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

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 |
| *Apium graveolens* L. (celery) | Apiaceae | Seed | 16.6 | 26.6 | 31.8 | 28.0 | 57.1 | 41.1 |
| *Cinnamomum cecidodaphne* Meissn. (Sugandh Kokila) | Lauraceae | Fruit | 53.4 | 33.3 | 47.8 | 54.6 | 47.6 | 47.0 |
| *Elsholtzia polystachya* Benth. (Bhangaria) | Lamiaceae | Leaves | 54.5 | 66.6 | 60.0 | 56.0 | 21.4 | 20.0 |
| *Juniperus communis* L. (juniper) | Cupressaceae | Leaves | 48.4 | 48.1 | 53.3 | 13.0 | 10.7 | 20.0 |
| *Lavandula officinalis* Chaix (lavender) | Lamiaceae | Leaves | 24.5 | 28.3 | 21.7 | 70.0 | 47.6 | 47.0 |
| *Mentha arvensis* L. (Japanese mint) | Lamiaceae | Aerial parts | 64.2 | 69.5 | 35.7 | 63.0 | 65.3 | 65.2 |
| *Pleurospermum angelicoides* (D.C.) Kl. (Moor) | Apiaceae | Fruit | 55.8 | 40.0 | 47.8 | 68.0 | 47.6 | 58.8 |
| *Salvia sclarea* L. (clarysage) | Lamiaceae | Aerial parts | 55.8 | 40.0 | 43.4 | 62.0 | 52.3 | 58.8 |
| *Schinus molle* L. (California pepper tree) | Anacardiaceae | Leaves | 100 | 100 | 100 | 80.0 | 53.5 | 53.5 |
| *Zanthoxylum alatum* 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.

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

| Conditions | MGI (%) against: | | |
|---|---|---|---|
| | *M. gypseum* | *T. mentagrophytes* | *T. rubrum* |
| 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. [.3175 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*, *Microsporum canis*, *Microsporum ferrugineum*, *Trichophyton equinum*, and *Trichophyton tonsurans*, from the group of 19 fungi tested (Table 3).

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

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

[a] Fungistatic activity.

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 |
| *S. molle* oil | —[b] | 300 | 200 | 200 | —[c] | 900 | 400 |
| Multifungin[d] (standard) | 2% 5-Bromosalicyl-4'-chlorani-lide–1% *N*-phenyl-*N*-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 |
| $[\alpha]_D^{20}$ | +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 |
| *p*-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-Terpineol (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. [.3175 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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