Natural Product Sciences
3(2) : 141 ~ 147 (1997)

Antimicrobial Activities of Volatile Essential Oils from Korean Aromatic Plants

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Abstract – Volatile essential oils obtained by steam distillation from 55 plant parts of 42 species of representative aromatic plants newly collected in Korean peninsula have been evaluated for antimicrobial activity against 5 microorganisms. The essential oils derived from 15 plant parts and 9 plant parts were found to exhibit very strong antimicrobial activities by more than 95% inhibition at 100 μg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. Essential oil components such as *l*-limonene, β-myrcene, linalool, γ-terpinene, α,β-phellandrene, 1,8-cineole, *l*-borneol and bornylacetate, as a whole, have primarily contributed to the manifestation of the antimicrobial activity.

Key words – Antimicrobial activity, essential oils, Korean aromatic plants, *Staphlococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, Enterococcus faecali, E. coli.*

Introduction

Naturally occurring essential oils possessing characteristic sweet odor and flavor have been of wide applications as health foods, beverage flavorings in folk medicines and fragrances in cosmetic products in various industrial fields such as perfumary, condiments and medical supplies. Recently, several aromatic plants and their essential oil components have been studied for their biological activity including antimicrobial, antioxidant and antimutagenic properties, (Sharma *et al.*, 1980; Onawunmi *et al.*, 1984; Paster *et al.*, 1990; Gundidza *et al.*, 1994; Soliman *et al.*, 1994; Marotti *et al.*, 1994; Jedickova *et al.*, 1992; Jansen *et al.*, 1984; Yashphe *et al.*, 1979; Park *et al.*, 1991; Wi, 1989) etc.

In Korean peninsula, more than 250 species belonging to 164 genera and 69 families of aromatic plants are known to be distributed (Chung and Ahn, 1995) and the necessities in the developments of traditional perfumaries suitable for our savor are gradually rising.

In the present study, as an attempt to obtain fundamental data for the development of new herbal medicines for aroma therapy as well as new health foods, etc., volatile oil components obtained by steam distillation from 55 plant parts of 42 species of representative aromatic plants newly collected in Korean peninsula have been evaluated for antimicrobial activities against 5 different microorganisms. Common essential oils which exhibited more than 50% inhibiton against microorganisms were also compared with the indi-

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vidual components of the active essential oils.

Materials and Methods

Plant materials – Representative aromatic native plants in the southern parts of Korean peninsula, which are estimated to be suitable for the development of new fragrances and/or flavors, remedies for aroma therapy etc., were selected and their geographical distribution were surveyed. (Shin, 1996) Based on the results of this survey, various plant parts such as flowers, leaves, stems and roots were systematically collected during April-October, 1995-1996. The botanical identities of the specimen were confirmed and voucher specimen were deposited at the herbarium of Natural Products Research Institute, Seoul National University.

Isolation of essential oils – The flowers, leaves, twigs and roots collected were air-dried in the shade for two or three days and the crushed materials were subjected to steam distillation for 4-6 hr in a modified Karlsruker's apparatus (Egon, 1973) equipped with a mantle heater. The essential oils were collected in diethylether, dried over anhydrous sodium sulfate and stored in sealed cap vials at 5℃ in a refrigerator until used.

Antimicrobial activity test – The antimicrobial activity was evaluated by the gradient agar plate diffusion procedures described by Brock (Brock, 1994) and Holt. (Holt, 1994) The essential oils and their fractions were diluted with ethanol-water(1:4) to a concentration of 1 μg/ml which was sterilized filtering through millipore membrane (0.43 μm), admixed with 20 μl of bacterial strains and were spread on petri plates containing standard nutrient agar. The plates were incubated at 36±0.5℃ for 48 hr. The control group consisted of 20 μl of bacterial strains in ethanol-water (1:4) alone. The growth inhibition of microorganism was estimated by counting the number of colony of sample to those of the control which were expressed as % inhibition of the rate of the bacterial growth. The bacterial strains used were *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (Colony used), *Enterococcus faecali* (ATCC 29212) and *E. coli* (ATCC 10536) which were supplied from Kon-Kuk University Hospital.

Results and Discussion

Table 1 shows the test results of the antimicrobial activity screening of total essential oils derived from 55 plant parts of 42 plant species against 5 microorganisms. The test microorganisms showed strong differential sensitivity to the plant derived essential oils.

Table I. Screening for antimicrobial activity of volatile essential oils derived from aromatic plants

Species	Family	Part used[a]	Microorganisms[b]				
			1	2	3	4	5
Acanthopanax koreanum	Araliaceae	st	+++	-	-	-	-
Ainsliama acerifolia	Compositae	rt	-	-	-	-	-
Akebia quinata	Lardizabalaceae	fl	-	-	-	-	-
Angelica dahurica	Umbelliferae	rt	-	-	-	-	-
Angelica gigas	Umbelliferae	lf	-	-	-	-	-
		rt	+++	+	-	-	-
Angelica koreana	Umbelliferae	rt	-	-	-	-	-
Angelica tenuissima	Umbelliferae	rt	-	-	-	-	-
Artemisia iwayomogi	Compositae	lf	+++	+++	-	-	-
Asiasarum sieboldii	Aristolochiaceae	st	-	-	-	-	-
		rt	-	-	-	-	-

Table 1. continued

Species	Family	Part used[a)]	Microorganisms[b)]				
			1	2	3	4	5
Aster scaber	Compositae	lf	-	-	-	-	-
Cinnamomum japonicum	Lauraceae	lf	-	-	-	-	-
Clerodendron trichotomum	Verbenaceae	lf	+++	++	-	-	-
Codonopsis lanceolata	Campanulaceae	lf	-	-	-	-	-
Dendropanax morbifera	Araliaceae	lf	+++	+++	-	-	-
Dictamnus dasycarpus	Rutaceae	st	-	-	-	-	-
		lf	-	+	-	-	-
		rt	-	-	-	-	-
Gardenia jasminoides	Rubiaceae	fl	-	-	-	-	-
Gardenia jasminoides	Rubiaceae	fl	-	-	-	-	-
Houttuynia cordata	Saururaceae	wp	-	+	-	-	-
Juniperus rigida	Cupressaceae	lf	+++	+++	-	++	-
Lindera obstusiloba	Lauraceae	fl	-	-	-	-	-
		st	+++	+++	-	++	-
		lf	++	-	-	-	-
Ligustrum japonicum	Oleaceae	fl	-	-	-	-	-
		st	-	-	-	-	-
		lf	-	-	-	-	-
Ligusticum wallichii	Umbelliferae	rz	-	+	-	+	-
Magnolia sieboldii	Magnoliaceae	fl	+++	+++	-	-	-
Neolitsea aciculata	Lauraceae	lf	-	-	-	+	-
Paulownia coreana	Scrophulariaceae	fl	-	-	-	-	-
Peucedanum japonicum	Umbelliferae	rt	+++	++	-	-	-
Philadelphus schrenkii	Saxifragaceae	st	-	-	-	-	-
		lf	-	+	-	-	-
		fl	-	-	-	-	-
Pinus densiflora	Pinaceae	lf	+++	+++	-	-	-
Pinus koraiensis	Pinaceae	lf	+++	+++	-	-	-
Pittosporum tobira	Pittosporaceae	lf	+++	++	-	-	-
Poncirus trifoliata	Rutaceae	fr	-	+	-	+	-
Prunus padus	Rosaceae	st	+++	++	-	-	-
		lf	-	-	-	-	-
Ptestyrax corymbasa	Styracaceae	fl	-	-	-	-	-
Rosa multiflora	Rosaceae	st	-	+	-	+	-
		lf	-	-	-	-	-
		fl	-	-	-	-	-
Rosa davurica	Rosaceae	fl	-	-	-	+	-
Sorbus alnifolia	Rosaceae	fl	-	-	-	-	-
Staphylea bumalda	Staphyleaceae	fl	-	-	-	-	-
Styrax japonicus	Styracaceae	fl	-	-	-	-	-
Syringa dilatata	Oleaceae	fl	-	-	-	-	-
Valeriana fauriei	Valerianaceae	rt	+++	+++	-	-	-
Vitex negundo var.incisa	Verbenaceae	fl	+	-	-	-	-
Zanthoxylum ailanthoides	Rutaceae	lf	-	-	-	-	-
Zanthoxylum piperitum	Rutaceae	lf	+++	+++	-	-	-

Data represent mean of triplicate determination at 100 μg/ml of each test extract. [a]wp, whole plant; rz, rhizome; st, stem; lf, leaf; fr, fruit; fl, flower; rt, root. [b](1) *Staphylococcus aureus* (ATCC 25923); (2) *Pseudomonas aeruginosa* (ATCC 27853); (3) *E. Coli* (ATCC 10536); (4) *Enterococcus faecali* (ATCC 29212); (5) *Streptococcus pneumoniae* (Coloning used). Culture growth inhibition: (-), less than 60% of the standard colony petri dish. (+), more than 60%; (++), more than 80%; dish (+++), more than 95%.

The most sensitive were *Staphylococcus aureus* (gram positive) and *Pseudomonas aeruginosa* (gram negative) which were inhibited by 17(30.9%) and 20(36.4%) of the test materials, respectively. *Enterococcus faecali* was inhibited by only 7(12.7%), but none of the essential oils tested showed growth inhibition against microorganisms such as *E. coli* and *Streptococcus pneumoniae*.

The essential oils derived from 15 plant parts such as Acanthopanax koreanae Lignum, Angelicae gigantis Radix, Artemisiae Folium, Juniperii Folium, Linderae obstilobae Lignum, Magnoliae Flos, Pinii densiflorae Folium, Pinii koraiensii Folium, Zanthoxylii Folium, Valerianae faureii Radix, Dendropanax morbiferae Folium, Prunus padii Folium, Clerodendrii Folium, Pittosporum tobirae Folium, Peusedanii japonicae Radix, and 9 plant parts such as Artemisiae Folium, Dendropanax morbiferae Folium, Juniperi Folium, Linderae obstilobae Lignum, Magnoliae Flos, Pinii densiflorae Folium, Pinii koraiensii Folium, Valerianae faureii Radix, Zanthoxylii Folium exhibited very strong anti-microbial activities by more than 95% inhibition at 100 μg/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. Against *Enterococcus faecali*, however, only the essential oils derived from 7 plant species such as Juniperii Folium, Linderae obstilobae Lignum, Ligusticii Rhizoma, Neolitseae aciculatae Folium, Poncirii trifoliatae Fructus, Rosae multiflorae Lignum and Rosae davuricae Flos exhibited relatively weak inhibition. The active essential oils which showed more than 95% inhibition at 100 μg/ml were subjected to test their dose dependent inhibition to evaluate their inhibitory potencies against the suseptible microorganisms according to the results indicated in Table 1. In the case of *Staphylococcus aureus*, essential oils from seven of 15 plant parts tested were shown to exhibit more than 50% inhibition at 0.1 ug/ml, among which, Linderae obstilobae Lignum and Valerianae faurei Radix showed the most potent inhibitory potencies, exhibiting more than 50% inhibition even at 0.01 ug/ml(Table 2). In the case of *Pseudomonas aeruginosa*, essential oils from five of 8 plant parts showed more than 50% inhibition at 1 μg/ml, among which those from Valerianae faureii Radix exhibited the most potent inhibitory potency, exhibiting more than 50%

Table 2. Antimicrobial activity of volatile essential oils derived from 15 plants against *Staphylococcus aureus*

Species	Family	Part used[a]	Inhibition(%)[b]					
			100 μg/ml	10 μg/ml	1 μg/ml	0.1 μg/ml	0.01 μg/ml	0.001 μg/ml
Acanthopanax koreanum	Araliaceae	st	100	69.5	50.2	20.3	-	-
Angelica gigas	Umbelliferae	rt	100	100	100	58.2	20.3	-
Artemisia iwayomogi	Compositae	lf	100	100	100	59.8	31.2	-
Clerodendron trichotomum	Verbenaceae	fl	100	95.5	13.0	-	-	-
Dendropanax morbifera	Araliaceae	lf	100	70.4	30.2	12.0	-	-
Juniperus rigida	Cupressaceae	lf	100	100	100	79.3	32.1	-
Lindera obstusiloba	Lauraceae	st	100	100	100	89.2	56.3	12.3
Magnolia sieboldii	Magnoliaceae	fl	100	100	100	43.2	5.3	-
Peucedanum japonicum	Umbelliferae	rt	100	100	100	55.7	22.1	-
Pinus densiflora	Pinaceae	lf	100	100	80.6	40.2	13.1	-
Pinus koraiensis	Pinaceae	lf	100	64.9	45.2	23.2	-	-
Pittosporum tobira	Pittosporaceae	lf	100	85.5	24.6	-	-	-
Prunus padus	Rosaceae	st	100	80.6	40.3	13.0	-	-
Valeriana fauriei	Valerianaceae	rt	100	100	100	96.5	56.3	12.3
Zanthoxylum piperitum	Rutaceae	lf	100	100	85.3	52.7	32.1	-

[a]st, stem; lf, leaf; fl, flower; rt, root. [b]Mean of duplicate determinations.

Table 3. Antimicrobial activity of volatile essential oils derived from 8 plants against *Pseudomonas aeruginosa*

Species	Family	Part used[a]	Inhibition (%)[b]			
			100 μg/ml	10 μg/ml	1 μg/ml	0.1 μg/ml
Dendropanax morbifera	Araliaceae	lf	96.8	96.3	73.6	35.6
Juniperus rigida	Cupressaceae	lf	99.2	58.3	21.3	-
Lindera obstusiloba	Lauraceae	st	100	82.3	62.5	12.3
Magnolia sieboldii	Magnoliaceae	fl	95.4	63.2	11.5	-
Pinus densiflora	Pinaceae	lf	96.8	86.5	56.3	6.8
Pinus koraiensis	Pinaceae	lf	100	100	53.2	2.6
Valeriana fauriei	Valerianaceae	rt	100	100	83.6	58.6
Zanthoxylum piperitum	Rutaceae	lf	100	89.3	43.2	23.2

[a]st, Stem; lf, leaf; fl, flower; rt, root. [b]Mean of duplicate determinations.

Table 4. Screening for antimicrobial activity of common standard essential oils

Essential oils	Microorganisms[a]		Essential oils	Microorganisms[a]	
	Staphylococcus aureus	*Pseudomonas aeruginosa*		*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Cinnamic alcohol	36.8[b]	16.2	Anethol	53.6	63.2
Eugenol acetate	12.8	0.0	1,8-Cineole	63.8	45.3
Thujone	46.3	0.0	Linalool oxide	18.3	0.0
Benzaldehyde	56.8	32.6	Menthone	27.3	0.0
Methyleugenol	8.9	0.0	Humulene	16.3	0.0
α-Phellandrene	82.6	53.2	*l*-Borneol	83.2	73.2
α-Ionone	57.3	0.0	*l*-Limonene	64.3	46.3
β-Phellandrene	46.2	0.0	β-Cardineol	17.3	0.0
Coumarin	76.3	0.0	Norfloxacin	43.2	63.2
Thymol	12.3	0.0	Isomenthone	23.8	0.0
Linalyl acetate	12.8	0.0	(-)Menthol	16.3	0.0
Perilla alcohol	16.8	0.0	α-Terpinene	12.3	0.0
Bornyl acetate	68.4	0.0	γ-Terpinene	48.2	25.9
Cinnamic aldehyde	0.0	16.3	α-Terpineol	23.6	16.5
Safrol	0.0	18.2	Myrcene	78.6	12.3
Leonurine	17.3	0.0	Linalool	63.2	36.2
Anis aldehyde	0.0	36.2	Eucalyptol	58.9	0.0
β-Ionone	48.6	16.9	Cedrol	59.6	68.9
p-Cymene	16.3	0.0	Acetic acid-farnesylester	26.8	18.6
Phenyl ethyl alcohol	43.2	0.0			

[a]*Staphylococcus aureus* (ATCC 25923); *Pseudomonas aeruginosa* (ATCC 27853). [b]Percent Inhibition: mean of duplicate determinations at 100 μg/ml of each test compound.

inhibition at 0.1 ug/ml (Table 3).

One hundred eighteen common essential oil compounds were tested for anti-microbial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* (data not shown) and among which those exhibited more or less significant inhibition against the microorganisms at 100 μg/ml (Table 4) were selected and compared with the individual componments of the active essential oils. It was found that monoterpenoids such as *l*-limonene, β-myrcene, linalool, γ-terpinene, α,β-phellandrene, 1,8-cineole, *l*-borneol and bornylacetate have primarily contributed to the manifestation of anti-microbial activity as compared by GC composition of essential oils from 13

Table 5. Gas chromatographic composition of the standard essential oils possessing antimicrobial activity against *Staphylococcus aureus* from 13 aromatic plants

Essential oil	1[a]	2	3	4	5	6	7	8	9	10	11	12	13
Thujone	-	-	-	-		0.05	-	-	-	-	-	-	-
Benzaldehyde	-		-	0.57	-	-	-	0.22	-	-	74.87	-	-
α-Phellandrene	0.55[b]	0.11	-	-	0.05	-	14.25	-	0.48	-	-	-	-
β-Phellandrene	0.58	-	-	-	5.25	-	0.52	13.16	-	-	-	-	-
Coumarin	-	-	-	-	-	-	-	-	-	-	0.04	-	-
Bornyl acetate	-	-	-	-	-	-	-	-	4.96	-	-	30.5	-
1,8-Cineole	4.73	-	8.81	0.04	-	1.02	-	-	-	-	-	-	19.64
Linalool oxide	-	-	-	-	-	-	0.08	-	-	-	0.23	-	-
l-Borneol	-	-	-	-	4.82	-	-	-	-	-	-	0.79	-
l-Limonene	17.01	10.72	-	-	2.93	0.99	0.12	2.49	5.27	13.54	-	4.68	5.47
α-Terpinene	0.85	0.05	-	-	-	4.83	-	-	-	-	-	-	-
γ-Terpinene	-	0.12	0.74	-	12.87	1.50	-	0.18	0.21	-	-	27.29	1.37
α-Terpineol	0.68	-	-	-	0.21	-	-	0.13	-	-	0.16	-	-
Myrcene	6.85	5.82	0.89	0.16	4.95	12.72	-	5.10	8.03	-	-	-	5.64
Linalool	0.21	0.26	-	29.0	0.05	-	-	-	-	-	0.19	-	1.03
Cedrol	-	-	-	-	-	-	-	-	-	1.20	-	-	

[a]1. *Acanthopanax koreanum* (stem); 2. *Angelica gigas* (root); 3. *Artemisia iwayomogi* (leaf); 4. *Clerodendron trichotomum* (leaf); 5. *Lindera obstusiloba* (stem); 6. *Magnolia sieboldii* (flower); 7. *Peucedanum japonicum* (root); 8. *Pinus densiflora* (leaf); 9. *Pinus koraiensis* (leaf); 10. *Pittosporum tobira* (leaf); 11. *Prunus padus* (stem); 12. *Valeriana fauriei* (root); 13. *Zanthoxylum piperitum* (leaf). [b]Peak area(%).

active aromatic plants against *Staphlococcus aureus* (Table 5). These common essential oils, however, can not be disclosed as key components of essential oils representing the antimicrobial activity of the active plants, as the inhibitory activities were shown by common essential oils only at relatively high concentration (i.e., 100 μg/ml). More thorough investigations on genuine active principles of the active aromatic plants remains to be elucidated.

Acknowledgement

This work was supported by the research grant from the Ministry of Science and Technology, Republic of Korea (1994-1996, Project No. 94-N-09-01-C).

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(Accepted December 15, 1997)