Phytochemistry 72 (2011) 1406–1413

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Composition and antifungal activity of the essential oil from the rhizome and roots of *Ferula hermonis*

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ARTICLE INFO

Article history:
Received 24 February 2011
Received in revised form 19 April 2011
Available online 24 May 2011

Keywords:
Ferula hermonis
Essential oil
α-Pinene
3,5-Nonadiyne
Jaeschkeanadiol benzoate
Antifungal activity
Dermatophytes

ABSTRACT

The analysis of the essential oil from rhizome and roots of *Ferula hermonis* Boiss. (Apiaceae) by GC–FID, GC–MS and ^{13}C NMR allowed the identification of 79 constituents, more than 90% of the oil, the major one being α-pinene (43.3%), followed by α-bisabolol (11.1%) and the unusual acetylenic compound 3,5-nonadiyne (4.4%).

The antifungal activity of the essential oil before and after fractionation was assayed against several yeasts and filamentous fungi. Purification of the active fractions afforded 3,5-nonadiyne, α-bisabolol, jaeschkeanadiol angelate, α-bisabolol oxide B and *trans*-verbenol, as well as two purified fractions, one of them (JB73) with 73% of jaeschkeanadiol benzoate and the other with 50% of spathulenol. Determination of MIC and MFC values of all these products evidenced strong antifungal activities for JB73 and 3,5-nonadiyne. Particularly, against the dermatophyte *Tricophyton mentagrophytes*, MIC and MFC values were 0.25 μg/ml for JB73, and 8 μg/ml for 3,5-nonadiyne, the former being more active than amphotericin B and nystatin.

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1. Introduction

During the last decades the incidence of fungal infections in humans, in particular those involving skin and mucosal surfaces, has increased considerably, especially in tropical and subtropical countries, dermatophytes and *Candida* sp. being the most frequent pathogens. Furthermore, a dramatic increase of cases of immunocompromised patients which frequently develop opportunistic and superficial mycoses has been also reported in the last years (Pfaller and Diekema, 2007). Fungi produce serious invasive mycoses in individuals submitted to organ transplants or antineoplastic chemotherapy, those with the acquired immunodeficiency syndrome (AIDS), extremely aged persons and patients in intensive care units, among others (Mathew and Nath, 2009). Thus, fungal diseases have emerged as important public health problems with a high economic cost. Although several antimycotic drugs are available at present, its use is limited by a number of factors such as low potency, poor solubility, drug toxicity and emergence of resistant strains due in part to their intensive prophylactic use (Bastert et al., 2001). Therefore, despite the progress in human medicine, there is a distinct need for the discovery of alternative new, safer and more effective antifungal agents (Abad et al., 2007).

Plants used in Traditional Medicine usually constitute an important source of new biologically active compounds (Portillo et al., 2001; Svetaz et al., 2010). Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos et al., 2006). In recent years, many studies on the evaluation of antifungal activities of plant extracts have been carried out and a number of reports on new antifungal agents from plants have been reported (Aqil et al., 2010; Freixa et al., 2001; Portillo et al., 2005; Thevissen et al., 2007). From all of them, essential oils constitute one of the most promising groups, not only for their own activity but also because of the synergism when administered in combination with synthetic drugs, allowing a dose reduction of the latter (Pyun and Shin, 2006; Rosato et al., 2008).

The genus *Ferula* (Apiaceae), known as a good source of biologically active compounds, comprises about 170 species widely distributed throughout the Mediterranean area and Central Asia (Pimenov and Leonov, 1993). Many of them have been employed since antiquity as sources of scented gum-resins used either for medicinal or culinary purposes. Several species of *Ferula* are appreciated in traditional medicine for the treatment of skin infections, stomach disorders, fever, dysentery, hysterias and as aphrodisiac (Al-Yahya et al., 1998; Gamal-Eldeen and Hegazy, 2010).

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0031-9422/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2011.04.013

Hormonal effects and, more recently, cytotoxic and cancer preventing properties have been also investigated in plants of this genus (Lhuillier et al., 2005). Some of these activities have been related to sesquiterpene derivatives, mainly to those of the daucane type, such as ferutinin (Zanoli et al., 2005; Zavatti et al., 2006), a potent phytoestrogen which acts as an agonist for both estrogen receptors (ERα and ERβ) (Ikeda et al., 2002).

Particularly, *F. hermonis* Boiss., a perennial shrub that grows on the Hermon mountain, between Lebanon and Syria commonly known as "Shirsh-el-Zallouh" which means hairy root, is used in the Middle East to improve sexual behaviour in the treatment of frigidity and impotence (Auzi et al., 2008; El-Taher et al., 2001; Lev and Amar, 2002). Although some papers dealing on the sesquiterpene composition of different extracts (Al-Shae'r et al., 2001; Auzi et al., 2008; Diab et al., 2001a,b; Galal, 2000; Galal et al., 2001; Lhuillier et al., 2005) as well as on its antibacterial and insecticidal activities (Al-Nahar et al., 2006; Al-Shae'r et al., 2001; Galal et al., 2001; Hilan et al., 2007) have been previously published, little is known about the composition of its essential oil and its antifungal activity. Only seven constituents (meaning *ca.* 61% of the oil) were identified by Al-Nahar et al. (2006) in a GC–MS analysis of the essential oil from root, α-bisabolol (23.0%) and α-farnesene (12.7%) being the major ones. A slight activity against *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum* was also reported. On the other hand, Hilan et al. (2007), based on a GC–FID analysis and correlation of the retention times with those of pure standards, showed α-pinene (31%) as the main constituent of the essential oil from the root of *F. hermonis*.

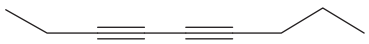
With the aim of searching for new antifungal agents and to potentiate the use of natural resources, the present work deals with the study of the essential oil from the rhizome and roots of *F. hermonis*, in particular its chemical composition and antifungal activity against several strains in view of characterising the active constituents.

2. Results and discussion

The rhizome and roots of *Ferula hermonis* submitted to hydrodistillation for 3.5 h gave an essential oil yield of 1.5% (v/w). A first screening of the antifungal activity of the oil by the paper disk agar diffusion method evidenced antimycotic activity against several fungi strains, which was confirmed by determination of the MIC values, ranging from 32 to 128 μg/ml (Table 1). The strongest activity was demonstrated against the dermatophytes *T. mentagrophytes* and *M. gypseum*, the latter showing the same MIC and MFC values (32 μg/ml). MIC and MFC were also coincident for *C. lactis-condensi* (64 μg/ml), *C. albicans* (128 μg/ml) and *S. cerevisiae* (128 μg/ml). The MFC values for *T. mentagrophytes*, *P. purpurogenum*, *A. niger* and *A. fumigatus* were about two-fold the MIC values.

Bioguided fractionation of the essential oil was performed with two aims: improvement of the identification of its constituents through GC–FID, GC–MS and ^{13}C NMR analyses of the fractions obtained, and assessment of the fractions and/or the constituents responsible for the antifungal activity. Preparative column chromatography using silicagel 60 as stationary phase eluted with a step gradient of hexane-EtOAc afforded 27 fractions.

GC, GC–MS and ^{13}C NMR analysis of the total oil and the fractions obtained by CC allowed the identification of 79 constituents, meaning more than 90% of the total sample (Table 2). From them, 55 compounds were identified as sesquiterpenes, whereas only 21 were monoterpenes. The major constituent was α-pinene (43.3%), followed by α-bisabolol (11.1%), compound **1** (4.4%), β-farnesene (3.2%) and δ-cadinene (2%).



1

The identity of compound **1**, which was the major constituent of the active fraction 10, could not be definitely assigned from the retention indices, the MS and the ^{13}C NMR spectrum, so further fractionation was needed in order to isolate this constituent whose structure was finally elucidated as 3,5-nonadiyne (**1**). This unusual acetylenic compound has only been reported twice before, in the essential oils from the roots of *Selinum tenuifolium* (Dev et al., 1984) and *Cachrys ferulacea* (Dokovic et al., 2004) (Apiaceae) where it reached percentages higher than 80%. 3,5-Nonadiyne has been found to selectively inhibit endogenous nitric oxide

Table 1
Antifungal activity of essential oil from the rhizome and roots of *Ferula hermonis* and some of its major constituents.

Fungi strains[a]		Samples[b]									Positive controls[c]			
		EOFH	αP	Nd	αB	αBO	tV	JA90	JB73	S50	A	N	K	C
C.a.	MIC[d]	128	128	>256	64	128	128	128	128	64	1	4	>8	8
	MFC[e]	128	128	>256	64	128	128	>256	128	64	1	2	>8	>8
C.l.	MIC	64	64	>256	64	64	128	128	128	32	0.5	2	0.25	0.125
	MFC	64	64	>256	64	128	128	128	128	64	1	2	0.5	0.25
S.c.	MIC	128	128	>256	128	128	128	128	128	128	4	8	0.125	0.125
	MFC	128	128	>256	128	128	128	128	128	128	>8	>8	2	2
T.m.	MIC	32	64	8	16	64	32	32	0.25	32	0.5	2	0.25	0.0625
	MFC	64	64	8	32	64	32	32	0.25	32	0.5	2	0.25	0.0625
M.g.	MIC	32	32	8	16	64	32	32	64	32	1	4	8	0.125
	MFC	32	32	16	16	128	32	64	128	32	1	8	>8	0.25
A.n.	MIC	64	128	>256	64	128	128	128	128	64	1	4	>8	2
	MFC	128	128	>256	128	128	128	>256	256	128	1	4	>8	2
A.f.	MIC	128	128	>256	64	128	128	128	128	64	1	4	8	1
	MFC	256	128	>256	128	128	128	>256	256	256	2	4	8	1
P.p.	MIC	64	>256	>256	>256	>256	>256	64	>256	32	2	4	4	2
	MFC	128	>256	>256	>256	>256	>256	128	>256	32	8	8	4	2

[a] *C.a.: Candida albicans; C.l.: C. lactis-condensi; S.c.: Saccharomyces cerevisiae; T.m.: Tricophyton mentagrophytes; M.g.: Microsporum gypseum; A.n.: Aspergillus niger; A.f.: A. fumigatus; P.p.: Penicillium purpurogenum.*

[b] EOFH: Essential oil of *Ferula hermonis*; αP: α-pinene; Nd: 3,5-nonadiyne; αB: α-bisabolol; αBO: α-bisabolol oxide B; tV: *trans*-verbenol; JA90: jaeschkeanadiol angelate 90%; JB73: jaeschkeanadiol benzoate 73%; S50: spathulenol 50%.

[c] **A**: amphotericin B; **N**: nistatyn; **K**: ketoconazole; **C**: clotrimazole.

[d] MIC: Minimum inhibitory concentration (μg/ml).

[e] MFC: Minimum fungicidal concentration (μg/ml).

Table 2
Composition of the essential oil from the rhizome and roots of *Ferula hermonis*.

Components	Retention indices[a]				%	Identification methods[b]
	A	B	C	D		
α-Pinene	1021	935	111	214	43.4	A, B, C, D, E, F
Camphene	1052	939	122	216	0.2	A, B, C, D, E
Hexanal	1091	<800	131	116	0.1	A, B, C, D, E
β-Pinene	1102	963	140	230	1.4	A, B, C, D, E, F
Verbenene	1116	942	150	218	0.4	A, B, C, D, E, F
3-Carene	1137	995	165	247	0.4	A, B, C, D, E, F
β-Myrcene	1159	980	180	239	0.2	A, B, C, D, E
Limonene	1189	1013	201	256	0.2	A, B, C, D, E, F
p-Cymene	1263	1004	239	251	0.2	A, B, C, D, E
3,5-Nonadiyne	1433	1059	322	278	4.4	A, B, C, D, E, F
α-Cubebene	1445	1354	328	419	0.2	A, B, C, D, E, F
α-Copaene	1476	–	344	–	0.3	A, C, E, F
Daucene	1478	–	345	–	0.4	A, C, E, F
α-Gurjunene	1514	1443	361	461	t	A, B, C, D, E
γ-Gurjunene	1533	1605	369	554	0.3	A, B, C, D, E
Aristolene	1552	–	379	–	0.2	A, C, E, F
Pinocarvone	1554	1135	381	316	0.2	A, B, C, D, E, F
cis-α-Bergamotene	1558	–	381	–	t	A, C, E
Bornyl acetate	1567	–	388	–	t	A, C, E
trans-α-Bergamotene	1575	–	390	–	0.4	A, C, E, F
β-Elemene	1579	1365	391	422	0.3	A, B, C, D, E
β-Gurjunene	1584	1369	394	435	0.1	A, B, C, D, E, F
Terpinen-4-ol	1587	1158	398	321	t	A, B, C, D, E, F
Aromadendrene	1590	1400	400	442	1.2	A, B, C, D, E, F
Myrtenal	1611	–	405	–	0.3	A, C, E, F
trans-Pinocarveol	1640	1110	416	303	0.5	A, B, C, D, E, F
cis-Verbenol	1643	–	417	–	0.6	A, C, E, F
trans-β-Farnesene	1660	1433	424	455	3.2	A, B, C, D, E, F
trans-Verbenol	1666	1121	426	307	1.9	A, B, C, D, E, F
β-Cubebene	1669	1459	428	467	t	C, D, E
γ-Muurolene	1676	–	429	–	0.3	A, C, E, F
Ledene	1677	–	430	–	0.2	A, C, E, F
α-Terpineol	1684	1169	432	324	t	A, B, C, D, E, F
Verbenone	1686	1167	434	332	1.0	A, B, C, D, E, F
β-Selinene	1704	1452	439	465	0.5	A, B, C, D, E, F
α-Selinene	1709	1462	442	472	0.4	A, B, C, D, E, F
α-Muurolene	1713	1466	444	475	0.3	A, B, C, D, E, F
β-Bisabolene	1717	1482	447	483	1.1	A, B, C, D, E, F
Bicyclogermacrene	1717	1462	447	472	1.0	A, B, C, D, E, F
γ-Cadinene	1737	1482	461	483	1.7	A, B, C, D, E, F
δ-Cadinene	1737	1500	461	491	1.5	A, B, C, D, E, F
Selina-3,7(11)-diene	1748	–	469	–	t	A, C, E, F
ar-Curcumene	1756	1450	472	465	0.6	A, B, C, D, E, F
trans-α-Bisabolene	1756	1522	472	508	0.5	A, B, C, D, E, F
α-Cadinene	1768	–	478	–	t	A, C, E, F
Myrtenol	1776	–	483	–	0.3	A, C, E, F
Germacrene B	1800	–		–	t	A, E
cis-Calamenene	1808	–	506	–	t	A, C, E
trans-Calamenene	1813	–	507	–	t	A, C, E, F
trans-Carveol	1828	1189	514	342	0.2	A, B, C, D, E, F
Thymyl acetate	1847	–	524	–	t	A, C, E
α-Calacorene	1891	1515	551	500	0.3	A, B, C, D, E, F
α-Copaene-11-ol	2031	–	611	–	0.4	A, C, E
cis,trans-Farnesol	2037	1539	613	526	0.4	A, B, C, D, E
trans-Dauc-8-en-4β-ol	2039	–	614	–	t	A, C, E, F
Epicubenol	2041	–	615	–	t	A, C, E, F
Germacradien-11-ol	2043	–	617	–	0.2	A, C, E, F
Globulol	2045	–	618	–	t	A, C, E
Viridiflorol	2050	1549	620	525	0.1	A, B, C, D, E, F
Cubenol	2065	–	624	–	t	A, C, E
Bisabolol oxide	2090	–	641	–	0.9	A, C, E
Spathulenol	2100	1542	646	521	1.1	A, B, C, D, E, F
α-Bisabolol oxide B	2107	1623	649	564	1.0	A, B, C, D, E, F
T-Cadinol	2148	1612	668	559	0.4	A, B, C, D, E, F
T-Muurolol	2164	1612	675	563	0.1	A, B, C, D, E, F
α-Bisabolol	2195	1658	693	580	11.1	A, B, C, D, E, F
α-Cadinol	2207	–	700	–	0.1	A, C, E, F
β-Eudesmol	2207	–	702	565	t	A, C, D, E
Myristicin	2224	1462	705	502	0.3	A, B, C, D, E
α-Humulen-10-ol	2280	1618	730	562	1.3	A, B, C, D, E, F
Jaeschkeanadiol angelate	>2300	–	866	–	0.1	C, E, F
Jaeschkeanadiol	>2300	1721	872	609	1.9	B, C, D, E, F
Jaeschkeanadiol benzoate	>2300	>2300	>1000	910	1.2	D, E, F

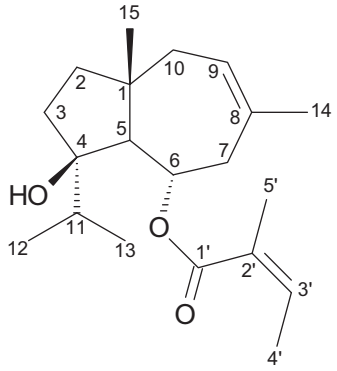
Table 2 (*continued*)

Components	Retention indices[a]				%	Identification methods[b]
	A	B	C	D		
p-Cymene-8-ol[c]	–	1154	–	325	0.4	B, D, E
γ-Selinene[c]	–	1350	–	417	0.1	B, D, E
Selina-4,11-diene[d]	–	–	–	–	t	F
β-Bisabolol[d]	–	–	–	–	t	F
Cadinadien-8-ol[d]	–	–	–	–	t	F
Selin-11-en-4α-ol[d]	–	–	–	–	t	F
Monoterpene hydrocarbons					46.4	
Oxygenated monoterpenes					5.5	
Sesquiterpene hydrocarbons					15.4	
Oxygenated sesquiterpenes					20.7	
Others					4.8	
Total identified					92.8	

Compounds listed by elution order in the polar column except the last six constituents.
t: traces (⩽0.05%).
[a] A: *n*-Alkane indices in SupelcowaxTM-10; B: *n*-Alkane indices in SPB-1™; C: FAME indices in Supelcowax™-10; D: FAME indices in SPB-1™.
[b] Identification methods: E: GC–MS, F: ^{13}C-NMR analysis of the total oil and/or after fractionation.
[c] Constituents only detected in the SPB-1™ column.
[d] Constituents identified from the ^{13}C NMR shift data after fractionation of the essential oil.

release (IC_{50} = 6.7 ± 0.8 μM) by rat peritoneal macrophages at doses that do not inhibit T cell proliferation, activity that has been related to the highly lipophilic nature of the compound (Dokovic et al., 2004).

It should be noted that some daucane sesquiterpene derivatives were identified in the essential oil investigated, in particular: jaeschkeanadiol (ferutinol), jaeschkeanadiol benzoate (teferidin) and jaeschkeanadiol angelate (**2**), which were identified from their MS and ^{13}C NMR spectral data. To our knowledge, in the case of jaeschkeanadiol angelate (**2**), it is the first time that this daucane ester is described in *F. hermonis* and that its ^{13}C NMR chemical shifts are reported. This sesquiterpene was firstly isolated from *Ferula elaeochytris* (Miski et al., 1983) and later on reported in other *Ferula* sp.



2

A number of papers dealing on the composition of the essential oils from different plant parts of *Ferula* sp. can be found in the literature, from which only two concerned preliminary analysis of the volatile oil from root of *F. hermonis* (Al-Nahar et al., 2006; Hilan et al., 2007). Particularly, in the essential oil from roots different composition patterns have been reported depending on the species. In some of them, like in our sample from *F. hermonis*, the main constituents identified were monoterpene hydrocarbons, such as α-pinene in *F. penninervis* (80.2%) (Goryaev et al., 1971) and *F. hermonis* (Hilan et al., 2007) (31%), and β-pinene in *F. galbaniflua* (58.8%) (Rustaiyan et al., 2002). Other compounds reported as major components in the volatile oil from subterranean parts were oxygenated monoterpenes like methyl thymol (41.2%) in *F. oopoda* (Karim et al., 1979), sesquiterpene hydrocarbons such as E-β-farnesene (8.4%) and α-zingiberene (6.9%) in *F. glauca* (Maggi et al., 2009), sulphur-containing compounds (25.8%) in *F. persica* (Iranshahi et al., 2006) as well as phenylpropanoids like myristicin in *F. oopoda* and *F. persica* (11.2% and 8.9%, respectively) or elemicin (9%) in *F. glauca*.

α-Pinene has also been reported as major constituent in the essential oils from aerial parts of several species of *Ferula*, particularly from the flowering tops and seeds of *F. hermonis* (Hilan et al., 2007).

The agar overlay bioautographic assay of the 27 fractions showed growth inhibition zones of different intensities in the case of fraction 2, fraction 10, and from fraction 17–27. In fraction 2 the activity was directly related to α-pinene, the major constituent of the oil, whereas 3,5-nonadiyne (**1**) was the main active constituent of fraction 10. Table 3 shows the main constituents of the active fractions 17–27. A new fractionation of a higher amount of essential oil was needed in order to isolate the major constituents of these fractions. In this way, α-bisabolol, α-bisabolol oxide B, *trans*-verbenol, jaeschkeanadiol angelate (**2**), and two purified fractions one of them (JB73) with 73% of jaeschkeanadiol benzoate and the other with 50% of spathulenol were obtained.

The antifungal activities of these constituents, as well as those of α-pinene and 3,5-nonadiyne (**1**) were determined against three yeasts and five filamentous fungi strains. Results are shown in Table 1. The human pathogen dermathophytes assayed, *M. gypseum* and *T. mentagrophytes*, were the most sensitive strains, MIC and MFC values ranging from 0.25 to 128 μg/ml. The most active products were found to be JB73, 3,5-nonadiyne (**1**) and α-bisabolol.

The most potent activity was demonstrated by JB73 against *T. mentagrophytes*, with the same MIC and MFC values of 0.25 μg/ml, equivalent to that one of ketoconazole (0.25 μg/ml) and superior to amphotericin B (0.5 μg/ml) and nystatin (2 μg/ml). On the contrary, the activity of this constituent against *M. gypseum* was quite lower, with MIC and MFC values of 64 and 128 μg/ml, respectively. Both dermatophytes were highly sensitive to 3,5-nonadiyne whose MIC and MFC's for *T. mentagrophytes* were 8 μg/ml, whereas in the case of *M. gypseum* MFC value was about two-fold the MIC value (8 and 16 μg/ml, respectively). 3,5-Nonadiyne did not show any activity against the other strains assayed. In the case of α-bisabolol, fungicidal and fungistatic activity against both dermatophyte strains was exhibited between 16 and 32 μg/ml.

α-Pinene, α-bisabolol oxide B, *trans-verbenol*, jaeschkeanadiol angelate (**2**) and the fraction with 50% spathulenol evidenced a

Table 3
Main constituents (percentages ⩾ 1%) of the active fractions of the essential oil from the rhizome and roots of *Ferula hermonis*.

Constituents	% in Fractions										
	17	18	19	20	21	22	23	24	25	26	27
cis-Verbenol	–	–	–	–	–	–	17.5	4.2	–	–	–
Unidentified oxygenated monoterpene	–	–	–	–	–	–	5.2	6.0	–	–	–
trans-Pinocarveol	–	–	–	–	12.8	14.6	–	–	–	–	–
trans-Verbenol	–	–	–	–	–	–	–	–	2.9	68.5	99.7
Verbenone	–	–	–	–	–	–	–	5.2	22.8	19.6	–
p-Mentha-1,5-dien-8-ol	–	–	–	–	–	–	–	7.3	17.2	5.9	–
Myrtenol	–	–	–	–	–	–	–	8.0	4.6	–	–
trans-Carveol	–	–	–	–	–	–	3.9	7.7	3.6	–	–
α-Copaene-11-ol	–	–	–	–	–	15.9	14.2	–	–	–	–
Germacradien-11-ol	–	–	–	1.7	2.3	–	–	–	–	–	–
Globulol	–	–	–	–	–	–	–	3.6	4.6	–	–
Viridiflorol	–	–	–	–	1.7	–	–	–	–	–	–
Spathulenol	–	–	–	22.8	33.3	2.5	–	–	–	–	–
α-Bisabolol oxide B	–	–	–	–	–	27.5	15.0	–	–	–	–
T-Cadinol	–	1.0	14.3	–	–	–	–	–	–	–	–
T-Muurolol	–	–	–	3.3	2.6	–	–	–	–	–	–
α-Bisabolol	75.7	93.6	66.2	–	–	–	–	–	–	–	–
α-Cadinol	–	–	–	–	–	–	–	6.7	4.7	–	–
β-Eudesmol	–	–	–	2.0	1.4	–	–	–	–	–	–
Cadinadien-8-ol	–	–	4.2	–	–	–	–	–	–	–	–
Selin-11-en-4α-ol	–	–	–	–	–	–	3.4	5.1	–	–	–
Unidentified oxygenated sequiterpene	–	–	–	–	–	–	21.9	22.7	–	–	–
α-Humulen-10-ol	–	–	–	47.8	35.9	–	–	–	–	–	–
Jaeschkeanadiol	–	–	–	–	–	–	–	–	15.8	5.9	–
Jaeschkeanadiol benzoate	20.7	–	–	–	–	–	–	–	–	–	–
Total	96.4	94.6	84.7	77.6	90.0	60.5	81.1	76.5	76.2	99.9	99.7

moderate activity against *T. mentagrophytes* and *M. gypseum* with MIC and MFC values ranging from 32 to 64 μg/ml, except in the case of α-bisabolol oxide B whose MFC against *M. gypseum* was 128 μg/ml. These values were comparable to the ones exhibited by the total essential oil against the same strains.

Furthermore, the MIC value against *C. lactis-condensi* and the MIC and MFC values against *P. purpurogenum* for the fraction with 50% spathulenol were 32 μg/ml.

The MIC and MFC values determined against the other fungi strains assayed ranged between 64 and >256 μg/ml, *P. purpurogenum* being the most resistant strain.

3. Conclusion

In conclusion, the composition and antifungal activity of the essential oil from *Ferula hermonis* evidence this species as a potential source of interesting antifungal agents. The exhaustive analysis of the essential oil combined with successive bioguided fractionations afforded different active products among which 3,5-nonadiyne (**1**), α-bisabolol and a fraction with 73% jaeschkeanadiol benzoate were the most active against the dermathophytes *M. gypseum* and *T. mentagrophytes*. Particularly, the fraction with the latter compound showed the strongest activity with MIC and MFC values superior or equivalent to those of the positive controls.

4. Experimental

4.1. General experimental procedures

Silica gel 60 (35–70 μm, SDS, France) was used for preparative column chromatography (CC), Sephadex® LH-20 (Pharmacia Biotech, Sweden) was used for separation and purification on CC and precoated silica gel 60 F_{254} plates (SDS, France) were used for TLC.

NMR spectra of **1** were recorded in $CDCl_3$, on a Varian VNMRS 400 MHz spectrometer using tetramethylsilane (TMS) as internal standard. MS were obtained with Agilent Technologies 5973 and 5975 Inert XL mass spectrometers.

NMR spectra of the essential oil and fractions were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer, equipped with a 5 mm probe, in deuterated chloroform, with all shifts referred to internal TMS.

Sabouraud dextrose agar (Panreac, Spain) and Sabouraud dextrose broth (Adsa, Spain) were used as culture media for the antifungal assays. Sterile paper disks were provided by Difco (diameter: 13 mm, Ref. 1571-33). Nystatin, amphotericin B, ketoconazole and clotrimazole were purchased from Sigma Chemical (USA).

4.2. Plant material

Air-dried rhizome and roots of *Ferula hermonis* Boiss. (Apiaceae) were provided by The Jordanian Pharmaceutical Manufacturing Co. (Naor, Jordan). Voucher specimens were identified and deposited at the Herbarium of the University of Barcelona under No. BCN 51579.

4.3. Isolation of the essential oil

The essential oil was obtained by hydrodistillation of plant material for 3.5 h, using a Clevenger apparatus, according to the standard procedure described in the European Pharmacopeia (Council of Europe, 2007). It was as dried over anhydrous Na_2SO_4, filtered and stored at 4 °C till use.

4.4. Fractionation of the essential oil

Essential oil (3.3 g) of *F. hermonis* were submitted to fractionation by preparative column chromatography of silicagel 60 (60 × 4.5 cm) eluted with a gradient of hexane-EtOAc from (100:0) to (50:50). Twenty-seven fractions were obtained and submitted to GC–FID, GC–MS and ^{13}C NMR analyses as well as to antifungal assays.

4.5. Isolation and identification of 3,5-nonadiyne (**1**)

Successive (3x) fractionation of 10.1 g of essential oil through silicagel 60 eluting with a gradient of hexane-EtOAc from (100:0) to (50:50), followed by final purification through Sephadex® LH-20 eluted with hexane-CH_2Cl_2 (50:50), afforded 17.8 mg of the unknown compound **1** which represented 4.4% of the total essential oil.

Structure elucidation of **1** was achieved using standard spectroscopic techniques: ^{1}H NMR, ^{13}C NMR, DEPT, EI–MS and CI–MS. For complete assignments of ^{1}H and ^{13}C NMR signals, COSY, HMBC and HSQC 2D-NMR methods were applied.

4.6. Isolation of major constituents of active fractions

Essential oil (20 g) of *F. hermonis* were fractionated over silicagel 60 (45 × 8 cm) using hexane-EtOAc from (100:0) to (50:50) as mobile phase affording 28 fractions which were analyzed by GC and GC–MS.

Fractions 12–14 were joined (1.56 g) and submitted to fractionation through silicagel (80 × 1.4 cm) with hexane: EtOAc from (100:0) to (50:50) yielding 748.3 mg of α-bisabolol.

Fractions 15–19 (585.5 mg) were chromatographed over silicagel (100 × 2 cm) with the same gradient as above. Ten fractions were obtained, from which fraction III afforded 8 mg of jaeschkeanadiol angelate (**2**). Fraction V (6.2 mg) was mainly constituted by spathulenol (50.5%) and T-muurolol (29%). Successive fractionation of fractions VI and VII (154 mg) over silicagel eluting with CH_2Cl_2 gave 15.2 mg of α-bisabolol oxide B and a fraction (JB73, 16.2 mg) with 73% of jaeschkeanadiol benzoate, 16% of α-bisabolol oxide B and 10% of eudesmol.

Finally, fraction 21 (420 mg) gave, after successive fractionation over silicagel (70 × 1 cm) using CH_2Cl_2 as mobile phase, 16.7 mg of *trans*-verbenol.

Prior to the GC–FID and GC–MS analyses, as well as to the antifungal assays, all products were submitted to a final purification over Sephadex® LH-20 eluting with hexane.

4.7. GC–FID and GC–MS analysis

The essential oil and its fractions were analysed by GC–FID and GC–MS using two fused silica capillary columns (60 m × 0.25 mm i.d.; 0.25 μm film thickness) of different stationary phases: Supelcowax™ 10 and SPB-1™. GC–FID analyses were performed on a Hewlett–Packard 6890 instrument, equipped with a HP ChemStation data processor software, using the following analytical conditions: carrier gas, Helium; flow rate, 1 ml/min; oven temperature programmed from 50 °C (initial isotherm of 8 min) to 120 °C (isotherm of 10 min) and to 230 °C (final isotherm of 17 min) at 4 °C/min; injector temperature, 250 °C; detector temperature, 270 °C; split ratio 1:80. The complexity of the composition of the essential oil, mainly of the sesquiterpene fraction, required a two step oven temperature program in order to achieve a good resolution, avoiding overlapping of the peaks as far as possible. The essential oil was injected undiluted (0.1 μl). Mass spectra were obtained with a computerized system constituted by a GC Hewlett–Packard 6890 coupled to a mass selective detector Hewlett–Packard 5973N, using the same analytical conditions as above. MS was operated in the EI mode at an ionizing voltage of 70 eV over an *m/z* range from 35 to 400 amu.

4.8. ^{13}C NMR analysis of the essential oil and fractions

^{13}C NMR spectra of the essential oil or the fractions obtained by column chromatography were recorded with the following parameters: pulse width = 4 μs (flip angle 45°); acquisition time = 2.7 s for 128 K data table with a spectral width of 25,000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 for each sample (20–40 mg of the sample depending of the available amount, in 0.5 mL of $CDCl_3$).

4.9. Identification and quantification

Identification of components was achieved from the data obtained from the analyses of the essential oil and the column chromatography fractions by means of:

(a) Comparison of their GC linear retention indices (RI) in two stationary phases, determined in relation to a homologous series of *n*-alkanes (8–23 carbons) and a homologous series of fatty acid methyl esters (FAME indices), with those of authentic compounds or literature data.
(b) Comparison of fragmentation patterns in the mass spectra with those stored in our own library or in the GC–MS mass spectral library.
(c) Comparison of the signals in the ^{13}C NMR spectra of the essential oil and fractions with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi and Casanova, 2006).

Quantification of each compound was performed on the basis of their GC peak areas on the two columns, using the normalisation procedure without corrections for response factor.

4.10. Spectral data of jaeschkeanadiol angelate (**2**)

^{13}C NMR ($CDCl_3$): δ 15.81 (C-4′), 17.46 (C-13), 18.51 (C-12), 20.12 (C-15, 5′), 26.45 (C-14), 31.61 (C-3), 37.03 (C-11), 40.95, 41.30, 41.52 (C-2,7,10), 43.38 (C-1), 59.94 (C-5), 70.35 (C-6), 86.21 (C-4), 125.20 (C-9), 127.84, 138.93 (C-2′,3′), 133.56 (C-8), 168.35 (C-1′). EIMS (GC–MS) 70 eV, *m/z* (rel. int.): 277 $[M–C_3H_7]^+$ (26), 220 $[M–C_5H_7O_2–H]^+$ (3), 177 $[M–C_3H_7–C_5H_7O_2–H]^+$ (99), 159 $[M–C_3H_7–C_5H_7O_2–H–H_2O]^+$ (90), 134 (58), 121 (40), 83 $[C_5H_7O]^+$ (100), 55 $[C_5H_7O–CO]^+$ (62).

4.11. Antifungal activity

The antifungal activity of the essential oil as well as of its fractions and major constituents was assayed against several yeasts and filamentous fungi strains.

4.11.1. Microorganisms, growth conditions, and preparation of fungal inocula

Aspergillus fumigatus CECT 2071, *A. niger* CECT 2574, *Candida albicans* CECT 1394, *C. lactis.condensi* CECT 1075, *Microsporum gypseum* CECT 2908, *Penicillium purpurogenum* CECT 20436, *Saccharomyces cerevisiae* CECT 1324 and *Trichophyton mentagrophytes* CECT 2795 were provided by the Colección Española de Cultivos Tipo (CECT), Valencia, Spain. Strains were grown on Sabouraud dextrose agar plates for 24 h at 30 °C. Cell suspensions of yeasts in sterile NaCl 0.9% were adjusted at 0.5 McFarland to obtain approximately 10^6 CFU/ml. Spore suspensions of dermatophytes were adjusted at 10^6 CFU/ml by plating 10 μl of 4- and 5-times serially diluted suspensions and counting germinated spores/dilution.

4.11.2. Paper disk-agar diffusion method

Antimycotic activity the essential oil of *F. hermonis* was first evaluated by the paper disk-agar diffusion method (Barry and Thornsberry, 1991). Test plates (diameter: 15 cm) were prepared with Sabouraud dextrose agar medium and inoculated in surface

with a cell suspension in sterile dissolution of 0.9% saline in the case of yeasts, and with a spore suspension in Tanquay buffer in case of filamentous fungi. In both cases, the concentration was adjusted to 10^6 CFU/ml.

Sterile paper disks impregnated with 100 µl of extract dilutions at concentrations of 50 and 100 mg/ml, as previously reported (Portillo et al., 2001), or with 20 µl of a solution of 1 mg/ml of amphotericin B or nystatin as standard compounds, were applied over the test plates. The diameters of the growth inhibition zones around each disk were measured after incubation at 30 °C for 48 h. For each extract, 3 replicate trials were conducted against each fungus.

4.11.3. Overlay bioautographic method

A bioautographic agar overlay method was used for the detection of the antifungal activity of the fractions (Rahalison et al., 1991). Developed TLC plates of each fraction on precoated silica gel 60 F_{254} plates eluted with hexane-EtOAc (6:4) and (9:1) or CH_2Cl_2:MeOH (98:2) were placed over an inoculated Sabouraud dextrose agar medium (test plates dimensions: 12 × 12 cm), during 1 h at 4 °C. After removing TLC, test plates were incubated at 30 °C for 48 h to visualize growth inhibition. Assays were performed against *T. mentagrophythes* and *M. gypseum*.

4.11.4. Minimal inhibitory concentration (MIC)

The MIC was determined by a standard two-fold dilution technique (Murray et al., 1999). Sabouraud dextrose broth was used as medium for yeasts and dermatophytes.

Samples were first dissolved in dimethylformamide (2.56 mg/ml) and the fungistatic activity was determined by measuring the minimum inhibitory concentration (MIC) from diluted aqueous samples of 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/ml. All the experiments were performed in triplicate and the results expressed as mean values. Nystatin, amphotericin B, ketoconazole and clotrimazole were included (at an initial concentration of 0.8 mg/ml) as positive antifungal controls.

4.11.5. Minimal fungicidal concentration (MFC)

The MFC was established technically by extending the MIC (microdilution method) (Balues et al., 1991). Mixtures of the fungus with samples which in MIC studies showed no visible growth in the last three wells after 48 h of incubation were subcultured onto Sabouraud dextrose agar medium plates using an inoculum of 0.01 ml. Plates were incubated at 37 °C for 48 h. The MFC was regarded as the lowest concentration that prevented the growth of any fungal colony on the solid medium.

Acknowledgements

Authors are grateful to AA Badwan (The Jordanian Pharmaceutical Manufacturing Co., Naor, Jordan) and TS El-Thaher (ARAGEN Biotechnology, Naor, Jordan) for providing plant material, and to Prof. M. Bou Dagher Kharrat (Département Sciences de la Vie et de la Terre, Université Saint Joseph, Beyrouth, Lebanon) for the botanical identification. Thanks are also due to the Fundación María Francisca de Roviralta (Barcelona, Spain) for financial support.

References

Abad, M.J., Ansuategui, M., Bermejo, P., 2007. Active antifungal substances from natural sources. Arkivoc 7, 116–145.

Al-Nahar, H.A., Ismaeil, A., Bishr, M.M., Gaafar, M.M., Abbass, A.A., 2006. GC–MS analysis and antimicrobial activity of the essential oil from *Ferula harmoni* L. (Zaloo). Alex. J. Pharm. Sci. 20, 17–19.

Al-Shae'r, M., Darwish, R.M., Aburjai, T., 2001. Antimicrobial sesquiterpene from the roots of *Ferula harmonis*. Acta Technologiae et Legis Medicamenti 12, 255–264.

Al-Yahya, M.A., Muhammad, I., Mirza, H.H., El-Feraly, F.S., 1998. Antibacterial constituents from rhizomes of *Ferula communis*. Phytother. Res. 12, 335–339.

Aqil, F., Zahin, M., Ahmad, I., Owais, M., Khan, M.S.A., Bansal, S.S., Farooq, S., 2010. Antifungal activity of medicinal plant extracts and phytocompounds: a review. In: Ahmal, I., Owais, M., Shahid, M., Aqil, F. (Eds.), Combating fungal infections. Problems and remedy (Chapter 19). Springer, Heidelberg, pp. 449–484.

Auzi, A.A., Gray, A.I., Salem, M.M., Badwan, A.A., Sarker, S.D., 2008. Feruhermonins A–C: three daucane esters from the seeds of *Ferula hermonis* (Apiaceae). J. Asian Nat. Prod. Res. 10, 701–707.

Balues, A., Hausler, W., Hevmann, L.K., Iyenbery, H.D., Shadomy, H.J., 1991. Manual of Clinical Microbiology, 5th ed.. American Society for Microbiology, ASM Press, Washington, DC.

Barry, A.L., Thornsberry, C., 1991. Susceptibility test: diffusion test procedures. In: Balows, A., Hausler, W.J., Herramann, K.L., Isenberg, H.D., Shadomy, H.J. (Eds.), Manual of Clinical Microbiology. American Society for Microbiology, Washington, DC, pp. 1526–1542.

Bastert, J., Schaller, M., Korting, H.C., Evans, E.G.V., 2001. Current and future approaches to antimycotic treatment in the era of resistant fungi and immunocompromised hosts. Int. J. Antimicrob. Agents 17, 81–91.

Cos, P., Vlietinck, A.J., Vanden Berghe, D., Maes, L., 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. J. Ethnopharmacol. 106, 290–302.

Council of Europe, 2007. European Pharmacopeia, vol. 1, 6th ed. Directorate for the Quality of Medicines and Health Care of the Council of Europe, Strasbourg, pp. 251–252.

Dev, V., Oka, M., Mathela, C.S., Murari, N.D., Stevens, T.H., 1984. The volatile constituents of the roots of *Selinum tenuifolium*. J. Nat. Prod. 47, 904–905.

Diab, Y., Dolmazon, R., Bessière, J.M., 2001a. Daucane aryl esters composition from the Lebanese *Ferula hermonis* Boiss (zallooh root). Flav. Fragr. J. 16, 120–122.

Diab, Y., Dolmazon, R., Fenet, B., 2001b. 2,3-Epoxyjaeschkeanadiol 5-benzoate from *Ferula hermonis* Boiss. Flav. Fragr. J. 16, 397–400.

Dokovic, D.D., Bulatovic, V.M., Bozic, B.D., Kataranovski, M.V., Zrakic, T.M., Kovacevic, N.N., 2004. 3, 5-Nonadiyne isolated from the rhizome of *Cachrys ferulacea* inhibits endogenous nitric oxide release by rat peritoneal macrophagues. Chem. Pharm. Bull. 52, 853–854.

El-Taher, T.S., Mtalka, K.Z., Taha, H.A., Badwan, A.A., 2001. *Ferula hermonis* "Zallouh" and enhancing erectile function in rats: efficacy and toxicity study. Int. J. Impot. Res. 13, 247–251.

Freixa, B., Vila, R., Ferro, E.A., Adzet, T., Cañigueral, S., 2001. Antifungal principles from *Piper fulvescens*. Planta Med. 67, 873–875.

Galal, A., 2000. Sesquiterpenes from *Ferula hermonis* Boiss. Pharmazie 55, 961–962.

Galal, A.M., Abourashed, E.A., Ross, S.A., ElSohly, M.A., Al-Said, M.S., El-Feraly, F.S., 2001. Daucane sesquiterpenes from *Ferula hermonis*. J. Nat. Prod. 64, 399–400.

Gamal-Eldeen, A., Hegazy, M.-E.F., 2010. A crystal lapiferin derived from *Ferula vesceritensis* induces apoptosis pathway in MCF-7 breast cancer cells. Nat. Prod. Res. 24, 246–257.

Goryaev, M.I., Sharipova, F.S., Tikhonova, L.K., El'chibekova, L.A., 1971. Substances present in essential oils. LIII. Comparative study of the chemical composition of essential oils of the above ground and root parts of *Ferula penninervis* plants. Izvest. Akad. Nauk Kazak. SSR Ser. Khim. 21, 49–53.

Hilan, C., Sfeir, R., El Hage, R., Jawich, D., Frem, M.F., Jawhar, K., 2007. Evaluation of the antibacterial activities of *Ferula hermonis* (Boiss.). Lebanese Sci. J. 8, 135–151.

Ikeda, K., Arao, Y., Otsuka, H., Nomoto, S., Horiguchi, H., Kato, S., Kayama, F., 2002. Terpenoids found in the Umbelliferae family act as agonists/antagonists for ER and ER: differential transcription activity between ferutinine-liganded ER and ER. Biochem. Biophys. Res. Commun. 291, 354–360.

Iranshahi, M., Amin, G., Sourmaghi, M.S., Shafiee, A., Hadjiakhoondi, A., 2006. Sulphur-containing compounds in the essential oil of the root of *Ferula persica* Willd. Var. persica. Flavour Fragr. J. 21, 260–261.

Karim, A., Ashraf, M., Bhatty, M.K., 1979. Studies of the essential oils of the Pakistani species of the family Umbelliferae. Part XXVI. *Ferula oopoda* Boiss Buhse, oil from seeds, stalks and roots. Pak. J. Sci. Ind. Res. 22, 198–201.

Lev, E., Amar, Z., 2002. Ethnopharmacological survey of traditional drugs sold in the Kingdom of Jordan. J. Ethnopharmacol. 82, 131–145.

Lhuillier, A., Fabre, N., Cheble, E., Oueida, F., Maurel, S., Valentin, A., Fourasté, I., Moulis, C., 2005. Daucane sesquiterpenes from *Ferula hermonis*. J. Nat. Prod. 68, 468–471.

Maggi, F., Lucarini, D., Tirillini, B., Sagratini, G., Papa, F., Vittori, S., 2009. Chemical analysis of the essential oil of *Ferula glauca* L. Apiaceae) growing in Marche (central Italy). (Biochem. Syst. Ecol. 37, 432–441.

Mathew, B.P., Nath, M., 2009. Recent approaches to antifungal therapy for invasive mycoses. ChemMedChem 4, 310–323.

Miski, M., Ulubelen, A., Mabry, T.J., 1983. Six sesquiterpene alcohol esters from *Ferula elaeochytris*. Phytochemistry 22, 2231–2233.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, R.H., 1999. Manual of Clinical Microbiology, seventh ed. American Society for Microbiology, ASM Press, Washington, DC.

Pfaller, M.A., Diekema, D.J., 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. 20, 133–163.

Portillo, A., Vila, R., Freixa, B., Adzet, T., Cañigueral, S., 2001. Antifungal activity of Paraguayan plants used in traditional medicine. J. Ethnopharmacol. 76, 93–98.

Portillo, A., Vila, R., Freixa, B., Ferro, E., Parella, T., Casanova, J., Cañigueral, S., 2005. Antifungal sesquiterpene from the root of *Vernonanthura tweedieana*. J. Ethnopharmacol. 97, 49–52.

Pimenov, M.G., Leonov, M.V., 1993. The genera of the Umbelliferae. Royal Botanic Gardens, Kew.

Pyun, M.S., Shin, S., 2006. Antifungal effects of the volatile oils from *Allium* plants against *Tricophyton* species and synergism of the oils with ketoconazole. Phytomedicine 13, 394–400.

Rahalison, L., Hamburger, M., Hostettmann, K., Monod, M., Frenk, E., 1991. A bioautographic agar overlay method for the detection of antifungal compounds from higher plants. Phytochem. Anal. 2, 199–203.

Rosato, A., Vitali, C., Gallo, D., Balenzano, L., Mallamaci, R., 2008. The inhibition of *Candida* species by selected essential oils and their synergism with amphotericin B. Phytomedicine 15, 635–638.

Rustaiyan, A., Monfared, A., Masoudi, S., Ameri, N., 2002. Essential oils of the stem and root of *Ferula galbaniflua* Boiss. et Busse. from Iran. J. Essent. Oil Res. 14, 286–287.

Svetaz, L., Zuljan, F., Derita, M., Petenatti, E., Tamayo, G., Cáceres, A., Cechinel Filho, V., Giménez, A., Pinzón, R., Zacchino, S.A., Gupta, M., 2010. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. J. Ethnopharmacol. 127, 137–158.

Thevissen, K., Kristensen, H.H., Thomma, B.P.H.J., Cammue, B.P.A., François, I.E.J.A., 2007. Therapeutic potential of antifungal plant and insect defensins. Drug Discovery Today 12, 966–971.

Tomi, F., Casanova, J., 2006. ^{13}C-NMR as a tool for identification of individual components of essential oils from Labiatae: a review. Acta Hort. 723, 185–192.

Zanoli, P., Rivasi, M., Zavatti, M., Brusiani, F., Vezzalini, F., Baraldi, M., 2005. Activity of single components of *Ferula hermonis* on male rat sexual behavior. Int. J. Impot. Res. 17, 513–518.

Zavatti, M., Montanari, C., Zanoli, P., 2006. Role of ferutinin in the impairment of female sexual function induced by *Ferula hermonis*. Physiol. Behav. 89, 656–661.