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Acaricidal Activity of *Paeonia suffruticosa* Root Bark-Derived Compounds against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

HYUN-KYUNG KIM, JUN-HYUNG TAK, AND YOUNG-JOON AHN*

School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Republic of Korea

The acaricidal activities of materials derived from the root bark of *Paeonia suffruticosa* against adults of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* were examined using direct contact and fumigation bioassays and compared with those of benzyl benzoate, dibutyl phthalate, and *N*,*N*-diethyl-*m*-toluamide (deet), widely used acaricides. The active constituents of *Paeonia* root bark were identified as paeonol and benzoic acid by spectroscopic analyses. On the basis of 24-h LD_{50} values, the acaricidal activities of paeonol (7.82 μg/cm^3) and benzoic acid (6.58 μg/cm^3) against adult *D. farinae* were comparable to that of benzyl benzoate (7.72 μg/cm^3) but higher than those of deet (36.34 μg/cm^3) and dibutyl phthalate (33.92 μg/cm^3). Against adult *D. pteronyssinus*, the acaricidal activities of paeonol (7.08 μg/cm^3) and benzyl benzoate (7.22 μg/cm^3) were comparable to that of benzyl benzoate (7.14 μg/cm^3). Deet and dibutyl phthalate were less effective. In fumigation tests with both mite species, paeonol and benzoic acid were much more effective in closed containers than open ones, indicating that the effect of these compounds was largely a result of action in the vapor phase. Neither benzyl benzoate, deet, nor dibutyl phthalate exhibited fumigant toxicity. *Paeonia* root bark-derived materials, particularly paeonol and benzoic acid, merit further study as potential acaricides or lead compounds for the control of *D. farinae* and *D. pteronyssinus*.

KEYWORDS: Natural acaricide; natural fumigant; house dust mite; *Dermatophagoides farinae*; *Dermatophagoides pteronyssinus*; *Paeonia suffruticosa*; paeonol; benzoic acid

INTRODUCTION

House dust mites are classified in the family Pyroglyphidae, which consists of 10 species (*1*). Pyroglyphid mites usually account for >90% of the mite populations in houses. The most important pyroglyphid mites are the American house dust mite, *Dermatophagoides farinae* Hughes, and the European house dust mite, *Dermatophagoides pteronyssinus* Trouessart, because of their cosmopolitan occurrence and abundance in homes (*2*, *3*), because they are a major source of multiple potent allergens (*1*, *3*, *4*), and because of 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, promote dust mite growth (*2*). Control of house dust mites depends primarily on multiple applications of chemicals, such as γ-benzene hexachloride (γ-BHC), benzyl benzoate, dibutyl phthalate, *N*,*N*-diethyl-*m*-toluamide (deet), natamycin, pirimiphos-methyl, pyrethrin, and pyrethroids (permethrin and *S*-bioallethrin) (*1*, *2*, *6*). Repeated use of these acaricides has resulted in resistance (*7*) and undesirable effects on nontarget organisms and fosters serious human health concerns (*2*, *8*). These problems substantiate the need for selective control alternatives for house dust mites, particularly for compounds with fumigant action for ease of application.

Plants have been suggested as an alternative source of materials for dust mite control, and much effort has been focused on plant extracts or phytochemicals as potential sources of commercial control agents or as lead compounds. The reported naturally occurring acaricidal compounds against house dust mites include *O*-anisaldehyde, citronellal, and perillaldehyde derived from perilla oil (*9*); sericealactone from the heartwood of *Neolitsea sericea* (*10*); isosericenine, caryophyllene oxide, and α-cadinol from leaf essential oil of *N. sericea* (*11*); pisiferic acid from the leaves of *Chamaecyparis pisifera* (*12*); butylidenephthalide from the rhizome of *Cnidium officinale* (*13*); eugenol and isoeugenol from the bud and leaf essential oils of *Eugenia caryophyllata* (*14*); liriodenine and verisuvanone from *Uvaria versicolor* (*15*); and cedrol and thujopsene from *Thujopsis dolabrata* var. *hondai* (*16*). In the Chinese Pharmacopoeia, the dry root bark of *Paeonia suffruticosa* Andrew (Paeoniaceae) has long been considered to have medicinal properties and is used as an analgesic, hemostyptic, and bacteriostatic agent (*17*). These properties are attributable to various compounds such as apiopaeonoside, benzoic acid, benzoxypaeoniflorin, benzoyloxypaeoniflorin, benzoylpaeoniflorin, campesterol, galloylpaeoniflorin, oxypaeoniflorin, paeoniflorin, paeonol, paeonolide, paeonoside, and 1,2,3,4,6-pentagalloylglucose (*17*, *18*). Little

* Author to whom correspondence should be addressed (telephone +82-2-8804702; fax +82-2-8732319; e-mail yjahn@snu.ac.kr).

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information exists with respect to managing house dust mites with *P. suffruticosa* root bark compounds, although *Paeonia* root extract is insecticidal against adult *Musca domestica* (L.) (*19*).

This paper describes a laboratory study aimed at isolating acaricidal constituents from the root bark of *P. suffruticosa* active against adults of *D. farinae* and *D. pteronyssinus* and determining their acaricide route of action. The acaricidal activity of *P. suffruticosa* root bark-derived compounds was also compared with those of the widely used acaricides benzyl benzoate, deet, and dibutyl phthalate.

MATERIALS AND METHODS

Chemicals. Benzyl benzoate and deet were purchased from Sigma-Aldrich (St. Louis, MO). Dibutyl phthalate was supplied by Junsei Chemical (Tokyo, Japan). 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. Mites were reared in plastic containers (12.5 × 10.5 × 5.0 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.

Isolation and Identification. Air-dried root bark (600 g) of *P. suffruticosa* was pulverized, extracted with 3 L of methanol twice at room temperature for 2 days, and filtered. The combined filtrate was concentrated under vacuum at 40 °C to yield 97.8 g of an extract. The extract (40 g) was sequentially partitioned into hexane (12.6 g), chloroform (1.4 g), ethyl acetate (1.9 g), butanol (6.1 g), and water portions (18.0 g). The organic solvent portions were concentrated to dryness by rotary evaporation at 40 °C, and the water portion was freeze-dried. For isolation of the active principles, 50.9 μg/cm^3 of each *P. suffruticosa* root bark-derived fraction in ethanol was applied by a fabric contact bioassay described below.

The hexane fraction (12 g) was chromatographed on a silica gel (70−230 mesh, 700 g, Merck) column (5.5 × 70 cm), and successively eluted with a stepwise gradient of hexane/ethyl acetate (100:0, 99:1, 90:10, 80:20, 70:30, and 0:100 by volume). Column fractions were monitored by thin-layer chromatography (TLC) on silica gel plates (Kieselgel 60 F_{254}, 0.20 mm thickness, Merck) with hexane/ethyl acetate (9:1). Fractions with similar R_f values on the TLC plates were pooled. Spots were detected by spraying with 30% H_2SO_4 and then heating on a hot plate. Two active H1 (8.14 g) and H3 (0.45 g) fractions were obtained among five fractions. The H1 fraction was rechromatographed on a silica gel column, using hexane/ethyl acetate (9:1 by volume) to give three fractions. The active H11 fraction (1.55 g) afforded compound **1** (1.52 g) by repeated recrystallization in hexane. Further separation of the constituents of the H3 fraction was achieved using a preparative HPLC (Spectra System P2000, Thermo Separation Products). The column was a 3.9 mm i.d. × 300 mm μPorasil (Phenomenex) using a mobile phase of hexane/ethyl acetate (4:1 by volume) at a flow rate of 3 mL/min. Chromatographic separations were monitored using a UV detector at 257 nm. Finally, a potent active principle, **2** (13 mg), at the retention time of 3.0 min was isolated.

The structures of the isolates were determined by spectroscopic analysis. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on 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). Mass spectra were obtained on a JEOL GSX 400 spectrometer.

Bioassays. A fabric contact bioassay (*13*) was used to determine the toxicity of *P. suffruticosa* root bark-derived materials and acaricides to adults of *D. farinae* and *D. pteronyssinus*. Mites were exposed to eight concentrations of materials (101.8, 50.9, 25.5, 12.7, 6.4, 3,2, 1.6, and 0.08 μg/cm^3), each of which was dissolved in 40 μL of ethanol and applied to disks (5-cm diameter) of black cotton fabric. Control fabric disks received 40 μL of ethanol. After drying in a fume hood for 2 min, each fabric disk was placed on the bottom of a Petri dish (5 cm diameter × 1.2 cm). Batches of 25−30 adult mites (7−10 days old) were placed on each Petri dish and covered with a lid.

Table 1. Mortality of *P. suffruticosa* Root Bark-Derived Materials against Adults of *D. farinae* and *D. pteronyssinus* Using the Fabric Contact Bioassay

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

[a] Means within a column followed by the same letter are not significantly different ($P = 0.05$, Scheffé test). [b] Exposed for 24 h at a dose of 50.9 μg/cm^3. [c] Number of mites tested.

Treated and control (ethanol only) mites were held at the same conditions used for colony maintenance. Mortalities were determined 24 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when they were probed with a wooden dowel. All treatments were replicated three to five times. Benzyl benzoate, deet, and dibutyl phthalate served as standard acaricides for comparison in toxicity tests. The LD_{50} values of the test compounds were calculated by probit analysis (*20*). Acaricidal activity was considered to be significant when 95% confidence limits were not overlapping with ethanol control values.

Acaricidal Route of Action. The vapor phase toxicity of the test compounds and acaricides against adults of *D. farinae* and *D. pteronyssinus* was investigated according to the method of Kwon and Ahn (*13*). Briefly, batches of 25−35 adult mites (7−10 days old) were placed on the bottom of a Petri dish (5 cm i.d. × 1 cm) and covered with a lid with a fine wire sieve (200 mesh, 4-cm diameter) attached to the central hole (3-cm diameter). Black cotton fabric disks (5-cm diameter) were treated either with 50.9 μg/cm^3 of paenol, benzoic acid, or benzyl benzoate or with 101.8 μg/cm^3 of deet or dibutyl phthalate in 40 μL of ethanol. The treated disk was placed on top of the wire sieve, which prevented direct contact of adult mites with the test compound and acaricide. Each Petri dish was then either sealed with another lid (method A) to investigate the potential vapor phase toxicity of the test compounds and acaricides or left unsealed (method B). Control fabric disks received 40 μL of ethanol.

Treated and control (ethanol only) mites were held at the same conditions used for colony maintenance. Mortalities were determined 24 h after treatment under a binocular microscope. All treatments were replicated three times.

Statistical Analysis. Percent mortality was determined and transformed to arcsine square root values for analysis of variance (ANOVA). The Scheffé test was used to test for significant differences among the test compounds and acaricides (*20*). A *t* test was used to test for significant differences between two treatment methods (*20*). Means [± standard error (SE)] of untransformed data are reported.

RESULTS

Lethal Activity of *P. suffruticosa* Root Bark Extract. Significant mortality differences were observed in adults of *D. farinae* and *D. pteronyssinus* (**Table 1**). At a dose of 50.9 μg/cm^3, the hexane fraction showed 100% mortality against both mite species 24 h after treatment, whereas the other fractions were ineffective. There was no mortality in the ethanol-treated controls.

Identification of Active Principles. Fabric contact bioassay-guided fractionation of *P. suffruticosa* root bark extract afforded two active principles identified by spectroscopic analyses, including MS and NMR. The two active principles were the aryl ketone, paeonol [1-(2-hydroxy-4-methoxyphenyl)ethanone] (**1**) and benzoic acid (**2**) (**Figure 1**). Paeonol was identified on the basis of the following evidence: white needles; EI-MS (70

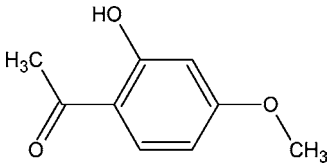

Figure 1. Structure of paeonol, the acaricidal constituent of the root bark from *P. suffruticosa*.

Table 2. Toxicity of Paeonol, Benzoic Acid, and Acaricides against Adult *D. farinae* Using the Fabric Contact Bioassay

compound[a]	*n*	slope ± SE	LD_{50} (μg/cm^3)	95% cl[b]
paeonol	526	2.71 ± 0.26	7.82	6.69–8.86
benzoic acid	549	3.21 ± 0.26	6.58	5.89–7.32
benzyl benzoate	544	3.12 ± 0.36	7.72	6.63–8.66
deet	872	2.67 ± 0.18	36.34	33.53–39.53
dibutyl phthalate	375	2.92 ± 0.26	33.92	30.05–38.20

[a] Exposed for 24 h. [b] cl denotes confidence limit.

Table 3. Toxicity of Paeonol, Benzoic Acid, and Acaricides against Adult *D. pteronyssinus* Using the Fabric Contact Bioassay

compound[a]	*n*	slope ± SE	LD_{50} (μg/cm^3)	95% cl[b]
paeonol	922	2.74 ± 0.16	7.08	6.46–7.73
benzoic acid	593	3.52 ± 0.26	7.22	6.58–7.89
benzyl benzoate	944	2.31 ± 0.15	7.14	6.32–7.95
deet	350	2.66 ± 0.26	44.58	39.10–51.30
dibutyl phthalate	396	2.75 ± 0.25	31.59	27.85–35.61

[a] Exposed for 24 h. [b] cl denotes confidence limit.

eV), *m*/*z* (% rel int) 166 $[M]^+$ (40), 151 (100, base peak), 149 (7), 108 (7), 95 (8), 88 (8), 61 (11), 57 (5); ^{1}H NMR (CD_3OD, 400 MHz) δ 2.52 (3H, s), 3.81 (3H, s), 6.38 (1H, d, J = 2.4 Hz), 6.46 (1H, dd, J = 9.0 and 2.4 Hz), 7.74 (1H, d, J = 9.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 26.3 q, 56.1 q, 101.7 d, 108.3 d, 115.0 s, 133.9 d, 166.2 s, 167.7 s, 204.5 s. Benzoic acid was identified by direct comparison with an authentic compound.

Acaricidal Activity of Test Compounds. The toxicities of paeonol and benzoic acid to adult *D. farinae* were compared with those of benzyl benzoate, deet, and dibutyl phthalate (**Table 2**). On the basis of 24-h LD_{50} values, the acaricidal activities of paeonol and benzoic acid were comparable to that of benzyl benzoate. Deet and dibutyl phthalate were less effective. Paeonol was 4.6- and 4.3-fold more toxic than deet or dibutyl phthalate, respectively. Benzoic acid was 5.5 and 5.2 times more toxic than deet or dibutyl phthalate, respectively. No mortality was observed in the ethanol-treated controls.

The toxicities of paeonol and benzoic acid along with those standard acaricides benzyl benzoate, deet, and dibutyl phthalate on adult *D. pteronyssinus* in the fabric contact bioassay are given in **Table 3**. The acaricidal activities of paeonol and benzoic acid were comparable to that of benzyl benzoate as judged by 24-h LD_{50} values. Deet and dibutyl phthalate were less active. Paeonol was 6.3- and 4.5-fold more toxic than deet or dibutyl phthalate, respectively. Benzoic acid was 6.2 and 4.4 times more toxic than deet or dibutyl phthalate, respectively.

Acaricidal Route of Action. The vapor phase toxicities of paeonol, benzoic acid, benzyl benzoate, deet, and dibutyl phthalate against adult *D. farinae* were investigated using a fumigant bioassay in two formats (**Table 4**). After 24 h of exposure to 50.9 μg/cm^3, there was a significant difference in acaricidal activity of paeonol between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted in 11% mortality against adult *D. farinae*. Similar differences in the response of adult *D. farinae* to benzoic acid in treatments A and B were likewise observed. Little or no fumigant toxicity was observed with benzyl benzoate, deet, and dibutyl phthalate. There was no mortality in the ethanol-treated controls.

Table 4. Fumigant Activity of Paeonol, Benzoic Acid, and Acaricides against Adults of *D. farinae* and *D. pteronyssinus*, Exposed for 24 h

		dose	mortality,[a] % (mean ± SE)			
compound	method[b]	(μg/cm^3)	*n*	*D. farinae*	*n*	*D. pteronyssinus*
paeonol	A	50.96	97	100 ± 0.0a	83	100 ± 0.0a
	B	50.96	83	19 ± 4.5b	73	11 ± 1.5b
benzoic acid	A	50.96	90	100 ± 0.0a	82	100 ± 0.0a
	B	50.96	87	24 ± 3.2b	74	8 ± 2.6b
benzyl benzoate	A	50.96	103	7 ± 2.9a	74	8 ± 1.6a
	B	50.96	102	8 ± 1.6a	76	7 ± 1.5a
deet	A	101.92	85	14 ± 2.7a	83	8 ± 2.1a
	B	101.92	75	5 ± 2.6a	74	4 ± 2.0a
dibutyl phthalate	A	101.92	90	0 ± 0.0a	66	5 ± 1.5a
	B	101.92	102	5 ± 0.8a	83	5 ± 1.8a

[a] Means within a column followed by the same letter are not significantly different (P = 0.05, t test). [b] A, vapor in closed containers; B, vapor in open containers.

Toxic effects of vapors of the test compounds and acaricides on adult *D. pteronyssinus* were also examined (**Table 4**). Both paeonol and benzoic acid elicited 100% mortality in closed containers but were less toxic in open containers. Neither benzyl benzoate, deet, nor dibutyl phthalate exhibited fumigant toxicity.

DISCUSSION

Natural product fumigants have potential as house dust mite control agents because some are selective to certain pests and have little or no harmful effects on nontarget organisms or the environment (*21*−*23*). They can be applied to dust mite nests such as beds, sofas, furnitures, and carpeted floors in the same way as other conventional acaricides. Many plant extracts and plant essential oils are known to possess ovicidal, antifeeding, repellent, and killing activities against arthropod pests (*21*−*23*). Additionally, some plant extracts or phytochemicals can be highly effective against insecticide-resistant insect pests (*24*, *25*). In the present study, *P. suffruticosa* root bark-derived materials exhibited potent acaricidal activity against adults of *D. farinae* and *D. pteronyssinus*.

Various compounds such as alkaloids, phenolics, and terpenoids exist in plants. Jointly or independently, they contribute to the generation of a variety of bioactivities. Many plant extracts and essential oils are known to possess acaricidal activity against house dust mites (*9*−*13*, *26*). In the present study, the acaricidal constituents of *P. suffruticosa* root bark were identified as paeonol and benzoic acid. This is apparently the first report on the acaricidal activity of paeonol. The acaricidal activities of these compounds against adults of *D. farinae* and *D. pteronyssinus* were comparable to that of benzyl benzoate but were more pronounced than those of deet and dibutyl phthalate. Additionally, there was no significant difference in the toxicities of paeonol, benzoic acid, and acaricides between *D. farinae* and *D. pteronyssinus*. Similar results have been also reported for butylidenephthalide (*13*). Paeonol is insecticidal against adult *M. domestica* (*19*). It also possesses antithrombotic and anti-inflammatory (*27*), analgesic (*28*), diuretic (*29*), antispasmodic (*30*), antiarrhythmic (*31*), and antihypertensive (*32*) activities in mammals. Paeonol is antibacterial (*33*) and antimutagenic (*34*) and promotes DNA adduct formation and *N*-acetyltransferase activity in colon tumor cells (*35*).

Elucidation of the mode of action of acaricidal natural products and acaricides is of practical importance for mite control because it may give useful information on the most appropriate formulation and delivery means. Volatile compounds of many plant extracts consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids, and exhibit fumigant activity (*13*, *14*, *36*). Fumigant activity against adults of *D. farinae* and *D. pteronyssinus* has been reported for butylidenephthalide (*13*) and for eugenol, isoeugenol, and methyleugenol (*14*). In the current study, paeonol and benzoic acid were much more effective in closed versus open containers against adults of *D. farinae* and *D. pteronyssinus*. These results indicate that the mode of delivery of these compounds was likely by vapor action via the respiratory system, although the exact mode of action of these compounds remains unknown.

Results of this study indicate that *P. suffruticosa* root bark-derived paeonol and benzoic acid could be useful as fumigants for *D. farinae* and *D. pteronyssinus*. Additionally, paeonol has low toxicity to mammals (LD_{50} orally, 3430 mg/kg rat; LD_{50} intravenously, 196 mg/kg rat; LD_{50} intraperitoneally, 781 mg/kg rat) (*28*). For the practical use of these compounds as novel fumigants to proceed, further research is necessary on safety issues of paeonol and benzoic acid on human health. Other areas requiring attention are formulations to improve the acaricidal potency and stability as well as to reduce cost.

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