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Ovicidal and Adulticidal Effects of *Eugenia caryophyllata* Bud and Leaf Oil Compounds on *Pediculus capitis*

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The toxicity of *Eugenia caryophyllata* bud and leaf oil-derived compounds (acetyleugenol, β-caryophyllene, eugenol, α-humulene, and methyl salicylate) and congeners of eugenol (isoeugenol and methyleugenol) against eggs and females of *Pediculus capitis* was examined using direct contact application and fumigation methods and compared with those of the widely used δ-phenothrin and pyrethrum. In a filter paper diffusion bioassay with female *P. capitis*, the pediculicidal activity of the *Eugenia* bud and leaf oils was comparable to those of δ-phenothrin and pyrethrum on the basis of LT_{50} values at 0.25 mg/cm^2. At 0.25 mg/cm^2, the compound most toxic to female *P. capitis* was eugenol followed by methyl salicylate. Acetyleugenol, β-caryophyllene, α-humulene, isoeugenol, and methyleugenol were not effective. Eugenol at 0.25 mg/cm^2 was as potent as δ-phenothrin and pyrethrum but was slightly less effective than the pyrethroids at 0.125 mg/cm^2. Against *P. capitis* eggs, methyl salicylate and eugenol were highly effective at 0.25 and 1.0 mg/cm^2, respectively, whereas little or no activity at 5 mg/cm^2 was observed with the other test compounds as well as with δ-phenothrin and pyrethrum. In fumigation tests with female *P. capitis* at 0.25 mg/cm^2, eugenol and methyl salicylate were more effective in closed cups than in open ones, indicating that the effect of the compounds was largely due to action in the vapor phase. Neither δ-phenothrin nor pyrethrum exhibited fumigant toxicity. The *Eugenia* bud and leaf essential oils, particularly eugenol and methyl salicylate, merit further study as potential *P. capitis* control agents or lead compounds.

KEYWORDS: Natural insecticide; pediculicide; ovicide; fumigant; *Pediculus capitis*; *Eugenia caryophyllata*; essential oil; GC-MS; eugenol; methyl salicylate; mode of action

INTRODUCTION

The human head louse, *Pediculus capitis* De Geer, is an ectoparasite, confined to the scalp and hair of humans. Infestations are prevalent worldwide and especially common among schoolchildren in both developed and developing countries (*1*). *P. capitis* infections cause skin irritation, pruritus, and sleep loss, as well as occasional secondary bacterial infections from scratching (*1*, *2*). Although the symptoms are relatively mild, infestation by *P. capitis* causes substantial degrees of social, mental, and economic problems. In recent years, *P. capitis* infestations have increased in Korea (*3*, *4*). Control of this insect worldwide primarily depends on continued applications of organachlorinates (DDT and lindane), organophosphates (malathion and temephos), carbamates (carbaryl and propoxur), and pyrethroids (permethrin and phenothrin) (*1*, *2*). Their repeated use has sometimes resulted in the development of resistance (*1*, *5*), and increasing levels of resistance to the most commonly used pediculicides have caused multiple and overdosed treatments, fostering serious human health concerns (*6*). These problems have highlighted the need for the development of selective *P. capitis* control alternatives, particularly with fumigant action for ease of application to hairs.

Plant essential oils may be an alternative source of materials for insect control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and as flavoring agents for food additives (*7*). Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial *P. capitis* control agents. As a traditional Chinese medicine, clove (*Eugenia caryophyllata* Thunberg), belonging to the family Myrtaceae, has long been considered to have medicinal properties such as a stimulant against digestive disorders and diarrhea. The *Eugenia* bud and leaf essential oils contain various compounds such as acetyleugenol, benzaldehyde, benzyl acetate, benzyl alcohol, β-caryo-

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phyllene, chavicol, eugenol, α-humulene, *m*-methoxy benzaldehyde, methyl-*n*-amyl ketone, methyl salicylate, and α-ylangene (*8*). Very little work has been done with respect to the pediculicidal activity of the *Eugenia* bud and leaf oils, although their biocidal activity against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motsch) (*9*), *Tyrophagus putrescentiae* (Schrank) (*10*), *Dermatophagoides farinae* (Hughes), and *Dermatophagoides pteronyssinus* (Trouessart) (*11*) to suppress progeny production of *T. castaneum* and *S. zeamais* (*9*) is well-known.

This paper describes a laboratory study in which we examined the insecticidal activity of *E. caryophyllata* bud and leaf oil-derived compounds (acetyleugenol, β-caryophyllene, eugenol, α-humulene, and methyl salicylate) and congeners of eugenol (isoeugenol and methyleugenol) against *P. capitis* females and eggs and investigated their pediculicidal route of action. The structure−pediculicidal activity relationships of the phenylpropenes (acetyleugenol, eugenol, isoeugenol, and methyleugenol) are also discussed.

MATERIALS AND METHODS

Chemicals. β-Caryophyllene and α-humulene were purchased from Aldrich (Milwaukee, WI). Acetyleugenol, eugenol, isoeugenol, methyleugenol, and methyl salicylate were supplied by Wako (Osaka, Japan), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), Merck (Mohenbrunn, Germany), and Kanto (Tokyo, Japan), respectively. δ-Phenothrin (92% purity) and pyrethrum extract (50%) were obtained from Hanil and Biomist (Seoul, Korea), respectively. All other chemicals were of reagent grade.

Head Lice. A colony of *P. capitis* was collected by combing from the hair of 78 infested children (7 boys and 71 girls) at a primary school in Songpa District, Seoul, in December 2001. The collected head louse specimens were immediately transferred to a Petri dish (5 cm diameter) with 0.01 and 1.0 mm mesh screens attached over the central holes (4 cm diameter) on the lid and bottom sides, respectively, and containing a few strands of human hair. To feed the head lice with bloodmeal, the Petri dish was placed on the leg skin of one of the authors (Y.-C.Y.), and maintained there for ca. 16 h everyday according to the method of Lee et al. (*12*). Eggs were held at 32 ± 1 °C and 60 ± 5% relative humidity (RH) in darkness. Under these conditions, longevity of eggs, nymphs, and adults was ca. 6.3, 12.7, and 7.3 days, respectively.

Chromatographic Analysis of Clove Bud and Leaf Oils. *E. caryophyllata* bud and leaf oils were purchased from Jin Aromatics, Anyang, Kyunggi Province, Korea. Chromatographic analyses were performed using a Hewlett-Packard 6890 series gas chromatograph (GC), equipped with a splitless injector and a flame ionization detection system. Analytes were separated with a 0.2 mm i.d. × 50 m CBP-20 column (Shimadzu) with a film thickness 0.25 μm. The temperature program used for the analysis was as follows: initial temperature at 50 °C, held for 10 min and ramped at 2 °C/min to 200 °C, held for 15 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. The detector gases were hydrogen and air, and their flow rates were regulated at 500 and 45 mL/min, respectively. The detector temperature was set to 250 °C, and the injector temperature was set to 210 °C.

GC-MS experiments were performed on a GC (HP 6890) MS (JMS-600W, JEOL, Japan). The capillary column and temperature conditions for the GC-MS analysis were the same as described above for GC analysis. Helium was used as the carrier gas (1 mL/min). The interface was kept at 230 °C, and mass spectra were obtained at 70 eV. The effluent of the capillary column was introduced directly into the ion source of the mass spectrometer. The sector mass analyzer was set to scan from 50 to 800 amu for every 1.3 s. *E. caryophyllata* bud and leaf oil compounds were identified by the comparison of mass spectra of each peak with those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectral Data, 6th ed.) and confirmed by the comparison of retention times obtained by GC with those of authentic samples.

Bioassay. To synchronize the developmental stages, adults were allowed to lay eggs for 24 h as mentioned above, after which time the adults were removed with a fine brush. The stages tested consisted of eggs and adult females. Female lice were separated from the colony based on the criteria as suggested by Ibarra (*13*).

A filter paper diffusion bioassay was used for toxicity of the test materials to female *P. capitis*. Amounts (0.125, 0.25, and 0.5 mg/cm^2) of each test material dissolved in 20 μL of acetone were applied to filter papers (Whatman No. 2, 5 cm diameter). Control filter papers received 20 μL of acetone. After they were dried in a fume hood for 2 min, each filter paper was placed on the bottom of a Petri dish (5 cm diameter × 1.2 cm). Then, batches of 20 females (7−9 days old) of *P. capitis*, fed with human blood meal 4 h prior to the test, were placed on each Petri dish containing a few strands of human hair and covered with a lid.

For *P. capitis* eggs, amounts (0.125−5 mg/cm^2) of each test compound dissolved in 20 μL of acetone were applied to filter papers. Control filter papers received 20 μL of acetone. After they were dried in a fume hood for 1.5 min, each filter paper was placed in the bottom of a Petri dish. Then, *P. capitis* eggs (3−4 days old) attached to hair were placed in each Petri dish and covered with a lid.

Treated and control (solvent only) females and eggs were held at 31 ± 1 °C and 65 ± 5% RH in darkness. Adult mortalities were determined every 5 min for 5 h. The toxicity of the test compounds to the eggs was based on the number of unhatched eggs 8 days after treatment. All treatments were replicated three times. δ-Phenothrin and pyrethrum served as a standard for comparison in toxicity tests. The LT_{50} values were calculated by probit analysis (*14*).

Pediculicidal Route of Action. Vapor phase toxicity of the test compounds against female *P. capitis* was investigated according to the method of Kwon and Ahn (*15*). Briefly, batches of 20 females (7−10 days old) were placed on 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 over the center hole (4.5 cm diameter). Each filter paper (5 cm diameter), treated with 0.25 mg/cm^2 of each test compound dissolved in 20 μL of acetone, was placed over the wire sieve. This prevented direct contact of test females with the test compound. Each Petri dish was then either covered with another lid (method A) to investigate the potential vapor phase toxicity of the test compounds or left uncovered (method B). Control filter papers received 20 μL of acetone.

Treated and control females were held under the same conditions used for colony maintenance. Mortalities were determined every 5 min for 5 h. All treatments were replicated three times. The LT_{50} values were calculated by probit analysis (*14*).

Statistical Analyses. The percentage of mortality and hatchability was determined and transformed to arcsine square root values for analysis of variance. Treatment means were compared and separated by the Scheffe test at $P = 0.05$ (*14*). Means (± SE) of untransformed data are reported.

RESULTS

Chemical Constituents of *E. caryophyllata* Bud and Leaf Oils. Differences in quantitative and qualitative composition were observed for the *Eugenia* bud and leaf oils by comparison of mass spectral data and retention times of authentic compounds (**Table 1**). *Eugenia* bud oil was mainly composed of eugenol, acetyleugenol, and β-caryophyllene, and also contained α-humulene, methyl salicylate, caryophyllene oxide, and chavicol as minor components. *Eugenia* leaf oil was mainly composed of eugenol, β-caryophyllene, and α-humulene, and caryophyllene oxide and chavicol as minor components. Methyl salicylate and acetyleugenol were not detected in the leaf oil.

Pediculicidal Activity of the *Eugenia* Bud and Leaf Oils. The pediculicidal activity of the *Eugenia* bud and leaf oils along with standard compounds δ-phenothrin and pyrethrum was evaluated by comparing the LT_{50} values estimated from direct contact bioassay (**Table 2**). As judged by the LT_{50} values at 0.25 mg/cm^2, the pediculicidal activity of the *Eugenia* bud and leaf oils was comparable to those of δ-phenothrin and pyrethrum

Table 1. Chemical Constituents of *E. caryophyllata* Bud and Leaf Oils Identified by GC-MS

	bud oil		leaf oil	
compound	RT (min)	relative (%)	RT (min)	relative (%)
β-caryophyllene	40.29	7.3	40.52	15.6
α-humulene	44.17	0.8	44.34	3.4
methyl salicylate	50.57	0.2	ND[a]	ND
caryophyllene oxide	62.06	0.2	62.14	0.6
eugenol	71.26	69.8	72.02	79.5
acetyleugenol	75.40	20.9	ND	ND
chavicol	78.38	0.4	78.36	0.1

[a] Not detected.

Table 2. Toxicity of *E. caryophyllata* Bud and Leaf Oils and Pyrethroids against Female *P. capitis* Using the Filter Paper Diffusion Bioassay

material[a]	dose (mg/cm^2)	n[b]	slope ± SE	LT_{50} (min)	95% cl[c]
bud oil	0.25	60	8.20 ± 0.89	18.80	17.78–19.81
leaf oil	0.25	60	6.49 ± 0.74	21.64	20.37–23.04
δ-phenothrin	0.25	60	5.17 ± 0.76	20.97	18.64–23.24
pyrethrum	0.25	60	4.01 ± 0.70	22.66	19.71–25.77

[a] Exposed for 5 h. [b] Number of females. [c] cl denotes confidence limit.

Table 3. Toxicity of Test Compounds against Female *P. capitis* Using the Filter Paper Diffusion Bioassay

compound[a]	dose (mg/cm^2)	n[b]	slope ± SE	LT_{50} (min)	95% cl[c]
eugenol[d]	0.125	60	7.58 ± 1.27	41.32	37.81–44.69
	0.25	60	6.19 ± 1.12	26.55	24.42–29.03
	0.50	60	5.21 ± 0.84	26.04	23.55–29.02
methyl salicylate[d]	0.25	60	15.26 ± 2.21	35.86	34.58–37.21
	0.50	60	14.90 ± 2.19	28.26	27.15–29.32
acetyleugenol[d]	0.50	60		>300	
isoeugenol	0.50	60		>300	
methyleugenol	0.50	60		>300	
α-humulene[d]	0.50	60		>300	
β-caryophyllene[d]	0.50	60		>300	
caryophyllene oxide[d]	0.50	60		>300	
δ–phenothrin	0.125	60	3.00 ± 0.40	34.57	30.49–39.41
	0.25	60	3.22 ± 0.38	25.22	22.34–28.53
pyrethrum	0.125	60	3.33 ± 0.46	36.73	32.26–41.04
	0.25	60	3.44 ± 0.39	27.93	24.57–31.32

[a] Exposed for 5 h. [b] Number of females. [c] cl denotes confidence limit. [d] *E. caryophyllata* bud and leaf oil compounds identified in this study.

against female *P. capitis*. The pediculicidal activity of the *Eugenia* bud and leaf oils was almost identical. There was no mortality in the untreated controls.

Toxicity of Test Compounds to Female *P. capitis*. The toxicity of the *Eugenia* essential oil compounds (acetyleugenol, β-caryophyllene, eugenol, α-humulene, and methyl salicylate) and congeners of eugenol (isoeugenol and methyleugenol) against female *P. capitis* was compared with those of δ-phenothrin and pyrethrum (**Table 3**). Responses varied according to compound. On the basis of 24 h LT_{50} values at 0.25 mg/cm^2, the most toxic compound against female *P. capitis* was eugenol followed by methyl salicylate. Acetyleugenol, β-caryophyllene, α-humulene, isoeugenol, and methyleugenol were almost ineffective. Eugenol at 0.25 mg/cm^2 was as potent as δ-phenothrin and pyrethrum but was slightly less effective than the pyrethroids at 0.125 mg/cm^2. No mortality was observed in the untreated controls.

Table 4. Effects of Test Compounds on Hatchability of *P. capitis* Eggs As Determined by the Filter Paper Diffusion Bioassay

compound[a]	dose (mg/cm^2)	n[b]	hatchability (%) (mean ± SE)
eugenol[c]	0.125	60	32 ± 1.7^{c-e}
	0.25	60	23 ± 1.7^{de}
	0.5	60	12 ± 1.7^{ef}
	1.0	60	3 ± 1.7^{fg}
methyl salicylate[c]	0.125	60	20 ± 2.9^{de}
	0.25	60	0 ± 0.0^{g}
	0.5	60	0 ± 0.0^{g}
acetyleugenol[c]	5.0	60	77 ± 1.7^{a}
isoeugenol	5.0	60	67 ± 1.7^{ab}
methyleugenol	5.0	60	68 ± 4.4^{ab}
α-humulene[c]	5.0	60	48 ± 1.7^{bc}
β-caryophyllene[c]	5.0	60	42 ± 1.7^{cd}
δ-phenothrin	5.0	60	83 ± 4.4^{a}
pyrethrum	5.0	60	75 ± 0.0^{a}
untreated		60	80 ± 2.9^{a}

[a] Exposed for 24 h. [b] Number of eggs. [c] *E. caryophyllata* bud and leaf oil compounds identified in this study.

Table 5. Fumigant Activity of Test Compounds against Female *P. capitis*

compound[a]	method[b]	n[c]	LT_{50} (min)	95% cl[d]
eugenol	A	60	21.19	19.52–22.71
	B	60	111.26	91.29–131.44
methyl salicylate	A	60	33.95	31.80–35.94
	B	60	>300	
δ-phenothrin	A	60	>300	
	B	60	>300	
pyrethrum	A	60	>300	

[a] Exposed for 5 h at 0.25 mg/cm^2. [b] A, vapor in close containers; B, vapor in open containers. [c] Number of females. [d] cl denotes confidence limit.

Ovicidal Effects of Test Compounds on *P. capitis* Eggs. Ovicidal activity, as measured by decreased hatchability, was concentration-dependent (**Table 4**). After 24 h of exposure, methyl salicylate and eugenol exhibited potent ovicidal activity against *P. capitis* eggs at 0.25 and 1.0 mg/cm^2, respectively. Little or no ovicidal activity at 5 mg/cm^2 was observed with acetyleugenol, β-caryophyllene, α-humulene, isoeugenol, and methyleugenol as well as with δ-phenothrin and pyrethrum.

Pediculicidal Route of Action. The responses of female *P. capitis* to vapors of eugenol, methyl salicylate, δ-phenothrin, and pyrethrum varied with treatment method (**Table 5**). On the basis of LT_{50} values at 0.25 mg/cm^2, there was a significant difference in pediculicidal activity of eugenol against female *P. capitis* between exposure in a closed container (method A) and exposure in an open container (method B). Similar differences in the response of female *P. capitis* to methyl salicylate in treatments A and B were also observed. No mortality was observed within 5 h of evaluation time by the treatment of δ-phenothrin or pyrethrum in a closed container (method A), suggesting little or no fumigant action of the pyrethroids.

DISCUSSION

Plant essential oils have the potential as products for *P. capitis* control because some of them are selective, have little or no harmful effects on nontarget organisms, and may be applied to humans in the same way as other conventional insecticides (*7*). Various compounds, including phenolics, terpenoids, and alkaloids, exist in plants. Jointly or independently, they contribute

to bioefficacy by having insecticidal, repellent, antifeeding, and ovicidal activities. Additionally, some plant-derived compounds are found to be highly effective against insecticide resistant insect pests (*16*, *17*). Much concern has been focused on the distribution, nature, and practical use of plant-derived chemical substances having insecticidal activity. In the present study, the insecticidal activity of *Eugenia* bud and leaf oils against female *P. capitis* was comparable to those of δ-phenothrin and pyrethrum. The bioactive constituents of both oils were identified as eugenol and methyl salicylate. The insecticidal activity of eugenol or methyl salicylate, respectively, was slightly less effective than δ-phenothrin or pyrethrum against female *P. capitis*. Additionally, eugenol and methyl salicylate were highly effective against *P. capitis* eggs. The exact adulticidal and ovicidal mode of action of eugenol and methyl salicylate remains unknown. Eugenol is an attractant for adult *Diabrotica barberi* (Smith and Lawrence) (*18*, *19*), has insecticidal activity against *T. castaneum* and *S. zeamais* (*9*), and has acaricidal activity against adult *T. putrescentiae* (*10*).

Structure−activity relationships of phytochemicals against arthropod pests have been well-studied (*10*, *20*−*22*). Rice and Coats (*20*) and Tsao et al. (*21*) attempted to enhance potency of selected monoterpenes and phenols through derivatization of the hydroxyl group. They found that enhanced bioactivity of the derivatives appeared to be resulted from increased vapor pressure, leading to greater fumigant action, and/or increased lipophilicity, leading to better penetration and bioavailability in the insect's body. In our study, eugenol exhibited potent insecticidal activity against *P. capitis* eggs and females, whereas acetyleugenol, isoeugenol, and methyleugenol were ineffective. These results indicate that vapor pressure and lipophilicity may play, in part, a crucial role in determining the toxicity of the phenylpropenes.

Volatile compounds of many plant extracts and essential oils are composed of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids, and exhibit fumigant activity (*23*−*26*). The monoterpene carvacrol is highly toxic to nymphs of *Reticulitermes speratus* (Kolbe), and adults of *Sitophilus oryzae* (L.), *Callosobruchus chinensis* (L.), and *Lasioderma serricorne* (F.) and acts as a fumigant (*24*). In the present study, eugenol and methyl salicylate were much more effective in closed containers than in open ones against female *P. capitis*. These results indicate that the mode of delivery for the compounds was largely in the vapor phase via the respiratory system. The insecticidal mode of action of eugenol and methyl salicylate may be related to the specific neurotoxic action such as the octopaminergic action, as previously described (*7*), although this remains to be proven.

Our results indicate that *E. caryophyllata* bud and leaf oil-derived materials could be useful as fumigants for *P. capitis* eggs and adults. Eugenol is found on the U.S. Food and Drug Administration's Generally Regarded as Safe list and is exempt from toxicity data requirements by the U.S. Environmental Protection Agency. Although this compound is carcinogenic in mice and rats (*27*), eugenol has been used as an antioxidant on oleaginous foods, an anticarminative, an antispasmodic, an antiseptic, and an antimicrobial agent (*28*, *29*). Methyl salicylate has low toxicity to mammals (LD_{50} orally, 887 mg/kg rat) and has been used as a flavoring agent (*30*). For the practical use of the *Eugenia* oil-derived materials as novel fumigants to proceed, further research is necessary on safety issues of these materials for human health and formulations for improving the insecticidal potency and stability.

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