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Schinus molle: a New Source of Natural Fungitoxicant†

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The oil of *Schinus molle* exhibited the maximum fungitoxic activity during the screening of some essential oils against some common storage and animal pathogenic fungi. It showed absolute toxicity against animal pathogens and mild activity against storage fungi. The effective concentrations of the oil varied from 200 to 900 ppm. The toxicity of the oil persisted up to 80°C and 90 days of storage but declined when autoclaved. It withstood heavy inoculum density. The oil exhibited a narrow range of activity and was found to be more effective than Multifungin, an antifungal drug. The oil was characterized by its various physicochemical properties. It was found to comprise 50 constituents. It appeared that some changes in the oil constituents during storage affected its fungitoxic potency.

Naturally occurring fungitoxicants described to date are mostly biodegradable (3) and are devoid of side effects (9) compared with commercially available fungitoxicants.

We report here results of our investigation of *Schinus molle* L., a member of the Anacardiaceae, as a new source of fungitoxicant. Essential oils extracted from higher plants were investigated for their fungitoxic activity; test fungi used were those which caused dermatomycoses in animals and fungal deterioration of foodstuffs during storage.

MATERIALS AND METHODS

Essential oils were extracted from different parts of 10 taxa of phanerogams by hydrodistillation with a Clevenger apparatus (4) and were evaluated at various concentrations

and storage fungi such as *Alternaria alternata* (Fries) Keissler, *Aspergillus flavus* Link, and *Penicillium italicum* Wehmer. The evaluations were performed by using the "poisoned food" technique (7) with Sabouraud dextrose agar medium.

Minimum fungistatic and fungicidal concentrations of the oils were evaluated by the method of Garber and Houston (10). The influence of temperature, storage, and inoculum density on the mycelial growth inhibition (MGI) of the oils at 500 ppm were observed as previously described by Dikshit and Dixit (6).

A total of 19 animal or phytopathogenic fungi were used to test the MGI of *S. molle* oil (500 ppm) by the conventionally used poisoned food technique. The fungistatic-fungicidal

TABLE 1. Inhibition of animal pathogenic and storage fungi by essential oils from higher plants

Species	Family	Oil source	MGI (%) against:					
			Animal pathogenic fungi ^a			Storage fungi ^b		
			Mg	Tm	Tr	Aa	Af	Pi
<i>Apium graveolens</i> L. (celery)	Apiaceae	Seed	16.6	26.6	31.8	28.0	57.1	41.1
<i>Cinnamomum cecidodaphne</i> Meissn. (Sugandh Kokila)	Lauraceae	Fruit	53.4	33.3	47.8	54.6	47.6	47.0
<i>Elsholtzia polystachya</i> Benth. (Bhangaria)	Lamiaceae	Leaves	54.5	66.6	60.0	56.0	21.4	20.0
<i>Juniperus communis</i> L. (juniper)	Cupressaceae	Leaves	48.4	48.1	53.3	13.0	10.7	20.0
<i>Lavandula officinalis</i> Chaix (lavender)	Lamiaceae	Leaves	24.5	28.3	21.7	70.0	47.6	47.0
<i>Mentha arvensis</i> L. (Japanese mint)	Lamiaceae	Aerial parts	64.2	69.5	35.7	63.0	65.3	65.2
<i>Pleurospermum angelicoides</i> (D.C.) Kl. (Moor)	Apiaceae	Fruit	55.8	40.0	47.8	68.0	47.6	58.8
<i>Salvia sclarea</i> L. (clarysage)	Lamiaceae	Aerial parts	55.8	40.0	43.4	62.0	52.3	58.8
<i>Schinus molle</i> L. (California pepper tree)	Anacardiaceae	Leaves	100	100	100	80.0	53.5	53.5
<i>Zanthoxylum alatum</i> Roxb. (Tomer)	Rutaceae	Seed	39.5	28.8	56.5	74.0	57.1	58.8

^a Mg, *M. gypseum*; Tm, *T. mentagrophytes*; Tr, *T. rubrum*; tested at 400 ppm.

^b Aa, *A. alternata*; Af, *A. flavus*; Pi, *P. italicum*; tested at 500 ppm.

for fungitoxic activity against common animal pathogenic fungi, namely, *Microsporium gypseum* (Bodin) Guiart and Grigorakis, *Trichophyton mentagrophytes* (Robin) Blanchard, *Trichophyton rubrum* (Castellani) Sabouraud,

nature of the oil against the pathogens that did not proliferate (100% MGI) was examined by using a conventional technique (10). The efficacy of the oil was also compared with that of Multifungin (synthetic lotion for the control of ringworm infections; Boehringer Knoll Ltd.) in Sabouraud dextrose agar by using the poisoned food technique.

The physicochemical characterization of *S. molle* oil was performed by the method of Langenau (13). Gas-liquid

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TABLE 2. Influence of temperature, storage, and inoculum density on MGI by *S. molle* oil^a

Conditions	MGI (%) against:		
	<i>M. gypseum</i>	<i>T. mentagrophytes</i>	<i>T. rubrum</i>
Temperature			
40°C	100	100	100
60°C	100	100	100
80°C	100	100	100
Autoclaving (15 lb/in ² , 20 min)	75.8	72.0	60.0
Storage at 4°C (days)			
0	100	100	100
30	100	100	100
60	100	100	100
90	100	100	100
120	76.8	72.0	87.5
150	70.3	68.4	81.8
180	68.0	63.1	79.3
210	61.9	58.3	75.8
240	57.8	54.5	68.9
Inoculum density (no. of fungal disks)			
2	100	100	100
4	100	100	100
8	100	100	100
16	100	100	100
32	100	100	100

^a Concentration, 500 ppm.

chromatographic analysis of the oil was done by using a Perkin-Elmer GC model 3920 equipped with TCD and an SS column (2 m by 0.125 in. [3.175 cm]) packed with 10% Carbowax 20 M on 80/100 Chromosorb WAW. The oven temperature was programmed from 60°C, with an initial hold of 8 min, to 180°C at a rate of 2°/min with a final hold time of 20 min. A hydrogen flow of 30 ml/min was maintained through the column. The relative retention and coinjection techniques were used to identify the constituents. A Hewlett-Packard integrator (HP 3390 A) attached to the detector output was used to calculate percent area.

RESULTS

The efficacy of the essential oils against animal pathogens and storage fungi varied. The oil of *S. molle* was the most effective, inhibiting the animal pathogens completely and exhibiting moderate activity against the storage fungi. All other essential oils showed either partial or poor activity (Table 1).

The minimum fungistatic concentrations of *S. molle* oil were 300, 200, and 200 ppm against *M. gypseum*, *T. mentagrophytes*, and *T. rubrum*, respectively. The minimum fungicidal concentrations were 900 ppm against *T. mentagrophytes* and 400 ppm against *T. rubrum*. *M. gypseum* was completely resistant to the fungicidal action of *S. molle* oil, even at concentrations of 900 ppm.

The toxicity of the oil persisted at temperatures as high as 80°C and for up to 90 days of storage but declined when autoclaved. It withstood heavy inoculum densities (Table 2). The oil exhibited a narrow range of activity as it fungistatically checked the mycelial growth of animal pathogens, namely, *Epidermophyton floccosum*, *Histoplasma capsulatum*, *Microsporium canis*, *Microsporium ferrugineum*, *Tricho-*

TABLE 3. MGI of fungi by *S. molle* oil

Fungus	Disease	MGI (%)
<i>Cladosporium cladosporioides</i> (Fresenius) de Vries	Leaf spot	41.9
<i>Cladosporium trichoides</i> Emmons	Cerebral chromomycosis	60.0
<i>Curvularia lunata</i> (Wakker) Boedijn	Leaf spot	76.4
<i>Epidermophyton floccosum</i> (Harz) Langeron and Mitchenko	Dermatomycosis	100 ^a
<i>Fonsecaea pedrosoi</i> (Brumpt) Negroni	Chromomycosis	62.5
<i>Fusarium moniliforme</i> Sheldon	Fruit rot	54.5
<i>Geotrichum candidum</i> Link	Geotrichosis	34.4
<i>Histoplasma capsulatum</i> Darling	Histoplasmosis	100 ^a
<i>Microsporium canis</i> Bodin	Dermatomycosis	100 ^a
<i>Microsporium ferrugineum</i> Ota	Dermatomycosis	100 ^a
<i>Nocardia asteroides</i> (Eppinger) Blanchard	Nocardiosis	0.0
<i>Nocardia brasiliensis</i> (Lindenberg) Castellani and Chalmers	Nocardiosis	25.0
<i>Phialophora jeanselmei</i> (Langeron) Emmons	Mycetoma	40.0
<i>Sporotrichum schenckii</i> Hektoen and Perkins	Sporotrichosis	40.0
<i>Trichophyton equinum</i> (Mastruchot and Dassonville) Gedoelst	Dermatomycosis	100 ^a
<i>Trichophyton simii</i> (Pinoy) Stockdale, Mackenzie and Austwick	Dermatomycosis	80.0
<i>Trichophyton terrestre</i> Durie and Frey	Dermatomycosis	46.6
<i>Trichophyton tonsurans</i> Malmsten	Dermatomycosis	100 ^a
<i>Trichophyton violaceum</i> Saboraud	Dermatomycosis	65.3

^a Fungistatic activity.

phyton equinum, and *Trichophyton tonsurans*, from the group of 19 fungi tested (Table 3).

The minimum fungistatic concentrations of *S. molle* oil were 60, 75, and 55 times more active against *M. gypseum*, *T. mentagrophytes*, and *T. rubrum*, respectively, when compared with Multifungin. In terms of minimum fungicidal concentrations, *S. molle* oil was 125 times more effective than Multifungin against *T. rubrum* and 55.5 times more effective against *T. mentagrophytes* (Table 4). Physicochemical properties of the oil were determined (Table 5). Of 50 components resolved by gas-liquid chromatography, 14 were found above 1% (vol/vol); however, only 10 components could be identified (Table 6).

DISCUSSION

Pharmacological, physical, and chemical properties of *S. molle* oil have already been reported by various workers (1, 2, 5, 8, 11, 12, 14-19). To our knowledge, its fungitoxic action against tested pathogenic organisms is being reported for the first time.

The fungitoxic character of the oil declined after 90 days of storage (Table 2), suggesting that some effective chemical changes in its composition had occurred (Table 6). Out of 14

TABLE 4. Efficacy of *S. molle* oil compared with Multifungin

Antimycotic substance	Active ingredient	Minimum fungistatic concn (ppm) ^a			Minimum fungicidal concn (ppm) ^a		
		Mg	Tm	Tr	Mg	Tm	Tr
<i>S. molle</i> oil	— ^b	300	200	200	— ^c	900	400
Multifungin ^d (standard)	2% 5-Bromosalicyl-4'-chloranilide-1% <i>N</i> -phenyl- <i>N</i> -benzyl-4-amino-1-methyl piperidine salicylate	18,000	15,000	11,000	50,000	50,000	50,000

^a Mg, *M. gypseum*; Tm, *T. mentagrophytes*; Tr, *T. rubrum*.

^b See Table 6.

^c Remained static.

^d Active ingredients are dissolved in polyethylene glycol.

TABLE 5. Physicochemical properties of *S. molle*

Property	Value
Leaves	
Moisture (%)	
Summer	62.76
Winter	67.45
Dry matter (%)	
Summer	37.24
Winter	32.57
Oil	
Yield (%) (fresh wt. basis)	
Summer	0.80
Winter	0.74
Yield (%) (dry wt. basis)	
Summer	2.148
Winter	2.272
Specific gravity at 20°C	0.9004395
[α] _D ²⁰	+37° 54'
Refractive index at 31°C	1.504
pH	4.0
Color	Light yellow

TABLE 6. Chemical constituents (above 1% [vol/vol]) detected in *S. molle* oil at intervals by gas-liquid chromatography

Constituent (retention time [min])	% Composition at day:		
	0 (fresh)	120	240
α-Pinene (2.36)	1.96	2.41	4.43
Myrcene ^a , α-phellandrene ^a (5.59)	12.59	9.92	29.06
Limonene ^a , β-phellandrene ^a (6.95)	15.46	18.03	24.13
<i>p</i> -Cymene (10.48)	8.41	9.31	6.90
β-Caryophyllene (33.87)	11.43	0.83	0.91
Cryptone (37.56)	2.93	3.93	1.66
α-Terpeneol (39.25)	5.96	8.11	7.01
Unidentified (54.7)	4.61	4.35	1.97
Unidentified (56.6)	4.87	4.28	2.15
Unidentified (58.3)	2.30	2.14	0.53
Carvacrol (61.1)	1.52	2.34	2.14
Unidentified (67.42)	10.39	6.85	2.17

^a Separated and confirmed on an SS column (2 m by 1/8 in. [3.175 cm]) packed with 10% OV-101 on 80/100 Chromosorb WHP. The temperature changes and the flow of hydrogen were the same as those used for the Carbowax 20 M column.

constituents only 10 could be identified. However, perceptible changes during storage occurred in α-pinene, limonene, and β-phellandrene in a defined pattern of gradual increase, but there was a decrease in the four unidentified constituents (Table 6). Therefore, it could be inferred that deterioration in

antifungal activity might have been caused by the relative changes in these four constituents.

Because of its potent and narrow spectrum of fungitoxicity and its superior effectiveness over Multifungin (standard), the oil from leaves of *S. molle* is an effective addition to the armory of antifungal agents. This effectiveness is even greater against animal pathogenic fungi and should be further studied in animals.

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