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Contact and Fumigant Toxicities of 3-Methylphenol Isolated from *Ostericum koreanum* and Its Derivatives against House Dust Mites

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ABSTRACT: The acaricidal activities of an active constituent derived from *Ostericum koreanum* roots and its derivatives were determined using fumigant and direct-contact toxicity bioassays against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. This was compared with that of a commercial acaricide (benzyl benzoate). In the fumigant toxicity bioassay, 4-chloro-6-isopropyl-3-methylphenol (0.29 $\mu g/cm^2$) was 37.17 times more effective than benzyl benzoate (10.78 $\mu g/cm^2$) against *D. farinae*, followed by 6-fluoro-3-methylphenol (0.57 $\mu g/cm^2$), 3-methylphenol (0.63 $\mu g/cm^2$), 4-chloro-3-methylphenol (0.75 $\mu g/cm^2$), and 4-isopropyl-3-methylphenol (0.78 $\mu g/cm^2$). In the direct-contact toxicity bioassay, 4-chloro-6-isopropyl-3-methylphenol (0.21 $\mu g/cm^2$) was 36.81 times more toxic than benzyl benzoate (7.73 $\mu g/cm^2$) against *D. farinae*, followed by 6-fluoro-3-methylphenol (0.40 $\mu g/cm^2$), 3-methylphenol (0.41 $\mu g/cm^2$), 4-isopropyl-3-methylphenol (0.56 $\mu g/cm^2$), and 4-chloro-3-methylphenol (0.60 $\mu g/cm^2$). The acaricidal effects of 3-methyphenol derivatives against *D. pteronyssinus* were similar to those against *D. farinae*. In structure−activity relationships, acaricidal activities could be related to the introduction of chloro, fluoro, and isopropyl functional groups onto the 3-methylphenol skeleton. These results indicate that naturally occurring 3-methylphenol and its derivatives are potential house dust mite control agents.

KEYWORDS: *acaricidal activity, Ostericum koreanum, Dermatophagoides farinae, Dermatophagoides pteronyssinus, structure−activity relationships*

INTRODUCTION

The most important domestic mites, *Dermatophagoides farinae* (Hughes) and *Dermatophagoides pteronyssinus* (Trouessart), are cosmopolitan species frequently found in human dwellings and a major source of indoor allergens.[1] Domestic mites of the family Pyroglyphidae are among the most common indoor pests, responsible for significant allergic disease, such as atopic dermatitis, bronchial asthma, rhinitis, and systemic anaphylaxis.[2,3] Control of these mites around the domestic environment has been principally through the application of synthetic chemicals such as benzyl benzoate, dibutyl phthalate, pirimiphos-methyl, and tannic acid.[4] Although effective, their repeated or continued use has resulted in the development of environmental and human health concerns.[5,6] These problems have highlighted the need for the development of efficient, safer, and selective alternatives for house dust in the indoor environment.[5−9] In particular, certain plants are being considered as an alternative source of mite control agents or as lead compounds, which exhibit biological activity against mites without harmful effects.[2,10−12]

Ostericum koreanum (Umbelliferae) roots have been widely used to treat arthritis, cold, fever, headache, and neuralgia in traditional Korean medicines.[13,14] This plant has various biological activities including antibacterial,[13] antitumor,[15] anti-inflammatory,[14] and antioxidant[16] activities. It contains coumarins (6-*O*-β-D-glucosyl-7-hydroxycoumarin, isoimperatorin, oxypeucedanin),[15,17] sesquiterpenes (bisabolangelone),[14] and volatile compounds (α-pinene, *p*-cresol, 4-methylacetophenone).[13] In a preliminary experiment, a bisabolangelone isolated from the methanolic extract of the *O. koreanum* had potent acaricidal activity against adults of *D. farinae* and *D. pteronyssinus*.[5] However, despite the many biological activities attributed to *O. koreanum*, the acaricidal activities of the essential oils and active constituents isolated from *O. koreanum* have not been investigated. Therefore, the main objective of our study was to evaluate the acaricidal activities of the oil and an isolated constituent from *O. koreanum* against *D. farinae* and *D. pteronyssinus*, the most important vector in asthma and allergic disease, to find a new natural acaricide. We also compared the acaricidal activities of the derivatives of the active compound with those of synthetic acaricides.

MATERIALS AND METHODS

Chemicals. 4-Amino-3-methylphenol, benzyl benzoate, 4-chloro-6-isopropyl-3-methylphenol, 4-chloro-3-methylphenol, 4′-hydroxy-3′-methylacetophenone, 4-isopropyl-3-methylphenol, 3-methylphenol, β-phellandrene, and α-pinene were purchased from Aldrich (Milwaukee, WI, USA). 6-Fluoro-3-methylphenol and limonene were supplied by Fluka (Buchs, Switzerland). α-Bisabolol, (+)-3-carene, isosafrole, and α-terpinolen were provided by Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade and commercially available.

Sample Preparation. The air-dried roots (2 kg) of *O. koreanum* were purchased from a local market (Jecheon, South Korea) and extracted using a steam distillation−extraction (SDE) method. The extracted oil (yield 0.28%) was dried over anhydrous sodium sulfate to remove the water and was concentrated by rotary evaporation (EYELA model Auto Jack NAJ-100, Japan) at 35 °C. The concentrated oil was stored in a sealed glass vial at 4 °C to minimize the volatilization of compounds.

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Isolation and Identification. The essential oil (5 g) of *O. koreanum* was isolated by silica gel column chromatography (Merck 70−230 mesh, 600 g, 550 mm i.d. × 700 mm; Rahway, NJ, USA) and then continuously eluted using a stepwise gradient of hexane/ethyl acetate (10:0 to 0:10, v/v), to give four fractions (KH1−KH4). Each fraction was leaded on a thin layer chromatography (TLC) plate to identify similar TLC patterns. The acaricidal activity of each of the four fractions was evaluated against *D. farinae* and *D. pteronyssinus* at a concentration of 78 $\mu g/cm^2$. The active KH2 fraction (1.3 g) was conducted on a silica gel column using hexane/ethyl acetate (9:1, 8:2, 7:3, and 1:1, v/v), and five fractions (KH21−KH25) were obtained. Among these fractions, KH22 had potent acaricidal activity against *D. farinae* and *D. pteronyssinus*. The active KH22 fraction (630 mg) was isolated by preparative high-performance liquid chromatography (HPLC) (Japan Analytical Industry Co., Ltd., Tokyo, Japan) using a Jai gel GS series column (GS 310 500 mm + GS 310 300 mm) with chloroform (100%) as the mobile phase at a flow rate of 3.5 mL/min and UV detection (288 nm). KH221 (375 mg) had the highest acaricidal activity among these fractions. Next, a Jai gel W series column (W 253 500 mm + W 252 500 mm) with chloroform (100%) as the mobile phase at a flow rate of 3.5 mL/min was used. Finally, the active component (KH2212, 220 mg) was isolated. The UV−visible absorption spectra were obtained using a UV spectrometer (DR/4000 spectrophotometer, HACH, Loveland, CO, USA), and EI/MS spectra were obtained on a JEOL GSX 400 mass spectrometer. The structure of KH2212 was determined using various spectroscopic data. ^1H and ^{13}C NMR were measured using a JNM-EX600 (Jeol Ltd., Tokyo, Japan) spectrometer in deuterochloroform ($CDCl_3$) with tetramethylsilane (TMS) as an internal standard at 600 and 150 MHz, respectively. Additionally, 1D NMR (DEPT) and 2D NMR ($^1H-^1H$ COSY and HMQC) were used to determine the connection between protons and carbons.

Chemical Analysis by GC-MS. The essential oil of *O. koreanum* was analyzed using a gas chromatograph (6890, Agilent)−mass spectrometer (5973 IV, Agilent) (GC-MS). A DB-5 (0.25 mm film) fused silica capillary column (30 m × 0.25 mm i.d., J&W Scientific, Folsom, CA, USA) was used as the GC column. The GC conditions were as follows: injector temperature, 210 °C; column temperature, isothermal at 50 °C for 15 min, then programmed to rise to 200 °C at 2 °C/min, and held at this temperature for 15 min; ion source temperature, 230 °C. Helium was used as the carrier gas at a rate of 0.8 mL/min. The effluent of the GC column was introduced directly into the source of the mass spectrometer. Spectra were obtained in the electron ionization (EI) mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 600 amu for 2 s. Compounds were then identified by comparison with retention times and mass spectra obtained when authentic standards were analyzed using the GC-MS system. When an authentic sample was not available, identification for the sample was conducted by comparing the mass spectra obtained experimentally with those of the mass spectra library (The Wiley Registry of Mass Spectral Data, 8th ed.).

Target Mites. Culture of *D. farinae* and *D. pteronyssinus* were maintained without exposure to any known acaricides. The mites were reared in plastic containers (25 × 25 × 20 cm) containing 20 g of sterilized diet (fry feed 1 and dried yeast, 1:1 by weight) at 27 ± 1 °C and 70 ± 5% relative humidity in dark conditions. The fry feed (Midopa), which was purchased from the Korea Special Feed Meal Co. Ltd., Jeonju, South Korea, consisted of crude protein (44.0%), cellulose (4.0%), crude lipids (3.0%), phosphate (2.0%), calcium (1.8%), and others (40.2%).

Bioassay. A fumigant toxicity bioassay slightly modified from that of Kim et al.[6] was used to determine the acaricidal activities of different concentrations (78, 39, 19.5, 9.8, 4.9, 2.4, 1.2, 0.6, 0.3, 0.15, and 0.075 $\mu g/cm^2$). Various concentrations were dissolved in acetone, after which 50 μL of the sample was applied to cotton fabric disks (5 cm diameter). Control disks received 50 μL of acetone. The disks were dried in a fume hood for 5 min, after which each disk was placed in the bottom of a Petri dish (55 × 12 mm). Thirty adult *D. farinae* and *D. pteronyssinus* mites (7−10 days old) were placed in the Petri dish, after which the lid was replaced.

A direct-contact toxicity bioassay was used to evaluate the contact toxicities of test materials against *D. farinae* and *D. pteronyssinus*. This was conducted using a modified from the assay described by Yang and Lee.[18] Various amounts (78, 39, 19.5, 9.8, 4.9, 2.4, 1.2, 0.6, 0.3, 0.15, 0.075, 0.038, and 0.019 $\mu g/cm^2$) of each material were dissolved in acetone and then applied to filter paper (5 cm diameter, 55 μm thick) (Whatman Co., UK), with the control filter paper being treated with acetone. Each piece was placed in the bottom of a Petri dish (5 cm diameter × 0.8 cm deep) after the treated and control filter papers had been dried in a fume hood for 5 min. After preparation of the Petri dish, 25−30 individual mites (7−10 days old) were separately placed in each Petri dish, and then the lid was sealed. The treated and control Petri dishes were then incubated at 27 ± 1 °C and 70 ± 5% relative humidity in the dark for 24 h. Mortality was determined under a binocular microscope (20×; Olympus, Tokyo, Japan) after 24 h. Mites were considered to be dead if their appendages did not move when prodded with an insect pin. All treatments were replicated three times, and the LD_{50} values were calculated using probit analysis.

Statistical Analysis. Mortality was transformed to arcsine square-root values for analysis of variance (ANOVA). The LD_{50} (lethal dosage needed to kill 50% of adult *D. farinae* and *D. pteronyssinus*) values were calculated using a standard probit analysis.[19] The relative toxicity (RT) was determined on the basis of the ratio of the benzyl benzoate LD_{50}/test material LD_{50}, as previously described.[18]

RESULTS AND DISCUSSION

The acaricidal activity of the essential oil extracted from *O. koreanum* against *D. farinae* and *D. pteronyssinus* was evaluated using a fumigant toxicity bioassay and compared with that of the commonly used benzyl benzoate, which served as positive control (Table 1). On the basis of the 24 h LD_{50} values, the

Table 1. Acaricidal Activities of *O. koreanum* Oil and Synthetic Acaricide against *D. farinae* and *D. pteronyssinus*, Using a Fumigant Toxicity Bioassay[a]

sample	mite species	LD_{50} ($\mu g/cm^2$)	95% CL	RT[b]
O. koreanum oil	*D. farinae*	3.09	2.44−3.85	3.49
	D. pteronyssinus	3.31	2.76−4.18	2.97
benzyl benzoate	*D. farinae*	10.78	9.25−11.71	1.00
	D. pteronyssinus	9.83	9.01−10.54	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of *O. koreanum* oil.

O. koreanum oil (3.09 and 3.31 $\mu g/cm^2$) was 3.49 and 2.97 times more effective than benzyl benzoate (10.78 and 9.83 $\mu g/cm^2$) against *D. farinae* and *D. pteronyssinus*, respectively). There was no mortality under the negative control (acetone-treated) against *D. farinae* and *D. pteronyssinus*. In this study, *D. farinae* was slightly more susceptible to the *O. koreanum* oil than *D. pteronyssinus*. Differences in the activity of mites may be explained on the basis of species-specific responses to the same plant extracts and phytochemicals.[2,20]

The volatile constituents of the essential oil extracted from *O. koreanum* were identified by GC-MS analyses (Table 2). A total of 16 volatile constituents were identified by comparing the retention indices, retention time, and mass spectra with libraries such as The Wiley Registry of Mass Spectral Data (8th ed.), the Adams library, and the mass spectral library (NIST 08) of authentic data established under identical experimental conditions. The composition of the volatile compounds was as follows; β-phellandrene (38.15%), α-bisabolol (9.37%), 3-methylphenol (6.70%), α-terpinolene (5.51%), 1-acetoxy-1,2-epoxycyclohexane

Table 2. Composition of Volatile Compounds Obtained from *O. koreanum* Oil

retention index[a]	compound	mass spectral data[b]	relative content (%)
688	2-methyl-3-ethylpentane	14, 27, 41, 43, 57, 70, 85, 99, 114	3.92
894	2,5-dimethyl-3-vinyl-1,4-hexadiene	41, 53, 67, 79, 93, 105, 121, 136	2.13
934	α-pinene	55, 93, 117, 136, 155, 193, 225	1.46
964	β-phellandrene	27, 41, 65, 77, 93, 107, 121, 136	38.15
976	(+)-3-carene	38, 67, 79, 93, 105, 121, 136	2.77
990	2,5-dimethyl-3-hexanol acetate	14, 27, 41, 43, 69, 70, 87, 111, 129	3.41
1014	3-methylphenol	26, 39, 51, 63, 79, 90, 108	6.70
1036	limonene	53, 68, 79, 93, 107, 121, 136	2.35
1052	terpinolen	27, 39, 53, 65, 79, 93, 105, 121, 136	5.51
1071	1-acetoxy-1,2-epoxycyclohexane	39, 43, 67, 70, 96, 113, 156	5.02
1104	isopentyl-3-methylbutanoate	27, 39, 55, 56, 83, 84, 100, 113, 170	3.65
1345	isosafrole	39, 51, 63, 78, 91, 104, 131, 147, 162	4.14
1363	4′-hydroxy-3′-methylacetophenone	39, 63, 77, 91, 107, 121, 135, 150	4.83
1415	4,7-dimethyl-5-decyne-4,7-diol	41, 43, 69, 95, 109, 123, 137, 155	1.34
1625	α-bisabolol	41, 69, 71, 93, 109, 134, 147, 161, 189, 204	9.37
1904	tridecanolide	39, 41, 55, 69, 82, 96, 113, 137, 152	2.33
	identification (%)		97.08

[a]The Kovats indices were determined on a DB-5 capillary column. [b]Major fragmentation ions, base peak, and other ions in decreasing order of relative abundance.

(5.02%), 4′-hydroxy-3′-methylacetophenone (4.83%), isosafrole (4.14%), 2-methyl-3-ethylpentane (3.92%), isopentyl-3-methylbutanoate (3.65%), 2,5-dimethyl-3-hexanol acetate (3.41%), (+)-3-carene (2.77%), limonene (2.35%), tridecanolide (2.33%), 2,5-dimethyl-3-vinyl-1,4-hexadiene (2.13%), α-pinene (1.46%), and 4,7-dimethyl-5-decyne-4,7-diol (1.34%). The essential oil of *O. koreanum* is composed of terpene hydrocarbons, oxygenated terpene hydrocarbons, phenols, alcohols, and aliphatic hydrocarbons. In particular, aromatic hydrocarbons were found to be the major components. In contrast with previous results, Shin[13] demonstrated that the main constituents in the *O. koreanum* oil were α-pinene (41.12%), *p*-cresol (17.99%), 4-hydroxy-2-methylacetophenone (7.90%), sabinene (7.60%), α-bisabolol (2.05%), *p*-cymen-8-ol (1.98%), and camphene (1.89%). This discrepancy suggested that the differential compositions are related to intrinsic and/or extrinsic factors, such as the plant species, parts of plant (flower, leaves, root, and stem), the different status of the plant sample used in essential oil extraction, and the culture conditions (geographical location, climate, or soil type).[13,21]

To evaluate the acaricidal toxicity of the 16 volatile compounds of *O. koreanum* oil and synthetic acaricide against *D. farinae* and *D. pteronyssinus*, the fumigant toxicity bioassay was examined (Table 3). When the acaricidal activities against *Dermatophagoides* spp. were evaluated, 3-methylphenol gave the highest mortality (100% at 2.5 μg/cm^2), followed by isosafrole (58.9 and 63.3% at 19.5 μg/cm^2), (+)-3-carene (34.2 and 43.8% at 39 μg/cm^2), and α-bisabolol (7.5 and 8.2% at 39 μg/cm^2). The other compounds (α-pinene, β-phellandrene, limonene, α-terpinolene, and 4′-hydroxy-3′-methylacetophenone) had no acaricidal activity against *D. farinae* and *D. pteronyssinus* at 39 μg/cm^2. Therefore, these results indicated that the acaricidal activity of *O. koreanum* oil can be mostly attributed to 3-methylphenol against *Dermatophagoides* spp.

To isolate the active constituent of the *O. koreanum* oil, silica gel column chromatography and preparative HPLC were carried out using a single or a variety of mixed solvents. As a result, KH2212 was successfully isolated and identified by spectroscopic analyses, including EI-MS, 1D NMR (^{1}H and ^{13}C NMR and DEPT-NMR), and 2D NMR (^{1}H−^{1}H COSY and HMQC). The various spectroscopic analyses resulted in the identification of 3-methylphenol by the following evidence. 3-Methylphenol (C_7H_8O; MW, 108.14; pale yellow liquid); EI-MS (70 eV) *m*/*z* (% relative intensity) M^+ 108 (100, base peak), 90 (18), 79 (55), 63 (13), 51 (24), 39 (38), 36 (7); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.032−7.058 (1H, t), 6.668−6.680 (1H, d), 6.581 (1H, s), 6.555−6.569 (1H, d), 4.895 (OH, s), 2.228 (3H, s); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 155.486 (C-3), 139.936 (C-1), 129.529 (C-5), 121.716 (C-6), 116.114 (C-2), 112.371 (C-4), 21.439 (C-7).[22]

The acaricidal activities of 3-methylphenol and its derivatives were compared with that of benzyl benzoate against *D. farinae* and *D. pteronyssinus* using a fumigant toxicity bioassay (Table 4; Figure 1). On the basis of the LD_{50} values against *D. farinae*, 4-chloro-6-isopropyl-3-methylphenol (0.29 μg/cm^2) was 37.17 times more toxic than benzyl benzoate (10.78 μg/cm^2), followed by 6-fluoro-3-methylphenol (0.57 μg/cm^2), 3-methylphenol (0.63 μg/cm^2), 4-chloro-3-methylphenol (0.75 μg/cm^2), and 4-isopropyl-3-methylphenol (0.78 μg/cm^2). Against *D. pteronyssinus*, 4-chloro-6-isopropyl-3-methylphenol (0.35 μg/cm^2) was 39.32 times more toxic than benzyl benzoate (9.83 μg/cm^2), followed by 6-fluoro-3-methylphenol (0.72 μg/cm^2), 3-methylphenol (0.75 μg/cm^2), 4-chloro-3-methylphenol (0.87 μg/cm^2), and 4-isopropyl-3-methylphenol (1.03 μg/cm^2).

In the direct-contact toxicity bioassay, 4-chloro-6-isopropyl-3-methylphenol (0.21 μg/cm^2) was the most active compound against *D. farinae* and was approximately 36.81 times more toxic than benzyl benzoate (7.73 μg/cm^2), followed by 6-fluoro-3-methylphenol (0.40 μg/cm^2), 3-methylphenol (0.41 μg/cm^2), 4-isopropyl-3-methylphenol (0.56 μg/cm^2), and 4-chloro-3-methylphenol (0.60 μg/cm^2) (Table 5). For *D. pteronyssinus*, 4-chloro-6-isopropyl-3-methylphenol (0.24 μg/cm^2) was 25.63 times more toxic than benzyl benzoate (6.15 μg/cm^2), followed by 6-fluoro-3-methylphenol (0.49 μg/cm^2), 3-methylphenol (0.52 μg/cm^2), 4-isopropyl-3-methylphenol (0.67 μg/cm^2), and 4-chloro-3-methylphenol (0.73 μg/cm^2). However, 4-amino-3-methylphenol had no activity against *D. farinae* and *D. pteronyssinus* in the fumigant and direct-contact toxicity bioassays at 39 μg/cm^2.

Table 6 shows a comparison of the acaricidal effects of 3-methylphenol and its derivatives in fumigant and direct-contact toxicity bioassays. Against *D. farinae* and *D. pteronyssinus*, the direct-contact toxicity of 3-methylphenol derivatives was about 1.19−1.54 times more toxic than the fumigant toxicity. Similarly, Oh et al.[23] reported that the acaricidal activities of acetophenone derivatives against *D. farinae*, *D. pteronyssinus*,

Table 3. Acaricidal Activities of Nine Volatile Compounds Identified by GC-MS in the Oil of *O. koreanum* and Synthetic Acaricide, Using a Fumigant Toxicity Bioassay[a]

compound	mite species	mortality (%, mean ± SE) at dose of				
		39 μg/cm²	19.5 μg/cm²	9.8 μg/cm²	4.9 μg/cm²	2.5 μg/cm²
α-pinene	*D. farinae*	0 ± 0	NT[b]	NT	NT	NT
	D. pteronyssinus	0 ± 0	NT	NT	NT	NT
β-phellandrene	*D. farinae*	0 ± 0	NT	NT	NT	NT
	D. pteronyssinus	0 ± 0	NT	NT	NT	NT
(+)-3-carene	*D. farinae*	34.2 ± 1.2	11.5 ± 0.9	0 ± 0	NT	NT
	D. pteronyssinus	43.8 ± 2.3	19.7 ± 1.8	6.7 ± 1.1	0 ± 0	NT
3-methylphenol	*D. farinae*	100	100	100	100	100
	D. pteronyssinus	100	100	100	100	100
limonene	*D. farinae*	0 ± 0	NT	NT	NT	NT
	D. pteronyssinus	0 ± 0	NT	NT	NT	NT
α-terpinolen	*D. farinae*	0 ± 0	NT	NT	NT	NT
	D. pteronyssinus	0 ± 0	NT	NT	NT	NT
isosafrole	*D. farinae*	83.5 ± 1.2	58.9 ± 1.9	46.8 ± 0.95	13.2 ± 0.8	0 ± 0
	D. pteronyssinus	91.2 ± 2.2	63.3 ± 1.7	33.8 ± 1.2	11.2 ± 1.1	0 ± 0
4′-hydroxy-3′-methylacetophenone	*D. farinae*	0 ± 0	NT	NT	NT	NT
	D. pteronyssinus	0 ± 0	NT	NT	NT	NT
α-bisabolol	*D. farinae*	7.5 ± 0.9	0 ± 0	NT	NT	NT
	D. pteronyssinus	8.2 ± 1.1	0 ± 0	NT	NT	NT
benzyl benzoate	*D. farinae*	100	100	51.2 ± 2.1	36.5 ± 3.1	0 ± 0
	D. pteronyssinus	100	100	61.7 ± 2.8	43.5 ± 2.2	0 ± 0

[a]Exposed for 24 h. [b]Not tested.

and *T. putrescentiae* were more effective in the contact toxicity bioassay than in the vapor phase toxicity method. Conversely, Yang and Lee[18] showed that 1-octen-3-ol and its derivatives with a fumigation bioassay were more toxic than in the filter paper bioassay against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*. These results indicate that the mode of action of 3-methylphenol derivatives was likely by contact action via the insect body. However, the acaricidal mode of action of these compounds is not yet understood.

To evaluate the structure−activity relationships between 3-methylphenol derivatives and acaricidal toxicities against *Dermatophagoides* spp., LD_{50} values of 3-methylphenol derivatives were compared to those of 3-methylphenol with various functional groups (amino, chloro, fluoro, and isopropyl groups). Introducing a chloro, fluoro, or isopropyl group into 3-methylphenol (4-chloro-3-methylphenol, 6-fluoro-3-methylphenol, and 4-isopropyl-3-methylphenol) exhibited potent acaricidal activity against *D. farinae* and *D. pteronyssinus*. Moreover, incorporating both a chloro group and an isopropyl group in 3-methylphenol (4-chloro-6-isopropyl-3-methylphenol) increased acaricidal activity against *D. farinae* and *D. pteronyssinus*. However, 4-amino-3-methylphenol (which results from the addition of an amino group into 3-methylphenol) had no acaricidal activity. Our results indicate that chloro, fluoro, and isopropyl functional groups added into 3-methyphenol seem to determine the acaricidal toxicity of compounds to *D. farinae* and *D. pteronyssinus*.

Table 4. Acaricidal Activities of 3-Methylphenol, Its Derivatives, and Synthetic Acaricide against *Dermatophagoides* spp., Using a Fumigant Toxicity Bioassay[a]

compound	mite species	LD_{50} (μg/cm²)	95% CL	RT[b]
3-methylphenol (isolated compound)	*D. farinae*	0.63	0.58−0.79	17.11
	D. pteronyssinus	0.75	0.69−0.83	13.11
4-amino-3-methylphenol	*D. farinae*	*c*	*c*	*c*
	D. pteronyssinus	*c*	*c*	*c*
4-chloro-3-methylphenol	*D. farinae*	0.75	0.68−0.87	14.37
	D. pteronyssinus	0.87	0.71−0.93	11.30
4-isopropyl-3-methylphenol	*D. farinae*	0.78	0.68−0.83	13.82
	D. pteronyssinus	1.03	0.99−1.12	9.54
4-chloro-6-isopropyl-3-methylphenol	*D. farinae*	0.29	0.21−0.36	37.17
	D. pteronyssinus	0.35	0.20−0.38	39.32
6-fluoro-3-methylphenol	*D. farinae*	0.57	0.49−0.64	18.91
	D. pteronyssinus	0.72	0.65−0.79	13.65
benzyl benzoate	*D. farinae*	10.78	9.25−11.71	1.00
	D. pteronyssinus	9.83	9.01−10.54	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c]No activity.

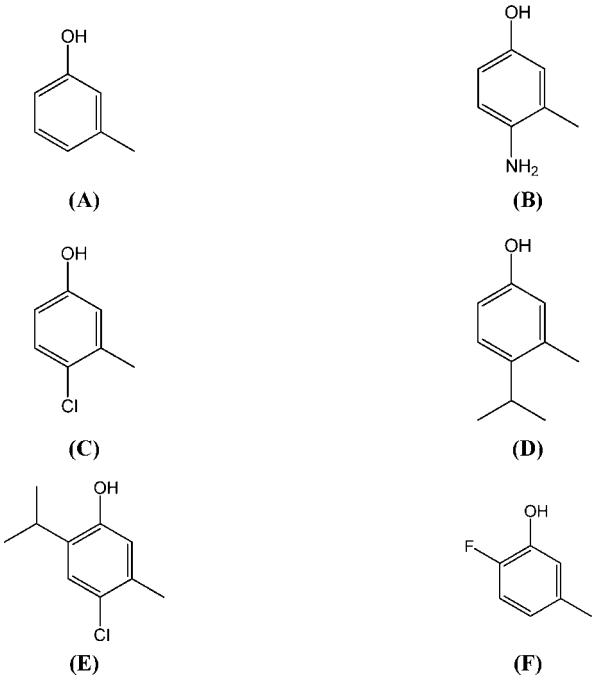

Figure 1. Structures of 3-methylphenol and its derivatives: (A) 3-methylphenol; (B) 4-amino-3-methylphenol; (C) 4-chloro-3-methylphenol; (D) 4-isopropyl-3-methylphenol; (E) 4-chloro-6-isopropyl-3-methylphenol; (F) 6-fluoro-3-methylphenol.

Table 5. Acaricidal Activities of 3-Methylphenol, Its Derivatives and Synthetic Acaricide against *Dermatophagoides* spp., Using a Direct-Contact Toxicity Bioassay[a]

compound	mite species	LD_{50} ($\mu g/cm^2$)	95% CL	RT[b]
3-methylphenol (isolated compound)	*D. farinae*	0.41	0.36−0.57	18.85
	D. pteronyssinus	0.52	0.48−0.59	11.83
4-amino-3-methylphenol	*D. farinae*	*c*	*c*	*c*
	D. pteronyssinus	*c*	*c*	*c*
4-chloro-3-methylphenol	*D. farinae*	0.60	0.52−0.69	12.88
	D. pteronyssinus	0.73	0.63−0.84	8.42
4-isopropyl-3-methylphenol	*D. farinae*	0.56	0.49−0.63	13.80
	D. pteronyssinus	0.67	0.59−0.74	9.18
4-chloro-6-isopropyl-3-methylphenol	*D. farinae*	0.21	0.09−0.19	36.81
	D. pteronyssinus	0.24	0.07−0.16	25.63
6-fluoro-3-methylphenol	*D. farinae*	0.40	0.34−0.52	19.33
	D. pteronyssinus	0.49	0.35−0.62	12.55
benzyl benzoate	*D. farinae*	7.73	6.38−9.05	1.00
	D. pteronyssinus	6.15	5.32−7.64	1.00

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical. [c]No activity.

According to the Materials Safety Data Sheet (MSDS) from Sigma-Aldrich,[24] the acute oral toxicities (LD_{50} values) of 3-methylphenol and 4-chloro-3-methylphenol in mouse are 242 and 1830 mg/kg, respectively, and the acute oral toxicity (LD_{50} values) of 4-isopropyl-3-methylphenol in rat is 6280 mg/kg. In addition, the acute topical toxicities (LD_{50} values) of 4-amino-3-methylphenol and 4-chloro-6-isopropyl-3-methylphenol in rat are 680 and 2460 mg/kg, respectively. However, the acute toxicity of 4-fluoro-3-methylphenol was not shown. These results indicate that 3-methylphenol and its derivatives from naturally occurring phenols have a relatively low acute toxicity for mammals.

Table 6. Comparison of Acaricidal Activities of 3-Methylphenol Derivatives against *Dermatophagoides* spp. by Fumigant and Direct-Contact Toxicity Methods[a]

compound	bioassay	LD_{50} ($\mu g/cm^2$) *D. farinae*	LD_{50} ($\mu g/cm^2$) *D. pteronyssinus*
3-methylphenol	fumigant	0.63	0.75
	contact	0.41	0.52
	fumigant/contact[b]	1.54	1.44
4-amino-3-methylphenol	fumigant		
	contact		
	fumigant/contact		
4-chloro-3-methylphenol	fumigant	0.75	0.87
	contact	0.60	0.73
	fumigant/contact	1.25	1.19
4-isopropyl-3-methylphenol	fumigant	0.78	1.03
	contact	0.56	0.67
	fumigant/contact	1.39	1.54
4-chloro-6-isopropyl-3-methylphenol	fumigant	0.29	0.35
	contact	0.21	0.24
	fumigant/contact	1.38	1.46
6-fluoro-3-methylphenol	fumigant	0.57	0.72
	contact	0.40	0.49
	fumigant/contact	1.43	1.47
benzyl benzoate	fumigant	10.78	9.83
	contact	7.73	6.15
	fumigant/contact	1.39	1.60

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of fumigant bioassay/LD_{50} value of direct-contact bioassay.

The *O. koreanum* roots have traditionally been used in oriental medicine as an analgesic in the treatment of arthritis, articular rheumatism, edema, headache, and perspiration.[5,15] They contain aesculin, bisabolangelone, caffeic acid, cimifugin, *p*-cresol, isoimperatorin, koreanin, 4-methylacetophenone, oxypeucedanin, α-pinene, and prangolarine.[5,13,14,17] In particular, volatile compounds of essential oils and plant extracts are plant secondary metabolites that have complex mixtures of alkaloids, coumarins, phenolics, quinones, terpenoids, and related substances. Moreover, these compounds exist in plants and jointly or independently have strongly influenced the bioactivity.[5,6] The reported plant-based substances and their derivatives that are acaricidal against *Dermatophagoides* spp. include 1-octen-3-ol, 1-octen-3-yl acetate, 1-octen-3-yl butyrate, 3,7-dimethyl-1-octen-3-ol,[18] 1-bromo-3,4-methylenedioxybenzene, 5-chloro-3,4-methylenedioxybenzene, 3,4-methylenedioxybenzonitrile,[25] 6-isovaleryl-2,2,4,4-tetramethyl-1,3,5-cyclohexanetrione, and 2,2,4,4,6,6-hexamethyl-1,3,5-cyclohexanetrione.[26] However, very few studies have assessed the acaricidal activities

of volatile compounds derived from *O. koreanum* oil against house dust mites. Our results are the first to demonstrate that active constituents derived from *O. koreanum* oil and its derivatives have acaricidal activity against *D. farinae* and *D. pteronyssinus*.

In conclusion, our results indicate that *O. koreanum* oil-derived 3-methylphenol and its derivatives could be highly useful in mite control agents for house dust mites and for protection of humans from allergic diseases. Further studies should be conducted to investigate safety issues of methylphenol derivatives with regard to human health and environment, the acaricidal mode of action, and formulations improving the acaricidal potency and stability.

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Notes

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