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

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATIONS ON *SCUTELLARIA HASTIFOLIA* L.

IN THE PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Hilal Bardakcı Altan, Pharm.

Advisor
Prof. Dr. Hasan Kırmızıbekmez

ISTANBUL
JUNE 2014

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DOKTORA TEZ SAVUNMASI

Doktora öğrencisi Ecz.Hilal BARDAKCI ALTAN'ın çalışması jürimiz tarafından Farmakognozi Anabilim Dalı doktora tezi olarak uygun görülmüştür.

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Üniversite : Yeditepe Üniversitesi .....

Üye : Prof. Dr. Erdem YEŞİLADA
Üniversite : Yeditepe Üniversitesi ----------

Üye : Prof. Dr. Fatih DEMİRCİ
Üniversite : Anadolu Üniversitesi ----------

Üye : Doç. Dr. Didem ŞÖHRETOĞLU
Üniversite :Hacettepe Üniversitesi .....

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Prof.Dr. Bayram YILMAZ
Müdür V.

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Dedicated to my grandmother and grandfather,

Muradiye and Mehmet Emin

I love you

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Abstract

Scutellaria L. (Lamiaceae) species have long been the subject of many phytochemical, analytical and biological studies. In the context of this thesis, four *Scutellaria* taxa, *S. hastifolia* L., *S. velenovskyi* Rech., *S. orientalis* L. subsp. *pinnatifida* and *S. albida* L. subsp. *albida* from Turkey were screened for their antioxidant profiles by calculating their total antioxidant capacities as well as total phenol and total flavonoid contents.

Based on the preliminary data, *S. hastifolia* was selected for the detailed phytochemical and biological investigations. From the acetone and MeOH extracts, 17 compounds were isolated, including a new one, by using several chromatographic techniques. Isolated compounds were categorized as ethylcyclohexane derivatives (isorengyol, isorengyoside, cleroindicin B, cleroindicin F), phenylethanoid glycosides (cornoside, calceolarioside D, hastifolioside, neocalceolarioside D, calceolarioside B, verbascoside), flavonoids (apigenin 7-*O*-β-D-glucopyranoside, scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside), sugar ester (6-*O*-caffeoylglucopyranose) and sugar molecules (glucose, saccharose). The pure compounds were then investigated for their effects on DPPH, LP and LOX inhibition similarly as crude extracts of *S. hastifolia*.

All crude extracts of *S. hastifolia*, highly interacted with DPPH, while in LP inhibition assay the acetone extract showed remarkable activity. In LOX inhibition assay, only MeOH extract showed significant inhibition while other extracts did not. Furthermore, all of the isolates highly reacted with DPPH while only glycosides of apigenin and scutellarein along with calceolarioside D showed moderate LP inhibition. Moreover, all of the isolates showed weak soybean LOX inhibition at the tested concentration.

Additionally, the qualitative and quantitative analysis of several phenylethanoid glycosides and flavonoids in all *Scutellaria* species were performed comparatively by using HPLC-DAD in order to characterize their main phenolic constituents.

Keywords: *Scutellaria hastifolia*, antioxidant and antiinflammatory activities, secondary metabolites, hastifolioside, HPLC-DAD

Özet

S. hastifolia Bitkisi Üzerinde Fitokimya ve Biyolojik Aktivite Araştırmaları

Scutellaria L. (Lamiaceae) türleri günümüze kadar pek çok fitokimyasal, analitik ve biyolojik aktivite çalışmalarına konu olmuştur. Bu tez kapsamında, Türkiye florasında yetişen dört *Scutellaria* taksonu (*S. hastifolia* L., *S. velenovskyi* Rech., *S. orientalis* L. subsp. *pinnatifida* ve *S. albida* L. subsp. *albida*) seçilerek ana ekstrelerin total antioksidan kapasiteleri, total fenol ve flavonoid miktarları hesaplanmıştır.

Yapılan ön çalışmalar neticesinde, *S. hastifolia* fitokimyasal ve ileri aktivite çalışmaları için seçilmiştir. *S. hastifolia* bitkisinin aseton ve MeOH ekstrelerinden çeşitli kromatografik teknikler kullanılarak bir tane yeni olmak üzere toplam 17 bileşik saflaştırılmıştır. Bu bileşikler etilsiklohekzan türevi bileşikler (izorengyol, izorengyozit, kleroindisin B, kleroindisin F), feniletanoit glikozitleri (kornozit, kalkeolariozit D, hastifoliozit, neokalkeolariozit D, kalkeolariozit B, verbaskozit), flavonoitler (apigenin 7-*O*-β-D-glukopiranozit, skutellarein 7-*O*-β-D-glukopiranozit, hispidulin 7-*O*-β-D-glukopiranozit, hispidulin 7-*O*-β-D-glukuronopiranozit), şeker esteri (6-*O*-kafeoil-α/β-glukopiranoz) ve şeker molekülleri (glikoz ve sakkaroz) olarak sınıflandırılmıştır. *S. hastifolia* ana ekstrelerinde olduğu gibi, saf bileşiklerin de DPPH radikal süpürme, LP ve soya LOX inhibisyonu araştırılmıştır.

S. hastifolia bitkisinin tüm ekstreleri önemli DPPH radikal süpürücü etki gösterirken sadece aseton ekstresi referansa yakın LP inhibisyonu göstermiştir. LOX inhibisyonu deneyinde sadece MeOH ekstresinde kuvvetli aktivite gözlenmiştir. İzole edilen tüm bileşikler yüksek DPPH radikal süpürücü etki gösterirken, apigenin ve skutellarein glikozitleri ile kalkeolariozit D bileşiği ortalama değerlerde LP inhibisyonu göstermiştir. Bunun dışında tüm bileşikler düşük LOX inhibisyonu göstermişlerdir.

Bunlara ilaveten, ekstrelerin temel fenolik bileşiklerinin karakterizasyonu için, bu dört *Scutellaria* türünde bazı feniletanoit glikozitlerin ve flavonoitlerin HPLC-DAD ile kalitatif ve kantitatif analizleri gerçekleştirilmiştir.

Anahtar Kelimeler: *Scutellaria hastifolia*, antioksidan ve antienflamatuvar aktivite, sekonder metabolitler, hastifoliozit, YPSK-DAD

Contents

Abbreviations

AA: Acetic acid

AAPH: 2,2'-Azobis (2-amidinopropane) dihydrochloride

Ac: Acetate

ACN: Acetonitrile

BuOH: Butanol

CH_2Cl_2: Dichloromethane

$CHCl_3$: Chloroform

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EtOAc: Ethyl acetate

EtOH: Ethanol

FA: Formic acid

Glc: Glucose

GluA: Glucuronic acid

H_2O: Water

HPLC: High Performance Liquid Chromatography

LOX: Lipooxygenase

LP: Lipid peroxidation

MeOH: Methanol

MPLC: Medium Pressure Liquid Chromatography

NDGA: Norhydroguairetic acid

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

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1. INTRODUCTION and AIM

This section consists of a brief introduction and the aim of the thesis.

INTRODUCTION AND AIM

World Health Organisation revealed that approximately 80% of the world's people count on medicinal plants in order to maintain their health or for treatment purposes. The genus *Scutellaria*, which belongs to the family Lamiaceae, is definitely one of the important genera that have multiple usages in traditional medicine in primary health care needs. In this context, antitumor, antiangiogenic, hepatoprotective, antioxidant, anticonvulsant and neuroprotective effects of *Scutellaria* species have been reported. Besides, the chemical composition of *Scutellaria* species have also been studied since 1889 and over 295 phytoconstituents (flavonoids, phenylethanoid glycosides, iridioids, diterpenes, triterpenes, alkaloids) have been isolated up to date (1).

Oxidative stress and inflammation are the defence mechanisms of the body against the external factors. Oxidative stress leads the formation of free radicals which are extremely hazardous for the living organisms causing detrimental diseases such as cardiovascular diseases, cancer and diabetes. Meanwhile, inflammation starts as an acute condition but in time it becomes chronic and causes serious diseases like rheumatoid arthritis and asthma. The seriousness of these two conditions compelled scientists to find effective drugs with less or no adverse effects. At this point, medicinal plants gained an importance. Based on *in vitro* and *in vivo* studies in this way, several *Scutellaria* species were found to have an antioxidant and antiinflammatory activities (2–4).

In the flora of Turkey, the genus *Scutellaria* L. is represented by 16 species, some of which are used for their hemostatic, tonic and wound healing properties (5–7). Four *Scutellaria* species namely *S. hastifolia*, *S. velenovskyi*, *S. orientalis* subsp. *pinnatifida* and *S. albida* subsp. *albida* were collected from different locations of Turkey and their acetone, MeOH and MeOH:H_2O (5:1) extracts were prepared from the aerial parts, consecutively. The crude extracts were then submitted to the antioxidant screening tests. The total antioxidant capacities, total phenolic and total flavonoid contents of the extracts were determined. Based on the results of the preliminary screening tests as well as the results of the initial thin layer chromatographic (TLC) screening of the extracts, *S. hastifolia* was found to worth further detailed studies.

A reference search revealed a limited number of phytochemical and biological activity studies on this species (8,9). Consequently, *S. hastifolia* was chosen for further detailed phytochemical and biological investigations.

The aim of this study was to isolate metabolites from the aerial parts of *S. hastifolia*. Furthermore, *in vitro* evaluation of antioxidant [diphenyl picrylhydrazine (DPPH) radical scavenging assay and lipid peroxidation inhibition assays] and antiinflammatory [soybean lipooxygenase (LOX) inhibition assay] activities of the crude extracts as well as the purified compounds were also targeted. Moreover, the qualitative and quantitative evaluation of the isolated and common phenylethanoid glycosides and flavonoids in the MeOH extracts of these *Scutellaria* species were also aimed by using newly developed high performance liquid chromatography (HPLC) methods. Thus, with the help of all results (activity, phytochemical and analytical), it was aimed to isolate new compounds with potent antioxidant and antiinflammatory activities from *S. hastifolia* as well as to provide contribution to the chemotaxonomy of the genus *Scutellaria*.

2. GENERAL DESCRIPTION

This section includes two parts.

First part consists of general information about *Scutellaria* species and divided into four subtitles. This section is constituted through an intense literature investigation. The subsections of the first part are botanical, pharmacological, phytochemical and analytical information concerning *Scutellaria* species.

Second part contains general information about the oxidative stress and inflammation.

2.1. General Information about *Scutellaria*

This section includes botanical, pharmacological, phytochemical and analytical information on *Scutellaria* species.

2.1.1. Botanical Information

In this section, botanical characteristics of the family Lamiaceae and the genus *Scutellaria* along with the botanical information on *S. hastifolia*, *S. velenovskyi*, *S. orientalis* and *S. albida* are given respectively.

2.1.1. Botanical Information

2.1.1.1. Lamiaceae

Lamiaceae members are herbs or shrubs, usually glandular and aromatic plants; and their stems are 4-angled or not. The leaves are exstipulate, simple, and sometimes pinnate, always opposite. Inflorescence is basically of cymes borne in the axils of bracts or upper leaves and usually contracted to form false whorls (verticillasters); the latter may also be arranged to form 'spikes', heads, racemes or cymes. Flowers are hermaphrodite, or male-sterile (functionally female) in gynodioecious plants. Bracts are clearly different from leaves or similar to them (when they may be termed 'floral leaves'); bracteoles present or not. Calyx is usually 5-lobed with an upper 3-toothed and lower 2-toothed part, rarely lobes or teeth 1 and 1 or 1 and 4, or actinomorphic; veins are 5-20. Corolla is gamopetalous, zygomorphic and bilabiate with usualy an indistinctly 2-lobed upper lip (hood or galea), falcate, straight or ± concave, and a 3-lobed lower lip (labellum); rarely upper lip reduced and lower lip 5-lobed, or with 1 upper and 4 lower lobes, or corolla actinomorphic. Stamens of the members are adnate to corolla, 4 and didynamous, or 2 (and staminodes usually present); posterior (upper) pair usually shorter than anterior (lower) pair; anther thecae 2- or 1-celled, parallel or divergent, rarely (in *Salvia*) separated by elongated connectives. Ovary is usually superior, 2-carpellate and 4-ovulate, 4-lobed. Style gynobasic, rarely not, shortly bifid above. Fruit of four (rarely fewer) dry (very rarely fleshy) nutlets, muscilaginous on wetting (myxospermic) or not (5).

The family includes many culinary or flavouring herbs, native to Turkey and the Mediterranean area, which are cultivated throughout the world (5).

Lamiaceae comprises 45 important genera (*Ajuga*, *Phlomis*, *Lavandula*, *Rosmarinus*, *Nepeta*, *Mentha*, *Stachys* etc.) including *Scutellaria.*

Dichotomous key for the genus *Scutellaria* in Lamiaceae can be illustrated as follows (5):

1. Fertile stamens 4 [or all reduced and sterile in female (male-sterile) flowers]

6. Upper lip of corolla absent or very reduced; style not fully gynobasic

8. Indumentum of simple hairs only, or absent

14. Fruiting calyx accrescent or not, never with membranous-reticulate lobes

17. Calyx 5-15 (-22)-veined or –ribbed; posterior (upper) stamens shorter than anterior (lower); stamens included or exserted beyond upper lip of corolla

21. Corolla tube long and slender, ± sigmoid, suberect, lips small; calyx upper and lower lips entire, upper with a small projecting flap

6. *Scutellaria*

2.1.1.2. *Scutellaria* L.

Scutellaria species are perennial herbs, often suffrutescent at base, ± lacking from an aromatic odour. Leaves are usually petiolate, at least below. Flowers are inflorescence of a raceme or a spike; arising singly in axils of bracts or floral leaves, on very short pedicels, secund or not. Calyx of *Scutellaria* members are bilabiate, tube ventricose-campanulate, upper part with a rounded, scale-like appendage (scutellum); lips entire, closed in fruit, lower part persistent, upper deciduous along with scutellum. Corolla with very long suberect sigmoid tube, dilated above, bilabiate, upper lip galeate, lower broader, flat to recurved. Stamens consist of 4 anthers included under hood, ciliate, lower pair longer, monothecous, upper pair with 2 divergent thecae. Style unequally bifid. Nutlets depressed-globose to broadly ellipsoid, often tuberculate, ± pubescent with stellate hairs (5).

Throughout the world *Scutellaria* genus comprises about 350 species, mostly distributed in Europe, the United States and East Asia (10). It is represented by 16 species in “Flora of Turkey” including *S. uzunderensis* (5,6). Moreover, in a PhD thesis prepared by Mehmet Çiçek, *Scutellaria* genus is represented by 24 species, 1 hybride species, 13 subspecies and 32 taxa (11).

Dichotomous key for Turkish *Scutellaria* species is as follows (5):

1. Flowers in a 4-sided inflorescense, not secund

2. Bracts exceeding calyx at anthesis; inflorescence a short dense spike, rarely a lax elongate spike

14. *orientalis*

2. Bracts equalling calyx at anthesis; inflorescence a lax raceme

15. *tomentosa*

1. Flowers secund

3. Rhizomatous; flowers in axils of floral leaves similar to, but smaller than, lower cauline leaves

4. Leaves hastate, entire in upper half; calyx densely glandular-hairy

1. *hastifolia*

4. Leaves cordate, weakly crenate; calyx glabrous or eglandular-pubescent

2. *galericulata*

3. Not rhizomatous; flowers in axils of bracts

5. Decumbent, often mat-forming; basal leaves ovate to elliptic, subentire to crenate, not cordate; corolla yellow or pinkish

6. Cauline leaves linear to oblong, lamina 20-30 mm; stems 30-60 cm

13. *heterophylla*

6. Cauline leaves ovate, to 15 mm; stems to 20 cm

7. Leaves glabrous, flat, margins weakly crenate to subentire

12. *pontica*

7. Leaves puberulous, rugose with impressed veins above, margins ± distincly crenate

8. Corolla yellow, 16-25 mm

10. *salviifolia*

8. Corolla pink, 12-15 mm

11. *diffusa*

5. Stems ascending to erect, not decumbent; basal leaves triangular-ovate to ovate, ± cordate; corolla white, cream, mauve, purplish, reddish, bluish or violet

9. Corolla with bluish upper lip and whitish or white or white-stripped lower lip

10. Inflorescence axis densely glandular-hairy in upper part; cauline leaves 7-12×3.5-6cm

3. *altissima*

10. Inflorescence axis densely eglandular-hairy in upper part; cauline leaves 3-5.5×1.5-3 cm

4. *tournefortii*

9. Corolla ± concolorous (lower lip usually stripped or spotted with contrasting color *in vivo*), whitish, dingy mauve, reddish-purple or violet

11. Corolla white or cream, rarely dingy mauve; bracts usually exceeding calyx at anthesis

12. Stem with short and long eglandular hairs and ± dense patent glandular hairs of intermediate length

6. *velenovskyi*

12. Stem shortly pubescent with antrorsely curved hairs; glandular hairs sparse or absent except within inflorescence

5. *albida*

11. Corolla violet to reddish-purple, rarely lilac or lavander-blue; bracts usually shorter than calyces at anthesis

13. Stems robust, 35-60 cm, main stem erect; lamina of cauline leaves 15-50×12-40 mm, crenate to crenate-serrate, each side with 6 or more lobes, acute to subacute

14. Calyx 5-9 mm in fruit; bracts subsessile to shortly petiolate, elliptic to ovate

7. *rubicunda*

14. Calyx to 13 mm in fruit; bracts long-petiolate, broadly ovate

8. *megalaspis*

13. Stems weak, 10-35 (-45) cm, main stem often creeping, horizontal; lamina of cauline leaves less than 15×12 mm, crenate with few (4-5) lobes pers ide, obtuse

15. Calyx villous with long straight eglandular hairs; axis of inflorescence ± densely long glandula

7. *rubicunda*

15. Calyx puberulous; axis of inflorescence eglandular

9. *glaphyrostachys*

***Scutellaria hastifolia* L.**

***Scutellaria velenovskyi* Rech.**

Scutellaria orientalis* L. subsp. *pinnatifida

Scutellaria albida* L. subsp. *albida

2.1.1.3. *Scutellaria hastifolia* L.

This species is similar to *Scutellaria galericulata* [stem 15-50 (-75) cm, erect or ascending, simple or branced, leafy, often purplish. Leaves are slightly discolorous, median to 5.5×2.5 cm, elliptic to lanceolate, ± cordate, weakly crenate-serrate, finely pubescent, very shortly petiolate. Flowers are secund, pedicels 1 mm. Calyx 4-5 mm, sparsely pubescent, eglandular. The color of the corrolla is lavender-to lilac-blue, with paler lower lip speckled blue in middle third, 14-20 mm, pubescent] in habit, but with narrower hastate leaves usually entire at least in upper half. The calyx is glandular-pubescent, and corolla tube strongly curved upwards at base (5).

Synonims: *Cassida hastifolia* (L.) Scop.

Scutellaria dubia Taliev & Širj.

Flowering season: May-June

Habitat: Streamsides, borders of lakes, inside of the *Rubus sp*. between the lakesides

Altitude: 80 to 800 m

Distribution in Turkey: Northwestern Anatolia (Edirne, Kırklareli, Istanbul, Bursa), partially western Anatolia (İzmir)

Worldwide distribution: From Europe to Caucasia (Europe, western part of the Siberia, Turkey and northern part of Caucasia (5,11).

2.1.1.4. *Scutellaria velenovskyi* Rech.

Plants are 20-40(-60) cm, robust, ± branched above; stems ± densely clothed with short and patent eglandular hairs and glandular hairs. Leaves are large, dentate, obtuse, puberulous. Inflorescence with numerous lateral branches, axis densely patent-glandular and –eglandular hairy. Bracts to 20 × 9 mm, broadly elliptic-oblong, obtuse to acuminate, petiolatei distinctly exceeding fruiting calyces (7-11 mm). Corolla is cream in colur and 14-17 mm in size.

Synonims: *Scutellaria pichleri* Velen.

S. albida L. f. *perhispida* Bornm.

S. velenovskyi Rech.

S. goulimyi Rech.

S. velenovskyi Rech.

S. naxensis Bothmer

S. albida L. subsp. *perhispida* (Bornm.) Bothmer

Flowering season: May-July

Habitat: Dry stream borders inside the *Pinus brutia* community, under the *Quercus* species near the stream borders, stone hills near the seaside

Altitude: 200 to 1950 m

Distribution in Turkey: Northwestern and West Anatolia

Worldwide distribution: From southeastern Europe to Turkey (Bulgaria, Greece, Romania, Yugoslavia, East Aegean Islands, Turkey) (5,11).

2.1.1.5. *Scutellaria orientalis* L.

A very variable suffrutescent perennial herb, usually repent at base. Stems are 5-45 cm, obscurely tetragonal. Leaves are distinctly petiolate, lamina ovate-elliptic to linear, 5-30 mm, usually obtuse, cuneate or attenuate (rarely truncate) at base, (with rare exceptions) 1.5-2 × as long as broad, weakly crenate to incised-pinnatifid or pectinate-pinnatifid, glabrous to densely canascent-tomentose, often markedly discolorous. Inflorescence a terminal spike with tetragonal ranks of flowers (not secund), usually dense with ± imbricate bracts; bracts very variable in size and shape but often either pale greenish or deeply purple-tinged, sessile. Corolla is 20-32 mm in size, usually yellow, sometimes reddish-spotted ır with red upper or lower lip or entirely pink, bright red, brick-red or purplish ± pubescent. Nutlets are oblong and tomentellous (5).

1. Cauline leaves less than 4× as long as broad

2. Leaves pinnatifid, divided at least to midway to midrib

3. Lower bracts entire, rarely lowermost pair with 1-2 teeth; stems not exceeding 20 cm tall

4. Leaves divided to distictly short of midrib, lobes ± revolute, concolorous or weakly discolorous, greenish above and beneath

subsp. *pinnatifida*

Synonims: *Scutellaria caucasica* A.Ham

S. hercegovinica Formánek

S. orientalis var. *taurica* (Juz.) N.P.Popov

S. hirtella Juz.

S. hypopolia Juz.

S. stevenii Juz.

S. taurica Juz.

S. heterochroa Juz.

S. subalbida Klokov

Flowering season: May-June

Habitat: Volcanic rock sides, clay hillsides, naked hills

Altitude: 300 to 1750 m

Distribution in Turkey: Northeastern Anatolia

Worldwide distribution: From the Crimea to Transcaucasia (Crimea, Middle Russia, Turkey, Transcaucasia) (5,11).

2.1.1.6. *Scutellaria albida* L.

Erect, usually robust plants (15-) 20-35 (-40) cm tall. Stems are usually branced, densely clothed with antrorsely adpressed hairs, eglandular below inflorescence. Leaves are 20-30×15-20 mm in size, triangular ovate, cordate, coarsely crenate, obtuse and adpressed-puberulous. Inflorescence branced, axis densely patent glandular- and eglandular-hairy and densely puberulous. Bracts are 9-20 mm, short-to long-petioled, lamina acute or acuminate to rounded, elliptic. Corolla is white, cream, or dingy mauve, 12-19 mm, lower lip usually streaked with violet or purple.

1. Corolla 12-15 (-16) mm; stems often branched from base at least to middle

2. Flowers White or cream; bracts long-petioled, with rounded or acuminate

subsp. *albida*

Synonims: *Scutellaria albida* Ledeb.

S. peregrina Ledeb.

S. cretica Mill.

S. nigrescens Spreng.

S. pallida M.Bieb.

S. decumbens Sieber ex Rchb.

S. hirta Sieber ex Rchb.

S. albida L. var. *typica* Stoj.

S. albida L. subsp. *eu-albida* Rech.

S. albida L. subsp. *pallida* Rech.

Flowering Season: May-June

Habitat: Hillsides, inside the *Quercus*, *Pinus*, *Abies*, *Fagus*, *Fagus-Abies* forests

Altitude: 50-1500 m

Distribution in Turkey: Northwestern and partially western Anatolia

Worldwide distribution: From southeastern Europe to Transcaucasia (Southeasttern Europe, Crimea, Transcaucasia, East Aegean Islands, Turkey) (5,11)

2.1.1.7. Local Names of the *Scutellaria species*

Scutellaria name comes from the *Latin* name *Scutella* meaning of plate or pot (11).

In Turkish: kaside (7).

In Other Languages: Scullcap, skullcap (English), Huang Qin, Ban-Zhi-Lian (Chinese) (1), Banjiryun (Korean), Hwang-gum (12,13), Scutellaire, toquie (French), Helmkraut, Fiberkraut, Schildkraut (German), Escutelaria (Spanish) (14).

2.1.1.8. Traditional Usage of *Scutellaria* species

In Turkey, *Scutellaria orientalis* is used for constipation and wound healing. Besides, it is also used for its hemostatic and tonic properties (7).

In East Asia, particularly in Korea, China and Japan, some species of the genus *Scutellaria* (*S. baicalensis* and *S. barbata*) are used in traditional medicine due to their sedative, ,antiinflammatory, antiviral, antithrombotic and antioxidant activities (1).

S. barbata is known as "Ban-Zhi-Lian" in Traditional Chinese Medicine and used as pain killer, to treat swelling throat, edema, and hemorrhoids (1).

The roots of *S. baicalensis* are used to eliminate stasis, activate blood circulation, induce diuresis and reduce edema as well as to clear away the heat evil and expel superficial evils in China (1).

In China, Korea and India, *S. indica* is consumed for analgesia, detoxification and promoting blood circulation activities (1).

Additionally in China, there are many other applications of using skullcap in traditional medicine, in combination with various other plants. Some of them are curing

menstruation diseases, thyphoid fever, diarrhea, dyspnea, headache, common cold, measles, toothache, abdominal pain, dysentery and eye diseases (1).

Among the herbal medicine practitioners in the United Kingdom and Ireland, herba *S. lateriflora* is generally used against anxiety and stress, also it is used for spasms, digestive disorders, hypertension, irritable bowel syndrome, insomnia and allergies (15)

The herb of the skullcap is primarily sold in the form of tea in health food stores as well as tonic or in combination with other medicinal plants such as valerian and passion flower in the form of tablets against sleeping disorders in Canada (16).

2.1.2. Bioactivity studies on *Scutellaria* species

In this part, pharmacological studies referring *Scutellaria* species were gathered and sorted with respect to the related topics.

2.1.2. Bioactivity studies on *Scutellaria* species

2.1.2.1. Studies on Antioxidant Activity

In a study performed by Kimuya et al., H_2O, MeOH and EtOAc extracts of *S. baicalensis* Georgi (ogon in Japanese) roots and its flavonoid components, especially oroxylin A, wogonin, skullcapflavone II, chrysin, baicalein, baicelin and wogonin 7-*O*-D-glucuropyranoside were investigated for their antioxidant activities. *In vivo* studies showed that oral administration of the three extracts and the pure compounds, wogonin, baicalein and baicalin reduced the lipid peroxide level in the liver of the rats treated with $FeCl_2$-ascorbic acid-adenosine 5'-diphosphate (ADP) mixture. Wogonin, skullcapflavone II, baicalein, baicalin and wogonin 7-*O*-D-glucuronide inhibited NADPH-ADP aroused lipid peroxidation in rat liver homogenate. However, oroxylin A and chrysin had no effect at the tested concentration. Also baicalein, baicalin and quercetin suppressed $FeCl_2$-ascorbic acid triggered lipid peroxidation in rat liver homogenate *in vitro* while other flavonoids had no inhibitory effect at the same concentration. As a result of this study the authors suggested that the inhibitory action of ogon on lipid peroxidation may be due to the flavonoid content of this plant (17).

In an another study, H_2O extract prepared from *S. baicalensis* roots, it was found that the extract extremely suppressed the oxidative stress in cardiomyces during ischemia, reperfusion, moderate hypoxia and antimycin A administration. Though, these effects led to an improvement in cardiomyocyte survival and contractile function in the ischemia-reperfusion models (18).

Schinella et al. reported that *S. baicalensis*, inhibited lipid peroxidation in rat liver microsomes and red blood cells. MeOH extract of *S. baicalensis* roots also exhibited inhibitory effect on aminopyrine N-demethylase and xanthine oxidase in addition to pro-oxidant effect in Fe^{3+}-EDTA-H_2O_2 system (19).

Sawicka et al. examined the antioxidant activity of *S. baicalesis* root extract from the different point of view. In this study chromium (III) and (VI) increased lipid peroxidation in erythrocytes measured by calculating malondialdehyde concentration. At the end of the study, positive effect on antioxidant mechanisim of *S. baicalensis*

extract was shown. The plant extract was found to be a good membrane protector against chromium exposure and also inhibited lipid peroxidation (20).

In 2008, Waisundara et al. demonstrated that *S. baicalensis* is known for its radical scavenging activity. In an *in vivo* study, the authors gave metformin 500 mg/kg to the first group, *S. baicalensis* 400 mg/ kg to the second group, metformin 500 mg/ kg + *S. baicalensis* extract 400 mg/kg to the third group for 30 days to the streptozocin induced diabetic rats. The results exhibited that, rats treated with both metformin and *S. baicalensis* had elevated hepatic activities of the antioxidant enzymes (SOD, catalase and gluthatione peroxidase). Based on the results of this work metformin and *S. baicalensis* improved the antioxidant status in rats when used simultaneously (21).

In 2003, Bochorakova et al. examined the radical scavenging activity of the MeOH extract of *S. baicalensis* roots and its main flavonoids (baicalein glucuronide, baicalein, wogonin glucuronide, wogonin and oroxylin) using the reaction with the stable DPPH radical. Baicalein glucuronide and baicalein showed higher scavenging activity among the other flavonoids. In addition to DPPH scavenging activity test, hydroxyl radical scavenging activity test was performed. According to these results, glucuronides of wogonin and baicalein were showed a great activity. They were more effective than one of the references ascorbic acid but not than rutin. According to the deep analyses, the DPPH radical scavenging activity of *S. baicalensis* extract was found to be due to its baicalein content (22).

H_2O extract of *S. baicalensis* roots were found to have synergistic antioxidant effect with the grape seed extract. Shao et al. suggested that co-administration of these extracts increases the ability to scavenge reactive oxygen species while decreasing the possible side effects, may be the result of high doses of single herb preparations (23).

In 1999, Lim et al. studied the flavonoids of *S. baicalensis* in order to examine their antioxidant effects. Briefly, they isolated wogonin, wogonoside and ganhuangenin. Regarding the lipid peroxidation tests induced by NADPH/ADP/Fe^{3+} in rat liver microsomes, ganhuangenin showed superior antioxidative effect than the control group α-tocopherol. Authors explained that the activity may come from the methoxyl substitution with its position in the polyphenolic rings (24).

Gao et al. studied baicalein, baicalin, wogonin and wogonoside which were isolated from *S. baicalensis*. According to electron spin resonance technique, baicalein and baicalin scavenged hydroxyl radical, DPPH radical and alkyl radical in a dose dependent manner, yet wogonin and wogonoside elicited a little or no effect on those radicals. Baicalin and baicalein at a concentration of 10 μmol/L inhibited lipid peroxidation of rat brain cortex mitocondria generated by Fe^{2+}- ascorbic acid, AAPH or NADPH, while wogonin and wogonoside showed its effect only on NADPH-induced lipid peroxidation. Baicalin and baicalein also protected human neuroblastoma SH-SY5Y cells against H_2O_2-induced injury. Due to *o*-trihydroxyl structure at the A ring of baicalin and baicalein, it was found as the most effective flavonoid among the tested flavonoids (25).

Shang et al. treated rat pheochromocytoma line PC12 with hydrogen peroxide (H_2O_2) for 30 min and observed the injury caused by superoxide dismutase (SOD) and Na^+-K^+-ATPase, increased malondialdehyde (MDA) and lactate dehydrogenase (LDH) production. Despite that when they pre-treated the PC12 cells prior to H_2O_2 exposure with *S. baicalensis* flavonoids, they found that pre-treatment with the *Scutellaria* flavonoid mixture, increased the cell survival, decreased the unwanted effects of SOD and Na^+-K^+-ATPase, as well as the MDA levels and LDH release (26).

Baicalin is one of the major flavonoids found in most of the *Scutellaria* species, especially in *S. baicalensis*. Hydroxyl group of the A ring in baicalein was alkylated at the position 6 with prenyl, geranyl and farnesyl groups in this study. Baicalein and its semisynthetic derivatives decreased the levels of cumene hydroperoxide and H_2O_2 in human acute monocytic leukemia cell lines (THP-1). They already prevented the intracellular gluthatione depletion induced by cumene hydroperoxide in THP-1 cells. The tests demonstrated that baicalin and its derivatives could be beneficial to human health and can be used as antioxidants (27).

Baicalin has many activities, but the most significant activity of this flavonoid is obviously its antioxidant activity. In Waisundara and colleague's study, the activity of baicalin in streptozocin-induced diabetic rats was investigated. The rats treated with baicalin and baicalin + metformin had significantly elevated hepatic activities of SOD,

catalase, and gluthatione peroxidase. The study revealed that baicalin mitigated oxidative stress in diabetic rats (28).

Liu et al. studied the activity of *S. baicalensis* flavonoids (SSF) on rat cortical neurons in order to explain the neuroprotective effect of this plant. In this study, primary cortical cells were damaged by exposure to 100 μmol/L H_2O_2 for 150 min. This procedure was preceded by decreased SOD, GSH-Px and Na^+-K^+-ATPase activities and increased MDA levels. SSF inhibited the increment of MDA levels and reducement in SOD, GSH-Px and Na^+-K^+-ATPase activities. As a result, it was explained that SSF has antioxidant activity due to their polyphenolic nature, by blocking the free radical attack and cytotoxicity (29).

In a research program conducted by Nguyen et al., aimed to explore the biologically active components of Vietnamese medicinal plants, they collected *S. barbata* and extracted with CH_2Cl_2. From the CH_2Cl_2 extract they isolated five diterpenes, barbatines A-D and scutebarbatine A. Barbatine A and scutebarbatine A were shown to posses an interesting capacity to prevent cell damage induced by H_2O_2 (30).

Ye and Huang performed an uncommon analysis. They extracted polysaccharides from the H_2O extract of *S. barbata* and conducted superoxide anion radical scavenging assay, DPPH radical scavenging assay and hydroxyl radical scavenging test. Superoxide radical scavenging assay showed that the IC_{50} values of polysaccarides and the standard ascorbic acid were 0.17 and 0.19 mg/mL respectively, which indicated that both had the similar activity. DPPH radical scavenging assay displayed that the IC_{50} values of polysaccarides and the standard ascorbic acid were 0.57 and 0.26 mg/mL, respectively. The scavenging activity was lower than that ascorbic acid despite that polysaccarides showed similar activity on hydroxyl radicals. Finally, polysaccarides displayed slightly greater activiy than the standard ascorbic acid in hydroxyl radical scavenging assay (31).

A very first study concerning the antioxidant activity of ganhuangenin, ganhuangemin and wogonin which were isolated from the acetone extract of *S. rehderiana*, revealed that ganhuangenin and baicalein displayed greater antioxidant

effect than butylated hydroxytoluene when added to canola oil than ganhuangemin, wogonin, baicalin and oroxylin A at the same conditions. At a concentration of 200 ppm, acetone, hexane and MeOH extracts of *S. rehderiana* were compared in inhibiting oxidation of canola oil. The results showed the acetone extract contains higher antioxidant substances than the other extracts (32).

Petroleum benzene, $CHCl_3$, acetone and MeOH extracts of *S. colebrookiana* and *S. violacea* were prepared. According to superoxide radical inhibiton assay, $CHCl_3$ and acetone extracts of both plants showed a remarkable inhibiton than that of petroleum benzene and MeOH extracts. Thus $CHCl_3$ and acetone extracts were selected for further tests. The $CHCl_3$ extracts of both plants elicited a significant activity. The IC_{50} values were 17.5 and 28 μg/mL for *S. colebrookiana* and *S. violacea,* respectively for hydroxyl radical scavenging activity. The higher values were obtained for acetone extracts. The IC_{50} values were 76.25 and 60.0 μg/mL for $CHCl_3$ and acetone extracts of *S. colebrookiana* and 69.7 and 65.5 μg/mL for *S. violacea* in scavenging of DPPH stable radical. According to Fe^{3+}-ascorbate system in rat liver homogenate, both $CHCl_3$ and acetone extracts were active for both medicinal plants. For *S. colebrookiana,* IC_{50} values for $CHCl_3$ and acetone extracts were 69.7 and 110 μg/mL and for *S. violacea* 64.6 and 120 μg/mL, respectively. Baicalin occurance in the $CHCl_3$ extracts were investigated and baicalin was detected in $CHCl_3$ extracts of the both *Scutellaria* species (33).

S. litwinowii which grows wild in Iran and Afghanistan was investigated in terms of its antioxidant activity. MeOH, CH_2Cl_2 and EtOAc extracts from the aerial parts of *S. litwinowii* were prepared. They used thiobarbituric acid reactive species (TBARS) and DPPH radical scavenging assays. The greatest activity in both assays was shown by MeOH extract, nevertheless all the extracts showed lower activity than those of the reference compounds vitamin E and butylated hydroxytoluene (34).

2.1.2.2. Studies on Antitumor Activity

S. baicalensis is widely used in Chinese traditional medicine as an antiinflammatory and anticancer drug. Wu et al., studied the mechanism of the anticancer activity of *S. baicalensis* on head and neck squamous cell carcinoma (HNSCC) and its effect on cyclooxygenase-2 (COX-2). *S. baicalensis* reduced the

prostaglandin E_2 (PGE_2) levels in SCC-25 cells which is a cell line of HNSCC. After 12 hours of treatment with *S. baicalensis*, the inhibition of PGE_2 synthesis was similar to a COX-2 inhibitor, celecoxib. Furhermore, the extract decreased the COX-2 protein expression. To sum up, it was found as an effective therapeutic agent in the treatment of HNSCC (35).

Antiproliferative and apoptotic activities of *S. baicalensis* were examined against acute lymphocytic leukemia, lymphoma and myeloma cell lines by Kumagai et al. *S. baicalensis* was shown to suppress the acute lymphocytic leukemia, lymphoma and multiple myeloma cell lines (36).

Ye et al. prepared aqueous extract from *S. baicalensis* roots for examining the antiprostate cancer activity. The study showed that *S. baicalensis* had considerable dose-dependent inhibitory effect versus both androgen-dependent and -independent types of prostate cancer with the IC_{50} values of 0.15 mg/mL and 0.1 mg/mL, respectively. The capability of the extract for cytotoxicity on prostate cancer cells was further confirmed by *in vivo* experiments (37). Authors also investigated the cytotoxic activity of *S. baicalensis* on hepatocellular carcinoma cell line (HepG2). *S. baicalensis* inhibited the growth of HepG2 cells with the IC_{50} value of 360 μg/mL in a dose dependent manner (38).

Saiboku-to (herb mixture) is a traditional Chinese medicine that suppresses leukotriene (LK) production and release. Murashima et al. found that Saiboku-to suppressed LK production and release. After exposure to estrogen-responsive Mouse Leydig tumor cell line (B-1F), the cell proliferation was determined by the yield of viable cells. However, when B-1F cells were treated with Saiboku-to in the absence of 17β-estradiol (E2), the cell proliferation was increased. The most effective herb in the mixture, *S. baicalensis*, totally suppressed B-1F cell proliferation at 50-100 μg/mL. Growth inhibitory effects of the plant was seen in both condition with or without E2 (39).

Wang et al. formerly investigated the activity of *S. baicalensis* on breast cancer cell lines and could not find any inhibitory effect. Therefore it was planned to study the fractions of *S. baicalensis*. The fraction without baicalin (SbF1) and another fraction

with baicalin (SbF3) was obtained in order to analyse the anti-breast cancer activity by using MCF-7 cancer cells. Exposure with 100 µg/mL of SbF1 for 3 days inhibited MCF-7 cell growth by 81.6%, however, 100 µg/mL of SbF3 increased cell growth by 22.6%. SbF1 was found to inhibit the cell growth by intervention to S- and G2/M phases, by increased induction of cell apoptosis. Furthermore, the flavonoids scutellarin, baicalin, baicalein and wogonin were evaluated. Baicalein and wogonin were found to suppress the MCF-7 cell growth, on the contrary, scutellarin and baicalin increased the cell growth (40).

Peng and colleagues enlightened that the *S. baicalensis* total flavonoids suppressed the growth of U14 cervical cancer *in vivo*. The compounds were orally administered to mice at a dose of 250, 500 and 1000 mg/kg of the body weight. The cell proliferation was inhibited by arresting cell cycle and the cell apoptosis by regulating the expression of Bax and Bcl-2 gene by the treatment of *Scutellaria* flavonoids (41).

Konoshima et al. investigated the antitumor properties of the flavonoids obtained from the acetone extract of *S. baicalensis* root. 5,7,2'-trihydroxyflavone and 5,7,2',3'-tetrahydroxyflavone elicited a significant inhibitory activity on mouse skin tumor promotion in *in vivo* two-stage carcinogenesis test. Both flavonoids also reduced and delayed the papillomas in mice (42).

Ikemoto et al. conducted *in vitro* and *in vivo* experiments in order to explain whether *Scutellaria* radix (*S. baicalensis*) and its main flavonoids baicalein, baicalin and wogonin have an antitumor effect or not. Healthy mice were subcutenously injected with murine bladder cancer cell lines (MBT-2). *Scutellaria* root was administered orally by mice. When *Scutellaria* root was given for 10 days as 10 mg/day, the tumor growth was remarkably inhibited. In *in vitro* studies, baicalein, baicalin, wogonin and *Scutellaria* roots were tested for their inhibitory effects on human bladder cancer cell lines (KU-1 and EJ-1) and MBT-2. All of the compounds inhibited cell proliferation in a dose-dependent manner. Among them baicalin showed the most significant antitumor activity. Also researchers gave 1 g of *Scutellaria* radix (oral) to the five healthy volunteers (one of them also) and serum samples were taken at 1, 3, 6, 12 and 26 hours after administration. Antitumor activity of the serum on EJ-1 cells were also

investigated with the pharmacokinetics studies and the results showed that the greatest activity was seen after six hours of administration (43).

Anticancer activity of wogonin, an active ingredient of *S. baicalensis*, was investigated by Dong et al. At the concentration of 10 μM, wogonin inhibited the proliferation of human gallbladder carcinoma GCB-SD cells, human prostate carcinoma LNCaP cells and human U-937 leukemia cells *in vitro*. Though, at the concentrations of 1-10 μM, wogonin did not affect the viability of the cells, but it suppressed the mobility and invasion activity of human gallbladder carcinoma GCB-SD cells. When the mechanism was investigated, it was found that wogonin upregulated the expression of metastasis suppressor maspin (44).

Sonoda et al., isolated 17 flavonoids from the roots of *S. baicalensis* and *S. rivularis* and tested them against human leukemia cell line HL-60 *in vitro*. 2',3',5,7-tetrahydroxyflavone was found to be the most active flavonoid against HL-60 cells with the IC_{50} value 9.5 μM. Apigenin, wogonin and viscidulin III followed 2',3',5,7-tetrahydroxyflavone with the IC_{50} values of 15, 17.4 and 17.4 μM, respectively in inhibiting the proliferation of HL-60 cells. Baicalein, scullcapflavone II, isocarthamidin, 4'-hydroxywogonin, luteolin and scutellarein showed moderate activity. On the other hand, only oroxylin A stimulated the proliferation of the cancer cell lines (45).

Yin et al., demonstrated that the EtOH extract of *S. barbata* remarkably inhibited the growth of human lung cancer cell line A549 with the IC_{50} value of 0.21 mg/mL via cell apoptosis and cytotoxic effects (46).

Kim et al., studied the effect of *S. barbata* on human leiomyomal cell culture. Antiproliferative activity of *S. barbata* was shown with 10^{-5} M buserelin (gonadotropin-releasing hormone) or with 20-40 μg/mL *S. barbata* rhizoma extract. When protein kinase C (PKC) was added, the inhibition of cell growth decreased and then the decrease in the viable cells caused by addition of *S. barbata* extract was reversed by pretreatment with PKC inhibitor (calphostin C). It is understood that *S. barbata* suppressed the cell proliferation in human uterine leiomyoma cells, accompanied by PKC activation (47).

In another experiment, *S. barbata* was examined for its chemopreventive effects. Development of preneoplastic lesions on carcinogen treated mouse mammary glands, was suppressed by *S. barbata.* Moreover, inhibiton of tumorigenesis in mouse skin cancer model was also observed *in vitro*. *S. barbata* had also an inhibitory activity on proliferation of gynecological cancer cell lines like HeLa cell and human ovary cancer cells (12).

Cha et al., explained that the CH_2Cl_2 extract of *S. barbata* suppressed the human U937 leukemia cells by apoptosis via the mitochondrea-mediated signalling pathway (48).

Powell et al. investigated the apoptotic activity of aqueous extract from the aerial parts of *S. barbata* which is traditionally used to treat ovarian and breast cancers. The experiments revealed that 100% of ovarian and 50% of breast cancer cell lines were killed by the dilutions of *S. barbata*. Besides at the end of the study, the resistant cell lines were present. This means that *S. barbata* killed the cell lines via a specific biological pathway. The authors concluded that *S. barbata* arrested the cell lines via apoptosis (49).

Bendong et al., studied the antitumor activity of the EtOH extract of *S. barbata in vivo.* Initially, mice were transplanted with human hepatocellular carcinoma (HepG2). Between the doses of 50-200 mg/kg, extract reduced the tumor weight in a dose dependent manner. Additionally, the levels of proliferating cell nuclear antigen (PCNA), vascular endothelial growth factor (VEGF) and CD13 in tumor cells were quantified and it was found that *S. barbata* EtOH extract reduced the protein expression of PCNA, VEGF and CD31. The authors concluded that the antitumor activity of the extract may come from targeting the VEGF protein (50).

Lee et al., prepared a decoction from *S. barbata* and investigated its effects on human leimyomal cells and myomatrial smooth muscle cells *in vitro*. H_2O extract inhibited the growth and induced the apoptosis of leiomyomal cell lines LM-1 and LM-2. When compared with the myomatrial smooth muscle cells, leimyomal cells had lower viability at low concentrations of *S. barbata* extract. As a result of a series of experiments, it was concluded that *S. barbata* suppressed the growth of the leiomyomal

cell lines and induced apoptosis. The apoptosis induced by the extract was related with the release of cytochrome C from the mitochondria followed by an increase in caspase 3-like activity (51).

In a study that the 12 Chinese traditional medicinal herbs were used, the herbs were analysed for their antiproliferative activity on eight cancer cell lines and on normal human mammary epithelial cells *in vitro*. Five human and three murine cancer cell lines (breast, lung, pancreas and prostate) were used. Among them, *S. barbata* showed a positive activity against all the cancer cell lines also on human breast cancer cell lines. Plus, *S. barbata* was inactive against normal human mammary epithelial cells. Authors indicated that *S. barbata* may have specificity against cancer cell lines versus normal cell lines (52).

Goh et al., demonstrated that standardized extract of *S. barbata* induced cell death in the human colon cancer cell lines (53).

Yu et al., examined the antitumor activity of several fractions obtained from EtOH extract and the active constituents of the most active fraction of *S. barbata*. EtOH extract was fractionated by *n*-hexane, $CHCl_3$, EtOAc and 1-BuOH, respectively. Among them, the $CHCl_3$ fraction displayed the most remarkable cytotoxicity on cancer cell lines with a lower cytotoxic activity on a normal liver cell lines. Also, $CHCl_3$ extract showed an antitumor activity *in vivo*. Four compounds isolated from the $CHCl_3$ extract were phytol, wogonin, luteolin and hispidulin. The cytotoxicity of the pure compounds tested on human liver carcinoma cell lines and all of them exhibited the cytotoxicity against the tested carcinoma cells (54).

Diterpenoids (Scutebata A, B, D, E and F) isolated from *S. barbata* by Zhu et al., were tested for their cytotoxicity against human cancer cell lines (HL-60, SMMC-7721, A-549, SK-BR-3,CACO-2 and PANC-1). Among them only scutebata A displayed low cytotoxic effect against SK-BR-3 cell lines with IC_{50} value of 15.2 μM (55).

The first report on cytotoxic activitiy of *S. litwinowii* was published in 2011. The plant was collected from Iran and extracted with MeOH and partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH by using Kupchan method. Total MeOH extract of the drug was investigated on several malignant cell lines (AGS, HeLa, MCF-7, PC-12). The

results displayed that the MeOH extract reduced the cell viability and the toxicity began at the concentration 80 μg/mL. *n*-hexane fraction of the plant also showed antiproliferative activity on those cell lines. CH_2Cl_2 fraction showed the most signifiant activity on the malignant cancer cell lines than the other fractions. This fraction displayed inhibitory effect on the proliferation of malignant but not on non-malignant cells indicating the specificity (56).

Scutelinquanine D and 6-acetoxybarbatin C were the two *ent*-clerodane diterpenoids from *S. barbata.* These two diterpenes were tested against the four human cancer cell lines (HONE-1 nasophyrngeal, KB oral epidermoid carcinoma and HT-29 colorectal carcinoma cells). *In vitro* tests showed that both were significantly active against all the cancer cell lines (57).

There are few studies concerning the polysaccharides of *S. barbata.* One of them belongs to Gaochen et al. They obtained polysaccharides from H_2O extract and alcohol precipitation with Molisch test. Hepatocarcinoma H22 bearing mice administered polysaccharide fraction for ten days. When the concentration of the polysaccharide fraction of *Scutellaria* was 50 mg/kg, H22 liver cancer suppression rate was found as 34.35%. The results revealed that *Scutellaria* polysaccharides elicited significant antitumour activity (58).

Dai et al. studied *S. barbata* diterpenoids in a very comphrehensive pattern. They isolated many diterpenoids from this plant and investigated their cytotoxic activities. The authors used the methylene blue dye assay in order to evaluate the activity of the tested substances on cell growth. Known chemotherapeutics etiposide and cisplatin were used as reference drugs and the activities of the isolates were compared with those of the references. The cancer cell lines used in the assays were human nasopharyngeal carcinoma, HONE-1, oral epidermoid carcinoma, KB, and colorectal carcinoma, HT29. In 2006, Dai and colleagues isolated barbatins A-C, in 2007 scutebarbatines C-H, 6,7-di-*O*-nicotinoylscutebarbatine G, 6-*O*-nicotinoyl-7-*O*-acetylscutebarbatine G and 7-*O*-nicotinoylscutebarbatine H, in 2008 scutebarbatine I-L, in 2009 scutebarbatine O and 6-*O*-nicotinoylscutebarbatine G, scutehenanines A-D, 6-*O*-scutehenanine A and 6-*O*-(2-carbonyl-3-methylbutanoyl) scutehenanine A, scutehenanine H and 6-(2,3-epoxy-2-isopropyl-n-propoxyl) barbatin C and finally in

2011 they obtained scutebarbatines M-N from *S. barbata*. The authors tested all of the purified phytoconstituens for their cytotoxic activity as mentioned and found all of them active to those cell lines (59–66). The same study was also performed by Nie et al. They isolated three new *neo*-clerodane diterpenoids named scutelinquanines A-C. Cytotoxic activities of this three phytochemicals against HONE-1, KB and HT29 cancer cell lines were tested and the IC_{50} values in the range 2.7-6.7 μM (67). In addition Dai et al. also studied the antitumor effect of *S. barbata* extract on murine liver cancer *in vitro* and *in vivo*. MTT assay was used to determine the growth inhibition of Mouse hepatoma H22 cells *in vitro*. *In vivo* tests were performed on H22 tumor bearing mice. The results indicated that the extract suppresses the growth of hepatoma H22 cells *in vitro* and *in vivo*. Besides, the extract improved the immune function of H22 tumor bearing mice. In conclusion, the plant was found to be safe and active for the use of antitumor therapy (68).

Özmen et al. studied the antileukemic acitivity of *S. orientalis* subsp. *carica.* The plant material was collected from Aydın, Turkey. Plant material was extracted with petroleum ether, CH_2Cl_2, EtOAc and MeOH. The most significant antileukemic effect was seen in MeOH extract, which contains apigenin, baicalein, chrysin, luteolin and wogonin. The activity was linked to the antiproliferative effect of baicalein and the genotoxic property of wogonin (69).

Najaran et al., demonstrated that the MeOH extract of *S. lindbergii* showed cytotoxic activity against AGSi HeLa, MCF-7 and PC-12 cell lines. Then total extract was fractionated by *n*-hexane, CH_2Cl_2 and EtOAc. Among them the CH_2Cl_2 fraction elicited the most remarkable antiproliferative activity *in vitro*. The activity was only shown on malignant cells not on non-malignant cells (70).

2.1.2.3. Studies on Antimicrobial Activity

In a screening study, antifungal activities of 56 medicinal plants of China, including *S. baicalensis*, were investigated. *Aspergillus fumigatus*, *Candida albicans*, *Geotrichum candium* and *Rhodotorula rubra* strains were used as target fungi. *S. baicalensis* radix was found to be active against all fungus strains. Among the tested 56

plants against *S. baicalensis* was found to be the most active medicinal plant on *Candida albicans* (71).

Plants with antifever activity were screened for their human immunodefficiency virüs type-1 protease (HIV-1 PR) inhibiton activities by Lam et al. They prepared the H_2O extract from the roots of *S. baicalensis* and tested for HIV-1 PR. *S. baicelensis* showed a great inhibition (90% <) at a concentration of 200 µg/mL (72).

C. albicans is one of the widespread human fungal pathogens and causes candiasis. In a study that seven traditional Chinese medicinal materials (*Paeonia suffruticosa, Lonicera japonica, Isatis indigotica*, *Sophora flavescens*, *Scutellaria baicalensis, Fraxinus rhyncophylla* and *Andrographis paniculata*) have been investigated, H_2O or EtOH extracts of those plants have been prepared. For the activity analysis, *C. albicans* and *C. tropicalis* strains were used. Among seven medicinal plants only *S. baicalensis* inhibited the growth of both strains. Minimum inhibitory concentrations of aquous extract of *S. baicalensis* on two different C. albicans strains were found as 5 mg/m L and 2.5 mg/mL, respectively. For further tests, four known compounds of *S. baicalensis* (baicalein, baicalin, wogonin and scutellarin) were examined for anticandidal activity and only baicalein inhibited the growth of *C. albicans* (MIC 25 µg/mL) (73).

In a clinical study performed by Arweiler et al. in 2011, 20 patients used toothpaste with *S. baicalensis* extract (0.5% plant extract) for 21 days. Gingival index (GI), plaque index (PI) and biofilm viability (VF%) were calculated at days 0, 14 and 21. GI, PI and VF% values were significantly reduced at the group using the toothpaste with *S. baicalensis*. According to the results, this formulation obviously reduced the extend of gingivitis, plaque development and vital flora (74).

Yang et al. studied the *in vitro* effects of *S. baicalensis* against *Toxoplasma gondii*. Aqueous extract of *S. baicalensis* displayed antitoxoplasmic effect and decreased replication treatment after 72, 96 and 120 h of treatment (75).

Chang and colleagues examined the synergistic acitivity of *S. baicalensis* with several antibiotics (penicillin G, gentamicin, ciprofloxacin and ceftriaxone). *S.*

baicalensis showed synergistic activity with those four antibiotics, though, with ciptofloxacin it exhibited the most effective antimicrobial activity (76).

Nan et al., prepared total flavonoid extract from *S. baicalensis* and tested their antibacterial activity on *Staphylococcus aureus* and *Klebsilla pneumonia* strains. The results showed that minimum inhibitory concentration of this special extract was 1.56 μg/mL for *S. aureus* and 3.13 μg/mL for *K. pneumonia* (77).

Scutellaria amonea has been used in traditional Chinese medicine in order to treat infectious diseases. Liu et al. isolated baicalin from this herb and studied its synergistic activity with β-lactam antibiotics against methicillin-resistant *Staphylococus aureus* (MRSA) and other β-lactam resistant strains of *S. aureus*. When the authors combined 16 μg/mL baicalin with benzylpenicillin against MRSA and penicillin resistant *S. aureus*, minimum inhibitory concentrations were decreased from 125 and 250 μg/mL to 4 and 16 μg/mL, respectively. Results showed that antibacterial activities of ampicillin, amoxycillin, benzylpenicillin, methicillin and cefotaxime against MRSA and β-lactam resistant *S. aureus* at 10 to 50 μg/mL, were increased by 25 μg/mL baicalin (78).

Essential oil of *S. grossa* was obtained by steam distillation. Antibacterial activity of the oil was tested against different stains of gram positive and gram negative bacteria. Essential oil showed a significant inhibitory activity against *K. pneumonia* and *B. subtilis*. Against *Enterococcus faecalis*, the oil showed the lowest minimum inhibitory concentration at 31.25 μL/mL wheras against *K. pneumonia*, *B. subtillis* and *S. enterica* the minimum inhibitory concentration was 62.5 μL/mL (79).

Skaltsa et al., isolated essential oil from the aerial parts of *S. albida* L. subsp. *albida*. The oil was then tested against two gram positive bacteria (*S. aureus* and *B. subtilis*), two gram negative bacteria (*E. coli* and *P. aeruginosa*) and a yeast (*S. cevisiae*). Essential oil was found to be active against all microorganisms tested (80).

The essential oil of *S. barbata* was obtained by hydrodistillation and tested it against several microorganisms. *Staphylococcus epidemidis* was the most susceptible pathogen tested with the strong inhibition zone (29 mm, MIC 0.77 mg/mL), followed by *S. aureus* (MIC 1.53 mg/mL) and *S. heamolyticus* (MIC 1.53 mg/mL) with stronger

inhibiton zones (21-26 mm), *Salmonella paratyphi A* (MIC >49.00 mg/mL), *Enterococcus faecalis* (MIC 12.25 mg/mL) and *Candida albicans* (MIC 24.50 mg/mL) exhibited weak inhibiton zones (7-9 mm). MIC results showed that except *S. parathypi* A, all the microorganisms were sensitive to essential oil (81).

The study performed by Li et al. was about antiviral activities (herpes simplex virüs type-1 and human respiratory syncytial virus) of 21 Chinese medicinal plants. Among the tested materials, aqueous extract of *S. indica*, showed anti-RSV activity with IC_{50} value lower than 50 μg/mL wheras it did not exhibit anti-HSV-1 activity *in vitro* (82).

2.1.2.4. Studies on Antidiabetic Activity

Nishioka et al. screened 27 traditional Chinese medicines for their α-glucosidase inhibiton. The enzyme α-glucosidase, catalysis the last step in the chemical process of carbohydrates and so α-glucosidase inhibitors can decelerate the absorption of carbohydrates to suppress postprandial hyperglycemia. Ogon, namely *S. baicalensis* MeOH extract showed 70% inhibiton of α-glucosidase. Chromatographic purification of the extract yielded baicalein as an active substance (83).

In a study by Waisundara et al., along with the antioxidant activity of *S. baicalensis*, antidiabetic activity of metformin + *S. baicalensis* were investigated by performing *in vivo* techniques. In the group that the diabetic rats were treated with metformin 500 mg/kg + *Scutellaria* 400 mg/kg, it was shown that insulin levels of plasma and pancreas were significantly elavated (21).

In a recent study conducted by Liu et al., baicalin, berberine, baicalin + berberine, and Radix *Scutellaria* + Rhizoma *Coptidis* extract were orally given to the streptozocin induced diabetic rats. Body weight, food intake, urine volume, urine sugars, fasting blood glucose and fasting plasma insulin were monitored. It was found that baicalin and berberine together ameliorated the increases in urine volume, urine sugar, food intake and fasting plasma glucose than they were administered alone. Also the combination of the *Scutellaria* + *Coptidis* extracts has shown to posses antihyperglycemic effect. It could be said that baicalin and berberine might have a synergistic effect (84).

In a study performed in order to identify the relation between antioxidant and antidiabetic activity of medicinal herbs, a strong antioxidant plant *S. baicalensis* and a strong antidiabetic plant American ginseng were used. H_2O extracts of the plants were prepared. American ginseng was given intraperitoneally at the concentration of 300 mg/kg for 12 days and it resulted in reduced body weight and reduced fasting blood glucose levels in obese mice. On the contrary, when the *S. baicalensis* extract was given to mice at the concentration 5-50 mg/kg, it did not show any significant effect on body weight and glucose levels (85).

Kuroda, Iwabuchi and Mimaki studied on the MeOH extracts of 37 plants in order to screen their possible α-glucosidase inhibition. Among the tested materials, *S. laterifolia* elicited a potent inhibitory activity for sucrase and maltase activities *in vitro*. Bioactivity guided fractionation ended in isolation of baicalein and baicalin as significant inhibitors. Other compounds such as luteolin, acteoside, leucoseptoside A and isoacteoside showed weak inhibitory activities (86).

2.1.2.5. Studies on Antiinflammatory Activity

Kubo et al., examined the antiinflammatory activity of *S. baicalensis* and main flavonoids by using several *in vivo* methods. First of all edema was induced by carrageenan in rats and edema at the hind paw was calculated, and then separately 70% MeOH extract (500 mg/kg), baicalein (100 mg/kg), baicalin (100 mg/kg) and wogonin (100 mg/kg) were given to the rats. Except wogonin, all the tested substances inhibited the edema formed by carrageenan. In whittle method, test substances were orally given to the mice 1h before the injection of dye. 15 min after the dye injection 1% acetic acid was injected intraperiotaneally. After 20 min mice were killed and and viscera from the abdomine was taken and absorbance was measured. By this method, vascular permeability induced by acetic acid was determined. All the tested materials decreased the dye leakages in a dose dependent manner. To sum up, MeOH extract of *S. baicalensis* and the main flavonoids were found to be effective in the treatment of inflammatory conditions (3).

The activity of wogonin on nitric oxide (NO) synthesis and cyclooxygenase-2 (COX-2) induction was studied by using macrophage cell line, RAW 264.7 by Chi et al.

The results showed that wogonin, one of the main flavonoids of *Scutellaria* radix, inhibited NO and prostaglandin E2 (PGE2) formation. When added to the media after induction of NO synthase and COX-2, wogonin suppressed the production of PGE2 but not NO. Wogonin was also found to have an inhibitory effect on COX-2 activity, directly on the homogenate of aspirin pretreated RAW cells, but not inhibited nitric oxide synthatese (NOS) or phospholipase A2 activity. According to the western blotting technique, wogonin was found to have inhibitory effect on the induction of both NOS and COX-2, like prednisolone. One of the steroid receptor antagonists (RU-486) reversed the inhibitory activity of prednisolone and did not affect the suppressive activiy of wogonin. Thus, it might be said that the activiy of wogonin on inhibitory conditions was not mediated by binding to a steroid receptor (87).

Kim et al., extracted *S. baicalensis* with H_2O and then partitioned with *n*-hexane, $CHCl_3$, EtOAc and BuOH, respectively. The BuOH fraction was concentrated and then tested against inflammatory mediator. The extract inhibited NO, inducible NOS (iNOS), COX-2, PGE2 as well as many different cytokines (IL-1β, IL-2, IL-6, IL12 and TNF-α). The inhibition mechanism was explained by down regulation of IKKαβ, IKβα, NF-KB activation via suppression of c-Raf-1/MEK1/2 and MAP kinase phosphorylation (88).

Cantharidin, the active principle of mylabris, causes cell death through COX-2 overexpression *in vitro*, as well as raising hemorrhagic cystitis. In traditional Chinese medicine, *S. baicalensis* is generally used for mylabris induced hematuria. *In vivo* tests showed that cantharidin induced cystitis through hematuria via c-Fos and COX-2 overproduction. Distinctly, aqueous extract of *S. baicalensis* suppressed PGE2 production and COX-2 expression in lipopolysaccharide induced RAW 264.7 cells. A decrease was observed in the levels of PGE2 and COX-2 after administration of *S. baicalensis* extract to cantharidin treated rats (89).

Zhang et al. acquired nasal mucosal tissues from 12 patients and preincubated with tissue culture medium and stimulated with anti-IGE for 30 min and 6 hours to imitate the early and late phases of allergy. *S. aureus* superantigen B (SEB) stimulation for 6 hours, was used to imitate T-cell activation. *S. baicalensis* and *Eleutherococcus senticosus* were investigated for their antiinflammatory activities alone and with combination. Inhibition of the release of PGD2, histamine and IL-5 was shown by the

combination of the two medicinal plants. The combination also suppressed the SEB-induced cytokines. The combination of *S. baicalensis* and *E. senticosus,* obviously blocked allergic early and late phase mediators and inhibited the release of proinflammatory and Th1-,Th2- and Th17- derived cytokines (90).

Lin and Shieh investigated the antiinflammatory effect of *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH and H_2O extracts of *S. rivularis* along with the main flavonoids of the $CHCl_3$ extract (baicalin, baicalein and wogonin). According to the carrageenan induced paw edema method, all extracts were found to be active. As the most potent one was $CHCl_3$ extract with the inhibiton of the edema of 51.5% inhibition at a dose of 100 mg/kg, purification studies performed on this extract led to the isolation of bioactive flavonoids, namely baicalin, baicalein and wogonin. The isolates were also tested for their antiinflammatory activities. All of the isolates were found to be active being baicalin the most active one (20 mg/kg) in this experimental method (4).

2.1.2.6. Studies on Hepatic System

Kimura et al. evaluated the effects of radix *S. baicalensis* and its flavone components on lipid metabolism *in vivo*. Rats were orally administrated with radix *Scutellaria* powder (2g/kg), H_2O extract (1g/kg), and several flavonoids from *Scutellaria* radix [wogonin, skullcapflavone II, baicalein and baicalin (100 mg/kg)]. At the end of three days, liver was weighed, 2 g of the liver tissue was homogenized and the blood was taken by cardiac puncture. Baicalin and baicalein decreased the serum free fatty acid and triglyceride levels and liver triglyceride content. Wogonin suppressed the triglyceride deposition in liver and serum HDL-cholesterol level. Skullcapflavone II decreased the total blood cholesterol level, liver triglyceride content and increased serum HDL-cholesterol (91). Same group also investigated serum and liver lipid levels after administration of *S. baicalensis* flavonoids (wogonin, baicalein and baicalin) and EtOH *in vivo.* They gave rats orally 60% EtOH (10 mL/kg) and flavonoids (100 mg/kg) for 8 days and then killed the rats 24 h later after the last administration. A portion of the liver tissue was homogenized with Krebs-Ringer-phosphate buffer (10 mL, 7.4 pH) and extracted with $CHCl_3$:MeOH (2:1). Blood was taken by cardiac puncture and serum was separated. As a result, it was found that wogonin reduced serum triglyceride level,

baicalin and baicalein decreased total cholesterol, free cholesterol and triglyceride in liver. Baicalin also increased the high density lipoprotein in the serum (92).

Nan et al. studied the antifibrolytic activity of radix *S. baicalensis*, in liver fibrosis, induced by bile duct ligation (BDL), scission and CCl_4 in rats. BDL rats displayed increased liver weight, serum bilirubin and the activities of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase along with hydroxyproline and MDA content in liver. Administration of MeOH extract of *S. baicalensis* for 28 days, reduced stellate cell activation, connective tissue deposition and lipid peroxidation in BDL rat livers and improved serum parameters. In CCl_4 induced toxicity test, treatment with the extract also decreased the degree of hepatocellular injury as evidenced by improved serum parameters and levels of hydroxyproline and MDA (93).

Kim et al. performed a research on hepatoprotective effects of baicalin from *S. baicalensis* against ischemia/reperfusion injury in rat liver. In order to produce injury, rats were subjected to 60 min of ischemia followed by 5h reperfusion and baicalin was administered intraperitoneally 24h and 1h before ischemia. Baicalin ameliorated the increase in serum alanine aminotransferase and hepatic lipid peroxidation and decreased the hepatic glutathione level. Baicalin also suppressed the increase of TNF-α and IL-6. As a result, rats treated with the extract significantly lowered the apoptotic cells against hepatocellular ischemia/reperfusion induced toxicity (94).

Lee et al., demonstrated that H_2O extract of *Scutellaria* radix (from the market) exerted a protective effect on the liver of mice against the high fat diet with chronic alcohol exposure (95).

In a study performed in order to demonstrate the activity of *S. baicalensis* on obesity and dyslipidemia, *S. baicalensis* extract was given to the rats and diabetis mellitus male mice. The extract was given at the doses of 10 mg/kg and 100 mg/kg. A decline in weight gain, reduced serum insulin, serum alanine aminotransferase and triglyceride levels were seen at the end of the study. In conclusion, *S. baicalensis* had the anti-obesitiy and anti-hypertriglyceride activity via improvement of insulin sensitivy and the activation of 5' AMP-activated protein kinase (96). In another study by the same

group, hepatoprotective activities of several fractions of *S. rivularis* (*n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH and H_2O) were assessed by measuring SGOT and SGPT levels and along with the histopathological investigations by producing APAP, CCl_4 and D-GaIN induced liver damages. The results revealed that the $CHCl_3$ and EtOAc subextracts were found to be the most potent hepatoprotector against the CCl_4 induced liver damage. Both extracts also showed the greatest hepatoprotective activity against D-GaIN induced intoxication. While the $CHCl_3$ extract was demonstrated as the most significant protector extract on APAP-induced hepatotoxicity, all five extracts improved the hepatic lesions by compared with glycyrrhizin and silymarin reference drugs (97).

Chiu et al. investigated the hepatoprotective effect of *S. rivularis* aqueous extract. Male wistar albino rats were treated with CCl_4 subcutenously in order to induce hepatotoxicity. After administration of CCl_4 for three days, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamicpyruvic transaminase (SGPT) were increased. Also hepatic fatty degeneration was observed at hepatocytes. *S. rivularis* extract was given to the rats at the concentration of 1g/kg intraperiotanaly. After *S. rivularis* administration, CCl_4 induced SGOT and SGPT levels were decreased and improvement at the hepatocytes fatty degenaration was observed (98).

In a very comprehensive investigation, *in vivo* hepatoprotective effect of baicalein, baicalin and wogonin (isolated from *S. rivularis*) were examined by using three different techniques. Acetominophen (APAP), CCl_4 and β-D-galactosamine (D-GaIN) was used to induce liver damage. The activity was measured by observing the SGOT and SGPT levels, as well as histopatological investigations. In APAP induced liver damage, which produced the most severe damage to the liver, wogonin (5, 10, 20 mg/kg) showed the most significant effect by lowering SGOT and SGPT levels. Baicalein (5, 10, 20 mg/kg) showed moderate effect however baicalin had no significant effect on SGOT levels. In CCl_4 induced liver damage, baicalin (10 mg/kg) and wogonin (5 mg/kg) significantly lowered the serum activities. All the compounds showed similar effects in decreasing SGPT levels. At the end of the assay, in D-GaIN induced liver damage, wogonin (5 mg/kg) was found as the most potent hepatoprotector. In histopatological investigations, all of the tested compounds elicited moderate changes of

hyaline degeneration and a little increase of fatty changes in Kupffer cells and lymphocytes (99).

2.1.2.7. Studies on Cardiovascular System

Wang et al. demonstrated that *S. baicalensis* extract did not change the cardiac output in rats, its hypotensive activity might be due to a decrease in total peripheral resistance in peripheral vascular beds. The decrease in heart rate after injection was because of an autonomic mechanism was involved in the change (100).

Chan et al. performed a research on the protective effect of *S. baicalensis* extract myocardial ischemia-reperfusion injury in rats. Animals received five day pretreatment of the extract (30 mg/kg), had a remarkable reduction in myocardial infarct size and a significant increase in the activity of catalase in hepatocytes (101).

Baicalein, baicalin, wogonin, wogonin 7-*O*-D-glucuronopyranoside, oroxylin A, skullcapflavone II and chrysin were isolated from the roots of *S. baicalensis* and tested for their antithrombic activity *in vitro*. At a concentration of 1 mM, all isolates suppressed collagen induced platalet aggragation. Chrysin, further inhibited adenosine diphosphate induced blood platalet aggregation. In arachidonic acid induced aggregation, baicalin and wogonin were found to be active. Moreover, baicalin and baicalein inhibited the conversion of fibrinogen to fibrin, induced by thrombin. Plus, baicalin and baicalein, on the experimental disseminated intravascular coagulation induced by endotoxin in rats, prevented the reduction of blood platalets and fibrinogen *in vivo* (102).

2.1.2.8. Studies on Neurological System

Wang, Liao and Chen studied the anticonvulsant activity of the H_2O extract of *S. baicalensis* root both using *in vivo* and *in vitro* experimental models. According to the results, the extract had a slight activity on pentylenetetrazol induced clonic seizures, and suppressed the maximal electroshock induced tonic seizures. In $^{36}Cl^-$ uptake analysis, the aqueous extract had no remarkable effect on 25 μM GABA-activated $^{36}Cl^-$ uptake but chlordiazepoxide (10 μM) which is a benzodiazepine agonist, increased the $^{36}Cl^-$ uptake to 125% of control. In conclusion, the aqueous extract of *S. baicalensis* radix

showed the anticonvulsant activity against maximal electroshock induced tonic seizures (103).

Jeong et al. investigated the effects of *S. baicalensis* and its main components on neuroinflammation and memorial impairments in artificial senescence. The mice were injected with D-galactose (120 mg/kg) and sodium nitrate (90 mg/kg) and artificial senescence was generated. The treatment groups were as follows baicalin + baicalein (200 mg/kg), *S. baicalensis* EtOH extract (50 mg/kg, 100 mg/kg) for 14 days. According to the results of Y maze test, the administration of baicalein obviously improved the induced impairment of spontaneous alternation behaviour yet baicalin did not. In addition, crystal violet staining displayed that there were typical neuropathological changes in hippocampus of the mice. Administration of *S. baicalensis* extract at concentrations of 50 mg/kg and 100 mg/kg remarkably attenuated the neuropathological changes. Furthermore, SOD, catalase and MDA levels in brain tissues were calculated as the biomarkers of oxidative stress. *S. baicalensis* at a dose of 100 mg/kg displayed significant antioxidative activity in SOD, catalase activities and MDA levels (104).

Kim et al. demonstrated that *S. baicalensis* has been used for the treatment of stroke in traditional oriental medicine. They extracted the roots with 70% MeOH. The extract was tested for its neuroprotective activity by using four-vessel occlusion model. MeOH extract (0.1-10 mg/kg) was given intraperitoneally, significantly protected CA1 neurons against 10 min transient forebrain ischemica. Neuronal cell density in extract treated ischemic animals was remarkably increased. The optimal neuroprotection was achived by 1 mg/kg extract. Moreover, the *in vitro* tests revealed that *S. baicalensis* inhibited microglial TNF-α and NO production and protected PC12 cells from H_2O_2-induced cytotoxicity. So, the inhibiton of TNF-α and NO production and protection of neuronal cells from the oxidative stress *in vivo* may account for the neutoprotective mechanism of the plant (105).

Choi et al. examined the activity of flavone rich aqueous extract of *S. baicalensis* roots on neuronal cells exposed to oxidative stress. Neuronal HT-22 cells were exposed to low levels of H_2O_2, generated from glucose oxidase under the conditions that caused cell death in 24 h. Cellular survival was remarkably increased in cells treated with

flavones prior to the oxidative insult. Flavones in the extract scavenged ROS in a dose dependent manner and the scavenging ability was maximal at a concentration of 50 μg/mL (106).

Hwang et al. examined the effects of aqueous extract of *S. baicalensis* on memorial impairments. The impairments were induced in male wistar rats by bilateral common carotid artery occlusion (BCCAo) and chronic lipopolysaccharide (LPS) infusion. After 20 days of the BCCAo, *S. baicalensis* was daily adminestered for 40 days. In addition, after seven days of LPS infusion into the fourth ventricle of Fisher-344 rat brains, *S. baicalensis* was given for 32 days. It was found that rats with chronic cerebral hypofusion or chronic LPS infusion displayed spatial memory impairments comparing to control group yet the symptoms were decreased by *S. baicalensis* administration. In addition administration of the extract alleviated the alterations of hippocampal mitogen-activated protein kinase signalling. As a result, it was discussed that *S. baicalensis* might be used for the prevention of alzheimer' s disease and vascular dementia (107).

Shin et al., raised intracerebral hemorrhage by stereotaxic intrastriatal injection of bacterial type VII collagenase in male spraque Dawley rats. This disruption to blood brain barrier led to the inflammatory response and edema formation in the brain and thus causing brain damage. However, administration of the aqueous extract of *S. baicalensis* orally three times (50 mg/kg) 48 h after the intracerebral hermorrhage, reduced the hemorrhage volume, edema percentage, brain H_2O content and inflammation degree. This may be the basis of usage of *S. baicalensis* extract after stroke and trauma (108).

Spinal cord injury leads the formation of proinflammatory factors such as TNF-α, IL-1β, IL-6, COX-2 and ROS leading the apoptosis of neurons and oligodendrocytes. Yune et al., investigated the neuroprotective effects of *S. baicalensis* EtOH extract after the spinal cord injury *in vitro*. *S. baicalensis* therapy suppressed the expression of some inflammatory mediators namely TNF-α, IL-1β, IL-6, COX-2 and nitric oxide syntase with reactive oxygen species. Furthermore, in the *in vivo* experiments, spinal cord injury was produced at T9 of the rats administered *S. baicalensis* extract orally at a dose 100 mg/kg. The extract also inhibited the expression of proinflammatory mediators, protein

carbonylation, nitration and microglial activation at 4h after injury. Lesion cavity and myelin loss were further reduced (109).

Heo et al. studied the inhibitory activity of *S. baicalensis* flavonoids on amyloid β protein induced neurotoxicity. Amyloid β protein increases the free radical production and lipid peroxidation in PC12 nerve cells and leads to apoptosis and cell death. Baicalein and baicalin, the two flavonoids from *S. baicalensis* inhibited the cellular damage caused by amyloid β protein induced reactive oxygen species. By using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction test, lactate dehydrogenase test and trypan blue exclusion test, baicalin and baicalein found to have a dose dependent anti-amyloid β protein toxicity activity. Thus, amyloid β protein may be involved in the pathogenesis of alzheimer' s disease, *S. baicalensis* might be useful in the chemoprevention of alzheimer' s disease (110).

Heo et al. demonstrated that EtOH extract of radix *S. baicalensis* improved spatial memory functions and had a neuroprotective activity in ibotenic acid induced rat models. Treatment with *S. baicalensis,* furthermore repressed the activated microglial cells in hipocampal area (111).

Shang et al. studied the effects of *S. baicalensis* flavonoids (SSF) on memory deficits, neuronal degeneration and abnormal energy metabolism induced by permanent global ischemia in rats. The ischemia was produced by permanent occlusion of the bilateral common carotid arteries. Oral administration of SSF at a concentration of 35 mg/kg for 19-20 days reduced the decrease in learning and memory, attenuated neuronal injury and improved abnormality of energy metabolites in rats induced by ischemia (112).

Zhang et al. demonstrated that SSF remarkably prolonged gasping time and survival time after carotid artery occlusion and decrease malondialdehyde content in damaged brain tissues. Further SSF increased the SOD content in brain tissues after ischemic mice and showed protective activity on cerebral hypoxia and reperfusion brain tissues in middle cerebral artery occlusion procedure. Moreover, SSF had inhibitory activity on platalet aggregation. The results displayed that SSF had a remarkable

protective activity on cerebral ischemia and ischemia-reperfusion induced brain injury (113).

Song et al. investigated the activity of SSF on age-related cognitive impairment and neuronal changes in aged rats. They used Morris H_2O maze task. In this method the aged rats took longer time to find the hidden platform and spent less time in swimming. Also the light/electron microscopic analysis found remarkable neuropathological changes in the aged rats' brain. Pre-treatment with SSF at a concentration of 35-140 mg/kg for 16-21 days significantly improved cognitive diysfunction and neuropathalogical changes (114).

Peredery and Persinger investigated the effect of *S. laterifolia*, *Gelsemium sempervirens* and *Datura stromonium* on the seizures inducted by lithium and pilocarpine *in vivo*. Status epilepticus was induced by single systemic injection of lithium (3 mEq/kg) and pilocarpine (30 g/kg). Rats were continuously administered the plant mixture through H_2O supply for 30 days. Numbers of spontaneous seizures were recorded per day during a 15 min observation interval. Group that received the fluid extract showed no seizures during the treatment. Besides when the treatment discontinued the rats showed numbers of spontaneous seizures (115).

Zhang et al. isolated 12 compounds from *S. laterifolia* and examined the anticonvulsant activity of the extract. Anticonvulsant activity was determined in rat models of acute seizures induced by pilocarpine and pentyletetrazol. In both models, American skullcap was found to have anticonvulsant activity in rodent models of acute seizures (116).

Şenol et al. screened the acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and tyrosinase inhibitory effect of four *Scutellaria* species at the concentrations of 250, 500 and 1000 μg/mL. All of the extracts were found to be inactive against AChE, wheras only *S. orientalis* subsp. *macrostegia* showed some degree of inhibition against BChE. In addition, *S. brevibracteata* subsp. *subvelutina*, *S. brevibracteata* subsp. *brevibracteata*, *S. brevibracteata* subsp. *pannosulo*, *S. orientalis* subsp. *pinchleri*, *S. orientalis* subsp. *carica*, *S. albida* subsp. *colchica* and *S. hastifolia* exerted a moderate inhibition on tyrosinase at 1000 μg/mL (117).

Wogonin, one of the main flavonoids found in *Scutellaria* species was tested by Park et al., in order to evaluate its anticonvulsant effect on strychnine, pentylenetetrazole and electroshock induced seizures *in vivo*. Wogonin was injected intraperitoneally to mice or rats 30 min before the testing. Wogonin did not cause and change in the locomotor activities, but showed sedation and myorelaxation activities. Wogonin blocked convulsion induced by pentylenetetrazole and electroshock. Wogonin, further decreased the electrogenic response score, besides flumazenil reversed this decrease to the level of control group. Results showed that anticonvulsive activity of wogonin were mediated by GABAergic neurons (118).

2.1.2.9. Studies on Psychiatric Disorders

Hui et al., extracted wogonin from *S. baicalensis* and tested its anxiolytic activity *in vivo*. The affinity of wogonin to the benzodiazepine site on the γ-aminobutyric acid$_A$ ($GABA_A$) receptor complex was found to be 0.94 μM. By electrophysiological techniques, it was understood that wogonin increased the GABA-activated ganglion neurons current in rat dorsal root and in *Xenopus laevis* oocytes expressing recombinant rat $GABA_A$ receptors, the enhancement was partially reversed by the co-application of a 1 μM benzodiazepine antagonist anaxate. The acute toxicity and behavioral differences were also inverstigated in mice. Acute lethal activity was found to be low. Oral administration of wogonin (7.5-30 mg/kg) showed an anxiolytic activity similar to diazepam in the elevated plus maze. The anxiolytic activity was also reversed with the benzodiazepine antagonist. As a result, the study was concluded with that the anxiolytic effect of wogonin came from its positive allosteric modulation of the $GABA_A$ receptor via interaction of the benzodiazepine site and has lower toxic effects than benzodiazepines (119).

Lee et al. tested the antistress activity of *S. baicalensis* separately or with the combination of *Schizandra chinensis.* Mice were administered herbal extracts and the combination (equal amounts) before the subjected to a stress by immobilization and electric foot shocks over the five days. Reduced locomotor activity and the percentage of time spent in the open arms of an increased plus maze under stress were alleviated by the combination of the extracts. The activity of the combination was more than the extracts separately. The treatment with the combination (100, 200 and 400 mg/kg)

further decreased the serum corticosterone, spleen size, serum IL-2 levels inducted by stress with alleviating the stress related behaviours (120).

Flavonoid mixture from the roots of *S. baicalensis* was purchased (81.27% baicalin containing) and the effects on depression like behaviours investigated. Flavonoids were injected intraperiotanelly to the mice and it decreased the duration in the tail suspension test and the forced swimming test, exacerbated the symptoms of eyelid ptosis, akinesia and mortality caussed by reserpine, prolonged climbing times, affected the conditioned placed preference and increased sugar consumption in mice. Besides, it did not affect the head twitches induced by 5-hydroxy-*L*-tryptophan, locomotor activity, the toxicity of yohimbine and the body temperature decrease caused by high dose of apomorphine. Taken together, *S. baicalensis* flavonoids were found to have an antidepression activity related to dopamine system (121).

Gafner et al., isolated nine flavonoids (baicalein baicalin, scutellarin, wogonin, 5,6,7-trihydroxy-2'-methoxyflavone, ikonnikoside I, oroxylin A 7-*O*-glucuronopyranoside, dihydrobaicalin and laterifolin from the EtOH extract of *S. lateriflora*. Whole isolates, along with the crude H_2O and EtOH extracts were tested for their 5-HT_7 receptor binding activities. Flavonoids were able to bind to the receptor and contributed to the activity of the extracts. H_2O extract was found to be more active than the EtOH extract. It was stated that glucuronides were more active than the aglycones. They all inhibited binding of [H3]-LSD to the receptor, describing its utilization in folk medicine as sedative (122).

Awad et al., tested the anxiolytic activity of *S. laterifolia* by using *in vivo* techniques. Rats were orally administered H_2O, 50% EtOH and 95% EtOH extracts. Extracts were dissolved in a vehicle containing sweetened condensed milk (100 mg extract/mL). Enhancement in the number of entries into the center of an "open-field arena"; number of unprotected head dips, number of entries and the length of time spent on the open arms of the Elevated Plus-Maze were seen. After the activity tests one of the main flavonoids found in *Scutellaria* species, baicalin and its aglycone baicalein together with GABA and glutamine were calculated in EtOH and H_2O extracts. The quantitiy of baicalin in 50% EtOH extract was found as 40 mg/g, baicalein in 95% EtOH extract was found as 33 mg/g, GABA in H_2O and EtOH extracts approximately

were calculated as 1.6 mg/g and finally glutamine in H_2O extract was found to be 31 mg/g by HPLC (16).

2.1.2.10. Studies on Dermatological System

Kim et al. studied the effect of *Scutellaria* roots alone or in combination with *Aloe vera* on levels of immunoglobulin E (IgE) and inflammatory cytokines in spontaneous atopic dermatitis (AD)-like skin lesions on mice. AD-like skin lesions were induced by the laboratory conditions without air filtration at ambient temperature, humidity and light. 50 mg/kg *Scutellaria* radix extract and the combination of both plants suppressed the IL-5 and IL-10 wheras had no effect on IgE levels (123).

Kimura and Sumiyoshi investigated the activities of the flavonoids isolated from *S. baicalensis* [2',5,5',7-tetrahydroxy-6',8-dimethoxyflavone (1), skullcapflavone II (2), 2(*S*)-2',5,6',7-tetrahydroxyflavanone (3) and 2(*R*),3(*R*)-2',3,5,6',7-pentahydroxyflavanone] on skin damage. UVB light was used to induce irritation and the effects of the flavonoids on skin thickness and elasticity were evaluated following irradiation. Flavonoids were administered orally twice a day for 14 days, at doses of 10-50 mg/kg. The UVB irradiation was given on days of 7 and 8. Skin and ear thicknesses were measured and on 15^{th} day, mice were sacrified and skin was removed for further analyses. Oral administration of 50 mg/kg of compounds 1, 3 and 4 inhibited the skin thickness while compounds 1, 2 and 3 did not show any effect on skin thickness at doses of 10 mg/kg. Ear thickness was also reduced by compounds 1, 2 and 4 at the concentration of 50 mg/kg. Also, the thickness of epidermis and extracellular matrix in the dermis were increased by UVB radiation. Compound 4 remarkably inhibited the increase in thickness. In conclusion, compounds 1 and 4 purified from *S. baicalensis* roots might be useful for preventing skin inflammation induced by acute UVB radiation (124).

2.1.2.11. Studies on Antifeedant Activity

Seven diterpenes (hastifolin A-G) were purified from S. *hastifolia*. The isolates were tested for their antifeedent activity. Hastifolin A-C were reported to display significant activity at the hastifolin A-C were reported to display signifacant activity at the concentrations of 100 ppm against larvae *S. littoralis* (8).

Formisano and friends obtained the essential oils from three *Scutellaria* species; *S. brevibracteata*, *S. hastifolia*, *S. orientalis* subsp. *alpina*. Binary choice bioassays were used to examine whether oil can modulate the feeding of final stadium larvae of *S. littoralis*. Four different concentrations (1-1000 ppm) were used. According to the results only *S. hastifolia* essential oil inhibited the *S. littoralis* larvae from feeding (9).

Acetone extract prepared from the aerial parts of *S. alpina* subsp. *javalambrensis* yielded seven *neo*-clerodane diterpenes (scutalpin B, scutalpin C, scutalpin D, scutalpin G, scutalpin I, scutalpin J and 11-deacetylscutalpin D). The antifeedant activities of the isolates were evaluated by choice and no choice bioassays against larvae of the lepidopteran *Spodoptera littoralis*. Jodrellin A and jodrallin B were used as positive control group and the results implied that scutalpin C was more active than jodrellin A (125).

In another study, again using the acetone extract of the aerial parts of *S. rubicunda* subsp. *linneana*, two diterpenoids; scutecypol B and scutalbin C were purified. According to the method described by Simmonds and Blaney, both diterpenoids showed a significant antifeedant activity, especially scutecyprol B showed the activity at 100 ppm (126,127).

Bruno et al., conducted a study on two Scilian *Scutellaria* species, namely *S. rubicunda* subsp. *rubicunda* and *S. columnae* subsp. *gussonei*. The acetone extract of the plants led to the isolation of seven compounds. Chromatographic studies on *S. rubicunda* gave jodrellin B, scutegrossin A, scutalsin, scutecyprol B, jodrellin A and scutelbin A, while the studies on *S. columnae* subsp. *gussonei* yielded only one diterpene scutecyprol A. *Spodoptera littoralis*, *Spodoptera frugiperda*, *Heliocoverpa armigera*, *Mamestra brassicae* and *Pieris brassicar* were used to evaluate the activity of the compounds against the final stadium larvae of Lepidoptera. Although jodrellin B, jodrellin A, scutalbin A and scutecyprol B showed a potent antifeedant activity against all five lepidoptera species, scutalsin and scutecyprol A did not deter feeding. The authors discussed that *S. rubicunda* subsp. *rubicunda* contains four potent antifeedants so it provides this plant a very powerful defence system against herbivory wheras *S. columnae* subsp. *gussonei* might be more susceptable to attack due to lack of those active *neo*-clerodanes (128).

2.1.2.12. Studies on Other Activities

Jung et al., explored the activity of 80% EtOH extract of *S. baicalensis* on passive cutaneous anaphylaxis (PCA) reaction in rats and the compound 48/80 induced histamine release from rat peritoneal mast cells *in vitro* and *in vivo*, respectively. The rats were injected with anti-DNP IgE at three dorsal skin sites. 47h later rats administered *S. baicalensis* extract orally and 1h later after administration DNP-HSA in saline with 4% evans blue was injected through the dorsal vein of the penis. Further rat periotaneal mast cells were cultured and purified to examine histamine release. Treatment with *S. baicalensis* led to 6.6% inhibition of the PCA reaction. Further, the extract suppressed the histamine release significantly on rat periotaneal mast cells (129).

Baicalin, baicalein and *S. baicalensis* 70% EtOH extract were tested against noise induced hearing loss in mice. The study lasted 35 days. Each sample was given to mice 30 min before the noise exposure. As a result of the experiments, it was found that *S. baicalensis* significantly decreased the hearing threshold shift, central auditory function damage and cochlear function deficits. Furthermore, the activity of the extract might be related to baicalein, not baicalin as baicalin did not significantly reduce the hearing threshold shift when compared with other samples (130).

2.1.3. Compounds Isolated from *Scutellaria* species

The phytochemical studies conducted on *Scutellaria* species revealed that the genus is mainly characterized by flavonoids, diterpenoids, iridoids and phenylethanoid glycosides. In this section, secondary metabolites isolated from *Scutellaria* species were compiled and depicted in the figures and tables (**A**: aerial parts, **F**: flowers, **L**: leaves, **R**: roots, **W**: whole plant).

2.1.3. Compounds Isolated from *Scutellaria* species

2.1.3.1. Phenolic Compounds

a) Phenolic acids and derivatives isolated from *Scutellaria* species

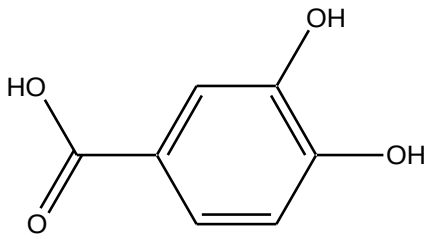


Table 1. Phenolic acids isolated from *Scutellaria* species

Compound	Species	Ref
Protocatechuic acid	*barbata* (W)	(131)

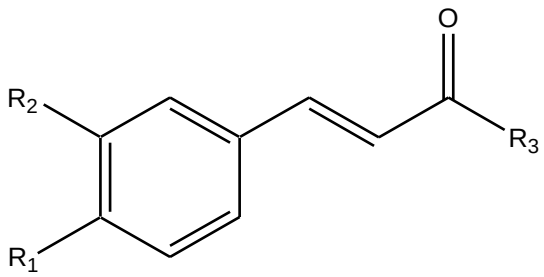


Table 2. Phenolic acids isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
E-caffeic acid	OH	OH	OH	*albida* (A)	(132)
E-ferulic acid	OH	OCH_3	OH	*albida* (A)	(132)
E-*p*-coumaric acid	OH	H	OH	*albida* (A)	(132)
E-*p*-coumaroylglucoside	OH	H	*O*-Glc	*albida* (A)	(132)

Glc: glucose

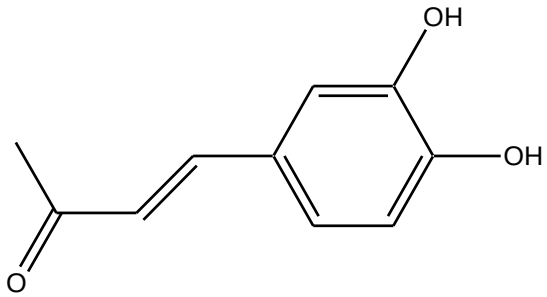


Table 3. Simple phenolics isolated from *Scutellaria* species

Compound	Species	Ref
4-(3,4-Dihydroxy-phenyl)-but-3-en-2-one	*barbata* (W)	(131)

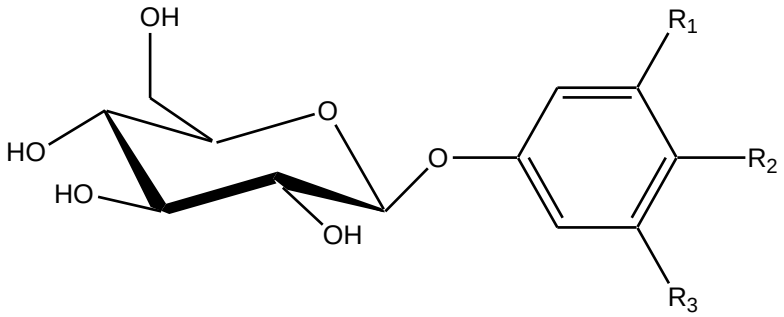


Table 4. Simple phenolics isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
Benzyl-*O*-β-D-glucopyranoside	H	H	H	*albida* (A)	(132)
3,5-Dihydroxyphenyl-*O*-β-D-glucopyranoside	OH	H	OH	*pontica* (A)	(133)
Vanilloside	OCH_3	OH	H	*albida* (A)	(132)

b) Flavonoids isolated from *Scutellaria* species

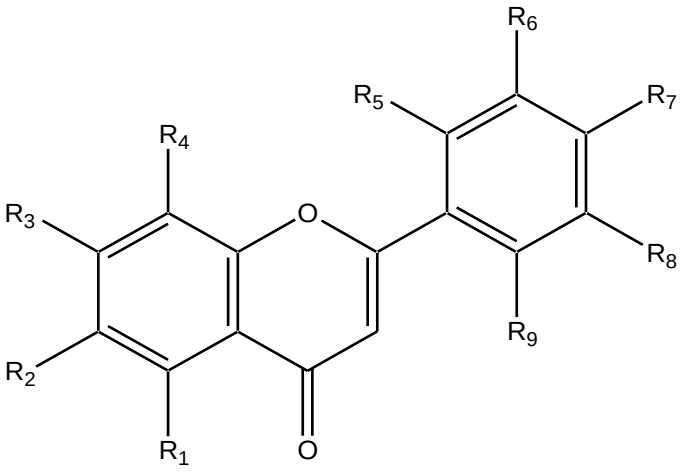


Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Chrysin	OH	H	OH	H	H	H	H	H	H	*discolor* (A) *grossa* (R) *scandens*(R) *indica* (A) *prostrata* (R) *ovata* (F) *linearis* (A) *alpina* (R+L) *lateriflora* (A)	(134–142)
Chrysin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	H	H	H	H	H	*prostrata* (R) *grossa* (R) *indica* (A) *scandens* (R) *discolor* (A) *alpina* (R+L) *ikonnikovii* (W)	(134–137,140, 141,143)
Chrysin 8-*C*-β-D-glucopyranoside	OH	H	OH	Glc	H	H	H	H	H	*amoena* *baicalensis*	(1)
Baicalin	OH	OH	*O*-GluA	H	H	H	H	H	H	*grossa* (R) *planipes* (R) *baicalensis* (R) *rivularis*(R) *prostrata* (R) *scandens* (R) *alpina* (R) *lateriflora* (A)	(20,84, 134,135, 137,141, 144–146)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Baicalein	OH	OH	OH	H	H	H	H	H	H	*alpina* (R) *grossa* (R) *planipes*(R) *prostrata* (R) *scandens* (R) *lateriflora* (A)	(84,134,135, 137,141, 144)
Baicalein 6-*O*-β-D-glucuronopyranoside	OH	*O*-GluA	OH	H	H	H	H	H	H	*grossa* (R)	(134)
Baicalein 7-*O*-β-D-glucopyranoside	OH	OH	*O*-Glc	H	H	H	H	H	H	*planipes* (R)	(144)
Baicalein 7-*O*-L-rhamnoside	OH	OH	*O*-Rha	H	H	H	H	H	H	*galericulata*	(1)
Wogonin	OH	H	OH	OCH_3	H	H	H	H	H	*alpina* (R) *amabilis* (R) *indica* (R) *grossa* (R) *lateriflora* (A) *discolor* (A+R) *linearis* (A) *planipes* (R) *prostrata* (R) *scandens* (R)	(134,135,137,139–142,144,147–149)
7-*O*-Methylwogonin	OH	H	OCH_3	OCH_3	H	H	H	H	H	*linearis* (A)	(139)
Wogonoside	OH	H	*O*-GluA	OCH_3	H	H	H	H	H	*indica* (R) *grossa* (R) *planipes* (R) *baicalensis* (R) *rivularis* (R) *discolor* (R) *scandens* (R) *linearis* (A) *alpina* (R)	(134,137, 139,141,144–148)
Wogonin 5-*O*-β-D-glucopyranoside	*O*-Glc	H	OH	OCH_3	H	H	H	H	H	*baicalensis*	(1)
4'-Hydroxywogonin	OH	H	OH	OCH_3	H	H	OH	H	H	*barbata* (W) *indica* (R) *prostrata* (R) *discolor* (A+R) *amabilis* (R)	(135,140, 147,149–151)
4'-Hydroxywogonin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	OCH_3	H	H	OH	H	H	*albida* (A)	(152)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Norwogonin	OH	H	OH	OH	H	H	H	H	H	*grossa* (R) *planipes* (R) *discolor* (R) *scandens* (R) *alpina* (R)	(134,137, 141,144, 148)
Norwogonin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	OH	H	H	H	H	H	*grossa* (R) *prostrata* (R) *ikonnikovii* (W) *discolor* (R)	(134,135, 143,150)
Norwogonin 8-*O*-β-D-glucuronopyranoside	OH	H	OH	*O*-GluA	H	H	H	H	H	*discolor* (A) *ikonnikovii* (W)	(140,143)
Oroxylin A	OH	OCH_3	OH	H	H	H	H	H	H	*grossa* (R) *racemosa* (W) *prostrata* (R) *scandens* (R) *lateriflora* (A) *baicalensis* (R) *seleriana* (A) *alpina* (R)	(45,134,135,137,141,142,153,154)
Oroxylin A 7-*O*-β-D-glucuronopyranoside	OH	OCH_3	*O*-GluA	H	H	H	H	H	H	*grossa* (R) *racemosa* (W) *prostrata* (R)	(134,135, 153)
Oroxylin A 7-*O*-β-D-glucopyranoside	OH	OCH_3	*O*-Glc	H	H	H	H	H	H	*ovata* (F+R)	(138)
Scutellarin	OH	OH	*O*-GluA	H	H	H	OH	H	H	*grossa* (R) *barbata* (W) *indica* (A) *alpina* (L) *ikonnikovii* (W)	(131,134, 136,141, 143)
Scutellarein	OH	OH	OH	H	H	H	OH	H	H	*grossa* (R) *indica* (A) *alpina* (L)	(134,136, 141)
Scutellarein 7 *O*-β-D-glucopyranoside	OH	OH	*O*-Glc	H	H	H	OH	H	H	*indica* (A)	(136)
Isoscutellarein	OH	H	OH	OH	H	H	OH	H	H	*indica* (A)	(136)
Isoscutellarein 8-*O*-β-D-glucuronopyranoside	OH	H	OH	*O*-GluA	H	H	OH	H	H	*indica* (A)	(136)
Hispidulin	OH	OCH_3	OH	H	H	H	OH	H	H	*racemosa* (W) *alpina* (R)	(141,153)
Hispidulin 7-*O*-β-D-glucuronopyranoside	OH	OCH_3	*O*-GluA	H	H	H	OH	H	H	*creticola*	(1)
Tenaxin I	OH	OCH_3	OCH_3	OCH_3	OH	H	H	H	H	*tenax* *baicalensis*	(1)
Tenaxin II	OH	OCH_3	OH	H	OH	H	H	H	H	*viscidula* *baicalensis*	(1)
Scutevurin	OH	H	OH	OCH_3	OH	H	H	H	H	*indica* (R)	(147)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Scutevurin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	OCH_3	OH	H	H	H	H	*prostrata* (R)	(135)
Apigenin	OH	H	OH	H	H	H	OH	H	H	*barbata* (W) *indica* (A) *pontica* (A) *ovata* (F) *linearis* (A) *discolor* (A) *alpina* (L)	(131,133, 136,138–141)
Apigenin 5-*O*-β-D-glucopyranoside	*O*-Glc	H	OH	H	H	H	OH	H	H	*barbata* (W)	(151)
Apigenin 7-*O*-β-D-glucopyranoside	OH	H	*O*-Glc	H	H	H	OH	H	H	*pontica* (A)	(133)
Apigenin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	H	H	OH	H	H	*prostrata* (R) *alpina* (L) *indica* (A)	(135,136, 141)
Luteolin	OH	H	OH	H	H	OH	OH	H	H	*barbata* (W) *discolor* (A) *grossa* (R) *indica* (A) *ovata* (F) *linearis* (A)	(131,134, 136,138–140)
6-Methoxyuteolin	OH	OCH_3	OH	H	H	OH	OH	H	H	*ovata* (F)	(138)
Luteolin 7-*O*-β-D-glucopyranoside	OH	H	*O*-Glc	H	H	OH	OH	H	H	*creticola*	(1)
Luteolin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	H	OH	OH	H	H	*prostrata* (R)	(135)
Luteolin 7-*O*-diglucuronide	OH	H	*O*-GluA (2-*O*-Glu A)	H	H	OH	OH	H	H	*barbata* (W)	(131)
Ikonnikoside I	OH	OH	*O*-GluA	H	OH	H	H	H	H	*ikonnikovii* (W)	(143)
Viscidulin II	OH	H	OCH_3	OCH_3	OH	H	H	H	OH	*baicalensis* (R)	(45)
Viscidulin II 2'-*O*-β-D-glucuronopyranoside	OH	H	OCH_3	OCH_3	*O*-GluA	H	H	H	OH	*barbata*	(1)
Viscidulin III	OH	H	OH	OCH_3	OH	OH	H	H	OCH_3	*planipes* (R) *rehderiana* (R)	(144,155)
Viscidulin III 2'-*O*-β-D-glucopyranoside	OH	H	OH	OCH_3	*O*-Glc	OH	H	H	OCH_3	*baicalensis*	(1)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Skullcapflavone I	OH	H	OCH_3	OCH_3	OH	H	H	H	H	*planipes* (R) *prostrata* (R)	(135,144)
Skullcapflavone II	OH	OCH_3	OCH_3	OCH_3	OH	H	H	H	OCH_3	*baicalensis* (R)	(145)
Rehderianin I	OH	H	OCH_3	OCH_3	OH	H	H	OH	H	*rehderiana* (R)	(155)
Salvigenin	OH	OCH_3	OCH_3	H	H	H	OCH_3	H	H	*baicalensis*	(1)
Acacetin	OH	H	OH	H	H	H	OCH_3	H	H	*prostrata* (R)	(135)
Acacetin 7-*O*-β-D-glucopyranoside	OH	H	*O*-Glc	H	H	H	OCH_3	H	H	*polydon*	(1)
Acacetin 7-*O*-diglucuronide	OH	H	*O*-GluA (2-*O*-GluA)	H	H	H	OCH_3	H	H	*barbata* (W)	(131)
Pectolinarigenin	OH	OCH_3	OH	H	H	H	OCH_3	H	H	*polydon*	(1)
Ganhuangenin	OH	H	OH	OCH_3	OCH_3	OH	H	H	OH	*rehderiana* (R)	(32)
Altisin	OH	H	OCH_3	OCH_3	OCH_3	H	H	H	OCH_3	*grossa* (R)	(134)
Ovatin	OCH_3	OCH_3	*O*-Glc	H	H	H	H	H	H	*ovata* (R)	(138)
Cirsilineol	OH	OCH_3	OCH_3	H	H	OCH_3	OH	H	H	*barbata*	(1)
Rivularin	OH	H	OCH_3	OCH_3	OH	H	H	H	OCH_3	*indica* (R) *prostrata* (R)	(135,147)
Isovitexin	OH	H	OCH_3	H	H	OCH_3	OCH_3	H	H	*pontica* (A)	(133)
Dinatin	OH	OCH_3	OH	H	H	H	OH	H	H	*ovata* (F)	(138)
5,8-Dihydroxy-6,7-dimethoxyflavone	OH	OCH_3	OCH_3	OH	H	H	H	H	H	*baicalensis*	(1)
5,4'-Dihydroxy-6,7,3',5'-tetramethoxyflavone	OH	OCH_3	OCH_3	H	H	OCH_3	OH	OCH_3	H	*barbata* (W)	(151)
5,7,2'-Trihydroxyflavone	OH	H	OH	H	OH	H	H	H	H	*prostrata* (R) *amabilis* (R)	(135,149)
5,7,2'-Trihydroxyflavone-7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	OH	H	H	H	H	*ikonnikovii* (W)	(143)
5,7,2'-Trihydroxyflavone-2'-*O*-β-D-glucopyranoside	OH	H	OH	H	*O*-Glc	H	H	H	H	*amabilis* (R)	(149)
5,7,2'-Trihydroxy-6-methoxyflavone-7-*O*-β-D-glucuronopyranoside	OH	OCH_3	*O*-GluA	H	OH	H	H	H	H	*amoena*	(1)
5,7,2'-Trihydroxy-8-methoxyflavone-2'-*O*-β-D-glucopyranoside	OH	H	OH	OCH_3	*O*-Glc	H	H	H	H	*amabilis* (R)	(149)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
5,7,3',6'-tetrahydroxy-6,8,2'-trimethoxyflavone	OH	OCH_3	OH	OCH_3	OCH_3	OH	H	H	OH	*planipes* (R)	(144)
5,7,2'-Trihydroxy-8,6'-dimethoxyflavone	OH	H	OH	OCH_3	OH	H	H	H	OCH_3	*discolor* (R)	(150)
5,8,2'-Trihydroxy-7-methoxyflavone	OH	H	OCH_3	OH	OH	H	H	H	H	*baicalensis*	(1)
5,7,8,2'-Tetrahydroxyflavone-7-*O*-β-glucopyranoside	OH	H	*O*-Glc	OH	OH	H	H	H	H	*barbata*	(1)
5,8,2'-Trihydroxy-6,7-dimethoxyflavone	OH	OCH_3	OCH_3	OH	OH	H	H	H	H	*baicalensis*	(1)
5,6,2'-Trihydroxy-7,8-dimethoxyflavone	OH	OH	OCH_3	OCH_3	OH	H	H	H	H	*grossa* (R)	(134)
5,6,2'-Trihydroxy-7,8,6'-trimethoxyflavone	OH	OH	OCH_3	OCH_3	OH	H	H	H	OCH_3	*prostrata* (R)	(135)
5,7,2'-Trihydroxy-6'-methoxyflavone	OH	H	OH	H	OH	H	H	H	OCH_3	*baicalensis*	(1)
5,2',5'-Trihydroxy-6,7,8-trimethoxyflavone	OH	OCH_3	OCH_3	OCH_3	OH	H	H	OH	H	*baicalensis*	(1)
5,2',6'-Trihydroxy-6,7,8-trimethoxyflavone-2'-*O*-β-D-glucopyranoside	OH	OCH_3	OCH_3	OCH_3	*O*-Glc	H	H	H	OH	*baicalensis*(R)	(145)
5,6,2',6'-Tetrahydroxy-7,8-dimethoxyflavone	OH	OH	OCH_3	OCH_3	OH	H	H	H	OH	*prostrata* (R)	(135)
5,2',6'-Trihydroxy-6,7-dimethoxyflavone-2'-*O*-β-D-glucopyranoside	OH	OCH_3	OCH_3	H	*O*-Glc	H	H	H	OH	*baicalensis*(R)	(145)
5,7,2',6'-Tetrahydroxy-8-methoxyflavone-2'-*O*-β-D-(2-caffeoyl)-glucopyranoside	OH	H	OH	OCH_3	*O*-Glc (2-caffeoyl)	H	H	H	OH	*discolor* (A)	(140)
7,2'-Dihydroxy-5-methoxyflavone	OCH_3	H	OH	H	OH	H	H	H	H	*planipes* (R)	(144)
5,7,8,2'-Tetrahydroxyflavone-7-*O*-β-D-glucuronopyranoside	OH	*O*-GluA	H	OH	OH	H	H	H	H	*prostrata* (R) *rivularis* (R)	(135,146)
5,7-Dihydroxy-2'-methoxyflavone	OH	H	OH	H	OCH_3	H	H	H	H	*prostrata* (R)	(135)

Table 5. Flavones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
5,7-Dihydroxy-8,2'-dimethoxyflavone	OH	H	OH	OCH_3	OCH_3	H	H	H	H	*indica* (R) *discolor* (R) *amabilis* (R)	(147–149)
5,7-Dihydroxy-2'-methoxyflavone-7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	OCH_3	H	H	H	H	*prostrata* (R)	(135)
5,7-Dihydroxy-8,2'-dimethoxyflavone-7-*O*-β-D-glucopyranoside	OH	H	*O*-Glc	OCH_3	OCH_3	H	H	H	H	*amabilis* (R)	(149)
5,7-Dihydroxy-8,2'-dimethoxyflavone-7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	OCH_3	OCH_3	H	H	H	H	*indica* (R) *amabilis* (R)	(147, 149)
5,7,2',6'-Tetrahydroxyflavone	OH	H	OH	H	OH	H	H	H	OH	*planipes* (R)	(144)
5,7-Dihydroxy-8,2',6'-trimethoxyflavone	OH	H	OH	OCH_3	OCH_3	H	H	H	OCH_3	*discolor* (R)	(150)
5,7,2',5'-Tetrahydroxyflavone	OH	H	OH	H	OH	H	H	OH	H	*amabilis* (R)	(149)
5,7,2',5'-Tetrahydroxy-8,6'-dimethoxyflavone	OH	H	OH	OCH_3	OH	H	H	OH	OCH_3	*baicalensis* (R)	(145)
5,7-Dihydroxy-6,8,2',3'-tetramethoxyflavone	OH	OCH_3	OH	OCH_3	OCH_3	OCH_3	H	H	H	*baicalensis*	(1)
5,2'-Dihydroxy-7,8,6'-trimethoxyflavone-2'-*O*-β-D-glucuronopyranoside	OH	H	OCH_3	OCH_3	*O*-GluA	OH	H	H	OCH_3	*rivularis* (R)	(146)
7-Hydroxy-5,8-dimethoxyflavone	OCH_3	H	OH	OCH_3	H	H	H	H	H	*discolor* (R)	(150)
7-Hydroxy-5,8-dimethoxyflavone-7-*O*-β-D-glucuronopyranoside	OCH_3	H	*O*-GluA	OCH_3	H	H	H	H	H	*rivularis* (R)	(146)
6,2'-Dihydroxy-5,7,8,6'-tetramethoxyflavone	OCH_3	OH	OCH_3	OCH_3	OH	H	H	H	OCH_3	*baicalensis*	(1)
7-Hydroxy-5,8,2'-trimethoxyflavone	OCH_3	H	OH	OCH_3	OCH_3	H	H	H	H	*discolor* (R)	(147, 150)
8-Methoxyflavone-5-*O*-β-D-glucopyranoside	*O*-Glc	H	H	OCH_3	H	H	H	H	H	*baicalensis*	(1)

GluA: glucuronic acid
Rha: rhamnose

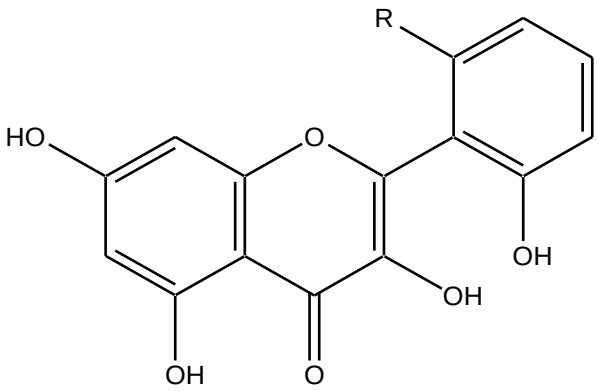


Table 6. Flavonols isolated from *Scutellaria* species

Compounds	**R**	**Species**	**Ref**
Viscidulin I	OH	*planipes* (R)	(144)
5,7,2',6'-Tetrahydroxyflavonol-2'-*O*-β-D-glucopyranoside	*O*-Glc	*amoena*	(1)

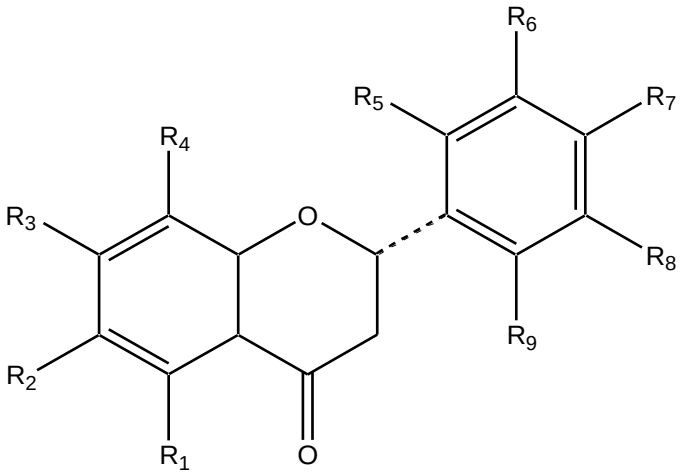


Table 7. Flavanones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Eriodictyol	OH	H	OH	H	H	OH	OH	H	H	*lateriflora* (A)	(86)
Pinocembrin	OH	H	OH	H	H	H	H	H	H	*discolor* (R)	(150)
Dihydrobaicalin	OH	OH	*O*-GluA	H	H	H	H	H	H	*scandens* (R)	(137)
Dihydrobaicalein	OH	OH	OH	H	H	H	H	H	H	*scandens* (R)	(137)
Dihydrooxylin A	OH	OCH_3	OH	H	H	H	H	H	H	*scandens* (R) *lateriflora* (A)	(137,142)
Dihydronorwogonin	OH	H	OH	OH	H	H	H	H	H	*amoena*	(1)
Dihydroscutellarein	OH	OH	OH	H	H	H	OH	H	H	*scandens*	(1)
Dihydroscutevurin	OH	H	OH	OCH_3	OH	H	H	H	H	*indica* (R)	(147)
Dihydrorehderianin I	OH	H	OCH_3	OCH_3	OH	H	H	OH	H	*indica* (R)	(147)
Carthamidin	OH	OH	OH	H	H	H	OH	H	H	*baicalensis* (R)	(145)
Carthamidin 7-*O*-β-D-glucuronopyranoside	OH	OH	*O*-GluA	H	H	H	OH	H	H	*baicalensis*	(1)
Isocarthamidin	OH	H	OH	OH	H	H	OH	H	H	*barbata* *baicalensis*	(1)
Isocarthamidin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	OH	H	H	OH	H	H	*rivularis* (R)	(146)
Dihydrohispidulin	OH	OCH_3	OH	H	H	H	OH	H	H	*baicalensis* (R)	(145)
Scuteamoenin	OH	H	OCH_3	H	OH	H	H	H	OH	*amoena*	(1)
Scuteamoenoside	OH	H	OCH_3	H	*O*-Glc	H	H	H	OCH_3	*amoena*	(1)
Dihydrorivularin	OH	H	OCH_3	OCH_3	OH	H	H	H	OCH_3	*discolor* (R)	(148)

Table 7. Flavanones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
Alpinetin	OCH_3	H	OH	H	H	H	H	H	H	*indica* (R) *amabilis* (R)	(147,149)
Naringenin	OH	H	OH	H	H	H	OH	H	H	*lateriflora* (R)	(86)
Naringenin 7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	H	H	OH	H	H	*lateriflora* (R)	(86)
6-Methoxynaringenin	OH	OCH_3	OH	H	H	H	OH	H	H	*barbata* (W)	(151)
5-Hydroxy-7,8,2',6'-tetramethoxyflavanone	OH	H	OCH_3	OCH_3	OCH_3	H	H	H	OCH_3	*grossa* (R)	(134)
(2*S*)-5,2',5'-Trihydroxy-7,8-dimethoxyflavanone	OH	H	OCH_3	OCH_3	OH	H	H	OH	H	*indica* (R)	(147)
(±)-5,2'-Dihydroxy-6,7,6'-trimethoxyflavanone	OH	OCH_3	OCH_3	H	OH	H	H	H	OCH_3	*indica* (R) *discolor* (R)	(147,148)
(2*S*)-7,2'-Dihydroxy-5-methoxyflavanone	OCH_3	H	OH	H	OH	H	H	H	H	*amabilis* (R)	(149)
(2*S*)-7,2'-Dihydroxy-5-methoxyflavanone-7-*O*-β-D-glucuronopyranoside	OCH_3	H	*O*-GluA	H	OH	H	H	H	H	*amabilis* (R)	(149)
(2*S*)-7,2'-Dihydroxy-5,8-dimethoxyflavanone	OCH_3	H	OH	OCH_3	OH	H	H	H	H	*barbata* (W)	(151)
(2*S*)-5,7-Dihydroxy-8,2'-dimethoxyflavanone	OH	H	OH	OCH_3	OCH_3	H	H	H	H	*discolor* (R)	(148)
(2*S*)-5,2'-Dihydroxy-7,8,6'-trimethoxyflavanone	OH	H	OCH_3	OCH_3	OH	H	H	H	OCH_3	*indica* (R)	(147)
(2*S*)-5,2'-Dihydroxy-7,8,6'-trimethoxyflavanone-2'-*O*-β-D-glucuronopyranoside	OH	H	OCH_3	OCH_3	*O*-GluA	H	H	H	OCH_3	*indica* (R)	(147)
(2*S*)-5,7,2',5'-Tetrahydroxyflavanone	OH	H	OH	H	OH	H	H	OH	H	*amabilis* (R)	(149)
(2*S*)-5,7,2',5'-Tetrahydroxy-6-methoxyflavanone	OH	OCH_3	OH	H	OH	H	H	OH	H	*scandens* (R)	(137)
(2*S*)-5,7,2',5'-Tetrahydroxy-6-methoxyflavanone-2'-*O*-β-D-glucopyranoside	OH	OCH_3	OH	H	*O*-Glc	H	H	OH	H	*scandens* (R)	(137)
(2*S*)-5,7,2',5'-Tetrahydroxyflavanone-7-*O*-β-D-glucopyranoside	OH	H	*O*-Glc	H	OH	H	H	OH	H	*amabilis* (R)	(149)
(2*S*)-5,7,2',5'-Tetrahydroxyflavanone-7-*O*-β-D-glucuronopyranoside	OH	H	*O*-GluA	H	OH	H	H	OH	H	*amabilis* (R)	(149)

Table 7. Flavanones isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	Species	Ref
(2*S*)-5,7,2',5',-Tetrahydroxy-6-methoxyflavanone-2'-*O*-β-D-(2-*O*-feruloyl)-glucopyranoside	OH	OCH_3	OH	H	*O*-Glc (2-feruloyl)	H	H	OH	H	*scandens* (R)	(137)
(2*S*)-5,7,2',5',-Tetrahydroxy-6-methoxyflavanone-2'-*O*-β-D-(2-Sinapoyl)-glucopyranoside	OH	OCH_3	OH	H	*O*-Glc (2-Sinapoyl)	H	H	OH	H	*scandens* (R)	(137)
(2*S*)-5,7,2',5'-Tetrahydroxy-6-methoxyflavanone-2'-*O*-β-D-(2-*O*-vanilloyl)-glucopyranoside	OH	OCH_3	OH	H	*O*-Glc (2-vanilloyl)	H	H	OH	H	*scandens* (R)	(137)
5,7,2'-Trihydroxy-6-methoxyflavanone-7-*O*-β-D-glucuronopyranoside	OH	OCH_3	H	OH	H	H	H	H	H	*amoena*	(1)
(2*S*)-5,7,2'-Trihydroxyflavanone	OH	H	OH	H	OH	H	H	H	H	*indica* (R) *amabilis* (R)	(147,149)
7-Hydroxy-5,8,2'-trimethoxyflavanone	OCH_3	H	OH	OCH_3	OCH_3	H	H	H	H	*discolor* (R) *barbata* (W)	(148,151)
5,6,7,2',3',4',5'-Heptamethoxyflavanone	OCH_3	OCH_3	OCH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	H	*indica* (A)	(136)

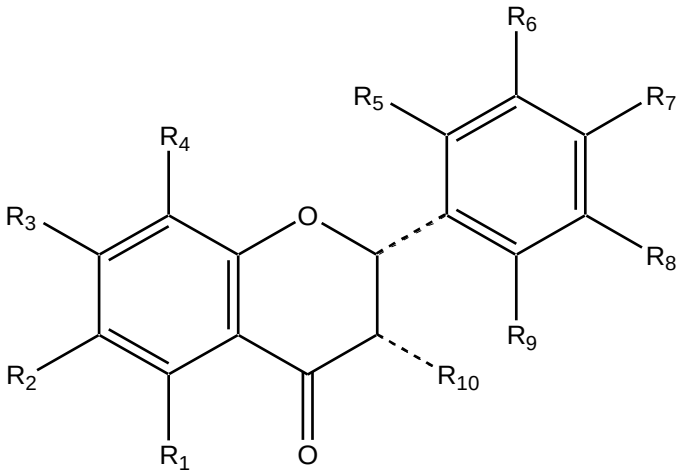


Table 8. Flavanonols isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**	**R_8**	**R_9**	**R_{10}**	**Species**	**Ref**
(2*R*,3*R*)-3,5,7,2'-Tetrahydroxyflavanone	OH	H	OH	H	OH	H	H	H	H	OH	*amoena* *viscidula*	(1)
Ganhuangemin	OH	H	OH	H	OH	H	H	H	OH	OH	*rehderiana* (R)	(32)
(2*R*,3*R*)-3,5,7,2',5'-Pentahydroxyflavanone	OH	H	OH	H	OH	H	H	OH	H	OH	*planipes* (R)	(144)
(*cis*)-3,5,7,2'-Tetrahydroxyflavanone-3-*O*-β-D-glucopyranoside	OH	H	OH	H	OH	H	H	H	H	*O*-Glc	*amoena*	(1)

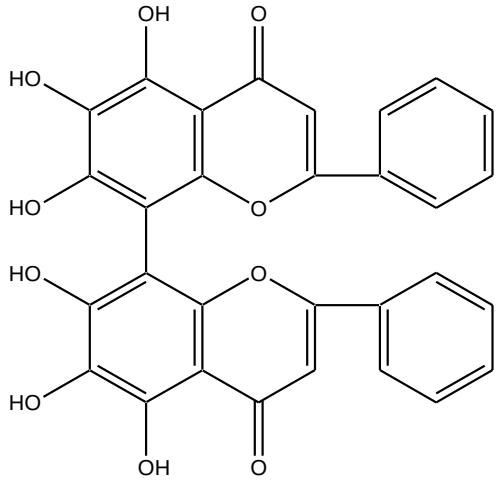


Table 9. Biflavonoids isolated from *Scutellaria* species

Compound	Species	Ref
8,8'-Bibaicalein	*discolor* (R)	(148)

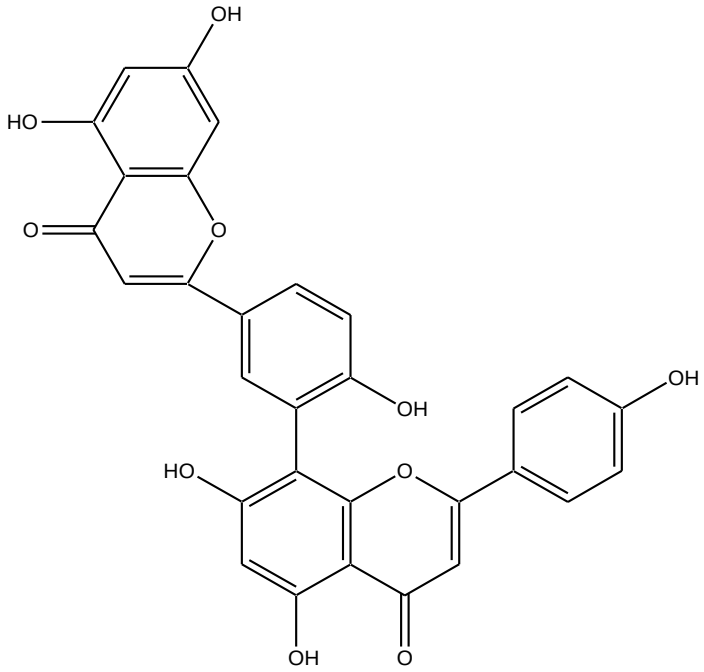


Table 10. Biflavonoids isolated from *Scutellaria* species

Compound	Species	Ref
Amentoflavone	*linearis* (A)	(139)

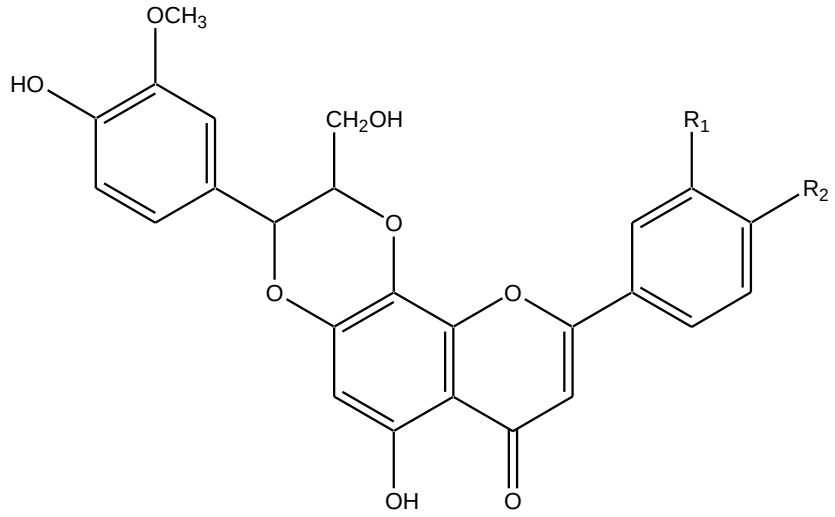


Table 11. Flavolignans isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Scutellaprostin A	H	H	*prostrata* (R)	(156)
Scutellaprostin B	H	OH	*prostrata* (R)	(156)
Scutellaprostin C	OH	OH	*prostrata* (R)	(156)

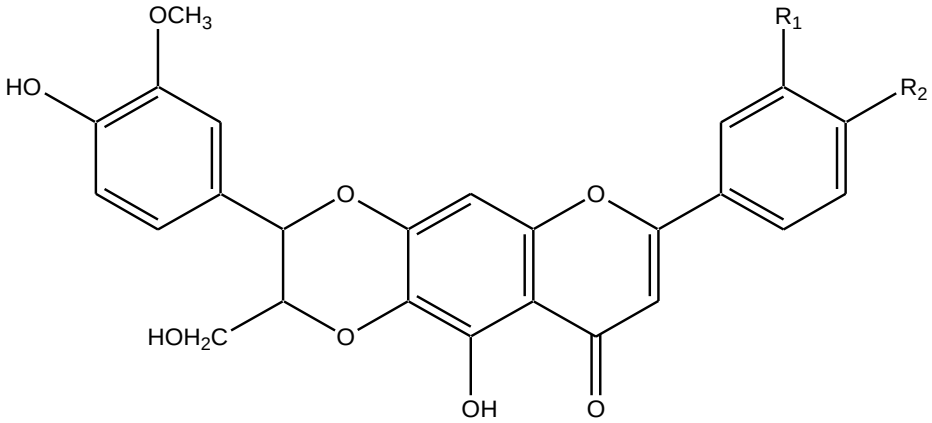


Table 12. Flavolignans isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Scutellaprostin D	H	H	*prostrata* (R)	(156)
Scutellaprostin E	H	OH	*prostrata* (R)	(156)
Scutellaprostin F	OH	OH	*prostrata* (R)	(156)

c) Chalcones isolated from *Scutellaria* species

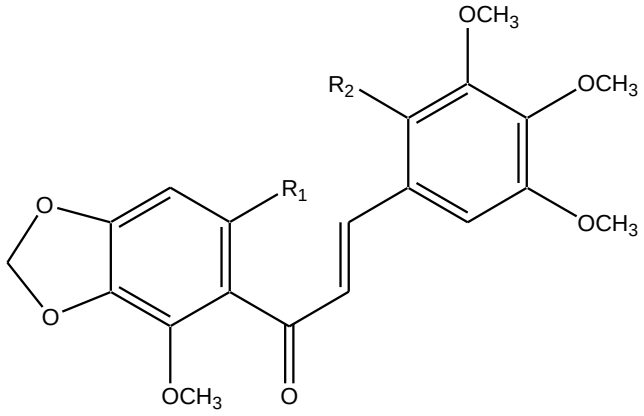


Table 13. Chalcones isolated from *Scutellaria* species

Compound	R_1	R_2	Species	Ref
2,2'-Dihydroxy-3,4,5,6'-tetrahydroxy-4',5'-methylenedioxychalcone	OH	OH	*indica* (A)	(136)
2,3,4,5,2',6'-Hexamethoxy-4',5'-methylenedioxychalcone	OCH_3	OCH_3	*indica* (A)	(136)
2'-Hydroxy-2,3,4,5,6'-pentamethoxy-4',5'-methylenedioxychalcone	OH	OCH_3	*indica* (A)	(136)

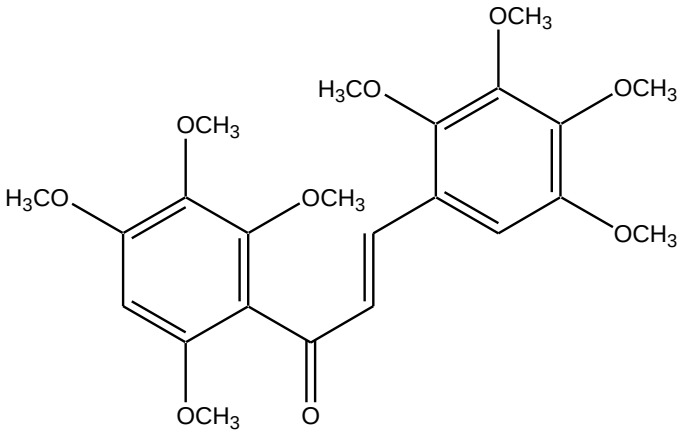


Table 14. Chalcones isolated from *Scutellaria* species

Compound	Species	Ref
2,3,4,5,2',4',5',6'- Octamethoxychalcone	*indica* (A)	(136)

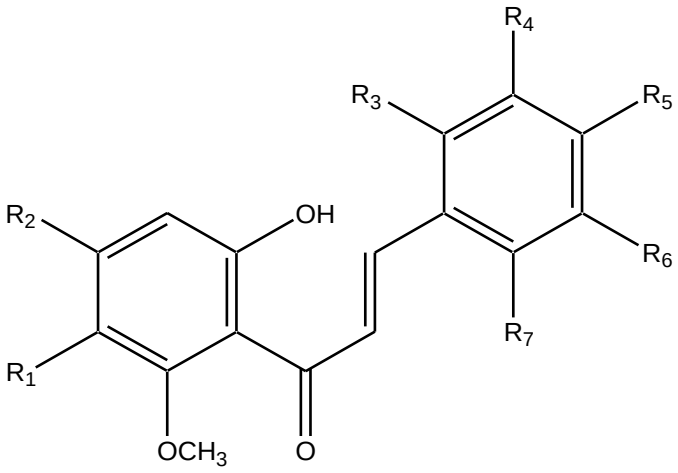


Table 15. Chalcones isolated from *Scutellaria* species

Compound	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Species	Ref
2'-Hydroxy-2,3,4,5,4',5',6'-heptamethoxychalcone	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H	*indica* (A)	(136)
2,4'-Dihydroxy-2,3,6'-trimethoxychalcone	H	OH	OCH_3	OCH_3	H	H	H	*discolor*	(1)
2,6,2',4'-Tetrahydroxy-6'-methoxychalcone	H	OH	OH	H	H	H	OH	*baicalensis*	(1)

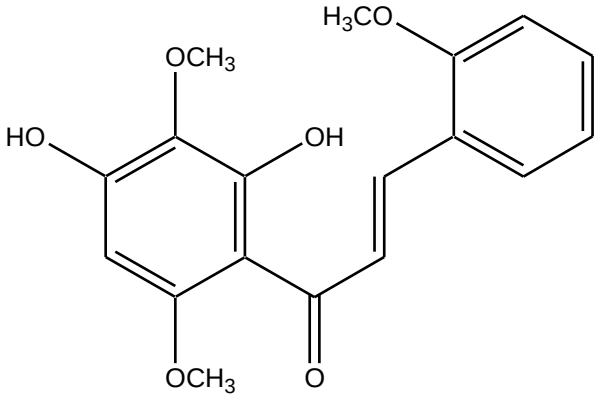


Table 16. Chalcones isolated from *Scutellaria* species

Compound	Species	Ref
2',4'-Dihydroxy-2,3',6'-trimethoxychalcone	*barbata* (W) *discolor* (R)	(148,151)

d) Coumarins isolated from *Scutellaria* species

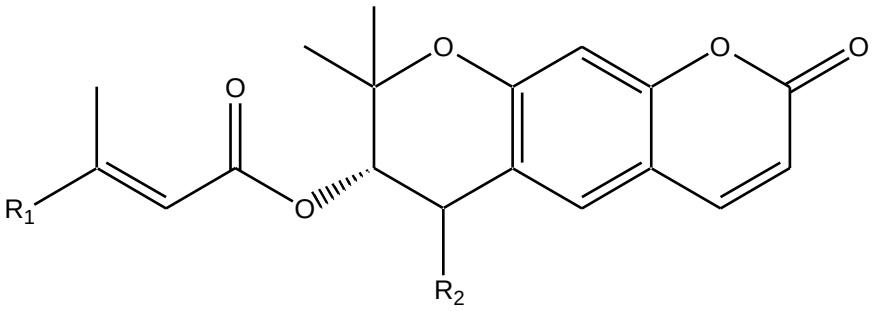


Table 17. Coumarins isolated from *Scutellaria* species

Compound	R_1	R_2	Species	Ref
Scuteflorin A	CH_3	=O	*lateriflora* (A)	(142)
Scuteflorin B	H	=O	*lateriflora* (A)	(142)
Decursin	CH_3	H	*lateriflora* (A)	(142)

e) Phenylethanoid Glycosides isolated from *Scutellaria* species

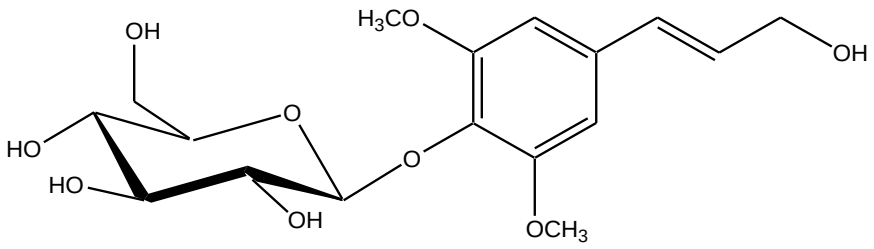


Table 18. Phenylpropanoid glycosides from *Scutellaria* species

Compound	Species	Ref
Syringin	*orientalis* (A)	(157)

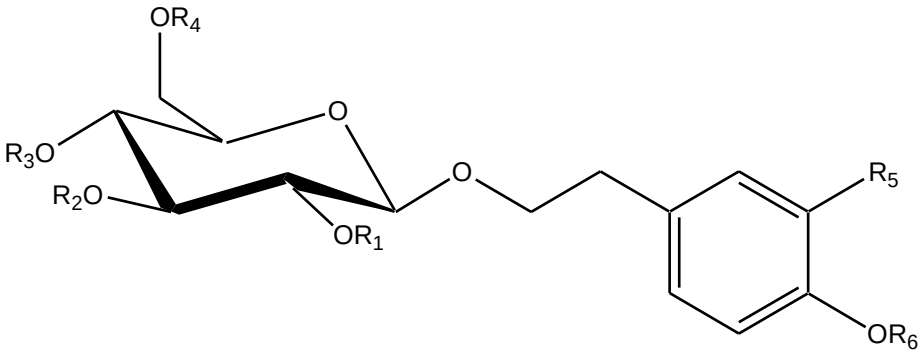


Table 19. Phenylethanoid glycosides isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**Species**	**Ref**
Verbascoside (=acteoside)	H	Rha	Caffeoyl	H	OH	H	*albida* (A) *pontica* (A) *planipes* (R) *prostrata* (R) *goulimyi* (A) *lateriflora* (A) *orientalis* (A)	(86,132,133,135,144,157,158)
Isoacteoside	H	Rha	H	Caffeoyl	OH	H	*lateriflora* (A)	(86)
Leucoseptoside A	H	Rha	Feruloyl	H	OH	H	*prostrata* (R) *lateriflora* (A) *orientalis* (A)	(86,135,157)
Martynoside	H	Rha	Feruloyl	H	OH	CH_3	*pontica* (A) *planipes* (R) *prostrata* (R) *galericulata* (A) *albida* (A) *orientalis* (A)	(132,133,135,144,158,159)
Isomartynoside	H	Rha	H	Feruloyl	OH	CH_3	*albida* (A)	(132)
Darendoside B (Deacyl-martynoside)	H	Rha	H	H	OH	CH_3	*orientalis* (A)	(157)
Salidroside	H	H	H	H	H	H	*baicalensis*	(1)
Jionoside D	H	Rha	Caffeoyl	H	OH	CH_3	*planipes* (R) *lateriflora* (A)	(86,144)
Calceolarioside B	H	H	H	Caffeoyl	OH	H	*galericulata* (A)	(159)
Osmanthuside E	H	H	H	Feruloyl	OH	H	*galericulata* (A)	(159)
Darendoside A	Api	H	H	H	H	H	*orientalis* (A)	(157)

Api: apiose

2.1.3.2. Terpenic Compounds

a) Iridoids isolated from *Scutellaria* species

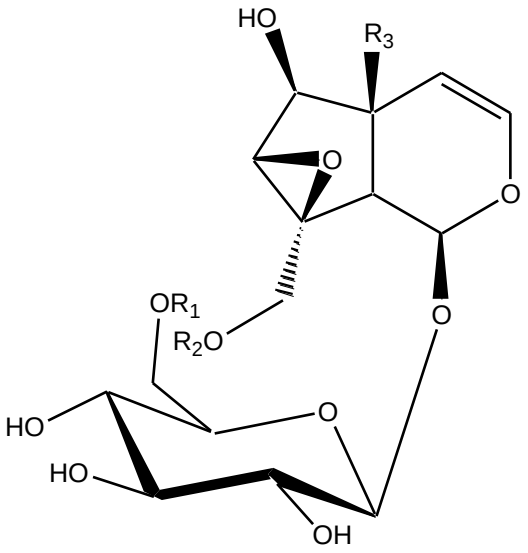


Table 20. Iridoid glycosides isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
Catalpol	H	H	H	*albida* (A) *grossa* (R)	(132,134,152)
Globularin (Scutellarioside I)	H	Cinnamoyl	H	*albida* (A) *columnae* (A) *goulimyi* (A) *grossa* (R)	(132,134,152, 158,160)
Macfadienoside	H	H	OH	*albida* (A)	(132,152)
6'-*O*-*E*-caffeoylcatalpol	Caffeoyl	H	H	*albida* (A)	(152)
6'-*O*-*p*-*E*-coumaroylcatalpol	*p*-coumaroyl	H	H	*albida* (A) *goulimyi* (A)	(152,158)

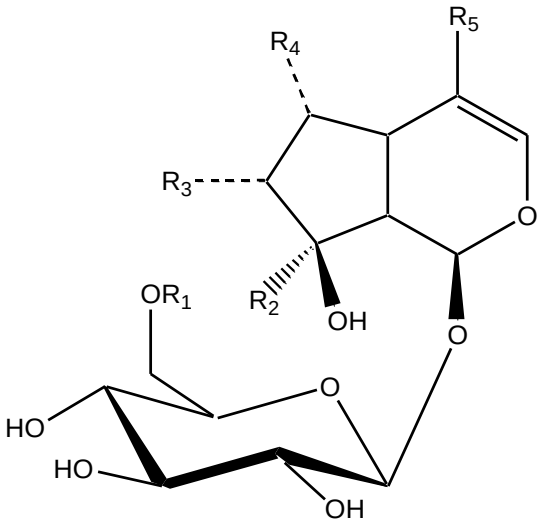


Table 21. Iridoid glycosides isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**Species**	**Ref**
Mussaenosidic acid	H	CH_3	H	H	COOH	*albida* (A) *goulimyi* (A)	(152,158)
Agnucastoside B	6,7-dihydrofoliamenthoyl	CH_3	H	H	COOH	*goulimyi* (A)	(158)
Caffeoyl- mussaenosidic acid	*E*-caffeoyl	CH_3	H	H	COOH	*albida* (A)	(152)
Albidoside	*p-E*-coumaroyl	CH_3	H	H	COOH	*albida* (A) *goulimyi* (A)	(132,152, 158)
10-descinnamoylglobularinin	H	CH_2OH	OH	OH	H	*albida* (A)	(132)

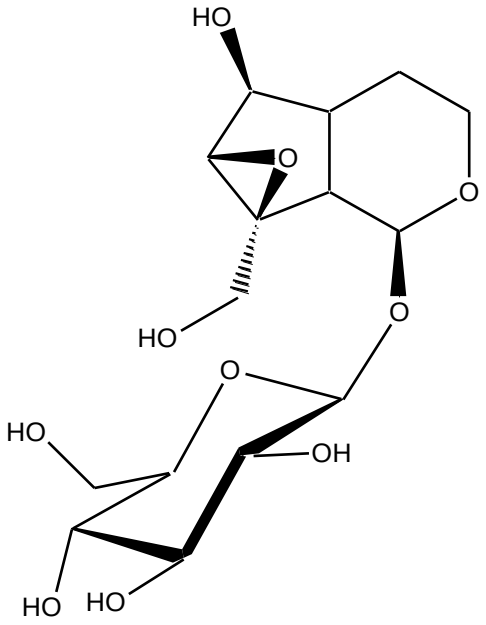


Table 22. Iridoid glycosides isolated from *Scutellaria* species

Compound	Species	Ref
Dihydrocatalpol	*albida* (A)	(132,152)

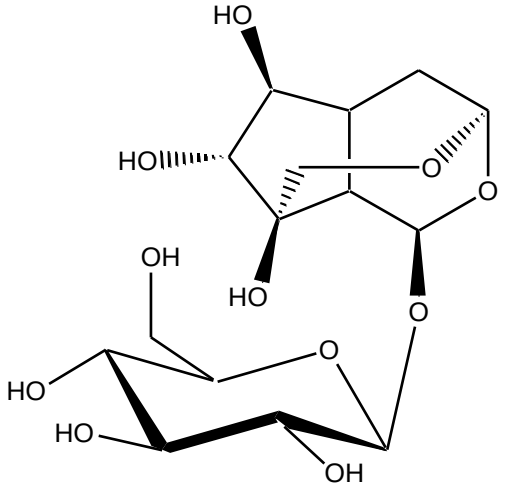


Table 23. Iridoid glycosides isolated from *Scutellaria* species

Compound	Species	Ref
Scutelloside	*albida* (A)	(132,152)

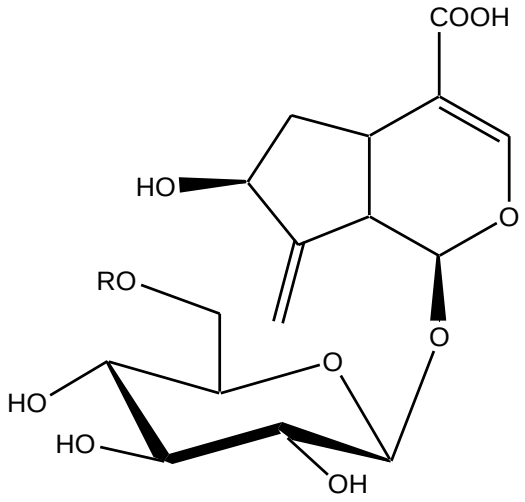


Table 24. Iridoid glycosides isolated from *Scutellaria* species

Compounds	**R**	**Species**	**Ref**
6'-*O*-*E*-*p*-coumaroylgardoside	*p*-coumaroyl	*albida* (A)	(132)
Gardoside	H	*albida* (A)	(132)

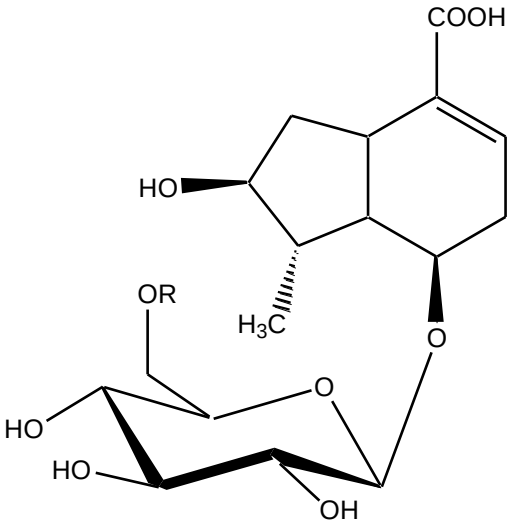


Table 25. Iridoid glycosides isolated from *Scutellaria* species

Compounds	R	Species	Ref
8-*ep*i-loganic acid	H	*albida* (A) *goulimyi* (A)	(132,158)
6'-*O*-*p*-*E*-coumaroyl-8-*ep*i-loganic acid	*p*-*E*-coumaroyl	*albida* (A) *goulimyi* (A)	(132,158)

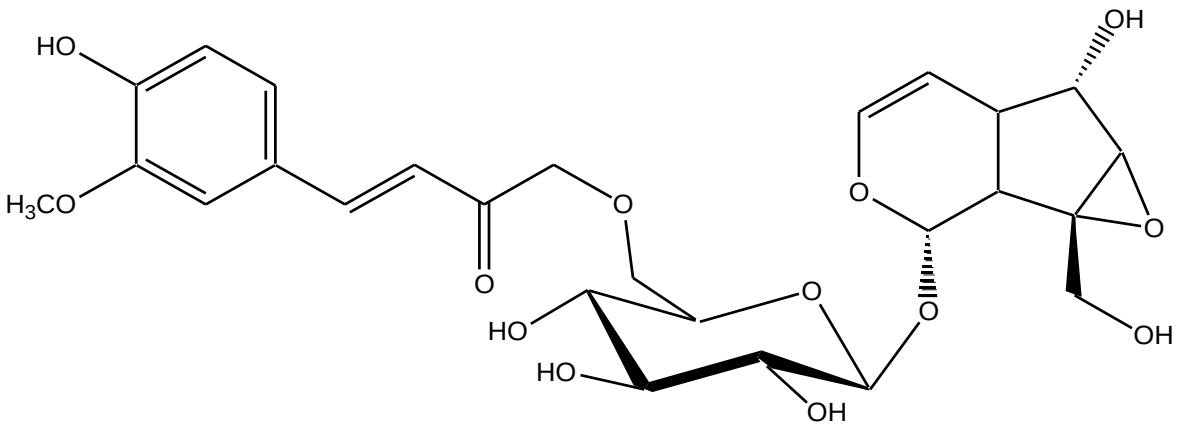


Table 26. Iridoid glycosides isolated from *Scutellaria* species

Compound	Species	Ref
Picroside III	*albida* (A)	(132)

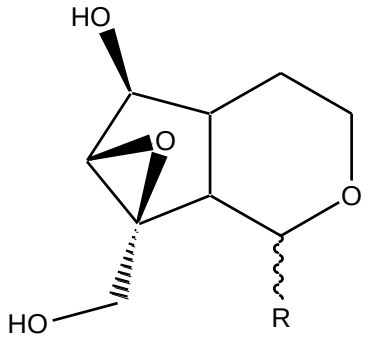


Table 27. Iridoids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Dihydrocatalpogenin α	α-OH	*albida* (A)	(152)
Dihydrocatalpogenin β	β-OH	*albida* (A)	(152)

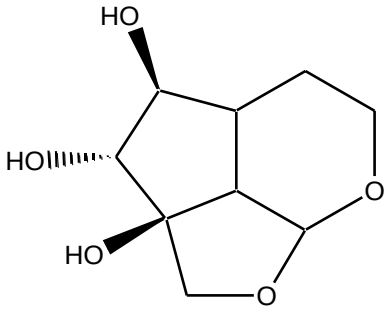


Table 28. Iridoids isolated from *Scutellaria* species

Compound	Species	Ref
Rehmaglutin	*albida* (A)	(152)

b)Diterpenoids isolated from *Scutellaria* species

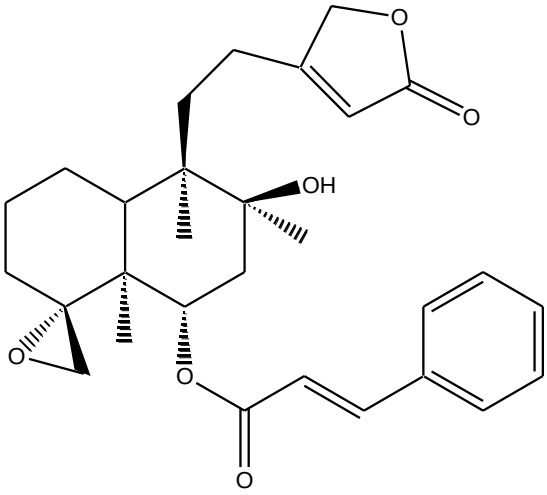


Table 29. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin A	*hastifolia* (A)	(8)

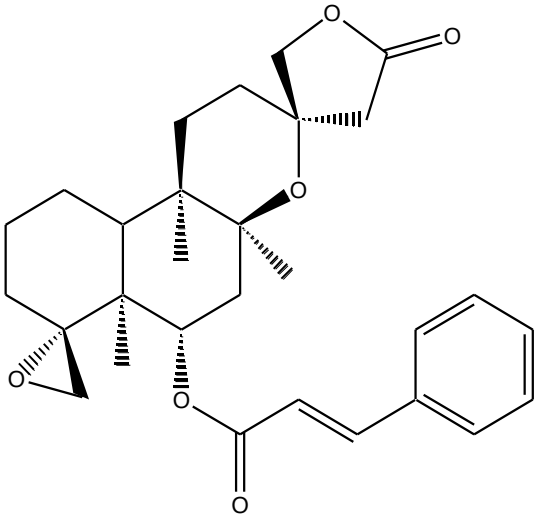


Table 30. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin B	*hastifolia* (A)	(8)

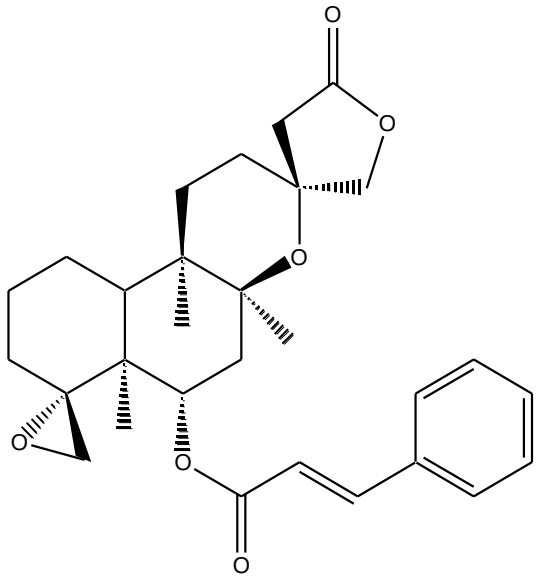


Table 31. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin C	*hastifolia* (A)	(8)

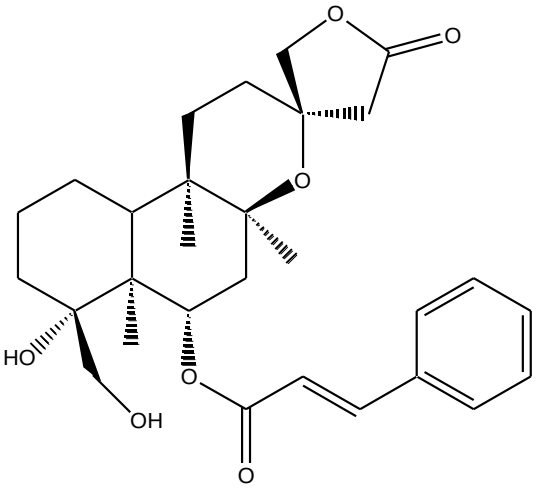


Table 32. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin D	*hastifolia* (A)	(8)

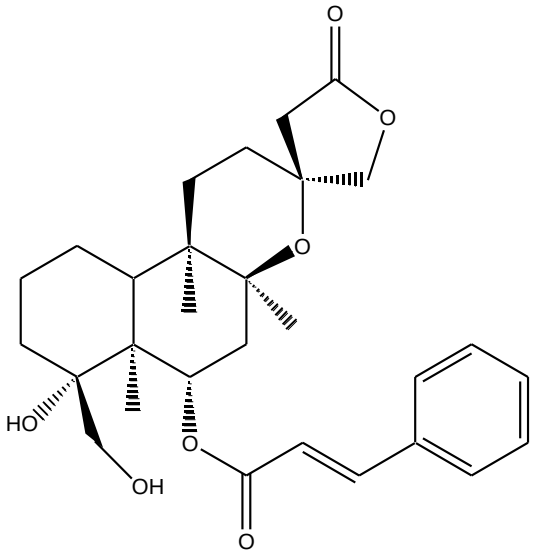


Table 33. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin E	*hastifolia* (A)	(8)

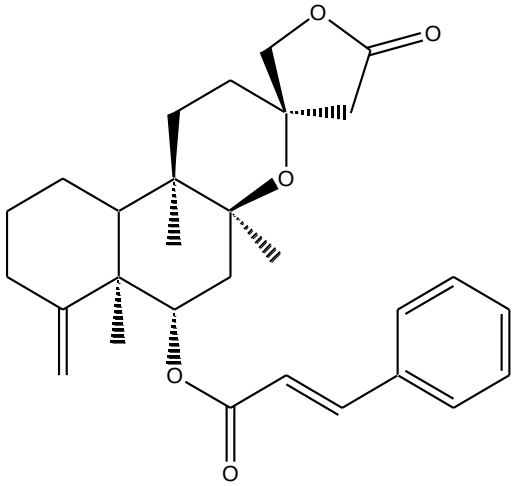


Table 34. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin F	*hastifolia* (A)	(8)

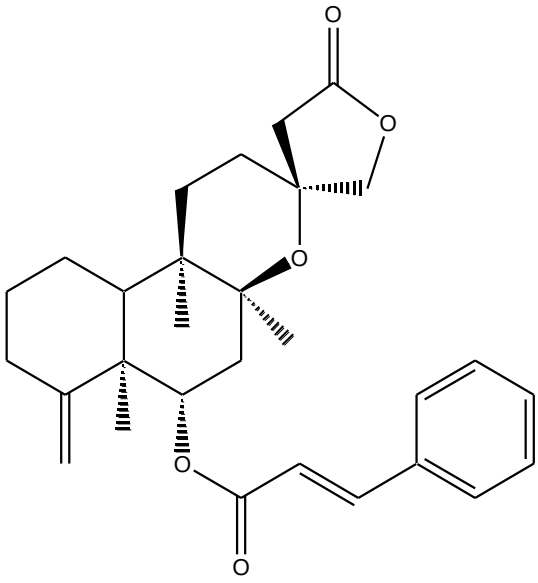


Table 35. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Hastifolin G	*hastifolia* (A)	(8)

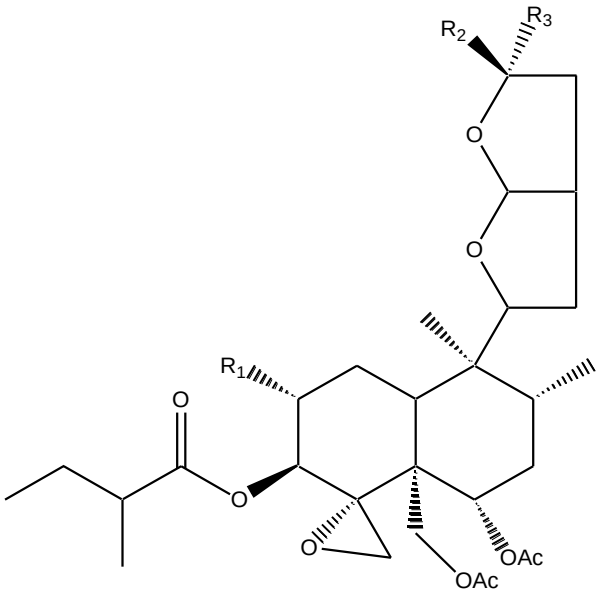


Table 36. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
Lupulin A	OH	OCH_3	H	*linearis* (A)	(139)
Lupulin B	H	H	OCH_3	*linearis* (A)	(139)

Ac: acetyl

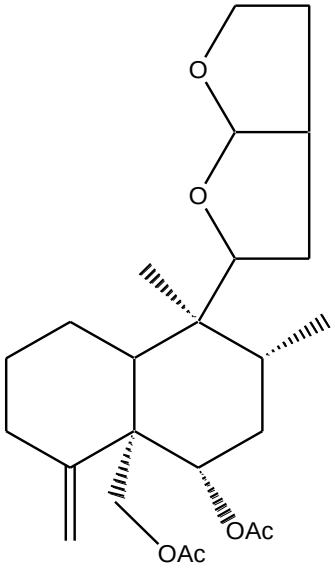


Table 37. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Lupulin C	*linearis* (A)	(139)

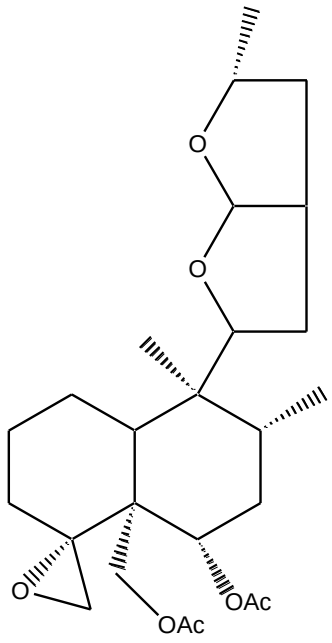


Table 38. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Lupulin D	*linearis*	(139)

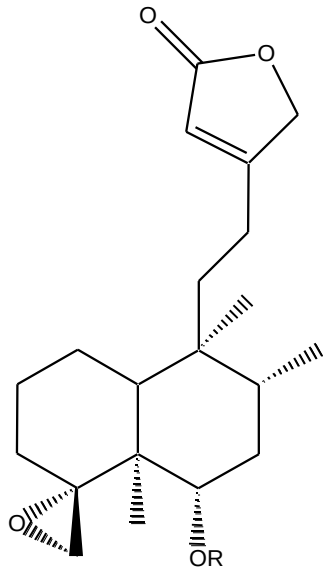


Table 39. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Ajugarin V	Ac	*orientalis* (A)	(161)
Scuteparvin	*trans*-cinnamoylester	*parvula* (A)	(162)

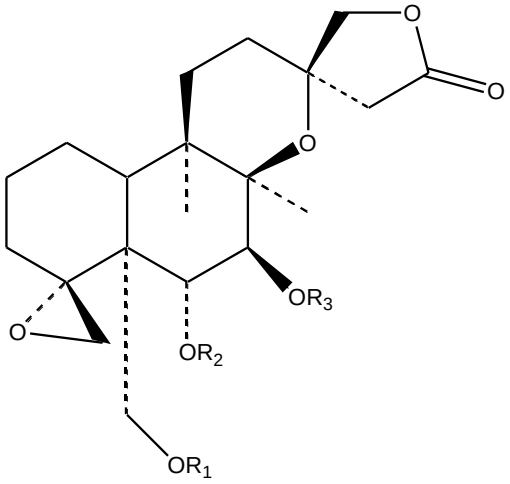
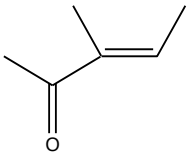


Table 40. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
Scutenisin	H	Isobutyryl	Isobutyryl	*orientalis* (A)	(161,163)
Scutalpin E	Ac		Ac	*alpina* (A)	(164)

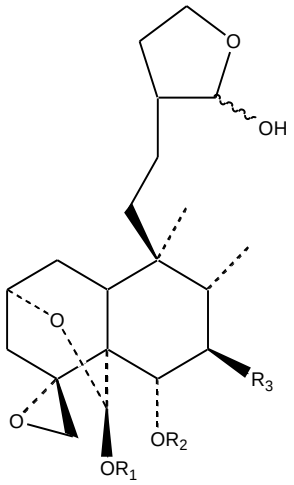


Table 41. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
Scutegalin C	Tig	Ac	OTig	*galericulata* (A)	(165)
Scutegalin D	Tig	Ac	H	*columnae* (A) *galericulata* (A)	(160,165)

Tig: tigloyloxy

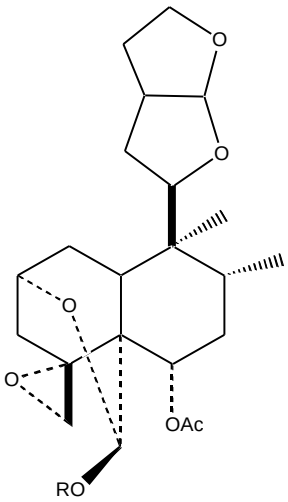
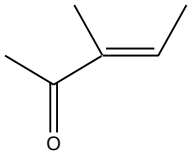


Table 42. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R**	**Species**	**Ref**
11-*epi*-scuteocolumnin C	H	*columnae* (A)	(160)
11-*epi*-scutecyprin		*columnae* (A)	(160,166)

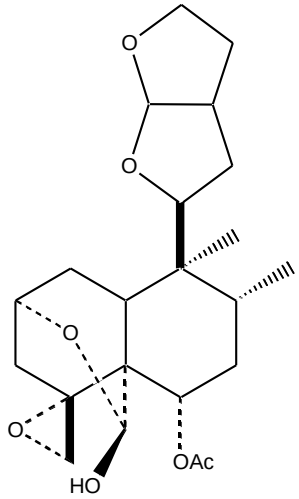


Table 43. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scutecolumnin C	*alpina* (A)	(167)

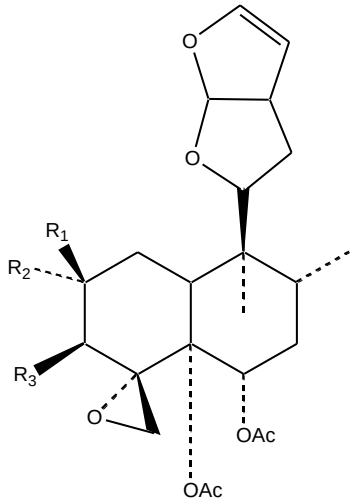
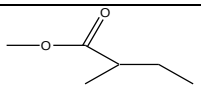
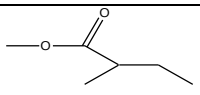


Table 44. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
Ajugapitin	H	OH		*lateriflora* (A)	(168)
Scutelaterin A	OAc	H	H	*lateriflora* (A)	(168)
Scutelaterin B		H	H	*lateriflora* (A)	(168)

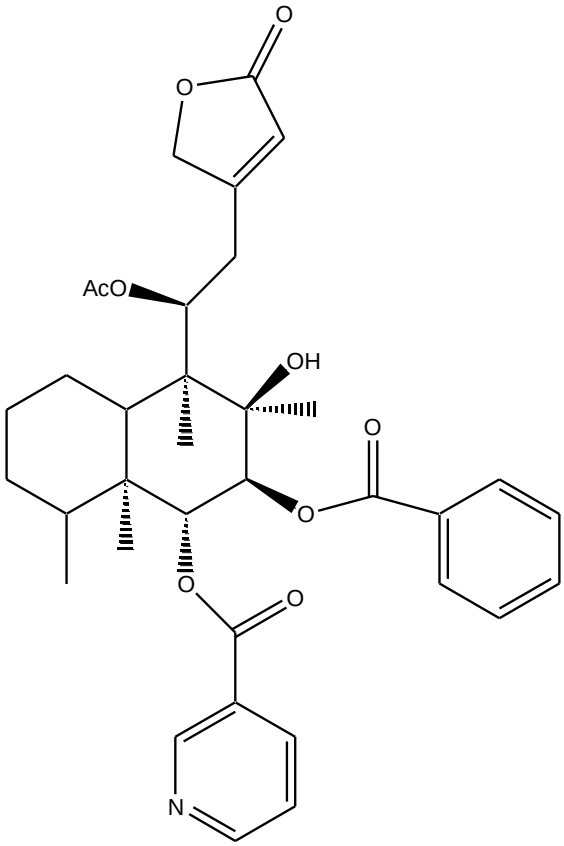


Table 45. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Barbatellarin B	*barbata* (A+W)	(169,170)

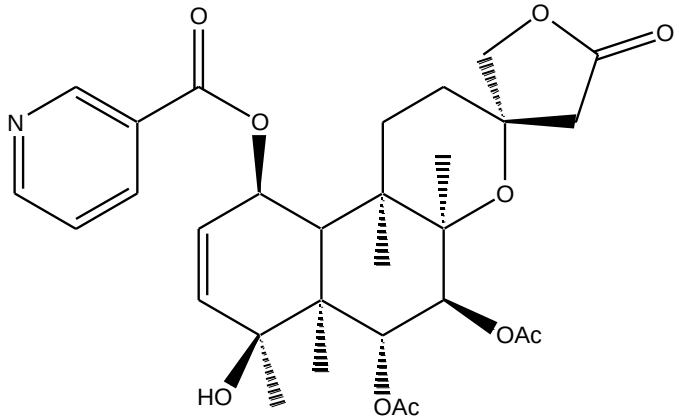


Table 46. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Barbatellarin C	*barbata* (A)	(169)

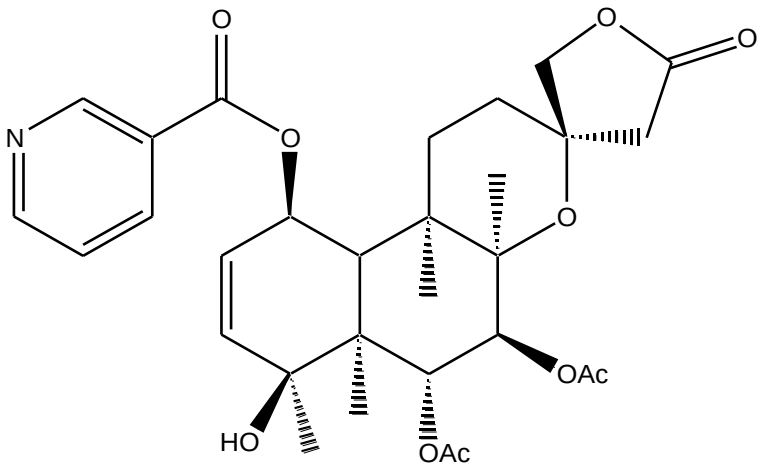


Table 47. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Barbatellarin D	*barbata* (A)	(169)

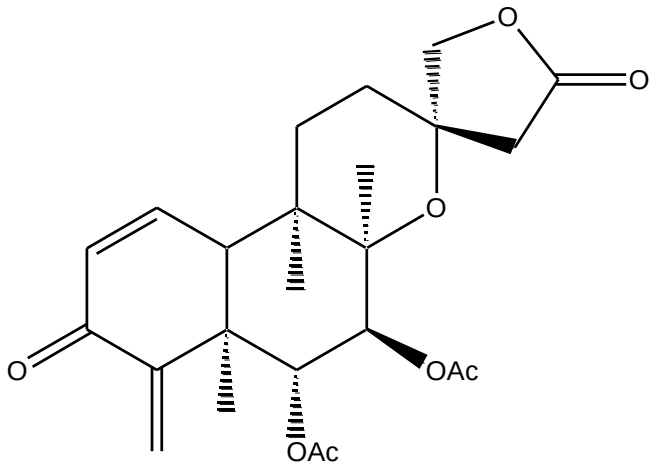


Table 48. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Barbatellarin E	*barbata* (A)	(169)

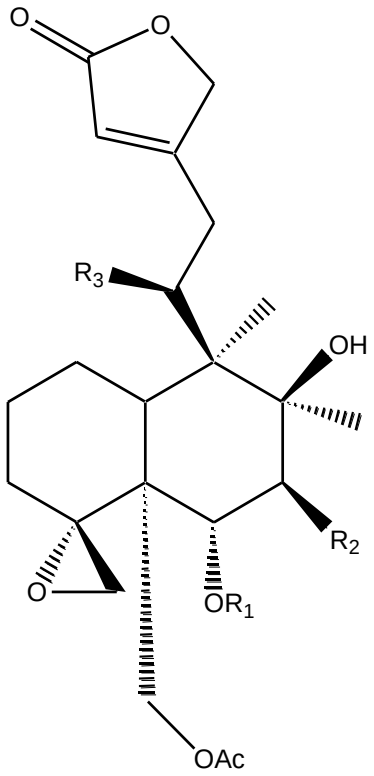
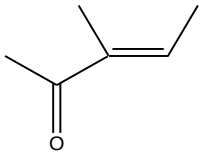
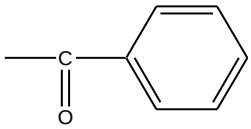
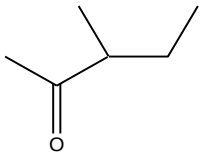


Table 49. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
Scutalpin B		H	*O*-Ac	*alpina* (A)	(167)
Scutalpin L	Bz	*O*-Bz	H	*alpina* (A)	(164,167)
Scutalpin N		H	H	*alpina* (A)	(164)
Scutalpin I	Bz	H	*O*-Ac	*alpina* (A)	(167)
Scutalpin H		H	*O*-Ac	*alpina* (A)	(167)
Scutorientalin E	*trans*-cinnamoylester	*O*-Ac	H	*orientalis* (A)	(171)

Bz: benzoyl

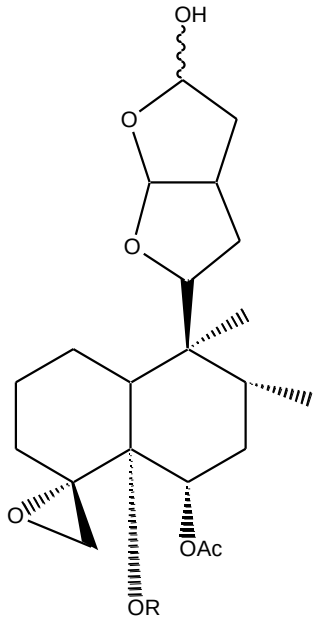


Table 50. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Scutalpin O	Isobutyryl	*alpina* (A) *polyodon* (A)	(164,172)

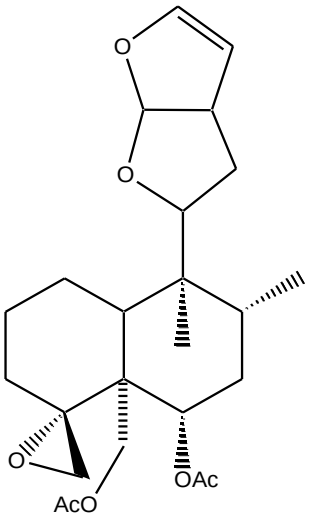


Table 51. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	Species	Ref
Clerodin	*albida* (A) *discolor* (A)	(173,174)

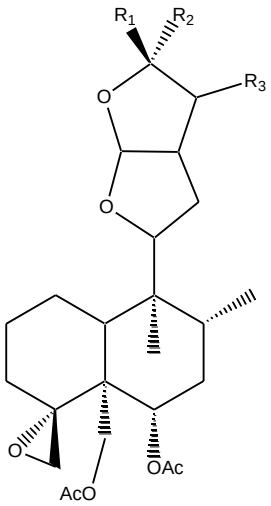


Table 52. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	R_3	Species	Ref
Dihydroclerodin-I	H	H	H	*discolor* (A)	(173)
Scutalpin M	H	H	*O*-Ac	*alpina* (A)	(167)
15β-ethoxy-14-hydroclerodin	*O*-Ethoxy	H	H	*discolor* (A)	(173)
15α-ethoxy-14-hydroclerodin	H	*O*-Ethoxy	H	*discolor* (A)	(173)

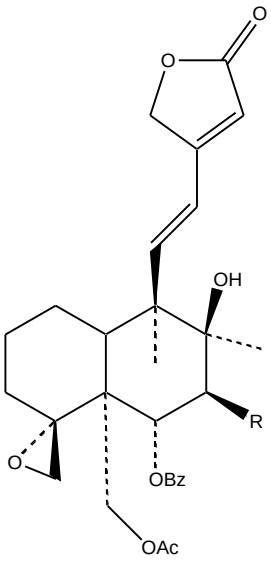


Table 53. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Scutalpin J	H	*alpina* (A)	(167)
Scutalpin K	*O*-Bz	*alpina* (A)	(167)

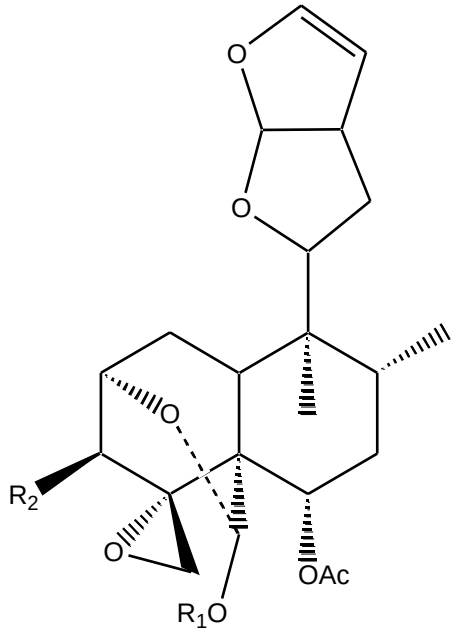
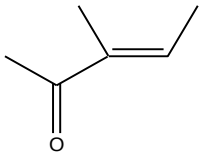
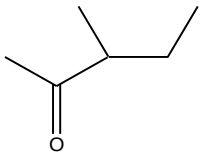


Table 54. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Jodrellin A	Ac	H	*discolor* (A) *rubicunda* (A)	(128,173)
19-*O*-deacetyljodrellin A	H	H	*discolor* (A)	(173)
Jodrellin B	Isobutyryl	H	*polyodon* (A) *rubicunda* (A)	(128,175)
Scutegrossin A		H	*grossa* (A) *rubicunda* (A)	(128,176)
Scupolin J	Isobutyryl	OH	*polyodon* (A)	(172)
Scutalbin A	H	H	*rubicunda* (A)	(128)
Scutecolumnin A		H	*polyodon* (A)	(175)
Scupolin H	CH_3	H	*polyodon* (A)	(175)

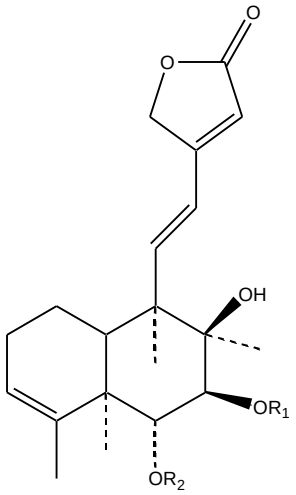
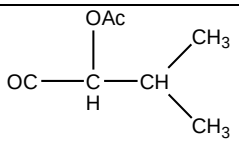
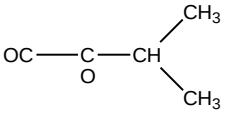


Table 55. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	Species	Ref
Barbatin D	Bz	Bz	*barbata* (W)	(177)
Barbatin E			*barbata* (W)	(177)

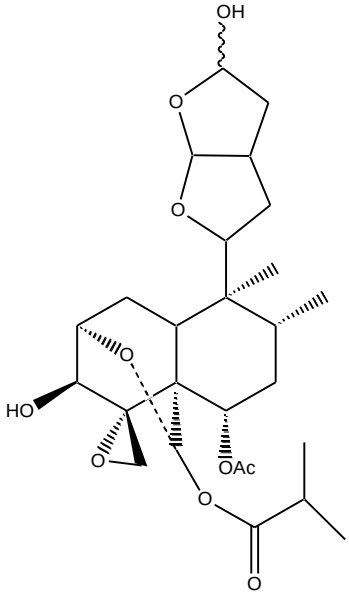


Table 56. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	Species	Ref
Scupolin K	*polyodon* (A)	(172)

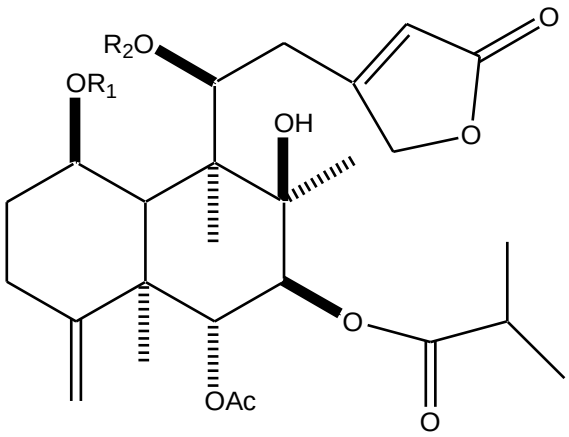


Table 57. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Scuterulein A	H	Isobutyryl	*caerulea* (A)	(178)
Scuterulein C	Ac	Ac	*caerulea* (A)	(178)
Deacetylscuterulein C	H	Ac	*caerulea* (A)	(178)

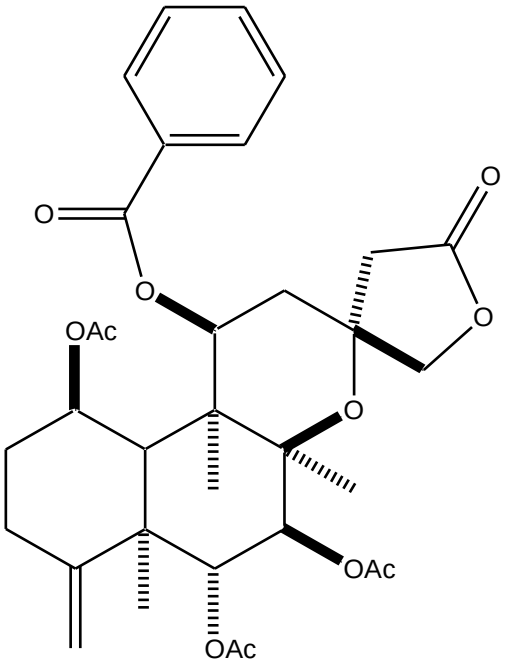


Table 58. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**Species**	**Ref**
Scuterulein B	*caerulea* (A)	(178)

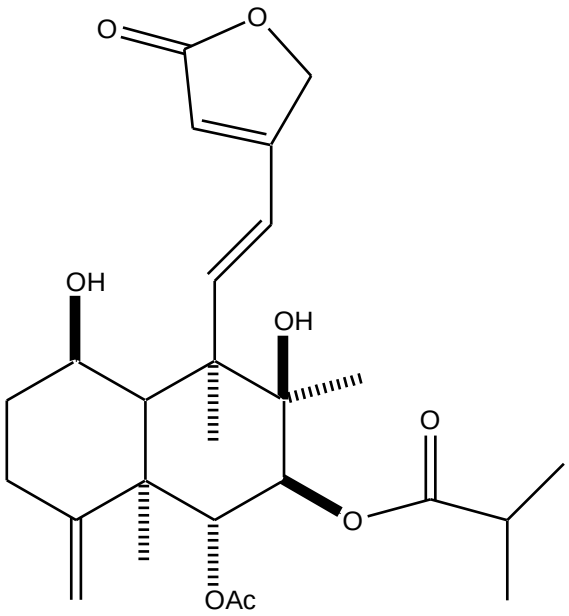


Table 59. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scuterulein D	*caerulea* (A)	(178)

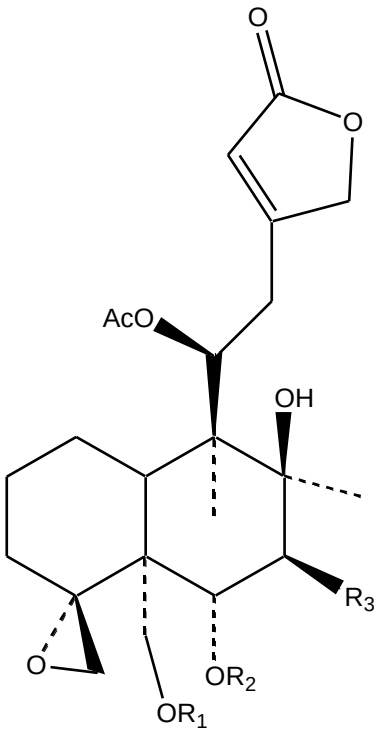
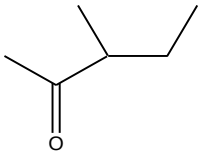
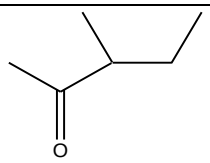
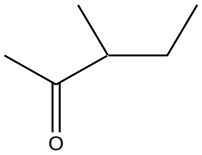


Table 60. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**Species**	**Ref**
Scupolin A	H		H	*polyodon* (A)	(175)
Scupolin B	Ac	Ac	H	*polyodon* (A)	(175)
Scupolin C	Bz	Bz	H	*polyodon* (A)	(175)
Scupolin D	H	H		*polyodon* (A)	(175)
Scupolin E	H		H	*polyodon* (A)	(175)

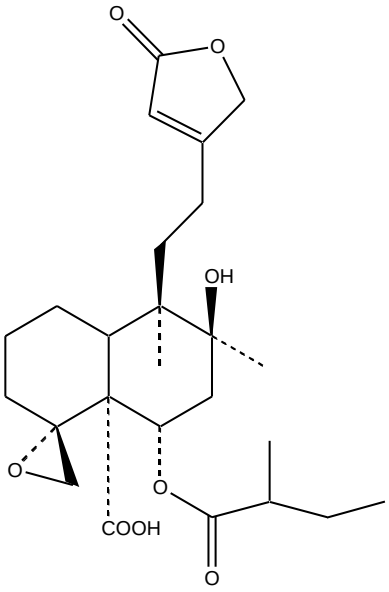


Table 61. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scutepolin F	*polyodon* (A)	(175)

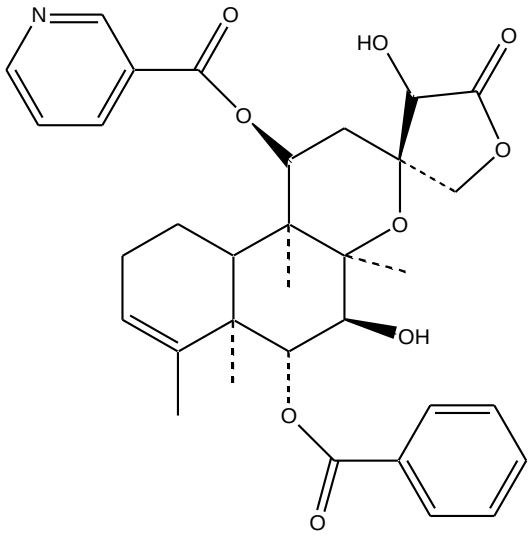


Table 62. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scutehenanine H	*barbata* (W)	(65)

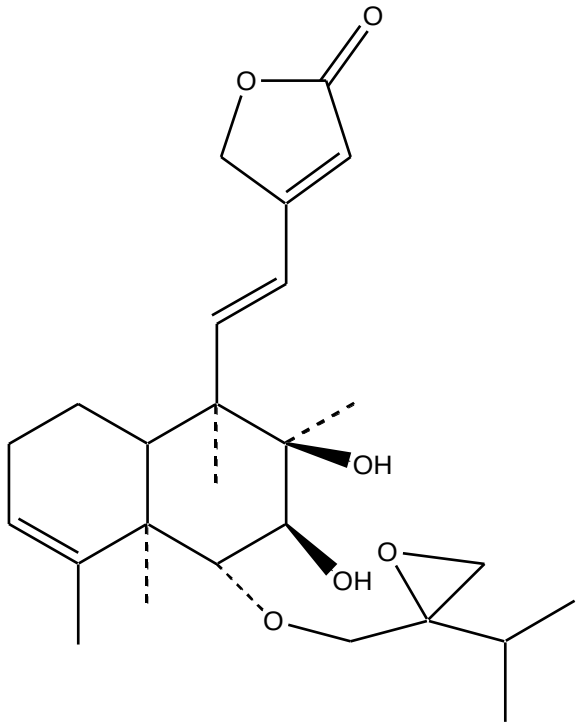


Table 63. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
6-(2,3-Epoxy-2-isopropyl-n-propoxyl) barbatin C	*barbata* (W)	(65)

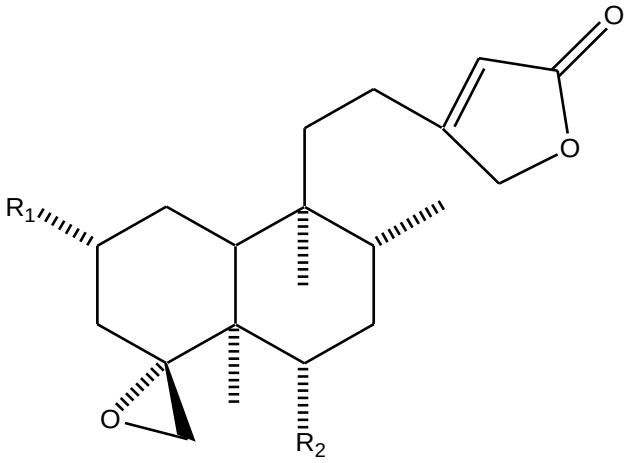


Table 64. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	Species	Ref
2α-Hydroxyajugarin V	OH	*O*-Ac	*drummondii* (A)	(179)
2α-Hydroxy-deacetylajugarin V	OH	OH	*drummondii* (A)	(179)

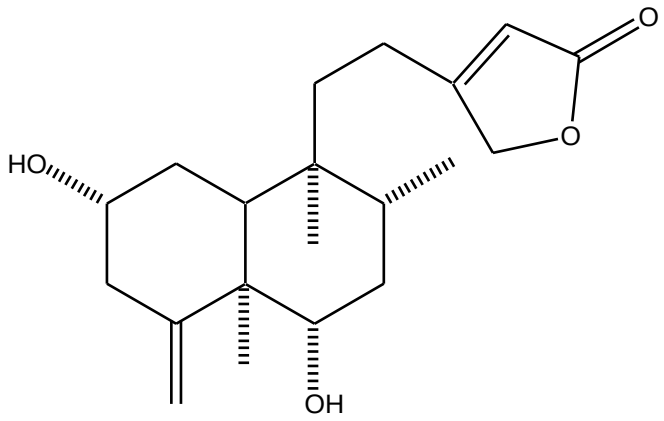


Table 65. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scutedrummonin	*drummondii* (A)	(179)

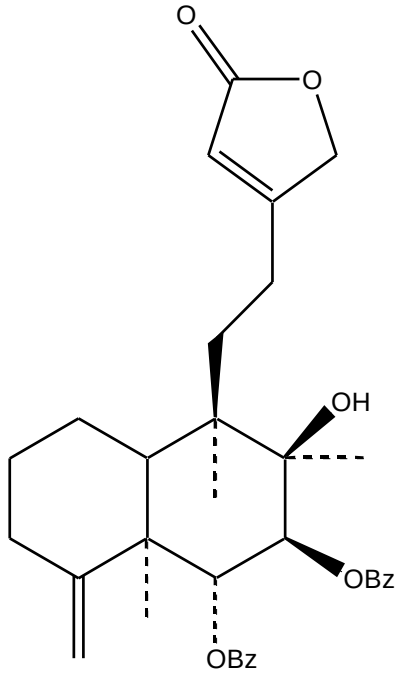


Table 66. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	Species	Ref
Scutebaicalin	*baicalensis*(A)	(180)

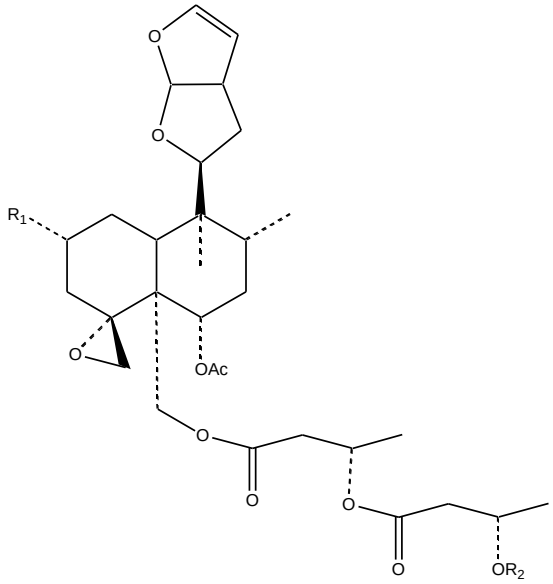
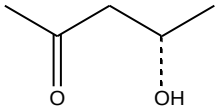


Table 67. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R_1	R_2	Species	Ref
Scupontin A	OH	Ac	*pontica* (A)	(181)
Scupontin B	H	Ac	*pontica* (A)	(181)
Scupontin E	OH		*pontica* (A)	(181)

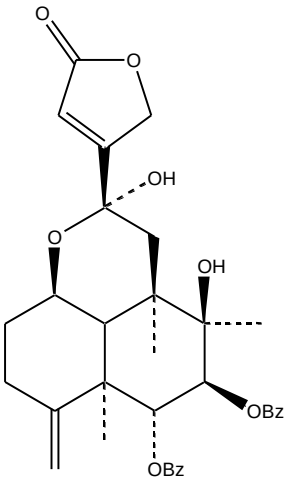


Table 68. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	Species	Ref
Scupontin G	*pontica* (A)	(181)

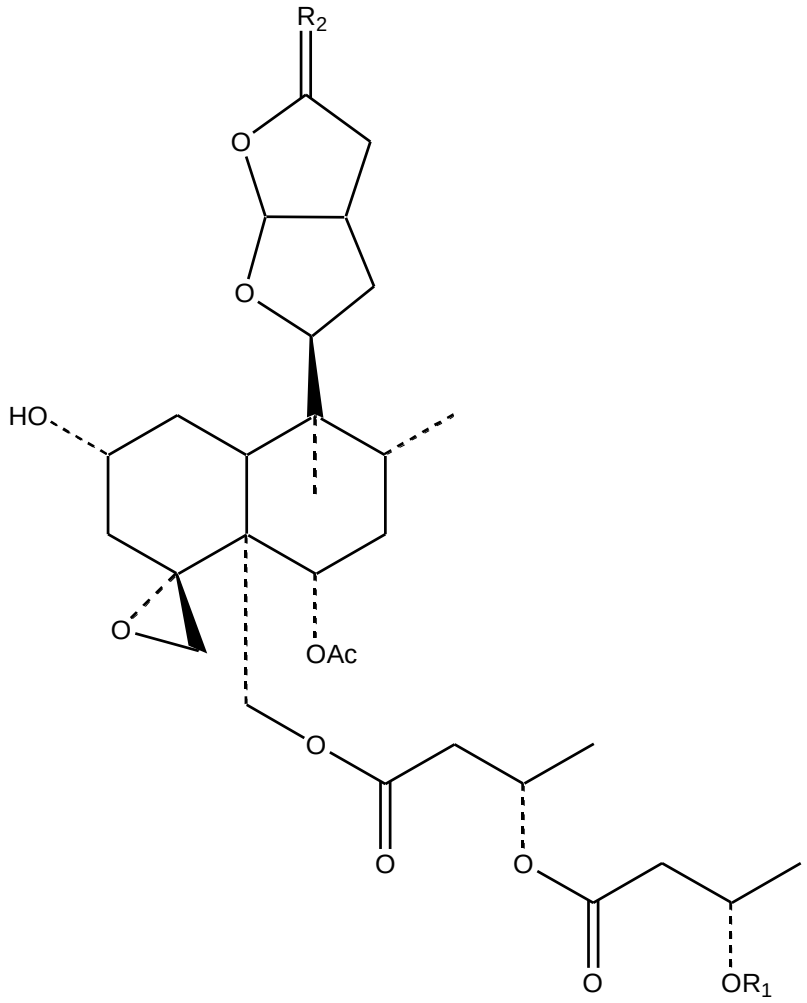
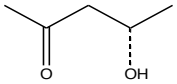


Table 69. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**Species**	**Ref**
Scupontin C	Ac	H,H	*pontica* (A)	(181)
Scupontin D	Ac	H,OH*	*pontica* (A)	(181)
Scupontin F		H,OH*	*pontica* (A)	(181)

*Mixture of 15*R* and 15*S* forms

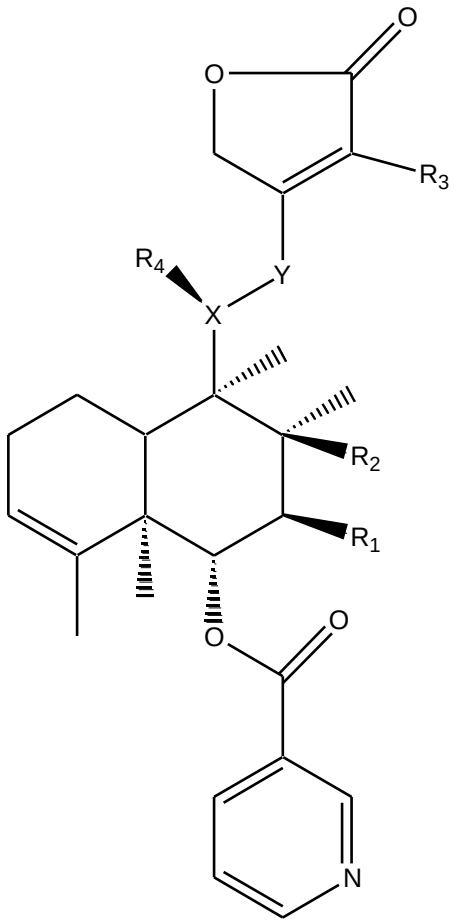
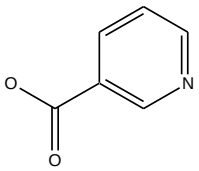
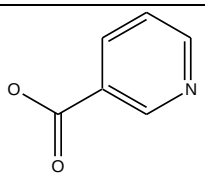


Table 70. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	**R_1**	**R_2**	**R_3**	**X-Y**	**Species**	**Ref**
Scutebarbatin X		OH	*O*-Ac	Single	*barbata* (W)	(182)
Scutebarbatin V		OH	H	Double	*barbata* (W)	(182)
Scutebarbatin Z	OH	H	H	Single	*barbata* (W)	(182)

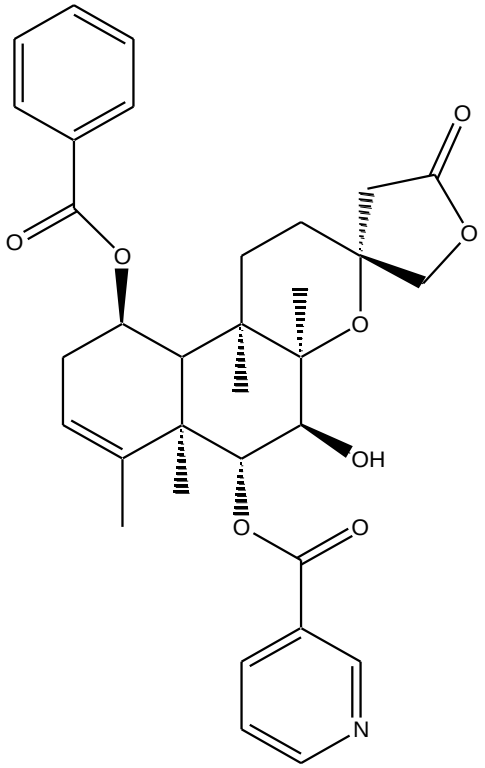


Table 71. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compound	Species	Ref
Scutebarbatin W	*barbata* (W)	(182)

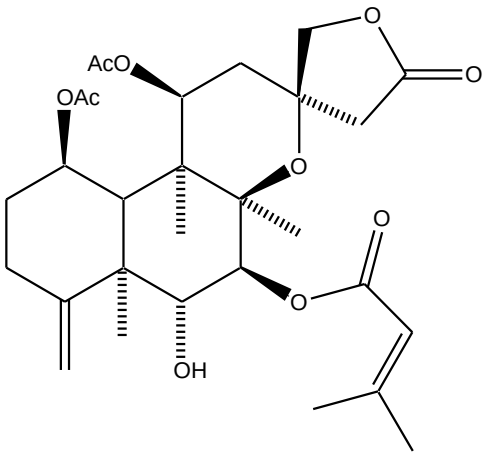


Table 72. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	Species	Ref
Scuteselerin	*seleriana* (A)	(154)

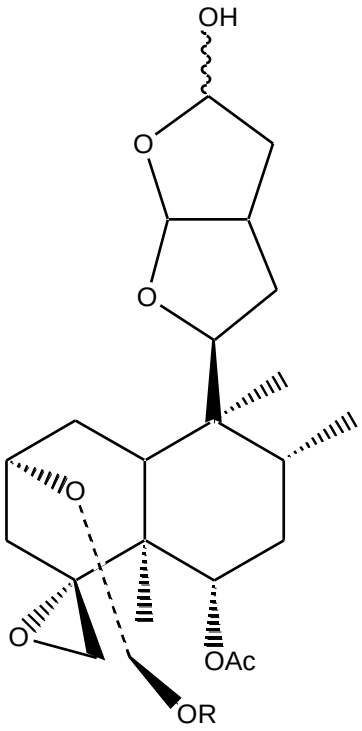
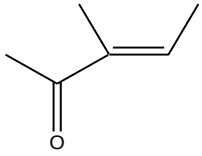
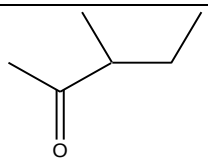


Table 73. Neoclerodane diterpenoids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Scutalsin	Isobutyryl	*altissima* (A) *grossa* (A) *polyodon* (A) *rubicunda* (A)	(128,172,174,176)
Scutecyprol B		*grossa* (A) *rubicunda* (A)	(127,128,176)
Scutalbin C	H	*rubicunda* (A)	(127)
Scutalbin B		*grossa* (A)	(176)

Regarding the diterpenoid components of *Scutellaria* species, many studies have been conducted. The structures of these compounds are given in the Tables 29-73. Besides the given diterpenoids, scupolin A-I and scutecolumnin A were isolated from *S. polyodon* (175), scuterepenin A_1, scuterepenin A_2, scuterepenin B, scuterepenin C_1, scuterepenin C_2, scuterepenin D_1, scuterepenin D_2, scuterepenin E, scuterepenin F_1, scuterepenin F_2, scuterepenin G_1, scuterepenin G_2, scuterepenoside A_1, scuterepenoside A_2, scuterepenoside A_3 and scuterepenoside A_4 were purified from *S. repens* (183), scutorientalin A-C were isolated from *S. orientalis* (184), scutebata H-M, scutebata O, barbatellarin A-B, scutelinquanin A-C and barbatin A were isolated from *S. barbata* (67,170,182,185), scuterivulactone A, scuterivulactone B, scuterivulactone C_1, scuterivulactone C_2, scuterivulactone D, scutellone D-E, scutellone B, scutellone G-I were isolated from *S. rivularis* (186–188)**.**

c) Triterpenoids isolated from *Scutellaria* species

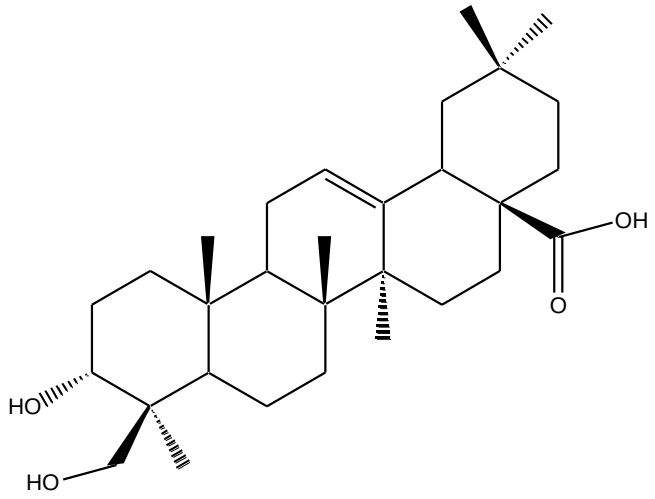


Table 74. Triterpenes isolated from *Scutellaria* species

Compound	Species	Ref
Scutellaric acid	*lateriflora* (A) *rivularis* (A)	(142,189)

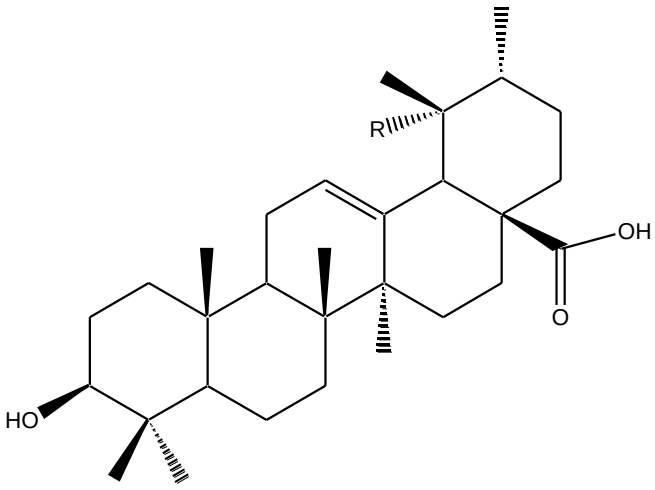


Table 75. Triterpenes isolated from *Scutellaria* species

Compounds	R	Species	Ref
Ursolic acid	H	*lateriflora* (A) *strigillosa* (L)	(142,190)
Pomolic acid	OH	*lateriflora* (A)	(142)

d) Steroids isolated from *Scutellaria* species

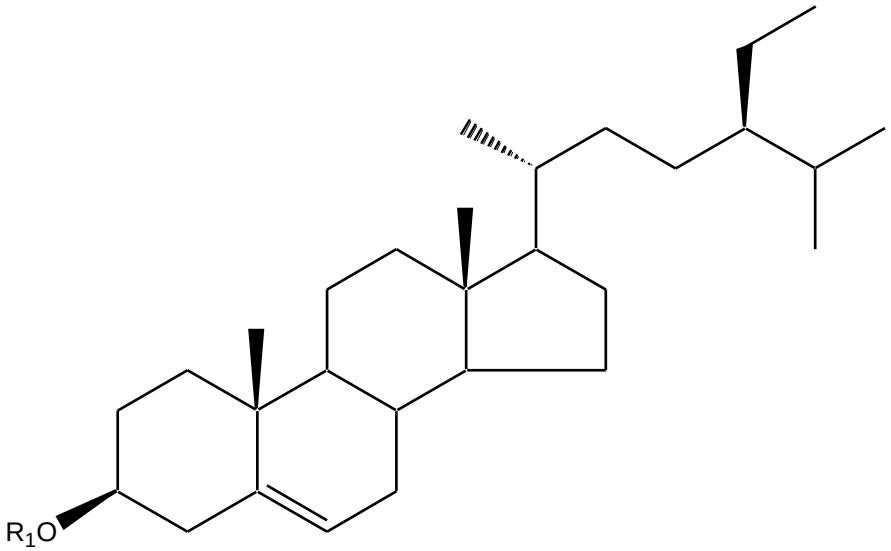


Table 76. Steroids isolated from *Scutellaria* species

Compounds	R	Species	Ref
Daucosterol	Glc	*lateriflora* (A)	(142)
β-Sitosterol	H	*lateriflora* (A) *linearis* (A)	(139,142)

e) Essential oils isolated from *Scutellaria* species

Table 77. Essential oils and their main components of *Scutellaria* species

Species	Yield (w/w)*	Main Components (%)	Ref
S. diffusa	-	Hexadecanoic acid (30%) Caryophyllene oxide (9%)	(191)
S. heterophylla	-	Germacrene D (21%) Hexadecanoic acid (16%) β-caryophyllene (13%)	(191)
S. salviifolia	-	Germacrene D (40%) Bicyclogermacrene (14%) β-caryophyllene (11%)	(191)
S. rubicunda	0.12%	Caryophyllene (28.7%) Linalool (27.8%) α-terpineol (6.7%)	(192)
S. laeteviolacea	0.02%	1-octen-3-ol (27.72%) Germacrene D (21.67%) β-caryophyllene (9.18%)	(193)
S. albida	-	Linalool (52.63%) Trans-nerolidol (9.03%) Nonanal (6.73%)	(80)
S. brevibracteata	0.14%	Caryophyllene (14.4%) Hexadecanoic acid (12.6%) 4-Vinylguaicacol (10.2%)	(9)
S. orientalis	0.18%	Hexahydrofarnesylacetone (11.7%) Hexadecanoic acid (7.6%) Caryophyllene (7.4%)	(9)
S. hastifolia	0.15%	Caryophyllene (12.9%) Hexadecanoic acid (6.3 %) Hexahydrofarnesylacetone (5.6 %)	(9)
S. barbata	-	1-Octen-3-one (6.21%) Caryophyllene (4.39%) Hexanal (1.91%)	(194)
S. orientalis	-	Hexahydrofarnesylacetone (11.7%) Hexadecanoic acid (7.6%) Caryophyllene (7.4%)	(195)
S. utriculata	-	Linalool (20.1%) 4-vinyl guaiacol (15.5%) α-terpineol (8.9 %)	(195)

*All of the essential oils were obtained by hydrodistillation from the aerial parts of the related species.

2.1.3.3. Alkaloids isolated from *Scutellaria* species

Alkaloids are rarely encountered in the genus *Scutellaria*. Only two species have been reported to contain alkaloids up to now. Scutebarbatine A-O, 6-*O*-nicotinoylscutebarbatine G, 6,7-di-*O*-nicotinoylscutebarbatine G, 6-*O*-nicotinoyl-7-*O*-acetylscutebarbatine G, scutebarbatine H, 7-*O*-nicotinoylscutebarbatine H, scutehenanine A-D and 6-*O*-acetylscutehenanine A were the neoclerodane diterpenoid and norditerpenoid alkaloids which were reported from *S. barbata* (60–64,66). Sophoranol, sophoridine, allmatrine, anagryne, cytosine, isomatrine, matrine, *N*-methylcistisine, oxymatrine, osysophocarpine and sophocarpine were the other alkaloids isolated from other species, *S. flavescens* (1).

2.1.4. Analytical studies on *Scutellaria* species

In this chapter, both qualitative and quantitative experiments performed on *Scutellaria* species were compiled.

2.1.4. Analytical studies on *Scutellaria* species

Most *Scutellaria* species have been used for different purposes (antiviral, antiinflammatory, antioxidant, anticancer, hepatoprotective etc.) all around the world for years. Crude extracts, subextracts as well as the pure compounds obtained from *Scutellaria* species were tested for their biological activities. Due to the broad pharmacological activity spectrum, the chemical composition of this genus has gained importance. The first study on the chemical composition of *Scutellaria* genus was conducted in 1889. Till today, more than 295 compounds were isolated from *Scutellaria* species (1). Since *Scutellaria* species are obviously important in the treatment of diseases, the need for quality assesment of these species procured. Many studies were performed for the evaluation of the marker and biomarker components (mainly flavonoids) up to now.

Yu et al., developed a new capillary electrophoresis method for the quantification of three major flavonoids (baicalin, baicalein and wogonin) found in *S. baicalensis*. 34 raw *S. baicalensis* materials were brought from the nine provinces of China. 1 gram of each powdered drug was extracted with 10 mL 70% aqueous solution of EtOH. Micellar electrokinetic capillary chromatograohy (MEKC) mode was applied in 50 cm × 75 μM i.d. fused silica capillary column with diode array detector. A supporting electrolyte mixture containing 15 mM borate, 40 mM phosphate, 15 mM sodium doceyl sulphate, 15% acetonitrile and 7.5% 2-propranol was employed. Electrophoretograms were obtained with a wavelength set at 280 nm. Finally baseline separation was completed in 15 min (196). Dong et al. also developed a MEKC method for the quantification of wogonin, oroxylin A, chrysin, 5,7,4'-trihydroxy-8-methoxyflavone and skullcaplavone II in *S. baicalensis* MeOH extract within 12 min. Same column used in previous study was used for the separation. Electrophoretograms were obtained with a wavelength set at 265 nm. Using this method, the contents of low abundent flavonoids skullcapflavone II, chrysin and 5,7,4'-trihydroxy-8-methoxyflavone were readily calculated in *S. baicalensis* as 38.2 mg/g, 10.6 mg/g and 6.7 mg/g, respectively (197).

Gao et al., also investigated the baicalin, baicalein and wogonin percentages in the tinctures of American (*S. lateriflora*) and Chinese skullcap (*S. baicalensis*) by high performance liquid chromatography (HPLC) with the assistance of UV detector. Luna C_{18} (5 μm, 150 × 4.6 mm) column at the room temperature was used. The UV detector was set to 270 nm. Gradient elution was employed using 0.1% FA (eluent A) and MeOH (eluent B). The separation was started with 45% B and ended at the 35^{th} min of the analysis with 99% B. The flow rate was adjusted to 1 mL/min. In conclusion, baicalin was found in the range of 0-12.66 mg/mL, baicalein 0-0.63 mg/mL and finally wogonin was calculated in the range of 0-0.16 mg/mL in *S. lateriflora* tinctures. Whereas, in *S. baicalensis* tinctures baicalin was found in the range of 0.12-10.61 mg/mL, baicalein 0.52-5.88 mg/mL and wogonin 0.08-1.61mg/mL (198).

Liu et al. investigated the flavonoid profile of the *S. baicalensis* roots by LC-UV/MS experiments. 0.5 g of the powdered root of *S. baicalensis* was extracted with 50 mL of 60% aqueous solution of EtOH for the analyses of flavonoid profiles. Chromatographic identification was performed by using 5 μm Ultimate XBC_{18} column (250 × 4.6 mm) at 25 °C. The mobile phase consisted of H_2O containing 0.06% AA and ACN. Flow rate was set at 1 mL/min. Analysis time was 55 min to achieve MS analysis. Only 0.2 mL/min of the effluent was delivered into the MS. The spectra were recorded in the range of *m/z* 100-1000.32 for full scan MS analysis, vonoids used for the test were chrysin, baicalin, baicalein, scutellarin, wogonoside, oroxylin A, tenaxin I, skullcapflavon I, wogonin and derivatives. All the 32 flavonoids tested were obviously detected in both UV and MS TIC chromatograms of the extract of *S. baicalensis* (199).

Another study concerning the phenolic composition of American skullcap was performed by Zhang et al. *S. lateriflora* has been traditionally used as a nerve tonic, sedative and anticonvulsant. Despite some previous studies, the quality and safety and the bioactive ingredients are not totally understood. Due to this fact, the researchers aimed to characterize the chemical profile of *S. lateriflora*. 12 phenolic compounds (viscidulin III 2'-*O*-glucopyranoside, chrysin 6-*C*-arabino-8-*C*-glucopyranoside, *trans*-verbascoside, viscidulin III, baicalin, *trans*-martynoside, oroxylin A 7-*O*-glucopyranoside, wogonoside, baicalein, wogonin, chrysin and oroxylin A) were examined. Accelerated solvent extraction was employed to prepare the sample solution

of ten batches of commercial American skullcap products. The experiment was carried out at 278 nm, using diode array detector (DAD). Analytical column was 5 µm Hypersil ODS column (250 × 4.6 mm) and the temperature of column was set at 36 °C was used. The H_2O with 0.05% AA and MeOH was used as a mobile phase. And the flow rate was set at 1 mL/min. Baicalin was reported to be the major flavonoid in all that nine extracts (116).

Baicalin, baicalein, wogonin, scutellarein and chrysin were quantified in 18 *S. baicalensis* extracts by Boyle et al. by using LC-DAD/MS. Separation was achieved in Zorbax SB C_{18} (5 µm) (150 × 4.6 mm) column at 30 °C. A gradient analysis was applied using 0.1 % FA in H_2O and MeOH at a flow rate 1 ml/min. Total analysis lasted for 45 min. Dedection was achieved at 278 nm by usind DAD. The mobile phase and gradient conditions applied for LC-DAD analysis were utilized for LC-MS determinations. Baicalin was found to be the main HPLC peak and was detected in every *Scutellaria* samples. Baicalein was also detected in all samples. In three samples scutallarein, in one sample both wogonin and chrysin were absent (200).

Qiau et al. developed a method in order to quantify seven flavonoids and four phenolic acids in *S. barbata* simultaneously by high performance liquid chromatography-tandem mass spectrometry. Protocatechuic acid, chlorogenic acid, *p*-hydroxybenzaldehyde, scutellarin, hesperidin, cinnamic acid, naringenin, luteolin, apigenin, baicalein and wogonin were used as standards. Zorbax SB C_{18} (5 µm, 150 × 4.6 mm) was used at 25 °C. The mobile phase consisted of MeOH and H_2O containing 0.1% AA. Mobile phase flow rate was set at 0.8 mL/min. MS analysis was performed using an electrospray ionisation source in negative mode. In general, whole standards were present in different *S. barbata* samples, however in some samples hesperidin, cinnamic acid and baicalein were not present (201).

2.2. General information about oxidative stress and inflammation

In this chapter of thesis, general information about the terms oxidative stress and inflammation are given.

2.2.1. Oxidative Stress

In this part oxidative stess, free radicals, lipid peroxidation and antioxidants are given respectively.

2.2.1. Oxidative Stress

2.2.1.1. Oxygen and Oxidative Stress

For the living beings and many biological systems, oxygen is an inevitable element for the nourishment (202). Oxygen free radicals or reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), are produced as a consequence of normal cellular metabolism. Both are destructive and beneficial for the living systems. For instance ROS are beneficial at low/moderate concentrations due to involving the physiological functions in cellular response to noxia (against infectious diseases etc) and the induction of a mitogenic responses (203).

The biological damage caused by free radicals (ROS and RNS), is called as "oxidative stress" or "nitrosative stress". This situation occurs in biological systems when there is an imbalance between production and catabolism of ROS/RNS. This may result from the overproduction of these free radicals and deficiency of enzymatic and non-enzymatic antioxidant enzymes (203).

The excess ROS can give harm to cellular lipids, proteins or DNA by inhibiting their normal functions. Because of this, oxidative stress has been involved in the occurance of many diseases as well as the aging process. The balance between the beneficial and harmful effects of free radicals is controlled by the mechanism called "redox regulation" and maintains with "redox homeostasis" by controlling the redox status *in vivo* (203).

2.2.1.2. Free Radicals

A free radical may be described as a molecule or molecular fragment containing one or more unpaired electrons in its outermost atomic or molecular orbital. It can be highly reactive and can initiate a chain reaction. ROS and RNS describe free radical and non radical reactive derivatives. Free radicals are produced from molecules by the homolytic cleavage of a chemical bond and via redox reactions. Once formed, they are highly reactive radicals and can start chain reactions. ROS and RNS includes radicals such as superoxide ($O_2^{\bullet}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$.), lipid peroxyl ($ROO^{\bullet}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), lipid

peroxyl ($LOO^{\bullet}$) and non radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1\Delta g$), peroxynitrate ($ONOO^-$), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) and lipid peroxide (LOOH). Non radical species are more stable than radical species and termed as oxidants that leading to formation of free radicals easily (204).

Radicals derived from oxygen represent the most important class of radical species in the living organisms. Molecular oxygen (dioxygen) has a unique electronic configuration and is itsef a radical. The addition of one electron to dioxygen produces the superoxide anion radical ($O_2^{\bullet -}$). Superoxide anion radical, is either produced through the metabolic processes or oxygen activation by physical irradiation, is defined as primary ROS and leads to the generation of secondary ROS either directly or prevalently through the enzyme- or metal- catalyzed processes (203). Superoxide oxygen is formed by direct auto-oxidation of O_2 during mitochondrial electron transport reaction. Mitocondrial electron transport chain is the primary sourse of ATP in the mammalian cell and thus is essential for life. During energy transduction a small number of electrons leak to oxygen prematurely, forming the oxygen free radical superoxide (202,203). $O_2^{\bullet -}$ is an important factor in toxicity of oxygen and the enzyme superoxide dismutase (SOD) protects the organisms toward the harmful effects of this radical. Because $O_2^{\bullet -}$ is a weak oxidant but a powerfull reductant, it is hard to give harm to cells is a rare incident. When produced $O_2^{\bullet -}$, reacts with thiol groups, thus reducing the GSH level and leading a further oxidative stress or reacting with thiols on enzymes or other cellular proteins leading their inactivation. Additionally $O_2^{\bullet -}$ can trigger a chain of reactions. One of the important reactions started with superoxide radical is Haber-Weiss reaction. In the presence of ironi on (Fe^{+3}) $O_2^{\bullet -}$ and H_2O_2 reacts to produce one of the extremely active radical hydroxyl radical ($OH^{\bullet}$). With the dismutation reaction superoxide radical turns into H_2O_2 and oxygen. The dismutation reaction is catalyzed by SOD (205).

Hydroxyl radical ($OH^{\bullet}$) is the neutral form of hydroxide ion. Althoug half-life of this radical is 10^{-9} miliseconds, this radical has very high reactivity. When it is formed the $OH^{\bullet}$ radical reacts close to its site of formation. The redox state of the cell is highly connected to an iron (and copper) redox couple and is maintained in strict conditions. It

has been known that there is no free intracellular iron. Despite that under stressful situations, an excess superoxide releases free iron from iron containing molecules. The generation of free iron (Fe^{+2}) starts the Fenton reaction. Fe^{+2} reacts with H_2O_2 to yield extremely active hydroxyl radical. To sum up Haber-Weiss and Fenton reactions yields hydroxyl radical in the presence of superoxide radical (203). Besides the main constituent of living organisms is H2O, under radiation (X radiation or gamma radiation), $OH^{\bullet}$ radical is formed from H2O molecule. As well as radiation, ultrasonication also can yield the hydroxyl radical from H_2O_2 fission. Hydroxyl radical can react almost by whole macromolecules. The main damage of this radical is seen on lipids, proteins, cytochromes and nucleic acids (205).

H_2O_2 is not a radical while it produces toxicity to cell by leading to DNA damage, membrane disruption and calcium ion release within the cells, causing calcium dependent proteolytic enzyme to be activated. Myeloperoxidase generates HOCl in activated neutrophils and intiates the deactivation of antiproteases and activation of latent proteases leading to tissue damage. HOCl can damage biomolecules and decomposes to toxic chlorine. Peroxyl radicals show their harmful effects by cyclisization reaction to endoperoxidases (204).

Free radicals lead to a series of a chain reactions starting with regeneration of new radicals and completed with destruction of radicals. Free radicals reactions take three distinct steps;

Initiation step: formation of radicals

Propagation step: in this step required free radical is regenerated repeatedly as a result of chain reaction

Termination step: destruction of radicals (204).

2.2.1.3. Lipid Peroxidation

Lipid peroxidation (LP) is the oxidative degradation of lipids with a number of carbon-carbon double bonds. A large number of toxic by-products are generated durig this process. Membrane lipids are vulnerable to LP. Because membranes form the basis of numerous cellular organelles like mitochondria, plasma membranes, endoplasmic reticulm, lysosomes, peroxisomes etc., the destruction caused by LP is highly hazardous to the functioning of the cell and its survival (206).

Polyunsaturated fatty acids (PUFAs) present in the phospholipids of the biological membranes, are the basis of their critical feature of fluidity. When LP attacks the components, it affects the biophysical properties of membranes (206). Free radicals in the presence of oxygen may cause degredation (peroxidation) of lipids within plasma and organellar membranes. Oxidative deterioration is started when double bonds in unsaturated fatty acids of membrane lipids are attacked by oxygen derived free radicals especially by $OH^{\bullet}$. The lipid-radical interactions generate peroxides, which are unstable and reactive and an autocatalytic chain reaction named propogation initiated that can result in extensive membrane, organellar and cellular damage (202). The reactive α, β-unsaturated carbony compounds likewise 4-hydroxynoneal and acrolein, which are the second products of lipid peroxidation, change the structures of biologically essential molecules such as proteins and DNA bases, resulting in various disorders and diseases (207)

LP has been associated in the pathogenesis of a number of diseases and clinical conditions. These are premature birth disorders, diabetes, adult respiratory distress syndrome, aspects of shock, parkinson disease, alzheimer disease, atherosclerosis, inflammation and etc (206).

The products of LP that can be measured include conjugated dienes, lipid hydroperoxides, aldehydes, isoprostanes, isofurans and exhaled gases. Furthermore, the progression of lipid peroxidation can be monitored by measuring the products of LP or the depletion of substrates like PUFAs or antioxidants (206).

2.2.1.4. Antioxidants

Disclosure to free radicals from various sources has led organisms to develop a series of defence mechanisms. The body has several mechanisms to protect itself from oxidative stress by producing antioxidants. According to the mechanistic functions, antioxidants may be classified into preventing antioxidants, scavenging antioxidants and repair and "*de novo"* antioxidants. Preventing antioxidants are the first line guardians, that they inhibit the formation of reactive species. For instance reducing hydrogen peroxide and lipid hydroxyperoxides to H_2O and lipid hydroxides, respectively or by sequestering metal ions. Scavenging antioxidants are the second line defense mechanism of the body against oxidative stress. The scavenging antioxidants remove reactive species rapidly before they attack other molecules. For example superoxide dismutase turns superoxide radical into hydrogen peroxide. Further, many phenolic compounds and aromatic amines act like a free radical scavengers. Several enzymes act like a third line defense by repairing destruction, clearing wastes, and reconstituting the lost function. Furthermore, the adaptation mechanism functions as the fourth line defense in which suitable antioxidants are produced at the right time and transferred to the right position in right concentration. Antioxidant compounds are either produced insitu (endogenous) or externally supplied through foods or supplements (exogeneous). The role of antioxidants is to neutralize the excess of free radicals to counteract against their toxic effects to contribute disease prevention. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This balance is necessary for the survival of organisms and their health (202,203,207).

Enzymatic (endogenous) antioxidants include superoxide dismutase, catalase and glutathione peroxidase. Besides non-enzymatic antioxidants are divided into two groups; metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants (endogenous) include glutathione, L-arginine, CoQ10, melatonin, uric acid and transferring. Nutrient antioxidants (exogenous) comprises vitamin E, vitamin C, carotenoids, trace elements (selenium, zinc, manganese), flavonoids, omega-3 and omega-6 fatty acids (202).

2.2.2. Inflammation

In this section of chapter 2.2., brief information about inflammation, eicosanoids, leukotrienes and lipoxygenases are given.

2.2.2. Inflammation

Human body is constantly subjected to bacteria, virüs, fungi and parasites, all of which normally found and to varying degrees in the skin, mouth, respiratory passageways, intestinal tract, lining membranes of the eyes an even in the urinary tract. Except normal microorganisms of the human body, we are also exposed to highly infectious microorganisms that can cause severe health problems for instance pneumonia, streptococcal infection and thyphoid fever. Our bodies have a specialized system for fighting to these infectious and toxic agents. This system is composed of certain blood leukocytes and tissue cells derived from leukocytes (208).

When tissue injury happens, whether caused by bacteria, trauma, chemicals, heat or any other reason, several substances that lead dramatic secondary changes in the tissues are released by the injured tissues. The entire complex of tissue changes is called inflammation. Inflammation is characterized by five changes at the inflammated area. These are (1) vasodilatation of the local blood vessels with consequent excess local blood flow, (2) increased permeability of the capillaries allowing leakage of large quantities od fluid into the interstital spaces, (3) often clotting of the fluid in the interstitial spaces because of excessive amounts of fibrinogen and other proteins leaking from the capillaries, (4) migration of large numbers of granulocytes and monocytes into the tissue and (5) swelling of the tissue cells (208).

2.2.2.1. Eicosanoids, Leukotrienes and Lipoxygenases

Eicosanoid term is used for a group of oxygenated, 20 carbon fatty acids. Arachidonic acid is the major precursor of this group of compounds (209). Eicosanoids like prostanoids and leukotrienes have a wide range of biological activities including potent effects on inflammation and immunity. Once liberated from the cell membrane, arachidonic acid may become a substrate for several pathways that produce biological inflammatory mediators. The most important of these pathways are the cyclooxygenase and the lipoxygenase enzymes. Lipoxygenases (LOXs) constitute a family of dioxygenases, which catalyze the oxygenation of free and esterified polyunsaturated fatty acids containing a (1*Z*,4*Z*)-penta-1,4-diene system to form the hydroperoxy derivatives. LOXs are monomeric proteins that contain a non-heme iron per molecule in

the active site as high spin Fe (II) in the native state and high spin Fe(III) in the activated state and they are categorized with respect to their positional specificity of arachidonic acid oxygenation. LOXs are the enzymes that have a role in the biosynthesis of leukotrienes. Leukotrienes are potent biological mediators in the pathopysiology of inflammatory diseases and host defense reactions. These properties imply an obvious role for leukotriene b4 in the pathogenesis of inflammatory diseases such as asthma, psoriasis, atherosclerosis and cancer. Specific natural products and synthetic compounds act by reducing the active site iron thereby uncoupling the catalytic cycle of the enzyme. Thus phenols like nordihydroguaretic acid, caffeic acid, flavonoids, coumarins or compounds like phenidone are effective 5-LOX inhibitors *in vitro* and *in vivo* (210).

3. MATERIALS AND METHODS

This section is divided into two parts. In the first part, chemicals and equipments used in this thesis are given. In the second part, the methods used in every step of this thesis are explained in details.

3.1. Materials

3.1.1. Plant Materials

Aerial parts of four *Scutellaria* taxa were collected from different regions of Turkey, between the years of 2011 and 2013. *S. hastifolia* was collected from Sakarya province of Turkey (from Kocaeli to Sakarya, at D-100 highway, after 11 km from Sapanca exit) in June 2011. Aerial parts of *S. velenovskyi* was collected on the limestones inside the *Pinus* forests, at the junction of the roads of Keltepe, Karaağaç and Küre villages, in Karabük province of Turkey (approximately at 1900 m) in July 2011. The aerial parts of *S. albida* were gathered from the ISKI (Istanbul Su ve Kanalizasyon İdaresi) terrain, neighbouring the campus area of Yeditepe University, Istanbul, Turkey in June 2013. Finally *S. orientalis* was collected from Çifteler, Çatmapınar village, Eskişehir in June 2013.

The above ground parts of each *Scutellaria* species were dried at shadow and fresh air and stored at 25 °C in air tight containers till further use.

Whole plant materials were authenticated by Prof. Dr. Hasan Kırmızıbekmez before any process. Voucher herbarium specimens for *S. hastifolia* L. (YEF 11005), *S. velenovskyi* Rech. (YEF 11006), *S. albida* L. (YEF 13008) and *S. orientalis* L. (YEF 13009) were deposited at the herbarium of the Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey.

3.1.2. Chemicals and Solvents

Table 78. Chemicals and Solvents

Chemicals & Solvents	Trademark
1,1-diphenyl-2-picryl hydrazil (DPPH)	Sigma-Aldrich
2,2′-Azobis (2-methylpropionamidine) dihydrochloride (AAPH)	Sigma-Aldrich
Acetic Acid	Riedel de Haen
Acetonitrile	Merck
Aluminum Chloride	Merck
Ammonium Molybdate	Riedel de Haen
Apigenin	Sigma-Aldrich
Ascorbic Acid	Sigma-Aldrich
Caffeic Acid	Sigma-Aldrich
Chloroform	Merck
Dichloromethane	Merck
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich
EtOH	Sigma-Aldrich
EtOAc	Sigma-Aldrich
Folin-Ciocalteu Reagent	Sigma-Aldrich
Formic Acid	Merck
Gallic Acid	Sigma-Aldrich
HPTLC Silica gel 60 F_{254} Aluminium sheet	Merck
Hydrochloric Acid (37%)	Sigma-Aldrich
Linoleic Acid Sodium Salt	Sigma-Aldrich
Lipoxidase from Glycine max (soybean)	Sigma-Aldrich
Luteolin	Sigma-Aldrich
MeOH	Sigma-Aldrich
n-Butanol	Fluka
n-Hexane	Sigma-Aldrich
Norhydroguairetic Acid (NDGA)	Sigma-Aldrich
Polyamide (for column chromatography)	Macharey-Nagel
Quercetin dihydrate	Sigma-Aldrich
Redisep MPLC columns	Teledyne Isco
Reverse phase (C_{18}) F_{254} Aluminium sheet	Merck
Silica gel 60 F_{254} Aluminium sheet	Merck
Sodium Phosphate Monobasic	Riedel de Haen
Sulphuric Acid (98%)	Riedel de Haen
Tris (hydroxymethyl) aminomethane	Sigma-Aldrich
Trolox	Sigma-Aldrich
Vanillin	Fluka

3.1.3. Equipments and Instruments

Table 79. Equipments and Instruments

Equipments	Trademark
Balance	Ohaus Explorer
Filter	Sartorius Stedim
Filter paper	Munktell
Glass basic laboratory equipments (beaker, test tubes…)	Isolab
Airdryer	Homend
HPLC	Agilent 1100
HPLC vials	Agilent
IR spectrometer	Perkin-Elmer
Laminar Flow Cabinet	Flores Valles
Lyophilizator	Christ Alpha 2-4 LD
Micropipette	Rainin
Micropipette Tips	Rainin
MilliQ H_2O device	Millipore
MPLC	Teledyne Isco, Buchi
MS	Waters
NMR device	Bruker DRX-400
Oven	Binder
Polypropylene tubes (16 x 100 mm)	Isolab
Refrigerator	Arçelik
Rotatory evaporator	Buchi, Heidolph
UV Spectrophotometer	Agilent, Spekol, Perkin-Elmer
TLC chamber	Camag
Ultrasonic bath	Sonorex
Vortex	Heidolph
H_2O bath	GFL

3.2. Methods

3.2.1. *In vitro* Activity Studies

3.2.1.1. Extraction of Plant Materials

The aerial parts of *S. hastifolia*, *S. velenovskyi*, *S. orientalis* and *S. albida* were subjected to subsequent extraction with acetone, MeOH and MeOH:H_2O (5:1), respectively. The details of the extraction will be explained in details in the section 3.2.2.1.

3.2.1.2. Antioxidant Activity Studies

3.2.1.2.1. Determination of the Reducing Activity of the Stable Radical DPPH

This assay was first reported by Brand-Williams and co-workers. The DPPH radical is one of the few stable organic nitrogen radicals, which have deep purple colour. It is commercially available, stable, easy to handle and have obvious visible absorption and hence the reaction with an antioxidant can be pursued readily by conventional UV/visible spectrophotometer. Antioxidants generally react with DPPH by hydrogen atom transfer or electron transfer, followed by proton transfer, depending on the antioxidant, radical and reaction environment. Calculations are performed after 20 and 60 min of vigorously shaking. To sum up, this assay is based on the measurement of the loss of DPPH color at 517 nm after the reaction with compounds. The reaction is monitored by spectrometer and the percentage of activity was calculated (207,211,212).

The scavenging activity of the extracts and isolated compounds against DPPH radical was calculated by using the method described by Neochoritis et al. (211). To an ethanolic solution of DPPH (freshly prepared) an equal volumes of the extracts and pure compounds dissolved in EtOH were added separately. The mixture was shaken vigorously and incubated at room temperature for 20 and 60 min. Absorbance was measured spectrophotometrically at 517 nm. NDGA was used as the reference

substance. All tests were performed in triplicate and the averages of the results were calculated.

3.2.1.2.2. AAPH Induced Linoleic Acid Lipid Peroxidation Assay

Azo compounds produce free radicals through spontaneous thermal decomposition. AAPH is one of the many azo compounds in nature, generating alkylperoxyl free radicals. In this assay, AAPH is used as a free radical initiator to follow oxidative changes of linoleic acid (LH) to conjugated diene hydroperoxide at 37 °C. Oxidation is monitored at 234 nm by spectrophometry (211,213,214).

AAPH induced lipid peroxidation inhibiton activities of the extracts and samples were calculated by using the method described by Neochoritis et al. (211). 10μL of the 16 mM linoleic acid dispersion was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4, which was previously prethermostated at 37 °C. The oxidation reaction was initiated under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of analytes (10 μL) in the assay without antioxidant, and lipid peroxidation was calculated in the presence of same level of EtOH. The oxidation rate was measured at 234 nm, which was related with the formation of conjugated diene hydroxyperoxides.

3.2.1.2.3. Determination of Total Antioxidant Capacity

Total antioxidant capacities of *S. albida, S. hastifolia, S. orientalis* and *S. velenovskyi* were measured by using the method described by Prieto et al. with slight modifications (215). The method depends on reduction of Mo (VI) to Mo (V) by the antioxidant compounds and subsequent formation of a green phosphate/Mo (V) complex at acidic pH.

300 μL of plant extract solution prepared in H_2O was mixed with 3000 μL of the reagent solution composed of ammonium molybdate (4 mM) and sodium phosphate monobasic (28 mM) which were dissolved in 600 mM sulphuric acid solution. The tubes containing the mixture were tightly capped and incubated at 95ºC for 90 min. After the incubation period, the samples were cooled to room temperature, and the absorbance was read at 695 nm. Ascorbic acid was used as the standard substance and a

calibration curve was plotted in the concentrations of 15.625, 31.25, 62.5, 125, 250 and 500 μg/mL. The results were given as mg ascorbic acid equivalent (AAE) per g material.

3.2.1.2.4. Determination of Total Phenolic Content

Total phenolic contents of *S. hastifolia, S. velenovskyi, S. orientalis and S. albida* were measured by using the method described by Singleton and Rossi (216). By using Folin-Ciocalteu Reagent, phenolic compounds form blue molybdenum-tungsten complex relying on single electron transfer. Formation of this complex was detected spectrophotometrically at 765 nm.

100 μL of Folin-Ciocalteu Reagent and 300 μL of Na_2CO_3 solution were added to 20 μL of sample in a closed cap polypropylene tubes, and the volume was adjusted to 2000 μL with doubly-distilled H_2O. The mixture was kept at 45 °C for 30 min and the absorbance was measured at 765 nm. Gallic acid dissolved in MeOH, was used as standard substance and the calibration curve was plotted in the concentrations of 50, 100, 150, 250 and 500 μg/mL. All experiments were performed in triplicate. The results were given as mg gallic acid equivalents per g material (mg GAE/g sample).

3.2.1.2.5. Determination of Total Flavonoid Content

Total flavonoid contents of *S. hastifolia, S. velenovskyi, S. orientalis and S. albida* were measured by using the method described by Woisky and Salatino (217). The method depends on the formation of acid stable complexed by $AlCl_3$ with the C-4 keto group and either C-3 or C-5 hydroxy groups of flavones or flavonols. Furthermore $AlCl_3$ also produces acid labile complexes with *o*-dihydroxyl groups in the A or B ring of flavonoids (218).

500 μL of samples dissolved in MeOH were combined with 1500 μL of 95% EtOH, 100 μL of 10% aluminum chloride solution, 100 μL of 1 M potassium acetate and the final volume of the mixture was adjusted to 5000 μL with double distilled H_2O. The mixture was kept at room temperature for 30 min and the absorbances of the samples were measured at 415 nm. Quercetin dissolved in MeOH, was used as standard substance and the calibration curve was plotted in the concentrations of 15.625, 31.25,

62.5, 125, 250 and 500 μg/mL. All of the experiments were performed in triplicate. The results were given as mg quercetin equivalents per g material.

3.2.1.3. Antiinflammatory Activity Studies

3.2.1.3.1. Soybean LOX Inhibiton Study

LOXs are important enzymes of inflammation process by leading lipid peroxidation and thus forming hydroperoxides in the lipid bilayer from the biotransformation of arachidonic acid. Inhibitors of this enzyme seen as potential agents for the treatment of inflammatory and allergic diseases. Most of the LOX inhibitors are antioxidants or free radical scavengers. LOXs carry a non-heme iron per molecule in the enzyme active site as high spin Fe^{2+} in the native state and the high spin Fe^{3+} in the activated state. The inhibition is related with the ability of the compounds to reduce the iron species in the active site to catalytically inactive ferrous form, although few LOX inhibitors are perfect ligands for Fe^{3+}. Lipophylicity is an important physicochemical property for LOX inhibitors (211). Soybean lipoxygenase assay is a universal test for assuming the antiinflammatory activities of the compounds. Sodium linoleate and enzyme solution is mixed and conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was measured in this assay and compared with the appropriate standard LOX inhibitor (152).

LOX inhibition activity of the extracts and samples were determined by using the method described by Pontiki and Hadjipavlou (219). The samples dissolved in EtOH were incubated at room temperature with sodium linoleate (100 μL) and 200 μL enzyme solution ($1/9 \times 10^4$ w/v in saline). The transformation of sodium linoleate to 13-hydroperoylinoleic acid was measured spectrophotometrically and compared with the appropriate reference substance caffeic acid at 234 nm.

3.2.2. Phytochemical Studies

3.2.2.1. Extraction

The isolation and structure elucidation studies were conducted only on air dried above ground parts of *S. hastifolia*. The powdered plant material was subjected to subsequent extraction with acetone, MeOH and MeOH:H_2O (5:1), respectively.

380 g powdered material was first extracted by macerating with acetone (2.5 L) over a night at room temperature and then filtered. The same procedure was repeated with the same amount of solvent. The pooled extracts were concentrated by using rotary evaporator under reduced pressure at 45 °C and lyophilized. The residue of the plant material after extraction with acetone was extracted twice with MeOH (2×2.4 L) at rotary evaporator at 45 °C for 4 hr. The combined MeOH extracts were concentrated as mentioned before and lyophilized. Finally, the plant material was extracted twice with MeOH:H_2O (5:1) mixture at rotary evaporator at 45 °C for 4 hr and left for maceration over a night. The pooled extracts were concentrated as mentioned before and lyophilized. This extraction procedure was valid for all other plant materials extracted for bioactivity studies.

3.2.2.2. Chromatographic Studies

3.2.2.2.1. Thin Layer Chromatography (TLC)

In order to estimate the chemical profile of the extract as well as the main fractions, to decide the mobile phases while fraction tracking during isolation studies and the preliminary purity controls of the isolates, thin layer chromatographic studies were performed. For that purpose, silica gel and reverse phase (C_{18}) coated aluminium plates were used. After preliminary TLC analysis of the crude extracts (acetone and MeOH), silica gel CC and polyamide CC were used for the main fractionation of the extracts, respectively.

Stationary Phases:

Silica gel 60 F_{254} aluminium plates 0.20 mm (Merck)

Reverse phase (C_{18}) F_{254} aluminium plates 0.20 mm (Merck)

Developing Solvents:

In order to separate compounds from each other, different proportions of solvent mixtures were utilized in TLC as mobile phase. Generally, in order to obtain a better resolution of spots on TLC plates and to prevent tailing, one or two drops of AA or FA were added in the mobile phase during the saturation process of the mobile phase.

CH_2Cl_2:MeOH:H_2O (90:10:1, 80:20:2, 70:30:3, 61:32:7)

EtOAc:MeOH:HCOOH:gCH_3COOH:H_2O (100:25:10:10:11)

H_2O:ACN (70:30)

Hexane:EtOAc (9:1, 1:2, 1:1)

Developing Distance:

7-10 cm

Derivatization:

5% H_2SO_4 (Δ)

1% Vanillin/H_2SO_4 (Δ)

Visualization:

The plates were observed under UV light (Camag UV lamp) at 254 nm and 366 nm.

3.2.2.2.2. Column Chromatography

3.2.2.2.2.1. Polyamide Column Chromatography

For the separation of phenolic and terpenic compounds from each other as well as for the preliminary fractionation of the crude MeOH extract polyamide column chromatography was preferred. Proper amount of polyamide was mixed with H_2O and kept at room temperature over a night. Then, polyamide was placed into the glass column and washed with a certain amount of H_2O in order to remove monomeric impurities.

In the main fractionation of MeOH extract of *S. hastifolia* polyamide CC was selected as told above. Elution was started with H_2O, in time MeOH proportion in H_2O was increased, and finally column was eluted with MeOH. In total 10 main fractions were obtained (Fr. A-J) based on their TLC profiles.

3.2.2.2.2.2. Sephadex Column Chromatography (Gel-Filtration)

Sephadex (LH-20) achives separation of molecules according to their molecular sizes. In this study, sephadex column chromatography was preferred in separation of different groups and generally in the last step of purification process. Amount of the gel was selected according to the quantitiy of the samples that will be loaded. Sephadex (LH-20) was mixed with MeOH and left for a maceration for a day prior to application of samples. Then, this suspension was poured into a proper glass column and left for allowing the stationary phase to settle properly in the column. MeOH was generally used as a mobile phase, whereas when working with the acetone extract CH_2Cl_2:MeOH (1:1) mixture was seldomly preferred as a mobile phase.

3.2.2.2.2.3. Silica Gel Column Chromatography

In order to fractionate and purify the samples with different polarities, silica gel column chromatography was choosen. Silica gel was suspended in the initial mobile phase and applied into the column. This adsorbant was used for the preliminary fractionation of crude acetone extract of *S. hastifolia*. With regard to the polarities of

the fractions, different solvent mixtures with various polarities were used as mobile phases.

Mobile phases:

$CHCl_3$:MeOH:H_2O (98:2:0, 97:3, 95:5:0, 90:10:0, 90:10:1, 87:13:1, 85:15:1, 80:20:2, 75:25:2, 70:30:3, 65:35:4, 61:32:7, 60:40:5,60:40:4, 50:40:10)

Hexane:EtOAc (9:1, 8:2, 2:1, 1:1, 0:1)

EtOAc:MeOH (1:1, 0:1)

As mentioned above, in the main fractionation of acetone extract of *S. hastifolia,* SiO_2 column chromatography was used. Elution was initiated with hexane:EtOAc mixture in the portions of 9:1 and ended with EtOAc:MeOH (1:1). In total 17 (Fr. A-R) fractions were obtained.

3.2.2.2.2.4. Medium Pressure Liquid Chromatography (MPLC)

In MPLC studies, both silica gel and reverse phase (LiChroprep C_{18}) columns were used. Just like mentioned in silica gel column chromatography, in MPLC purifications, compounds were separated according to their polarities. MPLC purification was preferred when the polarities of the compounds in the fractions were considerably similar. In this work, both Buchi and Combiflash MPLC instruments were utilized. As stationary phase, high performance gold columns of both normal and reverse phase adsorbents along with the standard silica gel and reverse phase columns in various sizes were frequently preferred. As mentioned before, with regard to the polarities of the fractions, different solvent mixtures with various polarities were used as mobile phases. In reverse phase MPLC studies, generally elution started with H_2O, went on MeOH (or ACN) in H_2O and purification was terminated with MeOH or ACN. In normal phase MPLC studies, elutions mainly initiated with CH_2Cl_2, sustained with different proportions of CH_2Cl_2:MeOH mixtures and concluded with 100% MeOH. In definitely apolar fractions, hexane:EtOAc mixtures in different ratios were preferred as mobile phase.

3.2.3. Analytical Studies

3.2.3.1. High Performance Liquid Chromatography (HPLC)

In the frame of this study, two new methods were developed for qualitative and quantitative analysis of the mostly encountered phenylethanoids and flavonoids of *Scutellaria* species. As well as main phenylethanoids and flavonoids of *Scutellaria* species, some of the isolated compounds from *S. hastifolia* were analysed in MeOH extracts of all plant samples (*S. hastifolia, S. velenovskyi, S. orientalis* and *S. albida*) with the help of HPLC.

3.2.3.1.1. Instrument

HPLC separations were performed on HP Agilent 1100 chromatographic system with quaternary pump and DAD dedector. The software of the HPLC system was Agilent Chem Station.

3.2.3.1.2. Preparation of Sample Solutions

All crude drug samples were powdered to a homogenous size by a mill. 1 g of each *Scutellaria* species were accurately weighed and extracted twice with 20 mL of HPLC grade MeOH in ultrasonic bath for 30 min at 30 °C for each time. The pooled extracts were concentrated by rotary evaporator at 45°C. The amounts of each extract and the yields of extractions were calculated. Each sample was dissolved in 5 mL of MeOH and filtrated through 45 µm Sartorius filters. Appropriate dilution processes were applied with MeOH when necessary. The working solutions were kept at -4°C until use.

3.2.3.1.3. Preparation of Standard Solutions

Stock solutions of the standards of both phenylethanoids and flavonoids were initially prepeared as 1000 µg/mL in MeOH, and then diluted with MeOH (1-120 µg/mL). At least five concentration levels were selected for calibration curve of each compound.

Phenylethanoid glycosides used in analysis were calceolarioside D, neocalceolarioside D, verbascoside, isoverbascoside, leucoseptoside A and martynoside (Table 80-81). Working concentrations were 3, 5, 15, 20, 40, 75 and 120 µg/mL for calceolarioside D and neocalceolarioside D, 3, 5, 15, 40, 75, 80 and 120 µg/mL for verbascoside, 3, 5, 15, 20, 40, 60, 75 and 120 µg/mL for isoverbascoside, 1, 3, 5, 15, 20, 40, 60, 75 and 120 µg/mL for leucoseptoside A, and finally 3, 5, 15, 20, 60, 75 and 120 µg/mL for martynoside. 10, 50 and 100 µg/mL concentrations of each standard phenylethanoid solution were selected as quality control concentrations during validation of the method developed.

Flavonoids utilized in the analysis were scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, luteolin and apigenin (Table 82). Working concentrations were 5, 15, 40, 75 and 120 µg/mL for scutellarein 7-*O*-β-D-glucopyranoside, 5, 15, 30, 40 and 75 µg/mL for hispidulin 7-*O*-β-D-glucuronopyranoside, 1, 5, 15, 30, 40, 75 and 120 µg/mL for apigenin 7-*O*-β-D-glucopyranoside, 1, 5, 15, 40 and 75 µg/mL for hispidulin 7-*O*-β-D-glucopyranoside and finally 1, 5, 15, 30, 40 and 75 µg/mL for the aglycones luteolin and apigenin. 10, 20 and 60 µg/mL concentrations of each standard flavonoid solution were selected as quality control concentrations during validation of the method developed. Quercetin was selected as internal standard.

3.2.3.1.4. Reference Substances

3.2.3.1.4.1. Phenylethanoid Glycosides

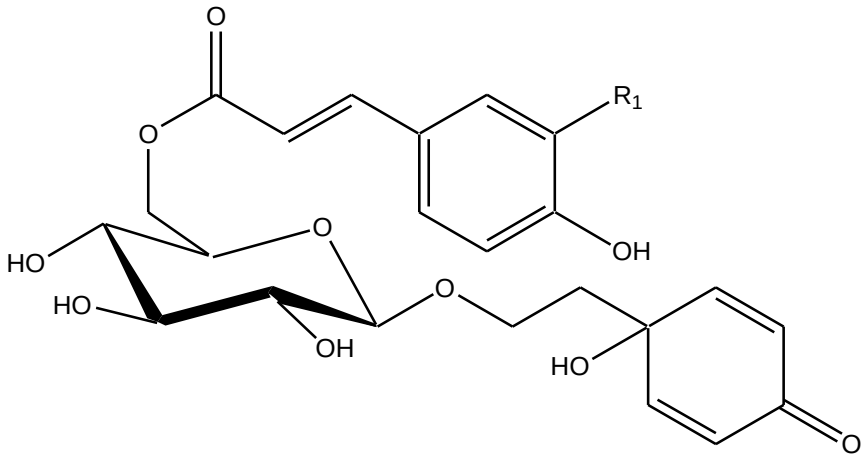


Table 80. Phenylethanoid glycosides

Phenylethanoids	R_1
Calceolarioside D **(1)**	OH
Neocalceolarioside D **(2)**	H

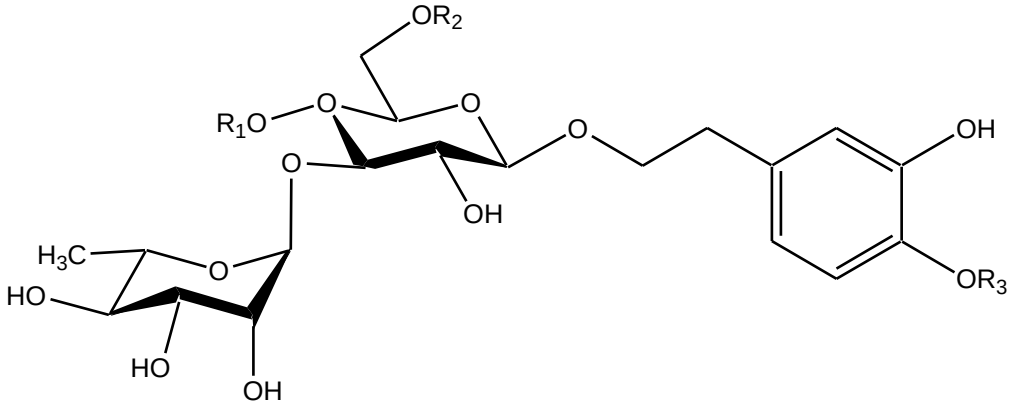


Table 81. Phenylethanoid glycosides

Phenylethanoids	R_1	R_2	R_3
Verbascoside **(3)**	Caffeoyl	H	H
Isoverbascoside **(4)**	H	Caffeoyl	H
Leucoseptoside A **(5)**	Feruloyl	H	H
Martynoside **(6)**	Feruloyl	H	CH_3

3.2.3.1.4.2. Flavonoids

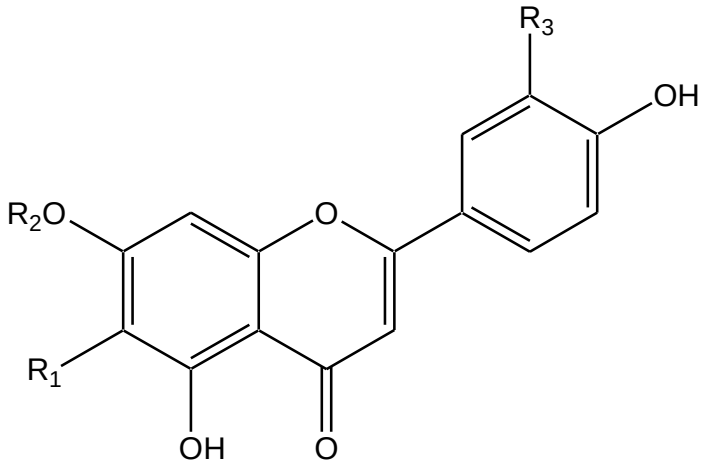


Table 82. Flavonoids

Flavonoids	R_1	R_2	R_3
Scutellarein 7-*O*-β-D-glucopyranoside **(1)**	OH	Glc	H
Hispidulin 7-*O*-β-D-glucuronopyranoside **(2)**	OCH_3	GluA	H
Apigenin 7-*O*-β-D-glucopyranoside **(3)**	H	Glc	H
Hispidulin 7-*O*-β-D-glucopyranoside **(4)**	OCH_3	Glc	H
Luteolin **(5)**	H	H	OH
Apigenin **(6)**	H	H	H

3.2.3.1.5. Method Validation

The quantification of phenylethanoid glycosides and flavonoids were accomplished by developing two different methods due to the complex composition of the plant extracts. The newly developed methods were validated in terms of linearity (calibration curve), sensitivity [limit of detection (LOD), limit of quantification (LOQ)], accuracy, precision, robustness and system suitability according to ICH and FDA guideliness (220–222).

Using developed optimum conditions, areas of the standard solutions were measured and linear concentration ranges were determined. Triplicate experiments of three independent samples were performed for 10, 50 and 100 µg/mL of standard solutions of each phenylethanoid glycoside and 10, 20 and 60 µg/mL of standard solutions of each flavonoid for validation studies. Linearity is the ability of the method to elicit the test results are directly proportional to analyte concentration within a given range. All calibration curves were obtained by plotting the peak areas of the standard compounds versus the corresponding concentrations of the injected standard solutions. Recovery values were calculated for each developed method by the help of the peak areas of standard substances. Accuracy of the method was calculated for both intra- and inter-day variations using at least triplicate analysis. Precision of the assay was determined by repeatability (intraday) and intermediate precision (interday). Intermediate precision was assessed by comparing the analyses on three different days. Robustness studies along with the measurements of system suitability of the developed method were evaluated according to the ICH guidelines (221). Temperature (23-27°C), acetic acid percent (0.015%-0.025%) and wavelenght (320–340 nm for phenylethanoid glycosides and 330-350 nm for flavonoids) were changed deliberately. Recovery values were again calculated for new conditions and compared. The system suitability is used to verify that the resolution and repeatability of the system are adequate for the analysis to be performed. Capacity factor ($k' > 2$), resolution ($R_s > 2$), tailing factor ($T \leq 2$) and theoretical plates ($N > 2000$) were determined and compared with the theoretical data (221). LOD, LOQ values were calculated by using standard deviation of calibration curve intercept values (S_a) and following equations were used:

$LOD=3S_a/m$

$LOQ=10S_a/m$

In these equations **m** value was slope of the calibration curve.

3.2.3.1.6. Method Development and Optimization for Phenylethanoid Glycosides

Different mobile phase compositions, column temperature and flow rates were compared in the view of achieving good peak shape, higher peak responses, shorter analysis time and better resolutions. Finally the best separation was achieved on a Zorbax XDB-C_{18} column (4.6×150 mm, 3.5 µm) with the flow rate of 0.8 mL/min. The injection volume was set at 10 µL, three injections were performed for samples and standard solutions. Wavelenght was selected as 330 nm due to the maximum absorption in spectra obtained by DAD detector of HPLC system for phenylethanoid glycosides. Chromatographic column was thermostated at 25°C during all experiments except robustness and ruggedness studies. Mobile phase was consisted of H_2O and ACN with 0.02% AA. AA was preferred as a buffer agent due to eliminate the peak tailing and for the high resolution of peaks. For the gradient elution, initial conditions were 15% of ACN with a linear gradient to 20% in 20 min, 20% of ACN was maintained for 5 min and in 10 min ACN proportion was increased to 45%. In total, qualitative and quantitative analysis of six phenylethanoid glycosides in four *Scutellaria* extracts, was performed in 31 min by using this newly developed and validated method.

3.2.3.1.7. Method Development and Optimization for Flavonoids

A variety of methods, solvents, times and column types were used in order to obtain the optimum separation of each standard in the extracts. MeOH:H_2O and ACN:H_2O combinations with different buffer agents (FA, AA), various flow rates (0.5 µL to 1.5 µL, several column temperatures (10 °C to 30°C) and different column types (zorbax cyano column, zorbax C_2 column, zorbax C_8 column and zorbax phenyl column with diverse pore and column sizes) were employed in order to obtain best resolution and the shortest analysis time. Eventually, the greatest separation was achieved on a Zorbax XDB-C_{18} column (4.6×150 mm, 3.5 µm) column with the flow rate of 0.8

mL/min. The injection volume was set at 10 μL, three injections were performed for each sample and standard solutions. Wavelenght was selected as 340 nm due to the maximum absorption in spectra obtained by DAD detector of HPLC system for flavonoids. Chromatographic column was thermostated at 25°C during all experiments except robustness and ruggedness studies. Mobile phase was consisted of H_2O and ACN with 0.02% AA. Gradient system was started with 15% ACN and in 10 min the proportion of ACN was increased to 20% and sustained for 3 min at 20%. In 2 min the ratio of ACN was raised to 25% and in 5 min to 30%. This concentration of ACN was retained for 5 min and terminally increased to 50% in 5 min. In total qualitative and quantitative analysis of six flavonoids in four *Scutellaria* species was lasted only for 29 min by this newly developed and validated method.

3.2.4. Statistics

Whole experiments were performed in triplicate. The results were expressed as mean ± standard deviation. When needed statistical comparisons were made using the Kruskal Wallis test. Statistically significant difference was defined as $p < 0.05$.

4. RESULTS

The results of the present study are compiled under three sections: Activity results, phytochemical results and analytical results. The first section includes the biological activity data performed on extracts. The second section comprises an elaborate discussion of the structure elucidation of the compounds which were obtained from *S. hastifolia*. The last section contains the results of the analytical studies on phenylethanoid glycosides and flavonoids of the MeOH extracts of four species.

4.1. Activity Results

In this part, the results of the activity assays are given. Results of total phenolic content, total flavonoid content and total antioxidant capacity assays on four *Scutellaria* extracts along with DPPH radical scavenging assay, AAPH linoleic acid lipid peroxidation assay as well as soybean LOX inhibition assay on *S. hastifolia* crude extracts and isolated compounds were declared.

4.1.1. Results of *in vitro* Activity Studies of Crude Extracts

4.1.1.1. Extraction Yields

10 g of each *Scutellaria* materials were extracted as described previously. The extraction yields are given in Table 83.

Table 83. Extraction yields

Plant Materials	Acetone Extract (%)	MeOH Extract (%)	MeOH:H_2O Extract (%)
S. hastifolia	3.6	14.7	7.6
S. velenovskyi	3.6	9.6	10.8
S. orientalis	2.6	12.3	7.3
S. albida	3.0	14.0	7.3

4.1.1.2. Total Antioxidant Capacities of *Scutellaria* Extracts

Total antioxidant capacities of the three different extracts of the four *Scutellaria* species are given in Table 84 and Figure 1. The results were expressed as mg ascorbic acid equivalents per g dry extract (mg AAE/g sample). According to results, among the acetone and MeOH extracts, *S. hastifolia*, among the MeOH:H_2O extracts, *S. velenovskyi* showed the highest total antioxidant capacity.

Table 84. Total Antioxidant Capacities of the *Scutellaria* extracts

	Total Antioxidant Capacities [A]			
Extracts/Plant Materials	***S. hastifolia***	***S. velenovskyi***	***S. orientalis***	***S. albida***
Acetone Extract	470.19±1.01[B*]	191.53±8.79	147.53±0.58	306.18±3.08
MeOH Extract	249.84±5.00	219.53±2.95	206.61±1.09	238.51±7.91
MeOH:H_2O Extract	203.10±11.52	260.32±6.57	181.73±5.25	231.89±9.38

[A]Total antioxidant capacity is expressed as mg ascorbic acid equivalents (AAE) in 1 g dry extract.
[B]Results are expressed as the mean of triplicates ± S.D.
*All of the results were found to be significantly different from each other ($p<0.05$)

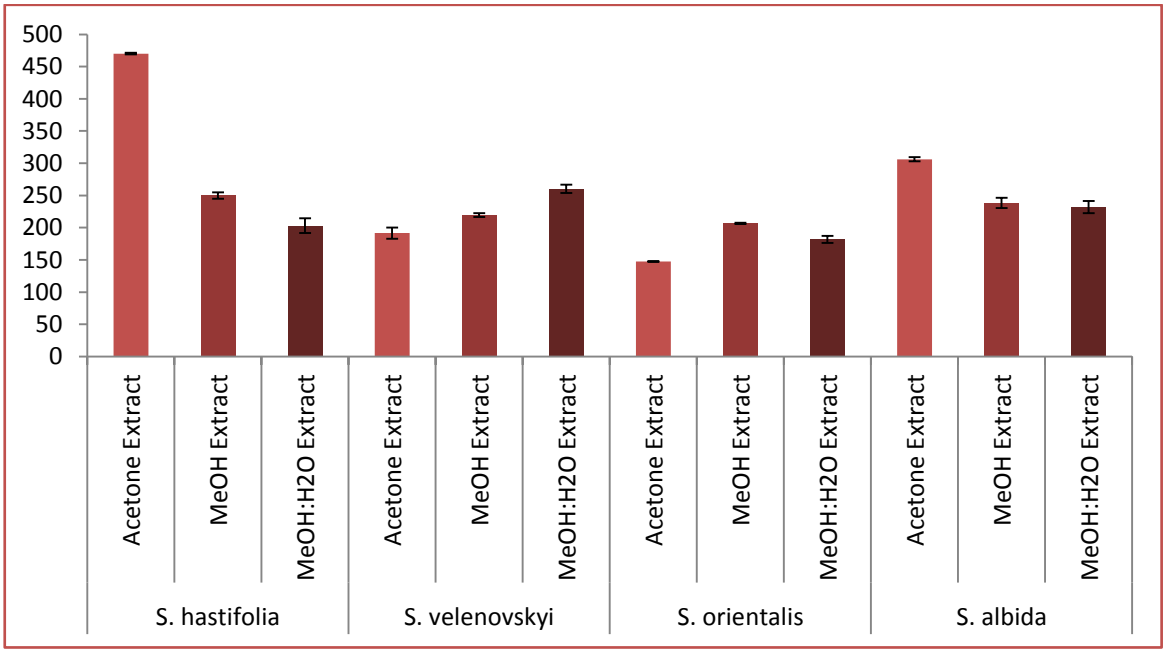


Figure 1. Total Antioxidant Capacities of *Scutellaria* extracts. Results are expressed as mg ascorbic acid equivalents (AAE) in 1 g dry extract.

4.1.1.3. Total Phenolic Contents of *Scutellaria* Extracts

The total phenolic contents of the three different extracts belonging to four *Scutellaria* taxa were calculated. The results were given as mg gallic acid equivalents per g material (mg GAE/g sample) and written in the Table 85 and Figure 2. Among the acetone extracts, *S. albida* showed the highest total phenolic content, among the MeOH extracts *S. orientalis* showed the highest total phenolic content, among the MeOH:H_2O extracts *S. hastifolia* showed the highest amount of total phenolic content.

Table 85. Total Phenolic Contents of the *Scutellaria* extracts

	Total Phenolic Content [A]			
Extracts/Plant Materials	***S. hastifolia***	***S. velenovskyi***	***S. orientalis***	***S. albida***
Acetone Extract	66.15±0.76[B*]	46.41±0.44	84.61±0.76	98.71±1.17
MeOH Extract	105.38±1.17	40.00±2.03	158.20±1.93	77.69±0.77
MeOH:H_2O Extract	127.94±1.33	48.46±1.53	124.61±2.03	82.30±0.76

[A]Total phenolic content is expressed as mg gallic acid equivalents (GAE) in 1 g dry extract.
[B]Results are expressed as the mean of triplicates ± S.D.
*All of the results were found to be significantly different from each other ($p<0.05$)

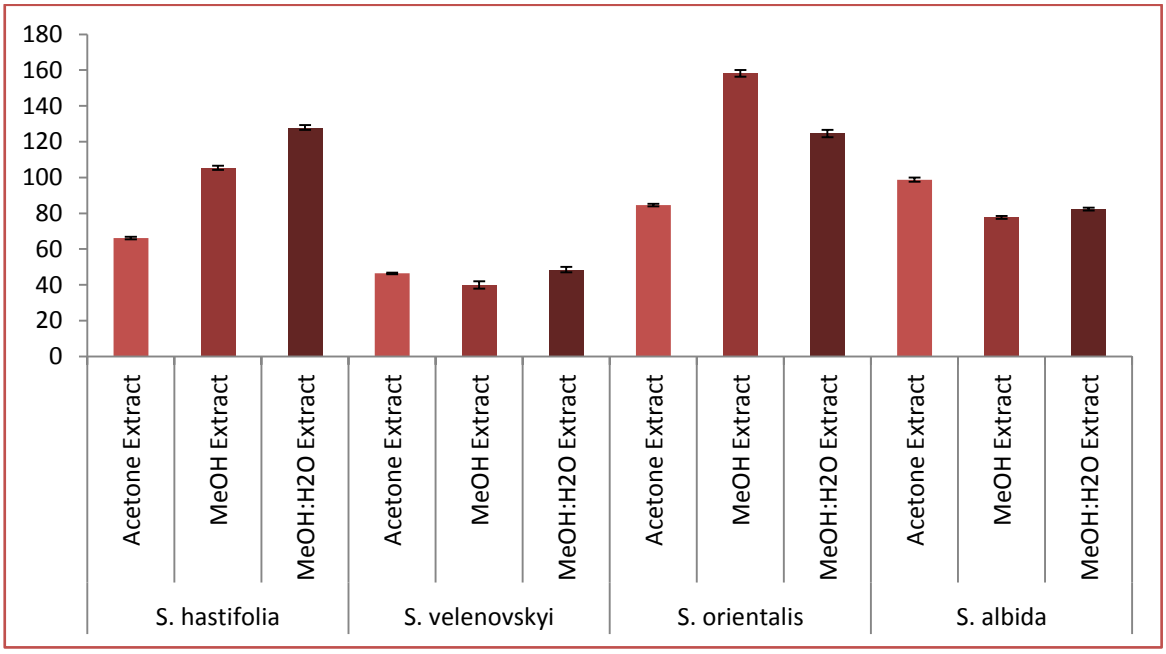


Figure 2. Total Phenolic Contents of *Scutellaria* extracts. Results are expressed as mg gallic acid equivalents (GAE) in 1 g dry extract.

4.1.1.4. Total Flavonoid Contents of *Scutellaria* Extracts

Three different extracts of the four *Scutellaria* species were investigated for their total flavonoid contents. The results were given as mg quercetin equivalents per g material (mg QE/g sample) in the Table 86 and Figure 3. Among the acetone extracts *S. velenovskyi* showed the highest total flavonoid content, while among the MeOH extracts *S. orientalis* showed the highest total flavonoid content, among the MeOH:H_2O extracts *S. hastifolia* showed the highest total flavonoid content.

Table 86. Total Flavonoid Contents of the *Scutellaria* extracts

	Total Flavonoid Content [A]			
Extracts/Plant Materials	***S. hastifolia***	***S. velenovskyi***	***S. orientalis***	***S. albida***
Acetone Extract	92.72±1.76[B*]	147.22±0.67	106.28±0.88	94.39±2.27
MeOH Extract	36.95±1.15	24.83±0.53	47.83±0.50	23.22±0.48
MeOH:H_2O Extract	53.33±1.53	20.39±0.48	41.17±0.19	27.06±0.41

[A]Total flavonoid content is expressed as mg quercetin equivalents (QE) in 1 g dry extract.
[B]Results are expressed as the mean of triplicates ± S.D.
*All the results were found to be significantly different from each other ($p<0.05$)

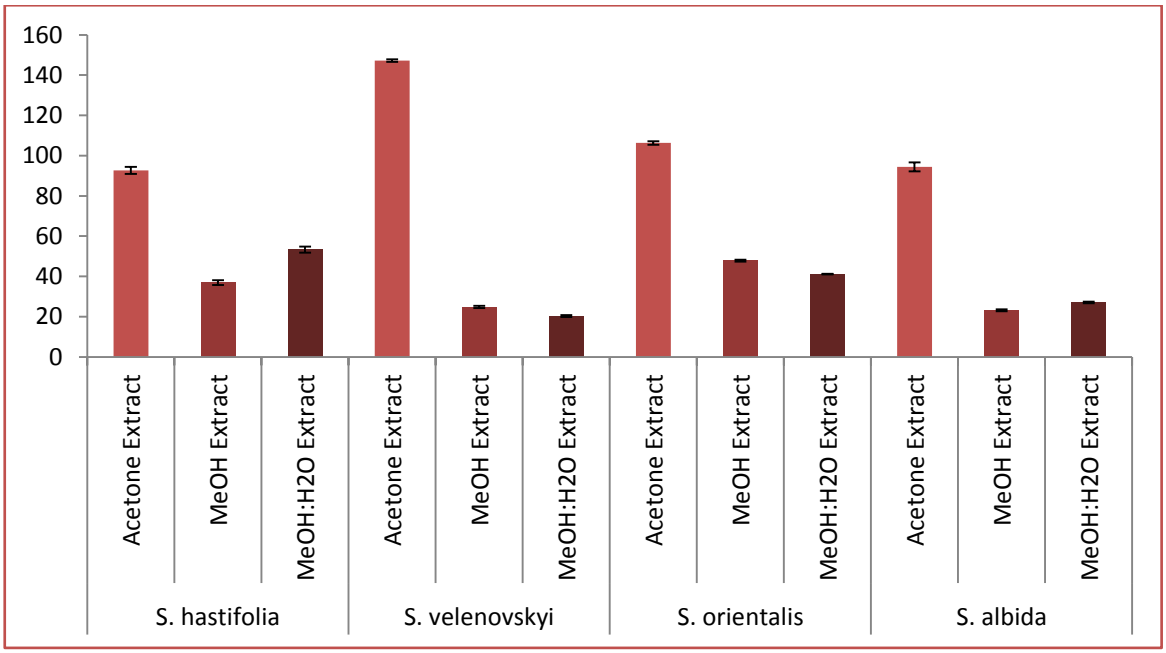


Figure 3. Total Flavonoid Contents *Scutellaria* extracts. Results are expressed as mg quercetin equivalents (QE) in 1 g dry extract.

4.1.1.5. DPPH Radical Scavenging Activity

Acetone, MeOH and MeOH:H_2O extracts of *S. hastifolia* were tested for their DPPH radical scavenging. Results of DPPH radical scavenging activities of the extracts are given in Table 87 and Figure 4. All the extracts highly interacted with DPPH, indicating equipotent antioxidant activity. No change in results is observed after 60 minutes.

Table 87. Percentage interaction of *S. hastifolia* extracts with DPPH

Extract (5 mg/mL EtOH)	DPPH RA % 20/60 min (20 µL/2 mL)
Acetone	63±2.0[A]
MeOH	68±1.8
MeOH:H_2O	66±1.1
NDGA	81/83±1.3

[A]Results are expressed as the mean of triplicates ± s.e.m.
NDGA: nordihydroquaeretic acid.

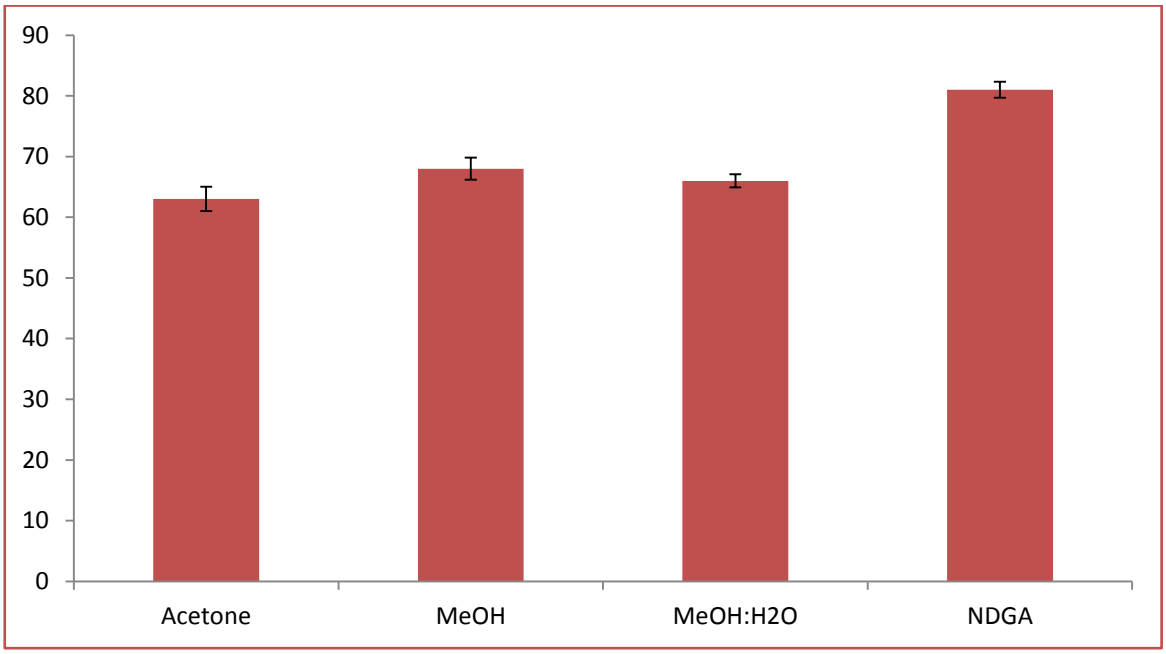


Figure 4. Percentage interaction of *S. hastifolia* extracts with DPPH

4.1.1.6. AAPH Induced Linoleic Acid Lipid Peroxidation Assay

Acetone, MeOH and MeOH:H_2O extracts of *S. hastifolia* were tested for their AAPH induced lipid peroxidation inhibiton as mentioned in the section 3.2.1.2.2. The results are given in the Table 88 and Figure 5. Among the three extracts, acetone extract exhibited the highest activity which was very close to activity of the reference compound Trolox.

Table 88. Percentage inhibition of *S. hastifolia* extracts on lipid peroxidation (AAPH %)

Extract (5 mg/mL EtOH)	AAPH % (10 µL/1 mL)
Acetone	57±0.5 [A]
MeOH	33±0.8
MeOH:H_2O	19±0.3
Trolox	64±0.8

[A]Results are expressed as the mean of triplicates ± s.e.m.
Trolox: 6-hydroxy-2,5,6-7,8-tetramethylchromane-2-carboxylic acid.

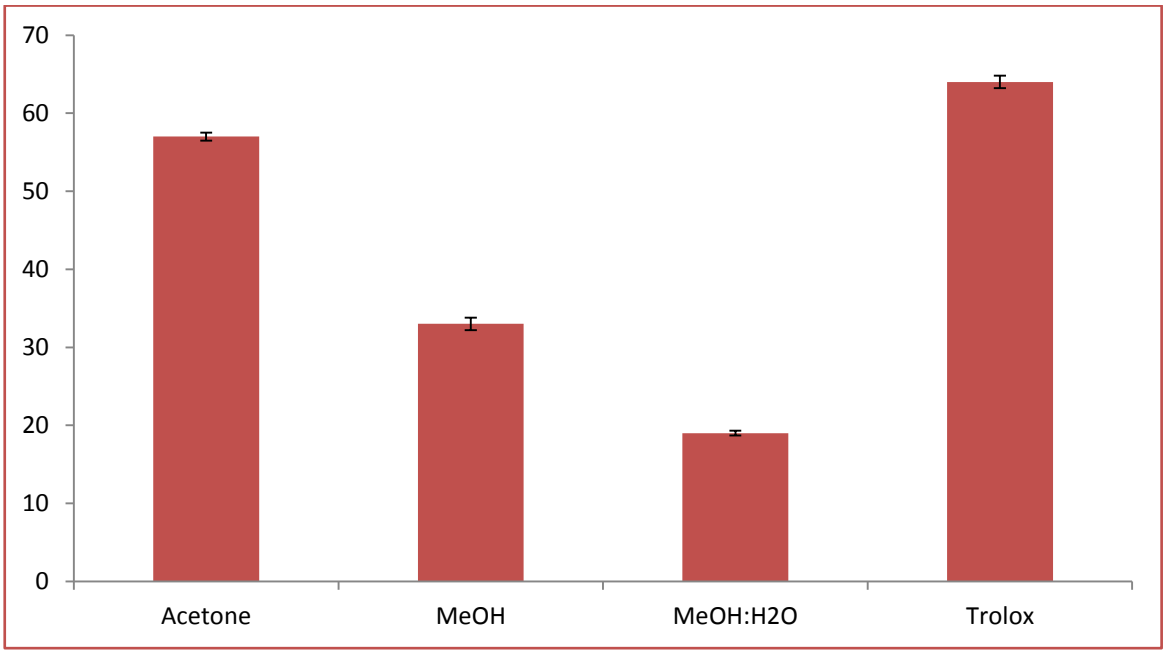


Figure 5. Percentage inhibition of *S. hastifolia* extracts on lipid peroxidation (AAPH %)

4.1.1.7. Soybean LOX Inhibiton Study

The soybean lipoxygenase (LOX) assay was used as an indication of the potent antiinflammatory activity as well as antioxidant activity. Most of the LOX inhibitors are also antioxidant substances. Acetone, MeOH and MeOH:H_2O extracts of *S. hastifolia* were tested for their soybean LOX inhibitions. The results are given in the Table 89 and Figure 6. Among the tested three extracts only MeOH extract inhibited the LOX enzyme. Acetone and MeOH:H_2O extracts did not showed any activity at the tested conditions and concentrations.

Table 89. Percentage inhibition of *S. hastifolia* extracts on soybean LOX (LOX %)

Extract (5 mg/mL EtOH)	**LOX % (10 µL/1 mL)**
Acetone	No
MeOH	100±1.9 [B]
MeOH:H_2O	No
NDGA	84±2.2

[A]Results are expressed as the mean of triplicates ± s.e.m.
NDGA: nordihydroquaeretic acid.
No means no activity observed under the experimental conditions.

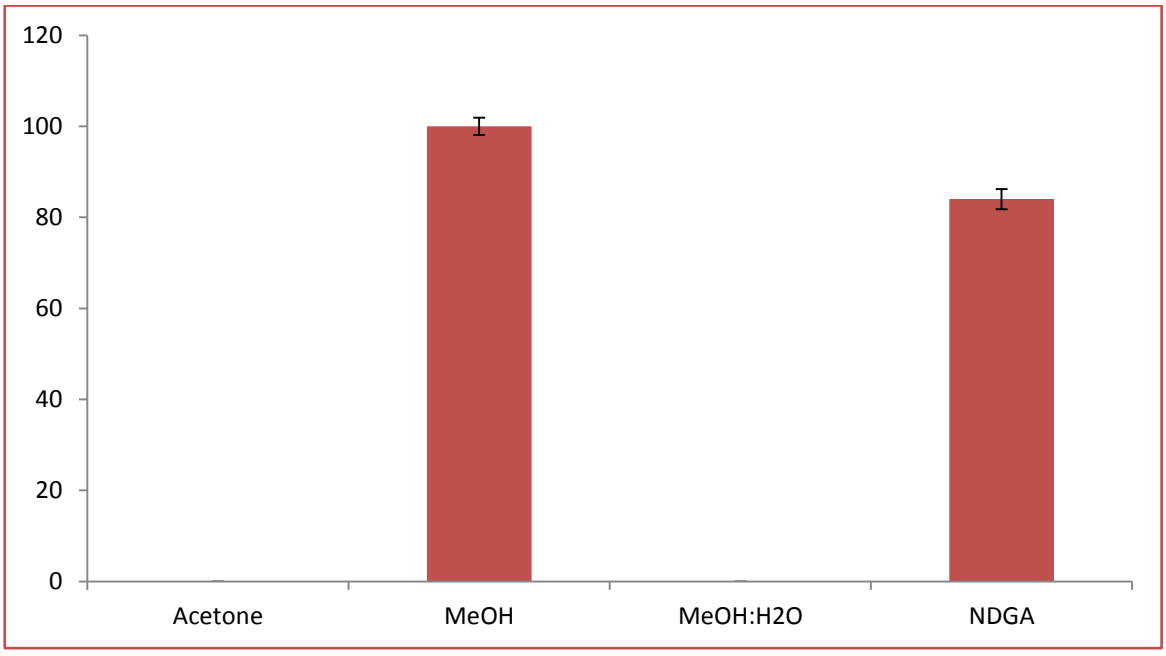


Figure 6. Percentage inhibition of *S. hastifolia* extracts on soybean LOX (LOX %)

4.1.2. Results of *In vitro* Activity Studies of Isolated Compounds from *S. hastifolia*

DPPH radical scavenging assay, lipid peroxidation inhibition assay and soybean LOX inhibition assays were also performed in order to estimate the antioxidant and antiiflammatory potentials of isolated compounds from the acetone and MeOH extracts of *S. hastifolia*. Isorengyol, isorengyoside, cleroindicin B and cleroindicin F are the etyhlcyclohexane derivatives isolated from the acetone and MeOH extracts of *S. hastifolia*, cornoside, calceolarioside D, hastifolioside, neocalceolarioside D, calceolarioside B and verbascoside are the phenylethanoid glycosides isolated from acetone and MeOH extracts of *S. hastifolia*, apigenin 7-*O*-β-D-glucopyranoside, scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside and hispidulin 7-*O*-β-D-glucuronopyranoside are the flavonoids isolated from acetone and MeOH extracts of *S. hastifolia*. Moreover, 6-*O*-caffeoylglucopyranose is one of the caffeic acid ester of glucose molecule, isolated from MeOH extract of *S. hastifolia.*

4.1.2.1. DPPH Radical Scavenging Activity

The DPPH radical scavenging of those compounds are given in the Table 90 and Figure 7. Among the ethylcyclohexane derivative compounds cleroindicin F and isorengyol elicited the highest DPPH radical scavenging activity, wheras the derivative of isorengyol, namely isorengyoside did not show any activity. Except hastifolioside and neocalceolarioside D, all the phenylethanoid glycosides highly reacted with DPPH. Among the four flavonoids isolated, scutellarein 7-*O*-β-D-glucopyranoside exhibited the highest DPPH radical scavenging activity. On the other hand hispidulin aglycone with different sugar moieties, elicited similar degrees of DPPH radical scavenging activity. All the flavonoid molecules showed higher DPPH interaction than the reference compound NDGA.

Table 90. Percentage interaction of the isolated compounds with DPPH

Compounds (5 mg/mL EtOH)	DPPH RA % 20 min (20 µL/2 mL)
Isorengyol	80±3.2 [A]
Isorengyoside	No
Cleroindicin B	75±1.5
Cleroindicin F	81±1.2
Cornoside	85±3.1
Calceolarioside D	97±1.3
Hastifolioside	10±1.1
Neocalceolarioside D	16±1.2
Calceolarioside B	97±1.8
Verbascoside	97±2.5
Apigenin 7-*O*-β-D-glucopyranoside	96±1.0
Scutellarein 7-*O*-β-D-glucopyranoside	98±1.2
Hispidulin 7-*O*-β-D-glucopyranoside	89±1.9
Hispidulin 7-*O*-β-D-glucuronopyranoside	90±2.0
6-*O*-caffeoyl-α/β-glucopyranose	52±2.0
NDGA	83±1.8

[A]Results are expressed as the mean of triplicates ± s.e.m.
NDGA: nordihydroquaeretic acid.
No means no activity observed under the experimental conditions.

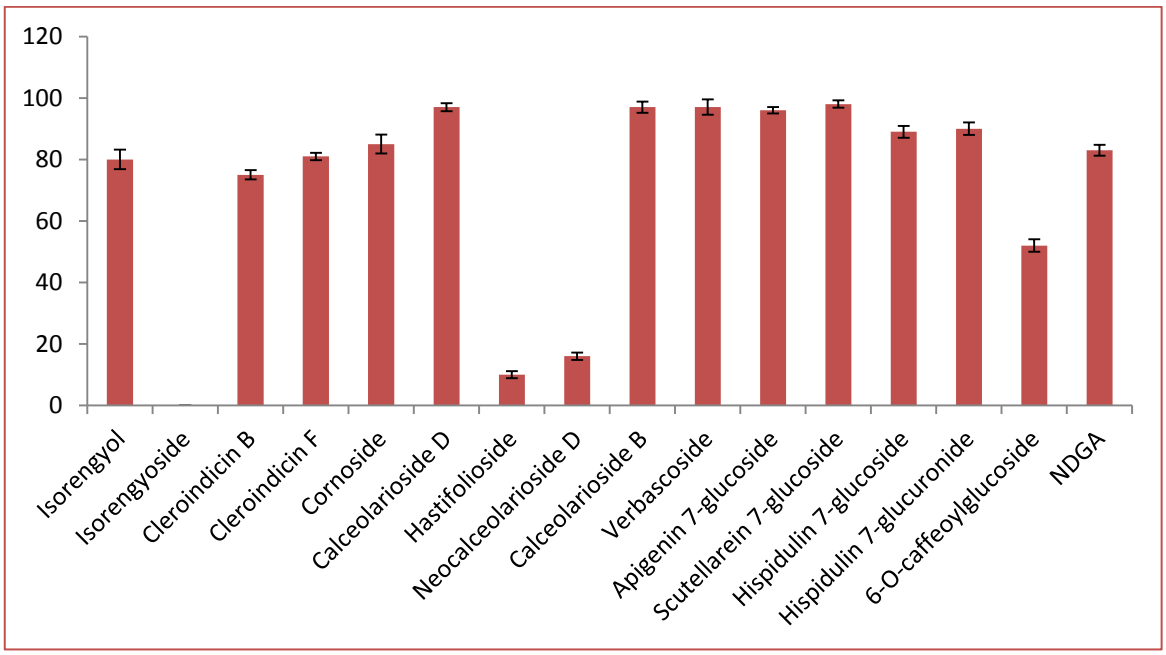


Figure 7. Percentage interaction of the isolated compounds with DPPH

4.1.2.2. AAPH Induced Linoleic Acid Lipid Peroxidation Assay

AAPH induced linoleic acid lipid peroxidation inhibition assay results of the isolated compounds are given in the Table 91 and Figure 8. The ethylcyclohexane derivatives did not show high lipid peroxidation inhibition, moreover they all showed weak inhibition response on AAPH induced linoleic acid lipid peroxidation. Among the phenylethanoid glycosides, only calceolarioside D exerted moderate inhibition while hastifolioside did not show any activity. Although all flavonoids were highly interacted with DPPH radical, half of them did not show any remarkable activity on lipid peroxidation inhibition. Among the pure compounds apigenin 7-*O*-β-D-glucopyranoside showed the highest inhibition on lipid peroxidation, wheras hispidulin 7-*O*-β-D-glucuronopyranoside did not show any effect under the experimental conditions.

Table 91. Percentage inhibition of the isolated compounds on lipid peroxidation (AAPH %)

Compounds (5 mg/mL EtOH)	**AAPH % (10 µL/1 mL)**
Isorengyol	22±1.3 [A]
Isorengyoside	10±0.5
SH-57	27±0.8
Cleroindicin B	22±2.0
Cleroindicin F	24±1.0
Cornoside	36±1.2
Calceolarioside D	48±1.5
Hastifolioside	No
Neocalceolarioside D	26±0.8
Calceolarioside B	19±0.8
Verbascoside	13±0.6
Apigenin 7-*O*-β-D-glucopyranoside	53±1.6
Scutellarein 7-*O*-β-D-glucopyranoside	40 ± 0.8
Hispidulin 7-*O*-β-D-glucopyranoside	29±0.5
Hispidulin 7-*O*-β-D-glucuronopyranoside	No
6-*O*-caffeoyl-α/β-glucopyranose	3±0.5
Trolox	64±0.8

[A]Results are expressed as the mean of triplicates ± s.e.m.
Trolox: 6-hydroxy-2,5,6-7,8-tetramethylchromane-2-carboxylic acid.
No means no activity observed under the experimental conditions.

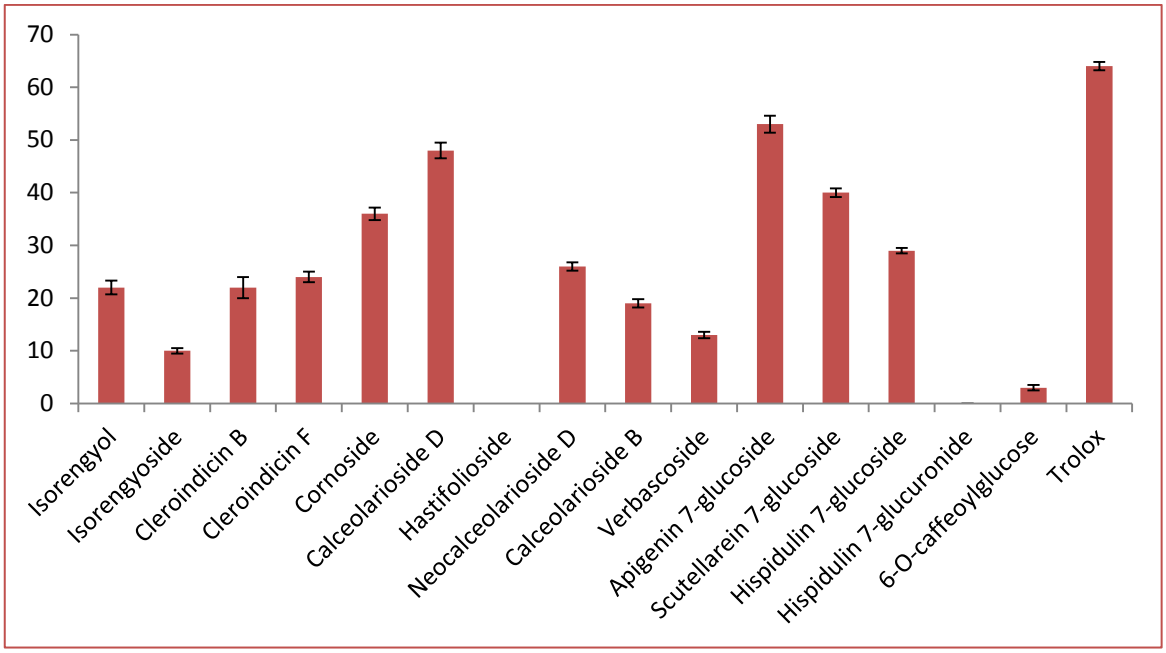


Figure 8. Percentage inhibition of the isolated compounds on lipid peroxidation (AAPH %)

4.1.2.3. Soybean LOX Inhibiton Study

The results of soybean LOX inhibition assay of the isolates are listed in Table 92 and Figure 9. Among the ethylcyclohexane derivatives, none of the molecules showed any remarkable LOX inhibition when compared with the reference substance NDGA. Only isorengyoside displayed moderate LOX inhibition. Additionally, phenylethanoid glycosides showed very low inhibition. Among the isolated flavonoids, apigenin 7-*O*-β-D-glucopyranoside elicited the highest LOX inhibition, wheras hispidulin 7-*O*-β-D-glucuronopyranoside did not show any activity under the experimental conditions.

Table 92. Percentage inhibition of the isolated compounds on soybean LOX (LOX %)

Compounds (5 mg/mL EtOH)	LOX % (10 µL/1 mL)
Isorengyol	12±0.4[A]
Isorengyoside	36±0.3
SH-57	12±1.9
Cleroindicin B	11±0.2
Cleroindicin F	19±0.2
Cornoside	29±1.0
Calceolarioside D	No
Hastifolioside	16±0.2
Neocalceolarioside D	32±1.9
Calceolarioside B	4±0.1
Verbascoside	9±1.0
Apigenin 7-*O*-β-D-glucopyranoside	55±1.3
Scutellarein 7-*O*-β-D-glucopyranoside	12±0.4
Hispidulin 7-*O*-β-D-glucopyranoside	18±0.4
Hispidulin 7-*O*-β-D-glucuronopyranoside	No
6-*O*-caffeoyl-α/β-glucopyranose	2±0.3
NDGA	84±2.2

[A]Results are expressed as the mean of triplicates ± s.e.m.
NDGA: nordihydroquaeretic acid.
No means no activity observed under the experimental conditions.

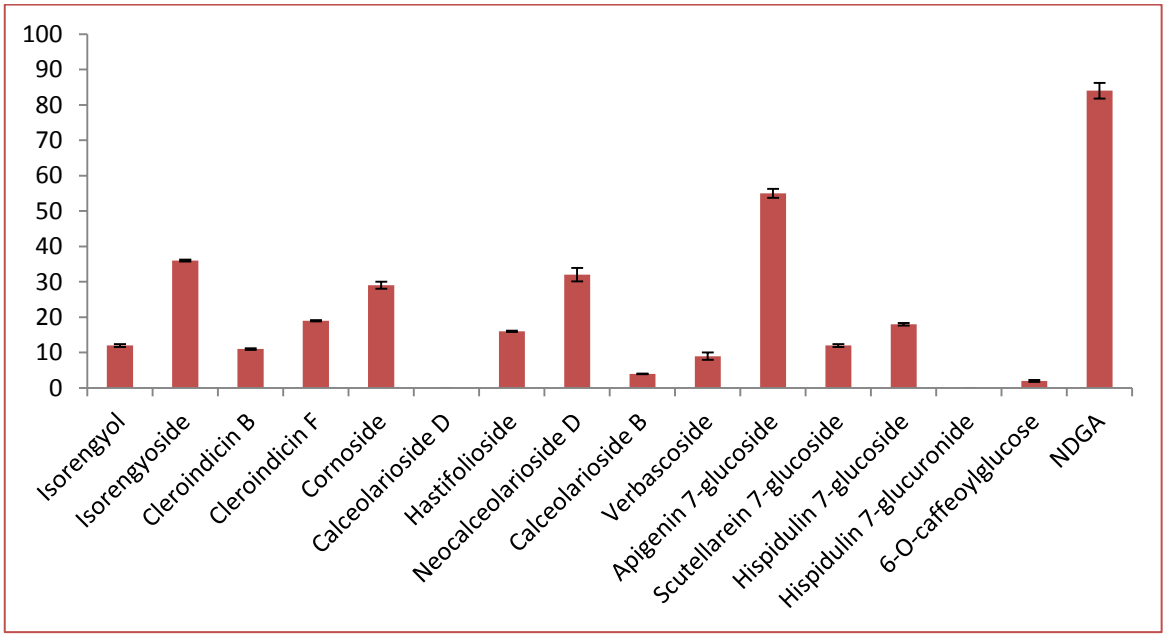


Figure 9. Percentage inhibition of the isolated compounds on soybean LOX (LOX %)

4.2. Phytochemical Results

This section includes the yields of extraction, isolation schemes, structure elucidation and TLC chromatograms of the isolated compounds from both acetone and MeOH extracts of *S. hastifolia* were given. Structure elucidation of the isolated compounds was carried out by spectroscopic techniques such as; UV, 1D (^{1}H, ^{13}C) and 2D (HSQC, HMBC, COSY) NMR and MS.

4.2.1. Extraction

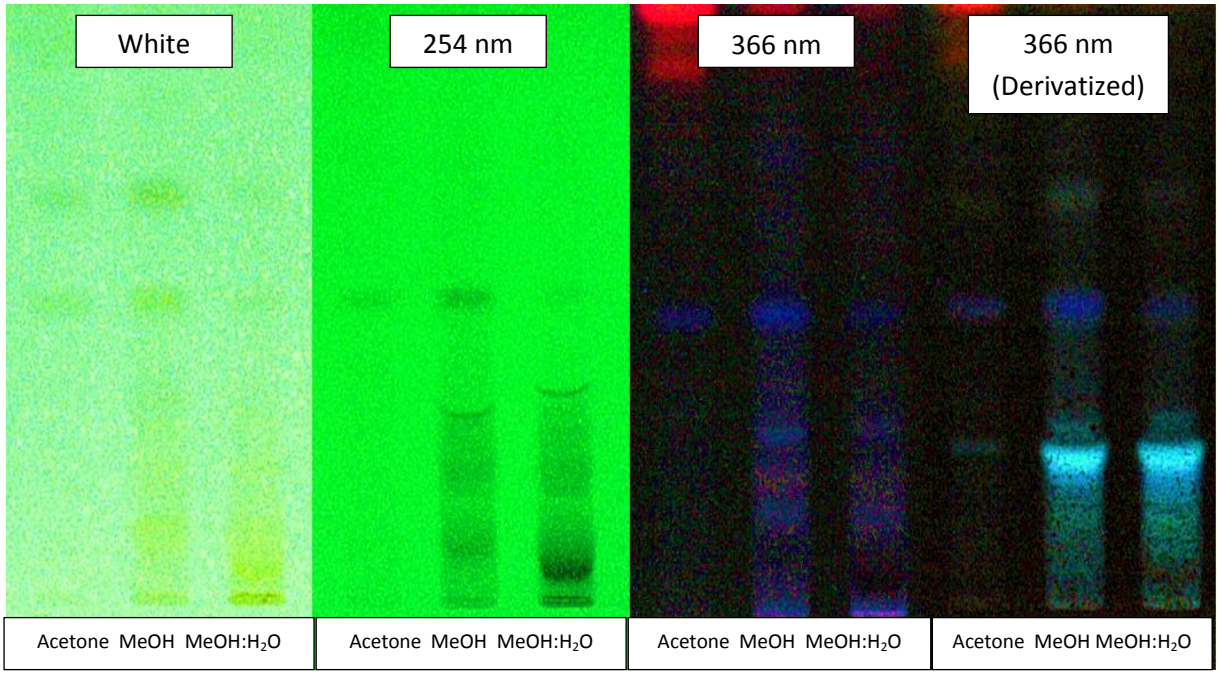
The phytochemical studies were conducted only on the above ground parts of *S. hastifolia*. The powdered plant material was subjected to subsequent extraction with acetone, MeOH and MeOH:H_2O (5:1), respectively. The extraction amounts and the yields are given in the Table 93.

Table 93. The extraction yields of acetone, MeOH and MeOH:H_2O extracts of *S. hastifolia*

Extract	Amount (g)	Yield (%)
Acetone extract	13.6	3.6
MeOH extract	55.8	14.7
MeOH:H_2O extract	28.8	7.6

4.2.2. TLC Chromatograms of Crude Extracts

Crude extracts were applied on SiO_2 aluminium plates before the purification studies. The developing solvent was the mixture of $CHCl_3$:MeOH:H_2O in the proportions of 80:20:2. The plates were photographed on white light, at 254 nm, 366 nm and after derivatization at 366 nm. After TLC studies, it was understood that MeOH:H_2O extract was rich in carbohydrates, so the isolation studies were conducted on remaining extracts which exerted higher antioxidant and antiinflammatory activity.



4.2.3. Main Fractionation

Phytochemical studies were conducted on acetone and MeOH extracts, relatively rich in secondary metabolite compositions which were figured out after preliminary TLC applications. Amounts of the main fractions obtained from acetone and MeOH extracts along with isolation schemes were given in Tables 94-95 and Figures 10-13.

Table 94. Main fractions of the MeOH extract of *S. hastifolia*

Fraction	Amount	Fraction	Amount
Fr. A	0.69 g	**Fr. F**	0.35 g
Fr. B	16.38 g	**Fr. G**	1.09 g
Fr. C	5.54 g	**Fr. H**	1.10 g
Fr. D	1.10 g	**Fr. I**	0.94 g
Fr. E	0.84 g	**Fr. J**	0.15 g

Table 95. Main fractions of the acetone extract of *S. hastifolia*

Fraction	Amount	Fraction	Amount
Fr. A	0.05 g	**Fr. J**	0.21 g
Fr. B	0.13 g	**Fr. K**	0.09 g
Fr. C	0.74 g	**Fr. L**	0.32 g
Fr. D	0.05 g	**Fr. M**	0.20 g
Fr. E	0.33 g	**Fr. N**	0.72 g
Fr. F	0.68 g	**Fr. O**	0.11 g
Fr. G	0.20 g	**Fr. P**	6.64 g
Fr. H	0.62 g	**Fr. R**	0.63 g
Fr. I	0.62 g		

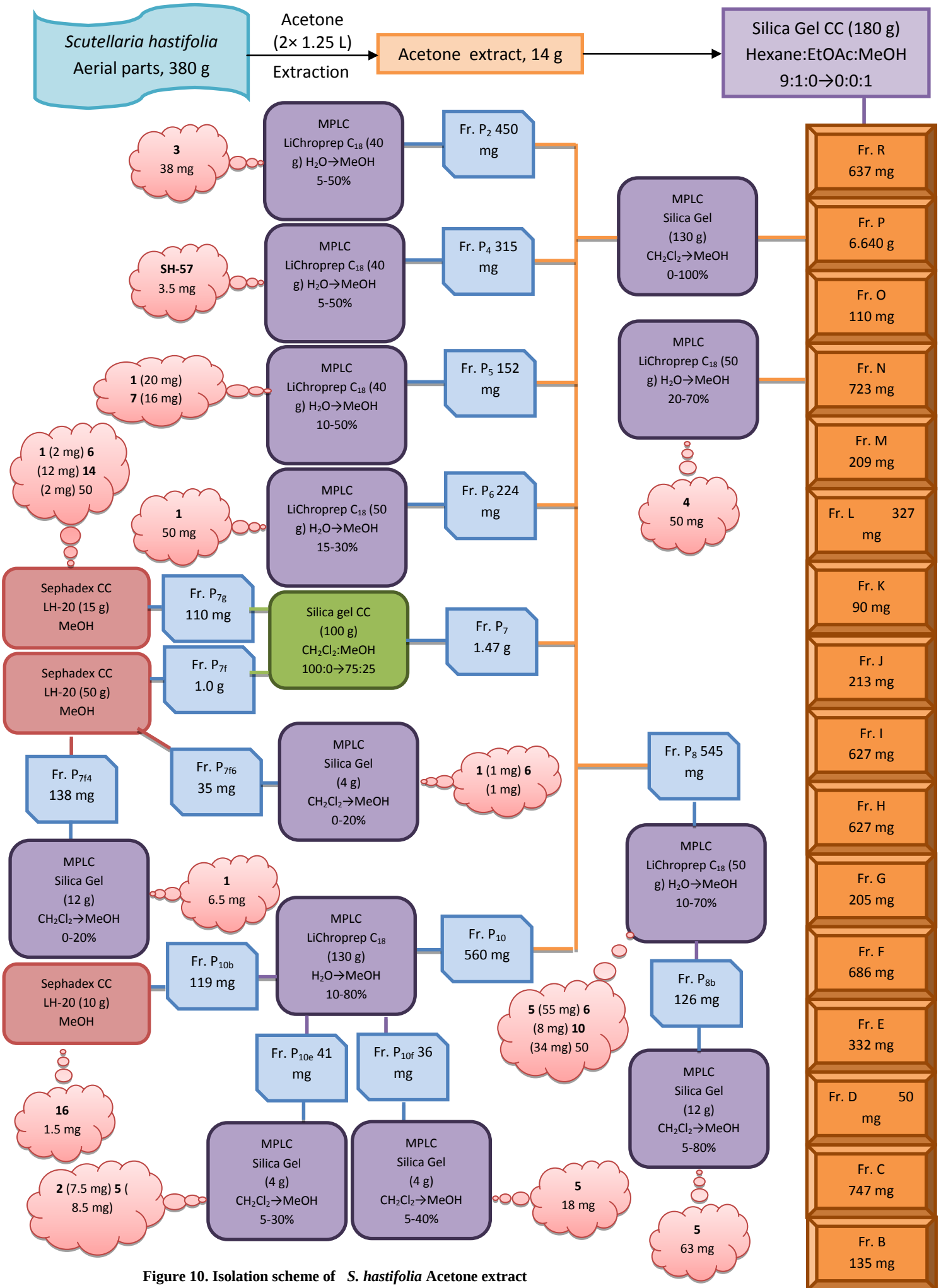


Figure 10. Isolation scheme of *S. hastifolia* Acetone extract

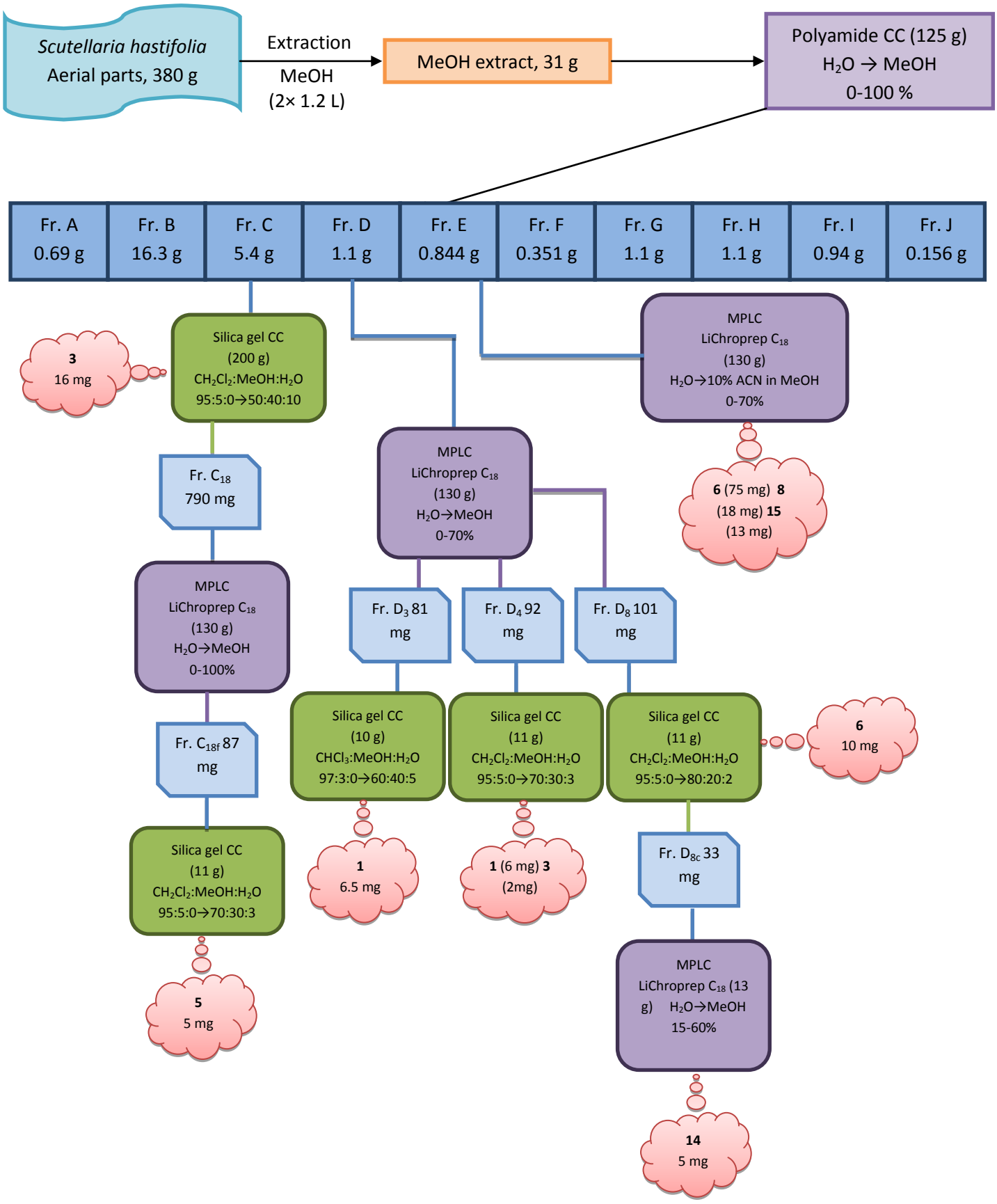


Figure 11. Isolation scheme of *S. hastifolia* MeOH extract

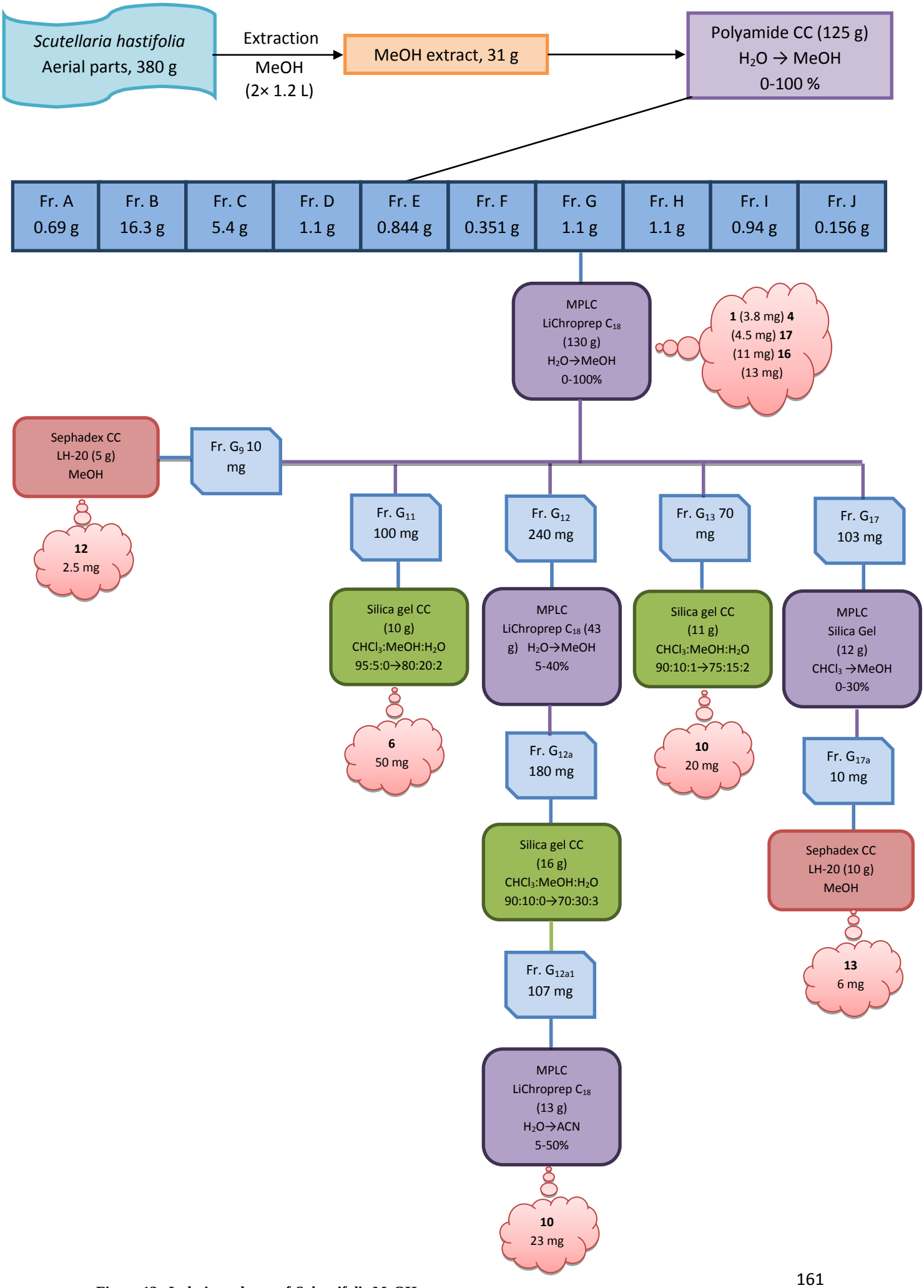


Figure 12. Isolation scheme of *S. hastifolia* MeOH extract

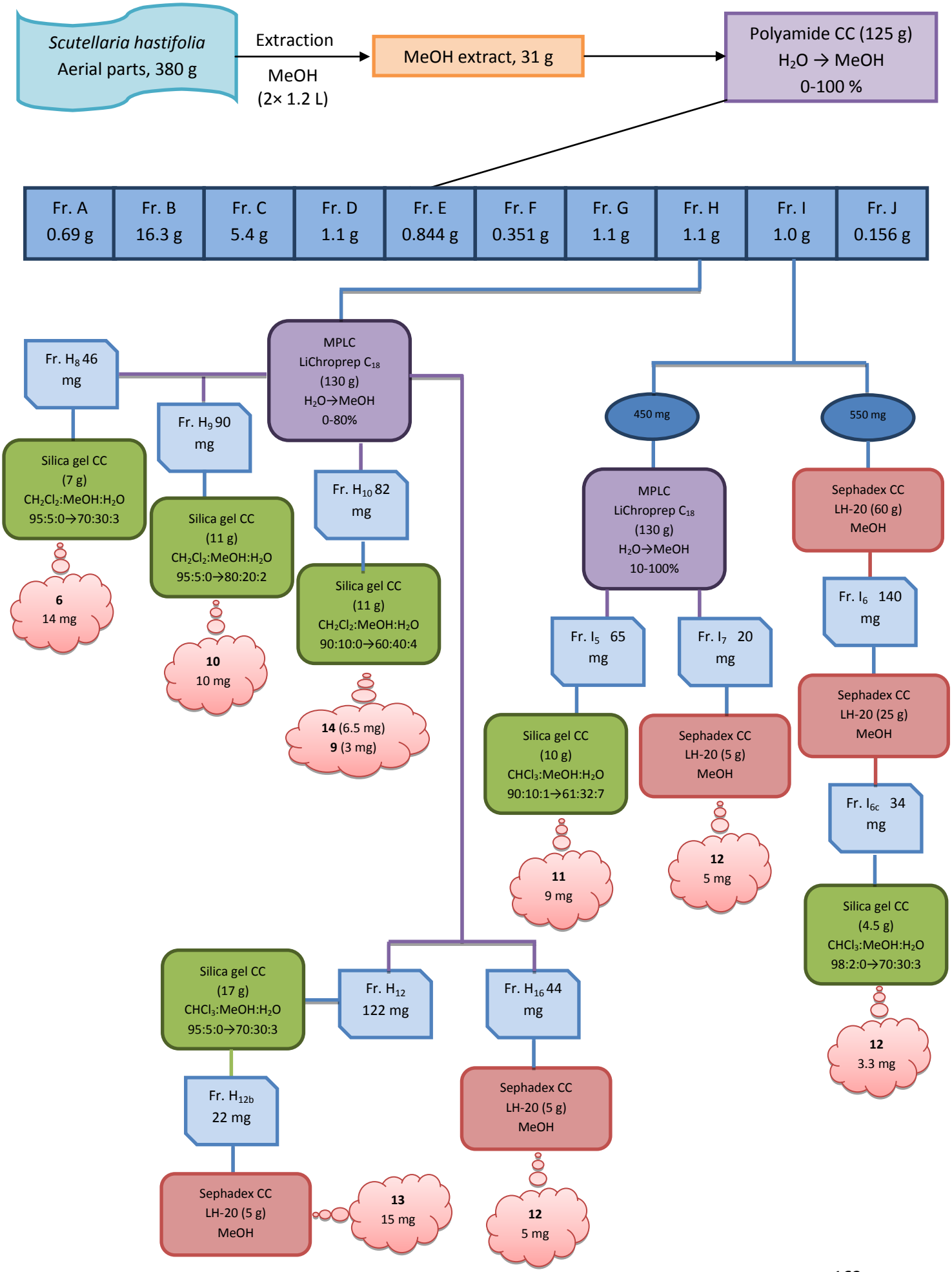
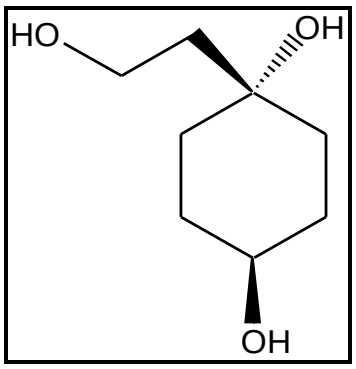


Figure 13. Isolation scheme of *S. hastifolia* MeOH extract

4.2.4. Structure Elucidation of Isolated Compounds

4.2.4.1. Ethylcyclohexane Derivative Compounds



ISORENGYOL (1): $C_8H_{16}O_3$ (160.21)

IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 2920 (aliphatic CH)

ESI-Mass *m/z*: 183 $[M+Na]^+$

^1H NMR: Table 96, Spectrum 1

^{13}C NMR: Table 96

HSQC: Spectrum 2

HMBC: Spectrum 3

ISORENGYOL (1)

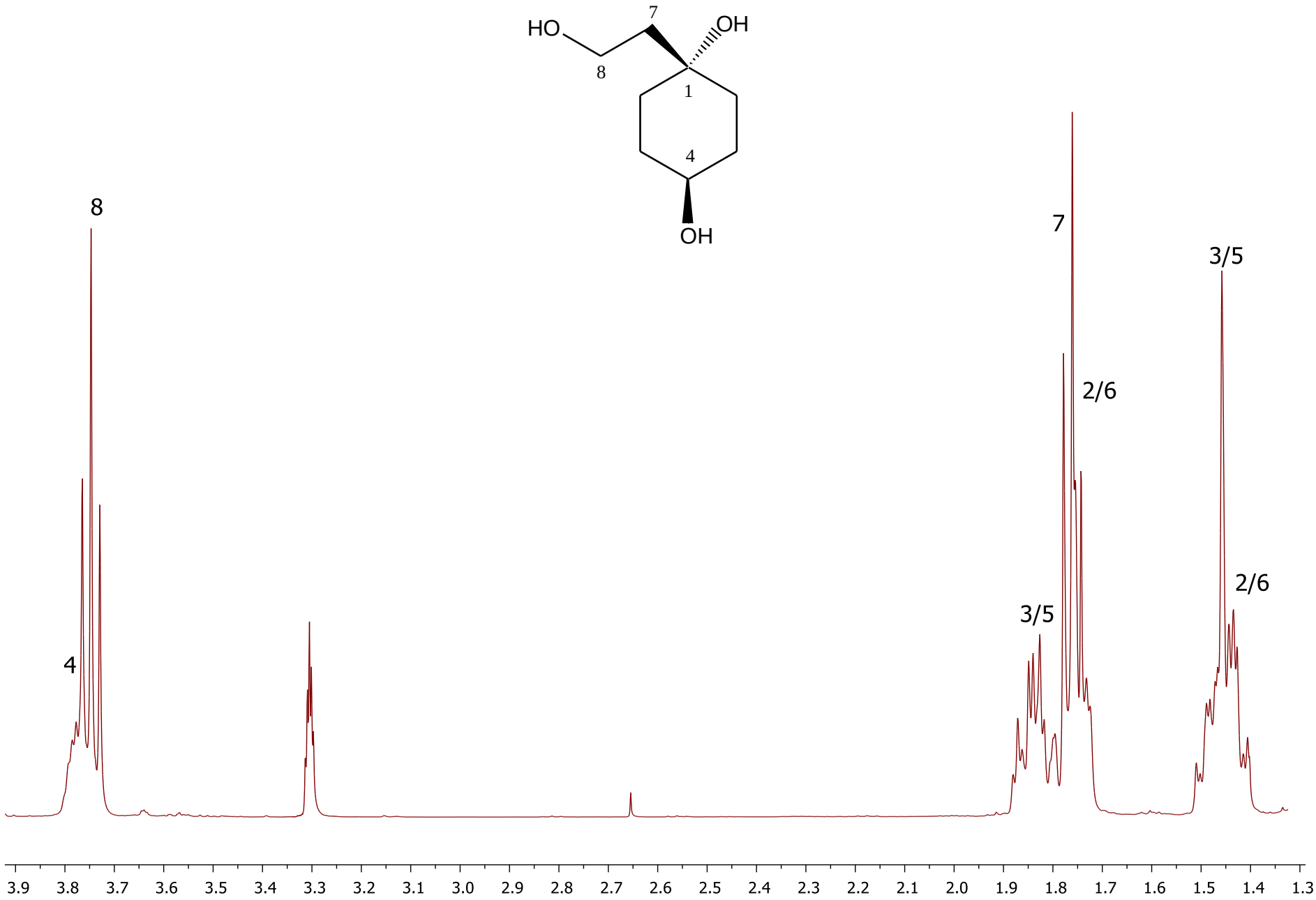
Compound **1** was obtained as brownish amorphous powder, obtained from both acetone and MeOH extracts. Its molecular formula was established to be $C_8H_{16}O_3$ (*m/z*: 183 $[M+Na]^+$) with an unsaturation degree.

In ^{1}H NMR (Table 96, Spectrum 1) spectrum of compound **1**, all the signals were observed between δ_H 3.8-1.4, suggesting that **1** to be a non-phenolic compound. The signals at 1.76 ppm (t, *J*=7.1 Hz) and 3.75 ppm (t, *J*=7.1 Hz), revealed the presence of methylene and oxymethylene groups, respectively in the structure of **1**. Furthermore, the spectrum contained the resonances of oxymethine at δ_H 3.78 (m), with four methylene signals (δ_H 1.8-1.39). These data together with the carbon resonances secured by 2D experiments, suggested the presence of tri-substituted cyclohexane moiety, with an ethanol side chain. The correlations between the carbons and directly bonded protons showed in the HSQC (Spectrum 2) spectrum. In HMBC spectrum (Spectrum 3), cross peaks between quaternary carbon (δ_C 71.3, C-1) atom and hydroxymethylene (δ_H 3.75, H-8) along with interactions of C-7 (δ_C 42.9) and H-2/6 (δ_H 1.76, 1.44) led to the location of side chain to be C-1 (δ_C 71.3), which is a quaternary carbon atom. Moreover, interactions of the quaternary carbon atom in HMBC spectrum with an oxymethylene (H-8), methylene (H-7) and symmetrical four methylenes (H-2, H-6, H-3 and H-5) along with its signal at downfield shield, indicated that the quaternary carbon atom was adjacent to an –OH group. Crosslinks between oxymethylene (C-4) signals with H-2/6 was seen in HMBC spectrum. Interpretations of ^{1}H NMR, HSQC and HMBC spectra together, suggested the structure as *trans*-1-(2-hydroxyethyl) cyclohexane 1,4-diol. Comparison of all the spectral data with the literature, all the data are overlapped with the spectral data of compound **isorengyol** (223).

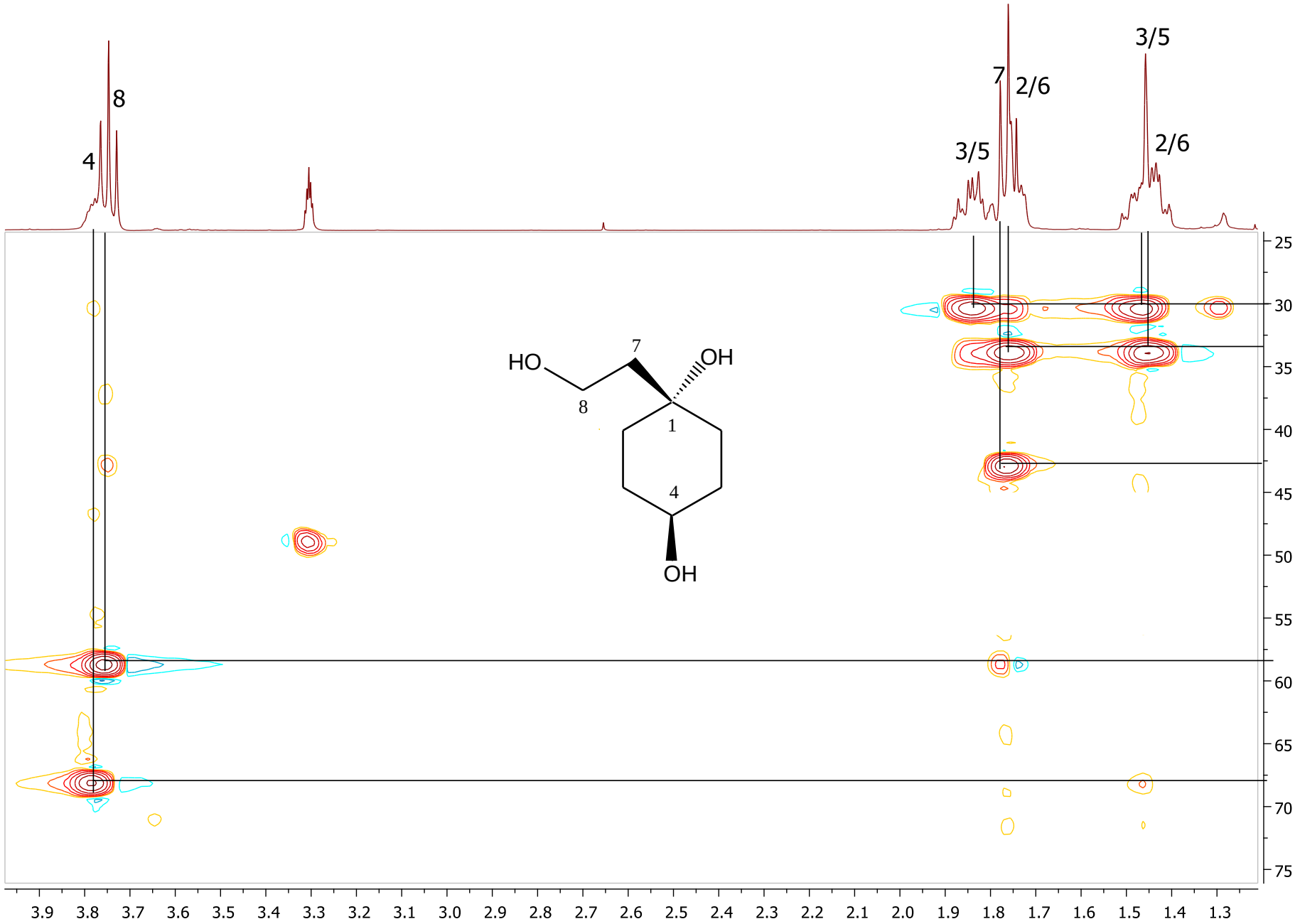
Table 96. ^{13}C and ^{1}H NMR Spectroscopic Data of Isorengyol (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
1	C	71.3	
2	CH_2	33.9	1.44 m 1.76 m
3	CH_2	30.2	1.46 m 1.84 m
4	CH	68.1	3.78 m
5	CH_2	30.2	1.46 m 1.84 m
6	CH_2	33.9	1.44 m 1.76 m
7	CH_2	42.9	1.76 t (7.1)
8	CH_2	58.7	3.75 t (7.1)

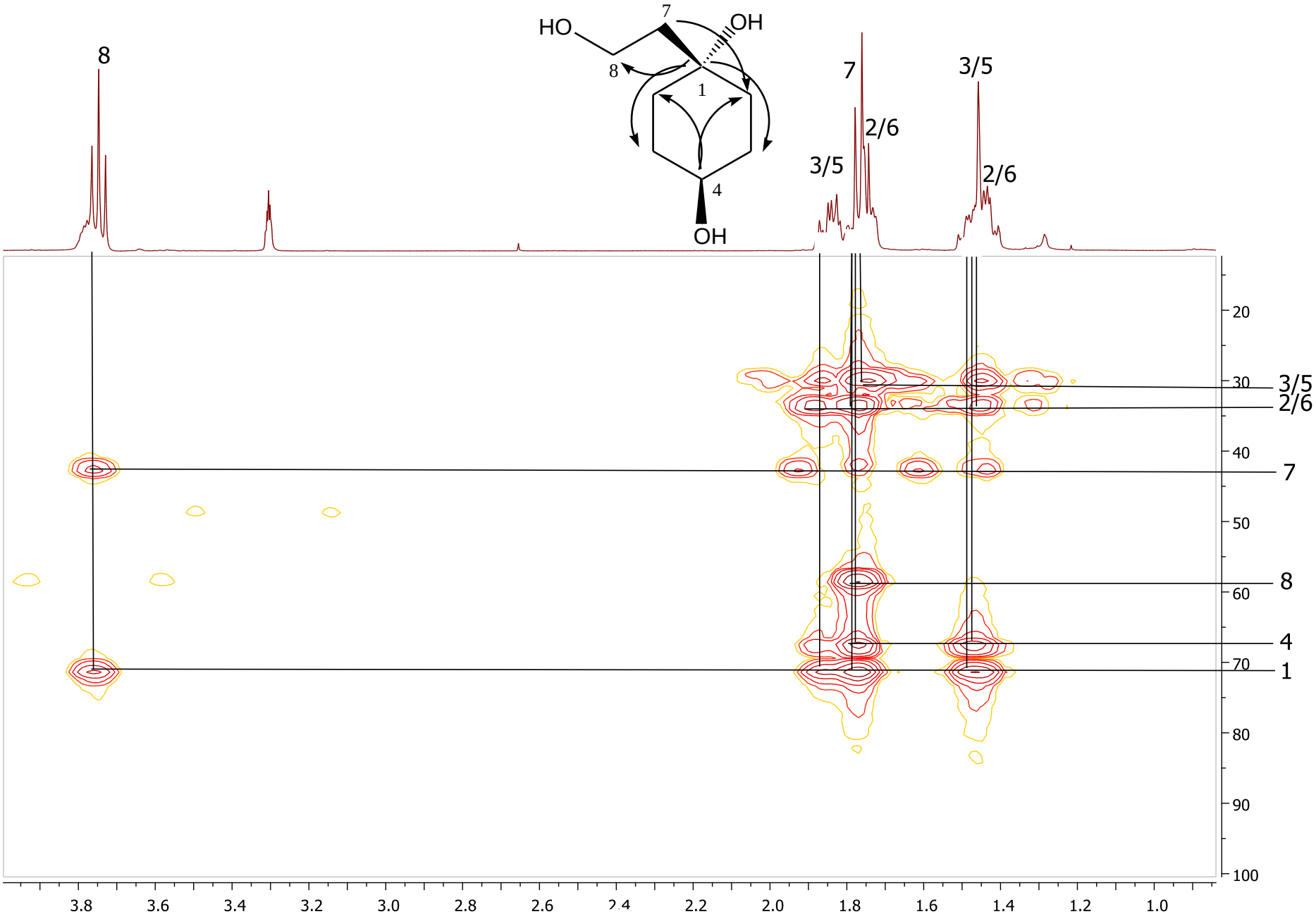
[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.



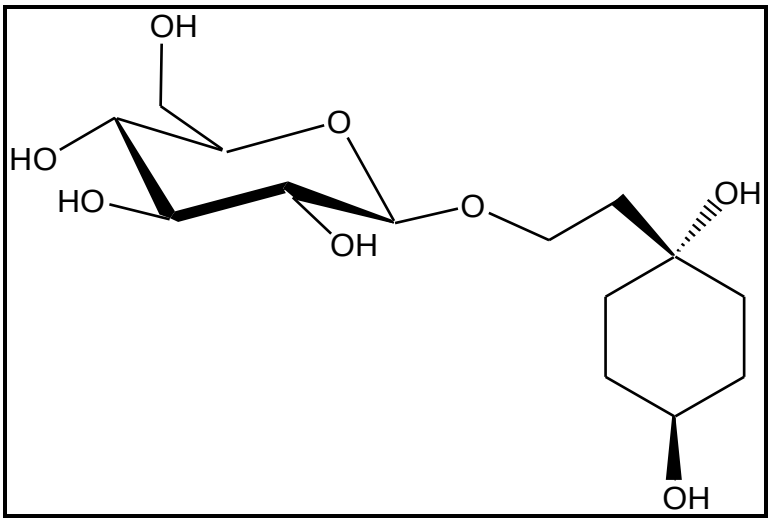
Spectrum 1. ^{1}H-NMR spectrum of Isorengyol (CD_3OD, 400 MHz)



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



Spectrum 3. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Isorengyol (HMBC)



ISORENGYOSIDE (2): $C_{14}H_{26}O_8$ (322.35)

IR ν_{max} (KBr) cm^{-1}: 3371 (OH), 2940 (aliphatic CH), 1085 (C-O-C)

ESI-Mass *m/z*: 323 $[M+H]^+$, 345 $[M+Na]^+$

^1H NMR: Table 97, Spectrum 4

^{13}C NMR: Table 97, Spectrum 5

HSQC: Spectrum 6

HMBC: Spectrum 7

COSY: Spectrum 8

ISORENGYOSIDE (2)

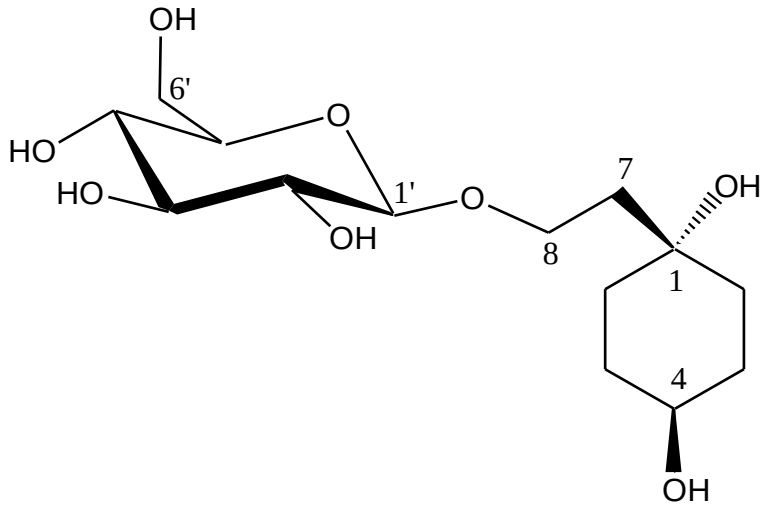
Compound **2** was obtained as brownish amorphous powder. It was obtained from acetone extract of *S. hastifolia*. Its molecular formula was found to be $C_{14}H_{26}O_8$ **(**323 $[M+H]^+$, 345 $[M+Na]^+$). Its IR spectrum showed the presence of hydroxyl (3371 cm^{-1}) and C-O-C groups (1085 cm^{-1}).

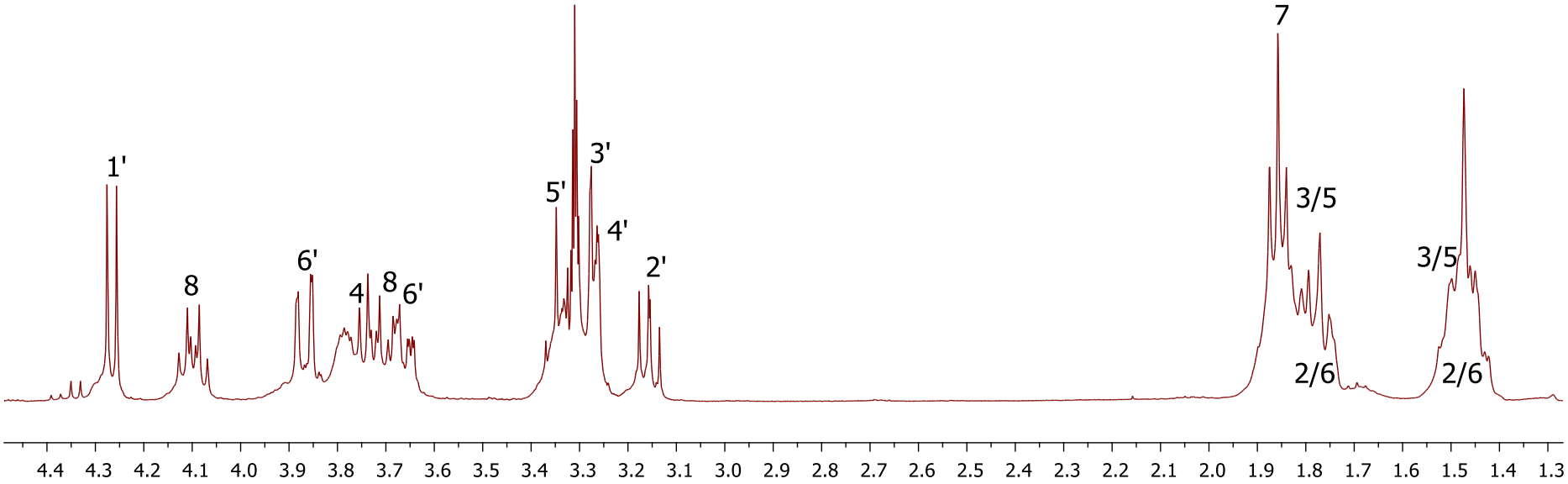
^1H NMR and ^{13}C NMR spectra (Table 97, Spectrum 4,5) showed signals between 3.75-1.44 ppm and 70.2-29.3 ppm suggesting that **2** to be a non-phenolic compound similar to **1.** The only difference between two compounds was from the signals of a sugar moiety. An equivalent and diastereotopic methylene and oxymethylene protons were seen at δ_H 1.86 (t, *J*=6.9 Hz), 4.11 (m) and 3.72 (m) belonging to H-7 and H-8, respectively. In COSY spectrum (Spectrum 8), three spin systems were seen; one of them was between H_2-7 and H_2-8, second was between H_2-2/H_2-3/H_2-4 and H_2-5, while the last one was between the sugar protons. Moreover, in HMBC spectrum (Spectrum 7), the correlations of C-4 (67.0 ppm) with H-2 and H-6, together with the correlations of C-1 (70.2 ppm) with H-3 and H-5 led to the presence of a 1, 4 trisubstituted cyclohexane ring as in **1**. All above data were superimposed the data belong to **isorengyol**, except the signals belonging to that sugar unit. The anomeric proton signal at δ_H 4.27 (d, *J*=7.9 Hz) with a large *J* coupling constant along with the signals (δ_H 3.87-3.11) and the carbon resonances (δ_C 103.1, 76.7, 76.6, 73.7, 70.3, 61.4) indicated that the sugar moiety on **isorengyol** structure was β-glucopyranose. Moreover, the long range correlation between C-1' (103.1 ppm) and H_2-8 (4.11 and 3.72 ppm) showed the glycosidation point. All these observations led to the identification of compound **2** as **isorengyoside** (224).

Table 97. ^{13}C and ^{1}H NMR Spectroscopic Data of Isorengyoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

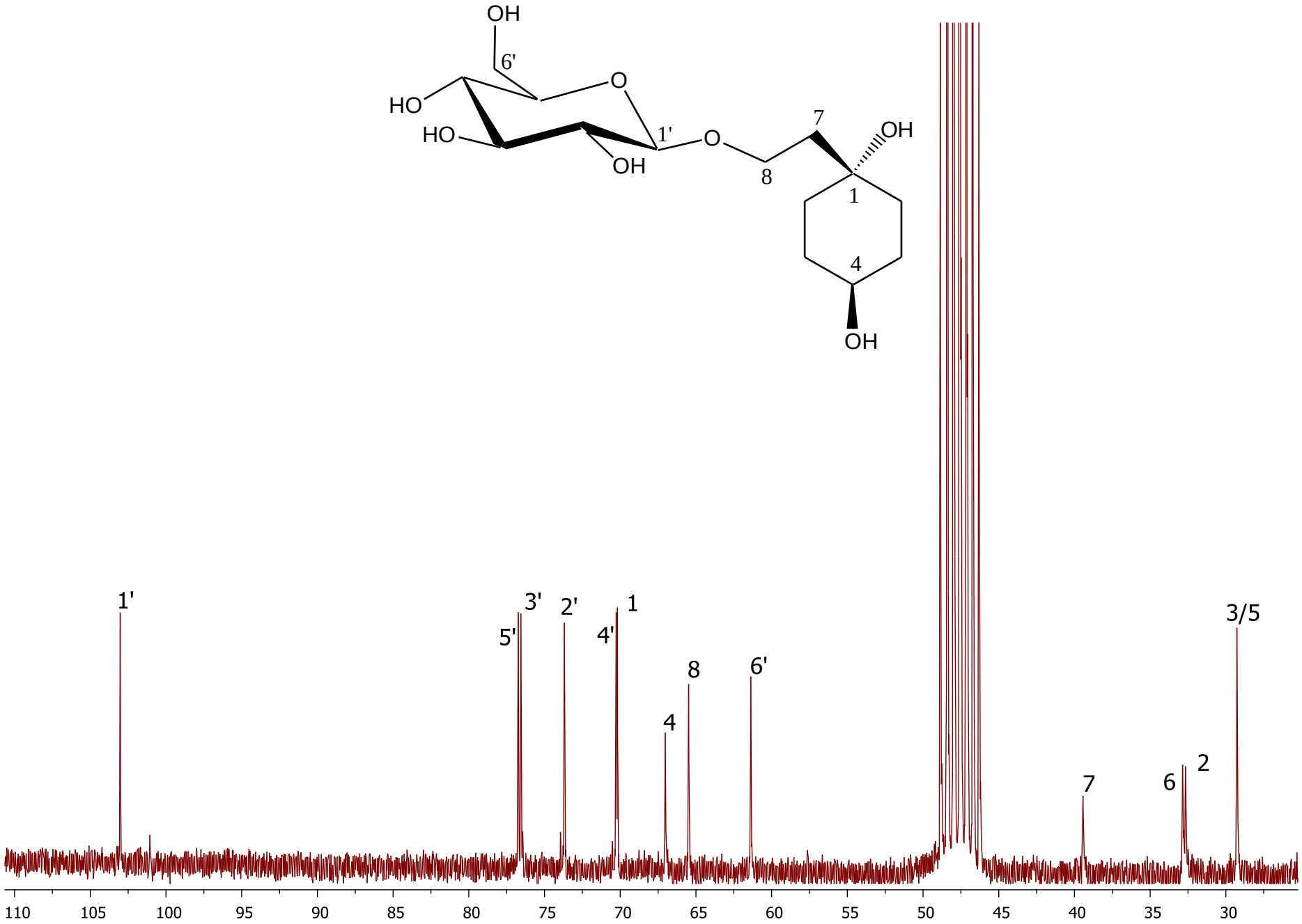
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
1	C	70.2	
2	CH_2	32.4	1.76 m 1.44 m
3	CH_2	29.3	1.80 m 1.47 m
4	CH	67.0	3.75 m
5	CH_2	29.3	1.80 m 1.47 m
6	CH_2	32.5	1.76 m 1.44 m
7	CH_2	39.5	1.86 t (6.9)
8	CH_2	65.5	4.11 m 3.72 m
Glucose			
1'	CH	103.1	4.27 d (7.9)
2'	CH	73.7	3.11 dd (7.9, 9.0)
3'	CH	76.6	3.23 m
4'	CH	70.3	3.22 m
5'	CH	76.7	3.33 m
6'	CH_2	61.4	3.87 dd (11.7, 1.3) 3.66 dd (11.7, 5.4)

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.

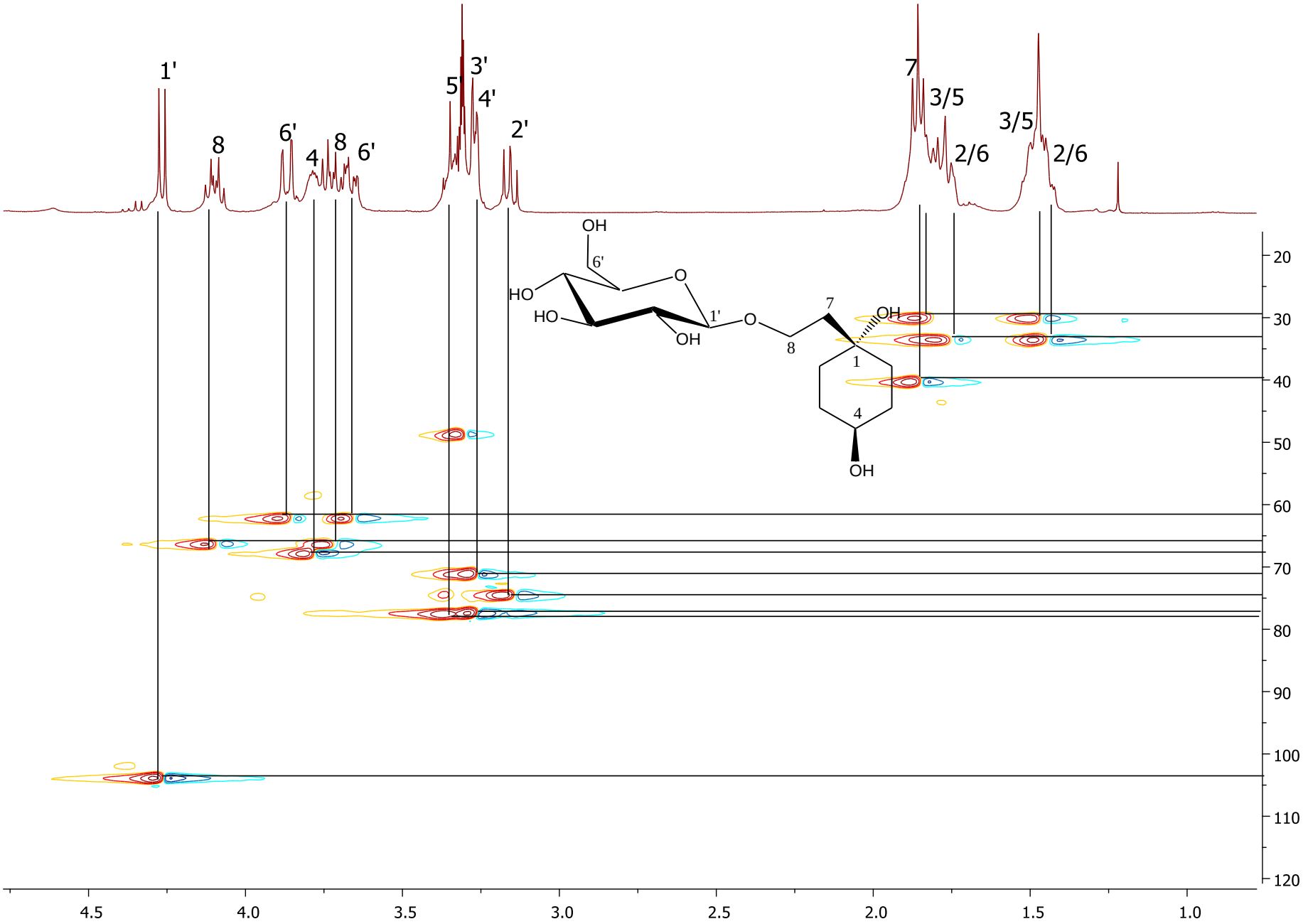




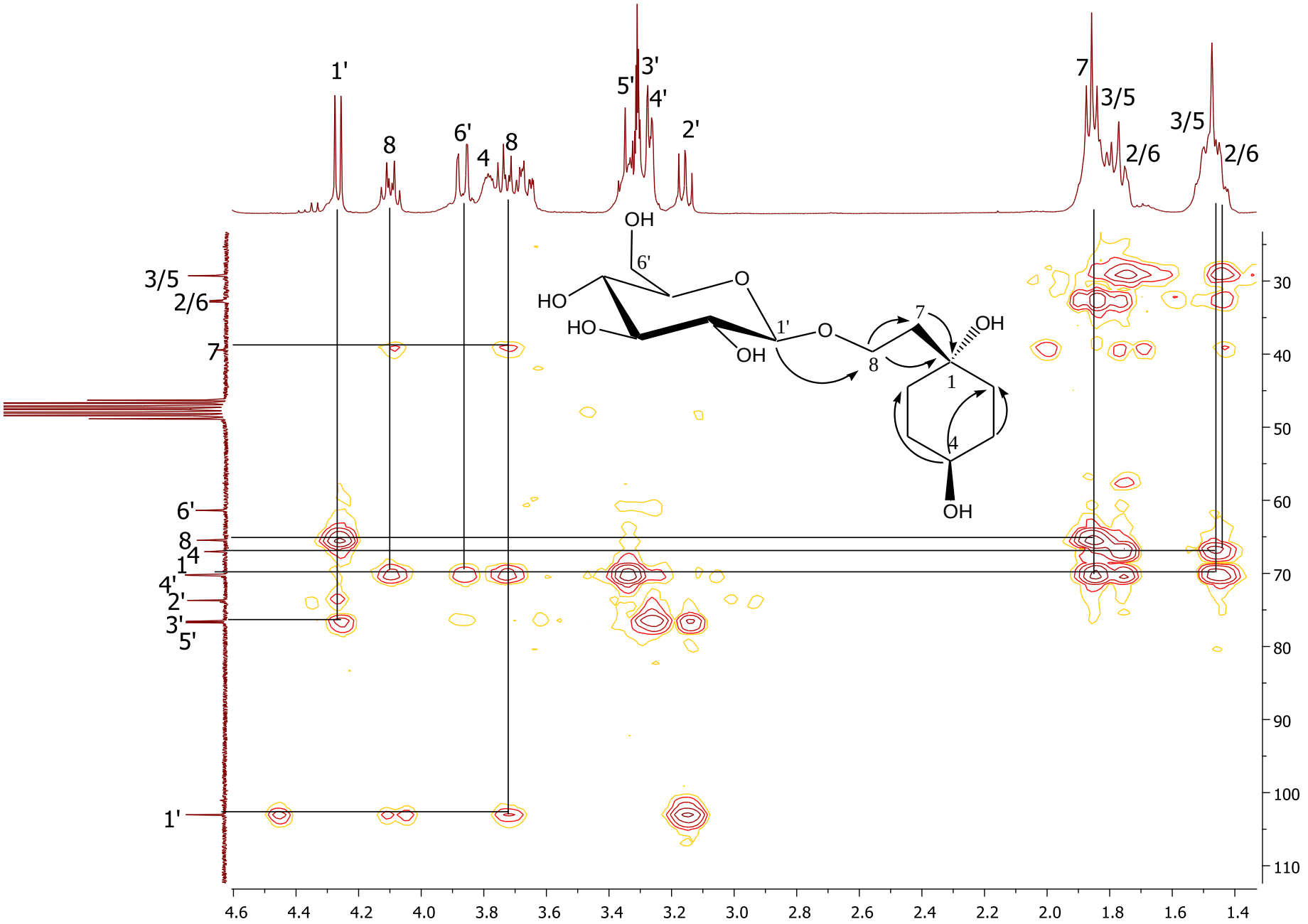
Spectrum 4. ^{1}H-NMR spectrum of Isorengyoside (CD_3OD, 400 MHz)



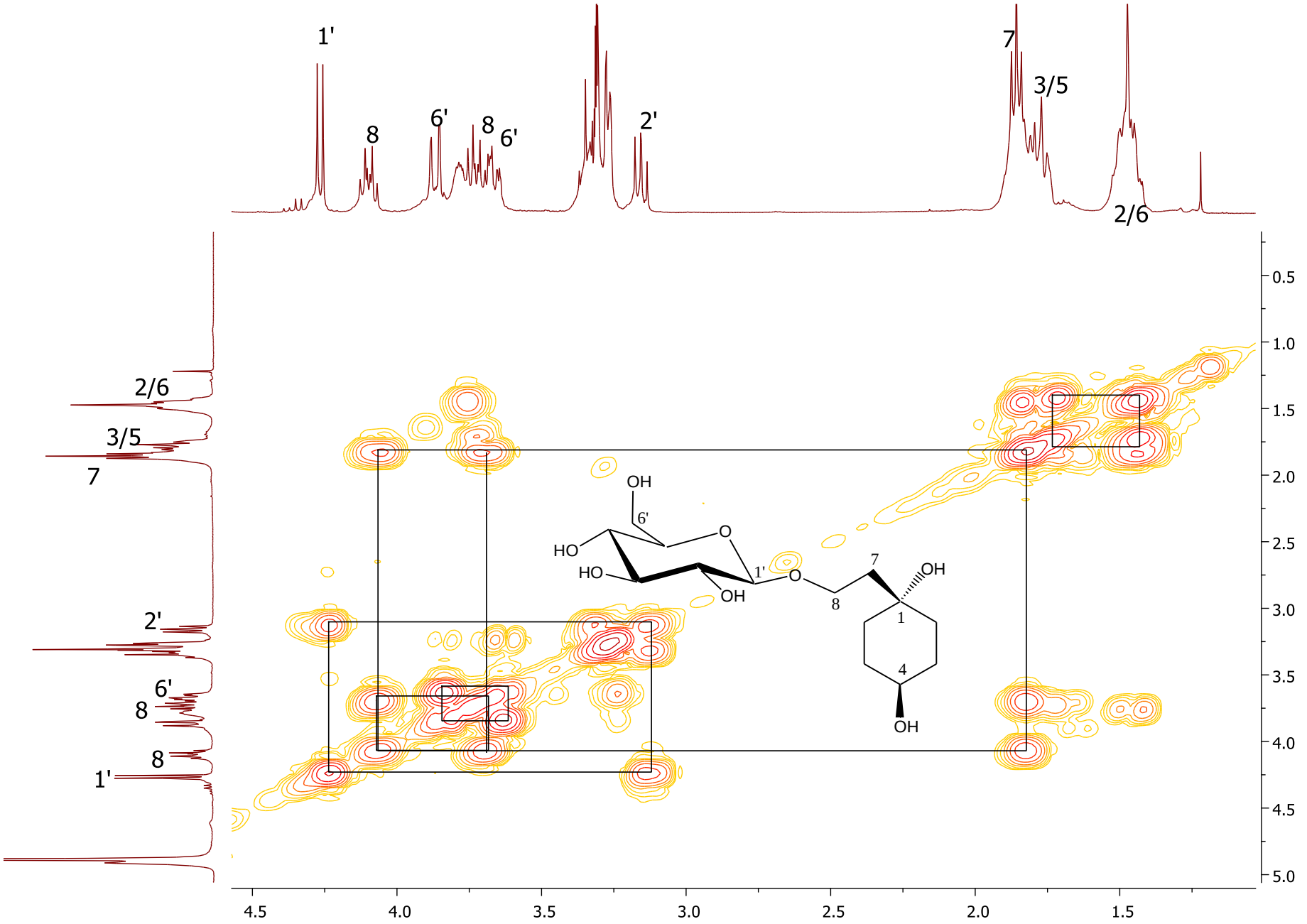
Spectrum 5. ^{13}C-NMR spectrum of Isorengyoside (CD_3OD, 100 MHz)



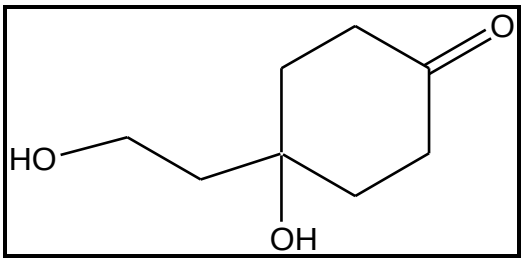
Spectrum 6. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Isorengyoside (HSQC)



Spectrum 7. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Isorengyoside (HMBC)



Spectrum 8. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum of Isorengyoside (COSY)



CLEROINDICIN B (3): $C_8H_{14}O_3$ (158.19)

IR ν_{max} (KBr) cm^{-1}: 3396 (OH), 2942 (aliphatic CH), 1707 (ketone C=O)

ESI-Mass *m/z*: 159 $[M+H]^+$

^1H NMR: Table 98, Spectrum 9

^{13}C NMR: Table 98

HSQC: Spectrum 10

HMBC: Spectrum 11

COSY: Spectrum 12

CLEROINDICIN B (3)

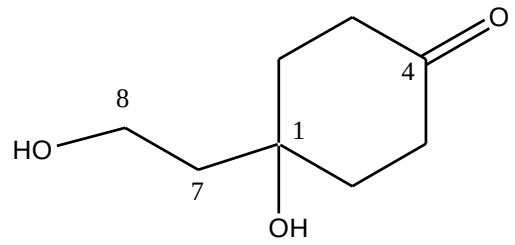
Compound **3** was colorless oil, purified from both MeOH and acetone extracts. The molecular formula of **3** was established as $C_8H_{14}O_3$ (158) from the pseudomolecular ion peak at m/z 159 $[M+H]^+$ on ESI-MS. Its IR spectrum indicated the presence of hydroxyl (3396 cm^{-1}) and carbonyl group (1707 cm^{-1}). Compound **3** had to have a ring and a carbonyl group according to two unsaturated degrees.

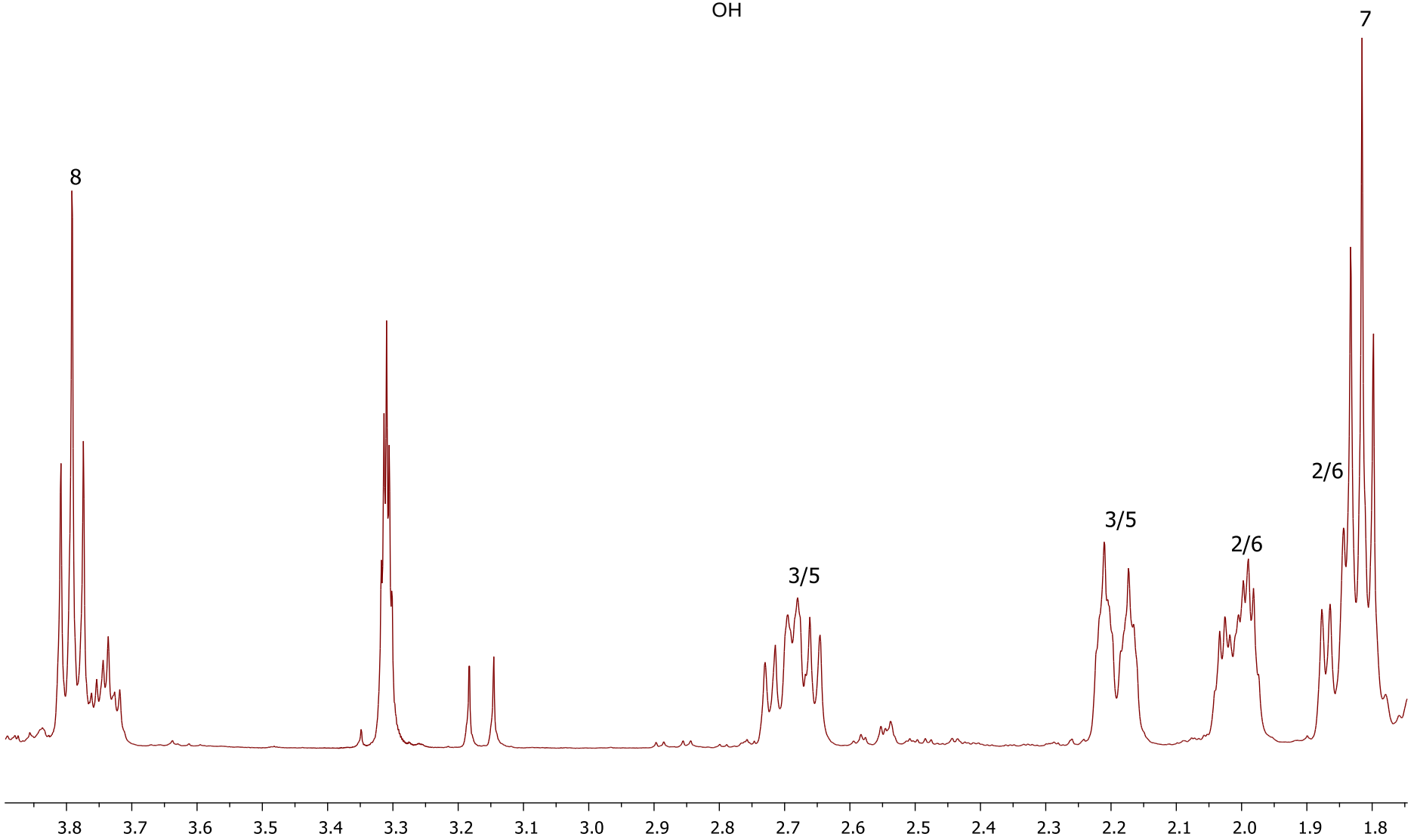
The ^1H NMR spectrum (Table 98, Spectrum 9) of **3**, displayed six groups of proton signals: δ_H 3.79 (t, *J*=6.9 Hz, 2H), 2.68 (dt, *J*= 6.3, 13.7 Hz, 2H), 2.18 (m, 2H), 2.01 (m, 2H), 1.85 (m, 2H), 1.82 (t, *J*=6.9 Hz, 2H). ^{13}C NMR data (Table 98) contained eight resonances, including a ketone carbonyl (δ_C 214.3) and a quaternary carbon signal (δ_C 69.6). Other signals and their corresponding proton resonances were determined from HSQC (Spectrum 10) spectrum. In COSY spectrum (Spectrum 12), two spin systems were seen; one between the side chain (-CH_2CH_2OH), the other was between the other methylene signals (δ_H 2.74-1.79). The location of ketone was understood from the cross-peaks between H_2-2/6 and H_2-3/5 with C=O from the HMBC spectrum. The NMR data of **3** were superimposible with those of **Cleroindicin B** (225).

Table 98. ^{13}C and ^{1}H NMR Spectroscopic Data of Cleroindicin B (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

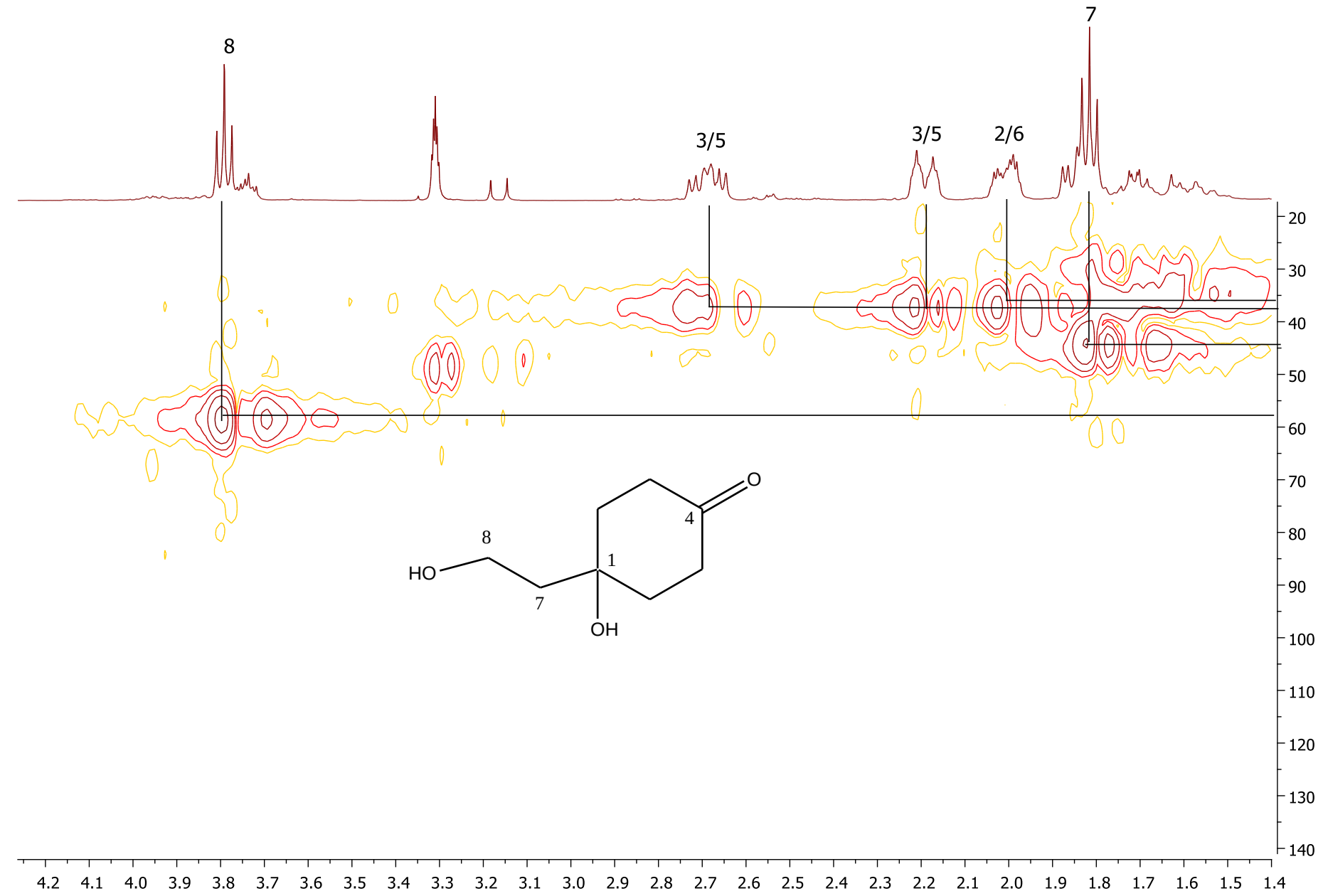
C/H Atom	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
1	C	69.6	
2	CH_2	35.3	2.01 m ł 1.85 m ł
3	CH_2	35.6	2.68 dt (6.3, 13.7) 2.18 m ł
4	C	214.3	
5	CH_2	35.6	2.68 dt (6.3, 13.7) 2.18 m ł
6	CH_2	35.3	2.01 m ł 1.85 m ł
7	CH_2	42.7	1,82 t (6.9)
8	CH_2	57.2	3.79 t (6.9)

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.

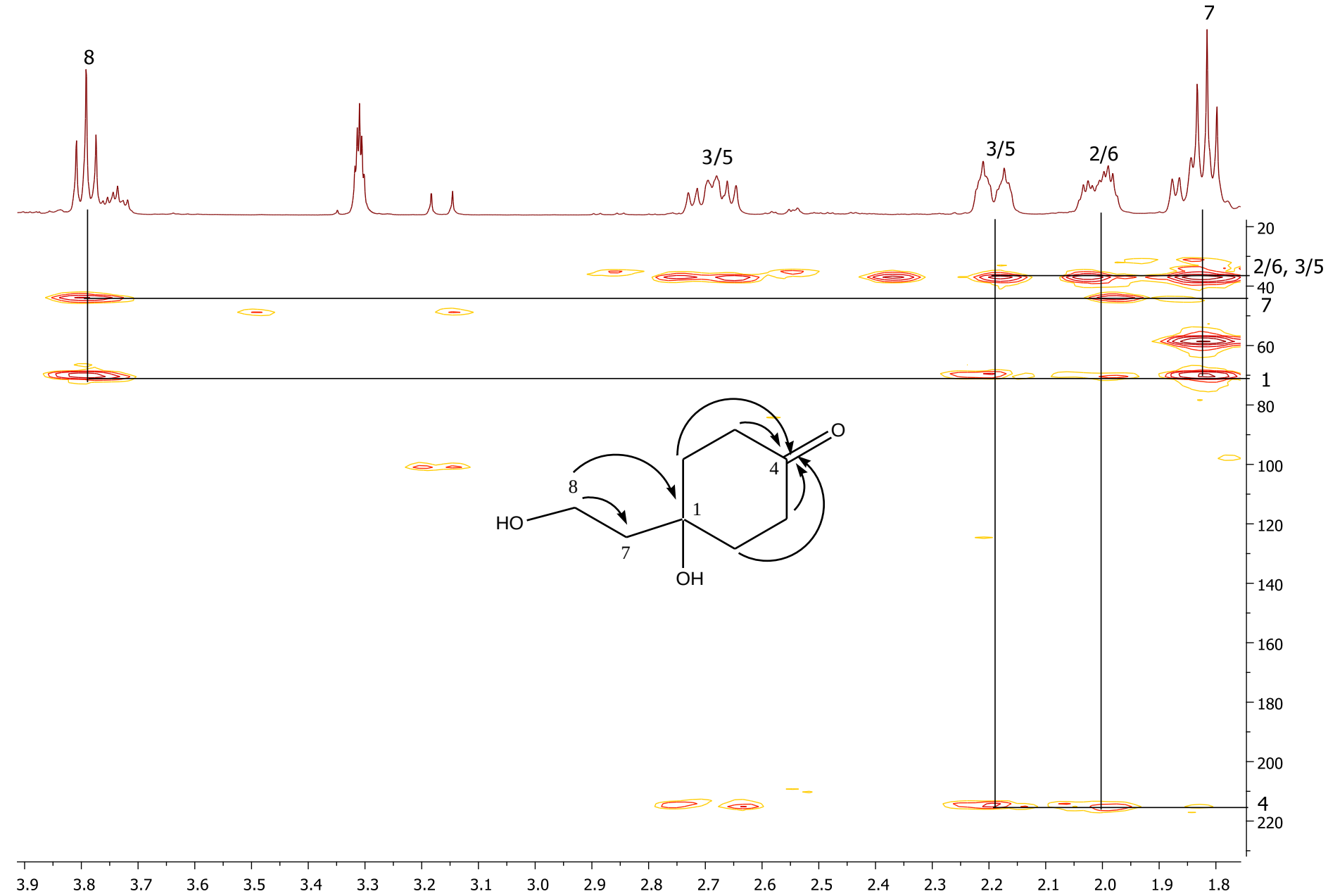




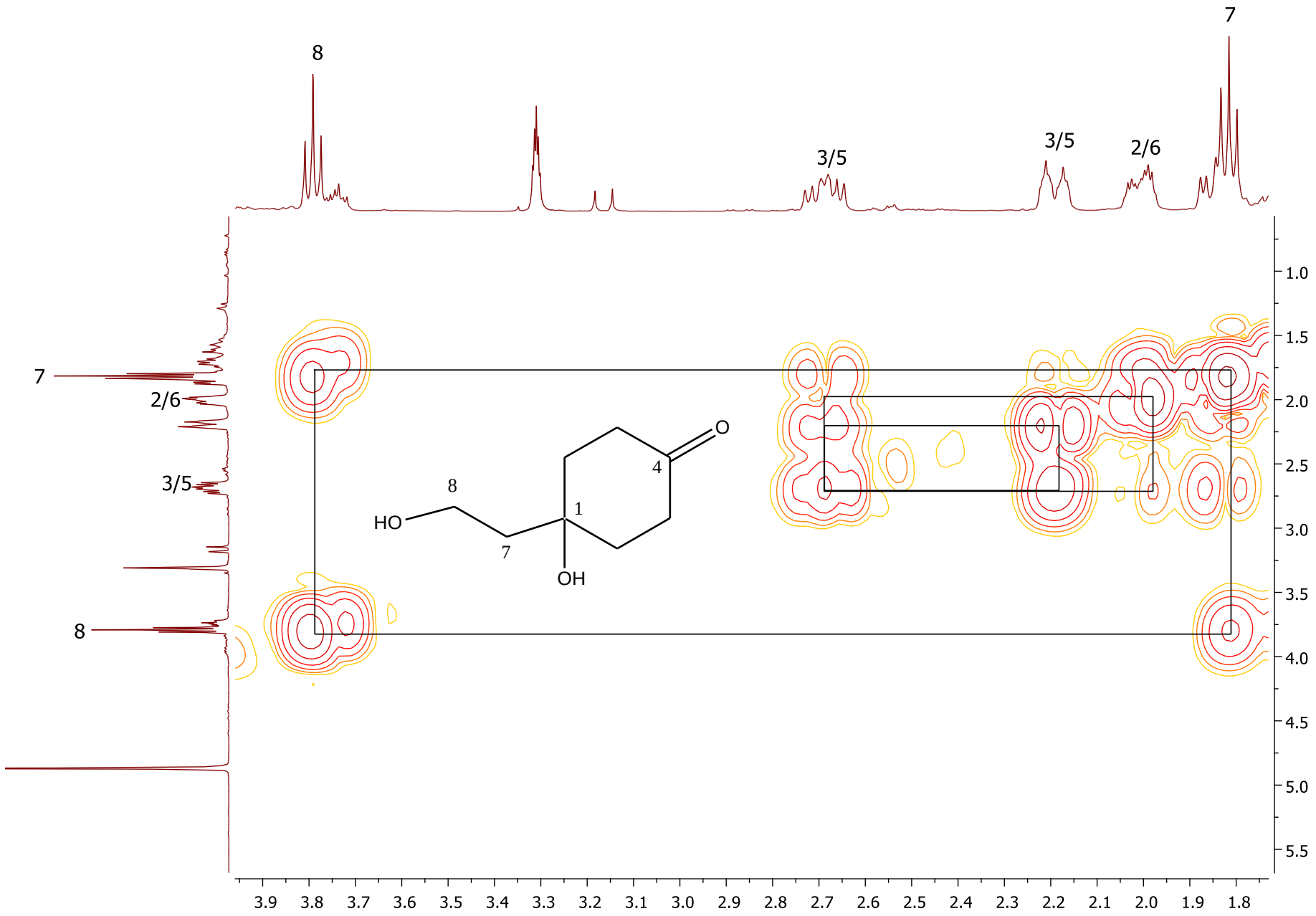
Spectrum 9. ^{1}H-NMR spectrum of Cleroindicin B (CD_3OD, 400 MHz)



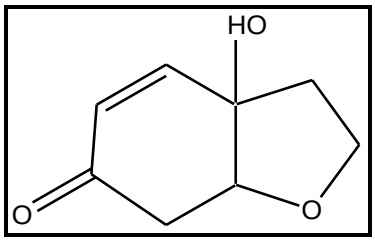
Spectrum 10. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Cleroindicin B (HSQC)



Spectrum 11. Heteronuclear 2D-1H,13C Correlation Spectrum (long range) of Cleroindicin B (HMBC)



Spectrum 12. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum (COSY) of Cleroindicin B



CLEROINDICIN F (4): $C_8H_{10}O_3$ (154.16)

UV λ_{max} (MeOH) nm: 206

IR ν_{max} (KBr) cm^{-1}: 3456 (OH), 1722 (ketone C=O)

ESI-Mass *m/z*: 155 $[M+H]^+$

^1H NMR: Table 99, Spectrum 13

^{13}C NMR: Table 99

HSQC: Spectrum 14

HMBC: Spectrum 15

COSY: Spectrum 16

CLEROINDICIN F (4)

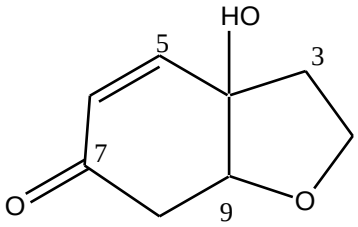
Compound **4** was obtained as colorless oil, from both MeOH and acetone extracts. From its UV and IR spectroscopy, bands indicating α,β- unsaturated ketone were seen. Its molecular formula was discovered as $C_8H_{10}O_3$ from the ESI-MS analysis with the help of pseudomolecular ion peak of *m/z* 155 $[M+H]^+$.

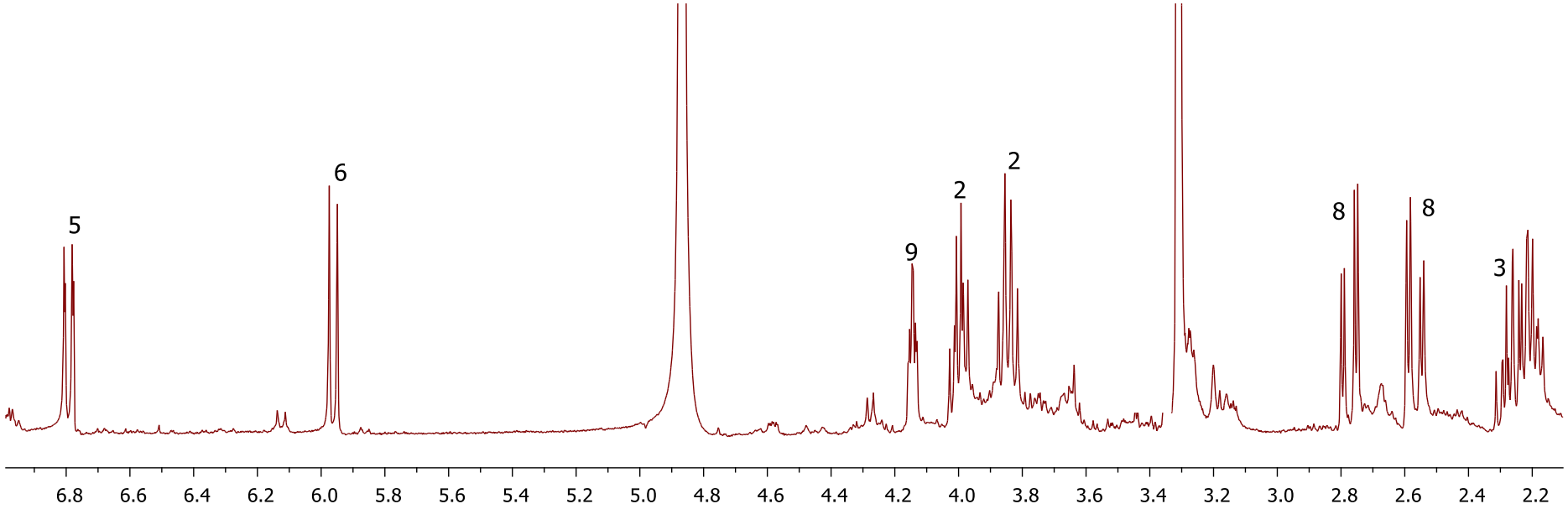
Its ^1H NMR spectrum (Table 99, Spectrum 13) displayed two olefinic signals at 6.79 and 5.96 ppm with a *J* value of 10.2 Hz. Additionally, one oxymethine (δ_H 4.14) and oxymethylene (δ_H 4.00 and 3. 85) signals as well as two methylene signals (δ_H 2.77, 2.57 and 2.28, 2.20) were seen in ^1H NMR spectrum. COSY spectrum (Spectrum 16) showed three spin systems; first between the olefinic signals, second between oxymethine and methylene (δ_H 4.14 and 2.77, 2.57) signals and last between oxymethylene and methine (δ_H 4.00, 3.85 and 2.28, 2.20) resonances. The ^{13}C NMR spectrum contained nine signals including a ketone carbonyl signal. Intense examinations of HSQC (Spectrum 14) and HMBC (Spectrum 15) spectra, led to the locations of the spin systems in **4**. Accordingly, **4** was identified as **Cleroindicin F** (225).

Table 99. ^{13}C and ^{1}H NMR Spectral Data of Cleroindicin F (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

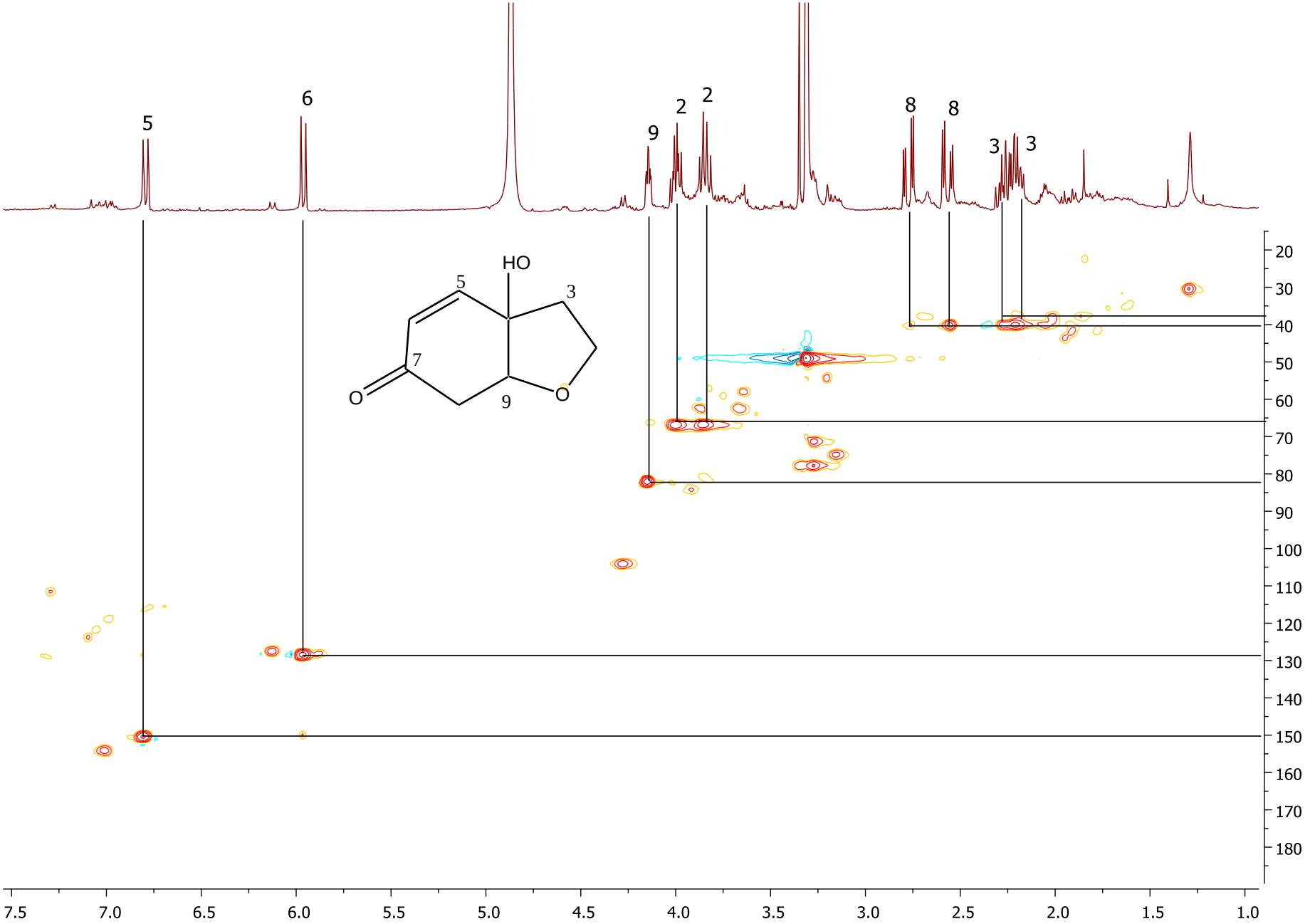
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
2	CH_2	66.8	3.85 m 4.00 m
3	CH_2	39.9	2.20 m 2.28 m
4	C	73.6	
5	CH	150.2	6.79 d (10.2)
6	CH	128.1	5.96 d (10.2)
7	C	197.4	
8	CH_2	40.3	2.57 dd (16.8, 4.3) 2.77 dd (16.8, 4.8)
9	CH	82.3	4.14 dd (4.8, 1.6)

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.

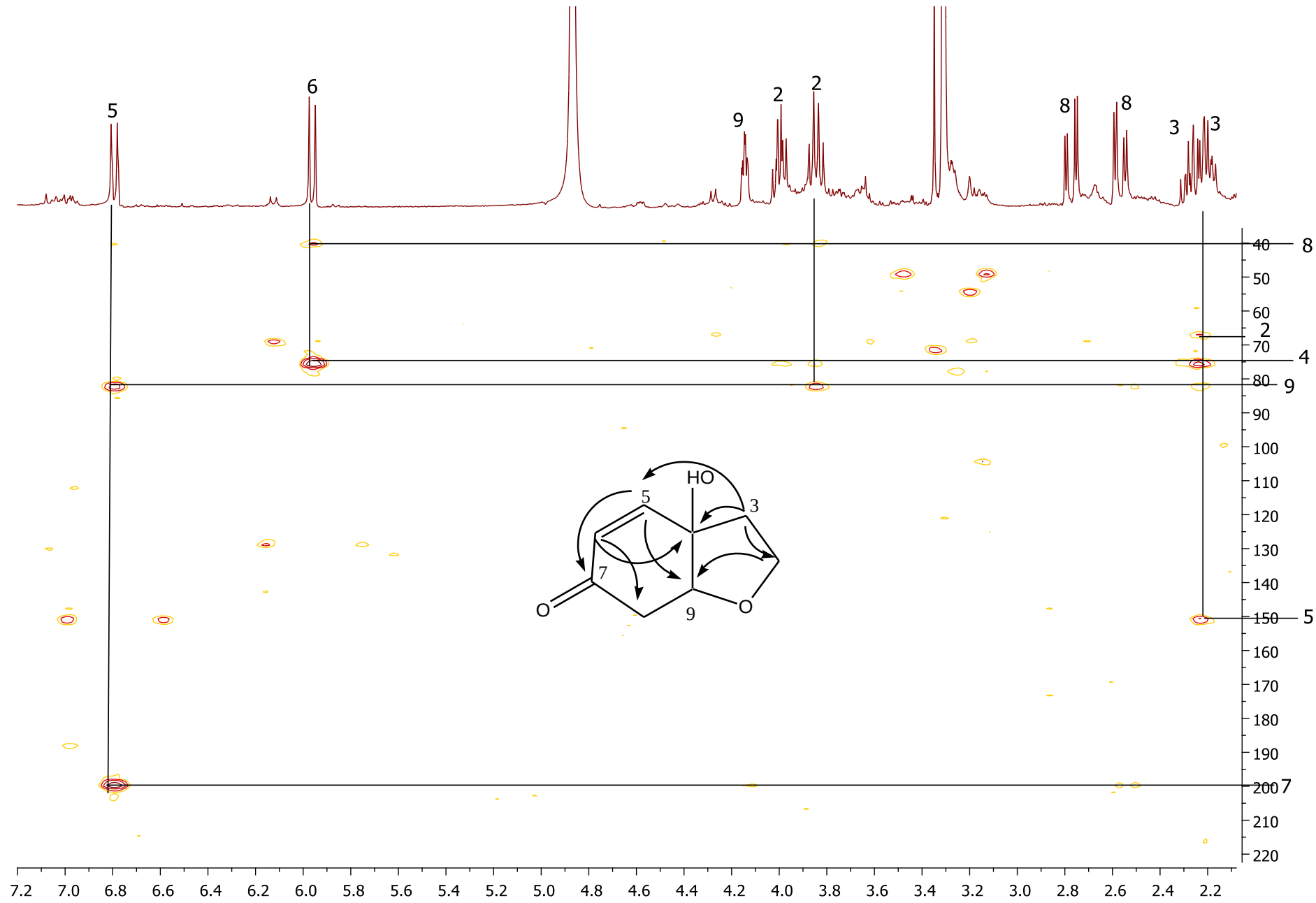




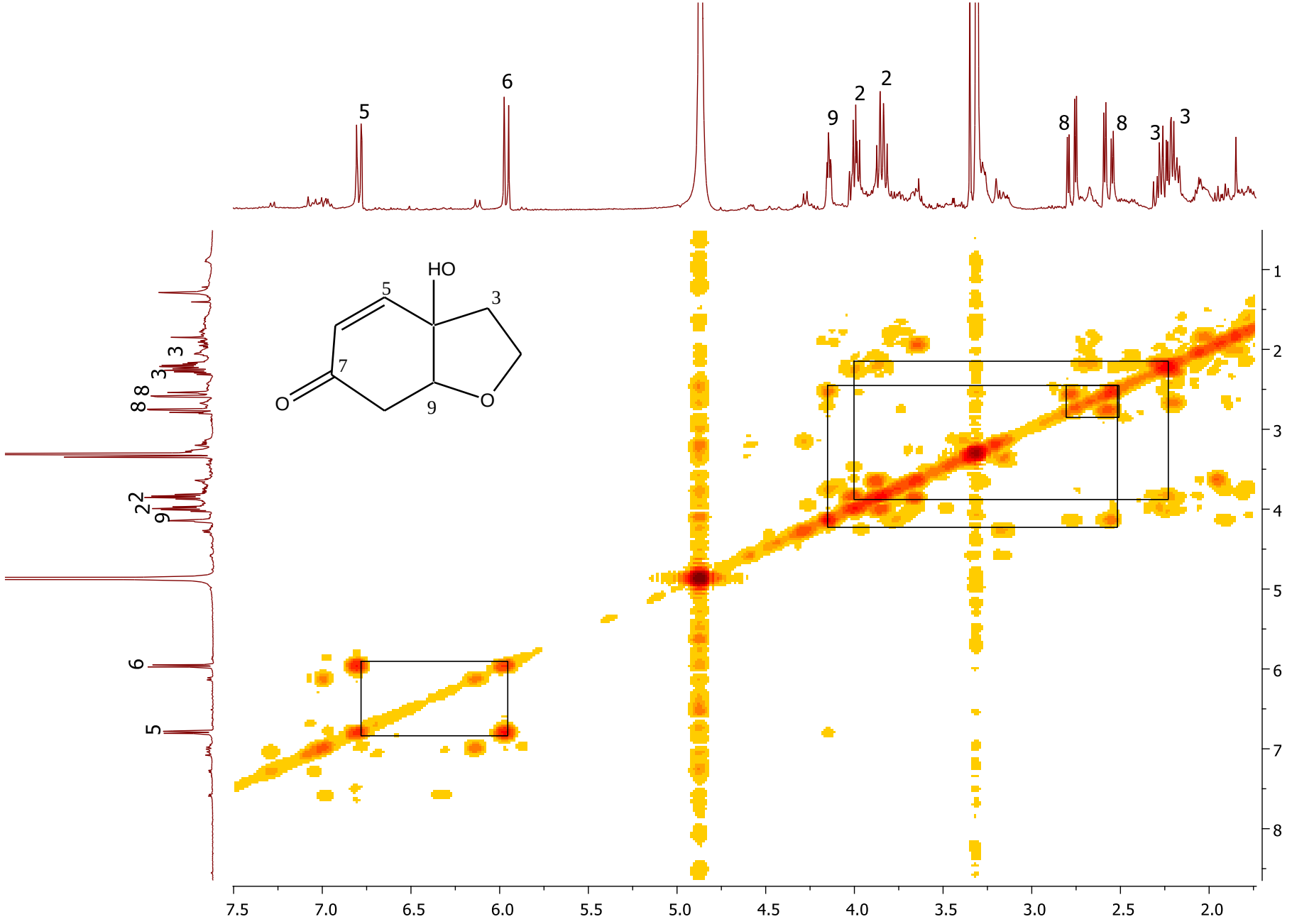
Spectrum 13. ^{1}H-NMR spectrum of Cleroindicin F (CD_3OD, 400 MHz)



Spectrum 14. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Cleroindicin F (HSQC)

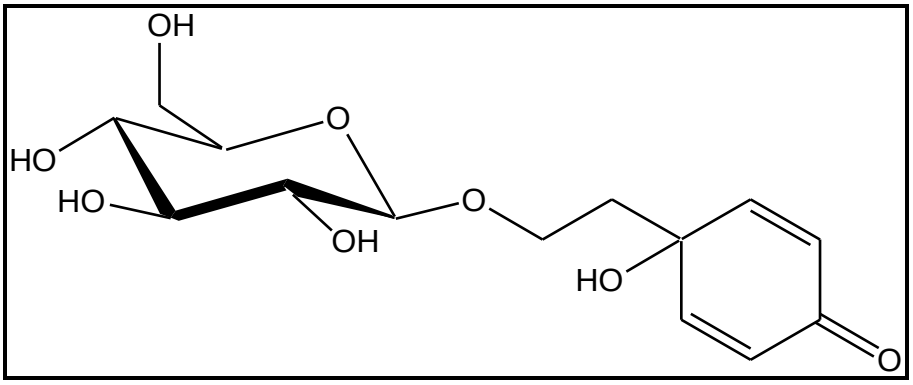


Spectrum 15. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Cleroindicin F (HMBC)



Spectrum 16. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum (COSY) of Cleroindicin F

4.2.4.2. Phenylethanoid Glycosides



CORNOSIDE (5): $C_{14}H_{20}O_8$ (316.30)

UV λ_{max} (MeOH) nm: 224, 278, 329

IR ν_{max} (KBr) cm^{-1}: 3434 (OH), 2923 (aliphatic CH), 1631 (conjugated ketone C=O), 1079 (C-O-C)

ESI-Mass *m/z*: 317 $[M+H]^+$, 339 $[M+Na]^+$

^{1}H NMR: Table 100, Spectrum 17

^{13}C NMR: Table 100

HSQC: Spectrum 18

HMBC: Spectrum 19

COSY: Spectrum 20

CORNOSIDE (5)

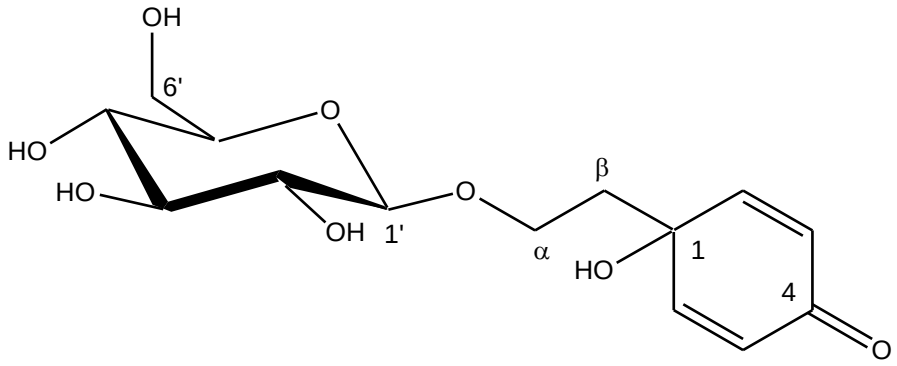
Compound **5** was obtained as white amorphous powder, from both MeOH and acetone extracts. Its molecular weight was calculated as 316 based on the pseudomolecular ion peaks at *m/z* 317 $[M+H]^+$, 339 $[M+Na]^+$ in ESI-Mass spectrum with five unsaturation degrees. The molecular formula was figured as $C_{14}H_{20}O_{8.}$ The IR spectrum revealed bands at 3434 cm^{-1} (OH), 2923 cm^{-1} (aliphatic CH), 1631 cm^{-1} (conjugated ketone C=O), 1079 cm^{-1} (C-O-C). The UV spectrum showed absorption bands at 220, 278, 329 nm.

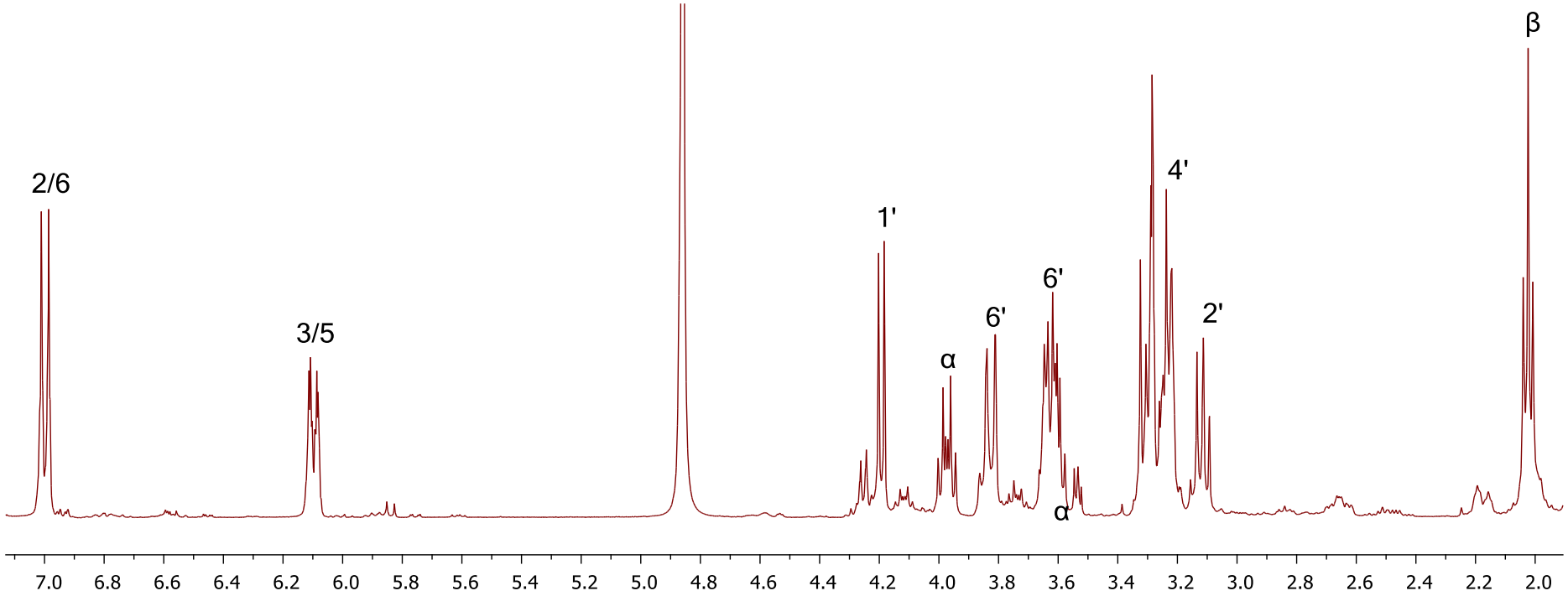
The ^1H NMR spectrum (Table 100, Spectrum 17), displayed two pairs of olefinic signals at δ_H 6.10 (dd, *J*= 1.8, 10.0 Hz, 2H) and δ_H 7.00 (dd, *J*=1.8, 10.0, 2H) elicited as AABB (A_2B_2) system. Moreover, the resonances at δ_H 2.02 (t, 2H) as well as two diastereotopic oxymethylene protons at δ_H 3.97 (m) and 3.61 (m) revealed the presence of of CH_2CH_2O- sequence in **5.** These findings along with the corresponding carbon resonances (δ_C 189.3, 154.0, 127.9, 69.2, 65.5, 41.0) which were established through HSQC (Spectrum 18) and HMBC (Spectrum 19) spectra, led to the identification of 1-(hydroxycyclohexa-2,5-dien-4-one)-ethyl unit (ethylcyclohexanone) in **5**. The ^{13}C spectrum (Table 100) displayed 14 carbon atoms, eight of which were ascribed to ethylcyclohexanone moiety. The remaining six resonances together with the corresponding proton signals, revealed the presence of a β-D-glucopyranose moiety in the structure of **5**. The above data were superimposible with those of 1-(hydroxycyclohexa-2,5-dien-4-one)-ethyl-β-D-glucopyranoside, which is also known as **cornoside** (226).

Table 100. ^{13}C and ^{1}H NMR Spectroscopic Data of Cornoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

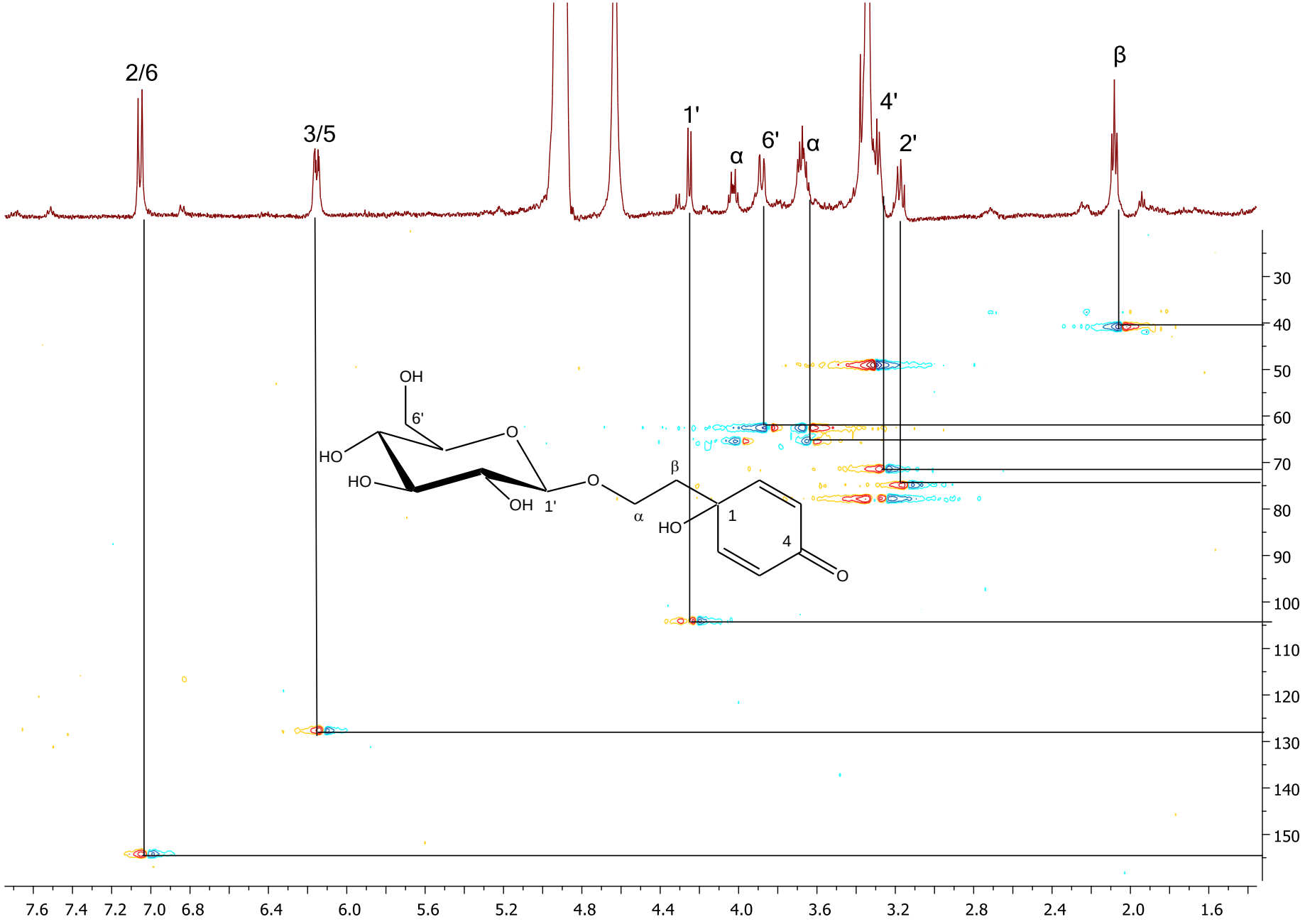
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
1	C	69.2	
2	CH	154.0	7.00 dd (1.8, 10.0)
3	CH	127.9	6.10 dd (1.8, 10.0)
4	C	189.3	
5	CH	127.9	6.10 dd (1.8, 10.0)
6	CH	154.0	7.00 dd (1.8, 10.0)
α	CH_2	65.5	3.97 m 3.61 m
β	CH_2	41.0	2.02 t (6.5)
Glucose			
1'	CH	102.9	4.19 d (7.8)
2'	CH	73.8	3.11 dd (7.8, 8.7)
3'	CH	76.5	3.20-3.34 ł
4'	CH	70.4	3.28 ł
5'	CH	76.7	3.20-3.34 ł
6'	CH_2	61.5	3.83 dd (11.5, 1.6) 3.65 dd (11.5, 6.1)

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.

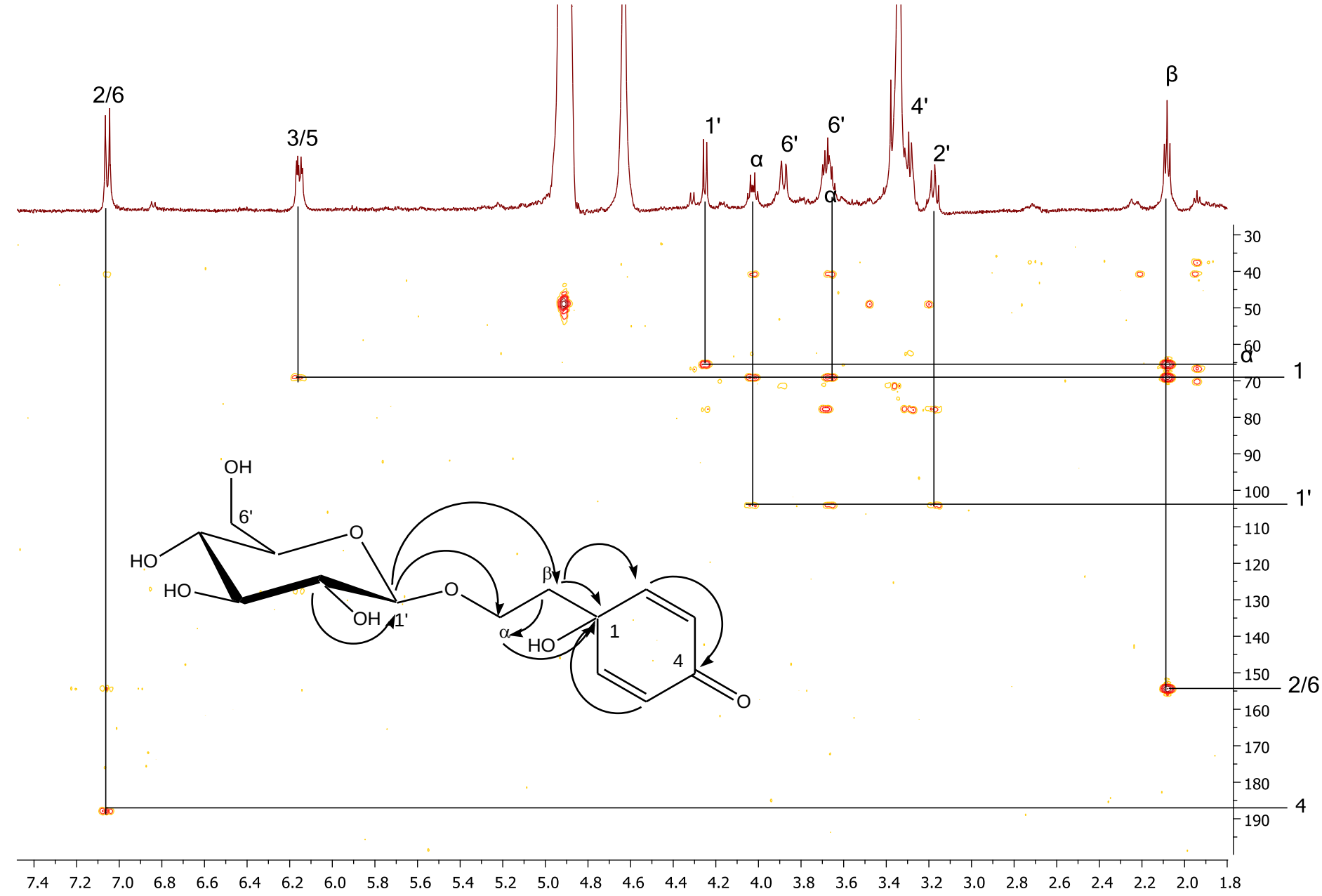




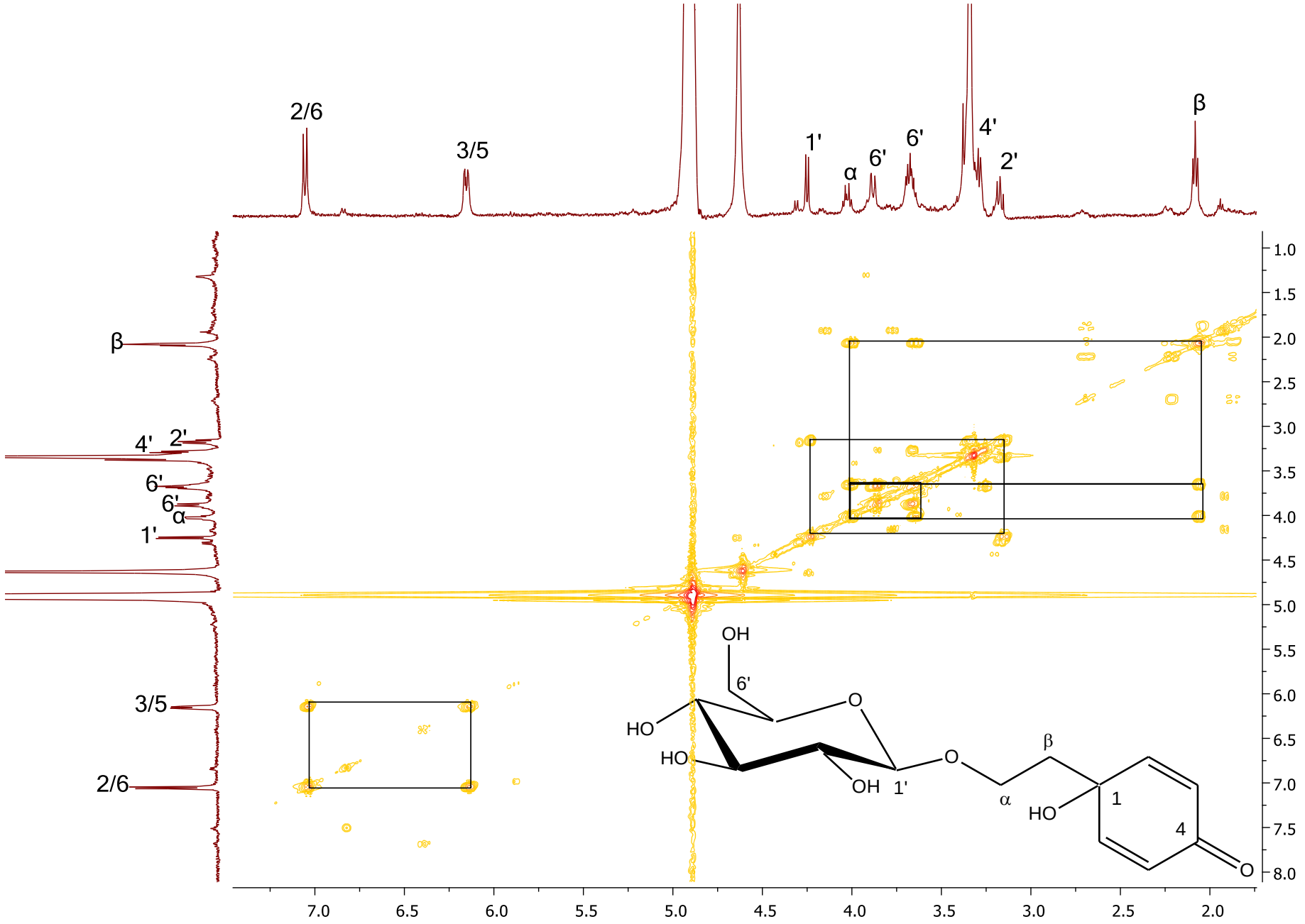
Spectrum 17. ^{1}H-NMR spectrum of Cornoside (CD_3OD, 400 MHz)



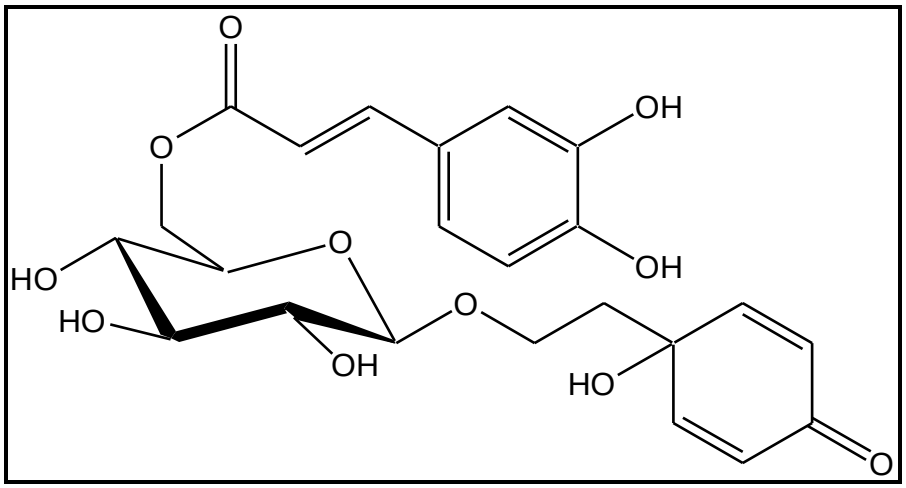
Spectrum 18. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Cornoside (HSQC)



Spectrum 19. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Cornoside (HMBC)



Spectrum 20. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum of Cornoside (COSY)



CALCEOLARIOSIDE D (6): $C_{23}H_{26}O_{11}$ (MW: 478.45)

UV λ_{max} (MeOH) nm: 221, 234 (sh), 302 (sh), 329

IR ν_{max} (KBr) cm^{-1}: 3429 (OH), 1700 (conjugated ester C=O), 1669 (conjugated ketone C=O), 1060 (C-O-C)

ESI-Mass *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$

^1H NMR: Table 101, Spectrum 21

^{13}C NMR: Table 101

HSQC: Spectrum 22

HMBC: Spectrum 23

CALCEOLARIOSIDE D (6)

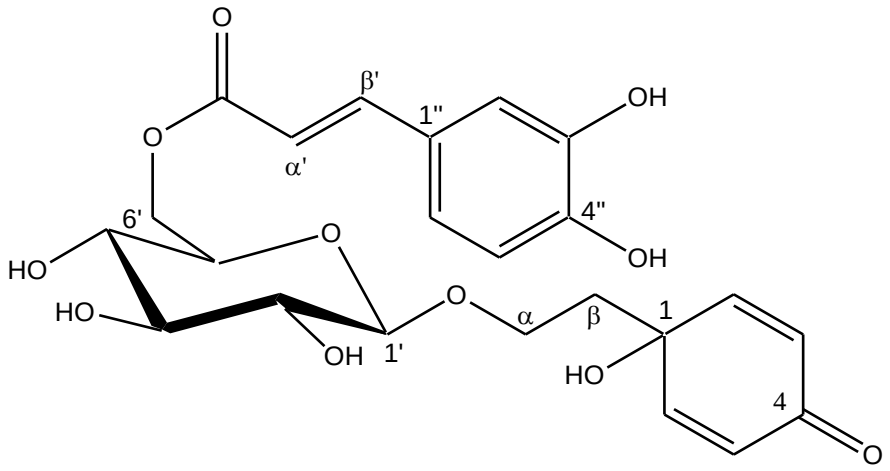
Compound **6** was obtained as whitish amorphous powder. The molecular formula $\mathbf{C_{23}H_{26}O_{11}}$, was deduced from the pseudomolecular ion peaks at 479 $[M+H]^+$, 501 $[M+Na]^+$ in positive ESI-MS spectrum and NMR data. The IR spectrum revealed bands at 3429 cm^{-1} (OH), 1700 cm^{-1} (conjugated ester C=O), 1669 cm^{-1} (conjugated ketone C=O), 1060 cm^{-1} (C-O-C). Its UV spectrum displayed maxima at 221 and 329 nm and two sholders at 234 and 295 nm, which are characteristic for the phenylethanoid structure.

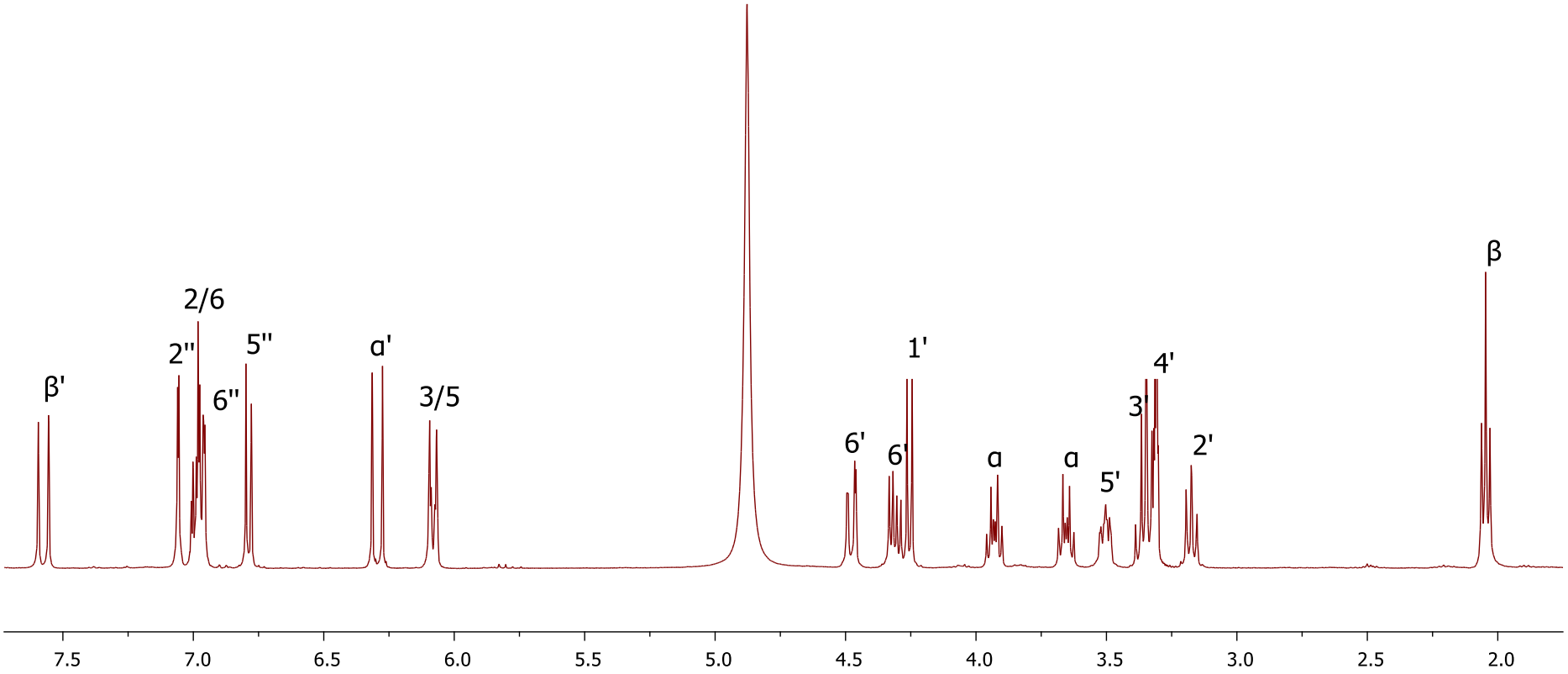
The ^{1}H NMR spectrum (Table 101, Spectrum 21) of **6** displayed two pairs of olefinic signals at δ_H 6.08 (H-3/5) and at δ_H 6.99 (H-2/6) assigning an AABB system along with signals at δ_H 2.05 (β) and δ_H 3.65 (m), 3.93 (m) as in cornoside suggesting the presence of same aglycone [1-(hydroxycyclohexa-2,5-dien-4-one)-ethyl]. Additionally, ^{1}H NMR spectrum contained an anomeric proton signal at δ_H 4.26 (d, *J*=7.8 Hz) indicating the presence of β-D-glucopyranose unit in **6**. However, additional signals and chemical shifting of the H_2-6' protons of glucose, pointed that **6** had another moiety on C-6' (OH) of glucose. Moreover, ^{1}H NMR spectrum contained two doublet signals at 7.06 ppm (H-2"), 6.79 ppm (H-5") and a doublet-doublet signal at 6. 97 ppm (H-6" which are characteristic signals of an ABX system, along with the two *trans*-olefinic resonances (δ_H 7.57 and 6.29) were matching with the signals of *trans*-caffeic acid. The C-6' of the glucose moiety was shifted downfield for 2 ppm (δ_C 64.3) which was understood from the HSQC spectrum (Spectrum 2). This was accounted for the α-effect of esterification. This revealed that the esterification point of *trans*-caffeic acid should be on C-6' (OH). This assumption was also confirmed by cross-peaks observed between C=O of caffeoyl (168.8 ppm) and H_2-6' (δ_H 4.48 and 4.31) in its HMBC spectrum (Spectrum 23). Further interfragmental connections were also determined by long-range correlations between anomeric carbon of β-D-glucopyranose (104.4 ppm) with α-methylene protons (δ_H 3.93 and 3.65). Based on the above data, the structure of **6** was elucidated as 1'-*O*-β-D-(1-hydroxy-4-oxo-2,5-cyclohexadien)-ethyl-6'-*O*-caffeoylglucopyranoside **(calceolarioside D)** (227).

Table 101. ^{13}C and ^{1}H NMR Spectral Data of Calceolarioside D (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

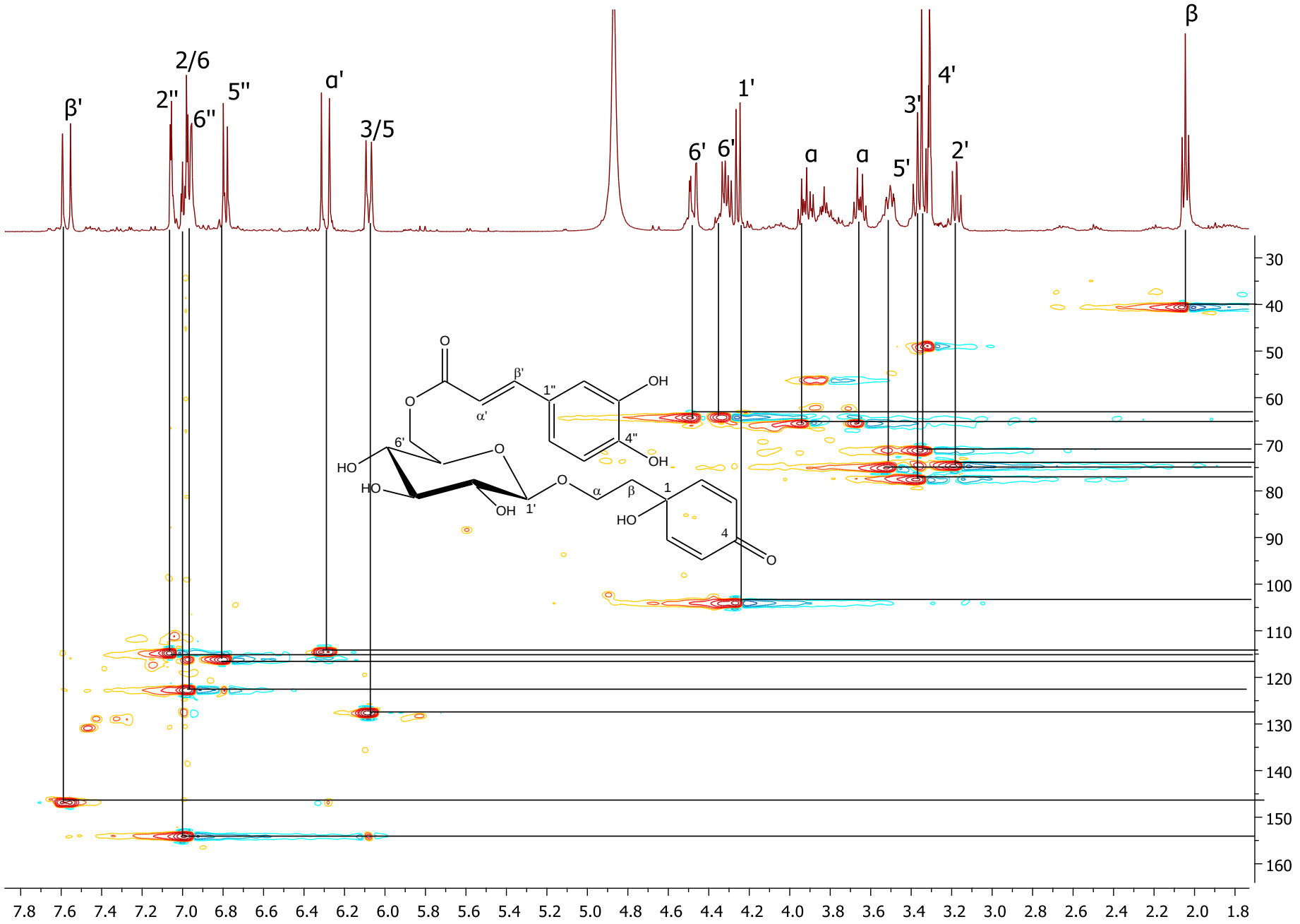
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
1	C	68.5	
2	CH	154.1	6.99 dd (2.3, 8.4)
3	CH	127.6	6.08 dd (2.3, 8.4)
4	C	187.6	
5	CH	127.6	6.08 dd (2.3, 8.4)
6	CH	154.2	6.99 dd (2.3, 8.4)
α	CH_2	65.5	3.93 m 3.65 m
β	CH_2	40.6	2.05 t (6.5)
Glucose			
1'	CH	104.4	4.26 d (7.8)
2'	CH	74.7	3.18 dd (7.8, 8.7)
3'	CH	77.7	3.32-3.39 ł
4'	CH	71.4	3.32-3.39 ł
5'	CH	75.0	3.50 ddd (1.9, 6.0, 8.2)
6'	CH_2	64.3	4.48 dd (1.9, 12.5) 4.31 dd (6.0, 12.5)
Caffeic Acid			
1"	C	127.7	
2"	CH	114.9	7.06 d (2.0)
3"	C	146.5	
4"	C	149.2	
5"	CH	116.2	6.79 d (8.0)
6"	CH	122.6	6.97 (2.0, 8.0)
α'	CH	114.6	6.29 d (15.9)
β'	CH	146.9	7.57 d (15.9)
C=O	C	168.8	

[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.
ł Overlapping signals.

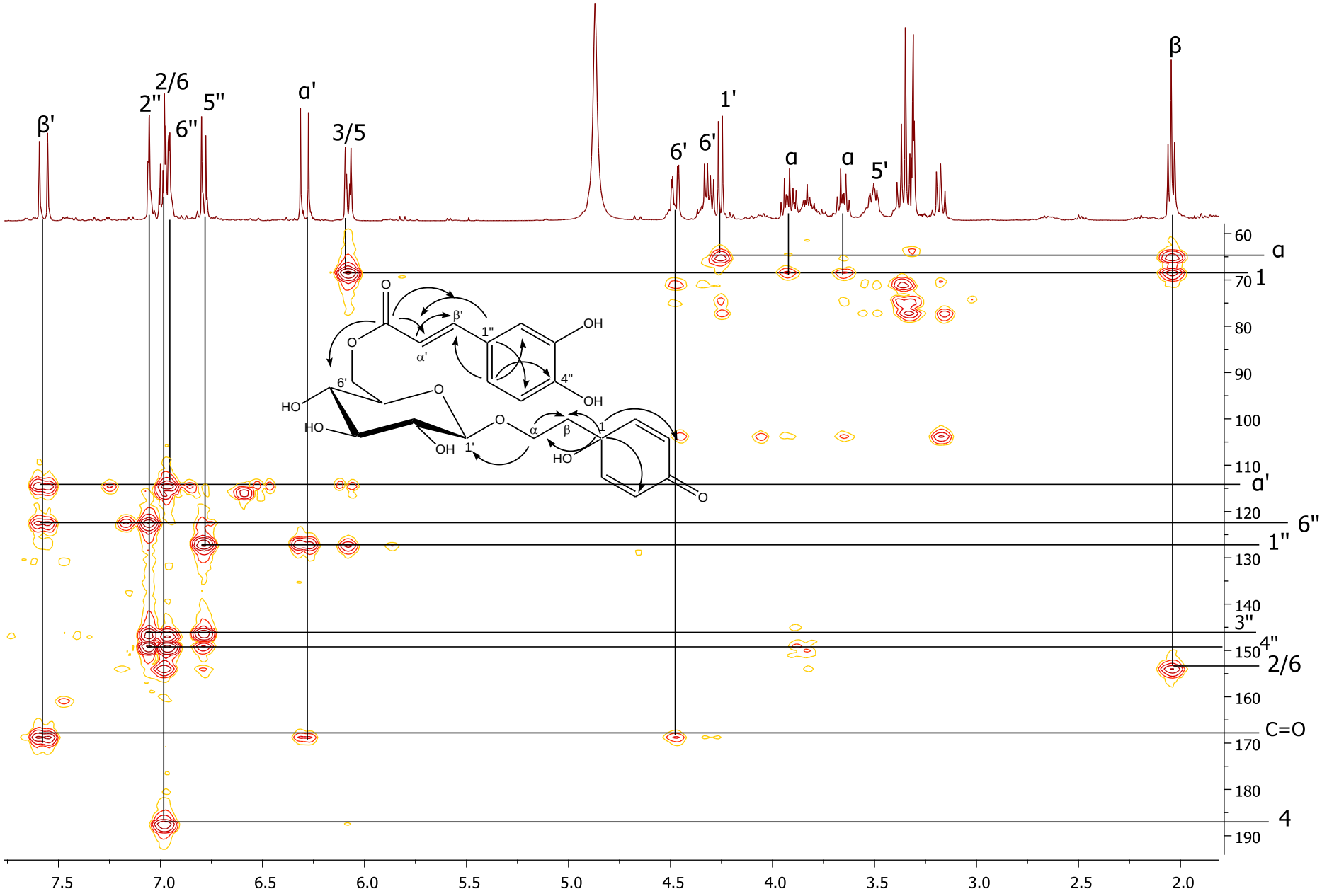




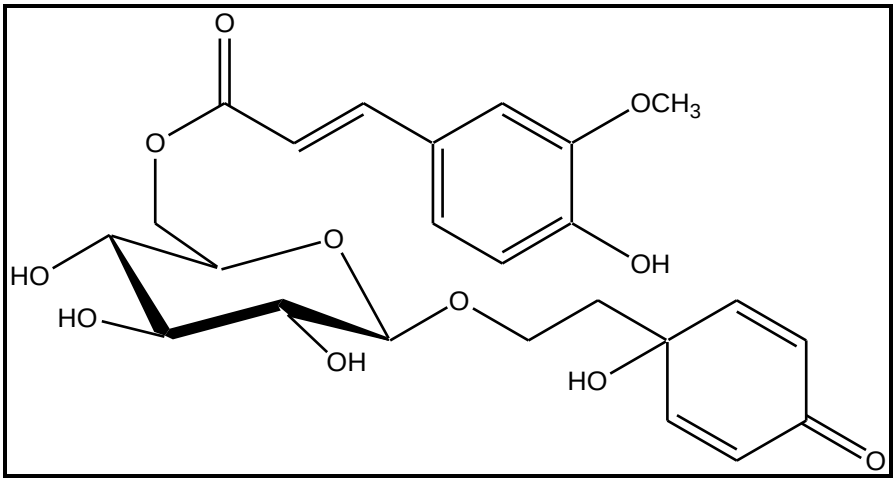
Spectrum 21. ^{1}H-NMR spectrum of Calceolarioside D (CD_3OD, 400 MHz)



Spectrum 22. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Calceolarioside D (HSQC)



Spectrum 23. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Calceolarioside D (HMBC)



HASTIFOLIOSIDE (7): $C_{24}H_{28}O_{11}$ (492.47)

UV λ_{max} (MeOH) nm: 288 (sh), 327 (max)

IR ν_{max} (KBr) cm^{-1}: 3387 (OH), 2857 (OCH_3), 1628 (conjugated C=C), 1600, 1516 (benzene)

ESI-Mass *m/z*: 491 $[M-H]^-$

^1H NMR: Table 102, Spectrum 24

^{13}C NMR: Table 102

HSQC: Spectrum 25

HMBC: Spectrum 26

HASTIFOLIOSIDE (7)

Compound **7** was isolated as yellowish amorphous powder from acetone extract with a molecular formula of $C_{24}H_{28}O_{11}$, as determined by data from negative ion ESI-MS, showing pseudomolecular ion peak at *m/z* 491 $[M-H]^-$ along with 1D and 2D NMR data. The UV (λmax 327) and IR (ν_{max} 3387, 2857, 1628, 1600, 1516) spectra of compound **7** were characteristical for the structures of cornoside derivative phenylethanoid glycosides.

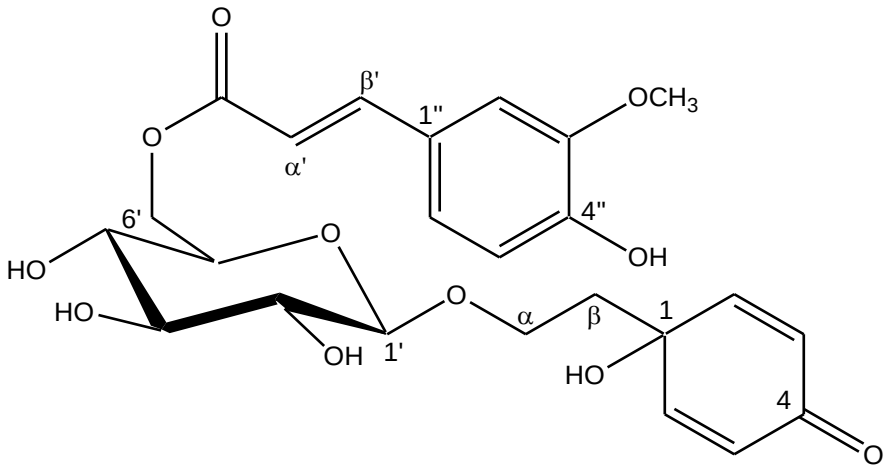
The ^{1}H NMR spectrum (Table 102, Spectrum 24) of **7** exhibited two pairs of olefinic signals at δ_H 6.96 (ł) and 6.07 (dd, *J*=1.0, 10.4 Hz) showing AABB system along with an equivalent and non equivalent oxymethylene signals at δ_H 2.04 (t, *J*=6.6 Hz), δ_H 3.92 (m) and 3.64 (m), respectively. These findings with corresponding carbon resonances (δ_C 186.9, 154.2, 127.8, 68.9, 65.7, 40.7) that were elicited with the help of short and long range 2D-heteronuclear spectra (Spectrum 25 and Spectrum 26), resulted in the identification of [1-(hydroxycyclohexa-2,5-dien-4-one)-ethyl] unit, which was also seen in the structure of **calceolarioside D** (227). Signals at δ_H 4.23 (d, *J*=7.8 Hz) and δ_C 104.3 (C-1') revealed its monoglycosidic structure as in the case of **cornoside** and **calceolarioside D**. Furthermore, an ABX sytem resonances in the aromatic region, led to the presence of a *trans*-cinnamoyl derivative similarly in **calceolarioside D**. Differently from **calceolarioside D**, signal at δ_H 3.86 (s, 3H) revealed that **7** bore a methoxyl group. In ^{13}C NMR, signal at δ_C 56.3 verified that the presence of methoxyl group. *Trans*-olefinic protons at the acyl region of the compound were shifted between 0.2-1 ppm through the downfield shield. Moreover the shifting of the aromatic protons of the acyl region through the downfield shield (especially H-2", δ_H 7.22, d, *J*=1.9 Hz) pointed that the methoxyl group located at C-3". Additionally, comparison of compound **7** with **calceolarioside D**, according to ^{13}C NMR spectrum, it was seen that resonances belonging to C-2" and C-3" were shifted 3.5 ppm to the downfield region and 3.1 ppm to the upfield region, respectively. This proved that the methoxyl group located at C-3". HMBC spectra was further verified that C-3" was substituted with methoxyl group. The above findings displayed that **7** contained ferulic acid as its acyl unit. C-6' of the glucose moiety was moved approximately two ppm to downfield (δ_C 64.3) region, assigning that the esterification point is between ferruloyl moiety. Based on these

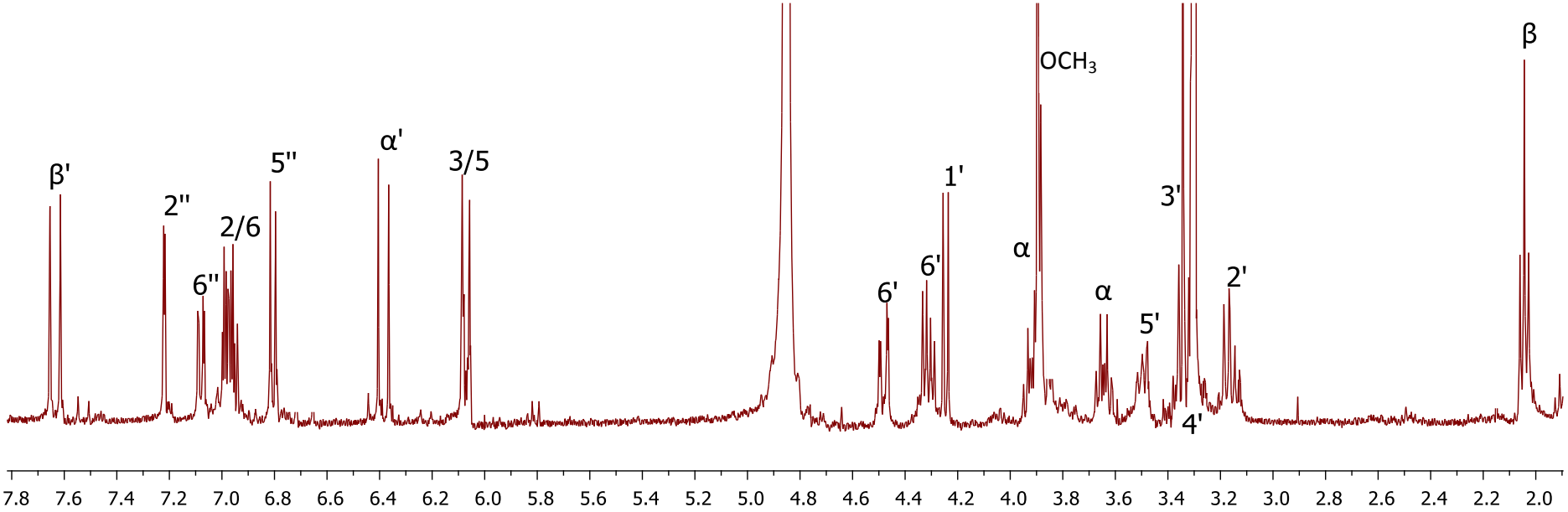
results, the structure of compound **7** was established as 1'-*O*-β-D-(1-hydroxy-4-oxo-2,5-cyclohexadien)-ethyl-6'-*O*-feruloylglucopyranoside. To the best of our knowledge, **7** is being reported for the first time and named as **hastifolioside**.

Table 102. ^{13}C and ^{1}H NMR Spectroscopic Data of Hastifolioside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

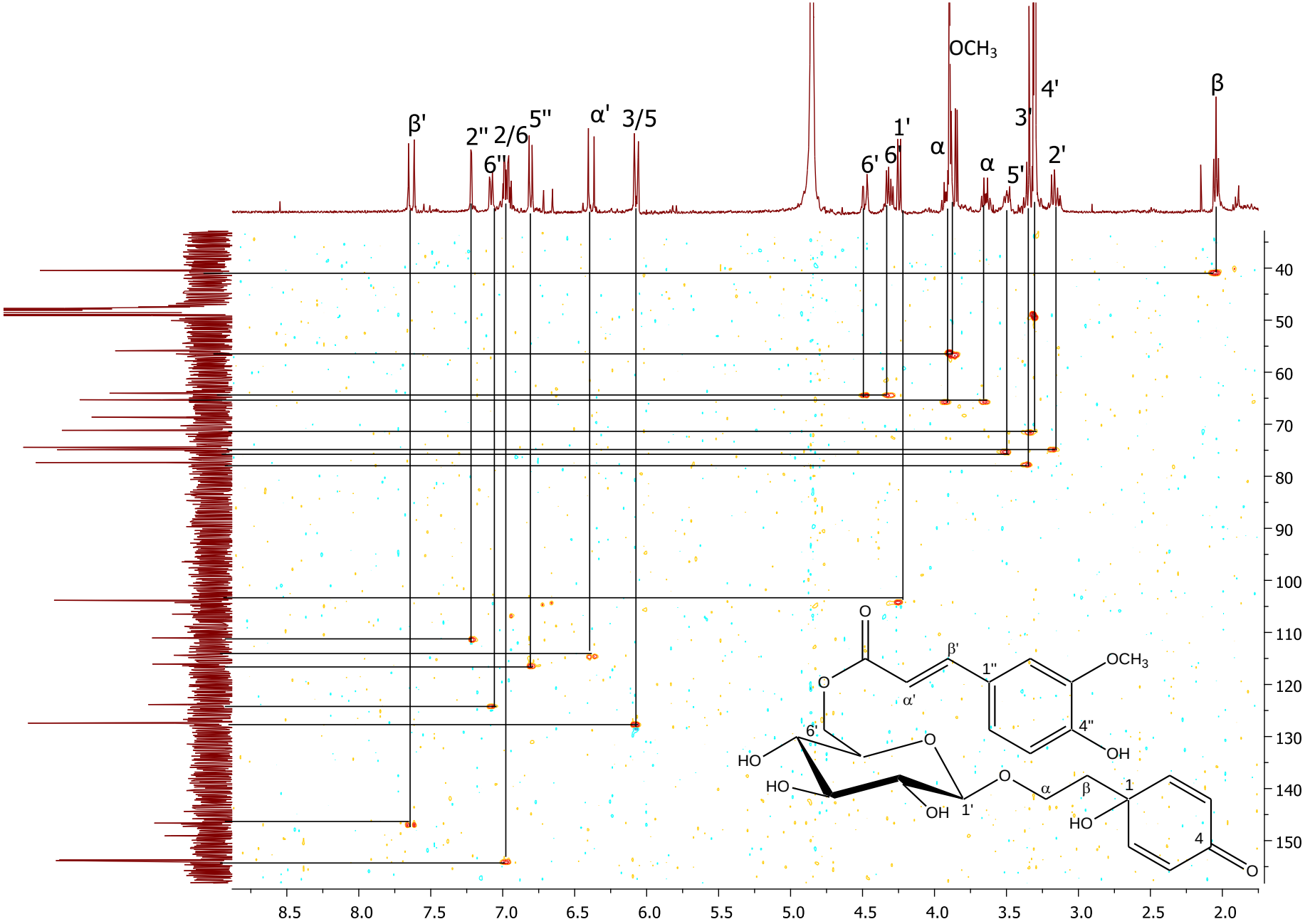
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
1	C	68.9	
2	CH	154.2	6.96 ł
3	CH	127.8	6.07 dd (1.0, 10.4)
4	C	186.9	
5	CH	127.8	6.07 dd (1.0, 10.4)
6	CH	154.2	6.96 ł
α	CH_2	65.7	3.92 m 3.64 m
β	CH_2	40.7	2.04 t (6.6)
Glucose			
1'	CH	104.3	4.23 d (7.8)
2'	CH	74.8	3.17 dd (7.8, 8.9)
3'	CH	77.8	3.34 ł
4'	CH	71.6	3.32 ł
5'	CH	75.3	3.49 m
6'	CH_2	64.4	4.48 dd (12.0, 2.1) 4.31 dd (12.0, 6.1)
Ferulic Acid			
1"	C	128.0	
2"	CH	111.4	7.22 d (1.9)
3"	C	149.6	
4"	C	151.6	
5"	CH	116.5	6.81 d (8.3)
6"	CH	124.3	7.08 dd (8.3,1.9)
α'	CH	114.7	6.39 d (15.9)
β'	CH	147.1	7.63 d (15.9)
C=O	C	169.3	
OCH_3	CH_3	56.3	3.86 s

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.

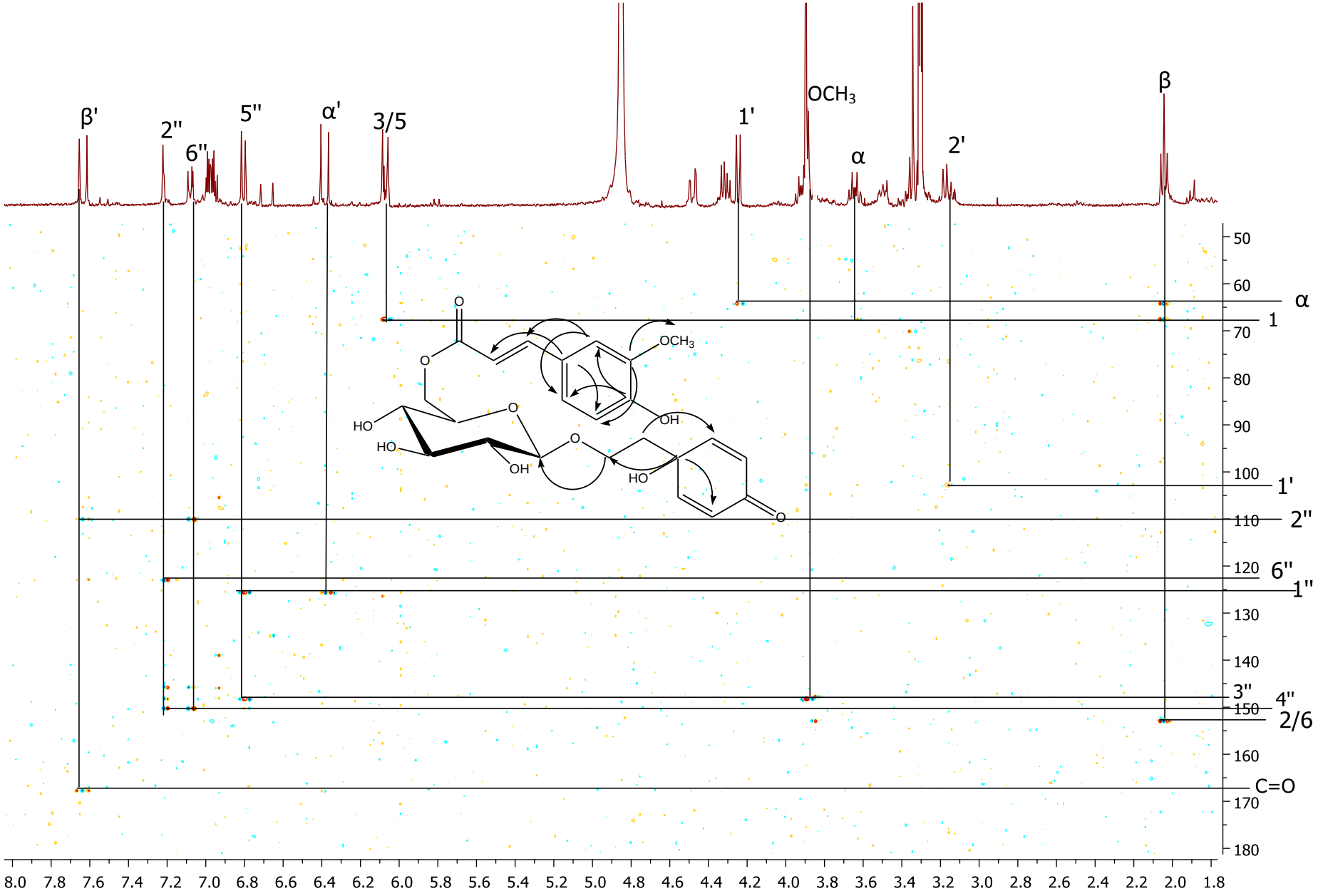




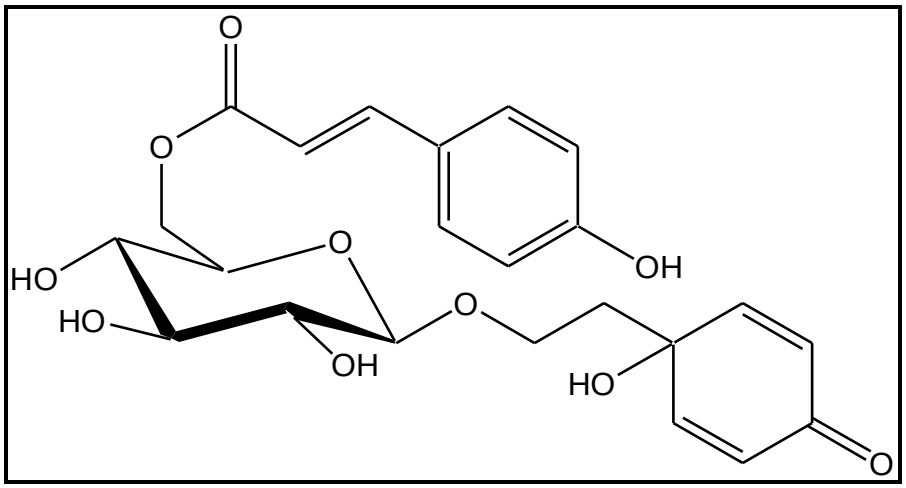
Spectrum 24. ^{1}H-NMR spectrum of Hastifolioside (CD_3OD, 400 MHz)



Spectrum 25. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Hastifolioside (HSQC)



Spectrum 26. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Hastifolioside (HMBC)



NEOCALCEOLARIOSIDE D (8): $C_{23}H_{26}O_{10}$ (462.45)

UV λ_{max} (MeOH) nm: 299 (sh), 315 (max)

IR ν_{max} (KBr) cm^{-1}: 3379 (OH), 1628 (conjugated C=C), 1600 (C=C), 1516 (benzene)

ESI-Mass *m/z*: 463 $[M+H]^+$, 485 $[M+Na]^+$

^1H NMR: Table 103, Spectrum 27

NEOCALCEOLARIOSIDE D (8)

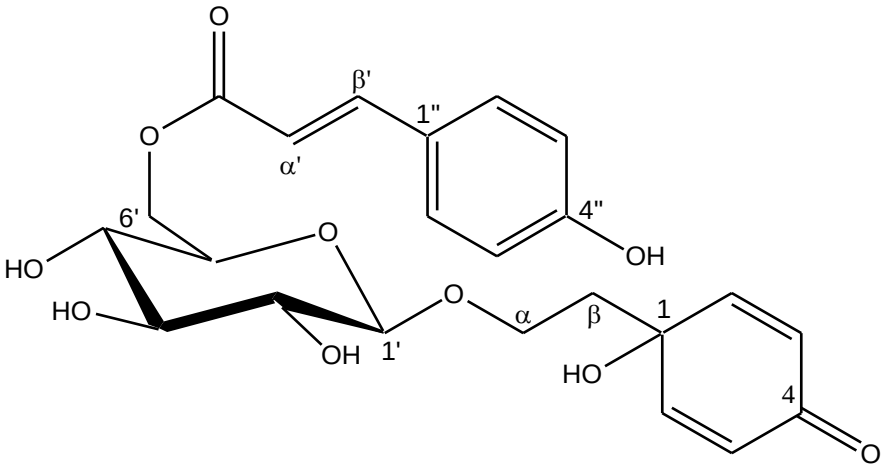
Compound **8** was obtained as yellowish amorphous powder from MeOH extract. Its molecular formula was assigned as $C_{23}H_{26}O_{10}$ by positive ion ESI-MS (463 $[M+H]^+$, 485 $[M+Na]^+$), requiring 11 degrees of unsaturation. The IR spectrum revealed bands at 3379, 1628, 1600, 1516. Its UV spectrum displayed maxima at 315 nm and shoulder at 299 nm which are characteristic for a phenylethanoid structure.

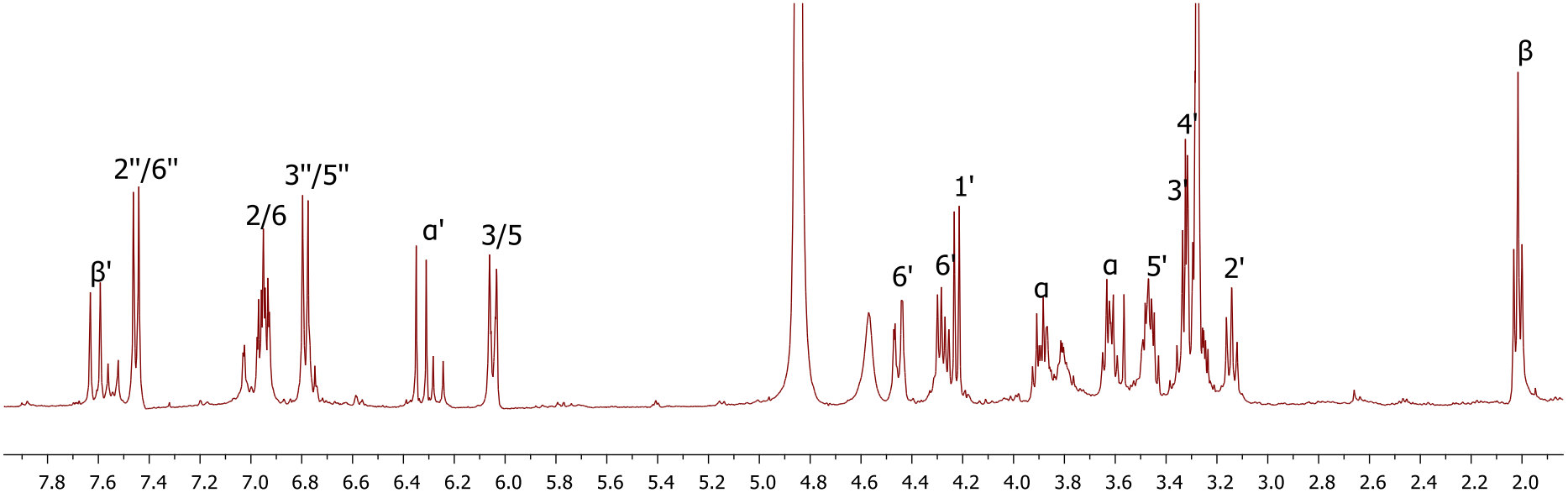
^{1}H NMR spectrum (Table 100, Spectrum 27) of compound **8** revealed the presence of two AABB systems in the molecule. Two pairs of olefinic signals at δ_H 6.08 (H-3/5) and at δ_H 6.99 (H-2/6) are the initial verification of this suggestion. Further, methylene signal at δ_H 2.02 (β), oxymethylene signal at δ_H 3.62 (m) and 3.89 (m) suggested the presence of CH_2CH_2O- unit. Taken together all of the findings indicate that the aglycone part of compound **8** was same as in **calceolarioside D** [1-(hydroxycyclohexa-2,5-dien-4-one)-ethyl]. Signals at δ_H 6.79 (d, *J*=8.6 Hz, H-3'/5') and 7.45 (d, *J*=8.6 Hz, H-2'/6') were the indication of another AABB system in the molecule. Moreover, an AX system at δ_H 6.33 (d, *J*=15.9 Hz, α') and 7.61 (d, *J*=15.9 Hz, β') denoted the *trans*-olefinic protons. A doublet proton resonance at δ_H 4.22 (d, *J*=7.8 Hz) was readily assigned to anomeric proton of β-D-glucopyranose unit, indicating the monoglycosidic structure of compound **8**. The appearance of a downfield signal at δ_H 4.28 (dd, *J*=11.9, 6.0 Hz) and 4.45 (dd, *J*=11.9, 2.0) for H-6' of glucose revealed the presence of a linkage between H-6' and coumaroyl unit. According to the complete inspection of ^{1}H NMR spectrum of **8** elicited that the compound was 1'-*O*-β-D-(1-hydroxy-4-oxo-2,5-cyclohexadien)-ethyl-6'-*O*-coumaroylglucopyranoside. The structure of this compound was established as **neocalceolarioside D** from the literature (228).

Table 103. ^{13}C and ^{1}H NMR Spectral Data of Neocalceolarioside D (CD_3OD, ^{1}H: 400 MHz)

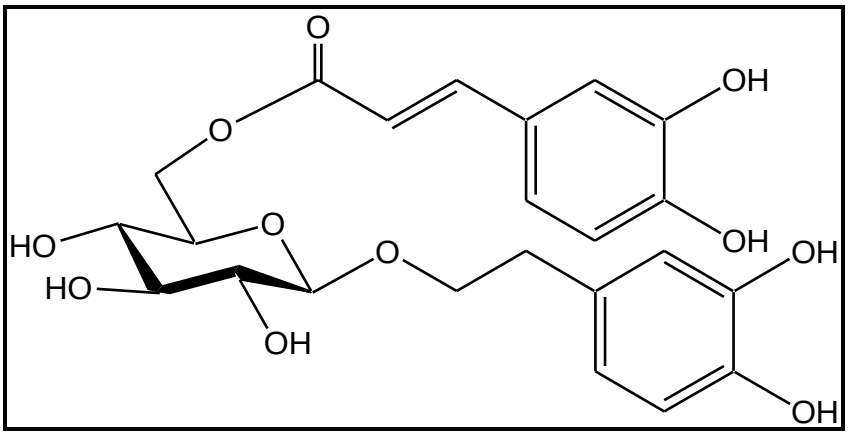
Position	Multiplicity	δ_H ppm, *J* (Hz)
Aglycone		
1	C	
2	CH	6.99 dd (2.1, 8.7)
3	CH	6.08 dd (2.1, 8.7)
4	C	
5	CH	6.08 dd (2.1, 8.7)
6	CH	6.99 dd (2.1, 8.7)
α	CH_2	3.62 m 3.89 m
β	CH_2	2.02 t (6.5)
Glucose		
1'	CH	4.22 d (7.8)
2'	CH	3.14 t (8.3)
3'	CH	3.32 dd (8.3, 5.3)
4'	CH	3.28 ł
5'	CH	3.46 m
6'	CH_2	4.28 dd (11.9, 6.0) 4.45 dd (11.9, 2.0)
p-Coumaric Acid		
1"	C	
2"	CH	7.45 d (8.6)
3"	CH	6.79 d (8.6)
4"	C	
5"	CH	6.79 d (8.6)
6"	CH	7.45 d (8.6)
α'	CH	6.33 d (15.9)
β'	CH	7.61 d (15.9)
C=O	C	

[α] Resonances are interpreted with the help of 1D NMR (^{1}H NMR) technique.
ł Overlapping signals.





Spectrum 27. ^{1}H-NMR spectrum of Neocalceolarioside D (CD_3OD, 400 MHz)



CALCEOLARIOSIDE B (9): $C_{23}H_{26}O_{11}$ (MW: 478.45)

UV λ_{max} (MeOH) nm: 203, 219 (sh), 246 (sh), 291, 329

IR ν_{max} (KBr) cm^{-1}: 3387 (OH), 1690 (conjugated ester C=O), 1080 (C-O-C), 1525 (aromatic ring)

ESI-Mass *m/z*: 479 $[M+H]^+$, 501 $[M+Na]^+$

^1H NMR: Table 104, Spectrum 28

^{13}C NMR: Table 104

COSY: Spectrum 29

CALCEOLARIOSIDE B (9)

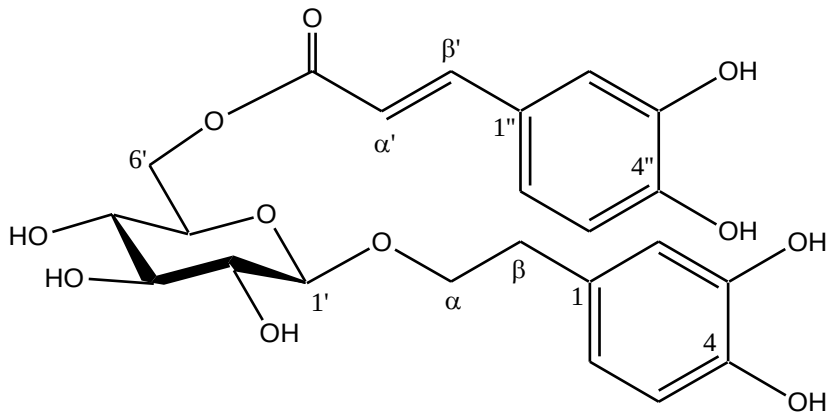
Compound **9** was obtained as brownish colored amorphous powder from the MeOH extract. Characteristic bands indicating the polyphenolic structure, were seen on UV spectroscopy (λ_{max} 203, 219 (sh), 246 (sh), 291 and 329). IR spectrum showed hydroxyl bands (3387 cm^{-1}), α, β-unsaturated ester (1690 cm^{-1}), saturated carbon-oxygen bonds (1080 cm^{-1}) and benzene (1525 cm^{-1}). ESI-Mass spectrum showed m/z 479 $[M+H]^+$ and 501 $[M+Na]^+$ peaks. Along with the ^{13}C and ^{1}H NMR spectroscopical data, the molecular formula of the compound **9** was found to be $C_{23}H_{26}O_{11}$ and the spectra were in agreement with those of phenylethanoid structure.

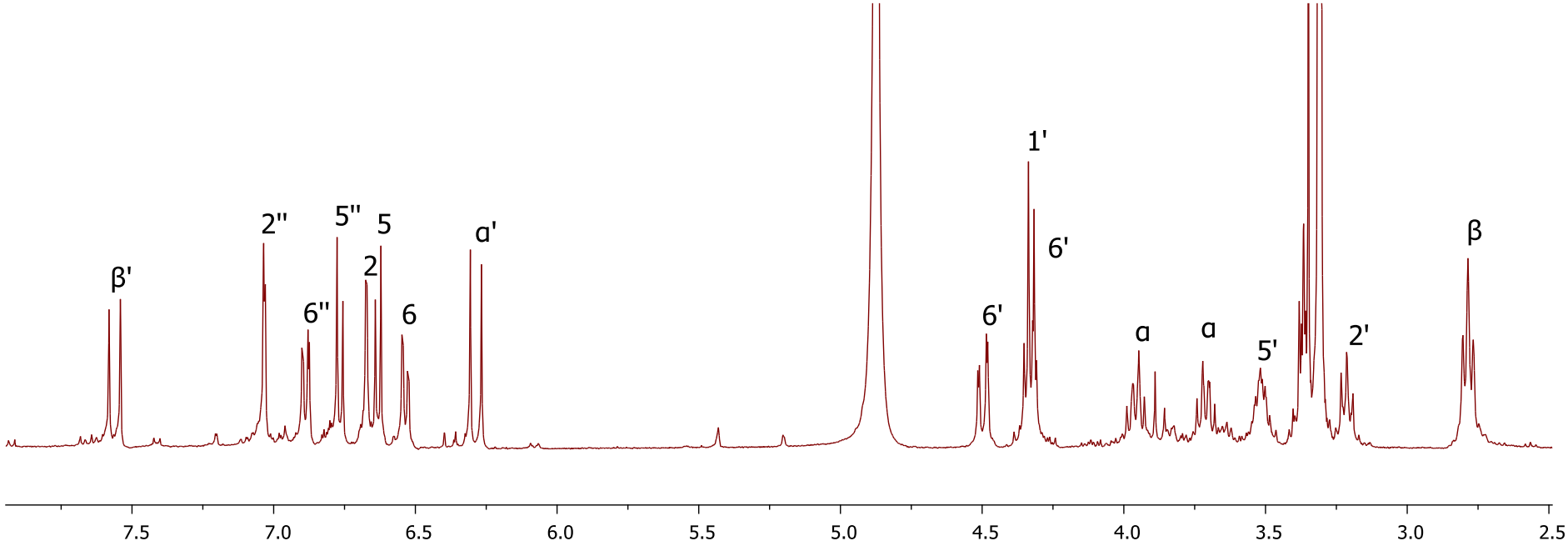
In ^{1}H-NMR spectrum (Table 104, Spectrum 28), six protons (δ_H 6.53-7.03) indicating two ABX system, exhibited that the compound contained two trisubstituted benzene ring. Additionally, two *trans*-olefinic proton signals (AX system) at 6.29 (d, *J*=15.9 Hz) and 7.56 (d, *J*= 15.9 Hz) ppm were seen. Benzylic β-methylene protons (δ_H 2.79, 2H, t, *J*=7.3 Hz) and two non-equivalent protons (δ_H 3.96 and 3.71, each 1H, m) were also seen in ^{1}H-NMR spectrum. All these findings showed that compound **9** contained *trans*-caffeic acid and 3,4-dihydroxyphenylethanol units in its structure. A doublet proton resonance at 4.33 ppm (1H, d, *J*=7.9 Hz) was readily assigned to the anomeric proton of a β-D-glucopyranose unit, indicating the monoglycosidic structure. The corresponding carbon resonance at 104.6 ppm confirmed this assumption. The caffeoyl moiety was considered to be positioned at the C-6' (OH) atom of glucose, on the basis of strong deshielding of the H_2-6' signals of the glucose unit (δ_H 4.50 dd, *J*=11.9, 2.0 Hz and 4.3 dd, *J*=11.9, 5.7 Hz) (α effect of esterification). Chemical shifting of the α-carbon atom of the 3,4-dihydroxyphenylethanol (aglycone) (δ_C 72.4) revealed the glucosidation point of the aglycone. All structural assignments, substantiated by the results obtained from the 2D-shift-correlated COSY and HSQC spectra and 1D-^{1}H-NMR spectrum, led the identification of the compound **9** as 2-(3,4-dihydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside. This compound was assigned as **calceolarioside B** referring the previous literatures (159).

Table 104. ^{13}C and ^{1}H NMR Spectral Data of Calceolarioside B (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

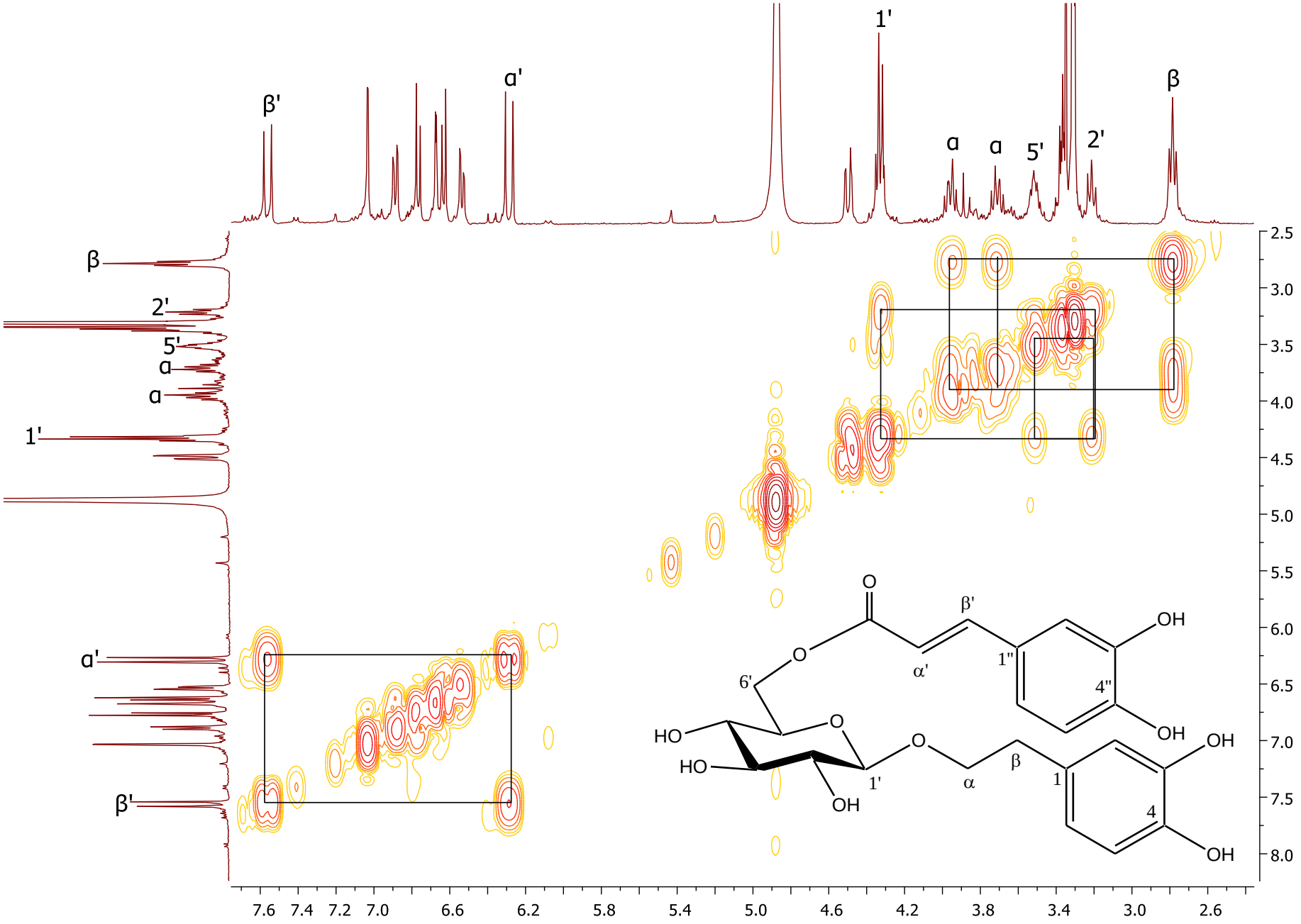
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
1	C	131.4	
2	CH	117.0	6.67 d (2.0)
3	C	146.2	
4	C	144.7	
5	CH	116.6	6.63 d (8.0)
6	CH	121.3	6.53 dd (8.0, 2.0)
α	CH_2	72.4	3.96 m 3.71 m
β	CH_2	36.7	2.79 t (7.3)
Glucose			
1'	CH	104.6	4.33 d (7.9)
2'	CH	75.1	3.21 dd (7.9, 8.8)
3'	CH	77.9	3.34-3.39 m ł
4'	CH	71.8	3.34-3.39 m ł
5'	CH	75.5	3.51 m ł
6'	CH_2	64.6	4.50 dd (11.9, 2.0) 4.30 dd (11.9, 5.7)
Caffeic Acid			
1"	C	127.8	
2"	CH	115.0	7.03 d (2.0)
3"	C	149.7	
4"	C	146.8	
5"	CH	116.4	6.77 d (8.2)
6"	CH	123.2	6.89 dd (8.2, 2.0)
α'	CH	114.9	6.29 d (15.9)
β'	CH	147.3	7.56 d (15.9)
C=O	C	169.2	

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.

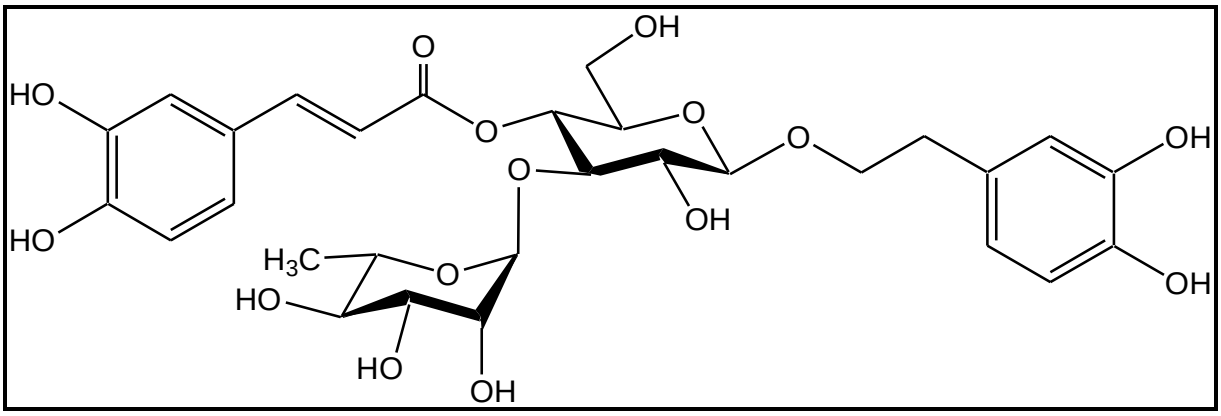




Spectrum 28. ^{1}H-NMR spectrum of Calceolarioside B (CD_3OD, 400 MHz)



Spectrum 29. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum (COSY) of Calceolarioside B



VERBASCOSIDE (=ACTEOSIDE) (10): $C_{29}H_{36}O_{15}$ (MW: 624.59)

UV λ_{max} (MeOH) nm: 204, 219, 247 (sh), 292, 332

IR ν_{max} (KBr) cm^{-1}: 3413 (OH), 2975 (aliphatic CH), 1693 (conjugated ester C=O), 1631 (conjugated C=C), 1525 (aromatic ring)

FAB-Mass *m/z*: 647 $[M+Na]^+$

^{1}H NMR: Table 105, Spectrum 30

^{13}C NMR: Table 105

VERBASCOSIDE (=ACTEOSIDE) (10)

Compound **10** was obtained as white amorphous powder. Molecular weight was calculated as 624 based on the pseudomolecular ion peak at m/z 647 $[M+Na]^+$ in FAB-MS spectrum with 11 unsaturation degrees. The IR spectrum revealed bands at 3413 cm^{-1} (OH), 2975 cm^{-1} (aliphatic CH), 1693 cm^{-1} (conjugated ester C=O), 1631 cm^{-1} (conjugated C=C), 1525 cm^{-1} (aromatic ring). Absorption bands at 204, 219, 247 (sh), 292, 332 showed that the compound was in polyphenolic nature and the spectra was in agreement with those phenylethanoid structure. In conjunction with the analysis of 1D and 2D NMR data, its molecular formula was deduced to be $C_{29}H_{36}O_{15}$.

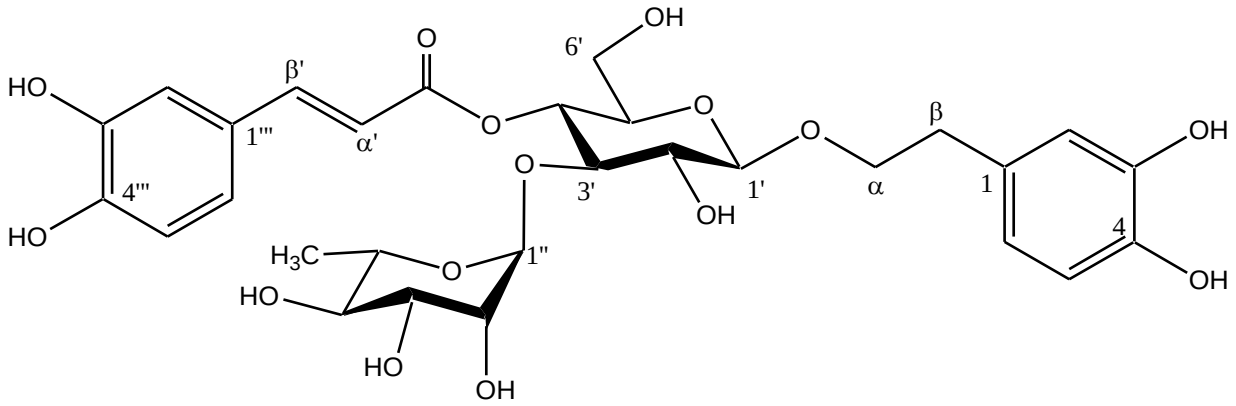
In ^{1}H NMR spectrum (Table 105, Spectrum 30) of compund **10**, in aromatic region (7.05-6.57 ppm) proton resonances of two ABX system of a 3,4 disubstituted aromatic ring were seen indicating two 3,4 disubstituted benzene rings. AX system signals indicating the acyl group of the molecule were seen at δ_H 7.59 (H-β') and δ_H 6.27 (H-α') with the *J* value of 15.9 Hz. At the side chain of the aglycone (3,4 disubstituted benzene ring) two non-equivalent oxymethylene signals δ_H 4.04 (m) and δ_H 3.72 (m) and olefinic methylene signal at δ_H 2.80 (t, *J*=7.5 Hz, 2H) were seen. All the findings up to now indicated the similarity of compound **10** with compound **9**. Distinctly from the compound **9**, two anometic proton signals revealed that the compound was diglycosidic. The *J* value (7.9 Hz) of the anomeric proton signal at 4.38 ppm (H-1') declared that one of the sugar moiety was β-D-glucopyranose, another anomeric proton signal at 5.19 ppm (*J*=1.8 Hz, H-1") and secondary methyl resonance at 1.09 ppm (d, *J*=6.2 Hz, 3H) indicated the other sugar molecule was α-rhamnose. Esterification effect seen on the H-4' proton signal of the glucose moiety (δ_H 4.93, t, *J*= 9.0 Hz), revealed that *trans*-caffeic acid was esterified at C-4'(OH) of the glucose. No chemical shifts were seen at the signals of the rhamnose molecule indicating that the rhamnose molecule was the terminal sugar. Glucosidation effect seen on the C-3' of the glucose (81.6 ppm) showed that rhamnose molecule was linked at the C-3' (OH) of the glucose. Chemical shift of the α-carbon atom (δ_C 72.3) indicated β-glucose was connected at this point. All the spectral data (1D and 2D) were compared and the structure of the compound **10** was found as 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-

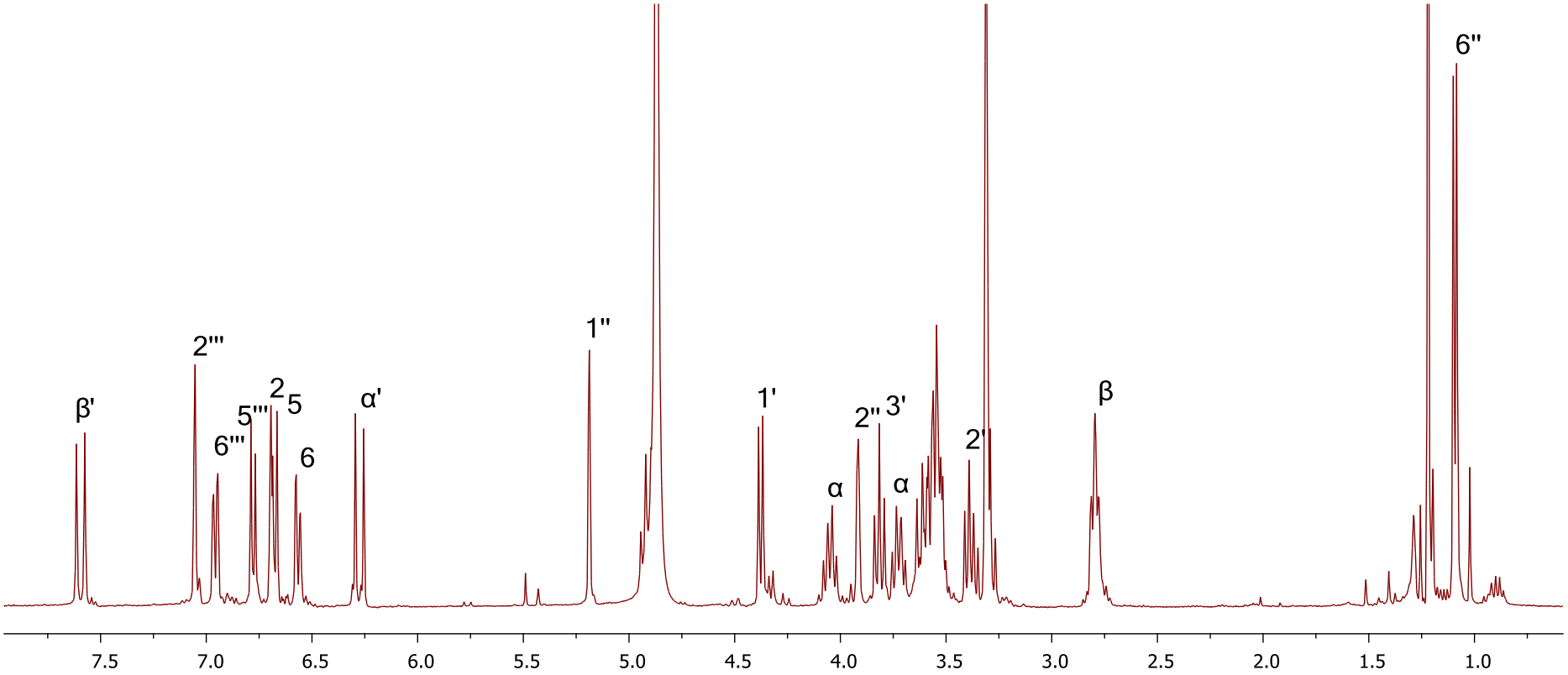
β-D-glucopyranoside. These data were compared with literature and the compound was assigned as **verbascoside(=acteoside)** (229).

Table 105. ^{13}C and ^{1}H NMR Spectral Data of Verbascoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
1	C	131.4	
2	CH	117.1	6.70 d (2.0)
3	C	146.1	
4	C	144.7	
5	CH	116.3	6.68 d (8.0)
6	CH	121.2	6.57 dd (2.0, 8.0)
α	CH_2	72.3	4.05 m 3.72 m
β	CH_2	36.6	2.80 t (7.5)
Glucose			
1'	CH	104.2	4.38 d (7.9)
2'	CH	76.2	3.39 dd (7.9, 9.0)
3'	CH	81.6	3.82 t (9.0)
4'	CH	70.4	4.93 t (9.0)
5'	CH	76.0	3.50-3.65 ł
6'	CH_2	62.3	3.50-3.65 ł
Rhamnose			
1"	CH	103.0	5.19 d (1.8)
2"	CH	72.3	3.92 dd (1.8, 3.1)
3"	CH	72.0	3.58 dd (9.5, 3.1)
4"	CH	73.8	3.39 t (9.5)
5"	CH	70.5	3.50-3.65 m ł
6"	CH_3	18.5	1.09 d (6.2)
Caffeic Acid			
1"'	C	127.6	
2"'	CH	115.2	7.05 d (2.0)
3"'	C	146.8	
4"'	C	149.8	
5"'	CH	116.5	6.78 d (8.2)
6"'	CH	123.2	6.96 dd (8.2, 2.0)
α'	CH	114.7	6.27 d (15.9)
β'	CH	148.0	7.59 d (15.9)
C=O	C	168.3	

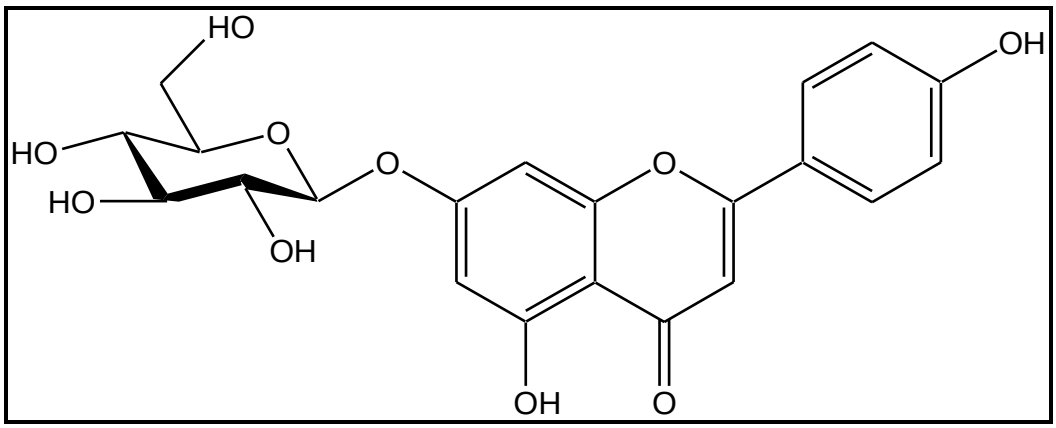
[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.
ł Overlapping signals.





Spectrum 30. ^{1}H-NMR spectrum of Verbascoside (CD_3OD, 400 MHz)

4.2.4.3. Flavonoids



APIGENIN 7-*O*-β-D-GLUCOPYRANOSIDE (11): $C_{21}H_{20}O_{10}$ (432.38)

UV λ_{max} (MeOH) nm: 269, 342

IR ν_{max} (KBr) cm^{-1}: 3367 (OH), 1655 (γ-pyrone C=O), 1606, 1598 (aromatic ring), 1073 (C-O-C)

ESI-Mass *m/z*: 433 $[M+H]^+$

^1H NMR: Table 106, Spectrum 31

^{13}C NMR: Table 106

APIGENIN 7-*O*-β-D-GLUCOPYRANOSIDE (11)

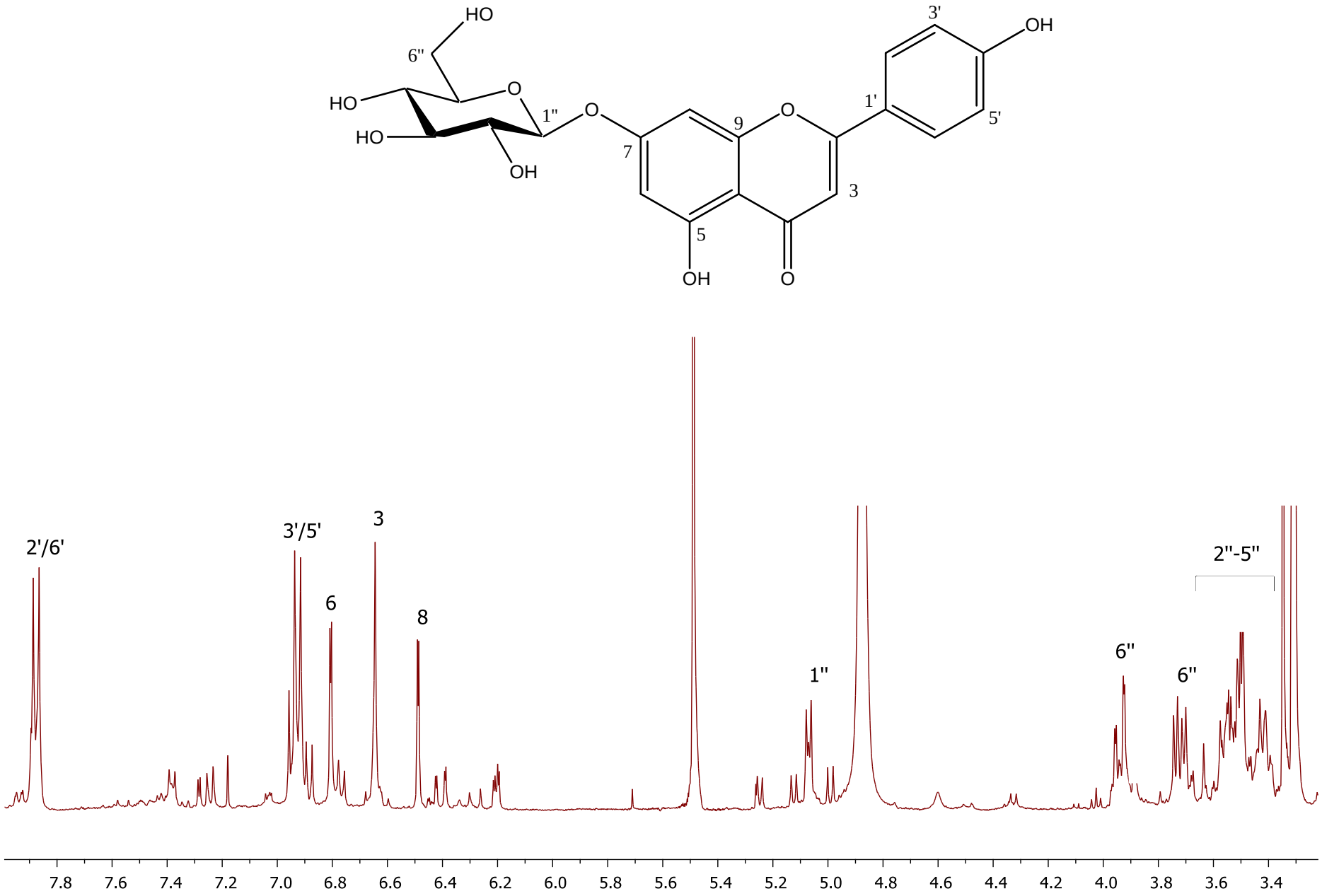
Compound **11** was isolated as yellow amorphous powder from the MeOH extract. The molecular formula was established as $C_{21}H_{20}O_{10}$ based on pseudomolecular ion peak at *m/z* 433 $[M+H]^+$ in its ESI-MS. UV spectra showed characteristic bands (λ_{max} 269, 342 nm) of flavonoidal structure. In its IR spectrum, distinctive hydroxyl group (3367 cm^{-1}), γ-pyrone carbonyl (1655 cm^{-1}), signals of aromatic ring (1606, 1598 cm^{-1}) and C-O-C bands (1073 cm^{-1}) were seen.

In ^{1}H NMR spectrum (Table 106, Spectrum 31), signals of an AABB system at δ_H 7.88 (d, *J*=8.8, H-2′/6′) and 6.93 (d, *J*=8.8, H-3′/5′) elicited the presence of *para*-substitution on B ring of a flavonoid. Moreover, aromatic proton signals of two *m*-coupled doublets at δ_H 6.81 (d, *J*=2.1 Hz) and 6.49 (d, *J*=2.1 Hz) in the ^{1}H NMR spectrum were the indicative of a 5,7-disubstituted A ring. Additionally, a singlet at 6.64 ppm together with the above findings led to the presence of a flavone core of **11**. Moreover, additional resonances arising from β-D-glucopyranose unit were observed. The appearence of an anomeric signal at δ_H 5.07 (d, *J*=7.6 Hz) along with the signals between 3.94-3.25 ppm, indicated its monoglucosidic structure. The large coupling constant of the anomeric proton (*J*=7.6 Hz), clarified its β-configuration. The data were superimposible with those reported for 5,7,3′-trihydroxyflavone-7-*O*-β-D-glucopyranoside namely **apigenin 7-*O*-β-D-glucopyranoside** in the literature (133).

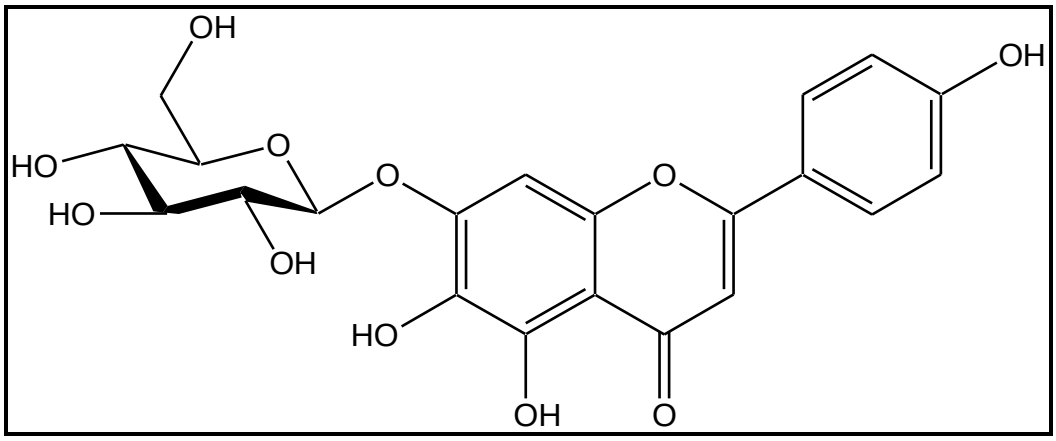
Table 106. ^{13}C and ^{1}H NMR Spectroscopic Data of Apigenin 7-*O*-β-D-glucopyranoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
2	C	164.4	
3	CH	106.6	6.64 s
4	C	180.5	
5	C	166.3	
6	CH	104.8	6.81 d (2.1)
7	C	160.2	
8	CH	99.3	6.49 d (2.1)
9	C	162.7	
10	C	109.4	
1'	C	123.3	
2'	CH	129.5	7.88 d (8.8)
3'	CH	117.1	6.93 d (8.8)
4'	C	162.6	
5'	CH	117.1	6.93 d (8.8)
6'	CH	129.5	7.88 d (8.8)
Glucose			
1"	CH	105.1	5.07 d (7.6)
2"	CH	74.8	3.25-3.50 ł
3"	CH	77.6	3.25-3.50 ł
4"	CH	71.8	3.25-3.50 ł
5"	CH	78.7	3.25-3.50 ł
6"	CH_2	62.6	3.72 dd (12.0, 5.7) 3.94 dd (12.0, 1.9)

[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.
ł Overlapping signals.



Spectrum 31. ^{1}H-NMR spectrum of Apigenin 7-*O*-β-D-glucopyranoside (CD_3OD, 400 MHz)



SCUTELLAREIN 7-*O*-β-D-GLUCOPYRANOSIDE (12): $C_{21}H_{20}O_{11}$ (448.38)

UV λ_{max} (MeOH) nm: 215, 230 (sh), 284, 334

IR ν_{max} (KBr) cm^{-1}: 3359 (OH), 1664 (conjugated C=O), 1075 (C-O-C)

ESI-Mass *m/z*: 449 $[M+H]^+$

^1H NMR: Table 107, Spectrum 32

^{13}C NMR: Table 107

HSQC: Spectrum 33

SCUTELLAREIN 7-*O*-β-D-GLUCOPYRANOSIDE (12)

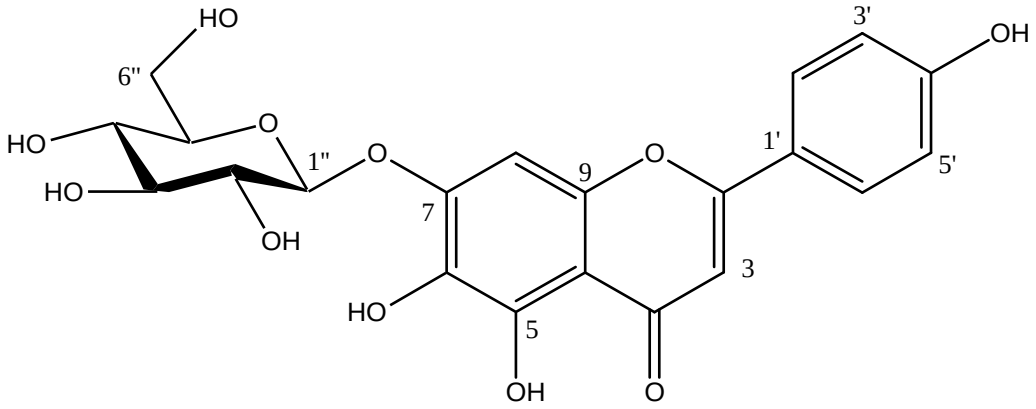
Compound **12** was obtained as yellow amorphous powder, showing the $[M+H]^+$ ion peak at m/*z* 449 in the positive ion ESI mass spectrum, indicating the molecular formula $C_{21}H_{20}O_{11}$. The absorption bands at 215, 230 (sh), 284 and 336 were the characteristics of a flavonoid backbone. The IR spectrum displayed bands at 3359 (OH), 1664 (conjugated C=O) and 1075 (C-O-C).

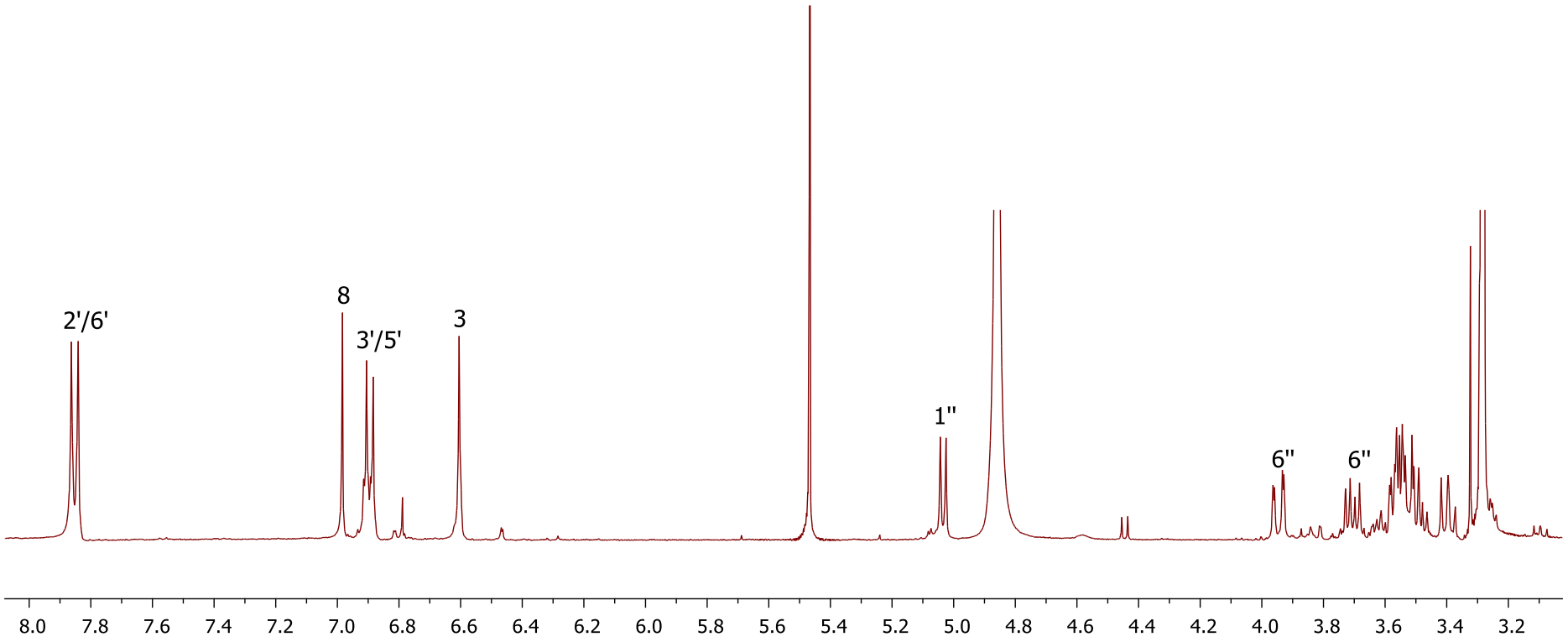
Inspection of the ^{1}H NMR spectrum (Table 107, Spectrum 32) revealed proton resonances of the AABB system of a *para*-substituted benzene ring B at δ_H 6.89 (d, *J*=8.8 Hz, H-3′/5′) and 7.85 (d, *J*=8.8 Hz, H-2′/6′). Two singlets at δ_H 6.98 (H-8) and 6.60 (H-3), together with the quaternary carbon resonance appeared at δ_C 130.6 (C-6) in the ^{13}C spectrum were consistent with the 6-hydroxyflavone skeleton as aglycone **scutellarein** (230). The ^{13}C NMR signal at δ_C 182.6 was assigned to be the carbon atom of the carbonyl function at the C-4 position while resonance at δ_C 149.2 was ascribed to C-7. Moreover, the anomeric signal at δ_H 5.03 (d, *J*=7.4 Hz) along with the other signals between 3.95-3.45 ppm and the corresponding carbon resonances secured by HSQC (Spectrum 33) led to the identification of β-D-glucopyranose unit. With the help of HMBC spectrum, it was found that β-D-glucopyranosyl unit was linked to flavone moiety at C-7. The structure of compound **12** was assigned as 5,6,7,3′-tetrahydroxyflavone-7-*O*-β-D-glucopyranoside. These data were compared with the literature and superimposed with those of **scutellarein 7-*O*-β-D-glucopyranoside** (230).

Table 107. ^{13}C and ^{1}H NMR Spectroscopic Data of Scutellarein 7-*O*-β-D-glucopyranoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

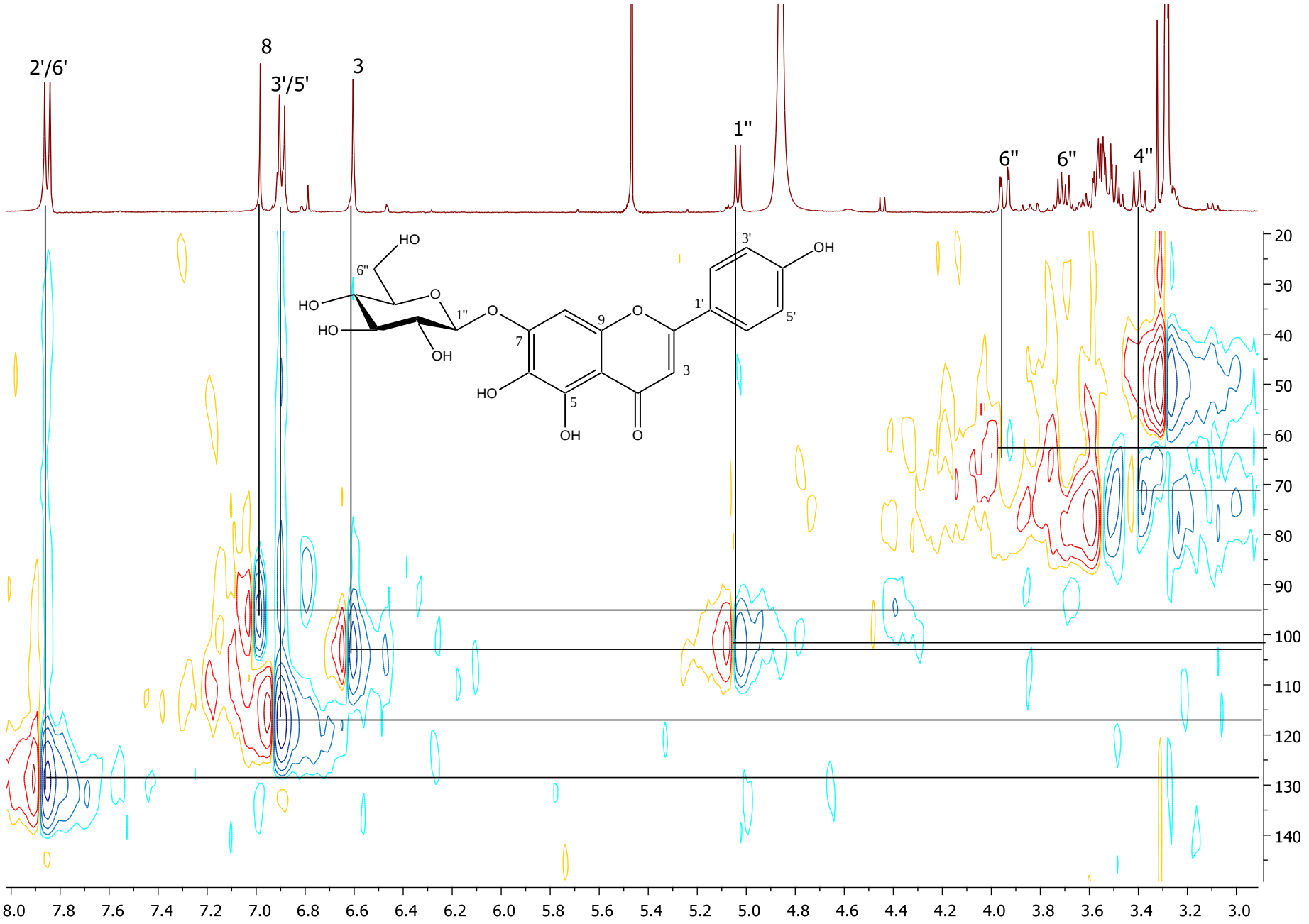
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
2	C	164.3	
3	CH	102.6	6.60 s
4	C	182.6	
5	C	146.8	
6	C	130.6	
7	C	149.2	
8	CH	94.3	6.98 s
9	C	151.5	
10	C	106	
1'	C	121.5	
2'	CH	128.5	7.85 d (8.8)
3'	CH	116.1	6.89 d (8.8)
4'	C	161.4	
5'	CH	116.1	6.89 d (8.8)
6'	CH	128.5	7.85 d (8.8)
Glucose			
1"	CH	101.2	5.03 d (7.4)
2"	CH	73.3	3.45-3.61 ł
3"	CH	76.0	3.45-3.61 ł
4"	CH	72.8	3.38 t (9.0)
5"	CH	77.4	3.45-3.61 ł
6"	CH_2	61.6	3.71 (12.1, 6.1) 3.95 dd (12.1, 2.1)

[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.

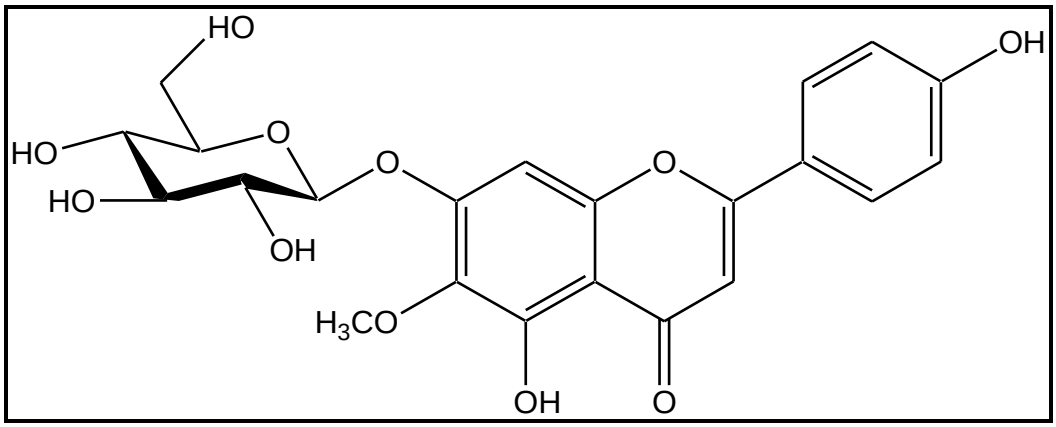




Spectrum 32. ^{1}H-NMR spectrum of Scutellarein 7-*O*-β-D-glucopyranoside (CD_3OD, 400 MHz)



Spectrum 33. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Scutellarein 7-*O*-β-D-glucopyranoside (HSQC)



HISPIDULIN 7-*O*-β-D-GLUCOPYRANOSIDE (13): $C_{22}H_{22}O_{11}$ (462.40)

UV λ_{max} (MeOH) nm: 218, 273, 331

IR ν_{max} (KBr) cm^{-1}: 3393 (OH), 1660 (γ-pyrone C=O) 1073 (C-O-C).

ESI-Mass *m/z*: 463 $[M+H]^+$

^{1}H NMR: Table 108, Spectrum 34

^{13}C NMR: Table 108

HSQC: Spectrum 35

HMBC: Spectrum 36

HISPIDULIN 7-*O*-β-D-GLUCOPYRANOSIDE (13)

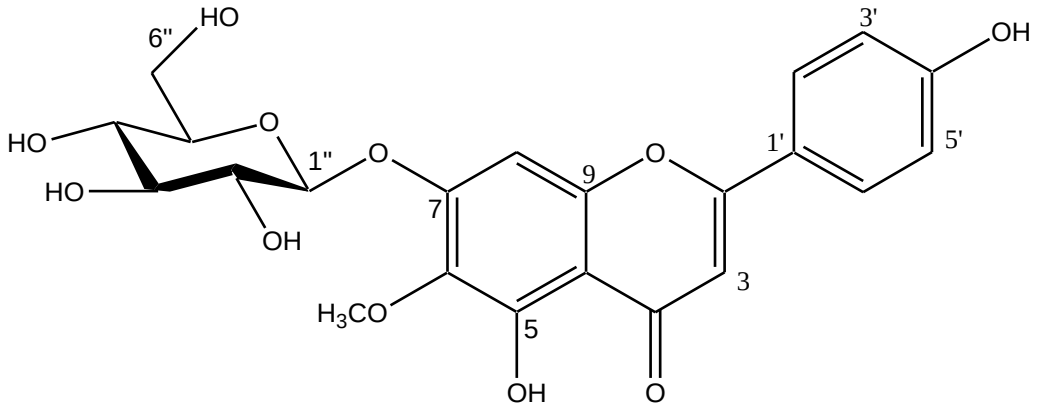
Compound **13**, isolated as pale yellowish powder, showed an $[M+H]^+$ peak at *m/z* 463 corresponding to the molecular formula $C_{22}H_{22}O_{11}$. The UV absorption maxima of **13** in MeOH at 273 nm (band II) and 331 nm (band I) were typical of flavonoids. Broad absorption band at 3393 cm^{-1} in the IR spectrum of **13** was attributed to the hydroxyl groups while a band at 1660 cm^{-1} was assigned to the α,β-unsaturated ketone carbonyl functional group.

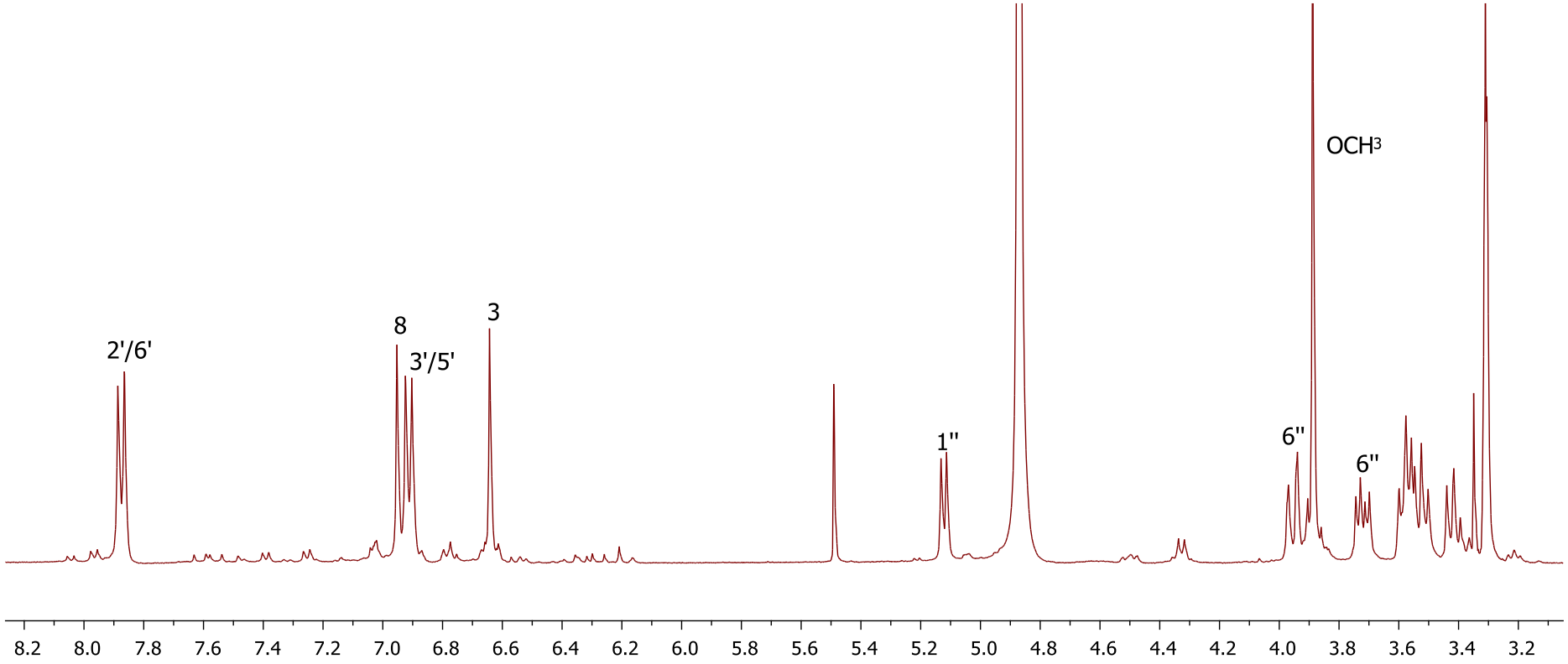
In ^1H NMR spectrum (Table 108, Spectrum 34), two *ortho* coupled doublets (*J*=8.7 Hz, 2H) at δ_H 7.88 and 6.91 integrating for two protons each, were attributed to H-2'/6' and H-3'/5' respectively of a *para*-disubstituted ring B. Two singlets at δ_H 6.95 and 6.64 were ascribed to H-3 and H-8 respectively with the help of HSQC (Spectrum 35) and HMBC (Spectrum 36) spectra. All the information together corresponding 6-*O*-substituted flavone ring is assigned as **scutellarein** (230). Moreover the proton signal at δ_H 5.12 with a relatively large coupling constant (*J*=7.3 Hz) along with the other signals between 4.00-3.30 ppm led to the presence of a β-D-glucopyranose unit. The cross-peak between C-7 and C-1'' elicited that the sugar unit was bonded to the flavone moiety at C-7. These data were very close to those of compound **13**, except for the sharp proton singlet at δ_H 3.89 with the three proton intensity, showing $^3J_{1''\text{-}2''}$ correlation with this carbon at δ_C 134.6 (C-6) in its HMBC spectrum, exhibiting **13** bore a methoxy group. All the findings indicated that compound **13** was 5,7,4'-trihydroxy-6-methoxyflavone-7-*O*-β-D-glucopyranoside which is known as **hispidulin 7-*O*-β-D-glucopyranoside** (231).

Table 108. ^{13}C and ^{1}H NMR Spectroscopic Data of Hispidulin 7-*O*-β-D-glucopyranoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

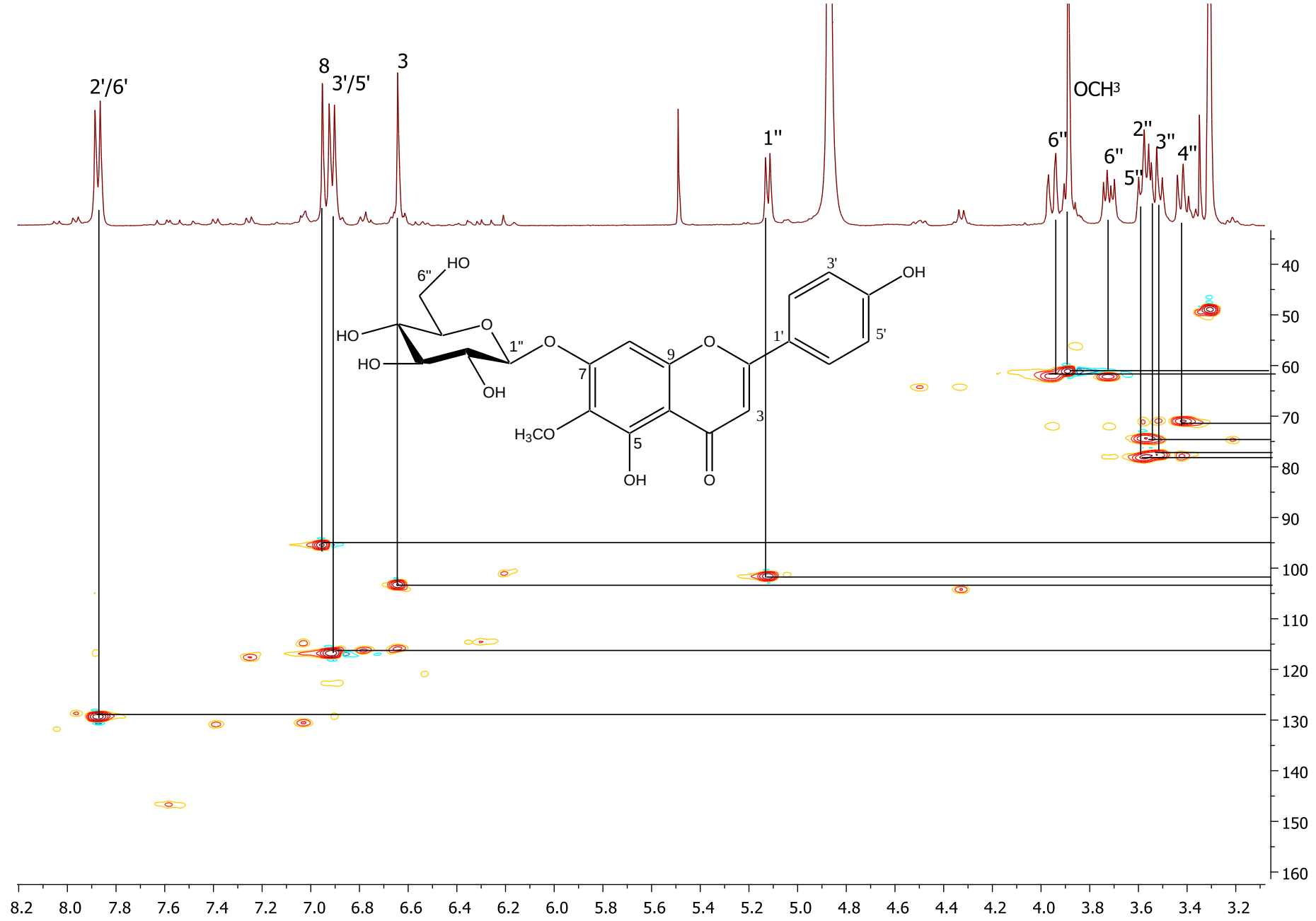
C/H Atom	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
2	C	166.1	
3	CH	102.7	6.64 s
4	C	184.9	
5	C	166.9	
6	C	134.6	
7	C	157.9	
8	CH	95.6	6.96 s
9	C	154.8	
10	C	106.9	
6-OCH_3		60.8	3.89 s
1'	C	121.9	
2'	CH	129.2	7.88 d (8.7)
3'	CH	116.9	6.91 d (8.7)
4'	C	162.8	
5'	CH	116.9	6.91 d (8.7)
6'	CH	129.2	7.88 d (8.7)
Glucose			
1"	CH	101.7	5.12 d (7.3)
2"	CH	74.4	3.56 ł
3"	CH	77.8	3.52 ł
4"	CH	71.0	3.41 t (9.1)
5"	CH	78.3	3.58 ł
6"	CH_2	62.0	3.72 dd (12.1, 6.0) 3.91 dd (12.1, 2.1)

[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.
ł Overlapping signals.

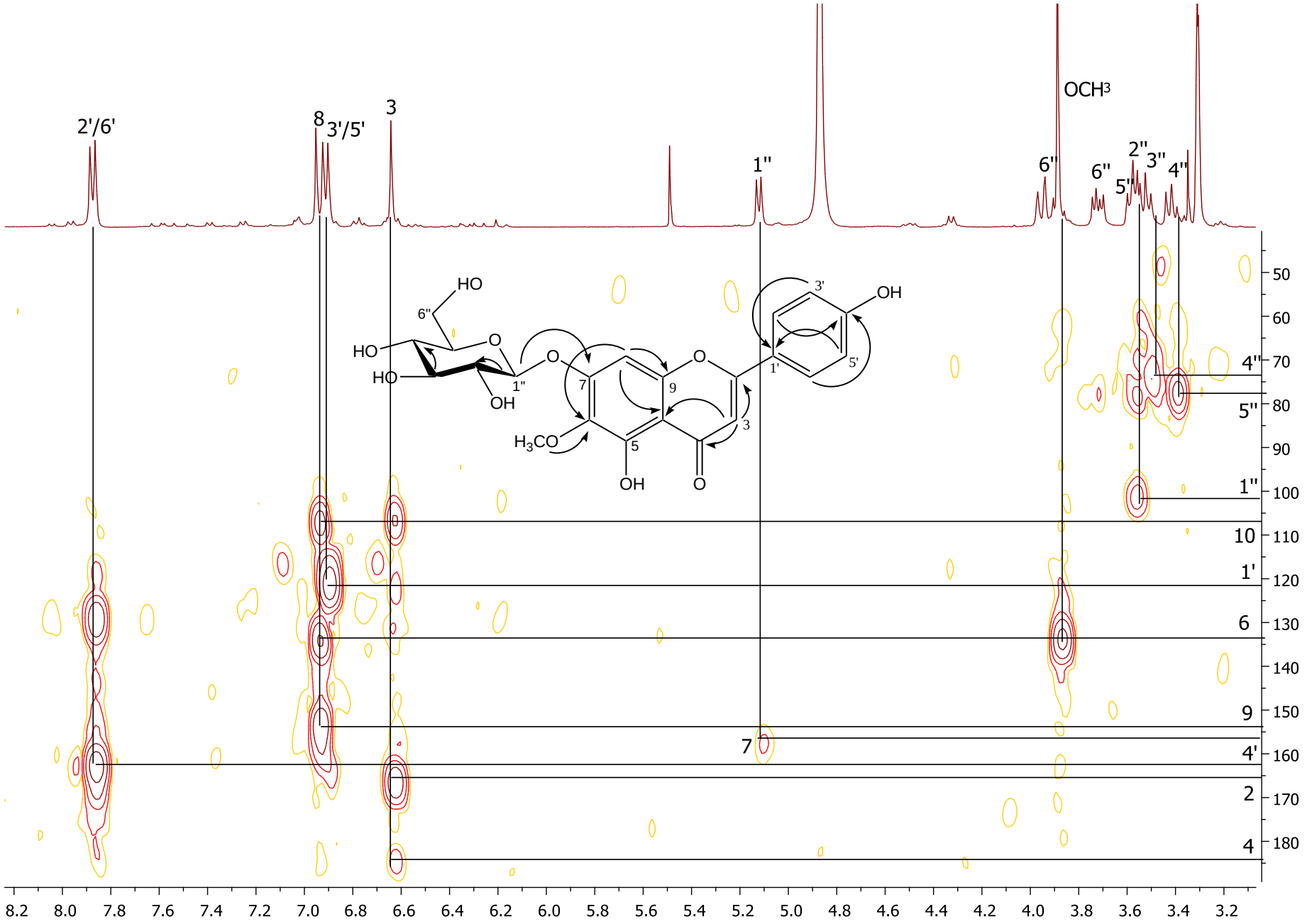




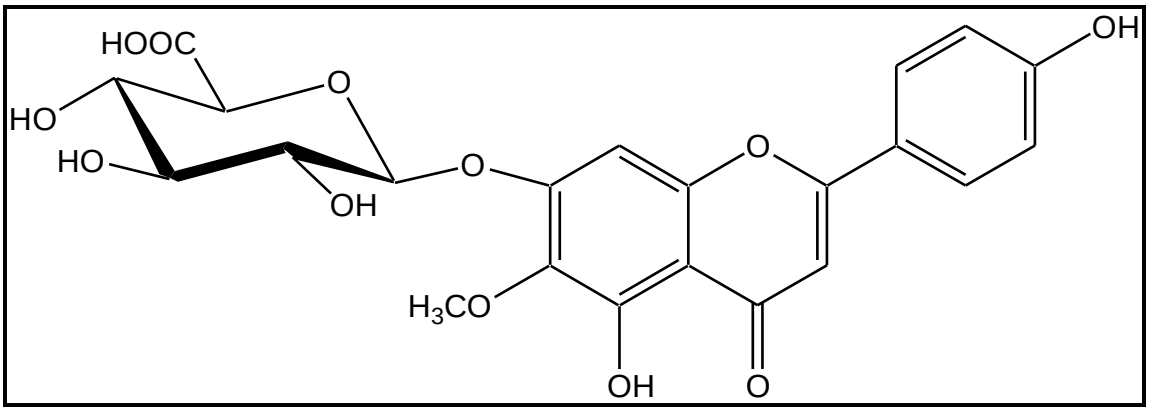
Spectrum 34. ^{1}H-NMR spectrum of Hispidulin 7-*O*-β-D-glucopyranoside (CD_3OD, 400 MHz)



Spectrum 35. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Hispidulin 7-*O*-β-D-glucopyranoside (HSQC)



Spectrum 36. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of Hispidulin 7-*O*-β-D-glucopyranoside (HMBC)



HISPIDULIN 7-*O*-β-D-GLUCURONOPYRANOSIDE (14): $C_{22}H_{20}O_{12}$ (476.39)

UV λ_{max} (MeOH) nm: 212 (sh), 270, 331

IR ν_{max} (KBr) cm^{-1}: 3422 (OH), 1660 (γ-pyrone C=O), 1067 (C-O-C)

ESI-Mass *m/z*: 477 $[M+H]^+$

^1H NMR: Table 109, Spectrum 37

^{13}C NMR: Table 109

HSQC: Spectrum 38

HISPIDULIN 7-*O*-β-D-GLUCURONOPYRANOSIDE (14)

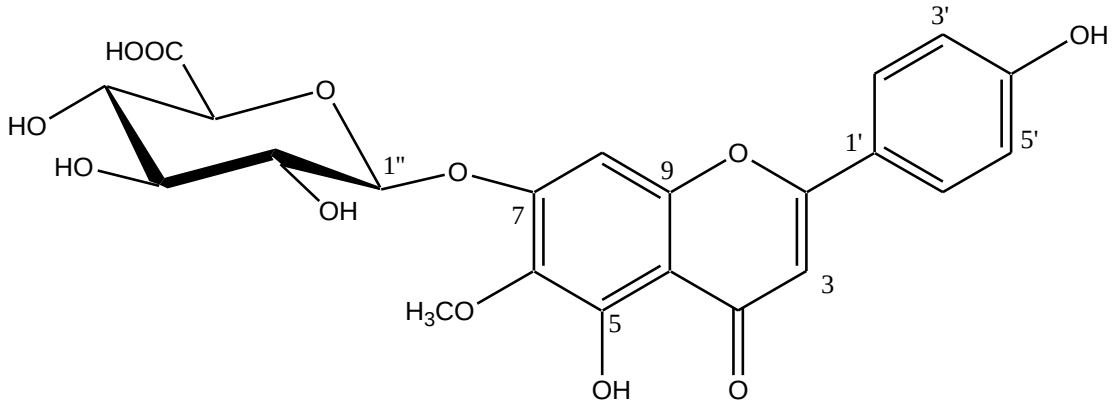
Compound **14,** a pale yellow powder, showed the signal of the $[M+H]^+$ ion at *m/z* 477 in the positive ion ESI-MS, indicating the molecular formula $C_{22}H_{20}O_{12}$ (476). Compound **14** exhibited UV maxima at 270 and 331 nm. The IR spectrum suggested the presence of hydroxyl (3422 cm^{-1}), γ-pyrone C=O (1660 cm^{-1}) and carbon-oxygen bonds (1067 cm^{-1}). All together they are the characteristic bands of a flavone skeleton.

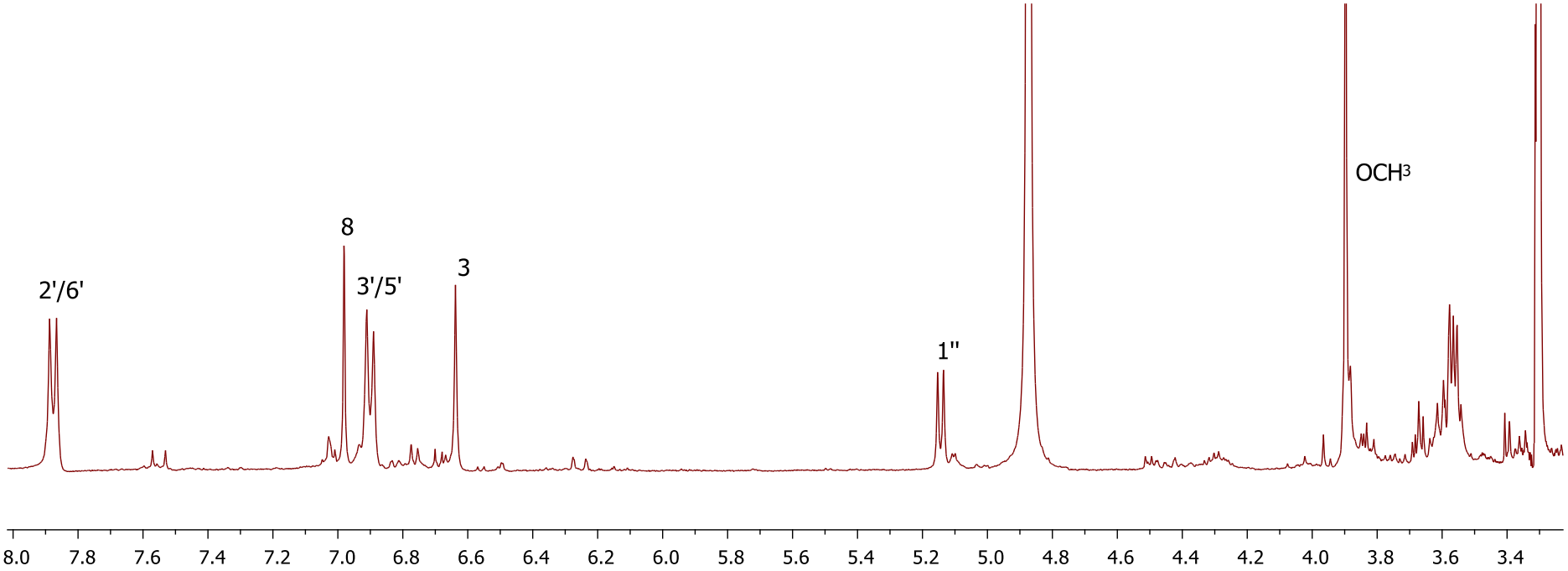
The ^1H NMR (Table 109 and Spectrum 37) and HSQC (Spectrum 38) spectra of compound **14** were similar to that belong to compound **13**. The ^1H NMR spectrum displayed two *ortho* coupled doublets (δ_H 6.90 and 7.87, d, *J*=8.5 Hz, 2H, H-3'/5' and H-2'/6', respectively), two singlet signals (δ_H 6.64 and 6.98, s, 1H, H-3 and H-8, respectively), moreover a methoxy signal at δ_H 3.88 (s, 3H). According to that data the aglycone was elucidated as **hispidulin**. The aromatic proton at 5.13 ppm (d, *J*=7.4 Hz) was the indicative of a sugar molecule. The absence of characteristic primary alcohol methylene signals revealed that the sugar moiety was an uronic acid. The complete 2D NMR analysis revealed the presence of β-D-glucuronic acid. Consequently, the structure of compound **14** was found to be 5,7,4'-trihydrox,6-methoxyflavone-7-*O*-β-D-glucuranopyranoside namely **hispidulin 7-*O*-β-D-glucuronopyranoside** (231).

Table 109. ^{13}C and ^{1}H NMR Spectroscopic Data of Hispidulin 7-*O*-β-D-glucuronopyranoside (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

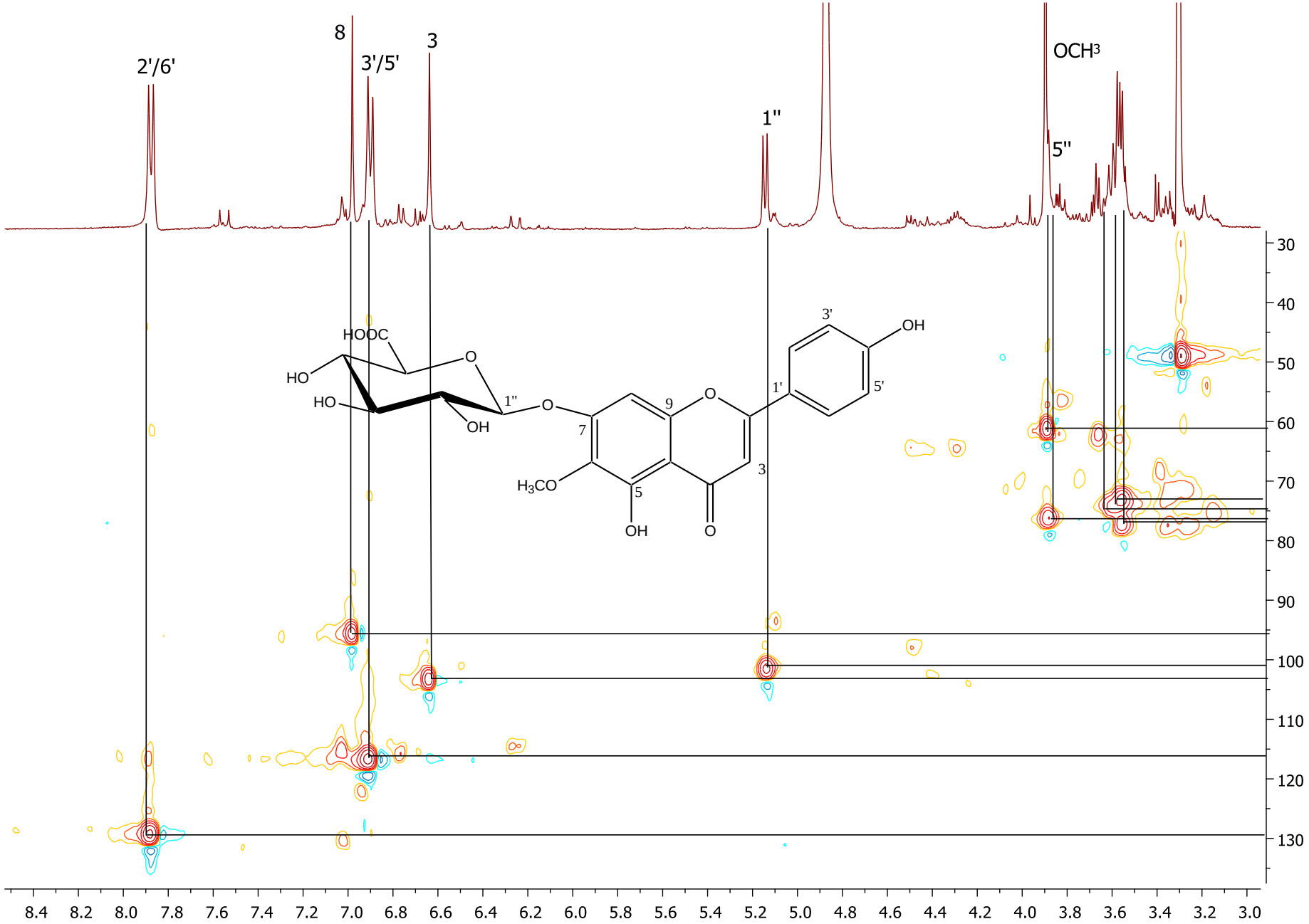
Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Aglycone			
2	C	166.4	
3	CH	103.4	6.64 s
4	C	184.7	
5	C	166.7	
6	C	134.2	
7	C	157.3	
8	CH	95.2	6.98 s
9	C	153.9	
10	C	106.8	
6-OCH_3		60.9	3.88 s
1'	C	121.9	
2'	CH	129.4	7.87 d (8.5)
3'	CH	116.8	6.90 d (8.5)
4'	C	162.1	
5'	CH	116.8	6.90 d (8.5)
6'	CH	129.4	7.87 d (8.5)
Glucuronic acid			
1"	CH	100.9	5.13 d (7.4)
2"	CH	74.4	3.50-3.70 ł
3"	CH	77.1	3.50-3.70 ł
4"	CH	73.0	3.50-3.70 ł
5"	CH	76.9	3.88 ł
6"	C	171.5	

[α] Resonances are interpreted with the help of 2D NMR (HSQC and HMBC) techniques.
ł Overlapping signals.



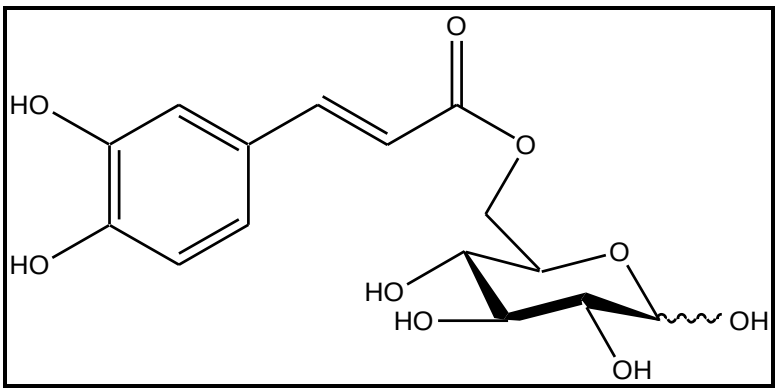


Spectrum 37. ^{1}H-NMR spectrum of Hispidulin 7-*O*-β-D-glucuronopyranoside (CD_3OD, 400 MHz)



Spectrum 38. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of Hispidulin 7-*O*-β-D-glucuronopyranoside (HSQC)

4.2.4.4. Sugar Ester



6-*O*-CAFFEOYL-α/β-GLUCOPYRANOSE (15): $C_{15}H_{18}O_9$ (342.30)

UV λ_{max} (MeOH) nm: 246, 297 (sh), 330

IR ν_{max} (KBr) cm^{-1}: 3320 (OH), 2925 (aliphatic CH), 1691 (conjugated ester C=O), 1631 (conjugated C=C), 1521 (aromatic ring)

ESI-Mass *m/z*: 343 $[M+H]^+$, 707 $[2M+Na]^+$

^{1}H NMR: Table 110, Spectrum 39

^{13}C NMR: Table 110

HSQC: Spectrum 40

HMBC: Spectrum 41

COSY: Spectrum 42

6-*O*-CAFFEOYL-α/β-GLUCOPYRANOSE (15)

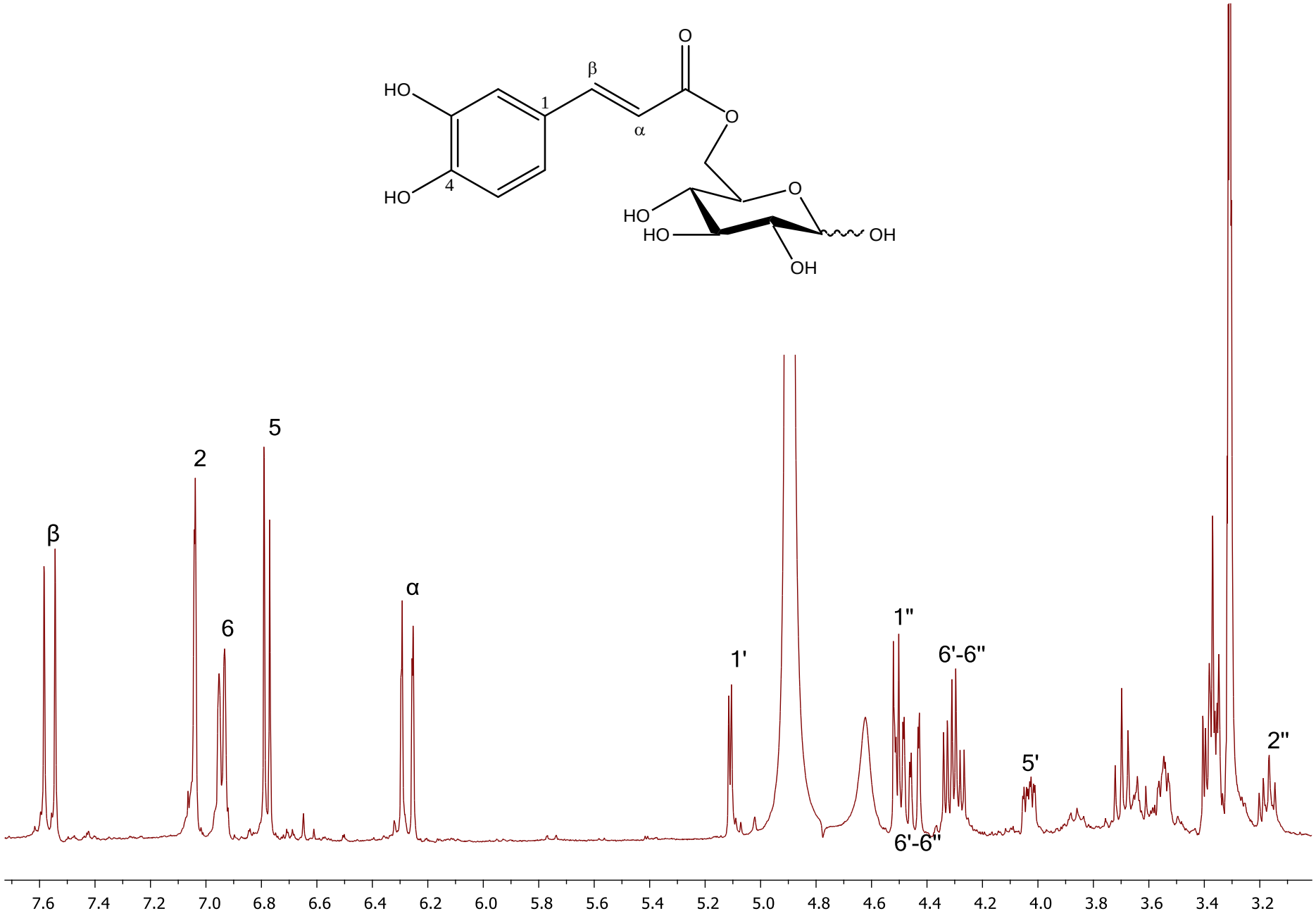
Compound **15** was isolated as as an amorphous powder from MeOH extract of *S. hastifolia* whose molecular $C_{15}H_{18}O_9$ formula was determined on the basis of pseudomolecular ion peaks at *m/z* 343 $[M+H]^+$ and 707 $[2M+Na]^+$ in ESI-MS. The IR spectrum revealed hydroxyl (3320 cm^{-1}), α/β-unsaturated ester (1691, 1631 cm^{-1}) and aromatic absorptions (1600, 1521 cm^{-1}). The UV spectrum in ethanol exhibited absorptions at 330, 297 and 246 nm.

The ^1H NMR (Table 110, Spectrum 39), HSQC, HMBC and COSY spectra (Spectrum 40-42) displayed characteristic signals for the caffeoyl and glucose moieties. The ^1H NMR spectrum displayed resonances at δ_H 7.56 (d, *J*=15.9 Hz, β), 7.04 (d, *J*=1.7 Hz, H-2), 6.94 (d, *J*=7.9 Hz, H-6), 6.78 (d, *J*=7.9 Hz, H-5), 6.27 (dd, *J*=15.9, 1.4 Hz, α), showing the presence of a caffeoyl group in **15**. The proton signal at δ_H 5.11 which was determined to be the anomeric proton of a sugar moiety and the coupling constant revealed the α-configuration (*J*=3.7 Hz). The other protons of the sugar moiety were determined by COSY spectrum and corresponding carbon atoms were determined by HSQC spectrum. The esterification point of caffeoyl moiety was found to be C-6' (OH) of glucose, based on crosspeak between H_2-6 and C=O in the HMBC spectrum. In total 21 carbon signals were counted, including 12 signals in sugar region. A second anomeric proton signal at δ_H 4.51(d, *J*=7.8 Hz) along with the number of carbon atoms in the spectra revelad that **15** was a mixture of 6-*O*-caffeoyl-α and β-glucopyranose. The ratio of intensities of the α and the β anomeric proton signal were compared and the proportions of 6-*O*-caffeoyl-α-glucopyranose to 6-*O*-caffeoyl-β-glucopyranose was found to be 0.45/0.56.

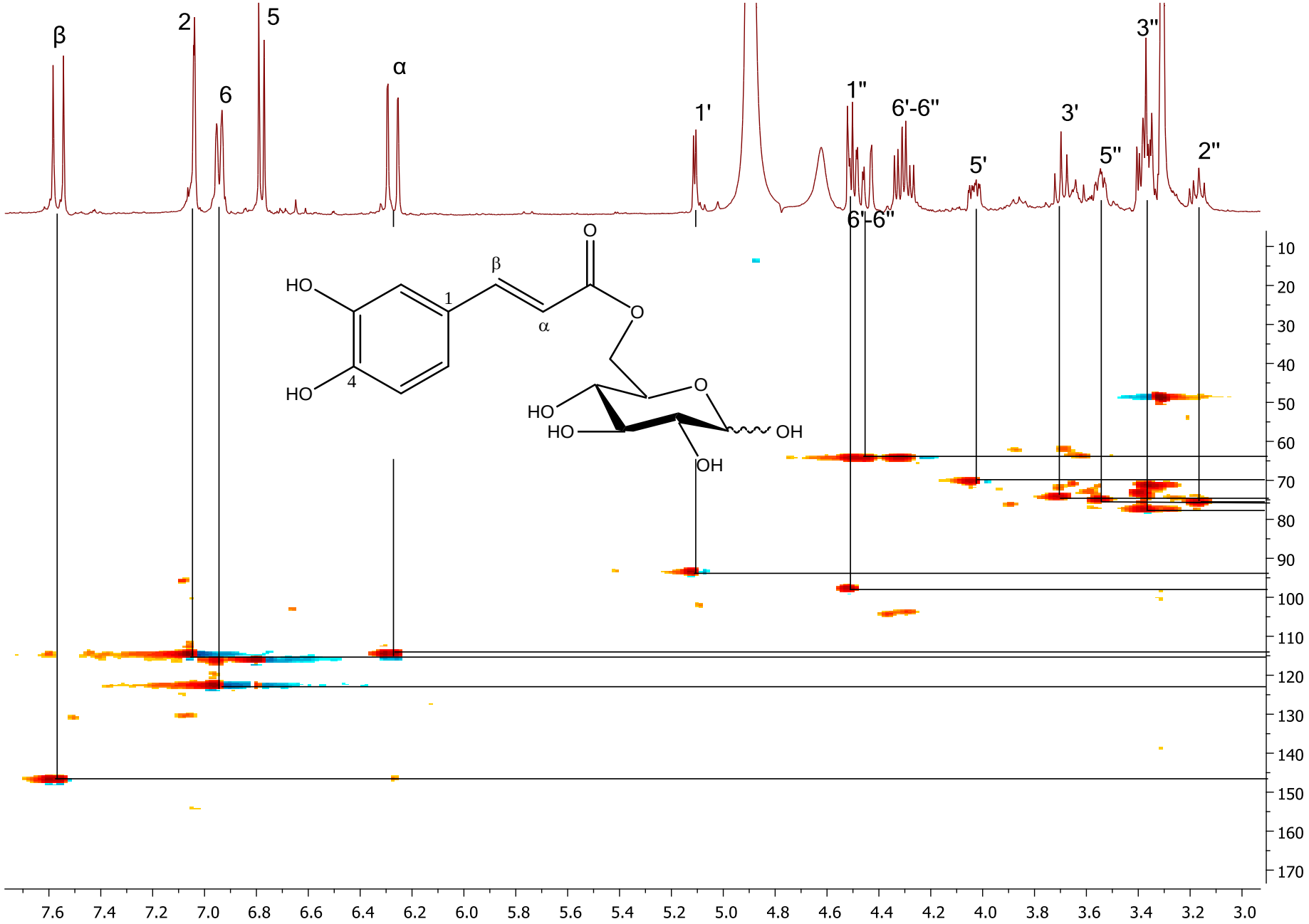
Table 110. ^{13}C and ^{1}H NMR Spectroscopic Data of 6-*O*-caffeoyl-α/β-glucopyranose (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
Caffeic Acid			
1	C	127.2	
2	CH	114.9	7.04 d (1.7)
3	C	149.2	
4	C	146.6	
5	CH	116.3	6.78 d (7.9)
6	CH	122.6	6.94 d (7.9)
α	CH	114.4	6.27 dd (15.9, 1.4)
β	CH	147.2	7.56 d (15.9)
C=O	C	169.1	
α-Glucose			
1'	CH	93.5	5.11 d (3.7)
2'	CH	73.2	3.34-3.42 ł
3'	CH	74.6	3.70 m
4'	CH	70.6	3.34-3.42 ł
5'	CH	70.5	4.03 m
6'	CH_2	64.5	4.44 dd (11.9, 2.0) 4.30 dd (11.9, 5.7)
β-Glucose			
1"	CH	97.8	4.51 d (7.8)
2"	CH	75.4	3.16 t
3"	CH	77.7	3.34-3.42 ł
4"	CH	71.4	3.34-3.42 ł
5"	CH	74.9	3.55 m
6"	CH_2	64.5	4.44 dd (11.9, 2.0) 4.30 dd (11.9, 5.7)

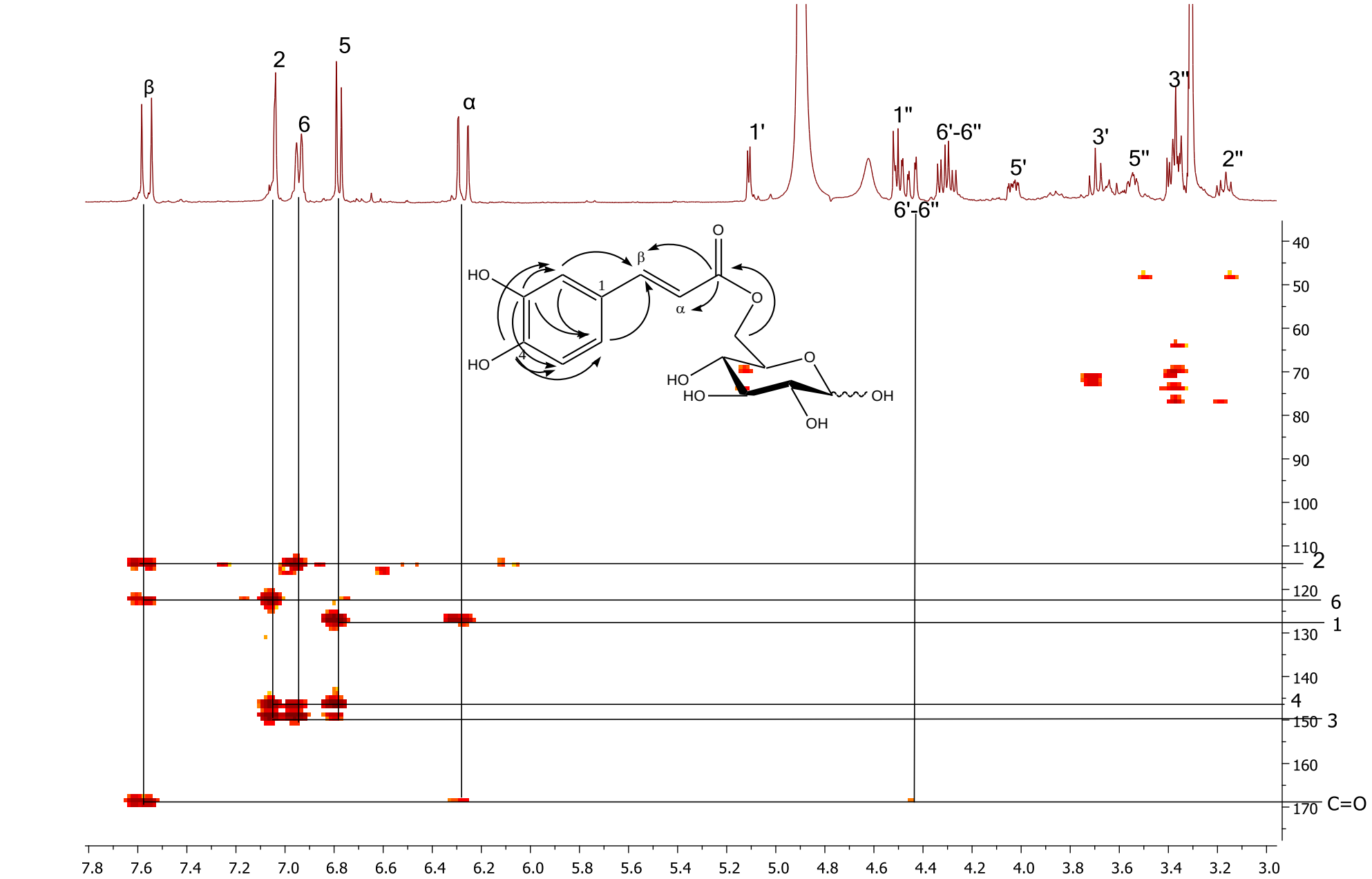
[α] Resonances are interpreted with the help of 2D NMR (COSY, HSQC and HMBC) techniques.
ł Overlapping signals.



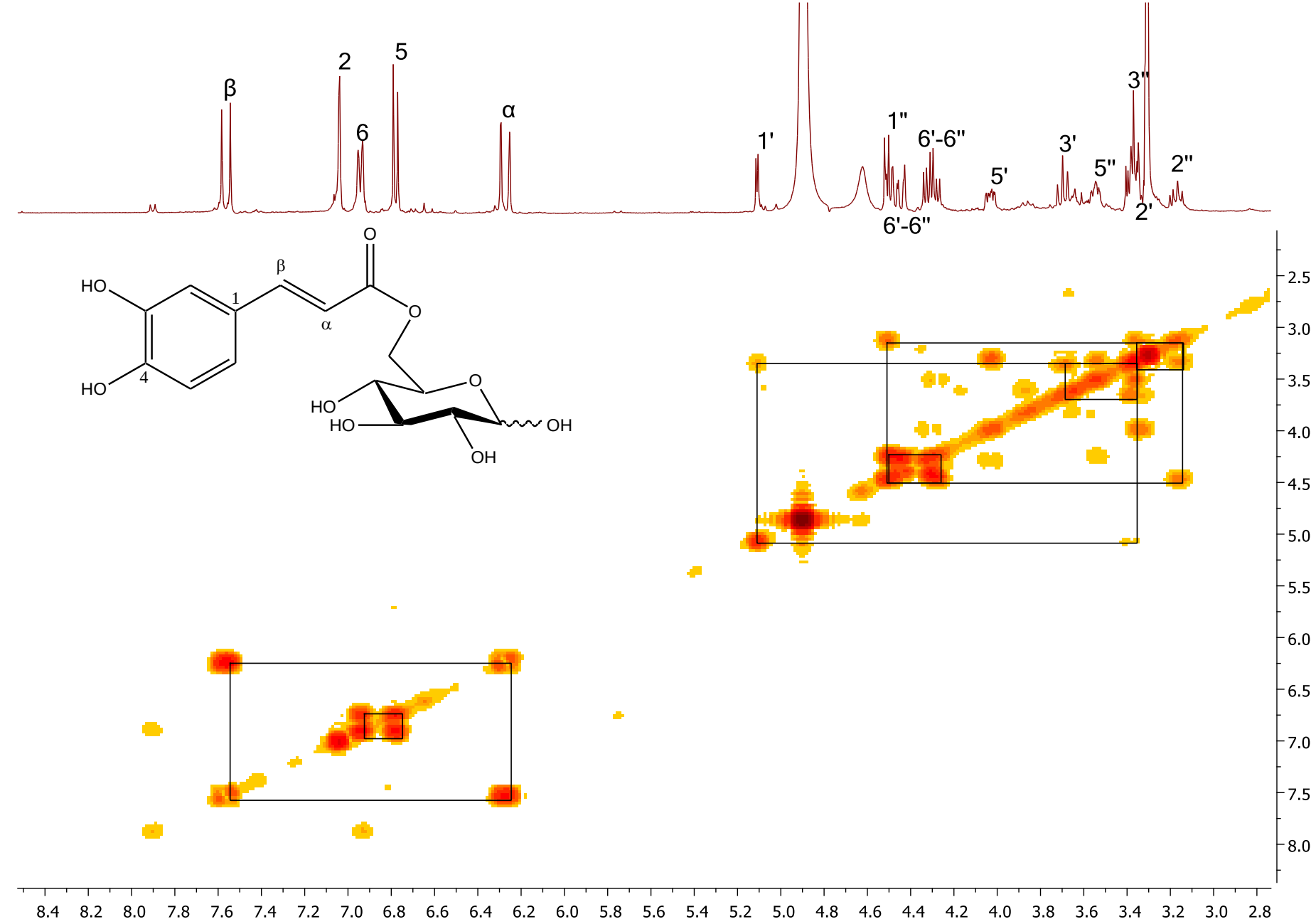
Spectrum 39. ^{1}H-NMR spectrum of 6-*O*-caffeoyl-α/β-glucopyranose (CD_3OD, 400 MHz)



Spectrum 40. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (short range) of 6-*O*-caffeoyl-α/β-glucopyranose (HSQC)

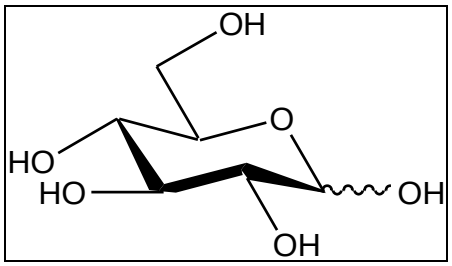


Spectrum 41. Heteronuclear 2D-^{1}H,^{13}C Correlation Spectrum (long range) of 6-*O*-caffeoyl-α/β-glucopyranose (HMBC)



Spectrum 42. 2D-^{1}H,^{1}H-Homonuclear Correlation Spectrum (COSY) of 6-*O*-caffeoyl-α/β-glucopyranose

4.2.4.5. Sugars



α/β-GLUCOSE (16): $C_6H_{12}O_6$ (180.16)

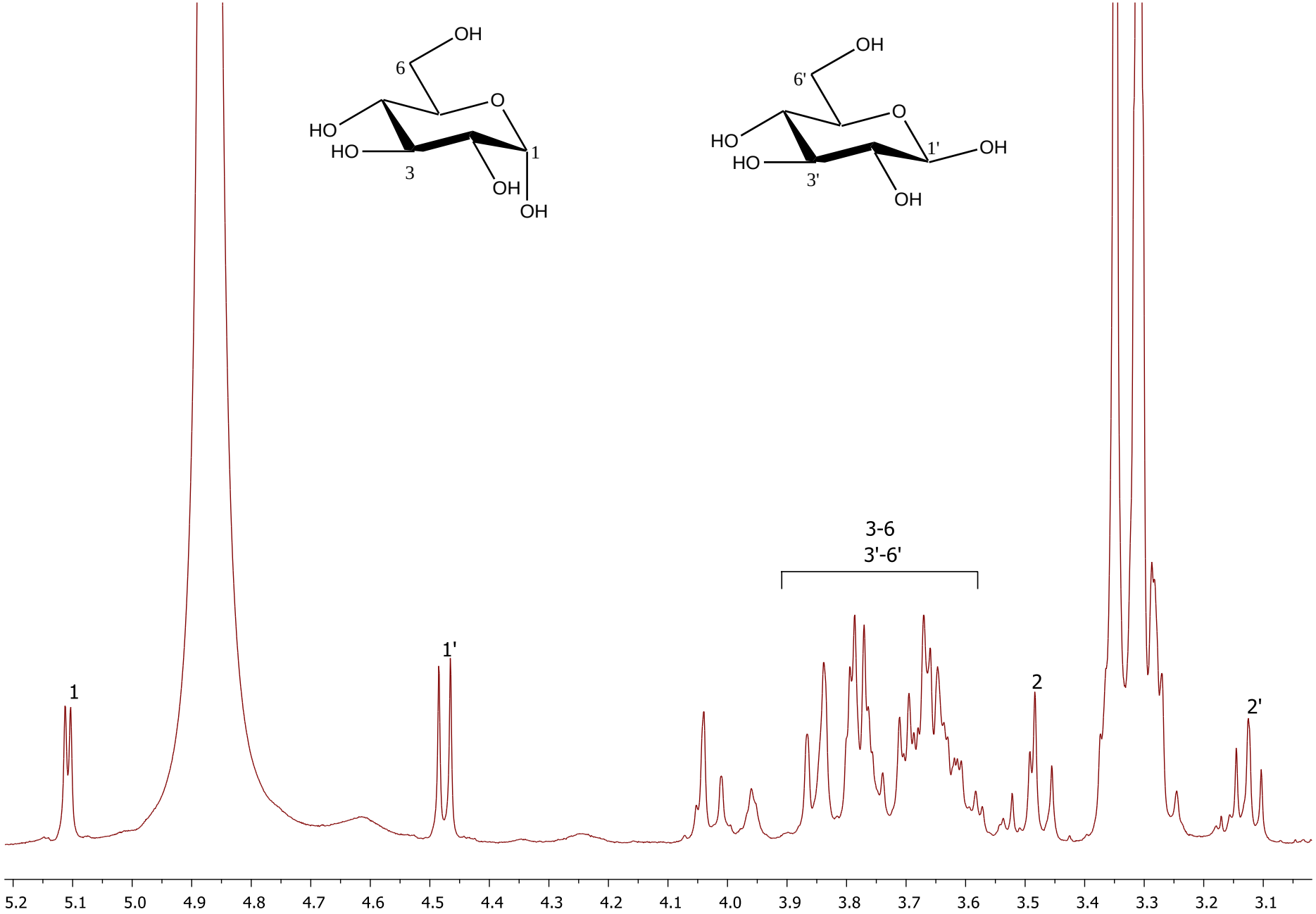
^{1}H NMR: Table 111, Spectrum 43

^{13}C NMR: Table 111

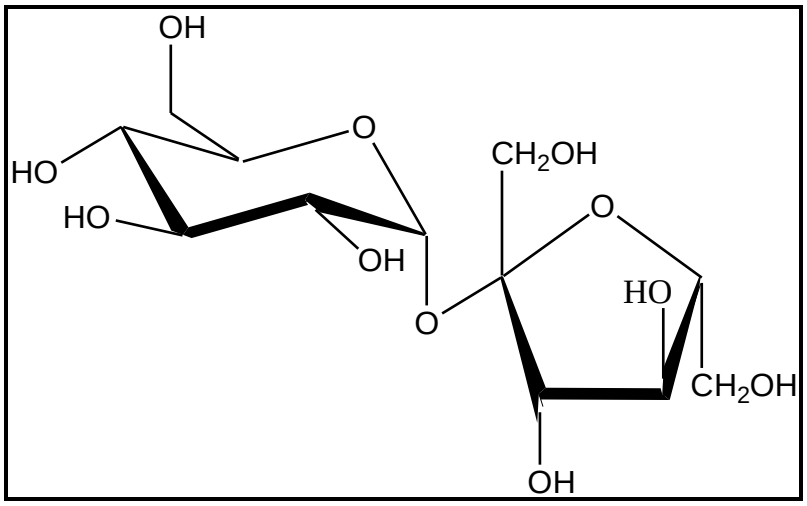
Table 111. ^{13}C and ^{1}H NMR Spectroscopic Data of α/β-Glucose (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
α-Glucose			
1	CH	92.5	5.11 d (3.6)
2	CH	72.6	3.49 dd (3.6, 9.7)
3	CH	73.5	3.65 ł
4	CH	70.5	3.29 ł
5	CH	71.6	3.75 ł
6	CH_2	61.5	3.71 ł 3.65 ł
β-Glucose			
1'	CH	96.9	4.48 d (7.8)
2'	CH	75.0	3.12 dd (7.8, 8.7)
3'	CH	76.7	3.31 ł
4'	CH	70.4	3.30 ł
5'	CH	76.8	3.35 ł
6'	CH_2	61.6	3.71 ł 3.65 ł

[α] Resonances are interpreted with the help of 1D NMR (^{1}H and ^{13}C) techniques.
ł Overlapping signals.



Spectrum 43. ^{1}H-NMR spectrum of α/β-Glucose (CD_3OD, 400 MHz)



SACCHAROSE (17): $C_{12}H_{22}O_{11}$ (342.30)

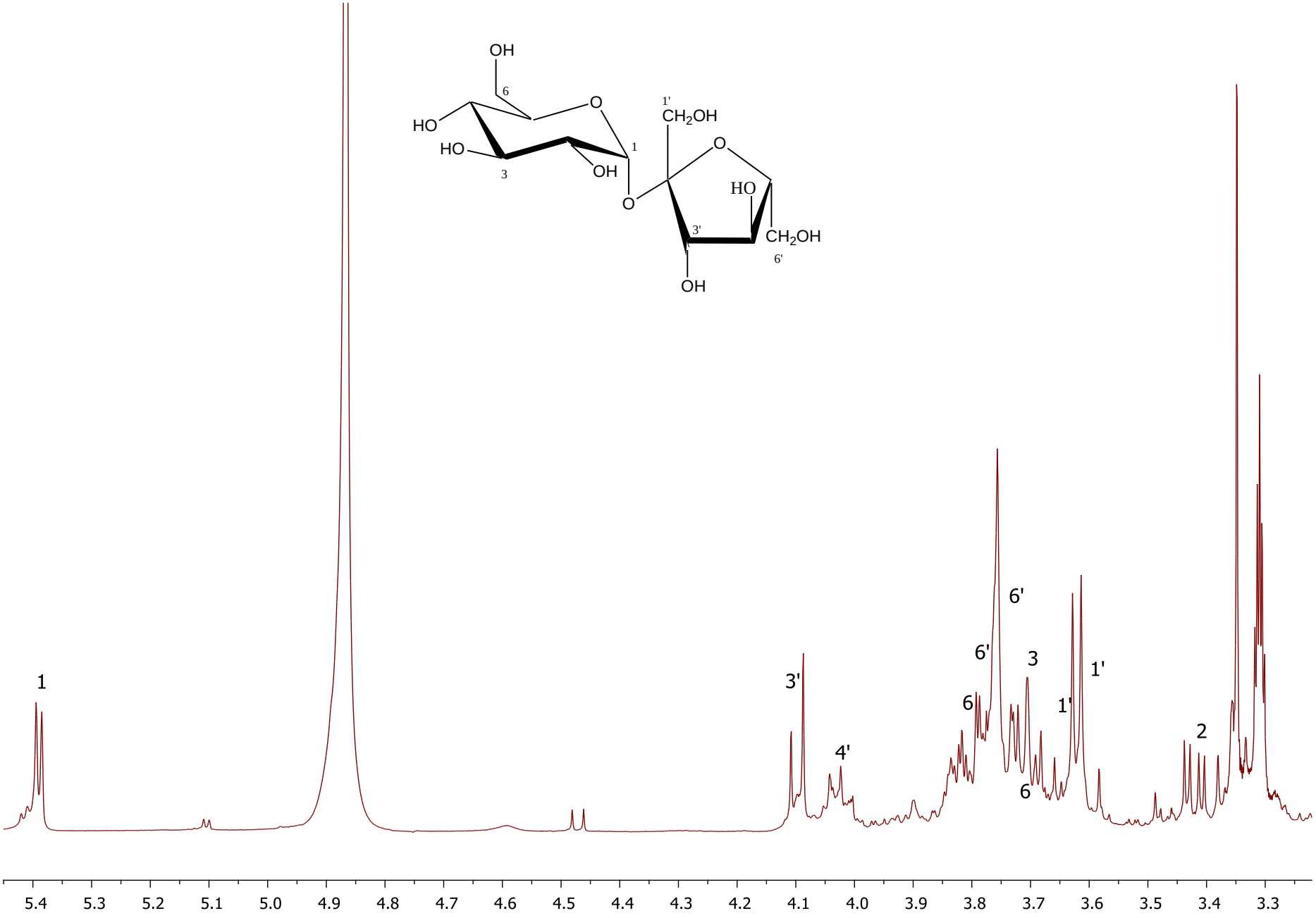
^{1}H NMR: Table 112, Spectrum 44

^{13}C NMR: Table 112

Table 112. ^{13}C and ^{1}H NMR Spectroscopic Data of Saccharose (CD_3OD, ^{1}H: 400 MHz, ^{13}C: 100 MHz)

Position	Multiplicity	δ_C ppm	δ_H ppm, *J* (Hz)
α-Glucose			
1	CH	93.3	5.39 d (3.8)
2	CH	73.2	3.43 dd (3.8,9,8)
3	CH	74.5	3.74 ł
4	CH		3.41 d (3.8)
5	CH		
6	CH_2	62.8	3.80 (11.9, 2.2) 3.70 (11.9, 5.5)
β-Fructose			
1'	CH	64.1	3.64 d (12.3) 3.62 d (12.3)
2'	C	105.2	
3'	CH	79.2	4.10 d (8.3)
4'	CH	76.0	4.03 dd (8.3, 7.5)
5'	CH	84.0	3.79 ł
6'	CH_2	64.3	3.80 ł 3.74 ł

[α] Resonances are interpreted with the help of 1D NMR (^{1}H and ^{13}C) techniques.
ł Overlapping signals.

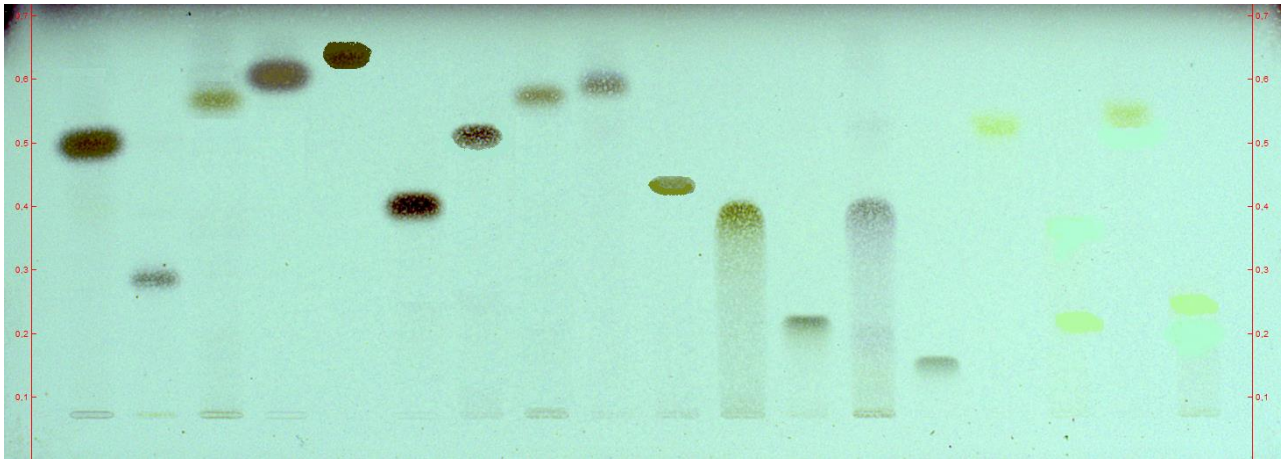


Spectrum 44 . ^{1}H-NMR spectrum of Saccharose (CD_3OD, 400 MHz)

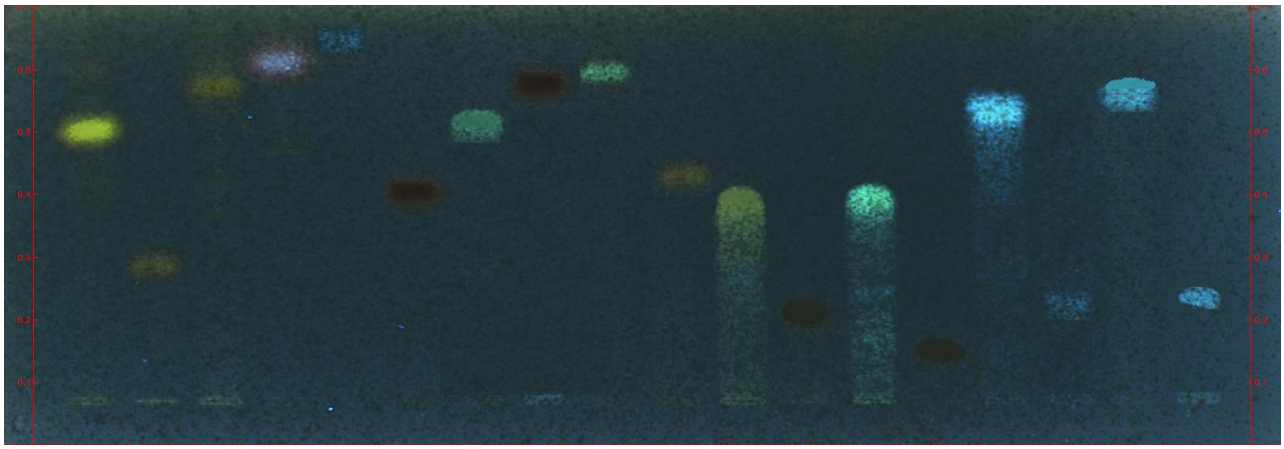
4.2.5. TLC Chromatograms of Isolated Compounds

Isolated compounds were applied on SiO_2 aluminium plates with the developing solvent which is a mixture of $CHCl_3$:MeOH:H_2O in the proportions of 80:20:2. The plates were photographed on white light and 366 nm after derivatization at 366 nm. R*f* values were written on both sides of the plates. Isolated compounds were applied in the sequence of isorengyol, isorengyoside, SH-57, cleroindicin B, cleroindicin F, cornoside, calceolarioside D, neocalceolarioside D, hastifolioside, calceolarioside B, verbascoside, α/β glucose, 6-*O*-caffeoyl-α/β glucopyranose, saccharose, apigenin 7-*O*-β-D-glucopyranoside, scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside.

White Light



366 nm



4.3. Analytical Results

In order to quantify the common and isolated phenylethanoid glycosides and flavonoids in different *Scutellaria* species, two new HPLC methods were developed. In this section results of the validation studies and the quantities of the analytes in test substances are given.

4.3.1. Yields of MeOH Extracts Prepared for HPLC Analyses

MeOH extracts of plant samples were prepared as described previously, and the yields of the extracts were utilized for the calculations of the quantities of the reference substances (Table 113).

Table 113. The extraction yields of the MeOH extracts of four *Scutellaria* species

Plant Samples	**Yield %**
S. hastifolia	22.4
S. velenovskyi	13.4
S. orientalis	18.1
S. albida	25.7

4.3.2. Phenylethanoid Glycosides

4.3.2.1. Validation Parameters

4.3.2.1.1. Linearity

Phenylethanoid glycosides selected for our analysis, were calceolarioside D (**1**), neocalceolarioside D (**2**), verbascoside (**3**), isoverbascoside (**4**), leucoseptoside A (**5**) and martynoside (**6**). Working concentrations were 3, 5, 15, 20, 40, 75 and 120 µg/mL for calceolarioside D and neocalceolarioside D, 3, 5, 15, 40, 75, 80 and 120 µg/mL for verbascoside, 3, 5, 15, 20, 40, 60, 75 and 120 µg/mL for isoverbascoside, 1, 3, 5, 15, 20, 40, 60, 75 and 120 µg/mL for leucoseptoside A, and finally 3, 5, 15, 20, 60, 75 and 120 µg/mL for martynoside. The calibration curves were constructed from peak areas of the reference compounds versus their concentrations and given in Figure 14. The correlation coefficient (r^2) of all the calibration curves were consistently greater than 0.999 (Table 114).

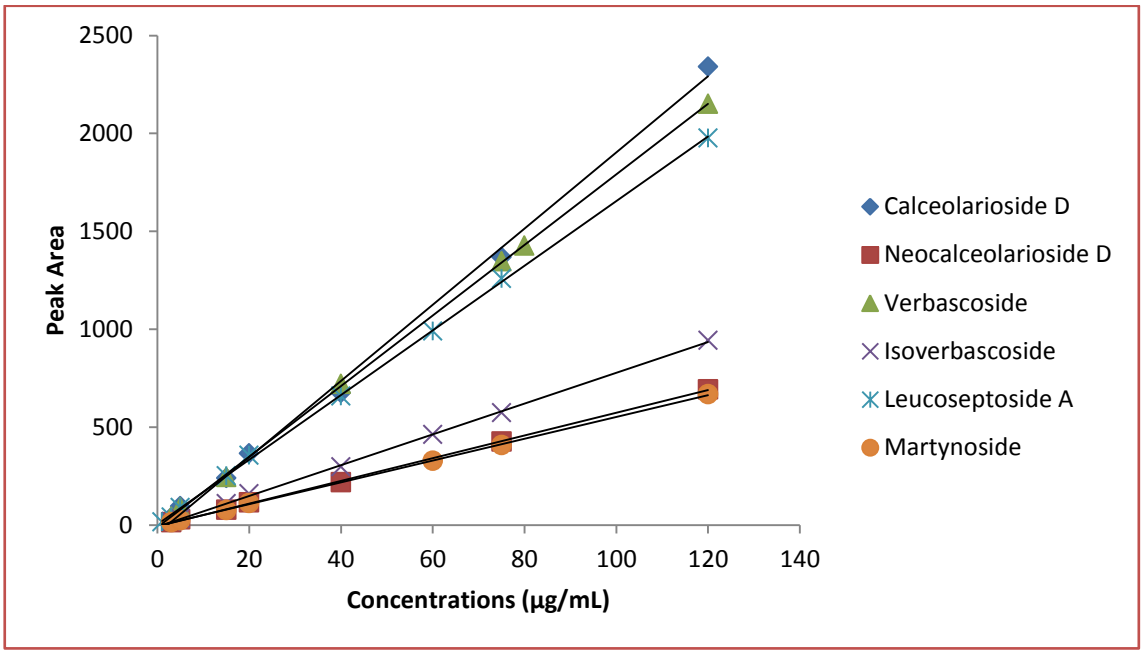


Figure 14. Calibration Curves for the standard substances

4.3.2.1.2. Calibration Curve Parameters

Using developed optimum conditions for standard solutions of phenylethanoid glycosides, linear concentration ranges were determined along with the LOD and LOQ values. Obtained results were given in Table 114.

Table 114. Calibration curve parameters for phenylethanoid glycoside analysis (n=3)

Parameter	**1**	**2**	**3**	**4**	**5**	**6**
Dynamic range (µg/mL)	3-120	3-120	3-120	3-120	1-120	3-120
Slope	19.459	5.786	18.010	7.849	16.503	5.571
Intercept	-14.964	-5.030	-9.912	-7.316	4.252	-4.543
S_a*	2.192	0.484	3.846	0.950	1.101	0.528
S_b**	0.378	0.079	0.413	0.219	0.098	0.080
LOD (µg/mL)	1.040	1.120	1.040	1.295	0.104	1.099
LOQ (µg/mL)	2.196	1.706	2.536	2.143	0.450	1.763
Correlation coefficient	0.999	0.999	0.999	0.999	0.999	0.999

*S_a standard deviation of slope
**S_b standard deviation of intercept

4.3.2.1.3. Accuracy

Recovery values were calculated for the developed method by using quality control concentrations of the standard substances. Calibration curve was used for this purpose. Triplicate experiments of three independent concentrations were performed for 10, 50 and 100 µg/mL of standard solutions of every substance. Obtained recovery values are presented in Table 115.

Table 115. Recovery and relative standard deviation values for phenylethanoid glycosides (n=9)

Analyte	Amount (µg/mL)	Intraday		Interday	
		Recovery %	RSD %	Recovery %	RSD%
1	10	110.58	1.826	112.11	3.404
	50	117.85	0.217	114.84	2.519
	100	111.80	3.705	113.83	2.064
2	10	100.04	1.216	98.23	4.257
	50	98.37	1.566	98.10	1.775
	100	104.71	2.423	101.60	3.222
3	10	102.84	0.424	105.30	4.184
	50	101.27	1.374	102.17	0.91
	100	102.43	2.960	101.42	3.587
4	10	98.46	1.747	96.49	2.607
	50	102.96	1.546	102.33	0.800
	100	99.20	1.289	99.62	1.959
5	10	100.05	0.088	97.44	2.494
	50	101.71	1.908	101.37	2.407
	100	102.39	2.517	102.21	1.926
6	10	98.27	0.694	94.034	4.165
	50	104.40	3.907	104.34	0.232
	100	97.46	0.866	97.71	0.718

In recovery studies, recovery values should be between 90-110%. All results obtained are in this range. It shows that, this method is appropriate at determined concentration range.

4.3.2.1.4. Precision

Instrument precision was evaluated by analysing the mixture standard solution in nine replicate injections. The retention times of the analytes were utilised to determine the intra- and inter-day variability of the method. The intra-day precision was performed using nine replications prepared from the phenlyethanoid glycosides within one day, while inter-day precision was performed over consecutive three days. The relative standard deviation (RSD) was taken as a measure of precision. Calculated intraday and interday precisions of the retention times are given in the Table 116.

Table 116. Intraday and interday precision values of retetion times of phenylethanoid glycosides (n=9)

	Intraday Retention Times ± RSD					
Amount (µg/mL)	**1**	**2**	**3**	**4**	**5**	**6**
10	7.79±0.320	12.34±0.318	13.22±0.378	16.43±0.845	20.63±0.601	30.82±0.035
50	7.80±0.644	12.44±0.333	13.28±0.271	16.42±0.284	20.64±0.314	30.88±0.010
100	7.77±0.711	12.55±0.581	13.22±0.610	16.38±0.499	20.59±0.366	30.79±0.070
	Interday Retention Times ± RSD					
10	7.76±0.066	12.25±0.614	13.28±0.884	16.39±0.689	20.57±0.276	30.79±0.055
50	7.77±0.103	12.25±0.827	13.22±0.066	16.41±0.818	20.52±0.499	30.80±0.018
100	7.77±0.092	12.55±0.581	13.23±0.584	16.45±0.330	20.58±0.121	30.83±0.037

In precision studies, standard deviation of results should be ≤ 2%. All values obtained were ≤ 2%. It shows that, the developed method is a precise method.

4.3.2.1.5. Robustness

Temperature (23-27 °C), acetic acid percent (0.015%-0.025%) and wavelenght (320–340 nm) were consciously changed during the study. Recovery values were calculated again for new conditions and obtained values were evaluated on Table 117.

Table 117. Results of robustness experiments (n=3)

Analytes	Amount µg/mL	Temperature 23 ºC	Temperature 27 ºC	0.015 % Acetic Acid	0.025 % Acetic Acid	Wavelength 320 nm	Wavelength 340 nm
1	10	113.80±0.333	119.62±0.212	113.96±2.776	115.38±0.273	117.40±0.505	115.69±2.389
	50	112.63±4.758	110.76±0.380	109.34±0.257	104.42±0.420	113.19±2.158	115.89±3.153
	100	111.77±2.591	109.78±4.106	113.26±0.164	106.82±0.235	118.61±2.738	118.26±2.812
2	10	93.02±0.808	101.47±1.171	109.21±2.488	104.55±0.984	106.39±0.382	99.39±1.761
	50	91.79±2.343	95.87±1.732	89.33±0.190	91.43±0.174	93.74±1.214	96.29±1.356
	100	94.05±1.370	100.30±2.144	96.04±1.261	101.22±0.765	95.63±1.367	98.31±1.799
3	10	97.76±0.228	105.62±0.233	109.01±1.195	106.81±0.627	107.89±0.171	103.88±0.102
	50	98.85±2.284	104.66±1.625	99.11±0.220	99.30±0.491	99.81±1.951	102.08±1.911
	100	98.16±2.705	99.25±0.194	98.95±1.251	101.10±0.813	99.48±2.448	100.33±2.495
4	10	100.18±1.100	98.91±0.526	96.48±1.904	99.68±1.506	98.01±0.382	97.34±0.834
	50	97.43±2.638	102.55±2.073	99.80±2.297	101.62±0.142	101.26±2.361	102.08±1.634
	100	98.06±0.370	99.05±1.583	97.43±0.681	98.93±0.397	98.15±0.139	98.29±1.463
5	10	99.09±0.770	98.40±0.333	100.71±2.325	99.03±0.404	101.83±0.038	101.81±0.198
	50	99.78±2.679	102.36±1.129	99.51±0.181	100.81±0.699	102.07±2.305	102.09±1.482
	100	101.44±2.525	99.78±2.156	102.05±0.232	99.97±0.306	99.51±1.970	98.5±2.091
6	10	96.64±0.096	101.70±0.174	100.28±1.58	98.45±0.179	99.25±0.105	98.61±0.119
	50	101.29±2.584	104.72±1.077	102.30±0.034	105.51±0.346	103.90±2.269	104.09±0.986
	100	98.15±2.569	96.67±0.699	97.13±0.230	99.05±0.472	97.09±1.301	99.78±1.099

Recovery values should be in the range between 90-110%.

4.3.2.1.6. System Suitability

System suitability results are compared with the values given in the guideliness (221). The capacity factor (k′>2), resolution (R_s >2), tailing factor (T≤2) and theroritical plates (N>2000) parameters are given in the Table 118.

Table 118. System suitability parameters of Phenylethanoid Glycosides

	1	**2**	**3**	**4**	**5**	**6**
k’	3,498	6,196	6,678	8,519	10,943	16,828
*R*s previous peak	5,692	2,173	2,223	2,921	7,725	7,886
*R*s next peak	1,983	2,223	3,762	1,29	7,398	2,752
T	0,869	0,822	0,744	0,894	0,692	1,87
N	7280	6317	12828	6837	15352	273889

Test results are suitable for the system suitability criteria.

4.3.2.2. UV Spectra of the Analytes

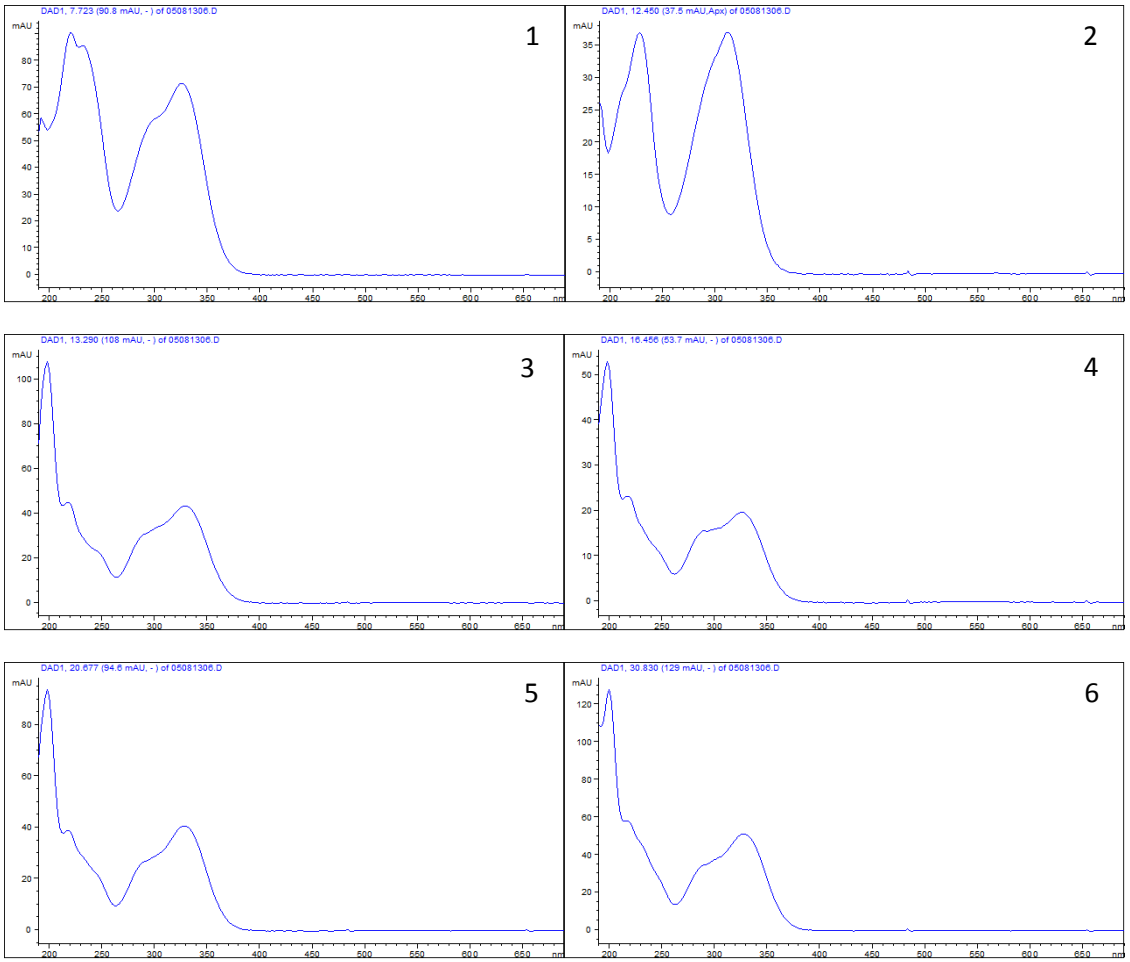


Figure 15. The UV Spectra of the analytes. The spectra were obtained from the maxima of the peaks.

4.3.2.3. HPLC Chromatograms of the Analytes and the Plant Materials

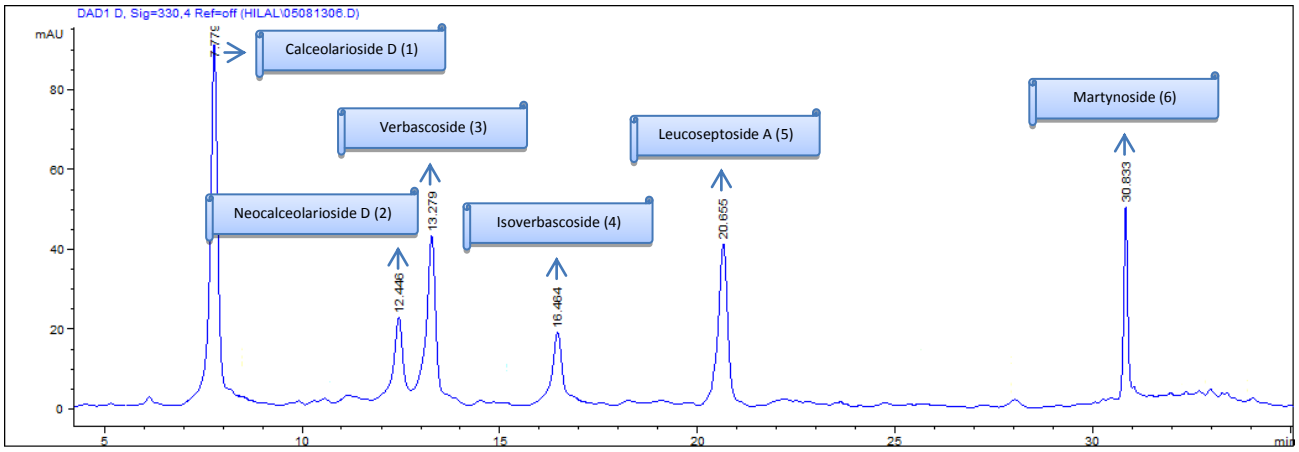


Figure 16. HPLC chromatogram of the standard substances. Column: Zorbax XDB-C18 (4.6×150 mm, 3.5 µm), column termostate: 25 ºC, flow rate: 0.8 mL/min, wavelength: 330 nm, eluents: ACN (0.02% AA) and H_2O (0.02% AA) (15-45%)

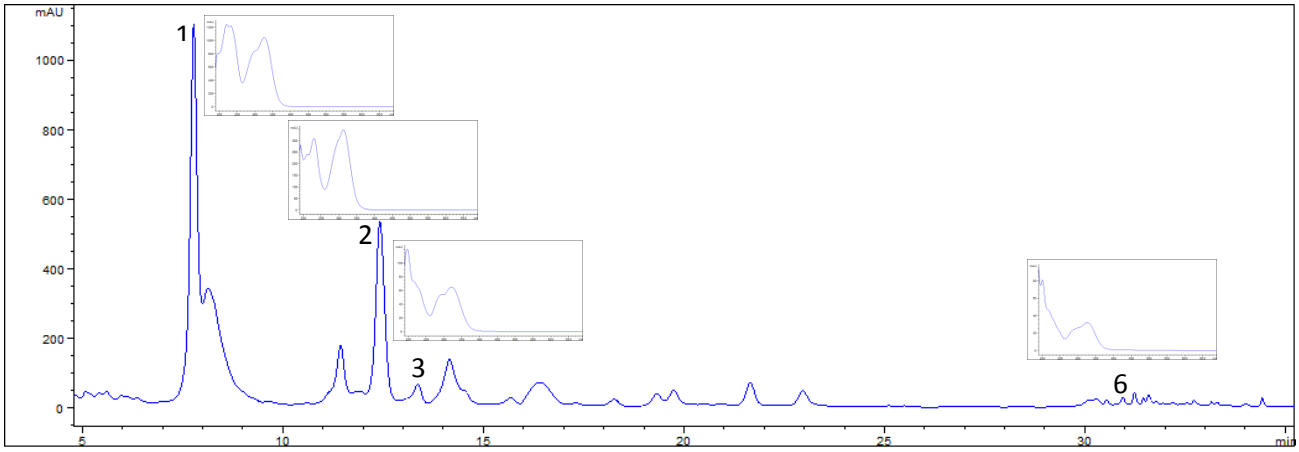


Figure 17. HPLC fingerprint of the methanolic extract of S. *hastifolia.* Conditions: same as Figure 16.

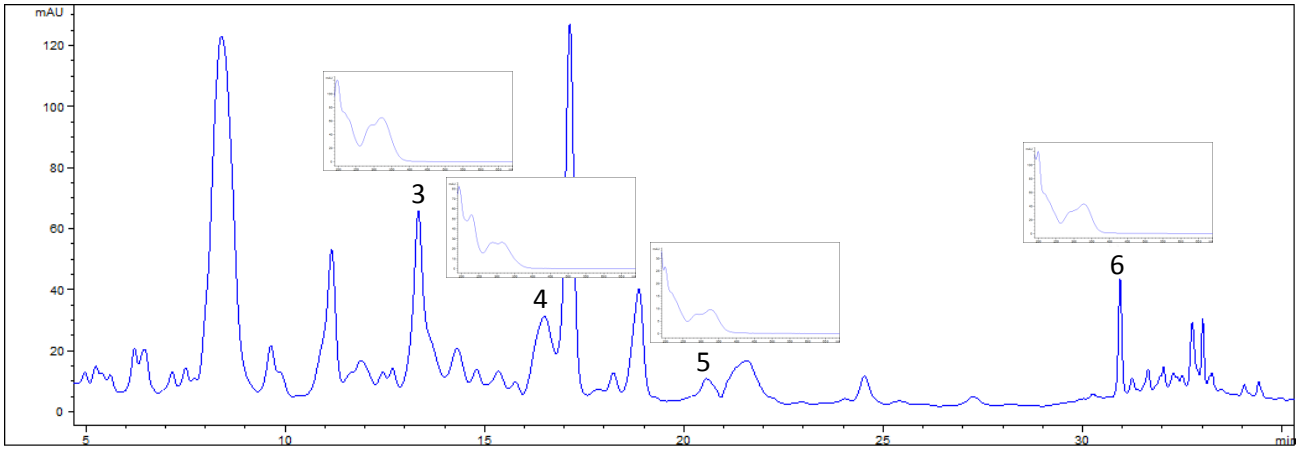


Figure 18. HPLC fingerprint of the methanolic extract of *S. velenovskyi.* Conditions: same as Figure 16.

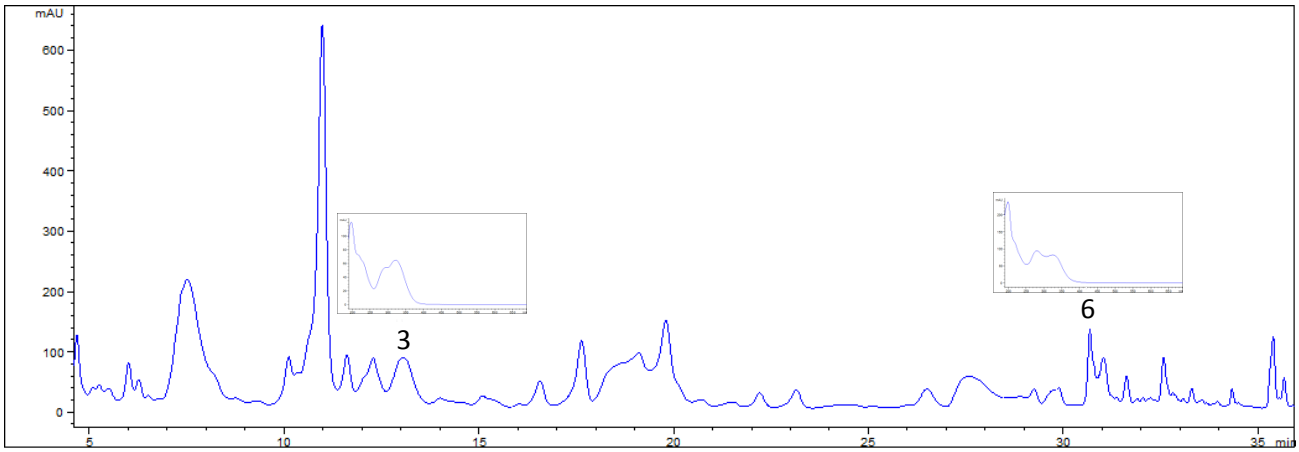


Figure 19. HPLC fingerprint of the methanolic extract of *S. orientalis.* Conditions: same as Figure 16.

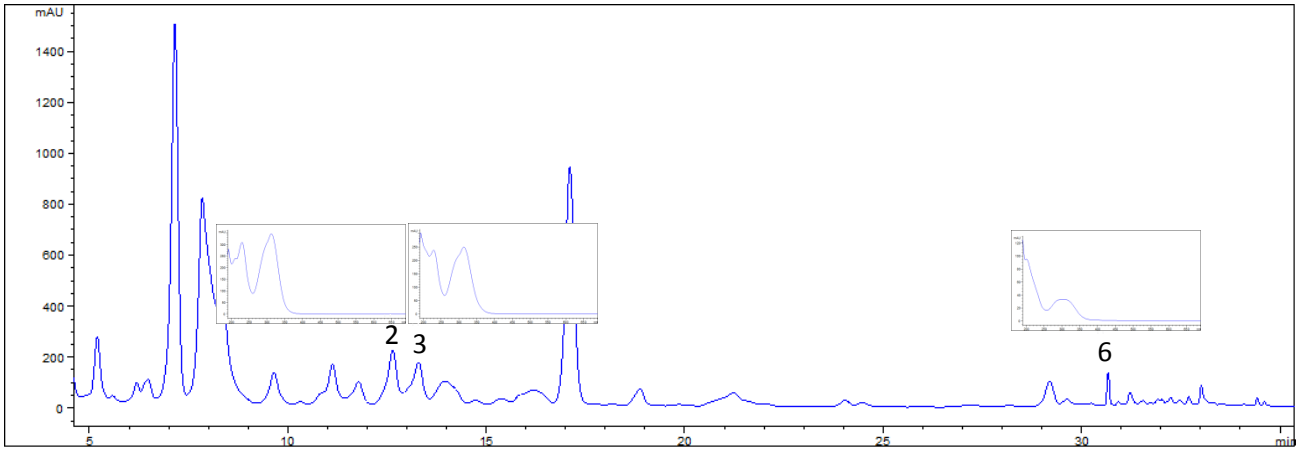


Figure 20. HPLC fingerprint of the methanolic extract *S. albida*. Conditions: same as Figure 16.

4.3.2.4. Quantities of the Phenylethanoid Glycosides in Plant Samples

The amounts of the analytes in MeOH extract of different *Scutellaria* species are given in Table 119 Figure 21 Among the four plant samples, *S. hastifolia* showed the highest calceolarioside D and verbascoside content, *S. velenovskyi* showed the highest leucoseptoside A content, *S. orientalis* showed the highest martynoside content and *S. albida* showed the highest neocalceolarioside D content.

Table 119. Contents of analytes in plant materials (w/w%). Results are given as the mean of triplicates ± S.D.

Plant Samples	1	2	3	4	5	6
S. hastifolia	2.58±0.05	2.49±0.09	0.30±0.005	n.d.	n.d.	0.27±0.005
S. velenovskyi	n.d.	n.d.	0.29±0.01	0.07±0.09	0.03±0.001	0.37±0.015
S. orientalis	n.d.	n.d.	0.17±0.0005	n.d.	n.d.	0.81±0.001
S. albida	n.d.	4.23±0.01	1.27±0.07	n.d.	n.d.	0.15±0.0005

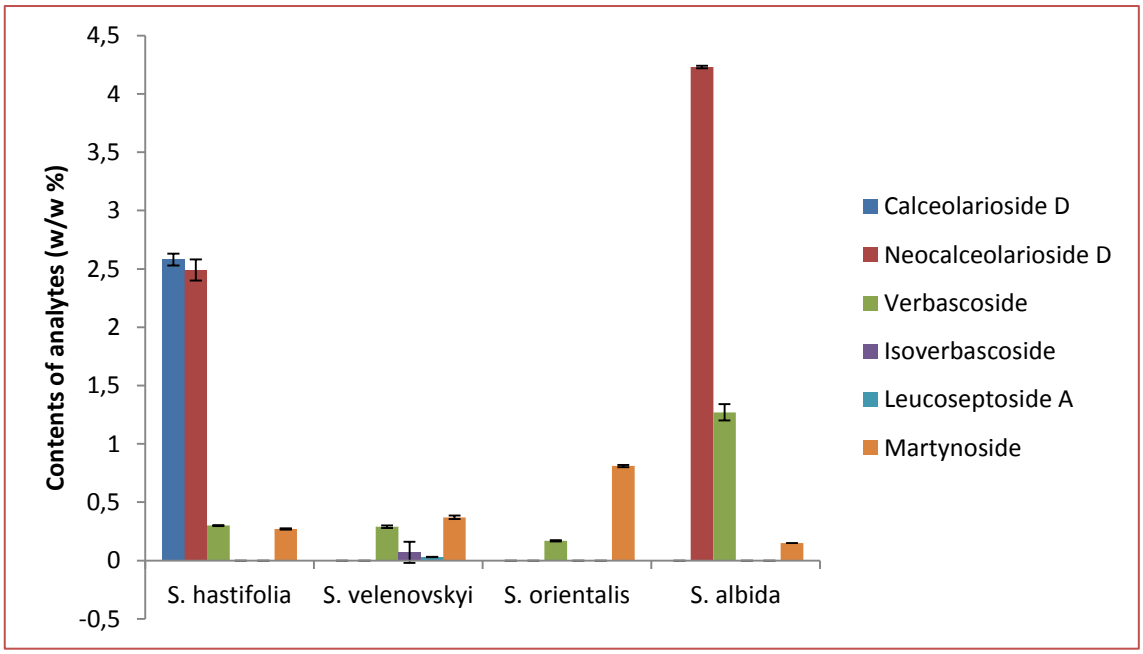


Figure 21. Contents of analytes in plant materials (w/w%). Results are given as the mean of triplicates ± S.D.

4.3.3. Flavonoids

4.3.3.1. Validation Parameters

4.3.3.1.1. Linearity

Flavonoids selected for our analyses were scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, luteolin and apigenin. Working concentrations were 5, 15, 40, 75 and 120 µg/mL for scutellarein 7-*O*-β-D-glucopyranoside, 5, 15, 30, 40 and 75 µg/mL for hispidulin 7-*O*-β-D-glucuronopyranoside, 5, 15, 30, 40, 75 and 120 µg/mL for apigenin 7-*O*-β-D-glucopyranoside, 1, 5, 15, 40 and 75 µg/mL for hispidulin 7-*O*-β-D-glucopyranoside, and finally 1, 5, 15, 30, 40 and 75 µg/mL for the aglycones apigenin and luteolin. For validation studies (RSD% calculations) 10, 20 and 60 µg/mL concentrations for each standard flavonoid solution were prepared. The calibration curves were constructed from peak areas of the reference compounds versus their concentrations and given at Figure 22. The correlation coefficient (r^2) of all the calibration curves were consistently greater than 0.999 (Table 120).

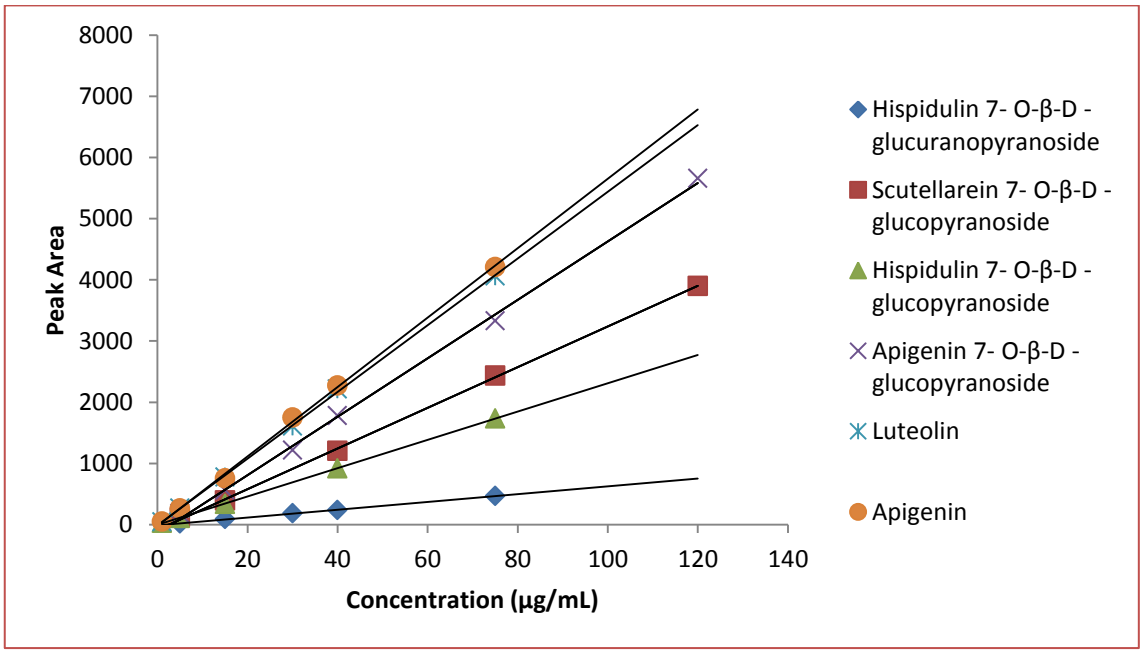


Figure 22. Calibration Curves for the standard substances

4.3.3.1.2. Calibration Curve Parameters

Using developed optimum conditions for standard solutions of flavonoids, linear concentration ranges were determined along with the LOD and LOQ values. Obtained results were given in Table 120.

Table 120. Calibration curve parameters for flavonoid analysis (n=3)

Parameter	**1**	**2**	**3**	**4**	**5**	**6**
Dynamic range (µg/mL)	5-120	5-75	5-120	1-75	1-75	1-75
Slope	33.231	6.3613	47.756	23.153	54.017	56.681
Intercept	-83.832	-9.401	-148.11	-4.229	-12.128	-17.866
S_a*	0.829	1.328	3.891	0.807	1.332	1.786
S_b**	0.453	0.759	0.982	0.159	0.789	0.326
LOD (µg/mL)	1.709	1.883	1.500	0.016	0.058	0.234
LOQ (µg/mL)	1.882	3.368	2.220	0.261	0.232	0.457
Correlation coefficient	0.999	0.999	0.998	0.999	0.999	0.999

*S_a standard deviation of slope
**S_b standard deviation of intercept

4.3.3.1.3. Accuracy

Recovery values were calculated for the developed method by using quality control concentrations of standard substances. Calibration curve was used for this purpose. Triplicate experiments of three independent concentrations were performed for 10, 20 and 60 µg/mL of standard solutions of every substance. Obtained recovery values were presented in Table 121.

Table 121. Recovery and relative standard deviation values for phenylethanoid glycosides (n=9)

Analyte	Amount (µg/mL)	Intraday		Interday	
		Recovery %	RSD %	Recovery %	RSD%
1	10	99.71	0.09	98.06	0.89
	20	98.58	0.14	98.25	1.08
	60	99.83	0.50	98.08	01.08
2	10	92.57	1.14	92.42	0.27
	20	99.61	0.65	100.47	4.51
	60	92.2	0.32	93.12	0.89
3	10	100.11	0.16	100.33	2.53
	20	100.83	0.17	102.16	1.80
	60	99.74	0.31	98.26	1.42
4	10	99.36	0.25	98.19	0.17
	20	99.30	0.21	99.47	0.94
	60	100.99	0.29	100.48	0.86
5	10	107.26	0.10	107.93	0.65
	20	99.74	15	100.09	0.64
	60	101.05	0.30	101.82	0.93
6	10	107.83	0.19	102.12	2.28
	20	104.65	0.15	104.99	1.36
	60	103.42	0.29	100.78	0.92

In recovery studies, recovery values should be between 90-110%. All results obtained are in this range. It shows that, this method is appropriate at determined concentration range.

4.3.3.1.4. Precision

Instrument precision was evaluated by analysing the mixture standard solution in nine replicate injections. The retention times of the analytes were utilised to determine the intra- and inter-day variability of the method. The intra-day precission was performed using nine replications prepared from the phenylethanoid glycosides within one day, while inter-day precision was performed over consecutive three days. The relative standard (RSD) was taken as a measure of precision. Calculated intraday and interday precisions of three retention times are given in the Table 122.

Table 122. Intraday and interday precision values of retetion times of flavonoids (n=9)

	Intraday Studies					
	Retention Times ± RSD					
Amount (µg/mL)	**1**	**2**	**3**	**4**	**5**	**6**
10	10.55±0.32	13.41±0.30	15.01±0.31	16.81±0.18	22.80±0.11	28.16±0.05
20	10.55±021	13.82±0.24	15.06±0.26	16.81±0.18	22.80±0.12	28.16±0.09
60	10.55±0.22	13.40±0.21	15.06±0.23	16.82±0.17	22.79±0.05	28.16±0.04
	Interday Studies					
	Retention Times ± RSD					
10	10.56±0.64	13.41±0.27	15.01±0.18	16.81±0.39	22.80±0.14	28.15±0.17
20	10.55±0.43	13.40±0.29	15.01±0.33	16.83±0.23	22.79±0.11	28.14±0.12
60	10.55±0.67	13.40±0.57	15.08±0.63	16.89±0.54	22.78±0.19	28.16±0.19

In precision studies, standard deviation of results should be ≤ 2%. All values obtained were ≤ 2%. It shows that, the developed method is a precise method.

4.3.3.1.5. Robustness

Temperature (23-27°C), acetic acid percent (0.015%-0.025%), wavelenght (330–350 nm) values were changed consciously during the study. Recovery values were calculated again for new conditions and obtained values were evaluated on Table 123.

Table 123. Results of robustness experiments (n=3)

Analytes	Amount µg/mL	Temperature 23 ºC	Temperature 27 ºC	0.015 % Acetic Acid	0.025 % Acetic Acid	Wavelength 330 nm	Wavelength 350 nm
1	10	92.209±0.213	92.636±0.52	93.738±0.14	92.375±0.527	93.514±0.934	94.073±1.241
	20	99.11 ±1.412	98.638±0.579	97.742±0.21	98.344±0.097	98.734±1.71	97.273±0.288
	60	95.374±0.639	97.135±1.153	96.964±1.028	97.3±0.224	98.565±1.804	98.644±2.018
2	10	93.864±0.271	92.301±3.489	91.293±0.241	95.77±0.251	95.77±0.215	94.296±1.027
	20	100.896±0.294	100.253±1.658	98.59±0.840	97.891±0.669	97.891±0.699	91.499±1.571
	60	90.767±1.074	92.39±4.222	94.572±1.232	94.861±0.188	95.861±0.188	93.811±1.702
3	10	99.069±0.477	101.056±0.553	101.399±0.299	99.946±0.561	102.301±0.575	102.984±0.695
	20	101.442±1.151	100.886±0.644	102.084±0.207	100.229±0.144	101.879±2.338	101.728±2.817
	60	100.134±0.606	101.68±1.602	101.047±0.823	101.996±0.204	104.972±1.506	105.095±1.655
4	10	93.395±0.085	93.059±0.393	94.537±0.213	94.535±0.075	94.518±0.529	94.426±0.733
	20	99.229±1.132	99.777±0.688	98.165±0.431	99.463±0.269	99.411±0.827	99.532±0.483
	60	100.493±0.622	100.745±1.176	101.965±1.356	101.078±0.413	102.447±1.427	102.971±1.37
5	10	106.598±0.133	107.126±0.777	107.267±0.108	106.842±0.397	107.539±0.837	107.369±0.709
	20	100.331±1.05	101.063±0.736	100.152±0.199	98.969±0.094	99.193±0.513	99.34±0.44
	60	100.902±0.66	101.622±1.024	102.703±0.698	102.142±0.236	102.766±1.58	102.805±1.53
6	10	107.901±0.086	108.74±0.67	107.652±0.146	106.736±0.43	106.961±0.597	107.062±0.685
	20	106.587±1.116	105.28±0.673	105.881±0.27	104.92±0.129	104.718±0.529	104.784±0.546
	60	102.599±0.652	102.253±0.932	102.316±0.468	101.861±0.194	101.995±1.624	102.056±1.525

Recovery values should be in the range between 90-110%.

4.3.3.1.6. System Suitability

System suitability results are compared with the values given in the guideliness (221). The capacity factor (k′>2), resolution (R_s >2), tailing factor (T≤2) and theroritical plates (N>2000) parameters are given in the Table 124.

Table 124. System suitability parameters of Flavonoids

	1	**2**	**3**	**4**	**5**	**6**
k'	4,696	6,241	7,105	7,962	11,314	14,204
***R*s previous peak**	31,32	1,625	4,697	5,137	14,277	16,405
***R*s next peak**	4,954	4,684	5,153	4,57	1,546	15,797
T	0,922	1,208	1,005	0,981	1,283	1,114
N	20875	16339	33206	38463	115251	855528

Test results are suitable for the system suitability criteria.

4.3.3.2. UV Spectra of the Analytes

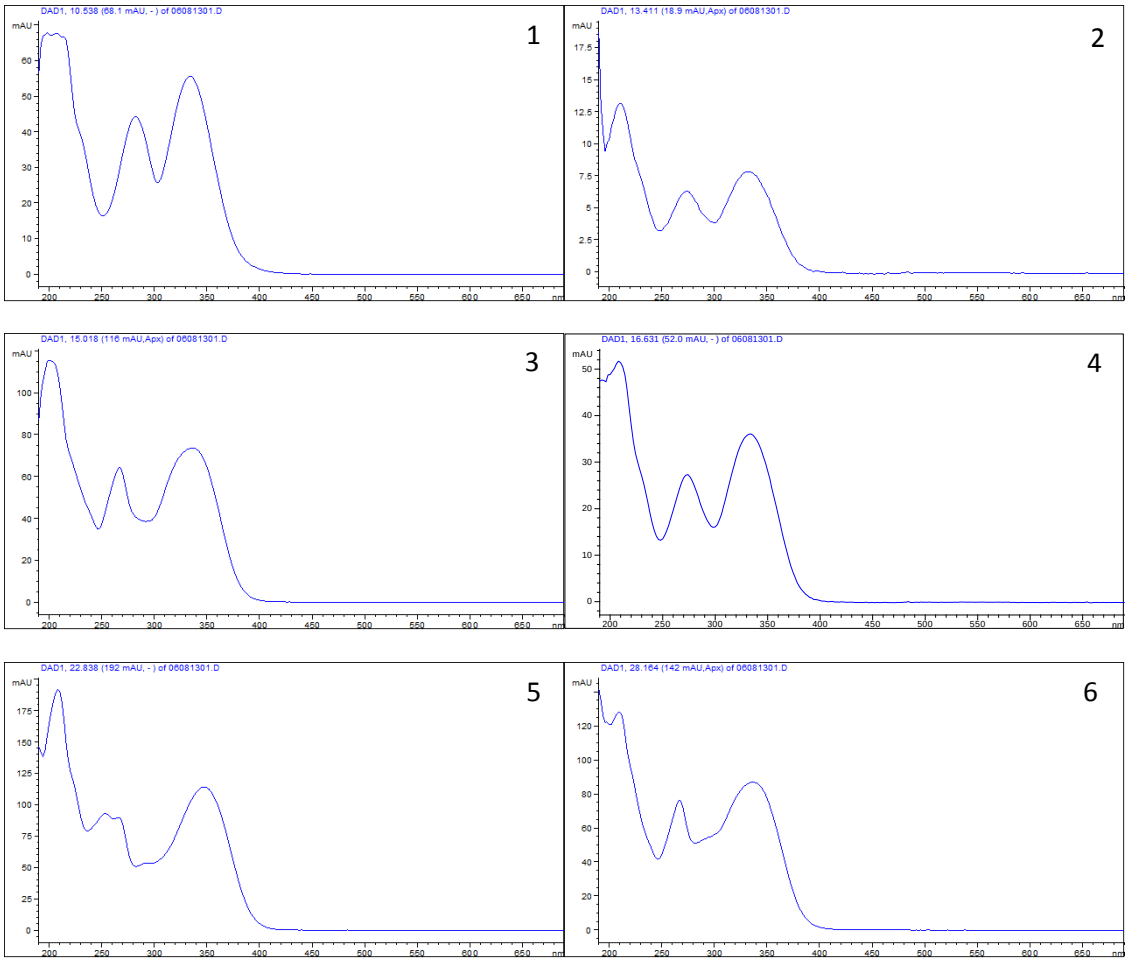


Figure 23. The UV Spectra of the analytes The spectra were obtained from the maxima of the peaks.

4.3.3.3. HPLC Chromatograms of the Analytes and the Plant Materials

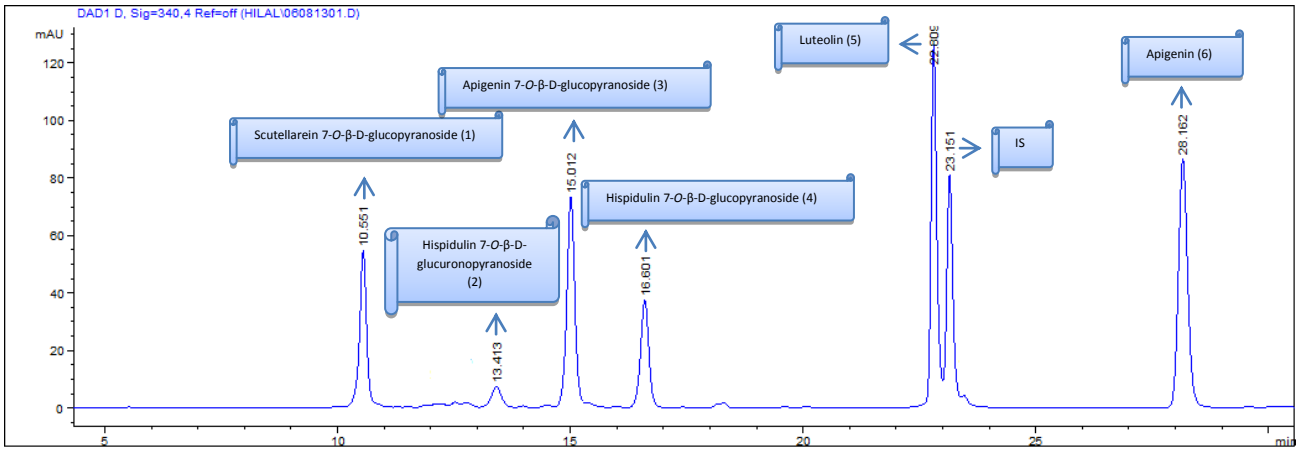


Figure 24. HPLC chromatogram of the standard substances. Column: Zorbax XDB-C18 (4.6×150 mm, 3.5 µm), column termostate: 25 ºC, flow rate: 0.8 mL/min, wavelength: 340 nm, eluents: ACN (0.02% AA) and H_2O (0.02% AA) (15-50%)

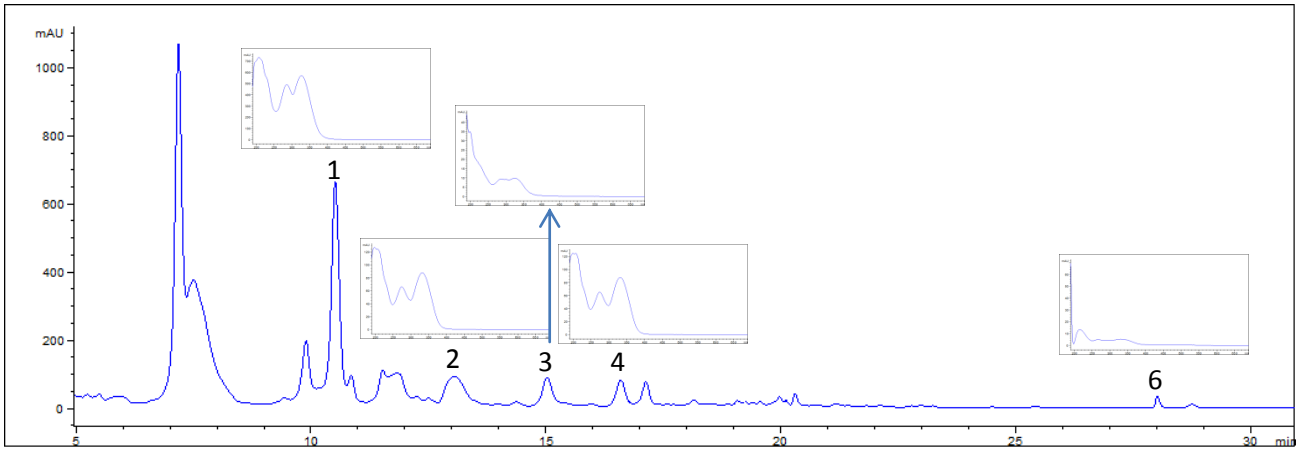


Figure 25. HPLC fingerprint of the methanolic extract of S. *hastifolia.* Conditions: same as Figure 24.

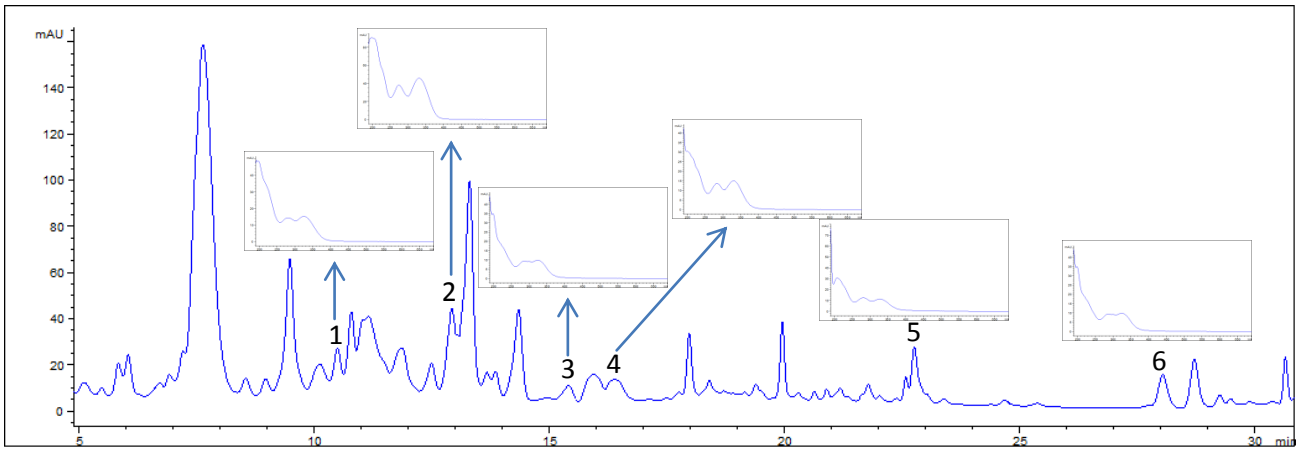


Figure 26. HPLC fingerprint of the methanolic extract of *S. velenovskyi.* Conditions: same as Figure 24.

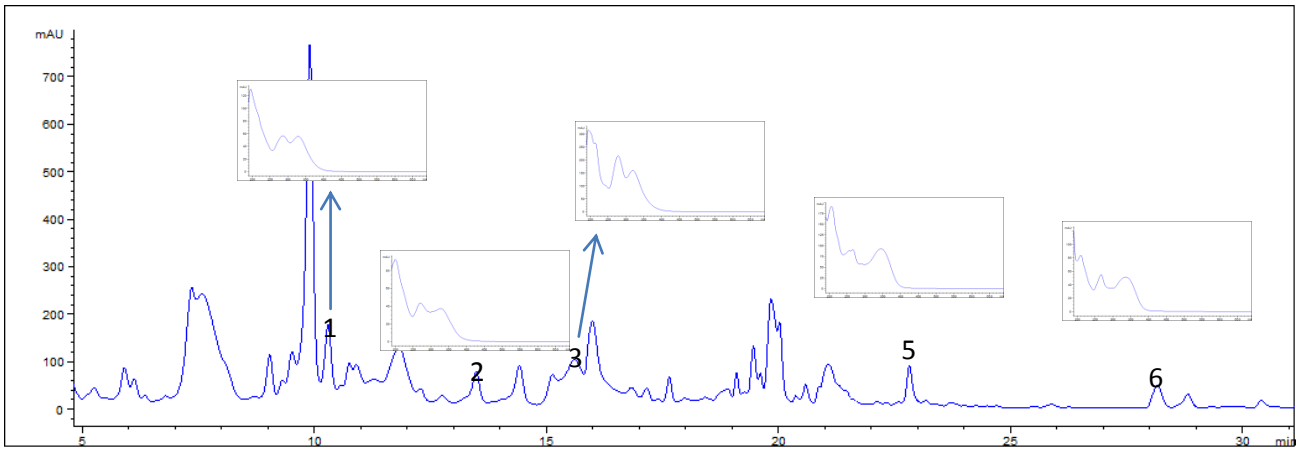


Figure 27. HPLC fingerprint of the methanolic extract of *S. orientalis.* Conditions: same as Figure 24.

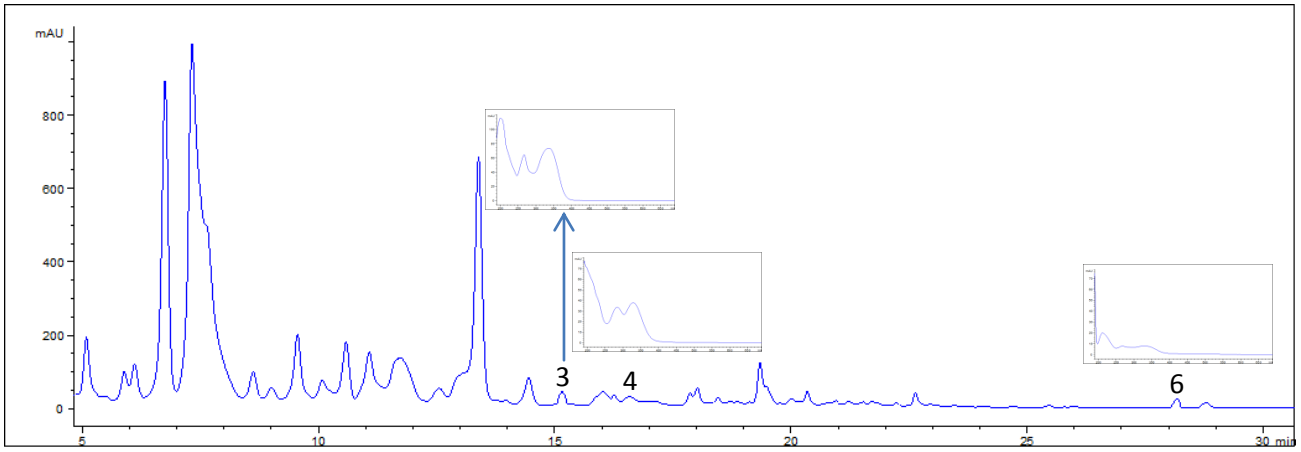


Figure 28. HPLC fingerprint of the methanolic extract of *S. albida.* Conditions: same as Figure 24.

4.3.3.4. Quantities of the Flavonoids in Plant Samples

The amounts of the analytes in MeOH extract of different *Scutellaria* species are given in Table 125 and Figure 29. Among the four plant samples, *S. hastifolia* showed the highest of hispidulin 7-*O*-β-D-glucuronopyranoside, scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside and apigenin 7-*O*-β-D-glucopyranoside content, *S. orientalis* showed the highest amount of luteolin content and finally *S. velenovskyi* showed the highest amount of apigenin content.

Table 125. Contents of analytes in plant materials (w/w%). Results are given as the mean of triplicates ± S.D.

Plant Samples	1	2	3	4	5	6
S. hastifolia	1.32±0.021	1.59±0.04	0.146±0.014	0.262±0.003	n.d.	0.005±0.00
S. velenovskyi	0.10±0.012	0.06±0.08	0.028±0.00	0.029±0.021	0.041±0.00	0.032±0.00
S. orientalis	0.038±0.00	0.029±0.05	0.358±0.00	n.d.	0.047±0.00	0.20±0.00
S. albida	n.d.	n.d.	0.036±0.00	0.098±0.021	n.d.	0.03±0.00

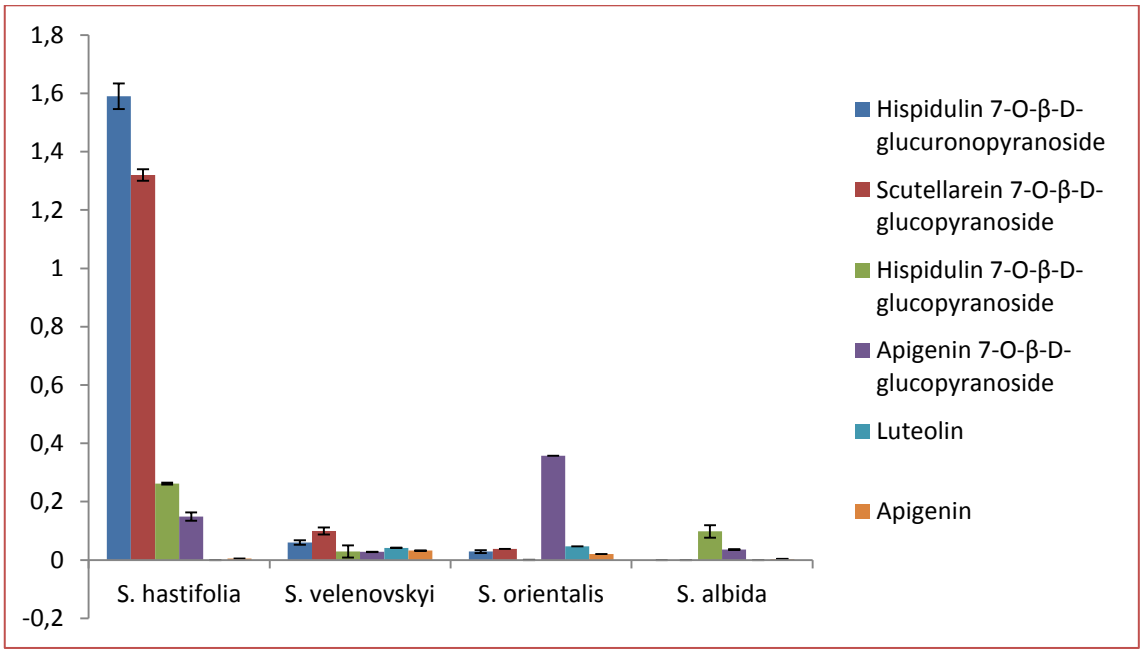


Figure 29. Contents of analytes in plant materials (w/w%). Results are given as the mean of triplicates ± S.D.

5. DISCUSSION

In this section, results of the all experiments were discussed in details in itself and compared with the previous studies.

DISCUSSION

According to the World Health Organization, about 80% of the world population depends on medicinal plants for primary healthcare. The achieved project is oriented towards the exploitation of the biological properties of the plant originated secondary metabolites and their application in human health. The desire for safer drugs with less environmental and mammalian toxicity is a major concern. The purpose of this project was to investigate some selected *Scutellaria* species in terms of their biological activities, mainly antioxidant and antiinflammatory activities and of course chemical components.

The genus *Scutellaria* is known to contain medicinally important species and therefore have been under an intense investigation. *Scutellaria* species are used for various medicinal purposes such as painkiller, sedative, to reduce edema, for swelling throat and hemorrhoids worldwide. Many of these usages were proven by scientific investigations and it is found that *Scutellaria* species and their metabolites possess significant antioxidant, antiinflammatory, antitumour activities as well as potent effects on cardiovascular and neurological systems (1). At the same time the chemical profile of the representatives within the genus *Scutellaria* were investigated in depth and a variety of compounds were isolated and detected.

As it is mentioned above, the fullfilled project is oriented towards the exploitation of *Scutellaria* sp. The choice of the plant material was justified due to the many reasons. The genus *Scutellaria* is known to contain medicinal plants, and is currently under investigation worldwide (1). It would be an optimal choice for the development of a medicinal model, because of a documented chemical profile and a relatively small genome size. Therefore, *Scutellaria* species are excellent model systems to study the biochemistry and genetic regulation of secondary metabolism in medicinal plants (232). Moreover, the chemical profile of the representative plants within the genus *Scutellaria* varies widely. Some members of the genus are characterized by the presence of iridoids, especially catalpol and its esters, while in others different metabolic routes are engaged. Due to this diversion, the infrageneric classification is less understood, although the position of the genus in the Lamiaceae family remains

established (1,233,234). Therefore, the chemical information provided by this thesis, could make a contribution to the chemotaxonomy of the genus.

The genus *Scutellaria* is represented by 16 species in the flora of Turkey (5,6). Among these species, *S. albida* subsp. *albida* (collected from Greece) has been deeply investigated and several bioactive secondary metabolites with antioxidant activity have been isolated, mainly iridoids and phenylethanoid glycosides (132,152). Regarding the bioactivity studies, 33 *Scutellaria* L. taxa collected from Turkey were screened for their acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition as well as their antioxidant potentials by Şenol et al. (117). On the other hand, *S. albida* subsp. *colchica*, *S. diffusa*, *S. galericulata*, *S. heterophylla*, *S. orientalis* subsp. *pinnatifida*, *S. orientalis* L. subsp. *porphyrostegia*, *S. pontica*, *S. salviifolia* growing in Turkey have been studied in terms of their secondary metabolites so far (133,157,159,161,191,235).

Four species of the genus *Scutellaria*, namely, *S. hastifolia*, *S. velenovskyi*, *S. orientalis* subsp. *pinnatifida* and *S. albida* subsp. *albida* were selected within the framework of this thesis. Based on a literature survey, there are few or no study on these species concerning their secondary metabolites as well as their antioxidant and antiinflammatory activities especially on *S. hastifolia* (1,8,152,236).

The utilization of plants as food and medicinal remedies since ancient times, is partially due to the biological efficacy of secondary metabolites that exerts antioxidant activities such as phenolic compounds, vitamins C and E, and carotenoids. Phenolic compounds constitute the most abundant class of antioxidants with an estimated total dietary intake as high as 1 g/day, which is 10 times higher than the intake of vitamin C and 100 times that of vitamin E. The antioxidant properties of plant extracts have been attributed to their phenolic contents. Many investigations have been performed since the early 1870s on the beneficial effects of phenolic compounds as natural antioxidants with well over 150.000 research papers related to antioxidants (237). The term phenolics involve approximately 8000 naturally occuring compounds. Phenolic acids, flavonoids and phenylethanoids are the few examples of the phenolic compounds. The best expressed property of phenolics is the ability to trap free radicals. Moreover, they are known to have the optimal chemical properties because they act both as hydrogen and electron donors and have the ability of chelating metal ions (238). Therefore, following

the collection of those four *Scutellaria* species, three different solvent extracts were prepared (acetone, MeOH and MeOH:H_2O). Then, the extracts were screened for their total antioxidant capacity, total phenolic and total flavonoid contents in order to select the species that encouraging the best expectations in their biological activities for further investigations.

Ascorbic acid equivalent total antioxidant capacity test gives general information about the whole reduction capacity of the samples. The results elicited that among the acetone and MeOH extracts, *S. hastifolia* (470.19±1.01 and 249.84±5.00 mg AAE/g extract, respectively) showed the highest total antioxidant capacity.

Total phenolic content test results showed that among the acetone extracts, the highest gallic acid equivalent phenolic content was seen in *S. albida* (98.71±1.17 mg GAE/g extract) which was followed by *S. orientalis* (84.61±0.76 mg GAE/g extract) and *S. hastifolia* (66.15±0.76 mg GAE/g extract). Among the MeOH extracts, *S. orientalis* (158.20±1.93 mg GAE/g extract) and *S. hastifolia* (105.38±1.17 mg GAE/g extract) displayed the highest total phenolic content. Among the MeOH:H_2O extracts, *S. hastifolia* (127.94±1.33 mg GAE/g extract) elicited the highest amount of gallic acid equivalent total phenol content. When all the extracts were considered, *S. orientalis* was found to contain the highest gallic acid equivalent phenolic content, and *S. hastifolia* was the second richest plant with regard to its phenolic content.

Total flavonoid content test results exhibited that among the acetone extracts, *S. velenovskyi* (147.22±0.67 mg QE/g extract) displayed the highest quercetin equivalent total flavonoid content. *S. orientalis* (106.28±0.88 mg QE/g extract), *S. albida* (94.39±2.27 mg QE/g extract) and *S. hastifolia* (92.72±1.76 mg QE/g extract) were following *S. velenovskyi*. Among the MeOH extracts, *S. orientalis* (47.83±0.50 mg QE/g extract) showed the highest total flavonoid content and *S. hastifolia* (36.95±1.15 mg QE/g extract), *S. velenovskyi* (24.83±0.53 mg QE/g extract) and *S. albida* (23.22±0.48 mg QE/g extract) were following *S. orientalis*, respectively. Among the MeOH:H_2O extracts, *S. hastifolia* (53.33±1.53 mg QE/g extract) showed the highest quercetin equivalent total flavonoid content and *S. orientalis* (41.17±0.19 mg QE/g extract), *S. albida* (27.06±0.41 mg QE/g extract) and *S. velenovskyi* (20.39±0.48 mg QE/g extract) were chasing them, respectively. When all the extracts were considered

as a whole, *S. orientalis* was found to contain the highest quercetin equivalent flavonoid content, and *S. velenovskyi* was the second richest plant with regard to its phenolic content.

Şenol et al. previously reported the total phenolic and flavonoid contents of *S. hastifolia*, *S. albida* subsp. *albida* and *S. orientalis* subsp. *pinnatifida*. According to the results obtained by Şenol et al., total phenol content of the MeOH extracts of *S. hastifolia* was found as 125.07±2.25 mg GAE/g extract, *S. albida* subsp. *albida* was found as 117.78±1.18 mg GAE/g extract and finally *S. orientalis* subsp. *pinnatifida* 160.0±1.91 mg GAE/g extract. When compared with current results, total phenol content of *S. hastifolia* and *S. orientalis* subsp. *pinnatifida* were similar and same, respectively. But the results differ slighlty in *S. albida* subsp. *albida*. The slight differences may be due to the difference of the extraction methods (117).

With respect to the results of *in vitro* tests mentioned, all extracts were found to possess potent antioxidant activity. Due to the results based on all tests especially total antioxidant capacity test and preliminary TLC studies along with the minority of investigations conducted on *S. hastifolia*, this plant was choosen for further phytochemical and bioactivity (antioxidant and antiinflammatory) studies. Accordingly, the crude extracts of *S. hastifolia* were screened for their DPPH radical scavenging, linoleic acid lipid peroxidation inhibition and soybean LOX inhibition activities.

Several methods are used for the prediction of efficiency of synthetic/natural antioxidants and antiinflammators, like the DPPH assay, AAPH/linoleic acid assay and soybean LOX assay. The antioxidant and antiinflammatory activities of the crude extracts of *S. hastifolia* along with the isolated 15 phytoconstituents have been evaluated in those three *in vitro* tests.

In view of the differences among the test systems available, the results of a single assay may give only a suggestion on the protective potential of phytochemicals. Among the plethora of the methods used for the evaluation of the antioxidant activity, the DPPH test is very useful in the micromolar range, requiring minutes to hours for both polar and apolar samples. In literature, many studies regarding the antioxidant activity of natural substances tested with DPPH have been reported. In the presence of

an antioxidant, which can donate an electron to DPPH, the purple color is typical of the free DPPH radical decays, a change which can be followed spectrophotometrically (517 nm). This interaction indicates its radical scavenging ability in an iron free system. Linoleic acid lipid peroxidation assay can be used to follow oxidative changes and to understand the contribution of each tested compound. Azo compounds generating free radicals through spontaneous thermal decomposition are useful for *in vitro* studies of free radical production. The assay is based on the inhibition of lipid oxidation that provides a measure of how efficiently antioxidants protect against lipid oxidation *in vitro*. Oxidation of exogenous linoleic acid by a thermal free radical producer (AAPH) is followed by UV spectrophotometry in a highly diluted sample. Oxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion is monitored at 234 nm. LOX assay was used as an indication of antiinflammatory activity. LOX is a key enzyme in the inflammatory process, whose inhibition is correlated to the ability of the inhibitors to reduce Fe^{+3} at the active site to the catalytically inactive Fe^{+2}. LOXs contain a "non-heme" iron per molecule in the enzyme active site as high-spin Fe^{+2} in the native state and the high-spin Fe^{+3} in the activated state. Several LOX inhibitors, such as phenolic compounds, are excellent ligands for Fe^{+3} (152).

DPPH scavenging activities of *S. hastifolia* EtOAc and MeOH extract was previously investigated by Şenol et al. (117). The activity was tested at 250, 500 and 1000 µg/mL concentrations and a significant DPPH scavenging activiy was found in all tested concentrations of MeOH extract. Besides, in EtOAc extract, the activity was remarkably decreased when compared with MeOH extract. Data obtaine by this study revealed similar results with previous one. MeOH extract displayed 68±1.8% reducing activiy, wheras acetone and MeOH:H_2O extracts showed 63±2.0 and 66±1.1% reducing activity, respectively. NDGA was used as a reference substance which showed 81/83±1.3% reducing activity at 20^{th} and the 60^{th} minutes. When compared with the reference substance, all extracts exerted significant DPPH scavenging activity. When results were compared with those of Şenol et al., MeOH extract of *S. hastifolia* was found to be the most active extract among the other extracts. The difference between the results of previous and the current study might be originated from the diversity of the methods applied, concentrations and extraction methods. Consequently, all the extracts of *S. hastifolia* were found to possess antioxidant activity. When compared with the

former tests, this activity showed a positive correlation with the extracts total antioxidant capacities and total phenolic contents.

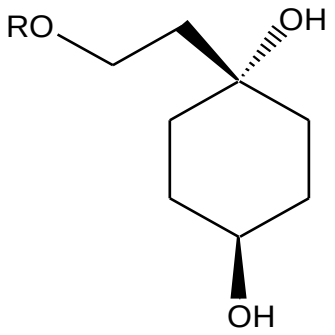
Linoleic acid lipid peroxidation assay findings elicited that acetone extract showed the highest inhibition (57±0.5%). Acetone extract was followed by MeOH and MeOH:H_2O (5:1) extracts with 33±0.8% and 19±0.3% inhibitions, respectively. Trolox was used as a reference substance in this assay and it showed 64±0.8% inhibition to lipid peroxidation. When, the data of this assay was compared, it was obvious that acetone extract inhibited the oxidation of linoleic acid higher than the other extracts and almost as the reference substance trolox. The data of this assay is furthermore displayed a slight correlation with total flavonoid contents of the extract. The higher total flavonoid content was calculated in acetone extract and the activity of acetone extract in this assay was approximately twice of MeOH and threefold of MeOH:H_2O extract.

The samples were further evaluated for their inhibition on soybean lipoxygenase (LOX). Although LOX interferes with the inflammatory cascade, chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. Then, many LOX inhibitors are also possible antioxidant agents. According to the results of this assay only MeOH extract of *S. hastifolia* elicited very high LOX inhibition (100±1.9%) even higher than the reference substance NDGA (84±2.2%). However, acetone and H_2O extracts did not show any inhibition at the tested concentrations.

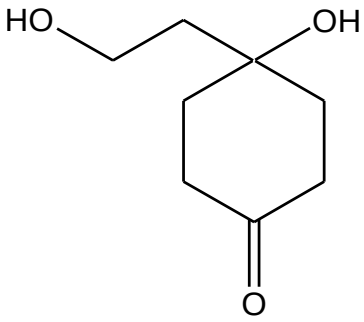
Following the results of the *in vitro* activity tests as well as the preliminary chromatographic studies, acetone and MeOH extracts of *S. hastifolia* were selected for further phytochemical studies. Acetone and MeOH extracts were mainly focused due to their rich secondary metabolite composition and their being more active rather than MeOH:H_2O extract in DDPH scavenging, AAPH induced lipid peroxidation inhibition and soybean LOX inhibition assays. Prefractionation and purification studies on the acetone and MeOH extracts by using common chromatographic methods (open column chromatography and medium pressure liquid chromatography) led to the isolation of 17 compounds, including a new one. The isolates can be catagorised as ethylcyclohexane derivatives (isorengyol, isorengyoside, cleroindicin B, cleroindicin F), phenylethanoid glycosides (cornoside, calceolarioside D, hastifolioside, neocalceolarioside D, calceolarioside B, verbascoside), flavonoids (apigenin 7-*O*-β-D-glucopyranoside,

scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside), sugar ester mixture (6-*O*-caffeoylglucopyranose) and sugar molecules (glucose, sucrose). The structures of the compounds were elucidated by spectroscopic techniques (UV, IR, 1D and 2D NMR) and MS.

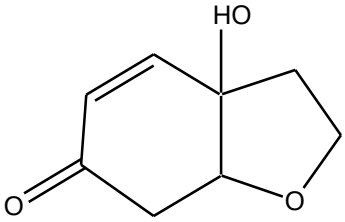
The genus *Scutellaria* is well known for the wide chemical variation within the representative plants (1). Due to this variations, the infrageneric classification is not well understood, although the position of the genus in the Lamiaceae family remains established (234). The distribution of catalpol and similar groups which gave same color reactions with catalpol, provide support for the classification that divides the genus into two subgenera, the subgenus *Scutellaria* and *Apeltanthus*. Moreover, the subgenus *Scutellaria* was divided into five sections: *Scutellaria*, *Salvifoliae*, *Perilomia*, *Anaspis* and *Salazaria*. Examinations of the distribution of catalpol and similar compound groups (which gave similar reactions with catalpol and categorised as E1, E2 and E3) supports the species group concept. The morphologically well-defined *Scutellaria albida* species group is also studied chemically, with members containing catalpol and compound groups E1 and E2. The other species groups from subgenus *Scutellaria* which were examined appear to be characterized by the absence of iridoid glycosides, e.g. the *S. angustifolia* and *S. lateriflora* species groups, or the presence of catalpol alone, e.g. the *S. galericulata* and *S. strigillosa* species groups. Of the subgenus *Apeltanthus*, species that belong to sect. *Lupulinaria* subsect. *Lupulinaria* was investigated and two chemical groups are found in this taxon. The first containing compound group E1, with or without the presence of catalpol, e.g. *S. alpina* and its varieties, and those which do not contain either, e.g. *S, orientalis, S. taurica* and S. *sosnovskyi*. Iridoids have been used as chemical markers for the Lamiiflorae superorder and in chemosystematics. Despite the fact that *S. hastifolia* belongs to the subgenus *Scutellaria*, even all the extracts, fractions and subfractions of *S. hastifolia* were investigated, iridoids are not encountered during this study (239).



		R
1	Isorengyol	H
2	Isorengyoside	β-Glc



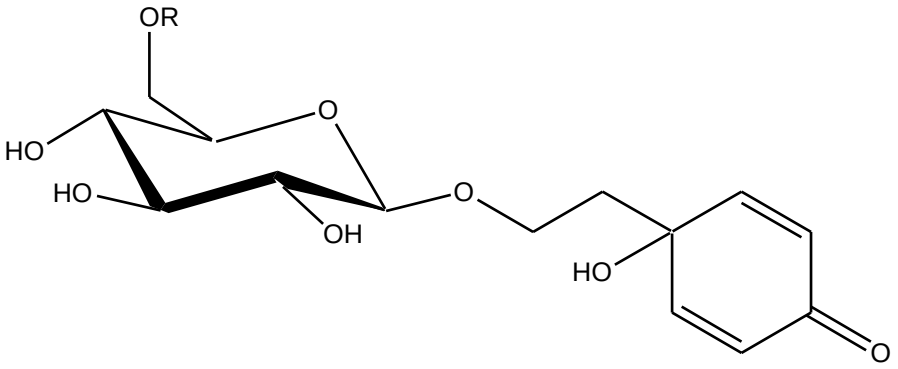
3 Cleroindicin B



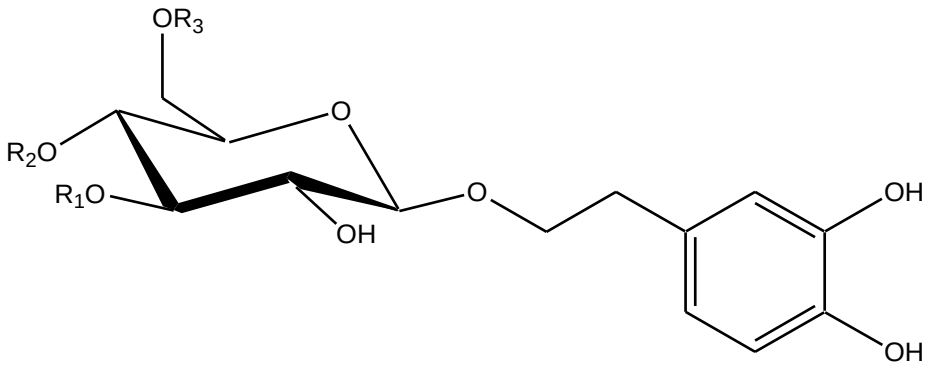
4 Cleroindicin F

Ethylcyclohexane derivatives whose structures are given above isolated from both acetone and MeOH extracts of *S. hastifolia* are isorengyol (**1**), isorengyoside (**2**), cleroindicin B (**3**) and cleroindicin F (**4**). Moreover, we also isolated another cleroindicin derivative (SH-57) which seems to be isocleroindicin A, in preliminary analysis. However, further spectroscopic studies are going on SH-57.

Ethylcyclohexanoids from the title plant can be classified into two groups, monocyclic (**1**-**3**) and bicyclic (**4**) ethylcyclohexanes. This group of compounds were previously reported from Bignoniaceae, Oleaceae, Theaceae and Gesneriaceae (240–244). Except isorengyoside, all isolated ethylcylochexane derivatives have been limitedly isolated from the plants of Lamiaceae, for example only from two *Clerodendrum* species (*C.indicum* and *C. trichotomum*) which were formerly a member of Verbenaceae but now placed under Lamiaceae. This is the first time that those group of compounds were isolated from the genus *Scutellaria* (225,245). The glycoside of isorengyol, namely isorengyoside only isolated from *Forsythia suspensa* fruits in 1989 and in this study, it is being reported for the second time from nature (241). Biogenesis like transformations of salidroside to many diverse cycloheylethanoids were reported and possible biogenetic route was suggested (246). In this pathway as well as the isolated ethylcyclohexanoid derivative compounds (**1**-**4**), a precursor of some phenylethanoid glycosides cornoside (**5**) were synthesised. According to the presence of ethylcyclohexanes in the *S. hastifolia*, meaning that this species resembles to various species such as *Peltanthera* sp., *Halleria* sp., *Oroxylum* sp., *Millitonga* sp., *Forsthyia* sp., from the families Gesneriaceae, Scrophulariaceae, Bignoniaceae, and Oleaceae respectively, for both bearing cyclohexanoids and not containing iridoids (240,244,247,248).



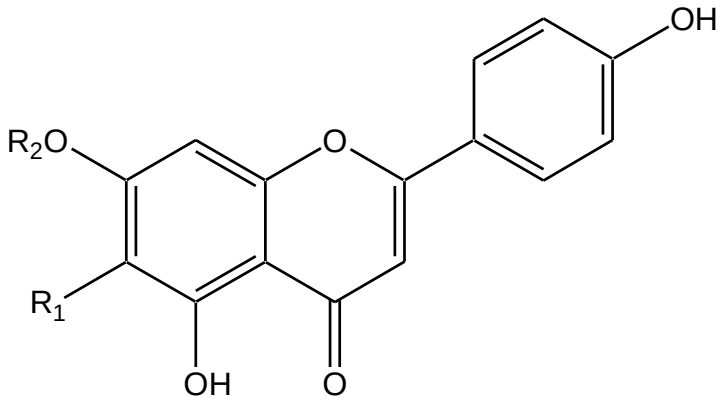
		R
5	Cornoside	H
6	Calceolarioside D	*trans*-caffeoyl
7	Hastifolioside	*trans*-feruloyl
8	Neocalceolarioside D	*p-trans*-coumaroyl



		R_1	**R_2**	**R_3**
9	Calceolarioside B	H	H	*trans*-caffeoyl
10	Verbascoside	α-Rha	*trans*-caffeoyl	H

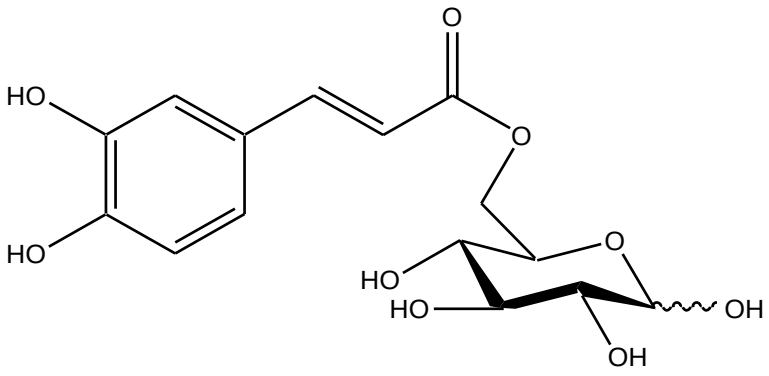
Phenylethanoid glycosides, obtained in this study can be categorized into two groups: cornoside derivatives [cornoside (**5**), calceolarioside D (**6**), hastifolioside (**7**), neocalceolarioside D (**8**)] and phenylethyl derivatives [calceolarioside B (**9**) and verbascoside (**10**)] as seen from the molecular structures above. The phenylethanoid glycosides obtained in this study were either monoglycosidic (**5**-**9**) or diglycosidic (**10**). Phenylethanoid glycosides are also accepted useful chemotaxomic markers due to their limited distribution. These compounds are not specific to any plant organ and have been previously purified from plant roots, barks, leaves, aerial parts and moreover from callus and suspension cultures. The majority of the phenylethanoid glycosides are found in Scrophulariaceae, Oleaceae, Plantaginaceae, Lamiaceae and Orobanchaceae families. For example verbascoside, has been isolated from number of genera within the Lamiaceae family, including *Stachys*, *Lamium*, *Marrubium*, *Phlomis*, *Sideritis* (249). Even more, verbascoside and calceolarioside B were already purified from various *Scutellaria* species as mentioned previously (133,159). Other phenylethanoids, rather than verbascoside have limited distribution among the plant kingdom. As mentioned above, it is biosynthetised from salidroside and is likely to belong to the same C6-C2 pool that gives rise to verbascoside. Rengyolone (helleridone) was considered as an artifact formed from cornoside and generally co-occuring with cornoside, hovewer in our study we did not encounter rengyolone. Cornoside is a compound with a limited distribution in plants, and found in many species of *Cornus*, *Eurya*, *Abeliophyllum*, *Forsythia* and *Olea*. Also it is found in the families Gesneriaceae, and Bignoniaceae (250). Moreover it was reported from *Digitalis* sp. as helleridone and also as cornoside (251). Besides those families, cornoside isolated from only two species of Lamiaceae, *Teucrium decipiens* and *Clerodendrum chinense* (252,253). When cornoside was obtained for the first time, in *Cornus* sp., the distribution was confined to the species that do not contain iridoids and this is totally consistent with the DNA sequence results. However some species of Bignoniaceae and Lamiaceae as well as Oleaceae are the exceptions. It is noteworthy that cornoside seems especially frequent in the first branching families of Lamiales (Plocospermataceae, Oleaceae, Tetrachondraceae, Gesneriaceae, Calceolariaceceae), but scattered to absent in most other families (250). Although cornoside was previously obtained from Lamiaceae plants, calceolarioside D and neocalceolarioside D were never isolated from any plant of Lamiaceae previously.

Cornoside, calceolarioside D and neocalceolarioside D were purified from *Abeliophyllum distichum* which belongs to Oleaceae, proving that the similarity between the genera *Abeliophyllum* and *Scutellaria* and the families Lamiaceae and Oleaceae in their chemistry (228). In addition to *Abeliophyllum* sp., from *Fraxinus ornus* (Oleaceae), verbascoside and calceolarioside B were also isolated (254). Moreover, neocalceolarioside D was additionally obtained from *Eurya japonica* (Theaceae) also verifying the similarities between *S. hastifolia* mentioned in ethylcyclohexane derivatives section (255). Even more, three phenylethanoids, calceolarioside D, calceolarioside B and verbascoside were initially detected from various *Calceolaria* sp. which was also revealing the similarities between Scrophulariaceae and Lamiaceae (256). This is the first study that cornoside, calceolarioside D and neocalceolarioside D were isolated from the genus *Scutellaria* while calceolarioside D and neocalceolarioside D were being isolated for the first time from Lamiaceae. In addition, a new cornoside derivative phenylethanoid glycoside was isolated from the acetone extract for the first time and named as hastifolioside.

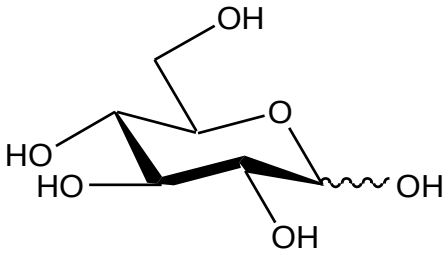


		R_1	R_2
11	Apigenin 7-*O*-β-D-glucopyranoside	H	β-Glc
12	Scutellarein 7-*O*-β-D-glucopyranoside	OH	β-Glc
13	Hispidulin 7-*O*-β-D-glucopyranoside	OCH_3	β-Glc
14	Hispidulin 7-*O*-β-D-glucuronopyranoside	OCH_3	β-GluA

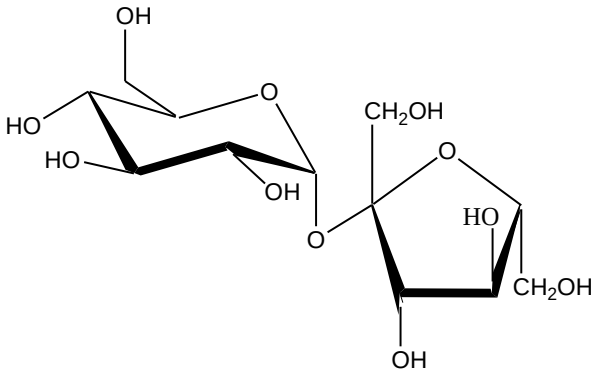
Isolated flavonoids were apigenin 7-*O*-β-D-glucopyranoside (**11**), scutellarein 7-*O*-β-D-glucopyranoside (**12**), hispidulin 7-*O*-β-D-glucopyranoside (**13**) and hispidulin 7-*O*-β-D-glucuronopyranoside (**14**). All of the isolated flavonoids were monoglycosidic (7-*O*-glycosidic) flavone derivative. Moreover, they are 4'-OH substitued and except for apigenin 7-*O*-glucopyranoside, all are 6-*O*-substituted flavon glycoside. Except hispidulin 7-*O*-β-D-glucopyranoside, all the flavonoids were previously obtained from various *Scutellaria* species as mentioned before.



15 6-*O*-caffeoyl-α/β-glucopyranose



16 α/β-glucopyranose



17 Saccharose

Moreover, a sugar ester epimer [6-*O*-caffeoyl-α/β-glucosepyranose (**15**)] and two sugar molecues [mixture of α/β-glucose (**16**) and saccharose (**17**)] were isolated from *S. hastifolia*. 6-*O*-caffeoyl-D-glucopyranose was previously isolated from *Prunus buergeriana*, *P. grayana* (Rosaceae) and *Caryopteris incana* (Lamiaceae) (257–259). Saccharose was previously isolated from *S. schacristanica* (260). But this is the first time that 6-*O*-caffeoyl-D-glucopyranose was isolated from the genus *Scutellaria*.

Except two sugar molecules (glucose and sucrose) all of the isolates were further investigated for their effects on DPPH scavenging, lipid peroxidation inhibition and LOX inhibition. Except for ethylcyclohexane derivative compounds, two phenylethanoid glycosides (hastifolioside and neocalceolarioside D) and sugar ester, all other isolates scavenged DPPH radical more than that the reference substance NDGA (83±1.8%). The highest activity was shown by scutellarein 7-*O*-β-D-glucopyranoside (98±1.2). Among the ethylcyclohexane derivatives, isorengyoside, the glycosidic derivative of isorengyol, did not show any activity however all other

ethylcyclohexanoids displayed significant activity. Among the phenylethanoid glycosides tested, calceolarioside D, calceolarioside B and verbascoside exerted equipotent DPPH radical scavenging. The high interaction values of the phenylethanoid glycosides could be attributed to the presence of the caffeoyl-group and to the *o*-dihydroxyphenyl group. They all have at least one caffeoyl moiety in common. Cornoside without caffeoyl moiety but cyclohexanone moiety also showed activity more than the reference substance. Distinctively, hastifolioside and neocalceolarioside D that bear feruloyl and *p*-coumaroyl moiety with an exception of an –OH group when compared with calceolarioside D did not show any activity. Furthermore, the presence of either glucose or glucuronic acid in the flavonoid structure, did not alter the activity displayed by these compounds in DPPH assay. Flavonoids with at least one OH group at ring B, presented a significant antioxidant activity. This behaviour is associated with the combination of the C_2–C_3 double bond and the 5-OH. On the contrary, whole compounds exerted weak inhibition on lipid peroxidation. In this assay, glycosidic ethylcyclohexane derivative, isorengyoside elicited the waekest inhibition, and apigenin 7-*O*-β-glucopyranoside exerted the highest activity. Whole ethylcyclohexane derivatives without sugar molecule, elicited similar but weak inhibition. Amongst phenylethanoid glycosides, except hastifolioside, all cornoside derivative phenylethanoid glycosides showed relatively high inhibition. But verbascoside and calceolarioside B showed very weak activity. Interestingly, the glucose derivative of hispidulin showed 29% inhibition, whereas glucuronic acid derivative did not display any activity. 4'-OH group in antioxidant activity of flavonoids, as seen in the results, all the flavonoids with 4'-OH group exerted significant DPPH radical scavenging. Moreover, in apigenin and scutellarein, the presence of more phenolic -OH groups rather than hispidulin derivatives resulted in the higher antioxidant activity. There is only one study related with antioxidant activities of ethylcyclohexane derivatives containing only cleroindicin B. In a former study conducted by Oh et al., DPPH radical scavenging activity of cleroindicin B was evaluated. Nevertheles the compound displayed very low activity when compared with the water soluble vitamin E derivative, trolox (242). In another study concerning verbascoside, it was found that DPPH scavenging activiy was found as similar as positive control, quercetin (223). Harput et al., isolated verbascoside and its desrhamnosyl derivative calceolarioside A from

Plantago lagopus and evaluated their radical scavenging activity. Calceolarioside A is the isomer of calceolarioside B. Both compounds showed strong activity similarly as the results obtained from this work (261). Additionally in another study, verbascoside was also found to be very active on DPPH radical scavenging even at 0.1 mM concentration. Yet, in lipid peroxidation inhibition assay, they found 77.4% inhibition which was higher than the reference compound trolox. It was found 13% inhibition in the present study (262). This might be due to the concentration differences between the experiments. The activity might decrease when the concentration increases. Antioxidant activity of hispidulin derivatives was investigated by Weng et al. and similar results to the findings (DPPH) above were reported, although the methods were different (263). Moreover, Yan et al., tested scutellarein 7-*O*-β-D-glucopyranoside for its DPPH radical scavenging activity, and same as results of this work, they found the highest antioxidant activity when compared with reference substance (264). In another study conducted by Ono et al., the scavenging effects of hispidulin 7-*O*-β-D-glucopyranoside and verbascoside were investigated. The results showed that the EC_{50} value of verbascoside was calculated almost thrice of the reference substance α-tocopherol. In addition, in the same study hispidulin 7-*O*-β-D-glucopyranoside also showed the scavenging activity. In this thesis both compounds elicited high scavenging activity to DPPH similar to previous studies (265).

The isolates were also evaluated for their antiinflammatory activity by using *in vitro* soybean LOX inhibition assay. The antioxidant activity (DPPH interaction and inhibition of lipid peroxidation) and the ability to act as ligands for the Fe in the active site of LOX, can express the LOX inhibition. Chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. Among the tested compounds, except apigenin 7-*O*-β-D-glucopyranoside, none of the compounds displayed significant activity at the tested concentration. However, in a study conducted by Chami et al., it was found that at different concentrations, verbascoside exerted a remarkable LOX inhibition which was found inactive in this study (262). The highest activity was seen in MeOH extract (100%) in LOX inhibition but acetone and MeOH:H_2O extracts did not show any inhibition at the tested concentrations. Separately each of the isolates from extract, showed weaker activity when compared with the crude extract. This might be due to the

synergistic activity of the metabolites in MeOH extract or improper working concentrations.

Based on the phytochemical studies, phenylethanoid glycosides and flavonoids constitue the major classes of *S. hastifolia*. We also aimed to quantify these molecules in the MeOH extract of *S. hastifolia*. Moreover the simultaneous detection and quantification of these compounds in the remaining *Scutellaria* species (*S. velenovskyi*, *S. orientalis* and *S. albida*) were also aimed. Due to the complex nature of the extracts, two different methods were developed and validated. Phenylethanoids analysed were calceolarioside D, neocalceolarioside D, verbascoside, isoverbascoside, leucoseptoside A and martynoside. In *S. hastifolia*, calceolarioside D was found as the major phenylethanoid glycoside (2.58±0.05%) and martynoside was found to be the minor one (0.27±0.005%). In *S. velenovskyi*, verbascoside, isoverbascoside, leucoseptoside A and martynoside were detected and well quantified their amounts. Martynoside was found with the highest concentration (0.37±0.015%) and leucoseptoside A was found in the lowest amount (0.03±0.001%). Moreover, most importantly, from *S. velenovskyi*, catalpol and two other iridoids were isolated. As mentioned above, catalpol is a chemotaxonomic marker in *Scutellaria* species. Where catalpol is found, cornoside and its derivatives are not encountered. This study also supports this assumption as calceolarioside D and neocalceolarioside D were not detected in this plant which are the derivatives of cornoside. Furthermore, in previous study conducted by Skaltsa et al., in MeOH extract of *S. velenovskyi* from Turkey, they have reported three iridoids; catalpol, albidoside and macfedienoside, and four phenylethanoid glycosides; verbascoside, leucoseptoside A, martynoside and a derivative of martynoside verifying the results obtained from this work (266). In *S. orientalis*, verbascoside and martynoside were found, both of which were previously isolated. Despite the fact that leucoseptoside A was isolated in former studies from *S. orientalis*, in this studt it could not detected in this study. Finally in *S. albida*, neocalceolarioside D, verbascoside and martynoside were detected. In former studies verbascoside and martynoside were isolated in *S. albida* that verifying this work. Amongst phenylethanoid glycosides, neocalceolarioside D is being reported for the first time from *S. albida*.

In addition to phenylethanoid glycosides, flavonoids were also analysed in those four *Scutellaria* sp. In *S. hastifolia*, the quantities of the isolated flavonoids, namely,

scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside were calculated, plus apigenin aglycone was detected and the amount was calculated. It was predictable because its glucose derivative was isolated in this plant. In *S. velenovskyi,* all the tested flavonoids were detected and quantified. As mentioned above, in a study concerning the chemical composition of the MeOH extract of *S. velenovskyi*, Skaltsa et al. isolated glucuronic acid derivatives of scutellarein and hispidulin similarly with this findings (266). In *S. orientalis*, among the tested flavonoids, except hispidulin 7-*O*-β-glucopyranoside, all of them were detected. Eventually, in *S. albida*, apigenin 7-*O*-β-glucopyranoside, hispidulin 7-*O*-β-glucopyranoside and apigenin were quantified.

6. CONCLUSION

In this part, the summary of this thesis and future prospects are given.

CONCLUSION

The genus *Scutellaria* contains medicinal species, and is currently under investigation worldwide. Overall, antioxidant activities of four *Scutellaria* taxa were compared by evaluating their total antioxidant capacity, total phenolic and total flavonoid contents. By considering these results along with the preliminary phytochemical results obtained by TLC analysis, for further detailed studies *S. hastifolia* was selected.

Totally 17 compounds were isolated from the acetone and MeOH extracts one of which was new (hastifolioside) from the aerial parts of *S. hastifolia*, Isolated compounds, can be categorized as ethylcyclohexane derivatives (isorengyol, isorengyoside, cleroindicin B, cleroindicin F), phenylethanoid glycosides (cornoside, calceolarioside D, hastifolioside, neocalceolarioside D, calceolarioside B, verbascoside), flavonoids (apigenin 7-*O*-β-D-glucopyranoside, scutellarein 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucopyranoside, hispidulin 7-*O*-β-D-glucuronopyranoside), sugar ester (6-*O*-caffeoylglucopyranose) and sugars (glucose, sucrose).

Along with the phytochemical studies, antioxidant and antiinflammatory activities of crude extracts and isolates were evaluated by photometric DPPH radical scavenging assay, lipid peroxidation inhibition assay and soybean LOX inhibition assay. In DPPH scavenging assay, all the extracts of *S. hastifolia* exerted remarkable antioxidant activity. Moreover, in lipid peroxidation inhibition assay, acetone extract displayed antioxidant activity as much as reference substance trolox. In addition to antioxidant activity experiments, the soybean LOX inhibition assay was performed in order to evaluate antiinflammatory potential as well as antioxidant potential of the title plant. MeOH extract exerted significant inhibition (100%) at the tested concentration. The isolates were further tested for their DPPH scavenging activity, linoleic acid lipid peroxidation inhibition and soybean LOX inhibition. Among them compounds with high antioxidant activities were identified. Although MeOH extract showed noteworthy LOX inhibition and the isolates showed weaker LOX inhibition. The activity of MeOH extracts might come from the synergism of the compounds or the low activity of the compounds might be due to the tested concentrations.

Subsequently, two different HPLC methods were developed for simultaneous quantification of six phenylethanoid glycosides as well as six flavonoids in four *Scutellaria* sp. which were tested for their antioxidant activities formerly. The results obtained by this analysis were consistent with previously published data.

The biological activiy (antioxidant and antiinflammatory) of this plant should further be examined thoroughly by *in vivo* assays and the mechanism of actions should be enlightened. The data acquired from this project will shed light on the future researches regarding the chemical composition and the bioactivities of *Scutellaria* species. As such the other *Scutellaria* species from flora of Turkey deserve attention interms of their potent biological activities and phytochemicals.

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8. CURRICULUM VITAE

Hilal Bardakcı Altan was born in June, 1985 in Uşak, Turkey. She graduated from Şehit Necati Sargın Anadolu Lisesi. She registered Yeditepe University Faculty of Pharmacy in the same year and graduated in July, 2008 and attended Yeditepe University Institute of Health Sciences Pharmacognosy PhD. Programme in August, 2008. She started to work as a research and teaching assistant in Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy in 2008. She is currently employed in the same job. She is married since 2011.