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Acaricidal Activity of Butylidenephthalide Identified in *Cnidium officinale* Rhizome against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

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The acaricidal activity of materials derived from the rhizome of *Cnidium officinale* against adults of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* was examined using direct contact application and fumigation methods and compared with that of benzyl benzoate and *N*,*N*-diethyl-*m*-toluamide (DEET). The active constituent of the *Cnidium* rhizome was identified as butylidenephthalide by spectroscopic analyses. Responses varied with dose. On the basis of 24-h LD_{50} values, the acaricidal activity of butylidenephthalide (6.77 μg/cm^2) against *D. farinae* adults was comparable to that of benzyl benzoate (8.54 μg/cm^2). Very low activity was observed with DEET (37.59 μg/cm^2). Against *D. pteronyssinus* adults, butylidenephthalide (6.46 μg/cm^2) and benzyl benzoate (6.68 μg/cm^2) were equitoxic. DEET (17.98 μg/cm^2) was relatively inactive. The typical poisoning symptom of butylidenephthalide was lethargy of treated mites, leading to death without knockdown, whereas benzyl benzoate and DEET caused death following uncoordinated behavior. In a fumigation test with both mite species, butylidenephthalide was much more effective in closed containers than open ones. Naturally occurring *C. officinale* rhizome-derived materials merit further study as potential house dust mite control agents or lead compounds.

KEYWORDS: Natural acaricide; natural fumigant; *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; *Cnidium officinale*; butylidenephthalide; mode of action; poisoning symptom

INTRODUCTION

The American house dust mite, *Dermatophagoides farinae* (Hughes), and the European house dust mite, *Dermatophagoides pteronyssinus* (Trouessart), are two of the most important pyroglyphid mites because of their cosmopolitan occurrence and abundance in homes (*1*, *2*), a major source of multiple potent allergens (*3*, *4*), and their causal association with sudden infant death syndrome (*5*). Changes in living environments such as a rise in the number of apartment households with centrally installed heating, space heating, tighter windows, and fitted carpets have improved conditions for mite growth (*1*). Control of these mite populations has been principally through development of chemical substances such as γ-benzene hexachloride (γ-BHC), pirimiphos-methyl, benzyl benzoate, diethyl-*m*-toluamide (DEET), and dibutyl phthalate (*1*). Although effective, their repeated use has sometimes resulted in the development of resistance (*3*), has undesirable effects on nontarget organisms, and has fostered environmental and human health concerns (*1*, *6*). These problems have highlighted the need for the development of new strategies for selective house dust mite control.

Plants may be an alternative source for dust mite control because they constitute a range of bioactive chemicals. Many of them are selective, often biodegrade to nontoxic products, and may be applied to dust mite nests such as mattresses, carpets, and sofas in the same way as conventional acaricides. Because of this, much effort has been focused on plant extracts or essential oils as potential sources of commercial pest control agents (*7*, *8*). In a preliminary experiment, a methanol extract of the rhizome from *Cnidium officinale* Makino had potent acaricidal activity against adults of *D. farinae* and *D. pteronyssinus*.

This paper describes a laboratory study aimed at isolating acaricidal principles from the rhizome of *C. officinale* active against adults of *D. farinae* and *D. pteronyssinus* and determining their acaricidal mode of delivery. The acaricidal activity of a *C. officinale* rhizome-derived compound was compared with those of benzyl benzoate and DEET.

MATERIALS AND METHODS

Chemicals. Benzyl benzoate and DEET were purchased from Sigma (St. Louis, MO). All other chemicals were of reagent grade.

Mites. Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for six years without exposure to any known acaricide. They were reared in plastic containers (17.5 × 17.5 × 17.5 cm) containing 25 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 was purchased from Korea Special Feed Meal Co., Ltd., Inchon, Korea.

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Isolation and Identification. Air-dried rhizome (5 kg) of *C. officinale* was purchased from Boeun medicinal herb shop, Kyungdong Market, Seoul, Korea. It was finely powdered with a blender, extracted with 10 L of methanol twice at room temperature for 2 days, and filtered (Whatman no. 2). The combined filtrate was concentrated in vacuo at 40 °C to yield a dark brownish tar. The yield was ~10% on the basis of the initial weight of the dried rhizome. The extract (80 g) was sequentially partitioned into hexane (16.8 g), chloroform (0.4 g), ethyl acetate (0.8 g), butanol (10.8 g), and water (51.2 g) for subsequent bioassay. The organic solvent fractions and water fraction were concentrated to dryness by rotary evaporation at 40 and 50 °C, rerspectively.

The hexane fraction (10 g) was chromatographed on a silica gel column (Merck 70−230 mesh, 600 g, 5.5 i.d. × 70 cm) and successively eluted with a stepwise gradient of hexane/ethyl acetate (99:1, 90:10, 70:30, 50:50, 30:70, and 0:100 by volume). Column fractions were analyzed by TLC (silica gel 60 F_{254}), and fractions with similar streaking patterns on the TLC plates were pooled. The bioactive 70:30 + 50:50 fraction (3.2 g) was successively rechromatographed on a silica gel column, using hexane/ethyl acetate (20:1 by volume). Preparative HPLC (Spectra System P2000, Thermo Separation Products) was used for further separation of the constituent. The column was a μPorasil (7.8 mm i.d. × 300 mm, Waters) using hexane/ethyl acetate (20:1 by volume) at a flow rate of 3 mL/min and detected at 313 nm. Finally, an active principle (113 mg) at the retention time of 9.13 min was isolated.

The structure of the active isolate was determined by spectroscopic analyses. ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform with a JNM-LA 400F7 spectrometer at 400 and 100 MHz (TMS as an internal standard), respectively, and chemical shifts are given in δ (parts per million). UV spectra were obtained in methanol with a JASCO V-550 spectrophotometer and mass spectra on a JEOL GSX 400 spectrometer.

Bioassay. A fabric bioassay was used for acaricidal activity of test materials. Amounts (76.4, 50.9, 25.5, 12.7, 9.6, 6.4, 4.8, and 3.2 μg/cm^2) of each test material dissolved in 100 μL of ethanol were applied to pieces of black cotton fabric (5 cm diameter). Control fabric pieces received 100 μL of ethanol. After drying in a fume hood for 1 min, each piece was placed in the bottom of a Petri dish (5 cm diameter × 1.2 cm). Then 20 individuals of *D. farinae* (7−10 days old) and *D. pteronyssinus* (7−10 days old) were separately 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, 48, and 72 h after treatment under a binocular microscope (20×). Mites were considered dead if appendages did not move when prodded with a pin. All treatments were replicated five times. LD_{50} values were calculated by probit analysis (*9*).

Poisoning symptoms of the *C. officinale* rhizome-derived isolate (12.7 μg/cm^2), benzyl benzoate (50.9 μg/cm^2), and DEET (50.9 μg/cm^2) to adults of *D. farinae* (7−10 days old) and *D. pteronyssinus* (7−10 days old) were examined by the fabric bioassay. The treated amounts of each test compound gave >90% mortality 3 h after treatment. Poisoning symptoms were determined at 15 min intervals under a binocular microscope (20×).

Acaricidal Route of Action. Susceptibility of adult *D. farinae* and *D. pteronyssinus* to the *C. officinale* rhizome-derived compound in the vapor phase was investigated according to the method of Kim (*10*). Briefly, groups of 20 adults (7−10 days old) were placed in the bottom of a Petri dish (5 cm diameter × 1.2 cm) and covered with a lid with a fine wire sieve (4.7 cm diameter) attached to the center hole (4.5 cm diameter). Each fabric piece (5 cm diameter), treated with 12.7 μg/cm^2 of the isolate dissolved in 100 μL of ethanol, was placed over the wire sieve. This prevented direct contact of test adults with the test compound. Each Petri dish was then either sealed with another lid (method A) to investigate the potential toxic effect of the vapor phase of the test compound or left unsealed (method B). Control fabric pieces received 100 μL of ethanol.

Treated and control mites were held under the same conditions used for colony maintenance. Mortalities were evaluated 24 h after treatment under a binocular microscope (20×). Test mites were considered dead if appendages did not move when prodded with a pin. All treatments were replicated five times.

Table 1. Acaricidal Activity of *C. officinale* Rhizome-Derived Materials against Adults of *D. pteronyssinus* and *D. farinae*, Using the Fabric Bioassay

material[b]	mortality,[a] % (mean ± SE)	
	D. farinae	*D. pteronyssinus*
methanol extract	100 ± 0.0a	100 ± 0.0a
hexane fraction	100 ± 0.0a	100 ± 0.0a
chloroform fraction	3 ± 3.3b	13 ± 3.3b
ethyl acetate fraction	0 ± 0.0b	0 ± 0.0b
butanol fraction	0 ± 0.0b	0 ± 0.0b
water fraction	0 ± 0.0b	0 ± 0.0b

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe test). [b] Exposed for 24 h at a dose of 50.9 μg/cm^2.

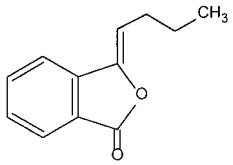

Figure 1. Structure of butylidenephthalide, an acaricidal constituent from *C. officinale* rhizome, against house dust mites.

Table 2. Toxicity of *C. officinale* Rhizome-Derived Butylidenephthalide and Acaricides against *D. farinae* Adults, Using the Fabric Bioassay

compound[a]	slope (± SE)	LD_{50}, μg/cm^2	95% confidence limit	RT[b]
butylidenephthalide	6.83 ± 0.71	6.77	6.41–7.17	1.3
benzyl benzoate	6.52 ± 0.70	8.54	8.03–9.03	1.0
DEET	5.02 ± 0.76	37.59	34.60–40.94	0.2

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

Statistical Analysis. The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe test at $P < 0.05$ (*9*). Means (±SE) of untransformed data are reported.

RESULTS

Identification of Active Principle. When fractions obtained from the methanol extract were bioassayed by direct contact, significant differences were observed in toxicity to mite adults (**Table 1**). At 50.9 μg/cm^2, the hexane fraction showed potent acaricidal activity against adults of both species.

Fabric bioassay-guided fractionation of the hexane fraction provided an active constituent identified as butylidenephthalide (**Figure 1**) by spectroscopic analyses, including MS and NMR. This compound was identified on the basis of the following evidence: UV λ_{max}^{MeOH}nm (ε) 313 (2700); EI-MS (70 eV), *m*/*z* (% relative intensity) M^+ 188 (34), 159 (100), 146 (39), 131 (16), 103 (9), 83 (12); ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, $J = 7.6$ Hz), 1.56 (2H, m, $J = 7.6$ Hz), 2.46 (2H, m, $J = 7.6$ Hz), 5.64 (1H, t, $J = 7.6$ Hz), 7.50−7.68 (3H, m), 7.89 (1H, d, $J = 7.6$ Hz); ^{13}C NMR ($CDCl_3$) δ 13.81 q, 22.55 t, 27.81 t, 109.46 d, 119.63 d, 124.52 s, 125.27 d, 129.34 d, 134.20 d, 139.62 s, 145.79 s, 167.22 s.

Acaricidal Activity. The toxicity of butylidenephthaldie against *D. farinae* adults was compared with those of benzyl benzoate and DEET (**Table 2**). The commonly used benzyl benzoate served as a standard of comparison in toxicity tests.

Table 3. Toxicity of *C. officinale* Rhizome-Derived Butylidenephthalide and Acaricides against *D. pteronyssinus* Adults, Using the Fabric Bioassay

compound[a]	slope (± SE)	LD_{50}, $\mu g/cm^2$	95% confidence limit	RT[b]
butylidenephthalide	6.94 ± 0.73	6.46	6.11–6.82	1.0
benzyl benzoate	2.93 ± 0.34	6.68	5.91–7.59	1.0
DEET	2.35 ± 0.38	17.98	15.08–22.77	0.4

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

Table 4. Cumulative Mortality of *C. officinale* Rhizome-Derived Butylidenephthalide and Acaricides against *D. farinae* Adults

compound	dose, $\mu g/cm^2$	mortality, % (mean ± SE) 24 h	48 h	72 h
butylidenephthalide	9.6	84 ± 1.9	90 ± 2.4	90 ± 2.4
	6.4	46 ± 4.0	50 ± 1.3	50 ± 1.3
benzyl benzoate	12.7	90 ± 2.9	98 ± 1.7	98 ± 1.7
	9.6	57 ± 2.6	60 ± 0.0	60 ± 0.0
DEET	50.9	79 ± 3.5	83 ± 2.3	83 ± 2.3
	38.2	41 ± 1.3	41 ± 1.3	41 ± 1.3

Table 5. Fumigant Activity of *C. officinale* Rhizome-Derived Butylidenephthalide against Adults of *D. pteronyssinus* and *D. farinae*

method[b]	dose, $\mu g/cm^2$	mortality,[a] % (mean ± SE) *D. farinae*	*D. pteronyssinus*[b]
A, vapor in open container	12.7	8 ± 1.7b	10 ± 0.0b
B, vapor in closed container	12.7	100 ± 0.0a	100 ± 0.0a

[a] Means within a column followed by the same letter are not significantly different ($P < 0.05$, Scheffe test). [b] Exposed for 24 h.

On the basis of 24-h LD_{50} values, butylidenephthalide (6.77 $\mu g/cm^2$) was most effective, but benzyl benzoate (8.54 $\mu g/cm^2$) was also toxic. Very weak activity was observed with DEET (37.59 $\mu g/cm^2$). No mortality was observed in the untreated controls.

Toxic effects in the fabric bioassay of the test compounds on *D. pteronyssinus* adults are reported in **Table 3**. On the basis of 24-h LD_{50} values, butylidenephthalide (6.46 $\mu g/cm^2$) and benzyl benzoate (6.68 $\mu g/cm^2$) were equitoxic. DEET (17.98 $\mu g/cm^2$) was relatively inactive. There was no mortality in the untreated controls.

The acaricidal activity of the test compounds to *D. farinae* adults was evaluated 24, 48, and 72 h after treatment (**Table 4**). Potencies varied according to dose. Similar results were also obtained from *D. pteronyssinus* adults (data not shown).

Poisoning Symptoms. Typical poisoning symptoms in both *Dermatophagoides* mites to butylidenephthalide were compared with those from benzyl benzoate and DEET using the fabric bioassay. All test compounds caused death without knockdown, but there were no distinguishable symptoms between *D. farinae* and *D. pteronyssinus*. Butylidenephthalide resulted in lethargy of treated mites, leading to death. Benzyl benzoate and DEET caused death following uncoordinated behavior, and in death the forelegs were extended forward in parallel.

Acaricidal Route of Action. The response of *D. farinae* adults to butylidenephthalide vapor varied with the treatment method (**Table 5**). After 24 h of exposure to 12.7 $\mu g/cm^2$, there was a significant difference ($P < 0.05$) in acaricidal activity of butylidenephthalide between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted in 8% mortality against *D. farinae* adults. There was no mortality in the untreated controls.

The toxic effects of butylidenephthalide vapor on *D. pteronyssinus* adults were examined at 12.7 $\mu g/cm^2$ (**Table 5**). There was a significant difference ($P < 0.05$) in toxicity of butylidenephthalide between closed (A, 100% mortality) and open containers (B, 10% mortality).

DISCUSSION

In East Asia, *C. officinale* (family Apiaceae) has long been considered to have medicinal properties such as an analgesic agent in the treatment of cold, headache, rheumatism, and traumatic pains and against menstrual disorders (*11*). It contains various compounds such as butylidenephthalide, butylphthalide, chrysophanoll, cnidilide, coniferyl ferulate, 4-hydroxy-3-butylphthalide, ligustilide, perlolyrine, pregnenolone, sedanonic acid, senkyunolide, and tetramethylpyrazine (*11*−*13*). Very little work has been done with respect to managing arthropod pests including house dust mites. In the present study, *C. officinale* rhizome-derived materials exhibited potent acaricidal activity against adults of *D. farinae* and *D. pteronyssinus*.

Many plant extracts and essential oils are known to possess acaricidal activity against house dust mites (*10*, *14*−*17*). The reported naturally occurring acaricidal compounds against house dust mites include *O*-anisaldehyde, citronellal, and perillaldehyde derived from perilla oil (*15*); isosericenine, acryophyllene oxide, and α-cadinol from the essential oil of the leaves from *Neolitsea sericea* Blume (*16*); sericealactone from the heartwood of *N. sericea* (*18*); pisiferic acid from the leaves of *Chamaecyparis pisifera* Sieb. et Zucc. (*19*); cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde from the bark of *Cinnamomum cassia* Blume (*10*); and eugenol and isoeugenol from the essential oil of the bud from *Eugenia caryophyllata* Thunb (*17*). It has been reported that susceptibility to some plant essential oils such as almond bitter oil, caraway oil, and perilla oil (*15*, *16*) was greater in *D. farinae* adults than in *D. pteronyssinus* adults. However, *D. farinae* adults are found to be more tolerant to the wood oils of *Thuga heterophylla* Sarg. and *Cryptomeria japonica* D. Don than *D. pteronyssinus* adults (*14*). Similar results have been also reported for eugenol, isoeugenol, and methyleugenol (*17*). Kim (*10*) reported no significant difference in toxicity of either cinnamaldehyde, cinnamyl alcohol, or salicylaldehyde between *D. farinae* and *D. pteronyssinus*. Additionally, the toxicity of cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde varied according to the dose (*10*). El-Nahal et al. (*20*) stated that exposure time appears to be a more important factor affecting the efficiency of the vapors of *Acorus calamus* L. essential oil to adults of five stored-product insect species than the dosage.

In the present study, the acaricidal constituent of *C. officinale* rhizome was identified as butylidenephthalide. The acaricidal activity of butylidenephthalide against *D. farinae* and *D. pteronyssinus* varied according to dose. This is the first report on the acaricidal activity of butylidenephthalide. The acaricidal activity of this compound against adults of *D. farinae* and *D. pteronyssinus* was comparable to that of benzyl benzoate. Additionally, there was no significant difference in toxicity of butylidenephthalide between *D. farinae* and *D. pteronyssinus*. Butylidenephthalide, a constituent of the rhizome from *Ligusticum chuanxiong* Hort., *Ligusticum sinensis* Oliv., *Ligusticum jeholense* Nakai et Kitagawa, *Angelica acutiloba* Kitagawa, and *Angelica sinensis* (Oliv.) Diels (*13*), possesses an antiplatelet effect

mainly due to an inhibitory effect on cyclooxygenase and partly due to interference with calcium mobilization (*21*), an inhibitory effect on the contraction of nonpregnant rat uterus in vitro induced by prostaglandin $F_2\alpha$, oxytocin, and acetylcholine (*21*, *22*), an antianginal effect (*23*), and antiarrhythmic effects and dilating activity on coronary arteries (*24*).

Observation of poisoning symptoms of chemicals is of practical importance for dust mite control. Five types of poisoning symptoms of chemicals against mites have been reported: a knockdown-type death caused by *N. sericea* leaf oil in adults of *D. farinae* and *D. pteronyssinus* (*16*); death related with uncoordinated behavior by (*E*)-cinnamaldehyde, salicylaldehyde, benzyl benzoate, and DEET in adults of *D. farinae* and *D. pteronyssinus* (*10*); death associated with desiccation by several monoterpenes such as fenchon, linalool, menthone, and pulegone in *Tyrophagus putrescentiae* (Schrank) adults (*25*); death related with a characteristic depression of the dorsal surface of the idiosoma by tricalcium phosphate and ferric phosphate in *T. putrescentiae* adults (*26*); and death associated with lethargy by isoeugenol amd methyleugenol in adults of *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (*17*). Compounds such as cinnamaldehyde, cinnamyl alcohol, salicylaldehyde, acetyleugenol, eugenol, methyleugenol, benzyl benzoate, and DEET are found to result in a similar death symptom of the forelegs extended forward together (*10*, *17*). In our study, the *Cnidium* rhizome-derived butylidenephthalide resulted in lethargy of treated *Dermatophagoides* mites, being directly connected to death without knockdown and with the forelegs extended forward together. Poisoning symptoms of butylidenephthalide are thus similar to those of isoeugenol.

Volatile compounds of many plant extracts and essential oils are composed of alkanes, alcohols, aldehydes, and terpenoids, especially monoterpenoids, and exhibit fumigant activity (*10*, *17*, *27*−*29*). Fumigant activity against adults of *D. farinae* and *D. pteronyssinus* has been reported for (*E*)-cinnamaldehyde, cinnamyl alcohol, and salicylaldehyde (*10*) as well as eugenol, isoeugenol, and methyleugenol (*17*). In this study, butylidenephthalide was much more effective in closed containers than in open ones against adults of *D. farinae* and *D. pteronyssinus*. These results indicate that the effects of the compound may be largely due to action in the vapor phase: the toxicity may be delivered by penetrating the dust mite body via the respiratory system. However, the exact acaricidal mode of action remains unknown.

Results of this and earlier studies indicate that *C. officinale* rhizome-derived butylidenephthalide could be of practical use as a fumigant for *D. farinae* and *D. pteronyssinus*, provided that a carrier producing a slow-release effect can be selected or developed. Further research should be done on safety issues of butylidenephthalide for human health, its acaricidal mode of action, and formulations improving the acaricidal potency and stability.

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