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ORIGINAL ARTICLE

Acaricidal activity of components of *Cryptomeria japonica* against spider mites

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Abstract The acaricidal activities of solvent extracts and essential oil obtained from *Cryptomeria japonica* were investigated. The two target spider mites (*Tetranychus kanzawai* and *T. urticae*), are known pests for various crops. The *C. japonica* leaves, barks, and heartwood were extracted by hexane, ethyl acetate and methanol successively, and acaricidal activities were tested by the leaf disc method. Acaricidal activity was observed on a hexane extract of *C. japonica* leaves. Next, the acaricidal activities of essential oil obtained from leaves by hot water distillation was tested and found to show stronger effects than the hexane extract. Elemol and ent-kaurene were found as the active components of essential oil. The LC_{50} values of ent-kaurene were quite lower than those of elemol and essential oil. Acaricidal activities of essential oil were mainly caused by ent-kaurene. Since other chemotypes of *C. japonica* are known in major diterpene hydrocarbon, we examined the acaricidal activity of phyllocladene and ent-sclarene. The activity of ent-sclarene was lower than ent-kaurene, and phyllocladene was much lower than ent-sclarene. Thus, the extract of *C. japonica* leaves containing ent-kaurene could be used as an interim pesticide when commercial pesticides are being changed or as a matrix of commercial pesticide.

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Introduction

Spider mites are known as the most common pests in agricultural fields with soybean, tea, and other vegetables including *Tetranychus kanzawai* and *Tetranychus urticae,* two typical agricultural pests in Japan [1]. These spider mites have been reported to cause serious damage to several agricultural products in various regions of Japan [2, 3]. Various commercial pesticides have been developed and used in agricultural fields because spider mites easily build up tolerance to pesticides [4]. The acaricidal activities of many essential oils of plants have been studied because of the need for environmentally safe pesticides [5]. However, the activities have not been evaluated for conifers of plantation trees in Japan such as *Cryptomeria japonica*, which accounts for a large proportion of Japanese plantation forests.

Currently, the forest industry considers most of the bark and leaves of *C. japonica* to be waste materials [6]; however, its bark, wood and leaves contain several bioactive components [7–12]. In particular, heartwood components have been found to have anti-mite activity against *Dermatophagoides pteronyssinus*, a house dust mite [13]. The leaves of *C. japonica* resist attacks from *T. kanzawai* and *T. urticae* in natural forest; therefore, some components of the leaves of *C. japonica* apparently have acaricidal or repellent activity that provides an effective defense against spider mites. In this study, the acaricidal activities of extracts obtained from *C. japonica* leaves, bark and heartwood were investigated against two spider mites, *T. kanzawai* and *T. urticae*.

Materials and methods

Spider mites

Spider mites (*Tetranychus kanzawai* and *T. urticae*) were supplied by the Life Science Research Institute of Kumiai Chemical Industry Co., Ltd. (Shizuoka, Japan). The mites were cultured on kidney bean (*Phaseolus vulgaris*) leaves at 25 ± 1 °C in the laboratory of Yamagata University.

Sample collection and extraction

Cryptomeria japonica leaves, heartwood, and bark samples were collected from the Yamagata Field Science Center, Faculty of Agriculture, Yamagata University, Japan. The bark was separated into inner and outer bark. The heartwood and bark samples were ground separately into powder using a Willey mill (Yoshida seisakusho Co., Ltd., Tokyo, Japan), and the leaves were cut into 5–10 mm sized pieces. Each sample was extracted at ambient temperature for one week by successive extractions with *n*-hexane, ethyl acetate, and methanol. Each solvent was removed by evaporation to yield the extracts. Essential oil was obtained from the leaves by hot water distillation at 100 °C according to the previous report [8].

Authentic compounds

β-Pinene and terpinen-4-ol were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). α-Pinene was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ent-kaurene, phyllocladene, and ent-sclarene were isolated from different chemotypes of *C. japonica* leaves by silica gel column chromatography with hexane according to a previous method [14]. Elemol was kindly provided by Dr. Shizuo Nagahama (Sojo University, Kumamoto, Japan). All isolated compounds were purified more than 95 % using gas chromatography (GC) by silica gel column chromatography before bioassay.

Analytical conditions

GC-flame ionization detection (GC-FID) was performed with a Hitachi G-3500 gas chromatograph (Hitachi Ltd., Tokyo, Japan) under the following conditions: Inert Cap 1 capillary column (30 m × 0.32 mm i.d.; 0.25 μm film thickness; GL Sciences, Tokyo, Japan); a column temperature from 40 °C (2 min) to 150 °C at 5 °C/min and from 150 °C (0 min) to 320 °C (2 min) at 15 °C/min; injection temperature of 170 °C; detection temperature of 200 °C. Helium was used as the carrier gas. GC-mass spectrometry (GC-MS) data were collected with a QP-5000 GC-mass spectrometer (Shimadzu, Kyoto, Japan) under the following conditions: DB-1 capillary column (0.32 mm i.d. × 30 m; 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA)); column temperature from 40 °C (2 min) to 150 °C (0 min) at 5 °C/min and from 150 °C (0 min) to 320 °C (2 min) at 15 °C/min; injection temperature of 170 °C; detection temperature of 250 °C; acquisition mass range of 450–50 amu using helium as the carrier gas. Components were identified by comparison of the experimental GC-MS data with authentic compounds or the NIST MS library.

Bioassay

The leaf disc method was used to investigate acaricidal activities based on previously published methods [15–17]. Each extract and compound was dissolved into acetone. A fixed amount of each acetone solution was added in distilled water containing an agrochemical spreader [3300 times diluted Kumiten® (Kumiai Chemical Industry, Co., Ltd., Tokyo, Japan)] to prepare 100–5000 μg/ml samples in aqueous solution. The control was treated only with acetone in an aqueous solution. Three commercial pesticides, cyflumetofen flowable (OAT Agrio Co., Ltd., Tokyo, Japan), emamectin benzoate emulsifiable concentrate (Syngenta Japan Co., Ltd., Tokyo, Japan) and pyridaben wettable powder (Nissan Chemical Industries, Ltd., Tokyo, Japan), were used as positive controls at each regulation concentration (cyflumetofen: 200 μg/ml, emamectin benzoate: 5 μg/ml, pyridaben: 200 μg/ml) in aqueous solutions. Leaf discs (20 mm in diameter) were prepared from kidney bean (*Phaseolus vulgaris*). Leaf discs were soaked in each solution for the application of samples covering the entire surface, and were picked up immediately. Then, each prepared leaf disc was placed on wet filter paper in a petri dish (40 mm diameter). Ten adult female mites were placed on each leaf disc. After 96 h, the numbers of dead mites were counted under a microscope. LC_{50} was calculated based on concentration (μg/ml) of extract or compound in each sample aqueous solutions.

Results and discussions

Acaricidal activity of extracts

Figure 1 provides the results of the analysis of acaricidal activity of solvent extracts from *C. japonica*. Only hexane extract from leaves showed clear activity against both of *T. kanzawai* and *T. urticae*. The activities of the 5000 μg/ml leaf hexane extract aq. were less than two positive controls of 200 μg/ml cyflumetofen and 5 μg/ml emamectin benzoate, and almost same as a positive control reagent of 200 μg/ml pyridaben. Other extracts did not show

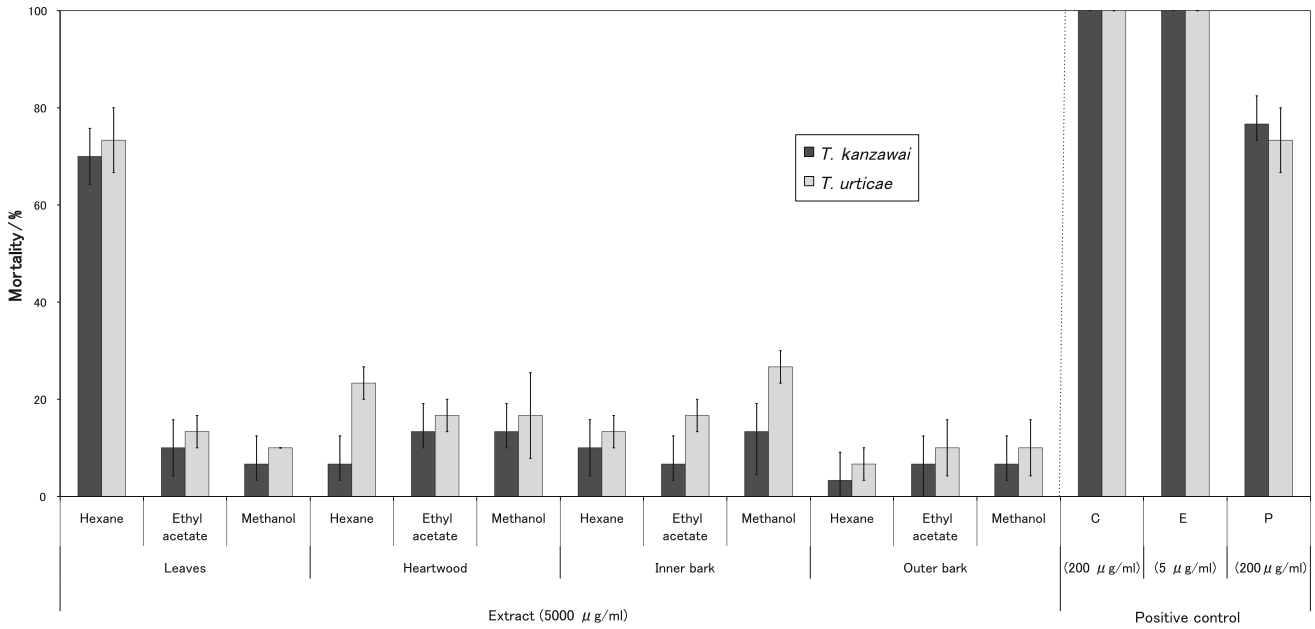

Fig. 1 Acaricidal activity of *C. japonica* solvent extracts. Positive control: *C* cyflumetofen, *E* emamectin benzoate, *P* pyridaben

remarkable activities. Because the activities were observed in low polar extract of the leaves, we subsequently examined the activities of leaf essential oil obtained by hot water distillation; the essential oil showed similar acaricidal activity as was observed with the leaf hexane extract. The activities of the hexane extract and essential oil differed because of the concentrations of extract; therefore, the LC_{50} values of the leaf hexane extract and essential oil were calculated. The LC_{50} values of the hexane extract and essential oil against *T. kanzawai* and *T. urticae* were 2002 and 1419 μg/ml, and 1150 and 1109 μg/ml, respectively. Essential oil showed slightly higher activity than hexane extract. These results suggested that the property of active components were low polar and distillable compounds in the leaves.

Acaricidal activity of essential oil components

Essential oil of *C. japonica* leaves was analyzed by GC, and the main components were identified (Table 1). Acaricidal activities of commercially available compounds and previously isolated components in our laboratory were tested together (Fig. 2). The results show that elemol and ent-kaurene exhibited potent activities at 5000 μg/ml sample concentration. Elemol was known as a common component together with α-, β- and γ-eudesmol in *C. japonica* leaf oil, and formed by rearrangement from hedycaryol during hot water distillation [18, 19]. The ent-kaurene was also commonly known as a main diterpene hydrocarbon component in *C. japonica* leaf extract [14]. The LC_{50} values of elemol and ent-kaurene against *T. kanzawai* and *T. urticae* were 1657 and 1749 μg/ml, and 161 and 247 μg/ml, respectively. The ent-kaurene had ca. 1/10 value of LC_{50} compared with elemol. Its values were also quite lower than that of the essential oil. Thus, the acaricidal activities of the essential oil were mainly caused by activities of ent-kaurene.

Table 1 Components in essential oil of *C. japonica* leaves

Component	Content[a] (%)
α-Pinene	1.98
β-Pinene	0.93
Terpinen-4-ol	21.01
Elemol	22.39
γ-Eudesmol	17.20
β-Eudesmol	8.00
α-Eudesmol	1.89
ent-Kaurene	22.54
Total	95.94

[a] Contents were calculated by calibration curve method with GC-FID analysis

However, different chemotypes are known that contain other diterpene hydrocarbons as the major component of *C. japonica* leaves. *C. japonica* has been classified by chemotypes of the leaves, which contained ent-kaurene, phyllocladene or ent-sclarene as a main diterpene hydrocarbon or mixtures of these [14, 20]. Thus, we examined acaricidal activities of phyllocladene and ent-sclarene. Phyllocladene showed weak activities or did not show acaricidal activities depending on the concentration; therefore, LC_{50} values cannot be calculated. The LC_{50} of ent-sclarene was 7457 μg/ml and 1173 μg/ml against *T. kanzawai* and *T. urticae*, respectively, and their activities

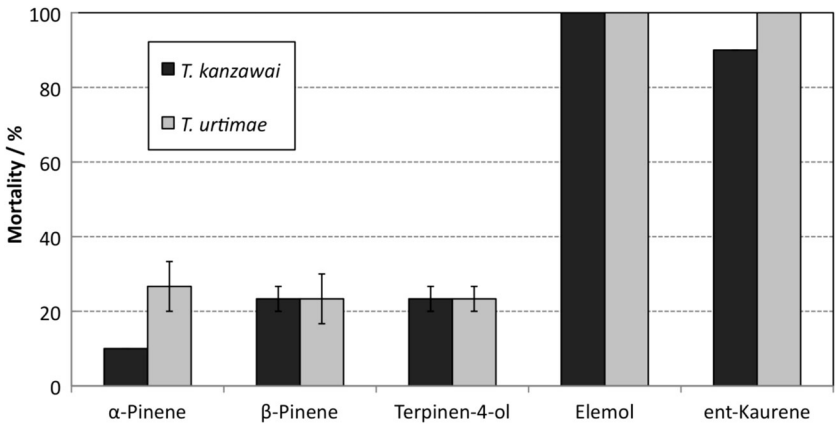

Fig. 2 Acaricidal activity of components in the essential oil of *C. japonica* leaves at 5000 μg/ml concentration

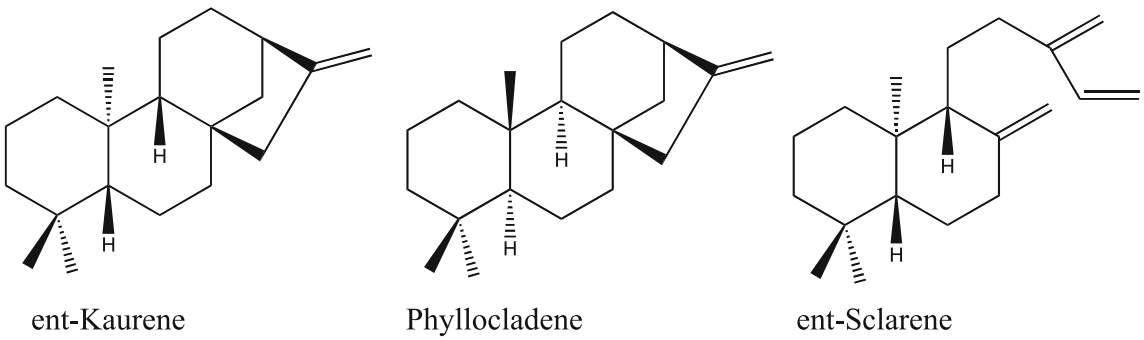

Fig. 3 Structure of diterpene hydrocarbons, the main components in *C. japonica* leaves

were remarkably lower than that of ent-kaurene. As shown in Fig. 3, the difference between ent-kaurene and phyllocladene was a diastereomer with the same planar structure; therefore, the differences of the activities between these two components were caused by this stereostructural difference.

The above results also suggested that chemotypes of the *C. japonica* leaves were important for considering the use of the extract. The activities of extracts and components obtained from *C. japonica* in this study were lower than those of commercial pesticides. However, the spider mites are well known to easily become resistant to pesticides [4]. Therefore, in agricultural fields, the types of pesticides used are periodically changed. The extracts of *C. japonica* leaves are a mixture of active components, and may provide a large supply of resources in Japanese forests that are useful as pesticides. Isman et al. [21] also reported that essential oil-based pesticides were not only used as "stand alone" products but can be also used in rotation or in combination with other conventional pesticide products. This study suggests that *C. japonica* leaf extracts could be used as pesticides during a changing period of commercial pesticides in an effort to minimize the resistance of spider mites to other pesticides or as a matrix designed to increase the effectiveness of current commercial pesticides.

Conclusion

Acaricidal activities of the extracts obtained from *C. japonica* were tested against two spider mite species, *T. kanzawai* and *T. urticae*, to find new uses for woody waste materials. Remarkable activities were observed in hexane extract and essential oil of the leaves. Ent-kaurene, a major component in the leaves, was detected as a strong and active component. However, phyllocladene and ent-sclarene, which are known as the major diterpene hydrocarbons in other chemotypes of *C. japonica* leaves, showed weak activities compared with ent-kaurene.

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