Phytotherapy – The role of an ancient tradition in modern times. PSE. Madeira, Portugal, 2005

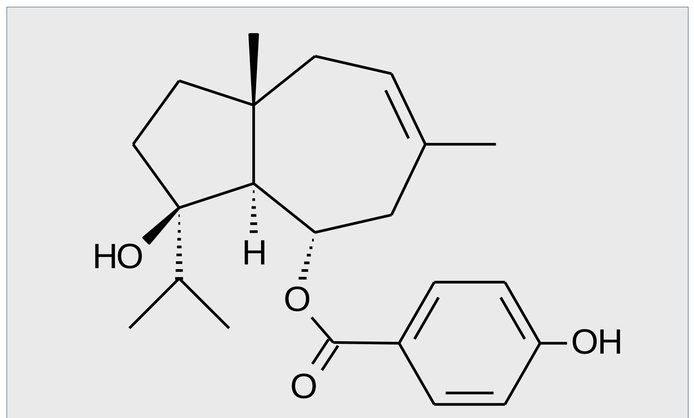
Antifungal principle from the root of *Ferula hermonis*

Abdel-Hadi Al-Ja'fari[a,b], Blanca Freixa[a], Roser Vila[a], Joan Costa[b] and Salvador Cañigueral[a]

[a] Unitat de Farmacologia i Farmacognòsia, Facultat de Farmàcia, Universitat de Barcelona. Av. Diagonal, 643. E-08028 Barcelona (Spain).

[b] Departament de Farmacologia, de Terapèutica i de Toxicologia, Facultat de Medicina, Universitat Autònoma de Barcelona, Unitat Docent Hospital Universitari Germans Trias i Pujol, E-08916 Badalona (Barcelona, Spain).

Figure 1. *Ferula hermonis* root.



Jaeschkeanadiol p-hydroxybenzoate (**1**)

Table 1. Antifungal activity of jaeschkeanadiol p-hydroxybenzoate.

Fungi	1		A		N	
	MIC	MFC	MIC	MFC	MIC	MFC
Cl	16	16	0.25	0.25	1	1
Sc	32	32	0.5	1	1	2
Ca	>256	>256	0.25	0.5	2	4

MIC and MFC values are in μg/ml.
1: Jaeschkeanadiol p-hydroxybenzoate, **A**: Amphotericin B, **N**: Nystatin.
Ca: *Candida albicans*, ***Cl***: *Candida lactis-condensi*, ***Sc***: *Saccharomyces cerevisiae*.

Introduction

Ferula hermonis Boiss. (Umbelliferae) grows above 6000 feet in the mountains between Lebanon and Syria. Its root (**Figure 1**), locally known as Zallouh root, is used in infusion as an aphrodisiac as it enhances male sexual behaviour (1).

In order to search for new antifungal agents, in this work we assayed the antifungal activity of its extracts.

Material and methods

Plant material and extraction

Air-dried roots of *F. hermonis* were provided by The Jordanian Pharmaceutical Manufacturing Co. (Naor, Jordan). Plant material (25 g) was successively submitted to extraction with increasing polarity solvents: hexane, Cl_2CH_2, MeOH and water.

Susceptibility test and microorganisms

The agar disk diffusion assay and overlay bioautographic method (TLC on Silicagel, hexane:AcOEt (6:4) and (9:1), CH_2Cl_2:MeOH (98:2), and Sabouraud Dextrose Agar medium) were used to assay the antifungal activity of the extracts and for the bioguided isolation of the active compound **1**. Minimal inhibitory concentration (MIC) was determined following the method described in (2). *Candida albicans* ATCC 10231, *Candida lactis-condensi* CECT 1075 and *Saccharomyces cerevisiae* CECT 1324 were provided by Colección Española de Cultivos Tipo (CECT, Valencia, Spain).

Isolation and structure determination

Isolation of compound **1** was performed from hexane extract by preparative TLC on silicagel plates, combined with column chromatography (CC) through silicagel and Sephadex LH-20 (**Figure 2**).

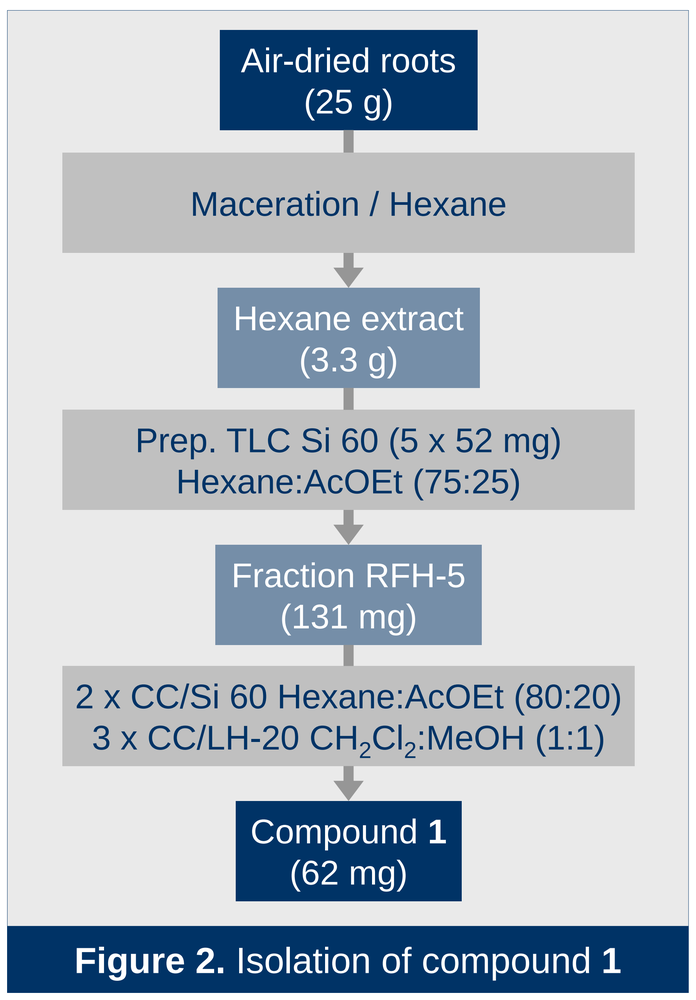


Figure 2. Isolation of compound **1**

Structure elucidation was done using standard spectroscopic techniques, such as ^{1}H-RMN, ^{13}C-RMN, DEPT, H,H-COSY, HSQC, HMBC, EI-MS and CI-MS, and literature data (3).

References

1. El-Thaher, TS *et al*. (2001) *Int J Impot Res* 13: 247-51. **2.** Jorgengen JH *et al*. (1999). In Murray, PR *et al*. *Manual of Clinical Microbiology*, 7th Edition. ASM Press. Washington. **3.** Diab, Y *et al*. (2001) *Flav Fragr J* 16: 120-2. **4.** Galal, AM *et al*. (2001) *J Nat Prod* 64: 399-400. **5.** Al-Sha'er, M *et al.* (2001) *Acta Tech Leg Medic* 12: 255-64. **6.** Appendino, G *et al.* (2004) *J Nat Prod* 67: 1557-1564. **7.** Saidkhodzhaev AI *et al.* (1974) *Khim Prir Soedin* 256.

Results and discussion

All the extracts assayed showed growth inhibition of some of the fungi strains tested. Particularly, the hexane extract was active against *C. lactis-condensi*.

Bioguided fractionation of this extract by preparative TLC afforded eight fractions from which only one (RFH-5) showed antifungal activity. Repeated alternate fractionation of RFH-5 by means of Si60 and Sephadex™ LH-20 column chromatography allowed the isolation of the active compound **1**.

Its structure was elucidated by standard spectroscopic techniques as jaeschkeanadiol p-hydroxybenzoate, a known daucane aryl ester (3,4) called ferutinin for which a weak activity against *Aspergillus niger* has been previously reported (5). It has also been revealed as a potent estrogenic compound (6). Ferutinin was isolated for the first time from the roots of *F. ovina* (7) and later on it has been found in several other species of the same genus.

Results on antifungal activity obtained in the agar dilution assay are shown in **Table 1**. MIC values of **1** against *C. lactis-condensi*, *S. cerevisiae* and *C. albicans* were 16, 32 and >256 μg/ml, respectively. MFC against the two first strains were the same as MIC values.

Acknowledgements

AA Badwan, The Jordanian Pharmaceutical Manufacturing Co. and TS El-Thaher, ARAGEN Biotechnology, (Naor, Jordan), for providing plant material.