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Antimycotic Activities of Selected Plant Flora, Growing Wild in Lebanon, against Phytopathogenic Fungi

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Petroleum ether (PE) and methanolic extracts of nine wild plant species were tested in vitro for their antimycotic activity against eight phytopathogenic fungi. The efficacy of PE extracts against all pathogens tested was higher than that of methanolic extracts. Wild marjoram (*Origanum syriacum*) PE extract showed the highest and widest range of activity. It resulted in complete inhibition of mycelial growth of six of eight fungi tested and also gave nearly complete inhibition of spore germination of the six fungi included in the assay, namely, *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., *Cladosporium* sp., *Fusarium oxysporum* f. sp. *melonis*, and *Verticillium dahlia*. The other plant extracts showed differential activities in the spore germination test, but none was highly active against mycelial growth. *Inula viscosa* and *Mentha longifolia* were highly effective (>88%) in spore germination tests against five of six fungi tested, whereas *Centaurea pallescens*, *Cichorium intybus*, *Eryngium creticum*, *Salvia fruticosa*, and *Melia azedarach* showed >95% inhibition of spore germination in at least two fungi. *Foeniculum vulgare* showed the least antimycotic activity. Fractionation followed by autobiography on TLC plates using *Cladosporium* sp. as a test organism showed that *O. syriacum* PE extracts contained three inhibition zones, and those of *Inula viscosa* and *Cichorium intybus*, two, whereas the PE extracts of the remaining plants showed each one inhibition zone. Some of the major compounds present in these inhibition zones were identified by GC-MS. The possibility for using these extracts, or their mixtures, to control plant diseases is discussed.

KEYWORDS: ***Centaurea*; *Cichorium*; *Eryngium*; fungal toxicity; *Inula*; *Mentha*; *Melia*; *Origanum*; *Salvia***

INTRODUCTION

During the past three decades there has been increased awareness of environmental pollution and its negative impact on all forms of life. The use of toxic pesticides, but mainly their misuse, has contributed significantly to this pollution. The trend, at present, is toward safer and more environmentally friendly pest management compounds. Pest populations are dynamic; they may develop resistance to the pesticides at differing times depending on the pest, the mode of action of the pesticide, and the frequency of use. Resistance to pesticides has been reported earlier (*1*−*3*). Therefore, there is a continuing need for the development of alternatives to chemicals used for pest control in agriculture.

Aromatic plants have been known since antiquity to possess biological activity, notably antibacterial and antifungal properties (*4*, *5*). They have been widely used to extend the shelf life of foods and in folk medicine (*6*). Fungitoxicants from higher plants may have a better future than synthetic fungicides largely due to their non-phytotoxic, systemic, and easily biodegradable nature (*7*, *8*). Therefore, interest in secondary metabolites from plant extracts and mainly essential oils as potential antimicrobial agents for use in food preservation, crop protection, and pharmacological applications has increased during the past decade (*9*). Furthermore, the rapid rise in demand for organically produced fruits and vegetables will increase the demand for natural pesticides.

Plant extracts and essential oils have been found to have antifungal activities against a wide range of plant pathogenic fungi. Extracts from neem, wild marjoram, and mint were shown to possess good fungicidal, bactericidal, or nematicidal activity (*10*−*14*). The production and quality of essential oils depend on two main factors, the vegetative stage and the geographic region (*15*, *16*). Lebanon is rich in plant flora, including several wild species of mint, wild marjoram, and tayyoun. The present study investigated the antifungal in vitro activity of nine wild plant species against eight genera of plant-pathogenic fungi that cause important diseases: root rots, flower and fruit rots, vascular wilts, and leaf spots or blights. It gives the first report of the antifungal properties of *Centaurea pallescens* and identifies some of the compounds that may be responsible for the antifungal activity.

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Table 1. Scientific Name, Common Name, and Family of Plant Species Used in This Study

scientific name	family	common name
Origanum syriacum Sieb. Exs. et L.	Labiatae	wild marjoram
Mentha longifolia (L.) Huds.	Labiatae	mint
Salvia fruticosa L.	Labiatae	sage
Cichorium intybus (L.)	Compositae	wild chicory
Inula viscosa (L.) Ait.	Compositae	inula
Centaurea pallescens Del.	Compositae	centaury
Eryngium creticum L.	Umbelliferae	eryngo
Foeniculum vulgare Mill.	Umbelliferae	fennel
Melia azedarach L.	Meliaceae	Chinaberry tree

MATERIALS AND METHODS

Plant Material and Extraction. Samples were collected from wild plants mostly at the flowering stage and identified by Professor C. Abou-Chaar (Plant Taxonomist, Emeritus Professor at the American University of Beirut) (**Table 1**). Preliminary screening tests for the evaluation of the antimycotic activity were performed with methanolic extracts of nine plant species, without a primary petroleum ether (PE) extraction. Because the autobiographic assays of direct methanolic extracts on TLC were difficult (not well resolved) with some samples, the technique was modified consequently to use a primary extraction with PE. Fifty grams of fresh leaves from each plant species was cut into small pieces, macerated with 250 mL of petroleum ether, left standing for 30 min, and filtered through a sintered glass funnel; the residue was extracted twice with PE in the same manner. The remaining plant residue was dried at room temperature and extracted with methanol dried over CaO. PE extracts were pooled and rotary evaporated at 40 °C; the oil fraction was collected with absolute ethanol, and the final volume brought to 5 mL. The methanol extract was concentrated in a similar manner and the residue taken up with methanol. All solvents used were of analytical grade.

Microorganisms. All fungi used in this study were isolated at the American University of Beirut from infected plant organs. *Botrytis cinerea*, *Alternaria solani*, *Penicillium* sp., and *Cladosporium* sp. cause flower, fruit, or seed rots; *Fusarium oxysporum* f. sp. *melonis* and *Verticillium dahlia* cause vascular wilts; *Phytophthora infestans* causes late blight of potato; and *Rhizoctonia solani* causes mainly root rots.

Antimycotic Tests. The toxicity of plant extracts against the eight fungi was tested using a poisoned plate technique. Plant extracts, diluted in either ethanol or methanol, were added to the culture medium (potato dextrose agar, PDA) after autoclaving, when the temperature of the medium reached about 50 °C, and mixed thoroughly. The final concentration was adjusted to 2 mL of extract in 98 mL of PDA, equivalent to 20 g of fresh material/100 mL. Unamended PDA plates and plates amended with either methanol or ethanol at concentrations equal to those used with the extracts served as controls.

Mycelial Inhibition Tests. Mycelial growth inhibition tests were performed by placing 5 mm mycelial agar disks, cut from the margin of expanding fungal colonies, in the center of amended or unamended PDA plates. Colony diameter was measured after incubation for 7 days, in the dark, at 22 °C. All treatments were replicated three times and repeated at least once. The percent inhibition was calculated after subtraction of the diameter of the initial inoculum disk.

Spore Germination Tests. Spore suspensions ($10^6 \times mL^{-1}$) of six fungi were prepared from actively growing colonies (7−11 days old). Ten microliter drops were added to PDA plates amended with plant extracts or with ethanol or methanol. Plates were incubated at 22 °C until the germination in the control reached >70% (6−15 h according to the rate of germination of each fungus), and then a drop of 37% formaldehyde was added to the medium to inhibit further development of germ tubes. A spore was considered to be germinated when the length of the germ tube was more than twice the length of the spore. Three replicates were conducted for each treatment, and a minimum of 200 spores were counted in each replicate. The percent inhibition was calculated according to Abbott's formula:

$$\frac{(\%\ \text{living in control} - \%\ \text{living in treatment})}{\%\ \text{living in control}} \times 100$$

Autobiography. Thin-layer chromatography (TLC) using silica gel G60 F254 (Merck) was performed with 10−50 μL of concentrated plant extracts as described above. The following solvent systems were tried: chloroform/methanol (9:1 v/v), *n*-propanol/water:ethyl acetate (7:1:2 v/v/v), choroform/methanol/triethylamine (90:9:1 v/v/v), ammonia/butanol (2:5 v/v), chloroform/methanol (6:4 v/v/v), and chloroform/methanol/acetic acid (90:10:1 or 0.5 v/v/v).

After development of the chromatograms, the silica plates were left to dry in a forced-air oven at 37 °C for 1 h. Half of the silica plates were sprayed with 2 mL of potato dextrose broth containing a spore suspension ($10^6 \times mL^{-1}$) of the test organism *Cladosporium* sp. Zones of inhibition could be visually detected following incubation at 100% relative humidity for 3−5 days at room temperature. Three replicates were conducted. From the other half of the developed TLC plate, zones that corresponded to the zones of inhibition were scraped, eluted with absolute ethanol, and centrifuged using an Eppendorf centrifuge (model 5419) at 10000*g* for 15 min, and the supernatant was used for GC-MS analysis after evaporation of the solvent and dilution with ethyl acetate.

Analytical Techniques. Eluted fractions from zones of inhibition of TLC plates were chromatographed and analyzed using a GC-MS gas chromatograph (HP 6890/HP 5972 with a mass selective detector) equipped with an HP-5 capillary column (5% diphenyl− 95% dimethylsiloxane copolymer, 30 m × 0.25 mm i.d.). The oven temperature was held at 70 °C for 5 min, programmed to 220 °C at 4 °C /min and then at 10 °C/min to 290 °C, and finally maintained isothermally for 10 min. Injector temperature was 300 °C. Components of the major peaks were identified by comparison of their mass spectra with those reported in the following libraries: Wiley 275.L, NBS 75K.L, and PMW-TOX 2.L.

RESULTS

Antimycotic Tests. Of the plants used in the preliminary screening tests using direct methanol extraction, only those that showed >60% spore germination inhibition of at least one fungus were used in further tests. *Malva* and *Calyctome* species were eliminated, whereas *Foeniculum vulgare* was retained as an example with a low level of antifungal activity.

In the two antimycotic tests performed, the mycelial growth inhibition test and the spore germination inhibition test, the PE extracts showed a higher efficacy for the control of fungi than the methanolic extracts. Consequently, the discussion will focus mainly on the PE extracts. Inhibition of spore germination was higher than inhibition of mycelial growth. Only extracts of wild marjoram (*O. syriacum*) showed a high activity against mycelial growth, with 100% inhibition of *A. solani*, *Cladosporium* sp., *F. oxysporum* f. sp. *melonis*, *P. infestans*, and *R. solani*, whereas its efficacy on *B. cinerea* and *Penicillium* sp. was 45 and 48%, respectively (**Table 2**). The other two Labiatae species tested, *Men. longifolia* and *S. fruticosa*, showed a lower activity than *O. syriacum. I. viscosa* also showed a moderate efficacy against mycelial growth of seven of the eight fungi tested, with efficacy ranging between 52 and 66%. *Ce. pallescens* showed a moderate activity against five fungi. The other plant extracts had low activity against mycelial growth.

In spore germination inhibition tests, the three species of Labiatae tested showed a high level of antimycotic activity. The PE extracts of *O. syriacum*, *Men. longifolia*, and *Ce. pallescens* (to a lesser extent) were highly active against the six fungi tested, whereas that of *S. fruticosa* was active on *V. dahlia*, *B. cinerea*, *F. oxysporum* f. sp. *melonis*, and *A. solani* but not on *Penicillium* sp. and *Cladosporium* sp. (**Table 3**). The PE extracts of *I. viscosa* and *Ci. intybus* showed a high inhibitory activity of spore germination of *A. solani*, *B. cinerea*, *Cladosporium* sp.,

Table 2. Antimycotic Activity of Plant Extracts As Determined by Mycelial Growth Inhibition Tests

	% inhibition of mycelial growth[a] of							
plant extract	*Botrytis cinerea*	*Alernaria solani*	*Cladosporium* sp.	*Fusarium oxysporum* f. sp. *melonis*	*Phytophthora infestans*	*Rhizoctonia solani*	*Penicillium* sp.	*Verticillium dahlia*
O. syriacum PE[b]	45[c]	100	100	100	100	100	48.3	100
O. syriacum M[b]	0	48	53	12	0	12	0	18
S. fruticosa PE	28	58	16	32	0	61	19	17
S. fruticosa M	0	48	0	17	0	0	0	0
I. viscosa PE	60	63	22	65	52	65	54	67
I. viscosa M	40	6	10	0	0	29	0	0
Ci. intybus PE	0	5	0	2	0	0	0	0
Ci. intybus M	0	0	0	10	0	0	0	0
E. creticum PE	0	10	0	17	0	0	0	16
E. creticum M	0	3	0	23	0	9	0	32
Mel. azedarach PE	0	1	0	16	0	57	38	0
Mel. azedarach M	0	0	0	3	0	31	0	0
Men. longifolia PE	59	7	0	0	0	70	0	8
Men. longifolia M	0	0	0	19	0	24	0	0
Ce. pallescens PE	0	60	62	72	33	51	60	0
Ce. pallescens M	0	5	52	47	12	9	0	0
F. vulgare M[d]	0	12	0	0	0	0	0	0

[a] Data collected after 7 days of incubation. The average colony diameters of the controls were 4.3, 4.8, 2.2, 3.7, 2.8, 5.2, 1.9, and 1.9 cm, respectively. [b] PE and M refer to petroleum ether and methanol extracts, respectively. [c] Average of three replicates. [d] Methanolic extract without primary extraction with petroleum ether.

Table 3. Antimycotic Activity of Plant Extracts As Determined by Spore Germination Inhibition Tests

	% inhibition of spore germination[a]					
plant extract	*B. cinerea*	*A. solani*	*Cladosporium* sp.	*F. oxysporum* f. sp. *melonis*	*Penicillium* sp.	*V. dahlia*
O. syriacum E[b]	100[c]	94	100	99	100	100
O. syriacum M[b]	85	89	77	98	90	79
S. fruticosa PE	100	86	22	98	20	100
S. fruticosa M	99	44	20	90	0	45
I. viscosa PE	100	89	100	97	66	100
I. viscosa M	98	9	47	79	10	72
Ci. intybus PE	99	90	80	97	40	80
Ci. intybus M	38	60	47	89	5	20
E. creticum PE	98	66	33	97	66	75
E. creticum M	43	3	36	69	75	27
Mel. azedarach PE	84	75	87	95	53	100
Mel. azedarach M	54	17	14	80	40	0
Men. longifolia PE	100	80	98	90	98	94
Men. longifolia M	65	30	40	70	5	43
Ce. pallescens PE	92	92	68	100	85	76
Ce. pallescens M	37	54	13	99	35	8
F. vulgare M[d]	47	0	56	53	0	64

[a] Percent inhibition was calculated according to Abbot's formula. The average spore germination of the controls ranged between 71 (*Botrytis*) and 91% (*Cladosporium*). [b] PE and M refer to petroleum ether and methanol extracts, respectively. [c] Average of three replicates. [d] Methanolic extract without primary extraction with petroleum ether.

F. oxysporum f. sp. *melonis*, and *V. dahlia* but not of *Penicillium* sp. *Mel. azaderach* showed a moderate to high level of activity against five of the six species of fungi tested, whereas *Eryngium creticum* showed a high level of activity against only two fungi, *B. cinerea* and *F. oxysporum* f. sp. *melonis*. *Foeniculum vulgare* showed the least antimycotic activity in the spore germination tests.

Autobiography. Between 10 and 50 μL of the concentrated plant extracts was analyzed by TLC. Six solvent systems were initially tried to develop the TLC plates. In a few instances, the addition of 1% acetic acid improved the separation into sharper bands. However, acetic acid was a potent inhibitor of fungal growth, even when TLC plates were incubated at 37 °C for 2 h before the fungal spore suspension was applied. The best resolution was obtained with chloroform/methanol (9:1 v/v). The size of the inhibition zones, and sometimes their number, varied

Table 4. Antimycotic Activity of Plant Extracts: R_f Values of Inhibitory Zones Obtained Following Chromatography on TLC Plates[a] Using Chloroform plus Methanol (9 + 1 by Volume) as Eluent and Inoculation of the TLC Plates with *Cladosporium* Species

	R_f of inhibition zones	
plant extract	PE extracts	methanol extracts
O. syriacum (30 μL)	0.61–0.68[b]	0.08–0.24
	0.75–0.84	0.72–0.76
	0.85–0.91	0.87–0.99
I. viscosa (20 μL)	0.38–0.55	0.60–0.75
	0.67–0.92	0.79–0.95
Men. longifolia (50 μL)	0.89–0.96	0.84–0.99
S. fruticosa (50 μL)	0.71–0.99	0.13–0.43
Ci. intybus (30 μL)	0.74–0.98	0.13–0.53
		0.87–0.99
E. creticum (30 μL)	0.80–0.99	
Ce. pallescens (30 μL)	0.46–0.58	0
		0.11–0.46
		0.92–0.99

[a] The volume of plant extract used on TLC plates is indicated in parentheses. [b] R_f values are reported based on the lower and upper border of theinhibition zone; this was done because some of the inhibition zones were quite large.

depending on the volume of extract applied. Between 10 and 20 μL of *O. syriacum* and *I. viscosa* extracts was enough to observe two good inhibitory zones in each case, whereas the number of inhibitory zones increased to three when >30 μL of *O. syriacum* extracts was tested. Between 30 and 50 μL was needed to observe good inhibitory zones with the other plant extracts. When 30−50 μL of plant extracts was applied on TLC plates, PE extracts of *O. syriacum* showed three inhibitory zones; those of of *I. viscosa*, two; and those of *Men. longifiola*, *S. fruticosa*, *Ci. intybus*, *Ce. pallescens*, and *E. creticum*, one (**Table 4**). Methanolic extracts of *O. syriacum* and *Ce. pallescens* showed three inhibition zones; those of of *I. viscosa* and *Ci. intybus*, two; those of of *Men. longifolia a*nd *S. fruticosa*, one; and those of of *E. creticum*, none. When more than one inhibition zone was observed, at least one had a different R_f than those of the PE extracts.

GC-MS Analysis. The natural products present in the inhibition zones (on TLC plates) of PE extracts were eluted and analyzed by GC-MS. In some cases, one compound was

Table 5. Major Compounds Detected in the Inhibition Zones of PE Extracts

plant extract	R_f	major compounds identified[a]
O. syriacum	0.61–0.68	azulene derivative; 1,7,7-trimethyl-bicyclo[2.2.1] heptan-2-one; octadecanoic acid; 3-phenyl-4-hydroxyacetophenone
	0.75–0.84	thymol; some other compounds at low concentrations
	0.85–0.91	naphthalenol; some other compounds at low concentrations
Ce. pallescens	0.46–0.58	alloaromadendrene; ledene (azulene compound); β-patchoulene
Ci. intybus	0.87–0.99	aristolone; hexatriacontane; decadiene-5,6-diol; tetracosanoic acid; piperazine derivative; menthol
E. creticum	0.82–0.95	β-bisabolene; caryophellene oxide; (+)-spathulenol; L-menthol
I. viscose	0.38–0.55	unidentified compounds
	0.67–0.92	azulene derivative, unidentified compounds
S. fruticosa	0.71–0.99	manool; xylometazoline (otrivin); viridiflorol; 5,7-dimethyl-1-naphthol; caryophellene oxide; phenanthrene derivative; stigmast-5-en-3-ol; β-eudesmol

[a] Compounds listed in order of their decreasing relative peak area.

present in much higher concentration than the others; for example, thymol and naphthalenol in *O. syriacum* (R_f 0.8 and 0.9, respectively), naphthalene propanol (manool) in *S. fruticosa* (R_f 0.71−0.99), and alloaromadendrene (R_f 0.461−0.58) in *Ce. pallescens*. However, in most cases several substances were usually detected in each inhibition zone (**Table 5**).

DISCUSSION

Among the nine species of plants tested for their potential use for the management of plant pathogenic fungi, *O. syriacum*, *Men. longifolia*, and *I. viscosa* were shown to possess the highest in vitro antimycotic activities. Only *O. syriacum* had high mycelial inhibitory efficacy, whereas most other plants tested showed only inhibition of spore germination. The level of inhibition varied according to the plant species and the fungus tested. Our results were in agreement with a few studies that showed that extracts of *Origanum* spp. had a higher level of antimicrobial activity than those of other plants tested, including other species of Labiatae such as *Salvia* spp. and *Mentha* spp. (*5*, *6*, *17*). *S. fruticosa* showed only a moderate to low level of activity against phytopathogenic fungi (*6*, *18*). *Men. longifolia* was reported to show a high antimicrobial activity (*19*). Similarly, *I. viscosa* was shown to possess a very wide spectrum of activity against many animal, human, and plant pathogens including fungi, yeast, and bacteria (*20*, *21*). On the other hand, only little is known about the antimicrobial activity of *E. creticum*, *Ci. intybus*, and *Ce. pallescens* (*22*). Our assays showed that *E. creticum* was mainly active against *B. cinerea*, and *F. oxysporum* f. sp. *melonis*, *Ce. pallescens*, and *Ci. intybus* were active against *B. cinerea*, *Alternaria* sp., and *F. oxysporum* f. sp. *melonis* (**Table 3**). *Ce. pallescens* showed also a moderate level of mycelial growth inhibition. To our knowledge, this is the first report of the antifungal properties of *Centaurea* sp. The activity of extracts of *Melia* and the related neem tree on insects is well documented (*23*−*25*). This study showed that *Melia* extracts may also improve preventive activity against infection by vascular wilt diseases such as *Verticillium* and *Fusarium* species. Because most of the extracts tested, except *O. syriacum*, showed significant activity only on spore germination, their use would be limited to preventive treatments.

To determine the compounds with antimicrobial activities in plant extracts, most previous studies focused on the activity of the extract and tried to correlate the antimicrobial activity to the major constituents of the extracts. For the Labiatae species used in this study, the major reported essential oils in *S. fruticosa* were 1,8-cineole (eucalyptol) and camphor (*6*); in *M. longifolia*, these were piperitone, 1,8-cineole (eucalyptol), and isoborneol (*19*); and in *Origanum* sp., thymol was the essential oil. In some instances it was found that the antimicrobial activities of some plant species such as *Majorana*, *Origanum*, and *Thymbra* may be correlated with high levels of thymol and/or carvacrol. Effectively, thymol and carvacrol have been demonstrated to possess high antimicrobial activity (*6*, *15*, *26*). However, in some plants, none of the major essential oil components (γ-terpenene, β-cymene, 1,8-cineole, camphor, pulgeone, and menthone) showed any antifungal activity (*15*). Therefore, the observed antifungal activity may often be associated with a compound that is present in minor quantity but possesses a high biological activity, but its presence may be masked by the high concentration of other compounds. This study aimed to reduce this masking effect by performing an additional step of TLC fractionation of the complex mixture of compounds present in extracts (*27*), followed by analysis of only the compounds present in the inhibition zones. This technique was effective in some cases and allowed good separation of thymol and naphthalenol in *O. syriacum*, manool in *S. fruticosa*, and alloaromadendrene in *Ce. pallescens*. However, in the other cases, this technique was only partially successful, because although it eliminated many compounds, few compounds were usually still present in each inhibition zone as determined by GC-MS analysis (**Table 5**). This is due to comigration of some substances with the major active substance under our chromatographic conditions. This technique may be further improved by trying other solvent systems or by conducting HPLC or bidimensional chromatography. Furthermore, in our assays we have tried to apply a large enough volume of plant extract to ensure obtaining good inhibition zones—the zones of inhibition were somewhat large (**Table 3**). For future studies, it is recommended to use the least volume of extract that will allow obtaining an observable biological activity, with smaller inhibition zones and possibly a better separation of the biologically active compounds from the other compounds.

The technique used demonstrated that *O. syriacum* and *I. viscosa* each contain at least two major compounds or two sets of comigrating compounds, and to a lesser extent a third compound in *Origanum*, that are responsible for the antifungal activity in PE extracts. How these inhibitory substances interact to provide the total activity is yet undetermined. The methanolic extracts of *O. syriacum* and *I. viscosa* also had each, at least, one additional inhibitory compound that is not present in the PE extracts, as could be determined from the R_f values of the inhibitory zones (0.08−0.24 in *O. syriacum* and 0.79−0.92 in *I. viscosa*) that were not detected in the PE extracts. Our results indicate clearly that in *O. syriacum* there are at least four active compounds, or groups of compounds (some lipohilic and one hydrophilic), that are responsible for the antimicrobial activity, that thymol and carvacrol are responsible for only part of this

activity, and that naphthalenol and other unidentified compounds may be responsible for the other part.

Thymol was reported to be a major antimicrobial agent in *Majorana* and *Origanum* spp. (*6*, *26*), but naphthalenol was not reported earlier, even though the presence of naphthalene derivatives was reported in two Labiatae species (*28*). We also detected naphthalene propanol in *S. fruticosa* (Labiatae). With regard to *I. viscose*, the results in **Table 4** show the presence of at least three active compounds or groups of compounds, which were not identified. Two reports showed that the antimicrobial activity of *Inula* sp. was associated with the sesquiterpenoid lactones alantolactone and isoalantolactone (*20*) or with a new sesquiterpene, tayunin (*29*).

PE extracts of *S. fruticosa*, *Ci. intybus*, and *Ce. pallescens* had each only one inhibition zone that had different R_f values from the R_f of inhibition zones in their respective methanolic extracts. These results indicate clearly that the plant extracts contained other inhibitory compounds that were not extracted with PE. In addition, the extraction technique with PE apparently was not 100% effective in some cases, because some alcoholic compounds such as thymol and naphthalenol were also identified in the methanolic extracts of *Origanum* (data not presented). This explains why two inhibition zones had similar R_f values in the PE and methanolic extracts (**Table 4**).

The antimicrobial activities of some essential oils have been well documented. It would be interesting to investigate and/or confirm the antimicrobial properties of the other major compounds detected in the plant extracts used in this study, namely, manool (*S. fruticosa*), alloaromadendrene (*Ce. pallescens*), azulene derivatives (*Ce. pallescens*, *I. viscosa*, *O. syriacum*, and *S. fruticosa*), and some of the other compounds listed in the results (**Table 5**) as well as a few other minor compounds that were detected but not reported such as bicyclogermacrene, β-selinene, 2,4,6-octatrienal, and undeca-7,10-dien-4-ol.

Plant extracts may be used directly or after partial purification. In our TLC assays, some plant extracts such as *I. viscosa* contained polar compounds that gave a much more abundant fungal growth at low R_f values as compared to higher R_f values. This suggests that sometimes partial purification may prove to be very beneficial because plant extracts may contain many growth factors that will favor the pathogen growth or interfere with the antifungal activity.

The presence of a wide variety of natural products with inhibitory activity against fungi calls for more research on the possible synergistic activity of some of these natural products, mainly between *Origanum* and *Inula* or *Centaurea* species. This may result in a wider range of plant disease control and/or lower effective concentrations, reducing costs (locally produced infusions) and allowing a safer and more effective management of agricultural pests.

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