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Verbenone Structural Analogues Isolated from *Artemesia aucheri* as Natural Acaricides against *Dermatophagoides* spp. and *Tyrophagus putrescentiae*

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ABSTRACT: The acaricidal activities of *Artemisia aucheri* oil and (1*S*)-(−)-verbenone structural analogues were evaluated using a fumigant method against *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Tyrophagus putrescentiae* and then compared to those of benzyl benzoate. On the basis of the LD_{50} values against *D. farinae*, (1*S*)-(−)-verbenone (1.38 $\mu g/cm^2$) was about 7.4 times more active than benzyl benzoate (10.15 $\mu g/cm^2$), followed by (+)-*trans*-myrtanol (2.27 $\mu g/cm^2$), (−)-*trans*-myrtanol (2.30 $\mu g/cm^2$), and *A. aucheri* oil (8.75 $\mu g/cm^2$). (1*S*)-(−)-Verbenone (1.25 $\mu g/cm^2$) was approximately 7.8 times more effective against *D. pteronyssinus* than benzyl benzoate (9.80 $\mu g/cm^2$), followed by (+)-*trans*-myrtanol (2.18 $\mu g/cm^2$), (−)-*trans*-myrtanol (2.22 $\mu g/cm^2$), and *A. aucheri* oil (8.46 $\mu g/cm^2$). In the case of *T. putrescentiae*, (1*S*)-(−)-verbenone (3.75 $\mu g/cm^2$) was roughly 3.5 times more toxic than benzyl benzoate (13.25 $\mu g/cm^2$), followed by (+)-*trans*-myrtanol (12.57 $\mu g/cm^2$), (−)-*trans*-myrtanol (12.95 $\mu g/cm^2$), and *A. aucheri* oil (11.55 $\mu g/cm^2$). These results indicate that *A. aucheri* oil and (1*S*)-(−)-verbenone structural analogues may be effective natural agents to control house dust and storage mites.

KEYWORDS: *Artemisia aucheri, essential oil, house dust mite, storage mite, structure−activity relationship, (1S)-(−)-verbenone*

INTRODUCTION

Dermatophagoides farinae (Hughes) and *Dermatophagoides pteronyssinus* (Trouessart) are the most important house dust mites because of their cosmopolitan occurrence.[1] They are a major source of allergens associated with atopic dermatitis, asthma, and perennial rhinitis.[1,2] The most significant storage mite is *Tyrophagus putrescentiae* (Schrank) because of its abundance in numerous stored foods with protein contents or high fats.[3] The storage mite is an etiological agent of allergic illness among farmers and workers handing contaminated stored products, and it causes acute enteritis and systemic anaphylaxis when ingested.[3] These mites have been primarily managed through use of various synthetic acaricides, such as avermectines, benzyl benzoate, and γ-benzene hexachloride.[4,5] However, the repeated use of synthetic acaricides has occasionally resulted in the appearance of resistance, human health concerns, and undesirable effects on non-target organisms.[3,5] Various problems indicate that it is necessary to develop new strategic alternatives for house dust and storage mites, particularly those with fumigant action, because powder or dust formulations are normally less active and are inconvenient to apply.[3,6]

Medicinal plants contain various metabolites and have been used for their naturally occurring acaricides and insecticides.[2,5,7,8] In particular, essential oils could be an alternative substance for managing house dust and storage mites because they are composed of a rich source of active chemicals.[2,5,6] They do not have toxic residues and have low toxicity to mammals.[9] Previous studies have focused on essential oils as acaricidal agents.[2,5,6] *Artemisia aucheri* (Asteraceae family) is mainly found in Asia, Europe, and North America.[10] *Artemisia* spp. are widely used as traditional medicinal agents for therapeutic applications, including angina, bronchial infections, cough, diarrhea, parasitism, pimples, stomach ache, and wounds.[10−12] Despite many previous studies, the acaricidal potential of the essential oil extracted from *A. aucheri* has not been evaluated. Therefore, a laboratory study was conducted to identify acaricidal constituents from *A. aucheri* oil against house dust and storage mites and determine their structure−activity relationships.

MATERIALS AND METHODS

Reagents. (1*S*)-(−)-Verbenone (97%) and (*S*)-*cis*-verbenol (98%) were purchased from Aldrich (St, Louis, MO). Benzyl benzoate (98%), (+)-*trans*-myrtanol (97%), and (−)-*trans*-myrtanol (98%) were purchased from Fluka (Buchs, Switzerland). All chemicals were reagent-grade.

Essential Oil Extraction. The aerial parts of *A. aucheri* (10 kg) were purchased from a local market (Jeonju, South Korea). A voucher specimen was authenticated by Prof. Jeong-Moon Kim and deposited in the herbarium at the Department of Landscape Architecture, Chonbuk National University. Dried plants were boiled in a Clevenger-type apparatus for 6 h. After cooling, the essential oil (yield of 0.23%) was added to anhydrous magnesium sulfate to remove the water and collect the oil by a rotary evaporator (model N-100, EYELA, Tokyo, Japan) at 30 °C. The concentrated oil was stored in a capped bottle at 4 °C to avoid volatilization of the constituents.

Gas Chromatography−Mass Spectroscopy (GC−MS) Analysis. The analysis of *A. aucheri* oil was performed using a model 6890 gas chromatograph and model 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA). The silica capillary columns (0.25 mm inner diameter × 3000 mm length × 0.25 μm film thickness, J&W Scientific, Folsom, CA) of different polarities (DB-5 and HP-Innowax)

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Table 1. Acaricidal Activities of *A. aucheri* Oil and a Commercial Acaricide against House Dust and Storage Mites Using a Fumigant Method

samples	mite species	LD_{50} ± SD ($\mu g/cm^2$)	95% CL	relative toxicity[a]
A. aucheri oil	*D. farinae*	8.75 ± 0.9	8.55−8.95	1.2
	D. pteronyssinus	8.46 ± 1.6	8.26−8.66	1.2
	T. putrescentiae	11.55 ± 1.4	11.35−11.75	1.2
benzyl benzoate	*D. farinae*	10.15 ± 2.1	9.95−10.35	1.0
	D. pteronyssinus	9.80 ± 1.8	9.60−10.00	1.0
	T. putrescentiae	13.25 ± 1.6	13.05−13.45	1.0

[a]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of *A. aucheri* oil.

were used, and the analytic conditions were as follows: injector temperature, 210 °C; column temperature, 50 °C; programmed temperature, rising to 200 °C at 2 °C/min; and ion source temperature, 230 °C. Helium was used as the carrier gas at a ratio of 0.8 mL/min. The effluent from the GC column was directly introduced into the mass spectrometer. Mass spectra were acquired in the electron ionization mode with 70 eV. The sector mass analyzer was arranged to scan from 50 to 600 for 2 s.

Determination of Volatile Constituents. The identification of volatile constituents isolated from *A. aucheri* oil was based on the retention indices on the DB-5 capillary column, which is related to the number of carbons in the *n*-alkanes and computer matching of mass spectra with the National Bureau of Standards library, as well as by comparison of the mass spectra fragmentation patterns to those reported in the literature[13] and, whenever possible, by co-injection with authentic compounds.

Isolation. *A. aucheri* oil (10 g) was loaded on an 800 × 50 mm inner diameter, 70−230-mesh silica gel column (Merck Rahway, NJ) and was gradually eluted using hexane/ethyl acetate (4 L, 9:1, 8:2, and 6:4, v/v). The identity of each fraction was determined by thin-layer chromatography, and five fractions (AJ 1−AJ 5) were obtained. The acaricidal activity of each fraction was evaluated using a fumigant method against house dust and storage mites at 40 $\mu g/cm^2$. The AJ 2 (4.12 g) fraction possessed excellent acaricidal activity against house dust and storage mites and then was subjected to rechromatography on a silica gel column using hexane/ethyl acetate (3 L, 6:4, v/v), to give five fractions (AJ 21−AJ 25). The AJ 23 (1.08 g) fraction had potent acaricidal activity against three mite species. Furthermore, preparative high-performance liquid chromatography (prep HPLC, model LC908-C60, Japan Analytical Industry Co., Ltd., Tokyo, Japan) was performed to subdivide the AJ 23 fraction. The AJ 232 (755 mg) fraction was separated into four fractions (AJ 231−AJ 234) using a Jaigel GS Series column (GS310 500 mm) with methanol (100%) at a rate of 5.0 mL/min. The AJ 232 fraction had the strongest acaricidal activity among these fractions. Then, a Jaigel W Series column (W252 500 mm + W253 500 mm) with methanol (100%) at a rate of 3.0 mL/min was used, to give three fractions (AJ 2321−AJ 2323). Finally, the active constituent (AJ 2321) was isolated. The structure of AJ 2321 (297 mg) was determined using nuclear magnetic resonance (NMR) spectroscopy. ^1H and ^{13}C NMR were obtained using a JNM-EX600 (Jeol, Ltd., Tokyo, Japan) spectrometer in deuterochloroform ($CDCl_3$) at 600 and 150 MHz, respectively, and tetramethylsilane served as an internal standard. EI−MS (70 eV) *m*/*z*: M^+ 150.22. ^1H NMR ($CDCl_3$, 600 MHz) δ: 0.99 (3H, s), 1.78−1.83 (3H, dd, J = 30.1 Hz), 1.85−1.94 (2H, m, J = 53.5 Hz), 2.15−2.18 (2H, t, J = 18.6 Hz), 2.61−2.65 (1H, d, J = 24.1 Hz), 2.78−2.81 (1H, d, J = 17.8 Hz), 5.85 (1H, s). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 203.5, 170.1, 122.8, 58.5, 54.0, 49.7, 40.8, 24.3, 22.9, 22.1.

Rearing of Test Mites. The cultures of house dust and storage mites were reared without exposure to commercial acaricidal agents, respectively. These mites were maintained on dried feed (Korea Special Feed Meal Co., Ltd., Jeonju, Korea) and yeast (1:1 by weight) in a plastic case (15 × 12 × 6 cm). The cages were kept in a 25 °C incubator at 75% relative humidity in the dark. The mixing proportions of the dried feed were protein (49.0%), lipid (4.0%), cellulose (3.0%), P (2.0%), and Ca (1.9%).

Fumigant Method. Acaricidal activities of the test samples were evaluated using the fumigant method against house dust and storage mites. This method was modified by Yang and Lee.[2] Different dosages (0.5−40 $\mu g/cm^2$) of each test sample were dissolved in 100% acetone, and a 20 μL sample was applied to a paper disk (8 mm diameter and 1 mm thickness, Tokyo Roshi, Japan). The negative control used only 100% acetone injected at the same volume. The treated paper disks were dried in a fume hood for 20 min and then placed in the cap of a microtube (2 mL, Greiner Bio-One GmbH, Frickenhausen, Germany). A total of 20 house dust and storage mites were inoculated in each microtube, which was sealed using the cap containing the treated paper disks. The processing and comparative groups were maintained under the same conditions as the mites for 24 h.

Data Analysis. Mortalities in each group were investigated though a binocular microscope (20×) after 24 h, because the house dust and storage mites, which were unsupplied with feed, were unaffected for 24 h. The mites were considered dead if they did not move when poked with a pin. All treatments were repeated 3 times, and the LD_{50} values were calculated by probit analysis. Relative toxicity was determined as the ratio of commercial acaricide LD_{50} values/sample LD_{50} values.

RESULTS AND DISCUSSION

Acaricidal Toxicities of the *A. aucheri* Oil. The acaricidal activities of the *A. aucheri* oil were evaluated using a fumigant method against house dust and storage mites. The LD_{50} values of *A. aucheri* oil were 8.75, 8.46, and 11.55 $\mu g/cm^2$ against *D. farinae*, *D. pteronyssinus*, and *T. putrescentiae*, respectively. The acetone treatment (negative control) resulted in no mortality of *D. farinae*, *D. pteronyssinus*, or *T. putrescentiae*. The essential oil of *A. aucheri* was approximately 1.2 times more active than benzyl benzoate as a commercial acaricide (positive control) (LD_{50} values of 10.15, 9.80, and 13.25 $\mu g/cm^2$) against the three mite species (Table 1). *Dermatophagoides* spp. were more sensitive to *A. aucheri* oil than *T. putrescentiae*. According to a previous study, the acaricidal activity of plant-derived materials is influenced by mite species and is attributed to differences in biological conditions (size and weight of the mite populations) as well as biochemical conditions (effect of detoxification enzymes).[2,14] Taken together, the essential oil extracted from *A. aucheri* may be a natural acaricide with fumigant action.

Composition of *A. aucheri* Oil. The volatile constituents of the *A. aucheri* oil were recognized by GC−MS and matched the retention indices and mass spectra of compounds in the literature[13] (Table 2). The identified by GC−MS constituents consisted of 89.1% of the total *A. aucheri* oil. The composition (%) of the volatile constituents was camphor (19.7%), carvone (1.9%), 1,8-cineol (15.5%), linalool (0.4%), myrcene (2.6%), myrtanol (2.4%), α-pinene (1.2%), pinocarvone (3.5%), piperitol (1.2%), spathulenol (5.2%), α-terpinene (0.7%), terpinen-4-ol (0.5%), (1*S*)-(−)-verbenone (23.5%), and (*S*)-*cis*-verbenol (10.8%). The volatile constituents were also grouped as monoterpene alcohols [1,8-cineol, linalool, myrtanol, piperitol, terpinen-4-ol, and (*S*)-*cis*-verbenol], mono-

Table 2. Analysis of *A. aucheri* Oil Volatile Components Identified by GC−MS

number	components	retention index DB-5	retention index HP-Innowax	relative composition (%)
1	α-pinene	942	1023	1.2
2	myrcene	988	1137	2.6
3	α-terpinene	1012	1174	0.7
4	1,8-cineol	1022	1203	15.5
5	linalool	1083	1213	0.4
6	(1*S*)-(−)-verbenone	1098	1425	23.5
7	vamphor	1120	1563	19.7
8	(*S*)-*cis*-verbenol	1126	1578	10.8
9	pinocarvone	1137	1589	3.5
10	terpinen-4-ol	1159	1613	0.5
11	myrtanol	1166	1740	2.4
12	piperitol	1188	1775	1.2
13	carvone	1212	1829	1.9
14	spathulenol	1562	1944	5.2

terpene hydrocarbons (myrcene, α-pinene, and α-terpinene), monoterpene ketones [camphor, carvone, pinocarvone, and (1*S*)-(−)-verbenone], and sesquiterpene alcohols (spathulenol). Sefidkon et al.[15] reported that the main compounds of *A. aucheri* oil were verbenone (21.5%), camphor (21.0%), 1,8-cineol (8.3%), (*S*)-*cis*-verbenol (8.1%), and ρ-cymene (3.5%), which was slightly different from our results. In comparison to the previous study, the major constituents of *A. aucheri* oil were camphor, 1,8-cineol, (1*S*)-(−)-verbenone, and (*S*)-*cis*-verbenol. Some constituents derived from plants are influenced by extrinsic or intrinsic elements, such as extraction methods, geographic location, harvest time, parts of herbs, and plant species.[2]

Identification of the Active Constituent Derived from *A. aucheri* Oil. To isolate the active component of *A. aucheri* oil, chromatographic analyses were performed using a silica gel column and prep HPLC. As a result, the AJ 2321 fraction was isolated and identified by various spectral analyses, such as EI−MS and ^{1}H and ^{13}C NMR. The isolated AJ 2321 fraction was characterized as (1*S*)-(−)-verbenone (Figure 1), and data were consistent with the previous study.[16]

Toxicity of (1*S*)-(−)-Verbenone Isolated from *A. aucheri* Oil. The acaricidal activities of (1*S*)-(−)-verbenone isolated from *A. aucheri* oil were evaluated using a fumigant method against house dust and storage mites and compared to that of benzyl benzoate (Table 3). On the basis of the LD_{50} values against *D. farinae*, (1*S*)-(−)-verbenone (1.38 μg/cm^2) was about 7.4 times more active than benzyl benzoate (10.15 μg/cm^2). (1*S*)-(−)-Verbenone (1.25 μg/cm^2) was approximately 7.8 times more effective than benzyl benzoate (9.80 μg/cm^2) against *D. pteronyssinus*. (1*S*)-(−)-Verbenone (3.75 μg/cm^2) was roughly 3.5 times more toxic than benzyl benzoate (13.25 μg/cm^2) in the case of *T. putrescentiae*. Therefore, the acaricidal activities of (1*S*)-(−)-verbenone were higher than those of a commercial acaricide under laboratory conditions.

Structure−Activity Relationships. To establish the relationships between (1*S*)-(−)-verbenone structural analogues and acaricidal activity, (+)-*trans*-myrtanol, (−)-*trans*-myrtanol, and (1*S*)-(−)-verbenone were selected as (1*S*)-(−)-verbenone structural analogues and their acaricidal toxicities were evaluated using a fumigant method against house dust and storage mites (Table 3). In comparison to benzyl benzoate against *D. farinae*, (*S*)-*cis*-verbenol (LD_{50} value of 1.97 μg/cm^2) was approximately 5.2 times more active than benzyl benzoate (LD_{50} value of 10.15 μg/cm^2), followed by (+)-*trans*-myrtanol (2.27 μg/cm^2) and (−)-*trans*-myrtanol (2.30 μg/cm^2). Against *D. pteronyssinus*, (*S*)-*cis*-verbenol (2.03 μg/cm^2) was about 4.8 times more toxic than benzyl benzoate (9.80 μg/cm^2), followed by (+)-*trans*-myrtanol (2.18 μg/cm^2) and (−)-*trans*-myrtanol (2.22 μg/cm^2). In the case of *T. putrescentiae*, (*S*)-*cis*-verbenol (10.55 μg/cm^2) was roughly 1.26 time more effective than benzyl benzoate (13.25 μg/cm^2), followed by (+)-*trans*-myrtanol (12.57 μg/cm^2) and (−)-*trans*-myrtanol (12.95 μg/cm^2). These results indicated that the differences of (1*S*)-(−)-verbenone structural analogues with fumigant toxicity were attributed to the functional groups (alcohol and ketone) on the skeletal structure of monoterpenes. (1*S*)-(−)-Verbenone, with a ketone group, appeared to be more toxic than (*S*)-*cis*-verbenol, which contains alcohol groups. The acaricidal activity of (*S*)-*cis*-verbenol, which is one of the unsaturated bicyclic ketones, was more pronounced than that of (+)-*trans*-myrtanol and (−)-*trans*-myrtanol, which are saturated bicyclic ketones. However, the difference between (+)-*trans*-myrtanol and (−)-*trans*-myrtanol was not observed. Taken together, structure−activity relationships were found among the skeletal structure types of each alcohol and ketone group.

A. aucheri has medicinal properties as an astringent and disinfectant agent and has antileishmanial, antiparasitic, and antioxidant activities.[17−19] These properties are contributed by various constituents, such as borneol, camphor, 1,8-cineol, ρ-cymene, lavandulol, linalool, (1*S*)-(−)-verbenone, and (*S*)-*cis*-verbenol.[17] Despite its excellent pharmacological action, very little work has been conducted to control house dust and

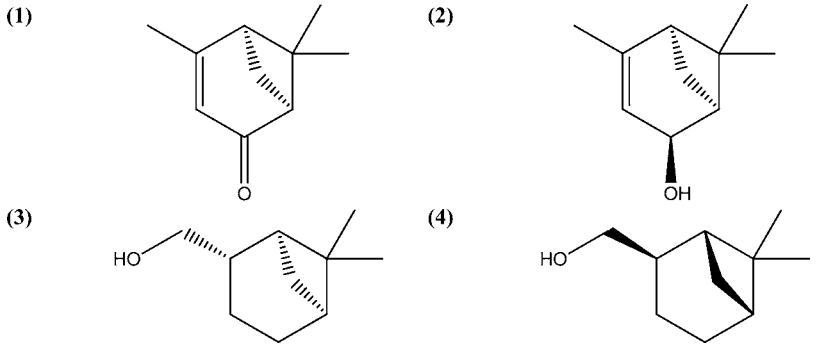

Figure 1. (1*S*)-(−)-Verbenone structural analogues: (**1**) (1*S*)-(−)-verbenone, (**2**) (*S*)-*cis*-verbenol, (**3**) (+)-*trans*-myrtanol, and (**4**) (−)-*trans*-myrtanol.

Table 3. Acaricidal Activities of Verbenone Structural Analogues and Commercial Acaricides against House Dust and Storage Mites Using a Fumigant Method

samples	mite species	LD_{50} ± SD ($\mu g/cm^2$)	95% CL	relative toxicity[a]
(1*S*)-(−)-verbenone	*D. farinae*	1.38 ± 1.6	1.18−1.58	7.4
	D. pteronyssinus	1.25 ± 2.5	1.05−1.45	7.8
	T. putrescentiae	3.75 ± 1.6	3.55−3.95	3.5
(*S*)-*cis*-verbenol	*D. farinae*	1.97 ± 1.8	1.77−2.17	5.2
	D. pteronyssinus	2.03 ± 0.9	1.83−2.23	4.8
	T. putrescentiae	10.55 ± 1.4	10.35−10.75	1.3
(+)-*trans*-myrtanol	*D. farinae*	2.27 ± 1.5	2.07−2.47	4.5
	D. pteronyssinus	2.18 ± 1.6	1.98−2.38	4.5
	T. putrescentiae	12.57 ± 2.8	12.37−12.77	1.1
(−)-*trans*-myrtanol	*D. farinae*	2.30 ± 2.5	2.10−2.50	4.4
	D. pteronyssinus	2.22 ± 1.8	2.02−2.42	4.4
	T. putrescentiae	12.95 ± 1.6	12.75−13.15	1.0
benzyl benzoate	*D. farinae*	10.15 ± 1.4	9.95−10.35	1.0
	D. pteronyssinus	9.80 ± 1.7	9.60−10.00	1.0
	T. putrescentiae	13.25 ± 1.6	13.05−13.45	1.0

[a]Relative toxicity = LD_{50} value of benzyl benzoate/LD_{50} value of each chemical.

storage mites. In the present study, *A. aucheri* oil-derived materials had potent acaricidal activities against *Dermatophagoides* spp. and *T. putrescentiae*. Numerous compounds, such as alkaloids, phenols, and terpenes, exist in medicinal plants and contribute to a variety of biological activities.[20] Therefore, interest has been focused to identify plant-based components with acaricidal activity. Papachristos et al.[21] reported that insecticidal activity of *Rosmarinus officinalis* depends upon the (1*S*)-(−)-verbenone-containing ketone functional groups. Taken together, *A. aucheri* oil and (1*S*)-(−)-verbenone structural analogues had potent acaricidal toxicities against the three mite species. Changes in the functional radicals on the monoterpene skeleton played a leading role in the acaricidal activities.

Various acaricide studies have sought to develop new and safe agents and to avoid unwanted problems, such as residual toxicity to the environment.[22] According to Lee et al.,[23] the acute toxicity of monoterpenoids is low relative to conventional insecticides. However, the acute toxicities of *A. aucheri* oil and (1*S*)-(−)-verbenone structural analogues have not been reported. Taken together, these results indicate that *A. aucheri* oil and (1*S*)-(−)-verbenone structural analogues could be suitable replacements for commercial acaricides as fumigant agents. Further studies should be conducted to evaluate their safety and effects on human health and the environment.

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Notes

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