

## Antimicrobial Activity and Components of Extracts from *Agastache rugosa* during Growth Period

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### Abstract

Antimicrobial activities of volatile flavor, water and methanol extracts from *Agastache rugosa* were investigated. The volatile flavor extract was obtained from *A. rugosa* by simultaneous distillation-extraction (SDE) method. Antimicrobial activity was investigated by disc diffusion method against several microorganisms, four species of Gram positive, three species of Gram negative and two species of yeast. The volatile flavor extracts had strong antimicrobial activity against *C. utilis* and *S. cerevisiae*. During the growth period, a difference in antimicrobial activity among volatile flavor extracts from *A. rugosa* was not shown. The water extract of above 10 mg/disc showed antimicrobial activity. Methanol extracts from *A. rugosa* harvested in June showed antimicrobial activity against tested Gram positive and Gram negative bacteria, showed weak antimicrobial activity against the bacteria from those harvested in July and August. In particular, antimicrobial activity against *V. parahaemolyticus* was stronger than that against other bacteria. Water and methanol extracts did not inhibit yeast, *C. utilis* and *S. cerevisiae*. To further elucidate the effective components, volatile flavor extracts was analyzed by GC/MS. Harvested in June, the components included 8 phenols (93.031%), 18 terpenes (5.230%), 12 alcohols (1.300%), 8 alkanes (0.181%), 1 ester (0.056%), 2 ketones (0.033%), 2 aldehydes (0.011%) and 1 pyrrole (0.007%). In July, the components included 6 phenols (94.366%), 19 terpenes (3.394%), 11 alcohols (2.045%), 1 ester (0.039%), 2 ketones (0.028%), 1 furan (0.005%) and 1 aldehyde (0.005%). And in August, the components included 7 phenols (95.270%), 19 terpenes (2.951%), 13 alcohols (1.399%), 1 ester (0.063%), 2 aldehydes (0.016%), 2 ketones (0.011%), 1 alkane (0.006%), 1 acid (0.005%) and 1 pyrrole (0.005%). The major component of volatile flavors was estragole, a phenolic compound.

**Key words:** *Agastache rugosa*, volatile flavor, water extract, antimicrobial activity, estragole

### INTRODUCTION

Plants have both volatile and nonvolatile compounds and confers odor and flavor as well as sensory impact. The volatile constituents give a plant its distinctive odor, whereas the non-volatile components are either inert or may be implicated in a diverse number of physiological effects when consumed. They can also serve in many cases as precursors for the formation of volatile flavor components. The intensity of the aroma and types of physiological effect show wide variation between plant families and species (1).

As a part of the continuing search for physiologically active substances toward a development of potential resources from traditionally medicinal materials, we have examined the antimicrobial properties of the volatile flavor components from *Agastache rugosa*. This plant, commonly known in Korea as Bang-Ah, is a perennial herb belonging to the Labiatae family and cultivated in whole boundary of Korea. This plant has been used as a traditional medicine for the common cold, perspiration, vomit and boils in addition to consumption as a spice in the country (2,3).

Wilson et al. (4) reported that a major component was methylchavicol in a headspace analysis of the volatile oil of

*Agastache* species, and *A. rugosa* produced more volatiles than an *A. foeniculum*, and had less diversity in its volatile composition. Also, methanol extracts of *A. rugosa* reportedly have antimicrobial activity against *E. coli*, *V. parahaemolyticus*, *S. aureus*, *B. subtilis*, *S. cerevisiae*, *A. oryzae* and *A. niger* (5,6) as well as antioxidative activity (7,8). In a previous study, we have investigated the antimicrobial activity of volatile flavors from fresh leaves and stem, and dried leaves and stem of *A. rugosa*, against microbes. Among them, the volatile flavor from fresh and dried leaves of *A. rugosa* exhibited a strong antimicrobial activity (9).

In the present experiments, we examined the antimicrobial activity of the volatile flavor, water and methanol extracts from leaves of *A. rugosa* during its growth period and identified and characterized volatile flavor components by GC/MS.

### MATERIALS AND METHODS

#### Material

The leaves of *A. rugosa* cultivated in Manduck-dong (Pusan, Korea) were harvested from June to August in 1998 and immediately transported to the laboratory, after harvest, and then extracted within 2h by SDE method.

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### Extractions

#### Volatile flavor extraction

The leaves of *A. rugosa* (1 kg) in distilled water (3.5 L) were extracted with 35 mL distilled diethyl ether for 3 h using a modified SDE (simultaneous distillation-extraction) apparatus of Liken and Nickerson type at atmospheric pressure (Fig. 1) (9). This procedure was repeated 2 times until the entire sample was utilized (2 kg). The extract was concentrated up to 25 mL under a gentle stream of nitrogen and stored at 4°C.

#### Water extraction

After extraction of volatile flavor compounds from leaves of *A. rugosa*, a part of the distilled water was filtered through filter paper (Whatman No. 2) and then dried by using a vacuum evaporator (Buchi R-114, Switzerland). The extract was stored in a desiccator.

#### Methanol extraction

After extraction of volatile flavor compounds from the leaves of *A. rugosa*, a part of the leaves was dried by dry oven at 70°C. Ten times volume of methanol was added to the dried leaves (50 g) for 24 h, at room temperature. The methanol extract was filtered through filter paper (Whatman No. 2) and then concentrated under a vacuum rotary evaporator (Buchi R-114, Switzerland). The extract was stored in a desiccator.

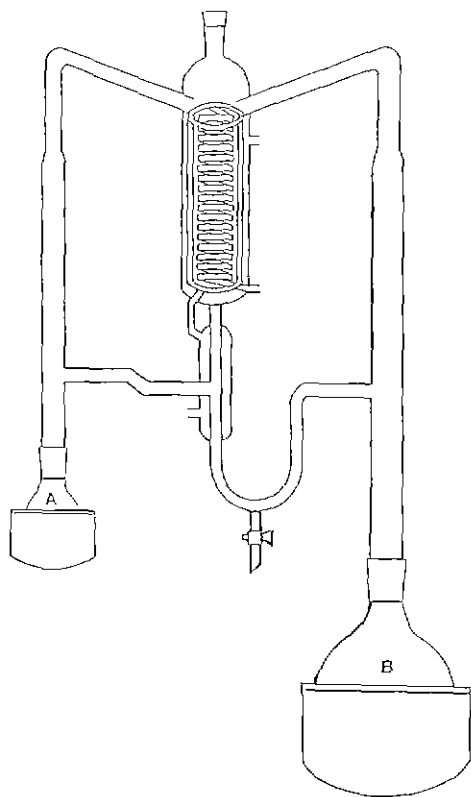


Fig. 1. Simultaneous distillation-extraction apparatus used for the extraction of volatile flavor components.  
A, diethyl ether; B, H<sub>2</sub>O + *Agastache rugosa*

### Test microorganisms and medium

Nine species of microorganisms were used for the assay of antimicrobial activity. Four species of Gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*), three species of Gram negative (*Escherichia coli*, *Salmonella typhi* and *Vibrio parahaemolyticus*) and two species of yeast (*Candida utilis* and *Saccharomyces cerevisiae*) were purchased from Korean Collection for Type Culture (KCTC).

According to the method by Song et al. (9), Tryptic soy broth (Difco, USA) and Mueller Hinton agar (Difco, USA) were used for cultivation of microorganisms and an antimicrobial activity test, respectively.

### Antimicrobial activity test

According to the disc diffusion method by Bauer et al. (10), sterilized filter paper discs (1.0 mm in thickness, 8.0 mm in diameter, Toyo Rochi Kaisha, Ltd., Japan) were saturated with volatile flavor extracts from *A. rugosa* for the sample and saturated with diethyl ether for the blank. Then, the disc was placed on the surface of the medium and the uniformly spread with 100 µL of each indicator. The plates were inverted and incubated at 36°C for 18 h. After incubation, antimicrobial activity was determined by diameter of a clear inhibition zone around the discs. Also, water and methanol extracts were tested using above same method.

### Instrumental analysis (GC/MSD)

A HP model 5890A series II GC interfaced to a HP model 5989A mass spectrometer was used for MS identification of GC components. The column used was a HP-5 crosslinked 5% Phenylmethyl Silicone capillary column (50 m × 0.32 mm I.d. × 1.05 µm film thickness, Hewlett-Packard Co.). Helium was used as the carrier gas (2.5 mL/min) and the split ratio was set to 10 : 1. Oven temperature was programmed from 50°C to 120°C at the rate of 2°C/min, and from 120°C to 220°C at the rate of 5°C/min with initial and final hold times of 5 and 20 min, respectively. In addition, injector and detector temperature was kept at 230°C and 260°C, respectively. Tentative identifications were based on standard MS library data (Wiley275.L).

## RESULTS AND DISCUSSION

### Antimicrobial activity

We have previously reported that volatile flavors from fresh and dried leaves of *A. rugosa* shows an inhibitory effect on antimicrobial activity (9). Therefore, in this study, we have examined the antimicrobial activity of the volatile flavors, water and methanol extracts from leaves of *A. rugosa* during its growth period. The volatile flavor extracts of *A. rugosa* were evaluated for antimicrobial activity against 4 Gram positive and 3 Gram negative bacteria and 2 yeast strains. The inhibitory effects of the volatile flavor extracts during its growth period are shown in Table 1. The volatile flavor extracts

**Table 1.** Antimicrobial activity of volatile flavor components from *Agastache rugosa* during growth period

Microorganisms	Period of harvest		
	Inhibition zone (mm)		
	June	July	August
<i>Bacillus cereus</i>	10.0	10.3	10.5
<i>Bacillus subtilis</i>	10.5	11.0	10.2
<i>Staphylococcus aureus</i>	9.3	11.0	9.5
<i>Staphylococcus epidermidis</i>	10.7	12.0	10.8
<i>Escherichia coli</i>	9.5	10.5	9.8
<i>Salmonella typhi</i>	10.8	11.0	10.5
<i>Vibrio parahaemolyticus</i>	12.3	12.2	11.2
<i>Candida utilis</i>	20.0	20.2	21.8
<i>Saccharomyces cerevisiae</i>	20.0	21.0	22.7

disc diameter, 8 mm

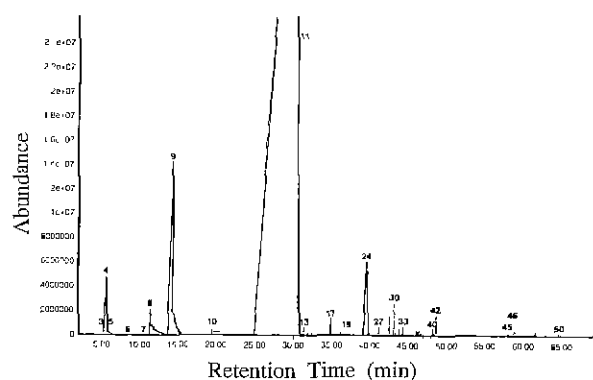
showed antimicrobial activity against tested all microorganisms and exhibited the strongest antimicrobial activity against *C. utilis* and *S. cerevisiae*. There was no significant difference among antimicrobial activity of extracts from *A. rugosa* during its growth period.

Also, the antimicrobial activity of the water and methanol extracts is shown in Table 2 and 3, respectively. The water extract above at 10 mg/disc concentration showed antimicrobial activity against Gram positive and Gram negative bacteria. The methanol extract from *A. rugosa* in June showed antimicrobial activity against tested Gram positive and Gram negative bacteria. In July and August, however, methanol extracts showed weak antimicrobial activity for bacteria. In particular, antimicrobial activity against *V. parahaemolyticus* is stronger than other bacteria. Water and methanol extracts

did not inhibit yeast, *C. utilis* and *S. cerevisiae*.

#### Identification of volatile flavor compounds in the extracts

Gas chromatograms of the volatile flavor extracts from *A. rugosa* during its growth period are shown in Fig. 2, 3 and 4. 51 volatile compounds were detected from *A. rugosa* harvested in June, including 8 phenols (93.031%), 18 terpenes (5.23%), 12 alcohols (1.3%), 8 alkanes (0.181%), 1 ester (0.056%), 2 ketones (0.033%), 2 aldehydes (0.011%) and 1 pyrrole (0.007%), and identification of these compounds is shown in Table 4. The presence of 41 volatile compounds from *A. rugosa* harvested in July was detected, including 6 phenols

**Fig. 2.** Gas chromatogram of volatile concentrate obtained from leaves of *Agastache rugosa* harvested in June. Peak numbers correspond to Table 4.**Table 2.** Antimicrobial activity of water extract from *Agastache rugosa* leaves during growth period

Microorganisms	The time of harvest											
	June (Inhibition zone, mm)				July (Inhibition zone, mm)				August (Inhibition zone, mm)			
	A	B	C	D	A	B	C	D	A	B	C	D
<i>Bacillus cereus</i>	+	10.0	11.3	12.0	-	10.0	10.7	11.7	-	9.3	10.2	11.2
<i>Bacillus subtilis</i>	+	9.7	10.8	11.8	-	9.3	10.2	11.0	-	8.5	10.0	11.0
<i>Staphylococcus aureus</i>	-	9.2	10.8	11.7	-	9.2	10.2	11.5	-	9.3	10.3	11.2
<i>Staphylococcus epidermidis</i>	+	9.8	11.7	13.0	-	9.2	10.5	11.7	-	9.0	10.5	11.5
<i>Escherichia coli</i>	+	10.2	11.5	13.0	-	9.0	10.0	11.0	-	9.0	10.2	11.7
<i>Salmonella typhi</i>	-	9.5	10.7	11.7	-	9.3	10.7	11.5	-	9.3	10.7	11.7
<i>Vibrio parahaemolyticus</i>	8.8	11.2	13.2	14.3	+	10.2	11.3	12.8	+	10.0	11.8	13.8
<i>Candida utilis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saccharomyces cerevisiae</i>	-	-	-	-	-	-	-	-	-	-	-	-

A, 5 mg/disc; B, 10 mg/disc; C, 15 mg/disc; D, 20 mg/disc; disc diameter, 8 mm; -, no inhibition; +, <8.5

**Table 3.** Antimicrobial activity of methanol extract from *Agastache rugosa* leaves during growth period

Microorganisms	The time of harvest											
	June (Inhibition zone, mm)				July (Inhibition zone, mm)				August (Inhibition zone, mm)			
	A	B	C	D	A	B	C	D	A	B	C	D
<i>Bacillus cereus</i>	9.0	9.7	10.0	10.5	+	+	8.5	+	+	+	9.3	9.0
<i>Bacillus subtilis</i>	8.7	9.2	9.3	9.3	+	+	+	+	-	+	+	+
<i>Staphylococcus aureus</i>	+	8.8	8.8	8.7	+	+	+	+	+	+	+	+
<i>Staphylococcus epidermidis</i>	8.7	9.3	9.7	9.5	+	+	+	+	+	+	8.5	+
<i>Escherichia coli</i>	+	8.8	9.3	9.3	+	+	+	+	+	+	+	+
<i>Salmonella typhi</i>	+	8.8	9.2	8.8	+	+	+	+	+	+	+	+
<i>Vibrio parahaemolyticus</i>	11.5	12.5	12.5	12.8	9.7	9.8	10.0	10.0	10.2	10.5	10.8	10.3
<i>Candida utilis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Saccharomyces cerevisiae</i>	-	-	-	-	-	-	-	-	-	-	-	-

A, 2.5 mg/disc; B, 5.0 mg/disc; C, 7.5 mg/disc; D, 10.0 mg/disc, disc diameter, 8 mm; -, no inhibition; +, <8.5

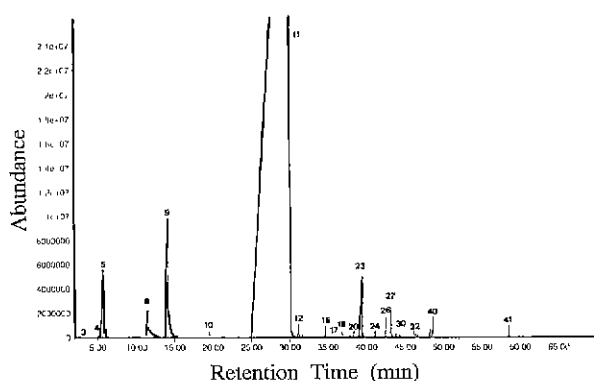


Fig. 3. Gas chromatogram of volatile concentrate obtained from leaves of *Agastache rugosa* harvested in July. Peak numbers correspond to Table 5.

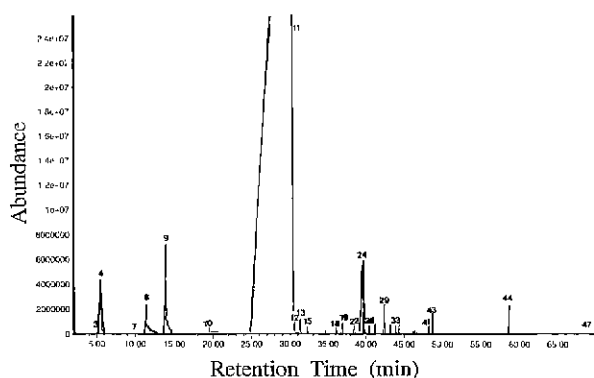


Fig. 4. Gas chromatogram of volatile concentrate obtained from leaves of *Agastache rugosa* harvested in August. Peak numbers correspond to Table 6.

(94.366%), 19 terpenes (3.394%), 11 alcohols (2.045%), 1 ester (0.039%), 2 ketones (0.028%), 1 furan (0.005%) and 1 aldehyde (0.005%) (Table 5). In August, the components were included 7 phenols (95.270%), 19 terpenes (2.951%), 13 alcohols (1.399%), 1 ester (0.063%), 2 aldehydes (0.016%), 2 ketones (0.011%), 1 alkane (0.006%), 1 acid (0.005%) and 1 pyrrole (0.005%) (Table 6).

The major compounds obtained from *A. rugosa* are phenols. Phenolic compounds were composed of estragole, trans-anethole, chavicol, anethole, eugenol, isoeugenol, 1-cyclopropyl-3,4-dimethoxyeugenol and 2,4-bis(dimethylbenzyl)-6-t-butylphenol. An amount of phenolic compound in *A. rugosa* increased during its growth period.

Wilson et al. (4) reported that estragole was the only compound to occur in the majority of *Agastache* spp. (4). These results were similar to our results in that estragole was the only compound to occur in the majority of the population during the growth period. Chun (11) reported that minimal inhibitory concentration (MIC) was shown at isoeugenol in the single component effect and estragole with isoeugenol in combination component effect. MIC for the microorganisms was found to be 200~400 µg/mL, 200~500 µg/mL, respectively (11).

Terpenes, the second predominant class of volatile flavor components identified, were composed of limonene, 1-methoxy-1-buten-3-yn, 5-methyl-3-[1-methylinyl]-1,4-hexadiene, cis-caryophyllene,  $\beta$ -caryophyllene, aromadendrene, trans-caryophyllene,  $\alpha$ -humulene, germacrene b, germacrene d, bicyclogermacrene,  $\alpha$ -muurolene,  $\delta$ -cardinene, caryophyllene oxide, [E]-farnesene, farnesene,  $\gamma$ -cardiene,  $\tau$ -muurolol, cis pinene hydrate and phytol. Terpene has been implicated in analgesic

Table 4. Volatile flavor compounds obtained from *Agastache rugosa* leaves harvested in June

Peak No.	RT min	Area %	Compound name	Peak No.	RT min	Area %	Compound name
1	2.114	0.009	1-penten-3-ol	27	41.179	0.051	$\alpha$ -humulene
2	3.117	0.005	2-penten-1-ol	28	42.313	0.025	germacrene b
3	5.159	0.051	trans-2-hexenol	29	42.538	0.099	germacrene d
4	5.681	0.731	cis-3-hexenol	30	43.254	0.208	bicyclogermacrene
5	5.885	0.031	2-hexen-1-ol	31	43.356	0.010	$\alpha$ -muurolene
6	5.946	0.043	1-hexenol	32	43.846	0.030	$\delta$ -cardinene
7	10.483	0.005	benzaldehyde	33	44.296	0.043	spathulenol
8	11.633	0.282	1-octen-3-ol	34	45.982	0.006	3-methoxy-cinnamaldehyde
9	14.325	3.473	limonene	35	46.145	0.032	1,6-germacradien-5-ol
10	19.731	0.056	1 octen 3 yl acetate	36	46.268	0.009	benzyl alcohol
11	28.963	92.788	estragole	37	46.391	0.022	[E]-farnesene
12	21.104	0.014	trans-anethole	38	46.636	0.017	globulol
13	31.390	0.062	chavicol	39	46.963	0.006	5-hepten-3-yn-2-one
14	31.850	0.012	1-methoxy-1-buten-3-yn	40	48.250	0.047	$\alpha$ -cardinol
15	32.371	0.009	anethole	41	48.353	0.008	cis-pinene hydrate
16	34.272	0.008	5-methyl-3-[1-methylinyl]-1,4-hexadiene	42	48.659	0.092	$\tau$ -muurolol
17	34.864	0.094	eugenol	43	52.941	0.007	nonacosane
18	36.152	0.022	isoeugenol	44	55.843	0.006	hexadecanoic acid
19	36.918	0.043	$\beta$ -bourbonene	45	57.999	0.026	pentacosane
20	37.358	0.007	1-methyl-1H-pyrrole	46	58.550	0.055	phytol
21	37.889	0.027	cis-jasmone	47	59.000	0.031	pentatriacontane
22	38.482	0.038	cis-caryophyllene	48	59.388	0.026	2,4-bis(dimethylbenzyl)-6-t-butylphenol
23	39.156	0.016	1-cyclopropyl-3,4-dimethoxyeugenol	49	61.493	0.032	triacontane
24	39.687	1.044	$\beta$ -caryophyllene	50	64.712	0.039	heptacosane
25	40.403	0.007	aromadendrene	51	66.020	0.031	2-methyl-tricosane
26	40.689	0.005	trans-caryophyllene				

**Table 5.** Volatile flavor compounds obtained from *Agastache rugosa* leaves harvested in July

Peak No.	RT min	Area %	Compound name	Peak No.	RT min	Area %	Compound name
1	2.111	0.005	1-penten-3-ol	22	38.999	0.022	1-cyclopropyl-3,4-dimethoxyeugenol
2	2.254	0.005	2-ethyl-furan	23	39.674	0.812	$\beta$ -caryophyllene
3	3.173	0.007	2-penten-1-ol	24	41.094	0.036	$\alpha$ -humulene
4	5.156	0.034	trans-2-hexenol	25	42.269	0.005	germacrene b
5	5.687	0.892	cis-3-hexenol	26	42.494	0.124	germacrene d
6	5.881	0.034	2-hexen-1-ol	27	43.209	0.223	bicyclogermacrene
7	5.953	0.064	1-hexenol	28	43.321	0.010	$\alpha$ -muurolene
8	12.079	0.859	1-octen-3-ol	29	43.802	0.027	$\delta$ -cardinene
9	14.128	2.019	limonene	30	44.251	0.035	spathulenol
10	19.666	0.039	1 octen 3 yl acetate	31	45.723	0.005	3-methoxy-cinnamaldehyde
11	28.694	94.125	estragole	32	46.111	0.041	germacrene-d-4-ol
12	31.172	0.117	chavicol	33	46.244	0.010	caryophyllene oxide
13	31.611	0.013	1-methoxy-1-buten-3-yn	34	46.356	0.021	[E]-farnesene
14	32.132	0.010	anethole	35	46.612	0.017	globulol
15	34.115	0.005	5-methyl-3-[1-methylinyl]-1,4-hexadiene	36	46.939	0.007	5-hepten-3-yn-2-one
16	34.677	0.064	eugenol	37	47.736	0.005	farnesene
17	36.046	0.028	isoeugenol	38	48.226	0.057	$\alpha$ -cardinol
18	36.853	0.068	$\beta$ -bourbonene	39	48.318	0.009	$\gamma$ -cardinol
19	37.876	0.021	cis-jasmone	40	48.615	0.103	$\tau$ -muurolol
20	38.406	0.053	cis-caryophyllene	41	58.526	0.044	phytol
21	38.519	0.007	trans-caryophyllene				

**Table 6.** Volatile flavor compounds obtained from *Agastache rugosa* leaves harvested in August

Peak No.	RT min	Area %	Compound name	Peak No.	RT min	Area %	Compound name
1	2.105	0.004	1-penten-3-ol	25	39.892	0.019	$\beta$ -cubebene
2	3.175	0.005	2-penten-1-ol	26	40.413	0.043	aromadendrene
3	5.129	0.019	trans-2-hexenol	27	40.659	0.007	trans-caryophyllene
4	5.610	0.601	cis-3-hexenol	28	41.149	0.054	$\alpha$ -humulene
5	5.834	0.033	2-hexen-1-ol	29	42.386	0.165	germacrene b
6	5.906	0.048	1-hexenol	30	42.626	0.014	germacrene d
7	9.809	0.009	benzaldehyde	31	43.142	0.057	bicyclogermacrene
8	12.047	0.524	1-octen-3-ol	32	43.336	0.009	$\alpha$ -muurolene
9	14.030	1.215	limonene	33	43.847	0.045	$\delta$ -cardinene
10	19.721	0.063	1 octen 3 yl acetate	34	44.296	0.046	spathulenol
11	28.578	95.011	estragole	35	45.972	0.007	3-methoxy-cinnamaldehyde
12	30.890	0.013	trans-anethole	36	46.425	0.010	1,6-germacradiene-5-ol
13	31.161	0.122	chavicol	37	46.258	0.014	benzyl alcohol
14	31.697	0.008	1-methoxy-1-buten-3-yn	38	46.371	0.019	[E]-farnesene
15	32.269	0.037	anethole	39	46.616	0.014	globulol
16	34.691	0.021	5-methyl-3-[1-methylinyl]-1,4-hexadiene	40	46.953	0.006	5-hepten-3-yn-2-one
17	34.967	0.011	eugenol	41	48.230	0.043	$\alpha$ -cardinol
18	36.111	0.043	isoeugenol	42	48.322	0.008	cis-pinene hydrate
19	36.939	0.097	$\beta$ -bourbonene	43	46.629	0.085	$\tau$ -muurolol
20	37.297	0.005	1-methyl-1H-pyrrole	44	58.551	0.104	phytol
21	37.654	0.017	cis-jasmone	45	60.564	0.005	benzenacetic acid
22	38.503	0.075	cis-caryophyllene	46	61.545	0.006	tricontane
23	39.105	0.033	1-cyclopropyl-3,4-dimethoxyeugenol	47	68.861	0.038	2-(4-methoxyphenyl) ethanol
24	39.606	0.906	$\beta$ -caryophyllene				

activity and antimicrobial activity was also found in Ginger (12-14), *Artemisia* sp. (15-17), *Poncirus trifoliata* (18) and *Aralia elata* (1).

In conclusion, the volatile flavors from *A. rugosa* showed strong antimicrobial activity against 7 species bacteria and 2 species yeast, and consisted of estragole, a phenolic compound. The antimicrobial activities of water and methanol extracts and the volatile components were not shown to be significantly different during the growth period. Also, the antimicrobial activity of extracts from *A. rugosa* could be the re-

sult of a combined action of several compounds. Further research is in progress by the authors to elucidate the bactericidal mechanism of volatile compounds either by single compound or by the mixture of the compounds to see the synergistic effects.

## ACKNOWLEDGMENTS

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