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Phytotoxicity and Volatile Monoterpenes of Leaves from *Artemisia capillaris* and *Artemisia iwayomogi* Used as Korean Herbal Injin

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ABSTRACT: *Artemisia capillaris* and *Artemisia iwayomogi* are weeds used as medicinal plants in Korea under the name "Injin". I collected leaves of *A. capillaris* and *A. iwayomogi*, examined them for phytotoxic effects from volatile substances and determined the composition of monoterpenes in the leaves. The effects of volatile substances from each species on seed germination and radicle elongation in each of the two *Artemisia* species were assessed. The volatile substances of *A. capillaris* did not negatively affect the seed germination of *A. capillaris*, but they did inhibit radicle elongation. Rates of seed germination of *A. iwayomogi* decreased when the seeds were exposed to high concentration of *A. capillaris* volatile substances. The inhibition of seed germination and radicle elongation by volatile substances from both *Artemisia* species was stronger for *A. iwayomogi* than for *A. capillaris*. I identified the monoterpenoids from the leaves with a gas chromatograph-mass spectrometer (GC-MS). The main constituents of *A. capillaris* were acenaphthylene (37.91%), β-pinene (12.08%), 4-carene (10.61%) and γ-curcumene (9.92%), while those of *A. iwayomogi* were germacrene-δ (32.15%), borneol (21.24%), camphor (20.45%) and trans-caryophyllene (7.75%).

Key words: *Artemisia capillaris*, *Artemisia iwayomogi*, Monoterpene, Phytotoxicity

INTRODUCTION

Artemisia species are known to be a rich source of volatile compounds with a variety of biological activities, and it has been suggested that *Artemisia* exhibits strong allelopathic properties (Rice 1984). Members of this genus have a characteristic scent or taste, and are of botanical and pharmaceutical interest (Kordali et al. 2006). Though *Artemisia capillaris* and *A. iwayomogi* are weeds, they have been used in traditional medicine, under the name "Injin". *Artemisia capillaris* is a common perennial herb and has been cultivated in Korea for the treatment of hepatitis. *A. capillaris* has been shown to have an antifungal effect (Choi et al. 2005) and an allelopathic effect (Kil 1999). *Artemisia iwayomogi* is an unique shrub of the genus in Korea. It inhibits hepatotoxicity and liver cirrhosis (Song et al. 2001).

Volatile monoterpenes exhibit a greater diversity of compounds than any of the other volatile terpenoid compounds. The biological activity of volatile compounds is dependent on the synergistic or additive effects of the constituent types present at different concentrations. Volatile components from aromatic plants can cause a number of positive or negative interactions (Vokou et al. 2003). The volatile constituents that play a prominent role as plant protection agents are being explored for their insecticidal, pesticidal, and herbicidal properties (Singh et al. 2002). Volatile compounds identified from *Artemisia* plants have been implicated as phytotoxic and are responsible in part for the allelopathic activity of these species (Rice 1984). The aim of the study was to assess the phytotoxic effect of volatile substances and the volatile monoterpene constituents in *A. capillaris* and *A. iwayomogi*.

MATERIALS AND METHODS

Plant Material

Aerial parts of *A. capillaris* and *A. iwayomogi* were collected from a cultivated population in the Jinan Medicinal Herbs Experiment Station, Jeollabuk-do Agricultural Research & Extension Services (35° 46' 15" N, 127° 22' 40" E), in Korea, on June 23, 2007. The fresh plant materials were taken, sealed in a plastic bag, transported to the laboratory and then used immediately for experiments. Seeds of the two *Artemisia* plants were collected from the Jinan Medicinal Herbs Experiment Station in the fall of 2006.

Phytotoxic Effects of Volatile Substances from *A. capillaris* and *A. iwayomogi*

To assess the phytotoxic activity of volatile substances from the two *Artemisia* species on germination and radicle elongation, 50 seeds of the test plants were placed in a 1.8 L glass chamber on filter paper that was layered on moist, absorbent cotton. Fresh leaves of the two *Artemisia* species were cut into 1-cm pieces, and different

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quantities of the cut *Artemisia* leaves (fresh masses of 5, 10, 15, 20, 25, or 30 g) were placed in a glass beaker within the chamber. The glass chambers were then covered with vinyl wrap and placed in a growth chamber that was maintained at 25℃ during the day (14 h) and 18℃ at night (10 h). The experiment was terminated after four days, when seed germination had ceased, and seed germination and radicle elongation were recorded. Seed germination rates and radicle elongation rates relative to control (RGR and RER) were calculated following Kil and Yun (1992) as (seed germination rate in treatment group) / (seed germination rate in control group) × 100 and (average radicle elongation in treated seedlings) / (average radicle elongation in control seedlings) × 100.

Analysis of Monoterpenoids

Fresh leaves of the two species of *Artemisia* plants were ground in a mortar with pure sand and extracted with 50 mL of pentane. After extraction, extracts from fresh leaves were concentrated to 8～10 mL or 0.5～1.0 mL under nitrogen gas. Each sample was analyzed by injection of a 1 μL aliquot onto a Gas Chromatography-Mass Spectrophotometer (HP 5890II-5972MS) with a flame ionization detector. Monoterpenoids were separated on a fused silica capillary column packed with HP5 (id, 0.25 mm, 30 m long) with oven temperature programmed from 37℃ for five minutes, increasing to 180℃ at a rate of 5℃ /min, and then at 20℃ /min to 320℃.

The individual peaks were identified by comparison with the spectral data in the internal spectral library of the instrument (Wiley library ver. 7.0) and by retention times, based on references. Because of the complexity of the chromatograms, a limited number of peaks were examined. The concentration of peaks at selected retention times were estimated from peak area using the internal standard curve of tetradecane (Kim et al. 2006).

Statistical Analysis

A randomized complete block design with four replications was applied in all the experiments. Each experiment was repeated three times. Statistical analysis was performed with the software program SPSS (Version 10.0). Comparisons between treatments were made at the 0.05 level using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Phytotoxic Effects of Volatile Substances from *Artemisia capillaris* and *Artemisia iwayomogi* on Each of the Two Artemisia plants

Biologically active compounds from higher plants are now known to be important components of a plant's interaction with its environment. Plant-produced compounds can act as phytotoxins if they are found to inhibit or halt growth of other organisms. In this case the interaction between plants is known as allelopathy (Beninger and Hall 2005). Seed germination in the two *Artemisia* species was influenced by the volatile substances from both *Artemisia* plants. In cases where the relative germination/elongation ratio is more than 100%, the plant compound has stimulated germination or elongation. Conversely, when the value is less than 100%, inhibition of germination or growth has occurred. Seed germination in *A. capillaris* was stimulated, rather than inhibited, by volatile substances from *A. capillaris* (i.e., from conspecifics), but *A. iwayomogi* was inhibited by concentrations of volatile substances of *A. capillaris* (i.e., from heterospecifics) that exceeded 25 g/1.8 L. Seed germination of *A. capillaris* and *A. iwayomogi* were both inhibited by concentrations of volatile substances of *A. iwayomogi* that exceeded 15 g/1.8 L (Fig. 1). Volatile substances from the two *Artemisia* species also affected radicle elongation in both *Artemisia* species (Fig. 2). Seed germination and radicle elongation of *A. iwayomogi* were dramatically inhibited by high concentrations of volatile substances from *A. capillaris* and *A. iwayomogi*. Perennial species in the genus *Artemisia* expend considerable energy to produce terpenoids that protect them from other organisms (Reid 1964). Aqueous extracts and volatile substances from *Artemisia princeps* var. *orientalis* affected seed germination and seedling growth depending on the concentrations of the extract and volatile substances and the species tested (Kil and Yun 1992, Yun et al. 1993). Plants of the genus *Artemisia*

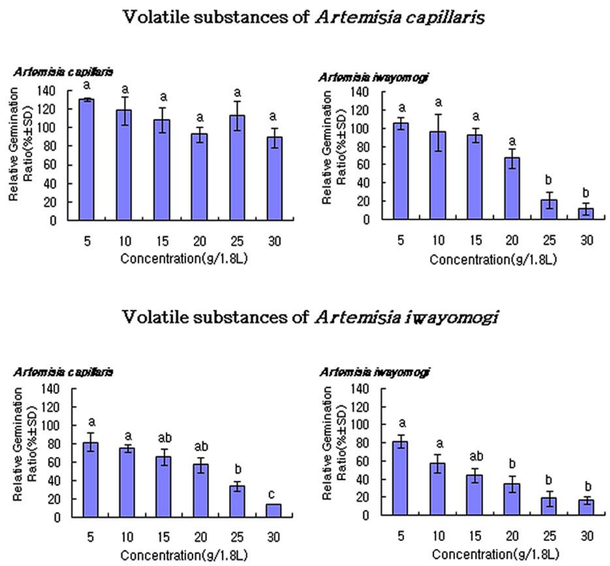

Fig. 1. Relative germination ratios of *Artemisia capillaris* and *A. iwayomogi* treated at different concentrations of volatile substances from the two *Artemisia* plants. Bars topped by different letters are significantly different according to Duncan's multiple-range test ($p \leq 0.05$)

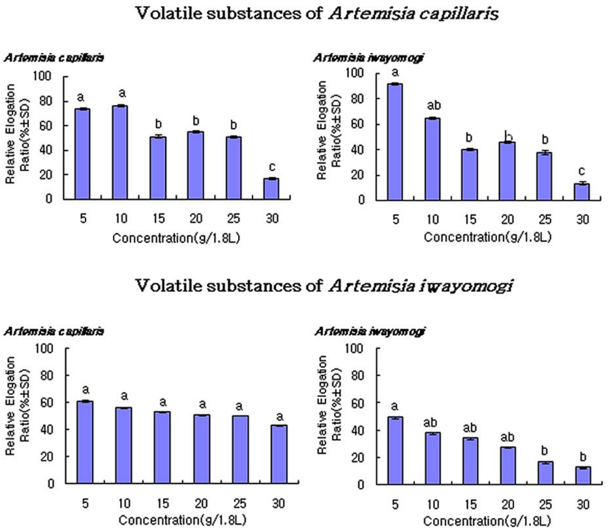

Fig. 2. Relative elongation ratios of *Artemisia capillaris* and *A. iwayomogi* treated at different concentrations of volatile substances from the two *Artemisia* plants. Bars topped by different letters are significantly different according to Duncan's multiple-range test ($p \leq 0.05$).

synthesize and emit water-extractable and volatile allelochemicals, which affect seed germination and seedling growth (Yun 1991).

The Composition of Monoterpenoides in *Artemisia capillaris* and *Artemisia iwayomogi*

Results of the GC/MS analysis of volatile constituents of *A. capillaris* and *A. iwayomogi* are shown in Table 1. Thirteen monoterpenes were identified from *A. capillaris*. The main components were acenaphthylene (37.91%), β-pinene (12.08%), 4-carene (10.61%) and γ-curcumene (9.92%). The monoterpene constituents of *A. iwayomogi* were different from those of *A. capillaris*. Only four of the same components were identified in both *Artemisia* plants. I identified 11 monoterpenes in *A. iwayomogi*. The main components for *A. iwayomogi* were germacrene-δ (32.15%), borneol (21.24%), camphor (20.45%) and *trans*-caryophyllene (7.75%). As has been reported previously, the major monoterpene constituents for the genus *Artemisia* are α- pinene, β-pinene, 1,8-cineol, bornyl acetate, camphor and caryophyllene (Weyerstahl et al. 1987, Simon et al. 1990, Yun 1991, Umano et al. 2000, Zheng et al. 2004, Kordali et al. 2006).

It has been known for some time that plant-produced allelopathic compounds are generally volatile toxic terpenes (Beninger and Hall 2005). In the present study, I have shown that differences in the monoterpene components isolated from the two *Artemisia* plants are responsible for at least some of the differences in the phytotoxic effects of volatile substances from these plants.

Table 1. Main monoterpenoid constituents (%) of leaves from *Artemisia capillaris* and *A. iwayomogi*

Components	% RA[a] *A. capillaris*	% RA *A. iwayomogi*
α-Pinene	3.25	–[b]
Camphene	0.88	2.83
β-Pinene	12.08	–
β-Myrcene	2.89	4.67
dl-Limonene	4.07	–
1,8-Cineole	–	4.58
4-Carene	10.61	–
1,3,6-Octatrene	1.54	–
cis-Ocimene	–	0.92
Camphor	–	20.45
Borneol	–	21.45
Bornyl acetate	0.99	–
trans-Caryophyllene	8.84	7.75
α-Humulene	–	0.90
γ-Curcumene	9.92	–
Germacrene D	5.94	32.15
α-Bergamotene	1.08	–
γ-Bisabolene	–	3.52
Acenaphthylene	37.91	–
Stahlianthusone	–	0.99

[a] RA: relative area (peak area relative to total peak area).
[b] –: Not found.

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