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**Chemical diversity and antimicrobial activity of the essential oils of four
Apiaceae species growing wild in Lebanon**

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Chemical diversity and antimicrobial activity of the essential oils of four Apiaceae species growing wild in Lebanon

The Apiaceae species are largely used in folk medicine to treat many diseases, including microbial infections. The essential oils (EOs) of *Prangos asperula* Boiss., *Daucus carota* L. subsp. *maximus*, *Ferula elaeochytris* Korovin and *Smyrniium olusatrum* L. were analysed by GC, GC-MS and NMR. The major components were as follows: sabinene (29.8 %) and β -phellandrene (19.2 %) were found in the *P. asperula* fruit oil; α -pinene (27.4%) and carotol (26.3%) were found in the *D. carota* flower oil; α -pinene (71.8%) was the major compound in the *F. elaeochytris* fruit oil; and the *S. olusatrum* flowerings tops EO was mainly composed of furanodiene (44.6%) and furanoeremophil-1-one (28.5%). *D. carota* and *S. olusatrum* were the most active oils especially against *Staphylococcus aureus* and the dermatophytes; the MIC values ranged from 8 to 64 $\mu\text{g/ml}$. Some of these EOs constituents hold promising antimicrobial potentials, confirming the traditional medical use of the plants.

Keywords: antimicrobials; essential oils; *Prangos asperula*; *Daucus carota*; *Smyrniium olusatrum*; *Ferula elaeochytris*.

1. **Introduction:**

In recent decades, the incidence of microbial infections in humans, particularly those involving the skin and mucosal surfaces, has increased considerably. A concomitant and dramatic increase of cases of immunocompromised patients which frequently develop opportunistic and superficial microbial infections has also been reported recently (1). In addition, the abuse or misuse of antibiotic and antimycotic drugs has led to appearance of multiresistant strains. Therefore, microbial diseases have emerged as an important public health problem associated with an increased rate of mortality and morbidity, as well as a rising economic burden (2, 3). Therefore, the development of new and effective antimicrobial compounds is urgent. In this matter, natural substances, particularly essential oils, have attracted a great deal of interest (4). In fact, essential oils may offer an alternative source of antimicrobial agents because they contain a wide range of bioactive chemicals with both specific and general antimicrobial potential (5, 6). They can be viewed as examples of evolution-optimized mixtures of natural products that can inspire the discovery of bio-inspired synthetic synergistic systems.

South-West Asia is a region with high Apiaceae diversity. This family is well represented in Lebanon with at least 56 genera and 120 species (7). Species in the Apiaceae family are very important in the pharmacopeia of the Middle East and the rest of the world (8).

For this study, the relationship between the antimicrobial potential and the chemical composition of four wild Apiaceae species essential oils used in the Lebanese traditional medicine (9, 10, 11) was investigated. The tested oils were those from *Ferula elaeochytris* Korovin (endemic to Lebanon, Syria and eastern Mediterranean Turkey) (12, 13), *Prangos asperula* Boiss. (endemic to Lebanon, Syria, Jordan and Palestine) (12, 14), *Smyrnum*

olusatrum L. (known as Alexanders or wild celery) and *Daucus carota* L. subsp. *maximus* (Desf.) Ball. (common names wild carrot, bird's nest, bishop's lace and Queen Anne's lace).

To the best of our knowledge, little information is known about the phytochemical composition and antimicrobial activity of *Ferula elaeochytris* and *Prangos asperula* EOs. Therefore, it was interesting to put into perspective those two oils in terms of composition and biological activity, as well as, the EO of other species widespread in Lebanon and belonging to the same family.

2. **Experimental:**

2.1. ***Plant material:***

Specimens of the four Apiaceae species were collected as follows:

- Flowers from *Daucus carota* were collected from a natural stand from the region of Adonis, Keserwan (33°58'05.91" N 35°36'35.20" E) at an altitude of approximately 50 m in May 2012.

- Fruits from *Ferula elaeochytris* were collected from a natural stand in Faraya, Mount Lebanon (34°00'33.21" N 35°50'02.50" E), at approximately 1500 m altitude, in July 2012.

- Fruits from *Prangos asperula* were collected from a natural stand in Laqlouq, Mount Lebanon (34°07'54.47" N 35°50'55.34" E), at approximately 1650 m altitude in June 2012.

- Flowering tops from *Smyrniium olusatrum* were collected from a natural stand in Zekrit, Metn, along the Nahr al-Kalb River (33°57'12.74" N 35°36'13.07" E), at approximately 50 m altitude in March 2012.

Plants have been collected according to the national biodiversity rights of Lebanon. Voucher specimens were deposited at the Herbarium of the Department of Botany and Medicinal plants, Holy Spirit University, Faculty of Agricultural and Food Sciences (USEK-

Lebanon), under the registry numbers MNU598, MNU640b, MNU618 and MNU615 respectively.

2.2. *Essential oil extraction:*

The essential oils (EOs) were obtained by hydrodistillation performed for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia (15). The *D. carota* flowers yielded 0.23 % EO (253.9 g of plant material were used to obtain 0.6 ml of EO), the *F. elaeochytris* fruits yielded 0.86 % EO (266.6 g of plant material were used to obtain 2.3 ml of EO), the *P. asperula* fruits yielded 0.52 % EO (341 g of plant material were used to obtain 1.8 ml of EO) and the *S. olusatrum* flowering tops yielded 0.12 % EO (457.8 g of plant material were used to obtain 0.7 ml of EO).

2.3. *Essential oils analyses:*

2.3.1. *GC analyses*

Analytical gas chromatography was carried out on a Thermo Electron Corporation gas chromatograph fitted with a DB-5 MS capillary column (30 m × 0.25 mm) with 0.1 µm film thickness or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 µm). Helium was the carrier gas (0.7 ml/minute). The column temperature was initially set to 35°C before being gradually increased to 85°C at 5°C/minute, held for 20 minutes at 85°C, raised to 300°C at 10°C /min and finally held for 5 minutes at 300°C. Diluted 1 µl samples (1/100, v/v) were injected at 250°C manually and in the splitless mode. Flame ionisation detection (FID) was performed at 310°C.

2.3.2. *GC/MS analyses*

The GC/MS analyses were performed using an Agilent gas chromatograph 6890 coupled with Mass Detector 5975. The 7683 B auto sampler injected 1 µL of each oil sample. A fused silica capillary column DB-5 MS (30 m × 0.25 mm internal diameter, film thickener 0.1 µm)

or a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm, film thickness 0.20 μm) was used. Helium was the carrier gas (0.7 ml/minute). The oven temperature program was identical to that described in 2.3.1. The mass spectra were recorded at 70 eV with an ion source temperature of 310°C and a transfer line heated to 320°C. The acquisition was recorded in full scan mode (50 – 400 amu).

2.3.3. *Identifications and quantifications*

Most constituents were identified by gas chromatography by comparing their retention indices (RI) with those from the literature (16, 17) or with those of authentic compounds obtained from Sigma-Aldrich (Lebanon). The retention indices were determined relative to a homologous series of *n*-alkanes (C8 to C24) analysed under the same operating conditions. Further identification was obtained by comparing their mass spectra on both columns with those provided in the NIST and Wiley 275 libraries, our home-made library constructed with pure compounds and EOs of known composition or with mass spectra from the literature (17, 18). The relative concentrations of the components were calculated based on the GC peak areas without correction (Figure 2); they are reported in Table 1.

2.3.4. *Identification of the main furanosesquiterpenes of *Smyrniium olusatrum* L. EO by NMR spectroscopy*

The nuclear magnetic resonance (NMR) spectra (¹H-NMR, ¹³C-NMR, ¹H-¹H COSY, HSQC and HMBC) of crude *Smyrniium olusatrum* L. EO allowed us to confirm the identification of the main constituents (furanodiene and furanoeremophil-1-one (Figure 1)) by comparison with the NMR data reported in the literature (19, 20).

The NMR spectra were recorded on a Bruker 500 MHz spectrometer equipped with a 1 mm inverse detection probe.

The characteristic signals are as follows:

Furanodiene (**11**): ^1H NMR (500 MHz, CDCl_3): δ (ppm) 1.27 (3H, s, H-15), 1.62 (3H, m, H-14), 1.93 (3H, d, $J = 1.2$ Hz, H-13), 3.43 (2H, d, $J = 15.9$ Hz, H-9a), 3.49 (2H, d, $J = 15.9$ Hz, H-9b), 4.76 (1H, br d, $J = 10.2$ Hz, H-5), 4.99 (1H, m, H-1), 7.15 (1H, m, H-12). ^{13}C -NMR (500 MHz, CDCl_3): δ (ppm) 9.0 (C-13), 16.4 (C-15), 16.6 (C-14), 41.5 (C-9), 128.5 (C-5), 129.5 (C-4), 130.0 (C-1), 134.9 (C-10), 137 (C-12).

Furanoeremophil-1-one (**12**): ^1H -NMR (500 MHz, CDCl_3): δ (ppm) 0.62 (3H, s, H-14), 1.06 (3H, d, $J = 6.7$ Hz, H-15), 1.92 (3H, d, $J = 1.2$ Hz, H-13), 2.05 (1H, dd, $J = 6.7$ and 3.7 Hz, H-4), 2.68 (1H, s, H-10), 7.15 (1H, m, H-12). ^{13}C -NMR (500 MHz, CDCl_3): δ (ppm) 8.2 (C-13), 12.2 (C-14), 15.2 (C-15), 42.0 (C-5), 42.7 (C-4), 54.5 (C-10), 137.0 (C-12), 209.5 (C-1).

2.4. **Antimicrobial activity:**

2.4.1. *Microorganisms*

The antimicrobial activity of the essential oils was investigated against Gram (–) bacterial strain *Escherichia coli* ATCC 25922; Gram (+) bacterial strain *Staphylococcus aureus* ATCC 29213; yeasts *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019 and *Cryptococcus neoformans* SNB-CN1; filamentous fungi *Trichophyton rubrum* SNB-TR1, *Trichophyton violaceum* SNB-TV1, *Trichophyton soudanense* SNB-TS1, *Trichophyton tonsurans* SNB-TT1, *Trichophyton mentagrophytes* SNB-TM1 and *Aspergillus fumigatus* SNB-AF1. The ATCC strains were purchased, while the others were clinical isolates kindly provided by Prof. Philippe Loiseau, Université Paris Sud. These strains were identified by Prof. Philippe Loiseau and Christian Bories; the molecular analyses were conducted by BACTUP. The ITS sequences were deposited in the NCBI GenBank database under the following registry numbers: KF360235 (*C. neoformans* SNB-CN1), KC692746 (*T. rubrum* SNB-TR1), KF360236 (*T. violaceum* SNB-TV1), KF360237 (*T. soudanense* SNB-TS1),

KF360238 (*T. tonsurans* SNB-TT1), KF360239 (*T. mentagrophytes* SNB-TM1) and KC692747 (*A. fumigatus* SNB-AF1).

2.4.2. *Microdilution method*

The broth microdilution method was used to determine the minimal inhibitory concentration (MIC) of the EOs according to the Clinical and Laboratory Standards Institute guidelines (21, 22, 23, 24). The investigated essential oils were diluted in DMSO and tested at concentrations ranging from 512 to 1 µg/ml. Oxacillin and gentamicin (16 - 0.03 µg/ml) were used as reference antibiotics, while itraconazole (16 - 0.03 µg/ml) and fluconazole (64 - 0.125 µg/ml) were used as positive controls for antifungal activity. These antimicrobial standards drugs were purchased from Molekula. The microplates were incubated at 37°C for 24 h for bacteria, 48 h for fungi and yeasts and five days for dermatophytes. The MIC values referring to the lowest concentration preventing visible microbial growth are reported in Table 2.

3. **Results and discussion:**

3.1. *Essential oils analyses:*

The chemical compositions of the four Apiaceae essential oils are reported in Table 1; the relative proportions of the components are given. The yields of essential oils obtained by hydrodistillation ranged from 0.12 % to 0.86 %, in *S. olusatrum* flowering tops and *F. elaeochytris* fruits, respectively.

The oils of *F. elaeochytris* and *P. asperula* were essentially composed of monoterpene hydrocarbons (80.2 % and 74.4 %). The two main constituents of the *F. elaeochytris* fruit oil were α -pinene (71.8%) and β -pinene (6.80%), representing 78.6% of the total oil . The *P. asperula* fruit EO was more complex with five components above 5%: sabinene (29.8 %), β -phellandrene (19.2 %), α -pinene (9.8 %), *trans*-nerolidol (9.2 %) and α -phellandrene (8.0 %). The *D. carota* flower EO was rich in monoterpene hydrocarbons (40.2 %) and

oxygenated sesquiterpenes (26.9 %). The main constituents were α -pinene (27.4 %), carotol (26.3 %), α -humulene (9.8 %), germacrene D (7.0 %) and myrcene (5.3 %) . Finally, the *S. olusatrum* flowering tops EO was characterised by the dominance of furanosesquiterpenoids, accounting for 77.3 % of the total volatile oil (Figure 1). Furanodiene (**1**) and its Cope rearrangement product curzerene (**2**) (together 44.6 %) and furanoeremophil-1-one (**3**, 28.5 %) were the most abundant compounds in the GC profile. Curzerene can be produced from furanodiene through a thermal Cope rearrangement due to the high temperatures in the injection insert during gas chromatographic analysis (25, 26).

1D and 2D NMR analyses were performed on the crude oil to differentiate these two isomers; curzerene could not be detected, while its precursor (furanodiene, **1**) was the major component. Therefore, the GC peak attributed to curzerene should be treated as a second peak for furanodiene (**1**), accounting for 44.6 % of the EO. NMR also confirmed the identification of the second major compound: furanoeremophil-1-one (**3**).

(Table 1)

(Figure 1)

The Lebanese EOs of the four studied Apiaceae species showed some differences in their chemical composition compared to those previously reported in the literature. Although *D. carota* EO has been the subject of several studies, the subspecies *maximus* remains poorly explored. A previous study reported the oil composition of *D. carota* subsp. *maximus* growing in Portugal and showed a markedly different composition compared to the Lebanese EO reported in our study (Valente). The Portuguese EO being mainly composed of α -pinene, geranyl acetate, β -bisabolene, α -asarone and E- methylisoeugenol doesn't share any major compound with the Lebanese EO, except for the α -pinene. However, high relative proportion of carotol, one of the main components of the Lebanese *D. carota* oil, has been reported in *D. carota* seeds cultivated in Turkey (Özcan and Chalchat). As for *F. elaeochytris*, few reports

are available on the chemistry and bioactivity of the oil given the limited geographical distribution of the species. One of the rare studies reported in turkey showed a considerably lower relative proportion of α -pinene and β -pinene (Baser et al). The chemical composition of the *P. asperula* EO has been mainly studied in Iran (Refs Iran). Compared to the Lebanese EO, several differences can be pointed out, in particular, the absence of sabine, α -phellandrene and nerolidol in these oils. And finally, all previous reports indicate that *S. olusatrum* EO is a rich source of furanosesquiterpenes with high relative proportions of curzerene (refs). Likewise, furanoeremophil-1-one, one of the main constituents of the Lebanese EO, has also been also reported as a major compound in Greece by Papaioannou et al. (ref). However, some differences are worth mentioning, like the absence of germacrone in our EO that is present in high relative proportion in some *S. olusatrum* oils (ref Greece; and Italy and Portugal).

3.2. **Antimicrobial activity:**

The minimum inhibitory concentrations (MICs) of the four Apiaceae essential oils are presented in Table 2. An oil was considered active if the minimal inhibitory concentration was 128 μ g/ml or below (27). The different pathogens were diversely sensitive to the EOs. Overall, the yeasts, Gram (–) bacterium *E. coli* and clinical isolates of the filamentous fungus *A. fumigatus* were resistant. However, the growth of Gram (+) bacterium *S. aureus* and the dermatophytic *Trichophyton* species was significantly inhibited. The most active inhibitors were the *D. carota* flowers and the *S. olusatrum* flowering tops oils. Notably, *D. carota* EO was only four times less active than oxacillin towards *S. aureus* with a MIC value of 8 μ g/ml. The significant antimicrobial effect of *D. carota* EO could be due to carotol, which is one of the major components of this oil. Carotol is known be involved in allelopathic interactions; it possesses antifungal, herbicidal and insecticidal potential (28). The antifungal activity of *D. carota* EO has been previously reported by Valente et al. and it was shown to be particularly

active against dermatophytic fungi and *Cryptococcus neoformans* (Valente et al.). The *S. olusatrum* EO was very active against dermatophytes with MIC values of 32-64 µg/ml, suggesting a potential antifungal property for the furanosesquiterpenes present in the oil. These results highlight the antifungal potential of this EO and are in full agreement with Marongiu et al. who also reported an antifungal activity of *S. olusatrum* EO against dermatophytic fungi (Marongiu et al.), thus, encouraging its use against dermatophytoses.

D. carota, *F. elaeochytris*, *S. olusatrum* and *P. asperula* are used in the traditional herbal medicine and are listed in the Lebanese pharmacopoeia. They are employed for wound disinfection and to cure several ailments, including microbial infections (9, 10, 11). Our results showed that the EOs from these plants possess antibacterial and antifungal potential and corroborate their traditional medical usage.

(Table 2)

4. **Conclusion:**

The present study demonstrated the effective antimicrobial activity of the EOs from the Apiaceae family, particularly against *S. aureus* and dermatophytic fungi. The antibacterial and antifungal potential observed may justify the use of these plants in herbal medicine as antimicrobial agents. In addition, this study highlighted the interesting antimicrobial potential of the EOs of *D. carota* rich in carotol and *S. olusatrum* mainly composed of furanosesquiterpenes (furanodiene and furanoeremophil-1-one). It is reasonable to hypothesize that these natural products are antimicrobial and could be used in the bio-inspired reconstruction of antimicrobial mixtures. Nevertheless, further studies are required to understand the mechanism of action and to obtain more information regarding the safety and toxicity of the oils and their constituents.

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References

1. M.A. Pfaller and D.J. Diekema, *Epidemiology of invasive candidiasis: a persistent public health problem*. *Clinical Microbiology Reviews*, **20**, 133–163 (2007).
2. M. Nucci and K.A. Marr, *Emerging fungal diseases*. *Clinical Infectious Diseases*, **41**, 521–526 (2005).
3. R. Smith and J. Coast, *The true cost of antimicrobial resistance*. *British Medical Journal*, **346**, f1493 (2013).
4. A.E. Edris, *Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: a review*. *Phytotherapy Research*, **21**, 308–323 (2007).
5. D. Kalemba and A. Kunicka, *Antibacterial and antifungal properties of essential oils*. *Current Medical Chemistry*, **10**, 813–829 (2003).
6. H.A.E. Shaaban, A.H. El-Ghorab and T. Shibamoto, *Bioactivity of essential oils and their volatile aroma components: Review*. *Journal Of Essential Oil Research*, **24**, 203–212 (2012).
7. M.G. Pimenov and M.V. Leonov, *The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to south-west Asian taxa*. *Turkish Journal of Botany*, **28**, 139–145 (2004).
8. R. Hegnauer, *Chemical Patterns and Relationships of Umbelliferae*. In: *The biology and chemistry of the Umbelliferae*. V.H. Heywood edn., Academic Press, London (1971).
9. M. El Beyrouthy, *Contribution à l'ethnopharmacologie libanaise et aux Lamiaceae du Liban*. Ph.D. Thesis, Université de Lille 2 (2008).

10. M. El Beyrouthy, N. Arnold, A. Delelis-Dusollier and F. Dupont, *Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon*. Journal of Ethnopharmacology, **120**, 315–334 (2008).
11. M. El Beyrouthy, *Contribution à l'ethnopharmacologie libanaise et aux Lamiaceae du Liban, Résumé de thèse*. Acta Botanica Gallica, **156**, 515–521 (2009).
12. Euro-Mediterranean plant diversity (Euro+Med) Website.
<http://ww2.bgbm.org/EuroPlusMed/> (25 August 2016)
13. P. Mouterde, *Nouvelle flore du Liban et de la Syrie*, pp. 640, Distribution Librairie Orientale Beyrouth, Liban (1983).
14. P. Mouterde *Nouvelle flore du Liban et de la Syrie*, pp. 618, Distribution Librairie Orientale Beyrouth, Liban (1983).
15. European Pharmacopoeia, Council of Europe Publishing, 3rd edn., Strasbourg (1997).
16. N.W. Davies, *Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20M phases*. Journal of Chromatography A, **503**, 1–24 (1990).
17. W. Jennings and T. Shibamoto, *Qualitative analysis of flavour and fragrance volatiles by glass capillary gas chromatography*. Academic Press, New York (1980).
18. R.P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th edn. Allured Publ. Corp., Carol Stream, IL (2007).
19. A. Dekebo , E. Dagne and O. Sterner, *Furanosesquiterpenes from Commiphora sphaerocarpa and related adulterants of true myrrh*. Fitoterapia, **73**, 48–55 (2002).
20. U. Mölleken, V. Sinnwell and K.H. Kubeczka, *Essential oil composition of Smyrniium olusatrum*. Phytochemistry, **49**, 1709–1714 (1998).

21. Clinical and Laboratory Standards Institute, *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*, Approved Standard, 8th edn. Document M7-A8, CLSI, Wayne, PA, USA (2009).
22. Clinical and Laboratory Standards Institute, *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi*, Approved Standard, 2nd edn. Document M38-A2, CLSI, Wayne, PA, USA (2008).
23. Clinical and Laboratory Standards Institute, *Reference method for broth dilution antifungal susceptibility testing of yeasts*, Approved Standard, 3rd edn. Document M27-A3, CLSI, Wayne, PA, USA (2008).
24. A.M.S. Rodrigues, P.N.E.T. Theodoro, C. Basset, M.R.R. Silva, J. Beauchêne, L.S. Espindola and D. Stien, *Search for Antifungal Compounds from the Wood of Durable Tropical Trees*. *Journal of Natural Products*, **73**, 1706–1707 (2010).
25. N. Baldovini, F. Tomi and J. Casanova, *Identification and quantitative determination of furanodiene, a heat-sensitive compound, in essential oil by ¹³C-NMR*. *Phytochemical Analysis*, **12**, 58–63 (2001).
26. F. Maggi, L. Barboni, F. Papa, G. Caprioli, M. Ricciutelli, G. Sagratini and S. Vittori, *A forgotten vegetable (*Smyrniolus atrum* L., Apiaceae) as a rich source of isofuranodiene*. *Food Chemistry*, **135**, 2852–2862 (2012).
27. P. Cos, A. Vlietinck and D. Vanden Berghe, *Anti-infective potential of natural products: How to develop a stronger in vitro “proof-of-concept”*. *Journal of Ethnopharmacology*, **106**, 290–302 (2006).
28. I. Jasicka-Misiak, J. Lipok, E.M. Nowakowska, P.P. Wieczorek, P. Młynarz and P. Kafarski, *Antifungal activity of the carrot seed oil and its major sesquiterpene compounds*. *Zeitschrift Naturforschung C*, **59**, 791–796 (2004).

Table 1. Composition of the essential oils of the four Apiaceae

					<i>D. carota</i> subsp. <i>maximus</i> Flowers EO	<i>F.</i> <i>elaeochytris</i> Fruits EO	<i>P. asperula</i> Fruits EO	<i>S. olusatrum</i> Flowering tops EO
	R _i ^a	R _i ^b	Compound ID	Identification ^c				
1	938	1076	α -Pinene	R _i , MS, CoGC	27.4	71.8	9.8	1.2
2	953	1076	Camphene	R _i , MS, CoGC	0.7	0.7	0.1	t
3	973	1132	Sabinene	R _i , MS, CoGC	-	-	29.8	-
4	980	1118	β -Pinene	R _i , MS, CoGC	3.1	6.8	-	0.2
5	993	1174	Myrcene	R _i , MS, CoGC	5.3	0.5	1.5	0.1
6	1005	1188	α -Phellandrene	R _i , MS, CoGC	t	t	8.0	0.1
7	1013	1159	δ -3-Carene	R _i , MS	t	-	1.7	0.2
8	1025	1280	<i>p</i> -Cymene	R _i , MS, CoGC	0.1	t	-	0.1
9	1030	1203	Limonene	R _i , MS, CoGC	3.1	0.4	-	-
10	1037	1218	β -phellandrene	R _i , MS, CoGC	-	-	19.2	1.7
11	1050	1253	<i>trans</i> - β -ocimene	R _i , MS	0.9	t	1.1	0.5
12	1057	1255	γ -Terpinene	R _i , MS, CoGC	0.1	t	3.2	0.2
13	1058	1556	<i>cis</i> -sabinene hydrate	R _i , MS	-	-	t	-
14	1075	-	<i>m</i> -Cresol	R _i , MS	-	-	1.2	-
15	1098	1553	Linalool	R _i , MS, CoGC	0.1	-	-	t
16	1140	1663	<i>cis</i> -Verbenol	R _i , MS	-	0.1	-	-
17	1176	1611	Terpinen-4-ol	R _i , MS	0.1	0.1	2.9	-
18	1182	1864	<i>p</i> -Cymen-8-ol	R _i , MS	-	t	-	-
19	1196	1804	Myrtenol	R _i , MS, CoGC	-	t	-	-
20	1217	1725	Verbenone	R _i , MS	-	t	-	-
21	1217	1845	<i>trans</i> -Carveol	R _i , MS	-	t	-	-
22	1215	1772	Citronellol	R _i , MS	-	-	-	t
23	1284	1597	Bornyl acetate	R _i , MS, CoGC	0.1	t	-	-
24	1227	1698	Myrtenyl acetate	R _i , MS	-	t	-	-
25	1352	1466	α -Cubebene	R _i , MS	-	t	-	-
26	1377	1497	α -Copaene	R _i , MS	0.2	0.1	t	t
27	1378	1600	β -Elemene	R _i , MS	0.4	0.3	t	0.9
28	1385	1535	β -Bourbonene	R _i , MS	0.1	-	-	0.1
29	1415	1612	β -Caryophyllene	R _i , MS, CoGC	2.9	-	t	0.4
30	1437	1628	Aromadendrene	R _i , MS	-	0.8	t	0.7
31	1452	1668	α -Humulene	R _i , MS	9.8	t	-	0.1
32	1455	1689	β -farnesene	R _i , MS	1.2	0.4	0.3	-
33	1463	1661	allo-Aromadendrene	R _i , MS	-	-	-	t
34	1477	1726	D-Germacrene	R _i , MS	7.0	0.7	0.3	2.9
35	1496	1743	Curzerene ^d	R _i , MS	-	-	-	31.5
36	1498	1744	α -Selinene	R _i , MS	0.7	-	0.3	-
37	1505	1758	α -Farnesene	R _i , MS	0.1	-	-	-
38	1508	1741	β -Bisabolene	R _i , MS	0.4	-	0.2	-
39	1510	1778	α -Bisabolene	R _i , MS	-	3.6	0.4	-
40	1526	1773	δ -Cadinene	R _i , MS	0.3	0.7	0.4	0.2
41	1566	2050	Nerolidol	R _i , MS	-	-	9.2	-
42	1577	2008	Caryophyllene oxide	R _i , MS, CoGC	0.2	-	-	-
43	1580	2152	Spathulenol	R _i , MS, CoGC	-	0.2	-	-
44	1593	2082	β -elemenone	R _i , MS	-	-	-	3.2
45	1595	2033	Carotol	R _i , MS	26.3	-	-	-
46	1640	2188	T-cadinol	R _i , MS	-	-	0.4	-
47	1643	2256	α -cadinol	R _i , MS	-	-	1.8	-
48	1688	2229	α -Bisabolol	R _i , MS, CoGC	0.4	4.3	3.1	2.5
49	1694	-	Furanodiene ^d	NMR	-	-	-	13.1
50	1704	2649	Furano-4(15)-eudesmen-1-one	R _i , MS	-	-	-	4.2
51	1875	2706	Furanoeremophil-1-one	NMR	-	-	-	28.5
52	1950	2614	Phytol	R _i , MS	0.1	-	-	-
53	1957	2993	Hexadecanoic acid	R _i , MS	0.1	-	0.2	-
			Monoterpene hydrocarbons		40.2	80.2	74.4	4.3
			Oxygenated monoterpenes		0.2	0.2	4.1	-
			Sesquiterpene hydrocarbons		23.1	6.6	1.9	5.3
			Oxygenated sesquiterpenes		26.9	4.5	14.5	5.7
			Furanosquiterpenes		-	-	-	77.3
			Other compounds		0.3	-	0.2	0
			Total identified		90.7	91.5	95.1	92.6

Notes: Bold numbers indicate the percentages higher than 2% to show main components; t = trace, less than 0.05.

^a Retention index on a HP-5MS column

^b Retention index on an Innovax column.

^c Ri Retention index identical to bibliography; MS: identification based on comparison of mass spectra. Co-GC: retention time identical to authentic compounds; NMR: comparison of NMR spectra with those reported in the literature.

^d Quantitative data are affected by thermal degradation.

Table 2. Antimicrobial activities (MIC in µg/ml) of the essential oils

Pathogens		EO							
		<i>Daucus carota</i> flowers	<i>Smyrniium</i> <i>olusatrum</i> fl. tops	<i>Ferula elaeochoytris</i> fruits	<i>Prangos asperula</i> fruits	Oxacillin	Gentamicin	Itraconazole	Fluconazole
<i>Bacteria</i> ^a	<i>S.a.</i> ATCC 29213	8	32	32	128	2	-	-	-
	<i>E.c.</i> ATCC 25922	> 512	> 512	> 512	> 512	-	8	-	-
<i>Yeasts</i> ^b	<i>C.a.</i> ATCC 10231	512	512	512	512	-	-	4	16
	<i>C.p.</i> ATCC 22019	> 512	> 512	> 512	512	-	-	0.5	2
	<i>C.n.</i> SNB-CN1	128	128	128	256	-	-	1	8
<i>Filamentous fungi</i> ^c	<i>A.f.</i> SNB-AF1	> 512	> 512	> 512	> 512	-	-	0.5	> 512
	<i>T.r.</i> SNB-TR1	32	64	128	64	-	-	0.03	2
	<i>T.v.</i> SNB-TV1	256	64	256	256	-	-	0.03	4
	<i>T.s.</i> SNB-TS1	64	32	128	128	-	-	0.03	4
	<i>T.t.</i> SNB-TT1	64	32	128	64	-	-	0.25	16
	<i>T.m.</i> SNB-TM1	32	32	64	128	-	-	0.125	64

^a *S.a.* = *Staphylococcus aureus*, *E.c.* = *Echerichia coli*, ^b *C.a.* = *Candida albicans*, *C.p.* = *Candida parapsilosis*, *C.n.* = *Cryptococcus neoformans*, ^c *A.f.* = *Aspergillus fumigatus*, *T.r.* = *Trichophyton rubrum*, *T.v.* = *Trichophyton violaceum*, *T.s.* = *Trichophyton soudanense*, *T.t.* = *Trichophyton tonsurans*, *T.m.* = *Trichophyton mentagrophytes*.

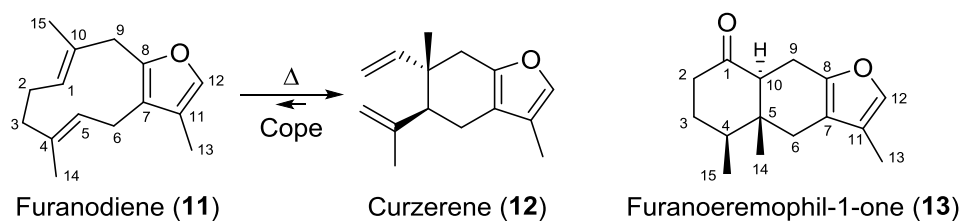


Figure 1. GC chromatograms of the four Apiaceae Eos with peak identification of the major compounds as listed in table 1.

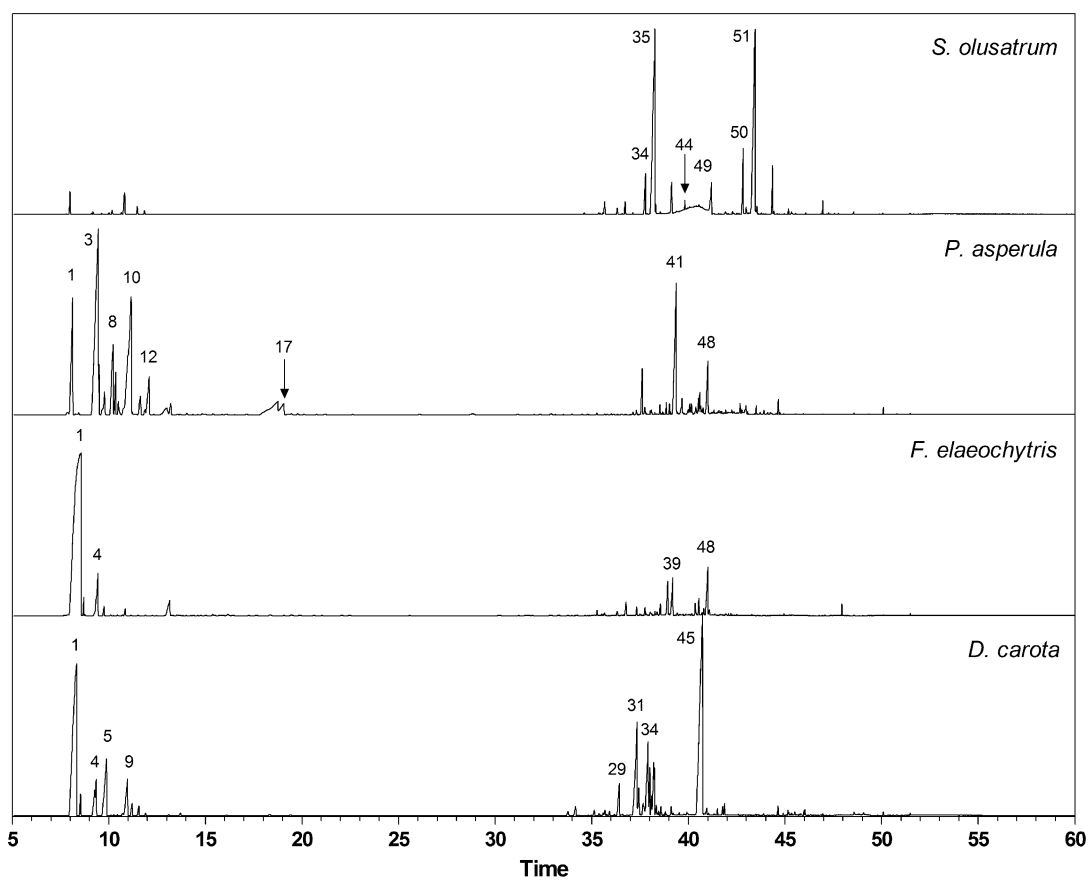


Figure 2. GC chromatograms of the four Apiaceae EOs with peak identification of the major compounds as listed in table 1.