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Acaricidal Activity of Constituents Identified in *Foeniculum vulgare* Fruit Oil against *Dermatophagoides* spp. (Acari: Pyroglyphidae)

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Acaricidal activities of components derived from *Foeniculum vulgare* fruit oil against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were examined using direct contact application and compared with that of the commercial repellent benzyl benzoate. The major biologically active constituent of *Foeniculum* fruit oil was characterized as (+)-fenchone by spectroscopic analyses. On the basis of LD_{50} values, the compound most toxic to *D. farinae* was *p*-anisaldehyde (11.3 mg/m^2) followed by (+)-fenchone (38.9 mg/m^2), (−)-fenchone (41.8 mg/m^2), benzyl benzoate (89.2 mg/m^2), thymol (90.3 mg/m^2), and estragol (413.3 mg/m^2). Against *D. pteronyssinus*, *p*-anisaldehyde (10.1 mg/m^2) was much more effective than benzyl benzoate (67.5 mg/m^2), thymol (68.5 mg/m^2), and estragol (389.9 mg/m^2). These results indicate that the acaricidal activity of *F. vulgare* fruit oil likely results from (+)-fenchone and *p*-anisaldehyde. (+)-Fenchone was 20.3 times more abundant in the oil than *p*-anisaldehyde. (+)-Fenchone and *p*-anisaldehyde merit further study as potential house dust mite control agents or as lead compounds.

KEYWORDS: Natural acaricide; house dust mite; *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; *Foeniculum vulgare*; (+)-fenchone

INTRODUCTION

Despite advanced techniques used for house dust mite control in recent decades, they continue to pose serious health problems (*1*). In industrialized countries, most individuals spend over 95% of their time within closed environments, where the air may contain pollutants and contaminants at higher concentrations than those found in the open air. For this reason, the quality of the air in closed environments is presently considered to be at least as important as that of the open air for health in general and for atopic dermatitis, bronchial asthma, rhinitis, and conjunctivitis in particular (*2*). It has been suggested that this increase is in response to provocative factors, such as house dust mites (*3*). Toward the development of diagnostics and a therapeutic vaccine, important house dust mite allergens have been explored and now classified as major house dust mite antigens (*4*−*6*). Two species in particular, *Dermatophagoides farinae* (Hughes) and *Dermatophagoides pteronyssinus* (Trouessart), are commonly found in house dust throughout the temperate regions of the world. Reducing the amount of dust or house dust mites in the home, or both, has been demonstrated to cause a correlated reduction in house dust allergy symptoms in sensitive persons (*6*). Interest in house dust mites and house dust allergies is rapidly growing due to an alarming increase in allergies over the last 10 years (*5*−*7*). Environmental control has been considered as one useful means of controlling house dust mite populations. Washing of bedding is only effective in killing mites at temperatures greater than 70 °C, and vacuuming of carpets should not be considered equivalent to replacing carpets with vinyl, as inadequate suction or leaking vacuum bags can exacerbate the problem by increasing the quantities of allergen that become airborne (*7*). In this regard, research into plant-derived acaricides is now being intensified as it becomes evident that these materials have enormous potential for management of mites for public health and agriculture.

Plant extracts or their constituents may provide an alternative to currently used acaricidal agents to control house dust mites (*8*, *9*). Because many of them are largely free from adverse effects and have excellent biological actions, they could lead to the development of new classes of possibly safer acaricides. In East Asia, *Foeniculum vulgare* belonging to the Apiaceae family have long been considered to have medicinal properties attributable to the terpenoids that they produce, e.g., *trans*-anethole, estragole, *d*-limonene, fenchone, α-pinene, terpinene, and *p*-cymene (*10*). Little work has been done with respect to managing house dust mites, although extractives and an essential oil of *Foeniculum* fruits are insecticidal agents (*11*). This paper describes a laboratory study to examine the oil of the fruits from *F. vulgare* for acaricidal constituents active against *D. farinae* and *D. pteronyssinus*. The acaricidal activities of the *Foeniculum* fruit oil-derived compounds were compared with that of the commonly used benzyl benzoate.

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MATERIAL AND METHODS

Chemicals. *trans*-Anethole, *p*-anisaldehyde, β-asarone, β-caryophyllene, *p*-cymene, estragole, (+)-fenchone, (−)-fenchone, α-pinene, γ-terpinene, and thymol were supplied by Sigma (St. Louis, MO). Benzyl benzoate was purchased from Aldrich (Milwaukee, WI). All other chemicals were of reagent grade.

Dust Mites. Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for 5 years without exposure to any known acaricide. They were reared in plastic containers (15 cm × 12 cm × 6 cm) containing 30 g of sterilized diet (fry feed no. 1/dried yeast, 1:1 by weight) at 25 ± 1 °C and 75% relative humidity in darkness. The fry feed (Miropa) was purchased from Korea Special Feed Meal Co. Ltd. (Chonju, Korea).

Isolation and Identification. The fruits (10 kg) of *F. vulgare* (Family Apiaceae) were purchased from a local market in Chonju and identified by Prof. Sang-Hyun Lee (Forestry Department, Chonbuk National University, Korea). The samples were washed three times with 500 mL of distilled water and dried in an oven at 40 °C for 2 days and then finely powdered. The essential oil (yield 6.1%) of *F. vulgare* fruits was extracted by steam distillation as previously described (*12*).

The oil (10 g) was chromatographed on a silica gel column (Merck 70−230 mesh, 720 g, 6.0 cm i.d. × 80 cm) and successively eluted with a stepwise gradient of hexane/ethyl acetate (90:10, 70:30, 50:50, and 0:100). The bioactive fraction (3.1 g) was successively rechromatographed on a silica gel column, using hexanes−ethyl acetate (80:20). Column fractions were analyzed by thin-layer chromatography (TLC) (silica gel 60 F_{254}), and fractions with similar streaking patterns on the TLC plates were pooled. Preparative high-performance liquid chromatography (HPLC) (Spectra System P2000, Thermo Separation Products) was used for further separation of the constituents. The column was a μPorasil (19 mm i.d. × 300 mm, Waters), using hexanes−ethyl acetate (95:5) at a flow rate of 3.5 mL/min and detection at 285 nm. One potent active principle (52 mg) was isolated.

The structure of the active isolate was determined by instrumental analyses. ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform with a JNM-LA 400F7 spectrometer, at 600 and 150 MHz (tetramethylsilane as an internal standard), respectively, and chemical shifts are given in δ (ppm). The unambiguous ^{1}H and ^{13}C NMR chemical shifts were obtained using a ^{1}H−^{1}H correlation spectroscopy spectrum as well as a ^{13}C−^{1}H correlation spectrum. UV spectra were obtained in methanol with a Uvikon 922 spectrometer and mass spectra on a JEOL GSX 400 spectrometer. Optical rotation was measured with an Autopol III polarimeter.

Gas Chromatography−Mass Spectrometry (GC-MS). The oil of *F. vulgare* fruits was analyzed on a gas chromatograph (HP6890)-mass spectrometer (JMS-600W, JEOL). The GC column was a 60 m × 0.25 mm i.d. DB-WAX (0.25 μm film) fused silica capillary column (J&W Scientific, Folsom, CA). The GC conditions were as follows: injector temperature, 210 °C; column temperature, isothermal at 50 °C for 15 min, then programmed to 200 °C at 2 °C/min, and held at this temperature for 15 min; ion source temperature, 200 °C. Helium was used as the carrier gas at the rate of 0.8 mL/min. The effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with 70 eV of ionization energy. The sector mass analyzer was set to scan from 50 to 800 amu for 2 s. Compounds were identified by comparison with retention times, and the mass spectra were obtained with the authentic standards on the GC-MS system used for analysis. When an authentic sample was not available, the identification was carried out by comparison of mass spectra with those in the mass spectra library (The Wiley Registry of Mass Spectral Data, 6th ed.).

Bioassay. An impregnated fabric disk bioassay was used to access acaricidal activity of test materials. Amounts (800, 400, 300, 200, 100, 50, 25, 20, 10, 5, and 2.5 mg/m^2) of each test material dissolved in 100 μL of ethanol were applied to disks of black cotton fabric (0.5 g, 5 cm diameter, 700 mesh). Control fabric disks received 1000 mL of ethanol. After the disks were dried in a fume hood (19 °C) for 30 s, each disk was placed in the bottom of a Petri dish (5 cm diameter × 1.2 cm). Then, 30 individuals of *D. farinae* (7−10 day old adults) or *D. pteronyssinus* (7−10 days old) were placed in each Petri dish and covered with a lid. Treated and control mites were held at 25 ± 1 °C and 75% relative humidity in darkness. Mortalities were determined 24 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times. LD_{50} values were calculated by probit analysis (*13*).

Table 1. Acaricidal Activity of *F. vulgare* Fruit Oil, Commercial Constituents Derived from *F. vulgare* Fruit Oil, and Syntheic Acaricide against *D. farinae* and *D. pteronyssinus*[a]

compound	mite species	LD_{50} (mg/m^2)	95% confidence limit	RT[b]
oil	*D. farinae*	119	114.7–123.1	0.8
	D. pteronyssinus	103	99.1–107.6	0.7
(+)-fenchone	*D. farinae*	38.9	34.6–43.9	2.3
	D. pteronyssinus	43.2	39.7–48.9	1.6
(–)-fenchone	*D. farinae*	41.8	37.8–47.8	2.1
	D. pteronyssinus	48.7	44.9–54.7	1.4
anisaldehyde	*D. farinae*	11.3	9.9–12.4	7.9
	D. pteronyssinus	10.1	8.9–11.0	6.7
estragol	*D. farinae*	413.3	410.3–449.8	0.2
	D. pteronyssinus	389.9	386.7–422.8	0.2
thymol	*D. farinae*	90.3	86.7–93.5	1.0
	D. pteronyssinus	68.5	66.1–71.5	1.0
benzyl benzoate	*D. farinae*	89.2	83.7–93.6	1.0
	D. pteronyssinus	67.5	58.6–73.9	1.0

[a] Exposed for 24 h. [b] Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical.

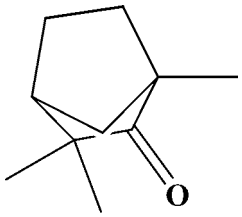

Figure 1. Structure of (+)-fenchone isolated from *F. vulgare* fruit oil.

RESULTS AND DISCUSSION

When the oil derived from *F. vulgare* fruits was bioassayed by direct contact, the acaricidal activity of the oil was observed in various doses against *D. farinae* and *D. pteronyssinus* (**Table 1**). The LC_{50} value of the oil was 119 and 103 mg/m^2 against *D. farinae* and *D. pteronyssinus*, respectively. There was no mortality in the untreated controls. Because of the strong activity of fruit oil, the isolation of the biologically active component was pursued. Bioassay-guided fractionation of the *F. vulgare* fruit oil afforded an active constituent identified by spectroscopic analyses, including EI-MS, ^{13}C NMR, and ^{1}H NMR, and by direct comparison with an authentic reference compound. The biologically active constituents were characterized as the monoterpene (+)-fenchone (**Figure 1**). This compound was identified on the basis of the following evidence. (+)-Fenchon: $C_{10}H_{16}O$; $[\alpha]_D^{20}$ + 67. UV (MeOH) λ_{max} nm (ϵ): 203 (17 478). EI-MS (70 eV), *m/z* (% rel int) M^+: 152 (16), 137 (20), 109 (27), 81 (100), 69 (49). ^{1}H NMR (CD_3OD, 600 MHz): δ 2.14 (1H, br, s), 1.77−1.81 (2H, m), 1.69−1.75 (2H, m), 1.52−1.58 (2H, m), 1.36−1.41 (2H, m), 1.14 (3H, s), 1.04 (6H, s). ^{13}C NMR (CD_3OD, 150 MHz): δ 223.52, 54.15, 47.39, 45.31, 41.65, 31.83, 24.94, 23.35, 21.71, 14.63.

The substances identified by GC-MS in the oil of the *Foeniculum* fruits are presented in **Table 2**. Analysis led to identification of 12 volatiles from the oil of the *Foeniculum* fruits. The main constituents were *t*-anethole (53.2%), anisaldehyde (0.7%), β-asarone (0.9%), β-caryophyllene (1.1%),

Table 2. Volatile Compounds in *F. vulgare* Fruit Oil Identified by GC-MS

compound	mass spectral data[a]	retention time (min)	relative (%)
α-pinene	93, 77, 41, 27, 121, 136	6:85	0.8
1,5,8-*p*-menthatriene	65, 77, 91, 105, 119, 134	11:70	0.6
p-cymene	93, 119, 121, 134, 154	13:69	3.1
d-limonene	39, 53, 68, 93, 107, 121, 136	14:23	0.7
γ-terpinene	65, 77, 93, 105, 121, 136	17:62	0.7
(+)-fenchone	69, 81, 91, 109, 137, 152	20:31	14.2
estragole	77, 91, 105, 121, 133, 148	30:65	12.7
p-anisaldehyde	39, 51, 63, 77, 92, 107, 135	34:85	0.7
trans-anethole	77, 91, 105, 117, 133, 148	37:65	53.2
thymol	77, 91, 115, 135, 150	39:25	1.4
β-caryophyllene	204, 176, 148, 133, 107	42:82	1.1
β-asarone	57, 137, 156, 165, 193, 208	54:79	0.9

[a] Major fragmentation ions, base peak (listed first), and other ions in decreasing order of relative abundance.

p-cymene (3.1%), estragole (12.7%), (+)-fenchone (14.2%), *d*-limonene (0.7%), 1,5,8-*p*-menthatriene (0.6%), α-pinene (0.8%), γ-terpinene (0.7%), and thymol (1.4%). Together, *t*-anethole, estragole, and (+)-fenchone made up 80.1% of the oil. Namba previously reported the main constituents of *F. vulgare* oil as *t*-anethole, *p*-cymene, estragole, fenchone, *d*-limonene, and terpinene (*10*).

The acaricidal activity of the *Foeniculum* oil-derived compounds against *D. farinae* and *D. pteronyssinus* adults was examined by direct contact (**Table 1**) and compared with that of benzyl benzoate, serving as a positive control. Responses varied according to compound and dose. On the basis of LD_{50} values, the compound most toxic against *D. farinae* was *p*-anisaldehyde (11.3 mg/m^2) followed by (+)-fenchone (38.9 mg/m^2), (−)-fenchone (41.8 mg/m^2), benzyl benzoate (89.2 mg/m^2), thymol (90.3 mg/m^2), and estragol (413.3 mg/m^2). Against *D. pteronyssinus*, *p*-anisaldehyde (10.1 mg/m^2) was much more effective than benzyl benzoate (67.5 mg/m^2), thymol (68.5 mg/m^2), and estragol (389.9 mg/m^2). However, no activity was observed for *t*-anethole, β-asarone, β-caryophyllene, *p*-cymene, *d*-limonene, α-pinene, or γ-terpinene at 800 mg/m^2 (not shown). These results indicate that the acaricidal activity of the oil of *F. vulgare* fruits can be mostly attributed to (+)-fenchone and *p*-anisaldehyde. For the acaricidal activity of the oil, (+)-fenchone is likely more important than *p*-anisaldehyde because (+)-fenchone is 20.3 times more abundant than anisaldehyde. (+)-Fenchone was about 2.3 and 1.6 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively, and *p*-anisaldehyde was about 7.9 and 6.7 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively. The acaricidal activity of thymol was comparable to that of benzyl benzoate. (+)-Fenchone and *p*-anisaldehyde merit further study as potential dust mite control agents or as lead compounds.

Plant products are potential sources for house dust mite control because many of them are selective to pests, with few if any harmful effects on nontarget organisms and the environment (*7*−*10*). Many plant extracts and phytochemicals are known to possess acaricidal activity against house dust mites (*8*, *9*, *14*). The reported naturally occurring acaricidal compounds against house dust mites include eugenol, isoeugenol, and methyleugenol from *Eugenia caryophyllata* (*8*), butylidenephthalide from *Cnidium officinale* (*9*), and cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde from *Cinnamomum cassia* (*15*). Our study is the first to report acaricidal properties of components derived from *F. vulgare* fruits against *D. farinae* and *D. pteronyssinus*. In a previous study, the oral LD_{50} value of fenchone for rats was reported as 6.16 g/kg indicating low acute toxicity to mammals (*16*). For practical use of *F. vulgare* fruit-derived materials as acaricidal agents, further research should be done on safety issues of this compound for human health, acaricidal mode of action, and formulations improving the acaricidal potency and stability.

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