SHORT COMMUNICATION

Antimite Activity of Essential Oils and Their Constituents from *Taiwania cryptomerioides*

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ABSTRACT Antimite activity of essential oils and their components obtained from *Taiwania cryptomerioides* Hayata heartwood against *Dermatophagoides pteronyssinus* (Trouessart) and *Dermatophagoides farinae* Hughes was investigated in this study. Results from antimite tests demonstrated that the essential oil extracted from *T. cryptomerioides* heartwood had miticidal activity against *D. pteronyssinus* and *D. farinae* with a mortality of 67.0 and 36.7% at the dosage of 12.6 μg/cm^2 after 48 h. Alpha-cadinol possessed the strongest antimite activity compared with other components of the *T. cryptomerioides* heartwood essential oil. The rectified mortalities of *D. pteronyssinus* and *D. farinae* were 100% for α-cadinol at the dosage of 6.3 μg/cm^2. The order of antimite activity of four dominant constituents was α-cadinol > T-muurolol > ferruginol > T-cadinol. Paired Student's *t*-tests showed that there were significant differences between the rectified mortality of α-cadinol, T-muurolol, ferruginol and that of T-cadinol at the dosage of 6.3 μg/cm^2 after 48 h.

KEY WORDS *Taiwania cryptomerioides,* essential oil, cadinane, α-cadinol, ferruginol, antimite activity

IT HAS BEEN confirmed that house dust mites are causative agents of many allergic diseases, such as bronchial asthma, allergic rhinitis, and atopic dermatitis. Particularly, *Dermatophagoides pteronyssinus* (Trouessart) and *Dermatophagoides farinae* Hughes were recognized as two major allergenic species of house dust mites (Wharton 1976, Furuno et al. 1994). In addition to reducing the relative humidity of living environment, using wooden floor or interior decorative panels can also decrease the population and activity of house dust mites. *Taiwania cryptomerioides* Hayata is an endemic tree that grows at elevations from 1,800 to 2,600 m in Taiwan's central mountains. The timbers of *T. cryptomerioides* are well recognized for their decay resistance and excellent durability in Taiwan. It has been proven that essential oils and extracts can inhibit the growth of microorganisms. Thus, the activity of essential oils from trees against house dust mites has become an interesting topic for several researchers (Miyazaki et al. 1989, Watanabe et al. 1989, Furuno et al. 1994, Yatagai and Nakatani 1994, Miyazaki 1996). Recently, we investigated the contribution of chemical constituents to the wood properties, including the photo-discoloration and antifungal activity in *T. cryptomerioides* heartwood (Chang et al. 1999a, 1999b, 2000a). Our research revealed that cadinane-type sesquiterpenoids contribute an excellent antifungal activity to the heartwood. In fact, alpha-cadinol completely inhibited the growth of *Coriolus versicolor* and *Laetiporus sulfureus* at levels as low as 100 ppm. Furthermore, we recently demonstrated the antibacterial activity of essential oils and extracts from *T. cryptomerioides* (Chang et al. 2000b). There is no research, however, discussing the relationship between antimite activities and chemical ingredients from *T. cryptomerioides.* The purpose of this study is to examine the antimite activity of extracts obtained from *T. cryptomerioides* and their major constituents against two house dust mite species, *D. pteronyssinus* and *D. farinae,* which are commonly found in Taiwan.

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Materials and Methods

Apparatus. High performance liquid chromatography (HPLC) was performed with a Jasco model PU980 pump equipped with a Jasco RI-930 RI detector and Hibar Lichrosorb Si 60 (25 by 1 cm i.d.) column (Jasco, Japan). FTIR (Fourier transform infrared spectrophotometer) spectra were recorded on a Bio-Rad model FTS-40 spectrophotometer (Bio-Rad, Hercules, CA). Mass spectrum (MS) was obtained on a Finnigan MAT-95S mass spectrometer (Finnigan, Bremen, Germany). ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance-300 MHz FT-NMR spectrometer (Bruker, Silberstreifen, Germany).

Extraction and Isolation. Twenty-seven-year-old *T. cryptomerioides* used in this study were collected from the Experimental Forest of National Taiwan University. *T. cryptomerioides* heartwood chips were prepared from a green cut tree. Essential oils were collected by water distillation. Air-dried heartwood chips (5.7 kg) were exhaustively extracted with methanol (MeOH). Condensed MeOH extracts ($\approx$286.4 g) were partitioned with *n*-hexane (*n*-C_6H_{14}), chloro-

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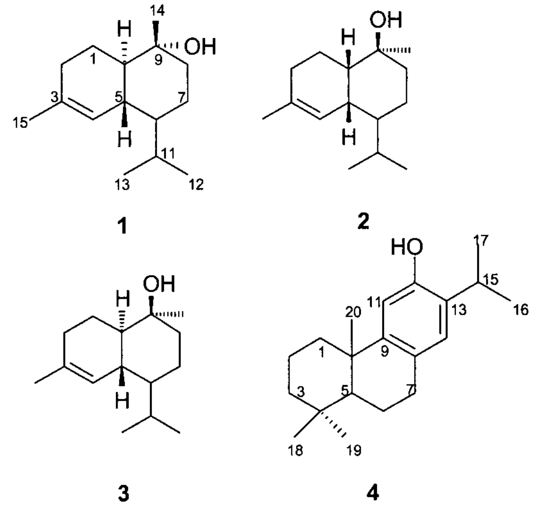

Fig. 1. Structures of alpha-cadinol (1), T-muurolol (2), T-cadinol (3), and ferruginol (4) from *Taiwania cyptomerioides* heartwood.

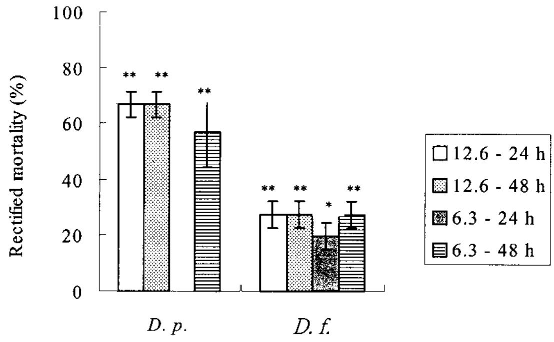

Fig. 2. Antimite activity of essential oils from *Taiwania cyptomerioides* heartwood. Bars are the standard derivation. *D.p., Dermatophagoides pteronyssinus; D.f., Dermatophagoides farinae;* 12.6–24 h, keeping mites in a concentration of 12.6 $\mu g/cm^2$ for 24 h; 12.6–48 h, keeping mites in a concentration of 12.6 $\mu g/cm^2$ for 48 h; 6.3–24 h, keeping mites in a concentration of 6.3 $\mu g/cm^2$ for 24 h; 6.3–48 h, keeping mites in a concentration of 6.3 $\mu g/cm^2$ for 48 h. Statistically different from the data of controls: **, $P < 0.01$; *, $P < 0.05$.

form ($CHCl_3$), ethyl acetate (EA), and methanol (MeOH), successively. After removing solvents from the combined extracts, the n-C_6H_{14}, $CHCl_3$, EA, and MeOH soluble fractions and MeOH insoluble fraction were obtained. Using chromatographing with a silicagel column (800 g) eluted with EA/n-C_6H_{14} (gradient elution was performed by changing from EA/n-C_6H_{14} = 0:100-100:0), the n-C_6H_{14} soluble fraction (5,000 mg) was divided into 41 subfractions (H1-H41). Alpha-cadinol (retention time, 27.6 min), T-muurolol (retention time, 24.0 min), and T-cadinol (retention time, 22.1 min) were isolated and purified from H16 to H22 by semipreparative HPLC (column, Si-60 column; Mobile phase, EA/n-C_6H_{14} = 30:70; flow rate, 1 ml/min). Ferruginol (retention time, 16.2 min) was collected from H2-H8 with the same HPLC system. The Mobile phase, however, was changed to EA/n-C_6H_{14} = 10:90. The compound's structures were confirmed by FTIR, MS, and NMR analyses (Chang et al. 1999a, 1999b, 2000a). Fig. 1 demonstrates the structures of compounds, which isolated from *T. cryptomerioides* evaluated in this study. Copies of the original spectra are obtainable from the corresponding author.

Antimite Test. Tests for antimite activity were conducted and modified with reference to the reports by Yatagai and Nakatani (1994). Briefly, *Dermatophagoides pteronyssinus* and *D. farinae,* the dominant species of house dust mites, were used for the antimite activity test. Test mites were cultured in a chamber maintained at 25°C and 75% RH on a mixture of powdered wheat and dry yeast (1:1, wt:wt). A 12-mm-diameter piece of filter paper was put between the 10-mm-diameter center holes of two acrylic resin plates (top 25 by 75 mm, thickness 3 mm; bottom 25 by 75 mm, thickness 1 mm). Ethylether solutions containing various concentrations (6.3 $\mu g/cm^2$ or 12.6 $\mu g/cm^2$) of essential oils or compounds were applied to the filter paper, only ethylether solution was used as the control. After air drying, 10 active female adult mites were put on the filter paper, and the top sides of the plates were covered with slide glass (25 by 75 mm, thickness 1 mm). Both edges of the plates were clamped with clips. Plates with mites were placed in a chamber maintained at 25°C and 75% RH for 48 h, and the number surviving were counted at 24 and 48 h. Each treatment was done with three replicates. The percentage rectified mortality was calculated by abbott's formula (Rosenheim and Hoy 1989):

Rectified mortality (%) =

$$[(mortality\ of\ testing\ plate - mortality\ of\ control)/(100 - mortality\ of\ control)] \times 100.$$

Student's t-test was used to evaluate the statistical significance of results at $P = 0.01$ and 0.05.

Results and Discussion

Essential oils and extracts obtained from trees have long been used as medicines. In addition, the fragrances of tree's essential oils stimulate or attenuate human senses. They have been studied for use as safe and natural insecticides instead of using organic phosphorous materials and other synthetic agents. Although many compounds have been isolated from *T. cryptomerioides,* investigations on the bioactivity of these constituents are limited. After previously demonstrating antifungal and antibacterial activities of *T. cryptomerioides,* essential oils and their constituents (Chang et al. 1999b, 2000a, 2000b), their activities against house dust mites are reported here. Fig. 2 is the rectified mortalities of *D. pteronyssinus* and *D. farinae* caused by *T. cryptomerioides*'s essential oils. Treatment decreased the survival of both *D. pteronyssinus* and *D. farinae.* The rectified mortalities of *D. pteronyssinus* and *D. farinae* after exposure to 12.6 $\mu g/cm^2$ after 48 h were 67.0 and 36.7%. Overall, we found that

Table 1. Rectified mortalities (%) of *D. pteronyssinus* and *D. farinae* caused by the cadinane-type sesquiterpenoids and ferruginol extracted from *Taiwania cyptomerioides* heartwood

Samples	*D. pteronyssinus*				*D. farinae*			
	12.6 μg/cm²		6.3 μg/cm²		12.6 μg/cm²		6.3 μg/cm²	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
α-Cadinol	100 ± 0*	100 ± 0*	100 ± 0**	100 ± 0**	100 ± 0**	100 ± 0**	100 ± 0**	100 ± 0**
T-Cadinol	50.0 ± 8.2	70.0 ± 8.2	26.7 ± 4.7	26.7 ± 4.7	13.3 ± 4.7	20.4 ± 7.8	13.3 ± 4.7	14.1 ± 4.2
T-Muurolol	93.3 ± 4.7*	100 ± 0*	73.3 ± 4.7**	80.0 ± 8.2*	80.0 ± 0**	83.3 ± 8.2**	40.0 ± 8.2	56.7 ± 4.7**
Ferruginol	73.3 ± 4.7	80.0 ± 0	16.7 ± 4.7	56.7 ± 4.7*	48.1 ± 5.7**	68.1 ± 5.0**	10.0 ± 0	36.7 ± 4.7**

D.p., Dermatophagoides pteronyssinus; D.f., Dermatophagoides farinae; 12.6 μg/cm², keeping mites in a concentration of 12.6 μg/cm²; 6.3 μg/cm², keeping mites in a concentration of 6.3 μg/cm². Statistically different from the data of T-Cadinol, **, $P < 0.01$; *, $< .05$.

D. pteronyssinus was more sensitive to essential oils than *D. farinae.* This is consistent with the results reported by Miyazaki et al. (1989).

Results from our previous study indicated that cadinane-type sesquiterpenoids were the major chemical constituents of essential oils from *T. cryptomerioides* heartwood (Chang et al. 2000a). In heartwood essential oils the total content of cadinane-type sesquiterpenoids was 66.7%, including 36.8% α-cadinol, 17.1% T-muurolol, and 12.8% T-cadinol, respectively. Moreover, these cadinane-type sesquiterpenoids and ferruginol (one of abietane-type diterpenes from *T. cryptomerioide* heartwood) have been demonstrated to have good antifungal and antibacterial activities (Chang et al. 2000a, 2000b). Therefore, HPLC equipped with Hibar Lichrosorb Si −60 column was performed to isolate and purify α-cadinol, T-muurolol, T-cadinol, and ferruginol. Table 1 is the rectified mortalities of *D. pteronyssinus* and *D. farinae* caused by the cadinane-type sesquiterpenoids and ferruginol. The results showed that, at either a high concentration (12.6 μg/cm²) or a low concentration (6.3 μg/cm²) dosage, the decreasing order of antimite activity was α-cadinol > T-muurolol > ferruginol > T-cadinol. Among them, α-cadinol possessed the strongest antimite activities. *D. pteronyssinus* and *D. farinae* were all killed at a low concentration (6.3 μg/cm²) dosage after 24 h of exposure. Student's *t*-test was performed to evaluate the statistical significance of results at $P = 0.01$ and 0.05 (Table 1). According to the paired Student's *t*-tests, there were significant differences between the rectified mortality of α-cadinol, T-muurolol, ferruginol and that of T-cadinol at the dosage of 6.3 μg/cm² after 48 h. As far as structure-activity relationships were concerned, an equatorial hydroxyl at C-9 (α-cadinol) seems to be an important factor for antimite activities. In contrast to the configuration of hydroxyl at C-9, the type of ring junction (C-5 connects to C-10) was less important for inhibiting the activity of house dust mites. Whether in *cis* configuration (T-cadinol) or *trans* configuration (T-muurolol) with axial hydroxyl at C-9, their antimite activities were lower than α-cadinol. The further activity of α-cadinol will be studied in the future. Moreover, because of these results, people allergic to house dust mites may consider *T. cryptomerioides* as a building material in their homes.

Acknowledgments

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