Essential Oil of *Daucus glaber* Forssk

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Z. Naturforsch. **59 c**, 373–378 (2004); received February 24/April 25, 2003

The composition of the essential oil of the fruits, leaves and stems of *Daucus glaber* Forssk has been studied by GC/MS. It was found that, the essential oil of the fruits consists of monoterpene hydrocarbons (limonene and sylvestrene are the majors) and phenylpropanoids (elemicin is the major). Sylvestrene has never been reported before in the essential oil of any *Daucus* species. The study of the essential oil of the leaves revealed the presence of monoterpene hydrocarbons; limonene and γ-terpinene are the majors and a small amount of sylvestrene. The essential oil of stems consists of monoterpene hydrocarbons (γ-terpinene is the major), terpene alcohols (mainly 4-terpineol) and phenylpropanoids (myristicin and elemicin are the majors). It is interesting that, the essential oil of the fruits is free from any oxygenated terpenes while that of the stems is free from limonene and sylvestrene which are present in the essential oil of the fruits and leaves in fairly large amounts. The essential oil of the fruits, leaves and stems shows broad antimicrobial activities against both gram positive and gram negative bacteria. In addition, the volatile oil of the stem, particularly, show activities against *Candida albicans* (yeast). Also, the prepared oils have variable cytotoxic activities with LC_{50} 21.52, 36.01 and 42.34 μg/ml, respectively.

Key words: Daucus glaber, Essential Oil, GC/MS

Introduction

The genus *Daucus*, Apiaceae, comprises about 60 annual and biennial species mostly distributed in Europe, Africa, West Asia, few ones in North America and Australia. The genus *Daucus* is represented in Egypt by 8 species (Tackholm, 1972). Many of these plants have been used by natives as diuretics, emollient, vermifuge, carminative and stomachic (Keith, 1965; Jafri El-Gadi, 1977) and some have edible roots, *Daucus carota*.

It is reported that the genus *Daucus* is the richest genus of the Apiaceae concerning its essential oil content. The essential oil pattern of the fruits was found to be very useful for separating and characterizing the genus within the family (Harborne, 1971; Williams and Harborne, 1972). Monoterpene hydrocarbons were found to be predominant (Lewis and Elvin-Lewis, 1977; Watt and Wijik, 1961). Limonene was reported in the essential oil of the above ground part of *Daucus carota* cultivated in Moldavia (Bakina *et al.*, 1972), *Daucus carota* var. *boissieri* (Halim *et al.*, 1988), *Daucus syrticus* Murb. (El-Alfy *et al.*, 1994) and *Daucus capillifolus* Gilli. (Haman *et al.*, 1989). Also, limonene was found in the essential oil of the fruits, leaves and stems of *Daucus carota* var. *maximus* (Saad *et al.*, 1995).

Phenylpropanoids, especially elemicin, were reported in the essential oils of the fruits of *Daucus syrticus* Murb. (El-Alfy *et al.*, 1994) and *Daucus capillifolius* Gilli (Haman *et al.*, 1989).

The monoterpene alcohol geraniol was reported in the essential oil of the fruits of *Daucus syrticus* Murb. (Halim *et al.*, 1988) and *Daucus capillifolius* Gilli (El-Alfy *et al.*, 1994).

The sesquiterpene alcohol carotol was found in the essential oil of the fruits of *Daucus syrticus* Murb. (El-Alfy *et al.*, 1994), but geraniol, nerol and carotol were detected in that of *Daucus carota* var. *boissieri* cultivated in Egypt (Halim *et al.*, 1988), wild red, black and yellow varieties of *Daucus carota* growing in Pakistan (Ashraf *et al.*, 1977).

The monoterpene ester, geranyl acetate was detected in the essential oil of the fruits of several varieties of *Daucus carota* (Pigulevskii and Kovaleva, 1955a; Pigulevskii and Kovaleva, 1955b; Pigulevskii *et al.*, 1960; Ashraf *et al.*, 1979), neryl acetate was found as a main constituent of the essential oil of the fruits of *Daucus carota* ssp. (wild carrot) and *Daucus carota* ssp. *sativus* (cultivated carrot) (Kilibarda *et al.*, 1996).

0939–5075/2004/0500–0373 $ 06.00

Table I. Composition of the essential oil of the fruit.

Peak scan #	Retention time t_R [min]	Relative composition (%)	M^+ peak	Base peak	Fragmentation peaks (*m/z*)	Component	Adams, 1995 (DB-S)
616	8:13	0.01	136.1	93.1	41.1,77.1, 91, 121a	α-thujene	0307
836	10:03	0.18a				α-pinene	0319
1248–1255	13:29–13:33	18.08	136.1	93.1	41.1, 53.1, 67, 68.1, 79, 107, 121.1	sylvestrene	0474
1461–1696	15:16–17:13	37.02	136.1	93.1	41.2, 53.1, 67, 68.2, 77, 79, 107, 121	limonene	0481
1725	17:28	2.91	136.1	93.1	41.1, 51, 53, 65.1, 77.1, 91, 105, 121.1 g	γ-terpinene	0545
3180	29:36	2.51	178.1	178.1	41.1, 51, 65,77.1, 91.1, 103.1, 107.1, 115, 135.1, 147.1, 163.1	methyleugenol	1403
3702–4200	33:58–38:07	32.69	208.1	208.1	41.1, 55.1, 77.1, 91.1, 105.1, 133.1, 150.1, 177.1, 193.1	elemicin	1772

Daucus glaber Forssk grows well in sand dunes and sandy sea shores in the Northern region of the Nile Delta and flowers from March to early May.

In previous publications, two triester phenylpropanoids, daucoglabrin and isodaucoglabrin were separated (Halim and Mansour, 1989, 1990). Nothing was reported about the composition of the essential oil of *Daucus glaber* Forssk, therefore, we are concerned with studying the composition of the essential oil content of *Daucus glaber* Forssk using GC/MS technique and also, studying its physical and biological properties.

Results and Discussion

The essential oil of the fruits, leaves and stems of *Daucus glaber* Forssk was separately prepared by steam distillation adopting the Egyptian pharmacopoeia method (1984). The essential oil of the fruits (4 % v/w) is colorless, has disagreable odour and optical rotation + 1.27°, while that of the leaves (0.67 % v/w) has pale yellow color, characteristic odour and optical rotation + 0.12° but the essential oil of the stems (0.10 % v/w) has yellow color, characteristic odour and optical rotation – 0.31°. Each oil was analyzed by GC/MS and the results are listed in Tables I–IV.

It was found that the essential oil of the fruits (Table I) consists chiefly of monoterpene hydrocarbons and phenylpropanoids (Table II). Monoterpene hydrocarbons are present in a significant amount (58.3 %) and consist mainly of limonene (37.0 %), sylvestrene (18 %) and a smaller amount of γ-terpinene (2.9 %). Sylvestrene has never been reported before in the essential oil of any *Daucus* species. Phenylpropanoids are also majors and consist mainly of elemicin (23.7 %) and methyl eugenol (2.5 %). It was found that the essential oil of the fruits does not contain any oxygenated terpenes, *viz*, monoterpene alcohols, sesquiterpene alcohols and monoterpene esters.

The essential oil of the leaves (Table II) consists of monoterpene hydrocarbons (61.5 %), total hydrocarbons (61.8 %), phenylpropanoids (19.7 %), monoterpene alcohols (8.2 %) and sesquiterpene alcohols (1.2 %). The monoterpene hydrocarbons

Table II. Different major constituents of the essential oil of fruits, leaves and stems.

Constituent	Fruit	Leaf	Stem
Monoterpene hydrocarbons	58.3%	61.5%	43.9%
Sesquiterpenc hydrocarbons	–	0.4%	3.0%
Total hydrocarbons	28.3%	61.8%	46.9%
Monoterpene alcohols	–	8.2%	25.8%
Sesquiterpene alcohols	–	1.2%	11.1%
Phenylpropanoids	35.2%	19.7%	1.1%
Terpene esters	–	–	41.7%

Table III. Composition of the essential oil of the leaves.

Peak scan #	Retention time t_R [min]	Relative composition (%)	M^+ peak	Base peak	Fragmentation peaks (*m/z*)	Component	Adams, 1995 (DB-S)
634	8:22	0.16	136.1	91.1	41.1, 77.1, 91, 121a	α-thujene	0307
950 to 959	11:00 to 11:04	2.04	136.1	93.0	41.1, 56.1, 69.1, 77.1, 79, 91, 105, 107, 121.1	sabinene	0379
1009 to 1021	11:29 to 11:35	1.52	–	–	–	β-terpinene	–
1278 to 1285	13:44 to 13:48	3.56	136.1	93.0	41.1, 53, 77.1, 79, 91, 107, 121.1	mentha-2,8-diene	0388 *trans-meta* 0395 *cis-meta*
1301	13:56	2.76	136.1	93.0	41.1, 53, 77.1, 79, 91, 107, 121.1	2-carene	0427
1346	14:18	2.51	136.1	93.0	41.1, 53, 77.1, 79, 91, 107, 121.1.	3-carene	0444
1363 to 1504	14:27 to 15:37	5.33	136.1	93.0	41.1, 53, 67, 68,1, 79, 91, 107, 121.1	sylvesterene	0474
1595	16:23	21.73	136	93.1	41.1, 3, 67.1, 68, 79, 91, 107, 121.0	limonene	0481
1647	16:49	21.88	136.0	93.0	43.1, 65.1, 77.1, 79, 91, 105, 121.0	γ-terpinene	0545
1661	16:56	1.60	154.1	71.1	43.0, 55.1, 69.1, 81, 111, 121.1, 136.1, 139	menth-2-en-1-ol (*cis-para*)	0682
1732	17:32	0.79	154	43.1	41, 55.1, 71.1, 79, 81.1, 93, 111.1, 121.1, 139.1	menth-2-en-1-ol (*trans-para*)	0725
2033 to 2090	20:02 to 20:31	5.31	154	71	41, 43.1, 55.1, 93.1, 111.1, 136.1	4-terpineol	0820
2112	20:42	0.45	–	–	–	γ-terpineol	–
3020 to 3027	28:16 to 28:20	1.55	178	178	41.1, 65.1, 177.1, 91.1, 103.1, 107, 1115.1, 135, 147.1, 163.1	methyleugenol	1403
3163	29:28	0.30	204	91.1 & 161.1	41.1, 55.1, 77, 79.1, 91.1, 93, 105, 107, 119, 133.1, 161.1, 189.2a	α-gurjunene	– 1421
3568 to 3775	32:51 to 34:35	18.16	208	208	41, 53, 65.1, 71.1, 91.1, 105, 118, 133.1, 150.1, 165, 177.1, 193.1	elemicin	1772
3794	34:44	0.39	220	43.1 & 91.1	41, 55.1, 67.1, 77, 79.1, 91.1, 93, 105, 119, 131, 147.2, 159.1, 162.2, 187.1, 205.2	spathulenol	1825
3931	35:53	0.83	222	59.1	41,43,55, 79.1, 91.1, 93, 109.1, 121.1, 149.2, 164.2, 189.2, 204.2b	β-eudesmol	1993
5198	46:28	90.30	296	71.1	41, 43.1, 55.1, 57, 81.1, 95.1, 123.1	phytol	2636

(61.5 %) consist mainly of limonene and γ-terpinene nearly in equal amounts (21.7 %) (Table III). Also, there are small amounts of sylvestrene (5.3 %), mentha-2,8-diene (3.6 %), 2-carene (2.8 %) and 3-carene (2.5 %). The phenylpropanoid fraction resembles that of the fruit and is characterized by the presence of elemicin (18.2 %) and a small amount of methyleugenol (1.6 %). It is evident that the essential oil of the leaves is free from myristicin. The sesquiterpene hydrocarbon fraction of the oil of the leaves consists of α-gurjunene (0.3 %), while the sesquiterpene alcohol fraction is represented by β-eudesmol (0.9 %) and spathulenol (0.4 %), which have never been reported before in the essential oil of any *Daucus* species.

The essential oil of the stems (Table II) consists chiefly of monoterpene hydrocarbons, terpene alcohols and phenylpropanoids (43.9 %, 25.8 % and 11 % of the oil composition, respectively), as well

Table IV. Composition of the essential oil of the stem.

Peak scan #	Retention time t_R [min]	Relative composition (%)	M^+ peak	Base peak	Fragmentation peaks (*m/z*)	Component	Adams, 1995 (DB-S)
666	8:37	0.13	136.1	93.1	41.1, 77.1, 91, 121	α-thujene	0307
983–994	11:16–11:21	1.84	136.1	93.1	41.1, 77.1, 91, 121	sabinene	0379
1013	11:31	0.84			–	β-terpinene	–
1060–1116	11:54–12:23	2.56	136.1	93.0	41.1, 77.1, 91, 121	2-carene	0427
1189	12:59	2.86	136.1	93.0	41.1, 77.1, 91, 121	3-carene	0444
1291	13:50	6.28	136.1	93.0	41.1, 77.1, 91, 121	β-phellandrene	0482
1367–1407	14:28–14:48	3.77	136	93.1 and 119.1	41.1, 68.1, 91, 121	4-carene	
1447–1468	15:08 – 15:19	6.18	136	119.1	41, 68.2, 91, 121, 134	ocimene	–
1589	16:20	19.48	136.1	93.0	41, 43.1, 65.1, 77.1, 79, 91, 105, 121.1	γ-terpinene	0545
1653	16:52	4.58	136.1	93.1	41, 53.1, 77, 79.1, 91, 105, 121.1	ocimene allo	0701
2230–2275	21:40–22:03	22.13	154	71.1	41, 43, 55.1, 69.1, 93.1, 111.1, 136.1	4-terpineol	0820
2291	22:11	1.77	–	–	–	γ-terpineol	–
2296	22:14	0.77	154	59.1	43, 81.1, 93.1, 95.1, 121.1, 136.1	α-terpineol	0852
2315–2336	22:23–22:34	1.09	154	84.1	41.1, 55.1, 79.1, 83, 93.1, 111.1, 139.1	piperitol	0865
2398	23:05	0.56	152 (M^+ acetate)	119.1	43.1, 79.1, 81.1, 91.1, 134.1	chysanthenyl ac-etate (*trans*)	0967
2432	23:22	0.52	152 (M^+-acetate)	91.1	43.1, 79.1, 92, 119.1	4-thujen-2α-yl-acetate	–
2785	26:18	0.36	204	161	41.1, 43, 55.1, 77.1,	α-copaene	1334
2828	26:40	0.66	204		91.1, 93, 105.1, 119.1, 133.1, 161.2, 189.2, 41.1, 43, 55.1, 79.1, 91.1, 105.1, 119.1, 133.1, 189.2	β-cubebene	1371
2949	27:40	0.45	204	41.1	55, 57.1, 79, 91.1, 93, 105, 133.1, 161.2, 178.1, 189.2	caryophyllene	1442
3254	30:13	0.55	204	121 & 193.1	41.1, 53, 67.1, 79.1, 91, 105, 107, 123.1, 147, 161.2, 189.2	γ-elemene	1476
3266	30:19	0.35	204		41.1, 55, 67.1, 77, 79.1, 91, 93.1, 105, 107, 119, 121, 137, 161.2	γ-gurjunene	1575
3281	30:26	0.65	204.2	93.1	41.1, 55, 67.1, 77, 79.1, 91, 105, 107, 119, 121, 137, 161.2, 189.	α-selinene	1631
3538	32:35	5.05	192	192	53, 65, 77.1, 191.1, 119.1, 131.1, 133.1, 147, 165.1	myristicin	1691
3589	33:01	6.04	208	208	65.1, 77.1, 91.1, 105, 150.1, 165, 177.1, 193.1	elemicin	1772
3679	33:46	1.98	220.2	43.1	41, 55.1, 69.1, 79.1, 91.1, 105, 119.1, 131, 147.1, 159.1, 162.2, 187.2, 205.2	spathulenol	1825
3920	35:47	1.78	222	59	41, 43, 62, 79.1, 91.1, 95.1, 109.1, 121.1, 149.2, 164.2, 189.2, 204.2	β-eudesmol	1993

Table V. Antimicrobial activity of the essential oil of fruits, leaves and stems.

Microorganism	Fruit oil 80 mg/ml	Leaf oil 80 mg/ml	Stem oil 80 mg/ml	Control (ampicillin) 5 mg/ml	Control (clotrimazole) 5 mg/ml
Staphylococcus aureus	2.5 mm	0.5 mm	0.5 mm	10.5 mm	–
Bacillus subtilis	2.0 mm	2.5 mm	–	13.0 mm	–
Escherichia coli	–	–	–	–	–
Candida albicans	–	–	3.5 mm	–	9.0 mm

Table VI. Cytotoxic activity of the essential oil of fruits, leaves and stems.

Concentration	Corrected mortality* (%)		
	Fruit oil	Leaf oil	Stem oil
1 mg/ml	100	100	96.4
0.1 mg/ml	56.7	59.6	76.4
0.01 mg/ml	46.6	54.1	20

* Using Abbot's formula.

as small amounts of sesquiterpenes: hydrocarbons (3 %), alcohols (3.8 %) and esters (1 %). It is evident from the composition of the stem oil (Table IV) that sylvestrene is absent from the essential oil of the stems while it is present in a significant amount in the essential oil of both the fruits and the leaves. Also, there are small amounts of chrysanthenyl acetate (0.6 %) and 4-thujen-2α-yl acetate (0.5 %), which have never been reported before in the essential oil of any *Daucus* species.

The essential oil of the fruits, leaves and stems showed a weak antimicrobial activity against both gram positive and gram negative bacteria (Table V). This was explained by the presence of high percentage of phenolic and/or oxygenated compounds. At the same time, the prepared oils showed cytotoxic activity with LC_{50} 21.52, 36.01 and 42.34 μg/ml, respectively (Table VI) which may be attributed also to the presence of high percentage of phenolic and/ or oxygenated compounds.

Experimental

Plant material

Ripe and mature fruits as well as leaves and stems of *Daucus glaber* Forssk, growing wild on El-Narges mountains, Balteem, Kafr El-Shiekh, north region of the Nile Delta, Egypt, were separately collected in May 2001, air-dried in shade and then finely powdered. The plant was kindly identified by Dr. I. Mashaly, Associate Professor of Systematic Botany, Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt and the identification was further confirmed by Botanical Center Kew, London, England. A voucher specimen is kept at the Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Preparation of the volatile oils

Powdered fruits, leaves and stems (100 g, each) were separately subjected to steam distillation for 8 h adopting the Egyptian pharmacopoeia (1984) method. Each oil was collected, dried over anhydrous sodium sulfate and kept in the freezer until analysis.

The GC/MS analysis was carried out at the National Research Center, Dokki, Cairo, Egypt on GC/MS Fenningan Mat SSQ 7000 with Digital DEC 3000 workstation fitted with a fused silica DB-5 (30 m × 0.25 mm ID, 5 % phenyl methyl polysiloxane) capillary column with helium as a carriergas at a flow rate of 1.6 ml/min, column head pressure 13 psi. The gas chromatography was coupled to a mass selective detector (MS) at 70 eV in EI ionization mode. The sample was injected in 1 μl size in splitless mode. The temperature was programmed initially at 50 °C for 1 min, and then increased with a rate of 4 °C/min up to 250 °C.

Identification of the components was based on matching their retention time and spectral indices with some reference samples and with those published in literature (Adams, 1989, 1995) and also by using NST mass spectral database of the gas chromatograph computer.

Determination of physical constants

Specific rotation was performed on the methanolic solution of the oil (0.1 %) and measured in

1 dM tubes at the sodium D line using Perkin-Elmer 141 polarimeter.

Biological activities of the essential oil

Screening for the antimicrobial activity of the prepared oils

The prepared oils under investigation were tested for their antimicrobial activity. The disc-agar diffusion method (Cruickshank *et al.*, 1975) was applied. Different bacteria and yeast (as test organisms) and ampecillin and clotrimazole (as control) were used. The susceptibility of various microorganisms to the inhibitory effect of the oils and the control is presented in Table I.

Screening for the cytotoxic activity of the oils

The brine shrimp eggs (*Artemia salina* Leach, available in pet shops, Cairo, Egypt) were hatched in a shallow rectangular dish (22 cm × 32 cm) filled with artificial sea water and double-distilled water. The eggs (*ca.* 50 mg) were sprinkled. After 48 h, the phototropic nauplii were collected and separated by the divider from their shells. The brine shrimp technique was applied (Meyer *et al.*, 1982). The rate of mortality was determined and corrected for the negative control mortality by Abbot's formula. The LC_{50} was obtained by making a linear regression of the corrected rate of mortality (Y) versus log concentration (X), then the X-intercept when Y = 50 % is found and the antilog was determined. The results are listed in Table II.

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