NEAR EAST UNIVERSITY

GRAGUATE INSTITUTE OF HEALTH SCIENCES

SECONDARY METABOLITES FROM *Phlomis floccosa* D. DON

Randa ALDABA

PHARMACOGNOSY

MASTER THESIS

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ÖZET

Phlomis (Lamiaceae) cinsi, Libya Florasında (Bitki Örtüsünde) sadece bir türle temsil edilmektedir, *Phlomis floccosa* D. Don. Bu çalışmada, bitkinin toprak üstü kısımları, fitokimyasal bileşikleri açısından araştırılmıştır.

Phlomis floccosa'nın açık havada kurutulmuş ve toz edilmiş topraküstü kısımlarının 300 g'ı 3500 ml %80 etanol ile oda ısısında, sık sık çalkalanarak 72 saat süreyle maserasyona bırakılmıştır. Vakumda süzülerek elde edilen ekstrakt, 50ºC'de vakum altında yoğunlaştırılarak ham sulu ekstre elde edilmiştir (HSE). Bu ekstrenin bir kısmı, bir seri kromatografik yöntemlere uygulandı [vakum likit kromatografisi (VSK), açık kolon kromatografisi (SK), jel kromatografisi (Sephadex LH-20), orta basınçlı sıvı kromatografisi (OBSK)]. Kromatografik çalışmalar sonunda, üç iridoit, lamiit (lamiide: PF-1&PF-9), ipolamit (ipolamiide: PF-5), ve aurozit (auroside: PF-6), üç feniletanoit glikoziti, verbaskozit (=akteozit) (verbascoside= acteoside: PF-4& PF-8), forsitozit B (forsythoside B: PF-3) ve alissonozit (alyssonoside: PF-7), ve bir flavon glikoziti, luteolin 7-*O*-glukuronit (luteolin 7-*O*-glucuronide: PF-2 &PF-10) izole edildi. Elde edilen bileşiklerin yapıları, UV, NMR [(1D NMR: ^{1}H NMR, ^{13}C NMR, DEPT-135 ve 2D NMR (COSY, HSQC, and HMBC)] gibi spektroskopik yöntemler yardımıyla tayin edildi. Kimyasal içerik sonuçları, diğer *Phlomis* türlerinden elde edilen sonuçlarla kısaca karşılaştırmalı olarak tartışıldı.

Anahtar kelimeler: *Phlomis floccosa, Lamiaceae,* İridoit glikozitleri, lamiit, ipolamiit, aurozit, feniletanoit glikozitleri, verbaskozit (=akteozit), forsitozit B, alissonozit, flavon glikozit, luteolin7-*O*-glukuronit.

ABSTRACT

The genus *Phlomis* (Lamiaceae) is represented by one species in the flora of Libya, *Phlomis floccosa* D.Don. In this study, above ground parts of this plant have been investigated phytochemically.

The air dried, 300g of powdered above ground parts of *Phlomis floccosa* were macerated with 3500 ml 80% of ethanol at room temperature. After evaporation of ethanol at 50ºC, under reduce pressure, 120 ml of crude water extract (WSE) was obtained. A series of chromatographic studies [(Vacuum liquid chromatography (VLC), Open column chromatography (CC), gel chromatography (Sephadex LH-20) and Medium pressure liquid chromatography (MPLC)] was carried out using *ca.* 100 ml of this crude extract. Three iridoid glycosides, lamiide (PF-1&PF-9), ipolamide (PF-5), and auroside (PF-6), three Phenylethanoid glycosides verbascoside (PF-4& PF-8), forsythoside B (PF-3), alyssonoside (PF-7), and one flavonoid glycoside, luteolin-7-*O*-glucuronide (PF-2 &PF-10) were isolated. Structures of the isolated compounds were elucidated by means of spectroscopic methods, UV, 1D NMR (^{1}H NMR and^{13}C NMR, DEPT-135) and 2D NMR (COSY, HSQC, and HMBC). A brief discussion is also given for the comparison of results with previous studies performed on other *Phlomis* species as to their chemical constituents.

Key words: *Phlomis floccosa*, *Lamiaceae,* Iridoid glycosides, lamiide, ipolamide, auroside, phenylethanoid glycosides, verbascoside, forsythoside B, alyssonoside, flavonoids, luteolin7-*O*-glucuronide.

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SPECTRA

1. INTRODUCTION:-

Medicinal plants have been used as an important source for biological active secondary metabolites to treat many health disorders such as inflammation, pain, healing wounds in many countries for centuries (Soltani-Nasab et al., 2014). Through drug research and chemical synthesis with the advances in modern medicine such plants have been established as primary sources of medicinal agents in industrialized countries as well as in developing countries such countries cannot afford pharmaceutical drugs and use their own plant-based indigenous medicines, due to their bioactive components traditionally used medicinal plants have received considerable attention for new drug discoveries (Limem Ben Amor et al., 2009a).

Labiatae (Lamiaceae) family has 180 genera and nearly 3200 species, growing mostly in the Mediterranean area. It is widely known that many species of the Lamiaceae are aromatic and often used as herbs, spices, folk medicines, and fragrances. It has aromatic herbs, sub shrubs and shrubs which often bear woolly leaves with arranged in opposing pairs, it have flowers resembling the lips of a mouth and four-lobed ovary, usually ball-like cluster where each lobe yields a seed (Ozdemir et al., 2014).

Phlomis L, is large and medicinal important genus of perennial herbs in the family Lamiaceae which comprises more than 100 different species spread in the Mediterranean region native to Turkey, North Africa, Europe and Asia (Harput et al., 2006; Soltani-Nasab et al., 2014; Sarkhail et al., 2006 and Marine et al., 2007). Their uses differ from one country to another. Several *Phlomis* species are consumed in the form of herbal teas as remedies for gastrointestinal problems and as prophylactics against liver, kidney, bone and cardiovascular diseases (Limem Ben Amor et al., 2009a and Lopez et al., 2010). They have been used for many decades as herbal remedies and used in traditional medicine for the treatment of various conditions such as stimulants, tonics, wound healers, pain relievers in gastrointestinal distress, anti-inflammatory, anti-diabetes, hemorrhoids and gastric ulcers (Sarkhail et al., 2006; Delzar et al., 2008 and Sarikukcu et al., 2014).

In addition to these uses, *Phlomis* species were described by Dioscorides as herbal drugs and used ethnopharmacologically in herbal medicine for the respiratory tract diseases or local treatment of wounds (Sarkhail et al., 2006).

Previous phytochemical investigations on several *Phlomis* species have been shown to contain different classes of secondary metabolites such as iridoids, flavonoids, phenolic compounds like phenyethanoids and phenypropanoids, monoterepenes, diterepenes, triterpenes, lignans, neolignans, as well as, their glycosides, alkaloids and essential oils (Sarkhail et al., 2006; Yalcin et al., 2005; Ersoz et al., 2001; Harput et al., 2006; Çalış et al., 2005b).

The iridoid glycosides such as lamiide, auroside and ipolamide are characteristic of this genus. Besides many flavonoids such as a luteolin 7-*O*-*β*-D-glucopyranoside and chrysoeriol 7-*O*-*β*-Dglucopyranoside have been reported. A wide variety of caffeic acid derivatives and phenylethanoid glycosides including verbascoside (acteoside) and forsythoside B have been identified in many species (Sarkhail et al., 2006 and Zhang et al., 2009). The biological and pharmacological activities of some *Phlomis* species have been investigated previously such as anti-inflammatory, anti-nociceptive (Shang et al., 2011), antimicrobial (Wafa et al., 2016), anti-malaria (Kirmizibekmez et al., 2004a), free radical scavenging and antioxidant (Yalcin et al., 2003 and Delzar et al., 2008), anti-diabetic (Sarkhail et al., 2007), anti-ulcerogenic (Limem Ben Amor et al., 2009b), recently considered as a potent anticancer agents (Soltani-Nasab et al, 2014).These activities are linked to their active constituents.

The *Phlomis* genus is represented by only one species in flora of Libya, which is *Phlomis floccosa* D. Don, commonly called (ALZHERIA), this plant is a rare stout tall perennial herb from 35-40 cm, which was native and distributed in the east of Libya, growing wild in Gebal - Akhdar, Wadi-Alkuf and Wadi of Baida (Siddiqi M.A., 1985). In folk medicine, it has been used as anti-diabetic and for treatment of metritis for honey production (El-Mokasabi et al., 2014).

A previous study on this species from Egypt showed the presence of some flavonoids such as Apigenin-7-glucoside, Apigenin-7-rutinoside, Apigenin-7-p-coumaroyl glucoside, Chrysoeriol-7-glucoside, Chrysoeriol-7-rutinoside, Chrysoeriol-7-p-coumaroyl glycoside, Luteolin-7-glucoside, Luteolin-7-rutinoside, Luteolin-7-O-diglucoside, Luteolin-7-p-coumaroylglucoside, Chrysoeriol-7-p-coumaroylglucoside (vicenin), 6,8-di-C-glucosyl apigenin, 6,8-di-Cglucosylluteolin (lucenin-2), which is similar in most *Phlomis* species (EL-Negoumy et al., 1986).

In 1992, Assaad with coworkers have reported the isolation and structure elucidation of two iridoid glycosides as lamiide and its 7-*O*-*p*-methoxycinnamate (Durantoside II) from aerial parts of *Phlomis floccosa*. However, no work has yet been reported on the isolation and elucidation of structures of phenylethaoid glycosides from this plant.

This study is aimed to photochemical investigations of the aerial parts of *Phlomis floccosa* D. Don to isolate and elucidate of the secondary metabolites including iridoid glycosides, phenylethanoid glycosides, and flavonoids. The structures of the isolated compounds have been determined by the help of spectral analysis such as UV and 1D (^{1}H-NMR, ^{13}C-NMR, DEPT), and 2D-NMR (COSY, HSQC and HMBC).

2. LITERATURE REVIEW:-

2. 1. Botanical Characters :-

2. 1.1. Lamiaceae Famialy:-

Shrubs, sub shrubs, annual and perennial herbs, commonly glandular and aromatic; stems often tetragonous; leaves generally opposite, decussate, simple, estipulate; flowers mostly hermaphrodite, often in more or less condensed cymes or verticillasters, sometimes in racemes or spikes; bracts foliaceous or reduced; bracteoles small or wanting; calyx often tubular or infundibuliform, persistent, commonly with prominent nervation, 4-5- toothed or lobed, occasionally 2-lipped with emarginate or toothed lips, or subentire; corolla tubular, sympetalous, the limb often 2-lipped, with the adaxial lip frequently emarginate, the abaxial 3-lobed; stamens usually 4, didynamous, sometimes 2, inserted on the corolla-tube; anthers 1-2- thecous, introrse; ovary superior, generally seated on a nectariferous disk, *2*-carpellate, but ultimately divided almost to base into 4 divisions; style generally gynobasic, arising from the base of the ovary-divisions; stigma commonly 2-lobed. Fruit usually consisting of 4, 1 -seeded nutlets enveloped by the persistent calyx, rarely drupaceous; seed without, or with very scanty endosperm; embryo straight or curved, the radicle pointing downwards.

About 180 genera and more than 3,000 species with a cosmopolitan distribution, but exceptionally well represented in the Mediterranean region. Many genera (*Salvia*, *Thymus*, *Origanum*, *Ocimum*, *Mentha*, etc.) furnish aromatic potherbs and are widely cultivated; others are valued for their fragrant oils.

Sexual dimorphism and cleistogamy are found in the flowers of many *Labiatae*, and may account for misleading differences within a single species.

DISTRIBUTION:

Mediterranean region, Pakistan, India, China, Central America, Australia.

2. 1.2. *Phlomis* L.

2. 1.3. *Phlomis floccosa* D. Don **:-**

Phlomis floccosa D. Don belonging to lamiaceae famialy is characterized as a *Dwarf shrub'* grows up to 35-40cm tall. The lower leaves 3-5cm, oblong ovate, cordate or subcordate at base, coriaceous, crenate, stellate-lanate on both sides, floral leaves are shorthlypetiolate, lanceolate, acute or acuminate. verticils 4-8- flowered. Braceoles 15-18 mm long, linear, uncinate, stellate-lanate, ciliate with hairs 2-3mm long. Calyx 15-19mm long, stellate-lanate, ciliate; teeth1-5mm long, subulate, uncinated. Corolla 25-32mm long, yellow. Nutlets oblong, trigonous, smooth, black, 1.5-1.8x4-4.5mm (Siddiqi, M.A., 1985).

DISTRIBUTION: Tunisia, Libya, Egypt, Syria, Crete.

Figure.2.1.3. Picture of the *Phlomis floccosa* D. Don

(www.ville-ge.ch/musinfo/bd/cjb/africa/images/data/images/Phlomis%20floccosa: A.Dobignard).

2. 2. Phytochemical Studies:-

One of extensive studies on the secondary metabolites from *Phlomis* species growing in Turkey has been carried by Çaliş, 2004a. This project was performed on the 33 *Phlomis* species, of which 21 are endemic. This study is the part of continuing research on the *Phlomis* species and other Lamiacae plants of the Mediterranean.

2.2.1. Monoterpenoids (Iridoid Glycosides):-

The iridoids appears to form a major group of compounds that have been isolated from various *Phlomis* species. They are a large group of monoterpenes that have been found to occur in a variety of animal species and as constituents of a number of orders and plant families within the dicotyledons. The name iridoid has been derived from iridodial, iridomyrmecin and related compounds isolated from the defence secretion of *Iridomyrmex* species, a genus of ants, which are characterized by a cyclopenta (C) pyranoid skeleton with a glucose moiety attached to C-1 in pyran ring (Junior, 1990).

Iridoids are present in a number of folk medicinal plants used as bitter tonics, sedatives, antipyretics,cough medicines, remedies for wounds, skin disorders and as hypotensives. This fact encouraged to investigate the bioactivities of these phytochemicals. Intensive study oftheir bioactivity revealed that these compounds exhibit awide range of bioactivities: cardiovascular, antihepatotoxic, hypoglycemic, hypolipidemic, antiinflammatory, antispasmodic, antitumor, antiviral, choleretic, immunomodulator and purgative activities (Dinda et al., 2007).

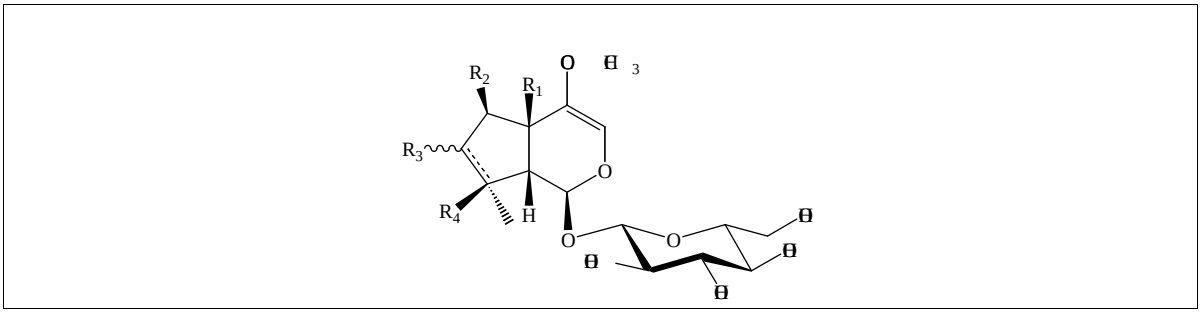


Figure 2.2.1.(A). Structure of Iridoid glycosides of some *Phlomis* species

Table 2.2.1.A. Iridoid glycosides obtained from some *Phlomis* species.

No	Compound	R_1	R_2	R_3	R_4	*Phlomis* species	references
1	5-Deoxypulchellosidel	H	OH	β–OH	H	*P. longifolia var.longifolia* *P. rigida*	Ersöz et al., 2001 Takeda et al., 2000
2	6-β-hydroxy-ipolamiide	OH	OH	H	OH	*P. rigida*	Takeda et al., (2000)
3	7-Epiphlomiol (Phloyoside I)	OH	OH	α-OH	OH	*P. rotate* *P. tuberosa* *P. umberosa*	Zhang et al., 1991 Çalış et al., 2005b Shang et al., 2011
4	8-Epiloganin	H	H	β-OH	H	*P. aurea* *P. grandiflora var.grandiflor*	Kamel et al., 2000 Takeda et al., 1999
5	Auroside	OH	H	β-OH	H	*P. linearis* *P. aurea* *Phlomis angustisssimia* *P. fruticosa*	Çalış et al., 1991 Kamel et al., 2000 Yalcin et al., 2005 Marin et al., 2007
6	Dehydropentstemoside	OH	OH	$\Delta^{6,7}$	H	*P. rotate*	Zhang et al., 1991
7	Lamalbide	H	OH	β-OH	OH	*P. longifolia* *P. tuberosa*	Ersöz et al., 2001 Çalış et al., 2005b
8	Ipolamiide	OH	H	H	OH	*P. linearia* *P. armeniaca* *P. aurea* *Phlomis brunneogaleata* *P. monocephala* *P. viscosa* *P. olivierii*	Çalış et al., 1991 Saracoglu et al., 1995 Kamel et al., 2000 Kirmizibekmez et al., 2004a Yalcin et al., 2003 Çalış et al., 2005a Delnavaz et al., 2016

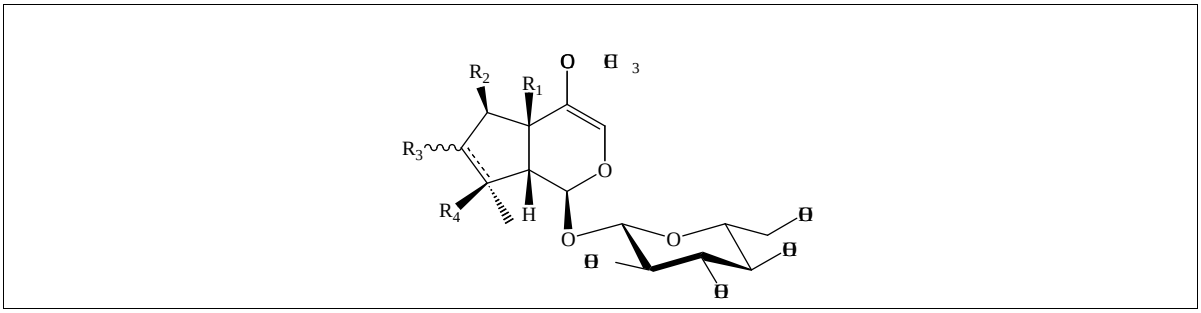


Figure 2.2.1(A). Structure of Iridoid glycosides of some *Phlomis* species

Table 2.2.1.A. Iridoid glycosides obtained from some *Phlomis* species (*Continuing*).

9	Lamiridoside	H	OH	β–OH	OH	*P. rigida* *P. spinidens*	Takeda et al., 2000 Takeda et al., 2001
10	Phlomiol	OH	OH	β–OH	OH	*P. longifolia var. longifolia* *P. fruticosa*	Ersöz et al., 2001 Marin et al.,(2007)
11	Phlomoside A	OH	H	β–OH	OH	*P. spinidens* *P. grandiflora var.grandiflora*	Takeda et al., 2001 Takeda et al., 1999
12	Lamiide	H	H	β-OH	H	*P. linearis* *P. aurea* *P. floccosa* *P. pungens var. pungens* *P. physocalyx* *P. angustissima* *P. longifolia var longifolia* *P. fruticosa* *P. monocephala* *P.oppositifloria* *P. syrica*	Çalış et al., 1991 Kamel et al., 2000 Assad et al., 1992 Ismailoglu et al., 2002 Ersöz et al., 2003 Yalcin et al., 2005 Ersöz et al., 2001 Marin et al., 2007 Yalcin et al., 2003 Çalış et al., 2005c Harput et al., 2006
13	Shanzhiside methyl ester	H	OH	H	OH	*P. rotate* *P. tuberosa* *P. samia* *P. rigida* *P. umberosa*	Zhang et al., 1991 Çalış et al., 2005b Yalcin et al. 2003 Takeda et al., (2000) Shang et al., 2011
14	Chlorotuberoside	H	OH	α–Cl	OH	*P. tuberose* *P. rotate*	Çalış et al., 2005b Zhang et al., 1991
15	Lamiidoside	OH	H	p-com	OH	*P. viscose*	Çalış et al., 2005a

p-com : p-coumaroyl α-Cl = chlore

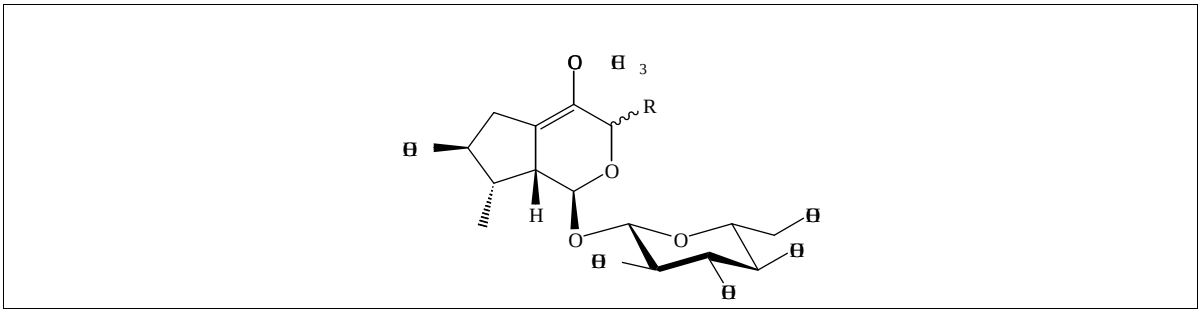


Figure .2. 2. 1. (B). Structure of iridoid from *Phlomis aurea*.

Table 2.2.1. B. Iridoid glycosides from *Phlomis aurea.*

No	Compound	R	References
16	3-*epi*-phlomurin	α-OCH_3	Kamel et al., 2000
17	Phlomurin	β-OCH_3	Kamel et al., 2000

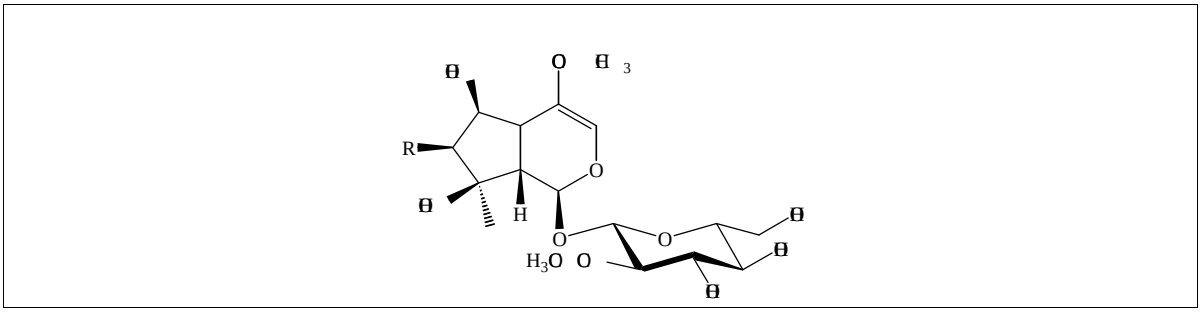


Figure .2. 2. 1. (C). Structure of Phlorigidoside A and B.

Table 2.2.1. C. Iridoid glycosides obtained from *Phlomis rigida.*

No	Compound	R	*Phlomis* species	Reference
18	*Phlorigidoside A (2-O-acetylamiridoside)*	$OCOCH_3$	*P. rigida*	Takeda et al., 2000
19	PhlorigidopisideB (8-O-acetyl-6-B-hydroxy ipolamide)	H		

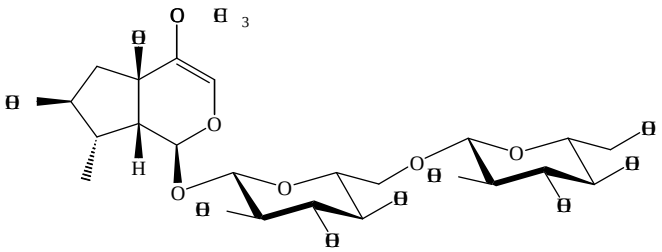
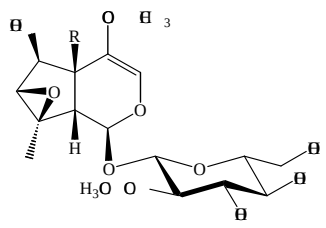


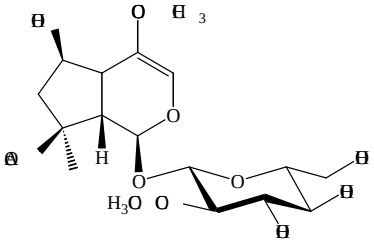
Figure 2. 2. 1. (D). Structure of Phlomiside

Table 2.2.1. D. Iridoid diglycosides from *P. aurea*

No	Compound	*Phlomis* species	Reference
20	Phlomiside	*Phlomis aurea*	Aboutable et al., 2002



[**21**] Sesamoside, [**21**] Phlorigoside C

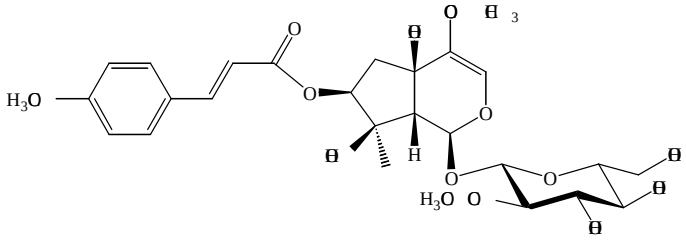


[**23**] 8-*O*-Acetyl shanzhiside Me ester

Figure 2.2.1. (E). Structure of some iridoid glycosides.

Table 2. 2. 1. E . Iridoid glycosides from some *Phlomis* species.

No	Compound	R	Species	References
21	Sesamoside	OH	*P.tuberosa* *P.rigida* *P. spinidens* *P.umberosa*	Çalış et al., 2005b Takeda et al., 2000 Takeda et al., 2001 Shang et al., 2011
22	5-deoxysesamoside (Phlorigidoside C *)*	H	*P.tuberosa* *P.rigida* *P. spinidens*	Çalış et al., 2005b Takeda et al., 2000 Takeda et al., 2001



[**24**] Durantoside II

Reviewing current literatures, it has been noted that the most frequent iridoids are mono-glycosides such as lamiide, ipolamiide, aurosid and shanziside methyl ester which were reported from the most species of *Phlomis* genus as C10 iridoids substituted with a methoxycarbonyl function group at C4 and a double bond between [C3=C4 bond], while *8-O-*acetylahnzhiside methyl esterwas a first iridoid glycoside substituted at C4 with carboxylic acid group [COOH] has been reported from some *Phlomis* species such as, *Phlomis tuberosa* (Çalış et al., 2005b); *P. rigida* (Takeda et al., 2000) ; *P. spinidens* (Takeda et al., 2001) and *P. umberosa* (Shang et al., 2011). In 1992, Assaad with coworkers isolated durantoside II from *Phlomis floccosa* of Egyptian flora.

Several new iridoid structures have been isolated from *Phlomis* species such as, Phlomiol, which was identified from *Phlomis longifolia var.longifolia* (Ersöz et al., 2001).

In 2000, Kamel characterized Phlomurin, 3-epiphlomurin and Phlomiside from the aerial parts of *Phlomis aurea*. In addition, new iridoid diglycosides [gentiobioside] was isolated from *Phlomis aurea* (Aboutabl et al., 2002). In addition, three new iridoids have been identified as Phlorigidosides A, B and C from *Phlomis rigida* (Takeda et al., 2000).

2.2. 2. Monoterpene Glycosides:-

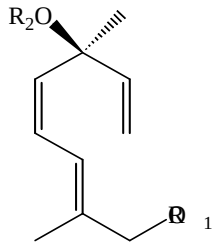


Figure 2.2.2. Structure of Monoterepene Glycosides.

Table 2. 2. 2. Monoterpene Glycosides obtained from some *Phlomis* species.

No	Compound	R_1R_2	Species	Reference
25	Betulalbuside A	β-D-Glu H	*P. armeniaca* *P. lunariifolia* *P.sieheana*	Saracoglu et al.,(1995) Çalış et al., (2004b) Ersöz et al., (2002)
26	Hydroxylinaloyl-3-*O*-β-D-glucopyranoside	H β-D-Glu	*P. armeniaca* *P. sieheana*	Saracoglu et al.,(1995) Ersöz et al., (2002)

β-D-Glu = β-D-glucose

2.2.3. Phenylethanoid Glycosides:-

Phenylethanoid glycosides (PhGs) are a group of water soluble natural products widely distributed in the plant kingdom, Structurally; they are characterized by cinnamic acid bacbone (e.g caffeic, ferulic,p-comaric acid) and phenylethyl alcohol moieties attached to a β-glucopyranose through ester and glycosidic linkages respectively. Rhamnose, xylose, apiose, etc. May also be attached to the glucose residue, which in most cases forms the core of the molecule. (Jimenez and Riguera., 1994).

Several phenylpropanoid glycosides were found to be active against bacteria and fungi some of them showed enzyme and hormone inhibitor activity, especially acteoside(verbascoside) and forsythoside B (Saracoglu et al., 1995).

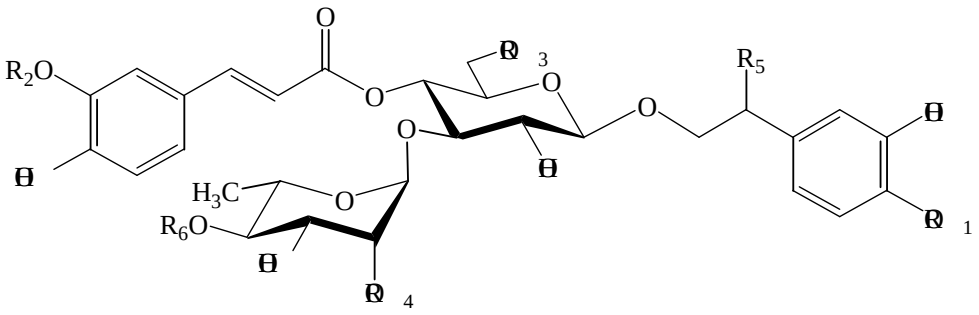


Figure 2. 2.3.(A). Structure of Phenylethanoid glycosides from some *Phlomis* species.

Table 2. 2. 3. A. Phenylethanoid glycosides obtained from some *Phlomis* species.

No	Compound	R_1 R_2 R_3 R_4 R_5 R_6	Species	References
27	β-hydroxy-acteoside	H H H H OH H	*P. sieheania* *P. syriaca*	Ersöz et al., 2002 Harput et al., 2006
28	Samioside	H H H H H Apio	*Phlomis angustissima* *P. samia* *P. syriaca*	Yalcin et al., 2005 Yalcin et al., 2003 Harput et al., 2006
29	Phlinoside A	H H H Glu H H	*P. linearis* *P. grandifolia var grandifloria*	Çalış et al., 1991 Takeda et al., 1999
30	Phlinoside B	H H H β-Xyl H H	*P. linearis* *P. armeniaca*	Calış et al., 1991 Saracoglu et al., 1995
31	Physocalycoside	CH_3 CH_3 glu Ram H H	*P. physoclayx*	Ersöz et al., 2003
32	Phlinoside F	CH_3 CH_3 HXylo H H	*Phlomis angustissimia*	Yalcin et al., 2005

Table 2. 2. 3. A. Phenylethanoid glycosides obtained from some *Phlomis* species. Continu..

No	Compound	R_1 R_2 R_3 R_4 R_5 R_6	Species	References
33	Verbascoside (acteosid)	H H H H H H	*P. armeniaca* *P. aurea* *P. longifolia var. longifolia* *P. monocephala* *P. physocalyx* *P. lunariifolia* *P. syriaca* *P.brunneoglata* *P. caucasica* *P. lanceolaate* *P. tuberosa* *P. fruticosa* *P. integrifolia* *P. sieheana* *P. viscosa*	Saracoglu et al., 1995 Kamel et al., 2000 Ersöz et al., 2001 Yalcin et al., 2003 Ersöz et al., 2003 Çalış et al., 2004b Harput et al., 2006 Kirmizibekmez et al., 2004a Delazar et al., 2008 Nazemiyeh et al., 2008 Çalış et al., 2005b Marin et al., 2007 Saracoglu et al., 2003 Ersöz et al., 2002 Çalış et al., 2005a
34	Phlinoside D	H CH_3 H α-Xyl H H	*P. linearis*	Çalış et al., 1991
35	Phlinoside E	H CH_3 H Ram H H	*P. linearis* *P. physocalyx*	Çalış et al., 1991 Yalcin et al., 2006

Table 2. 2. 3. A. Phenylethanoid glycosides obtained in some *Phlomis* species. continu…..

No	Compound	R_1 R_2 R_3 R_4 R_5 R_6	Species	References
36	Martynoside	CH_3 CH_3 H H H H	*P. physocalyx* *P. integrefolia* *P. armenica* *P. tuberosa* *P. samia* *P. sieheana* *P. viscosa*	Ersöz et al., 2003 Saracoglu et al., 2003 Saraocglu et al., 1995 Çalış et al., 2005b Yalcin et al., 2003 Ersöz et al., 2002 Çalış et al., 2005a
37	leucosceptosid A	H CH_3 H H H H	*P. armeniaca* *P. longifolia* var. *longifolia* *P. physocalyx* *P. tuberosa* *P. viscosa* *P. integrifolia* *P. sieheana* *P. oppostifloria* *P. physocalyx*	Saracoglu et al., 1995 Ersöz et al., 2001 Ersöz et al., 2003 Çalış et al., 2005b Çalış et al., 2005a Saracoglu et al., 2001 Ersöz et al., 2002 Çalış et al., 2005c Ersöz et al., 2003
38	Arenarioside	H cafeoyl β-xylose H H	*P. nissolii*	Kizmibekmez et al., 2004b

Table 2. 2. 3. A. Phenylethanoid glycosides obtained from some *Phlomis* species.

No	Compound	R_1 R_2 R_3 R_4 R_5 R_6	Species	References
39	Forsythoside B	H H Apio H H H	*P. sieheana* *P. armeniaca* *P. longifolia* *P. tuberosa* *P. spinidens* *P. physocalyx* *P. lunariifolia* *Phlomis bruneogaleata* *P. caucasica* *P. lanceolata* *P. fruticosa* *P. integrifolia* *Phlomis monocephala* *P. viscosa* *P. olivierii*	Takeda et al., 2001 Saracoglu et al.,1995 Ersöz et al., 2001 Çalış et al., 2005b Takeda et al.,2001 Ersöz et al., 2003 Çalış et al., 2004b Kirmizi-bekmez et al.,2004a Delazar et al., 2008 Nazemiyeh et al., 2008 Marine et al., 2007 Saracoglu et al., 2003 Yalcin et al., 2003 Çalış et al., 2005a Delnavazi et al., 2016
40	Integrifoliosides A	H CH_3 Apio H H H	*P. integrifolia*	Saracoglu et al., 2003
41	Integrifoliioside B	H CH_3 H Apio H H	*P. integrifolia* *P. brungeoleatea*	Saracoglu et al., 2003 Kirmizibekmez et al.,2004a

Table 2. 2. 3. A. Phenylethanoid glycosides obtained in some *Phlomis* species.

No	Compound	R_1 R_2 R_3 R_4 R_5 R_6	Species	References
42	Alyssonoside	H CH_3 Apio H H H	*P. pungens var pungens*	Ismailoglu et al., 2002
			P. integrifolia var. integrifolia	Saracoglu et al., 2003
			P. angustissima	Yalcin et al., 2005
			P. monocephala	Yalcin et al., 2003
			P. fruticosa	Marin et al., 2007
			P. viscosa	Çalış et al.,2005a
			P. syrica	Harput et al., 2006
43	Phlinoside C	H H H Ram H H	*P. linearis*	Çalış et al., 1990
			P. armeniaca	Saracoglu et al., 1995
			P. lanceolaata	Nazemiyeh et al., 2008
			P. olivierii	Delnavazi et al., 2016
			P. physocalyx	Yalcin et al., 2006
44	Lamio-phlomioside A	H ferul β-Api H H H	*P.nissoli*	Kirmizibekmez et al., 2004
45	Physocalycosid	CH3CH3 Glu Ram H H	*P. physocalyx*	Ersöz et al., 2003

Glu = glucose Apio = apiose Ram = rhamnose Xyl= xylose

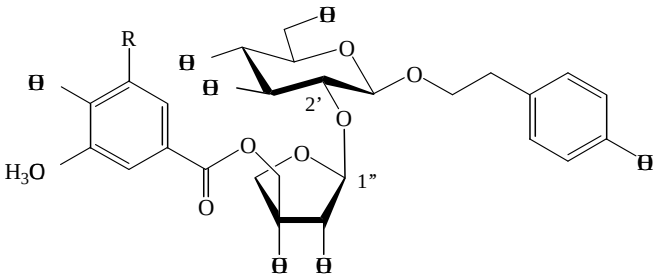


Figure 2.2.3.(B). Structure of a phenylethanoid glycosides.

Table 2. 2. 3. (B). Phenylethanoid glycoside from some *Phlomis* species

No	Compound	R	Species	References
46	Phlomisethanosid	H	*P.grandifolia var grandifolia*	Takeda et al., 1999
47	Hattushoside	OCH_3	*P.gandifolia var grandifolia* *P.armeniaca* *P.nissolii*	Takeda et al., 1999 Saracoglu et al., 1995 Kirmizibekmez et al., 2004b

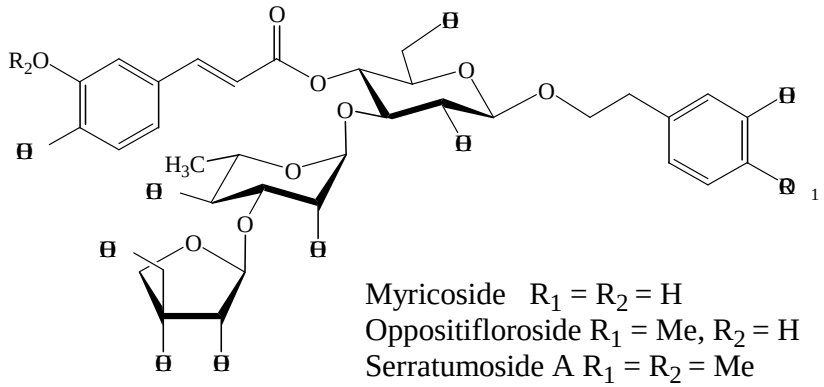


Figure 2.2.3.(C). Structure of Phenylethanoid glycosides from *P. oppositifloria*.

Furthermore, the genus *Phlomis* is rich in phenylethyl alcohol glycosides, which are a caffeic acid derivatives such as Verbascoside (acteoside), alyssenoside and forsythoside B

respectively. They have been reported from the famialy lamiaceae, also from other genera, including *Marrubium, Scutellaria, Lamium* (Delazar et al., 2008).

Several new phenylethanoid glycosides structures have been identified from *Phlomis* genus, five new structures were identified as (trigycosides) phlinosides A, B, C, D and E from *Phlomis linearis* (Çalış et al., 1991). In addition, phlinoside F was*reported* from *Phlomis angustissima* (Yalcin et al., 2005). Arenarioside and lamiphlomioside A have been first reported from *Phlomis nissolii* (Kirmizibekmez *et al*., 2004b). Moreover, From *Phlomis physocalyx* a rare tetraglycosides phenylethanoid (physocalycoside) was described by (Ersöz et al., 2003).

In *Phlomis longifolia var. longifolia*, another structure was elucidated and named as phlomisethanoside (Takeda et al., 1999). In *Phlomis oppositifoloria*, Myricoside and serratumoside A have been identified (Çalışet al., 2005c). Integrifoliiosides A and B were reported as new compounds from *Phlomis integrifolia* (Saracoglu et al., 2003).

2.2.4. Caffeic acid esters:-

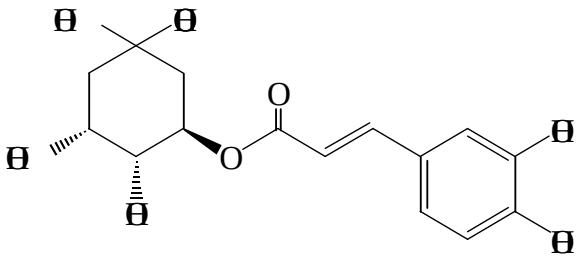


Figure 2. 2. 4.Structure of chlorogenic acid

Table 2. 2. 4. Caffeic acids from some *Phlomis* species.

No	Compound	Species	References
48	Chlorogenic acid	*Phlomis brunneogaleata* *P. longifolia* var.*longifolia* *P.olivierii*	Kirmizibekmez et al., 2004a Ersoz et al., 2001 Delnavazi et al., 2016

2.2.5. Benzyl alcohol glycosides:-

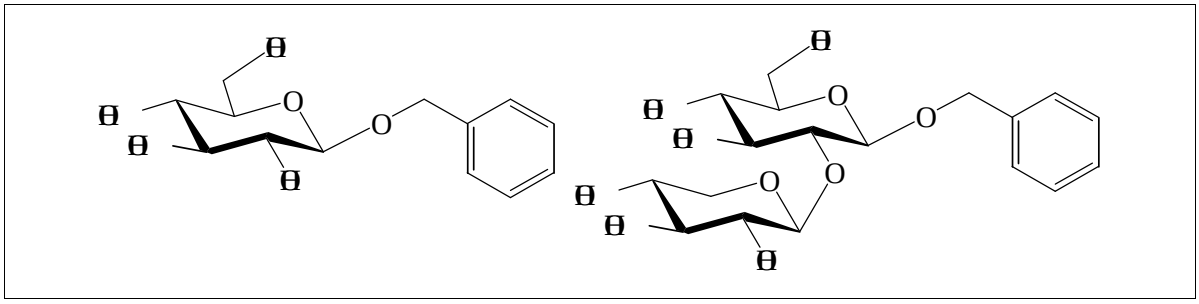


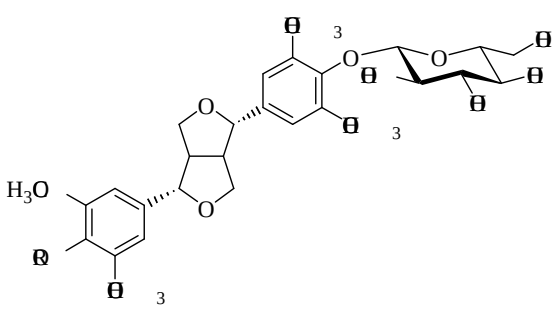
Figure 2.2.5. Structures of benzyl alcohol glycosides.

Table 2.2.5. Benzyl alcohol glycosides from some *Phlomis* species.

NO	Compound	Species	References
49	Benzyl alcohol-*O*-β-xylopyranosyl -(1→2)-β-glucopyranoside	*Phlomis aurea*	Kamel et al., 2000
50	Benzyl alcohol β-D-glucosides	*P.grandifolora var. grandifolora*	Takeda et al.,1999

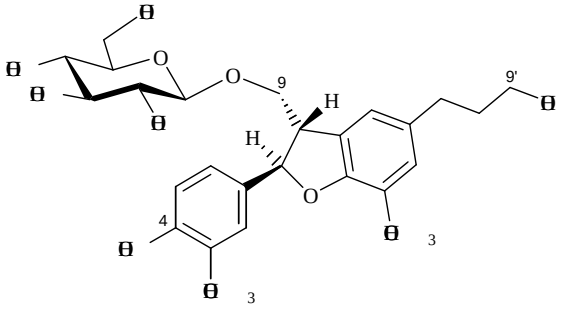
2.2.6. Lignans and Neolignans:-

Lignans are dimeric compounds formed essentially by the union of two molecules of a phenylpropene derivative, Neolignan are also derived from the same units as lignans but the C_6-C_3 moieties are linked "head to tail" or "head to head" and not through the β-β' carbons. They occur in the heart-woods of trees. Lignans and neolignans produced through a biosynthetic pathway starting from E-coniferyl alcohol, are widely distributed and structurally diverse phytochemical class (Evans, 2009).



[**51**] **Syringaresinol-4'-*O*- -D-glucopyranoside,** R = H

[**52**] **Liriodendrin,** R = β-D-glucopyranose



[**53**] **Dehydrodiconiferyl alcohol 9- *O*- -D-glucopyranoside**

Figure 2.2.6. Structure of some Lignans and Neolignans.

Table 2.2.6. Lignans and Neolignans obtained from some *Phlomis* species.

NO	Compound	Species	References
51	syringaresinol-4-*O*-β-D-Glucoside	*P. monocephala* *P. angustissima*	Yalcin etal., 2003 Yalcin et al., 2005
52	Liriodendrin	*P. aurea* *P. brunneogaleata* *P.capitata*	Kamel etal., 2000 Kirmizibekmez et al., 2004a Kimizibekmez et al., 2004b
53	Dihydrodehydrodiconiferyl – alcohol9-*O-β -D-glucopyranosid*	*P. lunariifolia* *P. tuberosa*	Çalış et al., 2004b Çalış et al., 2005b

2.2.7. Flavonoids:-

The flavonoids are a large class of water soluble polyphenolic compounds with a benzo-γ-pyrone structure (C6-C3-C6 Skelton) can occur both in Free State and as glycosides. They are structurally related compounds due to all these groups usually share a common chalcone precursor. They are classified according to the state of their oxygenation of the C_3 unit (Evans, 2009).

Flavonoids are the major phyto-constituents Isolated from the *Phlomis* genus it is more than forty flavonoids have been isolated from *Phlomis* species to date. These include apigenin, luteolin, naringenin, eriodictyol, chryseriol, kaempferol, and their glycosides (Hussain et al., 2010).

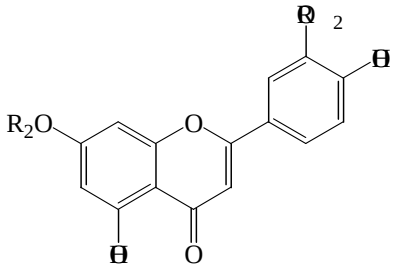


Figure 2.2. 7 (A). Structure of flavonoids from some *Phlomis species.*

Table 2. 2. 7. (A). Flavonoids obtained from some *Phlomis* species.

NO	Compound	R_1	R_2	Species	References
54	Apigenin	H	H	*P. lychnitis* *P. samia*	Tomas et al., 1986 Kyriakoput et al., 2001
55	Apigenin-7-glucoside	β-glu	H	*P. aurea* *P. floccosa* *P. lychnitis*	EL-Negomy et al., 1986 EL-Negomy et al., 1986 Tomas et al., 1986
56	Luteolin	H	OH	*P. lychnitis*	Tomas et al., 1986

Table 2. 2. 7. (A). Flavonoids obtained from some *Phlomis* species.

No	Compound	R_1 R_2	Species	References
57	Apigenin-7-rutinoside	Rut H	*P. aurea* *P. floccosa*	EL-Negomy et al., 1986 ELNegoumy et al., 1986
58	Apigenin-7-p-Coumaroyl-glucoside	p-comu-glu H	*P. aurea* *P. floccosa* *P. lychnitis*	EL-Negomy et al., 1986 ELNegomyet al., 1986 Tomas etal., 1986
59	Chrysoeriol-7-glucuronide	glu-A OCH3	*P. fruticosa*	Marin et al., 2007
60	Luteolin-7-p-coumaroyl-glucoside	p-com-glu OH	*P. aurea* *P. floccosa* *P. lychnitis* *P. fruticosa*	EL-Negoumy et al., 1986 EL-Negomy et al., 1986 Tomas etal.,1986 Marin et al.,2007
61	Luteolin-7-diglucoside	Di-glu OH	*P. aurea* *P. floccosa*	EL-Negoumy et al., 1986 EL-Negoumy et al., 1986
62	Luteolin-7-glucoside	glu OH	*P. aurea* *P. floccosa* *P. lychnitis* *P. fruticosa*	EL-negoumy et al., 1986 EL-negoumy et al., 1986 Tomas etal.,1986 Marin et al.,2007
63	Luteolin-7-glucuronide	glu-A OH	*P. fruticosa*	Marin et al., 2007

Table 2.2.7. (A). Flavonoids obtained from some *Phlomis* species.

No	Compound	R_1 R_2	Species	References
64	Chrysoeriol-7-O-glucoside	glu OCH_3	*P. aurea* *P. floccosa* *P. lychnitis* *P. fruticosa* *Phlomis caucasica*	El-negoumy et al., 1986 El-negoumy et al., (1986) Tomas et al., 1986 Marin et al., 2007 Delazar etal., 2008
65	Chrysoeriol-7-rutinoside	Rut OCH_3	*P.aurea* *P. floccosa* *Phlomis caucasica*	EL negoumy et al., 1986 EL-negoumy et al., 1986 Delazer et al., 2008
66	Chrysoeriol-7-p-Coumaroyl-glucoside	p-com-glu OCH_3	*P.aurea* *P. floccosa* *P. lychnitis* *P. fruticosa*	EL-negomy et al., 1986 EL-negoumy et al., 1986 Tomas et al., 1986 Marin et al., 2007
67	Chrysoeriol	H OCH_3	*P. lychnitis* *P.samia*	Tomas et al., 1986 Kyriakopoulos et al., 2001
68	Chrysoeriol-7-O-β glucopyranoside	glu-A OCH_3	*P. aurea* *P. integrifolia* *P. lunariifolia* *P.bruneogalea ata*	ELnegoumy et al., 1986 Saracoglu et al., 2003 Çalış et al., 2004b Kirmizibekmez et al., 2004a
69	Luteolin-7-rutinoside	RutOH	*P. aurea* *P. floccose*	EL-ngoumy et al., 1986 EL-negoumy et al., 1986

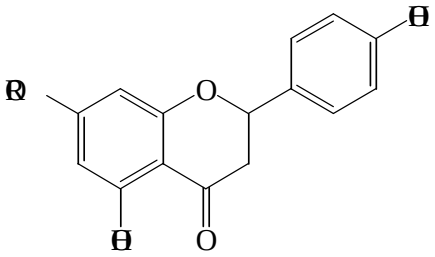


Figure 2.2.7.(B). Structure of flavonones.

Table 2.2.2.7. B. Flavonones obtained in some *Phlomis* species.

No	Compound	R	Species	References
70	Naringenin	H	*P. angustissima* *P. fruticosa* *P. caucasica*	Yalcin et al., 2005 Marin et al., 2007 Delazar et al., 2008
71	Naringenin-7-glucoside	Glu	*P.aurea*	EL- Ngoumy et al., 1986
73	Naringenin-7-p-coumaroylglucosid	p-com-glu	*P. aurea*	EL-Negoumy et al., 1986

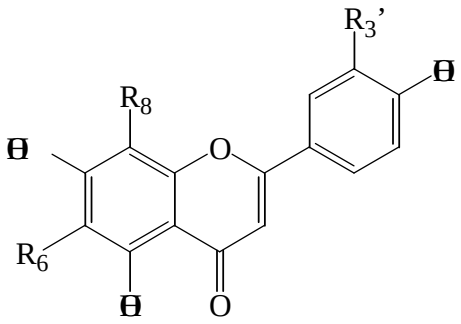


Figure 2.2.7(C). Structure of flavonoid -C- glucoside.

Table 2.2.7. C. Flavonoid -C-glycosides obtained from some *Phlomis* species.

No	Compound	R_3 R_6 R_8	Species	References
74	Lucenin-2	C-glu C-glu OH	*P. aurea*	EL-Negoumy et al., 1986
75	Luteolin-7-*O*-β-glucopyranoside	H C-glu OH	*P. aurea* *P. lunariifolia* *P.brunneo-galeatea*	EL -Negoumy et al., 1986 Çalış et al., 2004b Kirmizibekmez et al.,2004a
76	Vicenin-2	C-glu C-glu H	*P. aurea* *P. floccosa*	EL-Negoumy et al., 1986 EL-Negoumy et al., 1986

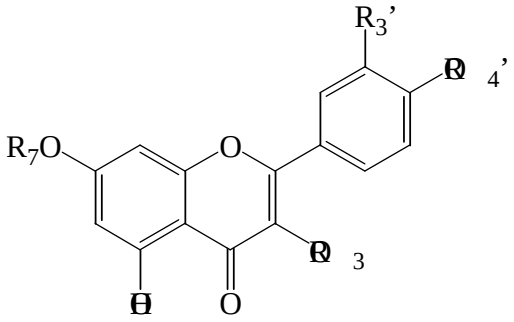


Figure 2.2.7(D). Structure of flavonols from some *Phlomis* species.

Table 2.2.7.D. Flavonols obtained from some *Phlomis* species.

No	Compound	R_3	R_7	R_3'	R_4'	Species	References
77	Astragalin	glu	H	H	H	*P. spinidens*	Takeda et al., 2001
78	Kaempferol-7,4di-Methylether)-3-glucosides	glu	CH_3	H	CH_3	*P. caucasica*	Delazar et al., 2008
79	Isoquercitrin	glu	H	OH	H	*P.spinidens*	Takeda et al., 2001
80	Phlomisflavosides A	glu	H	OH	Apio	*P. spinidens*	Takeda et al., 2001
81	Phlomisflavosides B	glu	H	OH	Apio	*P. spinidens*	Takeda et al., 2001
82	Kaempferol-3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside	p-com-gl	CH_3	H	CH_3	*P.aurea*	EL-Negomy et al., 1986

glu-A= glucuronoid acid Di-glu = diglucose p-com-glu = p-coumaroyl-glycoside

C-glu = C-glucosidic linkage Rut = rutinosyl Apio = Apiose Glu = glucose

Flavonoids have been isolated from *Phlomis* genus include flavonols and flavone, including apigenin, luteolin, chryseeriol and their 3 -O and/or 7-O- glycosides or 7-O-p coumaryl; either as monoglycosides or diglycosides, appears as a major phytoconstituents have been identified from most *Phlomis* species (Hussain et al., 2010).

Flavone-C-glycosides such as (vicenin and lucenin) and flavonone e.g. naringenin have been reported in some *Phlomis* species (Marin et al., 2007). Some flavonoids from this genus such as phlomisflavoside A and B have been identified for the first time in this genus from *Phlomis spinidens* (Takeda et al., 2001).

2.2.8. Other secondary metabolites:-

In the *Phlomis* genus, many other secondary metabolites such as Triterepenes, nortriterpenes, steroids, acetophenone glycosides, a megastigmine glycosides, essential oils, hydroquinone glycosides (phlomuroside), shikimic acid derivatives, aliphatic alcohol glycosides, caffeic-acide-derivatives and alkaloids (Ersöz et al., 2001; Kimizibekem et al., 2004 a,b; Harput et al., 2006; Çalış et al., 2005b and Ersöz et al., 2002).

2.3. Pharmacological activity:-

The selective extraction of bioactive molecules from natural sources such as endemics species, with appropriate techniques, can provide products with high biological activity that could be used as alternative of synthetic molecule in aims to reduce pollution and more healthy and economic levels (Soltani-Nasab et al., 2014).

2.3.1. Antioxidant and anti-radical activity:-

Plants are potential sources of natural antioxidants and radical scavenging substances because they contain phenolic compounds such as phenolic acids, flavonoids, tannins, and phenolic diterepenes (Zhang et al., 2009). They play a protective role in cardiovascular and neurological disorders, as well as certain types of cancer and ageing. (Lŏpez et al., 2010).

In many studies, the higher antioxidant activity of phenolic compounds was correlated with their chemical structures (a number and location) of free phenolic hydroxyl group and degrees of polymerization (Çalış et al., 2005a). Flavonoids contain conjugated ring

structures and hydroxyl groups that have the potential to function as antioxidants in vitro or cell free systems by scavenging superoxide anion, singlet oxygen, lipid peroxy radicals, and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species (Wafa et al., 2016; Delzar et al.,2008).

2. 3.1.1. Reduction of DPPH radical:-

Methanolic solutions (0.1%) of the phenylethanoid glycosides (verbascoside, forsythoside B and physocalycoside) were chromatographed on a Silica gel TLC plate using CHCl3: MeOH: H2O (61:32:7) mixture as solvent system. After drying, TLC plates were sprayed with a 0.2% DPPH (2, 2-diphenyl-1-picrylhydrazy) radical solution in MeOH. Compounds showing yellow-on-purple spot were regarded as antioxidant (Ersöz et al., 2003).

2.3.1.2. DPPH assay in vitro:-

One milliliter of the extracts at different concentrations was added to 0.5 mL of a DPPH-methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The antiradical dose required to cause a 50 % inhibition. IC50 was determined as the amount of the sample (µg) reducing the absorbance by 50%. A lower IC50 value corresponds to a higher antioxidant activity. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [(A0 – A1)/ A0] ×100

Where A0 is the absorbance of the control at 30 min and A1 is the absorbance of the sample at 30 min. βHT (β-hydroxy toluene, a-tocopherolal and ascorbic acid were used as standards and samples were analyzed in triplicate. This ability to scavenge free radicals dependent on the dose (Wafa et al., 2016).

The flavonoid compounds were isolated from *Phlomis bovei De Noe and P.caucasica* have been observed to possess a free radical scavenger activity in virto due to free radical chain breaking, metal chelating and singlet oxygen quenching with the inhibition of enzymatic activity to serve as potent antioxidants (Wafa et al., 2016 and Delzar et al., 2008).

The scavenging activity against 1,1- diphenyl-2-picrylhydrazyl (DPPH), 2,2-Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) (ABTS), super- oxide anion and nitric oxide radicals, have been determined in different fractions of *Phlomis pungens var. pungens and Phlomis*

nisolii L according to reported procedures. Water extracts that are rich in phenolic components of the both species exhibited the highest radical scavenging activity indicating 1.14 and 1.17mg/mL, IC_{50} values, respectively. Tocoferol (IC_{50}: 0.15 mg/mL) was used as reference (Sarikurku et al., 2014).

The phenolic compounds found in P. *umberosa, P. megalantha* and *P.olieverii* were showed a high scavenging activity to DPPH, superoxide free radicals, and inhibiting linoleic acid oxidation. Therefore, They could be considered as potential natural antioxidant sources for medicinal and food applications (Zhang et al., 2009 and Delnavazi et al., 2016).

The phenyletrhanoid glycosides such as (verbascoside, forsythoside B, martynoside, alyssenoside, leucosceptoside A also physocalycoside) have been isolated from some *Phlomis* species such as *P. carica, P. monocephlala , P. viscosa, P. syrica, P. physocalyx* also samioside isolated from*P. saima* were found to be potent dose-dependent scavengers to 2,2diphenyl-1-picrylhydrazyl (DPPH) radical where comparable to α-tocopherol as a reference. While their irridoid glycosides not shown any antioxidant activity as expected. (Yalcin et al., 2003; Çalış et al., 2005a and Ersöz et al., 2003; Harput et al., 2006).

In many *in vitro* studies, phenolic compounds, especially verbascoside, forsythoside B demonstrated higher antioxidant activities than vitamins and synthetic antioxidants. Their antioxidant effectiveness is mainly attributed to the different structural conformation, as well as the number and location of phenolic hydroxyl group of the compounds (Zhang et al., 2009).

2.3. 2. Antimicrobial activity:

Plants promising sources of natural antimicrobial agents. As reported, that the antimicrobial activity of plants is related with the defense mechanism against microorganisms (Wafa et al., 2016).

Agar disc diffusion method: - a suspension of the tested microorganism *(Escherichia coli*, *Salmonella typhimurium*and, *Staphylococcus aureus)* (0.1 ml 108 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of different concentration of the extracts and placed on the inoculated plates. These

plates were incubated at 37C° for 24 h. Gentamicin (10µg/disc) was used as a standards and dimethylsulfoxide DMSO as a control.

The antibacterial activity was determined by measuring of inhibition zone diameters (mm):-

<9 mm, inactive;

9–12 mm, less active;

13–18 mm, active;

>18 mm, very active.

The antifungal activity was tested by disc diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (*Aspergillumsinger*, *Aspergillus flavus, Candida albicans*), 8 days old by point inoculation. One hundred microliter of suspension was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 µl of each sample at different concentrations. Nystatin 100µg, clotrimazon 50 µg and amphotericin 100 µg were used as a standards and dimethylsulfoxide DMSO as a control. Inhibition zones were determined after incubation at 27 C° for 48h. Flavonoid compounds of *Phlomis bove* have been results a very weak antibacterial activity but a very strong antifungal activity against to tested microorganisms. This activity related to *their* capacity to form complexes with extracellular and soluble proteins and with the cell wall or by inhibition of germination or reduce sporulation of fungal pathogen (Wafa et al., 2016)

The phenylethanoid glycosides have been isolated from *Phlomis syriaca* and*Phlomis lanceolaate* exhibited a considerable antibacterial and antifungal activities against several microorganisms especially, Gram positive bacteria strains (*Staphylococcus aureus* and *Entercoccus faecÇalış*) also fungals suchas (*Candida albicans* and *C. krusei*) while they were inactive against Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) (Harput et al., 2006 and Nazemiyeh et al., 2008).

The methanol extracts of the aerial parts of *P. olivieri* exhibited concentration-dependent antibacterial activity agains *Stahpylococcus aureus, Streptococcus sanguis, Escherichia coli, Pseudomonas aeruginosa, Klebsielal pneumoniae*, while this extract did no showed antifungal activity against the tested fungi (Delnavazi et al., 2016).

Among glycosides, forsythoside B and verbascoside have shown considerable antibacterial activity against of multi-drug resistant of *Staphylococcus aureus* with minimum inhibitory concentration ranging from (MIC = 64 ug/L to 256 ug/L) when compared to positive control norfloxacine (Nazemiyeh et al., 2008).

2.3.3. Anticancer activity in vitro:

Patients with cancer worldwide is about 10 million, especially in developing countries, it is considered as a second leading cause of death after heart disease, the using of complementary and alternative medicine mainly by medicinal plants with less adverse effects and more efficacies is about 30-75% of drugs used to treat a cancer in this days (Soltani- Nasab et al., 2014).

2.3.3.1. MTT assay:-

The cells were seeded in 96-well plate at 1x104 cells/well and incubated for 24 hours. Thencells were washed and exposed to different concentration of the total extracts and fractions and incubated for 72 hours, under 5% CO_2 at 37ºC. The initial concentration of samples was 1000 µg/mL and serial dilution was made in culture medium to yield six different concentrations of samples.

The final concentration of DMSO (dimethysulfoxide) was less than 1% in all treatments. At the end of 72 hours incubation of treated cells, the medium in each well was replaced with MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 3-diphenyltetrazodium bromide) and plates were incubated for 4 hours. After this period, the medium was discharged and DMSO was added to dissolve formazan crystals produced by viable cells. Plates were gently shaken for 20 min and the absorbance was measured by a micro plate reader at 570 nm. IC_{50} was calculated as the concentration of samples, which inhibited 50% of cell viability (Sarkhail et al., 2017).

The all total extracts of six *Phlomis* species including P. *caucasica*, *P. anisodontea*, *P. bruguieri*, *P. oliveri*, *P. persica and P. kurdica*, exhibited a high cytotoxic activity ((IC_{50}< 1000 µg/mL) against MCF7 (breast adenocarcinoma), A549 (lung carcinoma), MDBK (bovine kidney cells) while did not showed cytotoxic activity against HepG2 (hepatocellular carcinoma), HT29 (colon carcinoma). (Sarkhail et al, 2017).

The highest cytotoxicity against A549 and MCF7 cancer cell lines, respectively, were found for ethyl acetate fractions of *P. caucasica, P. anisodontea*and *P. kurdica*. These results in presence of flavonoids and phenolic glycosides in this fraction. As well as, the cytotoxic activity of dichlormethan fraction of *P. anisodontea*and *P. kurdica* due to the toxic terepenoides and lipophilic compounds which exists in large amount in this fraction (Sarkhail et al., 2017)?

Phenylethanoid glycosides and phenylpropanoids especially forsythoside B and acteoside isolated from *Phlomis armeniaca* and *Phlomis viscose* showed cytotoxic activity against several kinds of cancer cells when investigated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl 2H-tetrazolium bromide (MTT) method. However, they did not affect the growth and viability of primary cultured cell line, while nonof theiridoid glycosides exhibited this activity (Çalış et al., 2005a; Saragolu et al., 1995).

These phenylethanoid glycosides showed both cytotoxic and cytostatic activities. These activities were found to mainly depend on the number of free hydroxyl phenyl moieties in their structures, type and location of different sugars within the molecule are also important for these activities (Saracoglu et al., 1995).

The total extract and different fractions of Phlomis *lanceolata* has cytotoxic activity against three cancer cell lines (colon carcinoma (HT 29), breast ductal carcinoma (T47D), Caco2) and normal cell NIH3T3, while the petroleum ether fraction had a highest cytotoxic activity due to the presence of triterpene and lipophilic compounds in this fraction. This activity was investigated by MTT assay where the cytotoxicity was expressed as the concentration of extract inhibiting the cell growth by 50% (IC_{50}) and (Methotrexate) as a positive control (Soltani-Nasab et al., 2014).

Flavonoid glycosides and phenylpropanoids which have been isolated from *Phlomis kurdica*, displayed a certain degree of hLDH5-inhibitory activities and these results may be useful for the perspective development of new anticancer agents starting from these natural products. An enzyme 5 (hLDH5) is up-regulated in human tumor tissues by catalyzing a crucial step in glycolysis, the reduction of pyruvate to lactate. Therefore, the inhibition of hLDH5 may be considered as a promising strategy in cancer treatment, since this inhibition activity cause a starvation of cancerous cells by reducing their conversion of glucose to lactate. (Bader et al., 2015).

Verbascoside, isoverbascoside, forsythoside B and 3-O-caffeoylquinic acid methyl ester isolated from *Phlomis bruneogaleate* showed cytotoxic activity against rat skeletal myoblasts L6 (Kirmizibekmez et al., 2004a).

2.3.4. Anti-diabetic activity:-

Several *Phlomis* species are recognized for their antidiabetic properties, i.e., *Phlomis aurea*, *Phlomis* floccosa. This activity may be due to their ability to protect liver and pancreas integrity by reducing the oxidative stress in diabetes or by stimulating the production of enzymes implicated in glucose metabolism (Kamel et al., 2000).

Alpha-glycosidase inhibitors have been used to lower blood glucose for about 20 years now, they regulating carob-hydrate metabolism and glucose release from food to suppress postprandial hyperglycemia. Recently, the investigations of herbal medicines have become increasingly important in the search for new, effective, and safe α-glycosidase inhibitors for diabetes treatment. The ethyl acetate fraction of *Phlomis tuberosa* extracts have shown significant inhibitory activity against α-glycosidase (with IC_{50} values in the range 0.067–1.203 mM) than the positive control, Acarbose (IC_{50} = 3.72 ± 0.113 mM) where investigated by a combined method using Sepbox chromatography and thin-layer chromatography (TLC) bio autography has been established to detect α-glycosidase inhibitors in plant extracts. This activity was related to phenolic compounds in this fraction such as phenyethanoides, flavonoids and especially diterepene glycosides (Yang et al., 2015).

Sarkhail et al. in 2007, investigated the effect of methanol extract of the aerial parts of *Phlomis ansiodonta* (PAME) which was rich in terepenoids, iridoid glycosides, flavonoids and phenolic compounds on streptozotocin (STZ)-induced diabetes in rats. Treatment of diabetic rats with oral administration of (PAME) at doses of 400mg/kg for 10 days showed a significant reduction in blood glucose, an increase in plasma insulin levels that lead to beneficial control of diabetes. This anti-hyperglycemic effect was the result of the ability of PAME to improve plasma ferric reducing antioxidant power, reduce liver lipid peroxidation, and combat oxidative stress through the activation of hepatic antioxidant enzymes.

2.3.5. Anti-ulcerogenic Activity:-

Herbal teas (decoction, infusion) prepared with *Phlomis* species in different countries are commonly used as digestive aid and to treat gastric ulcers and aches. Two different studies have confirmed the gastro protective activity of *Phlomis grandiflora* and *Phlomis crinitae* subsp. *mauritanica* aqueous extract (Limem-Ben Amor et al., 2009b).

Aqueous extracts of *P. grandiflora,* were shown to possess a high protective effect (100% inhibition) against ethanol-induced ulcerogenesis in rats, which stimulates leukotrienes, the 5-lipoxygenase pathway, mast cell secretion, and the breakdown of reactive oxygen species resulting in damage to the gastric mucosa. Stomachs in four out of six rats treated with the methanol extract of *P. grandiflora* were completely protected from any visible damage. Gastric protection provided by these plant extract was better than the reference drug misoprostol (400 ug/kg) (Gurbuz et al., 2003)

Limem-Ben Amor et al. (2009b) demonstrated that the aqueous extract (300mg kg−1) of *Phlomis crinitae* subsp. *mauritanica,* reduced ulcerogenesis induced with alcohol 50◦, in mice. It reduces ulcerogenesis by 91% in comparison to cimetidine (the positive control), which itself inhibits ulcerogenesis by 71%.that confirms the presence of gastro protective activity of this genus.

2.3.6. Anti-nociceptive Activity and Anti- inflammatory Activity:-

The inflammation is the normal result of host protective responses to tissue injury caused by numerous stimuli (e.g., physical trauma, chemicals and infectious agents to be due to overproduction of some mediators such as tumor necrosis factor alpha (TNF-a), interleukin-6 (IL-6), nitric oxide (NO), serotonin , histamine and prostaglandin E2 (PGE2) can be induced by cyclooxygenase-2 (COX-2) enzyme, commonly associated with pain as a secondary process, resulting from the secretion of analgesic mediators (Wang et al., 2014).

Some phenylethanoid and iridoid glycosides isolated from*Phlomis*species showed analgesic properties and anti-inflammatory activity due to inhibited the action of cyclooxygenase-2(COX-2) enzyme and/or decrease the production of prostaglandins (Ismailogu et al., 2002).

The total extract of *Phlomis oliverii and Phlomis ansiodonata* at a dose of 150 mg/kg and *Phlomis persica* at a dose of 100mg/kg reduced significantly the number of acetic acid induced visceral writhes in mice, revealing anti nociceptive properties comparable to indomethacin used as reference, that confirmed the analgesic properties of three *Phlomis* species and proved their pain reliving activity (Sarkhail et al., 2003).

The aqueous extract of *Phlomis umberosa* (25 and 50 mg/kg) was rich in irridoid glycosides and phenyethanoid glycosides were tested by acetic acid induced test and xylene induced ear swelling test, showed it has a significant anti-nociceptive and anti-inflammatory activities due to inhibit of (COX2) and inhibit the release of histamine, serotonin and cytokines. This activity was attributed to iridoid glycosides as the main components of this fraction (Shang et al., 2011). In addition, an irridoid glycoside in the methanol extract of Phlomis *younghusbandii* [MEAP] with dose dependent effect could decrease the number of writhings of mice induced by acetic acid and inhibit the inflammatory production induced by some agent. Therefore, can be considered as a potent peripheral analgesic with anti-inflammatory properties. The mechanisms of anti-inflammatory and analgesic effects of MEAP may be related to the decreased expression levels of TNF-a, IL-6, iNOS and COX-2 (Wang et al., 2014).

2.3.7. Anti-parasitic activity:

Several compounds purified from *Phlomis brunneogaleata have* beenshown anti-parasite activities against *Leishmania donovani*, *Plasmodium falciparum* and *Trypanosom abruceirhodesiens.* This activity was due to the inhibition ability of the purified compounds especially, luteolin-7-O-β-D-glycopyranoside to eonyl-ACP reductase (FabI) enzyme, which is crucial enzyme in the fatty acid biosynthesis of *Plasmodium falciparum* . hence, considerable significant as one of the a first potent anti-malarial natural product targatingFabI enzyme. (Kirmizibekmez et al., 2004a).

The chloroform extract soluble portion of P. *kurdica* and*P. leucophracta* exhibited potent anti-plasmodium and anti-leishmanial activity. Preliminary studies indicate that some polyphenolic compounds are responsible for FabI-inhibition effect of these extracts (Tasdemir et al., 2005).

2.3.8. Protection effect:-

The methanolic extract of *P.lychnitis* mainly phenolic contents from this species have shown a protective effect against a hydrogen peroxide induced toxicity, that lead to prevent some cardiovascular and neurological disorders, as well as, a certain types of cancer. This protective role is depended on doeses-dependent antioxidant properties of the phenolic compounds (Lopez et al., 2010). In addition, the protection causing phenylethylaclcohol glycosides have been purified from *P. pungens var.pungens*, throught prevented the inhibition of acetylcholine response induced by electrolysis lead to relaxation of aortic ring isolated from rats as a mechanism that, may be related to their free radical scavenging property. Electrolysis of a physiological solution has been shown to generate free radicals such as superoxide radical, hydrogen peroxide and hydroxyl radical that confirmed the traditional uses of some *Phlomis* species to protect the vascular system and as neuroprotective (Ismailoglu et al., 2002)

3. Experimental part

3.1. Plant Material

Phlomis floccosa L. was collected during flowering stage from El-Marj of Libya in 30 March 2016. A voucher specimen (NEUN 6884) has been deposited in the NEUN Herbarium of the Near East University, Cyprus.

3.2. Material and methods

3.2.1. Chemical Solid Materials

1% Vanillin (A) and 5% H_2SO_4 (B) were used as reagent for TLC studies.In addition, Silica gel 60 (Normal Phase and Reversed Phase -C18) powder (0.063-0.20 mm, mesh, Merck).

3.2.2. Solvents

Ethanol, t-butanol, distilled water, methanol, chloroform, dicloromethane,Ethylacetate. (Merck, Fluka Analytical, Sigma-Aldrich)

3.2.3.Chromatographic Methods

3.2.3.1. Thin Layer Chromatography;

Dichloromethane-Methanol-Water (DCM-MeOH-H_2O; 80:20:2, 70:30:3 and 61:32:7), Ethylacetate-Methanol-Water (EtOAc-MeOH-H_2O; 100:16,5:13,5) were used as a solvent system.

Normal Phase; Silica gel (Kieselgel 60 F_{254}Aluminium 20×20 cm, Merck 5554) was used as an adsorbent.However, for Reversed phase Silica gel 60F_{254} (25% Methanol in water) was used as a solvent system. 1% vanillin/H_2SO_4 was used as a reagent**.** TLC was used for control the procedures of this study**.**

3.2.3.2. Vacuum Liquid Chromatography

Water: Methanol 100:0 → 0:100 were used as a solvent system. Stepwise elution was carried out for fractionation.

LiChroprep RP-C18 (25-40µm), Merck about 100g was used as a stationary phase adsorbent, column dimension 4x12cm.

3.2.3.3. Silica gel Column Chromatography (liquid chromatography):-

Dichloromethane-Methanol-Water mixtures (90:10:1, 80:20:1, 80:20:2, 70:30:3, 60:40:5) were used as a solvent system.

Silica gel powder (Kieselgel 60, 0.063-0.2 mm, Merck 7734) was used as an adsorbent, Column dimension: 2x35cm.

3.2.3.4. Gel Chromatography

Sephadex (Lipophilic Sephadex Lit-20100 Bed size 25-100µm, Sigma-Aldrich) was used as a gel.

Methanol: Water (1:1) or MeOH were used as a solvent system.

3.2.3. 5. Medium pressure liquid chromatography

Adsorbent: Lichroprop, RP-C18 (Merck).

Solvent system: water: methanol (0%-30% methanol)

MPLC: Buchi (tow pumps C.605 and pump manger C.615)

Fraction collector: Buchi

Flow rate: 10m/min.

3.2.4. Instruments

Lyophilizator: CHRIST Alpha 1-4 LD Plus

NMR: Bruker (^{1}H-NMR; 400 MHz ; ^{13}C-NMR: 100 MHz)

UV Lamp: Camag

Rotary Evaporator: Büchi R-210 and Heidolph 4001

Vaccum : Rockk vacuum

Electrical Grinder : Retsch SK 100

Weight : Mettler Toledo PB 1502-S/FACT

Plate Heater : Camag TLC Plate Heater III

Separatory funnel 100ml

3.3. Plant Extraction:-

Completely air dried material (herba) was powdered with a electric grinder (Retsch SK 100) and stored in well-closed cellophane bags at room temperature.

Maceration procedure was used for extraction of the air-dried and powdered aerial parts of the plant material. 300g of the powdered aerial parts of *Phlomis floccosa* was macerated with about 3500ml of %80 ethyl alcohol for 72 hours by shaking at room temperature. After maceration, the extract was filtered with vacuum by using Buchner funnel.Filtrate was concentrated using rotary evaporator under reduced pressure at 50 °C to yield 120 ml crude extract (water soluble extract, WSE).During the concentration procedure, precipitated lipophilic compounds (chlorophil etc.) were removed by filtration.100 ml of water soluble part of the concentrated extract was fractionated by VLC (*see* 3.4.1).

3.4. Fractionation and Isolation Studies

3.4.1. Fractionation by Vacuum Liquid Chromatography

Concentrated extract in water (100 ml) was subjected to a RP-VLC (100 g LiChroprep RP-18) then fractionated by stepwise gradient elution with water-methanol mixtures with increasing amount of methanol in water by 5%. (Water: Methanol 100:0 → 0:100).Eighteen fractions were collected. Monitoring of the fractions was made by TLC (Kieselgel 60 F_{254}Aluminium 20×20 cm, Merck 5554). The mixtures of DCM-MeOH-H_2O (80:20:2, 70:30:3 or 61:32:7) was used as solvent system for development. For the revelation 1% Vanillin and 5% H_2SO_4 were used successively as a reagent. Fractions were combined into 13 fractions (FRS A – M)

3.4.2. Isolation of Lamiide [PF-1]:-

Fr.C (225mg) was subjected to Normal phase Silica gel column chromatography, eluted with Dichloromethane: methanol: water (80:20:1, 80:20:2, 70:30:3) to yield 50 fractions. Thesub fractions 14-15 are combined according to TLC profiles. Fr $_{C14-C15}$ was a pure compound (1), [PF1= 9mg].

3. 4. 3. Isolation of Ioplamide, Auroside and flavones glycoside:-

Fr. F (240mg) was subjected to Sephadex LH-20 gel chromatography using 100ml of Methanol 100%, to yield 20 fractions. Where the F $_{f13-f17}$ was Apure compounds (2) [PF_2 =32mg]. Also F $_{f6-f10}$ (183mg) was fractionated by dry applying to Normal phase Silica gel column chromatography, eluting with Dichloromethane: methanol: water (90:10:1, 85:15:1, 80:2, 75:25:2.5) to yield 40 fractions .F $_{f26-f27}$ [PF_5 =6mg] and F $_{f35-f38}$ [PF_6 =5mg] as pure compounds (3), (4).

3.4.4. Isolation of forsythoside B:-

Fr. H (677mg) was applied to Sephadex LH-20(3x37cm) using a mixture of methanol: water (1:1) total about 600ml, to yield 50 fractions, where F $_{H28-H32}$ [PF_3 =264mg] as pure compound (5).

3. 4. 5. Isolation of verbascoside:-

Fr. I (691mg) was fractionated on Sephadex LH-20, eluting with methanol: water (1:1) to yield 45 fractions. F $_{I24-I28}$ [PF_4 = 225mg) was a pure compound (6).

3.4.6. Isolation of verbasoside and alyssenoside:-

Fr. J (1000mg) was wet applied to Sephadex LH-20 using methanol: water (1:1) as a solvent system to yield 60 fractions. F $_{J18-J26}$ [PF_7 =208mg] was pure compound (7), and F $_{J35-J40}$ [PF_8 =92mg] was a pure compound (8).

3.4.7. Isolation ofluteolin7-O-β-D-glucuronoid:-

Fr. D (13030mg) was subjected to C18 medium pressure column chromatography eluting with water: methanol mixture (0%-30% of methanol) for 30 minute, gave 75 fractions. F $_{D45-D52}$ [PF_9 =828mg] and $F_{F-G68-F-G74}$ [PF_{10} =32mg] was a pure compounds (9) (10).

The structure elucidation of isolated compounds were carried out using H^1 ,C^{13} NMR [D1-D2].

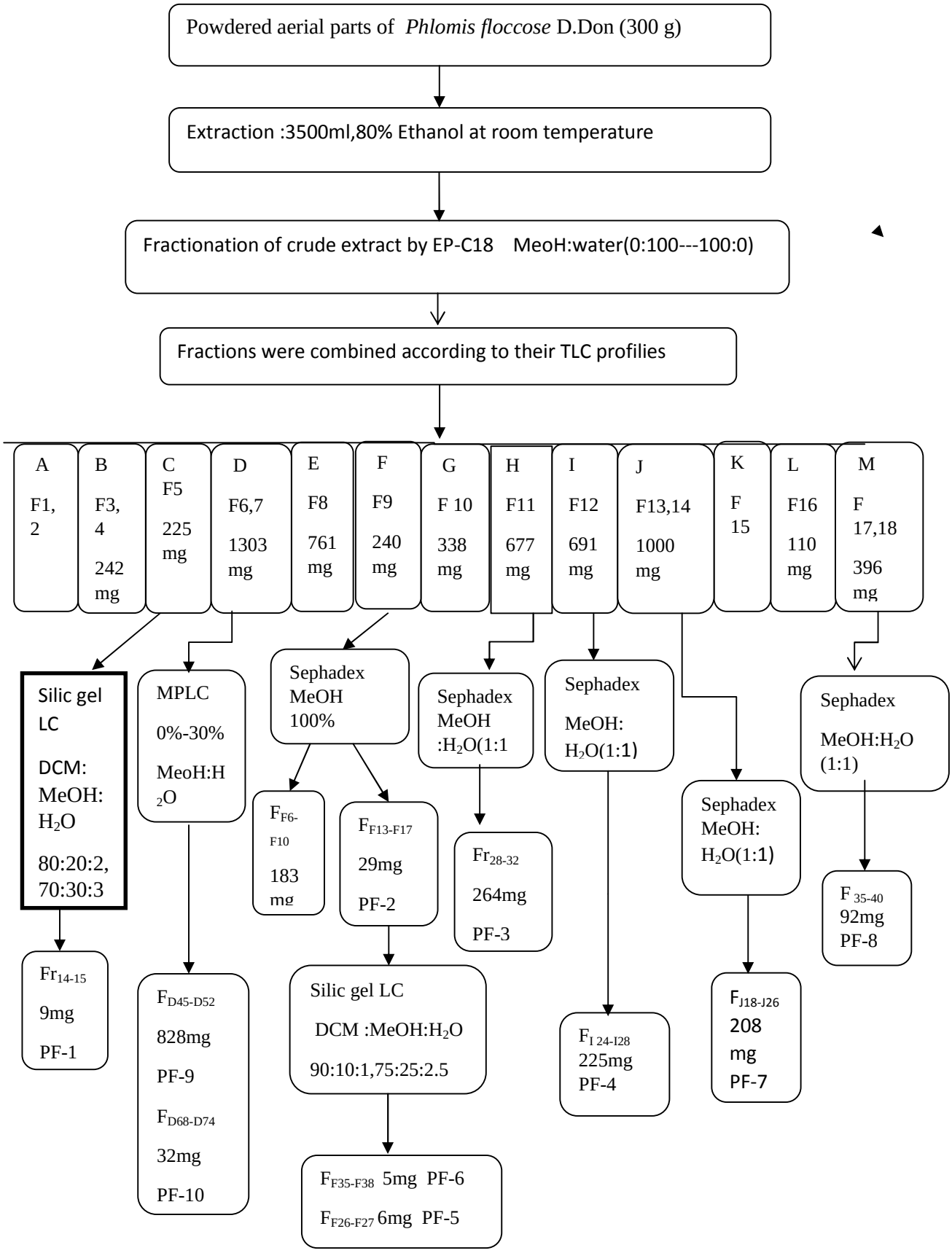


Figure 3.4.1. Extraction and fractionation of crude extract

4. RESULTS and DISCUSION

Chromatographical studies have resulted in the isolation of ten compounds **(PF-1 – PF-10).** Co-chromatography andNMR studies confirmed that three compounds,**PF-1&9, PF-4& PF8 and PF-2&10** were isolated twice from the successive fractions. Thus, the number of isolated compounds are seven and they can be classified into the three groups according to their UV and NMR spectra (*see* 4.1 – 4.3):

4.1. **Iridoids (PF-1&9, PF-5, PF-6)**

4.2. **Phenylethanoid Glycosides (PF-3, PF-4&8, PF-7)**

4.3. **Flavon Glycoside (PF-2 & 10)**

Their chromatographical behaviour and Rf values were given below,

Table 4.1.1. The colours and Rf values of the isolated compounds**(PF-1 – PF-10).**

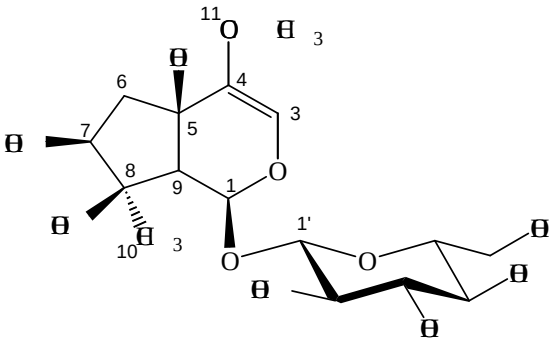
Groups	Compounds	Colours*	Solvent systems a&b	
			Rf_a	Rf_b
Iridoids	Lamiide **(PF-1&PF-9)**	Dark blue	0.48*	
	Ipolamiide **(PF-5)**	Pink	0.29**	
	Auroside **(PF-6)**	Pink	0.24**	
Phenyl ethanoides	Verbascoside (= Acteoside) (**PF-4& PF8**)	Cherry red turns to Brown in a few seconds	0.53*	0.50
	Forsythoside B (**PF-3**)		0.38*	0.39
	Alyssonoside (**PF-7**)		0.24*	0.44
Flavons	Luteolin 7-*O*-glucuronide (**PF-2&PF-10**)	Yellow		0.16

*) 1% Vanillin and 5% H_2SO_4, heating at 110°C, 3 – 5 min.
Solvent Systems for TLC: a&b

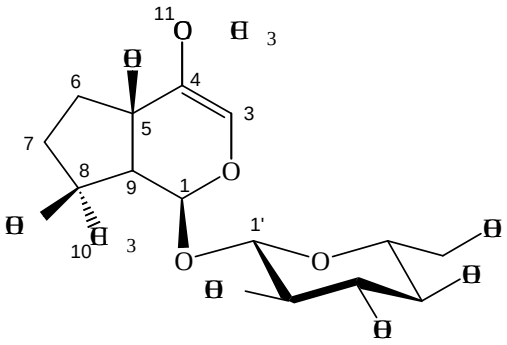
a) DCM – MeOH – H_2O (70:30:3)* (80:20:2)**
b) EtOAc – MeOH – H_2O (100:16.5:13.5)

4.1. Iridoids

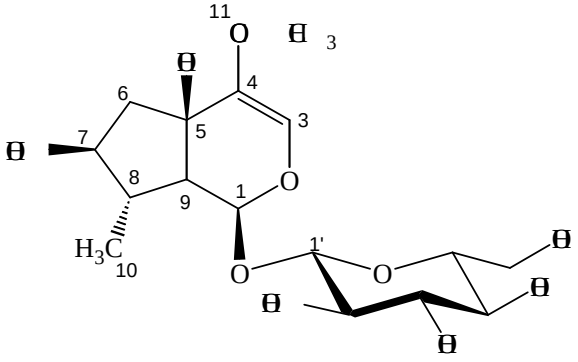
4.1.1. Lamiide (PF-1 and PF-9)

	UV $^{a}_{max}$ 230 nm $[\alpha]_D^{20}$ = -127 (c 0.1, MeOH)

4.1.2 Ipolamiide (PF-5)

	UV $^{a}_{max}$ 230 $[\alpha]_D^{20}$ = -136 (c 0.1, MeOH)

4.1.3. Auroside (**PF-6**)

	UV $^{a}_{max}$ $[\alpha]_D^{20}$ = (c 0.1, MeOH)

Compounds **PF-1, PF-5** and **PF-6** were obtained as colourless amorphous powder. Their UV spectra showed maxima at 224, 230, 230 nm, respectively, which were typical for C-4 substituted iridoids. This assumption have also been confirmed by the H-3 signal observed between 7.44 – 7.50 ppm.

4.1.1. Lamiide (PF-1 and PF-9)

The ^{1}H-NMR (Table 4.1.1, Spectrum 4.1.1.1) compound **PF-1**displayed signals due to a tertiary methyl (δ 1.09 s, Me-10) group. Additional methyl resonance was consistent for presence of a carbomethoxy group (δ 3.74 s). Two protons arising from a methylene (δ 2.36 and 2.04, both dd, H_2-6) group were observed as an AB part of an ABX system. The proton resonance observed at 3.54 ppm was assigned as the X part of this ABX system (H-7). These signals were observed in the same spin system (*see* Spectra 4.1.1.4A&B). The signals observed at δ 2.79 s, 5.82 s, and 7.44 s were atributed to a methine(H-9), an acetal(H-1) and an olefinic proton (H-3), respectively. An anomeric proton observed at δ 4.60 (H-1') was in the same spin system with the protons at δ 3.20 (H'-2'), 3.40 (H'-3'), 3.29 (H'-4'), 3. 34 (H-5'), 3.87 (H-6'a) and 3.68 (H-6'-b) (*see* Spectrum 4.1.1.4B). Corresponding carbon resonances with these protons were found to be at δ 99.6, 74.3, 77.4, 71.6, 78.3 and 62.7, respectively. Thus, the proton and carbon resonances indicated the presence of a β-glucopyranosyl.

The ^{13}C-NMR spectrum (Table 4.1.1, Spectrum 4.1.1.2) exhibited 17 carbon signals, two CH_3, two CH_2, nine CH and four quaternary C (*see* DEPT-135, Spectrum 4.1.1.3). Six of 17 were ascribed to a β-glucopyranosyl unit as mentioned above. The remaining 11 resonances were characteristic for an iridoid bearing a carbomethoxy ($COOCH_3$) group at C-4 position. All of the ^{1}H and ^{13}C chemical shifts were determined on the basis of 2D-NMR experiments (Table 4.1.1; COSY, HSQC and HMBC, Spectra 4.1.1.4 – 4.1.1.6). HMBC spectrum (Spectra 4.1.1.6A&B) showed the ^{1}H, ^{13}C-long range correlations from C-4 (δ 115.4) to an olefinic proton at δ 7.44, two methylene protons at δ 2.36 and 2.04 and a methine proton at δ 5.82 which were assigned as H-3, H_2-6, H-9, respectively. In the COSY spectrum (Spectrum 4.1.1.5), no other correlations were observed for H_2-6 and H-7 suggesting that both C-5 and C-8 were totaly substituted. The chemical shift value assigned to C-5 (δ 69.2) and the ^{1}H, ^{13}C-long range correlations from C-5 to H-1, H-3, H_2-6, H-7, H-9 signals indicated the presence of an hydroxyl function located at this position. Furthermore, the cross-peaks between H-1 and the anomeric carbon atom (C-1') of glucose unit and vice versa, revealed that the β-glucopyranosyl is attached to C-1(OH). These NMR data were in good accordance to those of lamiide (Yalçın et al., 2005). The Rf value and the NMR data of **PF-9** were same as those of PF-1.

On the basis of these spectroscopic data, the structures of **PF-1** and **PF-9** were determined as lamiide.

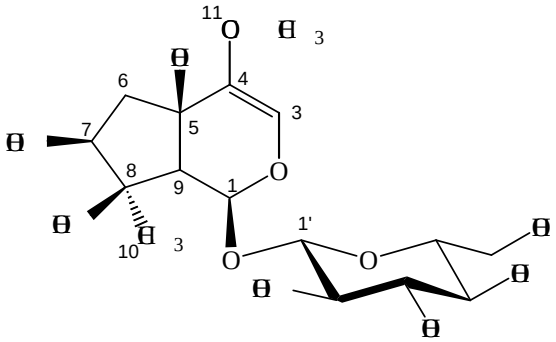
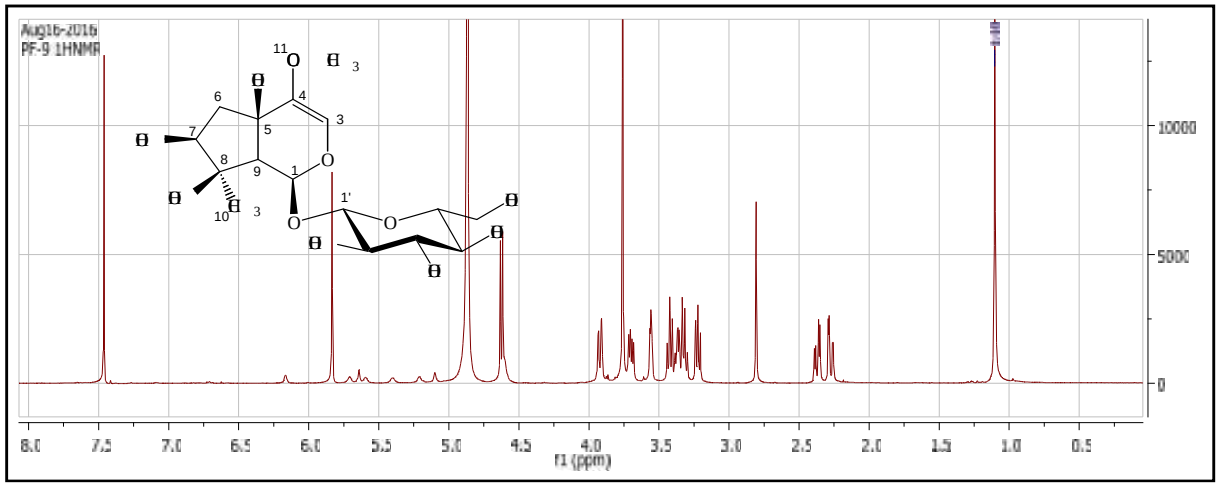


Table 4.1.1. H NMR and C NMR Data of lamiide (PF-1& 9)

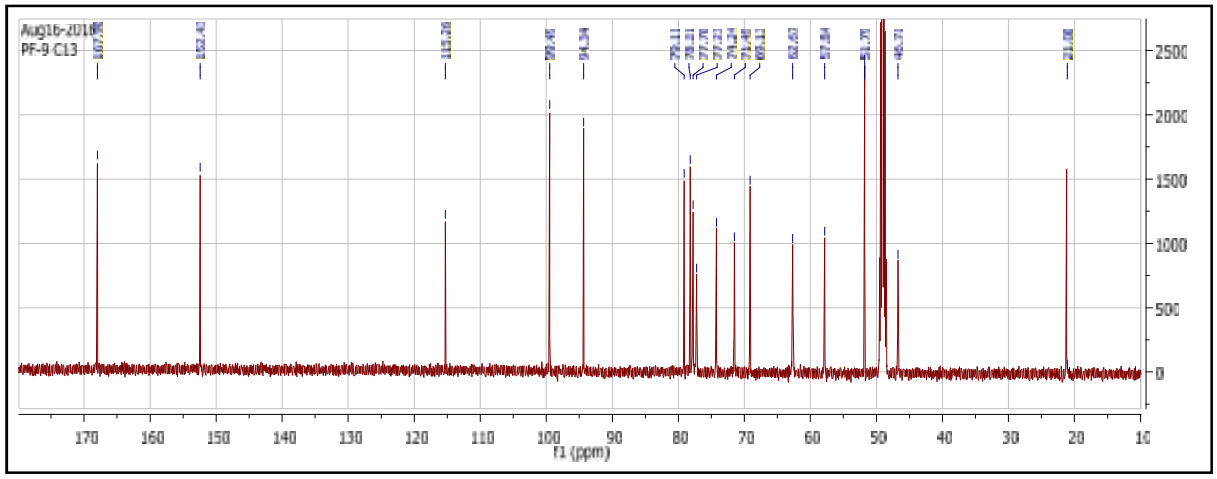
H:400MHz, C: 100MHz, CD30D

C No	**DEPT**	**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
Aglycone 1	CH	94.4	5.82 s	H-1', H-3, H-9
3	CH	152.4	7.44 s	H-1
4	C	115.4	-	H-3, H_2-6, H-9
5	C	69.2	-	H-1, H-3, H_2-6, H-7, H-9
6	CH_2	45.7	2.36 dd (15.4 / 6.4) 2.04 dd (15.4 / 2.9)	
7	CH	77.8	3.54 dd (5.0 / 2.9)	
8	C	79.1	2.27 m	H-1, H_2-6, H-9, H_3-10
9	CH	58.0	2.79 s	H-7, H_2-6, H_3-10
10	C	21.3	1.09 s	H-7, H-9
11	C	168.0	-	H-3, COOC$\underline{H}_3$
CO$\underline{C}H_3$	CH_3	51.7	3.74 s	-
Glucose 1′	CH	99.6	4.60 d (8.2)	H-1
2′	CH	74.3	3.20 dd (8.0 /9.0)	
3′	CH	77.4	3.40 dd "t" (9.0)	
4′	CH	71.6	3.29 dd "t" (9.0)	
5′	CH	78.3	3.34 m	
6′	CH_2	62.7	3.87 dd (12.0 /2.0) 3.68 dd (12.0 / 5.7)	H-4'

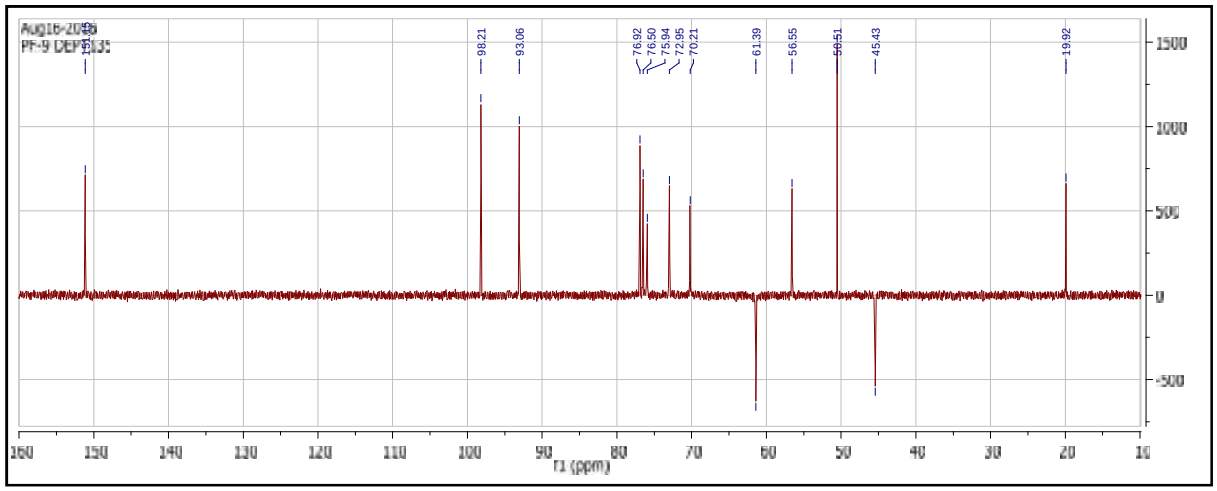
*) All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).



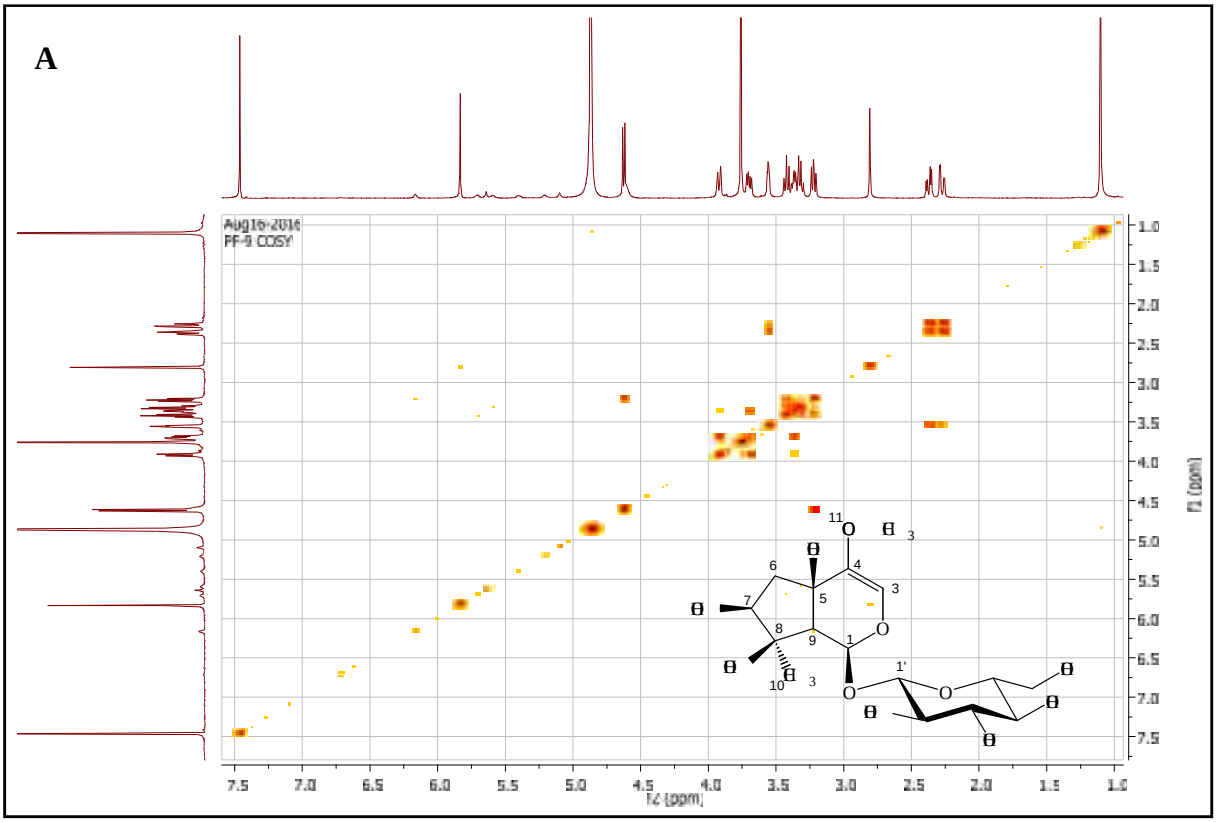
Spectrum4.1.1.1. The ^{1}H-NMR Spectrum of **Lamiide** (**PF-1&9**) (400 MHz, MeOD)

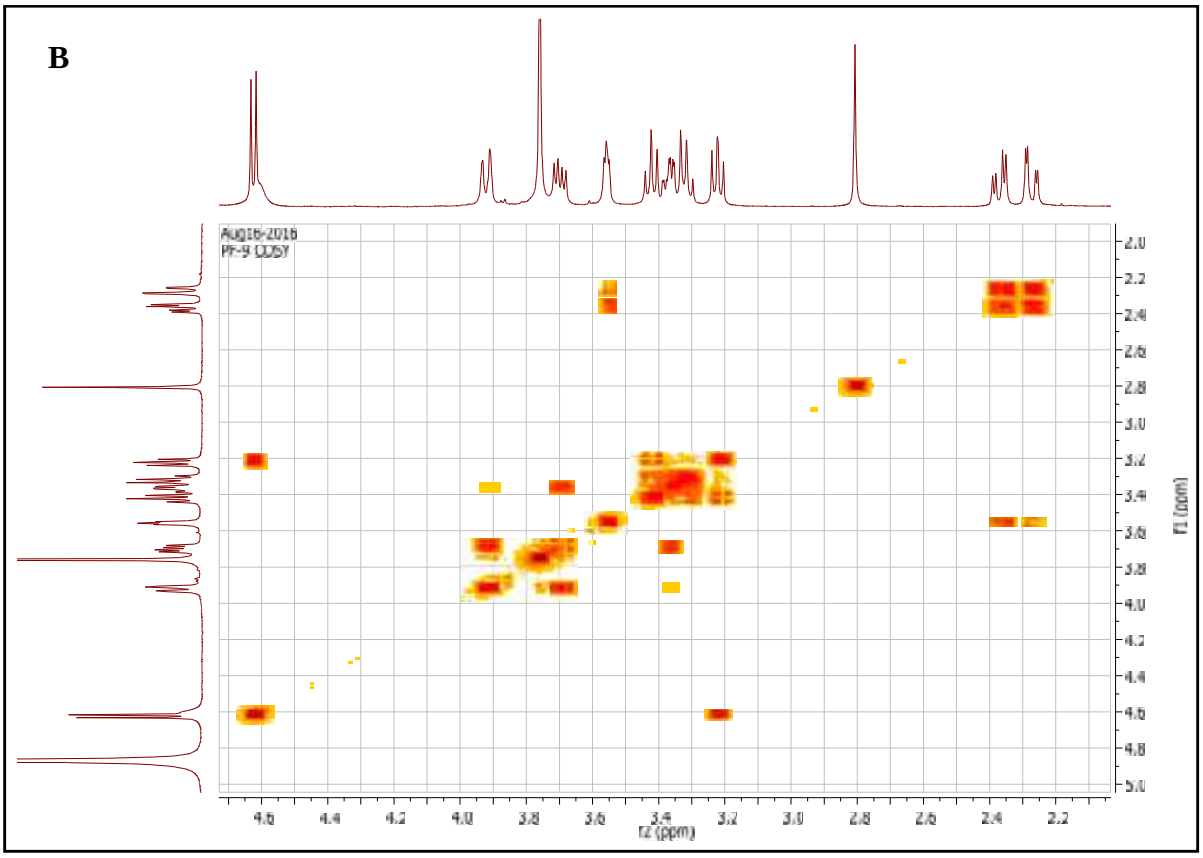


Spectrum4.1.1.2. The ^{1}C-NMR Spectrum of **Lamiide** (**PF-1&9**) (100 MHz, MeOD)

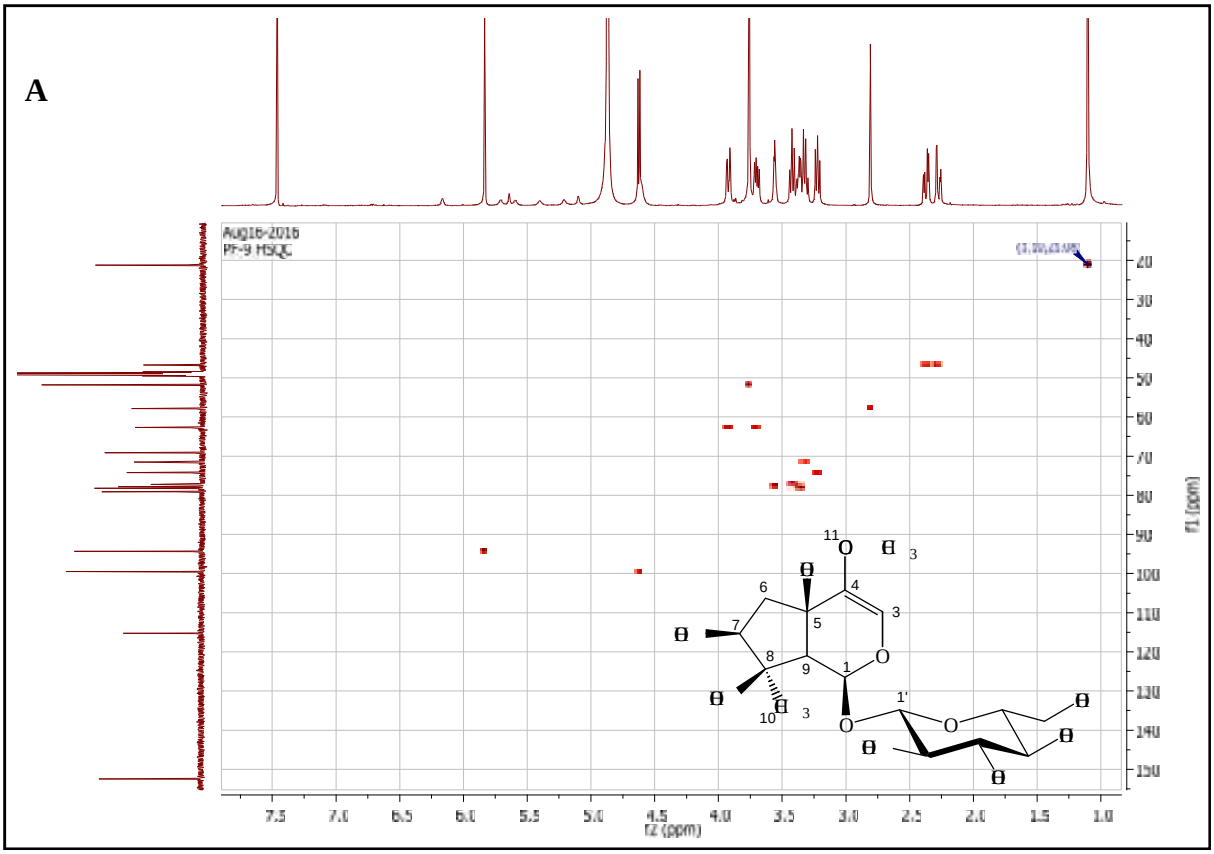


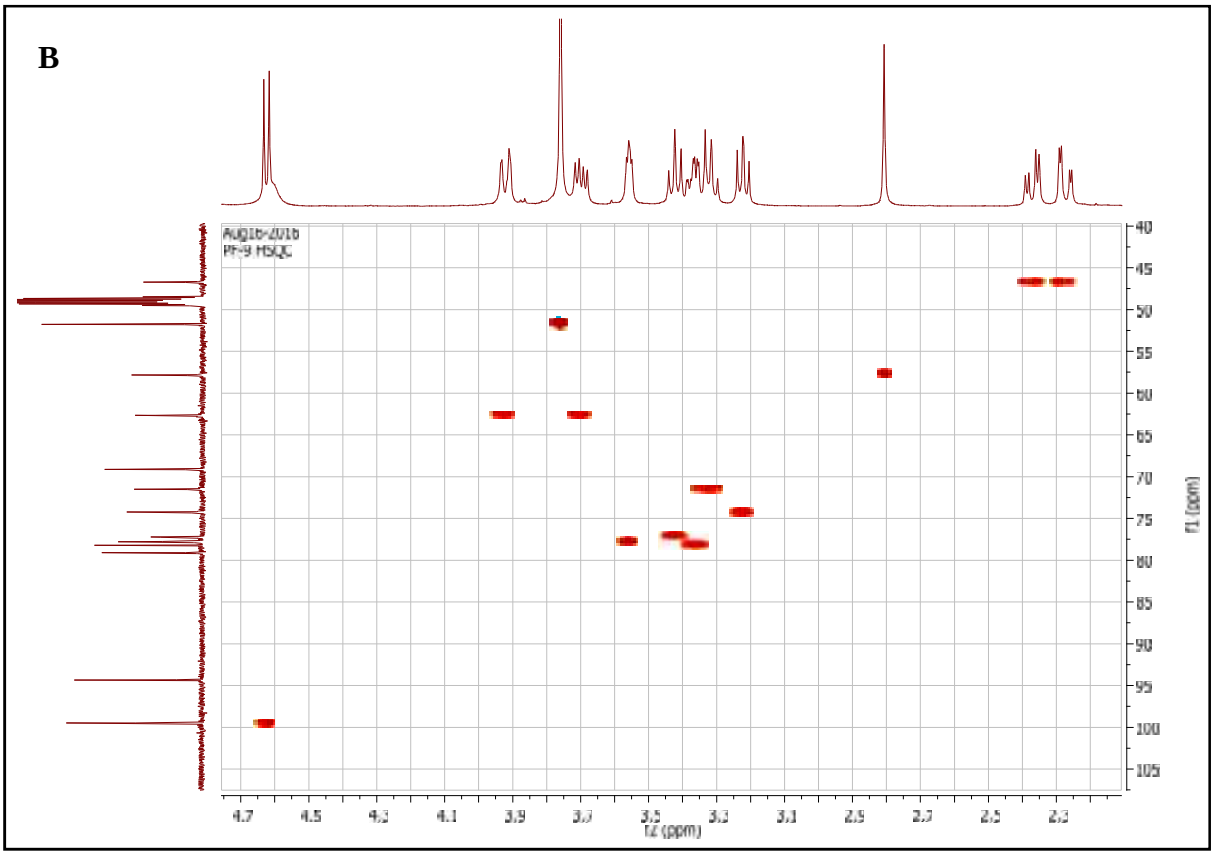
Spectrum4.1.1.3. The DEPT-135 Spectrum of **Lamiide** (**PF-1&9**)



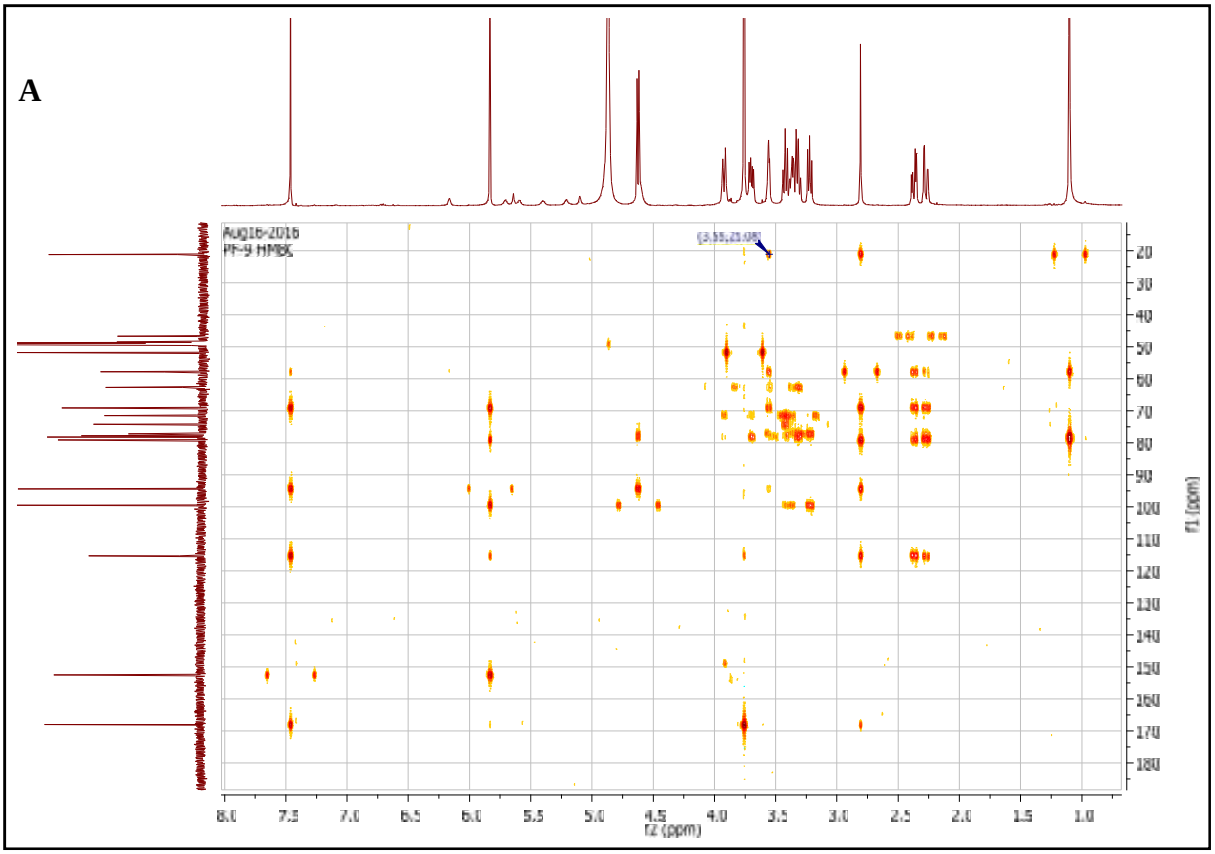


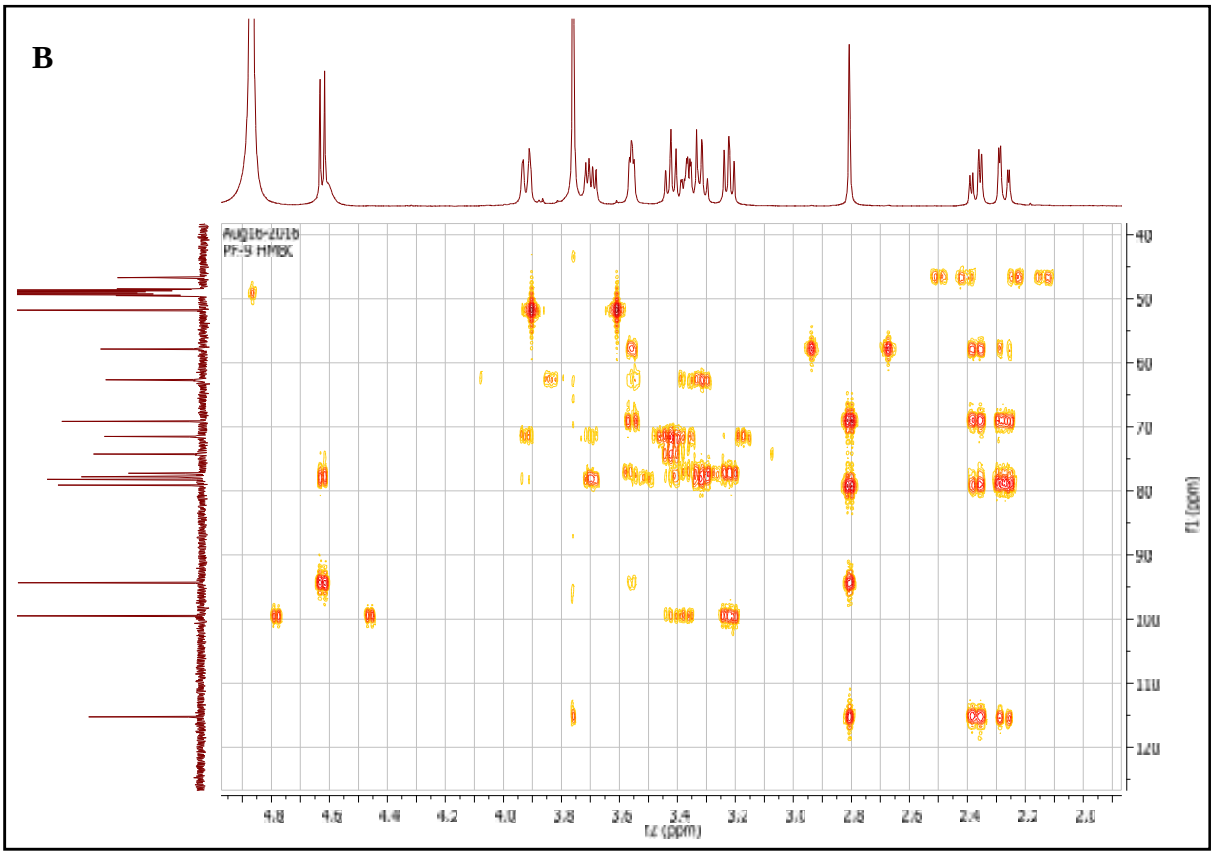
Spectrum4.1.1.4A&B. The COSY Spectra of **Lamiide** (**PF-1&9)**





Spectrum4.1.1.5A&B. The HSQC Spectra of **Lamiide** (**PF-1&9)**





Spectrum4.1.1.6.A&B. The HMBC Spectra of **Lamiide** (**PF-1&9)**

4.1.2. Ipolamiide (PF-5)

The ^{1}H-NMR (Table 4.1.2, Spectrum 4.1.2.1) compound **PF-5**exhibited signals due to a tertiary methyl (δ 1.15 s, Me-10) group, a three proton singlet methyl signal for a carbomethoxy group (δ 3.73 s), four protons arising from two methylene (δ 2.26 and 2.08, both ddd; (δ 1.94 and 1.57, both ddd; H_2-6 and H_2-7, resp.) groups. Two methylene protons were observed in the same spin system as an AA'BB' (see COSY; spectrum 4.1.2.4). The signals observed at δ 2.49 s, 5.81 s, and 7.50 s were similar to those of lamiide and atributed to a methine(H-9), an acetal(H-1) and an olefinic proton (H-3), respectively. The anomeric proton resonance at δ 4.59 (d, J = 8.2 Hz, H-1') indicated the presence of a β-glucopyranosyl in **PF-5**. Additional protons and the corresponding carbon resonances were almost similar to those of lamiide confirming the presence of a β-glucopyranose unit.

The ^{13}C-NMR spectrum (Table 4.1.2, Spectrum 4.1.2.2) exhibited 17 carbon signals, two CH_3, three CH_2, eight CH and four quaternary C (*see* DEPT-135, Spectrum 4.1.2.3). Six of 17 were ascribed to a β-glucopyranosyl. The remaining 11 resonances were characteristic for an iridoid bearing a carbomethoxy ($COOCH_3$) group at C-4 position. All of the ^{1}H and ^{13}C chemical shifts were determined on the basis of 2D-NMR experiments (Table 4.1.2; COSY, HSQC and HMBC, Spectra 4.1.2.4 – 4.1.2.6). HMBC spectrum (Spectrum 4.1.1.6) showed the ^{1}H, ^{13}C-long range correlations from C-4 (δ 115.2) to an olefinic proton at δ 7.50, one of the two pairs of methylene protons at δ 2.26 and 2.08 and a methine proton at δ 5.81 which were assigned as H-3, H_2-6, H-9, respectively. The chemical shift value assigned to C-5 (δ 71.7) and the ^{1}H, ^{13}C-long range correlations from this carbon (C-5) to H-1, H-3, H_2-6, H_2-7, H-9 signals indicated the presence of an hydroxyl function located at this position. Thus, the proton and carbon signals of compound **PF-5** were similar to those of **PF-1** (lamiide) except for the signals belonging to cylopentane ring. The resonances attributed to the C-7 (δ 40.4) and the corresponding protons at δ 1.94 and 1.57 revealed the presence of a methylene functionality instead of hydroxymethine. Furthermore, HMBC spectrum (Spectrum 4.1.2.6) displayed the cross-peaks between H-1 and the anomeric carbon atom (C-1') of glucose unit and vice versa, showing that the β-glucopyranosyl is attached to C-1(OH). These NMR data were in good accordance to those of ipolamiide (Damptof et al., 1984). On the basis of these spectroscopic data, the structures of **PF-5** was determined as ipolamiide.

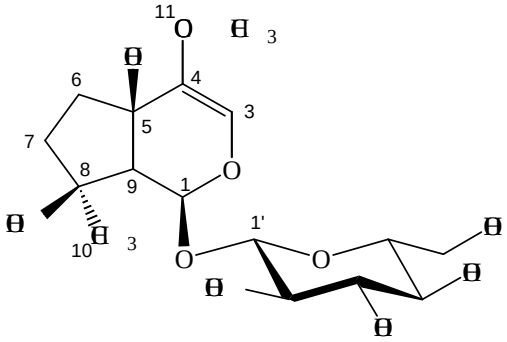
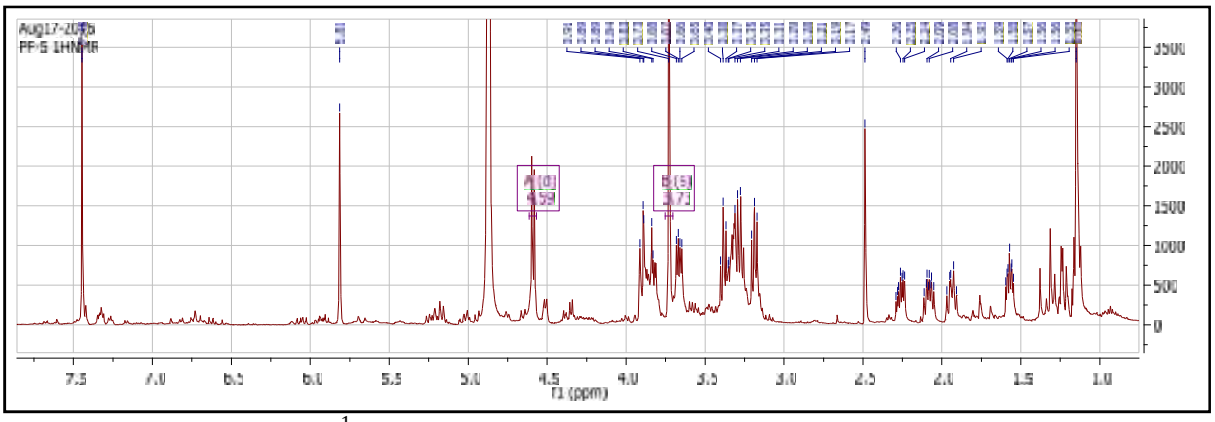


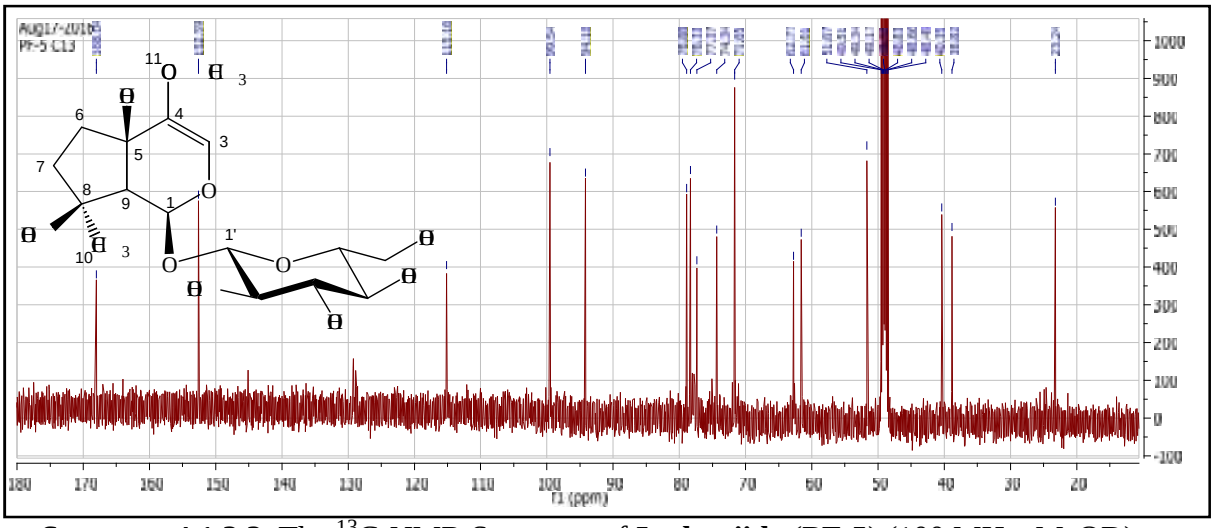
Table 4.1.2.1. **^{1}H-NMR and ^{13}C-NMR Data of Ipolamiide (PF-5)***
^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

C No	**DEPT**	**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
Aglycone				
1	CH	94.2	5.81 s	H-1', H-3, H-9
3	CH	152.6	7.50 s	H-1
4	C	115.2	-	H-3, H_2-6, H-9
5	C	71.7	-	H-1, H-3, H_2-6, H_2-7, H-9
6	CH_2	38.8	2.26 ddd (14.2 / 8.3/4.7) 2.08 ddd (14.2 / 9.8/7.6)	
7	CH_2	40.4	1.94 ddd (12.3/ 9.8/8.3) 1.57 ddd (12.3/4.7/7.6)	H_3-10
8	C	78.9	-	H-1
9	CH	61.6	2.49 s	H_3-10, H_2-6, H_2-7
10	CH_3	23.2	1.15 s	H-9, H_2-7
11	C	168.1	-	H-3, COOC$\underline{H}_3$
CO$\underline{C}H_3$	CH_3	51.7	3.73 s	-
Glucose				
1′	CH	99.5	4.59 d (8.2)	H-1
2′	CH	74.3	3.19 dd (8.2 /9.0)	
3′	CH	77.4	3.39 dd "t" (9.0)	
4′	CH	71.7	3.28 dd "t" (9.0)	
5′	CH	78.3	3.32 m	
6′	CH_2	62.8	3.90 dd (11.9 /1.7) 3.67 dd (11.0 / 6.0)	H-4'

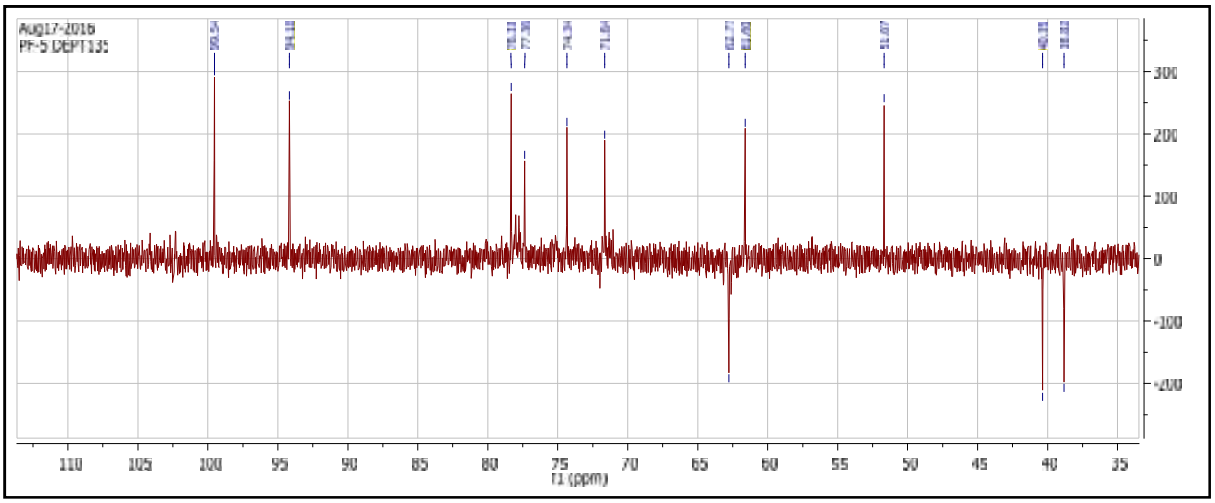
*) All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).



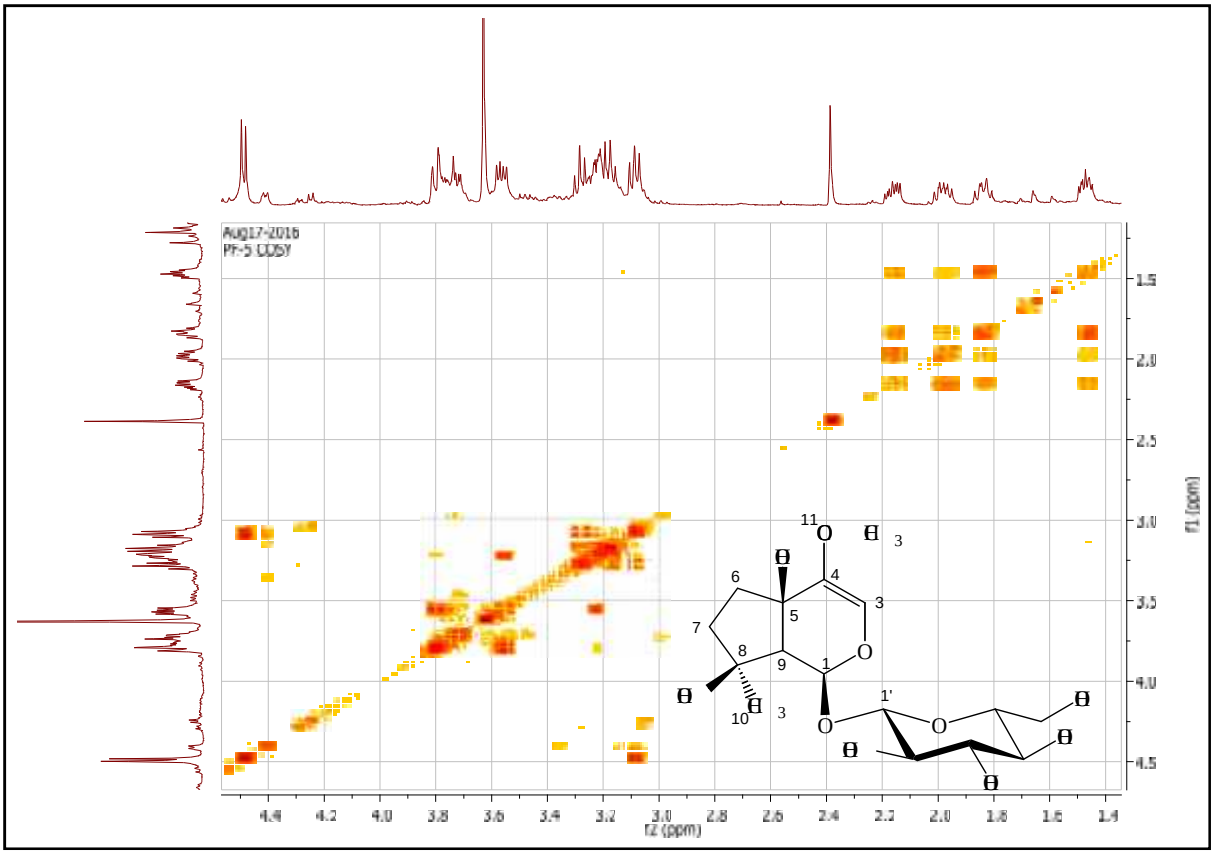
Spectrum4.1.2.1. The ^{1}H-NMR Spectrum of **Ipolamiide** (**PF-5**) (400 MHz, MeOD)



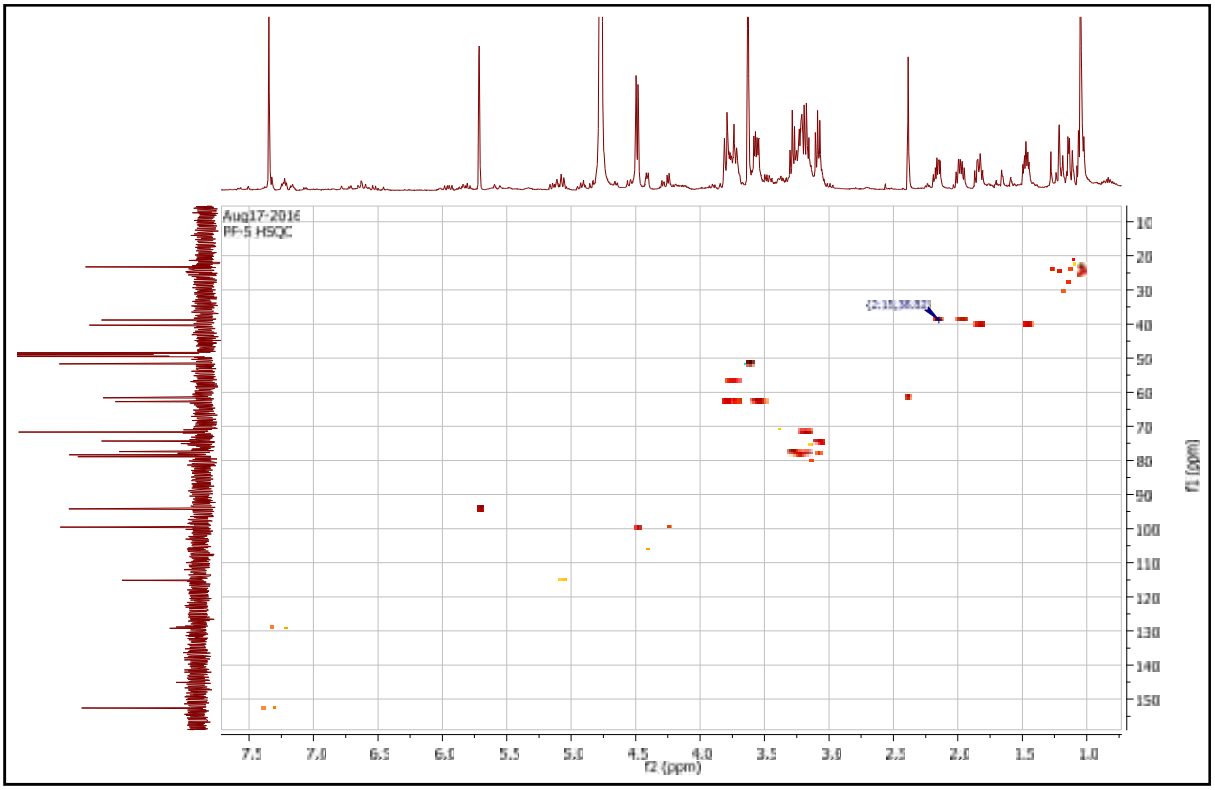
Spectrum4.1.2.2. The ^{13}C-NMR Spectrum of **Ipolamiide** (**PF-5**) (100 MHz, MeOD)



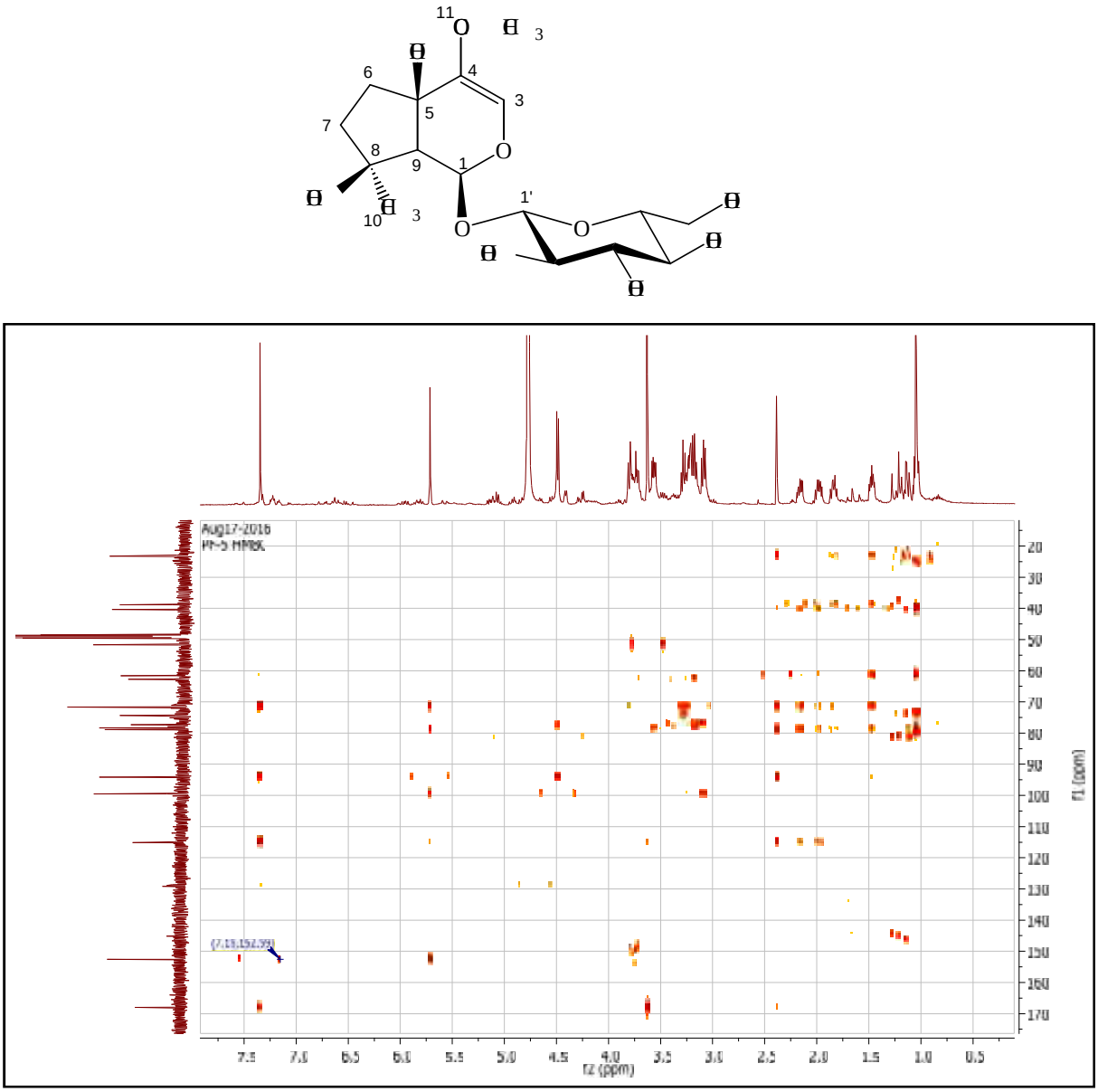
Spectrum4.1.2.3. DEPT-135 Spectrum of **Ipolamiide** (**PF-5**)



Spectrum4.1.2.4. COSY Spectrum of **Ipolamiide** (**PF-5**) (4.5 – 1.4 ppm)



Spectrum4.1.2.5. HSQC Spectrum of **Ipolamiide** (**PF-5**)



Spectrum4.1.2.6. HMBC Spectrum of **Ipolamiide** (**PF-5**)

4.1.3. Auroside (PF-6)

The most significant difference for compound PF-6 was a secondary methyl (δ 0.95 d, J = 7.49 Hz, Me-10) group observed in the ^{1}H-NMR spectrum (Table 4.1.3.1, Spectra4.1.3.1.A&B). Additionally, a three proton singlet signal for a carbomethoxy group (δ 3.73 s), a pair of protons of a methylene (δ 2.58 and 2.04, both dd; H_2-6), an oxymethine δ 3.56 (ddd "like q"; H-7) and two methine protons at δ 2.80 (dd, *J* = 10.4 and 1.6 Hz; H-9) and δ 2.27 (ddq"m", H-8) and an acetal proton at δ 5.75 (d, *J* = 1.6 Hz, H-1) were observed. These signals, assigned as H-1, H-9, H-8, Me-10, H-7 and H_2-6, were observed in the same spin system (*see* Figure 4.1.3.1; Spectra 4.1.3.4A.&B). Additional signal observed at δ 7.47 s was due to an olefinic proton (H-3). An anomeric proton observed at δ 4.57 (d, J = 8.0 Hz, H-1') was in the same spin system with the protons at assigned as H-2' – H_2-6'. Corresponding carbon resonances with these protons were found to be similar to those of lamiide and ipolamiide, indicating the presence of a β-glucopyranosyl (Table 4.1.3.1).

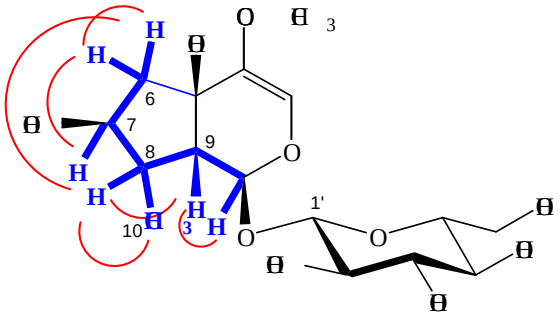


Figure 4.1.3.1. Spin system arising from the protons of cyclopentane moiety of **PF-6**

The ^{13}C-NMR spectrum (Table 4.1.3.1, Spectrum 4.1.3.2) exhibited 17 carbon signals, two CH_3, two CH_2, ten CH and three quaternary C (*see* DEPT-135, Spectrum 4.1.3.3). Six of 17 were ascribed to a β-glucopyranosyl. The remaining 11 resonances were characteristic for an iridoid bearing a carbomethoxy ($COOCH_3$) group at C-4 position. All of the ^{1}H and ^{13}C chemical shifts were determined on the basis of 2D-NMR experiments (Table 4.1.2; COSY, HSQC and HMBC, Spectra 4.1.3.4 – 4.1.3.6). HMBC spectrum (Spectrum 4.1.1.6) showed the ^{1}H, ^{13}C-long range correlations from C-4 (δ 115.4) to the olefinic proton at δ 7.47, methylene protons at δ 2.58 and 2.04 and methine protons at δ 2.80 which were assigned as H-3, H_2-6, H-9, respectively. The chemical shift value assigned to C-5 (δ 71.3) and the ^{1}H, ^{13}C-long range correlations from this carbon (C-5) to

H-1, H-3, H_2-6, H-7, H-9 signals indicated the presence of an hydroxyl function located at this position as observed for lamiide and ipolamiide. Furthermore, HMBC spectrum (Spectrum 4.1.2.6) displayed the cross-peaks between H-1 and the anomeric carbon atom (C-1') of glucose unit and vice versa, showing that the β-glucopyranosyl is attached to C-1(OH). These NMR data were in good accordance to those of auroside (Yalçın et al., 2005). On the basis of these spectroscopic data, the structures of **PF-6** was determined as auroside.

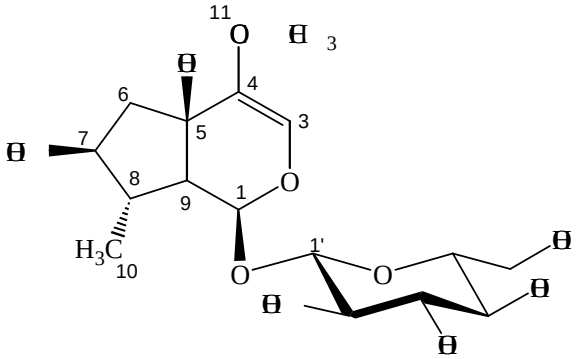
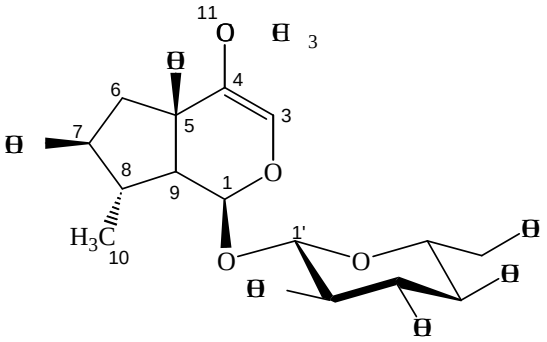
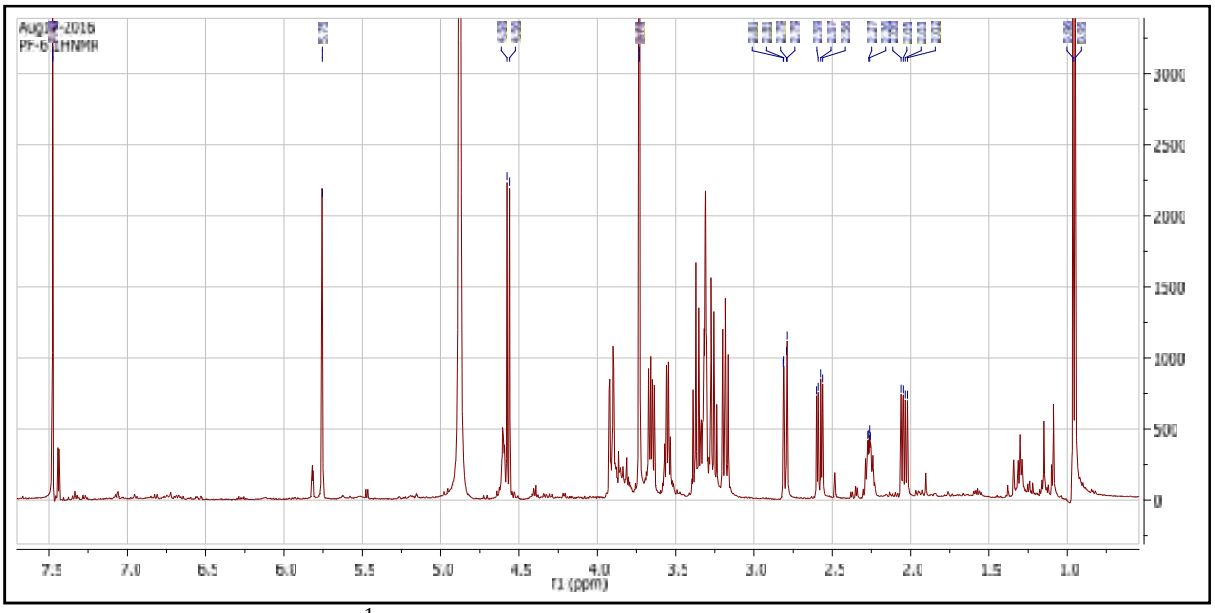


Table 4.1.3.1. **^{1}H-NMR and ^{13}C-NMR Data of Auroside (PF-6)***
^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

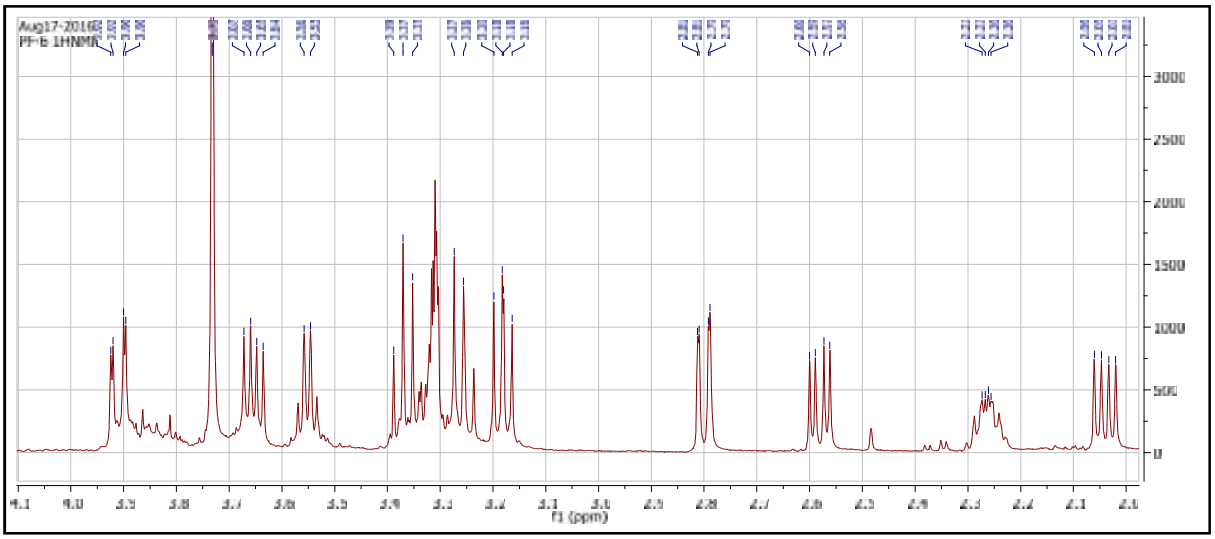
C No	**DEPT**	**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC** from C to H
Aglycone				
1	CH	95.6	5.75 d (1.6)	H-1', H-9
3	CH	153.6	7.47 s	H-1
4	C	115.4	-	H-3, H_2-6
5	C	71.3	-	H-1, H-3, H_2-6, H-7, H-9
6	CH_2	47.9	2.58 dd (13.5 / 5.6) 2.04 dd (13.5 / 7.1)	
7	CH	77.9	3.56 ddd "like q" (6.0)	
8	CH	43.5	2.27 m	H_2-7, H-9, H_3-10
9	CH	51.5	2.80 dd (10.4 / 1.6)	H_3-10
10	CH_3	13.9	0.95 d (7.49)	H-7, H-9
11	C	168.0	-	H-3, $COOC\underline{H}_3$
$CO\underline{C}H_3$	CH_3	51.7	3.73 s	-
Glucose				
1′	CH	99.7	4.57 d (8.0)	H-1
2′	CH	74.4	3.18 dd (8.0 /9.0)	
3′	CH	77.5	3.37 dd "t" (9.0)	
4′	CH	71.7	3.26 dd "t" (9.0)	
5′	CH	78.4	3.32 m	
6′	CH_2	62.8	3.90 dd (11.9 /2.1) 3.65 dd (11.9 / 5.2)	

*) All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).

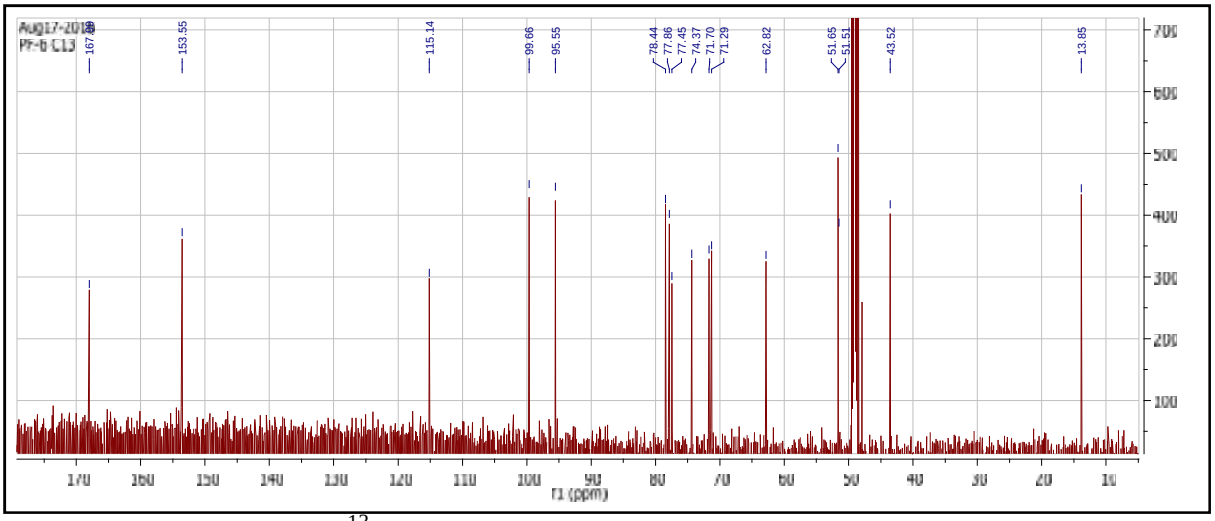




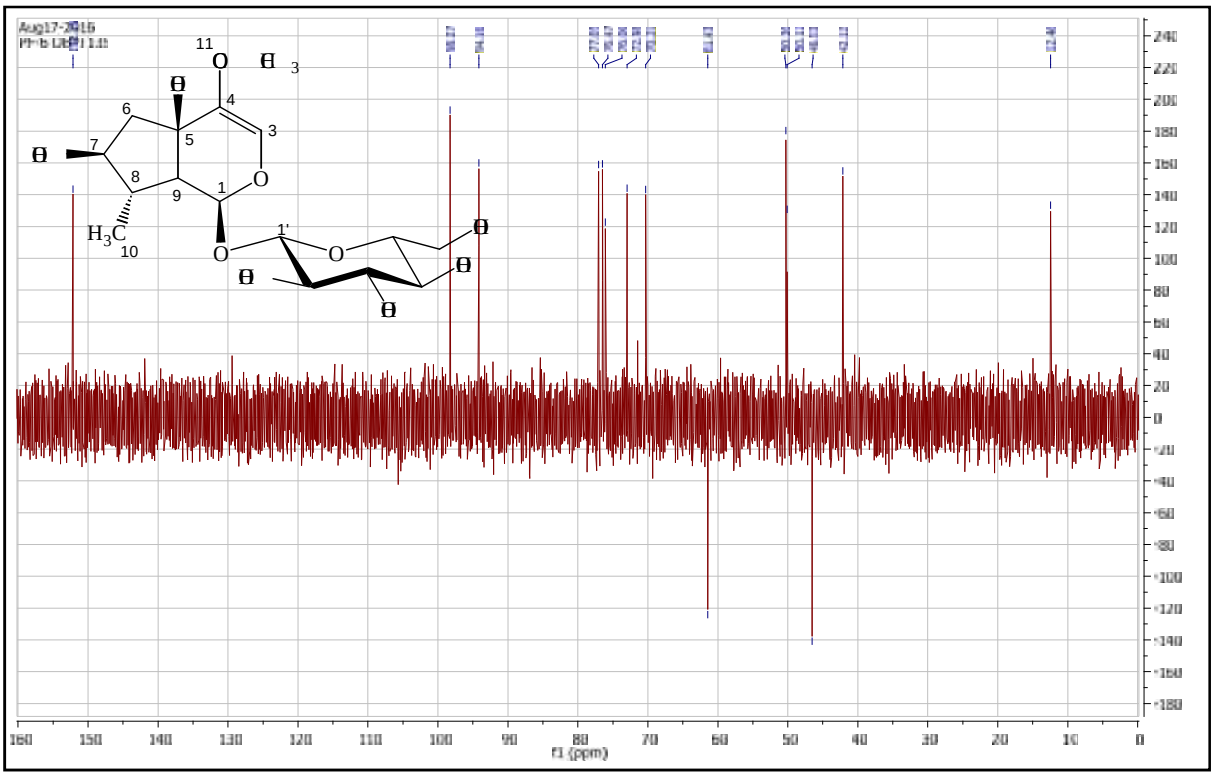
Spectrum4.1.3.1A. The ^{1}H-NMR Spectrum of **Auroside** (**PF-6**) (400 MHz, MeOD)



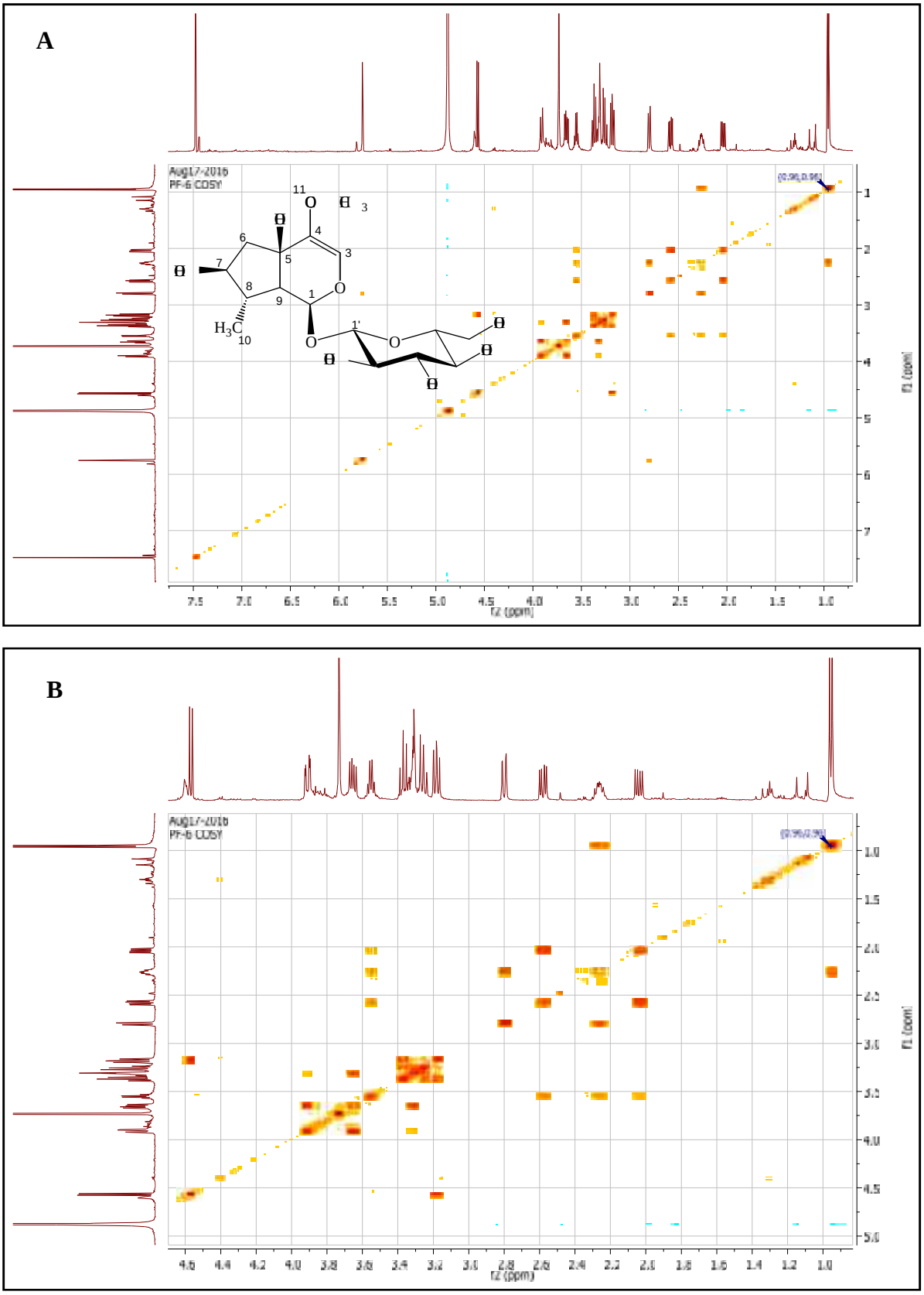
Spectrum4.1.3.1B. The ^{1}H-NMR Spectrum of **Auroside** (**PF-6**) (400 MHz, MeOD) (4.10 – 2.0 ppm)



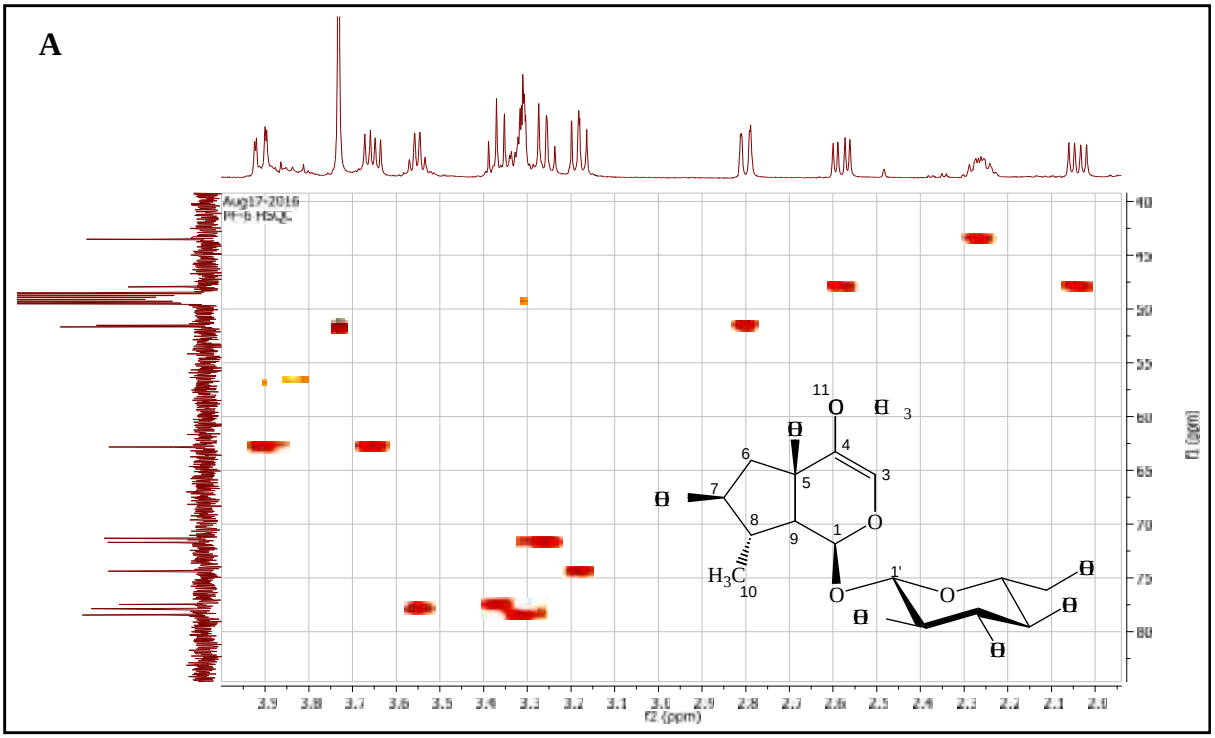
Spectrum4.1.3.2. The ^{13}C-NMR Spectrum of **Auroside** (**PF-6**) (100 MHz, MeOD)



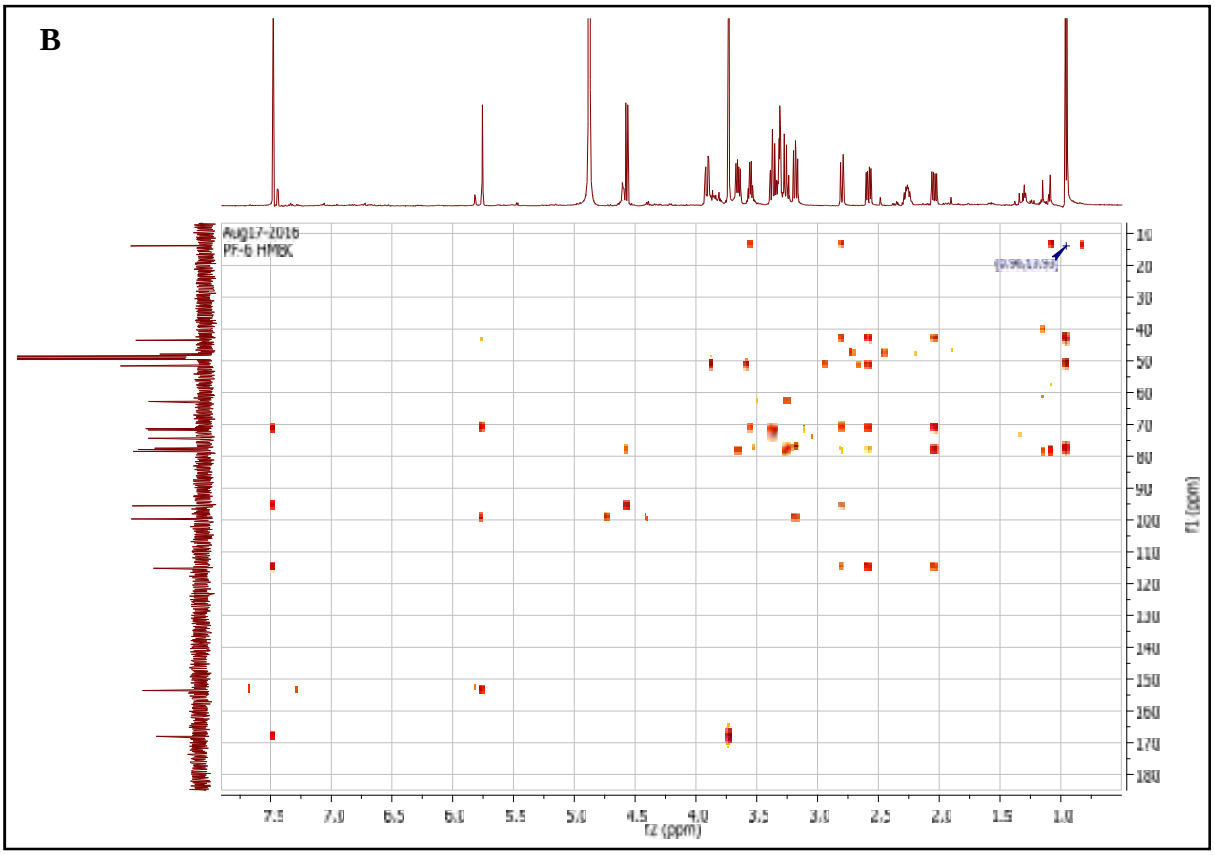
Spectrum4.1.3.3. DEPT-135 Spectrum of **Auroside** (**PF-6**)



Spectrum4.1.3.4.A&B. COSY Spectra of **Auroside** (**PF-6**)



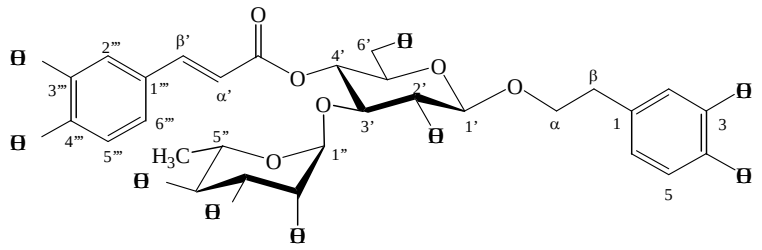
Spectrum4.1.3.5. HSQC Spectrum of **Auroside** (**PF-6**)



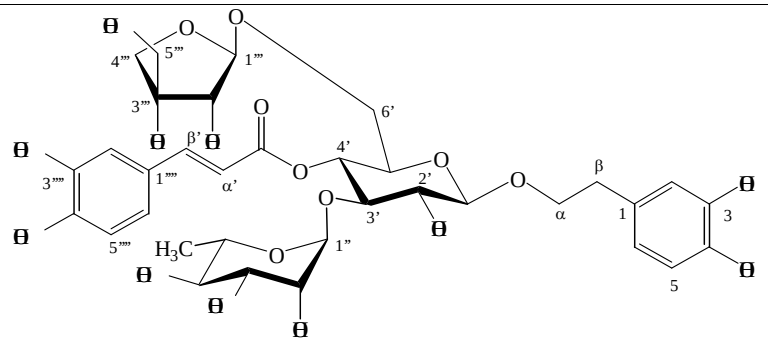
Spectrum4.1.3.6. HMBC Spectrum of **Auroside** (**PF-6**)

4.2. Phenylethanoid Glycosides (PF-3, PF-4&8, PF-7)

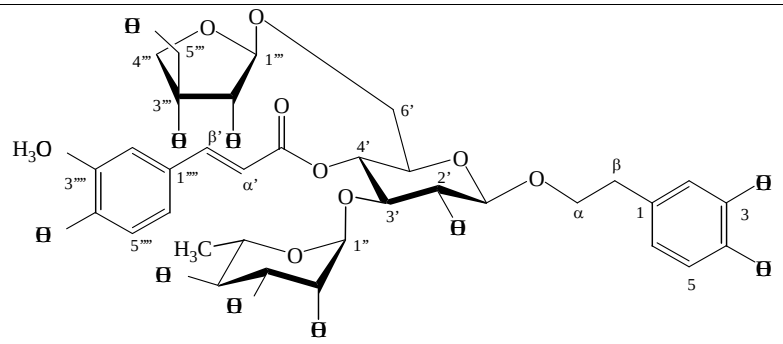
4.2.1.Verbascoside (PF-4 & PF-8)

	UV $^{a}_{max}$ 220, (235 sh), (250 sh), 332 nm $[\alpha]_D^{20}$ = - (c 0.1, MeOH)

4.2.2.Forsythoside B (PF-3)

	UV $^{a}_{max}$ 220, (290 sh), 330 nm $[\alpha]_D^{20}$ = - (c 0.1, MeOH)

4.2.3.Alyssonoside (PF-7)

	UV $^{a}_{max}$ 230, (290 sh), 330 nm $[\alpha]_D^{20}$ = - (c 0.1, MeOH)

These three compounds were obtained as pale yellow, amorphous powder. Their UV spectra confirmed their polyphenolic nature. Their behavior under UV and daylight were similar to those of flavonoids. On TLC plates, they were detected by successively using 1% vanillin (Reagent A) and 5% H_2SO_4 and heating at 110°C. In a few minutes, their colours emerge as a cherry reds which turns quickly to brown.

4.2.1.Verbascoside (PF-4 & PF-8)

The UV spectrum of PF-4 was consistent for its polyphenolic structure (λ_{max}220 and 332 nm). The NMR data of PF-4 (Table 4.2.1.1) were analyzed using 1D (^{1}H- and ^{13}C-NMR, DEPT-135: Spectra 4.2.1.1 – 4.2.1.3) and 2D NMR experiments (COSY, HSQC and HMBC: Spectra 4.2.1.4 – 4.2.1.6). In the ^{1}H-NMR spectrum, two olefinic protons as an AX system and the six aromatic proton resonances as two ABX systems between 7.60 – 6.20 ppm were observed (Spectrum 4.2.1.B). The signals one of the ABX system observed at δ 7.06 (d, *J* = 2.0 Hz, H-2‴), 6.78 (d, *J* = 8.2 Hz, H-5‴) and 6.95 (dd, *J* = 8.2 and 2.0 Hz, H-6‴) together with two olefinic protons at δ 6.27 (d, J_{AB} = 16.0 Hz, H-α') and 7.59 (d, J_{AB} = 16.0 Hz, H-β') were indicative for the presence of a caffeoyl unit. In the ^{1}H-NMR spectrum (Spectrum 4.2.1.1.A-C), the signals of the second ABX system were observed at δ 6.68 (d, *J* = 1.8 Hz, H-2), 6.68 (d, *J* = 8.2 Hz, H-5), 6.57 (dd, *J* = 8.2 and 1.8 Hz, H-6). Additionally, a benzylic methylene at δ 2.78 (m, H_2-β) and two non-equivalent protons at δ 4.03 and 3.73 (both m). These signals suggested the presence of a 3,4-dihydroxyphenylethyl unit as an aglycone for PF-4.

The ^{1}H-NMR spectrum (Spectrum 4.2.1.1.A-C) exhibited two anomeric protons at δ 4.37 (d, J = 8.2 Hz, H-1') and 5.15 (br.s, H-1"). The ^{13}C-NMR spectrum (Spectrum 4.2.1.2) displayed the two anomeric carbon resonances at δ 104.3 and 103.1. The protons resonances of both sugar units were assigned by the help of ^{1}H-^{1}H homonuclear correlated COSY experiment (Spectrum 4.2.1.4.A&B). The multiplicities of the sugar protons and their coupling constants were consistent for presence of a β-glucopyranosyl and an α-rhamnopyranosyl units (Table 4.2.1.1). The corresponding carbon resonances of the sugar protons were revealed by the help of ^{1}H-^{13}C heteronuclear correlated HSQC experiment (Spectrum 4.2.1.5.A&B). ^{13}C-NMR spectrum exhibited 29 carbon resonances. DEPT-135 experiment exhibited three CH_2 resonances at δ 62.4, 72.2 and 36.5 of which latter two were assigned ethyl part of the side chain of 3,4-dihydroxyphenylethyl unit. The proton resonance located at C-4' was observed as shifted to downfield (δ 4.91, dd"t", J = 9.0 Hz, H-4' of glucopyranosyl unit) due to the esterification indicating the site of acylation by caffeic acid. The secondary methyl resonance at δ 1.08 (d, *J* = 6.4 Hz) was indicative for presence of 6-deoxy-sugar unit, rhamnose as one of the two sugar units.

Intermolecular connectivites between the four molecular fragments, 3,4-dihydroxy-phenylethyl alcohol, caffeic acid, glucose and rhamnose, were determined by the help of ^{1}H-^{13}C long-range heteronuclear correlated HMBC experiment (Spectra 4.2.1.6.A&B). HMBC spectrum displayed the cross-peaks between the anomeric proton of glucopyranosyl unit (H-1', δ 4.37) and the alpha (α, δ 72.2) carbon atom of the aglycone, 3,4-dihydroxy-phenylethyl alcohol showing that the glucose unit was the core sugar in the structure.

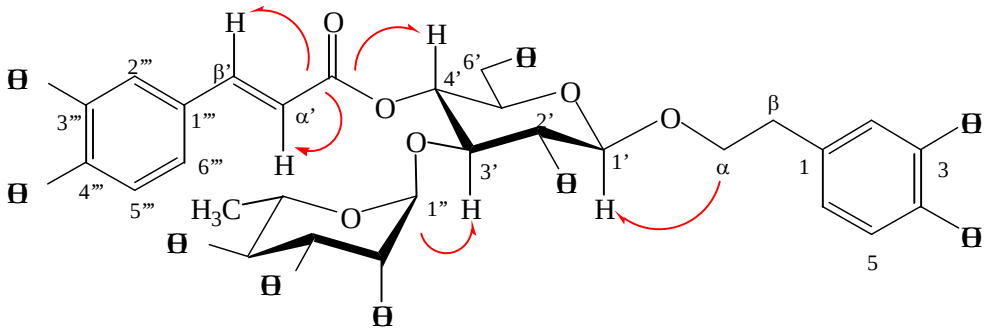


Figure 4.2.1.1. The significant ^{1}H, ^{13}C Long-range Heteronuclear Correlations showing intermolecular fragments (Arrows from C to H)

Further long-range correlations were observed between the carbonyl carbon of caffeic acid (δ 168.3) and H-4' (δ 4.91) of glucose and olefinic protons of caffeic acid at δ 7.59 (H-β') and 6.27 (H-α') confirming the acylation site on the glucose unit. Finally, the long-range correlations were observed between the anomeric carbon of rhamnose unit (δ 168.3) and the H-4' of glucose.

Based upon these observations, the structure of PF-4 was determined as a diglycosidic phenylethanoid glycoside, verbascoside (= acteoside) (Sticher & Lahloub, 1982). It was also reported from the 33 *Phlomis* species growing in Turkey (Çalış et al., 2004a).

Co-chromatographical studies (TLC) and NMR data of PF-8 indicated that the existence of the similar structure to those of verbascoside.

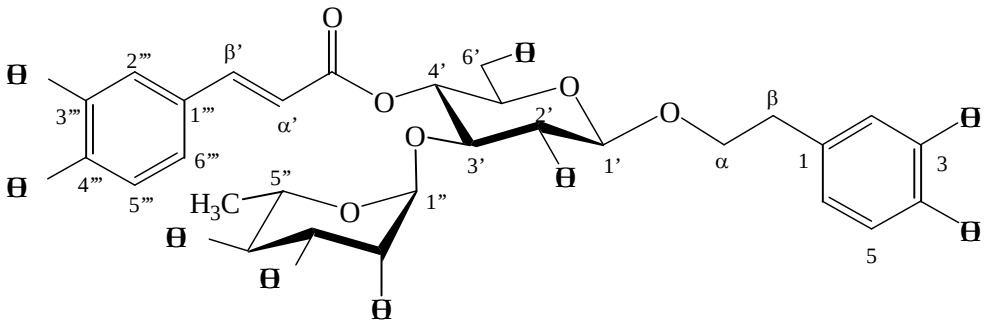
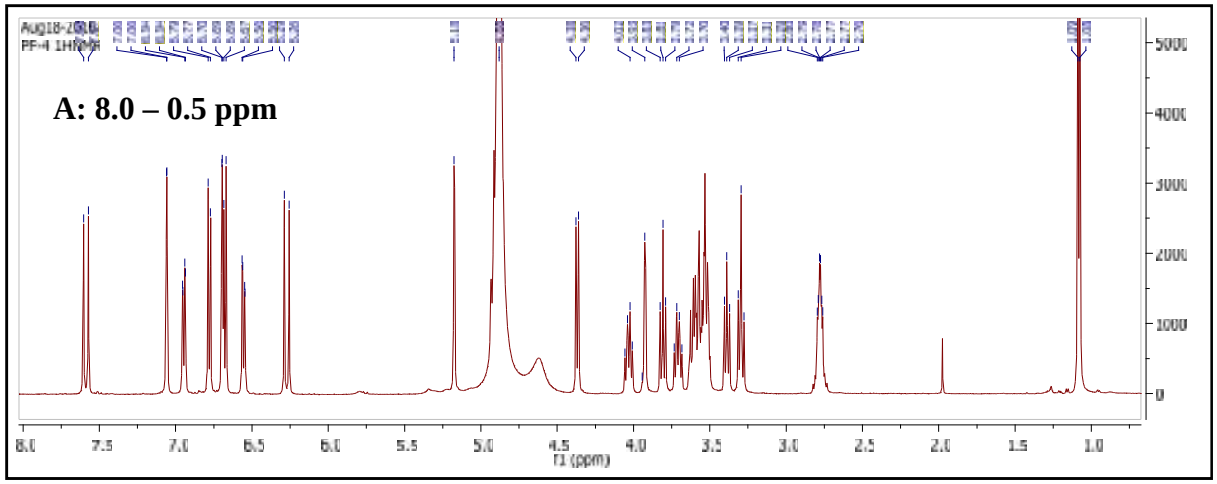
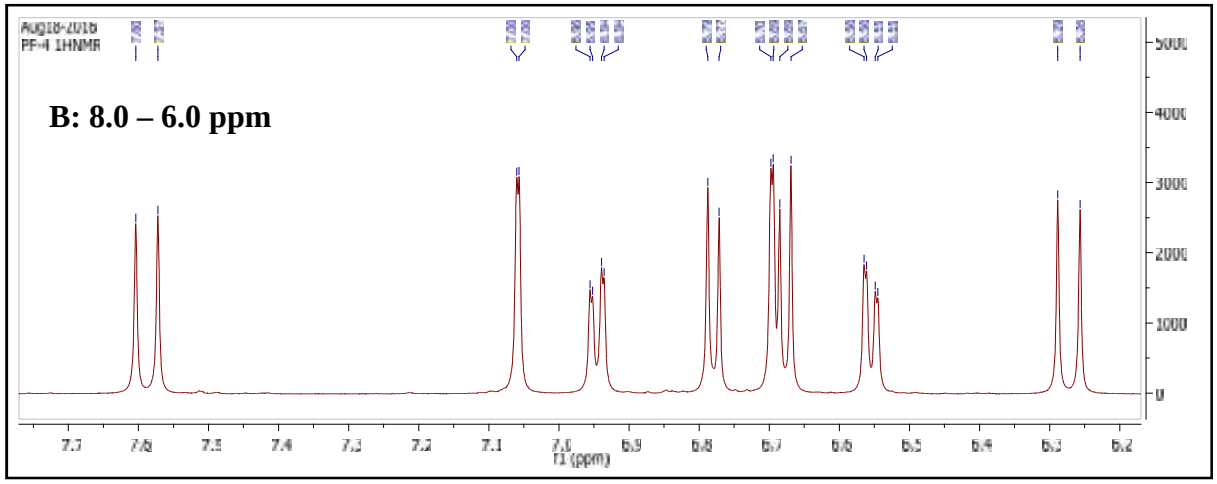


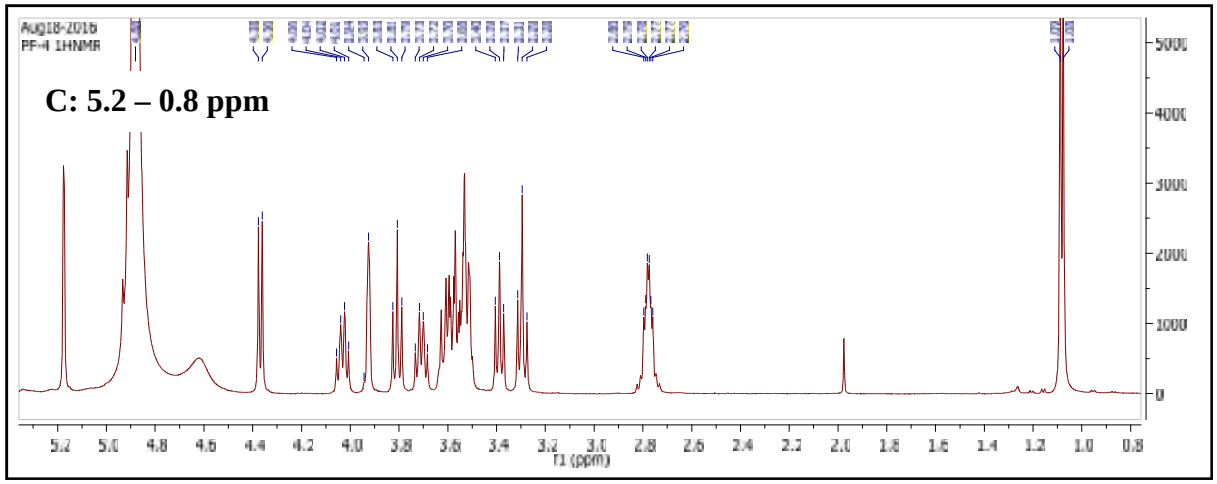
Table 4.2.1.1. **^{1}H-NMR and ^{13}C-NMR Data of Verbascoside (PF-4 & PF-8)**
^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

+C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
PhEthylalcohol 1	C	131.5	-	H_2-α, H_2-β, H-5
2	CH	117.2	6.68 d (1.8)	H_2-β, H-6
3	C	146.7	-	H-5
4	C	144.3	-	H-2, H-5, H-6
5	CH	116.4	6.68 d (8.2)	
6	CH	121.3	6.57 dd (8.2 / 1.8)	H-β
α	CH_2	72.2	4.03 m, 3.73 m	H-β, H-1’
β	CH_2	36.5	2.78 m	
Glucose 1’	CH	104.3	4.37 d (8.2)	H_2-α, H-2’
2’	CH	76.1	3.39 dd (8.2 / 9.0)	
3’	CH	81.7	3.80 dd”t” (9.0)	H-1”, H-1’, H-4’
4’	CH	70.5	4.91 dd”t” (9.0)	
5’	CH	74.6	3.53’	
6’	CH_2	62.4	3.62†, 3.52†	H-4’
Rhamnose 1”	CH	103.1	5.15 br s	H-3’
2”	CH	72.2	3.92	
3”	CH	71.9	3.58†	H_3-6”
4”	CH	73.7	3.29 dd”t” (9.6)	
5”	CH	70.4	3.55†	H-4”,
6”	CH_3	18.4	1.08 d (6.4)	
Caffeoyl 1”’	C	127.7	-	H-α’, H-β’, H-5”’
2”’	CH	115.3	7.06 d (2.0)	H-6”’
3”’	C	146.9	-	H-2”’, H-5”’
4”’	C	149.9	-	H-2”’, H-5”’, H-6”’
5”’	CH	116.6	6.78 d (8.2)	
6”’	CH	123.2	6.95 dd (8.2 / 2.0)	H-β’
α’	CH	114.8	6.27 d (16.0)	
β’	CH	148.1	7.59 d (16.0)	
C=O	C	168.3	-	H-α’, H-β’, H-4’

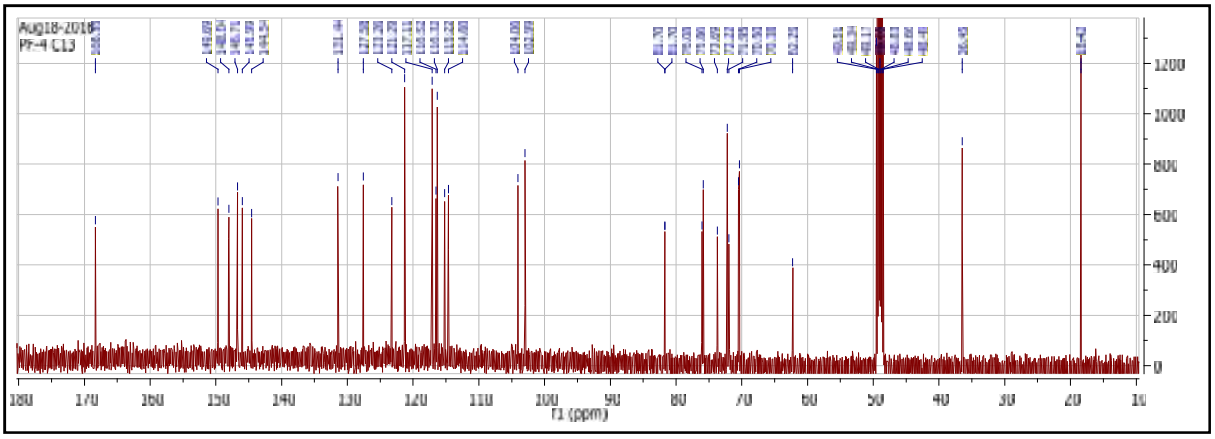
*All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).
†Signal pattern unclear due to overlapping.



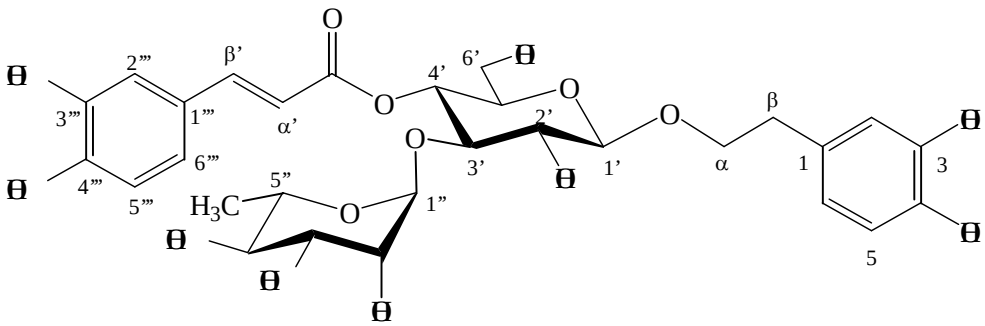


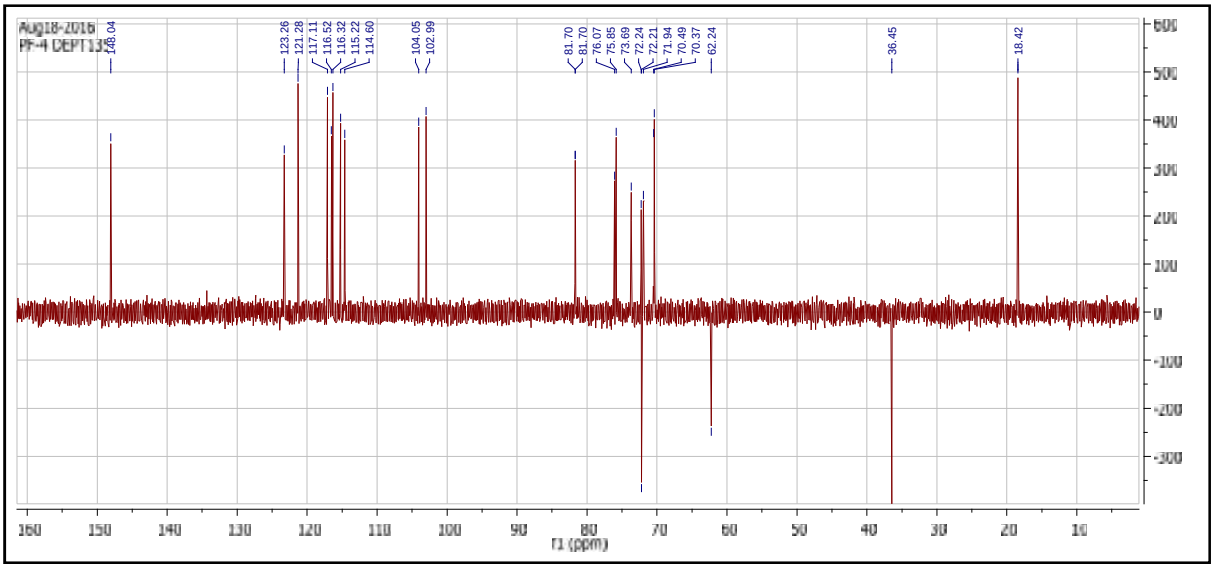


Spectrum4.2.1.1.A-C. The ^{1}H-NMR Spectrum of **Verbascoside** (**PF-4&8**) (400 MHz, MeOD)

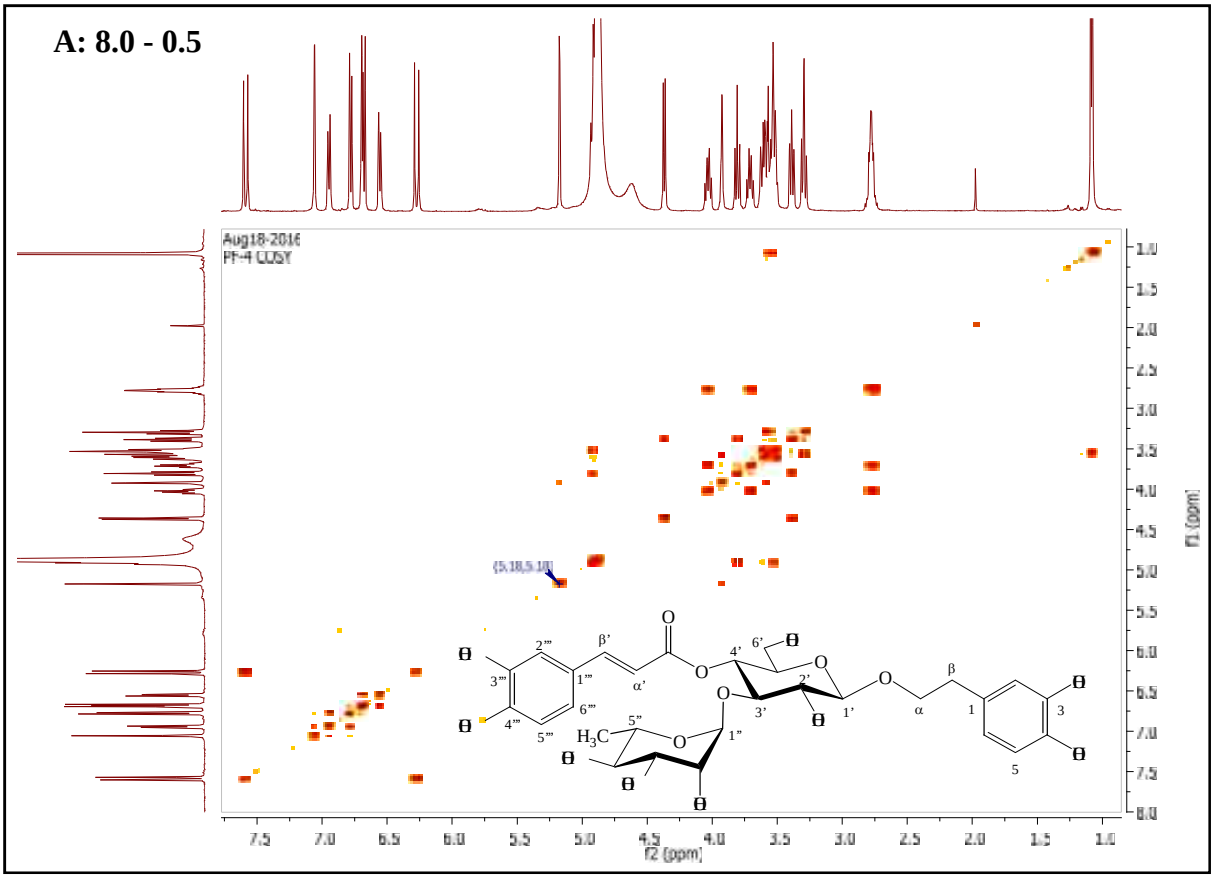


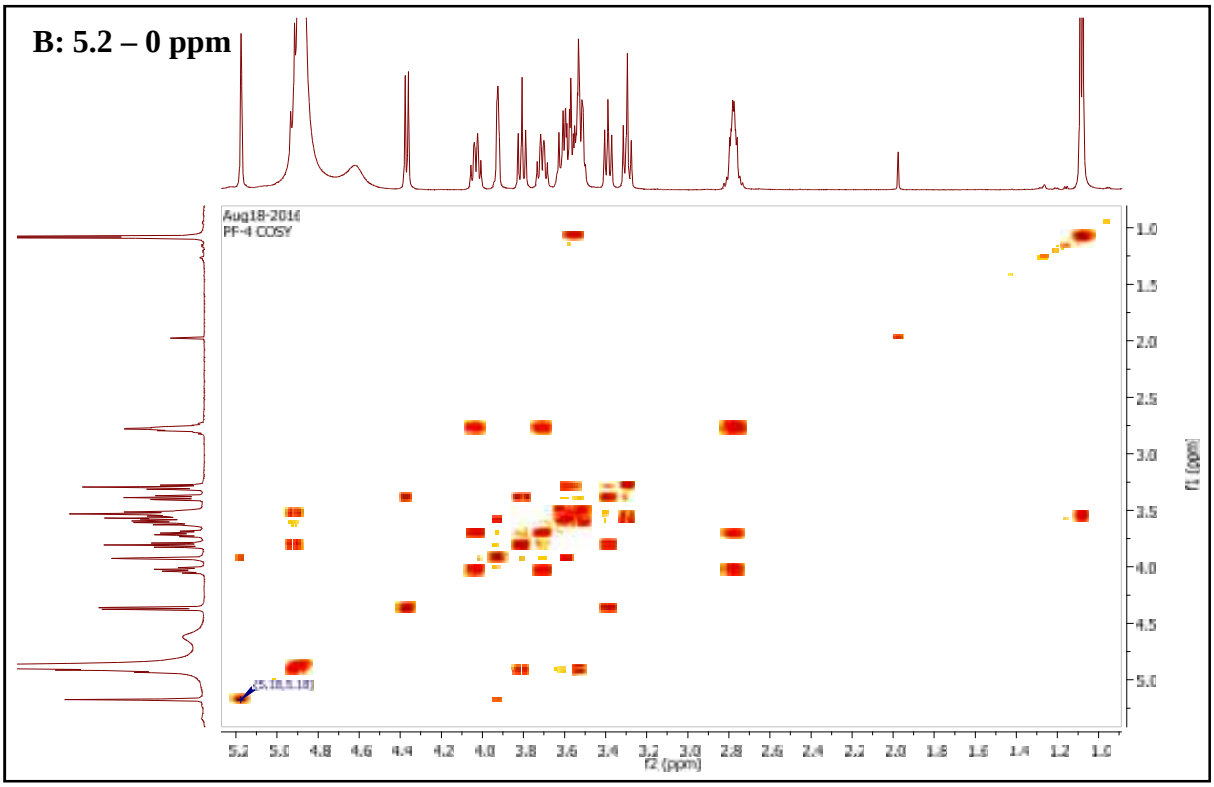
Spectrum4.2.1.**2**. The ^{13}C-NMR Spectrum of **Verbascoside** (**PF-4&8**) (100 MHz, MeOD)



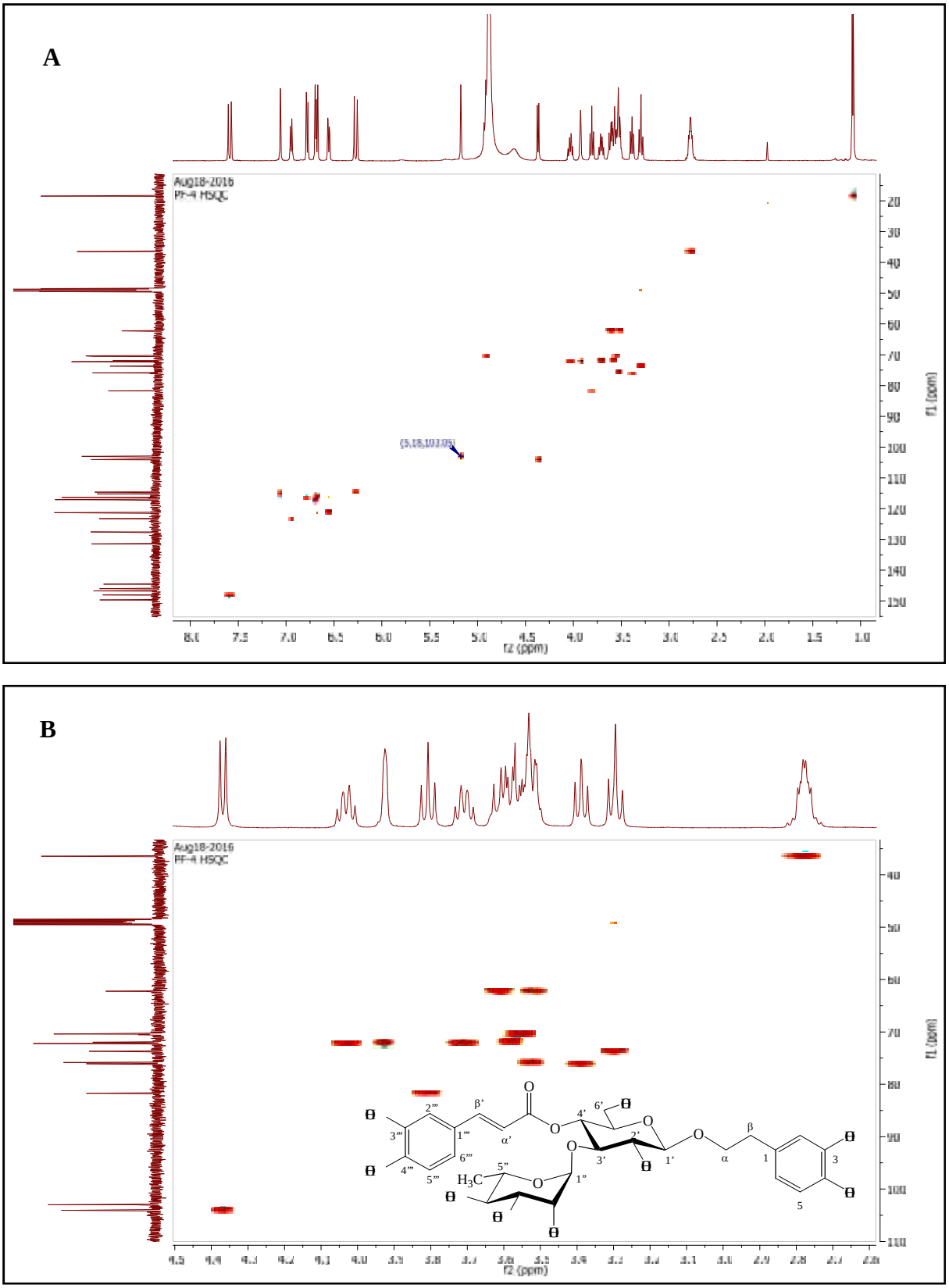


Spectrum4.2.1.3. DEPT-135 Spectrum of **Verbascoside** (**PF-4&8**)

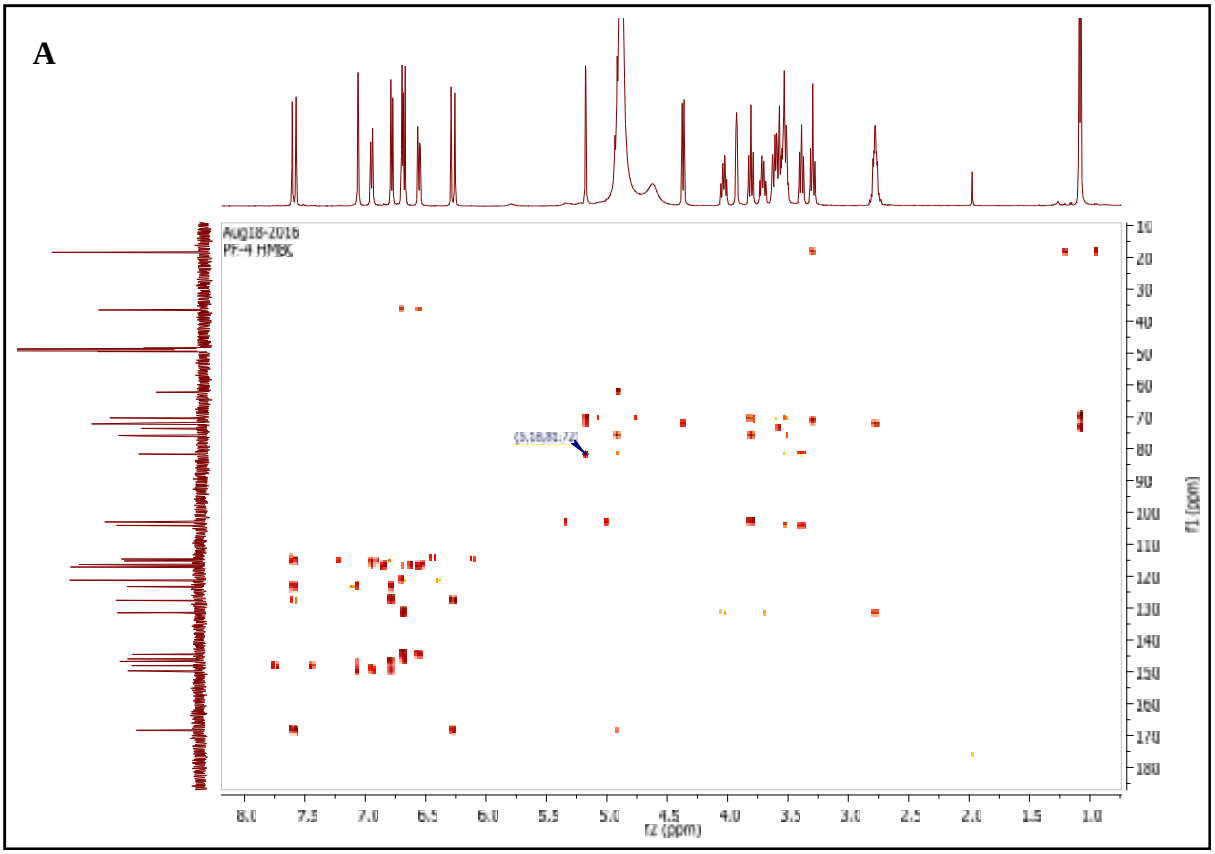




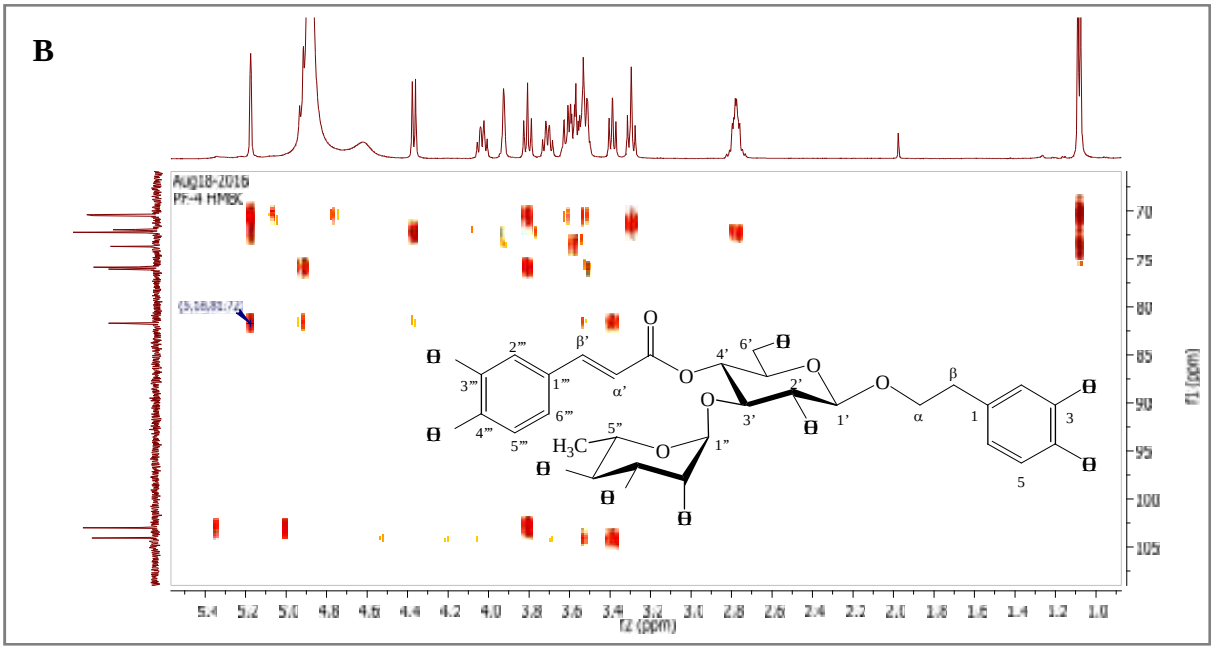
Spectrum 4.2.1.4.A&B. COSY Spectrum of **Verbascoside** (**PF-4&8**)



Spectrum 4.2.1.5.A&B. HSQC Spectrum of **Verbascoside** (**PF-4&8**)



A: All ^{1}H, ^{13}C- Long Range Correlations



B: ^{1}H, ^{13}C- Long Range Correlationsshowing interglycosidic linkages

Spectrum 4.2.1.6.A&B. HMBC Spectrum of **Verbascoside** (**PF-4&8**)

4.2.2. Forsythoside B (PF-3)

The UV spectrum of PF-3 was consistent for its polyphenolic structure (λ_{max} 220 and 330 nm). The NMR data of PF-3 (Table 4.2.2.1) was analyzed using 1D (^{1}H- and ^{13}C-NMR, DEPT-135: Spectra 4.2.2.1 – 4.2.2.3) and 2D NMR experiments (COSY, HSQC and HMBC: Spectra 4.2.2.4 – 4.2.2.6). The ^{1}H-NMR spectrum of PF-3 was very similar to those of verbascoside (PF-4) except for the presence of an additional sugar unit. The signals at the aromatic region were the six aromatic proton resonances observed as two ABX systems together with two olefinic protons. Moreover, a benzylic methylene at δ 2.79 (t, *J* = 7.4 Hz, H_2-β) and two non-equivalent protons at δ 4.00 and 3.71 (both m) were observed. These findings suggested the presence of a caffeic acid and the 3,4-dihydroxyphenylethyl alcohol units as acyl- and aglycone moieties as observed for verbascoside.

The ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra (Spectra 4.2.2.1, 4.2.2.2 and 4.2.2.3) exhibited three anomeric protons at δ 4.36 (d, J = 7.9 Hz, H-1’), 5.17 (br.s, H-1”), and 490 (overlapped) and three correponding anomeric carbon atoms δ 104.1 (C-1’), 103.0 (C-1”) and 110.9 (C-1”’) strongly suggested the presence of apiose unit in additon to glucose and rhamnose units. The protons resonances of three sugar units were assigned by the help of ^{1}H-^{1}H homonuclear correlated COSY experiment (Spectrum 4.2.2.4.A&B). The multiplicities of the sugar protons and their coupling constants were consistent for presence of a β-glucopyranosyl, an α-rhamnopyranosyl and a β-apiofuranosyl units (Table 4.2.2.1). The corresponding carbon resonances of the sugar protons were revealed by the help of ^{1}H-^{13}C heteronuclear correlated HSQC experiment (Spectrum 4.2.2.5.A&B). ^{13}C-NMR spectrum exhibited 34 carbon resonances. The five carbon atom resonances difference with those of verbascoside was consistent fort he presence of a pentose unit as a third sugar. DEPT-135 experiment displayed five CH_2resonances at δ 68.4, 72.3, 36.5, 75.0 and 65.6 of which latter two were assigned to a α-furanosyl unit. The former methylene carbon atoms were in good accordance with those of verbascoside. Exceptionally, 6 ppm chemical shift difference to the downfield for the signal of C-6 ’(68.4 – 62.4 = 6.0 ppm) of the glucose unit, was predictive for the site of further glycosidation at C-6’ (δ 68.4) for PF-3. The proton resonance located at C-4’ was observed as shifted to downfield (δ 4.94, dd”t”, J = 9.0 Hz, H-4’ of glucopyranosyl unit) due to the esterification indicating the site of acylation by caffeic acid as observed for verbascoside.

Intermolecular connectivites between the four molecular fragments, 3,4-dihydroxy-phenylethyl alcohol, caffeic acid, glucose, rhamnose and apiose were determined by the help of ^{1}H-^{13}C long-range heteronuclear correlated HMBC experiment (Spectra 4.2.2.6.A&B). HMBC spectrum displayed the cross-peaks between the anomeric proton of glucopyranosyl unit (H-1', δ 4.36) and the alpha (α, δ 72.3) carbon atom of the aglycone, 3,4-dihydroxy-phenylethyl alcohol showing that the glucose unit was the core sugar in the structure.

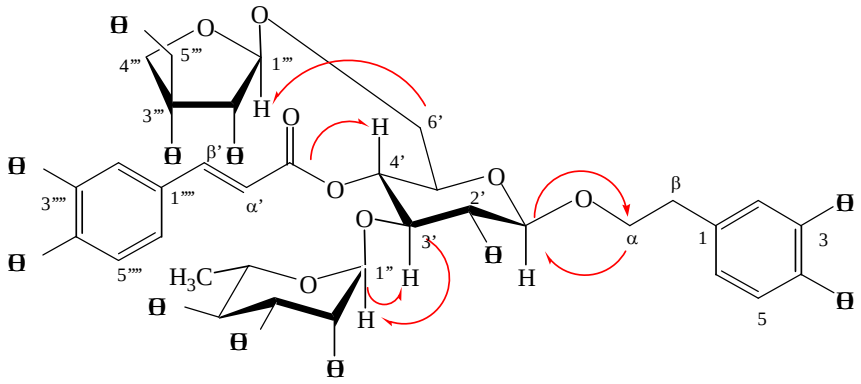


Figure 4.2.2.1. The significant ^{1}H, ^{13}C Long-range Heteronuclear Correlations showing intermolecular fragments (Arrows from C to H)

Further long-range correlations were observed between the carbonyl carbon of caffeic acid (δ 168.2) and H-4' (δ 4.94) of glucose and olefinic protons of caffeic acid at δ 7.65 (H-β') and 6.37 (H-α') confirming the acylation site on the glucose unit. Finally, the long-range correlations were observed between the anomeric carbon of rhamnose unit (δ 103.0) and the H-4' of glucose (δ 4.94) as well between the anomeric carbon of apiose unit (δ 110.9) and the H_2-6' methylene protons of the glucose unit (δ 3.72 and 3.47).

Based upon these observations, the structure of PF-4 was determined as a triglycosidic phenylethanoid glycoside, forsythoside B (Endo, K. *et al*, 1982). It was also reported from the 33 *Phlomis* species growing in Turkey (ÇALIŞ, I. *et al.*, 2004a)

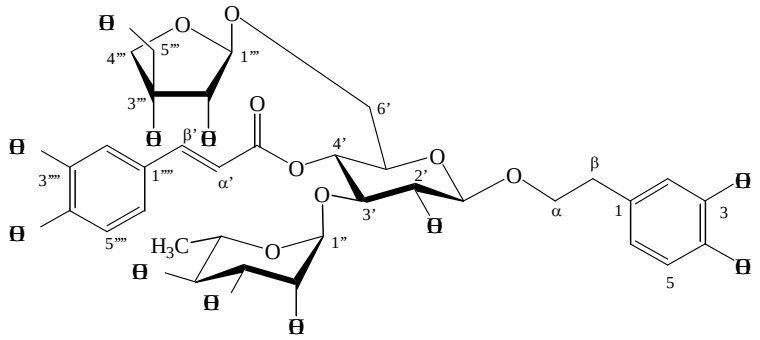
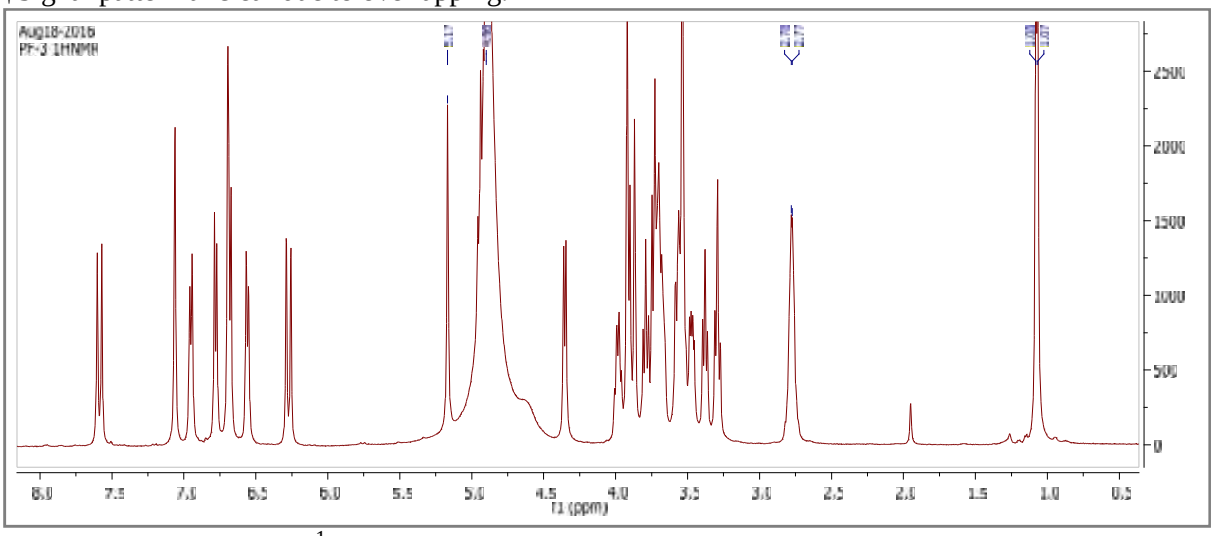


Table 4.2.2.1. **^{1}H-NMR and ^{13}C-NMR Data of Forsythoside B (PF-3)**
^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

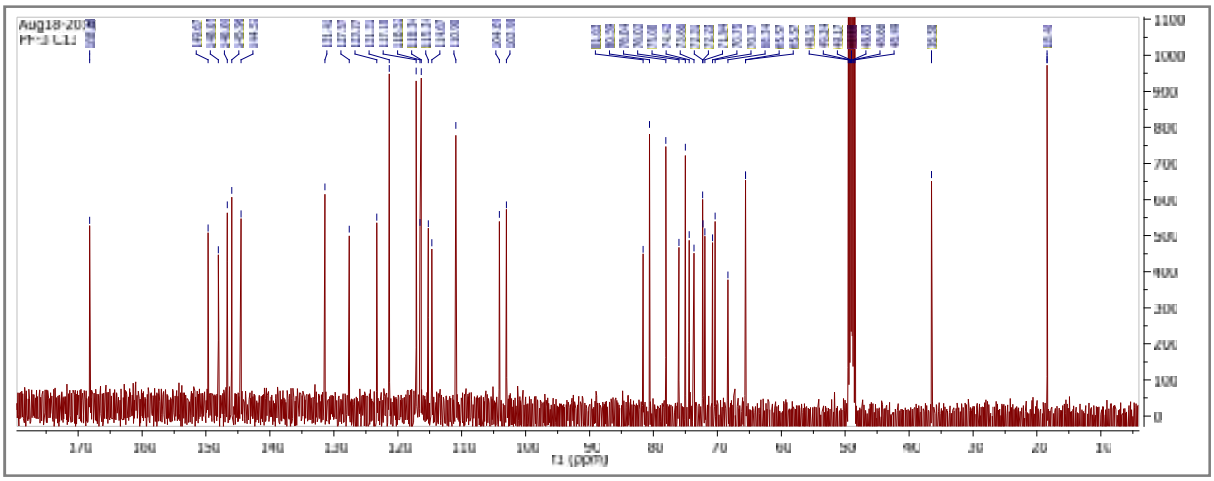
C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
PhEthylalcohol 1	C	131.4	-	H_2-α, H_2-β, H-5
2	CH	116.5	6.69 d (2.0)	H_2-β, H-6
3	C	146.0	-	H-5
4	C	144.5	-	H-2, H-5, H-6
5	CH	117.1	6.68 d (8.0)	
6	CH	121.3	6.55 dd (8.0 / 2.0)	H-β
α	CH_2	72.3	4.00 m, 3.71 m	H-β, H-1’
β	CH_2	36.5	2.79 t (7.4)	
Glucose 1’	CH	104.1	4.36 d (7.9)	H-2’
2’	CH	76.0	3.38 dd (7.9 / 9.0)	
3’	CH	80.1	3.79 dd”t” (9.0)	
4’	CH	70.8	4.94 dd”t” (9.0)	
5’	CH	74.4	3.67†	
6’	CH_2	68.4	3.72†, 3.47 dd (11.2 / 5.6)	
Rhamnose 1”	CH	103.0	5.17 br s	H-3’
2”	CH	72.2	3.92†	
3”	CH	71.9	3.57 dd (3.1 / 9.5)	
4”	CH	73.6	3.29 dd”t” (9.5)	
5”	CH	70.4	3.5	
6”	CH_3	18.4	1.08 d (6.2)	
Apiose 1”’	CH	110.9	4.90†	H_2-6’, H-2”’
2”’	CH	78.1	3.86 d (2.2)	
3”’	CH	80.6	-	
4”’	CH_2	75.0	3.91 d (9.6), 3.74 d (9.6)	
5”’	CH_2	65.5	3.54 br s	
Caffeoyl 1””	C	127.6	-	H-α’, H-β’, H-5””
2””	CH	111.8	7.06 d (2.0)	H-6””
3””	C	149.3	-	H-2””, H-5””
4””	C	150.6	-	H-2””, H-5””, H-6””
5””	CH	116.5	6.78d (8.2)	
6””	CH	124.3	6.95 dd (8.2 / 2.0)	H-β’
α’	CH	115.1	6.37 d (15.9)	
β’	CH	148.0	7.65 d (15.9)	
C=O	C	168.2	-	H-α’, H-β’, H-4’

*All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).

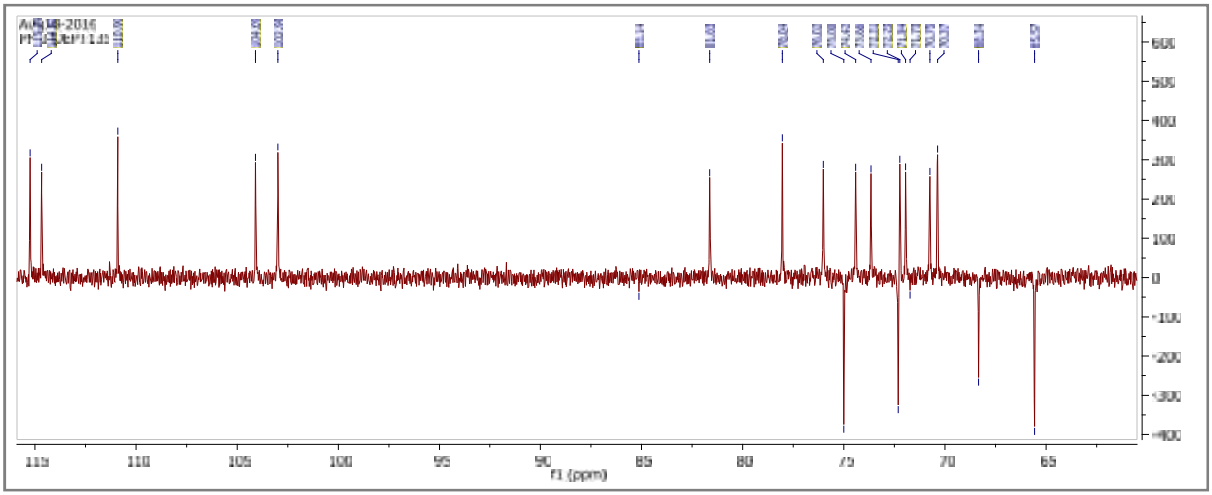
†Signal pattern unclear due to overlapping.



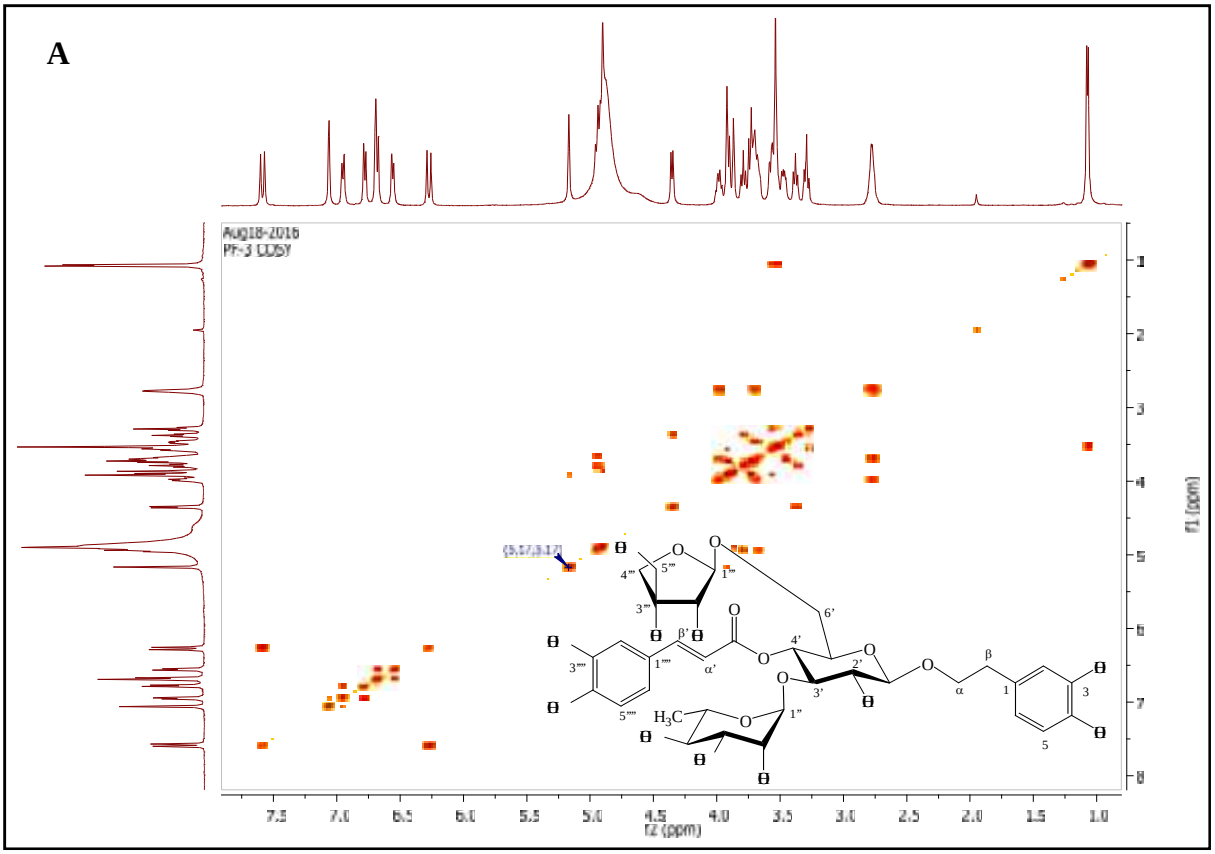
Spectrum 4.2.2.1. The ^{1}H-NMR Spectrum of **Forsythoside B** (**PF-3**) (400 MHz, MeOD)

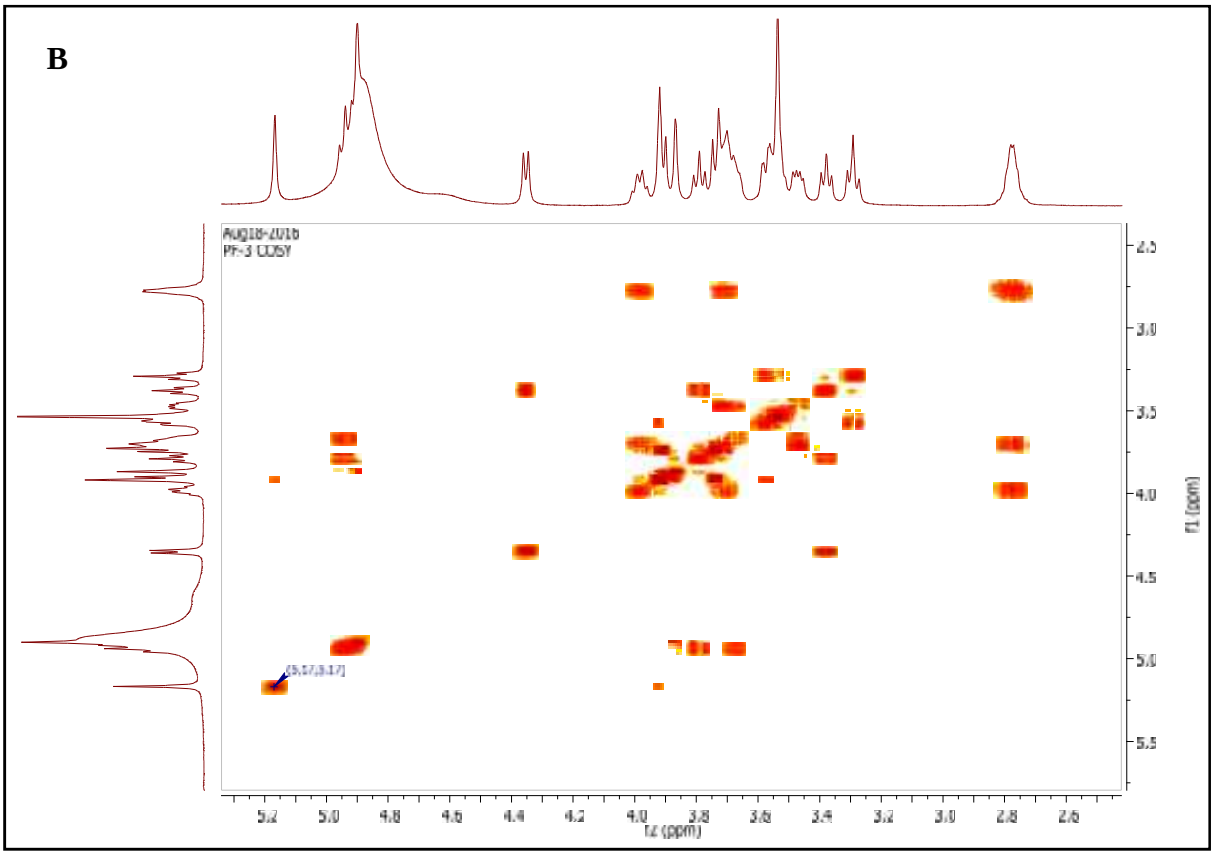


Spectrum 4.2.2.2. The ^{13}C-NMR Spectrum of **Forsythoside B** (**PF-3**) (100 MHz, MeOD)

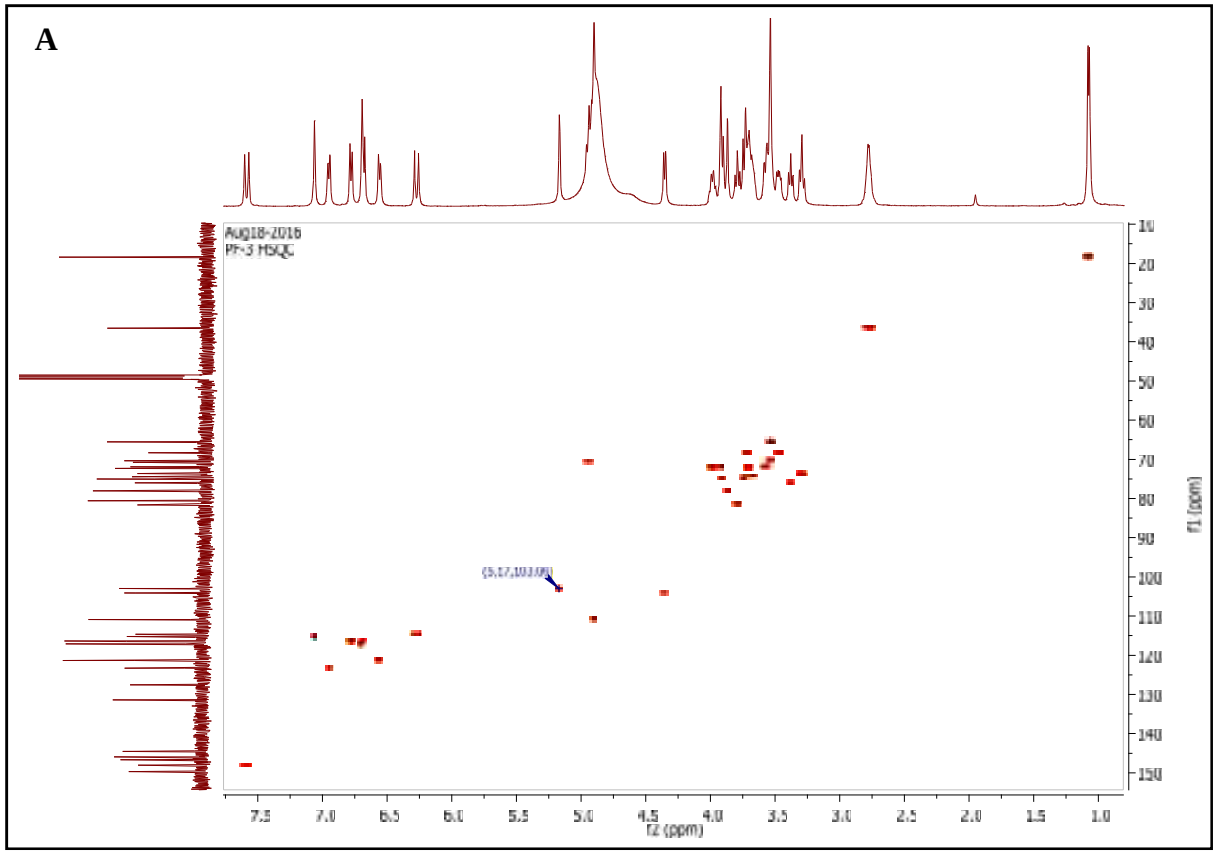


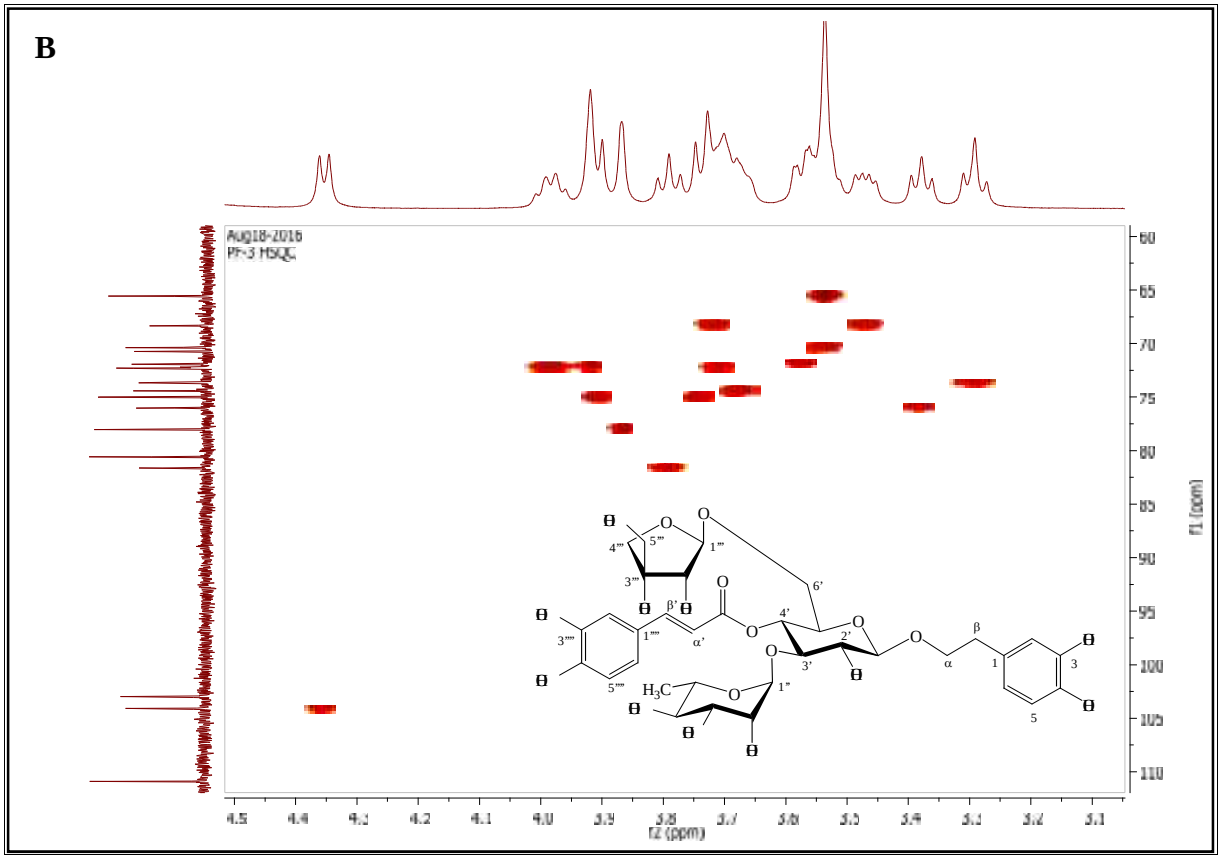
Spectrum 4.2.2.3. DEPT-135 Spectrum of **Forsythoside B** (**PF-3**)



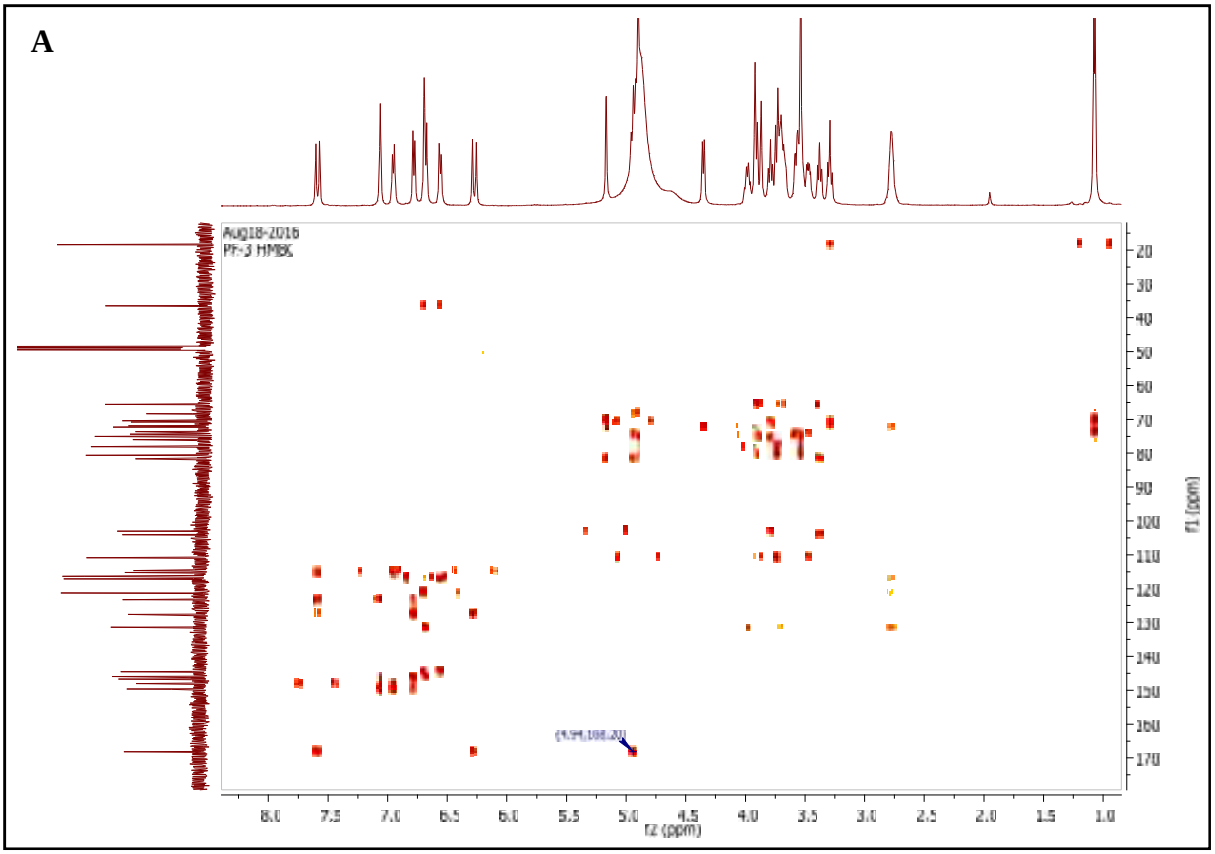


Spectrum 4.2.2.4.A&B. COSY Spectrum of **Forsythoside B** (**PF-3**)

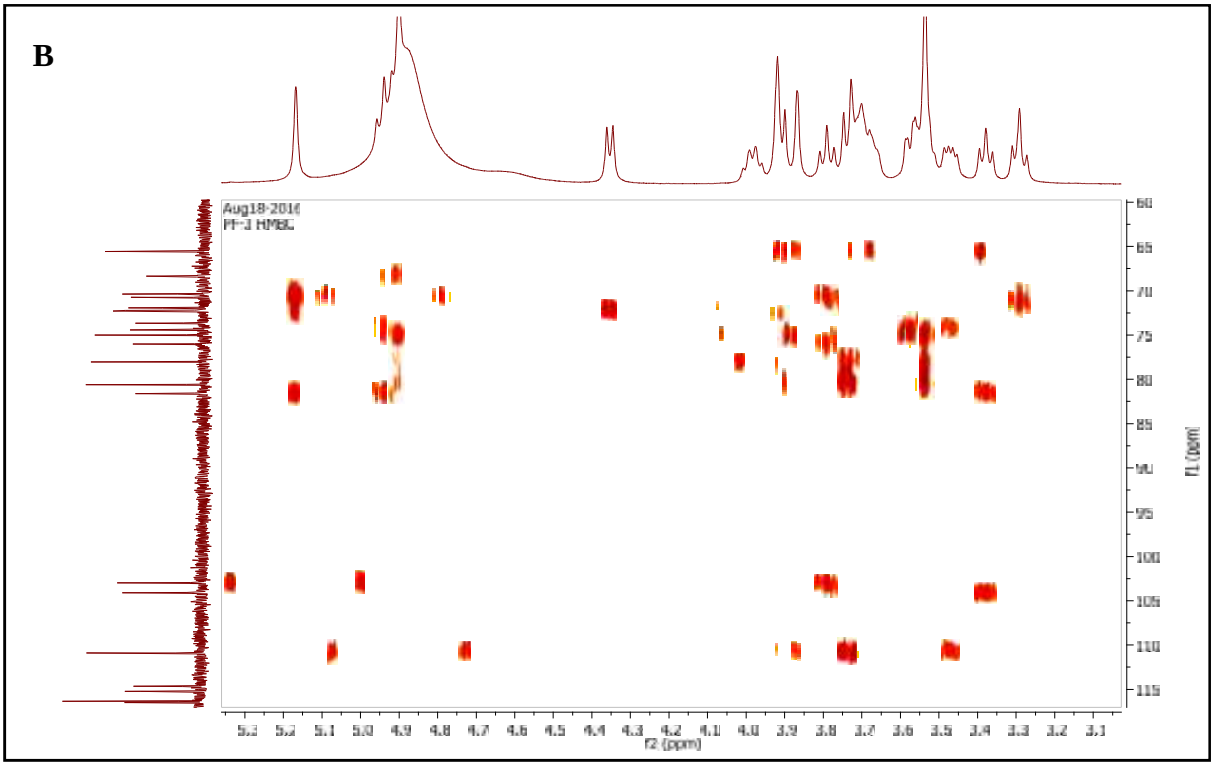




Spectrum 4.2.2.5.A&B. HSQC Spectrum of **Forsythoside B** (**PF-3**)



A: All[1]H, [13]C- Long Range Correlations



B: [1]H, [13]C- Long Range Correlationsshowing interglycosidic linkages

Spectrum 4.2.2.6.A&B. HMBC Spectrum of **Forsythoside B** (**PF-3**)

4.2.3. Alyssonoside (PF-7)

Chromatographical behavior, the UV spectrum (λ_{max} 230 and 330 nm) and the NMR data of PF-7 (Table 4.2.3.1b) were very similar to those of verbascoside and especially forsythoside B. The NMR data were analyzed using 1D (^{1}H- and ^{13}C-NMR, DEPT-135: Spectra 4.2.3.1 – 4.2.3.2) and 2D NMR experiments (COSY, HSQC and HMBC). Since the close similarity of its structure to those of forsythoside B, only the HMBC spectrum has been presented (spectrum 4.2.3.3).

The number and the type of sugar units (glucopyranosyl, rhamnopyranosyl and apiofuranosyl), aglycone (3,4-dihydroxy-phenylethyl) and acyl-moieties were almost same to those of forsythoside B. In the ^{1}H- and ^{13}C-NMR spectra of PF-3 (Forsythoside B) and PF-7 (Tables 4.2.2.1 and 4.2.3.1) the signals due to the sugar moiety of PF-7 were superimposable to those of PF-3, the major difference being the presence of a resonance for a methoxyl group in PF-7 (δ_H 3.88 s; δ_C: 56.5: OCH_3).

Table 4.2.3.1a. **^{1}H-NMR and ^{13}C-NMR Data of Acyl moieties of PF-3 and PF-7.**

Caffeoyl moiety of Forsythoside B (PF-3)				**Feruloyl moiety of Alyssonoside (PF-7)**	
1””	C	127.6	-	127.6	-
2””	CH	111.8	7.06 d (2.0)	111.8	7.20 d (2.0)
3””	C	149.3	-	149.3	-
4””	C	150.6	-	150.6	-
5””	CH	116.5	6.78d (8.2)	116.5	6.83 d (8.2)
6””	CH	124.3	6.95 dd (8.2 / 2.0)	124.3	7.09 dd (8.2 / 2.0)
α’	CH	115.1	6.37 d (15.9)	115.1	6.38 d (15.9)
β’	CH	148.0	7.65 d (15.9)	148.0	7.67 d (15.9)
C=O	C	168.2	-	168.2	-
$COOCH_3$	CH_3	-	-	56.5	3.88 s

The presence of a methoxyl group on one of the aromatic rings belonging to acyl and aglycone moieties resulted in the differences for chemical shifts for the aromatic protons of acyl units of PF-7 in comparison to those of forsythoside B (PF-3). In the HMBC experiment (Spectrum 4.2.3.3), the ^{1}H, ^{13}C-long range heteronuclear correlations were observed between the C-3"" (149.3) and the protons at δ 7.20 (H-2""), H-5"" of acyl unit and methoxyl signal at δ 3.88 confirming the presence of feruloyl unit as acyl moiety in PF-7. This data was in good aggreement with those alyssonoside reported from *Marrubium alysson* (Çalış et al, 1992). Alyssonoside have also been reported from many Phlomis species growing in Turkey (ÇALIŞ et al., 2004a).

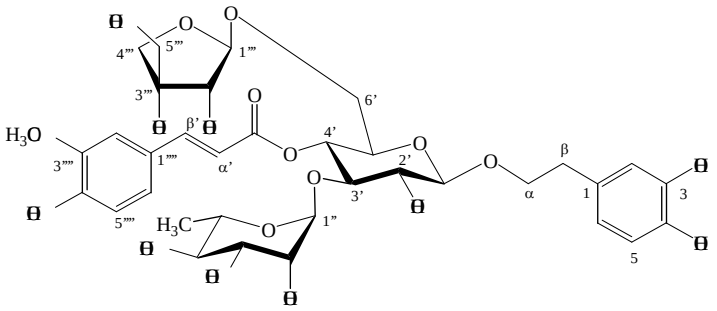
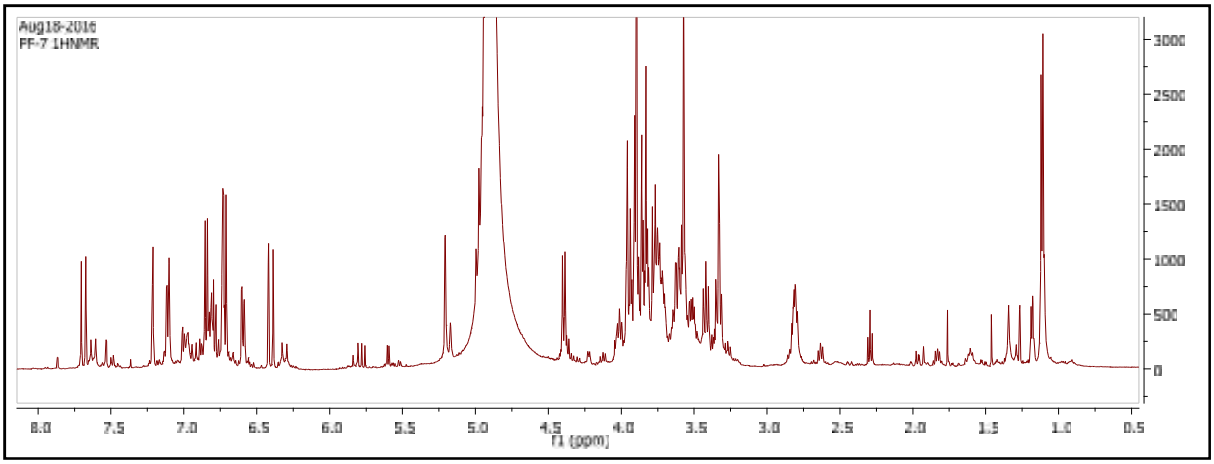


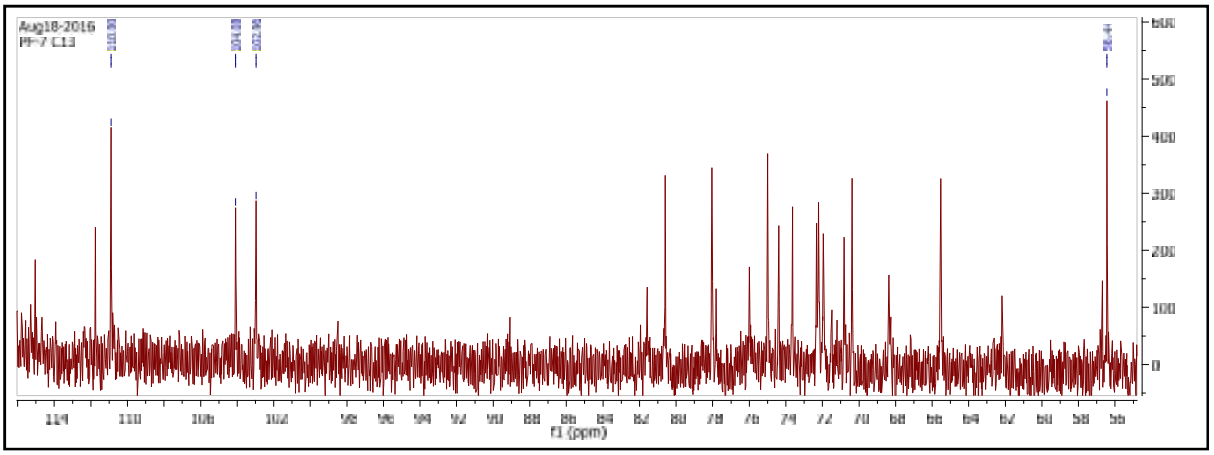
Table 4.2.3.1b. **^{1}H-NMR and ^{13}C-NMR Data of Alyssonoside (PF-7)***
^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
PhEthylalcohol 1	C	131.4	-	H_2-α, H_2-β, H-5
2	CH	116.4	6.72 d (2.0)	H_2-β, H-6
3	C	146.6	-	H-5
4	C	144.7	-	H-2, H-5, H-6
5	CH	117.1	6.70 d (8.0)	
6	CH	121.3	6.58 dd (8.0 / 2.0)	H-β
α	CH_2	72.3	4.04 m, 3.70 m	H-β, H-1’
β	CH_2	36.5	2.79 t (7.4)	
Glucose 1’	CH	104.1	4.36 d (7.9)	H_2-α, H-2’
2’	CH	76.0	3.38 dd (7.9 / 9.0)	
3’	CH	80.1	3.80 dd”t” (9.0)	
4’	CH	70.8	4.94 dd”t” (9.0)	
5’	CH	74.4		
6’	CH_2	68.4		
Rhamnose 1”	CH	103.0	5.18 d (1.4)	H-3’
2”	CH	72.0	3.90†	
3”	CH	71.9	3.56 dd (3.4 / 9.6)	
4”	CH	73.6	3.28 dd”t” (9.6)	
5”	CH	70.4	3.5	
6”	CH_3	18.4	1.08 d (6.2)	
Apiose 1”’	CH	110.9	4.90†	H_2-6’, H-2”’
2”’	CH	78.1	3.86 d (2.2)	
3”’	C	80.6	-	
4”’	CH_2	75.0	3.53 br s	
5”’	CH_2	65.5	3.91 d (9.6), 3.72 d (9.6)	
Feruloyl 1””	C	127.6	-	H-α’, H-β’, H-5””
2””	CH	111.8	7.20 d (2.0)	H-6””
3””	C	149.3	-	H-2””, H-5””, OCH_3
4””	C	150.6	-	H-2””, H-5””, H-6””
5””	CH	116.5	6.83 d (8.2)	
6””	CH	124.3	7.09 dd (8.2 / 2.0)	H-β’
α’	CH	115.1	6.38 d (15.9)	
β’	CH	148.0	7.67 d (15.9)	
C=O	C	168.2	-	H-α’, H-β’, H-4’
OCH_3	CH_3	56.5	3.88 s	

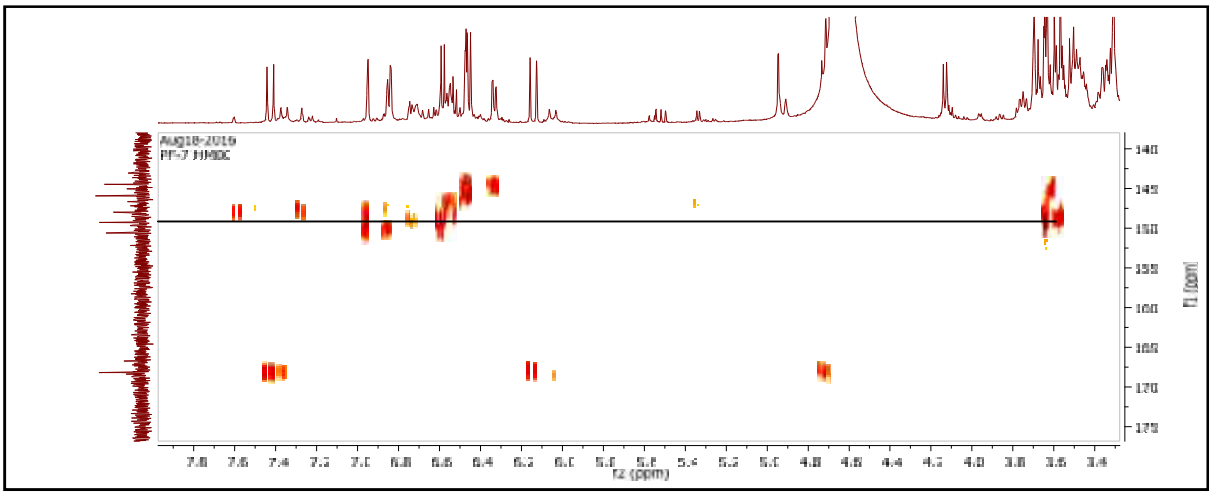
*All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).
†Signal pattern unclear due to overlapping.



Spectrum 4.2.3.1. The ^{1}H-NMR Spectrum of **Alyssonoside** (**PF-7**) (400 MHz, MeOD)



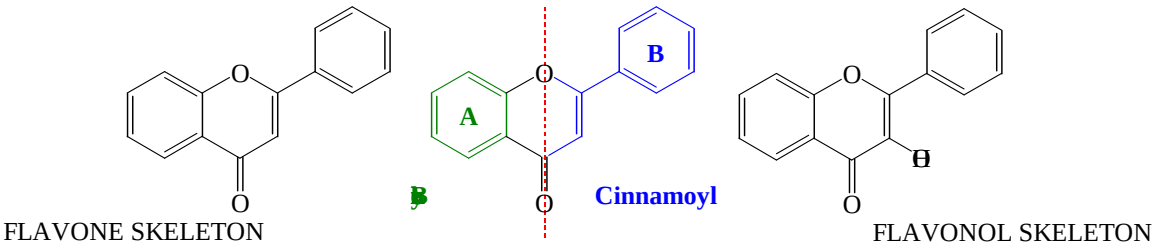
Spectrum 4.2.3.2. The ^{13}C-NMR Spectrum of **Alyssonoside** (**PF-7**) (100 MHz, MeOD)



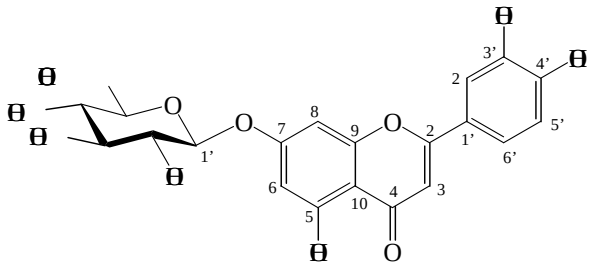
Spectrum 4.2.3.3 HMBC Spectrum of **Alyssonoside** (**PF-7**)

4.3. Flavon Glycoside (PF-2 & 10)

4.3.1. Luteolin 7-*O*-glucuronide



The UV spectra of flavones and flavonols exhibit two mainly two absorption peaks in the region 240 – 400 nm. These two absorptions are commonly referred to as Band I (300 – 380 nm) and Band II (240 – 280 nm). Band I is considered to be associated with absorption due to the B-ring cinnamoyl system, and Band II with absorption involving the A-ring benzoyl system. Band I lies in the 310 – 350 nm for flavones, while for flavonols itis between 350 and 385 nm (Mabry et al., 1970).

	UV $^{a}\lambda_{max}$ 350, 267, 255 nm $[\alpha]_D^{20}$ = - (c 0.1, MeOH)

Compound PF-2 showed the UV absorptions at 255, 267 and 350 nm which was typical for flavons. The NMR data of PF-2 (Table 4.3.1.1) was analyzed using 1D (^{1}H- and ^{13}C-NMR, DEPT-135: Spectra 4.3.1.1 – 4.3.1.3) and 2D NMR experiments (COSY, HSQC and HMBC: Spectra 4.3.1.4 – 4.3.1.6).

In the ^{1}H-NMR spectrum (Spectrum 4.3.1.1), three protons at δ 7.32 (d, *J* = 2.2 Hz, H-2’), 6.91 (d, *J* = 8.2 Hz, H-5’) and 7.45 (dd, *J* = 8.2 and 2.2 Hz, H-6’) were observed as an ABX system suggesting the presence of an *ortho*-disubstituted B ring. Moreover, two *meta*-coupled signals in the aromatic region at δ 6.46 and 6.81 (both d, J = 1.8 Hz) were assigned as H-6 and H-8, respectively. A singlet signal at δ 6.75 was attributed to the H-3

of the aglycone. All of these observations for the ^{1}H-NMR spectrum strongly suggested the presence of a 5,7,3',4'-tetrahydroxy-flavone (= luteolin) as aglycone moiety.

The ^{1}H-NMR spectrum displayed an anomeric proton signal at δ 5.26 (d, *J* = 7.4, H-1"). Apart from this signal the other signals arising the sugar unit were established by the help of COSY experiment (Spectrum 4.3.1.4). Thus, the signal observed at δ 3.31 (dd, *J* = 7.4 & 9.0 Hz, H-2"), 3.33 (dd"t", *J* = 9.0 Hz, H-3"), 3.40 (dd"t", *J* = 9.0 Hz, H-4") and 4.01 (d, *J* = 9.0 Hz, H-5").

The ^{13}C-NMR spectrum of PF-2 exhibited 21 carbon resonances. Of which 15 were assigned to the flavon skeleton, luteolin. The remaining six carbon resonances at δ 99.7 (CH), 73.3 (CH), 76.2 (CH), 71.8 (CH), 75.7 (CH) and 171.0 (C) were atributed to the β-glucuronic acid as sugar unit. This assumption was confirmed by the multiplicty of H-5 (d) and the ^{1}H, ^{13}C-long range heteronuclear correlation between the carbonyl carbon at δ 171.0 and proton signals at δ 3.40 and 4.01 which were assigned to the H-4" and H-5", respectively.

The^{1}H and ^{13}C-NMR assignments (Table 4.3.1.1) are based on to the 2D NMR experiments (COSY, HSQC, HMBC). All intermolecular connectivites and the glycosylation site of the β-glucuronopyranosyl unit was established by HMBC experiment (Spectrum 4.3.1.6.A&B). the ^{1}H, ^{13}C-long range heteronuclear correlation between the carbon resonance assigned as C-7 (δ 163.0) and anomeric proton signals H-1" (δ 5.26) showed the site of glycosylation to be C-7(OH). Based on these results, the structure of PF-2 was established as luteolin 7-*O*-β-glucuronide.

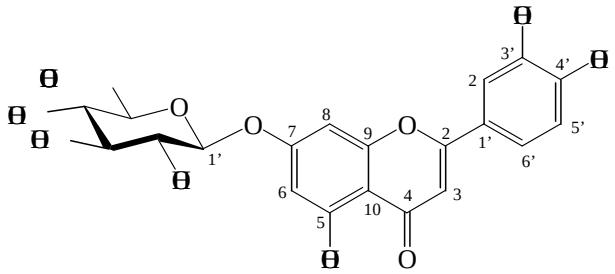
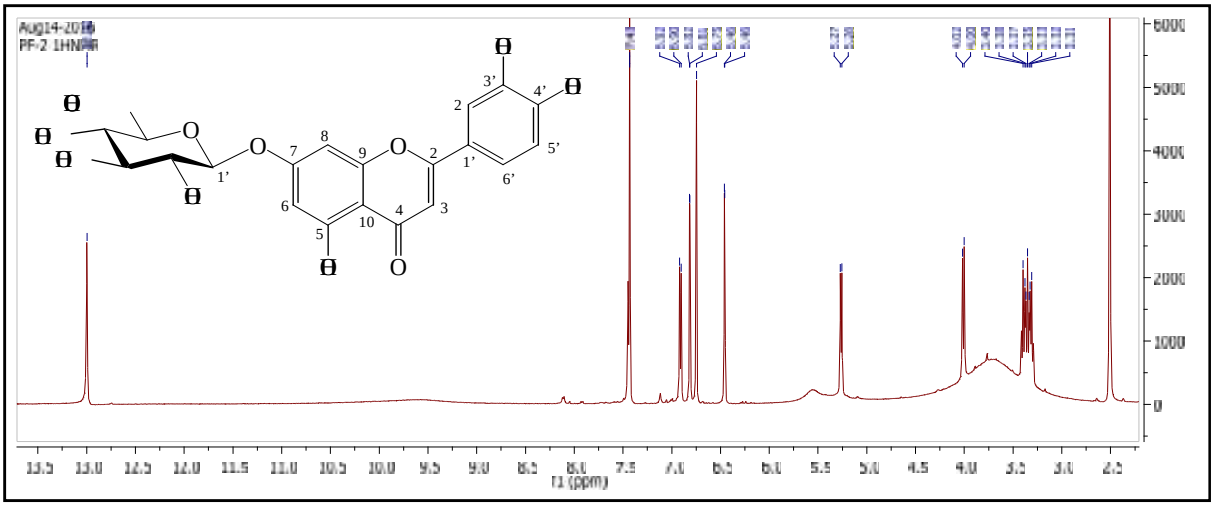


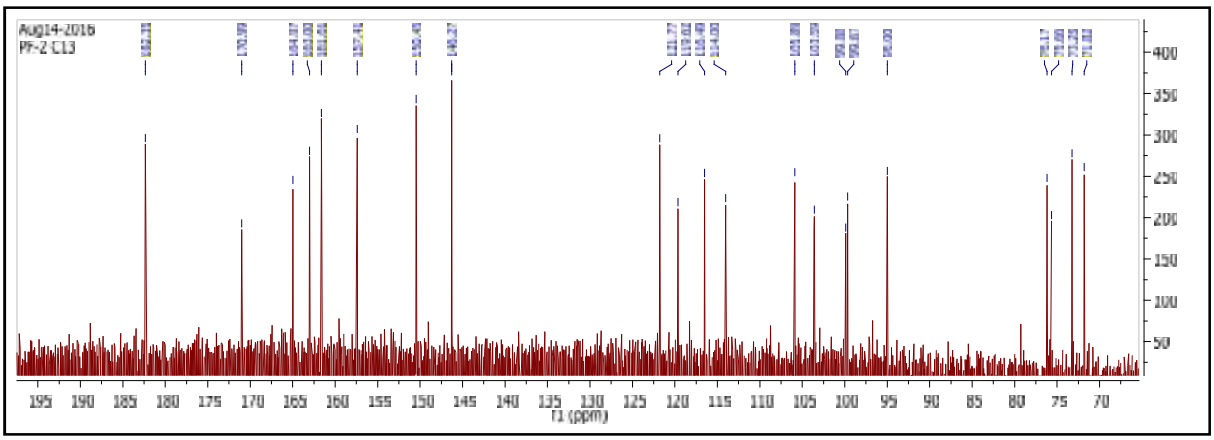
Table 4.3.1.1. **^{1}H-NMR and ^{13}C-NMR Data of Luteolin 7-*O*-glucuronide (PF-2&10)*** ^{1}H: 400 MHz, ^{13}C: 100 MHz, CD_3OD

C No		**δ_C** (ppm)	**δ_H**(ppm),***J***(Hz)	**HMBC (from C to H)**
Apigenin				
2	C	165.0	-	H-3, H-2', H-6'
3	CH	103.6	6.75 s	-
4	C	182.3	-	H-3
5	C	161.6	-	H-6, 5-OH
6	CH	99.9	6.46 d (1.8)	H-8, 5-OH
7	C	163.0	-	H-1", H-6
8	CH	95.0	6.81 d (1.8)	H-6
9	C	157.4	-	H-8
10	C	105.9	-	H-3, H-6, H-8, 5-OH
1'	C	121.8	-	H-3, H-6'
2'	CH	114.0	7.43 d (2.2)	H-5'
3'	C	146.1	-	H-2', H-5'
4′	C	150.5	-	H-2', H-5', H-6'
5′	CH	116.5	6.91 d (8.2)	
6'	CH	119.6	7.45 dd (8.2 / 2.2)	
5-OH	-	-	13.01 s	
Gluc. Acid				
1"	CH	99.7	5.26 d (7.4)	H-3", H-5"
2"	CH	73.3	3.31 dd (7.4 / 9.0)	
3"	CH	76.2	3.33 dd"t" (9.0)	
4"	CH	71.8	3.40 dd"t" (9.0)	
5"	CH	75.7	4.01 d (9.0)	
6"	C	171.0	-	H-4", H-5"

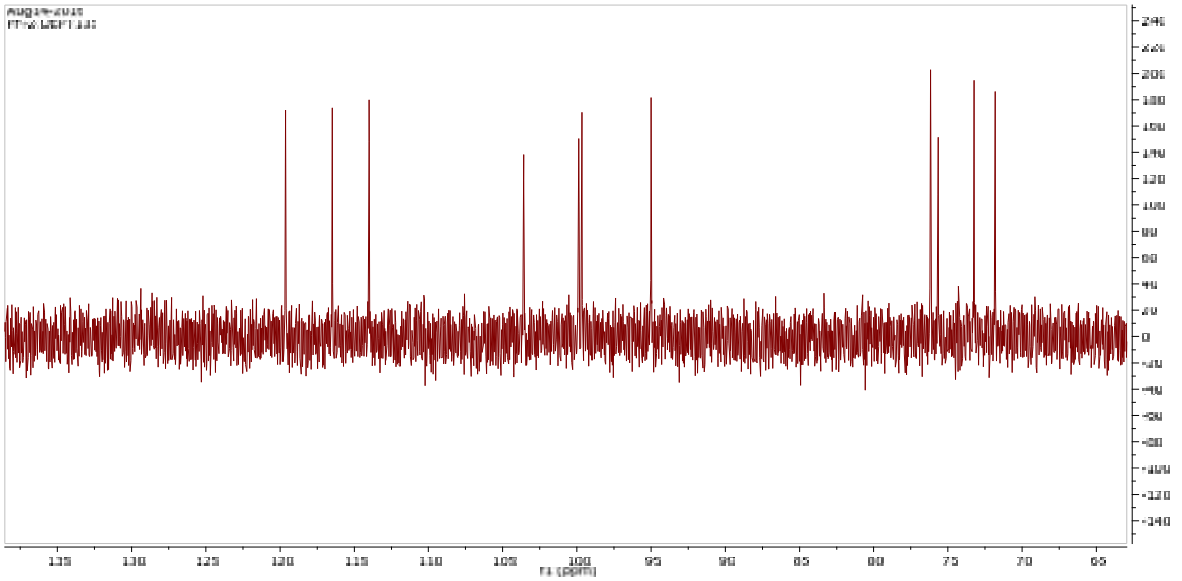
*) All assignments are based on to 2D NMR experiments (COSY, HSQC, HMBC).



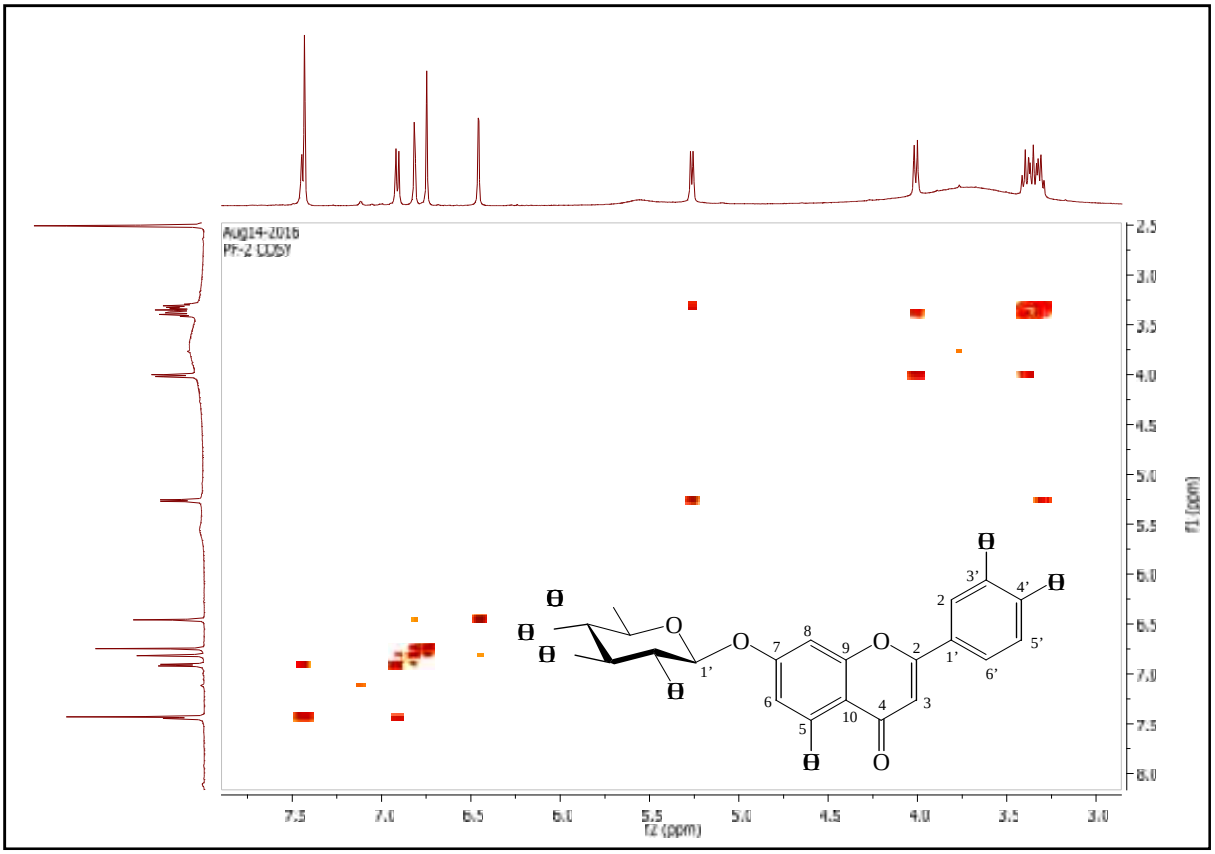
Spectrum 4.3.1.1. The ^{1}H-NMR Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)** (400 MHz, MeOD)



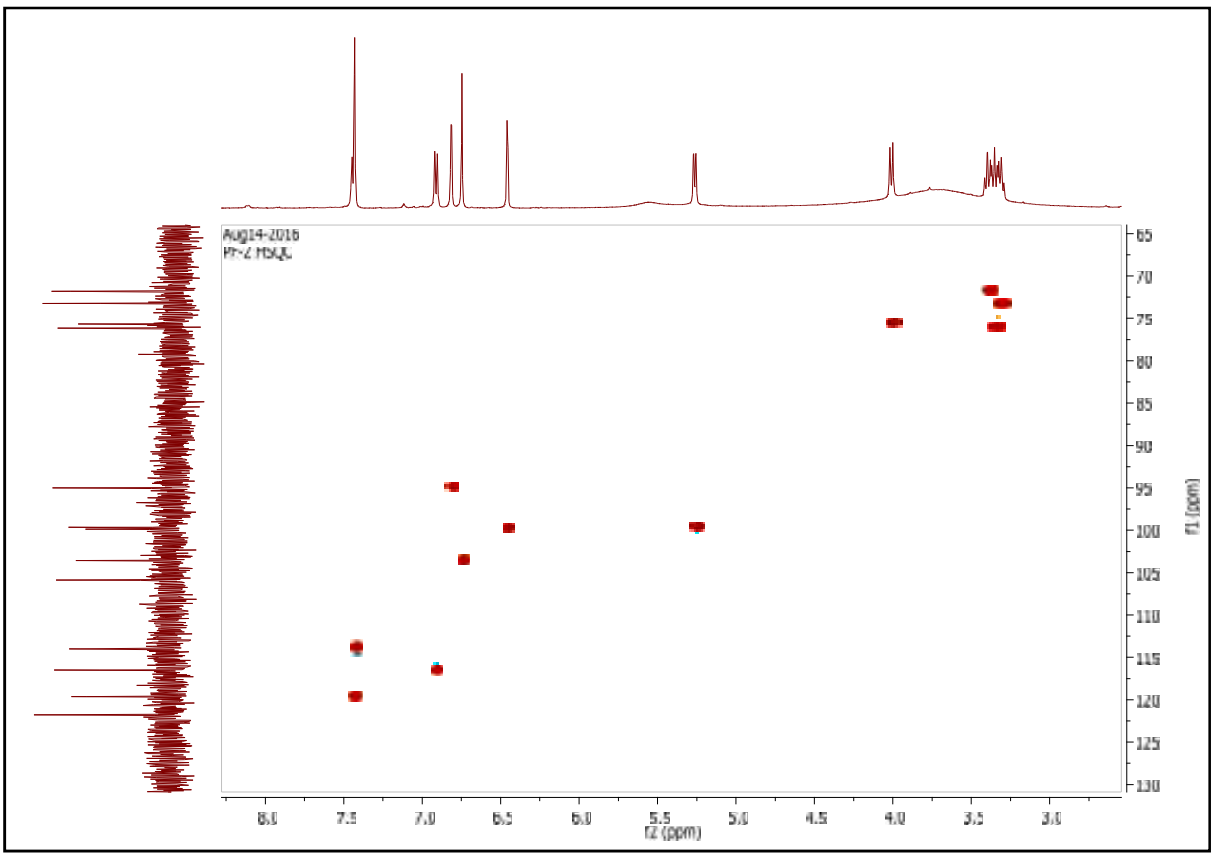
Spectrum 4.3.1.2. The ^{13}C-NMR Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)** (100 MHz, MeOD)



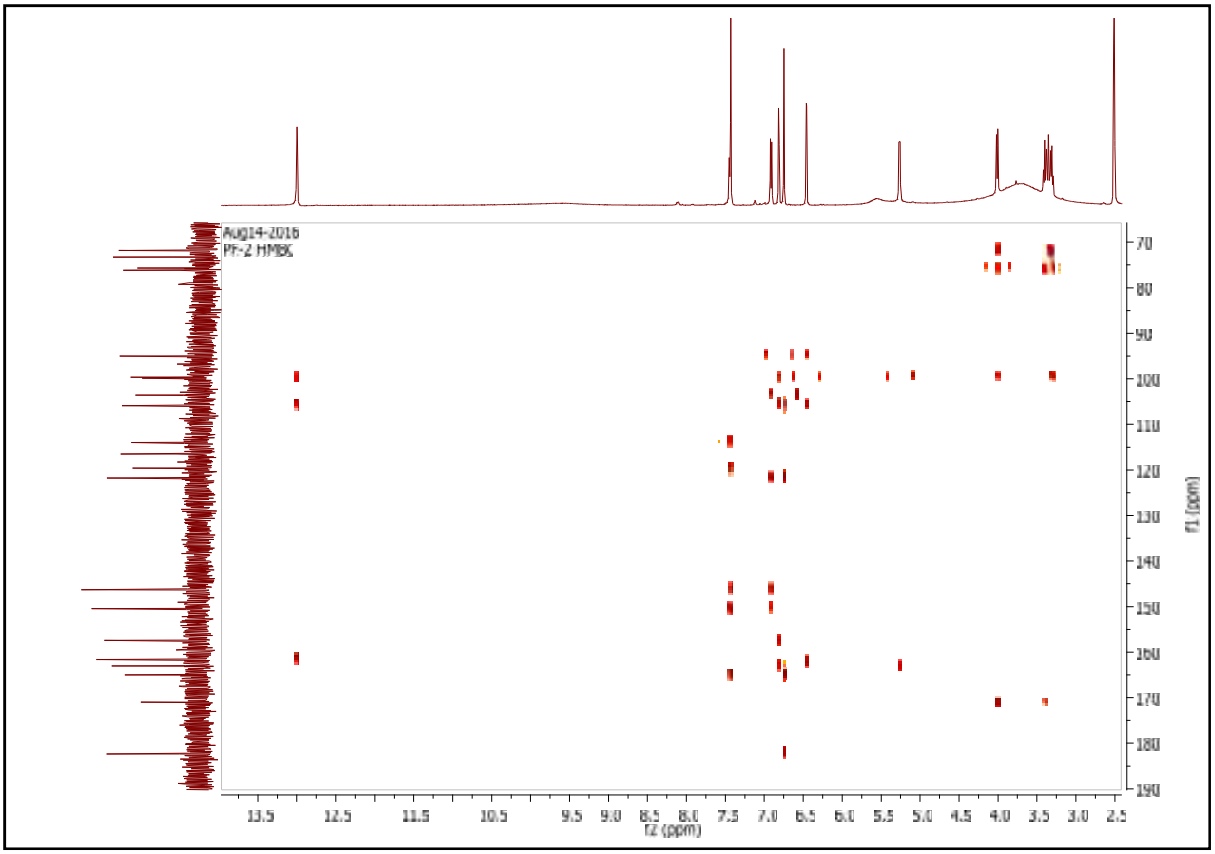
Spectrum 4.3.1.3. DEPT-135 Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)**



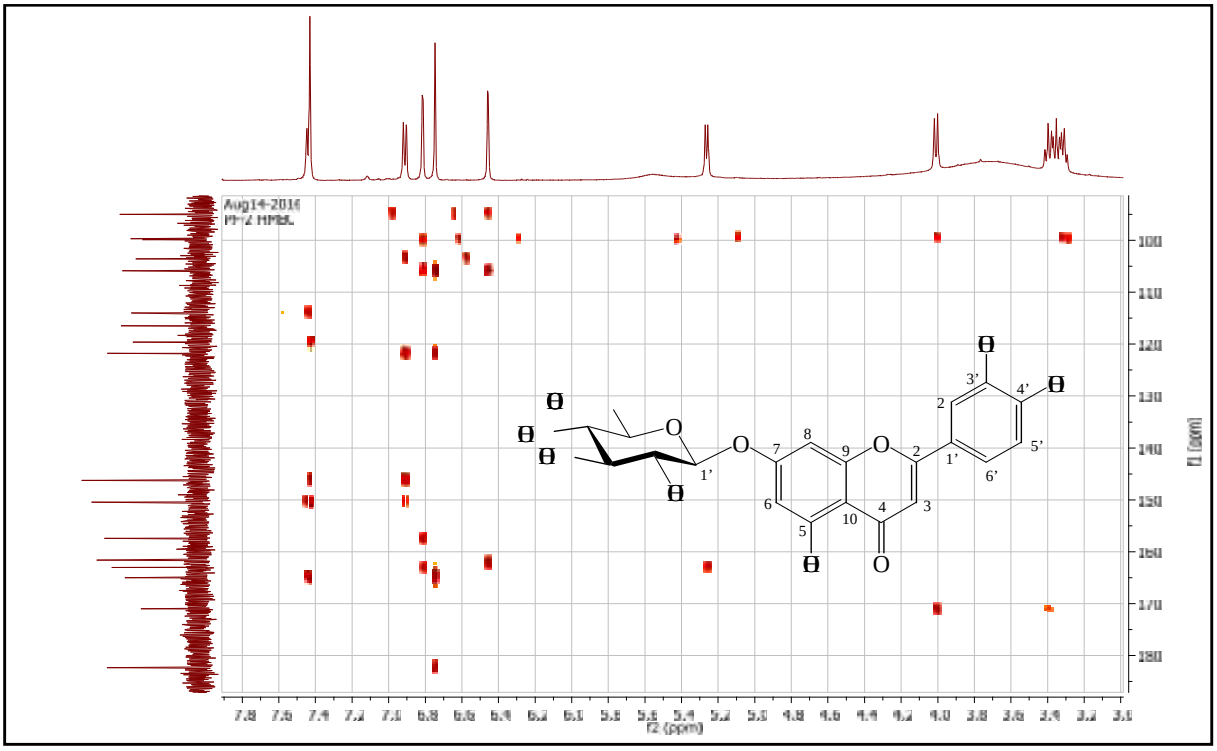
Spectrum 4.3.1.4. COSY Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)**



Spectrum 4.3.1.5. HSQC Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)**



A: All[1]H, [13]C- Long Range Correlations



B: [1]H, [13]C- Long Range Correlationsshowing intermolecularconnectivities

Spectrum 4.3.1.6.A&B. HMBC Spectrum of **Luteolin 7-*O*-Glucuronide PF-2&10)**

5. Conclusion:-

In this study, *Phlomis floccosa* D. Don was investigated from the view point of their phenylethanoid glycosides, iridoids and flavonoids.Three iridoid glycosides namely, lamiide (PF-1& 9), ipolamide (PF-5) and auroside (PF-6) together with three phenylethanoid glycosides, forsythoside B (PF-3), verbascoside (PF-4&8), alyssenoside (PF-7) and one flavonoid glycoside luteolin-7-*O*-glucurunoide were isolated and identified. All these compounds have also been reported from several *Phlomis* species.

The occurrence of iridoids and flavonoids in this plant collected from Egypt flora are somewhat different from that of *P. floccosa* of Libyan origin by comparing the results of reports. However, this is the first case of characterization of these compounds from *Phlomis floccosa* especially phenylethanoid glycosides

6. REFERENCES

Aboutabl, E.A., Meselhy, M.R., Afifi, M.S. (2002). "Iridoids from *Phlomis aurea* Decne growing in Egypt". Pharmazie. 57, PP: 646 - 647.

Assaad, A.M., EL-Sebakhy, N., Lahloub, M.F.I., EL-Khayat, S.A.A., (1992). "Iridoid Glucosides of *Phlomis floccosa* D. Don". Alex. J. Pharm. Sci. 6(2), PP: 183-185.

Bader, A., Tuccinardi, T., Granchi, C., Martinelli, A., Macchia, M., Tommasi, F.M.N., Braca, A., (2015). " Phenylpropanoid and Flavonoids from *Phlomis kurdica* as inhibitor of human lactate dehydrogenase". Phytochemistry, 116, PP: 262-268.

Çaliş, İ., Kirmizibekmez, H., Ersoz, T., Donmez, A.A., Gotfredsen, C.H., Jensen, S.R. .(2005b). "Iridoid glucosides from Turkish *Phlomis tuberosa*". Zeitschriftfur Naturfoschung. 60b, PP: 1295-1298.

Çalış, İ., Basaran, A.A., Saracoglu, I., Sticher, O., Ruedi, P., (1991). "Phlinosides A-Band C, three Phenypropanoid glycosides from *Phlomis linearis*". Phytochemistry. 30 (9), PP:3073-3075.

Çalış, İ., Hosny, M., Khalifa, T., Rüedi, P. (1992). "Phenylpropanoid Glycosides from *Marrubium alysson"*. Phytochemistry 31, 3624 – 3626.

Çalış, İ., Kirmizibekmez, H., Beutler, J.A., Donmez, A.A., Yalcin, F.N., Killic, E., Ozalp, M., Ruedi, P. (2005a). "Secondary Metabolites of *Phlomis viscosa* and Their biological activities". Turk. J. Chem. 29, PP: 71-81.

Çalış, İ., I., Bedir, E., Kirmizibekmez, H., Ersoz, T., Donmez, A.A., Khan, I.A. (2005c) "Secondary Metabolites from *Phlomis oppositiflora*". Natural. Product. Research. 19(5), PP:493-501.

Çalış, İ. and Kirmizibekmez, H., (2004b). "Glycosides from *Phlomis lunariifolia"*. Phytochemistry. 65, PP: 2619–2625.

Çalış, İ., Türkiye *Phlomis* L. (Lamiaceae) Cinsinin Kemotaksonomisi [Chemotaxomoy of Genus *Phlomis* L. (Lamiaceae) from the Flora of Turkey]. TUBITAK (Technical and Research Council of Turkey). (2004 a). Project No: SBAG 2304.

Damptof, S., Jensen, S.R., Nielsen, B.J. (1984). The Identity of the Iridoid Glucoside Tarphetalin with Ipolamiide. Phytochemistry 23 (4), PP: 907-908.

Delnavazi, M.R., Mohammadifar, F., Rustaie, A., Aghaahmadi, M., Yassa, N., (2016). "Phytochemical constituents, Antioxidants activity and cytotoxicity potential of *Phlomis olivierii-Benth* ". Research. J. Pharmacognosy. 3 (2), PP: 9-1.

Delzar, A., Sabzevari, A., Mojarrab, M., Nazemiyeh, H., Esnaashari, S., Nahar, L., Razavi, S.M., Sarker, S.D., (2008). "Free-radical-scavenging principles from *Phlomis caucasica* ". Natural Medicine. (Tokyo). 62, PP: 464-466.

Dinda, B., Dehanth, S., Harigayal., (2007). " Naturally occurring iridoids. A review, part I". Chem. Pharm. Bull. (Tokyo). 55 (2), PP: 159-222.

EL-Negoumy, S.I., Abdalla, M.F., Saleh, N.A.M., (1986). "Flavonoids of *Phlomis aurea and Phlomis floccosa* ". Phytochemistry. 25, PP: 772-774.

EL-Mokasabi, F.M., (2014). "Floristic Composition and Traditional Uses of Plant Species at Wadi ALkuf, Al-jabal Al-Akhder, Libya ". American-Eurasian. J. Agric and Environ. Sci. 14(8), PP : 685-697.

Endo, K., Takahashi, K., Abe, T. (1982). Structure of Forsythoside B, An Antibacterial Principle of *Forsythia koreana* Stems. Heterocycles 19 (2), pp. 261 – 264.

Ersöz, T., Schuhly, W., Popov, S., Handjieva, N., Sticher, O., (2001). "Iridoid and Phenylethanoid glycosides from *Phlomis longifolia var. longifolia* ". Natural. Product. Letters. 15, PP: 345-351.

Ersöz, T., Allipieva, K.I., Yalcin, F.N., Akbasy, P., Handjieva, .N., Dönmez, A.A., Popov, S., Çalış, İ., (2003). "Physocalycoside ,a new phenylethanoid glycoside from *Phlomis physocalyx Hub-Mor*". Z. Naturforsch. 58c, PP: 471-476.

Ersöz, T., Harput, U.S., Çalış, İ., Dönmez, A.A., (2002). "Iridoids and Phenylethanoid and Monoterpene glycosides from *Phlomis sieheana*". Turk. J. Chem. 26, PP: 1-8.

Evans, W.C., (2009). "Trease and Evans, Pharmacognosy ". 15th. Edition, Elsevier. Health. Sciences. Pages: 252, 246-248.

Gürbüz, I., Üstün, O., Yesilada, E., Sezik, E., Kutsal, O., (2003). "Anti-ulcerogenic activity of some plants used as folk remedy in Turkey". Journal of Ethnopharmacology. 88, PP: 93–97.

Harput, Ü.S., Çalış, İ., I., Saracoglu, I., Dönmez, A.A., Nagatsu, A., (2006). "Secondary Metabolites from *Phlomis syriaca* and their Antioxidant activities". Turk. J. Chem. 30, PP: 383-390.

Hussain, J., Bukhari, N., Hussain, H., Bano, N., Abdul Naeem and Green, I.R., (2010) "Flavonoids and Terpenoids from *Phlomis cashmeriana* and their chemotaxonomic significance". Rec. Nat. Produ. 4(4). PP: 242-249.

Ismailoglu, U.B., Saracoglu, I., Harput, U.S., Sahin-Erdemli, I., (2002). "Effects of phenylpropanoid and iridoid glycosides on free radical-induced impairment of endothelium-dependent relaxation in rat aortic rings". Journal Ethnopharmacology. 79, PP:193–197.

Jimenez, C and R, Riguera., (1994). " Phenylethanoid glycosides in Plants : Structure and biological Activity". Natural. Product. Reports, PP: 591-606.

Junior, P. (1990). Recent Develpoments in the isolation and structure elucidation of Naturally-Occurring Iridoid Compounds. Planta Med. 56, pp. 1 – 13.

Kamel, M.S., Khaled, M.M., Hassanean, H.A., Ohtani, K., Kasai, R., Yamasaki, K., (2000). "Iridoid and megastigmane glycosides from *Phlomis aurea*". Phytochemistry. 55, PP: 353-557.

Kirmizibekmez, H., Çalış, İ., Perozzo, R., Brun, R., Donmez, A., Linden, A., Ruedi, P., Tasdemir, D., (2004a). "Inhibiting activities of the secondary metabolites of *Phlomis brunneogaleata* against parasitic protozoa and plasmodialenoyl-ACP Reductase, a crucial enzyme in fatty acid biosynthesis". Planta. Medica. 70, PP: 711–717.

Kirmizibekmez, H., Piacente, S., Pizza, C., Dönmez, A.A., Çalış, İ., (2004b). "Iridoid and Phenylethanoid glycosides from *P.nissolii and P.capitata*". Z.Naturforsch. 59b, PP: 609-613.

Kyriakopoulou, I., Magiatis, P., Skaltsounis, A.L., Aligianis, N., Harvala, C., (2001). "Samioside, a new phenylpropanoid glycoside with free radical scavenging and antimicrobial activities from *Phlomis samia"*. Journal of Natural Product. 64, 1095–1097.

Limem-Ben Amor, I., Bouaker, J., Ben-Sgaier, M., Skandrani, I., Bhouri, W., Neffati, A., Kilani, S., Bouhlel, I., Ghedira, K., Ghedira, L.C., (2009a). "Phytochemistry and biological activities of *Phlomis* species". Journal of Ethanopharmacology . 125, PP:183-202.

Limem-Ben Amor, I., Skandrani, I., Boubaker, J., Ben Sghaïer, M., Neffati, A., Bhouri, W., Bouhlel, I., Chouchane, N., Soumaya Kilani, S., Guedon, E., Ghoul, M.,Ghedira, K., Chekir-Ghedira, L., (2009b). "Investigation of biological activity of polar extracts isolated from *Phlomis crinita* Cav ssp. *mauritanica* Mun". Drug and Chemical Toxicology. 32, PP: 38–46.

Lŏpez, V., Jager, A.K., Akerreta, S., Cavero, R.Y., Calvo, M.I., (2010). "Antioxidant Activity and phenypropanoids of *Phlomis lychnitis L.* A Traditional Herbal Tea". Plant. Foods. Hum. Nutr. 65,PP: 179- 185.

Mabry, T.J., Markham, K.R., Thomas, M.B. (1970). The Systematic Identification of Flavonoids. Springer, New York.

Marin, P.D., Veitch, N., Grayer, R.J., Kite, G.C., Sokovic, M., Janackovic, P., (2007). "Flavonoids from *Phlomis fruticosa (lamiaceae)* growing in Montengro" Biochemical, Systematics and Ecology. 35, PP: 462-466.

Nazemiyeh, H., Rahman, M.M., Gibbons, S., Nahar, L., Delazar, A., Ghahramani, M.A., Talebpour, H., Sarker, S.D., (2008). "Assessment of the antibacterial activity of phenylethanoid glycosides from *Phlomis lanceolata* against multiple-drugresistant strains of *Staphylococcus aureus"*. Natural Medicine. (Tokyo). 62,PP: 91–95.

Ozdemir, C., Durmuskahya, C., Sepet, H., Bozdag, B., (2014). "Karyological studies on some *Phlomis*. L Taxa (Lamiaceae)". Pak. J. Bot. 46(1), PP: 91-94.

Saracoglu, I., Inoue, M., Çalış, İ., Ogihara, Y., (1995). "Studies on constituents with cytotoxic and cystostatic activity of two Turkish medicinal plants *Phlomis armeniaca and Scutellaria salviifolia*". Biological and Pharmaceutical Bulltein. 18(10), PP: 1396-1400.

Saracoglu, I., Varel, M., Hada, J., Hada, N., Takeda, T., Donmez, A.A., Çalış, I., (2003). "Phenylethanoid glycosides from *Phlomis integrifolia* Hub-Mor". Zeitschriftfur, Naturforschung. 58C, PP: 820–825.

Sarkhail, P., Esfehani, H.R., Amin, G., Surmaghi, M.H.S., Shafiee, A., (2006). "Phytochemical study of *Phlomis olivierii-Benth* and *Phlomis persica Boiss*". DARUV. 14 (3), PP: 115-121.

Sarkhail, P., Rahmanipour, S., Fadyevatan, S., Mohammadirad, A., Dehghan, G., Amin, G., Shafiee, A., Abdollahi, M., (2007). "Antidiabetic effect of *Phlomis anisodonta*: Effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes". Pharmaceutical Research. 56, PP: 261–266.

Sarkhail, P., Sahranvard, S., Nikan, M., Gafari, S., Tehrani, B.E., (2017). “Evalution of the cytotoxic activity of extracts from six species of *Phlomis* genus”. Journal of Applied Pharmaceutical Sciences.7(2), PP: 180-184.

Sarikurkcu, C., Uren, M.C., Tepe, B., Cengiz, M., Kocak, M.S., (2014). “Phenolic content, Enzyme inhibitory and antioxidant activity potentials of *Phlomis nissolii and Phlomis pungens var. pungens*”. Industral Crops and Products. 62, PP: 333-340.

Shang, X., Wang, J., Li, M., Miao, X., Pan, H., Yang, Y., Wang, Y., (2011). “Antinociceptive and anti-inflammatory activity of *Phlomis umberosa* Turcz extract”. Fitoerpia. 82, PP: 716-721.

Siqqid, M.A., (1985) “Flora of Libya ”.The National Academy For Scientific Research. 118, PP: 70-71.

Sticher, O., Lahloub, M.F. (1982). Phenolic Glycosides of *Paulownia tomentosa* Bark. Planta Med. 46 (3), PP: 145 – 148.

Soltani-Nasab, F., Asparpanah, J., Majdzadeh, M., Ostad, S.N., (2014). “Investigation the effect of *Phlomis lanceolatae* Boiss and Hohen on cancer cell lines”. Acta. Medica. Iranicea, Tehran University of Sciences. 52 (5), PP: 333-336.

Takeda, Y., Isai, N., Masuda, T., Honda, J., Takaishi, Y., Ito, M., Otsuka, H., Ashurmetov,A.O., Khodzhimatov, K.O., (2001). “Phlomisflavosides A and B, new flavonolbisglycosides from *Phlomis spinidens”*. Chemical and Pharmaceutical Bulltein. 49, PP: 1039–1041.

Takeda,Y., Matsumur, H., Masud, T., Honda, G., Otsuka, H., Takaishi, Y., Sezik, E., Yesilada, E., (2000). “Phlorigidosides A-C, Iridoid glucosides from *Phlomis rigida*”. Phytochemistry. 53, PP: 931-935.

Takeda,Y., Kinougawa, M., Masuda, T., Honda, G., Otsuka, H., Sezik, E., Yesilada, E., (1999). "Phlomisethanoside, a phenyethanoid glycoside from *Phlomis grandifolora var.grandiflora*". Phytochemistry. 51, PP: 323-325.

Tasdemir, D., Brun, R., Perozzo, R., Dönmez, A.A., (2005). "Evaluation of antiprotozoal and plasmodial enoyl-ACP reductase inhibition potential of Turkish medicinal plants". Phytotherapy Research. 19, PP: 162–166.

Tomas, F., Nieto, J.L., Barberan, F.A.T., Ferreres, F., (1986). "Flavonoids from *Phlomis lychnitys"*. Phytochemistry. 25, PP: 1253–1254.

Wafa, N., Sofiane, G., Mouhamed, K., (2016). "The antioxidant and antimicrobial activities of flavonoids and tannis extracted from *Phlomis bovei"*. European. J of Experimental biology. 6(3), PP: 55-61.

Wang, Q.S., Yang, L., Cui, W.Y., Chen, L., Sing, Y.H., (2014). "Anti-inflamatory and Antinoceciptive activities of methanol extract from Aerialparts of *Phlomis younghusbandii* Mukerjiee". POLS ONE. 9(3), PP: 89-149.

Yalcin, F.N., Ersoz, T., Bedir, E., Sahpaz, S., Bailleul, F., Khan , I.A., Donmez, A.A., Çaliş, I.,(2005). "Phlinoside F, a new phenyethanoid glycoside from *Phlomis angustissima*". Turk. J. Chem. 29, PP: 417-423.

Yalcin, F.N., Ersoz, T., Akbay, P., Çalış, İ. (2003). "Iridoid and phenypropanoid glycosides from *Phlomis samia, Phlomis monocephala and Phlomis carica*". Turk. J. Chem. 27, PP: 295-305.

Yalcin, N.F., Erzos, T., Saracoglu, I., Donmez, A.A., Çalış, İ. (2006). "Further Phenylethanoid glycosides from *Phlomis physocalxy Hub-Mor*". Hacettepe university journal of the Faculty of Pharmacy. 26(1), PP: 13-21.

Yang,Y., Gu, L., Xiao, Y., Lin, Q., Hu, H., Wang, Z., Chen, K. (2015) "Rapid identification of α-glucosides inhibitor from *Phlomis tuberosa* by Sephox chromatography and TLC Biogautography". POLS ONE. 10(2). PP: 1371.

Zhang, C.Z., Li, C., Feng, S.I., Shi, J.G., (1991) "Iridoid glucoside from *Phlomis rotata*". Phytochemistry. 30, PP:4156-4158.

Zhang,Y and Wang , Z.Z., (2009). "Phenolic composition and Antioxidant activities of two *Phlomis* species, Acorrletation study". C. R. Biologies. 332, PP:816-826.

www.ville-ge.ch/musinfo/bd/cjb/africa/images/data/images/Phlomis%20floccosa: A.Dobignard).

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