ALTAHADI UNIVERSITY

FACULTY OF SCIENCE

CHEMISTRY DEPARTMENT

CHEMICAL AND BIOLOGICAL STUDY ON *TEUCRIUM ZANONII* GROWING IN LIBYA

A THESIS SUBMITED IN PARTIAL FULFILMENT FOR THE REQUIRMENTS OF THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

BY

MOHAMED ALI ABDUNNABI ALWAHSH

UNDER SUPERVISSION OF

Dr. KHALED ABD ELHADY ABD ELSHAFEEK

SIRTE-LIBYA

2005 - 2006

الجماهيرية العربية الليبية الشعبية الإشتراكية العظمى
جامعة التحدي
كلية العلوم
التاريخ .....
الموافق 18/2/2006 ف

G. S. P. L. A. J.
AL_TAHDI UNIVERSITY
الرقم الإشاري

Faculty of Science

Chemistry departement

M.Sc.Thesis

Chemical and biological stydy on *Teucrium zanonii* growing in Libya

Submitted by
Mohamed Ali Abdunnabi Alwahsh

Examinars :		Signature
Dr. Khaled Abdelhady Abd elshafek	Supervisor	
Dr. Kamel Hussein Shaker	Extrnal examinar	
Prof. Dr. Medhat Mahmoud Ali	Internal examinar	

Dr. Mohamed Ali Salem

Dean of Faculty of Science

674 ✉ 054 - 60361 62151 🖷 054 – 68089 ☎

بسم الله الرحمن الرحيم

وقضى ربك ألا تعبدوا إلا إياه وبالوالدين إحسانا

إما يبلغن عندك الكبر أحدهما أو كلاهما فلا تقل لهما أف ولا

تنهرهما وقل لهما قولا كريما واخفض لهما جناح الذل من الرحمة

وقل رب ارحمهما كما ربياني صغيرا

صدق الله العظيم

بسم الله الرحمن الرحيم

وقل رب زدني علما

صدق الله العظيم

الإهداء

إلى أبي ...
إلى أمي ...
إلى إخوتي ...
إلى أخواتي ...
إلى كل من يحمل لقب الوحش
...
إلى هند ...
إلى إبراهيم ...
مع خالص تمنياتي باالتوفيق

اهدي هذا العمل المتواضع

ناجي علي

ACKNOWLEDGMENT

*First of all profusely all thanks to **Allah** who enables to achieve and complete this study. Also the author would like to express his sincere gratitude to my supervisor, **Dr. Khaled Abd El-Hady Abd El-Shafeek**, Doctor of Organic Chemistry, Faculty of Science, Al-Tahadi University, for suggesting this study and his constant and continuous guidance throughout period of this work.*

I would like to express my thanks to the Dean of faculty of sciences, head of chemistry department and director of postgraduate studies office and their staffs.

I would like to express my thanks also to Dr. Ismail abdel Khalek for his helping in carring of insecticidal activity and his valuable discussion.

I owe deep gratitude to Dr. Mohammed Al-Sherif, Hussin Al-Tajouri and Abd El-Salam Almgasbi at Garyounis University for kindly help in collection and identification of the plant.

I owe deep gratitude to Dr. Khaled Kridan and Hend ben Hussin at Petroleum research center for their helping in NMR measurements.

My thanks also go to Dr. Mohamed Mimon for his helping in paper chromatography.

Also I would like to express my thanks to Osama Alammary and Abear Alshikhi at Ras Lanuf Oil & Gas Processing Co. for their helping in glassware and volatile oil analysis.

My thanks go also to Moftah Al-Gazzaly and Abdul Kader Al-Arabi who supplied me many facilities throughout my study.

*I am highly indebted to **my father, mother, brothers** and **sisters** for their continuous support, advices and prayers.*

I would like further to extend my grateful gratitude for all my colleagues for their kind co-operation and encouragement.

I like to express my thanks to Mostafa Altaher, Mahdi Edress, Abd Allah Almedhium and all my friends who helped me so much.

*Last but not least all thanks and grateful acknowledgment is expressed to the **AL-TAHADI UNIVERSITY, Faculty of science,** Chemistry department for offering me this opportunity to submit my **M.Sc. thesis** in chemistry.*

Mohamed Ali Abdunnabi Alwahsh

ABSTRACT

Name : Mohamed Ali Abdunnabi Alwahsh

Title of thesis : Chemical and biological study on *Teucrium zanonii* growing in Libya.

This work deals with the phytochemical investigation of *Teucrium zanonii*, Endemic to Libya with special emphasis to their volatile oil (in which β-Pinene is the main compound), lipids (fatty alcohols, fatty acids and unsaponifiable materials) and flavonoidal constituents (aglycones and glycosides) in addition to the studies of biological activity of different extracts of the plant concerning with antioxidant and insecticide activities.

Key words : *Teucrium zanonii*, *Lamiaceae* (*Labiatae*), volatile oil, lipids, flavonoids, antioxidant, insecticide activity.

Abbreviations

Paper chromatography	PC
Preparative paper chromatography	PPC
Two dimension paper chromatography	2DPC
Column chromatography	CC
Thin layer chromatography	TLC
Preparative thick layer chromatography	PTLC
High performance liquid chromatography	HPLC
Gas liquid chromatography	GLC
Ultraviolet	UV
Mass spectroscopy	MS
Nuclear magnetic resonance	NMR
Gas chromatography coupled with mass spectroscopy	GC/MS
Diphenyl picryl hydrazyl	DPPH
Reactive oxygen species	ROS
Structure activity relationship	SAR
Relative humidity	RH
Butanol : Acetic acid: Water	BAW
Dimethyl sulphoxide	DMSO
Acetic acid	AcOH

CONTENTS

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SCHEMES

Abbreviations

Paper chromatography	PC
Preparative paper chromatography	PPC
Two dimension paper chromatography	2DPC
Column chromatography	CC
Thin layer chromatography	TLC
Preparative thick layer chromatography	PTLC
High performance liquid chromatography	HPLC
Gas liquid chromatography	GLC
Ultraviolet	UV
Mass spectroscopy	MS
Nuclear magnetic resonance	NMR
Gas chromatography coupled with mass spectroscopy	GC/MS
Diphenyl picryl hydrazyl	DPPH
Reactive oxygen species	ROS
Structure activity relationship	SAR
Relative humidity	RH
Butanol : Acetic acid: Water	BAW
Dimethyl sulphoxide	DMSO
Acetic acid	AcOH

SUMMARY

SUMMARY

The thesis includes a study of the chemical constituents of one of the endemic plants *viz. Teucrium zanonii* belonging to family *lamiaceae* (*labiatae*) growing in Libya, Bengazi region.

The thesis includes three parts :-

1-Review of literature :

The available review of literature concerning the chemical constituents (volatile oil, terpenes, sterols, iridoids and flavonoids) as will as the biological activities of *Teucrium* genus.

2- Chemical studies of *T. zanonii* :

a- Preliminary phytochemical screening.

b- Study of the volatile oil :

The volatile oil was prepared by two methods as follow :

- **Hydrodistillation method:**

The study of the volatile oil by hydrodistillation method using GC/MS technique showed a mixture of 74 compounds. The main compounds were β-Pinene (14.13%), Linalyl acetate (11.10%), Linalool (11.00%) and Germacrene-D (8.81%).

- **Solvent extraction method:**

The study of the volatile oil extracted by light solvent (n-hexane–ether 1:1) using GC/MS technique showed a mixture of sixteen compounds. The main compounds were Germacrene-D (20.04%), β-Pinene (18.19%) and Linalyl acetate (7.93%).

c- Study of the lipid fraction :

The study of the lipid fraction using GLC and GC/MS analysis resulted in the isolation and identification of :

i- Fatty alcohols :

Tricosanol, tetracosanol, pentacosanol, nonacosanol, triacontene and tetratricontane, in which nonacosanol being the main constituent (26.21%).

ii- Unsaponifiable fraction :

The main constituents of the unsaponifiable fraction were identified by GLC analysis ; It consist mainly from a mixture of series of n-alkanes from n-C_3 to n-C_{32} (92.48%), cholesterol (4.48%), β-sitosterol (1.36%), campasterol (0.86%), stigmasterol (0.36%) and a triterpene β-amyrine (0.41%).

iii- Fatty acid methyl esters :

The GLC analysis of the total fatty acid methyl esters revealed the presence of lauric, myristic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, erucic, lignoceric and tetracosenoic. Linoleic acid was the major constituent (35.25%).

d- Investigation of the flavonoidal constituents :

Investigation of the flavonoidal constituents of the ethyl acetate fraction resulted in the isolation and identification of :

(1) Cirsiliol (2) Luteolin

(3) Chrysoeriol (4) Xanthomicrol

Investigation of the flavonoidal constituent of the butanol fraction resulted in the isolation and identification of

(5) Apigenin 6,8-di-*O*-glucoside

(6) Luteolin-7-*O*-rutinoside.

3- Biological studies :

A- Antioxidant activity :

The antioxidant activity measurement of different extracts was measured using DPPH. The ethyl acetate, butanol, total alcoholic and aqueous extracts were showed a highest antioxidant activities (93.6 %, 92.1 %, 87.6 % and 77.5 % respectively).

B- Insecticidal activity :

B.1- laboratory experiments:

The insecticidal activity measurements of different extracts against the adult of *Phloeotribus oleae* showed that the highest effect (86.67% mortality) was observed with the aqueous extract, while the unsaponifiable fraction was the least in this concern which give only 43.33% mortality,. Also, mortalities of 83.33%, 80.00%, 70.00% and 66.67% were obtained by using of alcoholic, butanol, ethyl acetate and chloroform extracts, respectively.

B.2- filed experiments :

The measurements after one week showed that aqueous, alcoholic and butanol extracts significantly lowered the percentage of infestation to 70.82%, 65.86% and 66.56%, respectively.

INTRODUCTION

INTRODUCTION

The use of medicinal plants for the treatment of many diseases dates back to the Ebers papyrus of about 1550 BC. Even after the discovery of synthetic drugs, the search for safer and more effective drugs of plant origin for many diseases like diabetes mellitus, hepatitis, HIV, arthritis, ...etc has been continued [1 1].

The plant kingdom consist of many families, one of them known as *Lamiaceae* (*Labiatae*) family. It is commonly called the mint or aromatic family due to high content of essential oils with aromatic odor. It is one of the flowering plant groups [1 2]. In the most recent classification the family comprises about 252 genera and 6700 species [1 3].

In Libya the family is represented by 22 genera and 65 species [1 2]. *Lamiaceae* species are important in the field of pharmacology, cosmology perfumes and food industry, the soap industry is the main consumer of their volatile oils. Many species are used as spices in flavoring meat and savory dishes. The volatile oils from *Lamiaceae* are also used in alcoholic drink and carbonated beverages as well as in the flavoring of candy, ice cream and packed food stuffs. Many species have great potential in the preparation of drugs in modern medicine. These species are also very popular for treating many diseases, especially in the rural area [1 4]. Many species of *Lamiaceae* family are used in folk medicine as anti-inflammatory, antibacterial, antiseptic and have effects on gastrointestinal disorders as carminative, appetizer and digestive and for treatment respiratory tract diseases like chronic cough and asthma and many others [1 4-5].

The genus *Teucrium* belonging to the *Lamiaceae* family is represented by about 300 species in the world. It represented by 13 species in the flora of Libya, five of them being endemic, viz.; *T. appollinis, T. barbeyanum, T.*

davaeanum, T. linivaccarii, and T. zanonii [2]. *Teucrium* species are rich source of volatile oils and neoclerodane diterpenoids, in addition to furanoid diterpenoids and flavonoids. The genus *Teucrium* is the most abundant natural source for these compounds, therefore *Teucrium* species are accepted as chemotaxonomic markers for neoclerodanes. Chemical investigation of this genus showed that some of species also contain sesquiterpenes, triterpenes, sterols, flavonoids, iridoids, phenolic acids and some alkaloids. Many *Teucrium* species have been used for more than 2000 years as medicinal plants. They exhibit some interesting biological activities like diuretic, diaphoretic, antiseptic, antipyretic, antispasmodic, hypoglycemic, antifeedant [6], besides some of *Teucrium* extracts are used in folk medicine to treat various aliments such as stomach and intestinal troubles, cold and as stimulant vermifuge, tonic, rheumatism, hemorroids and renal inflammatory [7].

In the frame of our chemical and biological investigation of Libyan medicinal plants, this work aim to study the chemical constituents and some biological activities of different extracts of *Teucrium zanonii*.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Here we will discuss about the chemistry of volatile oil, diterpenes, sesquiterpenes, triterpenes and sterols, flavonoids and iridoids, in addition to some biological activities of *Teucrium genus*.

1- Volatile oils

The mainly terpenoid essential oils comprise the volatile steam-distillable fraction responsible for the characteristic scent, odour or smell found in many plants. They are commercially important as the basis of natural perfumes and also of spices and flavourings in the food industry.

Chemically, the terpene essential oils can be divided into two classes. The mono- and sesquiterpenes, C_{10} and C_{15} isoprenoids, which differ in their boiling point range (monoterpenes b.p. 140-180 °C, sesquiterpenes b.p. >200 °C). First of all, with regard to monoterpenes, these substances can be further divided into three groups depending on whether they are acyclic (e.g. geraniol), monocyclic (e.g. limonene) or bicyclic (e.g. α- and β-pinene). Within each group, the monotepenes may be simple unsaturated hydrocarbons (e.g. limonene) or may have functional groups may be alcohols (e.g. menthol), aldehydes or ketones (e.g. menthone, carvone). Simple monoterpenes are widespread and tend to occur as components of the majority of essential oils. Some compounds are regularly found together in leaf oils, especially α- and β-pinene, limonene, Δ^3-carene, α-phellandrene and myrcene. Flower and seed oils tend to have more specialized monoterpenes present.

Like the monoterpenes, the sesquiterpenes fall chemically into groups according to the basic carbon skeleton; the common ones are either acyclic (e.g. farnesol), monocyclic (e.g. γ-bisabolene) or bicyclic (e.g. β-selinene, carotol). However, within each group there are several thousand sesquiterpenoids. The volatility of the simple terpenes means that they are

ideal subjects for separation by GLC. Many have fragrant odours and indeed can often be recognized in plant distillates directly , if present as the major constituent [8]. The volatile oils of *Teucrium* genus were studied by many investigator as follow :

The composition of the essential oil of *Teucrium flavum* was investigated by Petrici *et. al.* [9] using GC/MS. Thirty components were identified, in which α-, *β*-pinene (**1, 2**), caryophyllene (**3**) and *α*-aromadendren (**4**) were the major components.

The volatile oil of *Teucrium lusitanicum var. aureiformis* was investigated by Velasco and Perez in 1990 [10]. The main components were monoterpe-nes: *α*-pinene (**1**), *β*-pinene (**2**), p-cymene (**5**), limonene (**6**), fenchone (**7**), linalool (**8**), terpinen-4-ol (**9**) and *α*-terpineol (**10**). Sescoter-penes: *α*-copaene (**11**), *β*-bourbonene (**12**), *β*-caryophyllene (**3**), *β*-selinene (**13**), *t*-cadinene (**14**), *cis*-calamenene (**15**), spathulenol (**16**), caryophyllene epoxide (**17**), humulene epoxide, *t*-cadinol (**18**) and *α*-cadinol (**19**).

The essential oils of *Teucrium cyprium* suhsp. *cyprium*, *Teucrium micropodioides*, *Teucrium divaricatum* suhsp. *canescens* and *Teucrium kotschyanum* were extracted by steam distillation of dried flowers, leaves and stems and analyzed by GLC and GC-MS [11].

Two samples of *Teucrium arduini* were hydrodistilled to produce essential oil yields of 0.07 and 0.18 %(v/w) respectively. GC and GC/MS analysis revealed that, Germacrene-D (**20**) (23.4% and 57.8%) and Caryo-phyllene (**3**) (17.3% and 13,5%) were the main components of the oil [12].

The volatile component of *Teucrium polium* was extracted by Vokou and Bessiere using two methods; hydrodistillation and ether-pentane extraction. Thirty seven and thirty five compounds were separated respectively, whereas the most twenty four of the components were found in both. *α*-pinene (1), 1,8-cineole (**21**), borneol (**22**), *trans*-carvenol (**23**), *ε*-muuro-lene (**24**), *α*-curcumene (**25**), alloaromadendrene (**26**), *t*-(**14**) and *δ*-cadi-

nene (**27**), β-(**28**) and δ-calacorene and α-bisabolene (**29**) were found only in hydrodistillation sample and *cis*- and *trans*-linalyl oxide, undecane (**30**), pinocarvone (**31**), dodecane (**32**), bornylacetate (**33**), tridecane (**34**), tetradecane (**35**), ε-cadinene (**36**), pentadecane (**37**) and hexadecane (**38**) were found only in ether-pentane extracted [13].

Laura *et. al.* [14] were analyzed the volatile oil of two subspecies of *Teucrium flavum* subsp. *flavum and* subsp. *glaucum*. They found a rather similar pattern, mostly in the monoterpene fraction, while the sesquiterpene fraction is fairly richer in subsp. *flavum*. Very peculiar is the presence of a great amount of 4-methyl-4-hydroxy pentan-2-one (**39**) (diacetone alcohol) , a rather uncommon metabolite in plants.

The essential oils of *Teucrium heterophyllum* were investigated by Barroso *et. al.* [15] during the flowering period and the vegetative phase. The main components were sesquiterpenes (51% and 48% respectively), *t*-cadinol (**18**) and α-cadinol (**19**) were the main sesquiterpenes, the monoterpenes were (29% and 34% respectively), α-pinene (**1**) was the main monoterpere.

The volatile component from *Teucrinm polium* using supercritical CO_2 at 100 bar and 40°C was performed and compared with those obtained using hydrodistillation. The resultes showed that the major components identified were sesquiterpenes, Germacrene-D (**20**) (23.6% and 13.2%) and β-Caryophyllene (**3**) (16.5% and 18.0%) were the main components in the extract and oil respectively [16].

Assem *et. al.* [17] studied the water-distilled essential oil and n-hexane-ether extract of *Teucrium leucocladum* by GLC and GC/MS. They identified about seventy two compounds. The sesquiterpene alcohols; patchouli alcohol (**40**) (31.24% and 29.66%) and α-cadinol (**19**) (9.29% and 21.54%) were the main components in the oil and extract respectively.

Cavaleiro *et. al.* [117] were analyzed the essential oils of *Teucrium lusitanicum* and *Teucrium algarbiensis* by GC and GC/MS. They identified seventy one volatile compounds. The major component of *Teucrium algarbiensis* were, α-pinene (**1**), sabinene (**41**), β-pinene (**2**), limonene (**6**), and Germacrene-D (**20**), while the major constituents of the oil of *Teucrium lusitanicum* were α-pinene (**1**), sabinene (**41**), β-pinene (**2**), limonene (**6**), and elemol (**42**).

(Figure 1) shows the chemical structures of the most of these compounds.

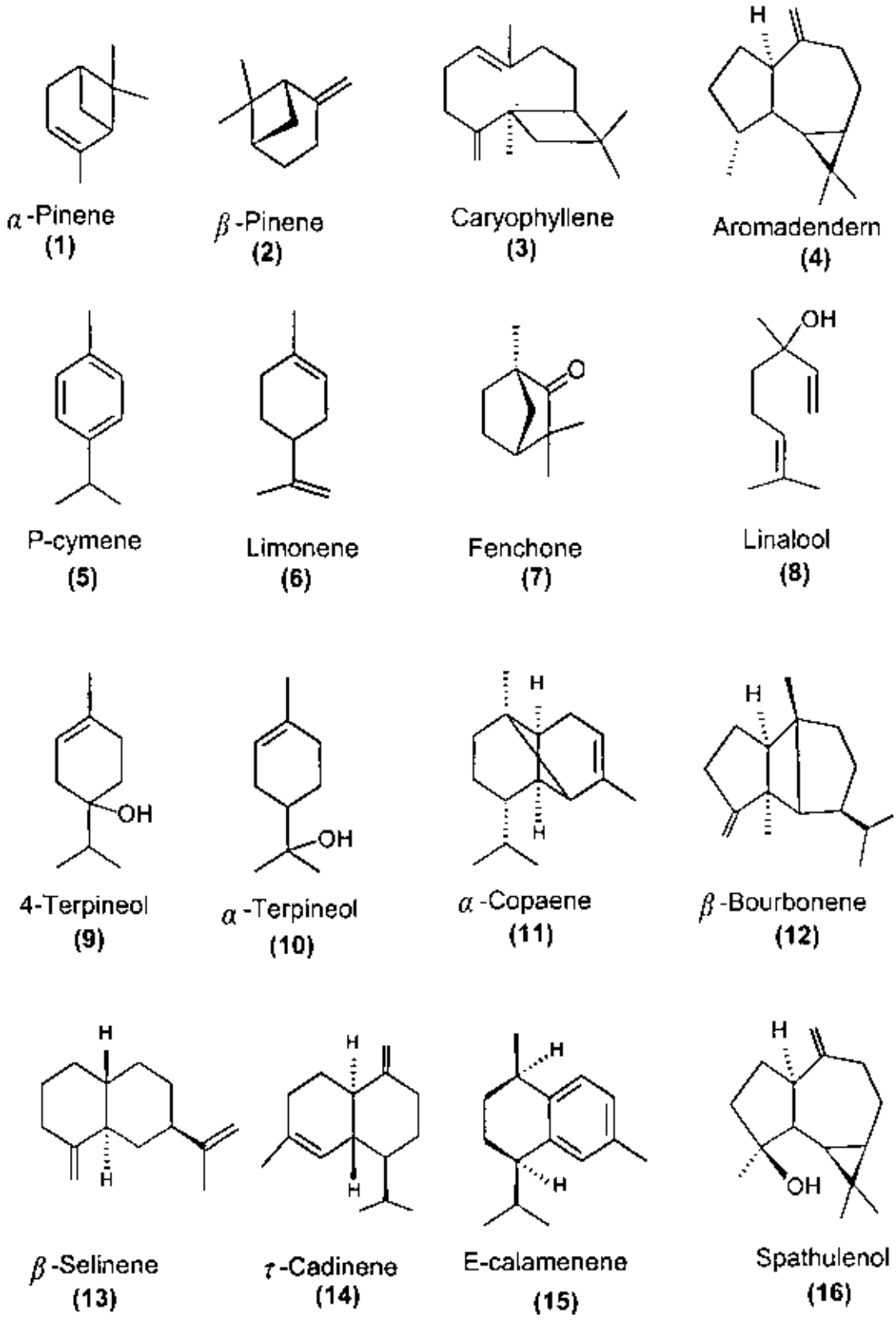
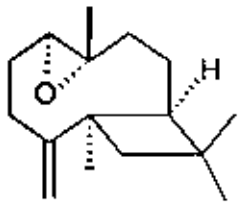
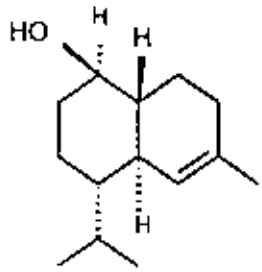


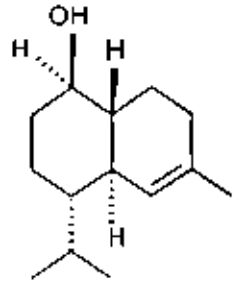
Fig. (1) : Chemical structures of some volatile oil compounds in *Teucrium* genus.



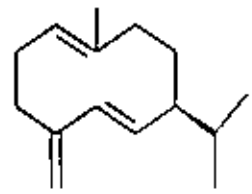
Caryophyllen oxide
(17)



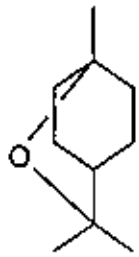
τ-Cadinol
(18)



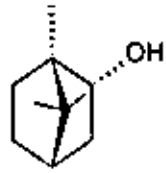
α-Cadinol
(19)



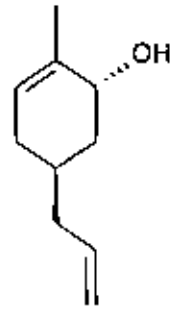
Germacrene-D
(20)



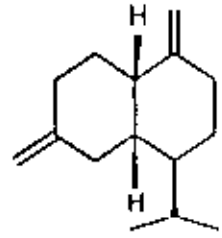
1,8-Cineole
(21)



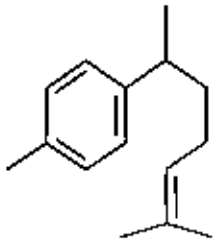
Borneol
(22)



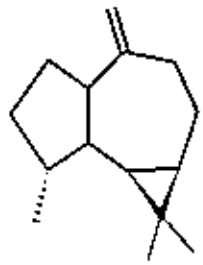
Trans-carvenol
(23)



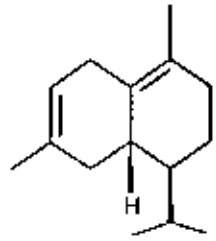
ε-Muurolene
(24)



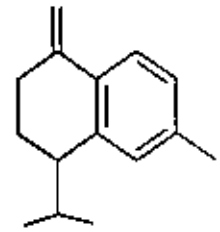
α-Curcumene
(25)



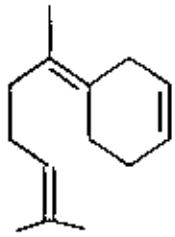
Alloaromadendrene
(26)



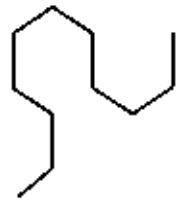
δ-Cadinene
(27)



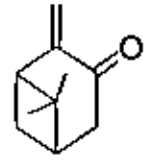
β-Calacorene
(28)



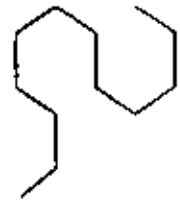
β-Bisabolene
(29)



Undecane
(30)

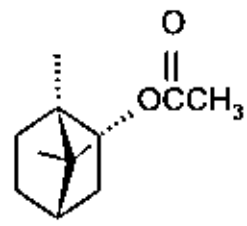


Pinocarvone
(31)



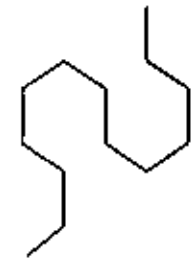
Dodecane
(32)

Fig. (1) : Cont.



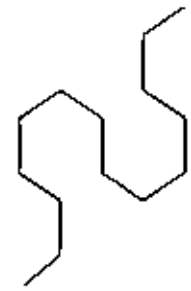
Bornyl acetate

(33)



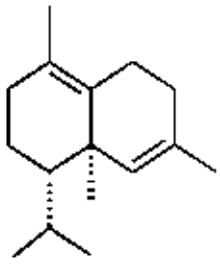
Tridecane

(34)



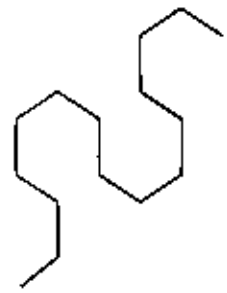
Tetradecane

(35)



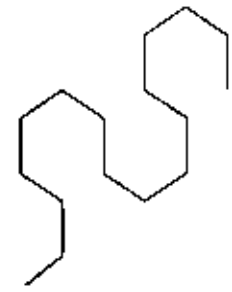
ε -Cadinen

(36)



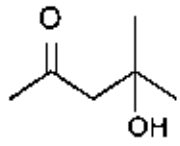
Pentadecane

(37)



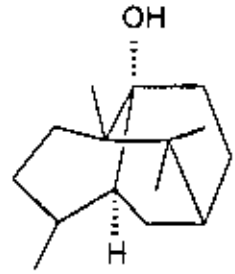
Hexadecane

(38)



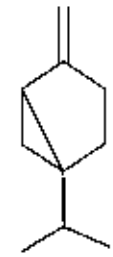
Diacetone alcohol

(39)



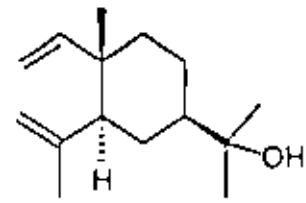
Patchouli alcohol

(40)



Sabinene

(41)



Elemol

(42)

Fig. (1) : Cont.

2- Diterpene compounds in *Teucrium* genus

Teucrium genus is consider as a rich source of diterpenoids, More than 200 diterpenoids having the neoclerodane skeleton have been isolated from the aerial parts of about 80 species and subspecies [18]. The *Teucrium* species (family labiatae) afforded a number of neoclerodane and 19-norn-coclerodane diterpenoids, some with unusual and fascinating structures [19]. Here we listed some of them in table (1) and Figure (2).

Table (1): Diterpens isolated from *Teucrium* genus

Plant species	Compounds	Reference
T. africanum	Tafricanin-A **(43)** and Tafricanin-B **(44)**.	[20]
T. alyssifolium	Alysine-A, B, C and 3-deactylalysine-B.	[21]
T. asiaticum	19-a-cetylgnaphalin **(45)**, Auropolin **(46)**, Teucr-in-A**(47)**, Teuflin **(48)**, Teucrasiatin **(49)** and Teucrasiolide.	[22-24]
T. betonicum	Teubetonin **(50)**.	[25]
T. bidentatum	Bidentatin **(51)**.	[26]
T. botrys	19-deacetylteuscorodol **(52)**, Teubotrin **(53)**, Teucvidin **(54)**, Montanin-D **(55)**, Teucham-aedrin-C and 6β-hydroxyteuscordin.	[27]

Table (1): Cont.

T. brevifolium	Teubrevins-A, B, C, D, E, F, G, H and I.	[28-29]
T. buxifolium	19-acetylteulepicin **(56)**, 19-acetylgnaphalin, Teulepicin **(57)**, Teulepicephin **(58)** and 19-acetylteulepicephin.	[30]
T. capitatum	Capitatin **(59)**, Teucapitatin **(60)**, Lolin **(61)** and 19-acetylgnaphalin **(45)**.	[31-32]
T. carolipaui	19-acetylgnaphalin **(45)**.	[33]
T. chamaedrys	Teucrin-A **(47)**, B **(62)**, E **(63)**, F **(64)**, G **(65)**, Teuchamaedryn-A **(66)** and B **(67)**, Dihydro-teugin **(68)**, Teugin **(69)**, Chamaedroxide **(70)**, Teucroxide **(71)**, 6-epiteucrin-A **(72)**, Teuflin **(48)**, Teuflidin **(73)**, Isoteuflidin **(74)**, Dihydroteugin **(68)**, Teuchamaedrin-C **(75)** and 6α-hydroxyteuscordin, 12(S)-15,16-epoxy-19-hydroxyneocleroda-13 (16),14-di-ene 18,6α:20,-12-diolid.	[34-46]
T. chamaedrys var. *syspirense*	Syspirensins-A and B.	[47]

Table (1): Cont.

T. corymbosum	Teucorymbin, 19-acetylgnaphalin (**45**), Teucjaponin-A (**76**) and 6-acetylteucjaponin-B (**77**).	[48]
T. cossonii	Teucossine-A, B and Montanin-H.	[49]
T. creticum	Teucretol (**78**), 6,19-diacetylteumassilin (**79**), 19-acetylgnaphalin (**45**), Teucjaponin-B (**80**).	[50]
T. cubense	Eugarzasudone (**81**) and Eugarzasadine (**82**).	[51-52]
T. divaricatum	2-deoxychamaedroxide (**83**), Teuflin (**48**), Teucrin-H_2 (**106**), Teuflidin (**73**), Teucrin-A(**47**), F(**64**), G (**65**), montanin-D (**55**), 6β-hydroxyteuscordin (**84**) and Dihydroteugin (**68**).	[53]
T. divaricatum ssp. *divaricatum*	Teucvidin (**54**) and Teucvin.	[23, 54]
T. divaricanum ssp. *villosianum*	Villosin-A (**85**), B (**86**) and C (**87**).	[55]
T. eriocephalum	Eriocephalin (**88**).	[56]
T. flavum	Teuflidin (**73**), Teuflin (**48**), Teupolin (**89**), Montanin-C (**90**) and 12-epiteucrin (**91**).	[57-59]

Table (1): Cont.

T. flavum subsp *glaucum*	Teuflavin (**92**), Teuflavoside (**93**), and Teuflin (**48**).	[60]
T. fragile	Teugin (**69**).	[61]
T. fruticans	Fruticolone (**94**), 7β-hydroxyfruticolone (**95**), 8β-hydroxyfruticolone (**96**), Isofruticolone (**97**), Teuvincenones-A, B, E, F, G, H, I, Ferruginol, Fruticolide (**98**), 11-hydroxyfruticolone (**99**), Deacetylfruticolone (**100**) and 6-acetyl-10-hydroxyteucjaponin-B (**101**)	[62-68]
T. gnaphalodes	Teugnaphalodin (**102**), Gnaphalin (**103**), 19-acetylgnaphalin (**45**), Teucrin-P_1 (**136**) and Gnaphalidin (**104**).	[69-71]
T. gracile	Teugracilin-A, B, C, D, E, Teumicropodin, 3-*O*-deacetylteugracilin-A and 19-acetylteulepicin (**56**).	[68, 72]
T. grisebachii	6-acetylteucjaponin-B (**77**).	[66]
T. haenseleri	19-acetylgnaphalin (**45**), Eriocephalin (**88**), Isoeriocephalin, 20-deacetyleriocephalin.	[73]
T. hyrcanicum	Teucrin H_1 (**105**), H_2 (**106**), H_3 (**107**)andH_4(**108**)	[74-77]
T. japonicum	Teucjaponin-A (**76**), B, Teucvin and Teuponin.	[78-79]

Table (1): Cont.

T. kotschyanum	Isoteucrin H_4, Teucrin H_4 **(108)**, 12-epiteufiin and Teukotschyn.	[80-81]
T. lamiifolium	12-epiteupolin II **(109)**, Teuscorodinon, Teuflin **(48)**, Montanin-C **(90)**, E, 19-acetylgnaphalin **(45)**, Teucroxide, Teulamifin-B **(110)**, Teulamioside, 19-deacatyloteuscorodol and Teuspinin.	[82-84]
T. lanigerum	20-deacetyleriocephalin, Isoeriocephalin, Eriocephalin, 7,8-dedydroeriocephalin **(111)**, Teulanigeral, Teulanigin, 20-epiteulanigin, Teulanigrin **(112)** and Teulanigeridin **(113)**.	[85-86]
T. lepicephalum	Teulepicin **(57)**, 19-acetylteulepicin **(56)** and Teulepicephin **(58)**.	[30]
T. leucocladum	Montanin-C **(90)**.	[87]
T. maghrebinum	12-epiteucjaponin-A **(115)**, 12-epimontanin-D **(116)**, 12-epimontanin-B **(117)**, Teucjaponin-A **(76)**, Montanin-B, D **(55)**, 19-deacetylteuscorodol, Teukotschyn, 12-epiteukotschyn **(118)**, Teusalvin-C, Teughrebin **(119)**, and 12-epiteughrebin.	[88-89]

Table (1): Cont.

T. marum	Teumarin (**120**).	[90]
T. massiliense	Teumassin, Montanin-C (**90**) and Teucjaponin-A (**76**),	[91-92]
T. micropodioides	Teumicropin, 3-acetylteumicropin (**121**), Teumicropodin (**122**), Deacetylteupyrenon, 3-deacetyl-20-epiteulanigin.	[93]
T. montanum.	Montanin-C (**90**), D (**55**) and G (**123**).	[94-97]
subp. *montanum.*	Montanin-H , 19-acetylgnaphalin (**45**), Montanin- B, D (**55**), E and Teubotrin (Teulamifin-B).	[98]
ssp. *pannonicum*	Auropolin (**46**) and Montanin-H.	[99]
subsp. *skorpillii*	Montanin-E, and Montanin-F (Teucjaponin-A (**76**))	[100]
T. nudicaule	6-acetylteucjaponin-B (**77**), Triacetylteumassilin and C-12 epimer of teupyreinin.	[101]
T. oliverianum	Teucrolivin-A (**124**), B (**125**), C (**126**), D (**129**), E (**130**), F, G (**127**), H (**128**), Teucrolin-E, F and G.	[102-106]
T. oxylepis subp. *montanum*	Teucroxylepin, 12-*O*-acetylcugnaphalodin	[107]

Table (1): Cont.

T. pernvi	Teupernin-A **(131)**, B **(132)** and C **(133)**.	[108]
T. pernyi	Teupernin-D, Teucvidin **(54)**, Montanin-D **(55)** Teuflin **(48)**, and Teuscorodonin.	[109]
T. pestalozzae	Teupestalins-A **(134)** and B **(135)**.	[110]
T. polium	Teucrin-P_1 **(136)**, Teupolin-I **(137)**, II **(138)**, III**(140)**, IV **(141)**, V **(142)**, Teucrin-H_3, Montanin-B **(117)**, Clerodanedione **(139)**, Auropolin **(46)**, Teulamifin-B **(110)**, 19-deacetyloteuscorodol, Teucroxide, 7-epicapitatin, Quassimin **(143)** 6-acetylmont-anin-F **(144)**, 6-acetyl-19-deacetylmontanin-F **(145)**, Teulolin-A **(146)** and B **(147)**.	[83], [111-118]
T. polium var. *album*	Montanin-C **(90)**.	[119]
T. polium var. *aurasianum*	3-deacetylteumicropodin **(148)**, Teumicropodin **(122)**, 3,20-bis- deacetylteupyreinidin and 6,20-bis-deacetylteupyreinidin.	[120]
T. polium subsp. *belion*	19-acetylgnaphalin **(45)**, Auropolin **(46)**, Teucrin-A **(47)** and Teuflin **(48)**.	[22]

Table (1): Cont.

T. polium subsp. *capitatum*	7-deacetylcapitatin **(149)**, 20-epiisoeriocephalin **(150)**, Picropolin, Picropolinol **(151)**, Teuflin **(48)**, Picropolinone **(152)**, 19-acetylgnaphalin **(45)**, Teucjaponin-B **(80)**, Auropolin **(46)** and Teucrin-A **(47)**.	[22, 121]
T. polium subsp. *pilosum*	19-acetylteupolin IV.	[122]
T. polium subsp. *vincentinum*	Teuvincentins-A, B, C, D, 19-acetylgnaphalin **(45)**, Eriocephalin **(88)** and Isoeriocephalin and 3-deacetyl-20-epiteulanigin.	[123-124]
T. pyrenaicum	Teupyrenone **(153)**, Teupyreinin **(154)**, Teupyreinidin **(155)**, Teupyrins-A **(156)**, B **(157)** , Teucvin, Teuflin **(48)**, Teucrin H_2 and 6α-hydroxyteuscordin.	[125-126]
T. queadrifarium	Teucvidin **(54)** and Teuflin **(48)**.	[127]
T. qudrifarium	Teucvidin **(54)**.	[128]
T. racemosum	Teuracemin **(158)**, Teutrifidin, 20-oxo-teuflavin and 14α,18-epoxytafricanin-A.	[129]
T. salviastrum	Teusalvin-A **(159)**, B, C, D, E, F, Teucvidin **(54)** and Teucroxide.	[130]

Table (1): Cont.

T. sandrasicum	Sandrasin-A (**160**), B (**162**) and 6-deacetylsandrasin-A (**161**), Teusandrin-A, B, C, D, E, F (**163**), Teucjaponin-B and 6-*O*-acetylteucjaponin-B.	[131-132]
T. scordium	6-Ketoteuscordin (**164**), 6α-hydroxyteuscordin (**165**), Teuscordinon (**166**), 2-keto-19Hydroxylteuscordin , Teucrins-E (**63**), H_4 (**108**) and 6-acetylteucjaponin-B (**77**).	[133-137]
T. scordium subsp. *scordium*	6*β*-hydroxyteuscordin (**167**) and 2*β*, 6*β*-dihydroxyteuscordin (**168**).	[138]
T. scorodonia	Teuscorolide (**169**), Teuscorodal (**170**), Teuscorodol (**171**), Teupolin-I (**137**), Teuscorodin (**172**), Teuscorodonin (**166**) and 2-hydroxyteuscorolide (**173**).	[139-140]
T. spinosum	Teuspinin (**174**), 19-acetylteuspinin (**175**) and 19-acetylgnaphalin (**45**).	[141]
T. tomentosum	Teuctosin (**176**), Teuflin (**48**), Teucrin H_2, Montanin-D (**55**), 6*β*-hydroxyteuscordin (**167**), 6*β*-acetylteuscordin.	[142]

Table (1): Cont.

T. trifidum	Teutrifidin and 4α-18-epoxytafricanin-A.	[143]
T. viscidum	Teucvin, Teucvidin (**54**), 6-epiteucvin and Teuflin (**48**).	[144-148]
T. viscidum var. *miquelianum*	Teucvidin (**54**).	[149-150]
T. webbianum	2*β*-hydroxyteucvidin, Teuflidin (**73**) and Teucrin-A (**47**).	[151]
T. yemense	6*β*-*O*-acetyl-3*β*-hydroxyteucroxylepin, Teucryemin, 19-*O*-acetylteucryemin and Teucryeminone.	[152]

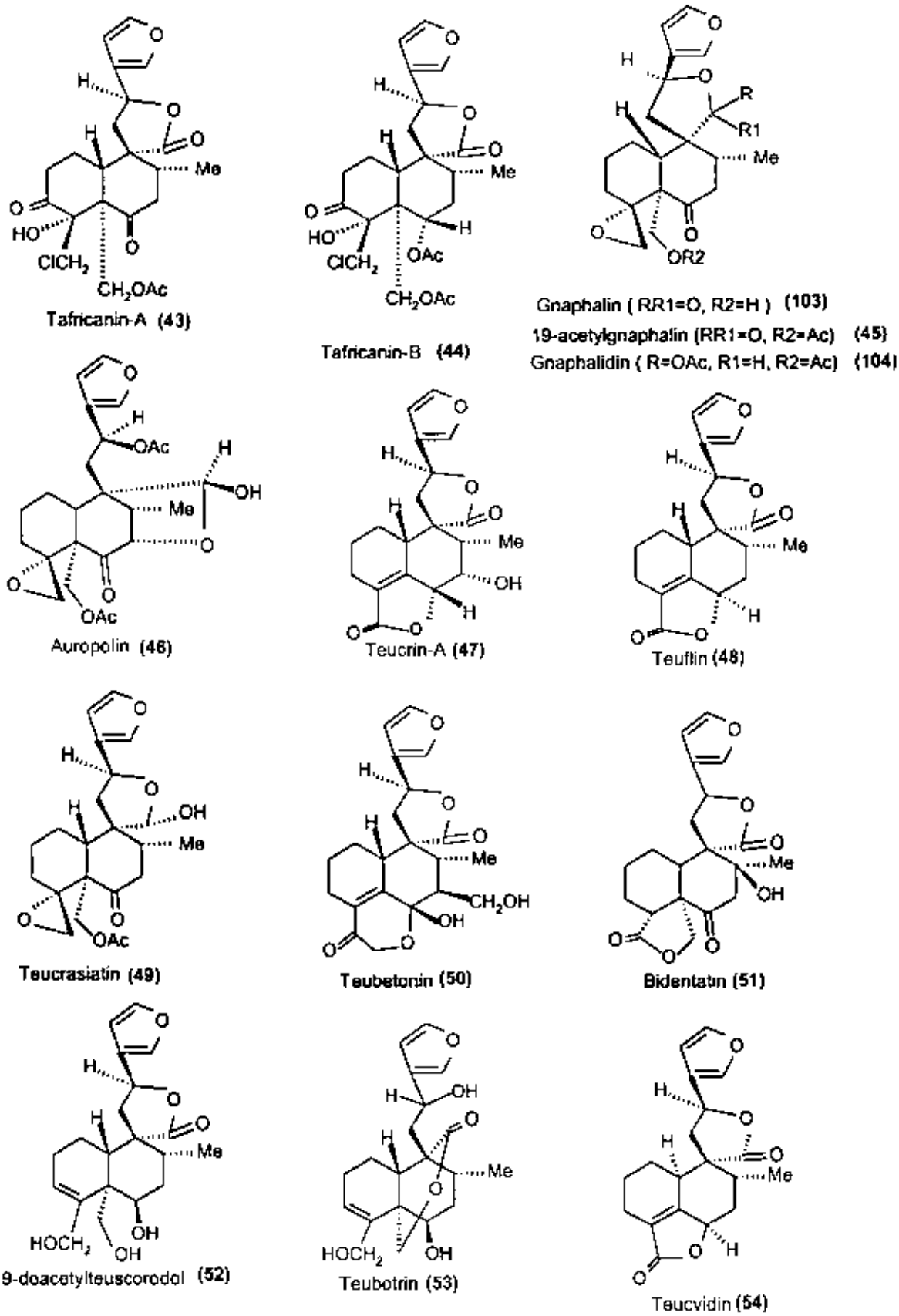
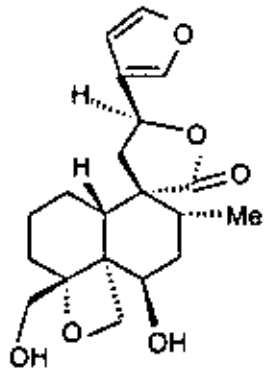
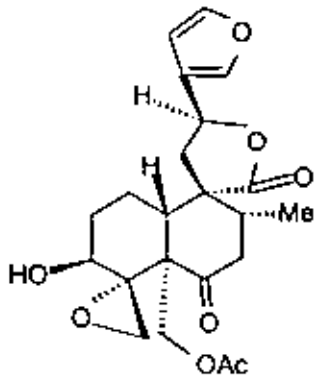


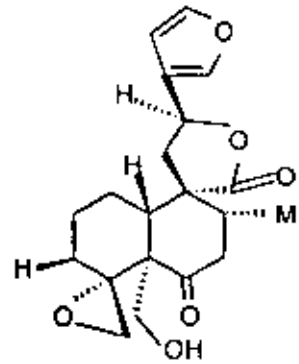
Fig. (2) : Chemical structures of some diterpenoid compounds in *Teucrium* genus.



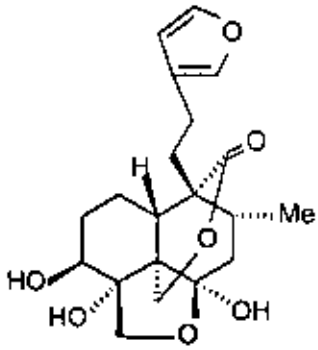
Montanin-D (55)



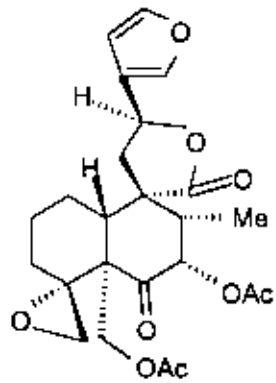
19-acetylteulepicin (56)



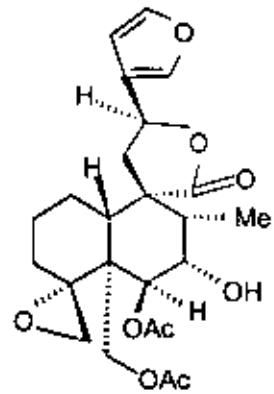
Teulepicin (57)



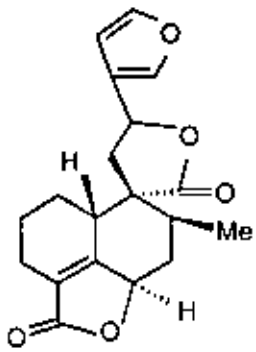
Teulepicephin (58)



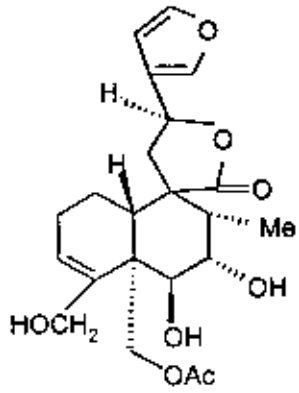
Capitatin (59)



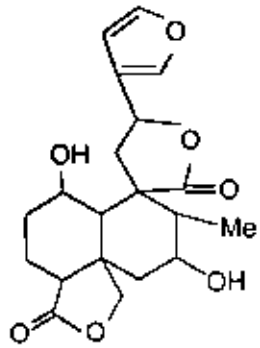
Teucapitatin (60)



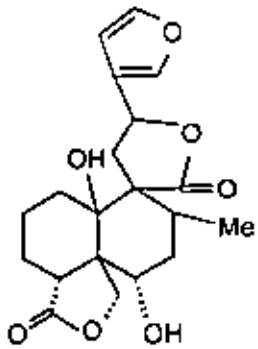
Teuchamaedryn-A (66)



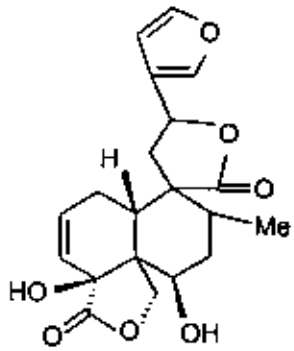
Lolin (61)



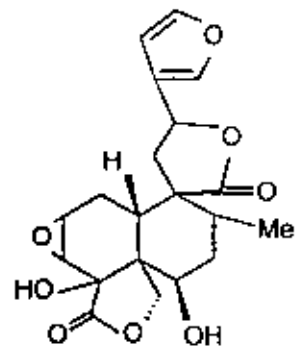
Teucrin-B (62)



Teucrin-E (63)

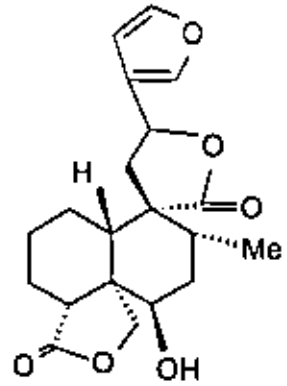


Teucrin-F (64)

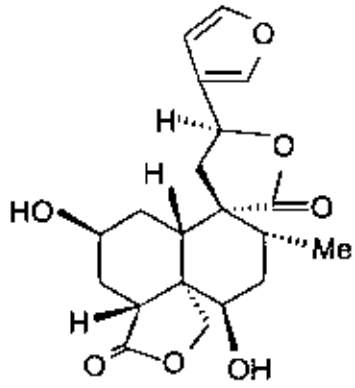


Teucrin-G (65)

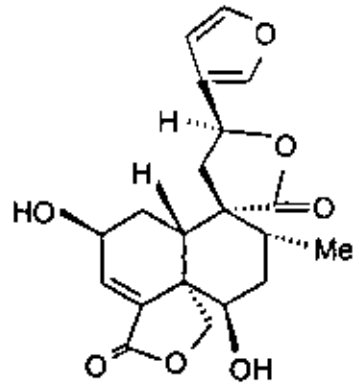
Fig. (2) : Cont.



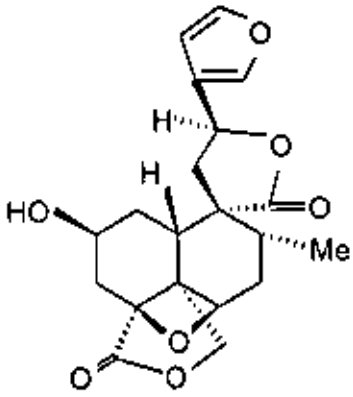
Teuchamaedryn-B (67)



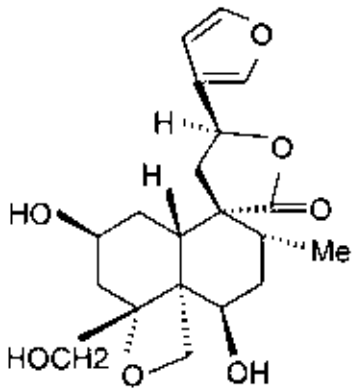
Dihydroteugin (68)



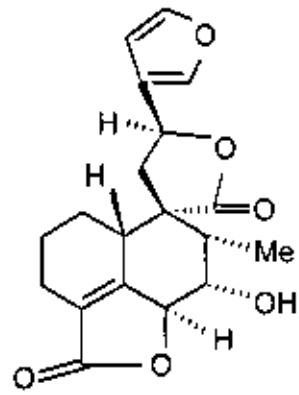
Teugin (69)



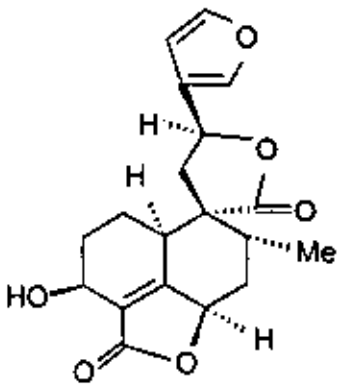
Chamaedroxide (70)



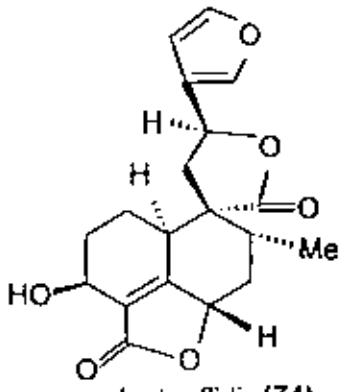
Teucroxide (71)



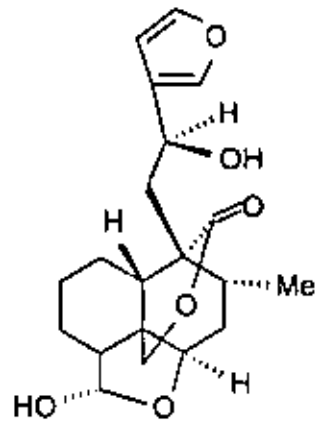
6-epiteucrin-A (72)



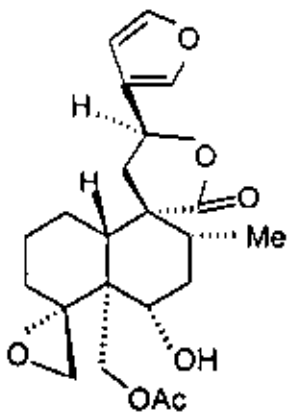
Teuflidin (73)



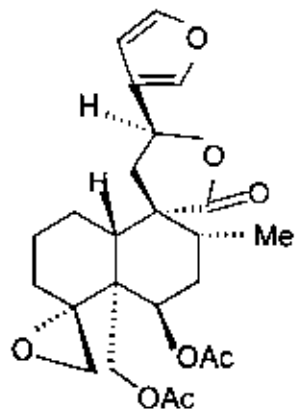
Isoteuflidin (74)



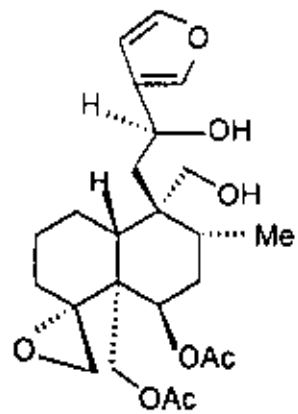
Teuchamaedrin-C (75)



Teucjaponin-A (76)

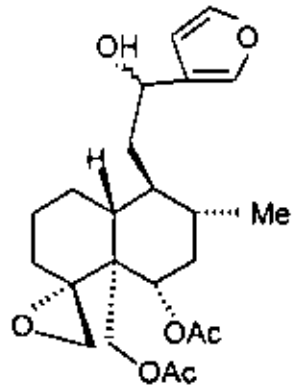


6-acetylteucjaponin-B (77)

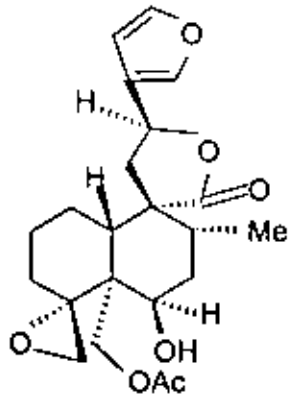


Teucretol (78)

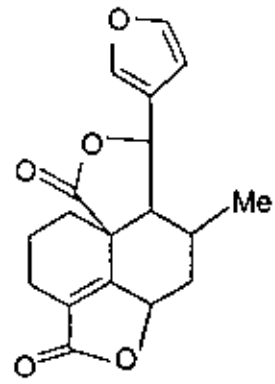
Fig. (2) : Cont.



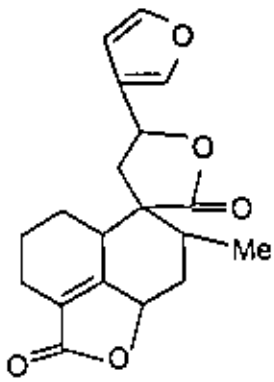
6,19-diacetylteumassilin(79)



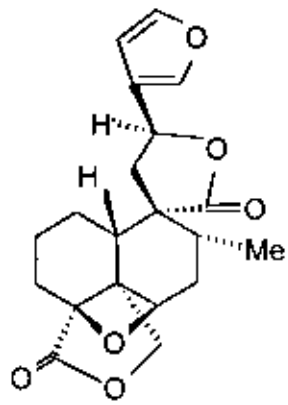
Teucgaponin-E(80)



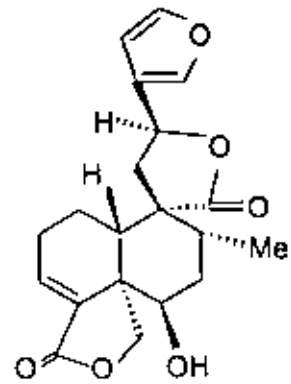
Eugarzasudone(81)



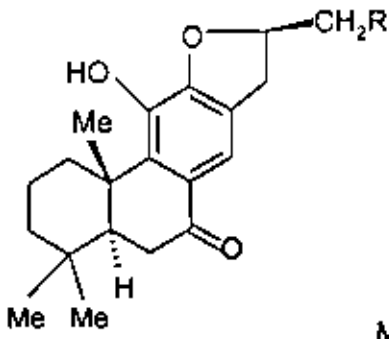
Eugarzasadine(82)



2-deoxychamaedroxide(83)

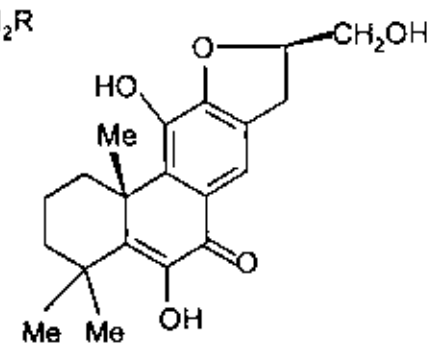


6β -hydroxyteuscordin(84)

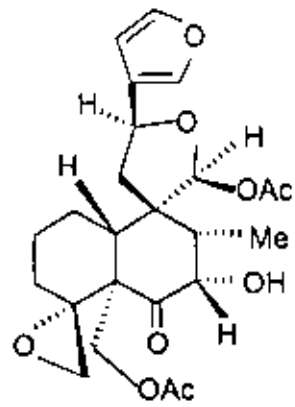


Villosin-A (R=H)(85)

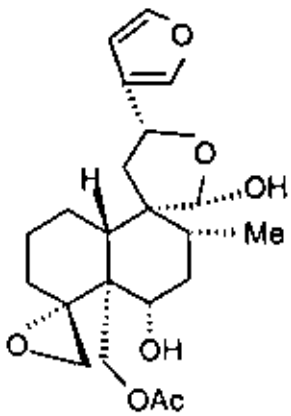
Villosin-B (R=OH)(86)



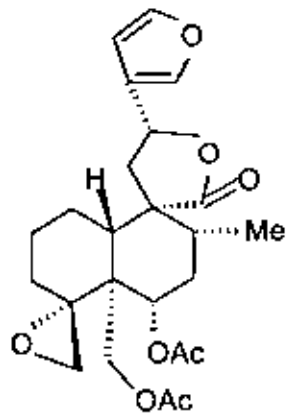
Villosin-C(87)



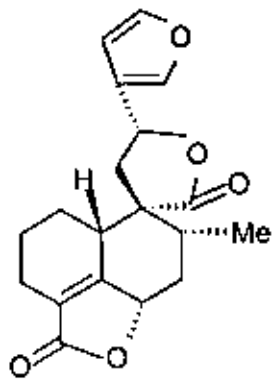
Eriocephalin(88)



Teupolin- I (89)

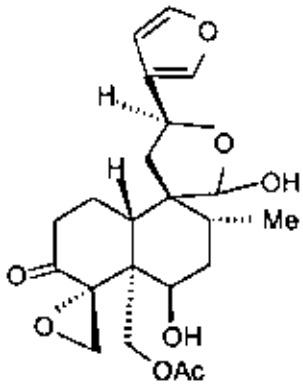


Montanin-C (90)

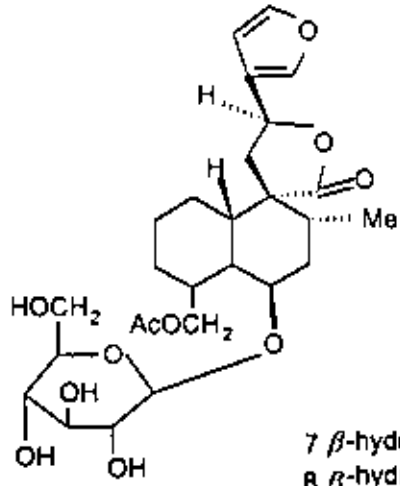


12-epiteucvir(91)

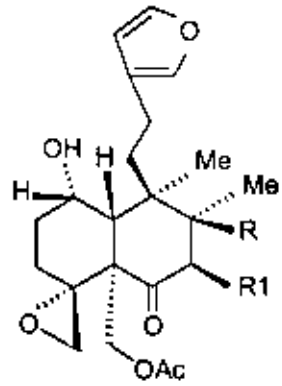
Fig. (2) : Cont.



Teuflavin (92)



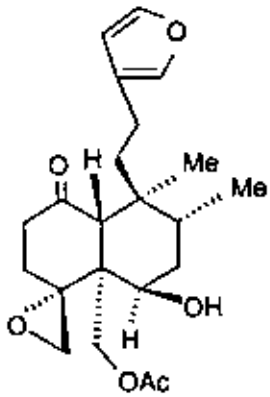
Teuflavoside (93)



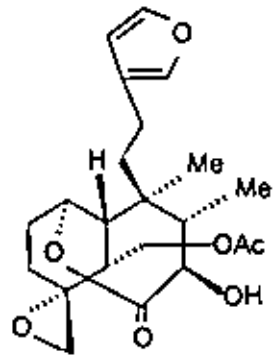
Fruticolone (R,R1=H) (94)

7 β-hydroxyfruticolone (R=H, R1=OH) (95)

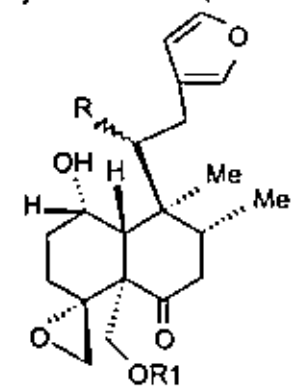
8 β-hydroxyfruticolone (R=OH, R1=H) (96)



Isofruticolone (97)

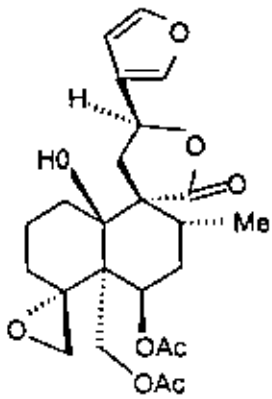


Fruticolide (98)

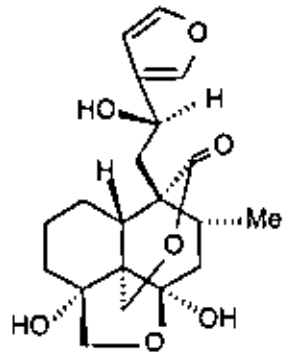


11-hydroxyfruticolone(R=OH, R1=Ac) (99)

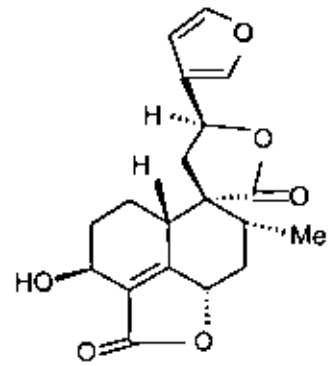
Deacetylfruticolone (R=R1=H) (100)



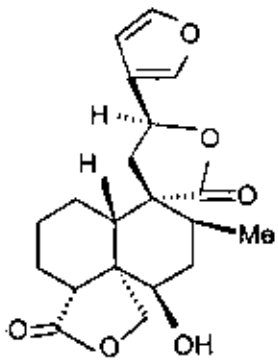
6-acetyl-10-hydro-xyteucjaponin-B (101)



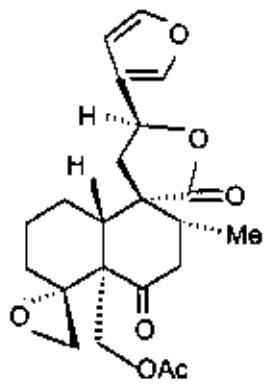
Teugnaphalodin (102)



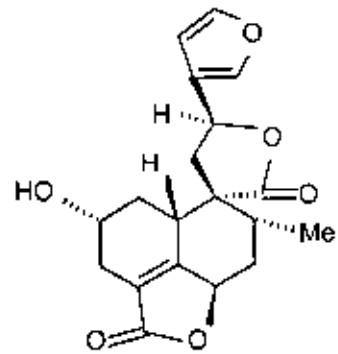
Teucrin-H1 (105)



Teucrin-H2 (106)

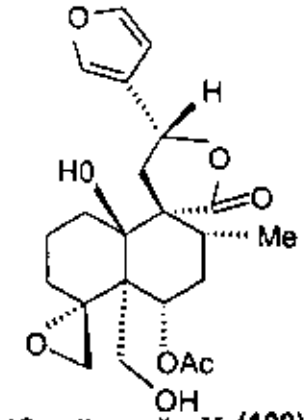


Teucrin-H3 (107)

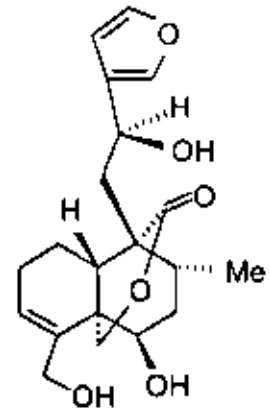


Teucrin-H4 (108)

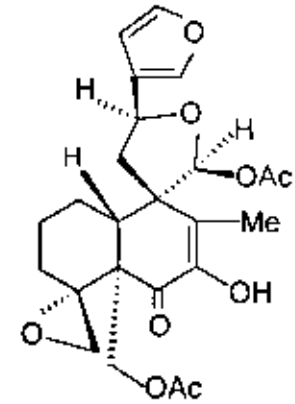
Fig. (2) : Cont.



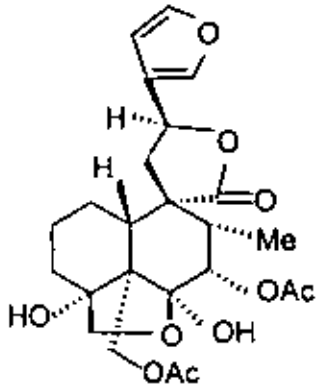
12-epiteupolin II (109)



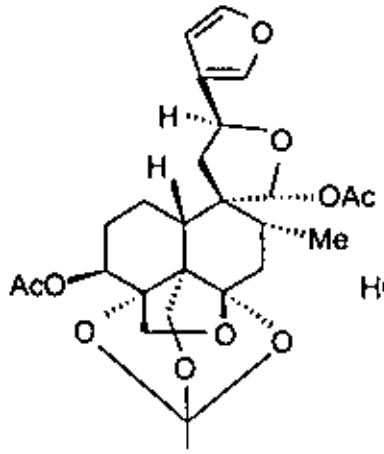
Teulamifin-B (110)



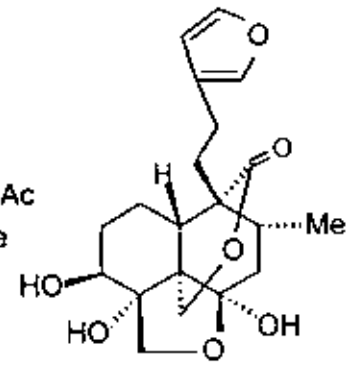
7,8-dehydroeriocephali (111)



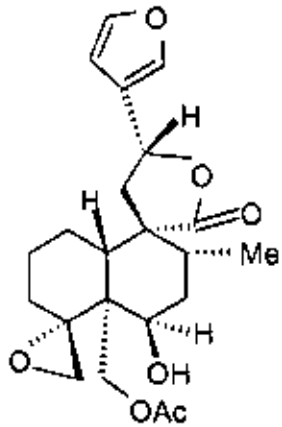
Teulanigerin (112)



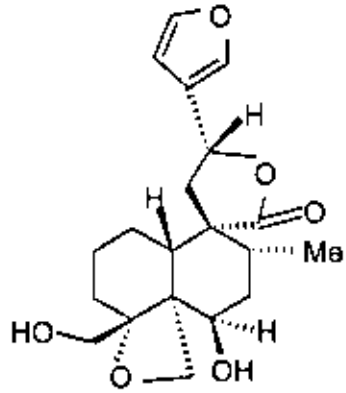
Teulanigeridin (113)



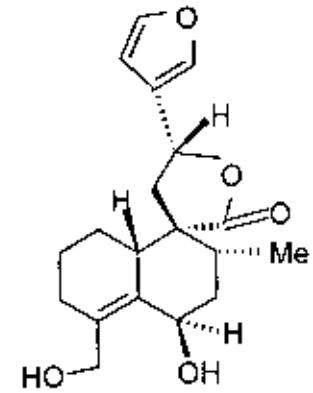
Teulepicephin (114)



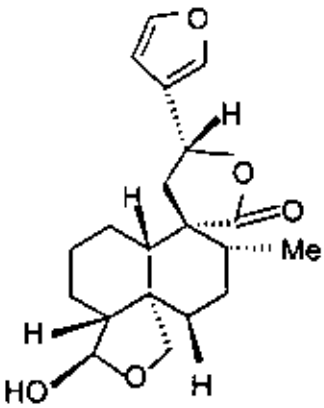
12-epiteucjaponin-A (115)



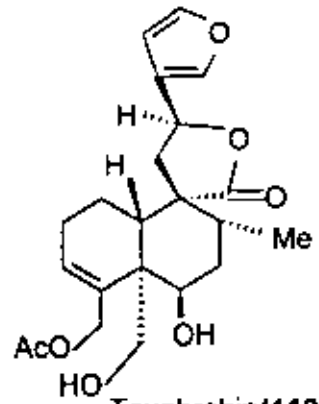
12-epi-montanin-D (116)



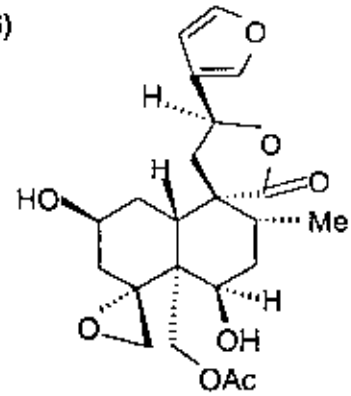
12-epi-montanin-B (117)



12-epi-teukotschyn (118)

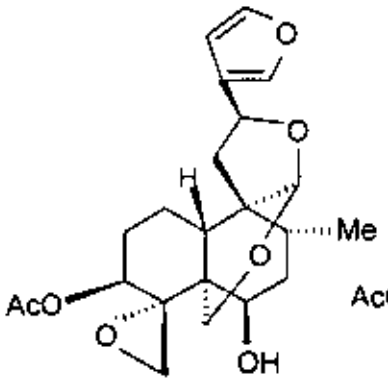


Teughrebin (119)

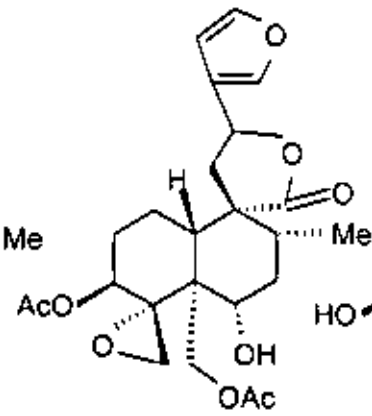


Teumarin (120)

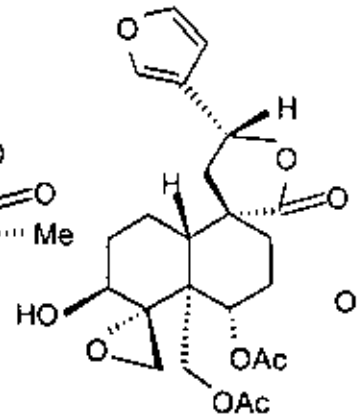
Fig. (2) : Cont.



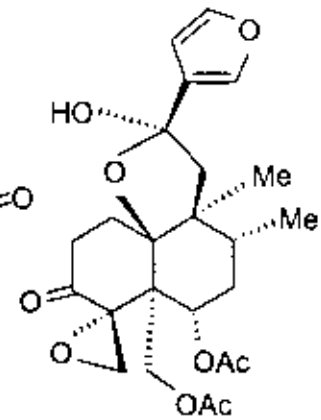
3-acetylteumicropin (121)



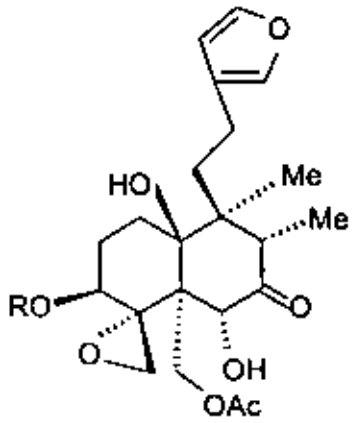
Teumicropodi (122)



Montanin-G (123)

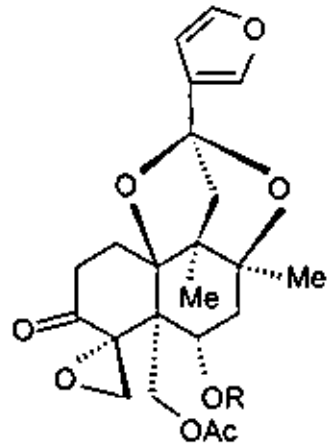


Teucrolivin-A (124)



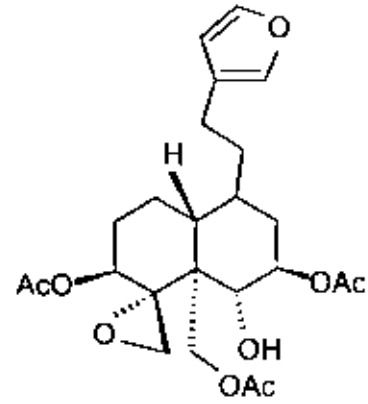
Teucrolivin-B (R=Ac) (125)

Teucrolivin-C (R=H) (126)

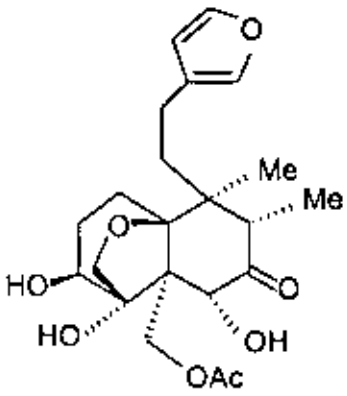


Teucrolivin-G (R=Ac) (127)

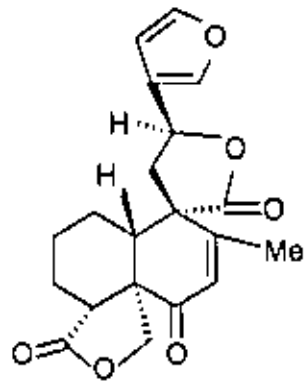
Teucrolivin-H (R=H) (128)



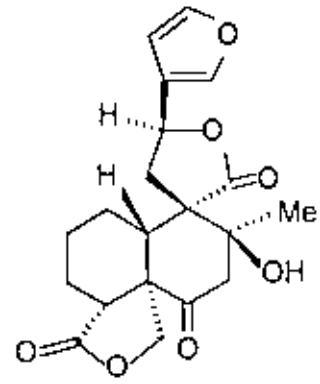
Teucrolivin-D (129)



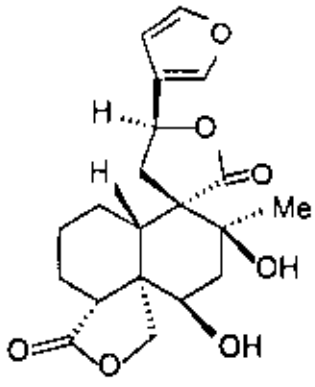
Teucrolin-E (130)



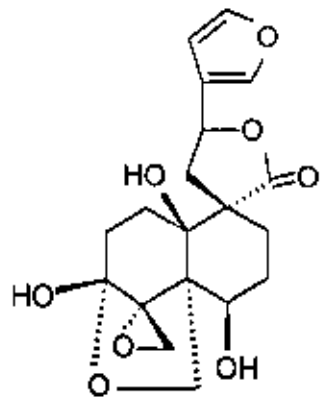
Teupernin-A (131)



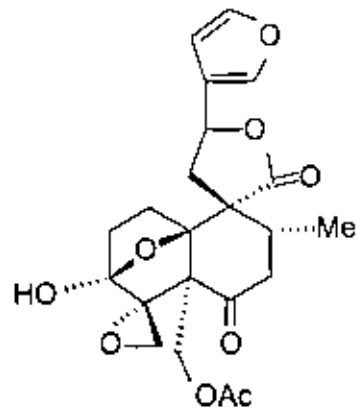
Teupernin-B (132)



Teupernin-C (133)

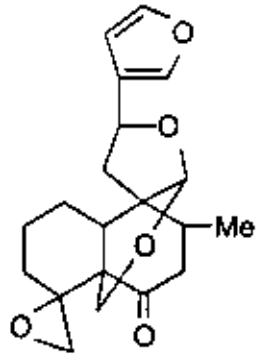


Teupestalin-A (134)

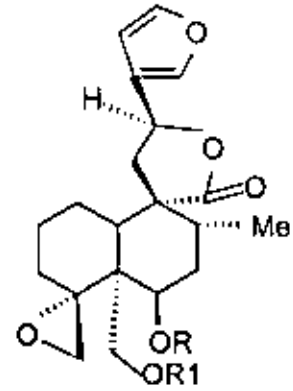


Teupestalin-B (135)

Fig. (2) : Cont.

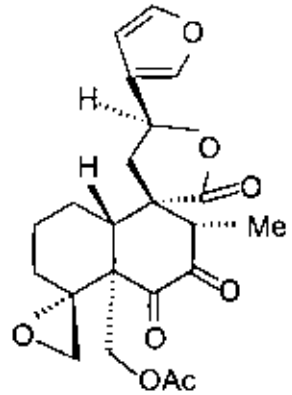


Teucrin-P **(136)**

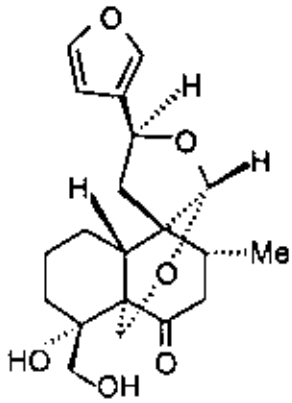


Teupolin-I (R=H, R1=Ac)**(137)**

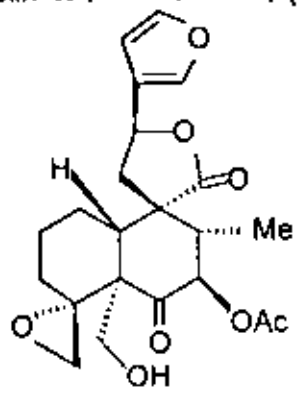
Teupolin- II (R=Ac, R1=H) **(138)**



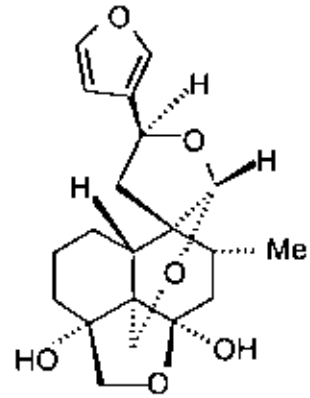
Clerodanedione **(139)**



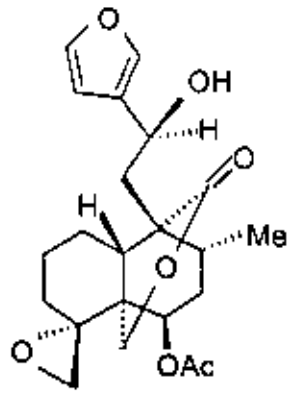
Teupolin III **(140)**



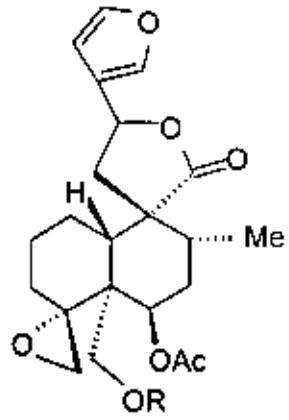
Teupolin IV **(141)**



Teupolin- V **(142)**

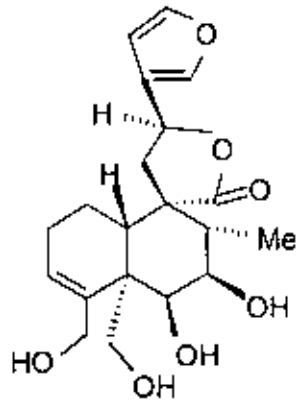


Quassimin **(143)**

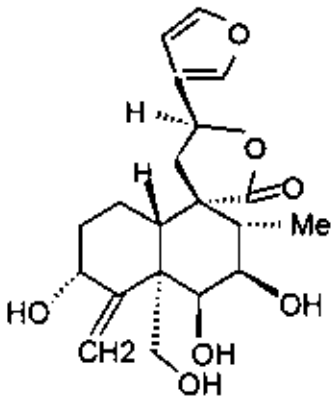


6-acetylmontanin-F (R=Ac)**(144)**

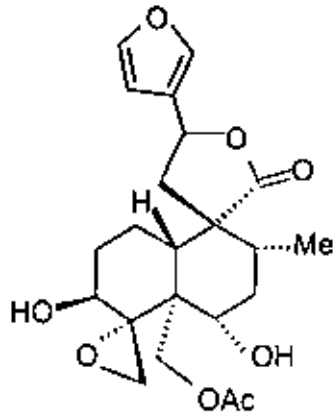
6-acetyl-19-deacetylmontanin-F (R=H) **(145)**



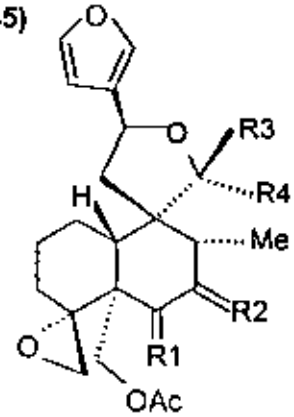
Teulolin-A **(146)**



Teulolin-B **(147)**



3-deacetylteumicropodin**(148)**



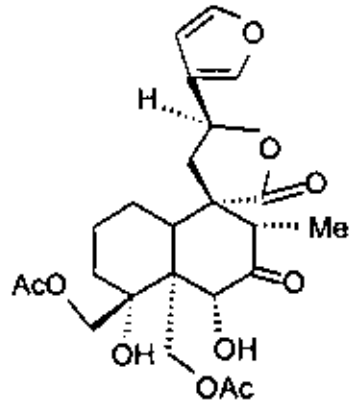
7-deacetylcapitatin**(149)**

(R1=O, R2=OH,H,R3R4=O)

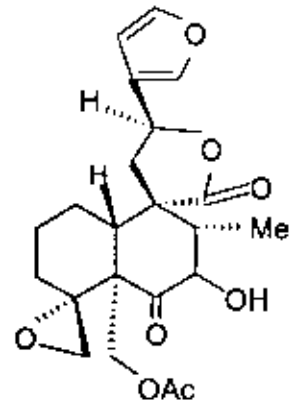
20-epiisoeriocephalin**(150)**

(R1=OH,OH,R2=O,R3=H,R4=OAc)

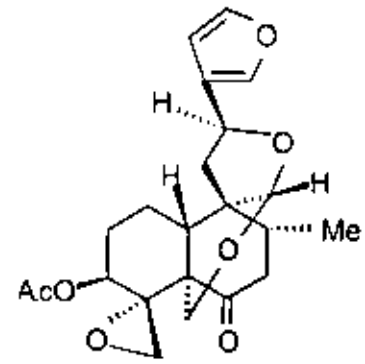
Fig. (2) : Cont.



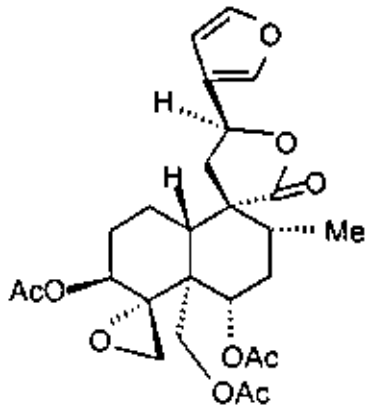
Picropolinol (151)



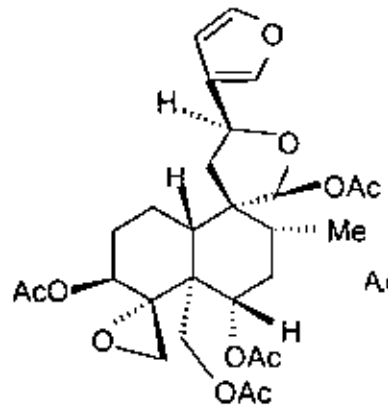
Picropolinone (152)



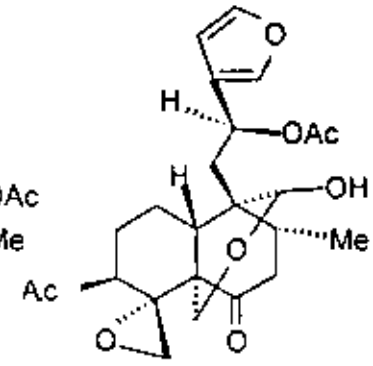
Teupyrenone (153)



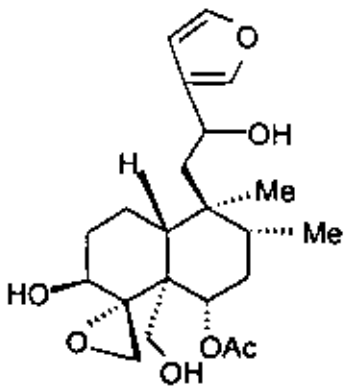
Teupyreinin (154)



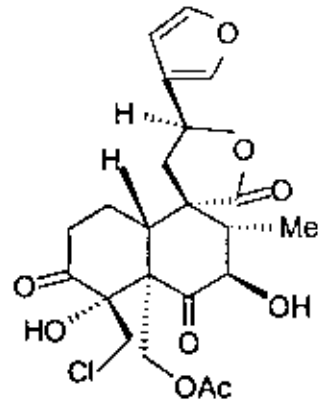
Teupyreinidin (155)



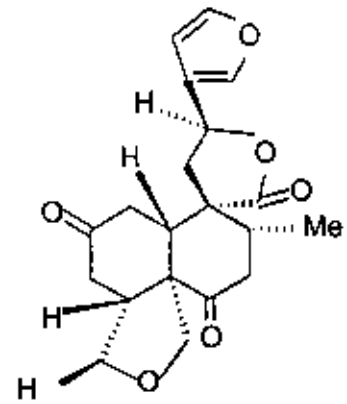
Teupyrrin-A (156)



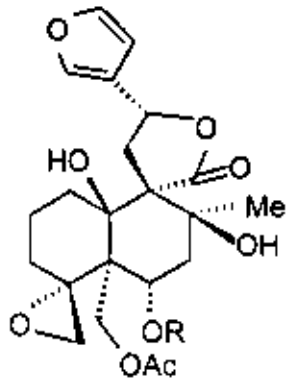
Teupyrin-B (157)



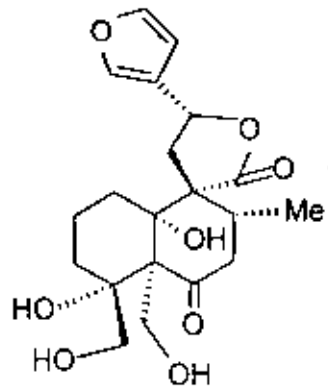
Teuracemin (158)



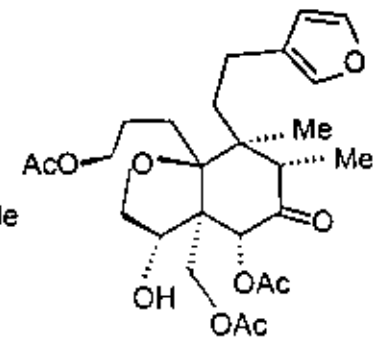
Teusalvin-A (159)



Sandrasin-A (R=Ac) (160)
6-deacetylsandrasin-A (R=H) (161)

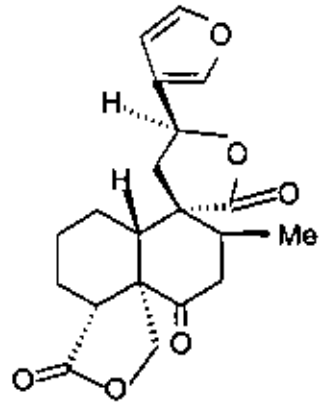


Sandrasin-B (162)

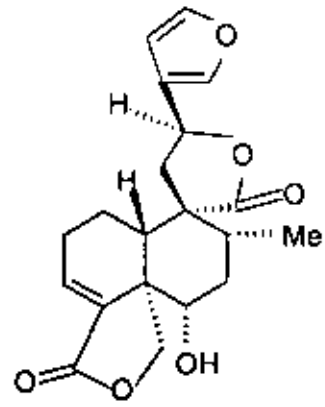


Teusandrin-F (163)

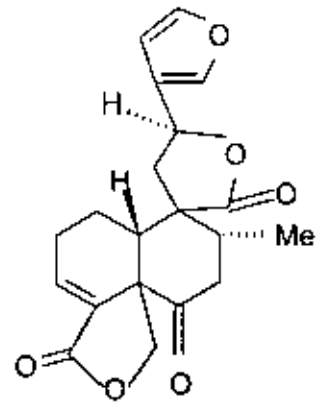
Fig. (2) : Cont.



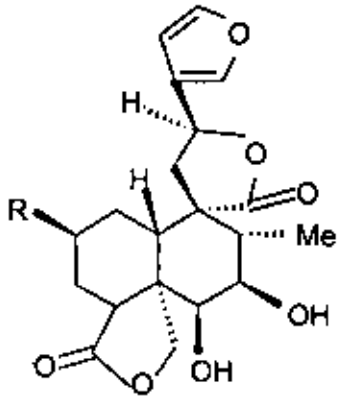
6-ketoteuscordin **(164)**



6 α-hydroxyteuscordin**(165)**

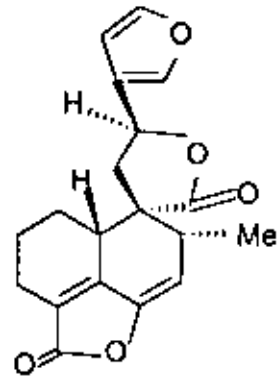


Teuscordinon **(166)**

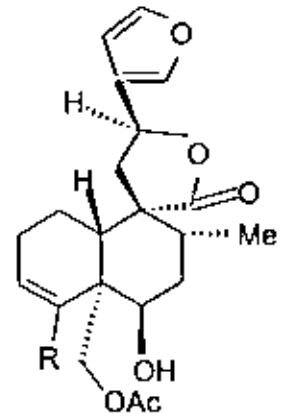


6 β-hydroxyteuscordin (R=H)**(167)**

2β,6 β-dihydroxyteuscordin (R=OH)**(168)**

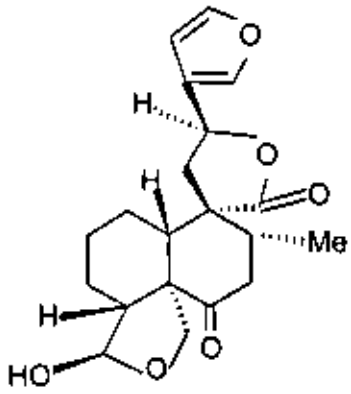


Teuscorolide **(169)**

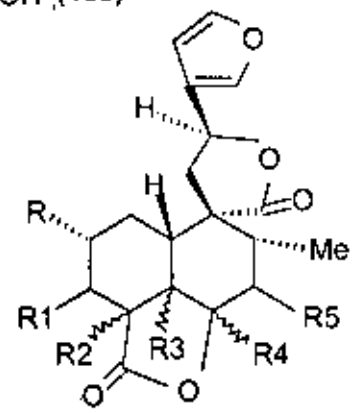


Teuscorodal (R=CHO)**(170)**

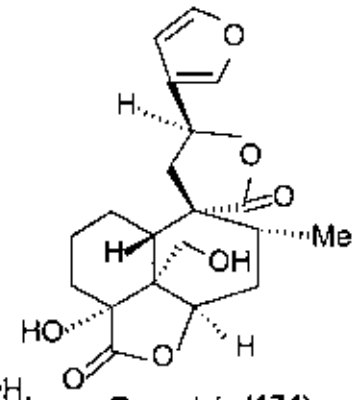
Teuscorodol (R=CH2OH)**(171)**



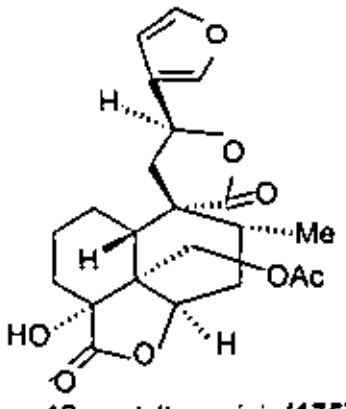
Teuscorodin **(172)**



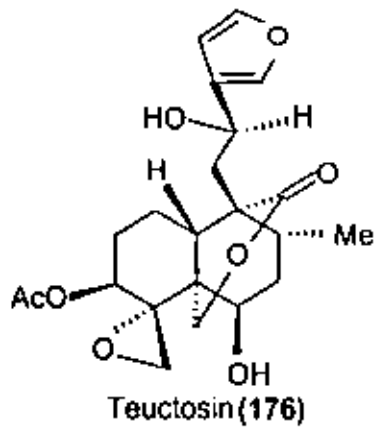
2-hydroxyeuscorolide (R=OH, R1=H,R2R3=R4R5=bond) **(173)**



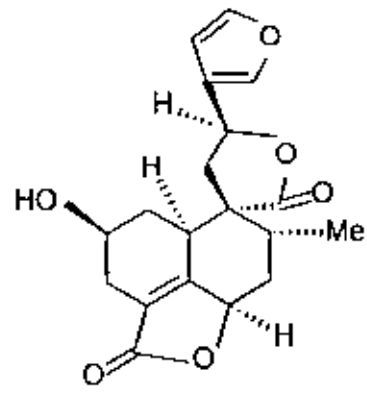
Teuspinin **(174)**



19-acetylteuspinin**(175)**



Teuctosin **(176)**



2 β-hydroxyteucvidin **(177)**

Fig. (2) : Cont.

3- Sesquiterpenes in *Teucrium genus* :-

The sesquiterpenoid, 11-hydroxyvalenc-1(10)-en-2-one was isolated from *T. carolipaui* by Savona *et. al.* in 1986[133].

The three guaiane derivatives, [1α, 5β-guai-10(14)-ene-4β,6β-diol (teucladiol), 1α, 5β-guaiane-4β,6β,10α-triol(teuclatriol), and 1α, 5β-guaiane-4β,6β,10β-triol (10-epiteuclatriol)] were isolated from the aerial parts of *T. leucocladum* by Maurizio *et. al*[87].

Braulio *et. al.*[153-154] were investigated the sesquiterpene fraction of *T. heterophyllum*, they identified teucdiol-A, teucdiol-B, teucrenone, 7-epiteucrenone, teuhetone, teuhetenone-A, teuhetenone-B, tephyllone, 9β-hydroxytephyllon, 9-oxo-tephyllone and 3,4-dehydroblumenol-C.

Two sesquiterpene diol, 7-epieudesm-4(15)-ene-1β,6α-diol and 7-epieudesm-4(15)-ene-1β,6β-diol, in addition to the sesquiterpene alcohols, β-eude-smol and α-cadinol were isolated from *T. polium* by Kamel, Alaa[155].

Maryam *et. al.*[156] were investigated *T. stocksianum*, they isolated sesquiterpenes shiromool 1,10-epoxides.

4- Triterpenes and Sterols in *Teucrium genus* :-

Triterpenoids are compounds with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C_{30} hydrocarbon, squalene. They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. They are colourless, crystalline, often high melting, optically active substances, which are generally difficult to characterized because of their lack of chemical reactivity.

Sterols are triterpenes which are based on the cyclopentane perhydrophenanthrene ring system. In recent years, an increasing number of such compounds have been detected in plant tissues[8].

Grzybek, Jan [157] was investigated *T. botrys* (A), *T. chamaedrys* (B), *T. montanum* (C), *T. scordium* (D) and *T. scordonia* (E). He found stigmasterol and β-amyrin in all the investigated species, β-sitosterol in species A, B and C and ursolic acid in B. Species C contain identified triterpenes which may constitute the sapogenin. In other species, saponins appeared in free form.

From *T. cubense* the clerosterol (stigmasta-5,25-dien-3β-ol) was isolated by Dominguez *et. al* in 1974 [51].

Stigmasterol, phytosterol and β-amyrin were isolated from *T. canadense* by Anderson *et. al* [158].

The triterpenes, ursolic, oleanolic, micrometric, maslinic, and 3-epi-maslinic acids were isolated from *Teucrium* species by Passannati *et. al* [159].

From the aerial parts of *T. kotschyanum*, the ursolic acid was isolated by Fatima *et. al.* [81]

In addition to the known sterols, 24α-ethylcholesta-5,25-dien-3β-ol, sitosterol, 3β-hydroxy stigmast-24(24'), 25-dien-24 2-al and 3β-hydroxy-24α-ethylcholesta-5,25-diene-7-one, the triterpene, ursolic acid and α-amyrin, were isolated from the aerial parts of *T. chamaedrys* subs. *chamaedrys* by Ulubelen *et. al.* [160]

Kisiel *et. al.* in 1995 [99] were isolated the most abundant steroids in *T. montanum* subsp. *pannonicum*, they identified clerosterol and clerosteryl acylglucosides in the aerial parts.

From *T. abutiloides* and *T. betonicum*, three steroids known as: 24-ethylcholestane derivatives, (24S)-24-ethylcholesta-5,22(E),25-trien-3β-ol, (24S)-24-ethylcholesta-5,25-dien-3β-ol (clerosterol) and (24R)-24-ethylcholesta-5,22(E)-dien-3β-ol (poriferasterol) were identified by Gaspar *et. al.* [161]

Chen *et. al.* in 2000 [162] were studied the terpenic fraction of *T. integrifolium*. They isolated a triterpene compound, which was identified as 3β-hydroxyfern-9-(11)-en-23-oic acid (integrifolin).

Flavonoids and their isolation:-

Plants of the family Lamiaceae are known for their high contents of flavonoids [163]. So, here we will discus some notes related to the structure and methods of isolation of flavonoids.

Flavonoids comprise a large group of secondary plant metabolites. Presently more than 5000 individual compounds are known, which are based on very few core structures, their multitude derives mainly from the various hydroxylation patterns (up to six hydroxy groups) and ether substitution by simple methylation or diverse mono- and di-saccharides [164].

The flavonoids are all structurally derived from the parent substance flavone which occurs as white mealy farina on *Primula* plants, and all share a number of properties in common [8]. All contain fifteen carbon atoms in their basic nucleus and these are arranged in a C6-C3-C6 configuration, that is, two aromatic rings linked by three carbon unit which may or may not form a third ring. For convenience the rings are labeled A, B and C and the individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A- and C-rings and "primed" numerals for the B-ring (Fig. 3), (but note modified numbering systems used for chalcones, Fig. 4)[165].

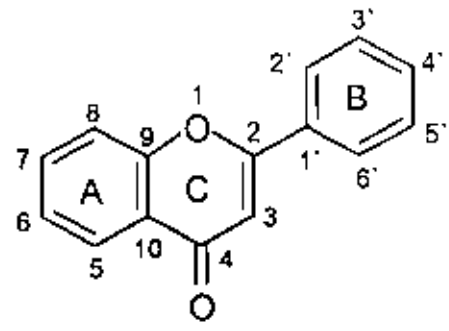


Fig. (3): Numbering pattern of the parent Flavonoid

The nomenclature of flavonoids proper is straight-forward with the aromatic ring-A condensed to the heterocyclic ring-C and the aromatic ring-B most often attached at the C-2 position. The various constituents are

listed first for the A and C ring and -as primed numbers for B ring [164].
Some ten classes of flavonoid are recognized as showed in Fig. (4) [8].

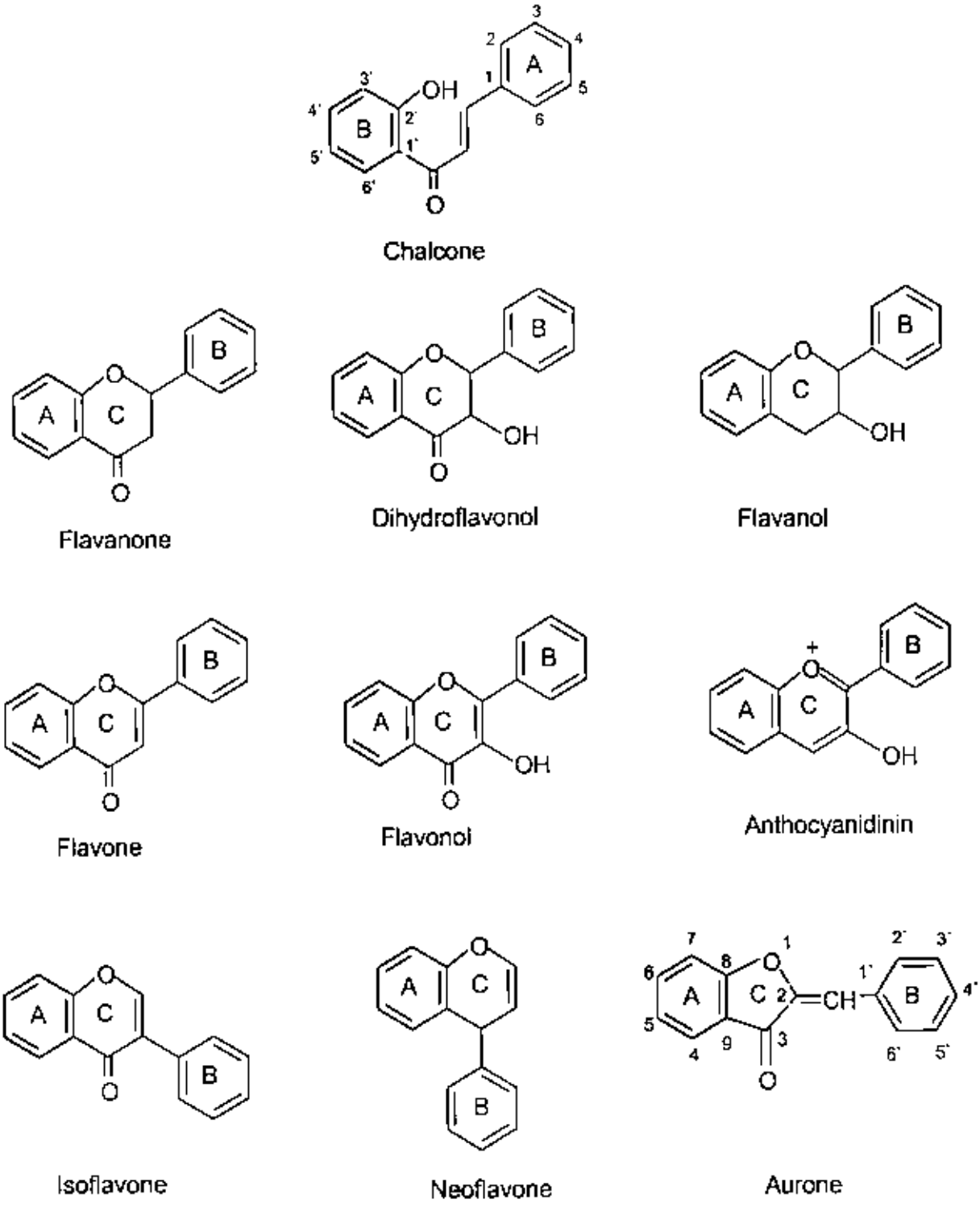


Fig. (4): Chemical structures of most important classes of flavonids

Flavonoids are mainly water-soluble compounds. They can be extracted with 70% ethanol and remain in the aqueous layer, following partition of this extract with organic solvent. Flavonoids are phenolic and hence change in colour when treated with base or with ammonia; thus they are easily detected on chromatograms or in solution romatic systems and thus show intense absorption bands in UV and visible regions of the spectrum [181].
Flavonoids are present in plants as mixtures and it is very rare to find only a single flavonoid component in a plant tissue [164].

Isolation techniques :

1. **Column chromatography :**

The column is simply a glass tube fitted with a tap at one end, with dimensions such that the diameter to length ratio is in the range 1:10 to 1:30. The size (volume) required for any particular separation can be roughly calculated once the weight of the flavonoid mixture is known. It is generally considered that for separations based on partitioning (i.e. most cellulose and silica chromatography), the sample to column ratio should be in the range 1:50 to 1:500, the latter ratio being more appropriate to complex mixtures and the former to simple mixtures. Column packings should be chosen that are marketed specifically for column chromatography as the particle size is important. Commercially available packings are usually in the 100-300 mesh range.
Packing of the column should be carried out with care, the objective being to produce a homogeneously packed column. It will be necessary first to plug the neck of the column with a wad of glass or cotton wool. This should then be covered to a height of about 10 cm with the eluting solvent. The column packing is then slurred in a beaker with the same solvent and poured carefully into the column, preferably all in one continuous process to avoid layering. The packing is then permitted to settle and the excess

solvent drained off.

The first step in column chromatography is to apply the sample solution to the top of the column in such a way that a narrow band is formed for further elution. To this end the sample should be dissolved in a minimum volume of solvent. The solvent used should be one of those selected for later elution. Application of the concentrated solution to the top of the column should be carried out with care to avoid disturbing the surface and use of a pipette is recommended for this purpose. The sample concentrate is then permitted to seep slowly into the column by opening the column tap slightly [165].

- **Column chromatography adsorbents :**

The range of available column packing is vast and the list below gives a number of the more useful types.

A-Polyamide column chromatography :-

Although a number of different adsorbents have been used for column chromatography of flavonoids (e.g. silica gel, magnesol, cellulose powder, polyamide, charcoal and starch), the best adsorbent for the chromatographic separation of all types of flavonoids appears to be polyamide. A polyamide-type adsorbent used in conjunction with various mixtures of water and methanol as eluents has been used successfully for the separation of complex mixtures of glycosides and aglycones of isoflavones, flavones, flavonols, dihydroflavonols and flavanones. Fractions produced from a large polyamide column often yield pure flavonoids or simple mixtures which may be further separated by additional column or paper chromatography. Two problems often associated with polyamide columns, namely, slow elution rates and the elution with the methanolic solvents of a mixture of flavonoids and low molecular weight polymer material.

B-Silica Gel column chromatography :-

Silica gel may be used for the separation of relatively non-polar flavonoid aglycones such as isoflavone, methoxylated flavones and flavonols. Silica gel column chromatography is not suitable for the separation of polar flavonoids such as polyhydroxyflavonols or glycosides but does provide a convenient method for the purification of many flavonoid aglycones obtained by the hydrolysis of glycosides. An increase in the methanol content of the eluting solvent will allow the removal of most flavonoid aglycones from silica gel. Isoflavone aglycones can be separated on silica gel by using as eluent chloroform which is gradually increased in polarity by the addition of ether or ethyl acetate.

C-Sephadex LH-20 column chromatography :-

Johnston, Stern and Waiss have described a procedure for the separation of flavonoids; both aglycones and glycosides, on Sephadex LH-20 columns. Generally the flavonoids were dissolved in methanol and then added to the column; however, in a few instances a 1:1 dioxane-methanol solution was used to dissolve the flavonoids. To illustrate the effectiveness of the procedure, the separation of a mixture of 166 mg of rutin and 75 mg of quercetin was described. Rutin was recovered in the 190-250 ml fraction and quercetin in the 390-460 ml fraction. Sephadex appears to be an efficient, high capacity medium for both analytical and preparative flavonoid work.[166] Since it produces residue-free eluant, LH-20 is ideally suited to final clean-up of flavonoid aglycones and glycosides which have been isolated from paper, cellulose, silica or polyamide. Methanol is generally a suitable solvent, although some water may be needed[165].

2. Paper Chromatography (PC) :

Paper chromatography (PC) is probably the most generally useful recognition chromatographic technique available to flavonoid chemists today [165].

On of the main advantages of PC is the great convenience of carrying out separations simply on sheets of filter paper, which serve both as medium for separation and as the support. Chromatography on paper usually involves either partition or adsorption chromatography. In partition, the compounds are partitioned between a largely water-immiscible alcoholic solvent (e.g. n-butanol) and water. The classic solvent mixture, n-butanol-acetic acid-water (4:1:5, top layer)(B A W). By contrast, adsorption forces are one of the main features of PC in aqueous solvent [8].

Most separation achieved with acetic acid (HOAc) at different concentrations, with BAW or with 2-methylpropan-2-ol–HOAc–Water (TBA) (3:1:1). Some other solvents have been used, namely butanol-1-ol –ethanol–water (5:1:4), butan-1-ol–pyridine–water (30:20:15), propan-2-ol–water (6:4), acetate–pyridine–water (2:1:2) or phenol saturated with water. These systems are also indicated for preparative paper chromatography [167]. The detection of flavonoid spots on paper usually by viewing the chromatogram under UV lamp (366 nm) with and without ammonia or other reagents [8].

3. Thin Layer Chromatography (TLC) :

Thin layer chromatography (TLC) remains an important method for the detection and separation of flavonoids in crude plant extracts [167]. The special advantages of TLC compared to PC include versatility, speed and sensitivity. Versatility is due to the fact that a number of different adsorbents beside cellulose may be spread on to a glass plate or other support and employed for chromatography. The greater speed of TLC is

due to the more compact nature of the adsorbent when spread on a plate. The sensitivity of TLC is such that separation on less than μg amounts of material can be achieved if necessary [18]. TLC is more commonly used for the analysis of mixtures than for the isolation of pure flavonoids. Polyamide is probably the best TLC adsorpant for all types of flavonoids; however, number of others (e.g. silica gel G, microcrystalline cellulose), may also be used. The detection of flavonoid spots on thin layer plates may be achieved, as in PC. A number of adsorbents are now available which contain UV-fluorescent phosphors and these provide a highly sensitive method for detection of flavonoids [166].

4. High Performance Liquid Chromatography (HPLC) :

High performance liquid chromatography is basically a form of column chromatography which utilized a column of packing material of small particle size and regular shape. The technique offers the researcher a method of quantitatively analyzing the flavonoid components of a mixture at a high level of resolution and sensitivity (<50 ng) and it is the quantitative aspect of the analysis in particular which sets it apart from other chromatographic methods. Quantification is achieved by automatically monitoring the eluant leaving the column by means of a variable wavelength UV spectromonitor and the chromatogram is traced out as a series of peaks on a chart. A wide range of packing/solvent combinations have been reported [165]. It is clear that for most applications reversed phase columns (in which a hydrocarbon is bonded to the silica packing) of the μ-Bondapak C-18 type are suitable. Solvents such as H_2O/MeOH, H_2O/MeOH/HOAc and H_2O/acetonitrile (in varying proportion) have been used successfully, and in some cases a changing solvent composition (solvent programming) has proven useful [165].

5- Flavonoids in *Teucrium genus* :-

The survey of the available literature about these compoundes in *Teucrium* genus shows the following results:

Brieskorn and Biechele in 1969 [168] were investigated *T. polium*. They isolated 6-methoxy genkwanin (**177**).

Grzybek, J. [169] was isolated the flavonoids diosmin (**178**), quercetin (**179**) and isoquercetin (**180**) from *T. botrys*, diosmin (**178**), isoquercetin (**180**) from *T. chamaedrys*, diosmin (**178**), isoquercetin (**180**) and quercetin (**179**) from *T. montanum*, isoquercetin (180), rutin (**181**) and quercetin (**179**) from *T. scordium* and isoquercetin (**180**) and rutin (**181**) from *T. scordonia*.

Raynaud and Chaouikha [170] were investigated the flavonoids of the flowering parts of *T. ramoissimum*. They found that only apigenin-6,7-diglucoside (**182**) is the main flavonoid.

Slyunkova *et. al.* [171-173] were investigated the flavonoidal constituents of *T. nuchens*. Six flavonoids were isolated and identified as nuchensein (**183**) ,luteolin (**184**), apigenin (**185**), luteolin-7-*O*-*β*-D-glucopyranoside (**186**), baicalein (5,6,7-trihydroxyflavone) (**187**) and 4'-hydroxybaicalein (**188**) .

From *T. gnaphalodes* the following flavonoidal aglycones and glycosides were isolated by Garcia *et. al.* [174-175] ; diosmin (**178**), cirsimaritin (4',5-dihydroxy-6,7-dimethoxyflavone) (**189**), salvigenin (5-hydroxy-6,7,4'-trimethoxyflavone) (**190**), cirsilineol (**191**), cirsiliol(4',5,3'-trihydroxy-6,7-dimethoxyflavone) (**192**), luteolin (**184**), apigenin (**185**), naringenin (apigenin-7-*O*-rhamnosylglucoside) (**193**), luteolin-7-*O*-*β*-D-glucoside (**186**), luteolin-7-*O*-*β*-D-rutinoside (**194**), luteolin-7-*O*-*β*-D-neohesperidoside (**195**) , luteolin-7-*O*-*β*-D-sambubioside and apigenin-7-*O*-*β*-D-glucoside (**196**).

From the aerial part of *T. scorodonia*, the known flavone, luteolin (**184**) was obtained by Macro *et. al.* [139]

A survey of the flavonoids of aerial parts of 42 European taxa of genus *Teucrium* has revealed the widespread presence of five surface flavonoids: cirsiliol **(192)**, cirsimaritin **(189)**, cirsilineol, salvigenin **(190)** and 5-hydroxy-6,7,3',4'-tetramethoxyflavone **(197)**[176].

Verykokidou *et. al.*[177-178] were investigated the phenolic components of leaves of *T. polium*. They found that it contain some flavonoidal aglycons identifid as; acacetin (5,7-dihydroxy-4'-methoxyflavone) **(198)**, salvigenin **(190)**, cirsimaritin **(189)**, eupatorin (3',5-dihydroxy-4',6,7-trimethoxyflavone) **(199)**, apigenin-4',7-dimethoxylether (5-hydroxy-4',7-dimethoxyflavone) **(200)**, cirsiliol **(192)**, in addition to some flavonoidal glycosides named acacetin-7-*O*-galactoside **(201)**, vicenin-2 **(202)**, rutin **(181)**, quercetin-3-*O*-glucoside **(180)**, luteolin-3-*O*-diglucoside **(203)** and apigenin-7-*O*-glucoside **(197)**.

The aerial part of *T. lepicephalum* was investigated by Savona *et. al.*[30] which result in isolation of known flavone , cirsiliol **(192)**.

Rizk *et. al.*[179] were studed the flavonoids of *T. polim* var. *polim* and var. *alba*. They isolated salvigenin **(190)** and cirsiliol **(192)** from both plants.

Ognesyan and Mnatsakanyan in 1987 [180] were investigated *T. hircanicum* and they isolated the flavonoids pedalitin (pedalin), luteolin-7-*O*-*ß*-D-glucopyranoside **(186)** and luteolin **(184)** from the aerial parts.

The flavonoid, diosmin **(178)** was isolated from *T. montanum* by Savin *et. al.* in 1988[181].

Maria *et. al.*[123] were isolated two flavones cirsiliol **(192)** and apigenin **(185)** from the aerial parts of *T. polium* subsp. *Vincentinum* .

From the aerial parts of *T. kotschyanum*, the flavones cirsimaritin **(189)** and cirsiliol **(192)** were isolated by Fatima *et. al.*[181]

Xie *et al* in 1990 [182] were isolated a flavone compound from *T. quadrifarium* which was identified as 5,4',5'-trihydroxy,2',6-dimethoxyflavone **(204)**.

The flavones, acacetin **(198)** and cirsimaritin **(189)** were isolated from the aerial parts of *T. japonicum* by Min *et. al.* [79]

Peter *et. al.* [98] were isolated the flavone cirsilol **(192)** from *T. montanum* subsp. *Montanum*.

Carmo and Nascimento in 1992 [183] were isolated and identified the flavones, viz. cirsimaritin **(189)** and cirsiliol **(192)** from *T. algarbiense*.

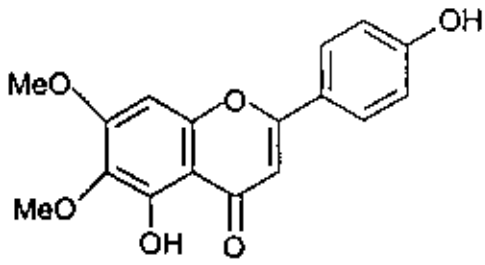
Kaloera *et. al.* [184-185] were investigated the flavonoids of *T. arduini*. They isolated luteolin-7-*O*-rutinosid, apigenin-7-*O*-glucoside **(197)**, querecetin-3-*O*-glucoside (isoquercitin) **(180)** and cirsimaritin **(189)** by means of column chromatography of Sephadex LH-20, TLC and HPLC.

The flavonoids, apigenin **(185)**, naringenin **(193)**, pectolinarigenin, and circiliol **(192)** were isolated from *T. chamaedrys* subsp. *chamaedrys* by Ulubelen *et. al.* [160]

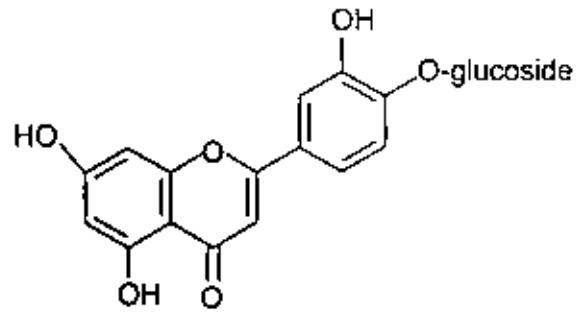
The flavone cirsilol **(192)** was isolated from *T. yemense* (aerial parts) in 1995 by Essam *et. al.* [152]

From the aerial parts of *T. nudicaula*, the flavones, cirsiliol **(192)** and eupatorin **(200)** were isolated by Gallardo *et. al.* [161]

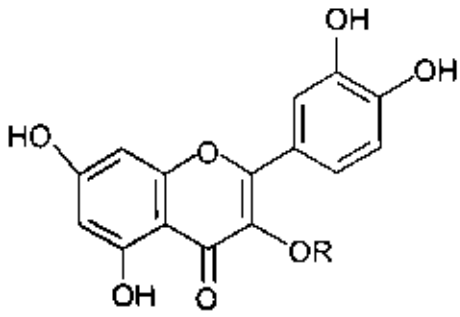
The flavonoids of both *T. leucocladum* and *T. polium* were studied by Kawashty *et. al.* in 1999 [186]. Apigenin-7-glucoside **(205)**, vicenin-2 **(202)**, luteolin-7-glucoside **(206)** and apigenin-5-galloylglucoside **(207)** as well as cirsimaritin **(189)** were identified. (Figure 5) shows some of these compounds in genus *Teucrium*.



6-methoxy genkwanin **(177)**

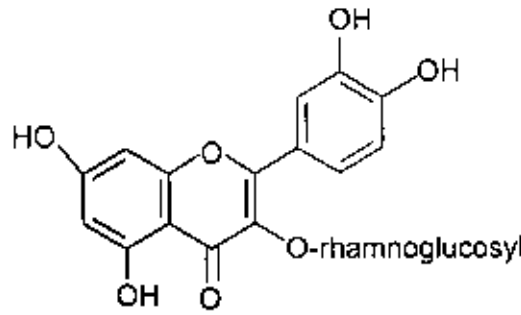


Diosmin **(178)**

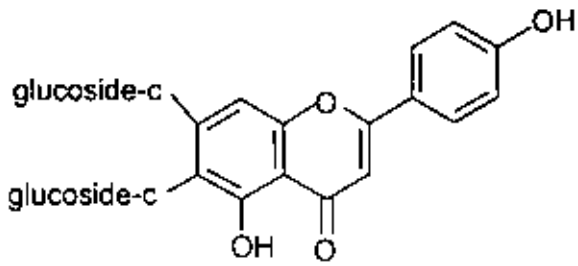


Quercetin, R = H **(179)**

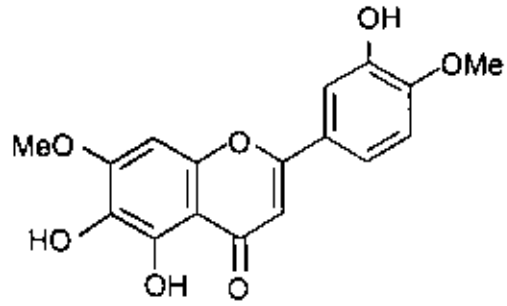
Isoquercetin, R = glucoside **(180)**



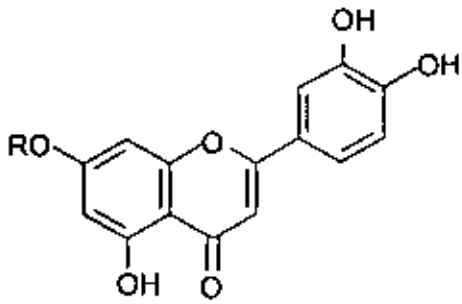
Rutin **(181)**



Apigenin-6,7-C-diglucosid **(182)**



Nuchensein **(183)**

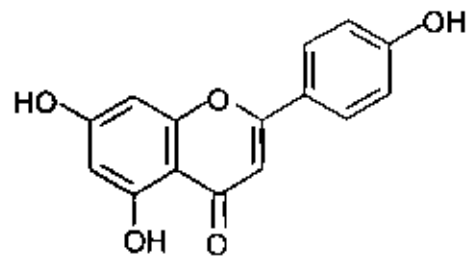


Luteolin, R = H **(184)**

Luteolin-7-O-glucopyranoside,
R = glucopyranosid **(186)**

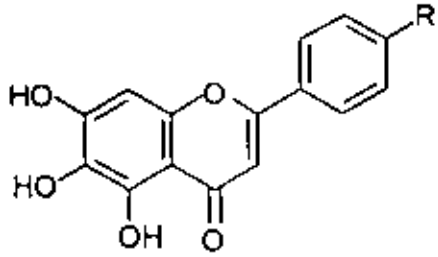
Luteolin-7-O-rutinoside,
R = rutinoside **(194)**

Luteolin-7-O-neohesperidoside,
R = neohesperidoside **(195)**

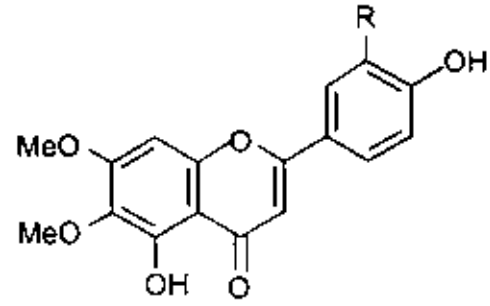


Apigenin **(185)**

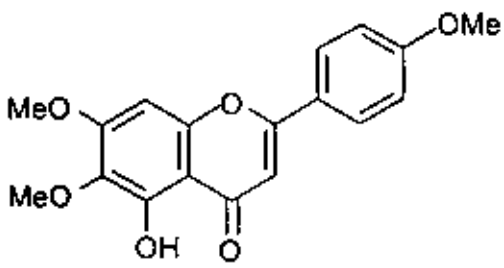
Fig. (5) : Chemical structures of flavonoidal compounds in *Teucrium* genus.



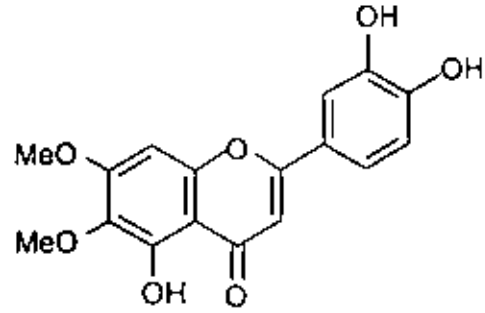
Baicalein, R =H **(187)**
4`-hydroxybaicalein, R = OH **(188)**



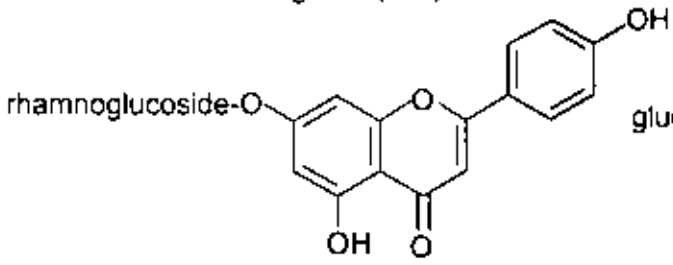
Cirsimaritin, R = H **(189)**
Cirsilineol, R = OMe **(191)**



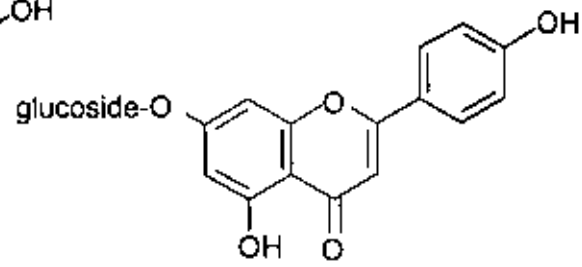
Salvigenin **(190)**



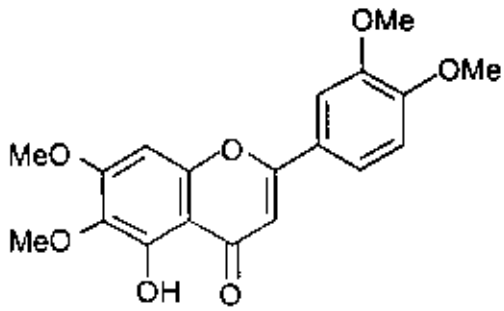
Cirsiliol **(192)**



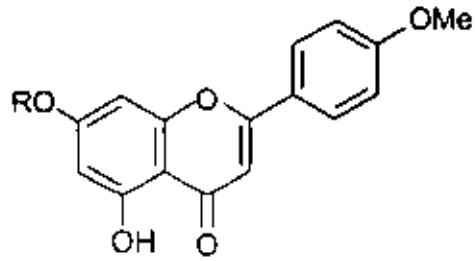
Naringenin **(193)**



Apigenin-7-*O*-glucoside **(196)**

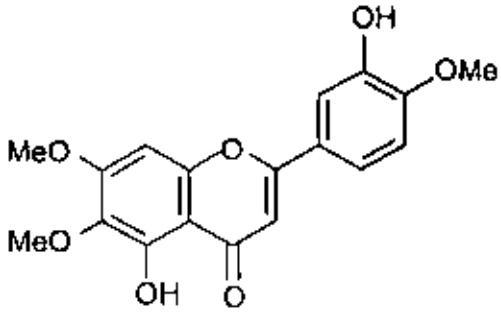


5-hydroxy-6,7,3`,4`-tetra-methoxyflavone **(197)**

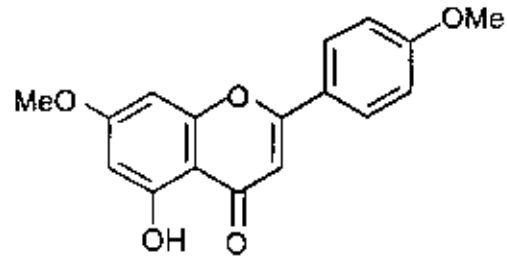


Acacetin, R = H **(198)**
Acacetin-7-*O*-galactoside, R = galactoside **(201)**

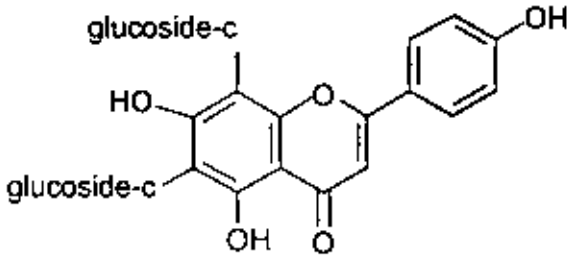
Fig. (5) Cont.



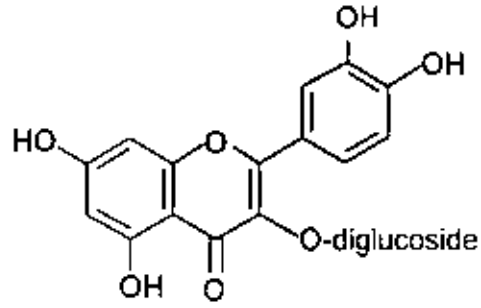
Eupatorin **(199)**



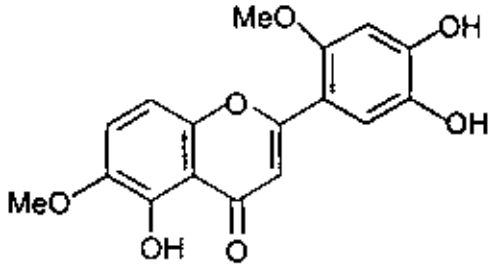
Apigenin-4`,7-dimethoxylether **(200)**



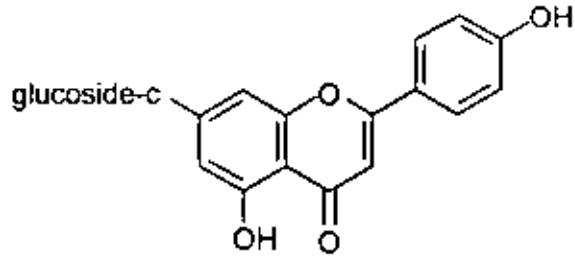
Vicenin-2 **(202)**



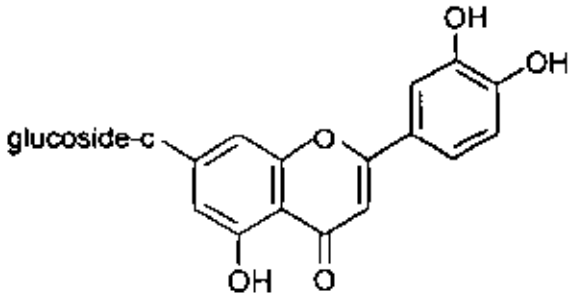
Luteolin-3-*O*-diglucoside **(203)**



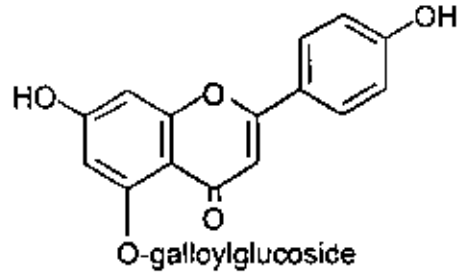
5,4`,5`-trihydroxy-2`,6-dimethoxyflavone **(204)**



Apigenin-7-glucoside **(205)**



Luteolin-7-glucoside **(206)**



Apigenin-5-galloylglucoside **(207)**

Fig. (3) Cont.

6- Iridoids in *Teucrium* genus :

Iridoids are a group of naturally occurring compounds. They are cyclopentanoid monoterpenes characterized by a cyclopentane nucleus attached to an α-pyrane nucleus [187]. Most frequently occur in plants combined with sugar as glucosides [8].

Many species of the genus *Teucrium* were investigated for their iridoidal content, most of species contained harpagide and harpagid acetate [188].

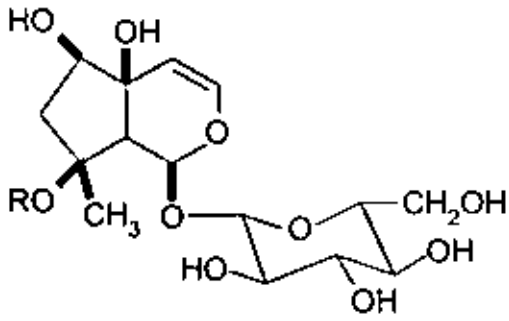
Some of these compounds were reported below in table (2) and figure (6).

Table (2) : Iridoids isolated from *Teucrium* genus

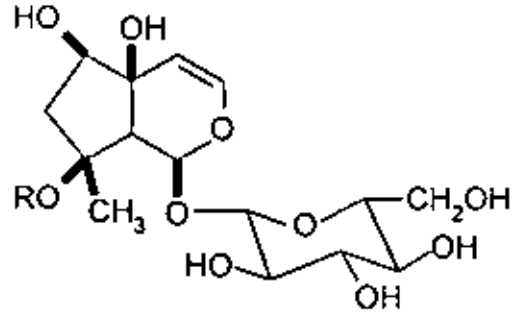
Plant species	Compound	References
T. arduini	Acetyl harpagide **(208)**, ajugol **(209)**, ajugoside **(210)**, reptoside **(211)** and teucardoside **(212)**.	[189-190]
T.aureum schreb	Harpagide **(213)** and acetyl harpagide **(208)**,	[190]
T. bicolor	Harpagide **(213)**.	[188-189]
T. botrys	Harpagide **(213)**, acetyl harpagide **(208)** and teucardoside **(212)**.	[188-189]
T. canadense	Harpagide **(213)** and acetyl harpagide **(208)**.	[188-189]
T. chamaedrys	Acetyl harpagide **(208)** and reptoside **(211)**.	[189, 191]
T. cubense	Acetyl harpagide **(208)** and reptoside **(211)**.	[189, 191]

Table (2) : Cont.

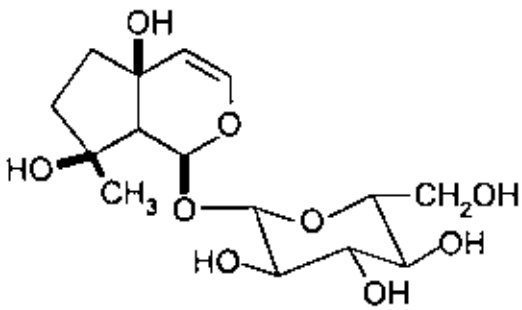
T. flavum	Harpagide **(213)** and acetyl harpagide **(208)**.	[188-189, 191]
T. fruticans	Harpagide **(213)** and acetyl harpagide **(208)**.	[188-189, 191]
T. hircanicum	Harpagide **(213)** and acetyl harpagide **(208)**, teucardoside **(212)** and teuhircoside **(214)**.	[188-189, 191]
T. lucidum	Harpagide **(213)**.	[188- 189]
T. massiliense	Harpagide **(213)** and acetyl harpagide **(208)**.	[188-189]
T. montanum	Harpagide **(213)** and acetyl harpagide **(208)**,	[188-189]
T. oriental	Harpagide **(213)**, fastigenin, and 8-O-acetyl harpagide **(208)**.	[192-193]
T. polium	Harpagide **(213)** and acetyl harpagide **(208)**,	[188-189, 191]
T. polium var. *alba*	Teucardoside **(212)**.	[179]
T. pyrenaicum	Harpagide **(213)**, acetyl harpagide **(208)** and teucardoside **(212)**.	[188-189]
T. scordium	Harpagide **(213)** and acetyl harpagide **(208)**.	[188-189]
T. scorodonia	Harpagide **(213)**, acetyl harpagide **(208)** and reptoside **(211)**.	[188-189]
T. taylori	Harpagide **(213)**.	[189, 193]



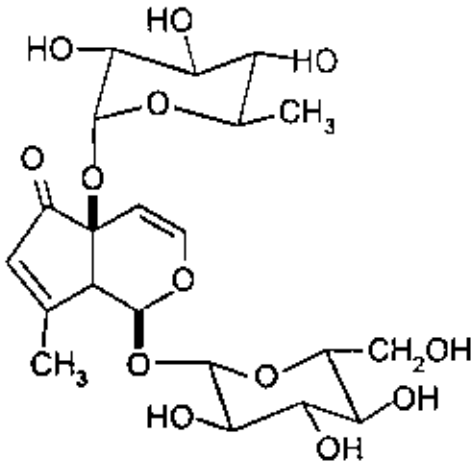
Harpagide, R = H (**213**)
8-O-acetyl harpagide, R = $COCH_3$ (**208**)



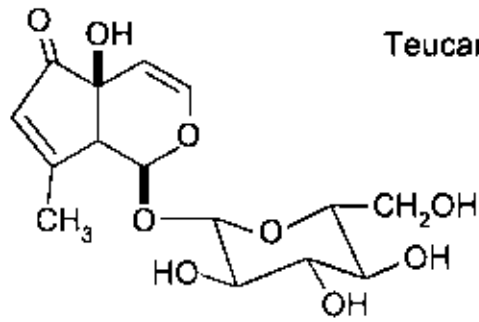
Ajugol, R = H (**209**)
Ajugoside, R = $COCH_3$ (**210**)



Reptoside (**211**)



Teucardoside (**212**)



Teuhircoside (**214**)

Fig. (6): Chemical structures of some iridoids in *Teucrium* genus.

6- Biological activities :-

Since the available analgesic drugs exert a wide range of side effects and are either too potent or too weak, the search for new analgestic compounds has been a priority of pharmacologists and pharmaceutical industries. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [192]. *Teucrium* species have been used as medicinal plants for more than 2000 years and some of them are still used in folk medicine as anti-inflammatory, antispasmodic, tonic, antipyretic and antiseptic [17]. Here we list some of these interesting biological properties :

Debat *et. al.* [193-194] stated that an extract of *T. marum* which was obtained by extracting the whole plant with boiling H_2O containing NH_3 has muscle relaxant, respiratory and analeptic and antianaphylactic activity, also showed spasmolytic activity in isolated rat duodenum, Uterus and pig ileum.

The furanoid diterpene teucjaponin-A which isolated from *T. japonicum* showed antifeedant activity for *Prodenia litura* [78].

Tafricanin-A and tafricanin-B which were isolated from *T. africanum* showed antiseptic and antifeedant activities [20].

Capasso *et. al.* in 1983 [195] showed that the alcoholic extract of *T. polium* has anti-inflammatory activity.

Omar *et. al.* [117, 196-200] reported that *T. polium* used as antidiabetic drug, also used in treatment of hemorrhoid, stomach pain and have effect on intestinal motility and blood pressure, also the aqueous decoction of the aerial parties showed significant reduction in the serum levels of cholesterol and triglycerides in hyperlipidemic rats.

T. flavum subsp. *glaucum* is chiefly found in Sardinia and used in popular medicine for healing wounds [14].

Simmonds *et. al.* [201] reported about the antifeedant activity of clerodane diterpenoides which isolated from *Teucrium* species against the larvae of *Spodoptera littoralis* and *Heliothis armigera*.

Kamel and Sandra in 1994 [202] suggested that the antispasmodic activity of *T. polium* oil could be attributed to its high content of sesquiterpene alcohols.

The methanolic extract of *T. pumillum* and MeOH-CH_2Cl_2 , CH_2Cl_2 extracts of *T. flavum* showed significant anti-inflammatory effect [203].

The study of the hepatoprotection of ethanolic extract of *T. stocksianum* indicates the presence of hepatoprotective constituents against paracetamol-induced hepatic damage in mice [142].

The antifeedant activity of the neoclerodane diterpenoids 6-acetylteucjaponin-B, triacetylteumassilin and C-12 epimer of teupyreinin against *Tenebrio molitor* larvae was studied by Gallardo *et. al.* in 1996 [101].

The norclerodane diterpenoid teucvidin which isolated from *T. quadrifarium* and its prepared derivatives showed significant antifeedant activities against larvae of *Leucania separate* [128].

Belen *et. al.* in 1997 [204] studied the traditionally using of *T. buxifolium* for treatment of rheumatic and other inflammatory affections. They found that it exhibited potent anti-inflammatory properties and significant antiulcer and cytoprotective activity.

Jesus *et. al.* [205] were studied the antifeedant activity of ten neoclerodane diterpenes isolated from *Teucrium* species, the results showed that these compounds have significant antifeedant activity against *Leptinotarsa decemlineata* larvae (*Colorado potato beetle* larvae).

The isofruticolone is a neoclerodane diterpene isolated from *T. fruticans* by Bruno *et. al.* [65]. It is one of the most potent antifeedant against larvae of *Spodoptra littoralis*.

The aerial parts of *T. divaricatum* were studied in 2000 [206] by Galati *et. al.* They reported that the extract of the plant showed a significant decreasing of ulcer index on rat.

The antifeedant activity of neoclerodane diterpenoids from *T. arduini* was confirmed by Bruno *et. al.* in 2002 [207].

Mohamed *et. al.* in 2004 [192, 208] were studied the total alcoholic extract and essential oil of *T. polium*. They concluded that the antinociception was mainly due to the essential oil. Also this study confirms the anti-visceral pain properties comparable to those of hyoscine and indomethacin and suggests a good place for it in antispasmodic therapies in human. Also anti-inflammatory activity of this plant was established and the data clearly showed that the plant extract reduces the high blood glucose levels through enhancing insulin secretion by pancreas.

In 2004 [17] the volatile oil, n-hexan-ether and crude ethanolic extracts of *T. leucocladum* were tested for their bacterostatic, antifungal and larvicidal activities. They showed potent activities against *Pseudomonas aeruginosa, Bacillus subtilis, Culex pipiens, Musca domestica* and *Ceratitis capitata* larvae.

Krishna *et. al.* [209] isolated teuctosin, teuflin, teucrin-H_2, 6β-hydroxyteuscordin, 6β-acetylteuscordin and montanin-D from *T. tomentosum*. All the compounds showed antifeedant activity against *Plutella xylostella* and *Spodoptera lituralis*.

Aim of the study

In the recent past, there has been a global trend towards revival of curative agent from indigenous plants.

The objectives of this study are to find out resources of potential biological active chemical constituents from an endemic plant viz.: *Teucrium zanonii*.

By reviewing the available literature on *T. zanonii*, no data was published about it's chemistry and/ or biological effects.

The aim of this study can be achieved by the following steps.

1- Complete literature survey.

2- Collection of the plant from it's growing region, drying and grinding to a fine powder.

3- Extraction of chemical constituents with different solvents.

4- Identification of isolated compounds by different chromatographic, chemical and spectroscopic techniques (UV, MS, NMR).

5- Investigation of the biological activity of different extracts and /or the isolated compounds.

STUDIED SPECIES

Plant description :-

Teucrium zanonii is an endemic to Libya and can be described as below: It is a subshrub, suffrutescent, branched from the base, branches prostrate, diffused, densely velutinous-tomentose. Leaves 6-7x 2-3 mm, oblong, obtuse, cuneate, usually coarsely 3-4 crenate in the middle, strongly revolute, woody above and beneath. Verticals forming many oblong terminal capitula which become densely spicate and cylindrical in fruit. Bracts oblong, narrowly attenuate, villous, shorter than calyx, 4 mm long, flat. Clayx 4 mm long, curved, ventricose, densely long villous, teeth triangular, acute, subequal. Corolla inferior lip glabrous, small, lateral lobes 1.5x 0.5 mm, lanceolate, obtuse, upper lip oblong, obtuse 2 x 1 mm. Stamens filaments glabrous rarely spreading hairy. Nutlets black, reticulate-faveolate, glossy [2]. (Figure 7) shows picture of *Teucrium zanonii* plant.

Plant Material

It was collected from Abo-fakhra region about (25 Km) from Benghazi city in April 2004 during the flowering stage. The plant was kindly identified by Dr. Mohamed Alsharif at Botany department, Faculity of science, Gariuones University. A voucher specimen has been deposited at the Herbarium of Biology department, Faculty of science, Altahady University, Sirt, Libya.

The aerial parts of the plant (leaves, flowers and branches) were air dried and ground altogether till it become as a fine powder.

Fig. (7) : Picture of *Teucrium zanonii* plant.

EXPERIMENTAL WORK & RESULTS

1- PRELIMINARY PHYTOCHEMICAL SCREENING.
2- VOLATILE OIL.
3- INVESTIGATION OF LIPID FRACTION.
4- INVESTIGATION OF FLAVONOIDS.

1-PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening was carried out on the powdered plant of *Teucrium zanonii*.

1-Volatile oils :

steam distillation [210-211]:

About 10 g of the powdered plant were subjected to steam distillation and the distillate was tested for the presence of volatile oils by saturation with sodium chloride, extraction with ether and evaporation of the ether spontaneously. The oily residue obtained indicates the presence of the volatile oil.

2-Unsaturated Sterols and/or Triterpenes :

The alcoholic extract (corresponding to about 2g plant material) was evaporated. The residue was treated with anhydrous chloroform (10 ml) and filtered. The filtrate was divided into two portions and tested by Liebermann-Burchardt and Salkowskis reactions.

a-Libermann-Burchardt's test [212]:

To the first part, 1ml of acetic anhydride was added followed by 2 ml of H_2SO_4 down the walls of the test tube. A reddish-violet ring was produced at the junction of the two layers and then the solution became bluish-green in colour in the acetic anhydride layer which indicate the presence of unsaturated sterols and/or triterpenes.

b-Salkowiski's test [213]:

To the second part, an equal volume of sulphuric acid was added. If a red

colour was produced, it indicates the presence of unsaturated sterols and/or triterpenes.

3-Carbohydrate and/or glycosides[210]:

About 2 g of the powdered plant were extracted with 50% ethanol and tested by Molisch's test.

a- Molisch's test:

About 5 ml of the ethanolic extract were mixed with 0.5 ml ethanolic α-naphthol. Sulphuric acid (1ml) was carefully poured down the walls of the test tube. The carbohydrate and/or glycosides are present when a violet ring was formed at the interface.

b-Reduction of Fehling's solution:

About 5 ml of the alcoholic extract were heated with 5 ml of Fehling's solutions. The colour changed form deep blue to green yellow or red indicating the presence of free reducing substances.

4-Flavonoids[214-215]:

Shinoda test:

The alcoholic extract corresponding to about 2 g of the plant material was tested with few drops of conc. HCl and magnesium turnings (~0.5g). The presence of flavonoids was indicated if a pink or magneta red colour is developed within 3 minutes

5-Coumarins [216]:

About 1 g of the moistened plant material was placed in test tube and the tube was covered with filter paper moistened with dilute NaOH solution. The tube was placed in a boiling water bath for few minutes. The filter paper was then removed and examined in UV light, any fluorescence was indicative for the presence of coumarins.

6-Saponins [217-218]:

a-Froth test:

About 3 g of the powdered plant were extracted with boiling water and filtered. After cooling, the extract was shacked vigorously until froth was obtained then allowed to stand for 15-20 minutes and classified for saponins content. (No froth = negative, froth lees than 1cm height = weakly positive, froth 1-2 cm height = positive, froth greater than 2 cm height = strongly positive)

b-Blood haemolysis:

About 5 g of the powdered plant were extracted with hot ethanol (95%). One ml aliquot portion was added to 10 ml of 1:4 suspension of erythrocytes in physiological saline solution and haemolysis was observed which indicates the presence of saponins.

7-Anthraquinones [216]:

About 2 g of the plant material were boiled for few minutes with 0.5 N KOH (10 ml) to which was added 1 ml of dilute H_2O_2 after cooling, the mixture was filtered and acidified with acetic acid. The acidified solution was extracted with benzene (10 ml) and the benzene extract was shacked with NH_4OH (5 ml). A positive reaction was evidenced by the formation of a red

colour in the alkaline layer.

8-Alkaloids [216]:

The alcoholic extract (corresponding to about 3 g plant material) was evaporated to dryness and the residue was heated on boiling water bath with 2N HCl (5ml). After cooling, the mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with few drops of Mayer's reagent [219] and the other with similar amounts of Wagner's reagent [220]. The appearance of turbidity or precipitation indicated the presence of alkaloids

9-Tannins [212, 221-222]:

About 10 g of the powdered plant were extracted with ethanol (50%) and tested for tannins by the following test:

Upon addition of ferric chloride, if a blue, blue black, green or blue green colour or precipitate would indicate the probable presence of tannins.

10-Iridoids [223]:

About 2 g of the fresh plant material were cut into small pieces and placed in a test tube with 5 ml of 1% aqueous HCl. After 3-6 hrs 0.1 ml of the macerate was decanted into another tube containing 1 ml of the Trim-Hill reagent (10 ml acetic acid, 1 ml 0.2 $CuSO_4.5H_2O$ in water and 0.5 ml conc. (HCl). When the tube is heated for a short time on a flame. If certain iridoids, are present, a blue colour is produced.

The results of the phytochemical screening are tabulated in (Tab. 3)

Table(3): The results of the phytochemical screening of *T. zanonii*

Constituents	Results
Volatile oil.	+ +
Sterols and/or Triterpenes.	+ + +
Carbohydrate and/or glycosides.	+ +
Flavonoids.	+ + +
Coumarins.	+
Saponins.	+ +
Anthraquinones.	-
Alkaloids.	+
Tannins.	+ +
Iridoids.	+

+ + + : Highly positive.

+ + : Modrately positive.

+ : Weakly positive.

- : Absent.

2-VOLATILE OIL

Preparation of the volatile oil of *T. zanonii* :

1- Hydro distillation method :

About 250 g of the fresh plant material (aerial parts) of *T. zanonii* were subjected to water distillation in all-glass apparatus for about three hours according to Gunther method [209].

The trapped oil in the side arm was removed after complete distillation and dried over anhydrous sodium sulphate to give a pale yellow oil having a characteristic odor (0.20% v/w)

GC/MS analysis of the volatile oil :

The volatile oil was subjected to GC/MS using the following conditions:

Gas chromatography:

Instrument : TRASC GC, Splitless Mode.

Column : DB-5 capillary column (30 m, 0.25 mm internal diameter, 0.25 μm film)

Temperature program : Injector 50°C, Initial Temp. 38°C, Rate, 2°C/min. to 200°C, Final Temp. 200°C for 5 min.

Flow gas : Helium at 10 ml/min.

Mass spectroscopy :

Instrument : TRACE DSQ.

Full scan 50-450, positive ion, Ion source 200 °C, mass transpher line 200 °C.

Library : NIST.

The mass spectra were measured in EI scan Mode at (70 e.v.) from 50-450 mass unit (Fig. 8 and Tab. 4). The results obtained revealed that the volatile oil (hydrodistillation method) consists of a mixture of seventy four compounds

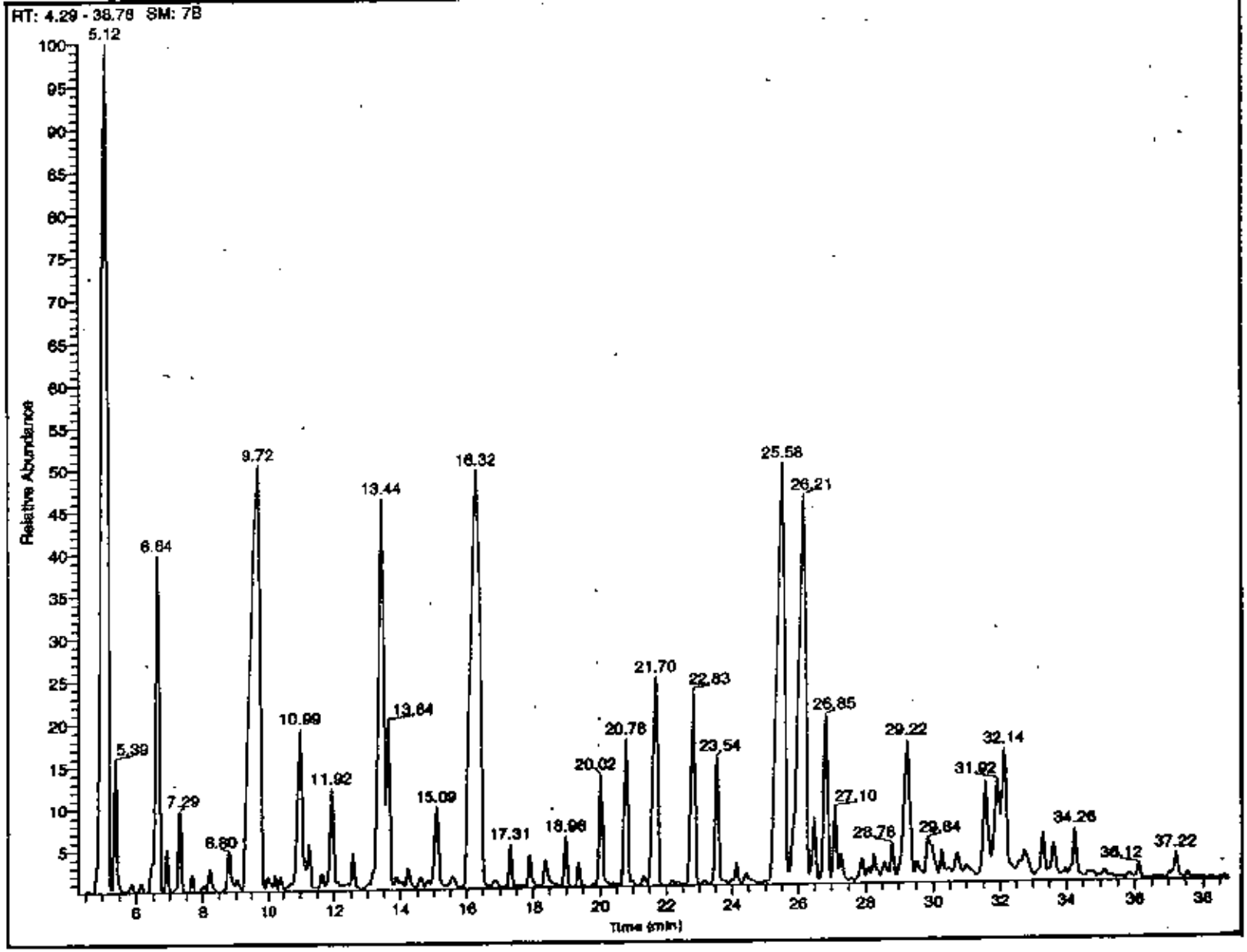


Fig. (8) : GC Chromatogram of the volatile oil of *Teucrium zanonii*

(prepared by hydrodistillation method)

Table (4): GC/MS data of volatile oil hydrodistelled of *T. zanonii*

No.	Components	R_t (min)	Relative %	Mass spectral data		
				M^+	B.P.	Fragments (%)
1	*β*-Pinene	5.12	14.13	138	93	53(10),69(23),77(14),79(12),91(26),94(12)
2	*β*-Myrcene	5.39	1.13	136	93	67(12),69(59),91(20),
3	3-Octanol	5.50	t	130	59	55(46),71(24),83(68),101(32)
4	α-Phellandrene	5.79	t	136	93	77(26),91(72),92(32),136(19)
5	1,1'-Bicyclopentyl	5.87	0.09	138	67	39(30),41(50),55(28).68(6).82(16),96(18)
6	Cyclohexane,1-methy-lene-4-(1-methylethenyl)	6.14	0.08	136	93	69(50),79(50),93(92),121(42),136(29)
7	P-Cymene	6.43	0.15	134	119	91(39),117(16),134(42)
8	D-Limonene	6.64	3.48	136	68	67(91),77(16),92(28),93(83),107(17),121(17)
9	E-Ocimene	6.95	0.34	136	93	77(20),79(25),91(46),92(42),105(11)
10	Benzene acetaldehyde	7.17	t	120	91	65(18),92(27),120(14)
11	*Z*-Ocimene	7.29	0.70	136	93	67(12),77(24),79(36),91(50),92(27),105(16)

Table (4): Cont.

12	γ-Terpinene	7.68	0.15	136	93	70(43),71(69),91(50),92(26),136(25)
13	Linalool oxide	8.18	0.24	170	59	55(27),67(62),68(61),81(28),93(78%),94(87),111(39)
14	Bicyclo[3,1,0]hexane,6-isopropylidene-1-methyl	8.80	0.42	138	93	77(26),79(30),91(56),105(20),121(50),136(64)
15	1-Pentanol,5-cyclopropylidene	9.0	0.11	126	79	59(38),67(46),71(56),91(28),93(30)
16	Linalool	9.72	11.0	154	93	55(30),67(27),69(54),79(14),93(96),121(20)
17	Octen-1-Ol, acetate	9.89	t	170	99	54(37),67(55),68(36),72(34),91(29),109(24),128(15)
18	β-Thujone	10.07	t	152	95	55(18),69(73),81(84),109(24),110(74)
19	3-Cyclopentene-1-acetyldehyde,-2,2,3-trimethyl	10.42	0.09	152	93	67(32),91(30),94(40),108(92),109(18)
20	E-Pinocarveol	10.99	2.22	152	92	55(34),69(46),70(67),83(44),91(95),93(28),119(27),
21	E-Verbenol	11.25	0.39	152	91	67(36),81(38),92(34),94(58),95(36),119(30)
22	2H-pyran,3,6-dihydro-4-methyl-2(2-methyl-1-propenyl)	11.62	0.12	152	68	67(99),69(34),83(52),85(22),91(13)
23	2(10)-Pinen-3-one	11.92	1.2	152	81	53(74),69(40),79(59),107(69),108(82)

Table (4): Cont.

24	Borneol	12.09	t	154	95	55(12),67(22).69(13),81(14),93(23)109(14)
25	Z-Terpineol	12.54	0.34	154	71	67(23),69(26),91(20)93(59),111(33)
26	3-Cyclohexene-1-methanol,-α,α,4-trimethyl	13.14	t	154	93	59(72),67(45),81(47),91(41),92(50),121 (49),136(48)
27	α-Terpineol	13.44	5.56	154	93	67(40),92(36),107(27),121(55),136(58)
28	Myrtenol	13.64	1.67	152	79	67(24)91(65),93(22),108(26)119(14)
29	2,6-Dimethyl-3,5,7-octatriene-2-Ol	13.88	t	152	91	55(26),67(55),68(32),77(40),79(49),81(43),93(48),109(49),119(44),134(26)
30	Verbenone	14.01	t	150	107	67(28),79(47),80(38),91(88),135(70),150(26)
31	2-Caren-4-Ol	14.24	0.22	152	94	67(46),77(34),79(71),91(87),119(44),134(26)
32	E-Carveol	14.61	0.10	152	84	56(16),69(52),83(60),108(32),109(66)
33	Nerol	14.81	0.10	152	69	67(44),68(28),77(17),79(26),91(23),93(38),97(67)
34	Geraniol	15.09	1.00	154	69	67(20),68(28),84(11),93(36)
35	Carvone	15.55	0.16	150	82	54(24),93(26),106(18),107(17),108(36)

Table (4): Cont.

36	Linalyl acetate	16.32	11.1	196	93	69(34),71(16),80(24),92(18),121(20)
37	Bornyl acetate	17.31	0.38	196	95	79 (14),93(48),108(12),121(24),136(25)
38	Myrtenyl acetate	17.88	0.12	194	91	92(47),119(30),134(12)
39	Thymol	18.35	0.36	150	135	91(14),115(8),150(32)
40	Verbenyl acetate	18.98	0.44	194	91	92(34),119(25),134(12)
41	δ-Elemene	19.35	0.21	204	121	79(38),91(50),93(81),94(36),107(47)
42	3-Cyclohexene-1-methanol, 4,5,5-trimethyl acetate	20.02	1.15	196	93	67(30),68(28),92(26),121(78),136(56)
43	Geranyl acetate	20.78	1.53	196	69	67(26),68(54),80(14),93(52)
44	α-Cubebene	20.95	0.05	204	105	119(98),133(15),161(88),162(15)
45	α-Bourbonene	21.31	0.10	204	81	79(29),80(68),123(61),161(29)
46	Undecane,4,7-dimethyl	22.18	0.05	170	71	56(10),57(83),70(14),85(36)
47	Caryophyllene	22.83	2.20	204	93	67(36),69(80),79(52),91(85),105(54),119(32),133(79)
48	α-Bergamotene	23.54	0.13	204	93	69(36),79(18),91(38),105(22),119(64)

Table (4): Cont.

49	z,z,z-1,4,7-Cycloundecatriene,1-,5,9,9-tetramethyl	24.13	0.23	204	93	80(22),91(14),147(16)
50	1-(3-Methylbutyl)-2,3,4,5-tetra-methylbenzene	24.41	0.16	204	133	91(49),105(80),119(52),147(62),148(46),161(71), 189(24),204(22)
51	Germacrene-D	25.58	8.81	204	161	79(28),81(30),91(57),105(67),119(34),
52	γ-Elemene	26.21	7.79	204	93	77(23), 91(46), 107(50),121(94),161(24)
53	α-Gurjunene	26.50	0.53	204	105	91(74), ,147(44),161(98),189(59),204(88),
54	Selinene	26.85	1.80	204	161	81(25),91(28),105(45),107(49),122(50)
55	δ-Cadinene	27.10	0.65	204	161	91(42),105(59),119(62),134(66),204(34)
56	Z-Bergamotol	27.30	0.30	220	93	59(20),77(32), 91(68), 105(51),132(36)
57	α -Farnesene	27.92	0.20	204	93	71(26),79(30),91(66),92(33),105(34),119(46),134(30)
58	Elemol	28.08	0.12	222	93	67(50),69(54),79(50),91(59),95(44),105(41),107(44)
59	Z-Farnesol	28.30	0.33	222	107	69(91),91(26),93(17),106(23),136(28)
60	Z-Nerolidol	28.56	0.15	220	69	55(31),67(64),71(37),91(71)93(94), 119(50)
61	E-Nerolidol	28.78	0.33	222	69	55(12),67(24),71(34),81(21),93(78)107(24)

Table (4): Cont.

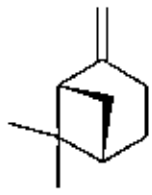
62	Spathulenol	29.22	2.30	220	43	67(46),79(58),105(61),131(40),159(44),205(36)
63	β-Elemenone	29.84	0.4	218	107	67(52),91(74),105(56),121(56),135(44),149(82)
64	Globulol	29.95	0.32	222	95	59(58),67(54),93(60),105(47),143(40),161(48),179(60)
65	Caryophyllene oxide	30.25	0.28	220	67	68(55),69(58),81(54),93(94),96(80),109(78),138(49)
66	Cubenol	31.03	0.21	222	119	67(51),69(64),91(86),93(72),95(83),105(78),161(88)
67	τ-Cadinol	31.56	1.27	222	161	81(28),91(30),93(33),105(40),119(28)
68	β-Eudesmol	31.92	1.33	222	59	67(38),91(41),93(52),95(42),108(39),149(79)
69	α-Cadinol	32.14	1.56	222	95	69(38),93(44),105(42),121(72),161(40)
70	Carotol	33.32	0.48	222	84	67(57),81(79),93(59),105(50),161(37)
71	Nerolidyl acetate	34.26	0.45	222	69	67(28),68(24),80(26),93(97),107(40)
72	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	36.12	0.12	218	91	67(52),79(52),81(34),93(84),105(65),107(49),119(44),132(60),133(58),147(74),161(46),175(40)

Table (4) : Cont.

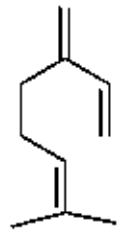
73	2(3H)-Naphthalenone,4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)	37.22	0.34	218	91	67(41),69(40),77(40),79(72),93(75),95(48),105(61),107(46),108(45),121(58),133(75),146(60),147(78),161(50),175(35)
74	2(H)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)	37.56	0.05	218	175	67(60),69(63),79(30),91(59),93(50),95(96),105(29),147(43),161(37),176(82)

R_t = Retention time, M^+ = Molecular ion peak, B.P. = Base peak, t = Traces (<0.05).

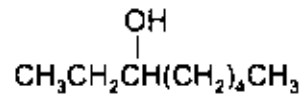
Note : The fragment abundance between parenthesis.



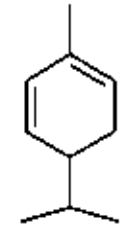
β-Pinene (1)



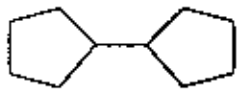
β-Myrcene (2)



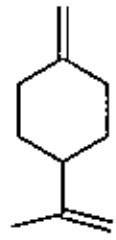
3-Octanol (3)



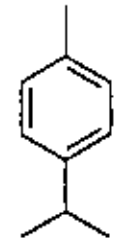
α-Phellandrene (4)



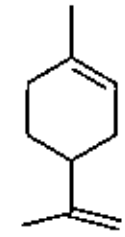
1,1`-Bicyclopentyl (5)



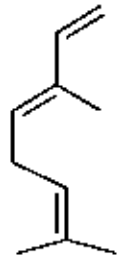
Cyclohexane,1-methy-lene-4-(methylethenyl (6)



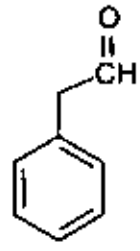
P-cymene (7)



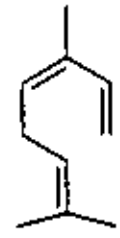
D-Limonene (8)



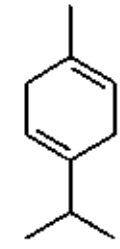
E-Ocimene (9)



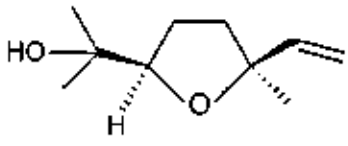
Benzene acet-aldehyde (10)



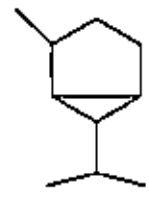
Z-Ocimene (11)



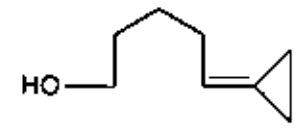
γ-Terpinene (12)



Linalool oxid (13)

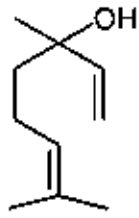


Bicyclo[3,1,0]hexane,6-isopropylidene-1-methyl (14)

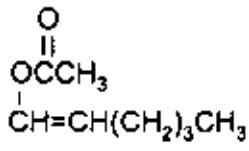


1-Pentanol,5-cyclopropylidene (15)

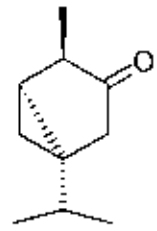
Fig. (9) : Chemical structures of the volatile oil compounds of *T. zanonii*. (prepared by hydrodistillation)



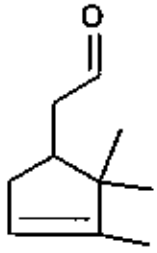
Linalool (16)



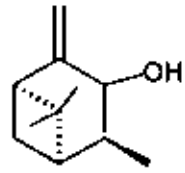
Octen-1-Ol, acetate (17)



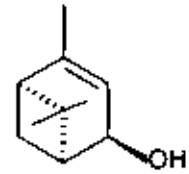
β-Thujone (18)



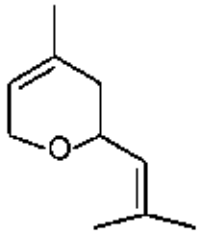
3-Cyclopentene-1-acet-aldehyde,2,2,3-trimethyl (19)



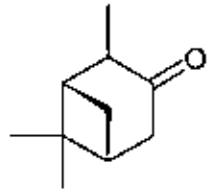
E-Pinocarveol (20)



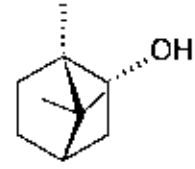
E-Verbenol (21)



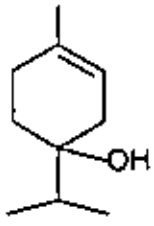
2H-pyran,3,6-dihydro-4-meth-yl-2-(2-methl-1-propenyl) (22)



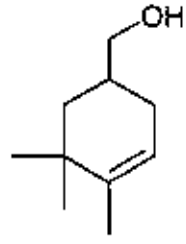
2(10)-Pinen-3-one (23)



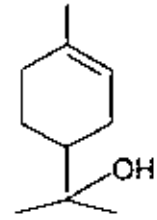
Borneol (24



Z-Terpineol (25)

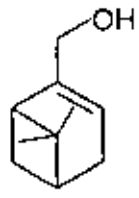


3-Cyclohexene-1-meth-anol,4,5,5-trimethyl (26)

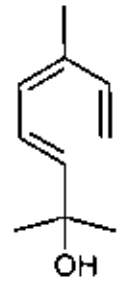


α-Terpineol (27)

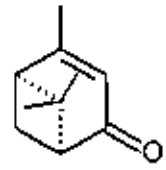
Fig. (9) : Cont.



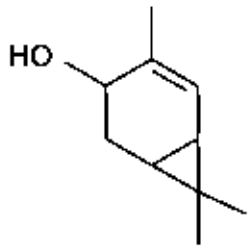
Myrtenol (28)



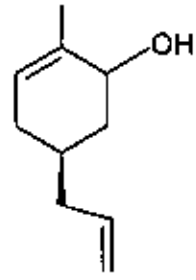
2,6-Dimethyl-3,5,7-octatriene-2-ol (29)



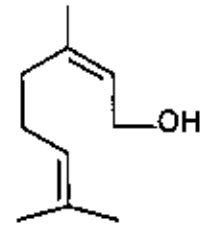
Verbenone (30)



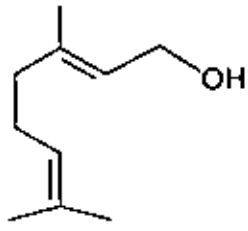
Caren-4-Ol (31)



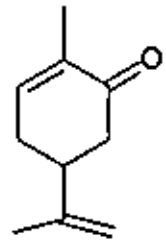
Carveol (32)



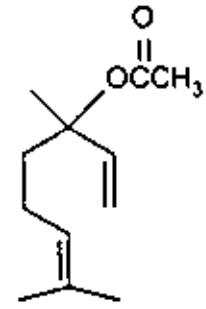
Nerol (33)



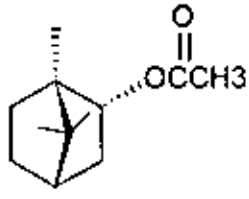
Geraniol (34)



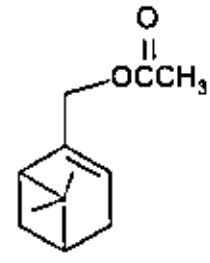
Carvone (35)



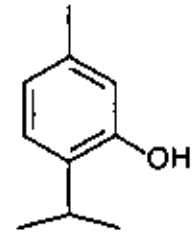
Linalyl acetate (36)



Bornyl acetate (37)

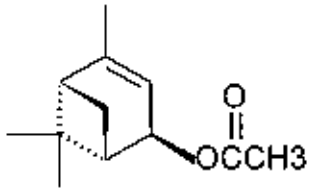


Myrtenyl acetate (38)

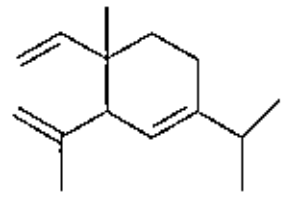


Thymol (39)

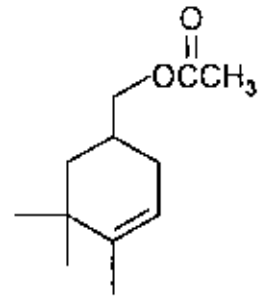
Fig. (9) : Cont.



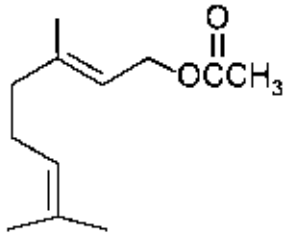
Verbenyl acetate **(40)**



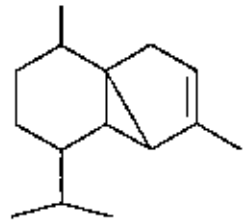
δ-Elemene **(41)**



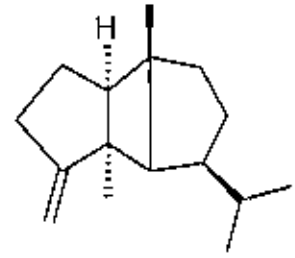
3-Cyclohexene-1-methanol-,4,5,5-trimethyl acetate **(42)**



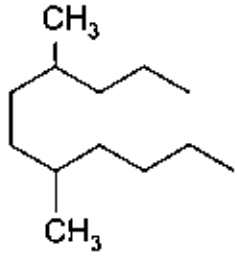
Geranyl acetate **(43)**



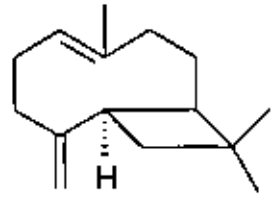
α-Cubebene **(44)**



α-Bourbonene **(45)**

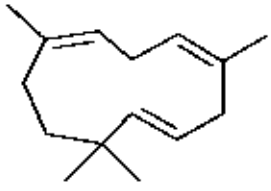


Undecane,4,7-dimethyl **(46)**

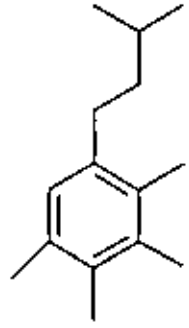


Caryophyllene **(47)**

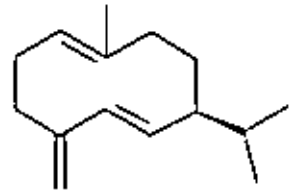
α-Bergamotene **(48)**



z,z,z1,4,7-Cycloundeca-triene1,5,9,9-tetramethyl **(49)**

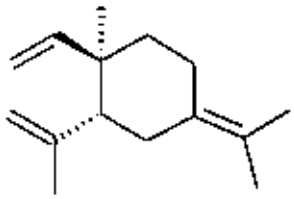


1-(3-Methylbutyl)-2,3,4,5-tetramethylbenzene **(50)**

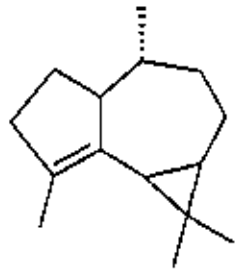


Germacrene-D **(51)**

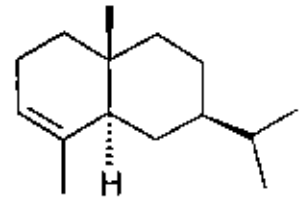
Fig. (9) : Cont.



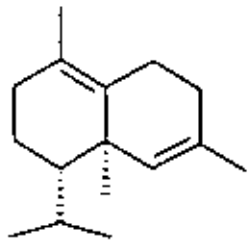
γ-Elemene **(52)**



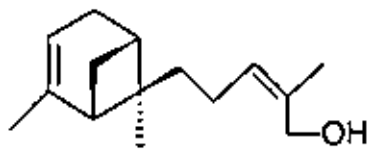
α-Gurjunene **(53)**



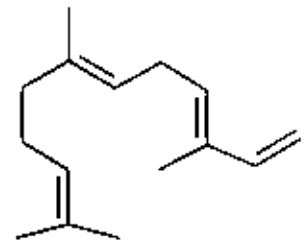
Selinene **(54)**



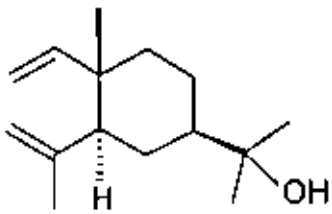
δ-Cadinene **(55)**



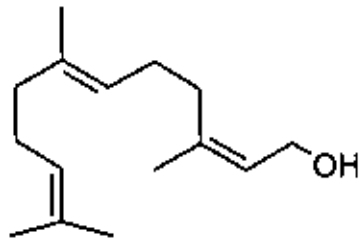
Z-Bergamotol **(56)**



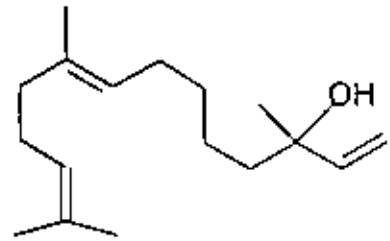
α-Farnesene **(57)**



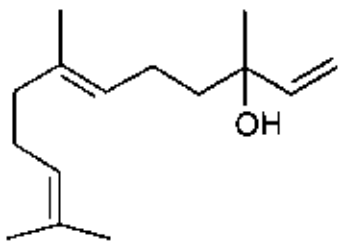
Elemol **(58)**



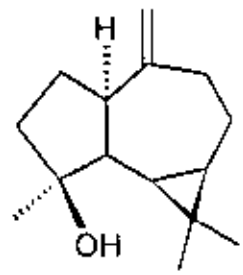
Z-Farnesol **(59)**



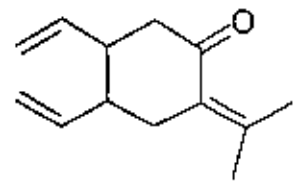
Z-Nerolidol **(60)**



E-Nerolidol **(61)**

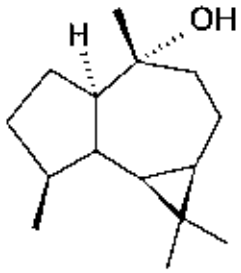


Spathulenol **(62)**

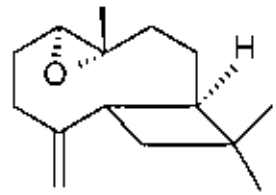


β-Elemenone **(63)**

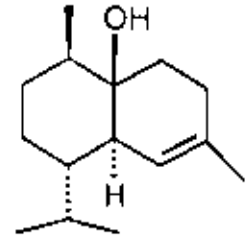
Fig. (9) : Cont.



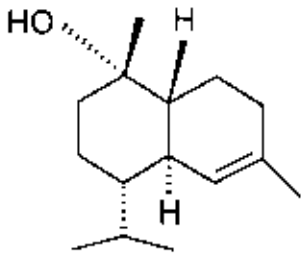
Globulol **(64)**



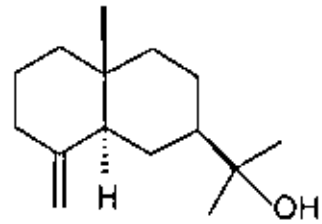
Caryophyllene oxide **(65)**



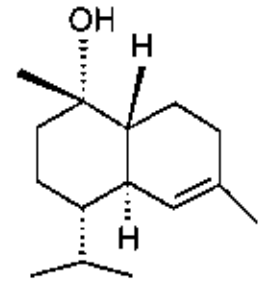
Cubenol **(66)**



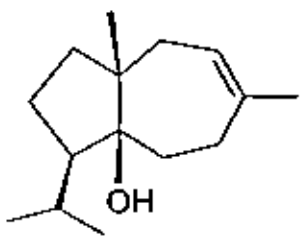
τ -Cadinol **(67)**



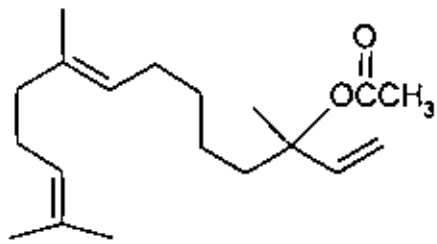
β-Eudesmol **(68)**



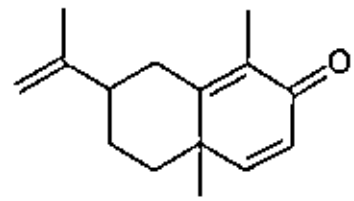
α -Cadinol **(69)**



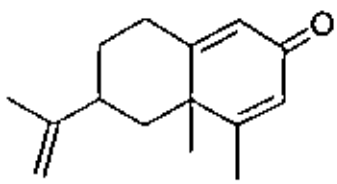
Carotol **(70)**



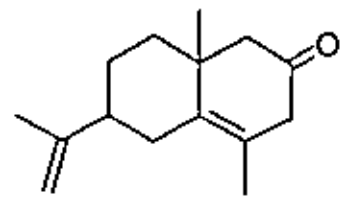
Nerolidyl acetate **(71)**



7-Isopropenyl-1,4a-dimethyl-
-4,4a,5,6,7,8-hexahydro-3H-
-naphthalen-2-one **(72)**



2(3H)-Naphthalenone,4,4a,-dimethyl-4,4a-
,5,6,7,18-hexahydro-6-(1-methylethenyl)
(73)



2(1H)-Naphthalenone-4,8a,-dimethyl-3,5-
,6,7,8,8a-hexahydro-6-(1-methylethenyl)
(74)

Fig. (9) : Cont.

belonging to many classes as follow (saturated hydrocarbons 0.56%, unsaturated hydrocarbons 41.79%, alcohols 31.68%, aldahydes 0.09%, ketones 2.39%, esters 15.16%, oxides 0.64%, aromatics 0.67% and about 7.02% unknowns and traces compounds).

2- Solvent extraction method (n-hexan–ether method):

about 100 g of *T. zanonii* were extracted with 300 ml n-hexan–ether (50 : 50) by percolation for 24 hour. The extract was filtered and the solvent was evaporated under reduced pressure at 30°C. The obtained semisold residue was subjected to GC/MS analysis using the condition shown in page 62. The results obtained (Fig. 10 and Tab. 5) revealed that the volatile oil consists of a mixture of sixteen compounds belonging to many classes as follow (saturated hydrocarbons 16.08%, unsaturated hydrocarbons 60.94%, alcohols 0.91%, ketones 1.24%, esters 7.93% and about 13.1% unknowns compounds).

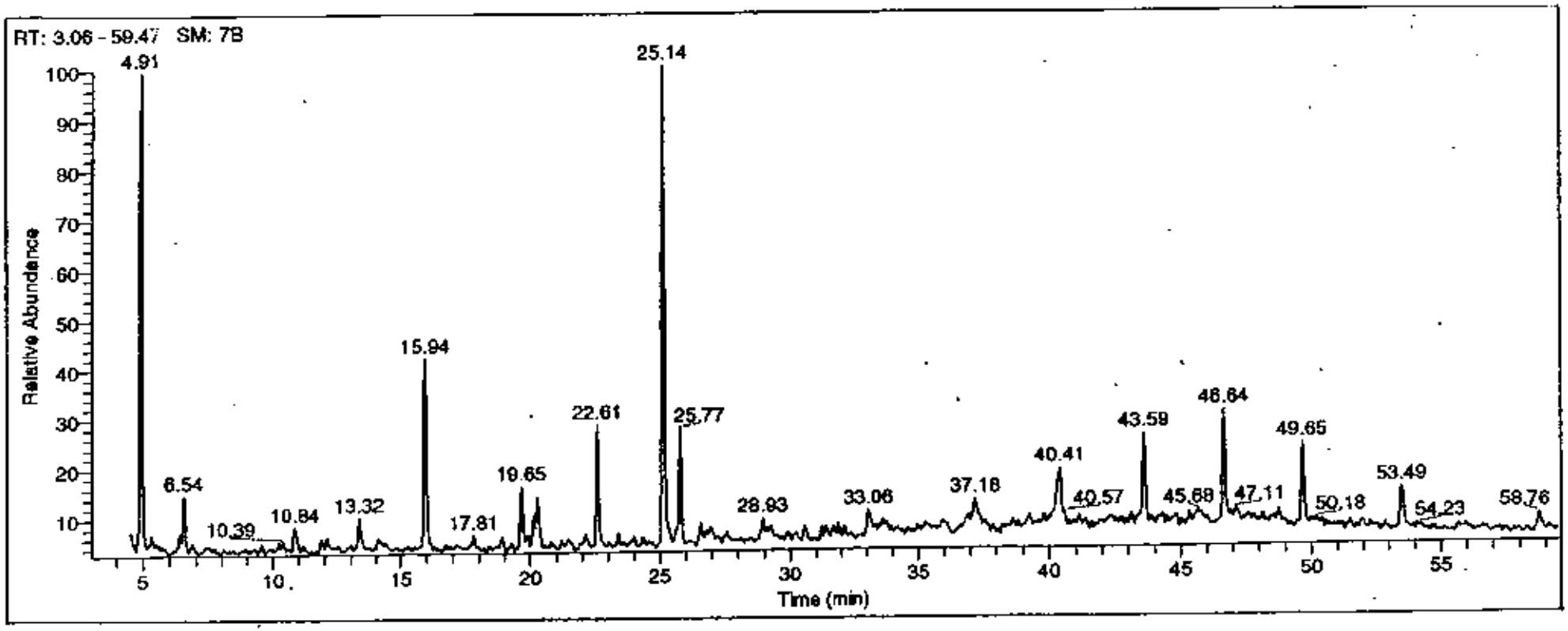


Fig. (10) : GC Chromatogram of the volatile oil (solvent extraction) of *T. zanonii.*

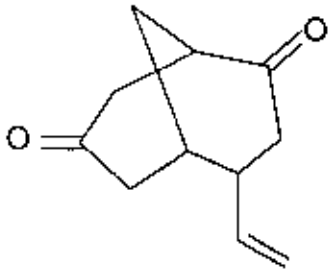
Table (5): GC/MS data of the volatile oil (solvent extract method) in *T. zanonii*

No.	Components	R_t (min)	Relative %	Mass spectral data		
				M^+	B.P.	Fragments (%)
1	*β*-Pinene	4.91	18.19	138	93	69(19),79(10),91(22),94(8%),121(8)
2	D-Limonene	6.54	2.77	136	68	67(74),69(26),91(40),92(54),93(94),136(32),137(22)
3	4-Vinylbicyclo[3.3.1]-nonane-2,7-dione	10.83	1.24	204	96	55(46),62(36),70(84),77(41),79(42),82(99),83(54), 92(38),95(48),105(50),134(60),154(48)
4	Linalyl acetate	15.94	7.93	196	93	67(12),69(26),77(11),80(17),91(14),92(12)
5	2,6-Dimethyl-1,3,5,7-octatetraene	19.65	2.65	134	119	71(40),77(22),82(34),91(95),92(54),117(32),120(30)
6	*γ*-Cadinene	20.25	2.56	204	161	69(16),79(42),93(31),115(24),119(25),158(27),204(25)
7	Caryophyllene	22.61	4.75	204	133	69(64),79(32),80(37),91(88)93(76),105(48),107(48)
8	Germacrene-D	25.14	20.04	204	161	67(16),79(25),81(33),91(64),93(18),105(68),119(27)
9	*γ*-Elemene	25.73	5.23	204	121	81(20),91(34),93(54),95(29),105(26),107(34),204(20)

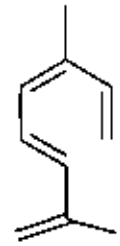
Table (5) : Cont.

10	6-isopropenyl-4,8a-dimethyl-1,2,3,5,-6,7,8,8a-octahydro-naphtha-len-2-Ol	28.93	0.91	220	79	66(47),91(56),92(72),128(81),159(60),163(82),202(44), 205(33)
11	Aromadendrene, dehydro	33.06	1.83	202	159	57(43),71(79),91(62),121(60),145(47),146(61),173(36)
12	2,15-Hexadecandione	37.18	2.92	254	71	58(59),83(43),85(30),92(27),95(25),109(21)
13	Tetradecane-2,6,10-trimethyl	43.59	4.79	238	71	55(15),57(68),83(14),85(45),155(12)
14	Heptadecane-2,6,10,15-tetramethyl	46.63	5.00	296	71	55(20),57(46),85(74),97(20),99(19)
15	Heptadecane-9-hexyl	49.65	3.86	324	71	55(15),57(44),70(22),85(46),86(34),113(26).
16	Eicosane-7-hexyl	53.49	2.23	366	71	57(64),69)22),85(54),97(14),99(20)

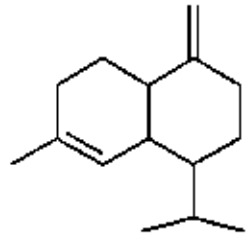
R_t = Retention time, M^+ = Molecular ion peak, B.P. = Base peak.
Note : The fragment abundance between parenthesis.



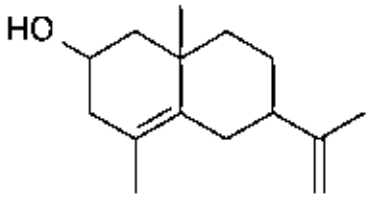
4-Vinylbicyclo[3.3.1]-nonane-2,7-dione **(3)**



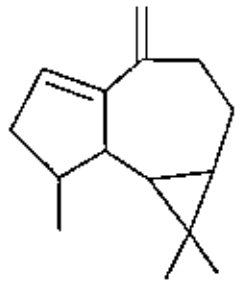
2,6-Dimethyl-1,3, 5,7-octatetraene **(5)**



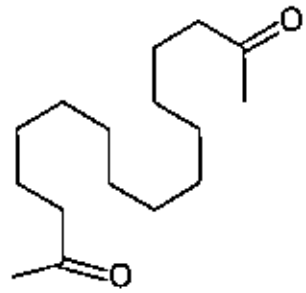
γ-Cadinene **(6)**



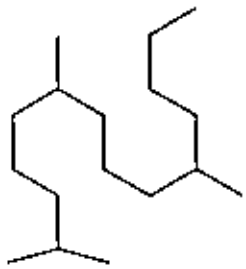
6-Isopropenyl-4a,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-nahthalen-2-Ol **(10)**



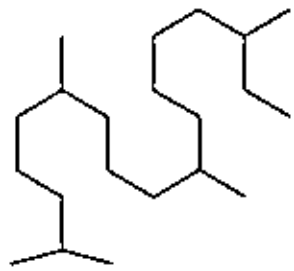
Aromadendrene, dehydro **(11)**



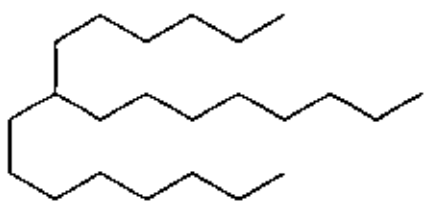
2,15-Hexadecanedione **(12)**



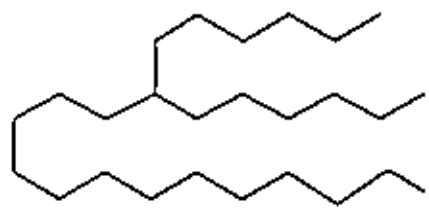
Tetradecane-2,6,10-trimethyl **(13)**



Heptadecane-2,6,10,15-tetramethyl **(14)**



Heptadecan,9-hexyl **(15)**



Eicosane,7-hexyl **(16)**

Fig. (11) : Chemical structures of some volatile oil compounds of *T. zanonii* (Prepared by solvent extraction)

3-INVESTIGATION OF THE LIPID FRACTION OF *TEUCRIUM ZANONII*

Extraction of lipids and related substances:

About 1.4 kg of the dried powdered plant of *T. zanonii* was extracted with petroleum ether (b.r.40-60°C) in a Soxhlet apparatus. The combined petroleum ether extract was passed through fuller's earth to remove the colored pigments, filtered, dried over anhydrous sodium sulphate and evaporated in *vacuo* at 40°C till dryness to give a pale yellow residue (12.3 g). The petroleum ether residue was dissolved in boiling acetone (300 ml) and left overnight at room temperature. An amorphous precipitate was filtered, washed with cold acetone and recrystallized from chloroform/methanol to gives bright white crystals (2.8 g) of acetone insoluble fraction (fatty alcohols). The filtrate (acetone soluble fraction) was evaporated till dryness (7.5 g).

GC/MS analysis of the acetone insoluble fraction (fatty alcohols):

The fatty alcohols mixture was subjected to GC/MS analysis using the following conditions and the results of GC/MS were shown in (fig. 12 and tab. 6)

Gas chromatography:

Instrument : Hewelett Packard Model 6890.

Column : HP-1, capillary, length 80 m, Thickness 0.3 µm

Temperature program : Oven 40-150°C, 4°C/min., 150-300, 10°C/min. final temperature for 15 min ; Detector 320 °C.

Carier gas : Helium at 0.8 cm/min.

Mass Spectroscopy:

Instrument : Hewelett Packard Model 5973 Mass Selective detector

Selective Ion Detector (SIM) AS Harvey (1981)

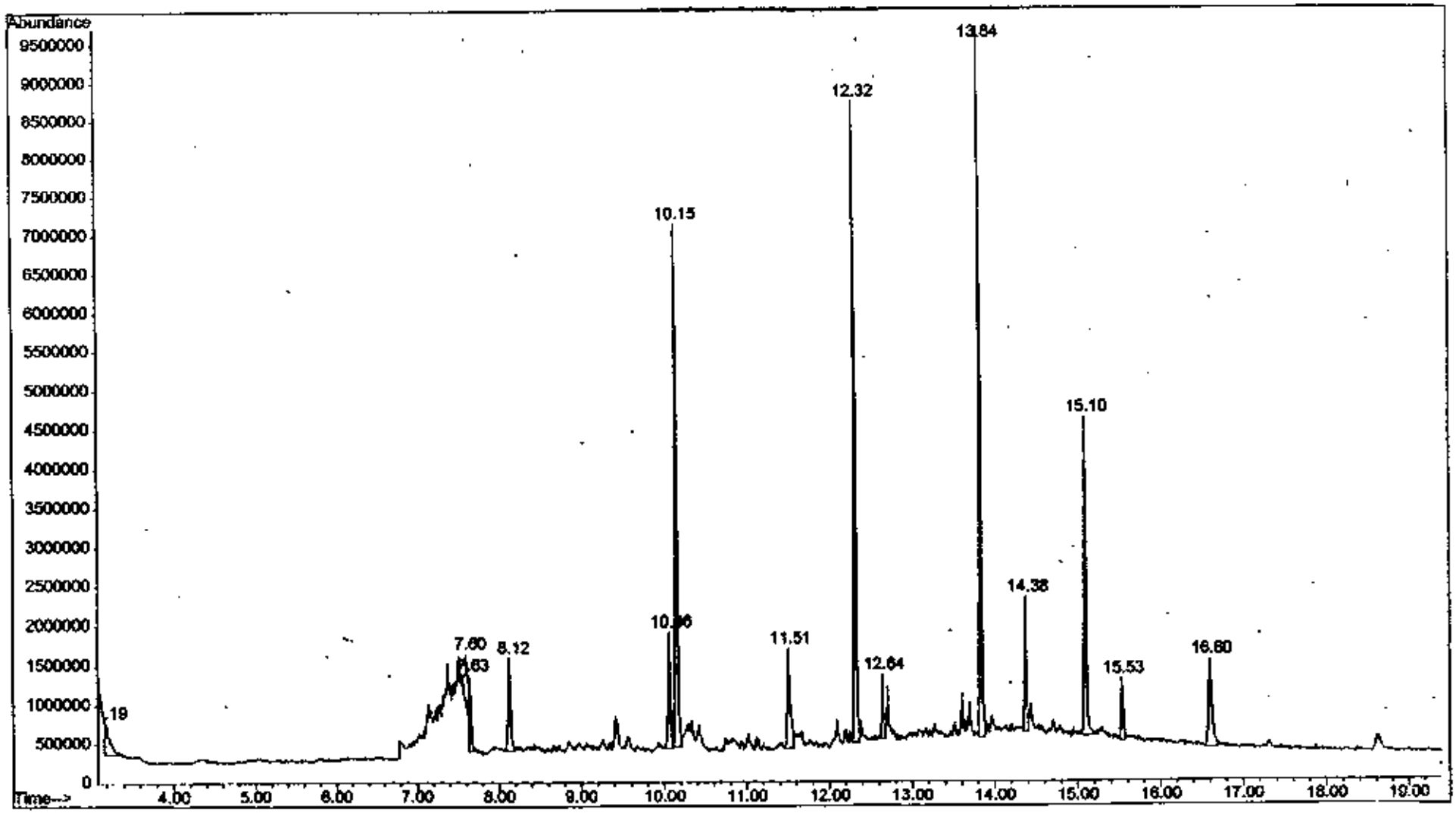


Fig. (12) : GC Chromatogram of the fatty alcohols fraction of *T. zanonii.*

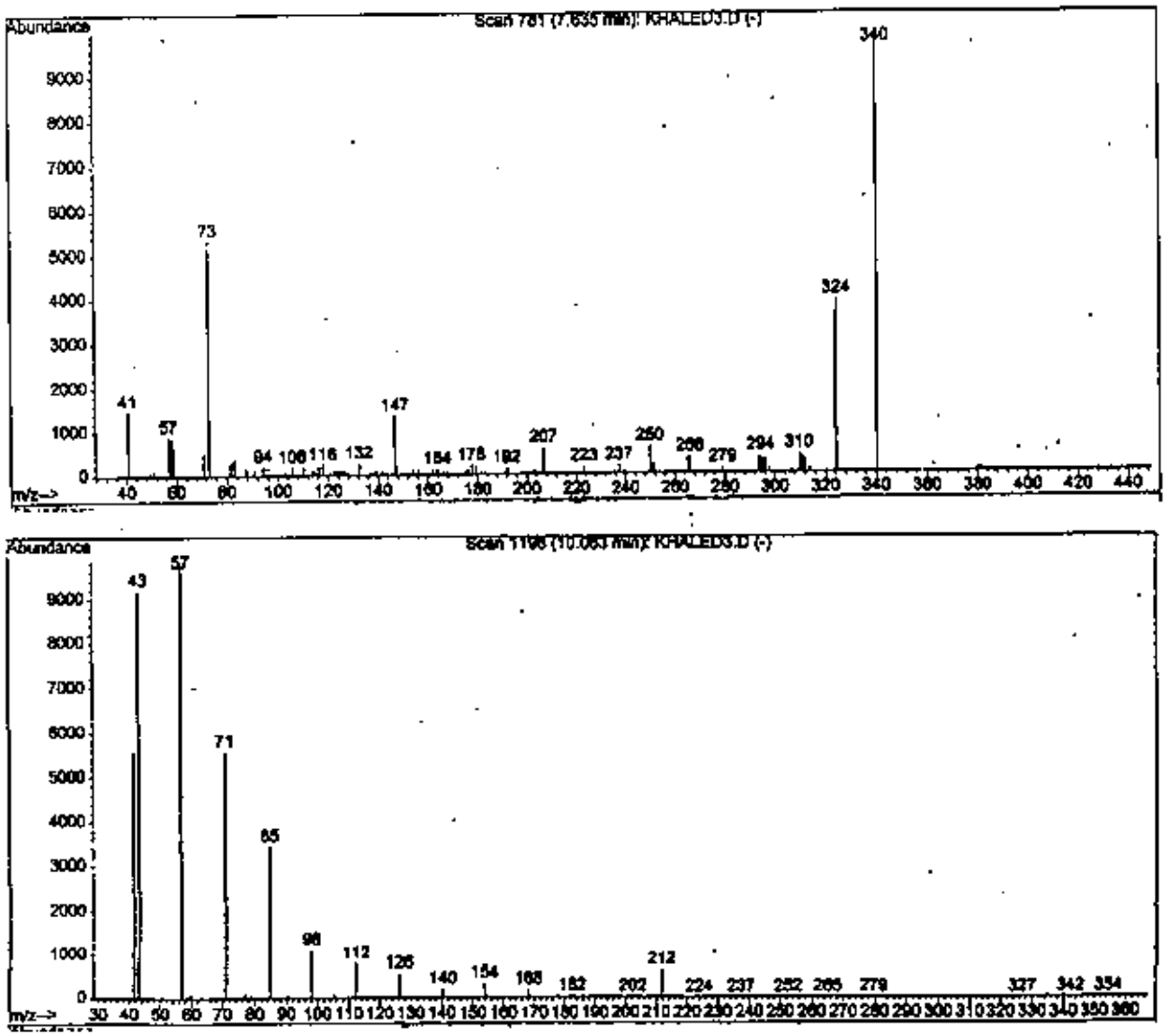


Fig. (13) : EI-mass spectrum of fatty alcohol and hydrocarbon compounds.

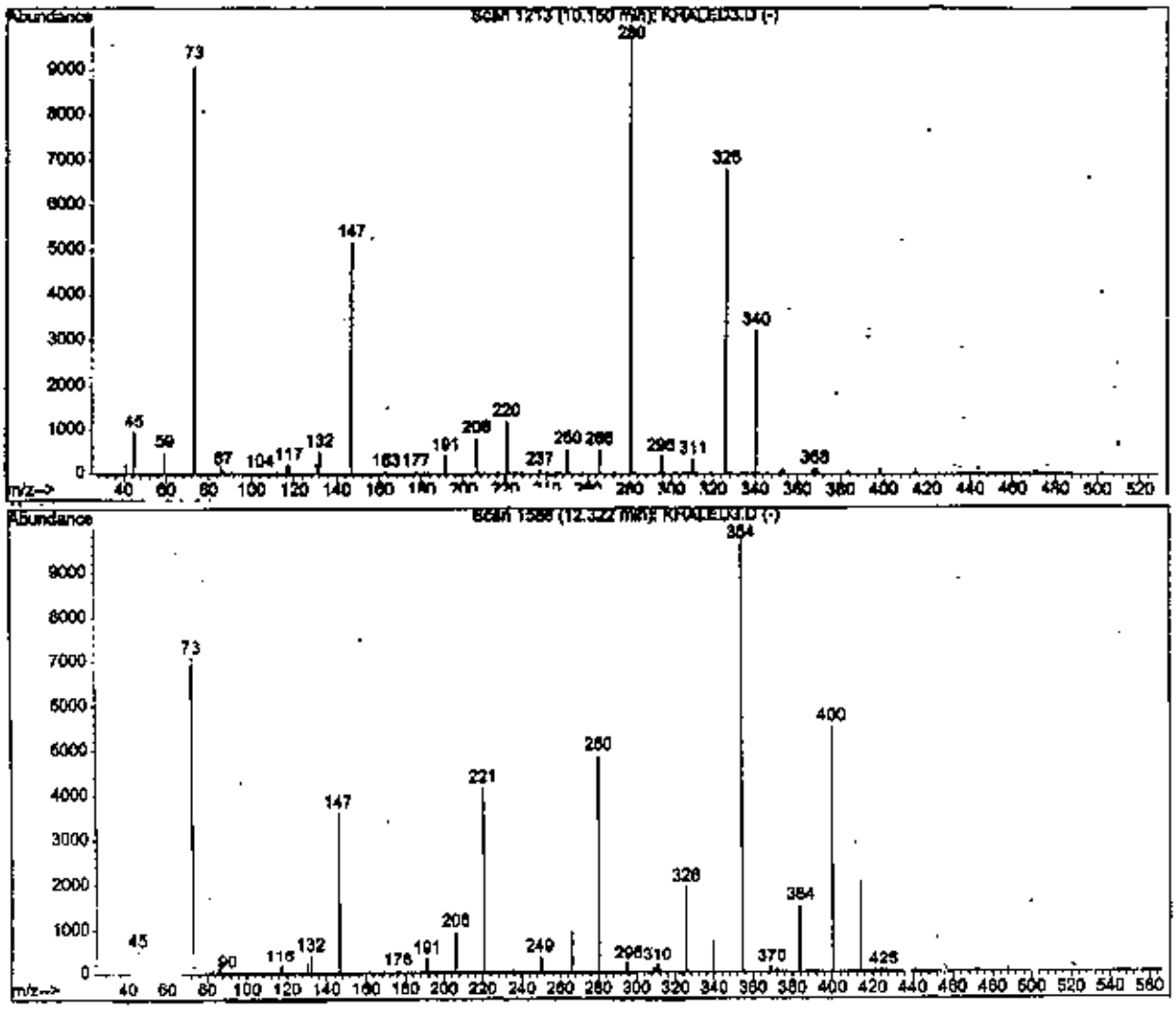

Abundance
Scan 1213 (10.150 min): KPDALE053.D (-)
73
280
325
147
340
45
59
87
104
117
132
163
177
191
208
220
237
260
286
296
311
368
m/z-->
Abundance
Scan 1586 (12.322 min): KPDALE053.D (-)
354
73
400
280
221
147
326
384
45
90
116
132
176
191
208
249
296
310
370
425
m/z-->

Fig. (13) : Cont.

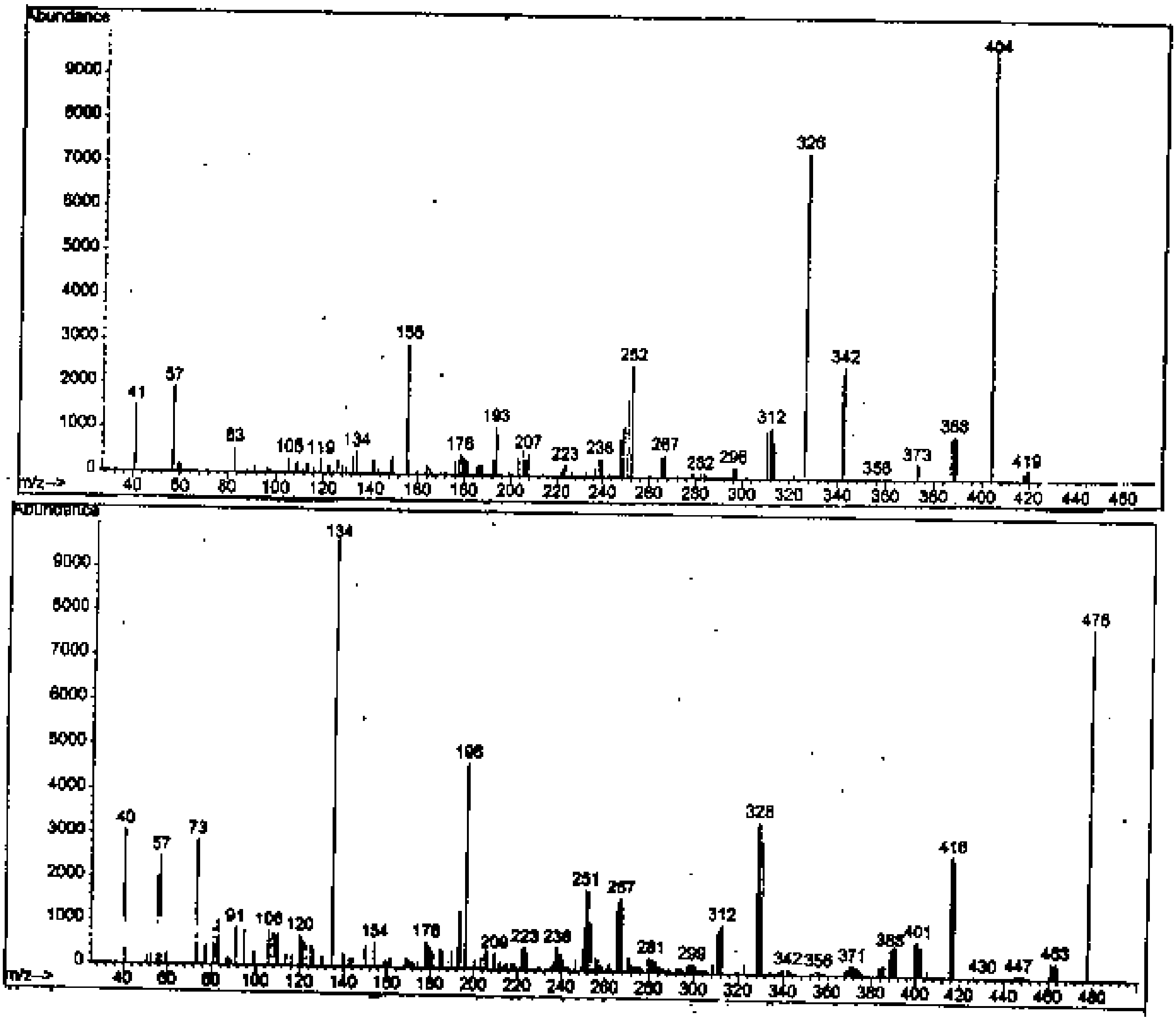

Abundance
m/z-->
404
326
156
252
342
41
57
83
105
119
134
176
193
207
223
238
267
282
296
312
358
373
388
419
Abundance
m/z-->
134
476
196
40
57
73
328
416
251
267
91
106
120
154
178
209
223
238
281
299
312
342
356
371
385
401
430
447
463

Fig. (13) : Cont.

Table (6): GC/MS analysis of the fatty alcohols mixture of *T. zanonii*

Peak No.	R_t (min.)	Relative %	Mass spectra data			Compounds	Chemical formula
			M^+	b. p.	Fragments		
1	7.63	5.10	340	340	324(39)310(6),294(6),250(8),73(54),57(10),41(15)	Tricosanol	$C_{23}H_{48}O$
2	10.06	4.62	354	57	212(7),140(4),126(6),112(8),98(12),71(56),43(92).	Tetracosanol	$C_{24}H_{50}O$
3	10.15	23.37	368	280	340(33), 326(68), 265(6), 250(6), 147(52), 73(92).	Pentacosanol	$C_{25}H_{52}O$
4	12.32	26.21	424	354	400(56),384(16), 370(2), 326(20), 280(50), 221(42), 147(37), 73(72).	Nonacosanol	$C_{29}H_{62}O$
5	13.84	24.73	420	404	388(12),373(4),326(74), 312(13), 296(3), 155(30).	Triacontene	$C_{30}H_{60}$
6	15.10	15.95	478	134	478(80), 463(4), 416(28), 328(35), 196(47).	Tetratricontane	$C_{34}H_{78}$

R_t = Retention time, M^+ = Molecular weight, b. p. = base peak. Note : The fragment abundance between parenthesis.

Identification of separated compounds was done by using: Standard library (NIST Version 2.0).

Saponification of acetone soluble fraction :-

The acetone soluble fraction (7.5 g) was saponified by refluxing with 50 ml N/2 alcoholic KOH for 6 hours. The alcoholic solution was concentrated to about 25 ml and diluted with cold distilled water. The unsaponified matter was extracted by shaking with successive portions of chloroform (3×100 ml). The combined chloroform extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and evaporated in *vacuo* till dryness to give a yellowish brown semisolid residue of unsaponified matter (4.1g).

Gas-liquid chromatographic analysis of unsaponifiable fraction:

The unsaponifiable matter was subjected to GLC analysis under the following conditions :-

Instrument	: Agient technologies 6890N Network GC system
Column	: capillary column (ZB-5), (length 30m, 530µm, Film-thickness 50 µm)
Temperature program:	
Oven	: initial temp.: 80°C, rate: 8C°/min., final temp.: 250°C, final time: 50 min.
Inlet	: 270°C, (split) =mode, Split ratio =15: 1
Detector	: (FID) 300°C
Carrier gas	: N2 30ml/min.
Hydrogen	: H2 30ml/min.
Air	: 300ml/min.

The results obtained are shown in (Fig. 14 and Tab. 7).

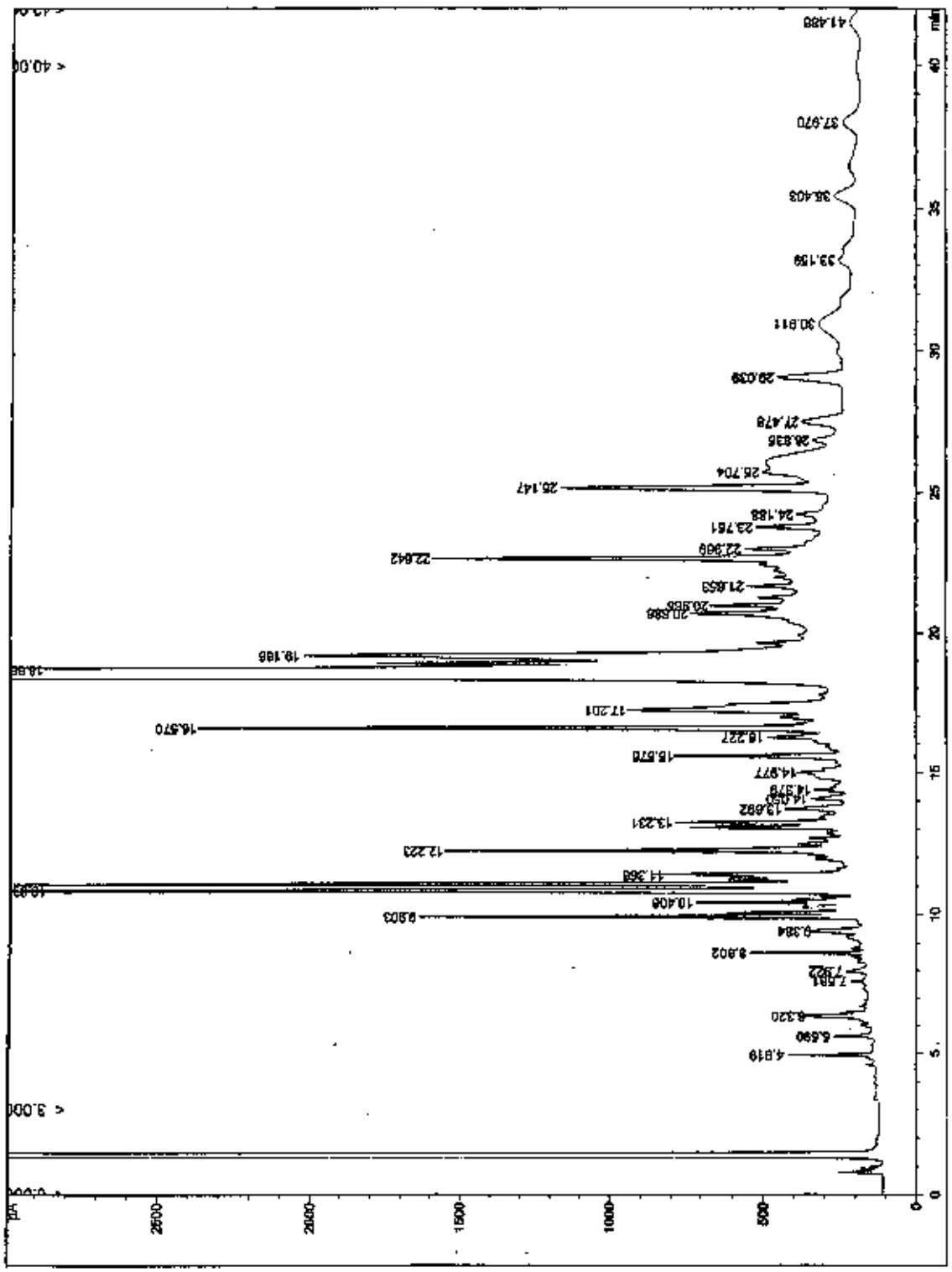


Fig. (14) : GLC Chromatogram of the unsaponifiable fraction of *T. zanonii.*

Table (7): GLC analysis of unsaponifiable fraction of *T. zanonii*

Compound	R_t (min.)	Relative %	Compound	R_t (min.)	Relative %
n-C_3	4.91	0.37	C_{25}	21.65	1.70
C_5	6.32	0.93	C_{26}	22.64	6.29
C_8	8.60	0.87	C_{28}	25.14	3.85
C_9	9.90	3.18	C_{29}	26.83	1.14
C_{10}	10.83	7.50	C_{30}	27.47	2.36
C_{12}	12.22	4.55	C_{32}	29.03	1.99
C_{14}	13.23	1.93	Cholesterol	30.91	4.48
C_{16}	14.37	0.83	β-Sitosterol	33.15	1.36
C_{17}	15.57	1.89	Campasterol	35.40	0.86
C_{18}	16.22	2.04	Stigmasterol	37.97	0.36
C_{20}	18.66	46.98	β-Amyrine	41.48	0.41
C_{22}	20.68	4.08			

R_t : Retention time.

Preparation of the total fatty acids:

The hydroalcoholic soap solution after saponification (*c.f.* page 88) was rendered acidic (PH = 2) with 5% sulphuric acid. The liberated fatty acids were thoroughly extracted several times with chloroform. The combined chloroform extract was washed with distilled water till free from acidity and dehydrated over anhydrous sodium sulphate. The solvent was evaporated *in vacuo* at 40°C till dryness (0.7 g).

Preparation of the fatty acid methyl esters:

About 0.5 g of the total fatty acids was dissolved in 30 ml dry methanol containing 4-5% dry HCl and refluxed on a boiling water bath for three hours. The reaction mixture was diluted with successive portions of chloroform (3×100 ml). The combined chloroform extract was washed with distilled water till free of acidity, dried over anhydrous sodium sulphate, filtered, and the solvent was evaporated in *vacuo* at 40°C (0.3 g).

Gas-Liquid Chromatography of the fatty acid methyl esters:

GLC analysis of the fatty acid methyl esters was carried out using the following conditions:-

Instrument	: Hewlett Packar DHP-6890 series.
Column	: capillary column HP-wax Bonded Polyethylene Glycol (Length : 60 m, Dimeter: 320µm, Film thickness: 0.25 µm.)
Temperature program	: 70 °C for 2min, rate 4°C/min Final Temp.200°C, Final time, 30 min.
Detector temp.	: 275°C (F I D)
Injector temp.	: 250°C
Flow rates: N2	: 30ml/min
H2	: 30ml/min
Air	: 350ml/min

The results obtained (Fig. 15 and Tab. 8) revealed the presence of 11 fatty acids.

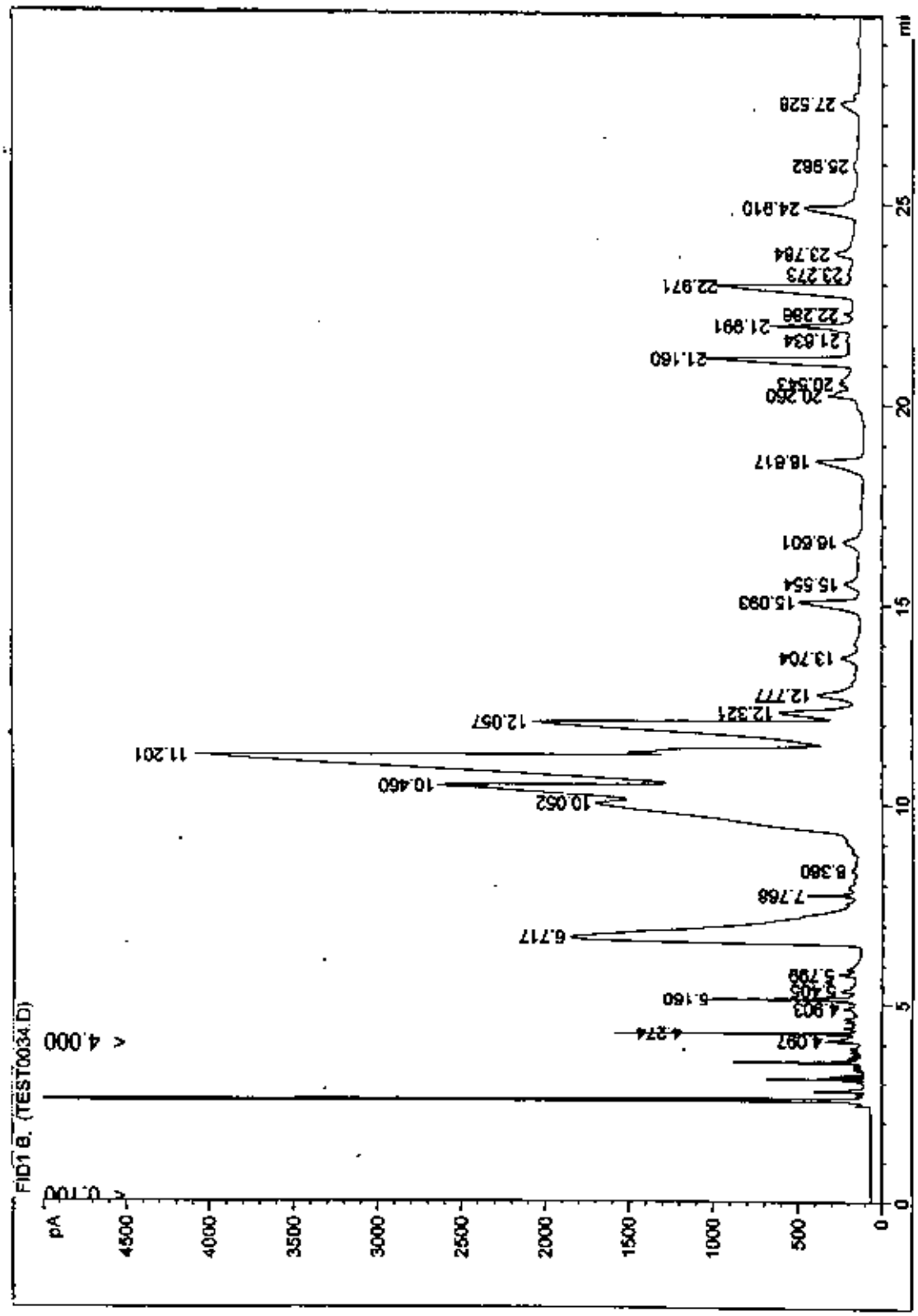


Fig. (15) : GLC Chromatogram of the fatty acid methyl esters of *T. zanonii.*

Table (8) GLC analysis of the fatty acid methyl esters

Peak No.	Fatty acid		R_t (min.)	Relative %
1	Lauric	($C_{12:0}$)	4.27	1.36
2	Myristic	($C_{14:0}$)	5.16	1.22
3	Palmitic	($C_{16:0}$)	6.71	13.95
4	Stearic	($C_{18:0}$)	10.05	15.05
5	Oleic	($C_{18:1}$)	10.46	13.69
6	Linoleic	($C_{18:2}$)	11.20	35.25
7	Linolenic	($C_{18:3}$)	12.05	11.21
8	Arachidic	($C_{20:0}$)	15.09	1.58
9	Erucic	($C_{22:1}$)	18.61	1.21
10	Lignoceric	($C_{24:0}$)	22.97	3.58
11	Tetracosenoic	($C_{24:1}$)	24.91	1.90

R_t = Retention time

4-INVESTIGATION OF THE FLVONOIDAL CONSTITUENTS OF *TEUCRIUM ZANONII*

Extraction and fractionation of the flavonoidal constituents:

About 1kg of the air dried powdered plant of *T. zanonii* was defatted with petroleum ether (b.r.40-60°C) (5 L). The defatted powder was macerated with 70% methyl alcohol till exhaustion. The alcoholic extract was evaporated in *vacus* at about 50°C (73.7 g), dissolved in hot distilled water (300 ml), left overnight in refrigerator and then filtered. The aqueous filtered was extracted with successive portion of ethyl acetate (5×500 ml) followed by butanol (5×500 ml). The solvents were dried; separately; over anhydrous sodium sulphate and evaporated in *vacuo* at 50 °C. The ethyl acetate and butanol free residues amounted to 3.5g and 6.5 g respectively.

Paper chromatography:

Paper chromatographies of ethyl acetate as well as the butanol extracts were carried out as follows:

Ascending paper chromatography (PC) was used for the detection, isolation and purification of the different flavonoidal components using chromatographic sheets (Whatman 3MM) and applying the following solvent systems:

1-Butanol-Acetic acid-Water (BAW) (3:1:1) [166].

2-Butanol-Acetic acid-Water (BAW) (4:1:5) (upper layer) [166].

3-15% and 25% Acetic acid (AcOH) [166].

Detection was carried out by examining chromatograms under UV light at 366 nm, before and after exposure to ammonia vapor and spraying with 1% alcoholic $AlCl_3$. [124].

Paper chromatography of the ethyl acetate fraction using Whatman 3MM irrigated with 15% acetic acid gave the best separation of the flavonoids (Tab. 9 and Fig. 16). It revealed the presence of four main flavonoids (R_f 0.05, 0.11, 0.31 and 0.36) while the butanol fraction contain main flavonods (R_f 0.48 and 0.60) (Tab. 10 and Fig. 17).

Table (9): Paper chromatography of the ethyl acetate fraction

compound	R_f	Colour under UV		
	15% HOAc	None	NH_3	$AlCl_3$
1	0.62	Sk. bl.	Sk. bl.	Sk. bl.
2	0.54	F.Y.	Y.	F.G.
3	0.43	Br.	F.G.	Br.
4	0.36	Br.	Y.G.	Y.G.
5	0.31	-	F.Y.	F.Y.
6	0.11	Br.	Y.	Y.
7	0.08	Br.	Br.	Y.
8	0.05	Br.	Y.	Fl. G.

Paper chromatography : (Whatman 3MM)

Solvent : 15% HOAc

Spray reagent : Alc. $AlCl_3$

Sk. bl. = Sky blue. Y. = Yellow.

Br. = Brown G. = Green.

F. = Faint Fl. = Fluorescent (bright)

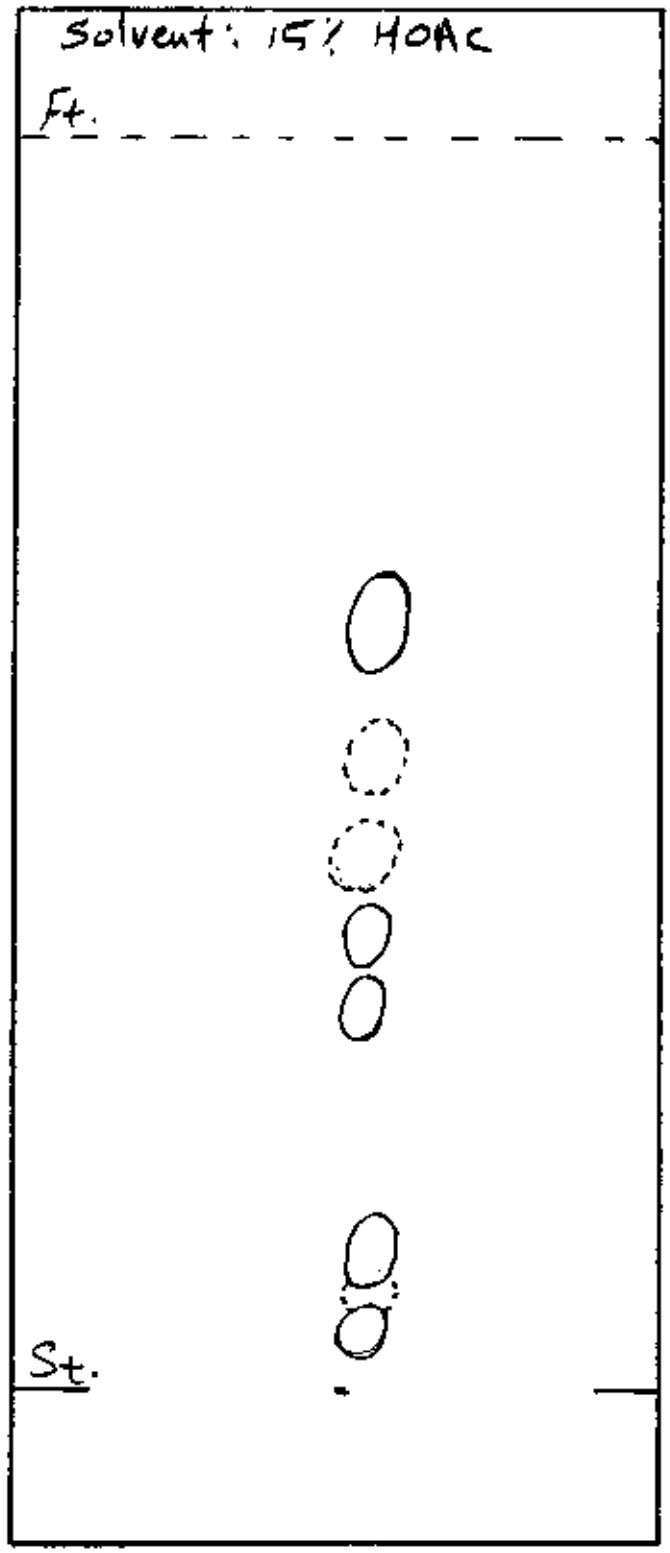


Fig. (16) : PC of ethyl acetate extract of *Teucrium zanonii*.

Table (10): Paper chromatography of the butanol fraction

compound	R_f	Colour under UV		
	15% HOAc	None	NH_3	$AlCl_3$
1	0.78	Sk. bl.	Sk. bl.	Sk. bl.
2	0.60	Br.	Y.	G.
3	0.48	Br.	F. Y.	F. Y.
4	0.26	Br.	F. Y.	G. Y.
5	0.12	-	F. Y.	F. Y.

Paper chromatography : (Whatmann 3MM)

Solvent : 15% HOAc

Spray reagent : Alc. $AlCl_3$

Sk. bl. = Sky blue. Y. = Yellow.

Br. = Brown G. = Green.

F. = Faint

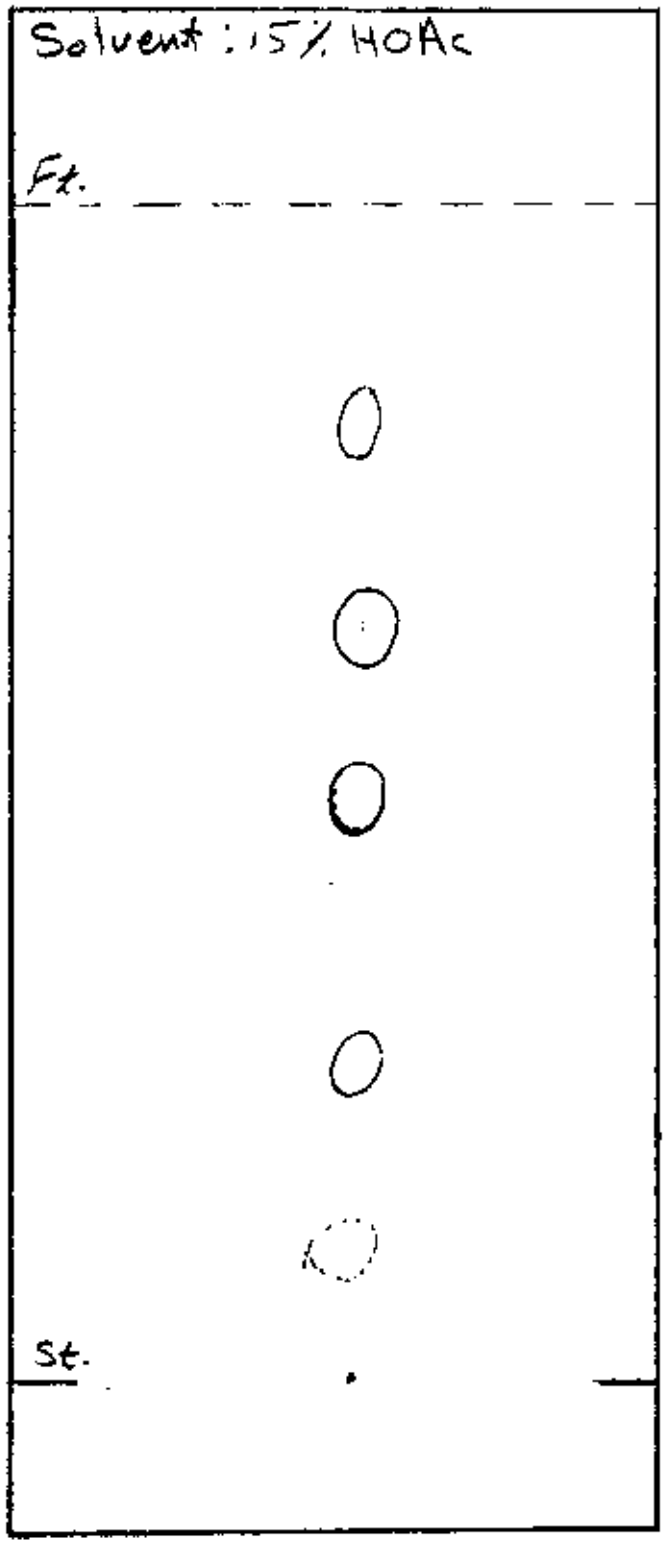


Fig. (17) : PC of butanol extract of *Teucrium zanonii*.

Fractionation of the ethyl acetate extract of *T. zanonii* :-

About 200 g of Sephadex LH-20 were swollen overnight in a mixture of methanol : water (80 : 20), then poured as a slurry in a glass column (70 x 3.5 cm). About 3 g of the ethyl acetate extract were dissolved in 5 ml of methanol : water (80 : 20) and applied on the top of Sephadex LH-20 column. Elution was affected with methanol : water with decreasing the polarity. Fractions 25 ml each were collected and the course of chromatographic fractionation was followed using PC in 15% acetic acid as a developing solvent. The column was summarized in (Tab. 11).

Table (11) : Column chromatography of ethyl acetate extract of *T. zanonii*

Solvent	Fraction	R_f	Colur in UV		Isolated compound
			NH_3	$AlCl_3$	
Methanol : Water	4-20	0.61	Sk. bl.	Sk. bl.	traces
80 : 20		0.52	F.Y.	G.	traces
		0.44	Br.	Y.G.	traces
Methanol : Water	21-26	0.36	G.	Br.	Comp.-4
90 : 10		0.31	Y.G.	G.Y.	Comp.-3
Methanol : Water	27-35	0.14	F.Y.	F.G.	traces
95 : 5		0.11	Y.	Y.	Comp.- 1
		0.08	Br.	F.Y.	traces
Methanol	36-50	0.08	Br.	Y.	traces
100%		0.05	Y.	Fl. G.	Comp.-2

Adsorbent : PC Whatmmn No. 3MM.

Solvent system : 15% acetic acid.

Spraying reagent : 1% $AlCl_3$ in methanol.

purification of compound -1 :

The fractions 27-35 (Tab. 11) containing compound-**1** were collected and rechromatographed over small column of Sephadex LH-20 eluted with methanol : water (90 : 10), and collecting small fractions (10 ml). The fractions containing compound-**1** in pure form (PC, 15% acetic acid) were collected and the solvent was evaporated in *vacuo* till dryness at 45 °C.

Identification of compound-1 :

The UV absorption spectra of compound-1 were carrid as follw:

UV spectroscopic measurements:

The UV absorption spectra of the isolated flavonoidal compounds were measured by preparation of a solution of 0.0001M of the flavonoidal compound in absolute spectroscopic methanol and measurements were carried out as follows:

a) preparation of reagent stock solutions and solids:

i. Sodium methoxide (NaOMe):

Freshly cut metallic sodium (2.5g) was added cautiously in small portions to dry spectroscopic methanol (100 ml). The solution was stored in a glass container with a tightly fitting stopper

ii. Aluminum chloride ($AlCl_3$):

About 5 grams of fresh anhydrous reagent grade $AlCl_3$ were added cautiously to spectroscopic methanol (100 ml).

iii. Hydrochloric acid (HCl):

Concentrated reagent grade HCl (50 ml) was mixed wih distilled water up 100 ml, the solution was stored in a glass stopper bottle.

iv. Sodium acetate (NaOAc):

Anhydrous powdered NaOAc reagent grade was used.

v. Boric acid (H_3BO_3):

Anhydrous powdered H_3BO_3 reagent grade was used.

b) Procedure of measurements:

1- The methanol spectrum was measured at normal scan speed (about 50 nm/min) using 2-3 ml of stock solution.

2- The NaOMe spectrum was measured immediately after the addition of three drops of the NaOMe stock solution to the methanolic solution used for step1, then after 5 min. the spectrum was rerun to check for flavonoid decomposition.

3- The $AlCl_3$ spectrum was measured immediately after the addition of six drops of the $AlCl_3$ stock solution to 2-3 ml of fresh stock solution of the flavonoid.

4- The $AlCl_3$/HCl spectrum was recorded immediately after the addition of three drops of the stock HCl to the solution used for step 3.

5- The NaOAc spectrum was determined by the addition of excess coarsely powdered anhydrous sodium acetate to 2-3 ml fresh stock solution of the flavonoid and shaking the cuvette (about 2 mm layer of NaOAc remained at the bottom of the cuvette) and the spectrum was recorded within two minutes, to cheek for flavonoid decomposition.

6- The NaOAc/H_3BO_3 spectrum was determined by the addition of sufficient powdered anhydrous H_3BO_3 to give a saturated solution to the cuvette from step 5 containing the NaOAc [166].

The UV absorption spectrum of the isolated flavonoidal compound-1 in methanol (Fig. 18 and Tab.12) showed band-I at 344 nm (flavone type) in addition to bathochromic shift band-I with NaOMe from 344 nm to 396 nm

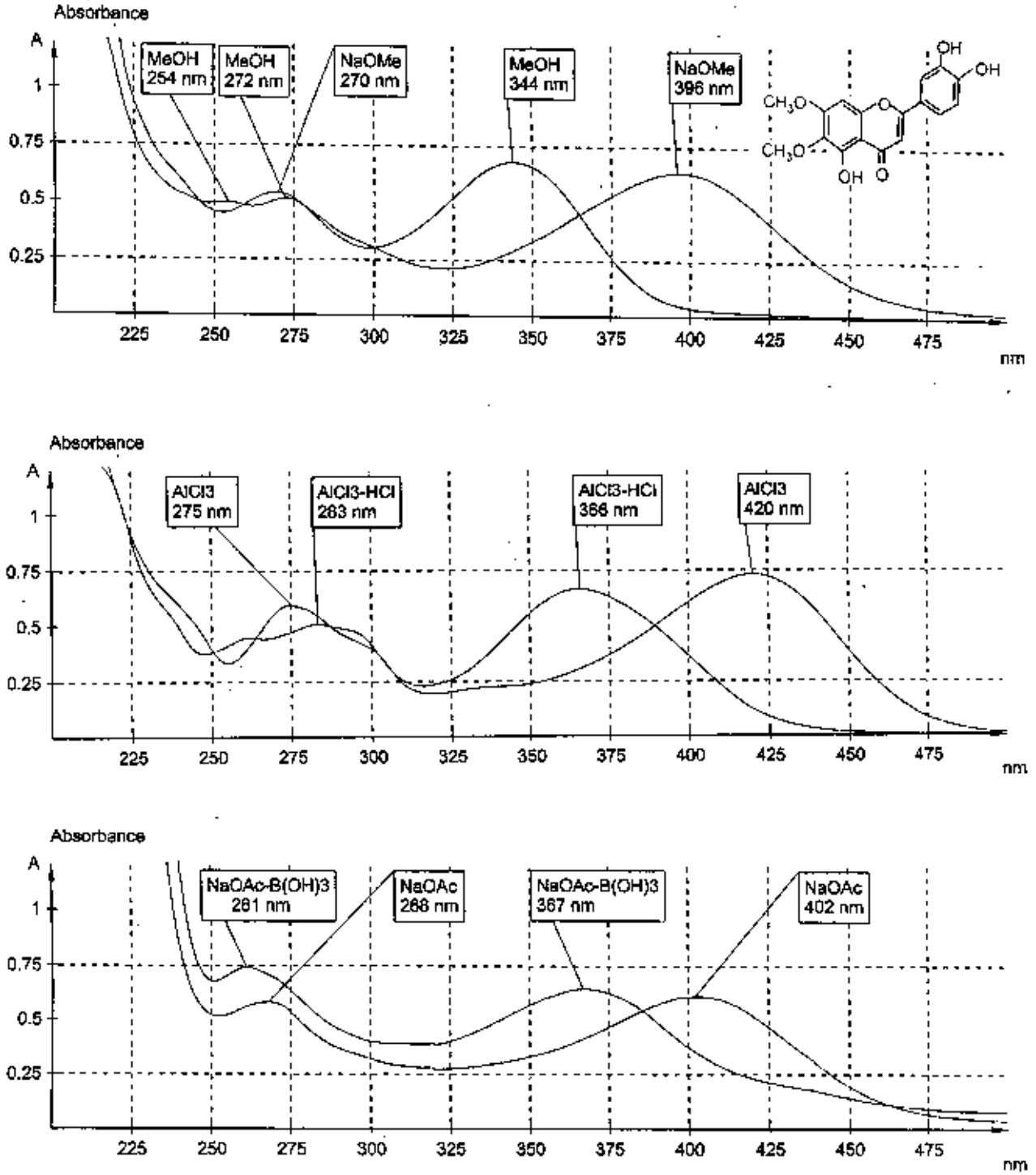


Fig. (18) : UV absorption spectra of compound-1 (Cirsiliol)

without decrease in intensity which indicates the presence of a free OH group at C-4'.

The $AlCl_3$ spectrum showed a bathochromic shift in band-I (76 nm) relative to methanol spectrum indicate the presence of free OH group at C-5. The presence of an *ortho*-dihydroxy system in ring-B was confirmed where there is a hypsochromic shift (54 nm) in band-I in $AlCl_3$/HCl spectrum relative to $AlCl_3$ spectrum. Also it was proved through NaOAc/H_3BO_3 spectrum where there is a bathochromic shift (22 nm) in band-I relative to methanol spectrum.

The NaOAc spectrum showed no bathochromic shift in band-II indicating the absence of free OH group at C-7.

Table (12): Ultraviolet absorption data of compound-1

Additions to Methanol	$\lambda_{max.}$ (nm)
None	254 (sh), 274, 344.
NaOMe	270, 396.
$AlCl_3$	239 (sh), 275, 300 (sh), 340 (sh), 420.
$AlCl_3$/HCl	263 (sh), 283, 366.
NaOAc	268, 402.
NaOAc/H_3BO_3	261, 367.

The EI-mass spectrum of compound-1 (Fig. 19 and scheme 1) showed a molecular ion peak at m/z = 330 (M^+; 58%) and others at 329 (M^+-1; 14%), 331 (M^++1; 16%), 315 (M^+-CH_3; 50%), 301 (M^+-CHO; 16%), 287 (M^+-(CO+CH_3); 26%) and 284 (M^+-(OCH_3+CH_3); 17%).

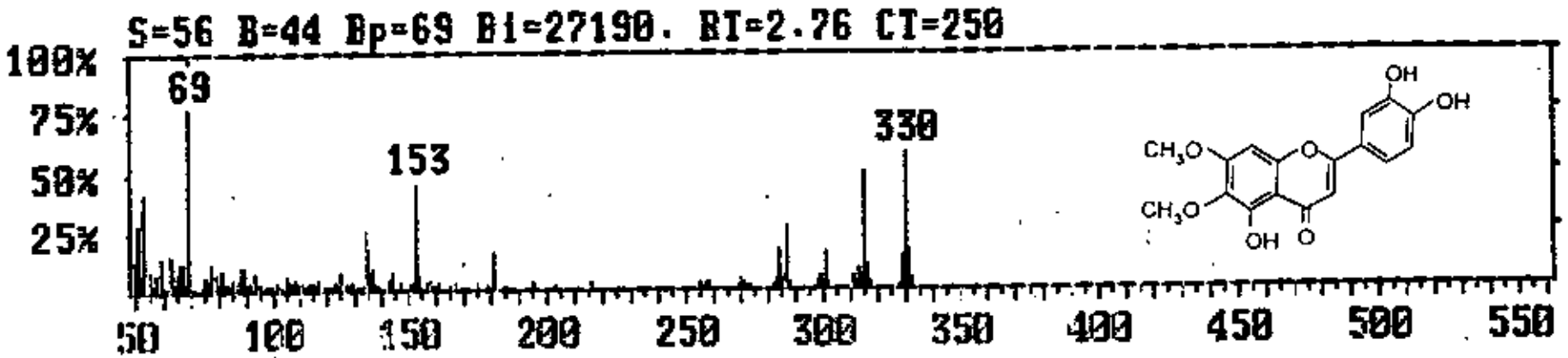
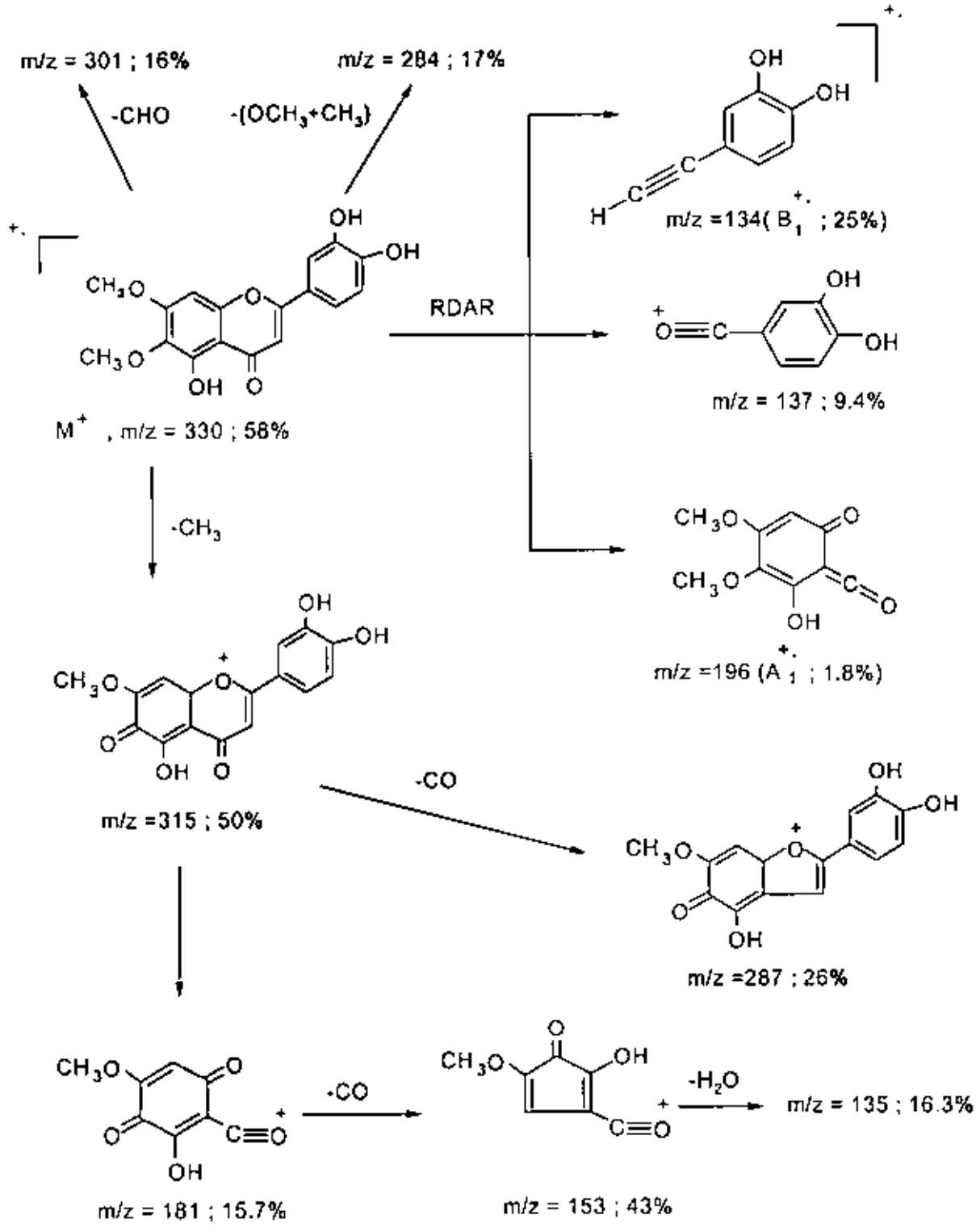


Fig. (19) : EI-mass spectrum of compound-1

(Cirsiliol)

The fragmentation pathway of compound-1 undergoes Retero Diel's Alder reaction (RDA) giving rise to fragments at m/z = 196 (A_1^{+} ; 1.8%) and 134 (B_1^{+}, 25%) as shown in scheme (1)



Scheme (1): Fragmentation pathways of compound-1

The ^{1}H-NMR spectrum of compound-1 in (CD_3OD) (Fig. 20) showed signals at δ in ppm 7.45 (1H, d, H-2'), 7.41 (1H, d, H-6'), 6.91 (1H, d, H-5') , 6.80 (1H, s, H-8), 6.61 (1H, s, H-3), 3.98 (3H, s, C-7-OCH_3), 3.83 (3H, s, C-6-OCH_3).

The ^{13}C-NMR spectrum of compound-1 (Fig. 21 Tab.13) displayed the most important peaks for 7,6-dimethoxylated flavones in addition to the carbonyl carbon at δ = 182.90 ppm. Also these data were coincided with that reported for cirsiliol as shown by Mouma *et. al.* [225]

Table (13): ^{13}C-NMR data of compound-1

Carbon No.	δ (ppm)
2	165.2
3	103.87
4	182.90
5	153.40
6	133.50
7	160.64
8	92.28
9	152.50
10	114.26
1'	123.58
2'	116.84
3'	145.60
4'	150.21
5'	120.48
6'	123.58
C-6-OCH_3	57.04
C-7-OCH_3	61.12

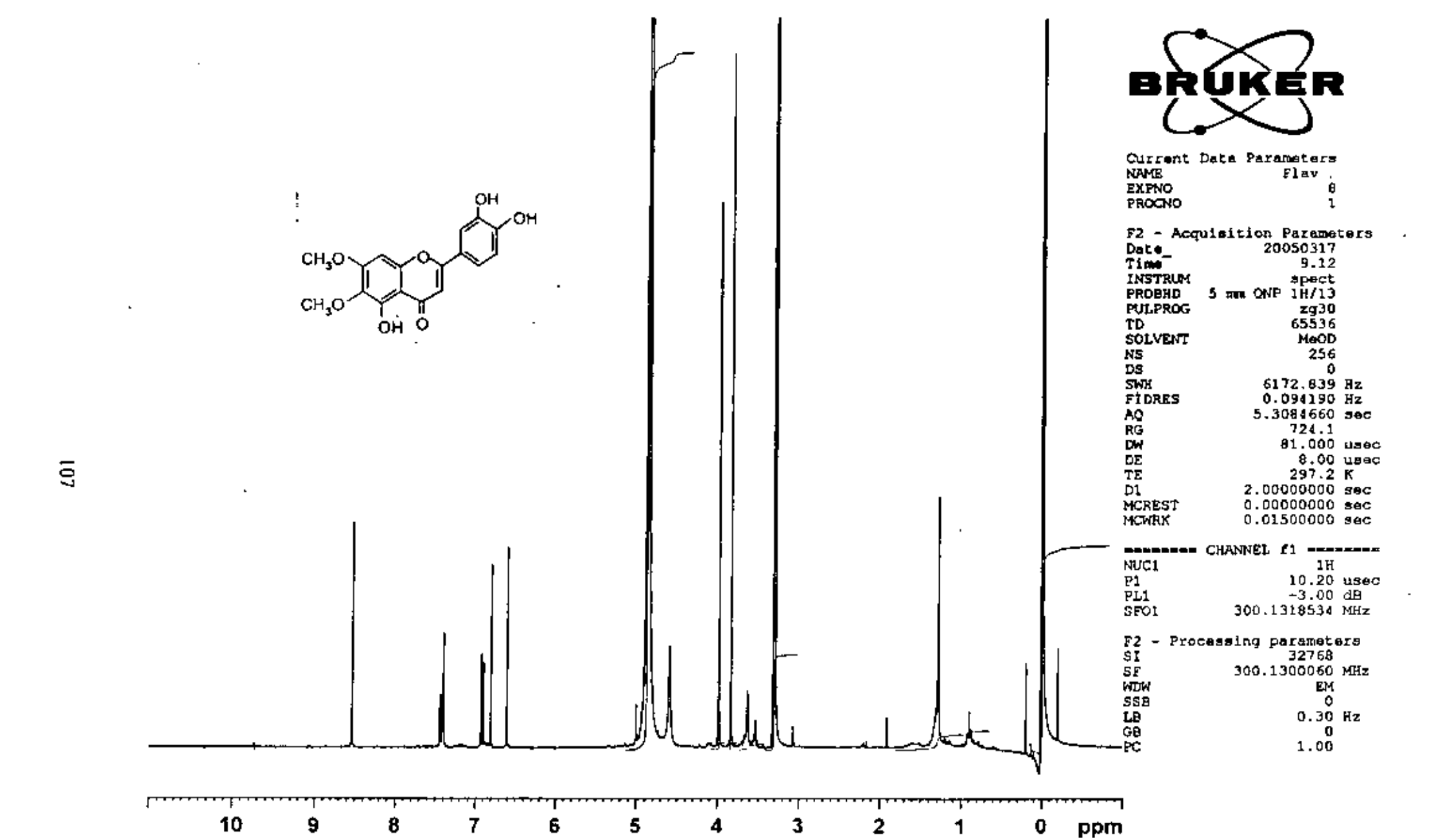


Fig. (20) : ^{1}H-NMR (MeOD) spectrum of compound-1 (Cirsiliol)

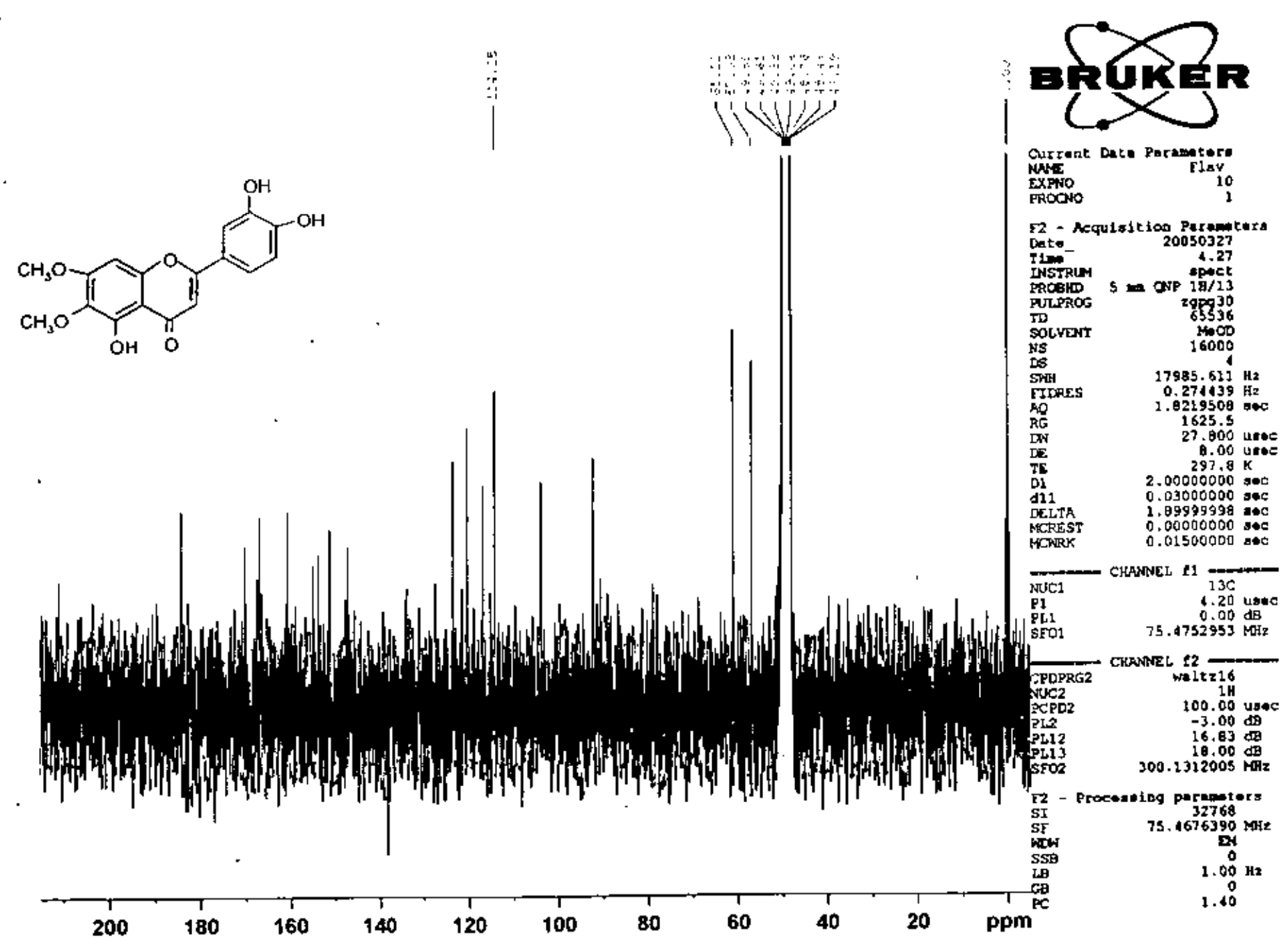
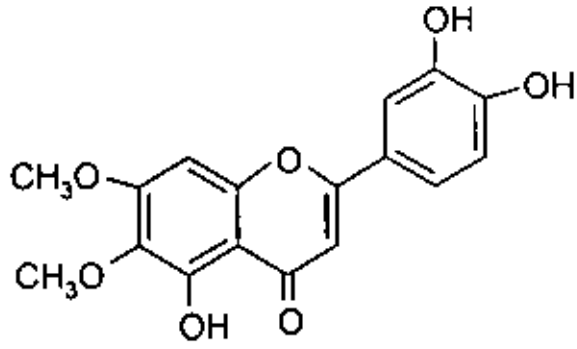


Fig. (21) : ^{13}C-NMR (MeOD) spectrum of compound-1 (Cirsiliol)

From the above chromatographic and spectroscopic data compound-**1** can be identified as cirsiliol (3',4',5-trihydroxy-6,7-dimethoxy flavone)



Cirsiliol (3', 4', 5-trihydroxy-6,7-dimethoxy flavone)

purification of compound-2 :

The fractions 36-50 (Tab. 11) containing compound-**2** were collected and rechromatographed over small column of Sephadex LH-20 eluted with methanol : water (90 : 10), and collecting small fractions (10 ml each). The fractions containing compound-2 in pure form (PC, 15% acetic acid) were collected and the solvent was evaporated *in vacuo* till dryness at 45°C.

Identification of compound-2 :

The UV absorption spectrum of the compound-**2** in methanol (Fig. 22 and Tab. 14) showed band-I at 348 nm (flavone type) in addition to a bathoc-hromic shift in band-**I** with NaOMe from 348 nm to 400 nm with increasing in intensity indicates the presence a free OH group at C-4' [166]. The $AlCl_3$ spectrum showed a bathochromic shift in band-I (74 nm) indicating the presence of free OH group at C-5. The bathochromic shift in band-Ia in $AlCl_3$/HCl spectrum relative to band-I in MeOH is (35 nm)

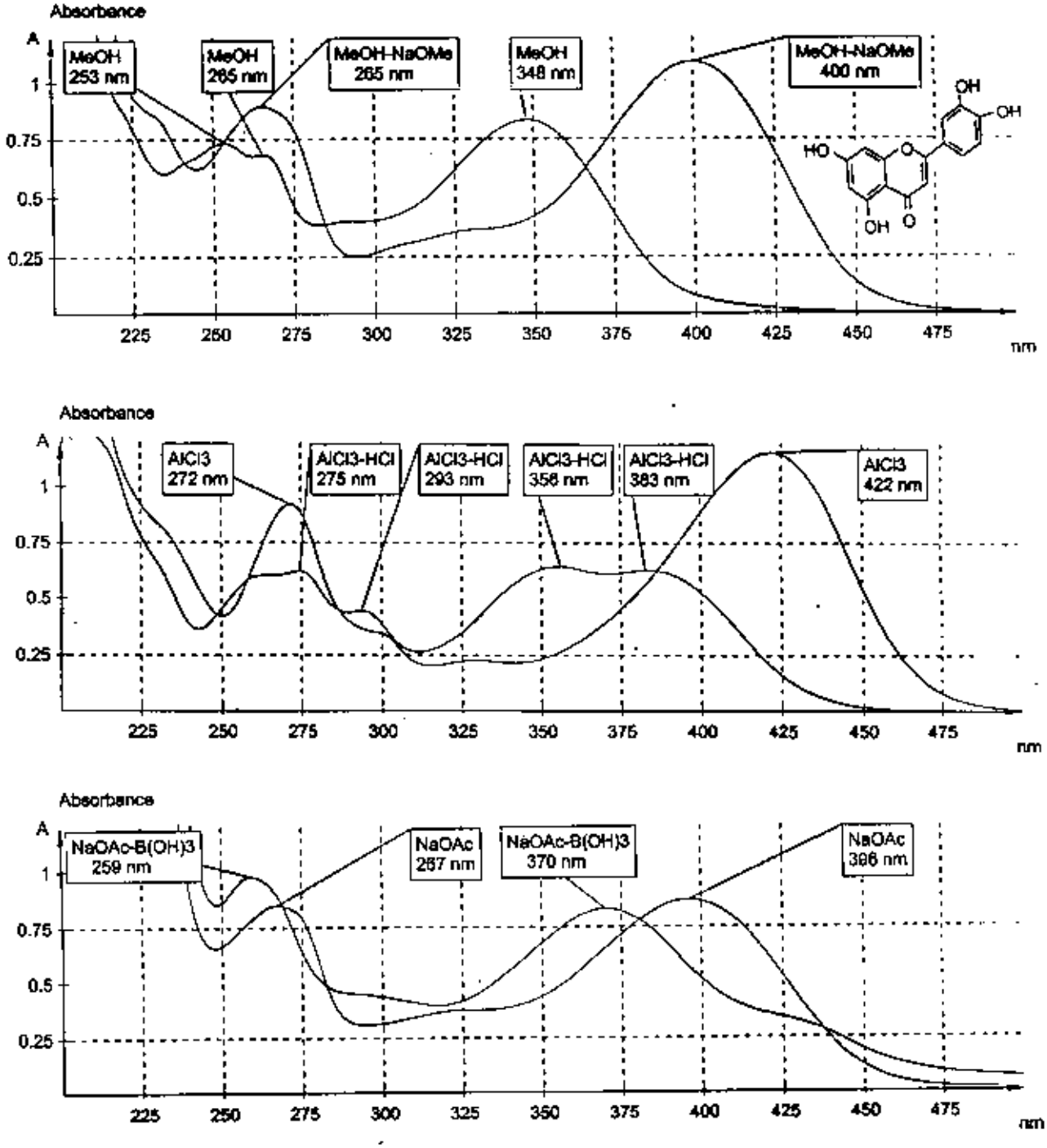


Fig. (22) : UV absorption spectra of compound-2

(Luteolin)

Indicating the presence of OH group at C-5 and not at C-3. Moreover, the $AlCl_3$/HCl spectrum exhibit hypthochromic shift (39 nm) in band-I relative to $AlCl_3$ spectrum indicating the presence of an *ortho*-dihydroxy system in ring-B.

The NaOAc spectrum showed bathochromic shift (14 nm) in band-II indicating the presence of free OH group at C-7.

An *ortho*-dihydroxy system is further proved to be present in ring-B as a bathochromic shift in band-I (22 nm) of NaOAc/H_3BO_3 spectrum was observed.

Table (14): Ultraviolet absorption data of compound-2

Addition to Methanol	**$\lambda_{max.}$ (nm)**
None	253, 265, 290 (sh), 348.
NaOMe	232 (sh), 265, 330 (sh), 400.
$AlCl_3$	232 (sh), 272, 300 (sh), 331 (sh), 422.
AlCl3/HCl	262, 275, 293 (sh), 356, 383.
NaOAc	267, 329 (sh), 396.
NaOAc/H_3BO_3	259, 300 (sh), 370, 430 (sh).

The EI-mass spectrum of compound-2 (Fig. 23 and scheme 2) showed a molecular ion peak at m/z = 286 (M^+ ; 100%) which corresponding to the molecular formula $C_{15}H_{10}O_6$, 287 (M^++1 ; 31%). Another important peak at m/z = 285 (M^+-1 ; 17%) and 258 (M^+- CO ; 34%).

The fragmentation pathway of compound-**2** undergoes Retero Diel's Alder reaction (RDAR) giving rise to fragments at m/z = 153 ($A_1'^+$+1, 57%) and 134 ($B_1'^+$, 49%) as shown in scheme (2).

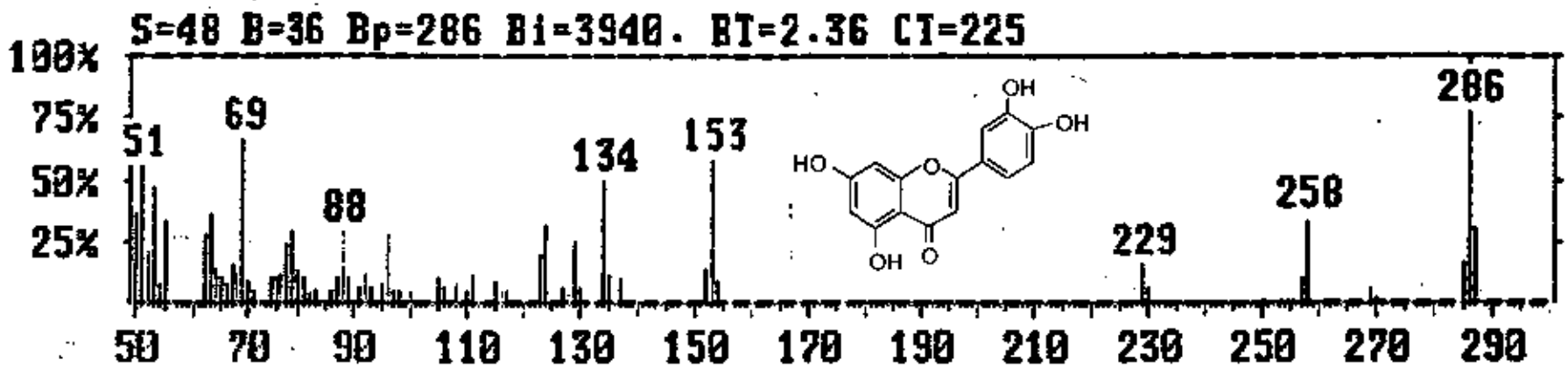
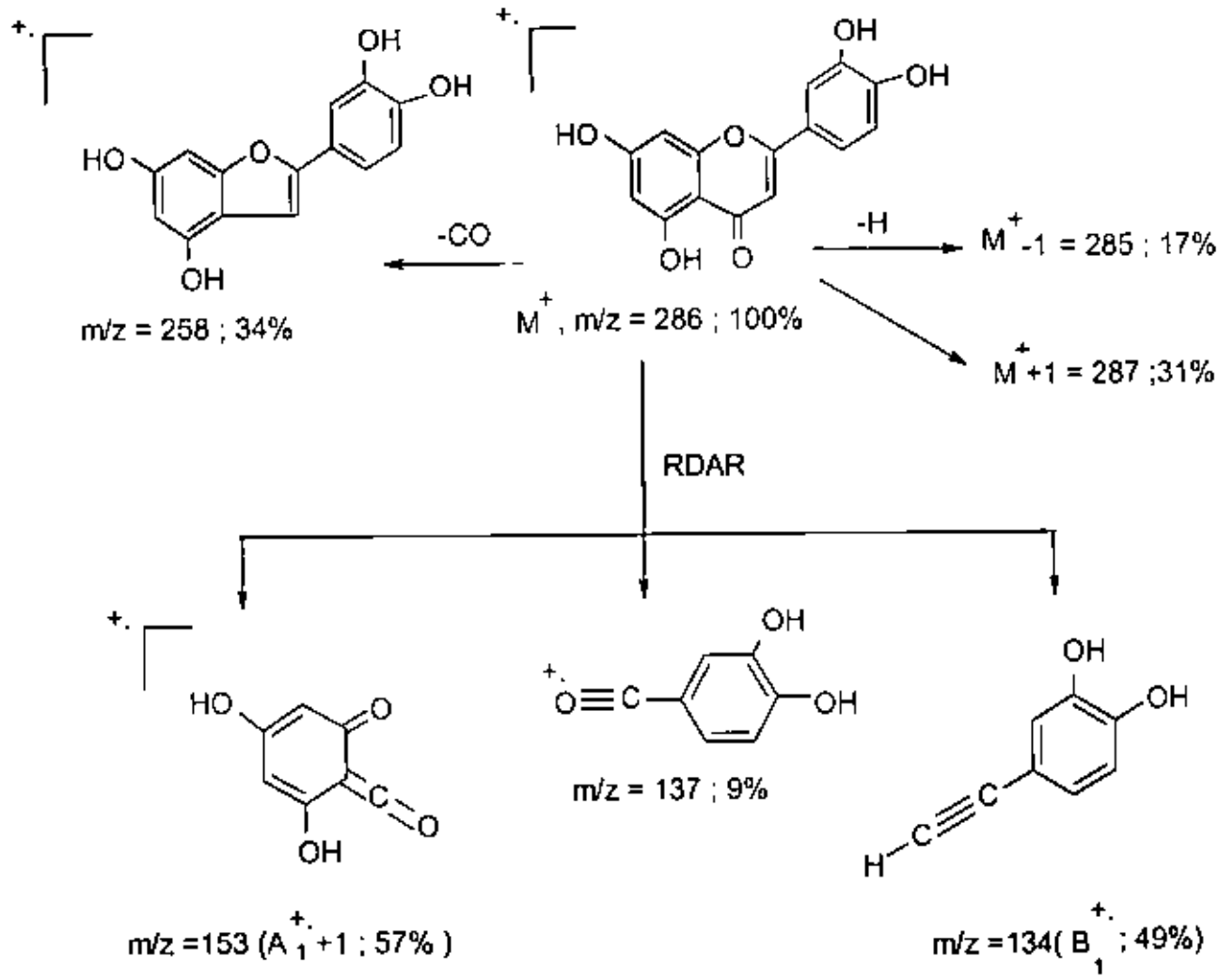


Fig. (23) : EI-mass spectrum of compound-2

(Luteolin)



Scheme (2): Frgmentation pattern of compound-2

The ^{1}H-NMR spectrum (CD_3OD) of compound-2 (Fig. 24) showed signals at δ in ppm 7.4 (1H, d, H-2'), 7.36 (1H, d, H-6'), 6.92 (1H, dd, H-5'), 6.55 (1H, s, H-3), 6.44 (1H, d, H-8) and 6.2 (1H, d, H-6) which are in agreement with those reported for Luteolin. [166]

The ^{13}C-NMR spectrum of compound-2 (Fig. 25 Tab. 15) displayed the carbonyl carbon at δ = 183.91 ppm and all the characteristic signal for flavone type structure. Also these data were coincided with that reported for Luteolin as shown by Kumari *et. al.*[226]

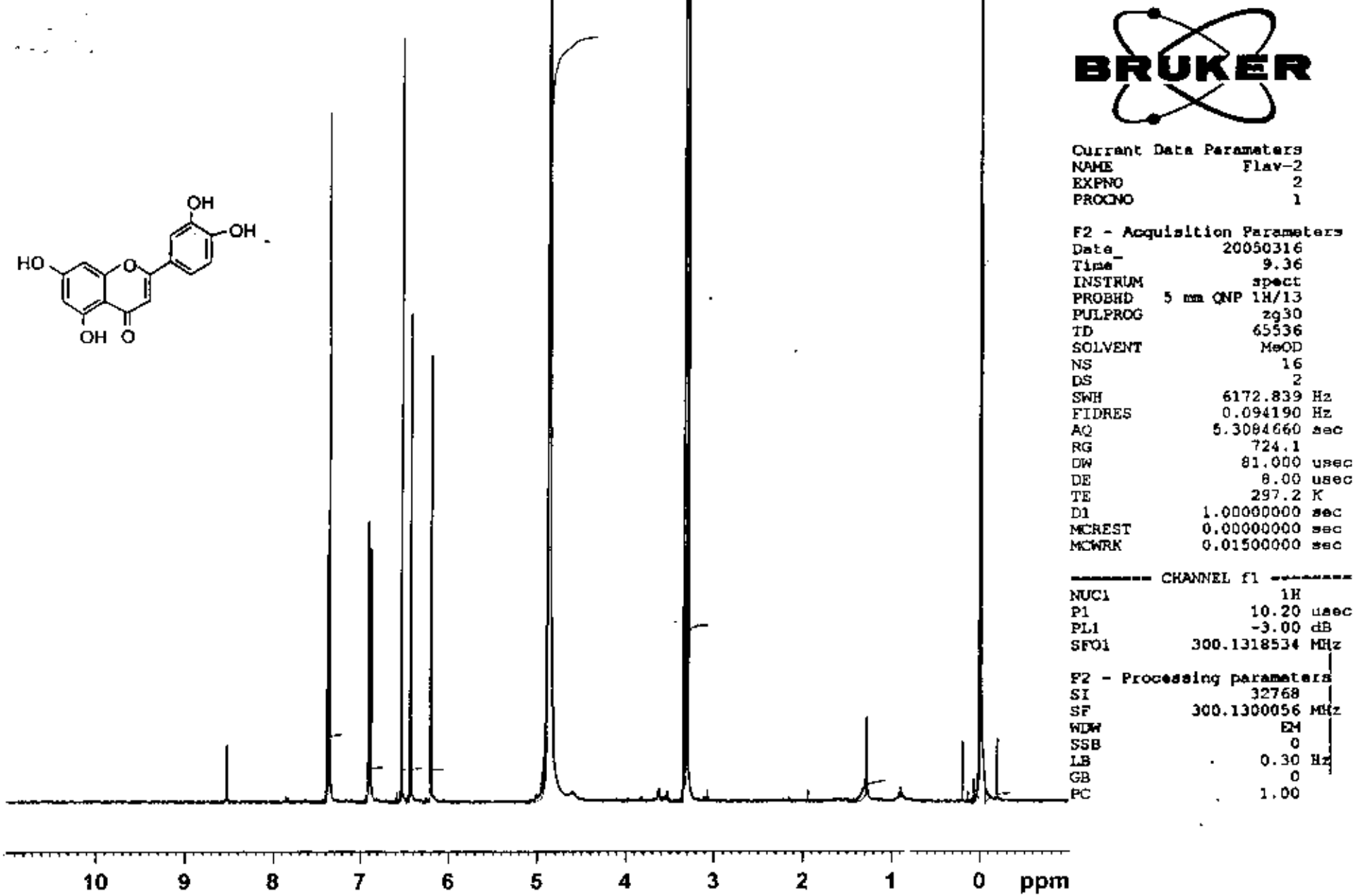


Fig. (24) : ^{1}H-NMR (MeOD) spectrum of compound-2 (Luteolin)

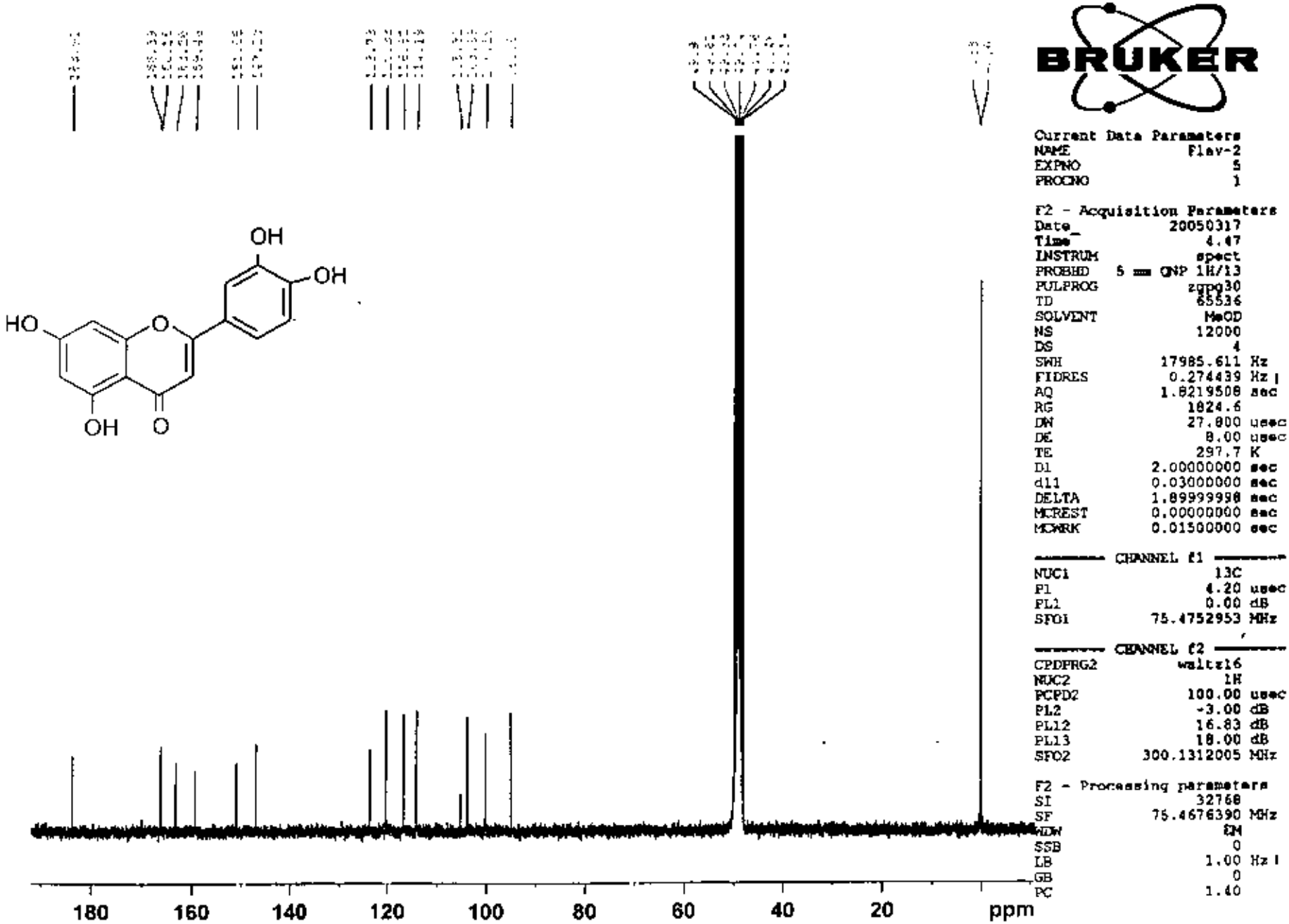


Fig. (25) : ^{13}C-NMR (MeOD) spectrum of compound-2 (Luteolin)

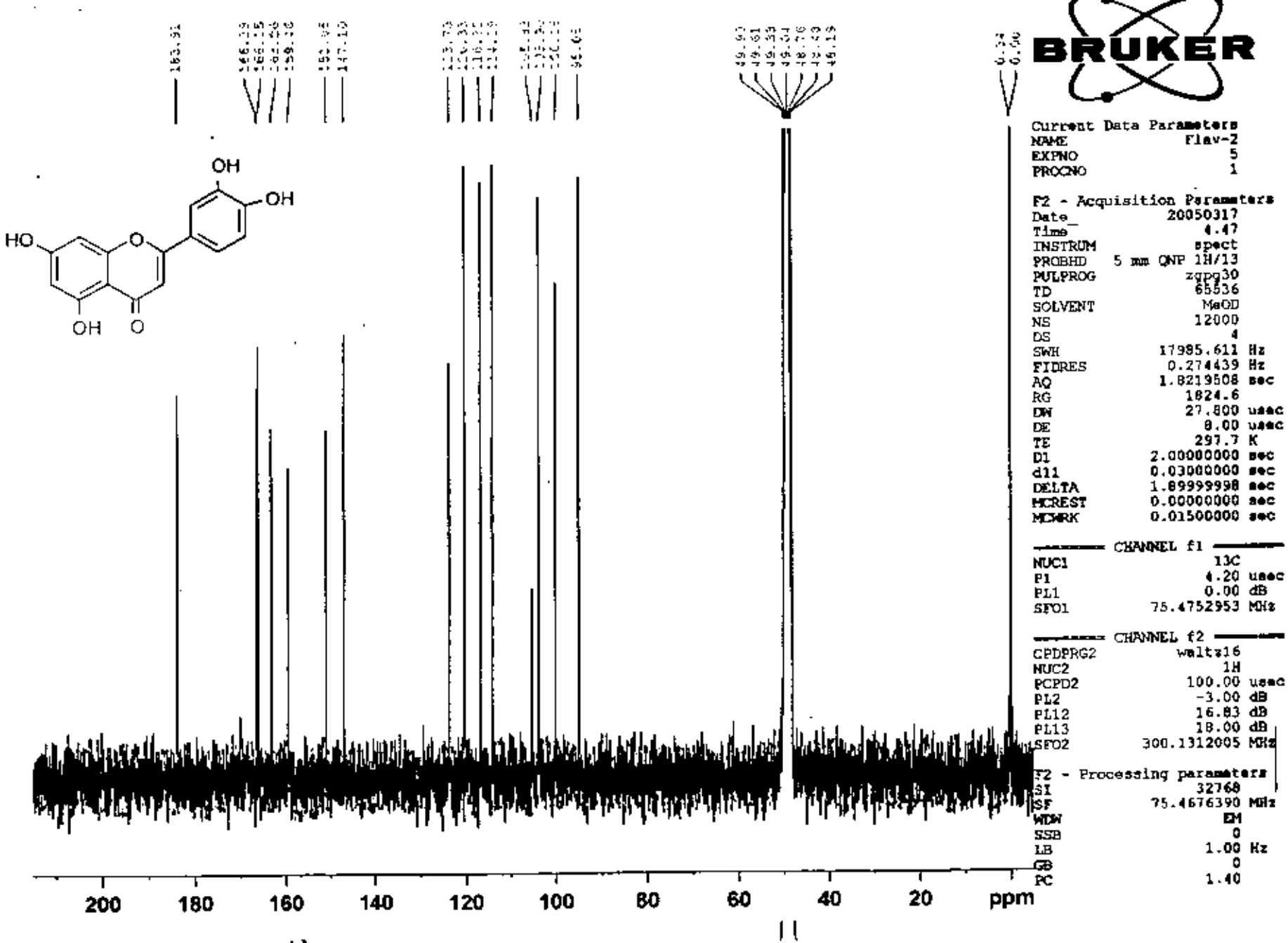
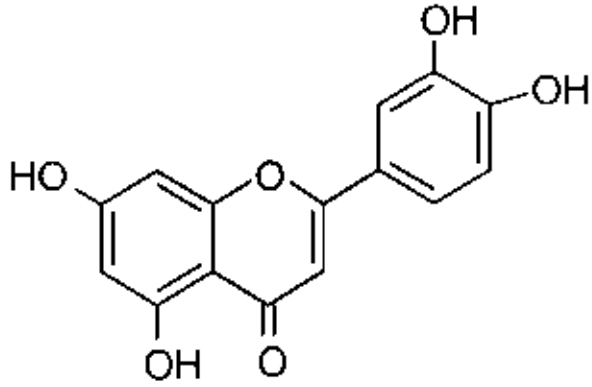


Fig. (25) : ^{13}C-NMR (MeOD) spectrum of compound-2 (Luteolin)

Table (15): ^{13}C-NMR data of compound-2

Carbon No.	δ (ppm)
2	166.39
3	103.90
4	183.91
5	163.26
6	100.18
7	166.15
8	95.05
9	159.46
10	105.33
1'	123.73
2'	114.19
3'	147.10
4'	151.05
5'	116.62
6'	120.33

All these data were coincided with that reported for luteolin so compound-2 could be identified as Luteolin.



Luteolin (3',4',5,7-tetrahydroxy flavone)

Purification of compounds (3 and 4) :

The fractions 21-26 (Tab. 11) were collected and tested by PC in 15% acetic acid, it was found to contain two main flavonoidal compounds (R_f 0.26, 0.60). The methanolic solution of fractions (21-26) was applied into a preparative thick layer chromatography (PTLC) using chloroform-methanol (80:20) as a developing solvent system. Two main zones (R_f 0.52 and 0.68) were localized under UV light, scrapped off and eluted with methanol (90%). The methanol was evaporated from each zone to afford two compounds (**3** and **4**) in pure form but in small quantities (0.7 mg and 0.5 mg respectively) so, we tentively identify these two compounds depending mainly on their UV spectra and mass spectrum for each one.

Identification of compound-3 :

The UV absorption spectrum of compound-3 in spectroscopic methanol (Fig. 26 and Tab. 16) displayed band-I at 340 nm which indicates the flavone nature of this compound. A bathochromic shift (48 nm) was noticed in band-I with increasing intensity on addition of NaOMe indicating the presence of a free OH group at C-4'.

The absence of an *ortho*-dihydroxy system was proved through $AlCl_3$/HCl spectrum as there is no hypsochromic shift in band-I was occur. Also, no bathochromic shift in band-I was observed in NaOAc/H_3BO_3 spectrum.

The presence of a free OH group at C-7 was confirmed through NaOAc spectrum, where there is a bathochromic shift in band-II (6 nm). So, we can concluded that compound-3 is flavone type have free OH groups at C-4', C-5 and C-7, also it does not contain *ortho*-dihydroxy system.

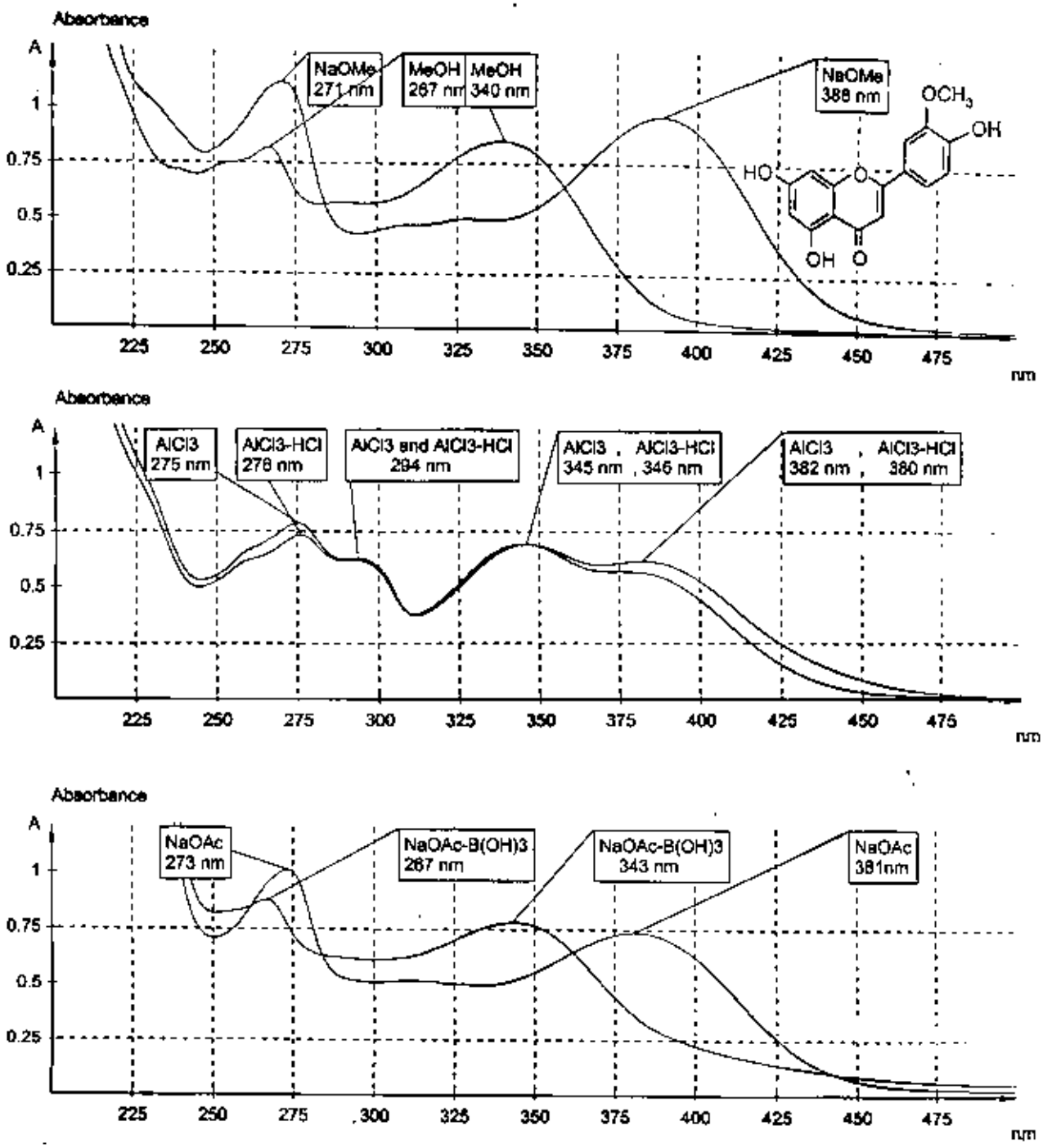


Fig. (26) : UV absorption spectra of compound-3 (Chrysoeriol)

Table (16): UV absorption data of compound-3

Addition to Methanol	$\lambda_{max.}$ nm
None	255 (sh), 267,291 (sh), 340.
NaOMe	371, 309 (sh), 330 (sh), 388.
$AlCl_3$	257 (sh), 275, 294, 345, 382.
$AlCl_3$/HCl	256 (sh), 276, 294, 346, 380.
NaOAc	273, 312 (sh), 381.
NaOAc/H_3BO_3	267, 343.

The El-mass spectrum of compound-3 (Fig. 27) showed a molecular ion peak (M^+) at m/z = 300 (60%) which constituted with the molecular formula $C_{16}H_{12}O_6$. Another peaks at m/z = 269 (M^+-OCH_3 ; 40%) and 241 (M^+- (OCH_3 + CO) ; 13%) were displayed.

The fragmentation pathway of compound-3 undergoes RDAR giving rise two peaks at m/z = 153 ; 36% and m/z = 148 ; 7% which correspond to $A_1^{'+}$+1 and $B_1^{'+}$ respectively as shown in scheme (3).

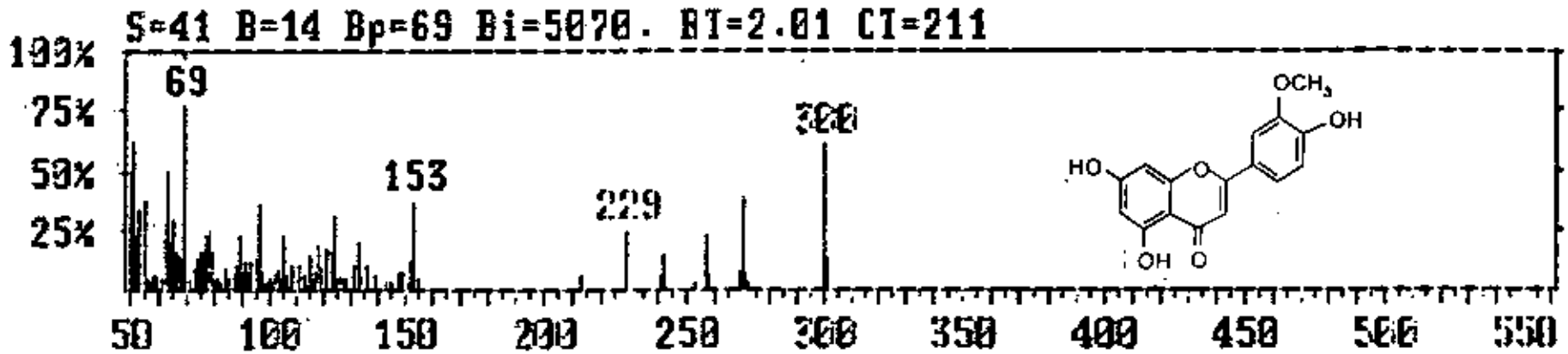
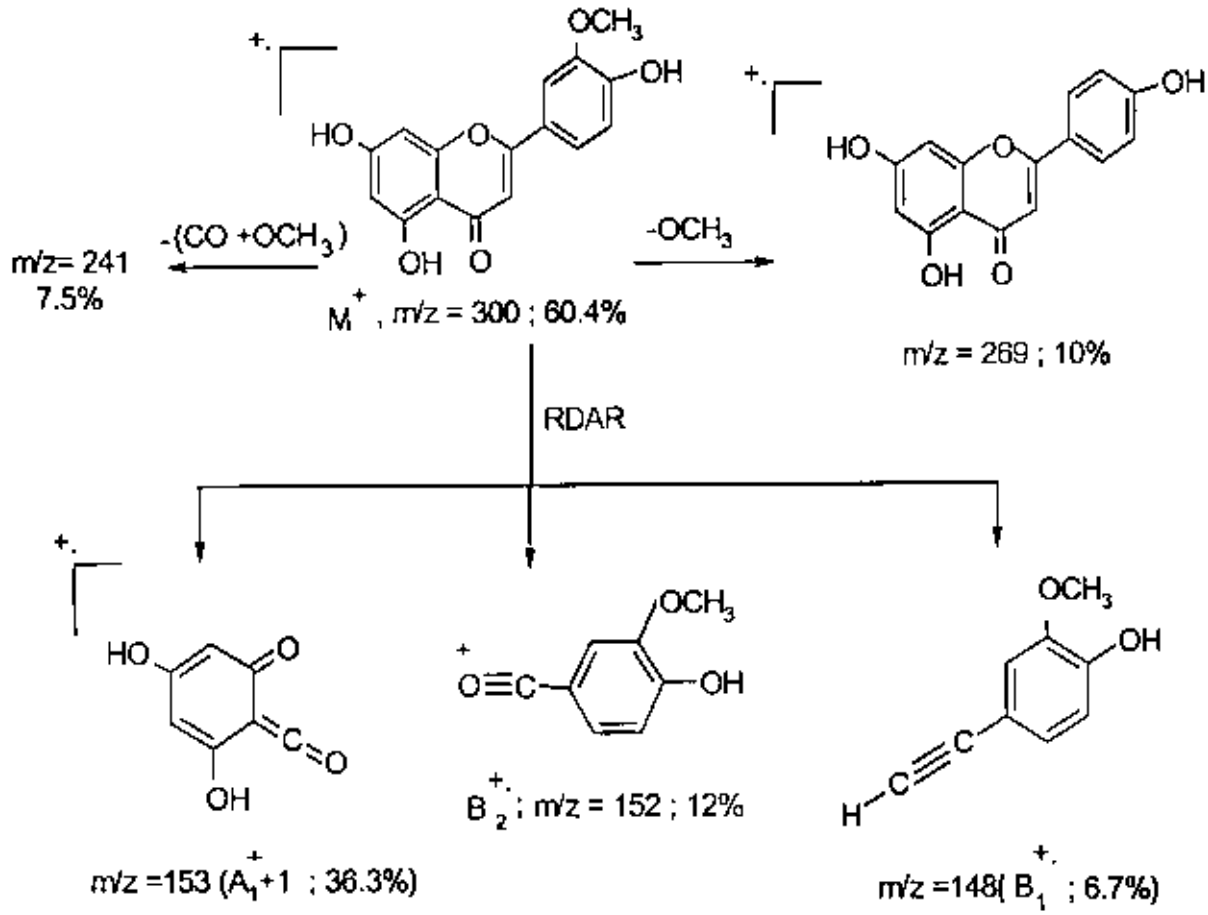


Fig. (27) : EI-mass spectrum of compound-3

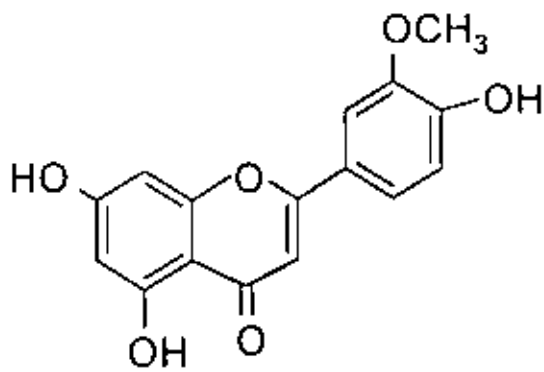
(Chrysoeriol)



Scheme (3) : Fragmentation pathway of compound-3

So, from this fragmentation pathway we can say that the methoxy group was present at C-3 in ring-B.

Finally, the chromatographic and the available spectroscopic data substantiated that compound-3 is chrysoeriol (4',5,7-trihydroxy-3'-methoxy flavone).[166]



Chrysoeriol (4',5,7-trihydroxy 3'-methoxy flavone)

Identification of compound-4 :

The UV absorption spectrum (Fig. 28, Tab. 17) in methanol gives band-I at 330 nm which prove the flavone nature of this compound. By addition of NaOMe a bathochromic shift (52 nm) in band-I with increasing in intensity was noticed which confirm the presence of free OH group at C-4'. Another free OH group at C-5 was proved through the $AlCl_3$ spectrum where there is a bathochromic shift (27 nm) in band-I was observed. No hypsochromic shift was occur in band-I in $AlCl_3$/HCl spectrum which confirm the absence of *ortho*-dihydroxy system which was confirmed through the NaOAc/H_3BO_3 spectrum. The NaOAc spectrum showed no bathochromic shift in band-II relative to methanol spectrum which prove the absence of free OH group at C-7.

Table (17): UV absorption data of compound-4

Addition to methanol	$\lambda_{max.}$ (nm)
None	274, 333.
NaOMe	272, 385.
AlCl3	263 (sh), 288, 298, 360.
AlCl3/HCl	263 (sh), 288, 298, 354.
NaOAc	371, 389.
NaOAc/H3BO3	373, 336.

The EI-mass spectrum of compound-4 (Fig. 29) showed a molecular ion peak (M^+) at m/z = 344 ; 6.3% which correspond to the molecular formula $C_{18}H_{16}O_7$. The most important fragments are at m/z = 329 (M^+- CH_3 ; 9.3%), 314 (M^+- HCHO ; 27.1%), 316 (M^+- CO ; 2.8%), 313 (M^+- OCH_3 ; 10%), 299 (M^+- (HCOH + CH_3) ; 38.3%), 298 (M^+- (OCH_3 + CH_3) ; 5.1%) and 271 (M^+- (CO + CH_3 + HCHO) ;18.6%).

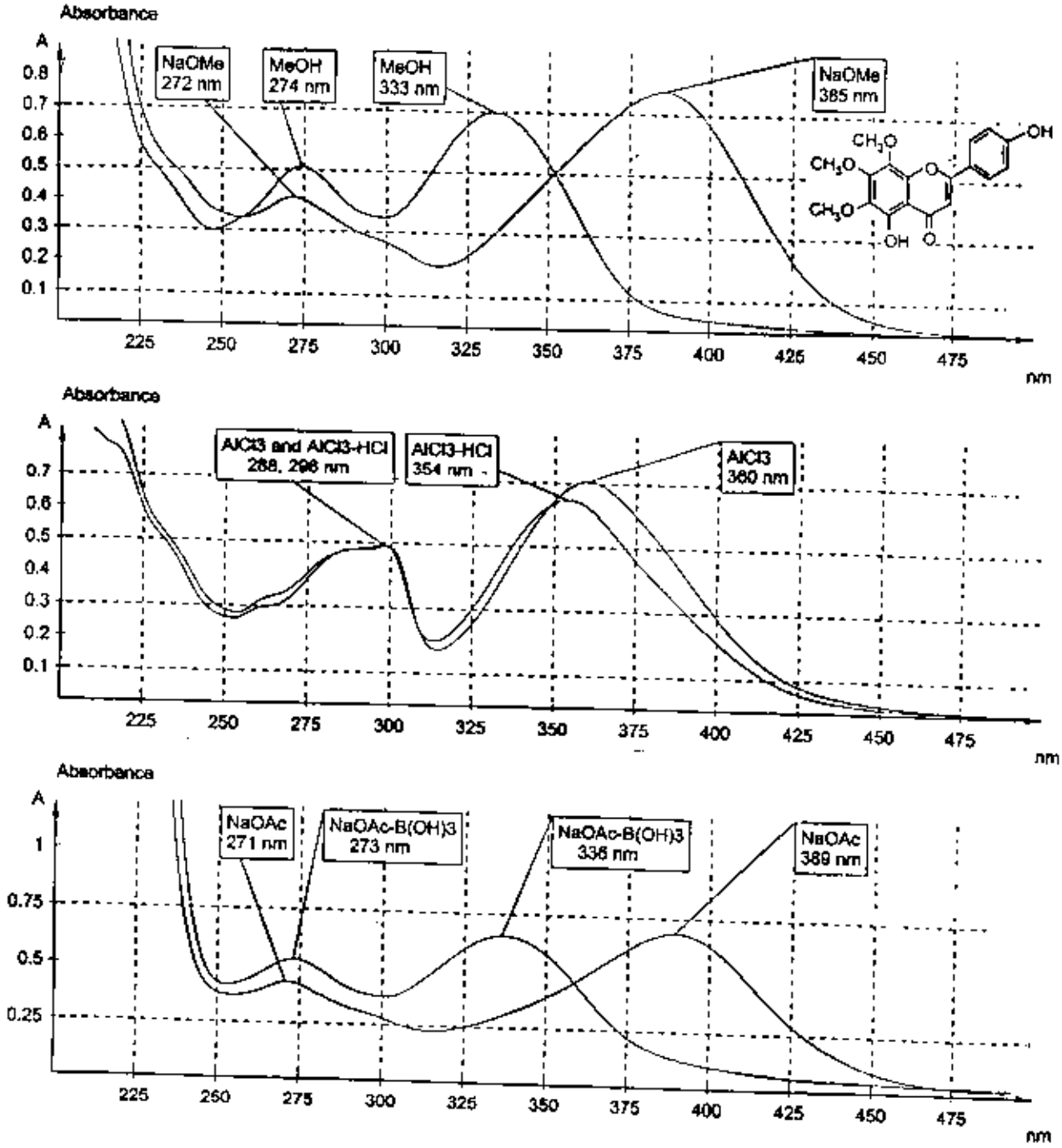


Fig. (28) : UV absorption spectra of compound-4

(Xanthomicrol)

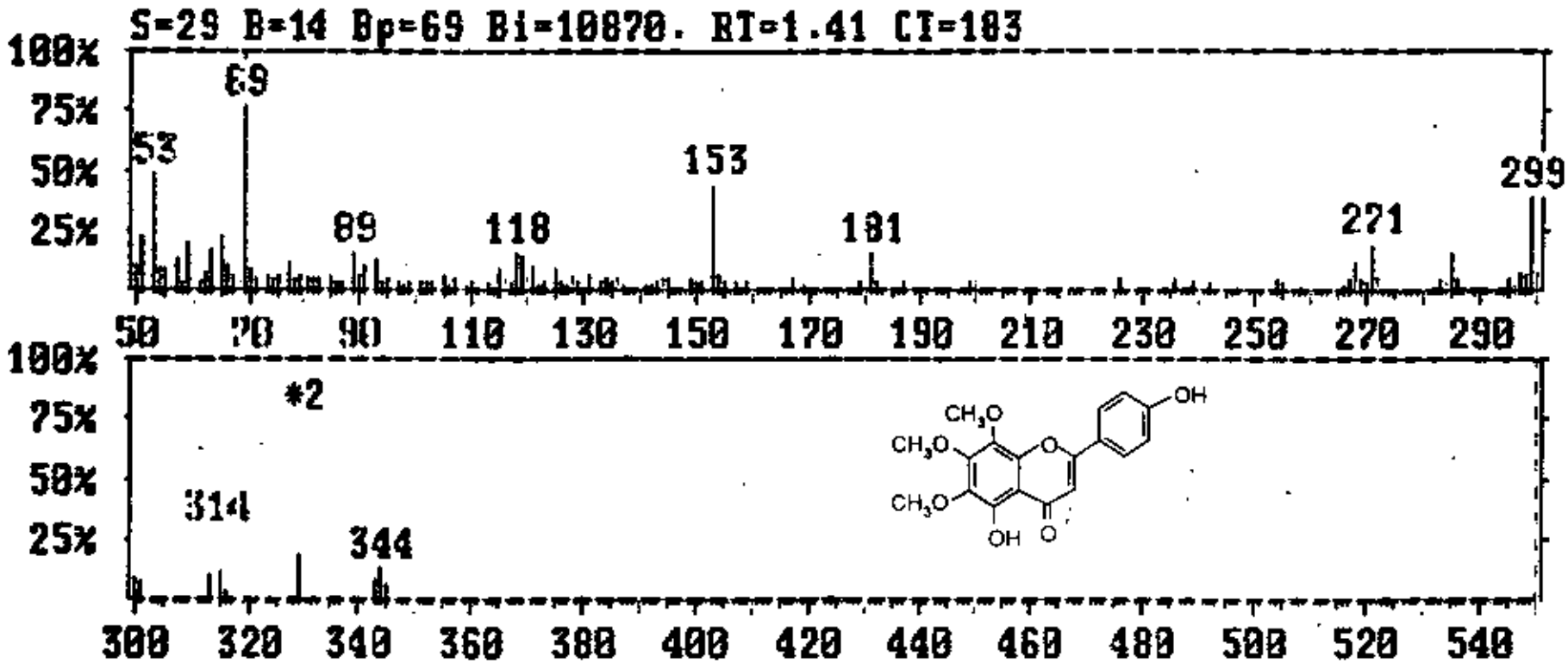
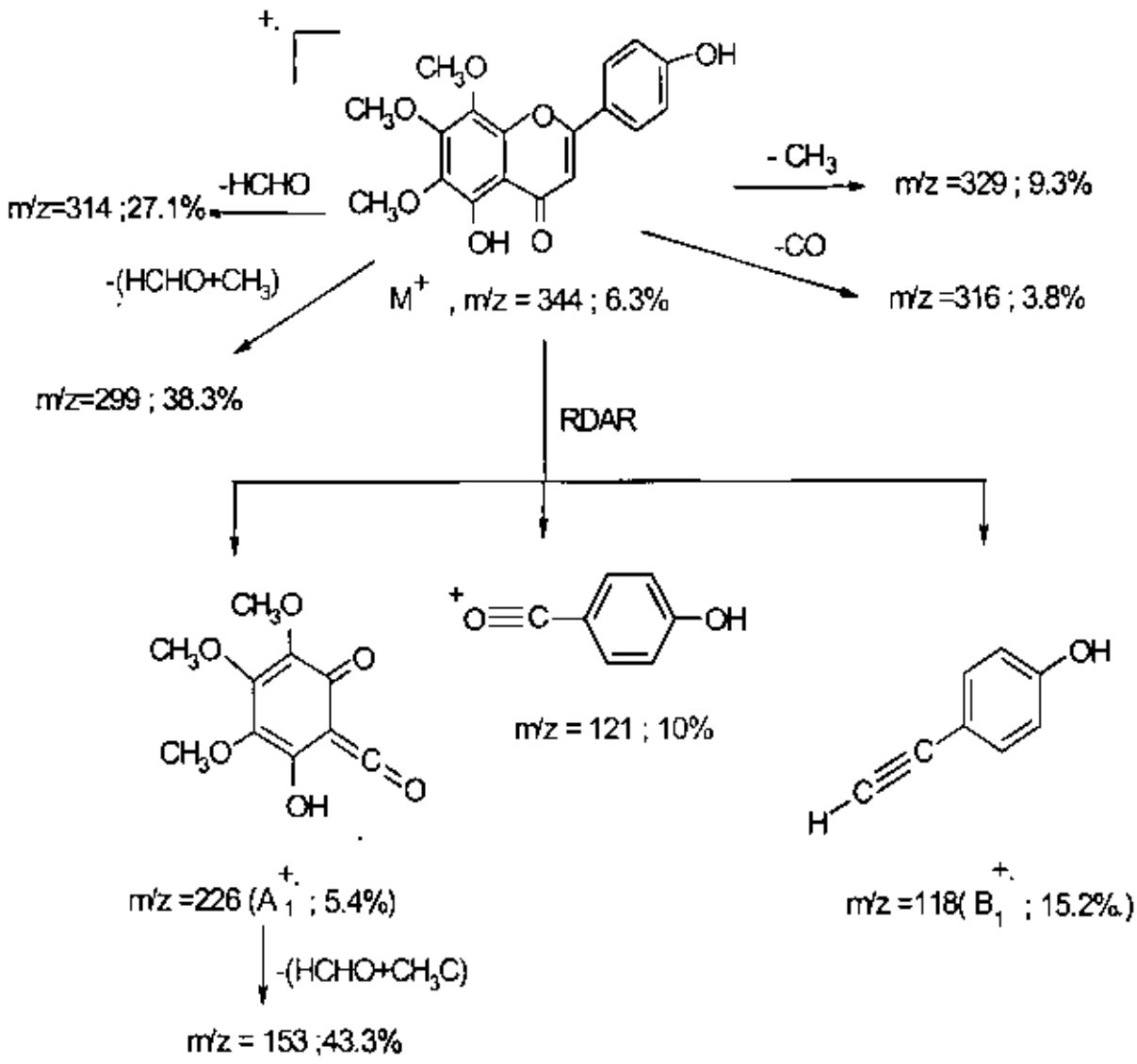


Fig. (29) : EI-mass spectrum of compound-4

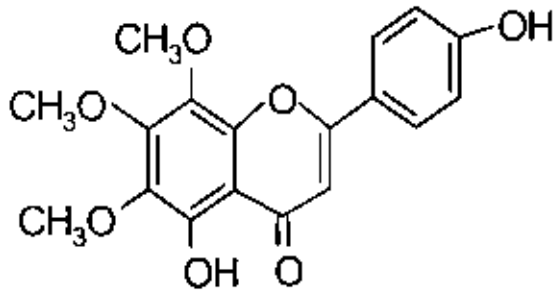
(Xanthomicrol)

The presence of a fragment at m/z = 118 ; 15.3% is indication to the B_1^{+} which means that only one OH group was present on ring-B and the other groups are at ring-A as shown in scheme (4)



Scheme (4) : Fragmentation pathway of compound-4

So, we can tentatively identify compound-**4** as Xanthomicrol (6,7,8-trimethoxy 5,4′-dihydroxy flavone).



Xanthomicrol (6,7,8-trimethoxy 5,4′-dihydroxy flavone)

Investigation of butanol extract:-

About 6.5 g of butanol fraction were subjected to preparative paper chromatography (PPC) by dissolving in about 15 ml 80% methanol and developing with 25% acetic acid. Two main zones (I, II) were localized under UV light, cut into small pieces and eluted, separately, with methanol 70%. The methanol was evaporated under reduced pressure at 45°C to afford two impure compounds (**5** and **6**).

Purification of compound-5 :

The residue obtained from zone-I (compound-**5**) was further purified by PPC, developed with BAW (4:1:5). Finally it was purified by passing through small Sephadex LH-20 column, eluted with 70% methanol to give compound-**6** in pure form (2DPC, different solvent system)

Identification of compound-5 :

The UV absorption spectrum of isolated flavonoidal compound-**5** in methanol (Fig. 30 and Tab. 18) showed band-I at 330 nm (flavone type) in addition to a bathochromic shift band-I with NaOMe from 330 nm to 399 nm without decrease in intensity which indicates the presence of a free OH group at C-4'.

The $AlCl_3$ spectrum showed a bathochromic shift in band-I (53 nm) indicating the presence of free OH group at C-3 and /or at C-5. The more intensity of band-Ia than band-Ib as well as band-IIb than band-IIa indicating the presence of free OH group at C-5 and not at C-3. Moreover the $AlCl_3$/HCl spectrum did not exhibit hypthochromic shift in band-I relative to $AlCl_3$ spectrum indicating the absence of an *ortho*-dihydroxy system in ring B. the NaOAc spectrum showed bathochromic shift (9 nm) in band-II indicating the presence of free OH group at C-7.

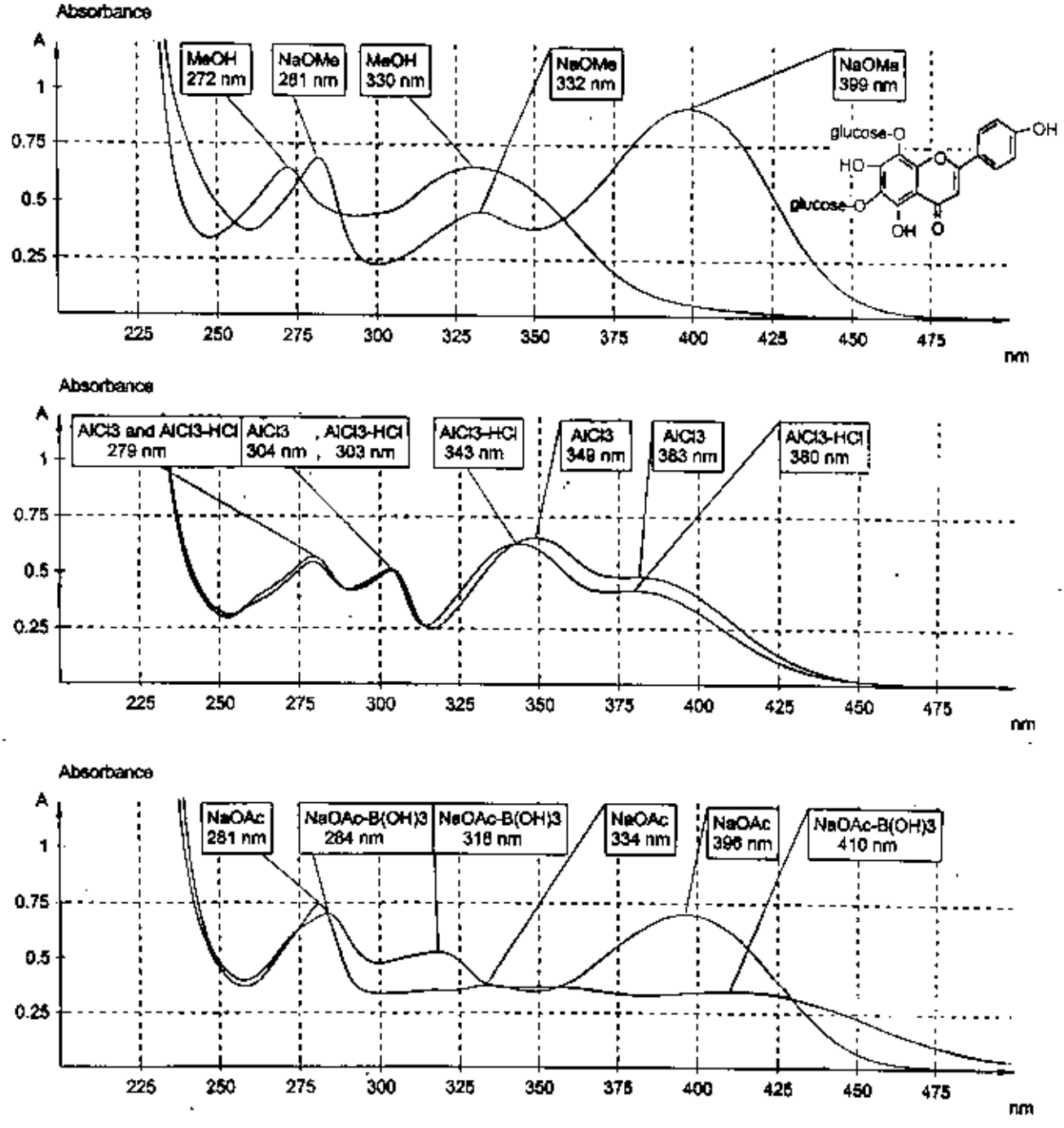


Fig. (30) : UV absorption spectra of compound-5

(Apigenin 6,8-di-*O*-glucoside)

Table (18): UV absorption data of compound-5

Addition to Methanol	λ_{max}(nm)
None	272, 330.
NaOMe	281, 332, 399.
$AlCl_3$	279, 304, 349, 383.
$AlCl_3$/HCl	279, 303, 343, 380.
NaOAc	281, 334, 396.
NaOAC/H_3BO_3	284, 318, 349, 410 (sh).

The ^{1}H-NMR spectrum (DMSO) (Fig. 31) showed signals at 8.02 (2H, d, H-2',6'), 6.91 (2H, d, H-3', H-5'), 6.79 (1H, s, H-3) and two anomeric protons for two glucose moieties at C-6, C-8, 5.1 (1H, d, H-1'') and 4.85 (1H, d, H-1''')

Acid hydrolysis :-

About 5 mg of the compound-**5** ware dissolved in 25 ml of 2N HCl : MeOH (1:1) and refluxed on boiling water bath for 2 hours. After complete hydrolysis, the solvent was evaporated and diluted with distilled water. The aglycone was extracted with ethyl acetate (3 X 50 ml). The ethyl acetate extract was washed with distilled water till free from acidity. The aglycone was obtained after evaporation of the solvent. The aglycone was further purified by passing over a small Sephadex LH-20 column, eluted with methanol.

The (+ve) FAB/MS of the aglycone of compound-**5** (Fig. 32) displayed a molecular ion peak at m/z = 271 corresponding to the molecular formula of $C_{15}H_{10}O_5$ +1 which coincided with that of apigenin. The aqueous layer after removal of the aglycone was rendered neutral with Barium carbonate,

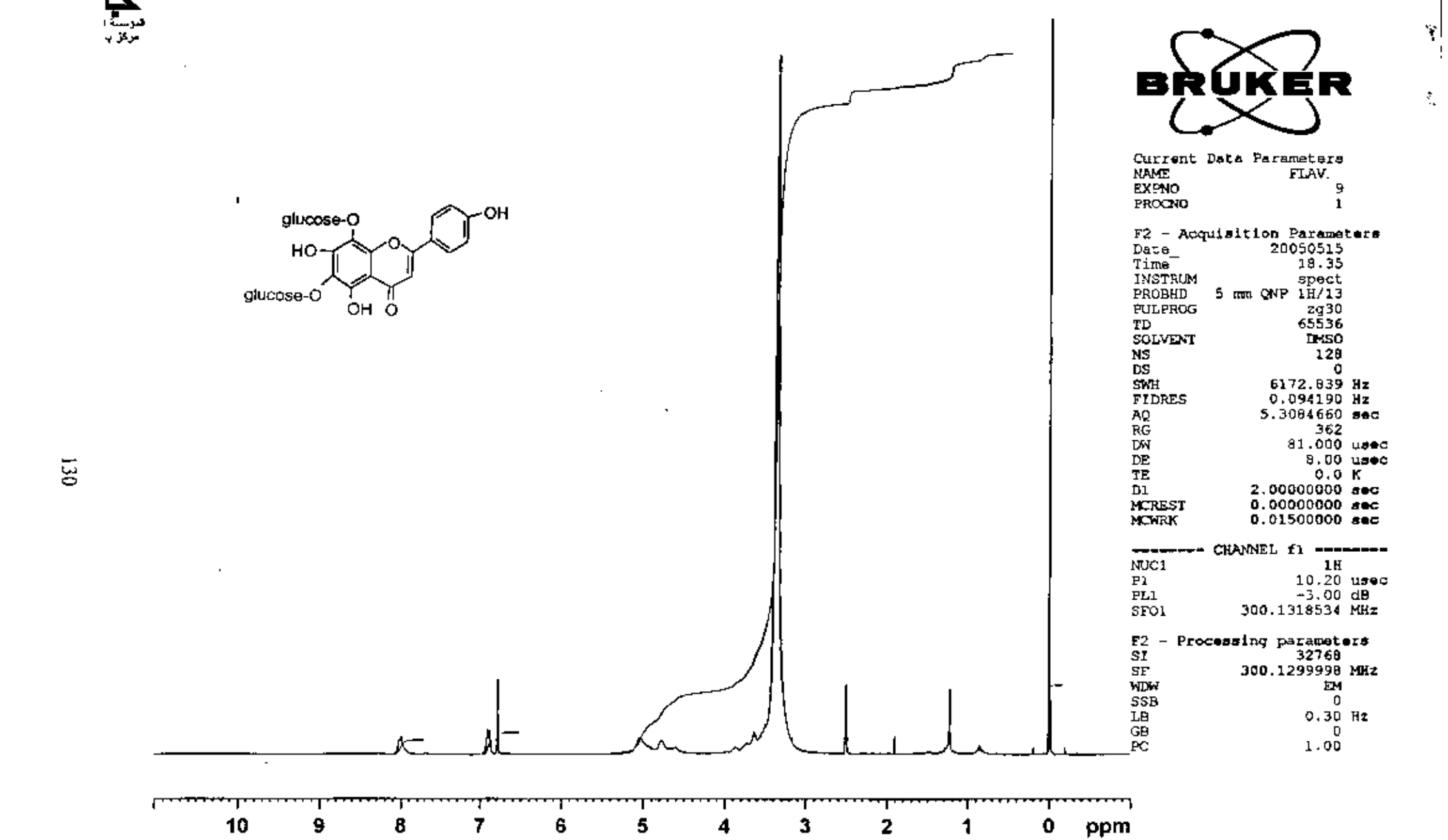


Fig. (31) : ^{1}H-NMR (DMSO) spectrum of compound-5 (Apigenin 6,8-di-*O*-glucoside)

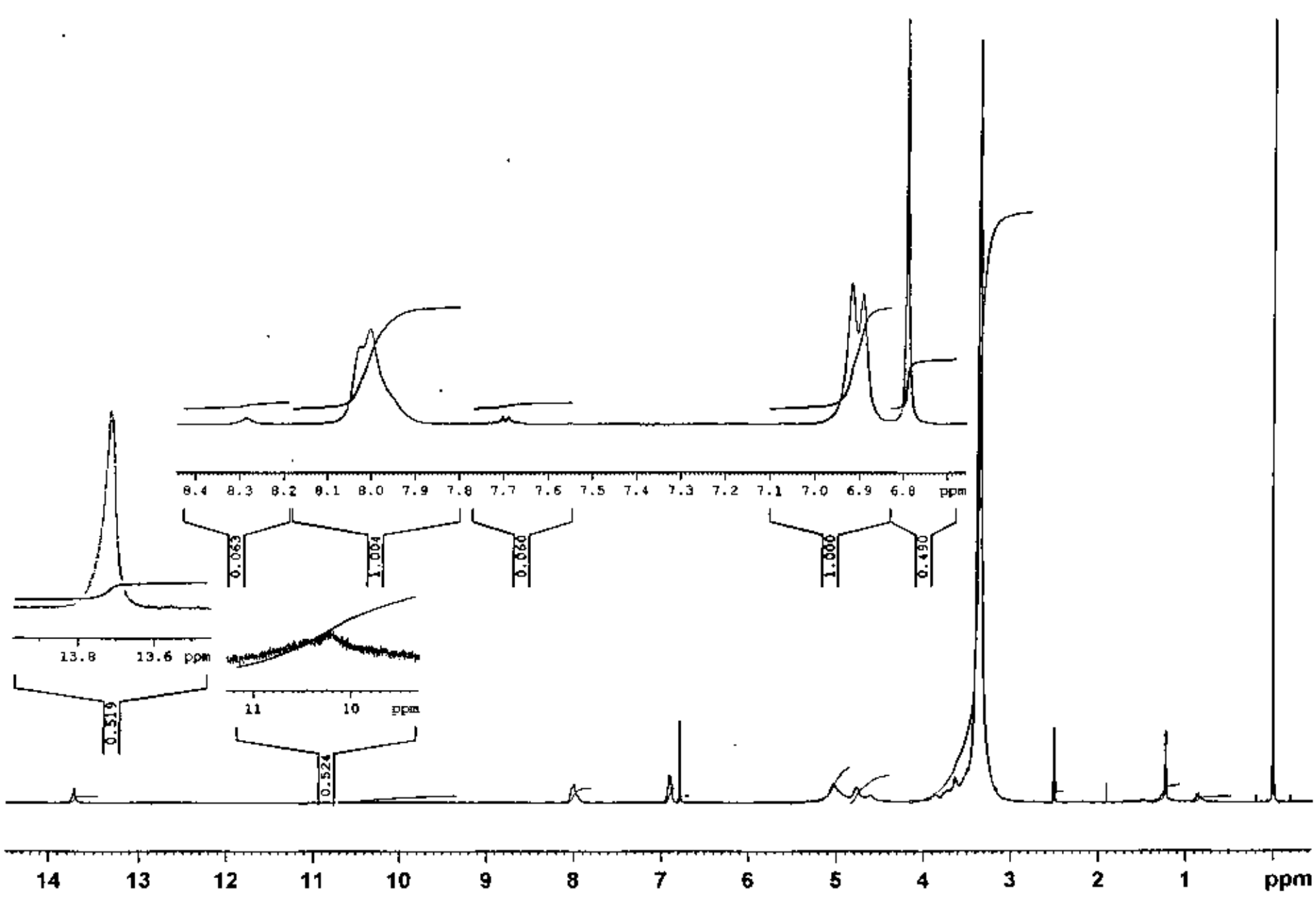

8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 ppm
0.063
1.004
0.060
1.000
0.490
13.8 13.6 ppm
0.519
11 10 ppm
0.524
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

Fig. (31) : Cont.

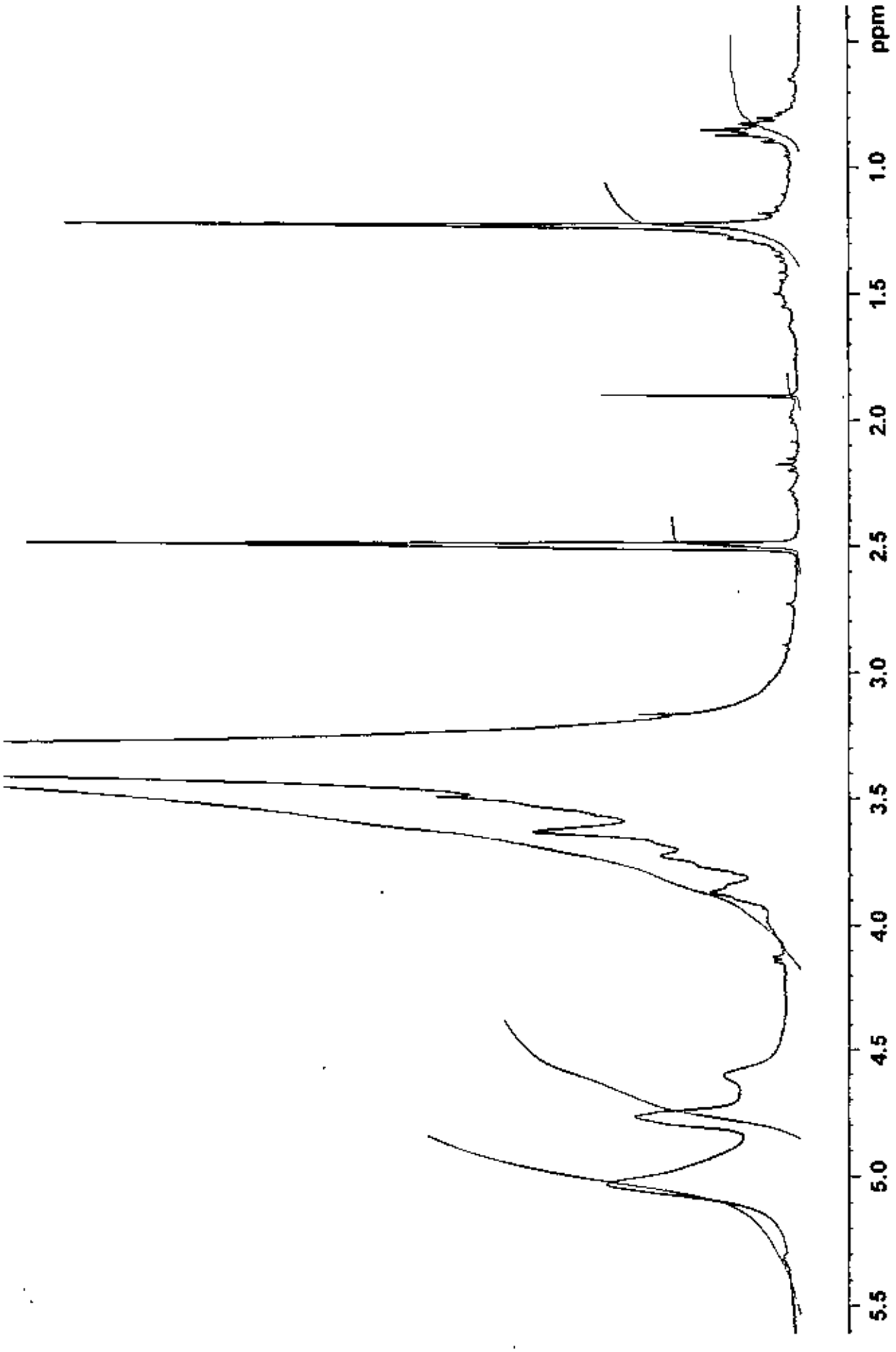


Fig. (31) : Cont.

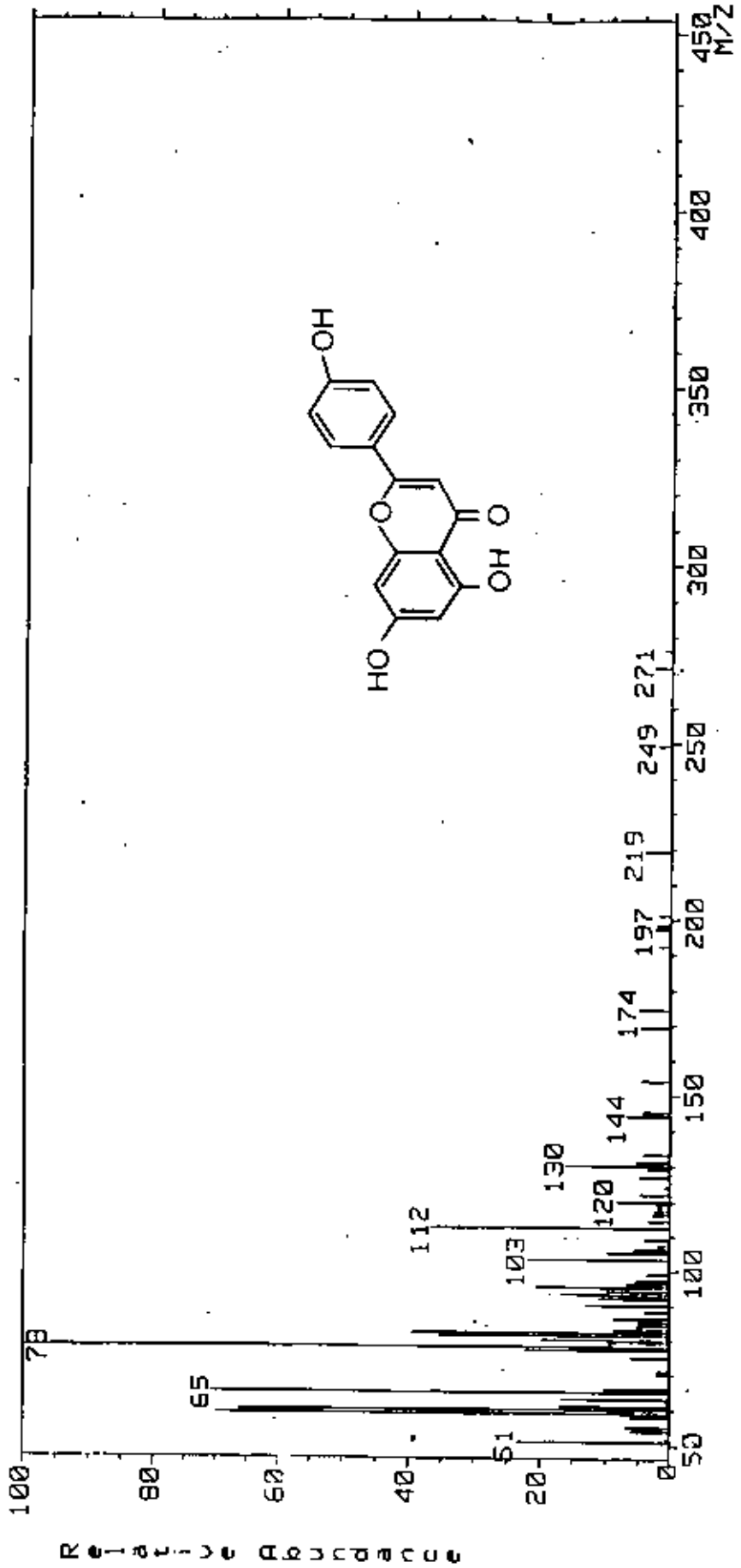
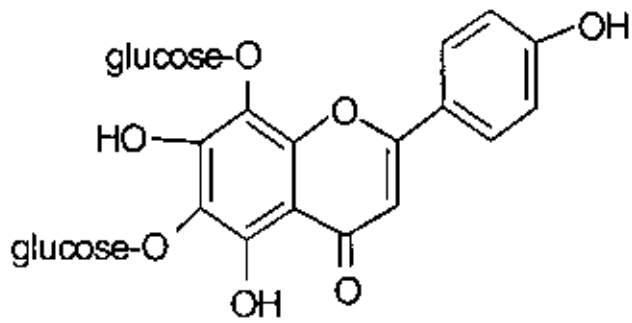


Fig. (32) : (+ve) FAB-mass spectrum of aglycone of compound-5.

filtered, evaporated and dissolved in isopropanol. The mixture of sugars was investigated by PC using Phenol saturated with water as developing solvent against some authentic sugars [165]. The chromatograms was dried and sprayed with aniline phathalate reagent and heated in an oven at 110°C for 5 min. only glucose was detected as sugar.

So, from the above chromatographic, spectroscopic data and acid hydrolysis, compound-5 can be identified as apigenin 6,8-di-*O*-glucoside.



Apigenin 6,8-di-*O*-glucoside

Purification of compound-6 :

The residue obtained from elution of zone-II was further purified using PPC developed with 20% acetic acid. The main zone was eluted as before and the obtained residue was further purified in another solvent system (B:A:W. 3:1:1). The pure compound-**6** was eluted and passed over small column of Sephadex LH-20 column eluted with methanol (70 %).

Identification of compound-6 :

The behaviors of compound-**6** in different solvents indicate it is highly glycosidic compound.

The UV spectra of compound-**6** showed band-I in methanol (Fig. 33 Tab. 19) at 344 nm which proves the flavone nature of this compound.

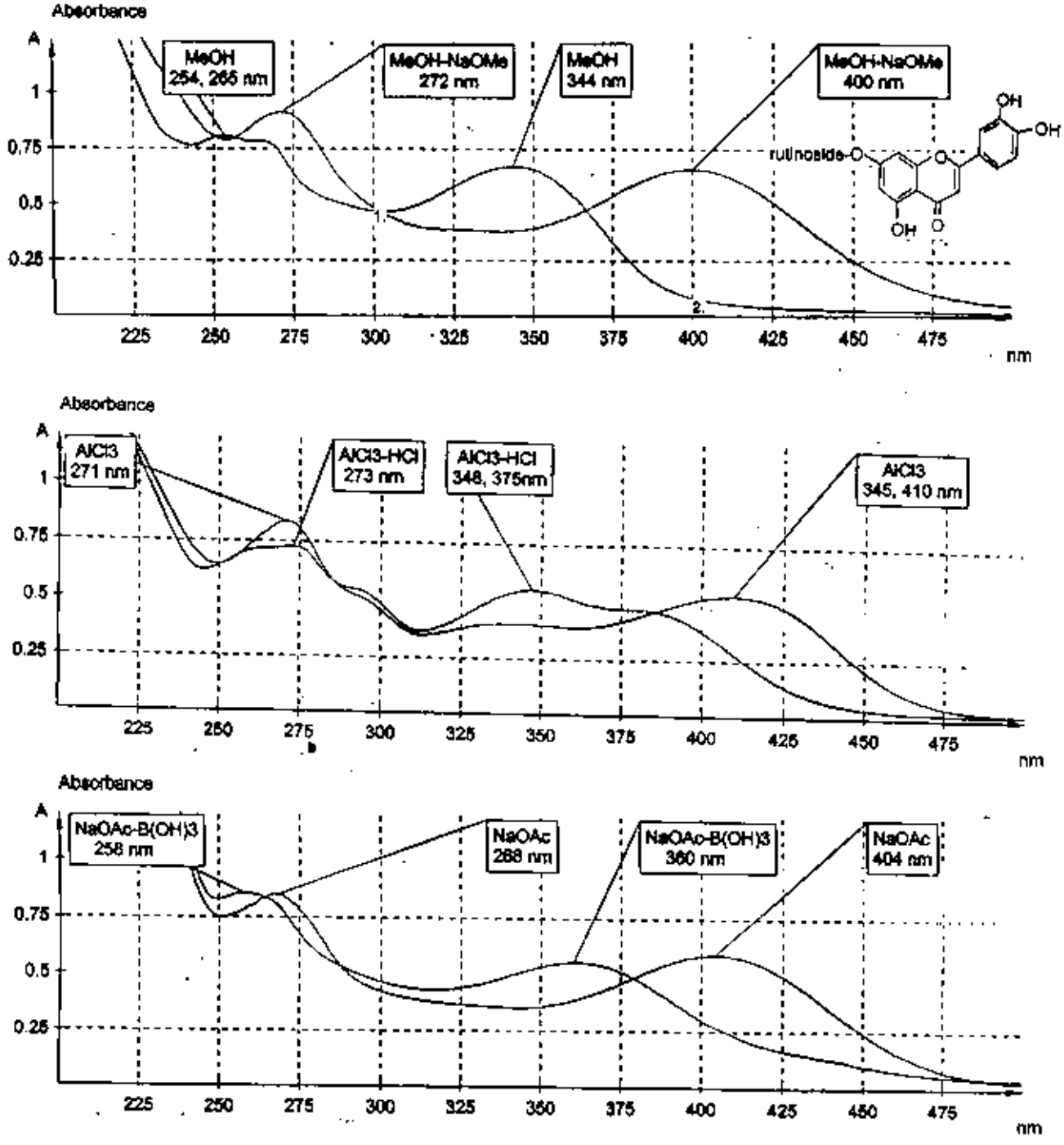


Fig. (33) : UV absorption spectra of compound-6

(Luteolin 7-*O*-rutinoside)

A bathochromic shift (56 nm) in band-I was noticed upon addition of NaOMe without decreas in intensity indicates the presence of a free OH group at C-4'.

The presence of an *ortho*-dihydroxy system was proved where there is a hypsochromic shift (35 nm) in band-I of $AlCl_3$ spectrum relative to $AlCl_3$/HCl spectrum, also there is a bathochromic shift (16 nm) in band-I of NaOAc/H_3BO_3 spectrum relative to methanol spectrum.

The absence of free OH group at C-7 was confirmed where there is no bathochromic shift in band-II of NaOAc spectrum.

Table (19): UV absorption data of compound-6

Addition to Methanol	$\lambda_{max.}$ (nm)
None	254, 265, 344.
NaOMe	272, 400.
$AlCl_3$	271, 345, 410.
$AlCl_3$/HCl	273, 348, 375, 361 (sh).
NaOAc	268, 404.
NaOAC/H_3BO_3	258, 360.

The ^{1}H-NMR spectrum of compound-6 (DMSO) (Fig. 34) showed signal at δ in ppm at 7.45 (2 H, d, H-2', H-6'), 6.95 (1 H, d, H-5'), 6.77 (1 H, d, H-8), 6.73 (1 H, s, H-3), 6.4 (1 H, d, H-6) in addition to two anomeric protons for two sugars at 5.1 (1 H, d, H-1'' for glucose), 4.55 (1 H, s, H-1''' for rhamnose) and the methyl protons of the rhamnose moiety at 1.07 (3 H, d, CH_3 protons). These data were in accordance with that reported for luteolin 7-*O*-rutinoside. [166]

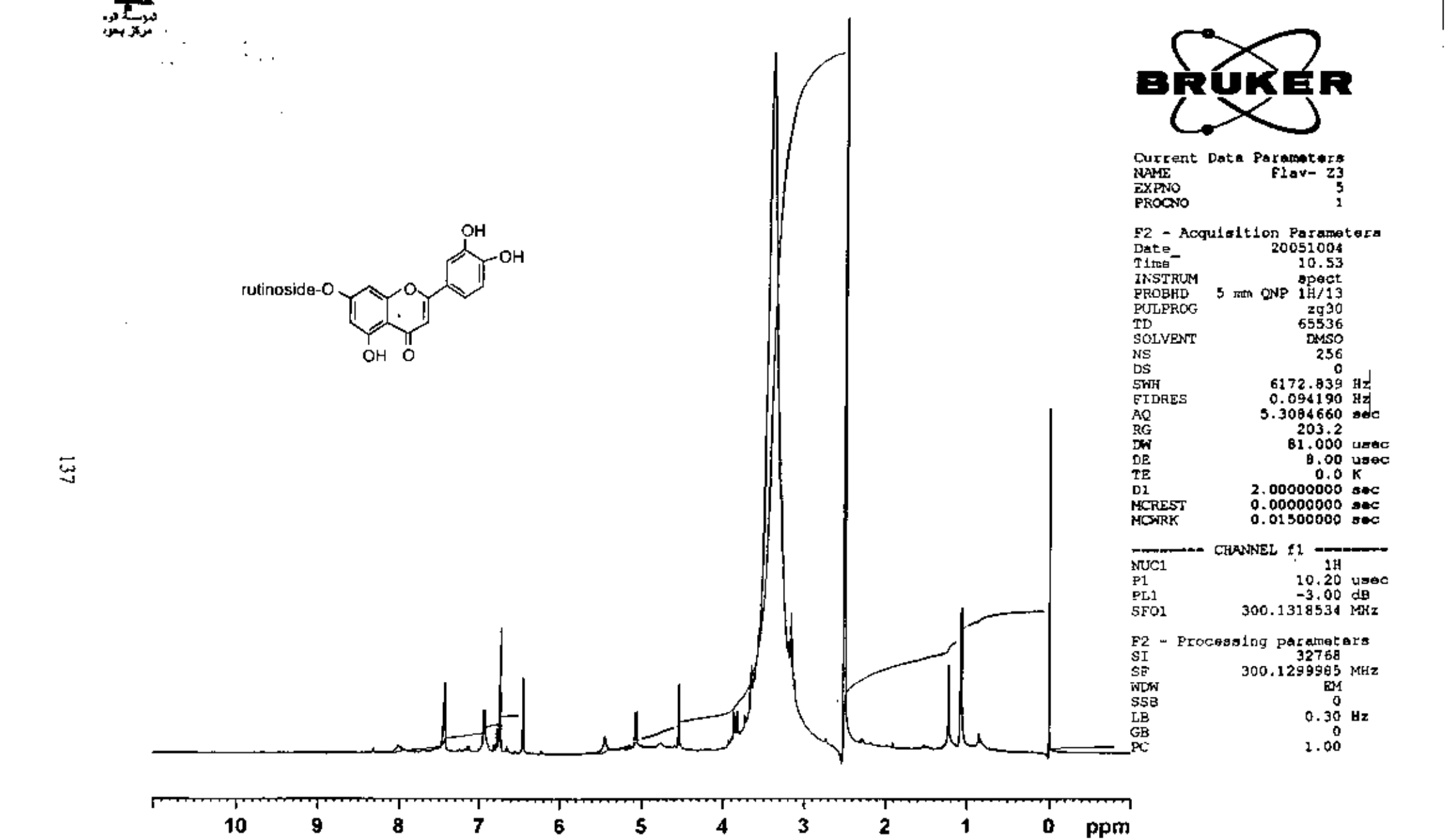


Fig. (34) : ^{1}H-NMR (DMSO) spectrum of compound-6 (Luteolin 7-*O*-rutinoside)

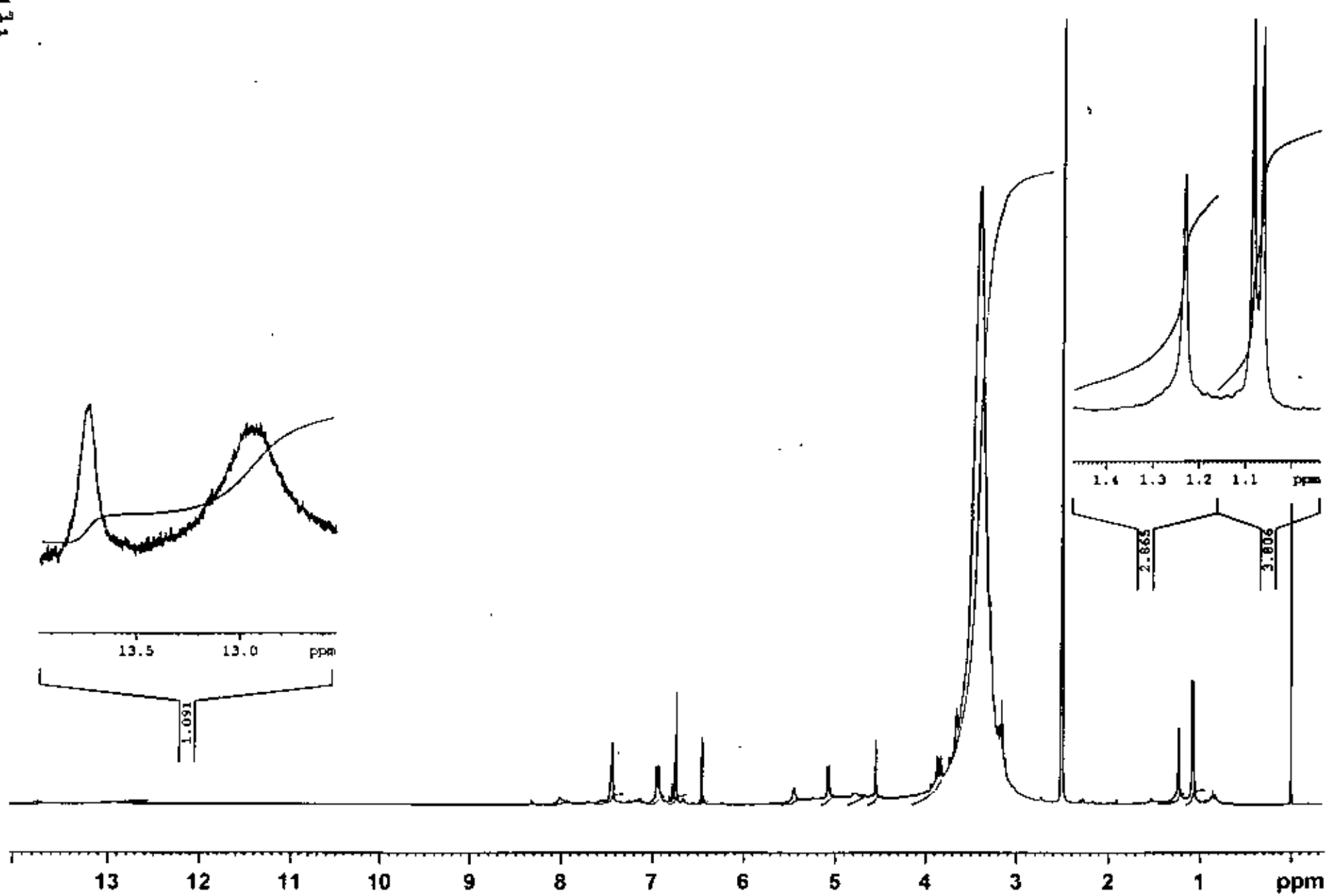

13.5
13.0
ppm
1.091
1.4
1.3
1.2
1.1
ppm
2.865
3.806
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

Fig. (34) : Cont.

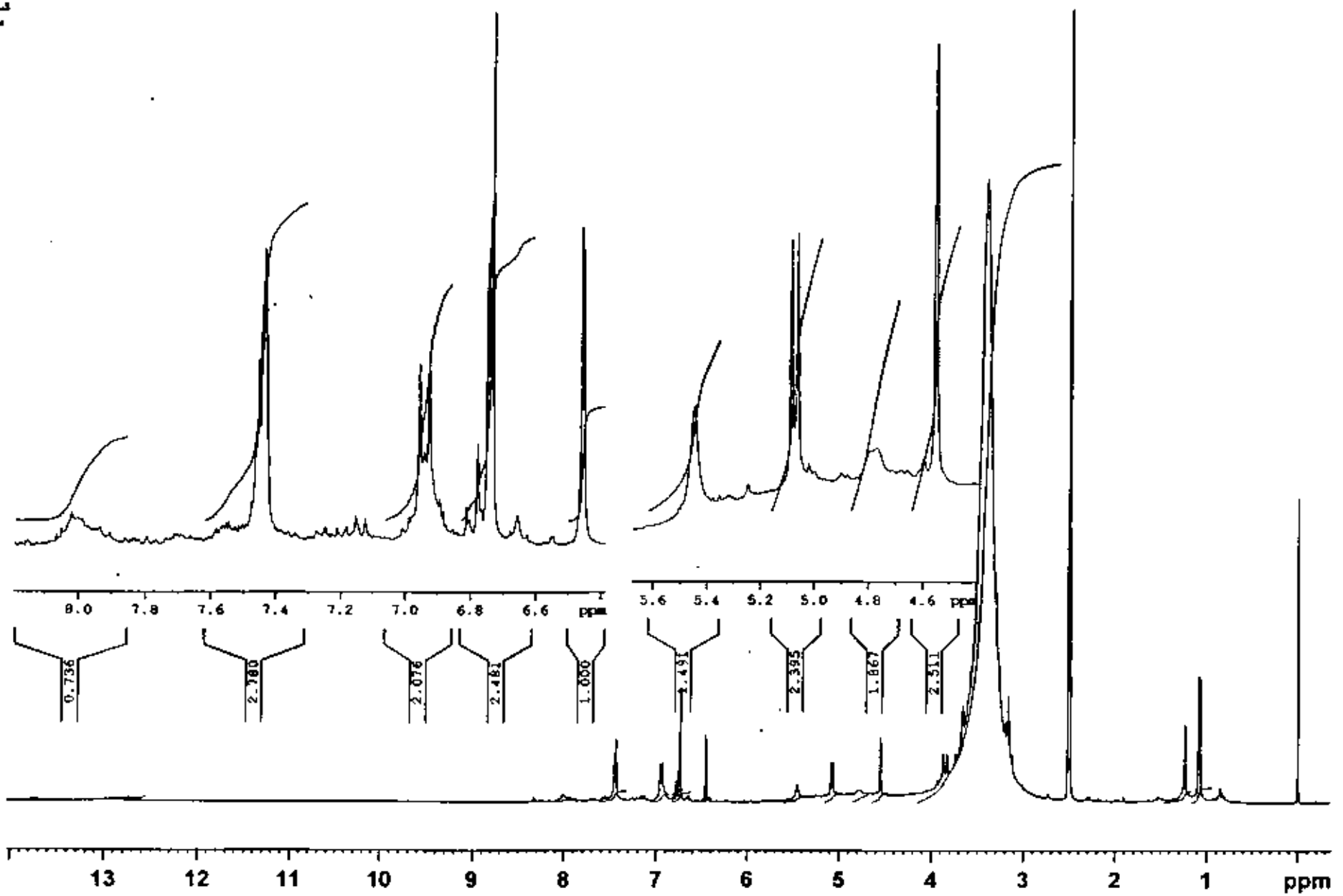

8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
ppm
0.736
2.780
2.076
2.482
1.000
5.6
5.4
5.2
5.0
4.8
4.6
ppm
2.491
2.395
1.867
2.511
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

Fig. (34) : Cont.

The ^{13}C-NMR spectrum (DMSO) of compound-**6** (Fig. 35) showed the most characteristic signals of flavone diglycoside like C-4 at δ = 181.76, C-1″ at 99.83, C-1‴ at 100.41 and C-6‴ of CH_3 group of rhamnose at 17.68. The down filed shift of C-6″ (65.94) and C-1‴ (100.41) indicates the two sugars are rutinoside i.e. gluco-(6 → 1) rhamnoside [227] and in accorda-nce with those of luteolin-7-*O*-rutinoside. The other data of ^{13}C-NMR were found in table (20) [166].

Table (20): ^{13}C-NMR data of compound-6

Carbon No.	δ (ppm)	Carbon No.	δ (ppm)
2	162.78	7-O-glucose	-
3	102.98	1″	99.83
4	181.76	2″	73.01
5	161.12	3″	76.20
6	99.42	4″	69.50
7	164.52	5″	75.47
8	94.69	6″	65.94
9	156.81	7-O-rhamnose	-
10	105.28	1‴	100.41
1'	121.14	2‴	70.19
2'	113.49	3‴	70.67
3'	145.74	4‴	71.97
4'	150.02	5‴	68.21
5'	116.07	6‴	17.68
6'	119.07		

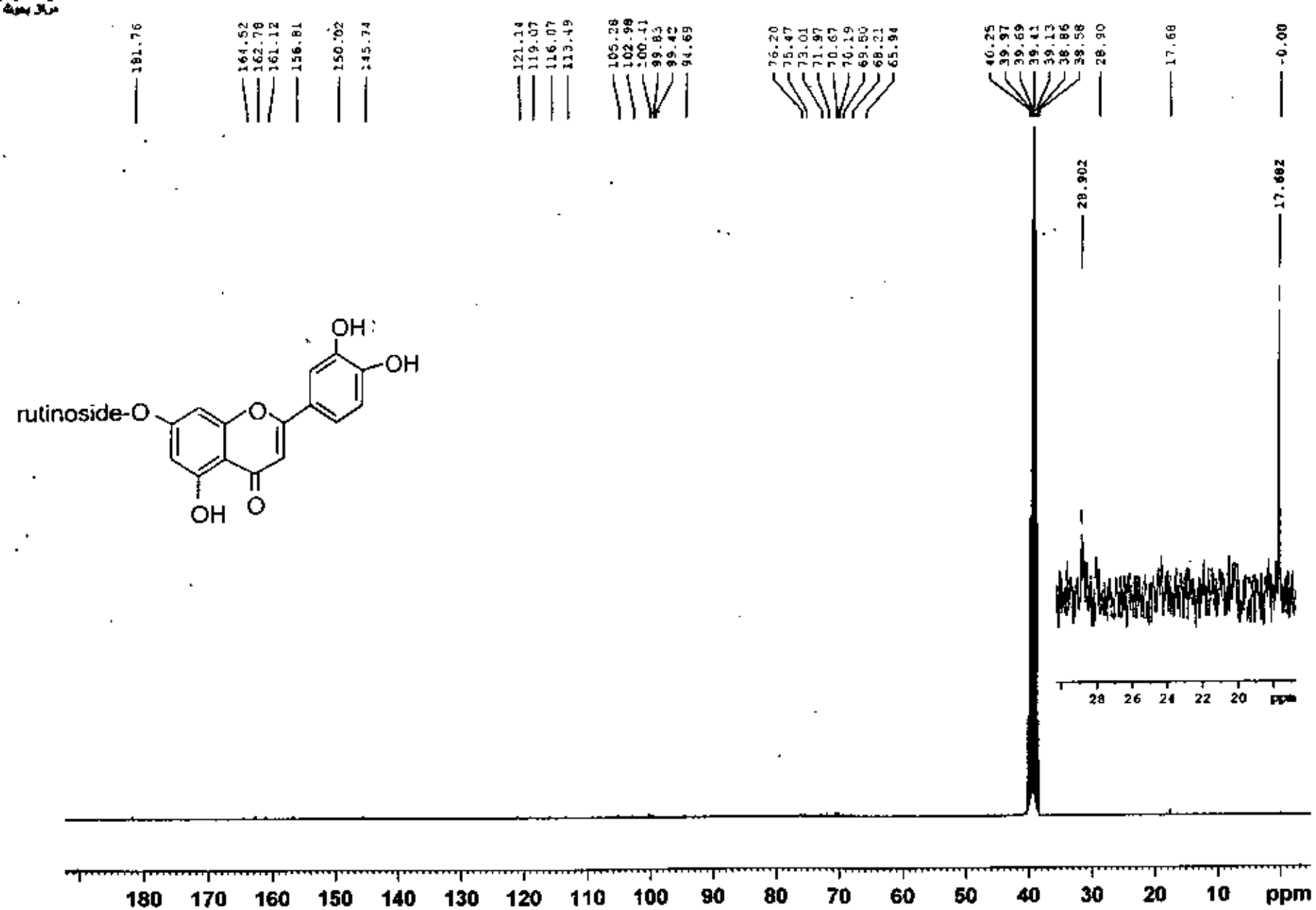


Fig. (35) : ^{13}C-NMR (DMSO) spectrum of compound-6 (Luteolin 7-*O*-rutinoside).

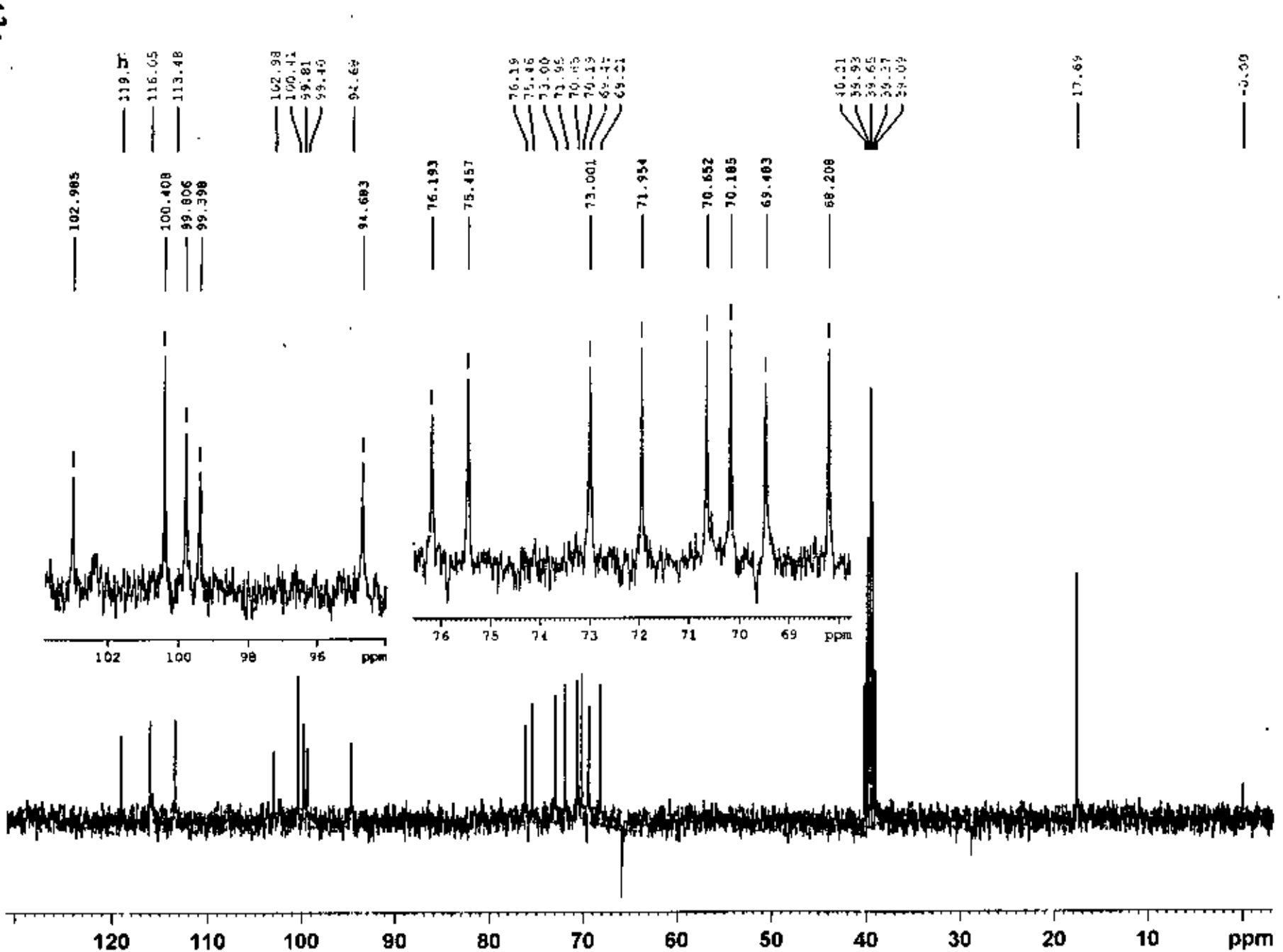

102.985
100.408
99.806
99.398
94.683
76.193
75.457
73.001
71.954
70.652
70.185
69.403
68.208
17.69
-0.39
102
100
98
96
ppm
76
75
74
73
72
71
70
69
ppm
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Fig. (35) : Cont.

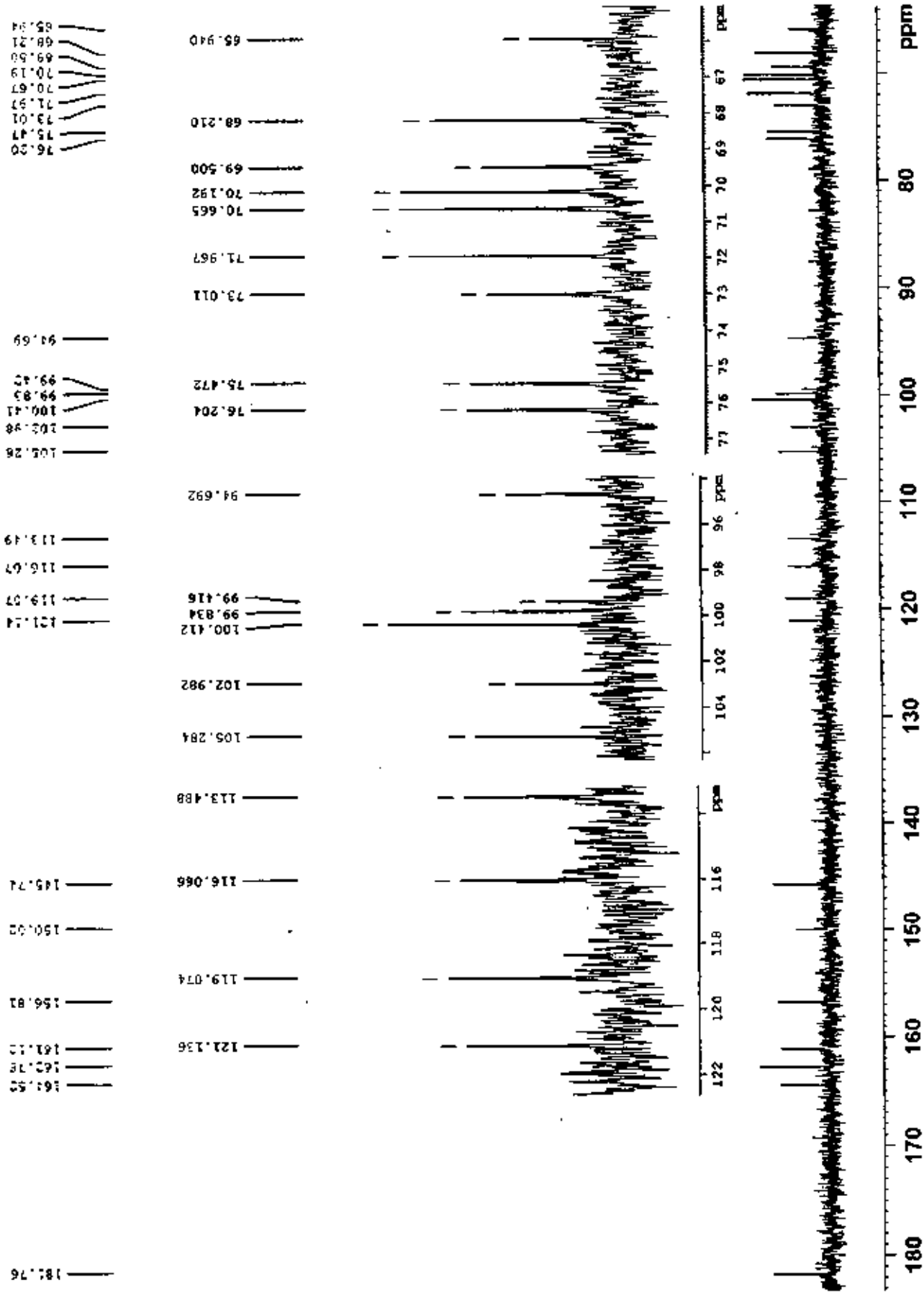

65.940
68.210
69.500
70.192
70.665
71.967
73.011
75.472
76.204
94.692
99.416
99.834
100.412
102.982
105.284
113.488
116.066
119.074
121.136
65.94
68.21
69.50
70.19
70.67
71.97
73.01
75.47
76.20
91.69
99.42
99.85
100.41
105.26
113.49
116.07
119.07
121.14
145.74
150.02
156.81
161.12
162.78
163.50
182.76
ppm
ppm
ppm

Fig. (35) : Cont.

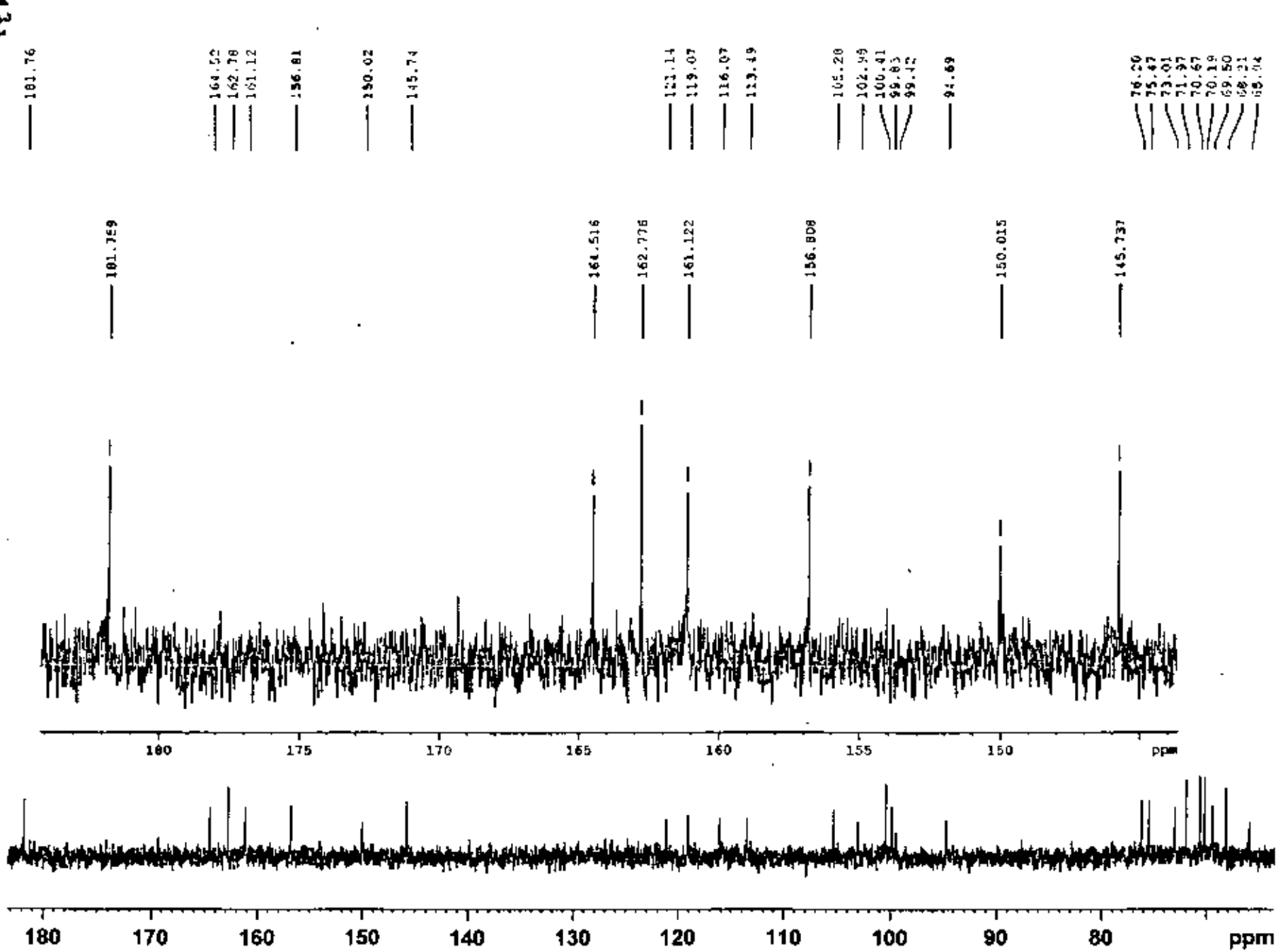
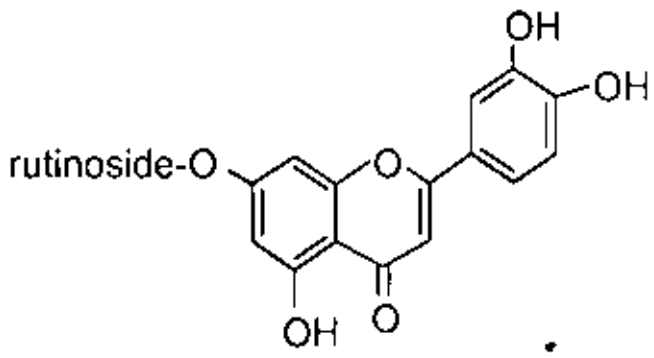

181.76
164.52
162.78
161.12
156.81
150.02
145.74
123.14
119.07
116.07
113.49
105.20
102.95
100.41
99.83
99.42
94.69
76.20
75.47
73.01
71.97
70.67
70.19
69.50
68.21
65.74
181.759
164.516
162.776
161.122
156.808
150.015
145.737
180
175
170
165
160
155
150
ppm
180
170
160
150
140
130
120
110
100
90
80
ppm

Fig. (35) : Cont.

Acid hydrolysis :-

About 5 g of compound-6 were subjected to acid hydrolysis as in page 129. Only glucose and rhamnose were detected as sugars and luteolin as an aglycone.

The position of the attachment of these sugars to the aglycone was confirmed at C-7 where the UV spectra of the aglycone (page 135) showed a bathochromic shift in band-II in NaOAc spectrum relative to methanol spectrum. Also the identity of luteolin was confirmed by the +ve FAB/MS (Fig. 36), where it displayed a molecular ion peak at m/z = 287. From all the above chromatographic and spectroscopic data, we can identify compound-6 as Luteolin-7-*O*-rutinoside.



Luteolin-7-*O*-rutinoside

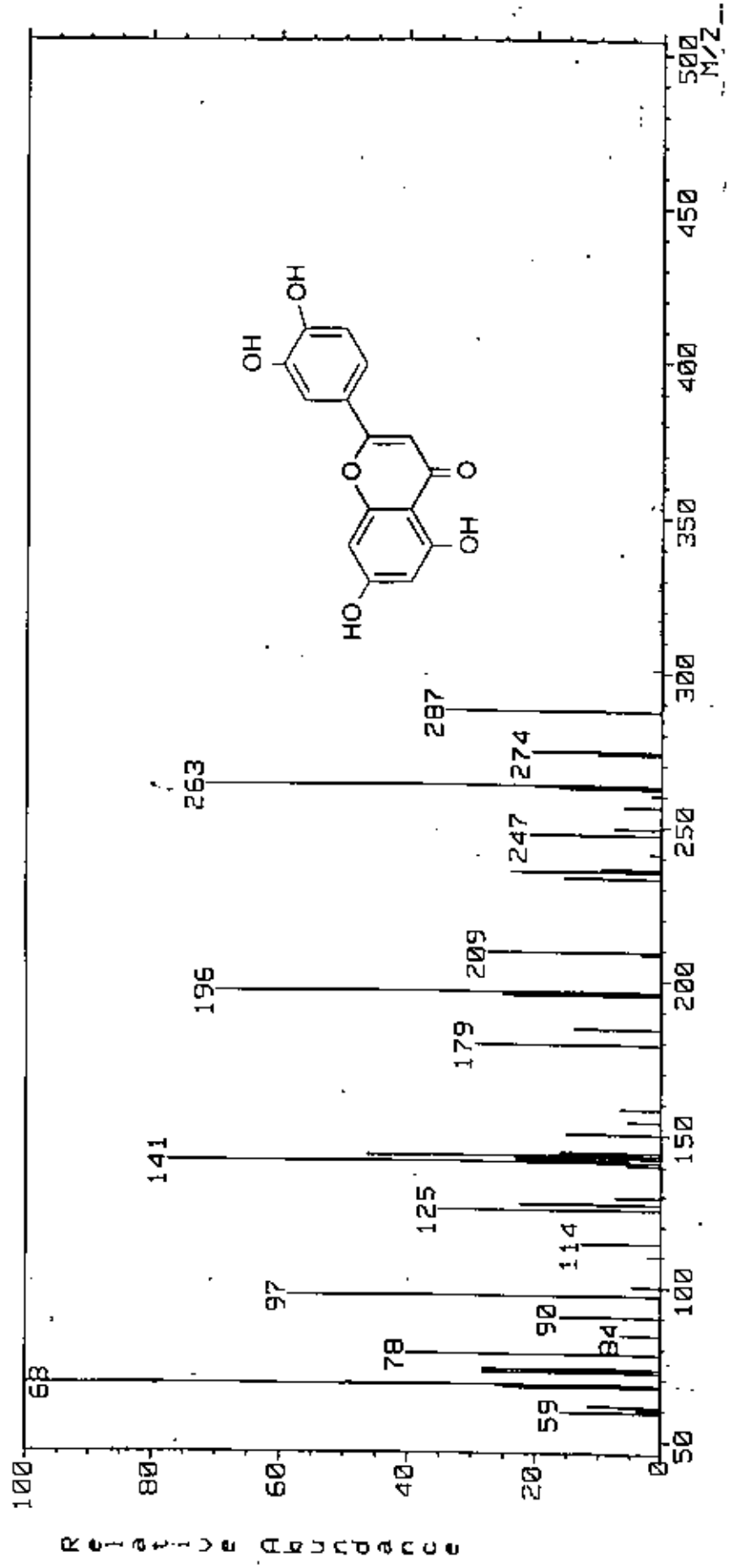


Fig. (36) : (+ve) FAB-mass spectrum of aglycone of compound-6

BIOLOGICAL ACTIVITY

ANTIOXIDANT ACTIVITY

1.1- Introduction

Oxidation is the transfer of electrons from one atom to another and represents an essential part of aerobic life and our metabolism, since oxygen is ultimate electron acceptor in the electron flow system that produces energy in the form ATP. However, problem may arise when the electron flow becomes uncoupled (transfer of unpaired single electrons), generating free radicals. Examples of oxygen-centered free radicals, known as reactive oxygen species (ROS), include superoxide ($O_2^{\bullet}$), peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroxyl ($HO^{\bullet}$), and nitric oxide ($NO^{\bullet}$).

The hydroxyl (half-life of 10^{-9} s) and the alkoxyl (half life of seconds) free radicals are very reactive and rapidly attack the molecules in nearby cells, and probably the damage caused by them is unavoidable and dealt with by repair processes. On the other hand, the superoxide anion, lipid hydroperoxides, and nitric oxide are less reactive. In addition to these ROS, nonradicals, such as the singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and hypochlorous acide (HOCl).

It is accepted that ROS play different roles *in vivo*. Some are positive and are related to their involvement in energy production, phagocytosis, regulation of cell growth and intercellular signaling and synthesis of biologically important compounds. However, ROS may be very damaging, since they can attack lipids in cell membranes, and DNA, to induce oxidations, which cause membrane damage, protein modification (including enzymes) and DNA damage. This oxidative damage is considered to play a causative role in aging and several degenerative diseases associated with it, such as heart disease, cataracts, cognitive dysfunction, and cancer.

Humans have evolved with antioxidant systems to protect against free radicals. These systems include some antioxidants produced in the body

(endogenous). The first include (a) enzymatic defenses, such as Se-glutathione peroxidase, catalase, and superoxide dismutase, which metabolize superoxide, hydrogen peroxide, and lipid peroxides, thus preventing most of the formation of the toxic $HO^{\bullet}$, and (b) nonenzymatic defenses. such as glutathione, histidine-peptides, the iron-binding proteins transferrin and ferritin, dihydrolipoic acid, reduced CoQ10, melatonin, urate, and plasma protein thiols, with the last two accounting for the major contribution to the radical-trapping capacity of plasma [228].

Flavonids have shown potential health benefits arising from the antioxidative effects of these phtochemicals, whose properties are attributed to the phenolic hydroxyl groups attached to the flavonoid structure. Scavenging of free radicals seems to play a considerable part in the antioxidant activity of flavonoids as potent free radical scavengers have attracted a tremendous interest as possible therapeutics against free radical mediated diseases. In general, the radical scavenging activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups, i.e., on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radicals via hydrogen bonding or by expanded electron delocalization. Previous structure-activity relationship (SAR) studies of flavonoids have pointed to the importance of the number and location of the phenolic OH groups present for the antiradical efficiency. The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3',4'-dihydroxy, i.e., an orthodihydroxy group (catechol structure) in the ring-**B**, possessing electron donating properties and being a radical target. Also, the 3-OH moiety of the ring-**C** is also beneficial for the antioxidant activity of flavonoids. The C2-C3 double bond conjugated with a 4-keto group, which is responsible for electron delocalization from the ring-**B**, enhances further the radical scavenging capacity, and saturation of the 2, 3-double bond is

believed to cause a loss of activity potential. Also the presence of both 3-OH and 5-OH groups in combination with a 4- carbonyl function and C2-C3 double bond increases the radical scavenging activity. In the absence of the orthodihydroxy structure in ring-**B**, hydroxyl substituents in a catechol structure on the ring-**A** were able to compensate and become a larger determinate of flavonoid antiradical activity (Fig. 37) summarizes the structural criteria that modulate the free radical scavenging activity of flavovoids. In summary, these structural features contribute to the increase of the phenoxyl radical stability, i.e., the radical scavenging activity of the parent flavonoid [229].

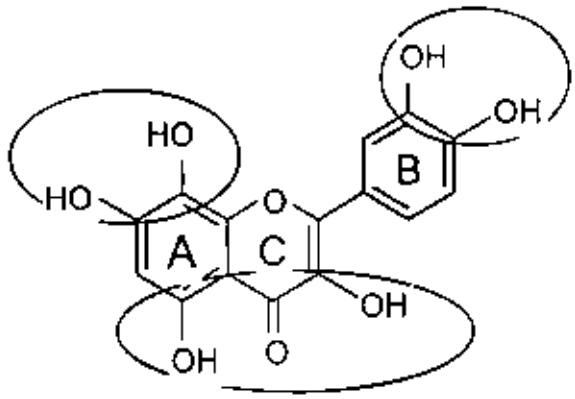


Fig (37) : Structural features of flavonoids with a high radical scavenging activity.

1.2- Principle :

The Radical Scavenging Activity (RSA) of the prepared plant extracts was tested using a methanolic solution of the stable free radical, 1,1-DiPhenyl Picryl Hydrazyl (DPPH). Unlike laboratory-generated free radicals such as the hydroxyl radical and super oxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal-ion chelation and enzyme inhibition, brought about by various additives [230].

DPPH has been widely used to test the scavenging ability of flavonoids. The scavenging of DPPH by flavonoid (free radical scavenger) can be represented as depicted in fig.(38) [229].

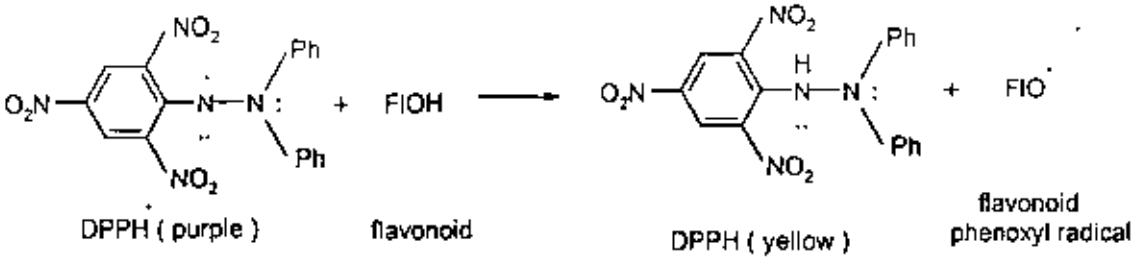


Fig. (38) : Scavenging of DPPH (free radical) by a flavonoid (free radical scavenger).

A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. The purple colour generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at the 517nm band [231] hence, the more rapidly the absorbance decrease, the more potent the antioxidant capacity of the extract in terms of hydrogen atom-donating capacity.

The DPPH test is a commonly employed assay in antioxidant studies and offers a rapid technique in which to screen the RSA of pure synthetic compounds, isolated natural compounds, crude plant extracts and foods [230].

2- Material and method :

2.1- preparation of plant extracts :

About 100 g of the aerial parts of the plant were dried in the shade and grinded. The petroleum ether extract was obtained by continuously extraction with petroleum ether (40-60°C) in soxhleth apparatus, the resulting extract was fractionated as before (*c.f.* page 82).

The defatted plant material was extracted at room temperature with 70% methanol, the extract (total alcoholic extract) was then partitioned with chloroform, ethyl acetate and butanol respectively.

The aqueous extract was prepared by maceration of 50 g of the plant material with water for 24 hr. The water was evaporated in *vacuo* at 50°C.

3- Assay :

Three hundreds microlitres of fixed concentrations of the extracts in methanol was added to 3 ml of a methanol solution of DPPH (20mg/L). After 30 minutes incubation period at room temperature the absorbance was read against a blank at 520 nm. Inhibition of free radical DPPH in percent (I%) was calculated in following way :

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the extract) and A_{sample} is the absorbance of the test extract.

4- Results :

The obtained results were shown in table (21).

Table (21) : Antioxidant activity of different extracts of *T. zanonii*.

Sample	Absorbance	I %
Blank (MeOH)	0.363	-
Total alcoholic extract	0.045	87.6
Chloroform extract	0.172	52.4
Ethyl acetate extract	0.023	93.6
Butanol extract	0.028	92.1
Petroleum ether extract	0.363	0
Unsaponifiabl fraction	0.361	2
Blank (H_2O)	0.358	-
Aqueous extract	0.080	77.5

INSECTICIDAL ACTIVITY

1- Introduction

Olive trees are liable to investigation by several destructive borers. The olive bark beetle, *phloeotribus oleae* Fab. (order : Coleoptera, Family : Scolytidae) is considered one of these borers, since it cause serious damage to olive trees which may lead them to death within few years. These losses start when the female beetle deposits her eggs beneath the bark of the tree. The hatching grubs complete their development in the cambium region, and then the beetles emerge through small round holes. Various conventional chemical insecticides are available which offer some protection against this pest, but they have created many problems such as resistance, secondary pests outbreaks, environmental contamination ...etc. A promising alternative in this regard is the application of plant extracts which can be both effective and inexpensive to produce.

The present study was undertaken to evaluate certain extracts as insecticide alternatives for controlling *Phloeotribus oleae* Fab.

2- Material & methods

2.1- Preparation the plant extracts:-

About 250 g of dry powered plant were extracted with petroleum (40-60°C) in a Soxhlet apparatus for eight hours. The petroleum ether was tested as a total extract and then fractionated to fatty alcohols, fatty acids and unsaponifide materials as in pages (82, 88 and 90). The defatted plant material was extracted with aqueous methanol (70%). The alcoholic extract was partitioned with chloroform, ethyl acetate and butanol respectively. Another 100 g of the plant material were macerated in distilled water for 24 hours. The tested extracts were petroleum ether, fatty alcohols, fatty acids, unsaponifide fraction, alcoholic, aqueous, chloroform, ethyl acetate and butanol extracts.

2.2- Stock culture :-

Cutting of olive branches severely infested with the olive bark beetle, *Phloeotribus oleae* Fab. Were collected from Burg-El-Arab region, Matrouh Governorate, Egypt.

A box of 60 x 60 x 100 cm, walls and floor was constructed of wooden frames covered with wire gauze and lined with cloth streamers while the ceiling and the door were made of glass. The collected cutting of olive branches were left under laboratory condition (25±2°C and 65± 5% R.H.), until emergence of the beetles at about the beginning of March. Newly emerged adducts were collected and classed according to sex , Cutting of fresh olive branches 10 cm long and 2.5 cm thick, each were used as an oviposition site. They were left for 1-3 days to be suitable to the entry of beetles.

3- Control experiments:-

3.1- Laboratory tests :

They were conducted using nine plant extracts (Tab. 22), the slide dip technique [232] was used. A small piece of a double faced adhesive tap was adhered on a glass slide and ten adult beetles of *P. oleae* of the same age, were transferred by means of a soft brush and placed with their backs on the surface of the tape. The slide with the adults on it was dipped for 5 seconds in each extract after which the slides were left to dry under room temperature. The beetles of the untreated control were dipped in water for comparison. Three replicates were used for each extract and untreated control, the dead and alive beetles were recorded after 24 hours.

3.2- Field experiment :-

An orchard of olive trees naturally infested with a fore mentioned insect was chosen at Burg El-Arab regin, Matrouh Governorate. The trees

were 6-8 years old and about 1.5-2.0 m in a height. For the individual extract, a randomized block design was used where nine treatments were arranged in three replicates, 4 trees each (12 trees/ treatment). Rows of olive trees were left, as borders among the treatments, to avoid any spray drift. All spray applications were made once on late March, 2005 using knapsack sprayer 20 L capacity, to cover stems, branches and twigs of all trees. Five of green branches, were randomly selected from each tree, and cut off. Then they were kept in plastic bags and transferred to the laboratory where they were examined. The number of living adults per branch both before and after application was recorded in each treatment (including control plats) and used as an index for the population density (infestation) of the borer. Pretreatment counts were taken immediately before spraying application, whereas post treatment counts were taken 1 ,2 and 3 weeks after application.

Evaluation of all treatments was based on the reduction of the population density of olive individuals per replicate according to Henderson and Tilton equation (1955)[233]. Data were statistically analyzed using Duncan's Multiple range test (1955)[234]. The results were shown in table (22) and (23).

Table (22): Insecticidal activity of different extracts of *T. zanonii* against the adult of *Phloeotribus oleae* (30 beetles/ treatment)

Extract	Number of dead beetles	% Percentage of mortality
	After 24 h	After 24 h
1- petroleum ether	18 de	60.00
2- fatty alcohols	15 bc	50.00
3- fatty acids M. E.	17 cd	56.67
4- Unsap. Fraction	13 b	43.33
5- Alcoholic	25 f	83.33
6- Aqueous	26 f	86.67
7- Chloroform	20 e	66.67
8- Ethyl acetate	21 e	70.00
9- Butanol	24 f	80.00
10- Untreated control (Without extract)	1 a	3.33

Means marked with the same letters are not significantly different ($P<0.05$).

Table (23): Efficiency of different treatments applied against *P. oleae* infesting olive trees.

Treatment	Mean number/ replicae and % reduction in infestation after sprayir								
	Before treatment	One week		Two weeks		Three weeks		Average	
		M.no	%R	M.no	%R	M.no	%R	M.no	%R
1-Pet. ether extract	14.95	12.00	30.20	13.05	25.59	13.90	24.93	12.98	26.91
2-Fatty alcohols fraction	14.75	12.20	28.07	12.20	29.49	14.00	23.36	12.80	26.97
3-Fatty acids fraction	13.25	11.10	27.16	12.20	21.52	14.00	14.69	12.43	21.12
4-Unsaponifid fraction	15.00	13.19	19.13	14.05	20.15	15.45	16.83	14.48	18.70
5-Alcoholic extract	14.90	5.85	65.86	6.00	65.67	7.00	62.07	6.28	64.53
6-Aqueous extract	14.75	4.95	70.82	4.95	71.39	5.00	72.63	4.97	71.61
7-Chloroform extract	15.00	6.15	64.35	7.05	59.93	9.40	49.40	7.53	57.89
8-Ethyl acetate extract	14.75	5.95	64.92	7.00	59.54	10.45	42.80	7.80	55.75
9-Butanol extract	13.00	5.00	66.56	5.85	61.64	6.30	60.87	5.72	36.02
10-Control	13.00	14.95	-	15.25	-	16.10	-	15.43	-

M. no =Mean number, %R = Percent reduction in infestation.

DISCUSSION

DISCUSSION

Family *Lamiaceae* (*Labiatae*) is known to be rich of medicinal plants, which are characterized by the presence of volatile oils, flavonoids, phenolic acids, terpens, iridoids and coumarins.

The studied species *Teucrium zanonii* is belonging to the family *Lamiaceae*. This plant was subjected to phytochemical investigation concerning with their volatile oils and lipids as well as the flavonoidal constituents.

The volatile oil of this plant was extracted using two methods (hydro-distillation and solvent extraction).

The GC/MS analysis of the volatile oil extracted by hydrodistillation method showed that it is a mixture of 74 compounds representing 92.98 % of the total oil. The identified compounds represent several chemical classes, *viz.:* saturated hydrocarbons 0.56%, unsaturated hydrocarbons 41.79%, alcohols 31.68%, aldahydes 0.09%, ketones 2.39%, esters 15.16%, oxides 0.64%, aromatics 0.67%, with the highest abundance of β-Pinene, linalyl acetate, linalool, germacrene-D in addition to γ-elemene (14.13%, 11.10%, 11.00%, 8.81% and 7.79% respectively). These results were coincided with that reported by Cavaleiro *et. al.* [17], where they reported the identification of more than seventy components from the oil of *T. lusitanium* and *T. algarbiensis* in which β-Pinene and germacrene-D are the major compounds.

The GC/MS analysis of the volatile oil extracted by solvent extraction (n-hexane-ether 50 : 50) showed a mixture of 16 compounds representing 86.90% of the total oil. The identified compounds represent several chemical

classes, *viz.*: saturated hydrocarbons 16.08%, unsaturated hydrocarbons 60.94%, alcohols 0.91%, ketones 1.24%, esters 7.93% and about 13.10% unknown compounds with the germacrene-D, β-Pinene and linalyl acetate as the main components, (20.04%, 18.19% and 7.93% respectively).

Investigation of the terpenoids and related substances of *T. zanonii* was carried out, revealing the identification of the fatty alcohols fraction using GC/MS technique. The result showed presence of tricosanole (5.10%), tetracosanol (4.62%), pentacosanol (23.37%), nonacosanol (26.21%), triacontene (24.73%), tetratricontene (15.95%). Nonacosanol was the major compound (26.21%).

The GLC analysis of the unsaponifiable fraction revealed that, the unsaponifiable matter consists mainly from a mixture of series of n-alkanes from n-C_3 to n-C_{32} (92.48%), sterol fraction (7.06%) [cholesterol (4.48%), β-sitosterol (1.36%), campasterol (0.86%), stigmasterol (0.36%)] and triterpene fraction contain β-amyrine (0.41%).

The study of the total fatty acids of *T. zanonii* was achieved by GLC analysis of their methyl esters. The results revealed the presence of lauric (1.36%), myristic (1.22%), palmitic (13.95%), stearic (15.05%), oleic (13.69%), linoleic (35.25%), linolenic (11.21%), arachidic (1.58%), erucic (1.58%), lignoceric (3.58%), tetracosenoic (1.90%). The saturated and unsaturated fatty acids represents 36.74% and 63.27% respectively. Also stearic and lineoleic acids are the major acids.

Flavonoids were obtained from the alcoholic extract (70%) by the conventional method, i.e. by treating the concentrated alcoholic extract with organic solvents (ethyl acetate and butanol).

Fractionation of the flavonoidal constituents was affected by applying column chromatography. Moreover, further purification was achieved using preparative TLC and/or PC as well as Sephadex LH-20 column chromatography.

The flavonoidal constituents either aglycons or glycosides was investigated. Six flavonoids were isolated from ethyl acetate and butanol viz. : cirsiliol, luteolin, chrysoeriol, xanthomicrol, apigenin 6,8-di-*O*-glucoside and luteolin 7-*O*-rutinoside, two of them *viz.* : chrysyoeriol and xanthomicrol were in minute amounts. However they were tentatively identified and will be subjected for further studies.
The same results was reported by Garcia *et. al.* [174-175], where they isolated cirsiliol, luteolin and luteolin 7-*O*-rutinoside from *T. gnaphalodes*.

Identification of the isolated flavonoids was achieved through chromatographic studies and spectroscopic measurements (*viz.* : UV, MS, ^{1}H-NMR and ^{13}C-NMR).

The studies of antioxidant activity of different extracts against DPPH showed that the ethyl acetate, butanol fractions and aqueous extract have the highest antioxidant activity. This activity may be mainly due to the presence of flavonoids (aglycones or glycosides) in these fraction. These observations were in accordance with that reported by Tiziane *et. al.* [235].

The insecticidal activity of different extracts on *Pholeotribus oleae* were measured in the laboratory. The results indicated that all extracts used proved to have various degrees of insecticidal effect on the adult beetles. Regarding to potency of the control agents tested, the aqueous extract showed the most effect (86.67% mortality) whereas that of unsaponifiable fraction was the least in this concern which give only 43.33% mortality. Also, mortalities of 83.33%, 80.00%, 70.00% and 66.67% were obtained by using of alcoholic, butanol, ethyl acetate and chloroform extracts, respectively. There is significance between aqueous and alcoholic extracts. These results clarify that aqueous extract was the most efficient as insecticide followed by alcoholic extract.

Filed experiments of insecticidal activity show that the mean numbers of *Pholeotribus oleae* Fab. on the olive trees before treatments, ranged from 13:00 to 15:00, indicating a relatively uniform distribution of insect infestation. One week after spraying, the treatments suppressed the levels of infestation to different degrees compared to that of untreated control. Aqueous, alcoholic and butanol extracts significantly lowered the percentage of infestation to 70.82%, 65.86% and 66.56%, respectively. Two weeks post-treatment, aqueous extract become more efficient and had almost similar activity as cidial 50% (conventional chemical insecticides, unpublished data) displaying 71.39% and 73.9% reduction in infestation respectively. Similar results were reported by Ismail and Abdalla [236].

As for the 3rd week after the treatment, both aqueous extract and alcoholic showed good bioresidual activities against *P. oleae* giving 72.63% and 62.07% reduction, respectively. This was in accordance with Ismail *et. al.* [237], also Masanori *et. al.* in 2000 [238] reported that some methoxylated flavones

have antifeedant activity. So the insecticidal activity of *T. zanonii* may be due to the presence of such compounds in the active extracts.

Accordingly, the present study showed that *T. zanonii* extracts was a good candidate to be considered for protecting olive trees against this pest in integrate pest management (IPM) program.

REFRENCES

[1] Lin, C. C.

Am. J. of Chinese Medicine, **1992,** 20, 269-279.

[2] Siddiqi, M. A.

Flora in Libya, Vol. 118 Lamiacea, **1985**, Revolution Printing Press, Tripoli, Jamahiriya.

[3] Jens, A. Pederen.

Biochem. Syst. and Ecol., **2000**, 28, 229-253.

[4] Githinji, G. W. and Kakwaro, J. O.

J. of Ethnopharm., **1992**, 39, 197.

[5] Barberan, F. A. T.

Fitoterapia, **1986**, 57, 67.

[6] Ulubelen, A.; Topcu, G. and Sonmez, U.

Stud. Nat. Prod. Chem., **2000**, 23(Bio. Nat. Prod.(Part D)), 591-648.

[7] Assem, M. E. and Karam T. H.

Biochem. Syst. and Ecol., **2004**, 32, 665-674.

[8] Harborne, J. B.

Phytochemical Methodes, , Chapman and Hall, New York, Second Edition, **1984.**

[9] Petriei, J.; Blaevi, N. and Plazibat, M.

J. Ess. Oil. Res., **1993**, 5(6), 625-627.

[10] Velasco N., A. and Perez-Alonso, M. J.

An. Bromatol., **1990**, 41(2), 241-8.

[11] Arnold, N.; Bellomaria, B.; Valentini G. and Rafaiani, S. M.

J. of Ethnoph., **1991**, 35(2),105-113.

[12] Blaevi, N.; Kaloera, Z.; Petriei, J and Plazibat, M.

J. Ess. Oil. Res.,**1992**, 4, 223-225.

[13] Vokou, D. and Bessiere, J. M.

J. Nat. Prod., 1985, 48(3),498-9.

[14] Laura, B. M.; Orietta, S. and Adriano, P.

Riv. Ital. EPPOS, 1993, 4(spec. Num), 664-71.

[15] Barroso, J. G.; Figueiredo A. C.; Pedro, L.; Antunes G. T.; Sevinate-Pinto, I.; Fontinha, Susan S. and Scheffer, Johannes J.C.

Flav. Frag. J., 1996, 11(2), 129-132.

[16] Eikani, M. H.; Goodarznia, I. and Mirza, M.

J. Essent. Oil Res. ,1999, 11(4), 470-472.

[17] Cavaleiro C.; Salgueiro L. R.; Miguel. M. G. and Proenca D. A.

J. of Chromatog., 2004, 1033(1), 187-190.

[18] Piozzi, F.; Bruno, M. and Rosselli, S.

Heterocycles, 1998, 48, 2185-2203.

[19] Jose, F.; Francisco, F.; Conrad, P. and Aurea, P.; Franco, P.; Manuel, R. Benjamin, R. and Giuseppe, S.

J. Org. Chem., 1984, 49, 1789-1793.

[20] Hanson, J. R.; Rive, H.; Douglas, E.; Les, S. and Williams, D.

J. Chem. Soc. Perkin Trans-1, 1982, (4), 1005-8.

[21] Topca, G.; Eris, C.; Ulubelen, A.; Krawiec, M. and Watson, W.

Tetrahedron, 1995, 51(43), 11793-800.

[22] Camps, F.; Coll, J.; Dargallo, O.; Rius, J, and Miravitlles, C.

Phytochemistry, 1987, 26(5), 1475-9.

[23] Rodriguez, B.; de la Torre, M. C.; Bruno, M. Pioozzi, F.; Vassallo, N.; Ciriminna, R. and Servettaz, O.

Phytochemistry, 1996, 43(2), 435-438.

[24] Benjamin, R.; Maria, C.de la Torre; Maurizio, Br.; Franco, P.; Nadia, V..; Rosaria, C. and Orietta, S.

Phytochemistry, 1997, 45(2), 383-385.

[25] Gaspar, H.; Brito, P.; Fernando M. S.; de la Torre, M. C.; Rodriguez, B. and Perales, A.

***Tetrahedron*, 1995**, 51(8), 2363-8.

[26] Son, H.; Chen. X.; Wang, T.; Pan, L.; and Zhongwen; C. D.

***Phytochemistry*, 1991**, 30(5), 1721-3.

[27] Dela Torre, M. C.; Fernandez-Gadea, F.; Michavila, A.; Rodriguez, B.; Piozzi, F. and Sanona, G.

***Phytochemistry*, 1986**, 25 (10), 2387.

[28] Benjamin, R.; Maria C. T.; Maurizio, B.; Caterina, F.; Franco, P.; Giuseppe, A. P. and Nelly, A. A.

***Tetrahedron*, 1991**, 50(7), 2289-2296.

[29] Rodriguez, B.; de la Torre C.; Jimeno, M.; Bruno, M.; Fazio, C.; Piozzi,F.; Savona G. and Perales, A.

***Tetrahedron*, 1995**, 51(3), 837-48.

[30] Sanona, G.; Piozzi, F.; Servettaz, O.; Rodriguez, B.; Mueso, J. A.; and Maria. D. C .

***Phytochemistry*, 1986**, 25(11), 2569-72.

[31] Marquez, C.; Rabanal, R. M.; Valverd, S.; Eguren, L. and Fayos, J.

***Tetrahedron Lett.*, 1980**, 21 (52) , 5039-5042.

[32] Marquez, C.; Rabanal, R.; Valverd, S.; Eguren, L. and Fayos ,J.

***Tetrahedron Lett.*, 1981**, 22(29), 2823-6.

[33] Savona, G.; Piozzi, F.; D. T., Maria C.; Servettaz, O. and Rodriguez, B.

***Phytochemistry*, 1987**, 26(2), 571-572.

[34] Popa, D. P. and Reinbold, A. M.

***Ref. Zh. Khim*, 1971**, Abstract no. 1R516.

[35] Popa, D. P. and Reinbold, A. M.

***Khim. Prir. Soedin.*, 1973**, 9(1), 31-35.

[36] Reinbold, A. M.; Popa, D. P.

Khim. Prir. Soedin., **1974**, (5). 589-598.

[37] Papanov, G.; Malakov, P.

Z. Nat., B: Anorg. Chem., Org. Chem., 1980 35 B(6), 764-766.

[38] Savona, G; Garcia-Alvarez, M.; Rodriguez, B.

Phytochemistry, **1982**, 21(3), 721-3.

[39] Eguren, L.; Perales, A. Fayos, J.; Rodriquez, B.;

Savona, G. and Piozzi, F.

J.Org.Chem., **1982**, 47(21), 4/57-60.

[40] Garcia-Alvarez, M.; Lukacs, G. N. A.; Piozzi, R. B. and Savona, G.

J.Org.Chem.,**1983**, 48(25), 5123-6.

[41] Fernadez-Gadea, F.; Pascual, C.; Rodriguez, B. and Savona, G.

Phytochemistry, **1983**, 22(3), 723-5.

[42] Rodriguez, M. C.; Barluenga, J.; Savona, G.; Piozzi, F.; Servettaz, O.

and Rodriguez, B.

Phytochemistry , **1984**,23(7),1465-9.

[43] Rodriguez, M. C.; Barluenga, J.; Pascual, C.; Rodriguez, B.; Savona, G.

and Piozzi; F.

Phytochemistry, **1984**, 23(12), 2960-1.

[44] Malakov, P.; Paparov, G.

Phytochemistry, **1985**, 24(2), 301-3.

[45] Erdal, B. ; Rangagvalli ,M. Y. and Tkhlas A. K.

Phytochemistry, **2003**, 63(8), 977-983.

[46] De Vaincenzi, M.; Maiale, F. and Silano M.

Fitoterapia, **2003**, 74 (7-8) , 746 -749.

[47] Calis. I.; Bedir, E.; Wright, A. D. and Sticher, O.

J.Nat.Prod., 1996, 54(4), 457-60.

[48] Bruno, M.; Fazio C.; Pioozzi, F.; Rodri, B. and De La Totte, M. C.
Phytochemistry, **1995**, 40(5), 1481-3.

[49] Roberto, A.; Maria, C. De La T.; Rodri, B.; Bruno, M.; Pioozzi, F.; Savona, G. and Nelly, A. A.
Phytochemistry, **1992**, 31(11), 3957-3960.

[50] Savona, G.; Piozzi, F.; Bruno, M.; Dominguez, G.; Rodriguez, B. and Servettaz, O.
Phytochemistry, **1987**, 26(12), 3285-8.

[51] Dominguez, X. A.; Merijanian, A. and Gonzalez, B. L.
Phytochemistry, **1974**, 13(4), 754-755.

[52] Dominguez, X. A.; Merijanian, A.; Gonzalez, B. L.;zamudio, A. and Salazar, A. L.
Rev. Latinoam. Quim., **1974**, 5(4), 225-229.

[53] Bruno, M.; Piozzi, F.; Savona, G. and Rodriguez, B.; De La Torre, M. C. and Servettaz, O.
Phytochemistry, **1987**, 26(10), 2859-61.

[54] Ahmed ,A. A.; Mahmoud, A.; A. and Ahmed, L. M.; Tzakou, O.; Couladis , M.; Spriug, O. and Mabry , T. J.
Rev .Latinoam .Quim., **2001**, 29(1), 23- 25.

[55] Lllubelen, A.; Topcu, G. and Olcol, S.
Phytochemistry, **1994**, 37(5), 1371-5.

[56] Fayos, J.; Martinez-Ripoll, M.; Paternostro, M.; Piozzi, F.; Rodriguez, B. and Savona, G.
J. Org., **1979**, 44(26), 4992-4.

[57] Savona, G.; Paternostro, M. P.; Piozzi, F.; Hanson, T.; Hitchcock, P. B. and Thomas, S. A .
J. Chem. Soc., Perkin Trans.1, **1978**, (9), 1080- 3.

[58] Savona, G.; Paternostro, M. P.; Piozzi, F.; Hanson, J. R.; Hitchcock, P. B. and Thomas. S. A.

J. Chem. Soc., Perkin Trans.1 1979, (7), 1915-1917.

[59] Fayos, J.; Fernandez-Gadea, F.; Pascual, C.; Perales, A.; Piozzi, F.; Rico, M.; Rodriguez, B. and Savona, G.

J.Org.Chem., 1984, 49(10), 1789-93.

[60] Savona, G.; Piozzi, F.; Servettaz, O.; Rodriguez, B.; Fernandez-Gadea, F. and Martin-Lomas, M.

Phytochemistry, 1984, 23(4), 843-8.

[61] Savona, G.; Pascual, C. and Rodrigues, B.

Phytochemistry, 1981, 20(9), 2259 -61.

[62] Savona, G.; Passannanti, S.; Paternostro, M. P.; Piozzi, F.; Hanson, J. R. and Siverns, M.

Phytochemistry, 1978, 17(2), 320-322.

[63] Savona, G.; Passannanti, S.; Paternostro, M. P.; Piozzi, F.; Hanson, J. R.; Hitchcock, P. B. and Siverns, M.

J. Chem. Soc., Perkin Trans.1, 1978, (4), 356-359.

[64] Maurizio, B.; De La Torre, M. C.; Savona, G.; Piozzi, F. and Rodrigues, B..

Phytochemistry, 1990, 29(8), 2710-2712.

[65] Bruno, M.; Ciriminna. R.; piozzi, F.; Rosselli, S. and Simmonds, M. S.

Phytochemistry, 1999, 52(6), 1055-1058.

[66] Josep, C. and Yudels, T.

Phytochemistry, 2004 ,65(4)387-39.

[67] Maria, J.; Sexmero, C.; Bruno, M.; De La Torre, M. C.; Piozzi, F.; Rodrigues, B. and Savona, G..

Phytochemistry, 1992, 31(5), 1697-1701.

[68] Maurizio, B.; Roberto, A.; De La Torre, M. C.; Savona, G.; Piozzi, F.; Rodrigues, B.; Aurea, P. and Nelly A. A.
Phytochemistry, **1992**, 31(10), 3531-3534.

[69] Savona, G.; Paternostro, M.; Piozzi, F. and Rodriguez, B.
Tetrahedron Lett., **1979**, (4), 379-382.

[70] Martinez-Ripoll, M.; Fayos, J.; Rodriguez, B.; Garcia-Alvarez, M. C.; Savona, G.; Piozzi, F.; Pastemostro, M. and Hanson, J. R.
J. Chem. Soc., Perkin Trans.1, **1981**, (4), 1186 -1190.

[71] De La Torre, M..; Rodriguez, B.; Savona, G. and Piozzi, F.
Phytochemistry, **1986**, 25(1), 171-3.

[72] Maurizio, B.; Gema, D.; Ana L.; Piozzi, F.; Rodriguez, B.; Savona, G.; De La Torre, M. C. and Nelly A. .
Phytochemistry, **1991**, 30(11), 3693-3697.

[73] Gaspar, H.; Palma, F. M.S. Brito; De La Torre, M. C. and Rodriguez, B.
Fitoterapia, **1996**, 67(2), 188-189.

[74] Organesyan, G. B. and Mnatsakanyan, V. A.
Khim. Prir. Soedin., **1977**, (2), 215-220.

[75] Organesyan, G. B. and Mnatsakanyan, V. A.
Arm. Khim. Zh., **1978**, 31(10), 768-75.

[76] Gacs B. E.; Radics, O. G. B. and Mnatsakanian, V. A.
Phytochemistry, **1978**, 17(11), 1967-1973.

[77] Oranesyan, G. B. and Mnatsakanyan, V. A.
Arm. Khim. Zh., **1978**, 31(10), 776-81

[78] Miyase, T.; Kawasaki, H.; Noeo, T.; Lleno, A.; Fukushima, S. and Takemoto, T.
Chem .Pharm. Bull. **1981**, 29(21), 3561-4.

[79] Min, Z.; Xie, N.; Zhang, P.; Zhao, S.; Wang, C. and Zheng, Q.

Phytochemistry, 1991, 30(12), 4175-4177.

[80] Simoes, F.; Rodriguez, B.; Piozzci, F.; Savona G.; Bruno, M. and Nelly, A. A.

Heterocycles 1989, 28(1), 111-15.

[81] Fatima, S.; Rodriguez, B.; Bruno, M.; Piozzci, F.; Savona, G. and Nelly A. A.

Phytochemistry, 1989, 28(10), 2763-2768.

[82] Boneva, I.; Malakov, P.; Papanov, G

Phytochemistry, 1988, 27(1), 295-7.

[83] Malakov,P.; Bonaeva, I.; Papanov, G.; Spasov, S

Phytochemistry, 1988, 27(4), 1141-3.

[84] Peter Y. Malakov; Georgi Y. Papanov; Iva, M. B.; Rodriguez, B. and De La Torre, M. C.

Phytochemistry, 1993, 34(4), 1095-1098.

[85] Fernandez-Gaea, F.; Rodriguez, B.; Savona, G. and Franco, P.

Phytochemistry, 1984, 23(5), 1113-18.

[86] Hueso-Rodriguez, J. A.; Fernandez-Godea, F.; Pascual, C.; Rodriguez, B.; Savona; G. and Piozzi, F.

Phytochemistry, 1986, 25(1), 175-80.

[87] Maurizio, B.; Maria C.De La T.; Rodriguez, B. and Abdallah, A. O.

Phytochemistry, 1993, 34(1), 245-247.

[88] Bruno, M.; Bond, M. L.; Rosselli, S.; Piozzi, F.; Al-Hillo, M. R. Y.; Lamara, K. and Ladjel, S.

J. Nat. Prod., 2000, 63(7), 1029- 1031.

[89] Bruno, M.; Bondi, M. L.; Rosselli, S.; Piozzi, F.; Al-Hillo, M. R. Y; Lamara , K. and Ladjel, S.

Eur. J. Org .Chem., 2001 ,(9) , 1669- 1671.

[90] Savona, G.; Piozzi, F.; Servettaz, O.; Fernandez-Gadea, F. and Rodriguez, B.

***Phytochemistry*, 1984**, 23(3), 611-3.

[91] Savona, G., Bruno, M.; Piozzi, F.; Servettoz, O. and Rodriguez, B.

***Phytochemistry*, 1984**, 23(4), 849-52.

[92] Bruno, M.; Piozzi, F.; Rodriguez, B.; Savona, G.; Maria C.De La T. and Servettoz, O.

***Phytochemistry*, 1992**, 31(12), 4366-4367.

[93] De La Torre, M. C.; Servettaz, O.; Rodriguez, B.; Bruno, M.; Savona G.; Piozzci, F. and Servettaz, O.

***Phytochemistry*, 1988**, 27(1), 213-16.

[94] Malakov, P.; Papanov, G. and Mollov, N.

***Z. Natur., B: Anorg. Chem. Org.Chem.*, 1978**, 33B (7),789-791.

[95] Malalov, P.; Papanov, G.; Mollov, N. and Spassov, S.

***Z. Natur., B: Anorg. Chem.,Org. Chem.*, 1978**, 33B(10), 1142-4.

[96] Malakhov, P.; Papanov, G.; Mollov, N. and Spasov, S.

***Symp. Pap.-IUPAC Int. Symp. Chem. Nat. Prod.*, 1978**, 2, 305 -8.

[97] Boneva, I.; Malakov, P. and Papanov, G.

***Z. Naturforsch.,B.;Chem.*, 1987**, 42(8), 1000-2.

[98] Peter Y. M.; Georgi Y. P. and Iva, M. B.

***Phytochemistry*, 1992**, 31(11), 4029-4030.

[99] Kisiel, W.; Piozzci, F. and Grzybek, J.

***Planta Med.*, 1995**, 61,(2), 191-192.

[100] Papanv, G.; Malakov, P.

***Phytochemistry*, 1983**, 22(12), 2787-9.

[101] Gallardo, O.V.; Tonn, C. E.; Nieto, M.; Morale, G. B.; Giordano, O. S.

***Nat. Prod. Lett.*, 1996**, 8(3), 189-197.

[102] Bruno, M.; Omar, A. A.; Perales, A.; Piozzi, F.; Rodriguez, B.; Savona, G. and Dela Torre, M. C.
Phytochemistry, **1991**, 30(1), 275-82.

[103] De La Torre, M. C.; Bruno, M.; Piozzi, F.; Savona, G.; Omar, A. A.; Perales, A. and Rodriguez, B.
Tetrahedron, **1991**, 47(20-21), 3463-70.

[104] De La Torre, M. C.; Bruno, M.; Piozzci, F.; Savona, G.; Rodriguez, B. and Omar, A. A .
Phytochemistry, **1991**,30(5),1603-6.

[105] Al-Yahya, M. A.; Muhammad, T.; Mirza, H. and El-Feraly, F. S.
J.Nat.Prod., **1995**, 58(4), 633.

[106] Mohammed, A. A.; Farouk S. E. Chuck, D. D. and Tlias, M.
Phytochemistry, **2002**, 59(4), 409-414.

[107] Sexmero, M. J. C.; De La Torre, M., C.; Benjamin, R; Bruno, M.; Piozzci, F.; Savona, G.
Phytochemistry, **1991**, 30(12), 4079-4082.

[108] Xie, N.; Min, Z.; Zhao, S.; Wu, B.; Zheng, Q. and Zhang, P.
Phytochemistry, **1991**, 30(6), 1963-6.

[109] Sun, D. and Li, G.
Phytochemistry, **1993**, 33(3), 716-717.

[110] De la Torre, M. C, Rodriguez, B.; Bruno, M.; Savona, G.; Piozzci, F.; Perales, A.; Torre, M. R. and Servettaz, O.
Phytochemistry, **1990**, 29(7),2229-33.

[111] Popa, D. P.; Phan, T. A. and Salei, L. A.
Khim. Prir. Soedin. **1977**, (1), 49-54.

[112] Malakhov, P.; Papanov, G. and Mollov, N.
Z. Natur., B: Anorg. Chem., Org. Chem. **1979**, 34B (11), 1570-1572.

[113] Marquez, C. and Valverde, S.

*J. Chem. Soc., Perkin Trans.*I, 1979, (10), 2526- 2527.

[114] Eguren, L.; Perales, A.; Fayos, J.; Savona, G.; Paternostro, M.; Piozzi, F. and Rodriguez, B.

J. Org. Chem. 1981, 46(16), 3364-7.

[115] Malakov, P.; Papanov, G. and Ziesche, J.

Phytochemistry, 1982, 21(10), 2597-8.

[116] Malakov, P. and Papanov, G.

Phytochemistry, 1983, 22(12), 2791-3.

[117] Omar, Abdallah, A.

Alexandria J. Pharm. Sci, 1987,1(2), 100-2.

[118] Erdal, B.; Deniz, T.; Ihsan, C.; Oliver, Z. and Otto, S.

Phytochemistry, 1999, 51(7), 921-925.

[119] De La Totte, M.; Rodriguez, B.; Rizk. A. F.; Bruno, M. and Piozzi, F

Fitoterapia, 1988, 59(2), 129-30.

[120] Ladjel, S.; Laamara, K.; Al-Hillo, M. R. Y. and Pais, Mary.

Phytochemistry, 1994,37(6),1663-6.

[121] Fernandez, P.; Rodriguez, B., Savona, G. and Piozzi, F.

Phytochemistry, 1986, 25(1), 181-4.

[122] De la Torre, M.; Piozzi, F.; Rizk, A.; ;Rodriguez, B. and Savona, G.

Phytochemistry, 1986, 25(9), 2239-40.

[123] Maria C. C.; Rodriguez, B.; Piozzei, F.; Savona, G.; Maria, R. T. and Aurea, P.

Phytochemistry, 1989, 28(5), 1453-1461.

[124] Maria C. C.; Rodriguez, B.; Piozzei, F.; Savona, G.; Maria, R. T.; Maria, C. D. and Aurea, P.

Tetrahedron, 1990, 46(3), 847-860.

[125] Garcia, A., M. C.; Marco, J. L.; Rodriguez, B.; Savona, G. and Piozzi, F.

***Phytochemistry*, 1982**, 21(10), 2559-62.

[126] Fernandez, P.; Rodriguez, B.; Villegas, J. A.; Perales, A.; Savona, G.; Piozzi, F. and Bruno, M.

***Phytochemistry*, 1986**, 25(6), 1405-9.

[127] Shen, X.; Tan, Z. and Sun, H.

***Yunnan Zhiwu Yanjiu*, 1990**, 12(2), 229-30.

[128] Chen, H.; Xie, N. and Min, Z.

***Gaodeng Xuexiao Huaxue Xuebao*, 1996**, 17(13), 405-408.

[129] Bruno, M.; Fazio, C.; Piozzi, F.; Sanona, G.; Rodriguez, B. and de la Torre, M. C.

***Phytochemistry*, 1995**, 40(2), 505-7.

[130] Dela Torre, M.; Pascual, C.; Rodriguez, B.; Piozzi, F.; Sauona, G. and Peales, A.

***Phytochemistry*, 1986**, 25(6), 1397-403.

[131] Topcu, G.; Eris, C.; Che, C.-T. and Ulubelen, A

***Phytochemistry*, 1996** ,42(3),775-778.

[132] Maria C. D. T.; Benjamin, R.; Maurizio, B.; Caterina, C.; Fazio; K. H. C. and Hayri Duman

***Phytochemistry*, 1997**,45(8),1653-1662.

[133] Papanov, G. and Malakok, P .

***Z. Natur., B: Anorg. Chem., Org.Chem* 1981**, 36B(1), 112-113.

[134] Papanov, G,; Malakov, P. and Bohlmann, F.

***Phytochemistry*, 1981**, 20(1), 170-171.

[135] Rapanov, G. and Malakov, P.

***Phytochemistry*, 1985**, 24(2), 297-9.

[136] Baruah, R. N.; Bohlmann, F. and QuacK, W.

Planta Med., **1985**, (4), 341-2.

[137] Josep, C. and Yudelsy, T.

Phytochemistry, **2004**, 65(4), 387-392.

[138] Papanov, G. and Malakok, P.

Z. Natur., B: Anorg. Chem ., Org. Chem. **1982**, 37 B(4),519 -20.

[139] Marco, J.; Rodriguez, B.; Savona, G. and Piozzi, F.

Phytochemistry, **1982**, 21(10), 2567-9.

[140] Marco, J.; Rodriguez, B.; Pascual, C.; Savona, G. and Piozzi, F.

Phytochemistry, **1983**, 22(3), 727-31.

[141] Savona, G.; Paternostro, M.; Piozzi, F. and Rodriguez, B.

Hetrocycles, **1980**, 14(2), 193 -195.

[142] Rasheed, R. A .; Ali, B. H. and Bashir, A. K.

General Pharmacology, **1995**, 26(2), 297-301.

[143] Michael T. D.; James R. H. and Douglas, E. A. R.

Phytochemistry, **1994**, 36(6), 1549-1550.

[144] Fujia, E.; Uchida, I.; Fujita, T.; Masaki, N. and Osaki, K.

J. Chem. Soc.,Chem. Commum. **1973**, (20), 793-794.

[145] Uchida, I.; Fujita, E.; Taira, Z. and Osaki, K.

Cryst. Struct. Commun. **1974**, 3(3), 569-572.

[146] Fujia, E.; Uchida, I. and Fujita, T.

J. Chem. Soc., Perkin Trans.1, **1974**, (13), 1547-1555.

[147] Node, M.; Sai, M.; Fujita, E. and Mc Phail, A. T.

J. Chem. Res., Synop., **1981**, (2), 32.

[148] Node, M.; Sai, M. and Fujita, E.

Phytochemistry, **1981**, 20(4), 757 -760.

[149] Uchida, I.; Fujia, T. and Fujita, E.

Tetrahedron, 1975, 31(7), 841-848.

[150] Uchida, I.; Fujia, T. and Fujita, E.

Tetrahedron, 1975, 31(13-14), 1512.

[151] Savona, G.; Piozzci, F.; Rodriguez, B.; Pascual, C. and Servettaz, O.

Phytochemistry, 1986, 25(12), 2857-9.

[152] Essam, A. S.; Jaber S. M.; Ilias, M. and Farouk S. E.

Phytochemistry, 1995, 40(6), 1737-1741.

[153] Braulio, M. F.; Melchor, G. H.; Teresa, M.; Arteaga, M. and Aurea, P.

Phytochemisry, 1993, 34(4), 1083-1086.

[154] Braulio, M. F.; Melchor, G. H.; Teresa, M.; Arteaga, M. and David, T.

Phytochemisry, 1995, 39(3), 617-619.

[155] Kamel, Alaa.

J. Nat. Prod., 1995, 58(3), 428-431.

[156] Maryam, H. A.; Ahmad, K. B.; Trevor, A. C.; Gerald, B. and Ming, Y.

Biochem. Syst. and Ecol., 1999, 27(1), 107-109.

[157] Grzybek, Jan.

Diss. Pharm. Pharmacol., 1969, 21(3), 253-260.

[158] Anderson, L. A.; Doggett, N.S. and Ross, M. S. F.

J. Liq. Chromatogr., 1979, 2(3), 455-561.

[159] Passannanti, S.; Paternostro, M. and Piozzi, F.

Phytochemistry, 1983, 22(4), 1044-5.

[160] Ulubelen, A.; Topcu, G. and Kaya.

Phytochemisry, 1994, 36(1), 171-173.

[161] Gaspar, H.; Brito, Palma, F. M.; De La Torre, M. C. and Rodriguez, B.

Phytochemisry, 1996, 43(3), 613-615.

[162] Chen, .; Wang, T.; Jian, B.; Lin Z.; Lu, Y.; Zheng, Q. and Sun, H.

Nat. Prod. Lett., 2000, 14(6), 459-462.

[163] Amani, M. D. E; Usama, W. H. and Sahar, A. M. H.

***Phytochemistry*, 2000**, 55, 927-931.

[164] Harborn, J. B. and Mabry, T. J.

"The Flavonoids: advances in Research", Chapman & Hall, New York **(1988)**.

[165] Markham, K. R.

"Techniques of Flavonoid Identification", Academic Press, New York, First edition **(1982)**.

[166] Mabry, T.J.; Markham, K. R. and Thomas, M. B.

"The Systematic Identification of Flavonoins", Springer-Verlag, Berlin, First edition **(1970)**.

[167] Harborn, J. B. and Mabry, T. J.

"The Flavonoids: advances in Research", Chapman & Hall, New York, First edition, **(1982)**.

[168] Brieskorn, C. H. and Biechele, W.

***Tetrahedron Lett.*, 1969**, 31, 2603.

[169] Grzybek, J.

***Diss. Pharm. Pharmacol.*, 1969**, 20(5), 563.

[170] Raynaud, J. and Chouikha, M.

***Plant. Med. Phytother*, 1976**, 10(3), 199-202.

[171] Slyunkova, O. V.; Dzhumyrko, S. F.; Kompantev, V. A.; Oganesyan, E. T. and Glyzin, V. I.

***Khim. Prir. Soedin.*, 1978**, (2), 268-269.

[172] Savona, G.; Paternostro, M. P.; Piozzi, F. and Rodrigez, B.

***An. Quim*, 1979**, 75(5), 433.

[173] Bhardwaj, D. K.; Bisht, M. S.; Gupta, A. K. and Kohli, R. M.

***Proc. Indian Natl. Sci. Acad., PartA*, 1982**, 48(3), 253-6.

[174] Garcia, M. C.; Rabanal, R. M.; Rodriguez, B. and Savona, G.
An. Quim., Ser. C., **1982**, 78(2), 271-3.

[175] Barberan, F. A.; Gil, M. I.; Tomas, F.; Ferreres, F. and Arques, A.
J. Nat. Prod.; **1985**, 48(5), 859-60.

[176] Jeffrey B. H.; Francisco A. T.; Christine A. W. and Maria I. Gil.
Phytochemistry, 1986.25(12),2811-2816.

[177] Verykokidou,V., E. and Vajias, K.
Plant. Med. Phytother., **1986**, 20(2), 109-14.

[178] Verykokidon, V. E. and Vajias, C.
Planta Med., **1986**, (5), 401-2.

[179] Rizk, A. M.; Hammouda, F. M.; Rimpler, H. and Kamel, A.
Planta Med., **1986**, (2), 87-88.

[180] Oganesyan, G. B and Mnatsakonyan, V. A.
Khim. Prir. Soedin., **1987**, (6), 910-11.

[181] Savin, K.; Ivanic, R.; Kilibarda, V.; Ralic, J.; Djurdjevic, G. and Kovacevic, N.
Arh. Farm., **1988**, 38(1-2), 7-12.

[182] Xie, N.; Min, Z. and Zhao, Z. S.
Zhongguo Yoake Daxue Xuebao, **1990**, 21(6), 376.

[183] Carmo, C. M. and Nasimento, J.
Fitoterapia, **1992**, Lx 111(3) , 277-278.

[184] Kaloera, Z.; Blaevi, N. and Stani, Z.
Planta Med., **1992**, 58(7), 690-691.

[185] Kalorera, Z.; Stani, Z. and Romi, E.
Farm.n Glas., **1993**, 49(3), 69-47.

[186] Kawashty ,S. A.; Gamal El-Din, E. M. and Swaleh, N. A. M.
Biochem. Syst. and Ecol., **1999**, 27(6), 657 -660.

[187] Hegnauer, R.

Pharm. Acta. Selv., **1966**, 41, 577.

[188] Fikenscher, L. H. and Hegnauer, R.

Planta Med. Phytother., **1969**, 3(3), 183-8.

[189] Ruhdorfer, J. and Rimpler, H.

Z. Natur. C: Biosci., **1981**, 36C(9-10),697-707.

[190] Ruhdorfer, J. and Rimpler, H.

Tetrahedron Lett., **1981**, 22(9), 839-842.

[191] Kooimann, P.

Acta Bot. Neer, **1972**, 21, 417.

[192] Mohamed, A.; Hoornaz, K. and Hamid, R. M.

Pharmaco. Rese., **2003**, 48(1), 31-35.

[193] Debat, J.; Lemoine, J.; Guay, B. and Crescioni, C.

Ger. Offen. 2,746,266 (cl. A61K35/78), 27Apr **1978**, Brit. Appl. 76/43,043, 15 Oct 1976; 36 pp.

[194] Debat, J.; Lemoine, J.; Guay, B. and Crescioni, C.

Belg. 859, 721 (cl. A61K), 01 Feb **1978**, Brit. Appl. 76/43,043, 15 Oct 1976; 24 pp.

[195] Capasso, F.; Cerri, R.; Morrica, P. and Senatore, F.

Boll-Soc. Ital. Biol. Sper, **1983**, 59(11), 1639-43.

[196] Zohara, Y.; Amots, D.; Jacob, F. and Dan P.

J. of Ethnoph., **1987**, 19(2), 145-151.

[197] Suleiman, M. S.; Abdul-Ghani, A. S.; Al-Khalil, S. and Amin, R.

J. of Ethnoph., **1988**, 22(1), 111-116.

[198] Munir N. G.; Hamzeh, H. E. and Abdul azim S. S.

J. of Ethnoph., **1988**, 24(1), 93-99.

[199] Erdal, B.; Deniz, T.; Ihsan, C.; Oliver, Z. and Otto, S.

Phytochemistry, 1999, 51, 921-925.

[200] Rasekh, H. R.; Khoshnool, M. M. J. and Kammalinejad, M.

Fitoterapia, 2001, 72(8), 937-939.

[201] Simmonds, M. S. J.; Blaney, W.M.; Ley, S. V.; Savona, G.; Bruno, M. and Rodriguez, B.

Phytochemistry, 1989, 28(4), 1069-1071.

[202] Kamel, A. and Sandra, P.

Biochem. Syst. and Ecol., 1994, 22(5),529-532.

[203] Barrachina, M. O.; Bello, R.; Martinez-Cuesta, M. A.; Esplugues, J. and Primo-Yufera, E.

Phytother. Res, 1995, 9(5), 368-371.

[204] Belen, F. P.; Irene, I. P. and Angel, M. V. F.

J. of Ethnoph., 1997, 55(2), 93-98.

[205] Jesus, L.; Maria, C. de la T.; Felix, O.; Pedro, C. and Benjamin, R..

Phytochemistry, 1999, 50(5), 749-753.

[206] Galati, E. M.; Mondello, M. R.; Aquino, A. D.; Miceli, N.; Sanogo, R.;Tza kou, O. and Monforte, M. T.

J. of Ethnoph., 2000, 72(1-2), 337-342.

[207] Bruno, M.; Piozzi, F.; Maggio, A. M.; Rosselli, S.; Simmonds, M. S. J. and Servettaz, O.

Biochem. Syst. and Ecol., 2002, 30(6), 595-599.

[208] Mohamed, A. E. and Razieh, Y.

J. of Ethnoph., 2004,

[209] Krishna, K. G. N.; Aravind, S.; Balachandran, J.; Ganesh, M. R.; Soundarya, D. S.; Rajan, S. S.; Malathi, R. and Ravikumar, K.

Phytochemistry, 2003, 64(6), 1119-1123.

[210] Balbaa, S. I.

"Medicinal Plant Constituents", Central Agency for University and School books, third edition **(1976)**.

[211] Vogel, A. I.

"Text book of practical organic chemistry", Longman's Green and Co., London-162 **(1961)**.

[212] Wall, M. E.; Krieder, M. M.; Krewson, C. F.; Eddy, C. R.; William, J.; Carel, D. S. and Centry, H. S.

J. Am. Pharm. Assoc., **1954**, 43, 1.

[213] Schmidt, J.

"Text book of Organic Chemistry", Oliver and Poyd Edinburgh and London **(1964)**.

[214] Kapoor, L. D.; Singh, A.; Kapoor, S. L. and Srivastava, S. N.

Lloydia, **1969**, 23, 279.

[215] Shinoda, J.

J. Pharm. Sco. Japan., **1928**, 48, 214.

[216] Farnswoth, N. R.

J. Pharm. Sci., **1966**, 55, 225.

[217] Arthur, H. R. and Chan, P. K.

R. Ttop. Sci., **1962**, 4, 147.

[218] Hungund, B. L. and Pathak, C. H.

U. S. D. A. Forest. Service Research Paper, **1971**, 201.

[219] Samolenski, S. J.; Silinis, H. and Farnsworth, N. R.

Lloydia, **1972**, 35; **1974**, 37, 506.

[220] Homon, N. N. and Hindmarch, K. W.

Can. J. Pharm. Sci., **1974**, 13, 39.

[221] Segelman, A. B.; Farnsworth, N. R. and Quinby, M. P.

Lloydia, **1969**, 23, 52.

[222] Gonalez, E. E. and Delgando, J. N.

J. Pharm. Sci., **1962**, 51, 76.

[223] Weiffering, J. H.

Phytochemistry, **1966**, 5, 1053.

[224] Neu, R.

Naturwissenschaften, **1965**, 43, 82.

[225] Mouma, M.; Matsuura, S. and Kusuda, K.

Chem. Pharm. Bull., **1980**, 28, 708.

[226] Kamari, G. N.; Rao, L. J. M. and Rao, N. S. P.

Proc. Indian, Acad. Sci., **1986**, 97, 171.

[227] Hira, Y.; Sanada, S.; Ida, Y. and Shoji, J.

Chem. Pharm. Bull., **1984**, 32, 295.

[228] Pier-Giorgio, Pietta.

J. Nat. Prod., **2000**, 63, 1035-1042.

[229] Dragan, A.; Dusanka, D.; Drago, B. and Nenad, T.

CROATIA CHEMIC ACTA, **2003**, 76(1), 55-61.

[230] Amarowicz, R.; Pegg, P.B.; Rahimi-Moghaddam, P.; Barl, B. and Weil, J.A.

Food Chemistry, **2004**, 84, 551-562.

[231] Yamaguchi, T.; Takamura, H.; Matoba, T. and Terso, J.

Biosci., Biotechn. and Biochem., **1998**, 62, 1201-1204.

[232] Ditrich, V.

J. Econ. Entom, **1962**, 55(55) : 644-648.

[233] Henderson, C.F. and Tilton, E.W.

J. Econ. Entom, **1955**, 48 (2) : 157-161.

[234] Duncan, D.B.

Biometrics, **1955**, 11 : 1-42.

[235] Tiziana, S.; Ammar, B.; Antonio, V.; Alessandra, B.;Ivaneo, M.; Cosimo, P. and Nunziatina, D. T.
***Bio. Syst. Ecolg.*, 2005**, 33, 341-351.

[236] Ismail, A. I. and Abdalla E. F.
***Annals Agric. Sci., Ain Shams Univ., Cairo*, 2001**, 46(1) : 355-364.

[237] Ismail, A. I.; Abd El-Salam, A. M. and Soliman, M.
***Egy. J. Biol. Pest. Control*, 2004**, 14(1) : 175-179.

[238] Masanori, M.; Sumiko, K. and Koichiro, K.
***J. Agric. Food Chem.*, 2000**, 48, 1888-94.

ARABIC SUMMARY

ملخص البحث

يهدف هذا البحث إلى دراسة المكونات الرئيسية لنبات التوكريم زانوني (الجعدة) وهو نبات يتبع العائلة الشفوية الواسعة الانتشار و ينمو محليا فقط في ليبيا بمنطقة سهل بنغازي.

وتشمل الدراسة الزيوت الطيارة والمواد الدهنية (الهيدروكربونات، الكحولات الدهنية، الأستيرويدات، والتربينات والأحماض الدهنية) والمركبات الفينولية (الفلافونويدات) وكذلك الفاعلية البيولوجية للخلاصات المختلفة للنبات.

و تتضمن هذه الدراسة ثلاثة أجزاء :-

الجزء الأول :

يبين عرض شامل للأبحاث السابقة فيما يتعلق بالمكونات الكيميائية (الزيوت الطيارة و التربينات و الستيرولات و الاريدودات والمركبات الفينولية) بالإضافة إلى الفعالية البيولوجية لهذا الجنس.

الجزء الثاني :

دراسة المكونات الكيميائية لنبات توكريم زانوني:-

1- دراسة الزيوت الطيارة للنبات :

- **استخلاص الزيوت الطيارة بطريقة التقطير البخاري:**

أسفرت نتائج تحليل مكونات الزيت الطيار باستخدام تقنية كروماتوجرافيا الغاز المتصل بمطياف الكتلة عن وجود 74 مركب تم التعرف عليها وتحديد نسب تواجدها. واتضح إن المركبات الرئيسية هي بيتا باينين بنسبة (14.13%)، خلات اللينالیل (11.10%)، اللينالول (11.00%) و الجرماكرين د (8.81%).

- **استخلاص الزيوت الطيارة باستخدام المذيبات العضوية:**

أسفرت نتائج تحليل الزيت الطيار المستخلص بمذيب الهكسان العادي و الأيثر(1:1) باستخدام تقنية كروماتوجرافيا الغاز المتصل بمطياف الكتلة عن وجود 16 مركب تم التعرف عليها

وتحديد نسب تواجدها. واتضح أن المركبات الرئيسية هي الجرماكرين د (20.04%) و بيتا بايفين (18.19%) وخلات الليناليل (7.93%).

2- دراسة المكونات الدهنية :

- أسفرت دراسة خليط الكحولات الدهنية باستخدام تقنية كروماتوجرافيا الغاز المتصل بمطياف الكتلة عن وجود الترايكوزانول والتتراكوزانول والبنتاكوزانول والنوناكوزانول والترياكونتين والتتراترايكونتين. واتضح أن النوناكوزانول هو المركب الرئيسي بنسبة (26.21%).
- أسفرت دراسة الجزء غير المتصبن باستخدام كروماتوجرافيا الغاز/سائل عن وجود خليط من الهيدروكربونات المشبعة (92.48%) تبدأ من ن-ك$_{19}$ إلى ن-ك$_{32}$ وجزء ستيرويدي مكون من الكوليستيرول (4.48%) والبيتاسيتوستيرول (1.36%) والكامباستيرول (0.86%) واستيجماستيرول (0.36%). أما الجزء التربيني فيحتوي على البيتاأميرين (0.41%).

- أسفرت دراسة خليط الأحماض الدهنية باستخدام كروماتوجرافيا الغاز/سائل انه يتكون من اللوريك, الميرستك, البالمتك, الاستياريك, الاوليك, اللنيوليك, اللينولينيك, الاراشيديك, الأريوسيك, الليغنوسيريك والتتراكوزينويك. وتبين إن اللنيوليك هو الأكثر تواجدا بنسبة (35.25%).

3- دراسة المكونات الفينولية :

دراسة مستخلص خلات الايثيل أسفر عن فصل وتعريف كل من:

1- السرسيليول.

2- الليتيولين.

3- الكريسوريول.

4- الاكزانثوميكرول.

أما دراسة مستخلص البيوتانول فأسفرت عن فصل وتعريف كل من:

5- الأبيجنين6,8-ثنائي-أ-جلوكوزيد.

6- الليتيولين7-أ-روتينوزيد.

الجزء الثالث :

دراسة الفاعلية البيولوجية

أ- الفاعلية ضد الأكسدة :

تم دراسة الفاعلية ضد الأكسدة للمستخلصات المختلفة باستخدام مادة ثنائي فينيل بيكريل هيدرازيل DPPH. وقد أظهرت النتائج أن مستخلصات خلات الايثيل والبيوتانول والمستخلص الكحولي والمستخلص المائي لها الفاعلية الأعلى بنسب (93.6%, 92.1% و 87.6% و77.5% على التوالي).

ب- **الفاعلية ضد الحشرات :**

1- التجارب المعملية:

أظهرت التجارب المعملية للمستخلصات المختلفة ضد حشرة خنفساء شجرة الزيتون أن المستخلص المائي هو الأعلى فعالية كقاتل لهذه الحشرة بنسبة (86.67%), بينما الجزء غير المتصبن هو الأقل فعالية (43.33%). وكانت باقي النتائج كالتالي: المستخلص الكحولي (83.33%), مستخلص البيوتانول (80.00%), مستخلص خلات الايثيل (70.00%), و مستخلص الكلوروفورم (66.67%).

2- التجارب الحقلية:

أظهرت التجارب الحقلية للمستخلصات المختلفة بعد أسبوع من الرش أن الفعالية الأعلى هي للمستخلصات المائي والكحولي والبيوتانول بنسب (70.82% و 65.86% و 66.56% على التوالي).

ملخص البحث
باللغة العربية

الإهداء

إلى أبي ...
إلى أمي ...
إلى إخوتي ...
إلى أخواتي ...
إلى كل من يحمل لقب الوحش
...
إلى هند ...
إلى إبراهيم ...
مع خالص تمنياتي باالتوفيق

اهدي هذا العمل المتواضع

ناجي علي

الجماهيرية العربية الليبية الشعبية الاشتراكية العظمى
جامعة التحدي
كلية العلوم
التاريخ: ....
الموافق: 2006/2/18 ف

G. S. P. L. A. J.
AL_TAHDI UNIVERSITY
الرقم الإشاري: ك ع .د ع. 6. 2006 ف

كلية العلوم

قسم الكيمياء

عنوان البحث

دراسة كيميائية وبيولوجية على نبات توكريم زانوني الذي ينمو في ليبيا

سرت / ليبيا

مقدمة من الطالب

محمد علي عبد النبي الوحش

لجنة المناقشة :		التوقيع
الدكتور / خالد عبد الهادي عبد الشفيق	(مشرف الرسالة)	[signature]
الدكتور / كامل حسين شاكر	(ممتحن خارجي)	[signature]
الدكتور / مدحت محمود علي الميض	(ممتحن داخلي)	[signature]

الدكتور / محمد علي سالم الرجباني
أمين اللجنة الشعبية لكلية العلوم