ALTAHADI UNIVERSITY
FACULTY OF SCIENCE, CHEMISTRY
DEPARTMENT

THESIS ENTITLED

CHEMICAL AND BIOLOGICAL STUDY ON *TEUCRIUM DAVAEANUM* GROWIN IN SIRTE REGION –LIBYA

FOR PARTIAL FULFILLMENT FOR REQUIRMENTS
OF THE DEGREE OF MASTER OF SCIENCE

SUBMITTED BY :
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B. Sc. (Chemistry, 2001)

UNDER SUPERVISSION OF :
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Chemistry departement

M.Sc.Thesis

Chemical and Biological study on *Teucrium davaeanum* Growing in Sirte region-Libya

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بسم الله الرحمن الرحيم

﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ
دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

صدق الله العظيم

سورة المجادلة الآية (11)

TO MY FATHER, MOTHER

TO MY FATHER, MOTHER
AND BROTHERS

AND BROTHERS

ABSTRACT

ABSTRACT

Name : Iegela Hussiene Mouhamed .

Title of Thesis : Chemical and biological study on *Teucrium davaeanum* growing in Sirte region, Libya .

Degree : M. Sc. of science, chemistry dept., Faculty of Science, Altahady university .

This work deals with the phytochemical investigation of *Teucrium davaeanum* growing in Sirte region Libya "Wadi Telal " with special emphesis to their volatile oil, Lipids (fatty alcohols, fatty acids and unsaponifiable matter) and flavonoidal constituents (aglycones as : 7, 3´, 4´- tetramethoxy 5- hydroxyl flavone, luteolin, 3, 5´dimethoxy myricetin and 5- hydroxyl, 3´,4´,6,7 tetramethoxy flavone and glycosides as : luteolin 7-*O*-gluco-glucosyl-3´-*O*-rhamnoside) addition to the studies of biological activity of defatted alcoholic extract of this plant concerning with pharmacotoxicity and antidiabetic activity .

Key words : *Teucrium davaeanum*, Labiatae, volatile oil, terpenoids, flavonoids, pharmacotoxicity and antidiabetic activity .

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SUMMARY

SUMMARY

This thesis includes a study of the chemical constituents of *Teucrium davaeanum* belonging to family Labiatae growing in Sirte region, Libya especially at Wadi Telal .

The thesis includes four parts :-

1- Review of literature:

A complete review of literature concerning the chemical constituents (volatile oils, diterpens, iridoids glycosides, flavonoids and other constituents) as well as the biological activity studies of the *Teucrium* genus.

2- Chemical studies of *Teucrium davaeanum* :-

a – The photochemical screening.

b – Study of the volatile oil:

The volatile oil was prepared by two methods (hydrodistillation and solvent extraction) . The study of the volatile oil (hydrodistillation) using GC/MS technique showed that it is a mixture of 80 compounds in which Spathulnol represents the main constituent (8.8%), while the volatile oil prepared by solvent extraction (ether/hexane 1:1) was found to contain 12 compounds in which Phytol (15%) is the main one .

c - Study of the lipid fraction:

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

Fatty alcohols:

Tetracosanol, octatriacotanol, tetratriacontene, and octatriacontane, in which tetracosanol is the main one (66.95%).

Unsaponifiable fraction :

n-Heptane (2.36%), octane (1.78 %), nonane (8.88 %), decane (15.35%), undecane (6.02%), dodecane (11.38%), tetradecane (21.27%), Hexadecane (7.16%), heptadecane (4.26%), octadecane (3.49%), decosane (2.07%), petacosane (1.31%), Heptacosane (6.39%), octacosane (3.79%), nonacosane (o.54%), hentricontane (0.74%), dotricontane (2.09%)], in addition to two sterols: [β - sitosterol (2.67%) and campasterol (1.49%)] .

Fatty acid methyl esters:

Lauric (2.83%), myristic (3.68%), palamitic (9.03%), oleic $C_{(18:0)}$ (12.33%), oleic $C_{(8:1)}$ (9.36%), linoleic (22.22%), Arachidic (2.71%), erucic (1.82%), lignoceric (1.60%), tetracosenoic (0.88%) . Linoleic acid was the major constituent (27.57 %), in which the saturated fatty acid methyl esters are represented by 15.54%, while the unsaturated one are represented by 82.49% .

d – Study of flavonoidal constituents:-

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

(1) 3, 7, 3', 4' tetramethoxy, 5 – hydroxyl flavone .

(2) 5,7, 3', 4' tetrahydroxy flavone (luteolin) .

(3) 3, 5' dimethyl myricetin .

(4) 5 – hydroxyl, 3', 4', 6, 7, tetramethoxy flavone) .

While investigation of the flavonoidal constituents of the butanol fraction resulted in the isolation and identification of :-

(5) Luteolin –7–*O*–gluco–glucosyl–3'–*O*–rhamnoside .

4 – Biological studies :

1 – Toxicity studies :

Acute toxicity studies of the defatted alcoholic extract of *T. davaeanum* showed that it have a wide marginal safty where it's LD_{50} for the intraperitoneal administration was calculated to be more than 5g / kg. b.wt. .

2 – Antidiabetic activity :

The study of the antidiabetic activity of the alcoholic extract of the studies plant revealed that it reduced the glucose level in the blood after daily administration for two weeks .

INTRODUCTION

INTRODUCTION

Plants have playing as curative and therapeutic agents in preserving human health against disease and decay since the beginning of man's life on the earth [1].

The plant kingdom consists of many families one of them is the Labiatae family. It is one of flowering plants that include 180 genera and nearly 3500 species , found chiefly in the Mediterranean region, Pakistan, India, Malaysia, China, central America and Australia[2] .

Most of the species of the Labiatae family are aromatic square stemmed herbaceous annuals or perennials, while some tropical are trees. Many members of this family have been employed as flavoring agents, spices, in the manufacture of perfumes and primitive medicinal practice[3].

The family is rich in essential oil bearing plants which contain a variety of mono, sesqui- and diterpenes. All twenty-six species of the Labiatae family investigated by Pluatova (1972) [4] contained essential oils up to 2.5 %, plants with high contents of essential oils were reported to accumulate more triterpene acids while plants with low levels of essential oils contained more sitosterol and triterpene saponins .

The pentacyclic triterpene oleanolic acid and/or ursolic acid has been reported in species representing approximately twenty genera[5].

In Libya the family is represented by 22 genera and 65 species[2]. Many studies showed that the Labiatate species have many activities like antioxidant, antibacterial, insect phagostimulant, antitumor, cytotoxic and cytostatic, vasoconstrictor, antifeedant and antifungal [6 - 15].

The genus *Teucrium* belonging to Labiatae family is represented by about 300 species in the world [2]. It represented by 13 species in Libya, six of them being endemic : *T. apollinis*, *T. barbeyanum*, *T. davaeanum*, *T. linivaccarii*, *T. zanonii* and *T. libyaca* [2].

The chemical constituents of *Teucrium* genus include, Neoclerodane diterpenoids , natural phenolic , (Flavonoids and phenolic acid), iridoids glycosides and terpenoidal compounds [16 - 19].

Teucrium species have been used in several countries, like infusion of the leaf of *T. capense* is used by the Europeans as a diabetes remedy and is said to be slightly purgative, *T. incanum* and *T. riparium* were used as a remedy for sore throat and snake bites [20]. The biological activities of *Teucrium* species includes anti-inflammatory [21] , hypolipidemic effects [22] , anti feedant [23] , anti ulcer [24] , and hypoglycemic effects [25] .

The present study deals with the investigation of some chemical constituents of *T. davaeanum* (volatile oils , lipids and flavonoids) in addition to some biological activities of different extracts .

REVIEW OF LITERATURE

monoterpenes were found to be 29 % and 34 %, in which α-pinene **(12)** (16%) of both oils being the major constituent [27] .

In 1997 the oils of *T. haenseleri* analyzed by GC-MS . The results revealed that the oil of flowering and vegetative stage consisted mainly of monoterpenes (84 % in the oil from the flowering and vegetative leaves, respectively). α-pinene **(12)** and β-pinene (1) were the dominate components in all the oils (42-54 %). The sesquiterpene fraction (8 – 10 %) was always dominated by δ -cadinene (3-5 %) [28] .

Eikani *et.al.* extracted the volatile oil of *T. polium* by two methods (super critical CO_2 extraction and hydrodistilation). They found that in both cases the major components were sesquiterpens. Germacrene- D **(13)** (23.6 % and 13.2 %) and β- caryophyllene **(14)** (16.5 % and 18.0 %) were the main components in the supercritical extraction and hydrodistilled essential oils respectively [29] .

Volatile compounds from *T. lepicephalum* and *T. carolipaui* studied by Isabel *et.al.* . The GC and GC-MS results showed that the main compounds were mono– and sesquiterpenes [30] .

Cavaleiro *et.al.* in 2004 studied the essential oils from four samples of *T. lusitanicum* and from one sample of *T. algarbiensis* by GC and GC-MS . Seventy one volatile compounds identified . The major compounds of *T. algarbiensis* oil were α – pinene **(12)** (8.3 %). sabinene **(15)** (7.2 %) , β-pinene **(1)** (10.2 %) , limonene **(2)** (11.8 %) , and germacrene-D **(13)** (7.6 %) , concerning *T. lusitanicum*, some quantitative differences were found with regards to the major constituents of the oils from four populations :α- pinene **(12)** (0.8 - 8.5 %) , sabinene **(15)** (2.1- 9.6 %) , β - pinene **(1)** (2.5 -11.9 %), limonene **(2)** (1.2 - 11.5 %) , and elemol (2.6 -12.0 %) [31].

Two sesquiterpene diols,7-epi-cudesm-4 (15)-ene-1-β-6-α-diol, and 7-epi-cudesm-4(15)-ene-1-β-6-β-diol, in addition to sesqualcohols ,β-eudesmol and α-cadinol isolated and identified from *T. polium* and their structure established by spectral data by Kamel [32].

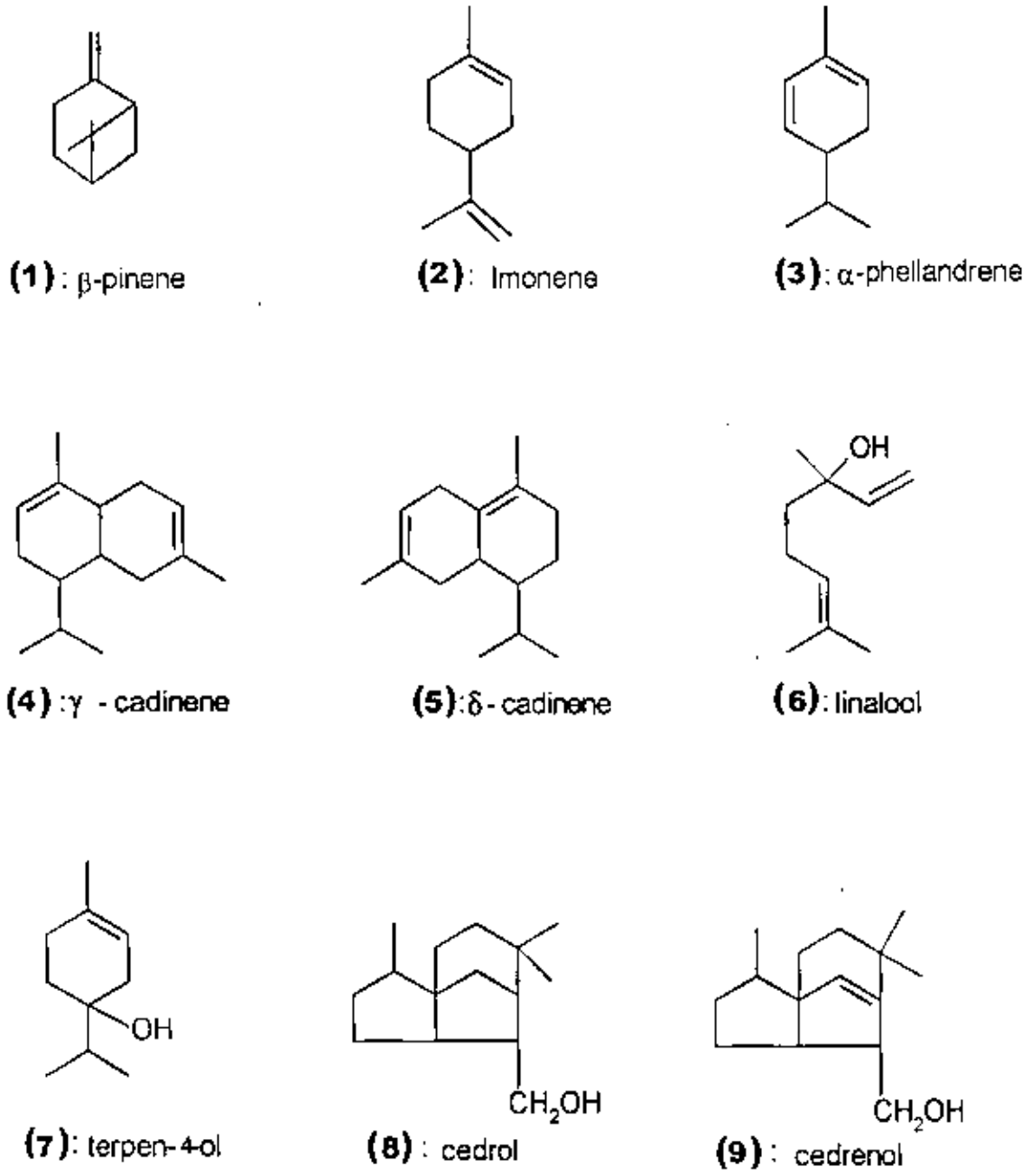
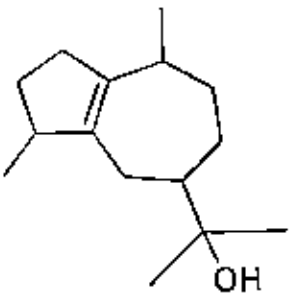
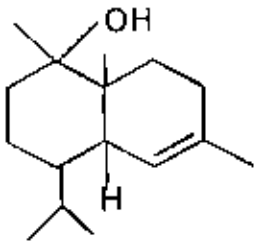


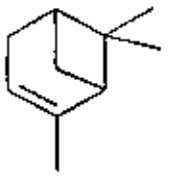
Fig. (1) : Chemical structures of some constituents of volatile oils isolated from *Teucrium* genus .



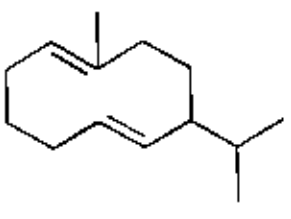
(10):Guaiol



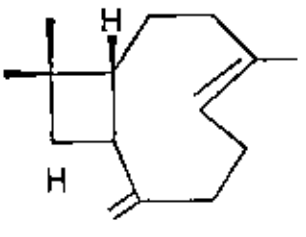
(11) : τ – cadienol



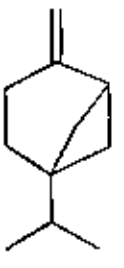
(12) : α - pinene



(13):Germacrene-D



(14):β-caryophyllene



(15): Sabinene

Fig. (1): Cont.

2- Diterpenoids :-

The genus *Teucrium* is 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 [33], Here we listed some of them in table (1) :-

Table (1) : Diterpenoids isolated from *Teucrium* species

Species	Diterpenoid	Reference
T. alyssifolium	Alysin A,B,C,D and 3 –deacetyl alysin D	[34 – 35]
T. arduini	Teucvin (16) , montanin –D (17) , diacetyl montamin D 6ß- hydroxy teuscordin (18), diacetyl teugin, isoteuflidin (19) , teugin (20) , diacetyl dihydro tengin. dihydroteugin (21), 19-deacetylteuscorodol (22) and teuscordinon (23) .	[36]
T. betonicum	Teubetonin .	[37]
T. bicolor	Montanin C (24) , teucvin (16) , 12-epi-teucvin (25) , teupolin (26) ,12-epiteupolin 1 and (12 S) teucrin H_2.	[38]
T. bidentatum	Teucvin (11) and teupernin A.	[39]
T. brevifolium	Teubrvins A , B, C , D , E , F ,G .	[40 - 41]
T. capitatum	Teucapitatin , 19- acetyl gnaphalin (27) and Lolin .	[42]
T. chamaedry	Teucrin ,Teucrin A (28) ,Teucrins B, C , D ,E , F , G , Teugin (20) , dihydroxy teugin (21) ,Teuchameadryn A (29, B (30),6-epiteucrin A (31) ,teucroxide (32) ,Isoteuflidin (19) , teucvidin (33) , tenflin (34) , Teuchamaedrin Syspirensine A , B 12(s)-15-16-epoxy-19-hydroxy-neo-cleroda-13,14-dien-18,6α ,20,12,diolide	[43 – 53]

Table (1) : Cont.

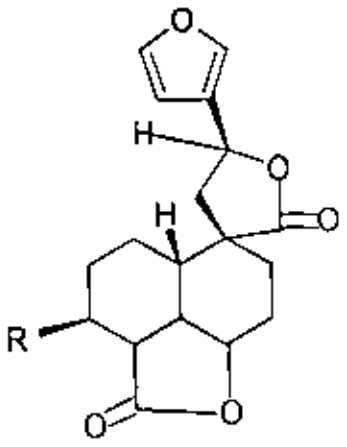
Species	Diterpenoid	Reference
T. eriocephalum	Eriocephalin (35) .	[54]
T. cubense	Eugarzasadine and eugarzasudone .	[55 - 56]
T. decemlineata	Teucrin A (28), teuscorolide (36) teucvin (16) ,teuflin (34) , teuflidin (37), Eriocepholin (35) , 20-deacetyl eriocephalin (38) , capitatin (39) 19-acetyl gnaphalin (27) and picroplinone (40) .	[57]
T. flavum	Teuflindin and Teuflin(34) .	[58]
T. flavum subs.glaucum	Teuflavin and 19-nor- clerodane glucoside teuflavin	[59]
T. fragile	Teugin (20) .	[42]
T. fruticans	Fruticolide , fruticolone (41), isofruticolone (42) 8-ß-hydroxy fruticolone (43) and its dehydration and oxidation derivative (44), 7-ß-hydroxy fruticolone, 11-hydroxy fruticolone, deacetyle fruticolone and 6-acetyl-10-hydroxy teucjapenin- B.	[60 - 63]
T. gnapholodes	Teugnaphalodin (45), gnaphalin,19-acetyl gnaphalin gnaphalidin, Teucrin P_1(46) and Isofruiticolone (42).	[64 - 66]
T. homotrichum	Eriocephalin and 19-acetyl gnaphalin.	[54]
T. hyrcanium	Teucrin H_1 (47) H_2 (48) , H_3 (49) and H_4 (50).	[67 – 68]
T. intricatum	Teucvin (16) .	[69]
T. kotschyaum	Isoteucrin H_4 and teucrin H_4 (50).	[70]
T. lanigrum	2-*O*-deacetyl eriocephalin, isoeriocephalin and eriocephalin (35) .	[69]

Table (1) : Cont.

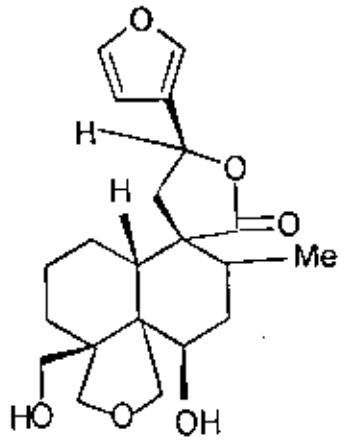
Species	Diterpenoid	Reference
T. maghrebinum	12-epiteucjaponin- A,12-epimontanin- D,12-epi-montanin-B,teucjaponin- A,montanin- D (17) , 19-deacetyl tenscorodol (22) , teusalvin- C and montanin- B (51).	[71]
T. marum	Teumarin.	[72]
T.massitiense	619-diacetyl teumassilin, montanin-C (24) , teucjaponin-A (52) , teumassilin deacetyl ajugarin and teumassilenins A , B , C ,D .	[73]
T. montanum	Montanin A (53) , B (51) , montanin C (24) , D (17) , *E* and F.	[74 - 79]
T. montbretti subsp lipaticum	3-ß-hydroxy teubutilin- A,12-epimontanin- G,20-epi-3-20-di-*O*-deacetly teupyreinidin, teuscordinon, (32) , 6-ketoteuscordin ,montanin- D(12),320-di-*O*-deacetyl teupyreinidin, montanin- G and 2-*O*-deacetyl teugracilin-A	[80]
T. oliverianum	Teucdivins G,H ,Teucrolins A,B,C,D,E, Teucrolins F,G.	[81 - 83]
T. pernyi	Teucvidin,teuflin (34) and teupernin D.	[84]
T. polium	19-acetyl gnaphalin, picripolin,6-acetyl picripolin teucrin P_1 (46), P_2, P_3, H_3 (49), teupolin I(26), II (54), IV (55), V (56) montanin B (51), isopocropolin, auropolin, and teulolin A , B.	[54 , 76] [85 - 92]
T. polium subsp. capitatium	7-deacetyl capitation (57), picropolinol (58) , 20-epi-isoeriocephalin (59), teupyrenone, teupyreinine and teupyreinidin .	[93– 94]
T. quadrifarium	12-epi-teucvidin, teuflin (34), 19-acetyl-teuspinin, eucvidin (33) and teuquadrin B .	[95]
T. salviastrum	teusalvins A(60) , B ,C , D , E and F .	[96]

Table (1) : Cont.

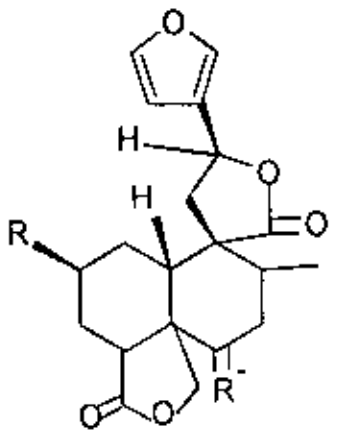
Species	Diterpenoid	Reference
T. scordium	6-ß-hydroxy-teuscordin, 2ß, 6ß,dihydroxyteuscordin6-keto-teuscordin, teuscordinon (23), 2-keto-19-hydroxyteuscordin, teucrin H_4 50) and montanin E (61) .	[97 - 98]
T. scorodonia	Teuflin, teuscordin, teuscordonin, teuscorolide,teuscorodin,2-hydroxy and teuscorolide .	[54] [99]
T. spinosum	Teuspinin ,19-acetyl teuspinin and 19-acetyl gnaphalin	[100]
T. tomentosum	Teuctosin (62),teuflin (34),teucrin H_2(48),6ß-hydroxy ,teuscrodin(18),6ß-acetyl teuscordin (63) and montanin-D(26)	[93]
T. viscidum	Teucvin (16), teucvidin (28),and its keto ester derivative (64) and teuflin (34) .	[101 - 104]



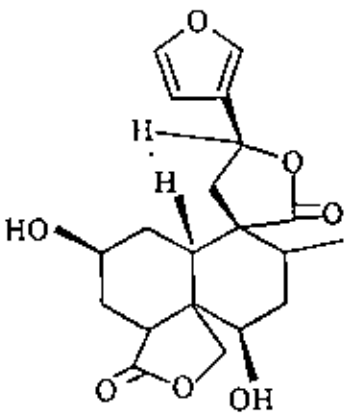
(16):Teucvin(R=H)
(19):Isoteuflidin(R=OH)



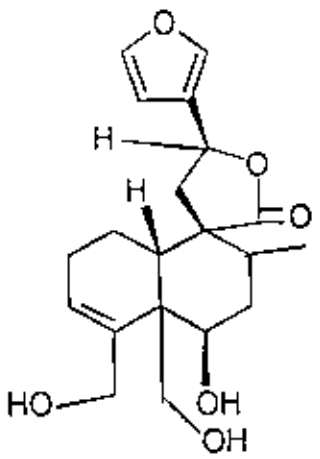
(17):Montanin-D



(18):6-β -hydroxyteuscordin(R=H ,R=β -OH)
(20):Teugin(R=OH , R= -OH)
(23):Teuscordinone(R=H ,R=O)

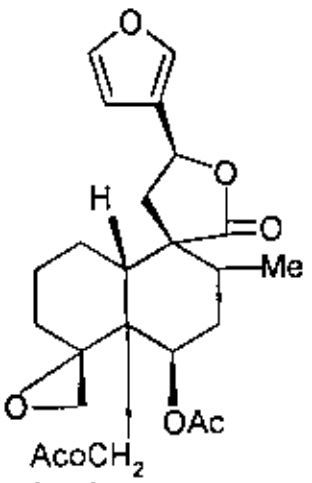


(21) :Dihydroxyteugin

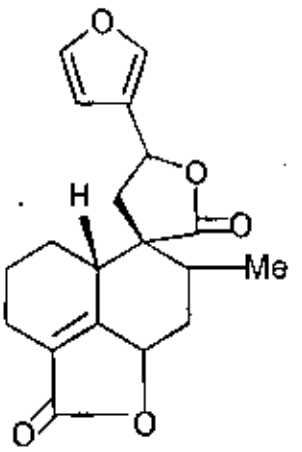


(22):19-deacetyl teuscorodol

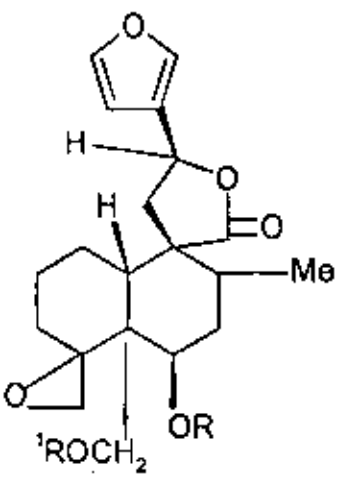
Fig. (2) : Chemical structures of diterpenoids isolated from *Teucrium* genus .



(24) :montanin-C

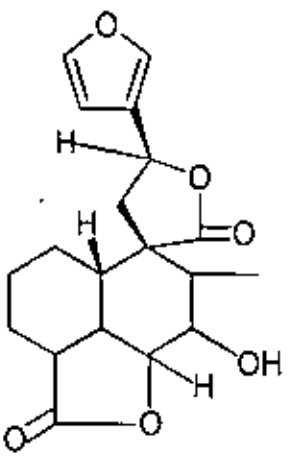


(25):12-epiteucvin



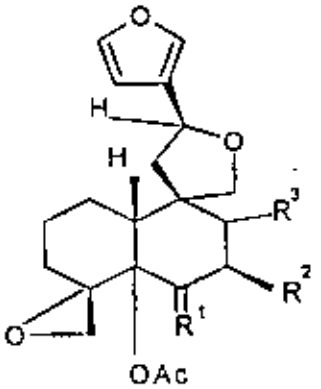
(26): Teupolin- I (R=H, R^1=AC)

(54): Teupolin- II (R=AC, R^1=H)

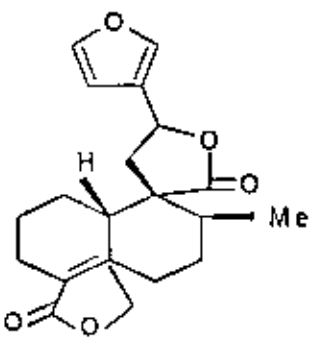


(28):Teucrin-A

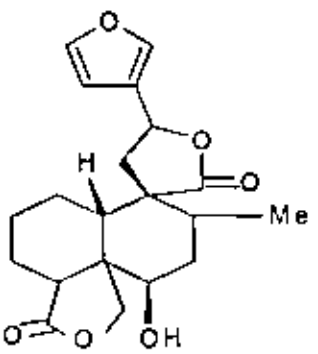
Fig. (2) : Cont



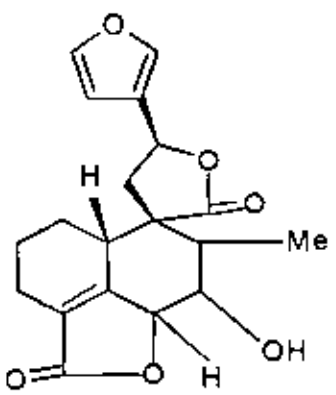
	R^1	R^2	R^3
(27):19 -acetyl gnaphlin	-O-	H , H	-O-
(35): Eriocephalin	-O-	α -OH , β- H	α -H , β - OAC
(38):20-deacetyl eriocephalin	-O-	α -OH , β- H	α -H , β -OH
(39): Capitatin	-O-	α -OAC, β-H	-O-



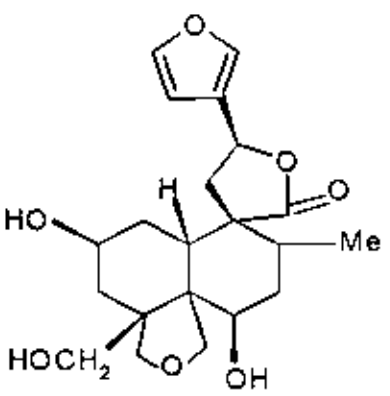
(29):Teucha meadryn-A



(30):Teucha meadryn-B

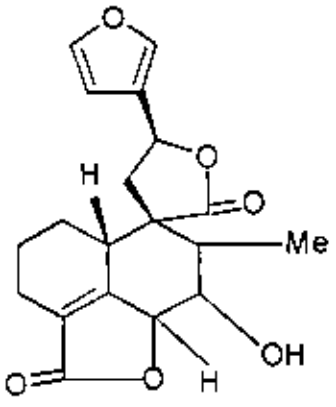


(31):6 -epiteucrin

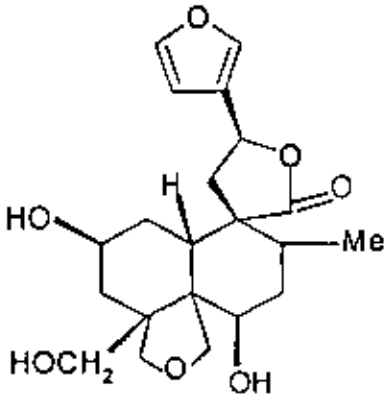


(32):Teucroxide

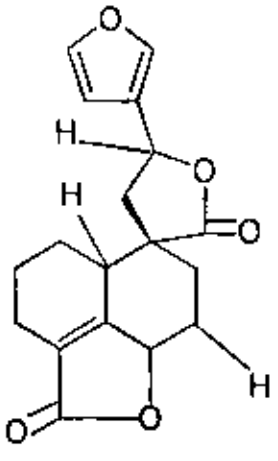
Fig. (2) : Cont.



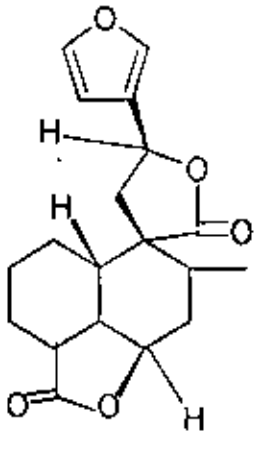
(31): 6 - epiteucrin



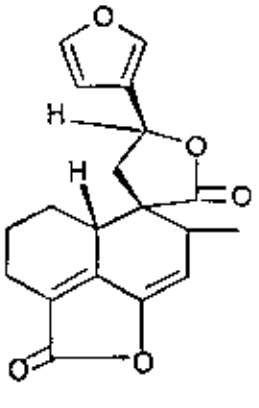
(32): Teucroxide



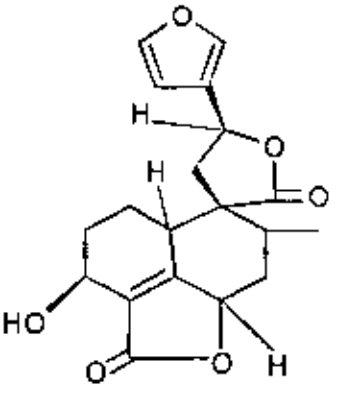
(33) :Teucvidin



(34) : Teuflin

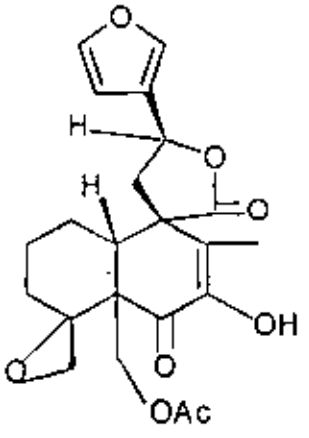


(36) : Teuscorolide

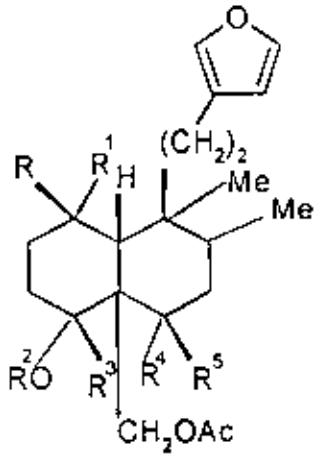


(37): Teuflidin

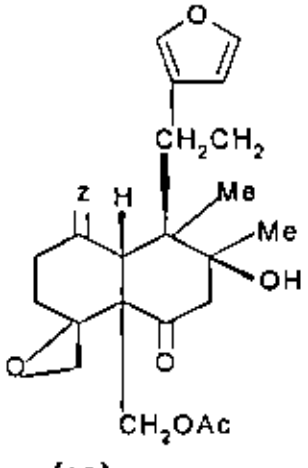
Fig. (2) : Cont.



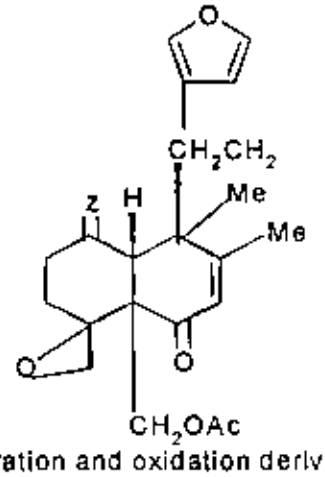
(40): picropolinone



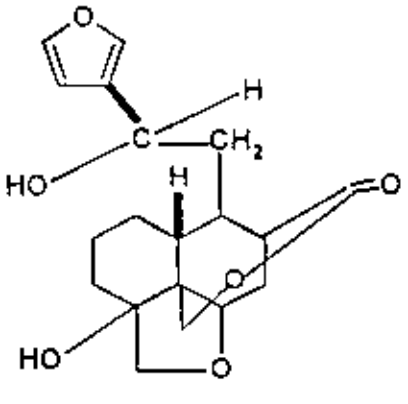
	R	R^1	$R^{2,3}$	R^4	R^5
(41): Fruiticolone	H	OH	CH_3	-O-	-O-
(42): Isofruiticolone	-O-	H	CH_3	H	OH



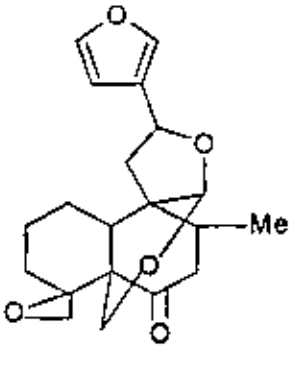
(43): 8 - β -hydroxy fruiticolone
(z=α -H , β -H)



(44) : Dehydration and oxidation derivative o
8 - β -hydroxy fruiticolone
(z=α -OH , H, O)

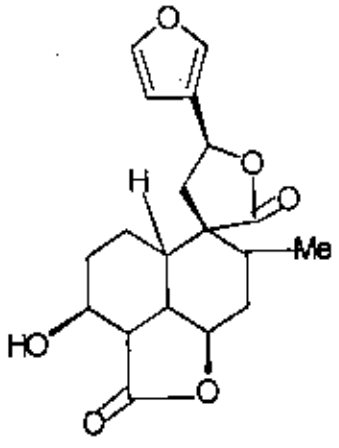


(45) :Teugnaphalodin

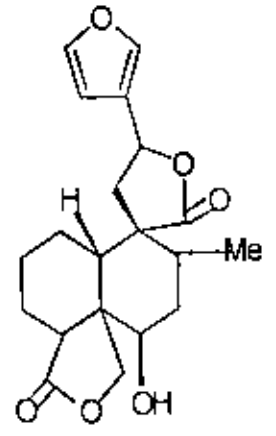


(46) : Teucrin - P_1

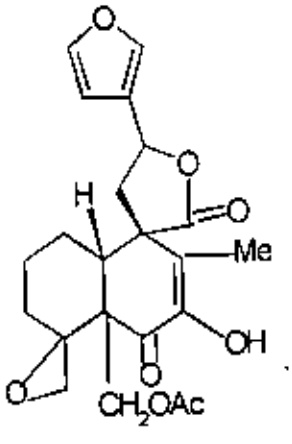
Fig. (2) : Cont.



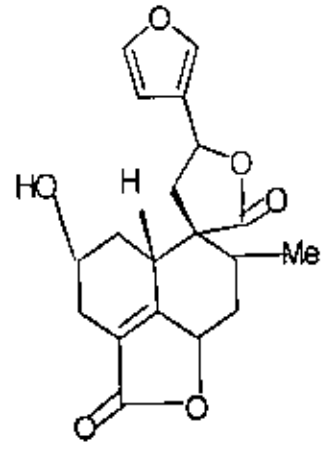
(47) :Teucrin -H_1



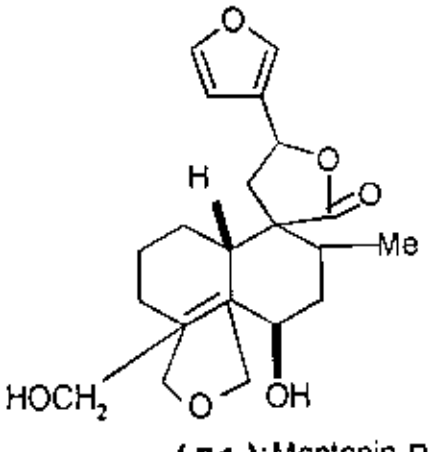
(48) :Teucrin-H_2



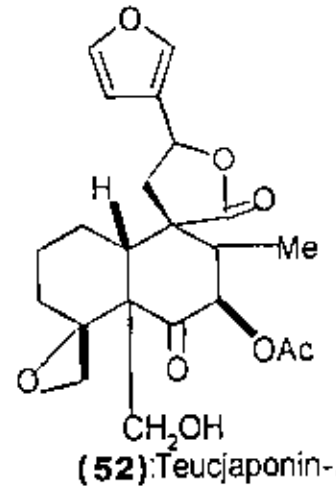
(49) :Teucrin-H_3



(50) :Teucrin-H_4

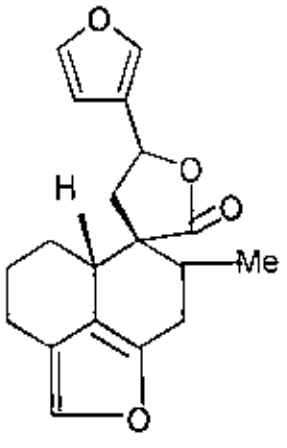


(51) :Montanin-B

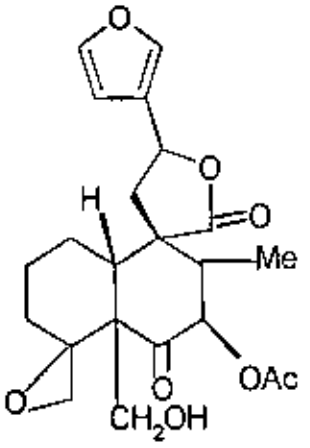


(52):Teucjaponin-A

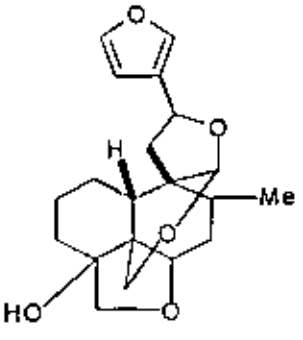
Fig. (2) : Cont.



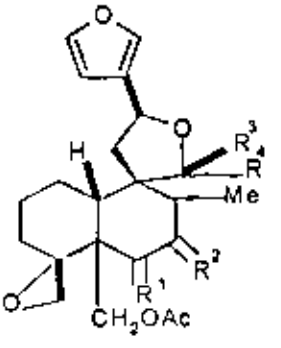
(53) :Montanin-A



(55) :Teupolin-IV

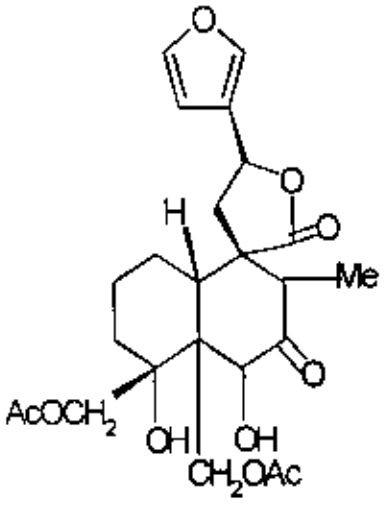


(56) :Teupolin-V

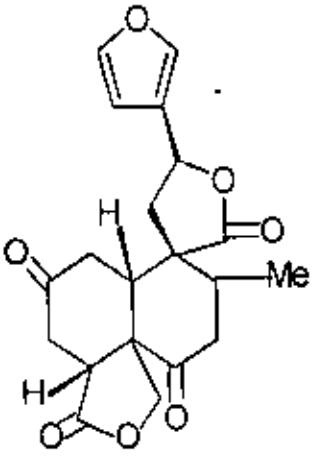


(57) : 7 -deacetyl capitatin ,
(R^1=O , R^2=α -OH , -H , R^3,R =O)

(59) :20- epi-Isoeriocephalin
(R^1=α -OH , -OHβ R^2 = O , R^3 = H, R^4= O,

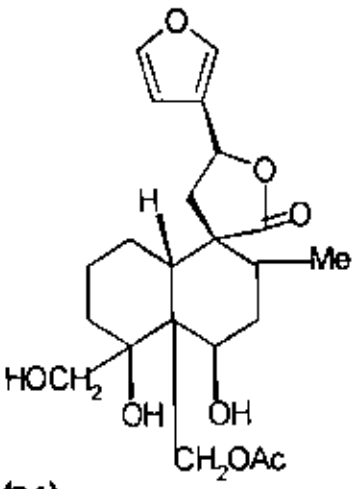


(58) : picropolinol

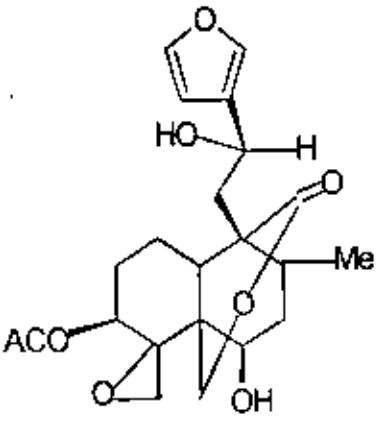


(60) :Teusalvins-A

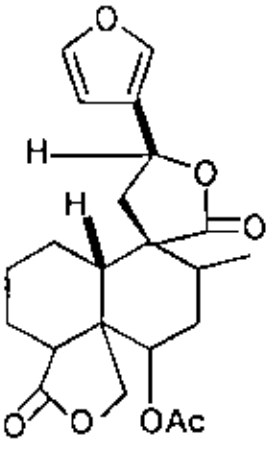
Fig. (2) : Cont.



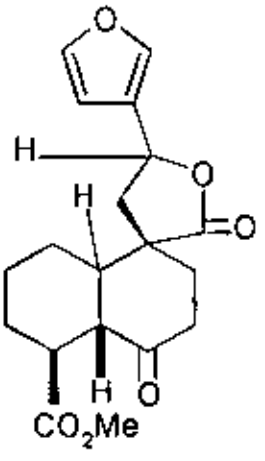
(61) : Montanin-E



(62) : Teuctosin



(63) : 6-β- acetyl teuscordin



(64): Ester derivative of -β- acetyl teusc

Fig. (2) : Cont.

3- Iridoid glycosides :-

The iridoids which have been isolated till now are mostly harpagide, and its acetate. However, reptoside, ajugol , ajugoside and teuhicosid were also reported . Iridoids diglycosides as teucardoside has been isolated from some *Teuerium* species[105] .

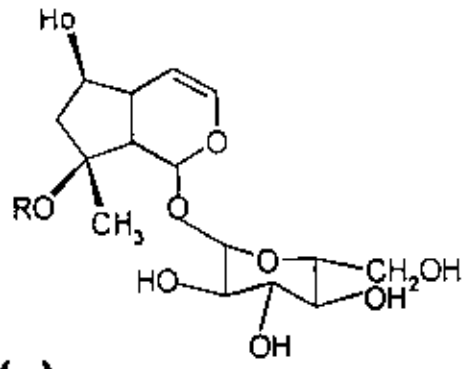
Table (2) represented the most common iridoid glycosides isolated from *Teucrium* species.

Table(2) : Iridoid glycosides isolated from *Teucrium* species

Species	Iridoid glycosides	References
T. arduini	Acetyl harpagide , ajugol (65) , ajugoside (66), reptosid (67) and teucardoside (68) .	[106]
T. aureum	Harpagide (69) and acetyl harpagide.	[106 - 107]
T. hicolor	Harpagide (69) and unidentified heteroside .	[106 - 107]
T. botrys	Harpagide (69), acetyl harpagide and teucardoside	[106 - 107]
T. canadense.	Harpagide (69) and acetyl harpagide	[106 - 107]
T. chamaedrys	Harpagide (69) and acetyl harpagide	[106 , 108]
T. cubense	Acetyl harpagide , reptoside (67) and unidentified iridoid .	[106 , 108]

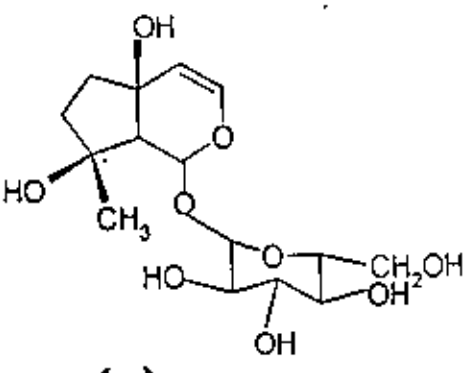
Table (2) : Cont.

Species	Iridoid glycosides	References
T. flavum.	Harpagide (69) , acetyl harpagide and unidentified heteroside..	[106 , 108]
T. fruiticans.	Harpagide (69) ,acetyl harpagide traces of 2 unidentified heterosides.	[106 , 108]
T. hircanicum	Harpagide (69) , acetyl herpagide teucardoside (68), teuhircoside (70)	[106 , 108]
T. lucidum.	Harpagide (69) .	[106 - 107]
T. massiliense.	Harpagide (69) and acetyl harpagide	[106 - 107]
T. montanum.	Harpagide(69) and acetyl harpagide	[106 - 107]
T. oliverianum	8-*O*- acetyl harpagide (71).	[82]
T. orientale	Harpagide (69).	[106 , 109]
T. polium	Harpagide (69) ,acetyl harpgide and teucardoside (68) .	[110]
T. pyrenaicum	Harpagide (69) ,acetyl harpagide and teucardoside (68) .	[107 - 109]
T. scordium	Harpagide (69) ,acetyl harpagide and unidentified heteroside .	[107 - 108]
T. scorodonia	Harpagide (69) ,acetyl harpagide reptoside (67), 2 unidentified iridoids .	[107 , 108]
T. taylori	Harpagide (69)	[106 , 108]
T. yemense	Teucardoside (68) and 8-*O*-acetyl harpagide (71).	[18]

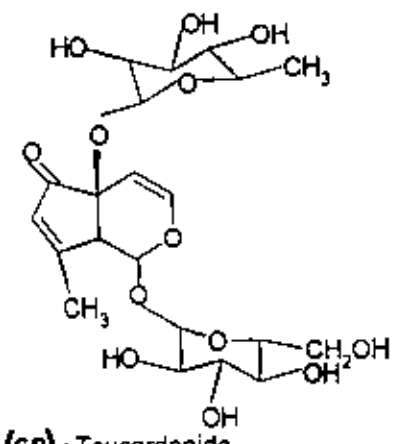


(65) : Ajugol R=H

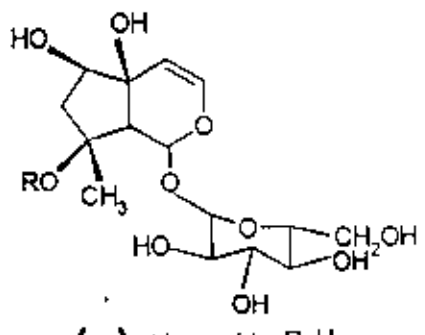
(66) : Ajugoside R=$COCH_3$



(67) : Reptoside

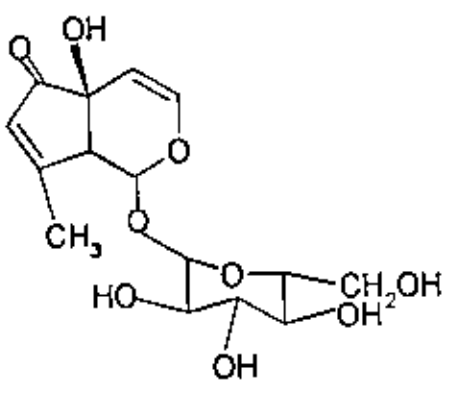


(68) : Teucardoside



(69) : Harpagide, R=H

(71) : 8-O-acetyl harpagide, R=$COCH_3$



(70) : Teuhircoside

Fig. (3) : Chemical structures of some iridoids isolated from *Teucrium* genus.

4- Flavonoids :-

Grzybele *et.al.* in 1968 studied the flavonoids from *T. montanum* , *T. chamaedrys* and *T. scordium* and identified four compounds : Diosmin **(72)**, quercitin **(73)**, isoquercitin **(74)** and rutin **(75)** by spectral data and chemical correlation [111] .

Briskorn and Biechele isolated one flavonoidal compound from *T. polium* which was identified as 6-methoxy genkwanin (salvigenin)**(76)** [112] .

Raynaud and Chauikha in 1976 isolated apigenin 6,8 di-*O*-glucoside **(77)** from *T. ramosissimum* ,its structure was established by thin layer chromatography, IR and UV spectroscopy [113] .

Six flavonoids Known as (3,5,6-trihydroxy -4,7-dimethoxy flavone **(78)**, luteolin-7-*O*-*β*-D-glycopyranosid, 3,4,5,7-tetrahydroxy flavone (Kaempferol) **(79)**, 4,5,7 trihydroxyflavone (Apigenin), 5,6,7-trihydroxy flavone **(80)** and 4,5,6,7-tetrahydroxy flavone **(81)** isolated and identified from *T. nuchense* by Slynkova *et.al.* [114] .

Savona *et.al.* in 1979 extracted the flavonoids from *T. pseudochamaepitys* and they found that, the main compounds are: Eupatorin **(82)** and 5-hydroxy -3,4,6,7- tetramethoxy flavone **(83)** [115] .

Barberan *et.al.* investigated the flavonoids of *T. gnaphalodes*. They isolated and identified narigenin, luteolin **(84)** , apigenin **(85)** , from ether extract. Also, they identified monoglycosides, luteolin 7-*O*-*β*-D-glycoside **(86)** and apigenin-7-*O*-*β*-D-glycoside and diglycosides luteolin-7-*O*-*β*-D-rutinoside, luteolin-7-*O*-*β*-D-neohesperiodoside and luteolin-7-*O*-*β*-D-sambubioside from the butauol extract [116] .

Methylated flavones known as: 5,7dihydroxy-4-methoxy flavone (acacetin) **(87)** and 5- hydroxy-6,7,4-trimethoxy flavone (salvigenin) isolated

from the chloroformic extract of leaves of *T. polium* , while the flavones apigenin and luteolin and the flavonol kaempferol isolated from ethyl acetate extract [117] .

The following four methylated flavones obtained from the leaves of *T. polium* (cirsimaritin, eupatorin **(82)**, 4',7-dimethoxy apigenin **(88)**, cirsiliol**(89)** by Verykokidou *et.al.* [118] .

From the *T. polium* var. *Pilosum* and *T. polium* var. *alba* the lipophilic flavonoids salvigenin **(76)** and cirsiliol **(89)** isolated and identified by Rizk *et.al.* [119] .

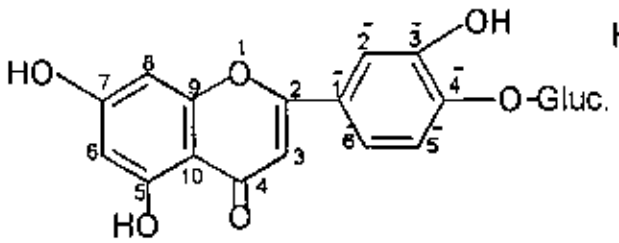
Xie *et.al.* in 1990 extracted the flavonoids from *T. quadrifarium* and identified: 6,2'- dimethoxy-5,4,5 triydroxy flavone **(90)** [120] .

Carmo and Nascimento isolated and identified two Flavone 5,4 dihydroxy -6,7- dimethoxy flavone (cirsimaritin) and 5,3,4, trihydroxy- 6,7 dimetoxy flavone (Cirsiliol) **(89)** from *T. algarbiense.* [121] .

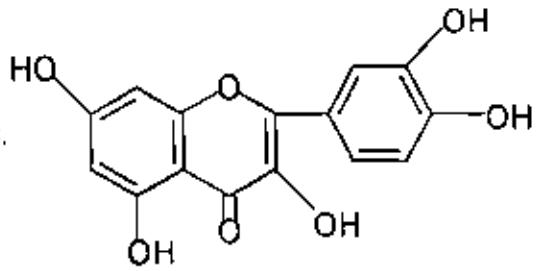
Kalogiera *et.al.* in 1992 extracted flavone compounds from *T. arduini* known as:(luteolin**(84)**, apigenin **(85)**,quercetin **(73)** and their structures were confirmed by UV and mass spectroscopy [122].

The identity of both (eupatorin **(82)** and cirsilliol **(89)** which isolated from *T. oliveranum* confirmed by Alyahya *et.al.* [82]

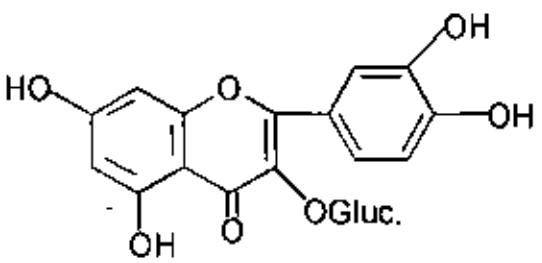
Kawashty *et.al.* in 1997 extracted three flavonoidal compounds (Apigenin 7-*O*-glucoside, apigenin 6,8 di-C-glucoside (vicenin-2), luteolin -7-*O*- glucoside**(86)** and apigenin 5-*O*-galloylglucoside) from *T. leucocladum* and *T.polium* [123] .



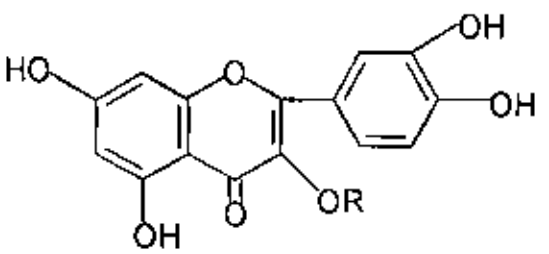
(72): Diosmin



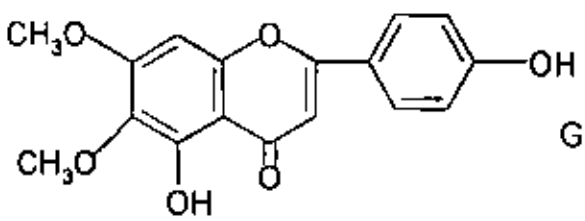
(73):Quercetin



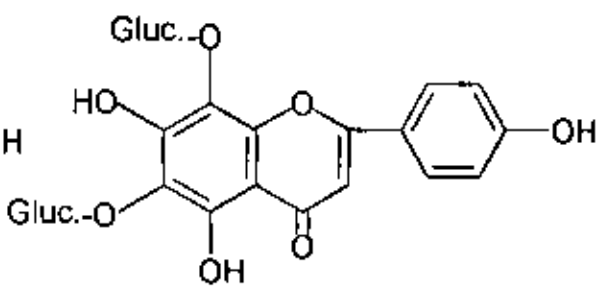
(74): Isoquercetin



(75): Rutin, R=Rutinose

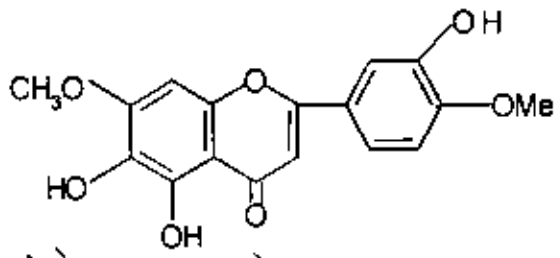


(76) : Salvigenin

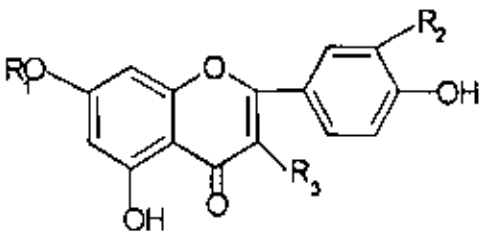


(77):Apigenin-6,8-di-O-glucoside

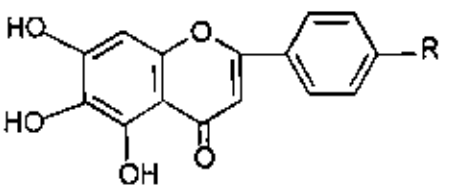
Fig.(4) :Chemical structures of some flavonoids of *Teucrium* genus .



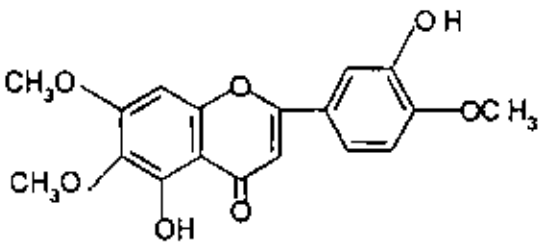
(78):3,5,6-trihydroxy 4,7-dimethoxy flavone



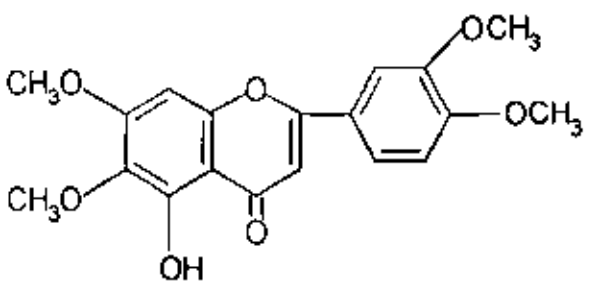
	R_1	R_2	R_3
(85): Apigenin	H	H	H
(79):5,7,3,4`tetra hydroxy flavone	H	H	OH



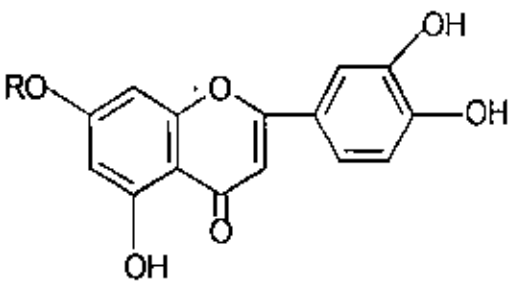
	R
(80):5,6,7 trihydroxy flavone	H
(81):4`,5,6,7 tetrahydroxy flavone	OH



(82):Eupatorin



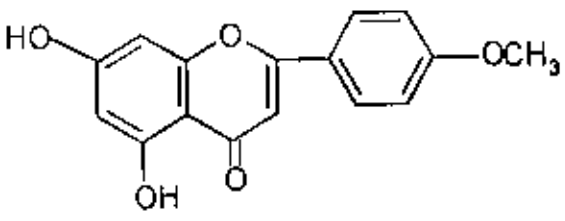
(83):5-hydroxy-3,4`,6,7- tetramethoxy flavone



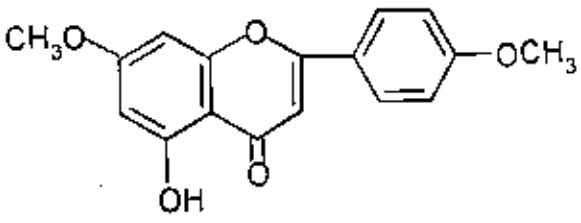
(84): Luteolin, R=H

(86): Luteolin-7-O-glucoside, R=Glu

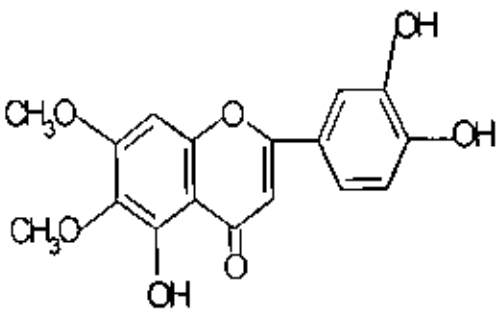
Fig. (4) : Cont.



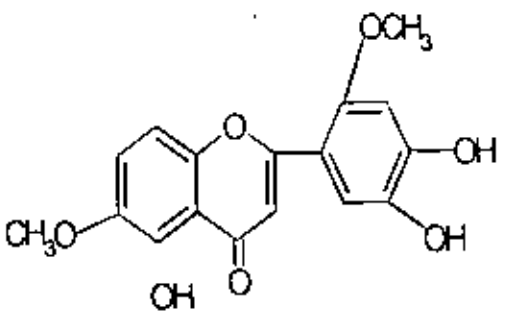
(87): Acacetin



(88): 4`, 7-dimethoxyapigenin



(89): Cirsiliol



(90): 6,2`dimethoxy5,4`,5`trihydroxy flavone

Fig. (4) : Cont.

5 - Triterpenes and Sterols :-

The triterpenes, ursolic acid **(91)** and *β*-amyrin **(92)** detected in *T. chamaedrys* , *T. scorodonia* and *T. polium* [124, 125, 126].

The fernane-type triterpeoid, named integrifolin isolated from the arerial parts of *T. integrifolium* and its structure confirmed by spectral properties and x-ray crystallography [127].

The sterols, *β*-sitosterol **(93)**, stigmasterol **(94)**, campesterol **(95)**,brassicasterol **(96)** and cholesterol **(97)** isolated from *T. polium* and identified using GC, MS, and NMR spectra by Capasso *et..al.* [128].

AL-Yahya *et.al.* in 1993 extracted the sterol fraction and identified one component known as 24(s)-stigmasta-5,22,25-trien-3-*β*-ol,from the aerial part of *T. oliverianum* [82].

Cholesterol **(97)**, and cholesteryl acylglucosides isolated by Kisiel *et.al.* from *T. montanum subsp ponnonicum* [129].

6 - Other constituents :-

Among the carbohydrates identified from *T. canadense* were sucrose, raffinose , planteose , stachycose, verbascose[130], Also, galactose, glucose, and fructose identified in *T. chamaedrys* in addition to arabinose ,raffinose, rhamnose and ribose [127, 131]. The PC and TLC examinations revealed the presence of rhamnose ,glucose,fructose, sucrose, raffinose and rhamnose in the hydrolysate of *T. polium* [132].

Alkaloids detected in number of *Teucrium* species: *T. marum,T. montanum*, *T. scordoides*, *T. eriocephalum*, *T. chamaedrys* and *T. orientale* by Petricic *et.al.* [133, 134]. Stachydrine isolated from *T. polium* the only identified alkaloid in *Teucrium* species [132].

The amino acid composition of *T. polium* cylindricum normal low levels of 8-containning compounds and significant levels of glycine-aspartic acid and glutamic acid . Alkanes and *β*-eudesmol observed in aq. and org. extracts of *Teucrium* plant [135] .

The cyclopentanoid monoterpenes , allodolicholactone and 2-formyl-3-methyl cyclopentyl acryaldehyde and its C_2-epimer were detected in *T. marnm* [136] .

Pagnoni *et.al.* in 1976 extracted and identified four cyclopentanoid monoterpenes , the same aldehyde (acryaldehyde) also isolated from *T. polium* as a cis-trans and trans- cis mixture [136] .

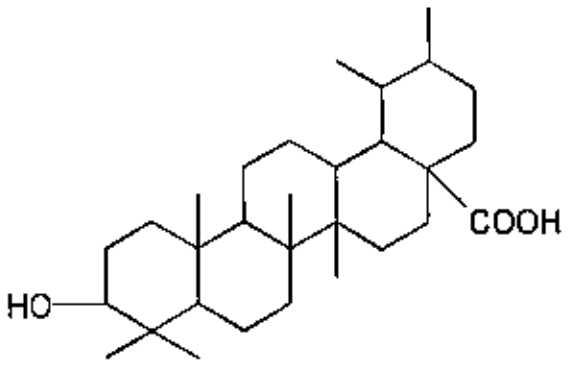
Poliumaside , a ceffeic acid glycoside ester was extracted by Andray *et.al.* [137] from *T. belion* and its structure elucidated by MS, ^1H and ^{13}C-NMR spectroscopy.

From the MeOH extract of *T. chamaedrys*, phenyl propanoid glycoside named teucrioside [3,4-dihydroxy-*β*-phenyl, ethyl-*O*-α-D-xylopyranosyl-(1→3)-α-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-D-glucopyranoside]was isolated and identified by Stiche r and Lahloub [138] .

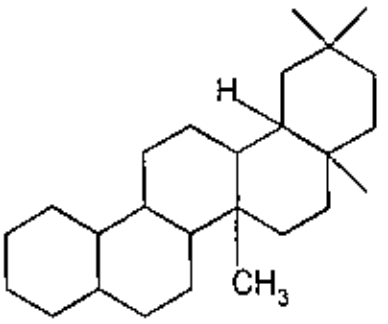
The phenolic acids and the phenyl ethanoid glycosides found to be mutually exclusive apart from one species *T. scorodonia* [139] .

From *T. pilosum*, two natural phenolics [Teucrol (98) , decarboxy rosmarinic acid and its triglycoside ,teucroside (99) (9-decarboxy rosmarinic acid –4–*O*–α-rhamnosyl (1→6)-*O*-*β*-galactosyl-(1→4)-*O*-α-rhamnoside)] were identified by Amani *et.al.* [140] .

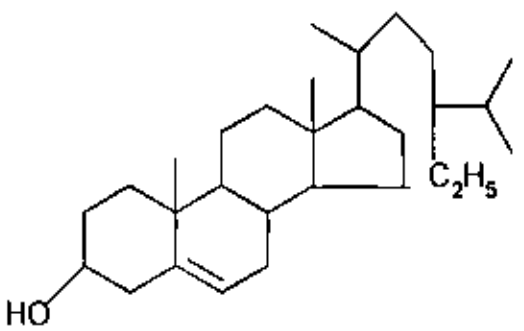
From the aerial parts of *T. chamaedrys* two phenyl ethanoid glycoside known as (teucrioside-3-*O*-methyl ether and teucrioside-3,4-*O*-dimethyl ether) were isolated and identified by Kawashty *et.al.* [123] .



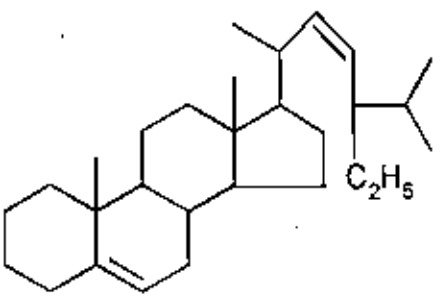
(91): Ursolic acid



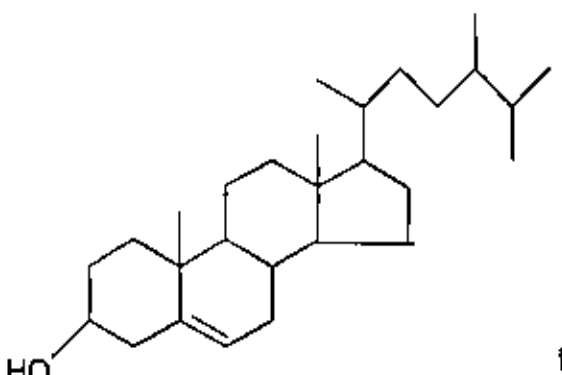
(92) : β- amyrin



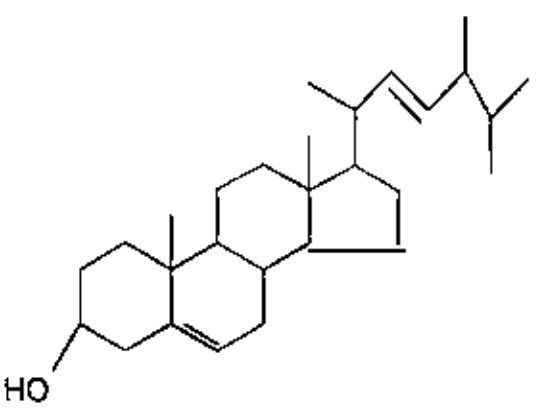
(93): β- sitosterol



(94): stigmasterol

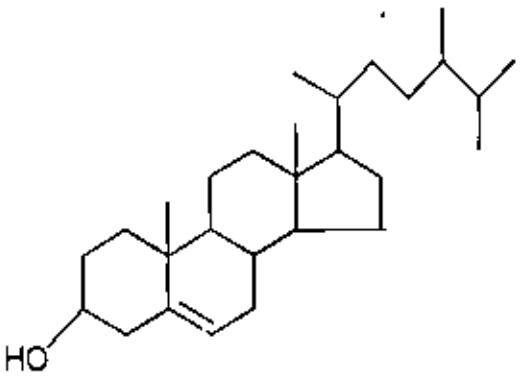


(95) :Campesterol

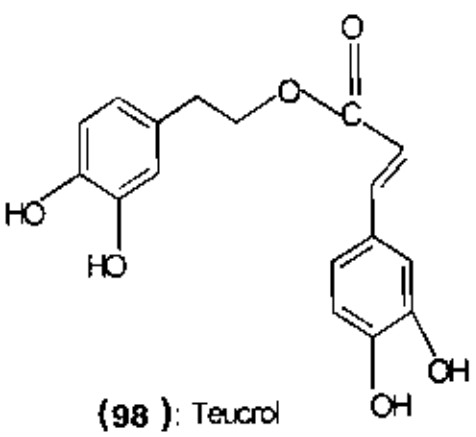


(96) :Brassicasterol

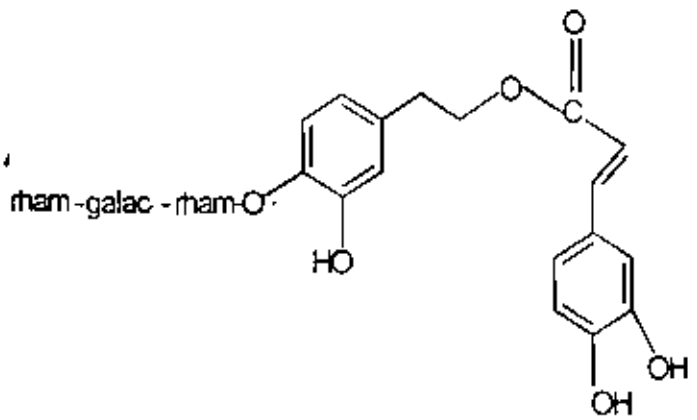
Fig. (5) : Chemical structures of some triterpens , sterols and other constituents isolated from *Teucrium* genus .



(97) : Cholesterol



(98): Teucrol



(99): Teucroside

Fig. (5) :Cont.

7- Biological activity :-

Debat *et.al.* studied the biological activity of the aldehydic compounds isolated from *T. polium*. They stated that these componds have bactericidal activity against *Staphylococcus aureus* at 1.75 mg/ml and against *Escherichia coli* at 1.5 mg/ml. It was also bronchodilator and have antianaphylactic activity at carrageenan odema in rats and they were muscle relaxant at 10 mg/ml *in Vitro* [141].

The antifeedant activity of clerodane diterpenoids from *Teucrium* species confirmed by Simmonds *et.al.* The results showed that these compounds are effective against larvae of *Spodoptera littoralis* and *Tteliothis armigera* [142].

The anti-inflammatory activity of the alcoholic extract of *T. polium* tested using male rats against carrageenan induced paw odema.The extract showed a sufficient pharmacological activity compared to that of indomenthacine [128].

The antipyretic and antibacterial activities of the ethanolic extract of the flowering tops of *T. polium* studied by Autora *et.al.* they found that the extract was effective against both yeast and carrageenan pyrexia in rats. Also, it exhibited a marked antibacterial action against both gram positive and gram negative organisms and was found to be nontoxic in acute studies [143].

The hypoglycemic (antidiabetic) activity of *T. oliveronum* studied by Mossa, where he injected the aqueous extract into albino male mice of (250 - 300 g). The results revelead that the extract has significant activity (more than 25% decreases in blood glucose) [144].

The hypoglycemic activity of an aqueous decoction of aerial parts of *T. polium* tested in normal glycemic and streptozacin hyperglycemic rats by Gharabeh *et.al.* .The results indicated that this extract caused significant

reductions in blood glucose concentration after one week of 4 hours intravenous (I.V) adminstration and after 24 hours intraperitonal (I.P) adminstration . This effect could be due to enhancement of peripheral metabolism of glucose rather than increase in insulin relase [145] .

The effect of ethanolic extract of *T. polium* on carrageenan-induced acute inflammation, cotton pellet granuloma and some of the biochemical parameters investigated by Tariq *et.al.*. They found that the ethanolic extract at a dose of 500 mg/kg body weight produced significant inhibition of carrageenan-induced inflammation and cotton-pellet granuloma. Biochemical studies showed a significant decrease in glucose level. The presence of flavonids and sterols might be responsible for the anti-inflammatory activity of this plant [146] .

Roman *et.al.* in 1991 studied the hypoglycemic effect of *T. cubense* . The studies realized in 27 rabbits submitted weekly to glucose tolerance tests after gastric adminstration of water extract of the plant The results showed that the plant caused 19.4 % decrease in glucose blood [147].

The clerodane diterpenoids isolated from *Teuerium* species assayed for insect antifeedant activity by Sosa *et.al.*. Among the tested compounds : furanoditerpenes with α-*β*-unsaturated-γ-lactone moieties, or C-4-epoxy substitution with C-5-methyl acetatoxy or C-12-acyloxy functionalities against *Tenebrio molitor* larvae . It exhibited maximal antifeedant and repellent activities [148] .

Ortego *et.al.* in 1995 studied the effects of neo-clerodane diterpenes from *Teucrium* species on feeding behavior of *Colorado potato beetle* larvae. They stated that the choice and no-choice tests suggested that the teuscorolide acts as feeding deterrent whereas the anti- feedant activity of teucrin-A,

teucvin and eriocephalin, was likely to be associated with a toxic mode of action [149].

Vincenzi *et.al.* confirmed the use of *T. chamaedrys* as flavouring material for foods [150].

The effect of *T. polium* extract on the growth and fatty acid composition of *Saccharomyces cerevisiae* and *Yarrowia lipolytica* confirmed by Aggelis *et.al.*. The results revealed that the aqueous extract slightly inhibits the growth of *Saccharomyces cerevisiae* (Ki = 0.029 [g / l] -1) and *Yarrowia lipolytica* (Ki = 0.061 [g / l] – 1), However this extract causes changes in the unsaturation degree (delta / mol) of the cellular lipids and increase of linolenic acid concentration and decrease of oleic one [151].

From the acetone extract of *T. tomentosum* six neo-cleodane diterpenoid isolated, teuctosin ، teuflin **(34)**, teucrin-H, 6-β-hydroxy teuscordin **(18)**,6-β-acetyl teuscordin **(63)**, and montanin-D **(17)**. All the compounds showed effective antifeedancy against *Plutella xylostella* and *Spodoptera litura*lis at [10 mg / cm^2] of leaf area [152].

The hypoglycemic effect of *T. polium* studied by Mohammed *et.al.*. They found that when the crude extract [0.5 g plant powder per kg body weight] was administered orally to a group of sterptozotocin diabetic rats for six consecutive weeks, a significant decrease (64 %) in blood glucose concentration , in treated animals compared to the untreated diabetic rats. In addition, the crude extract significantly enhanced the blood insulin level by almost 16 % compared to the untreated diabetic rats [153].

Josep and Yudesly [154] observed the antifeedant activity of the isolated diterpenoids from *T. fructican* .

Aseem *et.al.* in 2004 studied the bacterostatic and antifungal activities of the essential oil and n-hexane/ether extract of *T. leucocladum* they

showed that broad and potent activity against *Pseudomonas aeruginosa* , *Bacillus subatilis* and *Candida albicans* . A marked larvicidal activity of the essential oil, n-hexane / ether extract and crude ethanolic extract was also observed against *Culex pipiens*, *Musca domestice* and *Ceratitis capitata* larvae [155] .

AIM OF WORK

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Investigation of several *Teucrium* species resulted in the isolation of volatile oils, lipid fraction, flavonoids, diterpenoids, iridoid glycosides, and other constituents like alkaloids and coumarins .

On reviewing the literature, it was found that, there is no previous study on *Teucrinm davaeanum* , so this study aimed to investigate the chemical constituents viz : (volatile oils, lipid fraction and falvonoids) and biological activity of different extracts of *T. davaeanum* growing in Sirt region, Libya.

The aim of the present work can be achieved in the following steps:-

1- Collection of the plant from its growing region , drying and grinding.

2- Extractin of volatile oils by steam distillation and light solvents.

3- Isolation and identification of the constituents of lipid fraction (fatty alcohols, fatty acids , terpenes and sterols) with light solvent (petroleum ether 40 - 60 C°) .

4- Extraction of flavonoids (aglycones and glycosides) with ethyl acetate and butanol respectively.

5- Fractionation and purification of isolated compounds using different chromatographic techniques (PC , CC , TLC , PPC).

6- Identifition of isolated compounds using different chromatographic techniques and spectroscopic methods (UV, MS, ^{1}H , ^{13}C-NMR).

7- Study the biological activity of different extracts and /or the isolated compounds.

PLANT MATERIAL

THE STUDIED SPECIES *TEUCRIUM DAVAEANUM* :-

1- Plant description :-

Suffrutose shruble stem decumbent, subterete, much branched, younger branches covered with soft spreading branched hairs. Leaves ± sessile oblong - lanceolate, attenuate at base, tricrenate in the middle, obscurely nerved above, prominently nerved and densely bullate beneath, revolute margined, densely villose and wooly on both sides. Verticils forming dense ovate - subglobose terminal spikes. Calyx subsessile, membranous, tubular-campanulate, 10-nerved, teeth subequal, triangular,cute.Corolla pale yellowish, tube subincurved, villous, upper lip lobes oblong, suberect, lower lip trilobed, lateral lobes oblong-lanceolate, middle one 1.5 longer than lateral ones, deebly concave and almost conduplicate. Stamens arcuate, filaments sparsely villose. Nutlets black, smooth and rugose [2].

2-Plant material :-

Teucrium *davaeanum* was collected from Wadi Telal . Sirt region, in April 2004 during the flowering stage , the plant was kindly, identified by Dr. Mohammed ElSherif, Biology departement, faculty of science, Garyounis university.

The vavocher speciemen was deposited at the herbarium of Botany dept., faculty of science, Altahady university.

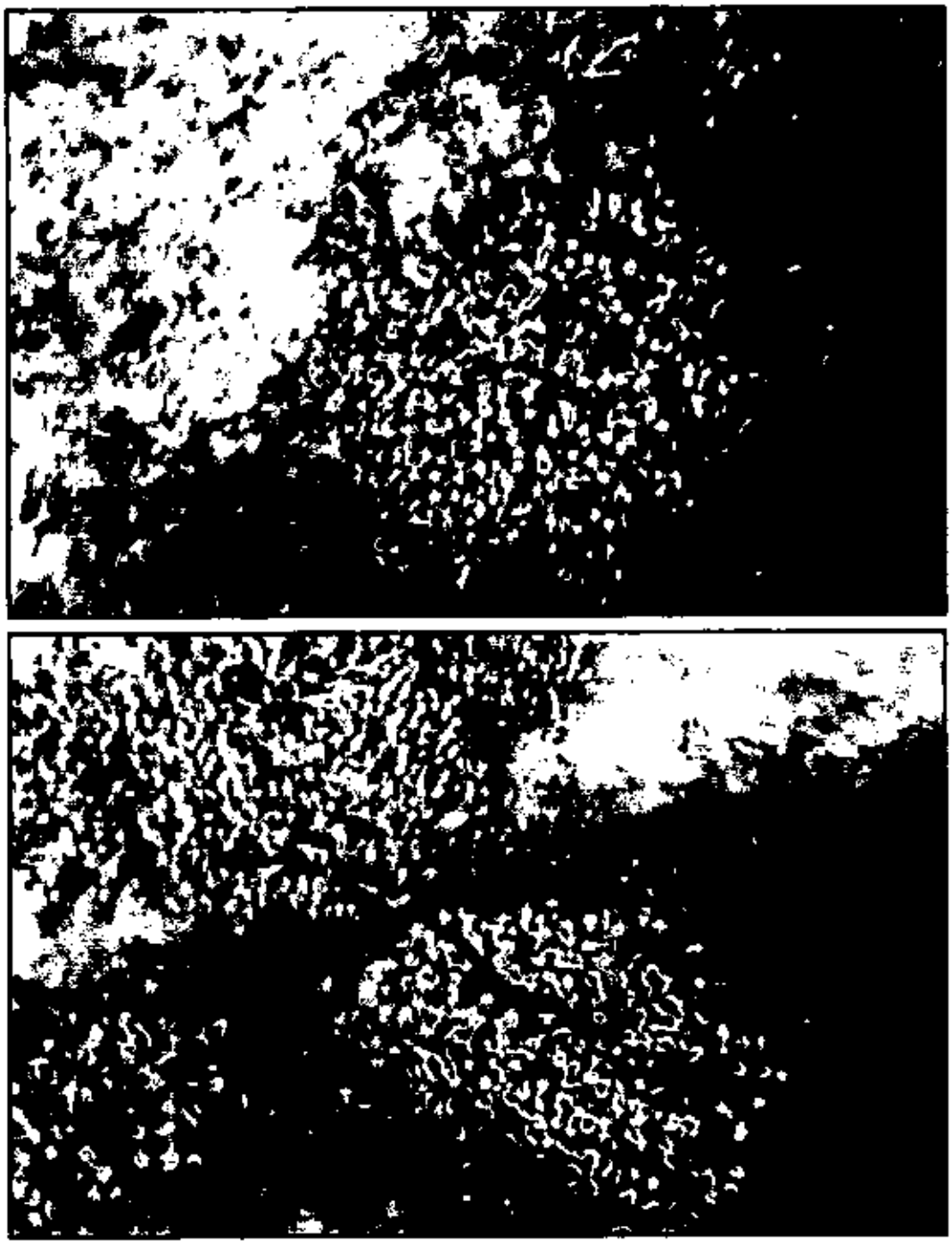


Fig . (6): *Teucrium davaeanum species*

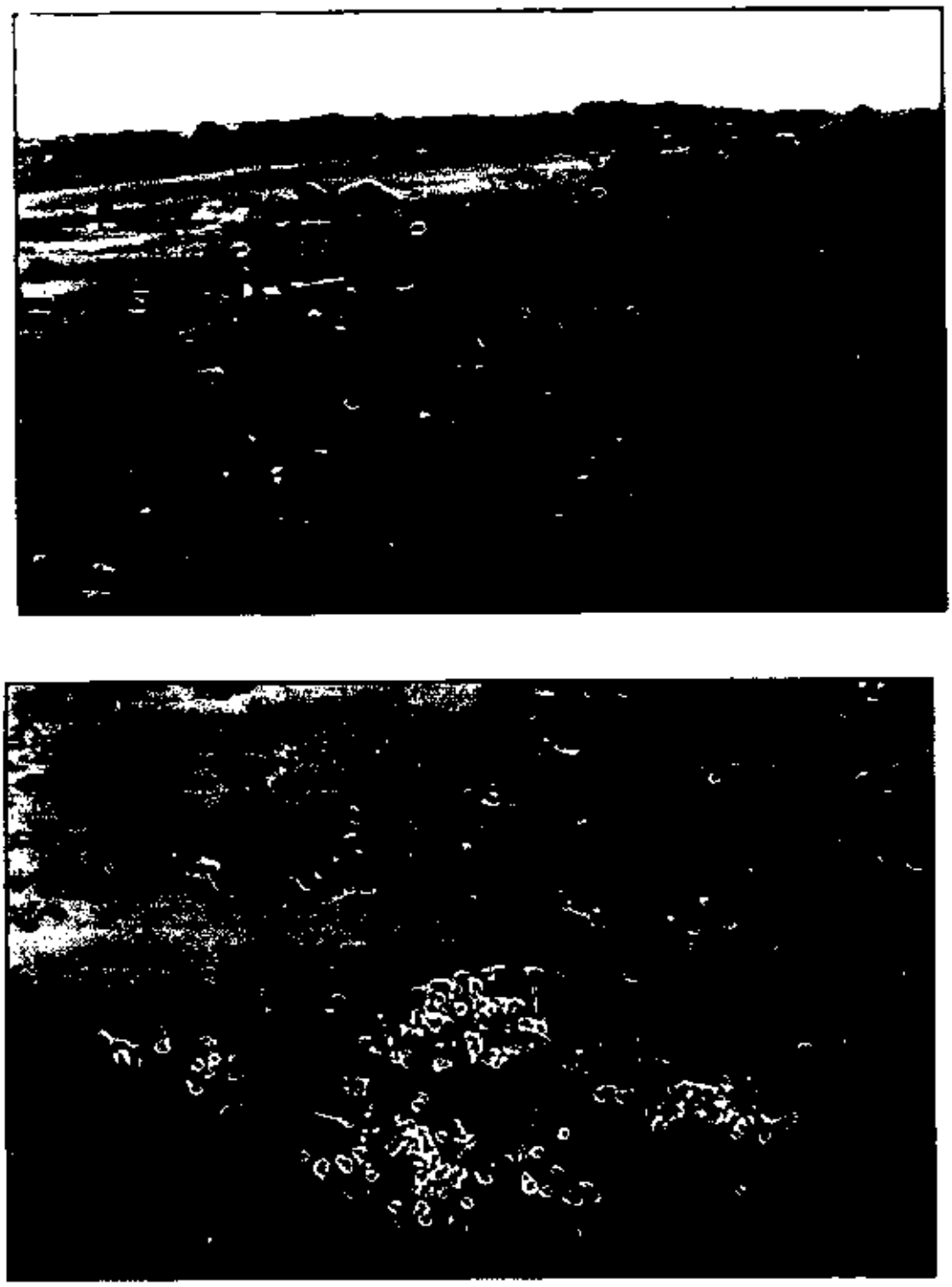


Fig . (6): Cont.

PRELIMINARY PHYTOCHEMICAL SCREENING

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening was carried out on the powdered plant of *Teucrium davaeanum*:-

1- Carbohydrates and/or glycosides [156] :

About 2g of powdered plant was extracted with 50 % ethanol and the following tests were carried out :

a- Molisch's test :-

About 5 ml of the ethanolic extract were mixed with 0.5 ml ethanolic α-naphthol . Sulphuric acid (1 ml) was carefully poured down the wall of the test tube. The carbohydrates 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 well mixed Fehling's solutions. The colour changed from deep blue to green, yellow or red indicating the presence of free reducing substances.

2- Volatile oils :

Steam distillation [156-157] :

About 10 g of powdered plant material are 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 indicated the presence of the volatile oils and vise versa.

3- Unsaturated sterols and/or Triterpenes :-

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

a- Libermann – Burchardt's test [158] :-

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

b- Salkowiski's test [159] :-

To the second part, an equal volume of sulphuric acid was added. If a red colour was produced, it indicate the presence of unsaturated sterols and / or triterpenes.

4- Coumarins[160] :-

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

5- Flavonoids [161-162] :-

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

6- Anthraquinones [160] :-

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

7- Alkaloids [160] :-

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 (5 ml). 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 and the other with similar amounts of Wagner's reagent the appearance of turbidity or precipitation indicates the presence of alkaloids.

8- Iridoids [163] :-

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 1ml of the Trim-Hill reagent (10 ml acetic acid,1ml 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

9- Saponins [164-165] :-

Forth test :-

About 3 g of the powdered plant were extracted with boiling water and filtered. After cooling, the extract was shaked vigorously until froth was obtained then allowed to stand for15 - 20 minutes and classified for saponins content (no froth = negative, froth less than 1cm height = weakly positive, forth 1–2 cm height = positive, froth greater than 2 cm height = strongly positive)

Blood Haemolysis :-

About 5 gm of the powdered plant were extracted with hot ethanol (95 %). One ml aliquet 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 .

10- Tannins[166- 167] :-

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

a- Upon a ddition of ferric chloride (T.S.),If a blue ,blue black, green or blue green colour or precipitate was produced, this would indicate the probable presence of catechol tannins.

b- A math stick was dipped in the alcoholic extract left to dry,then dipped again in hydrochloric acid, removed quickly and dried near a flame. A magneta red colour was formed, confirming the presence of catechol tannins.

c- To 5ml of the alcoholic extract, 2 ml of vanillin hydrochloric acid reagent were added, if a precipitate or red colour was formed, this would indicate the presence of gallic acid.

d- To 5 ml of the alcoholic extract, 2ml of acid phosphate solution were added. The mixture was warmed and cooled. To the filtrate, 2 ml of 2 % solution of phenazone were added, if a precipitate was observed this would indicate the prpsence of pyrogallol tannins [168].

The results of the phytochemical screening are summerized in table (3).

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

Constituents	Results
Carbohydrates and/or glycosids.	++
Volatiel oils.	+++
Unsaturated Sterols and/or triterpenes.	+++
Coumarins.	-
Flavoniods.	+++
Anthraquinones.	-
Alkaloids.	+
Iridoids.	++
Saponins.	+
Tanins.	+

+++ : High concentration.

++ : Moderate concentration.

+ : Low concentration.

- : Absent.

EXPERIMENTAL WORK AND RESULTS

I-VOLATILE OIL

Prepation of the volatile oil of *T. davaeanum* :-

1- By hydrodistillation method :-

About 200 g of the fresh plant (aerial parts) of *T. davaeanum* were subjected to water distillation in all glass apparatus for about three hours according to Gunther method .

The tarpped 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 odour (0.2 % v/w).

2- By solvent extraction :-

About 50 g of powdered plant material were macerated in 250 ml ether / hexane (50:50) for 24 hr. two times . The solvent was evaporated in vacuo at about 30 C° the pale yellow extract (1.5 g) was subjected to GC/MC analysis

GC/ MS analysis of the Volatile oils:-

The obtained volatile oil as well as ether / hexane extract were 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 dimeter, 0.25 µm film) .

Temperature program : Injector 50C° , Initial Temp. 38C° , Rate, 2 C°/ min to 200 C°,Final Temp.250 C° for 5 min .

Flow gas : Helium at 10 ml /min .

Mass spectroscopy

Instrument	: TRACE DSQ .
Full scan	: 50-450 , positive, Ion source 200 C° , mass transpher line 200 C° .
Library	: NIST .

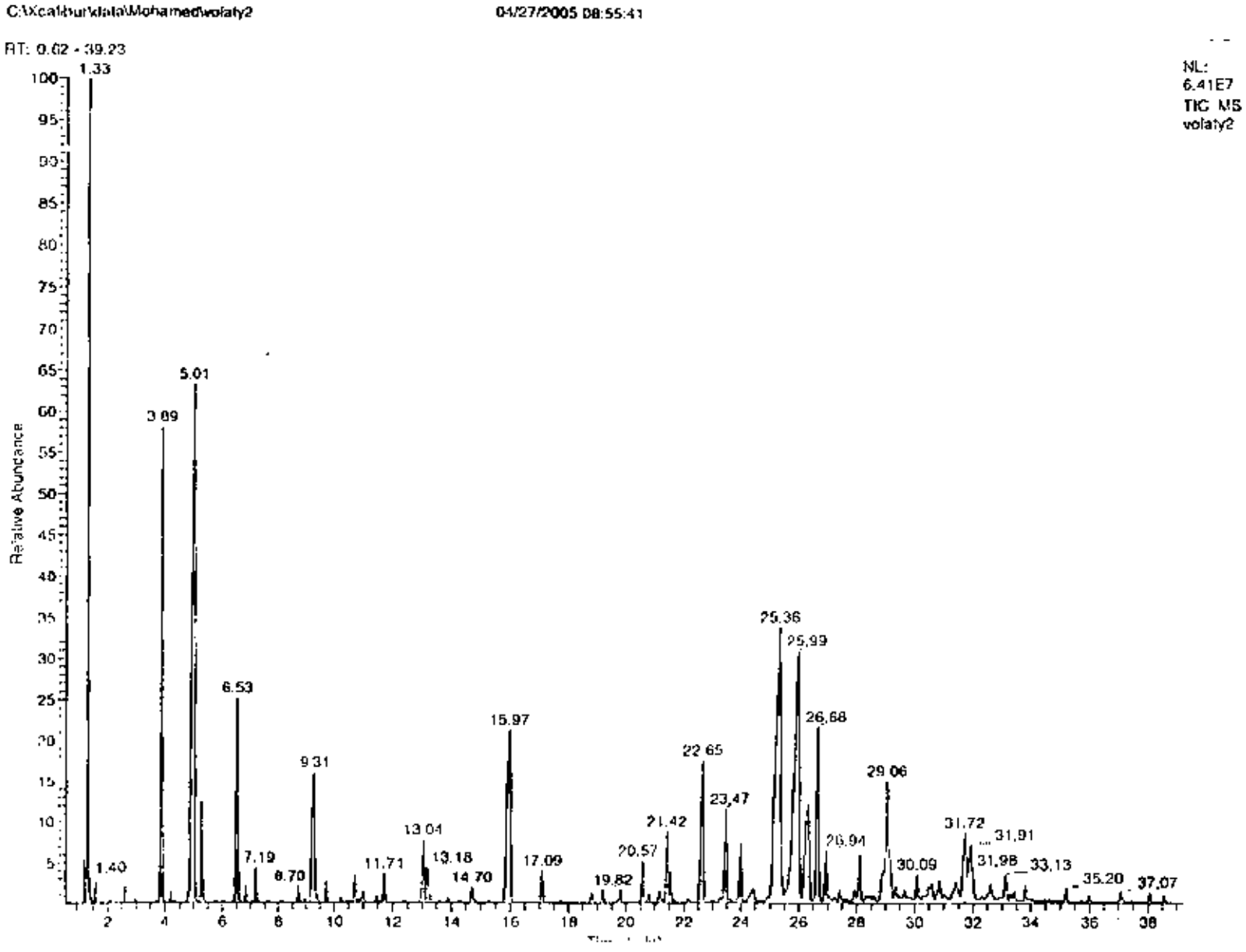


Fig.(7) : GC/MS chromatogram of the volatile oil of *T. davaeanum* prepared by hydrodistillation .

Table (4): GC/MS data of the volatile oil of *T. davaeanum* prepared by hydrodistillation

No	components	R_t (min.)	Relative (%)	Mass spectral data		
				M^+	B.P.	Fragments (%)
1	Furan -2-ethyle (100)	1.59	0.02	96	81	50 (2), 67 (10), 82 (5), 95 (10), 96 (5)
2	Hexanal (101)	2.10	0.10	100	56	53 (4), 67 (27)
3	2-Hexenal (102)	2.59	0.09	98	69	55 (51), 57 (3), 83 (6)
4	1-Hexanol (103)	2.75	0.01	102	56	55 (52), 57 (1), 69 (65)
5	1-Cyclohexene,3- (1-methylethyl) (104)	2.95	0.05	124	81	55 (12), 68 (21), 78 (55), 124 (10)
6	α – Pinene (12)	3.89	1.05	136	93	77 (21), 79 (24), 105 (9), 136 (4), 136 (9)
7	Camphene (105)	4.19	0.19	136	93	67 (25), 91 (30), 107 (20)
8	β– Pinene (1)	5.01	2.80	136	93	67 (10), 69 (28), 79 (17), 91 (26)
9	β– Myrcene (106)	5.29	0.71	136	93	65 (5), 67 (71), 91 (24)
10	Thujene (107)	5.71	0.10	136	93	65 (5), 67 (10), 77 (23), 91 (53), 136 (12)
11	2,4 – Heptadienal (108)	5.90	0.06	110	81	65 (9), 67 (18), 79 (13), 110 (10)
12	Limonene (2)	6.53	5.60	136	68	67 (80), 92 (26), 93 (90), 94 (30), 107 (18)
13	Bicyclo [3,1,1] hept – 2-ene,3,6,6-trimethyl (109)	6.82	0.76	136	93	67 (10), 91 (45)
14	Ocimene (110)	7.19	5.30	136	93	67 (14), 91 (56), 121 (11)

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

Table (4): Cont.

No	Components	R_t (min)	Relative (%)	Mass spectral data		
				M^+	B. P	Fragments (%)
15	Thujanol (111) .	7.57	0.10	154	93	77 (19), 91 (55), 136 (21)
16	Linalool oxide (112) .	8.11	0.02	154	59	68 (60), 93 (86), 94 (96), 111 (33)
17	1-pentanol, 5-cyclopropylidene (113)	8.93	2.40	124	79	59 (30), 67 (41), 71 (56)
18	Linalool-3,7-dimethyl (114)	9.30	6.40	154	93	55 (26), 69 (50) 71 (94), 80 (24)
19	Nonal (115)	9.40	2.10	142	57	56 (69), 69 (88), 70 (85), 95 (65)
20	Octan-1-Ol,acetate (116)	9.72	1.90	171	59	54 (30), 67 (48), 68 (33)
21	Nerol (117)	10.03	0.10	154	41	91 (50), 94 (94), 95 (40), 121 (4)
22	Carveol (118)	10.08	0.10	152	91	67 (22), 77 (25), 119 (40), 134 (24)
23	3- cyclopentene-1-acetaldhyde, 2,2,3-trimthyl (119)	10.23	0.47	152	93	67 (28), 91 (31), 108 (99)
24	Pinocarveol (120)	10.72	1.50	152	92	70 (65), 83 (44), 69 (41)
25	Verbenol (121)	11.03	0.19	152	91	67 (32), 81 (33), 109 (65), 119 (29),
26	2H- pyran,3,6-dihydro-4-methyl-2- (2-methyl-1-propenyl) (122)	11.45	0.28	152	68	67 (88), 69 (30), 83 (50)
27	Pinocarvone(123)	11.70	8.20	150	53	53 (66), 79 (49), 107 (65), 108 (79)
28	1-cyclohexene-3-acetoxy-4-(1-hydroxy-1-methyl ethyl)-1-methyl (124)	12.01	2.50	195	94	59 (63), 77 (15), 79 (61), 95 (11)
29	4-terpineol (7) .	12.40	1.05	154	71	67 (27), 69 (53), 91 (29), 93 (84)111(28)

Table (4): Cont.

No	Components	R_t (min)	Relative (%)	Mass spectral data		
				M^+	B.P.	Fragments (%)
30	3- cyclohexen-1-methanol, α,α, 4- trimethyl (125) .	13.04	3.50	112	93	59 (79), 121 (49), 136 (56)
31	Myrtenal (126)	13.18	3.30	150	79	77 (45), 91 (56), 106 (40), 107 (75)
32	Myrtenol (127)	13.23	1.10	152	79	77 (25), 91 (75), 93 (26), 107 (20),
33	Verbenone (128).	13.79	1.05	150	107	79 (38), 91 (93), 135 (56)
34	2- cyclohexen-1-one,2-methyl-5- (1-methyl ethyl) (129).	15.28	0.86	152	82	54 (22), 93 (55), 108 (39)
35	Citronellol (130) .	15.98	1.60	156	41	69 (66), 80 (23)
36	2H-1-benzapyran-3,4,4,5,6,8- hexahydro-2,5,5,8, tetramethyl (131) .	17.09	0.10	190	69	84 (27), 92 (31), 95 (63), 107 (48)
37	Trans-pinocarvyl acetate (132).	17.92	0.10	194	91	67 (13), 92 (38), 93 (15), 119 (21)
38	2,4-decadienal (133).	18.50	0.05	152	81	55 (5), 67 (20), 79 (7), 91 (11), 95 (50)
39	Myrtenyl acetate (134).	18.79	0.05	194	91	49 (13), 92 (30), 93 (12)
40	α-Bisabolene (135) .	19.19	0.38	204	41	79 (31), 91 (45), 107 (44), 121 (83)
41	α- Cubebene (136) .	19.72	0.10	204	105	81 (21), 91 (46), 93 (35), 119 (75),
42	Terpinenyl acetate (137).	19.82	0.47	196	43	67 (29), 121 (79), 136 (58)
43	Eugenol (138)	20.22	0.96	164	164	91(50), 103 (31), 131(36), 165(39),
44	Linalyl acetate (139).	20.57	1.10	196	43	67(29), 68(51), 92(16), 93(60), 121(13)

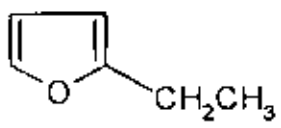
Table (4): Cont.

No	Components	R_t (min)	Relative (%)	Mass spectral data		
				M+	B.P.	Fragments (%)
45	Copaene (140).	20.79	0.28	204	105	91(55), 93(74), 119(79), 161(90)
46	Bourbonene (141).	21.15	0.38	204	81	79(39), 80(84), 123(73)
47	Cyclohexane,1-ethcyl-1-methyl-2, 4-bis (1-methyl ethenyl) (142).	21.54	0.76	196	93	67(64), 68(65), 79(45), 81(60), 107(45)
48	Azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl -7- (1-methyenyl) (143).	23.47	3.80	196	105	91 (78), 93 (89), 107 (75), 133 (55), 147 (80)
49	α-Caryophyllene (144).	24	2.10	204	41	67(22), 91(22), 93(71), 133(23)
50	Farnesene (145).	24.43	0.76	204	69	91 (61), 105(68), 119(31)
51	Germacrene-D (13).	25.34	2.4	202	161	91(45), 105(59), 119(50), 134(65)
52	δ- Cadinene (5).	26.93	0.38	206	124	81(53), 91(99), 122(98), 161(53)
53	Armodenorene (146).	27.05	0.97	204	41	59(44), 67(53), 79(43), 91(53), 107(41)
54	τ- Elemene (147)	28.09	1.24	204	93	67(81), 81(85), 91(99), 107(85), 133(53)
55	Viridiflorene (148) .	28.46	1.57	204	105	67(81), 81(85), 91(99), 107(85), 133(53)
56	Spathulnol (149).	29.05	8.80	220	91	93(80), 119(55), 159(53)
57	Caryophyllene (150).	29.14	1.35	204	93	79(65), 91(84), 95(65), 105(42), 107(41)
58	B-Ionene (151).	30.12	1.05	192	43	96(98), 109(81), 138(56)
59	α-Eudesmol (152)	30.47	0.97	222	93	67(64), 68(65), 79(45), 81(60), 107(45)

Table (4): Cont.

No	Components	R_t (min)	Relative (%)	Mass spectral data		
				M^+	B. P.	Fragments (%)
60	Cubenol (153).	30.86	3.77	222	119	91(40), 105(72), 161(83), 204(23)
61	Guaiene (154).	31.03	2.60	204	105	59(63), 91(86), 105(66), 218(36)
62	τ-Cadienol (11).	31.40	1.5	222	161	95(85), 105(70), 119(52)
63	β- Eudesmol (155).	31.72	2.85	222	59	93(46), 95(40), 164(28)
64	2,4,8,trimethy – 1,2,3,4,4 5,6,7octahydro-naphthalen,2-yl,) –α -2-prop-2-en-1-Ol (156).	32.61	2.28	222	91	67(60), 105(85), 122(76), 159(49)
65	Carotol (157).	33.12	1.05	222	41	81(76), 93(64), 121(36), 161(56)
66	1-Naphthalenol, decahydro-1,4-dimethyl-7- (1-methyl ethylidene) (158).	33.29	0.47	224	93	67(70), 81(90), 189(78)
67	Germacrone (159).	33.80	0.86	218	91	93(78), 105(74), 161(66), 218(64)
68	α- Muurolene (160).	35.20	0.47	204	91	79(55), 105(61), 147(74), 175(54)
69	2(3H)-naphthalenone,4,4,5,6,7,8-hexahydro-4,4-dimethyl-6-(1-methylethenyl) (161).	37.09	0.02	222	91	79(60), 93(76), 133(78), 174(91), 161(50)
70	Globulol (162).	37.43	0.03	222	43	67(73), 95(68), 147(54), 176(89)
71	Humulene (163) .	38.05	3.80	204	93	117(29), 209(73), 224(59)
72	2- pentadecanone,6,10,14- trimethyl (164)	38.11	1.90	254	71	58(99), 95(63), 85(44)
73	N – hexadecanoic acid (165)	42.73	2.80	256	93	60(63), 57(41), 129(38), 55(27)
74	Phytol (166)	46.98	0.95	296	71	69(32), 68(28), 81(26), 95(27)

R_t : Retention time , M^+ : Molecular ion peak , B.p. : Base peak .

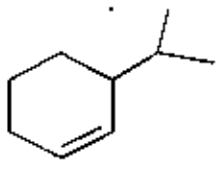


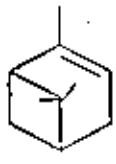
CH_3-$(CH_2)_4$CHO

CHO-CH=CH-$(CH)_2$-CH_3

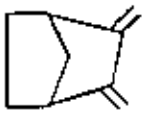
(100) : Furan-2-ethyl **(101)** : Hexanal **(102)** : 2-Hexenal

CH_2OH -$(CH_2)_4$-CH_3

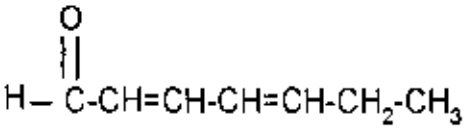


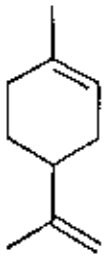


(103) : 1-Hexanol **(104)** : 1-Cyclohexene,3-(1-methyl ethyl) **(12)** : α-pinene



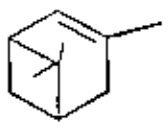
(105) :Camphene **(1)** : β-pinene **(106)** : β- Myrcene



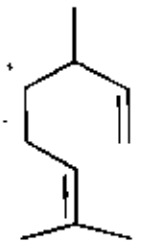


(107) :Thujene **(108)** : 2,4-heptadienal **(2)** : Limonene

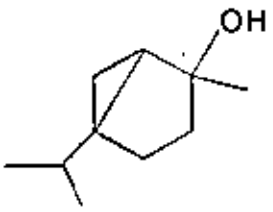
Fig. (8) : Chemical structures of some constituents of volatile oils of *T. davaeanum* prepared by hydrodistillation.



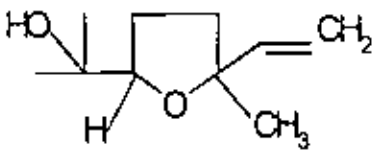
(109): Bicyclo[3,1,1]hept-2-ene,3,6,6-trimethyl



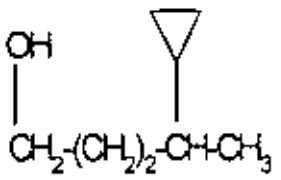
(110) : Ocimene



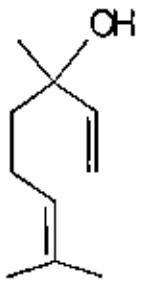
(111): Thujanol .



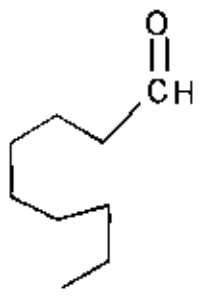
(112) : Linalool oxide



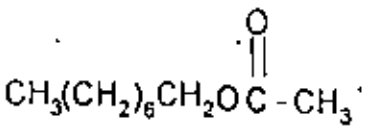
(113) : 1-pentanol,5- cyclopropylidene



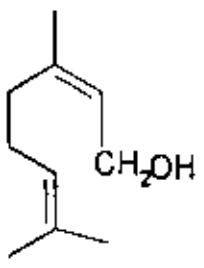
(114) : Linalool ,3, 7-dimethyl



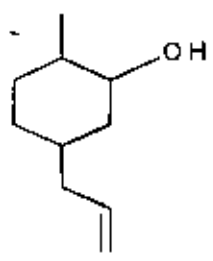
(115) : Nonal .



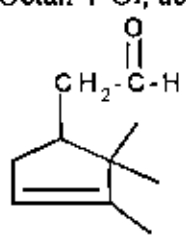
(116) : Octan-1-Ol, acetate .



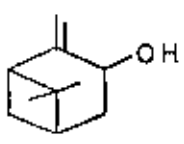
(117) : Nerol



(118) : Carveol .

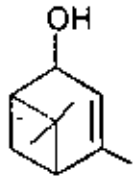


(119) : 3-cyclopentene-1-acetaldehyde,2,2,3-trimethyl .

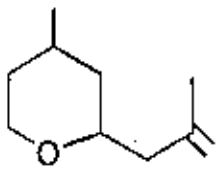


(120) : Pinocarveol

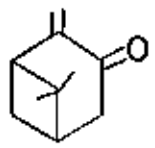
Fig. (8) :Cont.



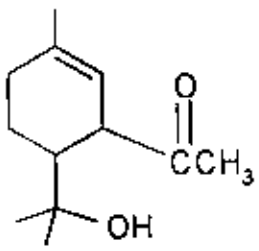
(121) : Verbenol.



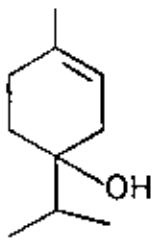
(122) : 2H-pyran,3,6-dihydro-4-methyl-2-(2- methyl-1-propenyl)



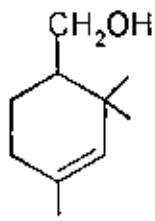
(123) : Pinocarvone



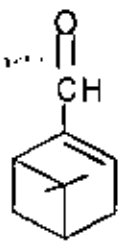
(124) : 1-cyclohexene-3-acetoxy-4 -(1-hydroxy-1-methyl ethyl)-1-methyl



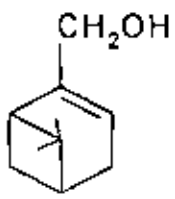
(7) : 4-terpineol



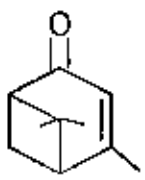
(125) :3-cyclohexene-1-methanol,α,α,4-trimehyl .



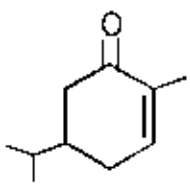
(126) : Myrtenal .



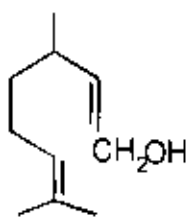
(127) : Myrtenol .



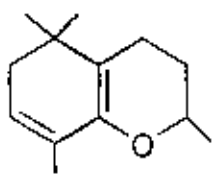
(128) : Verbenone



(129): 2-cyclohexen-1-one,2-methyl-5- (1-methylethyl)

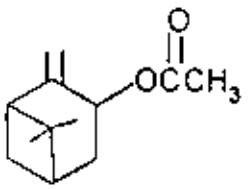


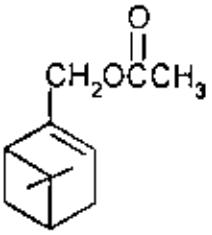
(130):Citronellene



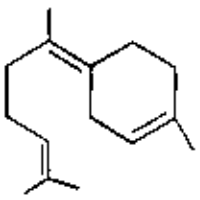
(131): 2H-1-benzopyran,3,4,4,5,6,8-hexahydro -2,5,5,8-tetrametyl

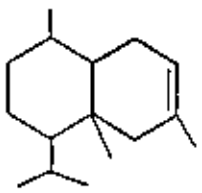
Fig. (8) : Cont.

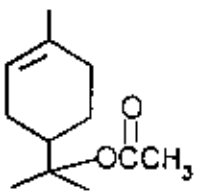




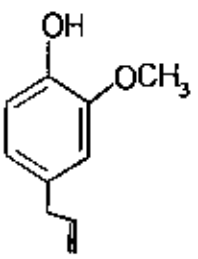
(132): Trans-pinocarvyl acetate . (133): 2,4 decadienal . (134): Myrtenyl acetate

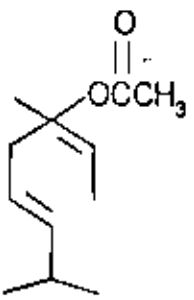


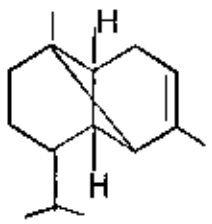




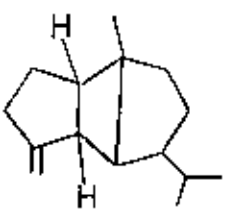
(135):α-Bisabolene . (136): α-Cubebene . (137):Terpinenyl acetate.

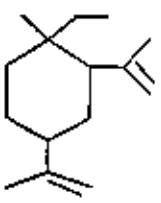


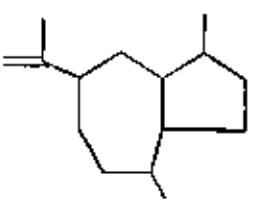




(138): Eugenol . (139): Linalyl acetate (140): Copaene

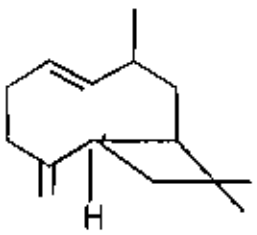




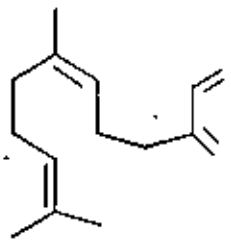


(141): Bourbonene . (142): Cyclohexane,1-etheyl-1-methyl-2,4bis (1-methylethenyl) (143):Azulene,1,2,3,4,5,6,7,8-octahydro-1,4 -dimethyl-7-(1-methylethenyl)

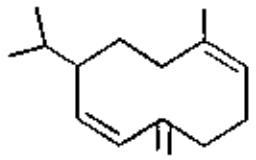
Fig. (8) : Cont.



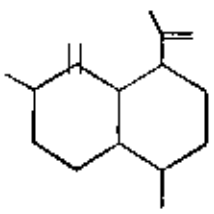
(144): α-Caryophyllene .



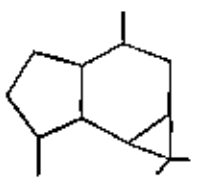
(145): Farnesene.



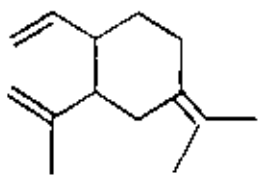
(13) : Germacrene-D .



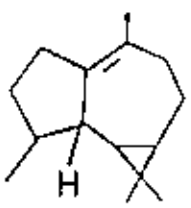
(5) : δCadinene .



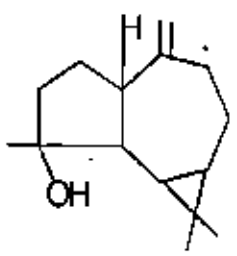
(146): Aromadenorene .



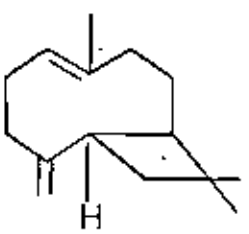
(147): τ-Elemene .



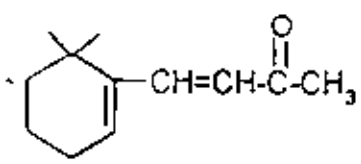
(148): Viridiflorene .



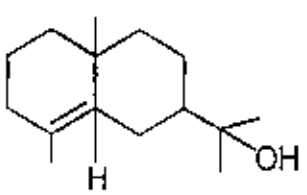
(149): Spathulenol



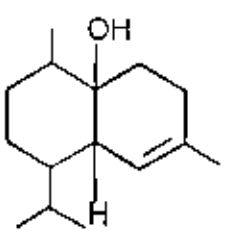
(150): Caryophyllene



(151): β- Ionone .

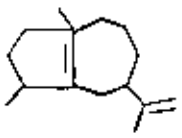


(152): α-Eudesmol.

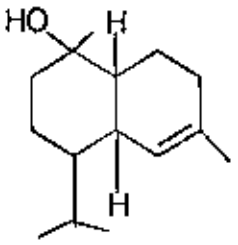


(153): Cubenol

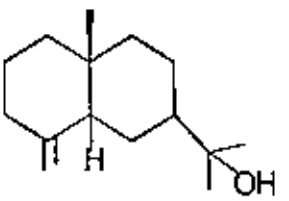
Fig. (8) : Cont



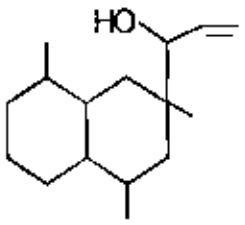
(154): Guaiene .



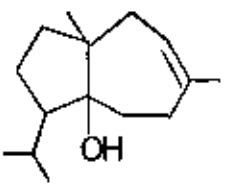
(11) : τ-Cadienol



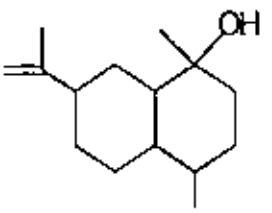
(155): β-Eudesmol .



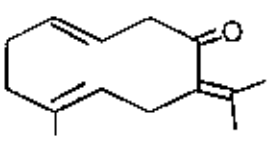
(156): 2,4,8-trimethyl-1,2,3,4,4,5,6,7 - octahydro-naphthalen,2-yl) -α-2-prop-2-en-1-Ol .



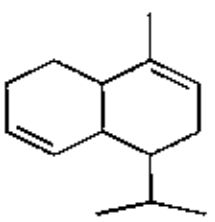
(157): Carotol .



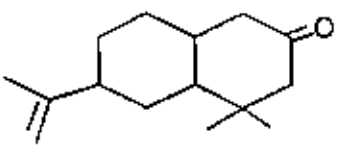
(158) :1-naphthalenol,decahydro-1,4 -dimethyl-7-(1-methyl ethylidene)



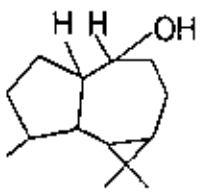
(159) : Germacrone .



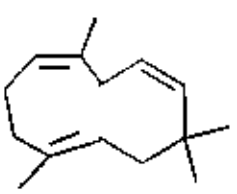
(160) : α- Muurolene .



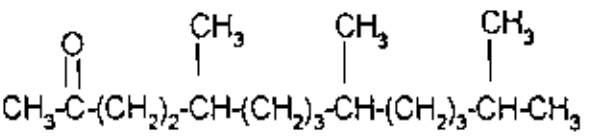
(161) : 2(3H)-naphthalenone,4,4,5,6,7,8 hexahydro-4,4-dimethyl-6- 1-methylethenyl) .



(162) : Globulene .



(163) : Humulene .

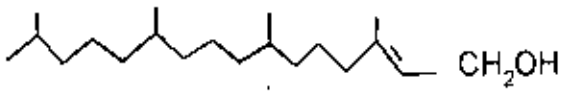


(164) : 2-pentadecanone ,6, 10,14-trimethyl .

Fig. (8) : Cont.

CH_3-$(CH_2)_{14}$-COOH

(165) : N-hexadecanoic acid



(166) : Phytol

Fig. (8) : Cont.

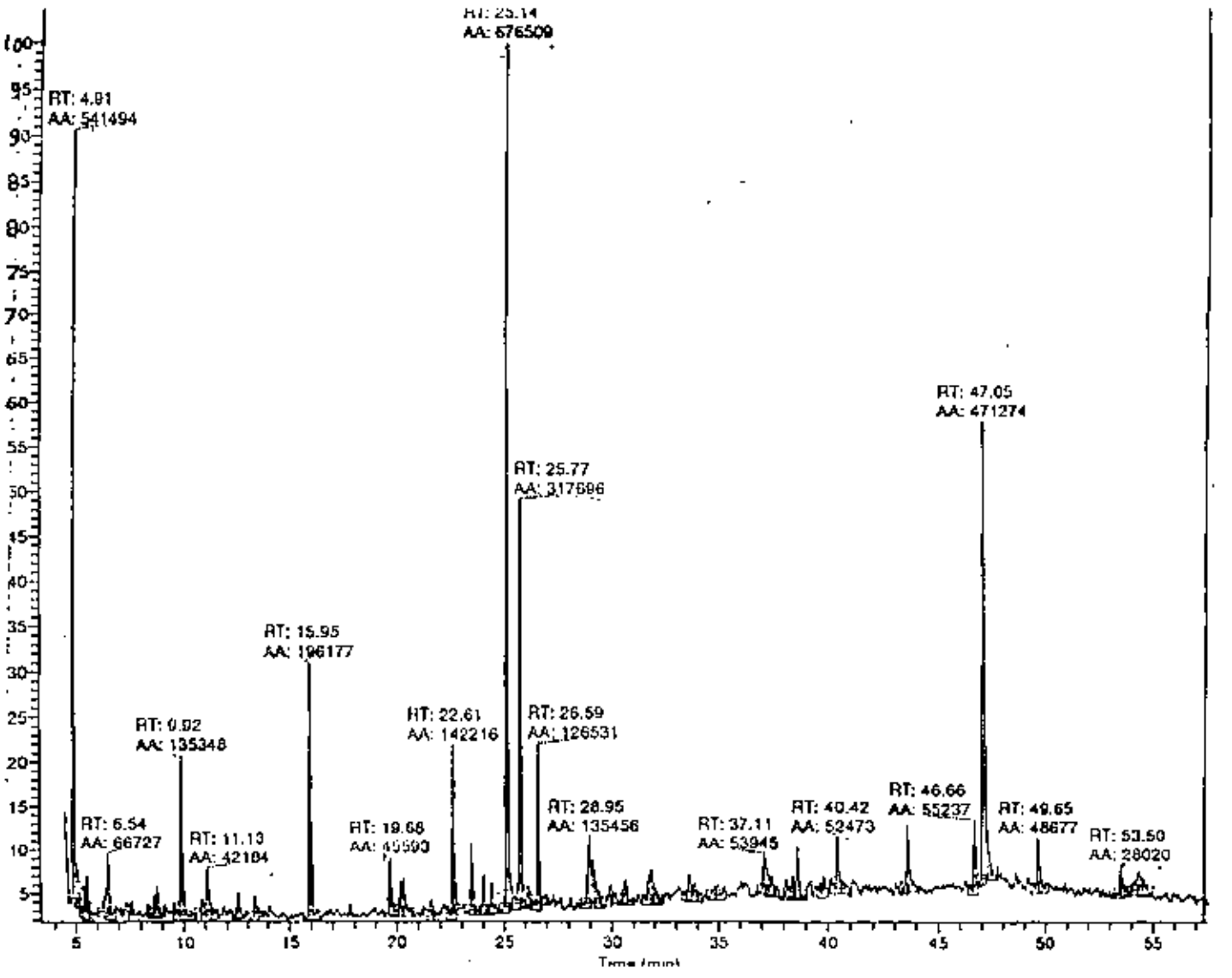


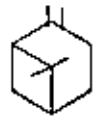
Fig. (9) : GC/MS chromatogram of the volatile oil of *T. davaeanum* prepared by solvent extraction .

Table (5): GC / MS data of the volatile oil of *T. davaeanum* prepared by solvent extraction.

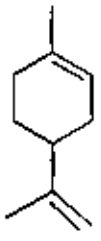
No	Components	R_t (min)	Relative %	Mass spectral data		
				M^+	B.P	Fragments %
1	Pinocarvane (123) .	4.95	1.7	136	93	6.9 (13), 91 (23), 94 (7)
2	Limonene (2).	6.63	2.1	136	93	67 (56), 68 (73), 77 (27), 95 (31)
3	Benzene, 1 – chloro – 3 – (chloromethyl) (167)	9.9	4.2	161	125	62 (19), 91 (21), 127 (11), 160 (66), 163 (10)
4	β – Myrcene (106)	15.96	1.3	136	93	68 (12), 69 (25), 91 (13).
5	2.6 – dimethyl – 1, 3, 5, 7 – octatetraene (168)	19.66	6.2	134	119	79 (29), 91 (85), 134 (44), 135 (29)
6	Farnesene (145) .	22.58	1.4	204	69	93 (64), 105 (35), 120 (85), 133 (88)
7	Caryophyllene (150)	24.03	4.5	204	93	80 (32), 91 (40), 94 (39)
8	Himachalene (169) .	26.59	10	204	41	91 (27), 119 (26), 120 (8), 204 (20)
9	Curcumene (170) .	28.95	4.29	202	132	81 (25), 105 (35), 1.7 (10), 146 (41), 158 (32)
10	Dodecane, 5, 8 – diethyl (171)	37.08	1.71	226	71	57 (76), 85 (65), 99 (15)
11	Phytol (166)	47.04	15	296	71	55 (22), 57 (32), 82 (50), 95 (84)
12	15% Unknown compound	-	-	-	-	-

R_t : Retention time , M^+ :Molecular ion peak , B.P. : Base peak .

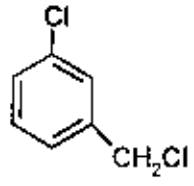
Note : The abundance of each fragment is between two parenthesis .



(123) : Pinocarvane .



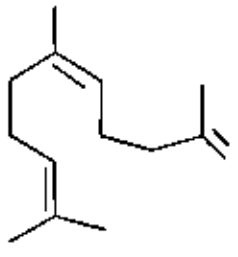
(2) : Limonene



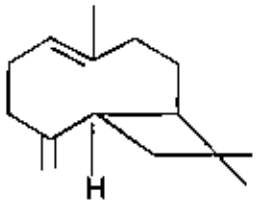
(167) : Benzene, 1-chloro-3-(chloro methyl) .

(106) : β – Myrcene

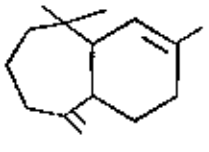
(168) : 2,6- dimethyl -1,3,5,7- octatetriene .



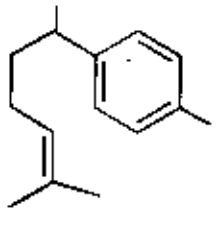
(145) : Farnesene



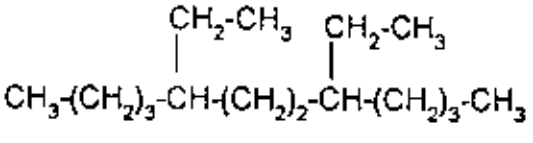
(150) : Caryophyllene



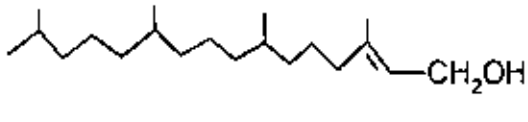
(169) : Himachalene



(170) : Curcumene



(171) : Dodecane ,5,8-diethyl .



(166) : Phytol

Fig. (10) : Chemical structures of constituent of volatile oils of *T. davaeanum* prepared by solvent extraction .

II- INVESTIGATION OF THE LIPID CONSTITUENTS OF *TEUCRIUM DAVAEANUM.*

Preparation of terpenoids and related substances:

About 1.5 kg of the dried powdered plant of *T. davaeanum* was extracted with petroleum ether (b.r.40–60C°) in a Soxhlet apparatus. The combined peteoleum ether extract was passed through fuller's earth to remove the coloured pigments, filtered, dried over anhydrous sodium sulphate and evaporated *in vacuo* at 40 C° till dryness to give a pale yellow residue (7 g).

The petroleum ether residue was dissolved in boiling acetone (400 ml) and left over night at room temperature. An amorphous precipitate was filtered, washed with cold acetone and recrystallized from chloroform : methanol to give a bright white crystals (1.1 g) acetone insoluble fraction (fatty alcohols). The filtrate (acetone soluble fraction) was evaporated till dryness (5.5 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:

Gas chromatography:

Insrument : Hewelett Packard Model 6890

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

Carrier gas: Helium at 0.8 cm/min

Column : Capillary column HP–1, Length 80 m, Thickness 0.3 µm

Mass Spectroscopy:

Instrument : Hewelett Packard Model 5973 Mass Selective detector

Detector : Selective Ion Detector (SIM) AS Harvey (1981)

Identification of separated compounds was by using Standard library (NIST Version 2.0) . The results were shown in Fig. (11-12) and Tab. (6) .

Saponification of acetone soluble fraction:

The acetone soluble fraction (5.5 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 unsaponifiable matter was extracted by shaking with successive portion of chloroform (3×150 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 unsaponifibale matter (3.8 g).

Gas liquid chromatographic analysis of the unsaponifiable fraction:-

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

Instrument : Agilent Technologies 6890 N, Network GC system .

Column : capillary column (ZB – 5)
(length 30m, 530 µm, film-thickness 50 µm).

Temprature

Oven : Initial temp.: 80 C°, rate : 8 C°/min., final temp.,250 C°, final time : 50 min. .

Inlent : 270 C, (split) = mode . split ratio = 15:1

Detector: typ : FID. 300 C

Carrier gas : N_2, H_2 30 ml / min.

Air 300 ml / min.

The results were summarized in Fig. (13) and Tab. (7) .

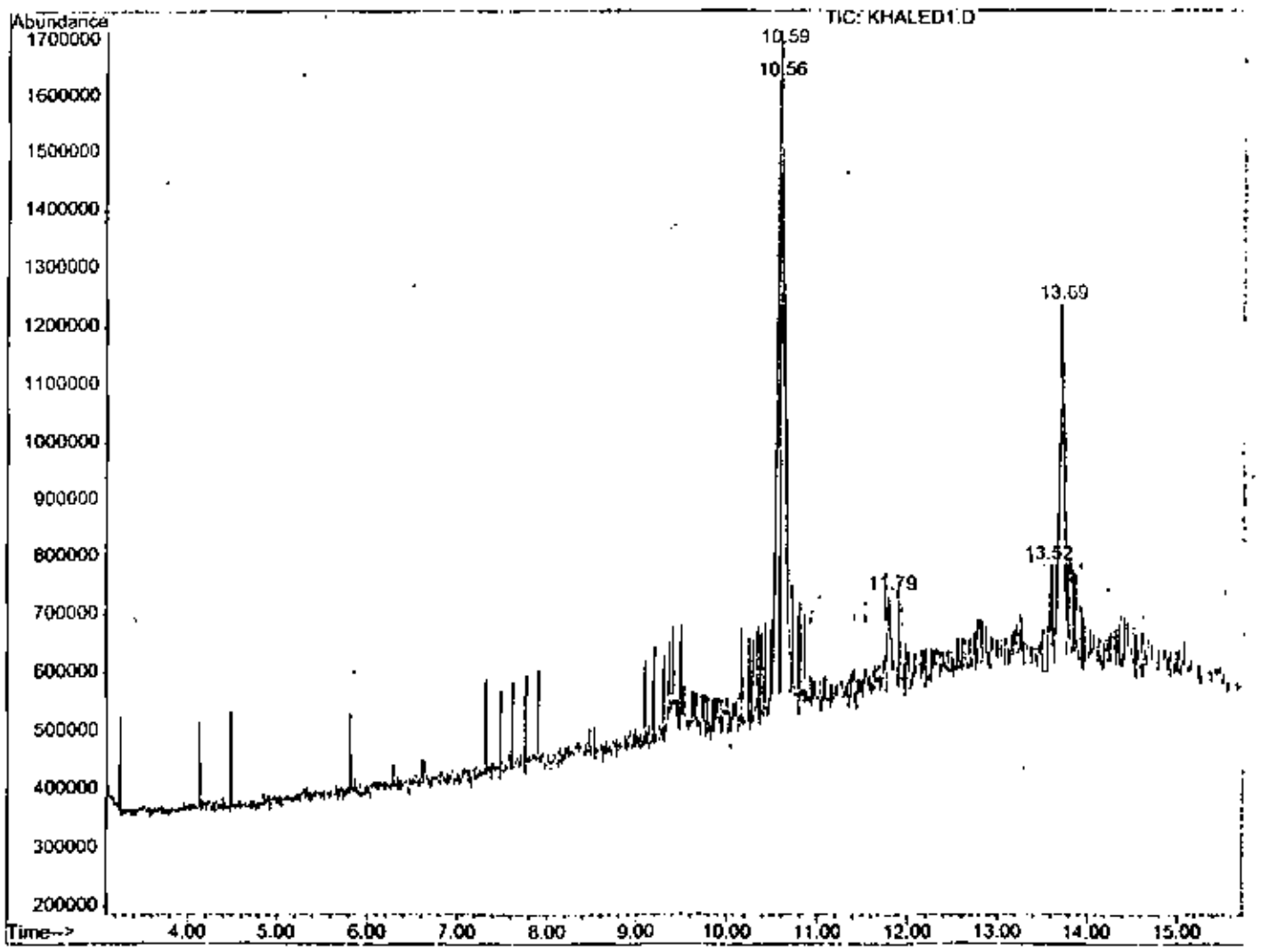


Fig. (11): GC chromatogram of the fatty alcohols of *T. davaeanum* .

Table (6): GC/MS data of fatty alcohols and hydrocarbon mixure of *T. davaeanun*

Peak No.	R_t min	Relative %	M^+	Fragments	b.p	Chemical formula	Compouud
1	10.59	66.95	354	339(11),325(12), 310 295,281(46),266 (8),221 (39),146 (36),73 (60),44 (9).	354	$C_{24}H_{56}O$	Tetracosanol
2	11.79	4.82	550	549 (26), 490 (9), 402 (60), 387 (10) ,355 (4), 340 (23), 207 (30) ,135 (61).	44	$C_{38}H_{78}O$	Octatriacontanol
3	13.52	4.34	476	429 (1), 281 (17), 206 (30), 97 (35), 71 (53), 57 (49).	44	$C_{34}H_{68}$	Tetratriacontene
4	13.69	23.88	534	518 (2), 502 (9), 428 (17) ,399(17), 353 (95), 280 (63) ,147 (58) ,73 (87), 44 (60).	428	$C_{38}H_{78}$	Octatriacontane

R_t : Retention time, M^+ : Molecular ion peak, B.P. : Base peak .

Note : The abundance of each fragment is between two parenthesis .

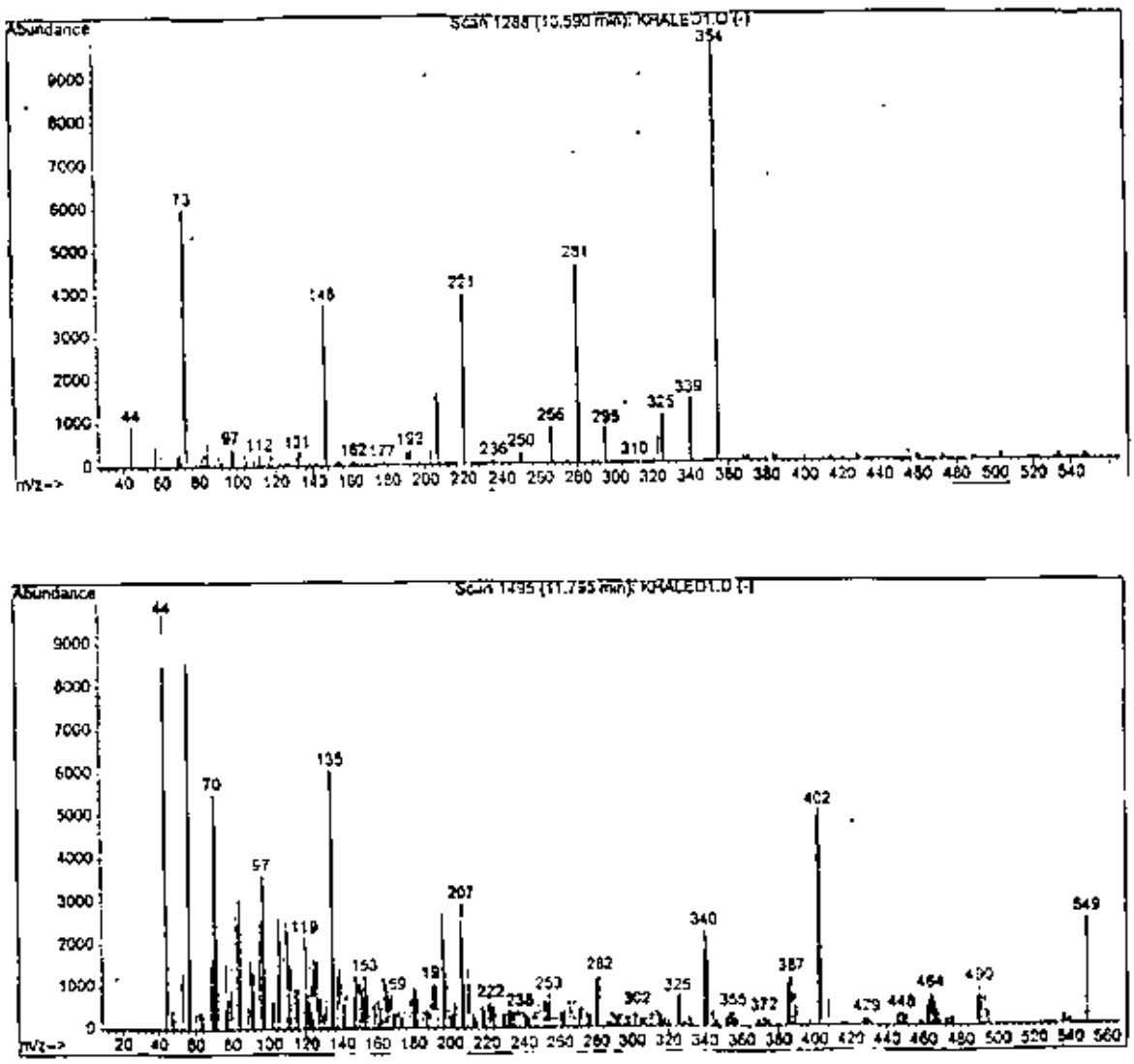


Fig. (12): EI- mass spectra of fatty alcohols and hydrocarbons of *T. davaeanum* .

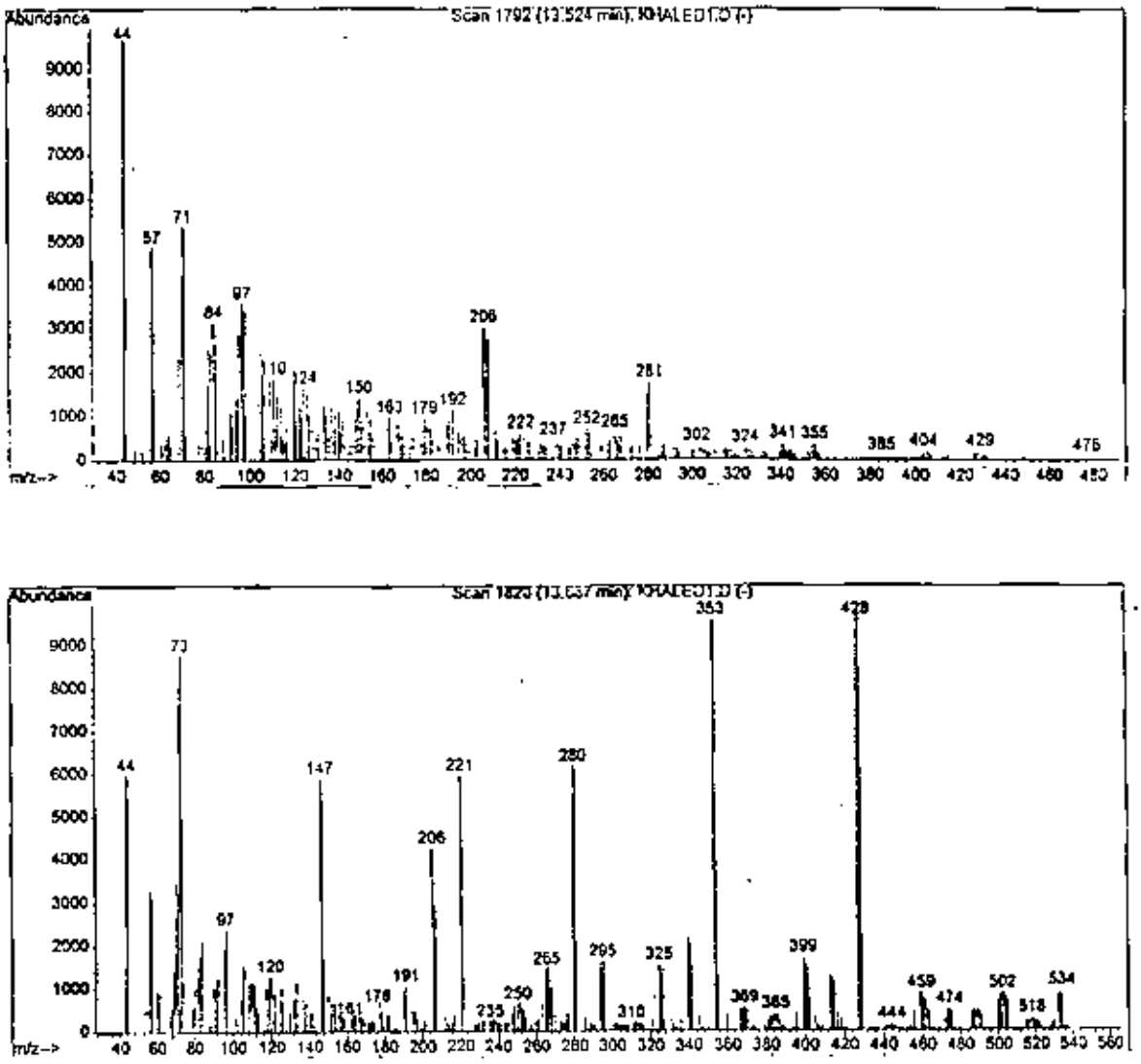


Fig. (12) : Cont.

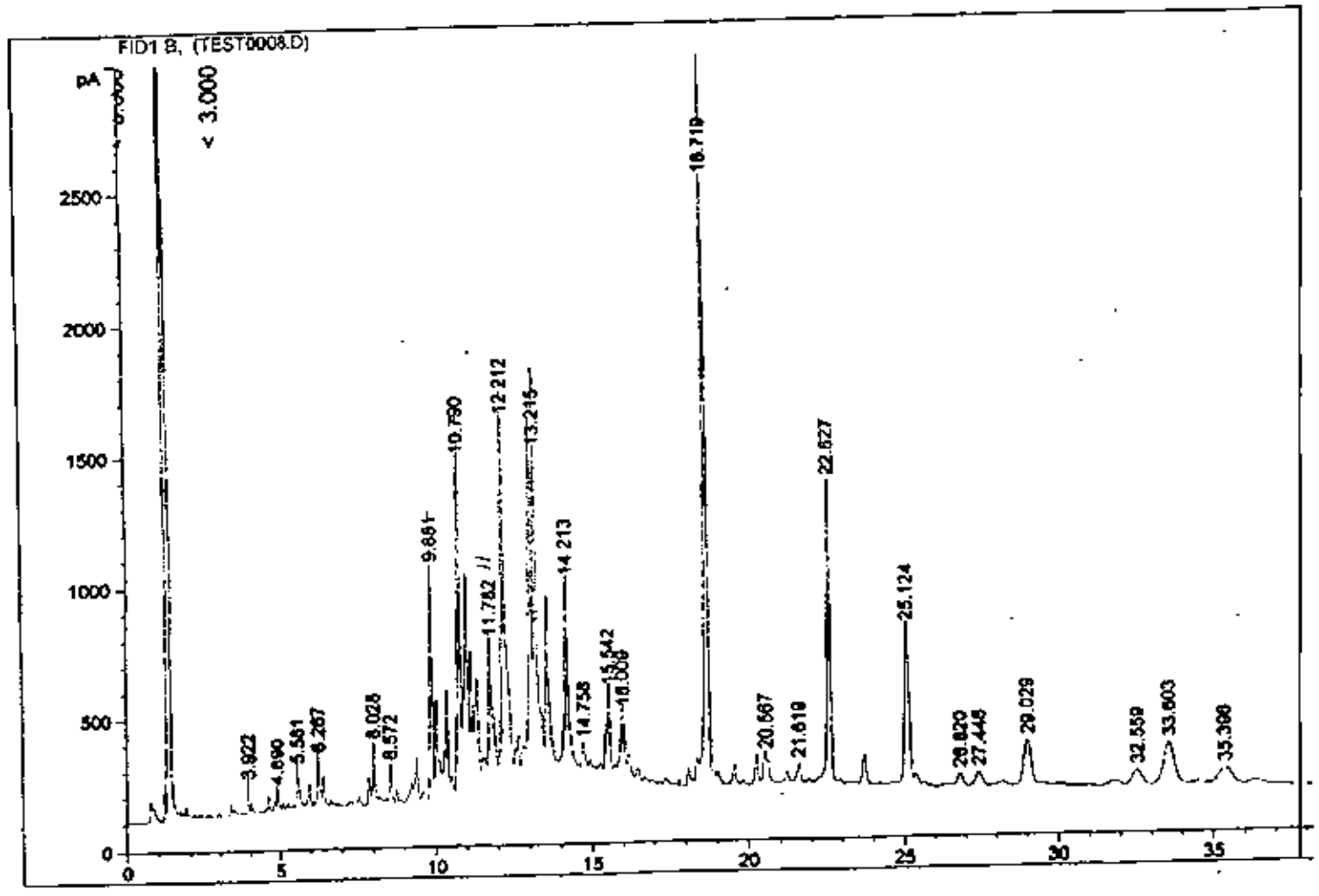


Fig. (13) : GLC analysis of the unsaponifiable fraction of *T. davaeanum* .

Table (7) : GLC data of the unsaponifiable fraction of *T. davaeanum*.

Peak no	constituents	R_t(min.)	Relative(%)
1	Heptane C_7	8.03	1.36
2	Octane C_8	8.57	0.78
3	Nonane C_9	9.88	8.88
4	Decane C_{10}	10.79	15.35
5	Undecane C_{11}	11.78	5.02
6	Dodecane C_{12}	12.21	11.38
7	Tetradecane C_{14}	13.22	21.27
8	Hexadecane C_{16}	14.21	6.16
9	Heptadecane C_{17}	15.54	3.26
10	Octadecane C_{18}	16.01	3.49
11	Docosane C_{22}	20.57	2.07
12	Petacosane C_{25}	21.62	1.31
13	Heptacosane C_{26}	22.63	6.39
14	Octacosane C_{28}	25.12	5.79
15	Nonacosane C_{29}	26.82	0.54
16	Hentricontane C_{30}	27.45	0.75
17	Dotricontane C_{32}	29.03	2.10
18	β-sitosterol	33.60	2.67
19	Camposterol	35.40	1.49

R_t : Retention time (min).

Preparation of the total fatty acids:-

The hydroalcoholic soap solution after saponification was rendered acidic (PH=2) with 5% sulphuric acid. The liberated fatty acids were throughly extracted several times with chloroform (3 × 150) . The combined ether extract was washed with distilled water till free from acidity and dehydrated over anhydrons sodium sulphate . The solvent was evaporated *in vacuo* at 40 C° till dryness(1.5 g).

Preparation of the fatty acid methyl esters:-

The total fatty acids (1.2 g) 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 distilled water and extrateed with successive portion of chloroform (3×100 ml). The combined chloroform extracted was washed with distilled water till free from acidity, dried over anhydrous sodium sulphate, filtered and the solvent was evaporated *in vacuo* at 40C° (1.1 g).

Gas liquid chromatography of the fatty acids methyl esters

GlC analysis of fatty acid methyl esters was carried out (The results shown in Fig. 14, Table 8) using the following conditions :-

Apparatus : HP – 6890 series.

Column : type: capillary column, Hp – wax, bonded polyethylene glycol (length 60 m, diameter 320 µm, film thickness 0.25 µm).

Temprature : Initial temp. 70 C°, time 2 min, Rate 4 C°/ mm

Final temp. 200 C°, time 10 min .

Detector : Flame Inization Detector, Temp. : 275 C°.

Injector temp: 250 C°.

Flow Rates : N_2, H_2 : 30 ml / min.

: Air 350 ml / min.

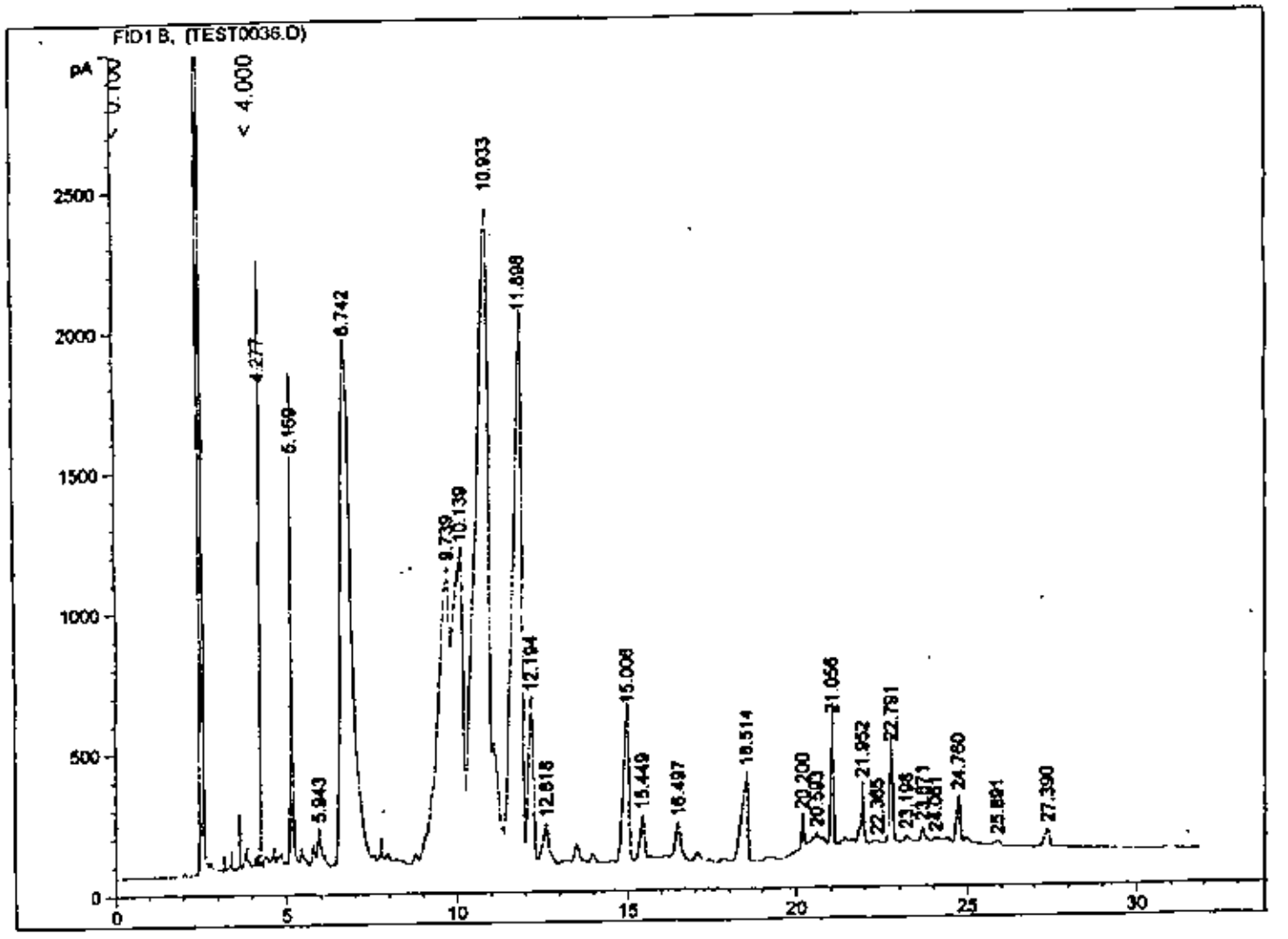


Fig. (14) : GLC analysis of the fatty acids methyl ester of *T. davaeanum*.

Table (8) : GLC data of the fatty acid methyl esters of *T.davaeanum*.

Paek no	Fatty acid	R_t(min.)	Relative%
1	Lauric C_{12}	4.28	1.83
2	Myristic C_{14}	5.17	2.68
3	Palmitic C_{16}	6.74	18.03
4	Stearic $C_{(18:0)}$	9.74	11.33
5	Oleic $C_{(18:1)}$	10.14	9.36
6	Linoleic $C_{(18:2)}$	10.93	27.54
7	Linolenic $C_{(18:3)}$	11.90	22.22
8	Arachidic $C_{(20:0)}$	15.01	2.71
9	Erucic $C_{(22:1)}$	18.51	1.82
10	Lignoceric $C_{(24:0)}$	22.79	1.60
11	Tetracosenoic $C_{(24:1)}$	24.76	0.88

R_t: Retention time.

III- INVESTIGATION OF THE FLAVONOIDOL CONSTITUENTS OF *T. DAVAEANUM*

Extraction and fractination of the flavonoidal constituents:-

About 1.4kg of the air-dried defatted powdered plant material were macerated with methyl alchohol (70%) till exhaustion. The alcoholic extract was evaporated *in vacuo* at about 45 C° (85 g), dissolved in hot distilled water (500 ml), left overnight in refrigerator and then filtered. The aqueous filtrate was extracted with successive portions of ethyl acetate(5×500 ml) followed by butanol (5×500 ml). The solvents were dried, separatly, over anhydrous sodium sulphate and evaporated *in vacuo* at 50 C° .

Paper chromatography :-

Both ethyl acetate and butanol fractions were investigated by PC and TLC using different solvent systems . It was found that the PC of the ethyl acetate extract in 15% acetic acid gave the best separation and revealed the presence of four main flavonoids (R_f = 0.11, 0.22, 0.33, 0.38) . The flavonoidal compounds were visualized by spraying the chromatogram with 1% Alc. $AlCl_3$ while PC of the butanol fraction in 25% acetic acid gave the best separation and revelead the presence of one main flavonoid at R_f = 0.77.

Investigation of ethyl acetate fraction:-

About 4.5 g of the ethyl acetate extract were dissolved in methanol and mixed with 5g silica gel . The methanol was evaporated *in vacuo* at 45C° till

dryness to give a homogenous powdered extract . This powder was transferred to silica gel column (5 × 80 cm, 60-120 mesh, BDH) packed as a dition of methanol up to 20% methanol . Fraction of 250 ml each were collected . The chromatographic fractionation was monitored using PC and 15% acetic acid as a developing solvent . The data were summarized in table (10) .

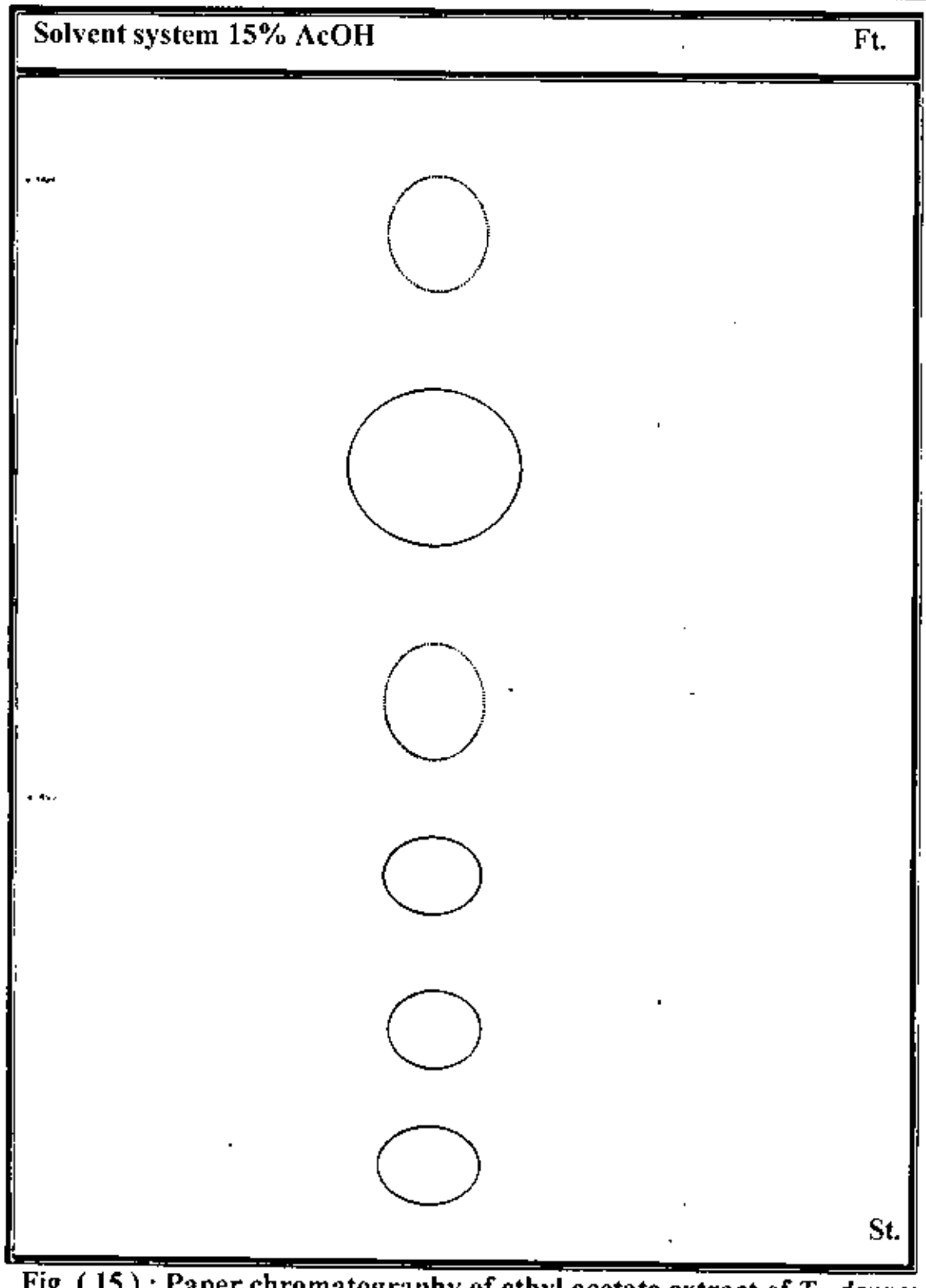


Fig. (15) : Paper chromatography of ethyl acetate extract of *T. davaeanum* .

Table (9): paper chromatography of the ethyl acetate fraction of *T. davaeanum.*

Compound	R_f*	Colour under UV		
			NH_3	$AlCl_3$
I	0.92	-	-	Y.G.
II	0.86	S.B.	S.B.	S.B.
III	0.72	B	B	B.Y
IV	0.66	F. Y.	Y.	Y. R
V	0.38	V.	B. V	V
VI	0.33	Br.	Y.	Y.
VII	0.22	-	Y.	Y.
VIII	0.11	F.Y.	Y.	Y.G.

Paper chromatography : (Whatmann No.1).

Solvent system : 15 % AcOH.

Spray reagent : $AlCl_3$ / MeOH.

B.V. = bluish violet. V. = Violet.

Br. = brown. Y. = Yellowish.

F. = faint. Y.G = Yellowish green.

S.B. = sky blue. Y.R = Yellowish red.

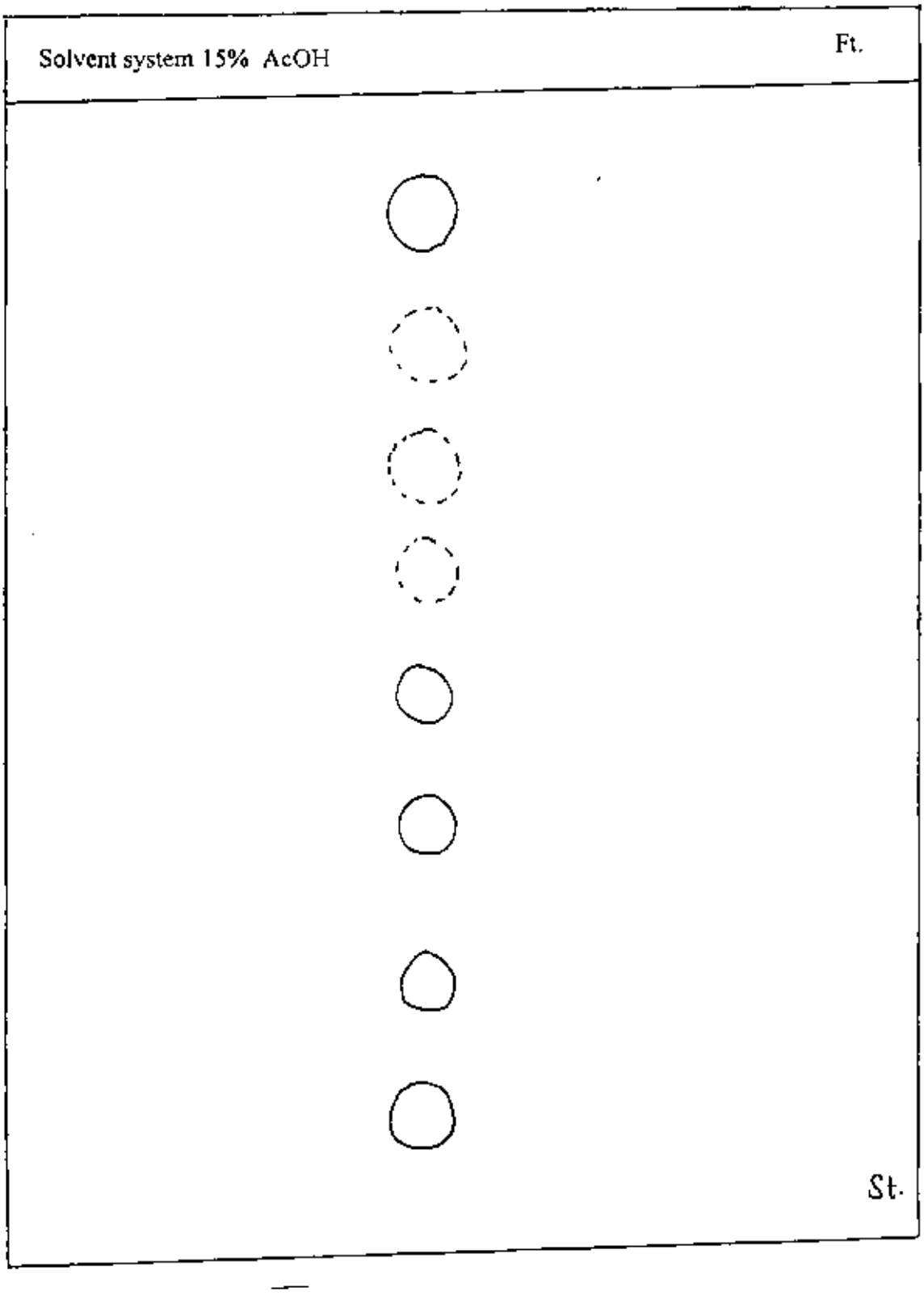


Fig. (16) : Paper chromatography of butanol extract of *T. davaeanum*

Table (10): Paper chromatography of butanol extract of *T. davaeanum*.

Compound	R_f*	Colour under UV		
			NH_3	$AlCl_3$
I	0.77	--	F.Y.	F.Y.
II	0.57	F.Br.	F.Br.	F.Br.
III	0.44	Br.	Y.	Y.
IV	0.36	F. Y.	F.Y.	F.Y.
V	0.30	Br.	Y.	Y.G.
VI	0.26	F.Bl.	F.Bl.	F.Bl
VII	0.20	Y.	Y.G.	Y.G.

* Paper chromatography: (Whatman No.1).

Solvent : 15% AcOH.

Spray reagent : $AlCl_3$ / MeOH

Bl. = Blue. Y = Yellow

Br. = Brown. Y.G = Yellowish green.

F. = Faint.

Table (11) : Column chromatography of ethyl acetate fraction of *T. davaeanum* .

Solvent	Fractions	R_f	Colour in UV		Isolated compounds
			NH_3	$AlCl_3$	
100% $CHCl_3$	1-5	0.80	Br.	Y.G.	-
		0.75	F.Br.	Y.G.	-
		0.72	Y.	Y.G.	
$CHCl_3$:MeOH 95:5	6-15	0.75	F.Y.	Y.	-
		0.65	Y.	Y.	-
		0.58	F.B.	F.B.	-
$CHCl_3$:MeOH 90:10	16-20	0.54	Y.G.	Y.G.	-
		0.45	S.B.	S.B.	-
		0.38	Br.	Br.	Compound-1
$CHCl_3$:MeOH 85:15	21-24	0.42	S.B.	S.B.	-
		0.38	F.S.B.	S.B.	-
		0.33	Br.	Y.	Compound-2
	25-30	0.25	Y.	Y.G.	
		0.22	F.Y.	Y.	Compound-3
		0.18	-	F.Y.	
$CHCl_3$:MeOH 80:20	31-37	0.33	Y.G.	Y.G.	-
		0.25	Y.G.	Y.G.	-
		0.18	-	F.Y.	-
		0.11	Y.	Y.G	Compound-4
		0.09	B.R.	Y.	-

- Paper chromatography : Whatmann 3MM .

Solvent system : 15% AcOH .

Spray reagent : $AlCl_3$ / MeOH .

B.V. = Bluish violet . V. = Violet .

Br. = Brown . Y. = Yellowish .

F. = Faint . Y.G. = Yellowish green .

S.B. = Sky blue . Y.R. = Yellowish red B. = Blue .

Purification of compound – 1

The fractions 16-20 (table 11) eluted with $CHCl_3$: MeOH 90:10 was found to contain one main compound (R_f = 0.38) so they were collected and purified using Sephadex LH-20 columns eluted with methanol (90%). The eluted fractions containing compound -1 in pure form (paper chromatography, 15% acetic acid and Butanol: acetic acid : water 3:1:1) were collected and evaporated *in vacuo* at 45C° . Compound -1 gave the characteristic colour and fluorescence in UV light for flavonoids.

Spectroscopic Measurements [169]:

The UV absorption spectra of the isolated flavonoidal compounds were measured by preparation of a solution of 0.0001M of the flavonoid 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 with distilled water up to 100 ml and, the solution was stored in a glass stoppered 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[169]:

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 three 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 solution 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 check 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.

Identification of compound -1

The UV absorption spectrum of compound -1 in methanol (Tab.12 and Fig.17) showed peak–I at 340 nm (flavone type) [169] structure or methoxylated flavonol at C-3 . A bathochromic shift (56 nm) in peak–I with low intensity was noticed on addition of NaOMe, which indicates the absence of free OH group at C-4. The $AlCl_3$ spectrum showed a bathochromic shift (40 nm) in peak–I indicating the presence of free OH group at C–5, Moreover the absence of *ortho* dihydroxy system was confirmed through the $AlCl_3$/HCl spectrum where, there is no hypsochromic shift in peak–I. The NaOAc spectrum showed no bathochromic shift in peak-II (272 nm) which indicates the absence of free OH group at C–7.

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

Addition to methanol	λ_{max} (nm)
None	275,340.
NaOMe	269,278,396.
$AlCl_3$	260,288,364,380.
$AlCl_3$/ HCl	259,289,360,380.
NaOAc	272,341,403.
NaOAc / H_3BO_3	274,343.

The EI Mass spectrum of compound –1(Fig.18) showed a molecular ion peak [M^+] at m/z = 358 (26.2%) which corresponding to the molecular formula $C_{19}H_{18}O_7$. Another peaks at m/z = 343 ($M^+ - CH_3$, 41%), 329 ($M^+ - CHO$, 59 %) and 298 ($M^+ - 2\ OCH_2$, 26 %). The fragmentation pathway undergoes

Retero Diels Alder reaction (RDAR) [169] giving rise to fragments at m/z = 164 (A^+ - 1, 185), and 194 (B^+ + 1, 9%). The presence of these two peaks confirms the presence of two methoxy groups at ring B, one methoxy group at ring A and one methoxy group at ring C.

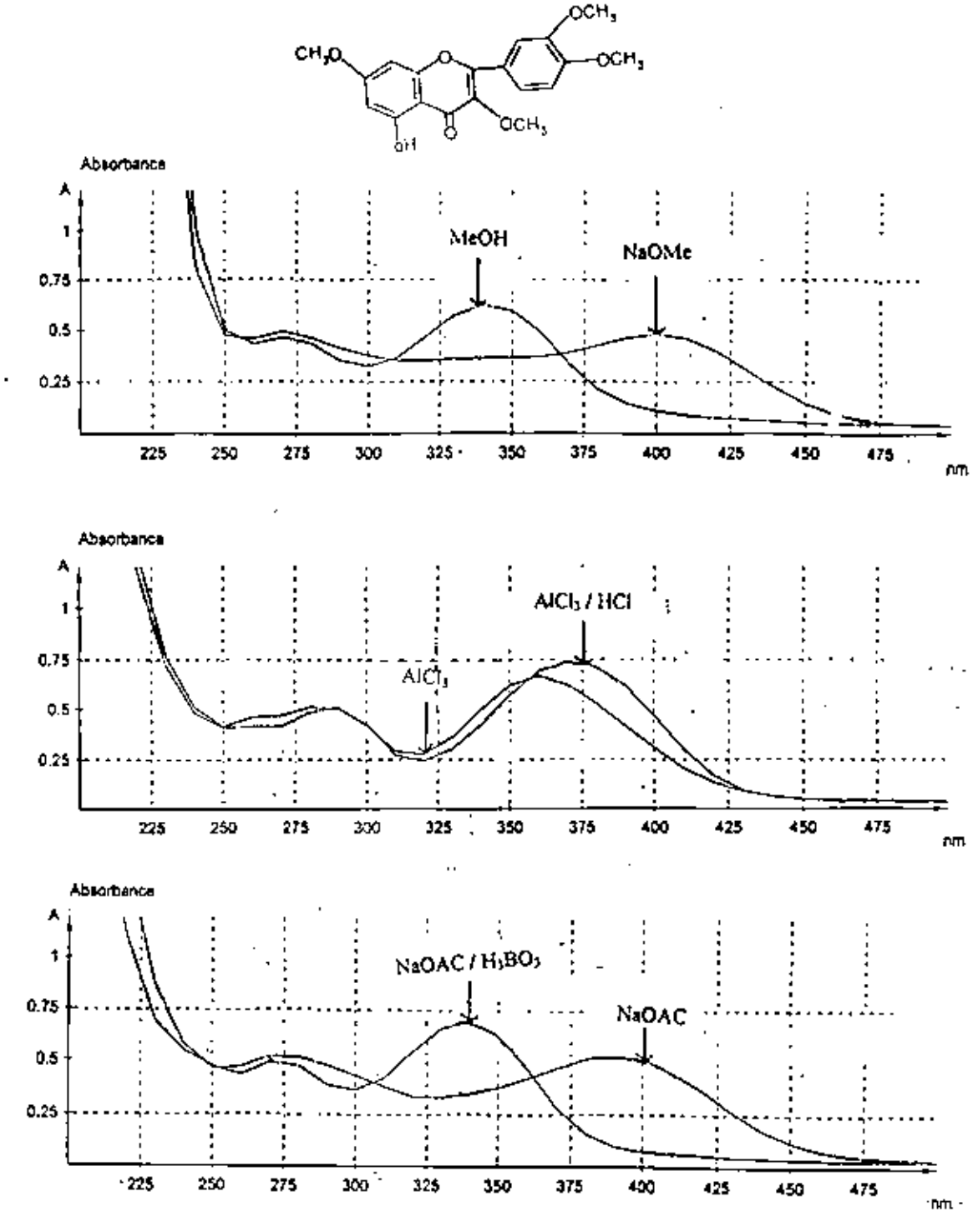


Fig. (17) : The UV absorption spectra of compound-1
(3,7,3',4' tetramethoxy, 5-hydroxyl flavone) .

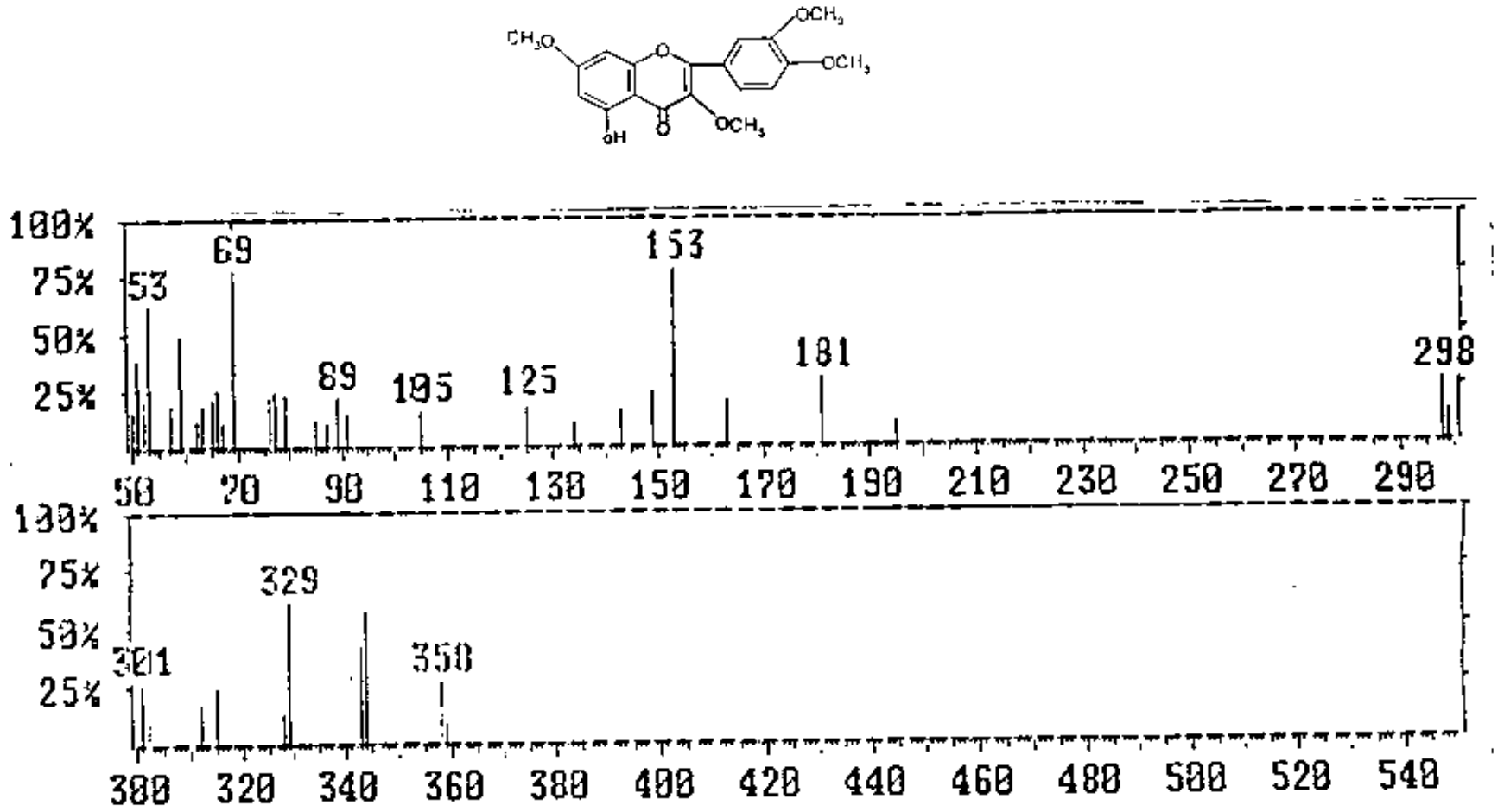
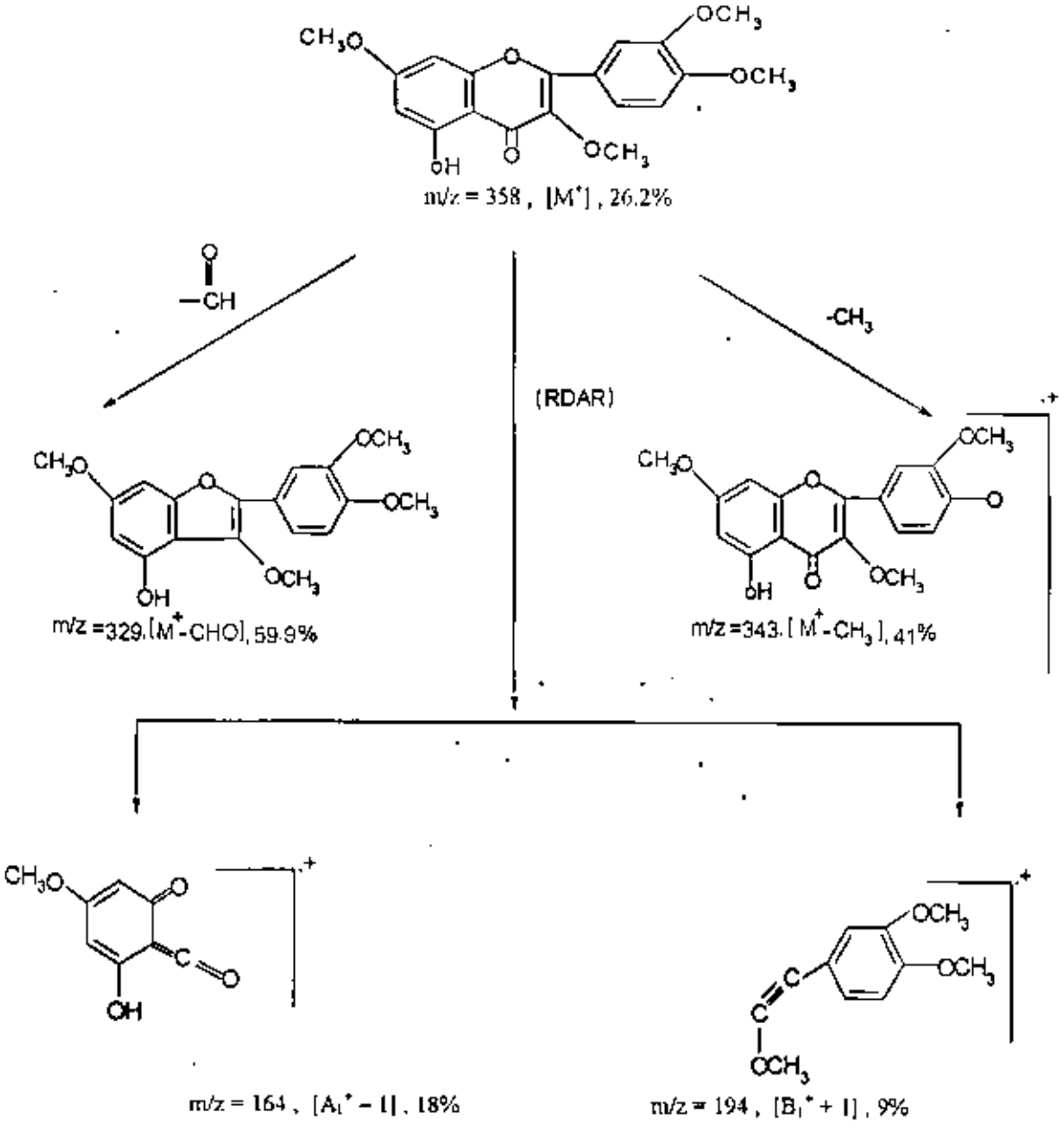


Fig. (18) : The EI mass spectrum of compound-1

(3, 7, 3, 4 tetramethoxy, 5-hydroxyl flavone).



Scheme (1) : Fragmentation pattern of compound-1 (3,7,3\`,4\` tetramethoxy , 5-hydroxy flavone) .

The ^{1}H-NMR spectrum of compound – 1 in CD_3OD (Fig.19) showed signal at δ in ppm 7.5 (2H, d, H – 2',6'), 6.9 (1H, d, H – 5'), 6,75 (1H, d, H – 8), 6.6 (1H, d, H – 6), 3.96 (3H, s, C -3 – OCH_3), 3.93 (3H, s, C– 3'–OCH_3), 388 (3H, s, C-4'– OCH_3) and 3.83 (3H, s, C – 7– OCH_3).

The ^{13}C-NMR spectrum of compound–1 in CD_3OD (Fig. 20) displayed the most important peaks characteristic to methoxylated flavonol where C-4 appears at δ = 184 ppm. The other data were shown in table (13) [170] .

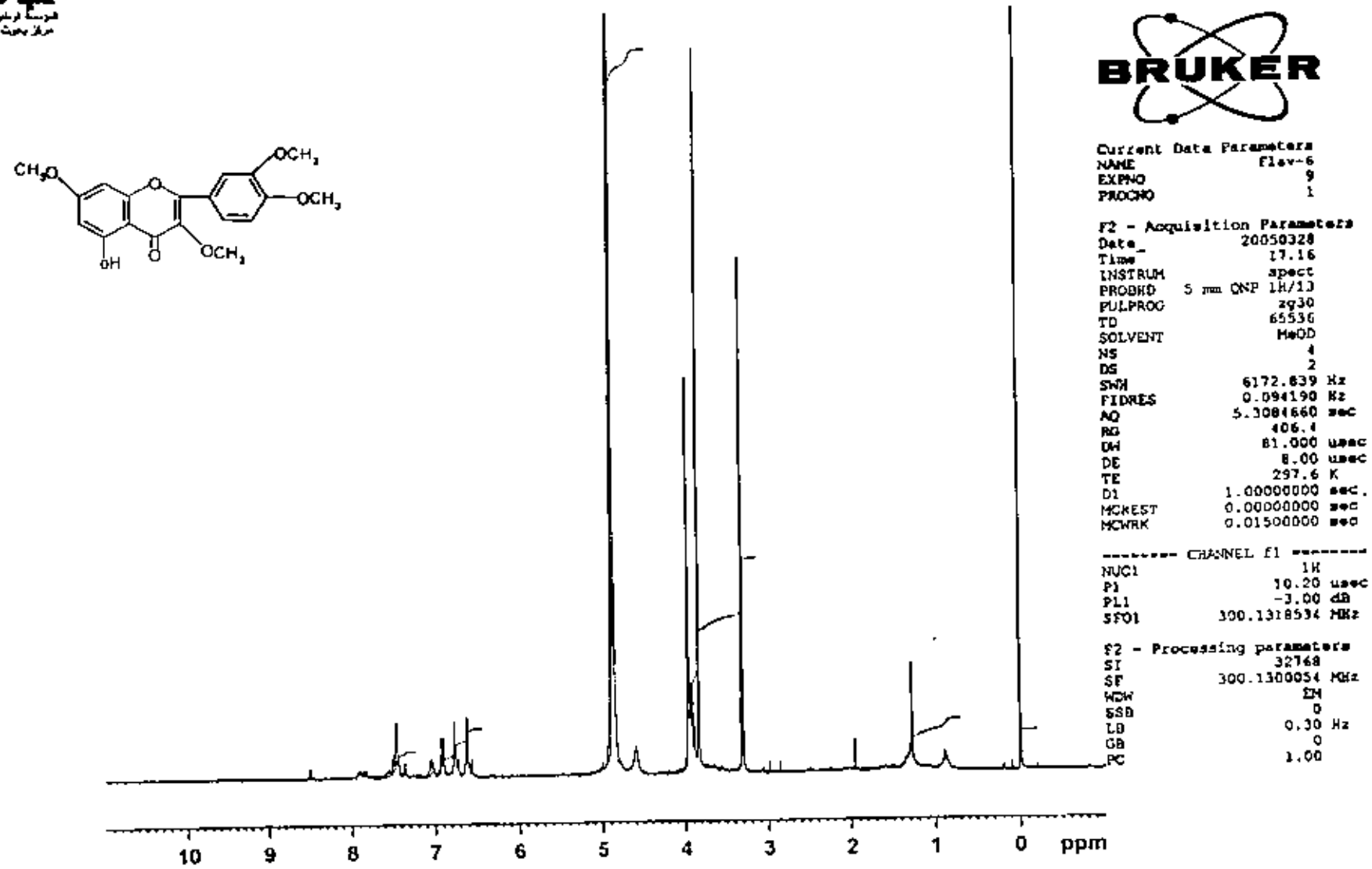


Fig. (19) : The ^{1}H NMR spectrum of compound-1

(3, 7, 3', 4' tetramethoxy, 5-hydroxyl flavone).

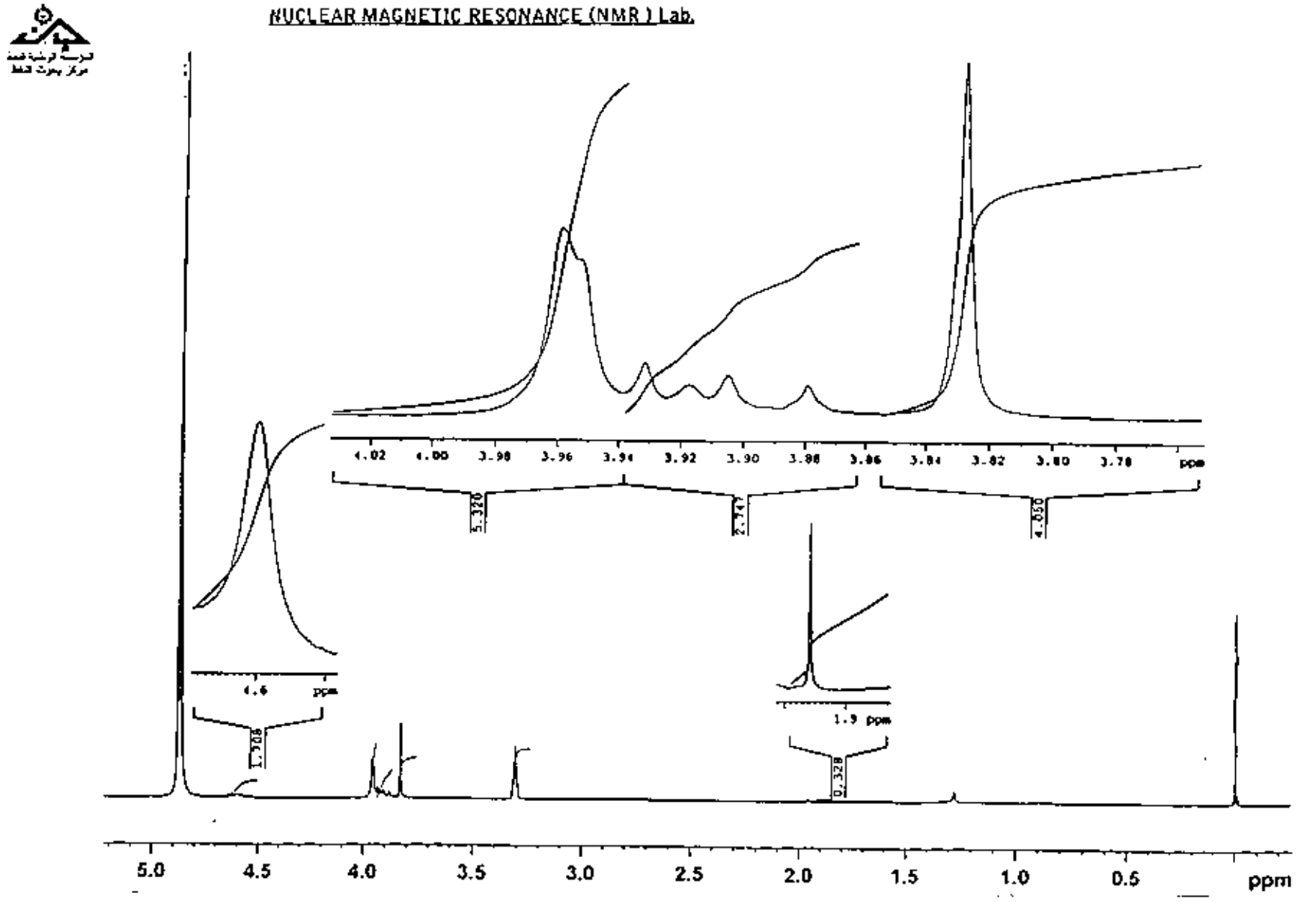

NUCLEAR MAGNETIC RESONANCE (NMR) Lab.
4.02
4.00
3.98
3.96
3.94
3.92
3.90
3.88
3.86
3.84
3.82
3.80
3.78
ppm
5.320
2.747
4.650
4.6
ppm
1.908
1.9 ppm
0.328
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

Fig. (19) : Cont.

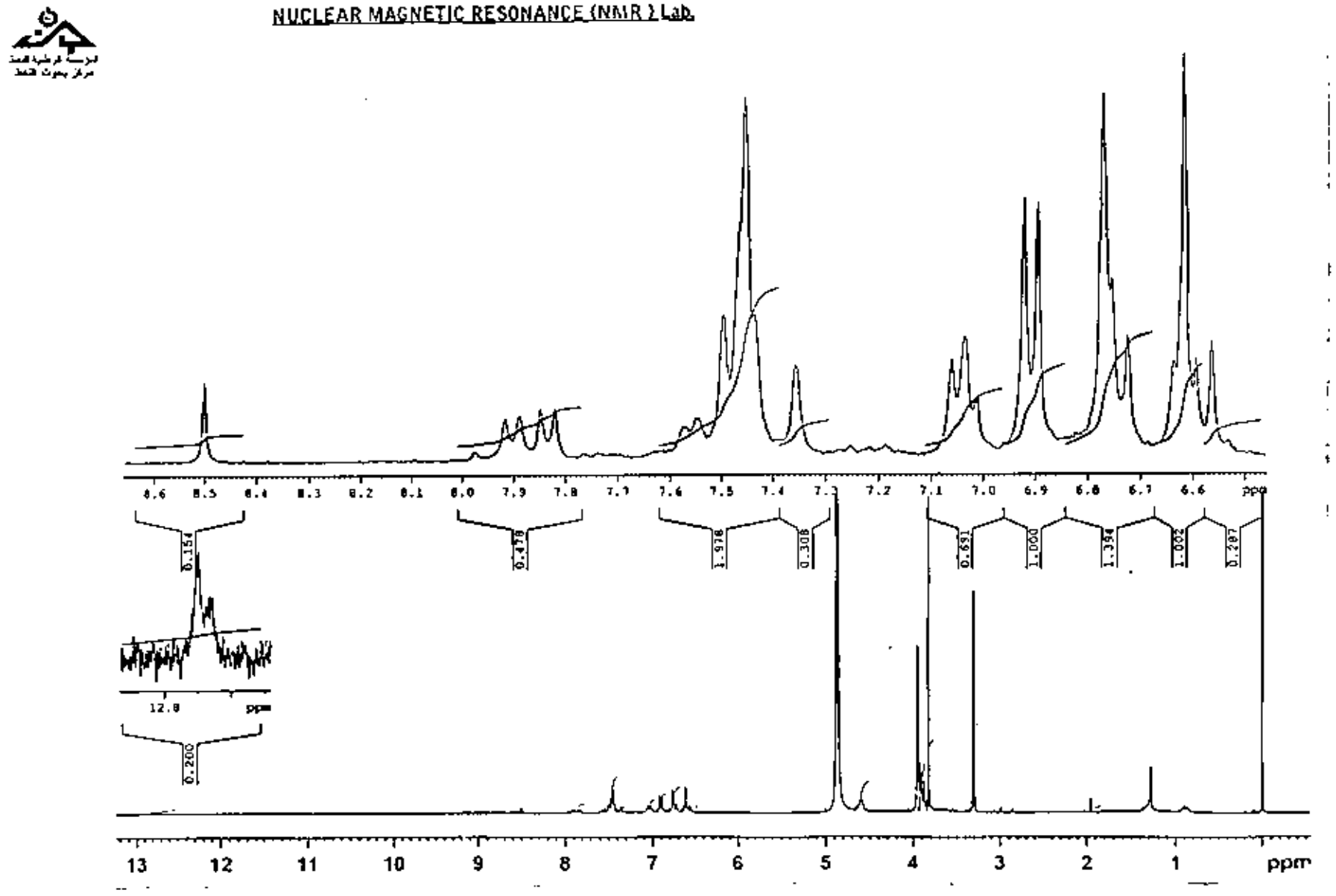
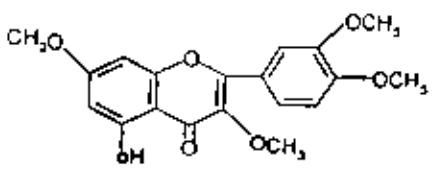

NUCLEAR MAGNETIC RESONANCE (NMR) Lab.
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

Fig. (19) : Cont.

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

Carbon no.	δ (ppm)
2	149.50
3	133.70
4	184.19
5	160.50
6	92.35
7	166.43
8	92.35
9	154.90
10	104.99
1'	123.2
2'	110.46
3'	152.25
4'	135.65
5'	113.97
6'	121.55
C3 – OCH_3	61.10
C3' – OCH_3	57.03
C4' – OCH_3	65.68
C7 – OCH_3	56.60



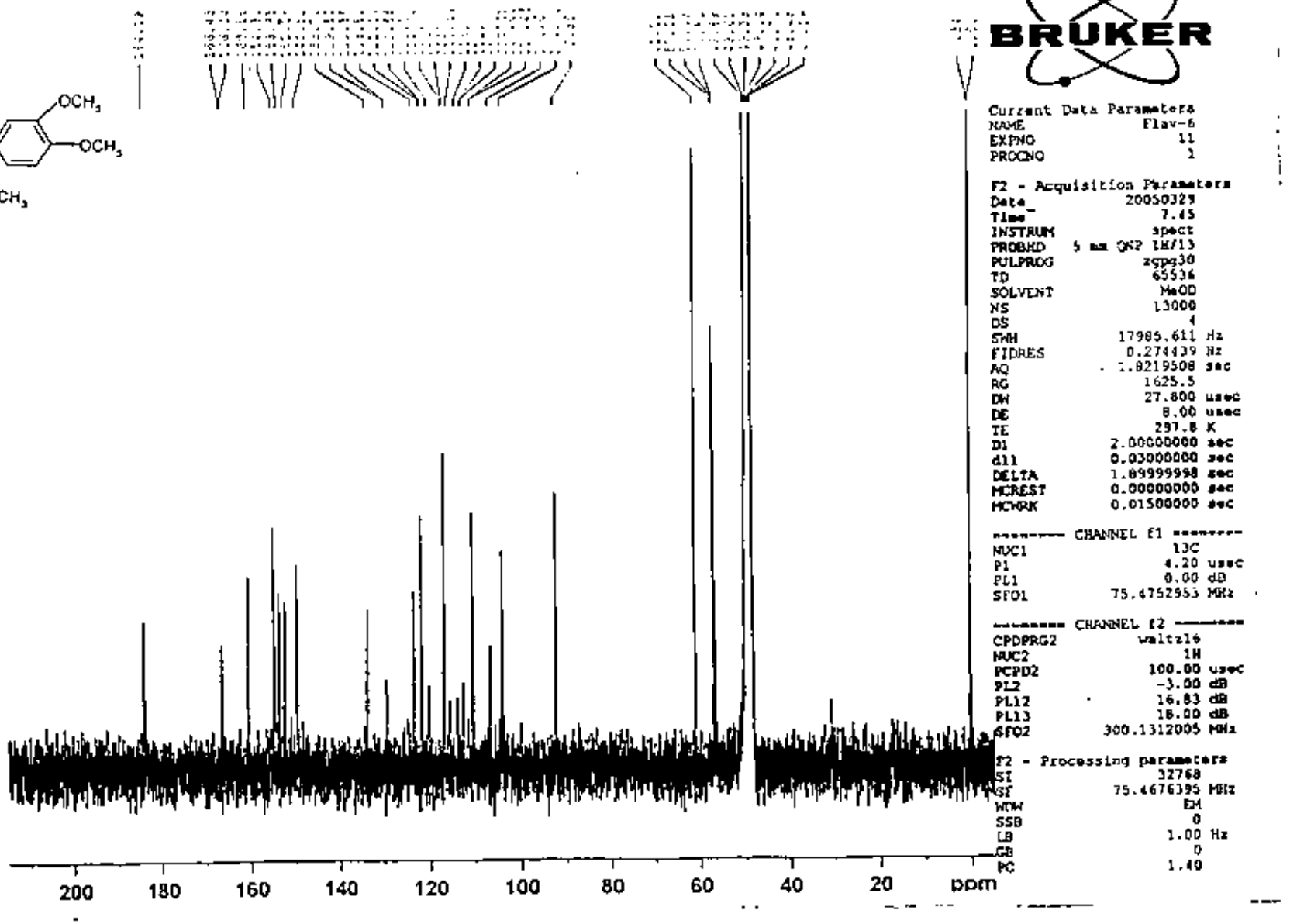
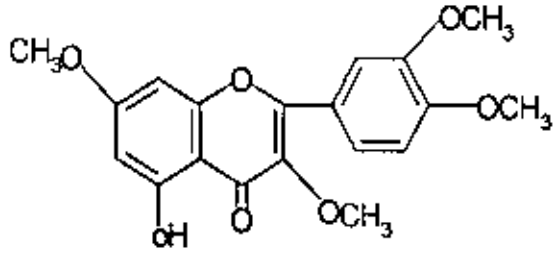


Fig. (20) : The ^{13}C NMR spectrum of compound-1

(3, 7, 3', 4' tetramethoxy, 5- hydroxyl flavone).

From all the above chromatographic and spectroscopic data, compound–1 could be identified as: 3, 7, 3', 4' tetramethoxy, 5 – hydroxy flavone:



3,7,3,4 tetramethoxy , 5-hydroxy flavone .

Purification of compound – 2:-

The fractions 21-24(table 11) eluted with $CHCl_3$: MeOH 85:15 were found to contain only one flavonoidal compound (Rf = 0.33) with some traces, so they were collected and further purified using preparative thick layer chromatography (PTLC) using silica gel plates and methanol : chloroform 85:15 as a devloping solvent. The main zone (R_f 0.52) was localized under UV light, scrapped off and eluted using methanol 95%. The solvent was evaporated *in vacou* at 45 C°. The purity of the compound – 2 was checked using two dimension paper chromatography (2DPC) using different solvent systems.

Identification of compound – 2:-

The UV absorption spectra of coumpound-2 (Fig. 21 and Tab. 14) showed peak–I at 349 nm which proved the flavone nature of the compound [169]. A bathochromic shift (50 nm) in peak–I with high intensity in NaOMe spectrum indicates the presence of a free OH group at C4'.

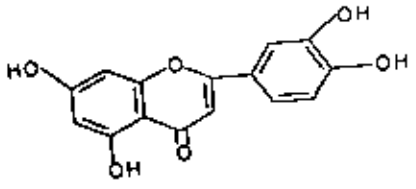
The bathochromic shift (71 nm) in peak–I in $AlCl_3$ spectrum showed the presence of a free OH group at C-5. The $AlCl_3$/HCl spectrum, displayed a hypsochromic shift (39 nm) in peak–I relative to the $AlCl_3$ spectrum proved the presence of an *ortho* dihydroxy system in ring B which was confirmed through the NaOAc/H_3BO_3 spectrum, where, there is a bathochromic shift in peak–I (24 nm) relative to methanol spectrum.

Moreover there is a bathochromic shift (16 nm) in peak–II in NaOAc spectrum relative to methanol which confirmed the presence of a free OH group at C – 7.

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

Addition to methanol	λ_{max} (nm)
None	254,267,293,349.
NaOMe	264,399.
$AlCl_3$	273,300,331,420.
$AlCl_3$ / HCl	262,275,294,355,381.
NAOAc	270,326,391.
NaOAc / H_3 BO_3	263,273.

The EI mass spectrum of compound – 2 (Fig.22) showed a molecular ion peak [M^+] at m/z = 286 (60.08%) which constitute with the molecular formula C_{15} H_{10} O_6 . Another fragments at m/z = 153 (54.9%) and 133 (35.8%) indicate that compound–2 react retero Diels Alder fragmentation as shown in Scheme (2) :



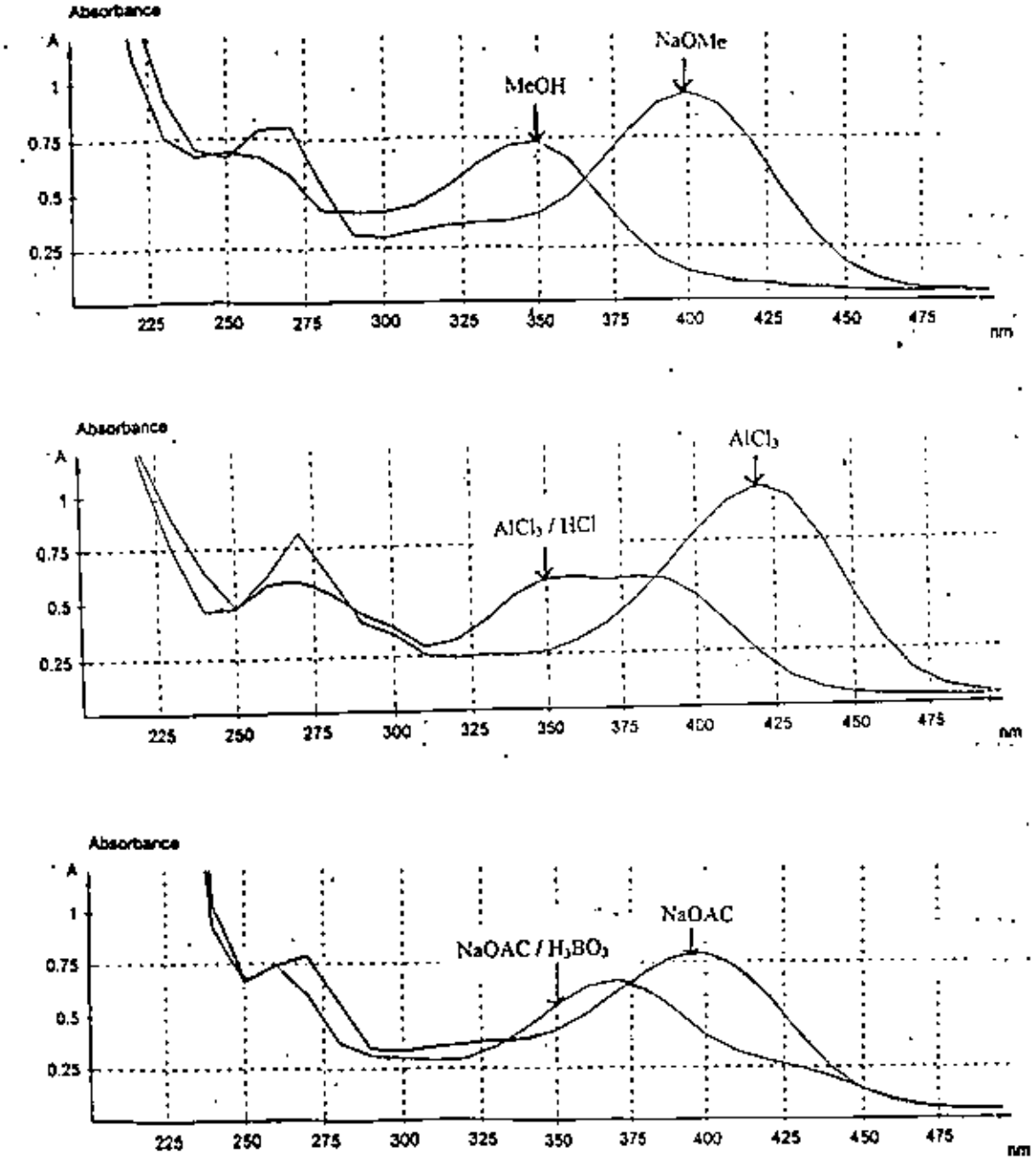
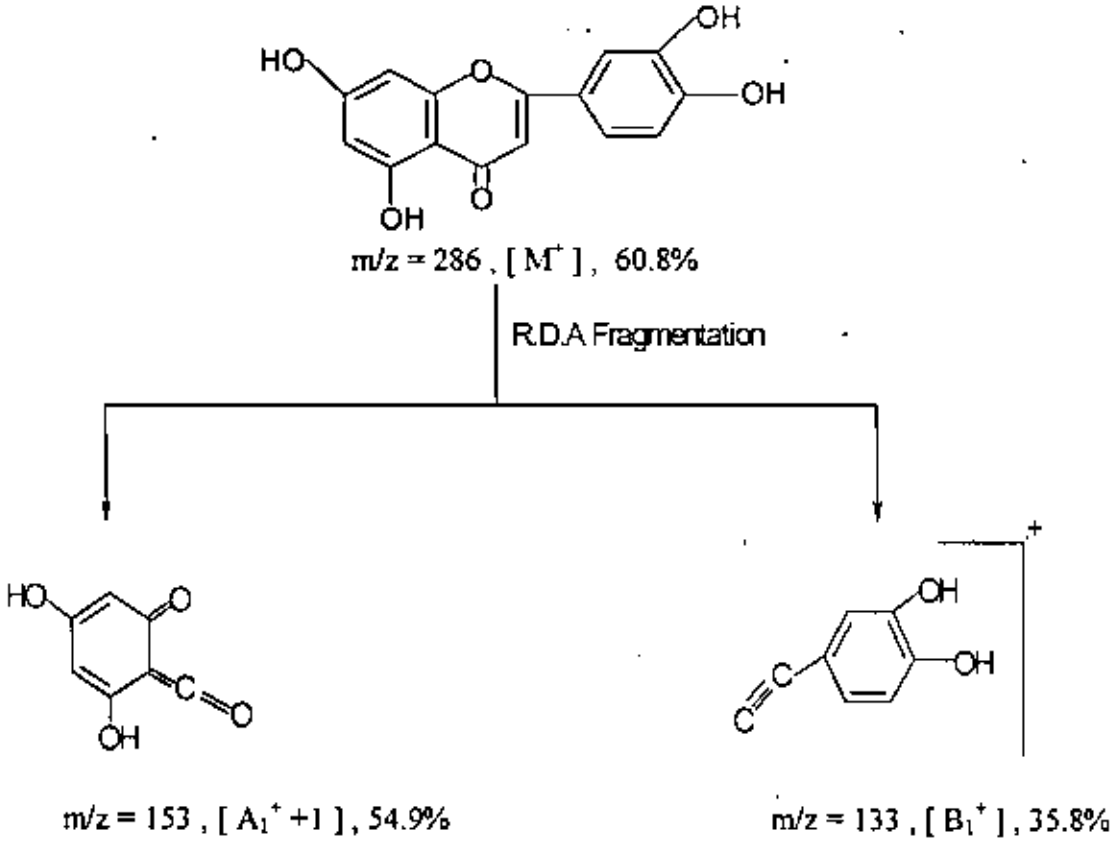


Fig. (21) : The UV absorption spectra of compound-2 (5,7,3-,4- tetrahydroxy flavone (luteolin) .



Scheme (2) : Fragmentation pathway of compound- 2 (luteolin) .

The ^{1}H-NMR spectrum of compound – 2 in CD_3OD Fig. (23) displayed signals at δ in ppm 7.39 (1H, d, J = 7 Hz, H – 2'), 7.36 (1H, d, J = 7 Hz, H – 6'), 6.93 (1H, d, J = 8Hz, H – 5'), 6.5 (1H, s, H–3), 6.4 (1H, d, J=3, H–8), 6.1 (1H, d, H–6), these data were in accordance with that reported for luteolin [169]. Also ^{13}C-NMR data were found in table (15).

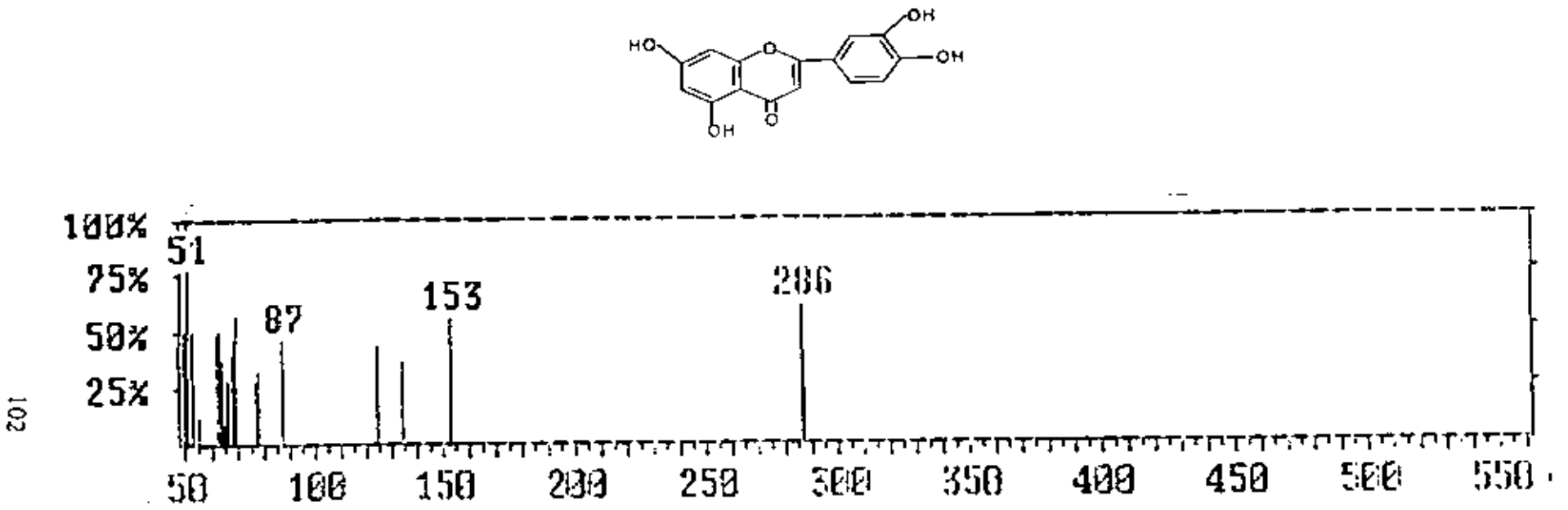


Fig. (22): The E1 mass spectrum of compound-2
(5,7,3-,4- tetrahydroxy flavone (luteolin) .

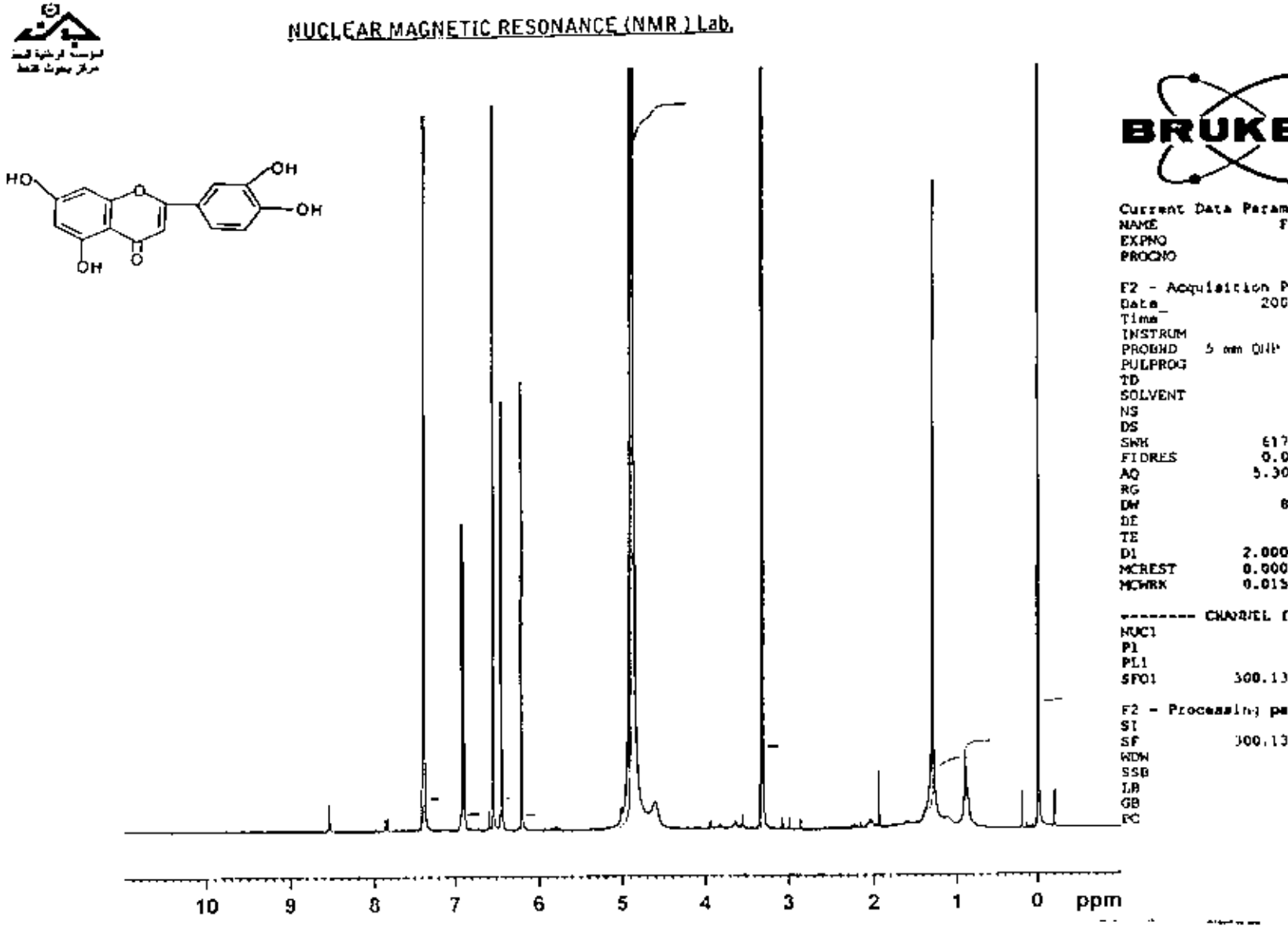


Fig. (23) : The ^{1}H NMR spectrum of compound-2 (5,7,3-,4- tetrahydroxy flavone (luteolin) .

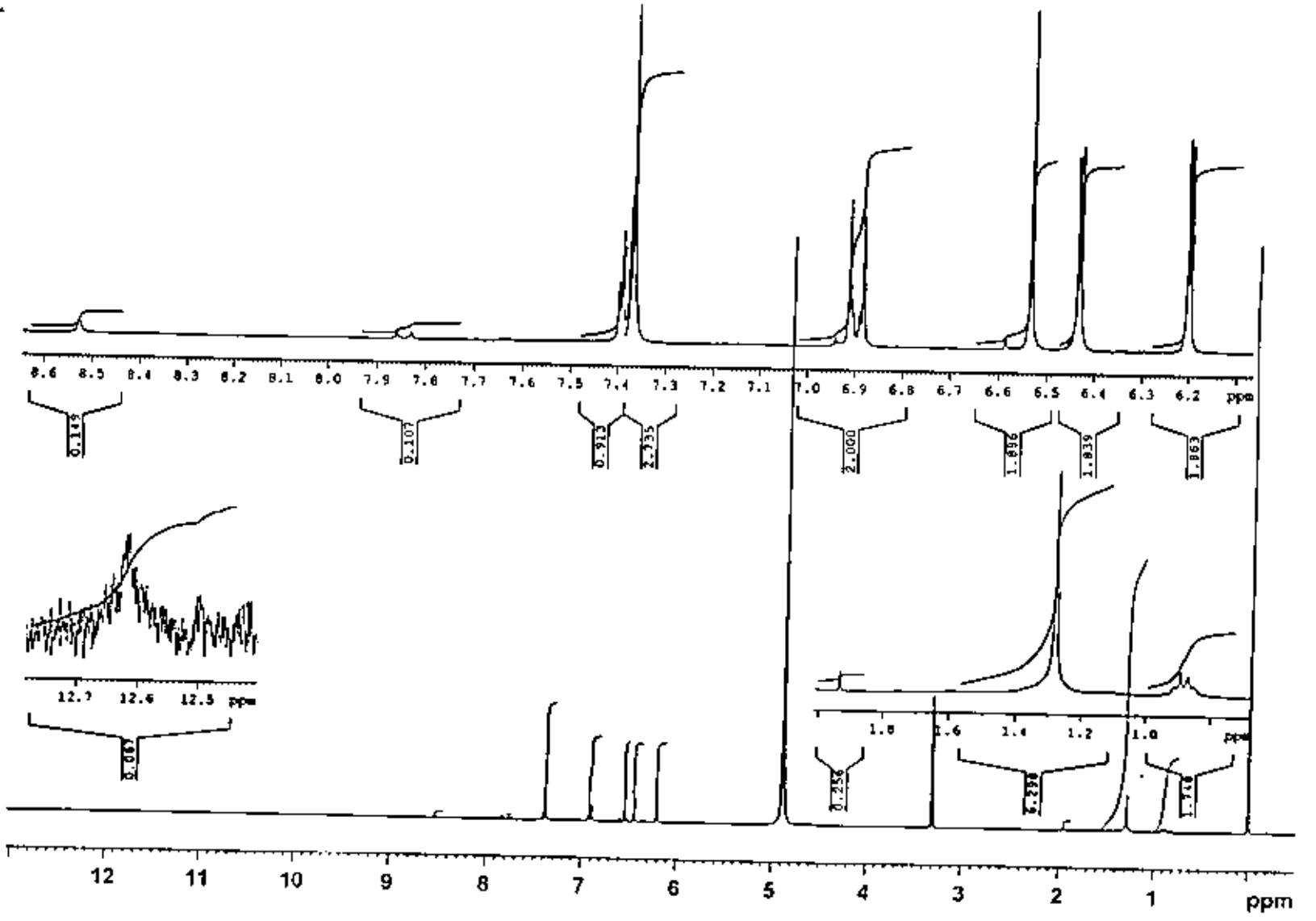


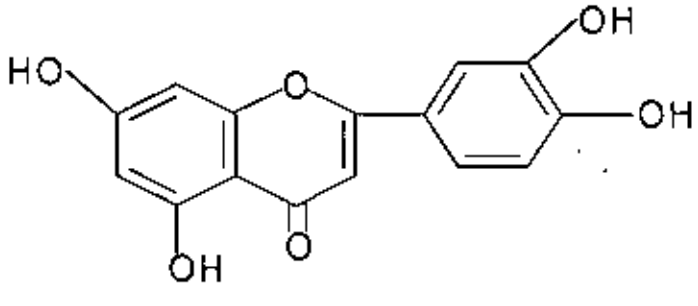
Fig. (23) : Cont.

Table (15): ^{13}C-NMR data of compound – 2 [171]

Carbon no.	δ (ppm)	Reported data*
2	166.15	165.00
3	103.99	103.30
4	182.9	182.20
5	163.25	162.10
6	100.19	99.20
7	166.39	165.00
8	95.06	94.20
9	159.40	157.90
10	105.32	104.20
1′	123.72	122.10
2′	114.19	113.80
3′	147.10	146.20
4′	151.05	150.10
5′	116.92	116.40
6′	120.43	119.30

*The reported data were measured in DMSO.

All the above data substantiate that compound – 2 is Luteolin



5,7,3,4- tetrahydroxy flavone (luteolin) .

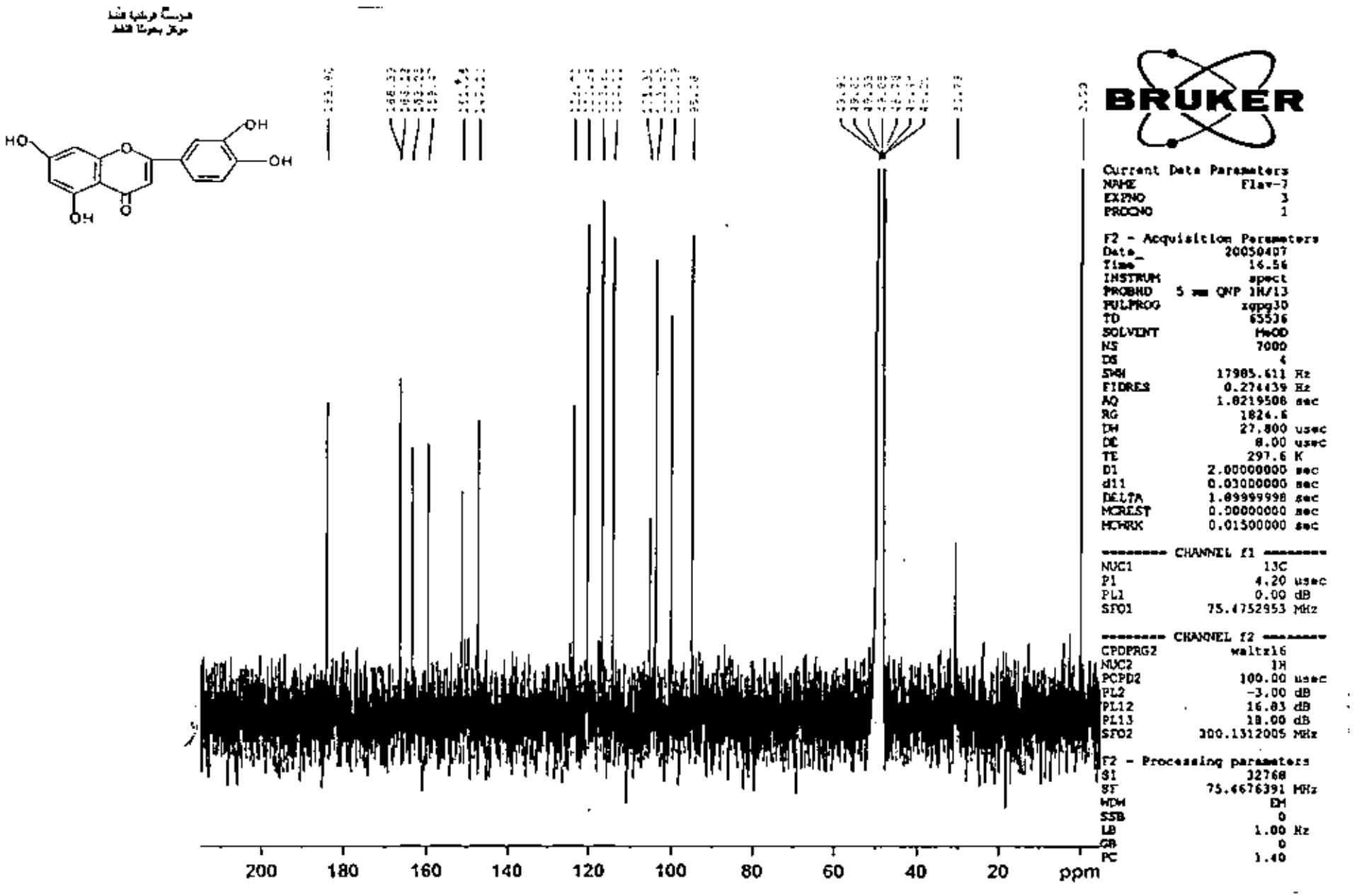


Fig. (24) : The ^{13}C NMR spectrum of compound-2 (5,7,3-,4- tetrahydroxy flavone (luteolin) .

Purification of compound – 3:-

The fractions 25-30 (table 11) eluted with $CHCl_3$: MeOH 85:15 were collected and rechromatographed on small silica gel column eluted with chloroform with increasing polarity using methanol. The fractions eluted with 85:15 was found to contain one main compound with some traces, which was further purified on Sephadex LH – 20 column eluted with methanol (100%). The fractions containing compound – 3 (R_f = 0.22) in pure form were collected and evaporated *in vacuo*.

Identification of compound – 3:-

The UV absorption spectra of compound-3 (Fig. 25 and Tab.16) showed peak–I in methanol at 345 nm (flavone type structure or substituted flavonol at C–3)[169] . A bathochromic shift (51 nm) in peak – I was noticed upon addition of NaOMe indicates the presence of free OH group at C -4'.

The $AlCl_3$ spectrum showed a bathochromic shift (76 nm) in peak–I relative to methanol indicates the presence of a free OH group at C–5. A hypsochromic shift (57 nm) in peak–I with $AlCl_3$ / HCl relative to $AlCl_3$ spectrum indicates the presence of an *ortho* dihydroxy system in ring-B. Also it was confirmed through NaOAc / H_3BO_3 spectrum where, there is a bathochromic shift (26 nm) in peak–I relative to methanol. The presence of free OH group at C– 7 was confirmed, where there is a bathochromic shift (14 nm) in peak– II was noticed in NaOAc spectrum.

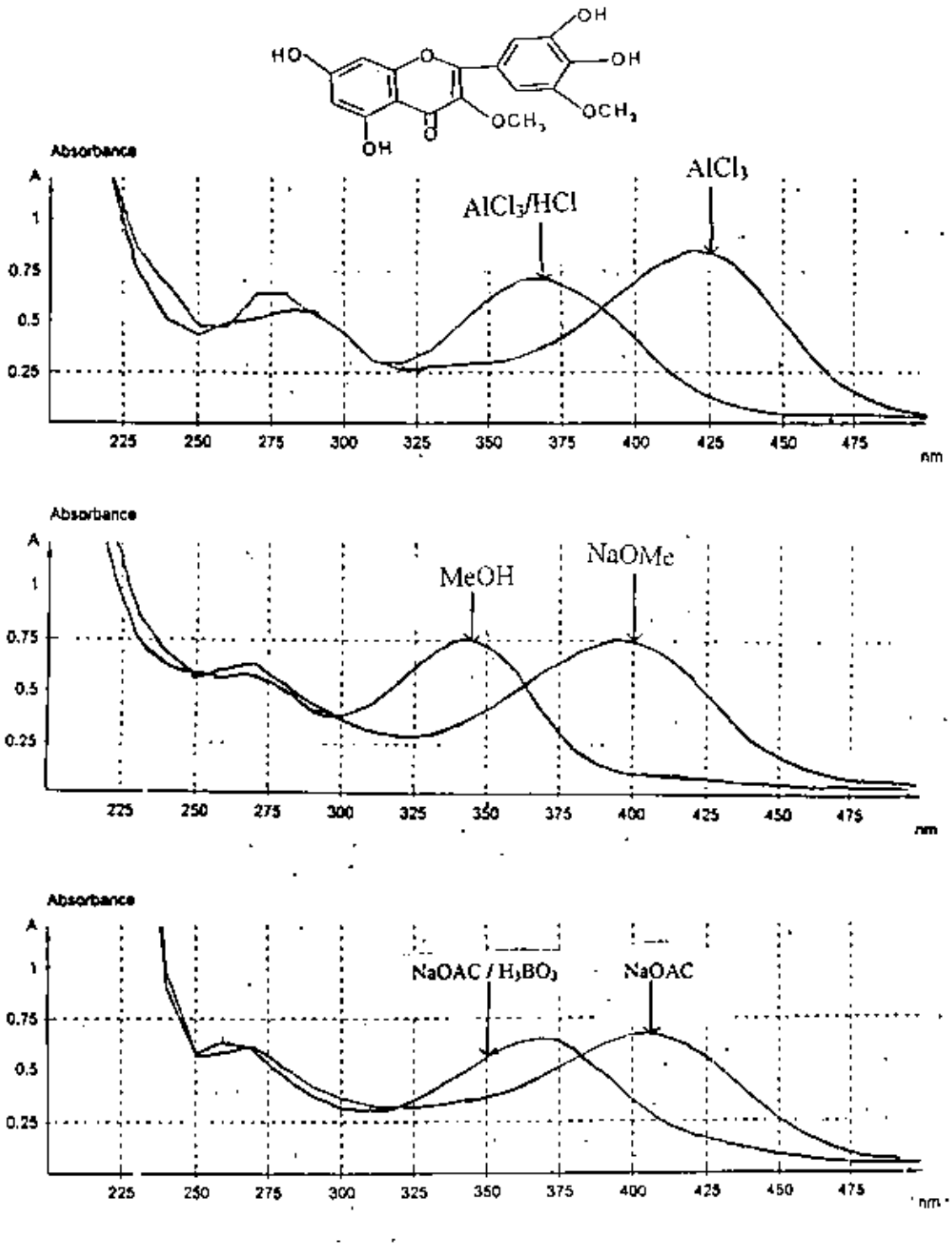


Fig. (25) : The UV absorption spectra of compound-3 (3,5 ˙dimethyl myricetin) .

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

Addition to methanol	λ_{max} (nm)
None	255,273,345.
NaOMe	266,396.
$AlCl_3$	264,275,380,421.
AlC_3 / HCl	$262,284,300_{(s)},364.$
NaOAc	269,400.
NaOAc / H_3BO_3	263,371.

The EI- mass spectrum of compound-3 (Fig. 26) showed a molecular ion peak M^+ at m/z =330 (29.25%) which constituted with the molecular formula $C_{17}H_{14}O_7$. Another peaks at m/z = 315 (M^+ - CH_3, 26.9%) , m/z = 269(M^+ - $2OCH_3$, 4%) , and 282 (M^+ - (OCH_3 + OH) 4%). The fragmentation pathway undergo RDA reaction giving rise to two peaks at m/z = 153 (A_1^+, 27%) and 134 (B_1^+ - $2OCH_2$) .

The ^{1}H-NMR spectrum of compound-3 in DMSO (Fig.27) displayed signals at δ in ppm 7.47 (1H, d, H-2'), 7.43(1H, d H-6') , 6.89(1H, d, H-8), 6.72(1H, d, H6), 3.95 (3H,s, C-3-OCH_3) and finally 3.73 (3H, s,C-5'OCH_3)

The ^{13}C-NMR spectrum in DMSO (Fig.28) showed the most important signal of flavonol type structure where C-4 appears at δ 181.95 ppm . Both C3 , C5' signal appears down field due to the presence of two OCH_3 groups at these carbons , other data were summarized in tabl (17) .

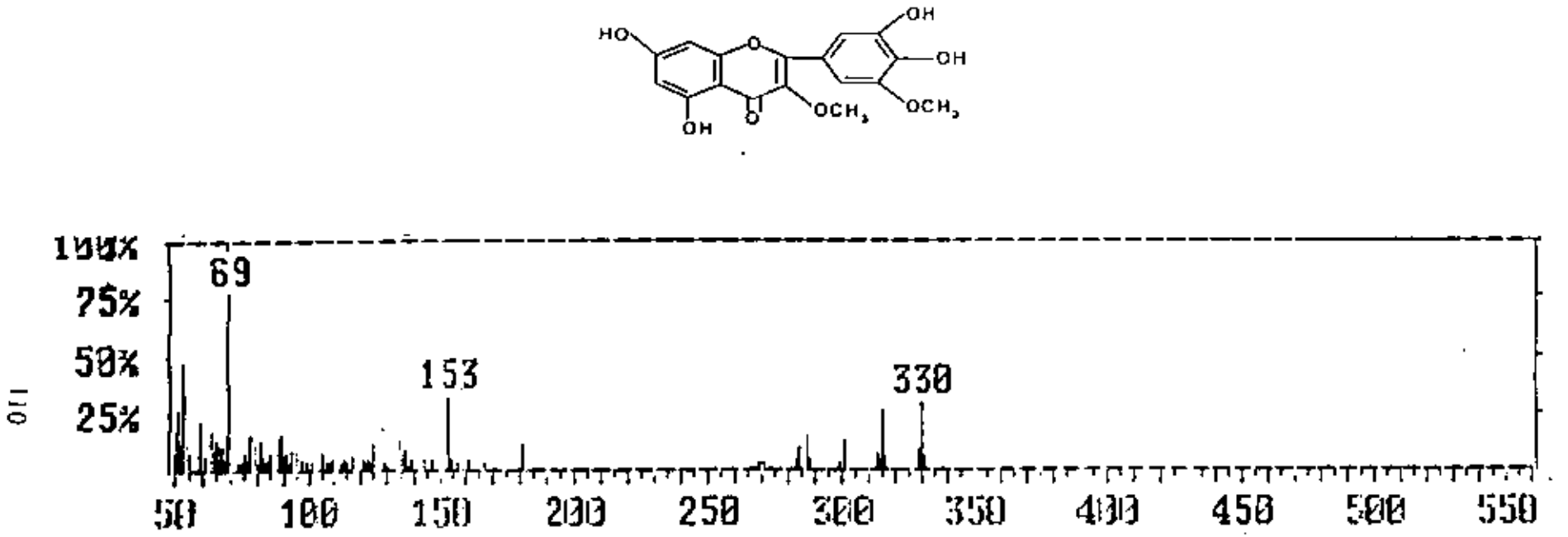
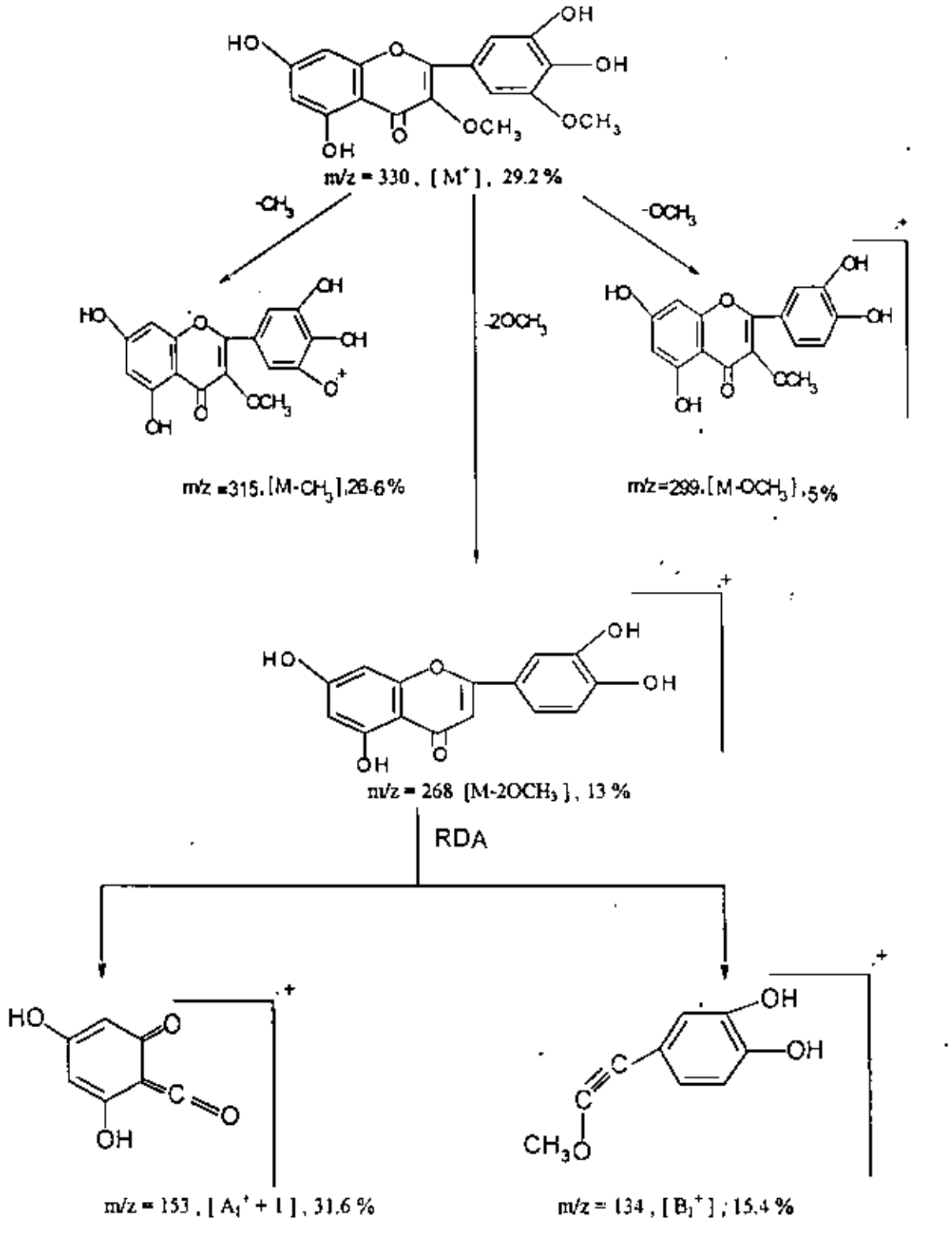
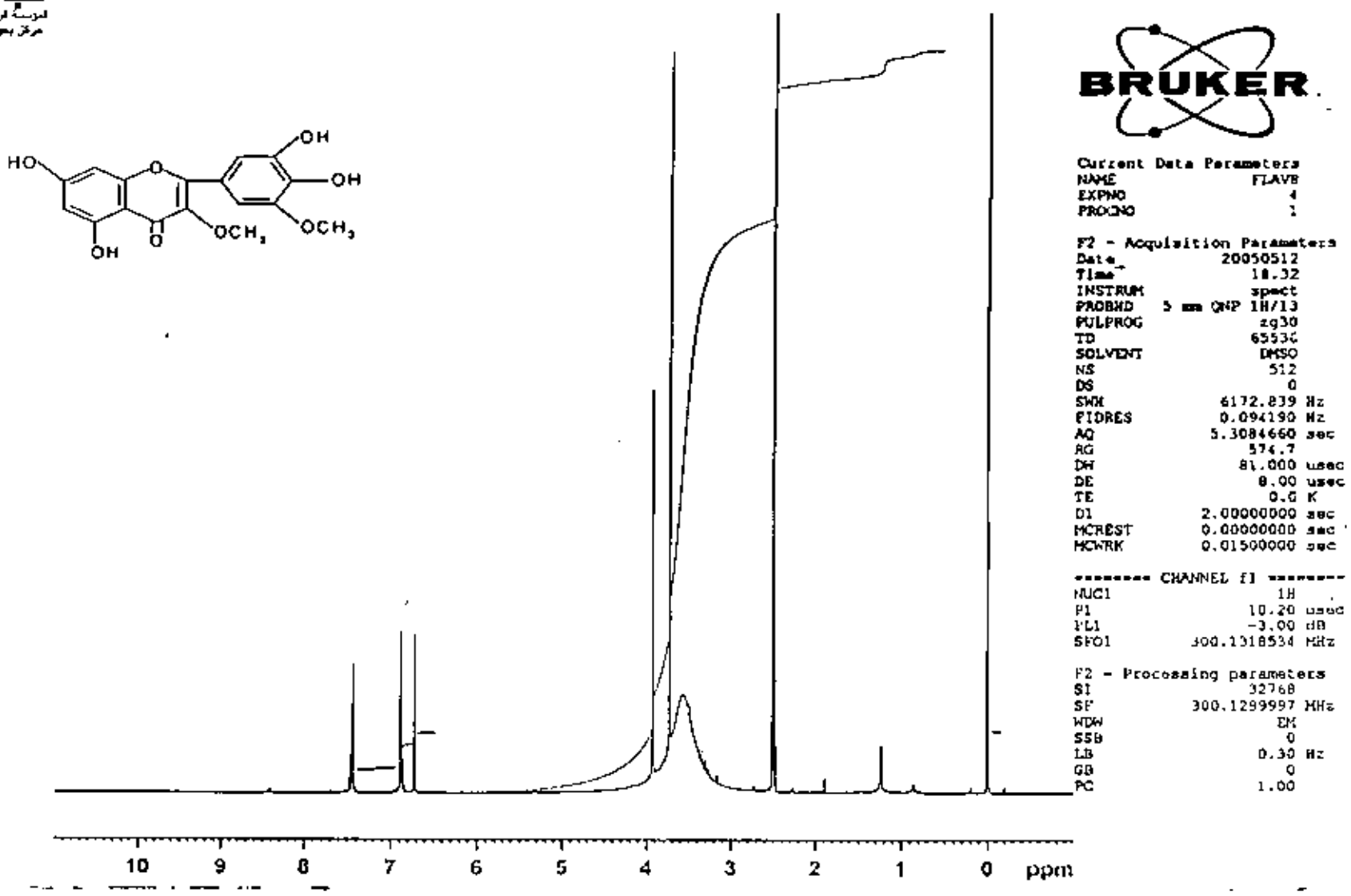


Fig. (26) : The EI mass spectrum of compound-3
(3,5 ` dimethyl myricetin) .

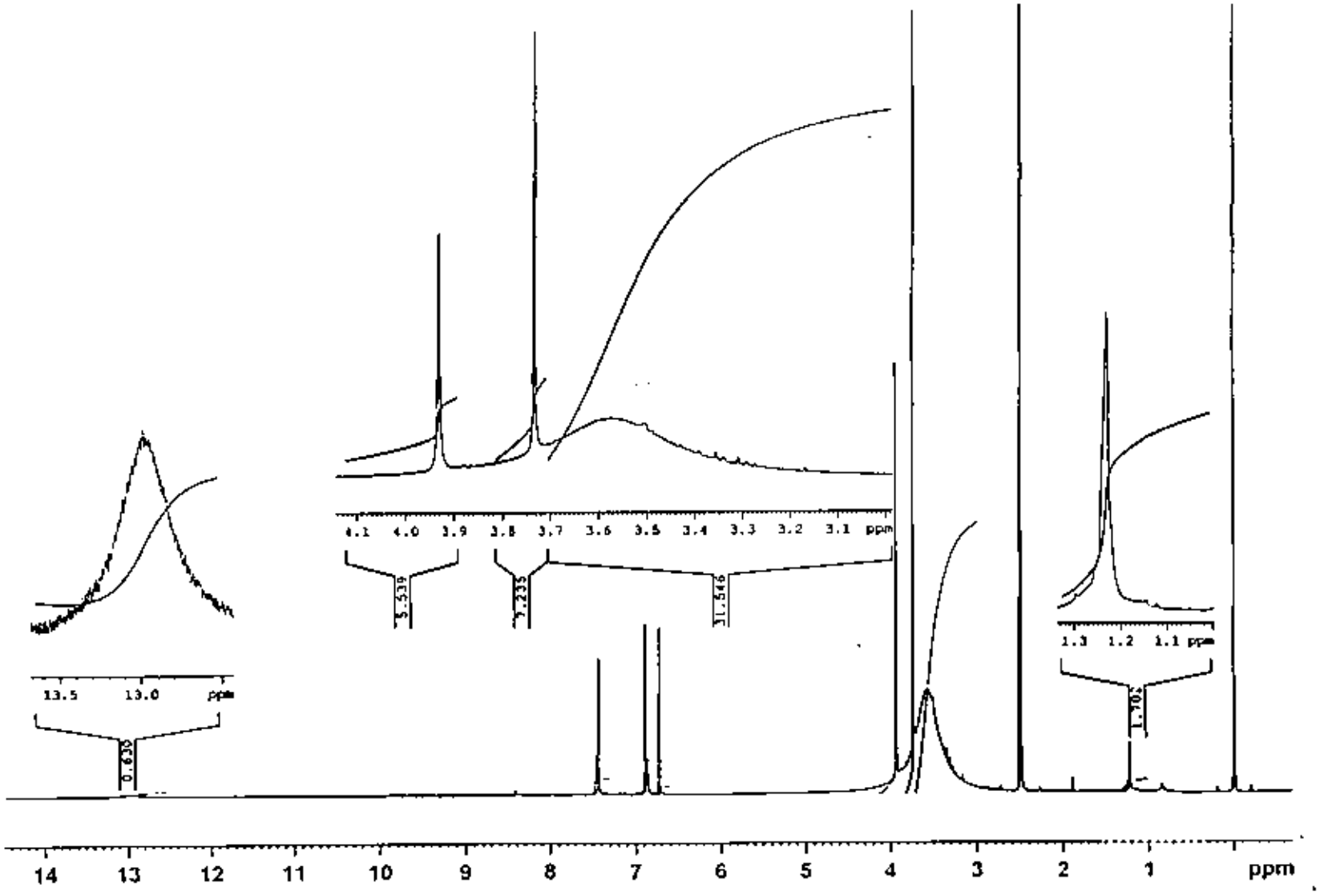


Scheme (3): Fragmentation pathway of compound -3 .



112

Fig. (27) : The ^{1}H NMR spectrum of compound-3 (3,5 ¯dimethyl myricetin) .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4.1
4.0
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3.8
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3.6
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3.4
3.3
3.2
3.1
ppm
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7.235
31.546
1.3
1.2
1.1
ppm
1.705
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11
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8
7
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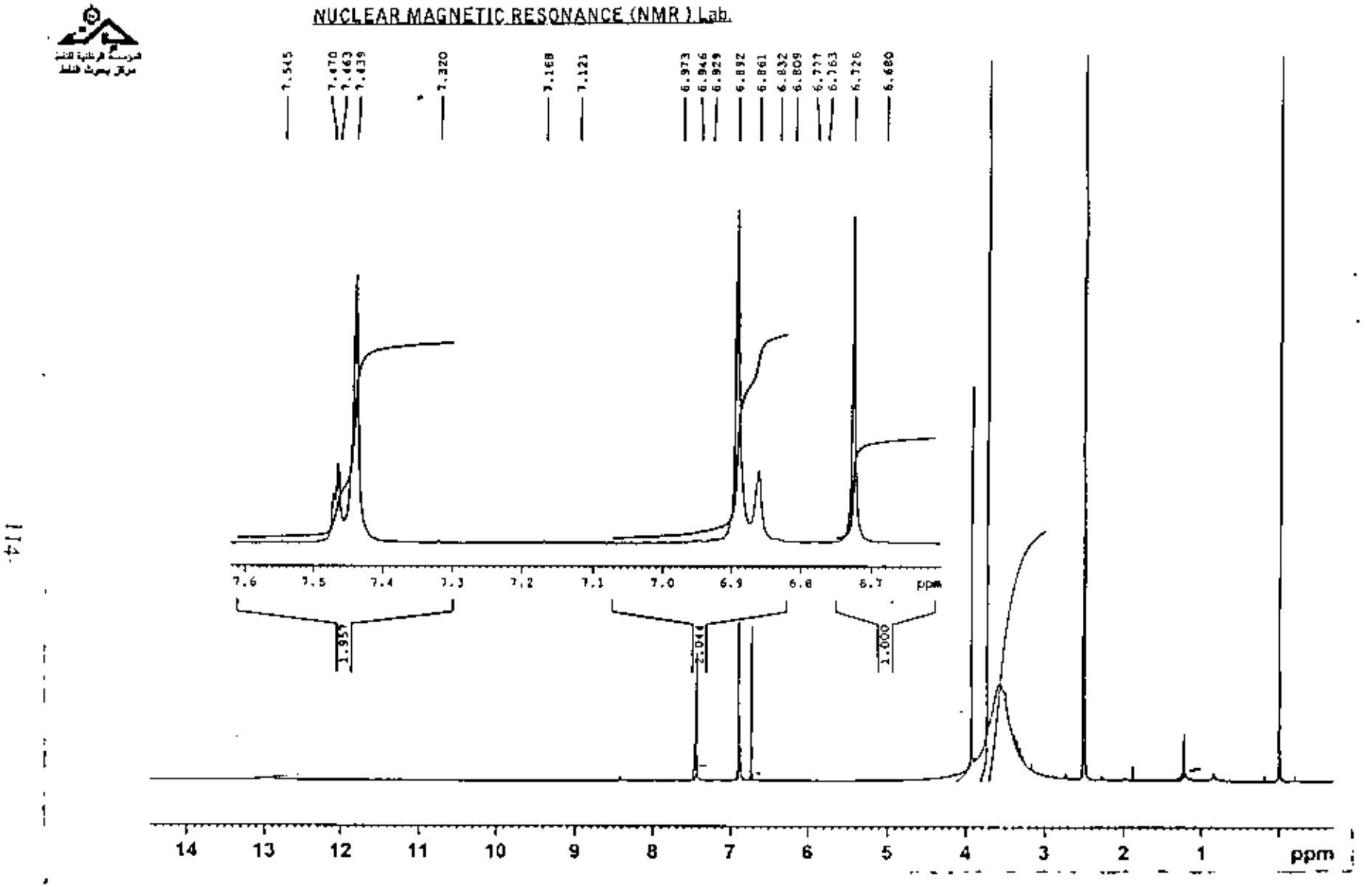
5
4
3
2
1
ppm

Fig. (27) : Cont.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6.832
6.809
6.777
6.763
6.726
6.680
1.957
2.044
1.000
ppm
14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm

Fig. (27) : Cont.

Table (17): ^{13}C-NMR data of compound – 3[172]

Carbon no.	δ (ppm)
2	152.49
3	131.14
4	181.95
5	158.44
6	102.34
7	164.26
8	91.35
9	161.92
10	191.42
1′	120.95
2′	113.14
3′	150.57
4′	145.82
5′	151.92
6′	115.83
C-3OCH_3	59.92
C-5′OCH_3	56.32

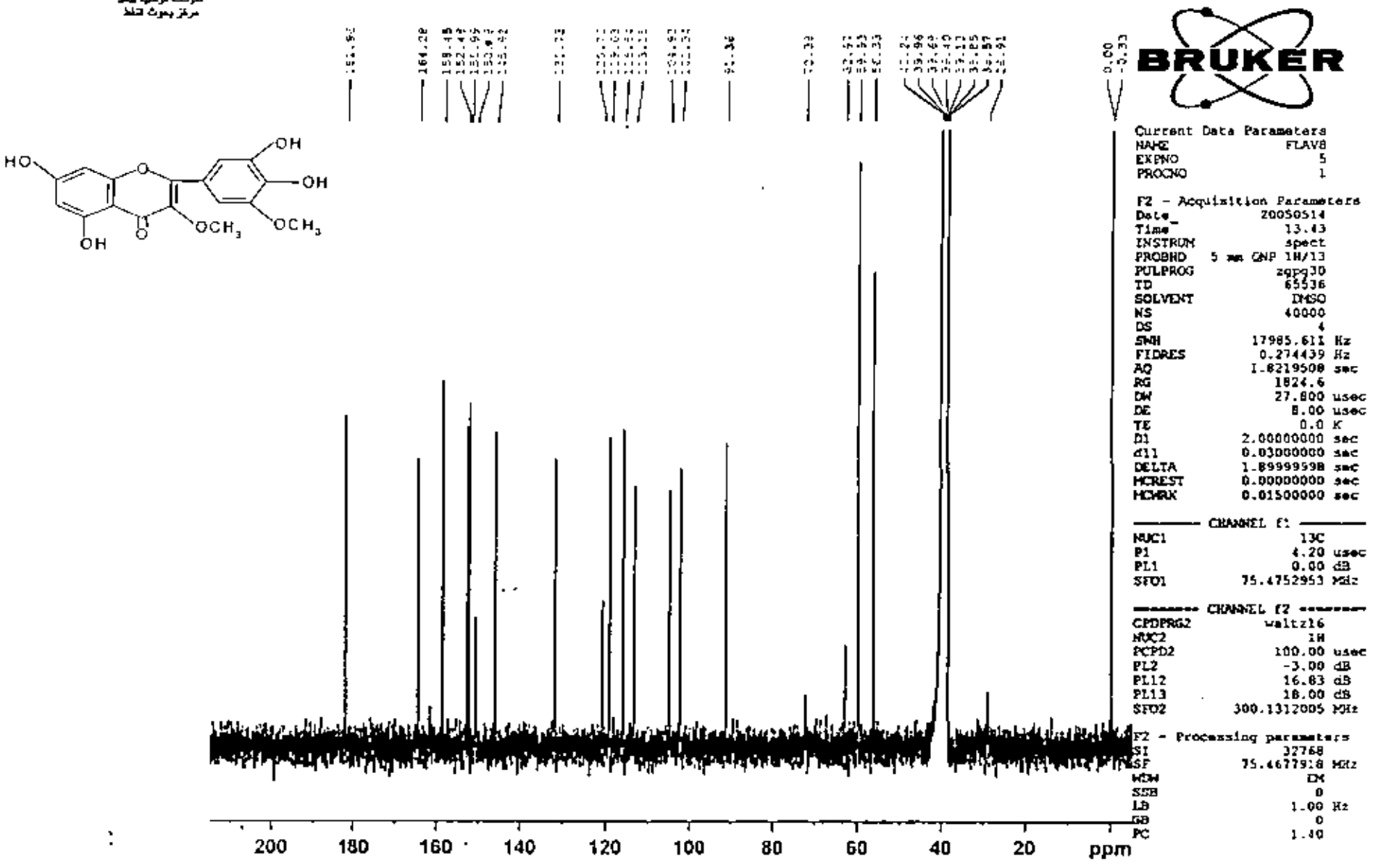
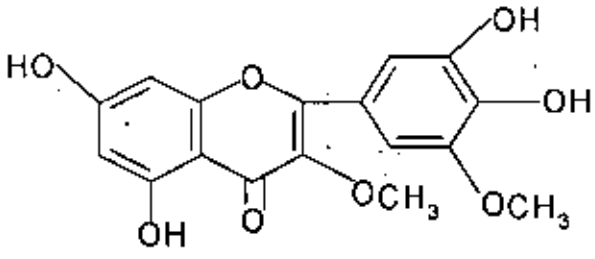


Fig. (28) : The ^{13}C NMR spectrum of compound-3 (3,5 dimethyl myricetin) .

From the above data we can identify compound-3 as 3,5' dimehyl myricetin



3,5' dimethyl myricetin .

Purification of compound – 4 :-

The fractions 31-37 (table 11) eluted with $CHCl_3$: MeOH 80:20 were subjected to preparative paper chromatography (whatmann 3 MM) using 15% AcOH as developing solvent. The main zone (R_f 0.11) containing compound – 4 was eluted and further purified using Sephadex LH – 20 column. Elution was afforded using 90% methanol. The eluant was evaporated *in vacuo* at 50 C°. The purity of the isolated compound was checked using two dimensional paper chromatography (15% AcOH and BAW 3:1:1). Moreover, the colour under UV light before and after spraying with the $AlCl_3$ reagent in addition to R_f values in different solvents were identical to those of 5 hydroxy 3', 4', 6, 7, tetramethoxy flavone [115] .

Identification of compound – 4 :-

The UV absorption spectra of compound-4 (Fig. 29 and Tab.18) showed peak – I at 338 nm (flavone type) [169]. The bathochromic shift (62 nm) in peak – I in the NaOMe spectrum with decreasing in it's intensity proved the absence of a free OH group at C – 4'. The bathochromic shift (32 nm) in peak – I in the $AlCl_3$ spectrum confirmed the presence of free OH group at C -5. The $AlCl_3$/ HCl spectrum showed no hypsochromic shift in peak – I relative to $AlCl_3$ spectrum indicates the absence of *ortho* dihydroxy

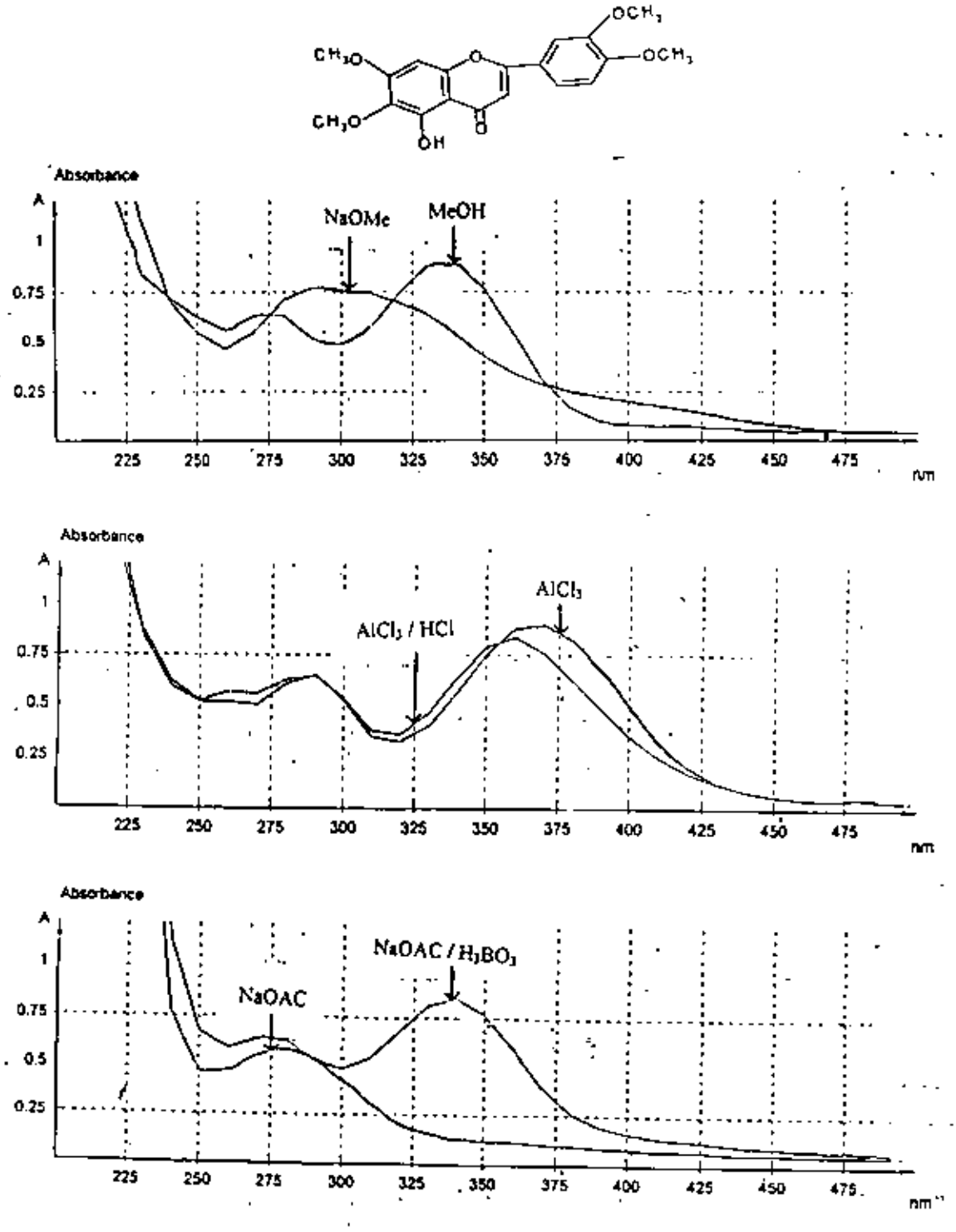


Fig.(29) : The UV absorption spectra of compound-4 (5- hydroxyl, 3, 4, 6,7-tatramethoxy flavone).

system in ring B. The NaOAc spectrum showed no bathochromic shift in peak – II indicates the absence of free OH group at C -7.

Table (18) : UV absorbtion data of compound-4

Addition to methanol	λ_{max} (nm)
None	275, 338
NaOMe	285, 400 (sh) .
$AlCl_3$	260, 285, 375 .
$AlCl_3$ / HCl	255, 285, 370 .
NaOAc	266, 375 (sh) .
NaOAc / H_3BO_3	275, 335 .

The El–Mass spectrum of compound – 4 Fig. (30) showed a molecular ion peak [M^+] at m/z = 358 (89.9 %) which crossponding to molecular formula $C_{19}H_{18}O_7$. The other important peaks at m/z = 343 (M^+ - CH_3, 67,6 %), 329 (M^+ - CHO, 20.9 %, 327 (M^+ - OCH_3, 9.4 %) and RDA fragments indicates the presence of four methoxy groups as shown in scheme (4) .

The ^{1}H-NMR spectrum of compound–4 in DMSO Fig. (31) gave signals at δ in ppm 7.76 (1H, d, H – 2′),7.60 (1H, d, H – 6′), 7.2 (1H, d,H-5′), 7.1 (1H, s, H-3), 6.99 (1H, d, H – 8), 3.94 (3H, s, C-7- OCH_3), 3.87 (3H, s, C, 4′– OCH_3), and 3.75 (3H, s, C – 3′– OCH_3), 3.72 (3H, s, C-6-OCH_3) . The ^{13}C NMR spectrum in DMSO (Fig.32) showed the signal at δ in ppm = 59.95 , 56.41 for C-6-OCH_3 and C-7-OCH_3 respectivily , other data were summarized in table (19) .

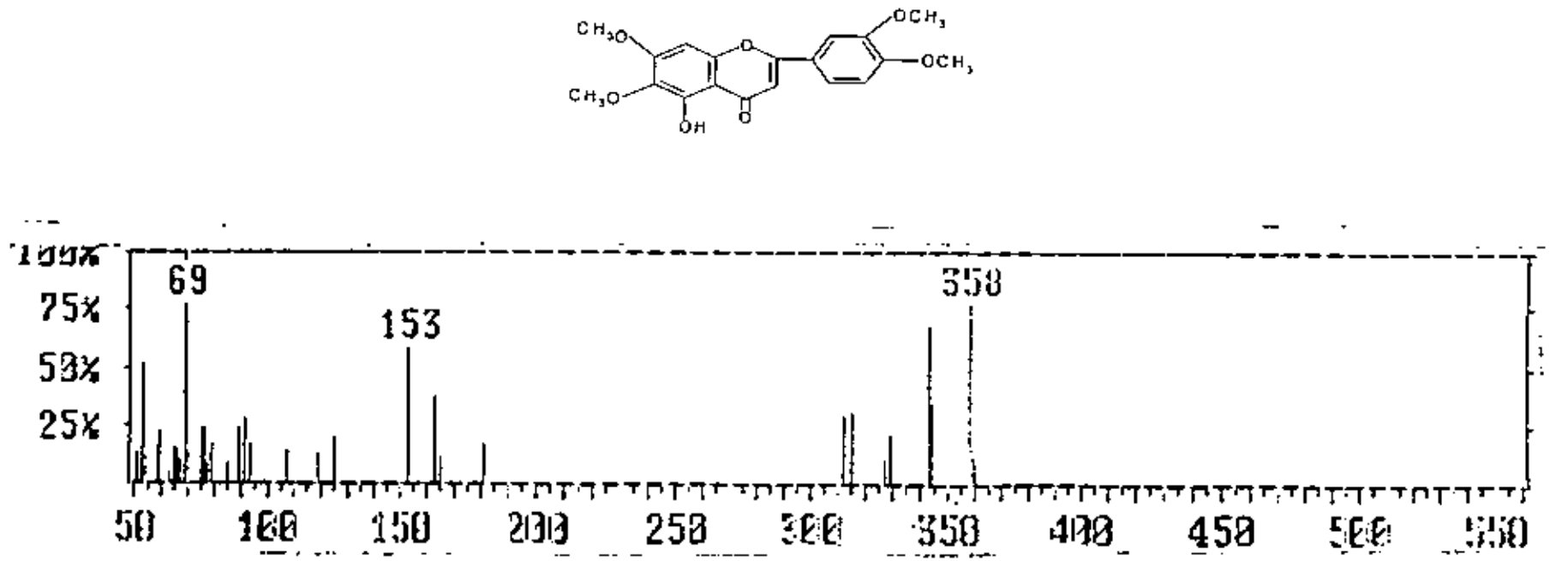
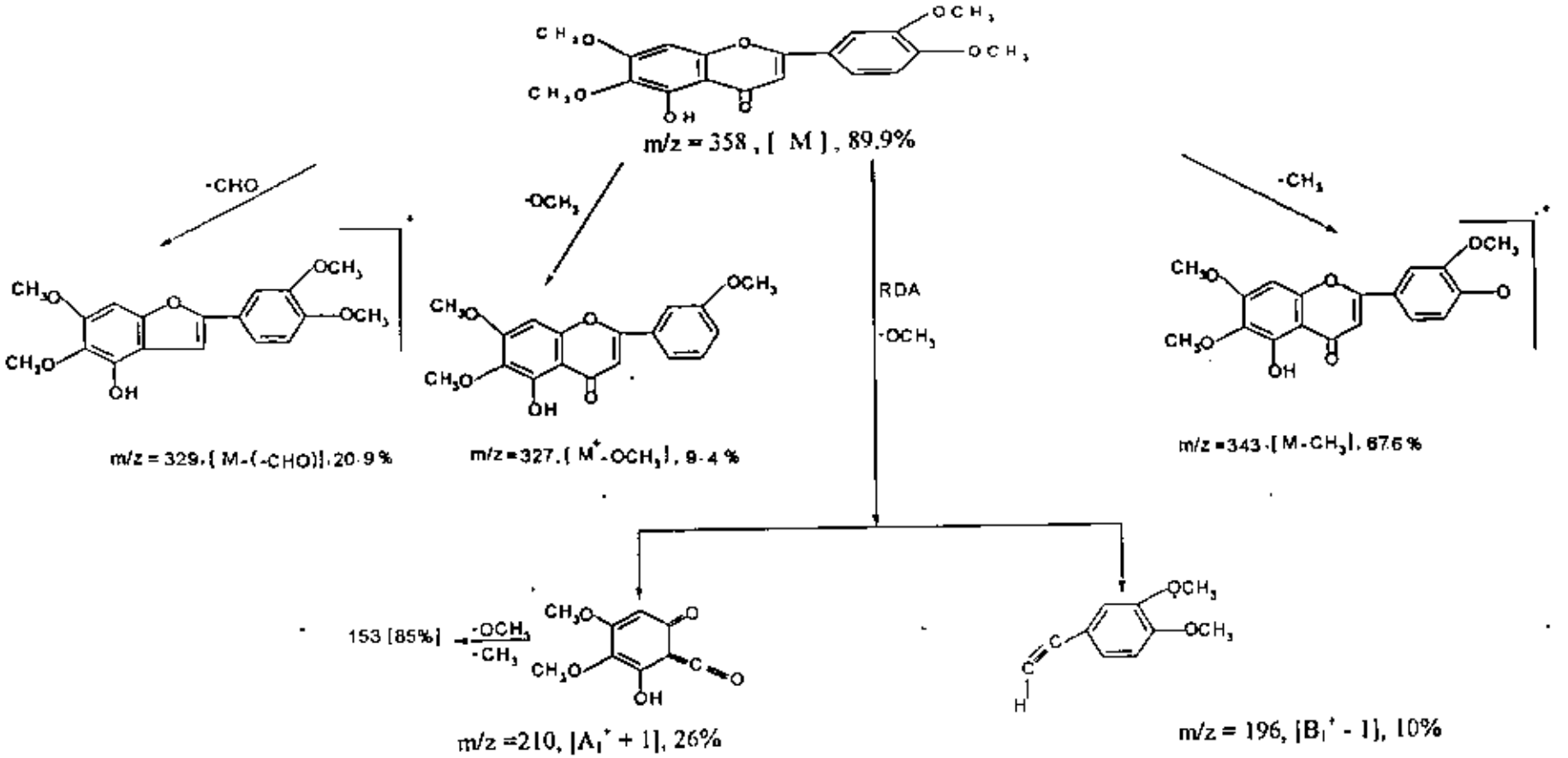
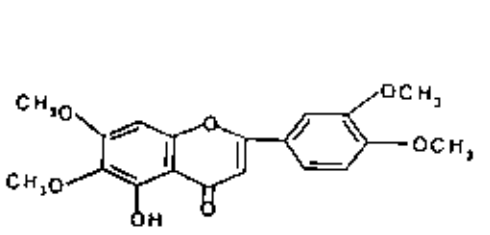


Fig. (30) : The EI mass spectrum of compound-4

(5- hydroxyl, 3, 4, 6,7- tatramethoxy flavone) .



Scheme (4) : Fragmentation pathway of compound-4



BRUKER

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PL1                    -3.00 dB
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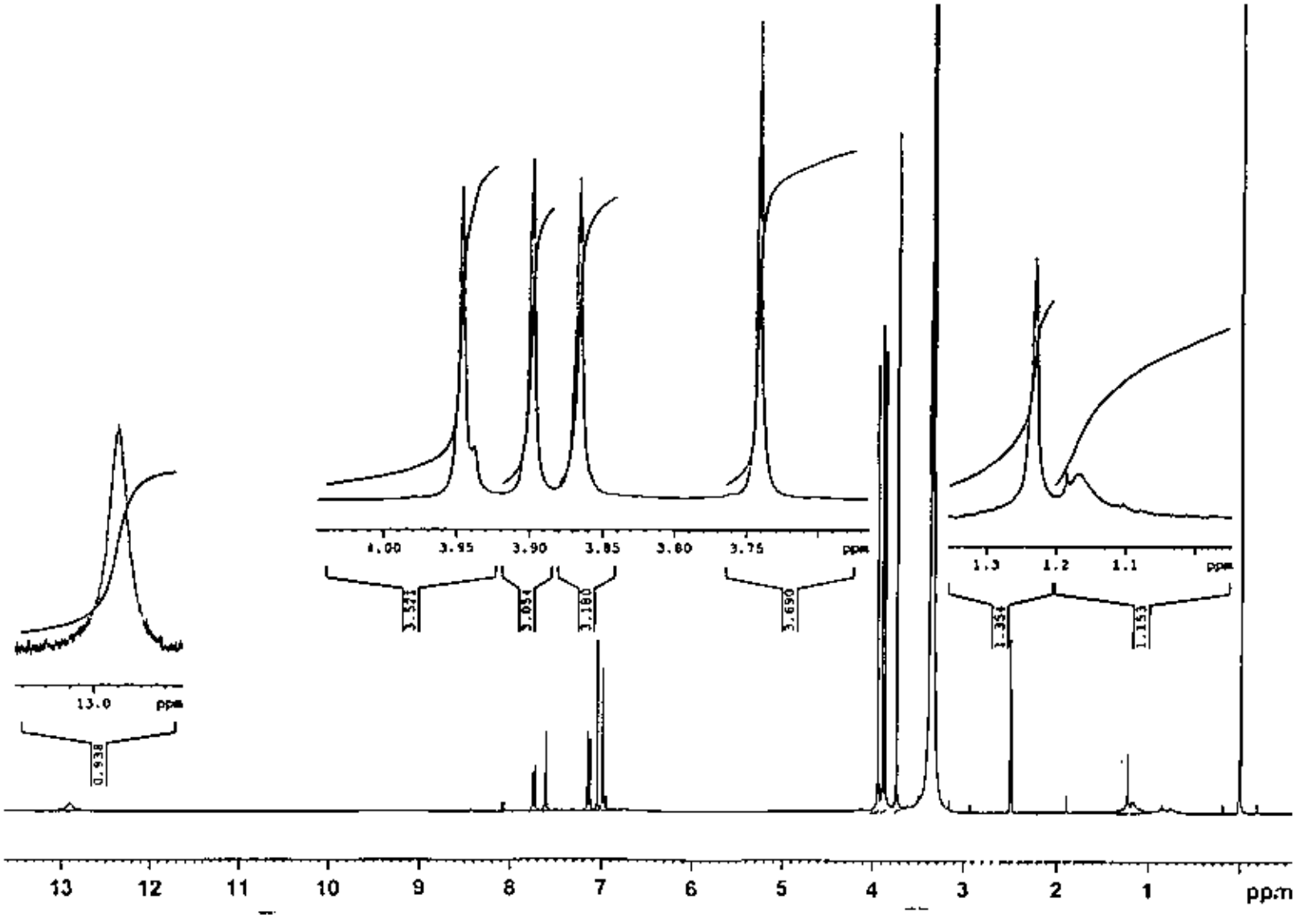
Fig. (31) : The ^{1}H NMR spectrum of compound-4

(5- hydroxyl, 3́, 4́, 6,7 - tatramethoxy flavone) .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ppm
1.354
1.153
13
12
11
10
9
8
7
6
5
4
3
2
1
ppm

Fig. (31) : Cont.

Table (19): ^{13}C-NMR data of compound – 4 .

Carbon no.	δ (ppm)
2	155.22
3	124.10
4	179.36
5	159.92
6	110.35
7	164.49
8	94.76
9	165.28
10	115.97
1´	126.5
2´	109.66
3´	151.70
4´	148.16
5´	120.57
6´	123.90
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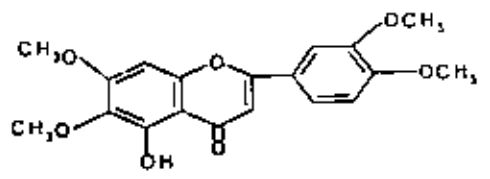
C-3´-OCH_3	55.69



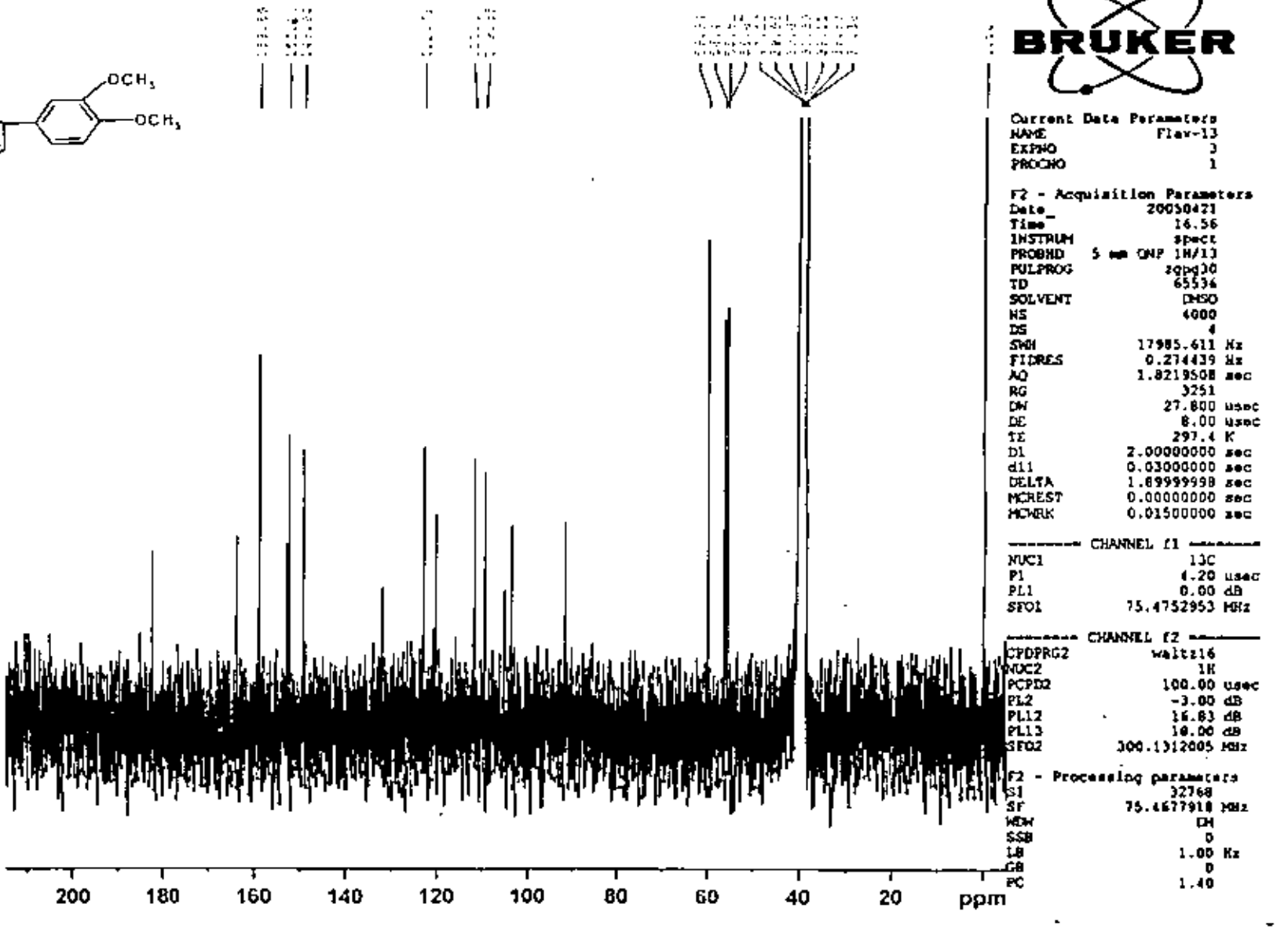
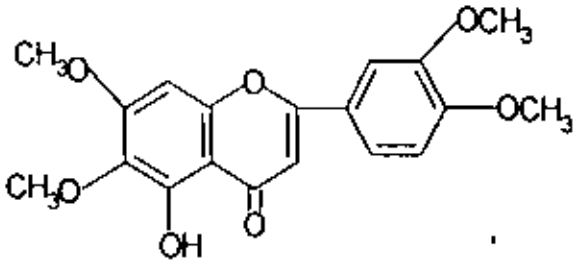


Fig. (32) : The ^{13}C NMR spectrum of compound-4

(5- hydroxyl, 3, 4, 6,7 - tatramethoxy flavone) .

According to the above chromatographic and spectroscopic data we can identified compound – 4 as : 5 – hydroxyl, 3', 4', 6,7 – tatra methoxy flavone.



5 – hydroxyl, 3', 4' 6,7 – tatra methoxy flavone.

Fractionation of butanol extract of *T. davaeanum*:-

The methanolic solution of butanol extract (≈ 5 g) was applied to preparative paper chromatography (Whatmann 3 MM using 25% acetic acid as a developing solvents. The main zone (R_f o.77) was localized under UV light and cut into small pecies and eluted with methanol (70%).

The methanol was evaporated *in vacuo* till dryness and further purified another time on PPC using B.A.W 4:1:5 (upper layer) as a developing solvent then eluted with methanol (70%) to give compound-5 in semi pure form.

Finally this compound (5) was passed through Sephadex LH-20 column eluted with methanol (80%) to give compound – 5 in a pure form (using 2DPC in different solvent systems).

Identification of compound – 5:-

The chromatographic behavour of the compound–5 on PC in different solvent systems indicated it's highly glycoside in nature [169], which is confirmed where it is easily soluble in water.

The UV absorption spectra of compound–5 (Fig.33 and Tab.20) displayed peak–I at 330 nm which indicates the flavone type structure[169].

A bathochromic shift (70 nm) in peak – I with increasing intensity with NaOMe spectrum indicate the presence of a free OH group at C-4'.

The presence of free OH group at C-5 was confirmed through $AlCl_3$ spectrum where there a bathochromic shift (50 nm) in peak–I was occur on addition of NaOAc . Also there is no hypsochromic shift in peak–I in $AlCl_3$ / HCl spectrum relative to the $AlCl_3$ spectrum. The absence of free OH group at C-7 was proved as no bathochromic shift in peak –II was occur. Also the absence of an *ortho* dihydroxy system was confirmed through the NaOAc / H_3BO_3 spectrum because there is no bathochromic shift in peak – I.

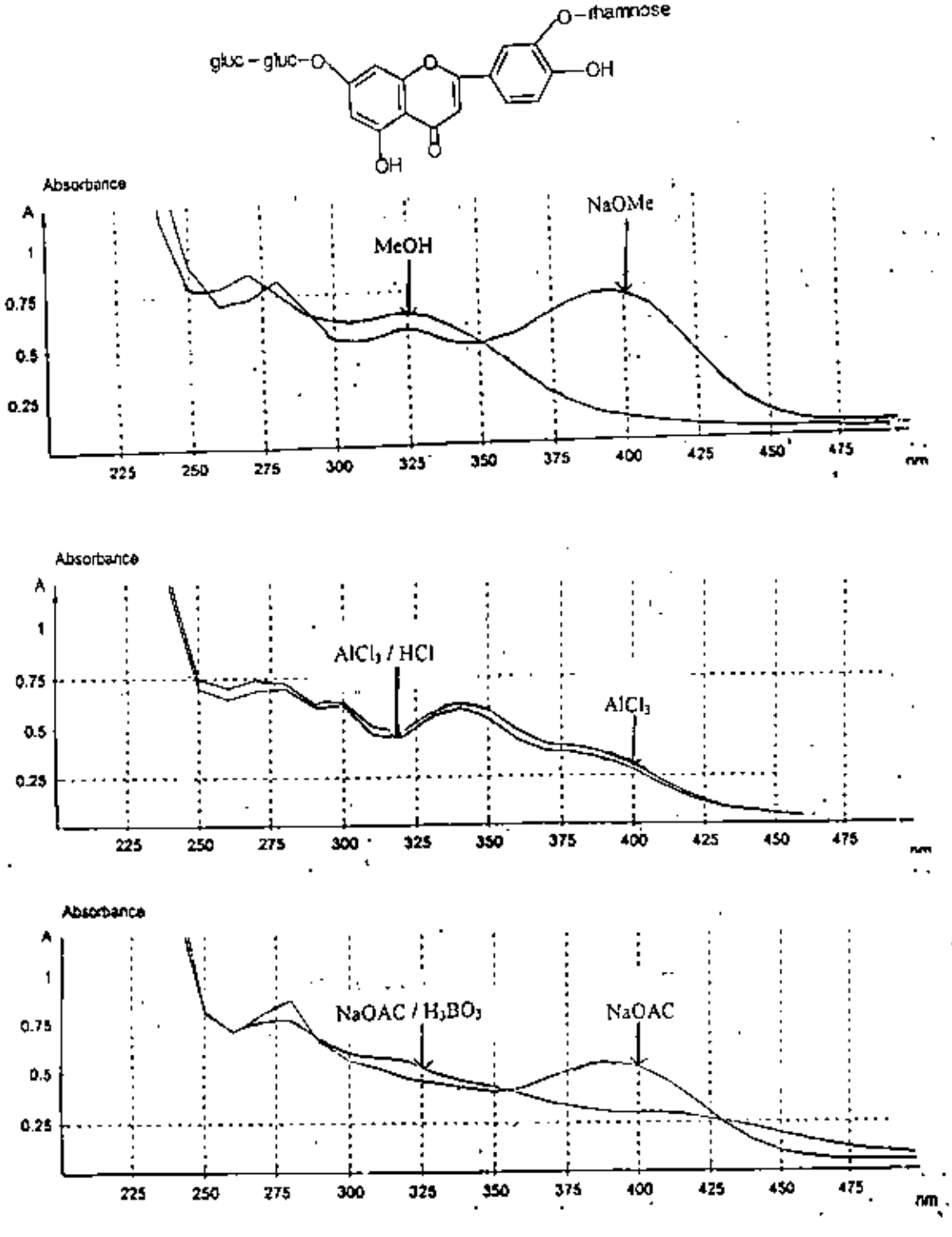


Fig. (33) : The UV absorption spectra of compound-5

(Luteolin -7-*O*-gluco-glucosyl-3'-*O*-rhamnoside) .

Table (20): Ultraviolet absorption data of compound – 5

Addition to methanol	λ_{max} (nm)
None	260,310,330.
NaOMe	280,340,400.
$AlCl_3$	280,300,340,350,390.
$AlCl_3$ / HCl	278,300,340,350,388.
NaOAc	260,315,390.
NaOAc / H_3BO_3	250,280,340.

The positive (+ve) (FAB) mass spectrum of compounds – 5 (Fig. 34) showed a molecular ion peak M^+ at m/z = 757 which constituted with the molecular formula C_{33} H_{40} O_{20} + 1, this peak also indicates the presence of three sugar moieties, two of them are hexoses (162 × 2 = 324) and the other is deoxyhexose (146) , in addition to Luteolin as an aglycone (286). Another important peaks at m/z = 432 (M^+ - 2 hexose (324 + 1) i.e deoxyhexose moiety is directly attached to the flavone nucleus at C-3´, m/z = 287 correspond to (M^+ - (2 hexoses +1 deoxyhexose) i.e molecular weight of the aglycone is 287 which is coinsided with that of Luteolin type structure.

The ^1H – NMR spectrum of compound – 5 in DMSO (fig.35) showed δ in ppm 8 (2H, d, H-2´, H-6´), 7.5 (1H, d, H-5´), 7.1 (1H, d, H-6), 6.9 (1H, d, H-8), 6,8 (1H, s, H-3). The anomeric protons (3 protons) appears as follow : 5.05 (1H, d, H´-1, C-7 glucose), 4.75 (1H, d, H-1^{III}, C-7 glucose), 4.6 (1H, s,H-1^{IV} C-3´ rhamonose), finally the CH_3 protons of the rhamonose moiety at δ = 1.2 (d).

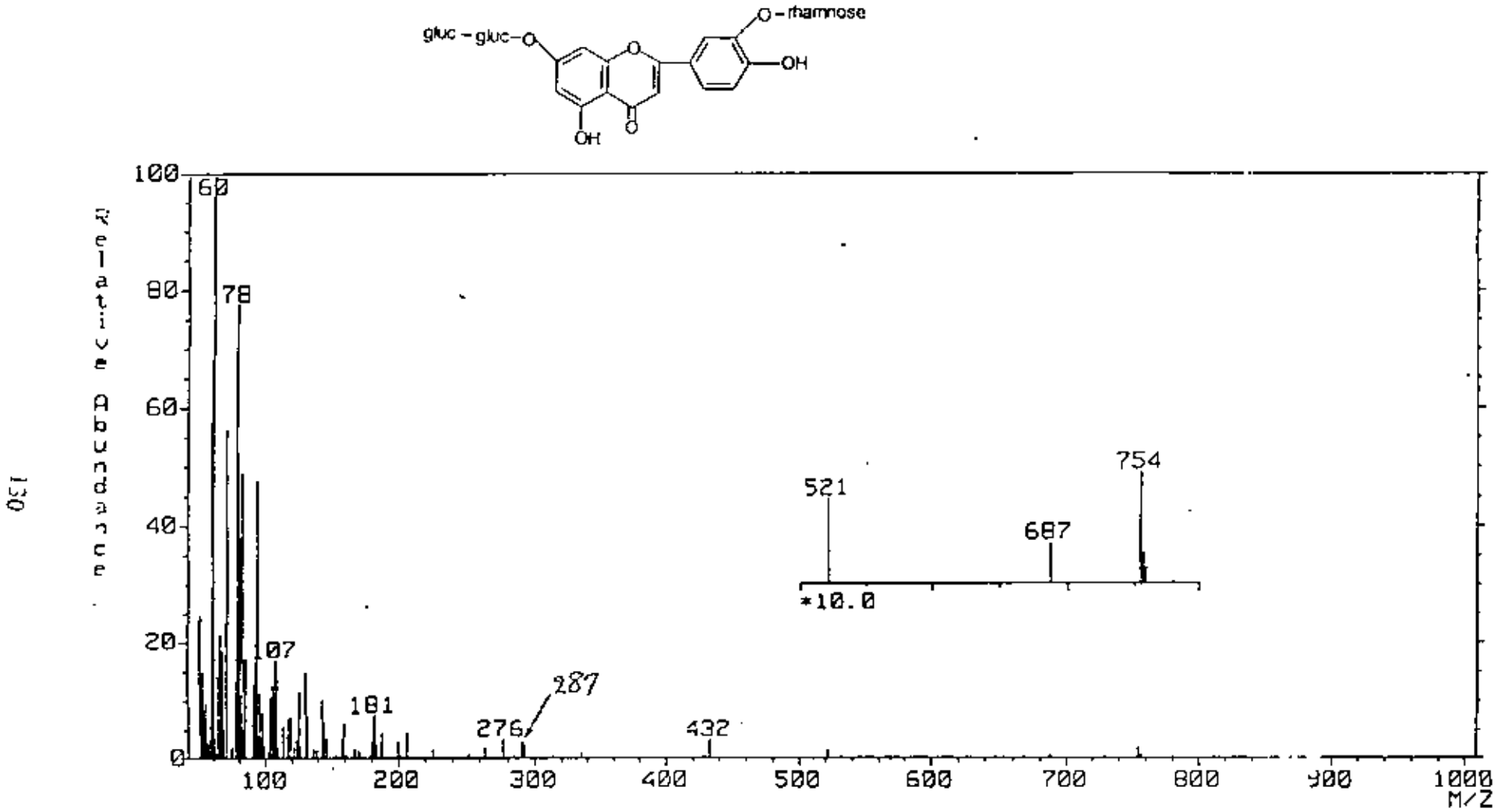


Fig. (34) : The FAB-mass spectrum of compound-5

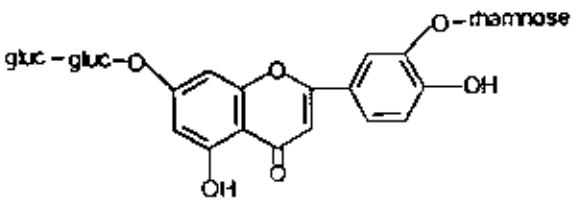
(Luteolin -7-*O*-gluco-glucosyl-3′ -*O*-rhamnoside) .

Acid hydrolysis:-

About 5 mg of compound – 5 were dissolved in 10 ml methanol mixed with 10% HCl refluxed on a boiling water bath for 2 hrs. The solution was diluted with distilled water and extracted with ethyl acetate (3×50ml). The ethyl acetate extract was washed with distilled water and evaporated in vacuo at 45C° till dryness, the obtained residue was chromatographed on PC with authentic sample of luteolin, it gave the same R_f values in different solvent systems and the same UV data. This means that the sugar residue were at C – 3 and C – 7 respectively.

The aqueous acidic solution after separation of the aglycone was neutralized with barium carbonate, filtered and evaporated till dryness. The residue was dissolved in isopropanol and subjected to PC using ethyl acetate: pyridine: water 12 : 5 : 4 as a developing solvent with authentic references from different sugars.

The chromatogram was visualized by spraying with aniline phthalate[170] and heated at 105 for few minutes. Glucose and rhamnose were the detected sugars.



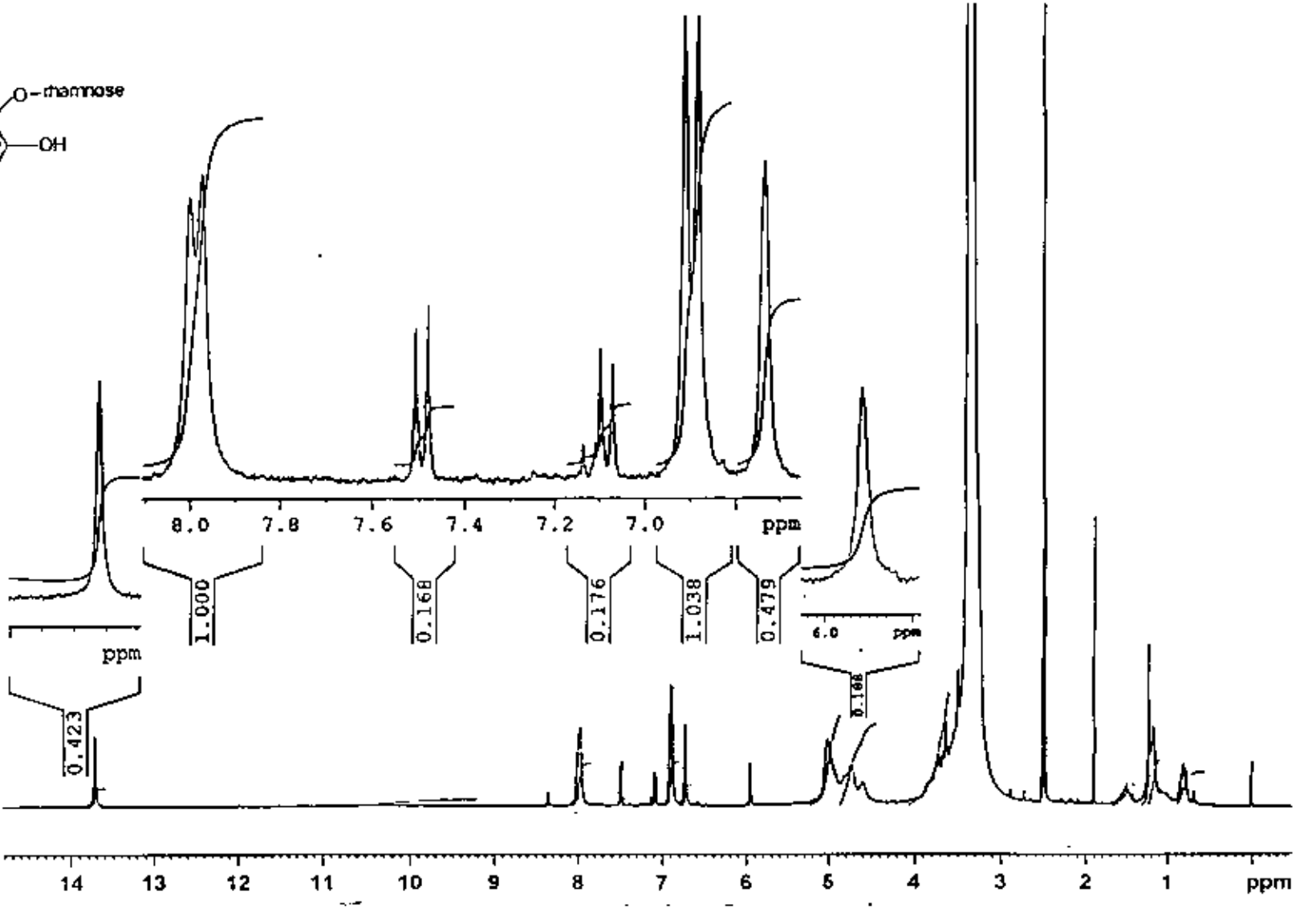


Fig. (35) : The ^{1}H-NMR spectrum of compound-5

(Luteolin -7-*O*-gluco-glucosyl-3`-*O*-rhamnoside).

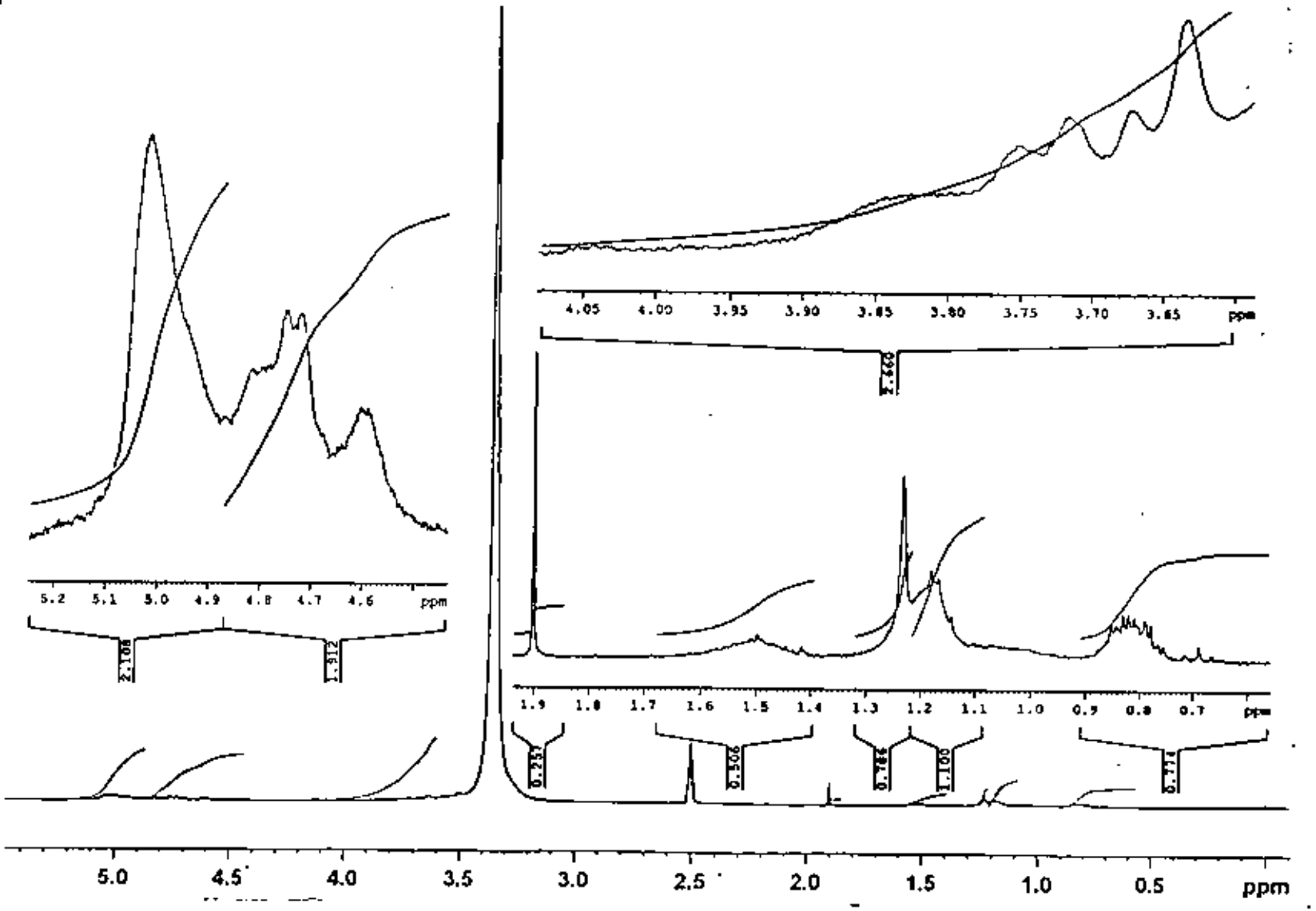
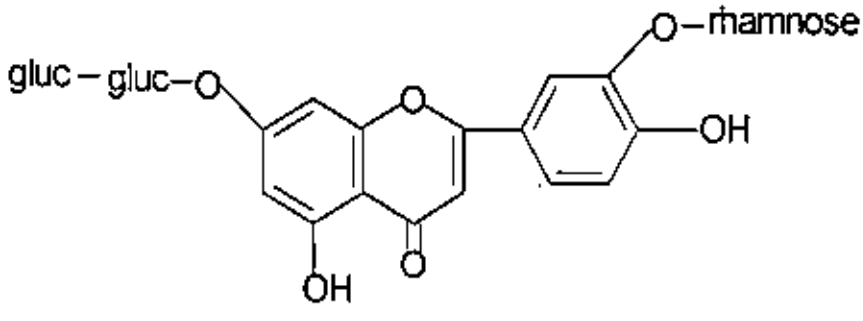

5.2 5.1 5.0 4.9 4.8 4.7 4.6 ppm
2.166
1.512
4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 ppm
2.660
1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 ppm
0.283
0.506
0.784
1.100
0.774
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

Fig. (35) :Cont.

From all the above data and the acid hydrolysis beside the mass spectrum of the aglycone (fig. 22) we can identify compound – 5 as Luteolin –7–*O*–gluco – glucoseyl – 3´–*O* – rhamnoside.

Note: there are further investigation by 2D NMR to know the exact position of linkage between the two glucose units and the aglycone.



Luteolin -7-*O*-gluco-glucosyl-3$^{\backslash}$ -*O*-rhamnoside .

PHARMACOTOXICITY STUDIES

According to the world health organization (WHO), over than 150 plants are known to be used for the treatment of diabetes mellitus and the study of hypoglycemic plants is then encouraged [173]

Many *Teucrium* species are known for their medicinal utilization and exhibit interesting biological properties such as hypoglycemic, hypolipidmic, hepatoprotection, antioxidant, antipyretic and anti-inflomatory [22] .

In the frame work of our chemical and biological investigation of Libyan medicinal plants, we will investigate the toxicity and antidiabetic activity of *T. davaeanum* growing in Sirt region .

Material and methods:-

I. Plant material:

The defatted powdered plant material (250g) were extracted three times with ethanol-water (7:3, V/V) at room temperature. The ethanol was evaporated *in vacuo* at 45C° and the aqueous extract was lypholized .

Animals: male albino Sprague dawely rats, body weight (130 – 150 g), from animal house unit, national research centre Cairo, Egypt . The animals were fed on a standard diet, and water ad libitum.

II: - Determination of LD_{50} :- Mice were injected with different doses of the plant extract intrapritoneally (i.p.), symptoms and mortalities were recorded.

The LD_{50} was calculated in mice injected intraperitonal administration (i.p) as shown in table (21) according to the following equation:

$$LD_{50} = D_m - \frac{\Sigma(a \times b)}{n}$$

Where :-

Dm: The highest dose which kills all animals in the group.

a: constant factor between two successive doses.

b: The mean number of dead animals in two successive groups.

n: The number of animals in each group.

Table (21): LD_{50} of alcoholic extracte of *T. davaeanum.*

NO	Dose alc. ext. g/kg.b.w	No. of anima group	No. of dead mice	Dose Differe-nce (a)	b	(a×b)
1	0.300	6	-	0.3	-	-
2	0.6	6	-	0.3	-	-
3	1.2	6	-	0.6	-	-
4	2.4	6	-	12	-	-
5	4.8	6	-	24	-	-
6	5	6	-	0	-	-

D_m = up to 5g / kg.b.w and no mortality $\Sigma (a \times b) = 0$, $n = 6$.

$LD_{50} = D_m - 0 = D_m = 5$ g / kg b. wt. .

Induction of diabetes in rats :-

The rats were injected with alloxan monohydrate dissolved in sterile normal saline in a dose of 150 mg/Kg.b.w, intraperitoneally. Since alloxan is capable of producing fatal glycemia as a result of massive pancreatic insulin release, rats were treated with 20 % glucose solution (15 – 20 ml) intraperitoneally after 6 hours. The rats were then kept for the next 24 hours

on 5 % glucose solution bottles in their cages to prevent hypoglycemia (Giupta et.al, 1984) [174] fasting blood glucose was estimated by glucose kits provided from Biodiagnostic company, Germany) . The injected dose of the extract was 5mg/100g body weight.

Results :

After injection of groups of mice (6 mice each) of a doses 25.50 and 100 mg/100g body weight, no mortalities were observed, symptoms were normal up to 5g/Kg body weight were safe.

Blood glucose level was determined in normal and fasted diabetic rats . The results were recorded in the following table (22):

Table (22) : Glucose level of normal diabetic and treated groups .

Normal group	Diabetic group	Extract treated group	
		After 1 week	After 2 weeks
Mean 99.18333	266.5656*	160.71*	90.85222
S.E ± 4.356263	± 8.216805	± 8.758343	± 3.13648

- The mean difference is significant at the 0.05 level.

Table (23) : Multiple comparisons of normal ,diabetic and treated groups.

Group	Group	Mean Difference
1	2	- 174,1380*
	3	- 71. 8330*
	4	- 3.1580
2	1	174.1380*
	3	102.3050*
	4	170.9800*
3	1	71.8330*
	2	102.3050*
	4	68.6750*
4	1	3.1580
	2	- 170.9800*
	3	- 68.6750*

* The mean difference is significant at the 0.05 level.

(Group 1) normal fasted rats.

(Group 2) diabetic rats after 72 hours of alloxan.

(Group 3) the same diabetic rats after administration of the extract for one week.

(Group 4) the same diabetic rats after administration of the extract for two weeks.

DISCUSSION

Discussion

Family Labiate is know 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 *(T. davaeanum)* is belonging to the family *Labiatae*, is common in Wadi Telal Sirt region, and it is used in folk medicine as antispasmodic, in wound healing and antidiabetic. The plant was subjected to phytochemical investigation concerning it's volatile oils and lipids as well as the flavonoidal constitutes. The GC/MS analysis of the volatile oil prepard by hydrodistillation of *T. davaeanum* revealed the presence of 74 compounds from many chemical classes, vis: hydrocarbons (0.46%), alcohols (0.33%), aldehydes (0.09%), esters (0.09%) and the most abundance compounds were α – pinene (1.05%), β– pinene (2.80%), limonene (5.60%), ocimene (5.30%), myrtenal (3..30%), gubenol (3.77%), humulene (3.80%) and the main compound were spathulnol (8.8%), while the GC/MS analysis of the volatile oil prepared by n-hexane / ether extract revealed the presence of 11compounds viz : pinocavane (1.7%), limonene (2.1%), benzene, 1-chloro-3-(chloromethyl) (4.2%), β-myrcene (1.3%), 2-6- dimethyl-1,3,5,7-octatetraene (6.2%), farnesene (1.4%), caryophllene (4.04%), Himachalene (10%), curcumene (4.29%), dodecane, 5,8-diethyl (1.71%), phytol (15%),

These data were in accordance with that reported by Hassan *et.al.*who isolated the [β-pinine, limonene, α-phellandrene, linalool and cedrol] from *T.polium* [19] .

The fatty alcohols fraction was isolated and identified using GC/MS technique. The obtained results revealed the presence of a mixture of fatty alcohols and hydrocarbons include : tetracosanol, octatriacotanol, tetratriacontene, and octatriacontane , in which tetracosanol is the main one

(66.95%) .The unsaponifiable fraction of the studied species was isolated and investigated by GLC technique. The results showed that the unsaponifiable fractions consists mainly from a mixture of n-alkanes include: [n-heptane (1.36%), octane (0.78 %), nonane (8.88 %), decane (15.35%), undecane (5.02%), dodecane (11.38%), tetradecane (21.27%), Hexadecane (6.16%), heptadecane (3.26%), octadecane (3.49%), decosane (2.07%), petacosane (1.31%), Heptacosane (6.39%), octacosane (5.79%), nonacosane (o.54%), hentricontane (0.74%), detricontane (2.10%)], in addition to two sterols: [β - sitosterol (2.67%) and campasterol (1.49%), this data were in agreement with Capasso *et.al.*where they isolated β-sitosterol, stigmasterol and campesterol from *T. polium* [128] .

The study of the total fatty acids was achieved by GLC analysis of their methyl esters. The obtained results revealed the presence of lauric (1.83%), myristic (2.68%), palamitic (18.03%), stearic $C_{(18:0)}$ (11.33%), oleic $C_{(18:1)}$ (9.36%), linoleic $C_{(18:2)}$ (27.54%), linolenic $C_{(18:3)}$ (22.22%), arachidic $C_{(20:0)}$ (2.71%), erucic $C_{(20:1)}$ (1.82%), lignoceric $C_{(24:0)}$ (1.60%), tetracosenoic $C_{(24:1)}$ (0.88%) . Flavonids present either in the free or in the glycoside form of this plant were studies . Flavonoids were obtained from the alcoholic extract (80%) by the conventional method, i.e by treating the concentrated alcoholic extract with hot water followed by extraction with organic solvents (ethyl acetate and butanol) .

Fractionation of the flavonoidal constituents was affected by applying column and paper chromatography using different adsorbents and solvent systems . Moreover, further purification was achieved using preparative TLC and/or PC as well as Sephadex LH – 20 column phromatography.

Five flavonoids have been isolated for the first time from *T. davaeanum*, four of them were aglycones viz (3, 7, 3', 4' tetramethoxy 5 – hydroxyl flavone, luteolin, 3, 5'dimethyl myricetin and 5 – hydroxyl, 3', 4', 6, 7, tetramethoxy flavone from the ethyl acetate extract and one of them was glycoside and known as: Luteolin–7–*O*–gluco–glucosyl–3'–*O*–rhamnoside from butanol extract .This is in accordance with Slynkova *et.al.*where they isolated (3',5,6-trihydroxy-4',7-dimethoxy flavone, apigenin 5,6,7-trihydroxy flavone, luteolin, luteolin-7-*O*-*β*-D-glycopyanoside from *T. nuchense* [114] .

Identification of the isolated flavonoids was achieved through chromatographic studies and spectroscopic measurements (Viz:UV,^{1}H,^{13}C NMR, MS (EI and/or FAB).

The acute toxicity studies of the alchoholic extract of *T. davaeanum* was studied according to Behrens and Karper (1953) using mice and rats. The study revealed that the extract have a wide marginal safly where it's LD_{50} for intraperitoned injection was calculated to be up to 5 g/kg weight i.e The extract have no toxicity and this is in accordance with that reported before for *T. polium* [143].

The study of antidiabetic activity of alchoholic extract showed that it decreases the glucose level in blood after daily administration of the extract for two weeks . This cofirm its using in the folk medicine for the treatment of diabetes mellitus. Also, these data were in agreement with that reported before for *Teucrium* species [145].

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ب- أوضحت النتائج أن المستخلص الكحولي له القدرة على خفض نسبة السكر في الدم في الفئران المصابة بالسكر (بعد حقن الاستربتو ذاسين) وذلك بعد الحقن اليومي للمستخلص لمدة اسبوعين متتاليين .

الـديكان (15.35%)، اللآنـديكان (5.02%)، الـدوديكان (11.38%)، التتراديكـان (21.27%)، الهكساديكان (6.16%)، الهبتاديكان (3.26%)، الاوكتاديكان (3.49%)، الدوكوسان (2.07%)، البينتاكوسان (1.31%)، الهبتاكوسان (6.39%)، الاوكتاكوسان (5.79%)، النوناكوسـان (0.54%)، الهنتریاكونتـان (0.75%)، الـدوتریاكونتان (2.10%)، و الجـزء الاسـيترويدى فهـو مخلـوط مـن البيتاسيتوسـتيرول (2.67%) و الكمباسيترول (1.49%) .

د- أسفرت دراسة خليط الأحماض الدهنية وذلك باستخدام تقنية كروماتوجرافيا الغاز/سائل عـن تكونـه مـن خلـيط مـن اللوريـك (1.83%)، الميريسـتيك (2.68%)، البالميتيـك (18.03%)، استيرك (11.33%)، الاوليك (9.36%)، اللينوليك (27.57%)، اللينولنيك (22.22%)، الارشـيديك (2.71%)، الاريسـك (1.82%)، ليجنوسـيرك (1.60%)، التتراكوسينويك (0.88%)، و ان نسبة الاحماض الدهنية المشبعة = 22.54%، وقد وجد أن البالميتيك يمثل الحمض الاساسى فى هذه الاحماض حيث تصل نسبته الى 18.03%، فى حين أن نسبة الاحماض الدهنية الغير مشبعة = 77.46%، و الحمض الاساسى فيها هو اللينوليك (27.54%) .

هـ - تحضير الفلافونيدات :-

اولا : من خلاصة خلات الايثيل :-

- 3، 7، 3´ ، 4´ -تترامیثوکسی فلافون-5-هیدروکسی فلافون .
- لوتيولين .
- 3، 5´- داى ميثوكسى ميريستين .
- 5- هيدروكسى، 3´، 4´، 6، 7 – تترامیثوکسی فلافون .

ثانيا : من خلاصة البيوتانول :-

- ليوتولين-7-أ- جلوكو- جلوكوزايل-3´-أ- رامونوزايد .

الجزء الثالث :

الدراسات البيولوجية

أ- تم تعيين الجرعة السامة المميتة الوسيطة لفئران التجارب للخلاصة الكحولية منزوعة المواد الدهنية لنبات الجعدة وقد وجد أنها تساوى 5 جرام / كجم من وزن الجسم أي أن النبات غير سام .

ملخص البحث

يهدف هذا البحث الى دراسة المكونات الرئيسية و الفاعلية البيولوجية لنبات الجعدة وهو أحد نباتات العائلة الشفوية الواسعة الانتشار و النمو في منطقة سرت " ليبيا" و خصوصا منطقة وادي تلال و قد وجد انه يتم استخدام هذا النبات في الطب الشعبي بواسطة أهالي المنطقة فى علاج الكثير من الامراض مثل مرض السكر و في علاج نزلات البرد و كمضاد للتقلصات .

وتشمل الدراسة بصفة خاصة الزيوت الطيارة و المواد الدهنية (الهيدروكربونات، الكحولات الدهنية، الأحماض الدهنية، الاستيرويدات، التربينات) و المركبات الفلافونيدية وكذلك دراسة الفاعلية البيولوجية للخلاصة الكحولية لهذا النبات .

وينقسم البحث إلى ثلاثة أجزاء يسبقهم مقدمة عامة متضمنة الغرض من البحث .

الجزء الأول :

يشتمل على عرض شامل للأبحاث التي أجريت على كل النباتات التي تتبع جنس الجعدة

الجزء الثاني :

دراسة المكونات الكيميائية لنبات تكريوم دافينيوم (الجعدة) :

أ – الزيوت الطيارة :

تم تحضير الزيت الطيار بطريقتين :-

1- طريقة الاستخلاص باستخدام التقطير البخارى أسفرت نتائج تحليل مكونات الزيت الطيار باستخدام تقنية كروماتوجرافيا الغاز المتصل بالطيف الكتلى عن وجود 74 مركب تم التعرف على هذه المركبات وتحديد نسبة تواجد كل واحد منها . واتضح أن مركب السباثينول هو المركب الرئيسي حيث انه يمثل نسبة 8.8% من هذا الزيت .

2- طريقة الاستخلاص باستخدام المذيبات العضوية الخفيفة (ايثر/هكسان) وقد اتضح أن مركب الفيتول هو المكون الرئيسي حيث انه يمثل نسبة 15% من هذا الزيت .

ب -أسفرت دراسة خليط الكحولات الدهنية بواسطة كروماتوجرافيا الغاز المتصل بالطيف الكتلى عن وجود التراكوزانول (66.95%)، الاوكتاترياكونتانول (4.82%)، تریاكونتين (4.34%) و الاوكتاترياكونتان (23.88%) .

ج- أسفرت دراسة الجزء الغير متصبن بواسطة تقنية كروماتوجرافيا الغاز/سائل المتصل بالطيف الكتلى عن وجود الهبتان (1.36%)، ألا وكتان (0.78%)، النونان (8.88%)،

الجماهيرية العربية الليبية الشعبية الاشتراكية العظمى

جامعة التحدي

كلية العلوم

التاريخ: ....

الموافق: 2006/3/8

إن الدراسة ليست غاية في حد ذاتها
[illegible]

G. S. P. L. A. J.

AL_TAHDI UNIVERSITY

الرقم الإشاري: ك.ع م.ع 17. 2006ف

كلية العلوم

قســم الكيمياء

<u>عنــوان البحــث</u>

دراسة كيميائية وبيولوجية على نبات الجعدة الذي ينمو في منطقة سرت – ليبيا

ســرت / ليبيا

مقدمة من الطالبة

أعقيلة حوسين محمد

<u>لجنة المناقشة :</u> **<u>التوقيـــع</u>**

الدكـــتور / خالد عبد الهادي عبد الشفيق (مشرف الرسالة) ....

الدكـــتور / فخـــري عبد الونيس العبار (ممتحن خارجي) ....

الدكـــتور / محمـــد طه عبد العـــال (ممتحن داخلي) ....

الدكتور / محمد علي سالم الفرجاني

أمين اللجنة الشعبية لكلية العلوم

جامعة التحدي

كلية العلوم

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" دراسة كيميائية و بيولوجية على نبات الجعدة الذي ينمو في منطقة سرت – ليبيا "

بحث مقدم كجزء من متطلبات استكمال درجة الماجستير في الكيمياء

للطالبة /

أعقيلة حوسبن محمد الغناى

(بكالوريوس كيمياء ، 2001)

تحت إشراف

د. خالد عبدا لهادى عبد الشفيق

جامعة التحدي

(2005 / 2004)