J. Food Sci. Nutr.
Vol. 4, No. 2, p. 97~102 (1999)

Antimicrobial Activity and Characterization of Volatile Flavor Extracts from *Agastache rugosa*

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Abstract

Antimicrobial activity and chemical composition of volatile flavor extracts from *Agastache rugosa* were investigated. The volatile flavor extracts were obtained from leaves and stems of *Agastache rugosa* by simultaneous distillation-extraction (SDE) method. Antimicrobial activity was investigated by disc diffusion and broth dilution methods against several microorganisms of *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Corynebacterium xerosis, Staphylococcus aureus, Staphylococcus epidermidis, Agrobacterium rhizogenes, Agrobacterium tumefaciens, Enterobacter cloacae, Escherichia coli, Salmonella typhi, Vibrio parahaemolyticus, Candida utilis and Saccharomyces cerevisiae*. Volatile flavor extracts from leaves have strong antimicrobial activity against *C. utilis* and *S. cerevisiae*. There was no significant difference in antimicrobial activity of extracts between fresh and dried leaves. Volatile flavor extracts from fresh and dried leaves inhibited the growth of *C. utilis* and *S. cerevisiae*. When 0.12% volatile flavor extract from fresh leaves were included in the medium, lag phase of *C. utilis* was extended 6 hr and that of *S. cerevisiae* extended 10 hr. When the same amount of extract from dried leaves was included, lag phase of *C. utilis* and *S. cerevisiae* was extended 2 hr. Further analyses were performed to elucidate the effective components of the extracts. The major component of volatile flavor was estragole, a phenolic compound. Minor components were determined to be terpenes, alcohols, acids, esters, ketones and aldehydes.

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

INTRODUCTION

Plants, either directly or indirectly, are probably most important for human beings as sources of food and medicine. There are thousands of different types of valuable plants throughout the world, that are utilized for the preparation of meals and medicines. Plants have both volatile and non-volatile compounds and confer odor and flavor as well as sensory impact (1). Numerous investigations have shown that several plant-emitted volatile compounds including phenols, aldehydes, ketones, alcohols, and other classes of natural products, exhibit antimicrobial properties against microorganisms such as *Aspergillus* (2,3), *Fusarium* (4), *Penicillium* (5) and other bacteria (6,7). Phenolic and terpene compounds are believed to be important for antimicrobial activity (8-11).

Agastache rugosa, commonly known in Korea as 'Bang-Ah', is a perennial herbs belong to the Labiatae family and cultivated throughout Korea (12,13). 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 (12-14). Several authors reported that methanol extract of *Agastache rugosa* had antimicrobial activity against *E. coli, V. parahaemolyticus, S. aureus, B. subtilis, S. cerevisiae, A. oryzae* and *A. niger* (15,16). Also, *Agastache rugosa* reportedly has potent antioxidative activity (17,18) and *Agastache rugosa* leaves were analyzed for moisture, proteins, fat, mineral, ascorbic acid and phenolic compound (19).

This research focused on the antimicrobial activity of the volatile flavor extracts from the leaves and stems of *Agastache rugosa* and identification and characterization of volatile flavor components by GC/MS analysis.

MATERIALS AND METHODS

Material

Leaves and stems of *Agastache rugosa* cultivated in Chungryong-dong (Pusan, Korea), were collected between July and August, 1997, and transported to the laboratory, immediately. Fresh *Agastache rugosa* were extracted within 2 hr by SDE and dried *Agastache rugosa* were extracted after air-drying. Volatile flavor extracts were stored at 4°C before antimicrobial activity test and analysis.

Volatile flavor extraction

The fresh leaves of *Agastache rugosa* (500 g) in 3.5 L of distilled water were extracted with 35 ml of redistilled diethyl ether for 3 hr using a modified SDE (simultaneous distillation-extraction) apparatus of Likens and Nickerson type at atmospheric pressure (20). This procedure was repeated 4 times until the entire sample was utilized (2 kg). Extracts were concentrated to 25 ml under a gentle stream of nitrogen and stored at 4°C. Fresh stems, dried leaves and stems were extracted by the same method.

Microorganisms and medium

Fourteen species of microorganisms were used for the

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antimicrobial activity test. Six species of Gram positive (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Corynebacterium xerosis, Staphylococcus aureus* and *Staphylococcus epidermidis*), six species of Gram negative (*Agrobacterium rhizogenes, Agrobacterium tumefaciens, Enterobacter cloacae, Escherichia coli, Salmonella typhi* and *Vibrio parahaemolyticus*) and two species of yeast (*Candida utilis* and *Saccharomyces cerevisiae*) were purchased from Korean Collection for Type Cultures (KCTC).

Tryptic soy broth (TSB, Difco, USA) and Mueller Hinton agar (Difco, USA) were used for cultivation of microorganisms and an antimicrobial activity test, respectively - *A. tumefaciens, B. cereus, B. megaterium, B. subtilis, C. xerosis, E. cloacae, E. coli, S. aureus, S. epidermidis* and *S. typhi*. For halophile such as *V. parahaemolyticus*, NaCl was added to the media to give a final concentration of 3%. Media for *A. rhizogenes* included 1% mannitol, 0.04% yeast extract, 0.05% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$ and 0.01% NaCl. And media for *C. utilis* and *S. cerevisiae* included 0.6% malt extract, 0.18% maltose, 0.6% dextrose and 0.12% yeast extract. The microorganisms were incubated at 30°C.

Antimicrobial activity test

According to the disc diffusion method by Bauer et al. (21), 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 *Agastache rugosa* for the sample and saturated with diethyl ether for the blank. Then the discs were placed on the surface of the medium and the uniformly spread with 100 µl of each indicator. The plates were inverted and incubated at 30°C for 18 hours. After incubation, antimicrobial activity was determined by the presence and diameter of a clear zone around the discs.

Effect of volatile flavor extracts on the growth of microorganisms

The bioassay was performed by a broth dilution method (22). Volatile flavor (40, 80, 120 µl) was first dissolved in 2 ml of N,N-dimethylformamide (DMF), and 50 µl of each mixture was added to 50 ml of appropriate media. Then, one-day old culture of the test microorganisms (*C. utilis* or *S. cerevisiae*) was inoculated and incubated by shaking (120 rpm) at 30°C.

Instrumental analysis (GC/MS)

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 (Willey275).

RESULTS AND DISCUSSION

Antimicrobial activity of volatile flavor extracts

The volatile flavor extracts from fresh and dried leaves of *Agastache rugosa* were evaluated for antimicrobial activity against 6 Gram positive and 6 Gram negative bacteria and 2 yeast strains. The inhibitory effects of the volatile flavor extracts from leaves are shown in Table 1. Volatile flavor extracts from leaves show antimicrobial activity against all the tested microorganisms. The volatile flavor extracts from leaves inhibited *C. utilis* and *S. cerevisiae* most effectively. Also, inhibition against *V. parahaemolyticus* was considerable. There was no significant difference in antimicrobial activity between extracts from fresh and dried leaves.

The inhibitory effects of the volatile flavor extracts from stems against test strains are shown in Table 2. Extracts from fresh and dried stems did not show any significant antimicrobial activity against the tested microorganisms.

Table 1. Antimicrobial activity of volatile flavor compounds from leaves of *Agastache rugosa*

Microorganisms	Inhibition zone, mm	
	Fresh leaves	Dried leaves
B. cereus	11.2	12.0
B. megaterium	12.7	12.0
B. subtilis	11.2	11.8
C. xerosis	10.7	10.7
S. aureus	11.0	12.3
S. epidermidis	12.5	12.3
A. rhizogenes	9.5	10.3
A. tumefaciens	10.5	11.7
E. cloacae	11.5	12.0
E. coli	11.0	12.3
S. typhi	12.5	11.2
V. parahaemolyticus	15.2	13.8
C. utilis	23.0	23.3
S. cerevisiae	22.8	22.0

disc diameter, 8 mm

Table 2. Antimicrobial activity of volatile flavor compounds from stem of *Agastache rugosa*

Microorganisms	Inhibition zone, mm	
	Fresh stem	Dried stem
B. cereus	—	—
B. megaterium	—	—
B. subtilis	—	—
C. xerosis	—	±
S. aureus	—	—
S. epidermidis	±	—
A. rhizogenes	9.0	—
A. tumefaciens	—	—
E. cloacae	±	—
E. coli	±	—
S. typhi	—	—
V. parahaemolyticus	±	±
C. utilis	±	—
S. cerevisiae	±	—

disc diameter, 8 mm; —, no inhibition zone; ±, trace (≦8.5 mm)

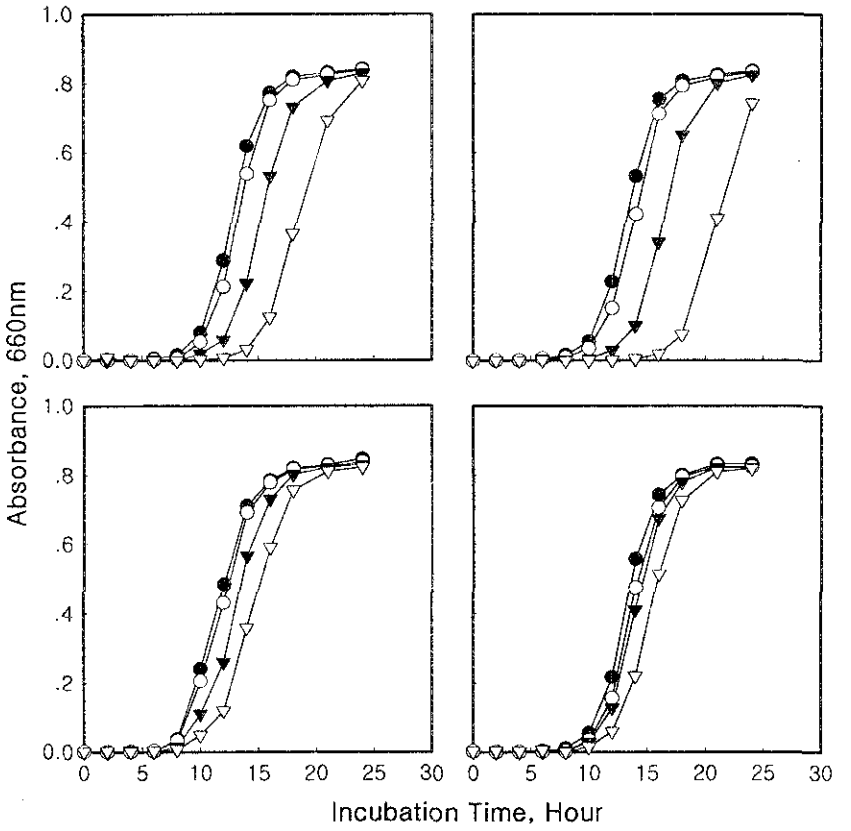

Fig. 1. Antimicrobial activity of volatile flavor components from *Agastache rugosa* on the growth of microorganisms.
A. Effect of volatile flavor from fresh leaves on *C. utilis*; B. Effect of volatile flavor from fresh leaves on *S. cerevisiae*; C. Effect of volatile flavor from dried leaves on *C. utilis*; D. Effect of volatile flavor from dried leaves on *S. cerevisiae*. control (●), 0.04% volatile flavor extracts (○), 0.08% volatile flavor extracts (▼), 0.12% volatile flavor extracts (▽).

Effect of volatile flavor extracts on the growth of microorganisms

The volatile flavor extracts from fresh and dried leaves inhibited the growth of *C. utilis* and *S. cerevisiae* (Fig. 1). When 0.12% volatile flavor extracts from fresh leaves were included in the medium, the lag phase of *C. utilis* was extended by 6 hr and that of *S. cerevisiae* extended by 10 hr. When the same amount of extracts from dried leaves were included, lag phase of *C. utilis* and *S. cerevisiae* was extended by 2 hr.

Identification of volatile flavor compounds in the extracts

Gas chromatograms of the volatile flavor extracts from fresh and dried leaves of *Agastache rugosa* are shown in Fig. 2 and 3, respectively.

67 volatile compounds were detected from fresh leaves, including 7 phenolics, 16 terpenes, 5 alcohols, 1 ester, 2 ketones, 2 acids, 1 aldehyde, and 5 others. Among them, 34 compounds were identified and shown in Table 3. Phenolic compounds, the predominant class of the volatile flavor components identified, were composed of estragole (92.11%), trans-anethole (4.53%), chavicol (0.18%), anethole (0.29%), eugenol (0.09%), isoeugenol (0.03%), and benzoic acid (0.01%). In the case of dried leaves, the presence of 47 volatile compounds was established, including 4 phenolics, 14 terpenes, 1 ester, 4 alcohols, 2 ketones, 2 acids, 1 aldehyde, and 6 miscellaneous. Among these, 28 compounds were identified and shown in Table 4. Phenolic compounds were composed of estragole (92.05%), trans-anethole (0.07%), chavicol (0.22%), and eugenol (0.06%). There was no significant difference in volatile flavor components between fresh and dried leaves.

Wilson et al. reported that estragole was the only compound to occur in the majority of *Agastache* spp. The analysis of volatile flavor extracts from *Agastache* populations were examined for some species, 5 populations of *A. foeniculum*, 2 populations of *A. rugosa*, and 3 putative hybrids. Headspace volatiles from *A. foeniculum* contained 0.0~97.0% estragole in the inflorescence and 0.0~90.2% estragole in their leaves. Estragole was also the dominant volatile in the headspace of the inflorescence and leaves of *A. rugosa* with 55.6~86.2% and 69.7~97.4%, respectively. The putative hybrids contained 0.8~74.0% estragole in the inflorescence and 0.0~8.4% in the leaves (23). These results nearly matched our results in terms that estragole was the only compound to occur in the majority of the population.

The gas chromatogram of volatile flavor extracts obtained from fresh stems is shown in Fig. 4 and that of dried stems

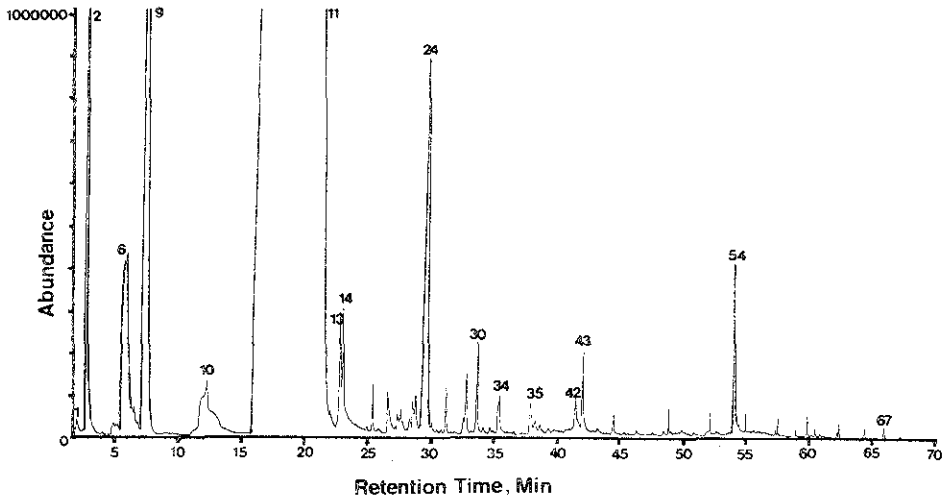

Fig. 2. Gas chromatogram of volatile flavor concentrate obtained from fresh leaves of *Agastache rugosa*. Peak numbers correspond to listed in Table 3.

Table 3. Volatile flavor compounds obtained from fresh leaves of *Agastache rugosa*

Peak No.	RT (min)	Area, %	Compound name
		Phenolics (92.78%)	
11	21.47	92.11	estragole
12	21.98	0.07	trans-anethole
13	22.73	0.18	chavicol
14	22.97	0.29	anethole
17	26.59	0.09	eugenol
19	27.36	0.03	isoeugenol
50	48.99	0.01	benzoic acid
		Terpenes (4.53%)	
4	5.13	0.01	sabinene
6	5.87	0.64	myrcene
9	7.64	2.19	limonene
16	25.41	0.05	bicycloelemene
21	28.34	0.02	β-elemene
23	28.79	0.06	cis-caryphyllene
24	29.74	1.02	β-caryophyllene
27	31.21	0.05	α-humulene
28	32.59	0.02	germacrene b
29	32.80	0.08	germacrene d
30	33.68	0.12	bicyclogermacrene
31	34.16	0.01	α-muurolene
32	34.69	0.01	γ-cardinene
34	35.40	0.08	δ-cadinene
46	46.29	0.01	γ-selinene
54	54.02	0.18	phytol
		Alcohols (1.47%)	
1	2.10	0.02	trans-2-hexenal
2	2.95	1.06	cis-3-hexenol
7	6.05	0.28	1-octen-3-ol
8	6.56	0.05	3-octanol
35	37.87	0.06	spathulenol
		Ester (0.51%)	
10	12.23	0.51	1-octen-3-yl-acetate
		Ketones (0.09%)	
5	5.28	0.05	4-octen-3-one
22	28.57	0.04	cis-jasmone
		Acids (0.03%)	
39	39.76	0.01	2-methyl-propanoic acid
53	52.11	0.02	hexadecanoic acid
		Aldehyde (0.03%)	
3	4.94	0.03	benzaldehyde
		Miscellaneous (0.31%)	
20	27.63	0.04	β-bourbonene
25	30.07	0.01	epi-bicyclosesqui phellandrene
42	41.45	0.10	α-cardinol
43	42.00	0.13	τ-muurolol
45	44.46	0.03	mintsulfide

is shown in Fig. 5.

There were 3 peaks found in the volatile flavor extracts obtained from the fresh stem (Table 5). These included estragole (87.04%), limonene (10.73%), and β-caryophyllene. In the case of the dried stem, the presence of 5 volatile compounds was including estragole (76.80%), limonene (6.19%), β-caryophyllene, and (+)spathulenol (Table 6).

In conclusion, considering various reports on biologically active volatile compounds from higher plants and herbs, the antimicrobial activity of the extracts from *Agastache rugosa* should be the results of combined action of several compounds. The volatile flavor compounds from *Agastache rugosa* described herein may be considered as potential antimicrobial agents for cosmetic and food products. Since *Agastache rugosa* has been consumed for centuries, either extracts or purified flavor compounds may be considered safe for practical use as oral products. Also, research is in progress by the authors to elucidate the antimicrobial mechanism of either a single or a mixture of volatile components to see the synergistic effects.

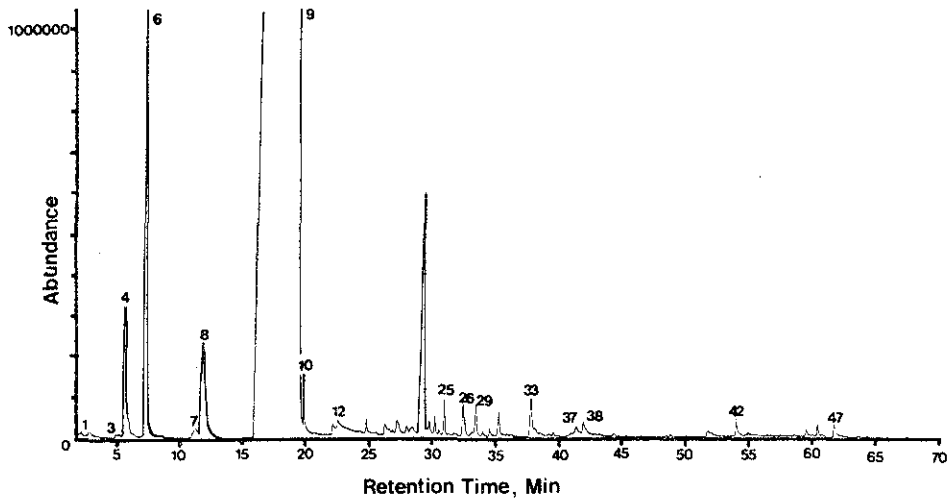

Fig. 3. Gas chromatogram of volatile flavor concentrate obtained from dried leaves of *Agastache rugosa*. Peak numbers correspond to listed in Table 4.

Table 4. Volatile flavor compounds obtained from dried leaves of *Agastache rugosa*

Peak No.	RT (min)	Area, %	Compound name	Peak No.	RT (min)	Area, %	Compound name
		Phenolics (92.40%)				**Alcohols (0.34%)**	
9	19.45	92.05	estragole	1	2.78	0.04	cis-3-hexenol
11	22.12	0.07	trans-anethole	5	6.24	0.02	3-octanol
12	22.54	0.22	chavicol	7	11.22	0.10	linalool
14	26.25	0.06	eugenol	33	37.79	0.18	spathulenol
		Terpenes (5.34%)				**Ketones (0.23%)**	
3	5.14	0.02	sabinene	10	19.80	0.16	pulegone
4	5.74	0.83	myrcene	19	28.42	0.07	cis-jasmone
6	7.42	2.30	limonene			**Acids (0.05%)**	
13	24.79	0.04	bicycloelemene	35	39.57	0.01	2-methyl-propqanoic acid
18	27.97	0.05	β-elemene	40	51.81	0.04	hexadecanoic acid
20	29.32	1.48	β-caryophyllene			**Aldehyde (0.02%)**	
25	30.96	0.10	α-humulene	2	4.92	0.02	benzaldehyde
26	32.43	0.12	germacrene b			**Miscellaneous (0.44%)**	
27	32.57	0.06	germacrene d	17	27.23	0.12	β-bourbonene
29	33.46	0.14	bicyclogermacrene	22	29.76	0.05	epi-bicyclosesqui -phellandrene
30	34.003	0.02	α-muurolene	23	30.19	0.06	aromadendrene
31	4.54	0.02	γ-cardinene	24	30.54	0.02	alloaromadendrene
32	35.26	0.10	δ-cadinene	37	41.39	0.09	α-cardinol
42	54.01	0.06	phytol	38	41.91	0.11	τ-muurolol
		Ester (0.89%)					
8	11.91	0.89	1 octen 3 yl acetate				

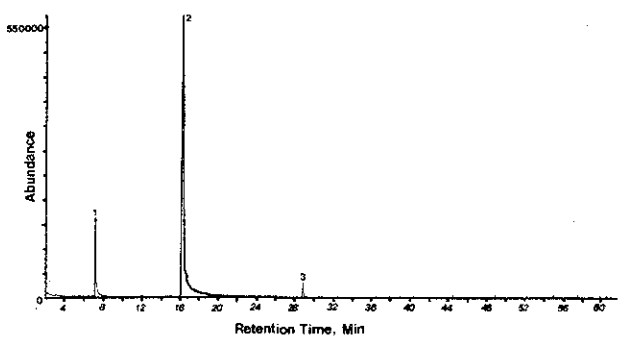

Fig. 4. Gas chromatogram of volatile flavor concentrate obtained from fresh stem of *Agastache rugosa*. Peak numbers correspond to listed in Table 5.

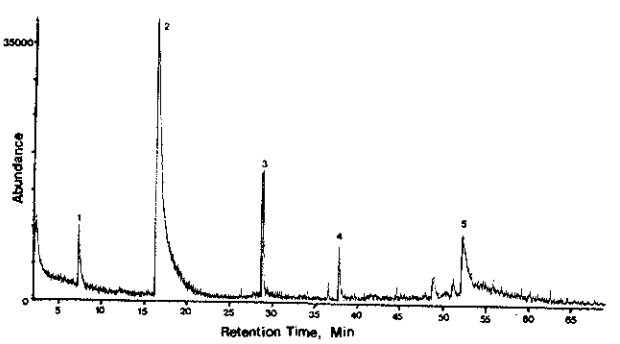

Fig. 5. Gas chromatogram of volatile flavor concentrate obtained from dried stem of *Agastache rugosa*. Peak numbers correspond to listed in Table 6.

Table 5. Volatile flavor compounds obtained from fresh stem of *Agastache rugosa*

Peak No.	RT (min)	Area, %	Compound name
		Phenolic (87.04%)	
2	16.23	87.04	estragole
		Terpenes (12.96%)	
1	7.18	10.73	limonene
3	28.81	2.23	β-caryophyllene

Table 6. Volatile flavor compounds obtained from dried stem of *Agastache rugosa*

Peak No.	RT (min)	Area, %	Compound name
		Phenolic (76.80%)	
2	16.30	76.80	estragole
		Terpenes (14.50%)	
1	7.31	6.19	limonene
3	28.83	8.31	β-caryophyllene
		Alcohol (4.79%)	
4	37.69	4.79	(+) spathulenol

ACKNOWLEDGEMENTS

This paper was supported by Dong-A University Research Fund in 1997.

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(Received April 15, 1999)