Insecticidal Activity of Plant Essential Oils Against *Pediculus humanus capitis* (Anoplura: Pediculidae)

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ABSTRACT The insecticidal activity of 54 plant essential oils against female *Pediculus humanus capitis* De Geer was examined using direct contact and fumigation methods, and compared with the lethal activity of δ-phenothrin and pyrethrum, two commonly used pediculicides. In a filter paper contact bioassay with female *P. humanus capitis*, the pediculicidal activity was more pronounced in eucalyptus, marjoram, pennyroyal, and rosemary oils than in δ-phenothrin and pyrethrum on the basis of LT_{50} values at 0.0625 mg/cm^2. At 0.125 mg/cm^2, pediculicidal activity of cade, cardamone ceylon, clove bud, myrtle, rosewood, and sage oils was comparable with that of the test insecticides. In fumigation tests with female *P. humanus capitis* at 0.25 mg/cm^2, eucalyptus, marjoram, pennyroyal, and rosemary oils were more effective in closed containers than in open ones, indicating that the effect of these oils was largely a result of action in the vapor phase. Neither δ-phenothrin nor pyrethrum exhibited fumigant toxicity. The essential oils described herein merit further study as potential control agents for *P. humanus capitis*.

KEY WORDS natural insecticide, natural pediculicide, fumigant, *Pediculus humanus capitis*, essential oil

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 (Gratz 1997). *P. humanus capitis* infections cause skin irritation, pruritus, and sleep loss, as well as occasional secondary bacterial infection from scratching (Gratz 1997, Rozendaal 1997). Unlike the human body louse, *Pediculus humanus humanus* L., *P. humanus capitis* has not been proven to be a vector of infectious disease agents (de Berker and Sinclair 2000). Although the symptoms are relatively mild, infestation by *P. humanus capitis* has resulted in various social, mental, and economic problems. *P. humanus capitis* infestations have been increasing in Korea in recent years (Ree et al. 1992, Hong et al. 1995). The control of human head lice worldwide depends primarily on the continued applications of organochlorine (DDT and lindane), organophosphorus (malathion), carbamate (carbaryl), pyrethrin, pyrethroid (permethrin and δ-phenothrin), and avermectin (ivermectin-originated from *Streptomyces avermitilis*) insecticides (Gratz 1997, Rozendaal 1997, Dolianitis and Sinclair 2002). Their repeated use has often resulted in the development of resistance (Gratz 1997, Downs et al. 1999, Dolianitis and Sinclair 2002), and increasing levels of resistance to the most commonly used pediculicides have caused multiple and excessive treatments, fostering serious human health concerns (Hayes and Laws 1991). These problems have highlighted the need for the development of selective *P. humanus capitis* control alternatives, particularly with fumigant action for ease of application to human hair.

Plant essential oils have been suggested as an alternative source of materials for insect control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and flavoring agents for foods and beverages (Isman 1999). Because of this, much effort has been focused on plant essential oils or phytochemicals as potential sources of commercial head lice control agents (Veal 1996, Morsy et al. 2000, Mumcuoglu et al. 2002, Yang et al. 2003). Little work has been done on pediculicidal activity of plant essential oils, although insecticidal activity of essential oils has been well described by Isman (1999).

This article describes a laboratory study that assesses the potential of plant essential oils as commercial pediculicides. Pediculicidal activity of 54 essential oils against adult female *P. humanus capitis* was compared with those of δ-phenothrin and pyrethrum. Pediculicidal mode of action was also investigated for the test plant essential oils.

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Table 1. Relative toxicity of 54 essential oils, δ-phenothrin, and pyrethrum against female *P. humanus capitis* by using the filter paper contact bioassay at 0.25 mg/cm²

Essential oil[a]	Source plant	Slope (± SEM)	LT_{50}, min	95% CL[b]	RT[c]
Basil	*Ocimum basilicum*	9.91 ± 1.34	27.9	26.57–29.40	0.8
Bay	*Pimenta racemosa*	8.60 ± 1.30	35.1	32.79–37.38	0.7
Bergamot	*Citrus bergamia*	13.50 ± 1.84	54.0	51.60–56.30	0.4
Bitter orange	*Citrus aurantium*	6.08 ± 1.00	57.3	52.16–62.97	0.4
Black pepper	*Piper nigrum*	10.54 ± 1.34	60.4	57.03–63.86	0.4
Cade	*Juniperus oxycedrus*	7.21 ± 1.05	19.2	17.79–20.55	1.2
Caraway seed	*Carum carvi*	13.01 ± 3.47	43.8	38.76–49.28	0.5
Cardamone ceylon	*Elettaria cardamomum*	16.43 ± 2.93	25.4	24.18–26.44	0.9
Cedar Atlas	*Cedrus atlantica*		>300.0		
Chamomille roman	*Anthemis nobilis*	16.83 ± 2.82	42.9	41.19–44.43	0.5
Cinnamon	*Cinnamomum cassia*	12.27 ± 1.72	36.3	34.85–37.79	0.6
Citronella java	*Cymbopogon nardus*	8.61 ± 1.34	72.3	67.97–77.24	0.3
Clary sage	*Salvia sclarea*	11.52 ± 1.99	52.7	49.39–55.51	0.4
Clove bud	*Eugenia caryophyllata*	7.37 ± 1.01	19.6	18.15–21.00	1.2
Clove leaf	*Eugenia caryophyllata*	6.49 ± 0.74	21.6	20.37–23.04	1.1
Coriander	*Coriandrum sativum*	12.93 ± 1.90	24.0	22.88–25.02	1.0
Cypress	*Cupressus sempervirens*	13.55 ± 2.84	24.7	23.34–25.81	0.9
Eucalyptus	*Eucalyptus globules*	3.39 ± 0.79	4.2	2.72–6.39	5.5
Fir needle	*Abies alba*	8.36 ± 1.34	44.7	41.79–47.60	0.5
Frankenscence	*Boswellia carterii*	10.12 ± 1.41	57.4	54.42–60.93	0.4
Geranium	*Pelargonium graveolens*	10.02 ± 1.64	70.4	66.60–74.21	0.3
Ginger	*Zinger officinale*		>300.0		
Grapefruit	*Citrus paradisi*	10.05 ± 1.54	58.1	54.68–61.27	0.4
Juniper berry	*Juniperus communis*	13.64 ± 1.99	53.3	51.26–55.76	0.4
Lavender	*Lavendula officinalis*	16.56 ± 3.03	31.6	30.20–32.77	0.7
Lemon eucalyptus	*Eucalyptus citriodora*	6.95 ± 0.76	26.8	25.11–28.62	0.9
Lemongrass	*Cymbopogon citratus*	5.87 ± 0.90	53.4	49.07–59.10	0.4
Lemon 10-Fold	*Citrus limonum*	5.47 ± 1.01	157.6	141.47–176.84	0.1
Lime dis 5F	*Citrus aurantifolia*	8.83 ± 1.41	48.4	44.94–51.43	0.5
Mandarine	*Citrus reticulata*	10.54 ± 1.44	47.1	44.64–49.74	0.5
Marjoram	*Origanum majorana*	11.34 ± 1.72	11.4	10.67–12.09	2.0
Myrtle	*Myrtus communis*	7.46 ± 1.29	19.2	17.34–20.77	1.2
Nutmeg	*Myristica fragrans*	8.33 ± 1.21	32.3	30.19–34.66	0.7
Orange	*Citrus sinensis*	8.53 ± 1.13	43.8	41.17–46.46	0.5
Oregano	*Origanum vulgare*	13.91 ± 2.33	57.6	55.18–59.92	0.4
Palmarosa	*Cymbopogon martinii*	3.48 ± 1.14	121.9	105.69–185.49	0.2
Patchouly	*Pogostemon cablin*		>300.0		
Pennyroyal	*Mentha pulegium*	4.69 ± 0.60	7.0	6.36–7.68	3.3
Peppermint	*Mentha piperita*	6.54 ± 1.08	18.8	17.67–20.14	1.2
Petitgrain	*Citrus aurantium*	4.21 ± 0.93	75.3	61.16–85.11	0.3
Pimento berry	*Pimenta officinalis*	10.33 ± 1.51	29.9	28.20–31.46	0.8
Rosemary	*Rosmarinus officinalis*	10.20 ± 1.78	14.3	13.26–15.16	1.6
Rosewood	*Aniba rosaeodora*	7.01 ± 0.92	22.4	20.91–24.16	1.0
Sage	*Salvia officinalis*	9.79 ± 1.95	18.0	16.78–19.08	1.3
Sandalwood	*Santalum album*		>300.0		
Spearmint	*Mentha spicata*	5.57 ± 0.74	22.9	21.01–24.73	1.0
Tagetes	*Tagetes glandulifera*	9.00 ± 1.68	54.0	49.85–57.50	0.4
Tangerine	*Citrus reticulata*	9.03 ± 1.24	70.6	66.79–74.15	0.3
Tea tree	*Melaleuca alternifolia*	12.12 ± 1.75	31.5	30.11–32.98	0.7
Thyme red	*Thymus vulgaris*	13.33 ± 2.21	47.6	45.11–49.87	0.5
Thyme white	*Thymus vulgaris*	15.38 ± 2.23	49.9	47.90–51.84	0.5
Vetiver haiti	*Vetiveria zizaniodes*		>300.0		
Wormwood	*Artemisia absinthium*	10.88 ± 1.48	47.0	45.19–49.04	0.5
Ylang ylang	*Cananga odorata*		>300.0		
δ-Phenothrin		4.19 ± 0.57	23.1	20.49–25.89	1.0
Pyrethrum		3.73 ± 0.55	25.3	22.14–28.55	0.9

[a] For each essential oil, the number of females tested was 60.
[b] Confidence limit.
[c] Relative toxicity, LT_{50} value of δ-phenothrin/LT_{50} value of each test material.

Materials and Methods

Chemicals and Essential Oils. In total, 54 plant essential oils were purchased from Jin Aromatics, Anyang, Kyunggi Province, Korea, and are listed in Table 1. δ-Phenothrin (92% purity) and pyrethrum extract (50% purity) were obtained from Hanil and Biomist (Seoul, Korea), respectively. All other chemicals were of reagent grade.

Head Lice. A colony of *P. humanus capitis* was collected by combing the hair of 78 infested children (seven boys and 71 girls) at a primary school in Songpa District, Seoul, in December 2001. Head lice were reared in petri dishes (5 cm in diameter, 1.2 cm in height) with 0.01- and 1.0-mm mesh screens attached over the central holes (4 cm in diameter) on the lid and bottom sides, respectively, and containing a few strands of human hair. To feed head lice with blood meals, the petri dish was placed on the bare lower leg of one of the authors (Y.C.Y.) and maintained there for ≈16 h every day according to the method of Lee et al. (2000). Eggs were held at 32 ± 1°C and 60 ± 5% RH in darkness. Under these conditions, longevity of eggs and adults was ≈6.3 and 7.3 d, respectively, and a head louse produces five to six eggs a day.

Bioassay. A filter paper contact bioassay was used to evaluate the toxicity of the essential oils and insecticides to female *P. humanus capitis.* In a preliminary experiment with cade, cinnamon, clove bud, eucalyptus, marjoram, and rosewood oils as well as δ-phenothrin and pyrethrum, 0.25 mg/cm^2 was an appropriate starting dose for a primary screening. If an essential oil gave similar or better activity than either δ-phenothrin or pyrethrum, further bioassays were conducted. Amounts (0.0625, 0.125, and 0.25 mg/cm^2) of each essential oil were applied to filter papers (Whatman No. 2, 4.5 cm in diameter) in 80 μl of acetone. 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 in diameter, 1.2 cm in height). Batches of 20 *P. humanus capitis* females (7–9 d old), given a human blood meal 4 h before the bioassay, were placed on each petri dish, containing a few strands of human hair, and the dish covered with a lid.

In a separate experiment, vapor phase toxicity of the test oils against female *P. humanus capitis* was investigated according to the method of Yang et al. (2003). Briefly, batches of 20 females (7–9 d old) were placed on the bottom of a petri dish (5 cm in diameter, 1.2 cm in height). The petri dish was then covered using a lid with a fine wire sieve (4.7 cm in diameter) attached over a central hole (4.5 cm in diameter). Each filter paper (4.25 cm in diameter), treated with 0.25 mg/cm^2 of each essential oil dissolved in 80 μl of acetone, was placed over the wire sieve. This prevented direct contact of female lice with the test oil. Each petri dish was then either covered with another lid (method A) to investigate the potential vapor phase toxicity of the test oils or left uncovered (method B). Control filter papers received 80 μl of acetone.

Treated and control (solvent only) females were held at 31 ± 1°C and 65 ± 5% RH in darkness. Mortalities were determined every 5 min for 5 h. Females were considered dead if they exhibited lethargic response or no movement. In fact, all the individuals scored as dead never recovered. All treatments were replicated three times. δ-Phenothrin and pyrethrum served as standards for comparison in toxicity tests. The LT_{50} values were calculated by probit analysis (SAS Institute 1996).

Results

The contact insecticidal activities of 54 plant essential oils at a dose of 0.25 mg/cm^2, against female *P. humanus capitis,* were compared with those of the commonly used insecticides δ-phenothrin and pyrethrum (Table 1). Significant differences were observed in the contact toxicity to female head lice. On the basis of LT_{50} values, eucalyptus followed by pennyroyal, marjoram, rosemary, sage, peppermint, cade, myrtle, and clove bud oils were more toxic than either δ-phenothrin or pyrethrum. In particular, eucalyptus, pennyroyal, and marjoram oils were 5.5-, 3.3-, and 2.0-fold more toxic than δ-phenothrin. No mortality was observed for solvent-treated lice over the observational interval of the contact bioassay.

Differential susceptibility of female *P. humanus capitis* to the essential oils from the same plant genus also was observed (Table 1). Of three *Mentha* species, pennyroyal oil (*M. pulegium*) was 2.7-fold more active than peppermint (*Mentha piperita* L.) and 3.3-fold more active than spearmint (*Mentha spicata* L.) oils. Similar differences in the response of female lice to seven *Citrus* species (bergamot, bitter orange, grapefruit, lemon 10-fold, lime dis 5 F, mandarine, orange, petitgrain, and tangerine oils), three *Cymbopogon* species (citronella java, lemongrass, and palmarosa oils), two *Eucalyptus* species (eucalyptus and lemon eucalyptus oils), two *Juniperus* species (cade and juniper berry oils), two *Origanum* species (marjoram and oregano oils), and two *Pimenta* species (bay and pimento berry oils) were likewise observed.

Because of the potent pediculicidal activity of 15 essential oils at 0.25 mg/cm^2, the activity of these oils was examined at 0.125 mg/cm^2 (Table 2). Of these, eucalyptus oil was most active and was 3.8- and 4.0-fold more toxic than δ-phenothrin and pyrethrum, respectively. Marjoram, pennyroyal, and rosemary oils were also highly effective and more toxic than either δ-phenothrin or pyrethrum. The pediculicidal activities of cade, cardamone ceylon, clove bud, myrtle, rosewood, and sage oils were comparable with those of δ-phenothrin and pyrethrum.

Eucalyptus, marjoram, pennyroyal, and rosemary oils were also toxic at 0.0625 mg/cm^2 (Table 3). The pediculicidal activity was more pronounced in eucalyptus and pennyroyal oils than marjoram and rosemary oils and all four essential oils were more potent than either δ-phenothrin or pyrethrum.

The vapor phase toxicities of the test essential oils and insecticides against female *P. capitis* were investigated using a fumigant bioassay in two formats (Table 4). The response of female *P. humanus capitis* to eucalyptus, marjoram, pennyroyal, and rosemary oils varied between exposures in a closed container (method A) versus exposures in an open container (method B) over a 5-h exposure. Eucalyptus oil was ≈50-fold more active in the fumigant assay (closed container) than when using method B (open container). Similar differences in the toxic response of female *P. humanus capitis* to marjoram, pennyroyal, and rosemary oils were likewise observed between method A versus method B exposures. No mortality was observed within the 5-h exposure interval to either δ-phenothrin or pyrethrum in the closed or open containers, suggesting little or no fumigant action of these insecticides.

Table 2. Relative toxicity of 15 selected essential oils, δ-phenothrin, and pyrethrum against female *P. humanus capitis* by using the filter paper contact bioassay at 0.125 mg/cm²

Essential oil[a]	Slope (± SEM)	LT_{50}, min	95% CL[b]	RT[c]
Cade	8.68 ± 1.18	32.9	30.72–34.79	1.0
Cardamone ceylon	9.97 ± 1.44	30.9	29.01–32.61	1.1
Clove bud	7.60 ± 0.96	29.1	27.43–30.77	1.1
Clove leaf	5.29 ± 1.08	63.0	54.77–76.70	0.5
Coriander	5.07 ± 1.03	58.4	52.77–65.21	0.6
Cypress	3.98 ± 0.73	43.9	38.68–51.77	0.8
Eucalyptus	5.41 ± 0.60	8.8	8.17–9.52	3.8
Marjoram	6.40 ± 0.73	15.1	13.98–16.19	2.2
Myrtle	11.36 ± 1.52	28.6	27.04–30.10	1.2
Pennyroyal	5.39 ± 0.72	11.5	10.63–12.34	2.9
Peppermint	3.87 ± 0.47	53.0	48.32–58.06	0.6
Rosemary	5.76 ± 0.68	16.5	15.24–17.74	2.0
Rosewood	5.99 ± 0.77	33.5	31.47–35.88	1.0
Sage	9.74 ± 1.50	33.3	31.20–35.18	1.0
Spearmint	4.35 ± 0.53	60.6	54.66–66.92	0.5
δ-Phenothrin	3.13 ± 0.33	33.2	28.41–38.73	1.0
Pyrethrum	3.25 ± 0.48	35.6	31.24–40.53	0.9

For each test material, the number of females tested was 60.
[a] Essential oils showing potent pediculicidal activity at 0.25 mg/cm² are recorded.
[b] Confidence limit.
[c] For explanation, see Table 1.

Discussion

Plant essential oils have potential as natural products for *P. humanus 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 (Hadfield-Law 2000, Morsy et al. 2000, Mumcuoglu et al. 2002). Many essential oils are known to possess ovicidal, repellent, antifeeding, and insecticidal activities against various insect species (Saxena 1989, Isman 1999). For example, neem, *Azadirachta indica* A. Juss, oil is found to have a variety of biological activities, including insecticidal activity against nearly 200 species of insects without any adverse effects on most nontarget organisms (Saxena 1989). Additionally, some plant extracts or phytochemicals can be highly effective against insecticide-resistant insect pests (Lindquist et al. 1990, Ahn et al. 1997). Pediculicidal activity has been reported for some essential oils such as aniseed, cinnamon leaf, thyme red, tea tree, and nutmeg oils (Veal 1996); neem oil (Morsy et al. 2000); and anise and ylang ylang oils (Mumcuoglu et al. 2002). In the current study, potencies varied according to oil type and dose. The contact pediculicidal activity was more pronounced in cade, cardamone ceylon, clove bud, eucalyptus, marjoram, myrtle, pennyroyal, rosemary, rosewood, and sage oils than to the insecticides δ-phenothrin or pyrethrum. These essential oils seem to be good candidates as naturally occurring control agents for *P. humanus capitis.*

Table 3. Relative toxicity of four selected essential oils, δ-phenothrin, and pyrethrum against female *P. humanus capitis* by using the filter paper contact bioassay at 0.0625 mg/cm²

Essential oil[a]	Slope (± SEM)	LT_{50}, min	95% CL[b]
Eucalyptus	5.63 ± 0.77	12.6	11.34–13.62
Marjoram	8.80 ± 1.26	19.6	18.23–20.92
Pennyroyal	5.07 ± 0.79	14.7	13.11–16.09
Rosemary	9.54 ± 1.38	22.4	20.98–23.68
δ-Phenothrin		>300	
Pyrethrum		>300	

For each test material, the number of females tested was 60.
[a] Essential oils showing potent pediculicidal activity at 0.125 mg/cm² are recorded.
[b] Confidence limit.

Variation in insect response to the essential oils related to plant species has been studied. Differences in the insecticidal effects on adult *Trialeurodes vaporariorum* Westwood among essential oils from seven *Citrus* species, three *Cymbopogon* species, two *Eucalyptus* species, two *Juniperus* species, three *Mentha* species, two *Origanum* species, and two *Pimenta* species have been reported (Choi et al. 2003). For example, *Citrus aurantifolia* Swingel oil was highly effective against adult *T. vaporariorum,* whereas the other *Citrus* oils were almost ineffective. These results suggest that qualitative and/or quantitative chemical composition among *Citrus* species may be different. In our study, differential susceptibility of female *P. humanus capitis* to the essential oils from the same plant genus was also observed in *Citrus, Cymbopogon, Eucalyptus, Juniperus, Mentha, Origanum,* and *Pimenta* species.

Elucidation of the modes of action of pediculicidal natural products and insecticides is of practical importance for insect control because it provides 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 (Coats et al. 1991, Kim and Ahn 2001, Chang and Ahn 2002, Yang et al. 2003). Fumigant activity against eggs and females of *P. humanus capitis* has been reported for eugenol and methyl salicylate derived from clove bud oil (Yang et al. 2003). In the current study, eucalyptus, marjoram,

Table 4. Fumigant activity of four selected essential oils, δ-phenothrin, and pyrethrum against female *P. humanus capitis* at 0.25 mg/cm²

Essential oil[a]	Method[b]	Slope (± SEM)	LT_{50}, min	95% CL[c]	RT[d]
Eucalyptus	A	4.03 ± 0.66	6.2	5.18–7.14	>48
	B		>300.0		
Rosemary	A	8.52 ± 1.22	12.8	11.75–13.69	>23
	B		>300.0		
Marjoram	A	9.53 ± 1.34	12.6	11.61–13.39	>24
	B		>300.0		
Pennyroyal	A	3.31 ± 0.63	7.5	6.29–9.13	>40
	B		>300.0		
δ-Phenothrin	A		>300.0		
	B		>300.0		
Pyrethrum	A		>300.0		
	B		>300.0		

[a] For each test material, the number of females tested was 60.
[b] A, vapor in close containers; B, vapor in open containers.
[c] Confidence limit.
[d] LT_{50} value of method B/LT_{50} value of method A.

pennyroyal, and rosemary oils were much more effective in closed versus open containers against female *P. humanus capitis*. These results indicate that the mode of delivery of the oils was likely by vapor action via the respiratory system, although the exact mode of action of these oils remains unknown.

Knockdown resistance to permethrin, the pyrethrins, and DDT has been well established (Lee et al. 2000), is widespread (Yoon et al. 2003), and is intensifying (Gao et al. 2003). Alternative control agents with novel modes of action, and low mammalian toxicity and environmental impact are badly needed. Results of this study indicate that plant essential oils, such as eucalyptus, pennyroyal, marjoram, and rosemary, are potent pediculicides and may be useful as fumigants for human head louse control. For the practical use of these oils as novel fumigants to proceed, further research is necessary on safety issues of these oils on human health as well as formulations for improving the pediculicidal potency and stability.

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