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Identification and Growth Inhibition of Phytotoxic Substances from *Artemisia scoparia*

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비쑥의 독성물질 확인과 생장억제작용

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ABSTRACT

Chemical substances from *Artemisia scoparia* were analyzed by gas chromatography. Seven phenolic compounds and thirty nine terpenoids were identified. Most abundant within each group were cinnamic acid and camphor, respectively. The chemicals were prepared as aqueous extracts and then used for germination, growth, and chlorophyll content tests. The extracts were inhibitory to germination and seedling growth of the receptor plants. This inhibitory effect was dependent on concentration. When the effect of the aqueous extract on chlorophyll content was assayed, both chlorophylls a and b were shown to be reduced. The reduction in seedling elongation and growth in dry weight paralleled the reduction in chlorophyll concentration. These chemical substances, including phenolic compounds and terpenoids, from *Artemisia scoparia* were responsible for the growth inhibition of the selected species.

Key words: *Artemisia scoparia*, Chlorophyll, Germination, Phenolics, Seedling growth, Terpenoids

INTRODUCTION

Among the different allelochemicals emitted from some plant species are water soluble, non-phytotoxic substances which are gradually degraded by soil microorganisms into harmful components (Balke *et al.* 1987). In these cases, it is difficult to pinpoint the interspecific actions of the allelochemicals because of their confounding abiotic and microorganismic factors (Hogan and Manners 1990). Sometimes plant growth is retarded

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by allelochemicals in the presence of mycorrhizal fungi (Becker and Bennett 1980). Allelochemicals are also known to hinder the biological activity of fungi (Kovacic *et al.* 1984).

There are many studies on the allelochemicals isolated from plants of genus *Artemisia* including: artemisin acid (Akhila *et al.* 1990), annulide, a sesquiterpene lactone (Brown 1993), artemisinin (Duke *et al.* 1987), two eudesmane acids (Marco *et al.* 1993), sesquiterpene lactones (Marco *et al.* 1993), sesquiterpene lactone dihydrolencodin (Pestchanker *et al.* 1990), cadinane derivatives (Sanz *et al.* 1991), sesquiterpene lactone (Tan *et al.* 1991), and functionalized silphiperfolenes (Weyerstahl *et al.* 1991).

Artemisia scoparia (oriental wormwood) plants are distributed around sandy areas along the seacoast forming perennial plant communities. It has a strong fragrance and there is sparse undergrowth in the habitat of the plant.

In surveying the areas surrounding oriental wormwood, one finds pioneer species such as *Aster tripolium* whose flying seeds have been dispersed within the *A. scoparia*, but few seeds are able to germinate or grow. Therefore we have hypothesized that phytotoxic chemicals are emitted from oriental wormwood plants that may be assayed to have a role in allelopathic activity. This study is focused on identifing the phytotoxic substances from *A. scoparia* and ascertaining their levels of involvement in the growth inhibition of selected receptor species.

MATERIALS AND METHODS

Experimental plants

For this study on the phytotoxic potential of the donor plant, *Artemisia scoparia* Waldst. et Kitamura, six receptor plants were selected: *Atriplex gmelini*, *Limonium tetragonum*, *Artemisia scoparia*, *Achyranthes japonica*, *Plantago asiatica* and *Artemisia princeps* var. *orientalis*.

Identification of phytotoxic substances

Isolation of water soluble substances from *A. scoparia* aqueous extracts and the procedures for detecting phenolic compounds were performed by the method of Kil and Yim (1983).

A gas chromatograph (Hewlett Packard 5890) using a J & W fused silica capillary column and a methylsilicone bonded column (B-1; 60 m × 0.25 mm i.d.) was used. The temperature program ranged from 100~300℃ with a 4℃ /min increase. The head pressure of the column was 30 psi and the split ratio was 1:50. The integrator was a Hewlett Packard 3392 A and sample injection volume was 0.5~1.0 μl.

Karlsruker's apparatus was used to isolate the volatile substances from the plants. The volatile substances were analyzed by a GC-MS (Hewlett Packard 5890) using a SE-54 column (50 m × 0.33 μ × 0.2 mm i.d.). Temperature was programmed from 45℃ (5 min) to 300℃ (3 min) at 4℃ /min. Carrier gas was helium, flow rate was 0.5 ml /min, with FID detector. Injector temperature was 250℃. Split ratio was 1:10 and head pressure was

34psi. Injection volume for all samples was 0.20 ㎕. Identification of each peak was achieved by comparing retention time and the mass spectrograph and operation conditions with those of commercial compounds.

Preparation of *Artemisia scoparia* extract

The plants of *Artemisia scoparia* were collected at the study area from December, 1991 to August, 1992. The seeds for the germination test were collected from the study area and the Iksan city vicinity. Fresh *A. scoparia* was dried in the shade for 7 days at 18℃.

The aqueous extract was prepared by soaking 100 g of dried material in 1 liter of distilled water for 24 hrs at 21℃. The extracts were then filtered through a sieve (0.105 mm, in diameter) and the filtrate stored at 4℃ until used. Filtrates were diluted to 10, 30, 50, and 70% extracts by mixing with distilled water. Filtrate was defined as 100 % extract. The pH of each extract was adjusted to 7.0±0.4 with a pH meter (Corning, ion analyzer 255).

Germination and growth test

A filter paper was placed in Petri dishes (12 cm in diameter), and fifty seeds of the six receptor species were spread evenly on the filter paper. The filter paper was moistened with 5 ml of extract. Control were treated identically except the Petri dishes were moistened with distilled water. The Petri dishes were kept at 20℃ at day and 15℃ at night to mimic natural growing conditions. At the end of the 10 day period, the germination percentage was determined, and the shoot and radicle lengths, and dry weights were measured.

For the growth test, seeds of the receptor plants were sown in plastic pots (i.d. 10 cm, h. 11 cm) containing vermiculite. The extracts (40 ml), diluted to various concentrations, were added to each pot every 2 to 3 days. After 16 days, the plants were thinned to five uniform seedlings per pot. The plants were grown for 30~40 days. Growth was determined by measuring the total length of the seedlings and the dry weight.

The relative ratios of germination, elongation and dry weight to the control were calculated according to Rho and Kil (1986).

Chlorophyll content analysis

Two species, *Achyranthes japonica* and *Plantago asiatica*, were selected. After being grown with the aqueous extracts of *Artemisia scoparia* for 4 to 5 weeks, the receptor plants were measured for elongation of the shoots and radicles. The veins of both unifoliate leaves of the test plants were excised. To eliminate the main vein and lateral vein, these tissues were washed with cold distilled water (4℃).

The content of chlorophyll in the extract was estimated by the method of Hiscox and Istraelstam (1979) using DMSO. The method was as follows; one hundred millgrams of unground leaf tissue were placed in a vial containing 7 ml of DMSO (Junsei Chemical Co.,

Ltd, Tokyo) and chlorophyll and incubated at 65℃ for 30 minutes. The liquid extract was transferred to a graduated tube and made up to a total volume of 10 ml with DMSO, then either assayed immediately or transferred to vials and stored at 0~4℃ until required for analysis. Before analysis the mixture was shaken by vortex.

A 3.0 ml sample of chlorophyll extract was transferred to a cuvette, and the OD (optical density) values at 645 and 663 nm were read in a spectrophotometer (UV-120-02, Shimadzu) against a DMSO blank. The OD values were calculated according to the equation of Arnon (1949) for the determination of chlorophyll content.

RESULTS

Identification of phytotoxic substances

Phytotoxic chemicals contained in the shoots of *A. scoparia* were identified by gas chromatography. They were identified by comparing the retention time to the standards. Seven compounds were identified as follows; cinnamic, ferulic, ρ-hydroxybenzoic, vanillic, gentisic, caffeic, and protocatechuic acids. Cinnamic acid was a major chemical in the aqueous extract of this species.

Fig. 1 shows the chromatograms of essential oil extracted from the shoots of the *A. scoparia* plant. The identification of each component was made by comparing retention times and mass spectra of standards.

Thirty-nine compounds were identified in the shoot essential oil as follows; α-thujene, α-pinene, campene, n-hexenal, β-pinene, sabinene, mycene, α-phellandrene, 1,8-cineol, γ-terpinene, ρ-cymene, terpinolene, cis-3-hexen-1-ol, α-thujone, β-thujone, 1-octen-3-ol-furfural, α-ylangene, α-copanene, camphor, α-bourbonene, α-berganotene, linalool, β-ylangene(+), β-caryophyllene, bornylacetate, terpinen-4-ol, γ-elemene, γ-gurjuneme, acetophenone, α-humulene, nerylacetate, borneol, α-mulorene, γ-bisabolene, geranylacetate, β-sesquiphellandrene, benzyl alcohol, calamenene, elemicine. The major constituent-cineol, camphor, β-ylangene(+), β-caryophyllene, +bornylacetate, terpinen-4-ol, γ-lemene, α-humulene, borneol, geranyl acetate and elemicine.

Growth inhibition

The pH value of the variously diluted extracts from *A. scoparia* varied between pH 6.6 and pH 7.4.

Seedling elongation was inhibited by the aqueous extract, but root elongation was inhibited more than shoot elongation (Fig. 2). In the case of *A. princeps* var. *orientalis* and *A. scoparia*, shoot length decreased gradually according to the extract concentration gradient, whereas radicle elongation was inhibited significantly even at the lowest concentration. The RER (relative elongation ratio) of the radicle was about 40% with the 10% extract in these two species. The inhibition percentages for radicle elongation of *A. princeps* var. *orientalis* and *A. scoparia* were 75% of the control with this extract and above

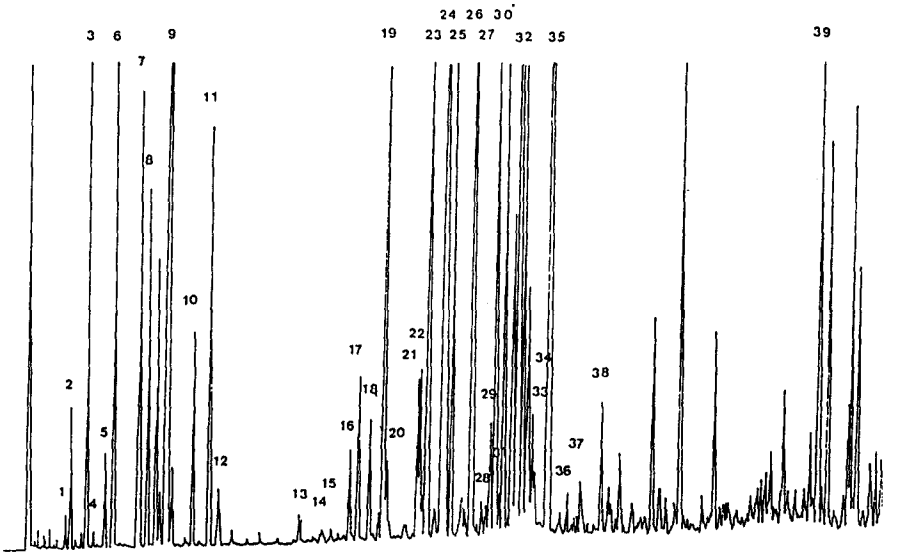

Fig. 1. Gas chromatogram of the essential oil from *Artemisia scoparia* shoot. Key to chemicals is: 1, α-thujene; 2, α-pinene; 3, campene; 4, n-hexenal; 5, β-pinene; 6, sabinene; 7, myrcene; 8, α-phellandrene; 9, 1,8-cineol; 10, γ-terpinene; 11, ρ-cymene; 12, terpinolene; 13, cis-3-hexen-1-ol; 14, α-thujone; 15, β-thujone; 16, 1-octen-3-ol + furfural; 17, α-ylangene; 18, α-copanene; 19, camphor; 20, α-bourbonene; 21, α-berganotene; 22, linalool; 23, β-ylangene(+); 24, β-caryophyllene; 25, bornylacetate; 26, terpinen-4-ol; 27, γ-elemene; 28, γ-gurjunene; 29, acetophenone; 30, α-humulene; 31, nerylacetate; 32, borneol; 33, α-mulorene; 34, γ-bisabolene; 35, geranylacetate; 36, β-sesquiphellandrene; 37, benzyl alcohol; 38, calamenene; 39, elemicine.

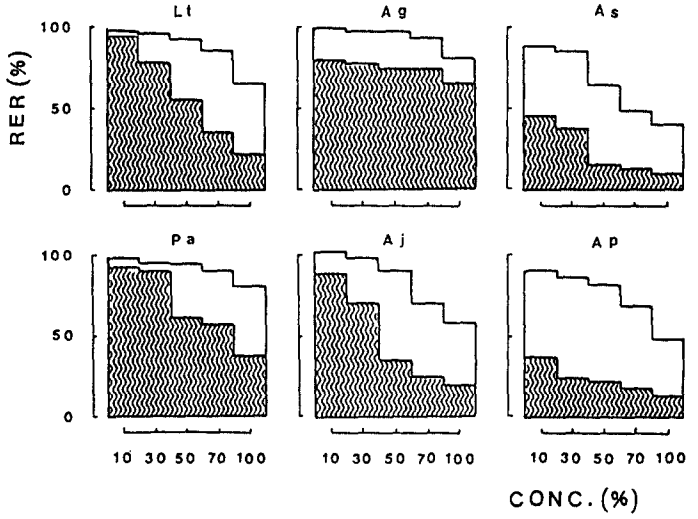

Fig. 2. Comparison between shoot and radicle elongation in germination tests with different concentrations of the aqueous extract from *Artemisia scoparia*. Key to species and symbols is as follows: Lt, *Limonium tetragonum*; Ag, *Atriplex gmelini*; As, *Artemisia scoparia*; Pa, *Plantago asiatica*; Aj, *Achyranthes japonica*; Ap, *Artemisia princeps* var. *orientalis*. Open (□) and filled (▨) areas represent the relative elongation ratio (RER) of shoots and that of radicles, respectively.

60% with the 30% extract. Elongation of *Limonium tetragonum* was slightly influenced by low concentrations, but the elongation of radicles and shoots was gradually inhibited with rising concentration. In the case of *Achyranthes japonica* the inhibitory effect on shoot and radicle elongation increased gradually along with the extract gradient. Shoot elongation of *P. asiatica* was inhibited little, with its radicle more so. *L. tetragonum, A. japonica* and *P. asiatica* were little influenced with the lower concentrations, and only slightly inhibited in shoot elongation, whereas radicle elongation was inhibited. *Atriplex gmelini* showed a high RGR independent of the extract concentration, shoot elongation was not affected at all, and the inhibition percentage of the radicle was below 50% with the 100% extract.

The relative dry weight ratio (RDR) of seedlings grown with the extrat is shown in Fig. 3. The dry weight of *A. scoparia* was reduced to 51.6% of the control with the 10% extract and to 3.2% with the 100% extract. *A. japonica* showed a reduction to 84% with 10% extract and 21% with 100% extract. *A. princeps* var. *orientalis* was 86.7% with 10% extract and 11% with 100% extract. This tendency of reducing dry weight was also found in *P. asiatica* and *L. tetragonum*.

Chlorophyll content

The contents of chlorophyll a and chlorophyll b of the seedlings of receptor plants, *A. japonica* and *P. asiatica*, was reduced in indirect proportion to the extract concentration (Table 1). These inhibitory effects were statistically significant at the 5% level. These

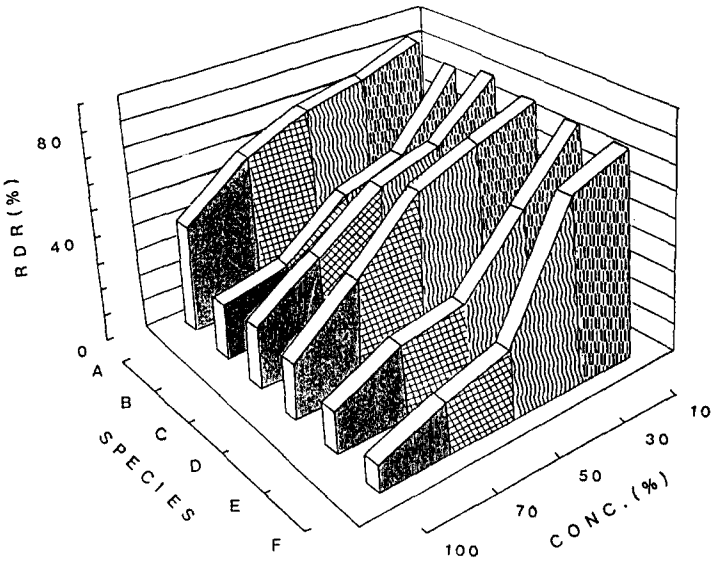

Fig. 3. The relative dry weight ratio (RDR) of receptor plants grown in pots for 28~35 days in various concentrations of the *Artemisia scoparia* extract. Key to species is as follows: A, *Artriplex gmelini*; B, *Achyranthes japonica*; C, *Limonium tetragonum*; D, *Artemisia princeps* var. *orientalis*; E, *Plantago asiatica*; F, *Artemisia scoparia*.

Table 1. Effect of various concentrations of aqueous extract on the chlorophyll contents of receptor plants

Extract concentration (%)	Chlorophyll content*					
	Achyranthes japonica			*Plantago asiatica*		
	Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.
Control(0%)	0.71 ± 0.04^{a}	1.45 ± 0.06^{a}	2.16 ± 0.10^{a}	0.50 ± 0.00^{a}	0.95 ± 0.01^{a}	1.44 ± 0.01^{a}
10%	0.57 ± 0.04^{b}	0.22 ± 0.07^{b}	1.79 ± 0.11^{b}	0.38 ± 0.03^{b}	0.77 ± 0.01^{b}	1.15 ± 0.02^{b}
30%	0.56 ± 0.04^{b}	1.19 ± 0.07^{b}	1.75 ± 0.11^{b}	0.34 ± 0.03^{c}	0.74 ± 0.01^{c}	1.09 ± 0.03^{c}
50%	0.45 ± 0.06^{c}	1.01 ± 0.14^{c}	1.46 ± 0.15^{c}	0.30 ± 0.01^{d}	0.59 ± 0.01^{d}	0.89 ± 0.01^{d}
70%	0.44 ± 0.06^{cd}	0.91 ± 0.09^{fc}	1.35 ± 0.12^{c}	0.26 ± 0.01^{e}	0.50 ± 0.01^{e}	0.75 ± 0.02^{e}
100%	0.37 ± 0.04^{d}	0.75 ± 0.08^{d}	1.12 ± 0.12^{d}	0.17 ± 0.01^{f}	0.33 ± 0.01^{f}	0.50 ± 0.01^{f}

* : Unit of chlorophyll content was mg /g fresh weight.

$^{a\sim f}$: Means followed by the same letter are not significantly different according to Duncan's multiple-range test ($p>0.05$)

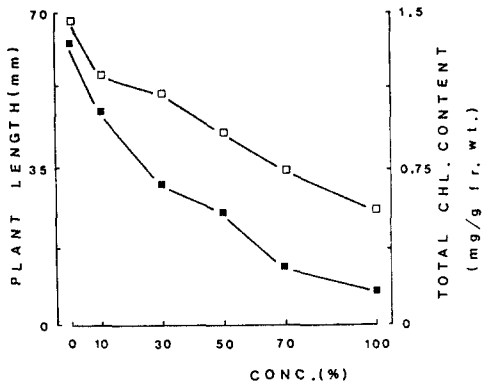

Fig. 4. Inhibitory effect of the aqueous extract as a comparison between chlorophyll content and elongation of the receptor plant, *Plantago asiatica*. Closed symbols(■-■) show plant length and open symbols(□-□), total chlorophyll concentration.

two receptor species showed a similar reduction in chlorophylls a and b.

The inhibitory effect as a relationship between chlorophyll content and seedling elongation was also studied (Fig. 4). Chlorophyll content was found to be reduced in proportion to the decrease of seedling elongation.

DISCUSSION

Numerous plants secrete chemical substances which inhibit seed germination and seedling elongation of competitive plants (Muller 1969, Rice 1984) and their own (autointoxication). In this study, we examined the phytotoxic potential of *Artemisia scoparia*. The identification by GC of cinnamic acid and camphor from *A. scoparia* plant supports the inhibition effect of *A. scoparia*. These chemical substances are verified as phytotoxic substances (Brown 1993, Rho and Kil 1986).

The phytotoxic aqueous extract from *A. scopaia* was tested on six receptor plants. Germination and seedling elongation were severely inhibited by the extract. The degree of inhibition increased in proportion to the extract concentration.

Mersie and Singh (1987) indicated that water soluble extracts of *Parthenium* had a toxic effect on the root growth of test species. At that time, the degree of toxicity depended on the extract concentration. Pardates and Dingal (1988), Hazebroek *et al.* (1989) and Heisey

(1990) also suggested that inhibitory activity strongly correlated to extract concentration.

There was a species specific toxic effect apparent in the RER of the receptor plants tested against the whole plant extract of the *A. scoparia* plant. This finding in Fig. 4 corresponds to the above-mentioned study.

The tested species may be classified into three types according to the sensitivity of the receptor plants to *A. scoparia* toxicity in this study. The first group, the susceptible species, showed low germination and seedling growth. The second group, the tolerant species, showed comparatively high germination and seedling growth. And the third group, the intermediate species, as the name implies, showed germination and seedling growth at levels between the first two.

The susceptible species, *Artemisia princeps* var. *orientalis, Artemisia scoparia, Limonium tetragonum, Plantago asiatica* and *Achyranthes japonica* grow well outside of *A. scoparia* stands. But they are so sensitive to the substances released from the *A. scoparia* plants that they cannot grow in the *A. scoparia* stand.

The quantitative analysis of chlorophyll concentration derived from plants with the aqueous extract of *A. scoparia* showed that the chlorophyll content of *A. japonica* and *P. asiatica* gradually decreased as extract concentration increased. According to Kumari and Kohli (1987), ragweed parthenium (*Parthenium hysterophorus*) showed autotoxicity, and leachates derived from the plant decreased cell life and chlorophyll content.

Einhellig and Rasmussen (1979) indicated that their experiments were designed to test the hypothesis that interference with chlorophyll metabolism may be one mechanism of inhibiting plant growth in allelopathic interactions. Sorghum seedling growth was reduced by each of the chemical compounds they used at the 50×10^{-4}M level, but leaf chlorophyll concentration was not less than that of the control plants. Also, according to Duke *et al.* (1987), artemisinin, a sesquiterpenoid lactone peroxide constituent of annual wormwood (*Artemisia annua*), inhibited lettuce seed germination and root and shoot growth, but chlorophyll content was not affected and chlorosis was not observed in any species tested. However, Alsaadawi *et al.* (1986) showed that chlorophyll a, total chlorophyll, and the ratio of chlorophyll a to chlorophyll b decreased with aqueous extract, but that chlorophyll b did not decrease. The result of our study showed that chlorophyll a and chlorophyll b decreased, being somewhat different from the above experiment.

In summary, numerous water soluble and volatile chemicals contained in *Artemisia scoparia* extracts were identified and may contribute to the growth inhibition of selected species.

적 요

비쑥에 함유되어 있는 화학물질을 가스크로마토그래피로 분석하여 7종류의 phenolic 화합물과 39종의 terpenoid를 확인해 냈다. 이들 중 cinnamic acid 와 camphor가 주요성분으로 나타났다. 비쑥의 수용추출액을 준비하여 종자발아, 유식물생장 그리고 엽록소함량을 실험해 보았더니

추출액의 농도가 높을수록 억제작용이 컸으나 그렇지 않은 경우도 있었다. 추출액이 엽록소 함량에 미치는 영향을 조사한 결과 엽록소 a와 b 양쪽 모두 억제되는 효과가 있었다. 또한 유식물의 신장과 건중량 생장도 엽록소의 경우와 비슷했다. 그래서 비쑥에 들어있는 phenolic 화합물과 terpenoid성분은 실험에 사용된 식물의 생장을 억제하는 작용이 있슴을 밝혀냈다.

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