

# Antimicrobial Activities of *Ferulago* Essential Oils<sup>Y</sup>

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Essential oils from *Ferulago asparagifolia* Boiss., *F. galbanifera* (Miller) W. Koch, *F. humilis* Boiss. (Endemic), *F. trachycarpa* Boiss. growing in Turkey were evaluated against 15 microorganisms for their antifungal and antibacterial activity using an agar tube dilution and micro-dilution broth susceptibility assay, respectively. The essential oil compositions were investigated by GC/MS. Inhibitory effects against *Escherichia coli*, *Enterobacter aerogenes*, *Candida albicans*, *Gaeumannomyces graminis* var. *tritici*, *Sclerotium rolfsii* and *Fusarium moniliforme* were remarkable. Results are discussed in comparison with the chemical composition of the essential oils.

## Introduction

The genus *Ferulago* (Umbelliferae) is widely distributed in Anatolia and comprises thirty species, sixteen of them being endemic. It is interesting that only about forty-five *Ferulago* species are described in the world, which suggests that the gene centre for this genus is Anatolia. *Ferulago* species are known as *kişniş*\*, kuzu başı, kuzu kemirdi, çakşır and resemble *Ferula* and *Prangos* species also widely abundant in Turkey (Davis, 1972 and 1988; Baytop, 1994; Akalin, 1999).

Since ancient times *Ferulago* species have been used as spice and flavoring. Furthermore they are used in folk medicine as sedative, tonic, digestive and as well as in the treatment of intestinal worms. Roots of *Ferulago* species are also used in Turkey as aphrodisiac, like those of *Ferula* and *Prangos* species (Akalin, 1999; Baytop, 1999).

Literature search showed the occurrence of only a few phytochemical investigations (Buckingham, 1998). The isolation of flavonoids, coumarins and quinones was reported from the aerial parts of two different *Ferulago* species (Doğanca *et al.*, 1991

and 1992). Sesquiterpenes were isolated from *F. antiochica* (Miski *et al.*, 1990). Essential oils of *F. trachycarpa* and *F. asparagifolia* have previously been investigated by our group (Başer, 1998; Başer *et al.*, 2000b). Most recently the essential oil composition of *F. contracta* from Iran has been reported.  $\alpha$ - and  $\beta$ -phellandrene were major constituents of the flower oil and *p*-cymene and  $\alpha$ -phellandrene were detected as major components of the stem oil (Rustaiyan, 1999).

Comprehensive research in our Research Centre (TBAM) into the essential oils of Umbelliferae growing in Turkey has recently been compiled by our group (Başer, 2000a).

This paper reports the main components of the essential oils of *Ferulago asparagifolia*, *F. galbanifera*, *F. humilis* and *F. trachycarpa*, collected from different regions of Turkey which were analyzed by GC/MS. We also report on their antimicrobial evaluation for the first time.

## Experimental

### Plant material and isolation of the oils

Plant materials were collected by the authors from different regions of Turkey. Voucher specimens are deposited at the Faculty of Pharmacy Herbarium, Anadolu University, Eskişehir (ESSE). Crushed plant materials were subjected

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\* *Coriandrum sativum* is commonly called *Kişniş* in Turkish.

Table I. Information on *Ferulago* Essential oils.

Code	<i>Ferulago</i> species	ESSE <sup>ψ</sup>	Part	Collection place, altitude	Collection period	Yield*(%)
<b>1</b>	<i>F. asparagifolia</i>	11644	fruit	Antalya: Gazipaşa-Alanya 8. km, 45 m	July 1995	6.9
<b>2</b>	<i>F. galbanifera</i>	12438	fruit	Eskişehir: Bozdağ, 600 m	Sep. 1999	1.3
<b>3</b>	<i>F. humilis</i> (E)	12925	fruit	Muğla: Sandras Dağı, Ağla Üzeri, 1430 m	July 1999	3.9
<b>4</b>	<i>F. trachycarpa</i>	12667	herb	Konya: Hadim Mihliçesme Korualan, 1890 m	July 1998	0.6

<sup>ψ</sup> Herbarium of the Faculty of Pharmacy at Anadolu University in Eskişehir. (E) Endemic.

\* Yields are given on moisture free basis.

to hydrodistillation using a Clevenger-type apparatus for 4 h. Oil yields and information on collection sites are given in Table I.

#### GC/MS analysis

The oils were analyzed by GC/MS using a Hewlett Packard GCD system. Innowax FSC column (60 m × 0.25 mm i.d., 0.25 µm film thickness) was used with helium as a carrier gas (1 ml/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, then kept constant at 220 °C for 10 min and subsequently programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 50:1. The injector temperature was at 250 °C. MS were recorded at 70 eV. Mass range was from *m/z* 35 to 425. Library search was carried out using the Wiley GC/MS Library and TBAM Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by the computer. The main compounds identified in the oils are listed in Table II.

#### Bioassays

Antifungal bioassay. Antifungal activity was determined using agar tube dilution technique (Paxton, 1991; Koneman, 1997). Stock solutions of the essential oils were freshly prepared in dimethylsulfoxide (DMSO) to reach a final concentration of 400 µg/ml using sterile molten Sabouraud dextrose agar (SDA-Acumedia, USA). Test tubes were kept at room temperature for solidification. Medium containing only DMSO was used as negative control. Fungi were cut to 4x4 mm from one week grown cultures and then inoculated onto the slant. After an incubation period of 7–10 days at

Table II. Main components of *Ferulago* essential oils.

	<i>Ferulago</i> species	Main components (%)
<b>1</b>	<i>F. asparagifolia</i>	myrcene (18.2) 2,3,6-trimethylbenzaldehyde (38.9)
<b>2</b>	<i>F. galbanifera</i>	α-pinene (31.8) sabinene (15.8) α-phellandrene (6.3) limonene (6.6)
<b>3</b>	<i>F. humilis</i>	(Z)-β-ocimene (32.4) limonene (17.3) α-pinene (12.1)
<b>4</b>	<i>F. trachycarpa</i>	trans-chrysanthenylacetate (12.1) (Z)-β-ocimene (34.1) α-pinene (8.0) α-phellandrene (5.0) <i>p</i> -cymene (4.9)

29 °C, tubes were examined for growth inhibition. Ketoconazole and Penconazole were used as reference antifungal drugs. Growth on the media containing compound was determined by measuring the linear growth (mm) of the fungal culture. Growth inhibition (%) was calculated with reference to the negative control (Table IV). The pathogenic fungi *Aspergillus flavus*, *Aspergillus niger*, *Drechslera sorokiniana*, *Fusarium moniliforme*, *Gaeumannomyces graminis* var. *tritici*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, and *Trichothecium roseum* used in this study were obtained from Anadolu University, Faculty of Sciences and HEJ Research Institute of Chemistry, Karachi, Pakistan.

Antimicrobial bioassay. Micro-dilution broth susceptibility assay was used for the evaluation of the essential oils (Koneman, 1997). Stock solution of essential oil was prepared in DMSO. Dilution series of essential oil was prepared in sterile dis-

Table III. Antimicrobial activity (MIC) of *Ferulago* sp. essential oils **1–4**.

Pathogen	Source	1	2	3	4	Standard µg/ml
<i>E. coli</i>	ATCC 25922	125	62.5	250	250	62.5 <sup>a</sup>
<i>S. aureus</i>	ATCC 6538	31.25	125	125	250	7.81 <sup>a</sup>
<i>P. aeruginosa</i>	ATCC 27853	500	250	500	500	250 <sup>a</sup>
<i>E. aerogenes</i>	NRRL 3567	125	250	500	250	125 <sup>a</sup>
<i>P. vulgaris</i>	NRRLB 123	125	62.5	250	500	31.25 <sup>a</sup>
<i>S. typhimurium</i>	NRRLB 4420	125	250	125	500	62.5 <sup>a</sup>
<i>C. albicans</i>	O. G. Ü.		62.5	62.5	500	125 <sup>b</sup>

<sup>a</sup> Chloramphenicol. <sup>b</sup> Ketoconazole.

tilled water in 96-well microtiter plate up to 1.94 µg/ml in sterile distilled water. Freshly grown bacterial suspensions in double strength Mueller-Hinton broth (Merck) and yeast suspension of *Candida albicans* in yeast medium were standardized to 10<sup>8</sup> CFU/ml. Sterile distilled water served as growth control. 100 µl of each microbial suspension was then added to each well. The last row containing only the serial dilutions of antimicrobial agent without microorganism was used as negative control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC) (Koneman, 1997). Human pathogens *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Salmonella typhimurium*, were obtained from the culture collection of the Microbiology Department in Anadolu University, and *Candida albicans* was obtained from the culture collection of Osmangazi University, Medical Faculty, Microbiology Department (Table III).

## Results and Discussion

Literature search showed that the essential oils of *Ferulago asparagifolia*, *F. galbanifera*, *F. humilis* (Endemic), and *F. trachycarpa* have not previously been subjected to any biological evaluation. Only ethnobotanic and folkloric data have been reported (Akalin, 1999).

We have previously investigated essential oil of the high oil yielding (6.9%) fruits of *F. asparagifolia* collected from Antalya by GC/MS (Başer et al., 2000b) and found that myrcene (18.2%) and 2,3,6-trimethylbenzaldehyde (38.9%) were the main components. This essential oil showed strong inhibition against *E. aerogenes*, the yeast *C. albi-*

*cans* and the wheat pathogenic fungi *G. graminis* var. *tritici*, and *T. roseum*. Also moderate activity against the other tested bacteria and fungi was observed (Tables III and IV).

*F. galbanifera* collected from Eskişehir showed a different essential oil profile with α-pinene (31.8%) and sabinene (15.8%) main components (Table II). However, it also showed significant inhibitory effects against *E. coli*, *P. aeruginosa*, *P. vulgaris* and especially against *C. albicans*. The plant pathogenic fungi *D. sorokiniana*, *G. graminis* var. *tritici* and *S. rolfsii* were also inhibited.

The endemic species *F. humilis* was less active in comparison to the other oils, however, it showed good activity against the yeast *C. albicans*, comparable with Ketoconazole. The fungi *G. graminis* var. *tritici* was inhibited moderately.

The fresh fruit oil of *F. trachycarpa* collected from Karaman was previously investigated by our group (Başer, 1998). The main components of this endemic species were in agreement with the recently collected species from Konya although the herbal parts were analyzed in this study. Good in-

Table IV. Growth Inhibition (%) at 400 µg/ml of some fungi by *Ferulago* sp. essential oils.

Fungus	(+)	(-)	1	2	3	4
<i>A. flavus</i>	100	0	0	0	0	0
<i>A. niger</i>	100	0	0	0	0	0
<i>D. sorokiniana</i>	95	0	0	<b>55</b>	5	40
<i>F. moniliforme</i>	66	0	30	—	5	<b>60</b>
<i>G. graminis</i> var. <i>tritici</i>	100	47	<b>90</b>	<b>50</b>	<b>50</b>	<b>100</b>
<i>R. stolonifer</i>	63	0	0	0	0	0
<i>S. rolfsii</i>	100	0	33	30	0	<b>65</b>
<i>T. roseum</i>	95	0	<b>90</b>	0	0	5

(+) Antifungal: Ketoconazole / Penconazole.

(-) DMSO.

hibitory activity was observed against the plant pathogenic fungi *G. graminis* var. *tritici*, *S. rolfsii*, *F. moniliforme* and moderate fungustatic activity against *D. sorokiniana*. This oil inhibited half of the fungi remarkably.

This study is encouraging to investigate the essential oils of other *Ferulago* species for further bioassays which may have stronger activities.

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