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Ovicidal and Adulticidal Activity of *Eucalyptus globulus* Leaf Oil Terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae)

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The toxic effects of *Eucalyptus globulus* leaf oil-derived monoterpenoids [1,8-cineole, *l*-phellandrene, (−)-α-pinene, 2-β-pinene, *trans*-pinocarveol, γ-terpinene, and 1-α-terpineol] and the known *Eucalyptus* leaf oil terpenoids (β-eudesmol and geranyl acetate) on eggs and females of the human head louse, *Pediculus humanus capitis*, were examined using direct contact and fumigation bioassays and compared with the lethal activity of δ-phenothrin and pyrethrum, two commonly used pediculicides. In a filter paper contact bioassay with female *P. h. capitis*, the pediculicidal activity was more pronounced with *Eucalyptus* leaf oil than with either δ-phenothrin or pyrethrum on the basis of LT_{50} values (0.125 vs 0.25 mg/cm^2). 1,8-Cineole was 2.2- and 2.3-fold more toxic than either δ-phenothrin or pyrethrum, respectively. The pediculicidal activities of (−)-α-pinene, 2-β-pinene, and (*E*)-pinocarveol were comparable to those of δ-phenothrin and pyrethrum. *l*-Phellandrene, γ-terpinene, and 1-α-terpineol were relatively less active than δ-phenothrin and pyrethrum. β-Eudesmol and geranyl acetate were ineffective. 1-α-Terpineol and (*E*)-pinocaveol were highly effective at 0.5 and 1.0 mg/cm^2, respectively, against *P. h. capitis* eggs. At 1.0 mg/cm^2, (−)-α-pinene, 2-β-pinene, and γ-terpinene exhibited moderate ovicidal activity, whereas little or no ovicidal activity was observed with the other terpenoids and with δ-phenothrin and pyrethrum. In fumigation tests with female *P. h. capitis* at 0.25 mg/cm^2, 1,8-cineole, (−)-α-pinene, (*E*)-pinocarveol, and 1-α-terpineol were more effective in closed cups than in open ones, indicating that the effect of the monoterpenoids was largely due to action in the vapor phase. Neither δ-phenothrin nor pyrethrum exhibited fumigant toxicity. *Eucalyptus* leaf oil, particularly 1,8-cineole, 1-α-terpineol, and (*E*)-pinocaveol, merits further study as potential pediculicides or lead compounds for the control of *P. h. capitis*.

KEYWORDS: Natural insecticide; pediculicide; ovicide; fumigant; *Pediculus humanus capitis*; human head louse; *Eucalyptus globulus*; 1,8-cineole; α-terpineol; (*E*)-pinocaveol; monoterpenoid

INTRODUCTION

The human head louse, *Pediculus humanus 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. h. capitis* infections cause skin irritation, pruritus, and sleep loss, as well as occasional secondary bacterial infection from scratching (*1*, *2*). Although the symptoms are relatively mild, infestation by *P. h. capitis* causes substantial degrees of social, mental, and economic problems. In recent years, infestations with *P. h. capitis* have been increasing in Korea (*3*, *4*). Control of this insect worldwide primarily depends on continued applications of organophosphates (malathion), carbamates (carbaryl), pyrethrin, and pyrethroids (permethrin and phenothrin) (*1*, *2*). Although these pediculicides are still effective, 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 excessive treatments, fostering serious human health concerns (*1*). These problems have highlighted the need for the development of selective control alternatives for *P. h. capitis*, particularly with fumigant action because formulations such as powder or dust are usually less effective and inconvenient (*2*).

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Plant essential oils may be an alternative source of materials for *P. h. capitis* control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and as flavoring agents for foods and beverages (*6*, *7*). Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial *P. h. capitis* control agents. In a preliminary experiment, the essential oil from the leaves of Australian *Eucalyptus globulus* Labillardière had potent insecticidal activity against female *P. h. capitis*. The leaf oil contains various compounds such as 1,8-cineole (65.0%), (−)-α-pinene (17.5%), β-eudesmol (2.3%), 1-methyl-3-(1-methyl)benzene (1.9%), globulol (1.7%), 1-α-terpineol (1.4%), α-eudesmol (1.3%), (*E*)-pinocarveol (1.2%), and valenecene (0.8%) (*8*). Little work has been done with respect to managing *P. h. capitis* with *E. globulus* leaf oil compounds, although *Eucalyptus* leaf oil is insecticidal (*8*) and ovicidal (*9*) and is used as a mosquito repellent (*10*).

This paper describes a laboratory study aimed at isolating insecticidal constituents from *E. globulus* leaf oil active against eggs and females of *P. h. capitis* and determining their pediculicidal route of action. The structure−pediculicidal activity relationships of the test terpenoids are also discussed.

MATERIALS AND METHODS

Chemicals. β-Eudesmol, geranyl acetate, (−)-α-pinene, 2-β-pinene, (*E*)-pinocarveol, γ-terpinene, and 1-α-terpineol were purchased from Sigma (St. Louis, MO). 1,8-Cineole was obtained from Wako (Osaka, Japan). *l*-Phellandrene was obtained from Fluka (Buchs, Switzerland). Values of hydrophobic and steric parameters for these compounds were calculated using ACD/log P DB (ACD lab, Montreal, Canada). δ-Phenothrin (92% purity) and 50% pyrethrins were obtained from Hanil and Biomist (Seoul, Korea), respectively. All other chemicals were of reagent grade.

Head Lice. A colony of *P. h. capitis* was collected by combing the hair of 78 infested children (7 boys and 71 girls) at a primary school in Songpa District, Seoul, in December 2001. The collected 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. The Petri dish was placed on the bare leg of one of the authors (Y.-C.Y) to provide the lice with bloodmeals and maintained there for ∼16 h everyday according to the method of Lee et al. (*11*). Eggs were held at 32 ± 1 °C and 60 ± 5% relative humidity (RH) in darkness.

Chromatographic Analysis of *E. globulus* Leaf Oil. Refined *E. globulus* leaf oil was supplied by Felton Grimwade & Bickford Pty. Ltd. (Oakleigh, South, Victoria, Australia). Chromatographic analyses were performed using a Hewlett-Packard 6890 series gas chromatograph (GC), equipped with a split injector (10:1) and a flame ionization detection system. Analytes were separated with a 0.25 mm i.d. × 50 m CBP-20 column (Shimadzu, Tokyo, Japan) with a film thickness of 0.20 μm. The temperature program used for the analysis was as follows: initial temperature at 30 °C, held for 5 min, and ramped at 1 °C/min to 110 °C, held for 5 min and ramped at 2 °C/min to 150 °C, and held for 30 min and ramped at 5 °C/min to 220 °C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. The detector gases were hydrogen and air, and their flow rates were regulated at 40 and 450 mL/min, respectively. The detector temperature was set to 250 °C, and the injector temperature was set to 230 °C.

E. globulus leaf oil compounds were identified using gas chromatography−mass spectral (GC-MS) analysis, which was performed using a GC (HP 6890)-MS (JMS-600W, JEOL). 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 (1.0 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 every 1.3 s. *E. globulus* leaf oil compounds were identified by 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 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.

A filter-paper contact lethal-time bioassay (*12*) was used for toxicity of the *Eucalyptus* leaf oil-derived materials and insecticides to female *P. h. capitis*. Lice were exposed to two concentrations of materials (0.125 and 0.25 mg/cm^2), each of which was dissolved in 80 μL of acetone and applied to filter papers (Whatman no. 2, 5 cm diameter). Control filter papers received 80 μL of acetone. After drying 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). Batches of 20 females (7−9 days old), fed with a human bloodmeal 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. h. capitis* eggs, one to four concentrations (0.125−1 mg/cm^2) of each test compound and insecticide were dissolved in 80 μL of acetone and applied to filter papers. Control filter papers received 80 μL of acetone. After drying in a fume hood for 2 min, each filter paper was placed in the bottom of a Petri dish. Eggs (3−4 days old) that were 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 standards for comparison in toxicity tests. The LT_{50} values of the test compounds for female *P. h. capitis* were calculated by probit analysis (*13*).

Pediculicidal Route of Action. Susceptibility of female *P. h. capitis* to the test compounds and insecticides in the vapor phase was investigated according to the method of Yang et al. (*12*). Briefly, batches of 20 females (7−9 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 central hole (4.5 cm diameter). Each filter paper (5 cm diameter), treated with 0.25 mg/cm^2 of each test compound and insecticide dissolved in 80 μL of acetone, was placed over the wire sieve. This prevented direct contact of test females with the test compound and insecticide. Each Petri dish was then either covered with another lid (method A) to investigate the potential vapor phase toxicity of the test compounds and insecticides or left uncovered (method B). Control filter papers received 80 μL of acetone.

Treated and control (solvent only) 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 (*13*).

Regression Analysis. Regression analyses between LT_{50} values and values of the physical parameters for the test compounds were performed using SAS (*13*). Compounds that produced LT_{50} values >300 min were excluded for regression analysis.

Statistical Analyses. The percentages of mortality and eclosion were 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 insecticides (*13*). Means [± standard error (SE)] of untransformed data are reported.

RESULTS

Chemical Constituents of *Eucalyptus* Leaf Oil. *Eucalyptus* leaf oil was composed of one major and eight minor constituents by comparison of mass spectral data and retention times of authentic compounds (**Table 1**). 1,8-Cineole comprised 90% of the oil.

Table 1. Chemical Constituents of *E. globulus* Leaf Oil Identified by GC-MS

compound	RT (min)	rel %
(−)-α-pinene	12.6	2.2
2-β-pinene	14.0	0.6
β-myrcene	14.3	0.6
l-phellandrene	14.9	0.5
1-isopropenyl-3-methylbenzene	15.7	1.5
1,8-cineole	16.2	90.0
γ-terpinene	17.2	0.7
(*E*)-pinocarveol	21.5	0.4
1-α-terpineol	25.0	1.7

Table 2. Toxicity of *E. globulus* Leaf Oil, δ-Phenothrin, and Pyrethrum against Female *P. h. capitis* Using the Filter-Paper Contact Lethal-Time Bioassay

material[a]	dose (mg/cm^2)	*n*	slope ± SE	LT_{50} (min)	95% cl[b]	rel toxicity
leaf oil	0.125	60	5.41 ± 0.60	8.83	8.17–9.52	4.2[c]
	0.25	60	3.18 ± 0.42	5.13	4.45–5.86	5.4[d]
δ-phenothrin	0.125	60	3.00 ± 0.40	34.57	30.49–39.41	1.1[c]
	0.25	60	3.22 ± 0.38	25.22	22.34–28.53	1.1[d]
pyrethrum	0.125	60	3.33 ± 0.46	36.73	32.26–41.04	1.0
	0.25	60	3.44 ± 0.39	27.93	24.57–31.32	1.0

[a] Exposed for 5 h. [b] cl denotes confidence limit. [c] LT_{50} value of test material at 0.125 mg/cm^2/LT_{50} value of pyrethrum at 0.125 mg/cm^2. [d] LT_{50} value of the material at 0.25 mg/cm^2/LT_{50} value of pyrethrum at 0.25 mg/cm^2.

Pediculicidal and Ovicidal Activities of *E. globulus* Leaf Oil. The adulticidal activities against female *P. h. capitis* of *Eucalyptus* leaf oil and the insecticides δ-phenothrin and pyrethrum were evaluated by comparing the LT_{50} values estimated from direct contact bioassay (**Table 2**). As judged by the LT_{50} values at 0.125 mg/cm^2, the insecticidal activity was much more pronounced with *Eucalyptus* leaf oil compared to either δ-phenothrin or pyrethrum. *Eucalyptus* leaf oil was 3.9- and 4.2-fold more toxic than either δ-phenothrin or pyrethrum, respectively. δ-Phenothrin and pyrethrum were equitoxic. No mortality was observed for solvent-treated lice over the observational interval of the contact bioassay.

Ovicidal activity in the direct contact bioassay of *Eucalyptus* leaf oil, as measured by decreased eclosion, was compared with that of δ-phenothrin and pyrethrum (data not shown). At 1.0 mg/cm^2, little or no activity was observed with *Eucalyptus* leaf oil, δ-phenothrin, or pyrethrum when compared to the control.

Toxicity of Test Compounds to Female *P. h. capitis*. The toxicity of nine terpenoids to female *P. h. capitis* was compared with that of δ-phenothrin and pyrethrum (**Table 3**). Potencies varied according to compound and dose. On the basis of 5-h LT_{50} values at 0.125 mg/cm^2, the compound most toxic to female *P. h. capitis* was 1,8-cineole followed by (−)-α-pinene, 2-β-pinene, and (*E*)-pinocarveol. 1,8-Cineole was 2.2- and 2.3-fold more toxic than either δ-phenothrin or pyrethrum, respectively. The pediculicidal activity of (−)-α-pinene, 2-β-pinene, and (*E*)-pinocarveol was comparable to that of δ-phenothrin and pyrethrum. *l*-Phellandrene, γ-terpinene, and 1-α-terpineol were relatively less active than δ-phenothrin and pyrethrum. β-Eudesmol and geranyl acetate were ineffective. There was no mortality in the controls.

Ovicidal Effects of Test Compounds on *P. h. capitis* Eggs. Toxic effects in the filter-paper contact bioassay of the test compounds on *P. h. capitis* eggs were compared with those of δ-phenothrin and pyrethrum (**Table 4**). Ovicidal activity was compound- and dose-dependent. After 24 h of exposure, no eclosion was observed with 0.5 mg/cm^2 1-α-terpineol and 1.0 mg/cm^2 (*E*)-pinocaveol. 1-α-Terpineol and (*E*)-pinocaveol gave 25 and 13% eclosion at 0.25 and 0.5 mg/cm^2, respectively. (−)-α-Pinene, 2-β-pinene, and γ-terpinene exhibited moderate ovicidal activity at 1.0 mg/cm^2. Little or no ovicidal activity at 1.0 mg/cm^2 was observed with 1,8-cineole, β-eudesmol, geranyl acetate, and *l*-phellandrene as well as δ-phenothrin and pyrethrum.

Table 3. Toxicity of Nine Terpenoids, δ-Phenothrin, and Pyrethrum against Female *P. h. capitis* Using the Filter-Paper Contact Bioassay

compound[a]	dose (mg/cm^2)	slope ± SE	LT_{50} (min)	95% cl[b]
1,8-cineole[c]	0.125	7.59 ± 1.41	16.0	14.5–17.2
	0.25	7.29 ± 0.89	14.4	13.6–15.2
β-eudesmol	0.25		>300	
geranyl acetate	0.25		>300	
l-phellandrene[c]	0.125	5.96 ± 1.72	60.4	52.0–71.7
	0.25	4.88 ± 1.03	41.0	37.0–45.1
(−)-α-pinene[c]	0.125	6.38 ± 1.24	30.9	27.9–34.2
	0.25	6.39 ± 1.06	24.9	22.9–27.2
2-β-pinene[c]	0.125	6.00 ± 1.97	32.4	30.0–36.0
	0.25	9.36 ± 1.29	30.4	29.0–32.0
(*E*)-pinocaveol[c]	0.125	6.42 ± 1.66	33.3	27.4–37.2
	0.25	8.55 ± 1.53	29.3	27.7–31.1
γ-terpinene[c]	0.125	8.19 ± 2.12	73.3	65.6–82.5
	0.25	11.62 ± 1.83	56.4	53.4–59.7
1-α-terpineol[c]	0.125	6.88 ± 1.78	59.1	51.7–67.7
	0.25	16.18 ± 2.43	29.6	28.6–30.8
δ-phenothrin	0.125	3.00 ± 0.40	34.6	30.5–39.4
	0.25	3.00 ± 0.46	25.2	22.3–28.5
pyrethrum	0.125	3.33 ± 0.46	36.7	32.3–41.0
	0.25	3.44 ± 0.39	27.9	24.6–31.3

[a] Exposed for 5 h. [b] cl denotes confidence limit. [c] Compounds identified in this study.

Table 4. Effects of Nine Terpenoids, δ-Phenothrin, and Pyrethrum on the Eclosion of *P. h. capitis* Eggs Using the Filter-Paper Contact Bioassay

compound[a]	dose (mg/cm^2)	*n*	eclosion (%) (mean ± SE)
1,8-cineole[b]	1	60	67 ± 1.7abc
β-eudesmol	1	60	82 ± 1.7a
geranyl acetate	1	60	82 ± 1.7a
l-phellandrene[b]	1	60	62 ± 1.7bcd
(−)-α-pinene[b]	1	60	37 ± 1.7ef
2-β-pinene[b]	1	60	30 ± 2.9f
(*E*)-pinocaveol[b]	1	60	0 ± 0.0h
	0.5	60	13 ± 1.7 g
	0.25	60	52 ± 4.4cde
γ-terpinene[b]	1	60	48 ± 1.7de
1-α-terpineol[b]	1	60	0 ± 0.0h
	0.5	60	0 ± 0.0h
	0.25	60	25 ± 2.9fg
	0.125	60	53 ± 1.7cde
δ-phenothrin	0.25	60	80 ± 2.9a
pyrethrum	0.25	60	77 ± 1.7ab
control		60	78 ± 1.7ab

[a] Exposed for 24 h. [b] Compounds identified in this study.

Structure−Activity Relationships. Linear regression analysis of the relative toxicity of six monoterpenoids was determined using their LT_{50} values (**Table 4**) and the values of the physical parameters for the test compounds (data not shown). Neither molecular weights ($r^2 = 0.34$), hydrophobicities ($r^2 = 0.13$), nor steric effects ($r^2 = 0.20$) parameters were significantly related to the observed monoterpenoid toxicities at 0.25 mg/cm^2.

Table 5. Fumigant Activity of Four Monoterpenoids, δ-Phenothrin, and Pyrethrum against Female *P. h. capitis*

compound[a]	method[b]	slope ± SE	LT_{50} (min)[c]	95% cl[d]
1,8-cineole	A	11.27 ± 1.67	13.22	12.46–14.04
	B		>300	
(–)-α-pinene	A	11.88 ± 2.21	27.05	25.57–28.40
	B		>300	
(*E*)-pinocarveol	A	10.64 ± 1.82	27.48	25.49–28.94
	B		>300	
1-α-terpineol	A	8.90 ± 1.31	39.59	37.15–42.06
	B		>300	

[a] Exposed for 5 h at 0.25 mg/cm^2. [b] A, vapor in close containers; B, vapor in open containers. [c] δ-Phenothrin and pyrethrum, LT_{50} > 300 min. [d] cl denotes confidence limit.

Pediculicide Route of Action. The responses of female *P. h. capitis* to vapors of 1,8-cineole, (−)-α-pinene, (*E*)-pinocarveol, 1-α-terpineol, 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 1,8-cineole against female *P. h. 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. h. capitis* to (−)-α-pinene, (*E*)-pinocarveol, and 1-α-terpineol in treatments A and B were likewise 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 these insecticides.

DISCUSSION

Plant essential oils have potential as products for *P. h. capitis* control because some of them are selective, have little or no harmful effects on nontarget organisms, and can be applied to humans in the same way as other conventional insecticides (*14*−*16*). Many essential oils are known to possess ovicidal, repellent, antifeeding, and insecticidal activities against various insect species (*6*, *7*). Pediculicidal activity against lice has been reported for some essential oils such as clove bud and leaf oils (*12*); anise and ylang ylang oils (*15*); and aniseed, cinnamon leaf, thyme red, tea tree, and nutmeg oils (*17*). In the present study, the insecticidal activity against female *P. h. capitis* was more pronounced with *E. globulus* leaf oil than with δ-phenothrin and pyrethrum.

Various compounds, including phenolics, terpenoids, and alkaloids, exist in plant essential oils. Jointly or independently, they contribute to bioefficacy such as insecticidal, ovicidal, repellent, and antifeeding activities. Additionally, some plant extracts or phytochemicals can be highly effective against insecticide-resistant insect pests (*18*, *19*). Much effort has been focused on the determination of the distribution, nature, and practical use of plant essential oil-derived chemical substances having insecticidal and ovicidal activities. In this study, the pediculicidal constituents of *E. globulus* leaf oil were identified as the monoterpenoids 1,8-cineole, (−)-α-pinene, 2-β-pinene, (*E*)-pinocarveol, *l*-phellandrene, γ-terpinene, and 1-α-terpineol by GC-MS analysis. 1,8-Cineole was found to be a more potent pediculicide than either δ-phenothrin or pyrethrum. The pediculicidal activity of (*E*)-pinocarveol and 1-α-terpineol against female *P. h. capitis* was equal to that of δ-phenothrin or pyrethrum. Additionally, 1-α-terpineol and (*E*)-pinocaveol were highly effective ovicides against *P. h. capitis* eggs, whereas neither δ-phenothrin nor pyrethrum exhibited ovicidal activity as reported earlier (*12*). The exact adulticidal and ovicidal mode of action of 1,8-cineole, (*E*)-pinocarveol, and 1-α-terpineol remains unknown. Neverthless, these monoterpenoids were less active against female *P. h. capitis* individually than in combination, indicating a possible synergy.

Structure−activity relationships of plant compounds against insect pests have been well studied. Rice and Coats (*20*) and Tsao et al. (*21*) attempted to enhance the potency of selected monoterpenes and phenols through derivatization of the hydroxyl group. They found that enhanced bioactivity of the derivatives appeared to result 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, neither molecular weights, hydrophobicities, nor steric effects parameters were significantly related to the observed monoterpenoid toxicities. These results indicate that other factors such as vapor pressure may play, in part, a role in determining the monoterpenoid toxicities to adults.

Elucidation of the mode of action of oils and their constituents is of practical importance for insect control because it may give useful information on the most appropriate formulation and delivery means. Volatile compounds of many plant extracts and essential oils consist of alkanes, alcohols, aldehydes, and terpenoids, particularly monoterpenoids, and exhibit fumigant activity (*12*, *22*−*24*). Fumigant activity against eggs and females of *P. h. capitis* has been reported with eugenol and methyl salicylate, derived from *Eugenia caryophyllata* bud oil (*12*). In our study, 1,8-cineole, (−)-α-pinene, (*E*)-pinocarveol, and 1-α-terpineol were more effective in closed than in open containers against female *P. h. capitis*. These results indicate that the mode of delivery of these monoterpenoids was likely by vapor action via the respiratory system.

In conclusion, *E. globulus* leaf oil and its constituents could be useful as fumigants for *P. h. capitis* eggs and adults. *E. globulus* leaf oil has been widely used as a perfumery oil, an industrial additive, medicines for inhalants, soaps, gargles, lozenges, and insect repellents (*25*). *E. globulus* leaf oil as well as the test terpenoids, except 1,8-cineole and β-eudesmol, are found on the U.S. Food and Drug Administration's GRAS list and are exempt from toxicity data requirements by the U.S. Environmental Protection Agency. It has been also noted that *Eucalyptus* oil and its constituents, including 1,8-cineole, are readily biodegradable, unreactive, and relatively nontoxic (*16*). For the practical use of *Eucalyptus* leaf oil and its constituents as novel fumigants to proceed, further research is necessary on the safety issues of these materials for human health and formulations for improving the insecticidal potency and stability and for reducing cost.

LITERATURE CITED

(1) Gratz, N. G. *Human Lice: Their Prevalence, Control and Resistance to Insecticides—a Review 1985−1997*; World Health Organization: Geneva, Switzerland, 1997; WHO/CTD/WHOPES/97.8.

(2) Rozendaal, J. A. *Vector Control. Methods for Use by Individuals and Communities*; World Health Organization: Geneva, Switzerland, 1997; pp 7−177.

(3) Ree, H. I.; Yong, T. S.; Shin, H. J.; Shin, C. O.; Lee, I. Y.; Seo, S. A.; Seo, J. H.; Chang, J. K.; Lee, D. H.; Im, K. I. Mass treatment of head louse infestation with Sumithrin powder in primary schools in Korea. *Korean J. Parasitol.* **1992**, *30*, 349−354.

(4) Hong, H. K.; Kim, C. M.; Lee, J. S.; Lee, W. J.; Yang, Y. C. Infestation rate of head lice in primary school children in Inchon, Korea. *Korean J. Parasitol.* **1995**, *33*, 243−244.

(5) Downs, A. M.; Stafford, K. A.; Coles, G. C. Head lice: prevalence in schoolchildren and insecticide resistance. *Parasitol. Today* **1999**, *15*, 1−4.
(6) Isman, M. B. Pesticides based on plant essential oils. *Pestic. Outlook* **1999**, 68−72.
(7) Isman, M. B. Plant essential oils for pest and disease management. *Crop Prot.* **2000**, *19*, 603−608.
(8) Choi, W. S.; Lee, H. S.; Lee, S. G.; Park, J. D.; Choi, D. H.; Lee, S. S. Bioactivity of Australian woody plant extracts against stored-product pests and sanitary microbials. *International Symposium on Development of Natural Pesticides from Forest Resources*; Korea Forest Research Institute: Seoul, Korea, 2001; pp 135−157.
(9) Tunç, I.; Berger, B. M.; Erler, F.; Dağli, F. Ovicidal activity of essential oils from five plants against two stored-product insects. *J. Stored Prod. Res.* **2000**, *36*, 161−168.
(10) Trigg, J. K. Evaluation of *Eucalyptus*-based repellent against *Anopheles* spp. in Tanzania. *J. Am. Mosq. Contr. Assoc.* **1996**, *12*, 243−246.
(11) Lee, S. H.; Williamson, M. S.; Yoon, K. S.; Takano-Lee, M.; Edman, J. D.; Devonshire, A. L.; Clark, J. M. Molecular analysis of *kdr*-like resistance in insecticide-resistant strains of head lice, *Pediculus capitis*. *Pestic. Biochem. Physiol.* **2000**, *66*, 130−143.
(12) Yang, Y. C.; Lee, S. H.; Lee, W. J.; Choi, D. H.; Ahn, Y. J. Ovicidal and adulticidal effects of *Eugenia caryophyllata* bud and leaf oil compounds on *Pediculus capitis*. *J. Agric. Food Chem.* **2003**, *51*, 4884−4888.
(13) SAS Institute. *SAS/STAT User's Guide*, version 6; SAS Institute: Cary, NC, 1990.
(14) Hadfield-Law, L. Head lice for A & E nurses. *Accid. Emerg. Nurs.* **2000**, *8*, 84−87.
(15) Mumcuoglu, K. Y.; Miller, J.; Zamir, C.; Zentner, G.; Helbin, V.; Ingber, A. The in vivo pediculicidal efficacy of a natural remedy. *Isr. Med. Assoc. J.* **2002**, *4*, 790−793.
(16) Barton, A. F. M. Industrial use of Eucalyptus oil. *Proceedings, The Oil Mallee Profitable Landcare Seminar*; Oil Mallee Association of WA: Perth, West Australia, 1999; pp 43−54.
(17) Veal, L. The potential effectiveness of essential oils as a treatment for headlice, *Pediculus humanus capitis*. *Complement Ther. Nurs Midwifery* **1996**, *2*, 97−101.
(18) Lindquist, R. K.; Adams, A. J.; Hall, F. R.; Adams, I. H. H. Laboratory and greenhouse valuations of Margosan-O against bifenthrin-resistant and -susceptible greenhouse whiteflies, *Trialeurodes vaporarium* (Homoptera: Aleyrodidae). *Proceedings of the USDA Neem Workshop*; USDA-ARS 86: Beltsville, MD, 1990; pp 91−99.
(19) Ahn, Y. J.; Kwon, M.; Park, H. M.; Han, C. G. Potent insecticidal activity of *Ginkgo biloba*-derived trilactone terpenes against *Nilaparvata lugens*. In *Phytochemical Pest Control Agents*; Hedin, P. A., Hollingworth, R., Miyamoto, J., Masler, E., Thompson, D., Eds.; ACS Symposium Series 658; American Chemical Society: Washington, DC, 1997; pp 90−105.
(20) Rice, P. J.; Coats, J. R. Insecticidal properties of several monoterpenoids to the house fly (Diptera: Muscidae), red flour beetle (Coleoptera: Tenebrionidae) and the southern corn rootworm (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1994**, *87*, 1172−1179.
(21) Tsao, R.; Lee, S.; Rice, P. J.; Jensen, C.; Coats, J. R. Monoterpenoids and their synthetic derivatives as leads for new insect-control agents. In *Synthesis and Chemistry of Agrochemicals IV*; Baker, D. R., Basarab, G. S., Fenyes, J. H., Eds.; ACS Symposium Series 584; American Chemical Society: Washington, DC, 1995; pp 312−324.
(22) Coats, J. R.; Karr, L. L.; Drewes, C. D. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. In *Naturally Occurring Pest Bioregulators*; Hedin, P. A., Ed.; ACS Symposium Series 449; American Chemical Society: Washington, DC, 1991; pp 305−316.
(23) Ahn, Y. J.; Lee, S. B.; Lee, H. S.; Kim, G. H. Insecticidal and acarcidal activity of carvacrol and β-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. *J. Chem. Ecol.* **1998**, *24*, 81−90.
(24) Kim, D. H.; Ahn, Y. J. Contact and fumigant activities of constituents of *Foeniculum vulgare* fruit against three coleopteran stored-product insects. *Pest Manag. Sci.* **2001**, *57*, 301−306.
(25) Boland, D. J.; Brophy, J. J.; House, A. P. N. *Eucalyptus Leaf Oils: Use, Chemistry, Distillation and Marketing*; Inkata Press: Melbourne, Australia, 1991.

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