J. Microbiol. Biotechnol. (2004), 14(3), 631–634

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Acaricidal Components of Medicinal Plant Oils Against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*

CHO, JANG-HEE[1], BO-KYUNG SUNG[1], MI-YOUN LIM[1], HYEON-JIN KIM[1], SANG-GUEI LEE[2], AND HOI-SEON LEE[1]*

[1]*Institute of Bio-Safety Research and Jinis Biopharmaceuticals Co. 452-32, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea*
[2]*National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon 441-707, Korea*

Received: August 18, 2003
Accepted: October 28, 2003

Abstract The oils of *Acorus gramineus*, *Cinnamomum sieboldii*, *Eugenia aromatica*, and *Inula helenium* were tested for their acaricidal activity against *Dermatophagoides farinae* and *D. pteronyssinus*. Responses varied according to dose and mite species. As compared to the oils, the oil most toxic to *D. farinae* and *D. pteronyssinus* was *E. aromatica*, followed by *C. sieboldii*, *A. gramineus*, and *I. helenium*. On the basis of LD_{50} values of the oils in *A. gramineus*, *C. sieboldii*, and *E. aromatica*, the compound most toxic against *D. farinae* and *D. pteronyssinus* was eugenol congeners (isoeugenol>eugenol> acetyleugenol) followed by benzyl benzoate, salicylaldehyde, safrol, DEET, cinnamyl alcohol, and 3-carene. As a naturally occurring acaricide, these oils and eugenol congeners could be useful as new acaricidal agents against *Dermatophagoides* spp.

Key words: Acaricidal agents, *Acorus gramineus*, *Cinnamomum sieboldii*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Eugenia aromatica*, eugenol congeners, *Inula helenium*

In homes, house dust mites, *Dermatophagoides farinae* (Hughes) and *D. pteronyssinus* (Trouessart) are a major source of multiple potent allergens in the cosmopolitan occurrence and abundance, and are associated with sudden infant death syndrome [7, 14, 16, 21]. Changes in living environments such as a rise in the number of apartment households with centrally installed heating, space heating, and fitted carpets have improved conditions for the growth of dust mites [16]. Control of these mite populations has been principally through the use of chemicals [16]. Although effective, their repeated use has sometimes resulted in the widespread development of resistance [14, 21], has undesirable effects on nontarget organisms, and has fostered environmental and human health concerns [6, 16]. These problems have highlighted the need for the development of new strategies for selective control of house dust mites.

Plant secondary metabolites have received considerable attention in the search for new pesticides [18, 19] and have been found to play the role of toxin, deterrents, repellents, arthropod growth regulators, and behavioral control agents against arthropod pests [5]. In previous investigations, the potential of medicinal plant extracts was assessed against the house dust mites to develop new and safer types of acaricidal agents. Among 20 medicinal plants, potent acaricidal activity of the oils extracted from *Acorus gramineus*, *Cinnamomum sieboldii*, *Eugenia aromatica*, and *Inula helenium* was detected against the house dust mites. In this study, further study of oils extracted from 4 medicinal plants is presented in relation to the results obtained.

Chemicals, Plant Preparation, and Dust Mites

Benzyl benzoate and DEET were purchased from Aldrich (Milwaukee, WI, U.S.A.). Acetyleugenol, asarone, 3-carene, caryophyllene, cinnamic acid, cinnamyl alcohol, eugenol, isoeugenol, α-phellandrene, α-pinene, safrol, and salicylaldehyde were supplied by Sigma (St. Louis, MO, U.S.A.). Plants were purchased from a local market in Seoul. The samples were dried in an oven at 40°C for 2 days, then finely powdered. The essential oil of each sample (200 g) was extracted by steam distillation as previously described by Hwang and Lee [9]. The yield of each extraction is given in Table 1. Cultures of *D. farinae* and *D. pteronyssinus* were maintained in the laboratory for 5 years without exposure to any known acaricide. They were reared in plastic containers (15×12×6 cm) containing

*Corresponding author
Phone: 82-63-270-2544; Fax: 82-63-270-2550;
E-mail: hoiseon@moak.chonbuk.ac.kr

Table 1. List of medicinal plants tested.

Species	Family	Tissue sampled	Yield (%)[a]
Acorus gramineus	Araceae	Flower	7.5
Cinnamomum sieboldii	Lauraceae	Root	9.1
Eugenia aromatica	Myrtaceae	Flower bud	30.6
Inula helenium	Compositae	Root	20.7

[a](Weight of crude methanol extract/100 g of dried weight of test materials)×100.

30 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., Chonju, Korea.

Gas Chromatography-Mass Spectrometry

The oils of *Acorus gramineus* flower, *Cinnamomum sieboldii* root, *Eugenia aromatica* bud, and *Inula helenium* root were analyzed on a gas chromatograph (HP6890)-mass spectrometer (JMS-600W, JEOL, Tokyo, Japan) (GC-MS). The GC column was a 60 m×0.25 mm i.d. DB-WAX (0.25 mm film) fused silica capillary column (J&W Scientific, Folsom, CA, U.S.A.). The GC conditions were as follows: injector temperature, 210°C; column temperature, isothermal at 50°C for 15 min, then programmed to 200°C at 2°C/min and held at this temperature for 15 min; ion source temperature, 200°C. Helium was used as a carrier gas at a rate of 0.8 ml/min. The effluent of the GC column was introduced directly into the source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 amu for 2 sec. Compounds were identified by comparison with retention times and the mass spectra obtained with the authentic standards on the GC-MS system used for analysis. When an authentic sample was not available, the identification was carried out by comparison of mass spectra with those in the mass spectra library (The Wiley Registry of Mass Spectral Data, 6th Ed.).

Bioassay and Statistical Analysis

An impregnated fabric disk bioassay was used for acaricidal activity of test materials. Amounts (80, 40, 20, 10, 5, 2.5, 1.25 μg/cm^2) of each test materials dissolved in 20 μl of ethanol were applied to disks of black cotton fabric (5 cm diameter). Control fabric disks received 20 μl of ethanol. Each piece was placed in the bottom of a Petri dish (5 cm diameter×1.2 cm). Then 20 individuals of *D. farinae* (7–10 days old) and *D. pteronyssinus* (7–10 days old) were separately placed in each Petri dish and covered with a lid. Treated and control mites were held at 25±1°C and 75% relative humidity in darkness. Mortalities were determined 24 h after treatment under a binolcular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times. The LD_{50} values were calculated by probit analysis [17]. The percentage of mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at $P<0.05$ [17]. Means (±SE) of untransformed data are reported.

The acaricidal activity of the essential oils extracted from *Acorus gramineus*, *Cinnamomum sieboldii*, *Eugenia aromatica*, and *Inula helenium* against *D. farinae* and *D. pteronyssinus* was bioassayed by direct contact method (Table 2). Responses varied according to plant species and dust mites. Titration studies were performed at 80, 40, 20, and 10 μg/cm^2. At concentrations of 80 and 40 μg/cm^2, strong activity (100%) was observed with the oil of *A. gramineus*, *C. sieboldii*, and *E. aromatica*. The oil of *A. gramineus* against *D. farinae* and *D. pteronyssinus* gave 76.7% and 79.3% mortality at 20 μg/cm^2 and 42.0% and 46.2% mortality at 10 μg/cm^2, respectively. The oil of *C. sieboldii* against *D. farinae* and *D. pteronyssinus* gave 80.7% and 82.3% mortality at 20 μg/cm^2 and 64.8% and 67.9% mortality at 10 μg/cm^2, respectively. At concentrations of 20 and 10 μg/cm^2, the oil of *E. aromatica*, gain 100% mortality against *D. farinae* and *D. pteronyssinus*. However, acaricidal activity of the oil of *I. helenium* was significantly reduced

Table 2. Acaricidal activities of medicinal plant oils against *D. farinae* and *D. pteronyssinus* adults.

Plant species	Dust mite	Mortality (mean±SE, %)[a]			
		80[b]	40	20	10
A. gramineus	*D. farinae*	100	100	76.7±6.0	42.0±1.3
	D. pteronyssinus	100	100	79.3±1.7	46.2±2.9
C. sieboldii	*D. farinae*	100	100	80.7±6.0	64.8±1.7
	D. pteronyssinus	100	100	82.3±1.7	67.9±6.0
E. aromatica	*D. farinae*	100	100	100	100
	D. pteronyssinus	100	100	100	100
I. helenium	*D. farinae*	100	52.7±1.7	20.0±2.3	8.3±6.0
	D. pteronyssinus	100	78.3±4.4	35.3±1.7	19.2±2.9

[a]Thirty adults per replicate; 3 replicates per treatment; n=90.
[b]Day after treatment (μg/cm^2).

Table 3. Acaricidal activity of currently used acaricides and constituents identified by GC-MS in the oils of *A. gramineus*, *C. sieboldii*, and *E. aromatica* against *D. farinae* and *D. pteronyssinus* adults.

Scientific name	Dust mite	Mortality[a] (%, mean±SE)						
		Dose (μg/cm^2)						
		40	20	10	5.0	2.5	1.25	LD_{50}
Asarone	*D. farinae*	0±0.0	NT[b]	NT	NT	NT	NT	0
	D. pteronyssinus	0±0.0	NT	NT	NT	NT	NT	0
3-Carene	*D. farinae*	49.3± 2.9	15.2±3.2	0±0.0	NT	NT	NT	42.1
	D. pteronyssinus	53.4±3.1	32.8±2.9	12.3±3.5	0±0.0	NT	NT	39.7
Caryophyllene	*D. farinae*	0±0.0	NT	NT	NT	NT	NT	0
	D. pteronyssinus	0±0.0	NT	NT	NT	NT	NT	0
Cinnamyl alcohol	*D. farinae*	83.2±3.5	27.9±4.2	0±0.0	NT	NT	NT	30.4
	D. pteronyssinus	90.1±2.7	40.1±3.4	0±0.0	NT	NT	NT	24.7
Cinnamic acid	*D. farinae*	0±0.0	NT	NT	NT	NT	NT	0
	D. pteronyssinus	0±0.0	NT	NT	NT	NT	NT	0
Eugenol	*D. farinae*	100	100	100	66.4±2.8	18.9±4.4	0±0.0	4.8
	D. pteronyssinus	100	100	100	87.2±3.2	33.8±3.6	0±0.0	3.7
Acetyleugenol	*D. farinae*	100	100	35.8±2.9	0±0.0	NT	NT	13.2
	D. pteronyssinus	100	100	100	42.6±3.2	8.9±2.3	0±0.0	5.7
Isoeugenol	*D. farinae*	100	100	100	70.7±3.7	24.2±3.6	0±0.0	4.1
	D. pteronyssinus	100	100	100	100	49.5±2.9	13.4±3.9	2.4
α-Phellandrene	*D. farinae*	0±0.0	NT	NT	NT	NT	NT	0
	D. pteronyssinus	0±0.0	NT	NT	NT	NT	NT	0
α-Pinene	*D. farinae*	0±0.0	NT[c]	NT	NT	NT	NT	0
	D. pteronyssinus	0±0.0	NT[c]	NT	NT	NT	NT	0
Safrol	*D. farinae*	100	100	45.3±2.9	15.9±3.8	0±0.0	NT	11.2
	D. pteronyssinus	100	100	50.8±3.6	24.1±2.7	0±0.0	NT	9.8
Salicylaldehyde	*D. farinae*	100	100	48.6±2.5	22.4±3.7	0±0.0	NT	10.3
	D. pteronyssinus	100	100	61.2±3.1	36.5±3.1	0±0.0	NT	9.4
Benzyl benzoate	*D. farinae*	100	100	69.3±3.2	21.5±1.9	0±0.0	NT	9.4
	D. pteronyssinus	100	100	86.7±2.8	43.5±2.2	0±0.0	NT	6.8
DEET	*D. farinae*	65.2±2.3	19.3±1.9	0±0.0	NT	NT	NT	36.9
	D. pteronyssinus	90.7±2.1	58.9±1.7	10.5±1.2	NT	NT	NT	18.1

[a]Means within a column followed by the same letter are not significantly different ($P<0.05$, Scheffe's test).
[b]NT: Not tested.

when used at 40 and 20 μg/cm^2 against *D. farinae* and *D. pteronyssinus*. These results suggest that *D. pteronyssinus* may be controlled more effectively by the application of the oils of *A. gramineus* and *C. sieboldii* than *D. farinae*.

It has been well recognized that plants or phytochemicals could be developed into mite-control agents because many of them have selective acaricidal activity, have no or little harmful effects on nontarget organisms and the environment, and may be applied to target sites such as wall, furniture, and warehouse in the same way as other conventional acaricides. Additionally, some plant-derived materials are found to be highly effective against insecticide-resistant insect pests [2, 3]. Derivatives of *Ginkgo biloba* L. are found to have potent insecticidal activity against three strains of *Nilaparvata lugens* (Stal.) resistant to carbofuran, fenobucarb, and diazinon [1]. Jacobson [10] has already pointed out that the most promising botanical arthropod-control agents are in the families Annonaceae, Asteraceae, Canellaceae, Compositae, Labiatae, Lauraceae, Meliaceae, Myrtaceae, and Rutaceae. Much concern has been, therefore, on the distribution, nature, and practical use of plant-derived chemical substances having acaricidal activity against house dust mites [12].

The substances identified by GC-MS in the oil of *A. gramineus*, *C. sieboldii*, and *E. aromatica* were examined (not shown). The acaricidal activity of the substances was bioassayed by direct contact method and compared with those of benzyl benzoate and DEET (Table 3). The commonly used benzyl benzoate and DEET served as a standard of comparison for the acaricidal activity. Analysis led to identification of 14 volatiles from three plant oils. The main constituents were asarone, caryophyllene, isoasarone, isoeugenol, and safrol from *A. gramineus* oil, cinnamic acid, cinnamyl alcohol, eugenol, α-phellandrene, and salicylaldehyde from *C. sieboldii* oil, and acetyleugenol, caryophyllene, 3-carene, eugenol, α-pinene, and β-pinene from *E. aromatica* oil (Table 3). Composition of the three plant oils was extensively investigated many years ago. In an earlier

study, the rhizome from *A. gramineus* Solander was found to contain various compounds such as (*Z*)-asarone (63-81%), (*E*)-asarone (8-14%), caryophyllene (1-4%), isoasarone (0.8-3.4%), isoeugenol (0.3-6.8%) and safrol (0.1-1.2%) [15, 20]. Some investigations have demonstrated that *Cinnamomum cassia*-derived materials have insecticidal and antifeeding effects against insect pests as well as rodent-repellent effects [4, 8, 13]. They reported that cinnamic acid, cinnamyl alcohol, eugenol, and salicylaldehyde derived from the bark of *C. cassia* possess potent activity against *Tyrophagus putrescentiae* adults [11]. The bud of *E. aromatica* contains various compounds such as acetyleugenol, α-caryophyllene, β-caryophyllene, 3-carene, chavicol, eugenol, furfural, α-pinene, and β-pinene [21]. On the basis of LD_{50} values of this study, the most toxic compound against *D. farinae* and *D. pteronyssinus* adults was eugenol congeners (isoeugenol and acetyleugenol) followed by benzyl benzoate, salicylaldehyde, safrol, DEET, cinnamyl alcohol, and 3-carene. In eugenol congeners, isoeugenol was more toxic than eugenol and acetyleugenol against *D. farinae* and *D. pteronyssinus*. However, no activity was observed for asarone, caryophyllene, cinnamic acid, α-phellandrene, and α-pinene.

In conclusion, the results indicate that medicinal plant-derived compounds could be useful as acaricidal agents against *D. farinae* and *D. pteronyssinus*. Research should be done on safety issues of this compound for human health, acaricidal mode of action, and formulations improving the acaricidal potency and stability. Additionally, further research to identify the biologically active substances and the acaricidal mode of action in the oils of *A. gramineus*, *C. sieboldii*, and *E. aromatica*, which showed the most potent acaricidal activity, is in progress.

Acknowledgments

This work was supported by Korea Research Foundation Grant (KRF-2002-041-F00010). We thank The Research Center for Industrial Development of BioFood Materials at Chonbuk National University for kindly providing the facilities for this research.

REFERENCES

1. Ahn, Y. J., C. O. Lee, J. H. Kwon, J. W. Ahn, and J. H. Park. 1998. Growth-inhibitory effects of galla rhois-derived tannins on intestinal bacteria. *J. Appl. Microbiol.* **84:** 439-443.
2. Ahn, Y. J., S. B. Lee, T. Okubo, and M. J. Kim. 1995. Antignawing factor of crude oil derived from *Thujopsis dolabrata* S. et Z. var. *hondi* sawdust against mice. *J. Chem. Ecol.* **21:** 263-271.
3. Arnason, J. T., B. J. R. Philogene, and P. Morand. 1989. *Insecticides of Plant Origin*. ACS Symp. Ser. 387, American Chemical Society, Washington, DC, U.S.A.
4. Ben-Yakir, D., A. Bazar, and M. Chen. 1995. Attraction of *Maladera matrida* (Coleoptera: Scarabaeidae) to eugenol and other lures. *J. Econ. Entomol.* **88:** 415-420.
5. Harbone, J. B. 1993. *Introduction to Ecological Biochemistry* 4th Ed. Academic Press, London.
6. Hayes, W. J. Jr. and E. R. Jr. Laws. 1991. *Handbook of Pesticide Toxicology*, Vol. 1. Academic, San Diego, CA, U.S.A.
7. Helson, G. A. H. 1971. House-dust mites and possible connection with sudden infant death syndrome. *N. Z. Med. J.* **74:** 209.
8. Huang, Y. and S. H. Ho. 1998. Toxicity and antifeedant activities of cinnammaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motsch). *J. Stored Prod. Res.* **34:** 11-17.
9. Hwang, Y. H. and H. S. Lee. 2002. Antibacterial activity of *Pinus densiflora* leaf-derived components toward human intestinal bacteria. *J. Microbiol. Biotechnol.* **12:** 610-616.
10. Jacobson, M. 1989. Botanical pesticides: Past, present, and future, pp, 1-10. *In* J. T. Arnason, B. J. R. Philogene, and P. Morand. (eds.), *Insecticides of Plant Origin*, ACS Symp. Ser. No. 387. American Chemical Society, Washington, DC, U.S.A.
11. Kim, H. K. 2001. Acaricidal activities of *Cinnamomum cassia* bark-derived materials against *Dermatophagoides* spp. (Acari: Pyroglyphidae). MS Thesis, Seoul National University, Korea.
12. Kwon, J. H. 2002. Acaricidal activity of phenylpropenes identified from *Cnidium officinale* against *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Tyrophagus putrescentiae*. MS Thesis, Seoul National University, Korea.
13. Lee, H. K., H. S. Lee, and Y. J. Ahn. 1999. Antignawing factor derived from *Cinnamomum cassia* bark against mice. *J. Chem. Ecol.* **25:** 1131-1139.
14. Lee, S. E., J. E. Kim, and H. S. Lee. 2001. Insecticide resistance in increasing interest. *Agric. Chem. Biotechnol.* **44:** 105-112.
15. Namba, T. 1993. *The Encyclopedia of Wakan-Yaku (Traditional Sino-Japanese Medicines) with Color Pictures*, Vol. I. p. 606. Hoikusha, Osaka, Japan.
16. Pollart, S. M., G. W. Jr. Ward, and T. A. E. Platts-Mills. 1987. House dust sensitivity and environmental control. *Immunol. Allergy Clin. North Am.* **7:** 447-461.
17. SAS. 1990. *SAS/STAT Users Guide*, version 6; SAS Institute, Cary, North Carolina.
18. Singh, D. P., A. Kumar, and M. B. Tyagi. 2003. Biotoxic cyanobacterial metabolites exhibiting pesticidal and mosquito larvicidal activities. *J. Microbiol. Biotechnol.* **13:** 50-56.
19. Singh, G. and R. K. Upadhyay. 1993. Essential oils: A potent source of natural pesticides. *J. Sci. Ind. Res.* **52:** 676-683.
20. Tang, W. and G. Eisenbrand. 1992. *Chinese Drugs of Plant Origin*. pp. 1056. Springer, New York, U.S.A.
21. Van Bronswijk J, E. M. H., and R. N. Sinha. 1971. Pyroglyphid mites (Acari) and house dust allery. *J. Allergy* **47:** 31-52.