i>n</i>-butyl morroniside		$\beta\text{-CH}_3$	H	H	H	<i>G. straminea</i>	(172,173)

Ac: acetyl Bz: benzoyl Van: vanilloyl A: 3-hydroxybenzoyl B: *p*-coumaryl C: 2,5-dihydroxybenzoyl

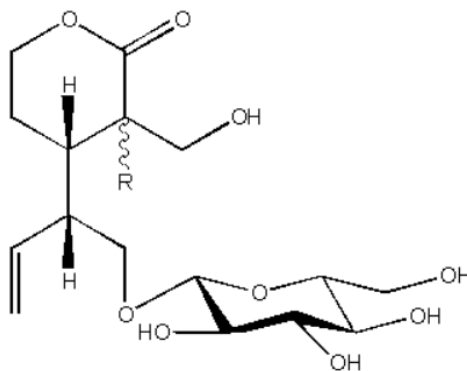


Table 22. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
1-<i>O</i>-β-D-glucopyranosyl amplexin	$\alpha\text{-H}$	<i>G. algida</i> <i>G. scabra</i>	(126), (127), (230) (223)
1-<i>O</i>-β-D-glucopyranosyl 4-epi-amplexin	$\beta\text{-H}$	<i>G. rhodantha</i> <i>G. scabra</i>	(86) (212)

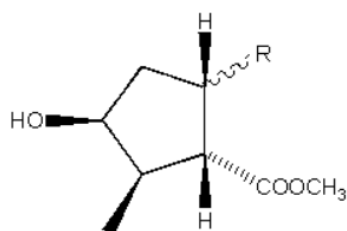


Table 23. Secoiridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Gentiolutelin	OH	<i>G. lutea</i>	(206)
Gentiolutelin dimethyl acetate	dimethyl acetate	<i>G. lutea</i>	(206)

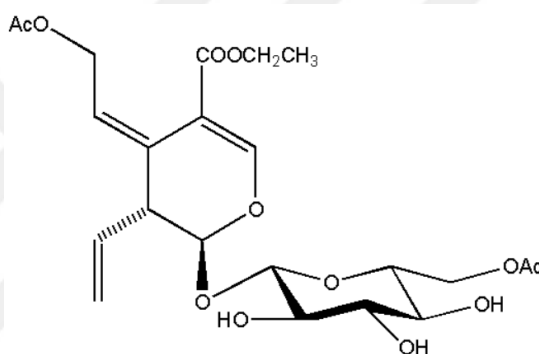


Table 24. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Gentiorigenoside A	<i>G. rigescens</i>	(231)

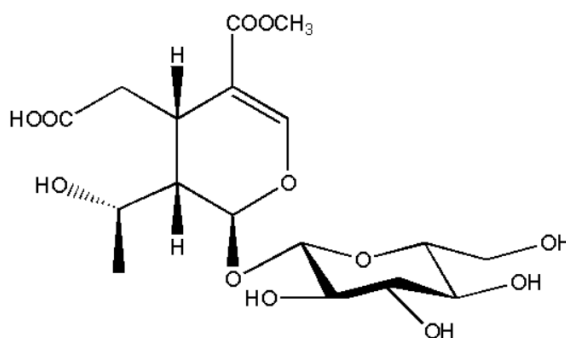


Table 25. Secoiridoid obtained from *Gentiana* sp.

Compound	Species	References
Alpigenoside	<i>G. rhodantha</i>	(226)

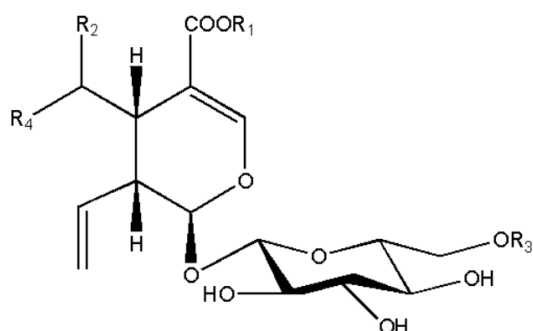


Table 26. Secoiridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
Depressine	CH ₃	A	H	H	<i>G. asclepiadea</i> <i>G. depressa</i>	(15) (232)
Secoxyloganin	CH ₃	COOH	H	H	<i>G. rhodantha</i>	(226)
Secologanoside	H	COOH	H	H	<i>G. rhodantha</i> <i>G. straminea</i>	(226) (191)
Secologanol	H	CH ₂ OH	H	H	<i>G. verna</i>	(233)
7-<i>O</i>-acetyl secologanol	H	CH ₂ OH	H	Ac	<i>G. verna</i>	(233)
7-<i>O</i>-(2,5- dihydroxy benzoyl) secologanol	H	CH ₂ OH	H	Gen	<i>G. verna</i>	(233)
6'-<i>O</i>-β- glucopyranosyl secologanoside	H	COOH	Glc	H	<i>G. rhodantha</i>	(226)
6'-<i>O</i>-β- glucopyranosyl secologanol	H	CH ₂ OH	Glc	H	<i>G. rhodantha</i>	(226)

A: 2-hidroksi-3-*O*-β-D-glycopyranosylbenzoyl Gen: genbiose

2.1.3.2. Iridoids

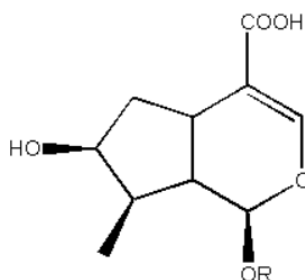


Table 27. Iridoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Loganetin	H	<i>G. verna</i>	(233)
Loganic acid	Glc	<i>G. algida</i> <i>G. asclepiadea</i> <i>G. crassicaulis</i> <i>G. cruciata</i> <i>G. dahurica</i> <i>G. davidii</i> <i>G. gelida</i> <i>G. lawrencei</i> <i>G. lourerii</i> <i>G. lutea</i> <i>G. macropylla</i> <i>G. manshurica</i> <i>G. officinalis</i> <i>G. paradoxa</i> <i>G. pneumonanthe</i> <i>G. rhodantha</i> <i>G. rigescens</i> <i>G. scabra</i> <i>G. septemfida</i> <i>G. siphonantha</i> <i>G. straminea</i> <i>G. tibetica</i>	(127), (234) (15), (131) (133), (135), (178) (46), (137) (133), (140), (216) (235) (131) (235) (143) (144,145), (206) (119), (133), (135), (152) (156) (133) (131) (131) (226) (156), (235) (156), (197), (236) (167) (133) (119), (133), (172,173), (191) (174)

Table 27. Iridoids obtained from *Gentiana* sp. (continue)

Loganic acid	Glc	<i>G. triflora</i>	(156)
		<i>G. veitchiorum</i>	(29)

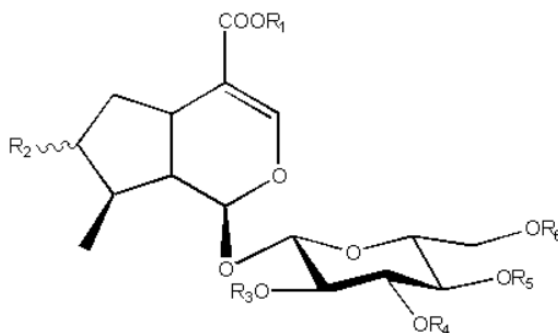


Table 28. Iridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	Species	References
2'- <i>O</i> -caffeoyl loganic acid	H	—OH	A	H	H	H	<i>G. lourerii</i>	(143)
2'- <i>O</i> - <i>p</i> -hydroxy benzoyl loganic acid	H	—OH	B	H	H	H	<i>G. lourerii</i>	(143)
2'- <i>O</i> - <i>cis-p</i> -coumaryl-loganic acid	H	—OH	C	H	H	H	<i>G. lourerii</i>	(143)
2'- <i>O</i> - <i>trans-p</i> -coumaryl loganic acid	H	—OH	D	H	H	H	<i>G. lourerii</i>	(143)
Loganic acid 6'- <i>O</i> -glucoside	H	—OH	H	H	H	Glc	<i>G. crassicaulis</i> <i>G. rhodantha</i> <i>G. macrophylla</i> <i>G. veitchiorum</i>	(178) (226) (237) (238)
Loganic acid 6'- <i>O</i> -(6''- <i>O</i> -β-D-glucopyranosyl)-β-D-glucopyranoside	H	—OH	H	H	H	E	<i>G. macrophylla</i>	(237)
Linearoside	H	—O-F	H	H	H	H	<i>G. linearis</i>	(195)

Table 28. Iridoids obtained from *Gentiana* sp. (continue)

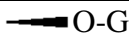
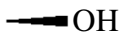
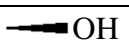
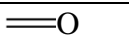
4''-O-β-D- gluco pyranosyl linearoside	H		H	H	H	H	<i>G. linearis</i>	(195)
4''-O-β-D-glucosyl-6'- O-(4-O-β-D-glucosyl caffeoyl) linearoside	H		H	H	H	I	<i>G. kurroo</i> <i>G. manshurica</i>	(125) (157)
Loganic acid 11-O-β- D-glucopyranosyl ester	Glc		H	H	H	H	<i>G. rhodantha</i>	(226)
Loganin (loganic acid methyl ester)	CH ₃		H	H	H	H	<i>G. dahurica</i> <i>G. depressa</i> <i>G. lourerii</i> <i>G. pedicellata</i> <i>G. straminea</i> <i>G. striata</i> <i>G. verna</i>	(140) (232) (143) (239) (191) (123) (240)
2'-p-coumaroyl loganin	CH ₃		J	H	H	H	<i>G. pedicellata</i>	(239)
2'-p-feruloly loganin	CH ₃		K	H	H	H	<i>G. pedicellata</i>	(239)
2'-p-caffeoyl loganin	CH ₃		L	H	H	H	<i>G. pedicellata</i>	(239)
4'-m-hydroxybenzoyl loganin	CH ₃		H	H	M	H	<i>G. verna</i>	(240)
4'-acetyl-2'-m- hydroxybenzoyl loganin	CH ₃		M	H	Ac	H	<i>G. verna</i>	(241)
6'-acetyl-2'-m- hydroxybenzoyl loganin	CH ₃		M	H	H	Ac	<i>G. verna</i>	(241)
7-ketologanin	CH ₃		H	H	H	H	<i>G. lourerii</i>	(143)

Table 28. Iridoids obtained from *Gentiana* sp. (continue)

2'-(2,3-dihydroxy benzoyloxy)-7-ketologanin	CH ₃	=O	N	H	H	H	<i>G. kurroo</i>	(26), (242)
2',3',6'-tri-O-acetyl-4'-O-trans-<i>p</i>-(<i>O</i>-β-D-glucopyranosyl)-coumaroyl-7-ketologanin	CH ₃	=O	Ac	Ac	O	Ac	<i>G. lourerii</i>	(143)

A: caffeoyl B: *p*-hydroxy benzoyl C: *cis-p*-coumaryl D: *trans-p*-coumaryl E: -(6"-*O*-β-D-glucopyranosyl)-β-D-glucopyranoside F: *p*-coumaryl G: 4"-*O*-β-D-glucopyranosyl-*p*-coumaroyl I: 4-*O*-β-D-glucopyranosyl caffeoyl J: 2'-*p*-coumaroyl K: 2'-*p*-feruloyl L: 2'-*p*-caffeoyl M: 4'-*m*-hydroxybenzoyl N: 2,3-dihydroxybenzoyl O: *trans-p*-(*O*-β-D-glucopyranosyl)coumaroyl

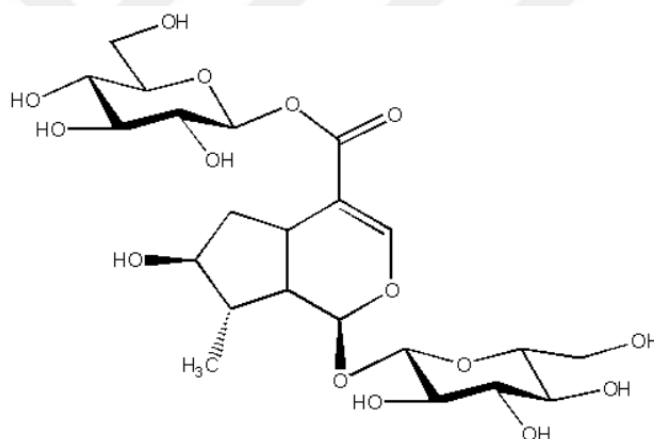


Table 29. Iridoid obtained from *Gentiana* sp.

Compound	Species	References
8-epi-loganic acid 11-O-β-D-glucopyranosyl ester	<i>G. straminea</i>	(191)

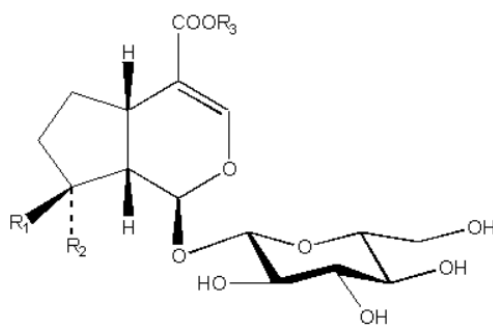


Table 30. Iridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
7-deoxyloganic acid	CH ₃	H	H	<i>G. apiata</i>	(175)
Asystasioside	H	CH ₃	Glc	<i>G. apiata</i>	(174)

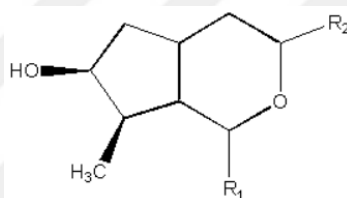


Table 31. Iridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Boonein	=O	H	<i>G. lourerii</i>	(143)
Isobonein	H	=O	<i>G. lourerii</i>	(143)

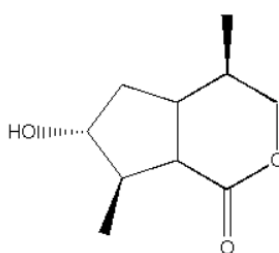


Table 32. Iridoid obtained from *Gentiana* sp.

Compound	Species	References
Nepetaside aglycone	<i>G. aristata</i>	(243)

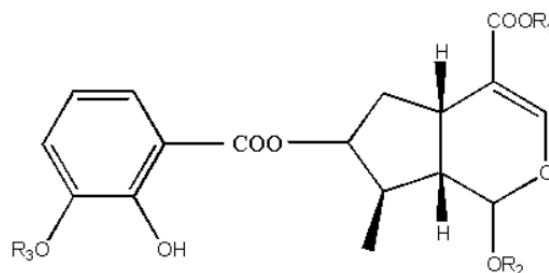


Table 33. Iridoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
Gentiournoside A (Depresteroside)	A	Glc	H	<i>G. depressa</i> <i>G. urnula</i>	(232), (244) (28), (245)
Gentiournoside B	A	Glc	Glc	<i>G. depressa</i> <i>G. urnula</i>	(232) (245)
Gentiournoside C	A	B	H	<i>G. urnula</i>	(245)
Gentiournoside D	H	Glc	H	<i>G. urnula</i>	(245)
Gentiournoside E	Glc	Glc	H	<i>G. urnula</i>	(28), (245)
Depressoside	C	Glc	H	<i>G. urnula</i>	(28)

A: secologanol B: glucose-glucose C: 6-(2,3-dihydroxybenzoyl)-β-D-glucopyranosyl

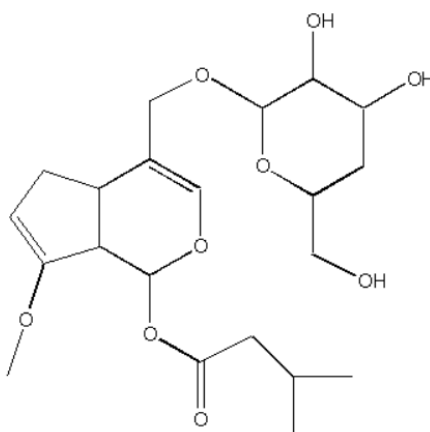


Table 34. Iridoid obtained from *Gentiana* sp.

Compound	Species	References
Deglucoserrulatoside	<i>G. macrophylla</i>	(152)

2.1.3.3. Flavonoids

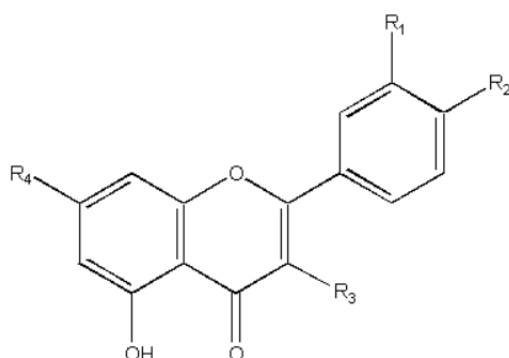


Table 35. Flavonoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
Apigenin	H	OH	H	OH	<i>G. asclepiadea</i> <i>G. delavayi</i> <i>G. kurroo</i> <i>G. scabra</i> <i>G. striata</i> <i>G. veitchiorum</i>	(14) (98) (246) (247) (123) (52)
Apigenin-7-O-β-D-glucoside (cosmosiin)	H	OH	H	OGlc	<i>G. asclepiadea</i> <i>G. cruciata</i> <i>G. gelida</i> <i>G. loureirii</i> <i>G. paradoxa</i> <i>G. pneumonanthe</i> <i>G. septemfida</i>	(131) (131) (131) (143) (131) (131) (131)
Apigenin-7-O-β-D-glucopyranosyl-(1$\rightarrow$3)-β-D-glucopyranosyl-(1$\rightarrow$3)-β-D-glucopyranosyl	H	OH	H	O-A	<i>G. rhodantha</i>	(86)
Luteolin	OH	OH	H	OH	<i>G. arisanensis</i> <i>G. kurroo</i> <i>G. piasezkii</i> <i>G. striata</i>	(82) (246) (57), (248,249) (123)

Table 35. Flavonoids obtained from *Gentiana* sp. (continue)

Luteolin-7-<i>O</i>-β-D-glucoside	OH	OH	H	OGlc	<i>G. arisanensis</i> <i>G. marcaillhouana</i>	(82) (250)
Chrysoeriol	OCH ₃	OH	H	OH	<i>G. asclepiadea</i> <i>G. macrophylla</i> <i>G. paradoxa</i>	(131) (154) (131)
Diosmetin	OH	OCH ₃	H	OH	<i>G. macrophylla</i>	(154)
Kaempferol	H	OH	OH	OH	<i>G. apiata</i> <i>G. makinoi</i> <i>G. kurroo</i>	(175) (251) (246)
Kaempferide	H	OCH ₃	OH	OH	<i>G. kurroo</i>	(246)
Quercetin	OH	OH	OH	OH	<i>G. arisanensis</i> <i>G. flavomaculata</i> <i>G. makinoi</i> <i>G. striata</i>	(82) (252) (251) (123)
Quercetin-3-<i>O</i>-β-D-glucoside (isoquercetin)	OH	OH	OGlc	OH	<i>G. pyrenica</i>	(253)
Quercetin 3-<i>O</i>-((2,3,4- triacetyl-α-rhamnopyranosyl) 1'''$\rightarrow$6'') 3-acetyl-β-galactopyranoside	OH	OH	O-A	OH	<i>G. spicata</i>	(61)
Quercetin 3-<i>O</i>-((2,3,4- triacetyl-α-rhamnopyranosyl) 1'''$\rightarrow$6'') 4-acetyl-β-galactopyranoside	OH	OH	O-B	OH	<i>G. spicata</i>	(61)
Rutin	OH	OH	Rut	OH	<i>G. flavomaculata</i> <i>G. striata</i>	(252) (133)

Rut: rutinose A: ((2,3,4-triacetyl- α -rhamnopyranosyl)1''' $\rightarrow$ 6'') 3-aceytl- β -galactopyranoside B: ((2,3,4-triacetyl- α -rhamnopyranosyl)1''' $\rightarrow$ 6'') 4-aceytl- β -galactopyranoside

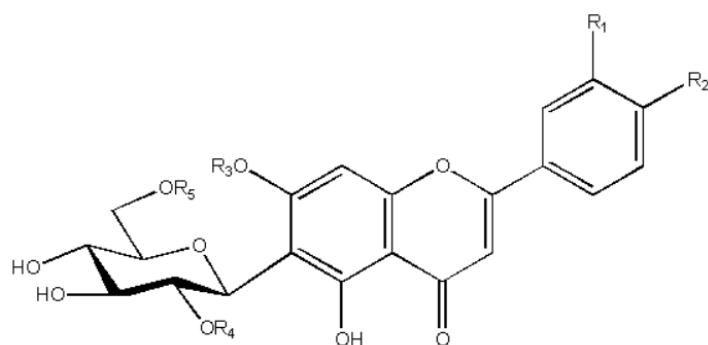


Table 36. Flavonoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
Isovitexin	H	OH	H	H	H	<i>G. algida</i>	(230)
						<i>G. arisanensis</i>	(82)
						<i>G. asclepiadea</i>	(15), (130), (254,255)
						<i>G. bavarica</i>	(256)
						<i>G. brachyphylla</i>	(256)
						<i>G. burseri</i>	(257)
						<i>G. ciliata</i>	(258)
						<i>G. favrati</i>	(256)
						<i>G. gelida</i>	(131)
						<i>G. linearis</i>	(195)
						<i>G. lutea</i>	(74), (183,184)
						<i>G. lutea subsp.</i>	(207)
						<i>symphyandra</i>	
						<i>G. macrophylla</i>	(155)
						<i>G. marcailhouana</i>	(259)
						<i>G. nivealis</i>	(256)
						<i>G. pannonica</i>	(257)
						<i>G. paradoxa</i>	(131)
						<i>G. pedicellata</i>	(260)
						<i>G. punctata</i>	(257)
						<i>G. purpurea</i>	(257)

Table 36. Flavonoids obtained from *Gentiana* sp. (continue)

Isovitexin	H	OH	H	H	H	<i>G. rostanii</i> <i>G. schleicheri</i> <i>G. straminea</i> <i>G. utriculosa</i> <i>G. veitchiorum</i> <i>G. verna</i> <i>G. villarsii</i>	(256) (256) (191) (261) (238) (256) (257)
Isovitexin-7-<i>O</i>-glucoside (saponarin)	H	OH	Glc	H	H	<i>G. asclepiadea</i> <i>G. cruciata</i> <i>G. gelida</i> <i>G. paradoxa</i> <i>G. piasezkii</i> <i>G. pyrenica</i> <i>G. septemfida</i>	(131) (131) (131) (131) (248) (253) (131)
Isovitexin-4'-<i>O</i>-β-D-glucopyranoside (isosaponarin)	H	OGlc	H	H	H	<i>G. algida</i> <i>G. asclepiadea</i> <i>G. burseri</i> <i>G. linearis</i> <i>G. lutea</i> <i>G. marcailhouana</i> <i>G. pannonica</i> <i>G. punctata</i> <i>G. purpurea</i> <i>G. villarsii</i>	(230) (254) (257) (195) (184) (259) (257) (257) (257) (257)
Isovitexin-2''-<i>O</i>-β-D-glucopyranoside	H	OH	H	Glc	H	<i>G. asclepiadea</i> <i>G. depressa</i> <i>G. lutea</i> <i>G. punctata</i>	(254) (232) (262) (263)
Isovitexin-6''-<i>O</i>-β-D-glucopyranoside	H	OH	H	H	Glc	<i>G. arisanensis</i> <i>G. piasezkii</i>	(82) (248)
Isovitexin-2'',4'-di-<i>O</i>-β-D-glucoside	H	OGlc	H	Glc	H	<i>G. asclepiadea</i>	(264)

Table 36. Flavonoids obtained from *Gentiana* sp. (continue)

Swertisin	H	OH	CH ₃	H	H	<i>G. algida</i>	(265)
						<i>G. campestris</i>	(266)
						<i>G. ottonis</i>	(185)
Isoorientin	OH	OH	H	H	H	<i>G. algida</i>	(230)
						<i>G. apiata</i>	(175)
						<i>G. arisanensis</i>	(82)
						<i>G. asclepiadea</i>	(15), (85), (130), (254,255)
						<i>G. bavarica</i>	(256)
						<i>G. brachyphylla</i>	(256)
						<i>G. burseri</i>	(257)
						<i>G. campestris</i>	(266)
						<i>G. ciliata</i>	(258)
						<i>G. dinarica</i>	(141)
						<i>G. favrati</i>	(256)
						<i>G. gelida</i>	(131)
						<i>G. karelinii</i>	(267)
						<i>G. lourerii</i>	(143)
						<i>G. lutea</i>	(183,184)
						<i>G. macrophylla</i>	(119), (152)
						<i>G. marcaillhouana</i>	(259)
						<i>G. nivalis</i>	(256)
						<i>G. olivieri</i>	(59), (70) (193), (268)
						<i>G. ottonis</i>	(185)
						<i>G. pannonica</i>	(257)
						<i>G. paradoxa</i>	(131)
						<i>G. pedicellata</i>	(260)
						<i>G. piasezkii</i>	(57), (248,249)
						<i>G. punctata</i>	(257)

Table 36. Flavonoids obtained from *Gentiana* sp. (continue)

Isoorientin	OH	OH	H	H	H	<i>G. purpurea</i> <i>G. rostanii</i> <i>G. schleicheri</i> <i>G. straminea</i> <i>G. utriculosa</i> <i>G. verna</i> <i>G. villarsii</i>	(257) (256) (256) (119) (256), (261) (256) (257)
Isoorientin-3'-O-β-D-glucoside	OGlc	OH	H	H	H	<i>G. nivalis</i> <i>G. pedicellata</i> <i>G. rigescens</i> <i>G. utriculosa</i>	(269) (260) (270) (261)
Isoorientin-4'-O-β-D-glucoside	OH	OGlc	H	H	H	<i>G. algida</i> <i>G. burseri</i> <i>G. dinarica</i> <i>G. macrophylla</i> <i>G. marcaillhouana</i> <i>G. pannonica</i> <i>G. punctata</i> <i>G. purpurea</i> <i>G. straminea</i> <i>G. villarsii</i>	(230) (257) (111), (141) (119) (259) (257) (257) (257) (119) (257)
Isoorientin-2''-O-β-D-glucopyranoside	OH	OH	H	Glc	H	<i>G. verna</i>	(271)
Isoorientin-6''-O-β-D-glucopyranoside	OH	OH	H	H	Glc	<i>G. arisanensis</i> <i>G. pedicellata</i>	(54), (82) (260)
Isoorientin-3',6''-di-O-β-D-glucoside	OGlc	OH	H	H	Glc	<i>G. pedicellata</i>	(260)
Isoorientin-4',2''-di-O-β-D-glucoside	OH	OGlc	H	Glc	H	<i>G. asclepiadea</i>	(264)
Isoscoparin	OCH ₃	OH	H	H	H	<i>G. ciliata</i> <i>G. depressa</i> <i>G. farreri</i>	(258) (232) (272)

Table 36. Flavonoids obtained from *Gentiana* sp. (continue)

Lutonarin	OH	OH	Glc	H	H	<i>G. punctata</i> <i>G. piasezkii</i>	(263) (57), (248,249)
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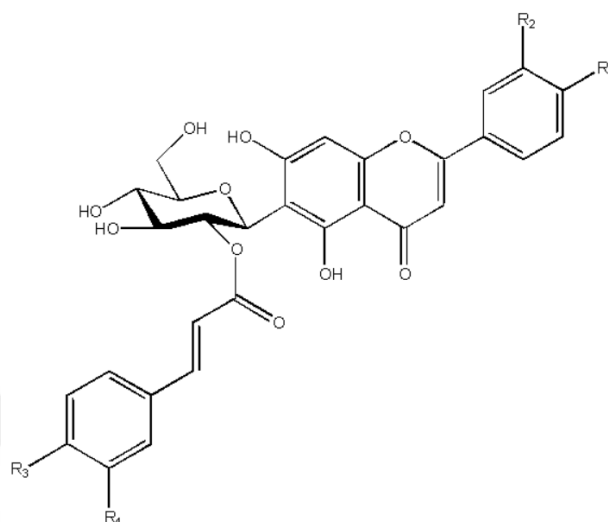


Table 37. Flavonoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
<i>Trans</i>-caffeoyl-2''-isoorientin	OH	OH	OH	OH	<i>G. marcaillhouana</i>	(259)
<i>Trans</i>-caffeoyl-2''-isoorientin-4'-O-β-D-glucopyranoside	OGlc	OH	OH	OH	<i>G. punctata</i>	(263)
<i>Trans</i>-feruloyl-2''-isoorientin	OH	OH	OH	OCH ₃	<i>G. burserii</i> <i>G. marcaillhouana</i>	(273) (259)
<i>Trans</i>-feruloyl-2''-isoorientin-4'-O-β-D-glucopyranoside	OGlc	OH	OH	OCH ₃	<i>G. burserii</i> <i>G. punctata</i>	(273) (263)
<i>Trans</i>-p-coumaroyl-2''-isoorientin	OH	OH	OH	H	<i>G. marcaillhouana</i>	(259)

Table 37. Flavonoids obtained from *Gentiana* sp. (continue)

<i>p</i>-O-β-D-Glucosyl-trans-caffeoyl-2''-isoorientin	OH	OH	OGlc	OH	<i>G. marcailhouana</i>	(259)
<i>Trans</i>-feruloyl-2''-isovitexin	OH	H	OH	OCH ₃	<i>G. punctata</i>	(263)

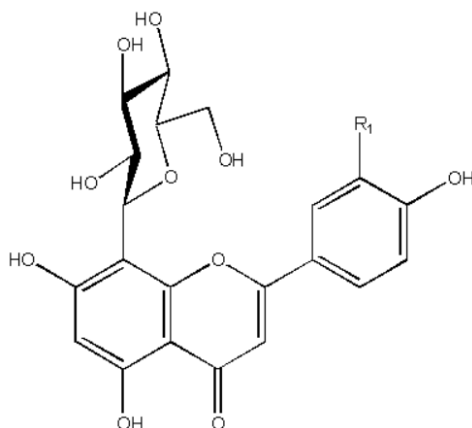


Table 38. Flavonoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Vitexin	H	H	<i>G. macrophylla</i> <i>G. straminea</i>	(119), (153) (119)
Homoorientin	OH	H	<i>G. asclepiadea</i> <i>G. lutea</i> subsp. <i>symphyandra</i>	(15) (207)

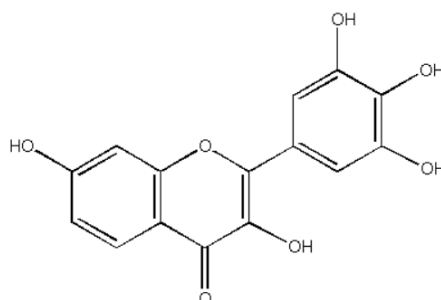


Table 39. Flavonoid obtained from *Gentiana* sp.

Compound	Species	References
Robinetin	<i>G. kurroo</i>	(26), (246)

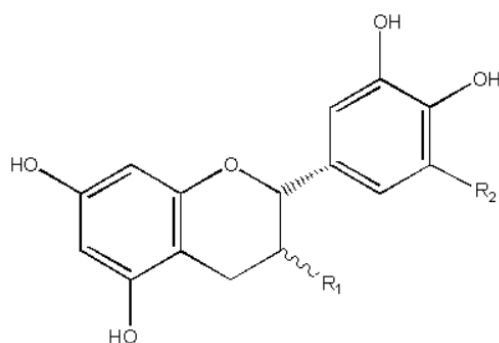


Table 40. Flavan-3-ols obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Catechin	—OH	H	<i>G. spicata</i>	(61)
Epicatechin	·····OH	H	<i>G. spicata</i>	(61)
Epicatechin-3- <i>O</i> -gallate	·····OGal	OH	<i>G. spicata</i>	(61)
Gallocatechin-3- <i>O</i> -gallate	—OGal	OH	<i>G. spicata</i>	(61)

Gal: Galloyl

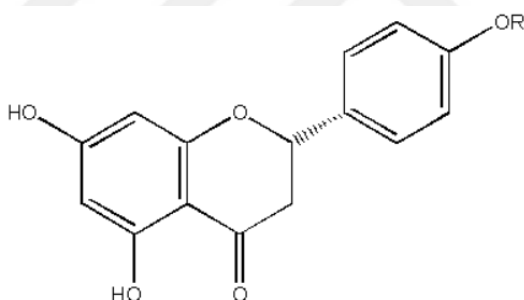


Table 41. Flavan-3-ols obtained from *Gentiana* sp.

Compounds	R	Species	References
Naringenin	H	<i>G. asclepiadea</i> <i>G. lutea</i>	(14) (51)
Naringenin-4'- <i>O</i> - β -glucopyranoside	Glc	<i>G. purpurea</i>	(40)

2.1.3.4. Anthocyanins

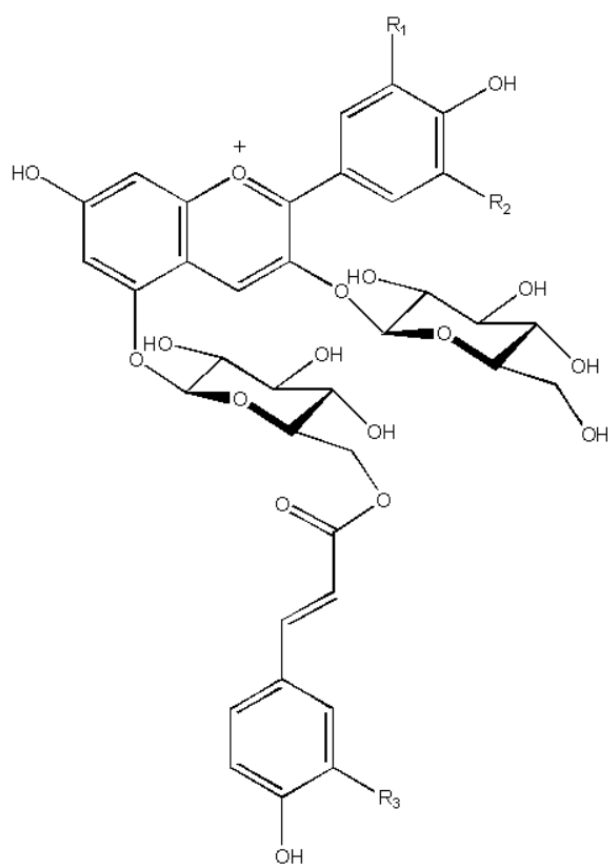


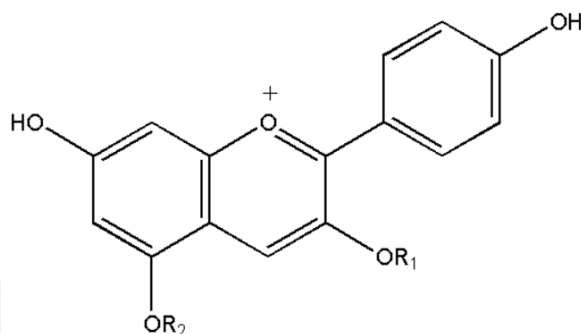
Table 42. Anthocyanins obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
Delphinidin 3-glucoside 5-caffeoyl glucoside	OH	OH	OH	<i>G. triflora</i>	(274)
Delphinidin 3-glucoside 5-caffeoyl glucoside 3'-glucoside	OGlc	OH	OH	<i>G. triflora</i>	(274)
Delphinidin 3-glucoside 5-caffeoyl glucoside 3'-caffeoyl glucoside (gentiodelphin)	O-A	OH	OH	<i>G. cv. albireo</i> <i>G. makinoi</i> <i>G. triflora</i>	(275) (276) (274)
Delphinidin 3-glucoside 5-caffeoyl glucoside 3'-<i>p</i>-coumaroyl glucoside	O-B	OH	OH	<i>G. triflora</i>	(274)
Delphinidin 3-glucoside 5-<i>p</i>-coumaroyl glucoside 3'-caffeoyl glucoside	O-A	OH	H	<i>G. triflora</i>	(274)
Albireodelphin A	OH	OGlc	OH	<i>G. cv. albireo</i>	(275)

Table 43. Anthocyanins obtained from *Gentiana* sp.

Albireodelphin B	OH	OGlc	H	<i>G. cv. albireo</i>	(275)
Albireodelphin C	OH	OH	H	<i>G. cv. albireo</i>	(275)
Albireodelphin D	OH	O-C	OH	<i>G. cv. albireo</i>	(275)
Albireodelphin E	OH	O-A	H	<i>G. cv. albireo</i>	(275)
Gentiocyanin A	H	OH	OH	<i>G. cv. albireo</i>	(275)

A: (*E*)-caffeoyl glucoside B: (*E*)-*p*-coumaryl glucoside C: (*E*)-*trans-p*-coumaroyl glucoside

**Table 44.** Anthocyanins obtained from *Gentiana* sp.

Compounds	R₁	R₂	Species	References
Pelargonidin 3-<i>O</i>-glucoside	Glc	H	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3,5-<i>O</i>-diglucoside	Glc	Glc	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-rutinoside	Rut	H	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-rutinoside-5-<i>O</i>-β-D-glucoside	Rut	Glc	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-(6-<i>p</i>-coumaroyl) glucoside	A	H	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-(6-<i>O</i>-malonyl-β-D-glucoside)	B	H	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-(6-<i>p</i>-coumaroyl-D-glucoside)-5-(4-<i>O</i>-malonyl-β-D-glucoside)	A	C	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)
Pelargonidin 3-<i>O</i>-(6-<i>O</i>-caffeoyl-D-glucoside)-5-<i>O</i>-(6-<i>O</i>-malonyl-β-D-glucoside)	D	B	<i>G. lutea</i> var. <i>aurantiaca</i>	(277)

Rut: rutinoside A: 6-*p*-coumaroyl B: 6-*O*-malonyl- β -D-glucoside C: 4-*O*-malonyl- β -D-glucoside D: 6-*O*-caffeoyl- β -D-glucoside

2.1.3.5. Xanthenes

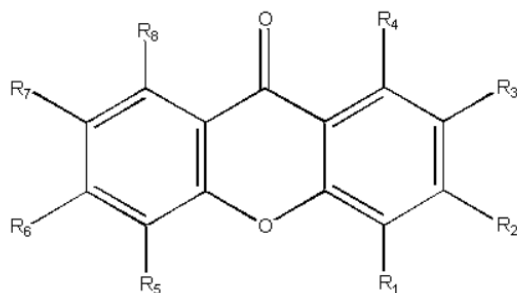


Table 45. Xanthenes obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	R ₈	Species	References
Gentisein	H	OH	H	H	H	H	OH	H	<i>G. lutea</i>	(206),(278)
Gentisin	H	OCH ₃	H	OH	H	H	OH	H	<i>G. asclepiadea</i> <i>G. cruciata</i> <i>G. lutea</i> <i>G. pneumonanthe</i>	(138) (136,137) (74),(138), (206),(251), (278) (138)
Isogentisine	H	OH	H	OH	H	H	OCH ₃	H	<i>G. asclepiadea</i> <i>G. cruciata</i> <i>G. burserii</i> <i>G. lutea</i> <i>G. marcailhouana</i>	(255) (136,137) (202) (183,184), (206),(251), (279) (259)
Gentioside	H	Prim	H	H	H	H	OCH ₃	H	<i>G. cruciata</i> <i>G. lutea</i> <i>G. marcailhouana</i>	(136,137) (145) (259)
Gentioside isomer	H	OCH ₃	H	H	H	H	Prim	H	<i>G. asclepiadea</i> <i>G. cruciata</i> <i>G. lutea</i> <i>G. pneumonanthe</i>	(138) (136,137) (138),(145) (147) (138)

Table 45. Xanthones obtained from *Gentiana* sp. (continue)

Bellidin	H	OH	H	OH	OH	H	H	OH	<i>G. campestris</i> <i>G. lutea</i> <i>G. ottonis</i>	(87),(280) (74) (185)
Norsvertianolin (bellidin-8-O-β-D-glucopyranoside)	H	OH	H	OH	OH	H	H	OGlc	<i>G. campestris</i> <i>G. lutea</i> <i>G. ottonis</i>	(87),(280) (74) (185)
Bellidifolin	H	OH	H	OCH ₃	OH	H	H	OH	<i>G. algida</i> <i>G. campestris</i> <i>G. cerastioides</i> <i>G. kochiana</i> <i>G. lutea</i> <i>G. ottonis</i> <i>G. strictiflora</i>	(265) (87) (281) (115) (74) (185) (281)
Desmethyl bellidifolin	H	OH	H	OH	OH	H	H	OH	<i>G. cerastioides</i> <i>G. kochiana</i> <i>G. strictiflora</i>	(281) (115) (281)
Swerchirin (methyl-bellidifolin)	H	OCH ₃	H	OH	OCH ₃	H	H	OH	<i>G. algida</i>	(282)
Swertianolin (Bellidifolin-8-O-β-D-glucopyranoside)	H	OCH ₃	H	OH	OH	H	H	OGlc	<i>G. algida</i> <i>G. campestris</i> <i>G. lutea</i>	(265) (74),(280) (74)
Isobellidifolin	H	OH	H	OH	OCH ₃	H	H	OH	<i>G. algida</i> <i>G. karelinii</i>	(282) (267)
1,3,5-trihydroxy-8-methoxy xanthone	H	OH	H	OH	OH	H	H	OCH ₃	<i>G. azurium</i>	(283)

Table 45. Xanthenes obtained from *Gentiana* sp. (continue)

3,5-dihydroxy-8-methoxy-1-<i>O</i>-β-D-glucopyranosyl xanthone	H	OGlc	H	OH	OH	H	H	OCH ₃	<i>G. azurium</i>	(283)
1-hydroxy-3-methoxy-7-<i>O</i>-primeverosyl xanthone	H	OCH ₃	H	OH	H	H	Prim	H	<i>G. lutea</i>	(284)
7-hydroxy-3-methoxy-1-<i>O</i>-primverosyl xanthone	H	OCH ₃	H	Prim	H	H	OH	H	<i>G. lutea</i>	(284)
Gentiacalein	H	OCH ₃	H	OH	H	H	OH	OCH ₃	<i>G. azurium</i> <i>G. bavarica</i> <i>G. brachyphyla</i> <i>G. ciliata</i> <i>G. favrati</i> <i>G. kochiana</i> <i>G. rostanii</i> <i>G. schleicheri</i> <i>G. utriculosa</i> <i>G. verna</i>	(283) (256) (256) (258) (256) (94) (115), (285) (256) (256) (256), (261) (256)
Genticaulein-1-<i>O</i>-glucoside	H	OCH ₃	H	OGlc	H	H	OH	OCH ₃	<i>G. ciliata</i> <i>G. kochiana</i>	(258) (115)
Isogentiacalein	H	OH	H	OCH ₃	H	H	OCH ₃	OH	<i>G. ciliata</i>	(258)
Isogentiacalein-8-<i>O</i>-primverosyl	H	OH	H	OCH ₃	H	H	OCH ₃	Prim	<i>G. ciliata</i>	(258)

Table 45. Xanthones obtained from *Gentiana* sp. (continue)

Isogentiacaulen-3-O-glucoside	H	OGlc	H	OCH ₃	H	H	OCH ₃	OH	<i>G. ciliata</i>	(258)
Decussatin	H	OCH ₃	H	OH	H	H	OCH ₃	OCH ₃	<i>G. azurium</i> <i>G. bavarica</i> <i>G. brachyphyla</i> <i>G. ciliata</i> <i>G. favrati</i> <i>G. kochiana</i> <i>G. rostanii</i> <i>G. schleicheri</i> <i>G. utriculosa</i> <i>G. verna</i>	(283) (256) (256) (258) (256) (115) (256) (256) (256), (261) (256)
Decussatin-1-O-primveroside	H	OCH ₃	H	Prim	H	H	OCH ₃	OCH ₃	<i>G. utriculosa</i> <i>G. verna</i>	(261) (286)
Decussatin-1-O-glucoside	H	OCH ₃	H	OGlc	H	H	OCH ₃	OCH ₃	<i>G. ciliata</i>	(258)
Isodecussatin	H	OCH ₃	H	OCH ₃	H	H	OCH ₃	OH	<i>G. ciliata</i>	(258)
Swertianin (gentiakochianin)	H	OCH ₃	H	OH	H	H	OH	OH	<i>G. bavarica</i> <i>G. brachyphyla</i> <i>G. ciliata</i> <i>G. favrati</i> <i>G. karelinii</i> <i>G. kochiana</i> <i>G. nivalis</i> <i>G. rostanii</i> <i>G. utriculosa</i> <i>G. verna</i>	(256), (287) (256) (258) (256) (267) (93), (115), (285) (256) (256) (261) (256)

Table 45. Xanthones obtained from *Gentiana* sp. (continue)

Swertianin-1-<i>O</i>-glucoside	H	OCH ₃	H	OGlc	H	H	OH	OH	<i>G. ciliata</i>	(258)
Swertianin-7-<i>O</i>-glucoside	H	OCH ₃	H	OH	H	H	OGlc	OH	<i>G. ciliata</i>	(259)
Swertianin-8-<i>O</i>-glucoside	H	OCH ₃	H	OH	H	H	OH	OGlc	<i>G. karelinii</i>	(282)
Swertianin-8-<i>O</i>-primveroside	H	OCH ₃	H	OH	H	H	OH	Prim	<i>G. karelinii</i> <i>G. kochiana</i>	(282) (115)
Swertsiperenin	H	OCH ₃	H	OH	H	H	OCH ₃	OH	<i>G. karelinii</i> <i>G. kochiana</i>	(267) (285)
Gentiakochianoside	H	OCH ₃	H	OH	H	H	OH	Prim	<i>G. ciliata</i>	(258)
Isogentiakochianoside	H	OCH ₃	H	Prim	H	H	OH	OH	<i>G. ciliata</i> <i>G. kochiana</i> <i>G. utriculosa</i>	(258) (115) (261)
Gentiabavaroside	H	OCH ₃	H	Prim	H	H	OH	OCH ₃	<i>G. barbata</i> <i>G. bavarica</i> <i>G. ciliata</i> <i>G. kochiana</i>	(287) (288) (258) (115)
Norswertianin	H	OH	H	OH	H	H	OH	OH	<i>G. bavarica</i> <i>G. verna</i>	(256) (256)
Norswertianin-1-<i>O</i>-primveroside	H	OH	H	Prim	H	H	OH	OH	<i>G. dinarica</i>	(141)
Norswertianin-8-<i>O</i>-primveroside	H	OH	H	OH	H	H	OH	Prim	<i>G. dinarica</i>	(141)
1,8-dihydroxy-3-methoxy-7-<i>O</i>-primveroside	H	OCH ₃	H	OH	H	H	Prim	OH	<i>G. utriculosa</i>	(261)

Table 45. Xanthenes obtained from *Gentiana* sp. (continue)

Corymbiferin	OCH ₃	OH	H	OH	OCH ₃	H	H	OH	<i>G. campestris</i>	(289)
1,5,8-tri hydroxy- 3,4-dimet hoxy xanthone	OH	OCH ₃	H	OH	OH	H	H	OH	<i>G. algida</i>	(265)
Rhodan tenone D	H	OH	OCH ₃	OH	H	H	OH	OH	<i>G. rhodantha</i>	(226)
Mangife rin	H	OH	Glc	OH	H	OH	OH	H	<i>G. asclepiadea</i> <i>G. favrati</i> <i>G. karelinii</i> <i>G. kurroo</i> <i>G. lutea</i> <i>G. lutea subsp. symphyandra</i> <i>G. nivalis</i> <i>G. rhodantha</i> <i>G. schleicheri</i> <i>G. utriculosa</i>	(15), (130), (290) (257) (267) (26) (110), (291) (207) (257) (86) (257) (261)
Mangiferi n-6-O-β- D-glucoside	H	OH	Glc	OH	H	OGlc	OH	H	<i>G. asclepiadea</i>	(290)
Neomangiferin	H	OH	Glc	OH	Glc	OH	OGlc	OH	<i>G. asclepiadea</i> <i>G. rhodantha</i>	(290) (86)
Lancerin	H	H	OH	H	Glc	OH	H	OH	<i>G. rhodantha</i>	(85)

Prim: Primverose

2.1.3.6. Benzophenones

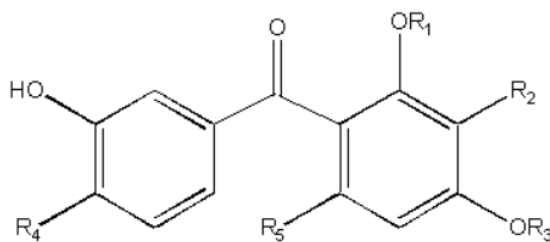


Table 46. Benzophenones obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
2,3'-dihydroxy-4-methoxy-benzophenone-6-O-β-glucopyranoside	H	H	CH ₃	H	Glc	<i>G. verna</i> subsp. <i>pontica</i>	(292)
2,4,3',4'-tetrahydroxy-benzophenone-6-O-β-glucopyranoside	H	H	H	OH	Glc	<i>G. verna</i> subsp. <i>pontica</i>	(292)
2,4,3'-trihydroxy-benzophenone-6-O-β-glucopyranoside	H	H	H	H	Glc	<i>G. verna</i> subsp. <i>pontica</i>	(292)
Rhodanthenone A (2,4,6,3'-tetrahydroxy benzophenone 4-O-β-D-glucopyranoside)	H	H	Glc	H	H	<i>G. rhodantha</i>	(86)
Rhodanthenone B (2,4,6,3',4'-pentahydroxy benzophenone 2-O-β-D-glucopyranoside)	Glc	H	H	OH	H	<i>G. rhodantha</i>	(86)
Rhodanthenone C (2,6,3'-trihydroxy-4-methoxy benzophenone 3-β-D-glucosyl pyranoside)	H	Glc	CH ₃	H	H	<i>G. rhodantha</i>	(86)

2.1.3.7. Coumarins

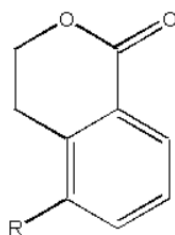


Table 47. Isocoumarins obtained from *Gentiana* sp.

Compounds	R	Species	References
Erythrocentaurin acid	COOH	<i>G. macrophylla</i>	(151), (153)
Erythrocentaurin	CHO	<i>G. lutea</i> <i>G. macrophylla</i> <i>G. pedicellata</i> <i>G. purpurea</i> <i>G. veitchiorum</i>	(206) (209), (213) (293) (40) (214)
Erythricin	CH ₂ OH	<i>G. macrophylla</i>	(213)
Erythrocentaurin dimethyl acetal	CH(OCH ₃) ₂	<i>G. lutea</i> <i>G. purpurea</i>	(206) (209)

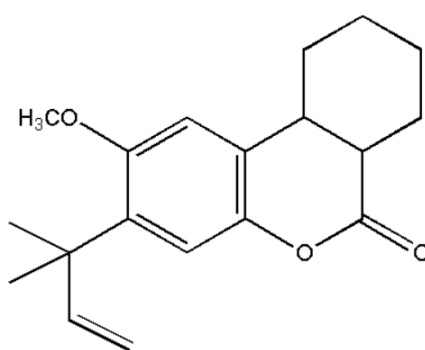


Table 48. Coumarins obtained from *Gentiana* sp.

Compound	Species	References
Methoxy-3-(1,10 -dimethylallyl)-6 α ,10 α -dihydrobenzo(1,2-c)chroman-6-one	<i>G. lutea</i>	(294)

2.1.3.8. Triterpenes

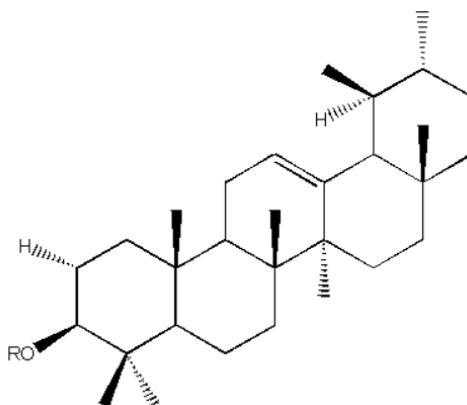


Table 49. Triterpenoids obtained from *Gentiana* sp.

Compounds	R	Species	References
α -Amirin	H	<i>G. aristata</i> <i>G. lutea</i> <i>G. scabra</i> <i>G. veitchiorum</i>	(243) (295) (296) (297)
α -Amirin-3- <i>O</i> -palmitate	Palm	<i>G. formosana</i>	(142)

Palm: Palmitoyl

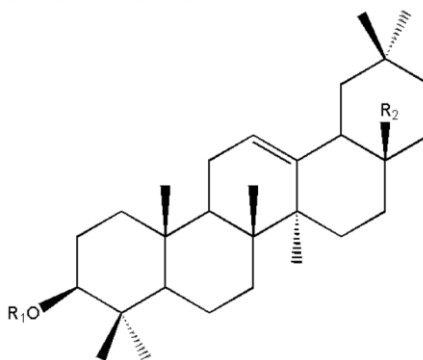


Table 50. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
β -Amirin	H	CH ₃	<i>G. lutea</i>	(295)
β -Amirin-3- <i>O</i> -palmitate	Palm	CH ₃	<i>G. formosana</i>	(142)
Erythrodiol-3- <i>O</i> -palmitate	Palm	CH ₂ OH	<i>G. lutea</i>	(295)

Table 50. Triterpenoids obtained from *Gentiana* sp. (continue)

Oleanolic acid	H	COOH	<i>G. apiata</i>	(175)
			<i>G. loureirii</i>	(143)
			<i>G. macrophylla</i>	(154), (153)
			<i>G. olivieri</i>	(298)
			<i>G. siphonantha</i>	(228)
			<i>G. veitchiorum</i>	(297)

Palm: Palmitoyl

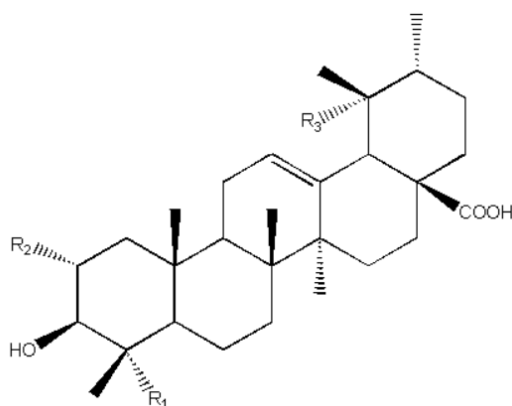


Table 51. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
Ursolic acid	CH ₃	H	H	<i>G. aristata</i>	(243)
				<i>G. asclepiadea</i>	(255)
				<i>G. dahurica</i>	(299)
				<i>G. farreri</i>	(300)
				<i>G. macrophylla</i>	(209)
				<i>G. manshurica</i>	(157)
				<i>G. pedicellata</i>	(293)
				<i>G. tibetica</i>	(221)
				<i>G. veitchiorum</i>	(214), (297)
(2α)-2-hydroxy ursolicacid	CH ₃	OH	H	<i>G. aristata</i>	(243)
				<i>G. dahurica</i>	(216)
				<i>G. farreri</i>	(300)
				<i>G. tibetica</i>	(221)

Table 51. Triterpenoids obtained from *Gentiana* sp. (continue)

(2α,19<i>R</i>)-2,19-dihydroxy ursolic acid (tormentic acid)	CH ₃	OH	OH	<i>G. aristata</i>	(243)
				<i>G. loureirii</i>	(143)
Rotundic acid	CH ₂ OH	H	OH	<i>G. loureirii</i>	(143)

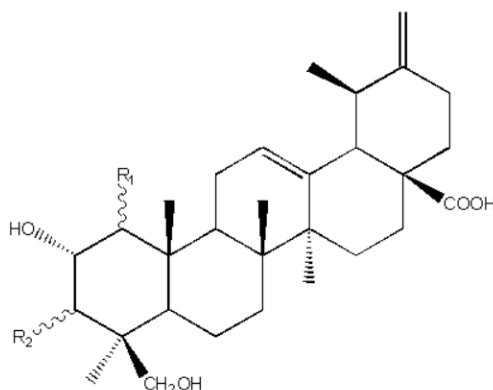


Table 52. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
1β,2α,3α,24-tetrahydroxyursa-12,20(30)-dien-28-oic acid	—OH	·····OH	<i>G. dahurica</i>	(216)
1α,2α,3β,24-tetrahydroxyursa-12,20(30)-dien-28-oic acid	·····OH	—OH	<i>G. tibetica</i>	(221)

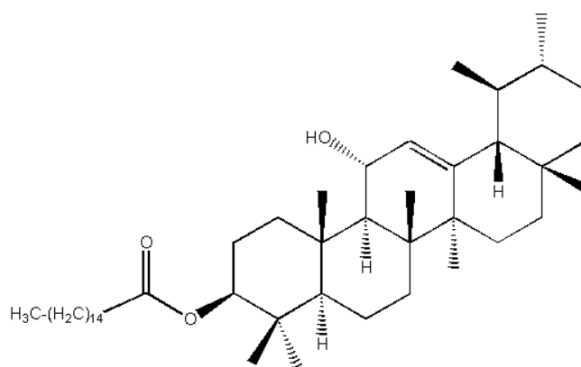


Table 53. Triterpenoid obtained from *Gentiana* sp.

Compound	Species	References
12-ursen-3β,11α-diol-3-<i>O</i>-palmitate	<i>G. lutea</i>	(301)

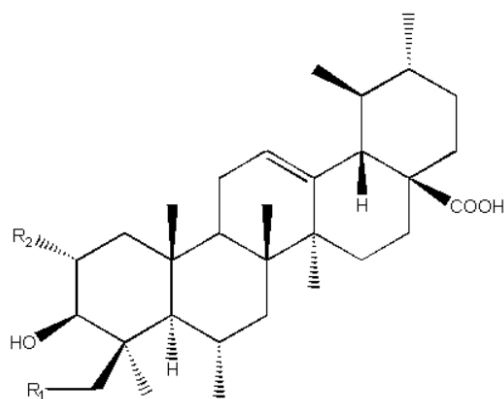


Table 54. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
3β,24-dihydroxyurs-12-en-28-oic acid	OH	H	<i>G. dahurica</i> <i>G. manshurica</i>	(140), (216) (157)
2α,3β-dihydroxyurs -12-en-28-oic acid	H	OH	<i>G. veitchiorum</i>	(297)
2α,3β,24-trihydroxy urs-12-en-28-oic acid	OH	OH	<i>G. dahurica</i>	(216)

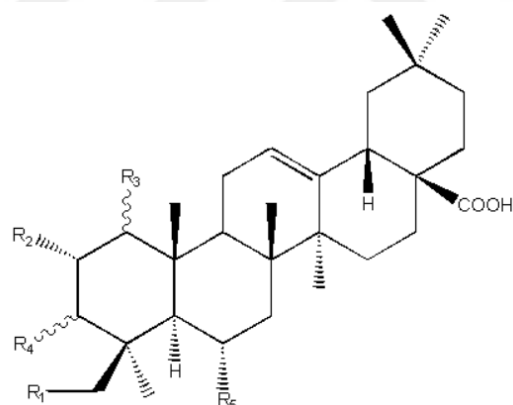


Table 55. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	R ₅	Species	References
Maslinic acid	H	OH	H	—OH	H	<i>G. dahurica</i>	(216)
2α,3β-dihydroxy oleanolic acid	H	OH	H	—OH	H	<i>G. farreri</i>	(300)
24-hydroxy oleanolic acid	OH	H	·····H	—OH	H	<i>G. aristata</i>	(243)

Table 55. Triterpenoids obtained from *Gentiana* sp. (continue)

3β,6α,24-trihydroxy olean-12-en-28-oic acid	OH	H	H	—OH	OH	<i>G. straminea</i>	(191)
(1α,2α)-1,2,24-trihydroxy-oleanolic acid	OH	OH	·····OH	—OH	H	<i>G. aristata</i> <i>G. straminea</i> <i>G. tibetica</i>	(243) (191) (221)
2α,3α,24-trihydroxy olean-12-en-28-oic acid	OH	OH	H	·····OH	H	<i>G. dahurica</i>	(139)
1α,2α,3α,24-tetrahydroxyolean-12-en-28-oic acid	OH	OH	·····OH	·····OH	H	<i>G. loureirii</i>	(302)
1β,2α,3α,24-tetrahydroxyolean-12-en-28-oic acid	OH	OH	—OH	·····OH	H	<i>G. dahurica</i>	(216)

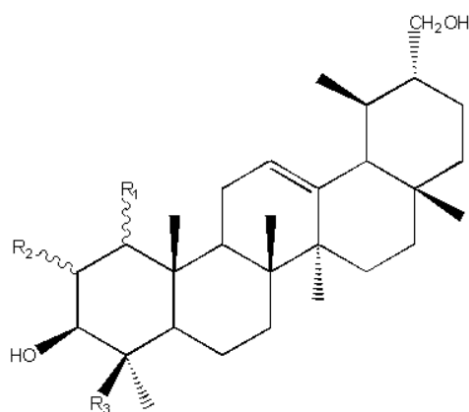


Table 56. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
1α,2α,3β,30-tetrahydroxyurs-12-ene	·····OH	·····OH	CH ₃	<i>G. neurotheca</i>	(175)
3β,24,30-trihydroxyurs-12-ene	H	H	CH ₂ OH	<i>G. neurotheca</i>	(174)

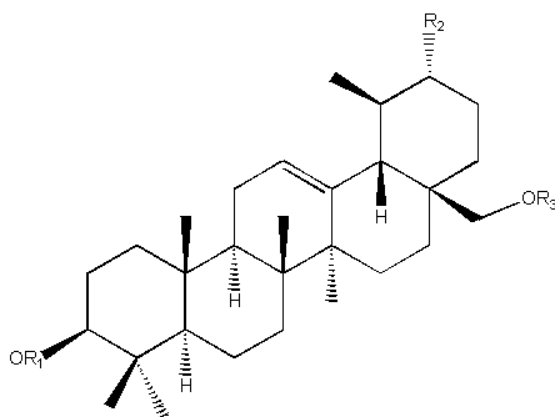


Table 57. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	Species	References
Uvaol	H	CH ₃	H	<i>G. davidii</i> var. <i>formosana</i>	(142)
Uvaol-3-O-palmitate	Palm	CH ₃	H	<i>G. davidii</i> var. <i>formosana</i> <i>G. lutea</i>	(142) (295)
3β,30-dihydroxy-12-ursen-28-oic acid 28-O-β-D-glucopyranoside ester	H	CH ₂ OH	Glc	<i>G. loureirii</i>	(302)

Palm: Palmitoyl

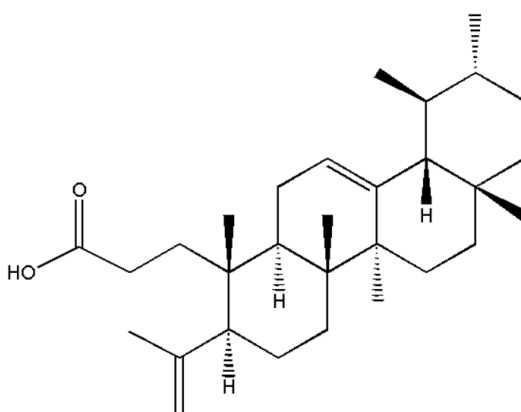
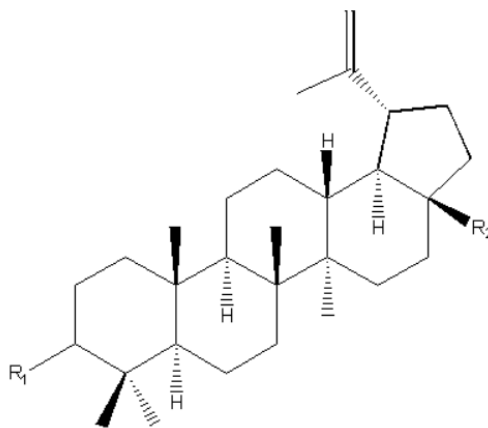


Table 58. Secotriterpenoids obtained from *Gentiana* sp.

Compounds	R	Species	References
Roburic acid	H	<i>G. crassicaulis</i> <i>G. dahurica</i> <i>G. macrophylla</i>	(303) (140), (303) (151), (153), (209), (303)

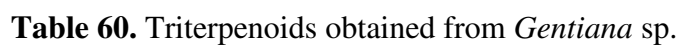
Table 58. Triterpenoids obtained from *Gentiana* sp. (continue)

		<i>G. officinalis</i>	(303)
		<i>G. siphonantha</i>	(303)
		<i>G. straminea</i>	(303)
		<i>G. tibetica</i>	(303)
		<i>G. waltonii</i>	(303)
4-hydroxy roburic acid methyl ester	CH ₃	<i>G. macrophylla</i>	(154)

**Table 59.** Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Lupeol	—OH	CH ₃	<i>G. farreri</i> <i>G. lutea</i> <i>G. manshurica</i> <i>G. scabra</i> <i>G. veitchiorum</i>	(300) (206), (295) (157) (296) (214), (297)
28-O-(3,4-dihydroxyl -benzyl)-lupeol	—OH	A	<i>G. veitchiorum</i>	(214)
Lup-20(29)-en-3-one	=O	CH ₃	<i>G. manshurica</i>	(157)
Lupeol palmitate	Palm	CH ₃	<i>G. formasana</i>	(142)
Betulinic acid	—OH	COOH	<i>G. veitchiorum</i>	(214)
Betulin-3-O-palmitate	Palm	CH ₂ OH	<i>G. lutea</i>	(295)

A: *O*-(3,4-dihydroxyl -benzyl) Palm: Palmitoyl



The chemical structure of Steroid 1 is a complex polycyclic molecule. It features a four-ring steroid nucleus. The leftmost ring (A) has a hydroxyl group (HO-) at the 3-position and a methyl group at the 10-position. The second ring (B) has a methyl group at the 13-position. The third ring (C) has a methyl group at the 14-position and a double bond between the 5 and 6 positions. The rightmost ring (D) has a methyl group at the 17-position. The stereochemistry is indicated by wedged and dashed bonds for the methyl groups and the hydroxyl group.

Table 61. Triterpenoid obtained from *Gentiana* sp.

85

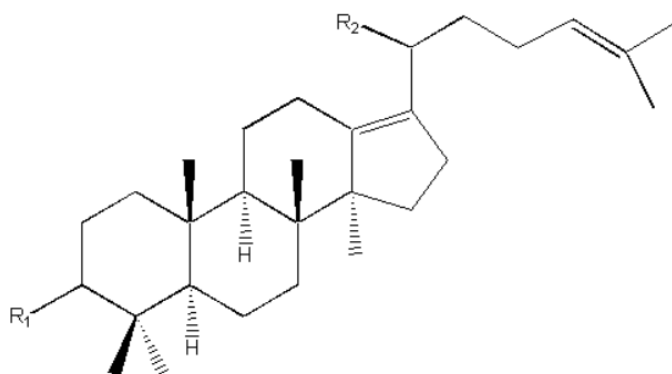


Table 62. Triterpenoids obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
(20 <i>S</i>)-dammara-13(17),24-dien-3-one	= O	—OH	<i>G. scabra</i>	(296)
(20 <i>S</i>)-dammara-13(17),24-dien-3β-ol	α-H, β-OH	—OH	<i>G. scabra</i>	(296)
(20 <i>R</i>)-dammara-13(17),24-dien-3-one	= O	·····OH	<i>G. scabra</i>	(296)
(20 <i>S</i>)-dammara-13(17),24-dien-3β-ol	α-H, β-OH	·····OH	<i>G. scabra</i>	(296)

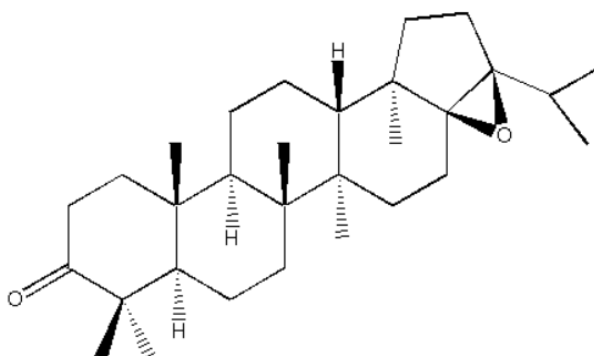


Table 63. Triterpenoid obtained from *Gentiana* sp.

Compound	Species	References
17β,21β-epoxyhopan-3-one	<i>G. scabra</i>	(296)

2.1.3.9. Phytosterols

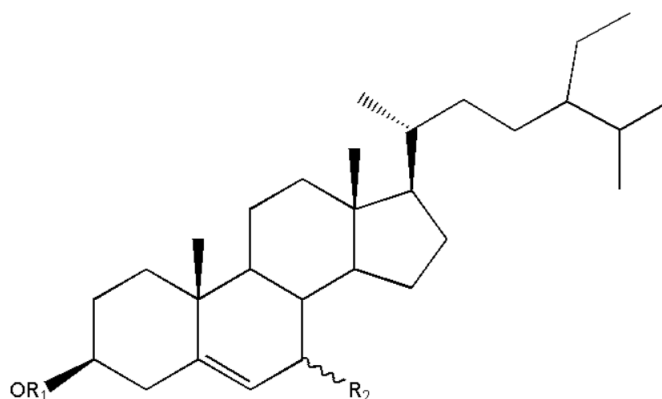


Table 64. Phytosterols obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
<i>β</i>-sitosterol	H	H	<i>G. apiata</i> <i>G. aristata</i> <i>G. asclepiadea</i> <i>G. crassicaulis</i> <i>G. lutea</i> <i>G. macrophylla</i> <i>G. siphonantha</i> <i>G. tibetica</i>	(175) (243) (255) (213) (301) (155) (228) (174)
<i>β</i>-sitosterol-3-<i>O</i>-genbioside	Gen	H	<i>G. crassicaulis</i> <i>G. macrophylla</i>	(177) (155)
Daucosterol (<i>β</i>-sitosterol-3-<i>O</i>-<i>β</i>-D-glucopyranoside)	Glc	H	<i>G. aristata</i> <i>G. lutea</i> <i>G. macrophylla</i> <i>G. scabra</i> <i>G. siphonantha</i> <i>G. tibetica</i>	(243) (301) (153,213) (166) (228) (174), (221)
(7α)-7-hydroxy sitosterol	H	—OH	<i>G. aristata</i>	(243)
(7β)-7-hydroxy sitosterol	H	·····OH	<i>G. aristata</i>	(243)

Gen: Gentibiose

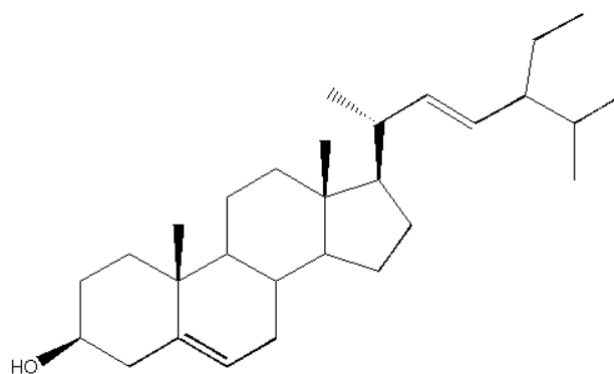


Table 65. Phytosterol obtained from *Gentiana* sp.

Compound	Species	References
Stigmasterol	<i>G. aristata</i>	(243)
	<i>G. lutea</i>	(301)
	<i>G. macrophylla</i>	(155)

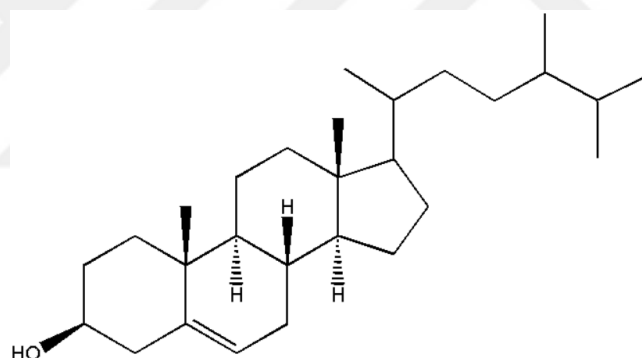


Table 66. Phytosterol obtained from *Gentiana* sp.

Compound	Species	References
Campesterol	<i>G. lutea</i>	(296)

2.1.3.10. Lignans

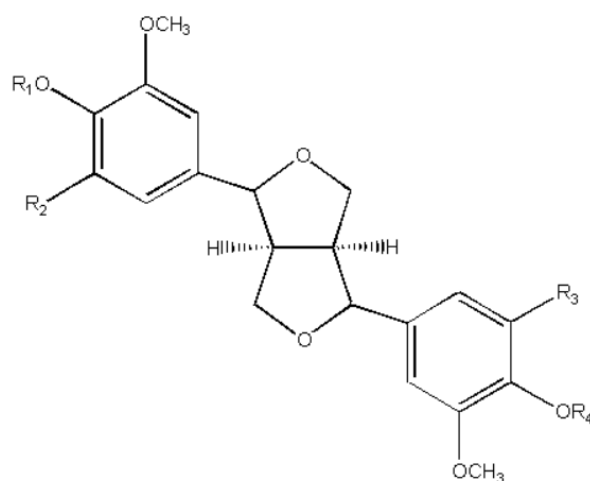


Table 67. Lignans obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
Syringaresinol	H	OCH ₃	OCH ₃	H	<i>G. lutea</i> <i>G. triflora</i>	(206) (201)
Syringaresinol-<i>O</i>-glucoside	Glc	OCH ₃	OCH ₃	H	<i>G. dahurica</i> <i>G. delavayi</i> <i>G. lutea</i> <i>G. macrophylla</i> <i>G. rigescens</i> <i>G. rhodantha</i>	(140) (98) (206) (154) (270) ,(304) (86)
Syringaresinol- 4, 4'-bis-β-<i>O</i>-D-glucopyranoside	Glc	OCH ₃	OCH ₃	Glc	<i>G. macrophylla</i>	(154)
Liriodendrin	Glc	OCH ₃	OCH ₃	Glc	<i>G. dahurica</i> <i>G. rigescens</i>	(140) (270) ,(304)
Pinoresinol	H	H	H	H	<i>G. lutea</i>	(206)
Pinoresinol-<i>O</i>-glucoside	Glc	H	H	H	<i>G. rigescens</i>	(270) ,(304)

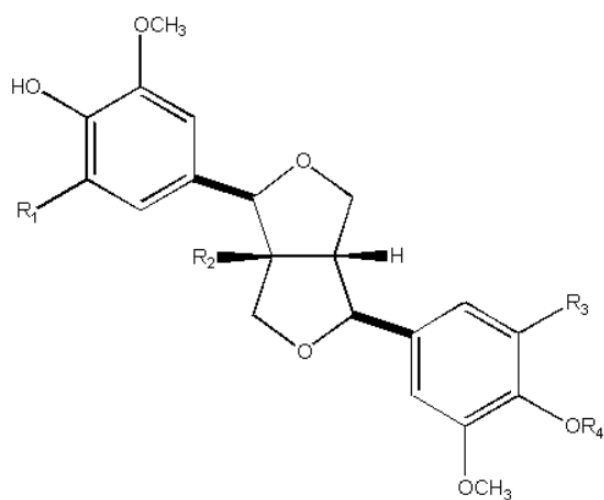


Table 68. Lignans obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	R ₃	R ₄	Species	References
(+)-1-Hydroxy syringaresinol	OCH ₃	OH	OCH ₃	H	<i>G. lutea</i>	(206)
(+)-Fraxiresinol	OCH ₃	H	OCH ₃	H	<i>G. lutea</i>	(206)
(+)-1-Hydroxy pinoresinol	H	OH	H	H	<i>G. lutea</i>	(206)
(+)-Medioresinol	OCH ₃	H	H	H	<i>G. lutea</i>	(206)
Acanthoside B	OCH ₃	H	OCH ₃	Glc	<i>G. purpurea</i>	(40)

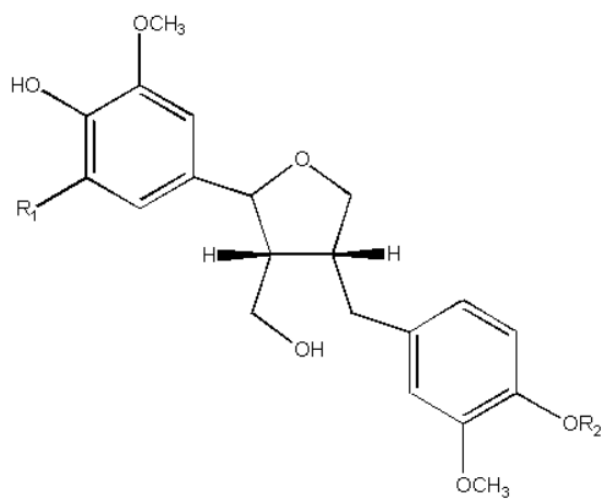


Table 69. Lignans obtained from *Gentiana* sp. (continue)

Compounds	R ₁	R ₂	Species	References
Berchemol	H	H	<i>G. lutea</i>	(206)
Berchemol-4'-O-β-D-glucoside	H	Glc	<i>G. crassicaulis</i> <i>G. macrophylla</i>	(178) (154)
Gentioluteol	OCH ₃	H	<i>G. lutea</i>	(206)

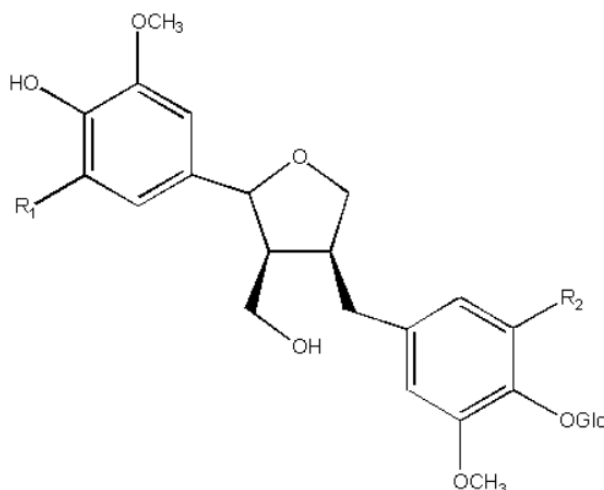


Table 70. Lignans obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Lariciresinol-4'-O-β-D-glucoside	H	H	<i>G. dahurica</i> <i>G. rigescens</i>	(140) (270) ,(304)
Tortoside B	OCH ₃	OCH ₃	<i>G. rigescens</i>	(270) ,(304)

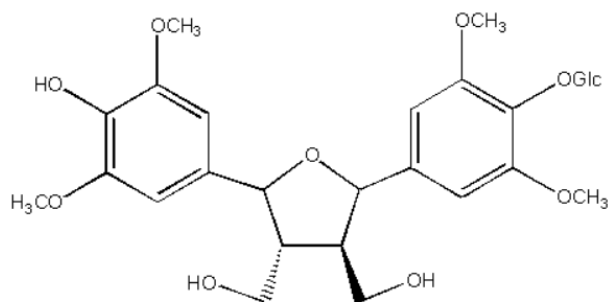


Table 71. Lignans obtained from *Gentiana* sp.

Compound	Species	References
Foliachinenoside C	<i>G. rhodantha</i>	(86)

2.1.3.11. Alkaloids

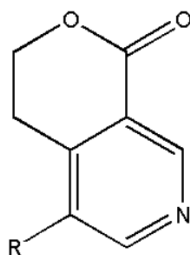


Table 72. Alkaloids obtained from *Gentiana* sp.

Compounds	R	Species	References
Gentianadine	H	<i>G. lutea</i>	(74)
		<i>G. olgae</i>	(305)
		<i>G. olivieri</i>	(306)
Gentianine	Vinyl	<i>G. dinarica</i>	(141)
		<i>G. kurroo</i>	(26), (71), (125), (307)
		<i>G. lutea</i>	(74), (308)
		<i>G. macrophylla</i>	(62), (75), (153), (211), (309)
		<i>G. olivieri</i>	(79), (306), (310), (311)
		<i>G. pedicellata</i>	(293)
		<i>G. purpurea</i>	(40)

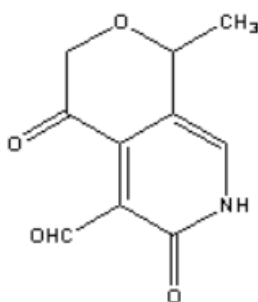


Table 73. Alkaloid obtained from *Gentiana* sp.

Compound	Species	References
Gentianal	<i>G. macrophylla</i>	(312)

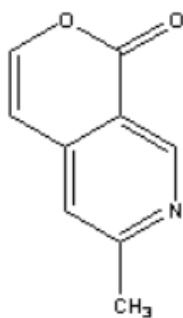


Table 74. Alkaloid obtained from *Gentiana* sp.

Compounds	Species	References
Gentianidine	<i>G. macrophylla</i>	(153)
	<i>G. tibetica</i>	(221)

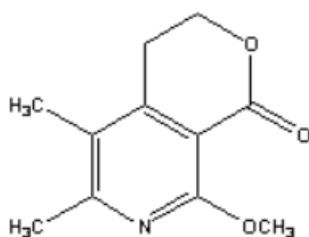


Table 75. Alkaloid obtained from *Gentiana* sp. (continue)

Compounds	Species	References
Gentiananine	<i>G. olivieri</i>	(311), (313)
	<i>G. olgae</i>	(305)
	<i>G. tianschanica</i>	(305)
	<i>G. vvedenskyi</i>	(305)

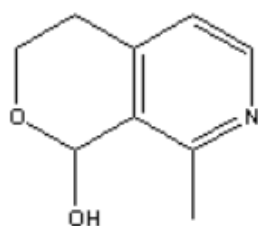


Table 76. Alkaloid obtained from *Gentiana* sp.

Compound	Species	References
Gentiotibetine	<i>G. olivieri</i>	(310)

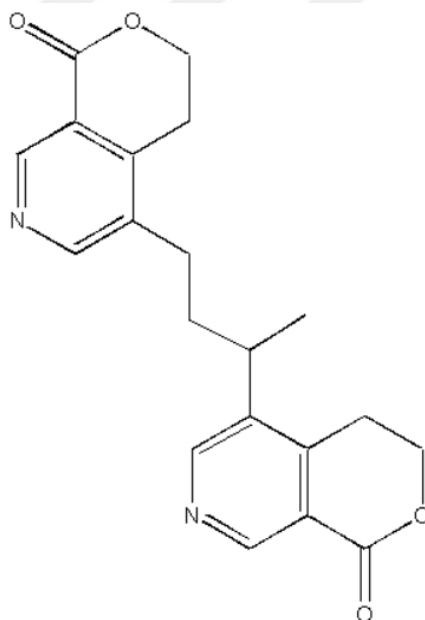


Table 77. Alkaloid obtained from *Gentiana* sp.

Compounds	Species	References
Oliveramine	<i>G. olivieri</i>	(313)
	<i>G. straminea</i>	(170)

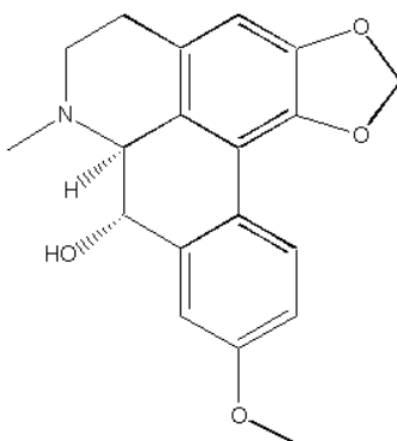


Table 78. Alkaloid obtained from *Gentiana* sp.

Compounds	Species	References
Oliveridine	<i>G. olivieri</i>	(306)

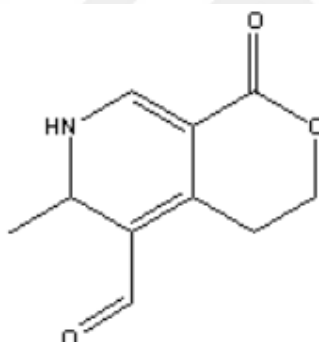


Table 79. Alkaloid obtained from *Gentiana* sp.

Compounds	Species	References
Gentioflavine	<i>G. olgae</i>	(305)
	<i>G. olivieri</i>	(306)
	<i>G. scabra</i>	(25)
	<i>G. tianschanica</i>	(305)

2.1.3.12. Other phenolics

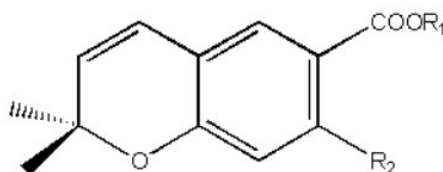


Table 80. Other phenolic compounds obtained from *Gentiana* sp.

Compounds	R ₁	R ₂	Species	References
Macrophylloside C	Glc	OCH ₃	<i>G. macrophylla</i>	(155)
Macrophylloside D	Gen	OCH ₃	<i>G. lutea</i> <i>G. macrophylla</i> <i>G. straminea</i>	(206) (155), (314) (191)
Anofinic acid	H	H	<i>G. algida</i> <i>G. lutea</i> <i>G. macrophylla</i>	(126) (206) (155)
2-hydroxyanofinic acid	H	OH	<i>G. lutea</i>	(206)
2-methoxyanofinic acid	H	OCH ₃	<i>G. lutea</i> <i>G. macrophylla</i>	(206) (155)

Gen: Gentiobiose

2.2. General Information About Inflammation and Pain

The word ‘inflammation’ is derived from the Latin word ‘inflammare’ which means ‘to set on fire’. Ancient Roman encyclopedist Cornelius Celsus defined the signs of inflammation as pain, swelling, redness, and heat in his only surviving work ‘De Medicina’. The last sign of the current five classical signs, ‘loss of function’ was added later by Rudolf Virchow, a German physician (315).

Inflammation is a complicated and multicomponent pathological process that includes several regulatory mechanisms, proteins, and enzymes (217) to protect the host by eliminating the cause and initiating tissue repair and recovery (1). It can be triggered by both internal and external factors such as pathogens, toxins, and tissue injuries. Inflammatory responses can be classified as acute or chronic due to the time and duration of the process that responds to the injury or trauma (2). Acute inflammation is the initial response of the immune system to ensure tissue repair and restoration of homeostasis. Thus, initiation and resolution are defined as two main parts of acute inflammatory response. Initiation can be characterized by tissue edema generated by increased blood flow and permeability, which is mediated by histamine, bradykinin, and lipid mediators such as leukotrienes and prostaglandins (316). Following these vascular and cellular responses, the resolution phase takes place to achieve homeostasis. Chemokine proteolysis and degradation by neutrophil extracellular traps are two mechanisms that restrict further neutrophil influx to the inflammation site. Downregulation of the production of inflammatory mediators is another important crucial step for restoring homeostasis (317). Persistent, unresolved inflammation may become chronic and lead to inflammation-related diseases including arthritis, autoimmune diseases, asthma, cardiovascular diseases, diabetes mellitus, and obesity. Chronic inflammation may be identified and characterized by both genetic (p53 genomic alteration) and physiological factors. Monocellular cell filtration (monocytes and lymphocytes), proliferation of fibroblasts, formation of collagen fibers and connective tissue are identified as characteristics of chronic inflammation (316).

Pain is defined as an unpleasant sensation associated with tissue and nerve injury. According to symptoms, underlying mechanisms, responsible receptors, and hormones, pain can be divided into three main categories: acute nociception, post-nerve injury and post-tissue injury/inflammation. Acute nociception is an evolutionary

protective function that is crucial for survival and pain resolves following to removal of the stimulus. Tissue injury and inflammation may lead to ongoing pain as a result of the secretion of various inflammatory mediators. Termination of inflammation is commonly result in resolution of pain (318).

Vasoactive amines (histamine, serotonin), peptides (bradykinin), eicosanoids (thromboxanes, leukotrienes, prostaglandins), cytokines (interleukins, $\text{TNF-}\alpha$), acute phase proteins (C-reactive protein, fibrinogen, haptoglobin, serum amyloid A), monocytes (macrophages) play key roles in the inflammatory processes. Cytokines regulate the immune system and inflammatory responses and the generation of the interleukins (IL-1 β , IL-6, IL-8, and IL-12) has been shown to induce acute-phase response (319). Among these cytokines, IL-6 contributes to host defense through inflammation, immune responses, and hematopoiesis. After its production as a part of the initial stage, it induces acute phase proteins and inhibits the generation of fibronectin, albumin, and transferrin. It also contributes to the regulation of serum iron and zinc levels (320).

Nitric oxide is a free radical that is also defined as an endothelial-derived vascular relaxation factor. NO diffuses across cell membranes and permeates through cell compartments to regulate vascular functions (321). It is produced by various inflammatory and immune cell types (macrophages, T lymphocytes, antigen-presenting cells, mast cells, neutrophils, and natural killer cells) and regulates their growth, death and functional activities (322).

Prostaglandins are generated through the cyclooxygenase pathway and metabolization of arachidonic acid, which contributes to inflammatory pain. PGE_2 is a potent inflammatory mediator and has a major impact on nociceptive processing. Therefore, COX-1 and COX-2 inhibitors are commonly utilized to suppress inflammatory pain by inhibiting the actions of prostaglandins (323). It is also reported that prostaglandin-cytokine crosstalk amplifies cytokine actions on different types of cells involved in inflammation and regulates gene expressions, which lead to processes that result in chronic inflammation (324).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Chemicals and Solvents

Table 81. List of chemicals and solvents

Chemicals and solvents	Trademark
3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT)	Sigma-Aldrich
Acetic Acid	Sigma-Aldrich
Acetone	Isolab
Chloroform	Isolab, Sigma-Aldrich
Diaion HP20	Sigma-Aldrich
Dichloromethane	Isolab, Sigma-Aldrich
Dimethyl Sulfoxide (DMSO) (molecular biology grade)	Sigma-Aldrich
Dulbecco's Modified Eagle Medium (DMEM)	Gibco Chemicals Thermo Fisher
Ethanol	Isolab, Sigma-Aldrich
Ethyl Acetate	Isolab, Sigma-Aldrich
Fetal Bovine Serum (FBS)	Gibco Chemicals Thermo Fisher
Formic Acid	Sigma-Aldrich
IL-6 Rat ELISA Kit (BMS625)	Invitrogen
Indomethacin	Fluka
Lipopolysaccharide from <i>Escherichia coli</i> 0111:B4	Sigma-Aldrich
L-NAME (L-N ^G -Nitro arginine methyl ester)	Sigma-Aldrich
Methanol	Isolab, Sigma-Aldrich
N-(1-naphthyl) ethylenediamine dihydrochloride	Fluka
<i>n</i> -Butanol	Isolab
<i>n</i> -Hexane	Isolab, Sigma-Aldrich
PGE ₂ ELISA Kit (ab287802)	Abcam
Phosphoric acid	Fluka
Polyamide (for CC)	Fluka

Table 81. List of chemicals and solvents (continue)

RediSep Rf MPLC Columns (SiO ₂ and C ₁₈)	Teledyne Isco
Reverse phase (C ₁₈) F254 Aluminium TLC plate	Merck
Sephadex LH-20	Fluka
Silica gel 60 (for CC)	Merck
Silica gel 60 F ₂₅₄ Aluminium TLC Plate	Merck
Spectrophotometric Plate Reader	BioTek
Streptomycin/Penicillin Antibiotic Solution	Gibco Chemicals Thermo Fisher
Sulphuric Acid	Riedel de Haen
<i>tert</i> -Butanol	Isolab
Vanillin	Fluka

3.1.2. Equipments and Instruments

Table 82. List of equipments and instruments

Equipments and Instruments	Trademark
96-Well Plate, 48-Well Plate	Isolab, TPP
Balance	Ohaus Explorer
Basic Laboratory Glass Equipments	Isolab
Centrifuge	Thermo Scientific SL 8R
Eppendorf Tubes	Eppendorf
Freezer	Siemens
Incubator	Binder CB-150
Infrared (IR) Spectrometer	Perkin-Elmer 2000 FT-IR
LiChroprep C ₁₈ columns (MPLC)	RediSep-Teledyne Isco
Lyophilizator	Christ Alpha 2-4 LD
Mass Spectrometry (HR-MS)	Agilent 6200 series TOF/6500
Medium Pressure Liquid Chromatography (MPLC)	Buchi- SepacoreVR Flash Systems X10/X50
Micropipette	Pipetman

Table 82. List of equipments and instruments (continue)

Multiskan Spectrophotometric Plate Reader	ThermoFischer
Nuclear Magnetic Resonance	Bruker Avance DRX 500 Spectrometer
Oven	Binder
Rotary Evaporator	Buchi, Heidolph
SiO ₂ columns (MPLC)	Merck
Spectrophotometer	Epoch (Biotek, Agilent)
TLC Chamber	Camag
TLC Plates (Silica Gel 60 F ₂₅₄ Precoated)	Merck
Ultrasonic Bath	Sonorex
Ultraviolet (UV) Spectrophotometer	HP Agilent 8453
UV Cabinet	Camag

3.1.3. Plant Materials

Gentiana cruciata and *G. asclepiadea* were gathered from Devrekani, Kastamonu, Türkiye, in August 2019. The plant materials were identified and authenticated by Prof. Dr. Hasan Kırmızıbekmez and voucher specimens (YEF 19041 and YEF 19042) have been stored at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, İstanbul, Türkiye.

3.2. Methods

3.2.1. Chromatographic Techniques

3.2.1.1. Thin Layer Chromatography (TLC)

TLC experiments were performed for the determination of phytochemicals existing in the main fractions, extracts and subextracts and also for the purity of the isolates. SiO₂-coated (Normal phase) and C₁₈-coated (Reverse phase) aluminum plates were used as stationary phases during chromatographic studies.

3.2.1.2. Mobile phases utilized in TLC experiments

Mobile phases with different ratios of solvents were prepared (Table 83) before analyses. In some instances, one or two drops of acetic acid or formic acid were added into the mobile phase mixture to prevent tailing and to acquire a better resolution of spots.

Table 83. Mobile phases prepared for TLC analyses

Stationary Phase	Mobile phases	Ratio
SiO ₂	CH ₂ Cl ₂ : MeOH:H ₂ O	90:10:1, 80:20:2, 70:30:3, 61:32:7
C ₁₈	MeOH: H ₂ O	1:1, 1:2, 1:3, 2:3

Developing Distance: 9 cm

Derivatization: TLC plates were derivatized with 1% Vanillin/Sulphuric acid and they were heated in an oven at 105 °C for 2-3 minutes.

Visualization: UV light at two different wavelengths (254 and 366 nm) under a UV cabinet.

3.2.1.2. Column Chromatography

3.2.1.2.A. Polyamide Column Chromatography

Polyamide column chromatography (CC) is a convenient tool for the separation of phenolic compounds in mixtures. Therefore, this technique was used for the main fractionation of EtOAc and *n*-BuOH subextracts. An adequate amount of polyamide material was weighed, dispersed with distilled water, and kept overnight. Then it was transferred to a suitable-sized glass column with cotton placed at the bottom, and washed with a certain amount of water in order to remove monomeric layers. After equilibration, the sample was suspended in distilled water and loaded onto the column. The elution was started with 100% distilled water and followed by increasing the methanol proportion in the water in a stepwise manner until reaching 100 % methanol.

3.2.1.2.B. Sephadex LH-20 (Gel Filtration) Column Chromatography

In gel filtration CC (also known as size exclusion chromatography), molecules are separated from a mixture according to their molecular size. Sephadex is cross-linked dextran and a porous material that enables preliminary fractionations and final-stage

purifications. An appropriate amount of Sephadex was weighed, dispersed with MeOH, and left for a day in order to swell the dextran pores, then transferred to an appropriately sized glass column. After the column was equilibrated with the starting mobile phase (MeOH), the sample was also dissolved in MeOH and loaded into the column. Mainly, isocratic elution was preferred in gel filtration separations.

3.2.1.2.C. Silica Gel Column Chromatography

In silica column chromatography, a mixture of molecules is separated based on their polarities. An adequate amount of silica was dispersed in starting mobile phase, loaded into the glass column with cotton placed bottom, and waited until it settled. Following that process, the dissolved sample (in starting solvent system) was applied to the column. Stepwise gradient elution was preferred mostly during the isolation process, with increasing polarities (Table 84) of solvent systems.

Table 84. Mobile phases prepared for silica gel CC.

Mobile phases	Ratio
CH ₂ Cl ₂ :MeOH	100:0, 97.5:2.5, 97.5:2.5:0, 95:5:0, 92.5:7.5:0, 95:5,
CHCl ₃ :MeOH	90:10:1, 90:10, 87.5:2.5, 87:12:1, 85:15, 85:13:2,
CH ₂ Cl ₂ :MeOH:H ₂ O	82.5:17.5, 80:20:2, 80:20, 75:25, 75:25:3, 70:30:3, 70:30,
	65:35, 61:32:7, 60:40, 65:45, 50:50, 50:50:2, 40:60

3.2.1.2.D. Medium Pressure Liquid Chromatography (MPLC)

MPLC is a type of column chromatography that was used for both fractionation and purification experiments. For this technique, both normal (SiO₂) and reverse (LiChroprep C₁₈) phase packed columns were used. Column choice is a critical aspect of the analyses and selections, regarding chemical/physical properties and retention characteristics of the compounds in the mixture. During chromatographic separations, a stepwise gradient elution system was preferred and conditioning was performed with starting mobile phase before the sample injection. MeOH-H₂O mixture was preferred as mobile phase in reverse phase separations, whereas CH₂Cl₂-MeOH mixture was applied in normal phase separations.

3.2.1.3. Extraction and Partition

3.2.1.3.A. Extraction and Partition of *G. asclepiadea*

The shade-dried and powdered aerial parts of *G. asclepiadea* (150 g) were macerated with 1.5 L of MeOH for 48 hours at room temperature and then extracted at 45 °C for 4 hours. After filtration, the same extraction procedure was repeated for the plant pulp with the same amount of solvent under the same conditions. The combined extracts were concentrated under reduced pressure in a rotary evaporator at 45 °C to obtain crude MeOH extract (56 g, yield 37%). Subsequently, the crude MeOH extract was dispersed in 100 mL of H₂O and partitioned against CHCl₃ (3 x 100 mL), EtOAc (3 x 100 mL), and *n*-BuOH (3 x 100 mL) to acquire CHCl₃ (13.33 g), EtOAc (1.65 g), *n*-BuOH (12.21 g) and rH₂O (23.49 g) subextracts.

3.2.1.3.B. Extraction and Partition of *G. cruciata*

The shade-dried and powdered aerial parts of *G. cruciata* (150 g) were macerated with 1.5 L of MeOH for 48 hours at room temperature and then extracted twice at 45 °C for 4 hours. The combined extracts (50.25 g, yield: 33.5%) were concentrated and dried in *vacuo* to afford crude extract of *G. cruciata*. The crude methanol extract was dispersed in 100 mL of H₂O and partitioned against CHCl₃ (3 x 100 mL), EtOAc (3 x 100 mL), and *n*-BuOH (3 x 100 mL) respectively, to obtain CHCl₃ (9.4 g), EtOAc (1.20 g), *n*-BuOH (7.32 g) and rH₂O (32.14 g) subextracts.

3.2.1.4. Main Fractionation and Isolation

3.2.1.4.A. Main Fractionation and Isolation Studies on *G. asclepiadea* (Schema 1)

EtOAc subextract (1.65 g) was loaded onto the polyamide column (20 g) eluting with H₂O (200 mL) and a stepwise gradient of MeOH in H₂O (0→100% in steps of 10%, each 100 mL) to yield ten fractions, frs. 1-10. Fr. 2 (663 mg) was subjected to SiO₂ (60 g) column eluting with CH₂Cl₂–MeOH (100:0 to 50:50) to give **5** (27 mg). *n*-Butanol subextract (12.21 g) was fractionated over polyamide column (70 g) eluting with H₂O (200 mL) and a stepwise gradient of MeOH in H₂O (0→100% in steps of 10%, each 100 mL) to yield nine fractions, frs. A-I. Fr. C (3 g) was applied to SiO₂ (150 g) CC eluting with CH₂Cl₂–MeOH (100:0 to 55:45) to obtain 12 fractions, frs. C₁₋₁₂. Fr. C₁₁ (173 mg) was subjected to C₁₈-MPLC (43 g) by using H₂O–MeOH (10 to 55% MeOH) to afford five subfractions, frs. C_{11a-11e}. Purification of **4** (10 mg) was carried out from fr.

C_{11e} (21 mg) by using SiO₂ (5 g) CC eluting with CH₂Cl₂–MeOH (95:5 to 75:25). Fr. C₁₂ (205 mg) was subjected to C₁₈-MPLC (43 g) by using H₂O–MeOH gradient (5 to 50% MeOH) to afford five subfractions, frs. C_{12a-12e}. Fr. C_{12a} (108 mg) was subjected to Sephadex LH-20 column (20 g) and eluted with MeOH to obtain **7** (13 mg). Likewise, fr. C_{12c} (17 mg) was loaded to the Sephadex LH-20 column (10 g) and eluted with MeOH to obtain **12** (2 mg). Fr. F (579 mg) was subjected to C₁₈-MPLC (130 g) by using H₂O–MeOH (0 to 45% MeOH) to afford seven fractions, frs. F₁₋₇. Fr. F₄ (76 mg) was subjected to SiO₂ (12 g) CC eluting with CH₂Cl₂–MeOH–H₂O (95:5:0.5 to 61:32:7) to obtain seven subfractions, frs. F_{4a-4g}. Purification of **11** (3 mg) was accomplished from Fr. F_{4f} (18 mg) by using Sephadex LH-20 column (6 g) eluted with MeOH. Fr. F₅ (50 mg) was subjected to SiO₂ (9 g) CC eluting with CH₂Cl₂–MeOH–H₂O (90:10:1 to 50:50:2) to obtain five subfractions (frs. F_{5a-5e}) and the latter F_{5e} (31 mg) was loaded to C₁₈-MPLC (43 g) by using H₂O–MeOH (20 to 50% MeOH) to give three subfractions, frs. F_{5e1-5e3}. Purification of **8** (2 mg) was carried out from fr. F_{5e3} (19 mg) by using Sephadex LH-20 column (6 g) eluted with MeOH. Fr. G (2063 mg) was subjected to SiO₂ (100 g) CC eluting with CH₂Cl₂–MeOH–H₂O (95:5:0 to 60:40:4) to obtain seven fractions, frs. G₁₋₇. Fr. G₄ (68 mg) was loaded to SiO₂ (12 g) CC eluting with CH₂Cl₂–MeOH (95:5 to 70:30) to purify **9** (44 mg).

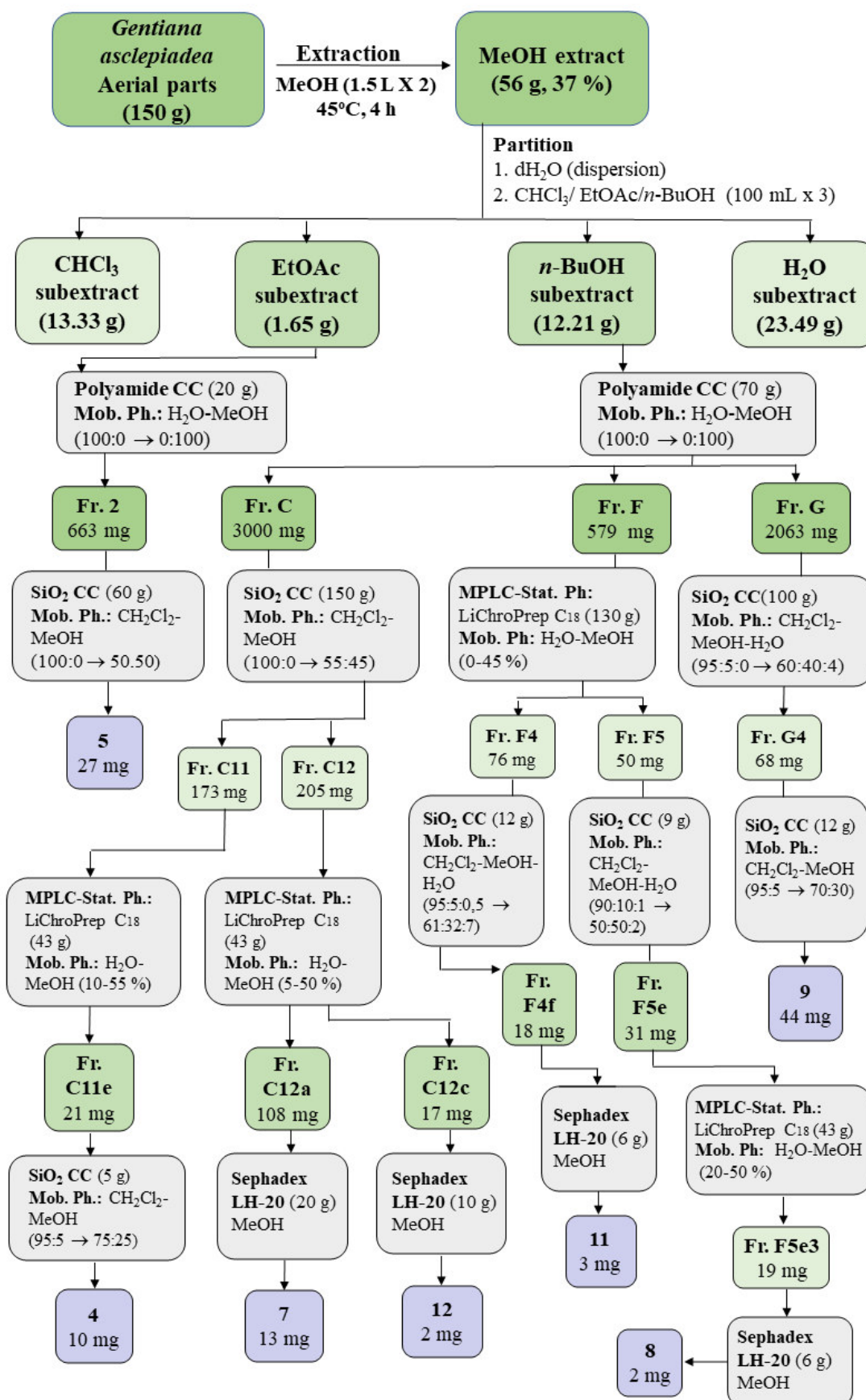
3.2.1.4.B. Main Fractionation and Isolation Studies on *G. cruciata* (Schema 2)

Ethyl acetate subextract (1.2 g) was loaded to a polyamide column (20 g) eluting with H₂O (200 mL) and a stepwise gradient of MeOH in H₂O (0→100% in steps of 10%, each 100 mL) to yield eight fractions, frs. A–H. Fr. F (73 mg) was subjected to C₁₈-MPLC (43 g) column by using H₂O–MeOH (5 to 45% MeOH) as the eluting solvent to give **8** (12 mg) and **11** (7 mg) along with fr. F₃ (32 mg) which was subsequently loaded to SiO₂-MPLC (12 g) by using CH₂Cl₂–MeOH (0 to 15% MeOH) to obtain **9** (4 mg). Compound **10** (3 mg) was isolated from fr. G (166 mg) by using C₁₈-MPLC (43 g) and eluting with H₂O–MeOH (10 to 60% MeOH). Purification of **5** (33 mg) was carried out from fr. A (252 mg) by using C₁₈-MPLC (43 g) eluting with H₂O–MeOH (0 to 50% MeOH). An aliquot of the *n*-Butanol subextract (7.32 g) was loaded to the polyamide column (40 g) eluting with H₂O (200 mL) and a stepwise gradient of MeOH in H₂O (0→100% in steps of 10%, each 100 mL) to afford seven fractions, frs. A–G. Fr. A (2241 mg) was fractionated with C₁₈-MPLC (150 g) by using H₂O–MeOH (0 to 90% MeOH) as the eluting solvent to obtain 13 fractions, frs. A₁₋₁₃. Fr. A₇ (226 mg) was fractionated with

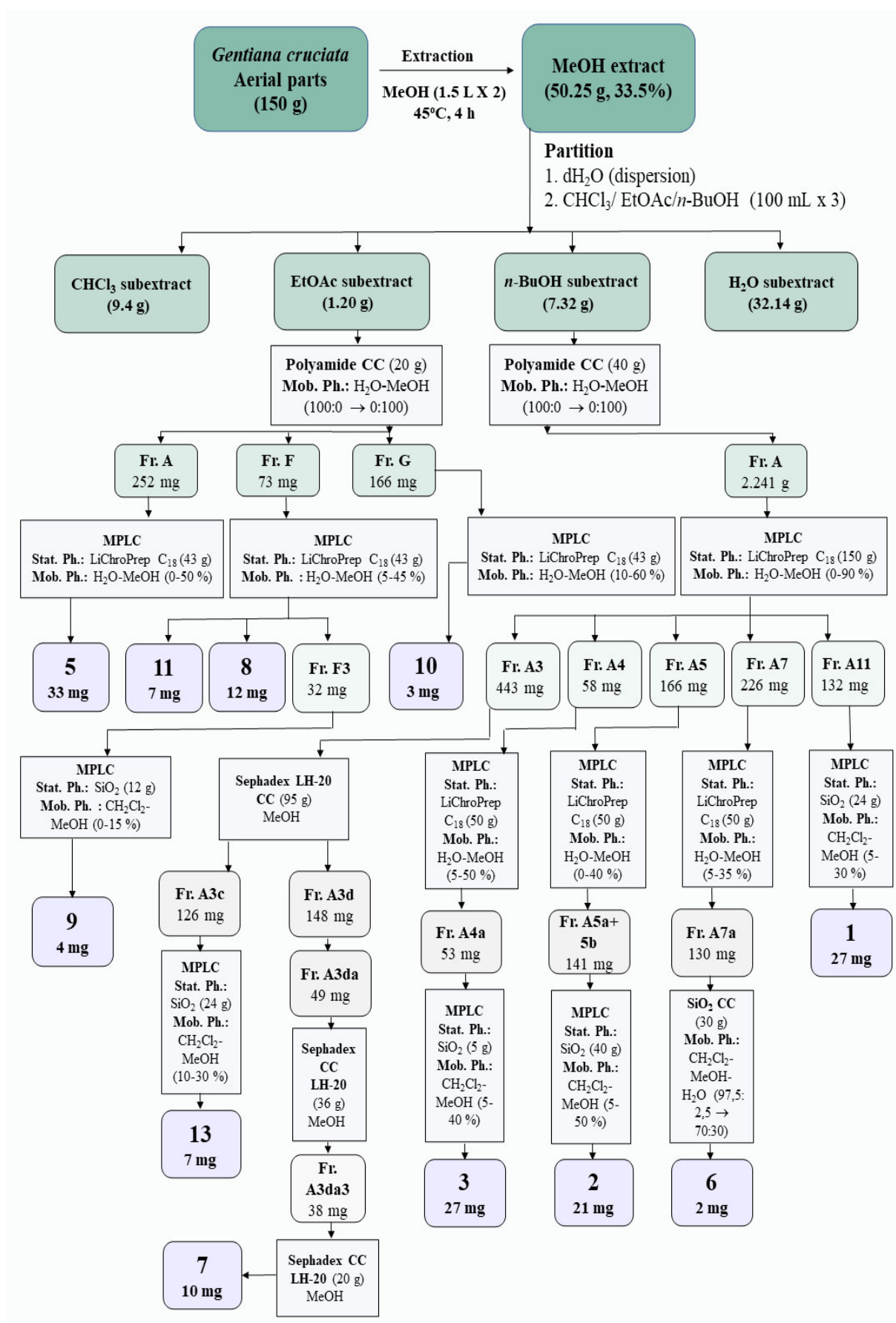
C₁₈-MPLC (50 g) by using H₂O–MeOH (5 to 35% MeOH) to yield five fractions, frs. A_{7a-7e}. Fr. A_{7a} (130 mg) was subjected to SiO₂ (30 g) CC eluting with CH₂Cl₂–MeOH (97.5:2.5 to 70:30) to obtain **6** (2 mg). Fr. A₃ (443 mg) was loaded to Sephadex LH-20 column (95 g) and eluted with MeOH to afford five fractions frs. A_{3a-3e}. Purification of fr. A_{3c} (126 mg) with SiO₂-MPLC (24 g) by using CH₂Cl₂–MeOH (10 to 30% MeOH) gave **13** (7 mg). Fr. A_{3d} (148 mg) was divided into two subfractions, frs. Fr. A_{3da-3db}. Fr. A_{3da} (49 mg) was subjected to Sephadex LH-20 column (36 g) eluting with MeOH to obtain four subfractions frs. A_{3da1-3da4}. Application of fr. A_{3da3} (38 mg) to another Sephadex LH-20 column (20 g) eluting with MeOH yielded **7** (10 mg). Fr. A₁₁ (132 mg) was fractioned with SiO₂-MPLC (24 g) by using CH₂Cl₂–MeOH (5 to 30% MeOH) to obtain **1** (27 mg). Fr. A₅ (166 mg) was loaded to C₁₈-MPLC (50 g) and eluted with H₂O–MeOH (0 to 40% MeOH) to give four fractions, frs. A_{5a-5d}. Frs. A_{5a} and A_{5b} were combined (141 mg) and loaded to SiO₂-MPLC (40 g) by using CH₂Cl₂–MeOH (5 to 50% MeOH) to give **2** (21 mg). Fr. A₄ (58 mg) was fractioned with C₁₈-MPLC (50 g) by using H₂O–MeOH (5 to 50% MeOH) to afford five fractions, frs. A_{4a-4e}. Fr. A_{4a} (53 mg) was loaded to SiO₂-MPLC (5 g) by using CH₂Cl₂–MeOH (5 to 40% MeOH) as the eluting solvent to give **3** (27 mg).

3.2.1.5. Structure Elucidation Studies on the Isolates

The exact structural elucidation of the purified compounds was performed with the help of spectroscopic techniques including UV, IR, 1D (¹H and ¹³C), and 2D (COSY, HSQC, HMBC, NOESY) NMR and ESI-MS/HR-ESI-MS.



Schema 1. Isolation of compounds from *G. asclepiadea*



Schema 2. Isolation of compounds from *G. cruciata*

3.2.2 Bioactivity Studies

3.2.2.1. Cell Lines and Cell Culture

The cytotoxicity of the isolates was evaluated on RAW 264.7 murine macrophage cell line obtained from the American Type Culture Collection (ATCC) which was cultured in DMEM (10% FBS, v/v; Gibco) and supplemented with 1% antibiotics (10.000 µg/mL streptomycin and 10.000 units/mL penicillin; Gibco) at 37°C in a humidified atmosphere of 5% CO₂.

3.2.2.2. MTT Assay

Prior to the assessment of anti-inflammatory and analgesic activities of the isolates obtained from *G. asclepiadea* and *G. cruciata*, cytotoxicity profiles of compounds have been assessed via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) test. Briefly, RAW 264.7 cells were plated in 48-well plates and incubated for twenty-four hours at 37°C in 5% CO₂ to form a confluent layer. After 24 h of incubation, cells were treated with serial dilutions of compounds at final concentrations between 25-200 µM. Following the 24 h of incubation, the used cell culture medium was discarded and MTT (0.5 mg/mL) was added to each well for an additional 2 h at 37°C. Following the incubation, the medium was discarded and produced formazan crystals were dissolved by 100 µL of isopropanol. Afterward, the optical density of the violet-colored chromophore was measured at 570 nm spectrophotometrically. The viability (%) was calculated by using the following equation (325):

$$\text{Viability \%} = ((\text{Absorbance})_{\text{treatment group}} - (\text{Absorbance})_{\text{control}}) \times 100\%$$

3.2.2.3. Anti-Inflammatory and Analgesic Activity

3.2.2.3.A. Determination of Nitrite Level

Nitrite is a stable metabolite of nitric oxide (NO) and is frequently used as an inflammation indicator, which was shown to be increased during an inflammatory response. NO levels were evaluated by measuring the accumulation of nitrite with Griess assay. The RAW 264.7 cells were cultured at 37° C in 5% CO₂ in a 48-well plate for 24 hours. The next day, the highest non-cytotoxic concentrations of the isolated compounds were added and treated for 2 h, then stimulated with 1 µg/mL of LPS for an additional 22 hours. After the incubation period, cell culture supernatants of each group were collected and mixed with the same volumetric amount of Griess reagent (1% sulfanilamide and

0.1% N-(1- naphthyl) ethylenediamine dihydrochloride in 5% phosphoric acid) for 10 minutes in the dark at room temperature. The absorbance of the yielded chromophore was measured at a microplate reader at 540 nm wavelengths whereas nitrite levels were calculated with the help of a sodium nitrite standard calibration curve. Indomethacin (100 μ M) was selected as a positive control (326).

3.2.2.3.B. Determination of IL-6 Level

Interleukin-6 (IL-6) is a pro-inflammatory cytokine and increased IL-6 levels are observed during infections and inflammatory diseases. Therefore, the anti-inflammatory activities of the isolates were also evaluated through the estimation of IL-6 levels in LPS-activated RAW 264.7 cells. IL-6 levels from cell supernatants of nitrite assay were determined via commercial IL-6 ELISA kit according to the manufacturer's protocol in duplicates. The absorbance was measured at 450 nm spectrophotometrically and results for IL-6 levels were expressed as pg/mL (327).

3.2.2.3.C. Analgesic Activity

Prostaglandin E₂ (PGE₂), is also known as dinoprostone, a cyclooxygenase (COX) product and a potent inflammatory mediator, which contributes to inflammatory pain. In order to evaluate the analgesic activity, the inhibition potential of the compounds on PGE₂ levels in LPS-activated murine macrophage cells was used. The release of PGE₂ was assessed from cell supernatants by a commercial ELISA kit according to the manufacturer's protocol in duplicates. The results were measured at 450 nm spectrophotometrically and expressed as pg/mL (326).

3.2.3. Statistical Analysis

The *in vitro* results obtained were statistically assessed by the GraphPad Prism 7.0 (GraphPad Software, La Jolla-CA, USA) program. $p < 0.05$ was considered significant. One-way analysis of variance (one-way ANOVA) was used to compare the statistical significance between groups.

4. RESULTS

The aim of this thesis was the purification of metabolites from aerial parts of *G. asclepiadea* and *G. cruciata* naturally grown in Türkiye. Thus, EtOAc and *n*-BuOH subextracts of these species, which were rich in phytochemicals (mostly iridoids and flavonoids) were submitted to multiple chromatographic analyses and isolates were assessed for their biological activities.

4.1. Extraction

4.1.1. Extraction and Isolation Studies of *G. asclepiadea*

The shade-dried and powdered aerial parts of *G. asclepiadea* (150 gr) were extracted with MeOH to afford crude MeOH extract (56 mg, yield 37 %). The crude MeOH extract was firstly dissolved in dH₂O and then partitioned against CHCl₃, EtOAc, and *n*-BuOH to yield four subextracts; CHCl₃ (13.33 g), EtOAc (1.65 g), *n*-BuOH (12.21 g) and rH₂O (23.49 g). As a result of sequential chromatographic methods (silica CC, polyamide CC, Sephadex, MPLC), seven compounds were purified from the EtOAc and *n*-BuOH subextracts. Their structures were established unambiguously by spectroscopic methods such as 1D and 2D NMR as well as HR-ESI-MS. These seven compounds were identified as, two secoiridoid glycosides **4** and **5**, one iridoid glycoside **7**, two flavone-C-glycosides **8** and **9**, one xanthone-C-glycoside **11** as well as a nucleoside **12** (Schema 1).

4.1.2. Extraction and Isolation Studies of *G. cruciata*

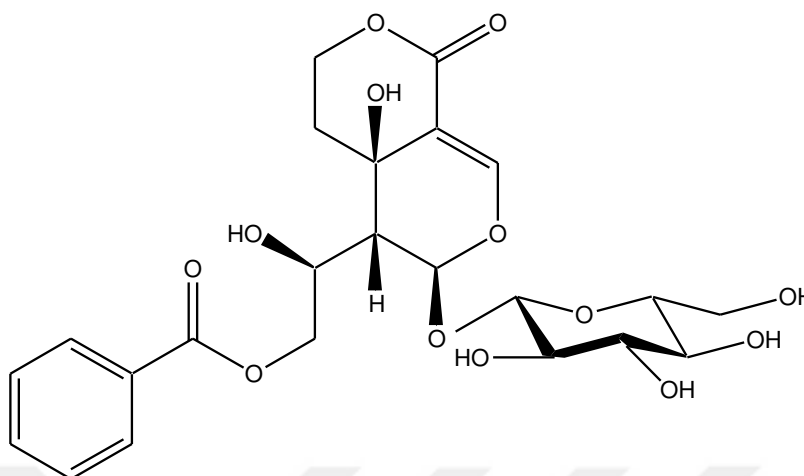
The shade-dried and powdered aerial parts of *G. cruciata* (150 gr) were extracted with MeOH to obtain MeOH extract (50.25 g, yield: 33.5 %). The crude methanol extract was dispersed in dH₂O and then partitioned with CHCl₃ (3 x 100 mL), EtOAc (3 x 100 mL), and *n*-BuOH (3 x 100 mL) to acquire four subextracts CHCl₃ (9.4 g), EtOAc (1.20 g), *n*-BuOH (7.32 g) and rH₂O (32.14 g). As a result of consecutive chromatographic separations (silica CC, polyamide CC, Sephadex, MPLC), eleven compounds were isolated from the EtOAc and *n*-BuOH subextracts. The chemical structures of the isolates were elucidated based on 1D and 2D NMR methods as well HR-ESI-MS. As a result of spectroscopic findings, these eleven compounds were identified as five secoiridoid glycosides **1**, **2**, **3**, **5**, and **6**, one iridoid glycoside **7**, three flavonoids **8**, **9**, **10**, one xanthone-C-glycoside **11**, and one cyclitol **13** (Schema 2).

Table 85. Isolated metabolites from *G. asclepiadea* and *G. cruciata*

Compound No	Compound Name	Species
1	Cruciatoside	<i>G. cruciata</i>
2	Eustomoside	<i>G. cruciata</i>
3	Eustomorusside	<i>G. cruciata</i>
4	Depressine	<i>G. asclepiadea</i>
5	Gentiopicroside	<i>G. asclepiadea</i> , <i>G. cruciata</i>
6	6'- <i>O</i> - β -D-glucosyl gentiopicroside	<i>G. cruciata</i>
7	Loganic acid	<i>G. asclepiadea</i> , <i>G. cruciata</i>
8	Isoorientin	<i>G. asclepiadea</i> , <i>G. cruciata</i>
9	Isovitexin	<i>G. asclepiadea</i> , <i>G. cruciata</i>
10	Isovitexin 2''-(<i>E</i>)-Ferulate	<i>G. cruciata</i>
11	Mangiferin	<i>G. asclepiadea</i> , <i>G. cruciata</i>
12	Adenosine	<i>G. asclepiadea</i>
13	2-Methyl Inositol	<i>G. cruciata</i>

4.2. Structure Elucidation of Isolated Compounds

4.2.1. Secoiridoid Glycosides



CRUCIATOSIDE (1), C₂₃H₂₈O₁₃ (MW: 512.46)

UV λ_{max} (MeOH) nm:	231, 282
IR ν_{max} (KBr) cm^{-1} :	3349 (OH), 1711 (ester C=O), 1617 (C=C)
¹ H NMR:	Spectrum 1, Table 86
¹³ C NMR:	Spectrum 2, Table 86
COSY:	Spectrum 3
NOESY:	Spectrum 4
HSQC:	Spectrum 5
HMBC:	Spectrum 6
HR-ESI-MS:	Spectrum 7

CRUCIATOSIDE (1)

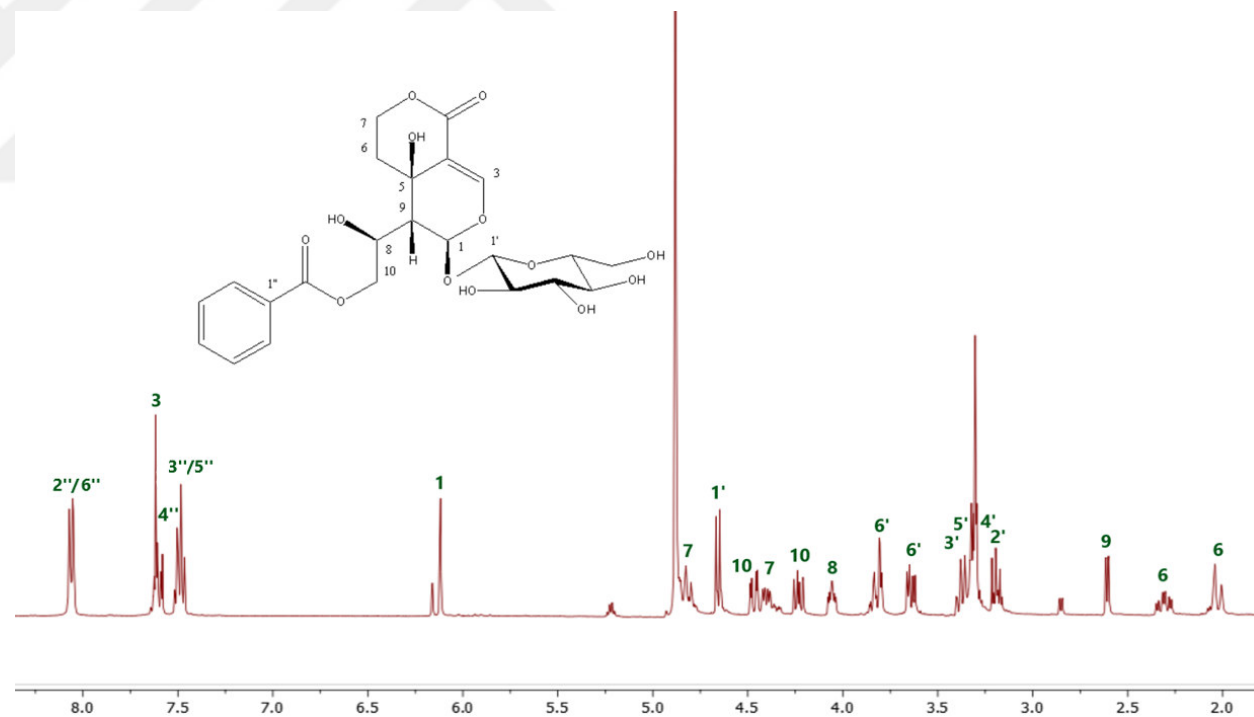
Compound **1** was isolated as a colourless amorphous powder. The molecular formula, $C_{23}H_{28}O_{13}$, was determined based on the sodiated ion peak at m/z 535.1425 (calcd for $C_{23}H_{28}O_{13}Na^+$, 535.1422) in its HR-ESI-MS, indicating 10 degrees of unsaturation. It showed UV absorption maxima at 231 and 282 nm, while its IR spectrum contained bands at 3349 (OH), 1711 (C=O) and 1617 (C=C), respectively. The ^1H NMR spectrum of **1** displayed the signals due to the presence of an olefinic ($\delta_H = 7.62$, s, H-3), a hemiacetal ($\delta_H = 6.12$, d, $J = 1.2$ Hz, H-1), two oxymethylenes ($\delta_H = 4.81$ (m), 4.40 (m), H₂-7; $\delta_H = 4.47$ dd ($J = 11.8$ and 2.9) and 4.43 dd ($J = 11.8$ and 7.4) H₂-10), one oxymethine ($\delta_H = 4.06$, m, H-8) one methine ($\delta_H = 2.61$, dd ($J = 5.7$ and 1.2), H-9), and one methylene ($\delta_H = 2.31$, m, and 2.02, br.d, $J = 13.0$, H₂-6) groups. Moreover, an anomeric proton signal was observed at $\delta_H = 4.65$ with a coupling constant of 7.9 Hz. All these findings suggested that **1** had a structure similar to that of secoiridoid glycosides (328). Besides, five additional aromatic signals at $\delta_H = 8.06$ (dd, $J = 7.8$, 1.3 Hz, 2H), 7.61 (m) and 7.48 (t, $J = 7.8$ Hz, 2H) were detected (Spectrum 1).

The ^{13}C NMR spectrum of **1** contained 23 resonances, six of which were arising from a β -glucopyranose unit while seven were from a benzoyl moiety. The remaining ones were characteristic of a C₁₀-secoiridoid skeleton (328). All of these data showed a signal pattern similar to eustomorusside (**3**) (329), except for the presence of a benzoyl unit (Spectrum 2). Furthermore, H₂-10 signals (δ_H 4.47 and 4.23) of **1** were found to be shifted downfield around 1 ppm compared to those of eustomorusside, implying the site of acylation. 2D NMR experiments (COSY, HSQC and HMBC) supported the proposed structure and the attachment points of β -glucopyranose and benzoyl units to the aglycone of **1**. Thus, the long-range correlation from C-1 (δ_C 96.0) to H-1' (δ_H 4.65) confirmed the glycosidation point to be C-1. The cross-peak between the benzoyl carbonyl signal at δ_C 167.9 to H₂-10 (δ_H 4.47 and 4.23) positioned the benzoyl moiety at C-10 (Spectrum 6). The relative configuration of **1** was established by the NOESY spectrum and coupling constant values (329). The NOE correlation between H-8 and H-1 and between H-8 to H α -10 (δ_H 4.23) positioned them on the same side of the molecule (α) (Spectrum 4). Moreover, $J_{9,1}$ (1.2 Hz) and $J_{9,8}$ (5.7 Hz) values of **1** were found to be superimposable with those of eustomorusside. According to these results, the structure of **1** was determined as a 10-*O*-benzoyleustomorusside and named cruciatoside based on the species name of *G. cruciata*.

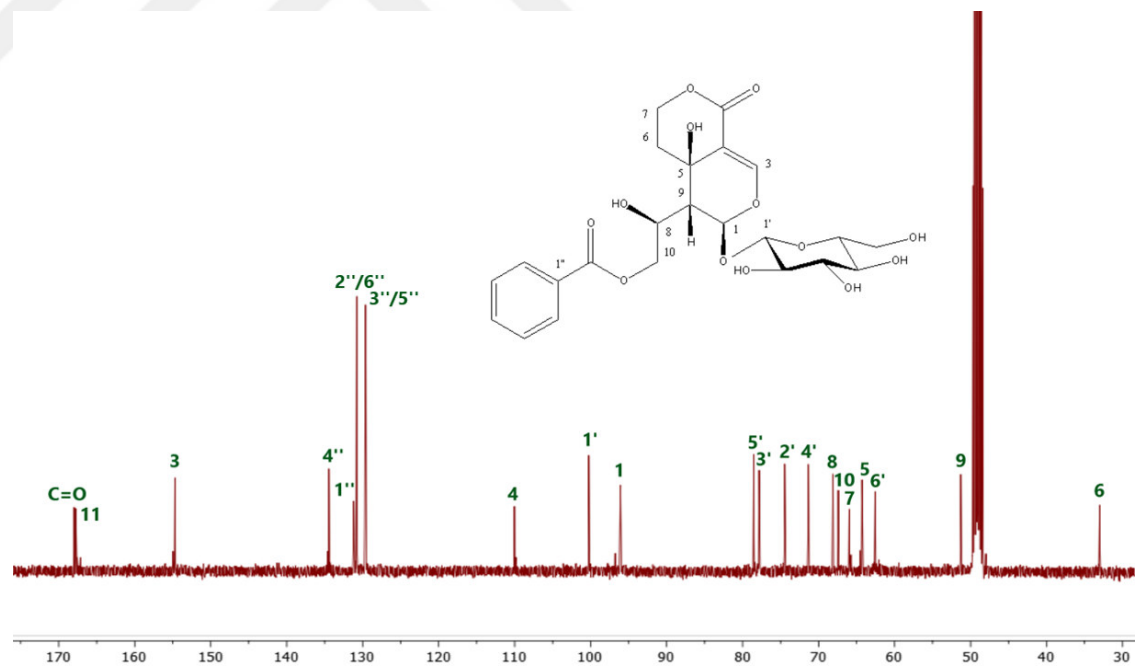
Table 86. ^1H and ^{13}C spectroscopic data of cruciatoside (**1**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	96.0	6.12 d (1.2)
3	CH	154.7	7.62 s
4	C	110.0	-
5	C	64.4	-
6	CH_2	32.9	2.31 m 2.02 br.d (13.0)
7	CH_2	65.8	4.81 m 4.40 m
8	CH	68.1	4.06 m
9	CH	51.2	2.61 dd (5.7, 1.2)
10	CH_2	67.4	4.47 dd (11.8, 2.9) 4.43 dd (11.8, 7.4)
11	C	167.7	-
<i>Glucose</i>			
1'	CH	100.2	4.65 d (7.9)
2'	CH	74.4	3.19 dd (9.0, 7.9)
3'	CH	77.8	3.38 t (9.0)
4'	CH	71.3	3.31 †
5'	CH	78.5	3.32 †
6'	CH_2	62.3	3.82 dd (12.0, 1.6) 3.64 dd (12.0, 5.2)
<i>Benzoyl</i>			
1''	C	131.1	-
2''	CH	130.8	8.06 dd (7.8, 1.3)
3''	CH	129.6	7.48 t (7.8)
4''	CH	134.4	7.61 m
5''	CH	129.6	7.48 t (7.8)
6''	CH	130.8	8.06 dd (7.8, 1.3)
C=O	C	167.9	-

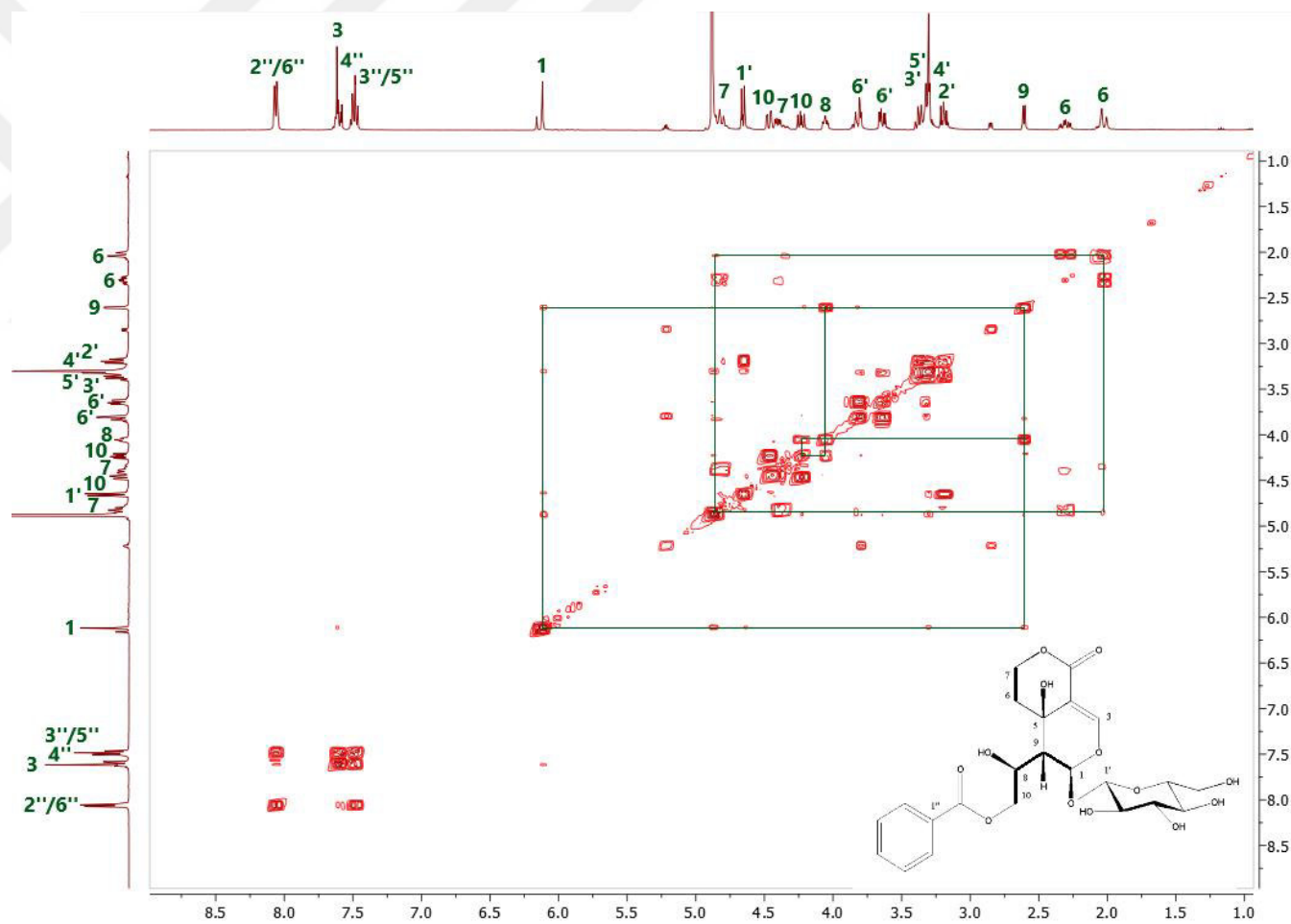
† Signal pattern unclear due to overlapping.



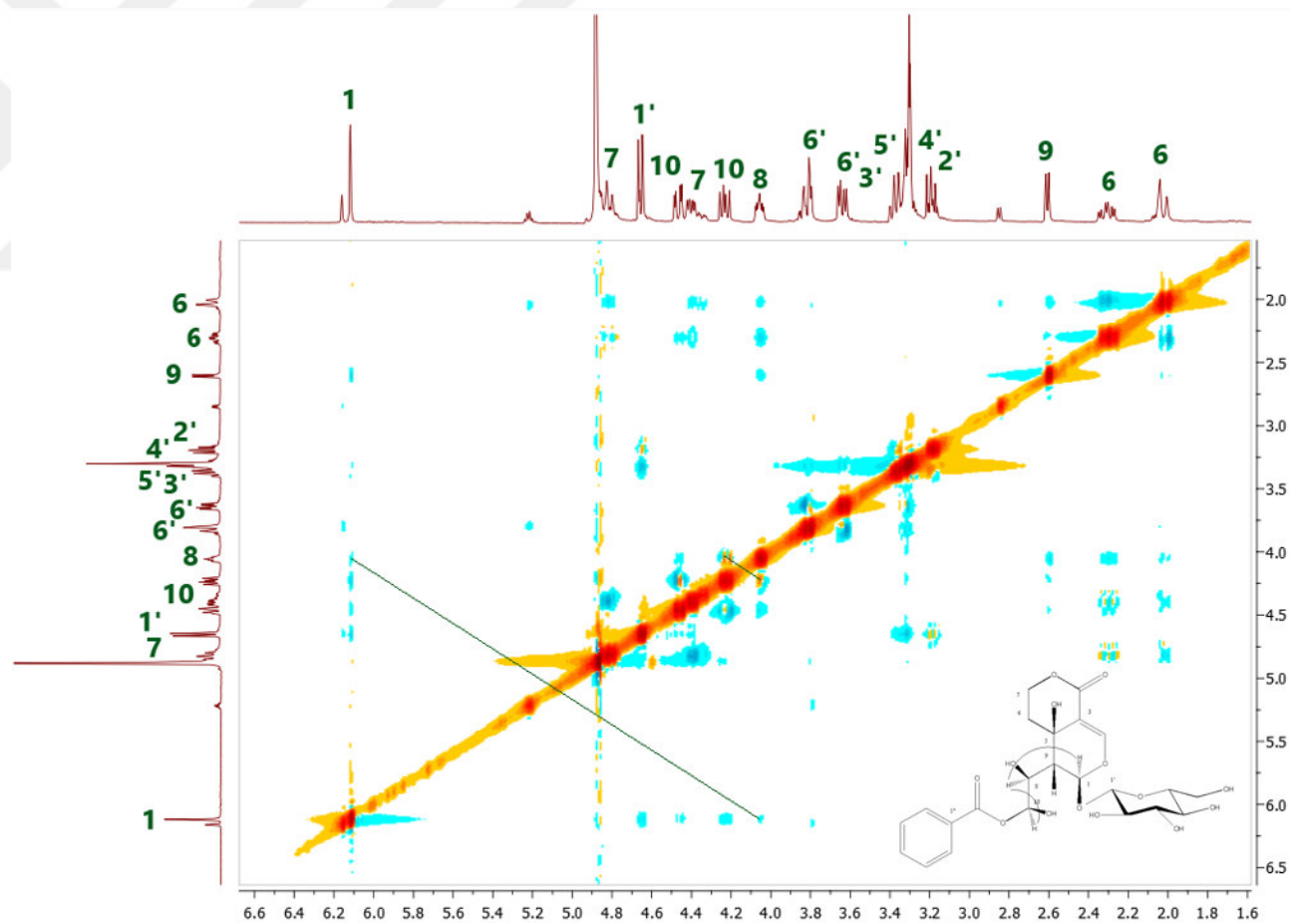
Spectrum 1. ^1H NMR spectrum of cruciatoside (**1**) (CD_3OD , 500 MHz)



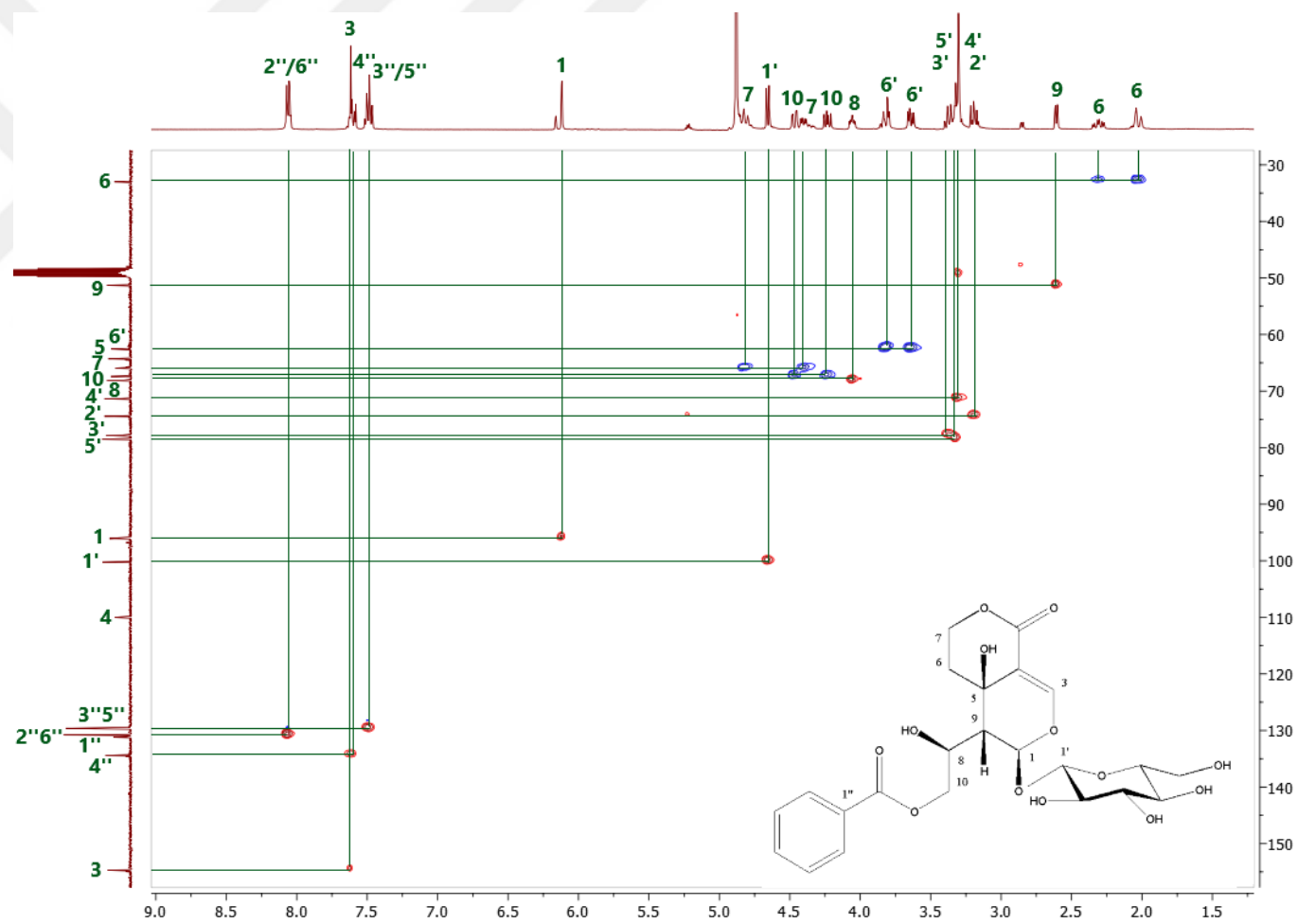
Spectrum 2. ^{13}C NMR spectrum of cruciatoside (1) (CD_3OD , 125 MHz)



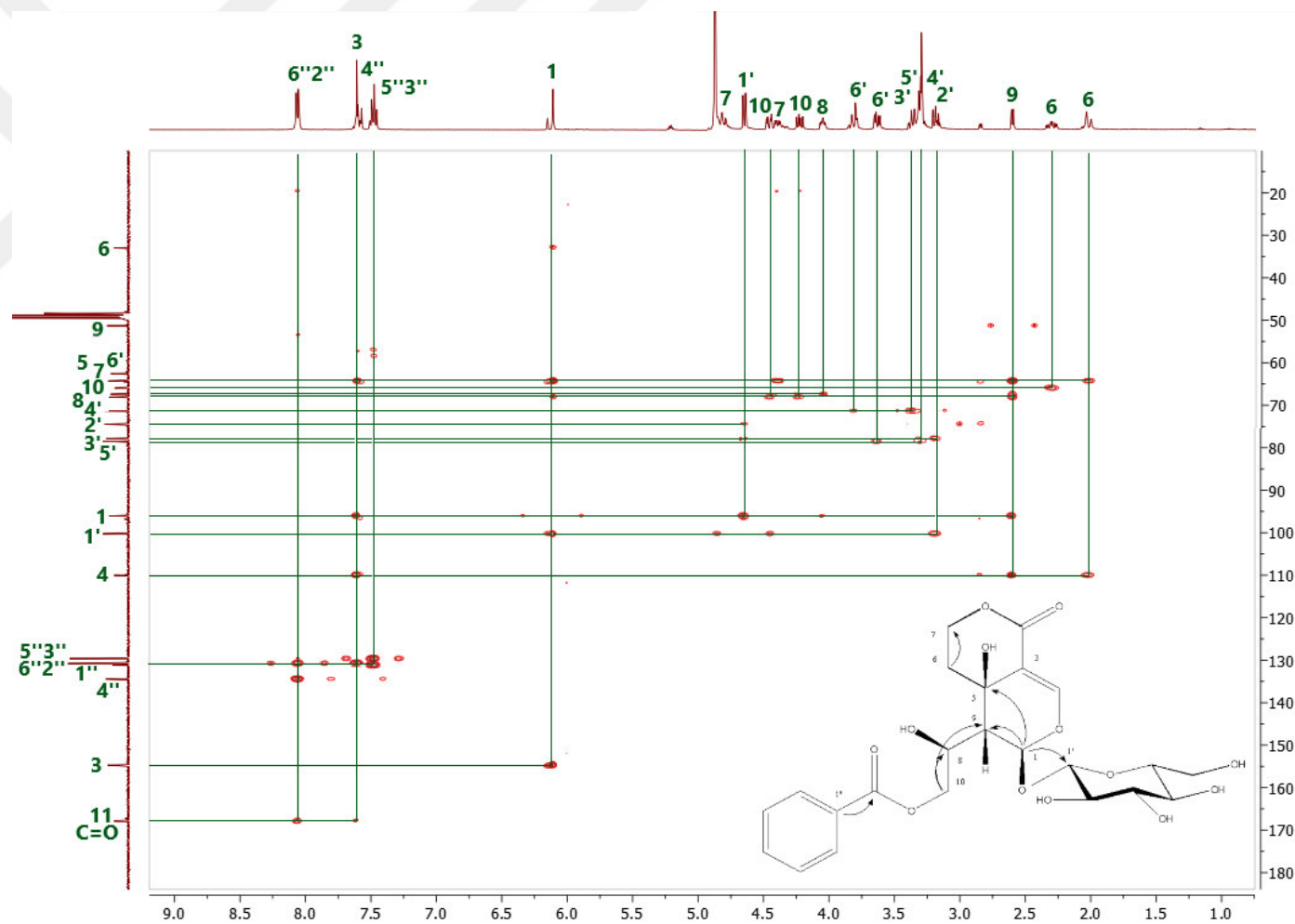
Spectrum 3. COSY spectrum of cruciatoside (**1**)



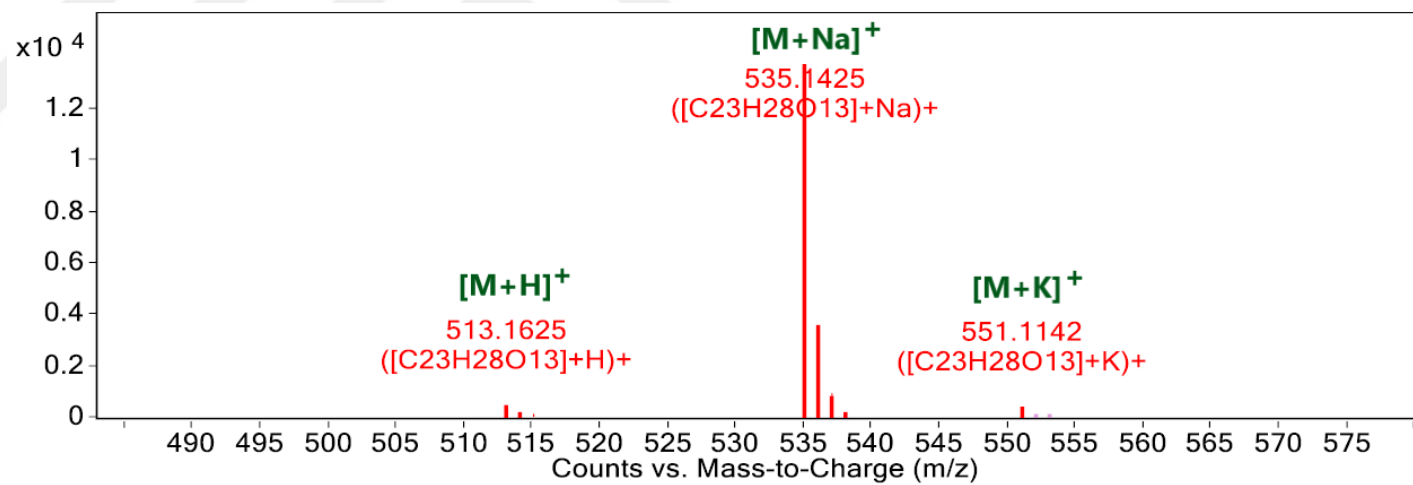
Spectrum 4. NOESY spectrum of cruciatoside (**1**)



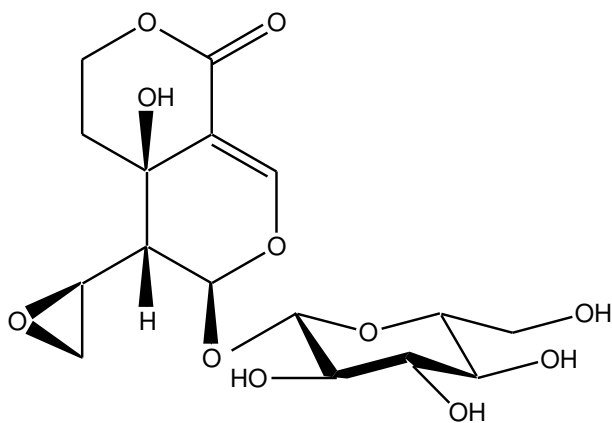
Spectrum 5. HSQC spectrum of cruciatoside (1)



Spectrum 6. HMBC spectrum of cruciatoside (**1**)



Spectrum 7. HR-ESI-MS spectrum of cruciatoside (**1**)



EUSTOMOSIDE (2), C₁₆H₂₂O₁₁ (MW: 390.34)

UV λ_{max} (MeOH) nm:	236
IR ν_{max} (KBr) cm ⁻¹ :	3362 (OH), 1694 (C=O), 1618 (C=C)
¹ H NMR:	Spectrum 8, Table 87
¹³ C NMR:	Spectrum 9, Table 87
COSY:	Spectrum 10
HR-ESI-MS:	Spectrum 11

EUSTOMOSIDE (2)

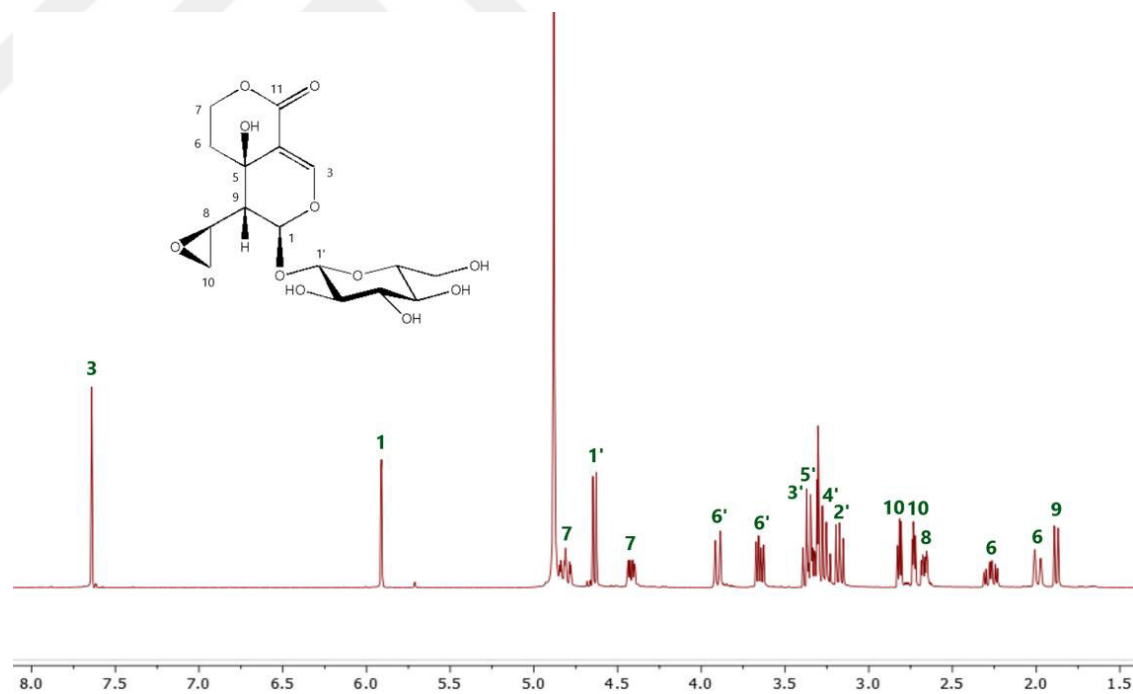
Compound **2** was purified as a colourless amorphous powder. The HRI-ESI-MS of **2** demonstrated (M+H)⁺ ion at m/z 391.1234 (calc. for C₁₆H₂₃O₁₁, 391.1240), (M+Na)⁺ ion at m/z 413.1051 (calc. for C₁₆H₂₂O₁₁Na, 413.1060), and (M+K)⁺ ion at m/z 429.0772 (calc. for C₁₆H₂₂O₁₁K, 429.0799). In its UV spectrum, band at 236 nm was observed, while absorption peaks at 3362 (OH), 1694 (C=O), and 1618 (C=C) were detected in the IR spectrum of **2**. The ¹H NMR spectrum of **2** showed the signals due to the presence of a trisubstituted olefinic (δ_H = 7.64, s, H-3), a hemiacetal (δ_H = 5.91, d, J = 1.2 Hz, H-1), two oxymethylenes (δ_H = 4.81 (m), 4.42 (m), H₂-7; δ_H = 2.82 dd (J = 4.8 and 4.1), 2.73 dd (J = 4.8 and 2.5) H₂-10), one oxymethine (δ_H = 2.67, m, H-8), one methine (δ_H = 1.88, dd (J = 9.0 and 1.2), H-9), and one methylene (δ_H = 2.27, m, and 1.99, br.d, J = 14.0, H₂-6) groups (Spectrum 8). These findings were consistent with a secoiridoid containing a lactone moiety (328). Besides, an anomeric proton signal was detected at δ_H = 4.64 with a coupling constant of 7.9 Hz. Likewise, proton signals of **2** showed a signal pattern similar to eustomorusside (**3**) (176), except for upfield shifted signals of C-8 and C-10 (δ_H 2.67, 2.73, 2.82) indicating the presence of epoxy unit. In addition to signals of H-8 and H-10, the further resonance at δ_H 1.88 (H-9) confirms the presence of a C-8/C-10 oxirane ring, which was previously detected in a swertiamarin-type secoiridoid glycoside, eustomoside (176).

The ¹³C NMR spectrum of **2** contained 16 resonances, six of which were arising from a β -glucopyranose unit (Spectrum 9). The remaining signals were characteristic of the C₁₀-secoiridoid skeleton (328). Moreover, the signals detected in the ¹³C NMR spectrum were in good agreement with those observed for eustomoside (176) and also support the presence of the C-8/C-10 oxirane ring structure. The COSY spectrum contained three spin systems; one between H-10 to H-1, the other between H₂-6 to H₂-7, and also between H-1' to H₂-6' (Spectrum 10). All the data in published literature overlapped with the spectral data of eustomoside (**2**) (329).

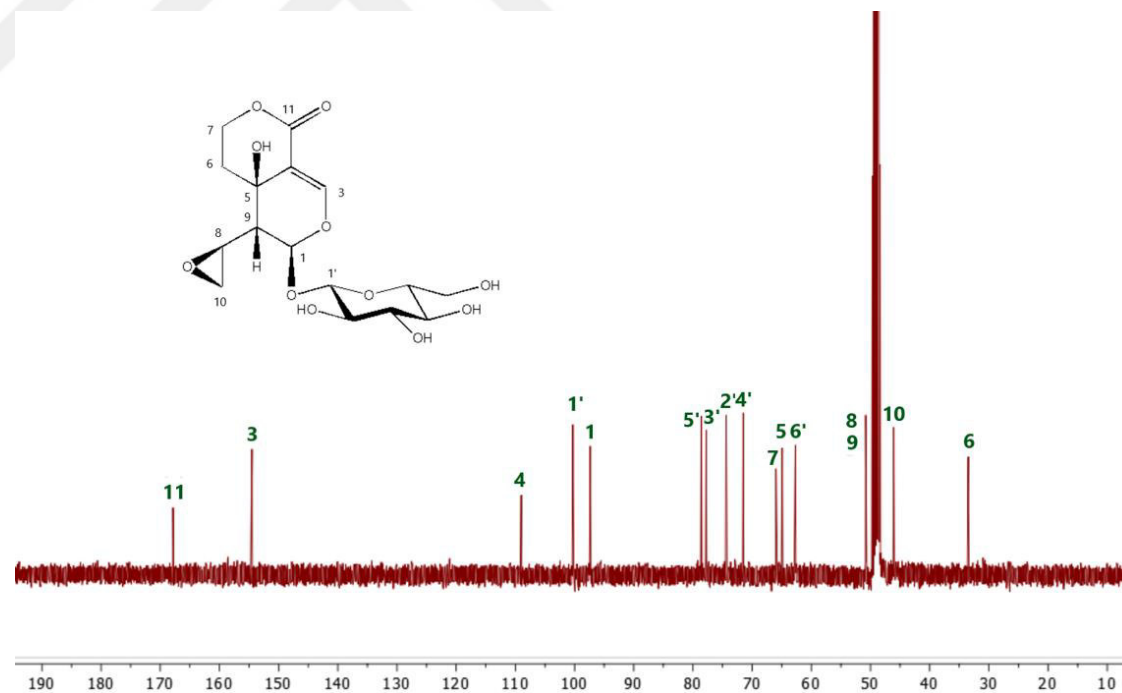
Table 87. ^1H and ^{13}C spectroscopic data of eustomoside (**2**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	97.3	5.91 d (1.2)
3	CH	154.5	7.64 s
4	C	109.1	-
5	C	64.9	-
6	CH ₂	33.4	2.27 m 1.99 br.d (14.0)
7	CH ₂	65.9	4.81 m 4.42 m
8	CH	50.8	2.67 m
9	CH	50.8	1.88 dd (9.0, 1.2)
10	CH ₂	46.0	2.82 dd (4.8, 4.1) 2.73 dd (4.8, 2.5)
11	C	167.8	-
<i>Glucose</i>			
1'	CH	100.2	4.64 d (7.9)
2'	CH	74.4	3.17 dd (9.0, 7.9)
3'	CH	77.7	3.37 t (9.0)
4'	CH	71.5	3.25 t (9.0)
5'	CH	78.5	3.32 †
6'	CH ₂	62.6	3.90 dd (12.0, 2.1) 3.65 dd (12.0, 6.2)

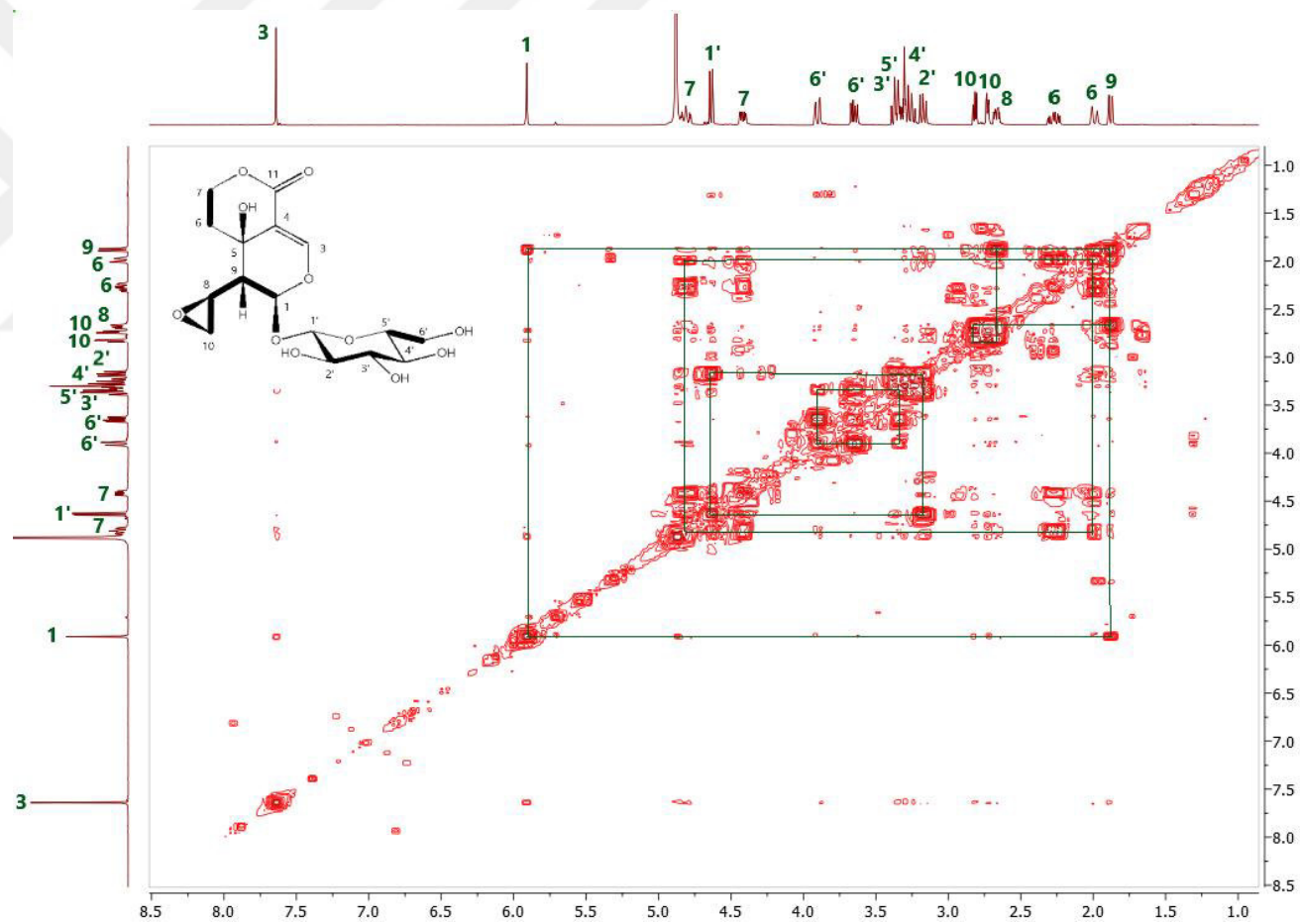
† Signal pattern unclear due to overlapping.



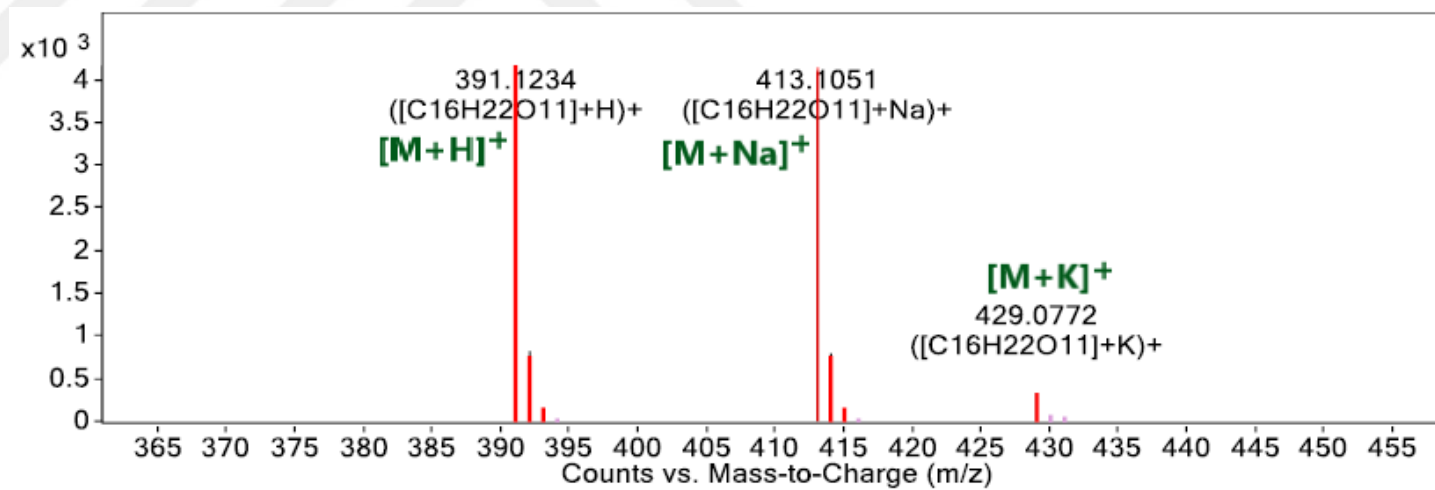
Spectrum 8. ^1H NMR spectrum of eustomoside (2) (CD_3OD , 500 MHz)



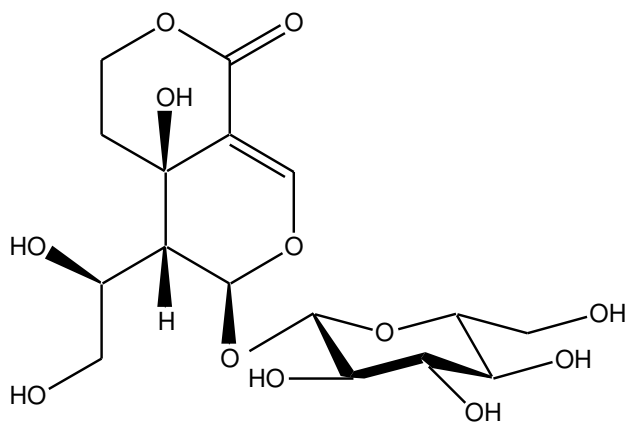
Spectrum 9. ¹³C NMR spectrum of eustomoside (**2**) (CD₃OD, 125 MHz)



Spectrum 10. COSY spectrum of eustomoside (2)



Spectrum 11. HR-ESI-MS spectrum of eustomoside (**2**)



EUSTOMORUSSIDE (3), C₁₆H₂₄O₁₂ (MW: 408.35)

UV λ_{max} (MeOH) nm:	238
IR ν_{max} (KBr) cm ⁻¹ :	3333 (OH), 1693 (C=O), 1617 (C=C)
¹ H NMR:	Spectrum 12, Table 88
¹³ C NMR:	Spectrum 13, Table 88
COSY:	Spectrum 14
NOESY:	Spectrum 15
HSQC:	Spectrum 16
HMBC:	Spectrum 17

EUSTOMORUSSIDE (3)

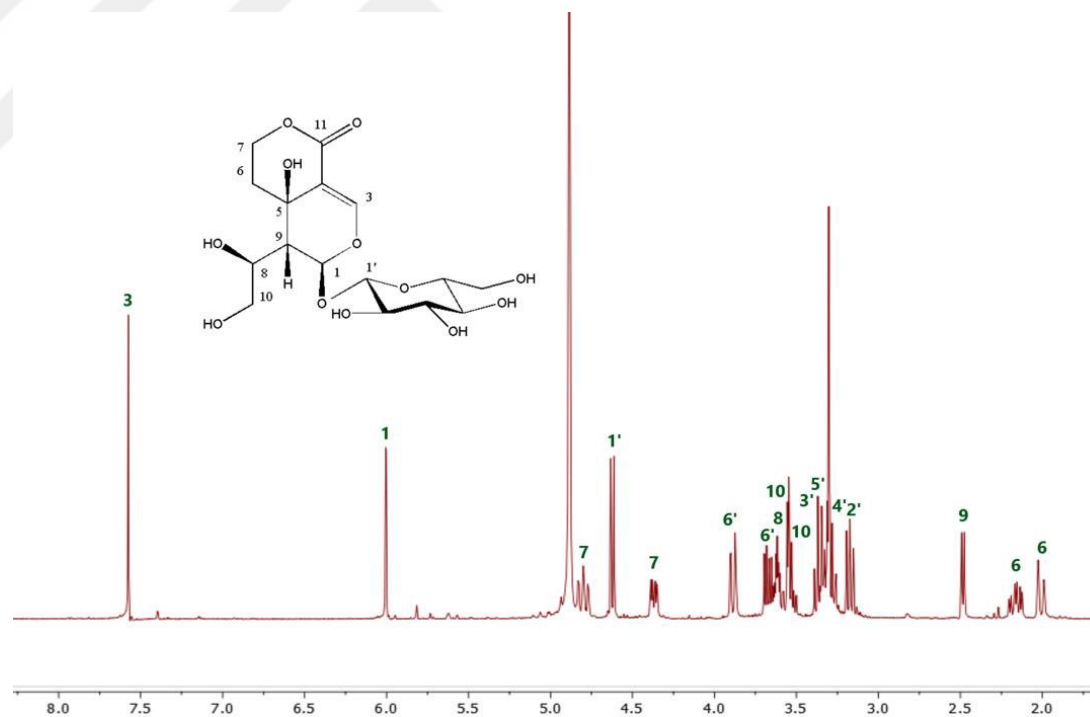
Compound **3** was purified as a colourless amorphous powder. The UV spectrum of **3** displayed absorption maxima at 238 nm, while its IR spectrum exhibited absorption bands of 3333 (OH), 1693 (C=O), and 1617 (C=C) cm^{-1} . In its ^1H NMR spectrum, proton signals of an olefinic ($\delta_{\text{H}} = 7.57$, s, H-3), a hemiacetal ($\delta_{\text{H}} = 6.00$, d, $J = 1.2$ Hz, H-1), two oxymethylenes ($\delta_{\text{H}} = 4.80$ (m), 4.37 (m), H₂-7; $\delta_{\text{H}} = 3.61$ dd ($J = 6.2$ and 3.2) and 3.55 (m) H₂-10), one oxymethine ($\delta_{\text{H}} = 3.62$, m, H-8), one methine ($\delta_{\text{H}} = 2.48$, dd ($J = 6.2$ and 1.2), H-9), and one methylene ($\delta_{\text{H}} = 2.16$, m, and 2.01, br.d, $J = 13.8$, H₂-6) were observed. Furthermore, an anomeric proton signal was detected at $\delta_{\text{H}} = 4.62$ with a coupling constant of 7.9 Hz (Spectrum 12). These NMR findings suggested that **3** had a structure similar to that of swertiamarin-type glycosides (330). The ^1H NMR spectrum of **3** was similar to that of **1**, except for the absence of proton signals of benzoyl moiety and the upfield shift of H₂-10 signals (δ_{H} 3.61 and 3.55) around 1 ppm.

In its ^{13}C NMR spectrum, 16 resonances were detected (Spectrum 13). Six of these signals were arising from a β -glucopyranose unit and the remaining ones were characteristic of a C₁₀-secoiridoid skeleton (328). Furthermore, $^{13}\text{C}/^1\text{H}$ correlations helped us determine the structure as depicted. The attachment site of the glucose unit was determined to be at C-1 with regard to the cross-peak between C-1 (δ_{C} 96.4) and the anomeric signal (δ_{H} 4.62) (Spectrum 17). Moreover, the NOE correlation between H-8 to H-1 and between H-8 to H α -10 (δ_{H} 3.55) positioned them on the same side of the molecule (α) (Spectrum 15) and $J_{9,1}$ (1.2 Hz) and $J_{9,8}$ (6.2 Hz) values of **3** were also observed. The data were superimposable with those reported for eustomorusside (329) in the literature.

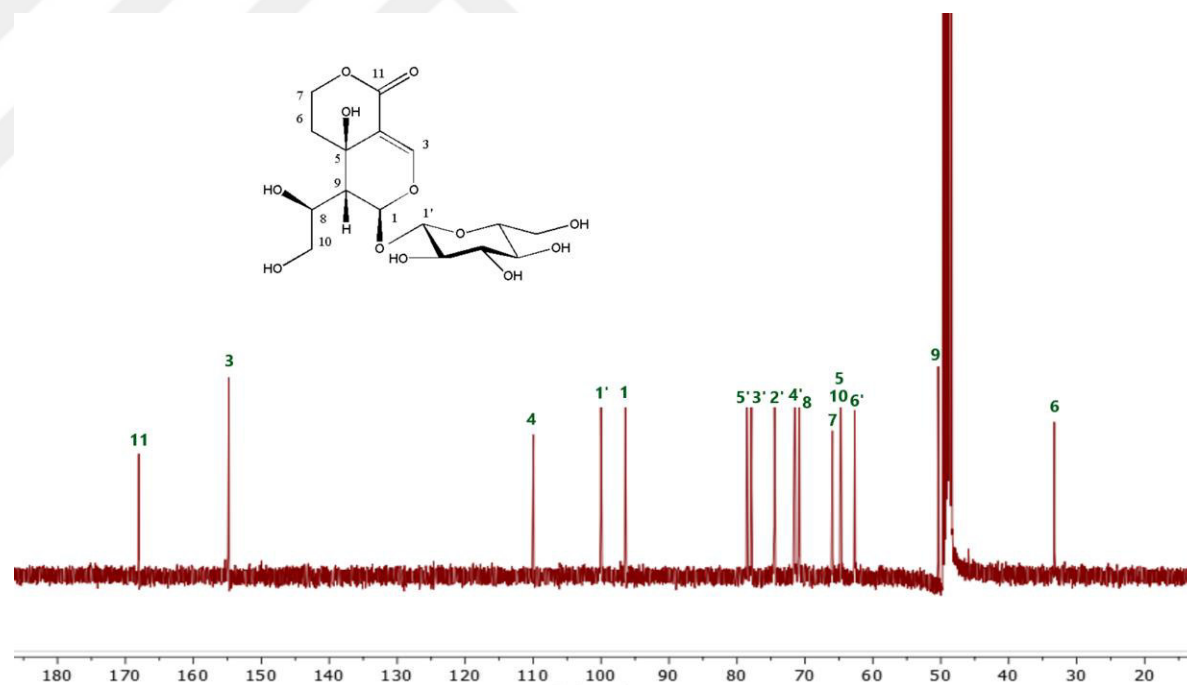
Table 88. ^1H and ^{13}C spectroscopic data of eustomorusside (**3**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	96.4	6.00 d (1.2)
3	CH	154.7	7.57 s
4	C	109.9	-
5	C	64.7	-
6	CH ₂	33.2	2.16 m 2.01 br.d (13.8)
7	CH ₂	65.9	4.80 m 4.37 m
8	CH	70.8	3.62 m
9	CH	50.9	2.48 dd (6.2, 1.2)
10	CH ₂	64.7	3.61 dd (6.2, 3.2) 3.55 m
11	C	168.0	-
<i>Glucose</i>			
1'	CH	100.0	4.62 d (7.9)
2'	CH	74.4	3.17 dd (9.0, 7.9)
3'	CH	77.8	3.37 t (9.0)
4'	CH	71.4	3.29 †
5'	CH	78.5	3.33 †
6'	CH ₂	62.6	3.89 dd (12.0, 2.0) 3.67 dd (12.0, 5.6)

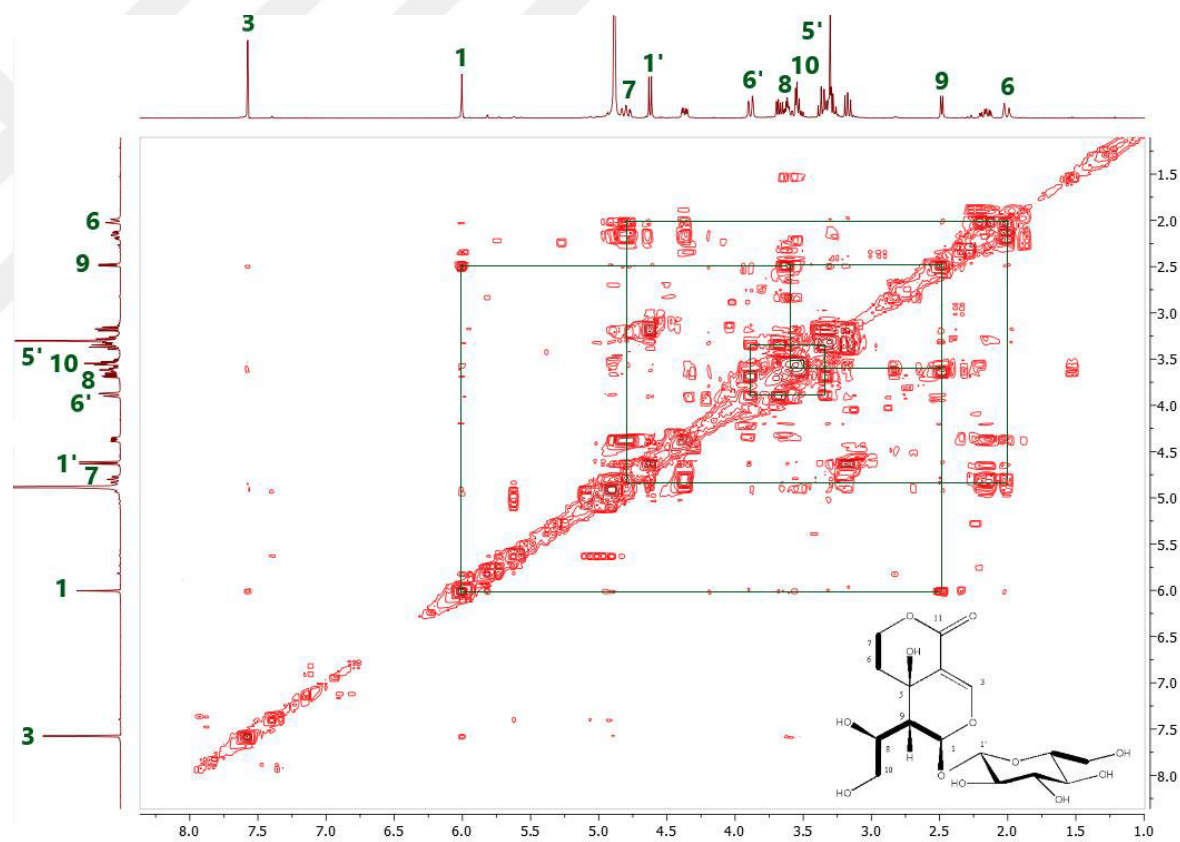
† Signal pattern unclear due to overlapping.



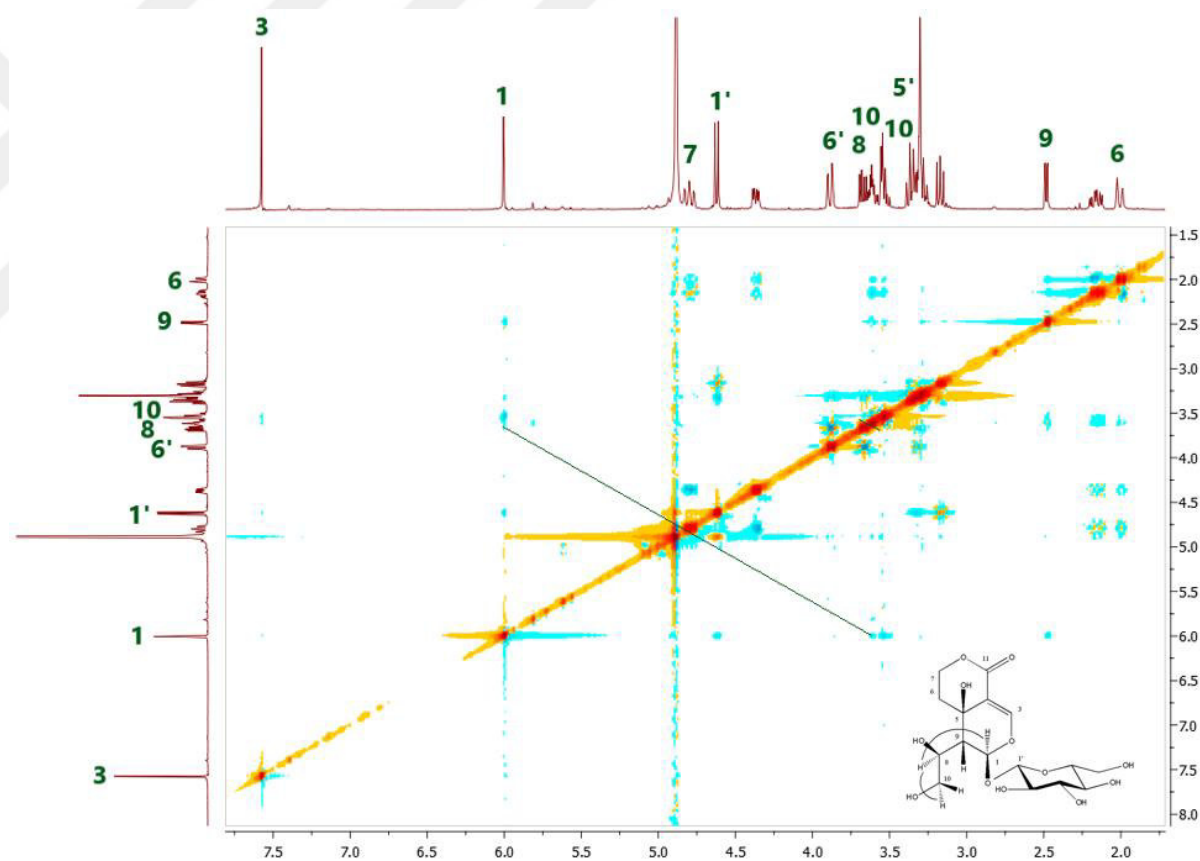
Spectrum 12. ^1H NMR spectrum of eustomorusside (**3**) (CD_3OD , 500 MHz)



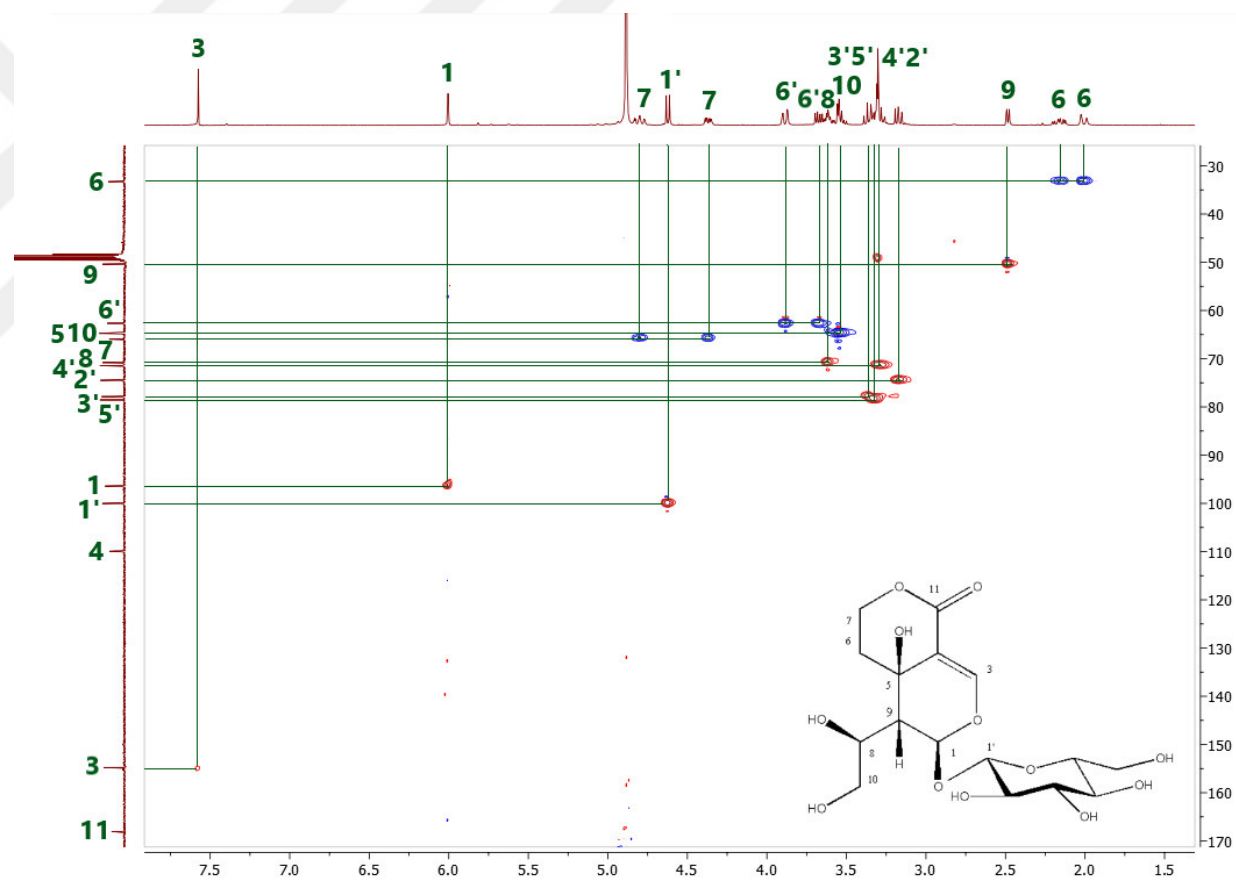
Spectrum 13. ^{13}C NMR spectrum of eustomorusside (3) (CD_3OD , 125 MHz)



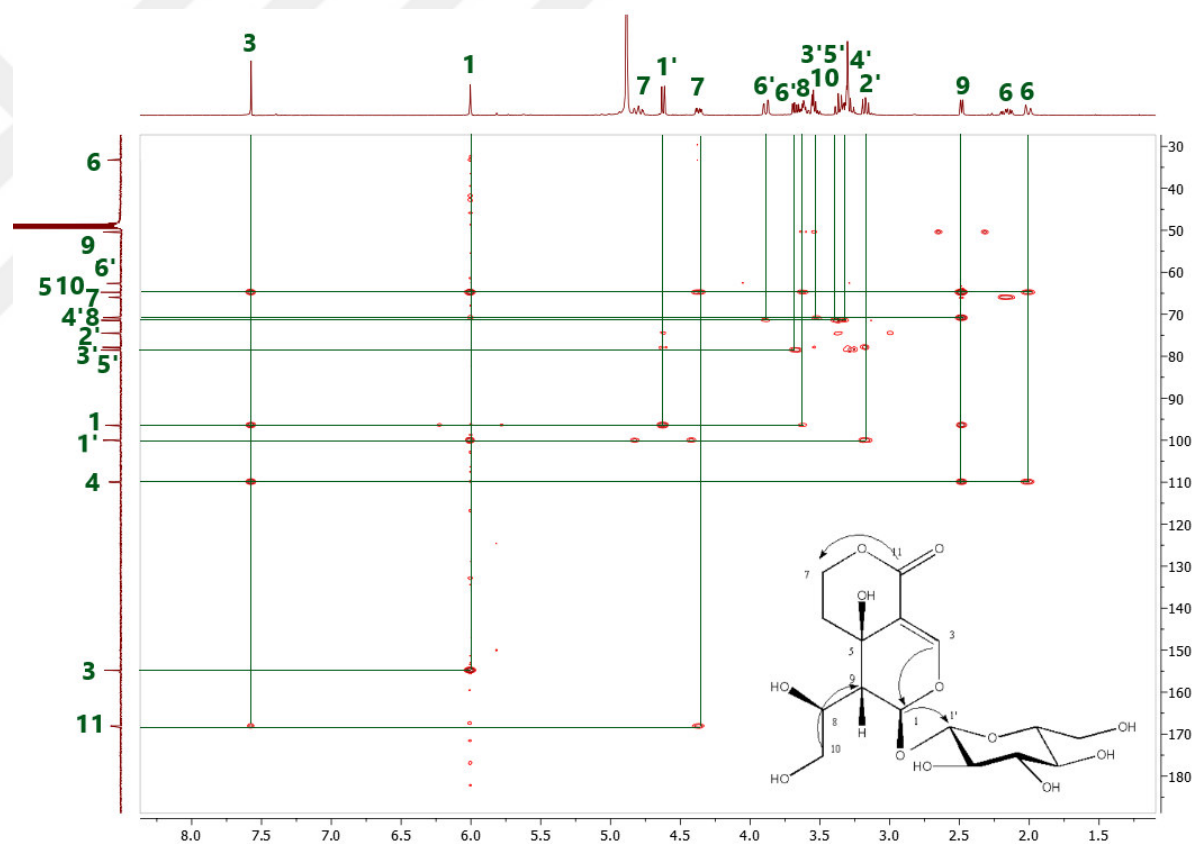
Spectrum 14. COSY spectrum of eustomorusside (3)



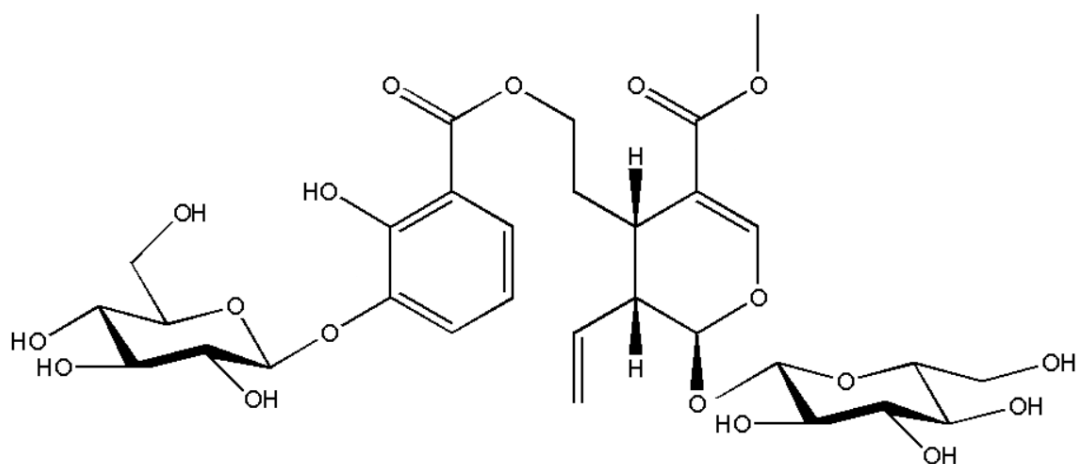
Spectrum 15. NOESY spectrum of eustomorusside (**3**)



Spectrum 16. HSQC spectrum of eustomorusside (**3**)



Spectrum 17. HMBC spectrum of eustomorusside (**3**)



DEPRESSINE (4), C₃₀H₄₀O₁₈ (MW: 688.63)

UV λ_{max} (MeOH) nm:	210, 240, 313
IR ν_{max} (KBr) cm^{-1} :	3357 (OH), 1702 (C=O), 1628 (C=C), 1468 (aromatic ring)
^1H NMR:	Spectrum 18, Table 89
^{13}C NMR:	Spectrum 19, Table 89
COSY:	Spectrum 20
HSQC:	Spectrum 21
HMBC:	Spectrum 22

DEPRESSINE (4)

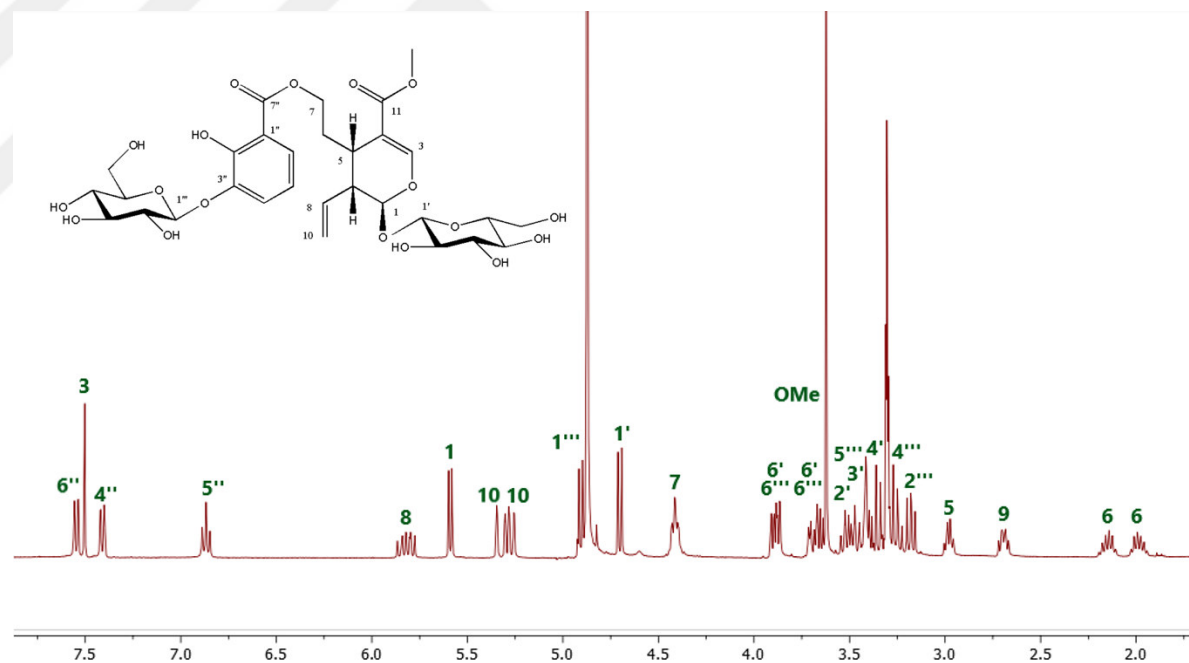
Compound **4** was purified from the *n*-BuOH subextract of *G. asclepiadea* as a white amorphous powder. ESI-MS of **4** exhibited (M+H)⁺ ion at *m/z* 688.27. UV spectrum of **4** exhibited bands at 210, 240, and 313 nm. Its IR spectrum displayed absorption bands at 3357 (OH), 1702 (C=O), 1628 (C=C), and 1468 (aromatic ring) cm⁻¹.

¹H and ¹³C NMR spectra of **4** revealed two β-glucopyranosyl units, one 1,2,3-trisubstituted benzene ring, and a secoiridoid moiety. Anomeric signals of sugar moieties were observed at (δ_H 4.70, d, *J* = 7.9), (δ_C 98.7; H-1'), (δ_H 4.91, d, *J* = 7.5) and (δ_C 101.6; H-1'') (Spectra 18-19). The signals related to the terpenoid part were ascribed to a secaloganol-like structure, except esterification on C-7 led to a shift of peak to the downfield in both proton and carbon NMR spectra. The COSY spectrum contained four spin systems: two of them belonging to sugar moieties, one of them between H-4'' to H-6'', the other between H-7 to H-10 (Spectrum 20). The protons were matched with the corresponding carbon resonances by the HSQC experiment, and connections between the subunits were unambiguously determined by the HMBC experiment (Spectrum 21-22). Long-range correlations from δ_C 167.7 to δ_H 7.50 (C-11 to H-3), δ_C 96.2 to δ_H 4.70 (C-1 to H-1'), and δ_C 145.7 to δ_H 4.91 to (C-3'' to H-1'') elicited the position of substitutions. The data were superimposable with those reported for depressine (331). It is reported for the third time from the genus *Gentiana*.

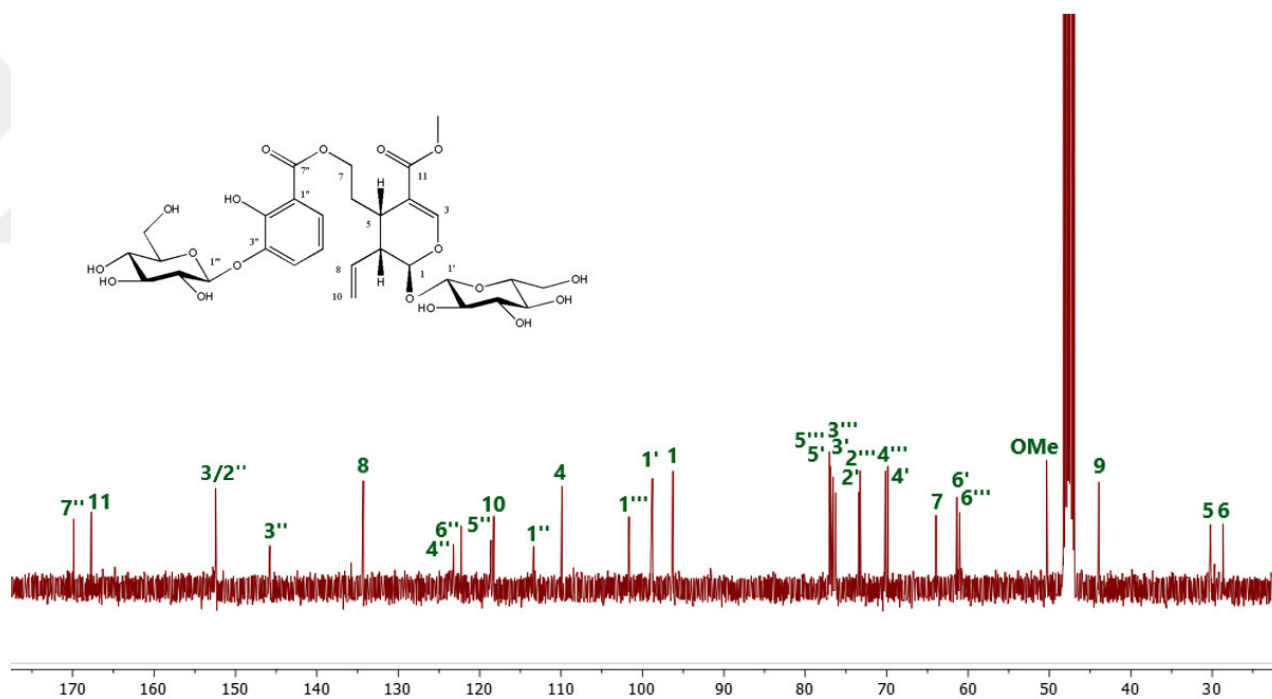
Table 89. ^1H and ^{13}C spectroscopic data of depressine (**4**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	96.2	5.59 d (6.6)
3	CH	152.7	7.50 d (0.6)
4	C	109.9	-
5	CH	30.2	2.98 dd (12.5, 6.5)
6	CH ₂	28.8	2.14 dd (14.0, 7.0) 1.98 dd (14.0, 7.0)
7	CH ₂	63.9	4.41 m
8	CH	134.3	5.82 ddd (17.0, 10.5, 8.5)
9	CH	43.9	2.69 dt (14.0, 8.3, 5.5)
10	CH ₂	118.4	5.32 dt (17.4, 1.3) 5.27 dd (10.3, 1.3)
11	C	167.7	-
OMe	CH ₃	50.3	3.62 s
<i>Glucose</i>			
1'	CH	98.7	4.70 d (7.9)
2'	CH	73.4	3.51 d (7.5)
3'	CH	76.3	3.48 d (8.1)
4'	CH	69.8	3.41 br.s
5'	CH	76.8	3.28-3.33 †
6'	CH ₂	61.3	3.89 dd (11.9, 2.0) 3.68 dd (11.9, 4.8)
<i>Benzoic acid</i>			
1''	C	113.3	-
2''	C	152.4	-
3''	C	145.7	-
4''	CH	123.2	7.55 dd (8.1, 1.5)
5''	CH	118.6	6.87 t (8.1)
6''	CH	122.2	7.41 dd (8.1, 1.5)
7''	C	169.8	-
<i>Glucose</i>			
1'''	CH	101.6	4.91 d (7.5)
2'''	CH	73.1	3.18 dd (9.1, 7.9)
3'''	CH	76.5	3.28-3.33 †
4'''	CH	70.1	3.25 t (9.2)
5'''	CH	77.0	3.42 m
6'''	CH ₂	61.0	3.89 dd (11.9, 2.0) 3.66 dd (11.9, 6.0)

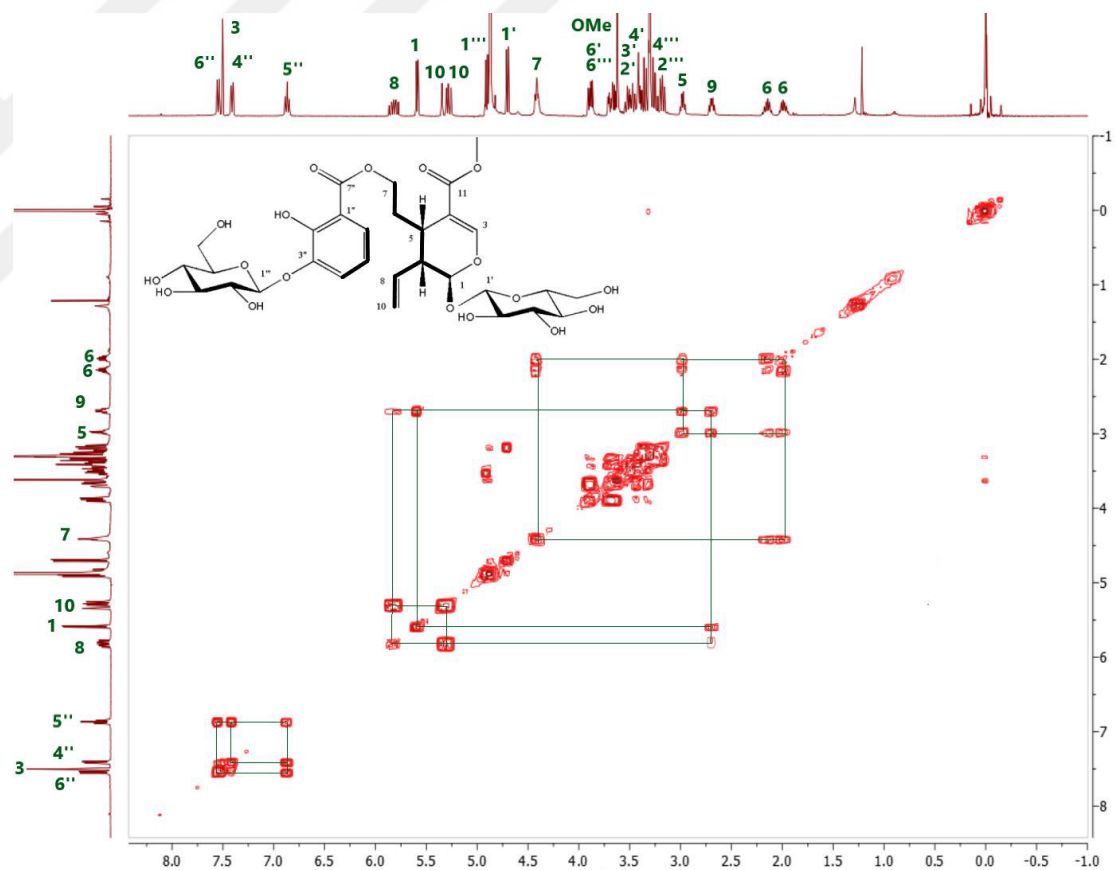
† Overlapping signals



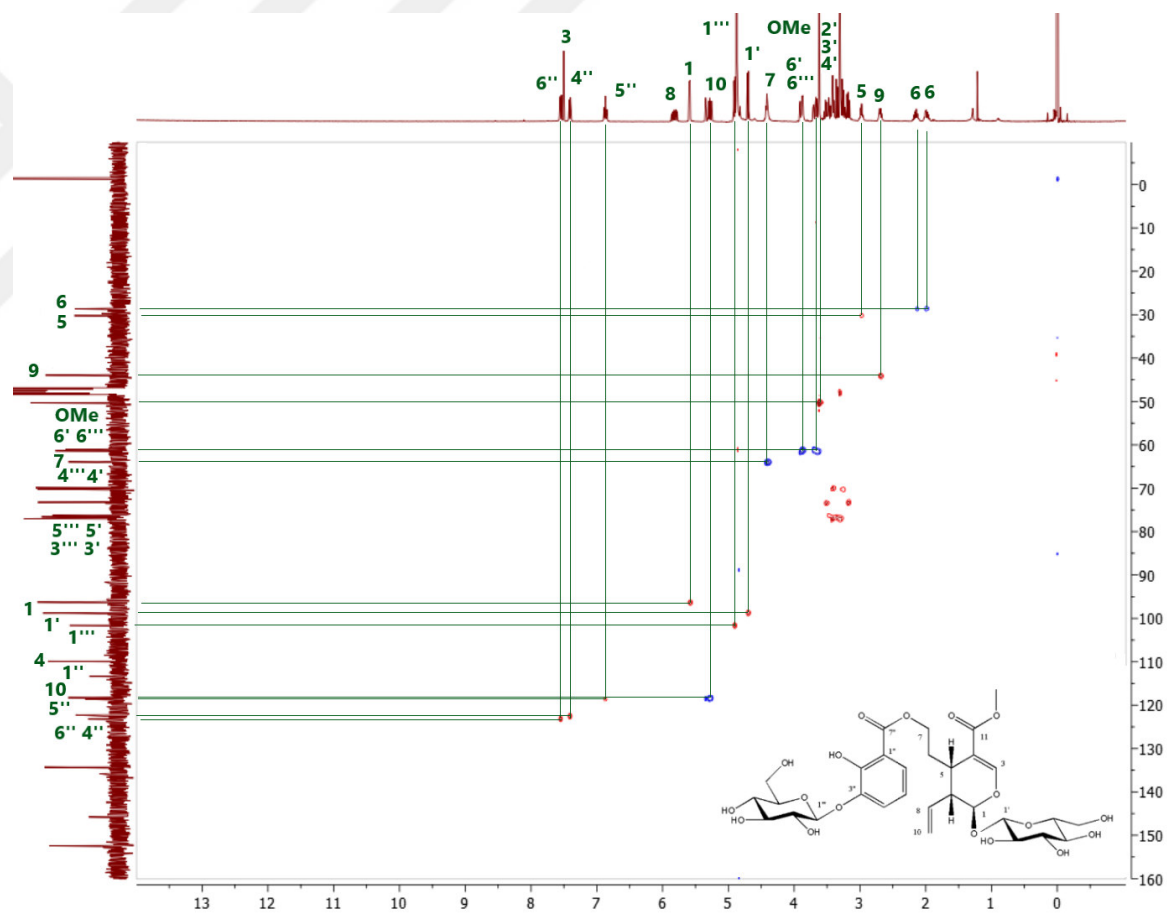
Spectrum 18. ^1H NMR spectrum of depressine (**4**) (CD_3OD , 500 MHz)



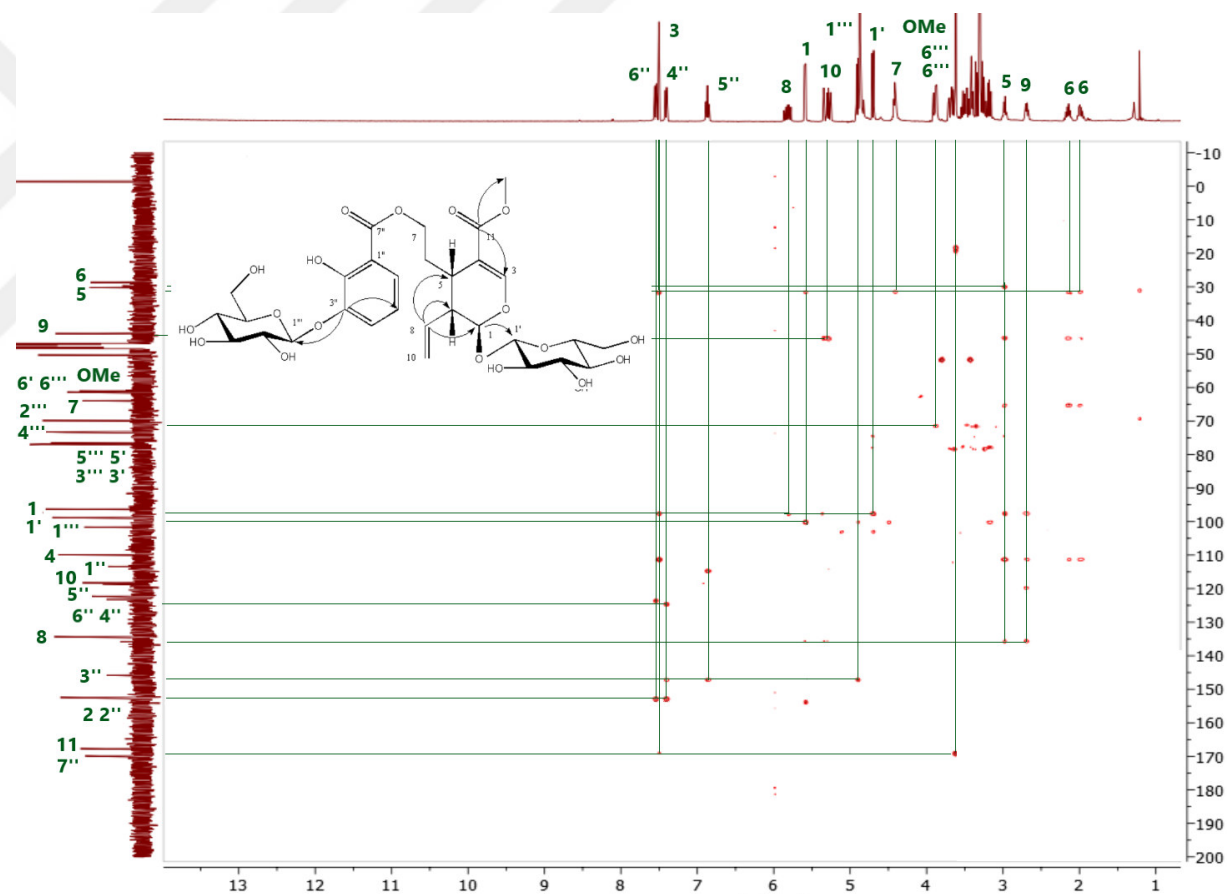
Spectrum 19. ^{13}C NMR spectrum of depressine (**4**) (CD_3OD , 125 MHz)



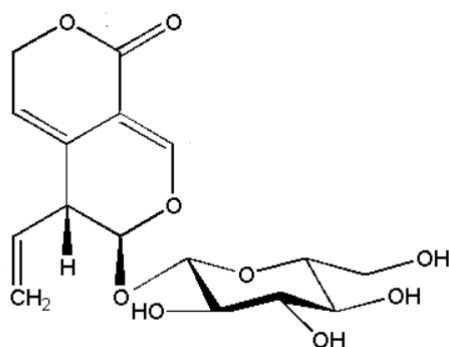
Spectrum 20. COSY spectrum of depressine (4)



Spectrum 21. HSQC spectrum of depressine (4)



Spectrum 22. HMBC spectrum of depressine



GENTIOPICROSIDE (5), C₁₆H₂₀O₉ (MW: 356.32)

UV λ_{max} (MeOH) nm:	253, 271
IR ν_{max} (KBr) cm ⁻¹ :	3412 (OH), 1703 (C=O), 1609 (C=C)
¹ H NMR:	Spectrum 23, Table 90
¹³ C NMR:	Spectrum 24, Table 90
COSY:	Spectrum 25
HSQC:	Spectrum 26
HMBC:	Spectrum 27
HR-ESI-MS:	Spectrum 28

GENTIOPICROSIDE (5)

Compound **5** was isolated from EtOAc subextracts of both *G. asclepiadea* and *G. cruciata* as a white amorphous powder. The HRI-ESI-MS of **5** exhibited (M+H)⁺ ion at m/z 357.1185 (calc. for C₁₆H₂₁O₉, 357.1186). The UV spectrum of **5** exhibited absorption maxima at 253 and 271, while its IR spectrum displayed absorption bands at 3412 (OH), 1703 (C=O), and 1609 (C=C) cm⁻¹.

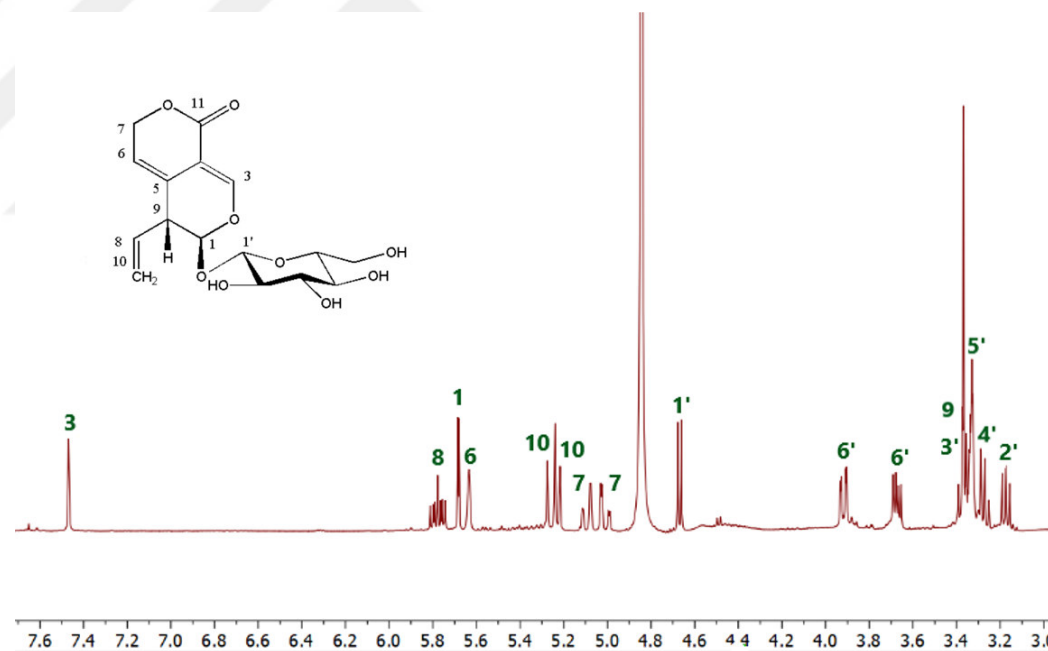
The ¹H NMR spectrum of **5** contained signals of vinyl protons (δ_H 5.78, ddd, J = 17.2, 10.3, 6.8 Hz, H-8; 5.27, dd, J = 17.0, 1.5 Hz, H-10a; 5.23 dd J = 10.5, 1.5 Hz, H-10b), one olefinic (δ_H 7.47, d, J = 1.5, H-3), one hemiacetal (δ_H 5.68, d, J = 2.9, H-1), one oxymethylene (δ_H 5.10, dd, J = 17.6, 1.4, H-7a; 5.01, dd, J = 17.6, 3.3, H-7b), and one methine signal (3.33, m, H-9). Moreover, an anomeric signal was observed at δ_H 4.67 with a large couple constant (J = 7.9). Taken together, these above signals were characteristic of a monoglycosidic secoiridoid (Spectrum 23).

In its ¹³C-JMOD NMR spectrum, 16 carbon signals were detected, six of them implying a β -glucopyranose unit including the anomeric signal at δ_C 98.8 (Spectrum 24). The remaining signals were ascribed to a C₉-secoiridoid skeleton with the help of 2D NMR experiments. The COSY spectrum contained three spin systems; one between H-10 and H-1, the other between H-6 and H₂-7, and also between H-1' and H₂-6' of glucose (Spectrum 25). HMBC spectrum helped us to determine the long-range correlations. Thus, cross-peak between C-11 and H₂-7 led to the presence of a lactone moiety in the structure. The location of the glucose unit was determined to be at C-1 by the HMBC long-range correlations between C-1 (δ_C 97.1) and H-1' (δ_H 4.67) (Spectrum 27). All these data led to the characterization of compound **5** as gentiopicroside (224).

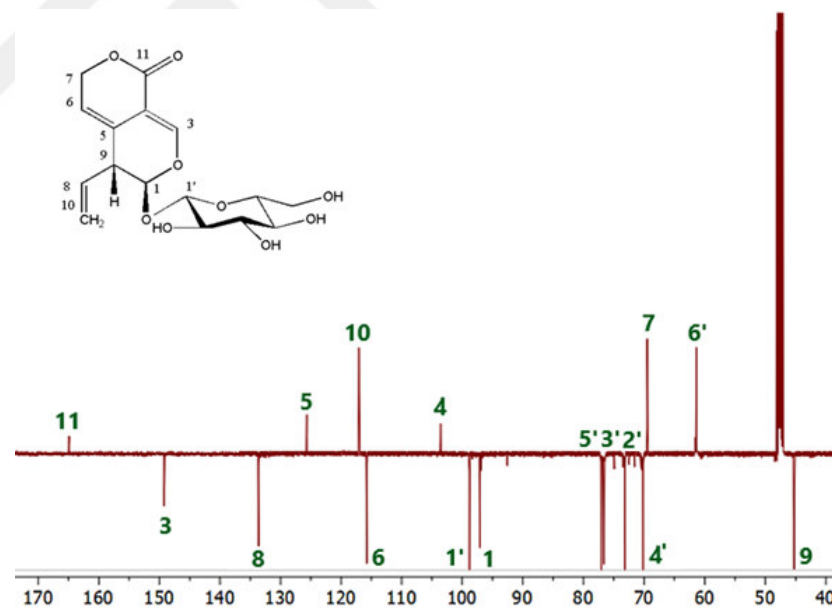
Table 90. ^1H and ^{13}C spectroscopic data of gentiopicroside (**5**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	97.1	5.68 d (2.9)
3	CH	149.2	7.47 d (1.5)
4	C	103.5	-
5	C	125.6	-
6	CH	115.7	5.63 m
7	CH ₂	69.4	5.10 dd (17.6, 1.4) 5.01 dd (17.6, 3.3)
8	CH	133.5	5.78 ddd (17.2, 10.3, 6.8)
9	CH	45.3	3.33 m
10	CH ₂	117.1	5.27 dd (17.0, 1.5) 5.23 dd (10.5, 1.5)
11	C	164.9	-
<i>Glucose</i>			
1'	CH	98.8	4.67 d (7.9)
2'	CH	73.2	3.17 t (8.5)
3'	CH	76.6	3.37 †
4'	CH	70.2	3.27 t (9.2)
5'	CH	77.2	3.34 †
6'	CH ₂	61.3	3.92 dd (11.9, 2.0) 3.67 dd (11.9, 6.1)

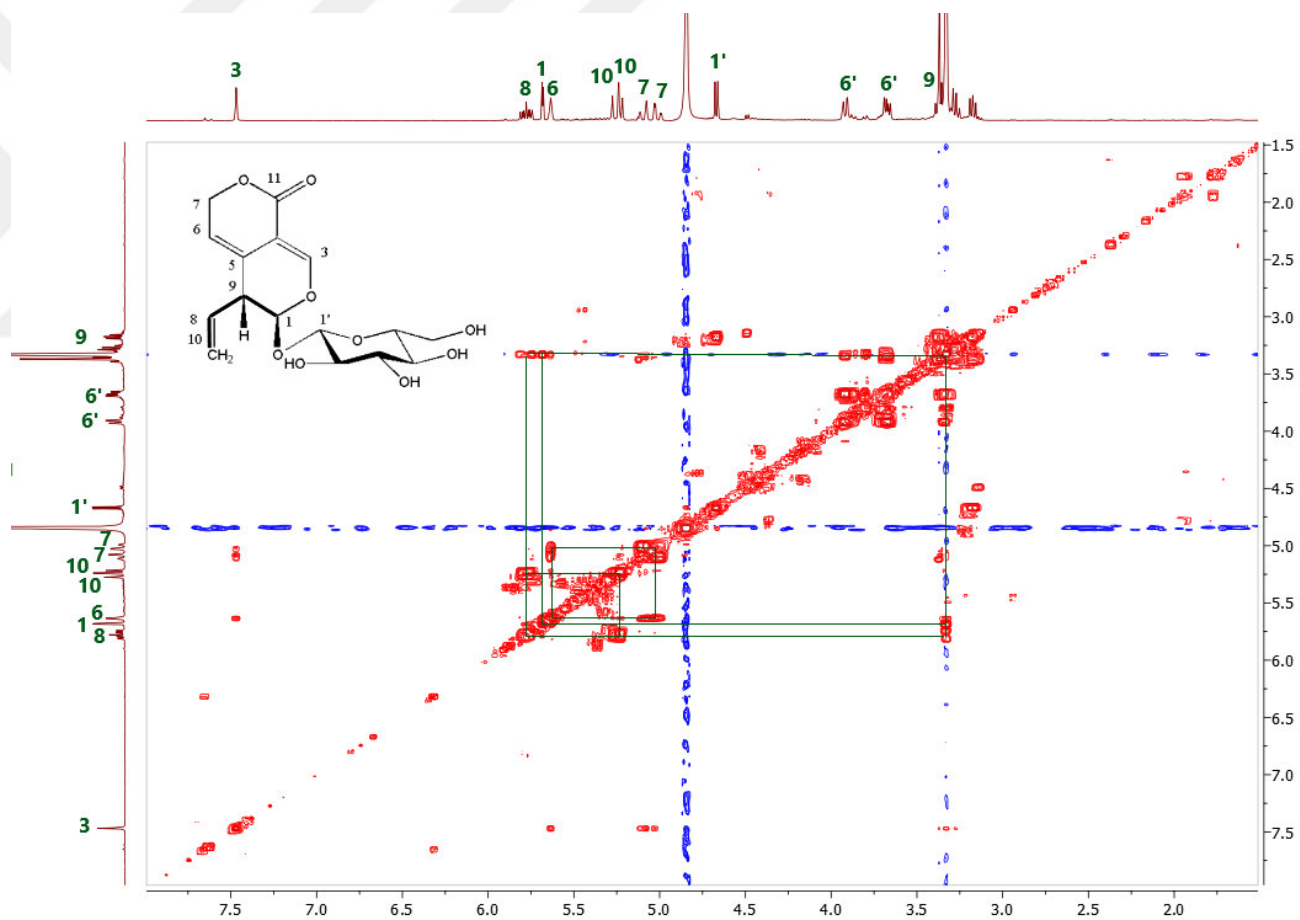
† Signal pattern unclear due to overlapping



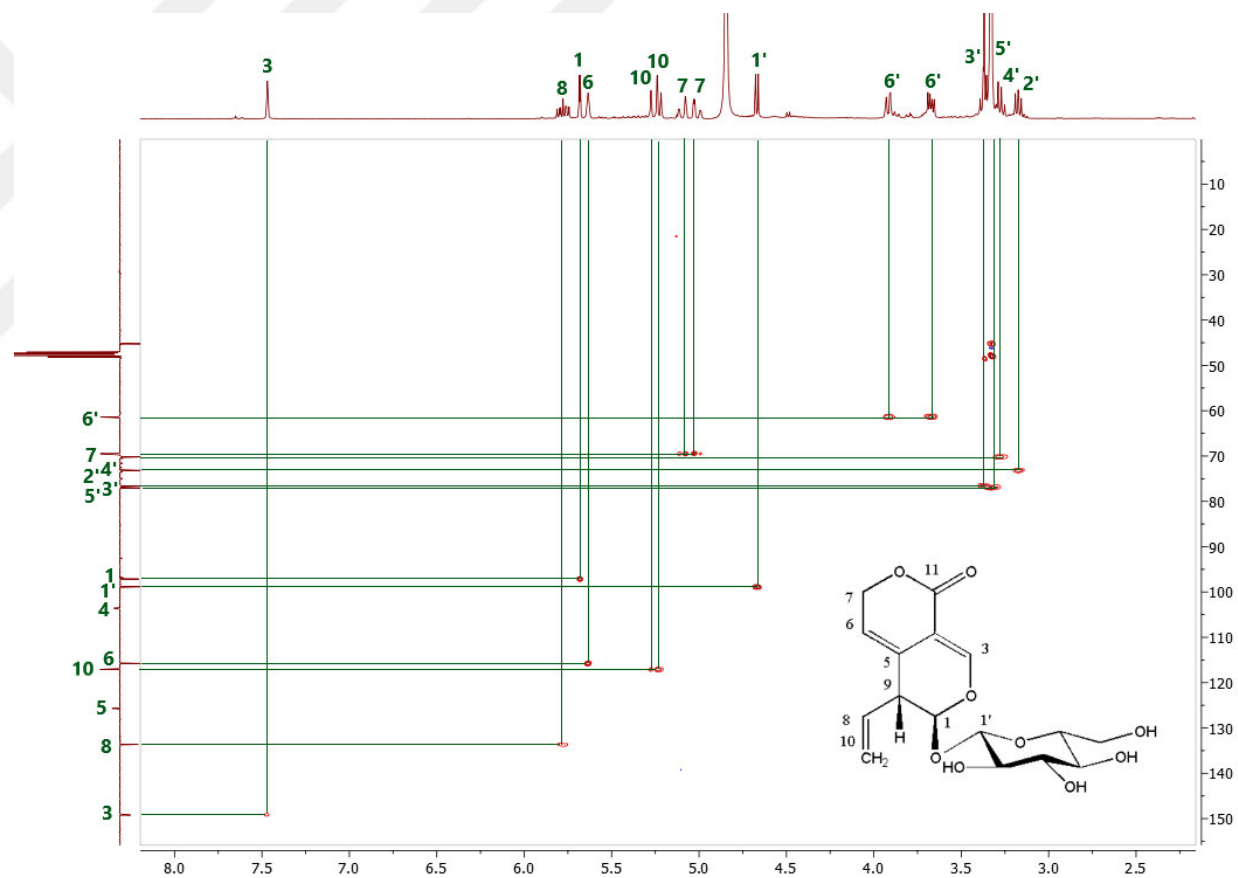
Spectrum 23. ^1H NMR spectrum of gentiopicroside (**5**) (CD_3OD , 500 MHz)



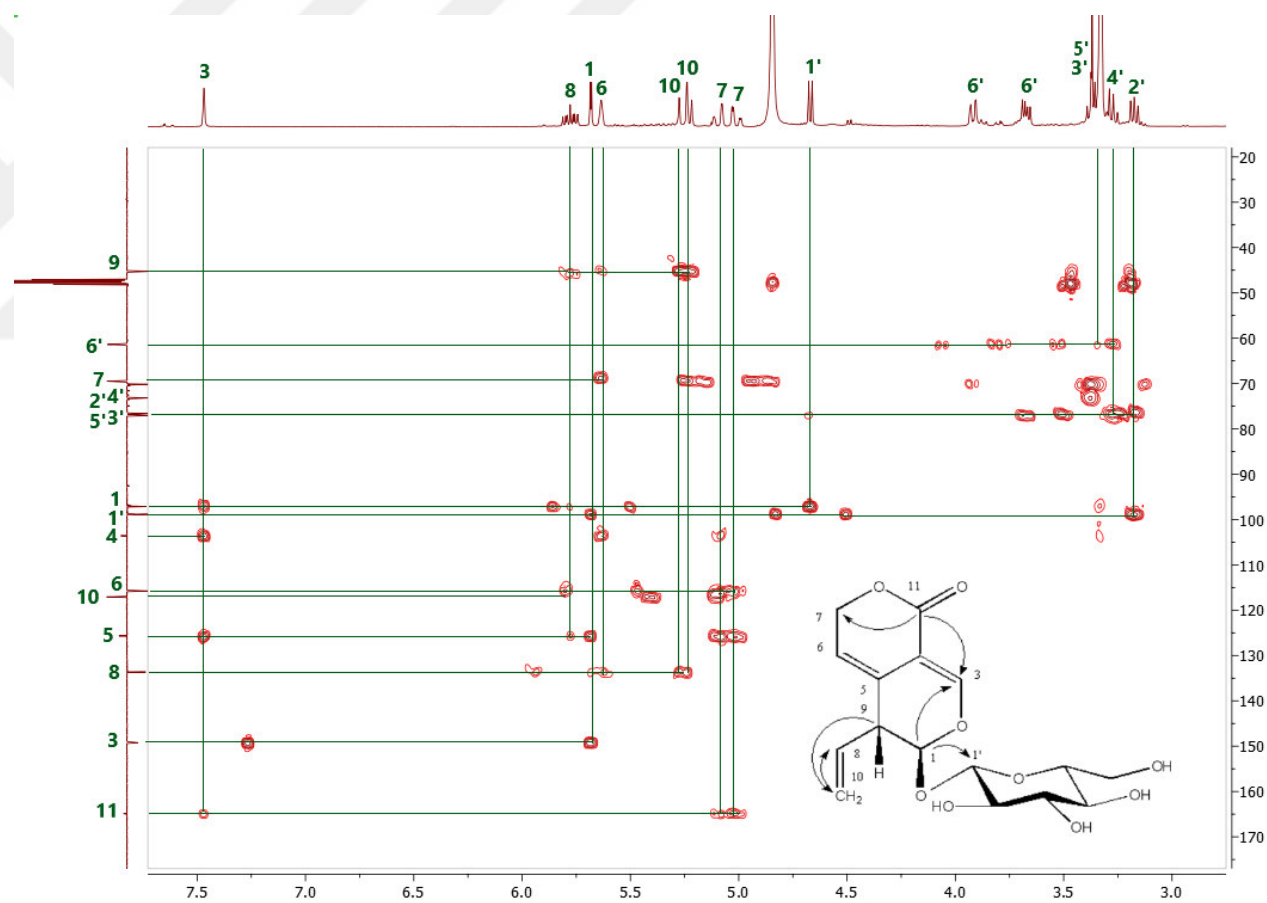
Spectrum 24. ^{13}C -JMOD NMR spectrum of gentiopicroside (**5**) (CD_3OD , 125 MHz)



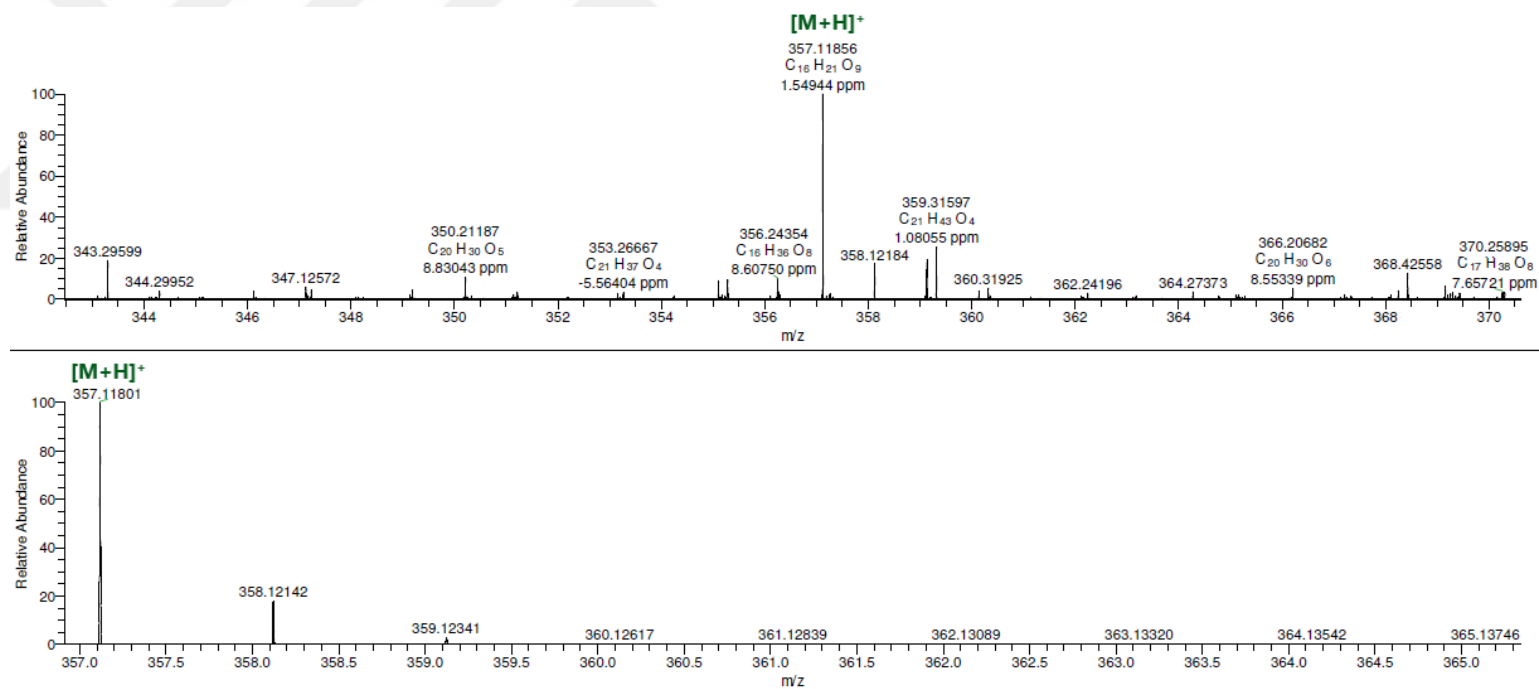
Spectrum 25. COSY spectrum of gentiopicroside (5)



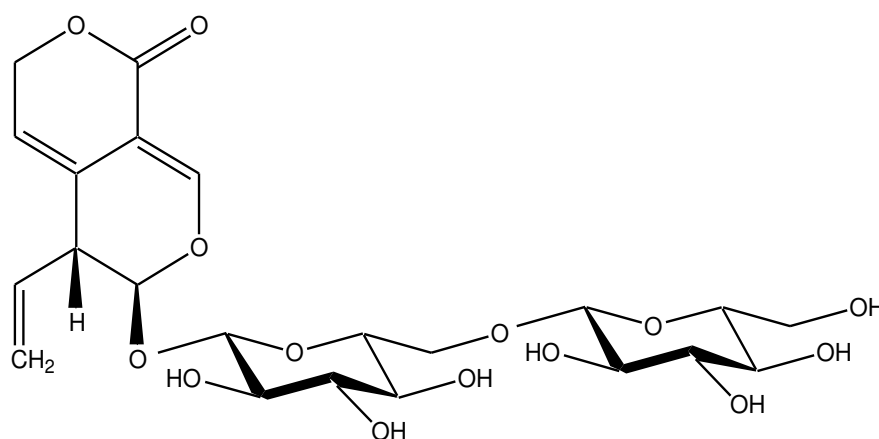
Spectrum 26. HSQC spectrum of gentiopicroside (**5**)



Spectrum 27. HMBC spectrum of gentiopicroside (5)



Spectrum 28. HR-ESI-MS spectrum of gentiopicroside (**5**)



6'-O- β -D-GLUCOSYL GENTIOPICROSIDE (6), C₂₂H₃₁O₁₄ (MW: 519.47)

UV λ_{max} (MeOH) nm:	231, 270
IR ν_{max} (KBr) cm ⁻¹ :	3668 (OH), 1705 (C=O), 1607 (C=C)
¹ H NMR:	Spectrum 29, Table 91
¹³ C NMR:	Spectrum 30, Table 91
COSY:	Spectrum 31
HSQC:	Spectrum 32
HMBC:	Spectrum 33

6'-O- β -D-GLUCOSYL GENTIOPICROSIDE (6)

Compound **6** was isolated from the *n*-BuOH subextract of *G. cruciata* as a white amorphous powder. Its UV spectrum showed bands at 231 and 270, while its IR spectrum exhibited absorption bands at 3668 (OH), 1705 (C=O), and 1607 (C=C) cm⁻¹.

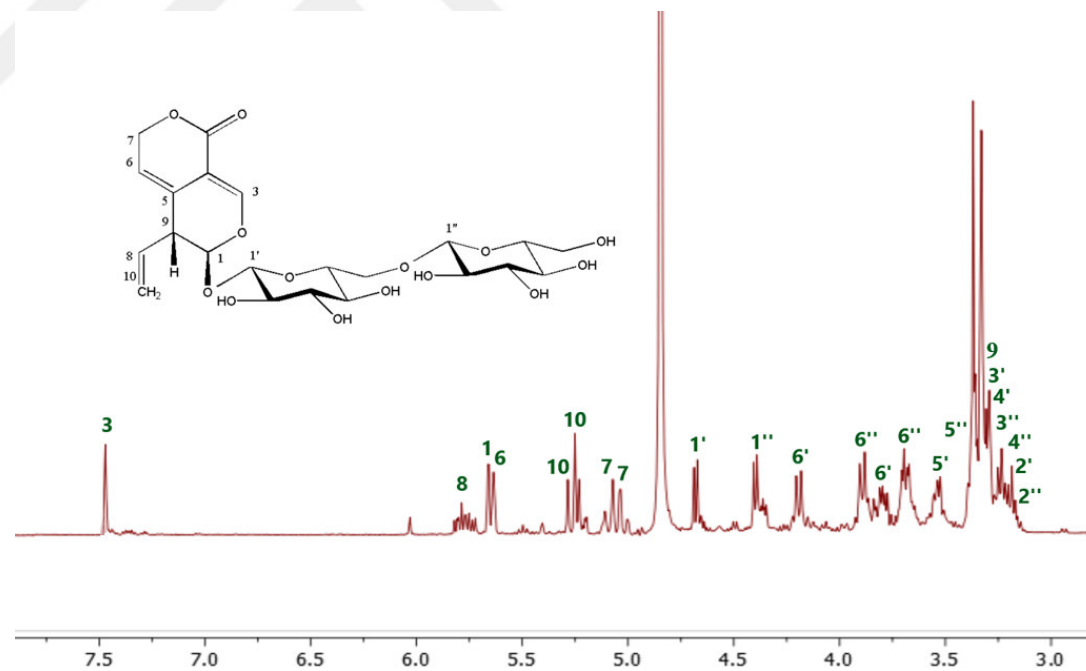
In the ¹H NMR spectrum of compound **6**, signals of vinyl protons (δ_{H} 5.79, ddd, $J = 17.3, 10.5, 6.7$, H-8; 5.28, br.s, H-10a; 5.24 d, $J = 10.0$, H-10b) were observed. Furthermore, an anomeric signal was observed at δ_{H} 4.68 with a large couple constant ($J = 8.0$). Besides, the H-6 signal was detected at the downfield (δ_{H} 5.64), indicating a double bond between C-5 and C-6. Also, H₂-7 signals imply the presence of a single proton at the C-6 (Spectrum 29). According to the above data, compound **6** showed structural similarity with gentiopicroside. However, an additional anomeric proton signal at δ_{H} 4.40 ($J = 7.6$) indicated the diglycosidic structure of **6**.

In its ¹³C-JMOD NMR spectrum, 22 carbon signals were detected and 16 of them were matched with those of gentiopicroside (Spectrum 30). Additional six signals in the spectrum were attributed to an additional β -glucosyl moiety in compound **6**. The attachment site of the secondary glycosidic unit was deduced with the help of 2D NMR experiments. ¹³C-¹H long-range correlations between δ_{C} 68.6 and δ_{H} 4.40 indicated the C-6' to H-1'' linkage between the two glucose units (Spectrum 33). The NMR findings of **6** were found to be superimposable with those of 6'-O- β -D-glucosyl gentiopicroside (224).

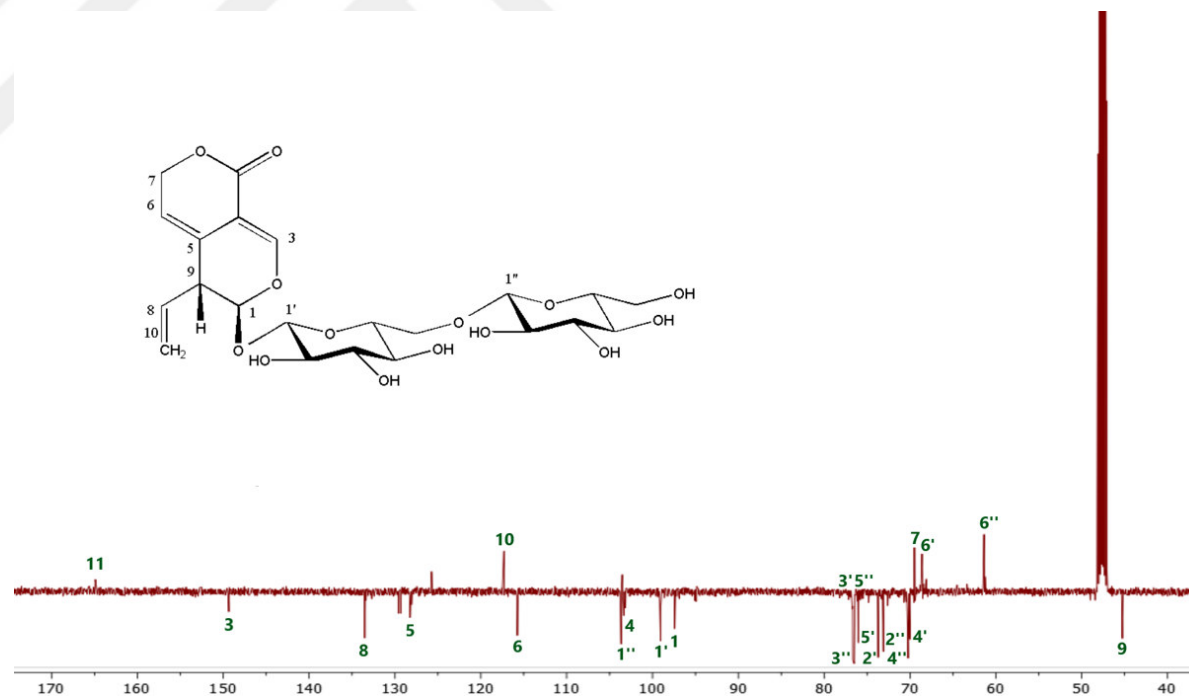
Table 91. ^1H and ^{13}C spectroscopic data of 6'-O- β -D-glucosyl gentiopicroside (**6**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	97.4	5.66 d (3.2)
3	CH	149.3	7.47 s
4	C	103.7	-
5	C	129.7	-
6	CH	115.8	5.64 m
7	CH ₂	69.5	5.09 d (17.5) 5.03 dd (17.5, 2.5)
8	CH	133.5	5.79 ddd (17.3, 10.5, 6.7)
9	CH	45.3	3.30 d (7.7)
10	CH ₂	117.3	5.28 br.s 5.24 d (10.0)
11	C	164.9	-
<i>Glucose</i>			
1'	CH	99.14	4.68 d (8.0)
2'	CH	73.7	3.23 t (8.3)
3'	CH	76.6	3.28-3.38 †
4'	CH	70.1	3.28-3.38 †
5'	CH	76.0	3.53 m
6'	CH ₂	68.6	4.19 d (11.5) 3.79 dd (11.5, 6.5)
<i>Glucose</i>			
1''	CH	103.5	4.40 d (7.6)
2''	CH	73.1	3.19 t (8.1)
3''	CH	76.7	3.28-3.38 †
4''	CH	70.2	3.28-3.38 †
5''	CH	76.5	3.28-3.38 †
6''	CH ₂	61.3	3.89 d (12) 3.69 dd (12, 4.8)

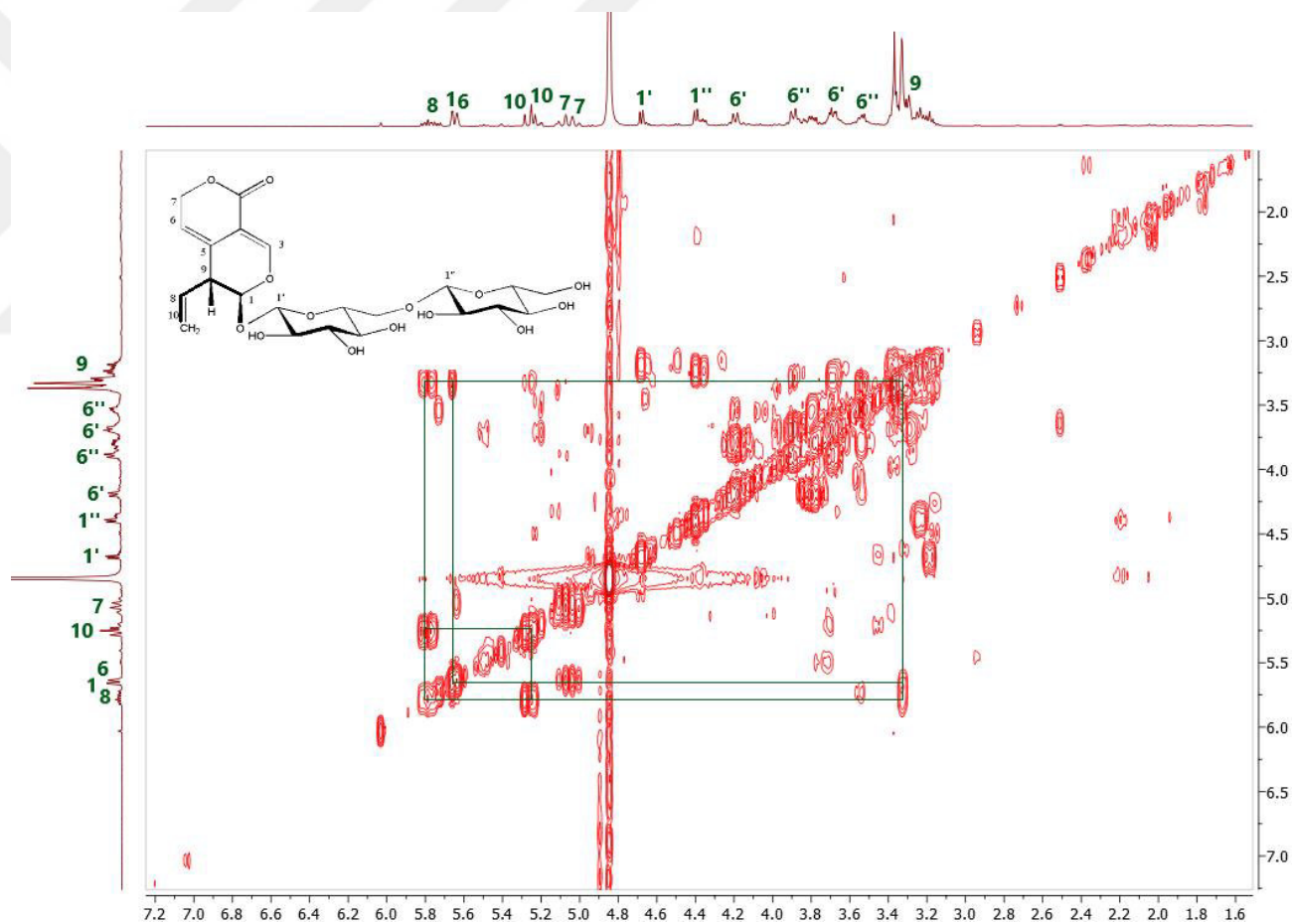
† Overlapping signals.



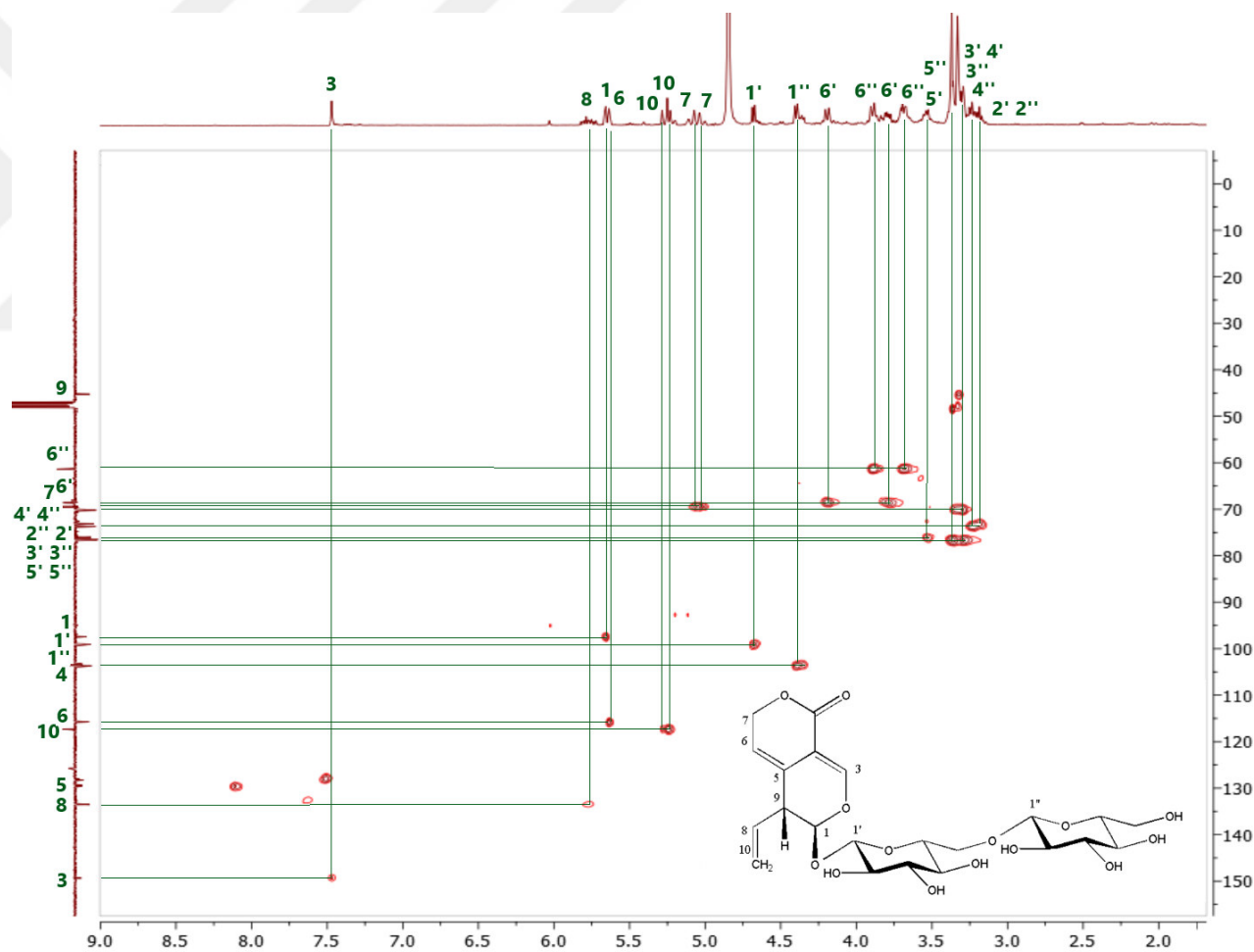
Spectrum 29. ^1H NMR spectrum of 6'-O- β -D-glucosyl gentiopicroside (**6**) (CD_3OD , 500 MHz)



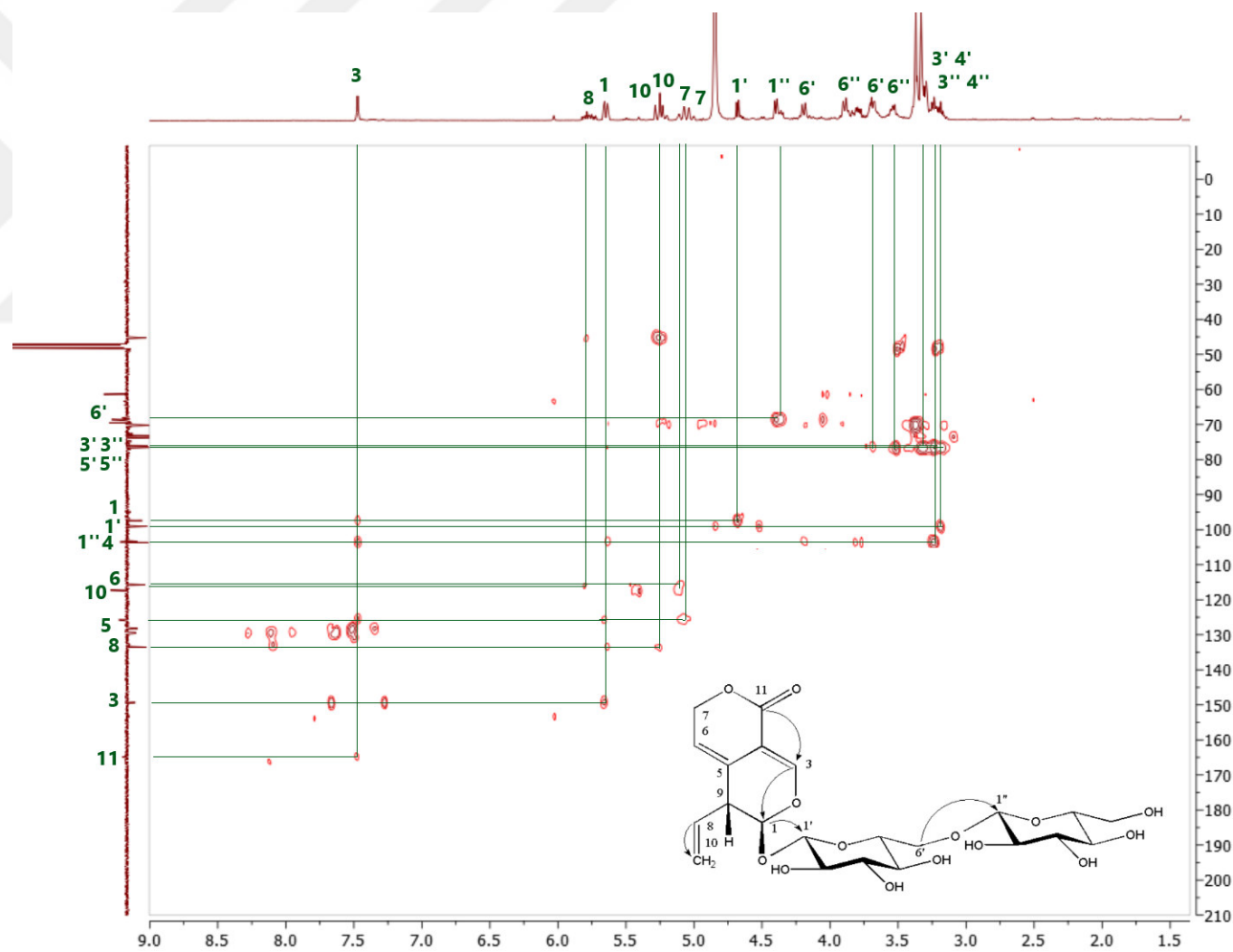
Spectrum 30. ^{13}C -JMOD NMR spectrum of 6'-O-β-D-glucosyl gentiopicroside (**6**) (CD_3OD , 125 MHz)



Spectrum 31. COSY spectrum of 6'-O- β -D-glucosyl gentiopicroside (6)

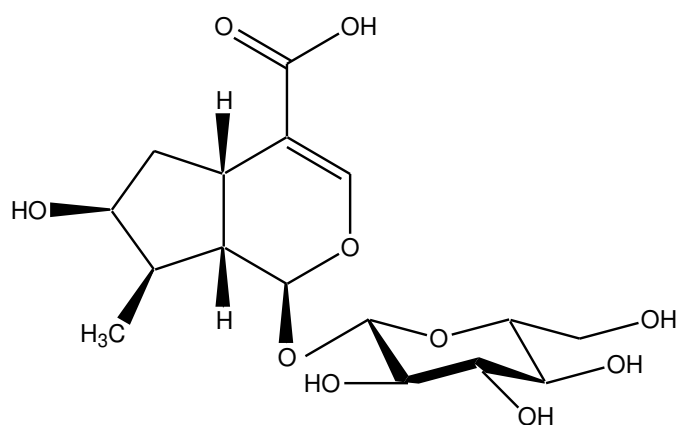


Spectrum 32. HSQC spectrum of 6'-O-β-D-glucosyl gentiopicroside (6)



Spectrum 33. HMBC spectrum of 6'-*O*- β -D-glucosyl gentiopicroside (**6**)

4.2.2. Iridoid



LOGANIC ACID (7), C₁₆H₂₄O₁₀ (MW: 376.35)

UV λ_{max} (MeOH) nm:	232
IR ν_{max} (KBr) cm ⁻¹ :	3330 (OH), 1702 (C=O), 1646 (conjugated enol ether, C=C)
¹ H NMR:	Spectrum 34, Table 92
¹³ C NMR:	Spectrum 35, Table 92
COSY:	Spectrum 36
HSQC:	Spectrum 37
HMBC:	Spectrum 38
HR-ESI-MS:	Spectrum 39

LOGANIC ACID (7)

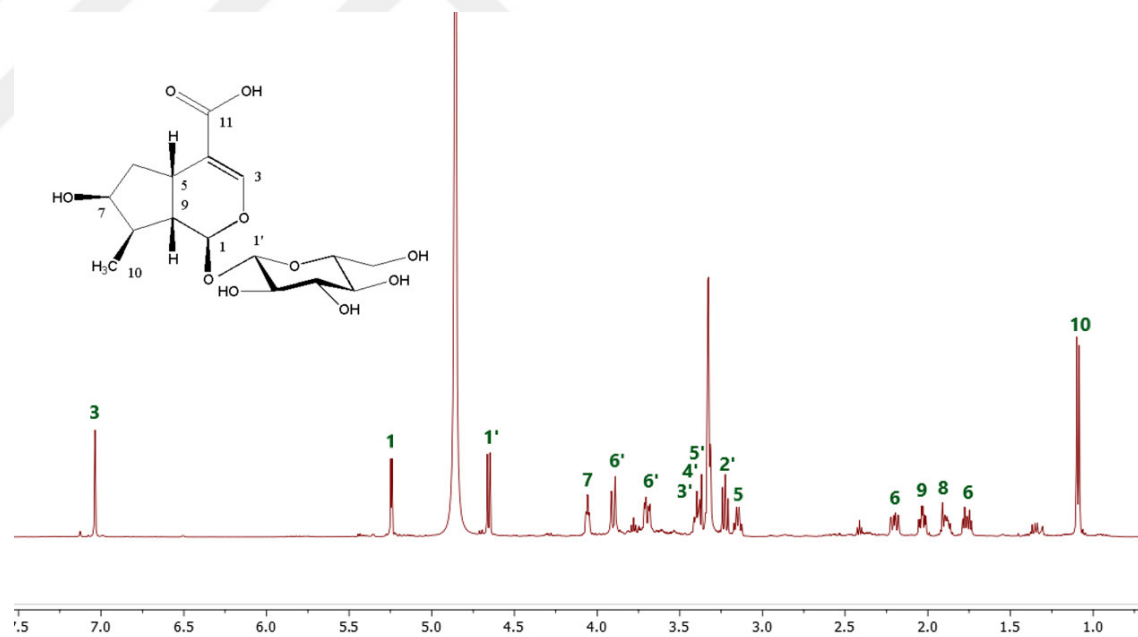
Compound **7** was isolated from *n*-BuOH subextracts of *G. asclepiadea* and *G. cruciata* as a white amorphous powder. Based on the ion peaks at m/z 377.1444 ($M+H$)⁺ (calcd for C₁₆H₂₅O₁₀, 377.1448) and at m/z 375.1296 ($M-H$)⁻ (calcd for C₁₆H₂₃O₁₀, 375.1291), the molecular formula was elucidated as C₁₆H₂₄O₁₀. UV spectrum of **7** contained a maximum at 232 nm characteristic for a C-4 substituted iridoid. Its IR spectrum showed bands at 3330 (OH), 1702 (C=O) and 1646 (C=C).

In the ¹H NMR and ¹³C NMR spectra of compound **7**, the signals detected at δ_H 4.66 and δ_C 98.5 were assigned to the anomeric proton/carbon belonging to a β -glucose moiety. In its ¹H NMR spectrum, signals observed at the highfield region were attributed to the three methines ((δ_H 3.15, H-5), (δ_H 1.89, H-8), and (δ_H 2.03 H-9)), a methylene group (δ_H 2.20, 1.76, H₂-6) for cyclopentane moiety (Spectrum 34). In the ¹³C-JMOD NMR spectrum, 16 signals were observed. Signals at δ_C 11.9 and δ_C 174.8 were ascribed to methyl and carbonyl groups, respectively, while resonances between 98.5-61.3 ppm were attributed to the β -glucopyranose moiety. The remaining nine signals suggested the presence of a cyclopentanopyran ring. The olefinic signal observed at δ_H 7.04 (d, $J = 1.2$) was assigned to the H-3, which also confirmed C-4 substitution (Spectrum 35). Further 2D NMR experiments were performed to elucidate the exact structure of **7**. 2D-¹H,¹H-Homonuclear Correlation Spectroscopy revealed two spin systems; one was in the aglycone starting from H-1 to H-5 and the other was among the protons of the β -glucopyranosyl unit (Spectrum 36). Long-range correlations between δ_H 7.04 and δ_C 174.8, δ_H 4.66 and δ_C 95.3 along with δ_H 1.89 and δ_C 11.9 confirmed substitutions on cyclopentanopyran structure (Spectrum 38). The data were superimposable with those reported for loganic acid (**7**) in the literature (332).

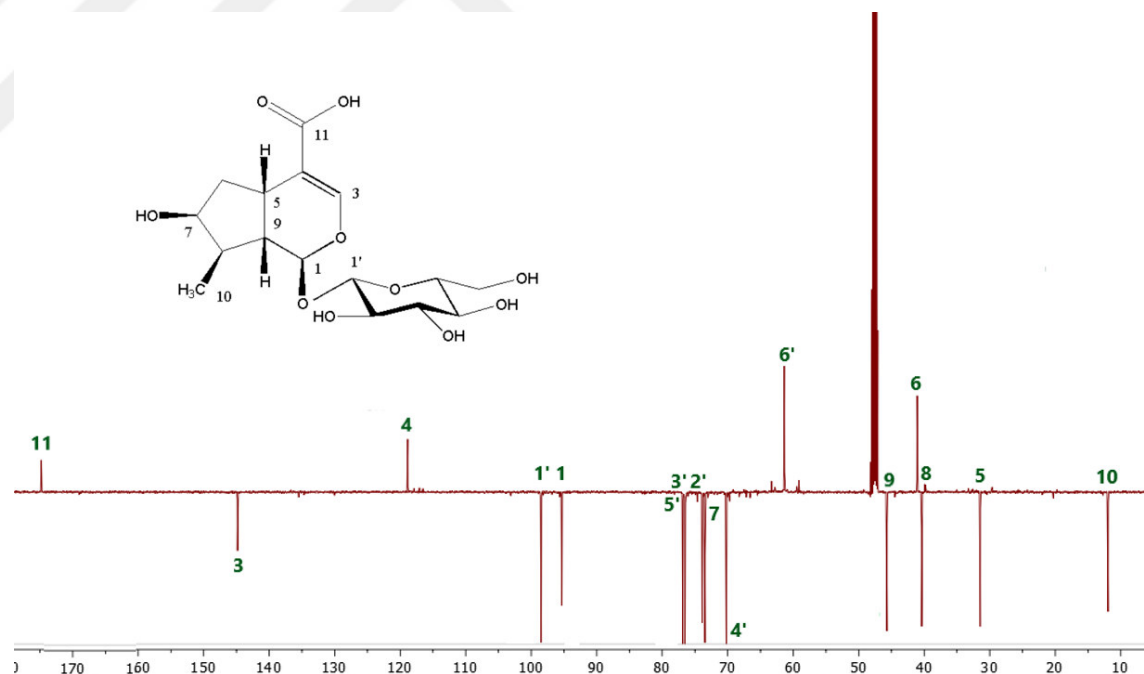
Table 92. ^1H and ^{13}C spectroscopic data of loganic acid (**7**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	CH	95.3	5.24 d (3.8)
3	CH	144.8	7.04 d (1.2)
4	C	118.8	-
5	CH	31.4	3.15 dd (15.4, 7.8)
6	CH_2	41.1	2.20 ddd (14.1, 8.2, 1.9) 1.76 dd (14.1, 5.8)
7	CH	73.9	4.06 m
8	CH	40.4	1.89 m
9	CH	45.7	2.03 m
10	CH_3	11.9	1.09 d (6.9)
11	C	174.8	-
<i>Glucose</i>			
1'	CH	98.5	4.66 d (7.8)
2'	CH	73.4	3.22 t (8.5)
3'	CH	76.4	3.40 m
4'	CH	70.2	3.33 †
5'	CH	76.8	3.32 m
6'	CH_2	61.3	3.90 dd (12, 1.5) 3.70 dd (12, 5.9)

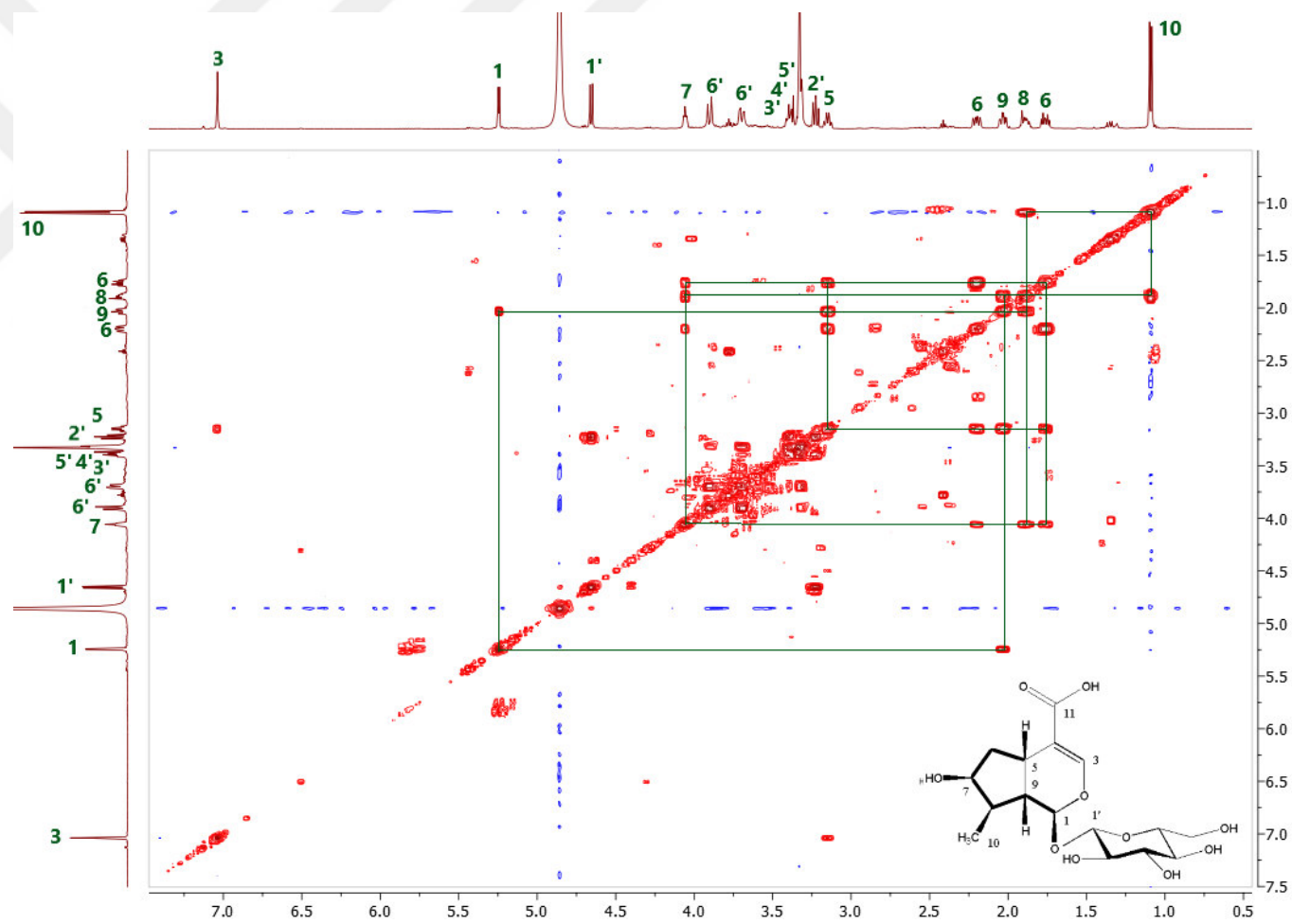
† Overlapped signals.



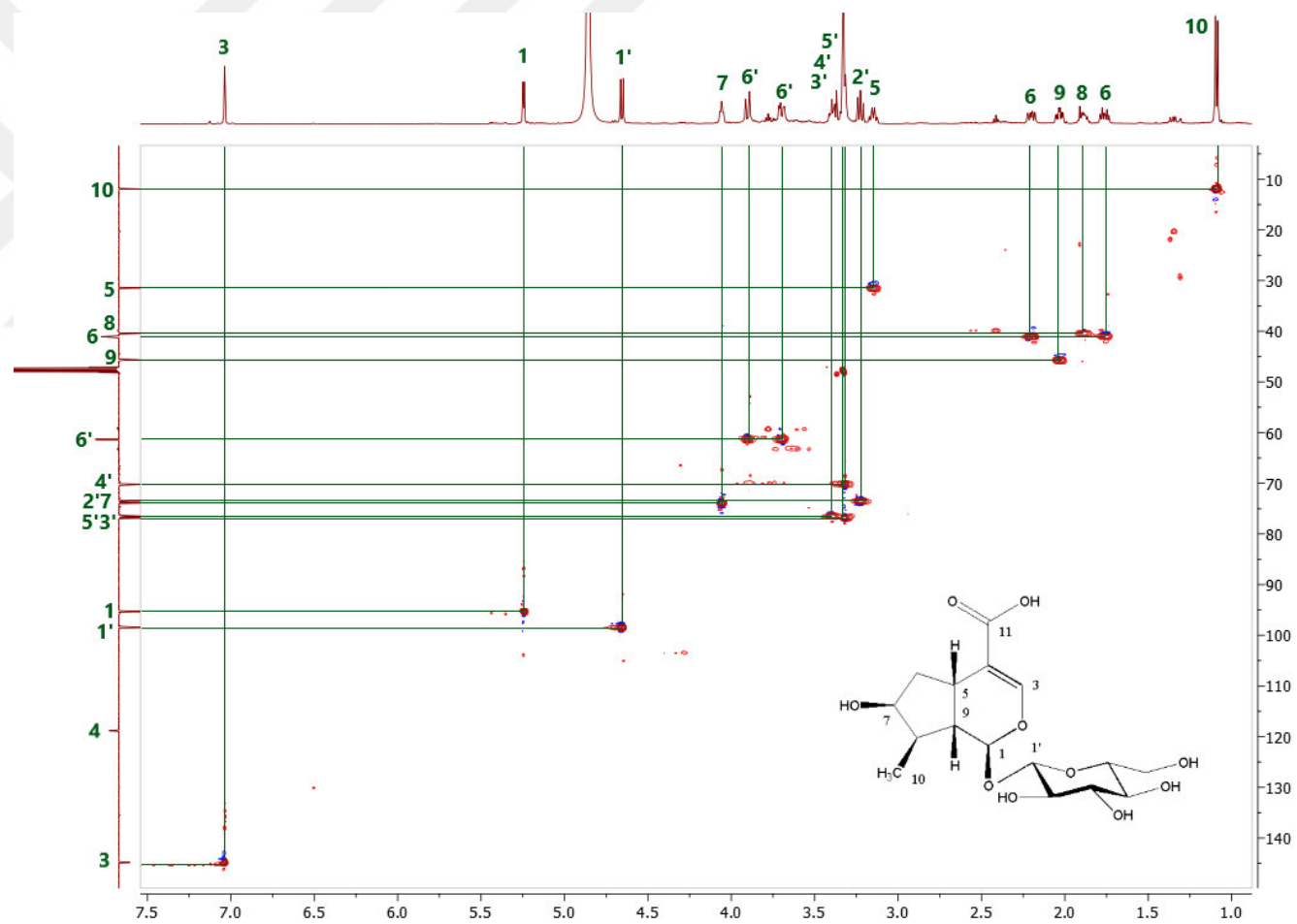
Spectrum 34. ^1H NMR spectrum of loganic acid (**7**) (CD_3OD , 500 MHz)



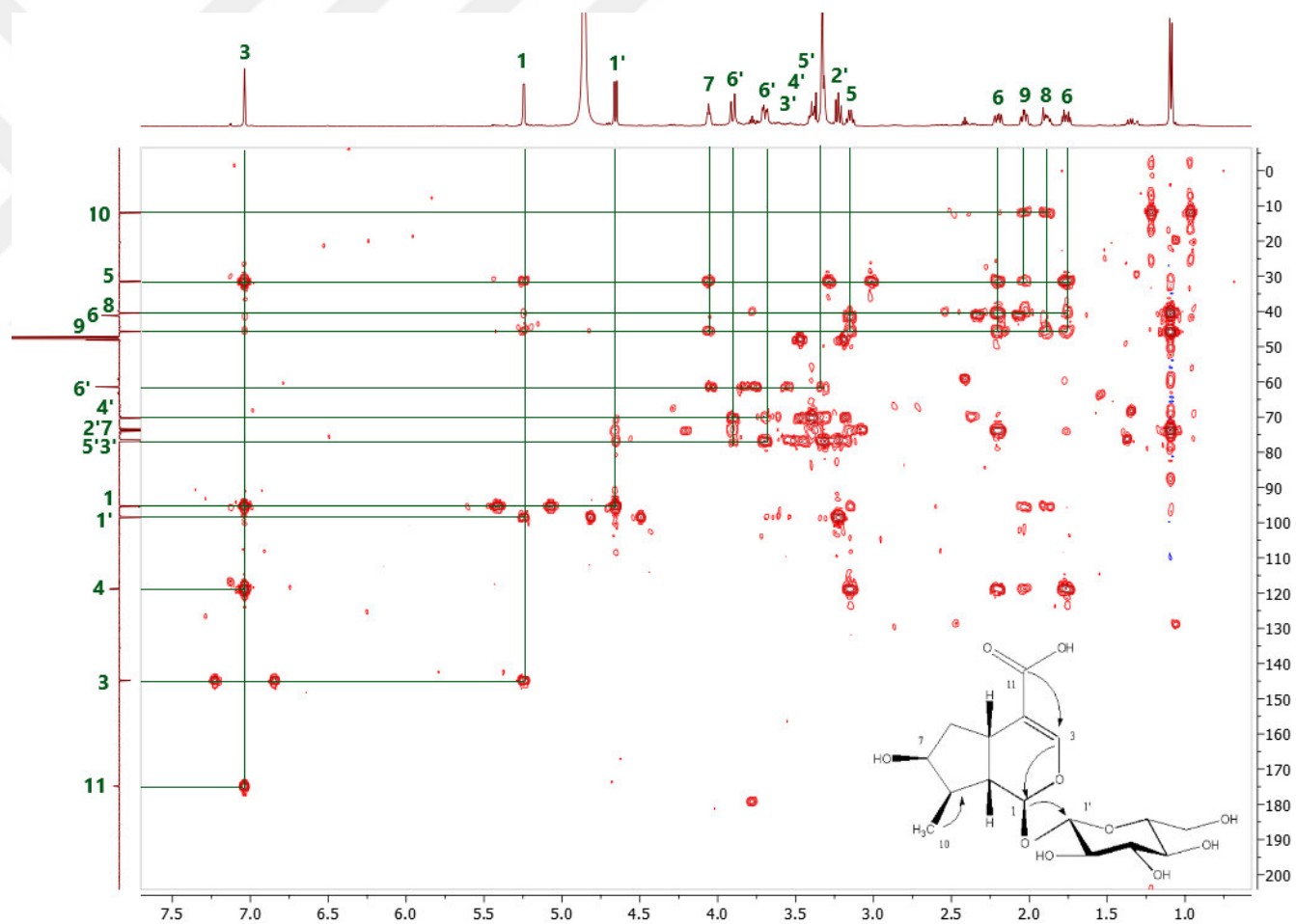
Spectrum 35. ^{13}C -JMOD NMR spectrum of loganic acid (**7**) (CD₃OD, 125 MHz)



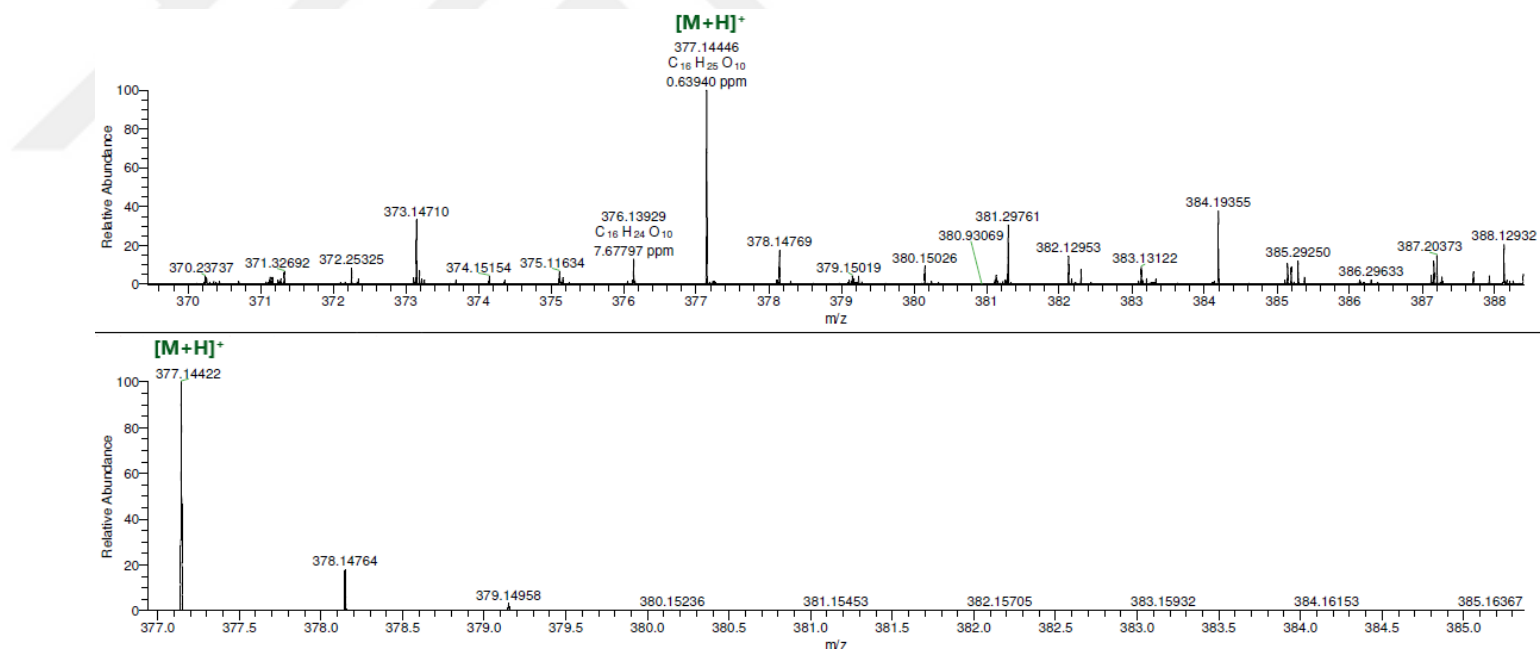
Spectrum 36. COSY spectrum of loganic acid (7)



Spectrum 37. HSQC spectrum of loganic acid (7)

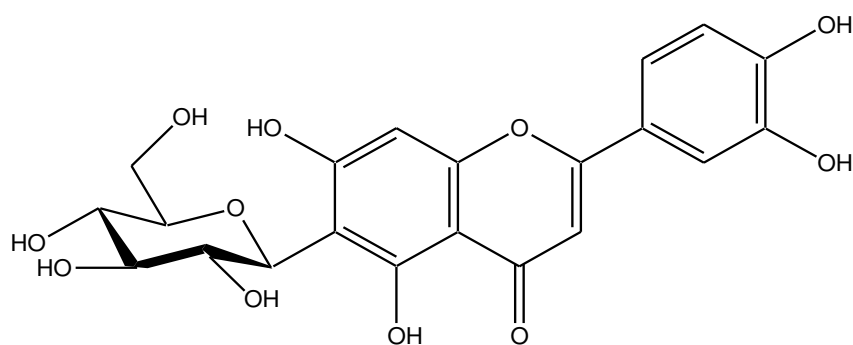


Spectrum 38. HMBC spectrum of loganic acid (7)



Spectrum 39. HR-ESI-MS spectrum of loganic acid (7)

4.2.3. Flavonoids



ISOORIENTIN (8), C₂₁H₂₀O₁₁ (MW: 448.38)

UV λ_{max} (MeOH) nm:	211, 270, 350
IR ν_{max} (KBr) cm^{-1} :	3222 (OH), 1650 (C=O), (C=C), 1452 (aromatic ring)
^1H NMR:	Spectrum 40, Table 93
^{13}C NMR:	Spectrum 41, Table 93
COSY:	Spectrum 42
HSQC:	Spectrum 43
HMBC:	Spectrum 44
HR-ESI-MS:	Spectrum 45

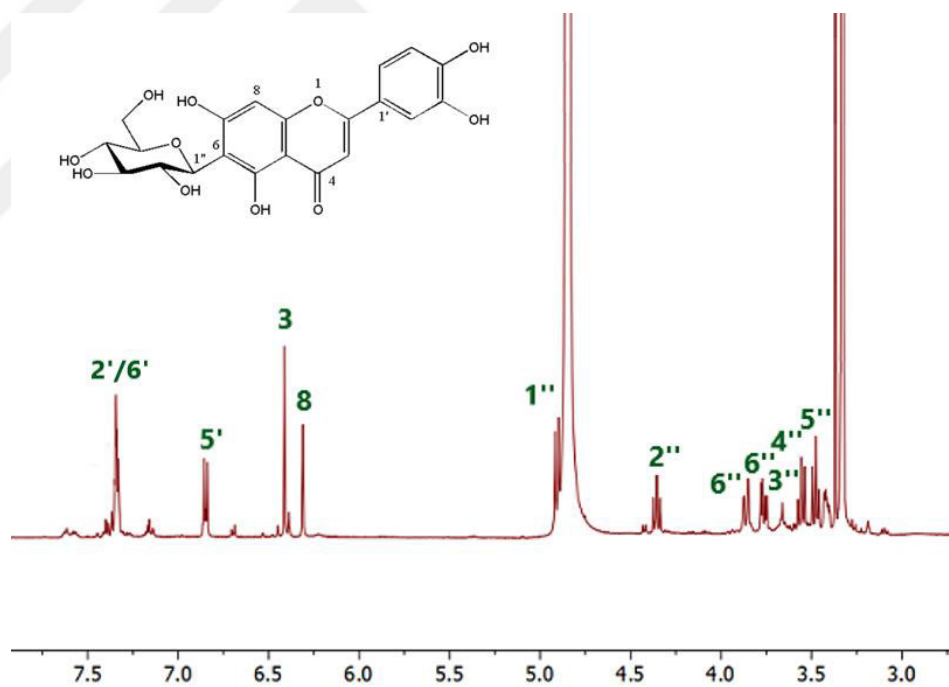
ISOORIENTIN (8)

Compound **8** was isolated from the *n*-BuOH subextract of *G. asclepiadea* and the EtOAc subextract of *G. cruciata* as an amorphous yellow powder. HR-ESI-MS of **8** showed ion peaks at m/z 449.1078 ($(M+H)^+$ (calcd for $C_{21}H_{21}O_{11}$, 449.1084) and at m/z 447.0932 ($(M-H)^-$ (calcd for $C_{21}H_{19}O_{11}$, 447.0927) that indicated the molecular formula of **8** as $C_{21}H_{20}O_{11}$. Its UV spectrum exerted bands at 211, 270, and 350 nm, which were characteristic of a flavone skeleton (333). The IR spectrum displayed absorption bands at 3222 (OH), 1650 (C=O), 1617 (C=C), and 1452 (aromatic ring) cm^{-1} .

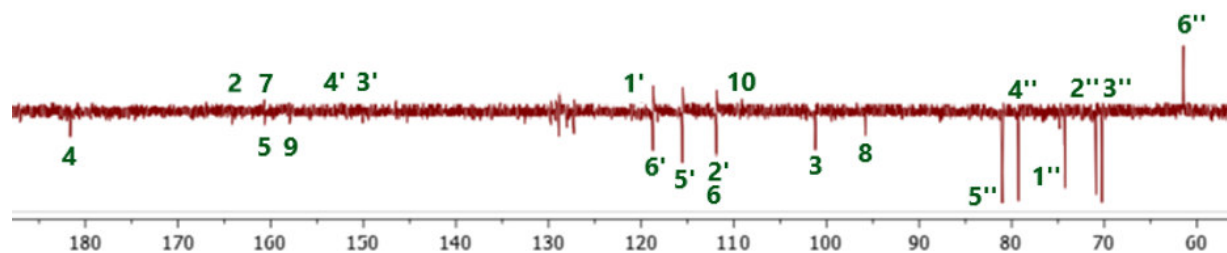
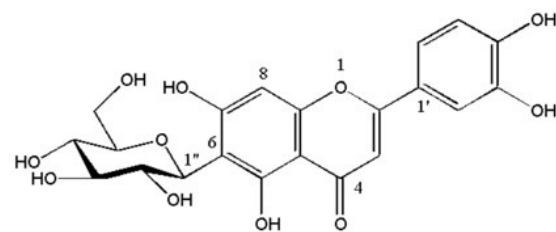
In the aromatic region of the ^1H NMR spectrum of compound **8**, five proton signals (δ_H 7.35-6.31) were observed. Three of these aromatic signals were ascribable to the ABX system (δ_H 7.35, 7.34, and 6.85) and revealed the presence of 3',4'-disubstitution in the B ring of flavonoid. Furthermore, the remaining singlet signals (δ_H 6.40 and 6.31) were assigned to H-3 and H-8 according to their chemical shift values (Spectrum 40). In its ^{13}C -JMOD NMR spectrum, 21 carbon resonances were detected. The signals belonging to C-5 (δ_C 160.6), C-7 (δ_C 161.2), C-3' (δ_C 145.2), and C-4' (δ_C 150.1) were compatible with the chemical shift values of oxygenated aromatic carbons (Spectrum 41). Anomeric signals in the proton (δ_H 4.91, d, $J = 9.9$) NMR spectrum indicated a β -glucopyranosyl unit in the molecule. The location of the H-2'' signal (δ_H 4.36) of glucose, as well as the C-1'' (δ_C 74.1), indicated the presence of a C-glucosidic band between the β -glucopyranose and flavonoid skeleton. The signal of aglycone indicated the presence of luteolin moiety in **8**. The attachment of the β -glycosidic linkage was determined with the help of the HMBC experiment. Besides, cross peaks of H-1'' (δ_H 4.91) with C-6 (δ_C 112.0) and C-7 (δ_C 161.2) indicated the glycosylation site of aglycone (Spectrum 44). The data were superimposable to those reported for isoorientin in the literature (334).

Table 93. ^1H and ^{13}C spectroscopic data of isoorientin (**8**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

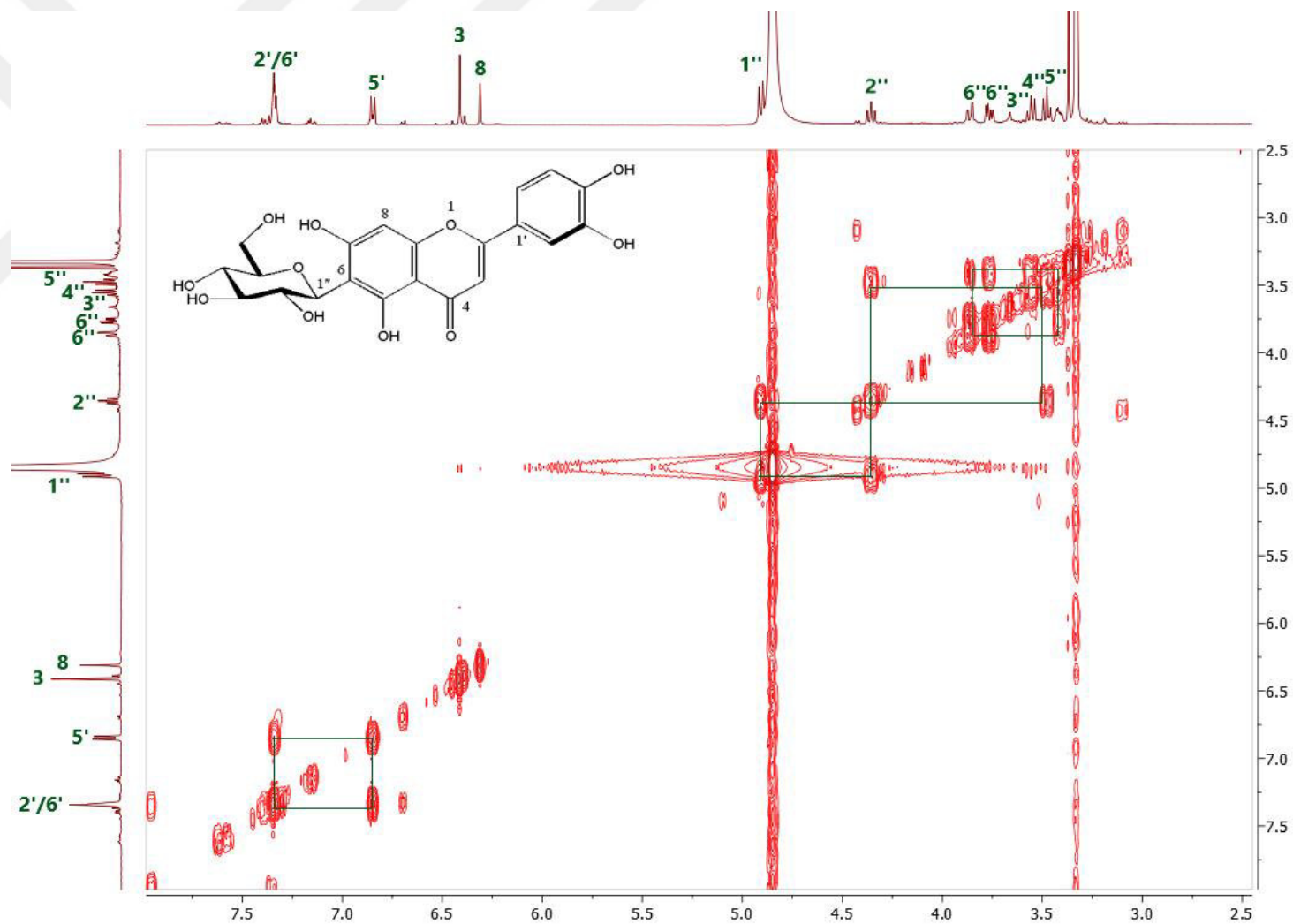
Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
2	C	164.1	-
3	CH	101.2	6.40 s
4	C	181.6	-
5	C	160.6	-
6	C	112.0	-
7	C	161.2	-
8	CH	95.6	6.31 s
9	C	158.1	-
10	C	109.2	-
1'	C	121.4	-
2'	CH	111.9	7.34 d (2.0)
3'	C	145.2	-
4'	C	150.1	-
5'	CH	115.6	6.85 d (8.9)
6'	CH	118.7	7.35 dd (8.9, 2.0)
<i>Glucose</i>			
1''	CH	74.1	4.91 d (9.9)
2''	CH	70.9	4.36 t (9.4)
3''	CH	70.2	3.56 t (9.2)
4''	CH	79.4	3.48 t (8.9)
5''	CH	80.9	3.41 m
6''	CH_2	61.5	3.86 dd (12, 2.4) 3.76 dd (12, 4.8)



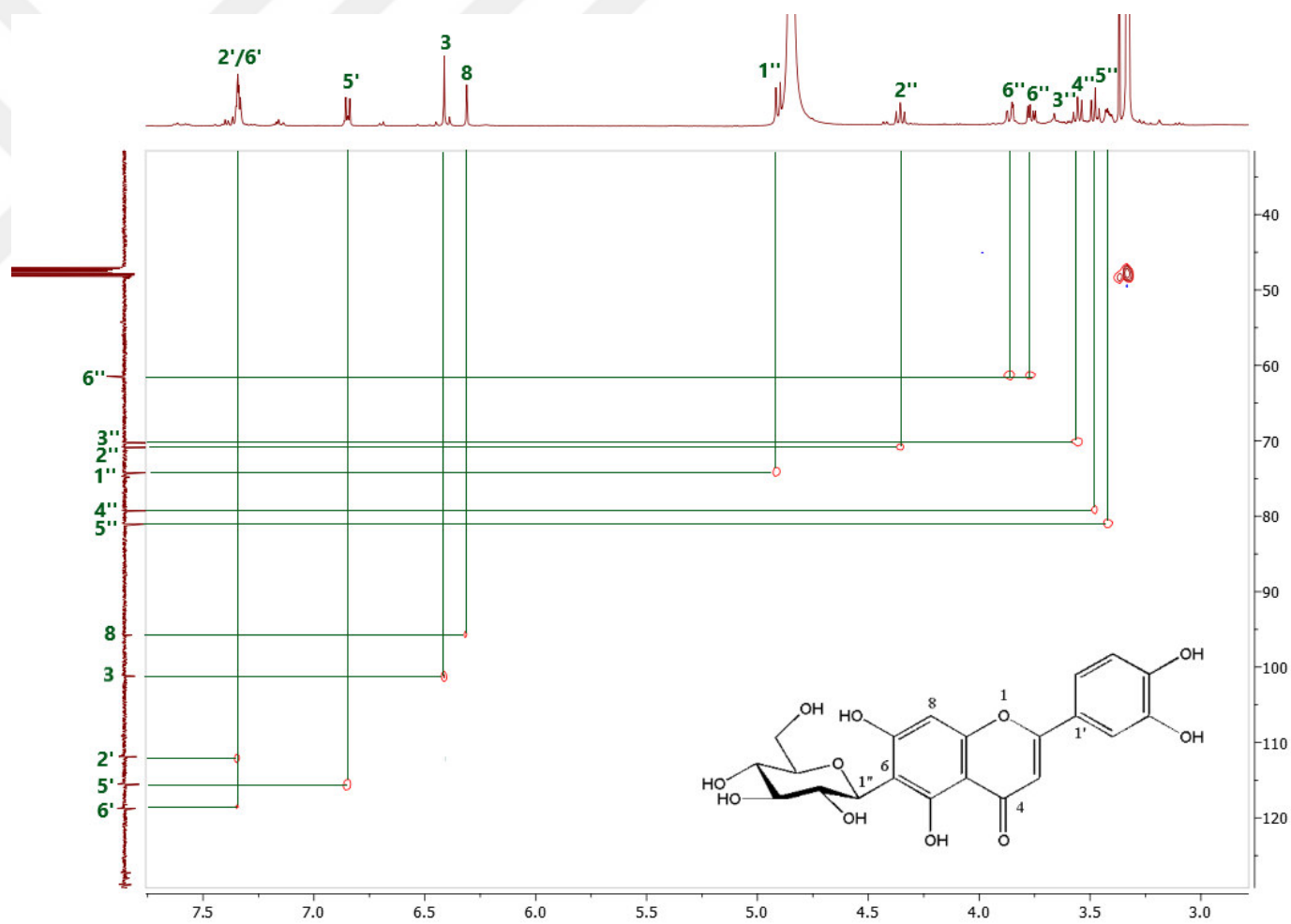
Spectrum 40. ^1H NMR spectrum of isoorientin (**8**) (CD_3OD , 500 MHz)



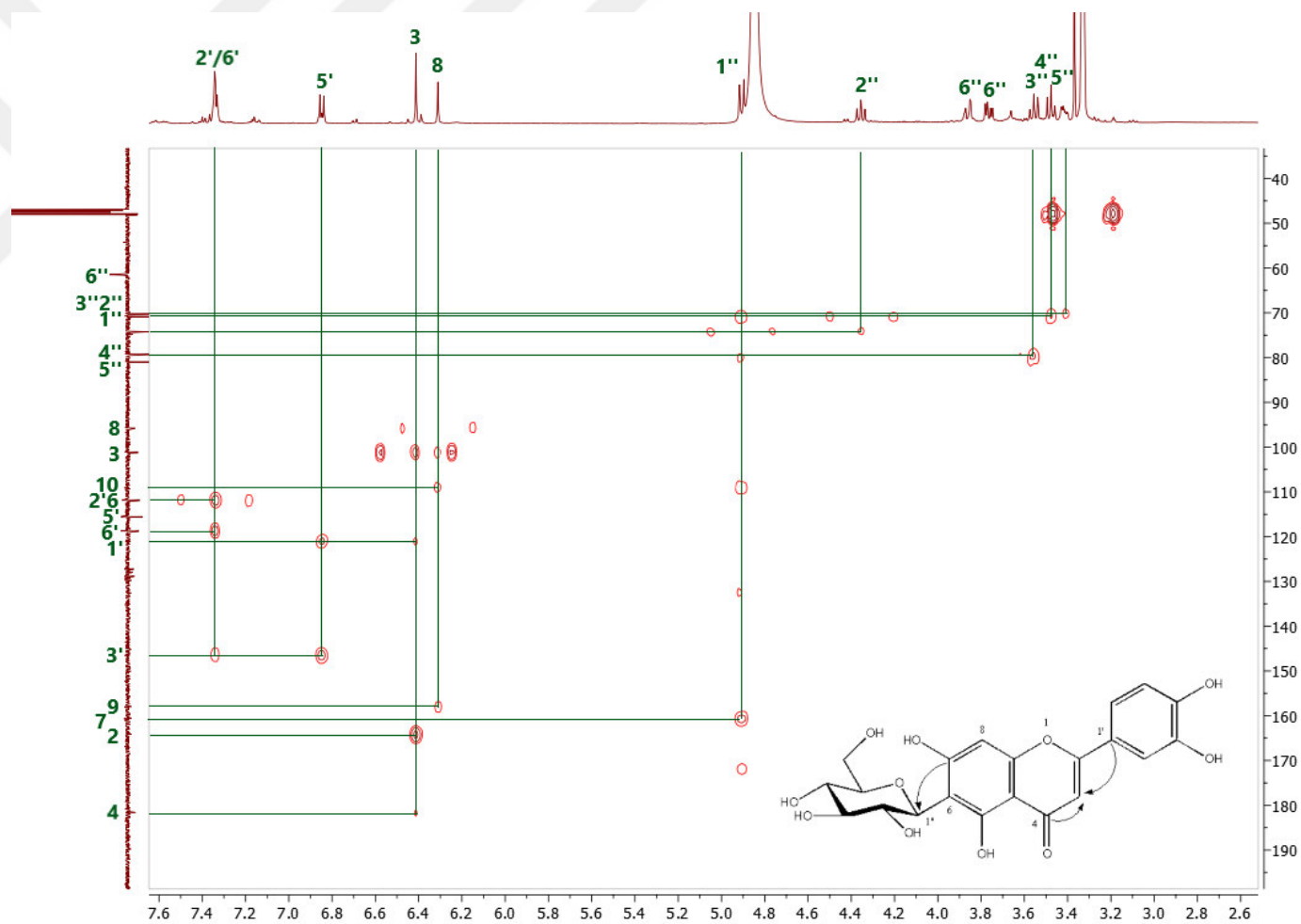
Spectrum 41. ^{13}C -JMOD NMR spectrum of isoorientin (**8**) (CD_3OD , 125 MHz)



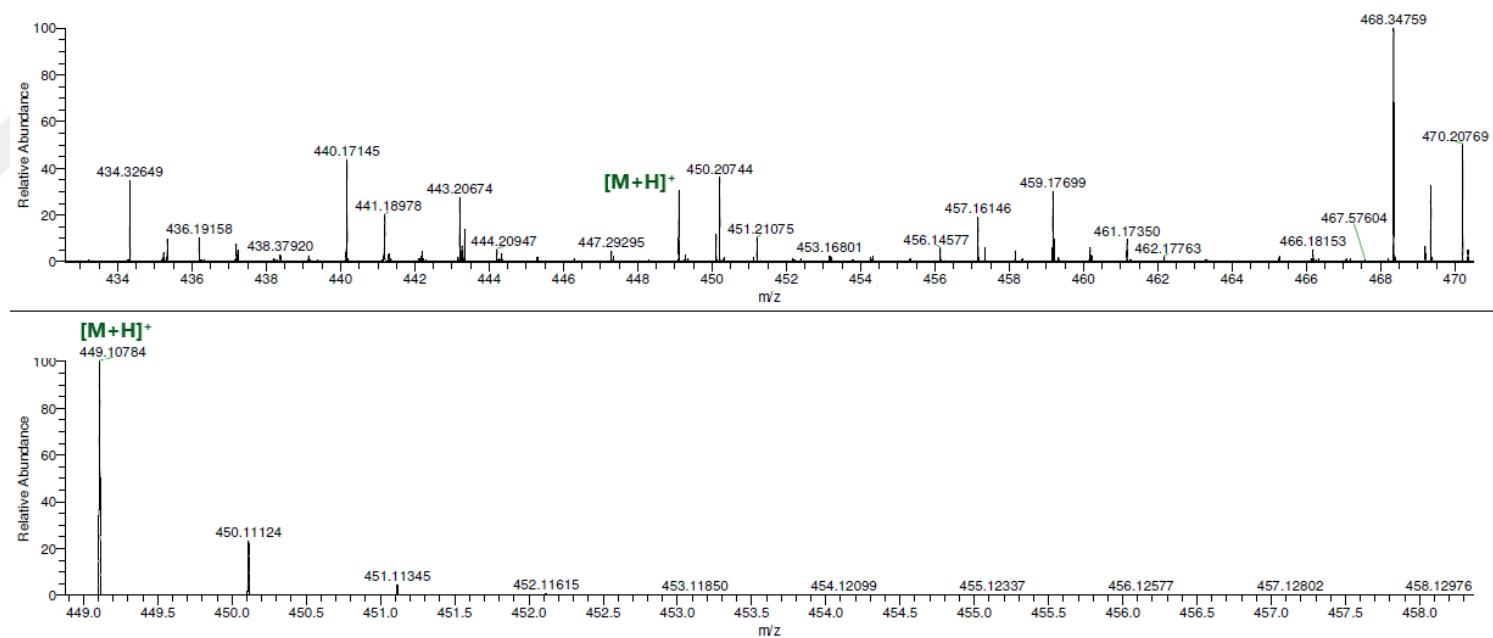
Spectrum 42. COSY spectrum of isoorientin (**8**)



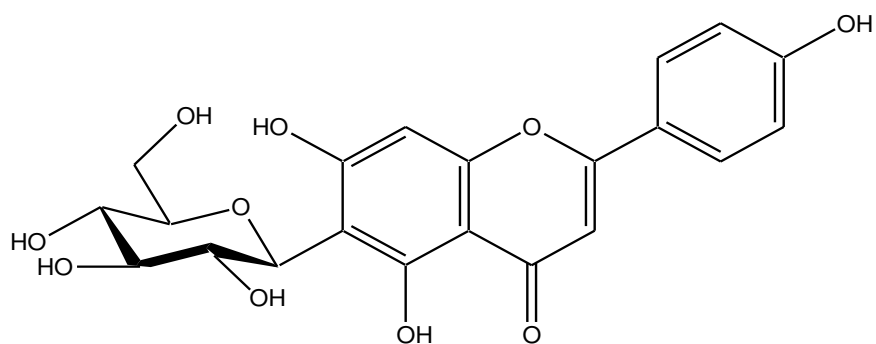
Spectrum 43. HSQC spectrum of isoorientin (**8**)



Spectrum 44. HMBC spectrum of Isoorientin (**8**)



Spectrum 45. HR-ESI-MS spectrum of isorientin (**8**)



ISOVITEXIN (9), C₂₁H₂₀O₁₀ (MW: 432.38)

UV λ_{max} (MeOH) nm:	214, 271, 334
IR ν_{max} (KBr) cm ⁻¹ :	3313 (OH), 1651 (C=O), 1609 (C=C)
¹ H NMR:	Spectrum 46, Table 94
¹³ C NMR:	Spectrum 47, Table 94
HR-ESI-MS:	Spectrum 48

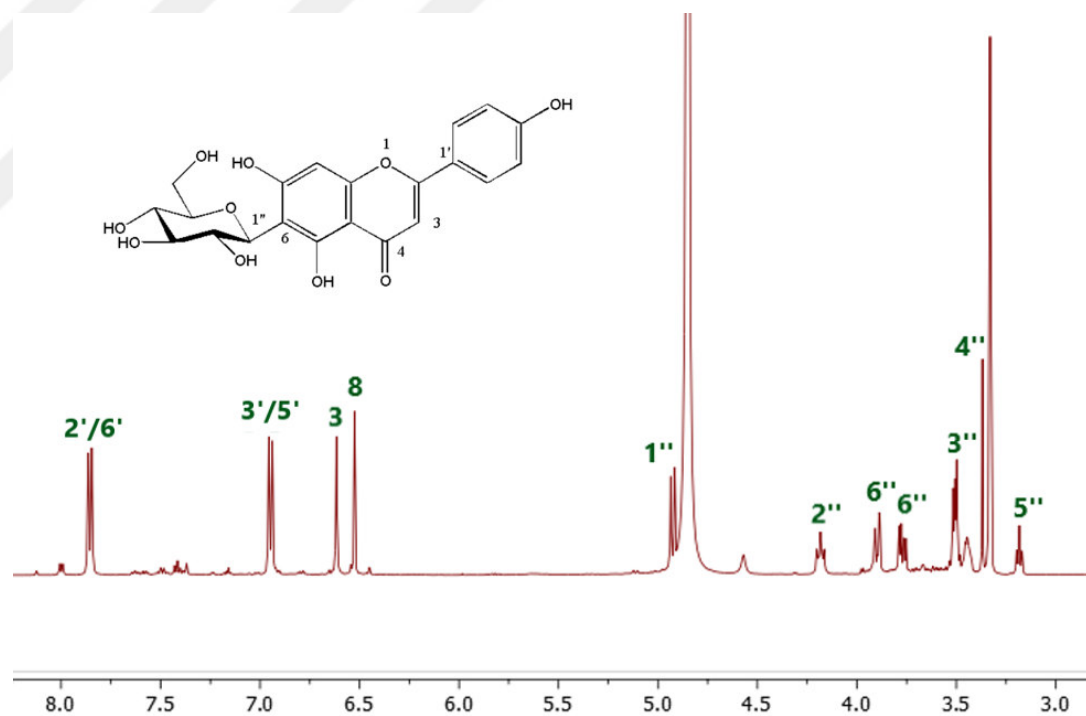
ISOVITEXIN (9)

Compound **9**, an amorphous yellow powder, was isolated from the *n*-BuOH subextract of *G. asclepiadea* and the EtOAc subextract of *G. cruciata*. The HR-ESI-MS of **9** displayed (M+H)⁺ ion at 433.1137 (calcd for C₁₉H₁₉O₁₁, 433.1135), while (M-H)⁻ ion was observed at 431.0988 (calcd for C₁₉H₁₇O₁₁, 431.0978). It showed characteristic absorption maxima bands for flavonoids at 214, 271, and 334 nm in the UV spectrum (333). The IR spectrum of **9** suggested the presence of hydroxyl (3313 cm⁻¹), C=O (1630 cm⁻¹), and C=C (1609 cm⁻¹).

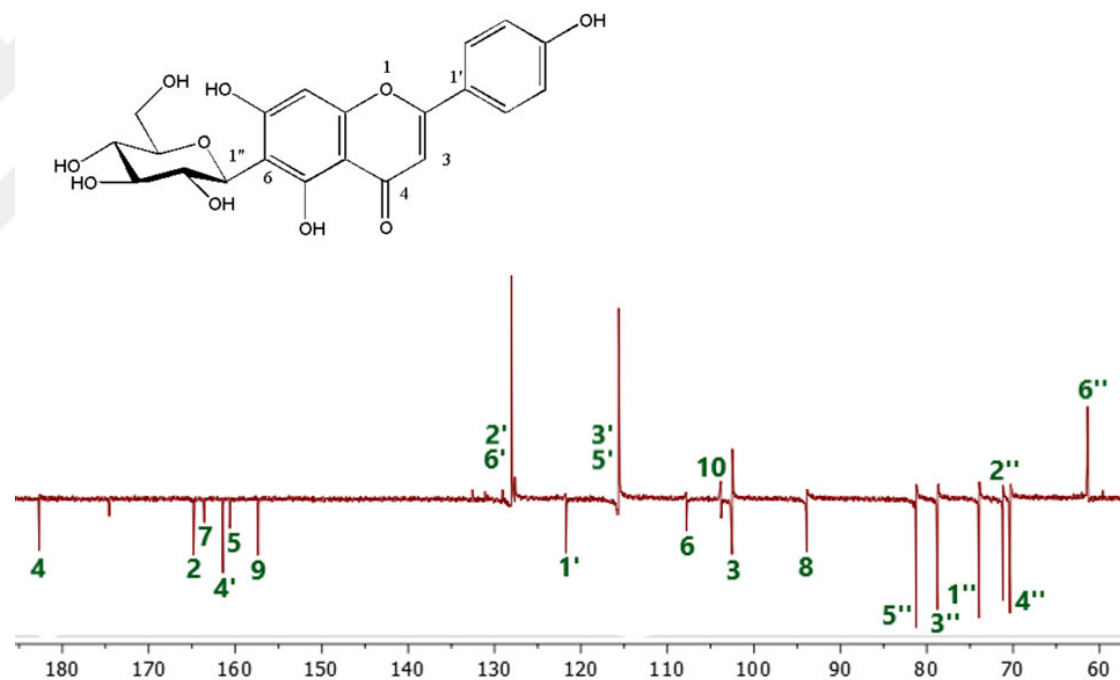
In the ¹H NMR spectrum of compound **9**, two aromatic signals were observed as an AA'BB' system at δ_H 7.85 (d, *J* = 8.5 Hz) and 6.95 (d, *J* = 8.5 Hz) revealed a *para*-substituted benzene ring and the signals were ascribed to H-2'/6' and H-3'/5', respectively (Spectrum 46). Other signals in the spectrum also showed similarity with those of isoorientin. The remaining singlet signals (δ_H 6.62 and 6.52) were assigned to H-3 and H-8 and an anomeric proton signal was detected at δ_H 4.93 with a large coupling constant (9.9). The ¹³C-JMOD NMR spectrum of **9** also showed similarity with those of **8**, except for carbon resonances belonging to the B ring ((δ_C 128.2, C-2/6), (δ_C 115.6, C-3/5)). (Spectrum 47). Thus, the NMR finding for aglycone was identified as apigenin. With regard to these findings, compound **9** was characterized as isovitexin (335).

Table 94. ^1H and ^{13}C spectroscopic data of isovitexin (**9**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

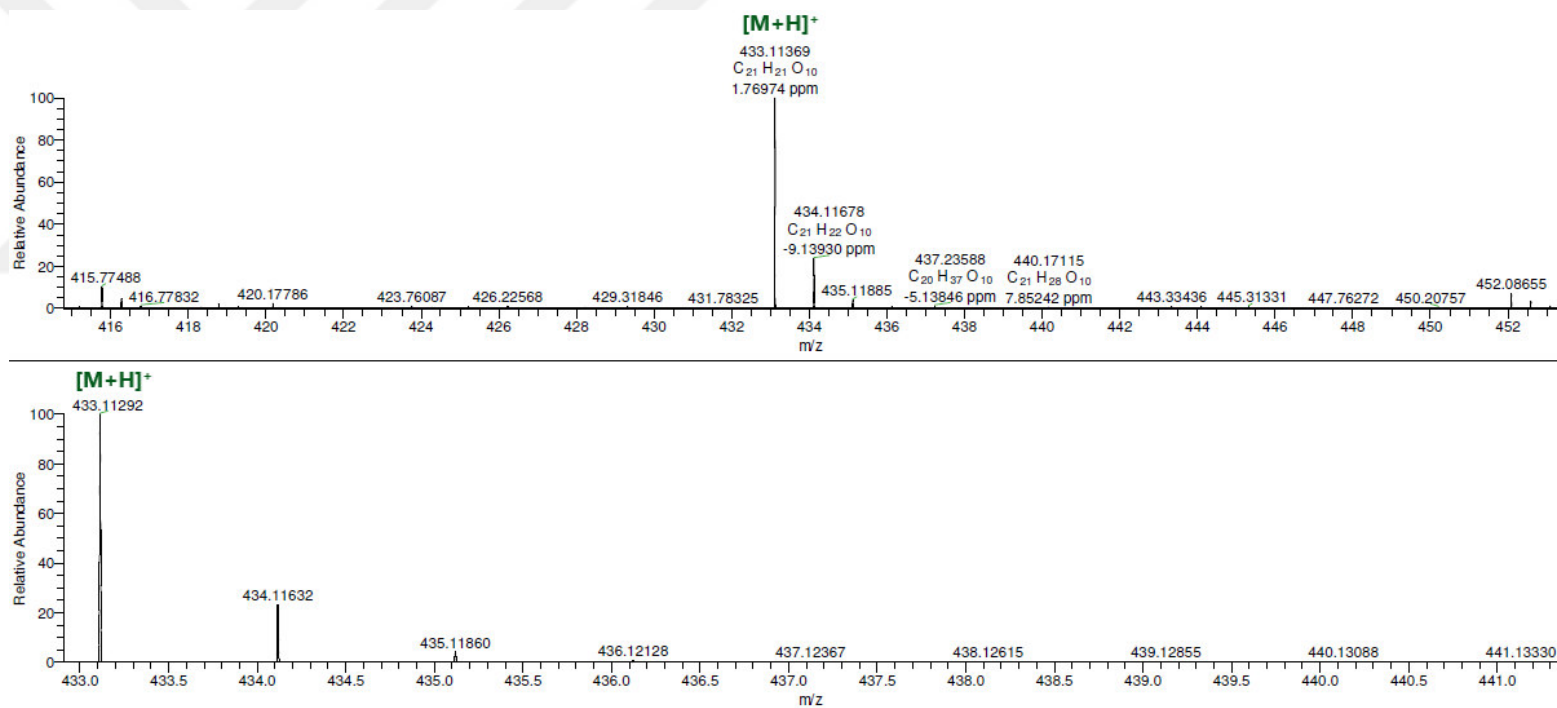
Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
2	C	164.9	-
3	CH	102.4	6.62 s
4	C	182.7	-
5	C	160.6	-
6	C	107.8	-
7	C	163.6	-
8	CH	93.9	6.52 s
9	C	157.4	-
10	C	103.7	-
1'	C	121.7	-
2'	CH	128.2	7.85 d (8.5)
3'	CH	115.6	6.95 d (8.5)
4'	C	161.5	-
5'	CH	115.6	6.95 d (8.5)
6'	CH	128.2	7.85 d (8.5)
<i>Glucose</i>			
1''	CH	73.9	4.93 d (9.9)
2''	CH	71.2	4.18 t (9.2)
3''	CH	78.7	3.51 t (4.3)
4''	CH	70.4	3.44 m
5''	CH	81.2	3.18 t (6.9)
6''	CH ₂	61.3	3.90 dd (12, 2.2) 3.77 dd (12, 5.2)



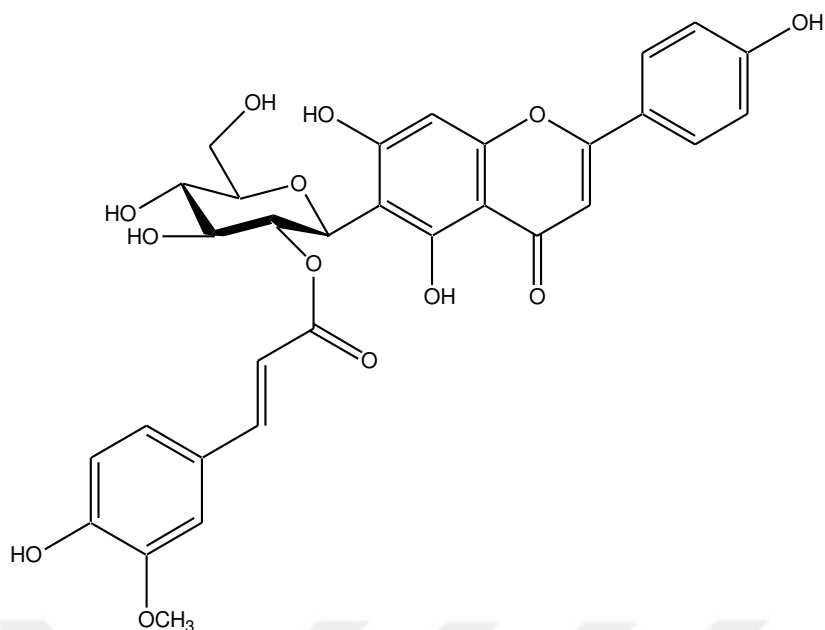
Spectrum 46. ^1H NMR spectrum of isovitexin (**9**) (CD_3OD , 500 MHz)



Spectrum 47. ^{13}C -JMOD NMR spectrum of isovitexin (**9**) (CD_3OD , 125 MHz)



Spectrum 48. HR-ESI-MS spectrum of isovitexin (**9**)



ISOVITEXIN 2''-(*E*)-FERULATE (10), C₃₁H₂₈O₁₃ (MW: 608.55)

UV λ_{max} (MeOH) nm:	201, 213, 272, 327
IR ν_{max} (KBr) cm ⁻¹ :	3278 (OH), 2972, 1651 (C=O), 1606 (C=C)
¹ H NMR:	Spectrum 49, Table 95
¹³ C NMR:	Spectrum 50, Table 95
COSY:	Spectrum 51
HSQC:	Spectrum 52
HMBC:	Spectrum 53
HR-ESI-MS	Spectrum 54

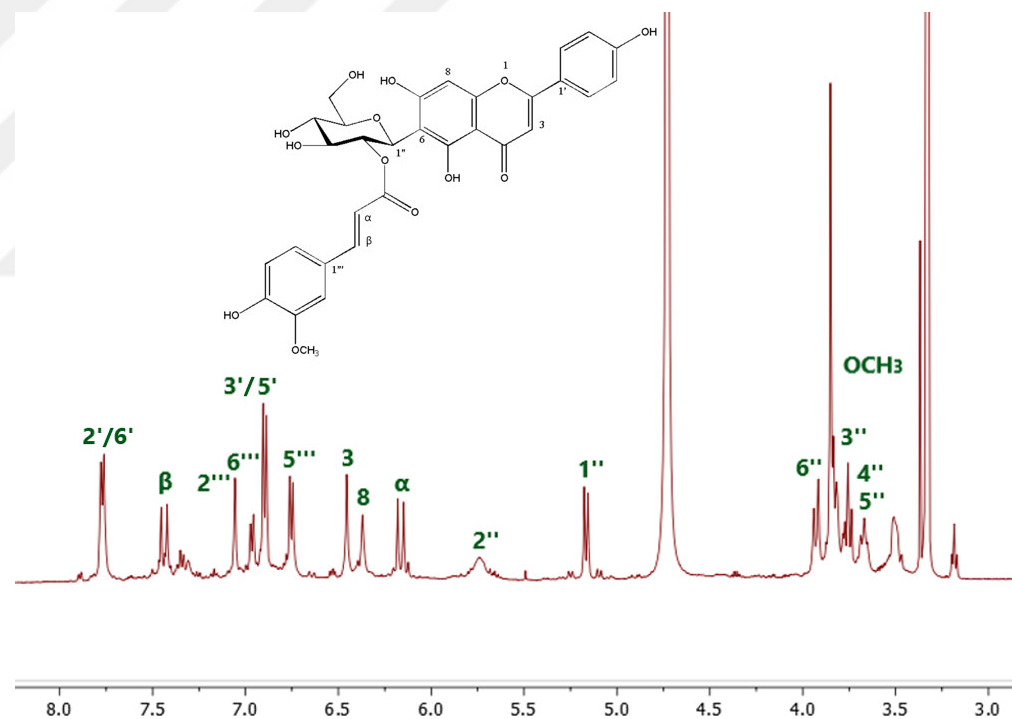
ISOVITEXIN 2''-(*E*)-FERULATE (10)

Compound **10** was isolated from the EtOAc subextract of *G. cruciata* as a yellow amorphous powder. The HR-ESI-MS of **10** exhibited (M+H)⁺ ion at m/z 609.1602 (calcd for C₃₁H₂₉O₁₃, 609.1608), and (M-H)⁻ ion at m/z 607.1458 (calcd for C₃₁H₂₇O₁₃, 607.1452), supporting a molecular formula of C₃₁H₂₈O₁₃. In its ¹H NMR spectrum, two aromatic signals were observed as an AA'BB' system at δ_H 7.77 (d, J = 8.4 Hz) and 6.90 (d, J = 8.4 Hz) revealed a *para*-substituted benzene ring that was assigned to H-2'/6' and H-3'/5', respectively. Singlet signals at δ_H 6.45 and δ_H 6.37 were ascribed to H-3 and H-8. Moreover, an anomeric proton signal was detected at δ_H 5.17 with a large coupling constant (9.9). Signals in the proton NMR spectrum of **10** showed similarities with those of **9**, however, additional signals were observed in both aliphatic and aromatic areas. Aromatic signals at δ_H 7.06 (br.s, H-2'''), δ_H 6.75 (d, J = 8.1, H-5'''), δ_H 6.96 (br.d, J = 8.1, H-6''') as an ABX system revealed *ortho*-substituted benzene ring of 3',4'-disubstitution. *Trans*-olefinic signals were also detected at 6.16 (d, J = 15.9) and 7.44 (d, J = 15.9). Moreover, proton resonance of a methoxy unit at 3.85 (3H, s) was detected (Spectrum 49). All these data concluded that a *trans*-feruloyl moiety was attached to isovitexin. Besides, the H-2'' signal belonging to β -glucopyranose moiety was found to be shifted downfield around 1 ppm compared to those of **9** due to the esterification, which also revealed the location of the (*E*)-feruloyl moiety.

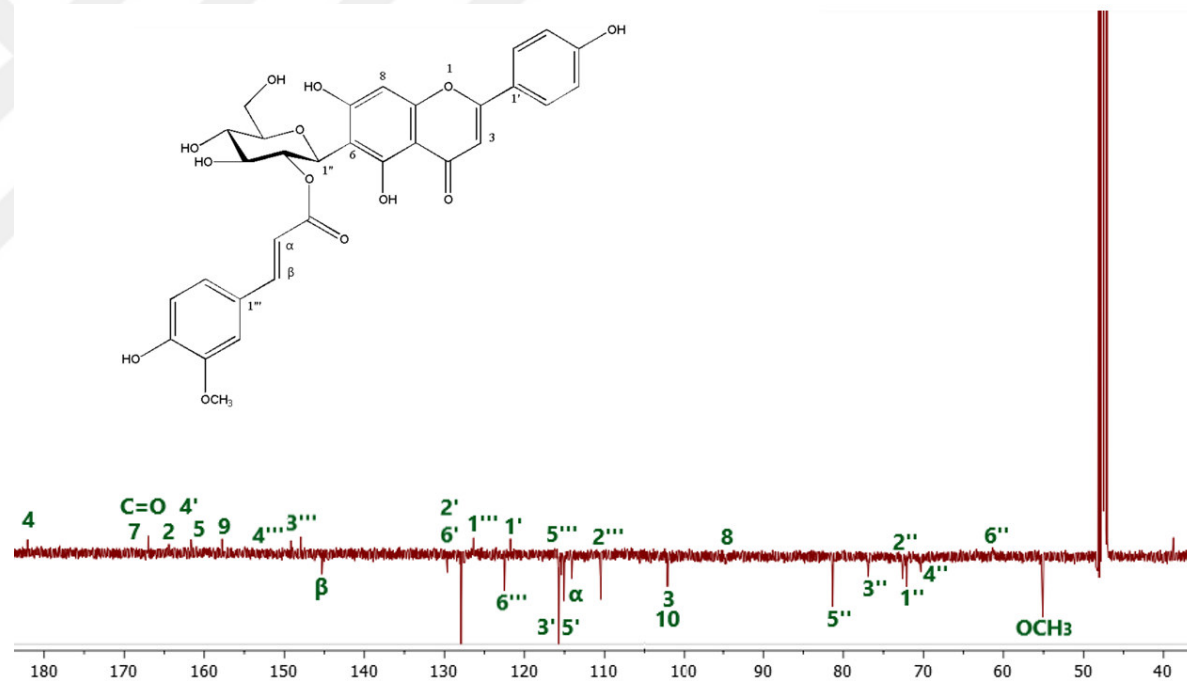
In its ¹³C-JMOD NMR spectrum, 31 carbon resonances were observed. Signals belonging to the flavon skeleton and sugar moiety showed similar carbon resonances to those of compound **9**. *Trans*-feruloyl signals were detected between δ_C 55.0-167.0 (Spectrum 50). 2D NMR experiments were conducted to confirm the exact structure. HMBC experiment permitted the determination of all the interfragmental connectivities of sugar and *trans*-feruloyl moieties. ¹³C/¹H correlations from δ_C 107.1 (C-6) to δ_H 5.17 (H-1''), δ_C 147.9 (C-3''') to δ_H 3.85 (OCH₃), δ_C 126.3 (C-1''') to δ_H 6.16 (H- α) and δ_C 122.5 (C-6''') to δ_H 7.44 (H- β) (Spectrum 53) secured the final structure of compound unambiguously as *trans*-feruloyl-2''-isovitexin, also known as isovitexin 2''-(*E*)-ferulate (250).

Table 95. ^1H and ^{13}C spectroscopic data of isovitexin 2''-(*E*)-ferulate (**10**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

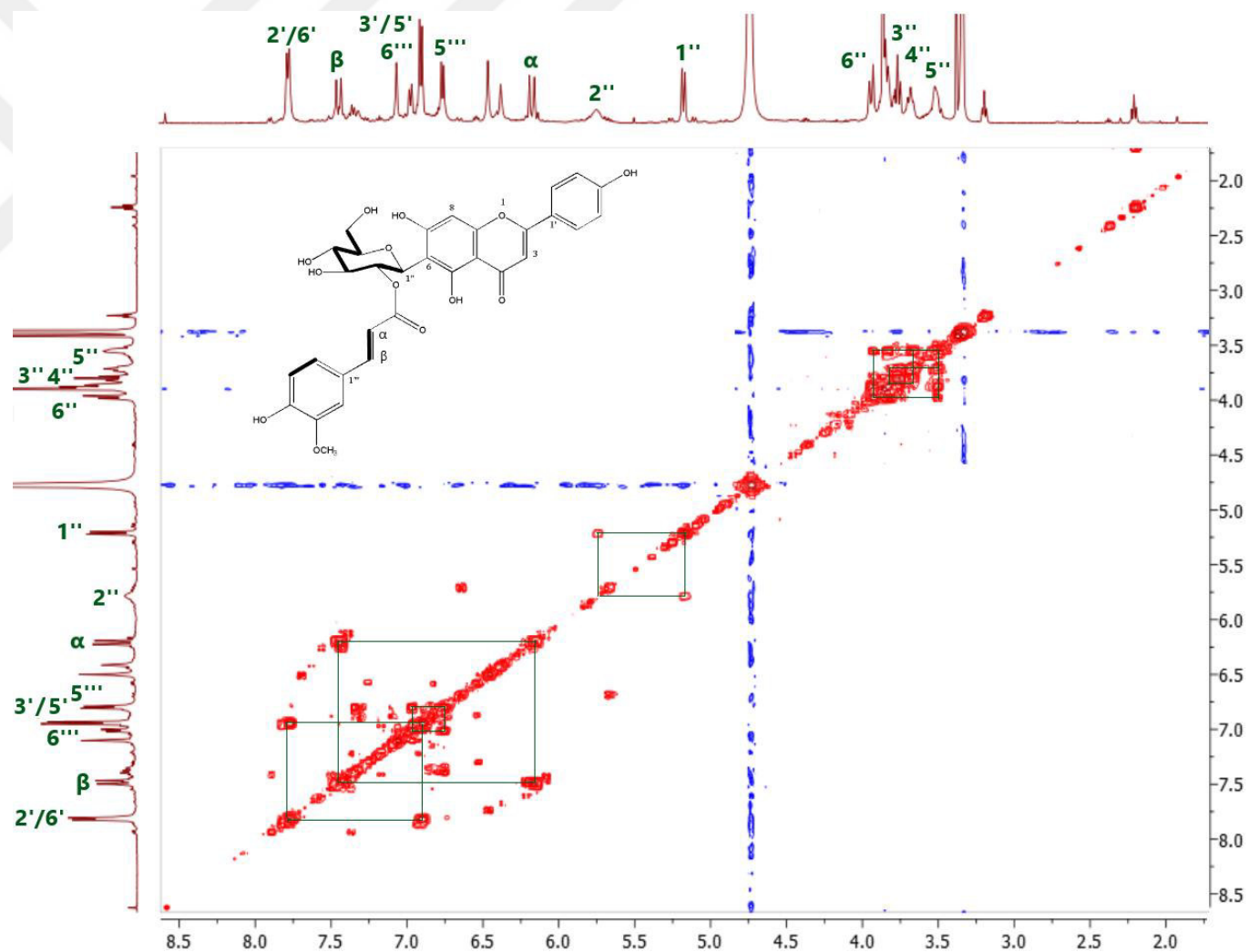
Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), <i>J</i> (Hz)
<i>Aglycone</i>			
2	C	164.5	-
3	CH	102.2	6.45 s
4	C	182.1	-
5	C	160.5	-
6	C	107.1	-
7	C	167.5	-
8	CH	94.8	6.37 s
9	C	157.8	-
10	C	102.6	-
1'	C	121.7	-
2'	CH	128.0	7.77 d (8.4)
3'	CH	115.7	6.90 d (8.4)
4'	C	161.7	-
5'	CH	115.7	6.90 d (8.4)
6'	CH	128.0	7.77 d (8.4)
<i>Glucose</i>			
1''	CH	72.0	5.17 d (9.9)
2''	CH	72.6	5.74 m
3''	CH	76.9	3.76 t (9.1)
4''	CH	70.3	3.67 t (9.1)
5''	CH	81.4	3.50 m
6''	CH ₂	61.3	3.93 br.d (12.1) 3.83 br.d (12.1)
<i>(E)-feruloyl</i>			
1'''	C	126.3	-
2'''	CH	110.5	7.06 br.s
3'''	C	147.9	-
4'''	C	149.2	-
5'''	CH	115.0	6.75 d (8.1)
6'''	CH	122.5	6.96 br.d (8.1)
α	CH	114.1	6.16 d (15.9)
β	CH	145.3	7.44 d (15.9)
C=O	C	167.0	-
OCH ₃	CH ₃	55.0	3.85 s



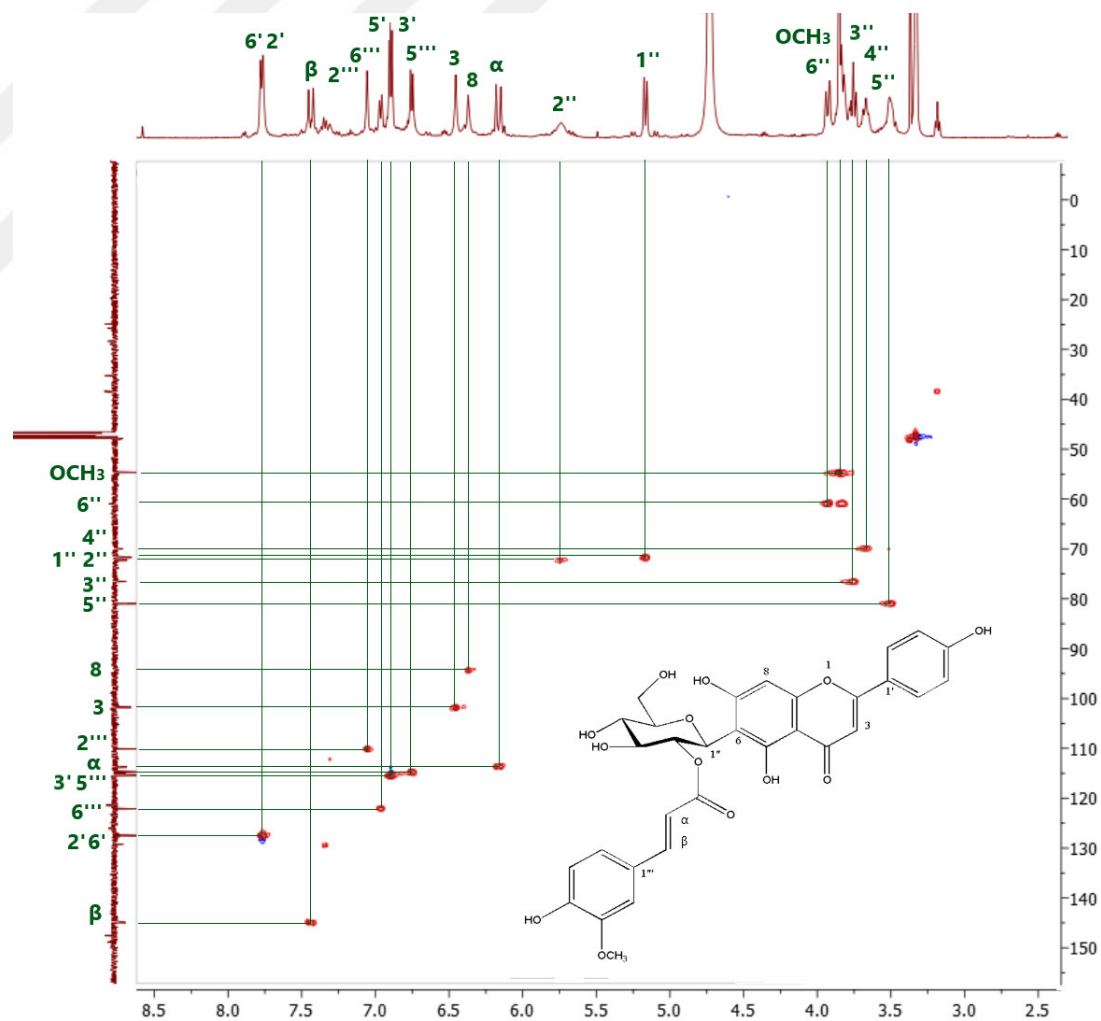
Spectrum 49. ^1H NMR spectrum of isovitexin 2''-(*E*)-ferulate (**10**) (CD_3OD , 500 MHz)



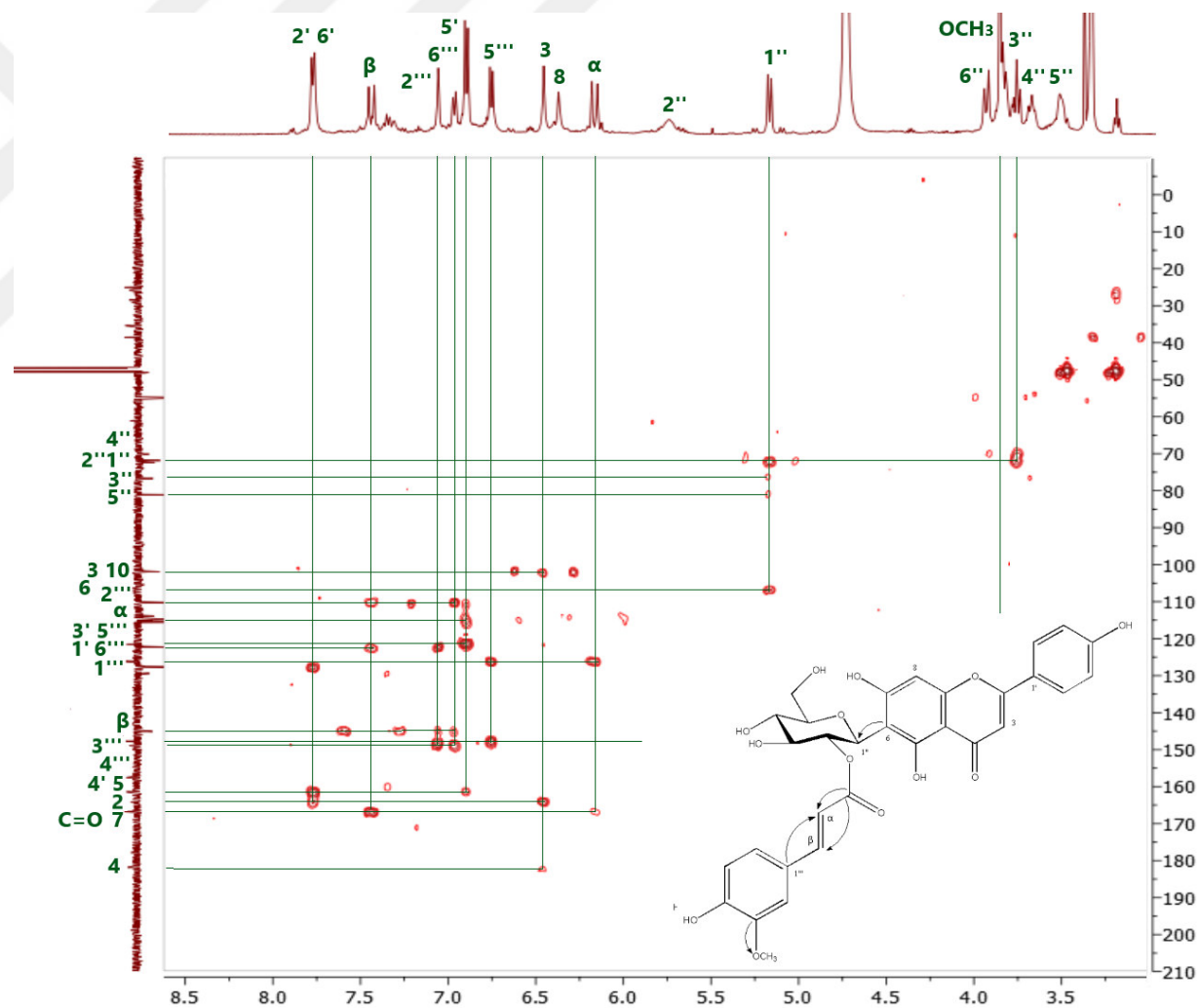
Spectrum 50. ¹³C-JMOD NMR spectrum of isovitexin 2''-(*E*)-ferulate (**10**) (CD₃OD, 125 MHz)



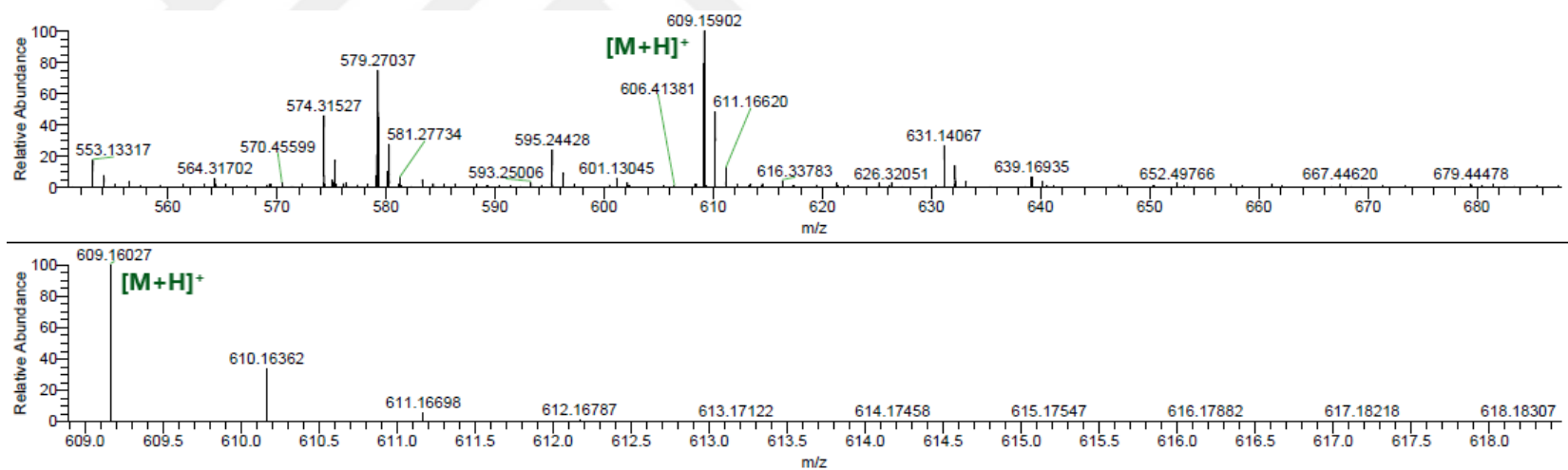
Spectrum 51. COSY spectrum of isovitexin 2''-(*E*)-ferulate (**10**)



Spectrum 52. HSQC spectrum of isovitexin 2''-(*E*)-ferulate (**10**)

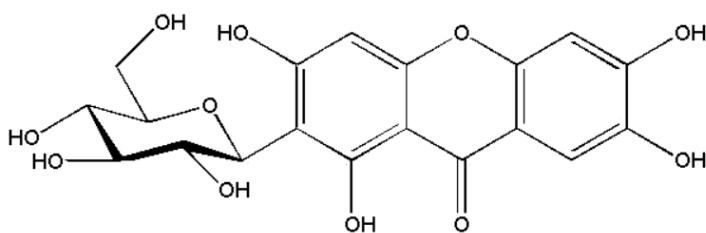


Spectrum 53. HMBC spectrum of isovitexin 2''-(*E*)-ferulate (**10**)



Spectrum 54. HR-ESI-MS spectrum of isovitexin 2''-(*E*)-ferulate (10)

4.2.4. Xanthone derivative



MANGIFERIN (11), C₁₉H₁₈O₁₁ (MW: 422.34)

UV λ_{max} (MeOH) nm:	240, 258, 316, 366
IR ν_{max} (KBr) cm^{-1} :	3368 (OH), 1626 (C=O), 1455 (aromatic ring)
^1H NMR:	Spectrum 55, Table 96
^{13}C NMR:	Spectrum 56, Table 96
COSY:	Spectrum 57
HSQC:	Spectrum 58
HMBC:	Spectrum 59
HR-ES-IMS:	Spectrum 60

MANGIFERIN (11)

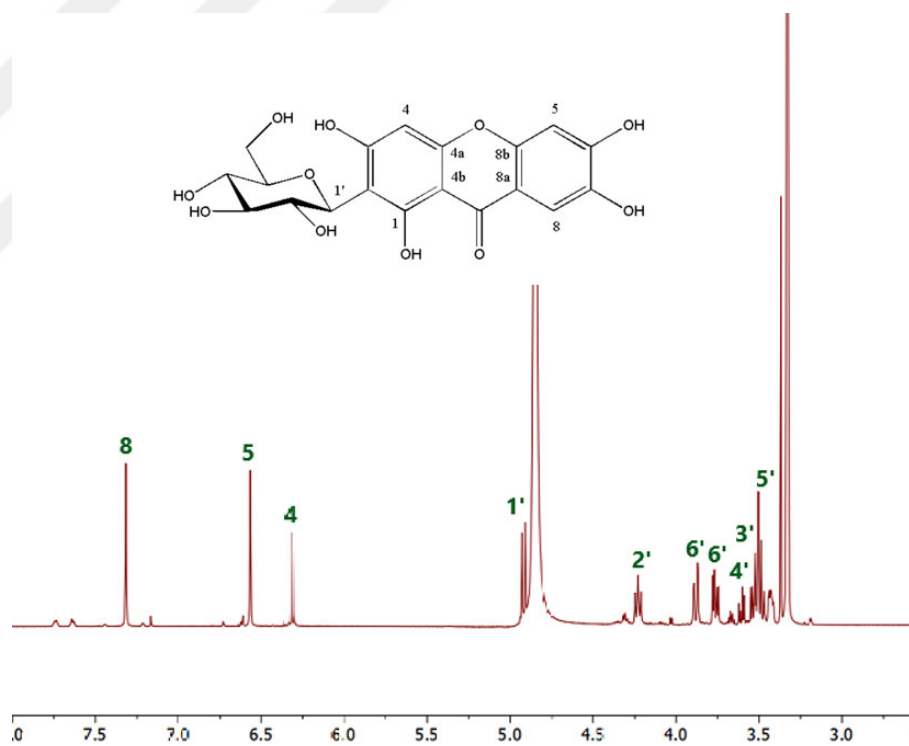
Compound **11** was purified from both the *n*-BuOH extract of *G. asclepiadea* and the EtOAc extract of *G. cruciata* as a yellow amorphous powder. The HR-ESI-MS of **11** exhibited (M+H)⁺ ion at 423.0921 (calcd for C₁₉H₁₉O₁₁, 423.0927) and (M-H)⁻ ion at 421.0776 (calcd for C₁₉H₁₇O₁₁, 421.0771). The ultraviolet (UV) spectrum maxima at 240, 258, 316, and 366 nm showed typical absorptions of xanthenes (Rahman et al., 2016), whereas its IR spectrum exhibited absorption bands at 3368 (OH), 1626 (C=O), 1455 (aromatic ring).

The ¹H NMR spectrum of **11** contained aromatic signals along with a sugar residue. Three singlets of aromatic signals (δ_{H} 7.32, 6.57, 6.31) were detected in the downfield area and signals belonging to a glucose moiety were observed between δ_{H} 4.92-3.43. The large couple constant ($J = 9.8$) of an anomeric signal at δ_{H} 4.92 indicated its β -configuration (Spectrum 55). In the ¹³C-JMOD NMR spectrum, 19 carbon resonances were detected. The carbon signal at δ_{C} 179.0 suggested the presence of a ketone function, which is also supported by the IR absorption band at 1626 cm⁻¹. Other signals in the downfield area ((δ_{C} 161.5, C-1), (δ_{C} 164.1, C-3), (δ_{C} 154.7, C-6), (δ_{C} 146.1, C-7)) were characteristic for oxygenated aromatic carbons (Spectrum 56). 2D NMR experiments (COSY, HSQC, and HMBC) were performed to identify the exact structure. Corresponding carbon data of proton signals were elucidated by HSQC and singlet proton signals were determined as H-4, H-5 and H-7 of the xanthone skeleton (Spectrum 58). With the help of the HMBC experiment, the attachment point of the sugar moiety was determined. ¹³C/¹H long-range correlations between δ_{C} 106.1 and δ_{H} 4.92 indicated that the sugar unit was attached through a β -C-glycosidic linkage to the C-2 of aglycone. Besides, other HMBC correlations between C-1' to H-1 and H-3 also confirmed the location of glycosidation (Spectrum 59). Comparison of all the spectral data with those published in the literature led to the elucidation of the compound **11** as 1,3,6,7-tetrahydroxy-2-C-glucopyranosylxanthone, which is also known as mangiferin (336).

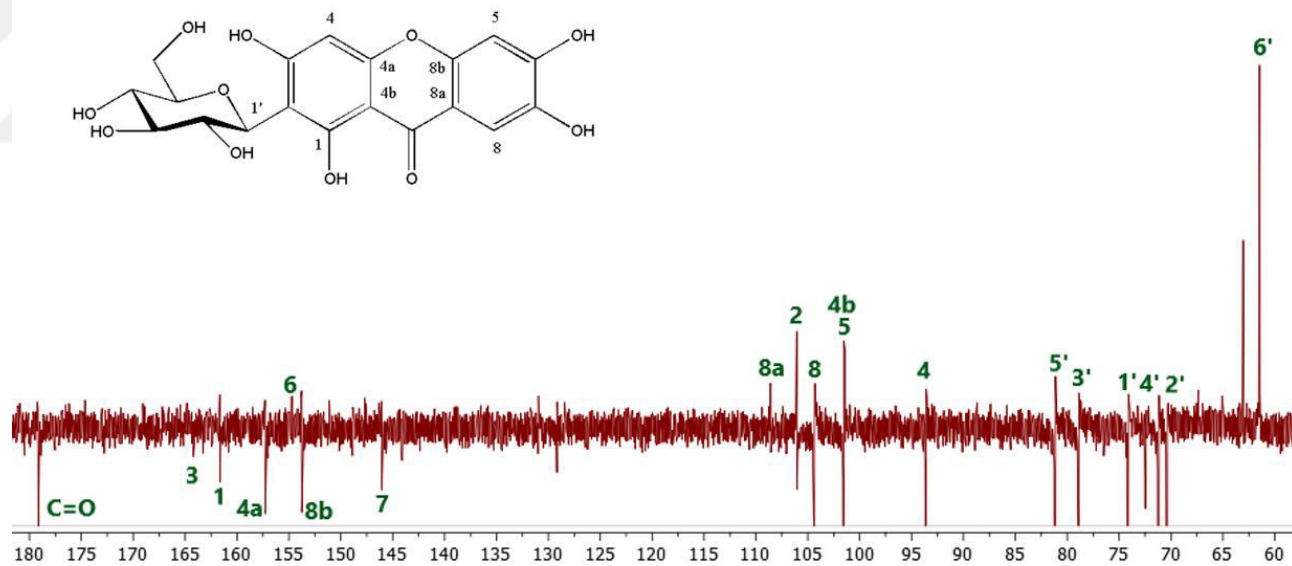
Table 96. ^1H and ^{13}C spectroscopic data of mangiferin (**11**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
1	C	161.5	-
2	C	106.1	-
3	C	164.1	-
4	CH	93.7	6.31 s
4a	C	157.2	-
4b	C	101.5	-
5	CH	101.6	6.57 s
6	C	154.7	-
7	C	146.1	-
8	CH	104.3	7.32 s
8a	C	108.7	-
8b	C	153.7	-
C=O	C	179.0	-
<i>Glucose</i>			
1'	CH	74.3	4.92 d (9.8)
2'	CH	71.3	4.23 t (9.1)
3'	CH	79.0	3.47-3.52 †
4'	CH	70.3	3.47-3.52 †
5'	CH	81.4	3.43 m
6'	CH ₂	61.5	3.88 dd (12, 2.3) 3.76 dd (12, 5.2)

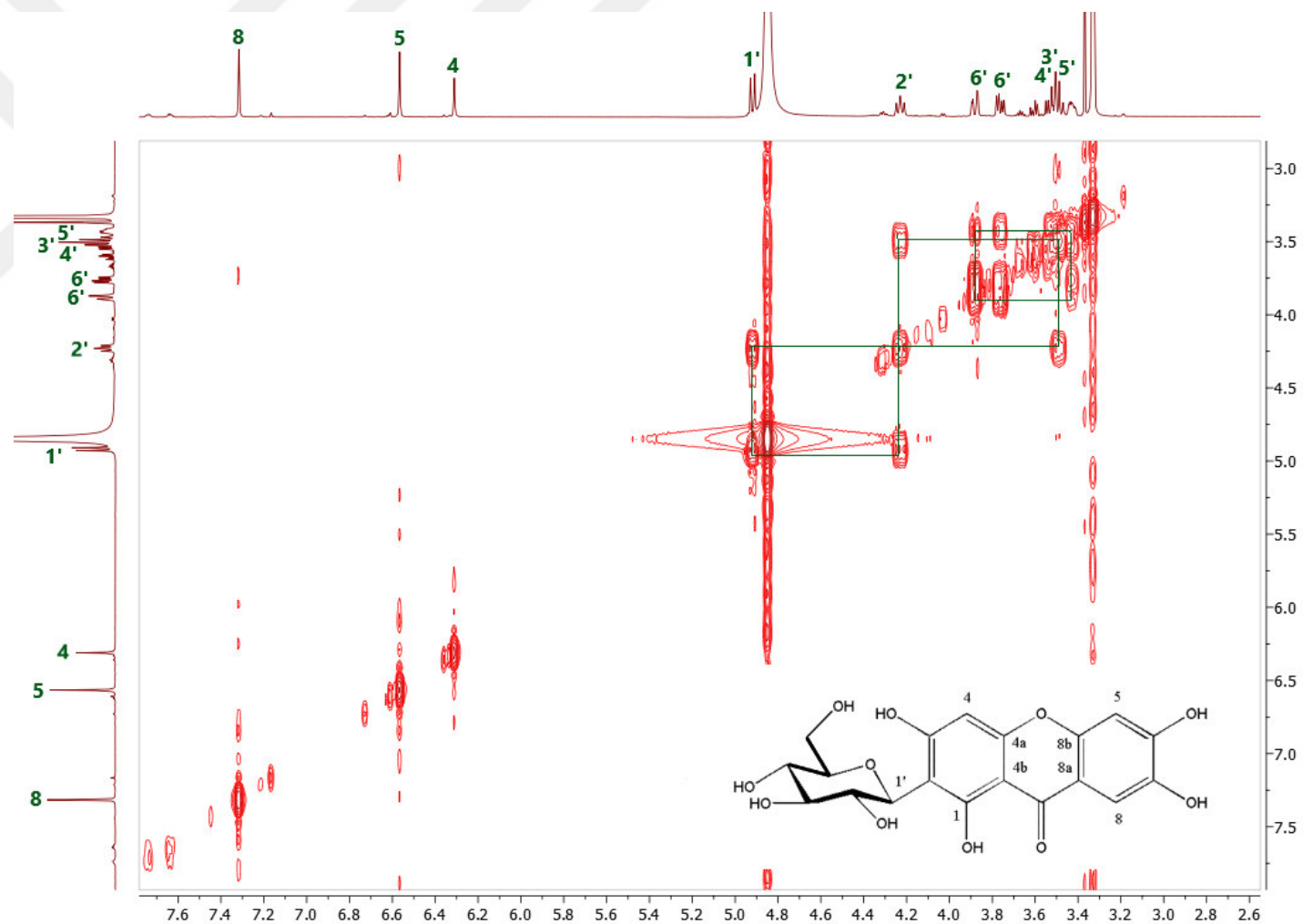
† Overlapping signals.



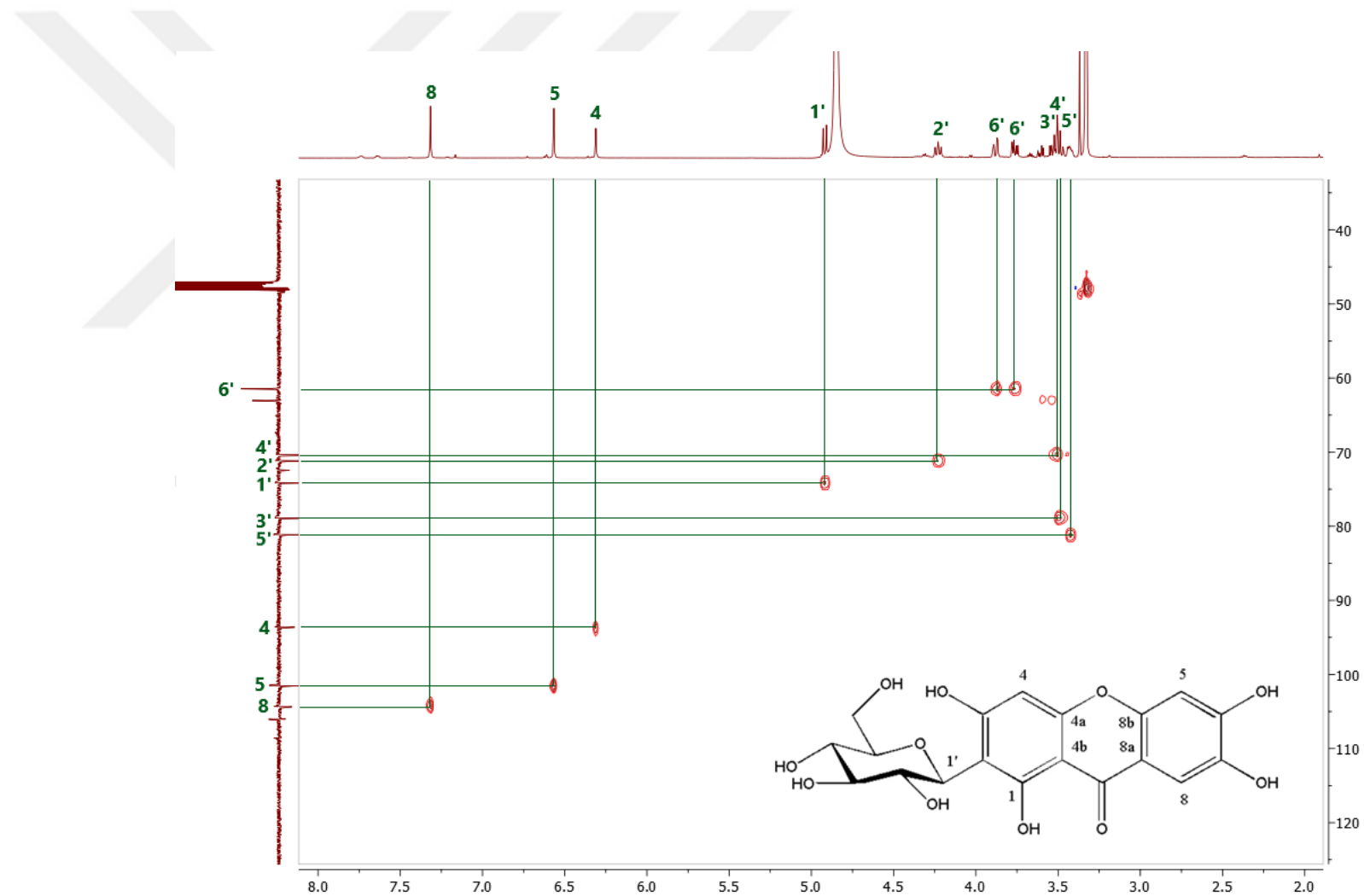
Spectrum 55. ^1H NMR spectrum of mangiferin (**11**) (CD_3OD , 500 MHz)



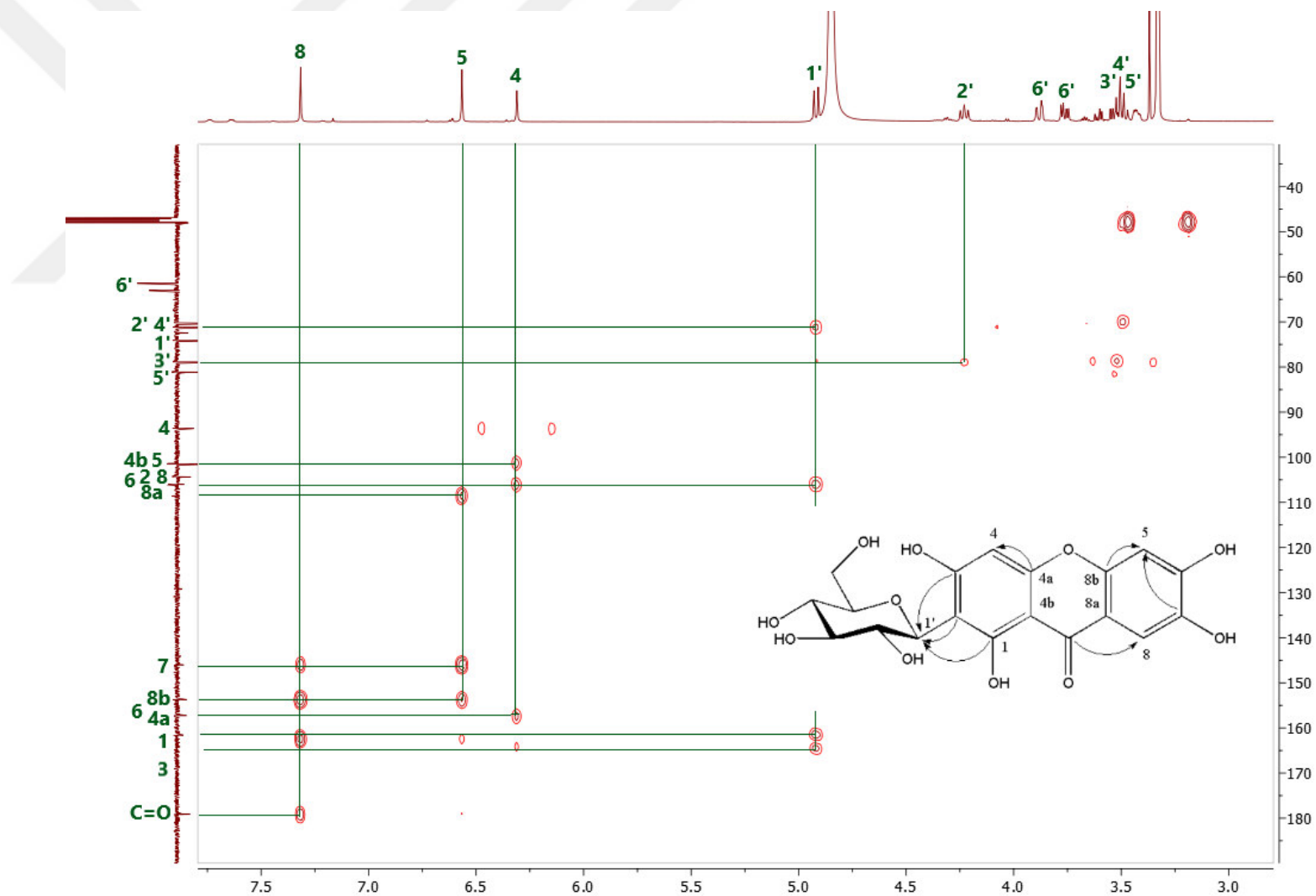
Spectrum 56. ¹³C-JMOD NMR spectrum of mangiferin (**11**) (CD₃OD, 125 MHz)



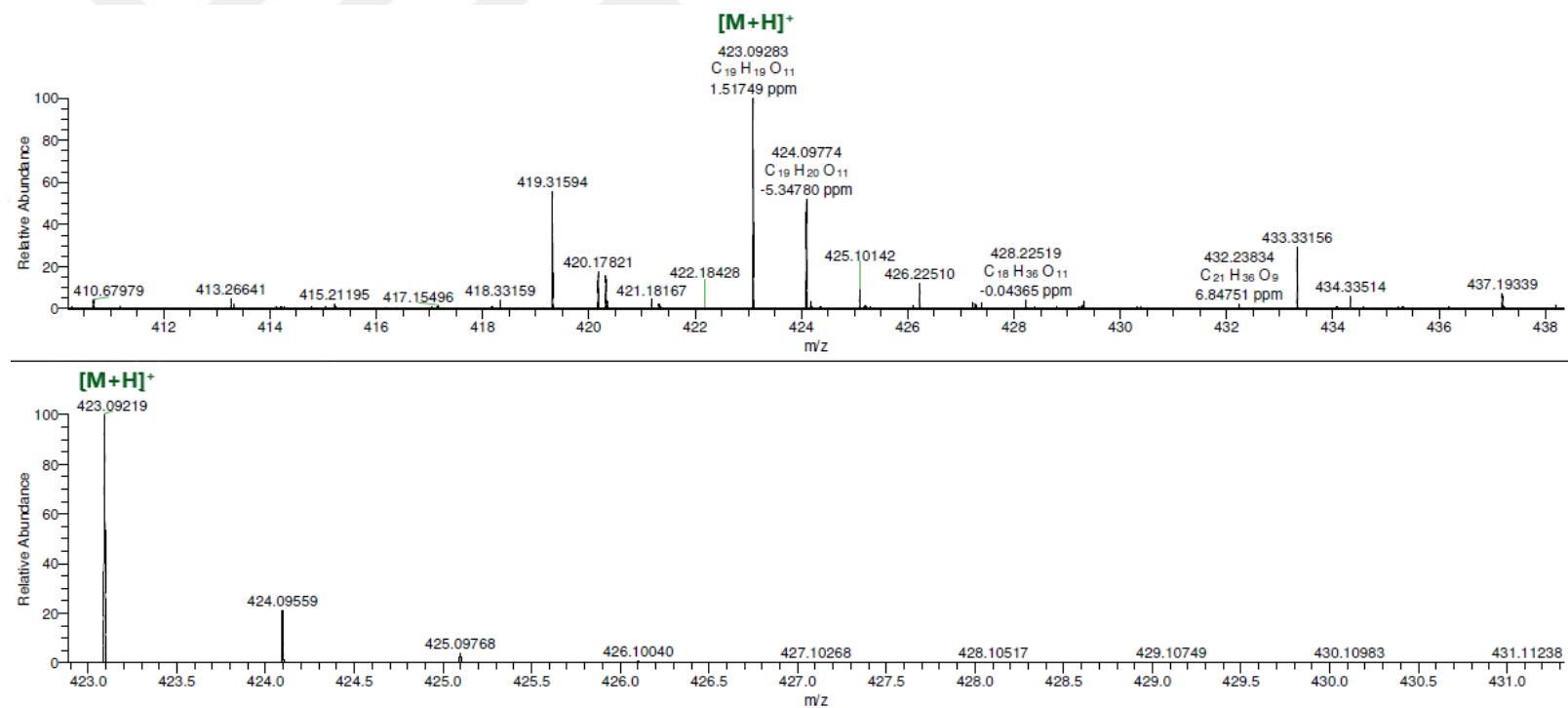
Spectrum 57. COSY spectrum of mangiferin (11)



Spectrum 58. HSQC spectrum of mangiferin (11)

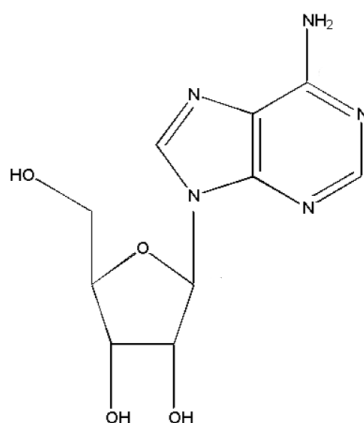


Spectrum 59. HMBC spectrum of mangiferin (**11**)



Spectrum 60. HR-ESI-MS spectrum of mangiferin (**11**)

4.2.5. Nucleoside



ADENOSINE (12), C₁₀H₁₃N₅O₄ (MW: 267.24)

UV λ_{max} (MeOH) nm:	260
¹ H NMR:	Spectrum 61, Table 97
¹³ C NMR:	Spectrum 62, Table 97
COSY:	Spectrum 63
HSQC:	Spectrum 64
HMBC:	Spectrum 65
HR-ESI-MS:	Spectrum 66

ADENOSINE (12)

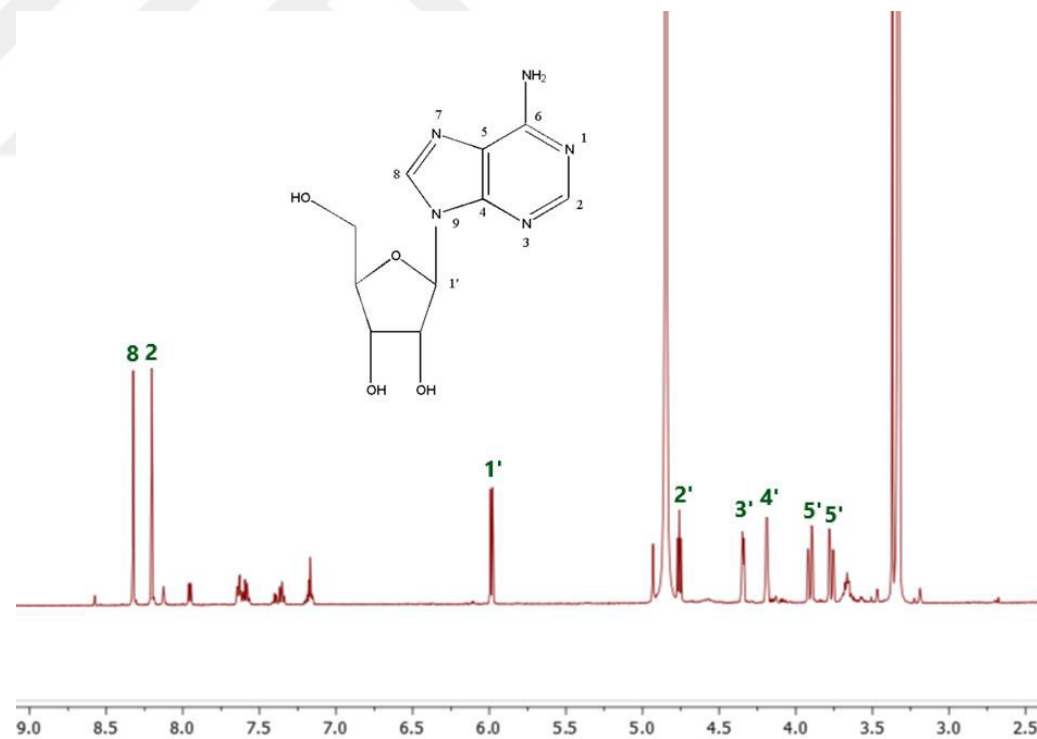
Compound **12** was obtained from the *n*-BuOH subextract of *G. asclepiadea* as a white powder. The HR-ESI-MS of **12** exhibited (M+H)⁺ ion at 268.1036 (calcd for C₁₀H₁₄N₅O₄, 268.1046), while its UV spectrum displayed absorption maxima at 260 nm.

In its ¹H NMR spectrum, two singlet signals were observed in the downfield area (δ_{H} 8.20, H-2 and δ_{H} 8.32, H-8) (Spectrum 60). The ¹³C-JMOD NMR spectrum of **12** displayed 10 carbon resonances. Five carbon resonances (δ_{C} 119.5-156.2) belonging to aglycone, and the remaining signals (δ_{C} 62.1-90.2) were attributed to a β -ribofuranosyl unit (Spectrum 61). In its COSY spectrum, a spin system between δ_{H} 5.98-3.77 (H-1' to H-5') was observed and ascribed to the sugar moiety (Spectrum 62). The HMBC spectrum of **12** also confirmed long-range correlations between carbons and atoms of the sugar moiety (Spectrum 64).

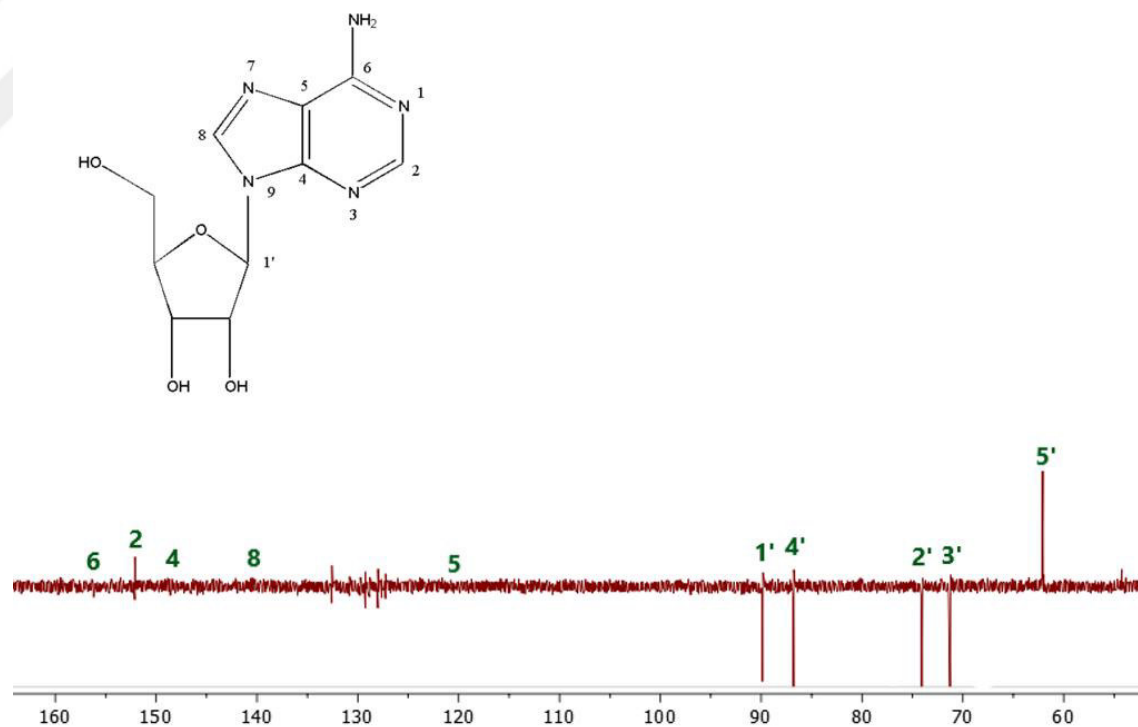
All the spectral data were compatible with those published and the structure of compound **12** was identified as adenosine (337).

Table 97. ^1H and ^{13}C spectroscopic data of adenosine (**12**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

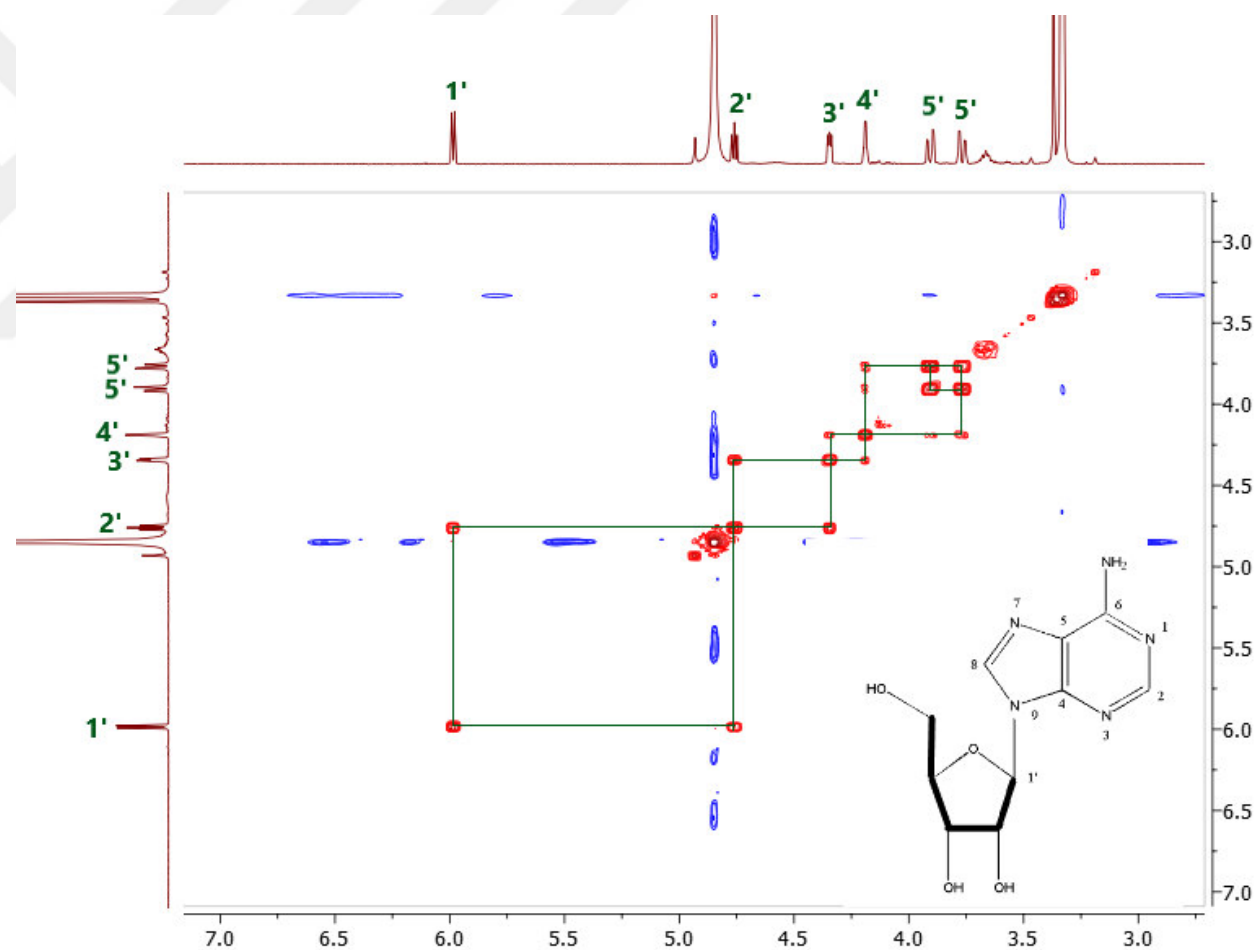
Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
<i>Aglycone</i>			
2	CH	152.1	8.20 s
4	C	148.6	-
5	C	119.5	-
6	C	156.2	-
8	CH	140.6	8.32 s
<i>Ribose</i>			
1'	CH	90.2	5.98 d (6.3)
2'	CH	74.1	4.76 t (5.7)
3'	CH	71.2	4.34 dd (5.0, 2.6)
4'	CH	86.8	4.19 m
5'	CH ₂	62.1	3.91 dd (12.6, 2.5) 3.77 dd (12.6, 2.6)



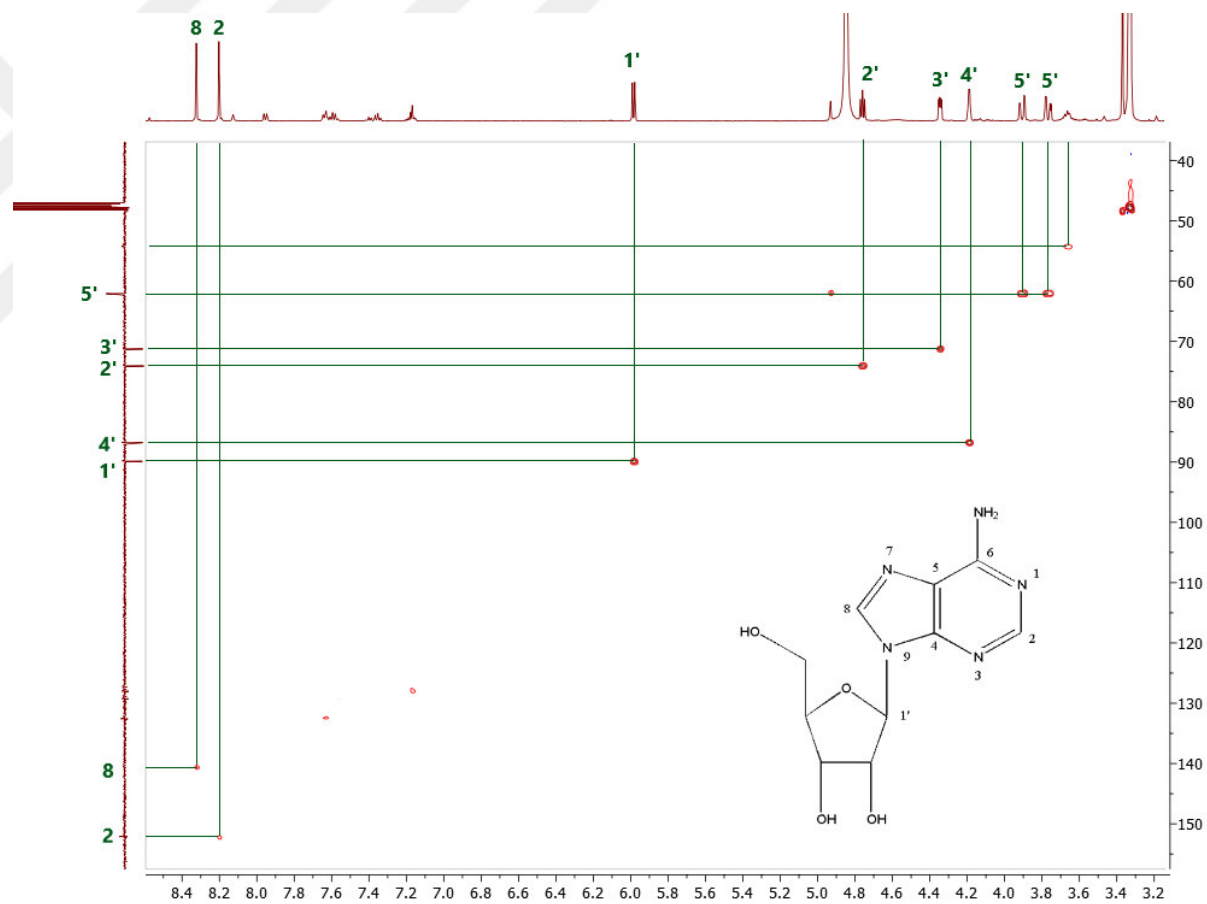
Spectrum 61. ^1H NMR spectrum of adenosine (**12**) (CD_3OD , 500 MHz)



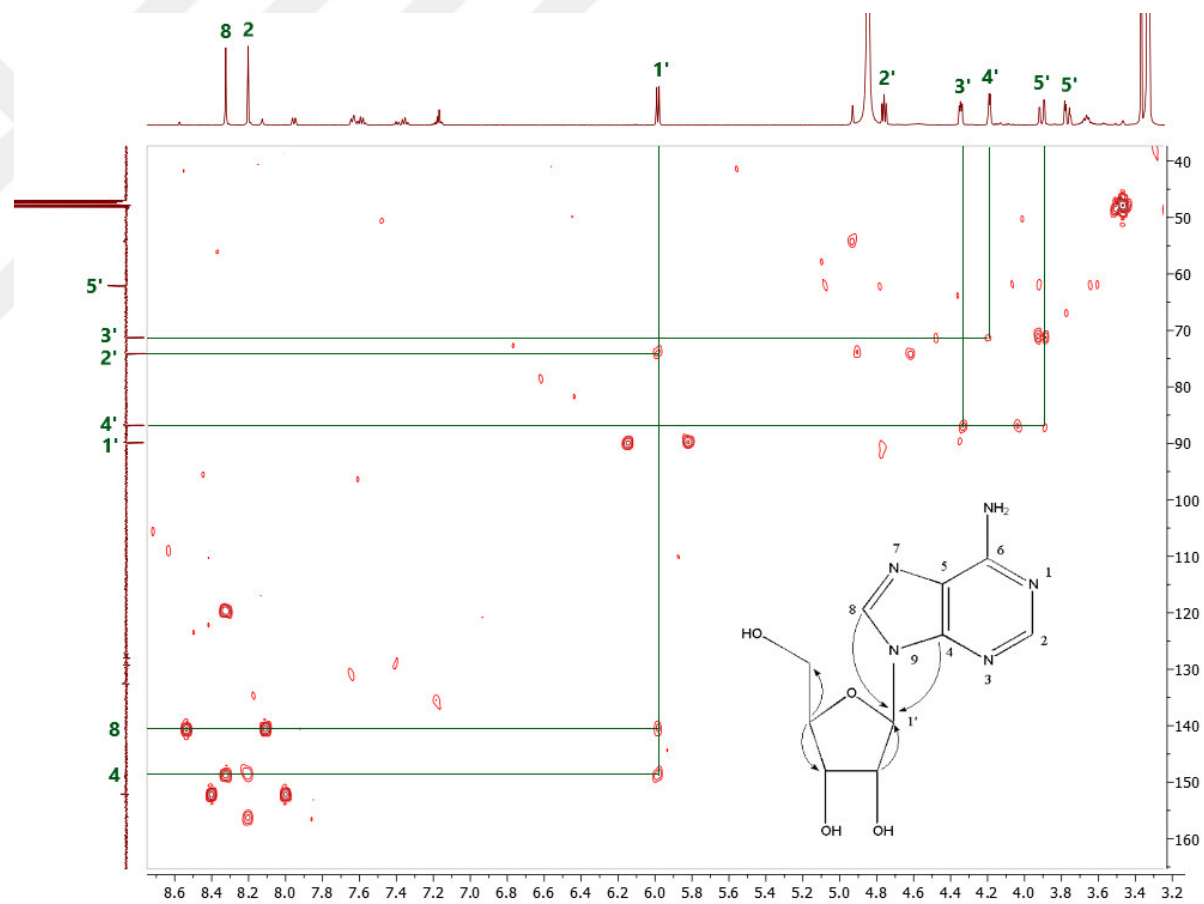
Spectrum 62. ^{13}C -JMOD NMR spectrum of adenosine (**12**) (CD_3OD , 125 MHz)



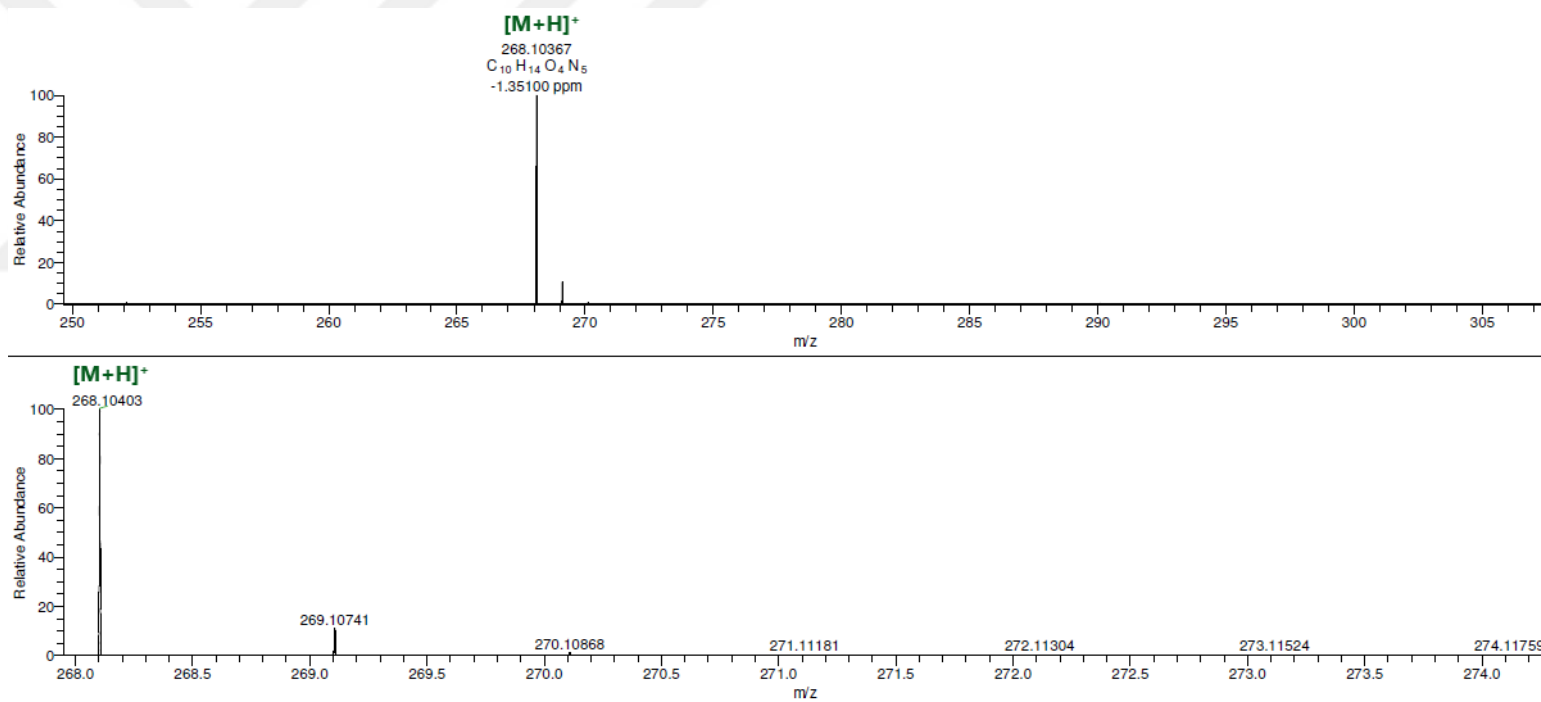
Spectrum 63. COSY spectrum of adenosine (12)



Spectrum 64. HSQC spectrum of adenosine (12)

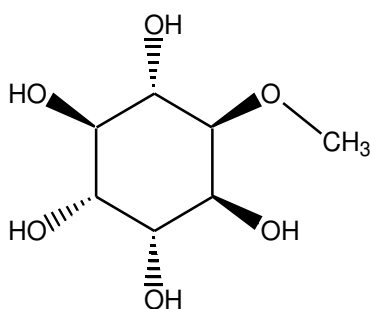


Spectrum 65. HMBC spectrum of adenosine (12)



Spectrum 66. HR-ESI-MS spectrum of adenosine (**12**)

4.2.3. Cyclitol



2- METHYL INOSITOL (13), C₇H₁₄O₆ (MW: 194.18)

IR $\nu_{\max}$ (KBr) cm ⁻¹ :	3412, 3321 (OH)
¹ H NMR:	Spectrum 67, Table 98
¹³ C NMR:	Spectrum 68
COSY:	Spectrum 69
HSQC:	Spectrum 70
HMBC:	Spectrum 71

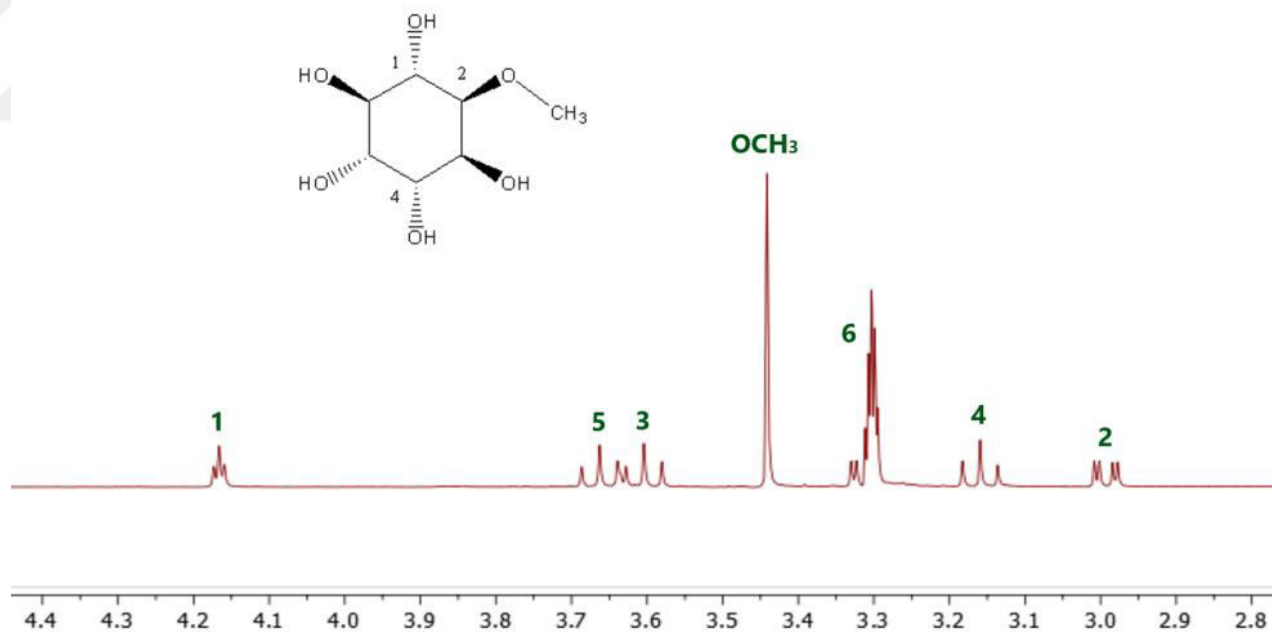
2- METHYL INOSITOL (13)

Compound **13** was purified from the *n*-BuOH fraction of the *G. cruciata* as a colorless crystalline powder. Its IR spectrum displayed absorption bands at 3412, and 3321 (OH).

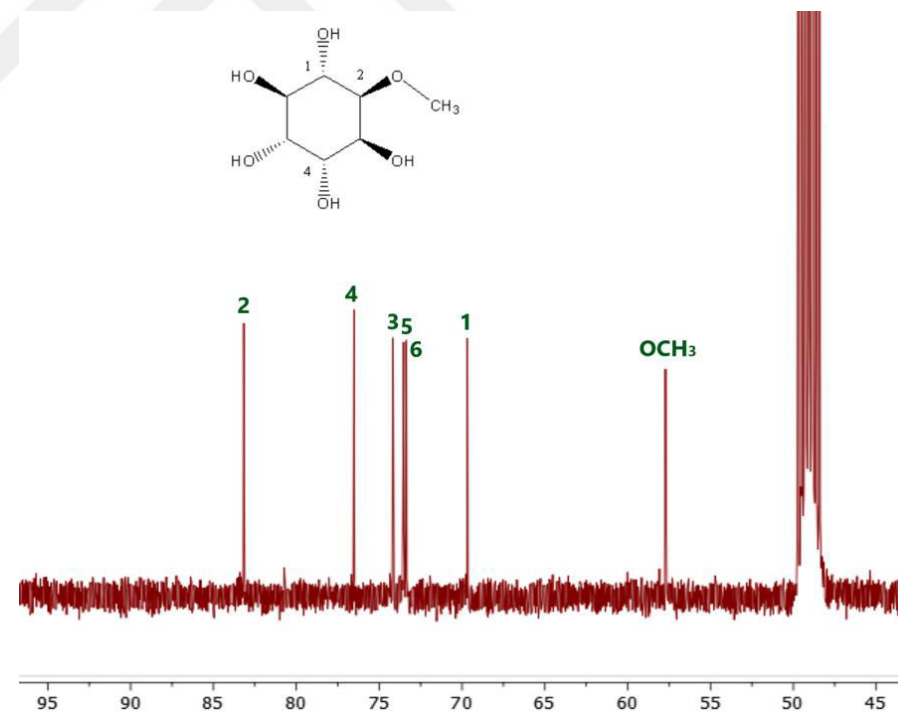
^1H NMR and ^{13}C NMR spectra of compound **13** displayed seven signals between δ_{H} 4.17-2.99 and δ_{C} 83.2-57.7, ascribed to a sugar derivative and a methoxy group. Singlet proton signal at δ_{H} 3.44 and carbon resonance at δ_{C} 57.7 was attributed to the methoxy unit (Spectra 66-67). The location of this unit was determined with the help of the HMBC experiment. The long-range couplings from δ_{C} 83.2 to δ_{H} 3.44 revealed the position of the methoxy unit at C-2 (Spectrum 70). The COSY spectrum indicated 2D- ^1H , ^1H homonuclear correlations between protons of the sugar unit (Spectrum 68). With regard to these findings, compound **13** was identified as 2-methyl inositol, a cyclic sugar alcohol, which was previously isolated from *Gentiana* species (338).

Table 98. ^1H and ^{13}C spectroscopic data of 2-methyl inositol (**13**) (CD_3OD , ^1H : 500 MHz, ^{13}C : 125 MHz).

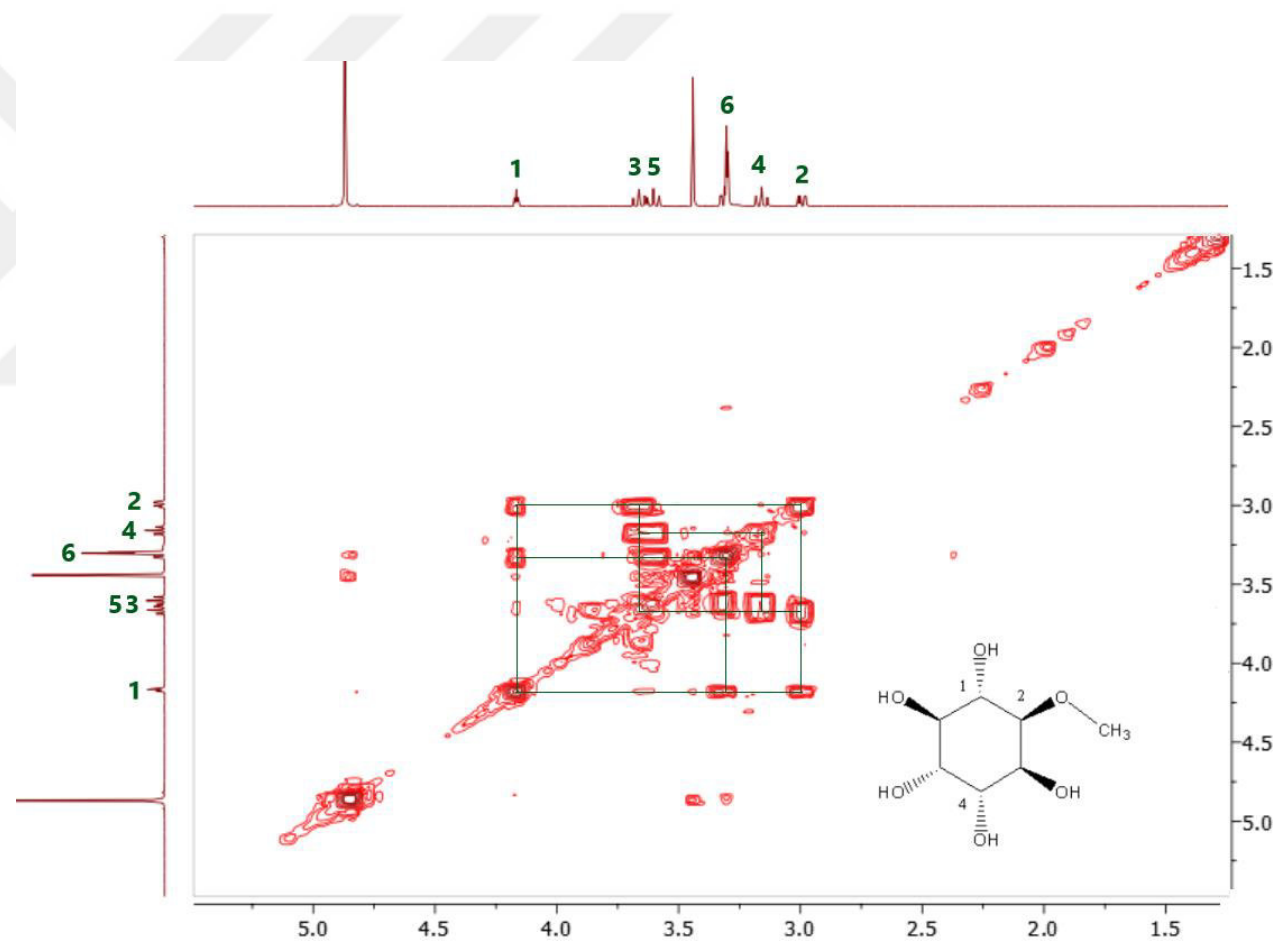
Position	Multiplicity	δ_{C} (ppm)	δ_{H} (ppm), J (Hz)
1	CH	69.6	4.17 t (2.7)
2	CH	83.2	2.99 dd (9.7, 2.7)
3	CH	74.1	3.60 t (3.6)
4	CH	76.5	3.16 t (9.3)
5	CH	73.6	3.66 t (9.5)
6	CH	73.3	3.30 m
OCH_3	CH_3	57.7	3.44 s



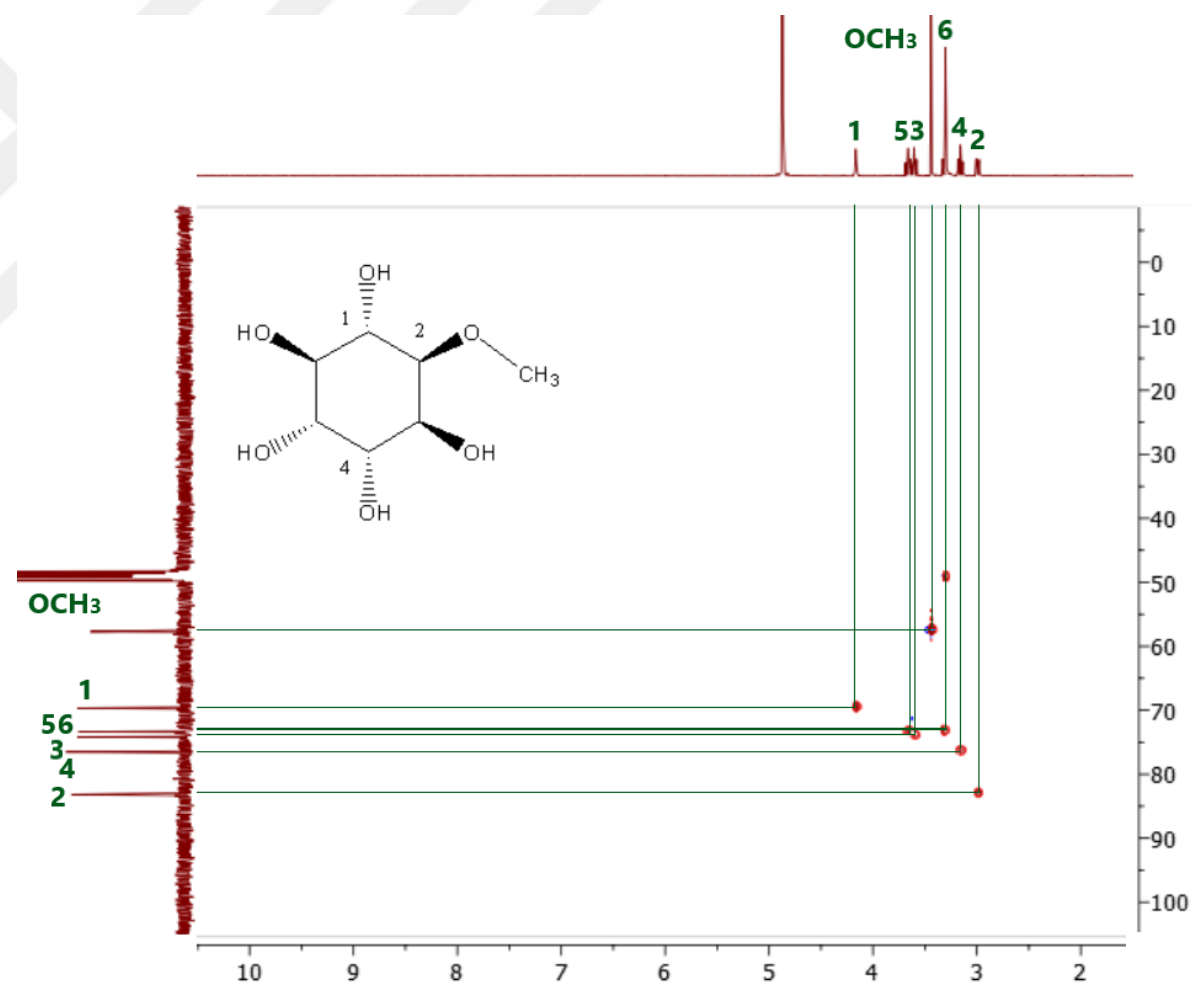
Spectrum 67. ^1H NMR spectrum of 2-methyl inositol (**13**) (CD_3OD , 500 MHz)



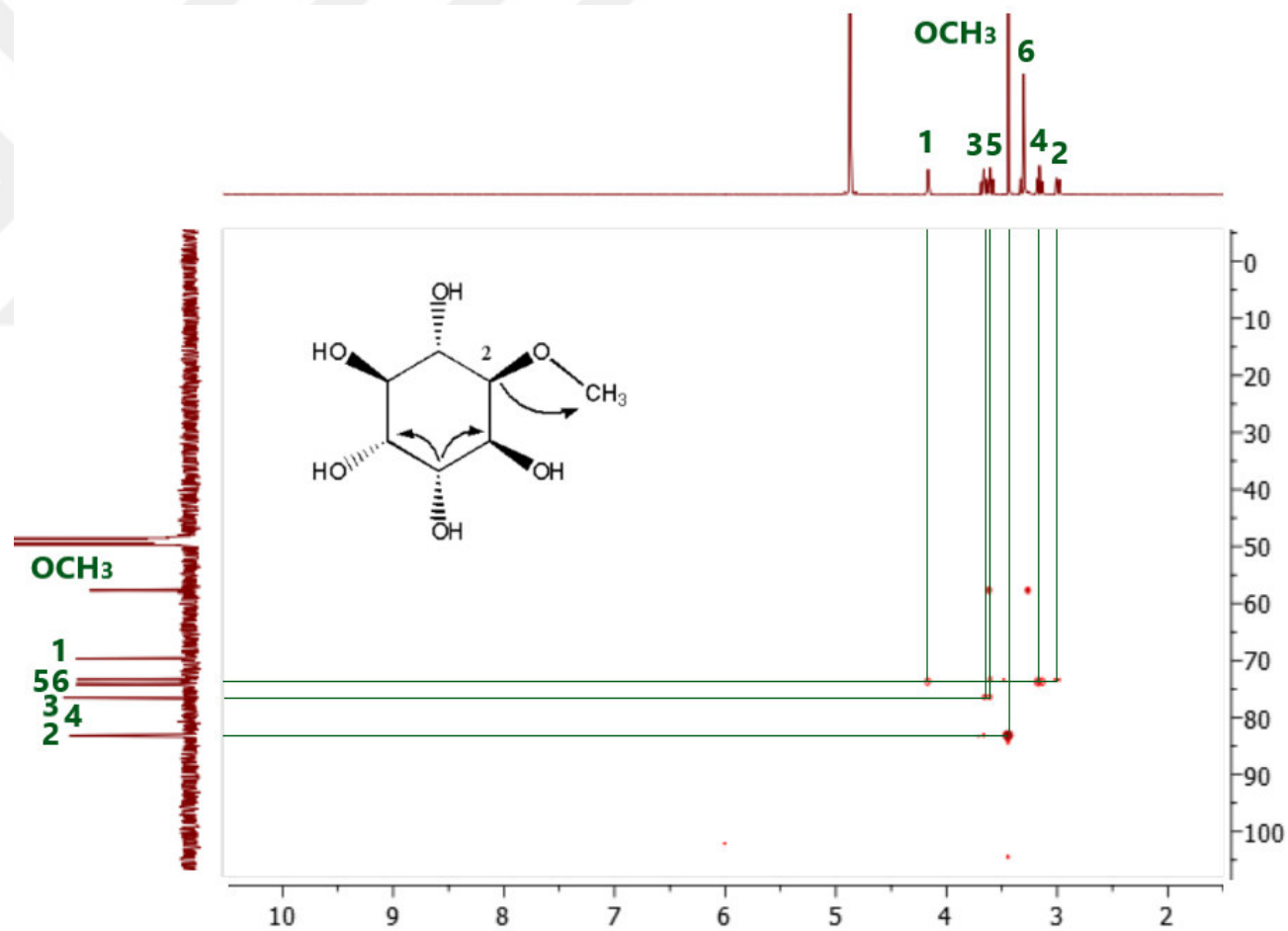
Spectrum 68. ^{13}C NMR spectrum of 2-methyl inositol (**13**) (CD_3OD , 125 MHz)



Spectrum 69. COSY spectrum of 2-methyl inositol (13)



Spectrum 70. HSQC spectrum of 2-methyl inositol (**13**)



Spectrum 71. HMBC spectrum of 2-methyl inositol (**13**)

4.3. Biological Activity Studies of Isolated Compounds

4.3.1. Cytotoxicity of the Isolated Compounds

Cytotoxicity of the isolates obtained from *G. asclepiadea* and *G. cruciata* was tested in RAW264.7 cells for 24 hours of exposure. According to the represented results, none of the isolated compounds showed remarkable toxicity at the highest dose 200 μ M, with relative cell viabilities above 70% compared to the control group. Therefore, 200 μ M was selected for further bioactivity studies. Among the tested isolates, only **7**, **13**, and **1** exerted dose-dependent cytotoxic activities with increasing doses, which were statistically insignificant.

Compound	Cell viability %	Nitrite level (μ M)	Nitrite inhibition %	PGE ₂ level (pg/mL)
<i>Ctrl</i>	102.98 $\pm$ 0.98	0.49 $\pm$ 1.72	-	49.43 $\pm$ 0.02
<i>Ctrl+LPS</i>	100.00 $\pm$ 2.24	39.00 $\pm$ 5.76	-	148.34 $\pm$ 2.04
1	91.51 $\pm$ 0.47	32.39 $\pm$ 2.55	16.64 $\pm$ 1.51	ni
2	92.66 $\pm$ 3.47	37.99 $\pm$ 2.51	3.78 $\pm$ 0.41	ni
3	97.19 $\pm$ 2.93	32.92 $\pm$ 3.62	15.49 $\pm$ 4.77	ni
4	89.58 $\pm$ 1.73	28.72 $\pm$ 7.36	23.97 $\pm$ 3.36	47.28 $\pm$ 0.51**
5	98.74 $\pm$ 0.64	28.86 $\pm$ 7.52	25.84 $\pm$ 3.22	100.18 $\pm$ 16.79*
6	102.31 $\pm$ 2.46	41.65 $\pm$ 5.62	ni	ni
7	89.86 $\pm$ 4.90	30.71 $\pm$ 2.91	18.11 $\pm$ 4.29	ni
8	95.01 $\pm$ 6.48	30.11 $\pm$ 2.12	22.90 $\pm$ 0.44	ni
9	92.81 $\pm$ 3.27	36.92 $\pm$ 5.92	5.68 $\pm$ 1.71	ni
10	94.31 $\pm$ 1.15	22.77 $\pm$ 5.37	39.46 $\pm$ 7.68	ni
11	95.82 $\pm$ 6.08	35.14 $\pm$ 5.16	10.44 $\pm$ 2.67	ni
12	100.58 $\pm$ 1.93	29.88 $\pm$ 3.24	18.93 $\pm$ 5.22	ni
13	93.67 $\pm$ 2.05	36.56 $\pm$ 4.56	5.92 $\pm$ 1.70	ni
<i>IND</i>	87.72 $\pm$ 5.28	28.18 $\pm$ 3.17	26.77 $\pm$ 4.19	94.45 $\pm$ 13.98**

Table 99. Relative cell viability, nitrite level, nitrite inhibition, and PGE₂ levels of isolated compounds from *Gentiana asclepiadea* and *G. cruciata* in LPS-activated RAW 264.7 cells.

Ctrl: Control group treated with culture medium; Ctrl+LPS: Control group activated with LPS; IND: Indomethacin (100 μ M), ni: No inhibition; LPS: Lipopolysaccharide from *Escherichia coli* 0111:B4. All isolated compounds were tested at 200 μ M. The results were expressed as mean $\pm$ SD. The significant differences between groups and Ctrl+LPS were defined with *p<0.05 **p<0.01.

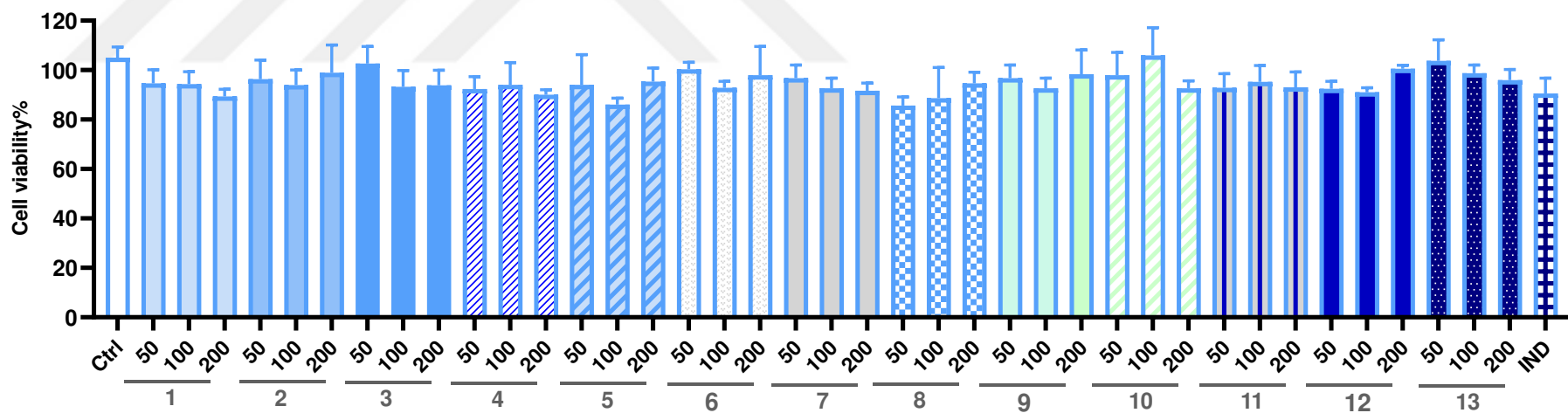


Figure 3. Cytotoxicity of compounds (1-13) from *Gentiana cruciata* and *Gentiana asclepiadea* in RAW264.7 cells. Ctrl: Control group treated with culture medium; IND: Indomethacin (100 μM).

4.3.2. Anti-inflammatory activity of the Isolated Compounds

4.3.2.1. Determination of Anti-Inflammatory Activity Through Nitrite Levels

The anti-inflammatory activities of the isolates yielded from *G. asclepiadea* and *G. cruciata* were assessed through nitrite, the stable metabolite of NO in RAW264.7 cells activated by LPS. Based on the results, LPS remarkably induced nitrite levels. Pretreatment with most of the compounds resulted in potent anti-inflammatory activities. Among all the tested compounds, **10** exerted the most potent nitrite inhibition with a $39.46 \pm 7.68\%$ relative nitrite inhibition rate, which is higher than the control IND (26.77 ± 4.19). Following the activity of **10**, compounds **5**, **4**, and **8** exhibited a significant nitrite inhibition over 20% compared to the control.

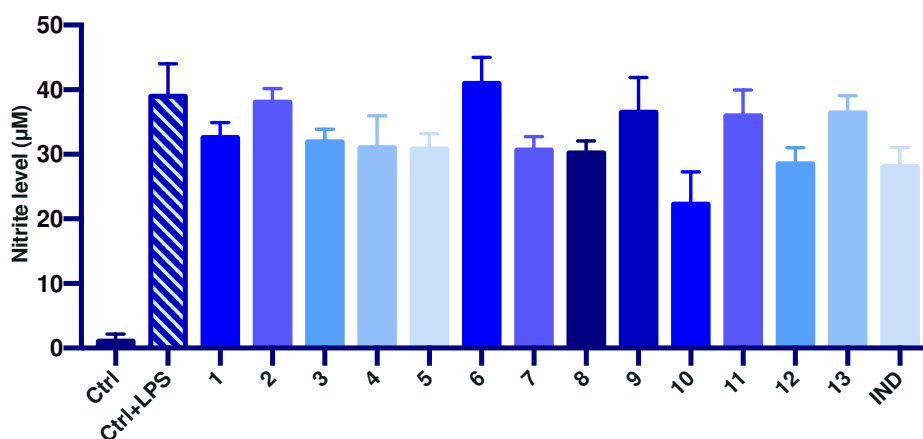


Figure 4. Nitrite level in LPS-activated RAW264.7 cells pre-treated with compounds (**1-13**). Ctrl: Control group treated with culture medium; Ctrl+LPS: Control group activated with LPS; IND: Indomethacin (100 µM); LPS: Lipopolysaccharide from *Escherichia coli* 0111:B4. All isolated compounds were tested at 200 µM. The results were expressed as mean $\pm$ SD. The significant differences between groups and Ctrl+LPS were defined with * $p < 0.05$; ** $p < 0.01$.

4.3.2.2. Determination of Anti-Inflammatory Activity Through IL-6 Levels

The anti-inflammatory activities of the isolates obtained from *G. asclepiadea* and *G. cruciata* were also evaluated through IL-6, a proinflammatory cytokine, in RAW264.7 cells activated by LPS. In comparison to nitrite inhibition production, IL-6 release demonstrated a different profile in the RAW264.7 cells pretreated with the isolated compounds. According to the results, compound **4** exhibited the highest decrease in IL-6 levels among all the isolated compounds, which was followed by compounds **5**, **8**, and **1**, respectively. In light of these results, compounds **1**, **4**, **8**, and **10** were determined as potent anti-inflammatory agents through both nitrite and IL-6 reduction.

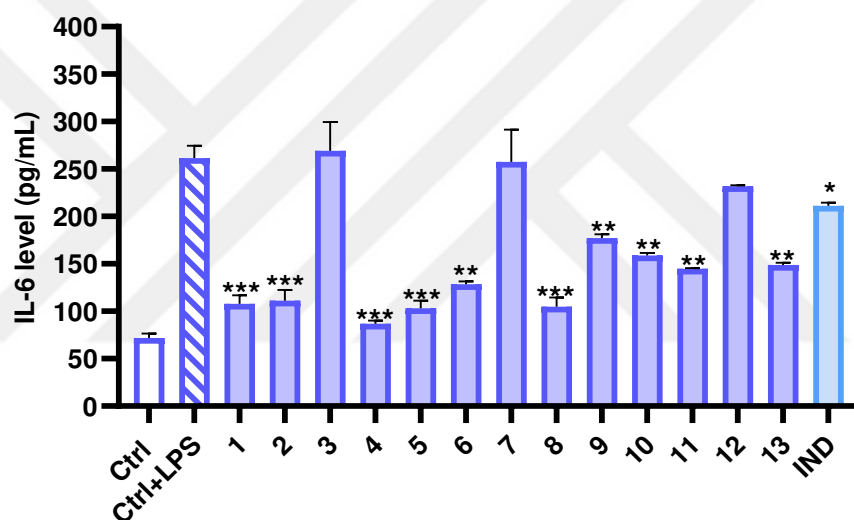


Figure 5. IL-6 level in LPS-activated RAW264.7 cells pre-treated with compounds (**1-13**). Ctrl: Control group treated with culture medium; Ctrl+LPS: Control group activated with LPS; IND: Indomethacin (100 μ M; LPS: Lipopolysaccharide from *Escherichia coli* 0111:B4. All isolated compounds were tested at 200 μ M. The results were expressed as mean $\pm$ SD. The significant differences between groups and Ctrl+LPS were defined with * p <0.05 ** p <0.01 and *** p <0.001.

4.3.3. Analgesic Activity of the Isolated Compounds

Analgesic activities of the compounds have been detected via a commercial ELISA kit in LPS-activated RAW264.7 cells through PGE₂ levels. Prostaglandin E₂ (PGE₂) is a potent mediator and plays a key role in acute and chronic inflammation. Results of the test revealed that compound **4** led to the highest decrease in the PGE₂ secretion. This activity was followed by compound **5**, which exhibited similar activity seen with the reference, IND. Besides, **7** also led to a slight decrease in the PGE₂ levels, however, it was statistically insignificant.

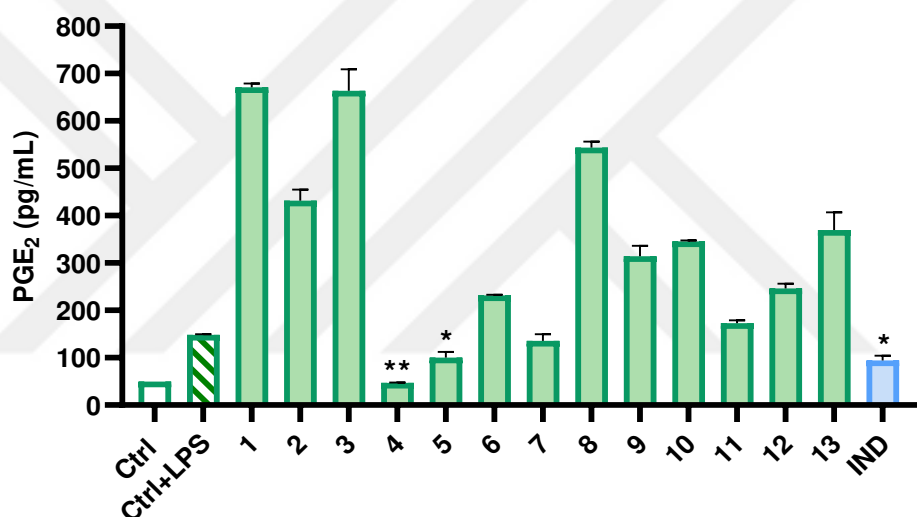


Figure 6. PGE₂ level in LPS-activated RAW264.7 cells pre-treated with compounds (**1-13**). Ctrl: Control group treated with culture medium; Ctrl+LPS: Control group activated with LPS; IND: Indomethacin (100 μ M); LPS: Lipopolysaccharide from *Escherichia coli* 0111:B4. All isolated compounds were tested at 200 μ M. The results were expressed as mean $\pm$ SD. The significant differences between groups and Ctrl+LPS were defined with * p <0.05; ** p <0.01.

5. DISCUSSION and CONCLUSION

Inflammation is the protective mechanism of the immune system to eliminate detrimental stimuli, clear damaged cells, and initiate tissue repair. Redness, swelling, heat, pain, and loss of tissue function are identified as five cardinal signs of inflammation. Inflammation can be caused by a number of factors, including infections, tissue injuries, and cardiac infarction (339). Inflammatory responses can be classified as either acute or chronic. Acute inflammation is the immediate host-protective response which is characterized by the rapid recruitment of granulocytes (neutrophils, eosinophils, and basophils) within a shorter resolution period (hours to days). Prostaglandins, leukotrienes, cytokines, chemokines, and other inflammatory mediators are responsible for the coordination of initial events of acute inflammation: regulation of vascular permeability and recruitment of leukocytes to the site of injury (340). Conversely, chronic inflammation represents a long-lasting inflammatory stimulus that is caused by aberrant activation of the immune systems. Chronic inflammation may be preceded by acute inflammation due to the failure of eliminating the agent causing inflammation (324).

Pain is defined as an uncomfortable physical sensation associated with illness or trauma. Inflammatory mediators contribute to the activation and sensitization of nociceptors, specialized nerve cell endings that relay noxious signals to initiate the sensation of pain. Several factors are associated with nociceptor sensitization, such as cytokines, chemokines, growth factors, ATP, H⁺, and hydroxynonenal. Dysregulated or unresolved inflammation may lead to chronic pain (341).

Today, chronic inflammatory diseases account for more than fifty percent of all deaths (2). Chronic inflammation is viewed as one of the major reasons of the development of serious illnesses including pulmonary disorders, cancer, cardiovascular diseases, metabolic syndrome, and many other autoimmune and neurodegenerative conditions. Inflammatory markers such as interleukins (IL-1, IL-6, IL-8), TNF- α , and CRP and transcription factors such as NF- κ B are identified as leading modulators and regulators in inflammation (342). NSAIDs and glucocorticoids are commonly utilized for their anti-inflammatory, antipyretic, and analgesic properties. However, their potentially harmful side effects led the pharmaceutical industry to develop safer alternatives (5).

Natural products are used as remedies for numerous health conditions since ancient times. Plant-derived products have made great contributions to drug discovery.

As an example, willow leaf extracts have long been used to relieve symptoms of inflammation and the development of formulas from salicylate-containing plants led to the discovery of acetylsalicylic acid, a globally used and known anti-inflammatory agent. Numerous plant extracts, remedies, and plant-derived semi-synthetic drugs were found effective against inflammatory diseases (343). Several plant metabolite groups, such as phenylpropanoids (polyphenols, stilbenoids, hydroxycinnamic acids, and derivatives), terpenes, and alkaloids were identified as anti-inflammatory agents (7).

The genus *Gentiana* (Gentianaceae) is a large genus containing around 400 species, mainly distributed in Southeast Asia, Europe, and North America (8). In traditional medicine, members of the genus *Gentiana* are consumed for stomach, liver, and gall diseases, and also against inflammation (19).

According to recent studies, extracts and isolated compounds from this genus were shown to possess antioxidant, antiatherosclerotic, anti-inflammatory, antityrosinase, cardioprotective, gastroprotective, hepatoprotective, and immunomodulatory activities (9). Furthermore, the roots of *G. lutea* (*Gentiana radix*) are described as an official drug and listed in many pharmacopeias, including European Pharmacopeia, British Pharmacopoeia, and Japanese Pharmacopoeia (10). TCM remedies, Longdan (*Gentianae Radix et Rhizoma*), Qin-Jiao (*Gentianae Macrophyllae Radix*) (21) and Honghualongdan are listed in Chinese Pharmacopoeia (9). More than 500 secondary metabolites were identified from *Gentiana* species, mainly belonging to xanthone, flavonoid, iridoid, triterpenoid, and alkaloid chemical classes (8).

In the flora of Türkiye, 12 species of the genus *Gentiana* were identified (11). Among these species, *G. asclepiadea* and *G. cruciata* are traditionally utilized as tonic and appetizer, also against fever and kidney pain (12,13). Recent biological activity studies on both species revealed their antioxidant, antibacterial, and hepatoprotective effects (14–18). Considering the anti-inflammatory and analgesic potentials of the isolates from *Gentiana* species, this thesis was designed to obtain the bioactive compounds from the two naturally grown *Gentiana* species, *G. asclepiadea* and *G. cruciata*. Therefore, aerial parts of these two species were extracted with MeOH. The crude extracts were dispersed in H₂O and partitioned against CHCl₃, EtOAc, and *n*-BuOH, respectively. EtOAc and *n*-BuOH subextracts of these species were submitted to multiple chromatographic separations to isolate potential bioactive metabolites. The

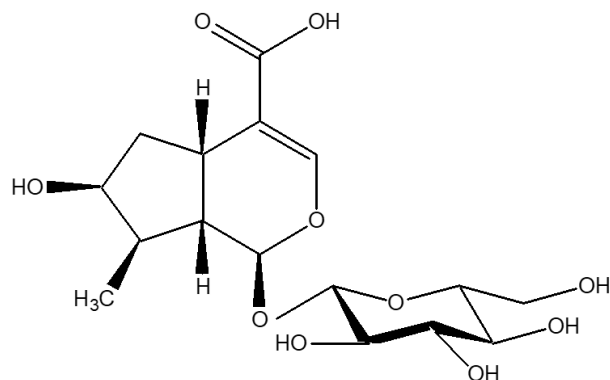
structures of the isolated compounds were determined by using NMR and HR-MS methods. Furthermore, *in vitro* anti-inflammatory and analgesic activities of the isolates were evaluated via LPS-induced RAW 264.7 murine macrophage cells.

EtOAc and *n*-BuOH subextracts of *G. asclepiadea* were subjected to Polyamide column to obtain main fractions. Further chromatographic methods (MPLC, SiO₂, and Sephadex LH-20) were used to purify bioactive compounds from these main fractions. Based on the isolation studies, depressine (**4**), gentiopicroside (**5**), loganic acid (**7**), isoorientin (**8**), isovitexin (**9**), mangiferin (**11**), and adenosine (**12**) were obtained. Our findings have just been published (344).

Similar chromatographic techniques were used to isolate metabolites from *G. cruciata*. EtOAc and *n*-BuOH subextracts of this species were loaded onto the Polyamide column to acquire main fractions. MPLC, SiO₂, and Sephadex LH-20 were used to obtain pure compounds from these main fractions. Accordingly, a new secoiridoid, named as cruciatoside (**1**) and 12 known compounds eustomoside (**2**), eustomorusside (**3**), gentiopicroside (**5**), 6'-*O*- β -D-glucosyl gentiopicroside (**6**), loganic acid (**7**), isoorientin (**8**), isovitexin (**9**), isovitexin 2''-(*E*)-ferulate (**10**), mangiferin (**11**), 2-methyl inositol (**13**) were yielded. The results have recently been published by us (345).

Iridoids include a broad group of cyclopenta(c)pyran monoterpenoids present in a limited number of plant genera. They are commonly found as glycosides and thus contributing to their water-soluble characteristic. There is a limited number of taxa that are reported to possess specific enzymes to form the cyclopentane ring, which is characteristic of carbocyclic iridoids. Therefore, iridoids are accepted as important chemotaxonomic markers (346).

The iridoids detected in Gentianaceae members are mainly loganin derivatives (deoxyloganin, loganin) which are also formed as intermediates in biosynthesis of indole alkaloids (330).

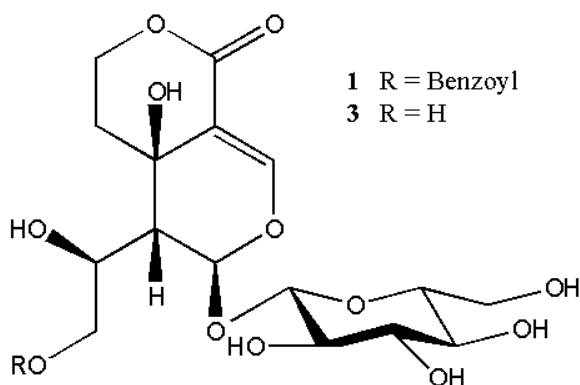


Loganic acid (**7**)

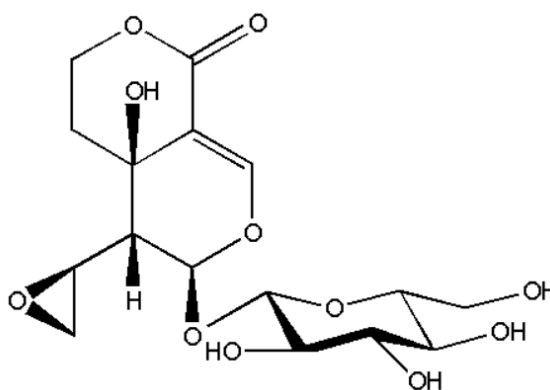
In this thesis, loganic acid (**7**) was obtained from EtOAc and *n*-BuOH subextracts of *G. asclepiadea* and *G. cruciata*. This loganin derivative was first isolated from *Lonicera periclymenum* by Çalış et al. (332). Based on the prior phytochemical studies, **7** was purified from several *Gentiana* sp., including *G. asclepiadea* (131) and *G. cruciata* (46).

Secoiridoid glycosides are commonly found in *Gentiana* and *Swertia* (Gentianaceae) species. Iridoid and secoiridoid glycosides have structural differences, such as oxidative cleavage at C7 and C8 (347). Secoiridoid glycosides are classified as sweroside-morroniside type, oleoside-10-hydroxyoleoside type, alkaloidal glycosides (containing a secoiridoid skeleton), and hydrangenosides (330).

In this study, cruciatoside (**1**), eustomoside (**2**), eustomorusside (**3**), depressine (**4**), gentiopicroside (**5**), 6'-*O*- β -D-glucosyl gentiopicroside (**6**) were identified as secoiridoid glycosides.



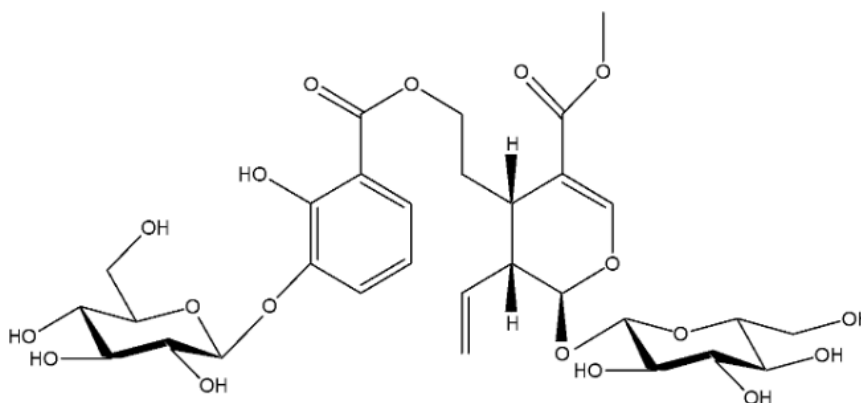
Cruciatoside (**1**) and eustomorusside (**3**)



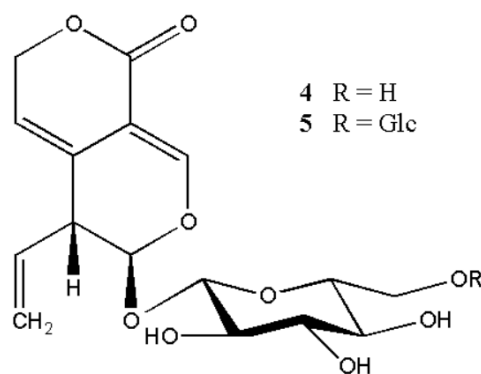
Eustomoside (2)

A new secoiridoid glycoside, cruciatoside (1) was obtained from the *n*-BuOH extract of *G. cruciata* along with secoiridoid glycosides eustomoside (2) and eustomorusside (3). Cruciatoside showed structural similarity to eustomorusside and all these three compounds can be classified as sweroside-morroniside type secoiridoid glycosides (330). Compounds 2 and 3 were first isolated from *Eustoma russellianum*, which is also a member of the Gentianaceae family (329). Therefore, the occurrence of such sweroside-morroniside type secoiridoid glycosides implies the chemotaxonomic relationship between the genera *Gentiana* and *Eustoma*. Compounds 2 and 3 were reported from various *Gentiana* sp. including *G. campestris*, *G. gelida*, *G. lutea*, *G. olivieri*, and *G. septemfida* (33,167,184).

Depressine (4) was yielded from *n*-BuOH extract from *G. asclepiadea*. This secoiridoid glycoside also bearing an aromatic acyl unit and was previously detected in *G. asclepiadea* and *G. depressa* (232). However, this study is the first record of the isolation of 4 from *G. asclepiadea*.

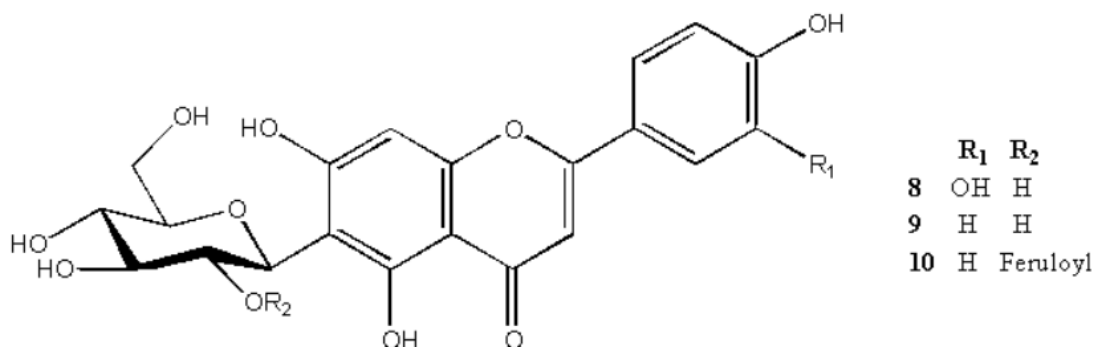


Depressine (4)



Gentiopicroside (**5**) and 6'-*O*- β -D-glucosyl gentiopicroside (**6**)

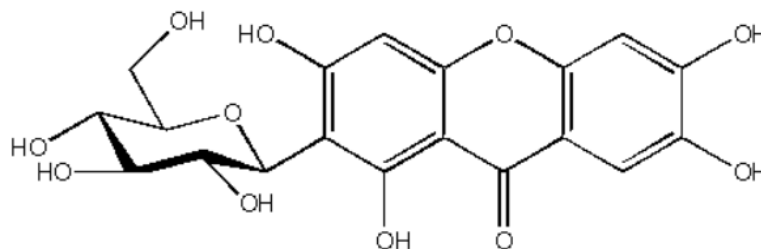
Gentiopicroside (**5**) was isolated from EtOAc subextracts of both species. It is a commonly detected secoiridoid in *Gentiana* species and was isolated from several species including *G. asclepiadea* (128–131) and *G. cruciata* previously (136–138). 6'-*O*- β -D-glucosyl gentiopicroside (**6**) was yielded from the *n*-BuOH extract of *G. cruciata*. This secoiridoid glycoside was previously detected in *G. cruciata* (131). However, this study is the first record of the isolation of **6** from this species.



Isoorientin (**8**), Isovitexin (**9**), Isovitexin 2''-(*E*)-ferulate (**10**)

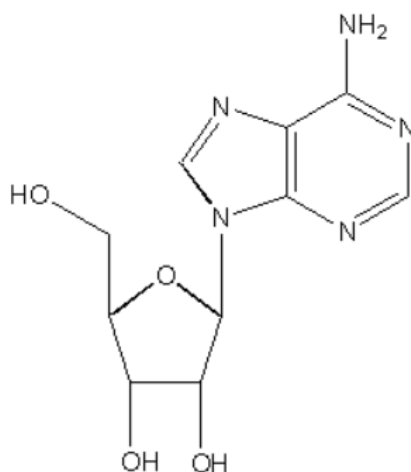
As a result of multiple chromatographic steps, compounds **8–10** were purified and identified as *C*-glycosides namely isoorientin, isovitexin, and isovitexin 2''-(*E*)-ferulate. Flavonoids from *Gentiana* species occur either in free or glycoside forms. The most commonly found flavonoids from this genus have been identified as isoorientin and isovitexin derivatives. Therefore, their widely distributed 4'-glycosylated derivatives indicate that isoorientin and isovitexin may be considered as key compounds in the flavonoid biosynthesis in this genus (8). Consistent with previous studies, structures of compounds **8–10** were determined as isoorientin, isovitexin, and isovitexin 2''-(*E*)-ferulate.

Isoorientin (**8**) is a flavone C-glycoside, also known as the 6-C-glucoside of luteolin. In this thesis, **8** was obtained from the *n*-BuOH subextract of *G. asclepiadea* and the EtOAc subextract of *G. cruciata*. It was previously isolated from *G. asclepiadea* (254,255) and *G. cruciata* (348). Isovitexin (**9**), another flavone C-glycoside, is known as apigenin-6-C-glucoside. In this study, it was yielded from the *n*-BuOH subextract of *G. asclepiadea* and the EtOAc subextract of *G. cruciata*. Isovitexin was also purified from *G. asclepiadea* (254,255) and *G. cruciata* (46) in prior studies. Isovitexin 2''-(*E*)-ferulate (**10**) was obtained from the EtOAc subextract of *G. cruciata*. It is a flavone C-glycoside esterified with (*E*)-ferulic acid. This compound was previously reported from other *Gentiana* species; however, this study is the first record of the isolation of **10** from *G. cruciata*.



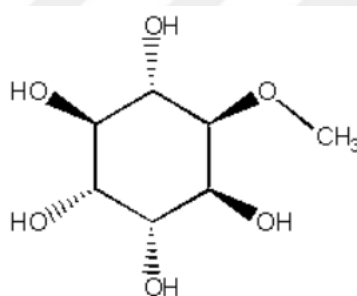
Mangiferin (**11**)

Xanthenes are oxygen-containing heterocyclic compounds with a basic skeleton of dibenzo- γ -pyrone. They are structurally related to flavonoids, and also they show similar chromatographic behavior. Flavonoids are widespread in kingdom Plantae, whereas xanthenes are distributed in a limited number of families, including Gentianaceae. Both *O*- and *C*-glycosidic xanthenes were detected in the genera *Swertia*, *Gentiana*, and *Canscora*. Xanthone-*C*-glycosides are considered as chemotaxonomic markers and they are closely related to *C*-glycoside flavonoids. Therefore, mangiferin is found commonly with isoorientin (349). Aerial parts of *Gentiana* members are reported to possess relatively higher amounts of xanthenes (9). Mangiferin (**11**) was yielded from *n*-BuOH extract of *G. asclepiadea* and the EtOAc extract of *G. cruciata*. Based on prior studies, it was isolated from both *G. asclepiadea* (15,130,264) and *G. cruciata* (350) species.



Adenosine (**12**)

Compound **12** was obtained from the *n*-BuOH subextract of *G. asclepiadea* and identified as adenosine, a nucleoside and primary metabolite. Adenosine was previously isolated from the *Gentiana* species (177).



2-Methyl inositol (**13**)

Compound **13** was obtained from the *n*-BuOH fraction of the *G. cruciata* and identified as 2-methyl inositol, a cyclitol. This compound was previously detected in *Gentiana* species (338).

Several chemical components, cells, and pathways are involved in the inflammatory processes (351). Immune system is designed to defend organism against foreign invaders or microorganisms, as well as damaged cells, which trigger inflammatory responses. The immune system consists of two main classes: innate and adaptive immune systems (352). Inflammatory responses are regulated through a class of receptors called peroxisome proliferator-activated receptors (PPARs), which also modulate COX-2 and NOS gene expressions.

Nitric oxide (NO) is produced by a reaction catalyzed by NOS and is a free radical with a short half-life. NO is an effector molecule of vasodilatation that plays key role in the inflammatory processes (353). However, when NO reacts with superoxide, this reaction yields peroxynitrate, a reactive nitrogen species (321). The generation of reactive oxygen and nitrogen species results in the response of cytokines and other signaling processes induced at the sites of inflammation (354).

Interleukin-6 (IL-6) is considered as a pleiotropic inflammatory cytokine that plays a pivotal role in inflammatory responses, hematopoiesis and immune system (320). It is produced in many tissues and various cell types such as monocytes, macrophages, fibroblasts, hepatocytes, and several types of tumor cells. Thus, IL-6 can induce a wide range of immune and physiological functions, including inflammation and antigen-specific immune responses. IL-6 production is also stimulated by inflammatory conditions and infections, and thus dysregulated or increased IL-6 levels are associated with chronic inflammation, autoimmune disorders, and tumor development (355).

Prostaglandins are lipid autacoids that are derived from arachidonic acid by the catalytic activity of COX isoenzymes. Prostaglandins play a crucial role in the generation of inflammatory responses and their biosynthesis is remarkably elevated in inflamed tissues (356). Both prostaglandins PGI₂ and PGE₂ have vasodilator effects and PGE₂ effectively stimulates hyperalgesia and fever (357). Nonsteroidal anti-inflammatory drugs are commonly used for blocking prostaglandin biosynthesis through selective inhibition of COX-2 (356).

Gentiana sp. have long been studied for their anti-inflammatory activities and numerous studies have been conducted to enlighten the underlying mechanisms of the effects of extracts, as well as isolated compounds. Extracts from different *Gentiana* species have been reported for their anti-inflammatory and analgesic properties, including *G. lutea* (76,308), *G. purpurea* (40), *G. kurroo* (124,125,246), and *G. striata* (123).

In this study, cytotoxicity of the isolates from *G. asclepiadea* and *G. cruciata* were tested in RAW264.7 cells. According to the results, none of the isolated compounds have shown significant toxicity at the highest concentration 200 µM, with relative cell viabilities above 70% compared to the control group. For this purpose, 200 µM was selected for further bioactivity tests. Among the tested compounds, **1**, **7**, and **13**, exerted

concentration-dependent cytotoxic activities with increasing concentrations, which were statistically insignificant (Fig. 3).

The anti-inflammatory activities of the compounds were evaluated through nitrite and IL-6 levels. Therefore, isolated compounds from *G. asclepiadea* and *G. cruciata* were assessed through nitrite in RAW264.7 cells activated by LPS. According to the represented results, LPS significantly induced nitrite levels. Most of the compounds were found to be effective after pretreatment. Among all the tested compounds, **10** exerted the most potent nitrite inhibition with a 39.46 % relative nitrite inhibition rate, which is higher than positive control indomethacin (26.77 % at 200 μ M). Following **10**, compounds **4**, **5**, and **8** exhibited a significant nitrite inhibition (over 20%) compared to the control (Fig. 4).

As the next step, isolated compounds from both species were assessed for their inhibitory effects through IL-6 level in RAW264.7 cells activated by LPS. In comparison to nitrite inhibition production, IL-6 release exhibited a different profile in the RAW264.7 cells pretreated with the isolated compounds. According to the results, compound **4** exhibited the highest decrease at 200 μ M in IL-6 levels among all the tested compounds, which was followed by compounds **5**, **8**, and **1**, respectively. In light of these results, compounds **1**, **4**, **5**, **8**, and **10** were determined as potent anti-inflammatory agents through both nitrite and IL-6 pathways (Fig. 5).

Analgesic activities of the compounds have been evaluated via a commercial ELISA kit in LPS-activated RAW264.7 cells through PGE₂ levels. Results of the test revealed that compound **4** led to the highest decrease (47.28 %) in the PGE₂ secretion at the tested concentration of 200 μ M. Following the effect of **4**, compound **5** exhibited similar activity (100.18 %) seen with the reference, indomethacin (94.45 %). Additionally, **7** also led to a slight decrease in the PGE₂ levels, however, it was statistically insignificant (Table 99, Fig.6).

Iridoids are structurally characterized as a six-membered ring with an O atom fused to a cyclopentane ring. Based on structure-activity relationship (SAR) studies, the double bond between C7 and C8 was identified as the principal structural characteristic of the anti-inflammatory activity (358). Iridoids were found effective against inflammatory diseases and showed inhibitory effects on COX-2, PGE₂, and thromboxane-B₂ (359). Ramírez-Cisneros et al. investigated the anti-inflammatory potentials of several

iridoids through COX-1 and COX-2 enzymes. Among the tested compounds, loganic acid exerted the highest level of inhibition of both COX enzymes (360). In another study, loganic acid exhibited anti-inflammatory activity by decreasing TNF- α and IL-6 activity (361). Simu et al. investigated the biological activity of a loganic acid derivative: 8-*epi*-7-deoxyloganic acid. According to the results, 8-*epi*-7-deoxyloganic acid suppressed NO generation, inhibited activation of MAPKs, and led to reduced production of NF- κ B, TNF- α , IL-1 β , IL-6, formation of NO and PGE₂ through inhibiting iNOS and COX-2 expression levels (362). In this study, loganic acid (**7**) exhibited a slight anti-inflammatory profile through nitrite inhibition (18.11 % at 200 μ M).

Secoiridoids are formed by the oxidative cleavage of the C7-C8 bond of the cyclopentane residue. Several secoiridoid glucosides were found effective against inflammatory diseases and pain in prior studies including amarogentin (363,364), amaroswerin (365), 8-*epi*-kingiside and kingiside derivatives (200), gentimilegenin A, gentimilegenin B and swerimuslactone A derivatives (154). Sweroside-morroniside type secoiridoids glycosides consist of a large group of secoiridoids including secologanin, sweroside, swertiamarin, gentiopicroside, eustomoside, eustomorusside and morroniside (330). In this study, all the isolated secoiridoids **1-6** can be classified as sweroside-morroniside type. Many members of this class were reported for their anti-inflammatory and analgesic properties.

Eustomoside (**2**) and eustomorusside (**3**) are structurally related to swertiamarin. Because, **2** is an epoxide of this compound and co-occurs with the diol **3** in *Eustoma russellianum* (330). Further studies confirmed their co-existence in *Gentiana* species, including *G. campestris* (176), *G. gelida* (181), *G. lutea* (184), *G. olivieri* (33), *G. septemfida* (167). Saravanan et al. reported that swertiamarin reduced secretion of the cytokines IL-1, TNF- α , IL-6, enzymes MMPs, iNOS, PGE₂, PPAR γ , COX-2, and also modulated NF- κ B/I κ B and JAK2/STAT3 signaling (366). In another study, swertiamarin decreased pro-inflammatory cytokines IL-6, TNF- α , IL-17, IFN- γ , and TGF- β levels effectively. There are no studies in the literature that confirm the anti-inflammatory and analgesic potencies of **2** and **3**, however aforementioned findings supported the potential of the swertiamarin against inflammation and pain, which is structurally related to **2** and **3**. Furthermore, cruciatoside (**1**) is the *O*-benzoyl derivative of **3** and is also structurally related to these compounds. Based on the results, isolates **1** and **2** exhibited a higher decrease in IL-6 levels, in comparison to **3**. On the other hand, **1** and **3** exhibited higher

nitrite inhibition rates, compared to **2**. Overall, newly identified compound **1** showed the most potent anti-inflammatory effect among these three secoiridoid glycosides. Furthermore, the presence of benzoyl moiety in **1** seems to have only a positive effect of its reduction on IL-6 levels when compared to **3**.

Depressine (**4**) is a secologanol derivative secoiridoid glycoside that was previously isolated from *G. depressa* (15,232), whereas it was also detected in *G. asclepiadea* (15). However, there are no prior studies conducted for evaluating the biological activity of **4**. In this study, compound **4** exerted the highest decrease in IL-6 levels among all the compounds. Moreover, it moderately inhibited NO levels (23.97 %). In addition, compound **4** led to the highest decrease (47.28 pg/mL) in PGE₂ level among all tested compounds, which represented a higher inhibition rate compared to the positive control IND (94.45 pg/mL). All these findings concluded that compound **4** is a promising compound for the development of new therapeutic alternatives for inflammatory diseases and pain.

Gentiopicroside (**5**) is one of the most commonly detected secoiridoids in the *Gentiana* species. Numerous studies have been conducted for evaluating its anti-inflammatory and nociceptive properties. Choi et al. reported that gentiopicroside downregulated cytokine gene expression of NFκB1, TNF-α, and IL-6 (220). In another study, gentiopicroside inhibited NO production in LPS-stimulated RAW264.7 cells concentration-dependently (213). Gentiopicroside and its effect on gastric tissue are investigated by Yang et al. and according to the results, pretreatment with gentiopicroside remarkably decreased TNF-α and IL-6 levels (367). Zhang et al. investigated the anti-inflammatory effect of gentiopicroside and activity was ascribed to the downregulation of NO, PGE₂, and IL-6, and also to the suppression of iNOS and COX-2 (122). The results of our research were consistent with the previous findings. Compound **5** exerted a potent inhibitor activity on NO (25.84± 3.22) and IL-6 levels. Moreover, **5** exerted analgesic activity (100.18± 16.79) similar to those seen with IND (94.45± 13.98). In light of these findings, compound **5** can be considered a potential therapeutic candidate for further activity studies.

6'-O-β-D-glucosyl gentiopicroside (**6**) is a β-glycoside derivative of **5**. There are no prior studies conducted for evaluating its anti-inflammatory activity. Based on the results of this research, it exhibited an inhibitory effect on IL-6 levels. From this

standpoint, it can be considered that β -glycosidation may diminish the anti-inflammatory and analgesic potencies of gentiopicroside derivatives.

Some structural modifications of flavonoids which are widely distributed in genus *Gentiana* have been identified as requirements for anti-inflammatory activity. Studies revealed that double bonds in the C ring, carbonyl group at C-4, the number and position of OH groups, and glycosylation determine the activity (368). Moreover, the hydroxylation pattern of the B ring led to the promotion of cytokine inhibition secreted by mast cells and macrophages, (369).

Various mechanisms of action were proposed to enlighten the anti-inflammatory and analgesic properties of flavonoids. Briefly, regulation of inflammation-related cellular activities (mast cells, macrophages, lymphocytes, and neutrophils), suppression of histamine release, inhibition of T-cell proliferation, modulation of enzyme activities such as arachidonic acid (AA), phospholipase A₂ (PLA₂), COX, LOX and NOS, and reduction in the generation of prostaglandins, leukotrienes and NO can be given as examples (370).

In this thesis, the flavonoids were elucidated as isoorientin (**8**), isovitexin (**9**), and isovitexin 2''-(*E*)-ferulate (**10**). Numerous studies conducted to confirm the biological activity of **8**. Anilkumar investigated the bioactivity of isoorientin and according to the results, isoorientin decreased TNF- α , 5-LOX, COX-2, IL-1 β , and IL-6 expression (371). In another study, isoorientin exerted a potent inhibition of COX-2 (372). In a study published by Li et al., isoorientin reduced generation of TNF- α , IL-6, and IL-1 β (373). It is also reported that isoorientin inhibited iNOS and COX-2 expression, as well as TNF- α and IL-6 release (374). In this study, isoorientin moderately inhibited NO (22.90 ± 0.44) and decreased IL-6 levels. The anti-inflammatory and analgesic potency of compound **9** has been also widely investigated. Lv et al. demonstrated that isovitexin decreased iNOS and COX-2 expression (375). In another study, isovitexin inhibited IL-2, IL-17A, TNF- α , and IFN- γ expressions (376). In also this study, isovitexin reduced IL-6 levels.

There are no prior studies conducted for evaluating the *in vitro* biological activity of isovitexin 2''-(*E*)-ferulate (**10**). Compound **10** is the feruloyl derivative of **9** and previous studies reported that ferulic acid inhibited LPS-induced NO generation and iNOS expression (377), and also IL-6 and TNF- α expression (378). In this study, compound **10** demonstrated the most potent nitrite inhibition (with a 39.46 % relative

nitrite inhibition rate) among all the compounds and showed higher activity than the positive control IND. Moreover, **10** decreased IL-6 levels. It can be concluded that, feruloyl moiety may increase anti-inflammatory activity.

Xanthones are hetero-tricyclic compounds and are considered as chemomarkers. Their biological activities were evaluated in previous studies. These studies reported that they suppressed iNOS expression, decreased NO and PGE₂ production, and inhibited TNF- α -induced activation of NF- κ B (379). Mangiferin (**11**) is a C-glycosidic xanthone that is also investigated for its anti-inflammatory and analgesic properties. Bhatia et al. stated that mangiferin potently decreased PGE₂ and COX-2 protein synthesis induced by LPS (380). In another study, mangiferin inhibited NO and PGE₂, suppressed protein levels of iNOS and COX-2, reduced TNF- α release and IL-6 expression levels and inhibited NF- κ B activation (381). Based on the results of this study, **11** reduced IL-6 levels.

As a part of this thesis, primary metabolites were also assessed for their anti-inflammatory and nociceptive properties. Adenosine (**12**) slightly inhibited NO (18.93 ± 5.22) and decreased IL-6 levels. Besides, 2-Methyl inositol (**13**) reduced IL-6 levels. Our results have recently been published (344,345).

In conclusion, this thesis represents some results that are conforming with current literature on both species, and also some results for contributing to existing literature. These include the first biological activity studies of some isolated compounds. According to the represented results, cruciatoside (**1**), depressine (**4**), gentiopicroside (**5**), isoorientin (**8**), and isovitexin 2''-(*E*)-ferulate (**10**) might be considered as potential candidates and deserve further *in vitro* and *in vivo* anti-inflammatory and analgesic activity studies on the way to discover new drug leads. Considerably more work will need to be performed to determine the mechanism of actions underlying these biological activities. A greater focus on *Gentiana* species growing wild in Türkiye could produce interesting findings that will contribute to the literature on drug discovery and adjuvant therapies from natural sources.

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7. CURRICULUM VITAE

Personal Information

Name	Rima	Surname	Konya
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Education

Level	Proficiency	Institution	Graduation
Ph.D.	Pharmacognosy	Yeditepe University	2023
M.Sc.	Phytotherapy	Yeditepe University	2017
B.Sc.	Pharmacy	Yeditepe University	2015
High School	-	TED Kdz Ereğli College	2010

Language Skills

English: C1 (Fluent), IELTS: March 2023 (7.5), YÖKDİL: March 2022 (90)
German: B1 (Intermediate)
Spanish: A1 (Beginner)

Computer Skills

Microsoft Office	Very Good
MestreNova	Very Good
Mendeley	Very Good
ChemDraw	Very Good

Scientific Works

Publications in SCI, SSCI, AHCI Indexed Journals

Konya, R., Reis, R., Sipahi, H., Barta, A., Hohmann, J., Kırmızıbekmez, H., 2023. Iridoids and flavonoids from the aerial parts of <i>Gentiana asclepiadea</i> L. with anti-inflammatory and analgesic activities. J. Res. Pharm. (in press)
Konya, R., Reis, R., Sipahi, H., Barta, A., Hohmann, J., Kırmızıbekmez, H., 2022. Secondary metabolites from <i>Gentiana cruciata</i> L. and their anti-inflammatory and analgesic activities. Nat. Prod. Res. 1–8. DOI: 10.1080/14786419.2022.2144301
Erdoğan, M., Konya, R., Özhan, Y., Sipahi, H., Çinbilgel, İ., Masullo, M., Piacente, S., Kırmızıbekmez, H., 2021. Secondary metabolites from <i>Scutellaria brevibracteata</i> subsp. <i>subvelutina</i> and their <i>in vitro</i> anti-inflammatory activities. S. Afr. J. Bot. 139, 12–18. DOI:10.1016/j.sajb.2021.01.028

International Conference Proceedings

Konya, R., Barta, A., Hohmann, J., Kırmızıbekmez, H., 2022. Secondary metabolites from <i>Gentiana cruciata</i> L. GA – 70th International Congress & Annual Meeting of the Society for Medicinal Plant and Natural Products, Thessaloniki, Greece (Poster Presentation)
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National Conference Proceedings

Konya, R., Barta, A., Hohmann, J., Kırmızıbekmez, H., 2022. <i>Gentiana asclepiadea</i> L. bitkisinden elde edilen sekonder metabolitler. XXIV. Bitkisel İlaç Hammeddeleri Toplantısı. 2022, June 23-26. Ankara, Türkiye. (Poster Presentation)

Awards & Certificates

Best Poster Presentation Award, XXIV. Bitkisel İlaç Hammeddeleri Toplantısı. 2022, June 23-26. Ankara, Türkiye
Certificate of Forensic Pharmacy Training, Forensic Sciences Association, Forensic Pharmacy Commission, June, 2022

Liquid Chromatography Systems (HPLC and LC-MS MS) Applied Basic Training, Istanbul University, Cerrahpaşa Continuing Education Application and Research Center, June 2022
Scientific Phytocosmetics Training, Yeditepe University, October 2021
Scientific Apitherapy Training, Yeditepe University, June 2021
Scientific Aromatherapy Training, Yeditepe University, March 2021
Goethe Institut, German Language, Teilnahmebestätigung (participation certificate), A2.1, A2.2, B1.1, B1.2, B1.3 level courses, October 2020-December 2021
Certificate of Participation to IPSF Student Exchange Programme- Research Internship- Medical University of Lodz – Bioinorganic Chemistry Department, July 2019