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Anti-dermatophyte constituents of the essential oil from the root of *Ferula hermonis*

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Introduction

In a previous work, the essential oil from the dried root of *Ferula hermonis* Boiss. (Umbelliferae) (**Figure 1**), whose major constituents were α-pinene (43.3 %), α-bisabolol (11.1 %) and the unusual acetylene compound 3,5-nonadiyne (4.4 %), showed moderate antifungal activity against *Trichophyton mentagrophytes*. MIC and MFC values of the essential oil and α-pinene suggested that other constituents were also responsible of the antifungal activity [1]. With the aim of characterizing the active constituents, a bioguided fractionation of the essential oil was performed.

Figure 1. *Ferula hermonis* root.

Results

Growth inhibition zones of different magnitudes were observed in the case of fraction 2, fraction 10 and from fraction 17 to 27. In fraction 2 the activity was directly related to α-pinene, whereas in fraction 10 the major constituent was 3,5-nonadiyne. Successive fractionation of the essential oil allowed the isolation of the main constituents of the active fractions 17 to 27 (**Figure 2**). The most potent activity (**Table 1**) was demonstrated by the fraction with 73% jaeschkeanadiol benzoate 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). Both dermatophytes were highly sensitive to the unusual compound 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).

Table 1. MIC and MFC (µg/ml) of the active constituents and fractions of the essential oil of *Ferula hermonis* root.

Substance	*Microsporum gypseum*		*Trichophyton mentagrophytes*	
	MIC	MFC	MIC	MFC
α-P	32	32	64	64
α-B	16	16	16	32
Ny	8	16	8	8
***t*-V**	32	32	32	32
α-Bo	64	128	64	64
Jb	64	128	0.25	0.25
Ja	32	64	32	32
S	32	32	32	32
A	1	1	0.5	0.5
N	4	8	2	2
K	8	>8	0.25	0.25
C	0.125	0.25	0.0625	0.0625

α-P: α-Pinene; **α-B**: α-Bisabolol; **Ny**: 3,5-Nonadiyne; ***t*-V**: *trans*-Verbenol; **α-Bo:** α-Bisabolol oxide B, **Jb**: Jaeschkeanadiol benzoate 73%, **Ja**: Jaeschkeanadiol angelate, **S**: Spathulenol 50%, **A**: Amphotericin B, **N**: Nystatin, **K**: Ketoconazole, **C**: Clotrimazol.

Material and methods

Plant material

Air-dried roots of *F. hermonis* were provided by The Jordanian Pharmaceutical Manufacturing Co. (Naor, Jordan).

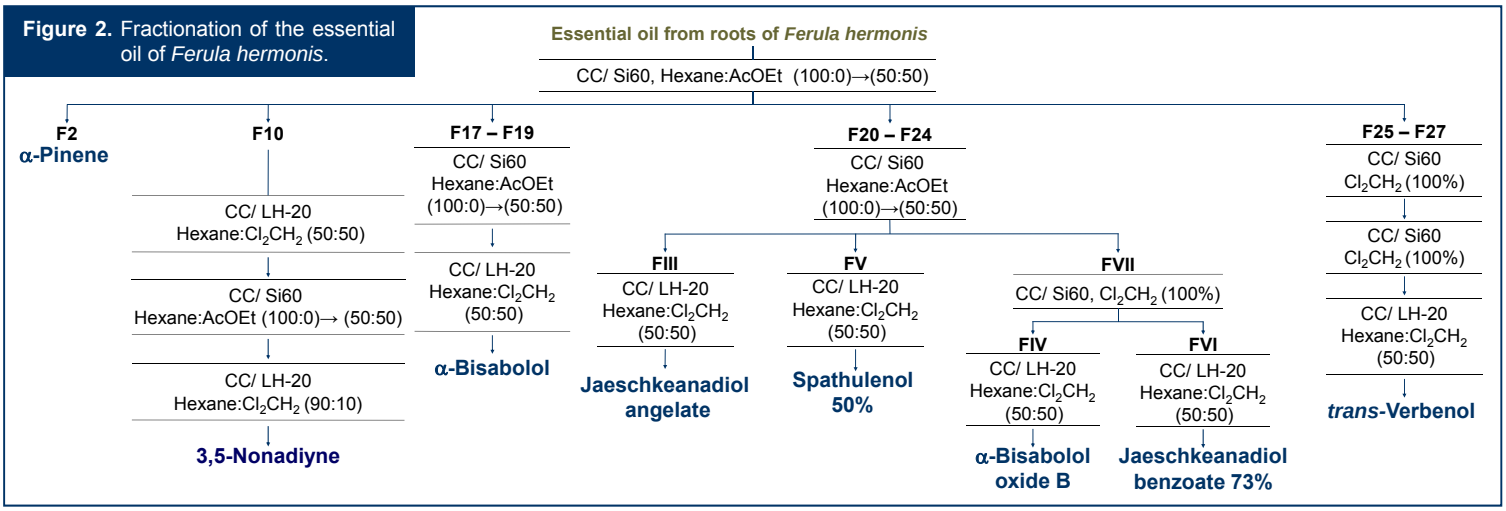
Obtention and fractionation of the essential oil

Plant material was submitted to hydrodistillation (3.5 h) according to the procedure described in the European Pharmacopoeia [2] and the essential oil obtained was dried over Na_2SO_4, filtered and stored at 4ºC till use. The oil was fractionated (**Figure 2**) through silicagel CC affording 27 fractions which were submitted to GC-FID, GC-MS and ^{13}C-NMR analyses, as well as to an agar overlay bioautographic assay. Successive fractionation of the active fractions allowed the isolation of 6 pure compounds and two purified fractions.

Anti-dermatophyte activity

Dermatophyte fungi *Microsporum gypseum* (CECT 2908) and *Trichophyton mentagrophytes* (CECT 2795) were provided by Colección Española de Cultivos Tipo (CECT, Valencia, Spain). An overlay bioautographic method was used for the detection of active fractions [3]. Developed TLC plates of each fraction on silicagel 60 F254 eluted with hexane:AcOEt (6:4) and (9:1) or Cl_2CH_2:MeOH (98:2), were placed over an inoculated Sabouraud Dextrose Agar medium (12x12 cm plates, 1 h, 4ºC). After removing TLC, test plates were incubated at (30ºC, 48 h) to visualize growth inhibition. MIC and MFC values of the active constituents of the essential oil were determined by an agar dilution assay following the method described in [4]. Amphotericin B, nystatin, ketoconazole and clotrimazol were used as positive controls.

Figure 2. Fractionation of the essential oil of *Ferula hermonis*.



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References

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