Korean J. Ecol., 23(2): 149~155, 2000

Allelopathic Effects of *Artemisia lavandulaefolia*

Kil, B. S., D. M. Han, C. H. Lee, Y. S. Kim*, K. Y. Yun** and H. G. Yoo

Division of Chemistry and Life Science, Wonkwang University, Iksan 570-749, Korea
*Iksan Health College, Iksan 570-749, Korea**
*Dept. of Medicine Resources, Sunchon National University, Sunchon 540-742, Korea***

ABSTRACT: The allelopathic effects of *Artemisia lavandulaefolia* were studied using several test plants and microbes. Aqueous extracts and volatile compounds of *A. lavandulaefolia* inhibited seed germination, seedling and root growth of the test species such as *Achyranthes japonica*, *Lactuca sativa*, *Artemisia princeps* var. *orientalis*, *Oenothera odorata*, *Plantago asiatica*, *Aster yomena*, *Elsholtzia ciliata*, and *Raphanus sativus* var. *hortensis* for. *acanthiformis*. The root growth of test species was more affected than shoot growth by allelochemicals of *A. lavandulaefolia*. Essential oil of *A. lavandulaefolia* had antibacterial and antifungal effects. However, the antimicrobial activity of the essential oil was dependent upon the microbial species and concentrations. Callus growth of *Oryza sativa*, *Brassica campestris* subsp. *napus* var. *pekinensis* and *Achyranthes japonica* was sensitive by the essential oil of *A. lavandulaefolia*. Twenty three chemicals were identified from *A. lavandulaefolia* essential oil by gas chromatography. Primary allelochemicals among them were 1, 8-cineole, 1-α-terpineol, α-terpinene, camphor, 2-buten-1-ol and azulene. We concluded that aqueous extract and essential oil of *A. lavandulaefolia* were responsible for allelopathic effects.

Key Words: Allelopathy, *Artemisia lavandulaefolia*, Aqueous extract, Essential oil, Antimicrobial activity.

INTRODUCTION

Allelopathy is an important competitive strategy of plants (Fischer *et al.* 1994, Langenheim 1994). Allelopathic compounds are released to inhibit the germination and/or growth, or increase the mortality of another species. Plant secondary compounds have great potential in the development of environmentally safe herbicides with novel molecular sites of action (Schulz and Friebe 1999). Many studies have been conducted on the chemical composition of the secretory products of *Artemisia* (Kelsey *et al.* 1984, Chen and Leather 1990).

Most secondary metabolites appear to have multiple functions (Vokou 1992). They may act on as antiherbivory, antiparasitic, antifungal and antibacterial compounds: phytotoxins: attractants (kairomones and synomones): internal regulators of growth and development: seed germination stimulants: and have other roles. Primary callus cultures were established from stem cambial tissues of cork tree by culturing on LS liquid medium with some chemical compounds. Some of them enhanced callus growth but others resulted in a significant depletion of cell growth (Park and Choi 1999). Although there are some reports about the fungitoxic properties of the volatile constituents (essential oils) of some higher plants (Pandey *et al.* 1982), there has been given little attention of the genus *Artemisia*. The phytotoxicity was dependent upon the concentration of essential oil, species and environmental conditions (Langenheim 1994).

The objectives of the present study was 1) to investigate the alleopathic potential of *A. lavandulaefolia* by bioassays as germination and seedling growth test, callus growth, and antimicrobial activity, and 2) to analyze essential oil of *A. lavandulaefolia* and identify chemicals with allelopathic potential.

MATERIALS AND METHODS

Test plants

To study the allelopathic potential of *Artemisia lavandulaefolia* DC, eight test plant species were selected. They were *Lactuca sativa* L., *Achyranthes japonica* (Miq.) Nakai, *Artemisia princeps* var. *orientalis* (Pampan.) Hara, *Oenothera odorata* Jacq., *Plantago asiatica* L., *Aster yomena* Makino, *Elsholtzia ciliata* (Thunb.) Hylander and *Raphanus sativus* var. *hortensis* for. *acanthiformis* Makino.

Aqueous extract and essential oil

Aqueous extract of *A. lavandulaefolia* was made by the following procedures: One liter of distilled water was added to 2-L Erlenmeyer

flask containing 200 g of explants. It was kept for 24 hr in an incubator at 20℃. After that, it was filtered through a 0.5 mm sieve. Osmotic potentials of aqueous extracts below 143 mosm/kg had no influence (Elakovich and Wooten 1991): the extracts in this study were diluted to osmotic potentials no greater than 90 mosm. The essential oil of *A. lavandulaefolia* was made by distillation using a Karlsruker's apparatus (Stahl 1973): The crushed leaves of *A. lavandulaefolia* were placed in 2-L round flask. The water-distillates were saturated with sodium sulfate and then extracted with ether. The ether extracts were concentrated in rotary evaporator and were transferred into a deep freezer (−70 ℃) to minimize the escape of volatile compounds.

Seed germination and seedling growth experiments

The germination experiments by aqueous extract and essential oil of *A. lavandulaefolia* were carried out in Petri dishes (diam. 12 cm). Germination test dishes contained two layers of filter paper wetted with the extract or essential oil. Thirty seeds of test plants were scattered in each dish. The control was treated with distilled water instead of aqueous extract or essential oil. They were incubated at 20℃/15℃ (day/night). The experiment continued for at least more than 10 days to allow maximum seed germination. The germination ratios were calculated by counting the number of germinated seeds. And on final day, the length of seedlings of test plants were measured and weighed. The nominal essential oil concentrations were 5, 10, 15, and 20 μl/260 ml (oil-distilled water, v/v). Petri dishes were rapidly sealed with polyvinyl wrap and parafilm within 30sec to limit volatilization. RER (relative elongation ratio) was calculated as follows: (mean length of the treatment/mean length of the control) × 100.

Antimicrobial activity assay

Microbes such as *Candida albicans* KCTC 1940 (SB media), *Pseudomonas aeruginosa* KCTC 1636 (NB), *Salmonella typhimurium* KCTC 1925 (BH), *Zymomonas mobilis* KCTC 1534 (YEP), *Pseudomonas putida* KCTC 8729 (NB), *Streptococcus aureus* ATCC 29213 (BH), *Staphylococcus epidermidis* ATCC 12228 (BH), *Aspergillus nidulans* (FGSC4) (CM), *Alternaria mali* (PD), and *Fusarium oxysporum* (CM) were used for antimicrobial activity assay. Bacterial colony of *C. albicans* KCTC1940, *P. aeruginosa* KCTC 1636, *S. typhimurium* KCTC 1925, *Z. mobilis* KCTC 1534, *P. putida* KCTC 8729, *S. aureus* ATCC 29213, and *S. epidermidis* ATCC 12228 were inoculated in microbial liquid media and incubated for 16 hr at 37℃ in shaking incubator (1000rpm). Dilutions for test solutions were prepared as for approximately 5×10^3 cell/ml in the cell suspension. Each essential oil (0~1000 ppm) of *A. lavandulaefolia* was added to in 25 ml of bacterial cell suspensions. The bacterial growth was examined as a function of turbidity (OD at 600 nm) using spectrophotometer (Hitachi U-1100, Japan). Fungal colonies of *A. nidulans* (FGSC4), *A. mali*, and *F. oxysporum* were inoculated in the center of agar plates. Each essential oil of different concentrations (0~1000 ppm) was added to in the center of plates and incubated at 24℃, 37℃ and 30℃. The radial extension was measured for the fungal growth. Colony diameters were measured on 5th days (Costilow 1981). IC_{50} value was calculated as the concentration of 50% inhibition to control by comparison.

Callus induction and growth

Each seed coat was removed from the seeds of *Oryza sativa* cv. *Dongjinbyeo*, *Brassica campestris* subsp. *napus* var. *pekinensis*, *Achyranthes japonica* and *Sesamum indicum*. Seeds were rinsed with 75% ethyl alcohol for 30 sec and then soaked in 5% sodium hypochlorite solution for 15 min. They were rinsed with sterile bidistilled water. The sterilized seeds were germinated on the surface of Murashige and Skoog's (MS) medium (Murashige and Skoog 1962) in test tubes (2 × 15 cm). Leaf explants (5 mm^2) of *A. japonica* were cultured in four-compartment Petri dishes containing MS 121 medium (Hogan and Mannners 1990) supplemented with different concentrations of the *A. lavandulaefolia* essential oil. Three explants were put on each compartment. The Petri dishes were sealed with parafilm (Whatman Co.) and incubated for 30 days at 25℃ in the dark to initiate callus induction. The Callus induction was estimated and graded according to callus volume: good, bad, and none (Krikorian and Katz 1968). Calli obtained from *O. sativa*, *B. campestris* subsp. *napus* var. *pekinensis* and *A. japonica* were placed in four-compartment Petri dishes containing 20 ml of MS 121 medium treated with 2,4-D 2 mg/L. Circular filter paper with *A. lavandulaefolia* essential oil (0, 10, 20 μl per Petri dish) were put in the central wall of the solid medium. The Petri dishes were sealed with parafilm and incubated at 25℃ in the dark. After 30 days, calli were dried in dry oven at 60℃ for 48 hr. Ten dried calli per treatment were weighed: (Dry weight of callus of the treatment/ Dry weight of callus of the control) × 100.

The results of the bioassays were based on

three or four replicates. Comparisons among treatments were made at the 5% or 1% level using Scheffe's method in one-way ANOVA.

Identification of essential oil

The essential oil of *A. lavandulaefolia* was analyzed by gas chromatography (Hewlett-Packard 5890) using a SE-54 column (50 m × 0.33 μl × 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 0.5 ml/min, with FID. Injector temperature was 250℃. Split ratio was 1:10 and head pressure was 34 psi. Injection volume for all samples was 0.20 μl. Identification of peaks was made by the comparison of retention times and mass spectra of the peaks with those of commercial compounds obtained from Sigma, Aldrich and Fluka Co.

Comparative bioassay with commercial chemicals

Seven commercial standard isoprenoid chemicals such as 1, 8-cineole, α-terpinene, β-pinene, myrcene, t-caryophyllene and camphor were used in seed germination and seedling growth experiments of two test plants, *A. japonica* and *L. sativa*. Procedures were the same as in the germination and growth tests by essential oil.

RESULTS

Germination and seedling growth test by aqueous extract

The allelopathic effects of *A. lavandulaefolia* aqueous extract on the germiantion of test plants such as *L. sativa, O. odorata, R. sativus* var. *hortensis* for. *acanthiformis* and *P. asiatica*. are shown in Fig. 1 and 2. The germination ratio of *L. sativa* was 75% in 10% concentration of aqueous extract, and sharply reduced in 50% extract. When *L. sativa* seeds were exposed to 100% (undiluted) extract, the germination ratio was 1.1% (Fig. 1).

Although the radicle elongations of *O. odorata* and *R. sativus* var. *hortensis* for. *acanthiformis* were stimulated by 10% aqueous extract, inhibitory effects on radicle elongation were proportoional to the concentration of agueous extract. It was shown that radicle growth of receptor species was actively inhibited at low concentration (Fig. 2).

Germination and seedling growth test by essential oil

Essential oil of *A. lavandulaefolia* inhibited seed germination of receptor plants. Seed germination of all species except *O. odorata, A. yomena* and *E. ciliata* was significantly reduced at 15 μl/260 ml ($P < 0.05$) (Table 1).

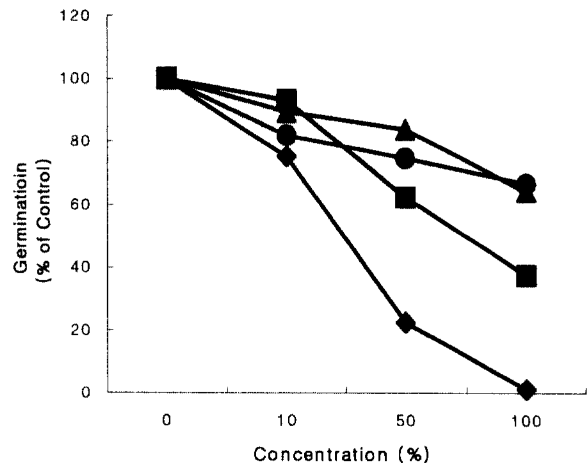

Fig. 1. Effects of *Artemisia lavandulaefolia* aqueous extracts on seed germination of receptor plants. Key to species: ◆---◆, *Lactuca sativa*; ■---■, *Oenothera odorata*; ▲---▲, *Plantago asiatica*; ●---●, *Raphanus sativus* var. *hortensis* for. *acanthiformis*.

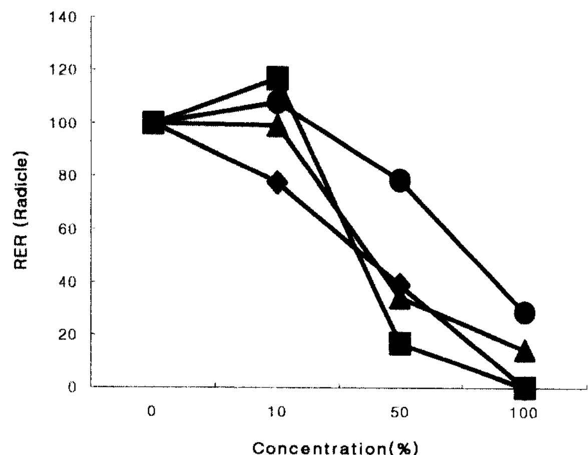

Fig. 2. Relative radicle elongation ratio of receptor plants grown at different concentrations of *A. lavandulaefolia* aqueous extract. Key to species: ◆---◆, *Lactuca sativa*; ■---■, *Oenothera odorata*; ▲---▲, *Plantago asiatica*; ●---●, *Raphanus sativus* var. *hortensis* for. *acanthiformis*.

The radicle growth in all of receptor species was more actively inhibited at low concentration. Especially, the radicle growth of *A. japonica* and *R. sativus* var. *hortensis* for. *acanthiformis* were significantly decreased in 63% and 54%, respectively, at 5 μl/260 ml (Table 2).

Antimicrobial activity

Fig. 3 shows that the essential oil extracted from *A. lavandulaefolia* inhibited the growth of some bacteria species tested. The growth of

Table 1. Germination percentage of receptor species by concentration of *A. lavandulaefolia* essential oil

Species	Control	Concentration (μl/260 ml)			
		5	10	15	20
Achyranthes japonica	85.8	75.8	74.2	55.8**	50.8**
Artemisia princeps var. *orientalis*	95.8	90.0	86.7	81.7*	75.0**
Aster yomena	78.3	74.2	67.5	60.0	56.7
Elsholtzia ciliata	91.7	90.8	83.3	60.8	60.0
Oenothera odorata	85.8	80.8	73.3	69.2	60.8
Plantago asiatica	89.2	74.2	66.7	56.7**	45.8**
Raphanus sativus var. *hortensis* for. *acanthiformis*	85.8	78.3	78.3	61.7**	55.8**

* Means are significantly different according to Scheffe's methods in one way ANOVA (*: P<0.05; **: P<0.01).

Table 2. Radicle elongation (mm) of receptor species by concentration of *A. lavandulaefolia* essential oil

Species	Control	Concentration (μl/260 ml)			
		5	10	15	20
Achyranthes japonica	4.099±0.341	2.621±0.386**	2.097±0.582**	1.879±0.262**	1.749±0.376**
Artemisia princeps var. *orientalis*	1.252±0.188	0.833±0.221	0.735±0.094	0.656±0.227*	0.492±0.109*
Aster yomena	2.189±0.182	1.651±0.383	1.237±0.707*	0.508±0.169**	0.410±0.089**
Elsholtzia ciliata	3.031±0.399	1.971±0.431*	1.652±0.395**	1.327±0.277**	1.078±0.423**
Oenothera odorata	1.662±0.132	1.246±0.146*	1.181±0.163*	1.027±0.133**	0.827±0.226**
Plantago asiatica	1.425±0.084	1.208±0.168	1.114±0.263	0.738±0.032**	0.551±0.039**
Raphanus sativus var. *hortensis* for. *acanthiformis*	3.726±0.637	2.026±0.304**	2.022±0.342**	1.506±0.422**	1.479±0.743**

* Means are significantly different according to Scheffe's methods in one way ANOVA (*: P<0.05; **: P<0.01).

some bacteria was severely inhibited with increasing concenctrations of essential oil. It was especially active aganist *P. putida* and *S. aureus* which 50% inhibition concentrations (IC_{50}) were below 200 ppm. IC_{50} was below 400 and 600 ppm in *S. epidermidis* and *P. aeruginosa*, respectively. It was shown that IC_{50} of *S. typhymurium* and *Z. mobilis* were below 800 ppm.

As shown in Fig. 4, *C. albicans* was severely inhibited below 200 ppm in IC_{50} concentration.

Changes of callus growth

The biomass of callus was reduced when the MS 121 medium with 1 mg/L of 2,4-D, 2 mg/L of

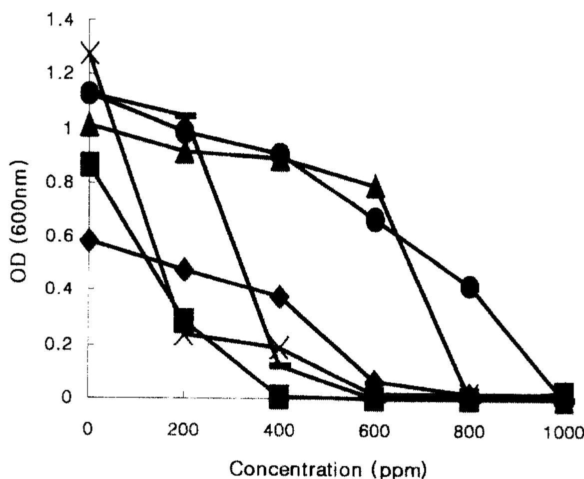

Fig. 3. Growth changes of bacteria cultured at different concentrations of *Artemisia lavandulaefolia* essential oil. Key to species. ◆---◆, *Pseudomonas aeruginosa*; ■---■, *Pseudomonas putida*; ▲---▲, *Salmonella typhymurium*; ×---×, *Staphylococcus aureus*; ▬ ----- ▬, *Staphylococcus epidermidis*; ●---●, *Zymomonas mobilis*.

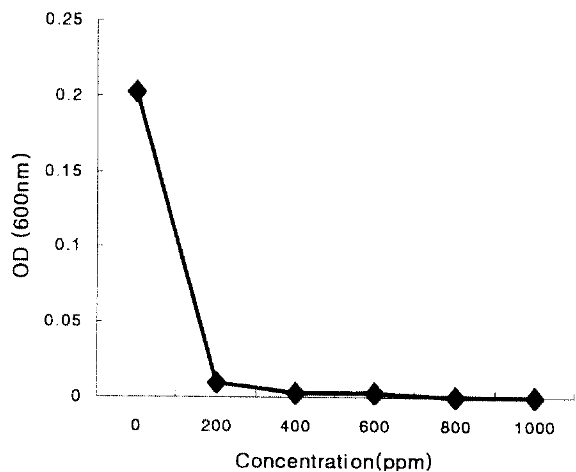

Fig. 4. Growth changes of *Candida albicans* cultured at different concentrations of *Artemisia lavandulaefolia* essential oil.

Table 3. Fresh and dry weight of test calli by concentration of A. lavandulaefolia essential oil at 30 days after inoculation[a]

Receptor plants	Fresh Weight (g)(% of Control) Essential oil (μl/80 ml)			Dry Weight (g)(% of Control) Essential oil (μl/80 ml)		
	0	10	20	0	10	20
Achyranthes japonica	100	34.1**	22.6**	100	25.2**	14.2**
Brassica campestris subsp. *napus* var. *pekinensis*	100	41.2**	22.2**	100	29.3**	14.8**
Oryza sativa	100	54.8**	23.2**	100	37.3**	21.4**

[a] MS medium supplemented with 2 mg/L 2, 4-D.
* Means are significantly different according to Scheffe's methods in one way ANOVA (*: $P<0.05$; **: $P<0.01$).

NAA and 1 mg of kinetin was supplemented with *A. lavandulaefolia* essential oil (Table 3). While *O. sativa* had the highest fresh and dry biomass, the biomass of *A. japonica* was reduced the most of the test plants In general, fresh and dry weight of calli or size of receptor plants were inversely proportional to the concentration of the essential oil.

Identification of allelochemicals

Phytotoxic chemicals contained in the *A. lavandulaefolia* were identified by gas chromatography. The identification of chemicals was carried out by comparing its retention time with that of authentic reference compounds and it was further confirmed by the peak enrichment technique, in which the retention time of chemicals from plant essential oil was identical to that of standard. Twenty-three compounds were identified in the essential oils as follows: 1, 8-cineole, 1-α-terpineol, α-terpinene, camphor, 2-buten-1-ol, azulene, 2-penten, camphene, β-myrcene, 1-phellandrene, myrcenol, benzene, sabinene, γ-terpinene, 2-pyrrolidinone, δ-3-carene, 2-β-pinene, endo-borneol, 3-cyclohexen-1-ol, cis-piperitol, trans-caryophyllene, α-capaene, patchulane. The major constituents were 1, 8-cineole, 1-α-terpineol, α-terpinene, camphor, 2-buten-1-ol and azulene.

Bioassay of standard chemicals

Five standard compounds which occurred commonly in the *A. lavandulaefolia* essential oils were used for the bioassay studied. Seed germination (Fig. 5) and seedling growth (Fig. 6) responded variously to each of these compounds. In germination test, α-terpinene and β-pinene exhibited strong inhibitory effects, whereas trans-caryophyllene and myrcene slightly inhibited seed germination of *A. japonica*. Inhibitory effects of these compounds were concentration dependent. Those 6 compounds affected also the seedling growth of *A. japonica*. The growth of shoot of receptor species was more inhibited in

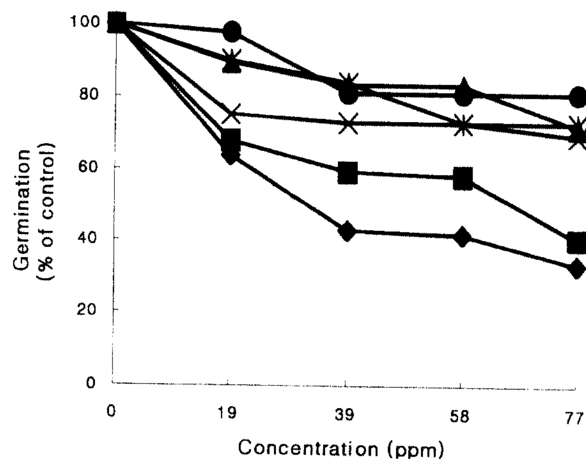

Fig. 5. The germination percentage of A. japonica tested at different concentrations of chemicals.
Keys to compounds are as follows: ◆—◆, α-Terpinene; ■—■, 2-β-Pinene; ▲—▲, Camphor; ×—×, 1,8-Cineole; ✳—✳, Myrcene; ●—●, t-Caryophyllene.

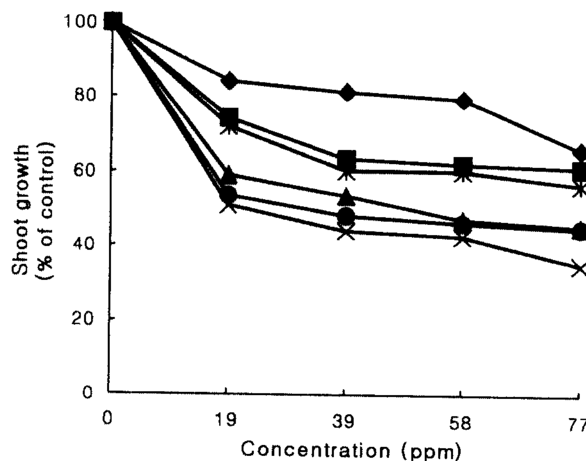

Fig. 6. The shoot growth of *A. japonica* tested at different concentrations of chemicals. Keys to compounds are the same as in Fig. 5.

1,8-cineole and t-caryophyllene than other compounds.

DISCUSSION

The aim of this study was to verify the allelopathic effects of *A. lavandulaefolia*. Aqueous extracts and essential oils from *A. lavandulaefolia* were provided using 9 receptor plant and 10 microbes. Germination, seedling growth and biomass of receptor plants were significantly inhibited by the extracts and essential oils.

In the genus *Artemisia*, over 260 species have been investigated to reveal that they contain many classes of secondary metabolites including terpenoids, flavonoids, coumarins, glycosides, steroids and polyacetylenes (Tan *et al.* 1998). A wide array of biologically active constituents are produced by plants in the genus *Artemisia* (Marco and Barbera 1990). For example, *A. scoparia* (Kil and Yoo 1996), *A. princeps* var. *orientalis* (Kil and Yun 1992), *A. annua* (Lydon *et al.* 1997), *A. princeps* (Ryu *et al.* 1997), *A. eriopoda* (Hu *et al.* 1998), *Artemisia* species (Tan *et al.* 1998), *A. capillaris* (Yamahara *et al.* 1989, Okuno *et al.* 1988), *A. argyi* (Yoshikawa *et al.* 1996), and *A. indica* (Chanphen *et al.* 1998) were investigated. The oils of *Artemisia* volatile constituents have been the subject of several investigations (Misra *et al.* 1991, Catalan *et al.* 1991).

During our investigation on seed germination and seedling growth of various plants (Figs. 1, 2, Tables 1, 2), we observed that radicles of the germinating seeds were significantly short at the high concentrations of aqueous extracts and essential oils. The aqueous leachates (1% w/v) of *Sicyos deppei* significantly inhibited (80%) radicle growth of *Cucurbita ficifolia* (Cruz-Ortega *et al.* 1998), too.

Essential oils are presently used for flavouring processed foods (Fenaroli 1995), antimicrobials substances (Lis-Balchin *et al.* 1996, 1997) as well as antioxidants (Paya 1993). The essential oils would, however, have to be used in considerable concentrations to achieve antibacterial potential, as preliminary studies have indicated that this would be between 500-100 ppm. Evidence that terpenes suppress cell elongation and cell division exists (Muller and Hauge 1967), but there is little support for one specific mode of terpene interference with normal plant metabolism.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Korea Research Foundation made in the Program Year 1998.

LETERATURE CITED

Catalan, C.A.N., M. Del Cuenca, J. Verghese, M.T. Joy, A.B. Guturrez and W. Herz. 1991. Sesquiterpene ketones related davanone from *Artemisia pallens*. Phytochemistry 30: 549-552.

Chanphen, R., Y. Thebtaranonth, S. Wanuppathamkul and Y. Yuthavong. 1998. Antimalarial principles from *Artemisia indica*. J. Nat. Proc. 61: 1146-1147.

Chen, P.K. and G.R. Leather. 1990. Plant growth regulatory activities of artemisinin and its related compounds. J. Chem. Ecol. 16: 1867-1876.

Costilow, R.N. 1981. Biophysical factors in growth. *In* P. Gerhardt (ed.), Mannual of methods for general bacteriology. American Society for Microbiology. Washington, DC. pp. 66-78.

Cruz-Oryega, R., A.L. Anaya, A.E. Hernandez-Bautista and G. Laguna-Hernandez. 1998. Effects of allelochemicals stress produced by *Sicyos deppei* on seedling root ultrastructure of *Phaseolus vulgaris* and *Cucurbita ficifolia*. J. Chem. Ecol. 24: 2039-2057.

Elakovich, S.D. and J.W. Wooten. 1991. Allelopathic potential of *Nuphar lutea* (L.) SIBTH. & SM. (Nymphaeaceae). J. Chem. Ecol. 17: 709-714.

Fenarolis. 1995. Handbook of flavour ingredients. 3rd ed. CRC Press. Vols. 1 and 2.

Fischer, N.H., G.B. Williamson, J.D. Weidenhamer and D.R. Richardson. 1994. In search of allelopathy in the Florida scrub: the role of terpenoids. J. Chem. Ecol. 20: 1355-1380.

Hogan, M.E. and G.D. Manners. 1990. Allelopathy of small everlasting (*Antennaria microphylla*) phytotoxicity to leaf spurge (*Euphorbia esula*) in tissue culture. J. Chem. Ecol. 16: 931-939.

Hu, J., Z. Jia and S. Bai. 1998. Two new polyacetylenes from *Artemisia eriopoda*. Planta Med. 64: 378-379.

Kelsey, R.G., G.W. Reynolds and E. Rodriguez. 1984. The chemistry of biologically active constituents secreted and stored in plant glandular trichomes. *In* E. Rodriguez, P.L. Healy and I. Metha (eds.) Biology and Chemistry of Plant Trichomes. Plenum Press. New York. pp. 187-214.

Kil, B.S. and H.G. Yoo. 1996. Identification and growth inhibition of phytotoxic substances from *Artemisia scoparia*. Kor. J. Ecol. 19: 295-304.

Kil, B.S. and K.W. Yun. 1992. Allelopathic effects of water extracts of *Artemisia princeps* var. *orientalis* on selected plant species. J. Chem. Ecol. 18: 39-51.

Krikorian, A.D. and G.M. Katz. 1968. The aseptic culture of onion roots and root tissue: A preliminary report. Phytomorphology 18: 207-211.

Langenheim, J.H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. J. Chem. Ecol. 20: 1223-1280.

Lis-Balchin, M., S.G. Deans and S. Hart. 1997. A study of the changes in the bioactivity of essential oils used singly and as mixtures in aromatherapy. J. Alt. Complement. Med. 3: 249-255.

Lis-Balchin, M., S. Hart, S.G. Deans and E. Eaglesham.

1996. Comparison of the pharmacological and antimicrobial action of commercial plant essential oils. J. Herbs Spices Med. Plants 4: 69-86.
Lydon, J., J.R. Teasdale and P.R. Chen. 1997. Allelopathic activity of annual wormwood (*Artemisia annua*) and the role of artemisinin. Weed Science 45: 807-811.
Marco, J.A. and O. Barbera. 1990. Natural products from the genus *Artemisia*. Stud. Nat. Prod. Chem. 7: 201-264.
Misra, L.N., A. Chandra and R.S. Thakur. 1991. Fragrant components of the oil from *Artemisia pallens*. Phytochemistry 30: 549-552.
Muller, W.H. and R. Hauge. 1967. Volatile growth inhibitors produced by *Salvia leucophylla*: effect on seedling anatomy. Bull. Torrey Bot. Club 94: 182-191.
Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
Okuno, I., K. Uchida, M. Nakamura and K. Sakurawi. 1988. Studies on choleretic constituents in *Artemisia capillaris* THUNB. Chem. Pharm. Bull. 36: 769-775.
Pandey, D.K, N.N. Tripathi and S.N. Dixit. 1982. Fungitoxic and phytotoxic properties of the essential oil of *Caesulia axillaris*. (Compositae). Angew. Bot. 56: 259-267.
Park, Y.G. and M.S. Choi. 1999. Allelopathic effects for cork tree (*Phellodendron amurense*) on microorganisms growth. *In* F.A. Macias, J.C.G. Galindo, J.M.G. Molinillo and H.G. Cutler (eds.) Recent Advances in Allelopathy. Vol. I. A Science for the Future. Servicio De Publicaciones Universiadad De Cadiz. Spain. pp. 81-88.
Paya, M., B. Halliwell and J. Hoult. 1993. Peoxyl radical scavenging by a series of coumarins. Free Rad. Res. Coumms. 17: 293.
Ryu, S.Y., J.O. Kim and S.U. Choi. 1997. Cytotoxic components of *Artemisia princeps*. Planta Medica 63: 384-385.
Schulz, M. and A. Friebe. 1999. Detoxication of allelochemicals in higher plants and enzymes involved. *In* F.A. Macias, J.C.G. Galindo, J.M.G. Molinillo and H.G. Cutler (eds.), Recent Advances in Allelopathy. Vol. I. A Science for the Future. Servicio De Publicaciones Universiadad De Cadiz. Spain. pp. 383-400.
Stahl, A.E. 1973. Thin-Layer Chromatography, 2nd ed. Allen and Unwin (eds.), Springer-Verlag. 208 p.
Tan, R.X., W.F. Zheng and H.Q. Tang. 1998. Biologically active substances from the genus *Artemisia*. Planta Medica 64: 295-302.
Vokou, D. 1992. The allelopathic potential of aromatics shrubs in phryiganic (east Mediterranean) ecosystems. *In* S.J.H. Rizvi and V. Rizvi (eds.). Allelopathy: Basic and applied aspects. Chapman and Hall, London. pp. 303-320.
Yamahara, J., G. Kobayashi, H. Matsuda, T. Katayama and H. Fujimura. 1989. The effect of scoparone, a coumarin derivative isolated from the Chinese crude drug *Artemisia capillaris* Flos, on the heart. Chem. Pharm. Bull. 37: 1297-1299.
Yoshikawa, M., H. Shimada, H. Matsuda, J. Yamahara and N. Murankami. 1996. Bioactive constituents of Chinese natural medicines. I. New sesquiterpene ketones with vasorelaxant effect from Chinese moxa, the processed leaves of *Artemisia argyi* LEVL. et VANT.: Moxartenene and Moxartenolide. Chem. Pharm. Bull. 44: 1656-1662.

(*Received January 4, 2000*)