Food Sci. Biotechnol. Vol. 14, No. 1, pp. 73 ~ 76 (2005)

Food Science and Biotechnology

Chemical Composition and Acaricidal Activities of Constituents Derived from *Eugenia caryophyllata* Leaf Oils

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Abstract The acaricidal activities of 12 commercial constituents derived from *Eugenia caryophyllata* leaf oils against *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae* adults were examined using an impregnated fabric disk application and compared with that of the commercial benzyl benzoate as synthetic acaricide. On the basis of LD_{50} values, the most toxic compound was methyl eugenol (4.13 μg/cm^2), followed by methyl isoeugenol (4.19 μg/cm^2), isoeugenol (4.29 μg/cm^2), eugenol (4.94 μg/cm^2), and acetyl eugenol (13.91 μg/cm^2) against *D. farinae*. In the case of *D. pteronyssinus*, isoeugenol (2.93 μg/cm^2) was the most toxic, followed by methyl isoeugenol (3.28 μg/cm^2), methyl eugenol (3.87 μg/cm^2), eugenol (3.92 μg/cm^2), and acetyl eugenol (7.21 μg/cm^2). These results suggest that *D. pteronyssinus* may be controlled more effectively by the application of eugenol congeners than *D. farinae*. In comparison with synthetic acaricides, the acaricidal activities of eugenol, isoeugenol, methyl eugenol, and methyl isoeugenol were about 1.9-2.2 times more toxic than benzyl benzoate. Furthermore, the most toxic constituent against *T. putrescentiae* was exhibited on eugenol (10.11 μg/cm^2), followed by methyl eugenol (38.67 μg/cm^2) and acetyl eugenol (70.09 μg/cm^2), but no activity was observed for isoeugenol and methyl isoeugenol. The results suggested that eugenol congeners may be useful as a new source for selective control of house dust mites and stored food mites.

Keywords: Eugenol congeners, *Eugenia caryophyllata*, natural acaricide, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Tyrophagus putrescentiae*

Introduction

The most common species of house dust mites and stored food mites all over the world are *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Tyrophagus putrescentiae* (1). House dust mites are a major cause of allergic diseases, including bronchial asthma, allergic rhinitis, conjunctivitis, and atopic dermatitis due to its ubiquity and abundance (2-3). House dust means have also been implicated in more serious conditions, such as sudden infant death syndrome (4-5). House dust mites have dramatically increased in number mainly due to changes in the living environment, such as a rise in the number of apartment households with central-heating, space-heating, tighter windows, and wall-to-wall carpeting (1, 2). House dust mites live in residential homes, but large numbers of *T. putrescentiae*, stored food mites, occur in farm buildings, food, and textile industries. The stored food mite is an urban species frequently found in a wide variety of stored products, particularly those with a high protein and fat content (5). House mites, therefore, particularly cause allergic asthma and rhinitis in farmers, grain handlers, bakers, and food industrial workers (6, 7). However, they are also now being recognized as important contributors to the allergen content in house dust in indoor urban dwellings (8). Recently, systemic anaphylaxis was reported from eating *T. putrescentiae*-infested food (9). Control of these mite populations has been principally through the use of synthetic acaricides (1, 2). Although effective, their repeated and widespread use has sometimes resulted in drug resistance, undesirable effects on non-target organisms, and environmental and human health consequences (10, 11). The research into plant-derived acaricides is now being intensified as evidence accumulates on their enormous potential in modern agrochemical research. In light of the importance of natural anti-mite agents to remove house dust and stored food mites, we investigated stored food and house dust mite-control activities by *Eugenia caryophyllata* leaf oil-derived materials. These effects were also compared with synthetic mite-control agents.

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Received August 26, 2004; accepted December 16, 2004

Materials and Methods

Chemicals Benzyl benzoate was purchased from Aldrich (Milwaukee, WI, USA). Acetyl eugenol, adinene, β-caryophyllene, α-copaene, α-cubebene, eugenol, α-humulene, isoeugenol, methyl eugenol, methyl isoeugenol, and valencene were supplied by Sigma (St. Louis, MO, USA). All other chemicals were of reagent grade.

Gas chromatography-mass spectrometry The essential oil of *E. caryophyllata* leaves was purchased from Jin Aromatics (Anyang, Korea). The essential oil of *E. caryophyllata* leaves was analyzed on a gas chromatograph (HP6890)-mass spectrometer (JMS-600W, JEOL) (GC-MS). The GC column was a 60 m × 0.25 mm i.d. DB-WAX (0.25 μm film) fused silica capillary column (J&W Scientific, Folsom, CA, USA). 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 the carrier gas at the 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 70eV ionization energy. The sector mass analyzer was set to scan from 50 to 800 amu for 2s. 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.).

Mites The *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* cultures were maintained at the laboratory for 5 years without exposure to any known acaricide. The mites were maintained on fry feed No.1 (Korea Special Feed Meal Co. Ltd., Chonju, Korea) and dried yeast (1:1 by weight) and reared in plastic containers (15 × 12 × 6 cm) containing 30 g of a sterilized diet. The raring cages were kept in an incubator at 27±1°C and 80% relative humidity in darkness, within a plastic tray (18 × 18 × 17 cm) where it was maintained by a saturated solution of potassium chloride to prevent mites escaping and to maintain high relative humidity.

Acaricidal activity An impregnated fabric disk bioassay was used for the acaricidal activity of the test materials. Ethanol solutions containing various concentrations of essential oils were applied to disks of black cotton fabric (5 cm diameter). Ethanol was applied as a control at the same dose to fabric disks (received 100 mL of ethanol). After air drying in a fume hood for 30s, each piece was placed in the bottom of a petri dish (5 × 1.5 cm). Then, 20 *D. farinae* (7-10 days old), *D. pteronyssinus* (7-10 days old), and *T. putrescentiae* (7-9 days old) individuals were separately placed in each petri dish and covered with a lid. The treated and control mites were held at 27±1°C and 80% relative humidity in darkness. Death was determined 24 hr after treatment under a stereomicroscope (20×). The acaricidal activity was evaluated by assessing the mobility of the mite. The mites were considered dead if the appendages did not move when stimulated with a fine pin. Each treatment was replicated three times. All assays were performed in an constant temperature (27±1°C) and humidity (80%) apparatus in dark conditions. The LD_{50} values were calculated by probit analysis (12).

Results and Discussion

When the essential oil derived from *E. caryophyllata* leaves was bioassayed by an impregnated fabric disk application, the acaricidal activity of the essential oil was observed in various doses against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* (Table 1). The LD_{50} values of the essential oil were 12.42, 14.46, and 20.43 μg/cm^2 against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively. There was no mortality in the untreated controls.

The substances identified by GC-MS in the oil of *E. caryophyllata* leaves are presented in Table 1. Analysis led to identification of 12 volatiles from the oil of *E. caryophyllata* leaves. The main constituents were α-amorphene (1.2%), β-bourbonene (3.5%), cadinene (9.5%), β-caryophyllene (6.4%), α-copaene (9.4%), α-cubebene (14.5%), eugenol (18.8%), germarene (9.2%), a-humulene (3.8%), methyl eugenol (12.6%), α-muurolene (3.3%), and valencene (2.3%). Cadinene, β-caryophyllene, a-copaene, a-cubebene, eugenol, germarene, and methyl eugenol made up 80.3% of *E. caryophyllata* leaf oil. For acaricidal activities of the substances identified in the essential oil of *E. caryophyllata* leaves, the LD_{50} values of eugenol and methyl eugenol were 4.94 and 4.13 μg/cm^2 against *D. farinae*, respectively. However, no or weak activity was observed for cadinene, β-caryophyllene, α-copaene, α-cubebene, α-humulene, and valencene against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*.

The acaricidal activities of eugenol congeners (acetyl eugenol, eugenol, isoeugenol, methyl eugenol, and methyl isoeugenol) against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* adults were examined by an impregnated fabric disk application (Table 2, Fig. 1) and compared with that of benzyl benzoate as synthetic acaricide. The commonly used benzyl benzoate served as a positive control. On the basis of LD_{50} values, the most toxic compound against *D. farinae* adults was methyl eugenol (4.13 μg/cm^2), followed by methyl isoeugenol (4.19 μg/cm^2), isoeugenol (4.29 μg/cm^2), eugenol (4.94 μg/cm^2), and acetyl eugenol (13.91 μg/cm^2). On the basis of LD_{50}

Table 1. Volatile compounds in *E. caryophyllata* leaf oil identified by GC-MS

compound	mass spectral data[a]	retention time (min)	relative (%)
α-cubebene	41, 91, 105, 161, 120, 204	41:63	14.48
eugenol	55, 77, 103, 149, 164	42:20	18.76
α-copaene	41, 91, 105, 119, 161, 204	43:22	9.44
β-bourbonene	81, 123, 161, 204	43:59	3.54
methyl eugenol	65, 77, 91, 107, 147, 163, 178	45:70	12.58
β-caryophyllene	107, 133, 148, 176, 204	45:81	6.44
α-humulene	80, 93, 121, 147, 204	47:87	3.77
valencene	79, 91, 105, 133, 161, 204	48:31	2.26
germacrene	41, 79, 91, 105, 119, 161, 204	49:74	9.15
α-amorphene	79, 93, 119, 161, 204	50:59	1.22
α-muurolene	41, 93, 105, 161, 204	51:03	3.27
cadinene	81, 105, 161, 204	52:52	9.52

[a]Major fragmentation ions, base peak (listed first) and other ions in decreasing order of relative abundance.

Table 2. Acaricidal activity of commercial constituents derived from *E. caryophyllata* leaf oils, and synthetic acaricide[a]

compound	mite species	LD_{50} (μg/cm^2)	95% confidence interval	RT[b]
oil	*D. farinae*	12.42	11.37-13.36	0.7
	D. pteronyssinus	14.46	13.23-15.41	0.5
	T. putrescentiae	20.43	18.39-22.32	0.5
acetyl eugenol	*D. farinae*	13.91	12.05-14.90	0.7
	D. pteronyssinus	7.21	6.72-7.69	1.0
	T. putrescentiae	70.09	66.23-75.02	0.2
eugenol	*D. farinae*	4.94	4.41-5.42	1.9
	D. pteronyssinus	3.92	3.43-4.45	1.9
	T. putrescentiae	10.11	9.25-11.06	1.0
isoeugenol	*D. farinae*	4.29	3.81-4.67	2.1
	D. pteronyssinus	2.93	2.56-3.31	2.5
	T. putrescentiae	-	-	-
methyl eugenol	*D. farinae*	4.13	3.66-4.69	2.2
	D. pteronyssinus	3.87	3.41-4.25	1.9
	T. putrescentiae	38.67	35.21-41.65	0.3
methyl isoeugenol	*D. farinae*	4.19	3.73-4.49	2.2
	D. pteronyssinus	3.28	2.93-3.54	2.2
	T. putrescentiae	-	-	-
benzyl benzoate	*D. farinae*	9.14	8.01-10.13	1.0
	D. pteronyssinus	7.35	6.56-8.02	1.0
	T. putrescentiae	10.27	5.38-16.19	1.0

[a]Exposed for 24 h. [b]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical.

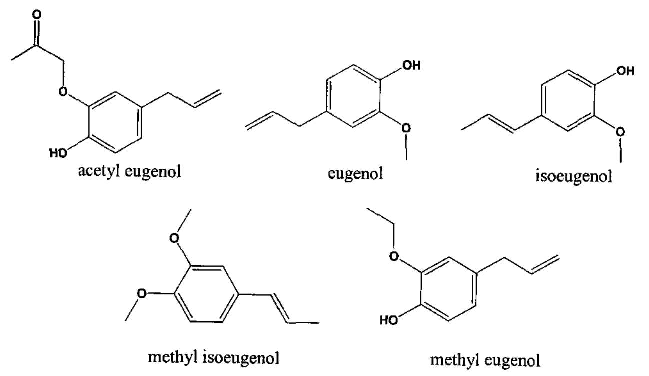

Fig. 1. Structure of eugenol congeners.

values, the most toxic compound against *D. pteronyssinus* adults was isoeugenol (2.93 μg/cm^2), followed by methyl isoeugenol (3.28 μg/cm^2), methyl eugenol (3.87 μg/cm^2), eugenol (3.92 μg/cm^2), and acetyl eugenol (7.21 μg/cm^2). Regarding acarcidal activities against house dust mites, these results suggest that *D. pteronyssinus* may be controlled more effectively than *D. farinae* by the application of acetyl eugenol, eugenol, isoeugenol, methyl eugenol, or methyl. Interestingly, acaricidal activities of eugenol, isoeugenol, methyl eugenol, and methyl isoeugenol were about 1.9-2.2 times more toxic than benzyl benzoate, a synthetic acaricide. On the basis of LD_{50} values, the most toxic compound against *T. putrescentiae* was eugenol (10.11 μg/cm^2), followed by methyl eugenol (38.67 μg/cm^2) and acetyl eugenol (70.09 μg/cm^2); however, no activity was observed for isoeugenol and methyl isoeugenol. The acaricidal activity of acetyl eugenol was less than that of benzyl benzoate. The acaricidal activity of eugenol was comparable to that of benzyl benzoate (10.27 μg/cm^2). These results indicate that the presence of the allyl moiety outside the ring may appear to be responsible for *T. putrescentiae* toxicity. However, on the basis of the acaricidal activities of iso-form compounds, of which an allyl moiety is replaced with a propenyl moiety, as is the case with isoeugenol and methyl isoeugenol, the propenyl moiety may have a reduced influence on *T. putrescentiae* toxicity. In this study, eugenol, isoeugenol, methyl eugenol, and methyl isoeugenol merit further study as potential house dust mite control agents or as primary compounds, but only eugenol in eugenol congeners merit further study as stored food mite control agents. Furthermore, these results indicate that the acaricidal activity of *E. caryophyllata* leaf oil can be mostly attributed to eugenol, methyl eugenol, and methyl isoeugenol because eugenol and methyl eugenol made up 31.3% of *E. caryophyllata* leaf oil.

It has been well recognized that plant extracts or phytochemicals could be developed into mite-control agents because many of them have selective acaricidal activity and have no or little harmful effects on non-target organisms and the environment (11). Furuno *et al.* (13) reported the acaricidal activity of leaf oils from six Lauraceae species at 1 mg/cm^2. They found that the leaf oil from *N. sericea* has potent activity against both *D. farinae* and *D. pteronyssinus*, but the *Machilus thunbergii* leaf oil is only active against *D. farinae*. Yatagai *et al.* (14) reported the acaricidal activity of six *Melaleuca* species against *D. pteronyssinus*. It was found that the leaf oil of *M. bracteata* has potent activity at 0.13 mg/cm^2 and the leaf oils of *M. argentea* and *M. dealbata* have moderate acaricidal activity. Recently, potent acaricidal activities against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae* have been reported in the barks of *Cinnamomum cassia*, clove bud, *Chamaecyparis obtusa*, and *Thuja orientalis* (1, 15, 16). Of the acaricidal activities of clove bud, the most toxic compound against *T. putrescentiae* was methyl eugenol (LD_{50}, 1.18 $\mu g/cm^2$), followed by isoeugenol (8.21 $\mu g/cm^2$), eugenol (12.11 $\mu g/cm^2$), and acetyl eugenol (28.72 $\mu g/cm^2$). In comparison with our data, the acaricidal activities of eugenol congeners isolated from clove bud oil are different (15). These results indicate that the differential responses are influenced by extrinsic and intrinsic factors, such as bioassay methods, the plant species, parts of the plant, and geographical location where the plants were grown (1, 13, 14, 17, 18). Furthermore, eugenol congeners may provide a new source for controlling stored food and house dust mites. Additionally, the control of stored food mites may reduce allergies among food industrial workers handling stored foods. Further research should be done on safety issues of eugenol congeners for human health, acaricidal mode of action, and formulations improving the acaricidal potency and stability.

Acknowledgments

This research was supported by the Program for the Training of Graduate Students in Regional Innovation which was conducted by the Ministry of Commerce, Industry and Energy of the Korean Government.

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