Ministry of Higher Education and Scientific Research
University of Mohamed Boudiaf-M'sila
Faculty of Sciences
Department of Chemistry
Order N°:

Doctoral Thesis in Sciences

Thesis submitted for the degree of Doctor of Sciences

Specialty: Chemistry

Presented By

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Theme

The phytochemical investigation of the Algerian medicinal Plant *Fraxinus xanthoxyloides*

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2017/2018

Abbreviations and symbols

EtOAc	Ethyl acetate
MeOH	Methanol
$CHCl_3$	Chloroform
CD_3OD	Deuterated methanol
DMSO-D_6	Deuterated dimethylsulfoxyde
D_2O	Deuterated water
TLC	Thin layer chromatography
PTLC	Preparative thin layer chromatography
CC	Column chromatography
SiO_2	Slica gel
LPLC	Low pressure liquid chromatography
HPLC	High pressure liquid chromatography
R_t	Retention time
TMS	Tetramethylsilane
δ	Chemical shift in ppm
J	Coupling constant in Hertz (Hz)
s	Singlet
brs	Broad singlet
d	Doublet
dd	Doublet doublet
ddd	Doublet doublet doublet
t	Triplet
dt	Doublet triplet
q	Quartet
dq	Doublet quartet
m	Multiplet
^{1}H NMR	Proton nuclear magnetique resonance
^{13}C NMR	Carbon nuclear magnetique resonance

HSQC	Heteronuclear single quantum correlation
HMBC	Heteronuclear multiple bond correlation
DEPT	Distortionless enhancement polarisation transfer
ROESY	Rotating-frame overhauser effect spectroscopy
MS	Mass spectroscopy
ESIMS	Electrospray ionisation mass spectroscopy
HRESIMS	High resolution electrospray ionisation mass spectroscopy
m/z	Masse/charge of ion
UV	Ultraviolet
$[\alpha]_D$	Optical activity referred to Sodium ray
IPP	Isopentyl pyrophosphate
GPP	Geranyl pyrophosphate
CoA	Coenzyme A as part of thioester
MVA	Mevalonic acid
Glc	Glucopyranosyl
Rha	Rhamnose
Api	Apiose
DPPH	2, 2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
TPC	Total phenolic content
TFE	Total flavonoid evaluation
FXB	*Fraxinus xanthoxyloides* stem bark
FXL	*Fraxinus xanthoxyloides* leaves
FAB	*Fraxinus angustifolia* stem bark
FAL	*Fraxinus angustifolia* leaves

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Summary

Chapter III: Phytochemical study

Chapter IV: Experimental section

Introduction

Introduction

Throughout the ages, humans have relied on nature for their basic needs: food-stuffs, shelters, clothing, means of transportation, fertilizers, flavours, fragrances and medicines.

Plants which have been the basis of traditional medicine systems for thousands of years in countries such as China and India, continue to provide mankind with new remedies. Even some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is still based on the empirical findings of hundreds and thousands of years [1, 2].

Although modern biomedicine to a significant degree employs synthetic drugs as therapeutic agents, plants still occupy a prominent place in contemporary pharmacy, either as sources of pharmaceutical drugs in the form of isolated plant compounds, as sources of precursors to drugs, or as sources of compounds that have served as models for synthetic or semisynthetic drugs. However, many compounds used in today medicine have a complex structure and synthesizing these bioactive compounds chemically at a low price is not easy. Moreover, with increasing realization of the health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, interest in the use of plants has revived throughout the world, and this can be attested from the fact that most of the pharmaceutical industry is highly dependent on wild population for the supply of raw material for extraction of medicinally important compounds.

Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world and in which higher plants contribute to no less than 25% [3]. During the last 40 years, at least a dozen potent drugs have been derived from flowering plants including: *Dioscorea* spp. derived diosgenin from which all anovulatory contraceptive agents have been derived; reserpine and other anti-hypertensive and tranquilizing alkaloids from Rauwolfia species; pilocarpine to treat glaucoma and dry mouth, derived from a group of south American trees (*Pilocarpus* spp.) in the Citrus family; two powerful anti-cancer agents from the *Catharanthus roseus*; laxative agents from *Cassia* sp. and as a cardiotonic agent to treat heart failure from *Digitalis* species.

The expansion of the market for herb demands strict standards for ingredients and manufacturing. The standardization of the herbal preparations requires a detailed study of their

chemical composition and finding of the active components. Herb remedies that enjoy the greatest popularity are generally those that have been the most thoroughly investigated.

In this context, and within the framework of the search for molecules with new biological activities of vegetable origin, it is thus preferable not to choose the plants to be studied randomly, but to circumscribe them according to their use in traditional or popular medicine that depends specifically on the empirical knowledge of peoples concerning medicinal substances.

The genus *Fraxinus* L. belongs to the Oleaceae family and comprises of about 43 species. In the flora of Algeria the genus is represented by two species *Fraxinus xanthoxyloides and Fraxinus angustifolia* [4, 5]. *Fraxinus* species have been used in folk medicine in different parts of the world for their diuretic and mild purgative effects as well as for the treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp [6, 7]. In recent years, an increased interest in the phytochemistry of the genus Fraxinus has been motivated by the discovery of the secoiridoid compounds that constitute the major secondary metabolites and shown an interesting spectrum of biological activities [8]. However, to the best of our knowledge, very few chemical studies has been carried out on the Algerian *Fraxinus* species to date.

The main objective of this work was to define the chemical constituents of *Fraxinus xanthoxyloides* and to evaluate the anti-oxidant capacity of this plant.

This thesis has been divided into four chapters as following described.

The first chapter, is devoted to the Oleaceae family and Fraxinus genus. The previous phytochemical studies reported in the literature for this genus are described by referring to the different groups of secondary metabolites.

In the second chapter, the different groups of the major secondary metabolites described in first chapter, their biosynthesis pathway and their biological interest are presented in a general way.

The third chapter describes the chemical results of the phytochemical investigation carried on *Fraxinus xanthoxyloides* species. The isolation and the structural elucidation of all pure compounds obtained are reported in details including the spectroscopic analysis conducted by using NMR and mass techniques.

The fourth chapter reports the experimental part describing all the steps of separation and purification used in this work, and the physicochemical data of all identified compounds. The manuscript includes a conclusive part that summarizes the contribution given by this thesis to the phytochemistry of plants.

CHAPTER I

Literature survey

I.1 Introduction

Many plants belonging to the Olive family, Oleaceae, have a history of medicinal use in systems of traditional medicine. With regard to chemical constituents, they have been quite extensively investigated. They are mainly characterized by the presence of iridoid glycosides and phenylethanoid derivatives. Coumarins and lignin glycosides are also common in the family, but they appear to have a more limited distribution [9].

This chapter provides background information on the Oleaceae family and the genus included in the present work, with emphasis on chemistry and pharmacology.

I.2 The Oleaceae family

The Oleaceae family, is a dicotyledonous family in the order Lamiales. The family comprises 24 extant genera with about 600 species [10]. The family is distributed on all continents except the Antarctic, from northern temperate to southern subtropical regions and from low to high elevations. The members of the family are trees, shrubs, or woody climbers. The taxonomy of the Oleaceae according to E. Wallander [11] is outlined in Table I.1.

Table I.1: Taxonomy of the family Oleaceae.

Phylum	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Oleaceae

In recent classification of the Oleaceae the subfamily level is abandoned, the family is split into five tribes (Table I.2).

Table I.2: Tribes and representative genera of the Oleaceae [12]

Tribe	Representative genera
Oleeae	Syringa, Ligustrum, Schrebera, Comoranthus, Fraxinus, Chionantus, Forestiera, Haenianthus, Hesperelaea, Nestegis, Noronhia, Notelaea, Olea, Osmanthus, Phyllurea, Picconia, Priogymnanthus.
Myxopyreae	Myxopyrum, Nyctanthes, Dimetra.
Fontanesieae	Fontanesia
Forsythieae	Abeliophyllum, Forsythia.
Jasmineae	Jasminum, Menodora.

I.3 Presentation of the genus Fraxinus

The genus Fraxinus, the ashes, comprises approximately 43 species occurring in temperate and subtropical regions of the northern hemisphere. The two main distribution areas are North America (20 species) and Eastern Asia (20 species). Three species occur in Europe and western Asia. Fraxinus is a member of the Oleaceae and sole member of the subtribe Fraxininae, in the tribe Oleeae [12, 13].

The genus is monophyletic and mostly having relatively large imparipinnate leaves and one seeded samaras. The small flowers have only one pistil and two stamens. The corolla may be lacking or consists of four (rarely two), white, linear, and free (rarely fused) petals. The synsepalous calyx is small, cup- shaped, and usually dentate, or lacking. The petaliferous and insect-pollinated flowers are born in large showy panicles that emerge together the leaves from terminal buds. The apetalous flowers, which are wind pollinated, occur in lateral or terminal inflorescences. The syncarpous ovary contains four ovules, two in each locule, but normally develops into one seeded samaras. Most of the species are large or medium sized trees, but some are shrubs in dry areas [4].

The genus is divided into six sections: Fraxinus, Sciadanthus, Pauciflorae, Melioides,Ornus and Dipetalae [11].

Fraxinus species have been used in folk medicine in different parts of the world for their diuretic and mild purgative, effects as well as for the treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp [7]

In Algeria, the genus Fraxinus is represented by two native species which are *Fraxinus xanthoxyloides* and *Fraxinus angustifolia* [5].

I.4 Fraxinus chemistry

The presence of coumarins, secoiridoids, and a phenylethanoids is a characteristic feature of fraxinus species [6].

I.4.1 Coumarins

The coumarins have been found in a free form or as glucosides (Figure I.1) in all investigated Fraxinus species so far [8, 14-16].

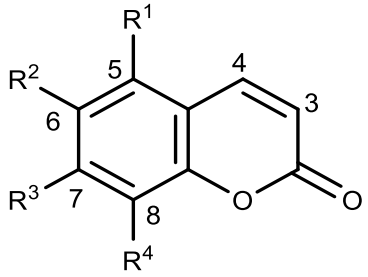
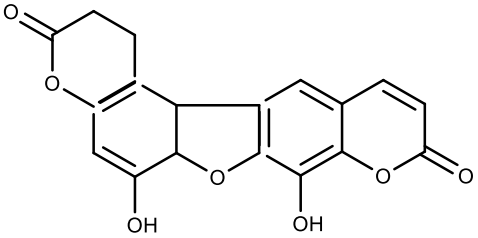


Figure I.1. Coumarins

	R^1	R^2	R^3	R^4	Trivial name
1	H	OH	OH	H	Esculetin
2	H	OGlc	OH	H	Esculin
3	H	OH	OGlc	H	Cichoriin
4	H	OMe	OH	H	Scopoletin
5	H	OMe	OGlc	H	Scopolin
6	H	OH	OMe	H	Isoscopoletin
7	H	OGlc	OMe	H	7-Methylesculin
8	H	OMe	OMe	H	Scoparon
9	H	OMe	OH	OH	Fraxetin
10	H	OMe	OH	OGlc	Fraxin
11	H	OMe	OMe	OH	Fraxidin
12	H	OMe	OMe	OGlc	Fraxidin-O-β-D-glucose
13	H	OMe	OH	OMe	Isofraxidin
14	H	OMe	OGlc	OMe	Calyncantoside

15	H	Me	OMe	OMe	6, 7, 8-Trimethoxycoumarin
16	H	OMe	OH	OAc	8-Acetyl-7-hydroxy-6-methoxycoumarin
17	OH	OH	OMe	H	Isofraxetin
18	OMe	OH	OMe	H	Fraxinol
19	OMe	OGlc	OMe	H	Mandshurin
20	H	H	H	OMe	8-Methoxycoumarin
21	OH	OMe	H	H	Floribin
22	OGlc	OH	OH	H	Esculetin-5-O-β-D-glucose
23					Xanthoxyloidin

The coumarins found in the genus Fraxinus are given in Table I.3.

Table I.3: Coumarins in Fraxinus species.

Plant source	Coumarins	References
F. americana	1,2,3,10,18	[6]
F. angustifolia Vahl	1,2,3,4,6,9,10,18	[17-19]
F. borealis Nakai	1,2,9,10	[6]
F. bungeana DC.	2,10	[18]
F. California Mill.	2,10	[18]
F.caroliniana Mill.	2,3	[19]
F. chinensis Roxb	1,2,3,4,10	[6, 17, 18]
F. excelsior L.	1,2,3,4,6,7,9,10,11,12,13,14,18,19	[17-20]
F. floribunda Wall	1,2,9,10,16,20,21	[21-23]
F. insularis	1,2,3	[24]
F. japonica Blume	1, 2,4,9,10,11,13,19.	[6, 25]
F. lanceolata Borkh.	1,2,3,10,18	[6]
F. mandshurica Rupr.	1,2,9,10,17,18,19	[6, 25, 26]
F. micrantha	1,10	[27]
F. nigra Marsh.	1,2,9,10	[6, 17]
F. ornus L.	1, 2, 3, 4, 7, 8, 9, 10, 15	[8, 17]
F. pallisiae Wilmott	1, 2, 4, 6, 9, 10	[17]

F. pennsylvanica March.	1, 2, 3, 10, 18	[6, 18]
F. quadrangulata Michx.	2, 3, 10	[18, 19, 28]
F. rotundifolia Mill.	2, 3, 10	[18]
F. rhynchophylla	1, 2, 3, 5, 10	[29]
F. sieboldiana	1, 2, 9, 10	[30, 31]
F. syriaca Boiss.	1, 2, 3, 10, 18	[18]
F. velutina	22	[7]
F. xanthoxyloides Wall.	3, 10, 23	[18, 19, 32]

The coumarin glucosides esculin **(2)** and Fraxin (**10**) occur in almost all Fraxinus species (Table I.3). However, their ratio varies for different plant sources [6, 8, 17]. For example, esculin predominates in *F. ornus* bark and *F. syriaca*, while in *F. excelsior and F. angustifolia* it is the opposite.

I.4.2 Secoiridoids

I.4.2.1 Secoiridoids of oleoside (24) and 10-hydroxyoleoside (25) type

Secoiridoids of oleoside (**24**) and 10-hydroxyoleoside (**25**) type having an ethylidene or hydroxyethylidene groupe at C-9 occur in Fraxinus species (Figure I.2) [9]. All natural secoiridoids of this type have the same configuration (H-1 is trans to H-5) and E-configuration of the C8/C9 double bond [33, 34]. The secoiridoids of oleoside type found in the genus Fraxinus are given in Table I.4.

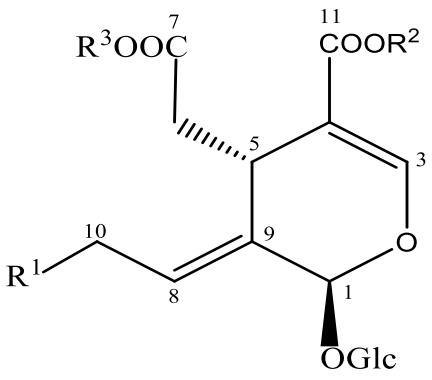
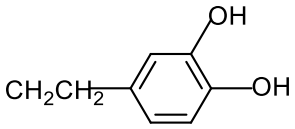
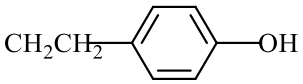
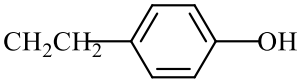
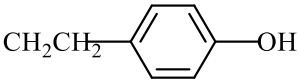
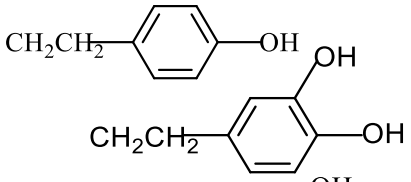
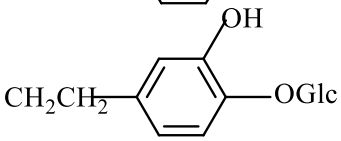
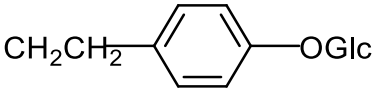
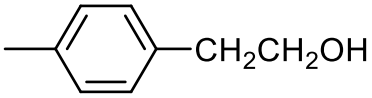
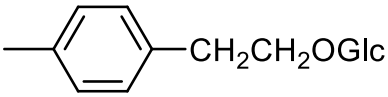
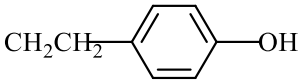
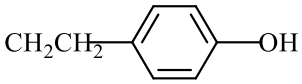
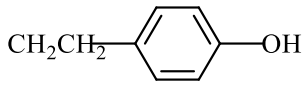
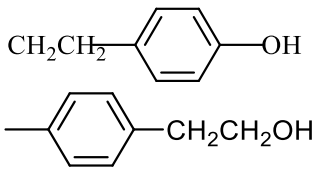
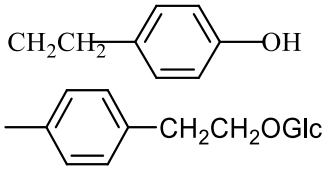
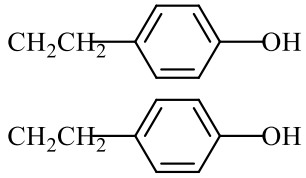
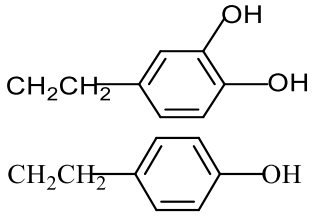
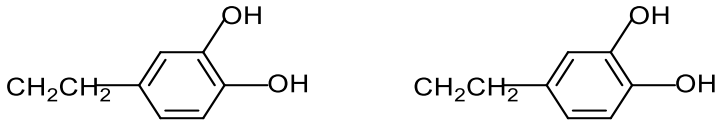
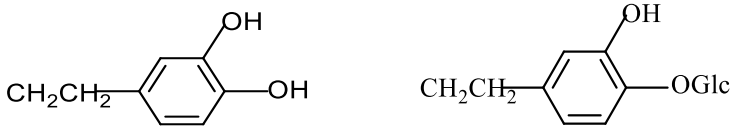
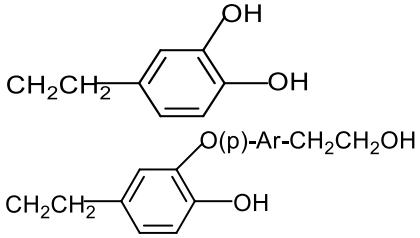


Figure I.2. Secoiridoids of oleoside type

Table I.4: Secoiridoids of oleoside type found in Fraxinus species

	R^1	R^2	R^3	Trivial name
24	H	H	H	Oleoside
25	OH	H	H	10-Hydroxyoleoside
26	H	H	Me	Oleoside-7-methylester
27	H	Me	H	Oleoside-11-methylester
28	H	Me	Me	Oleoside-7, 11-methylester
29	H	Me		Oleuropein
30	H	Me		Ligstroside
31	H	H		Demethylligstroside
32	OH	Me		10-Hydroxyligstroside
33	OH	H		Undenoside
34	OH	Me		10-Hydroxyoleuropein
35	H	Me		Angustifolioside A
36	H	Me		Angustifolioside B
37	H	Me		Formoside
38	H	Me		1‴-O-β-D-Glucosyl-Formoside
39	H		Me	Isoligstroside
40	H		H	Isoligstrosidic acid

41	H		—$CH_2CH_2CH_2CH_3$	Butylisoligstrosidate
42	H			Fraxiformoside
43	H			1‴-O-β-D-glucosyl fraxi formoside
44	H			Framoside
45	H			Hydroxyframoside A
46	H			Hydroxyframoside B
47	H			Neooleuropein
48	H			Angustifolioside C
49	H			Insuloside

I.4.2.2 Derivatives with O-function at C-2″

The 2″-hydroxyoleuropeins **50** (2″R) and **51** (2″S) and their 2″-methoxyderivatives **52** and **53** (Figure I.3) were isolated from *F. americana* [35].

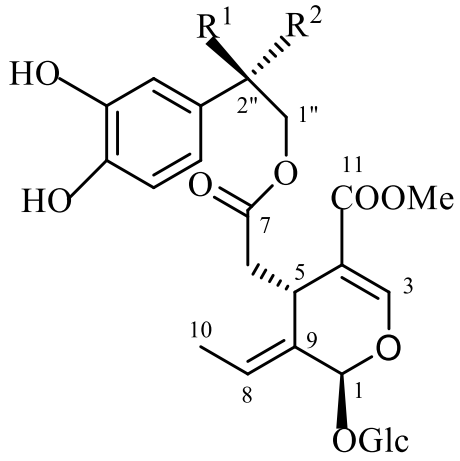


Figure I.3. Secoiridoids with O-function at C-2″

	R¹	**R²**
50	OH	H
51	H	OH
52	OMe	H
53	H	OMe

I.4.2.3 Acylated secoiridoid glucosides

The acylated derivatives of 10-hydroxyoleuropein (**32)** designated as fraxicarboside A (**54**), fraxicarboside B (**55**) and fraxicarboside C (**56**) (Figure I.4) have been isolated from *F. oxycarpa* [15].

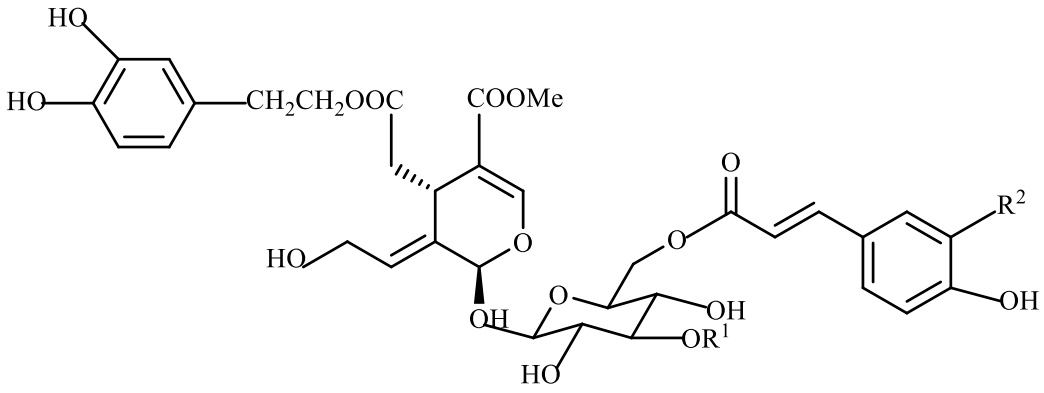


Figure I.4. **Acylated secoiridoid glucosides**

	R^1	R^2	**Trivial name**
54	H	H	Fraxicarboside A
55	H	OH	Fraxicarboside B
56	Ac	OH	Fraxicarboside C

I.4.2.4 Methylated secoiridoid glucosides

The methylated derivatives **57** and **58** of oleoside-7, 11-dimethylester (**28)** and ligstroside (**30**) (Figure I.5) were reported to occur in *F. angustifolia* [36].

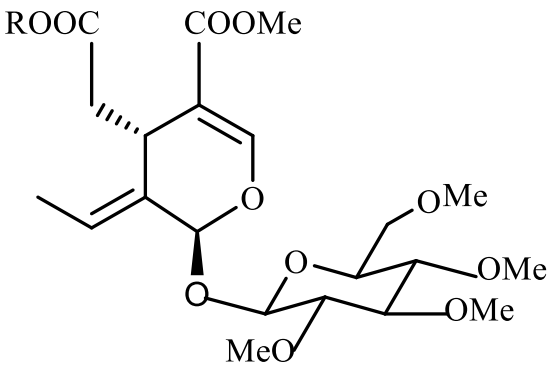
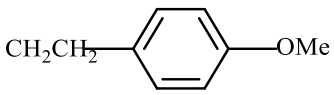


Figure I.5. Methylated secoiridoid glucosides

	R
57	Me
58	

I.4.2.5 Macrocyclic secoiridoids

The macrocyclic secoiridoids have been found only in *F. ornus*, *F. uhdei* and *F. insularis* [14, 24, 37-42]. In them, the two hydroxyphenylethyl units are coupled through a diphenylether linkage which gives rise to the formation of a flexible macrocyclic ring (Figure I.6).

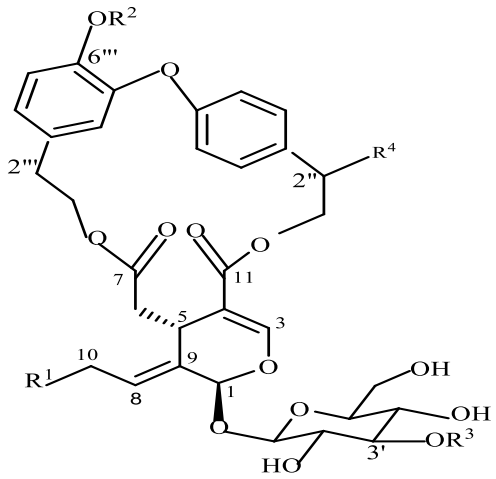
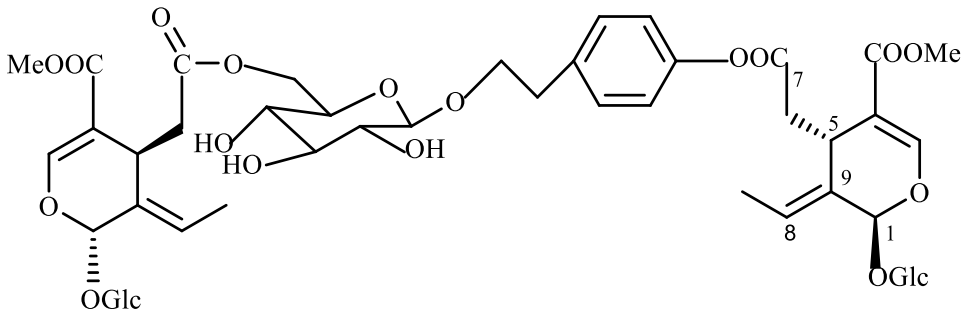


Figure I.6. Macrocyclic secoiridoids

	R^1	R^2	R^3	R^4	Trivial name
59	H	H	H	H	Insularoside
60	H	H	H	OH	Hydroxyornoside
61	OH	H	H	H	Uhdoside B
62	H	β-D-Glc	H	H	Fraxuhdoside
63	H	H	β-D-Glc	H	Insularoside-3′-O-β-D-Glc
64	H	β-D-Glc	β-D-Glc	H	Insularoside3′, 6‴-di-O-β-D-Glc

I.4.2.6 Dimeric secoiridoids

Complex dimeric secoiridoids consisting of two oleoside moieties linked to glucose and/ or tyrosol were also found in the genus Fraxinus. This type of secoiridoids is characteristic for Jasminum species and occurs rarely in other genera of Oleaceae. The secoiridoids Gl-3 (**66**) and Gl-5 (**67**) are reported from *F. americana* [43], while fraximalacoside (**68**) from *F. malacophyla* [44].



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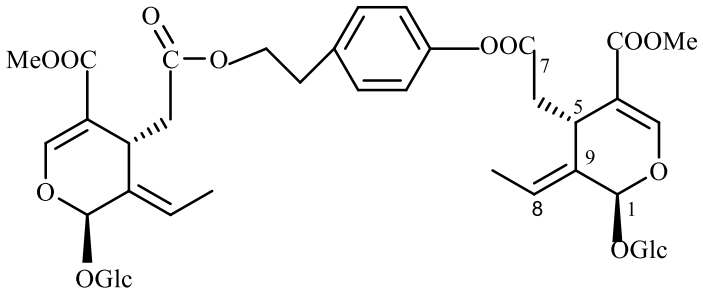

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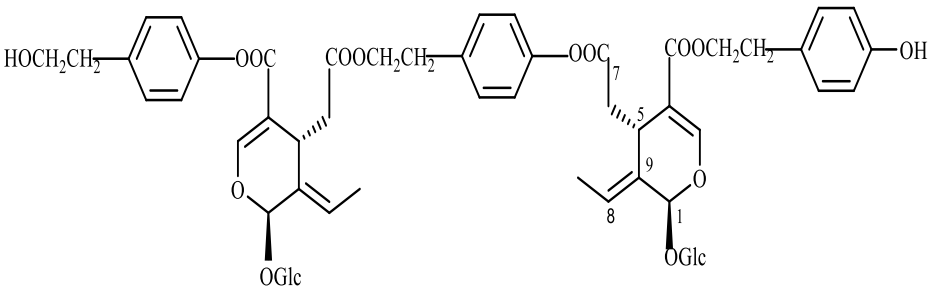

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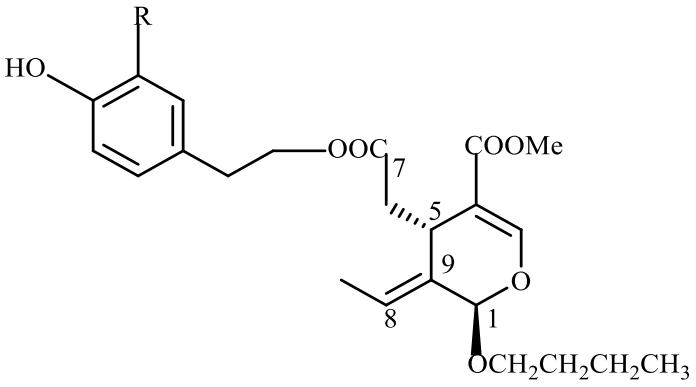
Figure I.7. Dimeric secoiridoids

I.4.2.7 Non-glucosidic secoiridoids

Oleobutyl (**69**) and Ligstrobutyl (**70**) (Figure I.8) have been found in *F. oxycarpa* [36].



	R
69	OH
70	H

Figure I.8. **Non-glucosidic secoiridoids**

I.4.2.8 Others

The unique compound frameroside (**71**), containing a cyclopentanoid monoterpene esterified with oleoside type secoiridoid glucoside, has been isolated from *F. Americana* [35]. Epiqingiside (**72**) is considered to be a biogenetic precursor of the oleoside-type secoiridoids [45]. Table I.5 summarizes the secoiridoids found in the genus Fraxinus.

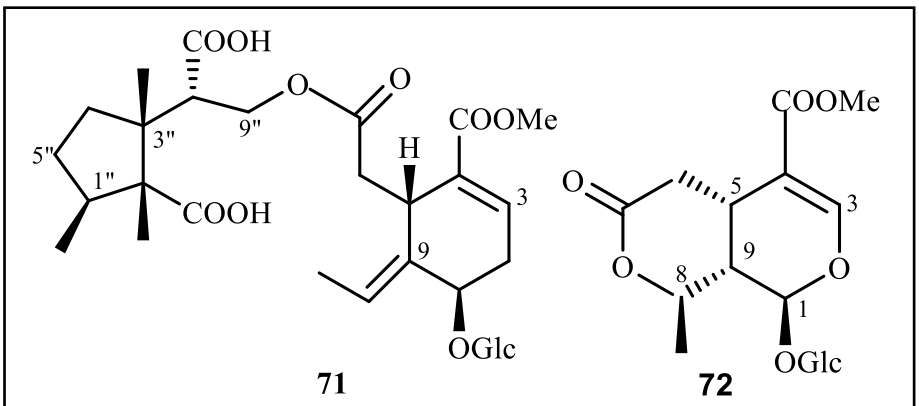


Figure I.9. Other secoiridoids

Table I.5 Occurrence of secoiridoids in Fraxinus species

Species	Compounds	References
F. Americana	**27, 28, 30, 31, 32, 50, 51, 52, 53, 65, 66, 67, 71**	[35, 43, 46]
F. angustifolia	**28, 29, 30, 32, 34, 35, 36, 48, 54, 55, 56, 57, 58, 66, 67, 69, 70**	[15-17, 36]
F. chinensis	**29, 47**	[47]
F.excelsior	**28, 29, 30, 32, 37**	[17, 20, 48]
F. formosana	**30, 37, 38, 39, 40, 42, 43, 44, 72**	[49]
F. griffithii	**30**	[50]
F. insularis	**29, 30, 49, 59, 62, 63, 64**	[24, 38, 39]
F. japonica	**29**	[51]
F. malacophyla	**39, 40, 41, 42, 43, 68**	**[**44**]**
F. mandshurica	**29, 30**	**[**6**]**
F. ornus	**29, 30, 44, 45, 46, 59, 60**	[17, 41, 42, 52, 53]
F. uhdei	**30, 32, 33, 34, 42, 59, 61, 62**	[14, 37, 40]
F. velutina	**26, 29, 30**	[7]
F. pallisiae	**28, 29, 30, 32**	[17]
F. rhynchophylla	**45, 47**	[29]

I.4.3 Phenylethanoid glucosides

Nine phenylethnoid glucosides (Figure I.10) were reported to occur in Fraxinus sepecies. Verbascoside (**73**) is found in *F. angustifolia* [15], *F. americana* [35, 47], *F. excelsior* [48], *F. formosana* [49], *F. insularis* [24], *F. malacophylla* [44], *F. ornus* [54], *F. sieboldiana* [30], *F. velutina* [7] and *F. uhdei* [40]. Salidroside (**74**) is isolated from *F. formosana* [49], while calceolarioside A (**75**) from *F. insularis* and *F. sieboldiana* [24, 30] and calceolarioside B (**76**) from *F. insularis, F. ornus* and *F. sieboldiana* [24, 30, 54]. The occurrence of lugrandoside (**77**), isolugrandoside (7**8**), isoacteoside (**79**) and 2-(4-hydroxyphenyl)-ethyl-(6-O-caffeoyl)-β-D-glucopyranoside (**80**) is reported from *F. ornus* [54]. Campneoside I (**81**) is isolated from *F. americana* [35]. Except salidroside (**74**), the phenylethanoids in Fraxinus are present as caffeoyl ester. The derivatives of dopaol (3, 4- dihydroxyphenyethylalcohol) predominate.

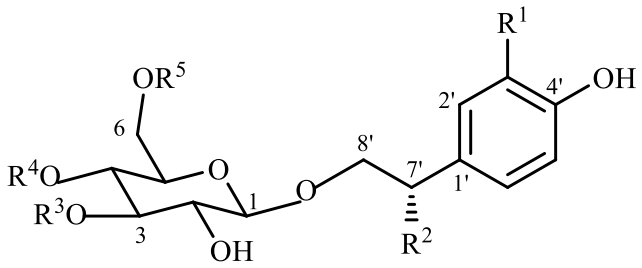


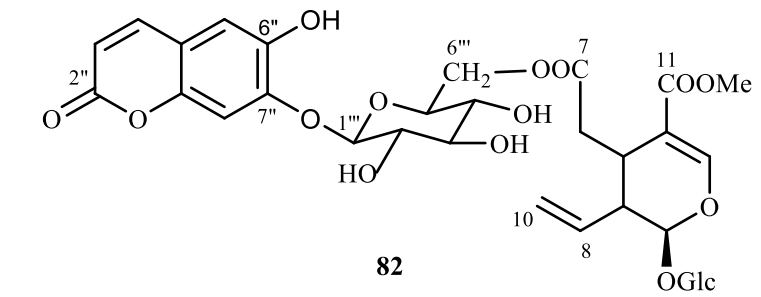
Figure I.10. Phenylethanoid glucosides

	R^1	R^2	R^3	R^4	R^5	Trivial name
73	OH	H	Rha	Caff	H	Verbascoside
74	H	H	H	H	H	Salidroside
75	OH	H	H	Caff	H	Calceolarioside A
76	OH	H	H	H	Caff	Calceolarioside B
77	OH	H	H	Caff	Glc	Lugrandoside
78	OH	H	Caff	H	Glc	Isolugrandoside
79	OH	H	Rha	H	Caff	Isoacteoside
80	H	H	H	H	Caff	
81	OH	OMe	Rha	Caff	H	Campneoside I

I.4.4 Compounds belonging to two different structural classes

Frachinoside (**82**), was isolated from *F. chinensis* [47], escuside (**83**), was isolated from *F. ornus* [53, 55] and fraxisecoside (**84**) from *F. rhynchophylla* [29], belong to this group. Their molecules consist of a coumarin glucoside and a secoiridoid glucoside, linked through the glucose of the coumarin part and the carboxyl group at C-7 of the secoiridoid (Figure I.11).

Desrhamnosyloleoacteoside (**85**) has been isolated from *F. insularis* [39]. It contains the ester linked phenylethanoid calceolarioside A (**75**) and the secoiridoid oleoside-11-methyl ester (**27**).



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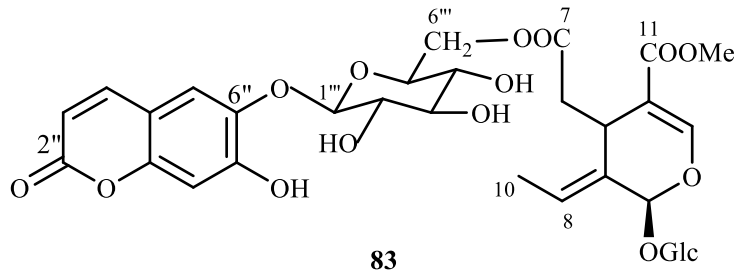

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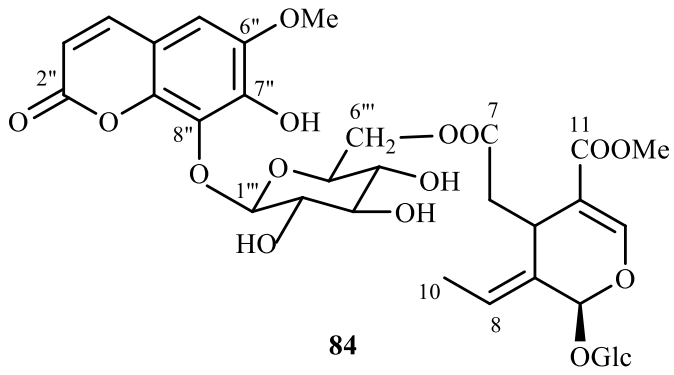

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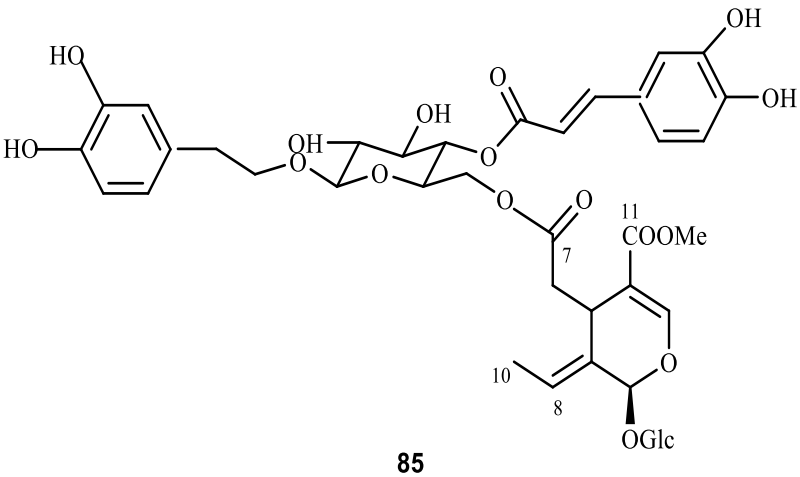


85

Figure I.11. Compounds with two structural classes

I.4.5 Lignans

The lignans in the Fraxinus are mainly of tetrahydrofurofurane (sesamine) type [56]. They have been found free or as glucosides (figure I.12). The lignans found in Fraxinus are given in Table I.6.

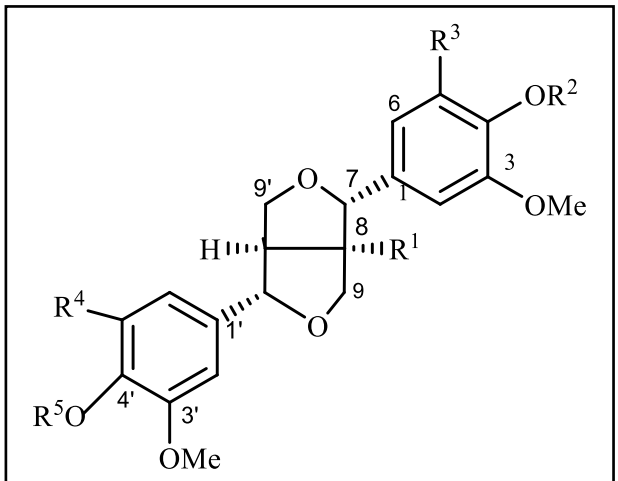


Figure I.12. Lignans

	R^1	R^2	R^3	R^4	R^5	**Trivial name**
86	H	H	H	H	H	Pinoresinol
87	OH	H	H	H	H	8-Hydroxypinoresinol
88	$OCOCH_3$	H	H	H	H	Acetoxypinoresinol
89	OH	H	OMe	H	H	Fraxiresinol
90	H	H	OMe	OMe	H	Syringaresinol
91	OH	H	OMe	OMe	H	8-Hydroxysyringaresinol
92	H	Glc	H	H	H	Pinoresinol-4-O-β-D-glucoside
93	OH	Glc	H	H	H	8-Hydroxy pinoresinol-4-O-β-D-glucoside
94	H	H	H	OMe	H	Medioresinol
95	OGlc	H	OMe	H	H	Fraxiresinol-8-O-β-D-glucoside

Table I.6: Lignans in the genus Fraxinus

Species	Compounds	References
F. angustifolia	**86, 92, 93, 95**	[6, 15]
F. chinensis	**86, 88, 92**	[6]
F. japonica	**86, 87, 89, 91, 92, 93**	[56]
F. mandshurica	**86, 87, 89, 90, 91, 92, 93, 94**	[6, 56]

I.4.6 Flavonoids

Flavones and flavonols are characteristic for the genus Fraxinus. Kaempferol (**105**) and quercetin (**96**) and their glucosides are frequently found. In the flavones the glucosidation is predominantly at C-7, while in the flavonols it is at C-3 (Figure I.13).

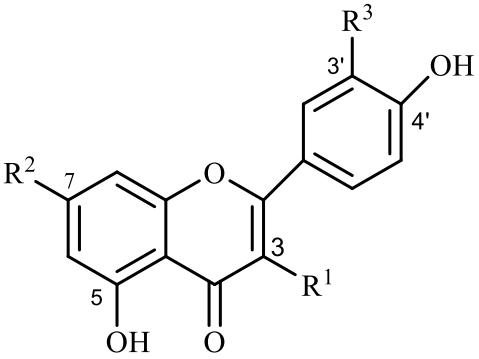
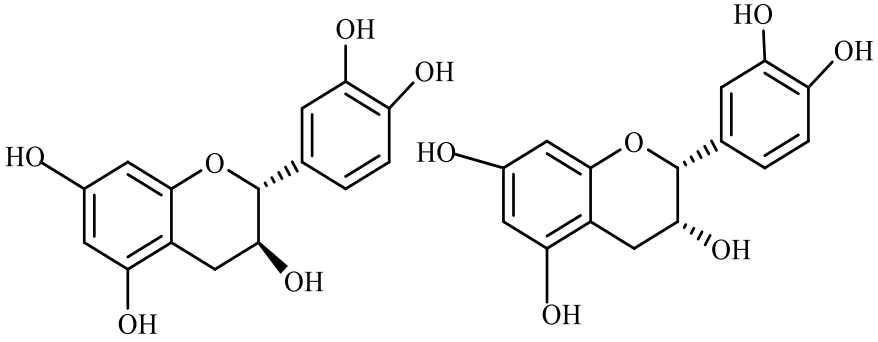


Figure I.13. Flavonoids

	R^1	R^2	R^3	Trivial name
96	OH	OH	OH	Quercetin
97	OH	OMe	OH	Rhamnetin
98	O-Glc^6-Rha	OH	OH	Rutin
99	O-Glc	OH	OH	Isoquercetrin
100	O-Rha	OH	OH	Quercetrin
101	O-Gal	OH	OH	Hyperoside
102	OGal	O-Gal	OH	Quercetin-3, 7-digalactoside

103	O-Glc^{6}-Rha	OH	OH	Quercetin-3-O-robinobioside
104	O-Glc^{2}-Glc	OH	OH	Quercetin-3-O-sophoroside
105	OH	OH	H	Kaempferol
106	O-Glc	OH	H	Astragalin
107	O-Glc^{6}-Rha	OH	H	Nicotiflorin
108	OH	Glc^{2}-Rha	H	Kaempferol-7-O-hesperidoside
109	O-Gal^{6}-Rha	OH	H	Kaempferol-3-O-robinobioside
110	H	OH	H	Apigenin
111	H	O-Glc^{6}-Rha	H	Rhoiflorin
112	H	O-Glc	H	Cosmosiin
113	H	O-Glc	OH	Luteolin-7-glucoside
114	H	O-Glc^{6}-Rha	OH	Luteolin-7-rutinoside
115	H	OH	O-Glc	Luteolin-3′-glucoside

(+)-Catechin (**116**) and (-)-epicatechin (**117**) have been isolated from F. excelsior [6]. The flavonoids found so far in Fraxinus are summarized in Table I.7.



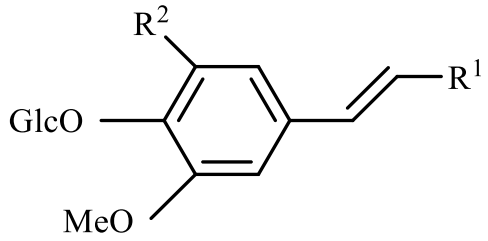
116 **117**

Table I.7: Flavonoids from Fraxinus species

Species	Compounds	References
F. Americana	**98, 103, 108, 109, 111, 112, 113, 114, 115**	[35, 57]
F. angustifolia	**98, 106, 107**	[15]
F. chinensis	**111, 112**	**[**6**]**
F.excelsior	**96, 98, 99, 100, 105, 106, 107, 110, 116, 117**	[6, 28, 58]
F. insularis	**98, 100**	[24]
F. lancheolata	**104**	[16]
F. malacophylla	**112**	**[**44**]**
F. ornus	**96, 97, 98, 99, 100, 101, 102, 110**	[59]
F. velutina	**98, 107**	[6]

I.4.7 Phenylpropanoid glucosides

Coniferin (**118**), syringing (**119**) and sinapic aldehyde-4-O-β-glucoside (**120**) (Figure I.14) were found in Fraxinus [16, 59].



	R^1	R^2
118	CH_2OH	H
119	CH_2OH	OMe
120	CHO	OMe

Figure I. 14: Phenylpropanoid glucosides

I.5 Biological activity

I.5.1 Antimicrobial activity

The ethyl ether fraction of the alcoholic extract of *F. excelsior* bark was inhibitory to *Bacillus subtilis* [60]. Extracts of the leaves of *F. excelsior* suppressed the growth of the fungi *Gloeosporum limetticolla* and *Alternaria tennis* [61].

Grujic-Vacic et al. tested the antimicrobial activity of aqueous extracts of the leaves and the barks of *F. ornus* and *F. excelsior* against 11 microorganisms and found that the leaves of both species showed strong inhibitionon the growth of *Candida albicans* with zones of inhibition of 25 and 22 mm, while the extracts of their barks expressed inhibitory activity against *Staphylococcus aureus* (zones of inhibition 13 and 15mm). Only the bark of *F. excelsior* was active against *Proteus mirabilis* and exhibited zone of inhibition 12mm [6].

The antimicrobial properties of three bark extracts of *F. ornus* and their main constituents **1, 2, 9, 10** against *S. aureus*, *Candida sp.*, *Esherichia coli* and *Pseudomonas aeruginosa* were studied [65]. All tested extracts and compounds were not active against *E. coli* and *P. aeruginosa*. Against Candida sp. Only fraxetin (**9**) and fraxin (**10**) exhibited some activity. Compound (**9**) caused a full inhibition of S. aureus, followed by esculetin (**1**) and fraxin (**10**). Esculin (**2**) was totally deprived of activity. The activity of the extracts against S. aureus was dependent on their hydroxycoumarin content.

The antimicrobial activity of different groups of bark constituent of F. ornus was investigated [63]. In the group of the coumarins **1, 2, 6-10, 15** a clear correlation between structure and microbial activity against *S. aureus* and *E. coli* was observed. Compared to the aglucones esculetin and fraxetin (MIC 500 and 125 μg/ml, respectively) the glucosides esculin and fraxin showed a negligible activity (MIC >1000μg/ml).The secoiridoid glucosides **30** and **59** inhibited the growth of *S. aureus* and *Cladosporum cucumerinum*. In another study the caffeoyl esters of phenylethanoid glucosides **73, 76-81** showed no activity against *Pseudomonas stutzeri*, while verbascoside (**73**) and isoacteoside (**79**) were inhibitors of *B.subtilis* [59].

I.5.2 Complement inhibition and anti-inflammatory activity

The water soluble and methanol extracts of the bark of *F. japonica* as well as the bark constituents esculetin (**1**) and esculin (**2**) were found to inhibit the rat edema induced by carrageenan, yeast and dextran. Esculin and esculetin were potent inhibitors of UV erythema in guinea pigs and decreased capillary permeability in mice [64]. The anti-inflammatory drugs Esqusan, Esflazid and Anavenol are based on esculin (**2**) [6].

Bark and leaves of *F. excelsior* have been used as rheumatic remedy since olden times. The ethanolic extract of the bark of this plant is a component of plant drug Phytodolor N. In vitro and in vivo studies proved its anti-inflammatory and anti- rheumatic properties often comparable to non- steroidal anti-inflammatories, but with little or no side effects [65].

The effects of the ethanolic extract of *F. ornus* bark and its main component esculin (**2**) on some, in vitro and in vivo, reactions related to acute inflammatory processes were studied [66]. The extract caused a more pronounced reduction of CP (classical pathway) hemolysis compared to esculin (**2**). The concentration causing 50% inhibition of CP was found to be 5 μg/ml for the extract and 10 μg/ml for esculin. In the AP (alternative pathway) assay they exhibited nearly equal dose dependent inhibition of complement-mediated hemolysis. The full inhibition of AP activity was achieved at esculin and total extract concentration of 50 μg/ml.

The in vitro effects of the coumarins **1, 2, 6-10** on the classical and alternative complement activity in normal human serum were examined at different concentrations. All the substances tested had a moderate or a weak ability to affect at least one of the complement pathways. The effect was not strictly dose dependent. Some of the compounds exhibited combined effect, activating one of the pathways and inhibiting the other [67].

Pure secoiridoid glucosides **29, 30, 44, 59, 60** were studied in vitro for their anti-complement action and for their ability to prevent cobra venom-induced complement activation in normal human serum [59]. The results showed that most of the secoiridoids possess the ability to suppress CP and AP activities.

I.5.3 Anti-oxidative activity

Investigations of Meyer et al. reveal the anti-oxidative activities of the alcoholic extract of *F. excelsior* bark, a component of the anti-inflammatory plant drug Phytodolor N [68]. Xanthine oxidase, diaphorase, lipoxygenase, riboflavin and rose Bengal, producing reactive oxygen species, were studied as model reactions.

The anti-oxidative action of the total extract of *F. ornus* bark and its main coumarin contituents 1, 2, 9, 10 was investigated using kinetically pure triacylglycerols of lard (TGL) and sunflower oil (TGSO) [69]. The activity of fraxetin (**9**) and esculetin (**1**) was higher than that of the corresponding glucosides fraxin (**10**) and esculin (**2**) and comparable to that of other well-known anti-oxidants such as caffeic acid.

Verbascoside (**73**), calceolarioside B (**76**) and isoacteoside (**79**) are also found to have anti-oxidative properties [4].

I.5.4 Skin-regenerating properties

Klouchek et al. investigated the skin-regenerating properties of the ethanolic bark extract and its main component esculin (**2**) on male rats having standard oval wounds [53]. The animals treated with the bark extract exhibited a more intense epithelization of the wounds in comparison with the control groups. On the third day from the beginning of the experiments 55.80% of epithelization was observed, the effect being more pronounced between the seventh (84.85%) and tenth (96.90%) days. A weaker regenerating effect was observed in the animals treated with esculin.

I.5.5 Photodynamic damage prevention

Lazarova et al. used the prevention of photodynamic yeast cell damage to investigate the protective activity of the coumarins **1, 2, 9, 10,** four bark extracts, caffeic acid and as a standard the sun screen p-aminobenzoic acid [71]. All of the tested pure compounds showed protective activity. The protective effect of compounds **1** and **9** at concentration of 20 mg/l was comparable to that of caffeic acid and p-aminobenzoic acid at concentration of 25 mg/l.

I.5.6 Antiviral activity

Galabov et al. investigated the antiviral properties of the coumarins **1, 2, 6, 7, 8, 9, 15** isolated from F. ornus against poliovirus 1, influenza virus, Newcastle disease virus (NDV) and pseudovirus [72]. Only esculetin (**1**) showed a significant activity against NDV.

I.5.7 Diuretic activity

Traditionally, leaf extracts from *F. excelsior* have been used to facilitate renal excretion. This diuretic activity is attributed to the presence of flavonoids. The spray dried powders prepared from the aqueous and ethanolic extracts from the leaves of this plant caused a significant dose dependent increase in the excretion of sodium and chloride ions and potassium and were qualified as potentially useful medicinal products [73].

I.5.8 Anti-hepatotoxic activity

The liver protecting properties of some coumarin and flavonoid components of Fraxinus species are reported in the reference [74]. Isofraxidin (**13**) and scopoletin (**4**) exert pronounced choleritic activity. Quercetin (**96**) was found to increase bile secretion and the detoxifying function of the liver in experimental animals and rutin (**98**) and nicotiflorin (**107**) showed low toxicity and marked choleritic effects when tested on rabbits, rats and mice.

CHAPTER II

Study of Iridoids, coumarins, flavonoids and phenylethanoids

II.1 Iridoids

II.1.1. Definition

Iridoids represent a large and still expending group of monoterpenoid compounds, being secondary plant metabolites with characteristic cyclopenta[C]pyran ring (Figure II.1), also described as iridane (2-oxabicyclo[4, 3, 0]nonane). Most of the known compounds till to date, have cis-linked rings and glucose as their sugar moiety [75-79].

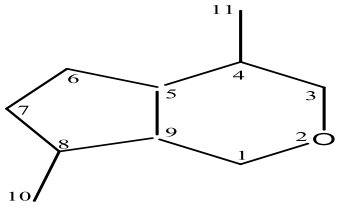


Figure II.1: Chemical structure of cyclopenta[C]pyrane (iridane)

However, the name iridoid is a generic term derived from the name iridodial (Figure II.2), compound isolated from some species of Iridomyrmex, a genus of ants, in which they occur as defensive secretions [80]. The older name of iridoids was pseudoindicans as they were known to react with acids giving bleu colored derivatives [81, 82].

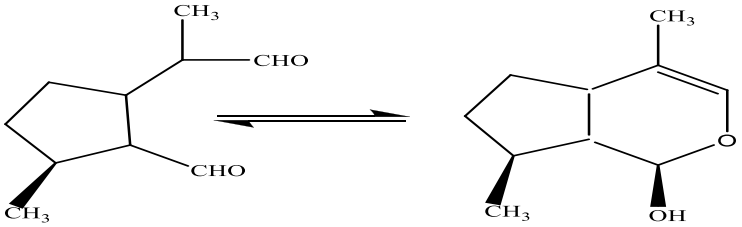


Figure II.2: The two isomeric forms of iridodial

Iridoids were first isolated in the latter part of the nineteenth century, but the detailed structure of these compounds had not been determined until 1963 [83]. Compounds formed by the cleavage of the cyclopentane ring of iridoids, as shown by the dotted line in Figure II.3, are called secoiridoids [84].

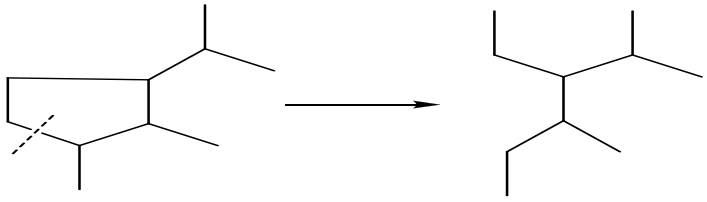


Figure II.3: Carbon skeleton of iridoids and secoiridoids

II.1.2 Classification of iridoid compounds

Naturally, occurring iridoids have been classified by several authors into different subgroups on the basis of their demonstrated or postulated biosynthesis as well as on the basis of chemical properties [85].

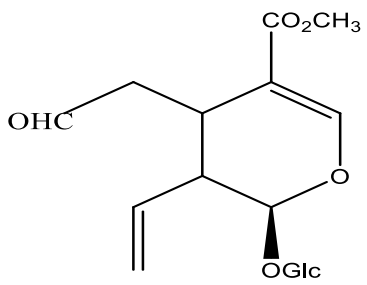
According to Sticher and Busch classification [86], natural iridoid compounds in the broadest sense are divided into five main groups:

1-Methylcyclopentanoid (monoterpenes of the nepetalactone type).
2-ridoids.
3-Monoterpene alkaloids.
4-Secoiridoids.
5-Nontryptophan portions of different indole and isoquinoline alkaloids

On the other hand, El-Naggar and Beal [87] have summarized only iridoid glucosides, secoiridoid glucosides and non glucosidic compounds, and omitting all nitrogen containing iridoids.

Secoiridoid glucosides are derived from iridoid glucosides by oxidative cleavage of the cyclopentane ring and they can be further divided into the following four subgroups [87]:

1- The simple secoiridoid glucosides: Secologanin (121), Sweroside (122).



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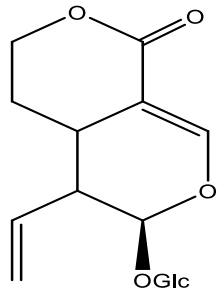
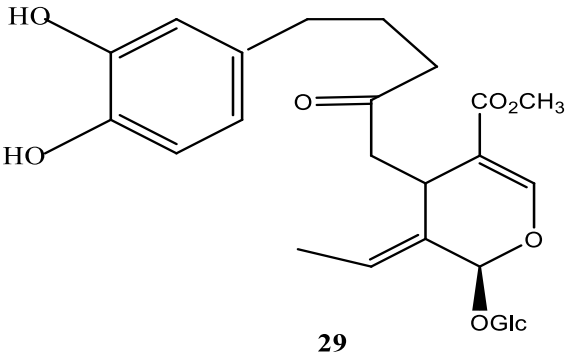


122

2- Secoiridoid glucosides carrying a phenolic moiety: oleuropein (29)



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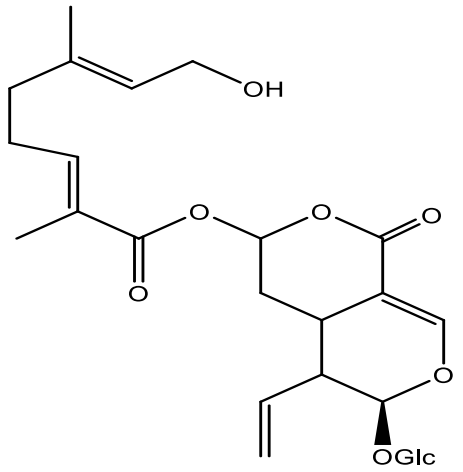
3- Secoiridoid glucosides conjugated with a terpene type moiety: Foliaenthin(123).



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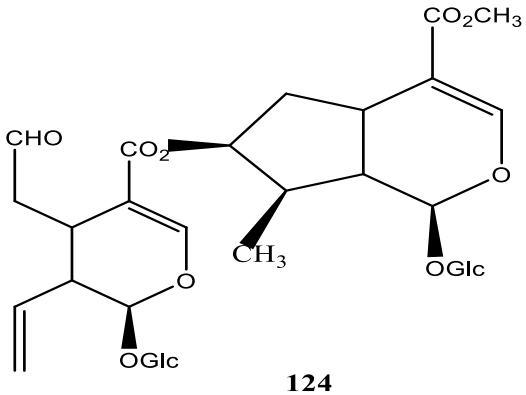
4- Bisiridoids: Sylvestroside III (124).

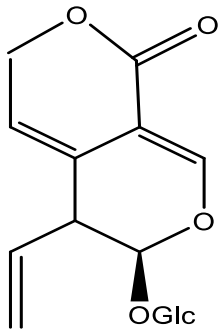


124

Inouyer and Uesato [88] have surveyed the biosynthesis of iridoids and secoiridoids and presented another classification from the biogenetic point of view and hence dividing the compounds conventionally into non glucosidic iridoids, iridoid glucosides and secoiridoid glucosides. The first two groups are not further subdivided, whereas the third group is subdivided

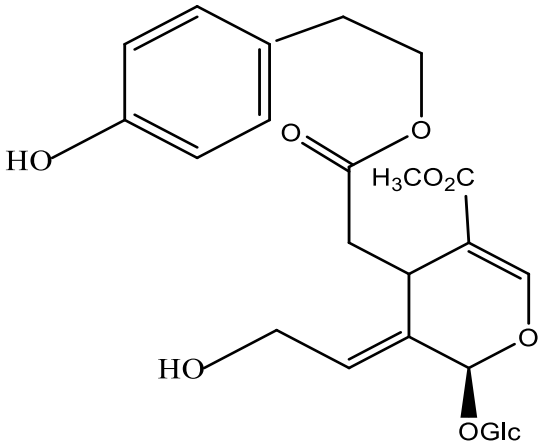
on the basis of biosynthetic pathways and structural similarities into the following four subgroups. For each subgroup, representative compounds are given.

1- The sweroside (122), gentiopicroside (125) type bearing a vinyl group at C-9.



125

2- The oleoside -10-hydroxyoleoside type: oleuropein (29), 10-hydroxyligustroside (32).



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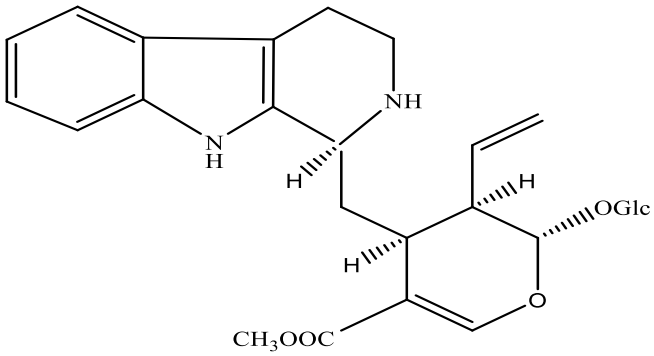
3- Alkaloidal glucosides containing a secoiridoid skeleton: strictosidine (126).



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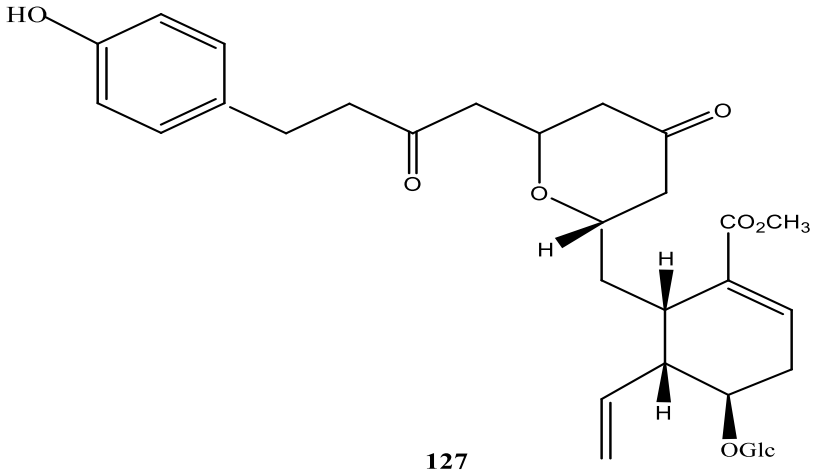
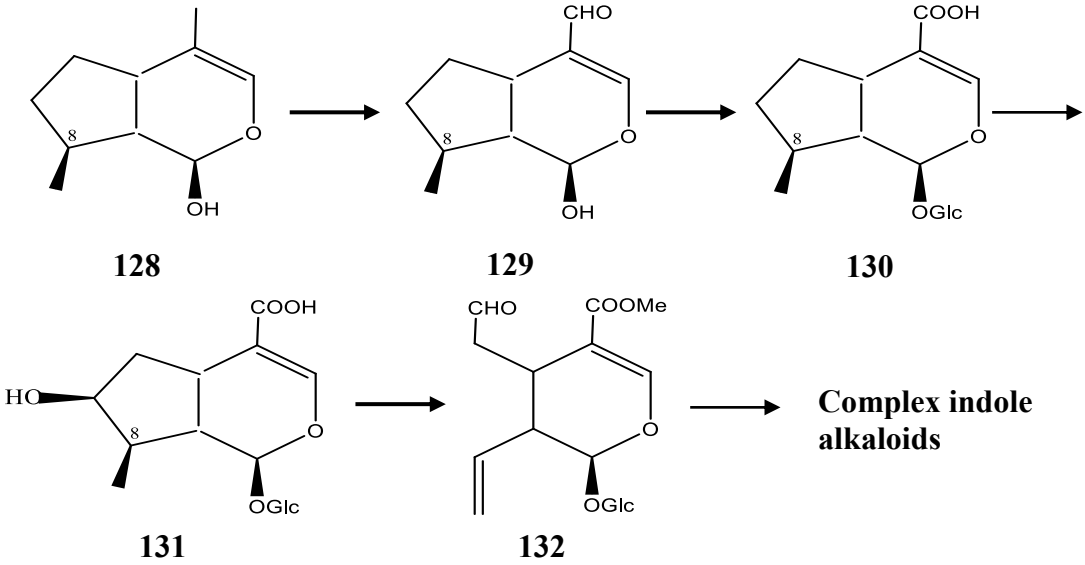
4- Hydrangenosides: hydrangenoside A (127).



127

II.1.3 Biosynthesis of iridoids

The iridoids are of terpenoid origin and their biosynthesis has been fairly well investigated [10, 88-90], thus it is known that two main routes exist. One main route (Figure II.4) is leading from iridodial (128) via iridotrial (129) to deoxyloganic acid (130) which is the known precursor of many carbocyclic iridoids having the 8β-stereochemistry including loganin and loganic acid (131), secologanin (132) and secologanic acid as well as the derived secoiridoids and complex indole alkaloids. Compounds from this route are found mainly in Cornales, Gentianales and Oleaceae.



128 **129** **130**

131 **132**

Figure II.4: Biosynthetic pathway (route I) to the common precursor deoxyloganic acid (130), secologanin (132) and to the complex indole alkaloids

Another main biosynthetic pathway (route II) involves 8-epi-iridodial, 8-epi-iridotrial and 8-epi-deoxyloganic acid; these are precursors for the decarboxylated carbocyclic iridoids such as

aucubin and catalpol. These compounds are almost exclusively found in Lamiales families and never in Gentianales or Oleaceae [90]. A few unusual secoiridoids are known to be formed by this route [48, 91] but they are different in structure from those derived from route I.

II.1.4 Biological activity of iridoids

As the fact that iridolactones (iridomyrmecin and isoiridomyrmecin), the first structurally elucidated iridoids, are defensive substances ejected by iridomyrmex ants suggests that there are several ecologically interesting iridoids. Dolicodial, a secretion of Iridomyrmex and Dolichoderus species, and anisomorphal, that of Anisomorpha species, are repellents of these insects [92, 93]. Iridolactones also occur in *Actinida polygama* and extracts are known as a powerful feline attractant [94].

On the other hand, iridoid glucosides often serve as feeding attractants and stimulants for larvae of some Lepidopterrae. The larvae of Ceratomia catalpa, the most destructive insect of Catalpa trees [95].

The two secoiridoid glucosides, oleuropein and its demethylated product demethyloleuropein, are known to stimulate oviposition of Dacus oleae Gmel., an insect which infects Mediterranean olive corps [96].

In 1969, Fleming et al. isolated a compound from green olives that appeared to have antimicrobial properties noted earlier during the fermentation of brined olives. The compound, a bitter phenolic material was considered to be an enzymatic degradation product of oleuropein. Latter, they found that oleuropein was not inhibitory, but its hydrolysis product, the aglucone which possess the antimicrobial properties [97]. In different pharmacological investigations including acute toxicity, effect on blood pressure, coronary action, antiarrhythmic action, effect on the intestinal smooth muscles, it was possible to demonstrate that oleuropein has a hypotensive action, as well as coronary dilating, antiarrhythmic and spasmolytic action [36, 97, 98].

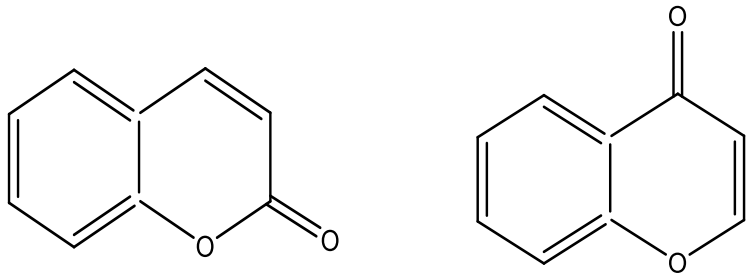
The secoiridoid glucosides, amarogentin, amaroswerin and amaropanin, isolated from gentianaceous plants should be mentioned that they rank among the most bitter known compounds and crude drugs containing them are used as bitter tonics [97].

In addition, the investigation carried out by Kostova et al. [99] proved that the secoiridoid glucosides, ligstroside, insularoside, hydroxyornoside, oleuropein, framoside and 10-hydroxyligstroside possess the anti-inflammatory action.

II.2 Coumarins

II.2.1 Introduction

Coumarins owe their class name to ‘Coumarou’ the vernaculair name of the tonka bean (Dipteryx *odorata* Willd, Fabaceae), from which the coumarin, it was isolated in 1820 [100]. Coumarin is classified as a member of the benzopyrone family of compounds. The benzopyrones, all members of which consist of a fused benzene and pyrone ring (Figure II.5), can be sub-classified on the basis of the position of the oxygen atom within the pyrone ring - the benzo-α-pyrones, to which the coumarins belong, and the benzo-γ-pyrones, of which the flavonoids are the principal members.



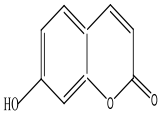
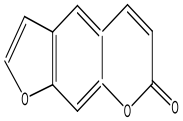
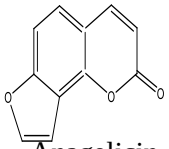
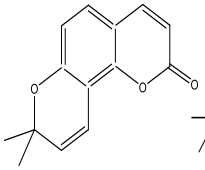
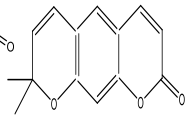
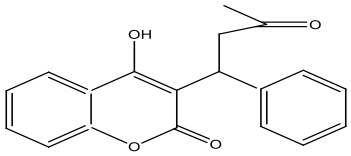
α-Benzopyrone **γ-Benzopyrone**

Figure II.5: Chemical structure of the benzopyrone sub-classes, with the basic coumarin structure (benzo-α-pyrone) and flavonoid (benzo-γ-pyrone) structure.

II.2.2 Occurrence

By virtue of its structural simplicity coumarin has been assigned as head of the benzo-α-pyrones, although it is generally accepted that 7-hydroxycoumarin (umbelliferone) be regarded as the parent compound (see below). Since 1820, when coumarin was first isolated from the tonka bean by Vogel, over one thousand coumarin derivatives have been described. These have been mainly isolated from natural sources. The derivatives range from simple coumarins with hydroxyl, alkoxyl and alkyl side chains, to more complex forms containing furanoyl, pyranoyl and benzoyl functions (Table II.1) [100,101].

Table II.1: The four main coumarin sub-types.

Classification	Features	Examples
Simple coumarins	Hydroxylated, alkoxylated or alkylated on benzene ring	 7-hydroxycoumarin
Furanocoumarins	5-membered furan ring attached to benzene ring. Linear or Angular	 Psoralen  Anagelicin
Pyranocoumarins	6-membered pyran ring attached to benzene ring. Linear or Angular	 Seselin  xanthyletin
Pyrone-substituted coumarins	Substitution on pyrone ring, often at C-3 or C-4 position	 Warfarin

Both Murray et al. [102], and Keating and O'Kennedy [103] have reviewed the botanical source of all naturally isolated coumarin compounds. Coumarins are usually found free, or in combination with sugars as glucosides, in many plants, especially those of the Umbelliferae, Rosaceae and Rutacecie families. They are found distributed throughout the roots, leaves, stems and fruits, occurring at highest levels in the fruits, but the levels tend to vary with seasonal and environmental changes [104]. Generally, a number of different coumarins are found within one plant. The role of coumarins in plants is still obscure, although their distribution appears to correlate with an ability to protect against disease or infection [104]. It has also been suggested that their role may be as plant growth regulators [105].

II.2.3 Biosynthesis of coumarins

Simple coumarins are the most common in all angiosperms, especially in Oleaceae and Asteraceae, and their occurrence is of 100% and 98, 68%, respectively [106]. Simple coumarins are biogenetically derived from shikimic acid, via cinnamic acid. The specificity of the process is the C-2 hydroxylation, producing a break (ß-oxidation) of the side chain, or chain isomerization and subsequent lactonization, generating the umbelliferone. Figure II.6 explains the entire process [107, 108].

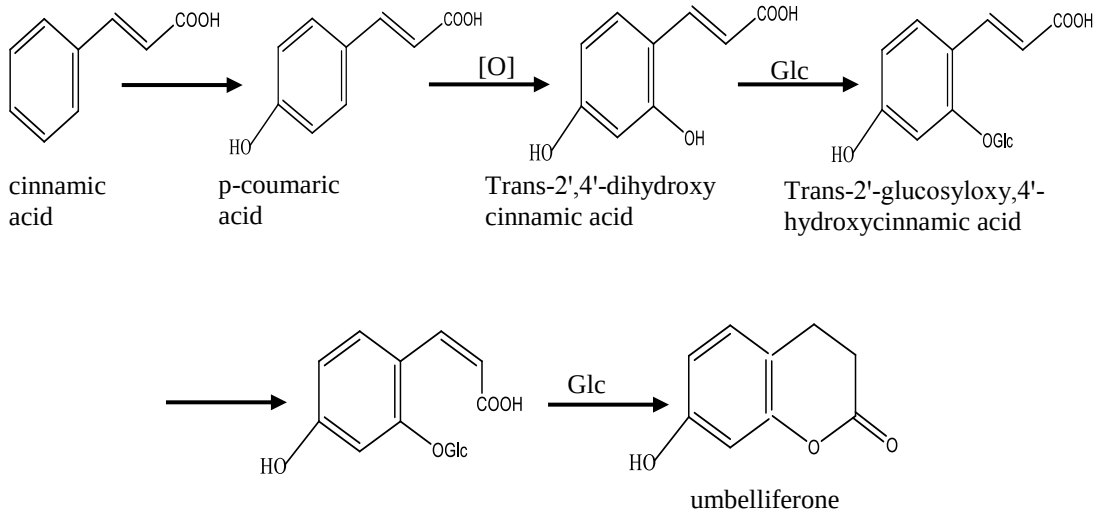


Figure II.6: Biosynthesis of simple coumarins

Pyrano and furanocoumarins are also biogenetically derived from shikimic acid. These coumarins could be divided in two groups—linear and angular—depending on the position where the isopentenyl pyrophosphate is condensed to further cyclize and form the heterocycle. The biosynthesis of these complex coumarins could also be the result of the cyclization of a simple coumarin previously prenylated [108-110].

II.2.4 Uses

II.2.4.1 Industrial uses of coumarins

Coumarin, with its strong, pleasant fragrance, is one of the most extensively used synthetic aroma chemicals [111]. It is applied in a variety of industrial settings, the most important being the perfumery industry. It is widely used by perfumists to enhance the fragrance of many essential oils such as lavender, rosemary and citrus, in addition to a more limited use in other cosmetics such as lotions, talcum powders, *etc.* 6-methylcoumarin, with a more subtle odour, is also a popular choice.

Coumarin has enjoyed only limited use as a food additive, but it has been included as an odour enhancer in tobacco products and is commonly used as an odour-masker, with applications in the paints, plastics and synthetic rubber industries.

II.2.4.2 Analytical uses of coumarins

The coumarin family of compounds has also found widespread use in many analytical scenarios [112-115]. In many cases this application arises from their inherent fluorescence properties, which offer increased sensitivity in detection, compared to absorbance/colourimetric measurements.This area has been extensively reviewed recently by Cooke *et* al. [104].

II.2.4.3 Clinical Uses of Coumarins

Coumarin compounds have been shown to possess a wide variety of useful pharmacological and physiological activities (Table II.2). The lead for the discovery of many such properties was taken from traditional medicines, and benzopyrone compounds are now known to be the active agents in many folk remedies [116, 117]. In the clinical arena, it is the activation of the immune system by coumarin, which has led to its use in a variety of disease states, including High Protein Oedemas, chronic infections and immune system disorders. Other coumarin derivatives have proven important in anti-coagulation therapy, and more recently as potential HIV therapies [118-120].

Table II.2: The important pharmacological and physiological activities of coumarins

Pharmacological activity	References
Analgesic	[121]
Anti-coagulant	[121, 106]
Anti-inflammatory	[123, 124]
Anti-microbial/Anti-viral	[121, 106]
Anti-oxidant	[125, 126]
Anti-pyretic	[127]
Sedatory	[125]

II.3 Flavonoids

II.3.1 Introduction

Flavonoids are molecules characterized by a C15 structure (C6-C3-C6) with a heterocyclic benzopyran ring (C ring), an aromatic ring (A ring) and a phenyl constituent as the B ring (Figure II.7), all of them with several structural variations. This family is divided in five major classes (flavones, flavonols, flavanones, flavanols and anthocyanidins), according to their oxidation state, the connection of an aromatic ring and the functional groups of the C ring. Up to this moment, more than 4000 flavonoids have been identified in plants, mainly occurring as glucosides [129,130].

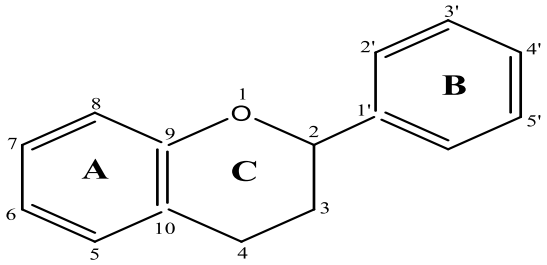


Figure II.7: General structure of flavonoids

II.3.2 Structure variation in flavonoids

Chemically, flavonoids consist of a benzopyran-4-one (rings A and C) carrying a phenyl unit (ring B) at C-2 position as substituent. Flavonoids are distinguished in different classes depending on the degree of oxidation at C-3 in ring C. The major classes of flavonoids are shown in Figure II.8.

In flavones and flavonols, the location of the hydroxyl/methoxyl group is usually at C-5, C-7 (ring A) and at C-3', C-4' (ring B) whereas it is rarely at C-2' and C-6' (ring B). The identification of complex mixtures of flavonoids is reported in the literature. This has been achieved by combination of standard techniques including spectroscopy (^{1}H and ^{13}C NMR), hydrolysis (acid, alkali and enzymic), TLC and comparison with authentic standards.

Some flavonoids contain alkyl groups (mainly methyl group) linked to C-5, C-6, C-7, and C-8 in the ring A; C-2', C-3', C-4' in the ring B; and C-3 in the ring C. Modifications to the hydroxylation patterns in the two aromatic rings may occur, generally at flavanone or dihydroflavonol stage, and methylation, glucosylation, and dimethylallylation are also possible, increasing enormously the range of compounds. Thus, a large number of flavonoids occur as *O*-glycosides (mainly glucose derivatives) in which one or more of the hydroxyl groups of the flavonoid are linked to one sugar or more via acid labile hemiacetal bonds. The effect of glucosylation is that flavonoids are less reactive and more soluble in water.

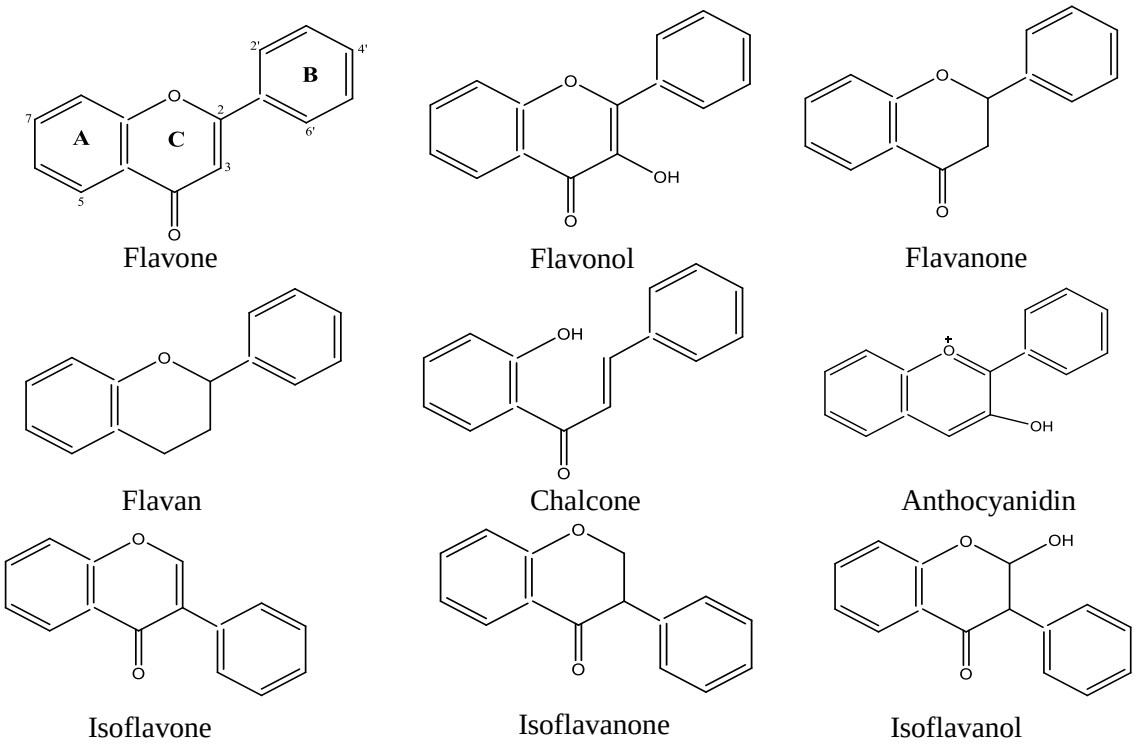


Figure II.8: Major classes of flavonoids

Several flavones and flavonols occur in a conjugated form, covalently linked to inorganic bisulphate (or sulphate). Such compounds contain one or more sulphate residues attached to a hydroxyl on the favonoid or sugar moiety. Sulphate flavonoids were found in some seagrasses such as Thalassioideae (*Thalassia testunidum*) and Zosteraceae (*Zostera marina*) [131, 132].

Acetyl and malonyl are the most common residues among the aliphatic acyl groups and have been reported in various naturally occurring *O*- and *C*-glucosyl flavonoids including flavones, isoflavones, flavonols and anthocyanins.

II.3.3 Biosynthesis of Flavonoids

Flavonoids are products from a cinnamoyl-CoA starter unit derived from L-tyrosine *via* the enzyme phenylalanine ammonialyase (PAL), that undergoes a chain extension by adding three molecules of malonyl-CoA. This gives a polyketide, which, according to the nature of the responsible enzyme, can be folded in two different ways generating aromatic rings by either aldol or Claisen–like reaction. Enzymes stilbene synthase and chalcone synthase couple a cinnamoyl-CoA unit with three malonyl-CoA units giving stilbenes, such as resveratrol or chalcones, such as naringenin–chalcone, respectively [133].

Chalcones act as precursors for a vast range of flavonoid derivatives found throughout the plant kingdom. Most contain a six membered heterocyclic ring, formed by Michael-type nucleophilic attack of a phenol group on the unsaturated ketone giving flavanones such as naringenin (Figure II.9). Flavanones can then give rise to many variants on this basic skeleton, such as flavones, flavonols, anthocyanidins, and catechins [134].

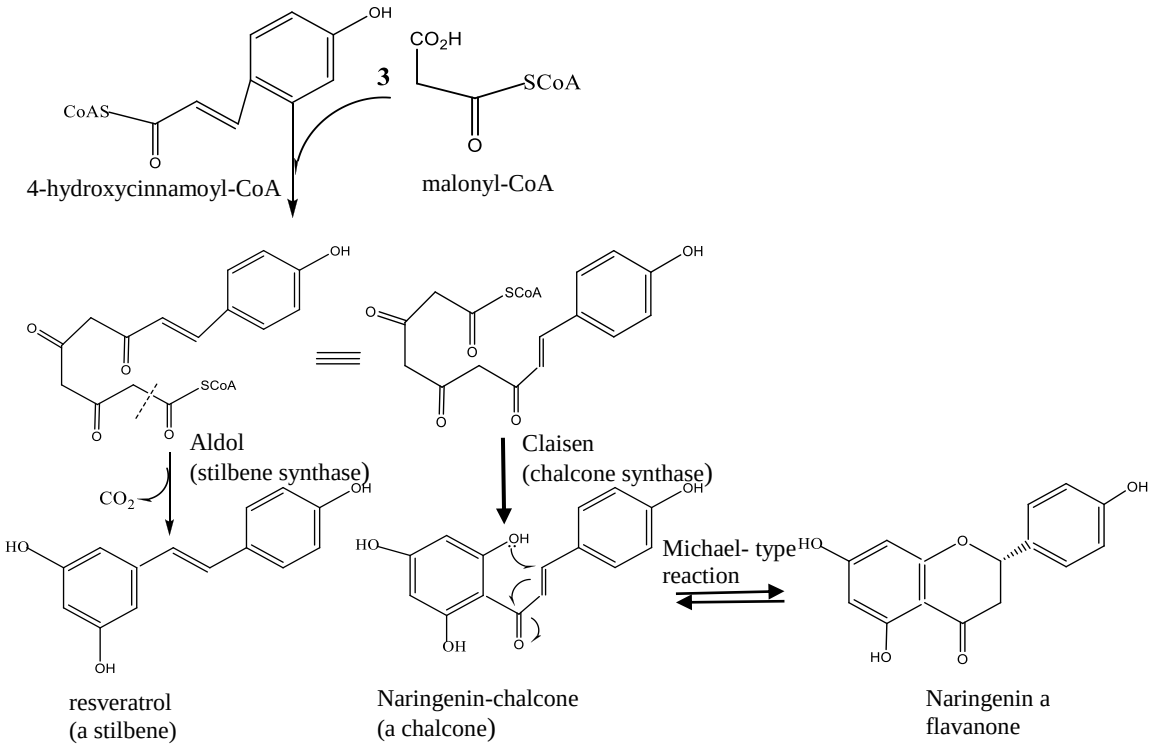


Figure II.9: Biosynthesis of flavanone: exemple of naringenin

II.3.4 Biological activities of Flavonoids

Flavonoids are particularly beneficial, acting as antioxidants, anti-inflammatory, antimicrobial, analgesic, neuroprotective and giving protection against cardiovascular disease, and certain forms of cancer [135-143]. Their polyphenolic nature enables them to scavenge injurious free radicals such as superoxide and hydroxyl radicals. In particular, quercetin, which is commonly present in substantial amounts in plants tissues, is a powerful antioxidant, chelating metals, scavenging free radicals, and preventing oxidation of low density lipoprotein. Kaempferol, anthocyanidins, and catechins are also demonstrated to be effective antioxidants.

Flavonoids have been previously shown to exhibit anti-inflammatory activity. Apigenin showed significant inhibition of fibroblast growth at all concentration from 0.01 to 100 µg/ml. Kaempferol is reported to inhibit granulation tissue formation induced by croton oil and to

protect against gastric ulcers induced by pyloric ligation and restraint stress in rats. Anthocyanins and their aglycone, cyanidin, have been also shown to have antiinflammatory properties. Anthocyanins were tested for their ability to inhibit prostaglandin endoperoxide hydrogen synthase-1 and 2 (PGHS-1 and 2). The glucosides showed little or no activity whereas the aglucone cyanidin displayed significant inhibitory activity against both enzymes with IC50 values of 90 and 60 μM, respectively compared with 1050 μM for aspirin in both tests.

II.4 Phenylethanoid glucosides

II.4.1 Introduction

The phenylethanoids are a diverse and widely distributed group of plant secondary metabolites characterised by the presence of a C_6C_2 moiety. Like the closely related phenylpropanoids (which feature a C_6C_3 moiety). Structuraly, they are cinnamic acids and hydroxyphenyl ethyl moieties attached to a ß-glucopyranose through ester linkages and glucosidic linkages, respectively (Figure II.10) [70]. Common hydroxycinnamic acids enclose the caffeic acid, cinnamic acid and ferulic acid, while rhamnose, apiose or arabinose are the most usual sugars found. Phenylethanoid derivatives, which are bonded to iridoid glycosides by ether or ester bonds, have been isolated recently [144]. To date several hundred compounds of this type have been isolated from medicinal plants and further pharmacological studies in vitro or in vivo have shown that these compounds possess a broad array of biological activities including antibacterial, antitumor, antiviral, anti-inflammatory, neuro-protective, antioxidant, hepatoprotective, immunomodulatory, and tyrosinase inhibitory actions [145].

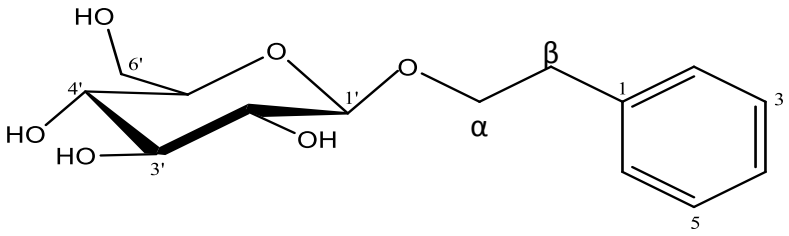


Figure II.10: General struture of phenylethanoid glucosides

II.4.2 Biosynthesis

The phenylethanoids are products of the shikimic acid biosynthetic pathway, with mixed C_6C_2/ C_6C_3 glucosides commonly occurring [146]. The shikimic acid pathway (Figure II.11) starts with the formal aldol coupling of the glucolysis product, phosphoenolpyruvate (PEP) (**133**), with erythrose-4-phosphate (**134**), from the pentose phosphate cycle, to give 3-deoxy-D-arabino heptulosonate7-phosphate (**135**) [147]. 3-Dehydroquinate (E2) synthase then mediates the conversion of **135** to 3-dehydroquinate (**136**) via a five step sequence. The C(5) alcohol is initially oxidised, followed by enolisation and E1cB-type elimination of phosphate. Subsequent reduction of the C(5) ketone, ring opening and aldol addition form the cyclohexane ring to give 3-dehydroquinate (**136)** [148-150]. 3-dehydroquinate (**136**) is then dehydrated to give 3-dehydroshikimate (**137**), which is subsequently reduced to give shikimic acid (**138**), the first key intermediate in the shikimic acid pathway. In plants these two steps are catalysed by a single bifunctional 3-dehydroquinate dehydrataseshikimate dehydrogenase enzyme (E4). Shikimic acid (**138**) is phosphorylated by Shikimate kinase/ATP (E5) to give shikimic acid 3-phosphate (**139**), which is then converted to 5-enolpyruvylshikimate-3-phosphate (**140**) via the 5-enolpyruvylshikimate 3-phosphate synthase (E6) mediated addition of an enolpyruvate moiety from PEP (**133**). The next key intermediate, chorismic acid (**141**) is formed via a chorismate synthase (E7) mediated 1, 4-eliminatation of hydrogen phosphate from **140**. Chorismic acid (**141**) serves as an important branch point, where the biosynthetic pathway diverges to ultimately give tryptophan on the one hand and tyrosine/phenylalanine on the other [147].

The phenylethanoids and phenylpropanoids are products of the tyrosine/phenylalanine branch of this biosynthesis. Along this branch, chorismic acid (**141**) undergoes a chorismate mutase (E8) mediated formal Claisen rearrangement to give prephenic acid (**142**). Several pathways may then operate to convert prephenic acid (**142**) to tyrosine (**143**) and phenylalanine (**144**); however, overall they all involve transamination to install the amino group, decarboxylation and aromatisation.[34,35] At this point the biosynthesis of phenylpropanoids and phenylethanoids diverge. In the case of the biosynthesis of phenylpropanoids and phenylethanoids diverge. In the case of the phenylpropanoids, tyrosine (**143**) and phenylalanine (**144**) feed directly in as the C_6 C_3 building blocks necessary to produce the diverse range of phenylpropanoid natural products. Alternatively, decarboxylation of tyrosine (**143**) or phenylalanine (**144**) can produce C_6C_2 units required to feed into the biosynthesis of phenylethanoid natural products [147].

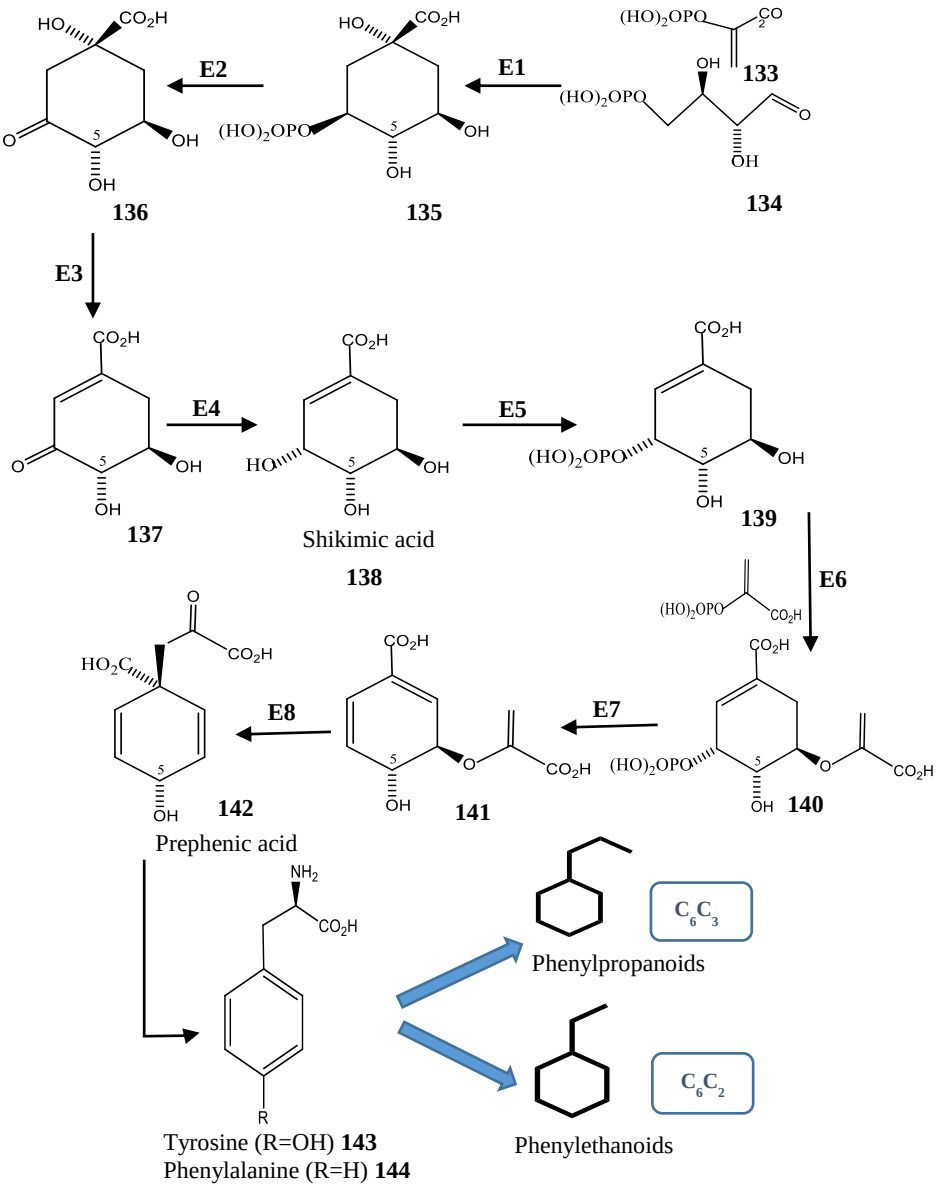


Figure II.11: The shikimic acid acid biosynthetic pathway to the phenylethanoids phenylpropanoids

CHAPTER III

Phytochemical study

III.1 Botanical reminder

Fraxinus xanthoxyloides (G.Don) Wall. ex A.DC. is a small tree (4-5m), characterized by apetalous flowers with calyx, except that the male flowers lack calyx. The flowers are polygamous and wind pollinated. The leaves are relatively small (7-15cm) with a more or less winged leaf-rachis. The fruit is a samara, normally one-seeded. Bark pale grey or whitish with reticulate carks (Figure III.1) [13, 151].

Figure III.1: Foliage with small compound leaves of the Algerian species *Fraxinus xanthoxyloides*.

Fraxinus xanthoxyloides is a mountain species. It is distributed in Northern areas of Pakistan, Afghanistan, India, Moroco and in Algeria [152]. According to Quezel and Santa [5], we find this ash in Aurès, Belezma, ountains of Hodna and in the Saharan Atlas. The common name is " Dimorphic Ash ", in Arabic is " Dardar "; In Tamazight " Touzzalt " in the Sousse region and the High Atlas in Moroco and " Imts or Imtese " in the the Middle Atlas [153].

This species is classified as below [13]:

Family: Oleaceae

Subfamily: Oleoideae

Tribe: Oleeae

Genus: Fraxinus

Section : Sciandanthus

Species: *Fraxinus xanthoxyloides* (G.Don) Wall. ex A.DC.

English name: Algerian Ash.

Synonyms: *Fraxinus dimorpha* Coss. & Dur. [154].

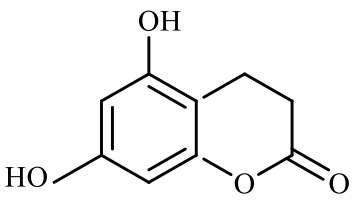
Fraxinus xanthoxyloides (G. Don) DC. [4].

III.2 Traditional uses of *Fraxinus xanthoxyloides*

Leaves and root bark of *Fraxinus xanthoxyloide* are used for the treatment of jaundice, malaria and pneumonia [155]. Stem bark is used in the form of decoction to reduce pain during labor [156], expulsion of pre-mature infant after death [157]. Decoction of stem/twigs is also used in wounds and bone fractures in cattle [158].

III.3 Previous phytochemical works

A few chemical studies has been carried out on the species *F. xanthoxyloides* growing in Pkistan and lead to the isolation of a new biscoumarin (xanthoxyloidin (**23)**) together with esculetin (**1**), 5, 7- dihydroxycoumarin (**121**) and 6,8-dihydroxy-7-methoxycoumarin (**122**) from the methanolic extract of the whole plant [32].



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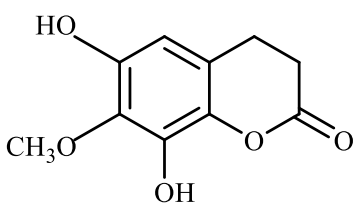


122

In 1968, the work done by Plauvier [18, 19] has allowed to isolate the following coumarins: cichoriin (**3**) and fraxin (**10**). Another phytochemical study has been carried out on the

Algerian species *F.xanthoxyloides* (G.Don.) DC. lead to the isolation of cichoriin (**3**), ligstroside (**30**) and formoside (**37**) [159].

III.4 Exractive chemistry

III.4.1 Extraction

From the dried and powdered material, extraction was carried out by using methanol solvent. After filtration and concentration, a viscous solid was obtained. The below diagram summarizes the different stages of extraction of both stem bark and leaves of *F. xanthoxyloides* species (Figure III.2).

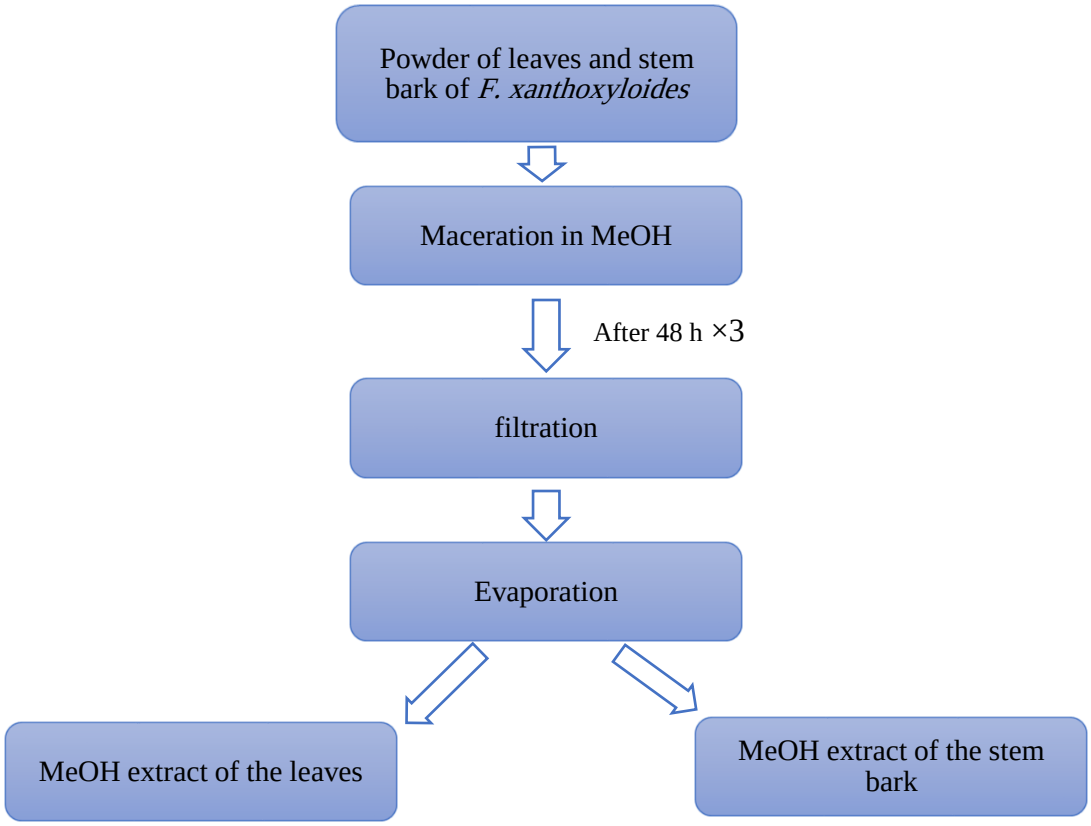


Figure III.2: **Scheme of extraction of the stem bark and the leaves of *Fraxinus xanthoxyloides***

III.4.2 Purification

The comparison of the TLC profiles of the two extracts obtained in different elution systems ($CHCl_3$:MeOH:H_2O, EtOAc) clearly shows their richness in secondary metabolites, particularly of the coumarins and secoiridoid compounds, which prompted us to chemically explore the two extracts.

III.4.2.1 *Fraxinus xanthoxyloides* stem bark (FXB)

10g of the methanolic extract were fractionated by using polyamide 6 column chromatography in reversed phase. The elution was carried out with Water:MeOH gradient system. Nine main fractions were collected according to their chromatographic profiles. These fractions were subjected to purification by using a combination of different chromatographic technics. Thus, ten compounds were isolated, one of which was a new coumarin-secoiridoid (Figure III.3).

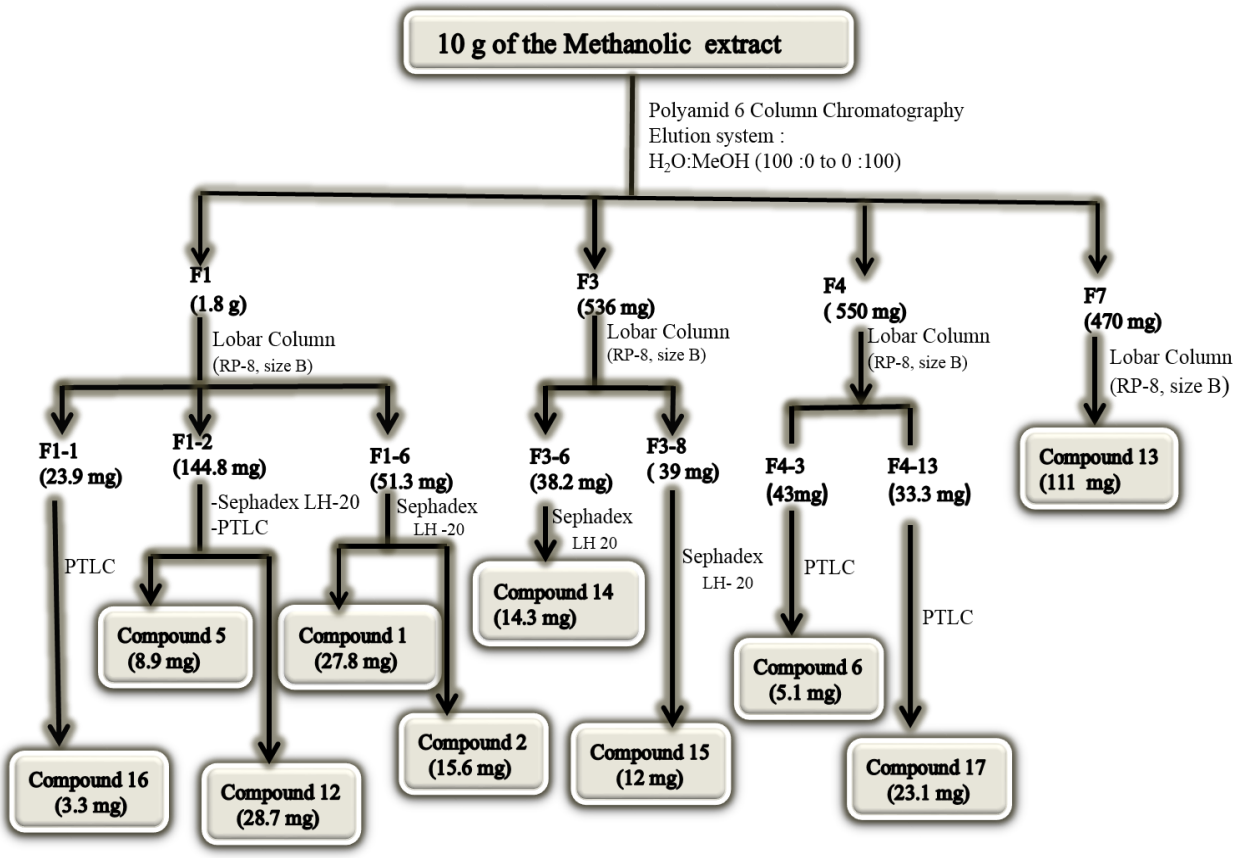


Figure III.3: Scheme of purification of the products of the MeOH extract of *F. xanthoxyloides* stem bark

III.4.2.2 *Fraxinus xanthoxyloides* leaves (FXL)

Fractionation of 15 g of the methanolic extract was carried out by column chromatography of polyamide 6 using H_2O:MeOH gradiant eluent ranging from (100:0) to (0:100). Thirteen fractions were collected according to their chromatographic profiles. Fractions F2, F3, F4, F6 and F8 (found to be interesting) were chromatographed using different chromatographic technics (TLC, Sephadex LH 20 Column chromatography and Lobar column). The purification lead to the isolation of nine compounds (Figure III.4).

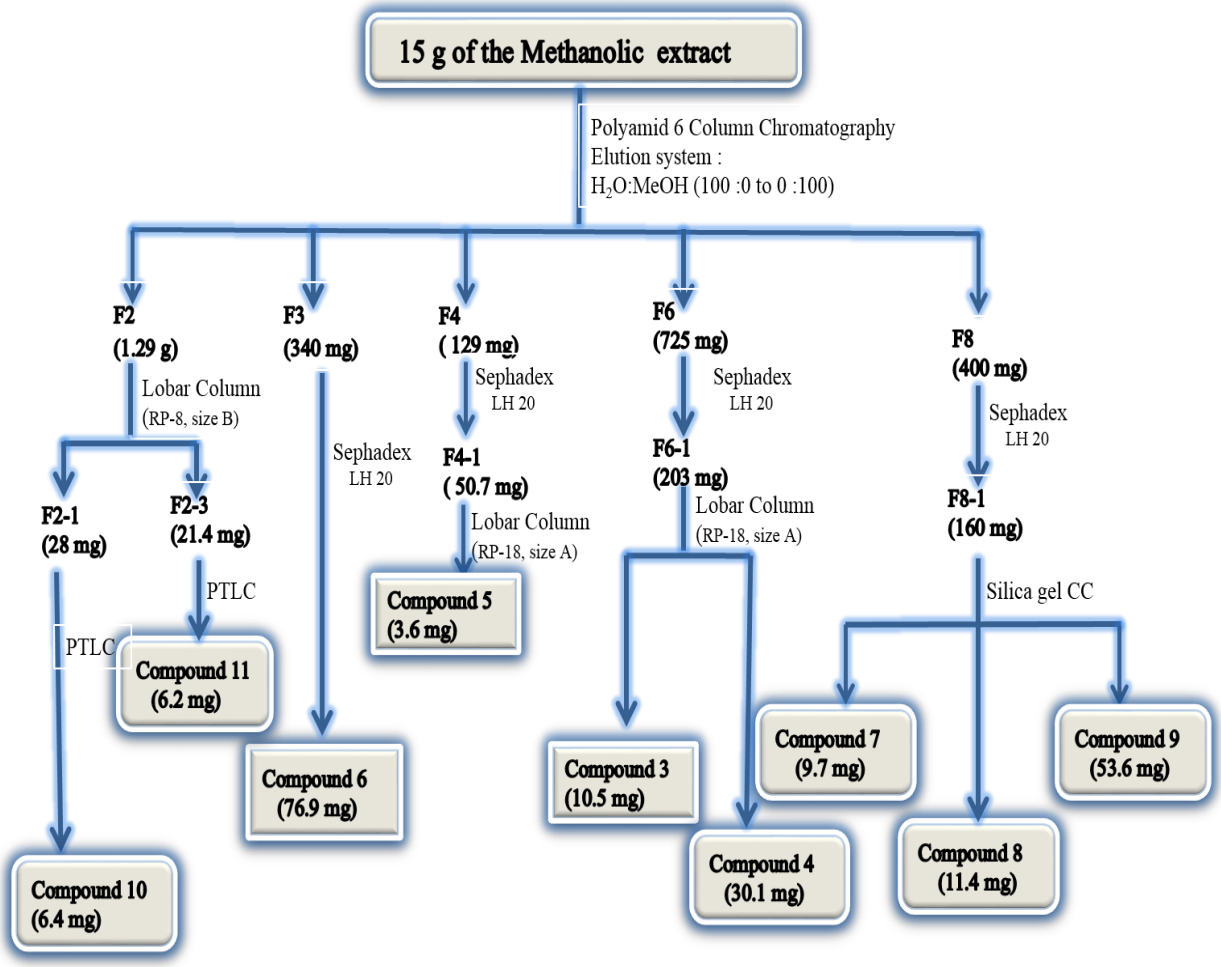
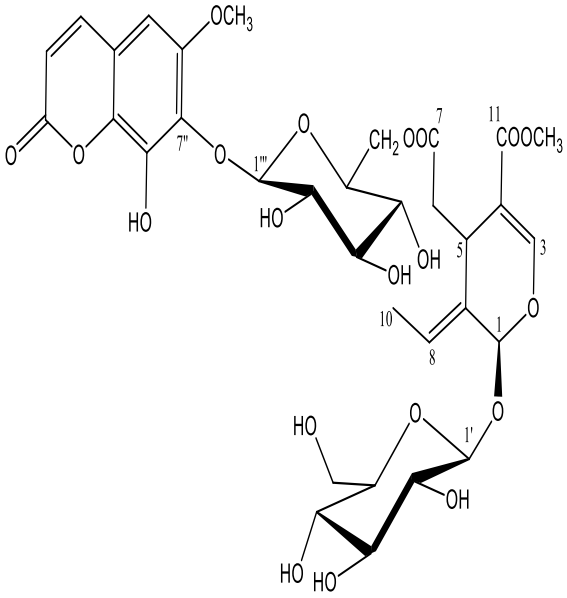
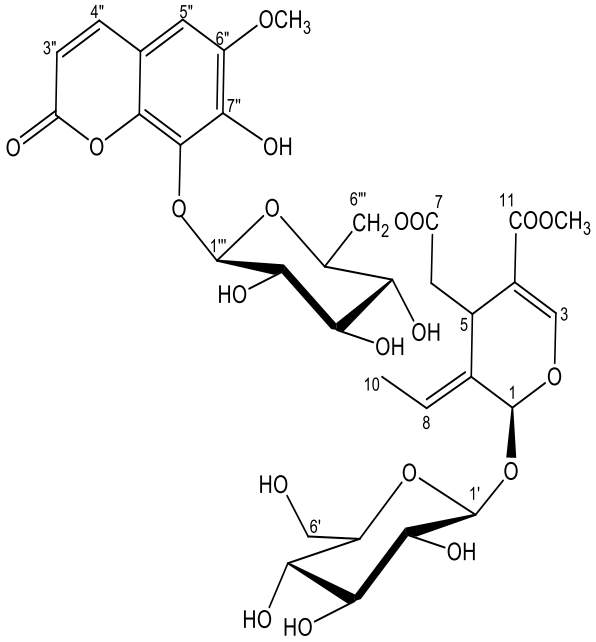


Figure III.4: Scheme of purification of the products of the MeOH extract of *F. xanthoxyloides* leaves.

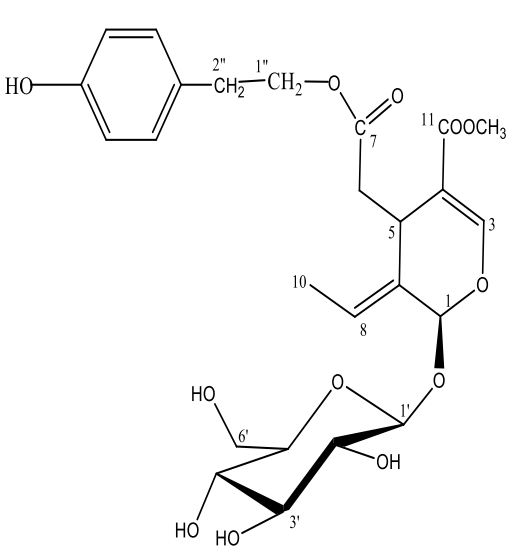
All the structures of the isolated compounds (**1-17**) from both leaves and stem bark are illustrated bellow.



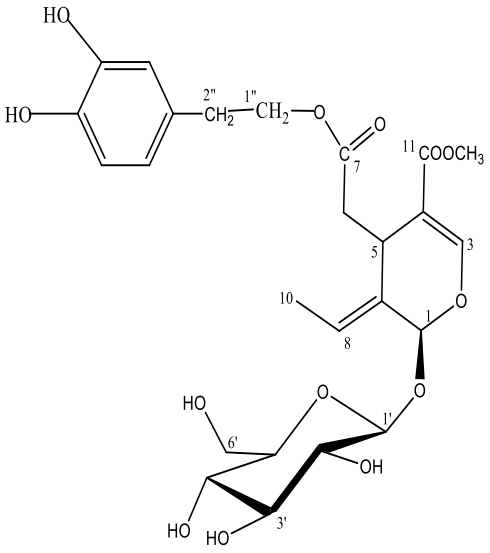
Isofraxisecoside (**1**)



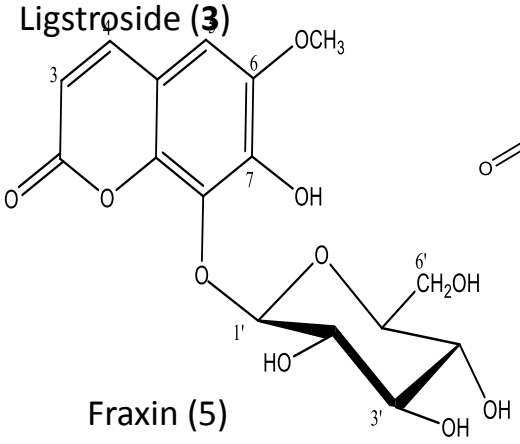
Fraxisecoside (**2**)



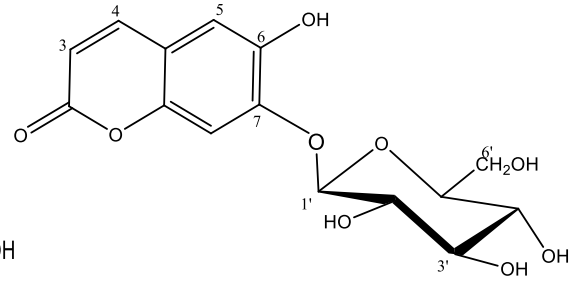
Ligstroside (**3**)



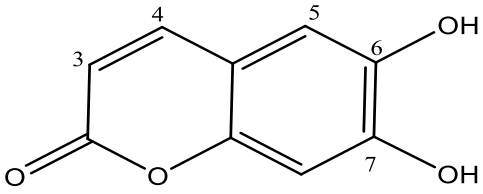
Oleuropein (**4**)



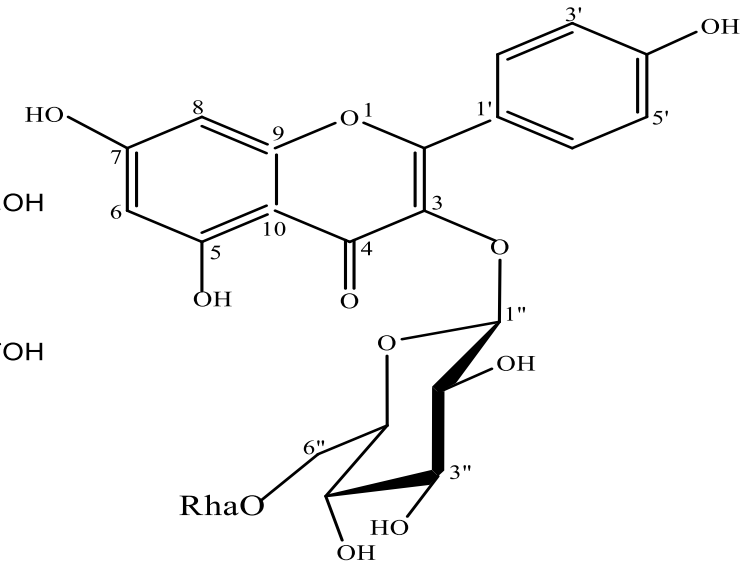
Fraxin (5)



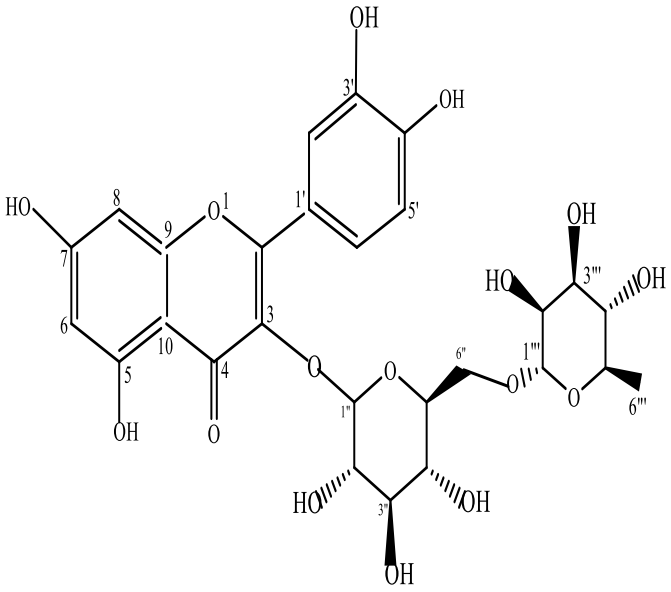
Cichoriin (6)



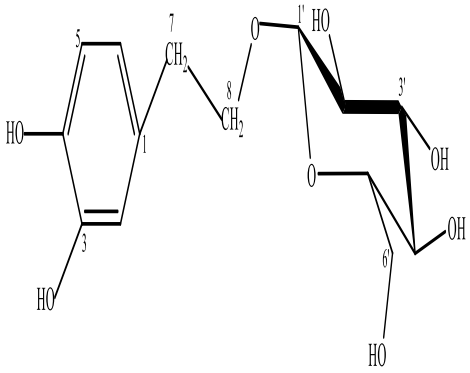
Esculetin (**7**)



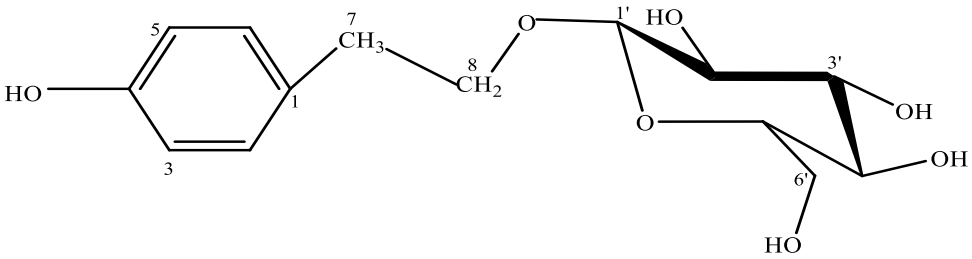
Nicotiflorin (**8**)



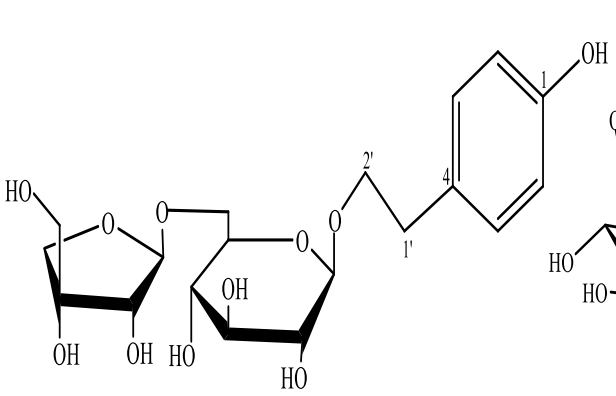
Rutin (**9**)



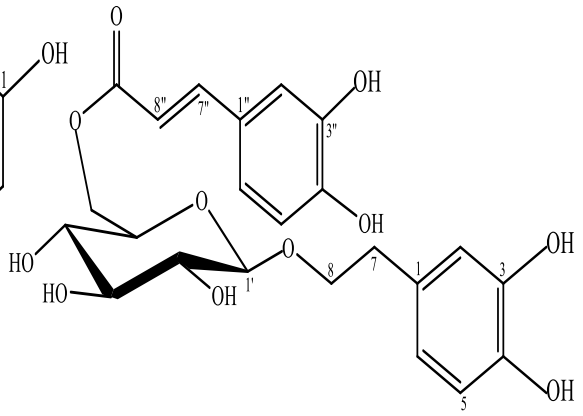
2-(3, 4-dihydroxy)phenylethyl-O-β-D-glucopyranoside (**10)**



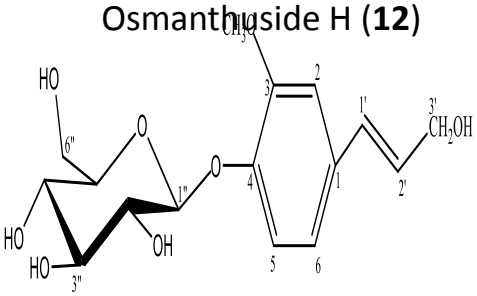
Salidroside (**11**)



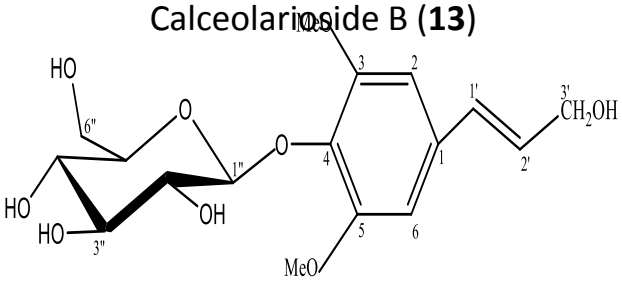
Osmanthuside H (**12**)



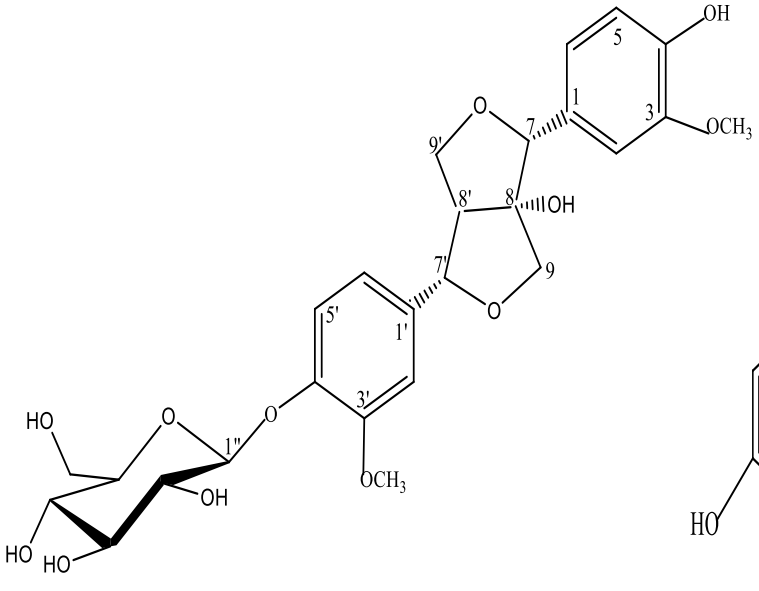
Calceolarioside B (**13**)



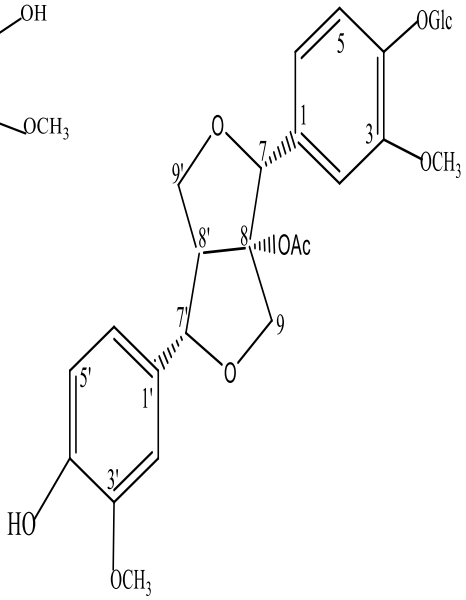
Coniferin (**14**)



Syringin (**15**)



8-hyroxypinoresinol-4’-O-β-D-glucoside (**16**)



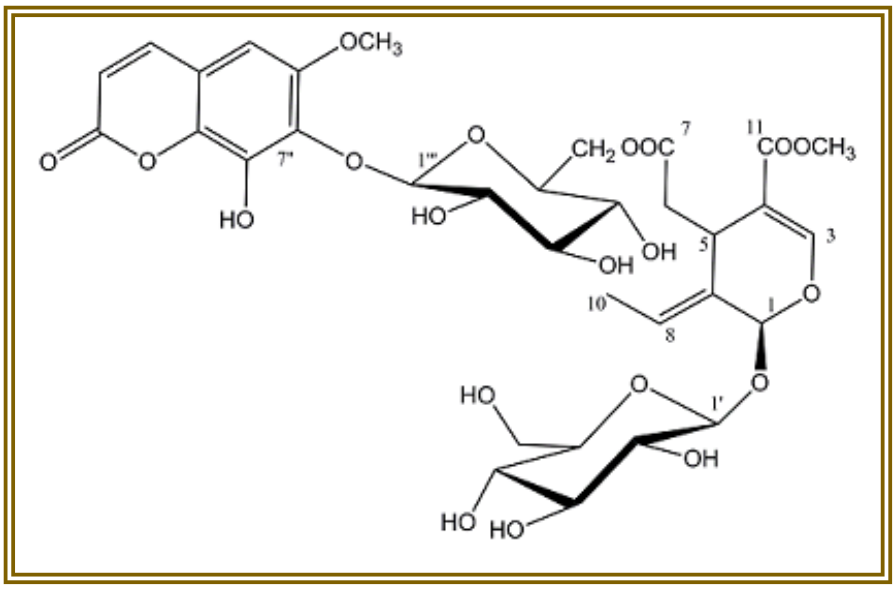
8-acetoxypinoresinol-4-O-β-D-glucoside (**17**)

III.4.3 Characterization of the obtained products

The structures of the isolated compounds have been determined on the basis of physical and spectroscopic analysis, including 1D and 2D NMR (^{1}H, ^{13}C, HSQC, HMBC and ROESY) and mass spectrometry (HRMS), measurement of the optical rotation $[\alpha]_D^{20}$ and by chemical transformations. The known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

III.4.3.1. Identification of coumarin-secoiridoid compounds

III.4.3.1.1 Structure elucidation of compound 1



Fraxetin-7″-O- [11-methyl-oleosidyl- (7⟶ 6‴)]-β-glucopyranoside

(Isofraxisecoside)

This compound was isolated from the stem bark as a yellow amorphous powder, $[\alpha]_D^{20}$ = -135.8 (c = 0.095, MeOH), with a dark blue fluorescence under UV light at 254 nm indicates a secoiridoid skeleton [160]. The UV spectrum (Figure III.5) recorded in methanol showed absorption maxima at 229.5, 328.5, 335.5, 345.0, suggesting a conjugated aromatic system [55].

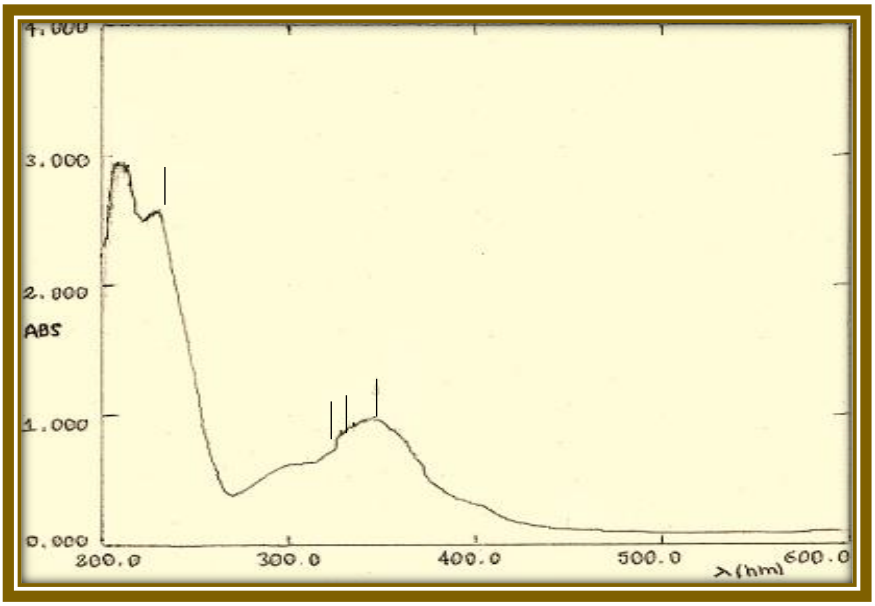


Figure III.5: UV spectrum of compound 1

The high resolution mass spectrum HR-ESI-MS (Figure III.6) obtained in negative electrospray mode showed deprotonated molecular ion $[M-H]^-$ at *m/z* 755.20404 pointing out molecular formula $C_{33}H_{40}O_{20}$ (calcd. for $C_{33}H_{39}O_{20}$: 755.2038).

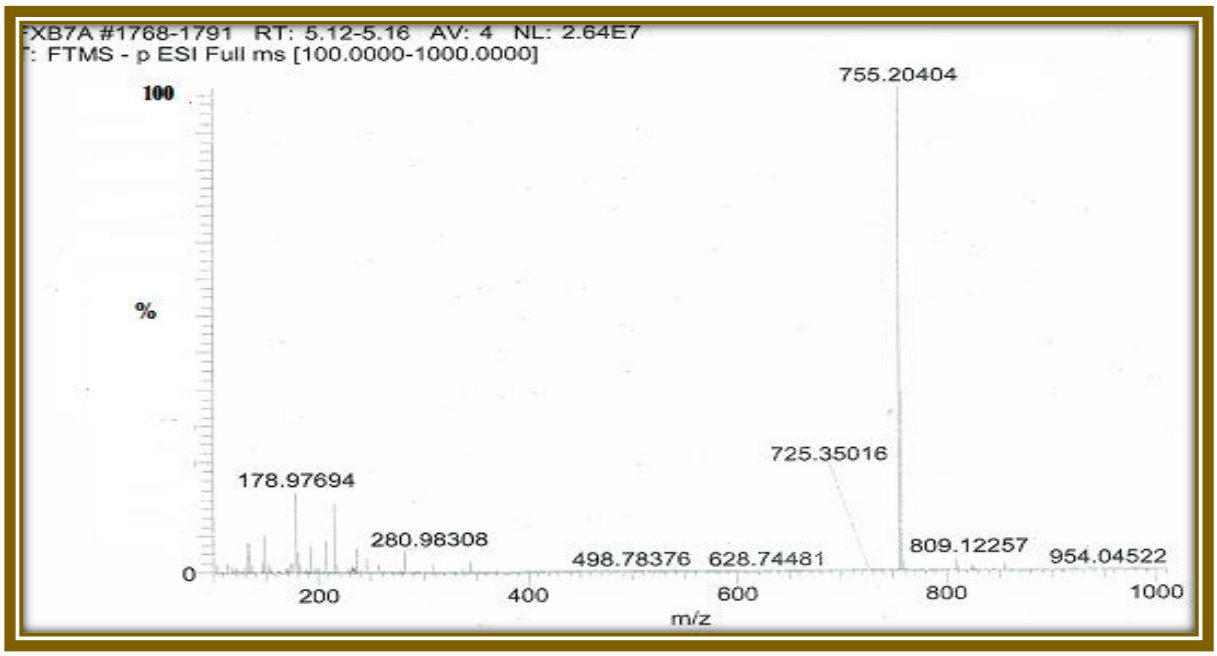


Figure III.6: Mass spectrum HR-ESI-MS of compound 1

The main diagnostic ion peaks in the ESI-MS/MS of compound **1** (Figure III.7) are formed by cleavage of the glucosidic linkage between the secoiridoid glucoside and the coumarin aglucone moiety. The first peak at *m/z* 755.204 for the pseudo-molecular ion and the second ion peak at *m/z* 207.029 for the coumarin aglucone unit. A proposed pathway for fragmentation of compound **1** is given in Figure III.8

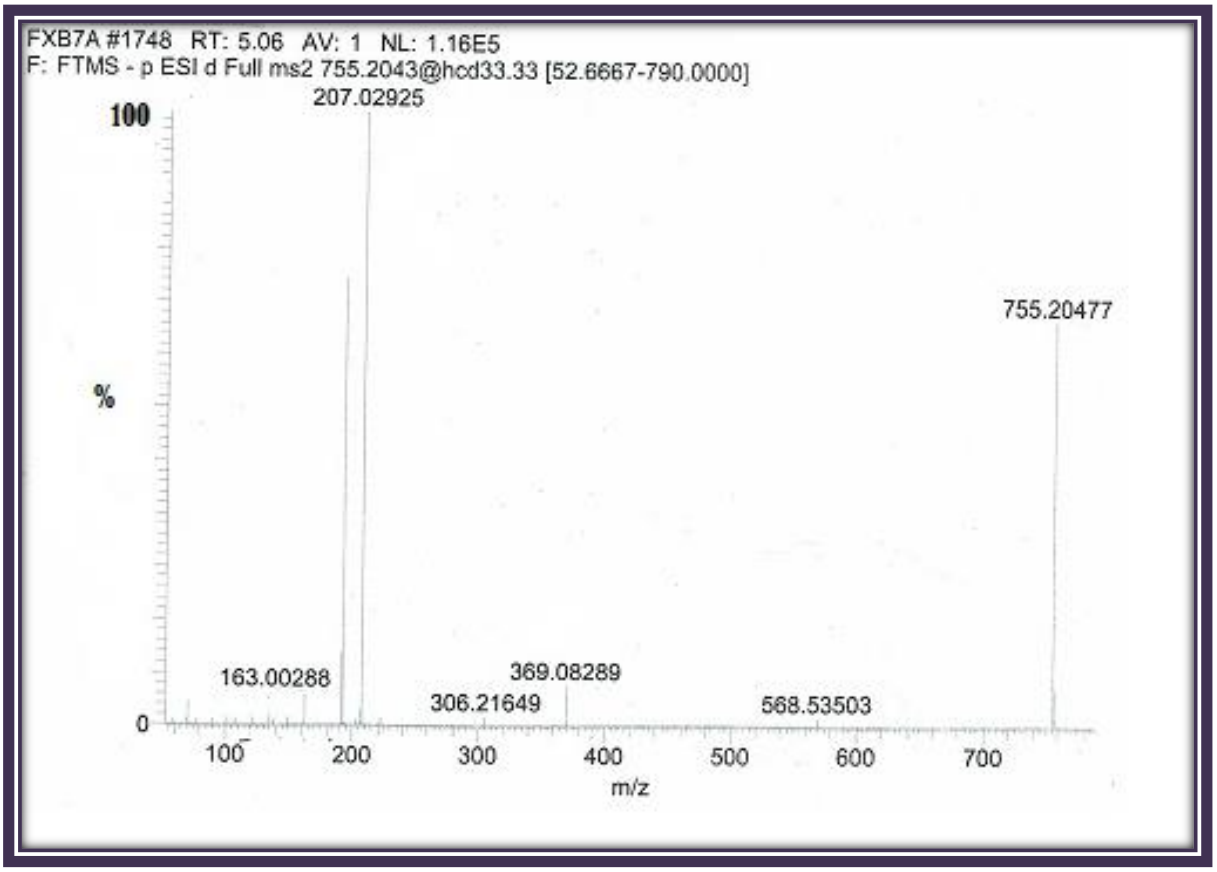


Figure III.7: Mass spectrum HR-ESI-MS/MS of compound 1

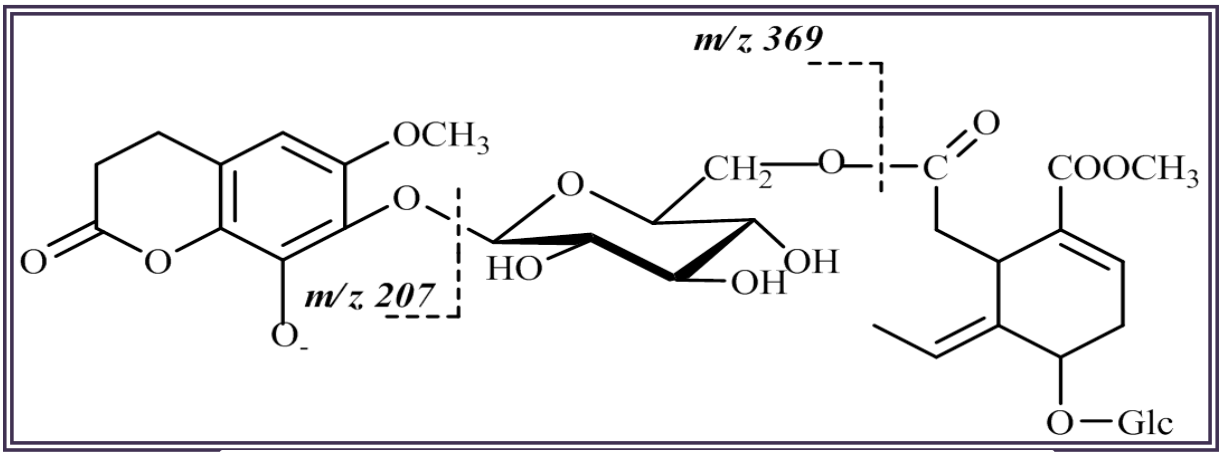


Figure III.8: Proposed fragmentation patterns for $[M-H]^-$

Detailed analysis of its ^{1}H, ^{13}C, HSQC, HMBC and ROESY spectra indicated the presence of two structural units: a secoiridoid glucoside and a coumarin glucoside.

The NMR spectrum (Figure III.9) of compound **1** recorded in CD_3OD shows signals of aromatic protons resonating between 6.04 and 7.83 ppm and glucosidic protons between 3.28 and 4.85 ppm.

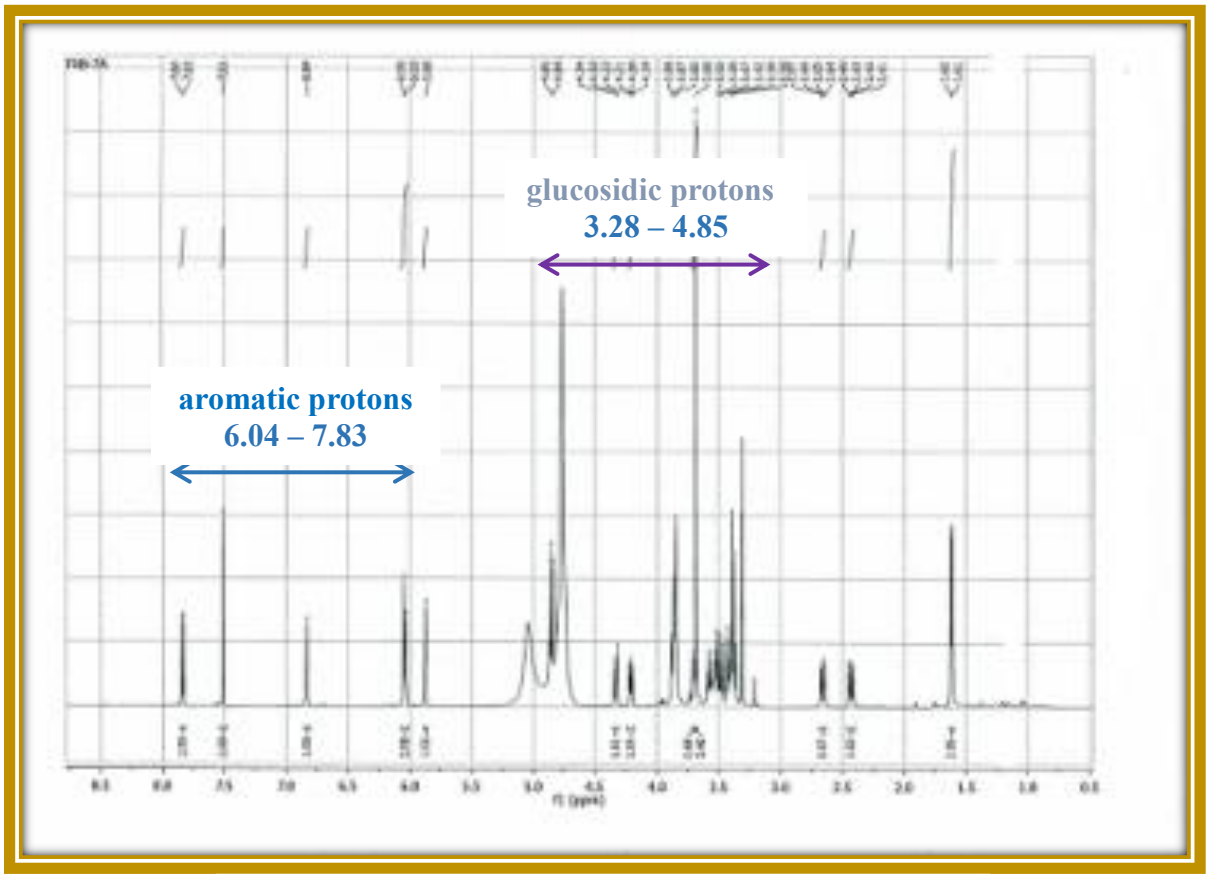


Figure III.9: ^{1}H NMR spectrum of compound 1

The structural analysis of the ^{1}H NMR spectrum (Figure III.10) reveals:

- Two signals with δ_H 6.04 and 7.83 of 1H integration of each, in the form of doublet. The value of the coupling constants between those protons (J = 9.3 Hz) indicates that the coupling is of ortho type.

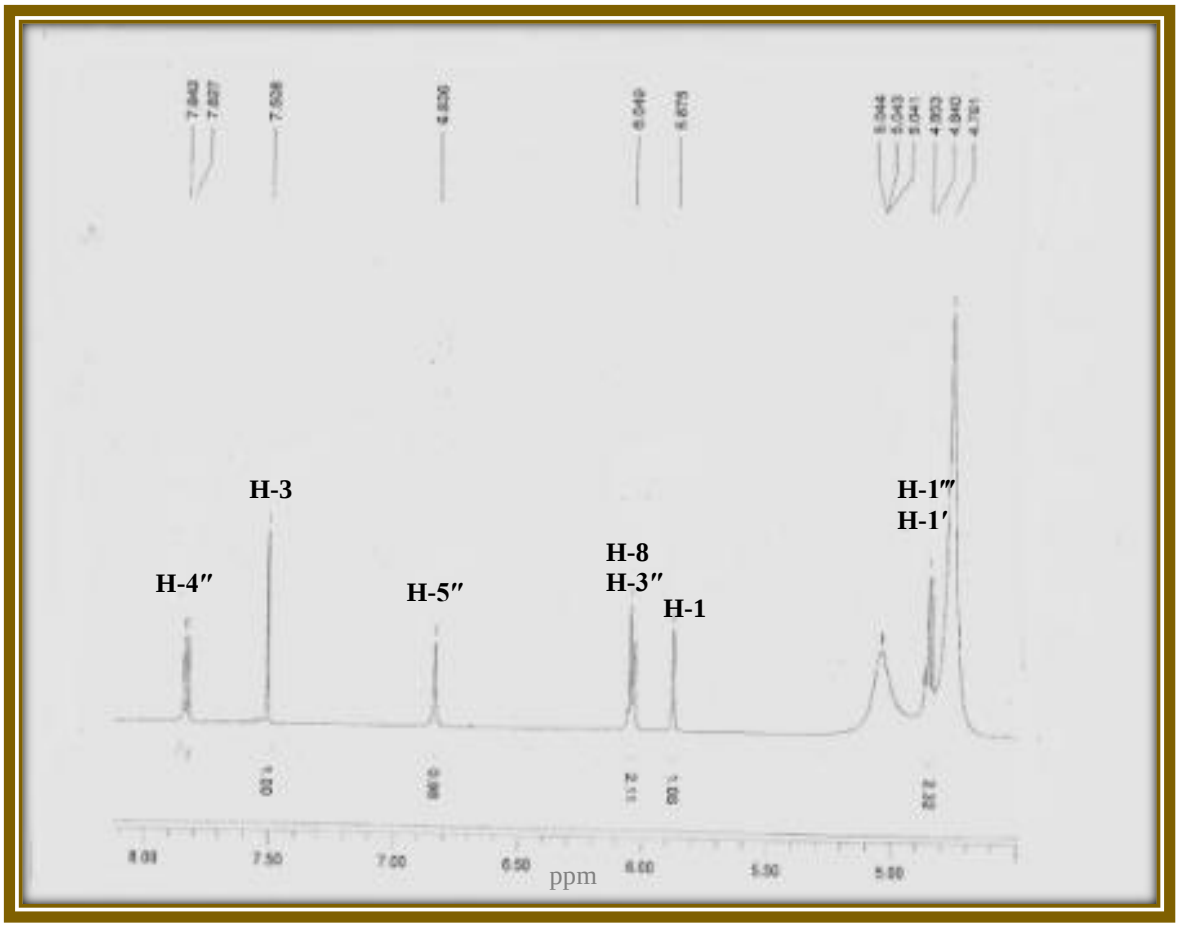


Figure III.10: ^{1}H NMR spectrum of compound 1

Moreover and according to the ROESY spectrum (Figure III.13), these two protons belong to the same spin system, corresponding to H-3″ and H-4″ protons of the coumarin moiety (Figure III.11).

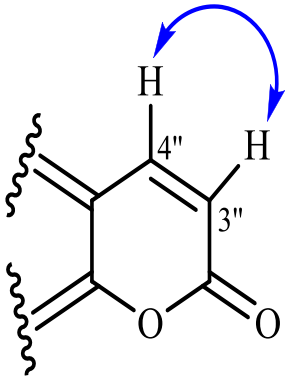


Figure III.11: ROESY correlation between H-4″ and H-3″

- One aromatic proton at δ_H 6.84 of 1H integration in the form of singlet (figure III.10). The ROESY experiment (Figure II.13), showed a cross peak between this signal and the proton H-4″, so it is assigned to H-5″.

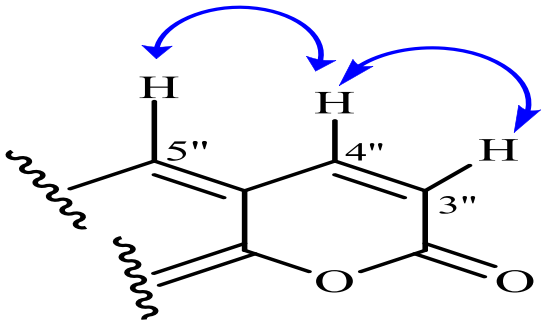


Figure III.12: ROESY correlation between H-4″ and H-5″

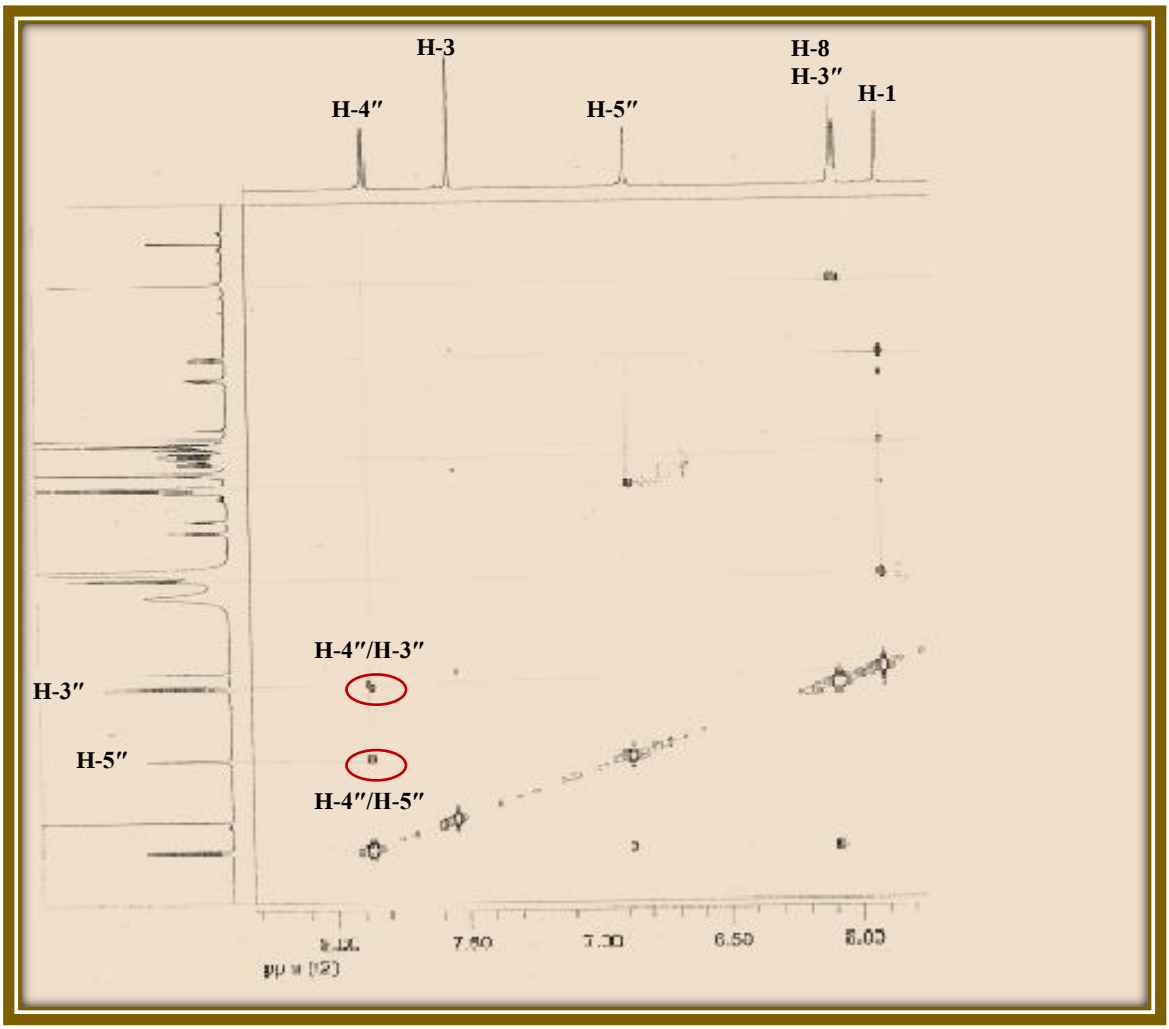


Figure III.13: ROESY spectrum of compound 1

The signals of three protons at δ_H 6.04 (1H, *d*, *J* = 9.3 Hz, H-3″), δ_H 7.83 (1H, *d*, *J* = 9.3 Hz, H-4″) and δ_H 6.84 (1H, s, H-5″) indicated a 6-, 7-, 8- substituted coumarin structure.

Also, the ^{1}H NMR spectrum (Figure III.10 and III.14) exhibited typical signals of an oleoside type secoiridoid which are:

- An olefinic signal at δ_H 7.50, correlating on HSQC spectrum with carbon C-3 resonating at 155.38 ppm.
- A signal at δ_H 5.87 in the form of singlet integrating for one proton was characteristic of the acetalic proton H-1.
- A methine proton resonating at 3.87 ppm corresponding to H-5.
- A signal at δ_H 6.05 corresponding to the olefinic methine proton H-8.

Besides these signals, the ^{1}H NMR spectrum (Figure III.10 and III.14) allows to show:

- Two methoxyls at δ_H 3.84 and 3.68.
- Two anomeric protons resonating at δ_H 4.85 (d, J = 7.8 Hz).
- An olefinic methyl double doublet at δ_H 1.61 (J = 1.1, 7.2 Hz) was characteristic for the protons H_3-10.
- Signals of glucosidic protons between 3.28 and 4.33 ppm were attributed to protons of at least two sugars.

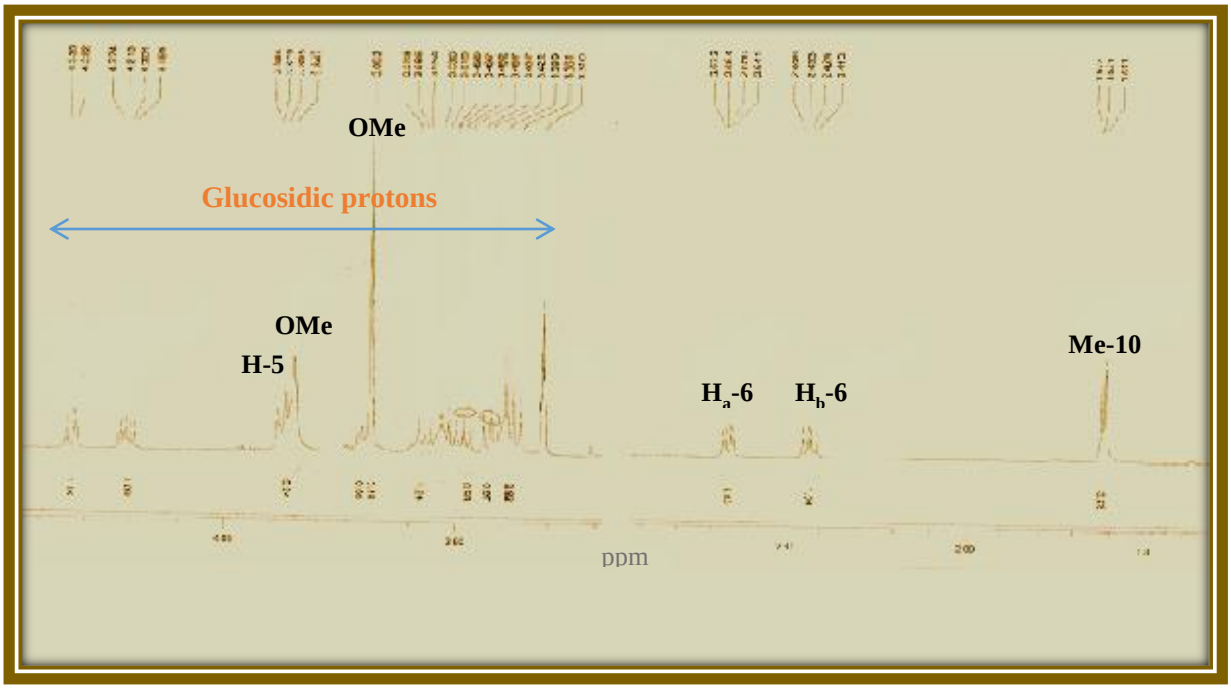


Figure III.14: ^{1}H NMR spectrum of compound 1

The ^{13}C NMR spectrum and DEPT 135 of compound **1** (Figure III.15 and III.16) make it possible to count 33 signals corresponding to 33 carbon atoms, which are distributed as follow:

- Three carbonyl carbons of an ester at δ_C 173.74, 169.32 and 165.34.
- Four oxygenated aromatic carbons resonating between 133 and 156 ppm.
- Three aromatic CH between 100 and 148 ppm
- Three quaternary carbons at δ_C 129.96, 109.26 and 108.28.
- Two olefinic carbons at δ_C 155.38 and 125.32.
- One acetalic carbon at δ_C 95.39.
- One methine carbon at δ_C 31.45.
- Ten CH resonant between 70 and 107 ppm corresponding to the carbons of two sugar units.
- Two oxymethylene groups at δ_C 65.01 and 62.12 and one methylene carbon at δ_C 41.33.
- A sp^3 carbon at δ_C 13.63.
- Two methoxyls (OCH_3) detected at δ_C 56.77 and 52.53.

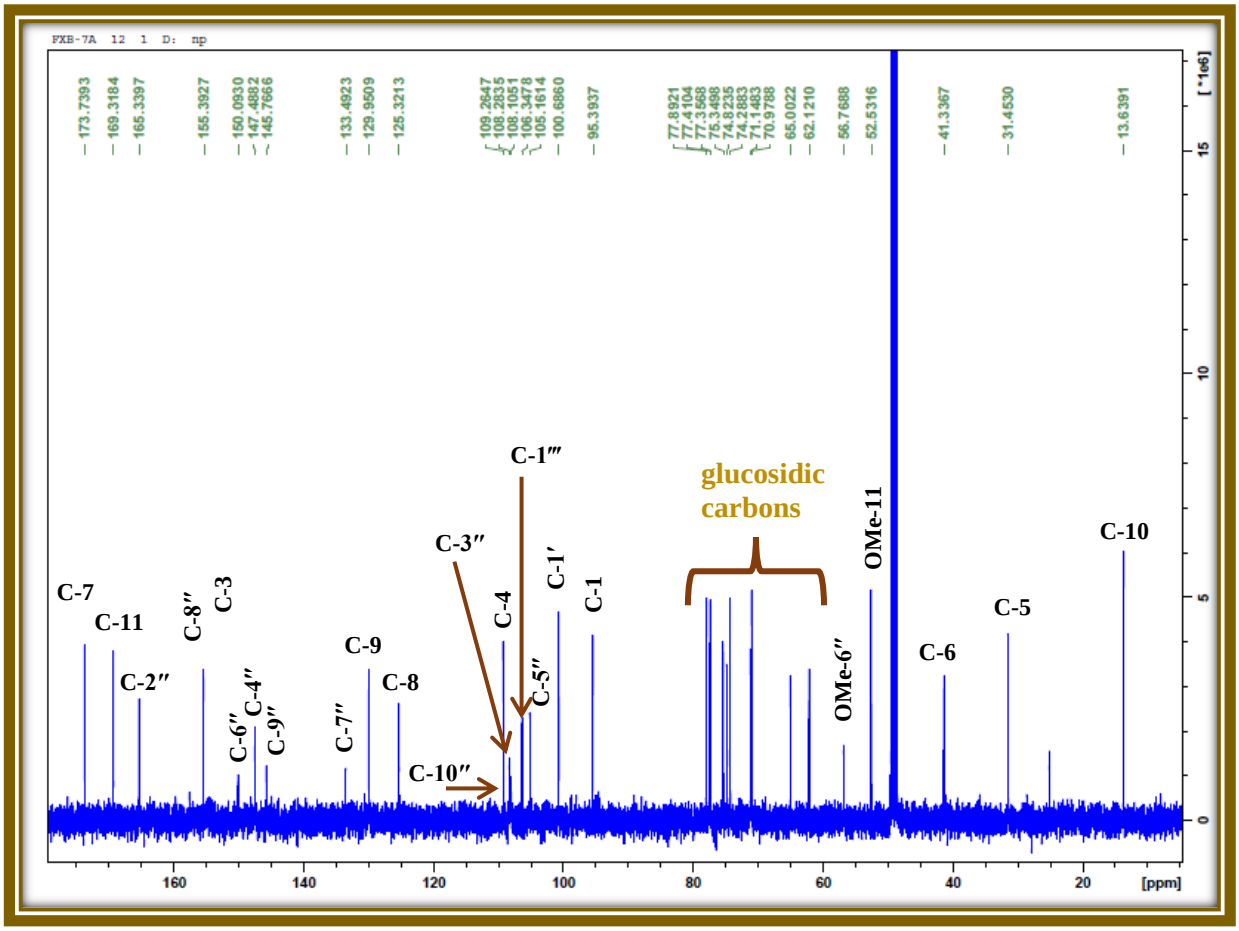


Figure III.15: ^{13}C NMR spectrum of compound 1

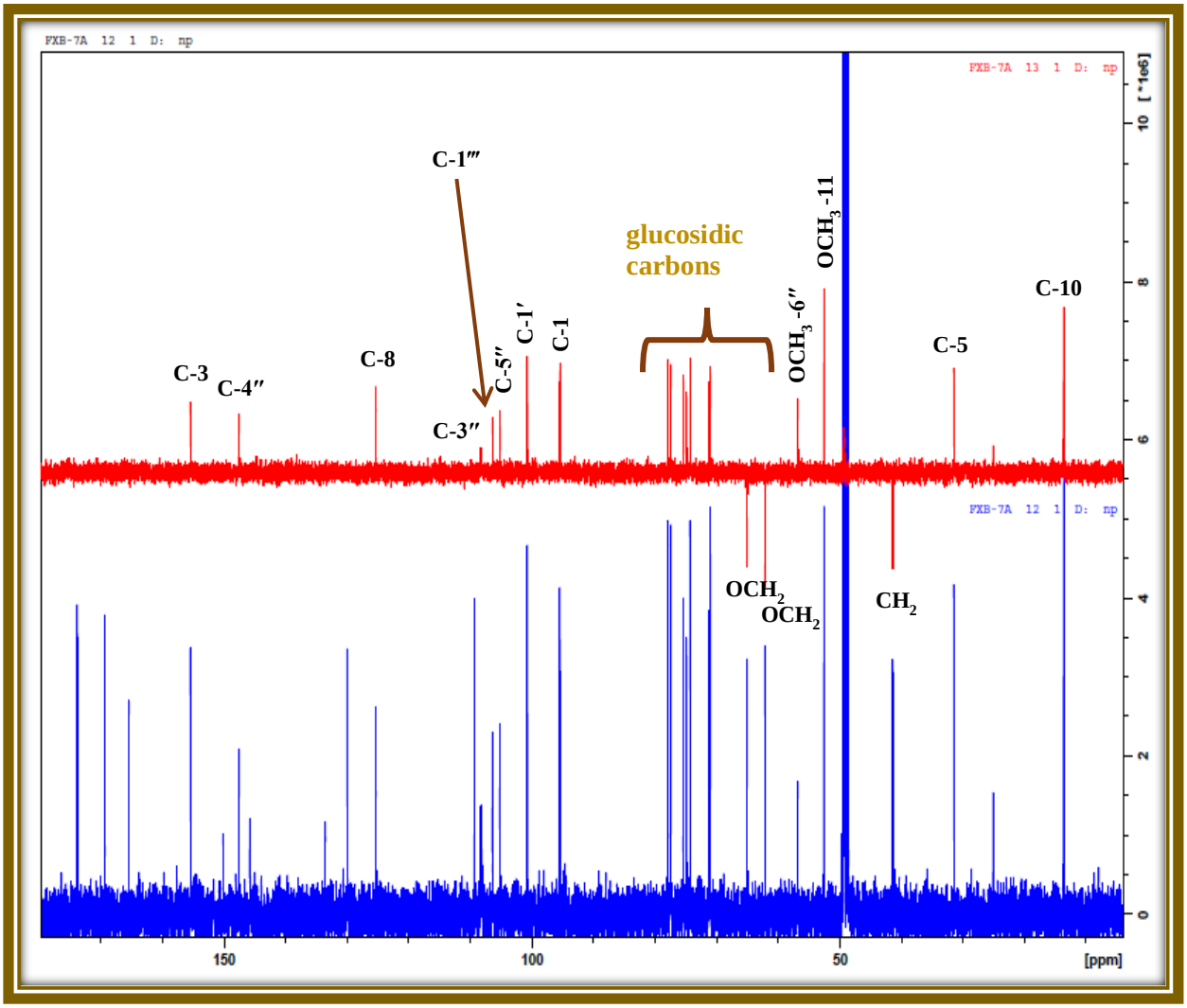


Figure III.16: DEPT experiment of compound 1

The combined analysis of the HMBC and HSQC spectra (Figure III.17, III.18, III.19 and III.20) allowed the elaboration of the following parts:

The coumarin glucoside unit:

- The H-3″ and H-4″ protons already identified coupling in HSQC with their carbons at δ_C 108.11 and 147.49 correlate on the HMBC spectrum in ^2J and ^3J with the carbonyl carbon at δ_C 165.34 (C-2″) and in ^3J and ^2J with the quaternary carbon at δ_C 108.28 (C-10″), respectively.
- The carbon C-9″ (δ_C 145.77) was identified further to the presented correlation in ^3J with the protons H-4″ and H-5″.

- The chemical shift of the C-7″ was localized at δ_C 133.49 following to the correlation which it had in ^3J with the anomeric proton H-1‴. The chemical shift of the anomeric carbon C-1‴ (δ_C 106.30) was determined by HSQC experiment.
- The proton H-5″ correlate on the HMBC spectrum in ^4J with the oxygenated aromatic carbon C-8″ at δ_C 155.39.

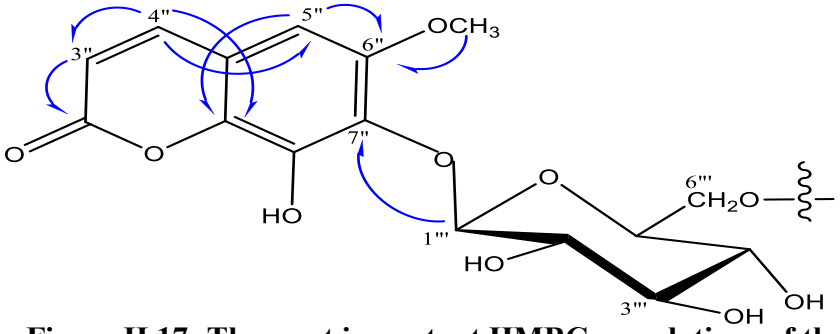


Figure II.17: The most important HMBC correlations of the coumarin unit of compound 1

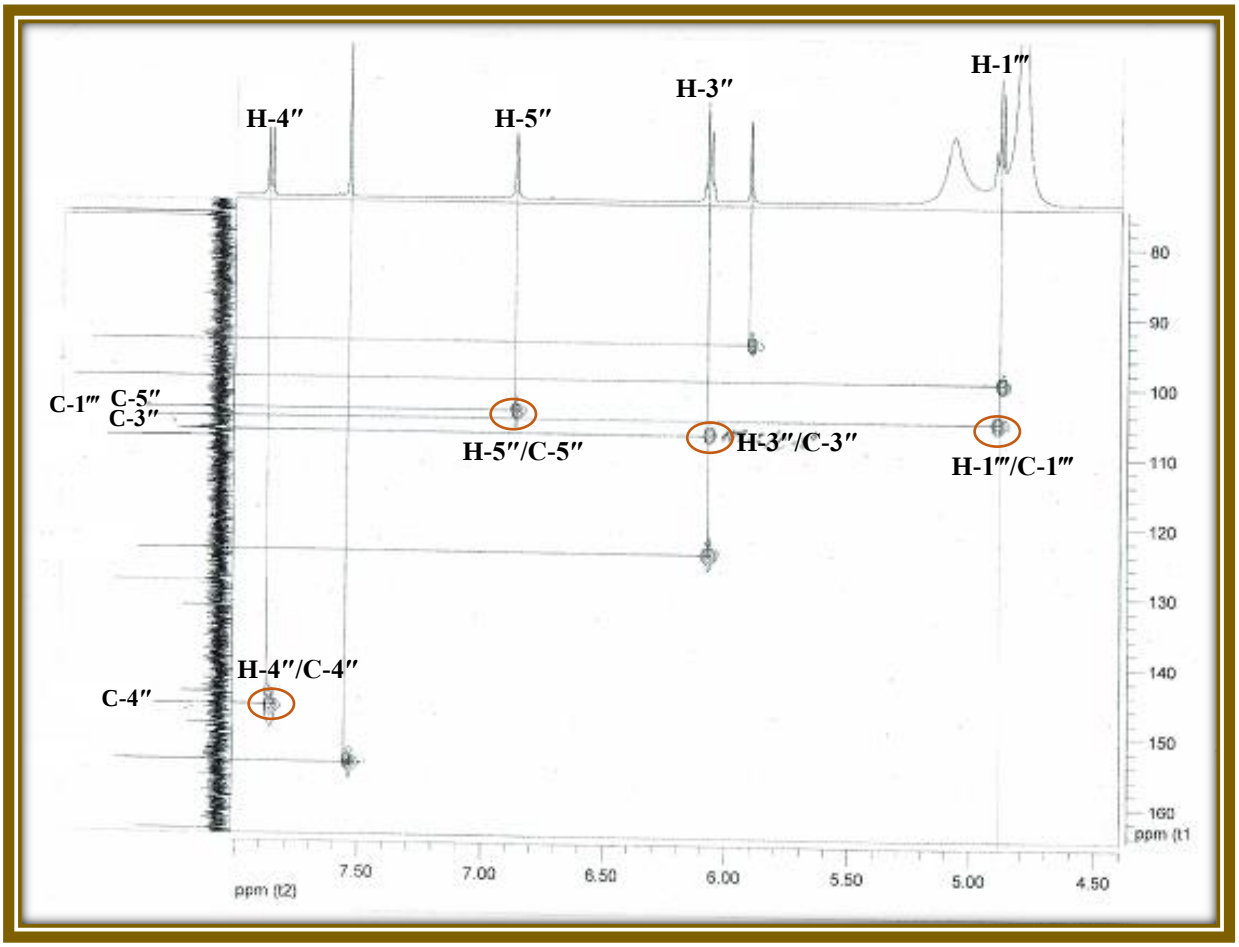


Figure III.18: The HSQC spectrum of the coumarin unit of compound 1

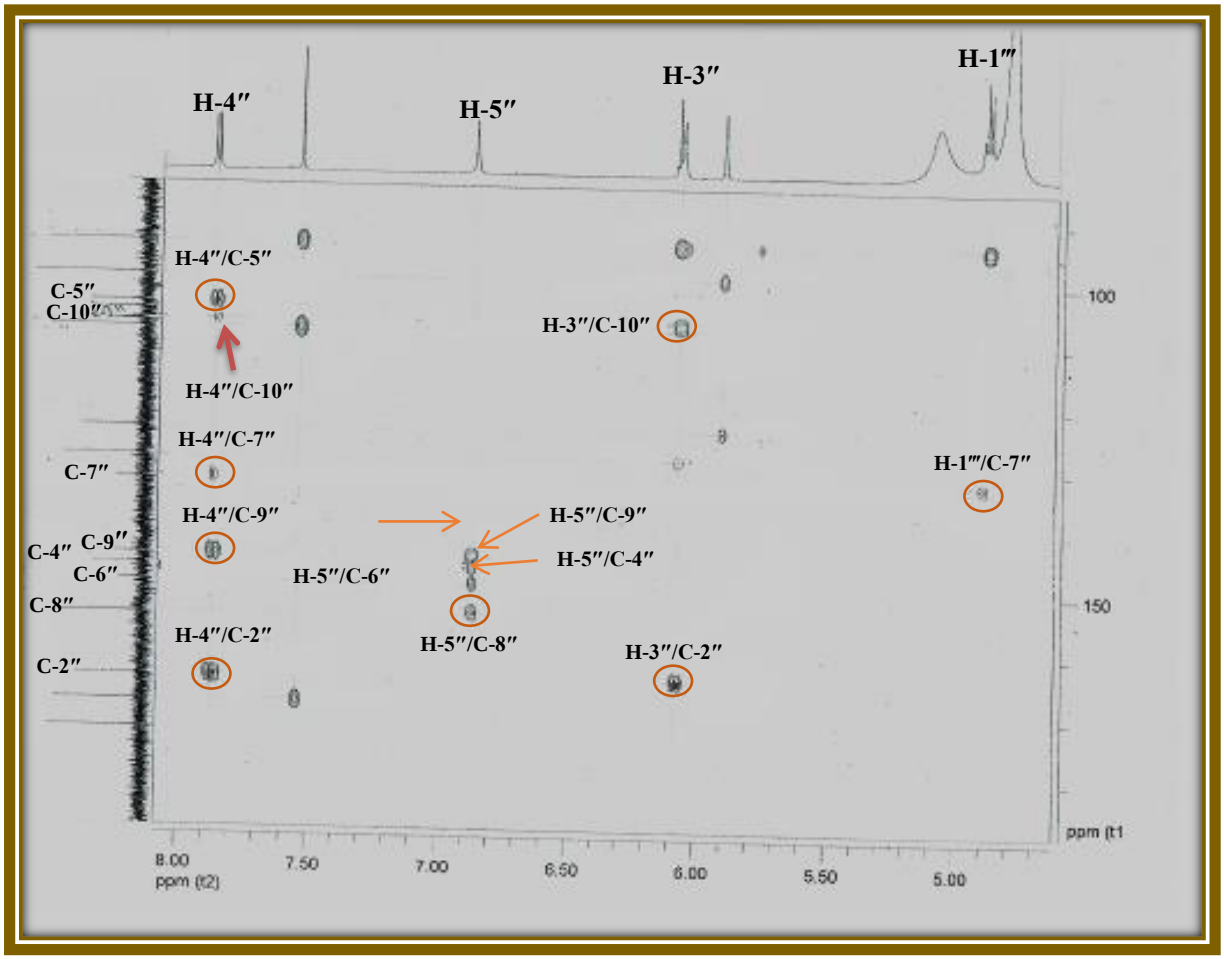


Figure III.19: The HMBC spectrum of the coumarin unit of compound 1

The signal at δ_C 56.78 corresponding to the aromatic methoxyl OCH_3 group (δ_H 3.84) was determined by HSQC and HMBC experiments (Figure III.20 and III.21).

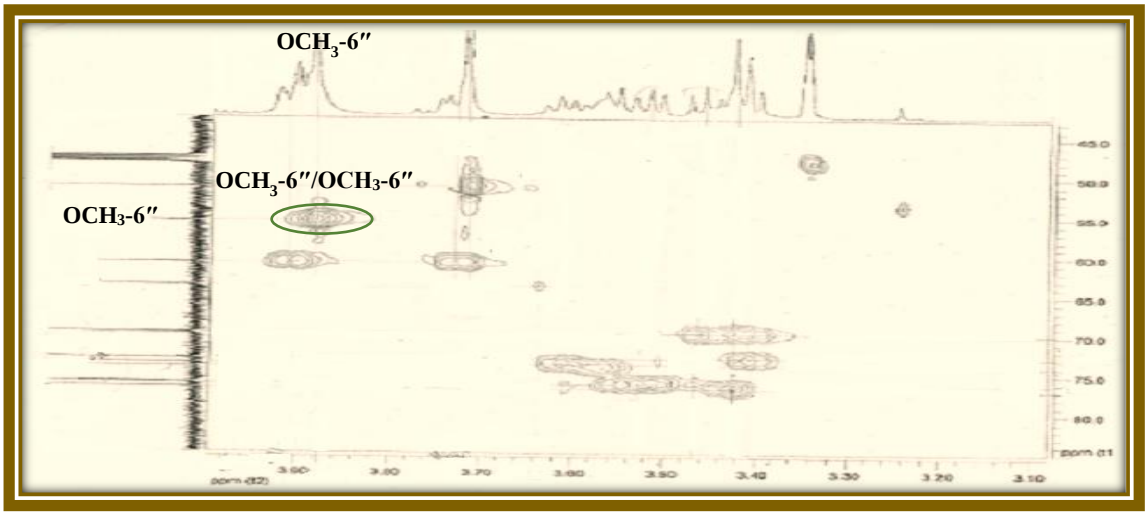


Figure III.20: The HSQC spectrum of the coumarin unit of compound 1

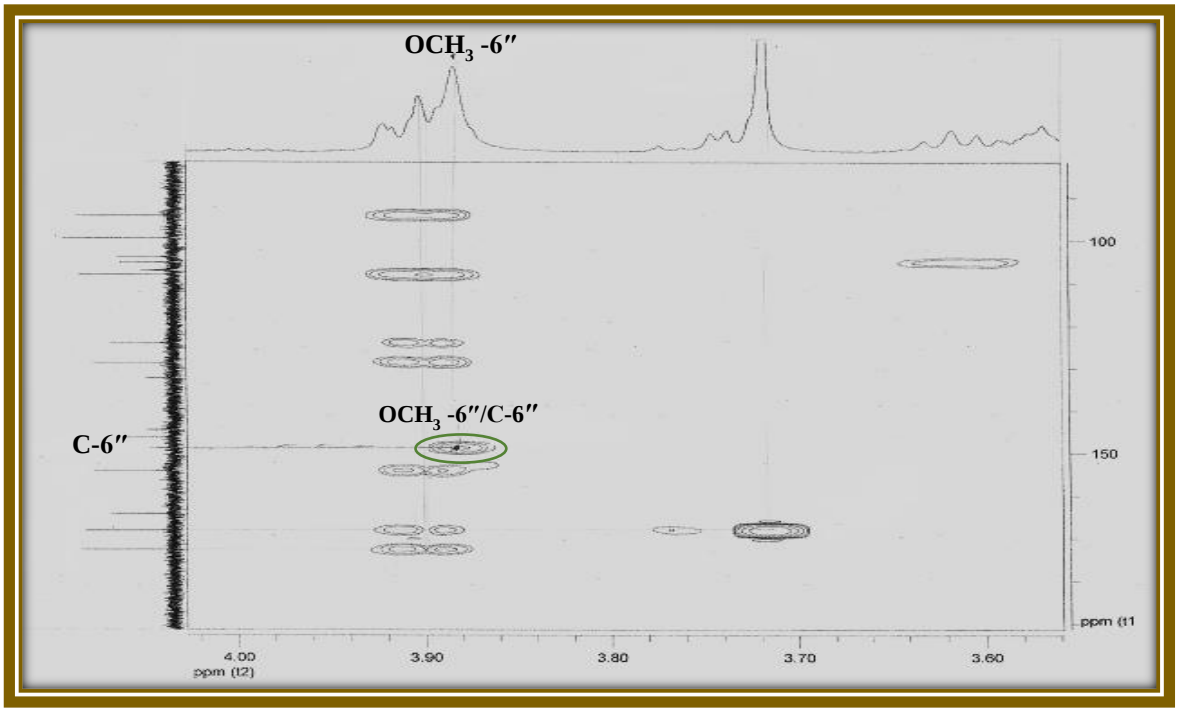


Figure III.21: The HMBC spectrum of the coumarin unit of compound 1

The ROESY association of the aromatic one proton singlet at δ_H 6.84 with both the olefinic H-4″ signal at δ_H 7.83 and the methoxy resonance at δ_H 3.84 provided strong evidence for the location of the single aromatic proton at C-5″ and the methoxyl adjacent at C-6″, consistent with its ^{13}C NMR chemical shift (δ_C 56.78) (Figure III.22).

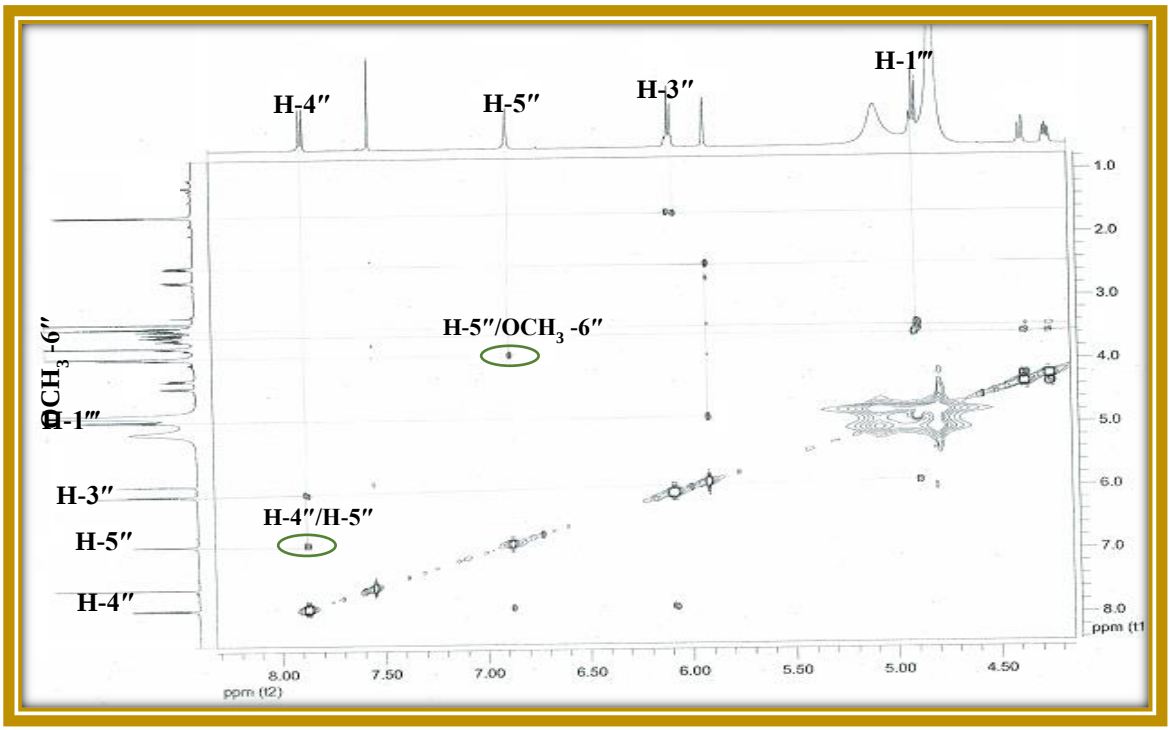


Figure III.22: The ROESY spectrum of the coumarin unit of compound 1

The doublet at 4.85 ppm was attributed to the anomeric proton of a sugar and the coupling constant (J = 7.8 Hz) indicates that it is of a β-configuration. The signal at δ_C 106.30 corresponding to the anomeric carbon C-1‴ was determined by HSQC experiment (Figure III. 18).

Also, the presence of the β-D-glucosyl group was confirmed by the ^{13}C NMR spectra which show a signal at δ_C 106.30, another signal of oxymethylene carbon at δ_C 65.01 (C-6‴) and other oxymethine carbon signals resonating at δ_C 74.82(C-2‴), δ_C 77.41 (C-3‴), δ_C 70.95 (C-4‴), δ_C 75.35 (C-5‴) [161, 162].

The glucoside moiety was linked to C-7″ of the coumarin nucleus as strongly evidenced by the HMBC correlation between the anomeric proton H-1‴ (δ_H 4.85) and C-7″ (δ_C 133.50) (Figure III.19).

Based on these findings, the coumarin part of compound **1** was identified as Isofraxoside (Fraxetin–7-O–β–glucoside) [163], a rarely found metabolite (Figure III.23).

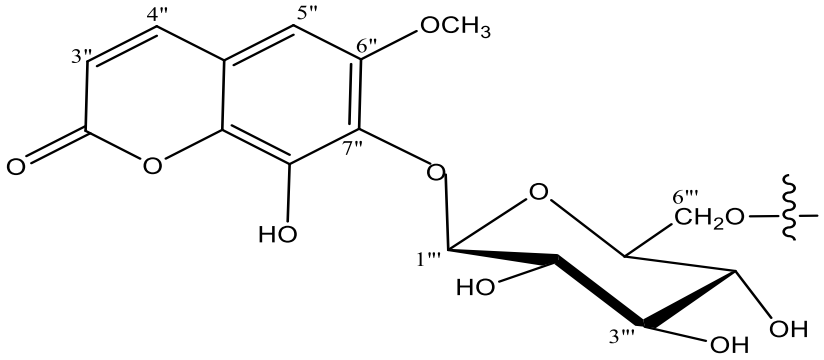


Figure II.23: The coumarin unit of compound 1

The secoiridoid glucoside unit:

- The ethylenic proton H-3 (δ_H 7.50) and the allylic acetal proton H-1 (δ_H 5.87), coupling in HSQC (Figure III.24) with their carbons resonating at δ_C 155.38 (C-3) and 95.39 (C-1). The proton H-3 correlates on the HMBC spectrum (Figure III. 25) with the acetalic carbon C-1 and with two quaternary carbons appearing at 169.32 and 109.26 ppm. The distinction between these two carbons was easy because it is a C-11 carbonyl (δ_H 169.32) and the quaternary olefinic carbon C-4 (δ_H 109.26).
- The two signals detected at δ_H 1.61 (dd, J = 1.1, 7.2 Hz, Me-10) and 6.05 (overlapped, H-8), coupling in HSQC experiment with the corresponding carbons C-10 (δ_C 13.63) and C-8 (δ_C 125.32).

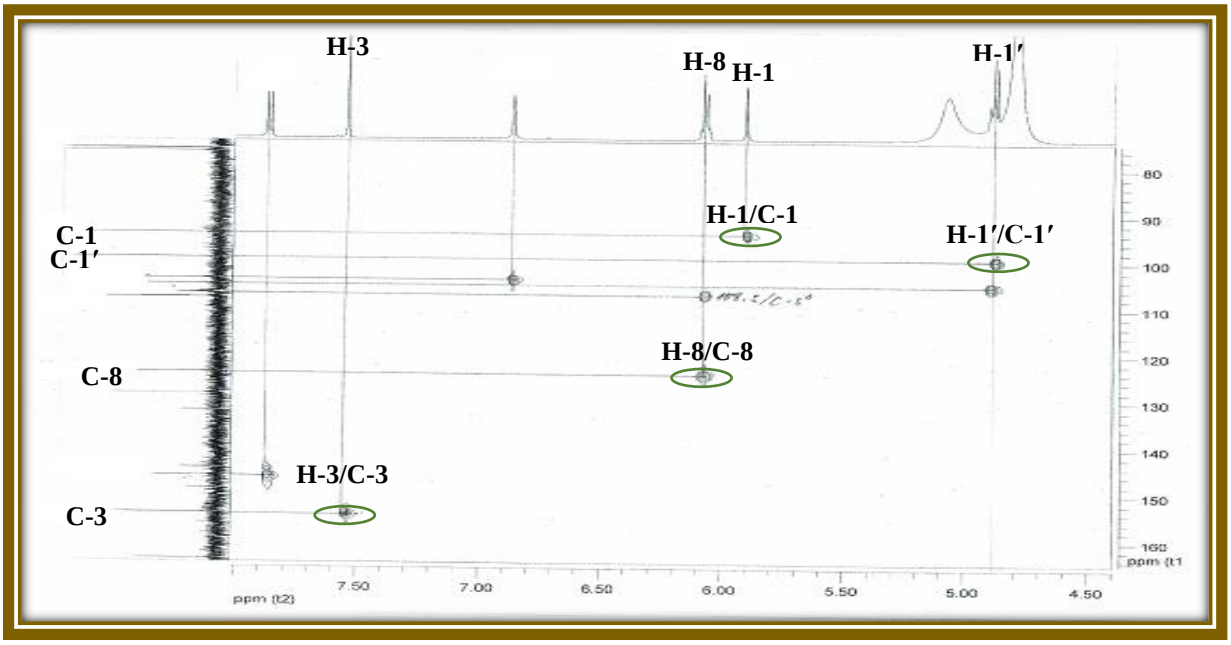


Figure III.24: The HSQC spectrum of the secoiridoid unit of compound 1

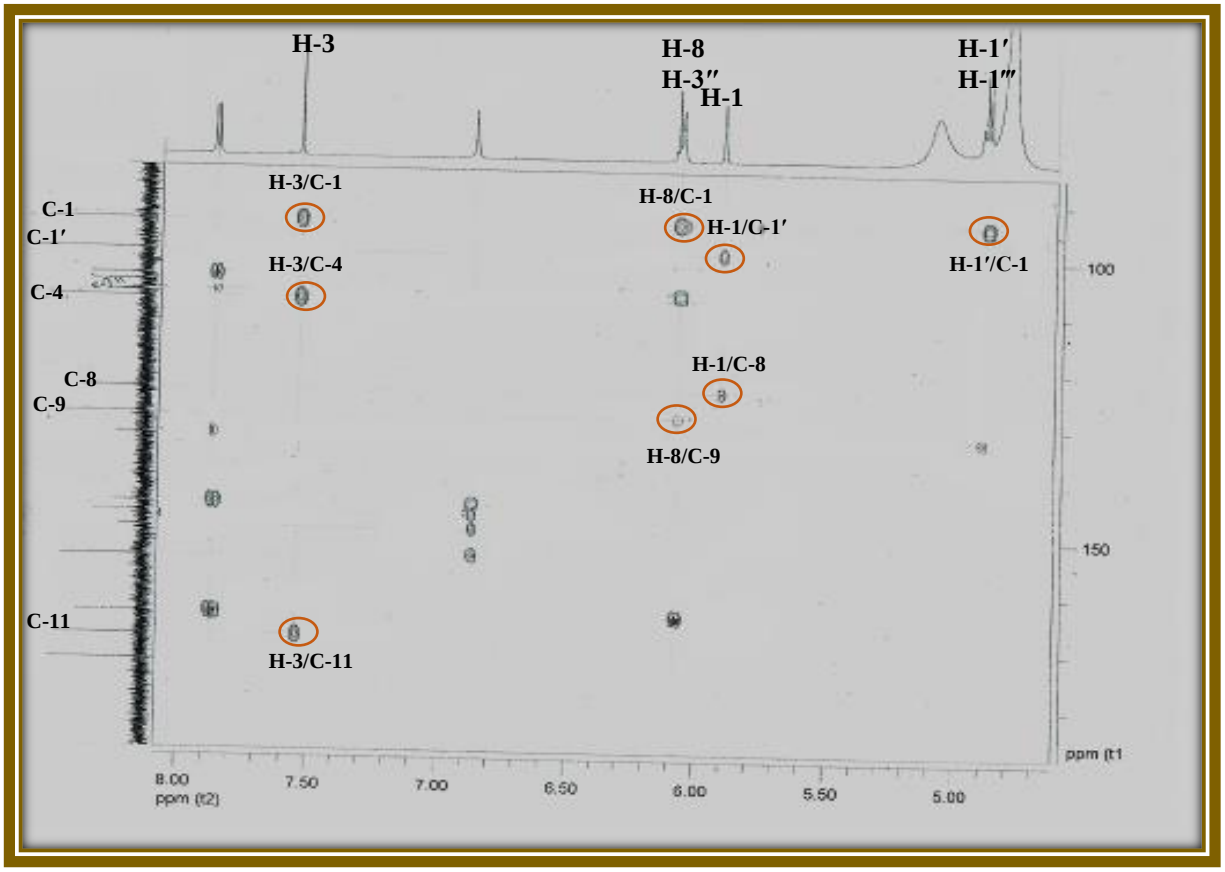


Figure III.25: The HMBC spectrum of the secoiridoid unit of compound 1

The two protons H_a-6 and H_b-6 resonating at δ_H 2.66 (1H, dd, J = 5.2, 14.1 Hz) and 2.43 (1H, dd, J = 8.6, 14.1 Hz), respectively, coupling with their corresponding carbon C-6 (δ_C 41.33) (Figure III.26). These two germinal protons correlate on the HMBC spectrum (Figure III.27) with

the methine carbon C-5 (δ_C 31.45) which was determined by HSQC experiment and with three quaternary carbons detected at δ_C 173.74 , 109.26 and 129.96. The significant HMBC ^{2}J correlation between these two geminal protons and the carbonyl ester signal (173.74) leads to assume that it is a C-7 carbon. Whereas, the two last signals were already identified as quaternary olefinic carbons (C-4 and C-9).

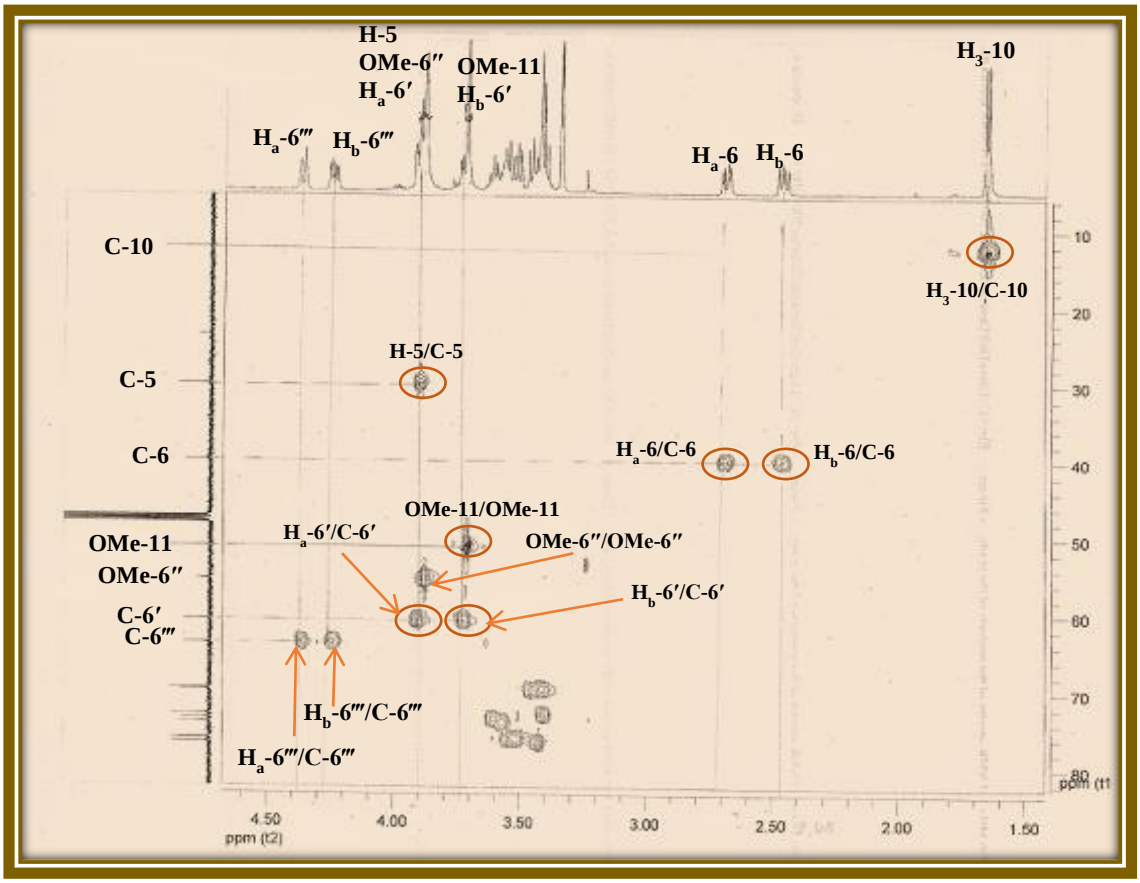


Figure III.26: The HSQC spectrum of the secoiridoid unit of compound 1

The analysis of the HSQC spectrum (Figure III.26) also shows a correlation spot between the mehoxy OCH_3 protons at 3.68 ppm and their corresponding carbon appearing at δ_C 52.53. The HMBC ^{2}J correlation between the methoxyl protons at δ_H 3.68 and carbonyl carbon at δ_C 169.32 indicated that the CH_3O group was linked with the carbonyl group at C-11 (Figure III.27).

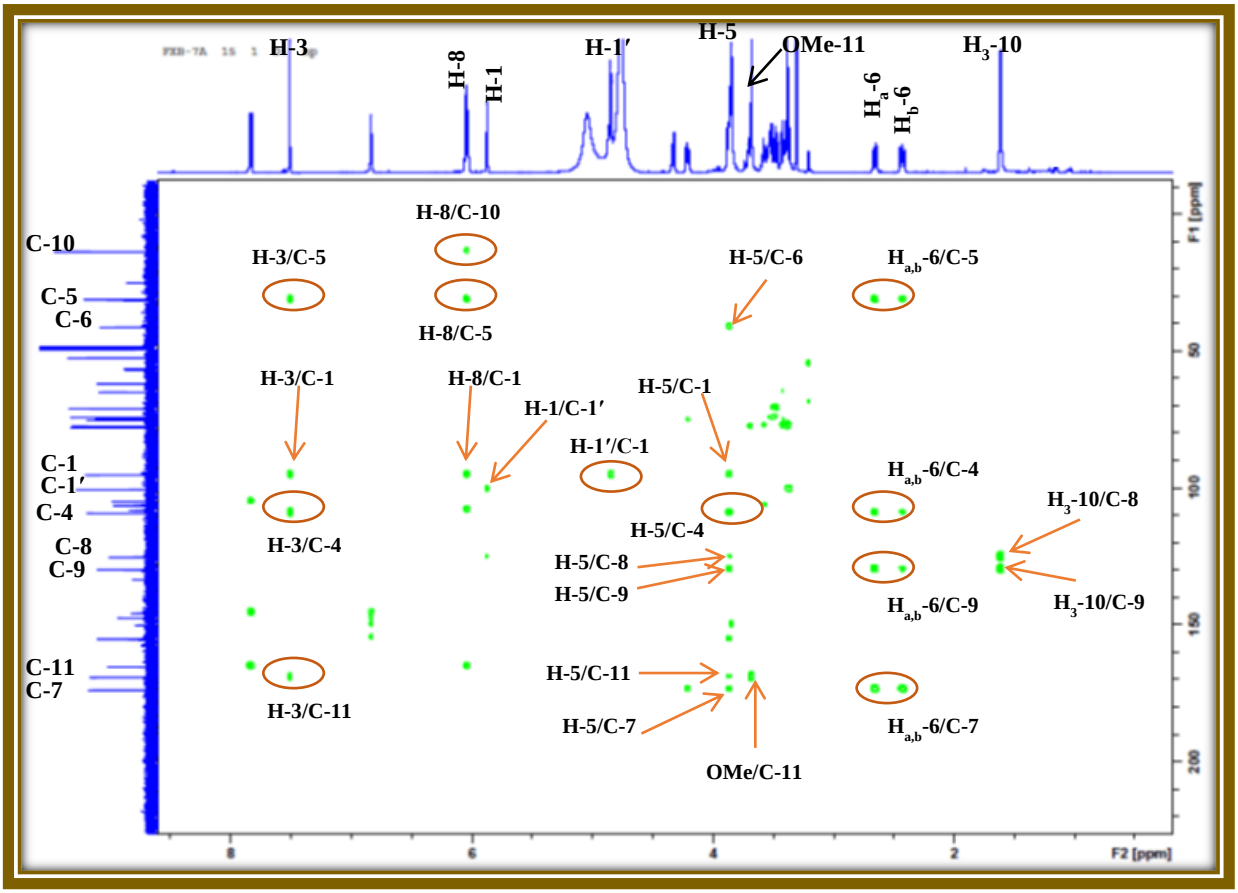


Figure III.27: The HMBC spectrum of the secoiridoid unit of compound 1

Then HSQC experiment (Figure III.24) showed a cross peak between the anomeric proton δ_H 4.85 (1H, d, J = 7.8 Hz, H-1′) and the anomeric carbon C-1′ at δ_C 100.69. The connection of the second glucoside was established by HMBC and ROESY experiments (Figure III.27 and III.28). In the HMBC experiment, the correlations between H-1′ (δ_H 4.85) and C-1 (δ_C 95.39) and between C-1′ (δ_C 100.69) and H-1 (δ_H 5.87) indicated that the glucoside moiety was linked to C-1 of the secoiridoid unit. The ROESY correlation between H-1 and H-1′ further confirmed the linkage. The signals of the secoiridoid part corresponded well to those of 7, 11-dimethyloleoside [48] (Figure III.29)**.**

According to HSQC experiment (Figure III.26), the carbon signal at δ_C 65.01 (C-6‴) correlates with the corresponding protons H_a-6‴ and H_b-6‴ resonating at δ_H 4.33 (dd, J = 1.6, 11.8 Hz) and 4.21 (dd, J = 6.3, 11.8 Hz), respectively. Furthermore, correlations between H_a-6‴, H_b-6‴ and C-7 (δ_C 173.74) (Figure III.30) gave clear evidence that the two structural units are bound through an ester linkage between OH group of C-6‴ and carboxyl group of oleoside at C-7 (Figure III.31). This was also evident from the downfield shifts of H_a-6‴, H_b-6‴ and C-6‴ in compound **1,** compared to the respective signals in Isofraxoside [164].

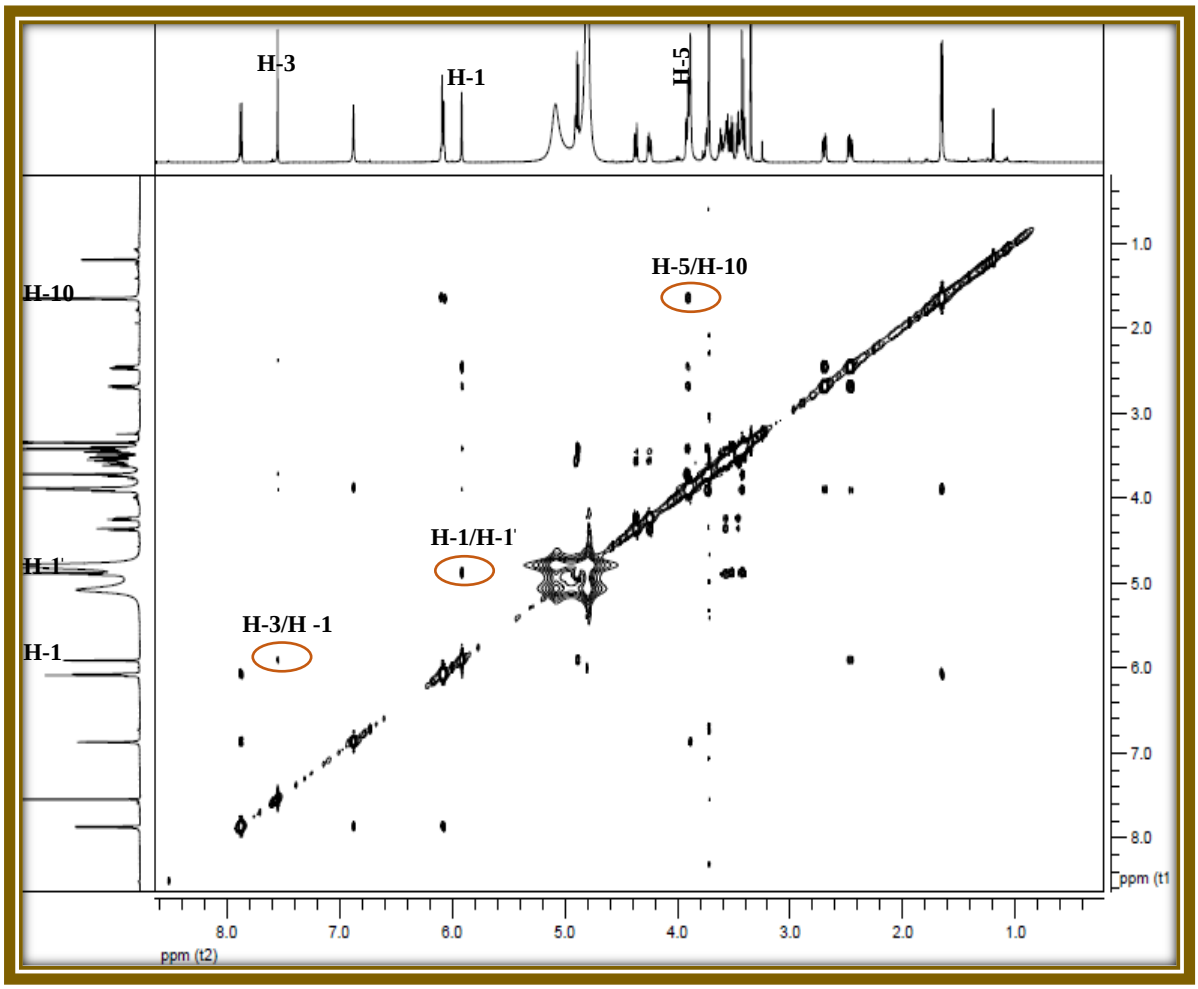


Figure III.28: The ROESY spectrum of the secoiridoid unit of compound 1

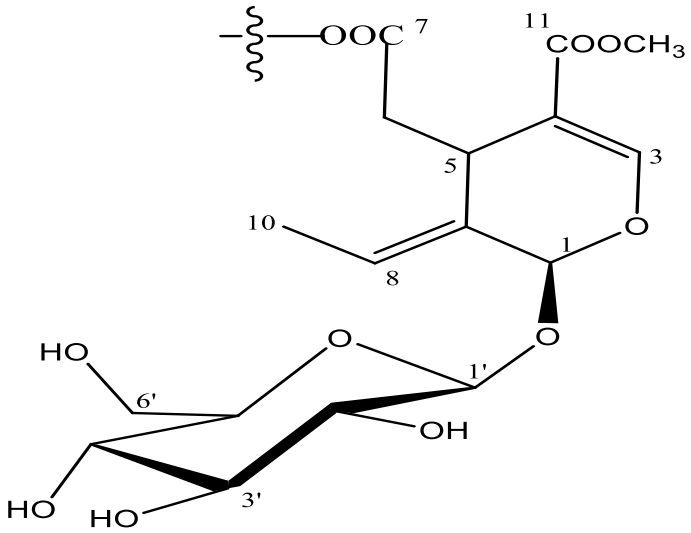


Figure III.29: Secoiridoid part of compoud 1

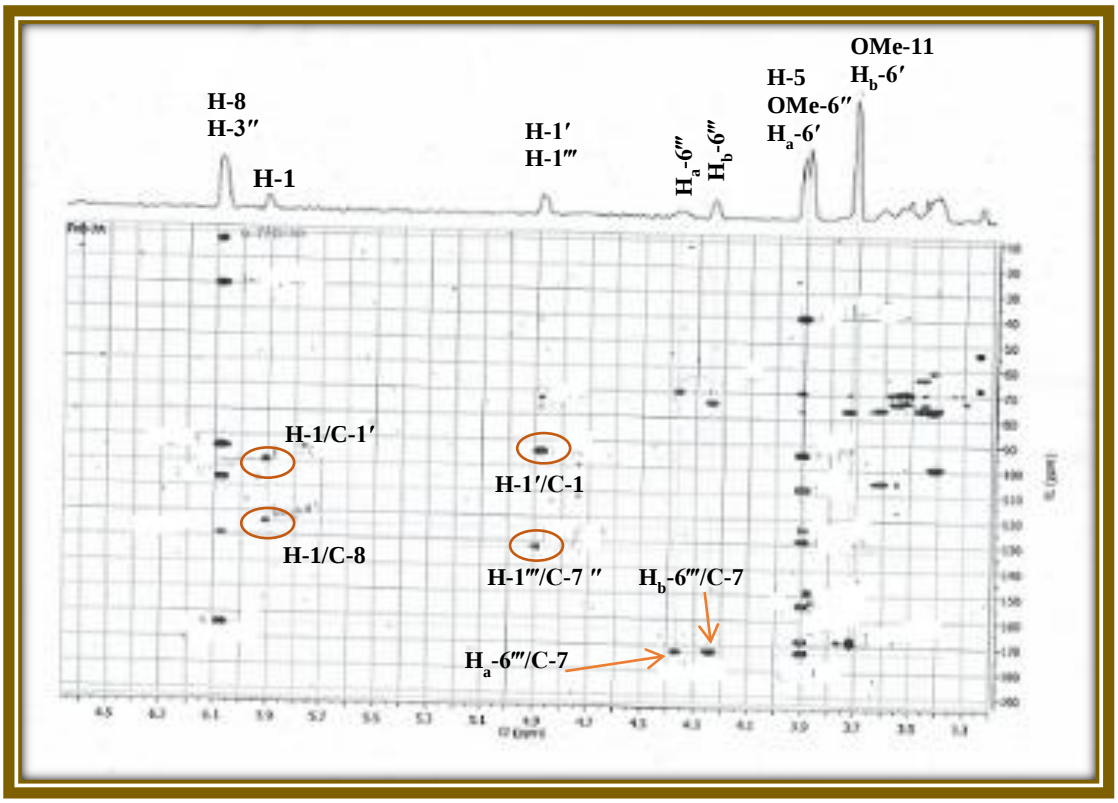


Figure III.30: The HMBC spectrum of compound 1

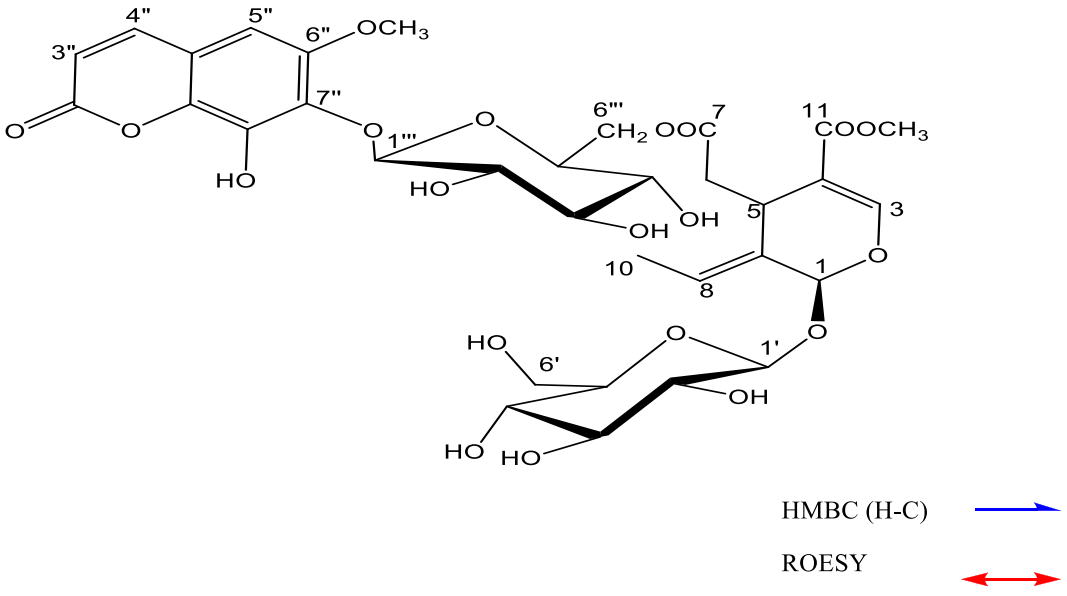


Figure III.31: The key HMBC and ROESY correlations of compound **1**

Additionally, the presence of D-glucose was authenticated by acid hydrolysis of compound 1 and synthesis of its tolylthiocarbamoyl-thiazolidine derivative [165].

On the basis of these evidences, compound **1** was identified as fraxetin-7″-O-[11-methyl-oleosidyl-(7-6‴)]-β-D-glucopyranoside, named isofraxisecoside and its ^{1}H and ^{13}C NMR data are given in Table III.1.

Isofraxisecoside (**1**) is the third example of a natural compound consisting of one coumarin glucoside unit linked to a secoiridoid moiety of oleoside type after previously described escuside [55] and fraxisecoside [29] isolated from the same genus.

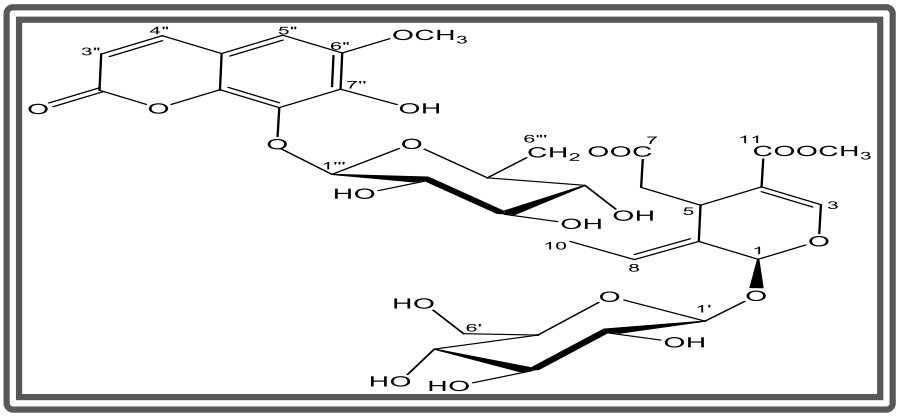
Table III.1: **^{1}H (600.11 MHz) and ^{13}C (150.91 MHz) data of isofraxisecoside 1 (CD_3OD; δ in ppm; *J* in Hz)** *

C/H	δ_H	δ_C	C/H	δ_H	δ_C
Aglycon			*Coumarin*		
1	5.87 (*s*)	95.39	1″	-	-
2	-	-	2″	-	165.34
3	7.50 (*s*)	155.38	3″	6.04** *d* (9.3)	108.11
4	-	109.26	4″	7.83 *d* (9.3)	147.49
5	3.87**	31.45	5″	6.84 (*s*)	105,20
6_a	2.66 *dd* (5.2/14.1)	41.33	6″	-	150.09
6_b	2,43 *dd* (8.6/14.1)		OMe	3.84 (*s*)	56.77
7	-	173.74	7″	-	133.49
8	6.05**	125.32	8″	-	155.39
9	-	129.96	9″	-	145.77
10	1.61 *dd* (1.1/7.2)	13.63	10″	-	108.28
11	-	169.32			
OMe	3.68 (*s*)	52.53			
Glucose-1			*Glucose-2*		
1′	4.85 *d* (7.8)	100.69	1‴	4.85 *d* (7.8)	106.30
2′	3.28 – 3.59 *m*	74.28	2‴	3.57 *m*	74.82
3′	3.28 – 3.59 *m*	77.35	3‴	3.48 *t* (9.0)	77.41
4′	3.28 – 3.59 *m*	71.14	4‴	3.42 *t* (9.0)	70.95
5′	3.28 – 3.59 *m*	77.89	5‴	3.53 *m*	75.35
$6'_a$	3.87 *d* (11.5)	62.12	$6'''_a$	4.33 *dd* (1.6/11.8)	65.01
$6'_b$	3.70 *d* (11.6)		$6'''_b$	4.21 *dd* (6.3/11.8)	

* Assignments were established by DEPT, HSQC and HMBC data.

** The signals are overlapped.

III.4.3.1.2 Structure elucidation of compound 2



Fraxetin-8″-O- [11-methyl-oleosidyl- (7 → 6‴)]-β-glucopyranoside

(Fraxisecoside)

Compound **2** was isolated from the stem bark as crystal like solid, isolated from the stem bark with dark blue fluorescence under UV light at 254 nm and a bleu spot when spraying with H_2SO_4 reagent. This was the first indication on the presence of secoiridoid skeleton [166]. The UV spectrum (Figure III.32) showed typical absorption at 226.0 nm of the iridoidic enol system conjugated with a carbonyl group and additional absorptions at 333.3, 339.0 and 343.5 nm due to a conjugated aromatic system.

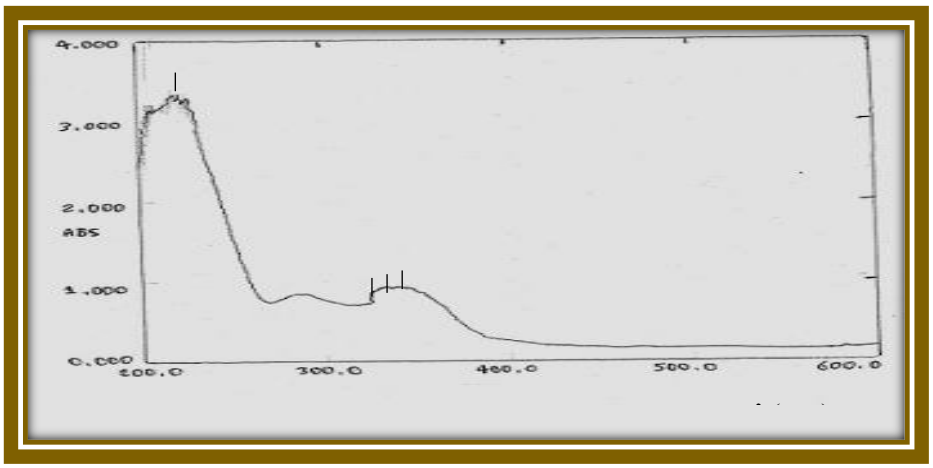


Figure III.32: UV spectrum of compound 2

The molecular formula $C_{33}H_{40}O_{20}$ was determined on the basis of its HR-ESI-MS spectrum (Figure III.33), obtained in negative electrospray mode which showed deprotonated molecular ion $[M-H]^-$ at *m/z* 755.20381 (calculated 755.2038 for $C_{33}H_{39}O_{20}$). Thus, compound **2** and **1** have been identified as isomers.

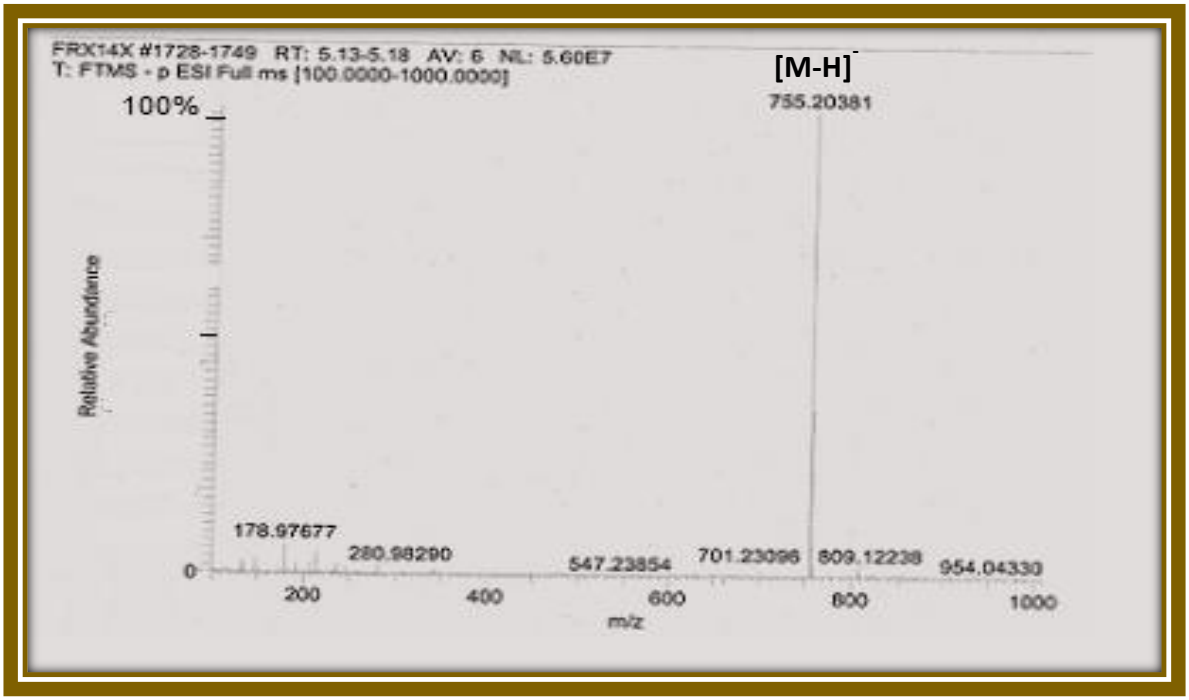


Figure III.33: Mass spectrum HR-ESI-MS of compound 2

The MS/MS spectrum of compound **2** (Figure III.35) yielded an intense ion at m/z 547, consistent with a neutral loss of a coumarin aglucone (208 Da). Also, the MS/MS spectrum showed ions at m/z 385 and 223, originating from successive losses of 162 Da, suggesting the presence of two hexosyl residues. A proposed fragmentation patterns for compound **2** is given in Figure III.34.

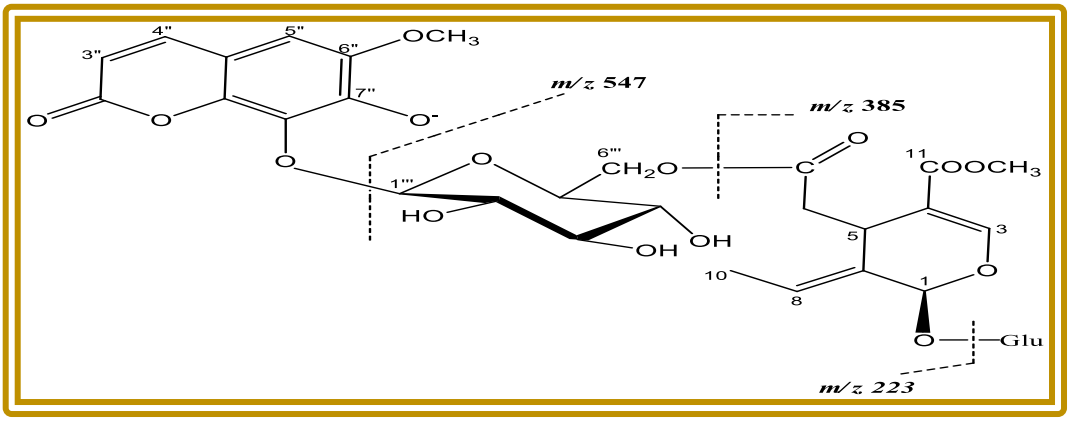


Figure III.34: Proposed pathway fragmentations for compound 2

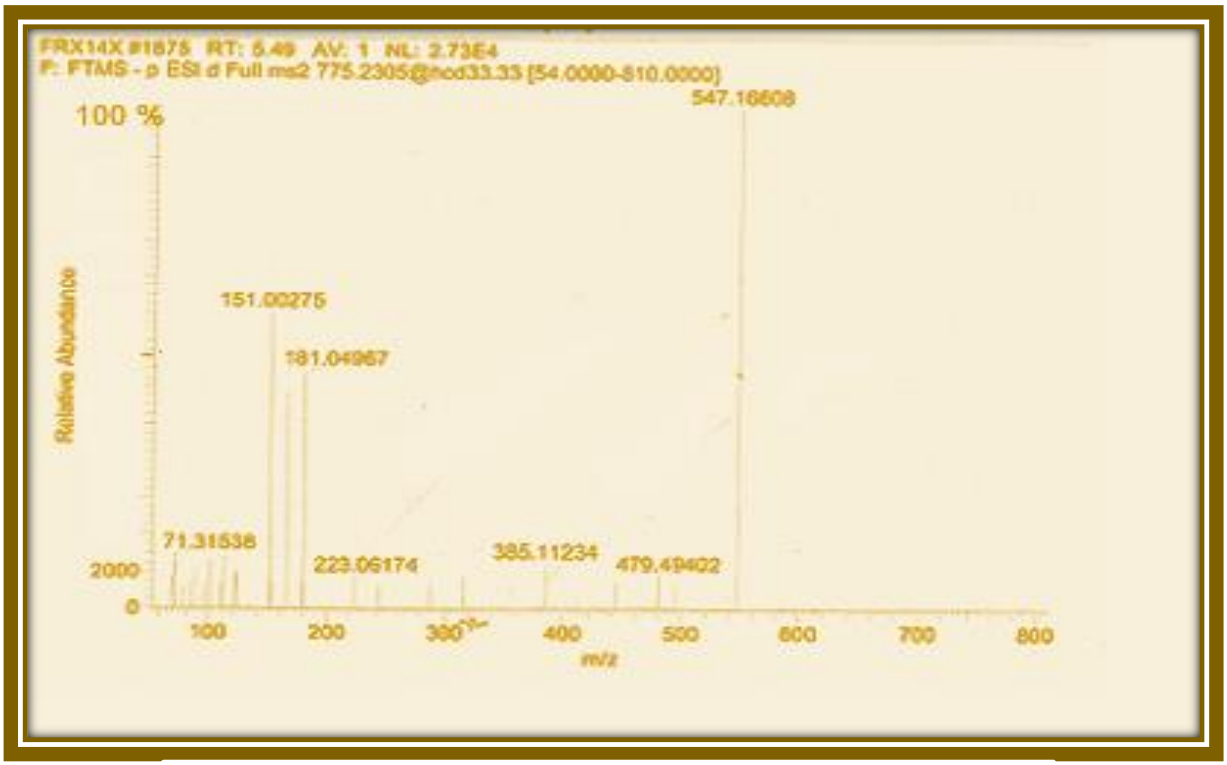


Figure III.35: Mass spectrum HR-ESI-MS/MS of compound 2

The NMR spectrum (Figure III.36) of compound **2** recorded in CD_3OD shows signals of aromatic protons resonating between 6.04 and 7.84 ppm and glucosidic protons between 3.30 and 5.05 ppm.

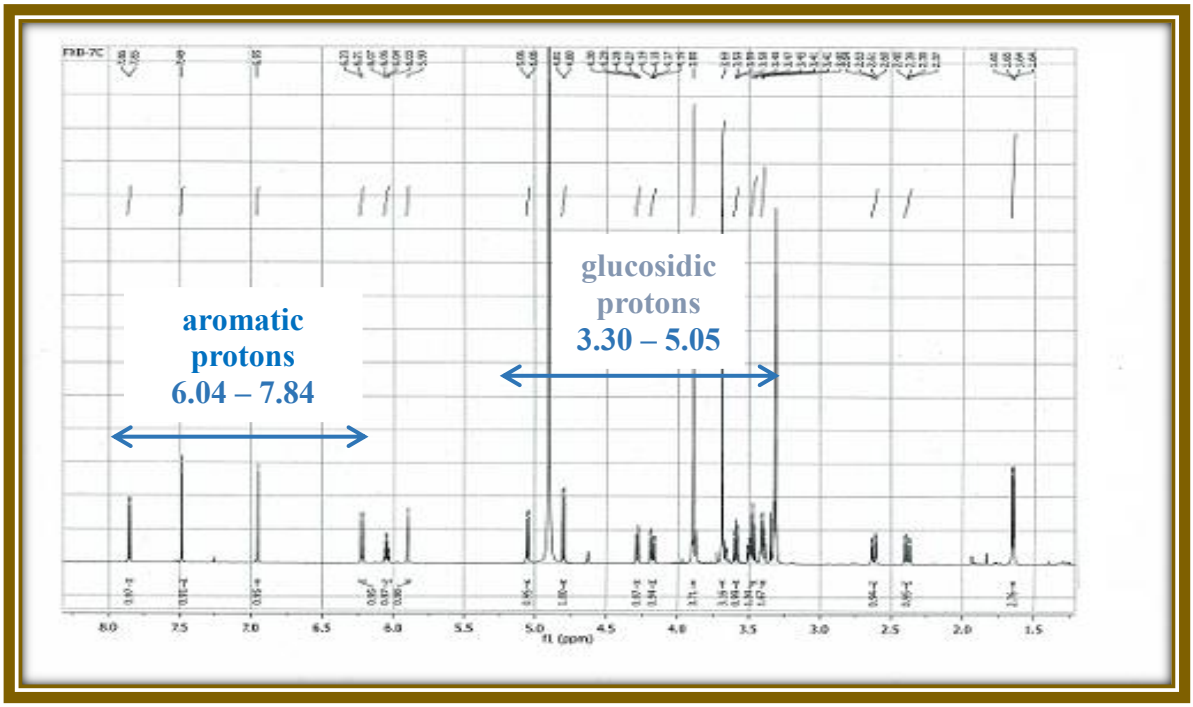


Figure III.36: ^{1}H NMR spectrum of compound 2

The structural analysis of the ^{1}H NMR spectrum (Figure III.37) reveals:

- The presence of a typical AB spin system for H-3″ and H-4″ detected at δ_H 6.22 (J = 9.5 Hz) and 7.85 (J = 9.5 Hz), respectively.

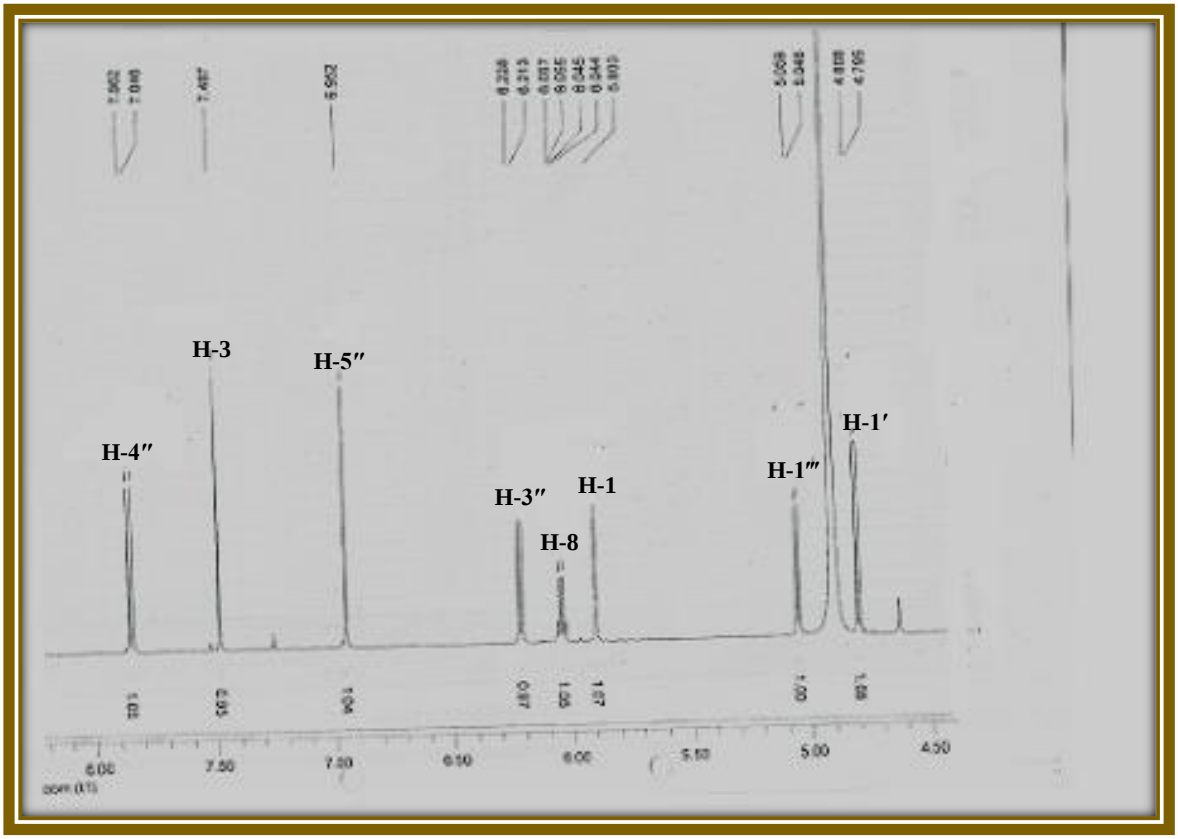


Figure III.37: ^{1}H NMR spectrum of compound 2

- An aromatic proton at δ_H 6.95 of 1H integration in the form of singlet (figure III.37). The HMBC experiment, showed a cross peak between this signal and the carbon C-4″, so it is assigned to H-5″. Thus, these findings indicated a 6, 7, 8- substituted coumarin structure.

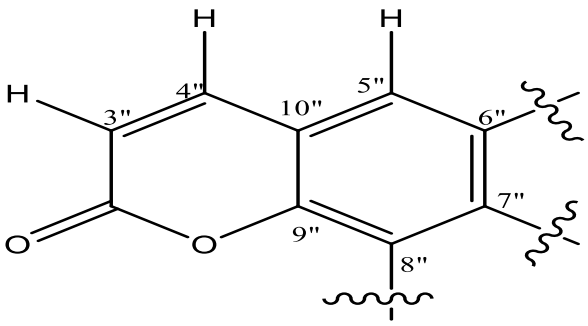


Figure III.38: 6″, 7″, 8″- substituted coumarin unit

Also, the ^{1}H NMR spectrum (Figure III.37 and III.39) exhibited typical signals of an oleoside type secoiridoid which are:

- A signal at δ_H 7.49, corresponding to the ethylenic proton H-3.
- The signal at δ_H 5.90 in the form of singlet integrating for one proton was characteristic of the acetalic proton H-1.
- A methine proton resonating at 3.89 ppm corresponding to H-5.
- Olefinic methine quartet detected at δ_H 6.05 (J = 7.0 Hz) is corresponding proton H-8.

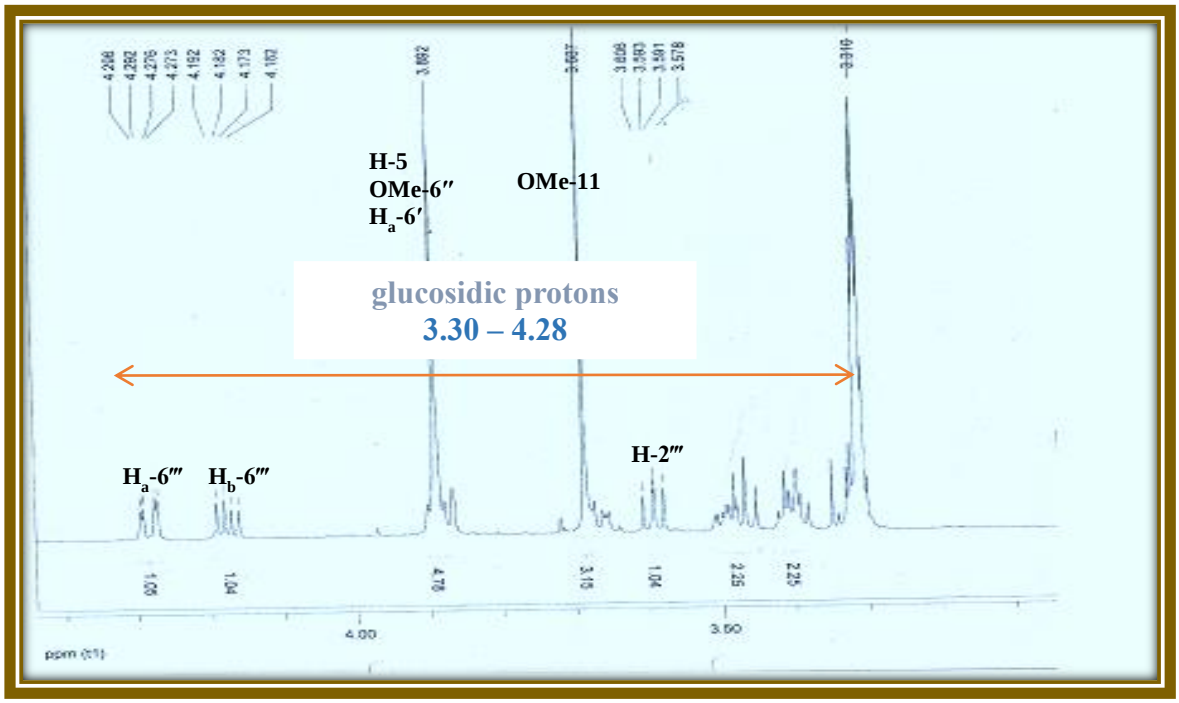


Figure III.39: ^{1}H NMR spectrum of compound 2

Besides these signals, the ^{1}H NMR spectrum (Figure III.39 and III.40) allows to show:

- Two methoxyls at δ_H 3.89 (s) and 3.69 (s).
- Two anomeric protons resonating at δ_H 5.05 (d, J = 7.8 Hz) and 4.80 (d, J = 7.8 Hz) indicating the presence of two glucoside residues in β-configurations.
- An olefinic methyl doublet at δ_H 1.65 (J = 7.0 Hz) was characteristic for the protons H_3-10.
- Signals of glucosidic protons between 3.30 and 4.28 ppm were attributted to protons of at least two sugars.

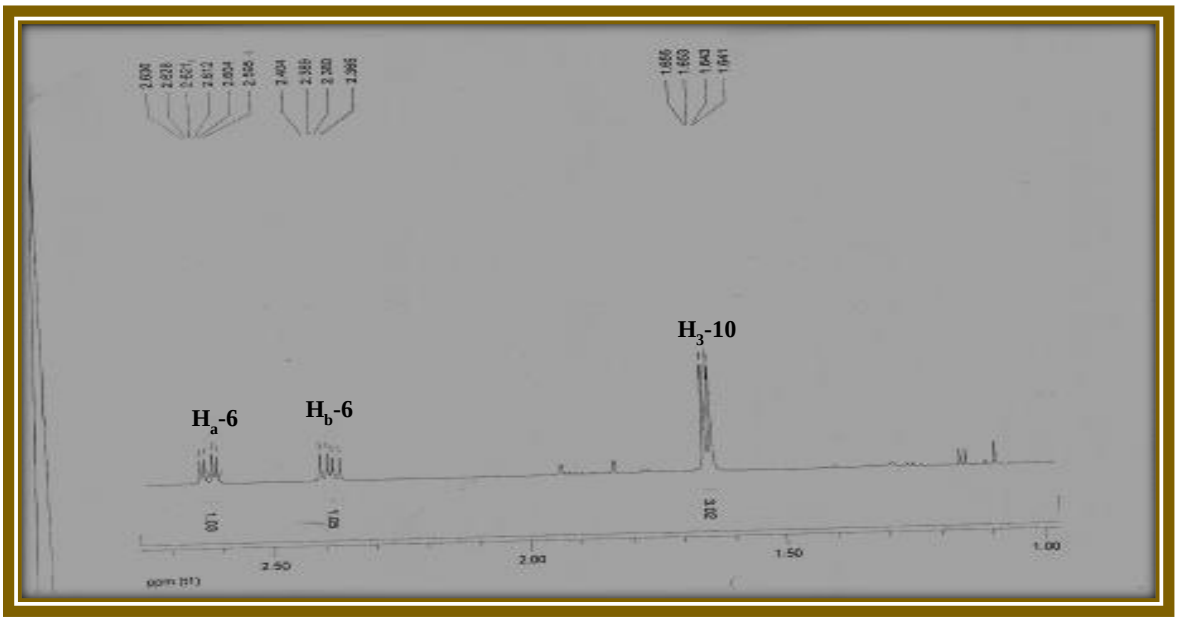


Figure III.40: ^{1}H NMR spectrum of compound 2

The ^{13}C NMR spectrum of compound **2** (Figure III.41) make it possible to count 33 signals corresponding to 33 carbon atoms.

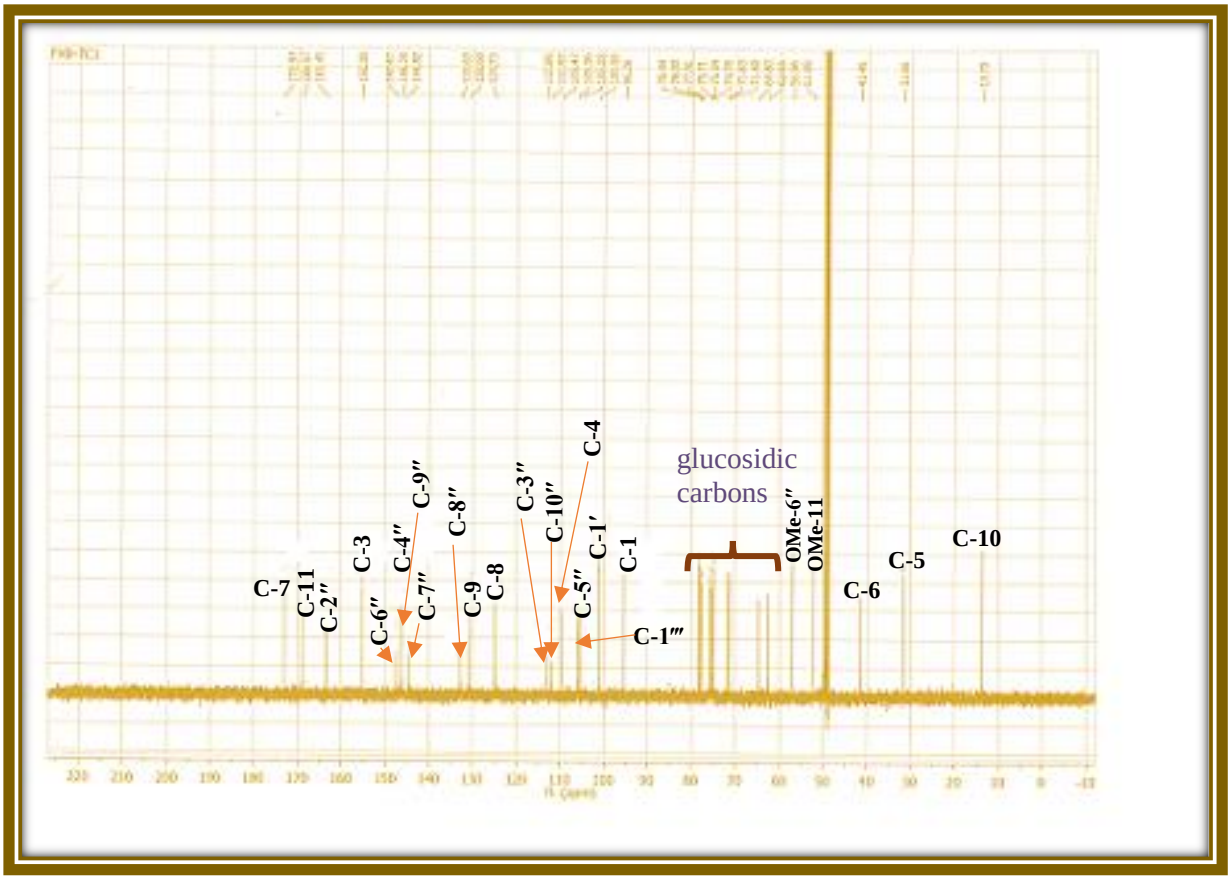


Figure III.41: ^{13}C NMR spectrum of compound 2

The ^{1}H and ^{13}C NMR spectra of compound **2 (**Figure III.42 and III.43**)** are very closely resembled to those of the novel natural compound Isofraxisecoside (**1**), consisting of a coumarin glucoside unit bound via an ester function to a secoiridoid glucoside unit.

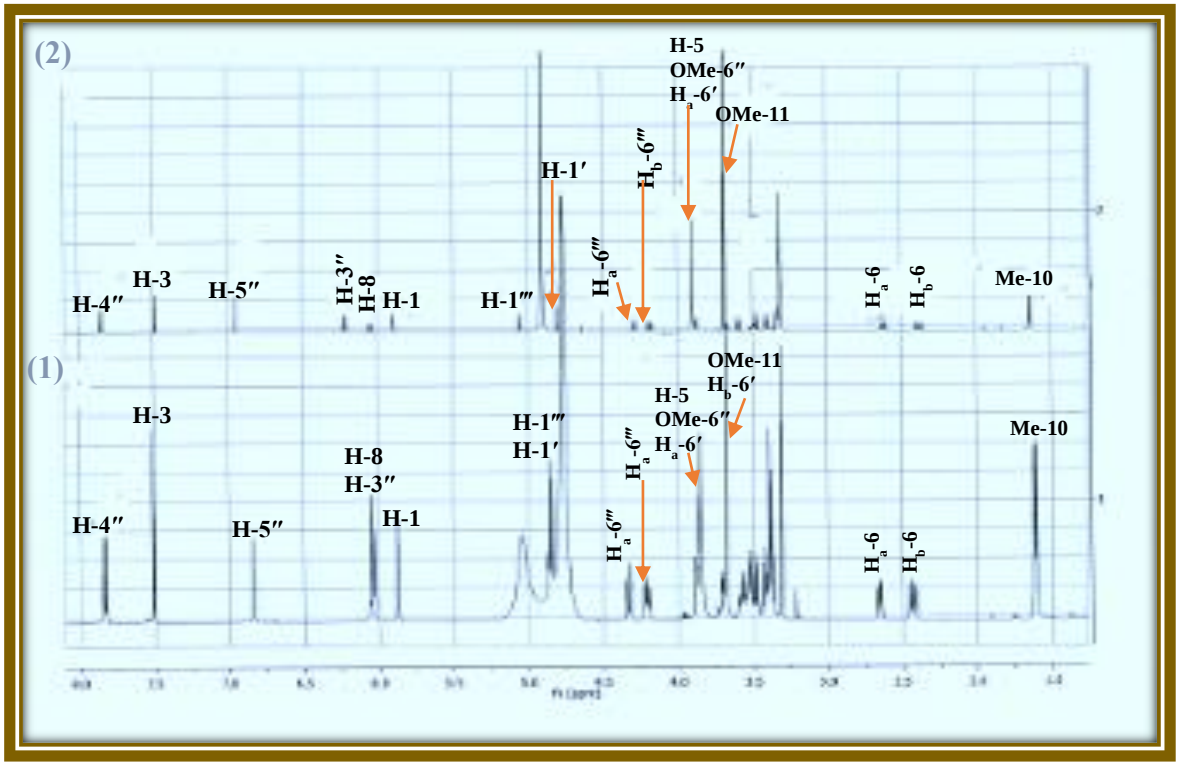


Figure III.42: (1) ^{1}H NMR spectrum of compound 1; (2) ^{1}H NMR spectrum of compound 2

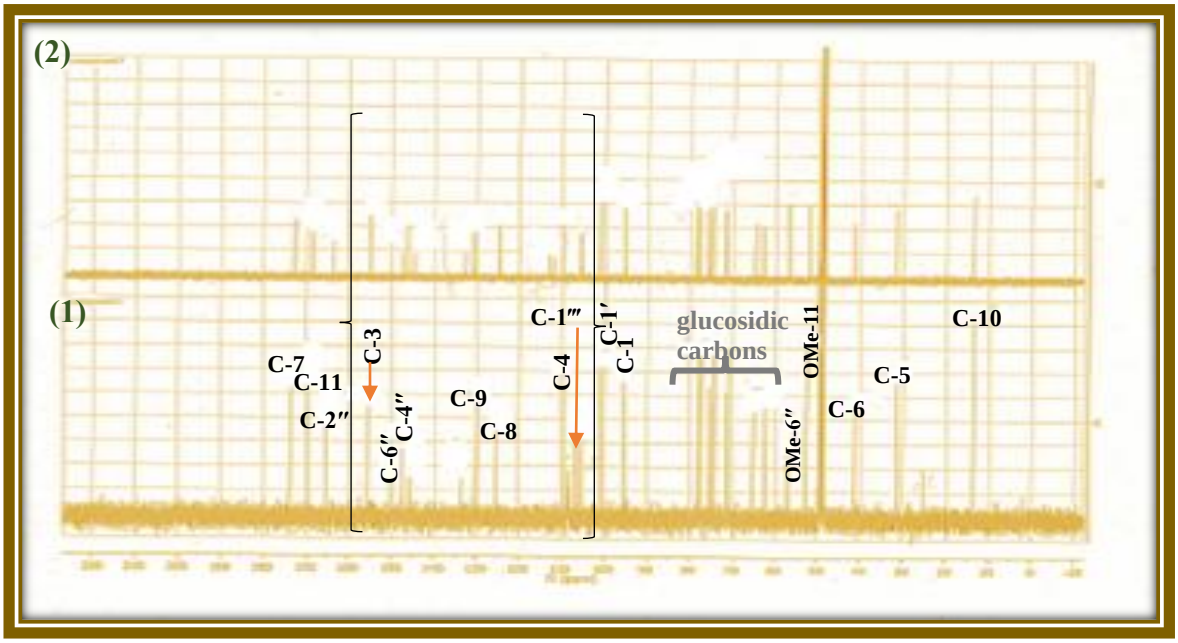


Figure III.43: (1) ^{13}C NMR spectrum of compound 1; (2) ^{13}C NMR spectrum of compound 2

The ^{13}C NMR spectroscopic signals due to the oleoside-11-methylester of compound **2** coincided well with those ascribable to the same part of the new compound isofraxisecoside (**1**) (Figure III.43) except that of the portion of the coumarin glucoside unit (Figure III.44).

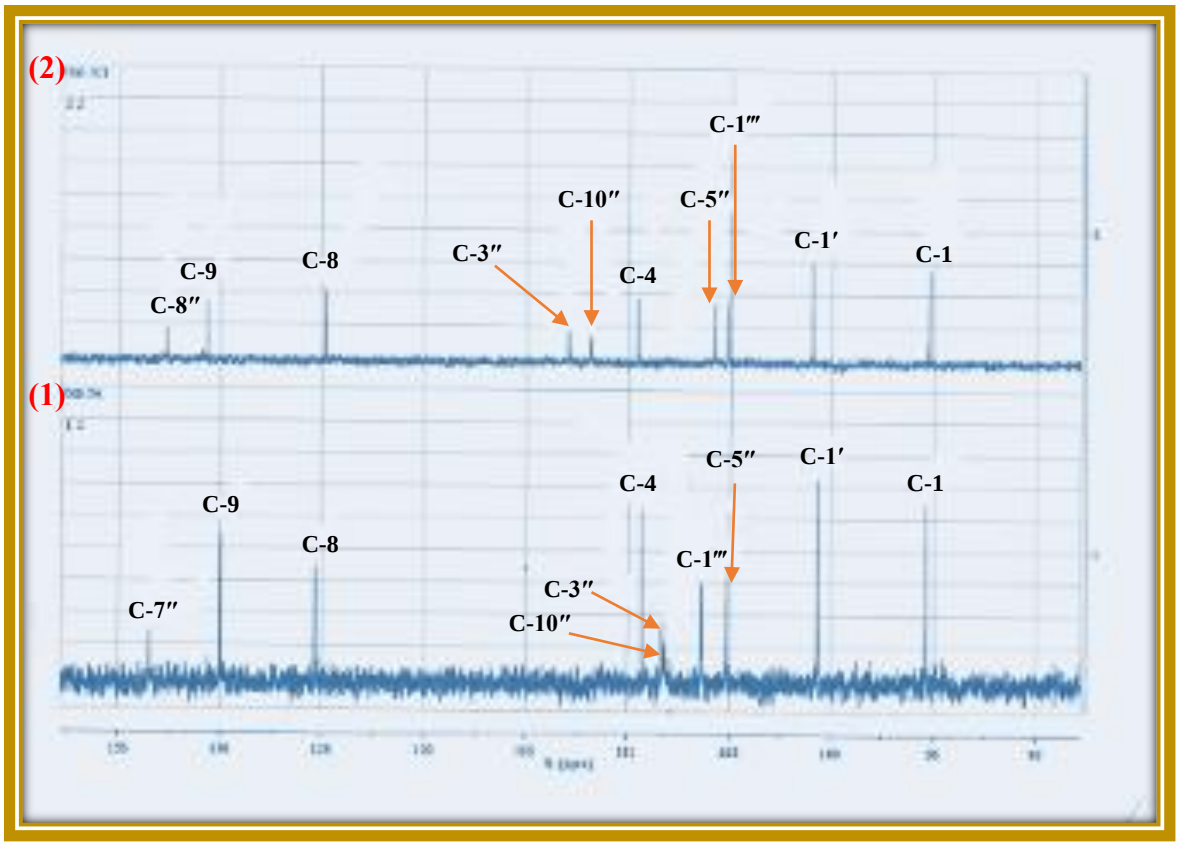


Figure III.44: (1) ^{13}C NMR spectrum of compound 1; (2) ^{13}C NMR spectrum of compound 2

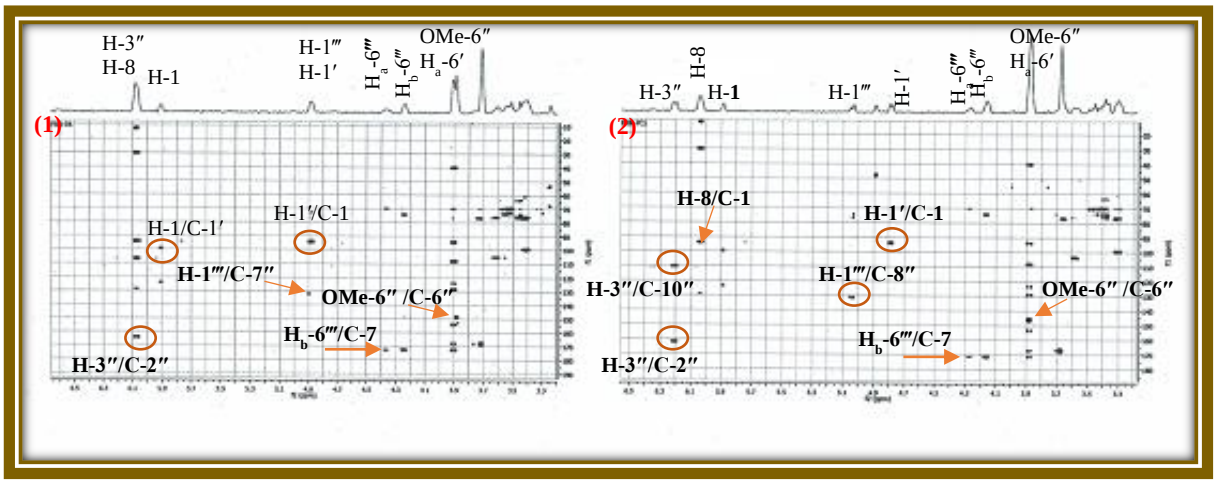


Figure III.45: (1) HMBC spectrum of compound 1; (2) HMBC spectrum of compound 2

The ^{13}C shift assignments, of the coumarin moiety of compound **2** were made through HMBC long-range correlations (Figure III.45 and III.46).

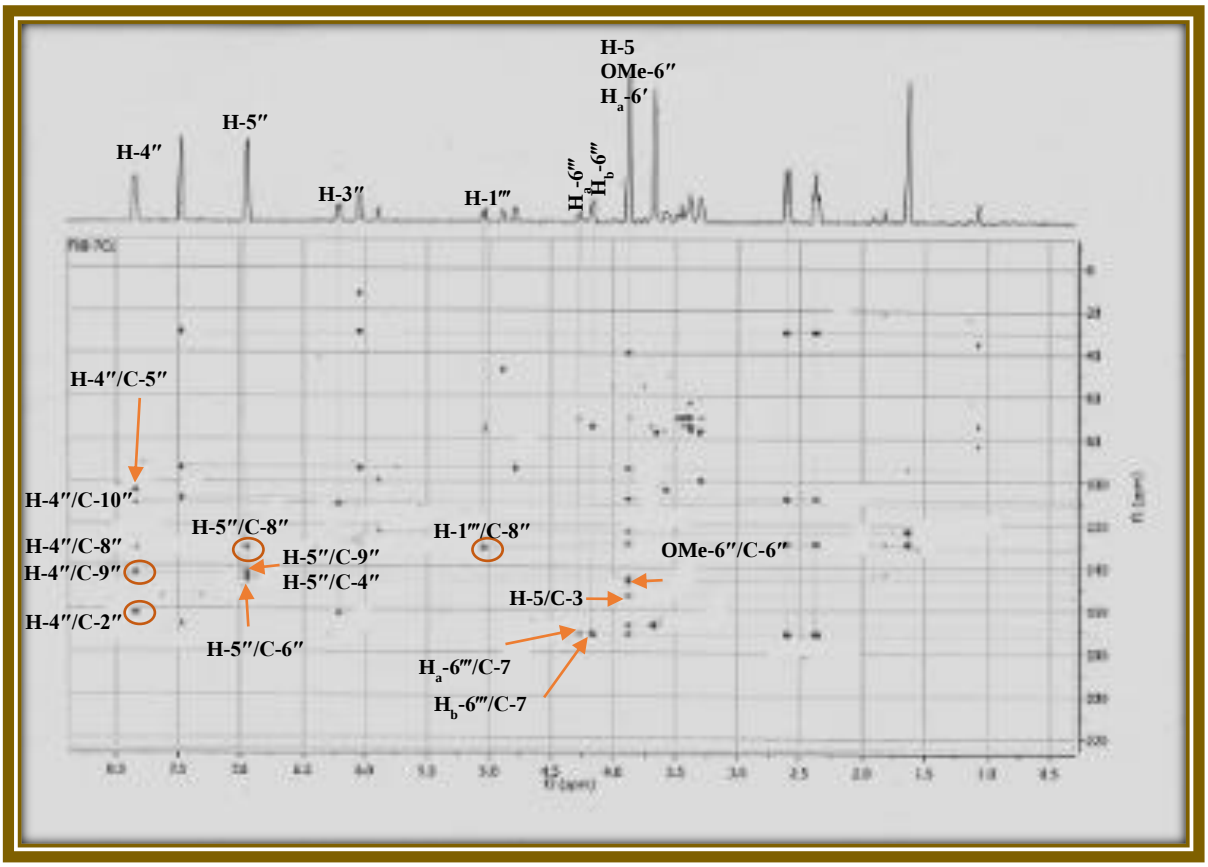


Figure III.46: The HMBC spectrum of the coumarin moiety of compound 2

The examination of the above HMBC spectra shows:

- The presence of an aromatic methoxy signal at δ_H 3.89 (δ_C 57.0) and the observed cross peak in HMBC spectrum between methoxyl protons and C-6″ (δ_C 147.6) provided strong evidence for the location of methoxyl at C-6″.
- Correlations between the aromatic one proton resonating at 6.95 ppm with the olefinic carbon C-4″ (δ_C 146.5) and the quaternary carbon C-6″ (δ_C 147.6) confirm the location of the aromatic proton at C-5″.
- Correlations across three –bond coupling between H-4″ (δ_H 7.85, d; δ_C 146.5) and H-5″(δ_H 6.95, s; δ_C 105.9) with the appearing signal at δ_C 146.1 assigned to carbon C-9″.
- Correlation between the anomeric proton H-1‴ (δ_H 5.05, d; δ_C 105.2) and the carbon C-8″ appearing at δ_C 132.7 confirmed glycosylation of coumarin part at C-8″.

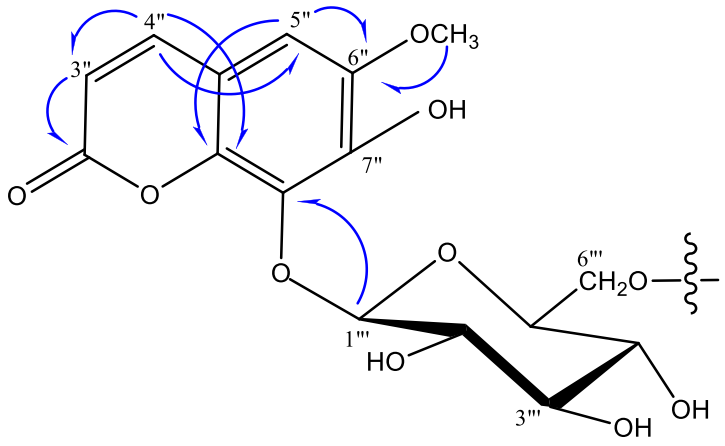


Figure III.47: The most important HMBC correlations of the coumarin unit of compound 2

Thus, the spectroscopic data of the coumarin part corresponded well with those of the coumarin glucoside fraxin (Fraxetin-8-O-β-D-glucoside) [167].

Furthermore, according to the HMBC experiment (Figure III.46) correlation between H_a-6‴ at δ_H 4.28 (dd, J = 2.1, 11.8 Hz) and H_b-6‴ at δ_H 4.18 (dd, J = 6.4, 11.8 Hz) with C-7 (δ_C 172.9) confirmed that the two structural units are bound through an ester linkage between the second carboxylic group of the oleoside at C-7 and the OH group at C-6‴ of the glucose in the coumarin part. This was also evident from the downfield shifts of H_a-6‴, H_b-6‴ and C-6‴ in compound **2** (Table III.2), compared to the respective signals in fraxin.

On the basis of these findings, and by direct comparison of these spectroscopic data with those reported in the literature [29], compound **2** was identified as fraxisecoside (fraxetin-8″-O-[11-methyl-oleosidyl-(7-6‴)]-β-D-glucopyranoside) and its ^{1}H and ^{13}C NMR data are given in Table III.2.

Table III.2: ^{1}H (600.11 MHz) and ^{13}C (150.91 MHz) data of fraxisecoside **2** (CD_3OD; δ in ppm; *J* in Hz)*

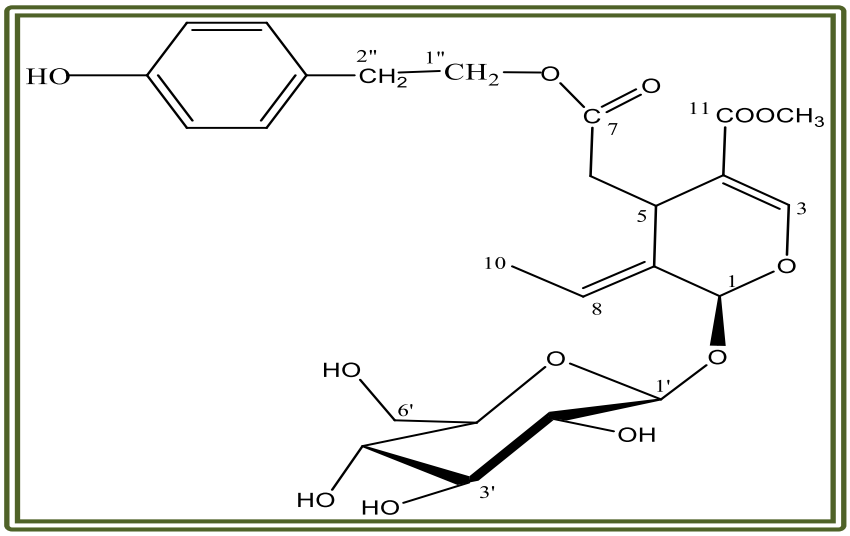
C/H	δ_H	δ_C	C/H	δ_H	δ_C
Aglycon			*Coumarin*		
1	5.90 (*s*)	95.30	1″	-	-
2	-	-	2″	-	163.50
3	7.49 (*s*)	155.10	3″	6.22** *d* (9.5)	112.90
4	-	109.60	4″	7.85 *d* (9.5)	146.50
5	3.89**	31.70	5″	6.95 (*s*)	105.90
6_a	2.62 *dd* (4.8/14.4)	41.40	6″	-	147.60
6_b	2,38 *dd* (9.0/14.4)		OMe	3.84 (*s*)	57.00
7	-	172.90	7″	-	144.70
8	6.05 *q* (7.0)	124.90	8″	-	132.70
9	-	130.70	9″	-	146.10
10	1.65 *dd* (1.2/7.2)	13.70	10″	-	111.90
11	-	168.70			
OMe	3.69 (*s*)	52.00			
Glucose-1			*Glucose-2*		
1′	4.80 *d* (7.8)	101.00	1‴	5.05 *d* (7.8)	105.20
2′	3.30 – 3.59 *m*	74.80	2‴	3.59 *dd* (7.9/9.1)	75.40
3′	3.30 – 3.59 *m*	77.80	3‴	-	75.70
4′	3.30 – 3.59 *m*	71.40	4‴	-	71.60
5′	3.30 – 3.59 *m*	78.40	5‴	-	78.00
6′	3.89**	62.60	$6‴_a$	4.28 *dd* (2.1/11.8)	64.90
			$6‴_b$	4.18 *dd* (6.4/11.8)	

* Assignments were established by direct comparison with the literature.

** The signals are overlapped.

III.4.3.2 Identification secoiridoids

III.4.3.2.1 Structure elucidation of compound 3



Ligstroside

Compound 3 was isolated from the leaves as a colourless amorphous powder. Its ^{1}H NMR spectrum (Figure III.48) recorded in CD_3OD displays signals of aromatic protons resonating between 6.71 and 7.05 ppm and glucosidic protons between 3.30 and 4.80 ppm.

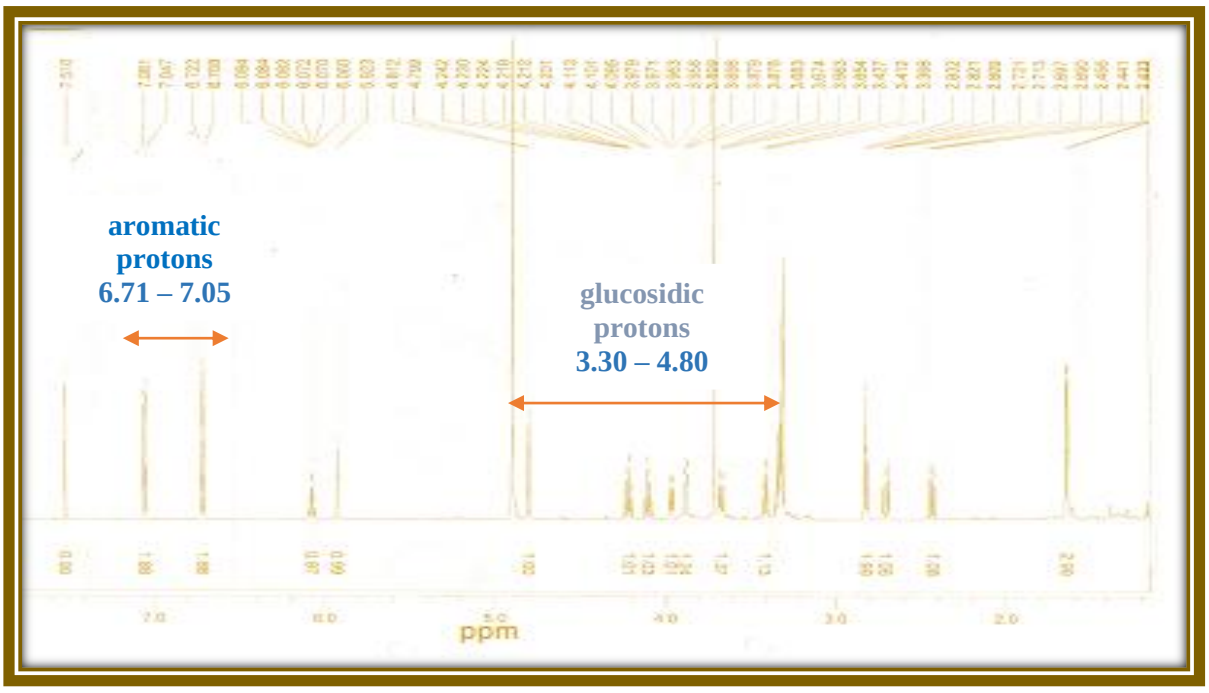


Figure III.48: ^{1}H NMR spectrum of compound 3

The structural analysis of the ^{1}H NMR spectrum (Figure III.49 and III.50) reveals typical signals of an oleoside nucleus:

- A singlet at 7.52 ppm characteristic for the vinylic proton H-3 of a secoiridoid glucoside.
- An allylic acetal proton appearing as a broad singlet at 5.92 ppm.
- A singlet signal detected at δ_H 4.80 (1H, d, J = 7.8 Hz) characteristic for the anomeric proton H-1′.
- Olefinic proton resonating at δ_H 6.08 (1H, dt, J = 6.0, 7.2 Hz).
- An ABX spin system of H_a-6, H_b-6 and H-5 protons resonating at δ_H 2.71 (1H, dd, J = 4.5, 14.1 Hz), δ_H 2.44 (1H, dd, J = 9.3, 14.1 Hz) and δ_H 3.97 (1H, dd, J = 4.5, 9.3 Hz), respectively.

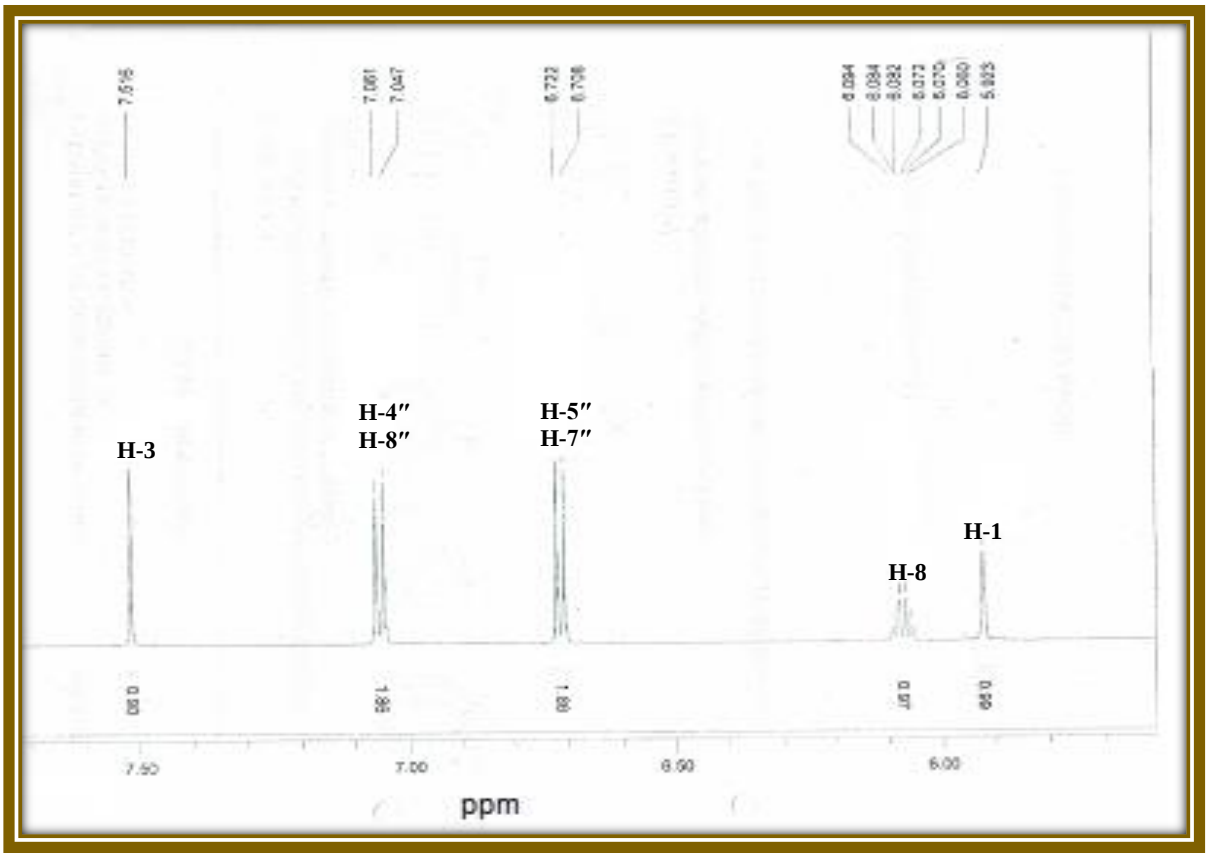


Figure III.49: ^{1}H NMR spectrum of compound 3

Besides these signals, the ^{1}H NMR spectrum (Figure III.49 and III.50) allows to show:

- The presence of an aromatic A_2X_2 spin system centered at δ_H 6.71 (2H, d, J = 8.4 Hz, H-5″ and H-7″) and at δ_H 7.05(2H, d, J = 8.4 Hz, H-4″ and H-8″), suggesting the presence of para-substituted phenolic ring.

- Another ABX_2 spin system appears at δ_H 4.22 (1H, dt, J = 6.9, 10.8 Hz), δ_H 4.10 (1H, dt, J = 7.1, 10.8 Hz), δ_H 2.82 (2H, t, J = 6.9 Hz), corresponding to H_a-1″, H_b-1″ and H-2″ respectively, of the two methylene groups of —OCH_2CH_2Ph moiety.
- An olefinic methyl protons resonating at δ_H 1.64 (3H, dd, J = 1.4, 7.2 Hz).
- A singlet signal appearing at δ_H 3.71 was assigned to the methoxyl protons (3H, s, OMe-11).

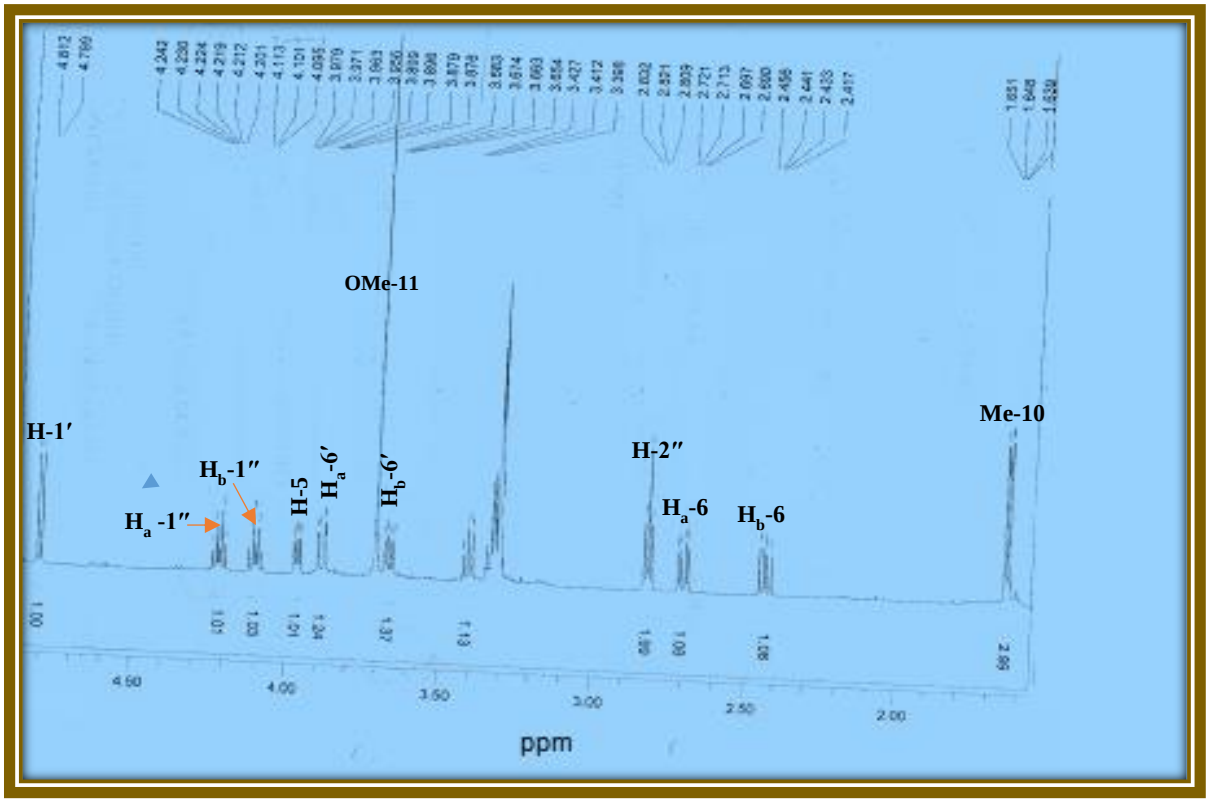


Figure III.50: ^{1}H NMR spectrum of compound 3

The spectral data of compound **3** (Table III.3) was in full agreement with those reported for ligstroside [16, 46]. Moreover, the co-TLC comparison with authentic samples gave proof that compound **3** is the secoiridoid glucoside ligstroside.

Table III.3: ^{1}H (600.11 MHz) data of ligstroside **3** (CD_3OD; δ in ppm; *J* in Hz)*

C/H	δ_H	C/H	δ_H
Aglycon		*Glucose*	
1	5.92 (*t-like*)	1′	4.80 *d (7.8)*
2	-	2′	3.30-3.43[a]
3	7.52 (*s*)	3′	3.30-3.43[a]

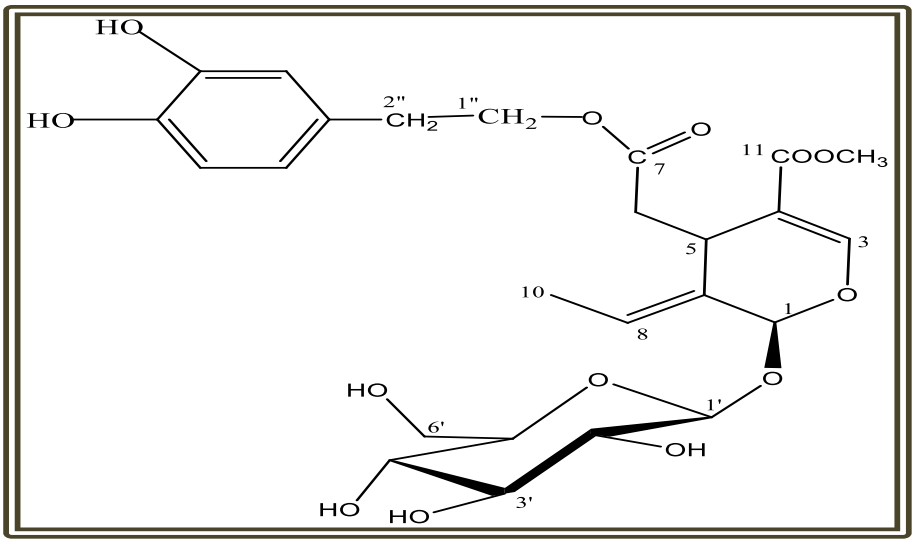
Table III.3: continued.

C/H	δ_H	C/H	δ_H
4	-	4′	3.30-3.43[a]
5	3.97 *dd* (4.5/9.3)	5′	3.30-3.43[a]
6_a	2.71 *dd* (4.5/14.1)	$6'_a$	3.89 *dd* (1.8/12.0)
6_b	2,44 *dd* (9.3/14.1)	$6'_b$	3.67 *dd* (5.4/12.0)
		Tyrosol	
7	-	$1''_a$	4.22 *dt* (10.8/6.9)
		$1''_b$	4.10 *dt* (7.2/10.8)
8	6.08 *dt* (6.0/7.2)	2″	2.82 *t* (6.9)
9	-	4″	7.05 *d* (8.4)
10	1.64 *dd* (1.4/7.2)	5″	6.71 *d* (8.4)
11	-	7″	6.71 *d* (8.4)
OMe	3.71 (*s*)	8″	7.05 *d* (8.4)

* Assignments were established by direct comparison with the literature.

[a] overlapped.

III.4.3.2.2 Structure elucidation of compound 4



Oleuropein

Compound **4** was obtained from the leaves as a powder. Its ^{1}H NMR spectrum (Figure III.51) showed signals due to aromatic protons resonating between 6.50 and 6.64 ppm and glucosidic protons between 3.28 and 4.80 ppm.

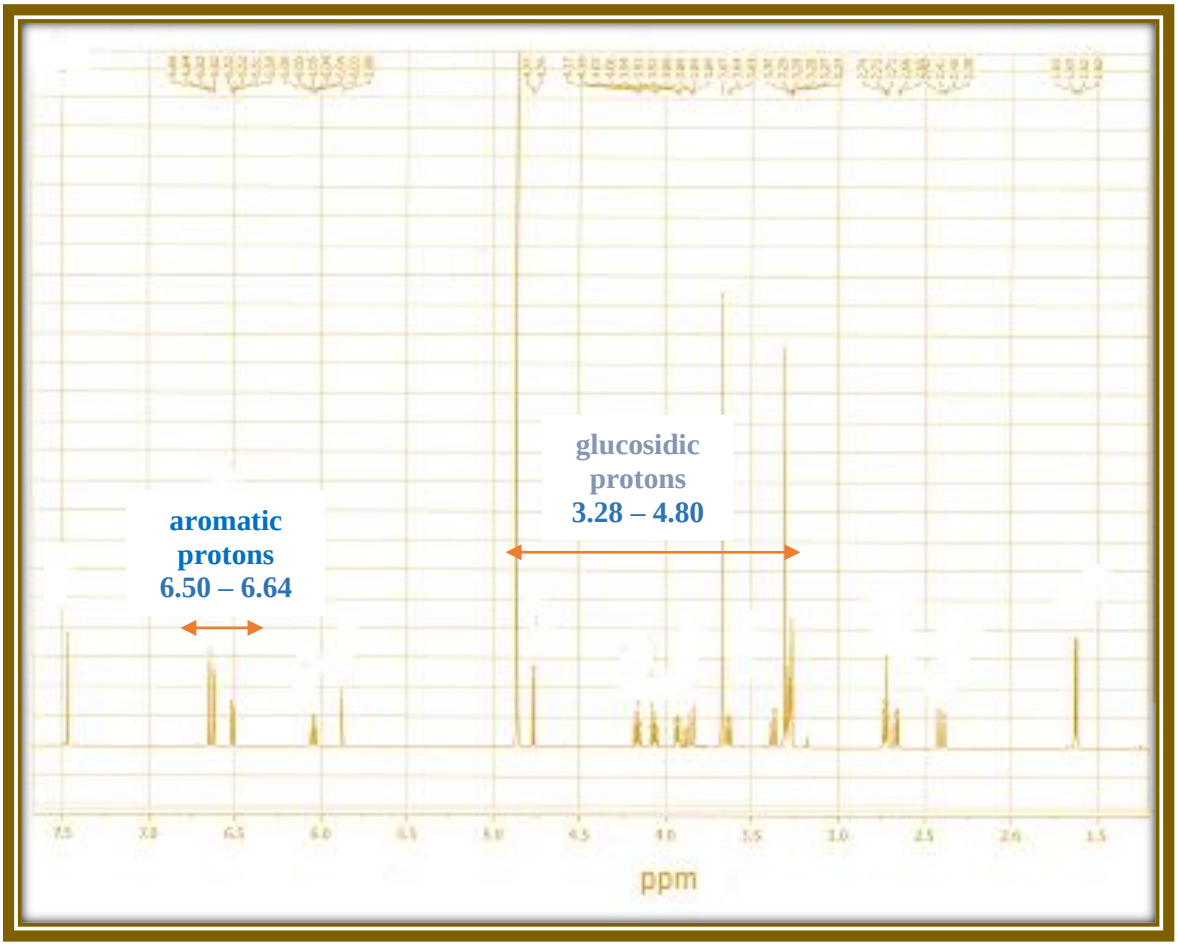


Figure III.51: ^{1}H NMR spectrum of compound 4 in CD_3OD

The structural analysis of the ^{1}H NMR spectrum (Figure III.52 and III.53) showed signals typical of a secoiridoid nucleus:

- An allylic acetalic proton resonating at δ_H 5.86, corresponding to H-1.
- A singlet signal appearing at δ_H 7.46 is corresponding to the vinylic proton H-3.
- An olefinic methine proton detected at 6.03 ppm of the proton H-8.
- An ABX spin system consisting of H_a-6, H_b-6 and H-5 protons resonating at δ_H 2.66 (1H, dd, J = 4.5, 14.1 Hz), δ_H 2.39 (1H, dd, J = 9.0, 14.1 Hz) and δ_H 3.92 (1H, dd, J = 4.8, 9.0 Hz) respectively.
- An ethylenic methyl protons was observed at δ_H 1.61 (1H, dd, J = 1.2, 7.2 Hz) of Me-10.

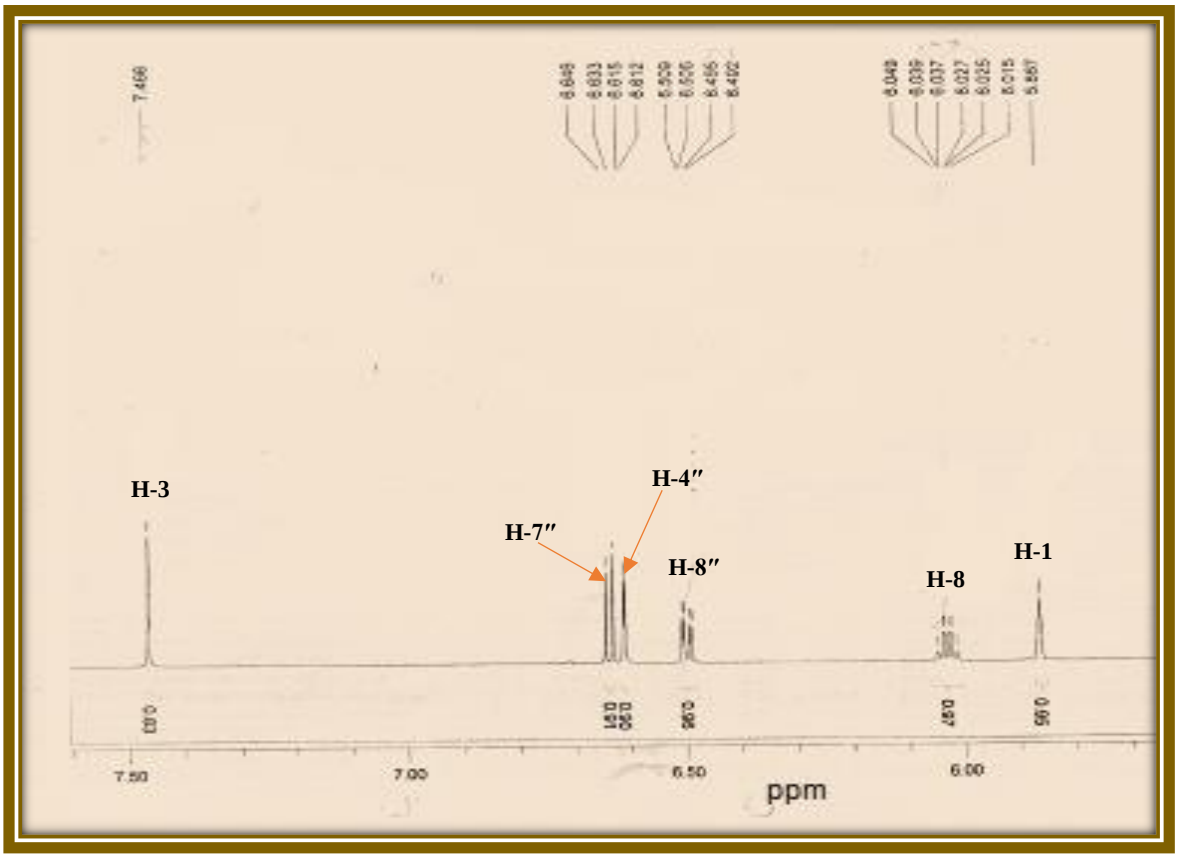


Figure III.52: ^{1}H NMR spectrum of compound 4

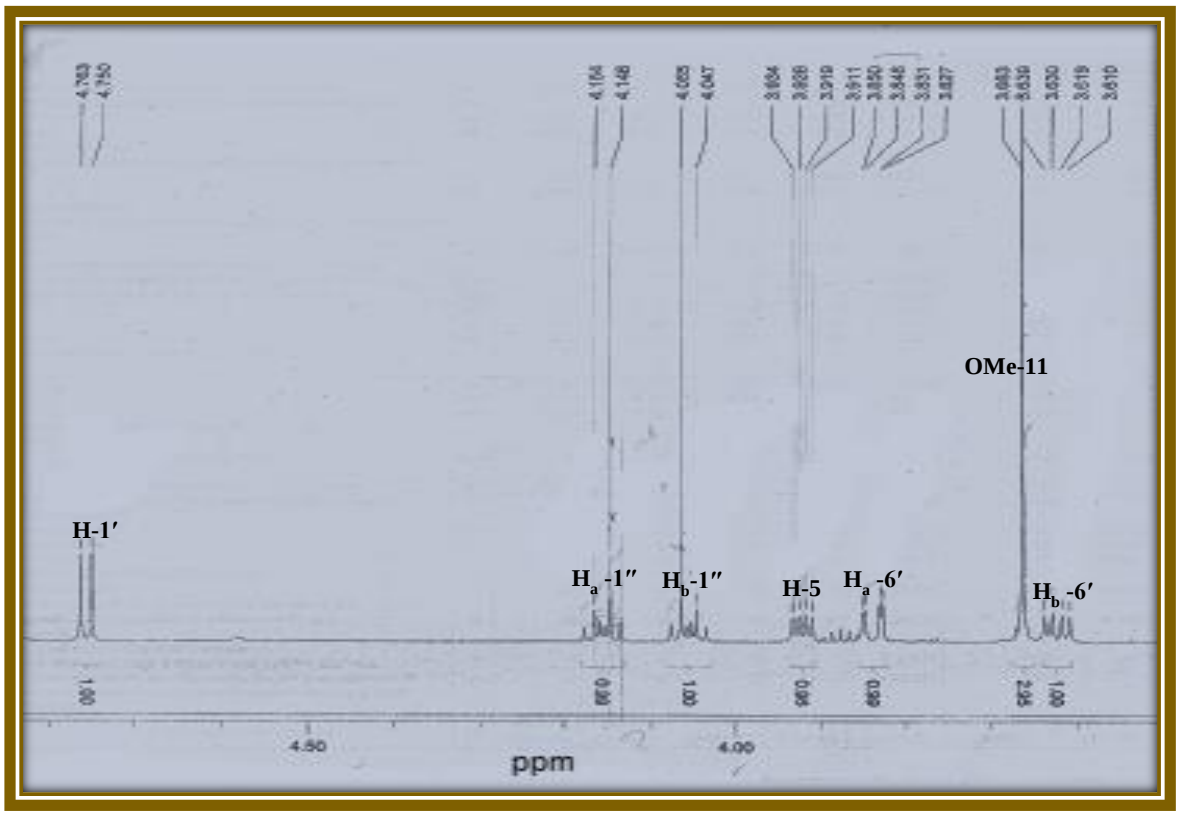


Figure III.53: ^{1}H NMR spectrum of compound 4

Additionally, The ^{1}H NMR spectrum (Figure III.52, III.53 and III.54) of compound **4** displayed characteristic signals of hydroxy tyrosol moiety:

- An ABX spin system appearing in the aromatic region at δ_H 6.64 (1H, d, J = 8.0 Hz, H-7″), δ_H 6.61 (1H, d, J = 2.0 Hz, H-4″) and δ_H 6.50 (1H, dd, J = 2.0, 8.0 Hz, H-8″), suggesting the presence of a catechol ring instead of an A_2X_2 spin system of a para-substituted phenolic unit in compound **3.**
- Signals appearing as an ABX_2 spin system at δ_H 4.15 (1H, dt, J = 7.2, 10.8 Hz), δ_H 4.06 (1H, dt, J = 7.2, 10.8 Hz), δ_H 2.71 (2H, t, J = 7.2 Hz), corresponding to H_a-1″, H_b-1″ and H-2″ respectively, of the two methylene groups of OCH_2CH_2Ph moiety.

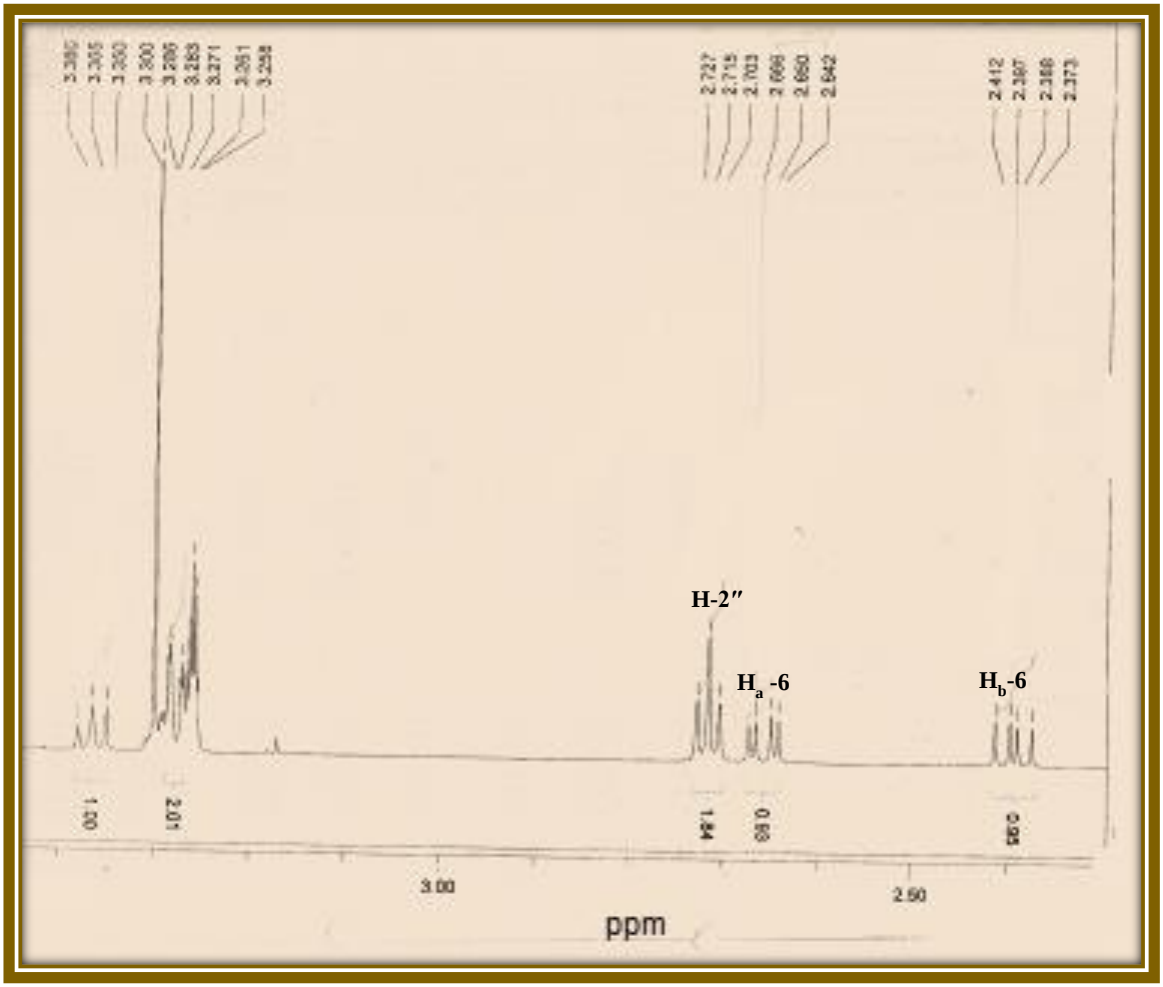


Figure III.54: ^{1}H NMR spectrum of compound 4

Also, we have observed the presence of characteristic signals of a glucoside unit (Figure III.53):

- A doublet signal detected at δ_H 4.76 with a coupling constant J = 7.8 was assigned to the anomeric proton H-1′ of a glucoside unit in β-configuration.

- A pair of double doublet signals appearing at δ_H 3.84 (1H, J = 1.8, 12.0 Hz) and δ_H 6.62 (1H, J = 5.4, 12.0 Hz) were assignable to the oxymethylene protons H_a-6′ and H_b-6′, respectively.

Finally, compound **4** was identified by direct comparison of its spectral data (Table III.4) with those reported for oleuropein [47, 168]. Moreover, the ^{1}H NMR comparison with oleuropein authentic sample (Figure III.55) gave proof that compound **4** is the secoiridoid glucoside oleuropein.

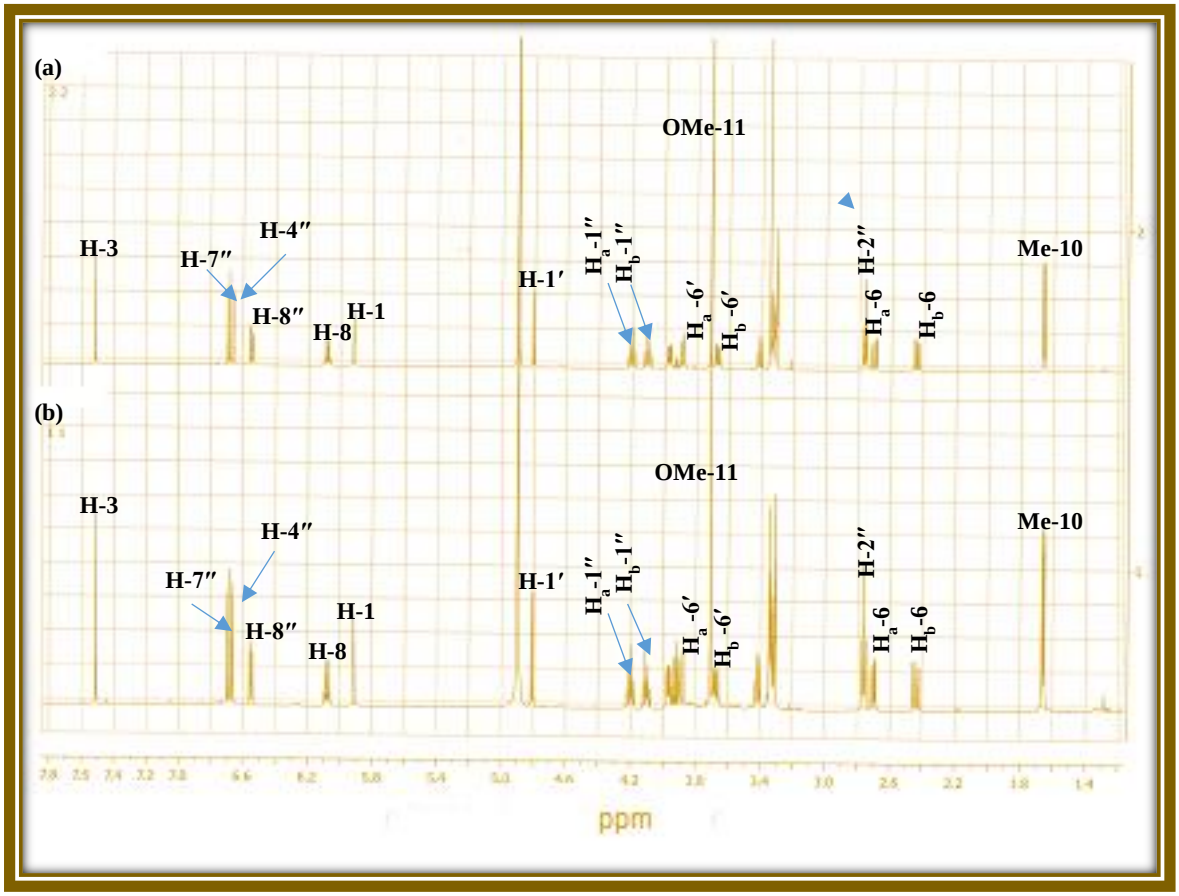


Figure III.55: (a) ^{1}H NMR spectrum of compound 4; (b) ^{1}H NMR spectrum of oleuropein authentic sample.

Table III.4: ^{1}H (600.11 MHz) data of oleuropein **4** (CD_3OD; δ in ppm; *J* in Hz)*

C/H	δ_H	C/H	δ_H
Aglycon		*Glucose*	
1	5.86 (*t-like*)	1′	4.76 *d (7.8)*
2	-	2′	
3	7.46 (*s*)	3′	

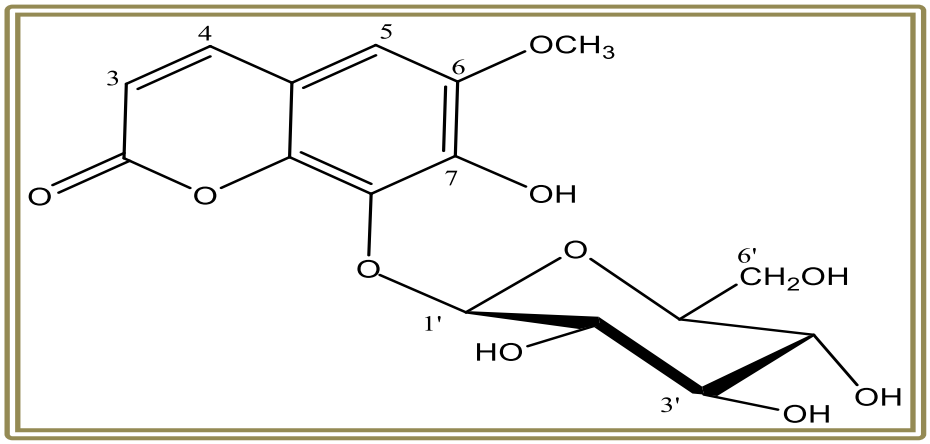
Table III.4: continued.

C/H	δ_H	C/H	δ_H
4	-	4′	
5	3.92 *dd*	5′	
	(4.8/9.0)		
6_a	2.66 *dd* (4.5/14.1)	$6'_a$	3.84 *dd* (1.8/12.0)
6_b	2,39 *dd* (9.0/14.1)	$6'_b$	3.62 *dd* (5.4/12.0)
		hydroxyTyrosol	
7	-	$1''_a$	4.15 *dt* (7.2/10.8)
		$1''_b$	4.06 *dt* (7.2/10.8)
8	6.03 (m)	2″	2.71 *t* (7.2)
9	-	4″	6.61 *d* (2.0)
10	1.61 *dd* (1.2/7.2)		
11	-	7″	6.64 *d* (7.8)
OMe	3.67 (*s*)	8″	6.05 *d* (2.0/8.0)

* Assignments were established by direct comparison with the literature.

III.4.3.3 Identification coumarins

III.4.3.3.1 Structure elucidation of compound 5



Fraxin

Compound **5** was obtained as a powder from the stem bark, it gave bleu fluorescence typical for coumarin compounds under $UV_{254\,nm}$, changing into yellow after spraying with H_2SO_4 in ethanol (20 %). The key features of the ^1H NMR spectrum (Figure III.56) were the presence of two doublets typical for AB spin system detected at 7.84 ppm and 6.14 ppm corresponding to the olefinic protons H-4 and H-3, respectively, with coupling constant J = 9.0 Hz. Moreover, the resonance of H-4 is found in the region δ_H 7.5 to 7.9 is an evidence that compound **5** is a coumarin lacking a C-5 oxygen functional group.

In addition, the ^1H NMR spectrum of compound **5** presents:

- A singlet signal at 6.90 ppm, which was characteristic for the aromatic proton H-5.
- An aromatic methoxy singlet signal appearing at 3.87 ppm.
- Signal arising at 4.89 ppm as doublet with coupling constant J = 7.8 Hz, provides evidence for the presence of β-glucoside residue.

Based on these findings (Table III.5) and according to the data given in reference [167] compound **5** corresponded to the coumarin glucoside fraxin.

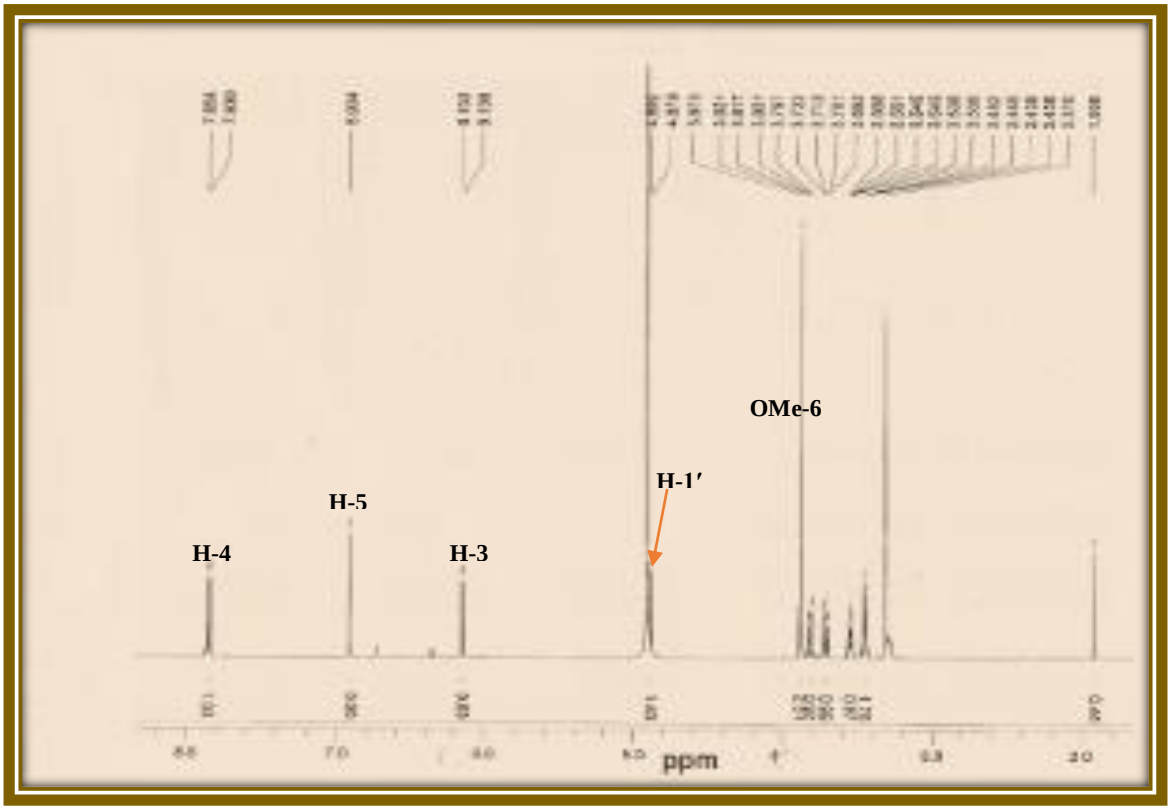


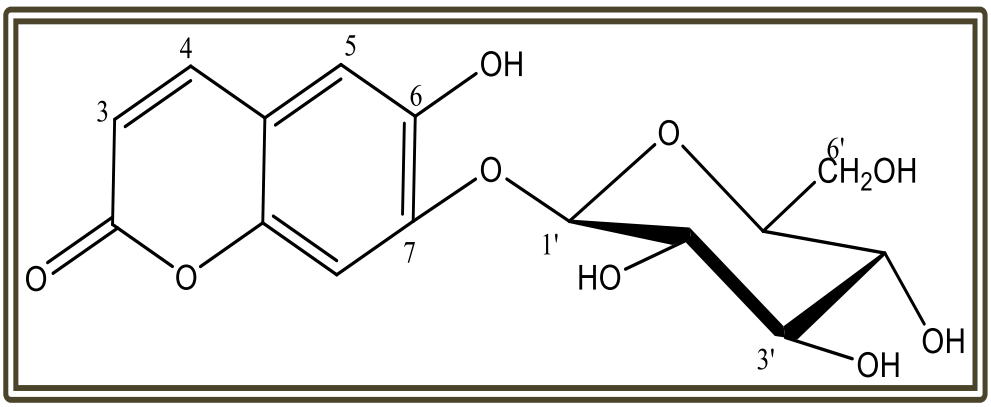
Figure III.56: ^1H NMR spectrum of compound 5

Table III.5: ^{1}H (600.11 MHz) data of fraxin 5 (CD_3OD; δ in ppm; *J* in Hz)*

C/H	δ_H	C/H	δ_H
Aglycon		*Glucose*	
1	-	1′	4.80 *d (7.8)*
2	-	2′	3.30-3.90
3	6.14 *d* (*9.0*)	3′	3.30-3.90
4	7.84 *d* (*9.0*)	4	3.30-3.90
5	6.90 (*s*)	5	3.30-3.90
6	-	6	3.30-3.90
OMe	3.87 (*s*)		
7	-		
8	-		
9	-		
10	-		

* Assignments were established by direct comparison with the literature.

III.4.3.3.2 Structure elucidation of compound 6



Cichoriin

Compound **6** was obtained as a powder from the stem bark and the leaves. It gave bleu fluorescence typical for coumarin compounds under $UV_{254\ nm}$, changing to yellow after spraying with H_2SO_4 in ethanol (20 %). The ^{1}H NMR spectrum (Figure III.57) showed signals due to aromatic protons resonating between 6.33 and 7.89 ppm and glucosidic protons between 3.30 and 5.04 ppm.

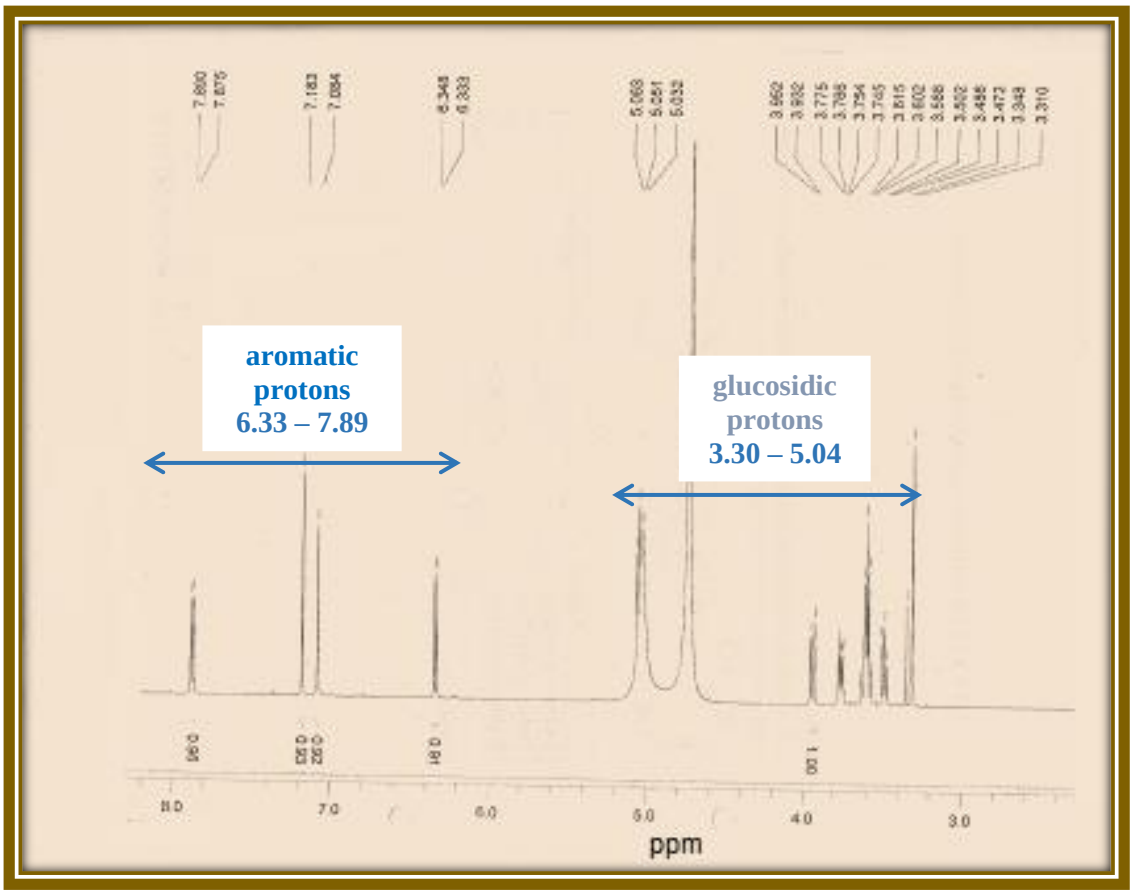


Figure III.57: ^{1}H NMR spectrum of compound 6 in CD_3OD

The ^{1}H NMR spectrum (Figure III.58 and III.59) allows to show :

- Two doublets have been observed at δ_H 7.89 and δ_H 6.33 with a coupling constant J = 9.4 Hz, corresponding to the methine protons H-4 and H-3 respectively, which are characteristic for benzo-α-pyrone derivatives.
- Two singlet signals at δ_H 7.18 and δ_H 7.06 corresponding to the aromatic protons H-5 and H-8, respectively.
- A doublet at δ_H 5.04 (J = 7.5 Hz) which is characteristic of the anomeric proton H-1′ of a β- glucosyl moiety.

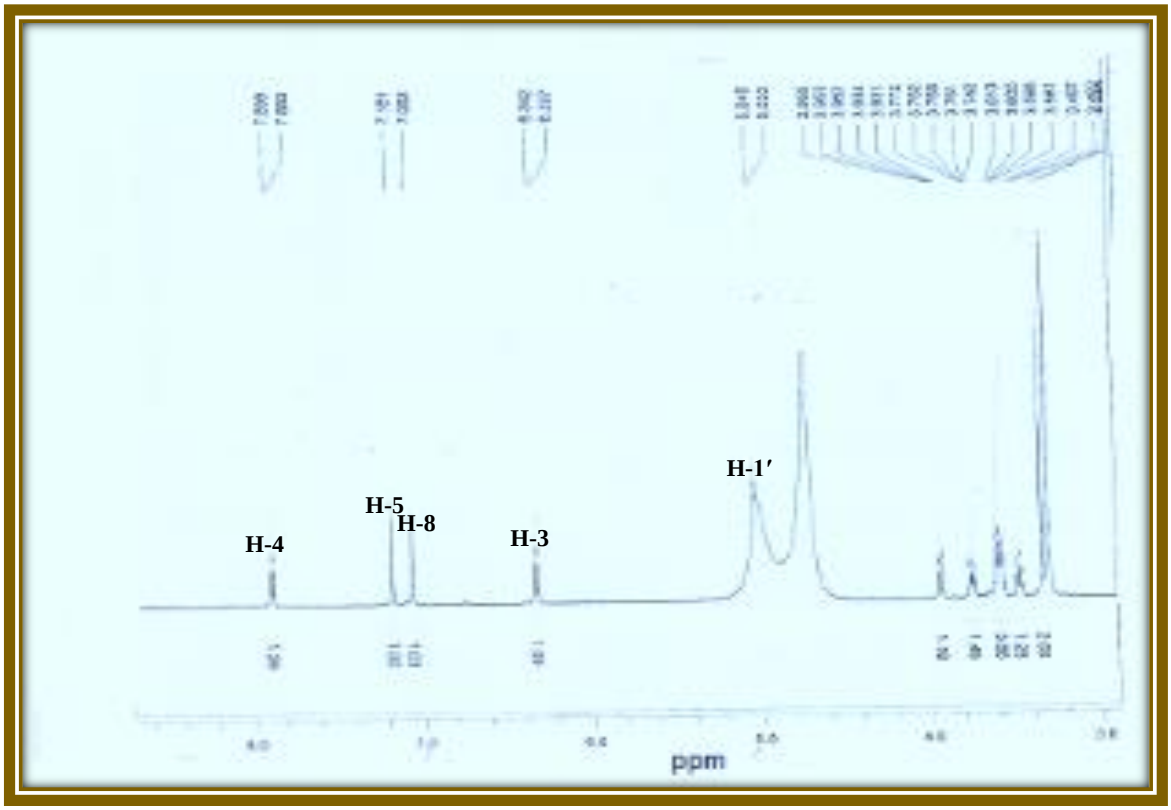


Figure III.58: ^{1}H NMR spectrum of compound 6

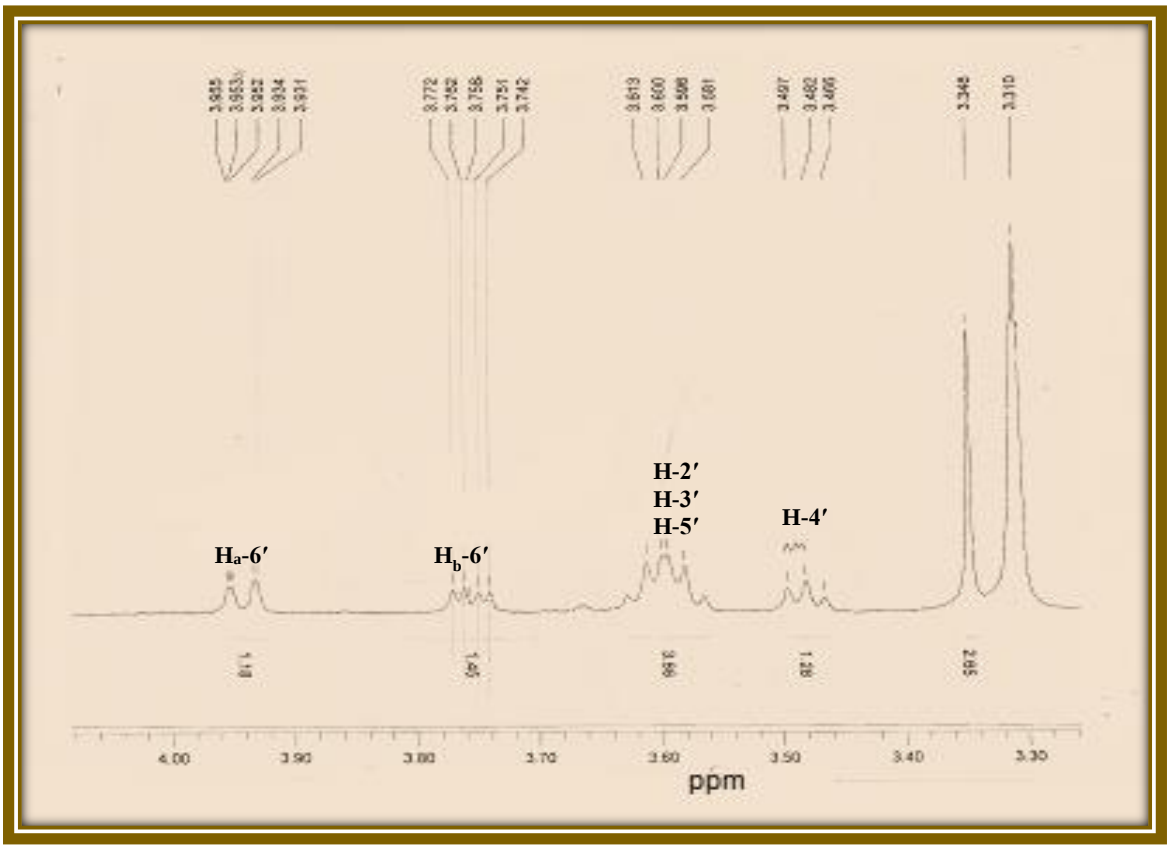


Figure III.59: ^{1}H NMR spectrum of compound 6

In the ^{13}C NMR spectrum (Figure III.60) fifteen carbon signals were resolved and support the suggested structure. Beside the carbon signals at δ_C 100.94 (C-1′), 73.17 (C-2′), 77.27(C-3′), 69.81 (C-4′), 75.85 (C-5′) and 60.74 (C-6′) which were attributed to β-glucoside moiety, there were also nine carbon signals comprising one typical signal for carbonyl carbon at δ_C 160.66 (C-2), four methine signals (δ_C 112.68 (C-3), 143.59 (C-4), 103.36 (C-5) and 103.34 (C-8)), and four quaternary carbons (δ_C 113.49 (C-6),148.83 (C-7), 147.82 (C-9) and 112.99 (C-10)). All of the signals above suggested that compound **6** is a coumarin glucoside.

These spectral features suggest that compound **6** should be either cichoriin or esculin. The identity of compound **6** was confirmed by comparison of the obtained ^{1}H and ^{13}C NMR data (Table III.6) with those reported in reference [169] and by comparison with cichoriin authentic sample, they were in full agreement. Thus, glucoside **6** proved to be cichoriin.

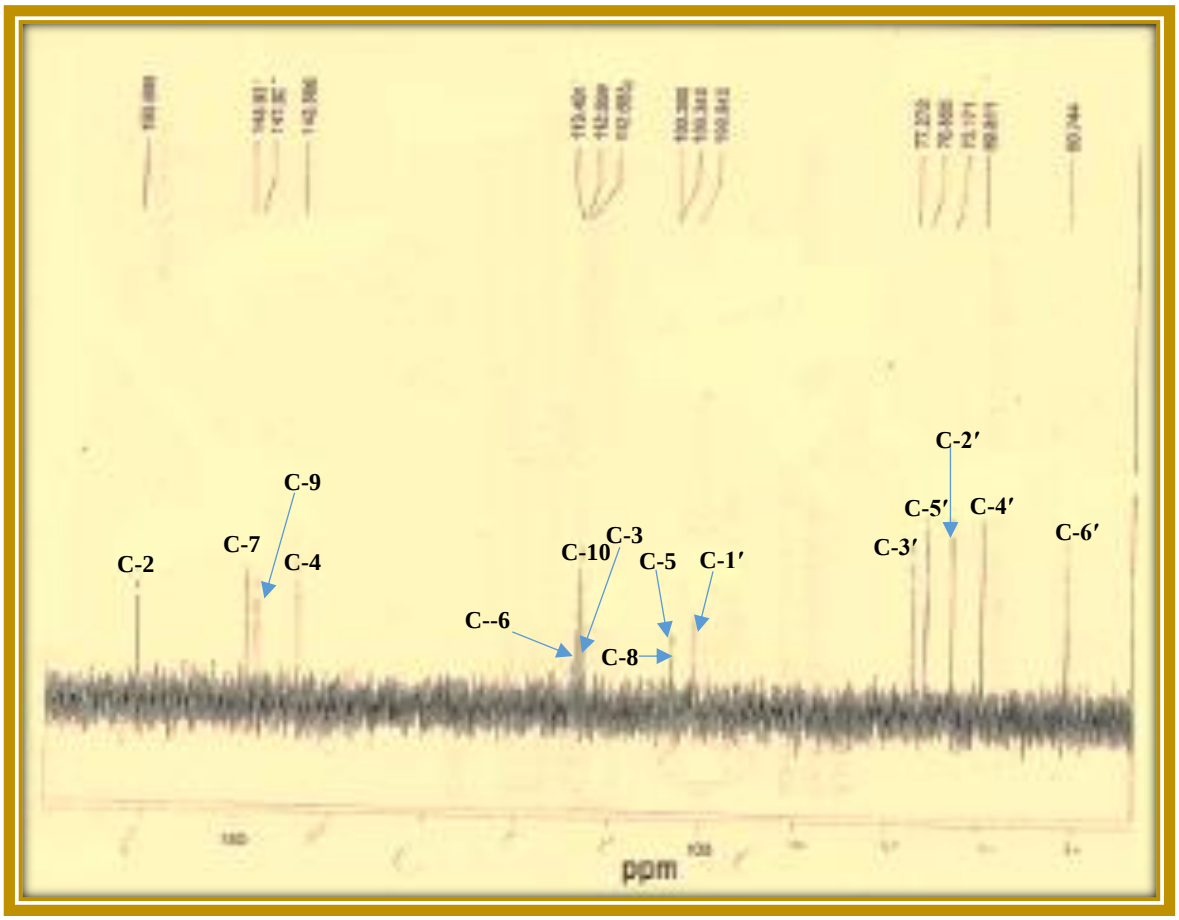


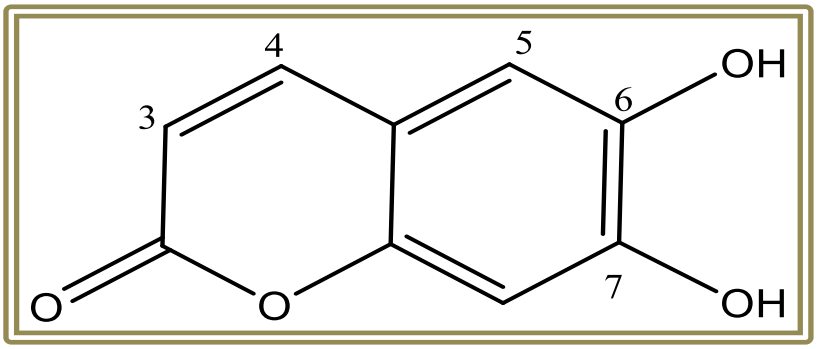
Figure III.60: ^{13}C NMR spectrum of compound 6 in DMSO-d_6

Table III.6: **^{1}H (600.11 MHz; CD_3OD) and ^{13}C (150.91 MHz; DMSO-d_6) data of cichoriin 6 (δ in ppm; *J* in Hz)***

C/H	δ_H	δ_C
Aglucone		
1	-	-
2	-	160.66
3	6.33 *d* (9.4)	112.68
4	7.89 *d* (9.4)	143.59
5	7.18 (*s*)	103.36
6	-	113.49
7	-	148.83
8	7.06 (s)	103.34
9	-	147.82
10	-	112.99
Glucose		
1′	5.04 *d* (7.5)	100.94
2′		73.17
3′		75.70
4′		69.81
5′		75.85
$6'_a$	3.94 *dd* (1.8/12.4)	60.74
$6'_b$	3.76 *dd* (5.7/12.4)	

* Assignments were established by direct comparison with the literature.

III.4.3.3.3 Structure elucidation of compound 7



Esculetin

Compound **7** was isolated as a powder from the leaves. It gave blue fluorescence, typical for coumarin compounds, under $UV_{254\ nm}$, changing into yellow after spraying with sulfuric acid reagent. The ^1H NMR spectrum (Figure III.61) of compound **7** showed two paires of a typical H-3 and H-4 signals appearing at δ_H 6.18 (1H, d, J = 9.6 Hz) and δ_H 7.78 (1H, d, J = 9.6 Hz), respectively, is a strong indication of a coumarin without substitution on the pyrone ring. Also, the the ^1H NMR spectrum revealed the presence of two singlet signals detected at δ_H 6.75 and 6.93, which were assigned to the aromatic protons H-5 and H-8, respectively.

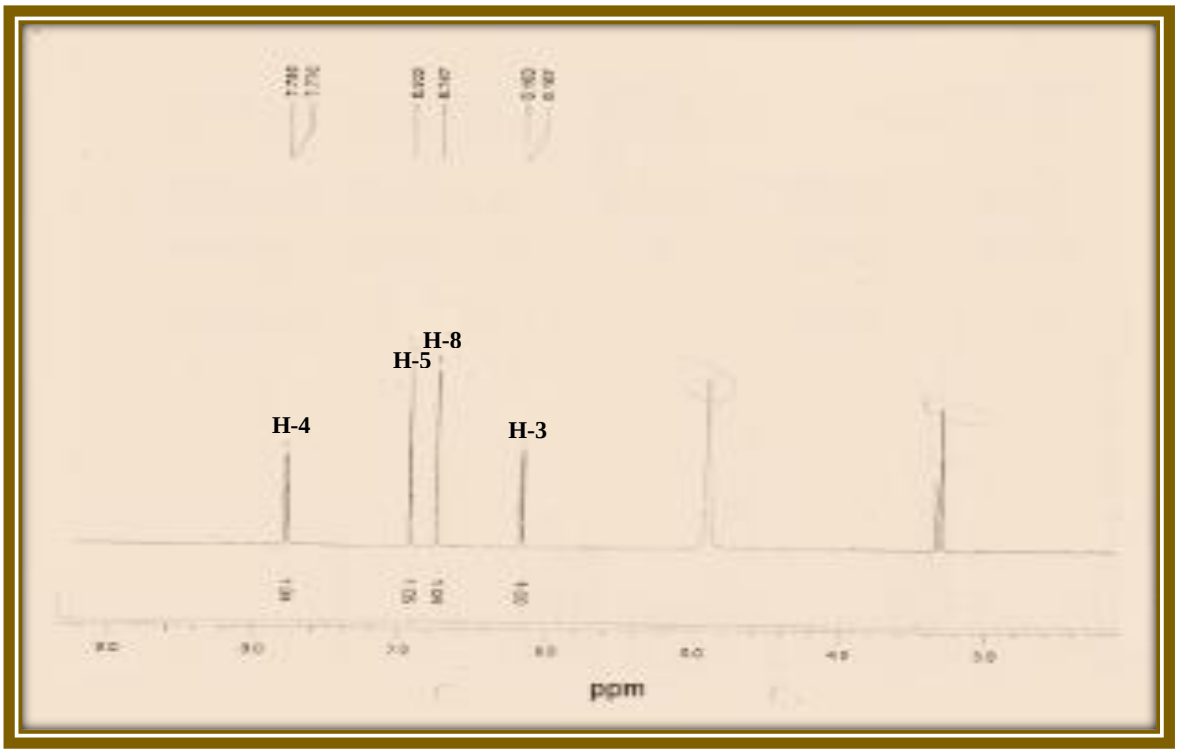


Figure III.61: ^1H NMR spectrum of compound 7

The obtained data and the ^{1}H NMR values (Table III.7), which were in full agreement to those in the literature [170], suggested that compound **7** is the coumarin esculetin.

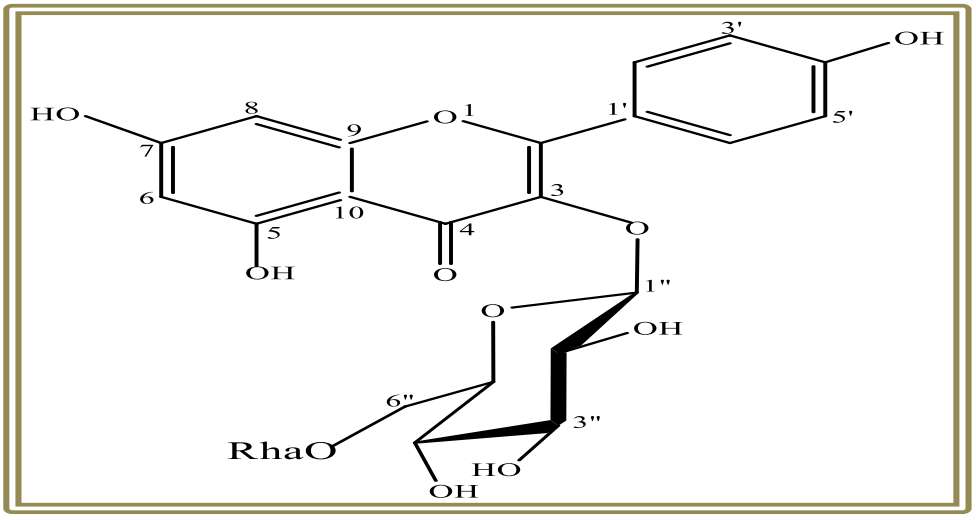
Table III.7: **^{1}H NMR (600.11 MHz) data of fraxin 5 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	-
3	6.18 *d* (*9.6*)
4	7.78 *d* (*9.6*)
5	6.75 (*s*)
6	-
7	-
8	6.93 (*s*)
9	-
10	-

* Assignments were established by direct comparison with the literature.

III.4.3.4 Identification of flavonoids

III.4.3.4.1 Structure elucidation of compound 8



Kaempferol-3-O-rutinoside

(Nicotiflorin)

Compound **8** was isolated as a yellowish powder from the leaves. The ^{1}H NMR spectrum (Figure III.62) of compound **8** displayed a set of signals resonating between δ_H 3.00 and 5.29 ppm, referring to two sugars at least as well as a characteristic aromatic protons suggesting the presence of flavonol framework.

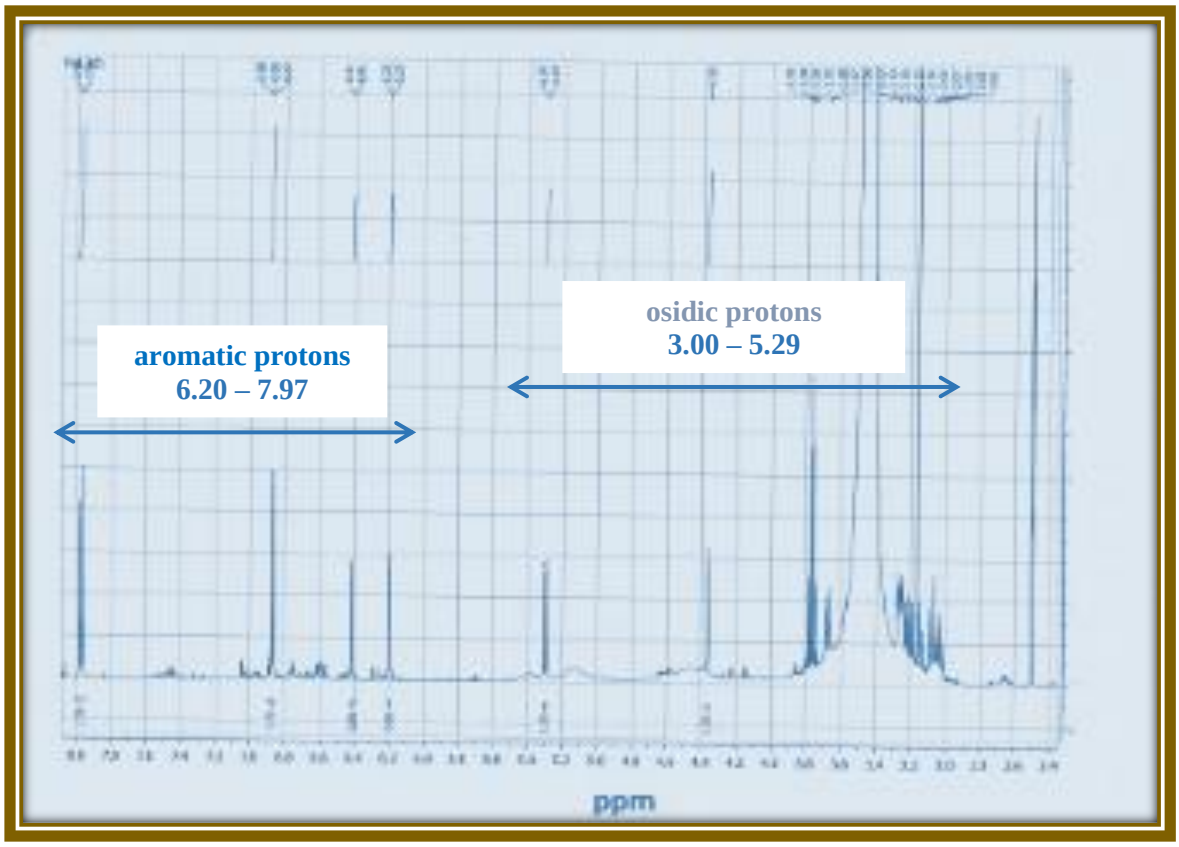


Figure III.62: ^{1}H NMR spectrum of compound 8

The structural analysis of the ^{1}H NMR spectrum (Figure III.63) of compound **8** revealed two doublets signals detected at δ_H 6.20 (1H, J = 2.0 Hz) and 6.41 (1H, J = 2.0 Hz) consistent with the meta protons H-6 and H-8 on A-ring. An A_2X_2 spin system at δ_H 7.97 (2H, d, J = 8.9 Hz, H-2′ and H-6′) and 6.87 (2H, d, J = 8.9 Hz, H-3′ and H-5′), suggesting the presence of para-substituted phenolic ring corresponding to B-ring. The comparison of the ^{1}H NMR data with the literature [171, 172] led us to identify the aglucone part of compound **8** as kaempferol.

In the saccharide region (Figure III.63) of the spectrum two anomeric proton signals were present as doublets at δ_H 5.30 (1H, J = 7.7 Hz) and 4.36 (1H, J = 1.3 Hz), which together with the overlapped proton signals at δ_H 3-4 (Figure III.62), demonstrating the glucosilated nature of compound **8**. The coupling constant (J = 7.7 Hz) of the anomeric proton H-1″ indicated the β-configuration of the glucopyranosyl moiety. Moreover, the attachment of the glucopyranosyl unit

to the C-3 position of the aglycone was confirmed by comparison of the anomeric proton chemical shift (δ 5.30) with the literature data [173].

The second sugar residue was suggested to be *α*-rhamnose by the disctint methyl protons signal at δ_H 0.97 (d, J= 6.22 Hz) in the high-field region (Figure III.64), further confirmed by the doublet signal at δ_H 4.36 ,which was assigned to the anomeric proton H-1‴ with a coupling constant (*J* = 1.6 Hz) characteristic for *α*-configuration. The anomeric proton of rhamnose at 4.36 ppm indicates that the glycoside moiety in the flavonoid is rhamnoglucose because of, when, the rhamnose is attached directly to the aglycon moiety in the flavonoid, the chemical shift of the rhamnosyl anomeric proton should be shown at a higher level (5.0~ 5.2) [174].

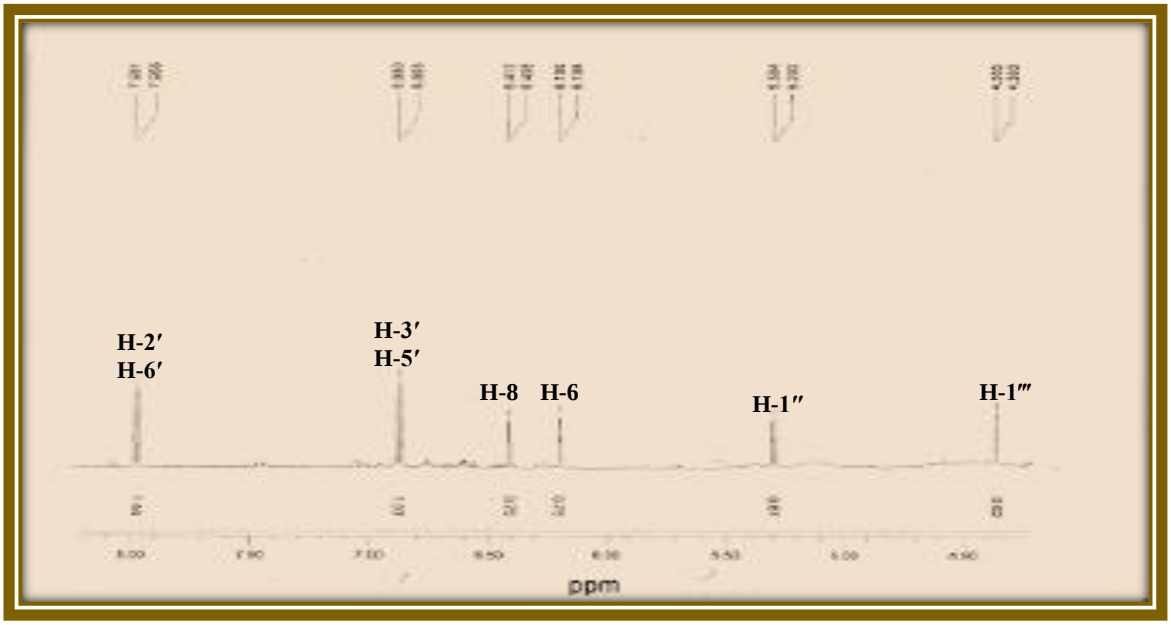


Figure III.63: ^{1}H NMR spectrum of compound 8

At this point, compound **8** was identified as kaempferol-3-O-rutinoside (nicotiflorin). The spectroscopic data (Table III.8) were identical with the literature [175, 176]. Nicotiflorin has been previously isolated from plant species like *Astragalus armatus* [177], *Edgeworthia chrysantha* [178], *Carthamus tinctorius* [179] and *Acalypha hispida* [180]. It has been demonstrated to have many interesting pharmacological activities, such as decreasing arterial blood pressure and heart rate [181], hepatoprotective effects on CCl_4 –induced liver injury [179], potent antioxidant, anti-inflammatory [182].

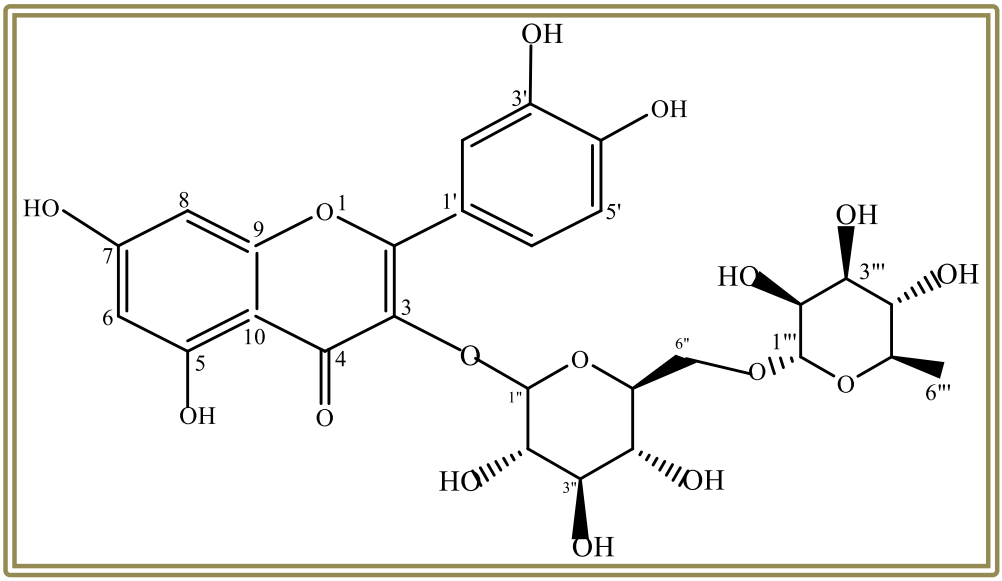
Table III.8: **^{1}H NMR (600.11 MHz) data of nicotiflorin 8 (DMSO-d_6; δ in ppm; *J* in Hz) ***

C/H	δ_H	C/H	δ_H
Aglucone		*Rutinoside*	
1	-	1″	5.30 *d* (*7.7)*
2	-	2″	3.00-3.85[a]
3	-	3″	3.20-3.85[a]
4	-	4″	3.20-3.85[a]
5	-	5″	3.20-3.85[a]
6	6.20 *d* (*2.0*)	6″	3.20-3.85[a]
7	-	1‴	4.36 *d* (*1.3*)
8	6.41 *d* (*2.0*)	2‴	3.20-3.85[a]
9	-	3‴	3.20-3.85[a]
10	-	4‴	3.20-3.85[a]
1′	-	5‴	3.20-3.85[a]
2′	7.97 *d* (*8.9*)	6‴	0.97 *d* (*6.22*)
3′	6.87 *d* (*8.9)*		
4′	-		
5′	6.87 *d* (*8.9)*		
6′	7.97 *d* (*8.9)*		

* Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.4.2 Structure elucidation of compound 9



Quercetin-3-O-rutinoside

(Rutin)

Compound **9** was obtained as a faint yellow powder from the leaves. The ^{1}H NMR spectrum (Figure III.65 and III.66) of this compound exhibited a great amount of similarity with that of compound **8**, except for the presence of an ABX spin system at δ_H 7.54 (1H, dd, J = 2.2, 8.3 Hz, H-6′), 7.52 (1H, d, J = 2.2 Hz, H-2′) and 6.83 (1H, d, J = 8.3 Hz, H-5′) corresponding to the catechol protons on B-ring, instead of an A_2X_2 spin system of a para-substituted phenolic unit in compound **8.**

The comparison of the ^{1}H NMR data (Table III.9) with the literature [183, 184] led us to identify compound **9** as quercetin-3-O-rutinoside (Rutin).

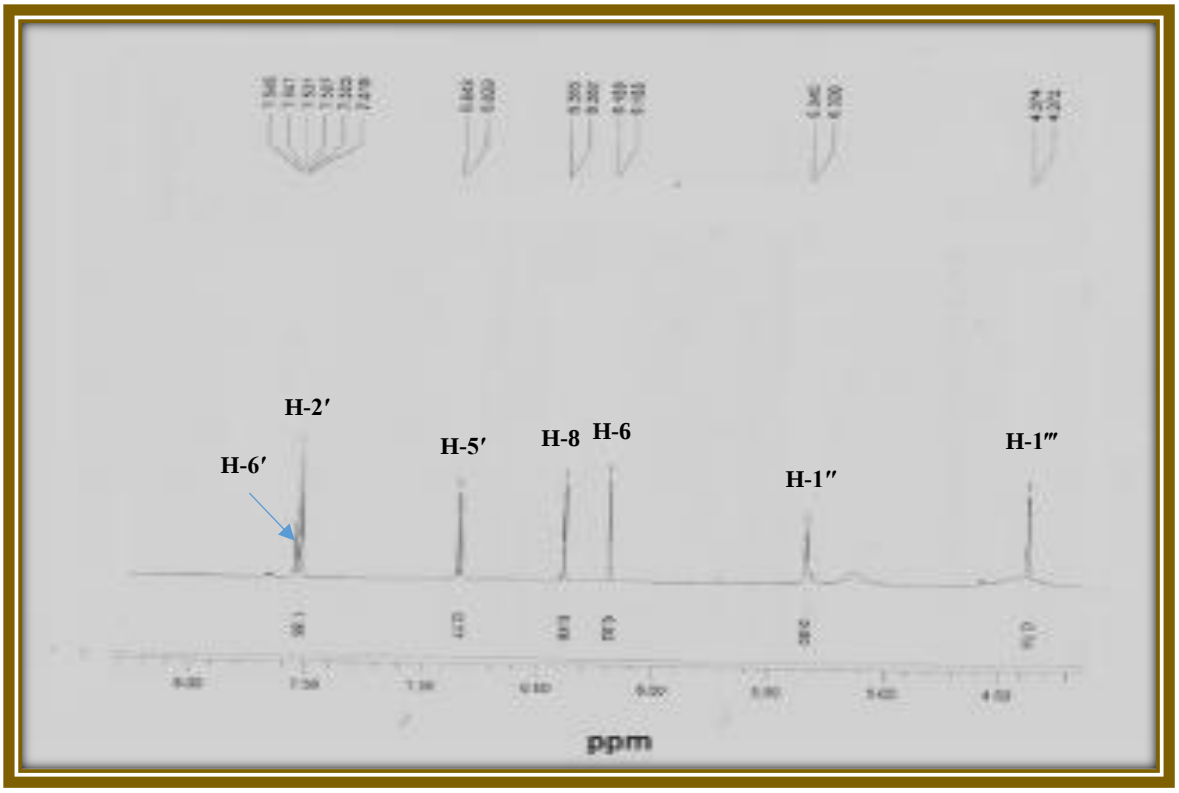


Figure III.65: ^{1}H NMR spectrum of compound 9

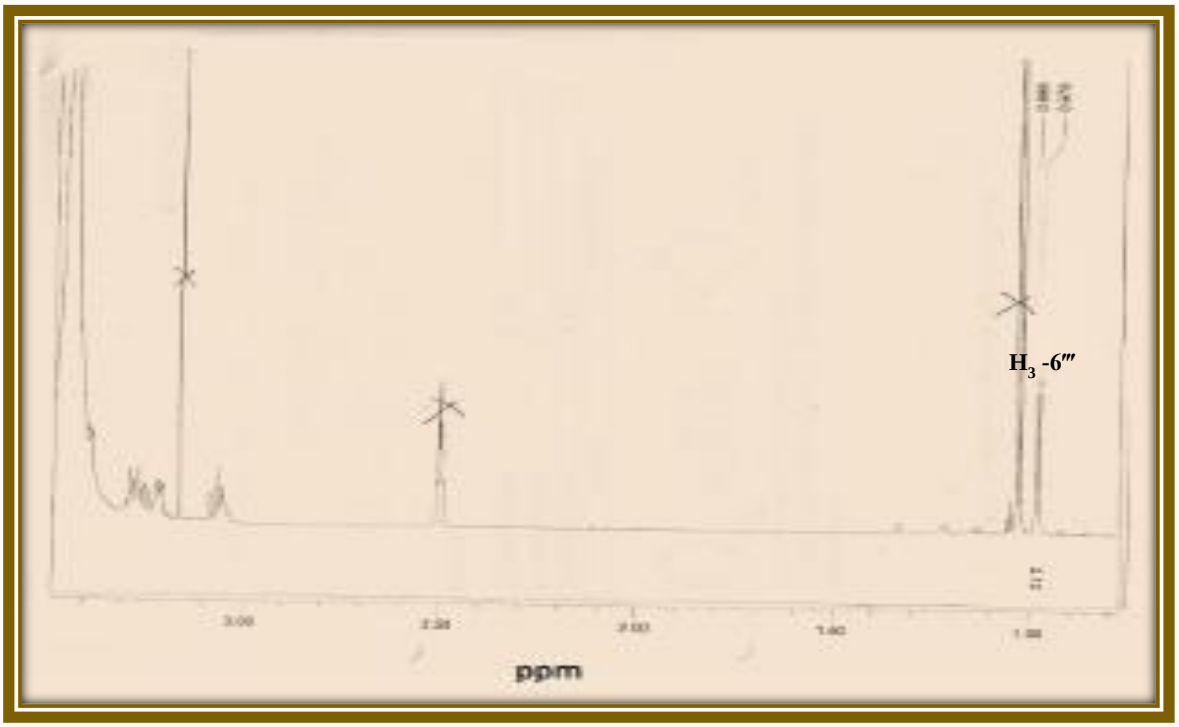


Figure III.66: ^{1}H NMR spectrum of compound 9

Table III.9: ^{1}H NMR (600.11 MHz) data of rutin 9 (DMSO-d_6; δ in ppm; *J* in Hz) *

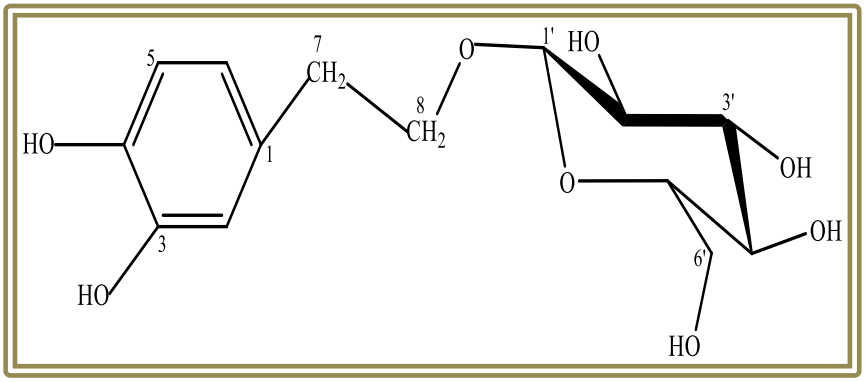
C/H	δ_H	C/H	δ_H
Aglucone		*Rutinoside*	
1	-	1″	5.33 *d* (*7.5)*
2	-	2″	3.00-3.80[a]
3	-	3″	3.20-3.80[a]
4	-	4″	3.20-3.80[a]
5	-	5″	3.20-3.80[a]
6	6.19 *d* (*2.0*)	6″	3.20-3.80[a]
7	-	1‴	4.37 *d* (*1.2*)
8	6.38 *d* (*2.0*)	2‴	3.20-3.80[a]
9	-	3‴	3.20-3.80[a]
10	-	4‴	3.20-3.80[a]
1′	-	5‴	3.20-3.80[a]
2′	7.52 *d* (*2.2*)	6‴	0.98 *d* (*6.21*)
3′	-		
4′	-		
5′	6.83 *d* (*8.3)*		
6′	7.54 *dd* (2.2/*8.3)*		

* Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.5 Identification of phenylethanoids

III.4.3.5.1 Structure elucidation of compound 10



2-(3,4-dihydroxy)phenylethyl-O-β-D-glucopyranoside

Compound **10** was isolated as a white amorphous powder from the leaves. The proton NMR spectrum (Figure III.67 and III.68) revealed the presence of an ABX spin system at δ_H 6.68 (1H, d, J = 2.1 Hz), 6.66 (1H, d, J = 8.0 Hz) and 6.55 (1H, dd, J = 2.1, 8.0 Hz) assignable to H-2, H-5 and H-6, respectively, suggesting the presence of catechol ring. Furthermore, the presence of two signals at δ_H 4.03 (1H, ddd, J = 6.6, 8.4 and 9.6 Hz), 2.78 (2H, J = 6.0, 8.4 Hz), corresponding to H-8 and H-7, respectively, indicating the presence of 3,4-dihydroxyphenyethyl moiety.

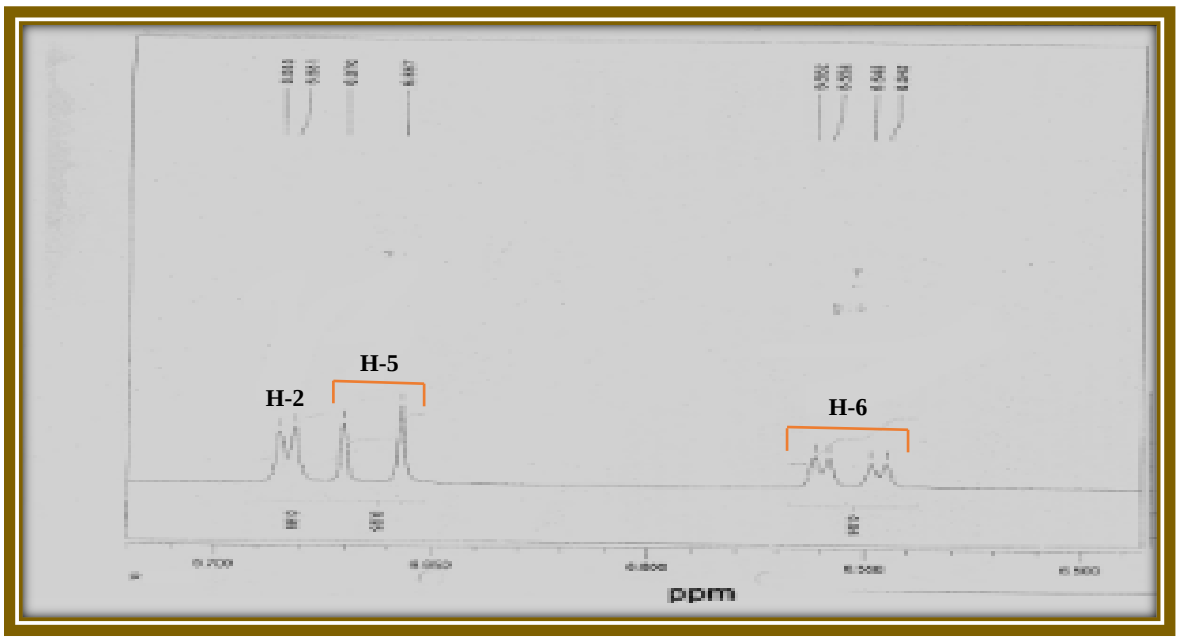


Figure III.67: ^{1}H NMR spectrum of compound 10

Additionally, the ^{1}H NMR spectrum (Figure III.68) also revealed the presence of a doublet signal resonating at δ_H 4.29 with a coupling constant J = 7.8 Hz, characteristic of the anomeric proton H-1′, together with the double doublet signal appearing at δ_H 3.86 (J = 1.8, 12.0 Hz) corresponding to the oxymethylene proton H_a-6 indicated the presence of a β- glucopyranoside unit. Comparison of the obtained ^{1}H NMR data (Table III.10) with those previously reported in the literature [185] led us to identify compound **10** as 2-(3, 4-dihydroxy)phenylethyl-O-β-D-glucopyranoside. This compound has been previously isolated from plant many plants like *Zantedeschia aethiopica* [186], *Forsythia Suspense* (Thunb.) [187], *Coptidis rhizoma* [188] and for the first time from Fraxinus species.

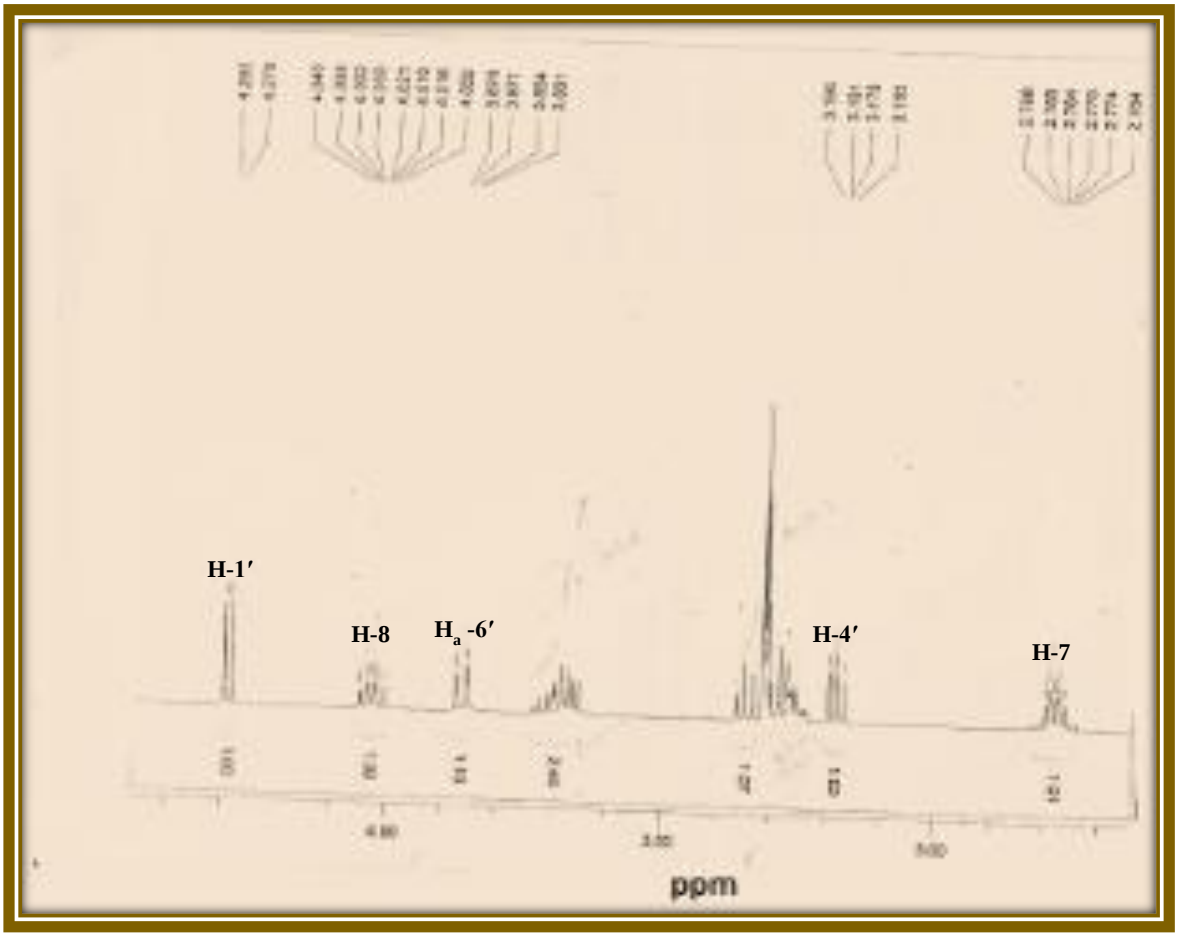


Figure III.68: ^{1}H NMR spectrum of compound 10

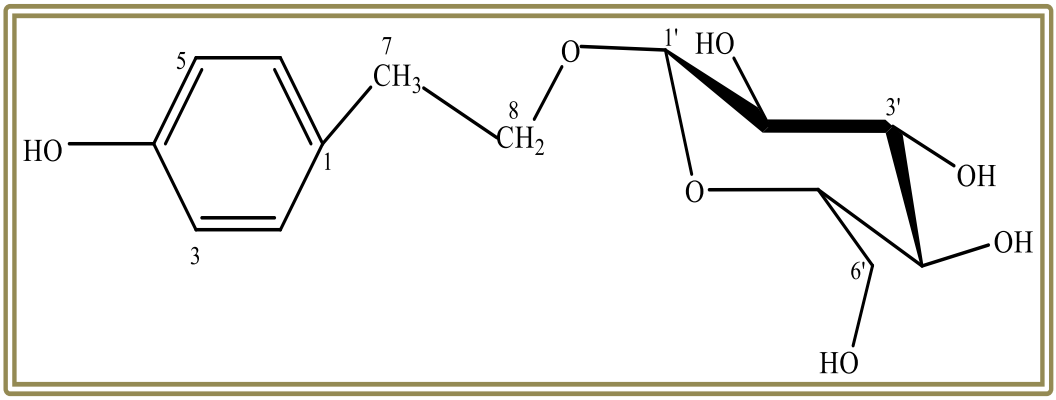
Table III.10: **^{1}H NMR (600.11 MHz) data of compound 10 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	6.68 *d* (*2.1*)
3	-
4	-
5	6.66 *d* (*8.0*)
6	6.55 *dd* (*2.1/8.0*)
7	2.78 *dt* (*6.0/8.4*)
8	4.03 *ddd* (*6.6/8.4/9.6*)
1′	4.29 *d* (*7.8*)
2′	3.28-3.35[a]
3′	3.28-3.35[a]
4′	3.18 *dd* (*7.8/9.6*)
5′	3.65-3.70[a]
H_a-6′	3.86 *dd* (*1.8/12.0*)
H_b-6′	3.65-3.70[a]

* Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.5.2 Structure elucidation of compound 11



Salidroside

Glucoside **11** was isolated as colorless needles from the leaves. Its ^{1}H NMR spectrum (Figure III.69 and III.70) very closely resembled those of 2-(3, 4-dihydroxy)phenylethyl-O-β-D-glucopyranoside (**10**). The only difference being that compound **11** had signals due to A_2X_2 spin system at δ_H 7.06 (2H, d, J = 8.4 Hz, H-2 and H-6) and 6.69 (2H, d, J = 8.4 Hz, H-3 and H-5), suggesting the presence of para-substituted phenolic ring instead of an ABX spin system of a catechol ring in compound **10.**

Compound **11** was identified by direct comparison of its ^{1}H NMR data (Table III.11) with those reported in the literature [189] as salidroside. This compound has been previously isolated from the roots of *Rodiola sachalinensis* and has been proved to possess broad pharmacological activities, such as neuroprotective effects [190], antiviral effects [187], anti-fatigue [191], anti-inflammation [192, 193], anti-cancer [194], and hepatoprotective [195] properties.

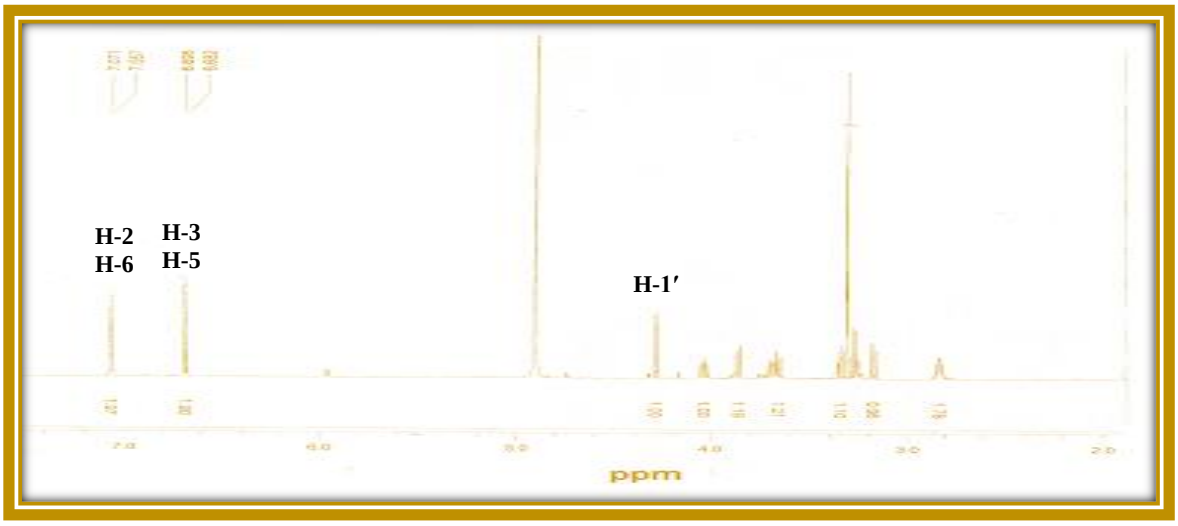


Figure III.69: ^{1}H NMR spectrum of compound 11

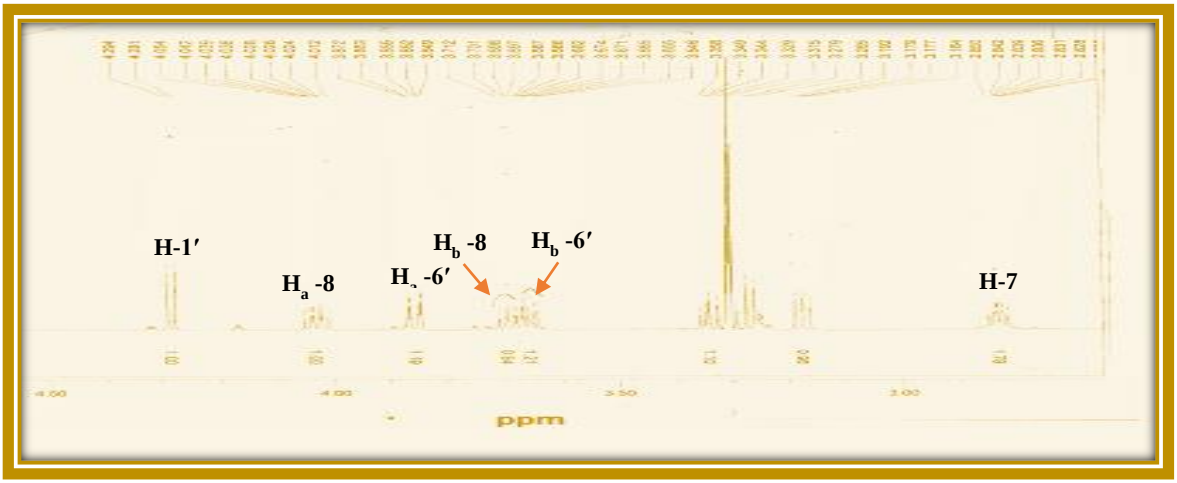


Figure III.70: ^{1}H NMR spectrum of compound 11

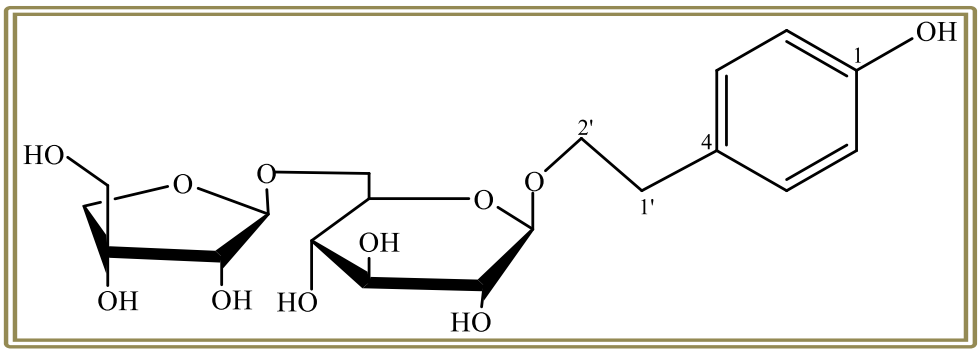
Table III.11: **^{1}H NMR (600.11 MHz) data of salidroside 11 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	7.06 *d* (*8.4*)
3	6.69 *d* (*8.4*)
4	-
5	6.69 *d* (*8.4*)
6	7.06 *d* (*8.4*)
7	2.83 *ddd* (*4.8/6.6/8.4*)
H_a-8	4.03 *ddd* (*7.2/8.7/9.6*)
H_b-8	3.69 *ddd* (*6.6/8.7/9.6*)
1′	4.29 *d* (*7.8*)
2′	3.18 *dd* (*7.8/9.2*)
3′	3.27-3.36[a]
4′	3.27-3.36[a]
5′	3.27-3.36[a]
H_a-6′	3.86 *dd* (*1.8/12.0*)
H_b-6′	3.66 *dd* (*5.4/12.0*)

* Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.5.3 Structure elucidation of compound 12



2-(4-hydroxyphenyl)ethanol-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside

(Osmanthuside H)

Compound **12** was isolated as brownish powder from the bark. The ^{1}H NMR spectrum (Figure III.71) showed the expected signals of aromatic protons at δ_H 7.07 (d, J = 8.4 Hz) for H-3, H-5 and δ_H 6.69 (d, J=8.4 Hz) for H-2, H-6. Additionally, the presence of two signals (Figure III.72 and III.73) at δ_H 3.70 (1H, ddd, J = 6.6, 8.1 and 9.6 Hz) and 2.84 (2H, m), corresponding to H-2′ and H-1′, respectively, indicating the presence of a p-hydroxyphenyethyl moiety.

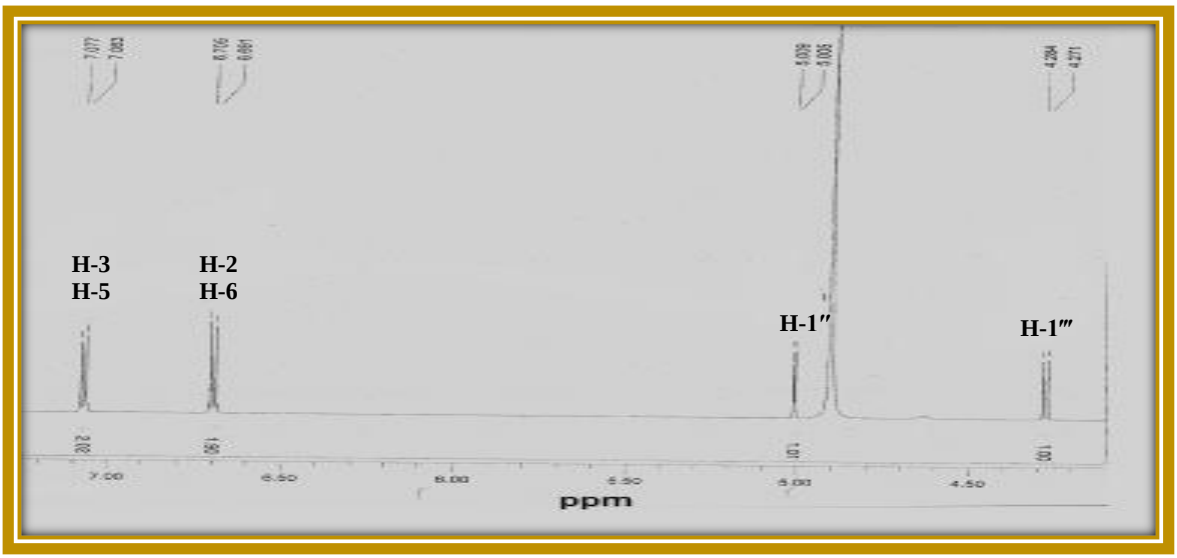


Figure III.71: ^{1}H NMR spectrum of compound 12

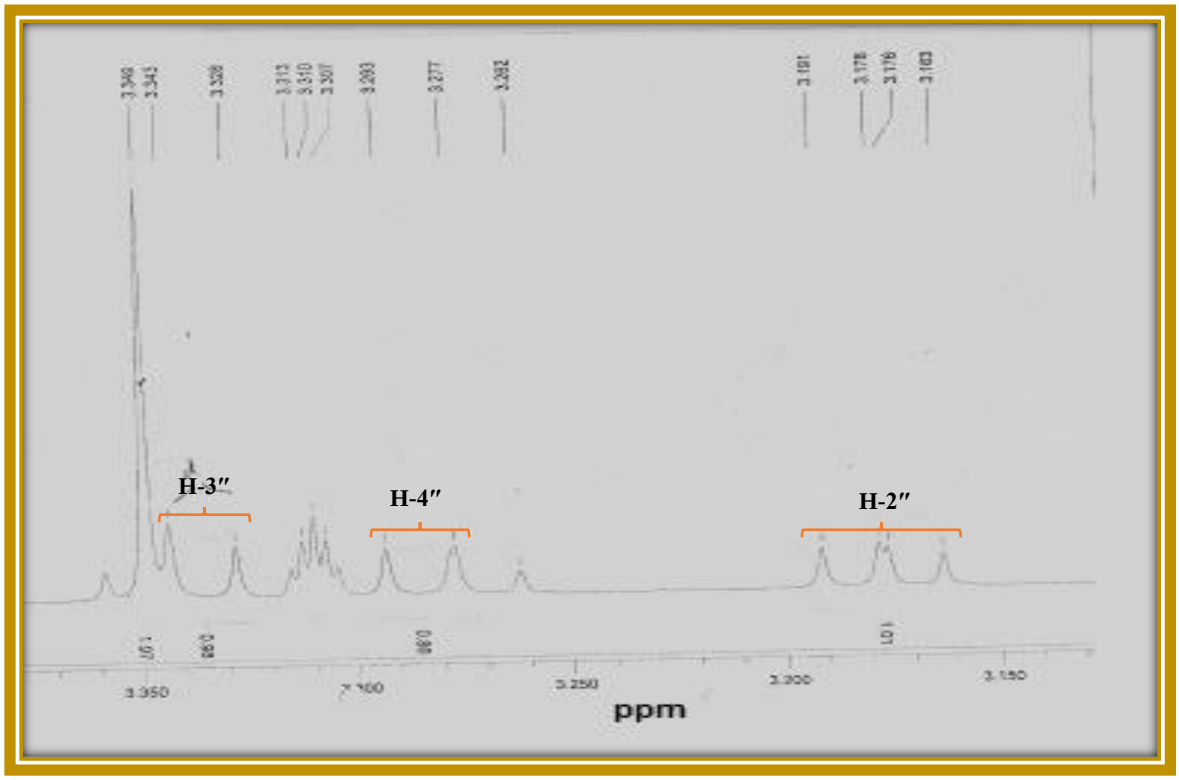


Figure III.73: ^{1}H NMR spectrum of compound 12

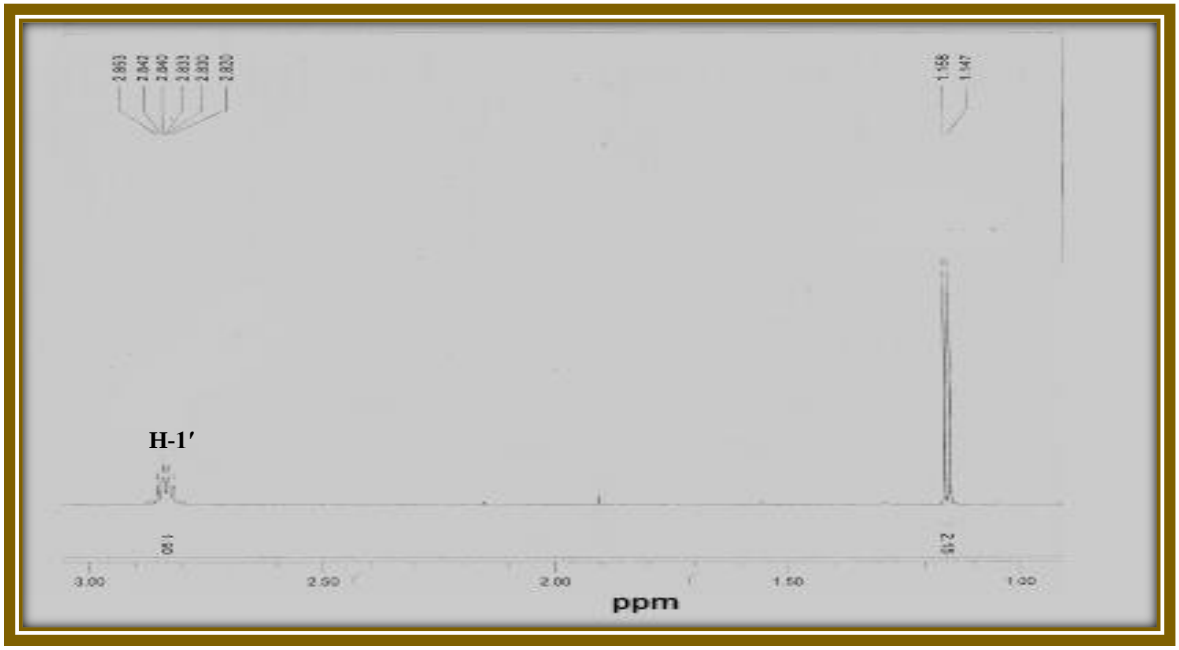


Figure III.74: ^{1}H NMR spectrum of compound 12

The presence of two sugars in compound **12** was apparent from a series of signals in the region of δ 3.18-5.00 (Figure III.71, III.72 and III.73). One of these sugars was β-D-glucose

suggested by the distinct anomeric proton at δ_H 4.28 (d). The second sugar was identified as β-D-apiose by the distinct anomeric proton at 5.0 (d). The β-configuration of the glucose unit was deduced from the coupling constant (7.8 Hz) of the anomeric proton. The β-configuration of the apiose unit was procured from the chemical shift (δ 111.0) of the anomeric carbon and the coupling constant (2.4 Hz) of the anomeric proton (quasiaxial) [196]. The coupling constant of the anomeric protons with α-configuration in apiofuranosides appears in 4.3-4.9 Hz compared to that of β-configuration [197].

In the ^{13}C NMR spectrum (Figure III.75, III.76 and III.77), four signals for a symmetrically substituted aromatic ring (δ_C 156.8, 131.0, 130.7 and 116.2) and two signals for methylene (δ_C 72.3 and 36.4) corresponding to the p-hydroxyphenylethyl alcohol moiety. The presence of the β-D-glucosyl group was confirmed by the ^{13}C NMR spectra which showed an anomeric carbon signal at δ_C 104.4 (C-1″), another signal of oxymethylene carbon at δ_C 68.7 (C-6″) and other oxymethine carbon signals resonating at δ_C 74.9 (C-2″), δ_C 77.9 (C-3″), δ_C 71.7 (C-4″) and δ_C 76.8 (C-5″). Also, the ^{13}C NMR spectra displayed characteristic signals of apiofuranose unit at δ_C 111.0 (C-1‴), 77.9 (C-2‴), 80.6 (C-3‴), 75.1 (C-4‴) and 65.5 (C-5‴).

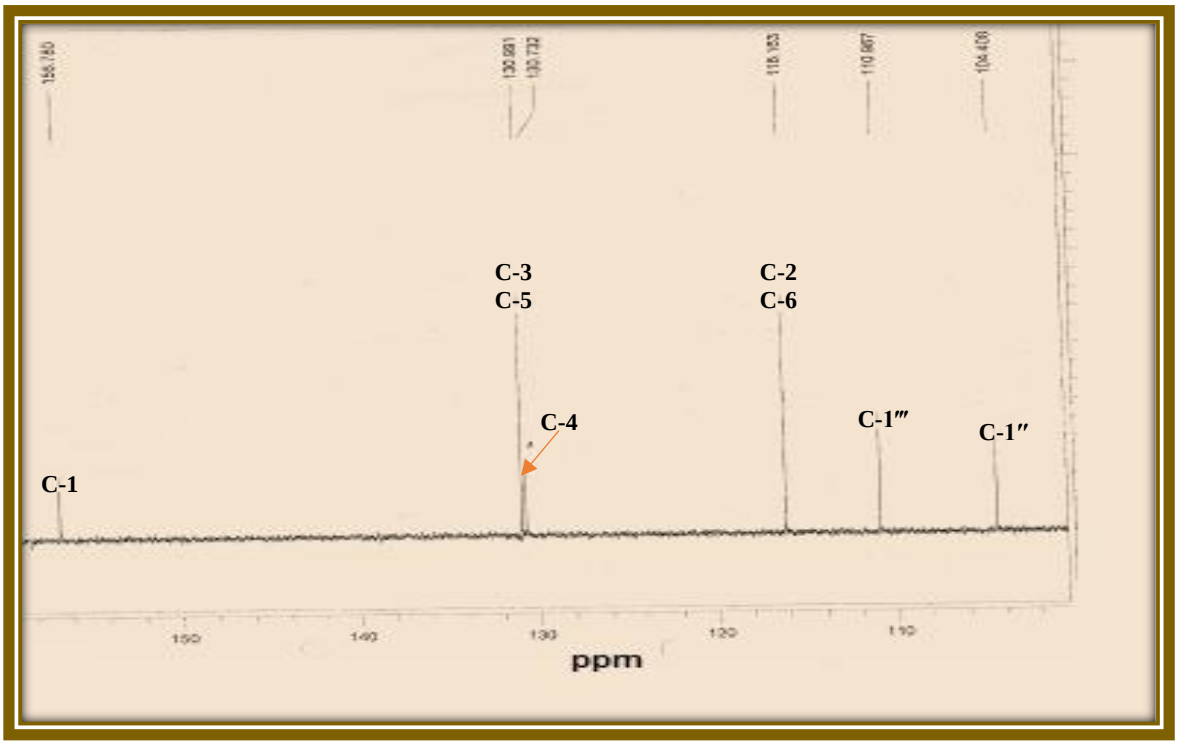


Figure III.75: ^{13}C NMR spectrum of compound 12

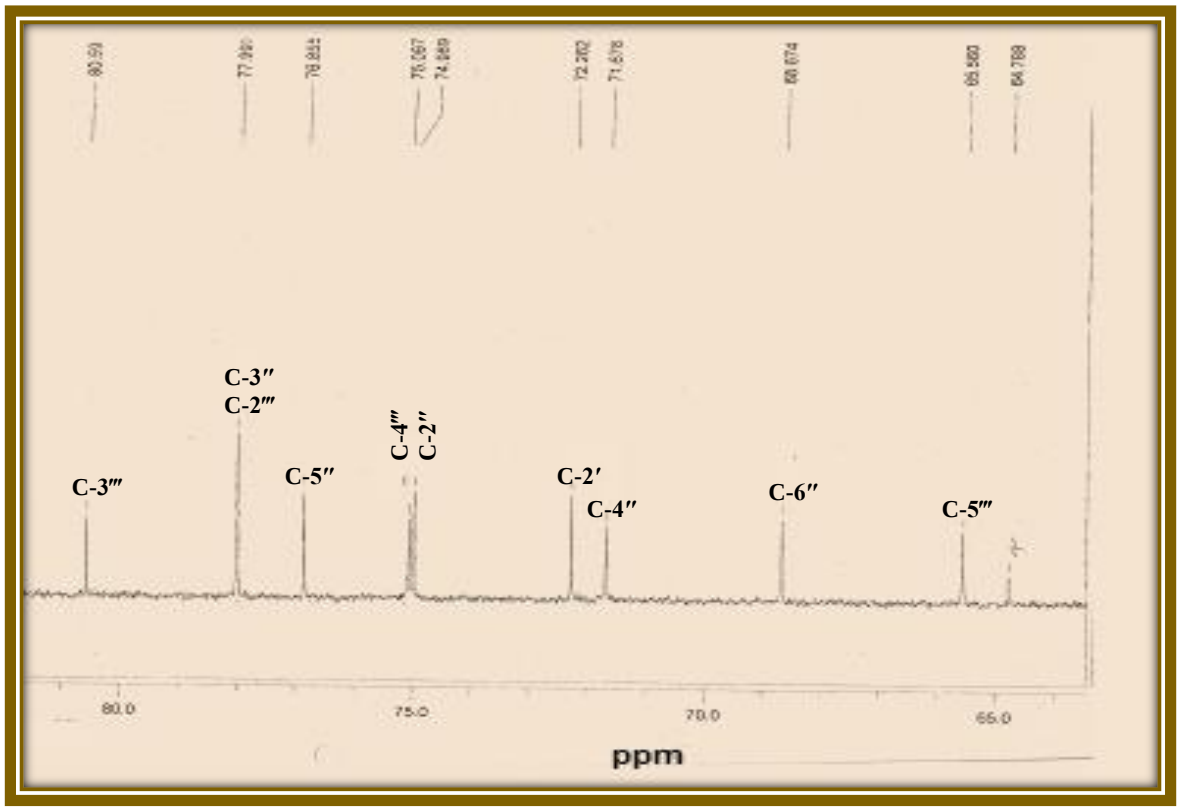


Figure III.76: ^{13}C NMR spectrum of compound 12

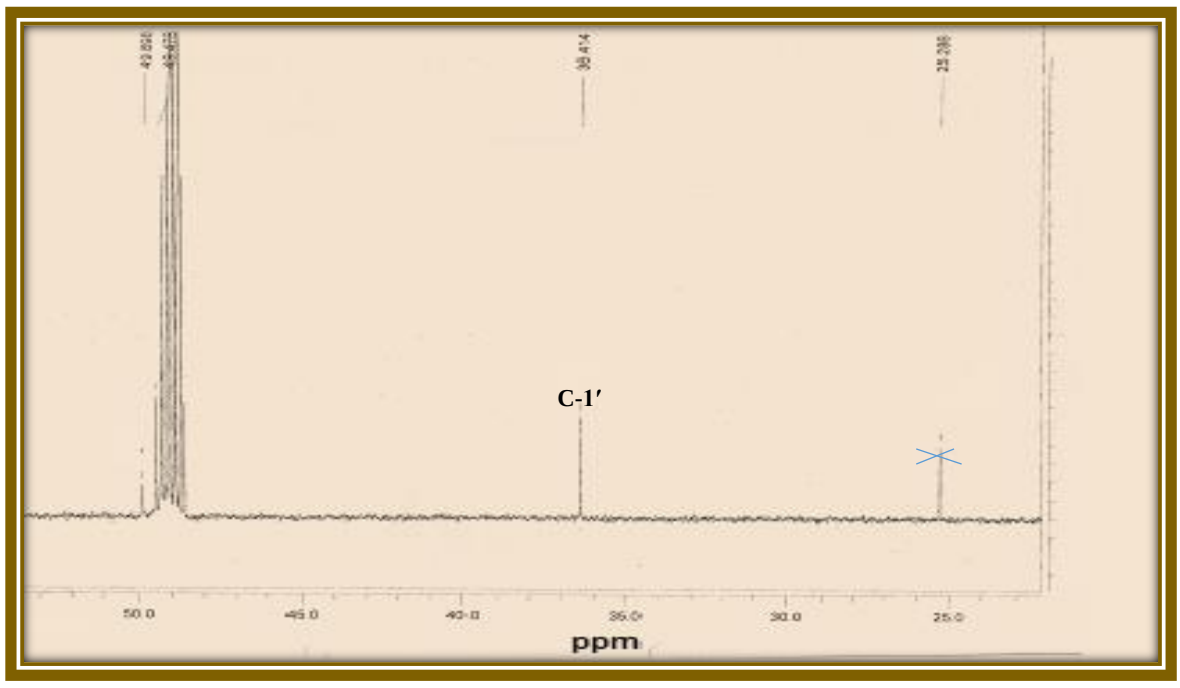


Figure III.77: ^{13}C NMR spectrum of compound 12

At this point, compound **12** was identified as osmanthuside, previously isolated from plants like *Tabebuia impetiginosa* [198] and *Fraxinus sieboldiana* [199]. The spectroscopic data (Table III.12) were identical with the literature [200].

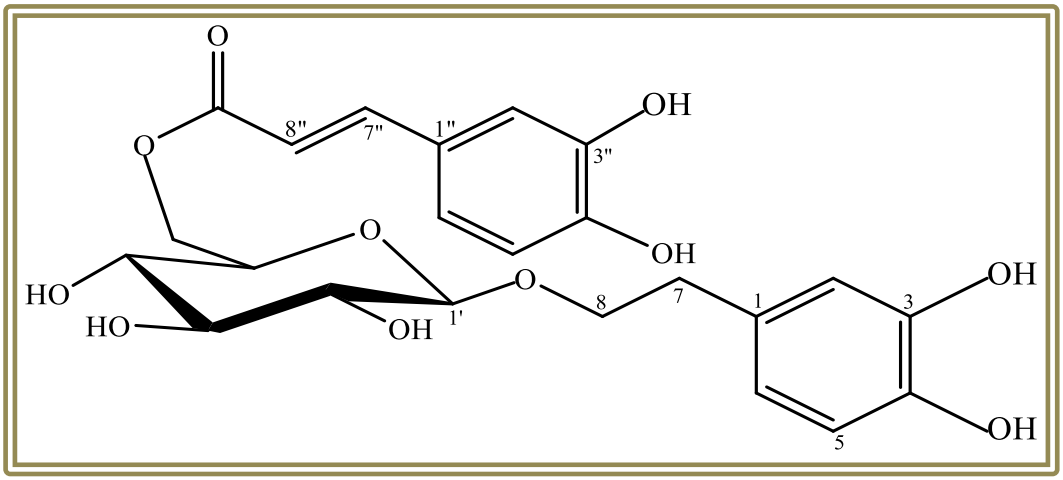
Table III.12: **^{1}H (600.11 MHz) and ^{13}C (150.91 MHz) data of osmanthuside 12 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H	δ_C
Aglucone		
1	-	156.8
2	6.69 *d* (8.4)	116.2
3	7.07 *d* (8.4)	131.0
4	-	130.7
5	7.07 *d* (8.4)	131.0
6	6.69 *d* (8.4)	116.2
1′	2.84 (m)	36.4
H_a-2′	3.98**	72.3
H_b-2′	3.70 *ddd* (6.6/8.1/9.6)	
Glucose		
1″	4.28 *d* (7.8)	104.4
2″	3.18 *dd* (7.8/9.0)	74.9
3″	3.34 *d* (9.0)	77.9
4″	3.28 *d* (9.6)	71.7
5″	3.40 *ddd* (1.9/6.1/9.6)	76.8
H_a-6″	3.99**	68.7
H_b-6″	3.60 *dd* (6.3/11.1)	
Apiose		
1‴	5.00 *d* (2.4)	111.0
2‴	3.90 *d* (2.4)	77.9
3‴	-	80.6
H_a-4‴	3.96 *d* (9.6)	75.1
H_b 4‴	3.76 *d* (9.6)	
5‴	3.57 (s)	65.5

* Assignments were established by direct comparison with the literature.

** The signals are overlapped

III.4.3.5.4 Structure elucidation of compound 13



Calceolarioside B

Compound **13** was isolated as an amorphous powder from the bark. The inspection of the ^{1}H NMR spectrum (Figure III.78) revealed the common key features suggestive of a phenylethanoid: signals (δ 6.4-6.7) for an aromatic ABX spin system and the presence of two mutually coupled methylene groups (δ 2.8-4.0) arising from a 3, 4-dihydroxyphenethyl moiety, and one anomeric proton assignable to glucosyl moiety.

As stated above the signals for ABX aromatic protons (δ 6.44, 6.55, 6.59), oxygenated methylene protons (δ 3.61, 3.87) and methylene protons (δ 2.70) were assigned to the 3, 4-dihydroxyphenylethanol moiety. Another set of signals of an aromatic ABX system (δ 6.68, 6.79 and 6.95) and a trans–olefinic protons (δ 6.20 and 7.46, each d, J = 15.9 Hz) suggested the presence of a (E)-caffeoyl moiety. The ^{1}H NMR spectrum also showed the presence of one anomeric proton detected at δ_H 4.25 (1H, d, J = 7.6 Hz) which is characteristic of a β-glucopyranosyl unit.

The comparison of these spectral data (Table III.13) with the literature [201], taking into account minor differences due to the presence of impurity, led us to identify compound **13** as calceolarioside B, previously isolated from *Fraxinus ornus* [54] and *Phlomis umbrosa* [202].

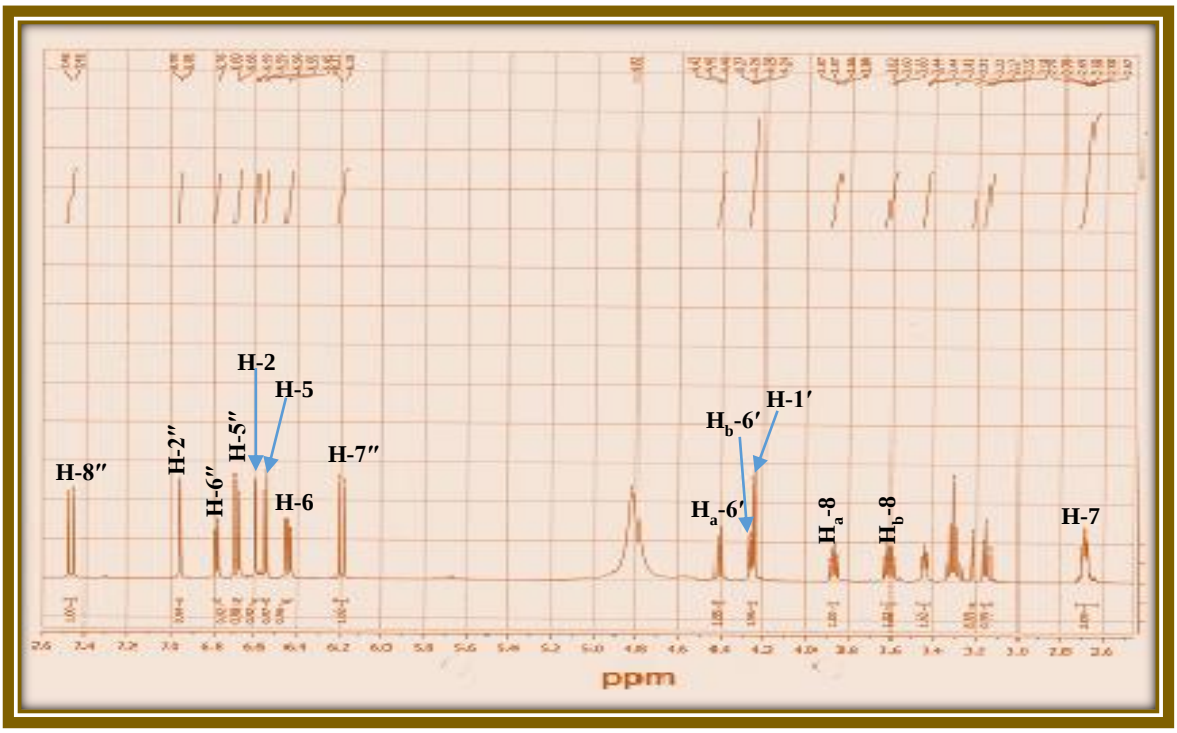


Figure III.78: ^{1}H NMR spectrum of compound 13

Table III.13: ^{1}H NMR (600.11 MHz) data of calceolarioside 13 (CD_3OD; δ in ppm; *J* in Hz)*

C/H	δ_H
Phenethyl alcohol	
1	-
2	6.59 *d* (1.9)
3	-
4	-
5	6.55 *d* (8.0)
6	6.44 *dd* (1.9/8.0)
7	2.70 (m)
H_a-8	3.87 *dd* (8.4/16.4)
H_b-8	3.61 *dd* (8.8/16.4)
Glucose	
1′	4.25 *d* (7.6)
2′	3.15-3.50[a]
3′	3.15-3.50[a]

Table III.13: continued.

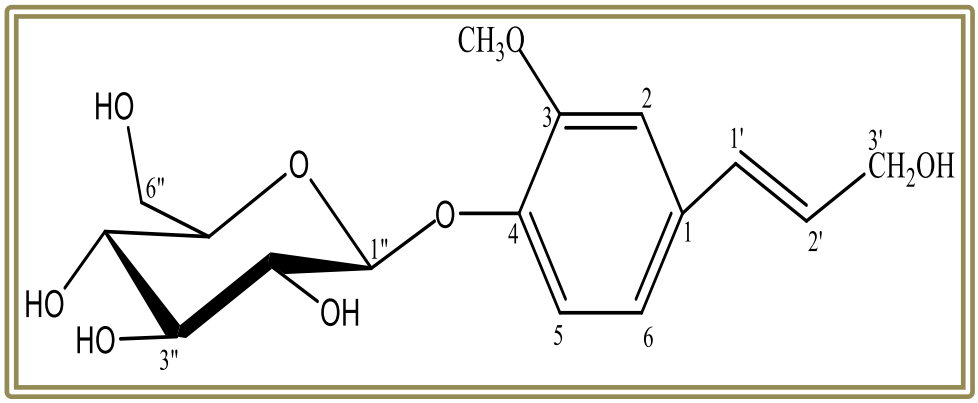
C/H	δ_H
4′	3.15-3.50[a]
5′	3.15-3.50[a]
H_a-6′	4.41 *dd* (1.9/11.9)
H_b-6′	4.26 *dd* (6.7/11.9)
Caffeoyl	
1″	-
2″	6.95 *d* (1.9)
3″	-
4″	-
5″	6.68 *d* (8.2)
6″	6.79 *dd* (1.9/8.2)
7″	6.20 *d* (15.9)
H-8″	7.46 *d* (15.9)

*Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.6 Identification of phenylpropanoids

III.4.3.6.1 Structure elucidation of compound 14



Coniferin

Compound **14** was isolated from the bark as an amorphous powder. The ^{1}H NMR spectrum (Figure III.79) exhibited the characteristic signals of aromatic protons (δ_H 6.28 -7.10) as well as an isolated double doublet at δ_H 4.21 (2H, dd, J = 5.7, 1.5 Hz), an anomeric proton signal at δ_H 4.88 (1H, d, J = 7.33 Hz) and overlapped proton signals of sugar residue (δ_H 3.33-3.90), altogether suggested the chemical structure of compound **14** as a glucosylated phenolic compound. Moreover, the observed singlet signal at δ_H 3.87 suggested the presence of an aromatic methoxyl group.

The spectroscopic data (Table III.14) of compound **14** were consistent with the common phenypropanoid glucoside coniferin [203]. Coniferin is a glycoside which has been firstly isolated from coniferous plant species, while it has been isolated later from a variety of plant species mainly some *citrus* and *asparagus* species [204, 205]. Coniferin has been reported to show different pharmacological effects including anti-cough and anti-asthmatic properties [206].

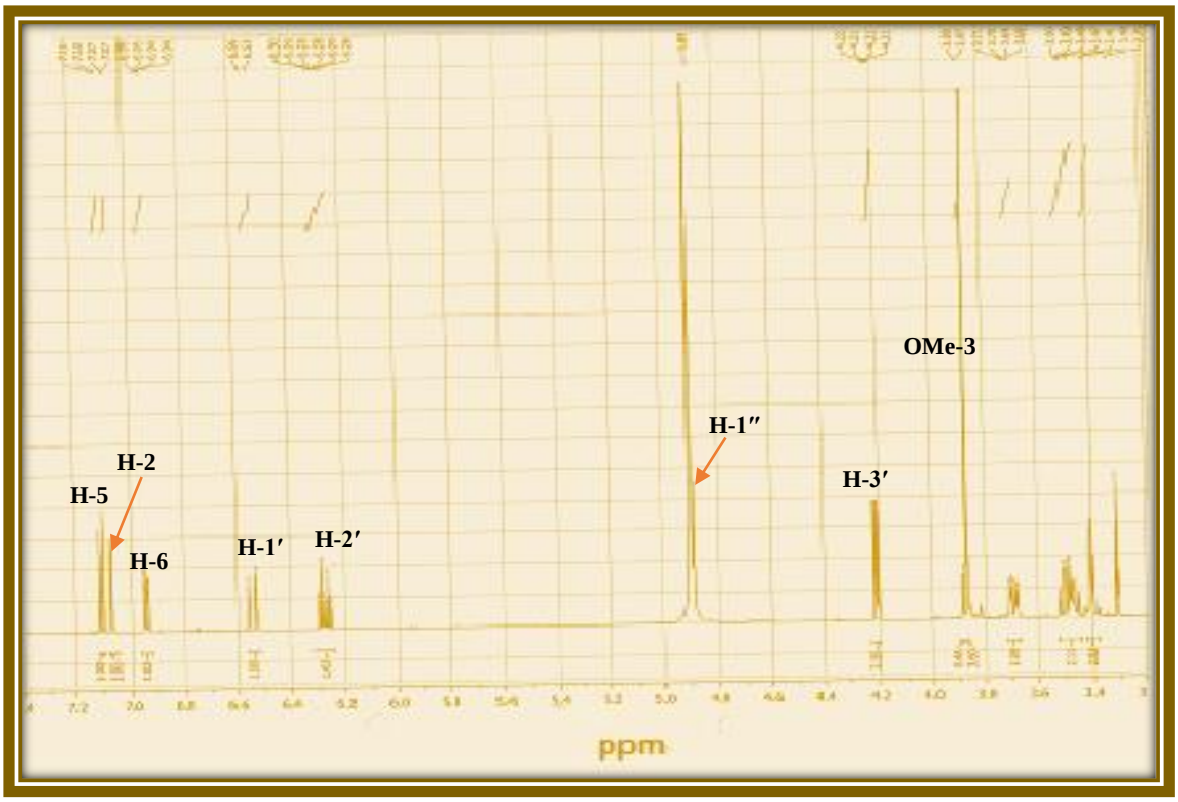


Figure III.79: ^{1}H NMR spectrum of compound 14

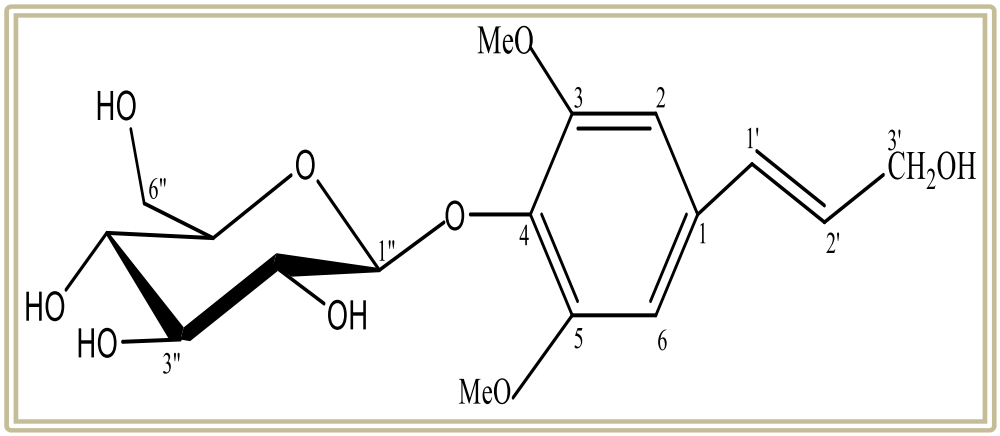
Table III.14: **^{1}H NMR (600.11 MHz) data of coniferin 14 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	7.07 *d* (*1.9*)
3	-
OMe-3	3.87 (s)
4	-
5	7.10 *d* (*8.4*)
6	6.95 *dd* (*1.9/8.4*)
1′	6.54 *d* (*15.9*)
2′	6.28 *dt* (*5.7/15.9*)
3′	4.21 *dd* (1.2/*5.7*)
1″	4.88 *d* (*7.33*)
2″	3.33-3.90[a]
3″	3.33-3.90[a]
4″	3.33-3.90[a]
5″	3.33-3.90[a]
6″	3.33-3.90[a]

*Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.6.2 Structure elucidation of compound 15



Syringin

Compound **15** was obtained as an amorphous powder from the bark. In the ^{1}H NMR spectrum (Figure III.80), displayed a singlet signal resonating at δ_H 6.82 (2H), characteristic of a symmetrically substituted aromatic ring and two signals for a trans-olefinic protons at δ_H 6.57 (1H, d, J = 15.9 Hz) and 6.38 (1H, dt, J = 5.7, 15.9 Hz), corresponding to H-1′ and H-2′, respectively. Also, the ^{1}H NMR spectrum showed a double doublet signals appearing at δ_H 4.26 (2H, dd, J = 1.2, 5.7 Hz), corresponding to H-3′. The presence of a β-glucopyranoside was confirmed by the presence of doublet signal at δ_H 4.95 with a coupling constant J = 7.2 Hz.

The spectral data of compound **15** (Table III.15) was in full agreement with those reported for syringing [207]. Syringin was isolated previously from *Osmanthus asiaticus* [208], *Centaurea crocodylium* [209], *Chartolepsis pterocaulea* [210], *Grossheimia macrocephala* [211]. The pharmacological properties of syringin includes anti-diabetic effect, anti-inflammatory potential, anti-nociceptive action, anti-allergic [212].

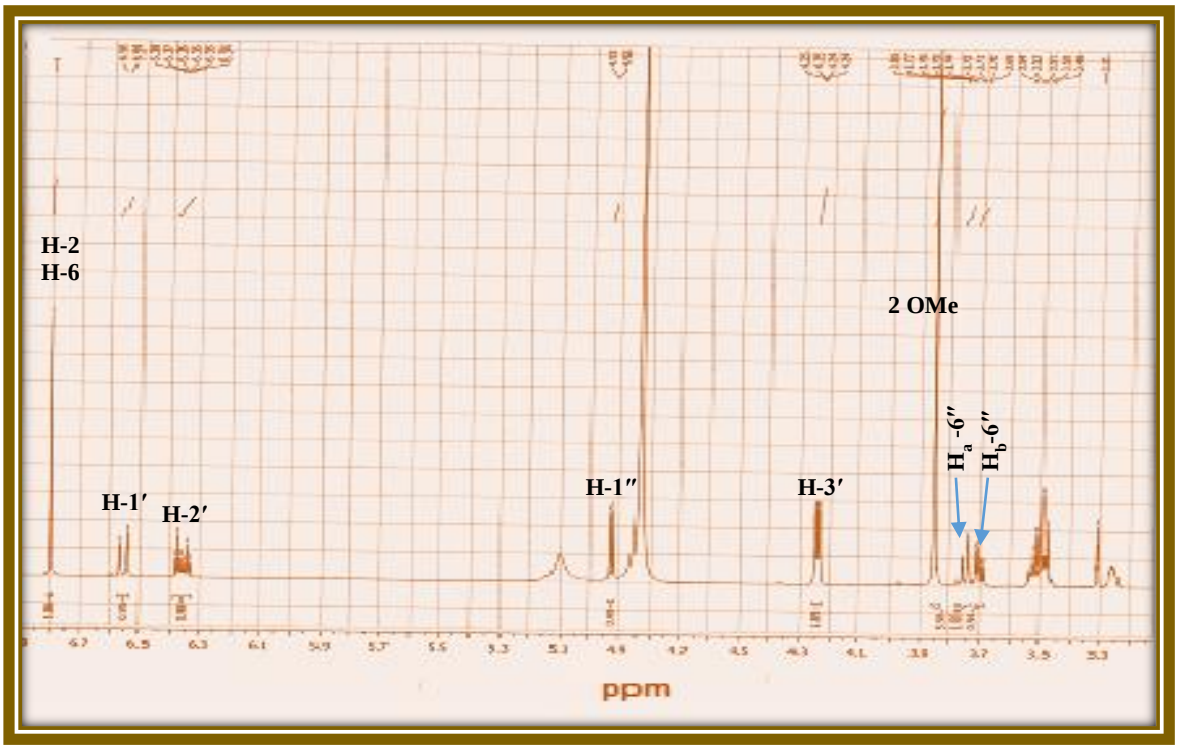


Figure III.80: ^{1}H NMR spectrum of compound 15

Table III.15: ^{1}H NMR (600.11 MHz) data of syringin 15 (CD_3OD; δ in ppm; *J* in Hz)*

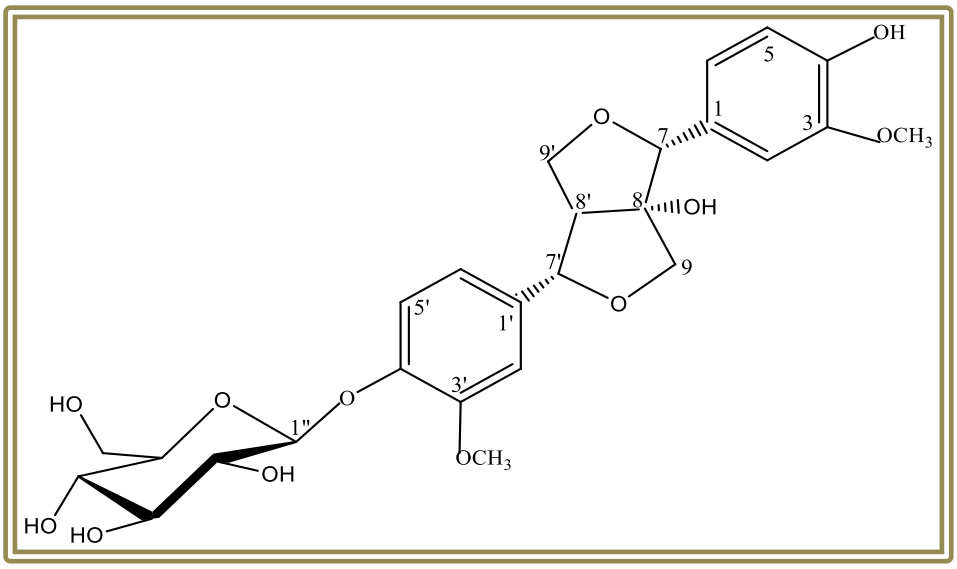
C/H	δ_H
1	-
2	6.82 (*s*)
3	-
OMe-3	3.87 (s)
4	-
5	-
OMe-5	3.87 (s)
6	6.82 (*s*)
1′	6.57 *d* (*15.9*)
2′	6.38 *dt* (*5.7/15.9*)
3′	4.26 *dd* (*1.2/5.7*)
1″	4.95 *d* (7.2)
2″	3.20-3.50[a]
3″	3.20-3.50[a]
4″	3.20-3.50[a]
5″	3.20-3.50[a]
H_a-6″	3.78 *dd* (*2.4/12.3*)
H_b-6″	3.72 *dd* (*4.8/12.3*)

*Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.7 Identification of lignans

III.4.3.7.1 Structure elucidation of compound 16



8-Hydroxypinoresinol-4′-O-β-D-glucoside

Compound **16** was obtained as an amorphous power from the bark. The ^{1}H NMR spectrum (Figure III.81) revealed the presence of two ABX type coupling patterns, thus indicating the presence of two 1, 3, 4-trisubstituted phenyl groups appearing at δ_H 7.12 (d, J = 1.8 Hz, H-2), 7.16 (d, J = 8.4 Hz, H-5), and 6.96 (dd, J = 1.8, 8.4 Hz, H-6); and at δ_H 7.05 (d, J = 1.8 Hz, H-2′), 6.78 (d, J = 8.4 Hz, H-5′), and 6.87 (dd, J = 1.8, 8.4 Hz, H-6′).

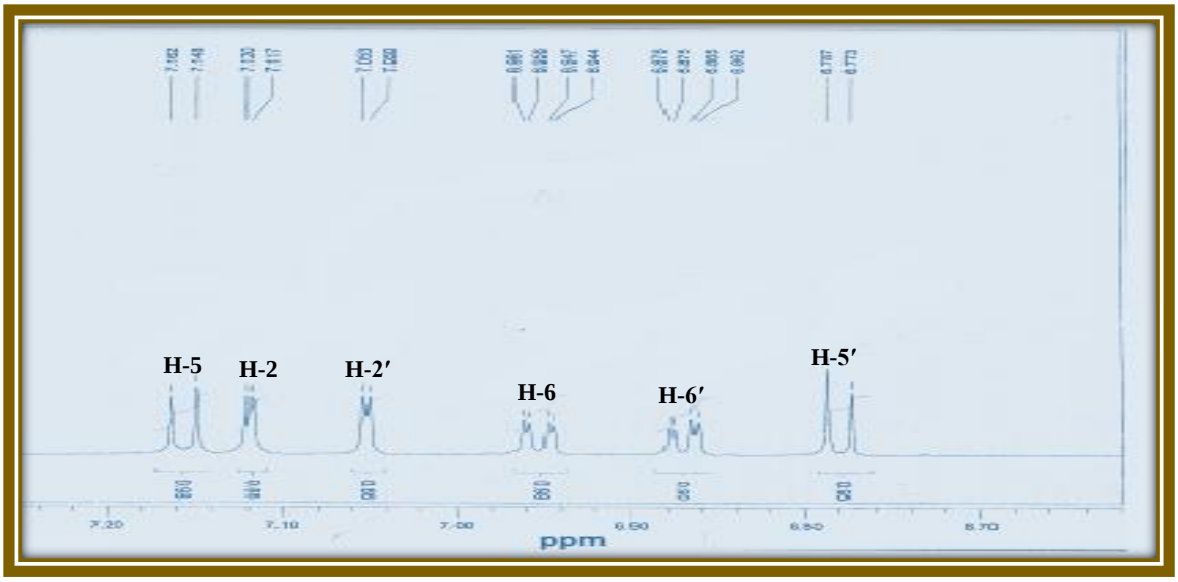


Figure III.81: ^{1}H NMR spectrum of compound 16

Also, the examination of the ^{1}H NMR spectrum (Figure III.82) showed:

- Two benzylic oxymethine protons resonating at δ_H 4.72 (1H, s) and 4.85 (1H, d, J = 5.4 Hz) corresponding to H-7 and H-7′, respectively.
- Two methylenes bearing an oxygen function at δ_H 3.87 (1H, d, J = 9.0 Hz, H_a-9), 4.06 (1H, d, J = 9.0 Hz, H_b-9), 3.78 (1H, dd, J = 6.3, 8.9 Hz, H_a-9′), and 4.47 (1H, t, J = 8.9 Hz, H_b-9′).
- Two O-methyl singlet signals detected at δ_H 3.88 (3H, s) and 3.86 (3H, s) corresponding to OMe-3 and OMe-3′, respectively.
- An anomeric proton at δ_H 4.88 with a coupling constant J = 7.5 Hz gave clear evidence on the presence of a β- glucoside unit.
- Signals in the δ_H 3.34-4.88 region attributable to a sugar moiety.

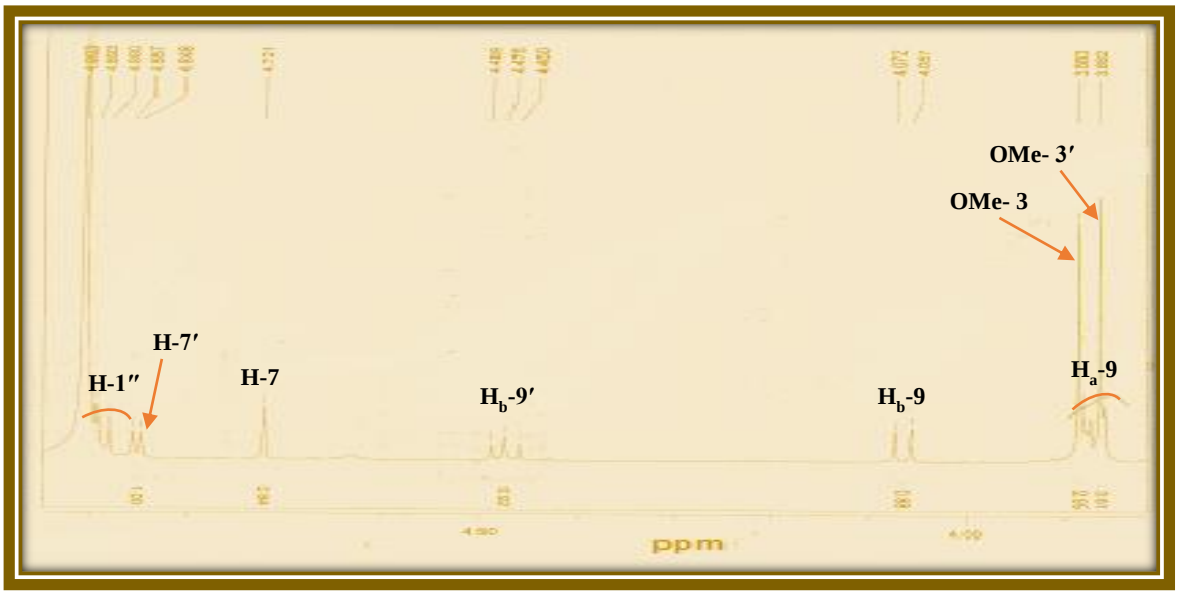


Figure III.82: ^{1}H NMR spectrum of compound 16

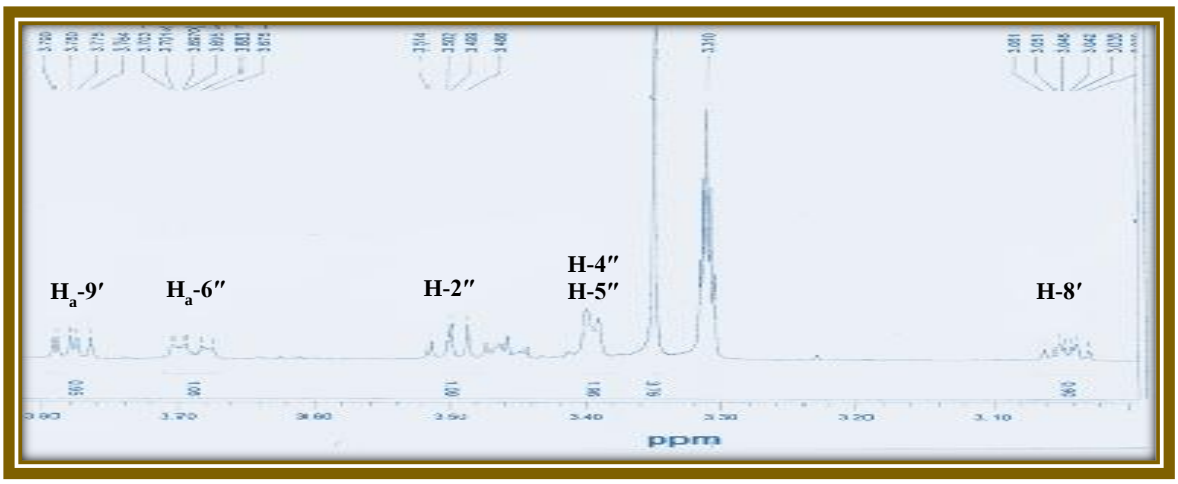


Figure III.83: ^{1}H NMR spectrum of compound 16

Comparison of spectral data (Table III.16) of glucoside **16** with the literature [213] led us to identify it as 8-Hydroxypinoresinol-4′-O-β-D-glucoside, previously isolated from plants like *Valeriana prionophylla* [214], *Valeriana officinalis* [215], *Eucommia ulmoides* [216] and for the first time from Fraxinus species.

Table III.16: **^{1}H NMR (600.11 MHz) data of 8-hydroxypinoresinol-4′-O-β-D-glucoside 16 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	7.12 *d* (*1.8*)
3	-
OMe-3	3.88 (s)
4	-
5	7.16 *d* (*8.4*)
6	6.96 dd (*1.8/8.4*)
7	4.72 (*s*)
8	-
H_a-9	3.87 *d* (*9.0*)
H_b-9	4.06 *d* (*9.0*)
1′	-
2′	7.05 *d* (*1.8*)
3′	-
OMe-3′	3.86 (s)
4′	-
5′	6.78 *d* (*8.4*)
6′	6.87 *dd* (*1.8/8.4*)
7′	4.85 *d* (*5.4*)
8′	3.05 (*m*)
H_a-9′	3.78 *dd* (*6.3/9.2*)
H_b-9′	4.47 *d* (*8.9*)
1″	4.88 *d* (*7.5*)

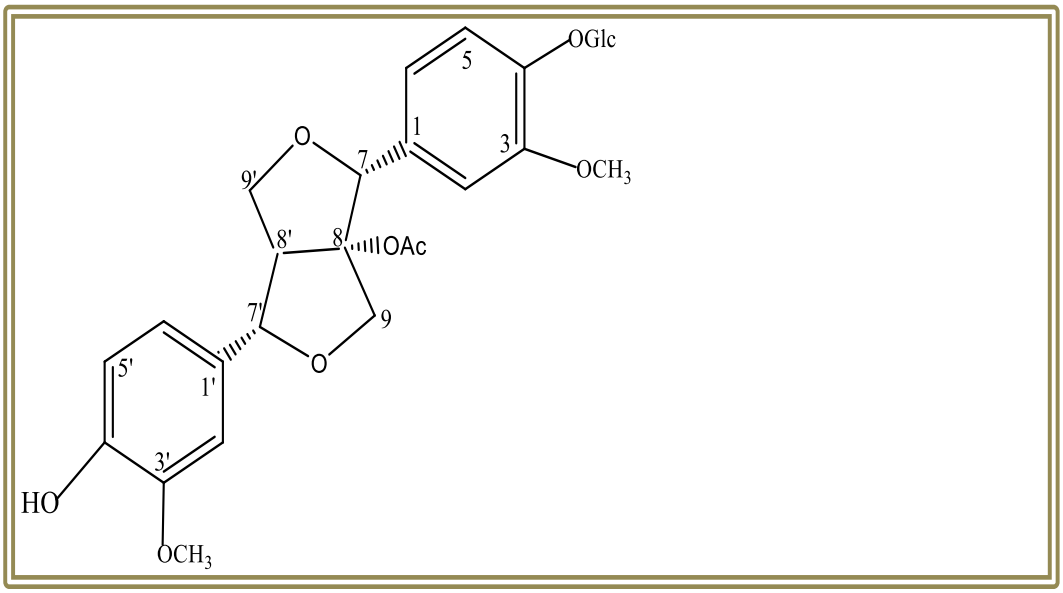
Table III.16: **continued.**

2″	3.50 *dd* (*7.5/9.3*)
3″	3.34-4.88[a]
4″	3.40[a]
5″	3.40[a]
6″	3.70 *dd* (*4.8/12.0*)

*Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.4.3.7.2 Structure elucidation of compound 17



8-Acetoxypinoresinol-4-O-β-D-glucoside

Compound **17** was obtained as colorless needles, from the bark. The ^{1}H NMR spectrum (Figure III. 84) exhibited signals of two 1, 3, 4-trisubstituted phenyl groups at δ_H 7.01 (d, J = 1.9 Hz, H-2), 7.13 (d, J = 8.4 Hz, H-5), and 6.99 (dd, J = 1.9, 8.4 Hz, H-6); and at δ_H 6.99 (d, J = 1.9 Hz, H-2′), 6.80 (d, J = 8.2 Hz, H-5′), and 6.87 (dd, J = 1.9, 8.2 Hz, H-6′).

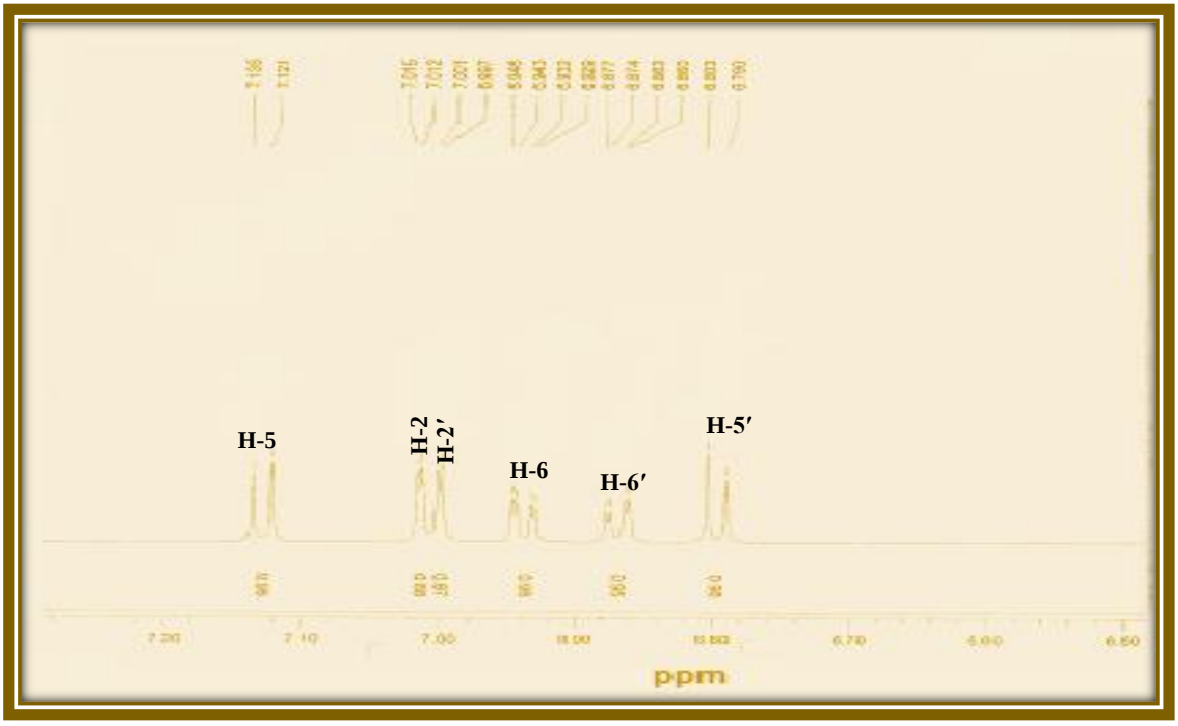


Figure III.84: ^{1}H NMR spectrum of compound 17

Beside these signals, the ^{1}H NMR spectra (Figure III.85 and III.86) showed:

- Two benzylic oxymethine protons resonating at δ_H 5.03 (1H, s) and 4.78 (1H, d, J = 4.8 Hz) corresponding to H-7 and H-7′, respectively.
- Two methylenes bearing an oxygen function at δ_H 4.26 (1H, d, J = 10.7 Hz, H_a-9), 4.32 (1H, d, J = 10.7 Hz, H_b-9), 3.76 (1H, dd, J = 5.4, 9.0 Hz, H_a-9′), and 4.46 (1H, dd, J = 7.8, 9.0 Hz, H_b-9′).
- Two methoxyl singlet signals detected at δ_H 3.85 (3H, s) and 3.86 (3H, s) corresponding to OMe-3 and OMe-3′, respectively.
- An anomeric proton at δ_H 4.91 with a coupling constant J = 7.8 Hz indicating the presence of a β- glucoside unit.
- Signals in the δ_H 3.34-4.91 region attributable to a sugar moiety.

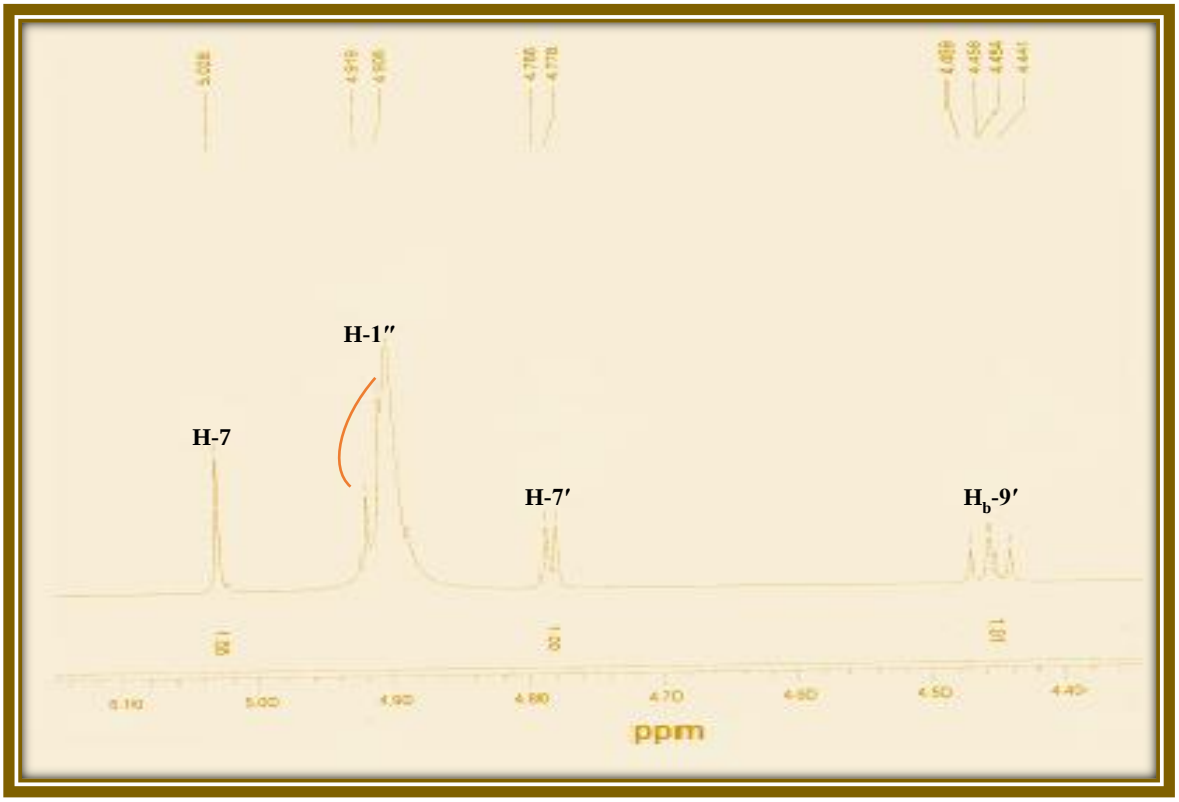


Figure III.85: ^{1}H NMR spectrum of compound 17

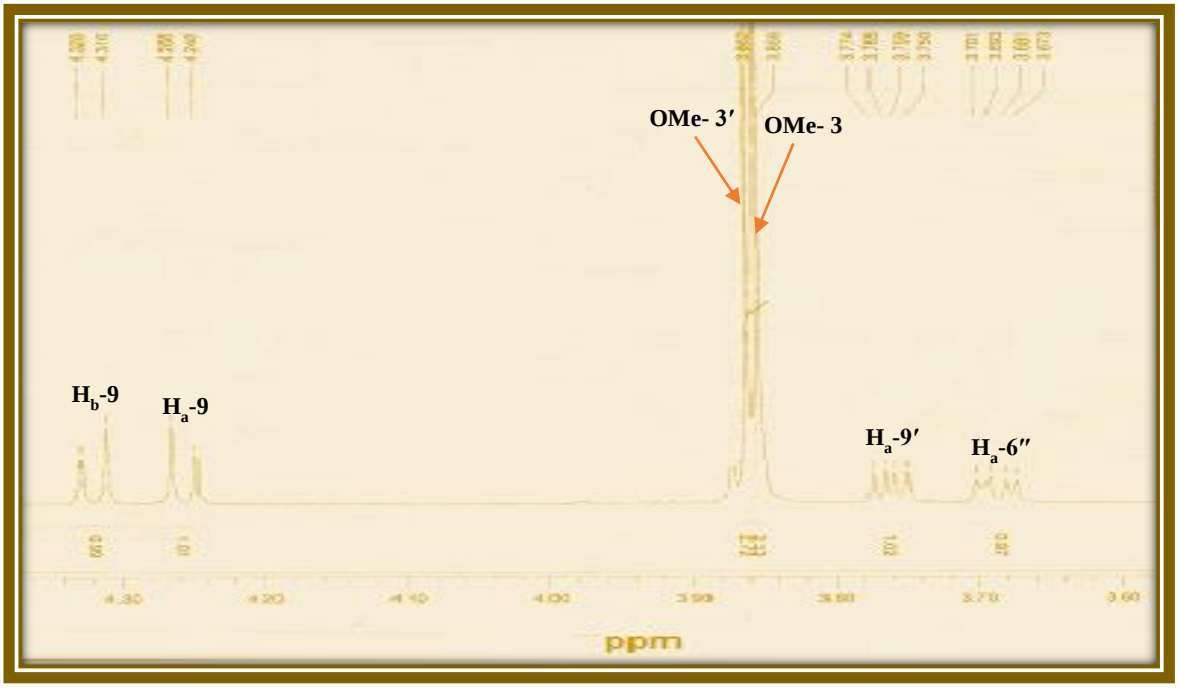


Figure III.86: ^{1}H NMR spectrum of compound 17

Additionally, the ^{1}H and ^{13}C NMR spectra (Figure III.87 and III.90), suggested the presence of one alcoholic acetoxyl group, which was confirmed by the appearance of a singlet signal at δ_H 1.68 (3H, s, δ_C 20.9, $\underline{CH_3}CO$).

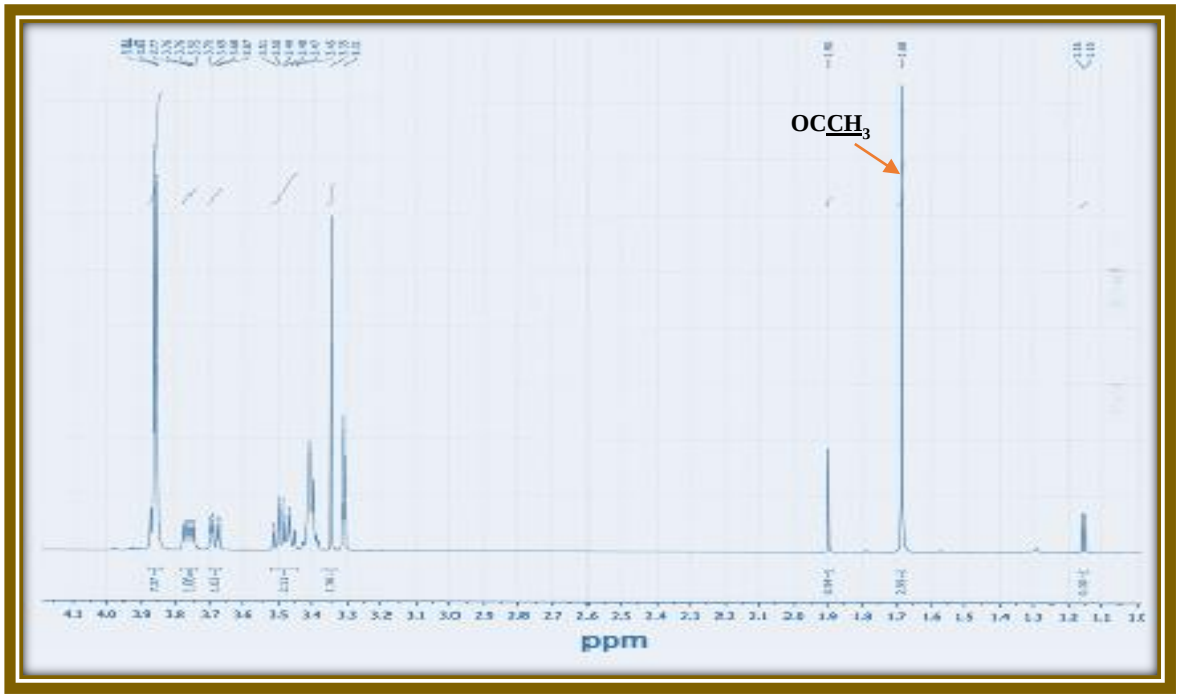


Figure III.87: ^{1}H NMR spectrum of compound 17

The ^{13}C NMR sepctra (Figure III.88, III.89 and III.90) showed 28 signals corresponding to the signals of 12 carbons for tow aromatic rings, two methylenes (δ 75.7 and 71.3, C-9 and C-9′), three methines (δ 60.1, C-8′, 88.6, C-7, 86.7, C-7′), a tertiary alcoholic carbon (δ 98.8, C-8), two methoxy carbons (δ 56.8 and 56.4, OMe-3 and OMe-3′), a carbonyl carbon (δ 171.2, $CH_3\underline{CO}$), and six carbon resonances corresponding to a sugar moiety.

By comparing the ^{13}C NMR spectrum of compound **17** with that of the known lignan, 8-hydroxypinoresinol-4′-O-β-D-glucoside (**16**) [213], it was clearly confirmed that glucoside **17** contains a 2, 6-diaryl-3, 7-dioxabicyclo [3, 3, 0] octane ring.

The appreciable differences of chemical shifts for the 8 and 8′ carbon atoms, at 92.7ppm and 62.4 ppm in **16**, 98.8 ppm and 60.1 ppm in **17**, respectively, also indicated that one alcoholic acetoxyl group of **17** is attached at the 8 carbon atom.

The aromatic carbon shifts of compound **17** suggested that the aryl groups are 3-methoxy-4-hydroxyphenyl units, and that one of them is linked to a β-D- glucosyl moiety.

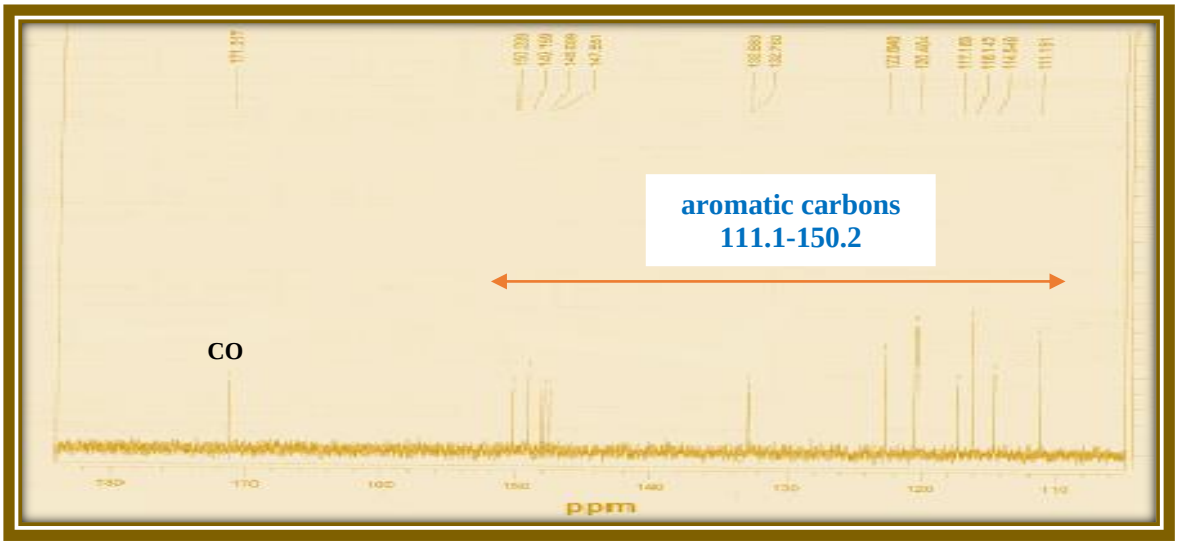


Figure III.88: ^{13}C NMR spectrum of compound 17

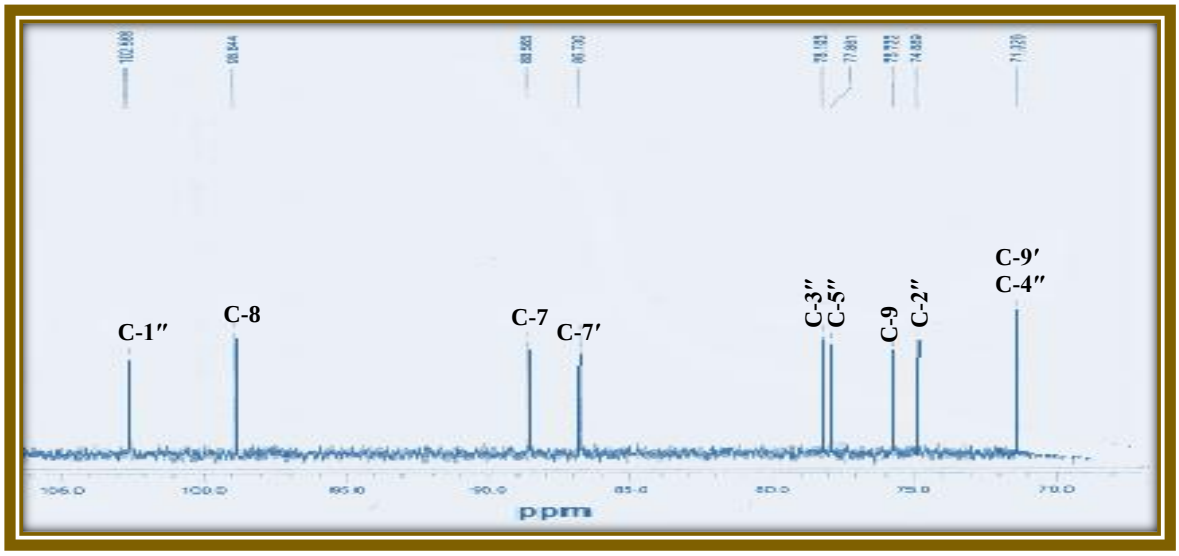


Figure III.89: ^{13}C NMR spectrum of compound 17

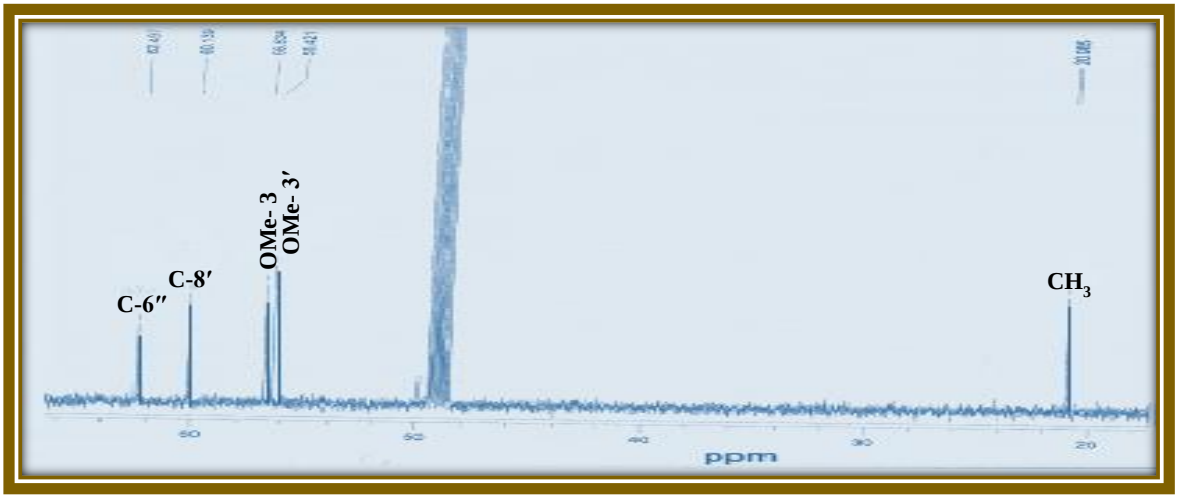


Figure III.90: ^{13}C NMR spectrum of compound 17

The upfield shift of the 1′ carbon atom of the 3′-methoxy, 4′-hydroxyphenyl unit is 4.5 ppm between **16** and **17**, indicating the presence of a free phenolic hydroxyl group at the 4′ carbon atom of **17**. The glucosidic linkage of **17** was determined by comparative study of ^{13}C NMR data of glucoside **17** and glucoside **16** (Table III.17)**.** The observed downfield shift of para- correlated C-1 (+5.5 ppm), in comparison with that observed in **16**, was indicative of glucosidation at C-4.

At this point, compound **17** was identified as 8-Acetoxypinoresinol-4-O-β-D-glucoside, previously isolated from *Olea europaea* [56] and for the first time from Fraxinus species. The spectroscopic data (Table III.17 and III.18) were identical with the literature [217]. Thus, inferring the identity also in the stereochemical data aspects as well as in the nature of the sugar moiety that was D-glucose.

Table III.17: **^{13}C NMR (150.91 MHz) data of componunds 17 and 16 in CD_3OD (δ in ppm; *J* in Hz)***

C/H	**17**	**16** [213]
1	132.8	127.3
2	114.5	112.7
3	150.2	149.5
OMe-3	56.8	56.3
4	148.0	147.7
5	116.1	116.4
6	122.6	121.7
7	88.6	89.4
8	98.8	92.7
$\underline{CH_3}CO$	20.9	-
$CH_3\underline{CO}$	171.2	-
9	75.7	76.3
1′	132.7	137.4
2′	111.2	112.0
3′	149.1	151.0
OMe-3′	56.4	56.7
4′	147.5	147.6
5′	117.1	118.1
6	120.4	120.2

Table III.17: **Continued.**

7′	86.7	87.4
8′	60.1	62.4
9′	71.3	72.0
1″	102.6	102.9
2″	74.9	74.9
3″	78.2	77.8
4″	71.3	71.4
5″	77.9	78.2
6″	62.5	62.5

Table III.18: **^{1}H NMR (600.11 MHz) data of acetoxypinoresinol-4-O-β-D-glucoside 17 (CD_3OD; δ in ppm; *J* in Hz)***

C/H	δ_H
1	-
2	7.01 *d* (*1.9*)
3	-
OMe-3	3.85 (s)
4	-
5	7.13 *d* (*8.4*)
6	6.94 dd (*1.9/8.4*)
7	5.03 (*s*)
8	-
H_a-9	4.26 *d* (*10.8*)
H_b-9	4.32 *d* (*10.8*)
$\underline{CH_3}CO$	1.68 (s)
1′	-
2′	6.99 d *(1.9)*
3′	-
OMe-3′	3.86 (s)
4′	-
5′	6.80 *d (8.2)*

Table III.18: **Continued.**

6′	6.87 *dd (1.9/8.2)*
7′	4.78 d (4.8)
8′	
H_a-9′	3.76 *dd (5.4/9.0)*
H_b-9′	4.46 *dd (7.8/9.0)*
1″	4.91 d (7.8)
2″	3.34-4.91[a]
3″	3.34-4.91[a]
4″	3.34-4.91[a]
5″	3.34-4.91[a]
6″	3.69 *dd (4.8/12.0)*

*Assignments were established by direct comparison with the literature.

[a] overlapped with other signals.

III.5 Anti-oxidant activity

Numerous techniques are available to evaluate the anti-oxidant activity of pure compounds or complex mixtures (as in the case of plant extracts). Herein, the methanolic extracts of the Algerian plants (*Fraxinus xanthoxyloides* and *Fraxinus angustifolia*), belonging to the Oleaceae family, were screened for their anti-oxidant activity by using two different tests in vitro: DPPH free radicals scavenging [218] and ABTS radical cation decolorization assay [219]. The results are expressed in percent of effectivity for 200 µg/mL of both extract as summarized in Table III.19. The studied plant species exhibited similar activity. Whereas, for the same plant, the studied plant parts (leaves and bark) showed differential activity, with *F. angustifolia* and *F. xanthoxyloides* bark as the extracts with the strongest activity in the two performed assays.

F. angustifolia and *F. xanthoxyloides* leaves showed slight difference DPPH scavenging activity (% inhibitions were 34.2 and 51.0 for *F. angustifolia* and *F. xanthoxyloide,* respectively) and a similar ABTS activity (% inhibitions were 27.1 and 28.6 *F. angustifolia* and *F. xanthoxyloides*, respectively).

In comparison to the leaves, the bark extracts of both species showed strong anti-oxidative activity with the DPPH scavenging *(*% inhibitions were 76.5 and 74.3 for *F. angustifolia* and *F. xanthoxyloides,* respectively) and ABTS activity (% inhibitions were 42.3 and 42.8 for *F. angustifolia* and *F. xanthoxyloides,* respectively).

Regarding the antioxidant activity developed by the positive controls, these plant extracts, used at relatively high concentrations, exhibited weak activity.

The weak anti-oxidative activity of the methanolic extracts of both *F. angustifolia* and *F. xanthoxyloides*, namely using the DPPH scavenging, was also reported for F. *chinensis* collected in China [220].

The activity shown in both assays demonstrates the electron donor properties of the molecules present in the extracts, particularly for neutralizing free radicals by forming stable products. Such activity may be provided by the presence of electron-donating or withdrawing groups at the aromatic system and glycosylation in the 7th position which strongly influence the redox potential of phenols [221].

Table III.19: **Anti-oxidant activity of the extracts of the Algerian plant species *F. angustifolia* and *F. xanthoxyloides*.**

	DPPH	ABTS
FAB	76,5 ± 1,9	42,3 ± 0,9
FAL	34,2 ± 1,4	27,1 ± 0,2
FXB	74,3 ± 2,2	42,8 ± 1,6
FXL	51,0 ± 1,1	28,6 ± 0,7

DPPH: Scavenging of DPPH

Results expressed as: % of effectivity at 200 µg/mL of extract.

ABTS: Scavenging of ABTS

Results expressed as: % of effectivity at 200 µg/mL of extract.

Considering the high levels of anti-oxidant activity for some of the studied extracts, a preliminary analysis on the bioactive compounds present in the extracts was also done. Given the polar nature of the extracts, the performed analysis was oriented for hydrophilic compounds, particularly phenolics. Furthermore, the anti-oxidant activity of plant species is often related to their phenolic content, since these compounds are known for their redox properties (as reducing agents, hydrogen donors, singlet oxygen quenchers or metallic elements chelators). In fact, the presence of phenols and many other groups of phenolic compounds (with different concentrations) in the plant extracts is a determining factor to prevent lipid oxidation [222], which constitutes one of the strongest types of anti-oxidant activity verified among the studied species. Plant phenolics can delay the onset of lipid oxidation and decomposition of hydroperoxides in food products as well as in living tissues [221].

The strong anti-axidative activity exhibited by *F. angustifolia* bark was explained with the phenolic and flavonoid content of the crude extract (TPC: 137.8 mg GAE/g of extract, TFE: 10.5 mg QE/g of extract). Contrariwise, the lowest level of total phenol was quantified in F. *xanthoxyloides* species (FXB: 22.1 mg GAE/g of extract; FXL: 20.6 mg GAE/g of extract). The bark of late species showed also minimum amounts of flavonoids (2.4 mg QE/g of extract), together with the leaves of *F. angustifolia* (5.9 mg QE/g of extract).

Table III.20: **Bioactive compounds quantified in the extracts prepared from the Algerian plant species *F. angustifolia* and *F. xanthoxyloides*.**

	TPC			TFE		
FAB	137,8	±	0,0	10,5	±	0,1
FAL	143,6	±	0,0	5,9	±	1,2
FXB	22,1	±	0,8	2,4	±	2,7
FXL	20,6	±	1,6	22,2	±	2,1

TPC: Total Phenolic Content

Results expressed as: mg of gallic acid equivalent per gram of extract (mg GAE/g of extract).

TFE: Total Flavonoid Evaluation

Results expressed as: mg of quercetin equivalent per gram of extract (mg QE/g of extract).

Overall, the studied species shown great heterogeneity regarding the evaluated parameters. Data in Table III19 and III.20 might be used to drawn some specific conclusions, but the selection of the best plants considering the contribution of all assayed parameters simultaneously might only be achieved using a more advanced statistical analysis tool.

CHAPTER IV

Experimental section

IV.1 Materials and methods

IV.1.1 Plant material

The stem bark and the leaves of *Fraxinus xanthoxyloides* were collected in June 2014 from the region of Khenchela, Algeria. The plant was kindly identified by Prof. Mohamed Kaabache, Ferhat Abbas Setif 1 University, Algeria. The leaves and the stem bark were separated and dried by air and then powdered.

IV.1.2 Chromatographic methods of analysis (analytical and preparative)

IV.1.2.1 Thin layer chromatography (TLC)

In the following experiments, analytical thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). Spots were detected under UV light first, then by spraying with 20% (v/v) H_2SO_4 in ethanol solution and heating. Preparative thin layer chromatography (PTLC) was performed on pre-coated plates 60 F_{254} 0.5x0.25 mm. All solvents used for chromatographic purposes were of analytical grade.

IV.1.2.2 Column chromatography (CC)

Column chromatography (CC) on Polyamide 6 (Fluka, Germany), Sephadex LH-20 and Silica gel 60, particle size 0.063-0.200, 70-230 mesh ASTM (Merck, Darmstadt, Germany) as well as Lobar chromatography (Lobar RP-8 and RP-18, Merck, Darmstadt, Germany) were used for separation and purification of the individual compounds.

IV.1.3 Spectroscopy

NMR spectra were acquired on Bruker AVII+ 600 spectrometer (Bruker, Karlsruhe, Germany), ^{1}H-NMR (600.11 MHz), ^{13}C-NMR (150.91 MHz) in D_2O, CD_3OD and DMSO-d_6 purchased from Deutero-GmbH (Kastellaun, Germany), with TMS as internal standard. UV spectra: Heλios Gamma UV spectrophotometer (Thermo Scientific, Bremen, Germany) in MeOH. HR-ESI-MS analysis was performed on a Thermo Scientific Q Exactive Plus (Bremen, Germany) in negative mode.

IV.1.4 Optical rotation

Optical rotation was recorded in MeOH using a Galan-Taylor Prism polarimeter.

IV.1.5 Acid hydrolysis of compound 1

The absolute configuration of the sugar was established using the method of Tanaka et al. [165] with some modifications [223]. Briefly, compound **1** (5 mg) was refluxed with 2 mL of 2N HCl-MeOH (1:1) for 2 h. The reaction mixture was filtered through Diaion HP-20SS followed by subsequent elution with H_2O and MeOH. The water portion was filtered through Amberlite IRC 86 resin and then evaporated to dryness. The dry water eluate was treated with a solution (0.1 mL) of L-cysteine methyl ester in pyridine (5 mg/mL) at 60°C for 1 h. A solution (0.1 mL) of o-tolylisothiocyanate in pyridine (5 mg/mL) was added to the mixture and heated at 60°C for 1 h. The resulting solution was analyzed using HPLC [Purospher STAR RP-18 5 µm column (Merck; 4.6×250 mm) with 25% ACN in 50 mM H_3PO_4, flow rate 1 mL/min, UV detection at 250 nm]. The presence of D-glucose (t_R value of the tolylthiocarbamoyl-thiazolidine derivative was 18.7 min) was found in the residue.

IV.1.6 Anti-oxidant activity

A. Anti-Oxidant capacity of extracts

A.1 Total Phenolic Content (TPC)

Folin–Ciocalteau reagent was first introduced by O. Folin and D. Ciocalteau in 1927. Chemically, it is a hetero polyacid that is phosphomolybdotungstic acid. It produces blue colour with phenolic group [224].

A standard curve of gallic acid in DMSO was made between 100 µg/mL to 2.5 µg/mL final concentration (1 mg/mL to 0.025 mg/mL of initial concentration). In each well were placed 25 µL of sample (extract or gallic acid standard curve point), plus 125 µL of Folin Ciocalteau reagent (10% v/v in H_20) and 100 µL/of sodium bicarbonate 7.5% w/v (250 µL final volume per well). Respective blank made replacing Folin Ciocalteau reagent with 125 µL of H_2O. The plates were incubated for 30 min protected of light and at room temperature. Measures were reading at 765 nm. Total phenolic concentration is expressed in mg gallic acid equivalents per gram of extract.

A.2 Total Flavonoid Evaluation (TFE)

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method [225]. $AlCl_3$ forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, it forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids.

A standard curve of quercetin was made in DMSO between 200 µg/mL to 1.25 µg/mL final concentration in the well (initial concentration from 1 mg/mL at 0.00625 mg/mL). In each well were placed 50 µL of sample (extract or quercetin standard curve point), plus 160 µL of EtOH, 20 µL of $AlCl_3$ (1.8 % w/v of $AlCl_3 \cdot 6\ H_2O$) and 20 µL of sodium acetate (820.3 mg in 100 mL H_2O). Respective blank were performed replacing the $AlCl_3$ and sodium carbonate with 40 µL of H_2O. The plates were incubated for 40 min at room temperature. Measures were reading at 415 nm. The flavonoid concentration is expressed in mg quercetin equivalents per gram of extract.

B. Anti-oxidant scavenging

Antioxidant activity was evaluated by using two different tests: DPPH free radical scavenging activity [226] and ABTS radical cation decolorization assay [219].

B.1 DPPH assay

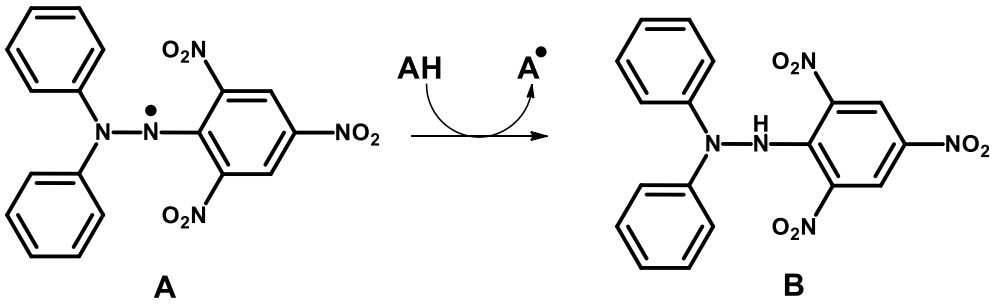


Figure III.1: Reaction of DPPH (A, λ_{max} 517) in presence of an antioxidant reagent (AH).

The reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared freshly just before the assay as follows: 12,4 mg of DPPH in 100 mL of ethanol. In each plate were placed 10 µL of sample solution (extract at 4 mg/mL of initial concentration, 200 µg/mL final concentration in the well) with 190 µL of DPPH solution. Negative (replacing sample with 10 µL of DMSO) and positive (replacing sample with 10 µL of gallic acid at 100 µg/ml initial concentration, final concentration of 5 µg/mL in the well with ~50% of efficacy. Blank of each sample and control were made. The plates were incubated for 30 min at room temperature protected from light. The absorbance was measured at 517 nm. The scavenging were calculated as follows:

$$\%\ DPPH\ Scavenging = \frac{(ODcontrol - ODblank\ control) - (OD\ sample - ODblank\ of\ sample)}{(OD\ control - OD\ blank\ of\ control)} \times 100$$

B.2 ABTS assay

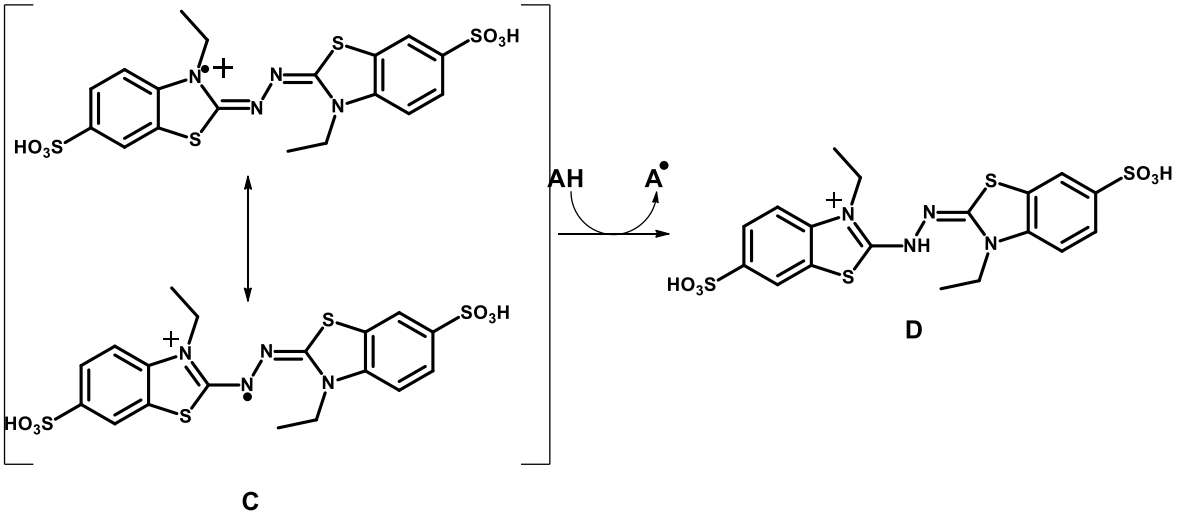


Figure III.2: reaction of ABTS$^{\cdot+}$ (C, λ_{max} 734) in presence of an antioxidant reagent (AH).

The reagent 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), was treated one day before the assay as follows: 10 mL of 7mM of ABTS solution (36.02 mg in 10 mL of H_2O) were mixed with 164 µL of 140 mM of potassium persulfate (37,84 mg in 1 mL of H_2O) and stored overnight (~ 16 h). The final solution after stored, was diluted in distilled H_2O (~1:20) until an absorbance of 0.70±0,02 (100 µL of ABTS solution plus 50 µL of DMSO). In each plate were placed 100 µL of final ABTS solution and 50 µL of sample (extract at 600 µg/mL as initial concentration, 200 µg/mL final concentration in the well). Negative control was measured by replaced 50 µL of sample solution with 50 µL of DMSO. Positive control was carried with 50 µL of 25 µg/mL trolox solution (final concentration in the well ~8 µg/mL with 50 % of efficacy). Blanc of each measured were made. The plates were incubated at room temperature, protected from light for 10 min. The absorbance was measured at 734 nm. The scavenging was calculated as follows:

$$\%\ ABTS\ Scavenging = \frac{(ODcontrol - ODblank\ control) - (OD\ sample - ODblank\ of\ sample)}{(OD\ control - OD\ blank\ of\ control)} \times 100$$

IV.2 Phytochemical study of *Fraxinus xanthoxyloides* stem bark

IV.2.1 Extraction

1 kg of the stem bark of *F. xanthoxyloides* was air dried and powdered by an electric mill. The plant material was subjected to exhaustive extraction with methanol (3 × 6 l) and left at room temperature for two days in each extraction. The combined extracts were concentrated to dryness by using a Rotavapor to afford 123.58 g of the methanolic extract 1 (ME1).

IV.2.2 Separation and purification

Part of methanolic extract (10 g) was dissolved in water and applied on polyamide 6 column chromatography with H_2O-MeOH gradient system (100:0 to 0:100). Using TLC analysis on silica gel ($CHCl_3$:MeOH:H_2O, 60:22:4) fractions having similar profiles were combined to give nine main fractions (1-9) (Figure IV.3; Table IV.1).

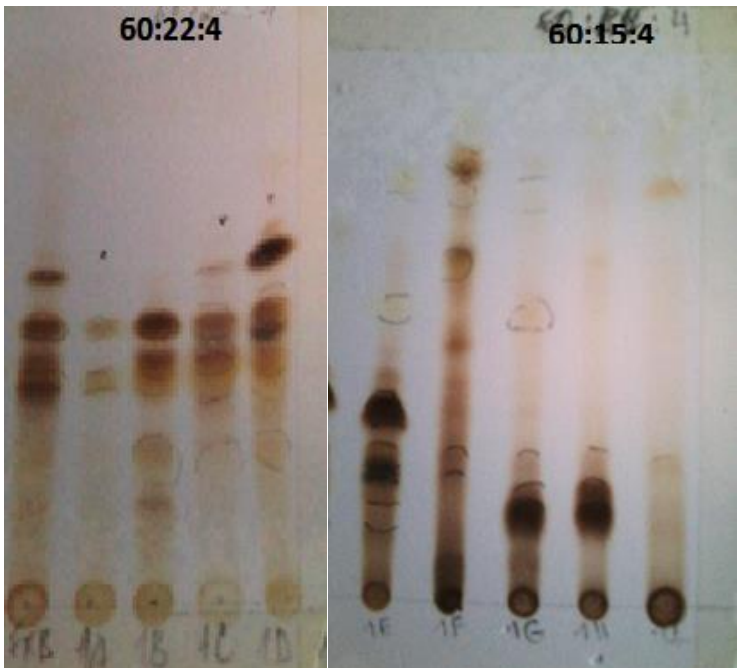


Figure IV.3: TLC of the main fractions of the methanolic extract of the stem bark.

Table IV.1: The main fractions collected from the methanolic extract (ME1)

Collected fractions	Weight (mg)
F1	2550
F2	3820
F3	570
F4	550
F5	500
F6	750
F7	470
F8	440
F9	80

A part of fraction F1 (1.8 g) was dissolved in water and chromatographed on Lobar column (RP-8, size B), using H_2O-MeOH (5% to 55%) gradient to afford seven sub-fractions (Figure IV.4; Table IV.2).

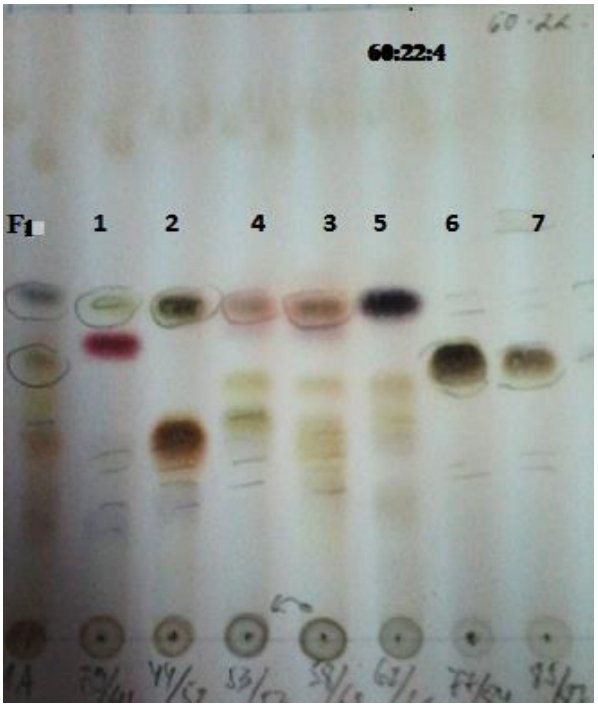


Figure IV.4 : TLC of the main fractions of sub-Fraction F1

Table IV.2. The main fractions collected from sub-fraction F1

Fractions	Weight (mg)
F1-1	23.9
F1-2	144.8
F1-3	536.6
F1-4	22.8
F1-5	21.4
F1-6	51.3
F1-7	30.9

Purification of sub-fraction F1-1 (23.9 mg) by PTLC using $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v) eluent yielded 3.3 mg of compound **16** (Figure IV.5).

Figure IV.5: TLC of compound 16

Sub-fraction F1-2 (144.8 mg) was subjected to Sephadex LH-20 column chromatography using MeOH:H_2O (2:1/v:v) as mobile phase to give two fractions (A and B) (Figure IV.6). Fraction B (50.9 mg) was then purified by PTLC with $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v) eluent to afford compound **5** (8.9 mg) and compound **12** (28.7 mg) (Figure IV.7).

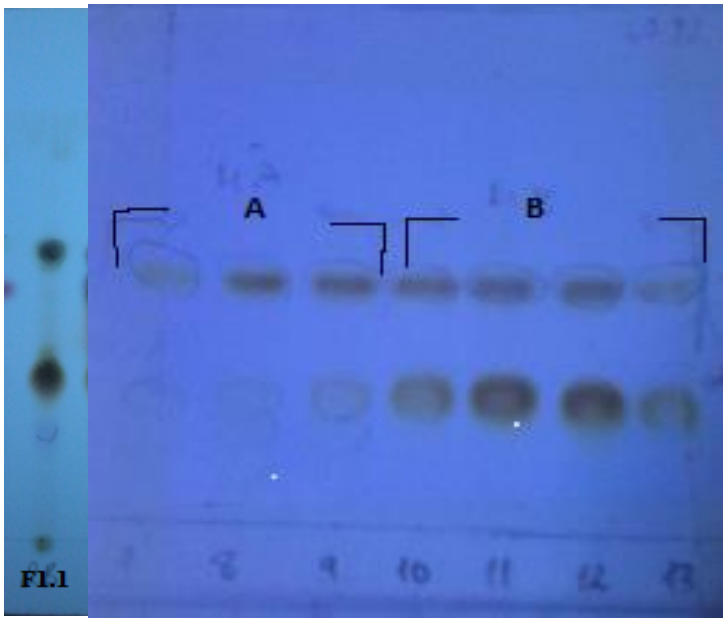


Figure IV.6: TLC of sub-fractions A and B

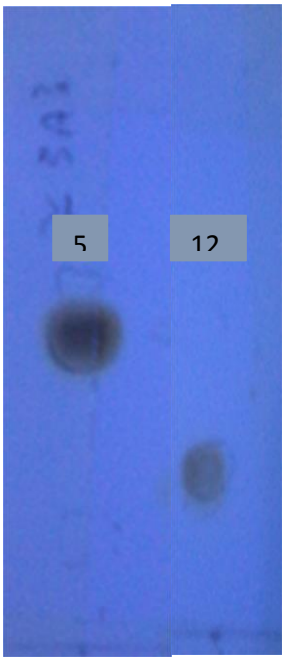


Figure IV.7: TLC of compounds 5 and 12

Sub-fraction F1-6 (51.3 mg) was subjected to Sephadex LH-20 column chromatography, using MeOH:H_2O (2:1/v:v) as eluent, to give compound **1** (27.8 mg) and compound **2** (15.6 mg) (Figure IV.8).

Figure IV.8 : TLC of compounds 1 and 2

Fraction F3 (536 mg) was dissolved in water and applied on Lobar column (RP-8, size B), using a gradient of H_2O:MeOH (5% to 55%) to obtain eight sub-fractions (Table IV.3).

Table IV.3. The main fractions collected from sub-fraction F3

Fractions	Weight (mg)
F3-1	10.8
F3-2	5.2
F3-3	13.9
F3-4	14.2
F3-5	60.4
F3-6	38.2
F3-7	5.8
F3-8	39

Sub-fraction F3-6 (38.2 mg) was subjected to Sephadex LH -20 CC and eluted with MeOH:H_2O (2:1/v:v) to provide compound **14** (14.3 mg) (Figure IV.9).

Figure IV.9 : TLC of compound 14

Compound **15** (12 mg) was isolated from fraction F3-8 (39 mg), which was submitted to CC over Sephadex LH-20 and eluted with MeOH:H_2O (2:1/v:v) (Figure IV.10).

Figure IV.10 : TLC of compound 15

Fraction F4 (550 mg) was dissolved in water and applied on Lobar column (RP-8, size B) eluting with H_2O:MeOH (5% to 55%) gradient to obtain thirteen sub-fractions (Figure IV.11; Table IV.4).

Table IV.4. The main fractions collected from sub-fraction F4

Fractions	Weight (mg)
F4-1	13
F4-2	19.8
F4-3	43.2
F4-4	19.8
F4-5	37.5
F4-6	9.7
F4-7	4.5
F4-8	27.5
F4-9	27.1
F4-10	37.5
F4-11	33.3
F4-12	55.8
F4-13	6.2

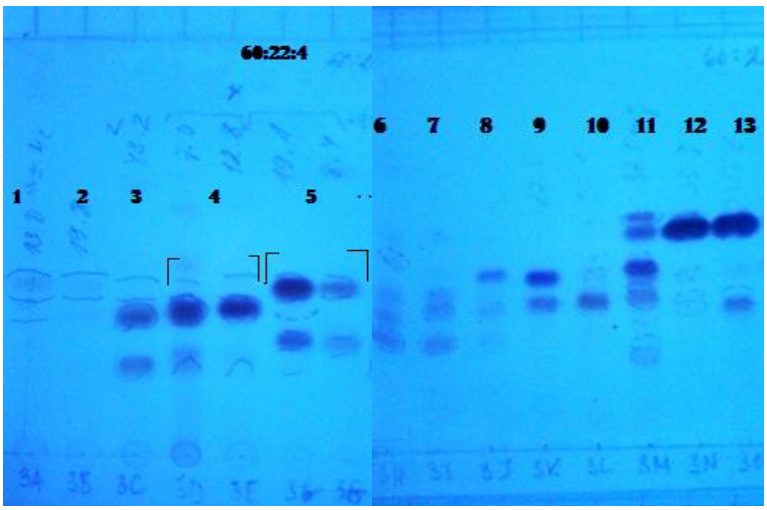


Figure IV.11: TLC of the main fractions of fraction F4

Sub-fractions F4-3 (43 mg) and F4-11 (33.3 mg), were separately purified by preparative TLC, developed with $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v), to afford compound **6** (5.1 mg) and compound **17** (23.1 mg), respectively (Figure IV.12)

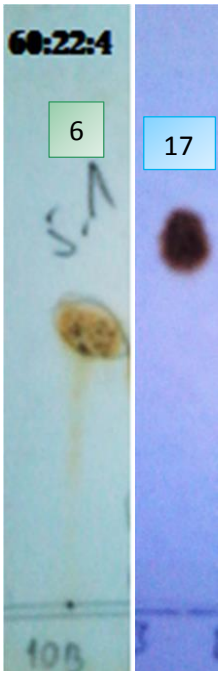


Figure IV.12: TLC of compounds 6 and 17

Fraction F7 (470 mg) was applied on Lobar column (RP-8, size B), using H_2O:MeOH (20% to 50%) gradient to yield compound **13** (111 mg) (Figure IV.13).

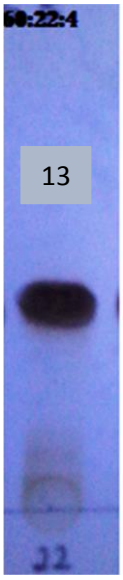


Figure IV.13: TLC of compounds 13

IV.3 Phytochemical study of *Fraxinus xanthoxyloides* leaves

IV.3.1 Extraction

A well air dried and powdered leaves (200 g) was extracted three times with methanol at room temperature for four days. The methanolic solutions were combined and concentrated to dryness under reduced pressure to yield 33.8 g of the methanolic extract 2 (ME2).

IV.3.2 Separation and purification

15 g of the methanolic extract was dissolved in water and subjected to polyamide 6 column chromatography eluting with H_2O, H_2O-MeOH mixtures (25-100%). Using TLC analysis on silica gel ($CHCl_3$:MeOH:H_2O, 60:22:4) fractions having similar profiles were combined to give nine fractions (1-9) (Figure IV.14; Table IV.5).

Table IV.5. The main fractions collected from the methanolic extract (ME2)

Fractions	Weight (mg)
F1	7700
F2	1290
F3	710
F4	129
F5	310
F6	725
F7	113
F8	1500
F9	4310

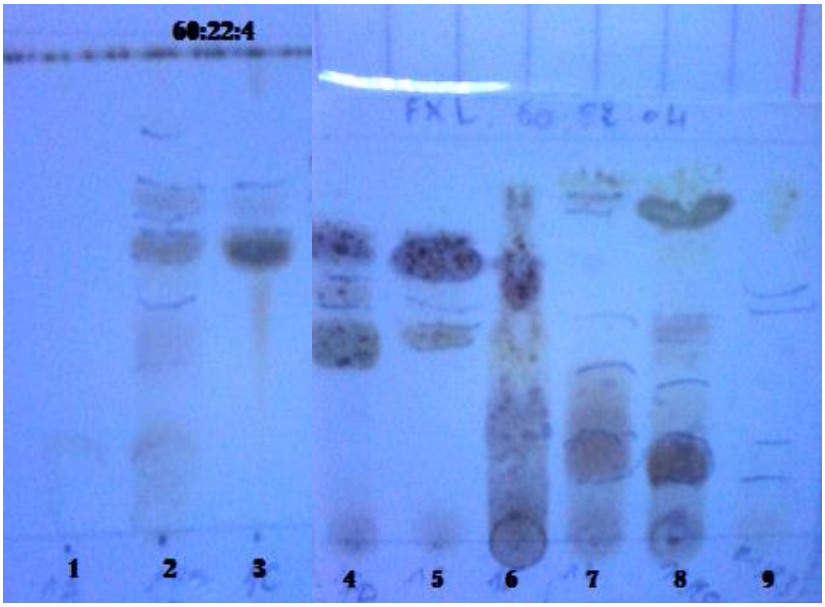


Figure IV.14: TLC of the main fractions of the methanolic extract of the leaves

Fraction F2 (1.23 g) was dissolved in water and was separated on a Lobar column chromatography (RP-8, size B) eluting with a gradient of increasing MeOH (0-50%) in H_2O to afford eight fractions (1-8) (Figure IV.15; Table IV.6).

Table IV.6. The main fractions collected from fraction F2

Fractions	Weight (mg)
F2-1	28.4
F2-2	08.4
F2-3	21.8
F2-4	51.6
F2-5	18.8
F2-6	13.8
F2-7	29.4
F2-8	108

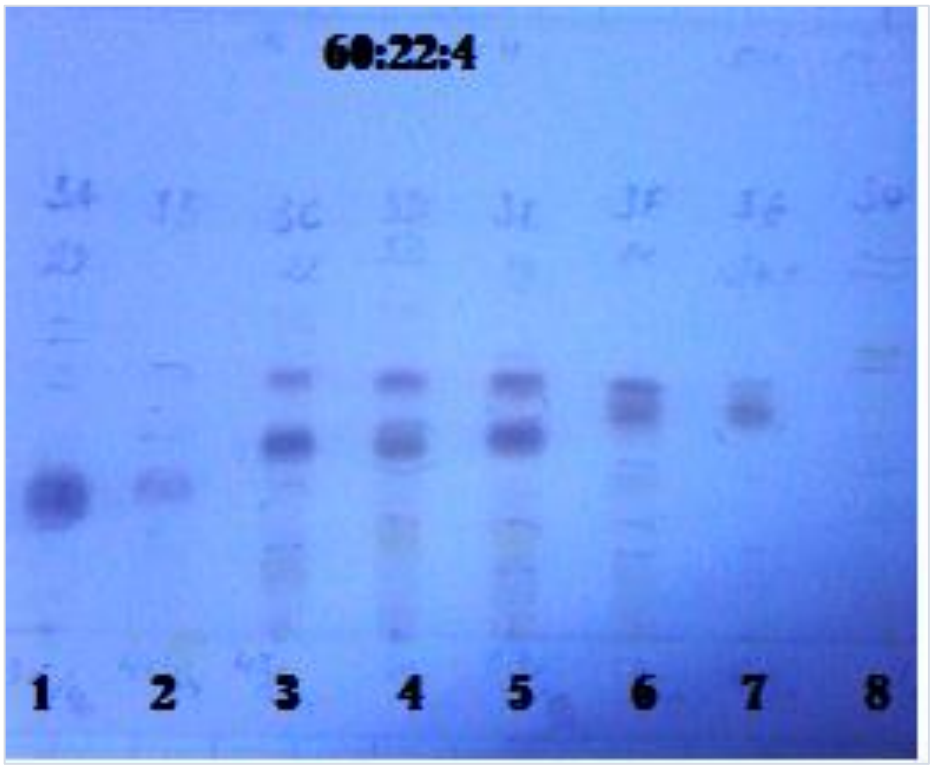


Figure IV.15: TLC of sub-fractions of fraction F2

Sub-fractions F2-1 (28 mg) and F2-3 (21.4 mg), were separately purified by PTLC with $CHCl_3$:MeOH:H_2O (60:22:4) to obtain compound **10** (6.4 mg) and compound **11** (6.2 mg), respectively (Figure IV.16).

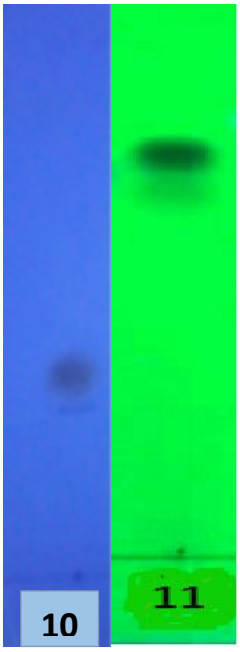


Figure IV.16: TLC of compounds 10 and 11

Fraction F3 (340 mg) was dissolved in MeOH:H_2O (4:1.5) and applied on Sephadex LH-20 column, eluting with MeOH:H_2O (4:1.5) to afford compound **6** (76.9 mg) (Figure IV.17).

Figure IV.17: TLC of compound 6

Fraction F4 (129 mg) was dissolved in MeOH and methanol soluble part was applied on Sephadex LH-20 column chromatography, eluting with MeOH and a sub-fraction (50.7 mg) was further purified on Lobar column (RP-18, size A, eluents 10-25% MeOH) to give compound **5** (3.6 mg) (Figure IV.18). The insoluble part was compared by TLC with compound **6** and it was cichoriin.

Figure IV.18: TLC of compound 5

Fraction F6 (720 mg) was applied on Sephadex LH-20 column chromatography eluting with MeOH and a sub-fraction (203 mg) was additionally purified by LPLC (Lobar RP-18, size A, eluents 10-50% MeOH) yielding compound **3** (10.5 mg) and compound **4** (30.1 mg) (Figure IV.19).

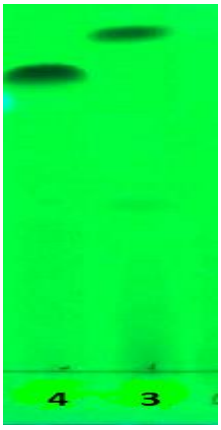


Figure IV.19: TLC of compounds 3 and 4

Fraction F8 (400 mg) was dissolved in MeOH and applied on Sephadex LH-20 column to obtain a sub-fraction which was additionally partitioned by silica gel column eluting with $CHCl_3$:MeOH:H_2O moxures (60:15:4, 60:22:4 and 61:32:7) to obtain compound **7** (9.7 mg), compound **8** (11.4 mg) and compound **9** (53.6 mg) (Figure IV.20).

The isolated compounds from *Fraxinus xanthoxyloides* leaves (FXL) are ordred on the chromatograms (Figure III.20 and III.21) according to their polarity. The solvent system used for TLC is $CHCl_3$:MeOH:H_2O (60:22:4).

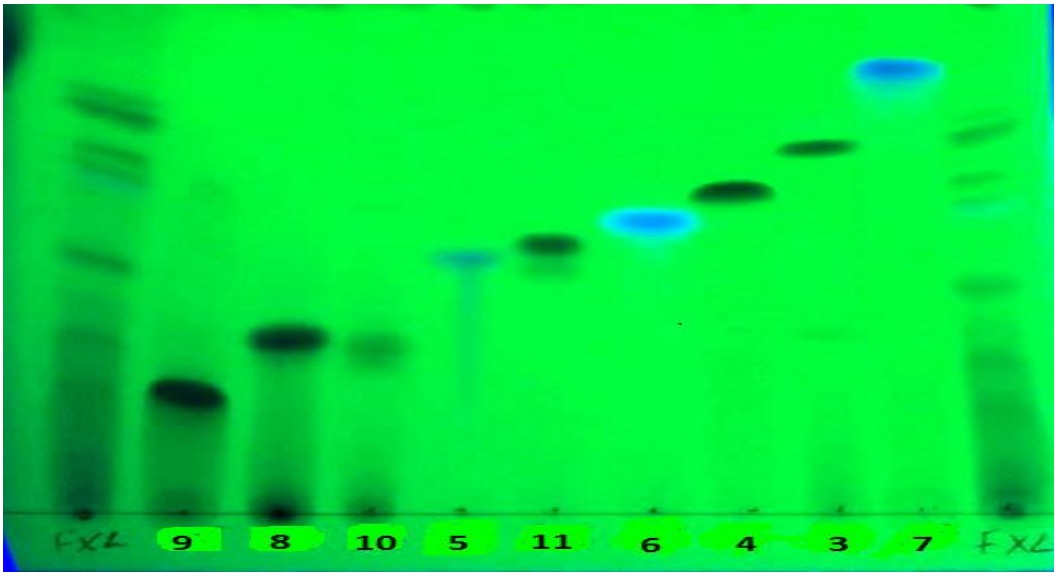


Figure IV.20: TLC of the isolated compounds from the leaves

under UV light (254 nm).

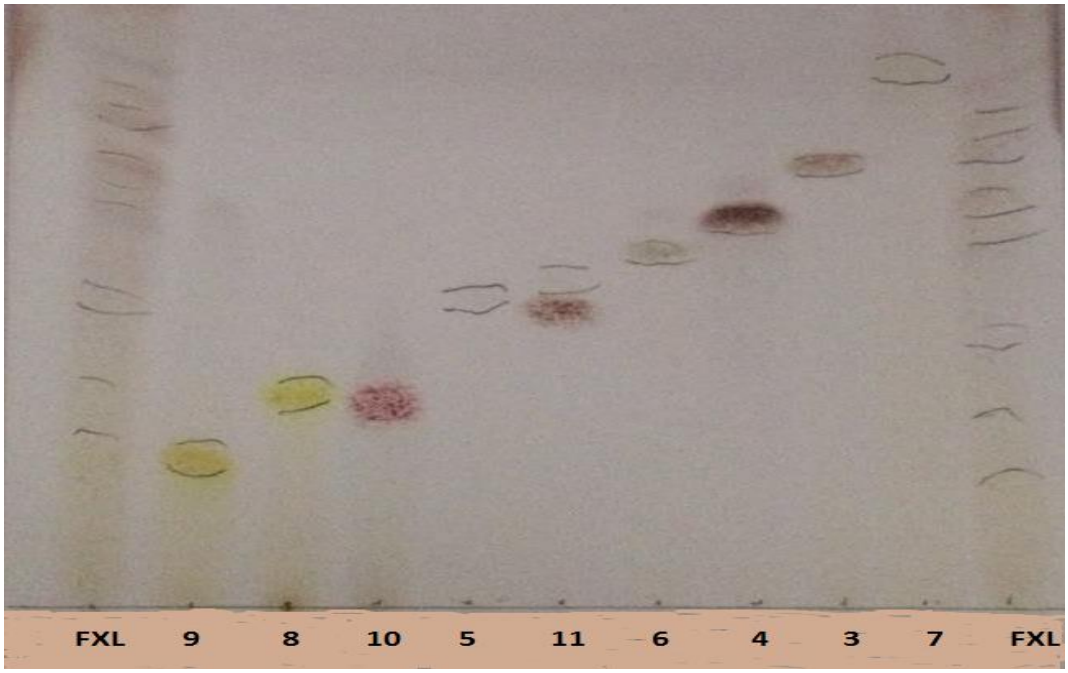
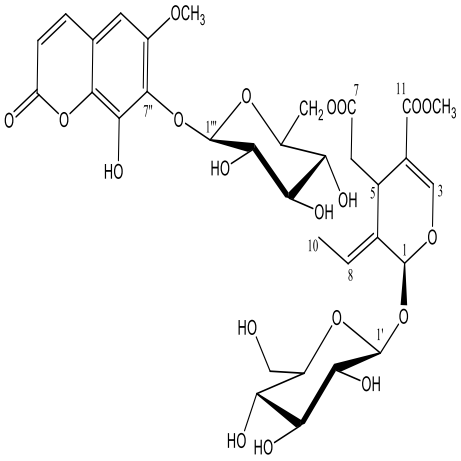


Figure IV.21: TLC of the isolated compounds from the leaves after spraying with H_2SO_4 reagent

IV.4 Spectral data and physical constants of the isolated compounds

IV.4.1 Coumain-secoiridoid compounds

Compound 1



Isofraxisecoside

Molecular formula: $C_{33}H_{40}O_{20}$

$[\alpha]_D^{20}$ = -135.8 (c = 0.095, MeOH)

HR-ESI-MS: *m/z* found: 755.20404 for $[M-H]^-$

^{1}H-NMR (600.11 MHz) and ^{13}C-NMR (150.91 MHz) in CD_3OD (Table III.1, page 69).

Compound 2

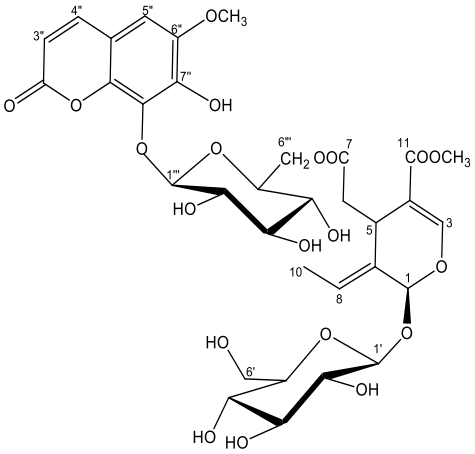
Fraxisecoside

Molecular formula: $C_{33}H_{40}O_{20}$

$[\alpha]_D^{20}$ = -144.16 (c = 0.115, MeOH)

HR-ESI-MS: *m/z* found: 755.20381 for $[M-H]^-$

^{1}H-NMR (600.11 MHz) and ^{13}C-NMR (150.91 MHz) in CD_3OD (Table III.2, page 80).



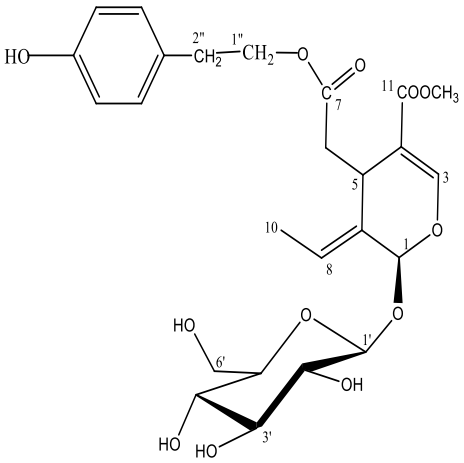
IV.4.2 secoiridoid compounds

Compound 3

Ligstroside

Molecular formula: $C_{25}H_{32}O_{12}$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.3, page 83).

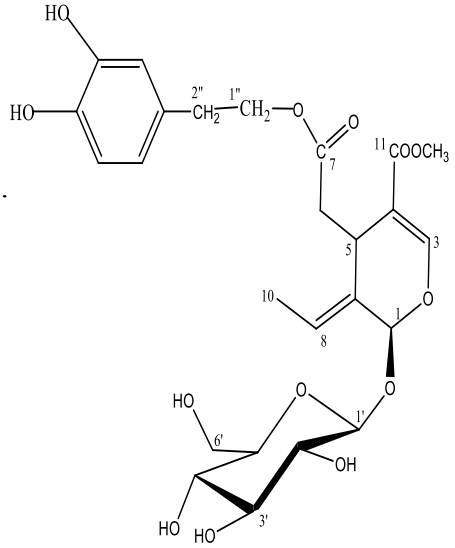


Compound 4

Oleuropein

Molecular formula: $C_{25}H_{32}O_{13}$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.4, page 88).



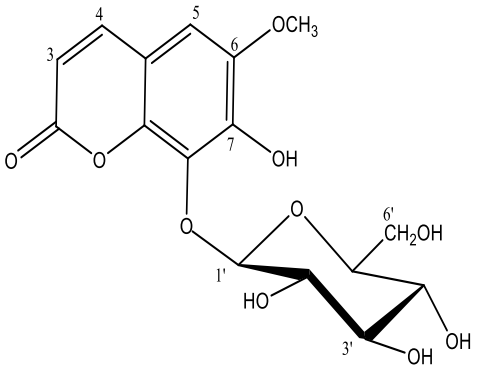
IV.4.3 Coumarin compounds

Compound 5

Fraxin

Molecular formula: $C_{16}H_{18}O_{10}$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.5, page 91).

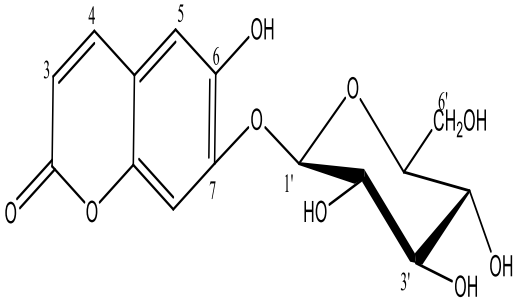


Compound 6

Cichoriin

Molecular formula: $C_{15}H_{16}O_9$

^{1}H-NMR (600.11 MHz, CD_3OD) and ^{13}C (150.91 MHz; DMSO-d_6) (Table III.6, page 95).

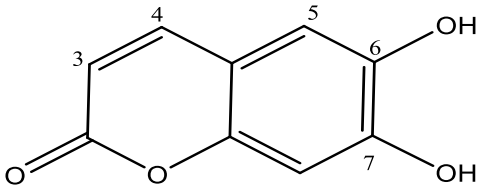


Compound 7

Esculetin

Molecular formula: $C_9H_6O_4$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.7, page 97).



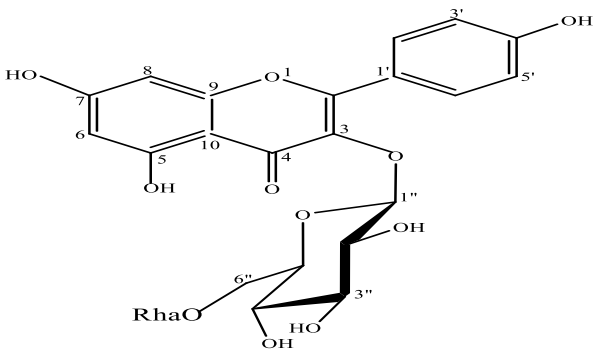
IV.4.4 Flavonoid compounds

Compound 8

Nicotiflorin

Molecular formula: $C_{27}H_{30}O_{15}$

^{1}H-NMR (600.11 MHz) in DMSO-d_6 (Table III.8, page 100).

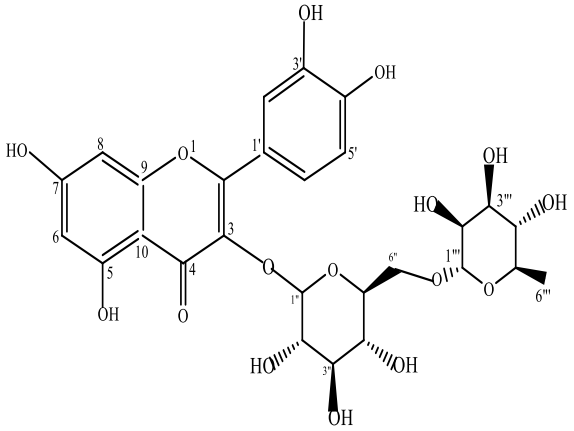


Compound 9

Rutin

Molecular formula: $C_{27}H_{30}O_{16}$

^{1}H-NMR (600.11 MHz) in DMSO-d_6 (Table III.9, page 103).



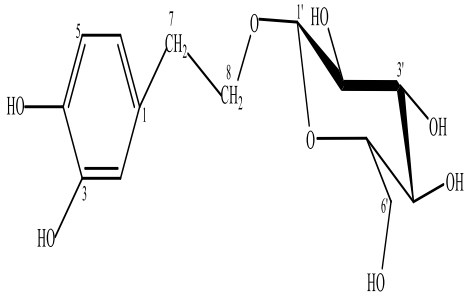
IV.4.5 Phenylethanoid compounds

Compound 10

2-(3, 4-dihydroxy)phenylethyl-O-β-D-glucopyranoside

Molecular formula: $C_{14}H_{18}O_8$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.10, page 106).

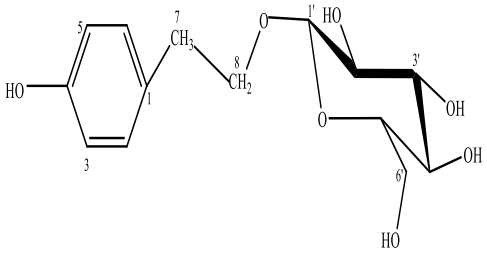


Compound 11

Salidroside

Molecular formula: $C_{14}H_{20}O_7$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.11, page 108).

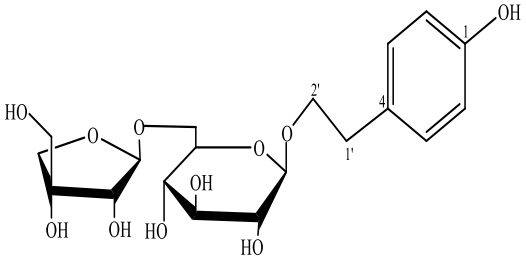


Compound 12

Osmanthuside

Molecular formula: $C_{14}H_{20}O_7$

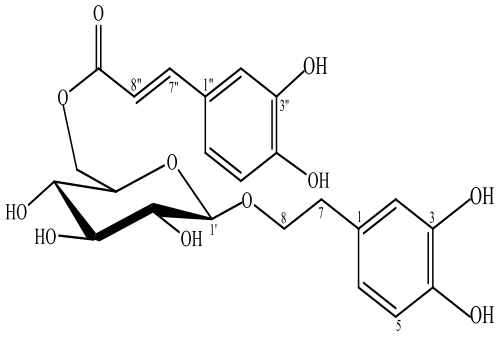
^{1}H-NMR (600.11 MHz) and ^{13}C-NMR (150.91 MHz) in CD_3OD (Table III.12, page 113).



Calceolarioside B

Molecular formula: $C_{23}H_{26}O_{11}$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.13, page 115).



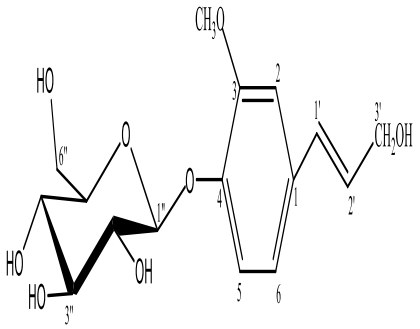
IV.4.6 Phenylpropanoid compounds

Compound 14

Coniferin

Molecular formula: $C_{16}H_{22}O_8$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.14, page 118).

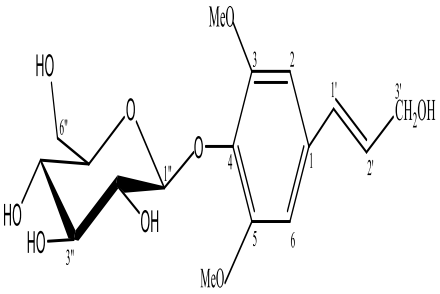


Compound 15

Syringin

Molecular formula: $C_{17}H_{24}O_9$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.15, page 120).



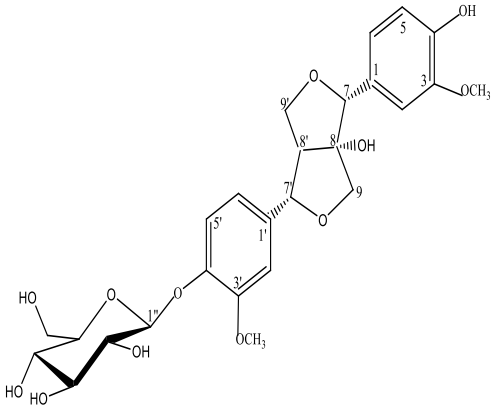
IV.4.7 Lignans

Compound 16

8-Hydroxypinoresinol-4′-O-β-D-glucoside

Molecular formula: $C_{26}H_{32}O_{12}$

^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.16, page 123).

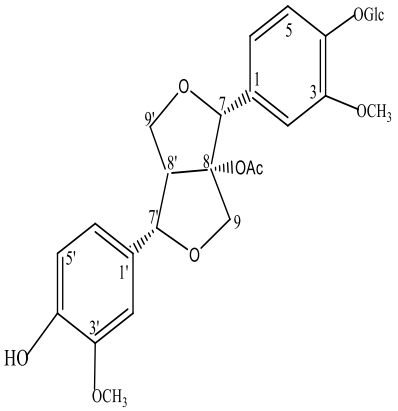


Compound 17

8-Acetoxypinoresinol-4-O-β-D-glucoside

Molecular formula: $C_{28}H_{34}O_{13}$

^{13}C-NMR (150.91 MHz) in CD_3OD (Table III.17, page 129). ^{1}H-NMR (600.11 MHz) in CD_3OD (Table III.18, page 130).



Conclusion

Conclusion

The present work concerns the chemical investigation of the Algerian plant: *Fraxinus xanthoxyloides*, belonging to the Oleaceae family. This species is commonly used in traditional medicine in different parts of the world. The occurrence of secoiridoids, coumarins and phenylethanoids is a characteristic feature of this *Fraxinus* species.

The chemical study of the secondary metabolism of the leaves and the stem bark of this medicinal plant has resulted in the isolation of natural products including one new coumarin-secoiridoid (Hadroug et al., 2018).

The structures of the new compound isolated from the stem bark was determined by spectroscopic methods, mainly 1D and 2D NMR techniques (^{1}H and^{13}C NMR, HSQC, HMBC and ROESY experiments), high resolution mass spectrometry (HR-ESI-MS), measurement of the optical rotation $[\alpha]_D^{20}$ and by chemical transformations. Known molecules were identified by comparing the spectroscopic data with the literature.

The methanolic extracts of both leaves and stem bark of *F. xanthoxyloides* were submitted to subsequent purification steps by using different chromatographic techniques, mainly the law pressure liquid chromatography (LPLC), to give seventeen pure compounds (**1-17**), including iridoids and phenolic compounds. One of them was unprecedented molecule. The composition of the two parts of the plant was different. In particular, the extract of the stem bark was found to contain coumarin-secoiridoid and phenypropanoid compounds whereas the leaves extract was found to contain flavonoid compounds, which was not detected in the bark.

Ten glucosides have been isolated from the stem bark extract and were identified as two coumarin-secoiridoid glucosides consist of a coumarin glucoside and a secoiridoid glucoside, linked through the glucose of the coumarin part and the carboxyl group at C-7 of the secoiridoid: fraxetin-7″-O-[11-methyl-oleosidyl-(7-6‴)]-β-glucopyranoside, named isofraxisecoside (**1**), which was the novel compound, and the known compound Fraxisecoside (fraxetin-8″-O-[11-

methyl-oleosidyl-(7-6‴)]-β-D-glucopyranoside) (**2**); two simple coumarins: fraxin (**5**) and cichoriin (**9**); two phenylethanoid: 2-(4-hydroxyphenyl)ethanol-β-D-apiofuranosyl(1-6)-β-D-glucopyranoside named osmanthuside H (**12**) and calceolarioside (**13**); two phenylpropanoids: coniferin (**14**) and syringing (**15**); two lignans: 8-Hydroxypinoresinol-4′-O-β-D-glucoside (**16**) and 8-Acetoxypinoresinol-4-O-β-D-glucoside (**17**).

From the leaves extract, we have isolated nine compounds identified as: two secoiridoid glucosides of oleoside type: ligstroside (**3**) and oleuropein (**4**); three simple coumarins: two glucosides isolated previously from the stem bark and one coumarin aglucone named esculetin (**7**); two flavonoid diglucosides having a flavonol framework: kaempferol-3-O-rutinoside named nicotiflorin (**8**) and quercetin-3-O-rutinoside named Rutin (**9**); two phenylethanoid glucosides: 2-(3, 4-dihydroxy)phenylethyl-O-β-D-glucopyranoside (**10**) and salidroside (**11**).

The methanolic extracts of both leaves and stem bark of the Algerian species (*Fraxinus xanthoxyloides* and *Fraxinus angustifolia*), were screened for their anti-oxidant activity by using two different tests in vitro: DPPH free radicals scavenging and ABTS radical cation decolorization assay.

It is noteworthy to indicate that among known compounds reported in the literature, one phenylethnoide and two lignan glucosides have been found for the first time in Fraxinus genus. Furthermore, this study contributes to determine the chemical composition of this genus. In general, further investigations on the Algerian Fraxinus species are required to complete the chemical scenario.

Accordingly, and in perspective, the Algerian medicinal plant *Fraxinus angustifolia*, that has an important biological activity reviewed in the literature, has been planned to be investigated in the next future.

References

References

[1]. Shravanthi, R., Sureshkumar, M., Veluswamy Bhuvaneshwari, V., Kalaiselvi, M., Amsaveni, R., 2016. Anti-urolithiatic activityof plant extracts. Inter. J. of Biosciences and Nanosciences. 3, 169-173.

[2]. Chang, H. M., But, P. P. H., 1986. Pharmacology and Applications of Chinese Materia Medica. World Sci. Pub., Singapore.

[3]. Ameenah, G. F., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 27, 1–93.

[4]. Wallander, E., 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Plant Syst. Evol.* 273, 25-49.

[5]. Quezel, P., Santa, S., 1963. New flora of Algeria and Southern desert regions, Tome 2. CNRS, Paris, France.

[6]. Kostova, I., Iossifova, T., 2007. Chemical components of Fraxinus species. Fitoterapia. 78, 85-106.

[7]. Si, C. L., Ren, X. D., Du, Z. G., Huang, X. F., Wu, L., 2015. Purification and spectrosc- opic elucidation of new coumarin glucoside in *Fraxinus velutina* Heartwood. Chem. Nat. Comp. 51, 1059-1061.

[8]. Kostova, I., 1992. Hydroxycoumarins from *Fraxinus ornus* bark. Planta Med. 58, 484-487.

[9]. Jensen, S. R., Franzyk, H., Wallander, E., 2002. Chemotaxonomy of the Oleaceae: iridoids as taxonomic markers. Phytochemistry. 60, 213-231.

[10]. Damtoft, S., Franzyk, H., Jensen, S. R., 1994. Biosynthesis of iridoids in Forsythia spp. Phytochemistry. 37, 173–178.

[11]. Wallander, E., 2013. Systematics and floral evolution in *Fraxinus* (Oleaceae). Belgische Dendrologie Belge. 2012, 38-58.

[12]. Wallander, E., Albert, V. A., 2000. Phylogeny and classification of Oleaceae based on rps16 and trnL-F sequence data. *Amer. J. Bot.* 87, 1827-1841.

[13]. Wallander, E., 2001. Evolution of wind-pollination in Fraxinus (Oleaceae) – an ecophylo-genetic approach. PhD thesis, Goteborg University, Sweden.

[14]. Shen, Y. C., Chen, C. H., Lee, K. H., 1993. Secoiridoid Dilactones from *Fraxinus uhdei*. Phytochemistry. 33, 1531-1533.

[15]. Hosny, M., 1998. Secoiridoid glucosides from *Fraxinus oxycarba*. Phytochemistry. 47, 1569-1576.

[16]. Calis, I., Hosny, M., Khalifa, T., Nishibe, S., 1993. Secoiridoids from *Fraxinus angusti- folia*. Phytochemistry. 33, 1453-1456.

[17]. Iossifova, I., Kostova, I., Evstatieva, L., 1997. Secoiridoids and hydroxycoumrains in Bulgarian Fraxinus species. Biochem. Syst. Ecol. 25, 271-274.

[18]. Plouvier, V., 1968. Hebd. Seances Acad. Sci. 267, p.1884.

[19]. Plouvier, V., 1968. Hebd. Seances Acad. Sci. 266, p.1526.

[20]. Jensen, S. R., Nielsen, B.J., 1976. A new coumarin, fraxidin 8-O-β-D-glucoside and 10-hydroxyligstroside from bark of *Fraxinus excelsior*. Phytochemistry. 15, 221–223.

[21]. Nagarajan, G. R., Rani, U., Parmar, V. S., 1980. Coumarins from *Fraxinus floribunda* leaves. Phytochemistry. 19, 2494-2497.

[22]. Nagarajan, G. R., Parmar, V. S., Satake, M., Katyal, M., 1984. Coumarins of *Fraxinus floribunda*. Indian J. Pharm. Sci. 46, 176-178.

[23]. Nagarajan, G. R., Rani, U., Parmer, V. S., 1983. Floribin- a new coumarin from *Fraxinus floribunda* Wall. Pharmazie. 38, 72-74.

[24]. Tanahashi, T., Shimada, A., Nagakura, N., Inoue, K., Kuwajima, H.,Takaishi, K., Chen, C. C., He, Z. D., Yang, C. R., 1993. Isolation of oleayunnanoside from *Fraxinus insul- aris* and revision of its structure to insularoside-6‴-O-β-D-glucoside. Chem. Pharm. Bull. 41, 1649–1651.

[25]. Reppel, L., 1954. Natural coumarins. Pharmazie. 9, 278-299.

[26]. Terazawa, M., Sasaya, T., 1986. Phenolic compounds in living tissues of woods. Res. Bull. College Experiment Forests. 43, 803–814.

[27]. Joshi, R. K., Prasad, D., Rawat, M. S., Pant, G., 1998. Constituents of *Fraxinus micrantha*. Fitoterapia. 69, 275-276.

[28]. Paris, R., Stambouli, A., 1961. Biochemical study of *Fraxinus oxyphylla* Bieb. Isolation of rutin from the leaves and esculin from the bark. C.R. Hebd. Seances Acad. Sci. 253, 313-317.

[29]. Xiao, K., Song, Q. H., Zhang, S. W., Xuan, L. J., 2008. Water-soluble constituents of the root barks of *Fraxinus rhynchophylla* (Chinese drug Qinpi). J. of Asian Nat. Prod. Res. 10, 205-210.

[30]. Kim, H. J., Yu, Y. G., Park, H., Lee, Y. S., 2002. HIV gp 41-binding phenolic components from *Fraxinus sieboldiana* var. angustata. Planta Med. 68, 1034–1036.

[31]. Lin, S., Wang, S., Liu, M., Gan, M., Li, S., Yang, Y., Wang, Y., He, W., Shi, J., 2007. Glucosides from the stem bark of *Fraxinus siebodiana*. J. Nat. Prod. 70, 817-823.

[32]. Iftikhar, M., Riaz, N., Saleem, M., Musaddiq, S., Jabbar, S. A., Tareen, R. B., 2015. Xanthoxyloidin, a new biscoumarin from *Fraxinus xanthoxyloides.* J. Chem. Soc. Pac. 37, 492-494.

[33]. Inouye, H., Yoshida, T., Tobita, S., Tanaka, K., Nishioka, T., 1970. Absolute structure of oleuropeins. Tetrahedron Lett. 28, 2459-2464.

[34]. Inouye, H., Ueda, S., Inoue, K., Takeda, Y., 1974. Studies on monoterpene glucosides and related natural products. XXIII. Biosynthesis of the secoiridoid glukosides gentiopicroside, morroniside, oleuropein, and jasminin. Chem. Pharm. Bull. 22, 676–686.

[35]. Takenaka, Y., Tanahashi, T., Shintaku, M., Sakai, T., Nagakura, N., Parida, S., 2000. Secoiridoid glucosides from *Fraxinus amiricana*. Phytochemistry. 55, 275-284.

[36]. Calis, I., Hosny, M., Lahloub, M. F., 1996. A secoiridoid glucoside from *Fraxinus angustifolia*. Phytochemistry. 41, 1557–1562.

[37]. Shen, Y. C., Chen, C. Y., 1995. Additional secoiridoid glucosides from *Fraxinus uhdei*. Planta Med. 61, 281–283.

[38]. Tanahashi, T., Shimada, A., Nagakura, N., Inoue, K., Kuwajima, H., Takaishi, K., Chen, C. C., 1993. A secoiridoid glucoside from *Fraxinus insularis*. Phytochemistry. 33, 397–400.

[39]. Tanahashi, T., Parida, S., Takenaka, Y., Nagakura, N., Inoue, K., Kuwajima, H., Chen, C. C., 1998. Four secoiridoid glucosides from *Fraxinus insularis*. Phytochemistry. 49, 1333–1337.

[40]. Shen, Y. C., Chen, C. H., 1993. New secoiridoid dilactones from *Fraxinus uhdei*. J. Nat. Prod. 56, 1905–1911.

[41]. Iossifova, T., Mikhova, B., Kostova, I., 1993. A secoiridoid glucoside and a phenolic compound from *Fraxinus ornus* bark. Phytochemistry. 34, 1373–1376.

[42]. Iossifova, T., Mikhova, B., Kostova, I., 1995. A secoiridoid dilactone from *Fraxinus ornus* bark. Monatshefte fur Chemie. 126, 1257–1264.

[43]. LaLonde, R. T., Wong, C., Tsai, A. I., 1976. Polyglucosidic metabolites of Oleaceae. The chain sequence of oleoside aglucon, tyrosol, and glucose units in three metabolites from *Fraxinus americana*. J. Am. Soc. Chem. 98, 3007–3013.

[44]. He, Z.-D., Ueda, S., Inoue, K., Akaji, M., Fujita, T., Yang, C.-R., 1994. Secoiridoid glucosides from *Fraxinus malacophylla*. Phytochemistry. 35, 177–181.

[45]. Damtoft, S., Franzyk, H., Jensen, S. R., 1993. Biosynthesis of secoiridoid glucosides in Oleaceae. Phytochemistry. 34, 1291–1299.

[46]. Nishibe, S., Sardari, S., Kodama, A., Horita, K., Kudo, M., Koike, K., Nikaido, T., 1997. Constituents of bark of *Fraxinus americana*. Nat. Med. 51, 482–485.

[47]. Kuwajima, H., Morita, M., Takaishi, K., Inoue, K., Fujita, T., He, Z. D., Yang, C. R., 1992. Secoiridoid, coumarin, and secoiridoid-coumarin glucosides from *Fraxinus chinensis*. Phytochemistry. 31, 1277–1280.

[48]. Damtoft, S., Franzyk, H., Jensen, S. R., 1992. Excelsioside, a secoiridoid glucoside from *Fraxinus excelsior*. Phytochemistry. 31, 4197–4201.

[49]. Tanahashi, T., Watanabe, H., Itoh, A., Nagakura, N., Inoue, K., Ono, M., Fujita, T., Morita, M., Chen, C. C., 1993. Five secoiridoid glucosides from *Fraxinus formosana*. Phytochemistry. 32, 133–136.

[50]. Sutarjadi, T., Malingre, M., Van, O., 1978. Iridoid and phenolic glycosides of *Fraxinus griffithii*. Phytochemistry. 17, 564-568.

[51]. Inouye, H., Nishioka, T., Kaniwa, M., 1975. Glucosides of *Fraxinus japonica*. Phytochemistry. 14, 304-309.

[52]. Iossifova, T., Vogler, B., Kostova, I., 1998. Secoiridoid glucosides from *Fraxinus ornus* bark. Phytochemistry. 49, 1329–1332.

[53]. Kostova, I., 2001. *Fraxinus ornus* L. Fitoterapia. 72, 471–480.

[54]. Iossifova, T., Vogler, B., Klaiber, I., Kostova, I., Kraus, W., 1999. Caffeic acid esters of phenylethanoid glycosides from *Fraxinus ornus* bark. Phytochemistry. 50, 297–301.

[55]. Iossifova, T., Vogler, B., Kostova, I., 2002. Escuside, a new coumarin-secoiridoid from *Fraxinus ornus* bark. Fitoterapia. 73, 386-389.

[56]. *Tsukamoto, H., Hisada, S., Nishibe, S., 1984*. Lignans from the bark of *Fraxinus mandshurica* and *F. japonica*. Chem. Pharm. Bull. *32, 4482*-4491.

[57]. Plouvier, V., 1967. Hebd. Seances Acad. Sci. 265, p. 1647.

[58]. Paris, R., Stambouli, A., 1960. Les hétérosides du frêne officinal (*Fraxinus excelsior*) et de quelques espèces voisines notamment *Fraxinus ornus*. Annales Pharmaceutiques Françaises. 18, 873-887.

[59]. Kostova, I., Iossifova, T., 2002. Chemical components of *Fraxinus* ornus bark- structure and biological activity. Studies in Nat. Prod. Chem. 26, 313-349.

[60]. *Naimie, H*., 1964. Attempts to isolate and purify substances inhibitory to *Bacillus subtilis* from tree bark. *Angew. Bot. 38, 1-34*.

[61]. *Martin, J. T., Baker, E. A., Byrde,* R. J., *1966*. The fungitoxicities of plant furocoumarins. *Ann. Appl. Biol. 57, 501*-508.

[62]. Kostova, I. N., Nikolov, N. M., Chipilska, L. N., 1993. Antimicrobial properties of some hydroxycoumarins of *Fraxinus ornus* bark extracts. J. Ethnopharmacol. 39, 205-208.

[63]. *Iossifova, T., Kujumgiev, A., Ignatova, A., Vassileva, E., Kostova,* I., 1994. Antimi- crobial effects of some hydroxycoumarins and secoiridoids from *Fraxinus ornus* bark. *Pharmazie. 49, 298*-299.

[64]. Yamagami, I., Suzuki, Y., Ito, K., 1968. Pharmacologic studies on the components of *Fraxinus japonica*. *Nippon Yakurigaki Zasshi. 64, 714*-729.

[65]. Kruedener, S., Schneider, W., Elstner, E. F., 1995. A combination of *Populus tremula, Solidago virgaurea* and *Fraxinus excelsior* as anti-inflammatory and anti-rheumatic drug. Drug Res. 45, 169–171.

[66]. Stefanova, Z., Neychev, H., Ivanovska, I., Kostova, I., 1995. Effect of a total extract from *Fraxinus ornus* stems bark and esculin on zymosan- and carrageenan-induced paw edema in mice. J. Ethnopharmacol. 46, 101-106.

[67]. *Ivanovska, N.,* Yossifova, *T., Vassileva, E.*, Kostova, I., 1994. Effect of some hydroxyl-coumarins on complement mediated hemolysis in human serum. *Methods Find Exp. Clin. Pharmacol. 16, 557*-562.

[68]. Meyer, B., Schneider W., Elstner, E. F., 1995. Anti-oxidative properties of alcoholic extracts from *Fraxinus* excelsior, *Populus tremula* and *Solidago virgaurea*. Drug Res. 45, 174–176.

[69]. *Marinova, E. M., Yanishlieva, N. V., Kostova,* I., 1994. Anti-oxidative action of the ethanolic extract and some hydroxy coumarins of *Fraxinus ornus* bark. *Food Chemistry. 51, 125*-135.

[70]. Jimenez, C., Riguera, R., 1994. Phenylethanoid glycosides in plants: structure and biological activity. Nat. Prod. Rep. 11, 591-606.

[71]. *Lazarova, G., Kostova, I., Neychev, H.,* 1993. Photodynamic damage prevention by some hydroxycoumarins. *Fitoterapia.* 64, 134-136.

[72]. *Galabov,* A.-S., Iosifova, *T., Vassileva, E., Kostova,* I., 1996. Antiviral activity of some hydroxycoumarin derivatives. *Z. Naturforsch. 51, 558*-562.

[73]. Casadebaig, J., Jacob, M., Cassanas, G., Gaudy, G., Baylac, G., Puech, A., 1989. Physicochemical and pharmacological properties of spray-dried powders from *Fraxinus excelsior* leaf extracts. J. Ethnopharmacol. 26, 211-216.

[74]. *Handa, S. S., Sharma, A., Chakraborti, K. K.,* 1986. Natural products and plants as liver protecting drugs. *Fitoterapia. 57, 307*–345.

[75]. Dinda, B., Debnath, S., Harigaya, Y., 2007. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. Chem. Pharm. Bull. *55*, 689–728.

[76]. Dinda, B., Chowdhury, D., 2009. Naturally occurring iridoids, secoiridoids and their bioactivity. A review, part 3. Chem. Pharm. Bull. 57, 765–796.

[77]. Dinda, B., Debnath, S., Banik, R., 2011. Naturally occurring iridoids and secoiridoids. A review, part 4. Chem. Pharm. Bull. 59, 803–833.

[78]. Bianco, A., 1990. The Chemistry of iridoids. Stud. Nat. Prod. Chem. 7, 439–497.

[79]. Franzyk, H., 2000. Synthetic aspects of iridoid chemistry. Prog. Chem. Org. Nat. Prod. 79, 1–114.

[80]. Cavill, G. W., Robertson, P. L., Brophy, J. J., Clark, D. V., Duke, R., Orton, C. J., Plant, W. D., 1982. Defensive and other secretions of the Australian cocktail ant, *Iridomyrmex nitidiceps*. Tetrahedron. 38, 1931-1938.

[81]. Emilia, A. G., 1995. The occurrence of iridoids in plants. Acta Societatis Botanicorum Poloniae. 64, 181-186.

[82]. Bianco, A., Jensen, S. R., Olesen, J., Passacantilli, P., Ramunno, A., 2003. Acid rearrangement of secoiridoids related to oleuropein and secologanin. Eur. J. Org. Chem. 22, 4349-4354.

[83]. Briggs, L. H., Cain, B. F., Lequesne, P. W., Shoolery, J. N., 1963. The structure of asperuloside. Tet. Lett. 4, 69–74.

[84]. Inouye, H., Ueda, S., Nakamura, Y., 1967. Zur biosynthese der bitteren glucoside der gentianazeen, des gentiopicrosids, des swertiamarins und des swerosids. Tet. Lett. 8, 3221–3226.

[85]. Hegnauer, R., 1966. Aucuba type glucosides. On their distribution and importance as systemic characteristics. Pharm. Acta Helv. 41, 577-587.

[86]. Sticher, O., Junod-Busch, U., 1975. The iridoid glucosides and their isolation. Pharm. Acta Helv. 50, 127-144.

[87]. El-Naggar, L. J., Beal, J. L., 1980. Iridoids. J. Nat. Prod. 43, 649-707.

[88]. Inouye, H., Uesato, S., 1986. Biosynthesis of iridoids and secoiridoids. Prog. Chem. Org. Nat. Prod. 50, 169-236.

[89]. Jensen, S. R., 1991. Plant iridoids, their biosynthesis and distribution in angiosperms. Proceedings of the Phytochemical Society of Europe. 31, 133-158.

[90]. Jensen, S. R., 1992. Systematic implications of the distribution of iridoids and other chemical compounds in the Loganiaceae and other families of the Asteridae. Ann. Missouri Bot. Gard. 79, 284–302.

[91]. Otsuka, H., 1993. Iridoid glucosides from *Linaria japonica*. Phytochemistry. 33, 617–622.

[92]. Inouye, H., 1991. Iridoids. Methods in Plant Biochemistry. 7, 99-100.

[93]. Meinwald, J., Chadha, S. M, Hurst, J. J., Eisner, T., 1962. Defense mechanisms of arthropods-9. Anisomorphal, the secretion of a phasmid insect. Tetrahedron Lett. 1, 29-33.

[94]. Bowers, M. D., Puttick, G. M., 1986. The fate of ingested iridoid glycosides in lepidopteran herbivores. J. Chem. Ecol. 12, 169-178.

[95]. Nishida, R., Fukami, H., 1989. Host plant iridoid-based chemical defence of an aphid, acyrthosiphon-nipponicus, against ladybird beetles. J. Chem. Ecol. 15, 1837-1845.

[96]. Ragazzi, E., Veronese, G., Guiotto, A., 1973. Demethyloleuropein, a new glycoside isolated from ripe olives. Ann. Chim. 63, 13-20.

[97]. Sticher, O., 1977. New natural products and plant drugs with pharmacological, biological or therapeutical activity, Eds. Wagner, H. and Wolff, P. Springer Verlag, Berlin. 137-176.

[98]. Omar, S. H, 2010. Oleuropein in olive and its pharmacological effects. Sci. Pham. 78, 133-154.

[99]. Ivanovska, N., Iossifova, T., Kostova, I., 1996. Complement- mediated anti-inflammatory action of extracts and pure secoiridoids isolated from Fraxinus species. Phytotherapy Research. 10, 555-558.

[100]. Bruneton, J., 1999. Immunotoxicity of epicutaneously applied anticoagulant rodenticide warfarin. Hampshire U K, Intercept Ltd. 2, 245-263.

[101]. Jain, P. K., Himanshu, J., 2012. Coumarin: chemical and pharmacological profile. Journal of Applied Pharmaceutical Science. 2, 236-240.

[102]. Murray, R. D. H., Mendez, J., Brown, S. A., 1982. The Natural Coumarins: occurrence, chemistry and biochemistry. John Wiley, Chichester.

[103]. Keating, G., O'Kennedy, R., 1997.The chemistry and occurrence of coumarins. In: Coumarins: Biology, Applications and Mode of Action, Eds. R O'Kennedy and R.D. Thornes, John Wiley and Sons, Chichester. 23-66.

[104]. Cooke, D., Fitzpatrick B., O' Kennedy, R., Cormack, T., Egan, D., 1997. Coumarin biochemical profile and recent developments. John Wiley and Sons. 3, 311-322.

[105]. Cooke, D., 1999. Recent advances on coumarin and its synthetic derivatives. Dublin City University, Dublin. 12, 125-139.

[106]. Feuer, G., 1979. The metabolism and biological actions of coumarins. Prog. Med. Chem. 10, 85-158.

[107]. Riordan, D. J., Daly, I., 1954. The growth-inhibiting properties of lactones. Ir. J. Med. Sei. 6, 157-161.

[108]. Ribeiro, C. V., Kaplan, M. A., 2002. Tendencias evolutivas de familias produtoras de cumarinas em angiospermae. Quim Nova. 25, 533-538.

[109]. Dewick, P. M., 2002. Medicinal natural products: a biosynthetic Approach. Eds. John Wiley and Sons Ltd, England.

[110]. Bruneton, J., 1993. Pharmacognosie -Phytochimie Plantes Médicinales. Tecnique et Documentation. Ed. Lavoisier, Paris.

[111]. Taylor, 1987. Perfumes. In: Encyclopedia of Chemical Technology, John Wiley and Sons, New York. 947-971

[112]. Stevenson, C. L., Tuan, V. D., 1995. Analysis of polynuclear aromatic compounds using laser excited synchronous fluorescence. Anal. Chim. Acta. 303, 247-253.

[113]. Dunges, W., 1977. 4-Bromomethyl-7-methoxycoumarin as a new fluorescence label for fatty acids. Anal. Chem. 49, 442-445.

[114]. Bourson, J., Borrel, M. N., Valeur, B., 1992. Cation complexation with coumarin 153 linked to monoaza-15-crown-5. Anal. Chim. Acta. 257, 189-193.

[115]. Casley-Smith, J. R., Foldi-Borcsok, E., Foldi, M., 1978. A fine structural study of the removal of the effectiveness of benzopyrone treatment of lymphoedema by the destruction of the macrophages by silica. Br. J. Exp. Path. 59, 116-127.

[116]. Piller, N. B., 1978. A morphological assessment of the stimulatory effect of coumarin on macrophages. Br. J. Exp. Path. 59, 93-96.

[117]. Piller, N. B., 1997. Mode of action of coumarin in the treatment of thermal injuries. In: Coumarins. Biology, Applications and Mode of Action, Eds: R O'Kennedy and R.D. Thornes, John Wiley and Sons, Chichester, 185-208.

[118]. Thornes, R. D., 1993. Prevention of recurrence of malignant melanoma by coumarin (1, 2-Benzopyrone. J. Ir. Coll. Phys. Surg. 22, 17-19.

[119]. Bourinbaiar, A. S., Tan, X., Nagorny, R., 1993a. Inhibitory effect of coumarins on H I V-1 replication and cell-mediated or cell-free viral transmission. Acta Virol. 37, 241-250.

[120]. Bourinbaiar, A. S., Tan, X., Nagorny, R., 1993b. Effect of the oral anti-coagulant, warfarin, on H I V - 1 replication and spread. AIDS. 7, 129-130.

[121]. Soine, T. O., 1964. Naturally occurring coumarins and related physiological activities. J. Pharm. Sci. 53, 231-264.

[122]. Link, K., 1959. The discovery of dicumarol and its sequels. Circulation. 19, 97-107.

[123]. Fontaine, L., Grand, M., Molho, D., Boschetti, E., 1967. Activité anti-inflammatoire experimentale de coumarine, indane-diones et acyl-mdane-diones apparentees aux anticoagulants oraux. Med. Pharmacol. Exp. 17, 497-507.

[124]. Lee, R. E., Bykadi, G., Ritschel, W. A., 1981. Inhibition of prostaglandin biosynthesis by coumarin, 4-hydroxycoumarin and 7-hydroxycoumarin. Drug Research. 31, 640-642.

[125]. Paya, M., Halliwell, B., Hoult, J. R. S., 1992. Peroxyl radical scavenging by a series of Coumarins. Free Rad. Res. Comm., 17, 293-298.

[126]. Chang, W. S., Lin, C. C, Chuang, S. C., Chiang, H. C., 1996. Superoxide anion scavenging effect of coumarins. Am. J. Chin. Med. 24, 11-17.

[127]. Ritschei, W. A., Alcom, G. J., Ritschel, G. B., 1984. Therapeutic concentration of coumarin as antipyretic. Meth. Find. Exp. Clin. Pharmacol. 6, 363-365.

[128]. Ritschel, W. A., Hardt, T. J., 1983b. Pharmacokinetics of coumarin, 7-hydroxycoumarin and 7-hydroxycoumarin glucuronide in the blood and brain of gerbils following intraperitoneal administration of coumarin. Drug Research. 33, 1254-1258.

[129]. Ghedira, K., 2005. Les flavonoïdes: structures, propriétés biologiques, rôles prophylactiques et emplois en thérapeutique. Phytothérapie. 4, 162-169.

[130]. De Rijke, E., Out, P., Niessen, W. M. A., Ariese, F., Gooijer, C., Brinkman, U. A. T., 2006. Analytical separation and detection methods for flavonoids. Journal of Chromatography. 1112, 31-63.

[131]. Buchsbaum, R.N., Short, F.T., Cheney, D.P. 1990. Phenolic–nitrogen interactions in eelgrass, *Zostera marina* L.: possible implications for diseases resistance. Aquat. Bot. 37, 291-297.

[132]. Rowley, D.C., Hansen, M.S.T., Rhodes, D., Sotrifler, C.A., Ni, H., Andrew, M. C., Bushman, F. D., Fenical, W., 2002. Thalassiolins A. C: New marine derived inhibitors of HIV c DNA integrase. Bioorgan. Med. Chem. 10, 3619-3625.

[133]. Calderon-Montano, J. M., Burgos-Moron, E., Pérez-Guerrero, C., Lopez-Lazaro, M., 2011. A review of the dietary flavonoid kaempferol. Medicinal Chemistry. 11, 298-344.

[134]. Winked-Shirley, B., 2001. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiology. 126, 485-493.

[135]. Miura, K., Nakatani, N., 1989. Antioxidative activity of flavonoids from Thyme (*Thymus vulgaris* L). Agricultural and Biological Chemistry. *53*, 3043-3045.

[136]. Rao, Y. K., Fang, S. H., Hsieh, S. C., Yeh, T. H., Tzeng, Y. M., 2009. The constituents of *Anisomeles indica* and their anti-inflammatory activities. J. Ethnopharmacol. 121, 292–296.

[137]. Shon, H. Y., Son, K. H., Kwon, C. S., Kang, S. S., 2004. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. Phytomedecine. 11, 666–672.

[138]. Ko, W. G., Kang, T. H., Lee, S. J., Kim, N. Y., Kim, Y. C., Sohn, D. H., Lee, B. H., 2000. Polymethoxyflavonoids from *Vitex rotundifolia* inhibit proliferation by inducing apoptosis in human myeloid leukemia cells. Food Chem. Toxicol. 38, 861–865.

[139]. Hirata, T., Fujii, M., Akita, K., Yanaka, N., Ogawa, K., Kuroyanagi, M., Hongo, D., 2009. Identification and physiological evaluation of the components from Citrus fruits as potential drugs for anti-corpulence and anticancer. Bioorg. Med. Chem. 17, 25–28.

[140]. Gurbuz, I., Yesilada, E., Ito, S., 2009. An anti-ulcerogenic flavonol diglucoside from *Equisetum palustre* L. J. Ethnopharmacol. 121, 360–365.

[141]. Sannomiya, M., Fonseca, V. B., Silva, M. A., Rocha, L. R. M., Dos Santos, L. C., HirumaLima, C. A., Britoc, A. R. M. S., Vilegas, W., 2005. Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. J. Ethnopharmacol. 97, 1–6.

[142]. Sutradhar, R. K., Rahman, A. K. M. M., Ahmad, M. U., Bachar, S. C., 2008**.** Bioactive flavones of *Sida cordifolia*. Phytochem. Lett. 1, 179–182.

[143]. Choi, H. J., Kim, J. H., Lee, C. H., Ahn, Y. J., Song, J. H., Baek, S. H., Kwon, D. H., 2009. Antiviral activity of quercetin 7-rhamnoside against porcine epidemic diarrhea virus. Antiviral Res. 81, 77–81.

[144]. Radev, R., 2010. Pharmacological effects of phenylethanoid glucosides. J. Clin. Med. 3, 20-23.

[145]. Guangmiao, F., Haihong, P., Yung, H. W., 2008. Naturally occurring phenylethanoid glucosides: potential leads for new therapeutics. Current Medicinal Chemistry. 15, 2592-2613.

[146]. Jimenez, C., Riguera, R., 1994. Phenylethanoid in plants: structure and biological activity. Nat. Prod. Rep. 11, 591-606.

[147]. Maeda, H., Dudareva, N., 2012. The shikimate pathway and aromatic amino Acid biosynthesis in plants. Annu. Rev. Plant Biol. *63*, 73-105.

[148]. Bender, S. L., Mehdi, S., Knowles, J. R., 1989. Dehydroquinate synthase: the role of divalent metal cations and of nicotinamide adenine dinucleotide in catalysis. Biochemistry. 28, 7555-7560.

[149]. Bender, S. L., Widlanski, T., Knowles, J. R., 1989. Dehydroquinate synthase: the use of substrate analogues to probe the early steps of the catalyzed reaction. Biochemistry. 28, 7560-7572.

[150]. Widlanski, T., Bender, S. L., Knowles, J. R., 1989. Dehydroquinate synthase: the use of iesubstrate analogues to probe the late steps of the catalyzed reaction. Biochemistry. 28, 7572-7582.

[151]. Chafai, C., 2016. Contribution à l'étude de la dynamique spatiale et de la biomasse du frêne dimorphe dans la cédraie de Ouled Yagoub W. de Khenchela. Thèse de Doctorat, Université de Batna 1, Algérie.

[152]. Emberger L., 1938. Les Arbres du Maroc et comment les reconnaître, Larose, paris, France.

[153]. Bellakhdar, J., 1997. La pharmacopée marocaine traditionnelle : médecine arabe ancienne et savoirs populaire. Ed. Ibis Press, Paris, France. p. 759.

[154]. Cosson, E., Durieu, M. D., 1855. Notes sur quelques espèces nouvelles d'Algérie. Bull. Soc. Bot. France. 2, 364-368.

[155]. Younis, T., Rashidkhan, M., Sajid, M., 2016. Protective effects of *Fraxinus xanthoxyloides* leaves against CCl_4 induced hepatic toxicity in rat. Complementary and Alternative Medicine. 16, 407-420.

[156]. Shah, S. M., Hussain, F., 2012. Ethnomedicinal plant wealth of Mastuj valley, Hindukush range, District Chitral, Pakistan. J. Med. Plant Res. 6, 4328–4337.

[157]. Hussain, F., Shah, S. M., Sher, H., 2007. Traditional source evaluation of some plants of Mastuj, District Chitral, Pakistan. Pak. J. Bot. 39, 339–354.

[158]. Sharma, P. K., Sethi, G. S., Sharma, S. K., Sharma, T. K., 2006. Ethnomedicinal observations among the inhabitants of cold desert area of Himachal Pradesh. Indian J. Trad. Knowl. 5, 358–361.

[159]. Hadroug, A., 2004. Sparation, purification, identification and biological activities of iridoid glucosides from the Oleaceae plants family. Master Thesis, M'sila University, Algeria.

[160]. Inouye H. 1991. Methods in plant biochemistry. Academic Press, Japan. 7, 99-100.

[161]. Agrawal, P. K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry. 31, 3307–3330.

[162]. Bock, K., Pedersen, C., 1983. Carbon-13 Nuclear Magnetic Resonance spectroscopy of monosaccharides Adv. Carbohydr. Chem. Biochem. 41, 27–49.

[163]. Godecke T, Kaloga M, H. Kolodziej H. 2005. A phenol glucoside, uncommon coumarins and flavonoids from *Pelargonium sidoides* DC. Z Naturforsch. 60b, 677-682.

[164]. Wu, T. S., Chang, F. C., Wu, P. L., 1995. Constituents of leaves of *Tetradium glabrifolium*. J. Chi. Chem. Soc. 42, 929-934.

[165]. Tanaka, T., Nakashima, T., Ueda, T., Tomii, K., Kouno, I., 2007. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem. Pharm. Bull. 55, 899-901.

[166]. Nicoletti, M., 1989. Iridoids. Rev. Latinoamer. Quim. 1, 131-159.

[167]. Yu, M., Sun, A., Zhang, Y., Liu, R., 2014. Purification of coumarin compounds from *Cortex fraxinus* by adsorption chromatography. Journal of Chromatographic Science. 52, 1033-1037.

[168]. Kikuchi, M., Yamauchi, Y., Yanase, C., Nagaoka, I., 1987. Structures of new secoiridoid glycosides from the leaves of *Syringa vulgaris*. Yakugaku Zasshi. 107, 245-248.

[169]. Gawronska-Grzywacz, M., Krazaczek, T., 2009. Flavonoids and coumarins from *Hieracum pilosella* L. Acta Societatis Botanicorum Poonaie. 78,189-195.

[170]. Huang, Y. H, Zeng, W. M., Li, G., 2014. Characterization of a new sesquiterpene and antifungal activities of chemical constituents from *Dryopteris fragrans* L. Molecules. 19, 507-513.

[171]. Adebayo, A. H., Tan, N. H., Akindahunsi, A. A., Zeng, G. Z., Zang, Y. M., 2010. Anticancer and antiradical scavenging activity of *Ageratum conyzoides* L. Pharmacogn. Mag. 6, 62-66.

[172]. Thakur, A., Jain, V., Hingorani, L., Ladha, K. S., 2009. Phytochemical studies on *Cissus quadrangularis* Linn. Pharmacogn. Res. 1, 213-215.

[173]. Ebada, S. S., Ayoub, N. A., Singab, A. N., Alaazizi, M. M., 2008. Phytophenolics from *Peltophorum africanum* with promising hepatoprotective activity. Pharmacogn. Mag. 4, 287-293.

[174]. Fiorentino, A., Darbrosca, B., Pacifico, S., Golino, A., Mastellone, C., Oriano, P., Monaco, P., 2007. Reactive oxygen species scavenging activity of flavone glucosides from *Melilotus neapolina*. Molecules. 12, 263-270.

[175]. Kasuma, K., Noda, N., Suzuki, M., 2003. Malonylated flavonol glucosides from the petals of *Clitoria ternatea*. Phytochemistry. 62, 229-237.

[176]. Nishina, A., Itagaki, M., Suzuki,Y., KoketsoM., Ninomiya, M., Sato, D., Suzuki, T., Hayakawa, S., Kuroda, M., Kimura, H., 2017. Effects of flavonoids and triterpene analogues from leaves of *Eleutherococcus sieboldianus* (Makino) Koidz. Himeukogi in 3T3-L1 Preadipocytes. Molecules. 22, 671-685.

[177]. Khalfallah, A., Karioti, A., Berrehal, D., Kabouche, A., Lucci, M., Bilia, A.-R., Kabouche, Z., 2014. A New triglycoside and other flavonol glucosides from *Astragalus armatus* Willd. *Rec. Nat. Prod.* 8, 12-18.

[178]. Tong, S. H., Yan, J., Chen, G., Lou, J., 2009. Purification of rutin and nicotiflorin from the flowers of *Edgeworthia chrysantha* Lindl. by high-speed Countercurrent Chromatography. J. Chromatogr. Sci. 47, 341-344.

[179]. Wang, Y., Tang, C. Y., Zhang, H., 2015. Hepatoprotective effects of kaempferol 3-O-rutinoside and kaempferol 3-O-glucoside from *Carthamus tinctorius* L. on CCl4-induced oxidative liver injury in mice. J. Food Drug Anal. 23, 310-317.

[180]. Adesina, S. K., Idowu, O., Ogundaini, A. O., Oladimeji, H., Olugbade, T. A., Onawunmi, G. O., Pais, M., 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytother. Res. 14, 371-374.

[181]. Ahmad, M., Gilani, A. H., Aftab, K. H., Ahmad, V. U., 1993. Effects of kaempfrol 3-O-rutinoside on rat blood pressure. Phytother. Res. 7, 314-316.

[182]. Gamal-Ideen, A. M., Kawashty, S. A., Ibrahim, L. F., Shabana, M. M., El-Negoumy, S. I., 2004. Evaluation of antioxidant, antiinflammatory and antinociceptive properties of aerial parts of *Vicia sativa* and its flavonoids. J. Nat. Remed. 4, 81-96.

[183]. Suzuki, H., Ikeda, T., Matsumoto, T., Noguchi, M., 1976. Isolation and identification of rutin from cultured cells of *Stevia rebaudiana* Bertoni. Agr. Biol. Chem. 40, 819-820.

[184]. Yoo, G., Park, S. J., 2015. Flavonoids isolated from *Lespedeza cuneata* G. and their inhibitory effects on nitric oxide production in lipopolysaccharide stimulated BV-2 microglia cells. Pharmacog. Mag. 11, 651-656.

[185]. Lee, T. H., Kuo, Y. C., Wang, G. J., Kuo, Y. H., Chag, C. I., Lu, C. K., Lee, C. K., 2002, Five new phenolics from the roots of Ficus beecheyana. J. Nat. Prod. 65, 1497-1500.

[186]. Greca, M. D., Ferrara, M., Fiorentino, A., Monaco, P., Previtera, L., 1998. Antianagal compounds from *Zantedeschia aethiopica*. Phytochemistry. 49, 1299-1304.

[187]. Wang, H., Ding, Y., Zhou, J., Sun, X., Wang, S., 2009. The in vitro and in vivo antiviral effects of salidroside from *Rhodiola rosea* L. against coxsackievirus B3. Phytomedicine. 16, 146–155.

[188]. Yahara, S., Satoshiro, M., Nishioka, I., Nagasawa, T., Oura, H., 1985. Isolation and characterization of phenolic compounds from *Coptidis rhizoma*. Chem. Pharm. Bull. 33, 527-531.

[189]. Zhang, A., Ma, Y., Yuan, Z., 2010. Chemical constituents from the roots of *Rhodiola algida* var. *tangutica*. Asian Journal of Traditional Medicines. 5, 138-144.

[190]. Chen, X., Liu, J., Gu, X., Ding, F., 2008. Salidroside attenuates glutamateinduced apoptotic cell death in primary cultured hippocampal neurons of rats. Brain Res. 1238, 189–198.

[191]. Li, M., Dong, C., Huai, L., Bende, T., Li, S., Ying, W., 2008. Anti-fatigue effects of salidroside in mice. J. Med. Coll. 23, 88–93.

[192]. Liu, S., Yu, X., Hu, B., Zou, Y., Li, J., Bo, L., Deng, X., 2015. Salidroside rescued mice from experimental sepsis through anti-inflammatory and anti-apoptosis effects. J. Surg. Res. 195, 277–283.

[193]. Diaz, L. A., Abad, M. M., Fernandez, M. L., Recuero, C. C., Villaescusa, C. L., Silvan, S. A., Bermejo, B. P., 2001. Lignan and phenylpropanoid glycosides from *Phillyrea latifolia* and their in vitro anti-inflammatory activity. Planta. Med. 67, 219–223.

[194]. Fang, D., Chen, Y., Xu, B., Ren, K., He, Z., He, L., Lei, Y., Fan, C., Song, X., 2014. Development of lipid-shell and polymer core nanoparticles with water-soluble salidroside for anti-cancer therapy. Int. J. Mol. Sci. 15, 3373–3388.

[195]. Nan, J., Jiang, Y., Park, E. J., Ko, G., Kim, Y. C., Sohn, D. H., 2003. Protective effect of *Rhodiola sachalinensis* extract on carbon tetrachloride-induced liver injury in rats. J. Ethnopharmacol. 84, 143–148.

[196]. Sugiyama, M., Kikushi, M., 1993. Phenylethanoid glucosides from *Osmanthus asiaticus*. Phytochemistry. 32, 1553-1555.

[197]. Ishii, T., Yanagisawa, M., 1998. Synthesis, separation and NMR spectral analysis of methylapiofuranoside. Carbohydr. Res. 313, 189-192.

[198]. Warashina, T., Nagatani, Y., Noro, T., 2004. Constituents from the bark of Tabebuia impetiginosa. Phytochemistry. 65, 2003-2011.

[199]. Lin, S., Liu, M., Wang, S., Li, S., Yang, Y., Shi, J., 2010. Phenolic and phenylethanoid glucosides from branch *of Fraxinus sieboldiana*. Zhongguo Zhong Yao Za Zhi. 35, 992-996.

[200]. Varughese, T., Rahaman, M., Kim, N. S., Cho, S. C., Moon, S. S., 2009. Gamnamoside, a phenylpropanoid glucoside from Persimmon leaves (Diospyros Kaki) with an inhibitory effect against an alcohol metabolizing enzyme. Bull. Korean Chem. Soc. 30, 1035-1038.

[201]. Ersoz, T., Tasmemir, D., Calis, I., 2002. Phenylethanoid glucosides from *Scutellaria galericulata*. Turk. J. Chem. 26, 465-471.

[202]. Liu, P., Deng, R. X., Duan, H. Q., Yin, W. P., Zhao, T. Z., 2009. Phenylethanoid glucosides from the roots of *Phlomis umbrosa*. J. Asian Nat. Prod. Res. 11, 69-74.

[203]. Ghaffari, H., Dinani, M. S., 2018. Isolation of phenylpropanoid glucosides from *Allium tripedale* Trautv. Journal of Reports in Pharmaceutical Sciences. 7, 116-122.

[204]. James, H., Robert, E., 1986. Coniferin-an astringent glycoside in citrus. Phytochemistry. 26, 322-323.

[205]. Kurkin, V. A., 2003. Phenylpropanoids from medicinal plants: distribution, classification, structural analysis, and biological activity. Chem. Nat. Comp. 39, 123-153.

[206]. Jun, H. I., Jang, H., Ahn, D., Kim, D. K., Yang, J. H., Yun, B. S., Kim, Y. S., 2015. Isolation and characterization of phenolic compound from *Allium hookeri* root for potential use as antioxidant in foods. Food Sci. Biotechnol. 24, 2031-2034.

[207]. Gohari, A. R., Saeidnia, S., Moghadam, M. B., Amin, G., Handjiakhoondi, A., 2009. Phytochemical and chemotaxonomic investigation of Stelleropsis iranica. Australian Journal of Basic and applied Sciences. 3, 3423-3427.

[208]. Sugiyama, M., Nagayama, E., Kikuchi, M., 1993. Lignan and phenylpropanoid glucosides from *Osmanthus asiaticus.* Phytochemistry. 33, 1215-1219.

[209]. Hassan, H. M., Nowak, G., 2001. Germ kranolidy W *Centaurea crocodylium* L. Herb Prolonicer. 47, 122-124.

[210]. Nowak, G., Drozoz, B., Holub, M., Lagodzinska, A., 1986. Sequisterpene lactonesxxxi. guaianolidos in the subgenus psephellus (Cass) selimalli genus centaurea L. Acta. Soc. Bot. Pol. 55, 629-637.

[211]. Daniewski, W., Wawrzun, A., Bloszyk, E., Drozdz, B., Holub, M., 1982. Sesquiterpenic lactones from *Grossheimia macrocephala*. Structure of grosheiminlo. Collect Czechoslov Chem. Commun. 47, 3160-3163.

[212]. Us, M. R., Zin, T., Abduarazak, M., Ahmad, B. A., 2015. Chemistry and pharmacology of syringing, a novel bioglucoside. Asian J. Pharm. Clin Res. 8, 20-25.

[213]. Schumacher, B., Scholle, S., Holzl, J., Khudeir, N., Hess, S., Muller, C. E., 2002. Lignans isolated from Valerian: Identification and characterization of a new Olivil derivative with partial agonistic activity at A1 adenosine receptors. J. Nat. Prod. 65, 1479-1485.

[214]. Piccinelli, A. L., Arana, S., Caceres, A., Bianka, R. E., Sorrentino, R., Rastrelli, L., 2004. New lignans from the roots of *Valeriana prionophylla* with antioxidative and vasorelaxant activities. J. Nat. Prod. 67, 1135-1140.

[215]. Bodesheim, U., Holzl, J., 1997. Isolation and receptor binding properties of alkaloids and lignans from *Valeriana officinalis*. Pharmazie. 52, 386-391.

[216]. Deyama, T., Ikawa, T., Kitagawa, S., Nishibe, S., 1986. The Constituents of *Eucommia ulmoides* OLIV. III. Isolation and structure of new lignan glucoside. Chem. Pharm. Bull. 34, 523-527.

[217]. Chiba, M., Okabe, K., Hisada, S., Shima, K., Takemoto, T., Nishibe, S., 1979. Elucidation of the structure of a new lignin glucoside from *Olea europaea* by Carbon-13 nuclear magnetic resonance spectroscopy. Chem. Pharm. Bull. 27, 2868-1873.

[218]. Blois, M. S., 1958. Antioxidant determinations by the use of a stable free radical. Nature. 26, 1199–1200.

[219]. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Evans, C. R., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Bio. Med. 26, 1231-1237.

[220]. Lee, B. C., Lee, S. Y., Lee, H. J., Sim, G. S., Kim, J. H., Cho, Y. H., Lee, D. H., Pyo, H. B., Choe, T. B., Moon, D. C., Yun, Y. P., Hong, J. T., 2007. Anti-oxidative and photo-protective effects of coumarins isolated from *Fraxinus chinensis*. Archives of Pharmacol. Res. 30, 1293-1301.

[221]. Carocho, M., Ferreira, I. C. F. R., 2013a. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem. Toxicol. 51, 15-25.

[222]. Rice-Evans, C. A., Miller, N. J., Paganga, G., 1996. Structure –anti-oxidant activity relationships of flavonoids and phenolic acids. Free Rad. Biol. Med. 20, 933-956.

[223]. Kokanova, N. Z., Kondeva, B. M., Zheleva, D. D, Tzankova, V., Nikolov, S., Heilmann, J., Nedialkov, P. T., 2015. 6-Methoxyflavonol glycosides with *in vitro* hepatoprotective activity from *Chenopodium bonus-henricus* roots. Nat. Prod. Commun. 10, 1377-1380.

[224]. Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152-178.

[225]. Zhishen, J., Mengcheng, T., Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 64, 555-559.

[226]. Hatano, T., Kagawa, H., Yasuhara, T., Okuda, T. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull. 36, 2090-2097.

Abstract

The nature was and still an interesting source of potential chemotherapeutic agents. Whatever the reasons for the presence of these compounds in nature, they provide invaluable resources that have been used to find new drug molecules. In this context, the Algerian medicinal plant *Faxinus xanthoxyloides* of the Oleaceae family has been selected for phytochemical investigation and anti-oxidant screening.

This work allowed the isolation by chromatographic methods (TLC, PTLC, CC and LPLC), and the characterization by spectroscopic analysis (NMR and MS) of 17 secondary metabolites including 2 coumarin-secoiridoid glucosides, 2 secoiridoids, 3 coumarins, 2 flavonoids, 4 phenylethanoids, 2 phenylpropanoids and 2 lignans.

10 compounds have been isolated from the methanolic extract of the stem bark from which one new structure was established as a coumarin-secoiridoid diglucosides. The known secondary metabolites consist of 1 coumarin-secoiridoid, 2 coumarins, 2 phenylethnoids, 2 phenylpropanoids and 2 lignans.

From the methanolic extract of the leaves 9 secondary metabolites have been identified. They were devided into 2 secoiridoids with oleoside skeleton, 3 coumarins among them 2 common glucosides with the bark and one coumarin aglucone, two flavonoid diglucosides and two phenylethanoids.

The molecular structures of the isolated compounds **1-17** were mainly elucidated by the use of NMR techniques 1D and 2D (^{1}H, ^{13}C, DEPT, HSQC, HMBC and ROESY), high resolution mass spectrometry (HR-ESI-MS), measurement of the optical rotation $[\alpha]_D^{20}$ and by comparison with the literature data.

The anti-oxidant activity of leaves and bark extracts was estimated by the DPPH free radicals scavenging and ABTS radical cation decolorization assay and the results showed that bark extracts had an interesting activity.

Finally, it is important to indicate that among known compounds reported in the literature, one phenylethanoide and two lignan glucosides have been found for the first time in Fraxinus genus. Furthermore, this study contributes to determine the chemical composition of this genus.

Keywords: Oleaceae, *Fraxinus xantoxyloides*, Coumarins-secoiridoids, Secoiridoids, coumarins, Flavonoids, Phenylethanoides, Phenylpropanoides, Lignans, NMR 1D and 2D, HRMS, Anti-oxidant activity.

Résumé

La nature comme source d'agents potentiels chimio-thérapeutiques suscite un intérêt considérable. Quelle que soit les raisons de la présence de ces composés dans la nature, ils fournissent des ressources de valeur inestimable qui ont été employées pour trouver de nouvelles drogues. Dans ce context, la plante médicinale algérienne *Faxinus xanthoxyloides* de la famille Oleaceae a été sélectionnée pour investigation phytochimique et screening anti-oxydant.

Ce travail a permis l'isolement par les méthodes chromatographiques (CCM, CCMP, CC, CLFP) et la caractérisation par les différentes méthodes spectroscopiques (RMN et HRMS) de 17 métabolites secondaires dont 2 composés de nature coumarin-secoiridoidique, 2 secoiridoides, 2 coumarines, 2 flavonoides, 4 phenylethnoides, 2 phenylpropanoides, 2 lignanes.

10 composés ont été isolés à partir de l'extrait methanolique de l'écorce de tige à partir duquel une nouvelle structure a été établie en tant que coumarine-secoiridoidique diglucosidique. Les métabolites secondaires connus au nombre de 9 se répartissent en 1 coumarine-secoiridoide, 2 coumarin, 2 phenylethanoides, 2 phenylpropanoides et 2 lignanes.

A partir de l'extrait methanolique des feuilles, 9 métabolites ont été identifiés. Il s'agit de 2 secoiridoides à squelette oleoside, 3 coumarines parmi eux 2 glucosides communs avec l'écorce et une coumarine aglucone, 2 flavonoides diglucosidiques et 2 phenylethanoides.

Les structures moléculaires des composés isolés **1-17**ont été élucidées principalement par l'utilisation des différentes techniques de RMN 1D et 2D (^{1}H, ^{13}C, DEPT, HSQC, HMBC et ROESY), la spectrométrie de masse de haute résolution (HR-ESI-MS), par la mesure du pouvoir rotatoire $[\alpha]_D^{20}$ et par la comparaison avec les données de la littérature.

L'activité anti-oxydante a été réalisée sur les extraits des feuilles et d'écorce par le piégeage des radicaux libres DPPH et le dosage de la décoloration des cations radicaux ABTS. Les résultats ont montré que les extraits d'écorce ont une activité intéressante.

Il est important de signaler que parmi les composés déjà cité dans la littérature, 1 phenylethanoide et 2 lignanes glucosylés ont été trouvés pour la première fois dans le genre Fraxinus, ce qui révèle l'importance de cette investigation permettant une meilleure connaissance de la composition chimique de ce genre.

Mots clés : Oleaceae, *Fraxinus xanthoxyloides*, Coumarines-secoiridoides, Secoiridoides, Coumarines, Flavonoides, Phenylethanoides, Phenylpropanoides, Lignanes, RMN 1D et 2D, HRMS, Activité anti-oxydante.

ملخص

تعتبر الطبيعة مصـــدر للمواد ذات القدرة العلاجية الكبيرة، .و مهما تكن أســباب وجود هذه المواد في الطبيعة فإنها تشـكل موارد مهمة تسـتعمل لاكتشـاف عقارات جديدة في هذا السـياق قمنا باختبار النبات الطبي الجزائري الدردار *Fraxinus xanthoxyloides* من عائلة Oleaceae لدراســة فيتوكيميائية وتشــخيص مضاد للأكسدة.

ســمح هذا البحث بعزل وتحديد بنية 17 مركب طبيعي وذلك باســتعمال الطرق الكروماتوغرافية وباسـتعمال طرق التحليل الطيفي كمطيافية الرنين المغنطيسـي النووي بجميع أنواعها، مطيافية الكتلة، حسـاب زاوية التدوير النوعي والمقارنة مع الدراسات السابقة. تنقسم هذه المركبات الي 2 مركب ذو طبيعة كومارينية-سـيكواريدويدية، 2 مركب ذو طبيعة ســيكواريدويدية، 3 مركب ذو طبيعة كومارينية، 2 مركب ذو طبيعة فلافونويدية، 4 مركب ذو طبيعة فينيلية ايثانويد ية، 2 مركب ذو طبيعة فينيلية بروبانويدية و 2 مركب من نوع اللينان.

تم عزل 10 مركبات من المســتخلص الميثانولي لقشــور الســاق منها مركب واحد جديد ذو طبيعة كومارينية –ســيكواريدويدية و 9 مركبات معروفة تشــمل المركبات المعروفة 2 كومارينات، 2 فينيل ايثانويدات، 2 فينيل بروبانووید و 2 لينان.

من خلال المســتخلص الميثـانولي للأوراق تم عزل 9 مركبـات طبيعيـة تم التعرف عليهـا منهـا 2 سـيكواريدويدية، 3 كومارينية منها 2 سـبق فصـلها من القشـور وواحد كومارين اغليكون، 2 فلافونويدية و2 فينيل ايثانويدات.

قدرت النشاطية المضادة للأكسدة لمستخلصات كل من الأوراق والقشور بواسطة تقنية الجذور الحرة DPPH و ABTS : وقد أظهرت النتائج ان مستخلصات القشور لها نشاطاً مثيرًا للاهتمام.

وأخيرا تجدر الإشـارة الي انه من بين المركبات التي تم ذكرها في الدراسـات السـابقة 1 مركب فينيل إيثانويدات و2 مركب من نوع اللينان تم عزلها لأول مرة من جنس Fraxinus الذي يســمح بإظهار أهمية هذا العمل في المعرفة الجيدة للتركيب الكيميائي لنباتات هذا النوع.

الكلمات المفتاحية:

Oleaceae، *Fraxinus xantoxyloides*، الكومارينات-السيكواريدويدية، السيكواريدويدات، الكومارينات، الفلافونويدات، فينيل ايثانوويدات، فينيل بروبانوويدات، أللينانات، مطيافية الرنين المغناطيسي، مطيافية الكتلة، الفعالية المضادة للأكسدة.

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Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Isofraxisecoside, a new coumarin-secoiridoid from the stem bark of Fraxinus xanthoxyloides

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To cite this article: Aldjia Hadroug, Rachid Belhattab, Kalina Alipieva & Paraskev T. Nedialkov (2018): Isofraxisecoside, a new coumarin-secoiridoid from the stem bark of Fraxinus xanthoxyloides, Natural Product Research, DOI: 10.1080/14786419.2018.1475379

To link to this article: https://doi.org/10.1080/14786419.2018.1475379

NATURAL PRODUCT RESEARCH, 2018
https://doi.org/10.1080/14786419.2018.1475379

Isofraxisecoside, a new coumarin-secoiridoid from the stem bark of *Fraxinus xanthoxyloides*

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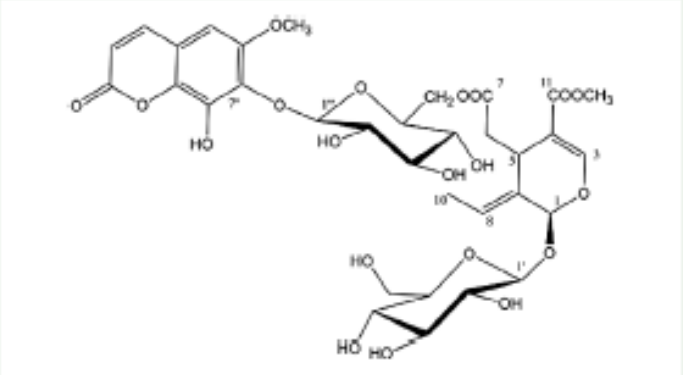
ABSTRACT

A new coumarin-secoiridoid diglucoside, named Isofraxisecoside, was isolated from the stem bark of *Fraxinus xanthoxyloides* (G. Don) Wall. ex A. DC. along with nine known compounds. The structure of new compound has been determined on the basis of ^{1}H, ^{13}C, 2D NMR and HRMS methods.



ARTICLE HISTORY
Received 23 January 2018
Accepted 8 May 2018

KEYWORDS
Fraxinus xanthoxyloides (G.Don) Wall. ex A. DC; Isofraxisecoside; new coumarin-secoiridoid

1. Introduction

The genus *Fraxinus* L. belongs to the Oleaceae family and comprises about 43 species. In the flora of Algeria the genus is represented by two species *Fraxinus xanthoxyloides and Fraxinus angustifolia* (Wallender 2008; Quezel and Santa 1963). *Fraxinus* species have been used in folk medicine in different parts of the world for their diuretic and mild purgative effects as well as for the treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp (Kostova and Iossifova 2007; Si et al. 2015). The occurrence of coumarins, secoiridoids and phenylethanoids is a characteristic feature of this *Fraxinus* species (Kostova

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1475379.

2001). However, no phytochemical investigation has been carried out on the Algerian *Fraxinus* species to date. In the present study, we describe the isolation and structure elucidation of Isofraxisecoside, a new natural compound consisting of a coumarin glucoside unit bound via an ester function to a secoiridoid glucoside unit together with nine known glucosides.

2. Results and discussion

In our investigation of the stem bark of *F. xanthoxyloides*, a novel coumarin secoiridoid diglucoside named Isofraxisecoside (**1**), together with nine known compounds were isolated. The known compounds were identified by comparison of their spectroscopic data with those reported in the literature as fraxisecoside (**2**) (Xiao et al. 2008), fraxin (**3**) (Yu et al. 2014), cichoriin (**4**) (Kuwajima et al. 1992), osmanthuside H (**5**) (Varughese et al. 2009), syringin (**6**) (Gohari et al. 2009), coniferin (**7**) (Sugiyama et al. 1993), calcelarioside B (**8**) (Ersoz et al. 2002), 8-hydroxypinoresinol-4′-*O*-β-glucoside (**9**) (Piccinelli et al. 2004), 1-acetoxypinoresinol-4′-β-glucoside (**10**) (Tsukamoto et al. 1984).

Compound (**1**) was obtained as a yellow amorphous powder. The HR-ESI-MS showed deprotonated molecular ion $[M-H]^-$ at *m/z* 755.20404 pointing out molecular formula $C_{33}H_{40}O_{20}$ which was confirmed by ^1H and ^{13}C NMR data. The UV spectrum showed absorption maxima at 229.5, 328.5, 335.5, 345.0 suggesting a conjugated aromatic system. Detailed analysis of its ^1H, ^{13}C, HSQC, HMBC and ROESY spectra indicated the presence of two structural units: a secoiridoid glucoside and a coumarin glucoside. The signals in ^1H NMR spectrum of three protons at δ_H 6.04 (1H, d, J = 9.3 Hz, H-3″), δ_H 7.83 (1H, d, J = 9.3 Hz, H-4″) and δ_H 6.84 (1H, s, H-5″) as well as the anomeric proton at δ_H 4.85 (1H, d, J = 7.8) indicated a 6-, 7-, 8-substituted coumarin glucoside structure. A singlet at δ_H 3.84 suggested the presence of OCH_3 group and the observed cross-peak in HMBC spectrum between methoxyl protons and C-6″ (δ_C 150.09) and correlation in ROESY experiment between H-5″ and the same protons confirmed the attachment of OCH_3 at C-6″. The spectroscopic data of the coumarin part were consistent with those of the uncommon coumarin isofraxoside (fraxetin–7-*O*–β–glucoside) (Godecke et al. 2005). Another set of signals at δ_H 7.50 (1H, s, H-3), δ_H 5.87 (1H, s, H-1), δ_H 6.05 (1H, overlapped, H-8), δ_H 1.61 (3H, **dd**, J = **1.1**, **7.2** Hz, Me-10) and δ_H 4.85 (1H, d, J = 7.8 Hz, H-1′) and the corresponding carbon signals at δ_C **155.38**, 95.39, 125.32, 13.63 and 100.69 were attributed to the secoiridoid glucoside moiety. The presence of another methoxy group was observed (δ_H 3.68, s; δ_C 52.53) and the position of methylester was determined at C-11 by correlation between the signal of OCH_3 and C=O (δ_C 169.32, C-11) in the HMBC spectrum. The signals of the secoirdoid part corresponded well to the previously reported oleoside-11-methylester (Damtoft et al. 1992). Further correlations between H-1‴ (δ_H 4.85) and C-7″ (δ_C 133.49) and between H_a-6‴ (δ_H 4.33), H_b-6‴ (δ_H 4.21) and C-7 (δ_C 173.74) confirmed glycosylation of coumarin part at C-7″ and ester linkage between OH group of C-6‴ and carboxyl group of oleoside at C-7. The presence of D-glucose was authenticated by acid hydrolysis and synthesis of its tolylthiocarbamoyl-thiazolidine derivative (Tanaka et al. 2007). On the basis of these evidences, compound **1** was identified as fraxetin-7″-*O*-[11-methyl-oleosidyl-(7–6‴)]-β-D-glucopyranoside, named isofraxisecoside and its structure is shown in Figure 1.

Isofraxisecoside (**1**) is the third example of a natural compound consisting of one coumarin glucoside unit linked to a secoiridoid moiety of oleoside type after previously described

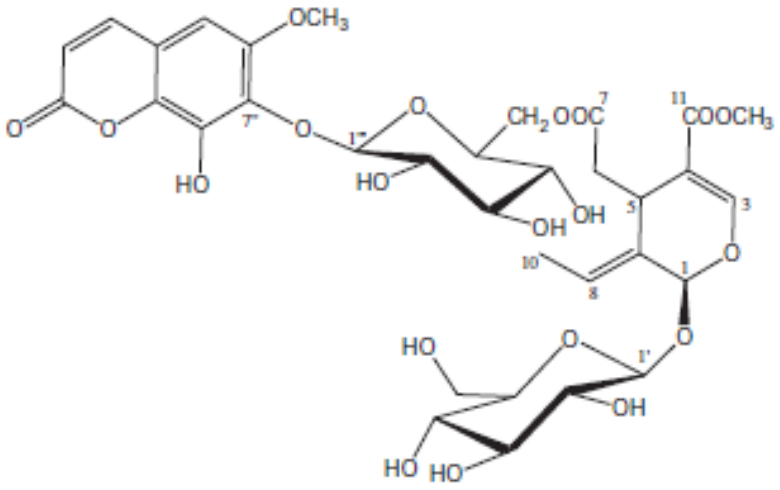

Figure 1. Structure of compound **1**.

escuside (Iossifova et al. 2002) and fraxisecoside (Xiao et al. 2008) isolated from the same genus.

3. Experimental

3.1. General experimental procedures

Optical rotation was recorded in MeOH using a Galan-Taylor Prism polarimeter. NMR spectra were acquired on Bruker AVII + 600 spectrometer (Bruker, Karlsruhe, Germany), ^{1}H NMR (600.11 MHz), ^{13}C NMR (150.91 MHz) in CD_3OD, purchased from Deutero-GmbH (Kastellaun, Germany), with TMS as internal standard. UV spectra: Helios Gamma UV spectrophotometer (Thermo Scientific, Bremen, Germany) in MeOH. HR-ESI-MS analysis was performed on a Thermo Scientific Q Exactive Plus (Bremen, Germany) in negative mode.

Column chromatography (CC) on Polyamide 6 (Fluka, Germany), Sephadex LH-20 and Silica gel 60, particle size 0.063–0.200, 70–230 mesh ASTM (Merck, Darmstadt, Germany) as well as Lobar chromatography (Lobar RP-8 and RP-18, Merck, Darmstadt, Germany) were used for separation and purification of the individual compounds. Preparative thin layer chromatography (PTLC) was performed on pre-coated plates 60 F_{254} 0.5 × 0.25 mm and thin layer chromatography (TLC) was performed on 60 F_{254} plates (Merck, Darmstadt, Germany). Separation was visualized by spraying with 20% H_2SO_4 in ethanol (v/v) solution. All solvents used for chromatographic purposes were of analytical grade.

3.2. Plant material

The stem bark of *F. xanthoxyloides* was collected in June 2014 from the region of Khenchela, Algeria. The plant was kindly identified by Prof Mohamed Kaabache, Setif University, Algeria. A voucher specimen, with the identification number 06/2014/KFX was deposited in the Herbarium of the Department of Biochemistry, Setif University.

3.3. Extraction and isolation

The stem bark material of *F. xanthoxyloides* was air dried and powdered by electric mill. The plant material was subjected to exhaustive extraction with MeOH at room temperature (3 × 48 h). The methanol extract was filtered and evaporated under reduced pressure to yield a residue (22.11 g). Part of methanolic extract (10 g) was dissolved in water and applied on polyamide 6 column chromatography with H_2O–MeOH gradient system (100:0 to 0:100). Using TLC analysis on silica gel ($CHCl_3$:MeOH:H_2O, 60:22:4) fractions having similar profiles were combined to give nine main fractions. A part of fraction F1 (1.8 g) was dissolved in water and chromatographed on Lobar column (RP-8, size B), using H_2O-MeOH (5% to 55%) gradient to afford seven sub-fractions. Purification of sub-fraction F1-1 (23.9 mg) by PTLC using $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v) eluent yielded 3.3 mg of compound **9**. F1-2 (144.8 mg) was subjected to Sephadex LH-20 CC using MeOH:H_2O (2:1/v:v) as mobile phase to give two fractions and a fraction was then purified by PTLC with $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v) eluent to afford compound **3** (8.9 mg) and compound **5** (28.7 mg). Sub-fraction F1-6 (51.3 mg) was subjected to Sephadex LH-20 column chromatography, using MeOH:H_2O (2:1/v:v) as eluent, to give compound **1** (27.8 mg) and compound **2** (15.6 mg). Fraction F3 was dissolved in water and applied on Lobar column (RP-8, size B), using a gradient of H_2O:MeOH (5% to 55%) to obtain eight sub-fractions. Sub-fraction F3-6 was subjected to Sephadex LH -20 CC and eluted with MeOH:H_2O (2:1/v:v) to provide compound **7** (14.3 mg). Compound **6** (12 mg) was isolated from fraction F3-8 (39 mg), which was submitted to CC over Sephadex LH-20 and eluted with MeOH:H_2O (2:1/v:v). Fraction F4 (550 mg) was dissolved in water and applied on Lobar column (RP-8, size B) eluting with H_2O:MeOH (5% to 55%) gradient to obtain thirteen sub-fractions. Sub-fractions F4-3 (43 mg) and F4-13 (33.3 mg), were separately purified by preparative TLC, developed with $CHCl_3$:MeOH:H_2O (60:22:4/v:v:v), to afford compound **4** (5.1 mg) and compound **10** (23.1 mg), respectively. Fraction F7 (470 mg) was applied on Lobar column (RP-8, size B), using H_2O:MeOH (20% to 50%) gradient to yield compound **8** (111 mg).

3.4. Acid hydrolysis of compound 1

The absolute configuration of the sugar was established using the method of Tanaka et al. (2007) with some modifications (Kokanova-Nedialkova et al. 2015). Briefly, compound **1** (5 mg) was refluxed with 2 mL of 2 N HCl-MeOH (1:1) for 2 h. The reaction mixture was filtered through Diaion HP-20SS followed by subsequent elution with H_2O and MeOH. The water portion was filtered through Amberlite IRC 86 resin and then evaporated to dryness. The dry water eluate was treated with a solution (0.1 mL) of L-cysteine methyl ester in pyridine (5 mg/mL) at 60 °C for 1 h. A solution (0.1 mL) of o-tolylisothiocyanate in pyridine (5 mg/mL) was added to the mixture and heated at 60 °C for 1 h. The resulting solution was analyzed using HPLC [Purospher STAR RP-18 5 µm column (Merck; 4.6 × 250 mm) with 25% ACN in 50 mM H_3PO_4, flow rate 1 mL/min, UV detection at 250 nm]. The presence of D-glucose (t_R value of the tolylthiocarbamoyl-thiazolidine derivative was 18.7 min) was found in the residue.

Isofraxisecoside (**1**): Yellow amorphous powder, $[\alpha]_D^{20} = -135.8$ (c = 0.095, MeOH); UV max (MeOH): (log ε) 229.5 (2.59), 328.5 (0.86), 335.5 (0.93), 345.0 (0.98) nm; ^1H (CD_3OD, 600.11 MHz) δ: 5.87 (H-1, s, 1H), 7.50 (H-3, s, 1H), 3.87 (H-5, overlapped, 1H), 2.66 (H_a-6, dd,

5.2, 14.1, 1H), 2.43 (H_b-6, dd, 8.6, 14.1, 1H), 6.05 (H-8, overlapped, 1H), 1.61 (H-10, dd, 1.1, 7.2, 3H), 3.68 (OMe-11, s, 3H), 4.85 (H-1′, d, 7.8, 1H), 3.28 – 3.59 (H-2′, H-3′, H-4′, H-5′, m, 4H), 3.87 (H_a-6′, d, 11.5, 1H), 3.70 (H_b-6′, d, 11.6, 1H), 6.04 (H-3″, d, 9.3, 1H), 7.83 (H-4″, d, 9.3, 1H), 6.84 (H-5″, s, 1H), 3.84 (OMe-6″, s, 3H), 4.85 (H-1‴, d, 7.8, 1H), 3.57 (H-2‴, m, 1H), 3.48 (H-3‴, t, 9.0, 1H), 3.42 (H-4‴, t, 9.0, 1H), 3.53 (H-5‴, m, 1H), 4.33 (H_a-6‴, dd, 1.6, 11.8, 1H), 4.21 (H_b-6‴, dd, 6.3, 11.8, 1H); ^{13}C (CD_3OD, 150.91 MHz) δ: 95.39 (C-1), 155.38 (C-3), 109.26 (C-4), 31.45 (C-5), 41.33 (C-6), 173.74 (C-7), 125.32 (C-8), 129.96 (C-9), 13.63 (C-10), 169.32 (C-11), 52.53 (OMe-11), 100.69 (C-1′), 74.28 (C-2′), 77.35 (C-3′), 71.14 (C-4′), 77.89 (C-5′), 62.12 (C-6′), 165.34 (C-2″), 108.11 (C-3″), 147.49 (C-4″), 105.20 (C-5″), 150.09 (C-6″), 56.77 (OMe-6″), 133.49 (C-7″), 155.39 (C-8″), 145.77 (C-9″), 108.28 (C-10″), 106.30 (C-1‴), 74.82 (C-2‴), 77.41 (C-3‴), 70.95 (C-4‴), 75.35 (C-5‴), 65.01 (C-6‴); HR-ESI-MS: *m/z* found: 755.20404 for $[M - H]^-$; calcd. for $C_{33}H_{39}O_{20}$: 755.2038 $[M - H]^-$.

Acknowledgements

All experiments were carried out in the laboratory of the Institute of Organic Chemistry with Center of Phytochemistry, Bulgarian Academy of Sciences.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Financial support was provided by the Algerian Ministry of Higher Education and Scientific Research is gratefully acknowledged.

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References

Damtoft S, Franzyk H, Jensen SR. 1992. Excelsioside, a secoiridoid glucoside from *Fraxinus excelsior*. Phytochemistry. 31:4197–4201.

Ersoz T, Tasdemir D, Calis I. 2002. Phenylethanoid glucosides from *Scutellaria galericulata*. Turk J Chem. 26:465–471.

Godecke T, Kaloga MH, Kolodziej H. 2005. A phenol glucoside, uncommon coumarins and flavonoids from *Pelargonium sidoides* DC. Z Naturforsch. 60b:677-682.

Gohari AR, Saeidnia S, Moghadam MB, Amin G, Hadjiakhoondi A. 2009. Phytochemical and chemotaxonomic investigation of *Stelleropsis iranica*. Aust J Basic Appl Sci. 3:3423–3427.

Iossifova T, Vogler B, Kostova I. 2002. Escuside, a new coumarin-secoiridoid from *Fraxinus ornus* bark. Fitoterapia. 73:386–389.

Kokanova-Nedialkova Z, Kondeva-Burdina M, Zheleva-Dimitrova D, Tzankova V, Nikolov S, Heilmann J, Nedialkov PT. 2015. 6-Methoxyflavonol glycosides with *in vitro* hepatoprotective activity from *Chenopodium bonus-henricus* roots. Nat Prod Commun. 10:1377–1380.

Kostova I. 2001. *Fraxinus ornus* (L.). Fitoterapia. 72:471–480.

Kostova I, Iossifova T. 2007. Chemical components of *Fraxinus* species. Fitoterapia. 78:85–106.

Kuwajima H, Morita M, Takaishi K, Inoue K, Fujita T, He ZD, Yang CR. 1992. Seciridoid, coumarin and secoiridoid-coumarin glucosides from *Fraxinus chinensis*. Phytochemistry. 31:1277–1280.

Piccinelli AL, Arana S, Caceres A, Bianca RE, Sorrentino R, Rastrelli L. 2004. New lignans from the roots of *Valeriana prionophylla* with antioxidative and vasorelaxant activities. J Nat Prod. 67:1135–1140.

Quezel P, Santa S. 1963. New flora of Algeria and Southern desert regions. Tome 2. Paris: CNRS.

Si CL, Ren XD, Du ZG, Huang XF, Wu L. 2015. Purification and spectroscopic elucidation of new coumarin glucoside in *Fraxinus velutina* Heartwood. Chem Nat Comp. 51:1059–1061.

Sugiyama M, Nagayama E, Kikuchi E. 1993. Lignan and phenylpropanoid glucosides from *Osmanthus asiaticus*. Phytochemistry. 33:1215–1219.

Tanaka T, Nakashima T, Ueda T, Tomii K, Kouno I. 2007. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem Pharm Bull. 55:899–901.

Tsukamoto H, Hisada S, Nishibe S, Roux DG. 1984. Phenolic glucosides from *Olea europaea* subsp. *africana*. Phytochemistry. 23:2839–2841.

Varughese T, Rahaman M, Kim NS, Cho SC, Moon SS. 2009. Gamnamoside, a phenylpropanoid glucoside from *Persimmon* leaves (*Diospyros kaki*) with an inhibitory effect against an alcohol metabolizing enzyme. Bull Korean Chem Soc. 30:1035–1038.

Wallender E. 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. Plant Syst Evol. 273:25–49.

Xiao K, Song QH, Zhang SW, Xuan LJ. 2008. Water-soluble constituents of the root barks of *Fraxinus rhynchophylla* (Chinese drug Qinpi). J Asian Nat Prod Res. 10:205–210.

Yu M, Sun A, Zhang Y, Liu R. 2014. Purification of coumarin compounds from *Cortex fraxinus* by adsorption chromatography. J Chromatogr Sci. 52:1033–1037.

ملخص

تعتبر الطبيعة مصدر للمواد ذات القدرة العلاجية الكبيرة .ومهما تكن أسباب وجود هذه المواد في الطبيعة فإنها تشكل موارد مهمة تستعمل لاكتشاف عقارات جديدة في هذا السياق قمنا باختبار النبات الطبي الجزائري الدردار **Fraxinus xanthoxyloides** من عائلة **Oleaceae** لدراسة فيتوكيميائية وتشخيص مضاد للأكسدة.

سمح هذا البحث بعزل وتحديد بنية 17 مركب طبيعي وذلك باستعمال الطرق الكروماتوغرافية وباستعمال طرق التحليل الطيفي كمطيافية الرنين المغنطيسي النووي بجميع أنواعها، مطيافية الكتلة، حساب زاوية التدوير النوعي والمقارنة مع الدراسات السابقة. تنقسم هذه المركبات الي 2 مركب ذو طبيعة كومارينية-سيكواريدويدية، 2 مركب ذو طبيعة سيكواريدويدية، 3 مركب ذو طبيعة كومارينية، 2 مركب ذو طبيعة فلافونويدية، 4 مركب ذو طبيعة فينيلية ايثانويد ية، 2 مركب ذو طبيعة فينيلية بروبانويدية و2 مركب من نوع اللينان.

قدرت النشاطية المضادة للأكسدة لمستخلصات كل من الأوراق والقشور بواسطة تقنية الجذور الحرة: **DPPH** و**ABTS** وقد أظهرت النتائج ان مستخلصات القشور لها نشاطً مثيرًا للاهتمام.

الكلمات المفتاحية: Oleaceae، Fraxinus xantoxyloides، الكومارينات-السيكواريدويدية، السيكواريدويدات، الكومارينات، الفلافونويدات، فينيل ايثانوويدات، فينيل بروبانوويدات، أللينانات، مطيافية الرنين المغناطيسي، مطيافية الكتلة، الفعالية المضادة للأكسدة.

Abstract

The nature was and still an interesting source of potential chemotherapeutic agents. Whatever the reasons for the presence of these compounds in nature, they provide invaluable resources that have been used to find new drug molecules. In this context, the Algerian medicinal plant *Faxinus xanthoxyloides* of the Oleaceae family has been selected for phytochemical investigation and anti-oxidant screening.

This work allowed the isolation by chromatographic methods (TLC, PTLC, CC and LPLC), and the characterization by spectroscopic analysis (NMR and MS) of 17 secondary metabolites including 2 coumarin-secoiridoid glucosides, 2 secoiridoids, 3 coumarins, 2 flavonoids, 4 phenylethanoids, 2 phenylpropanoids and 2 lignans.

The anti-oxidant activity of leaves and bark extracts was estimated by the DPPH free radicals scavenging and ABTS radical cation decolorization assay and the results showed that bark extracts had an interesting activity.

Keywords: Oleaceae, Fraxinus xantoxyloides, Coumarins-secoiridoids, Secoiridoids, coumarins, Flavonoids, Phenylethanoides, Phenylpropanoides, Lignans, NMR 1D and 2D, HRMS, Anti-oxidant activity.

Résumé

La nature comme source d'agents potentiels chimio-thérapeutiques suscite un intérêt considérable. Quelle que soit les raisons de la présence de ces composés dans la nature, ils fournissent des ressources de valeur inestimable qui ont été employées pour trouver de nouvelles drogues. Dans ce context, la plante médicinale algérienne Faxinus xanthoxyloides de la famille Oleaceae a été sélectionnée pour investigation phytochimique et screening anti-oxydant.

Ce travail a permis l'isolement par les méthodes chromatographiques (CCM, CCMP, CC, CLFP) et la caractérisation par les différentes méthodes spectroscopiques (RMN et HRMS) de 17 métabolites secondaires dont 2 composés de nature coumarin-secoiridoidique, 2 secoiridoides, 2 coumarines, 2 flavonoides, 4 phenylethnoides, 2 phenylpropanoides, 2 lignanes.

L'activité anti-oxydante a été réalisée sur les extraits des feuilles et d'écorce par le piégeage des radicaux libres DPPH et le dosage de la décoloration des cations radicaux ABTS. Les résultats ont montré que les extraits d'écorce ont une activité intéressante.

Mots clés : Oleaceae, Fraxinus xanthoxyloides, Coumarines-secoiridoides, Secoiridoides, Coumarines, Flavonoides, Phenylethanoides, Phenylpropanoides, Lignanes, RMN 1D et 2D, HRMS, Activité anti-oxydante.