Phytopathol. Mediterr. (2003) 42, 141–148

Fungitoxic activity of root extracts from *Ferula harmonis*

KHALIL I. AL-MUGHRABI[1] and TALAL A. ABURJAI[2]

[1] Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt 19117, Jordan
[2] Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman 11942, Jordan

Summary. Air-dried and finely powdered roots of *Ferula harmonis* F. (Umbelliferae) were extracted in a Soxhlet with several solvents in succession and the gummy extracts in each fraction were collected and tested for their fungitoxic effects. The antifungal activity of nine *F. harmonis* extracts against *Alternaria solani*, *Cladosporium* sp., *Colletotrichum* sp., *Fusarium oxysporum*, *Mucor* sp., *Penicillium italicum*, *Pythium* sp., *Rhizoctonia solani*, *Rhizopus stolonifer*, *Stemphylium solani*, and *Verticillium dahliae* is reported. The strongest fungitoxic effects were found against *V. dahliae*, *P. italicum* and *R. stolonifer.* The weakest effect was against *A. solani*. All extracts of *F. harmonis* had varying degrees of fungitoxicity against all the fungi tested, which makes it a potential source of antifungal compounds. Ferutinin and teferidine, two known sesquiterpenes, were isolated from the roots of *F. harmonis* and their structures were identified. The fungitoxic activity of the ethyl acetate extract might be due to the presence of ferutinin contained in it.

Key words: antifungal activity, ferutinin, sesquiterpenes, teferidine, Zallouh.

Introduction

The chemistry of the genus *Ferula* has been studied by various researchers (Valle *et al.*, 1987; Appendino *et al.*, 1990) and the genus is a good source of gum-resin (Frensh, 1971). Compounds from the roots of this genus include daucanes, humulanes, hemachalanes and guaianes (Miski and Mabry, 1985; Lamnaouer *et al.*, 1989; Ahmed, 1991; Abourashed *et al.*, 2001). In addition, monoterpenes and carotane sesquiterpenes have been identified (Miski *et al.*, 1983; Diaz *et al.*, 1986; Appendino *et al.*, 1990). Various pharmacological activities have been attributed to the genus *Ferula*. *F. communis* has been recognized as a medicinal plant in the eastern Mediterranean region since ancient times; however in the western Mediterranean area it is reputed to be poisonous, and many cases of livestock and human poisoning from ingestion, known as ferulosis, have been reported (Zohary, 1966; Miski and Jakupovic, 1990). *Ferula harmonis* F. (Umbelliferae) is a native plant of Syria and Lebanon. It grows at more than 2,500 meters on Mount Hermon, on the joint borders of Lebanon, Syria and Jordan. This plant, which is locally known as Zallouh, is a small shrub with thin leaves, small white or yellow flowers, and hairy roots (Zohary, 1966). Interest in *F. harmonis* in folk medicine has increased recently after the launching of Viagra® on the market, especially in Lebanon, Syria and Jordan. Many herbalists and laymen

Corresponding author: K.I. Al-Mughrabi
Fax: + 506 392 5102; E-mail: khalil.al-mughrabi@gnb.ca
Present address: New Brunswick Department of Agriculture, Fisheries and Aquaculture, 39 Barker Lane, Wicklow, New Brunswick E7L 3S4, Canada

claim that this plant has a strong aphrodisiac and anti-impotence activity similar to that of Viagra® and they call it "Lebanese Viagra". No scientific research has been found in the literature to support or contradict their claims. Preliminary results of investigations by the second author (data not shown) indicate that this plant may have a significant toxic effect on experimental animals. The present work reports on the isolation and identification of two known sesquiterpenes from the roots of *F. harmonis,* ferutinin and teferidine, and is the first study on the fungitoxic activity of *F. harmonis*. Nine root extracts of *F. harmonis* were tested for toxicity against eleven of the most destructive plant pathogenic fungi isolated from various diseased plants and infested soils in Jordan (Table 1).

Materials and methods

Plant material

Roots of *F. harmonis* were purchased, identified, and a voucher specimen, FH No. 1/99, was deposited at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan.

Extraction and purification

Air-dried and finely powdered roots of *F. harmonis* (700 g) were extracted in a Soxhlet successively with 2 l each of petroleum ether, ethyl acetate, methanol, and water for 15 hours. The solvents were then evaporated under reduced pressure. The petroleum ether extract gave a gummy residue (55 g) of a pleasant odor. A portion of the gummy residue was chromatographed over a silica gel column.

Column chromatography

The method of Al-Sha'er *et al*. (2001) was followed. Petroleum ether extract (55 g) was subjected to column chromatography (CC) over silica gel (550 g, 90×6 cm, column A) and eluted with petroleum ether, and with petroleum ether-ethyl acetate of increasing polarity content. The fractions collected (FHR1–FHR7) were monitored by thin layer chromatography (TLC) and further purified by CC. Elution of column A with petroleum ether-ethyl acetate (9:1, 1 l) gave fraction FHR5 (5 g).

This fraction was further purified by CC over silica gel (550 g, 65×4 cm, column B) and eluted using different proportions of chloroform:petroleum ether. Elution with chloroform:petroleum ether (9.5:0.5, 500 ml) gave FHR1 (750 mg), which was crystallized from chloroform to give ferutinin (**1a**) (675 mg). Transparent, needle-like crystals were formed when ferutinin recrystallized from ethanol. Ferutinin was also isolated from ethyl acetate crude extract and purified under the same conditions. Fraction FHR7 (350 mg) gave FH2 (**1b**), which was purified by CC (300 g, 60×3 cm) and eluted with chloroform:petroleum ether (1:1) to give a gummy, transparent residue (teferidine, **1b**, 250 mg).

Melting points were determined on a Stuart Scientific melting point apparatus (Stuart Scien-

Table 1. Fungal isolates used to study the fungitoxic activity of crude extracts from roots of *Ferula harmonis*.

Fungus	Source	Part sampled	I.D. #	Location in Jordan	Date collected
Fusarium oxysporum	Potatoes	Roots	517	Al-Balqa	June 1999
Rhizoctonia solani	Cucumber	Roots and stems	226	Jerash	June1999
Pythium sp.	Thyme	Roots and stems	580	Yadoda	June 1999
Verticillium dahliae	Tomatoes	Stems	305	Um Amad	August 1999
Alternaria solani	Potatoes	Leaves	070	Jordan Valley	July 1999
Stemphylium solani	Tomatoes	Stems and leaves	245	Jerash	June 1999
Rhizopus stolonifer	Tomatoes	Fruits	352	Yadoda	June 1999
Penicillium italicum	Beans	Stems and leaves	234	Al-Salt	June 1999
Cladosporium sp.	Petunia	Stems	250	Jerash	August 1999
Mucor sp.	Gerber	Roots	568	Baq'a	July 1999
Colletotrichum sp.	Dieffenbachia	Stems	304	Jerash	June 1999

Table 2. Crude extracts from roots of *Ferula harmonis* tested for the fungitoxic activity against phytopathogenic fungi.

Extract	Extract type
PE	Petroleum ether crude extract
PPE	Purified petroleum ether crude extract of extract PE utilizing 100% petroleum ether
W	Water crude extract
EA	Ethyl acetate crude extract
M	Methanol crude extract
PEA	Ethyl acetate purified crude extract of extract PE
EA-W	Ethyl acetate and water (50:50) purified crude extract of extract M
PE-EA (80:20)	Petroleum ether-ethyl acetate (80:20) purified crude extract of extract PE
PE-EA (90:10)	Petroleum ether-ethyl acetate (90:10) purified crude extract of extract PE

tific Co., Redhill, UK) and are uncorrected. IR spectra (KBr) were determined on a JASCO IR-810 Spectrometer (JASCO International, Tokyo, Japan), and UV spectra on a Unicam-810 Kontron Spectrophotometer (Fisher Scientific, Pearl River, NY, USA). ^{1}H-NMR spectra were determined at 300.13 MHz using Bruker DPX-300 spectroscopy (Brunker, Rheinstetten, Germany) and TMS as an external standard. ^{13}C-NMR spectra were determined at 75.46 MHz using Bruker DPX-300 spectroscopy and TMS as an external standard. Low-resolution MS spectra were recorded on a quadrupole Finnigan Mat 112, 70eV (Thermo Finnigan, Bremen, Germany). Kieselgel 60 silica gel (Merck, Darmstadt, Germany) was used for CC, and Kieselgel 60-F_{254} silica gel (Merck) for TLC. Spots were detected by UV fluorescence and sprayed with vanillin/H_2SO_4 followed by heating at 120°C for 5–10 minutes. Dry Na_2SO_4 was routinely used to dry solvents. All solvents were evaporated under reduced pressure at 40°C.

Ferutinin (**1a**): crystallized from chloroform; mp 121–122°C. UV _MeOH max nm: 325 FAB-MS (Positive) ($C_{22}H_{30}O_4$) *m/z:* 359 $[M+H]^+$ and *m/z* 365 $[M+Li]^+$; FD-MS shows the $[M]^+$ at *m/z* 358; EI-MS *m/z*: 358 $[M]^+$, 340 $[M-H_2O]^+$, 315 $[M-C_3H_7]^+$, 237 $[M-C_7H_5O_2]$, 221 $[M-C_7H_5O_3]^+$, 219 $[M-C_7H_5O_2-H_2O]^+$, 203 $[M-C_7H_5O_3-H_2O]^+$, 194 $[M-C_7H_5O_2-C_3H_7]^+$, 121 (100%) $[C_7H_5O_2]^+$, 93 $[C_7H_5O_2-CO]^+$. ^{1}H-NMR (300 MHz) $CDCl_3$: d 0.83 (3H, *d*, *J*=7 Hz, H-14), 0.94 (3H, *d,* *J*=7 Hz, H-13), 1.10 (3H, *s*, H-15), 1.81(3H, *s*, H-11), 5.32 (1H, *dt,* *J*=3 and 10 Hz, H-6), 5.56 (1H, *t*, J=6.5 Hz, H-9), 6.95 (2H, *d,* *J*=8.0 Hz, H-2‘, H-6‘), 7.9 (2H, *d,* *J*=8.0 Hz, H-3‘, H-5‘), 8.6 (1H, *s*, OH-4‘). ^{13}C-NMR data are shown in Table 3. Ferutinin structure is shown in Fig. 1.

Teferidine (**1b**): gummy, transparent and colorless substance. FAB-MS (Positive) ($C_{22}H_{30}O_2$) *m/z:* 342 $[M]^+$, 325 $[M-17]^+$, 249 $[M-93]^+$, 196 $[M-146]^+$. ^{1}H-NMR (300 MHz, $CDCl_3$): d 0.84 (3H, *d,* *J* =7 Hz,

Table 3. ^{13}C-NMR data for compounds **1a** and **1b**.

Carbon	Compound **1a**	Compound **1b**
C-1	44.0	44.1
C-2	31.3	31.7
C-3	40.9	40.9
C-4	87.3	86.3
C-5	60.1	59.9
C-6	71.3	71.4
C-7	41.1	41.3
C-8	133.4	133.5
C-9	125.3	125.3
C-10	41.3	41.3
C-11	26.3	26.4
C-12	37.0	37.2
C-13	17.6	17.5
C-14	18.5	18.5
C-15	20.2	20.2
C-1‘	121.5	130.5
C-2‘	132.0	129.6
C-3‘	115.6	128.5
C-4‘	161.6	133.1
C- 5‘	115.6	128.5
C-6‘	132.0	129.6
C-7‘	167.7	166.5

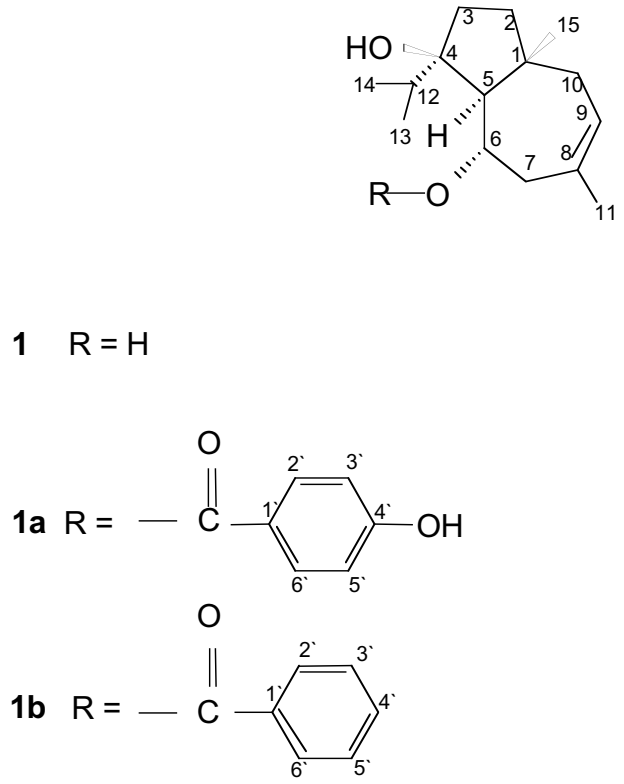

Fig. 1. Compounds extracted from the roots of *Ferula harmonis*. Based on the composition of R, structure **1** is jaeskeanadiol; **1a** is ferutinin; and **1b** is teferidine.

H-14), 0.96 (3H, *d, J*=7 Hz, H-13), 1.12 (3H*, s*, H-15), 1.83 (3H, *s,* H-11), 5.32 (1H*, dt, J*=3 and 10 Hz, H-6), 5.56 (1H, *t*, *J*=6.5Hz, H-9), 7.46 (2H*, dt, J*=2 and 9 Hz, H-2‘, H-6‘), 7.6 (1H, *dt*, *J*=2 and 9 Hz, H-4‘), 8.03 (2H*, d, J*=7.2 Hz, H-3‘, H-5‘). ^{13}C-NMR data are shown in Table 3. Teferidine structure is shown in Fig. 1.

Nine extracts were obtained when different solvents or combinations of solvents were used (Tables 2 and 4).

Microbial studies

Fungi used in this study were collected from various locations in Jordan. Table 1 lists each fungus and gives the plant and plant part from which it was isolated, the specimen identification number, and the location and date of collection. All fungal isolates were identified by the first author, and samples of each fungus were deposited in the fungal collection bank at the Department of Biotechnology of Al-Balqa’ Applied University, Al-Salt, Jordan.

As described by Al-Mughrabi *et al*. (2001) fungal isolates were maintained on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA) medium, and the cultures were stored at room temperature and subcultured once a month. The isolates were allowed to grow for 7–10 days before being used in the microbial studies.

Nine crude extracts of *F. harmonis* (Table 2) were diluted with sterile distilled water (SDW) to give a final concentration of 1000 mg l^{-1} (Carter, 1968). Two ml of each extract was evenly distributed on PDA in the Petri plates. Control plates received 2 ml of SDW each. Plates were left overnight in order for the solutions to be absorbed through the medium.

With a 10-cm-long spring-loaded plunger of 5 mm diameter, a plug of inoculum from the actively growing margin of a Petri plate culture of each fungal isolate (Table 1) was placed in the center of each Petri plate with the mycelium face down. Each isolate was inoculated onto four plates for each extract and incubated for 9 days at room temperature (~22°C). Four control plates receiving SDW only were run along each fungal isolate and crude extract. The same procedure was followed as in the samples.

Starting two days after inoculation, radial growth was marked every day for 7 days or until the plates were overgrown, whichever came first. The percentage of fungal growth inhibition due to each crude extract was calculated as follows: % inhibition = [(growth in control – growth in sample)/(growth in control) × 100]; growth was measured in mm of colony diameter. The values reported for percent inhibition (Table 4) were the means of four determinations each. Standard errors were calculated and are shown.

Results and discussion

Extensive column chromatography, TLC and preparative TLC of the petroleum ether extract of the roots of *F. harmonis* yielded two compounds which were characterized mainly by spectroscopic methods and by comparison with data from the literature (Miski *et al.*, 1983). The similarity of

Table 4. Inhibition of fungal growth by crude extracts from the roots of *Ferula harmonis*.

Fungus	Percent inhibition (%) [1]									
	PE[2]	PPE[3]	W[4]	EA[5]	M[6]	PEA[7]	EA-W[8]	PE-EA[9] (80:20)	PE-EA[10] (90:10)	Average
Verticillium dahliae	100	100	100	100	100	100	100	100	100	100
Fusarium oxysporum	26.6±2.9	100	0	49.6±2.0	0	0	0	0	0	19.6
Rhizopus stolonifer	84.6±4.9	67.9±5.4	2.1±0.1	69.6±2.9	11.3±0.1	12.5±0.1	91.7±0.1	86.7±0.3	37.1±2.3	51.5
Penicillium italicum	100	86.4±1.8	70.5±1.3	80.0±3.3	83.5±1.1	0	100	26.5±3.1	0	60.8
Rhizoctonia solani	48.4±3.5	69.7±4.0	42.4±3.1	60.1±3.4	38.3±3.1	40.1±5.7	51.1±1.8	64.7±4.4	40.9±3.9	50.6
Stemphylium solani	23.8±3.1	31.0±2.8	2.4±0.4	42.5±2.2	6.7±1.0	40.2±5.1	31.4±4.3	2.8±0.4	3.7±0.3	20.5
Alternaria solani	0	6.3±0.8	17.4±2.0	19.8±2.2	20.8±3.1	12.9±1.5	9.6±1.4	24.2±3.2	15.0±2.0	14.0
Cladosporium sp.	20.4±2.8	6.8±1.4	4.1±0.1	23.6±2.6	3.2±0.4	17.3±2.1	33.9±2.4	22.4±3.1	5.4±1.0	15.2
Mucor sp.	82.0±2.2	61.5±1.1	4.1±0.03	78.9±3.3	39.8±2.6	25.7±3.1	67.3±2.8	25.8±3.1	11.4±2.0	44.1
Colletotrichum sp.	49.0±5.1	41.5±6.1	3.3±0.2	79.5±3.1	5.6±0.8	79.5±2.9	47.0±5.3	26.5±2.2	21.0±3.3	39.2
Pythium sp.	0	0	68.5±4.9	77.5±1.1	37.9±2.0	75.0±1.9	19.4±2.0	0	52.6±6.1	36.8
Average	48.6	51.9	28.6	61.9	31.6	36.7	50.1	34.5	26.1	

[1] Percent inhibition is the mean ± SE of four determinations per fungus per extract. Values are averages of 2 experiments.
[2] Petroleum ether crude extract.
[3] Purified petroleum ether crude extract.
[4] Water crude extract.
[5] Ethyl acetate crude extract.
[6] Methanol crude extract.
[7] Ethyl acetate purified crude extract.
[8] Ethyl acetate and water (50:50) purified crude extract.
[9] Petroleum ether-ethyl acetate (80:20) purified crude extract.
[10] Petroleum ether-ethyl acetate (90:10) purified crude extract.

the spectral patterns of these compounds suggested that they were structurally related. The UV, IR, ^{1}H-NMR and ^{13}C-NMR of the isolated compounds showed that they were related to jaeskeanadiol (**1**) (Fig. 1) by the characteristic upfield methyl doublets in the ^{1}H-NMR spectra centered at σ 0.83–0.84 and 0.94–0.96 (*J*=7 Hz, H-14 and H-13). The ^{1}H-NMR spectra also suggested that both compounds carried a hydroxyl group at C-4. The IR and ^{13}C-NMR data revealed the presence of an ester function, σc 166.5–167.7, which was placed at the usual C-6 position on the basis of the chemical shift and multiplicity of the carbinilic proton, σ 5.32 (1H, *dt*, *J*=3, 10 Hz). The ^{1}H-NMR spectra of both compounds contained the signals due to vinylic proton at σ 5.56 (1H, *t, J*=6.5 Hz, H-9) showing the presence of the trisubstituted double bond as in compound **1**. This was confirmed by the ^{13}C-NMR data (Table 3), by mass spectra and by comparison with the literature data (Miski *et al.,* 1983). The IR spectrum of compound **1a** exhibited absorption for a hydroxyl group at 3360 cm^{-1} and for phenolic hydroxyl at 3450 cm^{-1}. The ^{1}H-NMR showed the signal of the phenolic hydroxyl proton at 8.6. The signals at σ 6.95 and 7.9 (*d*, *J*=7.8 Hz) indicated that the aromatic moiety was para substituted. The mass spectra showed the molecular ion $[M]^{+}$ at 358, which confirmed that this compound was ferutinin. This compound was obtained previously from the roots of *F. kuhistanica* and from the aerial parts of *F. elaeochytris* (Miski *et al.,* 1983).

The IR spectrum of compound **1b** exhibited absorption for a hydroxyl group (3360 cm^{-1}) and no absorption was detected in the region of the phenolic hydroxyl group. The ^{1}H-NMR revealed no signals at 8.6, which indicated that the aromatic ring was not substituted by the hydroxyl

group as in compound **1a**. The signals at σ 7.46 (2H, *dt*, *J*=2, 9 Hz) and 8.03 (2H, *dd*, *J*=2, 9 Hz) indicated that the aromatic moiety was ortho substituted. The mass spectra showed the molecular ion $[M]^+$ at 342, which confirmed that this compound was teferidine. This compound was previously obtained from the fruits of *F. tenuisecta* and *F. elaeochytris* (Miski *et al.,* 1983). Although compounds **1a** and **1b** were previously isolated, no antimicrobial studies on them are cited in the literature.

Extracts from the roots of *F. harmonis* used in this study demonstrated various degrees of fungitoxicity towards all fungi tested (Table 4). Complete inhibition of *V. dahliae* was achieved with all nine root extracts. *A. solani*, on the other hand, was the least responsive to the extracts, with an overall average inhibition of 14%. *P. italicum*, *R. stolonifer* and *R. solani* ranked second highest in their response to root extracts, with an approximate overall average growth inhibition of 50%. Growth of *Mucor* sp., *Colletotrichum* sp. and *Pythium* sp. was greatly inhibited (37–45%) when the extracts were added to the medium prior to inoculation with these fungi. A lower growth inhibition of 14–20%, was achieved against *F. solani*, *S. solani*, *Cladosporium* sp. and *A. solani*. The fungistatic effect of *F. harmonis* extract ranged between 14 and 100%.

Extract EA had the most extensive fungitoxic effect, with an overall average inhibition of 62% of all fungi studied. Inhibition values ranged from 19.8 to 100%. The fungitoxic effect of extract EA was greatest against *V. dahliae,* lowest against *A. solani* and *Cladosporium* sp. (Table 4). Extracts PPE, EA-W, and PE were the second most efficient, with inhibition averages of 52, 50, and 49% respectively. PPE completely inhibited *V. dahliae* and *F. solani* but failed to inhibit *Pythium* sp. The fungitoxic effect of EA-W was very high against *V. dahliae* and *P. italicum* (100%) and against *R. stolonifer* (92%), but was 0% against *F. solani* and *Pythium* sp. PE completely inhibited *V. dahliae* and *P. italicum* but did not inhibit *A. solani* or *Pythium* sp. at all. A third group of extracts comprised PEA, PE-EA (80:20), and M with inhibition percentages of 37, 34 and 32% respectively. These three extracts were highly effective against *V. dahliae* (100%) but not against *F. solani* (0%). PE-EA (80:20) was not effective against *Pythium* sp. and PEA was not effective against *P. italicum*. The least effective group included extracts PE-EA (90:10) (26% inhibition) and W (22%). Both these extracts were highly effective against *V. dahliae,* but not effective against *F. solani*. PE-EA (90:10) was also not effective against *P. italicum*.

Fusarium oxysporum was highly sensitive to PPE (100% inhibition) followed by EA (50%) and PE (27%). The other six extracts did not inhibit *F. oxysporum* in this experiment. PE and EA-W provided 100% inhibition of *P. italicum*. Against *F. oxysporum*, only PE, PPE and EA were effective. PEA and PE-EA (90:10) were ineffective not only against *F. oxysporum* but also against *P. italicum*; PE was ineffective against *A. solani* and *Pythium* sp.; and PPE and PE-EA (80:20) failed to inhibit *Pythium* sp.

The lowest overall activity (14%) was demonstrated against *A. solani* followed by *F. oxysporum* (19.6%). The least effective extract overall (26.1%) was PE-EA (90:10); the most effective EA (61.9%). More than 50% inhibition of various fungi was obtained with PPE, EA and EA-W. PE (48.6%) also exhibited good potential as a fungitoxic biocide.

In this first study on the fungitoxic effect of *F. harmonis* against some plant pathogenic fungi, root extracts were a promising source of fungitoxic compounds. Variations in the effect of different extracts on different fungi were most likely due to differences in the nature of the inhibitory materials they contained.

Of the nine extracts tested, ethyl acetate crude extract had the highest inhibitory effect (62%) against all fungi tested. Water crude extract was the least effective. Petroleum ether crude extract, ethyl acetate – water crude extract and petroleum crude extract gave approximately 50% growth inhibition overall. In general, crude extracts were ranked according to their fungitoxic ability as follows: 1. ethyl acetate crude extract; 2. purified petroleum ether crude extract; 3. ethyl acetate-water crude extract; 4. petroleum ether crude extract; 5. ethyl acetate purified crude extract; 6. petroleum ether-ethyl acetate (80:20) crude extract; 7. methanol crude extract; 8. petroleum ether-ethyl acetate (90:10) crude extract; and 9. water crude extract.

The fungitoxic activity of the ethyl acetate ex-

tract might be due to the ferutinin contained in it, which was isolated from it as described in the experimental part. Ferutinin was not found in the methanolic extract even though that extract exerted significant antimicrobial activity. This suggested that the methanolic extract contained other antimicrobial substance(s). Further studies on the ethyl acetate and methanol extracts are needed to isolate the compounds responsible for antimicrobial activity.

In the higher plants, the active principle is influenced by several factors such as plant age, age of the organism tested, method of extraction and time of harvesting the plant materials (Walker *et al.*, 1937; Gillver, 1947; Nicolls, 1970; Shekhawat and Pracada, 1971; Franje, 1984). The findings support the idea that these active substances are present in plants in an inactive form and become active only if the plant or its parts are properly processed. In addition, there is considerable diversity in the methods used in studies such as the present, and different results are obtained with different experimental techniques (Qasem, 1989). This emphasizes the need for more efficient, convenient and cheaper methods of extraction so as to encourage a more extensive use of fungicidal extracts, especially if greater quantities must be prepared for large-scale production.

The use of indigenous products for plant disease control is a very promising field, and encouraging results have been reported by different authors (Misra and Dixit, 1976; Chaudhuri, 1982; Mahmood, 1985; Akhtar *et al.*, 1986; Asthana, 1986; Al-Bana and Hijazi, 1987; Chaturvedi, 1987; Al-Abed *et al.*, 1993).

Our findings suggest that *F. harmonis* is a potential source of a fungitoxic biocide that can control the growth of many fungi (Table 1).

Acknowledgements

The authors acknowledge the technical assistance of Wesam Shahrour.

Literature cited

Ahmed A.A., 1991. Daucanes and other constituents from *Ferula sinaica*. *Phytochemistry* 30(4), 1207–1210.

Akhtar T., A. Sattar and I. Khan, 1986. Antifungal activity of some plant extracts against potato dry rot of *Fusarium*. *Sarbad Journal of Agriculture* 2, 187–191.

Al-Abed A.S., J.R. Qasem and H.A. Abu-Blan, 1993. Antifungal effects of some common wild plant species on certain plant pathogenic fungi. *Dirasat (Pure and Applied Sciences)* 20B(3), 149–158.

Al-Bana G.I. and A.A. Hijazi, 1987. *Evergreen Orchards*. Al-Dar Al-Arabia Lil Nashr Wa Al-Tawzeia, Egypt, 509 pp (in Arabic).

Al-Mughrabi K.I., T.A. Abujai, G.H. Anfoka and W. Shahrour, 2001. Antifungal activity of olive cake extracts. *Phytopathologia Mediterranea* 40, 240–244.

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

Appendino G., S. Tagliapietra, L. Paglino, G.M. Nano, D. Monti and V. Picci, 1990. Sesquiterpenoid esters from the fruits of *Ferula communis*. *Phytochemistry* 29(5), 1481–1484.

Asthana A, N.N. Tripathi and S.N. Dixit, 1986. Fungitoxic and phytotoxic studies with essential oil *Ocimum adscendens*. *Journal of Phytopathology* 117, 152–159.

Carter G.A., 1968. *Studies on Systemic Fungicides*. Ph.D. Thesis, University of London, UK, 217 pp.

Chaturvedi R., A. Dikshit and S.N. Dixit, 1987. *Adenocalymma allicea*: A new source of a natural fungitoxicant. *Tropical Agriculture* 64, 318–322.

Diaz J., B.M. Fraga, A.G. Gonzalez, M.G. Hernandez and A. Perales, 1986. Carotane sesquiterpenes from *Ferula linkii*. *Phytochemistry* 25(5), 1161–1165.

Franje N.S., 1984. *Evaluation of Medicinal Plant Extracts as Protectants and Therapeutants against Legume Pathogens*. Ph.D. Thesis, University of the Philippines, Diliman, Philippines.

Frensh D. M., 1971. Ethnobotany of Umbelliferae. In: *The Biology and Chemistry of The Umbelliferae* (V.H. Heywood, ed.), Academic Press, London, UK, 400–401.

Gillver K., 1947. The effect of plant extracts on the germination of conidia of *Venturia inaequalis*. *Annals of Applied Biology* 34, 136–143.

Lamnaouer D., M.T. Martin, D. Molho and B. Bodo, 1989. Isolation of daucane esters from *Ferula communis* var. *brevifolia*. *Phytochemistry* 28(10), 2711–2716.

Mahmood E.A.H., 1985. *Effect of Plant Extracts on Some Fungal Plant Pathogens*. M.Sc. Thesis, University of Baghdad, Iraq.

Miski M. and T.J. Mabry, 1985. Daucane esters from *Ferula communis* subsp. *communis*. *Phytochemistry* 24(8), 1735–1741.

Miski M., A. Ulubelen and T.J. Mabry, 1983. Six sesquiterpene alcohol esters from *Ferula elaeochytris*. *Phytochemistry* 22(10), 2231–2233.

Miski M. and J. Jakupovic, 1990. Cyclic faresyl-coumarin and farnesyl-chromone derivatives from *Ferula communis* subsp. *communis*. *Phytochemistry* 29(6), 1995–1998.

Misra S.B. and S.N. Dixit, 1976. Comparison of activity of the active principle of *Clematis gouriana* with commercial fungicides. *Indian Phytopathology* 29, 448.

Nicolls J.R., 1970. Antifungal activity in *Passiflora* species. *Annals of Botany* 34, 229–237.
Qasem J.R. and T.A. Hill, 1989. On difficulties with allelopathy methodology. *Weed Research* 29, 345–347.
Shekhawat P.S. and R. Pracada, 1971. Antifungal properties of some plant extracts. I. Inhibition of spore germination. *Indian Phytopathology* 24, 800–802.
Valle M.G., G. Appendino, G. Nano and V. Picci, 1986. Prenylated coumarins and sesquiterpenoids from *Ferula communis*. *Phytochemistry* 26(1), 253–256.
Walker J.C., S. Morrell, and H.H. Foster, 1937. Toxicity of mustard oil and related sulfur compounds to certain fungi. *American Journal of Botany* 24, 536–541.
Zohary M., 1966. Flora Palestina. The Israel Academy of Science and Humanities, Jerusalem, Israel, 58–60.

Accepted for publication: June 9, 2003