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Phytotoxic and Antimicrobial Activities and Chemical Analysis of Leaf Essential Oil from *Agastache rugosa*

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Seasonal variations in the chemical composition of leaves from *Agastache rugosa* were analyzed by gas chromatography-mass spectrometry (GC-MS). Phytotoxic and antimicrobial activities of its essential oil were investigated by characterizing 31 components, primarily methylchavicol (80.24%), *dl*–limonene (3.50%), linalool (4.23%), 5-methyl-2-(1-methylethylidene)-cyclohexanone (3.84%), and β-caryophyllene (2.39%). The contents of most of these chemical constituents varied significantly ($P<0.05$) over time, with the highest oil yield being early in the growing season (May). Their phytotoxic effects on the seed germination of *Majorana hortensis*, *Trifolium repens*, *Rudbeckia hirta*, *Chrysanthemum zawadskii* var. *latilobum*, *Melissa officinalis*, *Taraxacum platycarpum*, and *Tagetes patula* also were determined. These oils partially or completely inhibited germination, and prevented the growth of radicles and hypocotyls. Results of antimicrobial testing showed that the oils also entirely blocked the growth of four microorganisms (*Staphylococcus aureus* sub. *aures*, *Escherichia coli*, *Salmonella enteritidis*, and *Pseudomonas aeruginosa*). These inhibitory effects might depend on the activity of methylchavicol, a main compound in *A. rugosa*.

Keywords: Agastache rugosa, antimicrobial activities, essential oil, methylchavicol, phytotoxic

Agastache rugosa Kuntz is a perennial aromatic species in the Lamiaceae family, and is native to Korea, China, and Japan. This valuable plant has a variety of physiological and pharmacological activities (Hong et al., 2001; Lee et al., 2002; Oh et al., 2006), and is used for treating anorexia, vomiting, and other intestinal disorders. It is also commercially cultivated as a source of food spice. In Korea, it deodorizes the fishy aroma from fish pot stews.

Although the most abundant compound in the essential oil from *Agastache* is methylchavicol (Charles et al., 1991; Lee et al., 1994, 2002; Shin and Kang, 2003), its concentration along with those of others does vary according to the particular plant organ, season, location, and differences among individuals (Kim, 1997; Jordán et al., 2006).

Plant essential oils are involved in multiple ecological functions, such as protection against herbivores and microbial disease, attraction of pollinators, and in the phenomenon of allelopathy (Swain, 1977; Fischer, 1991). The phytotoxic effects by oils of unidentified composition were first described by Fischer (1991). Inderjit and Duke (2003) have reviewed the ecophysiological aspects of allelopathy, and have argued that allelochemicals play important roles both in inhibiting plant growth during interspecies interactions and in structuring the plant community. The most common allelochemicals are phenolic compounds and terpenoids (Einhellig and Leather, 1988; Inderjit and Keating, 1999). Among them, the latter are a major phytochemical class that is characterized by stereochemical diversity, exceeding that of any other group of plant products. Essential oils and pure monoterpenes strongly inhibit seed germination and plant growth. Unlike with other mechanisms of allelopathy, studies on the effects of volatiles in plant-plant interactions have been less often investigated. However, recent improved instruments, e.g., GC-MS and LCMS, have assisted researchers in gaining a better understanding of the chemical basis for this allelopathy phenomenon (Haig, 2001).

The antimicrobial effects of essential oils and their constituents have been examined in a wide variety of species (Koudou et al., 2005; Kim et al., 2006; Oliveira et al., 2007; Rota et al., 2008). Results from these studies have illustrated their potential uses in medical procedures and applications within the cosmetic, pharmaceutical, and food industries. Their biological activity depends upon their chemical composition, which is determined by genotype as well as influenced by environmental and agronomic conditions (Hussain et al., 2008; Yun et al., 2008). Secondary compounds, including essential oils, isolated from *A. rugosa* have such antimicrobial activity (Hong et al., 2001; Lee et al., 2002; Oh et al., 2006). However, no previous reports have been made on the particular essential oil and phytotoxic activities in that species, nor on seasonal variations. Therefore, the objective of this study was to investigate how its concentrations and components vary over time, and how it affects seed germination and sustainable growth as a source of allelochemical and antimicrobial activities.

MATERIALS AND METHODS

Plant Extraction and Chemical Analysis

During their growing season (April through September), plants of *Agastache rugosa* were collected from three sites that were environmentally similar within a cultivated area at Masan City. At two-week intervals, plant samples were sealed in plastic bags and transported to the laboratory. After their leaves and stems were separated, sub-samples (3 g each) were ground with pure sand, then extracted with n-pentane and 1 mL of 1% tetradecane as an internal standard. These extracts were filtered with sodium sulfate and

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concentrated by evaporation with a gentle stream of nitrogen gas.

Samples (1 μL) were analyzed by gas chromatography-mass spectrometry (GC-MS, HP 5890), on a 30-m-long HP5 capillary column (i.d. 0.25 mm; flame ionization detector). Helium was used as the carrier gas. The oven program comprised an initial 37°C for five min, increased first to 180°C at a rate of 5°C min^{-1}, then by 20°C $min^{-}1$, to a final temperature of 320°C. Individual terpenoids were identified by comparing them with both spectral data from the instrument's internal library (Wiley library 7.0) and their retention times, based on references. All tests were performed at least in triplicate; an ANOVA for variations in oil components with time was performed.

Essential Oil Isolation

Essential oils were isolated by steam-distilling 300 g of fresh leaves for 3 h in a Clevenger-type apparatus. Samples were then refrigerated. Pentane was used as the transfer solvent. The loading solvent, *n*-pentane, was allowed to evaporate completely at room temperature before being diluted with water. An added control with *n*-pentane was used to account for possible solvent effects. The influence of compounds on seedling growth was observed for 7 d (Kobaisy et al., 2001).

Phytotoxicity and Antimicrobial Activity Assays

A bioassay based on seed germination and subsequent radicle and hypocotyl growth was conducted to analyze any allelopathic effects by the extracts. Seeds of *Majorana hortensis*, *Trifolium repens*, *Rudbeckia hirta*, *Chrysanthemum zawadskii* var. *latilobum*, *Melissa officinalis*, *Taraxacum platycarpum*, and *Tagetes patula* were purchased from Korea Seed Co. (Masan, Korea). They were surface-sterilized in 95% ethanol for 15 s and sown in Petri dishes (90 mm diam.; 25 seeds each, 4 dishes per species) containing two layers of Whatman filter paper. Afterward, they were impregnated with either 10 mL of distilled water (control) or a paper disc (8 mm diam.; Toyo Roshi Kaisha) that contained one of three amounts of isolated *Agastache* oil (1, 5, or 10 μL). The dishes were placed in a growth chamber set at 22°C and a 12-h photoperiod. Germination was recorded as the percentage of seeds from which a radicle emerged, and relative germination was the treatment rate divided by that of the corresponding control. This process was observed directly in the dishes, at 24-h intervals, using a stereomicroscope. The Prompt Index (PI) was estimated according to the following formula:

$$PI = (D_1 \times 7) + (D_2 \times 6) + (D_3 \times 5) + (D_4 \times 4) + (D_5 \times 3) + (D_6 \times 2) + (D_7 \times 1)$$

D_n = No. of seeds germinating on the n^{th} day

After 7 d, the influence of the essential oil on radicle and hypocotyl elongation was determined. Relative elongation was defined as the treatment elongation divided by that of the corresponding control. All tests were repeated three times, and treatment effects were compared with their respective controls by a t-test.

Four microorganisms were purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea): *Pseudomonas aeruginosa* 2695, *Staphylococcus aureus* sub. *aures* 1927, *Escherichia coli* 1116, and *Salmonella enteritidis*. These strains, which are food spoilage organisms and food-borne bacteria, were maintained and tested on a nutrient agar (3 g of beef extract, 5 g of peptone, 15 g of agar, and 1 L of distilled water). Cells were grown in this medium for 24 h in a chamber at 37°C. For the antimicrobial testing (Disc diffusion test), approximately 1.0 x 10^8 cells mL^{-1} were streaked with a calibrated loop onto plates containing a solid medium. The stock solution, 20% DMSO, was prepared as described by Nostro et al. (2000). Filter paper discs (8mm diam.) were placed in the centers of the plates, on the inoculated agar surface, and were impregnated with 10, 20, or 30 μL of *Agastache* essential oil in 1:1 DMSO stock solutions. Pure DMSO (20 μL) was used as the control. All tests were performed at least in triplicate, and antimicrobial activity was expressed as the mean of inhibition diameters (mm) produced by the oil. A t-test was performed to compare the oil's potential inhibitory effect with that of the control.

RESULTS AND DISCUSSION

Chemical Composition and Seasonal Variation

Thirty-one compounds were detected from leaf extracts of *Agastache rugosa* (Table 1). Among these, methylchavicol was most abundant (80.24%), followed by *dl*-limonene (3.50%), linalool (4.23%), 5-methyl-2-(1-methylethylidene)-cyclohexane (5-mm-clohexane, 3.84%), and β-caryophyllene (2.39%). The essential oil was also dominated by oxygenated monoterpenoids (>90%) and some sesquiterpenoids. These results are in good agreement with those of Charles et al. (1991) and Lee et al. (1994), who reported that methylchavicol, β-caryophyllene, and limonene are the major oil constituents in that species. For example, Charles et al. (1991) showed that the main constituents of Hybrid 4992 are β-limonene (2.09%), β-caryophyllene (1.91%), and methylchavicol (93.7%), whereas Hybrid 5018 contains β-limonene (10.6%), isomenthone (8.50%), methylchavicol (55.9%), pulegone (8.72%), methyleugenol (9.08%), and β-caryophyllene (1.51%). Likewise, Lee et al. (1994) found that the essential oil of *A. rugosa* leaves consists of limonene, β-copaene, β-caryophyllene, and methylchavicol (79.1%). Other researchers also have determined that methylchavicol (42-79.83%) is most prominent (Lee et al., 2002; Wilson et al., 1992; Shin and Kang, 2003; Mallavarapu et al., 2004).

Methylchavicol, in particular, is used as an herbal medicinal product, under the product name of estragole. This compound also occurs in *Artemisia dranunculus* L. (172~7,000 ppm), *Ocimum basilicum* L. (238~6,500 ppm), *O. tenuiflorum* L. (39,950 ppm), and *Foeniculum vulgare* Mill. (70~4,018 ppm) (European Commission, 2005). Estragole has recently been recognized as safe (GRAS) by an expert panel from the flavor and extract manufacturer's association (FEMA), and has been approved by the US Food and Drug Administration (FDA) for food use. However, estragole and its metabolites are genotoxic and can produce hepatic tumors in susceptible strains of mice; i.e., it is considered to be a natu-

Table 1. Composition of essential oil in leaves of *Agastache rugosa* on 22 June 2006. Only compounds found at levels >0.05% are included.

No.	Compound	R.T.	%	mg g^{-1}
1	cis-3-hexenal	3.43	0.22	0.0070
2	2-hexenal	4.95	0.25	0.0057
3	sabinene	9.32	0.03	0.0006
4	1-octene-3-ol	9.69	0.20	0.0043
5	3-octanone	9.94	0.01	0.0002
6	β-myrcene	10.04	0.18	0.0037
7	*dl*-limonene	11.39	3.50	0.0798
8	1-octen-3-yl acetate	14.44	1.11	0.0263
9	linalool	16.21	4.23	0.0868
10	cis-isopulegone	16.69	0.15	0.0032
11	methylchavicol	17.76	80.24	2.0724
12	5-methyl-cyclohexanone	18.73	3.84	0.0788
13	trans-anethole	19.90	0.02	0.0004
14	8-hydroxy-δ-4(5)-p-menthen-3-one	20.04	0.06	0.0012
15	sabinyl acetate	20.12	0.02	0.0004
16	2-propyl cyclopentanone	21.21	0.09	0.0019
17	bicycloelemene	21.34	0.05	0.0011
18	cyclohexane	21.36	0.06	0.0012
19	2-cyclohexen-1-one	21.52	0.03	0.0006
20	eugenol	22.33	0.02	0.0004
21	β-bourbonene	22.72	0.07	0.0014
22	β-elemene	22.91	0.07	0.0016
23	β-caryophyllene	23.72	2.39	0.0559
24	1H-cyclopenta[1,3]cyclopropa[1,2]b	23.90	0.01	0.0003
25	β-humulene	24.59	0.10	0.0021
26	β-cubebene	25.30	0.58	0.0133
27	germacrene-D	25.34	0.83	0.0190
28	bicyclogermacrene	25.74	1.00	0.0229
29	E,E-α-farnesene	25.94	0.09	0.0019
30	naphthalene	26.14	0.02	0.0005
31	endo-1-bourbonanol	27.73	0.52	0.0112

rally occurring genotoxic carcinogen in some animals. However, no studies have been made of the long-term health effects from human exposure to estragole. Smith et al. (2008) and Rietjens et al. (2005) have reviewed its safety as a food additive, and have concluded that the metabolite 1′-hydroxyestragole is a supposed proximate carcinogen, and can be found in the urine of men dosed with 1 μg kg^{-1} of body weight (namely, within a high-dose diet).

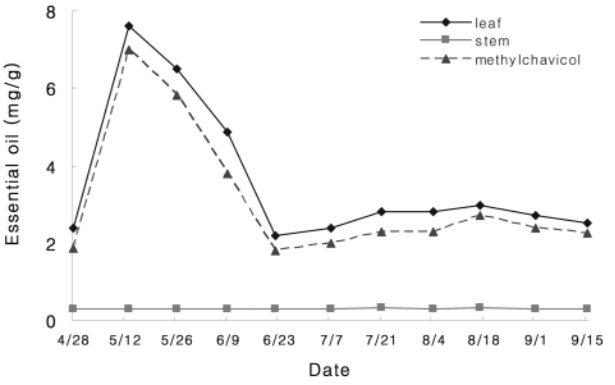

Figure 1. Seasonal variation in concentrations of methylchavicol and total essential oil in leaves and stems of Agastache rugosa.

In the current study, the total yields for essential oil from fresh *Agastache* leaves were 2.36∼7.51 mg g^{-1} tissue; those values varied with time ($p < 0.05$; Fig. 1), and were remarkably correlated with methylchavicol contents ($r = 0.98$). The total amount of *A. rugosa* leaf essential oil was very high compared with that measured in *Artemisia* sp. (Kim, 1997) and *Elsholtzia cilliata* (Kim and Jung, 2003). However, the use of steam-distillation isolation led to the lowest levels of essential oils obtained an outcome similar to that reported by Sefidkon et al. (2007). These results are in good agreement with the findings of van Vuuren et al. (2007), Rodilla et al. (2008), and Hussain et al. (2008), who also reported that the composition of essential oils obtained from plants in different seasons varied markedly.

Here, the amounts of essential oils were 5.7∼40.6 times greater in *Agastache* leaves than in stems, the latter tissues not showing any significant differences with time ($p > 0.05$). In contrast, the highest leaf oil content was obtained at the beginning of the growing season (13 May) before it decreased significantly ($p<0.05$) in the summer. The lower yield then might be expected because of high temperatures and the partial evaporation of some oil constituents. Moreover, dif-

Table 2. Seasonal variations in major monoterpene and sesquiterpene compositions (%) from leaves of *Agastache rugosa*.

	28 Apr	13 May	26 May	09 Jun	22 Jun	07 Jul	21 Jul	04 Aug	17 Aug	01Sep	15 Sep
Monoterpenes											
cis-3-hexenal**	0.3	0.9	0.8	0.5	0.2	0.3	1.2	0.6	0.7	0.9	1.0
1-octene-3-ol**	-	-	0.6	0.5	0.2	0.4	0.4	0.8	0.2	0.5	0.4
dl-limonene**	2.7	3.0	3.5	4.1	3.5	4.1	1.8	1.6	1.6	1.6	1.8
linalool**	2.2	-	0.9	0.8	4.2	-	5.2	4.2	-	0.9	-
5mm-cyclohexane**	1.2		0.43	2.05	3.84		4.3	3.6	1.8	0.7	0.4
methylchavicol*	88.2	93.3	90.9	77.5	80.2	90.1	88.2	88.0	94.9	91.3	93.7
Sesquiterpenes											
β-caryophyllene**	0.8	1.0	1.1	-	2.4	1.8	1.8	-	1.5	1.7	0.9
germacrene-D**	1.1	0.5	0.7	1.2	0.8	0.7	0.7	0.6	0.7	0.6	0.7

Variations with time are significantly different, based on ANOVA (* $P< 0.05$, ** $P<0.001$).

ferences in amounts might have been due to leaf shape, because younger tissues early in the year were not fully expanded, making its monoterpenoids less volatile than in the summer leaves. These results concur with those of Trivino and Johnson (2000), who showed that time within the growing season greatly, affected essential oil yields in *Origanum majorana*. Therefore, because of such seasonal fluctuations, it is very difficult to determine the best harvesting period for obtaining the highest quality and quantity of essential oils from *Agastache* and other species based on seasons (Kim, 1997; van Vuuren et al., 2007; Hussain et al., 2008).

Table 2 presents the changes in relative amounts of major compounds as they varied with time. For example, *dl*-limonene (4.14%), linalool (5.19%), and β-caryophyllene (2.39%) were most abundant during June and July, whereas the opposite trend was observed with linalool and β-caryophyllene, particularly from basil. In Pakistan, Hussain et al. (2008) also have recorded maximum values for these latter compounds in winter (60%) versus summer (12.4%). However, in the current study, the level of methylchavicol was always high, although variations were statically significant over time ($p<0.05$). This suggests that monoterpene metabolites, the major plant compounds, are somewhat regulated via gene expression (Mahmoud and Croteau, 2002; Wink, 2003). That is, the genetic constitution and environmental conditions for individual plants influence the yield and composition of volatile oils produced. Because flavor and monoterpenoid compositions differ markedly among species, monoterpenoids determine their chemotypes. In fact, knowledge of the genetic mechanisms of monoterpenes is widely used in taxonomic, phylogenic, and bioengineering studies (Mahmoud and Croteau, 2002; Wink, 2003; de Carvalho and da Fonseca, 2006; Herrero et al., 2008). For example, Wink (2003) has suggested that these monoterpenoid patterns might reflect differential expression of the corresponding genes that evolved earlier in plant development. Furthermore, because the total proportional quantities of monoterpenoids are dependent on species, inconsistent profiles of secondary metabolites apparently demonstrate

Table 3. Relative germination ratio (RGR) of receptor plants at different concentrations of essential oil from *Agastache rugosa* leaves.

Species	Concentration	Germination (%)		Prompt index	
		Germination	RGR	PI	% of control
Tagetes patula	control	54.7±4.8	100.00	265.7	100.00
	1 μL	40.0±2.3**	73.17	169.7	63.86
	5 μL	10.7±1.9**	19.51	36.7	13.80
	10 μL	0.00**	0.00	0.00	0.00
Chrysanthemum zawadskii var. *latilobum*	control	24.0±1.5	100.00	46.7	100.00
	1 μL	12.0±1.4**	50.00	25.0	53.57
	5 μL	6.6±1.3**	27.78	11.3	24.29
	10 μL	0.00**	0.00	0.00	0.00
Melissa officinalis	control	89.3±1.1	100.00	337.3	100.00
	1 μL	88.0±1.8**	98.51	302.7	89.72
	5 μL	84.0±3.8**	94.03	244.0	72.33
	10 μL	57.3±3.0**	64.18	105.0	31.13
Rudbeckia hirta	control	84.0±3.6	100.00	353.0	100.00
	1 μL	78.7±5.7**	93.65	259.7	73.56
	5 μL	26.7±3.8**	31.75	88.7	25.12
	10 μL	0.00**	0.00	0.0	0.00
Majorana hortensis	control	74.7±3.5	100.00	305.3	100.00
	1 μL	82.7±3.7**	110.71	311.7	102.07
	5 μL	61.3±2.8**	82.14	243.7	79.80
	10 μL	40.0±3.3**	53.57	120.0	39.30
Taraxacum platycarpum	control	16.0±1.0	100.00	21.0	100.00
	1 μL	10.7±0.8**	66.67	10.3	49.21
	5 μL	0.0**	0.00	0.0	0.00
	10 μL	0.0**	0.00	0.0	0.00
Trifolium repens	control	76.0±2.4	100.00	471.3	100.00
	1 μL	68.0±1.1**	89.47	412.7	87.55
	5 μL	74.7±2.1*	98.25	436.3	92.57
	10 μL	78.7±0.9	103.51	382.3	81.12

Data are means ± standard deviation for three replicates of experiments.
* Significantly different from the control, based on t-test (*$P<0.05$, ** $P<0.001$).

Table 4. Relative elongation of radicles and hypocotyls from receptor plants tested at different concentrations of essential oil from *Agastache rugosa*.

Species	Concentration	Radicle		Hypocotyl	
		Length (mm)	% of control	Length (mm)	% of control
Tagetes patula	control	38.8±2.3	100.00	11.24±1.1	100.00
	1 μL	37.17±1.7**	95.85	11.17±1.2	99.38
	5 μL	2.73±0.5**	7.04	1.96±0.5**	17.44
	10 μL	0.00**	0.00	0.00**	0.00
Chrysanthemum zawadskii var. *latilobum*	control	1.95±0.2	100.00	0.83±0.1	100.00
	1 μL	1.35±0.1*	69.59	0.83±0.3	100.00
	5 μL	2.70±0.9**	138.80	0.74±0.2*	89.10
	10 μL	0.00**	0.00	0.00**	0.00
Melissa officinalis	control	12.65±0.8	100.00	1.09±0.5	100.00
	1 μL	9.85±0.7**	77.84	0.94±0.1	86.52
	5 μL	3.13±0.3**	24.71	0.77±0.1**	70.83
	10 μL	1.10±0.1**	8.66	0.29±0.1**	26.68
Rudbeckia hirta	control	4.93±0.9	100.00	0.95±0.1	100.00
	1 μL	4.41±0.5*	89.40	0.85±0.3*	89.47
	5 μL	3.44±0.8**	69.75	0.72±0.1**	75.79
	10 μL	0.00**	0.00	0.00**	0.00
Majorana hortensis	control	5.62±0.9	100.00	0.65±0.2	100.00
	1 μL	5.09±0.8*	90.60	0.61±0.3	93.43
	5 μL	1.14±1.1**	20.25	0.38±0.1*	58.20
	10 μL	0.45±0.1**	8.07	0.09±0.1**	13.79
Taraxacum platycarpum	control	1.16±0.5	100.00	0.28±0.2	100.00
	1 μL	1.65±0.4*	141.89	0.00**	0.00
	5 μL	0.00**	0.00	0.00**	0.00
	10 μL	0.00**	0.00	0.00**	0.00
Trifolium repens	control	14.43±1.1	100.00	3.04±0.2**	100.00
	1 μL	11.80±0.9*	81.80	2.72±0.1*	89.42
	5 μL	3.47±0.5**	24.05	2.19±0.1**	71.99
	10 μL	1.38±0.3**	9.54	0.95±0.8**	31.23

Data are means ± standard deviation for three replicates of experiments.
* Significantly different from the control, based on t-test (*P<0.05, ** P<0.001).

their adaptation to various environmental conditions.

Phytotoxic and Antimicrobial Activities

Compared with the control, treatment with the *Agastache* essential oil components deterred or completely inhibited seed germination due to their allelopathic effects, with this influence being proportional to the oil concentration applied (Table 3). A significant delay in the Prompt Index (PI) was also observed for all tested species. Germination of *Tagetes patula*, *Chrysanthemum zawadskii* var. *latilobum*, *Rudbeckia hirta*, and *Taraxacum platycarpum* was entirely blocked when seeds were exposed to 10 μL of *Agastache* oil, but its allelopathic effect was weak when utilized with *Melissa officinalis*, *Majorana hortensis*, and *Trifolium repens*. In fact, such treatment actually increased the germination rates for *M. hortensis* (by 110.7% over the control) and *T. repens* (by 103.5%). Stebbing (1982) has shown that this phenomenon -- hormesis -- is typical of many phytotoxins at low inhibitor levels.

Nevertheless, the phytotoxic activity of volatile monoterpenoids can prevent plant growth (Kobaisy et al., 2001; Vokou et al., 2003; Salamci et al., 2007; Corrêa et al., 2008). In a separate investigation, Romagni et al. (2000) have compared the effects of 1,4- and 1,8-cineoles on two weedy plant species and have found that those compounds appear to have different modes of action even though their structures are similar. Here, the phytotoxic activity of *A. rugosa* essential oil observed at 1 μL suggested that methylchavicol was the main active monoterpenoid (see also Salamci et al., 2007), although its individual effect on germination and seedling growth was not investigated in the current study. The allelopathic effects of the *Agastache* oil was sustained throughout radicle and hypocotyl elongation (Table 4), following a linear trend with concentration. This result agrees with those of Corrêa et al. (2008). For example, treatment with *Agastache* oil accelerated the radicle elongation of *C. zawadskii* and *Taraxacum platycarpum* over that of the con-

Table 5. Antimicrobial activity of *Agastache rugosa* essential oil.

Organism tested	Concentration	Diameter of inhibition zone (mm)	
		24 h	48 h
Gram-positive	control	0	0
Staphylococcus aureus sub. *aures*	10 μL	15±0.9*	30±1.3**
	20 μL	22±1.1*	41±1.3**
	30 μL	29±1.2*	53±0.9**
Gram-negative	control	0	0
Escherichia coli	10 μL	10±0.7**	12±0.9**
	20 μL	12±0.8**	15±1.1*
	30 μL	14±1.0**	20±0.6**
Salmonella enteritidis	control	0	0
	10 μL	14±0.7**	17±0.8**
	20 μL	20±1.2**	26±1.1*
	30 μL	27±1.2*	33±0.9**
Pseudomonas aeruginosa	control	0	0
	10 μL	8±0.5*	8±0.6**
	20 μL	11±0.9*	11±0.8**
	30 μL	15±0.7*	15±1.1*

Data are means±standard deviation for three replicates of experiments. *Significantly different from the control , based on t-test (*$P<0.05$, **$P<0.001$).

trol (by 138.80% and 141.89%, respectively). Rizvi and Rizvi (1992) also have observed that some organic compounds, applied in small concentrations, may promote growth.

Finally, treatment with *Agastache* essential oil blocked the growth of bacterial strains, producing a zone diameter of inhibition ranging from 8 to 53 mm, depending on their degree of susceptibility. For all strains, the extent of this influence was in proportion to the oil concentration and length of culture period. Such antibiotic activity has been reported in other species (Wannissorn et al., 2005; Kim et al., 2006; Tepe et al., 2007; Deba et al., 2008). At all concentrations in the current study, the effect was strongest against *Staphyllococcus aureus* but moderate against *Salmonella enteritidis* and *Escherichia coli*. The lowest antimicrobial activity was seen with *Pseudomonas aeruginosa*. Differences were not significant between 24 h and 48 h.

These results agree with those reported by Lambert et al. (2001), who demonstrated that *P. aeruginosa* is less sensitive than *S. enteritidis* when treated with the main constituents of oregano essential oil, i.e., thymol and carvacrol. Helander et al. (1998) and Deba et al. (2008) also have found that the inhibitory activity of essential oils is significantly higher in Gram-negative bacteria compared with Gram-positive bacteria. In contrast, Bozin et al. (2006), Hussain et al. (2008), and Lopez et al. (2005) have shown that Gram-positive strains of bacteria have higher sensitivities. Likewise, in the current study, the *A. rugosa* oil was most inhibitory against Gram-positive bacteria (*S. aureus*) and least against the Gram-negative bacteria (*P. aeruginosa*). Therefore, such antimicrobial activity by essential oils is apparently related to its main component, methylchavicol. This was seen earlier in research by Fontenelle et al. (2008), who noted, when evaluating the antifungal activities of essential oils from Brazilian *Croton* species, that estragole (72.9%) was the main constituent in leaves of *C. zehntneri*, and that this compound was effective against *Microsporum canis*, *Candida albicans*, and *C. tropicalis*. Those investigators suggested that the activity of that oil could be attributed to its major constituent, estragole (i.e., methylchavicol). Nevertheless, even trace compounds might have possibly synergistic influences, playing an important role in the inhibition of microorganisms. Therefore, this antimicrobial activity by *A. rugosa* essential oil might be related to its main compound, methylchavicol alone, or in combination with ketoconazole (Shin and Kang, 2003).

In conclusion, as seen in this study, the phytotoxic effects and antibacterial activity of the essential oil from leaves of *A. rugosa* may be due to methylchavicol alone or in conjunction with small amounts of terpenoids, including *dl*-limonene, linalool, β-caryophyllene, 5-methyl-2-(1-methylethylidene)-cyclohexane (5-mm-cyclohexane), and germacrene-D (Table 2). Moreover, this activity against food-borne bacteria could be useful for preserving products in the food industry. Although seasonal fluctuations in the antibacterial properties of *A. rugosa* essential oil were not analyzed here, the outcome might be attributed to the consistent presence of a high concentration of methylchavicol in those tissues.

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LITERATURE CITED

Bozin B, Minica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile composition of essential oil of some lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54: 1822-1828

Charles DJ, Simon JE, Widrlechner MP (1991) Characterization of essential oil of *Agastache* species. J Agric Food Chem 39: 1946-1949

Corrêa LR, Soares GLG, Fett-Neto AG (2008) Allelopathic potential of *Psychotria leiocarpa*, a dominant understorey species of subtropical forests. S Afric J Bot Doi:10.1016/j.sajb

de Carvalho CCCR, da Fonseca MMR (2006) Biotransformation of terpenes. Biotech Adv 24: 134-142

Deba F, Xuan TD, Yasuda M, Tawata S (2008) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. Food Contr 19: 346-352

Einhellig FA, Leather GR (1988) Potentials for exploiting allelopathy to enhance crop production. J Chem Ecol 14: 1829-1844

European Commission (2005) Public statement on the use of herbal medicinal products containing estragole.

Fischer N (1991) Plant terpenoids as allelopathic agents, *In* JB Harborne, FA Tomas-Barberan, eds, Ecological Chemistry and Biochemistry of Plant Terpenoids. Oxford University Press, New York, pp 377-398

Fontenelle ROS, Morais SM, Brito EHS, Brihante RSN, Cordeiro

RA, Nascimento NRF, Kerntopf MR, Sidrim JJC, Rocha MFG (2008) Antifungal activity of essential oils of *Croton* species from the Brazillian *Caatinga* biome. J Appl Microbiol Doi:10.1111/j.1365-2672

Haig T (2001) Application of hyphenated chromatography-mass spectrometry techniques to plant allelopathy research. J Chem Ecol 27: 2363-2396

Helander IM, Alakomi HL, Latva-Kala K, Mattiala-Sandholm T, Pol I, Smid EJ (1998) Characterization of the action of selected essential oils components on Gram-negative bacteria. J Agric Food Chem 46: 3590-3595

Herrero Ó, Ramón D, Orejas M (2008) Engineering the *Saccharomyces cerevisiae* isoprenoid pathway for *de novo* production of aromatic monoterpenes in wine. Metabol Engr 10: 78-86

Hong JJ, Choi JH, OH SR, Lee HK, Park JH, Lee KY, Kim JJ, Jeong TS, Oh GT (2001) Inhibition of cytokine-induced vascular cell adhesion molecule-1 expression: Possible mechanism for anti-atherogenic effect of *Agastache rugosa*. FEBS Lett 495: 142-147

Hussain AI, Anwar F, Hussain Sherazi ST, Przybylski R (2008) Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem 108: 986-995

Inderjit SO, Duke (2003) Ecophysiological aspects of allelopathy. Planta 217: 529-539

Inderjit SO, Keating KI (1999) Allelopathy: Principles, procedures, processes, and promises for biological control. Adv Agron 67: 141-231

Jordán MJ, Martínez RM, Goodner KL, Baldwin EA, Sotomayor JA (2006) Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oil composition. Ind Crops Prod 24: 253-263

Kim JH (1997) Variation of monoterpenoids in *Artemisia feddi* and *Artemisia scoparia*. J Plant Biol 40: 267-274

Kim JH, Jung DH (2003) Variations in volatile compounds from *Elsholtzia cilliata*. J Plant Biol 46: 287-289

Kim JH, Jung DH, Park HK (2006) The composition of essential oil from *Nepeta cataria* and its effect on microorganism. J Ecol Field Biol 29: 381-387

Kobaisy M, Tellez MR, Webber CL, Dayan FE, Schrader KK, Wedge DE (2001) Phytotoxic and fungitoxic activities of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition. J Agric Food Chem 49: 3768-3771

Koudou J, Abena AA, Ngaissona P, Bessière JM (2005) Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. Fitoterapia 76: 700-703

Lambert RJW, Skandamis PN, Coote P, Nychas GJE (2001) A study of the minimum inhibitory concentration and mode of action of oregano essential oils, thymol and carvacrol. J Appl Microbiol 91: 453-462

Lee C, Kim H, Kho Y (2002) Agastinol and agastenol, novel lignans from *Agastache rugosa* and their evaluation in an apoptosis inhibition assay. J Nat Prod 65: 414-416

Lee JC, Choi YH, Kim YH (1994) Essential oils in aerial parts of *Agastache rugosa* O. Kuntze. Kor J Medic Crop Sci 2: 168-173 (Abstract only in English)

Lopez P, Sanchez C, Batlle R, Nerin C (2005) Solid and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected food borne bacterial and fungal strains. J Agric Food Chem 53: 6939-6946

Mahmoud SS, Croteau R (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7: 366-373

Mallavarapu GR, Kulkarni RN, Ramesh S (2004) The essential oil composition of anise hyssop grown in India. Flavour Frag J 19: 351-353

Nostro A, Germanò MP, D'Angelo V, Marino A, Cannatelli MA (2000) Extraction methods and bioautography for evaluation of medicinal plant microbial activity. Lett Appl Microbiol 30: 379-384

Oh HM, Kang YJ, Lee YS, Park MK, Kim SH, Kim HJ, Seo HG, Lee JH, Chang KC (2006) Protein kinase G-dependent heme oxygenase-1 induction by *Agastache rugosa* leaf extract protects RAW264.7 cells from hydrogen peroxide-induced injury. J Ethnopharmacol 103: 229-235

Oliveira D, Leitâo GG, Bizzo HR, Lopes D, Alviano DS, Alviano CS, Leitâo SG (2007) Chemical and antimicrobial analysis of essential oil of *Lippia origanoides* H.B.K. Food Chem 101: 236-240

Rietjens I MCM, Boersma MG, van der Woude H, Jeurissen SMF, Schutte ME, Alink GM (2005) Flavonoids and alkenylbenzenes: Mechanisms of mutagenic action and carcinogenic risk. Mutat Res 574: 124-138

Rizvi SJH, Rizvi V (1992) Allelopathy Basic and Applied Aspects. Chapman and Hall, London, pp 480

Rodilla JM, Tinoco MT, Morais JC, Gimenez C, Cabrera R, Martín-Benito D, Castillo L, Gonzalez-Coloma A (2008) *Laurus novocanariensis* essential oil: Seasonal variation and valorization. Biochem Syst Ecol 36: 167-176

Romagni J, Allen SN, Dayan FE (2000) Allelopathic effects of volatile cineoles on two weedy plant species. J Chem Ecol 26: 303-313

Rota MC, Herrea A, Martínez RM, Sotomayor JA (2008) Antimicrobial activity and chemical composition of *Thymus hyemalis* essential oils. Food Contr 19: 681-687

Salamci E, Kordali S, Kotan R, Cakir A, Kaya Y (2007) Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum*. Biochem Syst Ecol 35: 569-581

Sefidkon F, Abbasi K, Jamzad Z, Ahmadi S (2007) The effect of distillation methods and stage of plant growth on the essential oil content and composition of *Satureja rechingeri* Jamzad. Food Chem 100: 1054-1058

Shin S, Kang CA (2003) Antifungal activity of the essential oil of *Agastache rugosa* Kuntze and its synergism with ketoconzole. Lett Appl Microbiol 36: 111-115

Smith RL, Adams TB, Doull J, Feron VJ, Goodman JI, Marnett LJ, Portoghese PS, Waddell WJ, Wagner BM, Rogers AE, Caldwell J, Sipes IG (2008) Safety assessment of allylalkoxybenzene derivatives used as flavouring substances- methyl eugenol and estragole. Food Chem Toxicol 40: 851-870

Song JH, Kim MJ, Kwon HD, Park IJ (2001) Antimicrobial activity and components of extracts from *Agastache rugosa* during growth period. J Food Sci Nutr 6: 10-15

Stebbing ARD (1982) Hormesis – The stimulation of growth by low levels of inhibitors. Sci Total Environ 22: 213

Swain T (1977) Secondary compounds as protective agents. Annu Rev Plant Physiol 28: 479-501

Tepe B, Daferera C, Tepe A, Polissiou A, Sokmen A (2007) Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hud.-Mor. from Turkey. Food Chem 103: 1358-1364

Trivino MG, Johnson CB (2000) Season has a major effect on the essential oil yield response to nutrient supply in *Origanum majorana*. Hort Sci Biotech 75: 520-527

van Vuuren SF, Viljoen AM, Ozek T, Demirici B, Baser KHC (2007) Seasonal and geographical variation of *Heteropyxis natalensis* essential oil and the effect thereof on the antimicrobial activity. S Afric J Bot 73: 441-448

Vokou D, Douvli P, Blionis GJ, Halley JM (2003) Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. J Chem Ecol 29: 2281-2301

Wannissorn B, Jarikasem S, Siriwangchai T, Thubthimthed S (2005) Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia 76: 233-236

Wilson LA, Senechal NP, Widrlechner MP (1992) Headspace anal-

ysis of the volatile oils of *Agastache*. J Agric Food Chem 40: 1362-1366

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64: 3-19

Yun KW, Jeong HJ, Kim JH (2008) The influence of the growth season on the antimicrobial and antioxidative in *Artemisia princes* var. orientalis. Ind Crops Prod 27: 69-74