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PHYTOCHEMICAL STUDY OF THE FRUITS OF TORILIS ARVENSIS (Huds.) Link GROWING IN EGYPT

A.F.HALIM, H-E.A.SAAD, M.M.MASHALY*, M.F.LAHLOUB, A.F.AHMED

Faculty of Pharmacy and Faculty of Science (Damietta)*, University of Mansoura, El-Mansoura, Egypt.

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Faculty of Pharmacy and Faculty of Science (Damietta)*, University of Mansoura, El-Mansoura, Egypt.

Abstract: The essential fruit oil of Torilis arvensis (Huds.) Link was obtained by hydrodistillation (0.5%V/W) and analyzed by CGC-MS. The oil consists of at least 87 components, 20 of which accounting for about 28.5% of the oil composition were identified. The oil can be easily distinguished from those of the studied European species by the presence of significant amounts of β-bisabolene, β-caryophyllene, thymol, β-pinene and cresol. Moreover, bergapten, xanthotoxin, scopoletin, luteolin-7-o-glucoside and apigenin-7-o-diglucoside were isolated and their structures were established from their physico-chemical properties and spectral data. The coumarins and apigenin 7-o-diglucoside are reported for the first time in the genus Torilis.

Genus Torilis Adans(1), family Apiaceae(2), subfamily Apioideae(2), tribe Scandiceae(2), subtribe Caucalinae(2), includes about 10-15 species distributed in Europe, North Africa and South-West Asia(3). It is represented in Egypt by five species (1).

The Egyptian species, Torilis arvensis (Huds.) Link (Caucalis arvensis Huds., Torilis infesta Clairv.)(1) is an erect annual spiny-fruited herb growing wildly in the Mediterranean Coastal strip and the Nile Delta.

It was reported that the essential oils of the fruits of genus Torilis (European hedge- or bur-parsely)(3) are characterized by the presence of a dominant unknown sesquiterpene having the MS fragmentation pattern; 202,134,93,119,107,79,67, 105,91,55,159,187 (4). The five Torilis species viz; T.arvensis, T.japonica, T.leptophylla, T.nodosa and T.tenella were chemotaxonomicaly examined on the basis of the GC patterns of their fruit essential oils. However, very few components were identified in some of them as biphenyl and carotol(4).

The flavonoid patterns of the fruits of the European Torilis species are relatively uniform. They mainly contain luteolin derivatives with one, two, or three sugar residues at C-7(3,5, 6). It was also reported that the leaves of T.arvensis and T. nodosa contain the 5-glucosyl derivative of luteolin(7), while the aerial parts of T.arvensis are considered to be an economical source of D-mannitol (10-12%W/W)(8,9).

The fruits of the Japanese species, T.japonica, afforded germacrene, eudesmane, cycloeudesmane and oppositane type-sesquiterpenoids(10-12) in addition to novel germacranolides(13) and the humulene-type sesquiterpenoids were isolated from those of T.scabra(14,15).

On the other hand, the only work done on the Egyptian species, T.arvensis, revealed the presence of only flavone glycosides of apigenin and luteolin types with one sugar residue, as glucosides or glucuronides, in the alcoholic extract of leaves and stems(16).

Preliminary phytochemical screening of the fruits of Torilis arvensis (Huds.) Link growing in Egypt, revealed the presence of coumarins in addition to essential oil and flavonoids. Accordingly, the phytochemical study of the forementioned fruit constituents is presented.

EXPERIMENTAL

Plant material:

The ripe fruits of Torilis arvensis (Huds.) Link were collected in May 1988 from flowering and fruiting plants growing wildly in the sporadic rural areas near the University of Mansoura. The plant identity was kindly verified by Dr. I.Mashaly Department of Botany, Faculty of Science, University of Mansoura. A voucher specimen is deposited at the Pharmacognosy Department, Faculty of Pharmacy, University of Mansoura.

A- Essential oil:

Preparation of the oil:

The oil was obtained by subjecting the fruits (100g), immed-

iately after crushing, to hydrodistillation for 8 hours using the E.P. (1972) method.

Gas chromatographic analysis:

A Carlo Erba HRGC 5300 Mega Series gas chromatograph equipped with FID, a fused silica capillary column (25mx0.25mm ID) coated with bonded phase CW-20M of 0.25µ film thickness and a splitting injection mode with splitting ratio of 1/100 was used Operating conditions: injection temperature, 250°C; column temperature, programmed from 65-220°C at 2.5°C/min.; detector temperature, 250°C; carrier gas, hydrogen; inlet pressure, 0.5Kg/cm²; air, 1.0Kg/cm²; hydrogen, 0,5Kg/cm²; chart speed, 0.5cm/min. Quantitation and retention time determination were carried out with a Spectra Physics SP 42900 integrator.

Calculation of Kovats retention indices (R_I):

The oil sample was spiked with a standard mixture of a homologous n-alkane series (C_{10}-C_{28}) and then analyzed by CGC using the above mentioned conditions. Retention indices were directly obtained by application of Kovats procedure[17].

Gas chromatography-Mass spectrometry:

CGC-MS were obtained using HP 5992A system. The same column and operating conditions, as reported for CGC analysis, were used to obtain comparable results except that helium was used as a carrier gas at a flow rate of 4ml/min. Mass spectral analyses were run by EI technique at 70 eV.

Components identification:

The constituents of the oil were identified by matching their mass spectral and retention indices data with those reported in the literature[18,19].

B- Coumarins and flavonoids:

Preparation of extracts:

Air-dried and powdered fruits (2.5 Kg) of Torilis arvensis (Huds.) Link were exhaustively extracted at room temperature with ethanol 90%V/V (20L). The hydroethanolic extract was concentrated under vacuum and then successively partitioned with petroleum ether (br.60-80°C), ether and ethyl acetate. Evapo-

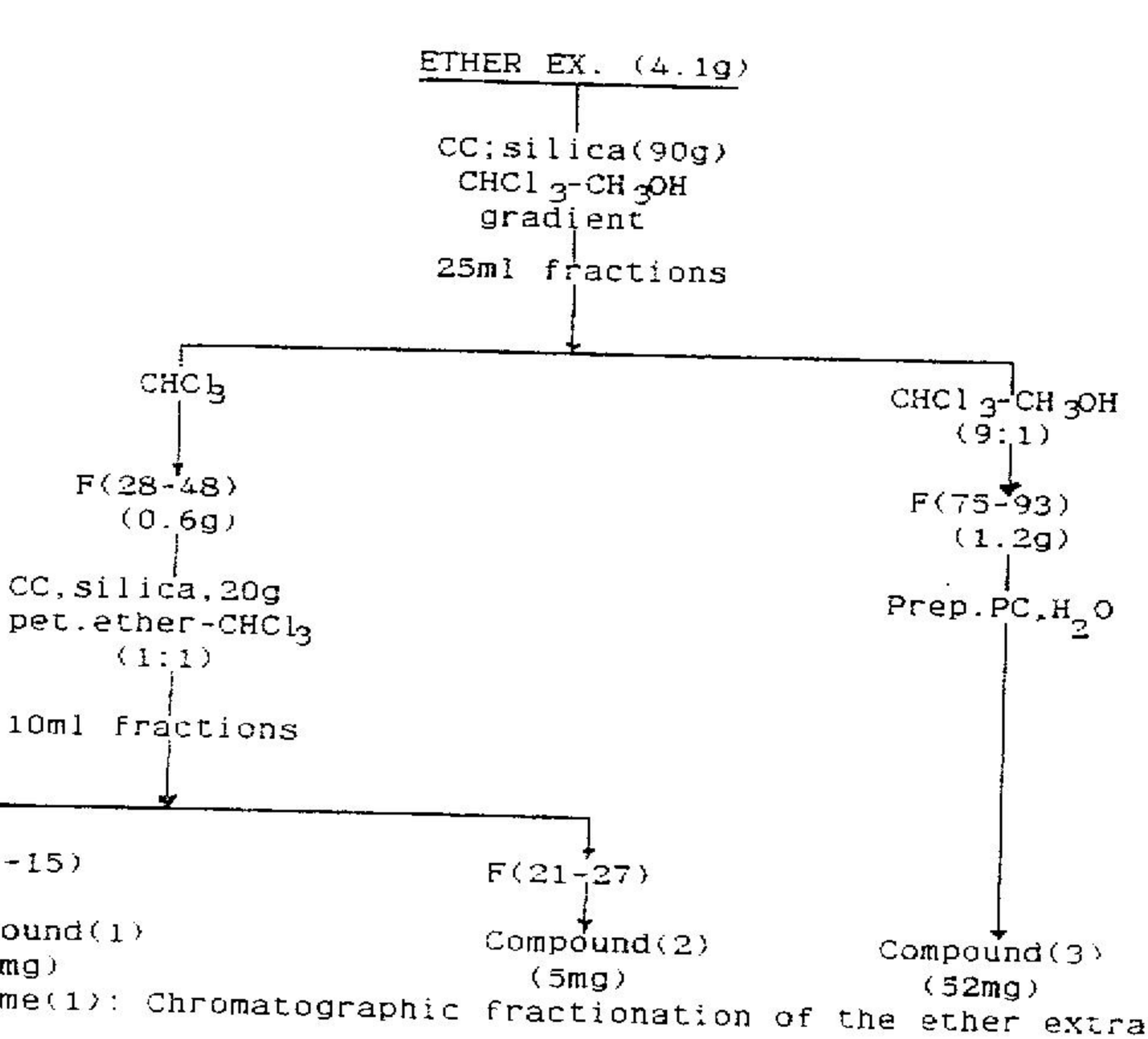
ration of organic solvent, in each case, left crude extracts weighing 203.7g (pet.ether), 4.2g (ether) and 7.5g (ethyl acetate).

Chromatographic investigation of the extracts*:

A- The ether extract:

TLC examination of the ether extract on silica gel-coated plate using chloroform as a solvent, revealed the presence of at least 3 major fluorescent spots (UV, 366nm) having R_f values 0.84 (yellowish-green), 0.74 (brownish-yellow) and 0.34 (blue to mauve).

The solvent free extract (4.1g) was fractionated over a silica gel-packed column (2.5cm ID, 90g) and gradiently eluted with chloroform-methanol. The effluent, in 25ml fractions, was monitored as mentioned above and similar fractions were combined. The obtained fractions were either subjected to further fractionation or direct crystallization as illustrated in scheme (1).



Scheme(1): Chromatographic fractionation of the ether extract.

*Investigation of the pet. ether extract will be discussed in a separate publication.

B- The ethyl acetate extract:

Examination of the ethyl acetate extract on a cellulose-coated plate, using 15% acetic acid for development, revealed the presence of 3 dull brown spots (UV,366nm) transformed into yellow upon exposure to ammonia vapour. They have the R_f values 0.35, 0.15 (major) and 0.07.

A part of the extract (2g) was dissolved in a minimum volume of methanol and subjected to preparative PC (3MM) using 15% acetic acid as a solvent (triple run). The UV-localized bands on the air-dried chromatogramms were separately eluted with hydromethanol (90%V/V), filtered, concentrated to small volumes and left for crystallization on cold. Three yellow deposites designated F_1 (15mg), F_2(93mg) and F_3(2mg) were obtained.

Spectral analysis:

UV spectra of flavonoids were recorded following the standard procedure of Mabry et al (1970)[20] and those of coumarins were recorded in methanol. IR were obtained in KBr pellet.

Acid hydrolysis:

Strong and mild acid hydrolysis were carried out following the standard procedure of Harborne (1965)[21].

RESULTS AND DISCUSSION

A- Essential oil:

The fruits of Torilis arvensis (Huds.) Link afforded on hydrodistillation a pale yellow oil lighter than water in a yield of 0.5%V/W.

CGC of the freshly distilled oil (Fig.1) revealed the presence of at least 87 components but more than half of them are present in trace amounts (less than 0.1%). Twenty components, accounting for about 28.5% of the oil composition, were identified (Table 1).

The fruit essential oil of the Egyptian species consists of oxygenated sesquiterpenes (64.22%), sesquiterpene hydrocarbons (19.92%), phenols (6.1%) and monoterpenes (4.71%). Its composition is distinctly different from those of the previously

Table(1): Major components of the essential oil of the fruits of Torilis arvensis.

Peak No:	Components	$t_{R(min.)}$ CW-20M	R_I	Relative percent composition
Monoterpene hydrocarbons (4.71%):				
1	α-pinene	2.20	1034	0.79
2	camphene	2.66	1080	0.03
3	β-pinene	2.93	1121	2.86
4	sabinene	3.05	1128	0.06
5	myrcene	3.55	1161	0.28
6	unknown	3.90	1192	0.07
7	limonene	4.17	1208	0.10
8	β-phellandrene	4.24	1213	0.20
9	p-cymene	5.50	1274	0.21
10	terpinolene	5.94	1290	0.11
Sesquiterpene hydrocarbons (19.92%):				
11	α-ylangene	11.97	1482	0.40
12	unknown	12.04	1484	0.24
13	unknown	14.82	1554	0.35
14	β-gurjunene	15.78	1577	0.70
15	β-caryophyllene	16.07	1584	5.47
16	unknown	16.19	1586	0.31
17	unknown	16.33	1589	0.10
18	α-humulene	18.90	1657	1.21
19	trans-β-farnesene	19.59	1674	1.47
20	unknown	21.41	1717	0.35
21	unknown	21.57	1721	0.45
22	β-bisabolene	21.94	1729	6.78
23	α-bisabolene	22.39	1750	1.36
24	δ-cadinene	23.50	1762	0.30
25	γ-cadinene	23.83	1769	0.43
Phenols (6.1%):				
26	cresol	29.55	1899	2.01
27	unknown	31.63	1951	0.29
28	unknown	35.10	2039	0.15
29	thymol	37.67	2106	3.65
Oxygenated sesquiterpenes (64.22%):				
30	unknown	43.92	2269	0.49
31	unknown	45.39	2309	1.02
32	unknown	46.89	2349	52.92
33	unknown	48.68	2395	0.76
34	unknown	49.40	2416	3.24
35	unknown	51.51	2480	0.45
36	unknown	52.23	2501	4.67
37	unknown	53.29	2532	0.67

studied European ones[4]. The oil is dominated by an oxygenated sesquiterpene (t_R,46.89; R_I,2349) having the same MS pattern of the major component reported before in the studied species[4].

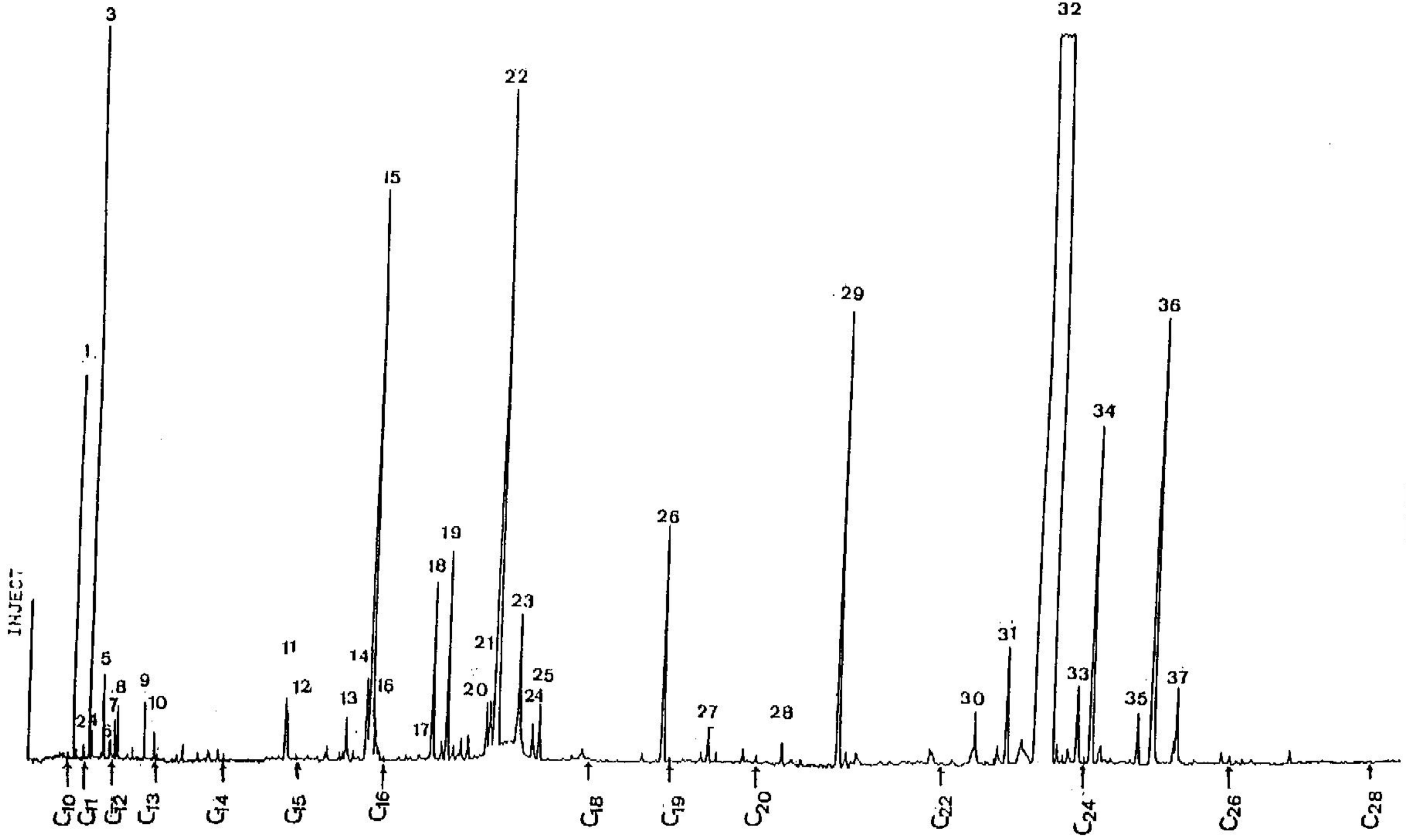


Fig.(1): CGC (CW-20M) of the essential oil of the fruits of Torilis arvensis (Huds.) Link.

It constitutes about 53% of the oil composition. It is accompanied by significant amounts of B-bisabolene (6.78%), B-caryophyllene (5.47%), two unidentified oxygenated sesquiterpenes (t_R,49.40,52.23; R_I,2416,2501; 3.24%,4.67%), thymol (3.65%), B-pinene (2.86%) and cresol (2.01%). However, carotol and biphenyl, the only previously identified components in this genus, could not be traced in the present study.

B- Coumarins and flavonoids:

From the fruit ether extract of Torilis arvensis,3 coumarinic compounds 1,2&3 were isolated and their structures were established as bergapten, xanthotoxin and scopoletin from their physico-chemical properties and spectral data (Table 2) as well as by comparison with authentic samples.

Table (2): Physico-chemical properties and spectral data of the isolated coumarins.

	Compound(1)	Compound(2)	Compound(3)
Crystal form	fine needles P.E.-$CHCl_3$ (2:8)	fine needles P.E.-$CHCl_3$ (2:8)	fine needles EtOAc-CH_3OH (8:2)
m.p.(°C)	187-189	146-147	204-206
R_f(silica,$CHCl_3$)	0.84	0.74	0.34
Fluorescence (UV,366nm)	yel-gr	br-yel	bl-mau
UV(CH_3OH.nm)	221,249,268, 312	215,247,263, 302	226,260.295, 343
NaOAc shift (nm)	——	——	244,275,390(-47)
IR(KBr,Cm^{-1})	1730,1630, 1610,1580, 1550	1740,1635, 1590,1540,	3330,1700 1605.1560, 1500

P.E.,petroleum ether; yel-gr, yellowish-green; br-yel, brownish-yellow; bl-mau, blue-mauve.

The ethyl acetate extract afforded 3 yellow amorphous compounds F_1, F_2 &F_3 gave positive tests for flavonoid glycosides (22). The UV spectral data (table 3) of F_1 and F_2 indicated that they are flavone in nature while those of F_3 show unusual behaviour. Its minute quantity precluded further investigation.

Table(3): UV spectral data of the isolated flavonoids.

Compound	Band	λmax(nm) (CH_3OH)	Shift in λmax (nm)				
			$NaOCH_3$	NaOAc	NaOAc/H_3BO_3	$AlCl_3$	$AlCl_3$/HCl
F_1	G I	335	+51*	+52	-5	+51	+47
	G II	270	-	-	-	+6	+7
	Ag I	336	+56*	+40	+2	+48	+45
	Ag II	268	+7	+7	-	+8	+8
F_2	G I	348	+46*	+57	+24	+84	+39
	G II	255	+8	+3	+4	+19	+18
	Ag I	349	-52*	+35	+21	+77	+36
	Ag II	252	+14	+17	+7	+22	+23
F_3	G I	363sh	-32*	+15	+1	+46	+29
	G II	330sh	-	-	+7	-15	+12

*, increase in intensity; G, glycoside; Ag, aglycone

UV-data of F_1 in the different ionizing and complexing reagents(20) indicated that it is a flavone of apigenin type with blocked hydroxy at C-7. Mild acid hydrolysis(21) of F_1 yielded the aglycone in two steps indicating its bioside nature. The monoside intermediate and the aglycone isolated after complete acid hydrolysis proved, through co-chromatography with authentic samples, to be respectively identical with apigenin-7-o-glucoside and apigenin. Glucose was the only detected sugar in the hydrolysate indicating that F_1 is apigenin-7-o-diglucoside

F_2 was found to be a flavone of luteolin type with blocked hydroxy at C-7. It was identified as luteolin-7-o-glucoside.

previously reported in Torilis species, by comparison with authentic sample.

Moreover, crystalline masses were deposited from the concentrated and refrigerated aqueous mother liquor remained after extraction with ethyl acetate. Repeated crystallization from methanol-water (1:1) afforded shiny needle-shaped crystals (2.63g), m.p. 165-166°C. It is soluble in water, hot methyl and ethyl alcohols, sparingly soluble in cold ones and reduces $KMnO_4$ solution. It was identified as D-mannitol by m.p. and IR comparison with authentic sample.

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دراسة كيميائية لنمار نبات تورلس ارفنز والذى ينمو فى مصر

أحمد فؤاد حليم • حسن الراضى على سعد • محمد محمد مشالى *
محمد فريد لهلوب • عطا الله فؤاد احمد
كلية الصيدلة وكلية العلوم (دمياط) * - جامعة المنصورة - المنصورة - مصر .

نبات التورلس ارفنز من نباتات العائلة الخبية واسعة الانتشار بمصر وينمو بمنطقة الساحل الشمالى ودلتا نهر النيل .

تناول هذا البحث دراسة مكونات الزيت الطيار لثمار النبات النامجة الذى تم تحضيره بطريقة التقطير البخارى • وقد تم تحليل الزيت الناتج (٥ر٠٪) باستخدام كروماتوجرافيا الغاز على أعمدة شعرية • وكذلك كروماتوجرافيا الغاز مع مطياف الكتلة .

وقد وجد أن الزيت الطيار يتكون من ٨٧ مركب على الاقل • تم التعرف على ٢٠ مكونا منهم تمثل مايقرب من ٥ر٢٨٪ من التركيب الكلى للزيت • وذلك لمقارنة طيف الكتلة لكل مركب بالعديد من أطياف الكتلة المنشورة بالمراجع • بالاضافة الى تعيين معامل كوفاتس للاحتجاز .

وقد لوحظ أن المكونات الرئيسية للزيت عبارة عن سيسكوتيرمينات أكسجينية (٢٢ر٦٤٪) • بالاضافة الى سيسكوتربينات هيدروكربونية (٩٢ر١١٪) • فينولات (٠ر١٦٪) • وتربينات أحادية (٢١ر٤٪) .

تناول هذا البحث ايضا دراسة المحتوى الكومارينى والفلافونولى لثمار هذا النبات • وتم فصل البرجابتين • والزانثوتوكسين • والكويرسلتين من الخلاصة الايثيرية والميثيولين - ٧ - أ - أحادى الجلوكوز • والابيجينين - ٧ - أ - ثنائى الجلوكوز من خلاصة خلات الايثيل • وتم اثبات التركيب الكيميائى لهم بدراسة الخواص الطبيعية وكذلك اطياف الاشعة تحت الحمراء وفوق البنفسجية .

وقد اثبتت هذه الدراسة لاول مرة أحتواء جنس التورلس على مركبات كومارينية والابيجينين - ٧ - أ - ثنائى الجلوكوز .