T.C.
YEDİTEPE UNIVERSITY
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PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATIONS ON *VERBASCUM BUGULIFOLIUM* L.

DOCTOR OF PHILOSOPHY THESIS

Ayşe Gökmen, Pharm.

İstanbul-2021

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SUPERVISOR
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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 01/03/2021 and numbered 2021/02-16.

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

11/02/2021

Ayşe Gökmen

DEDICATION

To my family with love….

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LIST OF SYMBOLS and ABBREVIATIONS

1D	1-Dimensional
2D	2-Dimensional
4-HNE	4-hydroxymonenal
AA	Arachidonic acid
ABTS	2,2′-Azinobis(3-ethylbenzthiazoline)-6-sulfonic acid
Ac	Acetyl
Acet	Acetoxy
BuOH	Butanol
CC	Column Chromatography
CCl_4	Carbon Tetrachloride
CD_3OD	Deuterated Methanol
$CDCl_3$	Deuterated Chloroform
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
COSY	Correlation Spectroscopy
CoQ10	Coenzime Q10
COX	Cyclooxygenase
COXIBs	COX-2 inhibitors
Cuprac	Cupric reducing antioxidant
d	Doublet
DPPH•	1,1-diphenypicryl-hydrazil radical
DMSO	Dimethyl Sulfoxide
EtOAc	Ethyl acetate
EtOH	Ethanol
GI	Gastrointestinal
Glc	Glucose
GlcA	Glucuronic acid
GSH	Glutathione
H_2O	Water
HMBC	Heteronuclear Multi-Bond Correlation
HPLC	High Performance Liquid Chromatography
ESI-MS	Electrospray Ionisation- Mass Spectrometry
HO_2•	Hydroperoxyl
HOCl	Hypochlorous acid
H_2O_2	Hydrogen peroxide
HNO_2	Nitrous acid
HSQC	Heteronuclear Single Quantum Coherence
IC_{50}	The Half Maximal Inhibitory Concentration

IL-1	Interleukin-1
IL-6	Interleukin-6
IR	Infrared Spectroscopy
LA	Linoleic acid
LP	Lipid peroxidation
LOO•	Lipid peroxyl
LOX	Lipooxygenase
LOOH	Lipid peroxide
m	Multiplet
MDA	Malondialdehyde
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
MPLC	Medium Pressure Liquid Chromatography
MS	Mass Spectrometry
MW	Molecular Weight
NA	Not Active
NDGA	Norhydroguairetic acid
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Enhancement Spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
NO•	Nitric oxide
NO_2•	Nitrogen dioxide
N_2O_3	Dinitrogen trioxide
O_2•	Superoxide
OH•	Hydroxyl
O_3	Ozone
ONOO-	Peroxynitrate
PDA	Potatoes Dextrose Agar
PUFA	Polyunsatured fatty acids
R•	Alkyl radical
RP-TLC	Reverse-phase TLC
Rha	Rhamnose
RNS	Reactive nitrogen species
RO•	Alkoxyl
ROO•	Lipid peroxyl
RO_2•	Peroxyl
ROS	Reactive oxygen species
s	Singlet
SiO_2	Silica gel
SOD	Super Oxide Dismutase

TLC	Thin Layer Chromatography
TNF	Tumor necrosis factor
IFNs	Type-1 interferons
UV	Ultraviolet

ABSTRACT

Gökmen, A. (2021). Phytochemical and biological investigations on *Verbascum bugulifolium* L. Yeditepe University, Institute of Health Sciences, Department of Pharmacognosy, Ph. D. Thesis, İstanbul. The genus *Verbascum* comprises about 255 species in the Flora of Turkey. The flowers and leaves of some *Verbascum* species are used for the treatment of rheumatic disorders, eczema, spasmodic coughs, as an antiseptic and expectorant agent in different traditional systems. In this study, the antioxidant (DPPH•, ABTS• and CUPRAC), anti-inflammatory (LOX inhibition) and antimicrobial activities of the crude MeOH extract and its subextracts obtained from the aerial parts of *Verbascum bugulifolium* were investigated by *in vitro* methods. Among the tested extracts, EtOAc and *n*-BuOH subextracts exhibited the highest antioxidant activity in DPPH, ABTS and CUPRAC assays (IC_{50} = 20-90 µg/mL). The *in vitro* anti-inflammatory activity of the extracts was measured using a LOX inhibition assay where *n*-BuOH subextract provided a 35.7% inhibition at 50 µg/mL. The antimicrobial activity of the extracts was also evaluated against three bacteria and three fungal strains. Only the *n*-hexane subextract indicated remarkable antibacterial activity against *P. aeruginosa* ATCC 10145 (MIC = 125 µg/mL), while $CHCl_3$ subextract showed moderate activity against *C. krusei* ATCC 6258 (MIC= 250 µg/mL). Subsequent chromatographic studies on the *n*-BuOH, EtOAc and $CHCI_3$ subextracts yielded five iridoid glycosides [catalpol (**1**), specioside (**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**)]; two phenylethanoid glycosides [verbascoside (**6**), and glucopyranosyl-(1→Gi-6)-martynoside (**7**)]; five flavonoids [luteolin (**8**), luteolin 7-*O*-*β*-D-glucopyranoside (**9**), luteolin 7-*O*-rutinoside (**10**), apigenin 7-*O*-rutinoside (**11**), quercetin 3-*O*-rutinoside (**12**)]; and one phytosterol [*β*-sitosterol (**13**)]. Compounds **2** and **6**-**8** elicited the equipotent or quite higher ABTS scavenging activity compared to the standard ascorbic acid in the range of 10-30 µg/mL. Compound **6** showed the highest CUPRAC activity, which was as good as gallic acid with IC_{50}=10 µg/mL. Compounds **6** and **8** showed the highest antioxidant activity in all tests where **8** showed a relatively remarkable anti-inflammatory activity compared to other samples with 54.1 % inhibition at 1 µg/mL. All the tested compounds indicated weak antimicrobial activity against tested microorganisms. This is the first phytochemical and bioactivity study on *V. bugulifolium*.

Key Words: *Verbascum bugulifolium;* Scrophulariaceae, secondary metabolites, iridoid glycosides; phenolic compounds; antimicrobial, antioxidant and anti-inflammatory activities

ÖZET

Gökmen, A. (2021). *Verbascum bugulifolium* L. üzerinde fitokimya ve biyolojik aktivite araştırmaları. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü. Farmakognozi ABD, Doktora Tezi, İstanbul. *Verbascum* cinsi Türkiye florasında yaklaşık 255 tür ile temsil edilmektedir. Bazı *Verbascum* türlerinin çiçek ve yaprakları çeşitli geleneksel tıp sistemlerinde romatizmal rahatsızlıklar, egzama ve öksürük tedavisinde, antiseptik ve balgam söktürücü olarak kullanılmaktadır. Bu çalışmada *Verbascum bugulifolium*'un topraküstü kısımlarından elde edilen MeOH ekstresinin ve alt ekstrelerin antioksidan (DPPH•, ABTS• ve CUPRAC), antienflamatuvar (LOX inhibisyon) ve antimikrobiyal aktiviteleri *in vitro* yöntemlerle araştırılmıştır. Test edilen ekstreler arasından EtOAc ve *n*-BuOH alt ekstreleri DPPH, ABTS ve CUPRAC deneylerinde en iyi antioksidan aktiviteyi göstermiştir (IC_{50} = 20-90 µg/mL). Ekstrelerin *in vitro* antienflamatuvar aktiviteleri LOX inhibisyon yöntemiyle araştırılmış olup *n*-BuOH alt ekstresi 50 µg/mL konsantrasyonda %35.7 inhibisyon yapmıştır. Ekstrelerin antimikrobiyal aktiviteleri üç bakteri ve üç fungus suşuna karşı test edilmiştir. Sadece *n*-hekzan alt ekstresi *P. aeruginosa'*ya karşı antimikrobiyal aktivite göstermişken (MIC = 125 µg/mL), $CHCl_3$ alt ekstresi *C. krusei* suşuna karşı orta derecede aktivite göstermiştir (MIC= 250 µg/mL). *n*-BuOH, EtOAc ve $CHCI_3$ alt ekstreleri üzerinde yapılan kromatografik çalışmalar sonucunda beş iridoit glikoziti [katalpol (**1**), spesiozit (**2**), ajugol (**3**), ajugozit (**4**), 8-*O*-asetilharpagit (**5**)]; iki feniletanoit glikoziti [verbaskozit (**6**), ve glukopiranozil-(1→Gi-6)-martinozit (**7**)]; beş flavonoit [luteolin (**8**), luteolin 7-*O*-*β*-D-glukopiranozit (**9**), luteolin 7-*O*-rutinozit (**10**), apigenin 7-*O*-rutinozit (**11**), kersetin 3-*O*-rutinozit (**12**)] ve bir fitosterol [*β*-sitosterol (**13**)] elde edilmiştir. **2** ve **6**-**8** ABTS deneyinde 10-30 µg/mL konsantrasyon aralığında askorbik asitle kıyaslandığında eşit ya da yüksek antioksidan aktivite göstermiştir. Bileşik **6** CUPRAC yönteminde IC_{50}=10 µg/mL değeriyle gallik asit kadar iyi bir aktivite göstermiştir. **6** ve **8** tüm antioksidan testlerde en yüksek aktiviteyi göstermişken, **8** diğer bileşiklere göre 1 µg/mL konsantrasyonda %54.1 inhibisyonla en iyi antienflamatuvar aktiviteyi göstermiştir. Bileşikler test edilen mikroorganizmalara karşı zayıf antimikrobiyal aktivite göstermiştir. Bu çalışma *V. bugulifolium* bitkisi üzerine yapılmış ilk fitokimyal ve biyoaktivite çalışmasıdır.

Anahtar Kelimeler: *Verbascum bugulifolium*, Scrophulariaceae, sekonder metabolitler, iridoit glikozitleri, fenolik bileşikler, antimikrobiyal, antioksidan ve antienflamatuvar aktivite

1. INTRODUCTION and AIM

The genus *Verbascum* (mullein), a member of family Scrophulariaceae, comprises about 360 species of flowering plants, distributed mainly in North America, Europe and West and Central Asia (1). The flowers and leaves of some *Verbascum* species such as *V. thapsus, V. densiflorum* and *V. phlomoides* are used in various traditional systems and regions as analgesic, anti-inflammatory, antiseptic, spasmolytic, diuretic, emollient, expectorant and for the treatment of rheumatic disorders, eczema, spasmodic coughs and as an antiseptic among others (2–5). Previous phytochemical studies on the other *Verbascum* species revealed the presence of secondary metabolites such as iridoids, flavonoids, phenylethanoid glycosides and lignans (3,6).

Scrophulariaceae family is one of the largest plant families in dicotyledonous angiosperms with 200 genera, including the genus *Verbascum* (7). In the Flora of Turkey, the genus *Verbascum* is represented by 255 species and 130 hybrids, among which around 200 species are endemic to Turkey (8).

Oxidative stress is a serious imbalance between oxidation and antioxidation mechanisms. Oxidative stress initiates with the formation of free radicals which are extremely dangerous for living organisms causing chronic diseases such as cardiovascular diseases, cancer and diabetes. Initially, inflammation leads to an acute condition, but in time it turns chronic and causes serious diseases like rheumatoid arthritis and asthma. The severity of these two conditions motivated the researchers to find effective drugs with less or no adverse effects. At this point, medicinal plants have gained importance (9).

Inflammation is a defence system of the body against injury and infections. Several mediators including macrophages, dendritic cells, mast cells, neutrophils and lymphocytes act as a part of inflammatory responses. The main inflammation symptoms are redness, pain, swelling and heat (10).

The use of *Verbascum* species in worldwide folk medicines, their diverse chemical compositions and lack of phytochemical and biological activity studies on *V. bugulifolium* has led us to perform phytochemical and bioactivity studies on this particular plant. The purpose of this study was to evaluate the *in vitro* antioxidant (DPPH, ABTS and CUPRAC), anti-inflammatory (LOX), and antimicrobial activities of the extracts and to isolate

secondary metabolites from the most active extracts. Furthermore, the pharmacological effects of the the isolates were also evaluated by the same test systems.

2. GENERAL INFORMATION

2.1. General Information about *Verbascum*

2.1.1. Botanical Information

2.1.1.1. Scrophuraliaceae

Scrophulariaceae family contains herbs or subshrubs, autotrophic or partially or rarely completely parasitic, without internal phloem. Shapes of the leaves are seen as exstipulate, alternate, opposite or whorled. The flowers are hermaphrodite, borne single in leaf axils, or in racemes or spikes, or in panicles of cymes. 4 – 5 sect to bilabiate or bilobed calyx is observed. Usually, zygomorphic and bilabiate type of gamopetalous corolla is seen on the members of this family. Sometimes corolla nearly actinomorphic which is always imbricate in bud. Stamens of the members are adnate to corolla, 4 and didynamous, or 2, rarely 5: anthers opening lengthwise, or confluent at apex and opening by a continuous slit: staminodes present (1-3) or absent. Ovary is usually superior, with a terminal style, usually bilocular with a horizontal septum: ovules are numerous or few on usually swollen axile placentae: ovary is rarely unicular 2 parietal bifid placentae (Lathraea). Fruit is usually a capsule, sometimes indehiscent. Seeds numerous (rarely few), often ornamented (11).

Scrophulariaceae consists of 30 important genera, including *Verbascum*. Dichotomous key for the genus *Verbascum* in Scrophulariaceae can be presented as follows (11).

1. Green herbs or subshrubs with chlorophyll; leaves neither scale-like nor fleshy.

2. Fertile stamens 4 or 5 (*Verbascum*); plants autotrophic or hemiparasitic.

7. Corolla not pouched or spurred at base.

13. Flowers in racemes (sometimes spile-like) or panicles.

17. Fertile stamens 5, without a staminode **1.*Verbascum***

18. Corolla ± rotate, or with ± inflated tube; flowers in panicles of cymes or glomeruli, or in racemes; capsule septicidal (rarely indehiscent); plants with or without stellate hairs staminode present or absent

19. Corolla ± rotate, with short tube and broad spreading sub actinomorphic limb; stamens long-exerted, filaments often villous; leaves usually alternate throughout **1. *Verbascum***

2.1.1.2. *Verbascum* L.

Verbascum species are annual, biennial or perennial herbs, rarely small shrubs, with alternate or very rarely opposite simple or divided leaves, the basal members forming a rosette. Plants are glabrous or with indumentum of eglandular or glandular, simple or branched hairs. Flowers are interminal racemes, spikes or panicles. Calyx of this species are yellow, rarely violet or purple, brown or yellowish or bluish green, rotate, ± actinomorphic or somewhat zygomorphic. Stamens are 4 or 5, sometimes 4 fertile and 1 staminode; filaments are villous, with yellowish or purple-violet hairs, or rarely glabrous, all equal or 2 anterior (lower) longer and thicker; anthers of 2 or 3 posterior (upper) stamens are always reniform and transversely medifixed, those of 2 anterior (lower) stamens are similar or ± elongate, longitudinally inserted and decurrent or rarely obliquely inserted. Style is single, filiform or scarcely club-shaped; stigma is hemispherical, obovate or spathulate. Capsule is septicidal, globose or oblong-ovoid or cylindrical; seeds are numerous, small, in Turkey obconical-prismatic, transversely pitted (11).

Verbascum (mullein) genus belonging to the family Scrophulariaceae, comprises about 360 species of flowering plants, predominantly in Europe, North America, West and Central Asia (especially Anatolia) (1).

The genus *Verbascum* bears a very complicated taxonomy. Therefore, there is no sufficient taxonomic scheme, reflecting the relationships between the taxa. The genus was divided into 13 informal groups by Huber-Morath. Lately, the taxonomic status of group A (*Verbascum*) in Turkey has been explained (1).

1. Fertile stamens 4, without or rarely with an antherless 5th staminode **Group A**

1. Fertile stamens 5, all perfect

2. Plant without branched hairs; hairs simple, eglandular or glandular, or plant glabrous **Group B**

2. Plant at least in part with branched hairs

3. Each bract with a single flower in its axil, rarely lower bracts with 2 flowers

4. Bracteoles absent, rarely lower bracts with bracteoles **Group C**

4. Bracteoles present **Group D**

3. Each bract with 2 or more flowers in its axil, rarely upper bracts with one flower only

5. Anthers of 2 anterior stamens elongate, longitudinally nor obliquely inserted, anthers of 3 posterior (upper) stamens reniform, transversely medifixed **Group E**

5. Anthers all reniform, transversely medifixed

6. Bracteoles absent **Group F**

6. Bracteoles present

7. Clusters of flowers sessile, with 2 bracteoles **Group G**

7. Clusters of flowers sessile, with 2 bracteoles

8. Connective of 2 anterior anthers glabrous, their filaments mostly glabrous near apex

9. Filament hairs violet (sometimes intermixed with white or yellow) **Group H**

9. Filament hairs white or yellow **Group I**

8. Connective of all anthers densely papillose inside, all filaments wooly right up to anthers

10. Filament hair violet **Group J**

10. Filament hairs whitish yellow

11. Longest pedicels 1/2 x calyx or shorter **Group K**

11. Longest pedicels scarcely shorter, as long as or longer than calyx

12. Longest pedicels at most a little longer than calyx **Group L**

12. Longest pedicels 2 x calyx or longer **Group M**

2.1.1.3. *Verbascum bugulifolium* L.

This species is usually perennial, 15-75 cm, densely covered with short glandular and aglandular hairs, later glabrescent. Stem is terete or subangular, simple or with few branches. Basal leaves are long-petiolate, lamina ovate or triangular-ovate, sub-entire, repand or crenate, 2.5-8(-12) x 1-3(-6) cm. cauline leaves are much smaller, bract-like, entire or denticulate. Inflorescence is simple, cylindrical, law, few-flowered. Bracts 7-14 mm, oblong-to linear-lanceolate, entire. Pedicels are 2-4 mm, ebracteolate. The calyx is 4-8 mm, 2 lower lobes oblong to ovate, others narrower. The color corolla is yellowish to bluish green, 20-35 mm diam., glandular outside, upper lobes with purplish lines. Filaments are with whitish yellow and purplish violet wool, 2 anterior anthers decurrent onto filament, 4.5-5 mm. Capsule is densely covered with glandular and eglandular hairs, broadly ellipsoid, 5-8 x 4-6 mm.

Synonyms : *Janthe bugulifolia* (Lam.)

Celsia bugulifolia (Lam.)

Flowering season : April-June

Habitat : Forests, bushy and waste places, s.l.-450 m.,

Distribution in Turkey : Northwestern Turkey (Kırklareli: Demirköy, İstanbul: Büyükdere, Alemdağ, Burgazada)

Worldwide distribution: Bulgaria. Euro-Sib. element. Without close allies.

Dichotomous key for *Verbascum bugulifolium* L. is as follows (11).

Group A

1. Capsule globose to elliptic, not more than 2 x as long as broad; placenta entire, sessile

2. Each bract with a single flower in its axil (inflorescence a raceme)

5. Plant without branched hairs

8. Plant with glandular and eglandular hairs or glabrous

9. Plant biennial or perennial (rarely annual); capsule not keeled

11. Basal leaves undivided or weakly lobed, never with obtuse eglandular hairs

17. Lamina of basal leaves longer than broad

20. Plant ± densely hairy

22. Basal leaves oblong-linear to ovate

24. Anterior anthers decurrent on filament

27. Cauline leaves bract-like, 5-10 mm broad, base ovate-cuneate **21. *bugulifolium***

Figure 1. *Verbascum bugulifolium* L. in its habitat

Figure 2. *Verbascum bugulifolium* L**.** flowers

2.1.1.4. Traditional Use of *Verbascum* species

The mullein flowers and leaves are reported as expectorant, astringent, diuretic, emollient, analgesic, anti-inflammatory, spasmolytic, vulnerary and antiseptic (2,4,12).

Fresh leaves of mullein are used in homoeopathic formulations for the management of headaches originating from the ear problems. Leaves of mullein are also used in ointment formulations for burns and earache externally. They are also reported as wound healing agents and and applied to treat ulcers, tumours and piles (13).

The flowers of mullein are used to treat ear problems. Herbal tea of mullein is indicated for abdominal and chest complaints such as diarrhea and productive cough (13). It is also used for rheumatic problems.

Decoction of the seeds is applied onto skin for dermatological problems. Powder of the dried roots of some *Verbascum* species as well as the juice of the fresh plant are indicated for the treatment of warts. Traditionally, the decoction is used to relieve toothache, cramps and convulsions (13).

In Turkish Traditional Medicine, the leaves and flowers of some *Verbascum* species are utilized as expectorant, diaphoretic, diuretic, sedative and to treat constipation (14).

2.1.2. Bioactivity studies on *Verbascum* Species

2.1.2.1. Antioxidant Activity

In a study performed by Georgiev et al., the antioxidant activities of methanolic extracts from *Verbascum xanthophoeniceum* were assessed by 2,2'-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), hydroxyl radical averting capacity (HORAC), ferric-reducing antioxidant power (FRAP), and superoxide anion radical scavenging methods. Additionally, *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChe) inhibitory activities were also tested. Moreover, the phenylethanoid glycosides isolated from the extracts were also tested in the same test system and forsythoside B, verbascoside and leucosceptoside B were showed potent radical scavengers and cholinesterases inhibitors (15).

In another study, Tatlı et al., studied free radical scavenging and cell aggregation inhibitory activities of secondary metabolites of Turkish *Verbascum* species were screened.

The isolated compounds from methanolic extracts showed a dose-dependent inhibition of bioautographic and spectrophotometric DPPH activities. Verbascoside found to be the most active (IC_{50} 4.0 μg/ml) metabolite when compared to vitamin C as a reference material (IC_{50} 4.4 μg/ml) to inhibit phorbol 12-myristate 13-acetate (PMA)-induced peroxide-catalyzed oxidation of 2,7-dichlorofluorescein (DCFH) by reactive oxygen species (ROS) within human promyelocytic HL-60 cells (16).

Mihailoviç et al. evaluated the antioxidant activities of methanolic and aqueous extracts of various *Verbascum* species from the Flora of Serbia. Differences in phytochemical content and antioxidant activities between *V. nigrum, V. phlomoides* and *V. thapsus* were assessed. Verbascoside was found as major secondary metabolite in examined plants which demonstrated a high DPPH scavenging activity. Moreover, remarkable correlation between verbascoside content and antioxidant activities was determined. Additionally, simulated *in vitro* digestion model was studied. According to the results, antioxidant activities of the extracts and verbascoside were remarkably diminished, while phenolic acids were remained during the simulated digestion. Based on the results of this work, *V. nigrum* (118.60 mg/g of dry extract) had more bioactive verbascoside and including harpagoside, the latter was not found *V. thapsus* and *V. phlomoides*. *V.nigrum* had higher ($p < 0.05$) antioxidant activity in DPPH assay (IC_{50} 60.7 μg/mL), ABTS assay (IC_{50} 90.0 7 μg/mL), NO radical scavenging activity (29.3 μg/mL) and OH radical scavenging activity (3.3 μg/mL) compare to *V. thapsus* and *V. phlomoides*. $DPPH^{\bullet}$ scavenging activities of VPM and VNW were not considerably different ($p > 0.05$) (17).

In a recent study, Boga et al. reported biological activities of *V. flavidum*. Rutin and chlorogenic acid were determined as the highest phenolic contents in *V. flavidum*. In DPPH free radical scavenging activity, the methanolic extract demonstrated higher activity than BHT (72.62% inhibition at 100 μg/mL concentration). The methanolic and aqueous extracts of *V. flavidum* showed 86.01 % and 87.39% inhibition in ABTS cation radical scavenging assay at 100 μg/mL concentration, respectively. The results indicated that the methanolic and aqueous extracts of *V. flavidum* displayed strong ABTS cation radical scavenging activity. Phenolic compounds or rutin was considered to be the components responsible for their antioxidant activity (12).

In 2016, phytochemical analysis of endemic *V. pinetorum* (Boiss.) O. Kuntze was investigated for the first time by Boga et al. Also, ABTS cation radical decolourization activity, cupric reducing antioxidant capacity were explored. Malic acid (47250.61+/-

2504.28 mu g/g) and luteolin (7651.96 +/- 527.98 mu g/g) were detected as the most abundant ingredients of acetone and methanol extracts. The main components of fatty acid were determined to be palmitic (27.1 %) and stearic acids (22.1%); while the major components of essential oils were found as cineole (16.9%) and α-selinene (16.4%). Acetone extract was detected as more active than BHT used as a standard in *β*-carotene-linoleic acid test method. Additionally, the acetone and methanol extracts exhibited higher activity than BHT in DPPH free radical scavenging activity test. The acetone, methanol and aqueous extracts demonstrated powerful inhibition while the acetone extract exhibited better activity than BHT and *α*-tocopherol which were used as reference materials in ABTS cation radical scavenging and cupric reducing antioxidant capacity assays, respectively. The outcomes of the study indicated that the acetone and methanol extracts of *V. pinetorum* show strong antioxidant activity in all activity assays namely *β*-carotene-linoleic acid test system, DPPH free radical scavenging, ABTS cation radical decolourisation and cupric reducing antioxidant capacity methods (18).

Shakeri and Frokh (2015) have isolated several phytochemicals from *V. sublobatum* leaves and also tested the antioxidant activity of the crude extracs. They prepared the methanolic extract and partitioned by chloroform, ethyl acetate, and butanol. According to these results, the ethyl acetate fraction exhibited the most powerful DPPH radical scavenging activity among the three fractions and was subjected to separation and identification. The isolated compounds which had flavonoid structure, were identified as apigenin and luteolin, which could be responsible for the antioxidant activity (4).

In a study, methanolic and aqueous extracts of various *Verbascum* species were evaluated to illuminate their possible antioxidant activities. For this purpose, DPPH free radical-scavenging and *β*-carotene/linoleic acid assays were utilized. Methanolic and aqueous extracts of *V. leptocladum, V. mucronatum* and *V. davisianum* were exhibited antioxidant activity. Both of the *in vitro* antioxidant assays indicated that the highest antioxidant activity was observed in *V. mucronatum* with 65.4 ± 0.5 μg/mL and 70.4 % inhibition ratio (2).

Various extracts of *V. pinetorum* were evaluated for their potential antioxidant activities by Ozcan et al. *In vitro* antioxidant activities were tested by using two complementary test methods which are DPPH free radical scavenging and *β*-carotene/linoleic acid test systems. The inhibitory activity of the methanolic extract of *V. pinetorum* on the free radical DPPH test was detected as 13.04 mg/ml. The *β*-

carotene/linoleic acid test system indicated that methanolic extract of *V. pinetorum* exerted an inhibitory activity on oxidation of linoleic acid. The inhibition ratio was 89.39% which is close to that of synthetic antioxidant, BHT 92.46%. Iridoid glycosides, flavonoids and saponins were found as the main contents in the methanolic extracts. The total phenolic components of *V. pinetorum* were obtained as 42.45 mg/g gallic acid equivalent. The outcomes of the study indicated that iridoid glycosides, phenolic compounds, flavonoids and saponins were considered to be responsible for the antioxidant activity (19).

Ozcan et al. (2010) studied possible antioxidant activity of *V. antiochium*. The activity studies were performed using two test systems, namely DPPH and *β*-carotene/linoleic acid assay. The outcomes of complementary test systems were found to be close to the value of synthetic reference antioxidant agent BHT. Methanolic extract of *V. antiochium* was specified as 4.80 mg/mL in DPPH and 79.92% inhibition in linoleic acid system. The total phenolic content of *V. antiochium* was determined as 92.71 mg of gallic acid equivalent. In this study, phytochemical analysis indicated that iridoid glycosides, flavonoids, and saponins were the main phytochemical ingredients (20).

The H_2O extract of *V. mucronatum* as well as its fractions and secondary metabolites were investigated for their *in vitro* antioxidant activities. The antioxidant activity was evaluated by various test systems such as DPPH radical scavenging activity, ferrous ion-chelating effect, and ferric-reducing antioxidant power (FRAP) tests. The aqueous extract and its fractions including the phenylethanoid glycoside exerted DPPH scavenger activity and showed the best FRAP activity. Verbascoside, one of the phenylethanoids displayed the best results (72.51 – 91.86%) at 200 μM in the DPPH radical scavenging test, fractions demonstrated the highest ferrous ion-chelating effect (21).

2.1.2.2. Antimicrobial Activity

Phytochemical analysis and biological activities of *V. flavidum* was assessed by Boga et al. The outcomes of phytochemical analysis indicated that rutin and chlorogenic acid were determined as main phenolics, palmitic and oleic acids were detected as major fatty acids, arachidic acid and *α*-selinene were found as essential oils. The moderate antimicrobial activity of methanolic extract was reported against *E. coli* (MIC 250 +/- 0.3, inhibition zone 15 mm at 30 mg/mL) (12).

Boga et al. evaluated phytochemical analysis and biological activities of *V. pinetorum*. This study indicated malic acid and luteolin were reported as the most abundant compounds for methanol and acetone extracts. Palmitic acid and stearic acid were reported as major fatty acids. Additionally, cineole and *α*-selinene were reported as volatile components of the extracts. The antimicrobial activity of the extracts against different microorganism were calculated according to inhibition warrying. The acetone, methanol and water extracts showed activity. Moderate antimicrobial activity of acetone extract was reported against *C. albicans* (inhibition zone< 20-16 mm). The other extracts displayed weak or no antimicrobial activity (18).

Ozcan et al. (2011) screened various extracts to find potential antimicrobial property of *V. pinetorum*. Antimicrobial activity of the extracts of *V. pinetorum* was detected with agar-well diffusion method. The hexane extract of plant demonstrated antimicrobial activity against few microorganisms. *Haemophilus influenzae* was detected as the most sensitive bacterial strain. Phytochemical evaluation indicated that iridoid glycosides, flavonoids and saponins were the essential natural contents in the methanolic extracts. (19).

Ozcan et al. (2010) evaluated possible antimicrobial effects of *V. antiochium,* which is endemic to Turkey. The extracts of *V. antiochium* were obtained by increased polarity and direct methanol extraction method. The agar well diffusion method was used to detect antimicrobial activity. For this purpose, extracts were applied to various Gram-positive and Gram-negative bacteria and one fungus. The aqueous methanolic extract demonstrated the most potent activity against Gram-negative and Gram-positive bacteria. *H. influenzae* was detected as the most sensitive bacterium among the bacteria tested (inhibition zone 24.7 mm methanol extract). *C. albicans* was only sensitive to the hexane extract of *V. antiochium* (20).

Anil et al. performed phytochemical analysis and isolation studies of *V. caesareum.* Results indicated that two flavonoid aglycones (luteolin and nepetin), two iridoid glycosides (verbaspinoside and saccatoside), two phenyl ethanoid glycosides (verbascoside and forsitoside-B), a phenolic acid (chlorogenic acid), and four flavonoid glycosides (luteolin 7-glucoside, quercetin 3,7-diglucoside, 6-hydroxy luteolin 7-glucoside, and apigenin 7-glucoside) were isolated. Additionally, the chloroform, ethyl acetate, methanol and aqueous extracts demonstrated antimicrobial activity towards both Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Pseudomonas aeruginosa, E. coli*, and *Klebsiella pneumoniae*), as well as a fungus (*C. albicans*). Results showed that the ethyl acetate (MIC 1250 µg/mL) and water extracts (MIC 2500 µg/mL for

S. aureus, S. epidermidis, and *E. coli* 1250 µg/mL for K. *pneumoniae, P. aeruginosa, P. mirabilis,* and *C. albicans*) showed antimicrobial activity against all microorganisms (22).

Sen et al. investigated *V. lagurus* to detect its phytochemical content and biological activity. For this purpose, chloroform, ethyl acetate, methanol and aqueous extracts were tested to detect their antimicrobial activity. *S. aureus, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa,* and *Proteus mirabilis* were used to test the possible antimicrobial activities of extracts. Additionally, possible antifungal activity was tested against *C. albicans*. Almost all extracts demonstrated antimicrobial activity against *S. aureus* and *C. albicans*, but the ethyl acetate extract had the most potent antimicrobial activity among the other *V. lagurus* extracts. Luteolin, luteolin-7-glucoside and verbascoside were considered to be the main contributors of antimicrobial activity (23).

Ghasemi et al. evaluated the antimicrobial activity of the aqueous-alcoholic extracts and the essential oil of *V. thapsus* against various bacterial and fungal strains namely *Streptococcus pyogenes, E. coli, S. aureus, C. albicans*, and *Aspergillus fumigatus*. The disk diffusion test indicated that the methanolic extract of *V. thapsus* had more powerful activity on *E. coli* and *S. pyogenes* than the those of aqueous and ethanolic extracts. For the methanol and ethanol extracts, MIC value were 31.25 µg/mL, 62.5-125 µg/mL and the maximal inhibition zones were in the range of 7-16.8 mm, 5.3-11 mm, respectively. Additionally, it was reported that the essential oil of *V. thapsus* did not demonstrate antibacterial and antifungal activity (24).

Morteza-Semnani et al. researched the chemical composition of the essential oil of *V. thapsus*. The major components were found to be 6,10,14-trimethyl-2-pentadecanone and (E)-phytol. Moreover, antimicrobial activity of essential oil was studied by the disk diffusion method. The essential oil demonstrated concentration-dependent antimicrobial effect against *B. subtilis* (MIC 0.625 mg/mL), *S. aureus* (20 mg/mL), *Salmonella typhi* (MIC 0.625 mg/mL), *P. aeruginosa* (MIC 5 mg/mL) and *Aspergilus niger* (MIC 2.5 mg/mL). The essential oil of *V. thapsus* did not display any antimicrobial activity against *E. coli* and *C. albicans* (25).

Saltan et al. evaluated antimicrobial potential of four *Verbascum* L. species, namely *V. bellum, V. detersile, V. myriocarpum*, and *V. pestalozzae*. Chloroform, ethylacetate and methanol extracts were prepared to evaluate the possible antimicrobial activity of plant materials. The extracts were tested against Gram-positive and Gram-negative bacterial

strains. The minimum inhibitory concentrations were 0.59-150 mg/mL. The results indicated that, ethyl acetate extract was effective against *E.coli* (1.88 mg/mL) and also, ethyl acetate extract of *V. pestalozzae* demonstrated the most potent effect on *P. aeroginosa* (0.59 mg/mL) (26).

Amirnia et al. studied possible antimicrobial activity of alcoholic and aqueous extracts of flowers of *V. speciosum*. Antimicrobial tests were performed by using the disc diffusion method, which included the *B. subtilis, B. cereus,* and *E. coli* bacteria strains, namely. The extracts exhibited concentration-dependent inhibitory effect on bacterial strains. Additionally, results showed that ethanolic extract exhibited more potent activity on bacterial strains. The maximum activity was observed against *B. cereus* followed by *B. subtilis*, and *E. coli* was the most resistant strain (27).

Sener et al. extracted leaves of *V. sinuatum* with alcohol to investigate the possible antimicrobial activity against urinary tract pathogens. The disk diffusion and microdilution methods were applied to illuminate the antimicrobial activity against *E. faecalis, E. coli, K. pnemoniae, P. aeruginosa, P. mirabilis* and *C. albicans* which were responsible for the urinary tract infections. The extracts exhibited potent antimicrobial actions against *E. faecalis, P. mirabilis* and *C. albicans* with inhibition zones of 20.0, 18.0 and 20.0 mm, with MIC's and MBC's of 4.0 (8.0), 8.0 (16.0) and 8.0 (16.0) mu g/mL, respectively. Also, the extracts demonstrated moderate activity against the other tested pathogens. According to the results, ethanol extract of the aerial parts of *V. sinuatum* has remarkable activity against infections (28).

Dulger et al. tested methanolic extracts of some endemic Scrophulariaceae members for their possible antimicrobial effects. The research contains wide range of Scrophulariaceae members including *V. protractum, V. bellum, V. dalamanicum, Scrophularia mersinensis, Scrophularia cryptophila, Pedicularis olympica* and *Veronica lycica.* Antimicrobial activity was evaluated by using the disk diffusion method which included the *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, Proteus vulgaris, B. cereus, Mycobacterium smegmatis, Listeria monocytogenes, Micrococcus luteus, C. albicans, Rhodotorula rubra,* and *Kluyveromyces fragilis* strains. The extracts of all Scrophulariaceae members exhibited that they have potent antimicrobial activity against the Gram-positive bacteria and yeast cultures with the inhition zone of 10.2-19.2 mm (29).

In another study, Dulger et al. studied methanolic extracts of *Verbascum* L. species including *V. cariense, V. adenophorum,* and *V. inulifolium* to illuminate their possible antimicrobial activities. For this purpose, disk diffusion method was used. Antimicrobial activity was tested on *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, P. vulgaris, B. cereus, M. smegmatis, L. monocytogenes, M. luteus, C. albicans, R. rubra, K. fragilis, D. hansenii* and *H. guilliermondii* strains. The extracts exhibited for their antimicrobial activity aginst Gram-positive bacteria strains but no antimicrobial activity was detected on Gram-negative bacteria strains (9.8-24.6 mm) (30).

Dulger et al. prepared methanolic extracts from endemic species including *V. pseudoholotrichum, V. cymigerum, V. cholorostegium, V. linguifolium* and *V. pellitum and* evaluated their antimicrobial activities against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, P. vulgaris, B. cereus, M. smegmatis, L. monocytogenes, M. luteus, C. albicans, R. rubra,* and *K. fragilis* by the disk diffusion method. *Verbascum* L. extracts showed potent antimicrobial activity against Gram-positive bacteria and yeast strains when Gram-negative bacteria were more resistant to extracts with the inhibition zone of 8.0-20.6 mm (31).

Dulger and Gonuz investigated that methanolic extracts of various endemic species for their probable antimicrobial activities. Antimicrobial activity was examined against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa, P. vulgaris, B. cereus, M. smegmatis, L. monocytogenes, M. luteus, C. albicans, R. rubra,* and *K. fragilis* by the disk diffusion method. The outcomes of the study indicated that methanolic extracts of *V. gypsicola* exhibited powerful antimicrobial activity against the Gram-positive bacteria and the yeast cultures showing inhibition zone of 9.2-17.6 mm (32).

2.1.2.3. Antiviral Activity

In a study, methanolic extract of *V. thapsus* was investigated for its potential *in vitro* antiviral activity against the pseudorabies virus strain RC/79 (pRv) by Escobar et al. Outcomes of this study indicated that the cells were pre-treated with the extract before the virus infection, the inhibition of plaque formation was detected as 70%. Moreover, the inhibition rate of pseudorabies virus was found to be high when it was incubated with plant extract or when the extract was added during the adsorption phase (%96). The 50% inhibitory concentration of the extract for PrV plaque formation was determined at 35 mu g/mL (-1), and selectivity indices were 31.4 (NRU) and 40.7 (MTT), respectively. However, no inhibitory effect was detected when the extract was added to the cells after the adsorption

period. The results indicated that the methanolic extract of *V. thapsus* might include bioactive compound(s) which has impacted pseudorabies virus mostly in the adsorption phase (33).

In a study, Rajbhandari et al. investigated methanolic extracts of various species for *in vitro* antiviral activity against *Herpes simplex* virus type 1 (HSV-1) and influenza virus A by dye uptake assay in the systems HSV-1/Vero cells and influenza virus A/MDCK cells. Results show that *V. thapsus* (IC_{50}< 6.25 µg/mL) possesses strong anti-influenza viral activity while other plants had either anti-herpes viral activity or anti-influenza viral activity (34).

In a phytotherapeutic research, Serkedjieva studied an infusion of *V. thapsiforme* and its combination with amantadine derivatives for antiviral activity. The outcomes of investigation indicated that the infusion prepared from the flowers of *Verbascum thapsiforme* diminished the infectious and haemagglutination yields of a number of infectious viruses in tissue cultures. The combined application of the infusion and various amantadine derivatives resulted in a significant augmentation of the inhibitory effect on the reproduction of influenza virus A/chicken/Germany/27 strain Weybridge (H7N7) in cell cultures of chicken embryo fibroblasts. The greatest antiviral activity was observed in the combination of herbal infusion and adamantamine glucuronide (35).

2.1.2.4. Antihelmintic Activity

In a study by Ali et al. methanolic extract of *V. thapsus* was investigated for its possible anthelmintic activity. *In vivo* test was performed perpormed by using adult roundworms (*Ascaridia galli*) and tapeworms (*Raillietina spiralis*). The extract was compared to albendazole, a reference drug. The comparison parameters included time of paralysis and death. For *V. thapsus* extracts, EC50 value was -1.9 +/- 0.06 vs. control EC50= -2.5 +/- 0.12. The results indicated that the methanolic extract had higher effect against *R. spiralis* than that of the reference drug (36).

Kozan et al. screened various *Verbascum* L. species to detect their possible anthelmintic activities. For this purpose, methanolic extracts of some endemic *Verbascum* L. species including *V. chionophyllum, V. cilicicum, V. dudleyanum, V. lasianthum, V. latisepalum, V. mucronatum, V. olympicum, V. pterocalycinum, V. pycnostachyum, V.*

salviifolium, V. splendidum, V. stachydifolium, and *V. uschackense* were investigated for their anthelmintic activities. *In vivo* tests were applied on mice. Results indicated that methanolic extracts of *V. lasianthum, V. latisepalum, V. mucronatum* and *V. salviifolum* demonstrated the most potent inhibition rates against *Aspiculuris tetraptera* at 100 mg/kg in mice (37).

2.1.2.5. *In vitro* Cytotoxic and Antiproliferative Activity

In a phytochemical investigation by Zhao et al, dry aerial parts of *V. thapsus* were extracted and isolation studies were performed. The chromatographic studies led to isolation and identification of a novel iridoid, which was named verbathasin A. The stereochemistry and structure of the verbathasin A were characterized by spectroscopic methods. All of the isolated compounds (except 10-deoxyeucommiol and ajugol) were studied for antiproliferative and antiangiogenic activities. Results showed that luteolin and 3-*O*-fucopyranosylsaikogenin F exhibited significant antiproliferative activity on A549 lung cancer cell line. The antiproliferative mechanism was determined to be induction of apoptosis in cancer cells (38).

Talib et al. studied various plant extracts which were used traditionally as anticancer agent for antiproliferative actions on Hep-2, MCF-7, and Vero cell lines. The antiproliferative action was evaluated by MTT assay. The apoptotic effect was determined by using TUNEL (terminal deoxynucleotidyl transferase (TdT) mediated-16-deoxyuridine triphosphate (dUTP) Nick-End Labelling system) colorimetric assay. Ethanolic extracts of five plants were reported as high antiproliferative activity including *V. siniaticum* ($p < 0.05$). Additionally, it was reported that extract of *V. siniaticum* flowers (% Viability ± SEM; MCF-7: 40 ± 1.30) are more potent than its aerial parts ((% Viability ± SEM; MCF-7: 60 ± 5.12) (39).

Papoutsi et al. isolated acteoside and martynoside from *V. macrurum*. Their estrogenic or antiestrogenic effects were evaluated by measuring IGFBP3 levels, cell viability and number of mineralized nodules in breast cancer cells (MCF7), endometrial cancer cells (Ishikawa) and osteoblasts (KS483). Acteoside and martynoside antagonized estrogen receptor isoforms (ER-alpha and ER-beta, $p<0.001$). Martynoside was found to be a potent anti-estrogen in MCF-7 cells. In osteoblasts, martynoside stimulated nodule mineralization. Moreover, its antiproliferative effect on endometrial cells suggests that

martynoside may be a very important natural selective estrogen receptor modulator. Additionally, acteoside was an anti-estrogen in breast cancer cells and osteoblasts, in the absence of any effect on endometrial cells (40).

2.1.2.6. Anti-Inflammatory Activity

Kupeli et al. designed a study to investigate *V. lasianthum* flowers for anti-inflammatory and antinociceptive activities. Methanolic extract of the flowers was exhibited remarkable inhibitory effect in the carrageenan mediated paw edema model and in *p*-benzoquinone-induced writhings in mice. Moreover, phytochemical analysis and isolation studies were performed to detect the source of biological activity. An iridoid glucoside, aucubin and a triterpenoid saponin, ilwensisaponin A were found to demonstrate remarkable antinociceptive, and anti-inflammatory activities without causing any acute toxicity or gastric damage (41).

Tatlı et al. performed another study to evaluate the possible activities of *V. salviifolium* for anti-inflammatory and antinociceptive effects. Carrageenan and PGE1 induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema models in mice were used to investigate the anti-inflammatory activity. The *p*-benzoquinone-induced writhing reflex model was used to investigate antinociceptive activity. Phytochemical analysis and isolation studies were performed and chemical structures were illuminated by spectral methods. Outcomes indicated that apigenin 7-*O*-glucoside, luteolin 3-*O*-glucoside, luteolin 7-*O*-glucoside and hydroxyacteoside were found to be potent anti-inflammatory agents for carrageenan induced paw edema. But, all the isolates exhibited no effect in the TPA induced ear edema. Luteolin 3-*O*-glucoside and luteolin 7-*O*-glucoside and also demonstrated remarkable antinociceptive activity (42).

Speranza et al. investigated possible anti-inflammatory activity of *V. mallophorum*. Verbascoside was isolated and evaluated for its bioactivities. An inflammatory state was reproduced by treating THP-1 (human myelomonocytic leukaemia) cells with pro-inflammatory stimuli (such as LPS and IFN-gamma), causing an up-regulation both in the expression and in the activity of inducible nitric oxide synthase (iNOS). The results showed a remarkable decline in the expression and activity of iNOS and extracellular O_2 when cells were treated with verbascoside. It is assumed that verbascoside possesses anti-inflammatory property by reducing the production of superoxide radicals and thus diminishes the action of iNOS (43).

Speranza et al. extracted aerial parts of *V. thapsus* and then verbascoside was isolated and purified. An inflammatory state was reproduced by treating THP-1 (human myelomonocytic leukaemia) cells with pro-inflammatory stimuli (such as LPS and IFN-gamma) which causing an up-regulation both in the expression and in the activity of inducible nitric oxide synthase (iNOS). Outcomes of the study demonstrated a remarkable reduction in the expression and activity of Zn-superoxide dismutase, iNOS, extracellular O_2 production activity, catalase activity, glutathione peroxidase activity when cells were treated with verbascoside (100μM). It can be presumed that verbascoside has anti-inflammatory properties because it diminishes the production of superoxide radicals and thus reduces the action of iNOS (44).

Akdemir et al. investigated *V. mucronatum* flower extracts for possible antiinflammatory, antinociceptive and wound healing activities. Carrageenan induced hind paw edema model in mice was used for evaluation of anti-inflammatory activity. Additionally, wound healing potential of the plant was evaluated by choosing *in vivo* experimental incision and excision models on mice, where positive control (Madecassol®) was used to compare the bioactivity. Results showed that *V. mucronatum* demonstrated antinociceptive, anti-inflammatory and wound healing effects. Phytochemical detection and isolation studies were also performed. According to the results four iridoid glucosides; ajugol, aucubin, lasianthoside I, catalpol, two saponins; ilwensisaponin A and C and a phenylethanoid glycoside, verbascoside were isolated and their structures were identified by spectral methods. Verbascoside was more effective than ibuprofen in the writhing test (67.6% and 50.0% at equimolar doses). It represented almost the same effects in the tail flick (topic and oral) near 25% at equivalent doses to ibuprofen. Verbascoside displayed remarkable antinociceptive, anti-inflammatory and remarkable wound healing activity (45).

Dimitrova et al. studied *V. xanthophoeniceum* for its possible *in vitro* and *in vivo* anti-inflammatory actions. Crude extract, various fractions and isolated compounds were tested. Methanolic extract of plant was applied in a paw oedema mice model for actions on NO and cytokine production by peritoneal macrophages on COX-1 and COX-2 expression. The methanolic extract exhibited inhibition on cobra venom factor induced oedema in mice. The most suppressive action was detected by nigroside VI on IL-6 and NO production and by forsythoside B on TNF-alpha production. Leucosceptoside B lowered NO release and COX-1 expression in macrophages. *V. xanthophoeniceum* could provide an excellent source of active compounds with anti-inflammatory action (46).

In a comparative study, crude extracts and isolated compounds of *V. xanthophoeniceum* were tested for its anti-inflammatory activity by Georgiev et al. The comparison was performed on *V. xanthophoeniceum* crude extract, its fractions, and isolates including aucubin, ajugol, forsythoside B, harpagide, harpagoside, nigroside III and nigroside VI and verbascoside in primary cultures of normal human keratinocytes (NHK). The synthesis, expression and release of proinflammatory chemokines (IL-8, MCP-1 and IP-10) were explored. IC_{50} values showed that verbascoside and forsythoside B were determined to be potent inhibitors of all the chemokines. The authors concluded that these compounds could be used for topical chronic inflammatory skin disorders like psoriasis and atopic dermatitis, because of overexpression of IL-8, MCP-1, and IP-10 (47).

Grigore et al. designed to explore possible correlation of antioxidant and anti-inflammatory potential of *V. phlomoides* extract. Aqueous extracts of the plant were prepared when subjected to *n*-butanol partition. By spectral techniques, total phenolics were determined and specific compounds were investigated. The antioxidant activity was defined by the DPPH assay. The anti-inflammatory capacity was assessed *in vitro* ELISA measurement of ICAM-1 expression in TNF-a-stimulated endothelial cells and *in vivo* by the rat paw edema assay. The main contents were identified as rosmarinic acid, caffeic acid, ferulic acid and quercetin. The extract (50–200 μg/mL) exhibited that significant inhibition of TNF-alpha induced ICAM-1 (55–58.8% at 100 and 200 μg/mL) expression but failed to reduce egg-white-induced rat paw edema. The polyphenols exhibited crucial antioxidant effect but had a weak anti-inflammatory activity that was correlated (48).

2.1.2.7. Studies on Wound Healing Activity

Demirci et al. studied wound healing effect of *V. speciosum in vitro* and its possible mechanism. The methanol extract was prepared, then cell viability was evaluated by the MTS-assay and then wound healing was evaluated by scratch assay. Apoptotic and wound healing gene expression levels were defined with Real Time PCR. According to the results, *V. speciosum* showed strong effects on wound healing activity (49).

2.1.3. Secondary metabolites isolated from *Verbascum* Species

Previous phytochemical studies on different *Verbascum* species revealed that the genus is rich in diverse group of secondary metabolites including iridoids, phenylethanoid glycosides, flavonoids, saponins and lignans. Furthermore, some phytosterols and alkaloids were also reported from some species of the genus *Verbascum*. The chemical structures of the previously isolated compounds from *Verbascum* species as well as and their species names are given in the following Tables.

2.1.3.1. Iridoid Glycosides

2.1.3.1.A. Catalpol type iridoid glycosides

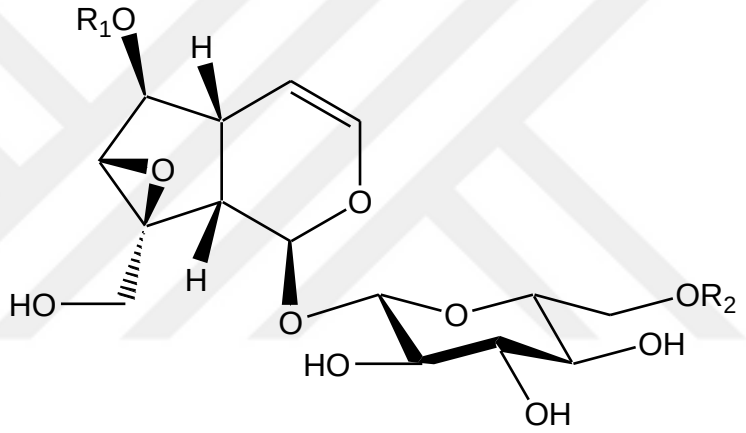


Table 1. Catalpol type iridoid glycosides obtained from *Verbascum* species

Compounds	R_1	R_2	Species	Ref

Catalpol	H	H	*V. bombyciferum* *V. blattarioides* *V. blattaria* *V. capitis-viridis* *V. cilicicum* *V. cheirantifolium* *V. densiflorum* *V. dudleyanum* *V. georgicum* *V. laxum* *V. lagurus* *V. leucophyllum* *V. lychnitis* *V. mucronatum* *V. niveum* *V. nigrum* *V. oreophilum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. roripifolium* *V. sinuatum* *V. spinosum* *V. songaricum* *V. thapsus* *V. thapsiforme* *V.wiedemannianum*	(3,45,50–66)
Methylcatalpol	CH_3	H	*V. lychnitis* *V. nigrum*	(3,53,64)
Catalposide	*p*-OH-benzoyl	H	*V. lychnitis*	(3,53)

Specioside	*(E)-p*-coumaroyl	H	*V. lychnitis* *V. phlomoides*	(3,67,68)
6-*O*-Rhamnopyranosylcatalpol	Rha	H	*V. dudleyanum* *V. georgicum* *V. lasianthum* *V. letourneuxii* *V. saccatum* *V. salviifolium*	(3,56,62,63,69–71)
6-*O*-β-Glucopyranosylcatalpol	Glc	H	*V. salviifolium*	(3,71)
6-*O*-β-Xylopyranosylcatalpol	Xyl	H	*V. lychnitis* *V. phoeniceum* *V. phlomoides* *V. songaricum* *V. thapsiforme*	(3,59,66)
6-*O*-(2''-(*E*)-*p*-coumaroyl)-3''-methiafoloyl-rhamnopranosylcatalpol	2''-(*E*)-*p*-coumaroyl)-3''-methiafoloyl-Rha	H	*V. nobile*	(72)
6-*O*-(2''-(*Z*)-*p*-coumaroyl)-3''-methiafoloyl-rhamnopranosylcatalpol	2''-(*Z*)-*p*-coumaroyl)-3''-methiafoloyl-Rha	H	*V. nobile*	(76)
6-*O*-(2''-*O-(E)*-Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol (Verbaspinoside)	2''-*O*-(E) Cinnamoyl-*α*-Rha	H	*V. cilicicum* *V. salviifolium* *V. spinosum*	(3,51,55,71)
6-*O*-(3''-*O-(E)*-Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol	3''-*O-(E)*-Cinnamoyl-*α*-L-Rha	H	*V. cilicicum*	(3,55)

6-*O*-(4"-*O*-*(E)*-Cinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-*(E)*-Cinnamoyl-*α*-Rha	H	*V. cilicicum*	(3,55)
Saccatoside	2"-*O*-*(E)*-*p*-coumaroyl-*α*-Rha	H	*V. cilicicum* *V. dudleyanum* *V. macrurum* *V. phlomoides* *V. saccatum* *V. thapsus* *V. virgatum*	(3,61,62,69,71,73–75)
6-*O*-(3"-*O*-*(E)*-*p*-Coumaryl)-*α*-L-rhamnopyranosylcatalpol	3"-*O*-*(E)*-*p*-Coumaryl-*α*-Rha	H	*V. cilicicum* *V. dudleyanum* *V. sinuatum* *V. thapsus* *V. virgatum*	(3,50,55,62,74,75)
6-*O*-(4"-*O*-*(E)*-*p*-Coumaryl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-*(E)*-*p*-Coumaryl-*α*-L-Rha	H	*V. thapsus*	(3,75)
6-*O*-(2 Verbascoside -*O*-*(E)*-*p*-Methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	2"-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(3"-*O*-*(E)*-*p*-methoxycinnamoyl)-*α*-rhamnopyranosylcatalpol	3"-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-Rha	H	*V. thapsus*	(3,75)
Verbascoside A = 6-*O*-(4"-*O*-*(E)*-*p*-Methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-*(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha	H	*V. densiflorum* *V. georgicum* *V. lasianthum* *V. thapsus*	(3,56,61,63,75)
6-*O*-(2"-*O*-*(E)*-Caffeoyl)-*α*-rhamnopyranosylcatalpol	2"-*O*-*(E)*-Caffeoyl-*α*-Rha	H	*V. thapsus*	(3,75)

6-*O*-(3"-*O*-*(E)*-Caffeoyl)-*α*-rhamnopyranosylcatalpol	3"-*O*-*(E)*-Caffeoyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(4"-*O*-*(E)*-Caffeoyl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-*(E)*-Caffeoyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(2"-*O*-*(E)*-Feruloyl)-*α*-L-rhamnopyranosylcatalpol	2"-*O*-*(E)*-Feruloyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(4"-*O*-*(E)*-Feruloyl)-*α*-rhamnopyranosylcatalpol	4"-*O*-*(E)*-Feruloyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(2"-*O*-*(E)*-Isoferuloyl)-*α*-L-rhamnopyranosylcatalpol	2"-*O*-*(E)*-Isferuloyl-*α*-L-Rha	H	*V. thapsus*	(3,75)
6-*O*-(3"-*O*-*(E)*-Isoferuloyl)-*α*-rhamnopyranosylcatalpol	3"-*O*-*(E)*-Isoferuloyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(4"-*O*-*(E)*-Isoferuloyl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-*(E)*-Isoferuloyl-*α*-L-Rha	H	*V. thapsus*	(3,75)
6-*O*-(2"-*O*-*(E)*-3,4-Dimethoxycinnamoyl)-*α*-rhamnopyranosylcatalpol	2"-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-Rha	H	*V. thapsus*	(3,75)
6-*O*-(3"-*O*-*(E)*-3,4-Dimethoxycinnamoyl)-*α*-rhamnopyranosylcatalpol	3"-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-Rha	H	*V. thapsus*	(3,75)
Buddlejoside A8	4"-*O*-*(E)*-3,4-Dimethoxycinnamoyl-*α*-L-Rha	H	*V. salviifolium*	(3,71)

Buddlejoside A5	2"-*O*-Acetyl-3"-*(E)-p*-Methoxycinnamoyl-*α*-L-Rha	H	*V. lasianthum*	(3,56)
6-*O*-(2", "3-*O*-Diacetyl)-*α*-rhamnopyranosylcatalpol	2",3"-*O*-Diacetyl-*α*-L-Rha	H	*V. lasianthum* *V. sinuatum*	(3,56,76)
Pulverulentoside I	3"-*O*-Acetyl-2"-*O-(E)-p*-Methoxycinnamoyl-*α*-Rha	H	*V. dentifolium* *V. letourneuxii* *V. pulverulentum* *V. salviifolium* *V. sinuatum* *V. thapsus*	(3,70,71,75–78)
6-*O*-(3"-*O*-Acetyl-2′′-*O-(Z)-p*-methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	3"-*O*-Acetyl-2"-*O-(Z)-p*-methoxycinnamoyl-*α*-Rha	H	*V. dentifolium*	(3,77)
6-*O*-(4"-*O*-Acetyl-2"-*O-(E)-p*-methoxycinnamoyl)-*α*-L-rhamnopyranosylcatalpol	4"-*O*-Acetyl-2"-*O-(E)-p*-methoxycinnamoyl-*α*-Rha	H	*V. thapsus*	(3,75)
Pulverulentoside II	2"-*O*-Acetyl-3"-*O-(E)*-isoferuloyl-*α*-Rha	H	*V. pulverulentum*	(3,78)
Scrospioside A	2",4"-di-*O*-Acetyl-2"-*O-(E)*-cinnamoyl-*α*-L-Rha	H	*V. ballii*	(3,79)

Scropolioside B	2"-*O*-Acetyl-3",4"-*O*-*(E)*-cinnamoyl-*α*-Rha	H	*V. ballii*	(3,79)
Picroside IV	H	(*E*)-*p*-coumoroyl	*V. pterocalycum* *V. thapsus.*	(3)

2.1.3.1.B. Acubine derivatives glycosides

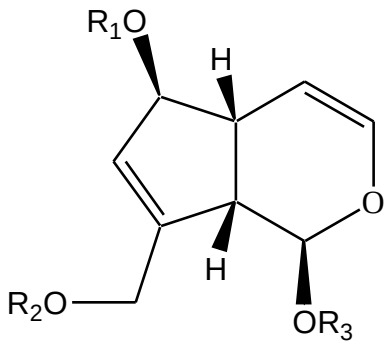


Table 2. Acubine-type iridoid glycosides obtained from *Verbascum* species

Compounds	**R1**	**R2**	**R3**	**Species**	**Ref**
Aucubin	H	H	Glc	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. cheirantifolium* *V. densiflorum* *V. dentifolium* *V. dudleyanum* *V. georgicum* *V. gnaphalodes* *V. lagurus* *V. laxum* *V. lasianthum* *V. leucophyllum* *V. lychnitis* *V. macrurum* *V. mallophorum*. *V. mucronatum*	(3,6,37,45,50–52,54,57–66,69,73,74,76,77,80–87)

				V. nigrum *V. niveum* *V. olympicum* *V. oreophilum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. pycnostachyum* *V. roripifolium* *V. saccatum* *V. sinuatum* *V. songaricum* *V. speciosum* *V spectabile* *V. spinosum* *V. thapsiforme* *V. thapsus* *V. undulatum* *V. virgatum* *V. wiedemannianum* *V. xanthophoeniceum*	
Sinuatol	α-L-Rha	H	Glc	*V. apentulium* *V. blattaria* *V. boerhaavii* *V. capitis-viridis* *V. chaixii* *V. laxum* *V. lasianthum* *V. leucophyllum* *V. mallophorum* *V. nigrum*	(3,50, 52,59, 81,85, 86,88)

				V. niveum *V. olympicum* *V. pyramidatum* *V. roripifolium* *V. sinuatum* *V. undulatum*	
6-*O*-*β*-D-Glucopyranosylaucubin	*β*-D-Glu	H	Glc	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. cheirantifolium* *V. georgicum* *V. gnaphalodes* *V. lychnitis* *V. mallophorum* *V. nigrum* *V. niveum* *V. olympicum* *V. oreophilum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. roripifolium* *V. salviifolium* *V. sinuatum* *V. songaricum* *V. speciosum* *V spectabile*	(3,59, 71,81)

				V. thapsiforme *V. thapsus* *V. undulatum*	
6-*O*-*β*-D-Xylopyranosylaucubin	*β*-D-Xyl	H	Glc	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. cheirantifolium* *V. georgicum* *V. gnaphalodes* *V. lagurus* *V. leucophyllum* *V. lychnitis* *V. mallophorum* *V. nigrum* *V. niveum* *V. olympicum* *V. oreophilum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. roripifolium* *V. sinuatum* *V. songaricum* *V. speciosum* *V spectabile* *V. thapsiforme* *V. thapsus*	(3,59, 61,66, 81,83)

				V. undulatum	
6-*O*-*(E)*-*p*-Coumaroylaucubin	*(E)*-*p*- Coumaroyl	H	Glc	*V. cheirantifolium* *V. macrurum*	(3,60, 73)
6-*O*-*(E)*-*p*-Methoxycinnamoyl aucubin	*(E)*-*p*-Methoxycinnamoyl aucubin	H	Glc	*V. cheirantifolium*	(3,60)
Nigroside I	3"-*O*-*(E)*-Cinnamoyl- *α*-L-Rha	H	Glc	*V. chaixii* *V. nigrum* *V. nigrum* *V. undulatum*	(3,59, 85,89)
Nigroside II	2"-*O*-*(E)*-Cinnamoyl- *α*-L-Rha	H	Glc	*V. nigrum* *V. undulatum*	(3,85, 88)
Nigroside III	2"-*O*-(*E*)-p-Coumaroyl-*α*-L-Rha	H	Glc	*V. nigrum*	(3,46, 90)
6-*O*-(3'-*O*-*(E)*-*p*-*C*oumaroyl)-*α*-L-rhamnopyranosylaucubin	3"-*O*-*(E)*-*p*- coumaroyl-*α*-L-Rha	H	Glc	*V. laxum* *V. macrurum* *V. mucronatum* *V. nigrum* *V. undulatum*	(3,45, 52,73, 89,90)
6-*O*-(4"-*O*-(E)-*p*-Coumaroyl)-*α*-L-rhamnopyranosylaucubin (Lasianthoside I)	4"-*O*-(*E*)-*p*-Coumaroyl-*α*-L-Rha	H	Glc	*V. lasianthum*	(3,56)
6-*O*-(6"-*O*-(E)-p-Coumaroyl)-β-D-glucopyranosylaucubin	6"-*O*-(*E*)-*p*-Coumaroyl)-β-D-Glu	H	Glc	*V. salviifolium*	(3,71, 88)
Phlomoidoside	4"-*O*-*(E)*-*p*-Coumaroyl-β-D-Xyl	H	Glc	*V. phlomoides*	(3,68)
Unduloside II	3"-*O*-(*E*)-*p*-Dimethoxycinnamoyl-*α*-L-Rha	H	Glc	*V. undulatum*	(3,91)

Unduloside III	3"-*O*-(E)-*p*-Methoxycinnamoyl-*α*-L-Rha	H	Glc	*V. lasianthum* *V. letourneuxii* *V. undulatum*	(3,56, 70,91)
6-*O*-(4"-*O*-(*E)*-*p*-Methoxycinnamoyl)-*α*-L-rhamnopyranosylaucubin	4"-*O-(E)*-*p*-Methoxycinnamoyl-*α*-L-Rha	H	Glc	*V. lasianthum*	(3)
Unduloside	2"-*O*-(*E*)-Feruloyl-*α*-L-Rha	H	Glc	*V. undulatum*	(3,88)
6-*O*-(3"-*O*-(*E)*-Feruloyl)-*α*-L-rhamnopyranosylaucubin	3"-*O*-(*E*)-Feruloyl-*α*-L-Rha	H	Glc	*V. nigrum* *V. undulatum*	(3,89, 90)
Nigroside IV	3"-*O*-(*E*)-*p*-Isoferuloyl-*α*-L-Rha	H	Glc	*V. nigrum*	(3,90)
Nigroside V	2"-*O*-(*E*)-Isoferuloyl-*α*-L-Rha	H	Glc	*V. nigrum*	(3)
6-*O*-(3"-*O*-Acetyl-2"-*O*-*p*-Methoxy-(*E)*-cinnamoyl)-*α*-L-rhamnopyranosylaucubin	3"-*O*-Acetyl-2"-*O*-*p*-Methoxy-(*E*)-cinnamoyl)-*α*-L- rha Acetyl-2"-*O*-*p*-Methoxy-(E)-cinnamoyl-*α*-L-Rha	H	Glc	*V. laxum*	(3,52)
Sinuatoside	3"-*O*-β-Xyl-*α*-D-Gal	H	Glc	*V. niveum* *V. sinuatum*	(3,59, 92)
Aucuboside	H	Glc	H	*V. chaixii* *V. lychnitis*	(6)

2.1.3.1.C. Ajugol and harpagide-type iridoid glycosides

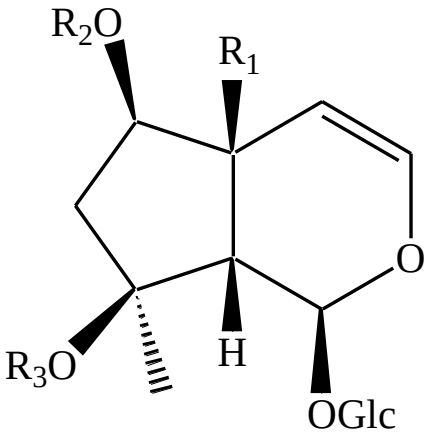


Table 3. Ajugol and harpagide-type iridoid glycosides obtained from *Verbascum* species

Compounds	**R1**	**R2**	**R3**	**Species**	**Ref**
Ajugol	H	H	H	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. cheirantifolium* *V. gnaphalodes* *V. lagurus* *V. lasianthum* *V. oreophilum* *V. leucophyllum* *V. lychnitis* *V. macrurum* *V. mallophorum* *V. mucronatum* *V. nigrum* *V. olympicum* *V. ovalifolium* *V. phoeniceum*	(3,38,45, 51,54,59, 73,74,84, 86,89,93, 94)

				V. phlomoides *V. pterocalycinum* *V. pulverulentum* *V. pyramidatum* *V. pycnostachyum* *V. roripifolium* *V. sinuatum* *V. songaricum* *V. speciosum* *V spectabile* *V. spinosum* *V. thapsiforme* *V. thapsus* *V. undulatum* *V. virgatum* *V. wiedemannianum* *V.xanthophoeniceum*	
6-*O*-Benzoylajugol	H	Benzoyl	H	*V. apentulium* *V. thapsus*	(3,75)
6-*O*-*p*-Hydroxybenzoylajugol	H	*p*-OH-Benzoyl	H	*V. apentulium* *V. thapsus*	(3,75)
6-*O*-*p*-Methoxybenzoylajugol	H	*p*-OH-Benzoyl	H	*V. apentulium* *V. thapsus*	(3,75)
6-*O*-Vanilloylajugol	H	Vanilloyl	H	*V. apentulium* *V. lasianthum*	(3,95)
6-*O*-Syringoylajugol	H	Syringoyl	H	*V. apentulium* *V. letourneuxii* *V. thapsus*	(3,75)
Ajugoside	H	Ac	H	*V. apentulium* *V. capitis-viridis* *V. chaixii* *V. cheirantifolium* *V. georgicum*	(3,16,59)

				V. gnaphalodes *V. lagurus* *V. leucophyllum* *V. lychnitis* *V. nigrum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pycnostachyum* *V. roripifolium* *V. sinuatum* *V. songaricum* *V. speciosum* *V spectabile* *V. thapsiforme* *V. thapsus* *V. undulatum*	
8-Cinnamoyl myoporoside	H	H	Cinnamoyl	*V. apentulium* *V. thapsus*	(3,38)
Harpagide	OH	H	H	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. densiflorum* *V. dentifolium* *V. georgicum* *V. gnaphalodes* *V. lagurus* *V. leucophyllum* *V. lychnitis*	(3,59,61, 66,77)

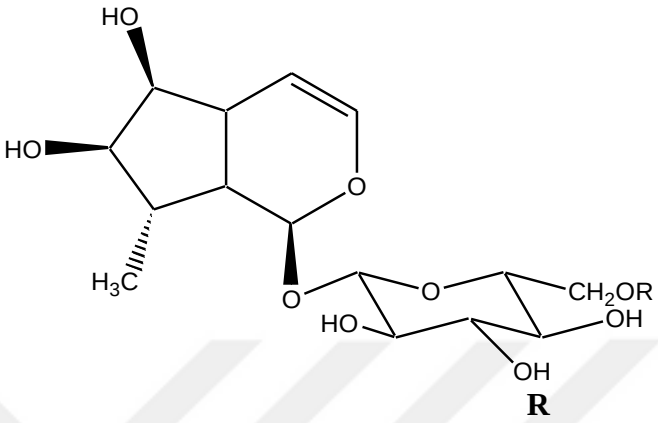
				V. mallophorum *V. nigrum* *V. niveum* *V. oreophilum* *V. ovalifolium* *V. phoeniceum* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. roripifolium* *V. sinuatum* *V. speciosum* *V spectabile* *V. thapsiforme* *V. thapsiforme* *V. thapsus* *V. undulatum*	
8-*O*-Acetylharpagide	OH	H	Ac	*V. apentulium* *V. blattaria* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. cheirantifolium* *V. densiflorum* *V. georgicum* *V. gnaphalodes* *V. lasianthum* *V. leucophyllum* *V. mallophorum* *V. nigrum* *V. niveum* *V. ovalifolium*	(3,59,61, 66)

				V. phoeniceum *V. phlomoides* *V. roripifolium* *V. songaricum* *V. thapsiforme* *V. thapsiforme* *V. thapsus* *V. undulatum*	
Harpagoside	OH	H	Cinnamoyl	*V. apentulium* *V. blattarioides* *V. boerhaavii* *V. bombyciferum* *V. capitis-viridis* *V. chaixii* *V. densiflorum* *V. dentifolium* *V. georgicum* *V. gnaphalodes* *V. laxum* *V. lasianthum* *V. letourneuxii* *V. leucophyllum* *V. lychnitis* *V. mallophorum* *V. nigrum* *V. niveum* *V. olympicum* *V. oreophilum* *V. ovalifolium* *V. phlomoides* *V. pulverulentum* *V. pyramidatum* *V. pycnostachyum*	(3,16,38, 46,52,59, 64,66,70, 75–78,84,85, 93,95)

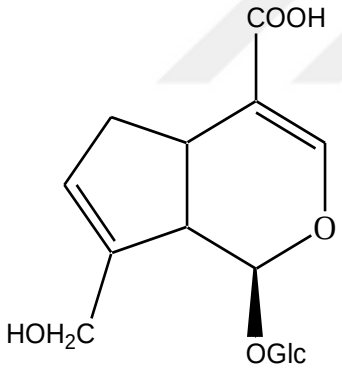
					V. roripifolium	
					V. sinuatum	
					V. songaricum	
					V. speciosum	
					V spectabile	
					V. thapsiforme	
					V. thapsiforme	
					V. thapsus	
					V. undulatum	
					V. undulatum	
					V. wiedemannianum	
					V. xanthophoeniceum	
Laterioside		H	H	Cinnamoyl	*V. apentulium*	(3)
					V. blattaria	
					V. blattarioides	
					V. chaixii	
					V. georgicum	
					V. lychnitis	
					V. nigrum	
					V. olympicum	
					V. phlomoides	
					V. pulverulentum	
					V. roripifolium	
					V. songaricum	
					V. speciosum	
					V. thapsiforme	
					V. thapsus	
					V. wiedemannianum	

2.1.3.1 D. Other iridoids

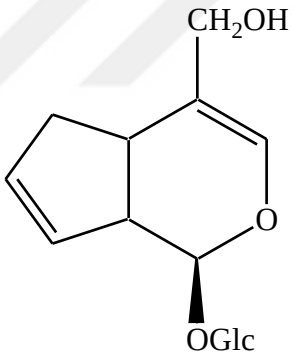
Some other unusual iridoids were also isolated and identified from different *Verbascum* species (3). The chemical structures of these iridoids are depicted below.



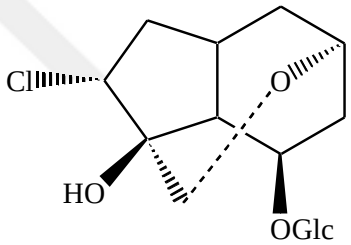
	R
Angeloside	H
6'-*O*-Vanilloylangeloside	Vanilloyl



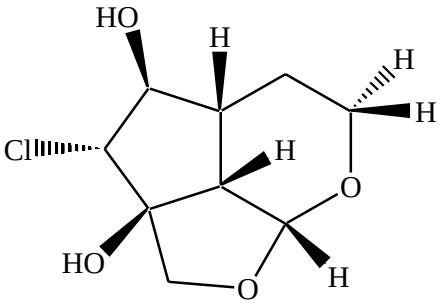
Geniposidic acid



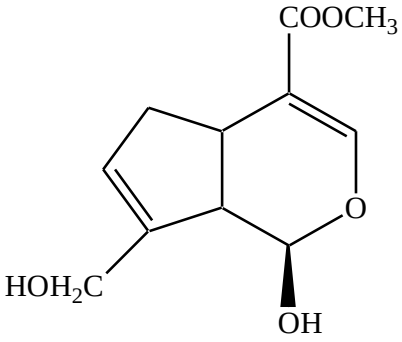
Lychnitoside



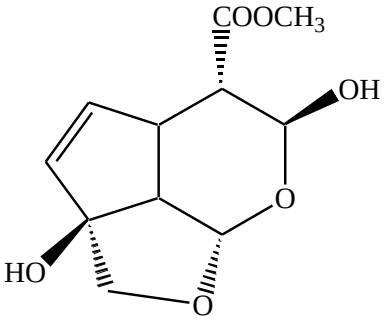
Rehmaglutin D



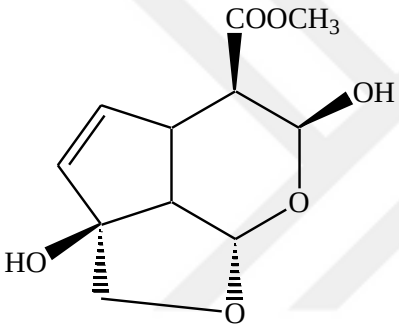
Glutinoside



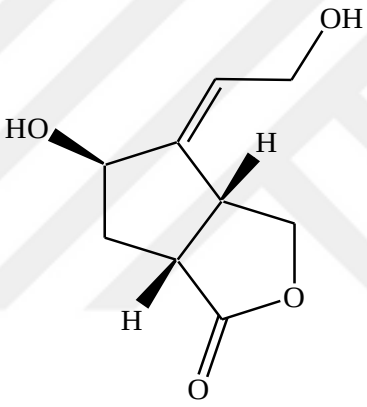
Genipin



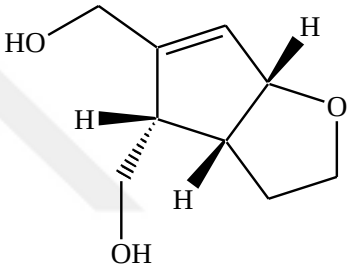
α-Gardiol



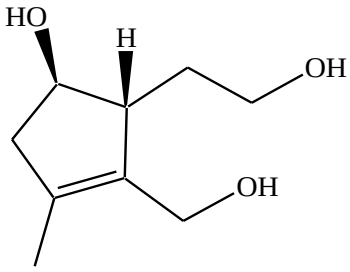
β-Gardiol



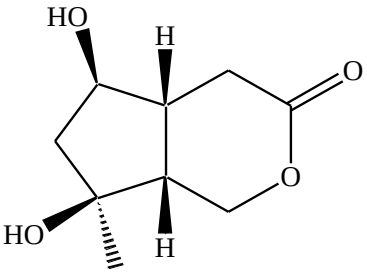
Verbathasin A



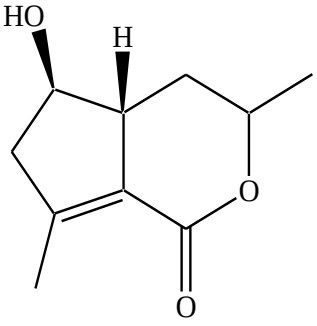
Ningpogenin



10-Deoxyeucommiol



Jioglutolide



6-Hydroxy-2-oxabicyclo [4.3.0] 8,9-none-1-one

2.1.3.2. Phenolic compouds

2.1.3.2.A. Phenylethanoid glycosides

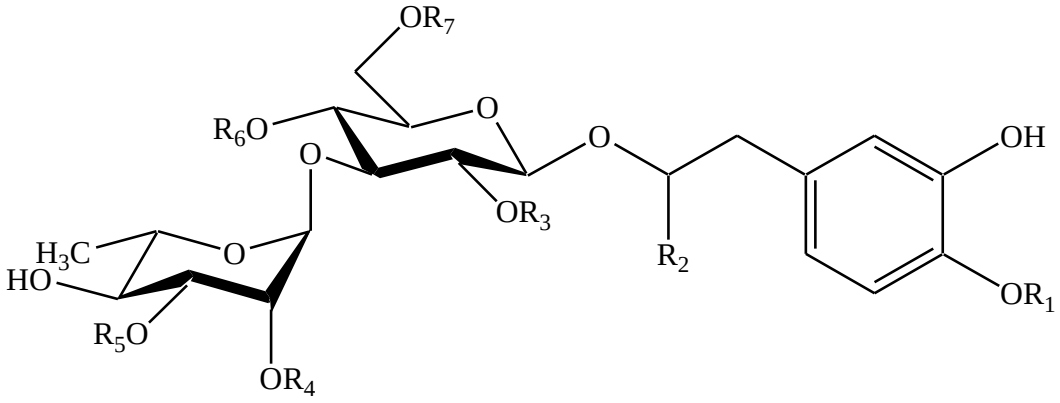


Table 4. Phenylethanoid glycosides obtained from *Verbascum* species

Compounds	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**Species**	**Ref**
Verbascoside (acteoside)	H	H	H	H	H	(*E*)-Caff	H	*V. georgicum* *V. lasianthum* *V. lychnitis* *V. macrurum* *V. mucronatum* *V. nigrum* *V. phlomoides* *V. pterocalycinum* *V. pycnostachyum* *V. salviifolium* *V. sinaiticum* *V. sinuatum* *V. spinosum* *V. thapsiforme* *V. thapsus* *V. undulatum* *V. wiedemannianum* *V. xanthophoeniceum*	(3,6,45,46,51,73,87,88,93,95–102)
Acetylacteoside	H	H	Ac	H	H	*(E)*-Caff	H	*V. sinaiticum*	(3,103)

β-Hydroxyacteoside	H	OH	H	H	H	*(E)*-Caff	H	*V. salviifolium*	(3,42)
Martynoside	CH_3	H	H	H	H	*(E)*-Fer	H	*V. letourneuxii* *V. macrurum* *V. salviifolium* *V. salviifolium* *V. undulatum* *V. wiedemannianum*	(3,42,70,89,96,102)
Poliumoside	H	H	H	H	H	*(E)*-Caff	Rha	*V. blattaria* *V. boerhaavii* *V. chaixii* *V. lasianthum* *V. phlomoides* *V. sinuatum* *V. thapsus*	(3,94,95)
Eukovoside	H	H	H	H	H	*(E)*-Isofer	H	*V. letourneuxii*	(3,70)
Angoroside A	H	H	H	H	H	*(E)*-Caff	Ara	*V. salviifolium* *V. salviifolium* *V. spinosum*	(3,42,51,102)
Arenarioside	H	H	H	H	H	*(E)*-Caff	Xyl	*V. sinaiticum* *V. thapsus* *V. undulatum*	(3,89,103,104)
Wiedemannioside A	CH_3	H	H	H	H	*(E)*-Fer	Ac	*V. wiedemannianum*	(3,96)
Wiedemannioside B	CH_3	H	H	Ac	Ac	*(E)*-Fer	Ac	*V. wiedemannianum*	(3,96)
Wiedemannioside C	H	H	H	H	H	*(E)*-Fer	Glc	*V. wiedemannianum*	(3,96)
Wiedemannioside D	H	H	Ac	H	H	*(E)*-Fer	Rha	*V. wiedemannianum*	(3,96)

Wiedemannioside E	H	H	Ac	Ac	H	*(E)*-Fer	Rha	*V. wiedemannianum*	(3,96)
Echinacoside	H	H	H	H	H	*(E)*-Caff	Glc	*V. wiedemannianum*	(3,96)
Forsytoside B	H	H	H	H	H	*(E)*-Caff	Api	*V. lychnitis* *V. nigrum* *V. phlomoides* *V. salviifolium* *V. thapsiforme* *V. thapsus* *V. xanthophoeniceum*	(3,42,100,102)
Angoroside C	CH_3	H	H	H	H	*(E)*-Fer	Ara	*V. macrurum* *V. spinosum*	(3,51,73)
Alyssonoside	H	H	H	H	H	*(E)*-Fer	Api	*V. thapsus*	(3,104)
Leucosceptoside B	CH_3	H	H	H	H	*(E)*-Fer	Api	*V. thapsus* *V. wiedemannianum* *V. xanthophoeniceum*	(3,46,73,96,104)
1'-*O*-*β*-D-(3,4-Dihydroxy-phenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')- *β*-D-xylopyranosyl-(1→6')-4'-*O*-*(E)*-feruloylglucopyranoside	H	H	H	H	H	*(E)*-Fer	Xyl	*V. thapsus*	(3,104)
1'-*O*-*β*-D-(3-Hydroxy-4-methoxyphenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')- *β*-D-xylopyranosyl-	CH_3	H	H	H	H	*(E)*-Fer	Xyl	*V. macrurum* *V. thapsus*	(3,73,104)

(1→6')-4'-*O*-*(E)*-feruloyl-glucopyranoside									
1'-*O*-*β*-D-(3-Hydroxy-4-methoxyphenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')- *β*-D-xylopyranosyl-(1→6')-4'-*O*-*(E)*-caffeoyloyl-glucopyranoside	CH_3	H	H	H	H	*(E)*-Caff	Xyl	*V. thapsus*	(3,104)
6''- *O*-*β*-D-Glucopyranosylmartynoside	CH_3	H	H	H	H	*(E)*-Fer	Glc	*V. thapsus*	(3,104)
1''-*O*-*β*-D-(3,4-Dihydroxy-phenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')- *β*-D-glucopyranosyl-(1→6')-4'-*O*-*(E)*-isoferuloyl-glucopyranoside	H	H	H	H	H	*(E)*-Isofer	Xyl	*V. sinaiticum*	(3,103)
1'-*O*-*β*-D-(3-Hydroxy-4-methoxyphenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')- *β*-D-glucopyranosyl-(1→6')-4'-*O*-*(E)*-isoferuloyl-glucopyranoside	CH_3	H	H	H	H	*(E)*-Isofer	Glc	*V. sinaiticum*	(3,103)

1'-*O*-*β*-D-(3,4-Dihydroxy-phenyl)-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→3')-3''-hydroxy-4''-*O*-*β*-D-glucopyranosyl-cinnamoyl-(1→6')-4'-*O*-*(E)*-isoferuloyl-glucopyranoside	H	H	H	H	H	H	4-*O*-Glc-Cinn	*V. thapsus*	(3,104)

Ac:Acetyl, Api: Apiofuranosyl, Ara: Arabinopyranosyl, Glc: Glucopyranosyl, Rha: Rhamnopyranosyl, Xyl: Xylopyaranosyl, Caff: Caffeoyl, Fer: Feruloyl, Isofer: Isoferuloyl, Cinn: Cinnamo

2.1.3.2.B. Phenylpropanoid glycosides

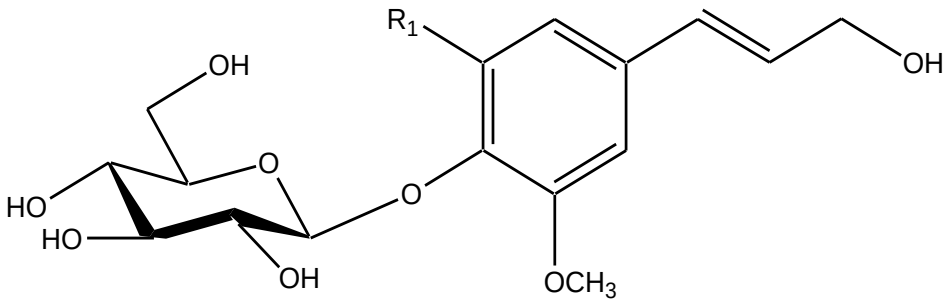


Table 5. Phenylpropanoid glycosides obtained from *Verbascum* species

Compounds	**R_1**	**Species**	**Ref**
Coniferin	H	*V. letourneuxii*	(3,70)
Syringin	OCH_3	*V. letourneuxii*	(3,70)

2.1.3.2.B. Flavonoids

2.1.3.2.B.1. Flavanones

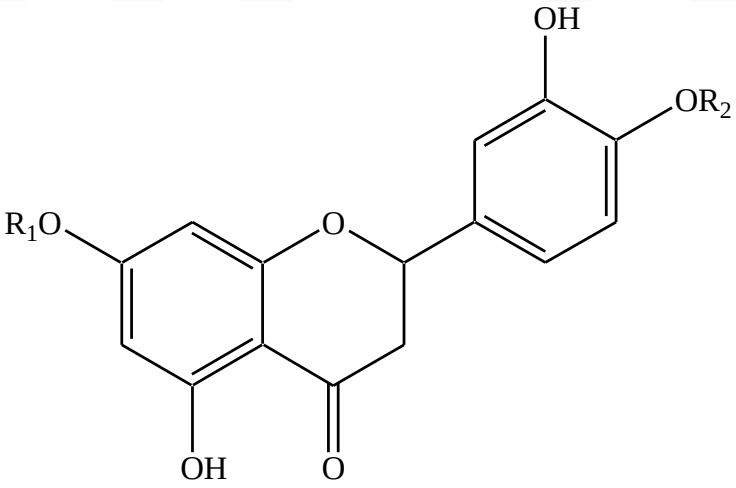


Table 6. Flavanones obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Eriodictiol	H	H	*V. phlomoides*	(3)
Hesperidin	Rut	CH_3	*V. phlomoides*	(3)

2.1.3.2.B.2. Flavones Derivatives

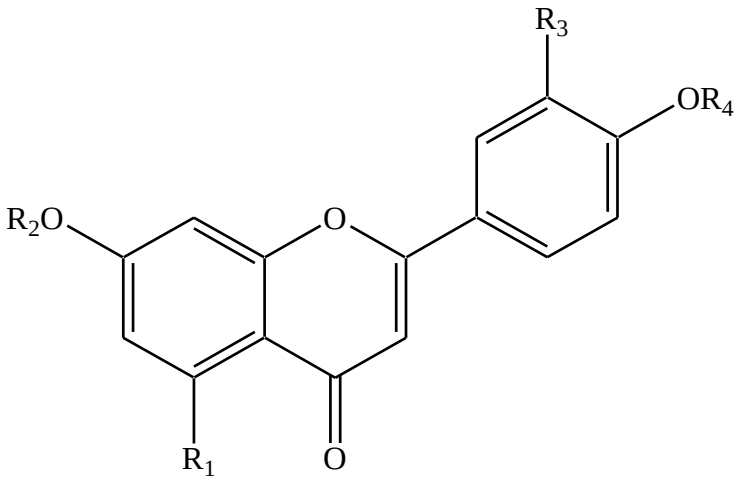


Table 7. Flavones obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**Species**	**Ref**
Apigenin	OH	H	H	H	*V. eremobium* *V. fruticulosum* *V. lychnitis* *V. phlomoides* *V. schimperianum* *V. songaricum* *V. thapsiforme*	(3,53,105–108)
Apigenin-4'-methylether	OH	H	H	CH_3	*V. lychnitis*	(3,53)
Luteolin	OH	H	OH	H	*V. fruticulosum* *V. lychnitis* *V. phlomoides* *V. sinaiticum* *V. songaricum* *V. thapsiforme* *V. thapsus* *V. wiedemannianum*	(3,38,53,96,105–110)
Chrysoeriol	OH	H	OCH_3	H	*V. phlomoides* *V. sinaiticum*	(3,107,109)

Apigenin-7-*O*-glucoside=apigetrin	OH	Glc	H	H	*V. eremobium* *V. fruticulosum* *V. letourneuxii* *V. phlomoides* *V. salviifolium* *V. schimperianum* *V. sinaiticum* *V. thapsiforme* *V. thapsus*	(3,38,42,105–107)
Apigenin-7-*O*-glucuronide	OH	Glc	H	H	*V. lychnitis* *V. nigrum*	(3,111)
Luteolin-5-*O*-glucoside	OGlc	H	OH	H	*V. lychnitis*	(3,53)
Luteolin-7-*O*-glucoside	OH	Glc	OH	H	*V. dudleyanum* *V. eremobium* *V. fruticulosum* *V. letourneuxii* *V. phlomoides* *V. salviifolium* *V. scadicolum* *V. scimperianum* *V. sinaiticum* *V. songaricum* *V. thapsiforme*	(3,42,105–108,112)
Luteolin-7-*O*-glucuronide	OH	Glc	OH	H	*V. lychnitis* *V. nigrum*	(3,111)
Luteolin-3'-*O*-glucoside	OH	H	OGlc	H	*V. salviifolium*	(3,42)
Luteolin-7-metylether	OH	CH_3	OH	H	*V. lychnitis*	(3,53)
Diosmetin	OH	H	OH	CH_3	*V. thapsus*	(3)
Diosmetin-7-*O*-glucuronide	OH	Glc	OH	CH_3	*V. nigrum*	(3,111)
Diosmetin-7-*O*-rutinoside	OH	Rut	OH	CH_3	*V. phlomoides*	(3,113)

7-Hydroxy-4'- *O*-α-L-rhamnopyranosylflavone	H	H	H	Rha	*V. thapsus*	(3,114)
Apigenin-4'-*O*-rhamnoside	OH	H	H	Rha	*V. thapsus*	(3,114)
Acacetin-7-*O*-glucoside	OH	Glc	H	CH_3	*V. fruticulosum* *V. schimperianum* *V. sinaiticum*	(3,105)
Acacetin-7-*O*-galactoside	OH	Gal	H	CH_3	*V. schimperianum* *V. sinaiticum*	(3,105)
Chrysoeriol-7-*O*-glucoside	OH	Glc	OCH_3	H	*V. eremobium* *V. salviifolium* *V. schimperianum* *V. sinaiticum*	(3,42,105)

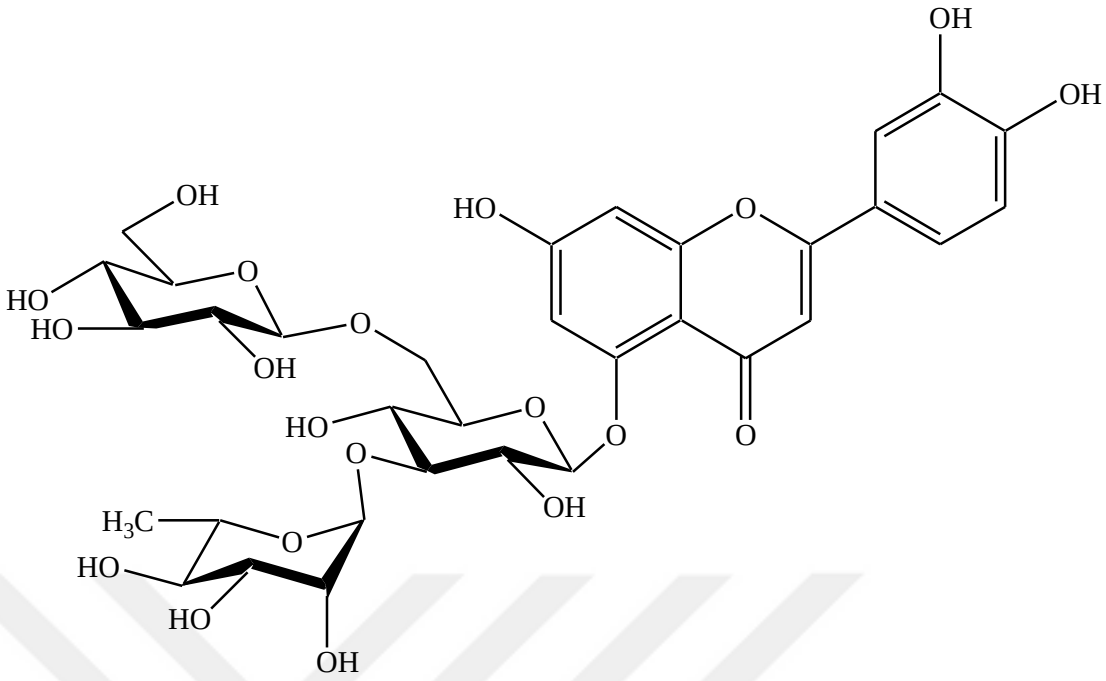


Table 8. Flavone obtained from *Verbascum* species

Compounds	**Species**	**Ref**
Verbacoside	*V. thapsus*	(3,110)

2.1.3.2.B.3. Flavonols

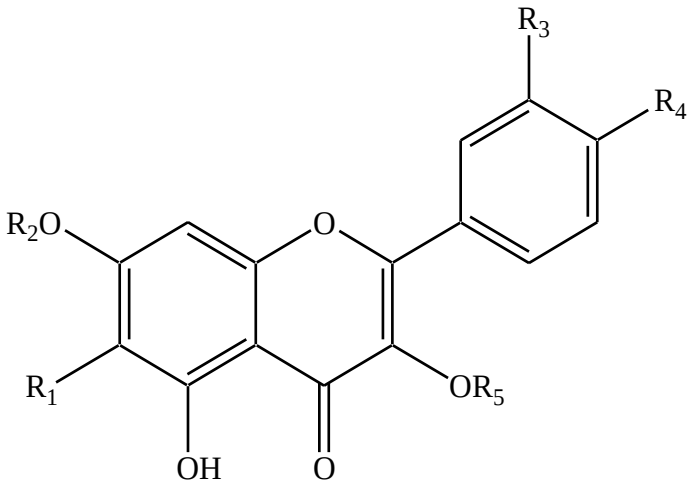


Table 9. Flavonol obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**Species**	**Ref**
Kaemferol	H	H	H	OH	H	*V. phlomoides*	(3,107)
Quercetin	H	H	OH	OH	H	*V. lychnitis* *V. phlomoides* *V. songaricum*	(3,107,108, 111)
3,5-Dixydroxy-7,7-dimethoxyflavone	OCH_3	CH_3	H	H	H	*V. thapsus*	(3,94)
Isorhamnetin	H	H	OH	OCH_3	H	*V. thapsus*	(3,114)
Qercetin-7-*O*-glucoside	H	Glc	OH	OH	H	*V. thapsiforme*	(3,106)
Qercetin-7-*O*-glucuronide	H	Gluc	OH	OH	H	*V. lychnitis* *V. nigrum*	(3,111)
Rutin	H	Rut	OH	OH	H	*V. phlomoides*	(3,107)
Qercetin-3,7-*O*-diglucoside	H	Glc	OH	OH	Glc	*V. thapsiforme*	(3,106)
Tamarixetin-7-*O*-glucoside	H	Glc	OH	OCH_3	H	*V. phlomoides*	(3,106,113)
Tamarixetin-7-*O*-rhamnoside	H	Rha	OH	OCH_3	H	*V. phlomoides*	(3,113)

Tamarixetin-7-*O*-rutinoside	H	Rut	OH	OCH_3	H	*V. phlomoides*	(3,106,113)
Patuletin	OCH_3	H	OH	OH	H	*V. lychnitis*	(3,53)

Rut: Rutinosyl Rha: Rhamnosyl

2.1.3.2.B.4. Isoflavonoids, flavone C-glycosides and bisflavonoids

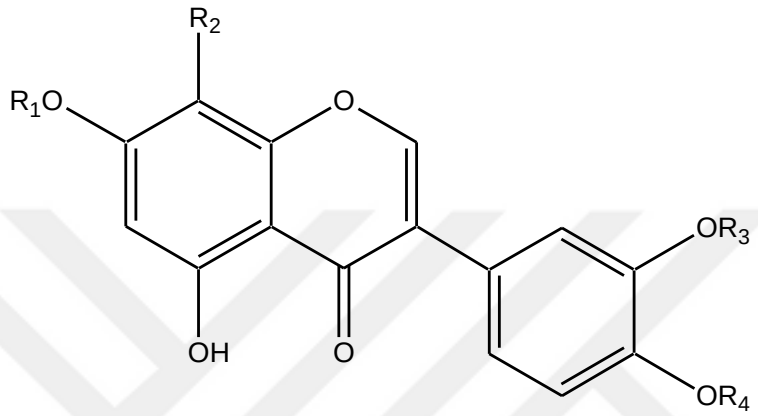


Table 10. Isoflavonoids obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**Species**	**Ref**
Orobol	H	H	H	H	*V. sinaiticum*	(3,98)
Orobol-7-*O*-glucoside	Glc	H	H	H	*V. sinaiticum*	(3,98)
5,3′,4′-Trihydroxy-8-methylisoflavone-7-*O*-glucoside	Glc	CH_3	H	H	*V. sinaiticum*	(3,98)
5-Hydroxy-3',4'-dimethoxyisoflavone-7-*O*-rhamnoside	Rha	H	CH_3	CH_3	*V. sinaiticum*	(3,98)

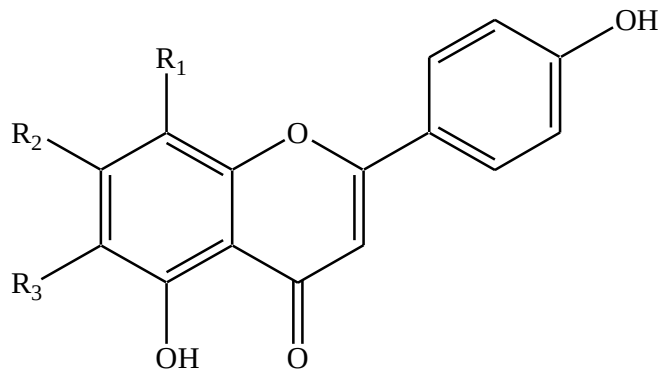


Table 11. Flavone *C*-glycosides isolated from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**Ref**
Vitexin	Glc	OH	H	(3)
Swetisin	H	OCH_3	Glc	(3)

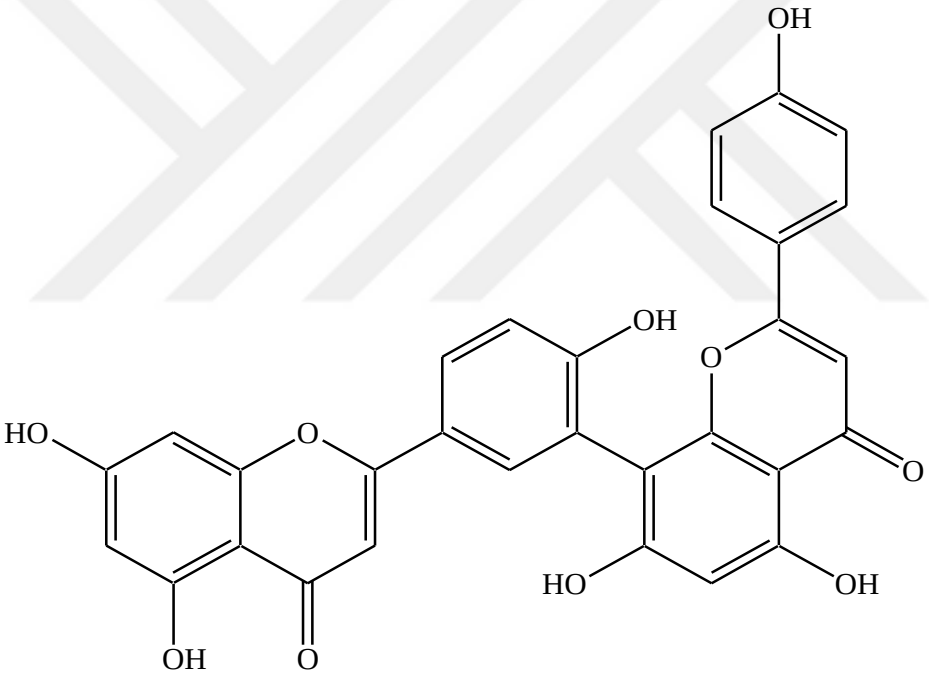


Table 12. Bisflavonoid obtained from *Verbascum* species

Compound	**Species**	**Ref**
Amentoflavone	*V. thapsus*	(3)

2.1.3.3. Neolignan glucoside

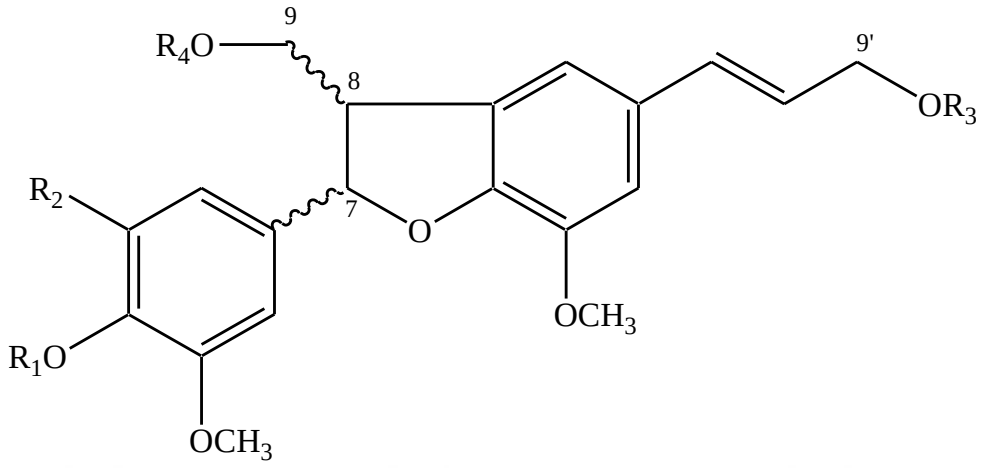


Table 13. Neolignan glucosides obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**Species**	**Ref**
Dehydrodiconiferyl glucoside E (*7R, 8S*)	H	H	H	Glc	*V. thapsus*	(3,104)
Dehydrodiconiferyl glucoside D (*7S, 8R*)	Glc	H	H	H	*V. thapsus*	(3,104)
Dehydrodiconiferyl glucoside G (*7S, 8R*)	H	OCH_3	H	Glc	*V. thapsus*	(3,104)
Dehydrodiconiferyl glucoside (*7S, 8R*)	H	OCH_3	H	Glc	*V. thapsus*	(3,104)
Dehydrodiconiferyl diglucoside	Glc	H	CH_3	Glc	*V. thapsus*	(3,104)
Dehydrodiconiferyl alcoho-9'-glucoside	H	H	Glc	H	*V. salviifolium*	(3,102, 104)
Dehydrodiconiferyl alcoho-9-glucoside	H	H	H	Glc	*V. salviifolium*	(3,102)
4-*O*-Methyl-dehydrodiconiferylalcohol-9'-glucoside	CH_3	H	Glc	H	*V. letourneuxi*	(3,70)

2.1.3.4. Flavonolignans

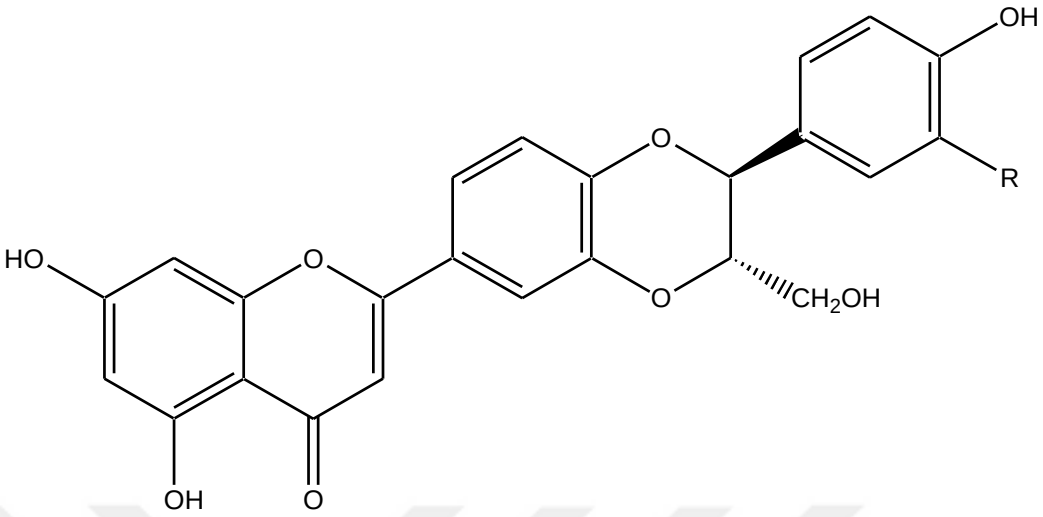


Table 14. Flavonolignans obtained from *Verbascum* species

Compounds	**R**	**Species**	**Ref**
Siniatisin	H	*V. siniaticum*	(3)
Hydrocarpin	OCH_3	*V. siniaticum*	(3)

2.1.3.5. Triterpene saponins

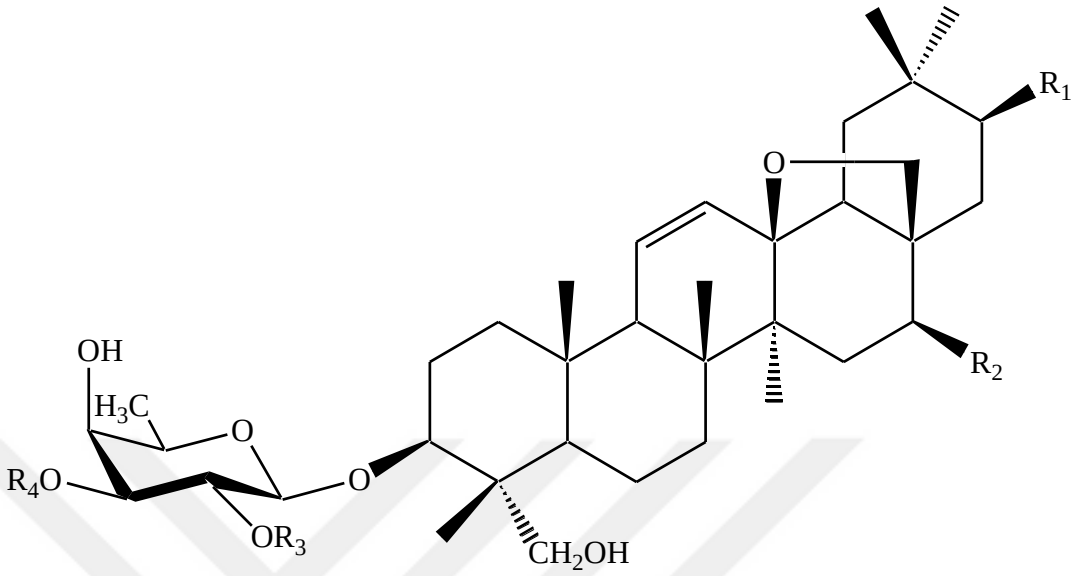


Table 15. Triterpene saponins obtained from *Verbascum* species

Compounds	**R1**	**R2**	**R3**	**R4**	**Species**	**Ref**
3-*O*-*β*-Fucopyranosylsaikogen in F	H	OH	H	H	*V. roripifolium*	(3,115)
Saikosaponin A	H	OH	H	Glc	*V. roripifolium* *V. sinaiticum*	(3,115)
Desrhamnosyl verbascosaponin	H	H	Glc	Glc	*V. phlomoides* *V. thapsiforme*	(3,68,115)
Ilwensisaponin A=Mimengoside A	H	H	Glc	Rha (1→4) Glc	*V. ballii* *V. dudleyanum* *V. lasianthum* *V. mucronatum* *V. nigrum* *V. phlomoides* *V. pterocalycinum* *V. roripifolium*	(3,45,62,79,94,115–119)

					V. sinaiticum *V. thapsiforme*	
Buddlejasaponin I (Verbascosaponin B)	H	OH	Glc	Rha (1→4) Glc	*V. fruticulosum* *V. roripifolium* *V. sinaiticum* *V. thapsiforme*	(3,115)
Buddlejasaponin IV	H	OH	Glc	Glc	*V. thapsiforme*	(3,115)
Mulleinsaponin I	H	H	H	Glc	*V. sinaiticum* *V. songaricum*	(3,115,120)
Mulleinsaponin II	H	H	H	Rha (1→4) Glc	*V. sinaiticum*	(3,115)
Mulleinsaponin III	H	OH	H	Rha (1→4) Glc	*V. fruticulosum* *V. roripifolium* *V. sinaiticum*	(3,115)
Mulleinsaponin IV	OH	OH	Glc	Rha (1→4) Glc	*V. fruticulosum*	(3,115)
Mulleinsaponin V	OAc	OH	Glc	Rha (1→4) Glc	*V. fruticulosum* *V. lychnitis*	(3,58,115)
Mulleinsaponin VI	H	H	Glc	Rha (1→4) Glc	*V. sinaiticum*	(3,115)
Mulleinsaponin VII	H	H	Glc	Rha (1→4) Glc	*V. sinaiticum*	(3,115)
Songarosaponin C	H	H	Glc	Glc (1→4) Glc	*V. songaricum* *V. thapsiforme*	(3,115,121)
Songarosaponin D	H	H	Glc	Glc (1→4) Glc	*V. songaricum*	(3,115,122)

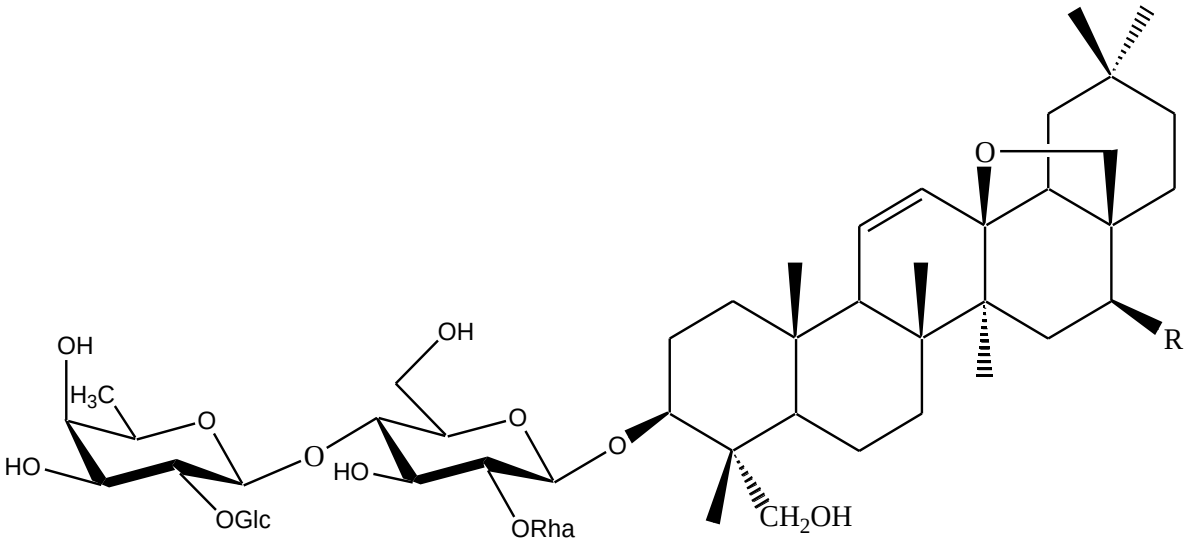


Table 16. Triterpene saponins obtained from *Verbascum* species

Compounds	**R**	**Species**	**Ref**
Thapsuin A	H	*V. lychnitis* *V. thapsus*	(3,58)
Hydroxythapsuin	OGlc	*V. lychnitis* *V. thapsus*	(3,58,115)

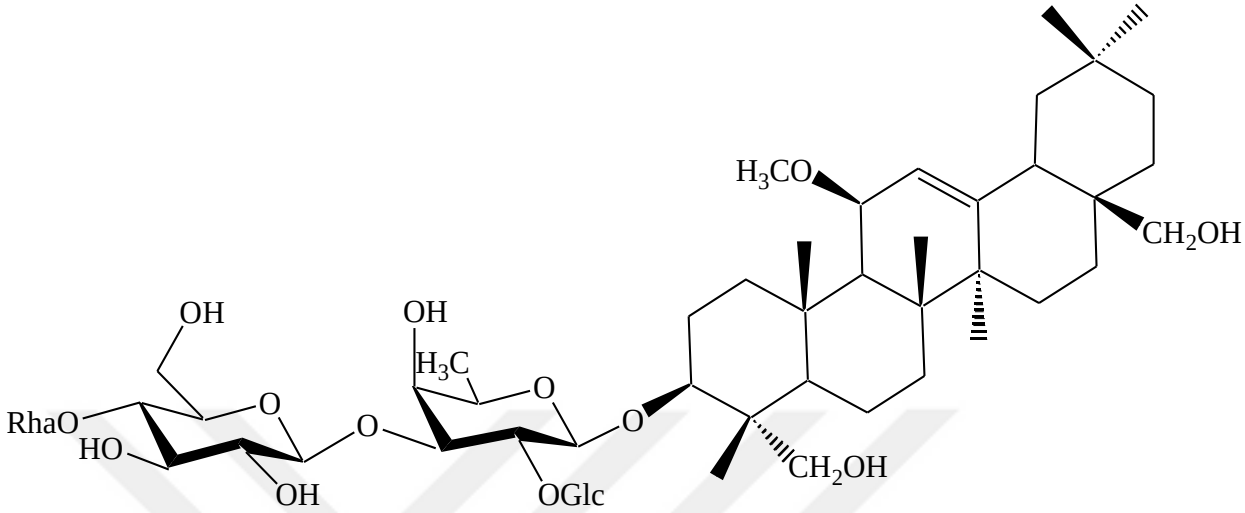


Table 17. Triterpene saponins obtained from *Verbascum* species

Compounds	**Species**	**Ref**
Ilwensisaponın C	*V. mucronatum*	(3,45,62,68,94,116,117)
	V. dudleyanum	
	V. phlomoides	
	V. pterocalicum	
	V. nigrum	
	V. nigrum	

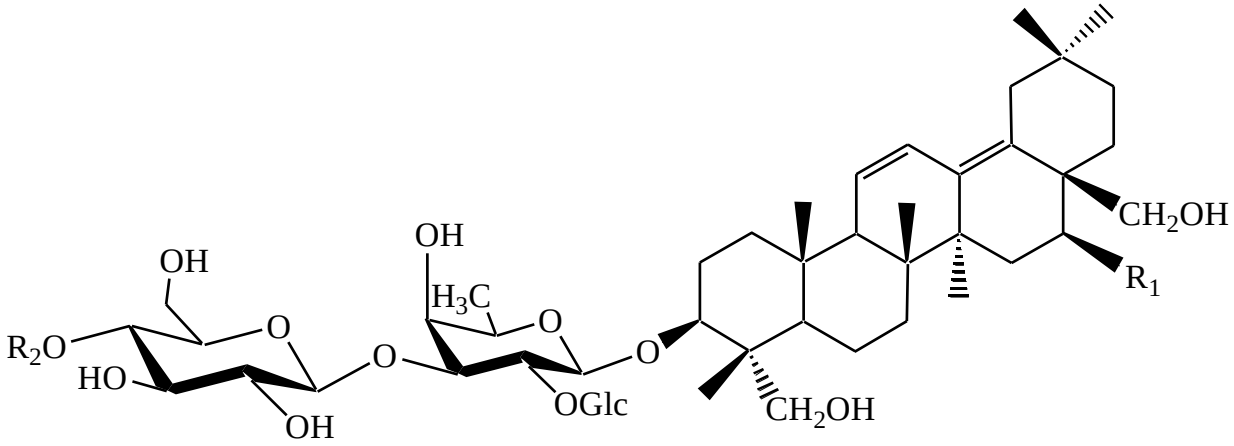


Table 18. Triterpene saponins obtained from *Verbascum* species

Compounds	**Species**	**Ref**
Songarosaponin B	*V. songaricum*	(3,121)

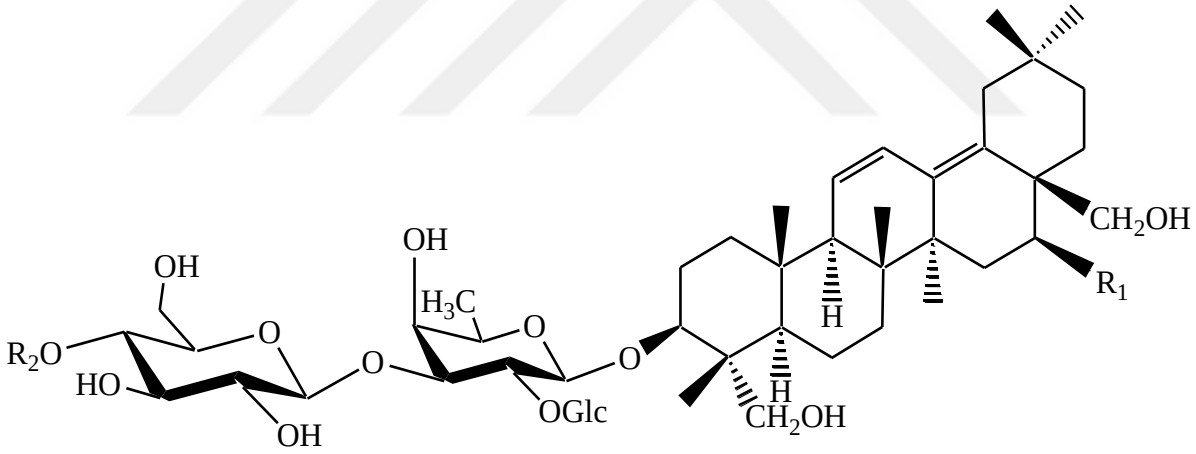


Table 19. Triterpene saponins obtained from *Verbascum* species

Compounds	**R1**	**R2**	**Species**	**Ref**
Ilwensisaponin B	H	Rha	*V. songaricum*	(3,121)
Songarosaponin E	H	Glc	*V. songaricum*	(3,120)
Songoarosaponin F	OH	Glc	*V. songaricum*	(3,120)

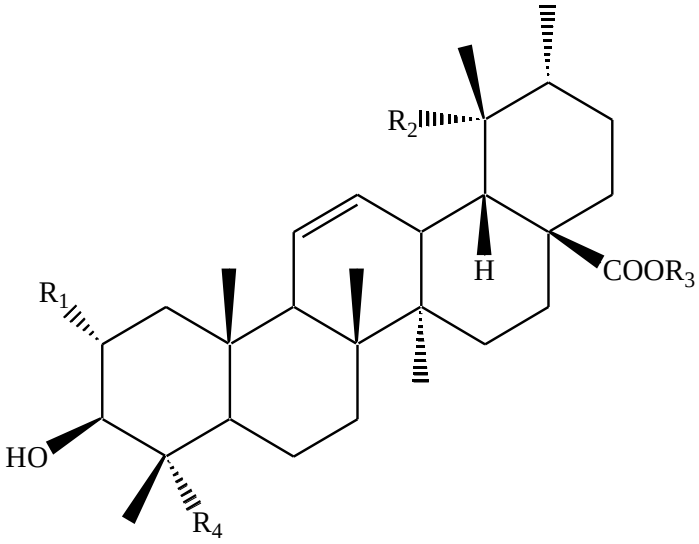


Table 20. Triterpene saponins obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**Species**	**Ref**
Ursolic acid	H	H	H	H	*V. lychnitis*	(3,58)
Rosamultin	OH	OH	Glc	H	*V. wiedemannianum*	(3,96)
Niga-ichigoside F1	OH	OH	Glc	CH_2OH	*V. wiedemannianum*	(3,96)

2.1.3.6. Alkaloids

2.1.3.6.A. Spermine alkaloids

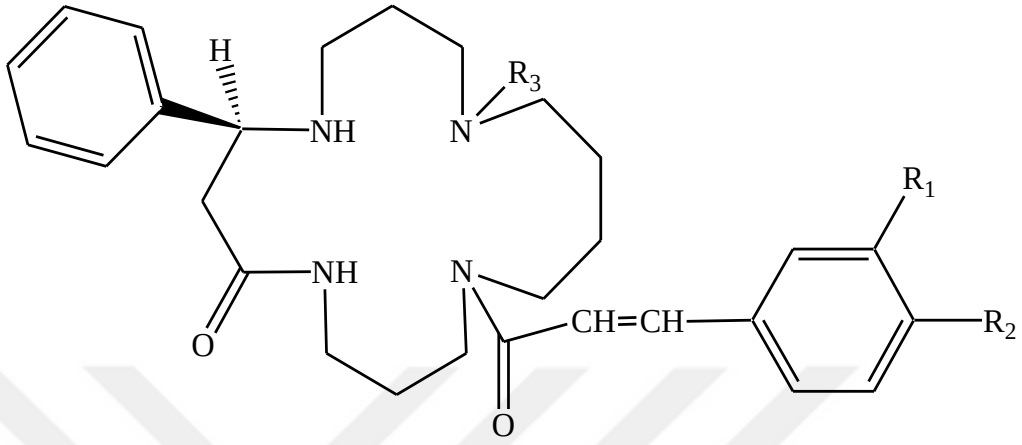
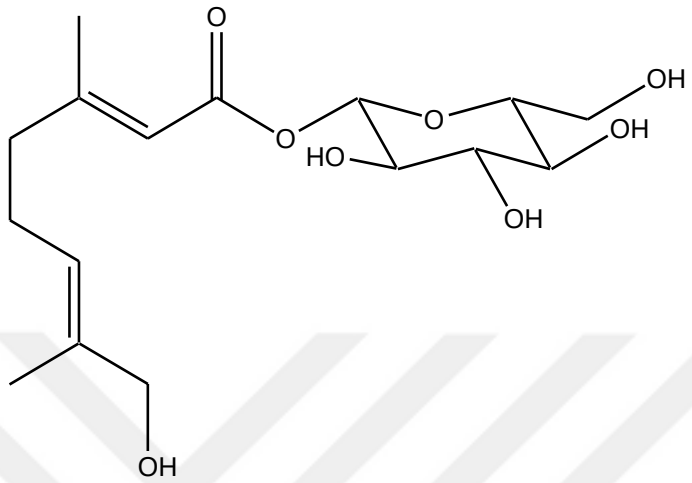


Table 21. Spermine alkaloids obtained from *Verbascum* species

Compounds	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
(*E*)-Verbacine	H	H	H	*V. pseudonobile* *V. phoeniceum* *V. nobile* *V. songoricum*	(3,123–129)
(*Z*)-Verballocine	H	H	H	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*E*)-Verbasitrine	OCH_3	OCH_3	H	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*Z*)-Isoverbasitrine	OCH_3	OCH_3	H	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*E*)-Verbasikrine	H	OCH_3	H	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*Z*)-Isoverbasikrine	H	OCH_3	H	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*E*)-Verbamedine	H	H	CHO	*V. pseudonobile* *V. phoeniceum*	(3,123–127)
(*Z*)-Isoverbamedine	H	H	CHO	*V. pseudonobile* *V. phoeniceum*	(3,123–127)

2.1.3.7. Terpenes

2.1.3.7.A. Monoterpene glycoside



T**able 22.** Monoterpene obtained from *Verbascum* species

Compounds	**Species**	**Ref**
1-*β*-D-glucopyranosyl-8-hydroxy-3, 7-dimethyl-2(*E*), 6(*E*)- octadienoate	*V. pterocalicinum*	(94)

2.1.3.7.B. Sesquiterpenes

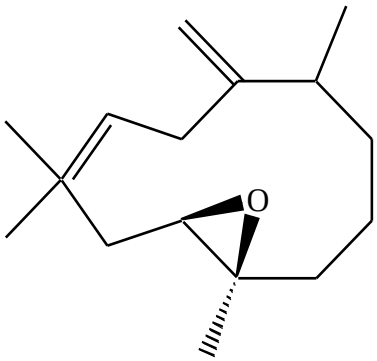


Table 23. Sesquiterpene obtained from *Verbascum* species

Compounds	**Species**	**Ref**
Buddlindeterpene A	*V. thapsus*	(3,93)

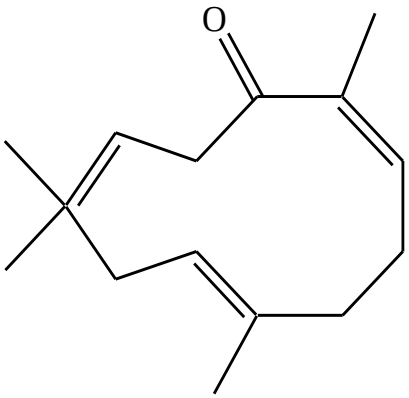


Table 24. Sesquiterpene obtained from *Verbascum* species

Compounds	**Species**	**Ref**
Buddlindeterpene B	*V. thapsus*	(3,79)

2.1.3.8. Phytosterols

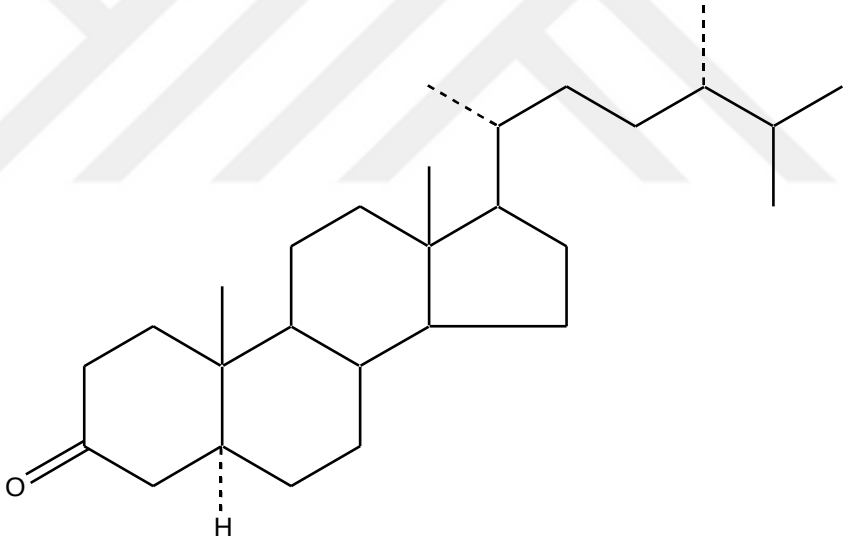


Table 25. Phytosterol obtained from *Verbascum* species

Compound	**Species**	**Ref**
24-*α*-methyl-5*α*-colestan-3-on	*V. thapsus*	(83)

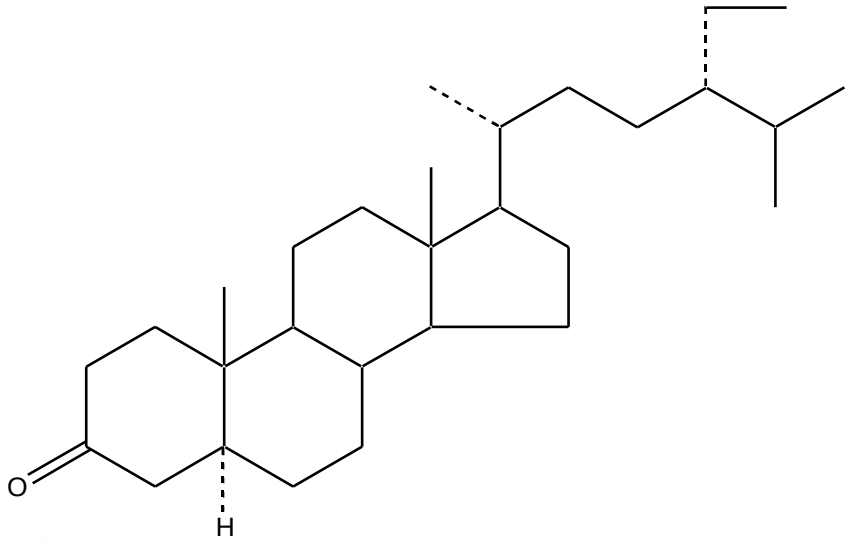


Table 26. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
24-*α*-ethyl-5*α*-colestan-3-on	*V. thapsus*	(83)

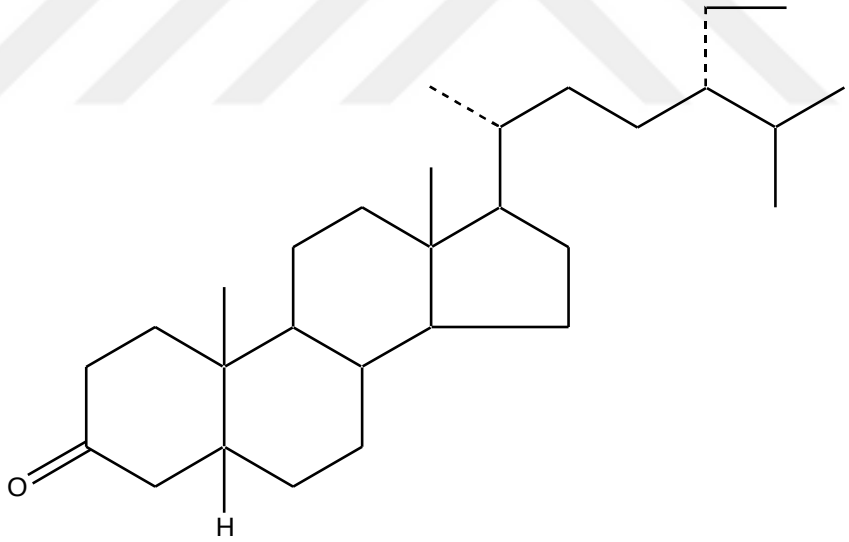


Table 27. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
24-*α*-ethyl-5*β*-colestan-3-on	*V. thapsus*	(83)

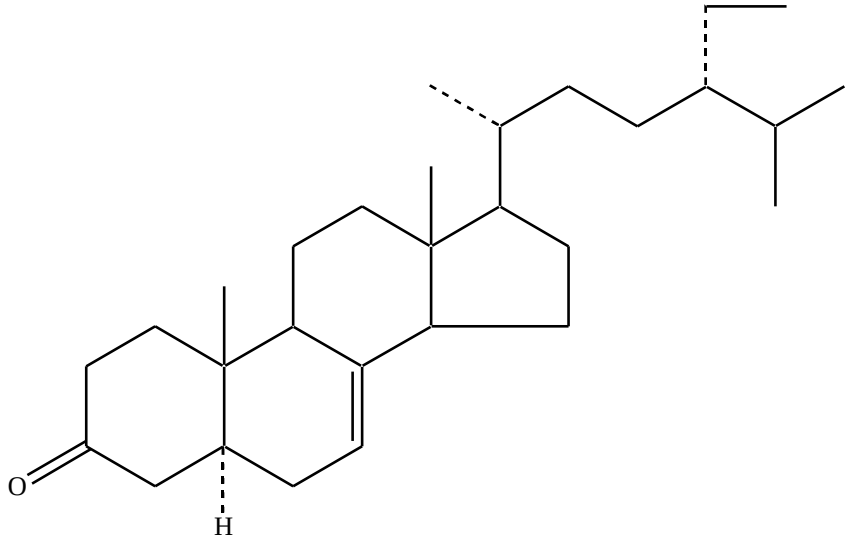


Table 28. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
24-*α*-ethyl-5*α*-colestan-7-en-3-on	*V. thapsus*	(83)

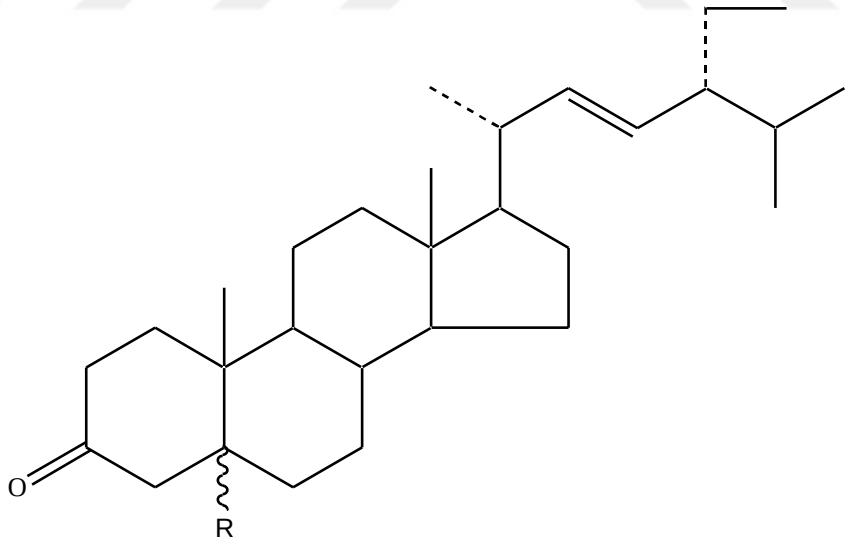


Table 29. Phytosterol obtained from *Verbascum* species

Compound	R_1	Species	Ref
24-*α*-ethyl-5*α*-colestan-22-en-3-on	*α*-H	*V. thapsus*	(83)
24-*α*-ethyl-5*β*-colestan-22-en-3-on	*β*-H	*V. thapsus*	(83)

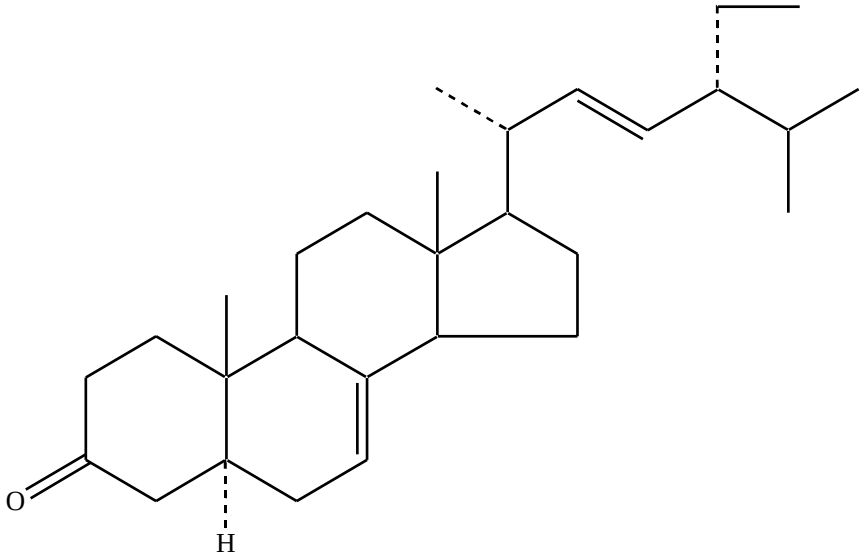


Table 30. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
24-α-ethyl-5α-colestan-$\Delta^{7,22}$-en-3-on	*V. thapsus*	(83)

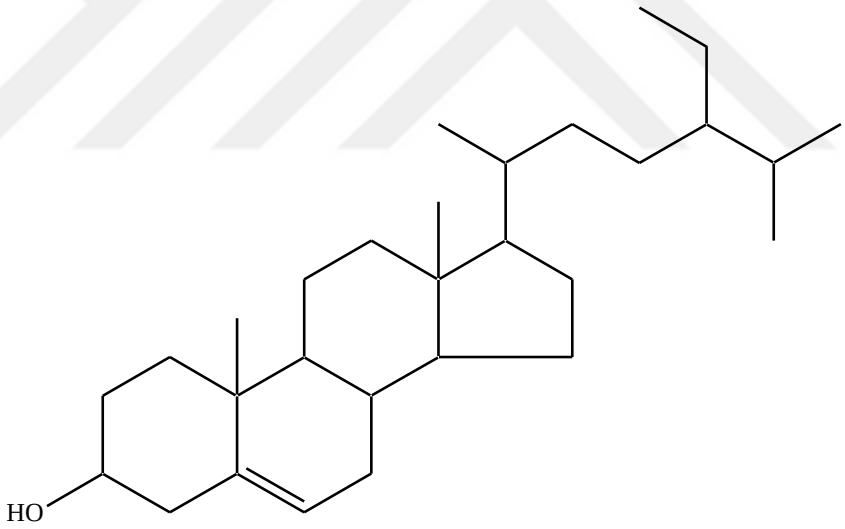


Table 31. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
β-Sitosterol	*V. lasianthum* *V. phlomoides* *V. pycnostachyum* *V. thapsiforme* *V. thapsus*	(6,65,83,130,131)

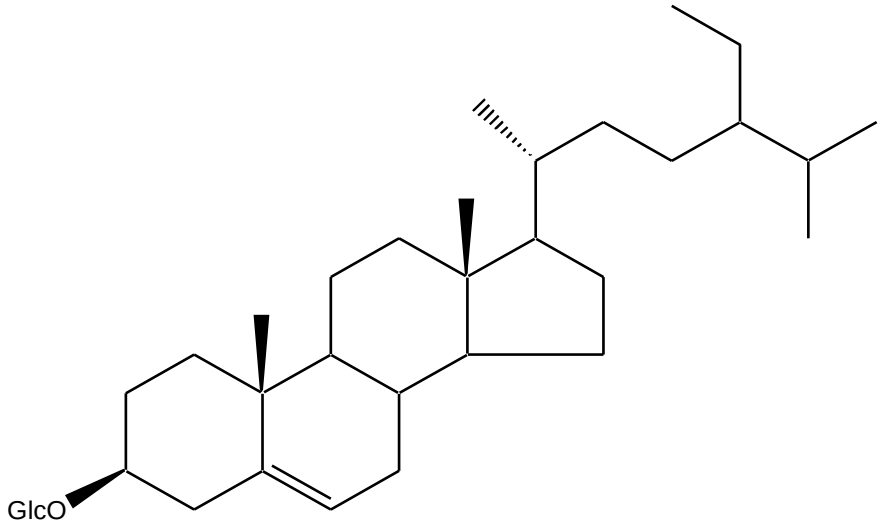


Table 32. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
β-Sitosterol-3-*O* -*β*-D-glucopyranoside	*V. wiedemannianum*	(6,54)

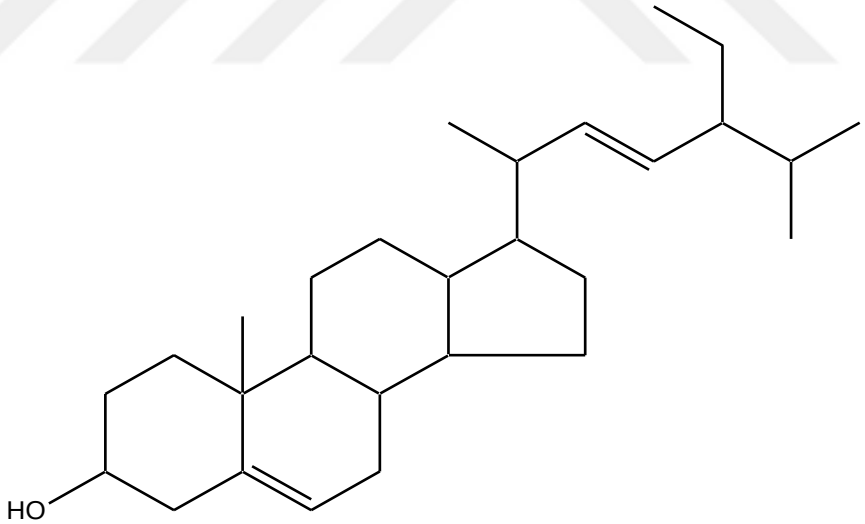


Table 33. Phytosterol obtained from *Verbascum* species

Compound	Species	Ref
Stigmasterol	*V. phlomoides* *V. pycnostachyum*	(6,65,132)

2.1.3.9. Other compounds

2.1.3.9.A. Acetophenone glucoside

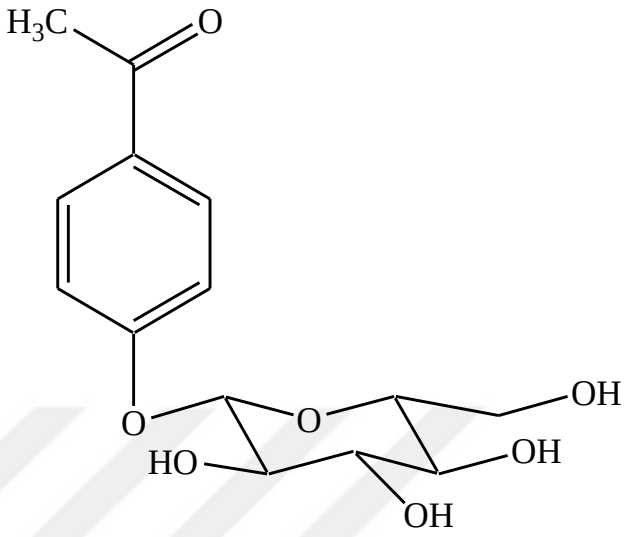
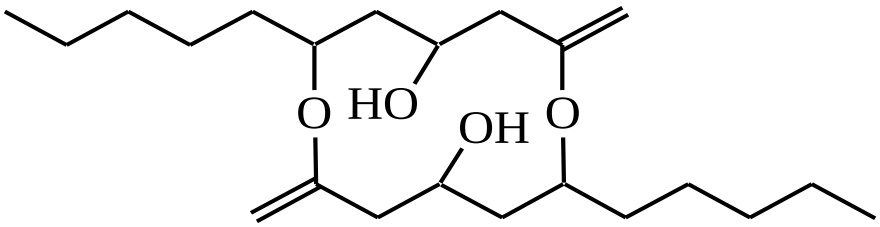


Table 34. Acetophenone glucoside obtained from *Verbascum* species

Compounds	Species	Ref
Picein	*V. dudleyanum*	(3,133)

2.1.3.9.B. Macrocyclic dimer lactone



T**able 35.** Macrocyclic dimer lactone obtained from *Verbascum* species

Compounds	Species	Ref
Verbalactone	*V. undulatum*	(3,62)

2.1.4. Analytical studies on *Verbascum* species

A validated reversed phase High Performance Liquid Chromatographic (RP-HPLC) method for the determination of two phenylethanoids and eight flavonoids in *V. densiflorum* and *V. phlomoides* was reported. In this method, a C_{18} Lichrosphere 100 column (5 μm, 250 mm × 4.6 mm) was used at room temperature. The UV–vis detector was set to 349 nm. An acetonitrile gradient elution was employed using aqueous 0.5% orthophosphoric acid solution containing 1% tetrahydrofurane. The flow rate was adjusted to 0.7 mL/min. Validation results of the method indicated that good linearity ($r > 0.997$), highly sensitive detection limits for 0.390 – 0.555 μg/mL for phenylethanoids, 0.156 – 0.336 μg/mL for flavonoid glycosides and 0.062 – 0.083 μg/mL for flavonoid aglycones. Moreover, intra-day and inter-day variations were less than 1.13 and 3.36%, respectively, and the average recoveries were between 93.5 and 101.9%. The analysis results concluded that diosmin and tamarixetin 7-*O*-rutinoside (2.327 – 2.392% of dry weight) were found in the flower of *V. phlomoides* at high level, but on the other hand verbascoside (0.688 – 0.742% of dry weight) and luteolin 7-*O*-glucoside (0.204–0.279% of dry weight) were demonstrated in the *V. densiflorum* flower. This study revealed that the developed method was suitable for quality assurance and the differentiation of *V. phlomoides* and *V. densiflorum* species (134).

Jamshidi et al. quantified caffeic acid content in four species (*V. macrophyllus, V. pseudo-digitalis, V. sinatum, V. songaricum)* of nine ecotypes mullein (*Verbascum* sp.) from southwest Iran. They used RP-HPLC method with UV PDA 2800 detector and C_{18} (250×4.6 mm) column. The results revealed that *V. sinatum* species and ecotype Sepidan (7.76 μg/mg extract) contained the highest level of caffeic acid, whereas *V. songaricum* species and ecotype Farokhshahr (0.54 μg/mg extract), contained the lowest caffeic acid. The outcomes showed that habitat and climate responded to high level of caffeic acid variation among *Verbascum* sp (135).

Georgiev et al., studied metabolic differentiation and classification of *Verbascum* species by NMR-based metabolomics by using principal component analyses (PCA) in five *Verbascum* species. They divided mulleins into two groups; group A (*Verbascum phlomoides* and *Verbascum densiflorum*) and group B (*Verbascum xanthophoeniceum, Verbascum nigrum* and *Verbascum phoeniceum*). The results showed that group B contained higher amounts of iridoid and phenylethanoid glycosides, whereas *V. xanthophoeniceum* and *V. nigrum* accumulate higher amounts of the pharmaceutically important harpagoside (0.5%

on dry weight basis) and verbascoside, forsythoside B and leucosceptoside B (in total 5.6–5.8% on dry weight basis) (15).

Grigore et al., examined the antioxidant and anti-inflammatory potential of *V. phlomoides* polyphenol-rich extract. Phenolic compounds were spectrophotometrically determined and specific compounds were measured by HPLC. A chromasil C_{18} analytical column (4.6 mm 100 mm) for polyphenols and a C_8 column (2.1 mm 100 mm) for polyphenols were used. LC-20AD pump, a CTO-20A column oven, a DAD-MS detector with SPD-M20A diode array and MS Shimadzu, a DGU-20As degasser and an LCMS solution software was used. In conclusion, major secondary metabolites of *Verbascum* sample quantified by HPLC. The amounts of rosmarinic acid (14.93 mg/g), caffeic acid (39.96 mg/g), ferulic acid (29.61 mg/g) and quercetin (17.29 mg/g) were calculated; on the contrary acteoside was not detected. The aucubine content was very low (0.028 mg/g) (48).

Torok and Varga (2015) identified the species of mullein (*V. phlomoides* L., *V. thapsus* L., *V. thapsiforme* Schrad., *V. speciosum* L.) and studied the plant's major active substances both qualitatively and quantitatively. It was understood that the plants were hybrids of *V. phlomoides* and *V. thapsiforme*. Any significant difference was not be found in microscopic analyses. Both stomata and pollen sizes were around 15-20 μm. Rutin and quercetin were determined as significant flavonoids. Total flavonoid aglycone content (hypericin) was 0.135% and total flavonoid glycoside content (rutinoside) was 1.3%. Saponins showed two spots in TLC. A hemolytic index was established as 13095. A year later the result repeated and it was found that an important decrease of flavonoid aglycon (0.006%), flavonoid glycoside (0.95) contents and hemolytic index (4000) (136).

Bom et al. determined hybrid affinity chromatography of a-galactosidase from *V. thapsus* L. Because of the presence of coloured contaminants purification of an *α*-galactosidase from the roots of *V. thapsus* L. was hard to succeed using conventional methods. Hybrid affinity chromatography attributed to a mixed matrix separation procedure. A substrate analogue and an immobilized metal afinity matrix as ligands were used to obtain the pure enzyme. (137).

Xue et al. (2015) studied pharmacokinetics and tissue distribution of aucubin, ajugol and catalpol in rats using validated simultaneous a high-performance liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) assay. C_{18} reversed-phase column (150mm×2.1mm, 5 m) was used at 40°C temperature.

Gradient elution was employed. The flow rate was adjusted at 0.2 mL/min. Total analysis time was 7 min. Samples were prepared by simple protein precipitation with methanol contained 0.05% formic acid. Detection was achieved by a triple quadrupole tandem mass spectrometer. In the multiple-reaction monitoring (MRM) mode to monitor the precursor-to-product ion transitions of m/z 364.3 → 149.0 for aucubin, m/z 366.5 → 151.1 for ajugol, m/z 380.0 → 183.3 for catalpol and m/z 530.3 → 183.1 for picroside-II (IS) in positive ionization. Good linearity of each calibration curve was generated over the concentration range of 1–1000 ng/mL. The lower limit of quantification (LLOQ) was 1 ng/mL for the three analytes. This method was successfully performed to the pharmacokinetic and tissue distribution researches of aucubin, ajugol and catalpol in rat. The results showed pharmacokinetic behaviours of the herb compound and ensured novel evidence of the presence of aucubin and catalpol in rat brain. The obtained data would be beneficial for the clinical application and further studies of Traditional Chinese Medicines (TCM) with active ingredients of iridoid glycosides (138).

2.2. Oxygen and Oxidative Stress

In 1777, Swedish scientist Carl Wilhelm Scheele discovered oxygen molecules. And then, it was found out that oxygen is necessary for breathing and it procures to take out the carbon dioxide form from cellular metabolism (9).

Oxidative stress was explained by Sies (1985, 1986) as a serious imbalance between oxidation and antioxidation, ‘a disturbance in the prooxidant-antioxidant balance in favour of the former, leading to potential damage’ (9).

Oxygen is an unreactive compound that can be metabolized *in vivo* to form highly reactive oxidants known as oxygen-free radicals. Oxygen-free radicals attack biological macro molecules such as lipids, proteins, and DNA and they cause the diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic disease, and malignancies (139).

Redox regulation controls the balance between the beneficial and harmful effects of free radicals (140).

2.2.1. Free Radicals

A free radical is described as any species that contain one or more unpaired electrons in its atomic or molecular orbitals (141). Free radicals may occur by electron-transfer reactions and energy-transfer reactions. ROS and RNS comprise radicals such as alkoxyl

(RO•.), hydroxyl (OH•), hydroperoxyl (HO_2•), lipid peroxyl (ROO•), lipid peroxyl (LOO•), peroxyl (RO_2•), superoxide (O_2•), nitric oxide (NO•), nitrogen dioxide (NO_2•), peroxynitrate ($ONOO^-$), and non-radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen, nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) and lipid peroxide (LOOH) (141).

Molecular oxygen is a radical. The addition of one electron to the molecular oxygen forms a superoxide anion radical (140).

Superoxide anion radical is a low reactive species. The enzyme superoxide dismutase (SOD) protects the organism toward the harmful effects of this radical (142).

Hydrogen peroxide produced two-electron reduction of molecular oxygen or one-electron reduction of superoxide anion. Molecular oxygen and superoxide anion are radicals because they have unpaired electrons. But, hydrogen peroxide has no unpaired electrons. Therefore, it is not a radical. Hydroxyl radical is generated with a reaction between superoxide anion radical and hydrogen peroxide. Hydroxyl radical is the most reactive oxygen species. Hydroxyl radical and water (hydroxyl anion) are yielded by one electron reduction of hydrogen peroxide (140).

Formation of free radicals consists of three steps that are initiation, propagation and termination steps. With propagation step, free radicals regenerate as a result of chain reaction. Destruction of radicals has occurred in the termination step (141).

2.2.2. Lipid peroxidation

Lipid peroxidation (LP) results from oxidation of polyunsaturated fatty acids (PUFA) by free radicals in biological systems (143).

Biomembranes and subcellular organelles are vulnerable to LP due to PUFA in their membrane phospholipids. When lipids undergo a chemical change with free radicals, the highly damaging chain reaction of LP occurs.

Initiation: Lipid-reactive oxidant interactions starts the LP. They generated a fatty alkyl free radical.

Propagation: The fatty alkyl radical reacts with molecular oxygen to form a fatty peroxyl radical (ROO•). ROO• attacks a neighboring unsaturated fatty acid (RH) to generate hydroperoxides and a new fatty alkyl radical (R•).

Termination: The peroxidation can be terminated by a number of reactions. The important one has required the reaction of ROO• or lipid radical L• with an antioxidant such as vitamin E or α-tocopherol. LP produces many toxic products such as malondialdehyde (MDA), 4-hydroxymonenal (4-HNE) and various 2-alkenals. Isoprostanes can be measured with a mass spectrometry and ELISA-assay kits. They are unique product of LP of arachidonic acid (144).

2.2.3. Antioxidants

Antioxidants are molecules that neutralize free radicals or their actions (145). Mammalian cells are not vulnerable against oxidant attack, but that has several complex and protective mechanisms. These mechanisms are preventing, limiting, or repairing oxidative damage.

Glutathione peroxidase, superoxide dismutase (SOD), glutathione reductase, thiols, disülfide bonding and thioredoxin are defending system in every cell. Vitamin E is an antioxidant that prevents cell membranes in the human body.

Antioxidants behave at the levels of preventing, interception and repair. Preventing is stopping the formation of ROS. In this step, SOD catalyses the dismutation of superoxide to H_2O_2 and breaks it down to water. Interception is radical scavenging that is the second line defense mechanism of peroxyl radicals are effected. The antioxidants such as vitamins E and C, carotenoids, flavonoids, etc. are used for this mechanism. Furthermore, enzymes are used for repairing and reconstitution levels. Superoxide dismutase, catalase and glutathione peroxidase are enzymatic antioxidants. Non-enzymatic antioxidants are classified into two groups: metabolic and nutrient. Metabolic antioxidants are uric acid, L-arginine, glutathione, CoQ10, melatonin. Nutrient antioxidants are trace elements (selenium, zinc, manganese), vitamin C, vitamin E, polyphenolic compounds, carotenoids, omega-3 and omega-6 fatty acids (144).

2.3. Inflammation

Inflammation is a protective and essential immune response that is necessary to maintain the vital events during the infection and tissue injury. Also, it is required for tissue homeostasis. Inflammation symptoms are redness, pain, swelling and heat (10).

Immune system cells like neutrophils, lymphocytes, macrophages, mast cells and dendritic cells act in inflammatory responses (146).

Also, epithelial cells, endothelial cells and fibroblasts are non-immune cells for inflammatory responses. Inflammatory pathways differ from nature of stimulant and target tissues. For example, pathogen-specific receptors induce inflammatory cytokine-like tumour necrosis factor (TNF), interleukin-1 (IL-1) and interleukin-6 (IL-6) and chemokines. In antiviral responses, Type-1 interferons (IFNs) are induced, and parasitic infections IL-4, IL-5, IL-13 and histamine (10).

2.3.1. Eicosanoids, Leukotrienes and Lipoxygenases

Eicosanoids biosynthesis starts with phospholipase A2 activation and the arachidonic acid (AA) release. It is found in mammalian cells and synthesis from membrane phospholipids. Prostaglandins, thromboxanes and leukotrienes that term as eicosanoids come from the AA by cyclooxygenase (COX, thromboxanes) and lipoxygenase (LOX) pathways. Eicosanoid production is an inflammation marker. Non-steroidal anti-inflammatory drugs (NSAIDs) target the COX pathway. But, because of gastrointestinal (GI) side effect of NSAIDs, COX-2 inhibitors (COXIBs) have been developped. Selective COX-2 inhibition causes cardiovascular side effects without reducing COX-1. It changes the prostacyclin and thromboxane balance (147).

The leukotrienes are synthesized by 5-lipoxygenase enzyme. Four leukotriene groups generated from AA. They are found in many inflammatory conditions. For example; in the skin (psoriasis), the lung (allergic asthma), joints (rheumatoid artritis), heart (myocardial infarction). When Leocotriene B4 is a chemotactic agent for leucocytes, Leucotriene C4, D4 and E4 are bronchoconstrictors and potent mediators of microvascular tone and permeability (148).

Lipoxygenases (LOX) are lipid peroxidizing enzymes. They oxygenate free and esterified polyenoic fatty acids. LOX are necessary for lipid hormones such as leukotrienes, lipoxide and hydroxy fatty acids. LOX subtypes are 15(S)-LOXs and 12(S)-LOXs. 5-LOX plays a major role in the biosynthesis of leukotrienes which are found at anaphylactic and

inflammatory diseases. 15-LOX are involved in monocytes/macrophage transitions and in macrophage function. Also, it is determined that 15-LOX plays a role in organelle degradation during differentiation of eye lens cell. 12/15-LOXs play a role in atherogenesis. 12-LOX requires for biosynthesis of hepoxilins and lipoxins. Hepoxilins are necessary for ion transport across biomembranes and lipoxins are involved in the regulation of immunological and hemodynamic processes (149).

3. MATERIAL and METHODS

3.1. Materials

3.1.1. Plant Material

Aerial parts of *Verbascum bugulifolium* L. were collected from Kayışdağı, İstanbul, Turkey (40°58'28.2"N 29°09'15.3"E) in May 2015. A voucher specimen (YEF 15003) is deposited at the Herbarium of the Faculty of Pharmacy, Yeditepe University, İstanbul, Turkey. Plant material was identified by Prof. Dr. Hasan Kırmızıbekmez. The above-ground parts of the plant were dried under shade at room temperature.

3.1.2. Chemicals and Solvents

Table 36. Chemicals and solvents used in the study

Chemicals & Solvents	**Trademark**
1,1-diphenylpicryl-hydrazil (DPPH)	Aldrich
2,2′-Azinobis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS)	Sigma
Ascorbic acid	Sigma-Aldrich
Chloroform	Merck
Dichloromethane	Merck
Dimethylsulfoxide (DMSO)	Sigma-Aldrich
EtOH	Sigma-Aldrich
EtOAc	Sigma-Aldrich
Gallic acid	Sigma-Aldrich
MeOH	Sigma-Aldrich
n-BuOH	Fluka
n-Hexane	Sigma-Aldrich
Norhydroguariretic Acid (NDGA)	Sigma-Aldrich
Polyamide (for column chromotography)	Sigma-Aldrich
Reverse phase (C_{18}) F_{254} Aluminium sheet	Merck
Sephadex LH-20 gel	Sigma-Aldrich
Silica gel F_{254} Aluminium sheet	Merck
Sodium persulfate ($Na_2S_2O_8$)	Roth
Neocuproine (2,9-dimethyl-1,10-phenanthroline)	Aldrich

Ammonium acetate (NH_4Ac)	Isolab
Copper (II) chloride ($CuCl_2$)	Acros organics
Lipoxygenase (1.13.11.12, Type I-B, 7 Unite/mg)	Fluco BioChemica
NaCI	Merck
Chloramphenicol	Sigma
Amphotericin B	Sigma-Aldrich
Resazurin	Sigma
Linoleic acid (LA)	Sigma
Dipotassium hydrogen phosphate	Acros organics
Potassium dihydrogen phosphate	Sigma-Aldrich
Tween 20	Sigma
t-BuOH	Isolab

3.1.3. Equipments and Instruments

Table 37. Equipment and Instruments

Equipments	**Brandname**
Air dryer	Homend
Autoclave	Hirayama
Balance	Ohaus Explorer
Filter	Sartorius Stedim
Filter paper	Munktell
Glass basic laboratory equipments (beaker, test tubes…)	Isolab
H_2O bath	GFL
HR-ESI-MS	Thermo Scientific Q-Exactive Plus Orbitrap
Incubator	Incucell
IR spectrometer	Perkin-Elmer
Laminar Flow Cabinet	Flores Valles
Freeze-dryer	Christ Alpha 2-4 LD
McFarland	Biosan
Micropipettes	Eppendorf and Brand
Mueller Hinton Agar (MHA)	Merck
Mueller Hinton Broth (MHB)	Merck
MPLC	Teledyne Isco, Sepacore® Flash Systems X10/X50 (Buchi Labortechnik AG, Flawil, Switzerland)
MS	Thermo Fisher Scientific, Waltham, MA, USA
NMR device (500 MHz for ^{1}H and 125 MHz for ^{13}C)	Bruker Avance
Oven	Binder
pH meter	Mettler Toledo
Polypropylene tubes (16x100 mm)	Isolab
Potatoes Dextrose Agar (PDA)	Merk
Refrigerator	Arçelik
Rotatory evaporator	Buchi, Heidolph

RPMI	Sigma
Sterile cabinet	Heal force
TLC chamber	Camag
UV lamb	Camag
Ultrasonic bath	Sonorex
UV Spectrophotometer	HP Agilent
Vortex	Ika vortex genius 3

3.2. Methods

3.2.1. Phytochemical Studies

3.2.1.1. Thin Layer Chromatography (TLC)

TLC is a simple, cheap, quick method with high sensitivity and good reproducibility. Mobile phase is liquid, and the stationary phase is a thin layer sorbent. Mobile phase carries the solutes through the stationary phase. Silica gel (SiO_2) is the most common layer coated to the glass plates. The sample is dissolved and applied to the plate. When the solvent rises to the top of the plate, the plate is taken out, dried and visualized. The other widely used TLC layer is reversed-phase (RP-TLC) in which polar solvents are used such as the mixtures of methanol and acetonitrile with water. In RP-TLC, hydrophobic sorbent binds nonpolar solutes, and they move more slowly but polar solutes migrate faster and closer to the solvent front.

The TLC analyses were used to choose the proper mobile phase mixtures during preparative chromatographic separations, to check the compositions of the fractions, as well as to control the purity of isolates.

Stationary Phase:

Normal Phase: Silica gel 60 F_{254} aluminium plates 9.5–11.5 μm (Merck)
Reversed-phase: (C_{18}) F_{254} aluminium plates 9.5–11.5 μm (Merck)

Developing solvents used in SiO_2-TLC analysis:

CH_2CI_2: MeOH: H_2O (90:10:1, 80:20:2, 70:30:3, 61:32:7)
n-Hexane: EtOAc (9:1, 1:2, 1:1, 4:1, 3:1, 2:1)
CH_2CI_2: MeOH (95:5)

Developing solvents used in reversed-phase TLC analysis:

MeOH: H_2O (1:1, 3:7, 4:6, 15:85, 1:3)

Developing Distance: 8 cm

Derivatization: 1% Vanillin/H_2SO_4 followed by heating at 105 °C for 3 min.

Visualization: UV light at 254 nm and 366 nm

3.2.1.2. Column Chromatography

3.2.1.2.A. Polyamide Column Chromatography

Polyamide CC was used to fractionate the *n*-BuOH subextract in this study.

Stationary Phase: Polyamide

Mobile Phase: H_2O: MeOH (1:0 to 0:1)

The sufficeint amount of polyamide was mixed with enough H_2O and kept at room temperature for 2 h. Then, small cotton was put on the bottom of glass column and the polyamide was placed into the glass column. And then, the column was washed with H_2O in order to place the material. The elution was maintained by increasing the amount of MeOH in H_2O. Polyamide column chromatography was used to separate phenolic compounds from non-phenolic ones in the extract. The sample was solved in H_2O and applied to the adsorbent with a pipette. A small amount of cotton was put on the column. The tap of the column was opened, and it was provided that the solution was passed through the column.

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

Sephadex (LH-20) was employed to separate the molecules according to their molecular sizes. In this study, it was used for the fractionation of $CHCl_3$ subextract as well as for the final purification steps of the isolates.

Stationary Phase: Sephadex LH-20

Mobile Phase: MeOH, MeOH-CH_2CI_2 (1:1)

Sephadex LH-20 was mixed with MeOH or MeOH:CH_2Cl_2 (1:1) and kept for a day. Then, it was placed into the glass column which its bottom was fixed with the cotton. After placing the gel into column, the sample was applied.

3.2.1.2.C. Silica Gel Column Chromatography

Silica gel column chromatography was used for fractionation and purification of the samples which were obtained from the other chromatographic studies.

Stationary Phase: Silica gel

Mobile Phases:

CH_2CI_2: MeOH (97.5:2.5, 95:5, 92.5:7.5, 90:10, 87.5:12.5, 85:15, 82.5:17.5, 80:20, 70:30, 75:25, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 30:70)

CH_2CI_2: MeOH: H_2O (80:20:2, 70:30:3, 61:32:7)

n-Hexane: EtOAc (95:5, 92.5:7.5, 90:10, 87.5:12.5, 85:15, 82.5:17.5, 80:20, 77.5:22.5, 75:25, 72.5:27.5, 70:30, 67.5:32.5, 65:35, 60:40, 55:35, 40:60, 45:55, 35:65, 30:70, 3:1, 2:1, 1:1)

Silica gel was first mixed with the starting mobile phase and then placed in the glass column. The cotton was put into the bottom and surface of the column again. After placing the SiO_2, the sample that was dissolved in starting mobile phase was applied onto column with a pipette and a cotton was placed on the SiO_2. Mostly, gradient elution was used for the separations.

3.2.1.2.D. Medium Pressure Liquid Chromatography (MPLC)

MPLC was used to separate the molecules in a fraction that possess similar polarities. Silica gel and reversed-phase (LiChroprep C_{18}) prepacked columns were used in the MPLC separations.

Stationary Phase: Silica gel, reversed-phase (LiChroprep C_{18})

Mobile Phases: MeOH (0→100), CH_2CI_2: MeOH

In C_{18}-MPLC, elution was started with H_2O, went on increasing amount of MeOH in H_2O. In SiO_2-MPLC, elution started with CH_2CI_2, went on with various proportions of CH_2CI_2: MeOH, ended with 100% MeOH.

For more apolar fractions, *n*-hexane:EtOAc mixtures were used as mobile phases. Flow rate, fraction volume, pressure, and the proportion and the time of solutions were arranged. The sample was dissolved in the elution solvent and injected into the column. The program was started after conditioning. The fractions were collected and monitored by TLC.

3.2.1.3. Extraction and Partition

The aerial parts of *V. bugulifolium* (300 g) were dried at shadow, at 25 °C and then powdered with plant crusher. The powdered aerial parts were extracted twice by macerating with MeOH (1.2 L x 2) for three days at room temperature. The pooled extracts were evaporated to dryness to give "VB-MeOH" (92.88 g) extract (yield: 30.96%). The crude MeOH extract was dispersed in H_2O (100 mL) and partitioned against three times with equal volumes of *n*-hexane, $CHCI_3$, EtOAc and *n*-BuOH, respectively.

3.2.1.4. Isolation of Compounds

The lyophilized EtOAc phase (590 mg) was subjected to C_{18}-Medium Pressure Liquid Chromatography (C_{18}-MPLC, 130 g) eluting with increasing amount of MeOH in H_2O (15–80%) to afford three fractions, frs. 1–3. Fraction 2 (250 mg) was likewise subjected to C_{18}-MPLC (43 g) (20-70%, MeOH) to afford fraction 2i (17.4 mg). Compound **(8)** (6 mg) was purified by Sephadex LH-20 CC (6 g) (MeOH) from fraction 2i.

The lyophilized *n*-BuOH phase (7.25 g) was fractionated over polyamide CC (35 g), using MeOH (0→100%) as eluent and afforded seven main fractions, A-G. An aliquot of fr. A (2 g) was subjected to C_{18}-MPLC (150 g) eluting with increasing amount of MeOH in H_2O (0→ 50%) to yield fraction A_7. Purification of fraction A_7 (23.2 mg) by MPLC (SiO_2, 4 g) eluting with CH_2CI_2-MeOH mixture yielded **3** (6.9 mg). The other aliquot of fr. A (2.4 g) was subjected to silica gel CC (120 g) eluting with CH_2Cl_2–MeOH–H_2O (90:10:1–61:32:7) to afford fr. A_h + A_i (97.8 mg) was subjected to C_{18}-MPLC (15.5 g) (MeOH, 5→70%) to yield **4** (5.1 mg). Fr. A_k (119.6 mg) was further applied to C_{18}-MPLC (43 g) (MeOH, 0-60%) to obtain **7**. Fr. A_i (89.6 mg) was similarly subjected to C_{18}-MPLC (43 g) (MeOH, 0-60%) to purify **5**. Fr. B (798.3 mg) was applied to silica gel (80 g) CC using CH_2Cl_2–MeOH–H_2O (95:15:0 to 61:32:7) mixtures to yield **1** (43 mg) along with fractions B_2 and B_5. Fraction B_5 was purified to yield **11** (2 mg) by C_{18}-MPLC (15.5 g) (MeOH, 5-30%). Fr. B_2 was applied to silica gel (12 g) CC using CH_2Cl_2–MeOH (95:5 and 90:10) to

afford fr. B_{2b}+ fr. B_{2c} (33 mg) which was further separated by C_{18}-MPLC (15.5 g) (MeOH, 20-50%) to obtain **2** (5 mg). Fr. C (103.1 mg) and D (120.6 mg) were combined (223.7 mg) and applied to C_{18}-MPLC (43 g) (MeOH, 15-70%) to obtain **6** (5.3 mg) and fraction CD_2. Fraction CD_2 (36.9 mg) was purified by MPLC (SiO_2, 12 g) eluting with increasing amounts of CH_2CI_2-MeOH (90:0→60:40) to obtain fraction CD_{2b}. Fraction CD_{2b} (9 mg) was further purified by Sephadex LH-20 CC (MeOH, 6 g) to give a mixture of **10** and **12** (9.8 mg). Compound **9** (7.9 mg) was purified by Sephadex LH-20 CC (MeOH, 10 g) from fraction G (147.6 mg).

The $CHCI_3$ extract (2.74 g) was fractionated over Sephadex LH-20 (100 g) using MeOH as eluent and afforded three fractions, 1-3. Fraction 2 was applied to silica gel (100 g) CC using CH_2Cl_2–MeOH (95:5-60:40) mixtures. Fraction 2e (433.9 mg) was further subjected to silica gel (50 g) CC by using CH_2Cl_2–MeOH (97.5:2.5→80:20) mixtures. Fraction 2e2 (365.7 mg) was similarly applied to silica gel (40 g) CC using *n*-hexane-EtOAc (80:20-30:70) mixtures. Fraction 2_{e2a} (209.9 mg) was further subjected to silica gel (25 g) CC using *n*-hexane-EtOAc (95:5-65:35) mixtures. Compound **13** (6.6 mg) was purified by silica gel (CC) (25 g) using *n*-hexane-EtOAc (100:0→67.5:22.5) mixtures. The isolation procedures for the compounds are shown in figures 3-5.

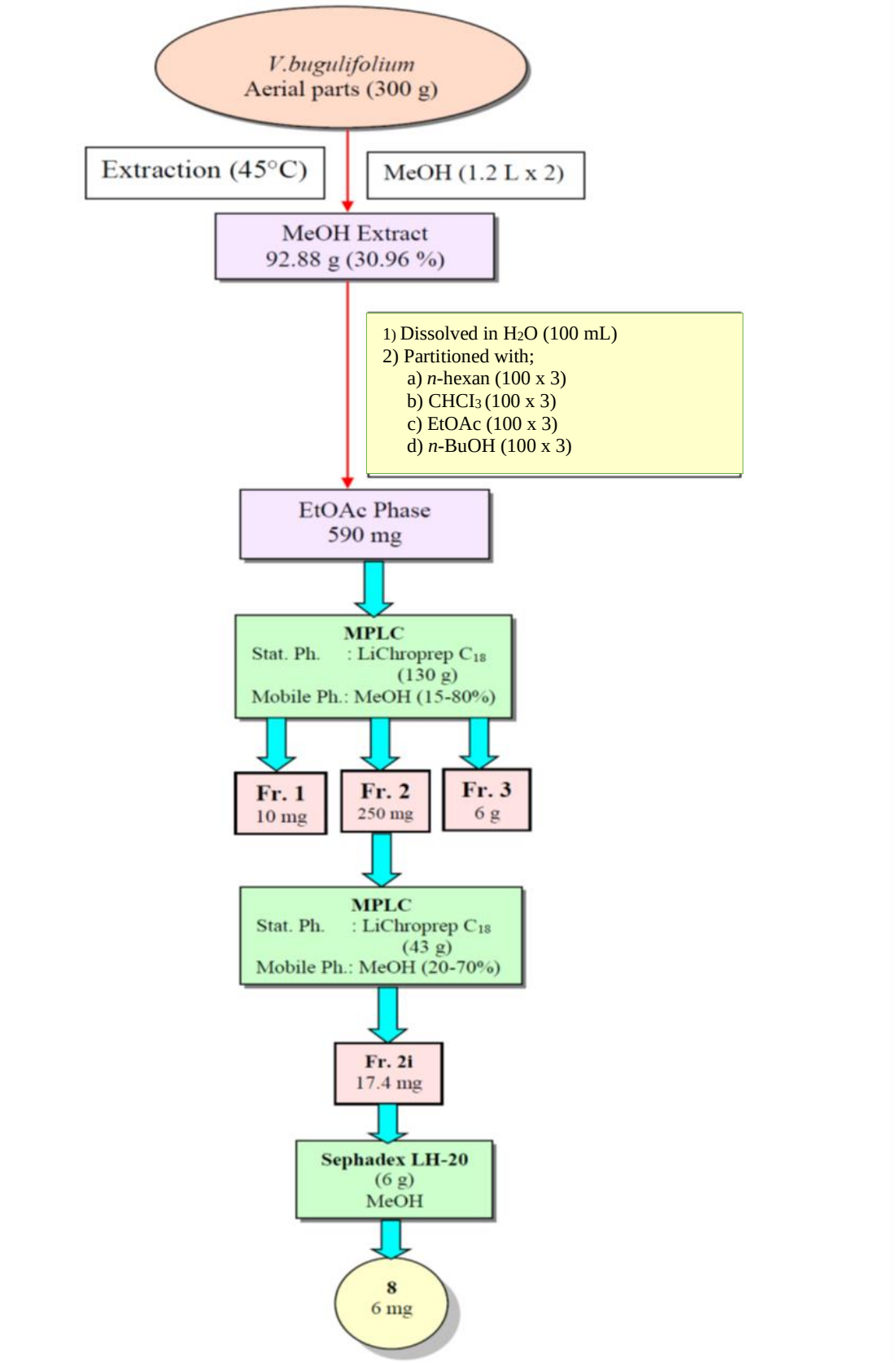


Figure 3. Isolation of **8** from *V. bugulifolium* EtOAc subextract.

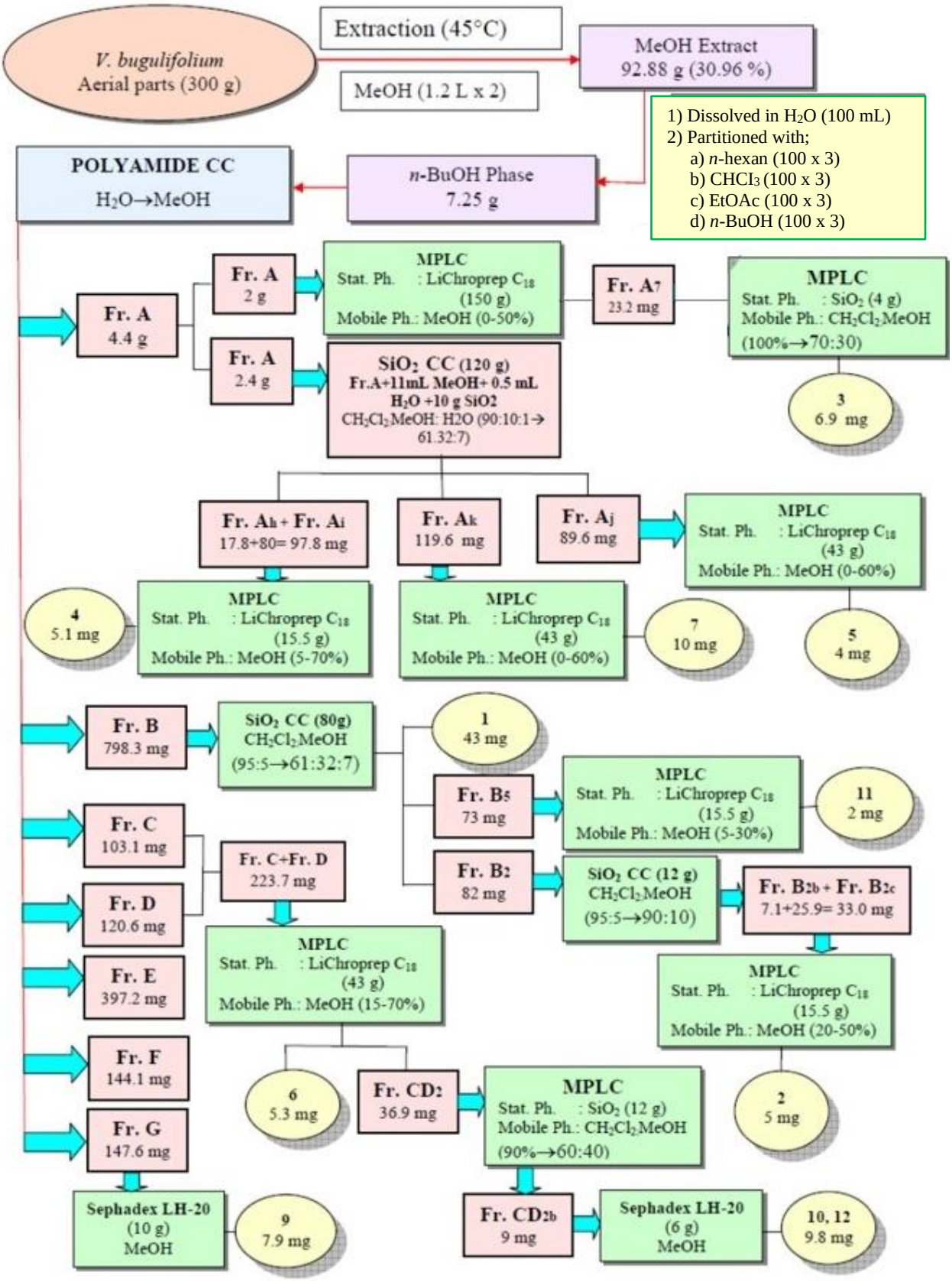


Figure 4. Isolation of compounds from *V. bugulifolium n*-BuOH subextract

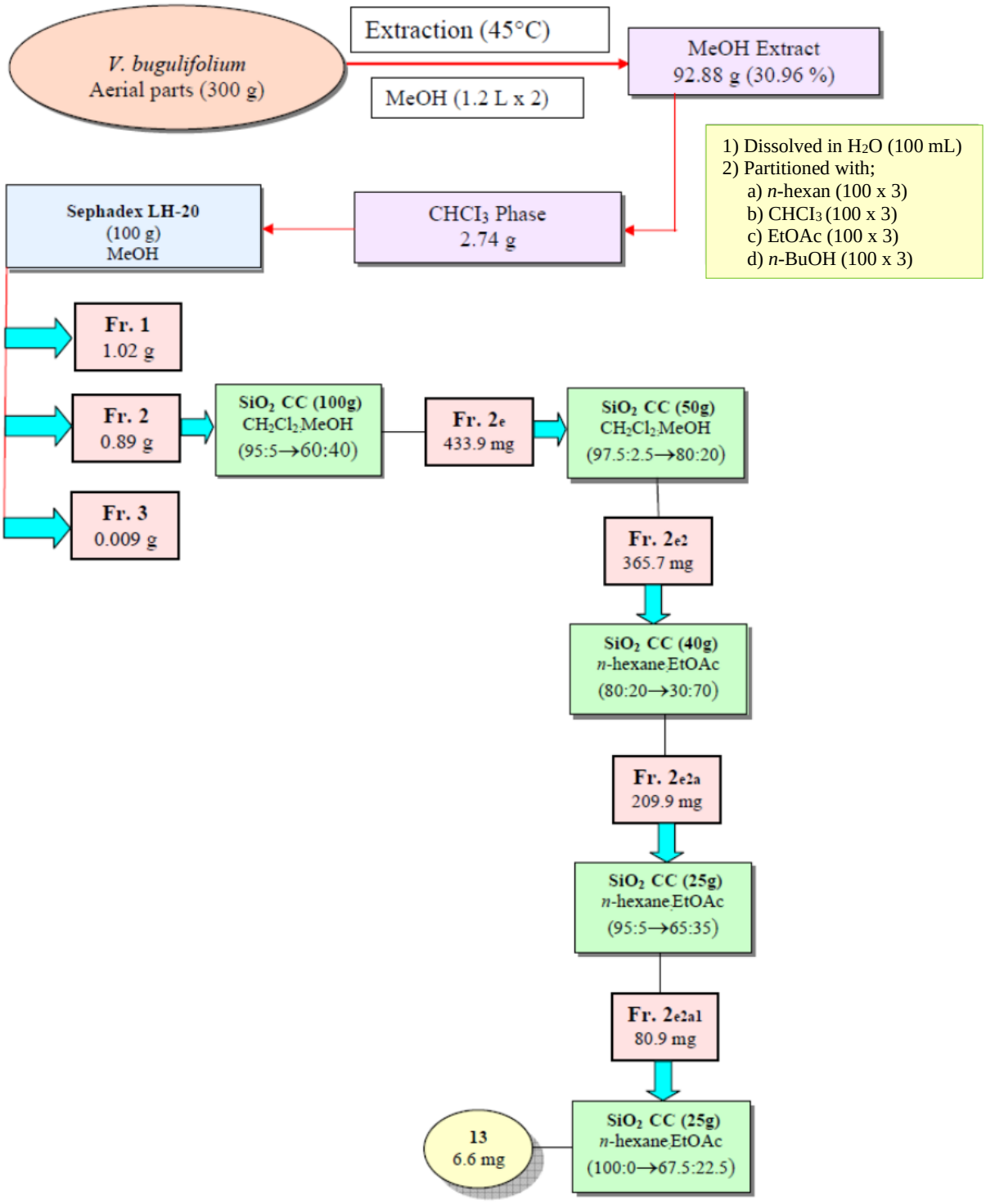


Figure 5 . Isolation of compound **13** from *V. bugulifolium* $CHCl_3$ extract

3.2.1.5. Structure Elucidation Methods

The chemical structures of the purified compounds were elucidated based on spectroscopic methods including UV, IR, 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC, NOESY) NMR and MS/HR-ESI-MS.

3.2.2. *In vitro* Activity Studies

3.2.2.1. Antioxidant Activity Studies

3.2.2.1.A. DPPH• Assay

The DPPH (1,1-diphenylpicryl-hydrazyl) methodology was first developed by Brand-Williams et al. in 1995. The DPPH radical is stable free radical. Also, it has a strong purple colour which makes it measurable spectrophotometrically. DPPH reacts with an antioxidant and gets discoloured by transferring an electron or donating hydrogen. After this reaction, absorbances changes and antioxidant capacity is determined with this change. Nitrogen is the radical of DPPH and it is found at the center of the surface (150). The DPPH radical is stable in methanol solution. The purple colour turns to yellow and absorbents losses when free radical is blocked by that antioxidants. Absorbance is monitored at 515 nm.

In the scavenging activity of the extracts and isolated compounds against DPPH radical, samples were dissolved in DMSO and the concentrations were adjusted 0.5-0.001 mg/mL. Then, 100 mL of 80 μg/mL DPPH was added. The mixture was shaken and incubated at room temperature for 60 min. After incubation, the absorbance was measured at 517 nm with UV spectrophotometer. Ascorbic acid was used as the positive control substance. % inhibition values were calculated, and the results were given as IC_{50} and standard deviation. All tests were repeated in triplicate (151,152).

$A_{control}$: Control absorbance

A_{sample}: Test sample absorbance

% inhibition = [$(A_{control} - A_{sample})/ A_{control}$] . 100

3.2.2.1.B. ABTS•$^{+}$ assay

The $ABTS^{\cdot+}$ (2,2′-Azinobis (3-ethylbenzthiazoline)-6-sulfonic acid) is an electron transfer-based assay. $ABTS^{\cdot+}$ molecule is a dark blue radical cation. It is reduced with an antioxidant into colorless. This reduction is measured spectrophotometrically at 734 nm. Antioxidants oxidate ABTS to form the $ABTS^{\cdot+}$ with manganese dioxide, potassium persulfate and horseradish peroxidase.

7 mM ABTS which was prepared with 0.1 M pH 7.4 phosphate buffer and 2.45 mM sodium persulfate ($Na_2S_2O_8$) was added at the ratio of 1:2 in the dark at room temparature for 12 h on the shaker. The absorbance of the ABTS solution at 734 nm was 0.700±0.025 nm. The working concentration range of the samples is 0.5-0.001 mg / mL. 150 µL of the sample was added to 30 µL ABTS solution and allowed to incubate at room temperature in the dark for 30 min. Ascorbic acid was used as a standard. The absorbance was measured at 734 nm and the results were given as IC_{50}. All tests were repeated in triplicate (153).

$A_{control}$: Control absorbance

A_{sample}: Test sample absorbance

% inhibition = [$(A_{control} - A_{sample})/ A_{control}$] . 100

3.2.2.1.B.1. Qualitative Radical Scavenging Assay of ABTS

Concentration of the samples was adjusted to 1 mg/mL and 4 µL of the samples applied to the TLC plate. 7 mM ABTS sprayed onto the TLC plate and incubated for 1 min in the dark. White spots on the dark-green background at 730 nm assigned the antioxidant activity.

3.2.2.1.C. Cupric Reducing Antioxidant (Cuprac) Assay

Cupric ion reducing is another method that is used for the determination of total antioxidant capacity. Cupric reducing antioxidant assay (CUPRAC) is developed by Apak et al. (154).

Antioxidants reduce the copper (II) neocuprine to copper (I) neucuprine, and the absorbance of the blue color is measured at 450 nm. Neocuprine is 2,9-dimethyl-1,10-phenanthroline and it is a heterophilic compound (155).

50 μL copper (II) chloride (CuCI2) solution (0.01 M), 50 μL ammonium acetate (NH4Ac) buffer at pH=7.0 and 50 μL neocuproine (Nc) solution ($7.5x10^{-3}$ M) were added to the 27.5 μL test samples (0.5-0.001 mg/mL). And this solution was shaken in the dark at room temperature for 30 min. Their A_{450} values were measured and results were given as $IC_{50.}$ All tests were repeated in triplicate (155,156).

3.2.2.2. Anti-inflammatory Activity Study

3.2.2.2.A. LOX Inhibition Study: 5-LOX enzyme activity inhibition

Lipoxygenases (LOXs) belong to a monomeric protein family that is non-sulphur non-heme and iron–containing dioxygenases which oxygenated free and esterified polyenoic fatty acids (157,158). LOXs play a major role in some inflammatory diseases like arthritis, allergic asthma, psoriasis, inflammatory bowel disease, cardiovascular disease, kidney disease, metabolic syndrome, skin diseases (158) and degenerative disorders such as Alzheimer's disease (159).

The substrates for LOXs are arachidonic acid, which is a precursor of prostaglandins, thromboxanes, leukotrienes and lipoxins. Antioxidants (such as polyphenolics, flavonoids) inhibit plant lipoxygenase. The anti-inflammatory (anti-lipoxygenase) activities of plant's extracts are based on their potent inhibitory effects of phenolic contents on arachidonic acid metabolism through the lipoxygenase pathway.

5-LOX catalyse the oxidation of unsaturated acids that is 1-4 pentadiene structures. Arachidonic acid is the substrate of 5-LOX, but linoleic acid is accepted. Reaction rate is measured by spectrophotometry, and this initial rate decreased by the inhibitory activity of the substance (160).

The inhibition of lipoxygenase activity was determined by a spectrophotometric method with 96 quartz plate (160,161). 1.94 mL 100 mM phosphate buffer (pH 8.0), 40 μL various concentration of samples and 20 μL lipoxygenase mixed and incubated for 10 min at 25℃. 300 μL of this mixture added to each well. And then, 7.5 μL substrate (linoleic acid) was added and mixed for 30 seconds. The absorbance change was measured at 243 nm for 10 minutes in 10 intervals. All tests were repeated four times. Nordihidroguairatic acid (NDGA) was used as a positive control compound. Results were given as inhibitions % and standard deviations.

The percentage inhibition (%) of lipoxygenase activity was calculated as:

$$\text{Inhibition (\%)} = (E-S) / E \times 100$$

E: the enzyme activity without sample

S: the enzyme activity with sample

3.2.2.3. Antimicrobial Activity Study

The antimicrobial studies aimed to establish the lowest concentration (minimal inhibitory concentration (MIC)) of the extracts. Antimicrobial agents inhibit the visible growth of the microbes. MIC values are used for antimicrobial drugs to evaluate the potential of new antimicrobial drugs. Antimicrobial activity tests are also important to establish resistance of microbial strains to antimicrobials, in ethnopharmacological studies and to determine the efficacy of various microorganisms (162). Some antimicrobial activity tests such as disk-diffusion, well-diffusion and agar or broth are the most known and used tests. Flow cytoflourometric and bioluminescent methods are not commonly used. Although they are fast, they require specific equipment and standardization.

Dilution methods: These methods are the most convenient methods to find MIC values that indicate the concentrations of the antimicrobial properties of the tested samples in the agar (agar dilution) or broth medium (macrodilution or microdilution). Two of them demonstrate *in vitro* quantitative measurements of the samples against bacteria and fungi. The most common standards are provided by the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (163).

The Growth of Microorganism Cultures: Potatoes Test Agar (PDA, Merck), RPIM for fungal cultures, Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) for bacterial cultures were used for microorganism growth. After 24 h incubation at 37 °C, mono colonies were taken and added to the tubes which contained 0.85 % NaCI. The densities of the colonies were arranged according to the Mc Farland No: 0.5 tube (164,165). Samples were weighed as 2 mg and 20 mg, dissolved in dimethyl-sulfoxide (DMSO) and diluted with distilled water.

Antimicrobial Activity: For this study, 96 flat bottom microtiter plates were used. 0.62-0.00125 mg/mL concentrations of samples were prepared. The density of microorganisms (m.o) was arranged, and 100 µL m.o added to every plate. Then, they incubated for 24 h

at 37 °C (166–168). Chloramphenicol was used as an antibacterial standard and amphotericin B was used as a fungal standard. The concentration of antimicrobials applied were 0.001-64 μg/mL. After incubation time, 20 μL %0.01 (w/v) resazurin solutions were added to give colour to every plate and incubated 3 h at 37 °C. After incubation time, the zones which were not staining showed that the growth did not occur. Results were given as minimum inhibitory concentration (MIC). Tests were repeated two times and average values were given.

4. RESULTS

4.1. Extraction

4.1.1. Extraction of *V. bugulifolium* aerial parts

The dried and powdered aerial parts of *V. bugulifolium* (300 g) were extracted with MeOH to yield crude MeOH extract (92.88 g, 30.96%). The crude MeOH extract was dispersed in H_2O (100 mL) and partitioned three times with equal volumes of *n*-hexane, $CHCI_3$, EtOAc and *n*-BuOH respectively. The extract amounts and the yields are presented in Table 38.

Table 38. Extract amounts and yield from *V. bugulifolium*

Extracts	Amount (g)	Yield (%)
VB-MeOH	92.88	30.96
VB-H_2O	53.09	57.15
VB-*n*-hexane	5.45	5.86
VB-$CHCl_3$	2.74	2.74
VB-EtOAc	0.59	0.63
VB-*n*-BuOH	7.25	7.80

4.2. *In vitro* Activity Results of Extracts

4.2.1. Antioxidant Activity Results of Extracts

4.2.1.1. DPPH Radical Scavenging Activity Results of Extracts

The crude VB-MeOH extract of *V. bugulifolium* as well as VB-H_2O, VB-*n*-hexane, VB-$CHCI_3$, VB-EtOAc and VB-*n*-BuOH subextracts were tested for their DPPH radical scavenging activities. Results of the assay are given in Table 39.

Table 39. DPPH activiy results (µg/mL)

Extracts/Reference	IC_{50} (µg/mL)
VB-MeOH	380±0.02
VB-H_2O	NA
VB-*n*-hexane	NA
VB-$CHCI_3$	220±0.01
VB-EtOAc	30±0.01
VB-*n*-BuOH	90±0.00
Ascorbic acid	10±0.00

NA: not active

4.2.1.2. ABTS Scavenging Activity Results of Crude Extracts

The crude MeOH extract and the subextracts were evaluated for their *in vitro* ABTS activities. Results of the experiments were presented in Table 40.

Table 40. ABTS activity results of the extracts

Extracts/Reference	IC_{50} (µg/mL)
VB-MeOH	200±0.03
VB-H_2O	370±0.03
VB-*n*-hexane	450±0.01
VB-$CHCI_3$	220±0.02
VB-EtOAc	20±0.01
VB-*n*-BuOH	70±0.01
Ascorbic acid	30±0.03

NA: not active

4.2.1.3. CUPRAC Activity Results of Crude Extracts

The crude MeOH extract and the subextracts were evaluated for their *in vitro* CUPRAC activities. Results of the assay were given in Table 41.

Table 41. CUPRAC activity results of the extracts

Extracts/Reference	IC_{50} (µg/mL)
VB-MeOH	170±0.01

VB-H_2O	250±0.01
VB-*n*-hexane	50±0.02
VB-$CHCI_3$	80±0.02
VB-EtOAc	20±0.03
VB-*n*-BuOH	20±0.01
Gallic acid	4±0.01

NA: not active

4.2.1.4. Anti-inflammatory Activity Results of the Extracts

VB-MeOH, VB-H_2O, VB-*n*-hexane, VB-$CHCl_3$, VB-EtOAc, VB-*n*-BuOH of *V. bugulifolium* were tested for their *in vitro* anti-inflammatory activities in 5-LOX inhibition assay. Results of the anti-inflammatory activities were given in Table 42 and Figure 6.

Table 42. Lipoxygenase inhibition % results of the extracts

Extracts/Reference	**Inhibition % (50 µg/mL)**
VB-MeOH	26.6±4.7
VB-H_2O	29.0±4.6
VB-*n*-hexane	25.8±3.3
VB-$CHCI_3$	22.6±4.9
VB-EtOAc	21.4±2.5
VB-*n*-BuOH	35.7±4.1
NDGA (3.1 µg/mL)	100

NA: not active

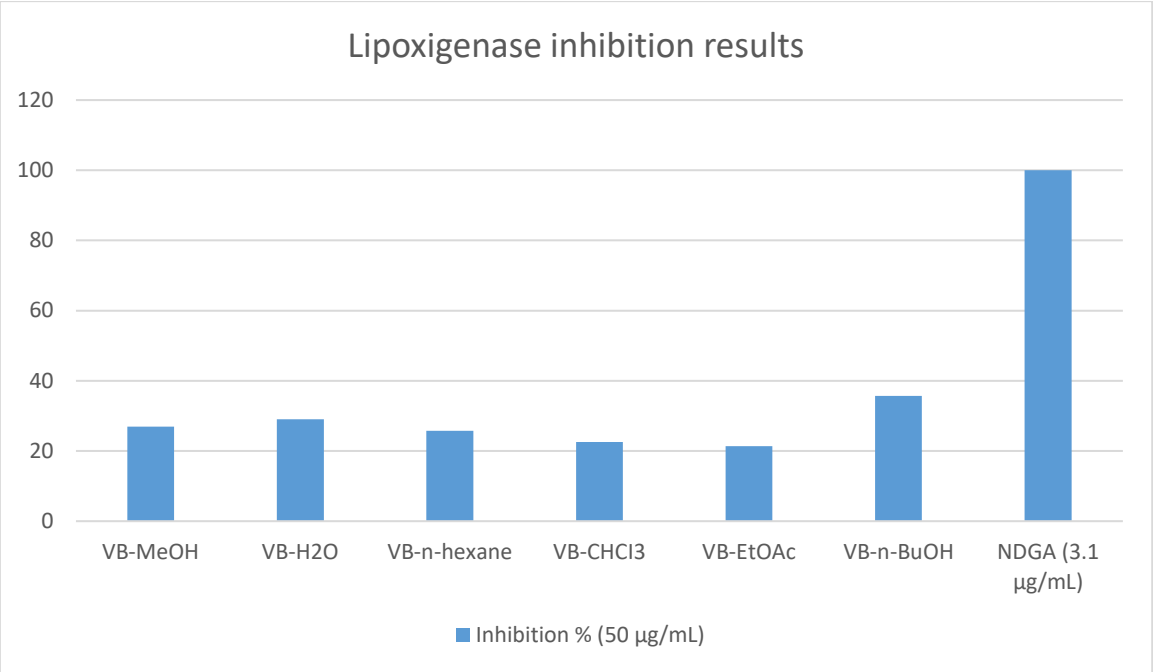


Figure 6. Lipoxygenase inhibition percentages of the extracts of *V. bugulifolium*

4.2.1.5. Antimicrobial Activity Results of the Extracts

VB-MeOH, VB-H_2O, VB-*n*-hexane, VB-$CHCI_3$, VB-EtOAc, VB-*n*-BuOH extracts prepared from the aerial parts of *V. bugulifolium* were tested for their antimicrobial activities against three bacteria and three *Candida* strains. Results of antimicrobial activities were given in Table 43.

Table 43. Antimicrobial activity results of extracts and standarts (μg/mL)

Extract/References	*Bacillus cereus* NRRLB 3711	*Pseudomonas aeruginosa* ATCC 10145	*Staphylococcus aureus* ATCC 6538	*Candida albicans* ATCC 90028	*Candida parapsilosis* ATCC 22019	*Candida krusei* ATCC 6258
VB-*n*-BuOH	NA	NA	NA	NA	1000	500
VB-MeOH	NA	NA	NA	NA	1000	500
VB-$CHCI_3$	NA	1000	NA	1000	1000	250
VB-*n*-hexane	NA	125	NA	NA	1000	500
VB-EtOAc	NA	125	NA	NA	NA	-
Amphotericin-B	-	-	-	0.031	0.062	0.125
Chloramphenicol	0.002	0.062	0.001	-	-	-

NA: Not active (>1000 μg/mL).

4.3. Phytochemical Studies

Successive chromatographic studies on the *n*-BuOH, EtOAc and $CHCl_3$ subextracts yielded 13 compounds (**1-13**) which were categorized into four groups according to their chemical structures as iridoid glycosides [catalpol (**1**), specioside (**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**)]; phenylethanoid glycosides [verbascoside (**6**), and glucopyranosyl-(1→Gi-6)-martynoside (**7**)]; flavonoids [luteolin (**8**), luteolin 7-*O*-β-D-glucopyranoside (**9**), luteolin 7-*O*-rutinoside (**10**), apigenin 7-*O*-rutinoside (**11**), quercetin 3-*O*-rutinoside (**12**)] and phytosterol [β-sitosterol (**13**)]. Since compounds **10+12** were obtained as mixtures, they were not subjected to bioactivity studies. Luteolin (**8**) was obtained from EtOAc subextract and *β*-sitosterol (**13**) was purified from $CHCl_3$ subextracts while all of the other compounds (**1-7** and **9-12)** were obtained from *n*-BuOH subextract. The chemical structures of the isolated compounds are presented in Tables 44-47.

4.3.1. Isolated Compounds

4.3.1.1. Iridoids

4.3.1.1.A. Catalpol type iridoid glucosides

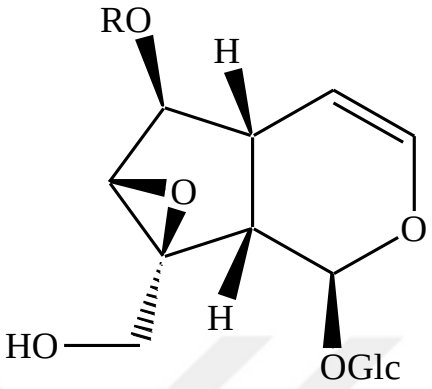


Table 44. Catalpol type iridoid glucosides obtained from *V. bugulifolium*

Iridoids	**R**
Catalpol **(1)**	H
Specioside **(2)**	*p*- Coumaroyl

4.3.1.1.B. Ajugol and harpagide type iridoids

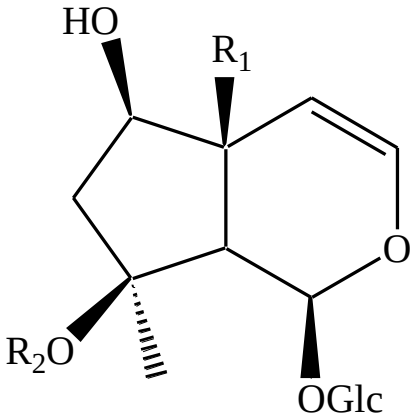


Table 45. Ajugol and harpagide type iridoids obtained from *V. bugulifolium*

Iridoids	**R_1**	**R_2**
Ajugol **(3)**	H	H
Ajugoside **(4)**	H	Ac
8-*O*-Acetylharpagide **(5)**	OH	Ac

4.3.1.2. Phenylethanoid glycosides

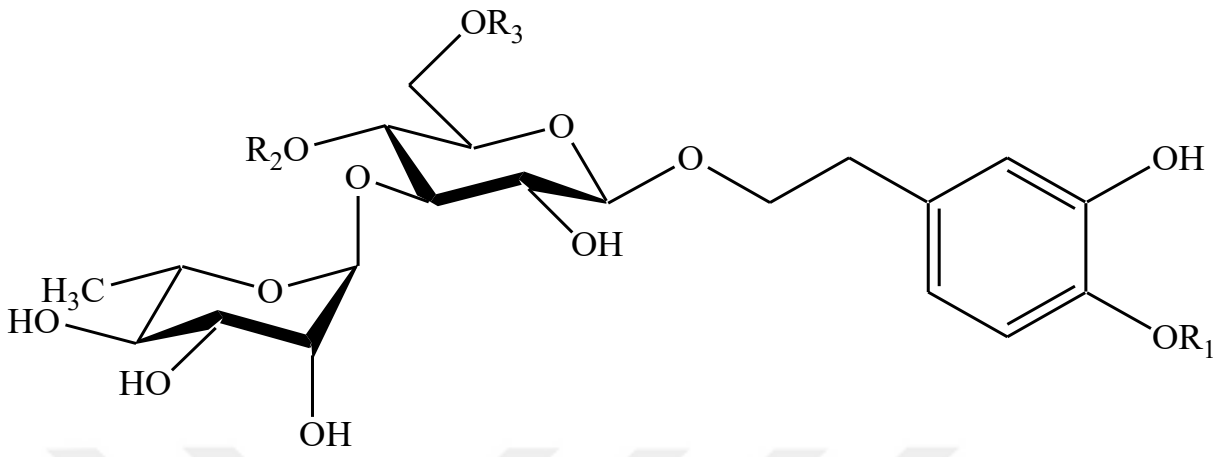


Table 46. Phenylethanoid glycosides isolated from *V. bugulifolium*

Phenyletanoid Glycosides	**R_1**	**R_2**	**R_3**
Verbascoside **(6)**	H	(*E*)-Caff	H
Glucopyranosyl (1→Gi6)-martinoside **(7)**	CH_3	(*E*)-Fer	Glc

4.3.1.3. Flavonoids

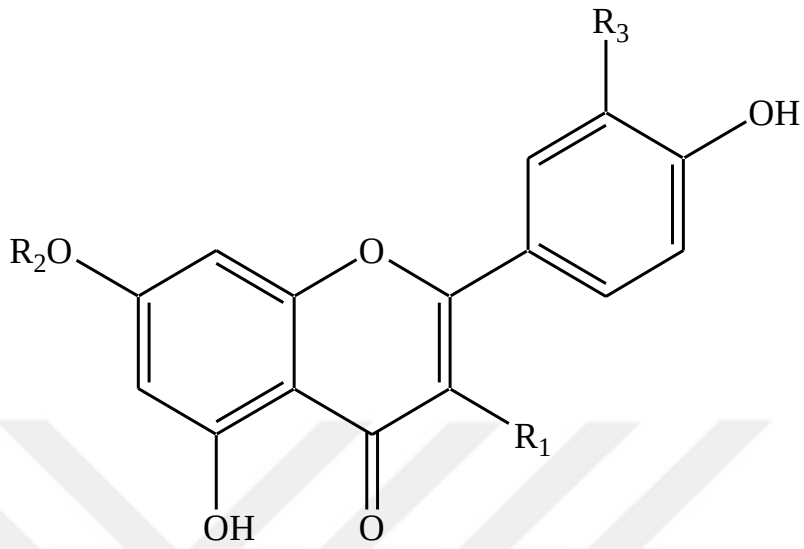
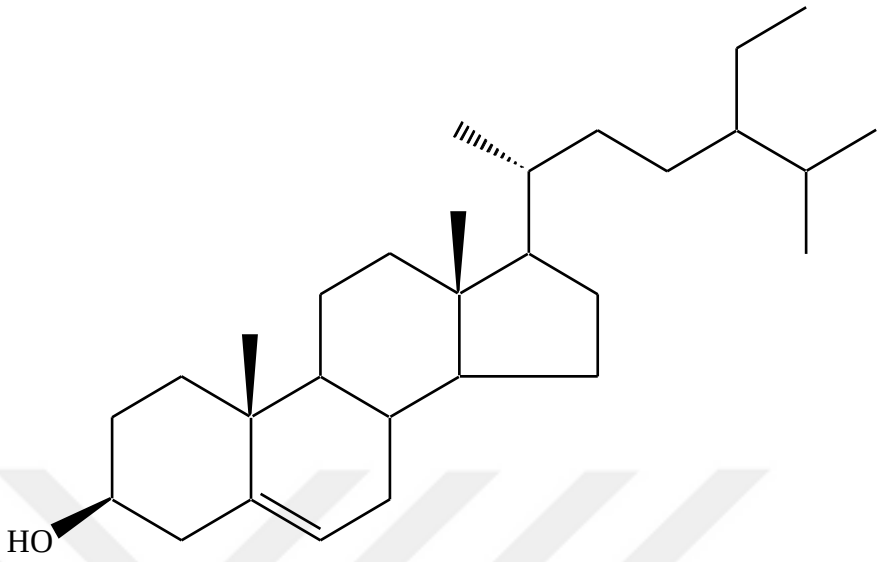


Table 47. Flavonoids obtained from *V. bugulifolium*

Flavonoids	**R_1**	**R_2**	**R_3**
Luteolin **(8)**	H	H	OH
Luteolin 7-*O*-*β*-D-glucopyranoside **(9)**	H	*β*-Glc	OH
Luteolin 7-*O*-rutinoside **(10)**	H	Rut	OH
Apigenin 7-*O*-rutinoside **(11)**	H	Rut	H
Quercetin 3-*O*-rutinoside **(12)**	*O*-Rut	H	OH

4.3.1.4. Phytosterol

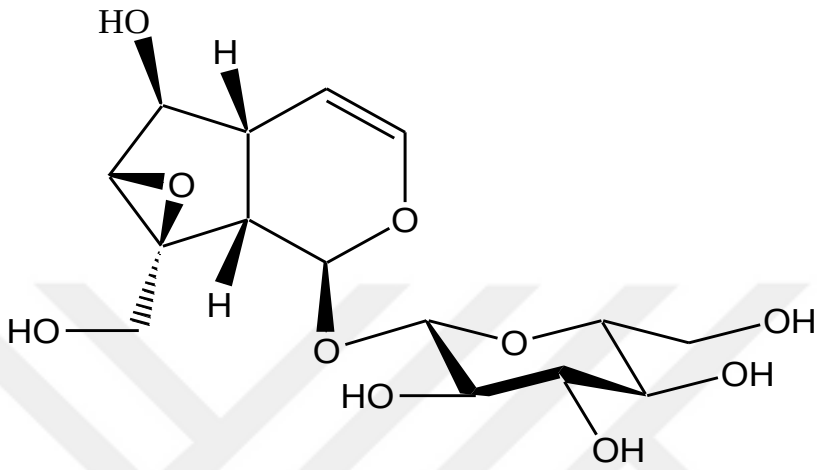


β-sitosterol **(13)**

4.4. Structure Elucidation of Isolated Compunds

4.4.1. Iridoids

4.4.1.1. Catalpol type iridoid glycosides



CATALPOL (1): $C_{15}H_{22}O_{10}$ (MW: 362.33)

UV λ_{max} (MeOH) nm:	207
IR ν_{max} (KBr) cm^{-1}:	3304 (OH), 1656 (C=C)
^{1}H NMR:	Spectrum 1, Table 48
^{13}C NMR:	Spectrum 2, Table 48

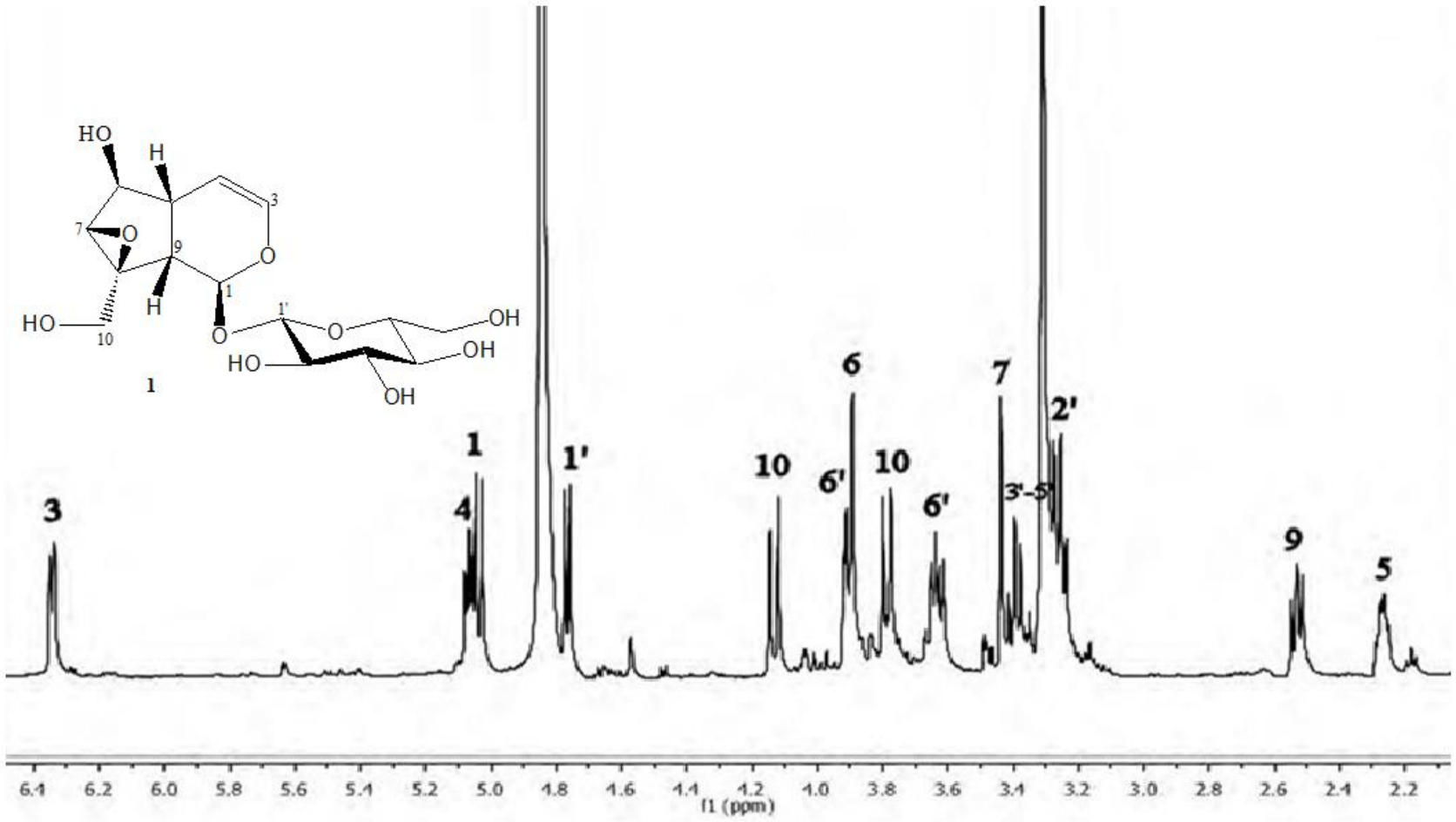
CATALPOL (1)

Compound **1** was obtained as amorphous powder from *n*-BuOH extract. UV spectra showed absorption bands at 207 nm, characteristic for iridoid enol-ether functional group. IR spectrum showed hydroxyl (3304 cm^{-1}) and olefinic (1656 cm^{-1}) groups.

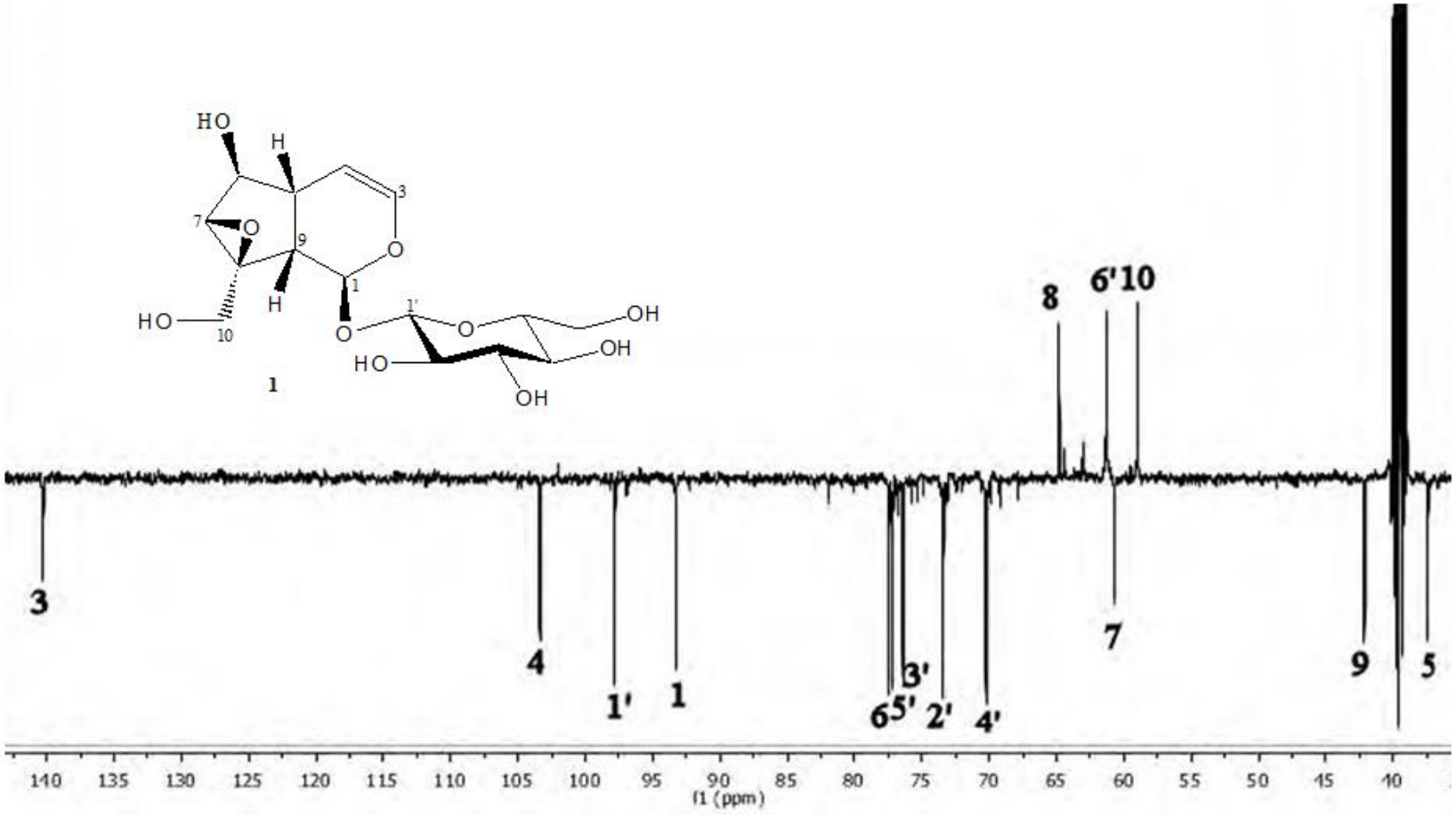
^{1}H and ^{13}C NMR spectra (Table 48, Spectrum 1 & 2) contained signals for anomeric proton at δ_H 4.75 (d, J= 7.8, H-1') and carbon at δ_C 97.8 (C-1') indicating the presence of β-linked sugar unit. ^{13}C NMR data contained 15 resonances, including a quaternary carbon signal (C), 12 methines (CH) and two methylenes (CH_2). The ^{13}C signals between δ_C 61.2-77.1 ppm and the anomeric carbon signal revealed the presence of a β-glucopyranose unit in **1**. The remaining nine carbon signals showed the presence of an aglycone consisting of a cyclopentan-pyran iridoid structure. Analysis of the ^{1}H and ^{13}C NMR spectra exposed the feature of an iridoid glucoside. ^{1}H NMR signals of compound **1** at δ_H 6.34, 5.03, 5.02, 3.89, 2.51 and 2.24 exhibited the precense of seven methine groups in cyclo-pentane pyran structure. The signals at δ_H 4.12 and 3.78 (J=13.1 Hz) assigned to hydroxymethylene group. The signals at δ_H 6.34 (H-3) and 5.07 (H-4) affirmed double bond between C-3 and C-4. Doublet doublet splitting of H-3 and H-4 represented non-substituted C-5. Thereby, the highest field at spectra attributed to H-5 (δ_H 2.24). The other signal at the high field was interpreted as H-9 (δ_H 2.51). In this case, the hemiacetal signal at δ_H 5.02 should belong to H-1 in the pyran ring. The existence of two oxymethine functions in the cyclopentane ring was understood from the chemical shift values of the H-6 and H-7 signals at δ_H 3.89 and 3.37 and the corresponding carbon resonances at δ_C 77.4 and 60.7 respectively. Furthermore, the chemical shift value of the quaternary carbon signal at δ_C 64.8 suggested the presence of tertiary hydroxyl at C-8. In this case the hydroxymethylene group must be bound to C-8. Since the findings obtained are similar to the recorded values for registered catalpol in the literature, it was understood that the compound is an epoxide-bearing iridoid glucoside, known as **catalpol** (169).

Table 48. ^{13}C and ^{1}H NMR spectroscopic data for catalpol (**1**) (CD_3OD, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

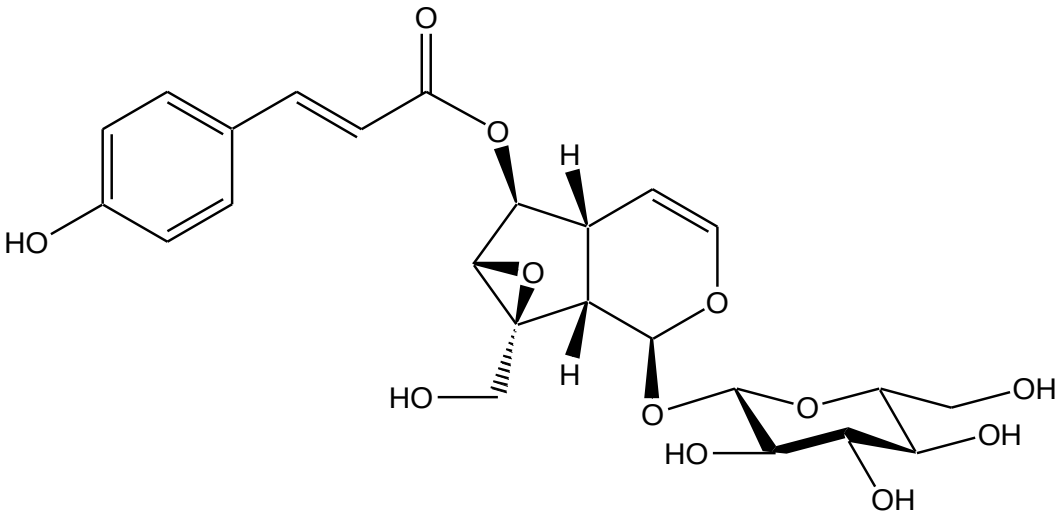
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**
Aglycone			
1	CH	93.2	5.03 d (9.8)
3	CH	140.2	6.34 dd (6.0, 1.2)
4	CH	103.3	5.07 t (5.1)
5	CH	37.4	2.27 m
6	CH	77.4	3.89 s
7	CH	60.7	3.43 s
8	C	64.8	-
9	CH	42.1	2.53 dd (9.6, 7.7)
10	CH_2	58.9	4.13 d (13.1)
			3.78 d (13.1)
Glucose			
1'	CH	97.8	4.76 d (7.8)
2'	CH	73.4	3.26 m
3'	CH	76.3	3.48-3.23 m
4'	CH	70.2	3.48-3.23 m
5'	CH	77.1	3.48-3.23 m
6'	CH_2	61.2	3.91 dd (11.8, 3.6)
			3.63 dd (11.8, 6.4)



Spectrum 1. ^{1}H NMR Spectrum of Catalpol (**1**) (500 MHz, CD_3OD)



Spectrum 2. JMOD-^{13}C-NMR Spectrum of Catalpol (**1**) (125 MHz, CD_3OD)



SPECIOSIDE (2): $C_{24}H_{28}O_{12}$ (MW: 508.47)

UV λ_{max} (MeOH) nm:	206, 313, 355
IR ν_{max} (KBr) cm^{-1}:	3300 (OH), 1654 (C=C), 1691 (conj. est. C=O), 1635 (conj. C=C), 1603 (aromatic ring)
^{1}H NMR:	Table 49, Spectrum 3
^{13}C NMR:	Table 49, Spectrum 4
HSQC:	Spectrum 5
HMBC:	Table 49, Spectrum 6
COSY:	Spectrum 7
HR-ESI-MS *m/z*:	531.1672 ($C_{24}H_{28}O_{12}$ Na)

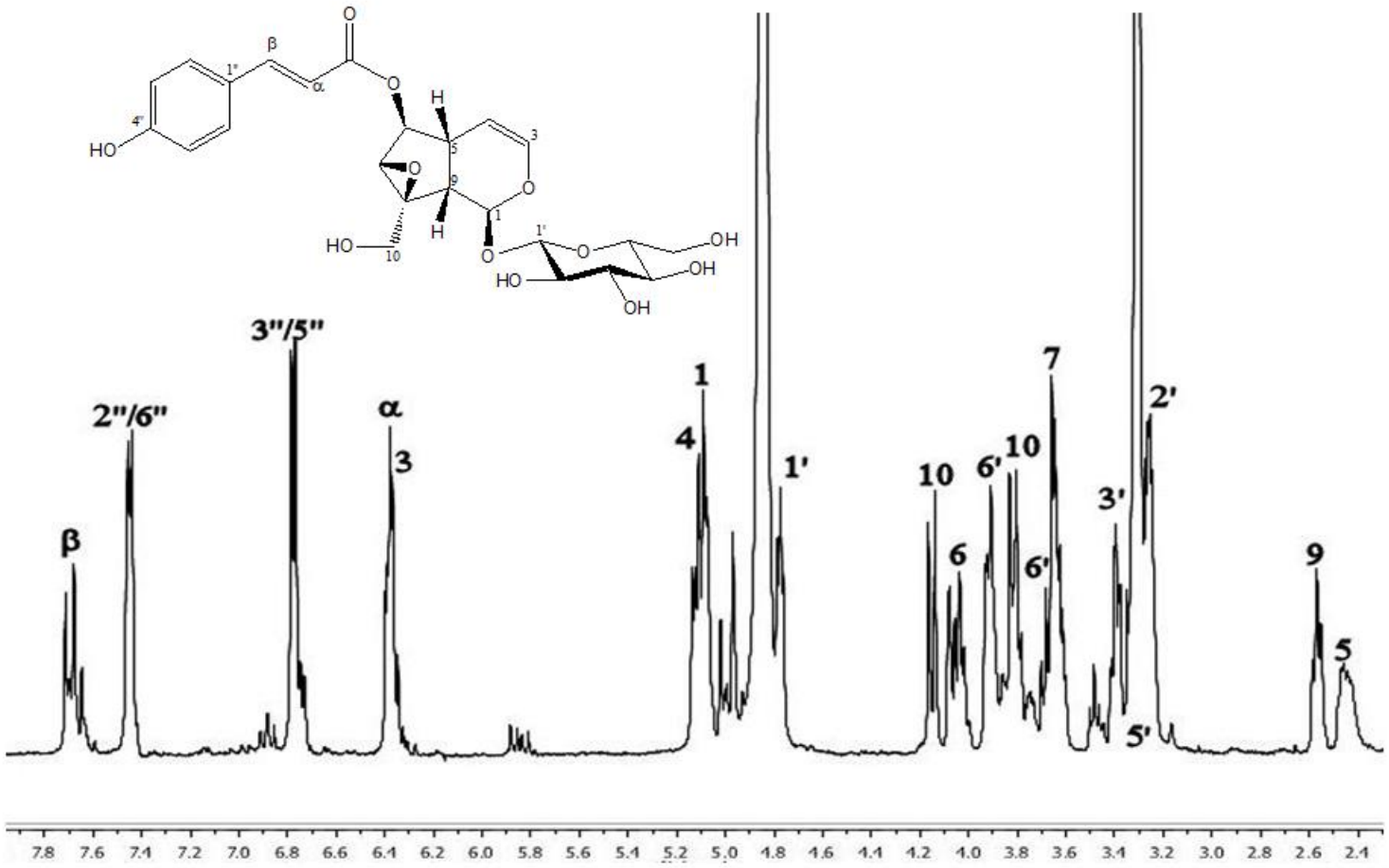
SPECIOSIDE (2)

Compound **2** was obtained as brown amorphous powder from *n*-BuOH extract. The absorption bands observed in the UV spectra of the compound (206 nm) indicated the iridoid enol-ether system as catalpol. The other bands at 313, 355 nm were indicative of a phenolic nature. IR spectrum revealed bands at 3300 (OH) and 1654 (C=C), 1691 (conj. est. C=O), 1635 (conj. C=C) and 1603 cm^{-1} (aromatic ring).

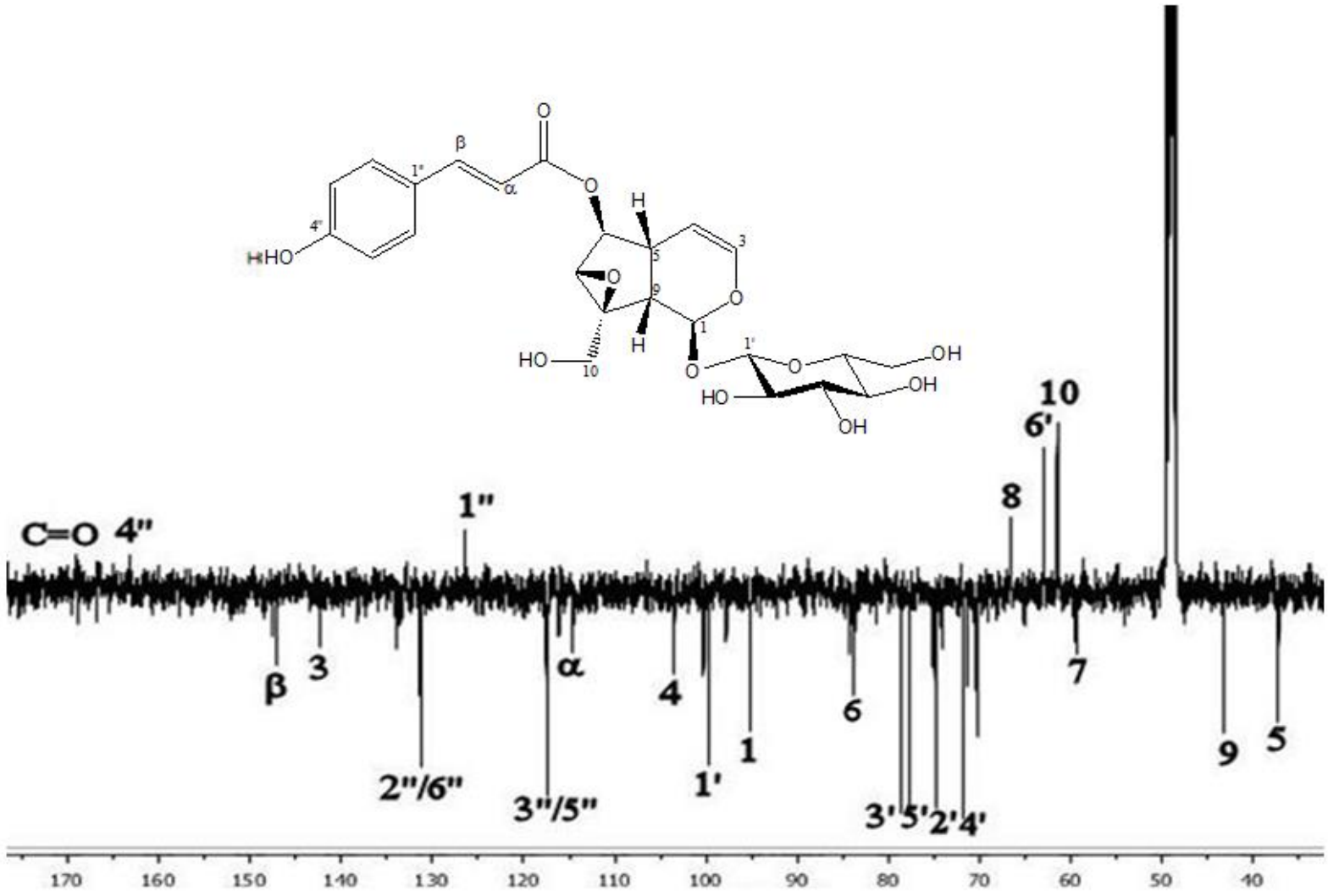
When the ^{1}H NMR data of **2** (Table 49, Spectrum 3) are compared with those of catalpol (**1**), it was observed that the aglycone and the sugar moiety of **2** seem to be quite similar to that of **1**. The ^{1}H NMR spectrum of **2** contained additional signals; two olefinic signals (δ_H 7.67 and 6.37) as *AX* systems and two aromatic signals (δ_H 7.45 and 6.77, each 2H). These signal together with the ^{13}C resonances at δ_C 167.8, 160.1, 147.2, 131.2 (2xC), 126.4, 117.5 (2xC) and 114.6 indicated the presence of *p*-coumaroyl unit in **2**. The chemical shift values of H-6 (4.05 ppm) and C-6 (84.1 ppm) were found to be shifted downfield compared to those of catalpol, indicating the site of esterification to be C-6 (OH). This assumption was also supported by the cross-reak between H-6 of aglycone and C=O of *p*-coumaroyl unit in the HMBC spectrum (Spectrum 6). Based on these findings compound **2** was identified as 6-*O*-*p*-coumaroylcatalpol, also known as **specioside** (68,170,171).

Table 49. ^{13}C and ^{1}H NMR spectral data of specioside (**2**) (CD_3OD, ^{1}H: 500 MHz, ^{13}C: 125 MHz) and HMBC correlations

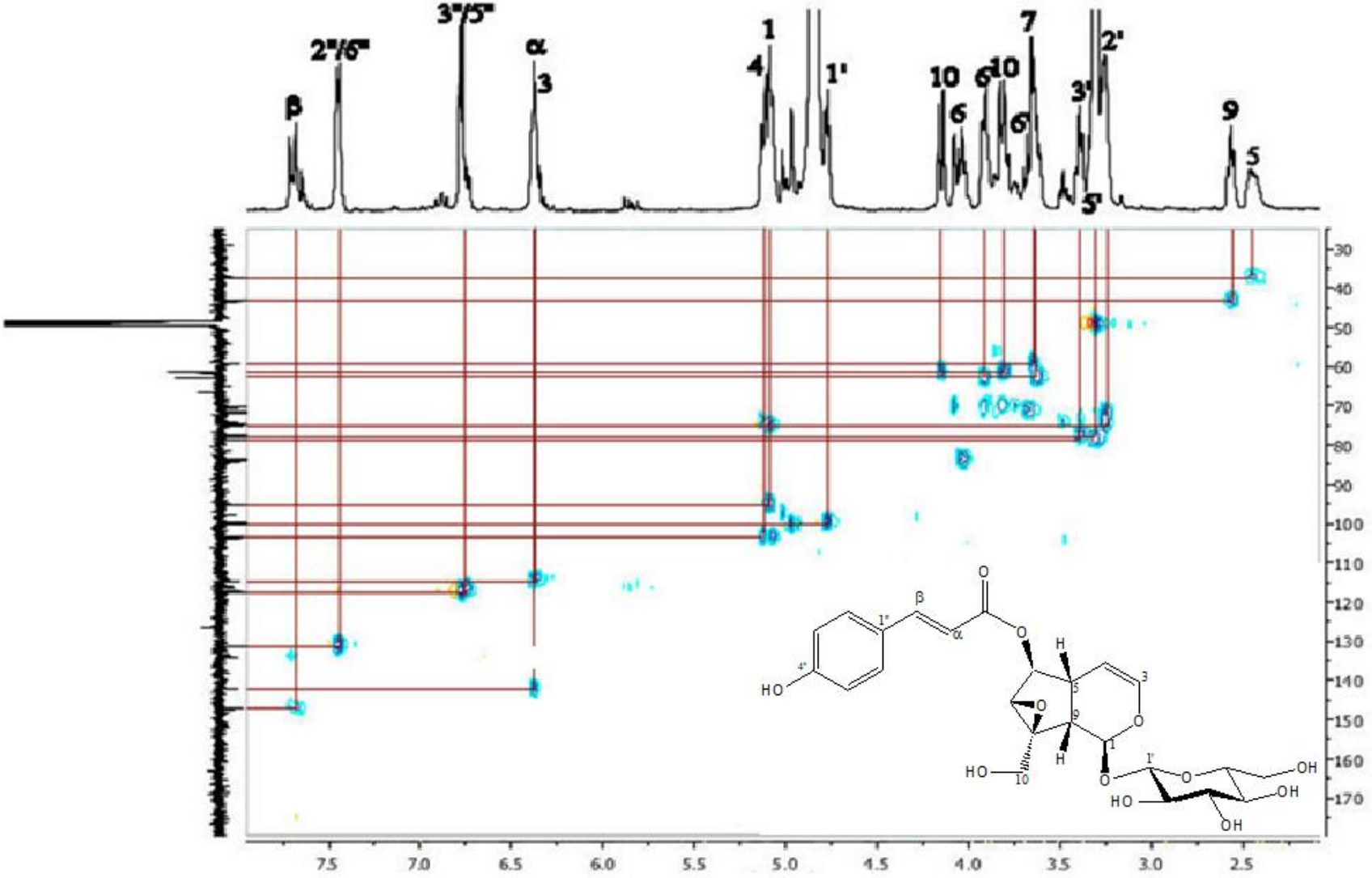
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**	**HMBC (H→C)**
Aglycone				
1	CH	95.2	5.09 m	C-1'
3	CH	142.2	6.38 m	C-5, C-1, C-4
4	CH	103.6	5.12 m	
5	CH	37.2	2.43 m	
6	CH	84.1	4.05 m	C-7, C-4, C=O
7	CH	59.3	3.65 m	C-5, C-6
8	C	66.5	-	
9	CH	43.3	2.57 t (8.4)	C-7, C-8, C-1
10	CH_2	61.5	4.15 d (13.0)	C-7
			3.82 d (13.0)	
Glucose				
1'	CH	99.7	4.77 d (7.8)	C-1
2'	CH	74.0	3.25 m	
3'	CH	77.7	3.30 m	
4'	CH	70.2	3.23 m	
5'	CH	78.2	3.38 m	
6'	CH_2	62.9	3.91 m	
			3.62 m	
p-Coumaroyl				
1"	C	126.4	-	
2", 6"	CH	131.3	7.45 d (8.5)	C-2"/6", C-β, C-4"
3", 5"	CH	117.5	6.77 d (8.5)	C-3"/5", C-1"
4"	C	160.1	-	
β	CH	147.2	7.67 d (16.0)	C-2"/6", C=O
α	CH	114.6	6.37 d (16.0)	C-1"
C=O	C	167.8	-	



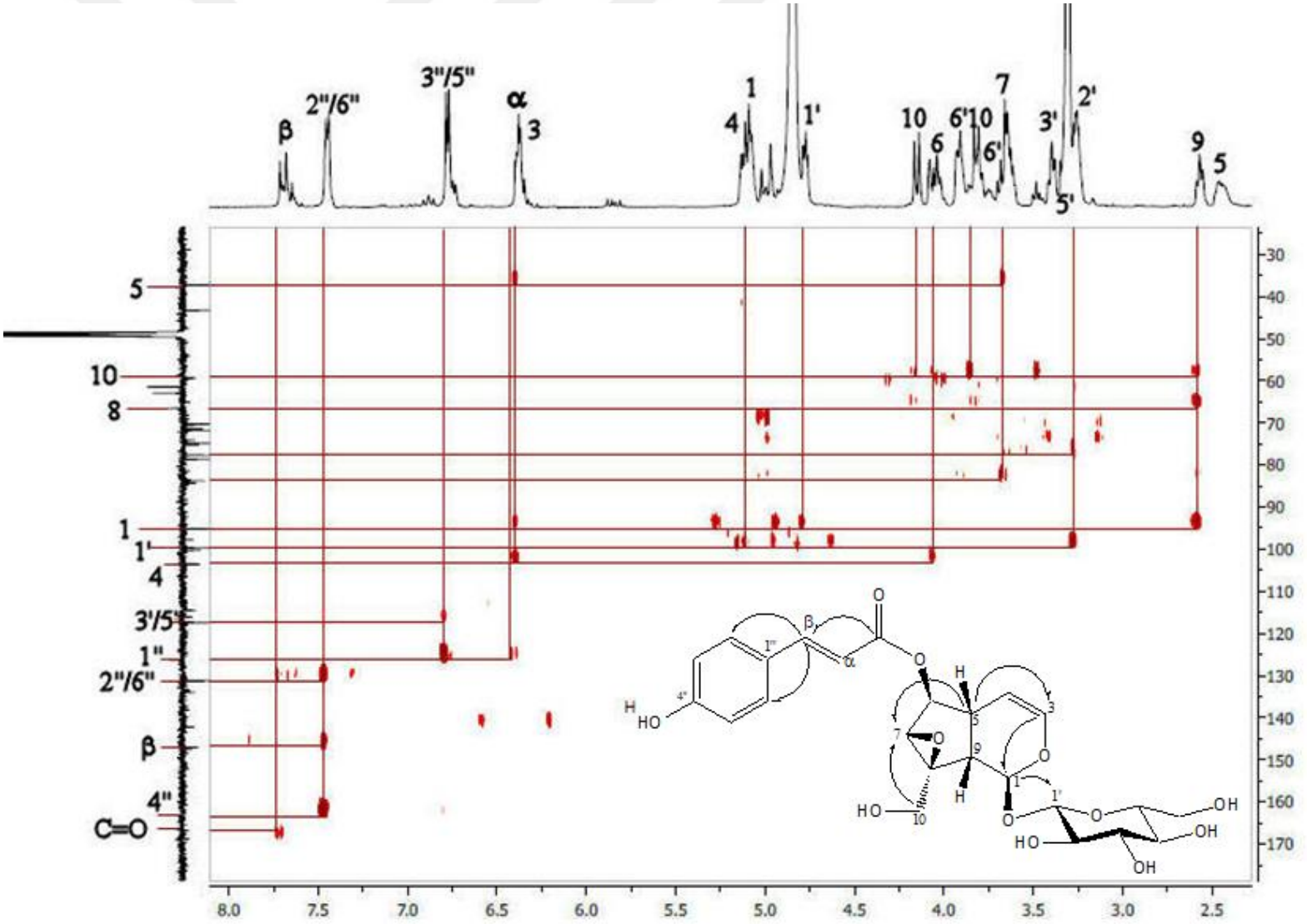
Spectrum 3. ^{1}H NMR Spectrum of Specioside (**2**) (500 MHz, CD_3OD)



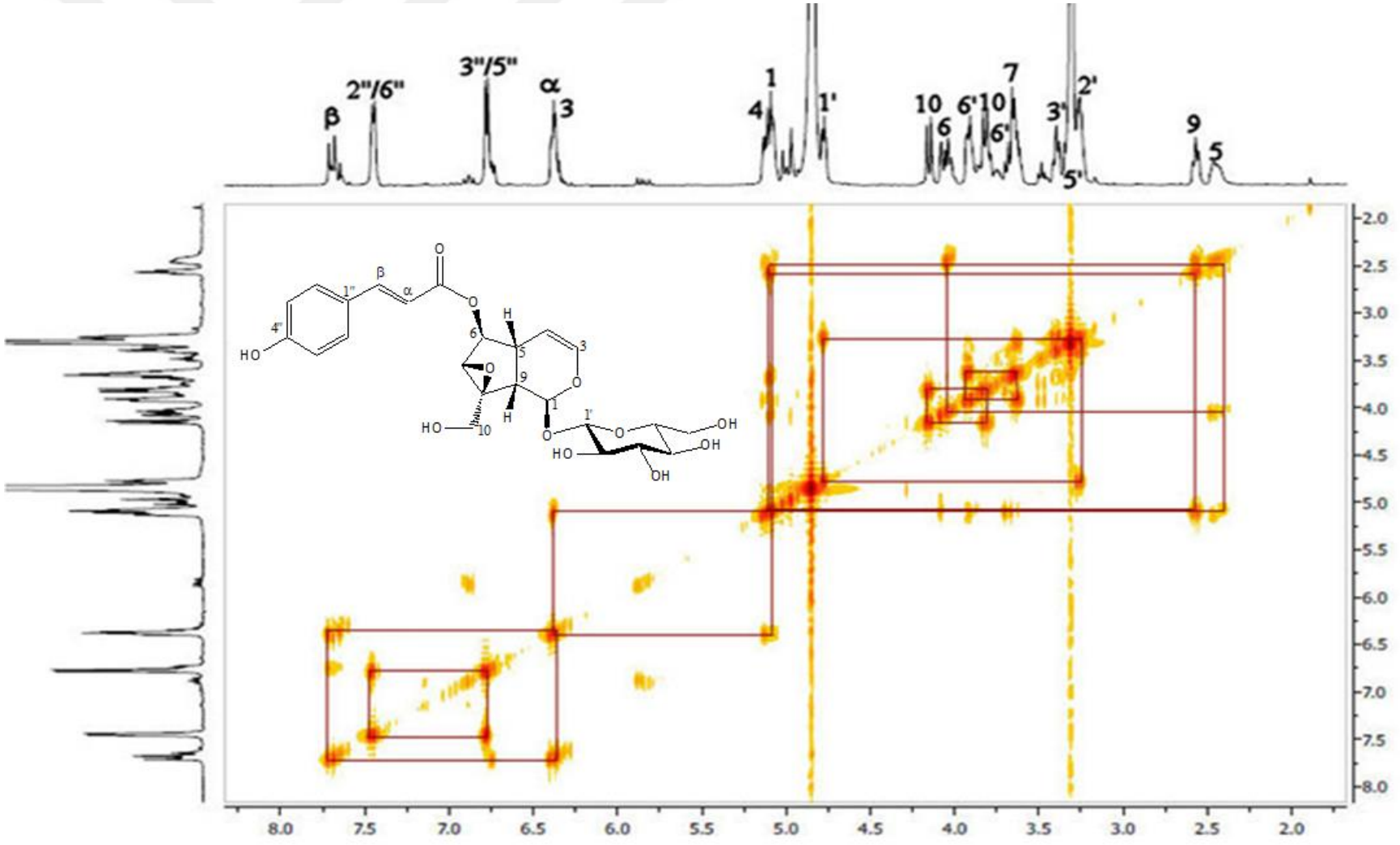
Spectrum 4. JMOD -^{13}C NMR Spectrum of Specioside (**2**) (125 MHz, CD_3OD)



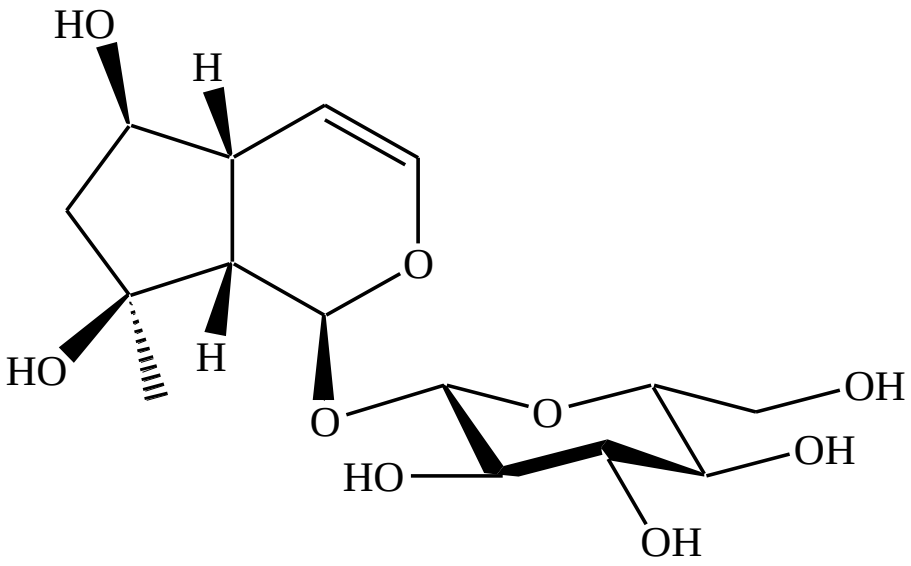
Spectrum 5. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (short range) (HSQC) of Specioside (**2**)



Spectrum 6. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (long range) (HMBC) of Specioside (**2**)



Spectrum 7. 2D-^{1}H, ^{1}H-Homonuclear Correlation Spectrum (COSY) of Specioside (**2**)



AJUGOL (3): $C_{15}H_{24}O_9$ (MW: 348.35)

UV λ_{max} (MeOH) nm:	208
IR ν_{max} (KBr) cm^{-1}:	3386 (OH), 1658 (C=C)
^1H NMR:	Table 50, Spectrum 8

AJUGOL (3)

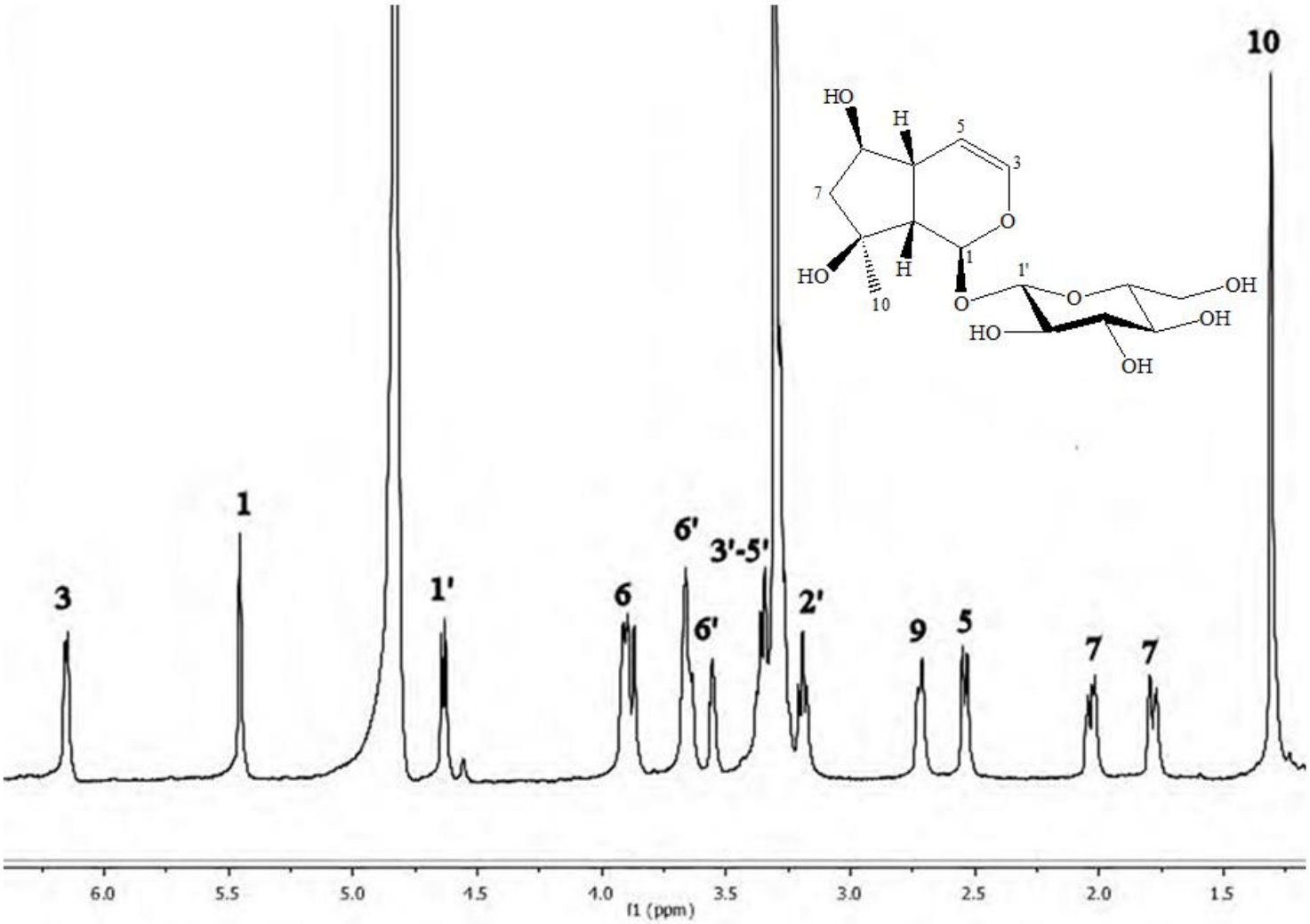
Compound **3** was obtained as amorphous powder from *n*-BuOH extract. The UV spectra of compound **3** showed absorption band of 208 nm. The IR spectrum displayed hydroxyl (3386 cm^{-1}) and olefinic double bands (1658 cm^{-1}).

The ^{1}H NMR spectrum (Table 50, Spectrum 8) of **3** contained one hemiacetal (δ_H 5.45), two olefinic (δ_H 6.15 and 4.63), one oxymethine (δ_H 3.89), two methines (δ_H 2.54, 2.72), one methylene (δ_H 2.03, 1.78) and one tertiary methyl (δ_H 1.31) signals due to the cyclopentanopyran nucleus. Moreover, the anomeric proton signal at δ_H 4.64 (d, J = 7.7 Hz) as well as the signals between 3.19 – 3.66 indicated the presence of a β-glucopyranose moiety in the structure. The spectroscopic data were found to be consistent with the values for **ajugol** in the literature (170). Ajugol was previously reported from several *Verbascum* species such as *V. thapsus* (84) and *V. xanthophoeniceum* (47).

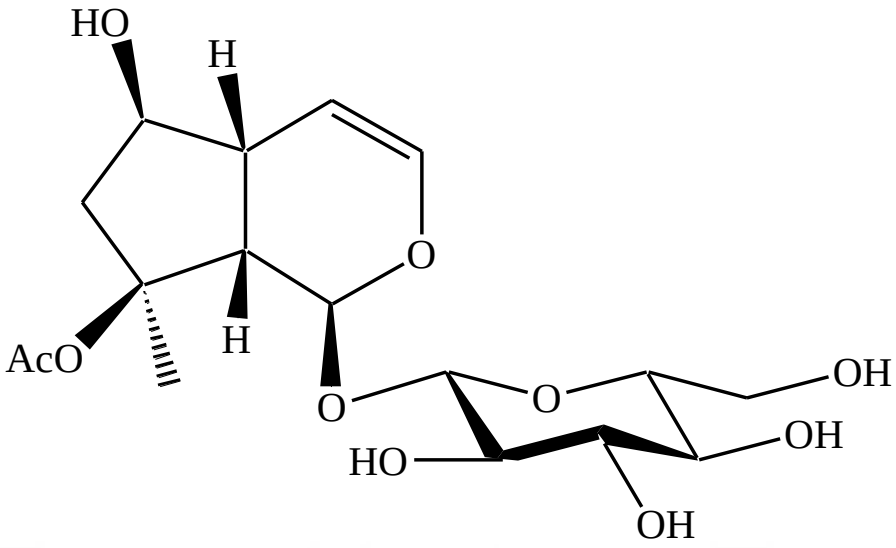
Table 50. ^{1}H NMR spectral data of ajugol (**3**) (CD_3OD, 500 MHz)

Position	**Multiplicity**	**δ_H ppm, *J* (Hz)**
Aglycone		
1	CH	5.45 br.s
3	CH	6.15 dd (6.2, 2.0)
4	CH	4.63 dd (8.0, 3.0)
5	CH	2.54 d (9.0)
6	CH	3.89 m
7	CH_2	2.03 m
		1.78 m
8	C	-
9	CH	2.72 d (9.0)
10	CH_3	1.31 s
Glucose		
1'	CH	4.64 d (7.7)
2'	CH	3.19 dd (9.0, 8.0)
3'	CH	3.36 m
4'	CH	3.34-3.23 m
5'	CH	3.34 m
6'	CH_2	3.66 dd (11.2, 2.2)
		3.54 †

† = overlapped



Spectrum 8. ^{1}H NMR Spectrum of Ajugol (**3**) (500 MHz, CD_3OD)



AJUGOSIDE (4): $C_{17}H_{26}O_{10}$ (MW: 390.38)

UV λ_{max} (MeOH) nm:	207
IR ν_{max} (KBr) cm^{-1}:	3378 (OH), 1655 (C=C)
^{1}H NMR:	Table 51, Spectrum 9
^{13}C NMR:	Table 51, Spectrum 10
HR-ESI-MS *m/z*:	413.1417 ($C_{17}H_{26}O_{10}Na$)

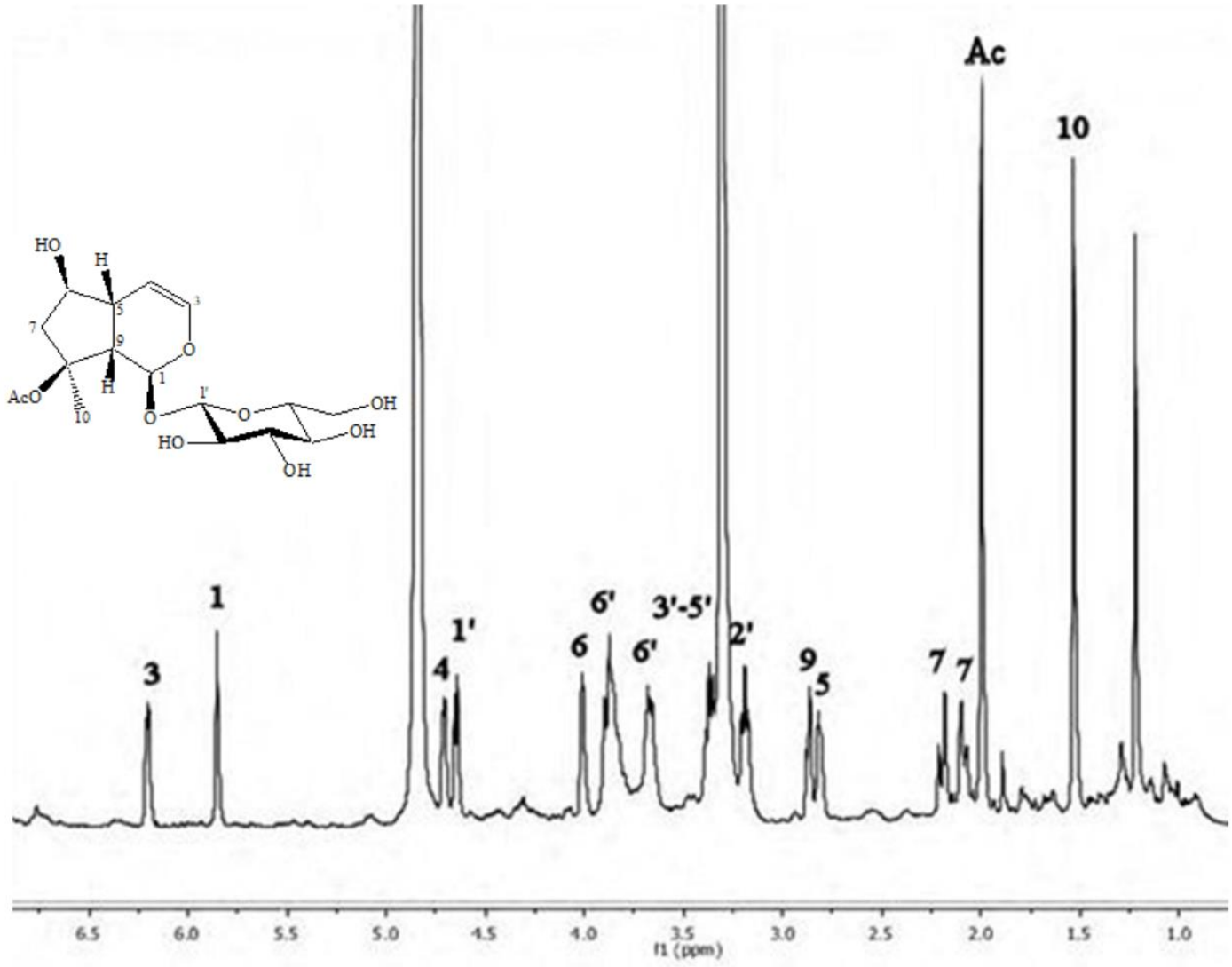
AJUGOSIDE (4)

Compound **4** was obtained as amorphous powder from *n*-BuOH extract. The UV spectrum of **4** showed an absorption band at 207 nm. The IR spectrum displayed absorption bands due to hydroxyl (3378 cm^{-1}) and olefinic (1655 cm^{-1}) functionalities. The molecular formula, $C_{17}H_{26}O_{10}$ were deduced from the pseudomolecular ion peak at *m/z* 413.1417 ($C_{17}H_{26}O_{10}Na$) in the HR-MS as well as NMR data.

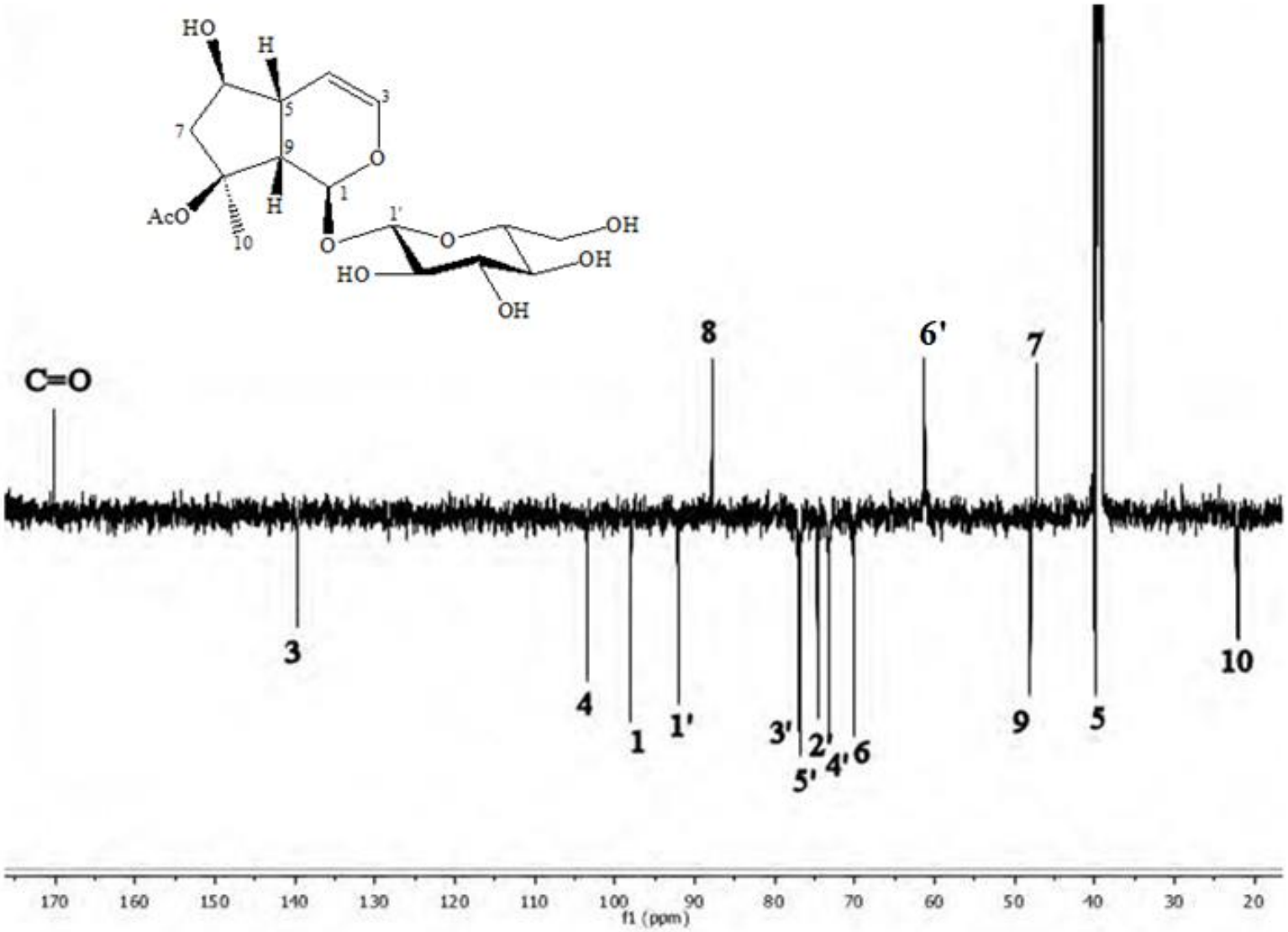
The ^{1}H and ^{13}C NMR spectra (Table 51, Spectrum 9 & 10) of **4** displayed one hemiacetal (δ_H 5.85, δ_C 92.0), two olefinic (δ_H 6.21 and 4.71, δ_C 139.9, 104.3), one oxymethine (δ_H 4.00, δ_C 77.0), two methines (δ_H 2.81, 2.87, δ_C 39.7, 48), two methylenes (δ_H 2.19 and 2.08, δ_C 47.2), one tertiary methyl (δ_H 1.53, δ_C 22.0), an acetyl (δ_H 1.99, δ_C 22.2 and 170.0) signals due to the acetylated iridoid core. Moreover, the anomeric proton (δ_H 4.65, d, J = 7.7 Hz) and anomeric carbon (δ_C 97.9) signals showed the presence of a β-glucopyranose moiety in the structure. The spectroscopic data of **4** were found to be similar to that of ajugol (**3**) except for the additional signals at and δ_H1.99 (s, 3H), δ_C 170.0 and 22.2 arising from an acetyl unit. The esterification site was found to be C-8 (OH) due to the downfield shift of C-8 (87.8 ppm) in the ^{13}C NMR spectrum. Based on the above findings compound **4** were elucidated as **ajugoside** (172).

Table 51. ^{13}C and ^{1}H NMR spectral data of ajugoside (**4**) (MeOD, 1H: 500 MHz, CD_3OD, ^{13}C: 125 MHz)

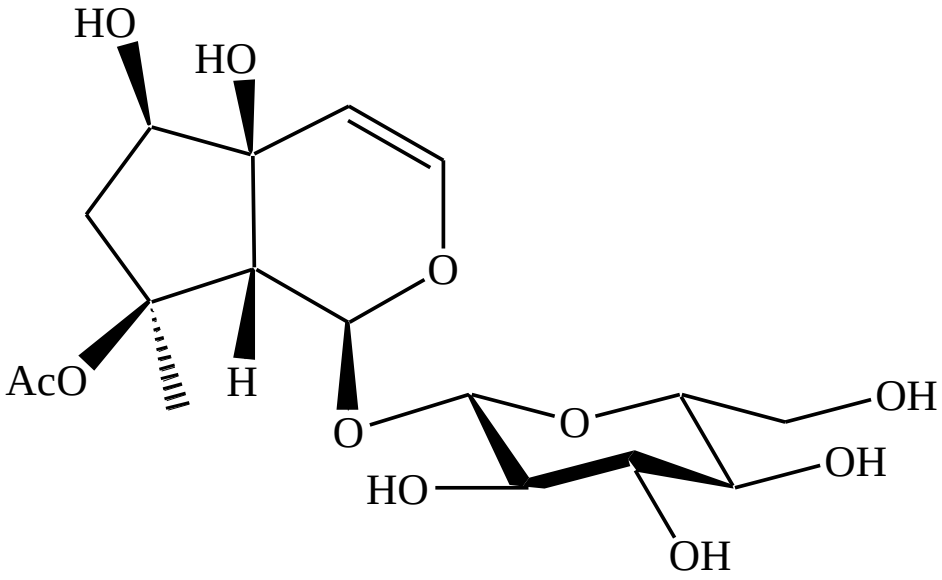
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm**
Aglycone			
1	CH	92.0	5.85 br. s
3	CH	139.9	6.21 d (5.5)
4	CH	104.3	4.71 br.d (5.5)
5	CH	39.7	2.81 d (6.8)
6	CH	77.0	4.00 s
7	CH_2	47.2	2.19 br. d (14.9)
			2.08 dd (14.9, 4.4)
8	C	87.8	-
9	CH	48.0	2.87 d (7.8)
10	CH_3	22.0	1.53 s
Glucose			
1'	CH	97.9	4.65 d (7.9)
2'	CH	73.1	3.19 t (8.5)
3'	CH	74.6	3.37 m
4'	CH	70.1	3.37-3.19 m
5'	CH	76.7	3.37-3.19 m
6'	CH_2	61.2	3.88 dd (12.0, 2.1)
			3.66 dd (12.0, 5.6)
COCH_3		170.0	-
CO*CH_3*		22.2	1.99 s



Spectrum 9. ^{1}H NMR Spectrum of Ajugoside (**4**) (500 MHz, CD_3OD)



Spectrum 10. JMOD-^{13}C NMR Spectrum of Ajugoside (**4**) (125 MHz, CD_3OD)



8-*O*-ACETYLHARPAGIDE (5): $C_{17}H_{26}O_{11}$ (MW: 406.38)

UV λ_{max} (MeOH) nm	204, 232
IR ν_{max} (KBr) cm^{-1}:	3389 (OH), 1653 (C=C)
^{1}H NMR:	Table 52, Spectrum 11
^{13}C NMR:	Tablo 52, Spectrum 12
HSQC:	Spectrum 13
HMBC:	Tablo 52, Spectrum 14
COSY:	Spectrum 15
HR-ESI-MS *m/z* :	429.1365 ($C_{17}H_{26}O_{11}Na$)

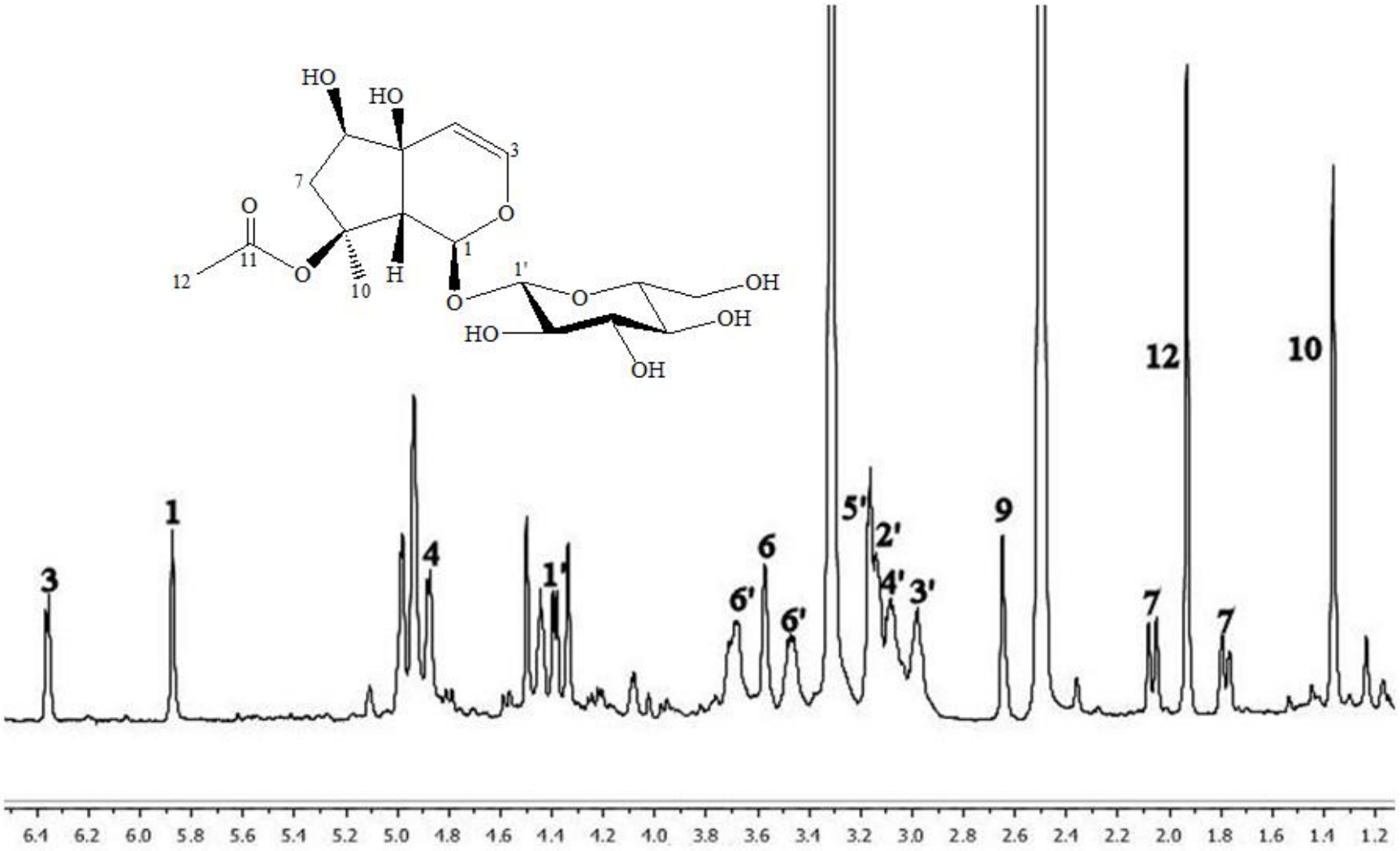
8-*O*-ACETYLHARPAGIDE (5)

Compound **5** was obtained as an amorphous powder from *n*-BuOH extract. Its UV (204, 232 nm) and IR (3389 and 1653 cm^{-1}) spectra were characteristic of an iridoid structure. The pseudomolecular ion peak at *m/z* 429.1365 ($C_{17}H_{26}O_{11}Na$), in the HR-MS and the NMR findings indicated the molecular formula, $C_{17}H_{26}O_{11}$ which contained an additional oxygen atom in **5** when compared to compound **4**.

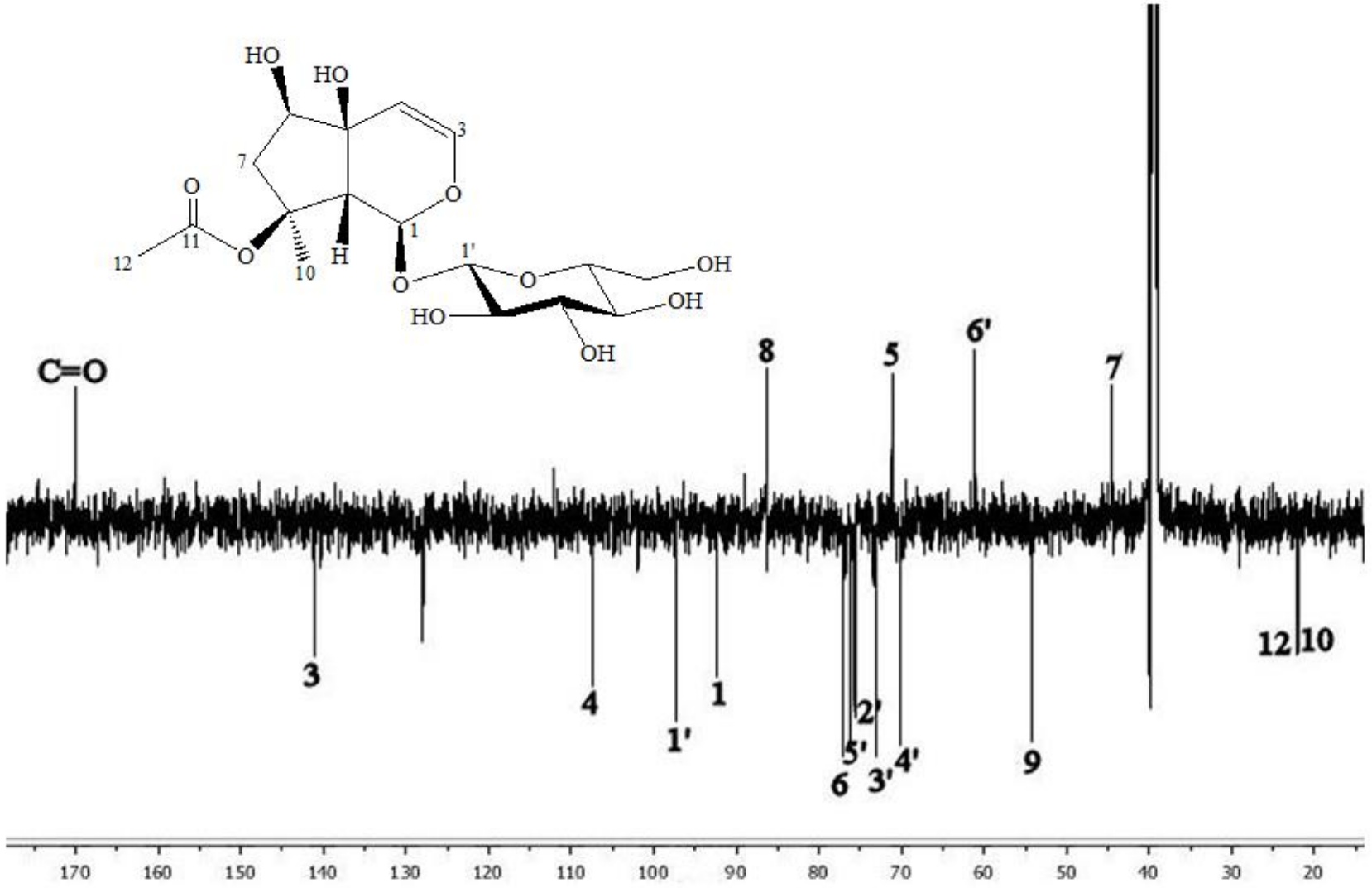
The ^{1}H NMR spectrum of **5** (Table 52, Spectrum 11) contained signals due to one hemiacetal (δ_H 5.87), two olefinic (δ_H 6.35 and 4.91), one oxymethine (δ_H 3.57), one methylene (δ_H 2.06 and 1.78) and one tertiary methyl (δ_H 1.36) functionalities. Furthermore, one anomeric signal (δ_H 4.58) and one acetyl methyl (δ_H 1.93) were also evident in the spectrum. When these findings were compared to those of **4**, only difference was due to the absence of H-5 signal in the ^{1}H NMR spectrum of **5** indicating that C-5 was oxygenated. This assumption was confirmed by the quaternary carbon signal at δ_C 77.1 in the upside of JMOD-^{13}C NMR spectrum of **5**. The structure was unambiguously identified with the help of 2D NMR spectra including COSY, HSQC and HMBC. Based on these results, the structure of compound **5** was identified as **8-*O*-acetylharpagide** (95,172).

Table 52. ^{13}C and ^{1}H NMR spectral data of 8-*O*-Acetylharpagide (**5**) (CD_3OD, ^{1}H: 500 MHz, ^{13}C: 125 MHz) and HMBC correlations

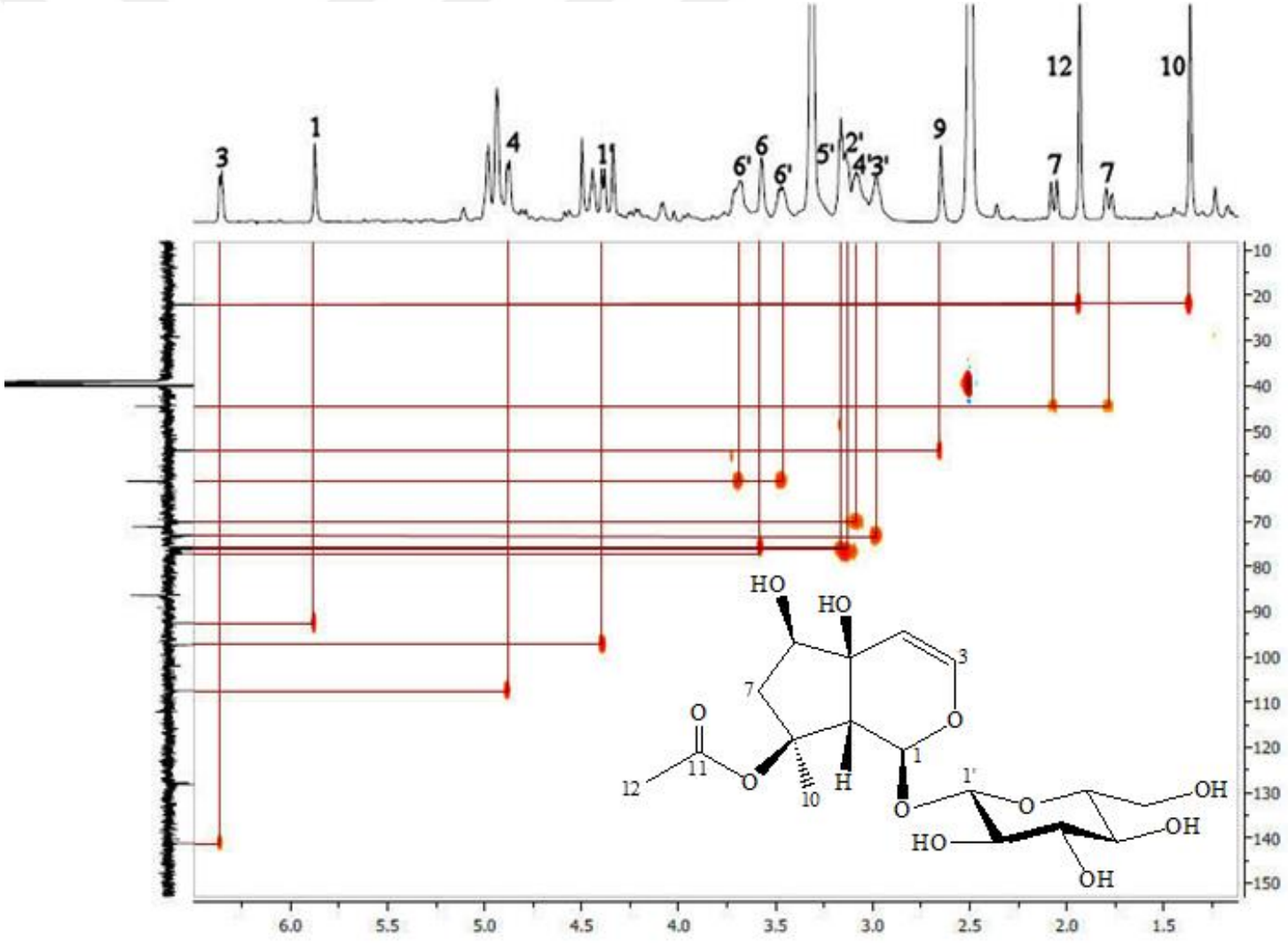
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**	**HMBC**
Aglycone				
1	CH	92.5	5.87 s	C-5, C-1, C-3
3	CH	141.1	6.35 d (6.4)	C-5, C-1, C-4
4	CH	107.4	4.91 d (6.4)	C-9, C-6, C-3
5	C	77.1	-	
6	CH	75.7	3.57 s	
7	CH_2	44.5	2.06 d (15.0)	C-10, C-5, C-6, C-8
			1.78 d (15.0)	C-10
8	C	86.4	-	
9	CH	54.3	2.65 s	C-5, C-8, C-1
10	CH_3	21.9	1.36 s	C-7, C-9, C-8
Glucose				
1'	CH	97.3	4.58 d (8.0)	C-1
2'	CH	75.6	3.12 m	C-4'
3'	CH	73.0	3.57 m	
4'	CH	70.1	3.08 m	
5'	CH	76.9	3.12 m	
6'	CH_2	61.1	3.69 m	
			3.47 m	
11		170.3	-	
12		21.9	1.93 s	



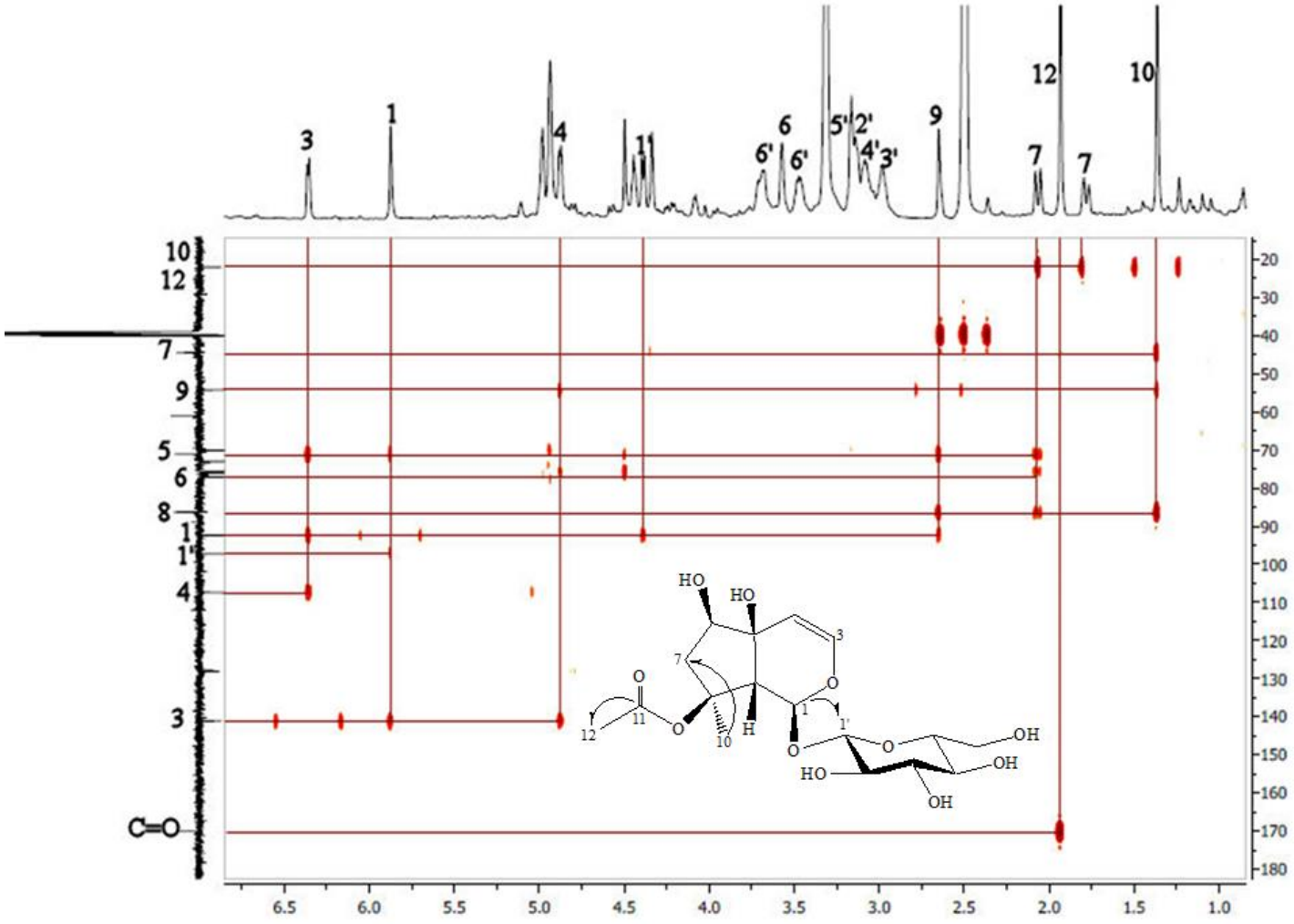
Spectrum 11. ^{1}H NMR Spectrum of 8-*O*-Acetylharpagide (**5**) (500 MHz, CD_3OD)



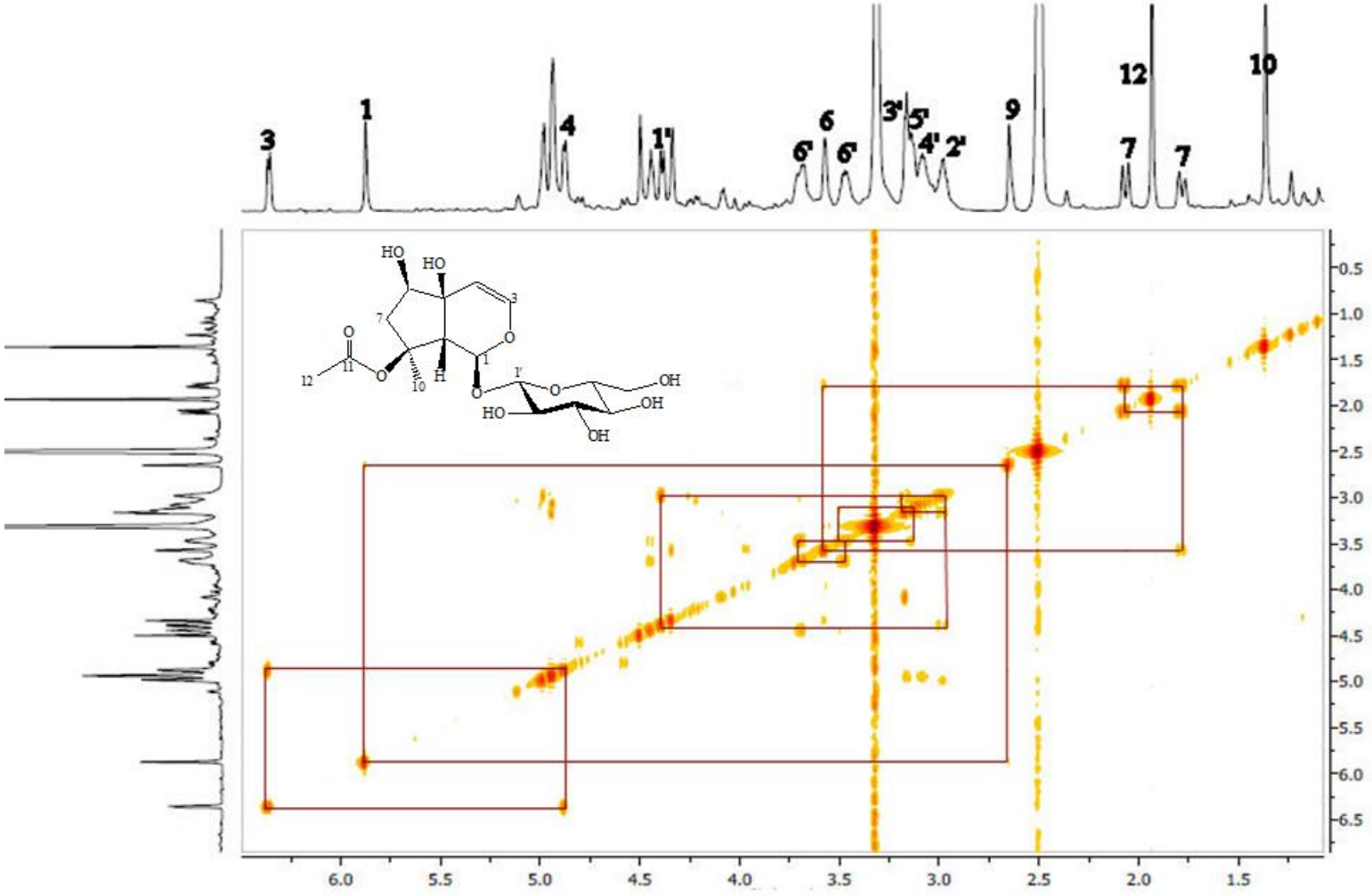
Spectrum 12. JMOD-^{13}C NMR Spectrum of 8-*O*-Acetylharpagide (**5**) (125 MHz, CD_3OD)



Spectrum 13. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (short range) (HSQC) of 8-*O*-Acetylharpagide (**5**)

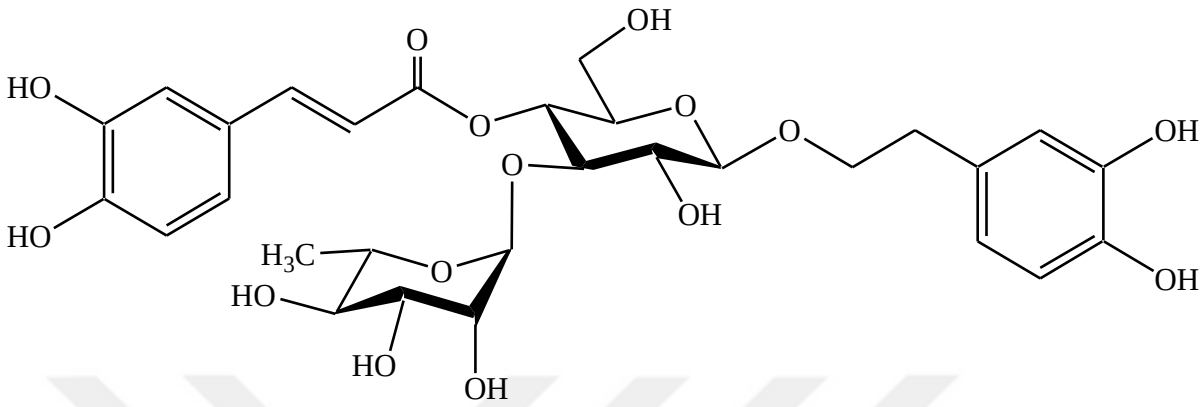


Spectrum 14. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (long range) (HMBC) of 8-*O*-Acetylharpagide (**5**)



Spectrum 15. 2D-^{1}H, ^{1}H-Homonuclear Correlation Spectrum (COSY) of 8-*O*-Acetylharpagide (**5**)

4.4.2. Phenylethanoid Glycosides



VERBASCOSIDE (=ACTEOSIDE) (6): $C_{29}H_{36}O_{15}$ (MW: 624.59)

UV λ_{max} (MeOH) nm	204, 288, 338
IR ν_{max} (KBr) cm^{-1}:	3278 (OH), 2971 (aliphatic CH), 1697 (conjugated ester C=O), 1655 (conjugated C=C), 1604, 1518 (aromatic ring)
^{1}H NMR:	Table 53, Spectrum 16

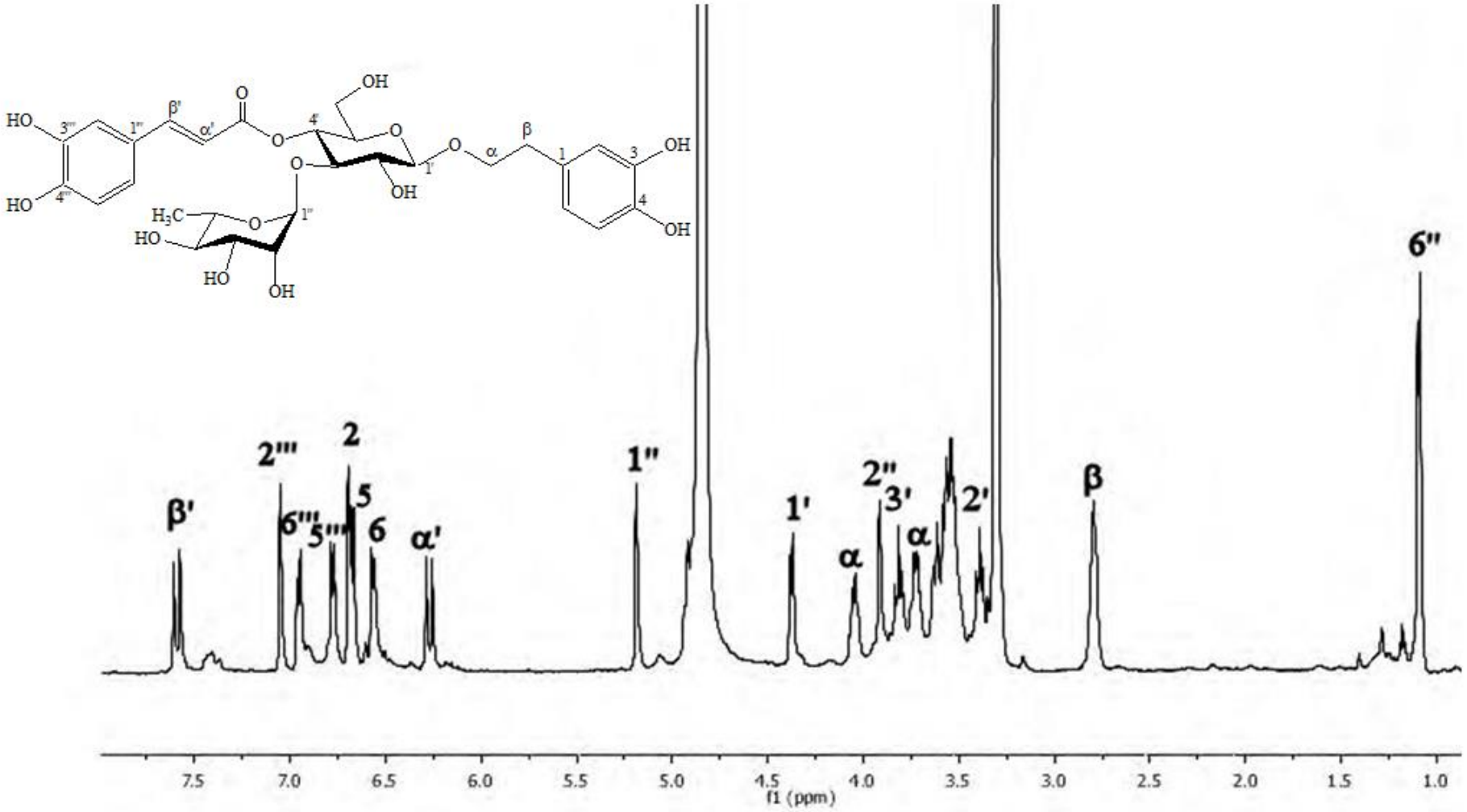
VERBASCOSIDE (=ACTEOSIDE) (6)

Compound **6** was obtained as an amorphous powder. The IR spectrum revealed bands at 3278 cm^{-1} (OH), 2975 cm^{-1} (aliphatic CH), 1697 cm^{-1} (conjugated ester C=O), 1655 cm^{-1} (conjugated C=C), 1604 cm^{-1} (aromatic ring). The UV spectrum (204, 288, 338) indicated its polyphenolic nature.

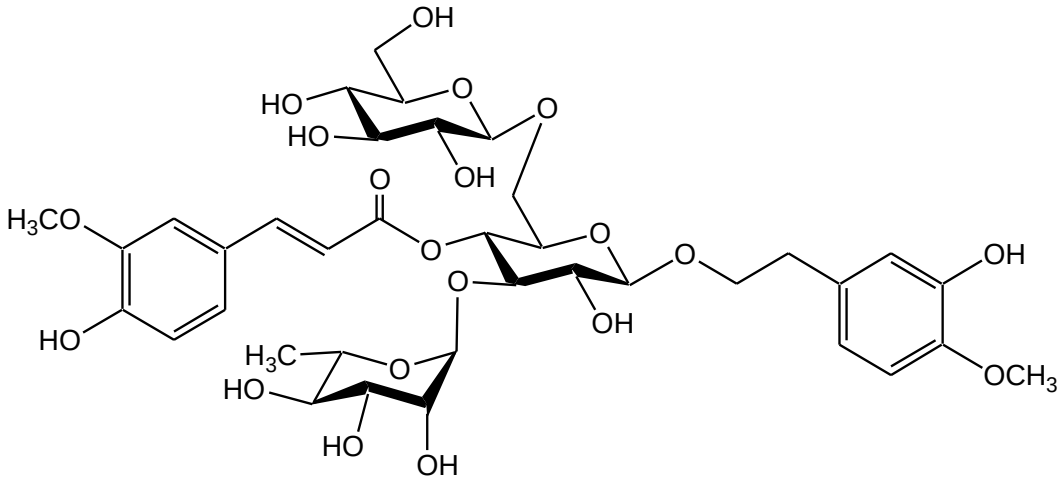
The ^{1}H NMR spectrum (Table 53, Spectrum 16) of compound **6**, exhibited six aromatic proton resonances as two *ABX* systems at 7.05-6.65 ppm arising characteristic of 3,4 disubstituted aromatic rings. Moreover, the spectrum also contained a pair of *trans* coupled (J = 15.9 Hz) olefinic signals at δ_H 7.59 (H-β') and 6.26 (H-α') as an *AX* system. Furthermore, one methylene signal at δ_H 2.79 (s, 2H) and two non-equivalent oxymethylene signals at δ_H 4.05 (m) and 3.81 (m) were also seen in the ^{1}H NMR spectrum. These findings revealed the presence of a *trans*-caffeic acid and 3,4-dihydroxyphenylethanol units in compound **6**. Two anomeric proton signals at 4.37 ppm (d, J=7.9, H-1') and 5.18 ppm (br. s, H-1") with a methyl resonance at 1.09 ppm (d, J = 6.1, 3H) determined its diglycosidic nature that was composed of one β-D-glucopyranose, and the α-L-rhamnopyranose. The downshift of H-4' signal of the glucose moiety (4.92 ppm) around 1 ppm indicated that *trans*-caffeic acid was esterified at C-4' (OH) of the glucose. The ^{1}H NMR spectroscopic data were compared with the literature and the structure of the compound **6** found as **verbascoside (=acteoside)** (95,172,173), which is widespread in the genus *Verbascum*.

Table 53. ^{13}C and ^{1}H NMR spectral data of verbascoside (**6**) (MeOD, ^{1}H: 500 MHz)

Position	**Multiplicity**	**δ_H ppm, *J* (Hz)**
Aglycone		
1	C	-
2	CH	6.68 d (2.0)
3	C	-
4	C	-
5	CH	6.66 d (8.0)
6	CH	6.56 dd (8.0, 2.0)
α	CH_2	4.05 m
		3.81 m
β	CH_2	2.79 t (9.1)
Glucose		
1'	CH	4.37 d (7.9)
2'	CH	3.39 t (8.6)
3'	CH	3.71 m
4'	CH	4.92 m
5'	CH	3.63-3.52 m
6'	CH_2	3.63-3.52 m
Rhamnose		
1"	CH	5.18 br. s
2"	CH	3.91 m
3"	CH	3.63-3.52 m
4"	CH	3.63-3.52 m
5"	CH	3.63-3.52 m
6"	CH_3	1.09 d (6.1)
Caffeoyl		
1"'	C	-
2"'	CH	7.05 d (2.0)
3"'	C	-
4"'	C	-
5"'	CH	6.77 d (7.9)
6"'	CH	6.96 dd (7.9, 2.0)
α'	CH	6.26 d (15.9)
β'	CH	7.59 d (15.9)
C=O	C	-



Spectrum 16. ^{1}H NMR Spectrum of Verbascoside (**6**) (500 MHz, CD_3OD)



GLUCOPYRANOSYL (1→Gi-6)-MARTYNOSIDE (7): $C_{37}H_{50}O_{20}$ (MW: 814.78)

UV λ_{max} (MeOH) nm	220, 283, 330
IR ν_{max} (KBr) cm^{-1}:	3338 (OH), 1703 (conjugated ester C=O), 1628 (conjugated C=C), 1594 (aromatic ring)
^{1}H NMR:	Table 54, Spectrum 17
^{13}C NMR:	Tablo 54, Spectrum 18
HSQC:	Spectrum 19
HMBC:	Spectrum 20
COSY:	Spectrum 21
HR-ESI-MS *m/z* :	837.2791 ($C_{37}H_{50}O_{20}Na$)

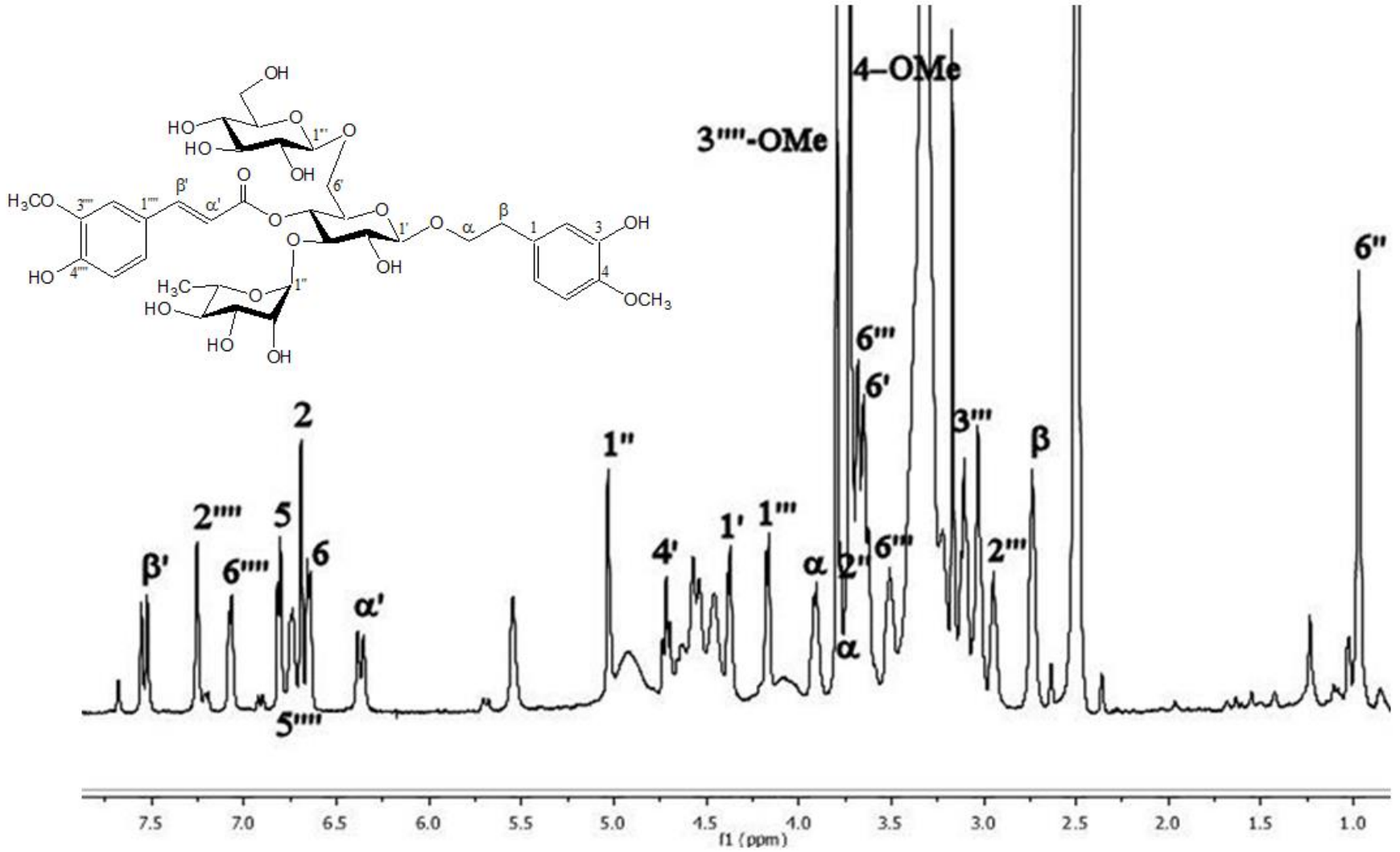
GLUCOPYRANOSYL (1→Gi-6)-MARTYNOSIDE (7)

Compound **7** obtained as white amorphous powder. The molecular formula was deduced to be $C_{37}H_{50}O_{20}$, from HR-MS and NMR data. The IR spectrum revealed bands at 3338 cm^{-1} (OH), 1703 cm^{-1} (conjugated ester C=O), 1628 cm^{-1} (conjugated C=C), 1594 cm^{-1} (aromatic ring). UV spectrum (220, 234, 330) was characteristic for a phenylethanoid structure.

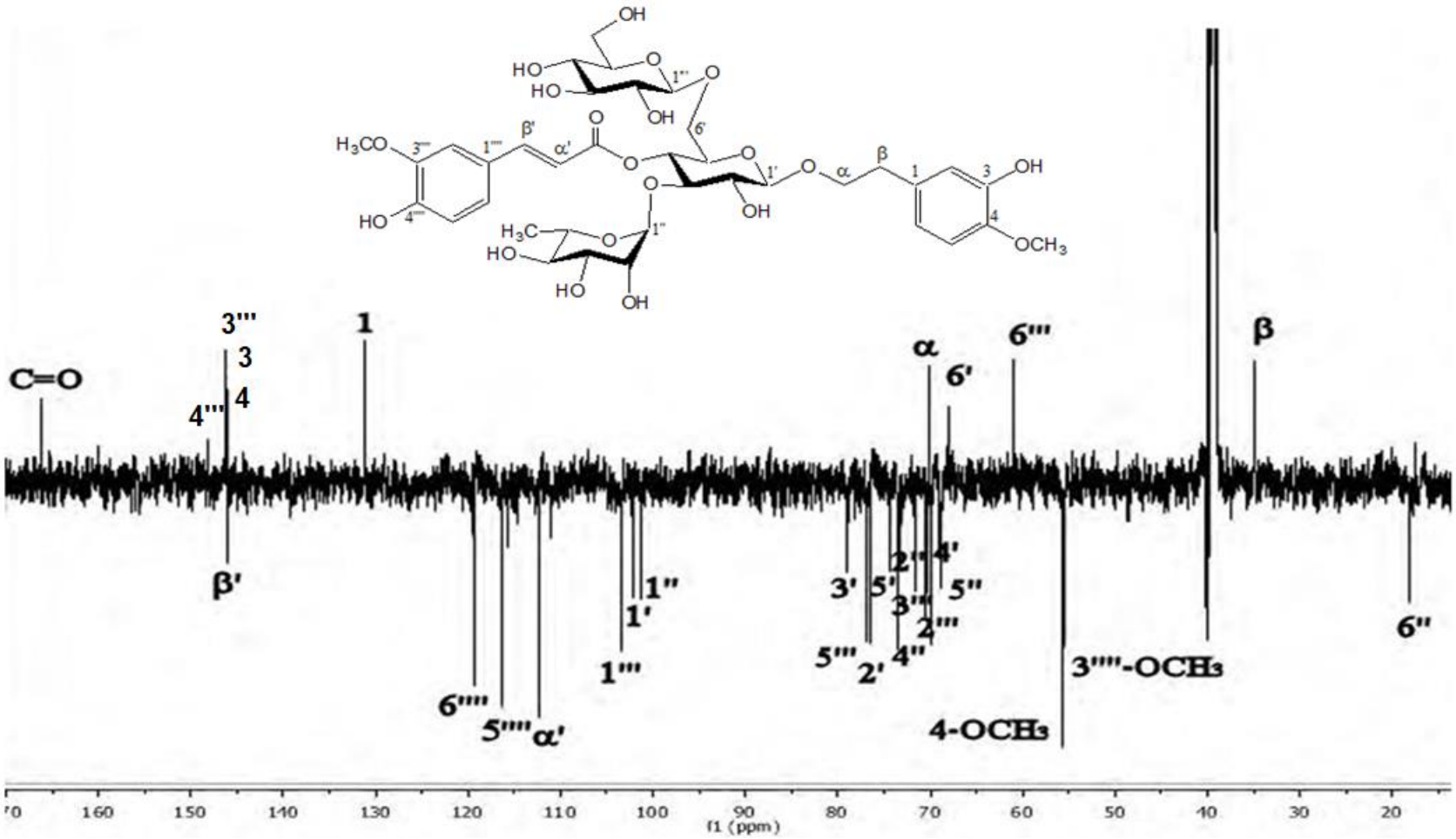
The ^{1}H NMR spectrum of **7** showed characteristic signals arising from a *trans*-hydroxycinnamoyl and 3,4-dihydroxyphenylethanol derivatives as that of verbascoside (**6**). However, the spectra contained two methoxy signals at δ_H 3.79 and 3.72 indicating that the aglycone was 3-hydroxy-4-methoxyphenylethanol and the aromatic acyl unit is (*E*)-feruloyl differing from verbascoside (**6**). The ^{1}H NMR spectrum of **7** (Table 54, Spectrum 17), contained three anomeric proton signals at δ_H 4.37 (d, H-1'), 5.08 (br. s, H-1") and 4.17 (d, H-1'"), indicating its triglycosidic structure. When the NMR spectra of **7** were compared to verbascoside, compound **7** contained an additional hexose unit in its structure. The anomeric proton signal at 4.17 ppm (d, J = 7.7 Hz, H-1'") together with the ^{13}C signals at δ_C 103.4, 74.8, 77.9, 69.9, 76.9, 60.9 showed the presence of a second β-D-glucopyranose unit as a terminal sugar in **7**. Glucosidation effects were seen for C-3' (δ_C 78.9) and C-6' (δ_C 68.0) of the inner glucose determined that the α-L-rhamnopyrnosyl and the second β-D-glucopyranosyl units were attached to core sugar. All the proton and carbon resonances were interpreted by using 1D and 2D NMR (COSY, HSQC and HMBC) techniques. In HMBC spectrum (Spectrum 20), the long-range correlations observed between the rhamnose H-1" (δ_H 5.08) and the glucose C-3' (δ_C 79.0) and between the glucose H-6' (δ_H 3.83-3.56) and the terminal glucose C-1'" (δ_C 103.4) signal. Similarly, the long-range correlation between H-1' (δ_H 4.37) of inner glucose and the C-α of the side chain of aglycone (δ_C 70.1) led us to determine the attachment point of glucose unit to aglycone. Furthermore, the cross-peak between the glucose H-4' (δ_H 4.71) signal and the carbonyl signal of the caffeoyl unit (166.2 ppm) confirmed the location of the (*E*)-feruloyl unit as C-4′(OH). Based on the above data and comparison with the literature values, **7** was identified as **glucopyranosyl-(1→6)–martynoside** (174).

Table 54. ^{13}C and ^{1}H NMR spectral data of glucopyranosyl-(1→Gi-6)-martynoside (**7**) (DMSO-d_6, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

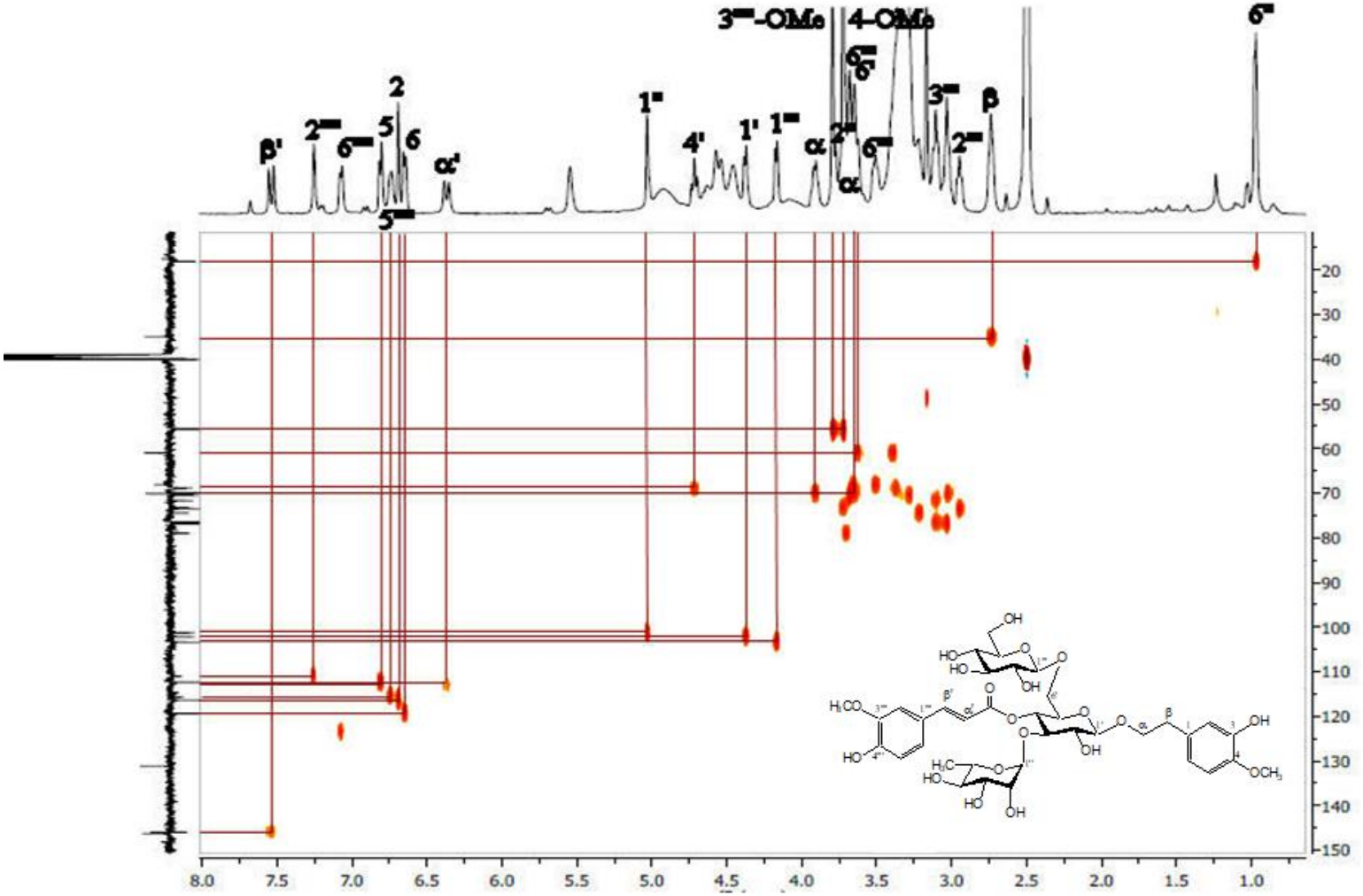
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm**
Aglycone			
1	C	131.1	-
2	CH	115.6	6.73 d (2.0)
3	C	146.2	-
4	C	146.0	-
5	CH	116.3	6.69 d (8.0)
6	CH	116.2	6.48 dd (8.0, 2.0)
α	CH_2	70.1	3.90 m
			3.76 m
β	CH_2	34.9	2.73 t (9.0 Hz)
OCH_3	CH_3	55.7	3.79 s
Glucose			
1'	CH	102.1	4.37 d (7.9)
2'	CH	76.5	3.39 m
3'	CH	81.6	3.82 m
4'	CH	69.0	4.71 m
5'	CH	74.4	3.64-3.50 m
6'	CH_2	68.0	3.83 m
			3.56 m
Rhamnose			
1"	CH	101.2	5.08 br.s
2"	CH	73.2	3.91 d (2.9)
3"	CH	70.3	3.64 m
4"	CH	73.4	3.22 m
5"	CH	68.8	3.56 m
6"	CH_3	18.1	0.96 d (5.6)
Glucose (ter)			
1"'	CH	103.4	4.17 d (7.7)
2"'	CH	74.8	3.03 dd (7.8, 9.0)
3"'	CH	77.9	3.76-3.42 m
4"'	CH	69.9	3.76-3.42 m
5"'	CH	76.9	3.76-3.42 m
6"'	CH_2	60.9	3.76-3.42 m
Feruloyl			
1""	C	-	-
2""	CH	111.1	7.19 d (2.0)
3""	C	146.9	-
4""	C	149.9	-
5""	CH	116.3	6.80 d (8.2)
6""	CH	119.4	7.07 dd (8.2, 2.0)
α'	CH	112.3	6.36 d (15.5)
β'	CH	145.9	7.66 d (15.5)
C=O	C	166.2	-
OCH_3	CH_3	55.5	3.72 s



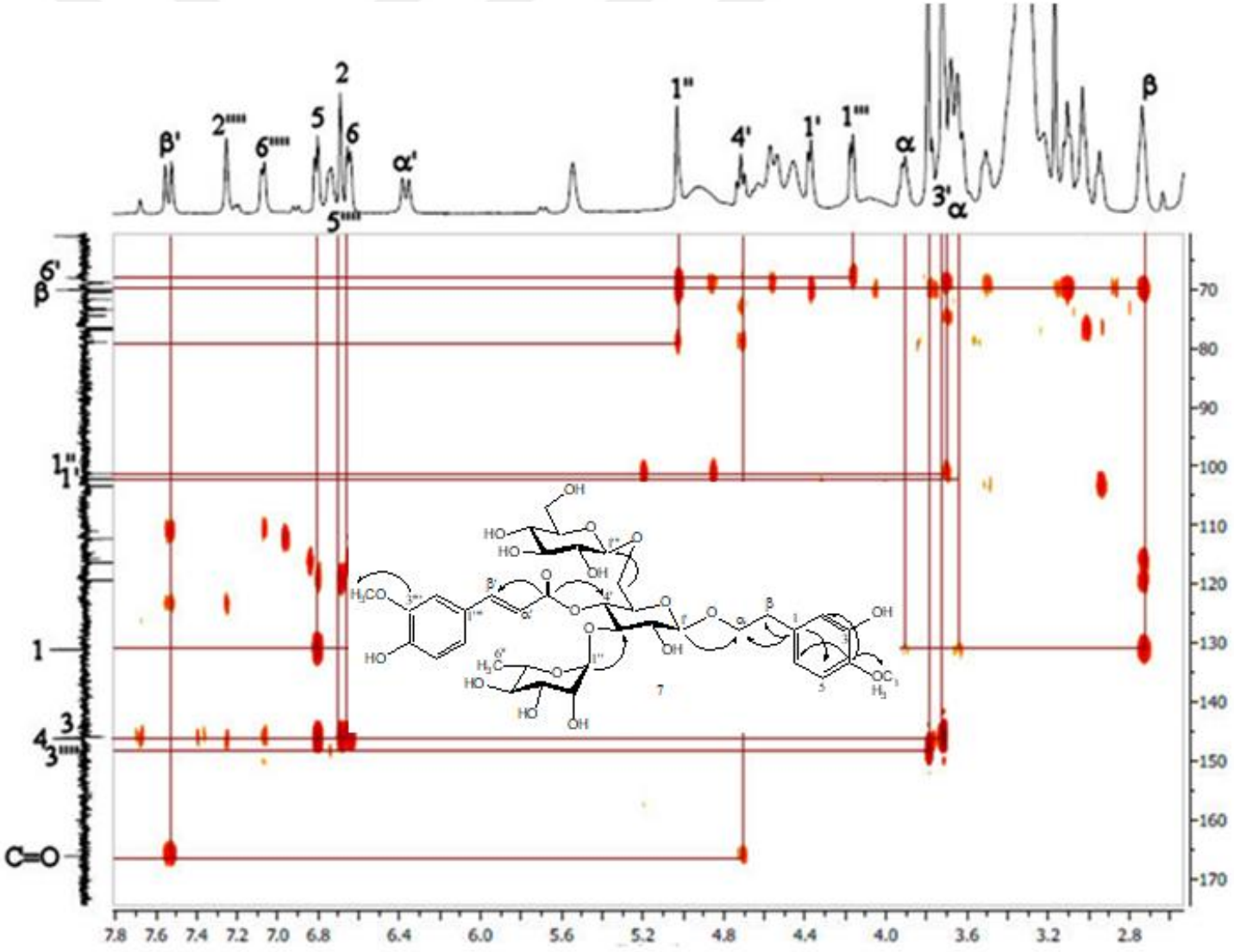
Spectrum 17. ^{1}H NMR Spectrum of Glucopyranosyl-(1→Gi-6)-martynoside (**7**) (500 MHz, DMSO-d_6)



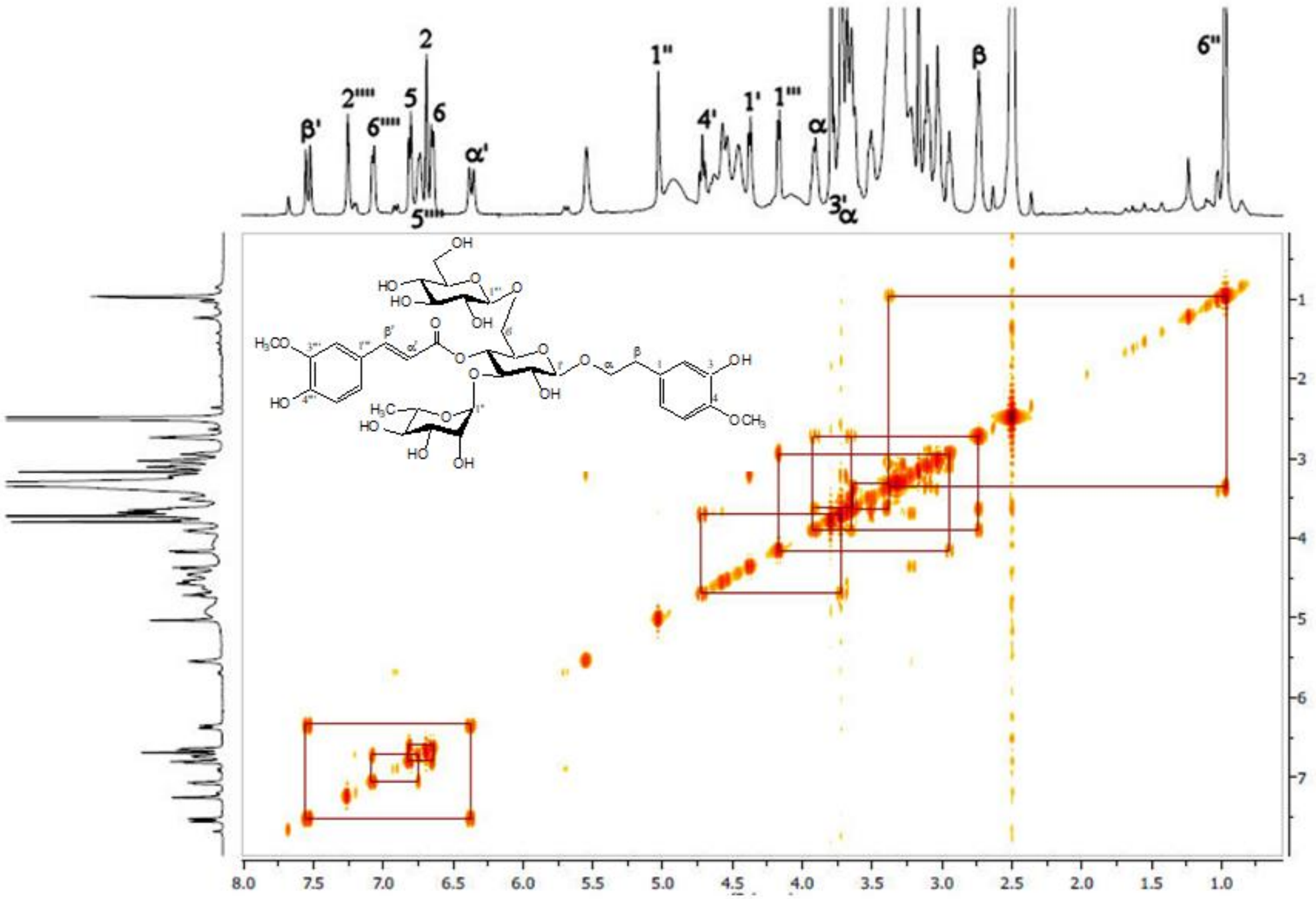
Spectrum 18. JMOD-^{13}C NMR spectrum of Glucopyranosyl-(1→Gi-6)-martynoside (**7**) (125 MHz, DMSO-d_6)



Spectrum 19. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (short range)(HSQC) of Glucopyranosyl-(1→Gi-6)-martynoside

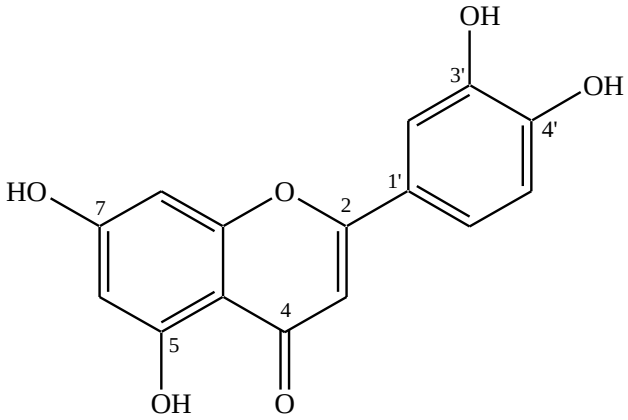


Spectrum 20. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (long range) (HMBC) of Glucopyranosyl-(1→Gi-6)-martynoside (**7**)



Spectrum 21. 2D-^{1}H, H-Homonuclear Correlation Spectrum (COSY) of Glucopyranosyl-(1→Gi-6)-martynoside (**7**)

4.4.3. Flavonoids



LUTEOLIN (8): $C_{15}H_{10}O_6$ (MW: 286.24)

UV λ_{max} (MeOH) nm	269, 333
IR ν_{max} (KBr) cm^{-1}:	3094 (OH), 1658 (γ-pyrone C=O), 1610 (aromatic ring), 1032 (C-O-C)
^{1}H NMR:	Table 55, Spectrum 22

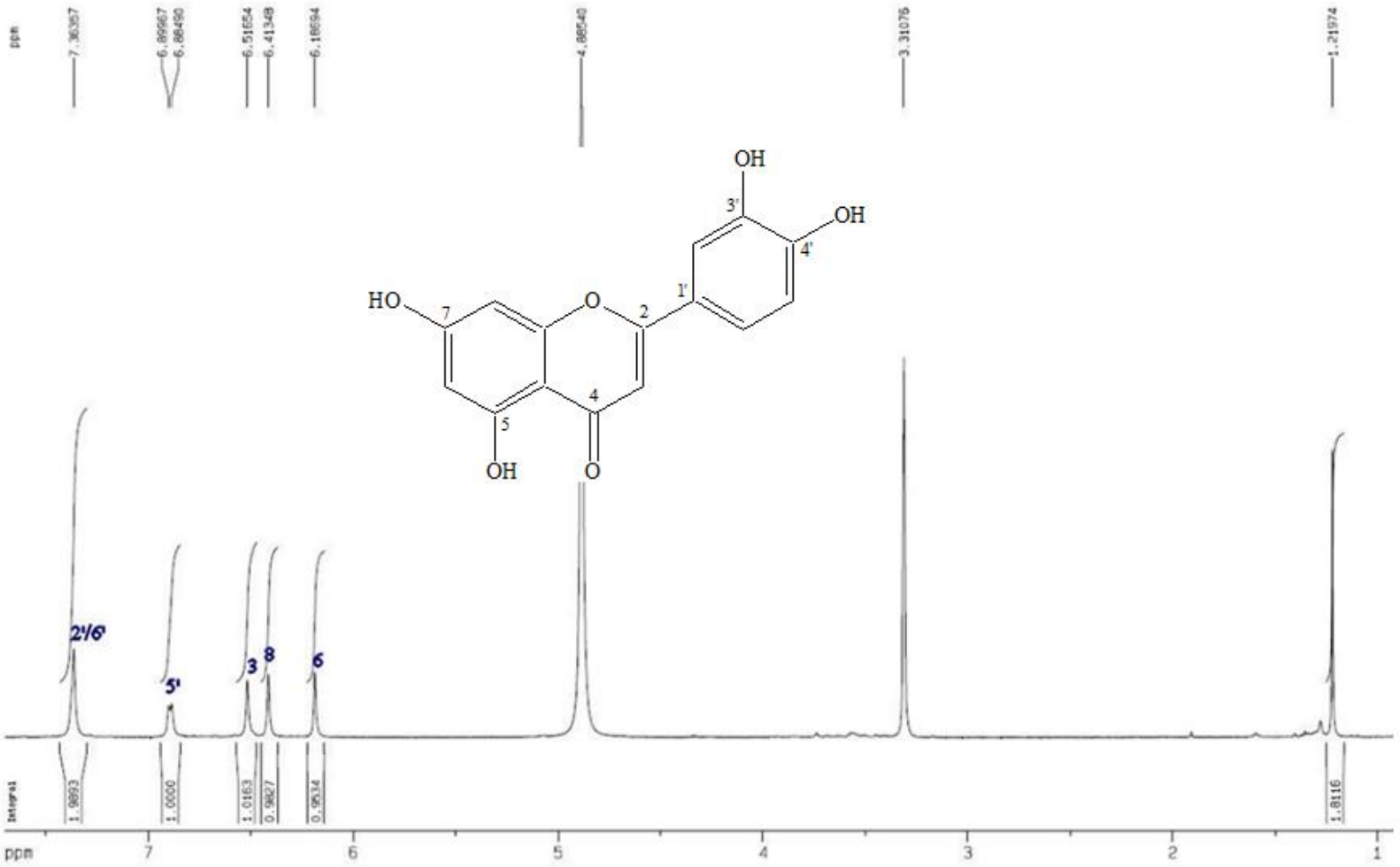
LUTEOLIN (8)

Compound **8** was isolated as a yellow amorphous powder from EtOAc extract. UV spectra showed characteristic bands (λ_{max} 333 nm) of flavonoid structure. In its IR spectrum, distinctive hydroxyl group (3094 cm^{-1}), γ-pyrone carbonyl (1658 cm^{-1}), signals of aromatic ring (1610 cm^{-1}) and C-O-C bands (1032 cm^{-1}) were observed.

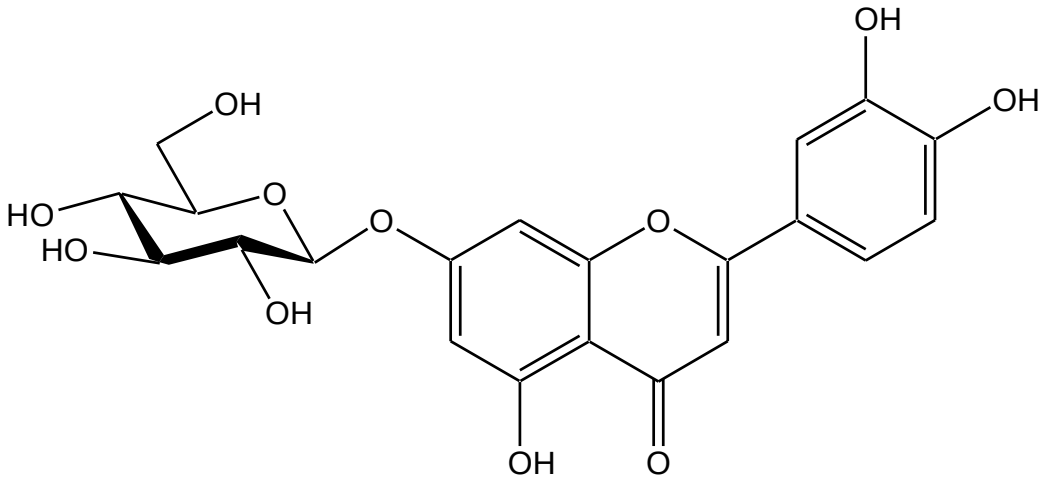
In ^{1}H NMR spectrum (Table 55, Spectrum 22) of **8**, three aromatic proton signals were observed as an *ABX*-type signal at 7.36 (2H) and 6.88 (d, J=8.0 Hz) a *meta*-coupled signal at δ_H 6.51 (d, J=1.8 Hz), 6.18 (d, J=1.8 Hz) as well as a singlet signal at δ_H 6.41. These findings were superimposable with those reported for 5,7-dihydroxyflavone namely **luteolin** in the literature (175,176)**.**

Table 55. ^{1}H NMR spectral data of luteolin (**8**) (500 MHz, CD_3OD)

Position	**Multiplicity**	**δ_H ppm, *J* (Hz)**
Aglycone		
2	C	-
3	CH	6.41 s
4	C	-
5	C	-
6	CH	6.18 d (1.8)
7	C	-
8	CH	6.51 d (1.8)
9	C	-
10	C	-
1'	C	-
2'	CH	7.36 d (2.0)
3'	C	-
4'	C	-
5'	CH	6.88 d (8.0)
6'	CH	7.36 dd (8.0, 2.0)



Spectrum 22. ^{1}H NMR Spectrum of Luteolin (**8**) (500 MHz, CD_3OD)



LUTEOLIN 7-*O*-*β*-D-GLUCOPYRANOSIDE (9): $C_{21}H_{20}O_{11}$ (MW: 448.38)

UV λ_{max} (MeOH) nm	260, 348
IR ν_{max} (KBr) cm^{-1}:	3154 (OH), 1659 (γ-pyrone C=O), 1606, 1608 (aromatic ring), 1089 (C-O-C)
^{1}H NMR:	Table 56, Spectrum 23
^{13}C NMR:	Tablo 56, Spectrum 24

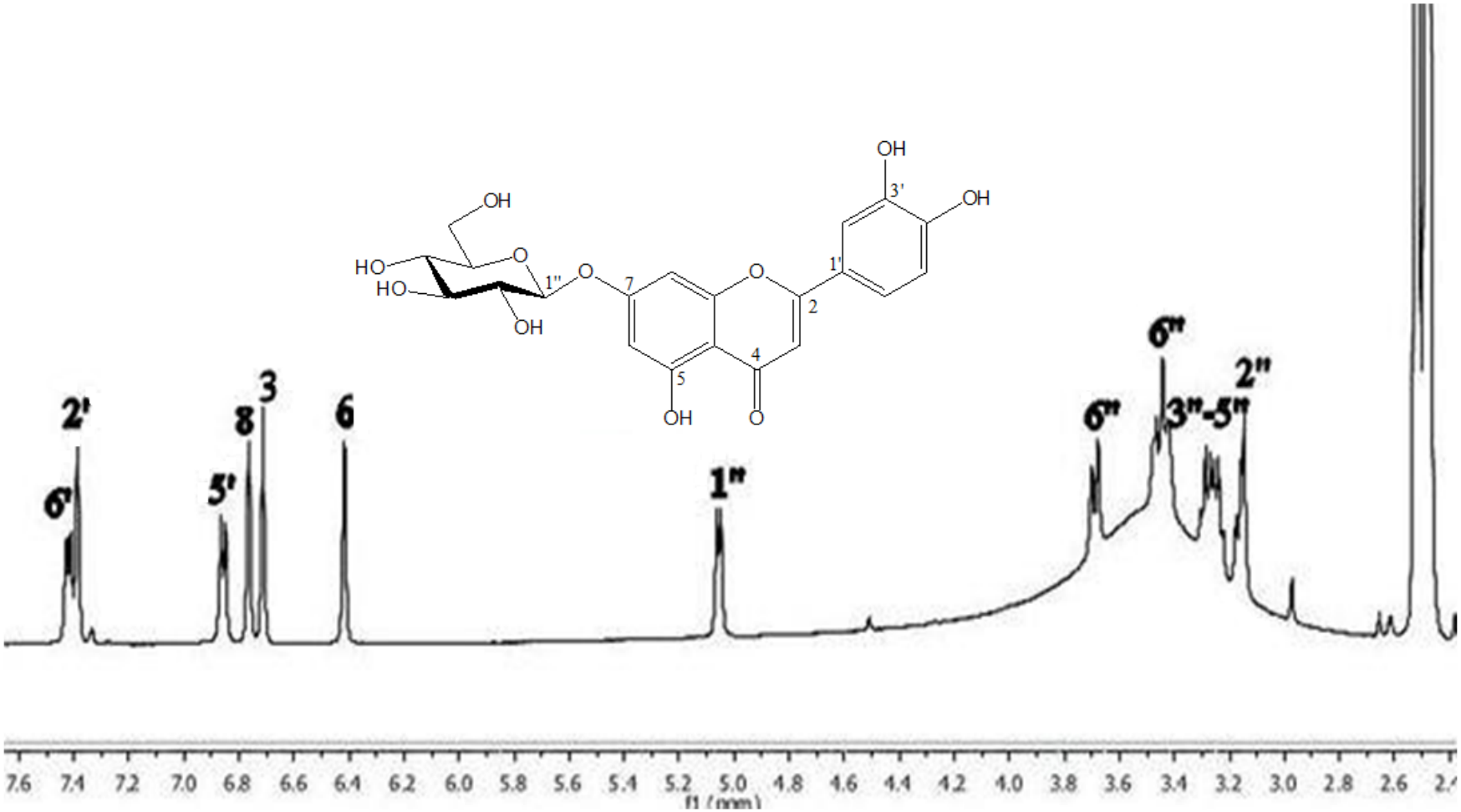
LUTEOLIN 7-*O*-*β*-D-GLUCOPYRANOSIDE (9)

Compound **9** was isolated as a yellow amorphous powder from *n*-BuOH extract. The molecular formula was established $C_{21}H_{20}O_{11}$. The absorption bands at 260, 348 were the characteristics of a flavonoid backbone. The IR spectrum displayed bands at 3154 (OH), 1659 (*γ*-pyrone C=O), 1606, 1608 (aromatic ring), 1089 (C-O-C).

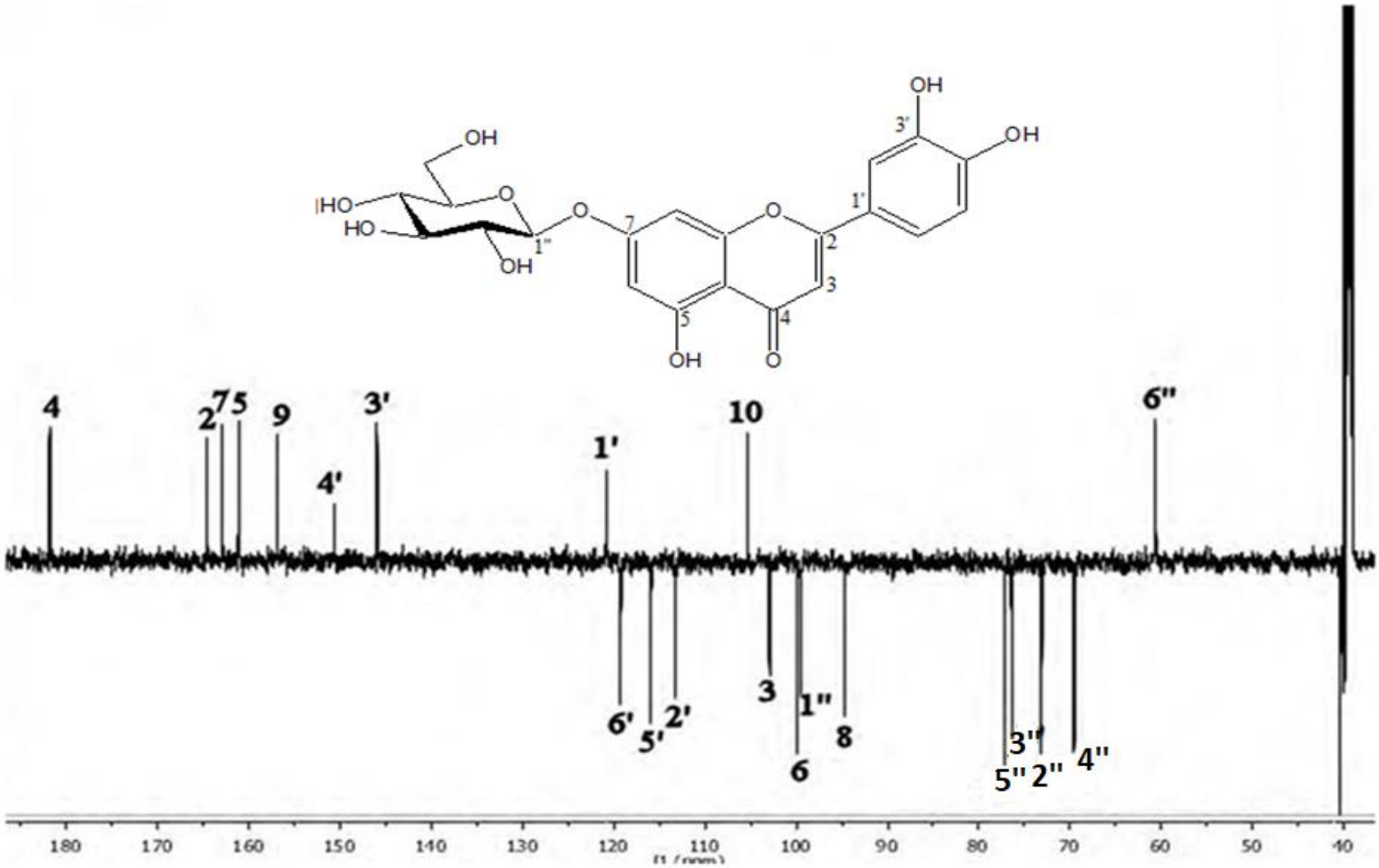
Inspection of the ^{1}H NMR spectrum (Table 56, Spectrum 23) revealed proton resonances of the 3,4-disubstituted benzene ring B of a flavonoid at δ_H 7.41 (dd, *J*= 8.0, 2.0 Hz), 7.39 (d, *J*= 2.0 Hz) and 6.89 (d, *J*= 8.0 Hz). Moreover, aromatic proton signals of two *m*-coupled doublets at δ_H 6.78 (H-8) and 6.48 (H-6) in the ^{1}H NMR spectrum were indicative of 5,7-disubstituted A ring. Additionally, a singlet at 6.71 ppm together with the above findings led to the presence of a flavone core of **9**. All of the findings indicated that aglycone of the **9** was luteolin. In addition to the signals of luteolin part, the appearance of *β*-D-glucopyranose signals; an anomeric signal at δ_H 5.06 (d, *J* =7.0, H-1″) along with the signals between 3.93-3.22 ppm, determined its monoglucosidic structure. The JMOD-^{13}C NMR spectrum displayed 21 signals. The anomeric carbon signal at δ_C 99.5 and the signals at 77.1, 76.4, 73.1, 69.5 and 60.6 ppm established the presence of a *β*-glucose in the structure. The remaining signals were typical for a luteolin aglycone. Based on the above findings, compound **9** was determined as 5,7,3',4'-tetrahydroflavone-7-*O*-*β*-D-glucopyranoside known as **luteolin 7-*O*-*β*-D-glucopyranoside** (177)**.**

Table 56. ^{13}C and ^{1}H NMR spectral data of luteolin 7-*O*-*β*-D-glucopyranoside (**9**) (DMSO-*d6*), ^{1}H: 500 MHz, ^{13}C: 125 MHz)

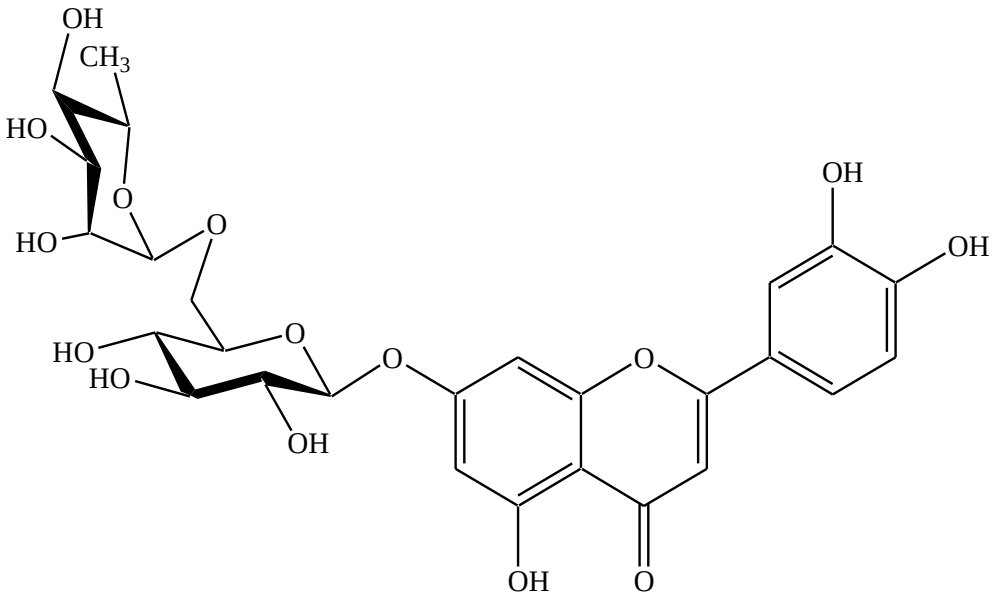
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**
Aglycone			
2	C	164.5	-
3	CH	102.9	6.71 s
4	C	181.8	-
5	C	161.1	-
6	CH	99.8	6.48 d (2.0)
7	C	162.9	-
8	CH	94.7	6.78 d (2.0)
9	C	156.9	-
10	C	105.3	-
1′	C	120.8	-
2′	CH	113.3	7.39 d (2.0)
3′	C	145.9	-
4′	C	150.6	-
5′	CH	115.9	6.89 d (8.0)
6′	CH	119.2	7.41 dd (8.0, 2.0)
Glucose			
1″	CH	99.5	5.06 d (7.0)
2″	CH	73.1	3.41 t (9.0)
3″	CH	76.4	3.30-3.22 m
4″	CH	70.0	3.30-3.22 m
5″	CH	77.1	3.30-3.22 m
6″	CH_2	60.6	3.93 br. d (12.0)
			3.72 dd (12.0, 6.0)



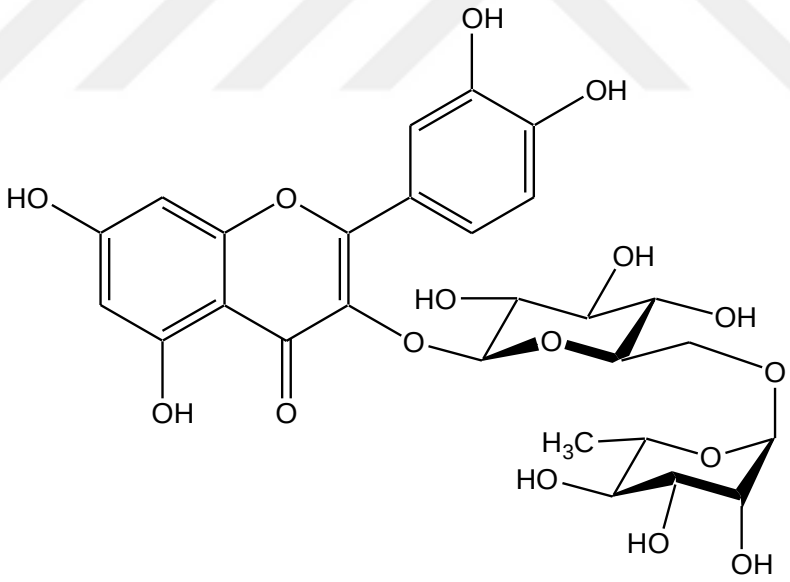
Spectrum 23. ^{1}H NMR Spectrum of Luteolin 7-*O*-*β*-D-glucopyranoside (**9**) (500 MHz, DMSO-d_6)



Spectrum 24. JMOD-^{13}C NMR Spectrum of Luteolin 7-*O*-β-D-glucopyranoside (**9**) (125 MHz, DMSO-d_6)



+



LUTEOLIN 7-*O*-RUTINOSIDE (10): $C_{27}H_{30}O_{15}$ (594.52)

and

QUERCETIN 3-*O*-RUTINOSIDE (12): $C_{27}H_{30}O_{16}$ (610.52)

LUTEOLIN 7-*O*-RUTINOSIDE (10): $C_{27}H_{30}O_{15}$ (594.52)

and

QUERCETIN 7-*O*-RUTINOSIDE (12): $C_{27}H_{30}O_{16}$ (610.52)

UV λ_{max} (MeOH) nm	208, 268, 353
IR ν_{max} (KBr) cm^{-1}:	3194 (OH), 1658, (γ-pyrone C=O), 1606 (aromatic ring), 1067 (C-O-C)
^{1}H NMR:	Table 57, 58, Spectrum 25
^{13}C NMR:	Tablo 57, 58, Spectrum 26
HSQC:	Spectrum 27
HMBC:	Spectrum 28
COSY:	Spectrum 29
HR-ESI-MS *m/z* :	595.1644 ($C_{27}H_{30}O_{15}H$) (**10**) 611.1691 ($C_{27}H_{30}O_{16}H$) (**12**)

LUTEOLIN 7-*O*-RUTINOSIDE (10) and QUERCETIN 3-*O*-RUTINOSIDE (12)

Compound **10** and **12** was obtained as a mixture from *n*-BuOH extract. Molecular formulas were found to be $C_{27}H_{30}O_{15}$ and $C_{27}H_{30}O_{16}$, on the basis of pseudomolecular ion peaks at *m/z* 595.1644 ($C_{27}H_{30}O_{15}H$) and 611.1691 ($C_{27}H_{30}O_{16}H$) respectively. The UV spectrum was typical for any flavonoid skeleton. IR spectrum displayed bands at 3194 (OH), 1658, (γ-pyrone C=O), 1606 (aromatic ring), 1067 (C-O-C) cm^{-1}.

The ^{1}H NMR spectrum (Table 57-58, Spectrum 25) contained 2 *ABX* type signals at δ_H 7.67 (2H, H-2'/6'), 7.40 (2H, H-2'/6') and 6.92 (d, H-5', *J*= 8.0), 6.87 (d, H-5', *J*=8.5) revealed the presence of 3,4-disubstituted B ring for both flavonoids. Aromatic proton signals of two pairs of meta-coupled protons at δ_H 6.74, 6.40, 6.51 and 6.21 in the ^{1}H NMR spectrum were indicative of a 5,7-disubstituted A ring for both molecules. A singlet at 6.60 ppm was ascribed to H-3 of one molecule while no additional singlet was observed in the spectrum indicated that one of them was flavone and the other was a flavonol derivative. Additionally, in ^{1}H NMR spectrum, four proton signals at δ_H 5.03 (d, H-1", *J*=6.0), 5.11 (d, H-1", *J*=7.4), 4.79 (s, H-1'"), 4.52 (s, H-1'") revealed that both compounds were diglycosidic flavonoids. The *J* values (7.0 and 7.4 Hz) of the anomeric proton signals at 5.03 (d, H-1"), 5.11 (d, H-1") ppm declared that one of the sugar moiety was β-D-glucopyranose and the other anomeric proton signals at 4.79 (br.s, H-1'"), 4.52 (br. s, H-1'") as well as the secondary methyl resonances at δ_H 1.19 (3H), δ_C 17.9 ve 1.12 (3H) δ_C 17.8 indicated the other sugar molecules were α-rhamnoses for both compounds. In the JMOD-^{13}C NMR spectrum, 54 resonances were observed, 31 CH and 2 CH_3 signals were at negative side while 19 C, 2 CH_2 were at positive side. Four anomeric carbon signals at δ_C 101.5, 104.7, 101.5 ve 102.4 and the other signals at 74.7, 75.7, 77.1, 77.2, 71.3, 71,4, 77.8, 78.1 and 72.2, 72.1, 72.4, 72.2, 74.0, 73.9, 69.8, 69.7 and 17.9, 17.8 showed that the disaccharide residue was rutinose for both compounds. The signals were unambiguously identified by using extensive 2D NMR experiemnts. The long-distance correlation between H-1'" and C-6" in HMBC showed that the rhamnose was linked to the C-6" of glucose. Other cross-peaks between H-1" and C-7 (for **10**), between H-1" and C-3 (for **12**), showed that attachment point of rutinose units as C-7(OH) and C-3(OH) for compounds **10** and **12** respectively. Thus, compounds **10** and **12** were identified as **luteolin 7-*O*-rutinoside** (173,178) and **quercetin 3-*O*-rutinoside**, respectively (173,179).

Table 57. ^{13}C and ^{1}H NMR spectral data of luteolin-7-*O*-rutinoside (**10**) (CD_3OD, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

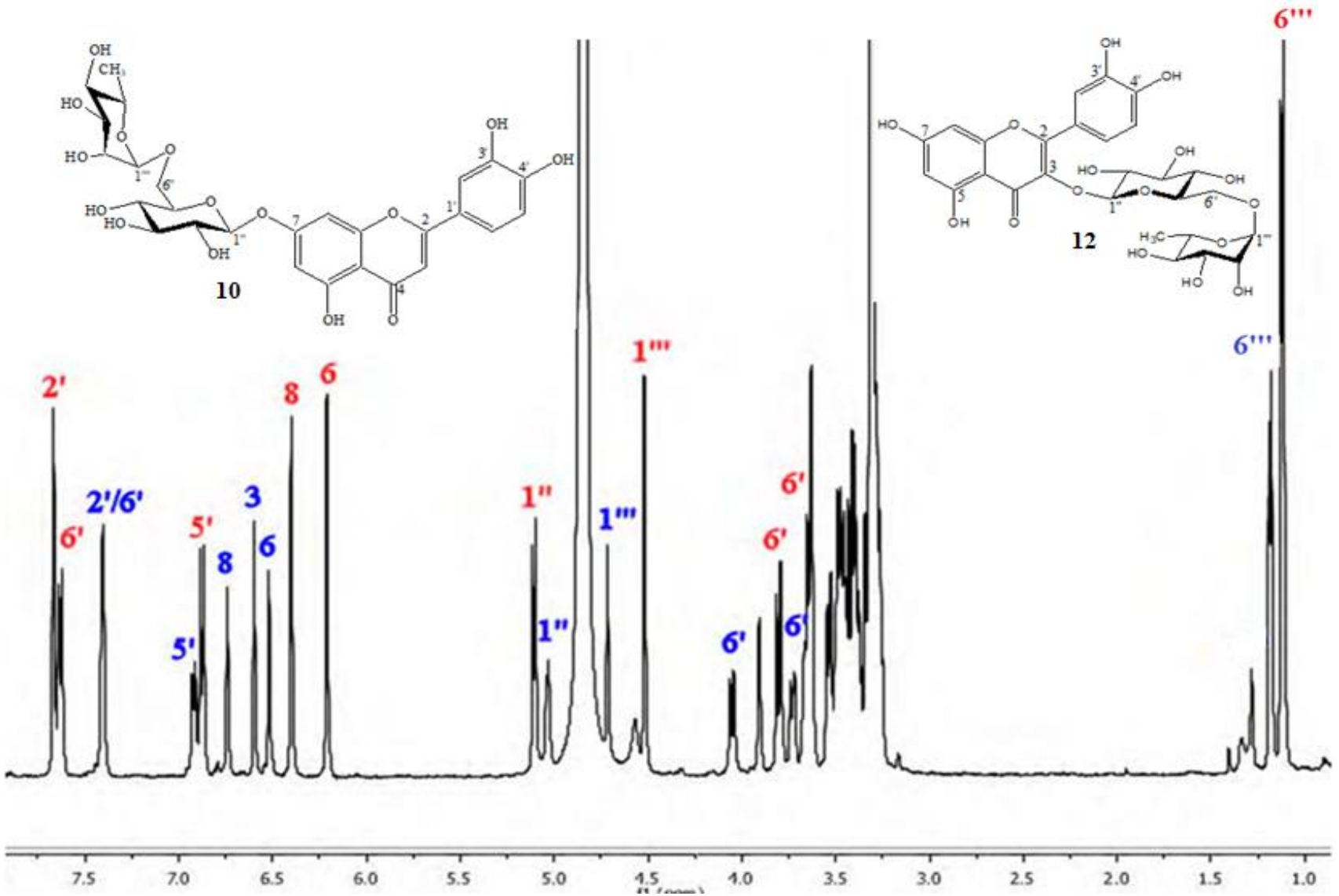
Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**
Aglycone			
2	C	164.8	-
3	CH	104.2	6.60 s
4	C	184.0	-
5	C	159.0	-
6	CH	101.1	6.51 d (2.0)
7	C	166.9	-
8	CH	96.1	6.74 d (2.0)
9	C	158.9	-
10	C	107.1	-
1'	C	123.2	-
2'	CH	114.3	7.40 †
3'	C	147.0	-
4'	C	151.1	-
5'	CH	116.8	6.92 d (8.0)
6'	CH	120.6	7.40 †
Glucose			
1"	CH	101.5	5.03 d (7.0)
2"	CH	74.7	3.45 m
3"	CH	77.1	3.65 s
4"	CH	71.3	3.51-3.29 m
5"	CH	77.8	3.64 m
6"	CH_2	67.5	4.04 br.d (11.9)
			3.73 dd (3.1, 11.9)
Rham			
1"'	CH	101.5	4.79 br.s
2"'	CH	72.2	3.90 s
3"'	CH	72.4	3.72 m
4"'	CH	74.0	3.90-3.20 m
5"'	CH	69.8	3.90-3.20 m
6"'	CH_3	17.9	1.19 d (6.0)

† = overlapped

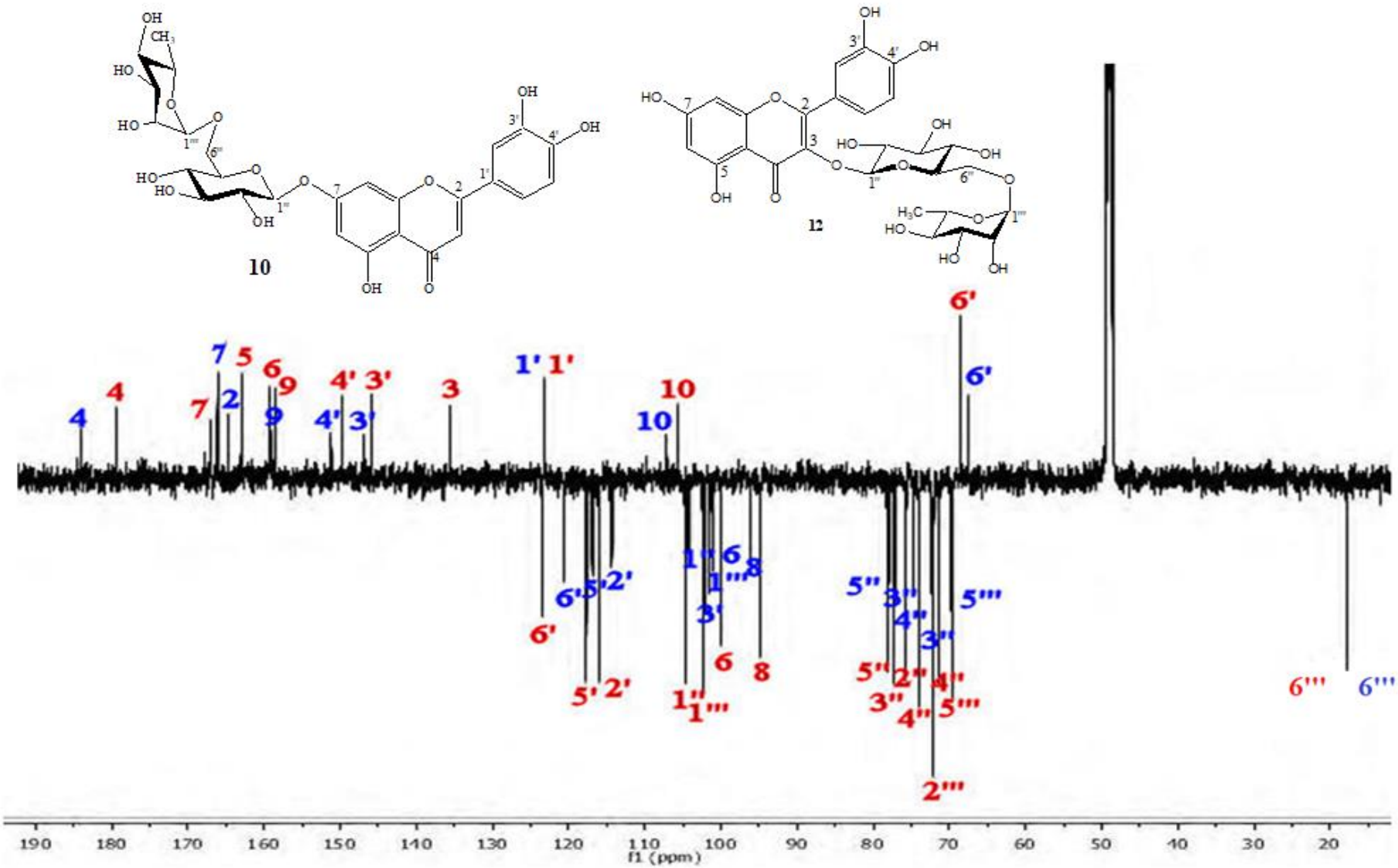
Table 58. ^{13}C and ^{1}H NMR spectral data of quercetin 3-*O*-rutinoside (Rutin) (**12**) (CD_3OD, ^{1}H: 500 MHz, ^{13}C: 125 MHz)

Position	**Multiplicity**	**δ_C ppm**	**δ_H ppm, *J* (Hz)**
Aglycone			
2	C	159.3	-
3	C	135.6	-
4	C	179.4	-
5	C	162.9	-
6	CH	99.9	6.21 d (2.0)
7	C	166.1	-
8	CH	94.8	6.40 d (2.0)
9	C	158.5	-
10	C	105.6	-
1'	C	123.1	-
2'	CH	116.0	7.67 d (2.0)
3'	C	145.8	-
4'	C	149.8	-
5'	CH	117.7	6.87 d (8.6)
6'	CH	123.5	7.65 dd (8.6, 2.0)
Glucose			
1"	CH	104.7	5.11 d (7.4)
2"	CH	75.7	3.45 †
3"	CH	77.2	3.65 †
4"	CH	71.4	3.44 †
5"	CH	78.1	3.51-3.29 †
6"	CH_2	68.5	3.80 d (10.9)
			3.67 d (10.9)
Rham			
1"'	CH	102.4	4.52 br.s
2"'	CH	72.1	3.62 †
3"'	CH	72.2	3.90-3.20 †
4"'	CH	73.9	3.90-3.20 †
5"'	CH	69.7	3.90-3.20 †
6"'	CH_3	17.8	1.12 d (6.0)

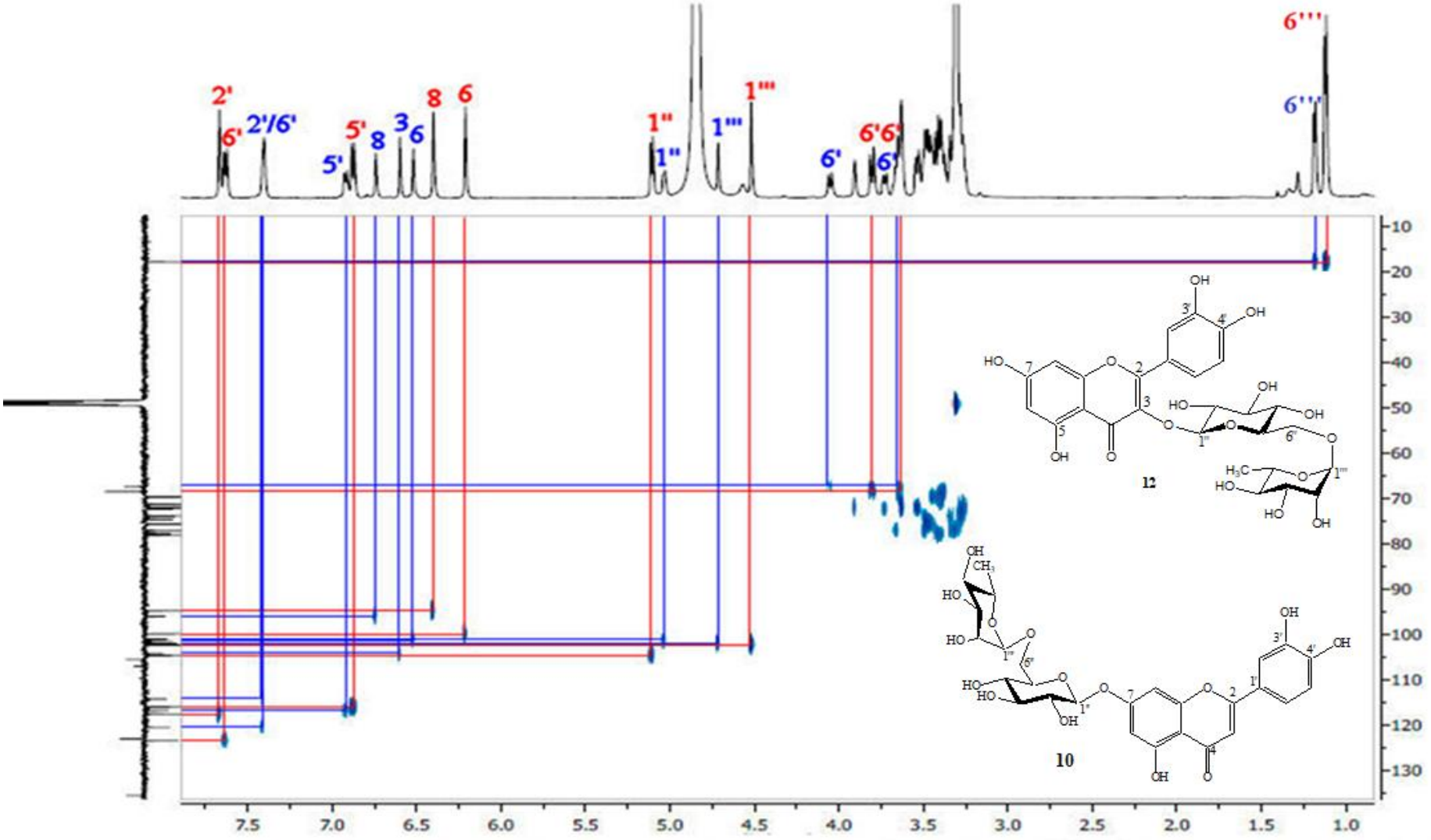
† = overlapped



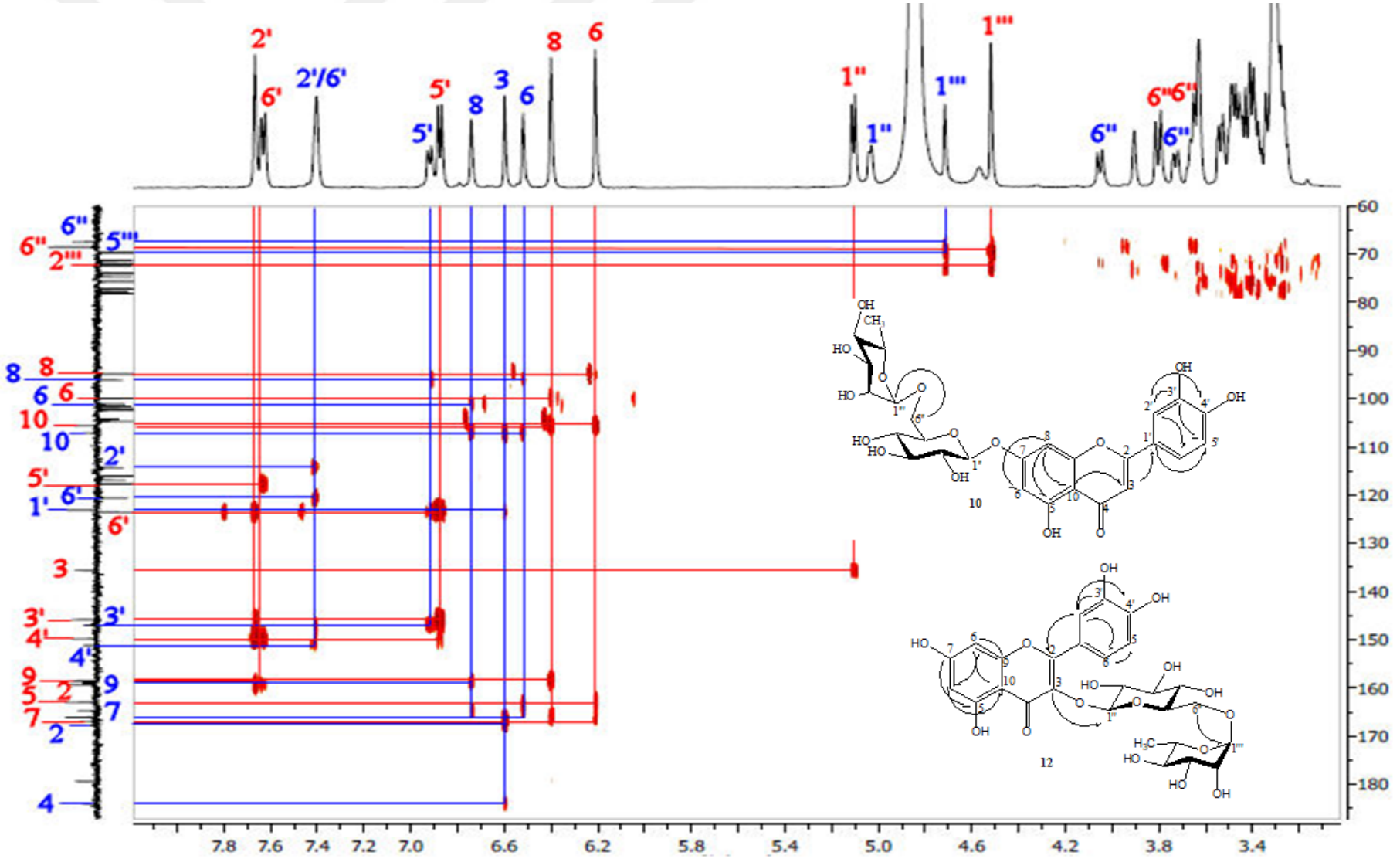
Spectrum 25. ^{1}H NMR Spectrum of **10** and **12** (500 MHz, CD_3OD) Red: 12, Blue: 10



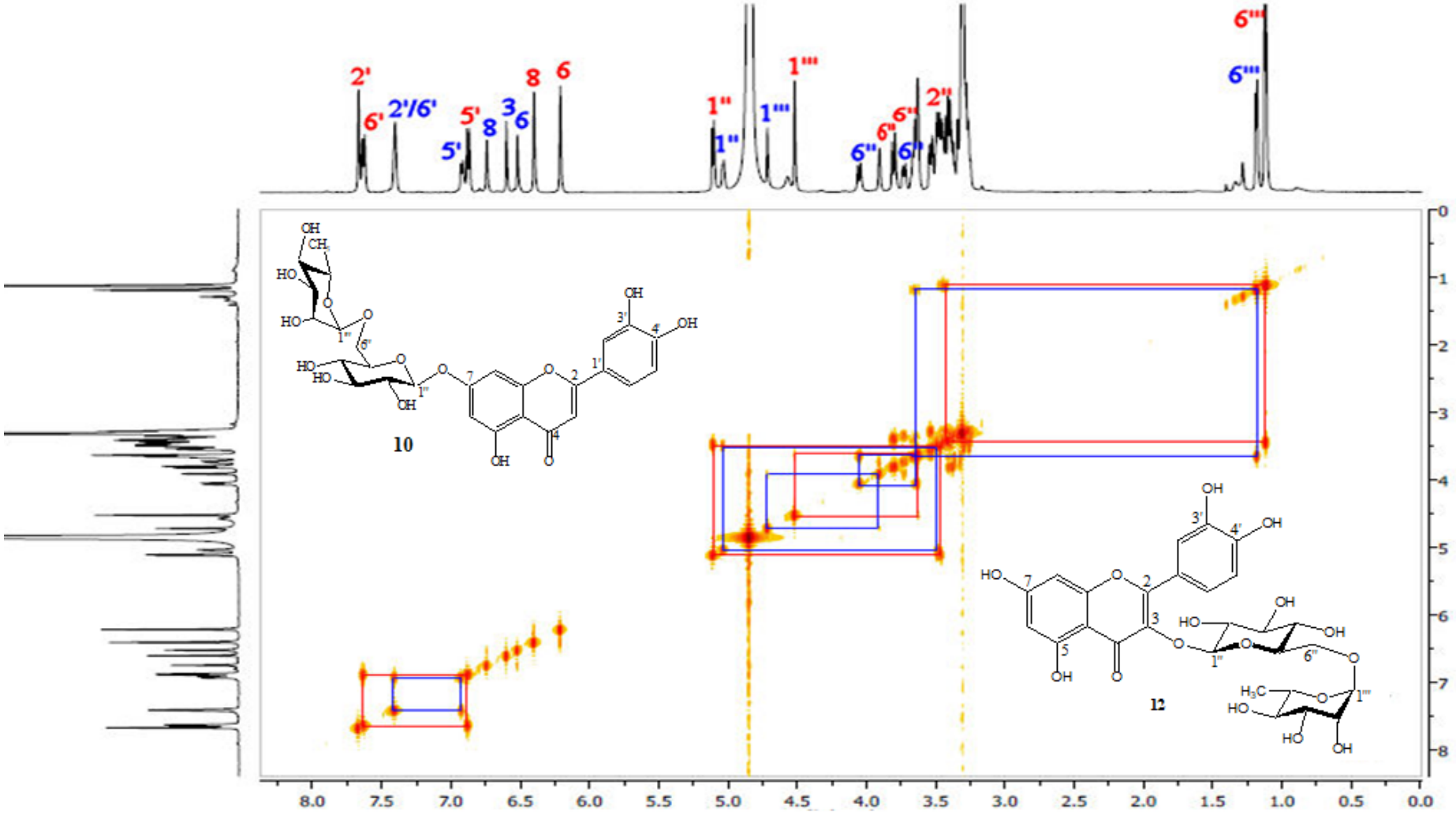
Spectrum 26. JMOD-^{13}C NMR spectrum of **10** and **12** (125 MHz, CD_3OD) Red: 12, Blue: 10



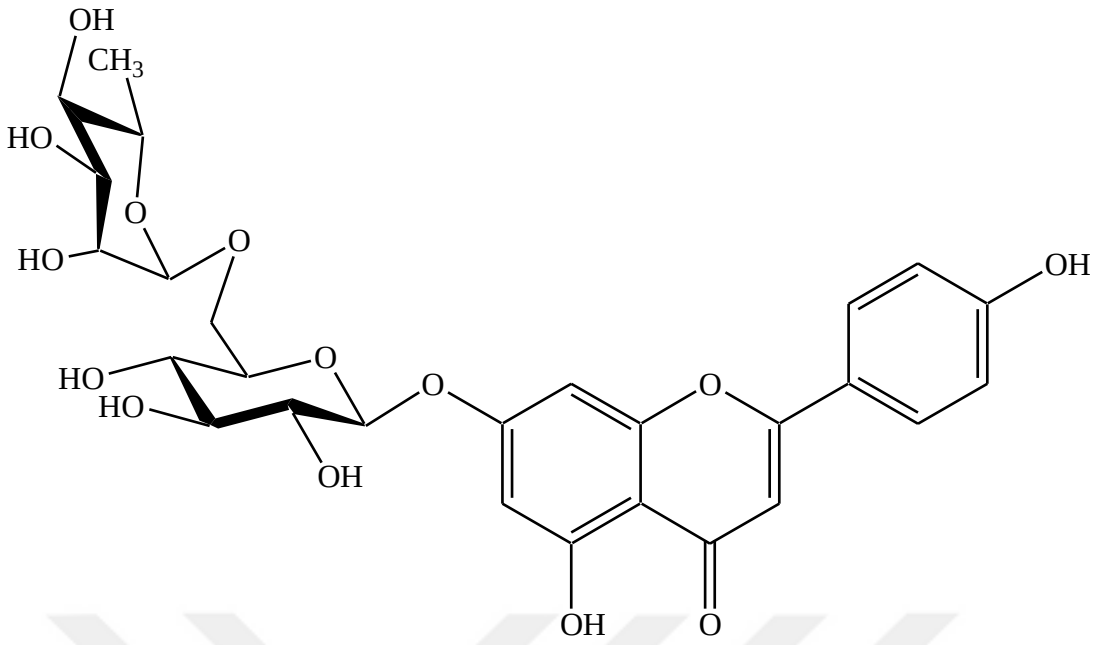
Spectrum 27. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (short range) (HSQC) of **10** and **12**. Red: 12, Blue: 10



Spectrum 28. Heteronuclear 2D-^{1}H, ^{13}C Correlation Spectrum (long range) (HMBC) of **10** and **12**. Red: 12, Blue: 10



Spectrum 29. 2D-^{1}H, Homonuclear Correlation Spectrum (COSY) of **10** and **12**. Red: 12, Blue: 10



APIGENIN 7-*O*-RUTINOSİDE (11): $C_{27}H_{30}O_{14}$ (MW: 578.52)

UV λ_{max} (MeOH) nm	208, 259, 328
IR ν_{max} (KBr) cm^{-1}:	3338 (OH), 1654 (γ-pyrone C=O), 1593 (aromatic ring), 1057 (C-O-C)
^{1}H NMR:	Table 59, Spectrum 30

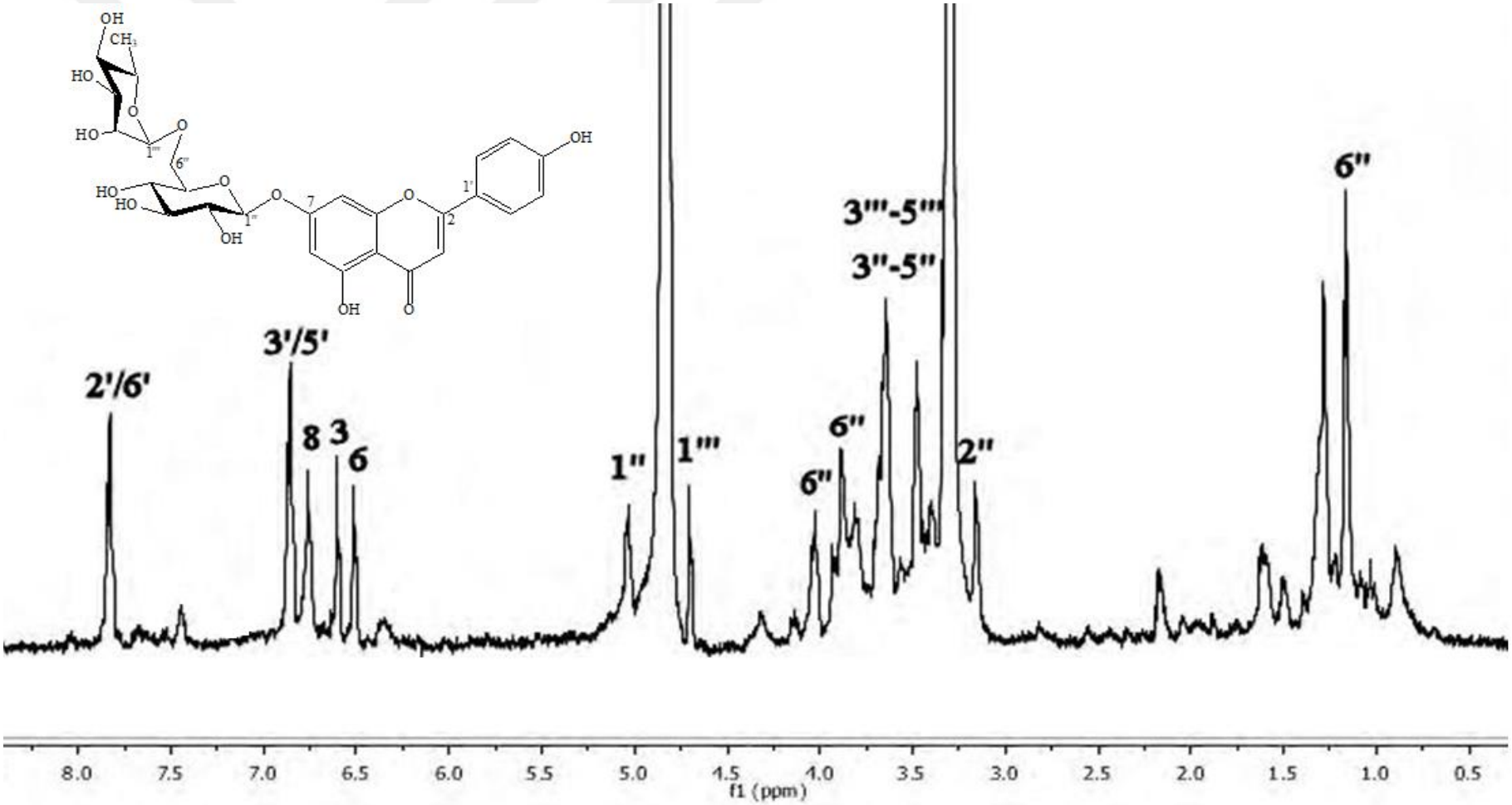
APIGENIN 7-*O*-RUTINOSİDE (11)

Compound **11** was isolated as a yellow amorphous powder from the *n*-BuOH extract. Compound **11** exhibited UV maxima at 208, 259 and 328 nm. The IR spectrum suggested the presence of hydroxyl (3154 cm^{-1}), γ-pyrone C=O (1659 cm^{-1}), aromatic ring (1593 cm^{-1}) and carbon-oxygen bonds (1057 cm^{-1}). All together they are the characteristic bands of a flavone skeleton.

^{1}H NMR spectrum (Table 59, Spectrum 30) clarified proton resonances of the *AABB* system of a *para*-substituted benzene ring B at δ_H 7.84 (2H, H-2'/6') and 6.87 (d, H-3'/5'). Moreover, *m*-coupled aromatic proton signals at δ_H 6.76 (H-8) ve 6.51 (H-6) in the ^{1}H NMR spectrum indicated to 5,7-disubstituted A ring. Additionally, a signal at 6.59 ppm was observed. Taken together, the aglycone was elucidated as **apigenin**. Two anomeric proton signals at δ_H 4.69 (d, *J*=8.2 Hz) and 5.04 br.s showed that the compound was diglycosidic. The anomeric signal at 5.04 ppm (H-1") along with the other signals in the region 3.16-4.02 ppm determined that one of the sugar moiety was β-D-glucopyranose, another anomeric proton signal at 5.04 ppm (H-1'") and secondary methyl resonance at 1.09 ppm (d, *J*=6.0 Hz) identified the other sugar molecule was α-rhamnose. These findings led to the identification of diglycosidic chain as rutinose which were also present in compounds **10** and **12**. Consequently, the structure of compound **11** was found to be 5,7,4'-trihydroxyflavone 7-*O*-rutinoside, which is known as **apigenin 7-*O*-rutinoside** (180)**.**

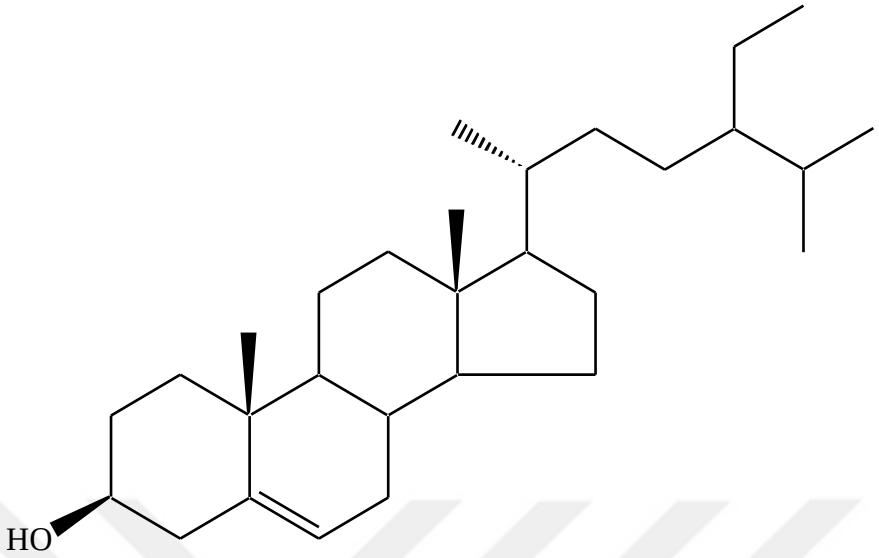
Table 59. ^{1}H NMR spectral data apigenin-7-*O*-rutinoside (**11**) (MeOD, ^{1}H: 500 MHz)

Position	**Multiplicity**	**δ_H ppm, *J* (Hz)**
Aglycone		
2	C	-
3	CH	6.59 s
4	C	-
5	C	-
6	CH	6.51 d (2.0)
7	C	-
8	CH	6.76 d (2.0)
9	C	-
10	C	-
1'	C	-
2'	CH	7.84 d (8.5)
3'	C	6.87 d (8.5)
4'	C	-
5'	CH	6.87 d (8.5)
6'	CH	7.84 d (8.5)
Glucose		
1"	CH	4.69 d (8.2)
2"	CH	3.16 m
3"	CH	3.64-3.40 m
4"	CH	3.64-3.40 m
5"	CH	3.64-3.40 m
6"	CH_2	4.02 m
		3.88 m
Rham		
1"	CH	5.04 br.s
2"'	CH	3.64-3.40 m
3"'	CH	3.64-3.40 m
4"'	CH	3.64-3.40 m
5"'	CH	3.64-3.40 m
6"'	CH_3	1.16 d (6.0)



Spectrum 30. ^{1}H NMR Spectrum of Apigenin 7-*O*-rutinoside (**11**) (500 MHz, CD_3OD)

4.4.4. Phytosterol



β- SITOSTEROL (13): $C_{29}H_{50}O$ (MW: 414.71)

IR ν_{max} (KBr) cm^{-1}:	3351 (OH), 2939 (aliphatic C-H), 1412 (C=C)
^{1}H NMR:	Table 60, Spectrum 31

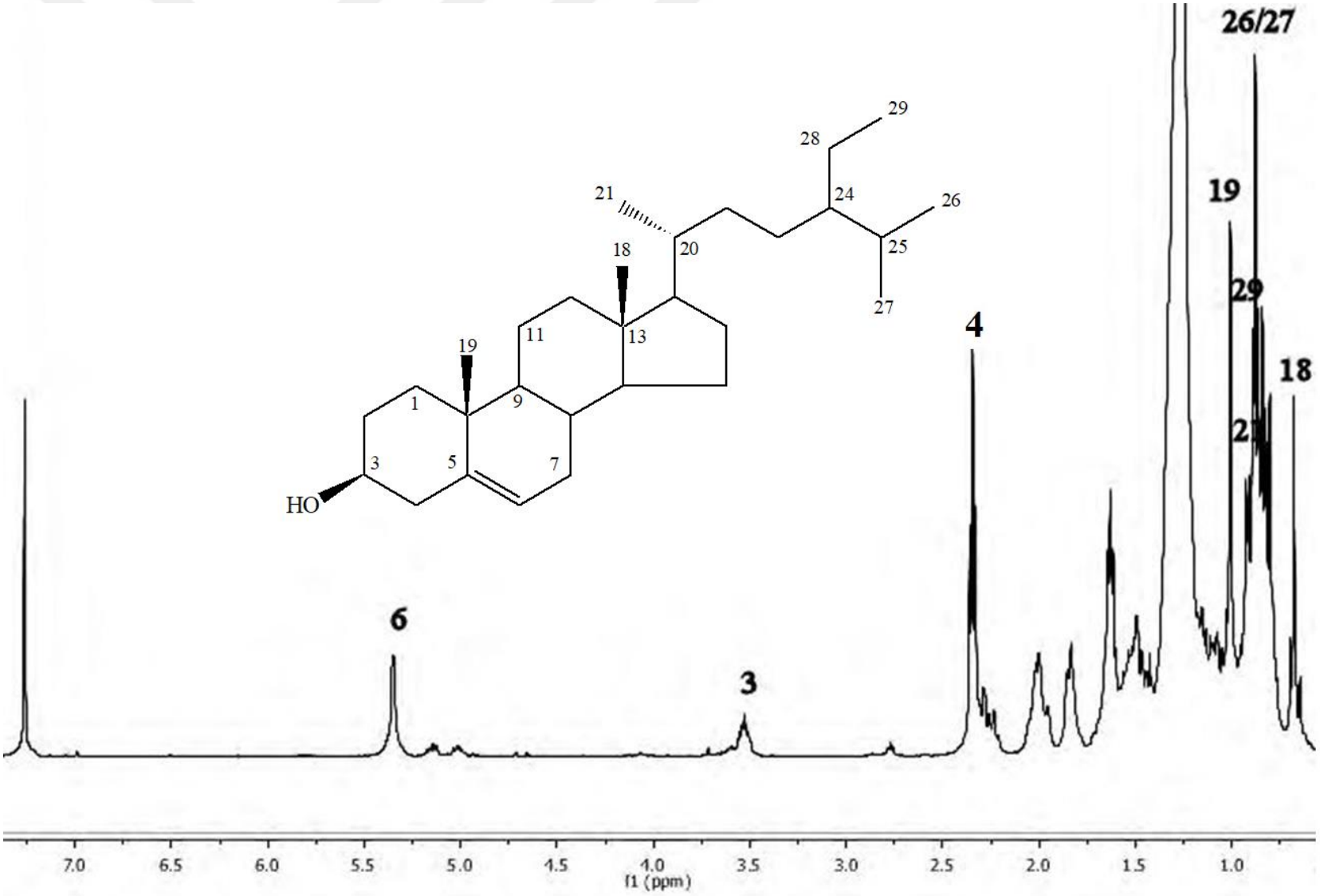
β- SITOSTEROL (13)

Compound **13** was isolated as a white powder from the chloroform extract. The absorption bounds for hydroxyl (3351 cm^{-1}), aliphatic CH (2939 cm^{-1}) and double bond (1412 cm^{-1}) were observed in the IR spectrum.

In ^{1}H NMR spectrum revealed one olefinic signal at δ_H 5.36 (br. s) and one oxymethine signal at δ_H 3.53 (m). Furthermore, in the upfield region of the spectrum six methyl signals were observed as two singlet methyls (δ_H 0.68, 1.01) three dublet methyls (δ_H 0.82 (6H) and 0.92) and one triplet methyl singlet (δ_H 0.89, J=6.9). These findings were superimposible with those for a common phytosterol, ***β*-sitosterol** (181). TLC analysis (stationary phase: SiO_2, mobile phase: *n*-hexane-EtOAc (3:1)) using *β*-sitosterol as a reference compound confirmed their assumption.

Table 60. ^{1}H NMR spectral data of *β*-sitosterol (**13**) (500 MHz, $CDCl_3$)

Position	**Multiplicity**	**δ_H ppm**
Aglycone		
1	CH_2	
2	CH_2	
3	CH	3.53 m
4	CH_2	
5	C	-
6	CH	5.36 s
7	CH_2	
8	CH	
9	CH	
10	C	-
11	CH_2	
12	CH_2	
13	C	-
14	CH	
15	CH_2	
16	CH_2	
17	CH	
18	CH_3	0.68 s
19	CH_3	1.00 s
20	CH	
21	CH_3	0.92 d (6.4)
22	CH_2	
23	CH_2	
24	CH	
25	CH	
26	CH_3	0.87 d (6.9)
27	CH_3	0.87 d (6.9)
28	CH_2	
29	CH_3	0.89 d (6.9)



Spectrum 31. ^{1}H NMR spectrum of *β*-Sitosterol (**13**) (500 MHz, $CDCl_3$)

4.5. *In vitro* Bioactivity Studies on the Isolated Compounds

Among the isolated compounds **1-9**, **11** and **13** were evaluated for their *in vitro* antioxidant, anti-inflammatory and antimicrobial activities. As compounds **10** and **12** were obtained as mixtures, their activities were not studied. Verbascoside (**6**) and luteolin (**8**) showed the highest antioxidant activity in all tests. Luteolin (**8**) also displayed the strongest anti-inflammatory activity compared to standards and other samples with 54.1±5.0 % inhibition at 1 µg/mL. All the tested compounds exerted weak antimicrobial activity against tested microorganisms compared to standard antibiotics.

4.5.1. Antioxidant Activity Results of the tested Compounds

4.5.1.1. DPPH Results of Isolated Compounds

Compound **1-9**, **11**, **13** were tested for their *in vitro* DPPH scavenging activities. Results of DPPH activities were given in Table 61. Luteolin (**8**) showed the highest DPPH radical scavenging activity as good as ascorbic acid, whereas the specioside (**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**), apigenin 7-*O*-rutinoside (**11**) and *β*-sitosterol (**13**) did not show any activity. Verbascoside (**6**) and luteolin 7-glucoside (**9**) showed the same activity which was less than luteolin. Catalpol (**1**) and glucopyranosyl (1→6)-martinoside (**7**) showed the least activity. Iridoids and *β*-sitosterol (**13**) did not show the DPPH activity.

Table 61. DPPH activity results of the tested compounds (1-9, 11,13)

Compound No	Compound Name	IC_{50} (µg/mL)[a]
1	Catalpol	490± 0.05
2	Specioside	NA
3	Ajugol	NA
4	Ajugoside	NA
5	8-*O*-acetylharpagide	NA
6	Verbascoside	10±0.01
7	Glucopyranosyl (1→6)-martinoside	50±0.00
8	Luteolin	5±0.00
9	Luteolin 7-glucoside	10±0.01
11	Apigenin 7-*O*-rutinoside	NA

13	*β*-sitosterol	NA
	Ascorbic acid	5±0.00

[a]Data expressed as mean ± SD (*n* = 4). NA: Not active

4.5.1.2. ABTS results of isolated compounds

Compound **1-9**, **11**, **13** were tested for their *in vitro* ABTS activities. Results of ABTS activities were given in Table 62. Verbascoside (**6**) and luteolin (**8**) elicited the highest ABTS activity. Verbascoside (**6**), luteolin (**8**), glucopyranosyl (1→6)-martinoside (**7**) showed higher activity than ascorbic acid. Specioside (**2**) showed the same activity with ascorbic acid. Apigenin 7-*O*-rutinoside (**11**) showed the least ABTS activity. Catalpol (**1**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**), and *β*-sitosterol (**13**) were found to be inactive.

Table 62. ABTS activity results of the tested compounds (1-9, 11,13)

Compound No	**Compounds Name**	**IC_{50} (μg/mL)[a]**
1	Catalpol	NA
2	Specioside	30±0.00
3	Ajugol	NA
4	Ajugoside	NA
5	8-*O*-acetylharpagide	NA
6	Verbascoside	10±0.00
7	Glucopyranosyl (1→G6)-martinoside	20±0.00
8	Luteolin	10±0.00
9	Luteolin 7-glucoside	40±0.00
11	Apigenin 7-*O*-rutinoside	90±0.01
13	*β*-sitosterol	NA
	Ascorbic acid	30±0.00

[a]Data expressed as mean ± SD (*n* = 4). NA: Not active

4.5.1.3. CUPRAC results of the isolated compounds

Compound **1-9**, **11**, **13** were tested for their *in vitro* CUPRAC activities. Results of CUPRAC activities were given in Table 63. Verbascoside (**6**) showed the highest CUPRAC

activity as good as gallic acid, whereas specioside (**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**), apigenin 7-*O*-rutinoside (**11**) and *β*-sitosterol (**13**) did not show the activity. The second highest activity was showed by luteolin (**8**), it was the same with ascorbic acid. Catalpol (**1**) showed the least CUPRAC activity. Luteolin 7-glucopyranoside (**9**) and glucopyranosyl (1→6)-martinoside (**7**) showed less activity than others. Except catalpol (**1**), iridoids and *β*-sitosterol (**13**) did not elicit CUPRAC activity.

Table 63. CUPRAC activity results of the tested compounds (1-9, 11,13)

Compound No	**Compounds Name**	**IC_{50} (μg/mL)[a]**
1	Catalpol	290±0.01
2	Specioside	NA
3	Ajugol	NA
4	Ajugoside	NA
5	8-*O*-acetylharpagide	NA
6	Verbascoside	10±0.00
7	Glucopyranosyl (1→G6)-martinoside	70±0.00
8	Luteolin	20±0.00
9	Luteolin 7-glucoside	50±0.01
11	Apigenin 7-*O*-rutinoside	NA
13	*β*-sitosterol	NA
	Ascorbic acid	20±0.00
	Gallic acid	4±0.01

[a]Data expressed as mean ± SD (n = 4). NA: Not active

4.5.2. Anti-inflammatory Activity results- 5-LOX inhibition Results of Isolated Compounds

Compound **1-9**, **11**, **13** were also evaluated for their anti-inflammatory activities through 5-LOX inhibition assay (Table 64). Luteolin (**8**) showed the highest anti-inflammatory activity with 54.1±5.0% inhibition at 1 μg/mL. Catalpol (**1**), ajugol (**3**), ajugoside (**4**), verbascoside (**6**), apigenin 7-*O*-rutinoside (**11**) were inactive. Specioside (**2**), 8-*O*-acetylharpagide (**5**), glucopyranosyl (1→6)-martinoside (**7**), luteolin 7-glucoside (**9**), and *β*-sitosterol (**13**) showed weak anti-inflammatory activity.

Table 64. Anti-inflammatory activity: Lipoxigenase inhibition results of the tested compounds (**1-9**, **11**,**13**)

Compound No (concentration)	**Compounds Name**	**%inhibition±std**
1 (1.6 µg/mL)	Catalpol	NA
2 (1.6 µg/mL**)**	Specioside	3.9±1.0
3 (1.6 µg/mL)	Ajugol	NA
4 (1 µg/mL)	Ajugoside	NA
5 (1.6 µg/mL)	8-*O*-asetylharpagide	6.5±0.8
6 (1.5 µg/mL)	Verbascoside	NA
7 (1.6 µg/mL)	Glucopyranosyl (1→G_6)-martinoside	5.0±1.2
8 (1 µg/mL)	Luteolin	54.1±5.0
9 (1.6 µg/mL)	Luteolin 7-glucoside	4.8±1.5
11 (1 µg/mL)	Apigenin 7-*O*-rutinoside	NA
13 (1.6 µg/mL)	*β*-sitosterol	6.1±2.4
	NDGA IC_{50}	2.95±0.21 µg/mL

[a]Data expressed as mean ± SD (*n* = 4). NA: Not active

4.5.3. Antimicrobial activity results of the tested compounds

Compound **1-9**, **11** and **13** were tested for their *in vitro* antimicrobial activities. Results of antimicrobial activities were given in Table 65. For antibacterial tests, results were commented against three bacteria strains. Glucopyranosyl (1→6)-martinoside (**7**) showed the highest antimicrobial activity which is followed by apigenin 7-*O*-rutinoside (**11**). Verbascoside (**6**) and luteolin 7-glucoside (**9**) did not show any activity against *Bacillus cereus NRRLB 371*. Only specioside (**2**) and ajugoside (**4**) displayed weak activity against *Pseudomonas aeruginosa* ATCC 10145 while the rest was inactive. Glucopyranosyl (1→6) martinoside (**7**) showed the highest activity against *Staphylococcus aureus ATCC 6538*. The tested compounds showed weak antimicrobial activity when compared to chloramphenicol. Verbascoside (**6**) and luteolin 7-glucoside (**9**) did not show activity against *Staphylococcus aureus ATCC 6538*. Specioside (**2**) showed the least activity against *Staphylococcus aureus ATCC 6538*.

For antifungal activity, against three fungi, results were commented. All the tested compounds were inactive against *Candida albicans ATCC 90028*. Apigenin 7-*O*-rutinoside (**11**) showed the highest antifungal activity against *Candida parapsilosis ATCC 22019* while the other tested compounds did not display promising activity. Luteolin 7-glucoside (**9**) showed the second highest antifungal activity against *Candida parapsilosis ATCC 22019*. Ajugoside (**4**), 8-*O*-acetylharpagide (**5**), glucopyranosyl (1→6)-martinoside (**7**) showed the less and the same activity, catalpol (**1**) showed the least activity against *Candida parapsilosis ATCC 22019*. Specioside (**2**), ajugol (**3**), verbascoside (**6**), luteolin (**8**), and *β*-sitosterol (**13**) did not show anfifungal activity against *Candida parapsilosis ATCC 22019*. Only compounds **3** and **6** displayed weak antifungal activity with MIC values of 310 and 620 µg/mL respectively against *Candida krusei* while the rest were inactive.

Table 65. Antimicrobial activities of the tested compounds (**1-9**, **11**,**13**) and standards (MIC: µg/mL)

Number	Compound Name	*B. cereus NRRLB 3711*	*P. aeruginosa ATCC 10145*	*S. aureus ATCC 6538*	*C. albicans ATCC 90028*	*C. parapsilosis ATCC 22019*	*C. krusei ATCC 6258*
1	Catalpol	16	NA	8	NA	310	NA
2	Specioside	31	620	31	NA	NA	NA
3	Ajugol	62	NA	16	NA	NA	310
4	Ajugoside	16	620	8	NA	620	NA
5	8-*O*-acetylharpagide	31	NA	31	NA	620	NA
6	Verbascoside	NA	NA	NA	NA	NA	620
7	Glucopyranosyl (1→G_6)-martinoside	2	NA	2	NA	620	NA
8	Luteolin	16	NA	8	NA	NA	NA
9	Luteolin 7-glucoside	NA	NA	NA	NA	80	20
11	Apigenin 7-*O*-rutinoside	8	NA	4	NA	20	NA
13	sitosterol	16	NA	16	NA	NA	NA
	Amphotericin-B	-	-	-	0.031	0.062	0.125
	Chloramphenicol	0.002	0.062	0.001	-	-	-

NA: Not active (>620 µg/mL)

5. DISCUSSION and CONCLUSIONS

Scrophulariaceae family is one of the largest plant families with 200 genera and 2500 species (7). The genus *Verbascum* (mullein), a member of family Scrophulariaceae, comprises about 360 species of flowering plants, distributed mainly in Europe, North America, West and Central Asia (1). The genus *Verbascum* (mullein) is represented about 255 species and 130 hybrids, among which around 200 species are endemic to Turkey (8). Turkey seems to be a major center for the genus *Verbascum* (3). *Verbascum* species are generally called as 'Sığırkuyruğu' in Turkey (57).

The usage of flowers and leaves of mullein are reported as analgesic, anti-inflammatory, antiseptic, spasmolytic, astringent, diuretic, emollient, expectorant and vulnerary in different traditional medicines (2–5). Moreover, fresh leaves of mullein are used in homoepathic formulations for the management of headaches originated from ear problems. Leaves of mullein are also used in ointment formulations for burns and earache. They are also reported as wound healing agents and are also applied to ulcers, tumours and piles (13). The flowers of mullein are used against bacteria-oriented ear problems. Herbal tea of mullein is indicated for chest and abdominal complaints such as productive cough and diarrhea (13). It is also used for the management of rheumatic problems.

Decoction of the seeds is applied to skin for dermatological problems. Powder of dried roots of plant and juice of plant is mentioned to the treatment of warts. Traditionally, a decoction of the roots is used to alleviate toothache, cramps and convulsions (13).

When the researches on *Verbascum* species (14,182) which are frequently used for the treatment among the population in Anatolia, are examined, it has been determined that there are many effects such as anti-inflammatory, antioxidant, antinociceptive, antimicrobial, antimalarial, antitumor, anticancer and cytotoxic effect (57) because of the biologically active compounds, such as flavonoids, phenylethanoid and neolignan glycosides, saponins and iridoid glycosides (57).

Tatlı et al. studied *V. cilicicum* (55), *V. pterocalycinum* var. *mutense* (94), *V. lasianthum* (56,95), *V. pycnostachyum* Boiss. & Helder (87), *V. salviifolium* Boiss (102), *V. dudleyanum* (62), *V. mucronatum* (21) growing in Turkey for their secondary metabolites.

Verbascum plants are known to possess a wide spectrum of biological activities and therefore have been used for centuries in the folk medicines. However, the knowledge of the

metabolites, accumulated in different mullein species is still limited and based mainly on determination of the major compounds (15).

In this study, it is aimed to investigate the phytochemical and bioactivities of *Verbascum bugulifolium* which has never been investigated in terms of its chemical composition and bioactivities. The aerial parts of *Verbascum bugulifolium* L. were collected, dried, powdered and extracted with MeOH to yield crude MeOH extract. The crude MeOH extract as well as its *n*-hexane, $CHCI_3$, EtOAc, *n*-BuOH and H_2O subextracts were screened initially for their *in vitro* antioxidant (DPPH•, ABTS• and CUPRAC), anti-inflammatory (5-LOX inhibition) (Table 39-42) and antimicrobial activities (Table 43). EtOAc and *n*-BuOH extracts exerted the best antioxidant activity in DPPH•, ABTS• and CUPRAC assays (IC_{50} = 20-90 µg/mL), while *n*-BuOH subextract was the most active in LOX inhibition assay (183).

Antioxidants protect the human body from the harmful effects of free radicals and the other reactive oxygene species. Cardiovascular disease, neural disorders, Alzheimer's disease, Parkinson's disease, alcohol induced liver disease, mild cognitive impairment, ulcerative colitis, aging and atherosclerosis are associated with free radicals. Antioxidants are effective in prevention from these degenerative diseases and improving the quality of life. There are different *in vivo* and *in vitro* methods to evaluate antioxidant activities of herbal extracts. *In vitro* antioxidant tests are comparatively comprehensible to qualify. Among the antioxidant tests, DPPH is quick, easy and cheap and it is widely used in the research laboratories. Also, DPPH is not comprised multiple steps and reagents. Another *in vitro* antioxidant method is ABTS, which is applicable to both hydrophilic and lipophilic substances (184). CUPRAC is fast, selective, more stable and accessible than other chromogenic reagents (DDPH, ABTS), easily and diversely applicable, insensitive to pH, air, light and humidity, the calibration curve is more linear than other methods over a wide concentration range (156).

Inflammation is a host defending mechanism with heat, redness, swelling, and pain. Septic shock, rheumatoid arthritis, gastritis, and atherosclerosis are among the inflammatory diseases. In the event of inflammation, arachidonic acid (AA) is excessively generated. Cyclooxygenase (COX) and lipooxygenase (LOX) pathways transform the AA to prostaglandins (PG) and leukotrienes (LT) which are strong inflammatory materials. LTs are produced by the 5-LOX enzyme in the mechanism of AA. Because of LTs are related with

some diseases such as carcinogenesis, allergy, inflammation, cerebral ischemia and brain edema, 5-LOX inhibitors which block the LTs are used as an option for treatment (185).

In this study, DPPH free radical-scavenging activity of VB-EtOAc (30±0.01 µg/mL) was found to be superior to other extracts tested. VB-EtOAc also showed the highest ABTS radical scavenging activity (20 ±0.01 µg/mL) better than gallic acid (30±0.01 µg/mL), whereas VB-*n*-hexane showed the lowest ABTS radical scavenging activity with 450 ±0.01 µg/mL. VB-*n*-BuOH and EtOAc displayed equipotent or higher activity against CUPRAC radical (20 ±0.01 µg/mL) comparable to gallic acid. VB-H_2O showed lowest CUPRAC radical scavenging activity (250 ±0.01 µg/mL). The *in vitro* anti-inflammatory activities of the extracts were evaluated using a LOX inhibition assay, where *n*-BuOH extract showed inhibition of 35.7% at 50 µg/mL concentration, which is followed by *n*-hexane (25.8%), $CHCI_3$ (22.6%) and EtOAc (21.4%) extracts (Table 39-42).

The aim of the antimicrobial studies is to establish the lowest concentration (minimal inhibitory concentration (MIC)) of the antimicrobial agents. Antimicrobial agents inhibit the visible growth of the microbes. MIC values are used for antimicrobial drugs in order to evaluate the potential of new antimicrobial agents. Antimicrobial activity tests are also important to establish resistance of microbial strains to antimicrobials, in ethnopharmacological studies and to determine the efficacy of various microorganisms (162). Some antimicrobial activity tests such as disk-diffusion, well-diffusion and agar or broth are the most known and used tests. Flow cytoflourometric and bioluminescent methods are not commonly used. Although they are fast, they require specific equipment and standardization (162).

The extracts were also screened for their antimicrobial activities against a panel of three human pathogenic bacteria including *Bacillus cereus* NRRLB 3711, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 36538 as well as three *Candida* strains. Only the *n*-hexane extract showed remarkable antibacterial activity against *P. aeruginosa* (MIC=125 µg/mL), while $CHCl_3$ extract showed moderate activity against *C. krusei* (MIC=250 µg/mL), followed by *n*-BuOH, MeOH and *n*-hexane extracts with a MIC value of 500 µg/mL, each (Table 43).

The *n*-BuOH, EtOAc and $CHCl_3$ extracts showed activity in the antioxidant and anti-inflammatory evaluation, and thus were subjected to chromatographic separation and purifications. As a result of successive chromatographic studies, totally 13 secondary metabolites (**1**-**13**) were isolated (183). The structures of these compounds were elucidated by spectroscopic techniques (UV, IR, 1D and 2D NMR) and HR-MS. The isolates were categorized as iridoid glycosides (**1**-**5**), phenylethanoid glycosides (**6**-**7**), flavonoids (**8**-**12**) and phytosterol (**13**). The structures were established as catalpol (**1**) (169), specioside (**2**), ajugol (**3**) (170), ajugoside (**4**) (172), 8-*O*-acetylharpagide (**5**), verbascoside (**6**) (95), glucopyranosyl-(1→G_i-6)-martynoside (**7**) (174), luteolin (**8**) (176), luteolin 7-*O*-β-glucopyranoside (**9**) (177), luteolin 7-*O*-rutinoside (**10**) (178), apigenin 7-*O*-rutinoside (**11**) (180), quercetin 3-*O*-rutinoside (**12**) (173) and *β*-sitosterol (**13**) (181) by comparison of their NMR data with those published previously.

Compounds **8** and **13** were purified in a row from the EtOAc and $CHCI_3$ extracts, respectively, while the remaining were isolated from *n*-BuOH extract. Five iridoid glycosides were isolated from the aerial parts of *V. bugulifolium n*-BuOH extract in this study for the frist time. They were categorized into two groups as catalpol type iridoid glycosides [catalpol (**1**), specioside (**2**)], and ajugol and harpagide-type iridoid glycosides [ajugol **(3)**, ajugoside **(4)**, 8-*O*-Acetylharpagide **(5)**].

Iridoids are common secondary metabolites in *Scrophulariaceae* family. They are found in *Verbascum* spp. as the largest group of compounds. Iridoids in *Verbascum* can be classified into two groups; C_9-type iridoids and C_{10}-type iridoids. C_9-type iridoids are catalpol type, aucubin and their derivatives, ajugol and harpagide and their derivatives. C_{10}-type are geniposidic acid, lychnitoside, genipin, *α*-gardiol and *β*-gardiol (3,54). In *Verbascum* spp. over 70 iridoid glycosides and several non-glycosidic analogous have been isolated (3).

Iridoids are accepted as as important chemotaxonomic markers are abundant different genera such as *Plantago* (Plantaginaceae), *Galium* (Rubiacea), and *Scrophularia* and *Verbascum* (Scrophulariaceae) (186). Catalpol is found in some families of the order Lamiales, such as Plantaginaceae, Lamiaceae, and Bignoniaceae. It was first discovered in 1962 after plants of genus *Catalpa* and it was first isolated from *Rehmannia glutinosa* by Kitagawa Hiroshi in 1971. Catalpol is distributed in Radix Rehmanniae, *Lancea tibetica,* and Radix Scrophulariae before (187).

In *Verbascum* species, catalpol was isolated from *V. blattaria, V. blattarioides,V. bombyciferum, V. capitis-viridis, V. cheirantifolium, V. cilicicum, V. densiflorum, V. dudleyanum, V. georgicum, V. lagurus, V. laxum, V. leucophyllum, V. lychnitis, V. mucronatum,V. nigrum, V. niveum, V. oreophilum, V. ovalifolium, V. phoeniceum, V. phlomoides, V. pulverulentum, V. pyramidatum, V. roripifolium, V. sinuatum, V. songaricum, V. spinosum, V. thapsiforme, V. thapsus, V.wiedemannianum* (3).

Specioside was first identified from *Catalpol speciosa* Warder (Bignoniaceae) (171). Specioside (**2**) is being reported for the third time in this study, which was previously isolated from *V. phlomoides* and *V. lychnitis* L. (67,68).

Ajugol was isolated from *V. apentulium, V. blattaria, V. blattarioides, V. boerhaavii, V. bombyciferum, V. capitis-viridis, V. chaixii V. cheirantifolium, V. gnaphalodes, V. lagurus, V. lasianthum, V. oreophilum, V. leucophyllum, V. lychnitis, V. macrurum, V. mallophorum, V. mucronatum, V. nigrum, V. olympicum, V. ovalifolium, V. phoeniceum, V. phlomoides, V. pterocalycinum, V. pulverulentum, V. pyramidatum, V. pycnostachyum, V. roripifolium, V. sinuatum, V. songaricum, V. speciosum, V spectabile, V. spinosum, V. thapsiforme, V. thapsus, V. undulatum, V. virgatum, V. wiedemannianum, V.xanthophoeniceum* (3)**.**

Ajugoside was found from *V. apentulium, V. capitis-viridis, V. chaixii, V. cheirantifolium , V. georgicum, V. gnaphalodes, V. lagurus* Fisch., *V. leucophyllum, V. lychnitis, V. nigrum, V. ovalifolium, V. phoeniceum, V. phlomoides, V. pycnostachyum, V. roripifolium, V. sinuatum, V. songaricum, V. speciosum, V spectabile, V. thapsiforme, V. thapsus, V. undulatum* (3,16,59). 8-*O*-Acetylharpagide was isolated from *V. apentulium, V. blattaria, V. blattarioides, V. boerhaavii, V. bombyciferum, V. capitis-viridis, V. cheirantifolium, V. densiflorum, V. georgicum, V. gnaphalodes, V. lasianthum, V. leucophyllum, V. mallophorum, V. nigrum, V. niveum, V. ovalifolium, V. phoeniceum, V. phlomoides, V. roripifolium, V. songaricum, V. thapsiforme, V. thapsiforme, V. thapsus, V. undulatum* (3).

In this study, two phenylethanoid glycosides, verbascoside (**6**) and glucopyranosyl (1→G6) martynoside (**7**) were isolated from *V. bugulifolium.* They were diglycoside (**6**) and triglycosidic structures (**7**). In this study, glucopyranosyl-(1→6)-martynoside (**7**) is being reported for the first time from the genus *Verbascum*.

From *Verbascum* species, more than 20 phenylethanoid (C_6-C_2) and two phenylpropanoid (C_6-C_3) glycosides were isolated (3). Phenylethanoid glycosides are particularly found in Scrophuiariaceae, Oleaceae, Plantaginaceae, Lamiaceae and Orobanchaceae families (188).

Verbascoside (acteoside) is a widespread compound which is found in a lot of species especially the species of *Stachys, Eremostachys, Faradaya, Lamium, Leonurus, Marrubium, Phlomis, Prostanthera, Oxera, Scutellaria,* and *Sideritis* from Lamiaceae family (188) and nearly all *Verbascum* species contain verbascosides (3). Verbascoside was first reported in 1963 from *V. sinuatum* and later determined more than 15 different mullein species. It has antioxidant, anti-inflammatory, antineoplastic, wound-healing and neuroprotective properties (189).

Verbascum species are all rich in flavonoids. In our study, we isolated five flavonoids namely luteolin (**8**), luteolin 7-*O*-*β*-glucoside (**9**), luteolin 7-*O*-rutinoside (**10**), apigenin 7-*O*-rutinoside (**11**), quercetin 3- *O*-rutinoside (**12**) from *V. bugulifolium*. Luteolin which is an aglycone was isolated from EtOAc extract while the other flavonoids were obtained from *n*-BuOH extract. Luteolin 7-*O*-glucoside is monoglycosidic whereas the others are diglycosidic flavonoids. Luteolin was previously reported from *V. fruticulosum, V. lychnitis, V. phlomoides, V. sinaiticum, V. songaricumSchrenk, V. thapsiforme, V. thapsus, V. wiedemannianum* (3). Luteolin 7-*O*-β-D-glucopyranoside was previously reprted from *V. dudleyanum, V. eremobium, V. fruticulosum, V. letourneuxii, V. phlomoides, V. salviifolium, V. scadicolum, V. scimperianum, V. sinaiticum, V. songaricum Schrenk., V. thapsiforme* (3). Quercetin 3-*O*-rutinoside (rutin) (**12**) was previously isolated from *V. phlomoides* (3,107). Also, it was isolated from *Verbascum cheiranthifolium* var. *cheiranthifolium* in 2014 by Dalar et al. (190).

In this study, one phytosterol namely β-sitosterol (**13**) was isolated from $CHCI_3$ extract. *β*-sitosterol is a common phytosterol which was also isolated from different *Verbascum* species such as *V. lasianthum, V. phlomoides, V. pycnostachyum, V. thapsiforme and V. thapsus* previously (3).

Except compounds **10**-**12,** all of the other isolates (**1**-**9**,**11**,**13**) were also tested for their *in vitro* antioxidant (DPPH•, ABTS• and CUPRAC), anti-inflammatory (5-LOX inhibition) and antimicrobial activities (Table 61-65). As **10** and **12** were obtained as a 1:1 mixture, bioactivity evaluation was not performed at this stage. Among the tested

compounds, luteolin (**8**) showed the highest DPPH radical scavenging activity comparable to ascorbic acid with an IC_{50} value of 5 μg/mL, whereas the specioside, ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**), apigenin 7-*O*-rutinoside (**11**) and *β*-sitosterol (**13**) did not show any activity. Verbascoside (**6**) and luteolin 7-glucoside (**9**) showed equipotent activity (10±0.01 μg/mL) which was moderate compared to luteolin (5±0.00 μg/mL). Glucopyranosyl (1→G6)-martinoside (**7**) showed weak activity. Iridoids and *β*-sitosterol did not exhibit the remarkable DPPH activity (Table 61).

Mihailoviç et al. (17) evaluated the methanolic and aqueous extracts of various *Verbascum* species from the flora of Serbia for their antioxidant activities. Differences in phytochemical content and antioxidant activities between *V. nigrum, V. phlomoides* and *V. thapsus* were assessed. Verbascoside was found to be a major secondary metabolite in examined plants which demonstrated high DPPH scavenging activity. Moreover, remarkable correlation between verbascoside content and antioxidant activities was determined. Tatlı et al. studied free radical scavenging and cell aggregation inhibitory activities of secondary metabolites of Turkish *Verbascum* species were screened (16). The isolated compounds from methanolic extracts indicated a dose dependent inhibition of bioautographic and spectrophotometric DPPH activities. Verbascoside found to be the most active (IC_{50} 4.0 μg/ml) metabolite when compared to vitamin C (as a reference material (IC_{50} 4.4 μg/ml)) to inhibit phorbol 12-myristate 13-acetate (PMA)-induced peroxide-catalyzed oxidation of 2,7-dichlorofluorescein (DCFH) by reactive oxygen species (ROS) within human promyelocytic HL-60 cells. Shakeri and Frokh have isolated phytochemicals of the *Verbascum sublobatum* and have evaluated for their antioxidant activity of the extracts from leaves of the plant. They prepared the methanolic extracts and partitioned by chloroform, ethyl acetate, and butanol. According to these results, the ethyl acetate fraction exhibited the most powerful DPPH radical scavenging activity among the three fractions and was subjected to separation and identification. The isolated compounds which had flavonoid structure, were identified as apigenin and luteolin. These phytochemicals were considered to be responsible for the antioxidant activity, which is in accordance with our findings (4).

Verbascoside (**6**) and luteolin (**8**) elicited the highest ABTS activity with 10±0.00 μg/mL. Verbascoside, luteolin, glucopyranosyl (1→6)-martinoside (**7**) (20 ±0.00 μg/mL) showed higher activity than ascorbic acid (30±0.00 μg/mL). Specioside (**2**) displayed the same activity with ascorbic acid. Apigenin 7-*O*-rutinoside (**11**) were less scavengers against

ABTS radical (90±0.01 µg/mL), while catalpol, ajugol, ajugoside, 8-*O*-acetylharpagide, and *β*-sitosterol were inactive (Table 62).

Mihalioviç et al. studied chemical composition and antioxidant activities of three *Verbascum* species; *V. nigrum*, *V. phlomoides* and *V. thapsus*. Verbascoside, harpagoside, phenolic acids and flavonoids contents and antioxidant activities of the extracts were remarkably diminished, whereas phenolic acids were stable during simulated digestion (17). Based on the results of this work, *V. nigrum* (118.60 mg/g of dry extract) had higher amounts of bioactive verbascoside and contained harpagoside which was not present in *V. thapsus* and *V. phlomoides*. *V.nigrum* had higher ($p < 0.05$) antioxidant activity in DPPH assay (IC_{50} 60.7 µg/mL), ABTS assay (IC_{50} 90.0 7 µg/mL), NO radical scavenging activity (29.3 µg/mL) and OH radical scavenging activity (3.3µg/mL). DPPH• scavenging activities of VPM and VNW were not significantly different ($p > 0.05$).

In 2016, phytochemical analysis of endemic *Verbascum pinetorum* (Boiss.) O. Kuntze was investigated for the first time by Boga et al. Also, ABTS cation radical decolourisation activity, cupric reducing antioxidant capacity were explored. Malic acid (47250.61+/- 2504.28 µg/g) and luteolin (7651.96 +/- 527.98 µg/g) were detected as the most abundant ingredients of acetone and methanol extracts. The acetone extract was detected as more active than BHT used as a standard in *β*-carotene-linoleic acid test method. Additionally, the acetone and methanol extracts exhibited higher activity than BHT in DPPH free radical scavenging activity test. The acetone, methanol and aqueous extracts demonstrated powerful inhibition while the acetone extract exhibited better activity than BHT and *α*-tocopherol which were used as reference materials in ABTS cation radical scavenging and cupric reducing antioxidant capacity assays, respectively. The outcomes of the study indicated that the acetone and methanol extracts of *V. pinetorum* exhibited strong antioxidant activity in all activity assays namely *β*-carotene-linoleic acid test system, DPPH free radical scavenging, ABTS cation radical decolourisation and cupric reducing antioxidant capacity methods (12).

In our study, verbascoside (**6**) showed the highest CUPRAC activity (10±0.00 µg/mL), which was comparable to gallic asid, whereas specioside (**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-acetylharpagide (**5**), apigenin 7-*O*-rutinoside (**11**) and *β*-sitosterol (**13**) did not show the activity. The second highest activity was showed by luteolin which was the same with ascorbic acid (20±0.00 µg/mL). Catalpol (**1**) showed the least CUPRAC activity (290±0.01

µg/m). Luteolin 7-glucoside (**9**) and glucopyranosyl (1→6)-martinoside (**7**) showed less activity than others. Except catalpol, iridoids and β-sitosterol did not show CUPRAC activity (Table 63). Verbascoside (**6**) and luteolin (**8**) showed the highest antioxidant activity in all tests.

Phenylethanoid glycosides have been established to possess antioxidant properties. Presence of the caffeoyl moiety and the ortho-dihydroxyphenyl group, the steric hindrance, the number as well as the position of phenolic hydroxyl is important for antioxidant activity of the phenylethanoid glycosides (191). Verbascoside (**6**) was considered major phenylethanoid glycoside that accommodates great antioxidant capacities.

Studies have established antioxidant activities of flavonoids. Flavonoids are used as antioxidant for oxidative stress diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases Flavonoids show their antioxidant activities with quenching free radical elements, chelating metal by donating a hydrogen atom or by single-electron transfer, suppressing the enzymes associated with free radical generation, and stimulation of internal antioxidant enzymes. The free hydroxyl groups, 4-carbonyl group and the C2-C3 double bond are related to their antioxidant activities (192). In our study, luteolin (**8**) was also found to be another major compound contributing the antioxidant capacity of *V. bugulifolium*.

Previous studies showed that aucubin and verbascoside (acteoside) are the main compounds that have anti-inflammatory activity (193). But, in some studies the anti-inflammatory and antimicrobial activities were attributed to the phenolic content (46,48). Anti-inflammatory effect of *V. pycnostachyium* (87), *V. salviifolium* (42) *V. latisepalum*, *V. mucronatum* (45), *V. pterocalycinum* var. *mutense* (94), *V. lasianthum* (41) were reported previously. Phenolic compounds such as flavonoids and phenylethanoid glycosides were reported to be responsible for their anti-inflammatory and antimicrobial activities (194).

In this study, luteolin (**8**) showed the highest anti-inflammatory activity compared to other isolates with 54.1±5.0 % inhibition at 1µg/mL. Catalpol (**1**), ajugol (**3**), ajugoside (**4**), verbascoside (**6**), apigenin 7-*O*-rutinoside (**11**) did not show any activity. Specioside (**2**), 8-*O*-acetylharpagide (**5**), glucopyranosyl (1→6)-martinoside (**7**), luteolin 7-glucoside (**9**), and *β*-sitosterol (**13**) showed less anti-inflammatory activity. Luteolin glucosides were determined to be bioactive compounds that are responsible for the *in vivo* anti-inflammatory

activities of different plant species (42,195). It is previously reported that the presence of *ortho*-dihydroxy groups on B-ring in flavonoids like luteolin could significantly contribute to their anti-inflammatory activities (42). Luteolin, on the other hand also exhibited significant antioxidant activity, which seems to contribute its anti-inflammatory activity (Table 64).

For antibacterial tests, results were commented against three bacterias as well as three *Candida* strains (Table 65). Glucopyranosyl (1→G_6)-martinoside (**7**) showed the highest activity with 2 µg/mL against *Bacillus cereus NRRLB 3711*, apigenin 7-*O*-rutinoside (**11**) showed the second highest antimicrobial activity (8 µg/mL). Verbascoside (**6**) and luteolin 7-glucoside (**9**) did not exert any activity against *Bacillus cereus NRRLB 371*. Except for specioside (**2**) and ajugoside (**4**)'s less activity any compound showed antimicrobial activiy against *Pseudomonas aeruginosa ATCC 10145*. Glucopyranosyl (1→ G_6) martinoside (**7**) showed the highest activity against *Staphylococcus aureus* ATCC 6538 (2 µg/mL). Compounds were all showed less antimicrobial activity than chloramphenicol. Verbascoside (**6**) and luteolin 7-glucoside (**9**) did not display activity against *Staphylococcus aureus ATCC 6538*. Specioside (**2**) showed the least activity against *Staphylococcus aureus ATCC 6538* (Table 65) (183). All the tested compounds exerted weak antimicrobial activity against tested microorganisms.

In 2016, phytochemical analysis and biological activities of *V. flavidum* was assessed by Boga et al. The outcomes of phytochemical analysis indicated that rutin and chlorogenic acid were determined as main phenolics, palmitic and oleic acids were detected as major fatty acids. The moderate activity of methanolic extract was reported against *E. coli* (MIC 250 +/- 0.3, inhibition zone 15 mm at 30 mg/mL) (12).

In a research, Ozcan et al. screened various extracts to find possible antimicrobial property of *Verbascum pinetorum*. Antimicrobial activity of the extracts of *V. pinetorum* was determined with agar-well diffusion method. The hexane extract of plant demonstrated antimicrobial activity against few microorganisms. *Haemophilus influenzae* was detected as the most sensitive bacterial strain. Phytochemical evaluation indicated that iridoid glycosides, flavonoids and saponins were the major constituents in the methanolic extracts. According to the results, those phytochemicals may be responsible for the crucial antimicrobial actions (19).

Ozcan et al. evaluated possible antimicrobial effects of *Verbascum antiochium* which is endemic to Turkey. The extracts of *V. antiochium* were obtained by increased polarity and direct methanol extraction method. The agar well diffusion method was used to detect antimicrobial activity. For this purpose, extracts were applied to various Gram-positive and Gram-negative bacteria and one fungus. The aqueous methanolic extract demonstrated the most potent activity against both the Gram-negative and Gram-positive bacteria. *Haemophilus influenzae* was detected as the most sensitive bacterium among the bacteria tested (inhibition zone 24.7 mm direct methanol extract) (20).

For antifungal activity studies, three fungi were used in our study. None of the compounds showed antifungal activity against *Candida albicans ATCC 90028*. Apigenin 7-*O*-rutinoside showed the highest antifungal activity against *Candida parapsilosis ATCC 22019*. Luteolin 7-glucoside showed the second highest antifungal activity against *Candida parapsilosis* ATCC 22019. Ajugoside, 8-*O*-asetylharpagide, glucopyranosyl (1→6)-martinoside showed the less and the same activity, catalpol showed the least activity against *Candida parapsilosis ATCC 22019*. Specioside, ajugol, verbascoside, luteolin, and *β*-sitosterol did not show anfifungal activity against *Candida parapsilosis ATCC 22019*. Luteolin 7-glucoside showed the highest anticandidal activity against *Candida krusei ATCC 6258*. Verbascoside showed the least antifungal activity, ajugol showed the less activity, and the other compounds did not show any antifungal activity against *Candida krusei ATCC 6258*. Sen et al. investigated *V. lagurus* to detect its phytochemical content and biological activity. For this purpose, chloroform, ethyl acetate, methanol and aqueous extracts were tested to detect their antimicrobial activities. *S. aureus, S. epidermidis, E. coli, K. pneumoniae, P. aeruginosa*, and *P. mirabilis* were used to test the possible antimicrobial activities of extracts. Additionally, possible antifungal activity was tested against *C. albicans.* Almost all extracts demonstrated antimicrobial activity against *S. aureus* and *C. albicans*, but the ethyl acetate extract had the most potent antimicrobial activity among the other *V. lagurus* extracts. Luteolin, luteolin-7-glucoside and verbascoside were considered to be main contributors of antimicrobial activity (23).

In conclusion, this thesis was aimed to evaluate both phytochemical and biological properties of one *Verbascum* species, *V. bugulifolum* which was not studied before. Totally **13** secondary metabolites were isolated from the *n*-BuOH, EtOAc and $CHCI_3$ subextracts including five iridoid glycosides; catalpol (**1**), specioside(**2**), ajugol (**3**), ajugoside (**4**), 8-*O*-

acetylharpagide (**5**), two phenylethanoid glycosides; verbascoside (**6**), and glucopyranosyl-(1→Gi-6)-martynoside (**7**), five flavonoids; luteolin (**8**), luteolin 7-*O*-*β*-D-glucopyranoside (**9**), luteolin 7-*O*-rutinoside (**10**), apigenin 7-*O*-rutinoside (**11**), quercetin 3-*O*-rutinoside (**12**) and one phytosterol; *β*-sitosterol (**13**). The isolates were also evaluated for their *in vitro* antioxidant, anti-inflammatory and antimicrobial activities by using the same test methods. Verbascoside (**6**) and luteolin (**8**) showed the highest antioxidant activity in all tests. Luteolin (**8**) also displayed the strong anti-inflammatory activity compared to other samples with 54.1±5.0 % inhibition at 1 µg/mL. All the tested compounds exerted weak antimicrobial activity against tested microorganisms. This is the first phytochemical and bioactivity study on *V. bugulifolium*. The results of bioactivities of the subextracts and compounds obtained from *V. bugulifolium* are summarized in Figure 7.

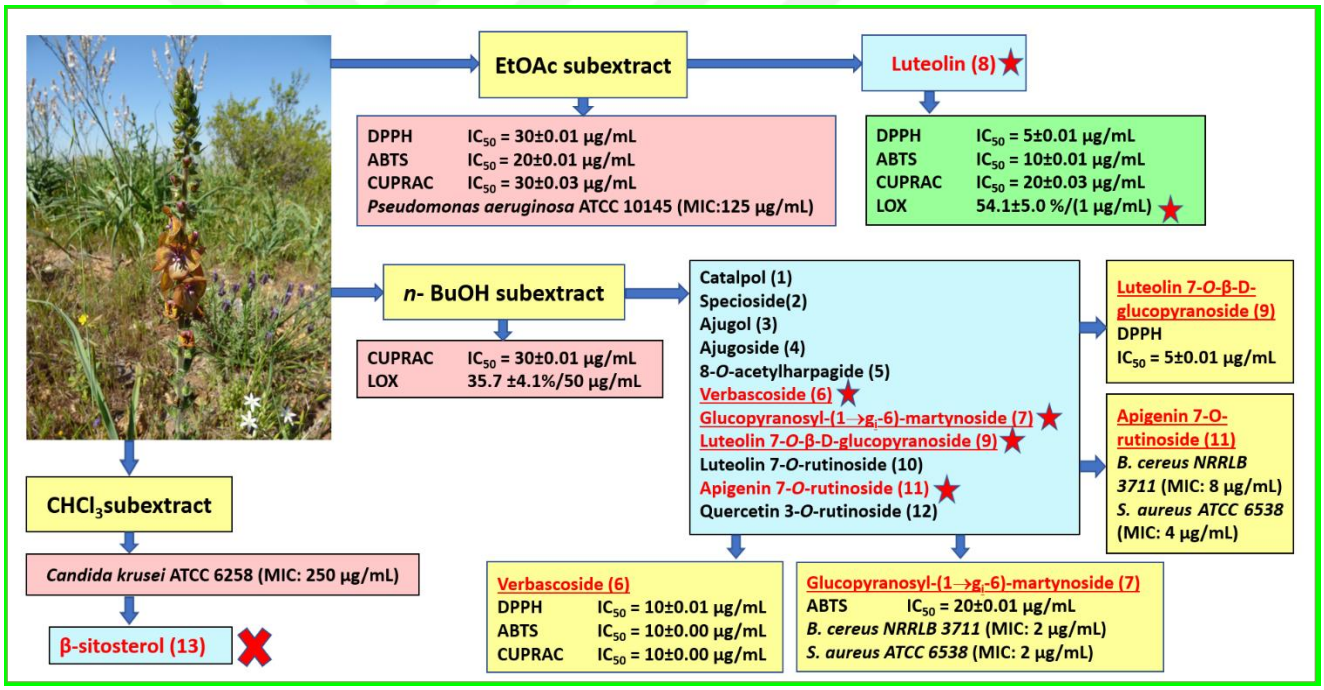


Figure 7. Summary of the bioactivity results of subextracts and compounds obtained *V. bugulifolium*

Although the extracts and isolated compounds displayed some *in vitro* antioxidant and anti-inflammatory activities, further detailed *in vitro* and *in vivo* studies are required to reveal the potential of the extracts and isolated compounds.

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6. CURRICULUM VITAE

Personal Information

Name	Ayşe	**Surname**	GÖKMEN
Place of birth		**Date of birth**	
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Education

Level	**Proficiency**	**Institution**	**Graduation Year**
Ph.D.	Pharmacognosy	Yeditepe University	2021
B.A.	Faculty of Pharmacy	Hacettepe University	2009
	Department of Nutrition and Dietetics	Hacettepe University	2003-2006
High School	-	Umraniye High School	2003

Language Skill

Language	**Exam Degree**
English	YDS-2014 (66.25)

Work Experience

Position	**Institution**	**Dates**
Pharmacist	Gokmen Pharmacy	2009-2021

Computer Skills

Programs	Degree
Microsoft Office	Good
MestReNova	Good
Mendeley	Good
ChemOffice	Good

Scientific Works

Publications in SCI, SSCI, AHCI Indexed Journals

Gökmen A, Kúsz N, Karaca N, Demirci F, Hohmann J, Kırmızıbekmez H. "Secondary metabolites from *Verbascum bugulifolium* Lam. and their bioactivities." *Nat Prod Res*. 2020:1-5.

International Conference Proceedings

Ayse Gokmen, Norbert Kúsz, Nursenem Karaca, Fatih Demirci, Judit Hohmann, Hasan Kirmizibekmez. "Bioactivities of *Verbascum bugulifolium* and isolation of secondary metabolites." 65th Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Basel, Switzerland, 2017.