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약학석사 학위논문

**Chemical constituents isolated from roots of  
*Glycyrrhiza uralensis* and its activities to  
LPS induced RAW 264.7 cells and  
C2C12 myoblasts**

감초 뿌리 추출물의 화학적 조성과 LPS 유도  
RAW 264.7 세포 및 C2C12 근원세포에 대한 생리활성

2023 년 2 월

서울대학교 대학원

약학과 생약학 전공

윤 상 준

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이 논문을 약학석사 학위논문으로 제출함  
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## Abstract

*Glycyrrhiza uralensis* Fisch is belong to Fabaceae Family, native to E. European Russia to Siberia and Pakistan. It is a perennial and grows primarily in the temperate biome. Licorice has been widely used in oriental medicine mainly in East asia, anti-inflammatory effect by isoliquiritigenin and antioxidant activities by glycy coumarin are well known. Aside from these licorice possesse variety bioactive compounds like triterpenoids, coumarins, flavonoids, chalcones, isoflavans, stilbenoids. Although licorice radix is one of the major medicinal herb, domestic requirements still largely depend on imports. To meet domestic demand, three of unique species Dagam, Sinwongam, Wongam had been developed but studies related with contents of secondary metabolites and morphological differences for breeding varieties are insufficient. In accordance with these needs fourty compounds containing triterpenoid, saponin, coumarin, flavonoid, chalcone type were isolated from roots of *G. uralensis* and their chemical structures and properties analyzed by NMR, UV, HRMS.

Among fourty compounds isolated from *G. uralensis* compound **11** (isoderrone), **24** (allolicoisoflavone B), **25** (3'-( $\gamma$ ,  $\gamma$ -dimethyl)-kievitone), **30** (glycyrrhizin) showed proliferatory activity to C2C12 myoblast and compounds **12** (glisoflavanone), **20** (glyurallin A), **22** (glabrene), **24** (allolicoisoflavone B) inhibited the nitrite accumulation without cytotoxicity.

**Keyword:** *Glycyrrhiza uralensis*, triterpenoid, coumarin, flavonoid, chalcone, saponin, anti-inflammation, anti diabetic

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## List of abbreviations

br s: broad singlet

*n*-BuOH: *n*-butanol

CC: column chromatography

MeCN: acetonitrile

d: doublet

dd: doublet doublet

ddd: doublet doublet doublet

dt: doublet triplet

DMEM: Dulbecco's modified Eagle's medium

DMSO: dimethyl sulfoxide

EtOAc: ethyl acetate

EtoH: ethanol

FBS: fetal bovine serum

HPLC: high pressure liquid chromatography

HRESIMS: high resolution electron spray ionization mass spectrometry

Hz: hertz

IHD: index of hydrogen deficiency

m: multiplet

MeOH: methanol

MPLC: medium pressure liquid chromatography

MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide

*m/z*: mass to charge ration

NMR: nuclear magnetic resonance

NP: normal phase

PBS: phosphate buffered saline

RP: reverse phase

s: siglet

t: triplet

TLC: thin-layer chromatography

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# 1. Introduction

## 1.1. Licorice properties and distribution

Licorice is a flowering plant of the bean family Fabaceae and is perennial growing to 0.6 m (2ft) by 0.4 m (1ft 4in). it is in flower from June to August, and the seeds ripen from July to October. The species is hermaphrodite.

Requires a deep well cultivated fertile moisture-retentive soil for good root production. Prefers a sandy soil with abundant moisture. Slightly alkaline conditions produce the best plants. This species is widely cultivated in China as a medicinal plant. Unless seed is required, the plant is usually prevented from flowering so that it puts more energy into producing good quality roots. This species has a symbiotic relationship with certain soil bacteria, these bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby. The plant growth habit is a runner spreading indefinitely by rhizomes or stolons. The root pattern is rhizomatous with underground stems sending roots and shoots along their length (Jacke et al., 2005). There are more than 30 species of *Glycyrrhiza* genus extensively spread worldwide (Rizzato et al., 2017). Licorice has been planted since the 16th century in Europe. It was the most prescribed herb in Ancient Egyptian, Roman, Greek, East China, and the West from the Former Han era (Shibata, S et al., 2000). Various species of licorice are cultivated in Europe, the USA, SouthWestern Asia and Central Africa, the Middle East, Afghanistan, and the North part of India. In addition, England, Spain, Iraq, Turkey, China, and Sicily commercially cultivate licorice (Tohma et al., 2010). Other countries which are also producing the licorice are Pakistan, Azerbaijan, Turkmenistan, and Uzbekistan. On the world map, licorice-

producing areas are shown in Figure1.

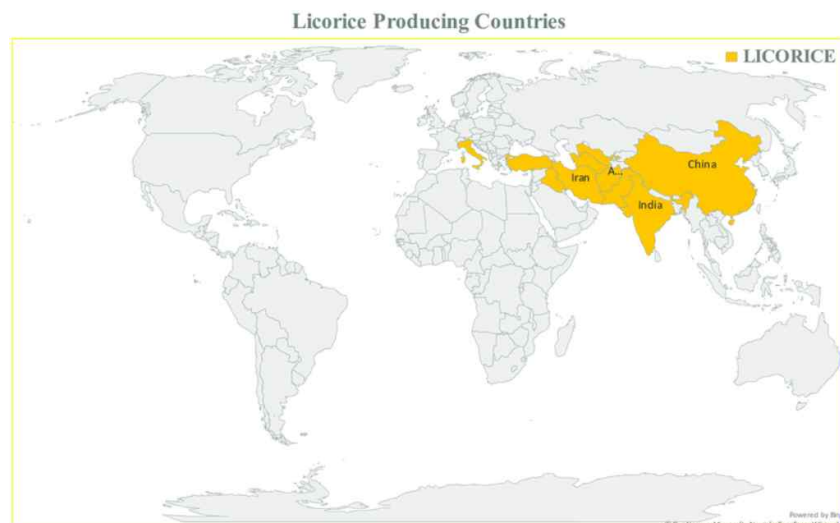


Figure 1. Licorice cultivated countries

(Shadma Wahab et al., 2021)

## 1.2. Morphology

As for the originated plants of licorice described in the KP, three species indicated *Glycyrrhiza uralensis*, *Glycyrrhiza glabra*, and *Glycyrrhiza inflata*.

In cross-section under a microscope, the licorice with the shell yellowish brown in layers and has one to three layers inside have a cork skin. The skin has a bundle of thickened, less cotton infibrils, and bundle of fibers mainly crystalline cells in a row. The old one not close to the formation layer generally degraded and visible penetrates the formation layer to the skin, and the cells are full of starch. Fibers surrounded by crystalline cell lines. Bundles are scattered between ducts. The rootstock can be male, and the skin and cells contain calcium oxalate starchpowder, the licorice without peel has no periderm.

*Glycyrrhiza uralensis* has a cylindrical shape sprinkled with roots, and is 25-100 cm

long and 5-35 mm in diameter. On the outside, it is reddish-brown or yellowish-brown, and has vertical wrinkle patterns, recessed patterns and sparse root marks. The cut side is fibrous, yellowish-white powdery and the formation layer has a distinct ring, and the line is radial. The rhizome is cylindrical and has sprouting marks on the outside.

*Glycyrrhiza glabra* is woody, thick and strong roots, and sometimes branches are split. The outer shell rough, most of them are grayish brown, and the shell eyes are thin and indistinct.

*Glycyrrhiza inflata* is relatively robust in stem and sometimes branches are split. It's rough on the outside, usually grayish-brown, and its shell eyes thin and indistinct (Choi, 2015).



Figure 2. Aerial and root part of licorice

### 1.3. Biological activities of licorice

According to a traditional Chinese medicine belief, “nine out of ten formulae contain licorice,” and licorice is one of the most effective herbal medicines for reducing toxicity and increasing the efficacy of other herbal medicines when used together. It may also be a health food product and natural sweetener because it is a “medicine food homology” herbal medication (Jiang et al., 2020).

Amino acids, proteins, simple sugars, polysaccharides, mineral salts, pectin, starches, sterols, gums, and resins are all found in licorice (Wang et al., 2015).

Prenylflavonoid type like glycycomarin exhibit antioxidant activities by inducing peroxisome proliferation-activated receptor (PPAR)- $\gamma$  ligand bond. And isoliquiritigenin alleviate vascular endothelial inflammation by inhibiting expression of vascular cell adhesion molecule (VCAM)-1 also restraining nuclear factor-KappaB (NF- $\kappa$ B), cyclooxygenase-2 (COX-2) lead to anti-inflammatory activities. And licochalcone A arouse osteoblast differentiation (Ahn *et al.*, 2006; Wu et al., 2011; Cao *et al.*, 2017).

Flavonoids are the main effective constituents extracted from rhizomes and the roots of licorice and Several studies have exhibited that these components contain properties that suppress the expansion of cells derived from different cancers (Tungmunthum et al., 2018; Zhou et al., 2016), such as gastric cancer (Zhang et al., 2018; Wei et al., 2017), breast cancer (Kwon et al., 2013).

Glycyrrhetic acid suppress the enzyme 11 $\beta$ -hydrogenase type II (11 $\beta$ -HSD2) that changes cortisol to cortisone. High cortisol levels result from a modest mineralocorticoid abundance in the kidney and boost systemic vascular resistance by provoking mineralocorticoid receptors.

Isoliquiritigenin (2',4',4'-trihydroxychalcone, ISL) has a chalcone structure that

exhibits a strong anticancer effect. Glycyrrhizin, glycyrrhizinic acid, isoliquiritin, and glycyrrhizic acid are other main chemicals in this plant with antiatherogenic, anti-cancer, anti-diabetic, anti-microbial, antispasmodic, anti-inflammatory, and anti-asthmatic properties (Gaur, R et al., 2014). Licorice has also been documented to help with weariness and debilitation in China. In addition, licorice acts as an anti-inflammatory, reducing allergic responses and preventing liver damage. According to the World Health Organization, licorice is used as a demulcent for sore throats and an expectorant for bronchial catarrh and coughs (Bagchi, D., 2017). There have been no reports of potentially toxic compounds from the taxa that have been studied so far. However, some adverse consequences are recognized, such as using high dosages over a prolonged period, resulting in serious illnesses. Nevertheless, the plant may be used for a medicinal purpose in small dosages for significant ailments, and there are no known side effects.

#### 1.4. Structural diversities from licorice extract

Considered as an excellent crude drug and plant model by primitive societies, due to its impressive pharmaceutical and culinary (as a sweetener) properties, studies have been carried out in order to determine the main responsible active principles for those benefits and investigate the modes of action. Among liquorice phytochemical constituents, glycyrrhizin (also known as glycyrrhizic or glycyrrhizinic acid), an oleanane-type triterpene saponin, is the major constituent (Hayasi et al., 2009). In addition, liquiritin apioside is the most abundant flavonoid compound in liquorice roots (Kondo et al., 2007) with significant antioxidant properties (Guan et al., 2012). However, other constituents, not of lesser importance but also contributors to important biological properties, are present, namely phenolic

compounds. Compounds such as glabridin, hispaglabridin (A and B), 4'-O-methylglabridin, isoprenylchalcone, liquiritigenin, isoliquiritigenin and formononetin are among the most abundant bioactive phenolic compounds that have been identified so far, with several bioactivity properties (Chin et al., 2007; Jiang et al., 2013; Martins et al., 2015; Vaya et al., 1997; Xie et al., 2014). The chemical structure of the main ingredients of liquorice, including glycyrrhizin and glabridin. Moreover, 15 different saponins and 49 phenolic compounds (including their glycosides) have been already identified in liquorice roots (Kitagawa et al., 2002). So, apart from the previously highlighted major components, there are other compounds that in spite of their low content have also significant properties. Among them, many flavonoids (5,8-dihydroxy-flavone-7-O- $\beta$ -D-glucuronide (glychionide A), 5-hydroxy-8-methoxyl-flavone-7-O- $\beta$ -D-glucuronide (glychionide B), glabrene, glabrone, glabraisoflavanone A, glabraisoflavanone B, isoviolanthin, 5,7-dihydroxyflavanone, rhamnoliquiritin), (Frag et al., 2012; Li et al., 2005; Simons et al., 2011; Suman et al., 2009) as well as saponins (licorice saponin A3, licorice saponin G2, licorice saponin J2, licorice saponin C2), (Farag et al., 2012) coumarins (i.e. glycy coumarin) (Lee et al., 2003) and chalcones (isoliquiritin, neolicucuroside, licochalcone B), are included (Farag et al., 2012).



two as well as an absorption valley at approximately 254 nm, the maximum absorption of compounds in group one. In view of this, compounds from different groups can be distinguished from each other by detecting at different wavelengths, 254, 280, and 355 nm, when they are coeluted (Zhang et al., 2013).

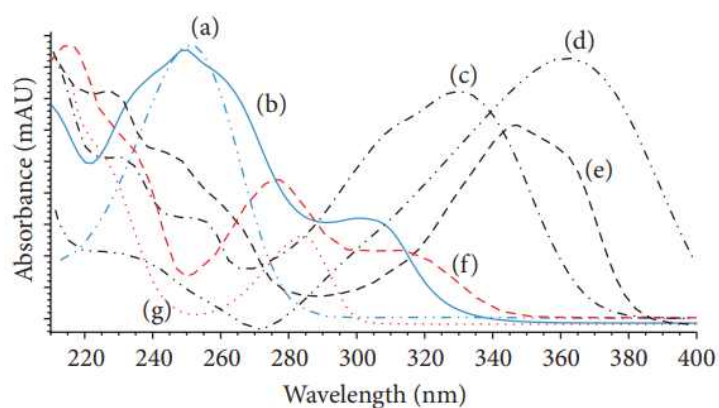


Figure 4. Ultraviolet spectra for different types of compounds. (Zhang et al., 2013)

Representative chromatogram of all 16 analytes in licorice is shown in Figure 4. All 16 compounds can be the baseline separated from the background constituents in licorice in a related short run time (60 min) when the detector response was recorded at a certain wavelength for each type of compound. The selectivity of the method was further proved by HPLC ESI MS analysis and purity analyses of HPLC UV peaks. The MS/MS data of 16 target analytes is shown in Table 1. Peak purities of all target analytes were larger than 950 at the range of their detection wavelengths. Peak purities of compounds 2, 3, 5, 6, 7, 8, 14, 15, and 16 were also proved by the MS spectra obtained at the range of the peaks (Zhang et al., 2013). The amount of glycyrrhizin in wild licorice was consistent with the results in the literature (Wang et al., 2007; Tan et al., 2010).

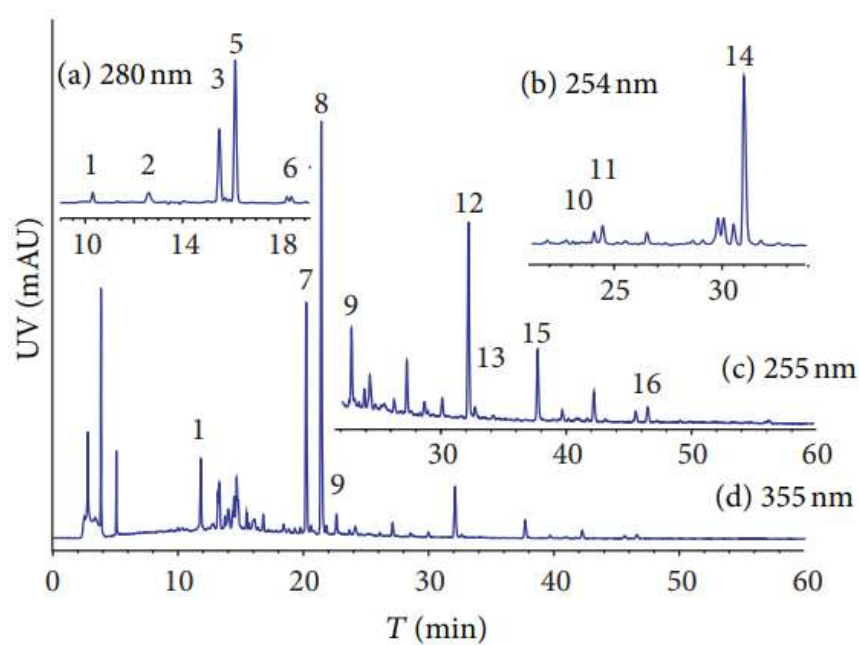


Figure 5. Representative chromatograms for licorice.

(Zhang et al., 2013)

Comp no.	Name	$M_r$	$R_t$ /min	MS/MS data	UV spectrum
1	Liquiritigenin; 4'-O-glucopyranyl-(1-2)-glucopyranoside	580	10.4	489, 417, 255	F
2	Sophoraflavone B	416	14.2	415, 253	C
3	Liquiritin apioside	550	15.5	549, 417, 295, 255	F
4	Liquiritigenin; 7,4'-O-diglucopyranoside	580	15.8	417, 255	F
5	Liquiritin	418	16.2	255, 135, 119	F
6	6''-O- $\alpha$ -hydroxypropynoylliquiritin	490	18.4	417, 399, 327, 255, 135	F
7	IsoLiquiritin apioside	550	19.6	549, 429, 417, 357, 327, 297, 255	D
8	Isoliquiritin	418	20.7	297, 255, 135, 119	D
9	Kumatakenin B	254	21.8	253	C
10	Medicarpin-3-O-glucoside	432	23.5	477, 431, 311, 269	G
11	Liquiritigenin	256	24.3	255, 153, 135, 119	F
12	Isoliquiritigenin	256	30.9	255, 153, 135, 119	D
13	Formononetin	268	31.7	267, 252	B
14	Glycyrrhizin	822	32.4	803, 777, 645, 627, 351	A
15	Neoglycyrol	366	41.0	365, 350, 307, 295	E
16	Isoglycyrol	366	45.0	365, 350	E

Table 1. Electrospray ionization mass and ultraviolet spectra data of 16 compounds

(Zhang et al., 2013)

## 1.6. Inflammation

In response to tissue injury, a multifactorial network of chemical signals initiate and maintain a host response designed to 'heal' the afflicted tissue. This involves activation and directed migration of leukocytes (neutrophils, monocytes and eosinophils) from the venous system to sites of damage, and tissue mast cells also have a significant role. For neutrophils, a four-step mechanism is believed to coordinate recruitment of these inflammatory cells to sites of tissue injury and to the provisional extracellular matrix (ECM) that forms a scaffolding upon which fibroblast and endothelial cells proliferate and migrate, thus providing a nidus for reconstitution of the normal microenvironment (Chettibi, S. & Ferguson, M. W. J., 1999).

The relationship between inflammation and cancer is not new. Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma, and DNA-damage-promoting agents, certainly potentiates and/or promotes neoplastic risk. During tissue injury associated with wounding, cell proliferation is enhanced while the tissue regenerates; proliferation and inflammation subside after the assaulting agent is removed or the repair completed. In contrast, proliferating cells that sustain DNA damage and/or mutagenic assault (for example, initiated cells) continue to proliferate in microenvironments rich in inflammatory cells and growth/survival factors that support their growth. In a sense, tumours act as wounds that fail to heal (Nathan, 2006). As a result, developing anti inflammatory from natural resources based on bio-guided assay seems to need for tentative approaches for treating inflammation.

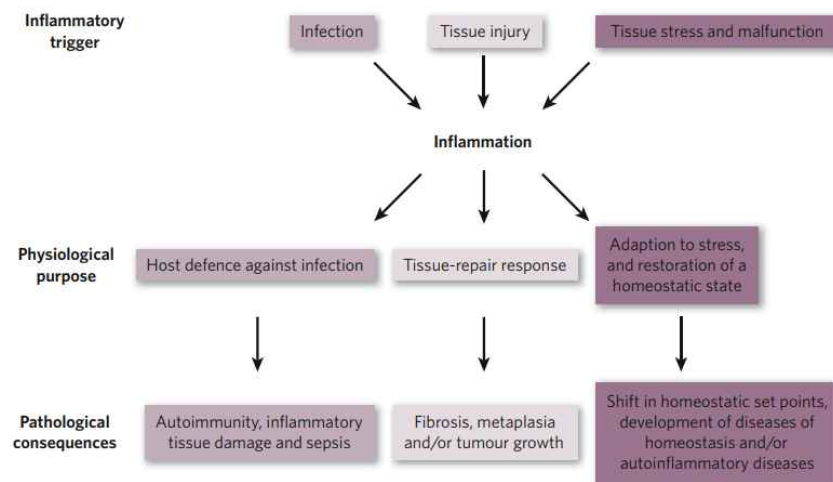


Figure 6. Causes and physiological and pathological outcomes of inflammation

(Ruslan Medzhitov, 2008)

### 1.7. Type 2 diabetes

Type 2 diabetes is characterised by relative insulin deficiency caused by pancreatic  $\beta$ -cell dysfunction and insulin resistance in target organs. Between 1980 and 2004, the global rise in obesity, sedentary lifestyles, and an ageing population have quadrupled the incidence and prevalence of type 2 diabetes (smolen et al., 2016). As the sixth leading cause of disability in 2015 (Vos et al., 2015), diabetes places considerable socioeconomic pressures on the individual and overwhelming costs to global health economies, estimated at US\$825 billion (Seuring et al., 2015). Cardiovascular disease is the greatest cause of morbidity and mortality associated with type 2 diabetes (WHO, 2014) and needs intensive management of glucose and lipid concentrations as well as blood pressure to minimise risk of complications and disease progression (Gæde et al., 2003). The benefits of intensive glucose management on microvascular complications, such as retinopathy, nephropathy, and neuropathy, have been shown in several large randomised controlled trials, including

the United Kingdom Prospective Diabetes Study (UKPDS, 1998), Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE, 2008), and Veterans Association Diabetes Trial (Duckworth et al., 2009). Hypoglycaemia is a major barrier to optimising glucose-lowering therapy, and results of an observational study showed that severe hypoglycaemia was associated with increased mortality at 12 months even in people not receiving insulin (Elwen et al., 2015). Type 2 diabetes is especially challenging in patients younger than 25 years for whom complex phenotypes might necessitate many decades of intensive management to minimise development and progression of microvascular and macrovascular complications. Intensive management of type 2 diabetes in elderly patients (65 years of age or older) must be balanced against management of other comorbidities, cognitive impairment, and hypoglycaemia risk. In this Seminar, we review the existing management strategies, discuss new developments in diagnosis, treatment, and cardiovascular benefits, highlight controversies and uncertainties, and address outstanding research questions (Chatterjee et al., 2017).

#### 1.8. Purpose of research

Industrial uses of licorice include a sweetener in cigarettes, chewing tobacco, chocolate candy, smoking mixes, and chewing gum. In cosmetology, licorice is used as a depigmenting agent. Health product containing glycyrrhizinic acid includes licorice tea, flavoured diet gum, cough mixtures, throat pearls, and herbal cough mixtures. Alcoholic drinks contain all types of licorice root extracts; also, it is included in chewing tobacco. In confectionery, licorice cakes, bricks, sticks, toffee, balls, bars, and gums are used. Glycyrrhiza is frequently utilized in the manufacture

of biomass, bioenergy, and pulp. Furthermore, licorice may be converted into a popular livestock feed (ASL et al., 2008). “Glabridin-40” is a glabridin-rich extract of *G. glabra* recognized in the International Nomenclature of Cosmetic Ingredients, which is employed rationally in cosmetics compositions (Husain et al., 2021).

Although *Glycyrrhiza radix* is one of the major medicinal herb, still domestic requirements largely depend on imports. In accordance with these needs recently the area of licorice cultivation in Korea has expanded. At the same time to resolve early deciduousness, low quantity, unequal contents of metabolites unique cultivars have been developed called ‘Dagam, Wongam, Sinwongam’ and showed superior regional adaptability, resistant to brown spotted diseases and lodging. In addition, Measuring the glycyrrhizin content, new varieties ‘Dagam’ contains 3.0% glycyrrhizin and ‘Wongam’ and ‘Sinwongam’ contain 3.95%, 3.63% each more than 2.5%, which is the standard of the Korean Pharmacy. In case of liquiritigenin ‘Dagam’ and ‘Wongam’ contain at least 0.7% which meets the pharmacological standard also it was 1.4 times higher than *G. uralensis* but the problem of supply occurred as it was different from the original plants of the Korean Pharmacy also studies related with contents of secondary metabolites and morphological differences for breeding varieties are insufficient. As a first step for second metabolites standardization and quantification of new breeds. Established chemical library from roots of *Glycyrrhiza uralensis* extract based on MS, NMR spectrum. As a fundamental reference for comparison with content of new varieties.

## 2. Materials and methods

### 2.1. Plant materials

The roots of *Glycyrrhiza uralensis* were purchased from Dukhyundang of orient medicine, seoul, Korea in September 2021. The raw materials were identified based on morphological characteristics by Prof. Oh of college of pharmacy at Seoul National University.

A botanical specimen was deposited in the herbal exhibition of the college of Pharmacy at Seoul National University.

### 2.2. Chemicals, Equipments

- Silica gel TLC plates: 60 F254, 60 RP-18 F<sub>254</sub>S (Darmstadt, Germany)
- Silica gel (particle size: 60–200  $\mu$ m, Zeochem AG, Rüti, switzerland)
- RP-C18 (particle size: 75  $\mu$ m Nacalai Tesque, Kyoto, Japan)
- Sephadex LH-20 (GE Healthcare, Little Chalfont UK)
- Analytical and industrial grade methanol, ethanol, ethyl acetate, *n*-hexane, *n*-butanol used for extraction & isolation and hydrochloride, sulfuric acid (Daejung pure chemical engineering Co. Ltd. Siheung, South Korea)
- Dulbecco's modified Eagles' medium (DMEM) were purchased from Hyclon (Logan, UT, USA)
- Dimethyl sulfoxide (DMSO) (Junsei Chemical CO. Ltd, Tokyo, Japan)
- Formic acid, 3-(4,5-dimethyl-2-thizolyl)-2,5-diphenyl-2*H*-tetrazoilum bromide (MTT) (Sigma-Aldrich, St. Louis, MO, USA)
- Autoclave: LAC-5080S (DAIHAN LABTECH CO. Ltd, South Korea)
- Centrifuge: MICRO17TR (Hanil Scientific Inc., Korea)

- Clean bench: ESCO Class II Biological Safety Cabinet (Esco Technologies, Inc, USA)
- Incubator: Forma Series II water jacketed CO<sub>2</sub> incubator (Thermo Sci, USA)
- Evaporator: EYELA KSB-202 (Eyela Singapore Pte. Ltd, Singapore)
- Freeze dryer: Operon FDCF-12012 (Operon Co. Ltd, South Korea)
- HPLC: Agilent Technologies 6530 qTOF MS spectrometer, Binary pump(G1312C), Degasser (G1322A), Thermostatically controlled column oven (G1316), Autosampler (G1329B) (Agilent Technologies, Inc, USA)
- Microplate reader: VersaMax (Molecular Devices, USA)
- Microscope: Primovert (Carl Zeiss Microscopy, LLC, USA)
- NMR: 400 MHz JNM-ECZ400s (JEOL, Japan)
- Preparative HPLC: Gilson HPLC purification system (Gilson Inc, USA)
- Preparative HPLC column  
 YMC-Triart phenyl column (particles: 5  $\mu$ m, 10 x 250 mm; YMC Co., Ltd., Japan)  
 Optima Pak C<sub>18</sub> column (particles: 10  $\mu$ m, 10 x 250 mm; RS Tech, Korea)
- Sonicator: Power Sonic 420 (Hwashin Tech CO. Ltd, South Korea)
- Speed vacuum: Modulspin40 (Hanil Scientific Inc, Korea)

### 2.3. Extraction and isolation

The roots of *Glycyrrhiza uralensis* (1.5 kg) were extracted with 10% EtOH (2 L x 3 times, for 90 min each) at 55 °C. Extract was condensed under lowered pressure using evaporator to yield 140.3 g of crude extract. Crude extract was suspended in distilled water and then consequently partitioned with *n*-hexane, EtOAc, *n*-BuOH (3 times each) and water layers. Condensed EtOAc layer (22.8 g) was subjected to normal phase open column chromatography (CC) with gradient from *n*-

hexane:EtOAc (v:v, 80:20) to *n*-hexane:EtOAc (v:v, 0:100), to generate 8 subfractions (F.1 – F.8). The fraction F.8 (2.4 g) was subjected to MPLC (Medium pressure liquid chromatography) with gradient system from MeOH:H<sub>2</sub>O (v:v, 25:75) to MeOH:H<sub>2</sub>O (v:v, 90:10) with 0.05% formic acid (8mL/min, flow rate) to yield eight subfractions (F.8.1 – F.8.8). Then F.8.2 (373.3 mg) was subjected to semipreparative HPLC with MeOH:H<sub>2</sub>O (v:v, 28:72) with 0.05% formic acid (3mL/min, flow rate) resulting in Compound **1** (13.2 mg, *t<sub>R</sub>* = 60.2 min), Compound **2** (6.2 mg, *t<sub>R</sub>* = 72.5 min), Compound **3** (11.3 mg, *t<sub>R</sub>* = 42.3 min). F.8.5 (903.1 mg) was subjected to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 24:76) with 0.05% formic acid (3mL/min, flow rate) to yield Compound **4** (11.9 mg, *t<sub>R</sub>* = 34.4 min), Compound **5** (8.1 mg, *t<sub>R</sub>* = 38.8 min), Compound **6** (7.2 mg, *t<sub>R</sub>* = 43.5 min), Compound **7** (6.7 mg, *t<sub>R</sub>* = 47.6 min). The fraction F.3 (1.04 g) was subjected to MPLC with gradient solvent system from MeOH:H<sub>2</sub>O (v:v, 55:45) to MeOH:H<sub>2</sub>O (v:v, 100:0) with 0.05% formic acid (8mL/min, flow rate) to yield Compound **10** (24.4 mg, *t<sub>R</sub>* = 32 min) and nine subfractions (F.3.1 – F.3.9). F.3.3 (96.5 mg) was subjected to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 52:48) isocratic with 0.05% formic acid (3mL/min, flow rate) resulting in compound **8** (7.3 mg, *t<sub>R</sub>* = 28.6 min). From F.3.7 (36.3 mg) compound **9** (15.1 mg, *t<sub>R</sub>* = 31.3 min) was isolated by semipreparative HPLC under MeCN:H<sub>2</sub>O (v:v 65:35) isocratic condition with 0.05% formic acid (3 mL/min, flow rate). F.3.5 (127.0 mg) was loaded to semipreparative HPLC with MeOH:H<sub>2</sub>O (v:v, 72:28) solvent condition with 0.05% formic acid (2.7 mL/min, flow rate) resulting in compound **11** (6.3 mg, *t<sub>R</sub>* = 36.3 min), compound **12** (29.5 mg, *t<sub>R</sub>* = 41.2 min). In the same manner compound **13** (11.3 mg, *t<sub>R</sub>* = 40.9 min) was isolated from F.3.6 (84.1 mg) by semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 60:40) solvent with 0.05% formic acid (2.7 mL/min, flow rate). Compound **14** (4.7 mg, *t<sub>R</sub>* = 42.9 min), **15** (4.9

mg,  $t_R = 46.9$  min) were isolated from F.3.8 (33.2 mg) by semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 69:31) solvent with 0.05% formic acid (2.7 mL/min, flow rate). The fraction F.4 (1.90 g) was subjected to MPLC with gradient solvent system from MeOH:H<sub>2</sub>O (v:v, 45:55) to MeOH:H<sub>2</sub>O (v:v, 100:0) with 0.05% formic acid (8 mL/min, flow rate) to yield Compound **16** (21.4 mg,  $t_R = 52.0$  min), compound **32** (30.5 mg,  $t_R = 152.0$  min) and ten subfractions (F.4.1 – F.4.10). F.4.3 (63.5 mg) was loaded to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 44:56) solvent condition with 0.05% formic acid (2.7 mL/min, flow rate) resulting in compound **17** (7.2 mg,  $t_R = 31.1$  min), compound **18** (29.4 mg,  $t_R = 36.7$  min).

From F.4.5 compound **19** (6.9 mg,  $t_R = 34.8$  min) and F.4.5.2 (52.0 mg) were isolated with semipreparative HPLC from MeCN:H<sub>2</sub>O (v:v, 49:51) to MeCN:H<sub>2</sub>O (v:v, 50:50) with 0.05% formic acid (2.7 mL/min, flow rate). Consecutively F.4.5.2 (52.0 mg) was loaded to semipreparative HPLC with MeOH:H<sub>2</sub>O (v:v, 70:30) solvent condition with 0.05% formic acid (2.7 mL/min, flow rate) resulting in compound **20** (2.5 mg,  $t_R = 31.6$  min), compound **21** (46.2 mg,  $t_R = 46.2$  min). F.4.4 (271.0 mg) was loaded to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 45:55) solvent condition with 0.05% formic acid (2.7 mL/min, flow rate) resulting in compound **22** (4.8 mg,  $t_R = 43.0$  min). From F.4.6 (84.1 mg) compound **31** (4.1 mg,  $t_R = 35.6$  min) and compound **23** (3.5 mg,  $t_R = 45.4$  min) and compound **24** (7.4 mg,  $t_R = 51.8$  min) were isolated with semipreparative HPLC from MeCN:H<sub>2</sub>O (v:v, 50:50) with 0.05% formic acid (2.7 mL/min, flow rate). From F.4.7 (36.3 mg) compound **25** (6.8 mg,  $t_R = 49.2$  min) were isolated with semipreparative HPLC from MeCN:H<sub>2</sub>O (v:v, 56:44) with 0.05% formic acid (2.7 mL/min, flow rate). From F.4.8 (33.2 mg) compound **26** (6.8 mg,  $t_R = 49.2$  min) and F.4.8.2 (29.1 mg) were isolated with semipreparative HPLC from MeOH:H<sub>2</sub>O (v:v, 75:25) with 0.05% formic acid (2.7 mL/min, flow rate).

Consecutively F.4.8.2 (29.1 mg) was loaded to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 61:39) solvent condition with 0.05% formic acid (2.7 mL/min, flow rate) resulting in compound **27** (6.2 mg,  $t_R$  = 28.3 min), compound **28** (2.8 mg,  $t_R$  = 35.3 min), compound **29** (28.8 mg,  $t_R$  = 38.7 min).

Condensed water layer (68.2g) was subjected to MPLC with gradient solvent system from MeOH:H<sub>2</sub>O (v:v, 20:60) to MeOH:H<sub>2</sub>O (v:v,90:10) with 0.05% formic acid (8mL/min, flow rate) to yield compound **30** (50.6 mg,  $t_R$  = 52.0 min). The fraction F.5 (3.3 g) was subjected to MPLC with gradient solvent system from MeOH:H<sub>2</sub>O (v:v, 40:60) to MeOH:H<sub>2</sub>O (v:v, 100:0) with 0.05% formic acid (8mL/min, flow rate) to yield Compound **33** (29.3 mg,  $t_R$  = 40.0 min). F.5.3 (46.0 mg) was loaded to semipreparative HPLC with MeCN:H<sub>2</sub>O (v:v, 38:62) solvent condition with 0.05% formic acid (2.5 mL/min, flow rate) resulting in compound **34** (5.6 mg,  $t_R$  = 28.6 min), compound **35** (7.4 mg,  $t_R$  = 30.7 min). From F.5.2 (19.5 mg) compound **36** (4.3 mg,  $t_R$  = 27.7 min), compound **37** (2.5 mg,  $t_R$  = 37.8 min) were isolated with semipreparative HPLC from MeOH:H<sub>2</sub>O (v:v, 53:47) with 0.05% formic acid (2.7 mL/min, flow rate). compound **38** (6.3 mg,  $t_R$  = 44.3 min) was isolated from F.4.9 (42.8 mg) with semipreparative HPLC from MeOH:H<sub>2</sub>O (v:v, 80:20) with 0.05% formic acid (2.7 mL/min, flow rate). From F.5.4 (107.2 mg) compound **39** (12.3 mg,  $t_R$  = 27.3 min), compound **40** (5.6 mg,  $t_R$  = 33.2 min) were isolated with semipreparative HPLC from MeCN:H<sub>2</sub>O (v:v, 46:54) with 0.05% formic acid (2.7 mL/min, flow rate).

## 2.4. Spectral properties of compounds

### *Liquiritin (1)*

White amorphous powder

ESIMS  $m/z$  417.1238 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, CD<sub>3</sub>OD-*d*<sub>4</sub>):  $\delta_{\text{H}}$  5.42 (1H, dt,  $J = 12.8, 2.7$  Hz, H-2), 2.70 (1H, dt,  $J = 16.9, 2.8$  Hz, H-3a), 3.01 (1H, dd,  $J = 16.9, 12.9$  Hz, H-3b), 7.70 (1H, d,  $J = 8.7$  Hz, H-5), 6.47 (1H, dd,  $J = 8.7$  Hz, 2.0 H-6), 6.34 (1H, d,  $J = 2.0$  Hz, H-8), 7.41 (2H, d,  $J = 8.6$  Hz, H-2', 6'), 7.11 (2H, d,  $J = 8.6$  Hz, H-3', 5'), 4.60 (1H, s, H-1''), 3.41 (1H, m, H-2''), 3.44 (2H, m, H-3'', 4''), 3.37 (1H, m, H-5''), 3.67 (1H, dd,  $J = 12.1, 5.4$  Hz, H-6'' $\alpha$ ), 3.86 (1H, m, H-6'' $\beta$ ).

<sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD-*d*<sub>4</sub>):  $\delta_{\text{C}}$  80.7 (C-2), 45.0 (C-3), 193.2 (C-4), 129.9 (C-5), 111.9 (C-6), 166.9 (C-7), 103.8 (C-8), 165.4 (C-9), 115.0 (C-10), 134.5 (C-1'), 128.8 (C-2'), 117.8 (C-3'), 159.3 (C-4'), 117.8 (C-5'), 128.8 (C-6'), 102.2 (C-1''), 78.0 (C-2''), 78.2 (C-3''), 74.9 (C-4''), 71.3 (C-5''), 62.5 (C-6'').

### *Liquiritin apioside (2)*

Yellowish gum

ESIMS  $m/z$  549.1597 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, CD<sub>3</sub>OD-*d*<sub>4</sub>):  $\delta_{\text{H}}$  5.43 (1H, m, H-2), 2.70 (1H, dt,  $J = 16.9, 3.0$  Hz, H-3a), 3.02 (1H, dd,  $J = 16.9, 12.8$  Hz, H-3b), 7.71 (1H, d,  $J = 8.7$  Hz, H-5), 6.48 (1H, dd,  $J = 8.7, 2.2$  Hz, H-6), 6.34 (1H, d,  $J = 2.2$  Hz, H-8), 7.43 (2H, d,  $J = 8.7$  Hz, H-2', 6'), 7.10 (2H, d,  $J = 8.7$  Hz, H-3', 5'), 4.99 (1H, d,  $J = 7.4$  Hz, H-1''), 3.68 (1H, m, H-2''), 3.59 (1H, m, H-3''), 3.38 (1H, m, H-4''), 3.43 (1H, m, H-5''), 3.64 (1H, m, H-6'' $\alpha$ ), 3.88 (1H, dd,  $J = 12.1, 1.9$  Hz, H-6'' $\beta$ ), 5.46 (1H, d,  $J = 1.5$  Hz, H-1'''), 3.94 (1H, d,  $J = 1.4$  Hz, H-2'''), 3.53 (2H, m, H-4'''), 3.78 (1H, d,  $J = 9.6$  Hz, H-5''' $\alpha$ ), 4.05 (1H, d,  $J = 9.6$  Hz, H-5''' $\beta$ ).

<sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD-*d*<sub>4</sub>):  $\delta_{\text{C}}$  80.8 (C-2), 45.0 (C-3), 193.2 (C-4), 129.9 (C-5), 112.1 (C-6), 167.5 (C-7), 103.9 (C-8), 165.5 (C-9), 114.7 (C-10), 134.4 (C-1'),

128.8 (C-2'), 117.6 (C-3'), 159.1 (C-4'), 117.6 (C-5'), 129.9 (C-6'), 100.8 (C-1''), 78.1 (C-2''), 78.6 (C-3''), 71.4 (C-4''), 78.6 (C-5''), 62.5 (C-6''), 110.8 (C-1'''), 78.1 (C-2'''), 80.8 (C-3'''), 66.0 (C-4'''), 75.5 (C-5''').

### *Neoliquiritin (3)*

Yellowish gum

ESIMS  $m/z$  417.1176 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  5.46 (1H, dt,  $J = 12.3, 3.2$  Hz, H-2), 2.63 (1H, dd,  $J = 14.4, 2.2$  Hz, H-3 $\alpha$ ), 3.17 (1H, m, H-3 $\beta$ ), 7.67 (1H, d,  $J = 8.8$  Hz, H-5), 6.67 (1H, d,  $J = 8.9$  Hz, H-6), 6.61 (1H, d,  $J = 2.3$  Hz, H-8), 7.30 (2H, d,  $J = 8.4$  Hz, H-2', 6'), 6.75 (2H, d,  $J = 8.4$  Hz, H-3', 5'), 4.95 (1H, d,  $J = 7.5$  Hz, H-1''), 3.20 (2H, m, H-2'', 5''), 3.34 (overlap, H-3''), 3.12 (1H, m, H-4''), 3.62 (1H, d,  $J = 11.6$  Hz, H-6'' $\alpha$ ), 3.41 (overlap, H-6'' $\beta$ ).

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  79.3 (C-2), 43.1 (C-3), 190.7 (C-4), 128.4 (C-5), 111.0 (C-6), 163.6 (C-7), 115.2 (C-8), 163.0 (C-9), 103.5 (C-10), 128.5 (C-1'), 128.0 (C-2'), 115.2 (C-3'), 157.9 (C-4'), 115.3 (C-5'), 128.0 (C-6'), 99.8 (C-1''), 73.1 (C-2''), 77.1 (C-3''), 69.5 (C-4''), 76.4 (C-5''), 60.6 (C-6'').

### *Isoliquiritin apioside (4)*

Yellow amorphous powder

ESIMS  $m/z$  549.1636 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, CD<sub>3</sub>OD-*d*<sub>4</sub>):  $\delta_{\text{H}}$  6.30 (1H, s, H-3), 6.43 (1H, d,  $J = 8.1$  Hz, H-5), 7.98 (1H, d,  $J = 8.5$  Hz, H-6), 7.71 (overlap, H-2', 6'), 7.13 (2H, d,  $J = 8.2$  Hz, H-3', 5'), 7.80 (1H, d,  $J = 15.2$  Hz, H- $\alpha$ ), 7.67 (overlap, H- $\beta$ ), 5.06 (1H, d,  $J = 7.3$  Hz, H-1''), 3.48 (1H, m, H-2''), 3.70 (1H, m, H-3''), 3.65 (1H, m, H-4''), 3.42 (1H, m, H-

5"), 3.72 (1H, d,  $J = 5.4$  Hz, H-6"a), 3.91 (1H, d,  $J = 10.8$  Hz, H-6"b), 5.48 (1H, d,  $J = 1.2$  Hz, H-1""), 3.96 (1H, H-2""), 3.61 (1H, m, H-3""), 3.81 (1H, d,  $J = 9.6$  Hz, H-4""a), 4.06 (1H, d,  $J = 9.6$  Hz, H-4""b), 3.56 (2H, s, H-5"").

$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}-d_4$ ):  $\delta_{\text{C}}$  114.7 (C-1), 166.6 (C-2), 103.8 (C-3), 167.6 (C-4), 109.2 (C-5), 133.5 (C-6), 130.5 (C-1'), 131.5 (C-2'), 117.9 (C-3'), 160.9 (C-4'), 117.9 (C-5'), 131.5 (C-6'), 120.0 (C- $\alpha$ ), 144.8 (C- $\beta$ ), 193.4 (C=O), 100.5 (C-1"), 78.1 (C-2"), 78.6 (C-3"), 78.6 (C-4"), 71.3 (C-5"), 62.5 (C-6"), 110.8 (C-1""), 78.1 (C-2""), 80.8 (C-3""), 75.5 (C-4""), 66.0 (C-5").

### *Isoliquiritin (5)*

Yellow amorphous powder

ESIMS  $m/z$  417.1197  $[\text{M} - \text{H}]^-$

$^1\text{H}$  NMR data (400 MHz,  $\text{CD}_3\text{OD}-d_4$ ):  $\delta_{\text{H}}$  6.26 (1H, d,  $J = 2.1$  Hz, H-3), 6.40 (1H, dd,  $J = 8.9, 2.0$  Hz, H-5), 7.96 (1H, d,  $J = 8.9$  Hz, H-6), 7.69 (overlap, H-2', 6'), 7.13 (2H, d,  $J = 8.7$  Hz, H-3', 5'), 7.78 (1H, d,  $J = 15.2$  Hz, H- $\alpha$ ), 7.69 (overlap, H- $\beta$ ), 4.97 (1H, d,  $J = 7.3$  Hz, H-1"), 3.46 (1H, m, H-2"), 3.46 (1H, m, H-3"), 3.38 (1H, m, H-4"), 3.47 (1H, m, H-5"), 3.69 (1H, dd,  $J = 12.1, 5.5$  Hz, H-6"a), 3.87 (1H, m, H-6"b).

$^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}-d_4$ ):  $\delta_{\text{C}}$  114.6 (C-1), 167.6 (C-2), 103.9 (C-3), 166.8 (C-4), 109.3 (C-5), 133.5 (C-6), 130.6 (C-1'), 131.4 (C-2'), 118.0 (C-3'), 161.1 (C-4'), 118.0 (C-5'), 131.4 (C-6'), 120.1 (C- $\alpha$ ), 144.8 (C- $\beta$ ), 193.3 (C=O), 101.8 (C-1"), 77.9 (C-2"), 78.3 (C-3"), 71.3 (C-4"), 74.8 (C-5"), 62.5 (C-6").

### *Licuraside (6)*

Yellow amorphous powder

ESIMS  $m/z$  549.1636  $[\text{M} - \text{H}]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.75 (3H, overlap, H-2, 6,  $\beta$ ), 6.84 (2H, d,  $J = 8.6$  Hz, H-3, 5), 6.53 (1H, d,  $J = 2.4$  Hz, H-3'), 6.57 (1H, dd,  $J = 9.0, 2.4$  Hz H-5'), 8.25 (1H, d,  $J = 9.0$  Hz, H-6'), 7.80 (1H, d,  $J = 15.5$  Hz, H- $\alpha$ ), 5.10 (1H, d,  $J = 7.4$  Hz, H-1''), 5.29 (1H, br s, H-1'''), 3.87 (1H, d,  $J = 9.4$  Hz, H-4'''a), 3.62 (1H, d,  $J = 9.3$  Hz, H-4'''b), 3.27 – 3.69 (10H, Glycosyl, H-2'', 3'', 4'', 5'', 6'', 2''', 3''', 5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  125.7 (C-1), 131.5 (C-2), 115.9 (C-3), 160.5 (C-4), 115.9 (C-5), 131.5 (C-6), 114.9 (C-1'), 165.2 (C-2'), 103.4 (C-3'), 163.4 (C-4'), 108.1 (C-5'), 132.6 (C-6'), 117.4 (C- $\alpha$ ), 145.1 (C- $\beta$ ), 192.2 (C=O), 97.9 (C-1''), 75.7 (C-2''), 76.1 (C-3''), 69.9 (C-4''), 77.1 (C-5''), 60.5 (C-6''), 108.8 (C-1'''), 76.8 (C-2'''), 79.3 (C-3'''), 64.3 (C-4'''), 74.0 (C-5''').

#### *Neoisoliquiritin (7)*

Yellowish gum

ESIMS  $m/z$  417.1197 [ $\text{M} - \text{H}$ ] $^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.79 (4H, overlap, H-2, 6,  $\alpha$ ,  $\beta$ ), 6.84 (2H, d,  $J = 8.4$  Hz, H-3, 5), 6.57 (1H, d,  $J = 2.3$  Hz, H-3'), 6.63 (1H, dd,  $J = 8.9, 2.2$  Hz H-5'), 8.27 (1H, d,  $J = 9.1$  Hz, H-6'), 5.03 (1H, d,  $J = 7.4$  Hz, H-1''), 3.26 (overlap, H-2''), 3.29 (overlap, H-3''), 3.16 (1H, t,  $J = 8.9$  Hz, H-4''), 3.43 (overlap, H-5'', 6''a), 3.69 (1H, d,  $J = 10.9$  Hz, H-6''b), 5.40 (1H, br s, 2''-OH), 4.61 (1H, br s, 6''-OH).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  114.8 (C-1), 131.5 (C-2), 116.0 (C-3), 160.6 (C-4), 116.0 (C-5), 131.5 (C-6), 125.6 (C-1'), 165.2 (C-2'), 103.5 (C-3'), 163.6 (C-4'), 108.2 (C-5'), 132.5 (C-6'), 117.3 (C- $\alpha$ ), 145.1 (C- $\beta$ ), 192.1 (C=O), 99.6 (C-1''), 73.1 (C-2''), 76.5 (C-3''), 69.6 (C-4''), 77.2 (C-5''), 60.6 (C-6'').

*Licoisoflavanone (8)*

Brownish gum

ESIMS  $m/z$  355.1203  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  4.40 (1H, dd,  $J = 10.8, 5.5$  Hz, H-2 $\alpha$ ), 4.28 (1H, dd,  $J = 11.0, 5.5$  Hz, H-2 $\beta$ ), 4.51 (1H, t,  $J = 10.9$  Hz, H-3), 5.88 (2H, s, H-6, 8), 6.27 (1H, d,  $J = 8.3$  Hz, H-5'), 6.82 (1H, d,  $J = 8.3$  Hz, H-6'), 6.68 (1H, d,  $J = 10.1$  Hz, H-1''), 5.68 (1H, d,  $J = 10.0$  Hz, H-2''), 1.34 (6H, s, H-4'', 5''), 12.29 (1H, br s, 5-OH).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  69.7 (C-2), 46.3 (C-3), 197.2 (C-4), 163.9 (C-5), 96.1 (C-6), 166.9 (C-7), 95.0 (C-8), 163.1 (C-9), 101.9 (C-10), 117.0 (C-1'), 150.8 (C-2'), 107.9 (C-3'), 152.8 (C-4'), 110.2 (C-5'), 130.2 (C-6'), 115.6 (C-1''), 129.1 (C-2''), 75.1 (C-3''), 27.4 (C-4'', 5'').

*6,8-diprenylgenistein (9)*

Brownish gum

ESIMS  $m/z$  405.1740  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.37 (1H, s, H-1), 7.37 (2H, d,  $J = 8.5$  Hz, H-2', 6'), 6.81 (2H, d,  $J = 8.6$  Hz, H-3', 5'), 3.42 (2H, d,  $J = 6.5$  Hz, H-1''), 5.14 (1H, q,  $J = 7.2$  Hz, H-2''), 1.76 (3H, s, H-4''), 1.63 (3H, s, H-5''), 3.32 (2H, d,  $J = 6.9$  Hz, H-1'''), 5.14 (1H, q,  $J = 7.4$  Hz, H-2'''), 1.73 (3H, s, H-4'''), 1.62 (3H, s, H-5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  153.9 (C-2), 121.5 (C-3), 180.5 (C-4), 159.6 (C-5), 106.3 (C-6), 152.9 (C-7), 111.6 (C-8), 156.7 (C-9), 104.4 (C-10), 121.9 (C-1'), 130.2 (C-2'), 115.1 (C-3'), 157.4 (C-4'), 115.1 (C-5'), 130.7 (C-6'), 21.4 (C-1''), 122.4 (C-2''), 131.0 (C-3''), 17.8 (C-4''), 25.3 (C-5''), 21.4 (C-1'''), 122.4 (C-2'''), 131.0 (C-3'''), 17.8 (C-4'''), 25.5 (C-5''').

*Medicarpin (10)*

Brownish gum

ESIMS  $m/z$  271.0963  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz,  $\text{CD}_3\text{OD}-d_4$ ):  $\delta_{\text{H}}$  7.28 (1H, d,  $J = 8.4$  Hz, H-1), 6.43 (1H, dd,  $J = 8.2, 2.4$  Hz, H-2), 6.30 (1H, d,  $J = 2.4$  Hz, H-4), 4.20 (1H, m, H-6 $\alpha$ ), 3.51 (2H, m, H-6 $\beta$ , 6a), 7.15 (1H, d,  $J = 8.2$  Hz, H-7), 6.48 (1H, dd,  $J = 8.4, 2.4$  Hz, H-8), 6.37 (1H, d,  $J = 2.2$  Hz, H-10), 5.45 (1H, d,  $J = 6.3$  Hz, H-11a), 3.73 (3H, s, 9-OCH<sub>3</sub>).  
 $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{OD}-d_4$ ):  $\delta_{\text{C}}$  133.2 (C-1), 107.2 (C-2), 160.1 (C-3), 104.1 (C-4), 158.1 (C-4a), 67.6 (C-6), 40.9 (C-6a), 120.9 (C-6b), 126.0 (C-7), 110.7 (C-8), 162.1 (C-9), 97.6 (C-10), 162.6 (C-10a), 80.0 (C-11a), 112.9 (C-11b), 55.9 (9-OCH<sub>3</sub>).

*Isoderrone (11)*

Brownish gum

ESIMS  $m/z$  335.0927  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.36 (1H, s, H-2), 6.21 (1H, d,  $J = 2.1$  Hz, H-6), 6.38 (1H, d,  $J = 2.1$  Hz, H-8), 7.29 (2H, m, H-2', 6'), 6.80 (1H, d,  $J = 8.0$  Hz, H-5'), 6.44 (1H, d,  $J = 9.8$  Hz, H-1''), 5.79 (1H, d,  $J = 9.8$  Hz, H-2''), 1.39 (6H, s, H-4'', 5'').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ): 152.4 (C-2), 121.9 (C-3), 180.0 (C-4), 162.0 (C-5), 99.2 (C-6), 164.9 (C-7), 93.8 (C-8), 157.7 (C-9), 104.3 (C-10), 123.2 (C-1'), 127.0 (C-2'), 120.7 (C-3'), 154.3 (C-4'), 115.7 (C-5'), 129.8 (C-6'), 121.7 (C-1''), 131.4 (C-2''), 76.4 (C-3''), 27.7 (C-4'', 5'').

*Glisoflavanone (12)*

Brownish gum

ESIMS  $m/z$  423.1823 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  4.45 (2H, m, H-2), 4.26 (1H, dd,  $J = 10.2$ , 5.7 Hz, H-3), 5.97 (1H, s, H-8), 6.30 (1H, d,  $J = 8.3$  Hz, H-5'), 6.67 (1H, d,  $J = 8.3$  Hz, H-6'), 3.11 (2H, d,  $J = 7.0$  Hz, H-1''), 5.12 (2H, d,  $J = 6.9$  Hz, H-2'', 2'''), 1.61 (2H, s, H-4''), 1.68 (3H, s, H-5''), 3.25 (2H, d,  $J = 6.8$  Hz, H-1'''), 1.61 (3H, s, H-4'''), 1.70 (3H, s, H-5''').

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  70.2 (C-2), 45.9 (C-3), 198.0 (C-4), 164.4 (C-5), 107.1 (C-6), 161.6 (C-7), 95.4 (C-8), 159.9 (C-9), 102.1 (C-10), 116.0 (C-1'), 153.5 (C-2'), 113.9 (C-3'), 155.4 (C-4'), 106.8 (C-5'), 127.0 (C-6'), 21.1 (C-1''), 122.9 (C-2''), 130.3 (C-3''), 17.8 (C-4''), 25.6 (C-5''), 22.4 (C-1'''), 123.4 (C-2'''), 129.8 (C-3'''), 17.7 (C-4'''), 25.5 (C-5''').

### *Glycyuralin H* (**13**)

Brownish gum

ESIMS  $m/z$  437.1612 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  4.61 (1H, d,  $J = 11.9$  Hz, H-2 $\alpha$ ), 4.06 (1H, d,  $J = 11.9$  Hz, H-2 $\beta$ ), 5.98 (1H, s, H-8), 6.35 (1H, d,  $J = 8.5$  Hz, H-5'), 7.25 (1H, d,  $J = 8.5$  Hz, H-6'), 3.12 (2H, d,  $J = 7.1$  Hz, H-1''), 5.13 (1H, t,  $J = 7.2$  Hz, H-2''), 1.69 (3H, s, H-4''), 1.62 (3H, s, H-5''), 6.61 (1H, d,  $J = 10.1$  Hz, H-1'''), 5.67 (1H, d,  $J = 10.0$  Hz, H-2'''), 1.35 (3H, s, H-4'''), 1.33 (3H, s, H-5''').

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  73.5 (C-2), 73.5 (C-3), 194.7 (C-4), 161.6 (C-5), 107.8 (C-6), 164.0 (C-7), 94.2 (C-8), 159.9 (C-9), 100.1 (C-10), 119.3 (C-1'), 153.2 (C-2'), 109.8 (C-3'), 149.2 (C-4'), 107.5 (C-5'), 127.6 (C-6'), 20.7 (C-1''), 122.7 (C-2''), 130.3 (C-3''), 17.7 (C-4''), 25.5 (C-5''), 116.7 (C-1'''), 129.2 (C-2'''), 75.2 (C-3'''), 27.4 (C-4'''), 27.5 (C-5''').

*Angustone B (14)*

Yellow amorphous powder

ESIMS  $m/z$  419.1502  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.16 (1H, s, H-2), 6.45 (1H, s, H-8), 6.32 (1H, d,  $J = 8.3$  Hz, H-5'), 6.88 (1H, d,  $J = 8.3$  Hz, H-6'), 3.23 (2H, d,  $J = 7.0$  Hz, H-1''), 5.17 (1H, t,  $J = 7.1$  Hz, H-2''), 1.62 (3H, s, H-4''), 1.72 (3H, s, H-5''), 6.67 (1H, d,  $J = 10.0$  Hz, H-1'''), 5.68 (1H, d,  $J = 10.0$  Hz, H-2'''), 1.37 (6H, s, H-4''', 5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  153.6 (C-2), 122.3 (C-3), 180.6 (C-4), 162.3 (C-5), 109.8 (C-6), 158.7 (C-7), 93.0 (C-8), 155.4 (C-9), 111.5 (C-10), 104.2 (C-1'), 155.5 (C-2'), 111.5 (C-3'), 151.2 (C-4'), 107.0 (C-5'), 131.4 (C-6'), 21.0 (C-1''), 120.3 (C-2''), 128.9 (C-3''), 17.7 (C-4''), 25.5 (C-5''), 117.0 (C-1'''), 128.9 (C-2'''), 75.4 (C-3'''), 27.5 (C-4'''), 27.5 (C-5''').

*Isochandalone (15)*

Brownish gum

ESIMS  $m/z$  403.1562  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.33 (1H, s, H-2), 6.45 (1H, s, H-8), 7.28 (2H, m, H-2', H-6'), 6.80 (1H, d,  $J = 8.2$  Hz, H-5'), 3.22 (2H, d,  $J = 7.0$  Hz, H-1''), 5.17 (1H, t,  $J = 7.0$  Hz, H-2''), 1.62 (3H, s, H-4''), 1.72 (3H, s, H-5''), 6.44 (1H, d,  $J = 10.3$  Hz, H-1'''), 5.78 (1H, d,  $J = 9.8$  Hz, H-2'''), 1.39 (6H, s, H-4''', 5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  154.0 (C-2), 122.3 (C-3), 180.0 (C-4), 162.0 (C-5), 111.2 (C-6), 158.8 (C-7), 93.1 (C-8), 155.5 (C-9), 103.9 (C-10), 123.4 (C-1'), 127.0 (C-2'), 120.7 (C-3'), 152.4 (C-4'), 121.7 (C-5'), 129.8 (C-6'), 21.1 (C-1''), 121.7 (C-2''), 130.7 (C-3''), 17.8 (C-4''), 25.5 (C-5''), 115.7 (C-1'''), 131.4 (C-2'''), 76.4 (C-3'''), 27.8 (C-4'''), 27.8 (C-5''').

*Vestitol (16)*

Brownish gum

ESIMS  $m/z$  273.1117  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  3.90 (1H, t,  $J = 10.1$  Hz, H-2 $\alpha$ ), 4.13 (1H, ddd,  $J = 10.7, 3.3, 2.2$  Hz, H-2 $\beta$ ), 3.30 (1H, m, H-3), 2.87 (1H, dd,  $J = 15.6, 10.9$  Hz, H-4a), 2.71 (1H, dd,  $J = 15.4, 4.7$  Hz, H-4b), 6.86 (1H, d,  $J = 8.2$  Hz, H-5), 6.28 (1H, dd,  $J = 8.2, 2.4$  Hz, H-6), 6.17 (1H, d,  $J = 2.3$  Hz, H-8), 6.41 (1H, d,  $J = 2.5$  Hz, H-3'), 6.35 (1H, dd,  $J = 8.5, 2.5$  Hz, H-5'), 6.98 (1H, d,  $J = 8.5$  Hz, H-6'), 3.66 (3H, s, 4'-OCH<sub>3</sub>).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  69.2 (C-2), 31.1 (C-3), 29.8 (C-4), 130.1 (C-5), 107.9 (C-6), 156.5 (C-7), 102.5 (C-8), 154.6 (C-9), 112.8 (C-10), 119.7 (C-1'), 155.9 (C-2'), 101.3 (C-3'), 158.8 (C-4'), 104.4 (C-5'), 127.8 (C-6'), 54.9 (4'-OCH<sub>3</sub>).

*Glyasperin F (17)*

Brownish gum

ESIMS  $m/z$  353.1028  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, Acetone- $d_6$ ):  $\delta_{\text{H}}$  4.53 (1H, t,  $J = 11.0$  Hz, H-2 $\alpha$ ), 4.39 (1H, dd,  $J = 10.9, 5.6$  Hz, H-2 $\beta$ ), 4.18 (1H, dd,  $J = 11.0, 5.6$  Hz, H-3), 5.94 (1H, d,  $J = 2.0$  Hz, H-6), 5.96 (1H, d,  $J = 2.0$  Hz, H-8), 6.40 (1H, d,  $J = 8.4$  Hz, H-5'), 6.85 (1H, d,  $J = 8.4$  Hz, H-4''), 6.67 (1H, d,  $J = 10.0$  Hz, H-1''), 5.62 (1H, d,  $J = 10.0$  Hz, H-2''), 1.34 (3H, s, H-4''), 1.33 (3H, s, H-5''), 12.41 (1H, s, 5-OH).

$^{13}\text{C}$  NMR (100 MHz, Acetone- $d_6$ ):  $\delta_{\text{C}}$  71.5 (C-2), 48.1 (C-3), 198.6 (C-4), 166.0 (C-5), 97.4 (C-6), 168.0 (C-7), 96.2 (C-8), 164.9 (C-9), 103.9 (C-10), 115.2 (C-1'), 152.8 (C-2'), 110.7 (C-3'), 154.2 (C-4'), 108.8 (C-5'), 131.6 (C-6'), 118.2 (C-1''), 129.5 (C-2''), 77.4 (C-3''), 28.4 (C-4''), 28.1 (C-5'').

*Dihydrolicoisoflavanone (18)*

Brownish gum

ESIMS  $m/z$  355.1196  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  4.48 (1H, t,  $J = 10.7$  Hz, H-2 $\alpha$ ), 4.39 (1H, dd,  $J = 10.9, 5.4$  Hz, H-2 $\beta$ ), 4.27 (1H, dd,  $J = 10.4, 5.4$  Hz, H-3), 5.89 (2H, s, H-6, 8), 6.30 (1H, d,  $J = 8.3$  Hz, H-5'), 6.66 (1H, d,  $J = 8.4$  Hz, H-6'), 3.25 (2H, d,  $J = 6.7$  Hz, H-1''), 5.12 (1H, t,  $J = 7.0$  Hz, H-2''), 1.69 (3H, s, H-4''), 1.61 (3H, s, H-5''), 12.31 (1H, s, 5-OH).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  70.1 (C-2), 45.9 (C-3), 197.7 (C-4), 163.9 (C-5), 95.9 (C-6), 166.6 (C-7), 94.8 (C-8), 163.1 (C-9), 102.2 (C-10), 113.8 (C-1'), 153.5 (C-2'), 116.0 (C-3'), 154.4 (C-4'), 107.1 (C-5'), 127.0 (C-6'), 22.4 (C-1''), 123.4 (C-2''), 129.8 (C-3''), 17.8 (C-4''), 25.6 (C-5'').

*Isowighteone (19)*

Brownish gum

ESIMS  $m/z$  339.1232  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.27 (1H, s, H-2), 6.20 (1H, d,  $J = 2.0$  Hz, H-6), 6.36 (1H, d,  $J = 2.1$  Hz, H-8), 7.21 (1H, d,  $J = 2.3$  Hz, H-2'), 6.82 (1H, d,  $J = 8.2$  Hz, H-5'), 7.18 (1H, dd,  $J = 8.2, 2.3$  Hz, H-6'), 3.24 (2H, d,  $J = 7.2$  Hz, H-1''), 5.29 (1H, m, H-2''), 1.68 (6H, s, H-4'', 5'').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  153.9 (C-2), 121.3 (C-3), 180.2 (C-4), 162.0 (C-5), 99.1 (C-6), 164.6 (C-7), 93.7 (C-8), 157.7 (C-9), 104.3 (C-10), 130.1 (C-1'), 127.3 (C-2'), 127.5 (C-3'), 155.1 (C-4'), 114.6 (C-5'), 122.5 (C-6'), 28.2 (C-1''), 122.9 (C-2''), 131.2 (C-3''), 17.7 (C-4''), 25.6 (C-5'').

*Glyurallin A (20)*

Pale red gum

ESIMS  $m/z$  353.1392  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  5.39 (2H, s, H-2), 6.28 (1H, s, H-8), 6.96 (1H, d,  $J = 2.1$  Hz, H-3'), 6.75 (1H, dd,  $J = 8.4, 2.1$  Hz, H-5'), 7.30 (1H, d,  $J = 8.4$  Hz, H-6'), 3.19 (2H, d,  $J = 7.0$  Hz, H-1''), 5.16 (1H, t,  $J = 7.1$  Hz, H-2''), 1.73 (3H, s, H-4''), 1.63 (3H, s, H-5''), 3.74 (3H, s, 5-OCH<sub>3</sub>).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  64.1 (C-2), 106.2 (C-3), 144.9 (C-4), 155.2 (C-5), 115.2 (C-6), 152.9 (C-7), 99.8 (C-8), 156.7 (C-9), 102.5 (C-10), 117.1 (C-1'), 155.9 (C-2'), 98.0 (C-3'), 153.3 (C-4'), 112.3 (C-5'), 118.9 (C-6'), 21.9 (C-1''), 123.6 (C-2''), 129.8 (C-3''), 17.7 (C-4''), 25.5 (C-5''), 61.5 (5-OCH<sub>3</sub>).

*Glabridin (21)*

White amorphous powder

ESIMS  $m/z$  325.1445  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  3.93 (1H, t,  $J = 10.3$  Hz, H-2 $\alpha$ ), 4.23 (1H, ddd,  $J = 10.3, 3.3, 2.1$  Hz, H-2 $\beta$ ), 3.28 (1H, m, H-3), 2.89 (1H, dd,  $J = 15.6, 11.3$  Hz, H-4a), 2.69 (1H, ddd,  $J = 16.3, 4.9, 1.9$  Hz, H-4b), 6.83 (1H, d,  $J = 8.2$  Hz, H-5), 6.28 (1H, d,  $J = 8.5$  Hz, H-6), 6.32 (1H, d,  $J = 2.4$  Hz, H-3'), 6.18 (1H, dd,  $J = 8.3, 2.4$  Hz, H-5'), 6.86 (1H, d,  $J = 8.4$  Hz, H-6'), 6.53 (1H, d,  $J = 10.0$  Hz, H-1''), 5.64 (1H, d,  $J = 9.9$  Hz, H-2''), 1.34 (3H, s, H-4''), 1.33 (3H, s, H-5'').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  69.8 (C-2), 30.9 (C-3), 30.0 (C-4), 129.3 (C-5), 108.1 (C-6), 151.3 (C-7), 109.1 (C-8), 149.3 (C-9), 114.8 (C-10), 117.4 (C-1'), 155.9 (C-2'), 102.5 (C-3'), 156.9 (C-4'), 106.3 (C-5'), 127.6 (C-6'), 116.5 (C-1''), 129.4 (C-2''), 75.3 (C-3''), 27.3 (C-4''), 27.4 (C-5'').

*Glabrene (22)*

Brownish gum

ESIMS  $m/z$  323.1287  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  4.88 (2H, d,  $J = 1.2$  Hz, H-2), 6.52 (1H, d,  $J = 0.9$  Hz, H-4), 6.92 (1H, d,  $J = 8.2$  Hz, H-5), 6.33 (1H, dd,  $J = 8.2, 2.3$  Hz, H-6), 6.23 (1H, dd,  $J = 2.3, 0.7$  Hz, H-8), 6.40 (1H, d,  $J = 8.5$  Hz, H-5'), 7.00 (1H, d,  $J = 8.4$  Hz, H-6'), 6.56 (1H, d,  $J = 9.9$  Hz, H-1''), 5.64 (1H, d,  $J = 9.9$  Hz, H-2''), 1.37 (6H, s, H-4'', 5'').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  67.7 (C-2), 127.9 (C-3), 119.9 (C-4), 127.5 (C-5), 108.7 (C-6), 158.1 (C-7), 102.4 (C-8), 154.0 (C-9), 115.4 (C-10), 117.7 (C-1'), 150.6 (C-2'), 108.9 (C-3'), 153.1 (C-4'), 108.1 (C-5'), 127.6 (C-6'), 116.9 (C-1''), 128.4 (C-2''), 75.9 (C-3''), 27.3 (C-4''), 27.3 (C-5'').

*Licoflavonol (23)*

Yellow amorphous powder

ESIMS  $m/z$  353.1037  $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  6.45 (1H, s, H-8), 7.99 (2H, d,  $J = 8.6$  Hz, H-2', 6'), 6.88 (2H, d,  $J = 8.8$  Hz, H-3', 5'), 3.20 (2H, d,  $J = 7.0$  Hz, H-1''), 5.15 (1H, d,  $J = 7.0$  Hz, H-2''), 1.70 (3H, s, H-4''), 1.59 (3H, s, H-5''), 12.68 (1H, s, 5-OH).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  146.5 (C-2), 135.5 (C-3), 175.9 (C-4), 159.1 (C-5), 110.2 (C-6), 161.9 (C-7), 92.8 (C-8), 154.0 (C-9), 102.7 (C-10), 121.8 (C-1'), 129.4 (C-2'), 115.4 (C-3'), 157.4 (C-4'), 115.4 (C-5'), 129.4 (C-6'), 21.0 (C-1''), 122.3 (C-2''), 130.5 (C-3''), 17.7 (C-4''), 25.5 (C-5'').

*Alloicoisoflavone (24)*

Brownish gum

ESIMS  $m/z$  353.1021  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, Acetone- $d_6$ ):  $\delta_{\text{H}}$  8.19 (1H, s, H-2), 6.22 (1H, d,  $J = 2.0$  Hz, H-6), 6.39 (1H, d,  $J = 2.0$  Hz, H-8), 6.32 (1H, d,  $J = 8.4$  Hz, H-5'), 6.88 (1H, d,  $J = 8.3$  Hz, H-6'), 6.66 (1H, d,  $J = 10.1$  Hz, H-1''), 5.68 (1H, d,  $J = 10.0$  Hz, H-2''), 1.37 (6H, s, H-4'', 5''), 12.85 (1H, s, 5-OH).

$^{13}\text{C}$  NMR (100 MHz, Acetone- $d_6$ ):  $\delta_{\text{C}}$  155.7 (C-2), 120.5 (C-3), 180.6 (C-4), 161.9 (C-5), 99.0 (C-6), 164.6 (C-7), 93.8 (C-8), 157.8 (C-9), 104.5 (C-10), 111.3 (C-1'), 151.3 (C-2'), 109.8 (C-3'), 153.6 (C-4'), 107.4 (C-5'), 131.4 (C-6'), 117.0 (C-1''), 128.9 (C-2''), 75.5 (C-3''), 27.5 (C-4''), 27.5 (C-5'').

*3'-( $\gamma$ ,  $\gamma$ -dimethylallyl)-kievitone (25)*

Brownish gum

ESIMS  $m/z$  425.1966  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  4.43 (1H, t,  $J = 10.5$  Hz, H-2 $\alpha$ ), 4.36 (1H, dd,  $J = 10.9, 5.4$  Hz, H-2 $\beta$ ), 4.32 (1H, dd,  $J = 10.0, 5.4$  Hz, H-3), 5.96 (1H, s, H-6), 6.30 (1H, d,  $J = 8.3$  Hz, H-5'), 6.65 (1H, d,  $J = 8.3$  Hz, H-6'), 3.11 (2H, d,  $J = 7.0$  Hz, H-1''), 5.13 (2H, m, H-2'', 2'''), 1.61 (3H, s, H-4''), 1.69 (3H, s, H-5'', 5'''), 3.24 (2H, d,  $J = 6.8$  Hz, H-1'''), 1.61 (3H, s, H-4'''), 1.69 (3H, s, H-5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  70.1 (C-2), 45.9 (C-3), 197.6 (C-4), 164.8 (C-5), 107.7 (C-6), 160.9 (C-7), 94.4 (C-8), 160.7 (C-9), 101.8 (C-10), 116.0 (C-1'), 153.5 (C-2'), 114.1 (C-3'), 155.4 (C-4'), 107.1 (C-5'), 126.9 (C-6'), 20.7 (C-1''), 122.8 (C-2''), 130.1 (C-3''), 17.8 (C-4''), 25.6 (C-5''), 22.4 (C-1'''), 123.4 (C-2'''), 129.7 (C-3'''), 17.7 (C-4'''), 25.5 (C-5''').

*Gancaonin H (26)*

Brownish gum

ESIMS  $m/z$  421.1663  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.31 (1H, s, H-2), 6.45 (1H, s, H-8), 6.91 (1H, d,  $J = 2.0$  Hz, H-2'), 6.71 (1H, d,  $J = 2.0$  Hz, H-6'), 6.38 (1H, d,  $J = 9.9$  Hz, H-1''), 5.75 (1H, d,  $J = 9.8$  Hz, H-2''), 1.39 (6H, s, H-4'', 5''), 3.23 (2H, d,  $J = 7.1$  Hz, H-1'''), 5.18 (1H, t,  $J = 7.2$  Hz, H-2'''), 1.62 (3H, s, H-4'''), 1.72 (3H, s, H-5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  155.4 (C-2), 122.2 (C-3), 180.1 (C-4), 158.8 (C-5), 111.1 (C-6), 162.4 (C-7), 93.0 (C-8), 154.0 (C-9), 104.1 (C-10), 122.1 (C-1'), 122.0 (C-2'), 121.5 (C-3'), 140.1 (C-4'), 145.1 (C-5'), 117.6 (C-6'), 130.7 (C-1''), 123.0 (C-2''), 76.0 (C-3''), 21.1 (C-4''), 21.1 (C-5''), 25.5 (C-1'''), 131.3 (C-2'''), 117.1 (C-3'''), 17.7 (C-4'''), 27.5 (C-5''').

*Isoangustone A (27)*

Brownish gum

ESIMS  $m/z$  423.1814  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.23 (1H, s, H-2), 6.44 (1H, s, H-8), 6.65 (1H, d,  $J = 1.9$  Hz, H-2'), 6.87 (1H, d,  $J = 1.7$  Hz, H-6'), 3.23 (4H, d,  $J = 7.0$  Hz, H-1'', 1'''), 5.17 (1H, t,  $J = 7.1$  Hz, H-2''), 1.67 (3H, s, H-4''), 1.62 (3H, s, H-5''), 5.28 (1H, m, H-2'''), 1.67 (3H, s, H-4'''), 1.72 (3H, s, H-5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  153.7 (C-2), 121.1 (C-3), 180.3 (C-4), 158.8 (C-5), 111.0 (C-6), 161.9 (C-7), 92.9 (C-8), 155.3 (C-9), 104.2 (C-10), 122.5 (C-1'), 120.5 (C-2'), 144.5 (C-3'), 143.2 (C-4'), 128.0 (C-5'), 114.0 (C-6'), 21.1 (C-1''), 122.2 (C-2''), 130.7 (C-3''), 17.7 (C-4''), 25.6 (C-5''), 28.3 (C-1'''), 123.1 (C-2'''), 131.0 (C-3'''), 17.7 (C-4'''), 25.5 (C-5''').

*Gancaonin G (28)*

Brown amorphous powder

ESIMS  $m/z$  353.1391  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.43 (1H, s, H-2), 6.74 (1H, s, H-8), 7.39 (2H, d,  $J = 8.7$  Hz, H-2', 6'), 6.82 (2H, d,  $J = 8.7$  Hz, H-3', 5'), 3.25 (4H, d,  $J = 7.0$  Hz, H-1''), 5.13 (1H, t,  $J = 7.2$  Hz, H-2''), 1.72 (3H, s, H-4''), 1.62 (3H, s, H-5''), 3.91 (3H, s, 7-OCH<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  154.3 (C-2), 121.4 (C-3), 180.5 (C-4), 157.8 (C-5), 111.7 (C-6), 162.9 (C-7), 90.3 (C-8), 156.0 (C-9), 105.3 (C-10), 121.9 (C-1'), 130.2 (C-2'), 115.1 (C-3'), 157.5 (C-4'), 115.1 (C-5'), 130.2 (C-6'), 21.0 (C-1''), 122.5 (C-2''), 131.1 (C-3''), 17.7 (C-4''), 25.5 (C-5''), 56.5 (7-OCH<sub>3</sub>).

*Glycyrrhetinic acid (29)*

White amorphous powder

ESIMS  $m/z$   $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  2.18 (2H, dd,  $J = 13.3, 3.2$  Hz, H-2), 3.24 (1H, dd,  $J = 10.6, 5.5$  Hz, H-3), 0.70 (1H, d,  $J = 11.4$  Hz, H-5), 2.34 (1H, s, H-9), 5.71 (1H, s, H-12), 2.78 (1H, m, H-18), 1.00 (3H, s, H-23), 0.83 (3H, s, H-24), 1.22 (3H, s, H-25), 1.12 (3H, s, H-26), 1.36 (3H, s, H-27), 0.80 (3H, s, H-28), 1.12 (3H, s, H-29).

$^{13}\text{C}$  NMR (100 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  39.2 (C-1), 27.4 (C-2), 79.0 (C-3), 39.2 (C-4), 55.0 (C-5), 17.6 (C-6), 32.9 (C-7), 43.3 (C-8), 61.9 (C-9), 37.2 (C-10), 200.8 (C-11), 128.6 (C-12), 169.7 (C-13), 45.6 (C-14), 26.6 (C-15), 26.5 (C-16), 32.0 (C-17), 48.4 (C-18), 41.0 (C-19), 43.9 (C-20), 31.0 (C-21), 37.8 (C-22), 28.2 (C-23), 15.7 (C-24), 16.5 (C-25), 18.8 (C-26), 23.5 (C-27), 28.6 (C-28), 28.7 (C-29), 181.8 (C-30).

***Glycyrrhizin (30)***

White amorphous powder

ESIMS  $m/z$  821.3979 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, Pyridine-*d*<sub>5</sub>):  $\delta_{\text{H}}$  4.29 (1H, m, H-3), 2.45 (1H, s, H-9), 5.97 (1H, s, H-12), 0.78 - 1.43 (3H, s, 7 X CH<sub>3</sub>), 5.06 (1H, d,  $J = 7.2$  Hz, H-1'), 5.53 (1H, d,  $J = 7.4$  Hz, H-1'').

<sup>13</sup>C NMR (100 MHz, Pyridine-*d*<sub>5</sub>):  $\delta_{\text{C}}$  40.3 (C-1), 27.0 (C-2), 89.6 (C-3), 37.5 (C-4), 55.7 (C-5), 19.1 (C-6), 32.9 (C-7), 43.8 (C-8), 62.4 (C-9), 37.5 (C-10), 199.9 (C-11), 129.0 (C-12), 170.0 (C-13), 45.8 (C-14), 28.4 (C-15), 27.0 (C-16), 32.5 (C-17), 49.0 (C-18), 41.9 (C-19), 44.4 (C-20), 31.9 (C-21), 39.0 (C-22), 28.4 (C-23), 17.0 (C-24), 17.2 (C-25), 19.1 (C-26), 23.9 (C-27), 29.0 (C-28), 29.1 (C-29), 179.5 (C-30), 105.0 (C-1'), 83.5 (C-2'), 76.5 (C-3'), 71.5 (C-4'), 76.5 (C-5'), 169.5 (C-6'), 106.3 (C-1''), 75.7 (C-2''), 77.7 (C-3''), 72.0 (C-4''), 75.7 (C-5''), 172.8 (C-6'').

***Lupiwighteone (31)***

Brownish gum

ESIMS  $m/z$  339.1239 [M + H]<sup>+</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  8.39 (1H, s, H-2), 6.30 (1H, s, H-6), 7.37 (2H, d,  $J = 8.7$  Hz, H-2', 6'), 6.81 (2H, d,  $J = 8.7$  Hz, H-3', 5'), 3.32 (overlap, H-1'), 5.15 (1H, t,  $J = 7.1$  Hz, H-2''), 1.63 (3H, s, H-4''), 1.75 (3H, s, H-5'').

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  154.0 (C-2), 121.9 (C-3), 180.5 (C-4), 159.6 (C-5), 98.6 (C-6), 162.0 (C-7), 105.7 (C-8), 154.9 (C-9), 104.2 (C-10), 122.3 (C-1'), 130.2 (C-2'), 115.1 (C-3'), 157.4 (C-4'), 115.1 (C-5'), 130.2 (C-6'), 21.1 (C-1''), 121.4 (C-2''), 131.1 (C-3''), 25.5 (C-4''), 17.8 (C-5'').

*Betulinic acid (32)*

White crystal

ESIMS  $m/z$  455.3588 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, CDCl<sub>3</sub>):  $\delta_{\text{H}}$  3.19 (1H, dd,  $J = 11.3, 4.9$  Hz, H-3), 2.99 (1H, m, H-19), 0.96 (3H, s, H-23), 0.75 (3H, s, H-24), 0.82 (3H, s, H-25), 0.97 (3H, s, H-26), 0.93 (3H, s, H-27), 4.74 (1H, br s, H-29a), 4.60 (1H, br s, H-29b), 1.69 (3H, s, H-30).

<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta_{\text{C}}$  39.0 (C-1), 27.5 (C-2), 79.2 (C-3), 38.8 (C-4), 55.5 (C-5), 18.4 (C-6), 34.4 (C-7), 40.8 (C-8), 50.6 (C-9), 37.3 (C-10), 21.0 (C-11), 25.6 (C-12), 38.5 (C-13), 42.6 (C-14), 30.7 (C-15), 32.3 (C-16), 56.4 (C-17), 47.0 (C-18), 49.4 (C-19), 150.6 (C-20), 29.8 (C-21), 37.2 (C-22), 28.1 (C-23), 15.5 (C-24), 16.2 (C-25), 16.3 (C-26), 14.8 (C-27), 180.3 (C-28), 109.9 (C-29), 19.5 (C-30).

*Liquiritigenin (33)*

Brownish gum

ESIMS  $m/z$  255.0655 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  5.43 (1H, dd,  $J = 12.9, 2.8$  Hz, H-2), 3.14 (1H, dd,  $J = 17.1, 4.1$  Hz, H-3 $\alpha$ ), 2.62 (1H, dd,  $J = 16.8, 2.9$  Hz, H-3 $\beta$ ), 7.64 (1H, d,  $J = 8.7$  Hz, H-5), 6.50 (1H, dd,  $J = 8.7, 2.2$  Hz, H-6), 6.33 (1H, d,  $J = 2.2$  Hz, H-8), 7.32 (2H, d,  $J = 8.6$  Hz, H-2', 6'), 6.79 (2H, d,  $J = 8.6$  Hz, H-3', 5').

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  79.0 (C-2), 43.2 (C-3), 190.2 (C-4), 128.5 (C-5), 110.6 (C-6), 164.8 (C-7), 102.6 (C-8), 163.2 (C-9), 113.5 (C-10), 129.4 (C-1'), 128.5 (C-2'), 115.2 (C-3'), 157.7 (C-4'), 115.2 (C-5'), 128.3 (C-6').

*Isoliquiritigenin (34)*

Yellowish gum

ESIMS  $m/z$  255.0661 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  6.26 (1H, d,  $J$  = 2.0 Hz, H-3), 6.39 (1H, dd,  $J$  = 8.8, 2.0 Hz, H-5), 8.15 (1H, d,  $J$  = 8.9 Hz, H-6), 7.75 (4H, overlap, H-2', 6',  $\alpha$ ,  $\beta$ ), 6.84 (2H, d,  $J$  = 8.6 Hz, H-3', 5').

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  112.8 (C-1), 165.9 (C-2), 102.7 (C-3), 165.9 (C-4), 132.9 (C-6), 191.4 (C=O), 117.4 (C- $\alpha$ ), 144.2 (C- $\beta$ ), 125.8 (C-1'), 131.3 (C-2'), 115.9 (C-3'), 160.4 (C-4'), 115.9 (C-5'), 131.3 (C-6').

*Isovestitol (35)*

Brownish gum

ESIMS  $m/z$  271.0967 [M - H]<sup>-</sup>

<sup>1</sup>H NMR data (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{H}}$  3.90 (1H, t,  $J$  = 10.1 Hz, H-2 $\alpha$ ), 4.13 (1H, ddd,  $J$  = 10.3, 3.3, 1.9 Hz, H-2 $\beta$ ), 3.42 (1H, m, overlap), 2.87 (1H, dd,  $J$  = 15.6, 10.9 Hz, H-4a), 2.70 (1H, dd,  $J$  = 15.8, 3.7 Hz, H-4b), 6.86 (1H, d,  $J$  = 8.2 Hz, H-5), 6.28 (1H, dd,  $J$  = 8.2, 2.4 Hz, H-6), 6.17 (1H, d,  $J$  = 2.4 Hz, H-8), 6.42 (1H, d,  $J$  = 2.6 Hz, H-3'), 6.34 (1H, dd,  $J$  = 8.5, 2.6 Hz, H-5'), 6.98 (1H, d,  $J$  = 8.5 Hz, H-6'), 3.66 (3H, s, 2'-OCH<sub>3</sub>).

<sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>):  $\delta_{\text{C}}$  69.2 (C-2), 31.1 (C-3), 29.8 (C-4), 130.1 (C-5), 108.0 (C-6), 156.5 (C-7), 102.5 (C-8), 154.6 (C-9), 112.8 (C-10), 119.7 (C-1'), 156.0 (C-2'), 101.3 (C-3'), 158.8 (C-4'), 104.3 (C-5'), 127.8 (C-6'), 54.9 (2'-OCH<sub>3</sub>).

*Dihydrogenistein (36)*

Colorless gum

ESIMS  $m/z$   $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  5.43 (1H, dd,  $J = 12.8, 2.9$  Hz, H-2), 2.66 (1H, dd,  $J = 17.1, 3.1$  Hz, H-2a), 3.25 (1H, dd,  $J = 17.1, 12.8$  Hz, H-2b), 5.86 (2H, s, H-6, 8), 7.31 (2H, dd,  $J = 8.5$  Hz, H-2', 6'), 6.79 (2H, d,  $J = 8.5$  Hz, H-3', 5').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  78.4 (C-2), 42.0 (C-3), 196.2 (C-4), 163.5 (C-5), 95.9 (C-6), 167.3 (C-7), 95.1 (C-8), 162.9 (C-9), 101.6 (C-10), 128.9 (C-1'), 128.3 (C-2'), 115.2 (C-3'), 157.7 (C-4'), 115.2 (C-5'), 128.3 (C-6').

### *Genistein (37)*

White amorphous powder

ESIMS  $m/z$   $[M - H]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.30 (1H, s, H-2), 6.20 (1H, d,  $J = 2.1$  Hz, H-6), 6.36 (1H, d,  $J = 2.1$  Hz, H-8), 7.37 (2H, d,  $J = 8.6$  Hz, H-2', 6'), 6.81 (2H, d,  $J = 8.6$  Hz, H-3', 5').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  153.9 (C-2), 121.3 (C-3), 180.1 (C-4), 162.0 (C-5), 99.2 (C-6), 164.3 (C-7), 93.8 (C-8), 157.4 (C-9), 104.2 (C-10), 122.2 (C-1'), 130.2 (C-2'), 115.1 (C-3'), 157.7 (C-4'), 115.1 (C-5'), 130.2 (C-6').

### *Glyasperin A (38)*

Yellow amorphous powder

ESIMS  $m/z$  423.1808  $[M + H]^+$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  6.47 (1H, s, H-8), 7.88 (2H, m, H-2', 6'), 6.93 (1H, d,  $J = 8.5$  Hz, H-5'), 3.23 (2H, d,  $J = 7.0$  Hz, H-1''), 5.19 (1H, d,  $J = 7.1$  Hz, H-2''), 1.71 (3H, s, H-4''), 1.63 (3H, s, H-5''), 3.28 (2H, d,  $J = 7.3$  Hz, H-1'''), 5.30 (1H, t,  $J = 7.3$  Hz, H-2'''), 1.73 (3H, s, H-4'''), 1.70 (3H, s, H-5''').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  146.6 (C-2), 135.6 (C-3), 175.8 (C-4), 157.4 (C-5), 110.2 (C-6), 162.1 (C-7), 92.7 (C-8), 154.0 (C-9), 102.6 (C-10), 121.8 (C-1'), 129.0 (C-2'), 127.6 (C-3'), 156.9 (C-4'), 114.9 (C-5'), 127.0 (C-6'), 21.0 (C-1''), 122.4 (C-2''), 130.5 (C-3''), 17.7 (C-4''), 25.5 (C-5''), 28.1 (C-1'''), 122.5 (C-2'''), 131.8 (C-3'''), 17.7 (C-4'''), 25.5 (C-5''').

*Glycycoumarin (39)*

Yellow amorphous powder

ESIMS  $m/z$  367.1188  $[\text{M} - \text{H}]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  7.80 (1H, s, H-4), 6.60 (1H, s, H-8), 6.36 (1H, d,  $J = 2.3$  Hz, H-3'), 6.26 (1H, dd,  $J = 8.4, 2.2$  Hz, H-5'), 7.10 (1H, d,  $J = 8.4$  Hz, H-6'), 3.26 (2H, d,  $J = 6.8$  Hz, H-1''), 5.15 (1H, t,  $J = 6.8$  Hz, H-2''), 1.73 (3H, s, H-4''), 1.63 (3H, s, H-5''), 3.76 (3H, s, 5-OCH<sub>3</sub>).

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  159.8 (C-2), 120.1 (C-3), 136.5 (C-4), 156.1 (C-5), 118.4 (C-6), 160.1 (C-7), 98.0 (C-8), 153.0 (C-9), 106.2 (C-10), 113.5 (C-1'), 155.3 (C-2'), 102.7 (C-3'), 158.4 (C-4'), 105.9 (C-5'), 131.6 (C-6'), 22.4 (C-1''), 122.8 (C-2''), 130.7 (C-3''), 17.8 (C-4''), 25.5 (C-5''), 62.9 (5-OCH<sub>3</sub>).

*Semilicoisoflavone B (40)*

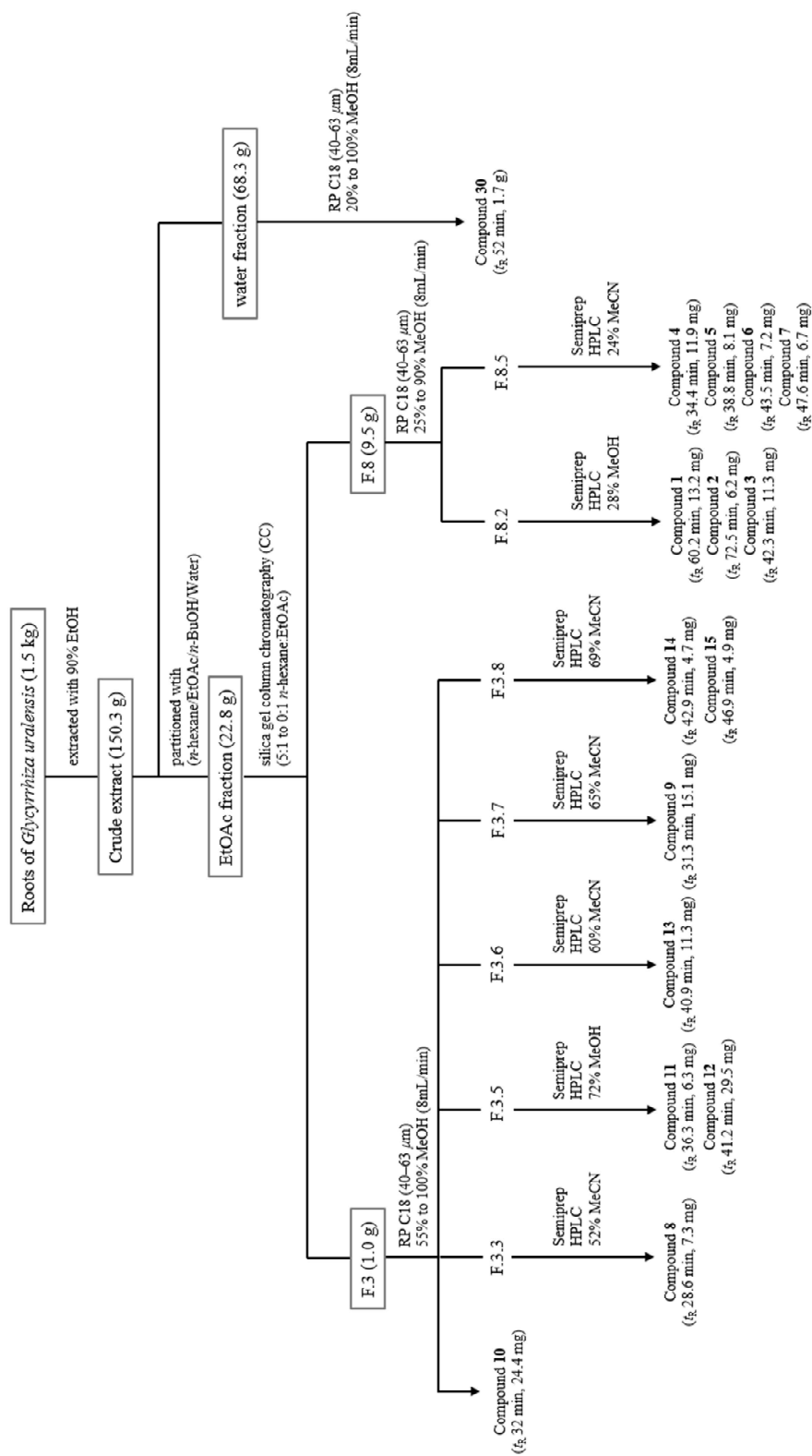
Yellow amorphous powder

ESIMS  $m/z$  351.0869  $[\text{M} - \text{H}]^-$

$^1\text{H}$  NMR data (400 MHz, DMSO- $d_6$ ):  $\delta_{\text{H}}$  8.32 (1H, s, H-2), 6.21 (1H, d,  $J = 2.1$  Hz, H-6), 6.37 (2H, m, H-8, 1''), 6.72 (1H, d,  $J = 2.1$  Hz, H-2'), 6.91 (1H, d,  $J = 2.1$  Hz, H-6'), 5.75 (1H, d,  $J = 9.8$  Hz, H-2''), 1.39 (6H, s, H-4'', 5'').

$^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta_{\text{C}}$  154.2 (C-2), 122.1 (C-3), 180.0 (C-4), 162.0

(C-5), 99.2 (C-6), 164.8 (C-7), 93.8 (C-8), 157.6 (C-9), 104.3 (C-10), 122.8 (C-1'),  
122.0 (C-2'), 145.2 (C-3'), 140.1 (C-4'), 121.5 (C-5'), 117.6 (C-6'), 117.0 (C-1''),  
131.3 (C-2''), 76.0 (C-3''), 27.5 (C-4''), 27.5 (C-5'').



Scheme 1. Isolation scheme from *G. uralensis*



## 2.5. C2C12 cells MTT assay

Myoblast cells from mouse (C2C12) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone), 1% penicillin-streptomycin (Gibco) under 37 °C, 5% CO<sub>2</sub> humidified atmosphere and used at passages 10–25. A 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay was used to evaluate cytotoxicity. C2C12 cells were seeded in 96-well plates at density 5000 cell/well each sample treated triplicate. After overnight incubation, cells were treated 100 µL of sample containing serum free media for 24h. 20 µL of MTT solution (2mg/ mL) was directly added to each well. After four hours, purple MTT formazan precipitate was then dissolved in 100 µL of DMSO, and the absorbance at 540 nm was measured on a multiwall reader (VersaMax).

## 2.6. RAW264.7 cells NO assay

RAW264.7 cells from mouse macrophages (American Type Culture Collection) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Hyclone), 1% penicillin-streptomycin (Gibco) under 37 °C, 5% CO<sub>2</sub> humidified atmosphere. Concentration of nitrite produced by A549 cells as a measure of the production of NO was determined. DMEM without phenol red (WelGene, Korea) was used for the NO production assay. RAW 264.7 cells were seeded in 96-well plates at density  $4.0 \times 10^4$  cell/well each and incubated for 1 day at 37 °C under a 5% CO<sub>2</sub> atmosphere. Nitric oxide production was evaluated by using the Griess reagent method, and the absorbance at 540 nm was measured on a multiwall reader (VersaMax). For the NO assay stimulated by lipopolysaccharide (LPS), RAW 264.7 cells were pretreated with the 100 µL of

sample containing serum free media for 1 h, and 1 ug/mL LPS was added to the cells. After 24 h of incubation, NO production was measured as described above. Also Cell viability was tested same as C2C12 MTT assay.

## 2.7. Statistical analysis

Data were calculated as the means  $\pm$  standard deviations (SD). The GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA) was used to establish statistical analysis. *P* value less than 0.05 was considered to be statistically significant.

### 3. Results and discussion

#### 3.1. Elucidation of compounds from roots of *G. uralensis*

##### 3.2.1. Compound 1

Compound **1** was obtained as white amorphous powder and its empirical formula was established as  $C_{21}H_{22}O_9$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  417.1184  $[M - H]^-$  (calcd for  $C_{21}H_{21}O_9$ , 417.1186) and 11 degrees of unsaturation.

The  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.41 (2H, d,  $J = 8.6$  Hz, H-2', 6') /  $\delta_C$  128.8 (C-2', 6')  $\delta_H$  7.11 (2H, d,  $J = 8.6$  Hz, H-3', 5') /  $\delta_C$  117.8 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.70 (1H, d,  $J = 8.7$  Hz, H-5) /  $\delta_C$  129.9 (C-5)  $\delta_H$  6.47 (1H, dd,  $J = 8.7, 2.0$  Hz, H-6) /  $\delta_C$  111.9 (C-6)  $\delta_H$  6.34 (1H, d,  $J = 2.0$  Hz, H-8) /  $\delta_C$  103.8 (C-8)] and one anomeric carbon [ $\delta_C$  102.2 (C-1'')] with 5 number of oxygenated carbons [ $\delta_C$  78.2 (C-3''), 78.0 (C-2''), 74.9 (C-4''), 71.3 (C-5''), 62.5 (C-6'')] indicated presence of glucose as hexose, deshielded methine [ $\delta_H$  2.70 (1H, dt,  $J = 16.9, 2.8$  Hz, H-3 $\alpha$ ), 3.01 (1H, dd,  $J = 16.9, 12.9$  Hz, H-3 $\beta$ )] and one carbonyl carbon [ $\delta_C$  193.2 (C-4)]. Based on these properties flavanone type compound with 1 sugar could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **1** was determined as liquiritin (Young Bae Ryu et al., 2010).

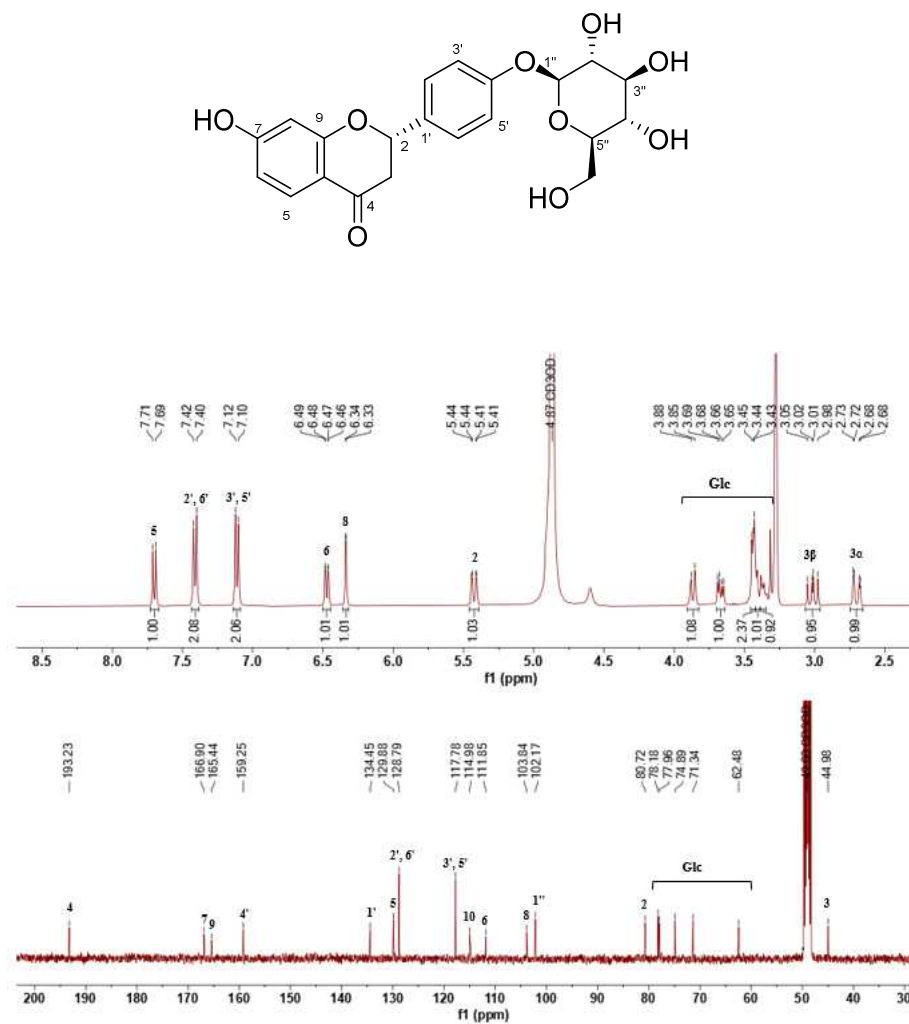


Figure 7. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **1** in CD<sub>3</sub>OD-*d*<sub>4</sub> (400, 100 MHz)

### 3.2.2. Compound 2

Compound **2** was obtained as yellowish gum and its empirical formula was established as  $C_{26}H_{30}O_{13}$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  549.1597  $[M - H]^-$  (calcd for  $C_{26}H_{29}O_{13}$ , 549.1608) and 12 degrees of unsaturation.

The  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.43 (2H, d,  $J = 8.7$  Hz, H-2', 6') /  $\delta_C$  129.9 (C-6'), 128.8 (C-2')]  $\delta_H$  7.10 (2H, d,  $J = 8.7$  Hz, H-3', 5') /  $\delta_C$  117.6 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.71 (1H, d,  $J = 8.7$  Hz, H-5) /  $\delta_C$  129.9 (C-5)  $\delta_H$  6.48 (1H, dd,  $J = 8.7, 2.2$  Hz, H-6) /  $\delta_C$  112.1 (C-6)  $\delta_H$  6.34 (1H, d,  $J = 2.2$  Hz, H-8) /  $\delta_C$  103.9 (C-8)] and two anomeric protons and carbons [ $\delta_H$  4.99 (1H, d,  $J = 7.4$  Hz, H-1'') /  $\delta_C$  100.8 (C-1'')  $\delta_H$  5.46 (1H, d,  $J = 1.5$  Hz, H-1''') /  $\delta_C$  110.8 (C-1''')] with 9 number of oxygenated carbons that distinct difference from compound **1** [ $\delta_C$  80.8 (C-3'''), 78.6 (C-3'', 5''), 78.1 (C-2'', 2'''), 75.5 (C-5'''), 71.4 (C-4''), 66.0 (C-4'''), 62.5 (C-6'')] indicated presence of glucose and apiose, deshielded methine [ $\delta_H$  2.70 (1H, dt,  $J = 16.9, 3.0$ , H-3 $\alpha$ ), 3.02 (1H, dd,  $J = \beta$ )] and one carbonyl carbon [ $\delta_C$  193.2 (C-4)]. Based on these properties flavanone type compound with 2 sugar could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **2** was finally determined as liquiritin apioside (Young Bae Ryu et al., 2010).

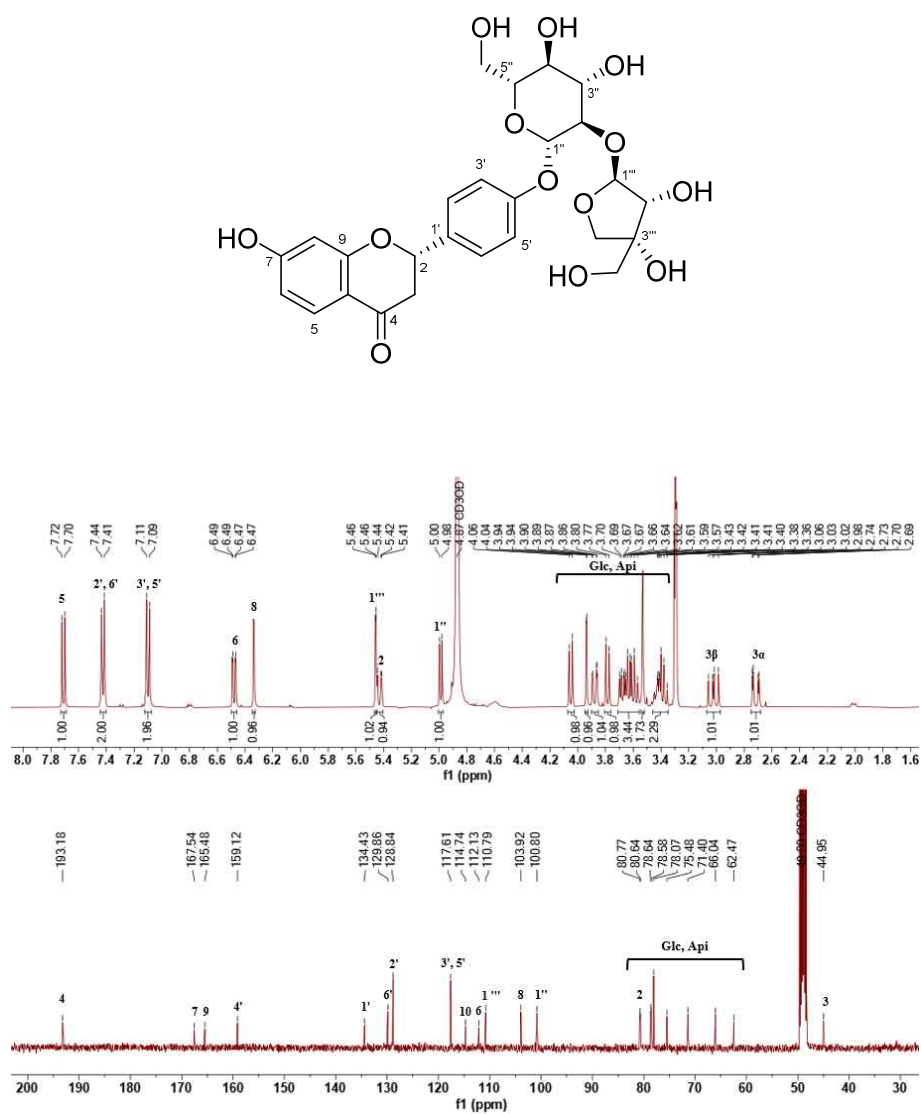


Figure 8. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **2** in CD<sub>3</sub>OD-*d*<sub>4</sub> (400, 100 MHz)

### 3.2.3. Compound **3**

Compound **3** was obtained as pale yellow gum and its empirical formula was established as  $C_{21}H_{22}O_9$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  417.1176  $[M - H]^-$  (calcd for  $C_{21}H_{21}O_9$ , 417.1186) and 11 degrees of unsaturation. The  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.30 (2H, d,  $J = 8.4$  Hz, H-2', 6') /  $\delta_C$  128.0 (C-2', 6')  $\delta_H$  6.75 (2H, d,  $J = 8.4$  Hz, H-3', 5') /  $\delta_C$  115.3 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.67 (1H, d,  $J = 8.8$  Hz, H-5) /  $\delta_C$  128.4 (C-5)  $\delta_H$  6.67 (1H, dd,  $J = 8.9$  Hz, H-6) /  $\delta_C$  111.0 (C-6)  $\delta_H$  6.61 (1H, d,  $J = 2.3$  Hz, H-8) /  $\delta_C$  115.2 (C-8)] and one anomeric proton and carbon [ $\delta_H$  4.95 (1H, d,  $J = 7.5$  Hz, H-1'') /  $\delta_C$  99.8 (C-1'')] 5 number of oxygenated carbons [ $\delta_C$  77.1 (C-3''), 76.4 (C-5''), 73.1 (C-2''), 69.5 (C-4''), 60.6 (C-6'')] indicated presence of glucose, deshielded methine [ $\delta_H$  2.63 (1H, dt,  $J = 14.4, 2.2$ , H-3 $\alpha$ ), 3.17 (1H, m, H-3 $\beta$ )] and one carbonyl carbon [ $\delta_C$  190.7 (C-4)]. Based on these properties flavanone type compound with glucose could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **3** was determined as neoliquiritin (Jun Li et al., 2010).

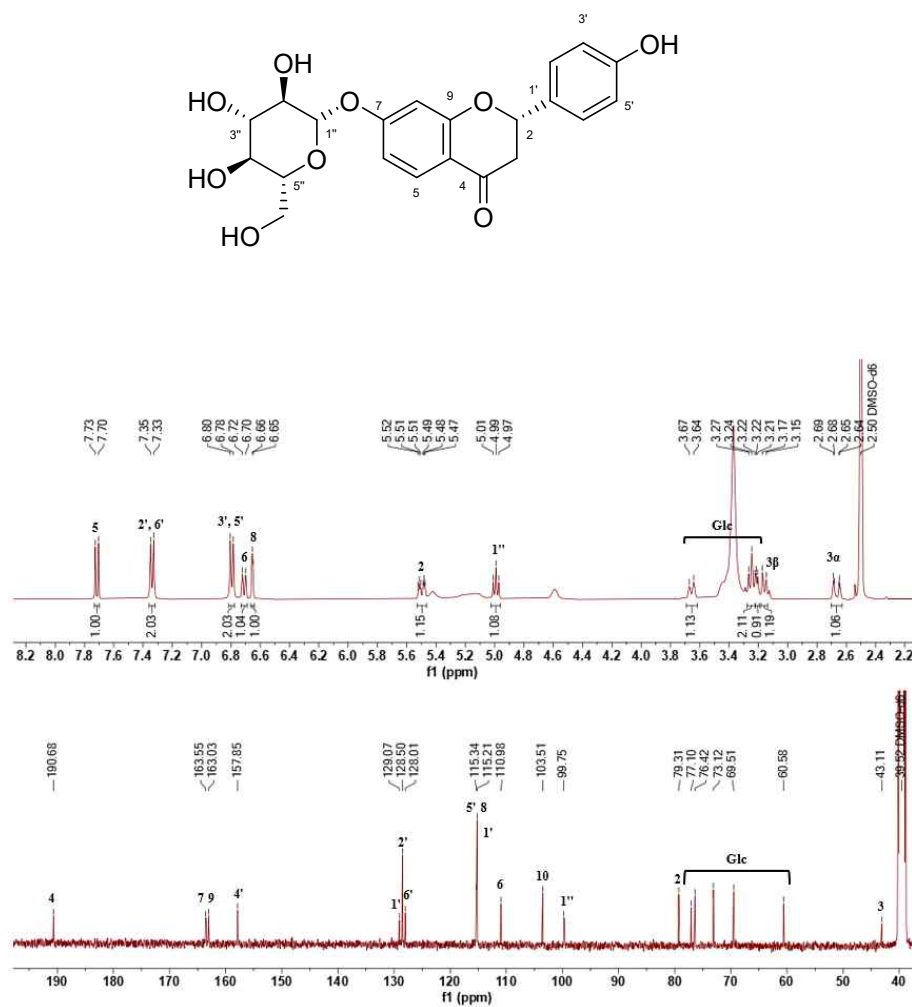


Figure 9.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **3** in  $\text{DMSO-}d_6$  (400, 100 MHz)

### 3.2.4. Compound 4

Compound **4** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{26}H_{30}O_{13}$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  549.1636  $[M - H]^-$  (calcd for  $C_{26}H_{29}O_{13}$ , 549.1608) and 12 degrees of unsaturation.

Same as liquiritin apioside the  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.71 (2H, overlap, H-2', 6') /  $\delta_C$  131.5 (C-2', 6')  $\delta_H$  7.13 (2H, d,  $J$  = 8.2 Hz, H-3', 5') /  $\delta_C$  117.9 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.98 (1H, d,  $J$  = 8.5 Hz, H-6) /  $\delta_C$  133.5 (C-6)  $\delta_H$  6.43 (1H, d,  $J$  = 8.1 Hz, H-5) /  $\delta_C$  109.2 (C-5)  $\delta_H$  6.30 (1H, s, H-3) /  $\delta_C$  103.9 (C-3)] and two anomeric protons and carbons [ $\delta_H$  5.48 (1H, d,  $J$  = 1.2 Hz, H-1''') /  $\delta_C$  110.8 (C-1''')  $\delta_H$  5.06 (1H, d,  $J$  = 7.3 Hz, H-1'') /  $\delta_C$  100.5 (C-1'')] 9 number of oxygenated carbons [ $\delta_C$  80.8 (C-3'''), 78.6 (C-3'', 4''), 78.1 (C-2'', 2'''), 71.3 (C-5''), 66.0 (C-5'''), 62.5 (C-6'')] indicated presence of 2 sugars, and one carbonyl carbon [ $\delta_C$  193.4 (C=O)]. The only distinctive differences from liquiritin apioside were  $^1H$  NMR,  $^{13}C$  NMR signals [ $\delta_H$  7.80 (1H, d,  $J$  = 15.2 Hz, H- $\alpha$ ) /  $\delta_C$  120.0 (C- $\alpha$ )  $\delta_H$  7.67 (overlap, H- $\beta$ ) /  $\delta_C$  144.8 (C- $\beta$ )] around 15.2 Hz coupling constant implied presence of *trans*-olefinic moiety. Based on these properties chalcone type compound could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **4** was determined as isoliquiritin apioside (Young Bae Ryu et al., 2010).



### 3.2.5. Compound **5**

Compound **5** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{21}H_{22}O_9$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  417.1197  $[M - H]^-$  (calcd for  $C_{21}H_{21}O_9$ , 417.1186) and 11 degrees of unsaturation.

Same as liquiritin the  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.69 (2H, overlap, H-2', 6') /  $\delta_C$  131.4 (C-2', 6')  $\delta_H$  7.13 (2H, d,  $J$  = 8.7 Hz, H-3', 5') /  $\delta_C$  118.0 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.96 (1H, d,  $J$  = 8.9 Hz, H-6) /  $\delta_C$  133.5 (C-6)  $\delta_H$  6.40 (1H, dd,  $J$  = 8.1, 2.0 Hz, H-5) /  $\delta_C$  109.3 (C-5)  $\delta_H$  6.26 (1H, d,  $J$  = 8.1, 2.0 Hz, H-3) /  $\delta_C$  103.9 (C-3)] and one anomeric proton and carbon [ $\delta_H$  4.97 (1H, d,  $J$  = 7.2 Hz, H-1'') /  $\delta_C$  101.8 (C-1'')], 5 number of oxygenated carbons except anomeric carbon [ $\delta_C$  78.3 (C-3'''), 77.9 (C-2'''), 74.8 (C-5'''), 71.3 (C-4'''), 62.5 (C-6''')] indicated presence of glucose as a sugar, and one carbonyl carbon [ $\delta_C$  193.3 (C=O)]. Like liquiritin apioside  $^1H$  NMR,  $^{13}C$  NMR signals [ $\delta_H$  7.78 (1H, d,  $J$  = 15.4 Hz, H- $\alpha$ ) /  $\delta_C$  120.1 (C- $\alpha$ )  $\delta_H$  7.69 (overlap, H- $\beta$ ) /  $\delta_C$  144.8 (C- $\beta$ )] of 15.4 Hz coupling constant implied the presence of *trans*-olefinic moiety. Based on these properties chalcone type compound could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **5** was determined as isoliquiritin (Young Bae Ryu et al., 2010).

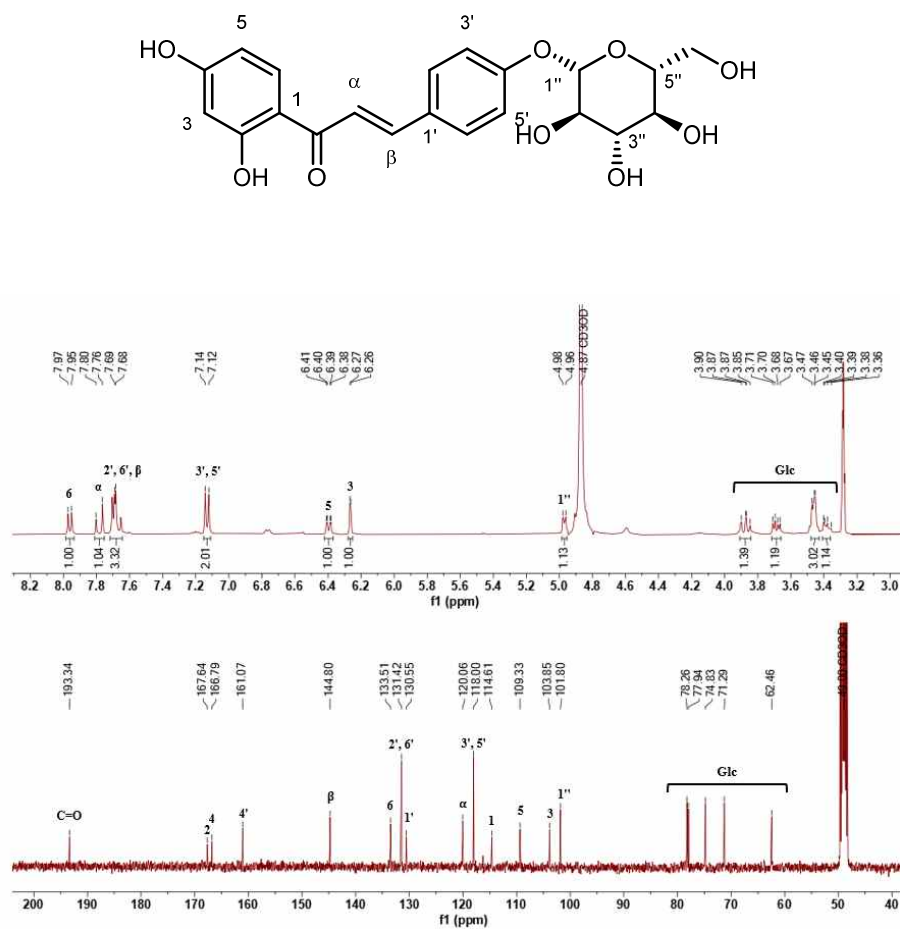


Figure 11.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **5** in  $\text{CD}_3\text{OD}-d_4$  (400, 100 MHz)

### 3.2.6. Compound 6

Compound **6** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{26}H_{30}O_{13}$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  549.1636  $[M - H]^-$  (calcd for  $C_{26}H_{29}O_{13}$ , 549.1608) and 12 degrees of unsaturation.

Same as isoliquiritin apioside the  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.75 (3H, overlap, H-2, 6,  $\beta$ ) /  $\delta_C$  131.5 (C-2, 6)  $\delta_H$  6.84 (2H, d,  $J$  = 8.2 Hz, H-3, 5) /  $\delta_C$  115.9 (C-3, 5)] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  8.25 (1H, d,  $J$  = 9.0 Hz, H-6') /  $\delta_C$  132.6 (C-6')  $\delta_H$  6.57 (1H, dd,  $J$  = 9.0, 2.4 Hz, H-5') /  $\delta_C$  108.1 (C-5')  $\delta_H$  6.53 (1H, d,  $J$  = 2.4 Hz, H-3') /  $\delta_C$  103.4 (C-3')] and two anomeric protons and carbons [ $\delta_H$  5.29 (1H, br s, H-1'') /  $\delta_C$  108.8 (C-1'')  $\delta_H$  5.10 (1H, d,  $J$  = 7.4 Hz, H-1'') /  $\delta_C$  97.9 (C-1'')] 9 number of oxygenated carbons [ $\delta_C$  79.3 (C-3''), 77.7 (C-5''), 76.8 (C-2''), 76.1 (C-3''), 75.7 (C-2''), 74.0 (C-5''), 69.9 (C-4''), 64.3 (C-4''), 60.5 (C-5'')] indicated presence of 2 sugars, and one carbonyl carbon [ $\delta_C$  192.2 (C=O)]. Based on these properties chalcone type compound could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **6** was determined as licuraside (Holger Miething et al., 1989).

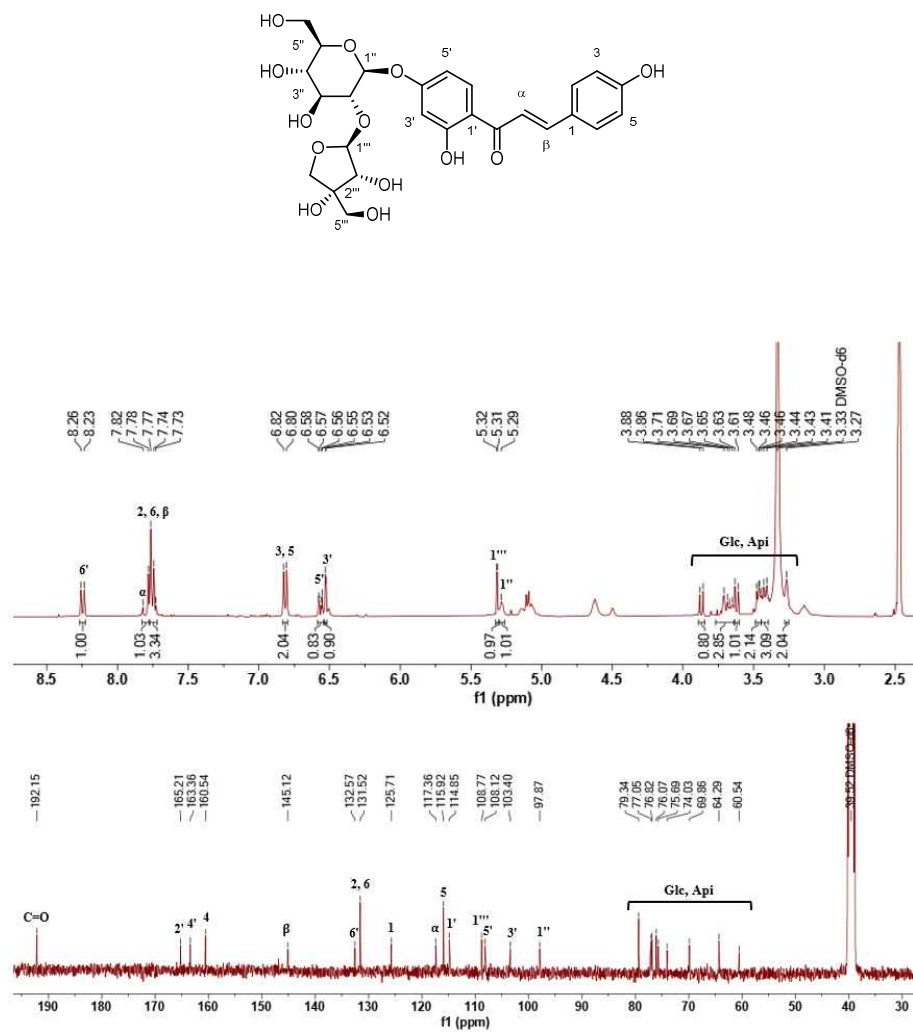
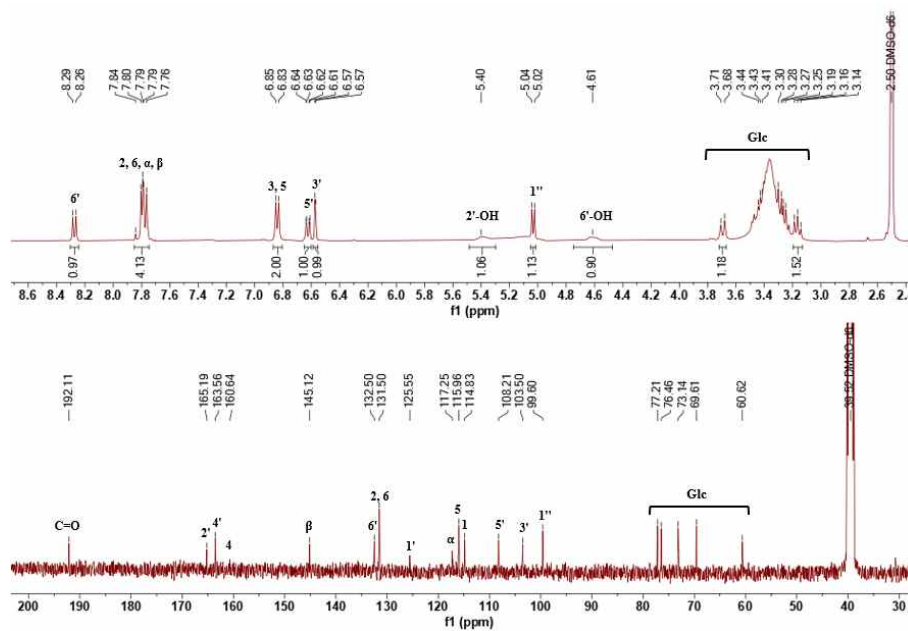


Figure 12.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **6** in  $\text{DMSO-}d_6$  (400, 100 MHz)

### 3.2.7. Compound 7

Compound **7** was obtained as yellowish gum and its empirical formula was established as  $C_{21}H_{22}O_9$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  417.1197  $[M - H]^-$  (calcd for  $C_{21}H_{21}O_9$ , 417.1186) and 11 degrees of unsaturation. Same as liquiritin apioside the  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.79 (4H, overlap, H-2, 6,  $\alpha$ ,  $\beta$ ) /  $\delta_C$  131.5 (C-2, 6)  $\delta_H$  6.84 (2H, d,  $J$  = 8.4 Hz, H-3, 5) /  $\delta_C$  116.0 (C-3, 5)] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  8.27 (1H, d,  $J$  = 9.1 Hz, H-6') /  $\delta_C$  132.5 (C-6')  $\delta_H$  6.63 (1H, dd,  $J$  = 8.9, 2.2 Hz, H-5') /  $\delta_C$  108.2 (C-5')  $\delta_H$  6.57 (1H, d,  $J$  = 2.4 Hz, H-3') /  $\delta_C$  103.5 (C-3')] and one anomeric proton and carbon [ $\delta_H$  5.03 (1H, d,  $J$  = 7.4 Hz, H-1'') /  $\delta_C$  99.6 (C-1'')] 5 number of oxygenated carbons [ $\delta_C$  77.2 (C-5''), 76.5 (C-3''), 73.1 (C-2''), 69.6 (C-4''), 60.6 (C-6'')] indicated presence of glucose, and one carbonyl carbon [ $\delta_C$  192.1 (C=O)]. By comparing above listed information with NMR data from already reported previous literature, Compound **7** was determined as neoisoliquiritin (Holger Miething et al., 1989).



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### 3.2.8. Compound **8**

Compound **8** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{18}O_6$  by HRESIMS, which showed a protonated ion peak at  $m/z$  355.1203  $[M + H]^+$  (calcd for  $C_{20}H_{18}O_6$ , 355.1182) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  197.2 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.40 (1H, dd,  $J$  = 10.8, 5.5 Hz, H-2 $\alpha$ ), 4.28 (1H, dd,  $J$  = 11.0, 5.5 Hz, H-2 $\beta$ ) /  $\delta_C$  69.7 (C-2), 75.1 (C-3'')] with two benzene ring [ $\delta_H$  6.82 (1H, d,  $J$  = 8.3 Hz, H-6') /  $\delta_C$  130.2 (C-6')  $\delta_H$  6.27 (1H, d,  $J$  = 8.3 Hz, H-5') /  $\delta_C$  110.2 (C-5')], [ $\delta_H$  5.88 (2H, overlap, H-6, 8) /  $\delta_C$  96.1 (C-6), 95.0 (C-8)] implied isoflavanone type. Two *cis*-olefinic peaks [ $\delta_H$  6.68 (1H, d,  $J$  = 10.1 Hz, H-1'') /  $\delta_C$  115.6 (C-1'')  $\delta_H$  5.68 (1H, d,  $J$  = 10.0 Hz, H-2'') /  $\delta_C$  129.1 (C-2'')] with two methyls [ $\delta_H$  1.34 (6H, s, H-4'', 5'') /  $\delta_C$  27.4 (C-4'', 5'')] indicated presence of pyran moiety with two methyls. By comparing above listed information with NMR data from already reported previous literature, Compound **8** was determined as licoisoflavanone (Shuai Ji et al., 2016).

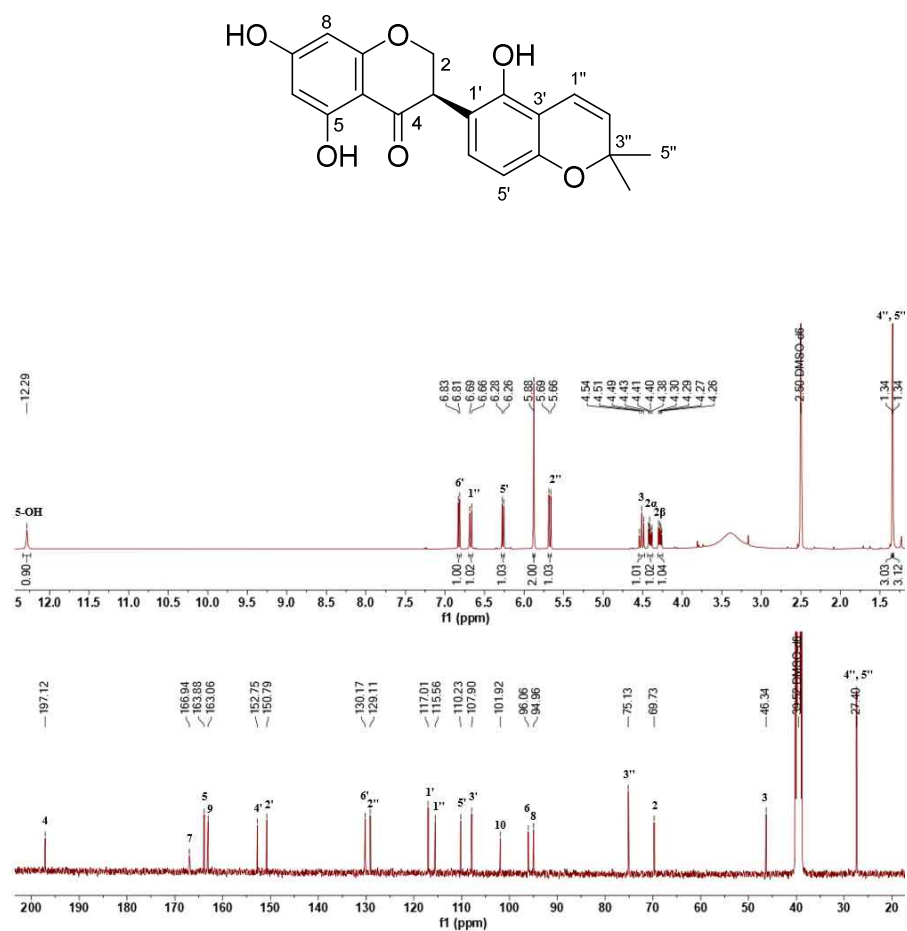


Figure 14. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **8** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.9. Compound **9**

Compound **9** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{26}O_5$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  405.1740  $[M - H]^-$  (calcd for  $C_{25}H_{25}O_5$ , 405.1702) and 13 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.5 (C-4)] and three olefinic protons and carbons [ $\delta_H$  8.37 (1H, s, H-2) /  $\delta_C$  153.9 (C-2), 131.9 (C-3", 3'')]  $\delta_H$  5.14 (2H, q,  $J = 7.4$  Hz, H-2", 2'') /  $\delta_C$  122.4 (C-2", 2''), 121.5 (C-3)] with 1,4-disubstituted benzene ring [ $\delta_H$  7.37 (2H, d,  $J = 8.5$  Hz, H-2', 6') /  $\delta_C$  130.2 (C-2'), 130.7 (C-6')]  $\delta_H$  6.81 (2H, d,  $J = 8.6$  Hz, H-3', 5') /  $\delta_C$  115.1 (C-3', 5')] and four methyls [ $\delta_H$  1.76 (3H, s, H-4'') /  $\delta_C$  17.8 (C-4'')  $\delta_H$  1.73 (3H, s, H-4''') /  $\delta_C$  17.8 (C-4''')  $\delta_H$  1.63 (3H, s, H-5'') /  $\delta_C$  25.3 (C-4'')  $\delta_H$  1.62 (3H, s, H-5''') /  $\delta_C$  25.5 (C-5''')] implied isoflavone type with 2 prenyl groups. By comparing above listed information with NMR data from already reported previous literature, Compound **9** was determined as licoisoflavanone (Shuai Ji et al., 2016).

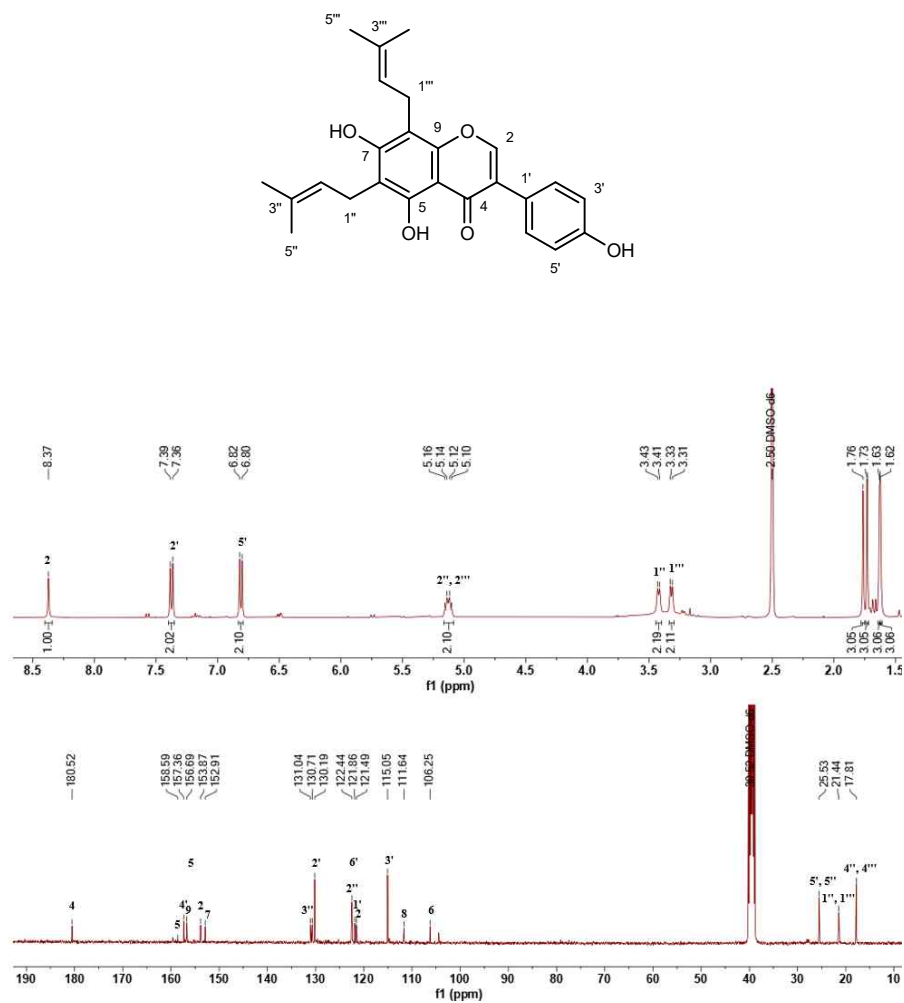
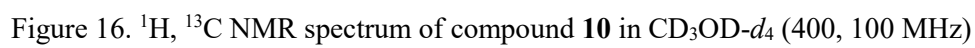


Figure 15.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound 9 in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.10. Compound **10**

Compound **10** was obtained as brownish gum and its empirical formula was established as  $C_{16}H_{14}O_4$  by HRESIMS, which showed a protonated ion peak at  $m/z$  271.0963  $[M + H]^+$  (calcd for  $C_{16}H_{15}O_4$ , 271.0970) and 10 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated presence of two 1,2,4-trisubstituted benzene rings [ $\delta_H$  7.28 (1H, d,  $J = 8.4$  Hz, H-1) /  $\delta_C$  133.2 (C-1)  $\delta_H$  6.43 (1H, dd,  $J = 8.2, 2.4$  Hz, H-2) /  $\delta_C$  107.2 (C-2)  $\delta_H$  6.30 (1H, d,  $J = 2.4$  Hz, H-4) /  $\delta_C$  104.1 (C-4)], [ $\delta_H$  7.15 (1H, d,  $J = 8.2$  Hz, H-7) /  $\delta_C$  126.0 (C-7)  $\delta_H$  6.48 (1H, dd,  $J = 8.4, 2.4$  Hz, H-8) /  $\delta_C$  110.7 (C-8)  $\delta_H$  6.37 (1H, d,  $J = 2.2$  Hz, H-10) /  $\delta_C$  97.6 (C-10)] and one methoxy [ $\delta_H$  3.73 (3H, s, 9-OCH<sub>3</sub>) /  $\delta_C$  55.9 (9-OCH<sub>3</sub>)] with oxygenated proton and carbon [ $\delta_H$  4.20 (1H, m, H-6 $\alpha$ ) /  $\delta_C$  67.6 (C-6 $\alpha$ )  $\delta_H$  3.51 (1H, m, H-6 $\beta$ ) /  $\delta_C$  67.6 (C-6 $\beta$ )]. One deshielded oxygenated peak [ $\delta_H$  5.45 (1H, d,  $J = 6.3$  Hz, H-11a) /  $\delta_C$  80.0 (C-11a)] without  $^{13}C$  NMR signal at carbonyl carbon region and 10 degrees of unsaturation implied structural modification of isoflavone. By comparing above listed information with NMR data from already reported previous literature, Compound **10** was determined as medicarpin (Chen, A-H et al., 2018).



### 3.2.11. Compound **11**

Compound **11** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{15}O_5$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  335.0927  $[M - H]^-$  (calcd for  $C_{16}H_{15}O_4$ , 335.0927) and 13 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.0 (C-4)] and deshielded olefinic proton and carbon [ $\delta_H$  8.36 (1H, s, H-2) /  $\delta_C$  152.4 (C-2)] with trisubstituted benzene ring [ $\delta_H$  7.29 (2H, m, H-2', 6') /  $\delta_C$  129.8 (C-6'), 127.0 (C-2')  $\delta_H$  6.80 (1H, d,  $J = 8.0$  Hz, H-5') /  $\delta_C$  115.7 (C-5')] and two methyls [ $\delta_H$  1.39 (6H, s, H-4'', 5'') /  $\delta_C$  27.7 (C-4'', 5'')] two *cis*-olefinic protons and carbons [ $\delta_H$  6.44 (1H, d,  $J = 9.8$  Hz, H-1'') /  $\delta_C$  121.7 (C-1'')  $\delta_H$  5.79 (1H, d,  $J = 9.8$  Hz, H-2'') /  $\delta_C$  131.4 (C-2'')] implied isoflavone type with pyran ring moiety. By comparing above listed information with NMR data from already reported previous literature, Compound **11** was determined as isoderrone (Shuai Ji et al., 2016).



Figure 17. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **11** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.12. Compound **12**

Compound **12** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{28}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  423.1823  $[M - H]^-$  (calcd for  $C_{25}H_{27}O_6$ , 423.1808) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  198.0 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.45 (2H, m, H-2) /  $\delta_C$  70.2 (C-2)] with two benzene ring [ $\delta_H$  6.67 (1H, d,  $J = 8.3$  Hz, H-6') /  $\delta_C$  127.0 (C-6')  $\delta_H$  6.30 (1H, d,  $J = 8.3$  Hz, H-5') /  $\delta_C$  106.8 (C-5')  $\delta_H$  5.97 (1H, s, H-8) /  $\delta_C$  95.4 (C-8)] implied isoflavanone type. Two deshielded methines [ $\delta_H$  3.25 (2H, d,  $J = 6.9$  Hz, H-1''') /  $\delta_C$  22.4 (C-1''')  $\delta_H$  3.11 (2H, d,  $J = 7.0$  Hz, H-1'') /  $\delta_C$  21.1 (C-1'')] and two olefinic [ $\delta_H$  5.12 (2H, d,  $J = 6.9$  Hz, H-2'', 2''') /  $\delta_C$  123.4 (C-2'''), 122.9 (C-2'')] with four methyls [ $\delta_H$  1.70 (3H, s, H-5''') /  $\delta_C$  25.5 (C-5''')  $\delta_H$  1.68 (3H, s, H-5'') /  $\delta_C$  25.6 (C-5'')  $\delta_H$  1.61 (6H, s, H-4'', 4''') /  $\delta_C$  17.8 (C-4''), 17.7 (C-4''')] implied the presence of two prenyls. By comparing above listed information with NMR data from already reported previous literature, Compound **12** was determined as glisoflavanone (Yan Lin et al., 2017).

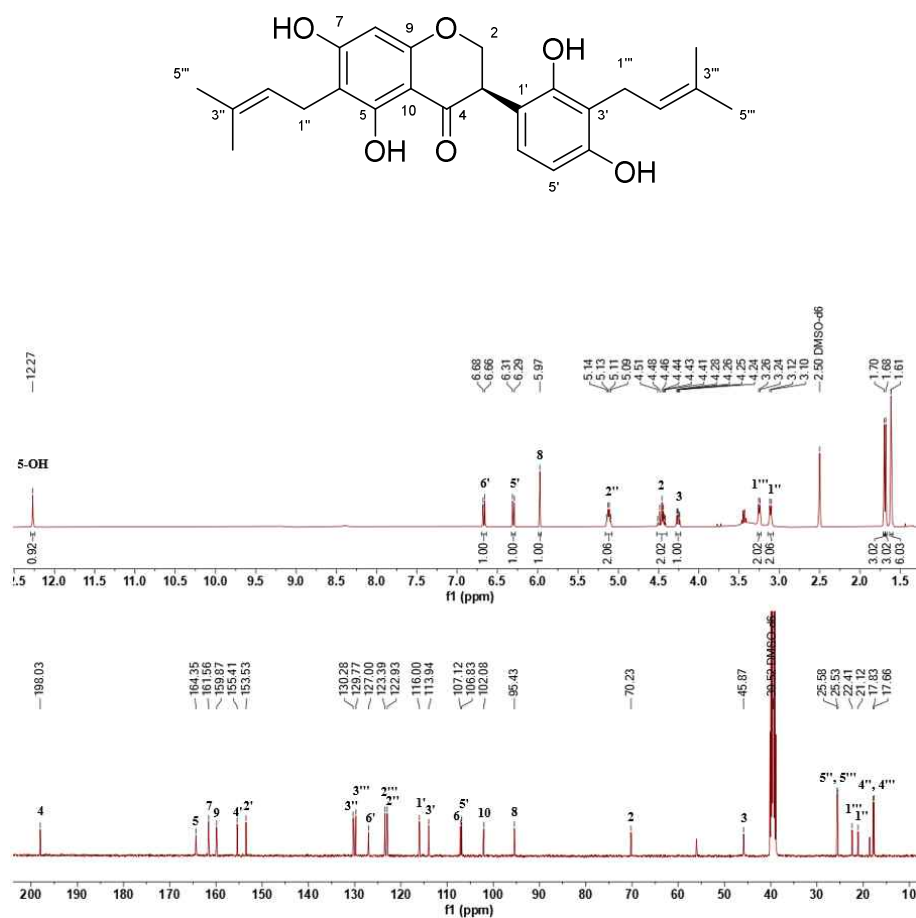


Figure 18. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **12** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.13. Compound **13**

Compound **13** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{26}O_7$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  437.1612  $[M - H]^-$  (calcd for  $C_{25}H_{25}O_7$ , 437.1600) and 13 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  194.7 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.61 (1H, d,  $J = 11.9$  Hz, H-2 $\alpha$ ), 4.06 (1H, d,  $J = 11.9$  Hz, H-2 $\beta$ ) /  $\delta_C$  73.5 (C-2)] with two benzene ring [ $\delta_H$  7.25 (1H, d,  $J = 8.5$  Hz, H-6') /  $\delta_C$  127.6 (C-6')  $\delta_H$  6.35 (1H, d,  $J = 8.5$  Hz, H-5') /  $\delta_C$  107.5 (C-5')  $\delta_H$  5.98 (1H, s, H-8) /  $\delta_C$  94.2 (C-8)]. Unlike isoflavanone type compounds  $^1H$  NMR of H-2 splitted in a doublet implied no protons adjacent to H-2. Two methyls [ $\delta_H$  1.35 (3H, s, H-4'') /  $\delta_C$  27.4 (C-4'')  $\delta_H$  1.33 (3H, s, H-5'') /  $\delta_C$  27.5 (C-5'')] and two *cis*-olefinic protons and carbons [ $\delta_H$  6.61 (1H, d,  $J = 10.1$  Hz, H-1'') /  $\delta_C$  116.7 (C-1'')  $\delta_H$  5.67 (1H, d,  $J = 10.0$  Hz, H-2'') /  $\delta_C$  129.2 (C-2'')] implied pyran ring moiety and two methyls [ $\delta_H$  1.69 (3H, s, H-4'') /  $\delta_C$  17.7 (C-4'')  $\delta_H$  1.62 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] and one deshielded methine [ $\delta_H$  3.12 (2H, d,  $J = 7.1$  Hz, H-1'') /  $\delta_C$  20.7 (C-1'')] and one olefinic [ $\delta_H$  5.13 (1H, t,  $J = 7.2$  Hz, H-2'') /  $\delta_C$  122.7 (C-2'')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **13** was determined as glyurallin H (Jing-ran Fan et al., 2020).

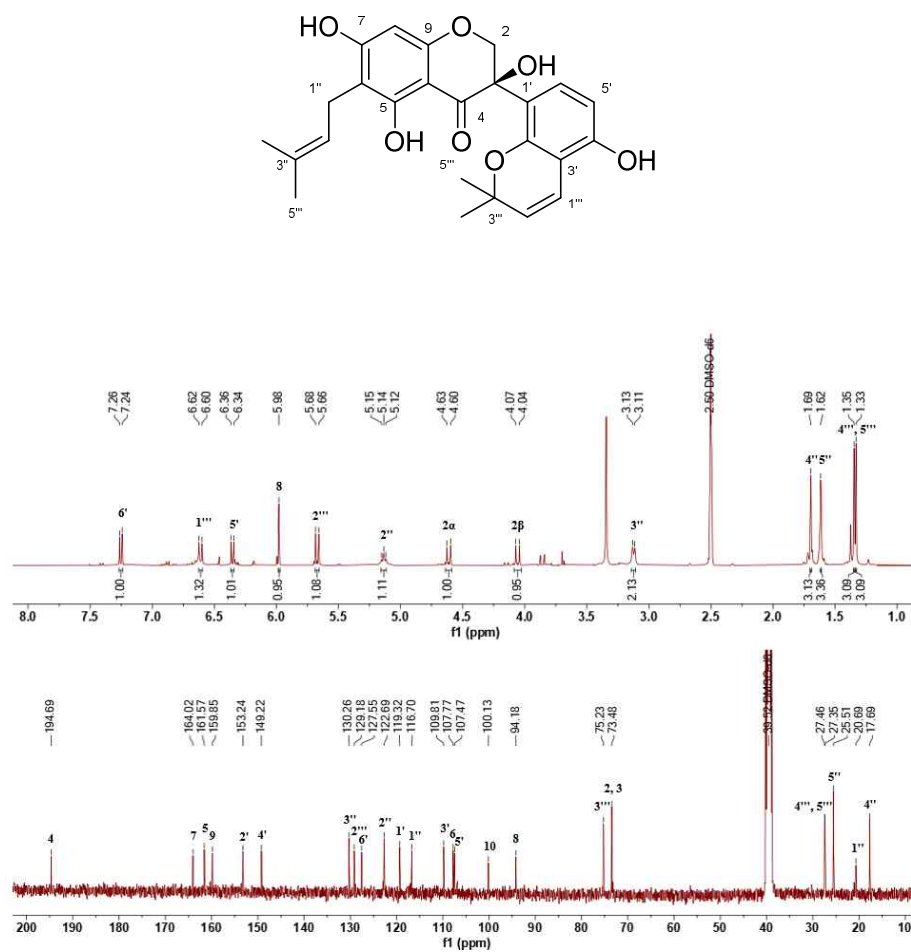


Figure 19.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **13** in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.14. Compound **14**

Compound **14** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{25}H_{24}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  419.1502  $[M - H]^-$  (calcd for  $C_{25}H_{23}O_6$ , 419.1495) and 14 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.6 (C-4)] and deshielded olefinic proton and carbon [ $\delta_H$  8.16 (1H, s, H-2) /  $\delta_C$  153.6 (C-2)] with two benzene rings [ $\delta_H$  6.88 (1H, d,  $J$  = 8.3 Hz, H-6') /  $\delta_C$  131.4 (C-6')  $\delta_H$  6.45 (1H, s, H-8) /  $\delta_C$  93.0 (C-8)  $\delta_H$  6.32 (1H, d,  $J$  = 8.3 Hz, H-5') /  $\delta_C$  107.0 (C-5')] implied isoflavone type compound. And two methyls [ $\delta_H$  1.37 (6H, s, H-4'', 5'') /  $\delta_C$  27.5 (C-4'', 5'')] and two *cis*-olefinic protons and carbons [ $\delta_H$  6.67 (1H, d,  $J$  = 10.0 Hz, H-1'') /  $\delta_C$  117.0 (C-1'')  $\delta_H$  5.68 (1H, d,  $J$  = 10.0 Hz, H-2'') /  $\delta_C$  128.9 (C-2'')] implied pyran ring moiety and two methyls [ $\delta_H$  1.72 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')  $\delta_H$  1.62 (3H, s, H-4'') /  $\delta_C$  17.7 (C-4'')] and one deshielded methine [ $\delta_H$  3.23 (2H, d,  $J$  = 7.0 Hz, H-1'') /  $\delta_C$  21.0 (C-1'')] and one olefinic [ $\delta_H$  5.17 (1H, t,  $J$  = 7.1 Hz, H-2'') /  $\delta_C$  120.3 (C-2'')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **14** was determined as angustone B (Yan Lin et al., 2017).

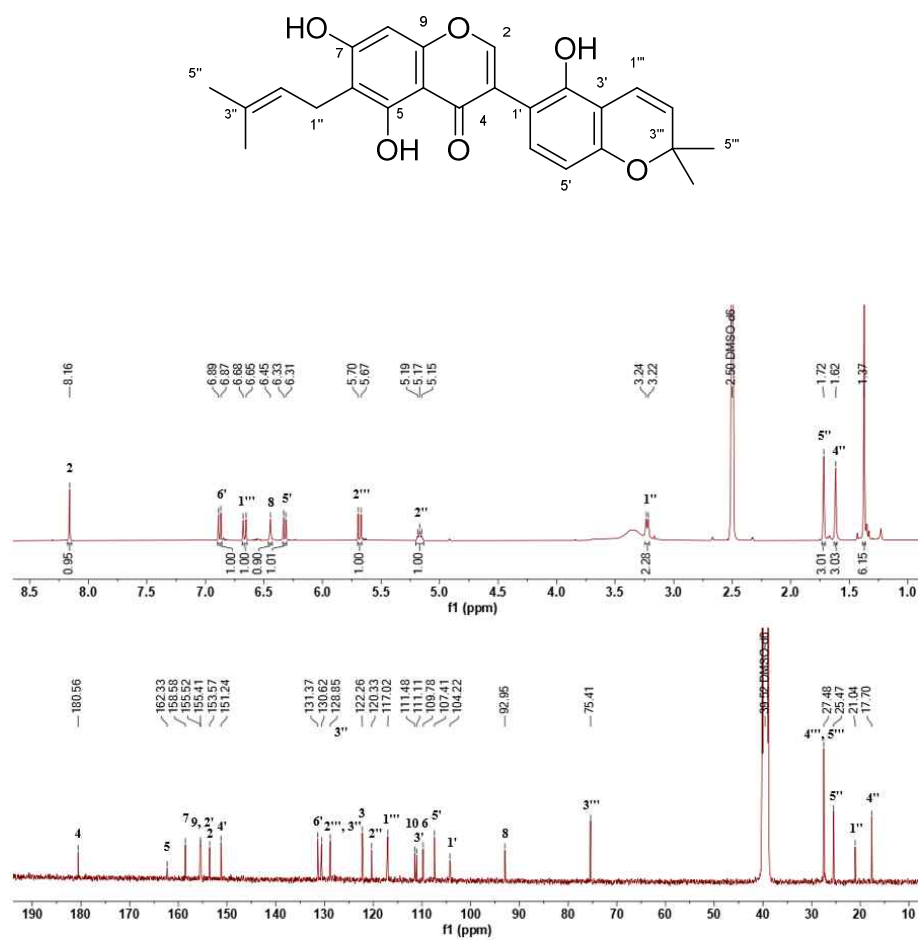


Figure 20.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **14** in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.15. Compound **15**

Compound **15** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{24}O_5$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  403.1562  $[M - H]^-$  (calcd for  $C_{25}H_{23}O_5$ , 403.1546) and 14 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.0 (C-4)] and deshielded olefinic proton and carbon [ $\delta_H$  8.33 (1H, s, H-2) /  $\delta_C$  154.0 (C-2)] with two benzene rings [ $\delta_H$  7.28 (2H, m, H-2', 6') /  $\delta_C$  129.8 (C-6'), 127.0 (C-2')  $\delta_H$  6.45 (1H, s, H-8) /  $\delta_C$  93.1 (C-8)] implied isoflavone type compound. And two methyls [ $\delta_H$  1.39 (6H, s, H-4'', 5'') /  $\delta_C$  27.8 (C-4'', 5'')] and two *cis*-olefinic protons and carbons [ $\delta_H$  6.44 (1H, d,  $J$  = 10.3 Hz, H-1'') /  $\delta_C$  115.7 (C-1'')  $\delta_H$  5.78 (1H, d,  $J$  = 9.8 Hz, H-2'') /  $\delta_C$  131.4 (C-2'')] implied pyran ring moiety and two methyls [ $\delta_H$  1.72 (3H, s, H-5') /  $\delta_C$  25.5 (C-5')  $\delta_H$  1.62 (3H, s, H-4') /  $\delta_C$  17.8 (C-4')] and one deshielded methine [ $\delta_H$  3.22 (2H, d,  $J$  = 7.0 Hz, H-1') /  $\delta_C$  21.1 (C-1')] and one olefinic [ $\delta_H$  5.17 (1H, t,  $J$  = 7.0 Hz, H-2') /  $\delta_C$  121.7 (C-2')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **15** was determined as isochandalone (Yan Lin et al., 2017).

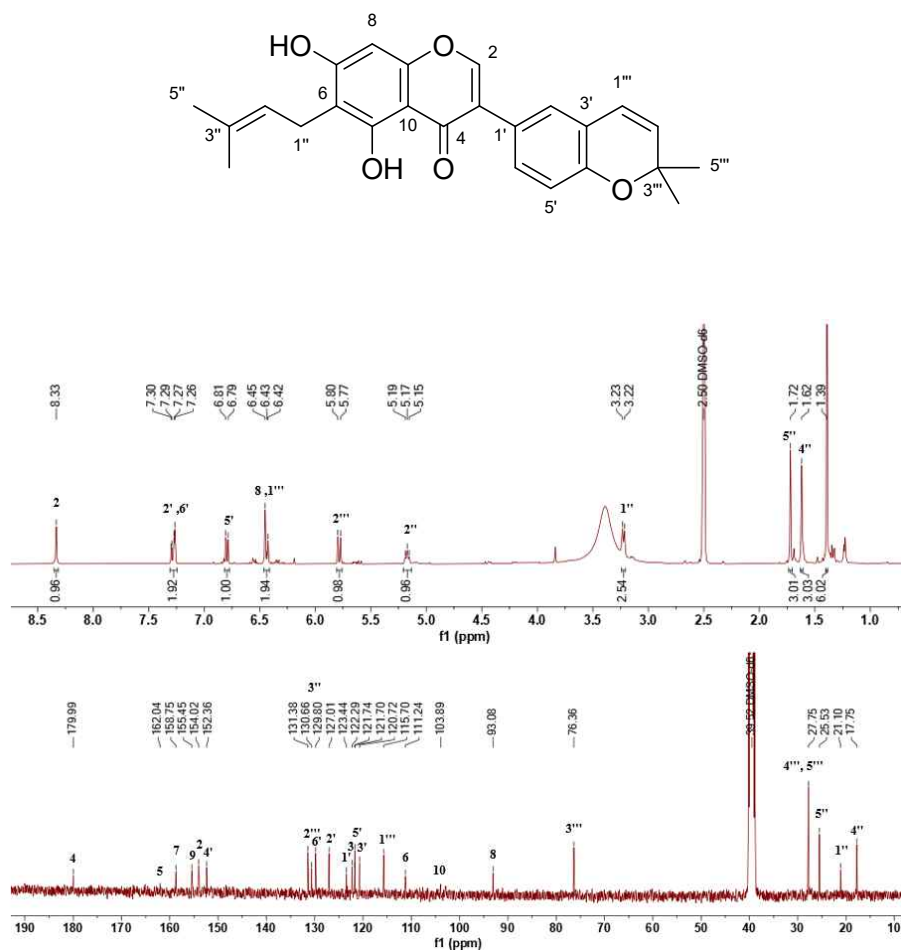


Figure 21.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **15** in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.16. Compound **16**

Compound **16** was obtained as brownish gum and its empirical formula was established as  $C_{16}H_{16}O_4$  by HRESIMS, which showed a protonated ion peak at  $m/z$  273.1117  $[M + H]^+$  (calcd for  $C_{16}H_{17}O_4$ , 273.1127) and 9 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated oxygenated protons and carbons [ $\delta_H$  3.90 (1H, t,  $J = 10.1$  Hz, H-2 $\alpha$ ), 4.13 (1H, ddd,  $J = 10.7, 3.3, 2.2$  Hz, H-2 $\beta$ ) /  $\delta_C$  69.2 (C-2)  $\delta_H$  3.66 (3H, s, 4'-OCH<sub>3</sub>) /  $\delta_C$  54.9 (4'-OCH<sub>3</sub>)] with two benzene ring [ $\delta_H$  6.98 (1H, d,  $J = 8.5$  Hz, H-6') /  $\delta_C$  127.8 (C-6')  $\delta_H$  6.86 (1H, d,  $J = 8.2$  Hz, H-5) /  $\delta_C$  130.1 (C-5)  $\delta_H$  6.41 (1H, d,  $J = 2.5$  Hz, H-3') /  $\delta_C$  101.3 (C-3')  $\delta_H$  6.35 (1H, dd,  $J = 8.5$  Hz, H-5') /  $\delta_C$  104.4 (C-5')  $\delta_H$  6.28 (1H, dd,  $J = 8.2, 2.5$  Hz, H-6) /  $\delta_C$  107.9 (C-6)] implied isoflavan type with one methoxy. By comparing above listed information with NMR data from already reported previous literature, Compound **16** was determined as (-)-vestitol (Abdallah et al., 2021).

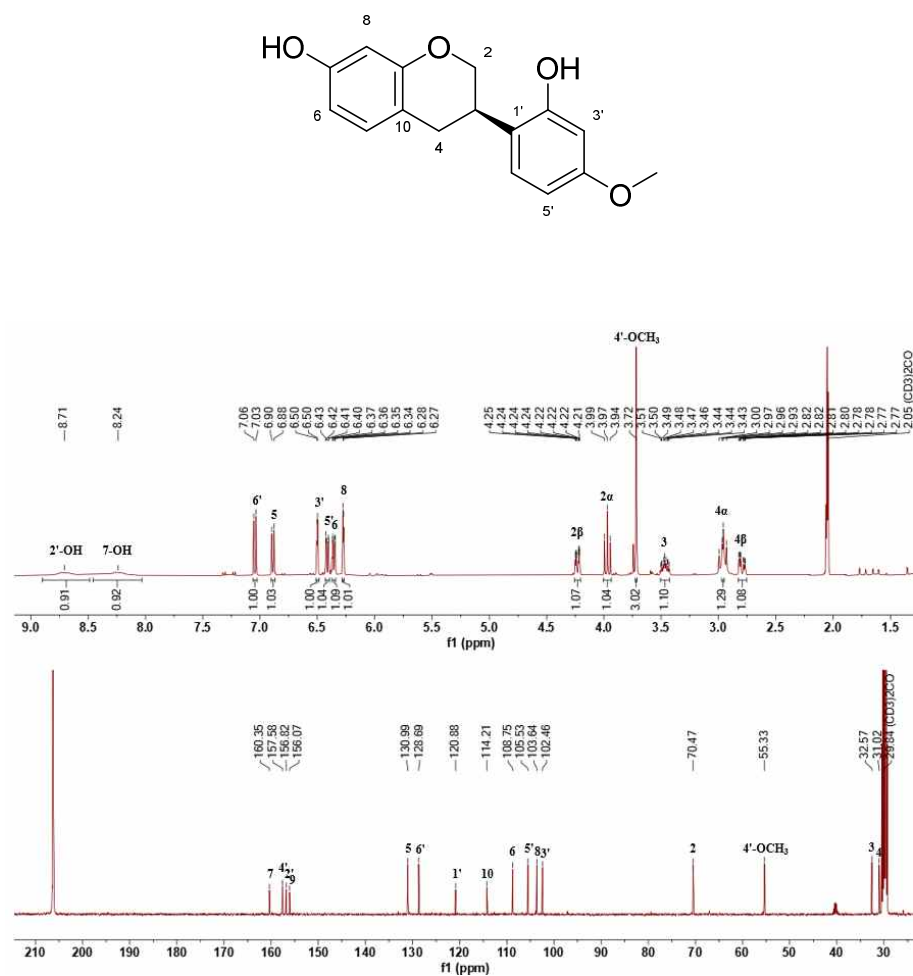


Figure 22.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **16** in  $\text{Acetone-}d_6$  (400, 100 MHz)

### 3.2.17. Compound **17**

Compound **17** was obtained as pale brownish gum and its empirical formula was established as  $C_{20}H_{18}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  353.1028  $[M - H]^-$  (calcd for  $C_{20}H_{18}O_6$ , 353.1025) and 12 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  198.6 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.53 (1H, t,  $J = 11.0$  Hz, H-2 $\alpha$ ), 4.39 (1H, dd,  $J = 10.9, 5.6$  Hz, H-2 $\beta$ ) /  $\delta_C$  71.5 (C-2), 77.4 (C-3'')] with two benzene ring [ $\delta_H$  6.85 (1H, d,  $J = 8.4$  Hz, H-6') /  $\delta_C$  131.6 (C-6')  $\delta_H$  6.40 (1H, d,  $J = 8.4$  Hz, H-5') /  $\delta_C$  108.8 (C-5')  $\delta_H$  5.96 (1H, d,  $J = 2.0$  Hz, H-8) /  $\delta_C$  96.2 (C-8)  $\delta_H$  5.94 (1H, d,  $J = 2.0$  Hz, H-6) /  $\delta_C$  97.4 (C-6)] implied isoflavanone type. Two *cis*-olefinic peaks [ $\delta_H$  6.67 (1H, d,  $J = 10.0$  Hz, H-1'') /  $\delta_C$  118.2 (C-1'')  $\delta_H$  5.62 (1H, d,  $J = 10.0$  Hz, H-2'') /  $\delta_C$  129.5 (C-2'')] with two methyls [ $\delta_H$  1.34 (3H, s, H-4'') /  $\delta_C$  28.4 (C-4'')  $\delta_H$  1.33 (3H, s, H-5'') /  $\delta_C$  28.1 (C-5'')] indicated presence of pyran moiety with two methyls. By comparing above listed information with NMR data from already reported previous literature, Compound **17** was determined as glyasperin F (Gumula et al., 2012).

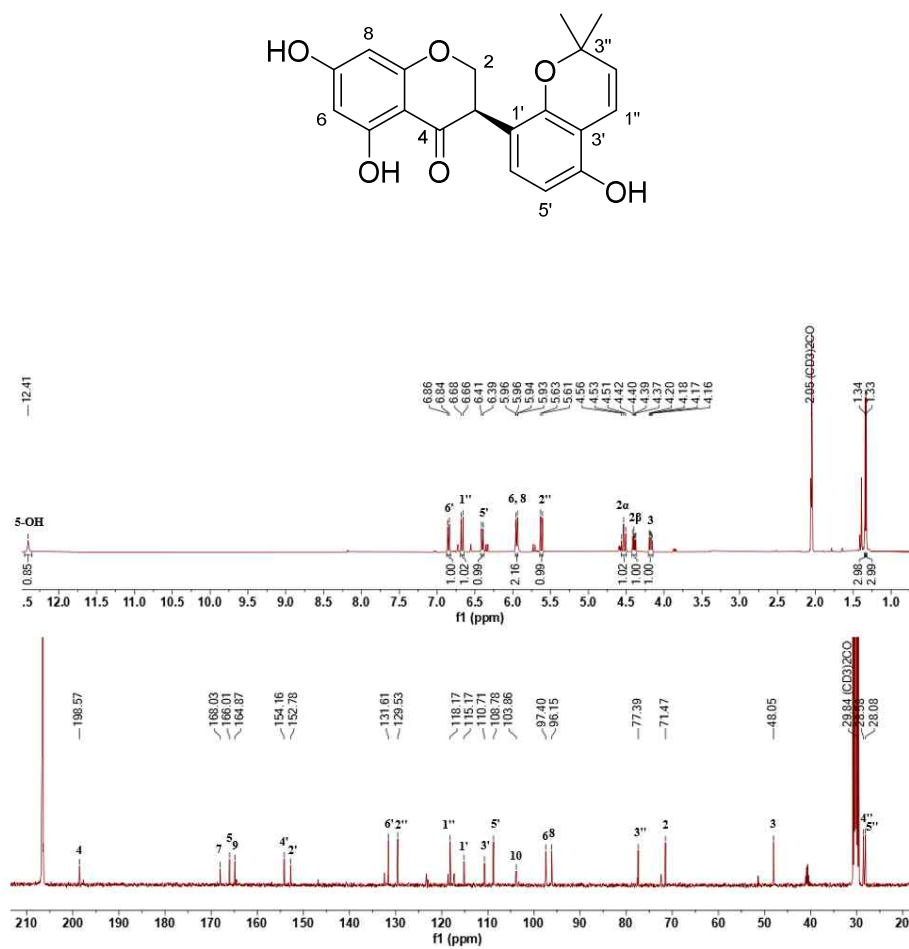


Figure 23. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **17** in Acetone-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.18. Compound **18**

Compound **18** was obtained as pale brownish gum and its empirical formula was established as  $C_{20}H_{20}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  355.1196  $[M - H]^-$  (calcd for  $C_{20}H_{19}O_6$ , 355.1182) and 11 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  197.7 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.48 (1H, t,  $J = 10.7$  Hz, H-2 $\alpha$ ), 4.39 (1H, dd,  $J = 10.9, 5.4$  Hz, H-2 $\beta$ ) /  $\delta_C$  70.1 (C-2)] with two benzene ring [ $\delta_H$  6.66 (1H, d,  $J = 8.4$  Hz, H-6') /  $\delta_C$  127.0 (C-6')  $\delta_H$  6.30 (1H, d,  $J = 8.3$  Hz, H-5') /  $\delta_C$  107.1 (C-5')  $\delta_H$  5.89 (2H, s, H-6, 8) /  $\delta_C$  95.9 (C-6), 94.8 (C-8)] implied isoflavanone type. And two methyls [ $\delta_H$  1.69 (3H, s, H-4'') /  $\delta_C$  17.8 (C-4'')  $\delta_H$  1.61 (3H, s, H-5'') /  $\delta_C$  25.6 (C-5'')] and one deshielded methine [ $\delta_H$  3.25 (2H, d,  $J = 6.7$  Hz, H-1'') /  $\delta_C$  22.4 (C-1'')] and one olefinic [ $\delta_H$  5.12 (1H, t,  $J = 7.0$  Hz, H-2'') /  $\delta_C$  123.4 (C-2'')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **18** was determined as dihydrolicoisoflavone (Fan et al., 2020).

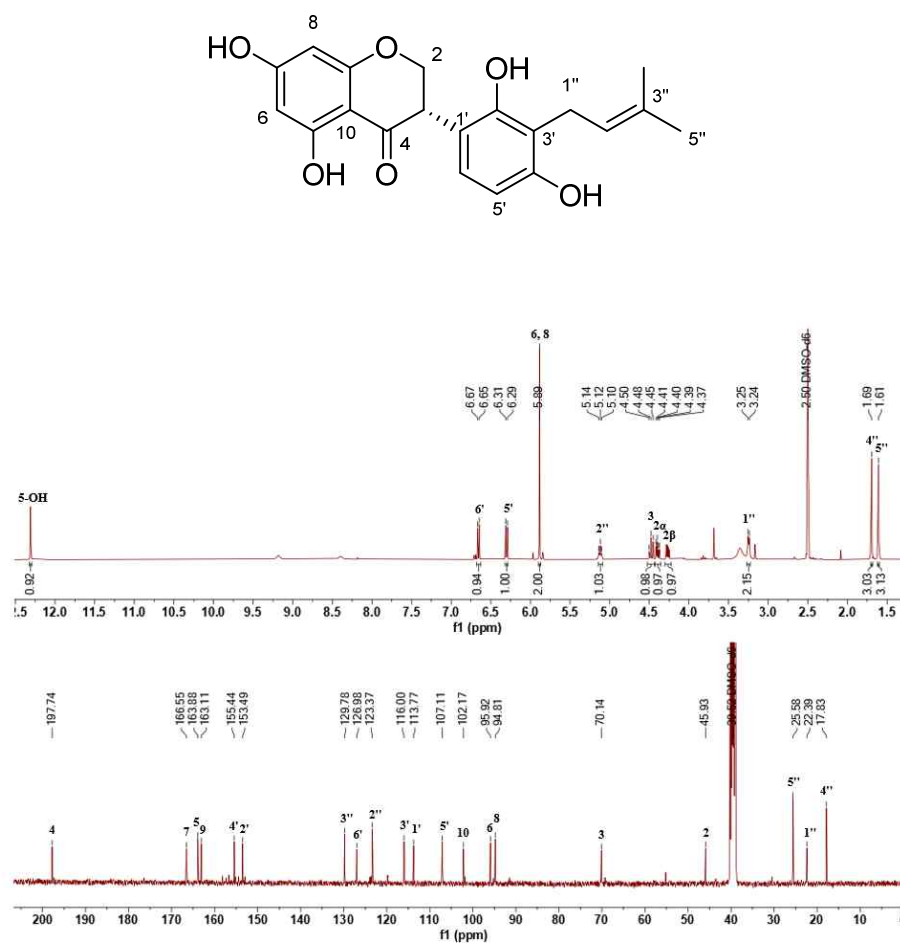


Figure 24. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **18** in DMSO-d<sub>6</sub> (400, 100 MHz)

### 3.2.19. Compound **19**

Compound **19** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{18}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  339.1232  $[M + H]^+$  (calcd for  $C_{20}H_{19}O_5$ , 339.1232) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.2 (C-4)] and two olefinic protons and carbons [ $\delta_H$  8.27 (1H, s, H-2) /  $\delta_C$  153.9 (C-2),  $\delta_H$  5.29 (1H, m, H-2'') /  $\delta_C$  122.9 (C-2'')] with two benzene rings [ $\delta_H$  7.21 (1H, d,  $J = 2.3$  Hz, H-2') /  $\delta_C$  127.3 (C-2')  $\delta_H$  7.18 (1H, dd,  $J = 8.2, 2.3$  Hz, H-6') /  $\delta_C$  122.5 (C-6')  $\delta_H$  6.82 (1H, d,  $J = 8.2$  Hz, H-5') /  $\delta_C$  114.6 (C-5')  $\delta_H$  6.36 (1H, d,  $J = 2.1$  Hz, H-8) /  $\delta_C$  93.7 (C-8)  $\delta_H$  6.20 (1H, d,  $J = 2.0$  Hz, H-6) /  $\delta_C$  99.1 (C-6)] and two methyls [ $\delta_H$  1.68 (6H, s, H-4'', 5'') /  $\delta_C$  25.6 (C-5''), 17.7 (C-4'')] implied isoflavone type with prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **19** was determined as isowighteone (Yo, J et al., 2021).

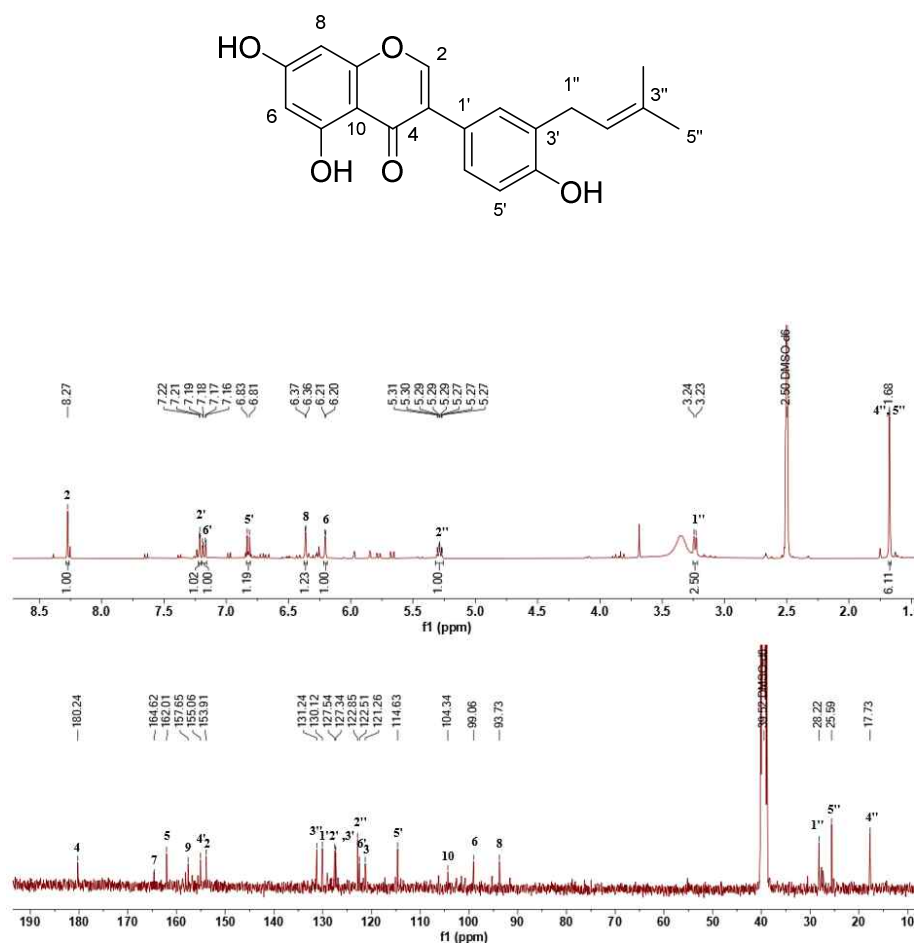


Figure 25. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **19** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.20. Compound **20**

Compound **20** was obtained as pale red gum and its empirical formula was established as  $C_{21}H_{20}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  353.1392  $[M + H]^+$  (calcd for  $C_{21}H_{21}O_5$ , 353.1389) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated oxygenated proton and carbon [ $\delta_H$  5.39 (2H, s, H-2) /  $\delta_C$  64.1 (C-2)] with two benzene ring [ $\delta_H$  7.30 (1H, d,  $J = 8.4$  Hz, H-6') /  $\delta_C$  118.9 (C-6')  $\delta_H$  6.96 (1H, d,  $J = 2.1$  Hz, H-3') /  $\delta_C$  98.0 (C-3')  $\delta_H$  6.75 (1H, dd,  $J = 8.4, 2.1$  Hz, H-5') /  $\delta_C$  112.3 (C-5')  $\delta_H$  6.28 (1H, s, H-8) /  $\delta_C$  99.8 (C-8)] and one methoxy [ $\delta_H$  3.74 (3H, s, 5-OCH<sub>3</sub>) /  $\delta_C$  61.5 (C-5')] and two methyls [ $\delta_H$  1.73 (3H, s, H-4'') /  $\delta_C$  17.7 (C-4'')  $\delta_H$  1.63 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] and one deshielded methine [ $\delta_H$  3.19 (2H, d,  $J = 7.0$  Hz, H-1'') /  $\delta_C$  21.9 (C-1'')] and one olefinic [ $\delta_H$  5.16 (1H, t,  $J = 7.1$  Hz, H-2'') /  $\delta_C$  123.6 (C-2'')] implied the presence of prenyl group. 12 degrees of unsaturation with deshielded olefinic  $^{13}C$  peak [ $\delta_C$  144.9 (C-4)] without  $^1H$  peak indicated one more ring joined to heterocyclohexane ring. By comparing above listed information with NMR data from already reported previous literature, Compound **20** was determined as glurallin A (Ji, S et al., 2016).

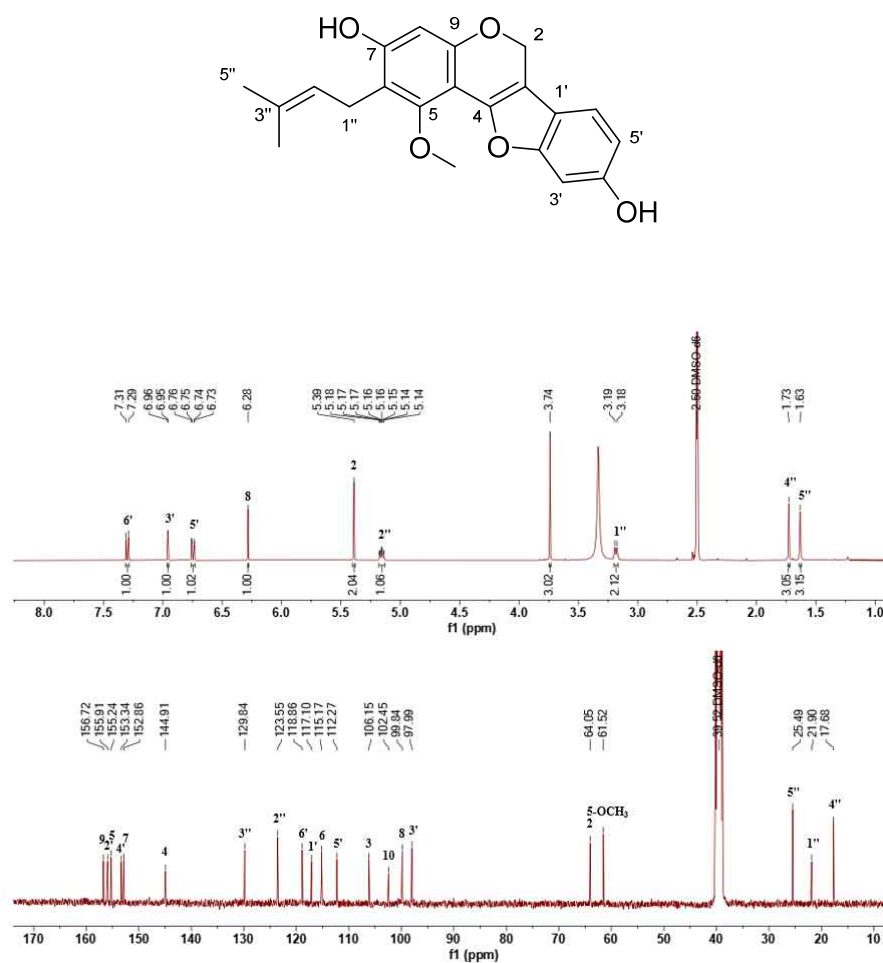


Figure 26. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **20** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.21. Compound **21**

Compound **21** was obtained as white amorphous powder and its empirical formula was established as  $C_{20}H_{20}O_4$  by HRESIMS, which showed a protonated ion peak at  $m/z$  325.1445  $[M + H]^+$  (calcd for  $C_{20}H_{21}O_4$ , 325.1440) and 11 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated oxygenated protons and carbons [ $\delta_H$  3.93 (1H, t,  $J = 10.3$  Hz, H-2 $\alpha$ ), 4.23 (1H, ddd,  $J = 10.3, 3.3, 2.1$  Hz, H-2 $\beta$ ) /  $\delta_C$  69.8 (C-2),  $\delta_C$  75.3 (C-3'')] with two benzene ring [ $\delta_H$  6.86 (1H, d,  $J = 8.4$  Hz, H-6') /  $\delta_C$  127.6 (C-6')  $\delta_H$  6.83 (1H, d,  $J = 8.2$  Hz, H-5') /  $\delta_C$  129.3 (C-5')  $\delta_H$  6.32 (1H, d,  $J = 2.4$  Hz, H-3') /  $\delta_C$  102.5 (C-3')  $\delta_H$  6.28 (1H, d,  $J = 8.5$  Hz, H-6) /  $\delta_C$  108.1 (C-6)  $\delta_H$  6.18 (1H, dd,  $J = 8.3, 2.4$  Hz, H-5') /  $\delta_C$  106.3 (C-5'')] and no carbonyl carbon peak in  $^{13}C$  NMR spectrum implied isoflavan type. Two *cis*-olefinic peaks [ $\delta_H$  6.53 (1H, d,  $J = 10.0$  Hz, H-1'') /  $\delta_C$  116.5 (C-1'')  $\delta_H$  5.64 (1H, d,  $J = 9.9$  Hz, H-2'') /  $\delta_C$  129.4 (C-2'')] with two methyls [ $\delta_H$  1.34 (3H, s, H-4'') /  $\delta_C$  27.3 (C-4'')  $\delta_H$  1.33 (3H, s, H-5'') /  $\delta_C$  27.4 (C-5'')] indicated presence of pyran moiety with two methyls. By comparing above listed information with NMR data from already reported previous literature, Compound **21** was determined as glabridin (Li, K et al., 2017).

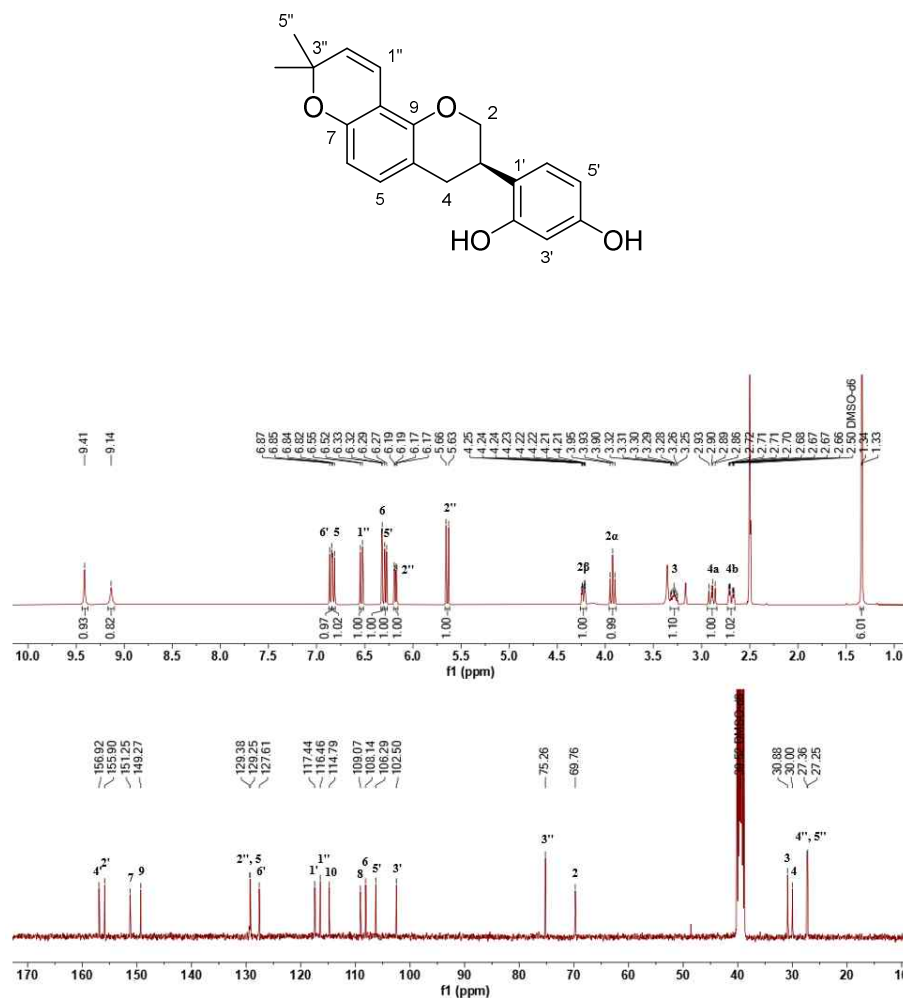


Figure 27. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **21** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.22. Compound **22**

Compound **22** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{18}O_4$  by HRESIMS, which showed a protonated ion peak at  $m/z$  323.1287  $[M + H]^+$  (calcd for  $C_{20}H_{19}O_4$ , 323.1283) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated oxygenated protons and carbons [ $\delta_H$  4.89 (2H, m, H-2) /  $\delta_C$  67.7 (C-2),  $\delta_C$  75.9 (C-3'')] with two benzene ring [ $\delta_H$  7.00 (1H, d,  $J$  = 8.4 Hz, H-6') /  $\delta_C$  127.6 (C-6')  $\delta_H$  6.92 (1H, d,  $J$  = 8.2 Hz, H-5) /  $\delta_C$  127.5 (C-5)  $\delta_H$  6.33 (1H, d,  $J$  = 8.2, 2.3 Hz, H-6) /  $\delta_C$  108.7 (C-6)  $\delta_H$  6.40 (1H, d,  $J$  = 8.5 Hz, H-5') /  $\delta_C$  108.1 (C-5')  $\delta_H$  6.23 (1H, dd,  $J$  = 2.3, 0.7 Hz, H-5') /  $\delta_C$  102.4 (C-5')] no carbonyl carbon peak in  $^{13}C$  NMR spectrum and implied isoflavan type. Two olefinic peaks [ $\delta_H$  6.56 (1H, d,  $J$  = 9.9 Hz, H-1'') /  $\delta_C$  116.9 (C-1'')  $\delta_H$  6.52 (1H, d,  $J$  = 0.9 Hz, H-4) /  $\delta_C$  119.9 (C-4)  $\delta_H$  5.64 (1H, d,  $J$  = 9.9 Hz, H-2'') /  $\delta_C$  128.4 (C-2'')] with two methyls [ $\delta_H$  1.37 (6H, s, H-4'', 5'') /  $\delta_C$  27.3 (C-4'', 5'')] indicated reduced isoflavan type with pyran moiety. By comparing above listed information with NMR data from already reported previous literature, Compound **22** was determined as glabrene (Li, K et al., 2017).

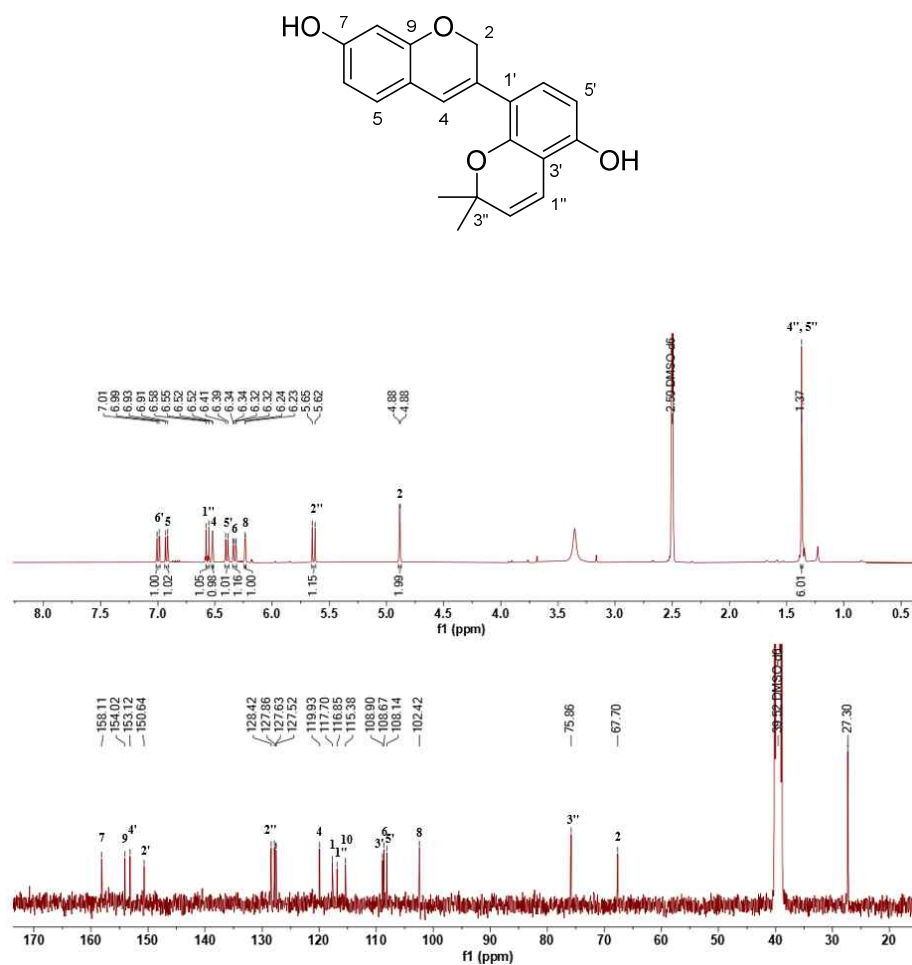


Figure 28. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **22** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.23. Compound **23**

Compound **23** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{20}H_{18}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  353.1037  $[M - H]^-$  (calcd for  $C_{20}H_{17}O_6$ , 353.1025) and 12 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated 1,4-disubstituted benzene ring [ $\delta_H$  7.99 (2H, d,  $J = 8.6$  Hz, H-2', 6') /  $\delta_C$  129.4 (C-2', 6')  $\delta_H$  6.88 (2H, d,  $J = 8.8$  Hz, H-3', 5') /  $\delta_C$  115.4 (C-3', 5')] carbonyl carbon peak [ $\delta_C$  175.9 (C-4)] and two deshielded olefinic  $^{13}C$  NMR peaks [ $\delta_C$  146.5 (C-2), 135.5 (C-3)] signify flavanone type compound contains hydroxyl at C-3. Two methyls [ $\delta_H$  1.70 (3H, s, H-4'') /  $\delta_C$  17.7 (C-4'')  $\delta_H$  1.59 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] and one deshielded methine [ $\delta_H$  3.20 (2H, d,  $J = 7.0$  Hz, H-1'') /  $\delta_C$  21.0 (C-1'')] and one olefinic [ $\delta_H$  5.15 (1H, t,  $J = 7.0$  Hz, H-2'') /  $\delta_C$  122.3 (C-2'')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **23** was determined as licoflavonol (Ji, et al., 2016).

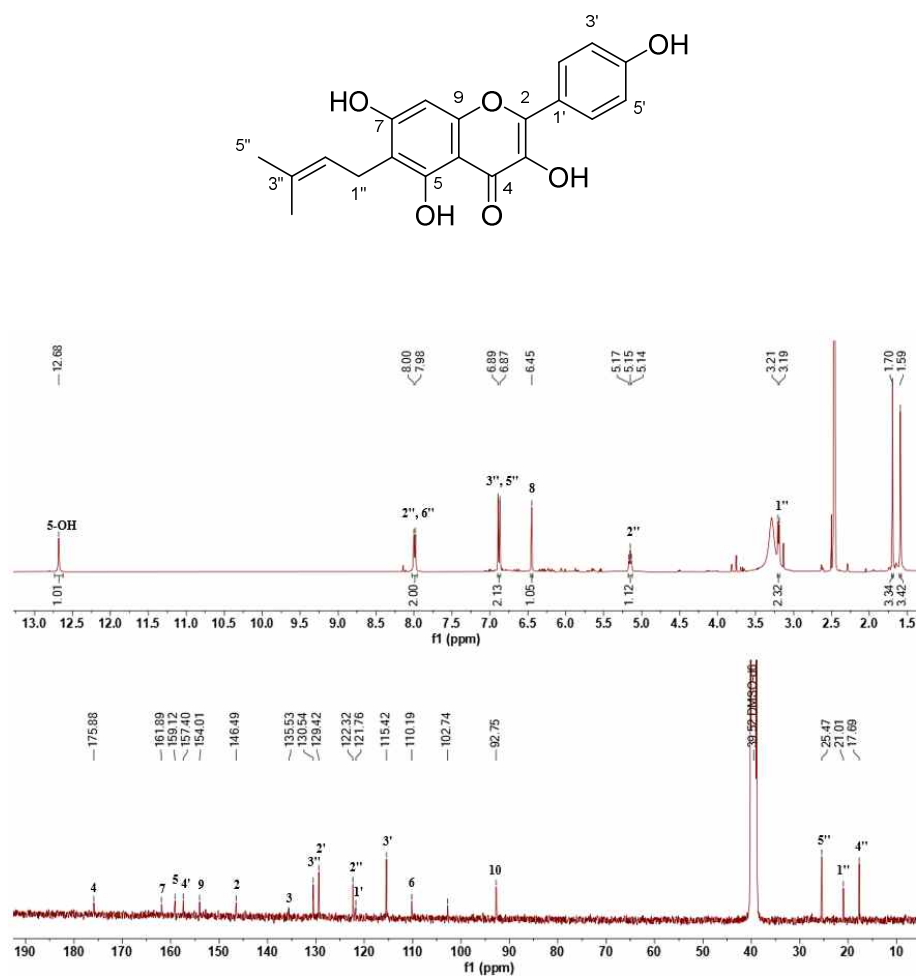


Figure 29. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **23** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.24. Compound **24**

Compound **24** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{16}O_6$  by HRESIMS, which showed a protonated ion peak at  $m/z$  353.1021  $[M + H]^+$  (calcd for  $C_{20}H_{17}O_6$ , 353.1025) and 13 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.6 (C-4)] and two deshielded olefinic protons and carbons [ $\delta_H$  8.19 (1H, s, H-2) /  $\delta_C$  155.7 (C-2), 120.5 (C-3)] with two benzene rings [ $\delta_H$  6.39 (1H, d,  $J = 2.0$  Hz, H-8) /  $\delta_C$  93.8 (C-8)  $\delta_H$  6.32 (1H, d,  $J = 8.4$  Hz, H-5') /  $\delta_C$  107.4 (C-5')  $\delta_H$  6.22 (1H, d,  $J = 2.0$  Hz, H-6) /  $\delta_C$  99.0 (C-6), 151.3 (C-2'')] and two methyls [ $\delta_H$  1.68 (6H, s, H-4'', 5'') /  $\delta_C$  25.6 (C-5''), 17.7 (C-4'')] implied isoflavone type compound. Two *cis*-olefinic peaks [ $\delta_H$  6.66 (1H, d,  $J = 10.1$  Hz, H-1'') /  $\delta_C$  117.0 (C-1'')  $\delta_H$  5.68 (1H, d,  $J = 10.0$  Hz, H-2'') /  $\delta_C$  128.9 (C-2'')] with two methyls [ $\delta_H$  1.37 (6H, s, H-4'', 5'') /  $\delta_C$  27.5 (C-4'', 5'')] indicated presence of pyran moiety with two methyls. By comparing above listed information with NMR data from already reported previous literature, Compound **24** was determined as allolicoisoflavone B (Li, K et al., 2017).

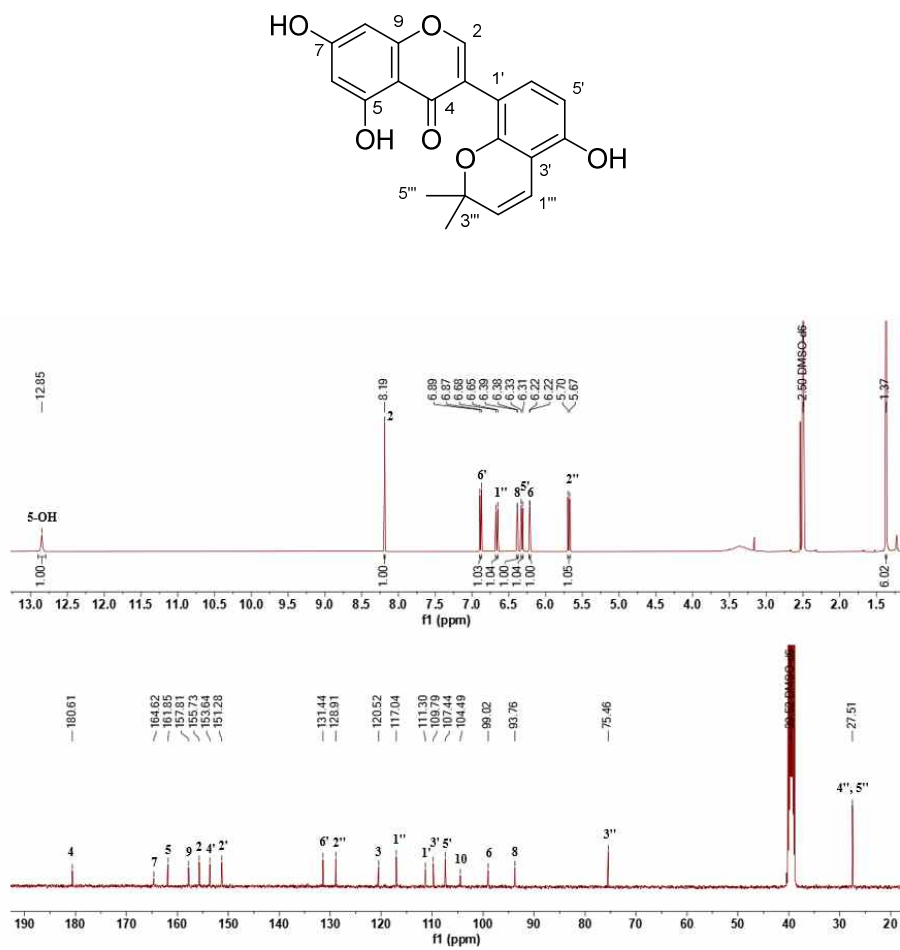


Figure 30. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **24** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.25. Compound **25**

Compound **25** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{28}O_6$  by HRESIMS, which showed a protonated ion peak at  $m/z$  425.1966  $[M + H]^+$  (calcd for  $C_{25}H_{29}O_6$ , 425.1964) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  197.6 (C-4)], oxygenated protons and carbons [ $\delta_H$  4.43 (1H, t,  $J = 10.5$ , H-2 $\alpha$ ), 4.36 (1H, dd,  $J = 10.9$ , 5.4, H-2 $\beta$ ) /  $\delta_C$  70.1 (C-2)] with two benzene ring [ $\delta_H$  6.65 (1H, d,  $J = 8.3$  Hz, H-6') /  $\delta_C$  126.9 (C-6')  $\delta_H$  6.30 (1H, d,  $J = 8.3$  Hz, H-5') /  $\delta_C$  107.1 (C-5')  $\delta_H$  5.96 (1H, s, H-6) /  $\delta_C$  107.7 (C-6)] implied isoflavanone type. Two deshielded methines [ $\delta_H$  3.24 (2H, d,  $J = 6.8$  Hz, H-1''') /  $\delta_C$  22.4 (C-1''')  $\delta_H$  3.11 (2H, d,  $J = 7.0$  Hz, H-1'') /  $\delta_C$  20.7 (C-1'')] and two olefinic [ $\delta_H$  5.13 (2H, m, H-2'', 2''') /  $\delta_C$  123.4 (C-2'''), 122.8 (C-2'')] with four methyls [ $\delta_H$  1.69 (6H, s, H-5'', 5''') /  $\delta_C$  25.6 (C-5''), 25.5 (C-5''')  $\delta_H$  1.61 (6H, s, H-4'', 4''') /  $\delta_C$  17.8 (C-4''), 17.7 (C-4''')] implied the presence of two prenyls. By comparing above listed information with NMR data from already reported previous literature, Compound **25** was determined as 3'-( $\gamma$ ,  $\gamma$ -dimethyl)-kievitone (Yan Lin et al., 2017).

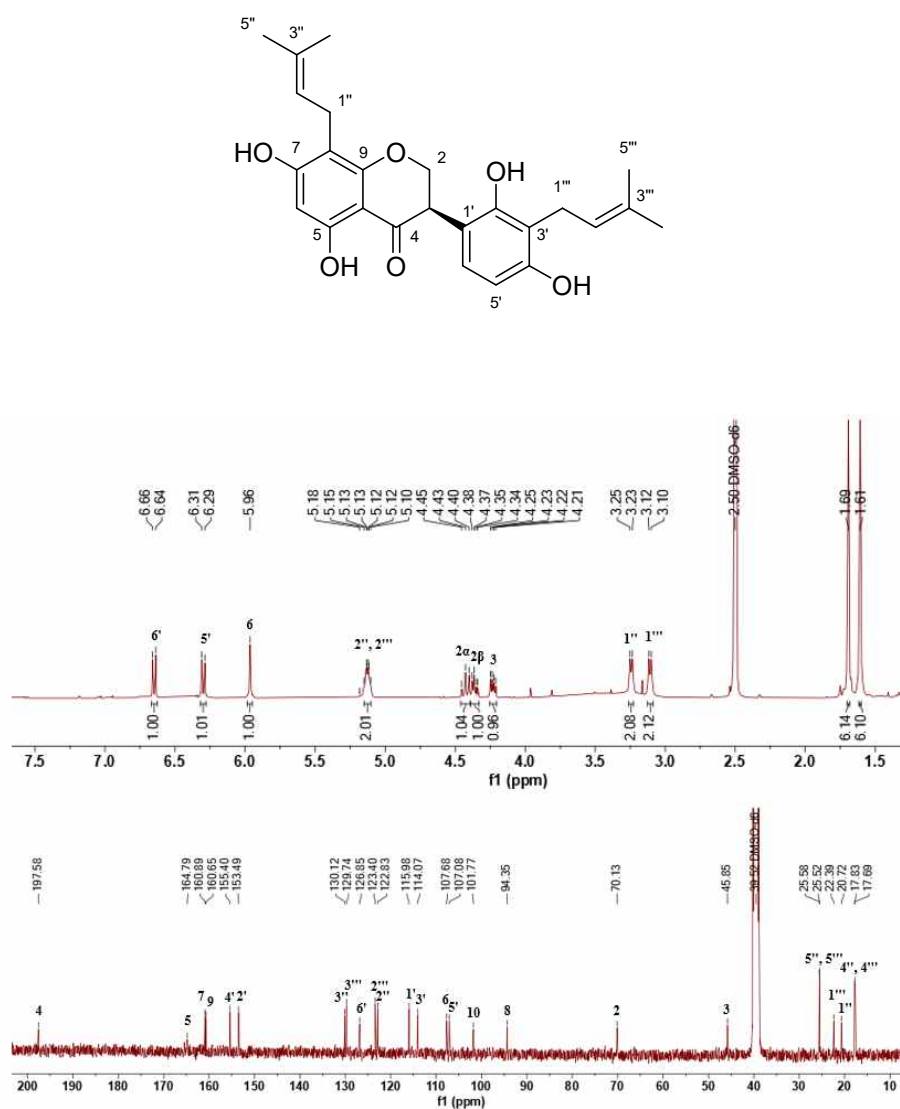


Figure 31. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **25** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.26. Compound **26**

Compound **26** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{24}O_6$  by HRESIMS, which showed a protonated ion peak at  $m/z$  421.1663  $[M + H]^+$  (calcd for  $C_{25}H_{25}O_6$ , 421.1651) and 14 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.1 (C-4)] and one deshielded olefinic proton and carbon [ $\delta_H$  8.31 (1H, s, H-2) /  $\delta_C$  155.4 (C-2)] with two benzene rings [ $\delta_H$  6.91 (1H, d,  $J = 2.0$  Hz, H-2') /  $\delta_C$  122.0 (C-2'), 127.0 (C-2')  $\delta_H$  6.71 (1H, d,  $J = 2.0$  Hz, H-6') /  $\delta_C$  117.6 (C-6')  $\delta_H$  6.45 (1H, s, H-8) /  $\delta_C$  93.0 (C-8)] implied isoflavone type compound. And two methyls [ $\delta_H$  1.39 (6H, s, H-4'', 5'') /  $\delta_C$  21.1 (C-4'', 5'')] and two *cis*-olefinic protons and carbons [ $\delta_H$  6.71 (1H, d,  $J = 9.9$  Hz, H-1'') /  $\delta_C$  130.7 (C-1'')  $\delta_H$  5.75 (1H, d,  $J = 9.8$  Hz, H-2'') /  $\delta_C$  123.0 (C-2'')] implied pyran ring moiety and two methyls [ $\delta_H$  1.72 (3H, s, H-5''') /  $\delta_C$  27.5 (C-5''')  $\delta_H$  1.62 (3H, s, H-4''') /  $\delta_C$  17.8 (C-4''')] and one deshielded methine [ $\delta_H$  3.23 (2H, d,  $J = 7.1$  Hz, H-1''') /  $\delta_C$  25.5 (C-1''')] and one olefinic [ $\delta_H$  5.18 (1H, t,  $J = 7.2$  Hz, H-2''') /  $\delta_C$  131.3 (C-2''')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **26** was determined as gancaonin H (Yan Lin et al., 2017).

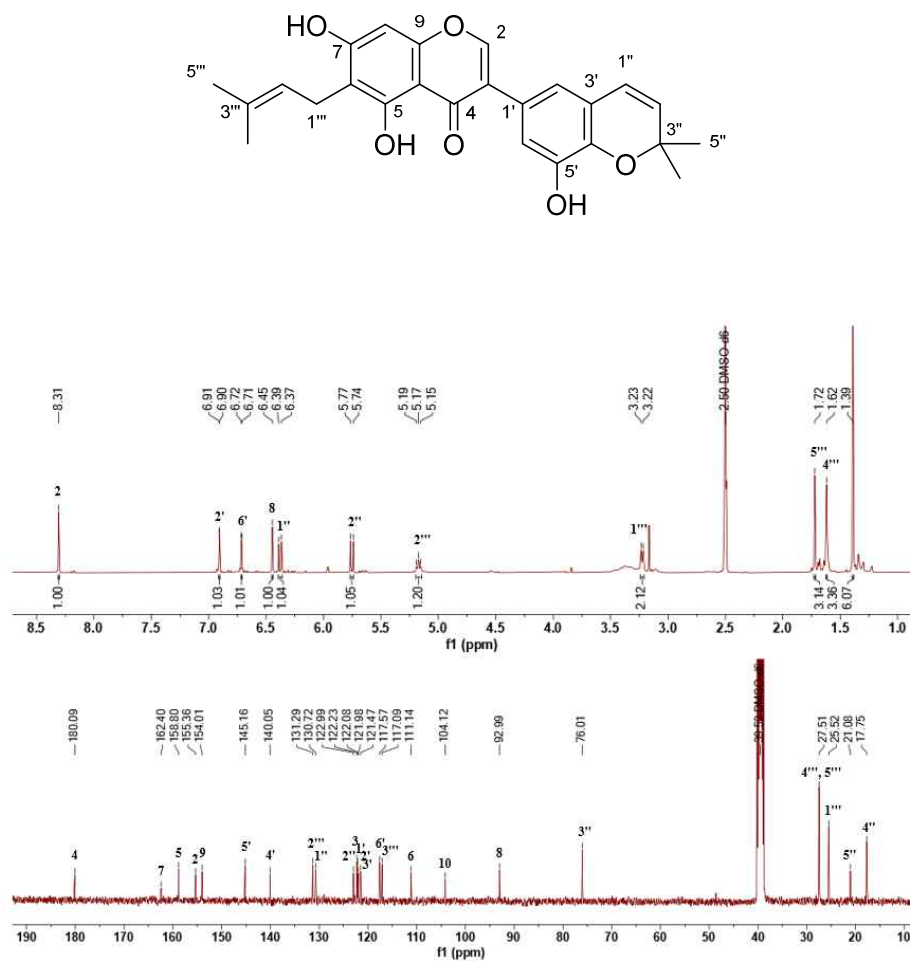


Figure 32.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **26** in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.27. Compound **27**

Compound **27** was obtained as brownish gum and its empirical formula was established as  $C_{25}H_{26}O_6$  by HRESIMS, which showed a protonated ion peak at  $m/z$  423.1814  $[M + H]^+$  (calcd for  $C_{25}H_{27}O_6$ , 423.1808) and 13 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.3 (C-4)] and deshielded olefinic protons and carbons [ $\delta_H$  8.23 (1H, s, H-2) /  $\delta_C$  153.7 (C-2)] with two benzene rings [ $\delta_H$  6.87 (1H, d,  $J = 1.7$  Hz, H-6') /  $\delta_C$  114.0 (C-6')  $\delta_H$  6.65 (1H, d,  $J = 1.9$  Hz, H-2') /  $\delta_C$  120.5 (C-2')  $\delta_H$  6.44 (1H, s, H-8) /  $\delta_C$  92.9 (C-8)] implied isoflavone type compound. Four methyls [ $\delta_H$  1.72 (3H, s, H-5''') /  $\delta_C$  25.5 (C-5''')  $\delta_H$  1.67 (6H, s, H-4'', 4''') /  $\delta_C$  17.7 (C-4'', 4''')  $\delta_H$  1.62 (3H, s, H-5'') /  $\delta_C$  25.6 (C-5'')] with olefinic protons and carbons [ $\delta_H$  5.28 (1H, m, H-2''') /  $\delta_C$  123.1 (C-2''')  $\delta_H$  5.17 (1H, t,  $J = 7.1$  Hz), H-2'') /  $\delta_C$  122.2 (C-2''), 131.0 (C-3'''), 130.7 (C-3'')] indicated presence of two prenyl groups. By comparing above listed information with NMR data from already reported previous literature, Compound **27** was determined as isoangustone A (Shuai Ji et al., 2016).

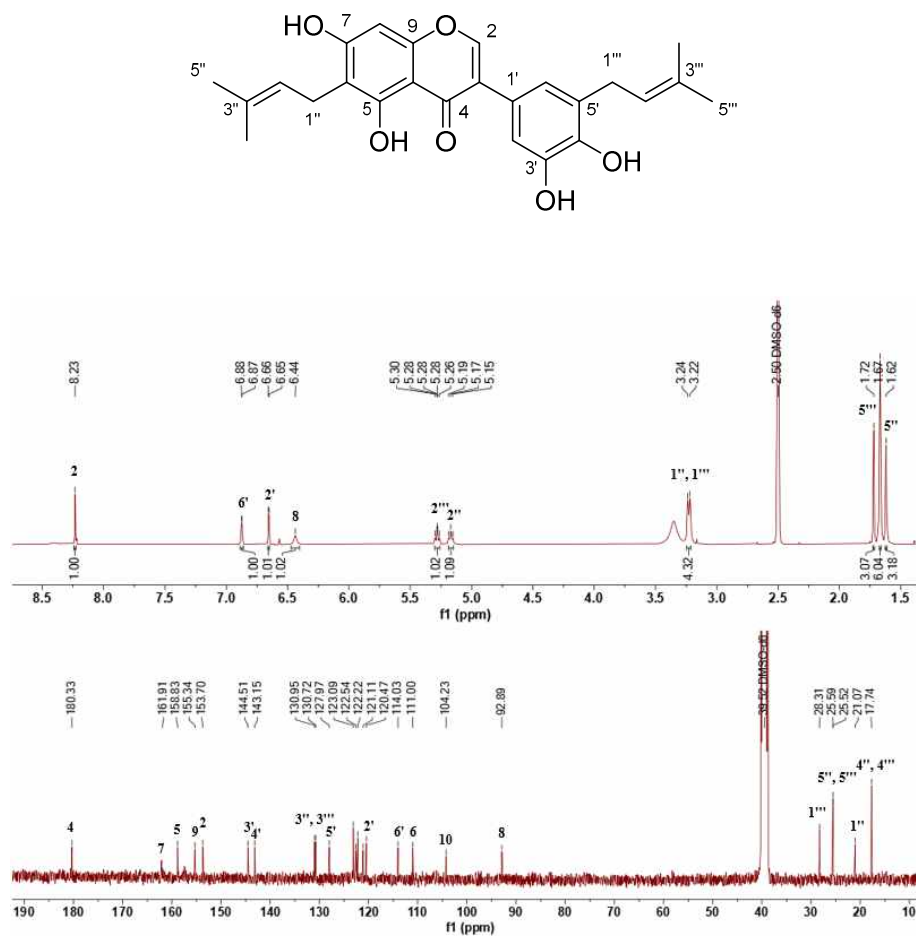


Figure 33. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **27** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.28. Compound **28**

Compound **28** was obtained as brown amorphous powder and its empirical formula was established as  $C_{21}H_{20}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  353.1391  $[M + H]^+$  (calcd for  $C_{21}H_{20}O_5$ , 353.1389) and 12 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.5 (C-4)] and deshielded olefinic protons and carbons [ $\delta_H$  8.43 (1H, s, H-2) /  $\delta_C$  154.3 (C-2)] with two benzene rings [ $\delta_H$  7.39 (2H, d,  $J = 8.7$  Hz, H-2', 6') /  $\delta_C$  130.2 (C-2', 6')  $\delta_H$  6.82 (2H, d,  $J = 8.7$  Hz, H-3', 5') /  $\delta_C$  115.1 (C-3', 5')  $\delta_H$  6.74 (1H, s, H-8) /  $\delta_C$  90.3 (C-8)] one oxygenated methyl [ $\delta_H$  3.91 (3H, s, 7-OCH<sub>3</sub>) /  $\delta_C$  56.5 (7-OCH<sub>3</sub>)] implied isoflavone type compound with one methoxy. Two methyls [ $\delta_H$  1.72 (3H, s, H-4'') /  $\delta_C$  17.7 (C-4'')] [ $\delta_H$  1.62 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] with olefinic protons and carbons [ $\delta_H$  5.13 (1H, d,  $J = 7.2$  Hz, H-2'') /  $\delta_C$  122.5 (C-2''), 131.1 (C-3'')] indicated presence of two prenyl groups. By comparing above listed information with NMR data from already reported previous literature, Compound **28** was determined as gancaonin G (Fukai et al., 1989).

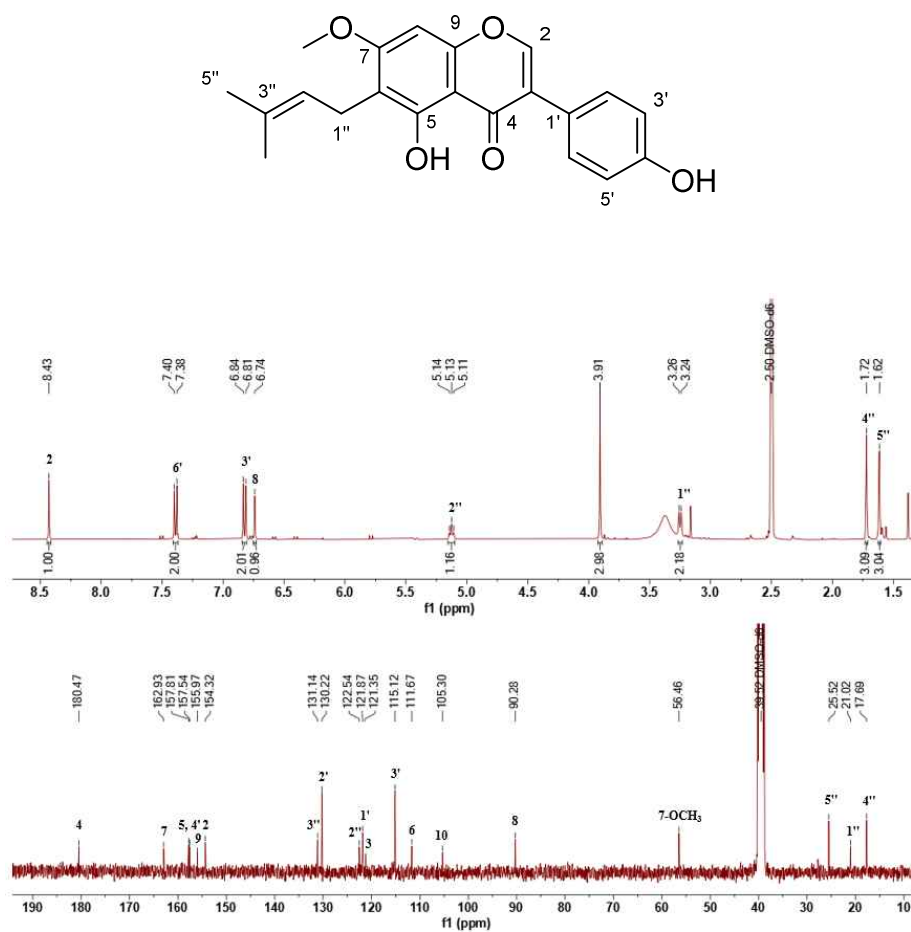


Figure 34. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **28** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.29. Compound **29**

Compound **29** was obtained as white amorphous powder and its empirical formula was established as  $C_{30}H_{46}O_4$  by HRESIMS, which showed a protonated ion peak at  $m/z$  471.3486  $[M + H]^+$  (calcd for  $C_{30}H_{47}O_4$ , 471.3474) and 8 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated presence of 7 methyls [ $\delta_H$  1.36 (3H, s, H-27) /  $\delta_C$  23.5 (C-27)  $\delta_H$  1.22 (3H, s, H-25) /  $\delta_C$  16.5 (C-25)  $\delta_H$  1.12 (3H, s, H-26) /  $\delta_C$  18.8 (C-26)  $\delta_H$  1.12 (3H, s, H-29) /  $\delta_C$  28.7 (C-29)  $\delta_H$  1.00 (3H, s, H-23) /  $\delta_C$  28.2 (C-23)  $\delta_H$  0.83 (3H, s, H-24) /  $\delta_C$  15.7 (C-24)  $\delta_H$  0.80 (3H, s, H-28) /  $\delta_C$  28.6 (C-28)] and two carbonyl carbons [ $\delta_C$  200.8 (C-11), 181.8 (C-30)] olefinic protons and carbons [ $\delta_H$  5.71 (1H, s, H-12) /  $\delta_C$  128.6 (C-12), 169.7 (C-13)]  $^{13}C$  chemical shift gap between C-12 and C-13 implied presence of conjugated system one additional oxygenated proton and carbon [ $\delta_H$  3.24 (1H, dd,  $J = 10.6, 5.5$  Hz, H-3) /  $\delta_C$  79.0 (C-3)] signified triterpenoid contains one olefinic bond with two carbonyl carbons. By comparing above listed information with NMR data from already reported previous literature, Compound **29** was determined as glycyrrhetic acid (Kalani et al., 2013).

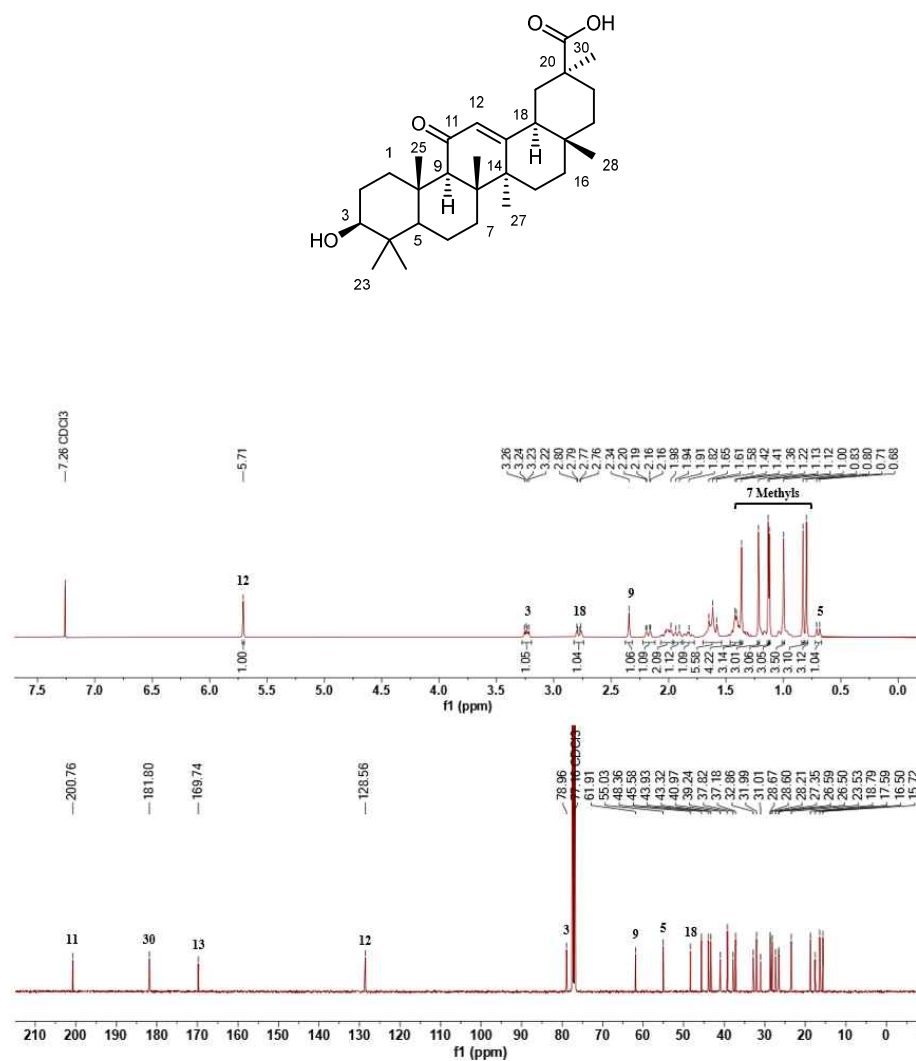


Figure 35. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **29** in CDCl<sub>3</sub> (400, 100 MHz)

### 3.2.30. Compound **30**

Compound **30** was obtained as white amorphous powder and its empirical formula was established as  $C_{42}H_{62}O_{16}$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  821.3979  $[M - H]^-$  (calcd for  $C_{42}H_{61}O_{16}$ , 821.3960) and 12 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated presence of 7 methyls [ $\delta_H$  0.78 – 1.43 (3H, s, 7 x  $CH_3$ ),  $\delta_C$  29.1 (C-29), 29.0 (C-28), 28.4 (C-23), 23.9 (C-27), 19.1 (C-26), 17.2 (C-25), 17.0 (C-24)] and four carbonyl carbons [ $\delta_C$  199.9 (C-11), 179.5 (C-30), 169.5 (C-6'), 172.8 (C-6'')] olefinic protons and carbons [ $\delta_H$  5.97 (1H, s, H-12) /  $\delta_C$  129.0 (C-12), 170.0 (C-13)]  $^{13}C$  chemical shift gap between C-12 and C-13 implied presence of conjugated system one additional oxygenated proton and carbon [ $\delta_H$  4.29 (1H, m, H-3) /  $\delta_C$  89.6 (C-3)] signified triterpenoid similar with Compound **29**. Two anomeric protons and carbons [ $\delta_H$  5.53 (1H, d,  $J = 7.4$  Hz, H-1'') /  $\delta_C$  106.3 (C-1'')  $\delta_H$  5.06 (1H, d,  $J = 7.2$  Hz, H-1') /  $\delta_C$  105.0 (C-1')] with 8 number of oxygenated carbons that distinct difference from compound **29** [ $\delta_C$  83.5 (C-2'), 77.7 (C-3''), 76.5 (C-3', 5'), 75.7 (C-2'', 5''), 72.0 (C-4''), 71.5 (C-4')]. Implied presence of two glucoses with carboxylic acid. By comparing above listed information with NMR data from already reported previous literature, Compound **30** was determined as glycyrrhizin (Shukla et al., 2020).

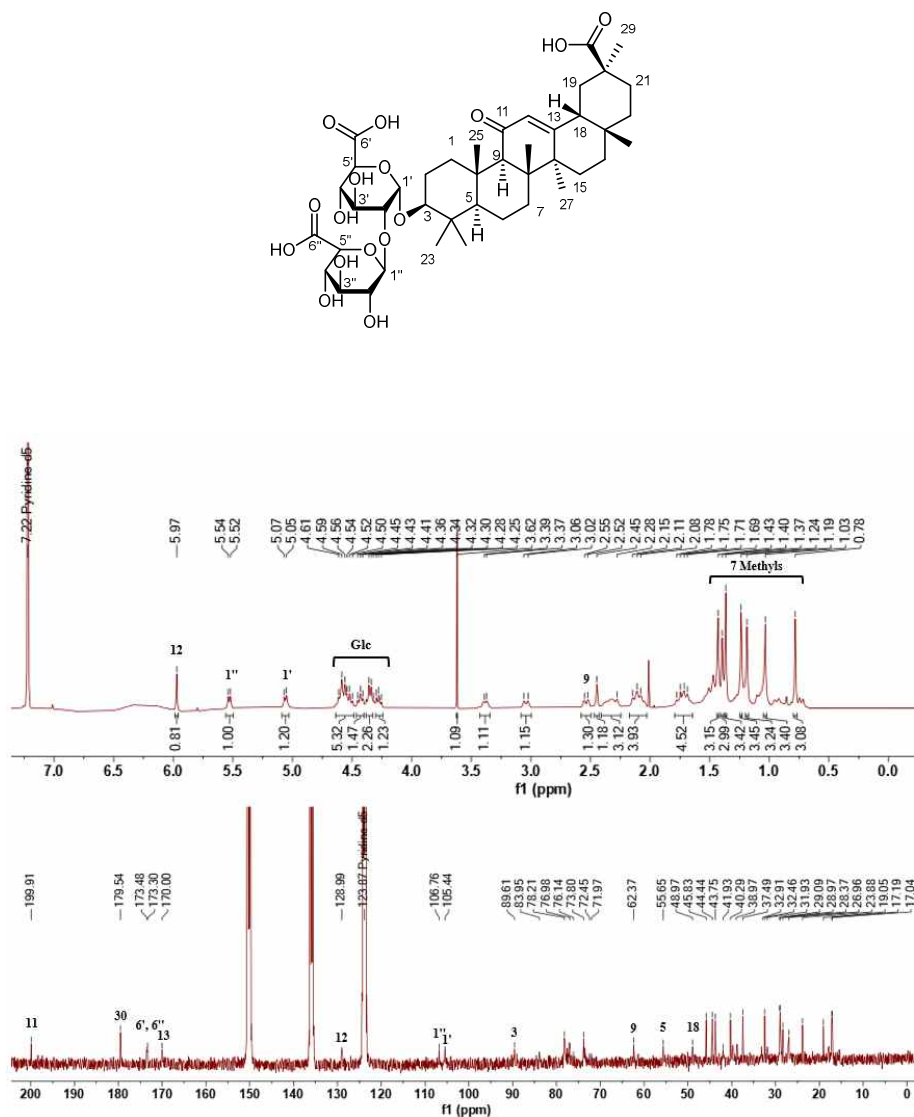


Figure 36. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **30** in Pyridine-*d*<sub>5</sub> (400, 100 MHz)

### 3.2.31. Compound **31**

Compound **31** was obtained as brownish gum and its empirical formula was established as  $C_{20}H_{18}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  339.1239  $[M + H]^+$  (calcd for  $C_{20}H_{19}O_5$ , 339.1233) and 12 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.5 (C-4)] and four olefinic protons and carbons [ $\delta_H$  8.39 (1H, s, H-2) /  $\delta_C$  154.0 (C-2),  $\delta_H$  5.15 (1H, t,  $J = 7.1$  Hz, H-2'') /  $\delta_C$  121.4 (C-2''), 131.1 (C-3'') 121.9 (C-3)] with two benzene rings [ $\delta_H$  7.37 (2H, d,  $J = 8.7$  Hz, H-2', 6') /  $\delta_C$  130.2 (C-2', 6')  $\delta_H$  6.81 (2H, d,  $J = 8.7$  Hz, H-3', 5') /  $\delta_C$  115.1 (C-3', 5')  $\delta_H$  6.30 (1H, s, H-6) /  $\delta_C$  98.6 (C-6)] and two methyls [ $\delta_H$  1.75 (3H, s, H-4'') /  $\delta_C$  25.5 (C-4'')  $\delta_H$  1.63 (3H, s, H-5'') /  $\delta_C$  17.8 (C-5'')] implied isoflavone type with prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **31** was determined as lupiwighteone (Cicek et al., 2022).

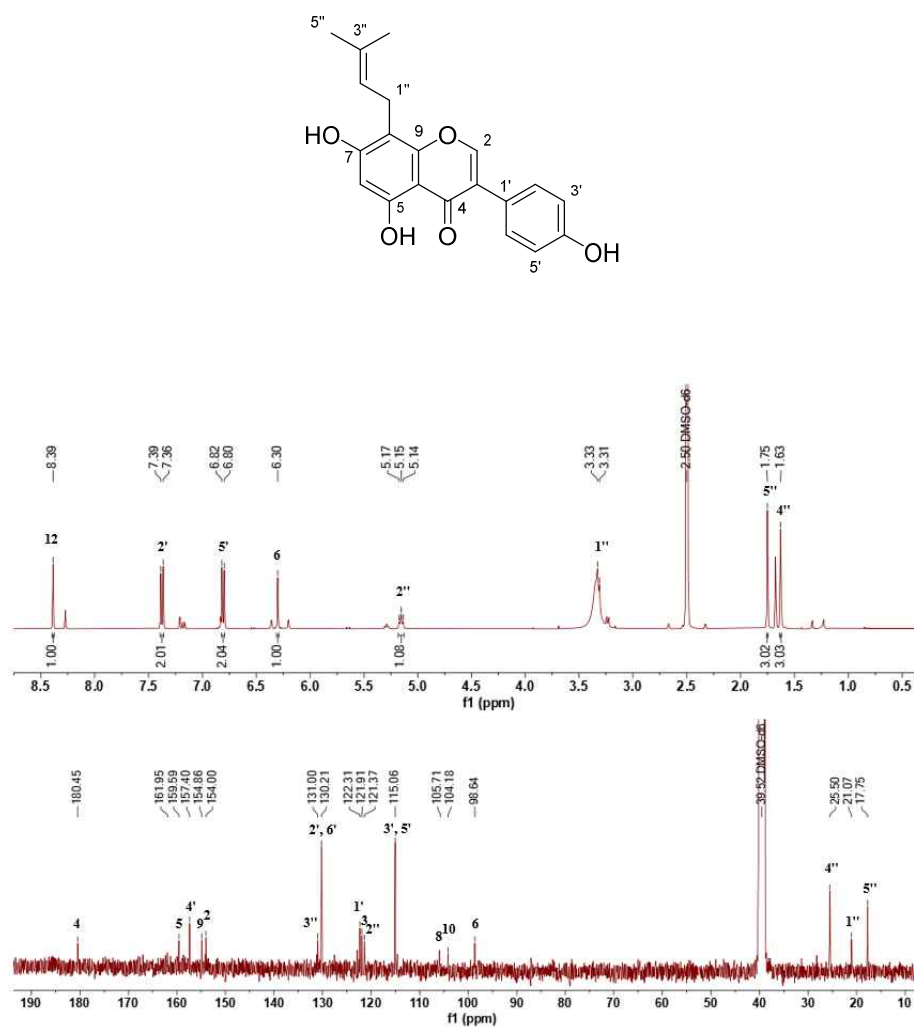


Figure 37. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **31** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.32. Compound **32**

Compound **32** was obtained as white crystal and its empirical formula was established as  $C_{30}H_{48}O_3$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  455.3588  $[M - H]^-$  (calcd for  $C_{30}H_{47}O_3$ , 455.3525) and 7 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated presence of 6 methyls [ $\delta_H$  1.69 (3H, s, H-30) /  $\delta_C$  19.5 (C-30)  $\delta_H$  0.97 (3H, s, H-26) /  $\delta_C$  16.3 (C-26)  $\delta_H$  0.96 (3H, s, H-23) /  $\delta_C$  28.1 (C-23)  $\delta_H$  0.93 (3H, s, H-27) /  $\delta_C$  14.8 (C-27)  $\delta_H$  0.82 (3H, s, H-25) /  $\delta_C$  16.2 (C-25)  $\delta_H$  0.75 (3H, s, H-24) /  $\delta_C$  15.5 (C-24)] and carbonyl carbon [ $\delta_C$  180.3 (C-28)] olefinic protons and carbons [ $\delta_H$  4.74 (1H, br s, H-29a), 4.60 (1H, br s, H-29b) /  $\delta_C$  109.9 (C-29), 150.6 (C-20)] one additional oxygenated proton and carbon [ $\delta_H$  3.19 (1H, dd,  $J$  = 11.3, 4.9 Hz, H-3) /  $\delta_C$  79.2 (C-3)] signified triterpenoid contains one olefinic bond with one carbonyl carbons. By comparing above listed information with NMR data from already reported previous literature, Compound **32** was determined as betulinic acid (Fomogne-Fodjo et al., 2017).

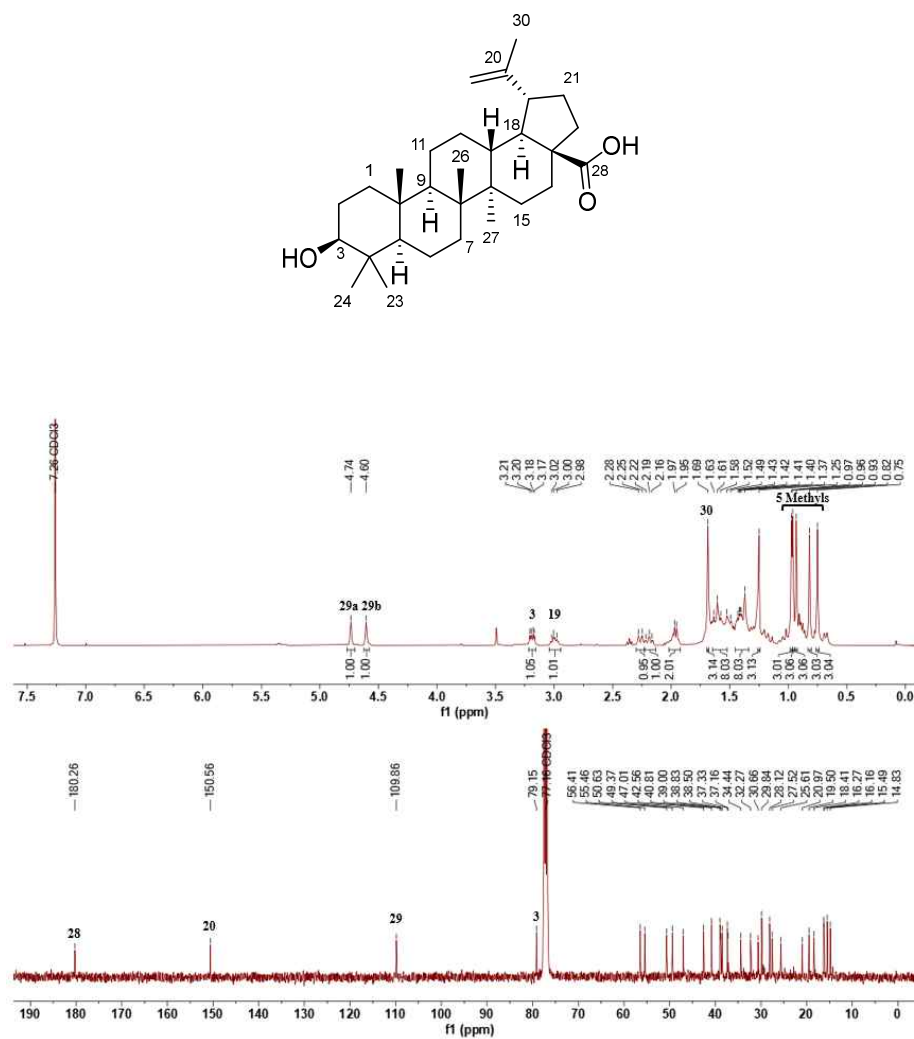


Figure 38.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **32** in  $\text{CDCl}_3$  (400, 100 MHz)

### 3.2.33. Compound **33**

Compound **33** was obtained as brownish gum and its empirical formula was established as  $C_{15}H_{12}O_4$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  255.0655  $[M - H]^-$  (calcd for  $C_{15}H_{11}O_4$ , 255.0657) and 10 degrees of unsaturation. The  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.32 (2H, d,  $J = 8.6$  Hz, H-2', 6') /  $\delta_C$  128.5 (C-2', 6')  $\delta_H$  6.79 (2H, d,  $J = 8.6$  Hz, H-3', 5') /  $\delta_C$  115.2 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  7.64 (1H, d,  $J = 8.7$  Hz, H-5) /  $\delta_C$  128.5 (C-5)  $\delta_H$  6.50 (1H, dd,  $J = 8.7, 2.2$  Hz, H-6) /  $\delta_C$  110.6 (C-6)  $\delta_H$  6.33 (1H, d,  $J = 2.2$  Hz, H-8) /  $\delta_C$  102.6 (C-8)] and one deshielded methylene [ $\delta_H$  3.14 (1H, dd,  $J = 17.1, 2.8$  Hz, H-3 $\alpha$ ), 2.62 (1H, dd,  $J = 16.8, 2.9$  Hz, H-3 $\beta$ ) /  $\delta_C$  43.2 (C-3)] and one carbonyl carbon [ $\delta_C$  193.2 (C-4)] one deshielded methine [ $\delta_H$  5.43 (1H, /  $\delta_C$  79.0 (C-2)]. Based on these properties flavanone type compound could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **33** was finally determined as liquiritigenin (Li, K et al., 2017).

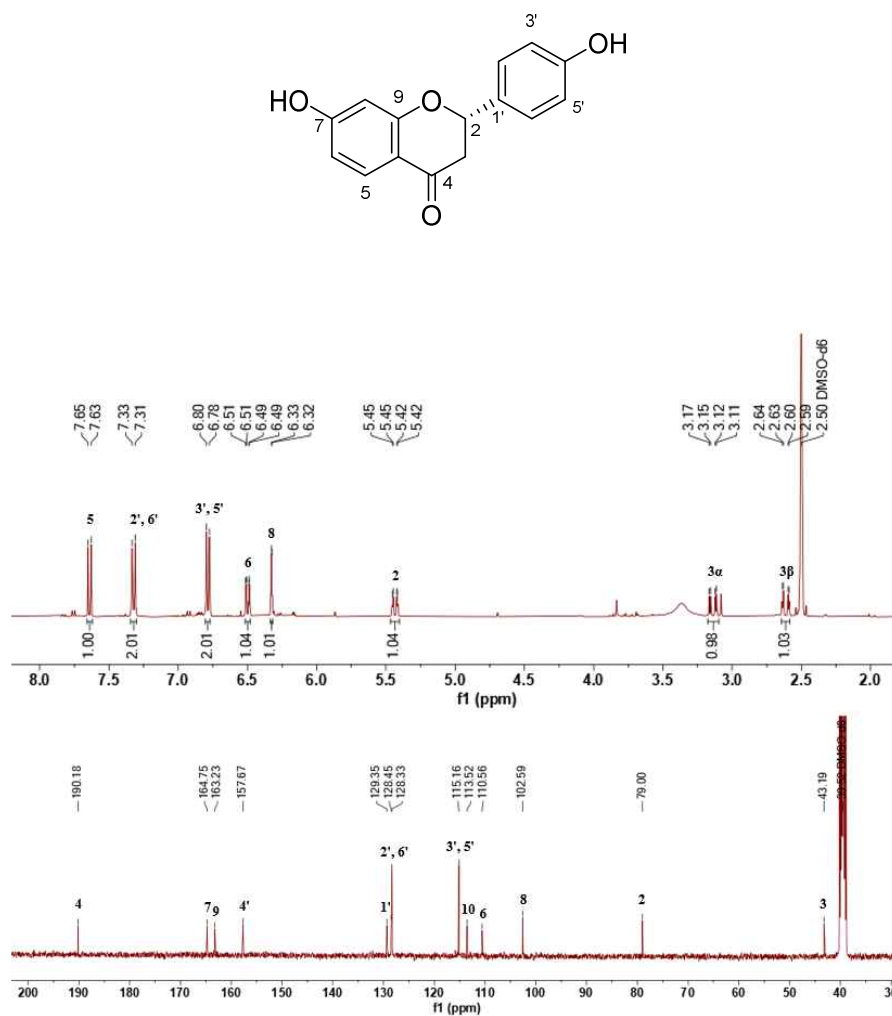


Figure 39. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **33** in DMSO-d<sub>6</sub> (400, 100 MHz)

### 3.2.34. Compound **34**

Compound **34** was obtained as yellowish gum and its empirical formula was established as  $C_{15}H_{12}O_4$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  255.0661  $[M - H]^-$  (calcd for  $C_{15}H_{11}O_4$ , 255.0657) and 10 degrees of unsaturation. Same as isoliquiritin the  $^1H$  and  $^{13}C$  NMR data indicated *para*-disubstituted benzene ring [ $\delta_H$  7.75 (4H, overlap, H-2', 6') /  $\delta_C$  131.3 (C-2', 6')  $\delta_H$  6.84 (2H, d,  $J$  = 8.6 Hz, H-3', 5') /  $\delta_C$  115.9 (C-3', 5')] and 1,2,4-trisubstituted benzene ring [ $\delta_H$  8.15 (1H, d,  $J$  = 8.9 Hz, H-6) /  $\delta_C$  132.9 (C-6)  $\delta_H$  6.39 (1H, dd,  $J$  = 8.8, 2.0 Hz, H-5) /  $\delta_C$  108.4 (C-5)  $\delta_H$  6.26 (1H, d,  $J$  = 2.0 Hz, H-3) /  $\delta_C$  102.7 (C-3)] and one carbonyl carbon [ $\delta_C$  191.4 (C=O)]. Like isoliquiritin except glucose  $^1H$  NMR,  $^{13}C$  NMR signals [ $\delta_H$  7.75 (4H, overlap, H- $\alpha$ ) /  $\delta_C$  117.4 (C- $\alpha$ )  $\delta_H$  7.75 (4H, overlap, H- $\beta$ ) /  $\delta_C$  144.2 (C-  $\beta$ )] could be established as a olefinic moiety. Based on these properties chalcone type compound could be confirmed. By comparing above listed information with NMR data from already reported previous literature, Compound **34** was determined as isoliquiritigenin (Ma CJ et al., 2005).

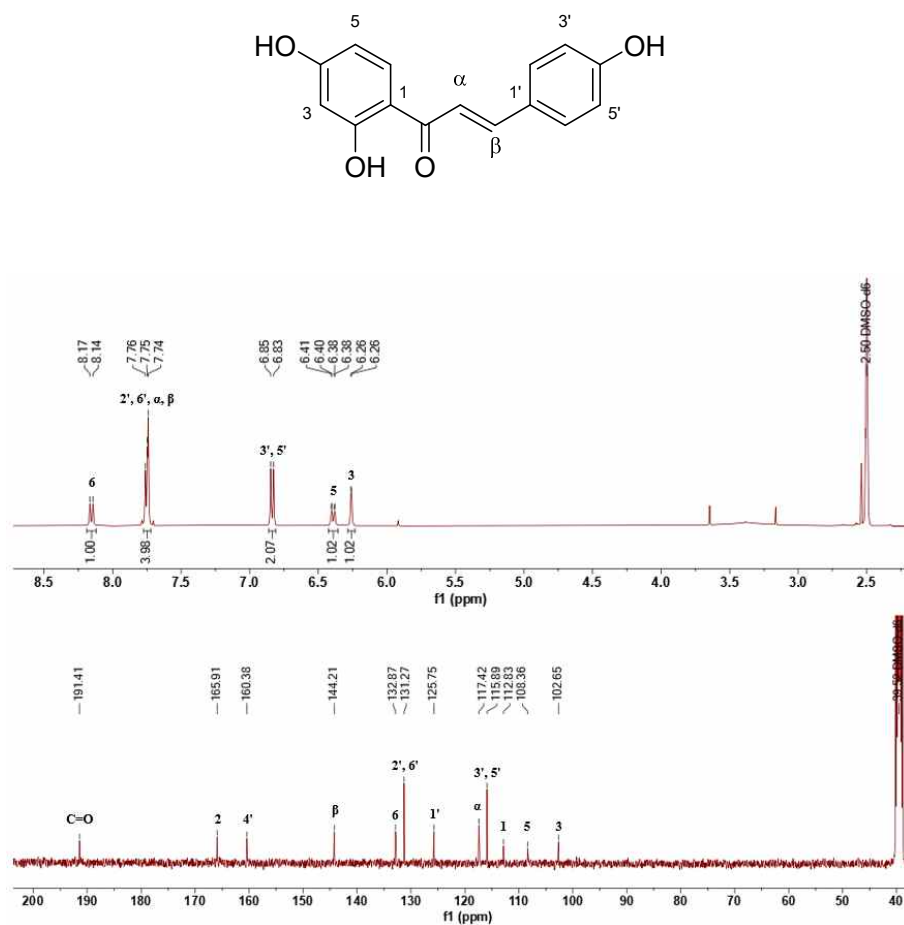


Figure 40. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **34** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.35. Compound **35**

Compound **35** was obtained as brownish gum and its empirical formula was established as  $C_{16}H_{16}O_4$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  271.0967  $[M - H]^-$  (calcd for  $C_{16}H_{15}O_4$ , 271.0970) and 9 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated oxygenated protons and carbons [ $\delta_H$  3.90 (1H, t,  $J = 10.1$  Hz, H-2 $\alpha$ ), 4.13 (1H, ddd,  $J = 10.7, 3.3, 1.9$  Hz, H-2 $\beta$ ) /  $\delta_C$  69.2 (C-2)  $\delta_H$  3.66 (3H, s, 2'-OCH<sub>3</sub>) /  $\delta_C$  54.9 (2'-OCH<sub>3</sub>)] with two benzene ring [ $\delta_H$  6.98 (1H, d,  $J = 8.5$  Hz, H-6') /  $\delta_C$  127.8 (C-6')  $\delta_H$  6.86 (1H, d,  $J = 8.2$  Hz, H-5) /  $\delta_C$  130.1 (C-5)  $\delta_H$  6.42 (1H, d,  $J = 2.6$  Hz, H-3') /  $\delta_C$  101.3 (C-3')  $\delta_H$  6.34 (1H, dd,  $J = 8.5, 2.6$  Hz, H-5') /  $\delta_C$  104.3 (C-5')  $\delta_H$  6.28 (1H, dd,  $J = 8.2, 2.4$  Hz, H-6) /  $\delta_C$  108.0 (C-6)] implied isoflavan type with one methoxy. By comparing above listed information with NMR data from already reported previous literature, Compound **35** was determined as 3,4-dihydro-3-(4-hydroxy-2methoxyphenyl)-2H-1-benzopyran-7-ol (Herath et al., 1998).

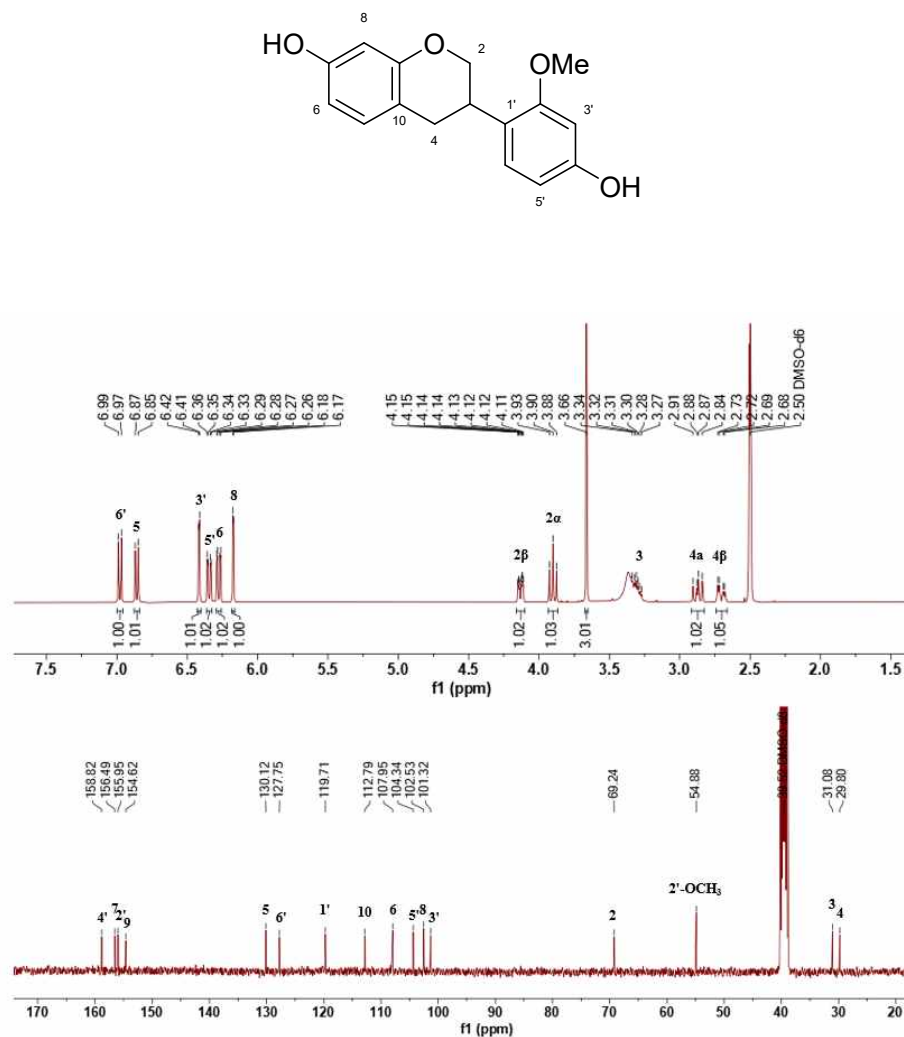
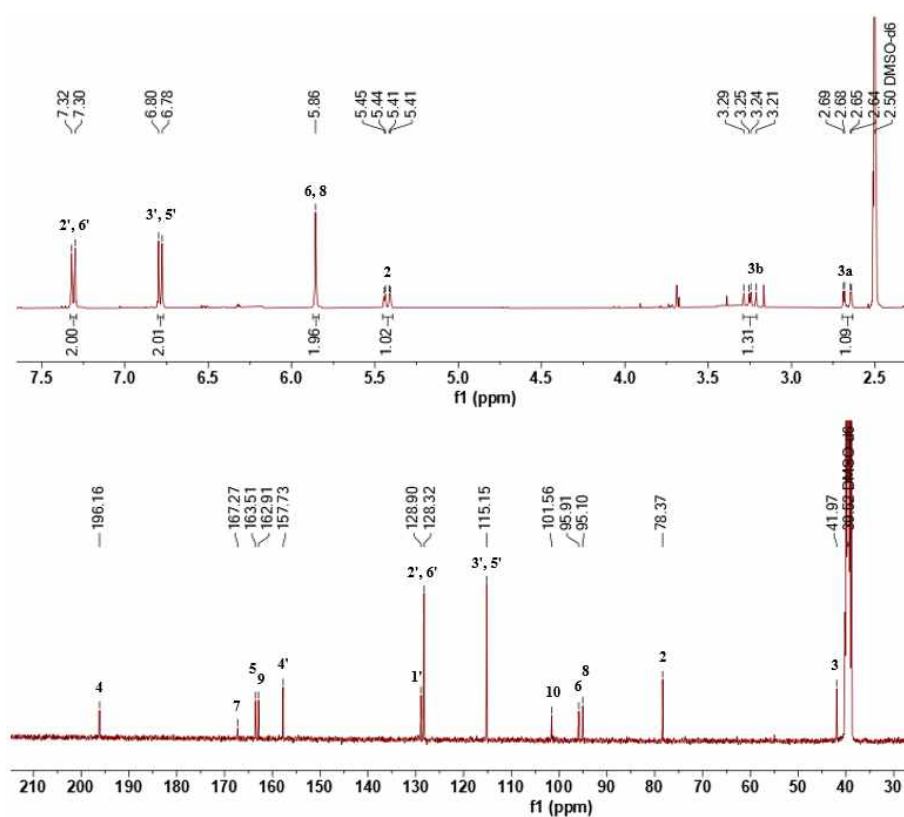


Figure 41. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **35** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.36. Compound **36**

Compound **36** was obtained as colorless gum and its empirical formula was established as  $C_{15}H_{12}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  273.0764  $[M + H]^+$  (calcd for  $C_{15}H_{13}O_5$ , 273.0763) and 10 degrees of unsaturation.  $^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  196.2 (C-4)], oxygenated protons and carbons [ $\delta_H$  5.42 (1H, dd,  $J = 12.8, 2.9$  Hz, H-2) /  $\delta_C$  78.4 (C-2)] with two benzene ring [ $\delta_H$  7.31 (2H, d,  $J = 8.5$  Hz, H-2', 6') /  $\delta_C$  128.3 (C-2', 6')  $\delta_H$  6.79 (2H, d,  $J = 8.5$  Hz, H-3', 5') /  $\delta_C$  115.2 (C-3', 5')  $\delta_H$  5.86 (2H, s, H-6, 8) /  $\delta_C$  95.9 (C-6), 95.1 (C-8)] implied isoflavanone type. By comparing above listed information with NMR data from already reported previous literature, Compound **36** was determined as dihydrogenistein (Yan Lin et al., 2017).



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### 3.2.37. Compound **37**

Compound **37** was obtained as white amorphous powder and its empirical formula was established as  $C_{15}H_{10}O_5$  by HRESIMS, which showed a protonated ion peak at  $m/z$  271.0611  $[M + H]^+$  (calcd for  $C_{15}H_{13}O_5$ , 271.0607) and 11 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.1 (C-4)] and two olefinic protons and carbons [ $\delta_H$  8.30 (1H, s, H-2) /  $\delta_C$  153.9 (C-2), 121.3 (C-3)] with two benzene rings [ $\delta_H$  7.37 (2H, d,  $J = 8.6$  Hz, H-2', 6') /  $\delta_C$  130.2 (C-2', 6')  $\delta_H$  6.81 (2H, d,  $J = 8.6$  Hz, H-3', 5') /  $\delta_C$  115.1 (C-3', 5')  $\delta_H$  6.36 (1H, d,  $J = 2.1$  Hz, H-8) /  $\delta_C$  93.8 (C-8)  $\delta_H$  6.20 (1H, d,  $J = 2.1$  Hz, H-6) /  $\delta_C$  99.2 (C-6)] implied isoflavone type compound. By comparing above listed information with NMR data from already reported previous literature, Compound **37** was determined as genistein (Li et al., 2016).

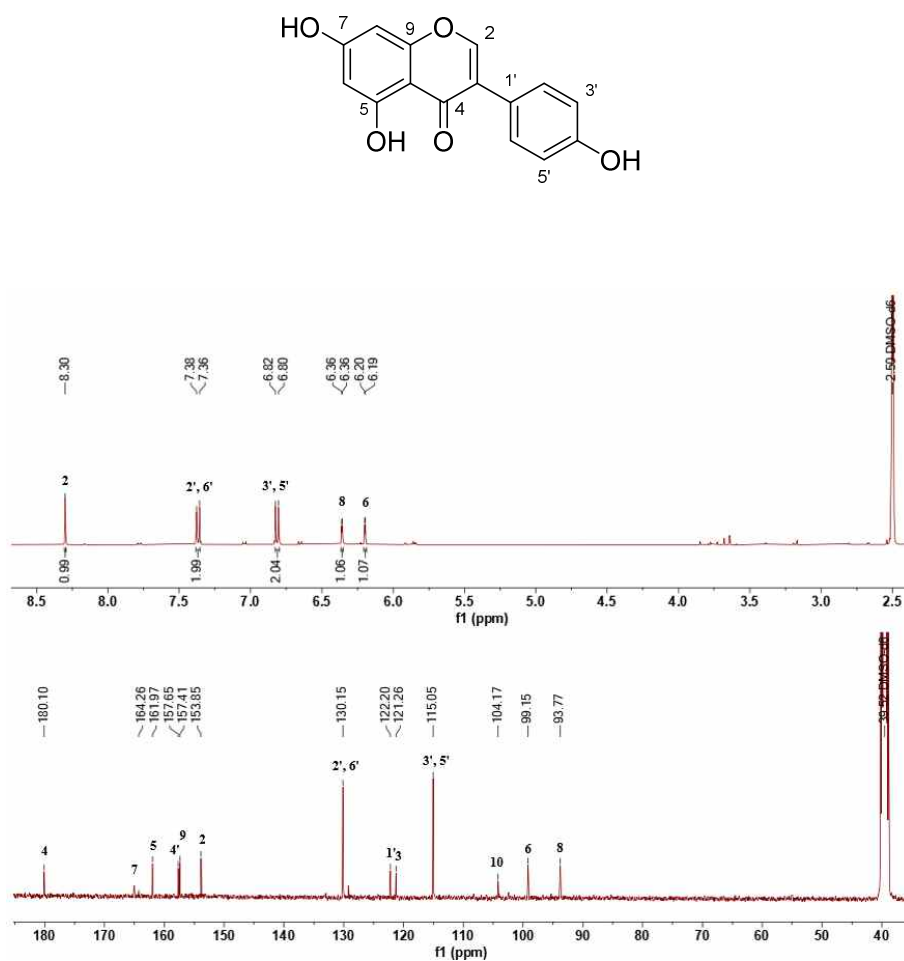


Figure 43.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectrum of compound **37** in  $\text{DMSO}-d_6$  (400, 100 MHz)

### 3.2.38. Compound **38**

Compound **38** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{25}H_{26}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  423.1808  $[M + H]^+$  (calcd for  $C_{25}H_{27}O_6$ , 423.1808) and 13 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated three aromatic ring peaks [ $\delta_H$  7.88 (2H, m, H-2', 6') /  $\delta_C$  129.0 (C-2'), 127.0 (C-6')]  $\delta_H$  6.47 (1H, s, H-8) /  $\delta_C$  92.7 (C-8)] carbonyl carbon [ $\delta_C$  175.8 (C-4)] and two deshielded olefinic  $^{13}C$  NMR peaks [ $\delta_C$  146.6 (C-2), 135.6 (C-3)] signified flavone type compound contains hydroxyl at C-3. Four methyls [ $\delta_H$  1.73 (3H, s, H-4''') /  $\delta_C$  17.7 (C-4'')  $\delta_H$  1.71 (3H, s, H-4'') /  $\delta_C$  25.5 (C-4'')  $\delta_H$  1.70 (3H, s, H-5''') /  $\delta_C$  25.5 (C-5''')  $\delta_H$  1.63 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] and two deshielded methines [ $\delta_H$  3.28 (2H, d,  $J = 7.3$  Hz, H-1''') /  $\delta_C$  28.1 (C-1''')  $\delta_H$  3.23 (2H, d,  $J = 7.0$  Hz, H-1'') /  $\delta_C$  21.0 (C-1'')] and two olefinics [ $\delta_H$  5.30 (1H, t,  $J = 7.3$  Hz, H-2''') /  $\delta_C$  122.5 (C-2''')  $\delta_H$  5.19 (1H, d,  $J = 7.1$  Hz, H-2'') /  $\delta_C$  122.4 (C-2'')] implied the presence of two prenyl groups. By comparing above listed information with NMR data from already reported previous literature, Compound **38** was determined as glyasperin A (Ji, et al., 2016).

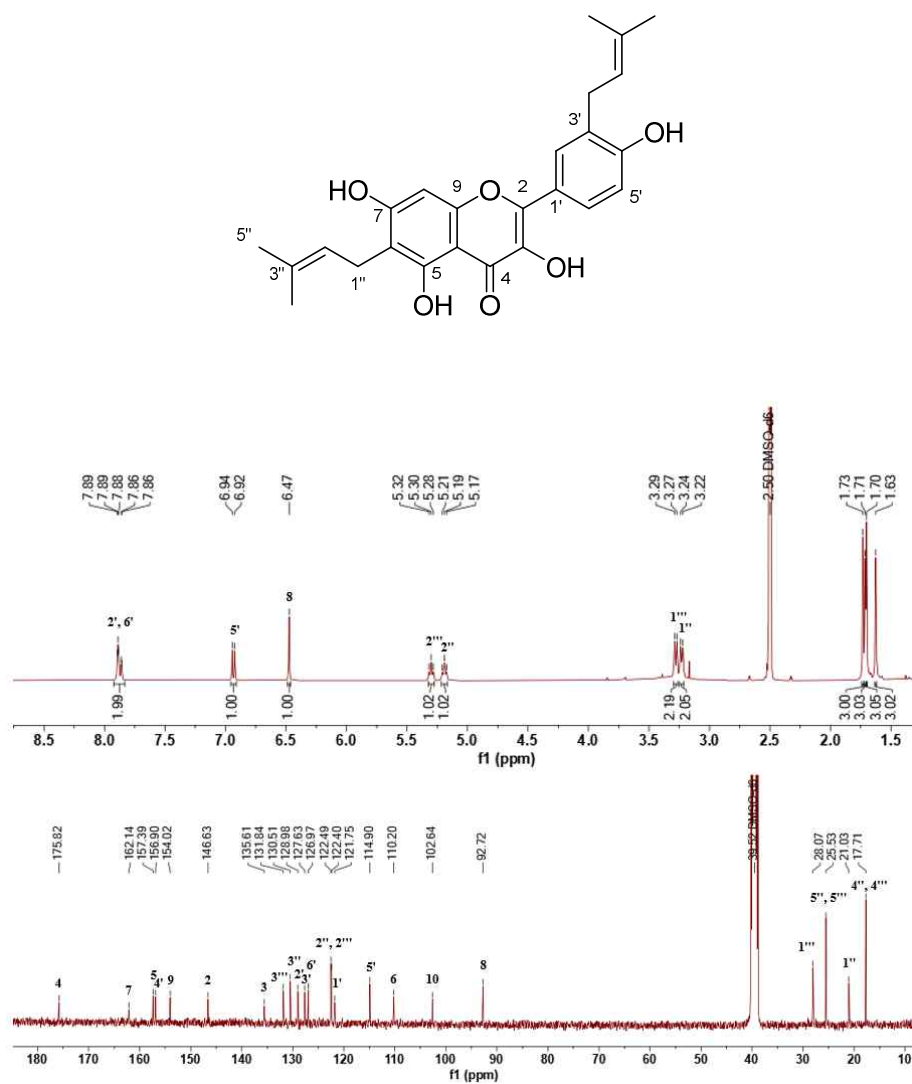


Figure 44. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **38** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.39. Compound **39**

Compound **39** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{21}H_{20}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  367.1188  $[M - H]^-$  (calcd for  $C_{21}H_{19}O_6$ , 367.1182) and 12 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one shielded carbonyl carbon [ $\delta_C$  159.8 (C-2)] and one deshielded olefinic proton and carbon [ $\delta_H$  7.80 (1H, s, H-4) /  $\delta_C$  136.5 (C-4)] and two benzene rings [ $\delta_H$  7.10 (1H, d,  $J$  = 8.4 Hz, H-6') /  $\delta_C$  131.6 (C-6')  $\delta_H$  6.60 (1H, s, H-8) /  $\delta_C$  98.0 (C-8)  $\delta_H$  6.36 (1H, d,  $J$  = 2.3 Hz, H-3') /  $\delta_C$  102.7 (C-3')  $\delta_H$  6.26 (1H, dd,  $J$  = 8.4, 2.2 Hz, H-5') /  $\delta_C$  105.9 (C-5')]. And two methyls [ $\delta_H$  1.73 (3H, s, H-4'') /  $\delta_C$  17.8 (C-4'')  $\delta_H$  1.63 (3H, s, H-5'') /  $\delta_C$  25.5 (C-5'')] with one olefinic [ $\delta_H$  5.15 (1H, t,  $J$  = 6.8 Hz, H-2'') /  $\delta_C$  122.8 (C-2'')] and one deshielded methine [ $\delta_H$  3.26 (2H, d,  $J$  = 6.8 Hz, H-1'') /  $\delta_C$  22.4 (C-1'')] implied the presence of prenyl group. By comparing above listed information with NMR data from already reported previous literature, Compound **39** was determined as glycycomarin (Ji et al., 2016).

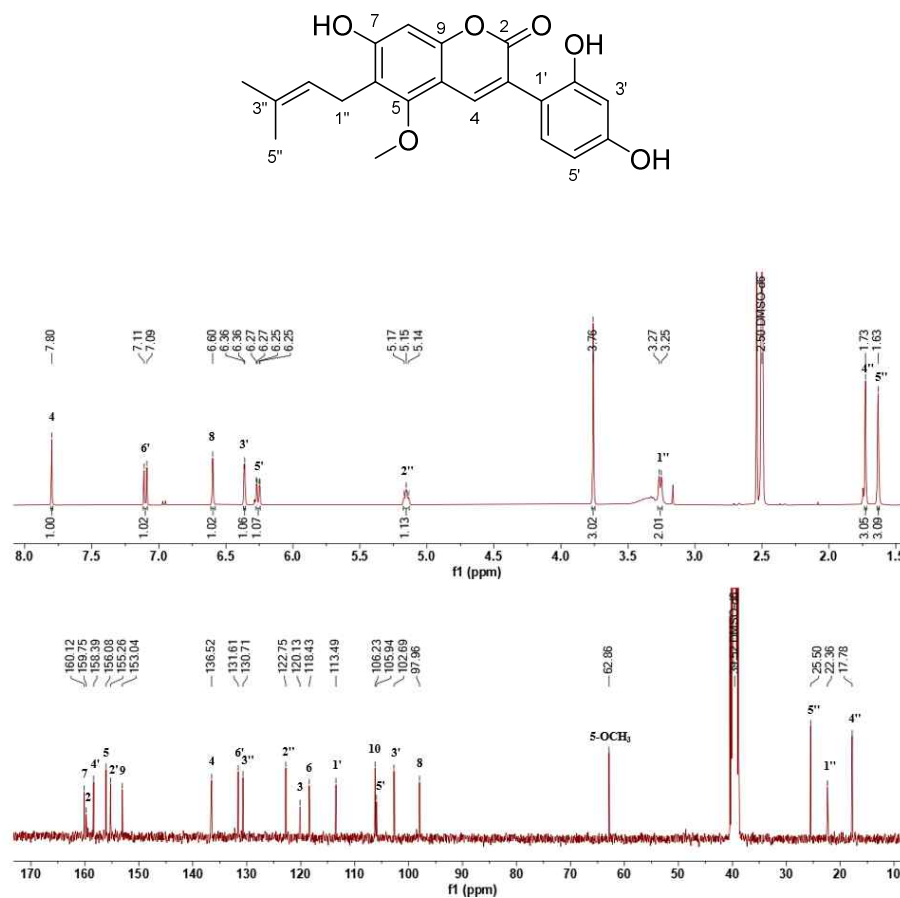


Figure 45. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **39** in DMSO-*d*<sub>6</sub> (400, 100 MHz)

### 3.2.40. Compound **40**

Compound **40** was obtained as yellow amorphous powder and its empirical formula was established as  $C_{20}H_{16}O_6$  by HRESIMS, which showed a deprotonated ion peak at  $m/z$  351.0869  $[M - H]^-$  (calcd for  $C_{20}H_{15}O_6$ , 351.0869) and 13 degrees of unsaturation.

$^1H$  and  $^{13}C$  NMR data indicated one carbonyl carbon [ $\delta_C$  180.0 (C-4)] and one deshielded olefinic proton and carbon [ $\delta_H$  8.32 (1H, s, H-2) /  $\delta_C$  154.2 (C-2)] with two benzene rings [ $\delta_H$  6.91 (1H, d,  $J = 2.1$  Hz, H-6') /  $\delta_C$  117.6 (C-6')  $\delta_H$  6.72 (1H, d,  $J = 2.1$  Hz, H-2') /  $\delta_C$  122.0 (C-2')  $\delta_H$  6.37 (1H, m, H-8) /  $\delta_C$  93.8 (C-8)  $\delta_H$  6.21 (1H, d,  $J = 2.1$  Hz, H-6) /  $\delta_C$  99.2 (C-6)] implied isoflavone type compound. And two methyls [ $\delta_H$  1.39 (6H, s, H-4'', 5'') /  $\delta_C$  27.5 (C-4'', 5'')] and two *cis*-olefinic protons and carbons [ $\delta_H$  6.37 (1H, m, H-1'') /  $\delta_C$  117.0 (C-1'')  $\delta_H$  5.75 (1H, d,  $J = 9.8$  Hz, H-2'') /  $\delta_C$  131.3 (C-2'')] implied presence of pyran ring moiety. By comparing above listed information with NMR data from already reported previous literature, Compound **40** was determined as semilicoisoflavone B (Ji, et al., 2016).

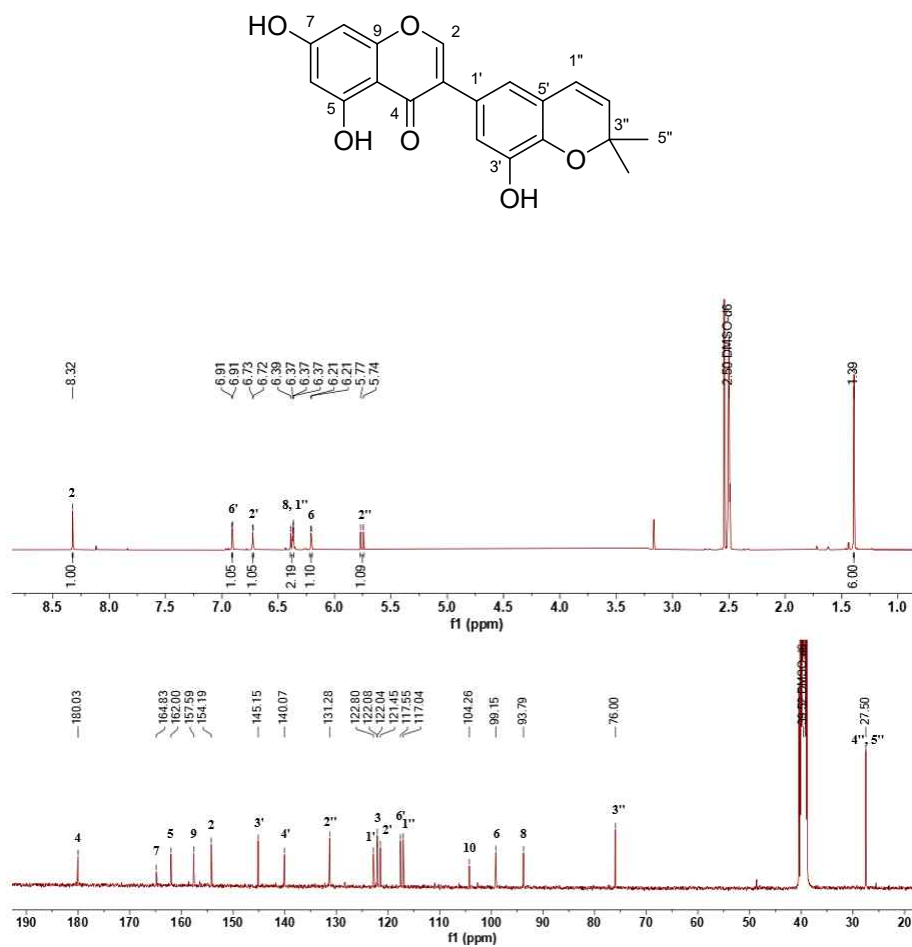
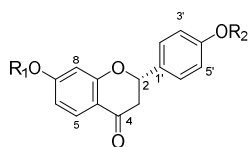
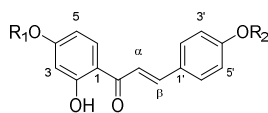


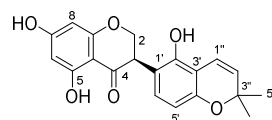
Figure 46. <sup>1</sup>H, <sup>13</sup>C NMR spectrum of compound **40** in DMSO-*d*<sub>6</sub> (400, 100 MHz)



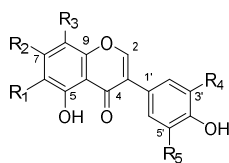
	R <sub>1</sub>	R <sub>2</sub>
<b>1</b>	H	Glc
<b>2</b>	H	Glc-(2,1)-O-Api
<b>3</b>	Glc	H
<b>33</b>	H	H



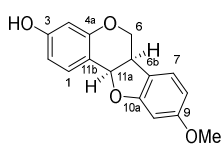
	R <sub>1</sub>	R <sub>2</sub>
<b>4</b>	H	Glc-(2,1)-O-Api
<b>5</b>	H	Glc
<b>6</b>	Glc-(2,1)-O-Api	H
<b>7</b>	Glc	H
<b>34</b>	H	H



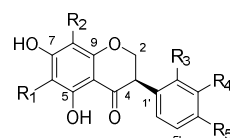
**8**



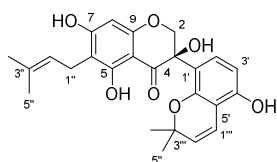
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<b>9</b>	Prenyl	OH	Prenyl	H	H
<b>19</b>	H	OH	H	Prenyl	H
<b>27</b>	Prenyl	OH	H	Prenyl	OH
<b>28</b>	Prenyl	OMe	H	H	H
<b>31</b>	H	OH	Prenyl	H	H
<b>37</b>	H	OH	H	H	H



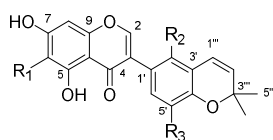
**10**



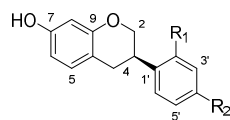
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<b>12</b>	Prenyl	H	OH	Prenyl	OH
<b>25</b>	H	Prenyl	OH	Prenyl	OH
<b>36</b>	H	H	H	H	OH



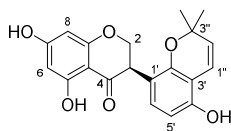
**13**



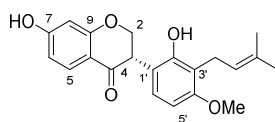
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
<b>11</b>	H	H	H
<b>14</b>	Prenyl	OH	H
<b>15</b>	Prenyl	H	H
<b>26</b>	Prenyl	H	OH
<b>40</b>	H	H	OH



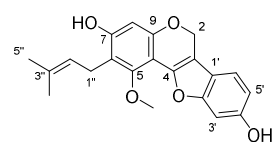
	R <sub>1</sub>	R <sub>2</sub>
<b>16</b>	OH	OMe
<b>35</b>	OMe	OH



**17**



**18**



**20**

Figure 47. Compound structures isolated from *G. uralensis*

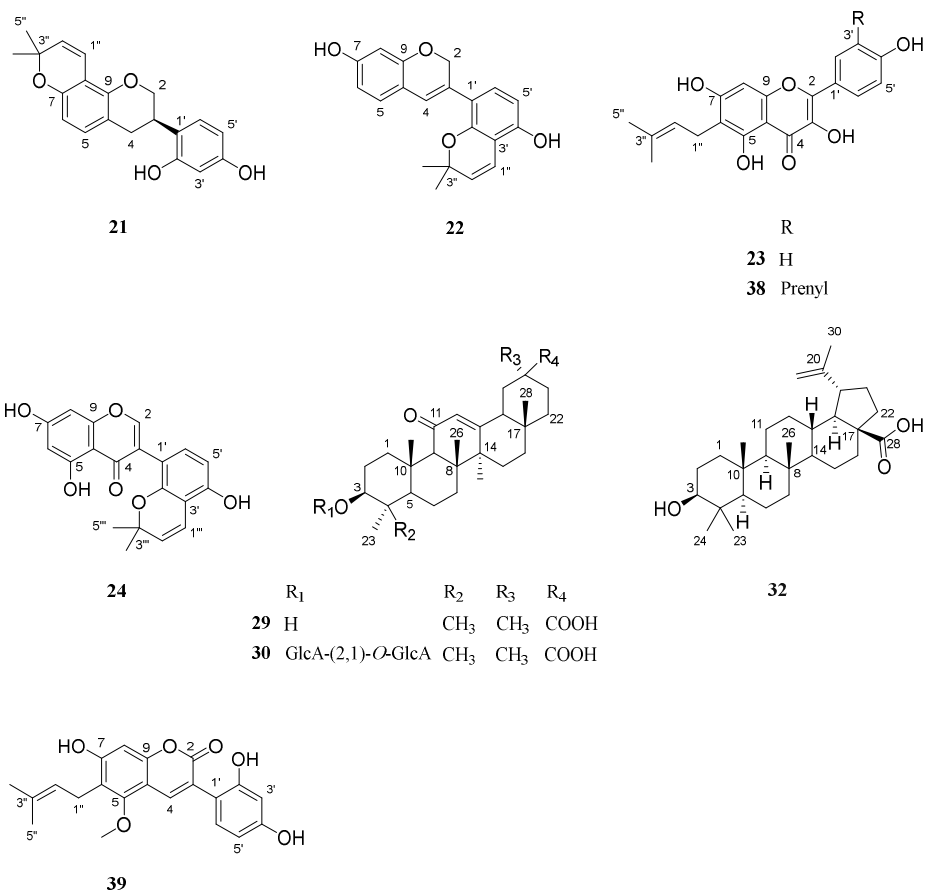


Figure 48. Compound structures isolated from *G. uralensis*

### 3.3. C2C12 myoblast proliferatory activity of Compounds from *G. uralensis*

To assess the cytotoxicity and proliferatory activities to C2C12 myoblast Compounds **1-40** were evaluated by MTT colorimetric assay when insulin (100 nM) treated as a positive control. In a cell viability test, compounds **14, 15, 21, 26, 27** showed cytotoxicity to C2C12 myoblast, except them most of compounds didn't showed significant cytotoxicity. Compared to positive control (100 nM insulin), compound **11**, **24, 25, 30** exhibited comparable effects at 10  $\mu$ M. Especially compound **11** (isoderrone) known as PTP1B negative regulator which showed insulin sensitizing effect in C2C12 implied that compound **24, 25** shared same flavonoid structure with compound **11** also can repress PTP1B enzyme activity. In the fact that compounds **15** showed cytotoxicity to C2C12 myoblast, prenyl group substituted at A ring of iosflavone could be related with C2C12 proliferation.

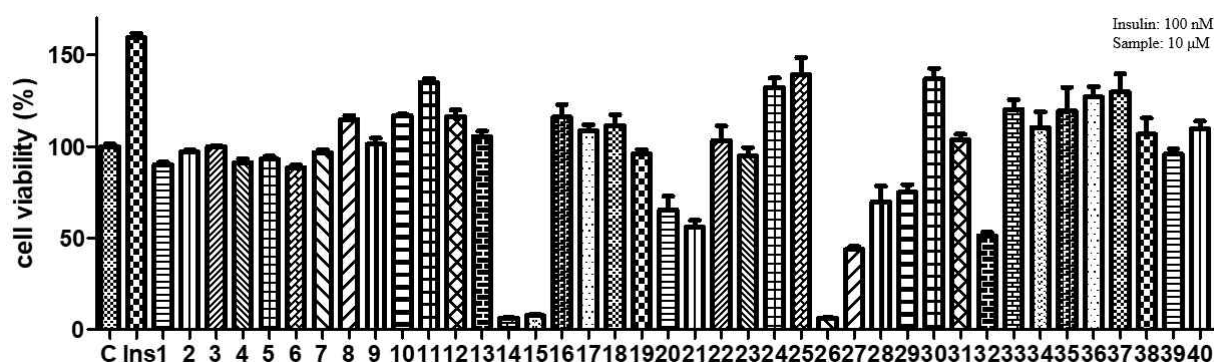


Figure 49. cytotoxicity and proliferatory activity of compounds **1-40**. After treatment compounds incubated for 24 hours. The viability evaluated by MTT assay.

### 3.4. NO assay to LPS induced A549 cells of Compounds from *G. uralensis*

To evaluate the anti-inflammatory activity to LPS induced A549 cells Compounds **1-40** were treated for 24 hours. In a NO assay, when quercetin (20  $\mu$ M) treated as a positive control. compound **12**, **20**, **22**, **24** inhibited the nitrite accumulation at 10  $\mu$ M without cytotoxicity. Although compound **39** effectively inhibited the nitrite accumulation also showed cytotoxicity. In the fact that nitrite (NO) one of inflammatory cytokine among ILs, TNF- $\alpha$ , PGs. Compounds **12**, **20**, **22**, **24** can be latent candidate for anti-inflammation.

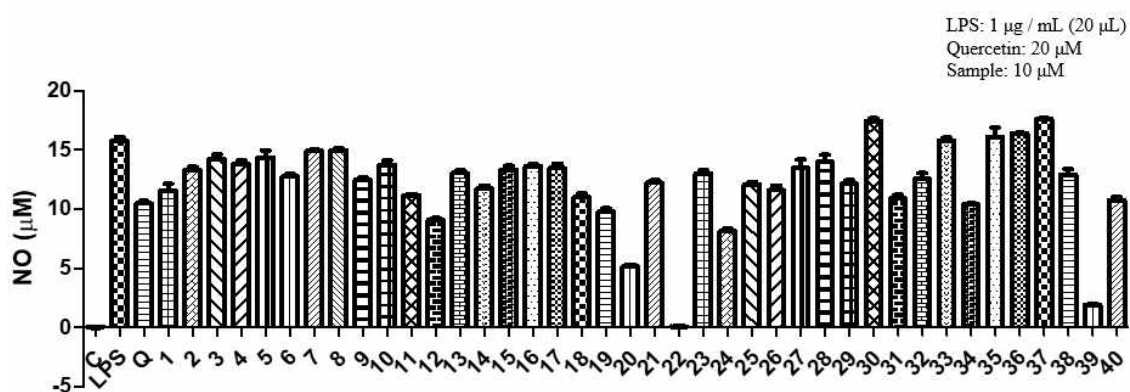


Figure 50. NO assay of compounds **1-40**. After 1 hour treated compounds LPS treated then incubated for 24 hours.

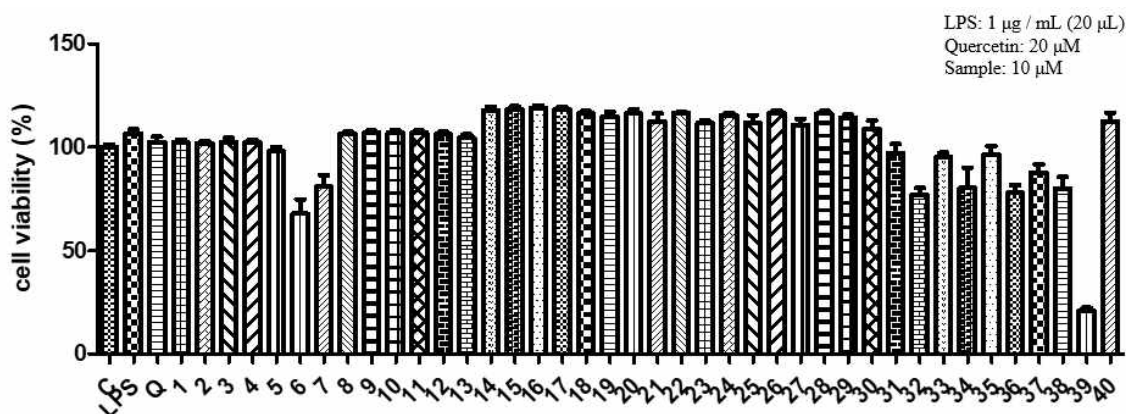


Figure 51. cytotoxicity of compounds **1-40**. The viability evaluated by MTT assay.

## 4. Conclusion

The purpose of ensuring fundamental spectroscopic data for analysing of new varieties of licorice fourty compounds were isolated from roots of *Glycyrrhiza uralensis* including triterpenoid, saponin, coumarin, flavonoid, using various chromatography including MPLC and HPLC. Structures of compounds were elucidated based on NMR and HRMS spectroscopy. The isolated compounds were evaluated their cytotoxicity, proliferation, and anti-inflammatory effects by *in vitro* C2C12 myoblast MTT assay and LPS induced A549 cells NO assay. In C2C12 myoblast MTT assay result compound **11** (isoderrone), **24** (allolicoisoflavone B), **25** (3'-( $\gamma$ ,  $\gamma$ -dimethyl)-kievitone), **30** (glycyrrhizin) showed proliferatory activity of C2C12 myoblast, among them compound **25** (3'-( $\gamma$ ,  $\gamma$ -dimethyl)-kievitone) showed most promising effect compare with positive control (insulin, 100 nM). In LPS induced A549 cell NO assay compounds **12** (glisoflavanone), **20** (glyurallin A), **22** (glabrene), **24** (allolicoisoflavone B) inhibited the nitrite accumulation without cytotoxicity among them compound **22** (glabrene) showed most promising inhibitory effect of nitrite accumulation.

Interestingly compound **24** (allolicoisoflavone B) showed potential both C2C12 myoblast MTT assay and LPS induced A549 cell NO assay.

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## 국문초록

감초 (*Glycyrrhiza uralensis*)는 콩과 (Fabaceae)에 속하는 다년생 식물로 동유럽을 비롯하여 시베리아 및 파키스탄에 걸쳐 분포하고, 온대 기후대의 사질성 토양에서 자생하는 식물이다. 감초에서 분리 가능한 계열의 화합물로는 트리테르페노이드 (Triterpenoid), 쿠마린 (Coumarin), 찰콘 (Chalcone), 플라보노이드 (Flavonoid), 스틸베노이드 (Stilbenoid) 계열 등이 있다. 대표적인 지표성분인 글리시리진 (Glycyrrhizin)은 항산화 효과, 항 궤양 효과, 항바이러스 효과, 항암 효과를 가지는 것으로 알려졌으며, 플라보노이드 (Flavonoid) 계열의 화합물 중 대표적인 지표성분인 리퀴리티게닌 (Liquiritigenin)은 해독 효과, 간세포 손상 저해 효과, 항산화 효과, 피부질환 억제 효과, 면역 조절 및 증진 효과를 가지는 것으로 보고되어 한약재뿐만 아니라 화장품 및 건강기능식품의 원료로 사용하는 등 그 쓰임새가 다양하다.

국내 소비량이 많은 감초의 자급률은 약 5% 수준에 불과한데, 국내 재배 시 감초의 성분 함유량이 적고 생리장해에 따라 수확량이 적기 때문에 기존의 품종을 그대로 도입해 재배하기 어려운 실정이다. 따라서 국내의 기후에 대한 적응성과 질병 저항성을 가진 신품종 육종의 필요성이 대두함에 따라 2014년부터 원감 (Wongam), 신원감 (Sinwongam), 다감 (Dagam) 등의 품종이 개발되었으나 대한민국 약전에 기재된 기원식물과 다르다는 문제가 있어 재배 농가의 보급에 어려움을 겪고 있다. 따라서 본 실험은 감초로부터 지표성분을 계열별로

분리 및 확보하고 chemical library를 조성하여 신품종의 지표성분 분석 및 성분 프로파일링에 필요한 참고 데이터를 확보함에 있다.

감초 (*Glycyrrhiza uralensis*) 뿌리 1.5 kg을 90% 에탄올로 3회 추출한 후 n-헥산, 에틸 아세테이트, 뷰탄올, 물 분획으로 나누었다. 에틸 아세테이트 분획을 이용하여 순상 크로마토그래피를 거쳐 8개의 분획으로 나누었고, HPLC를 비롯한 크로마토그래피를 이용하여 트라이테르페노이드, 플라보노이드, 찰콘 및 쿠마린 계열의 화합물을 포함한 총 40종의 화합물을 분리한 뒤, NMR 데이터를 이용하여 구조를 확인하였다. 분리된 화합물에 대해 C2C12 myoblast cell MTT assay와 LPS induced A549 cell NO assay를 이용하여 화합물의 PTP1B 억제제로서의 기능을 할 수 있는 후보 물질과, 항염 활성을 가지는 후보 물질을 제시하였다.

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**주요어:** 감초, 트라이테르페노이드, 찰콘, 플라보노이드, 쿠마린, 2형 당뇨병, 인슐린 감수성, 항염활성

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