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Comparison on Volatile Flavor Compounds in Cultivated and Wild *Pimpinella brachycarpa*

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ABSTRACT The volatile flavor compounds of wild and cultivated *chamnamul* (*Pimpinella brachycarpa*), an aromatic medicinal plant, were isolated via the simultaneous distillation extraction method and analyzed by GC and GC-MSD. From the oils of the wild *chamnamul*, 56 volatile flavor compounds were identified, and the major constituents were found to be sabinene (58.37 ppm) and germacrene-D (45.73 ppm). From the oils of cultivated *chamnamul*, 36 volatile flavor compounds were identified--the major constituents were identified as β-selinene (38.41 ppm) and myrcene (12.76 ppm).

KEYWORDS: *Pimpinella brachycarpa, chamnamul*, volatile flavor compounds, GC, wild, cultivated

INTRODUCTION

Chamnamul is a wild edible plant in Korea, which is widely consumed in the early spring. This species grows wild in Korean mountain regions, such as *Odae* mountain, and recently began to be cultivated on farms. The scientific name of this plant is *Pimpinella brachycarpa* KITAGAWA, a member of the Umbelliferae family, and is also referred to as *Pandinamul*. *Chamnamul* has been used as Namul with sauce in its raw state or after blanching at early bud (Choi 1995).

Chamanmul was also used medicinally. In 『Korean resources plants』, it is reported to have been used as a folk remedy for the treatment of fever, hypertension, stanching, pneumonia, paralysis, etc (Kim 1996). In 『*Dong Ei Bo Gam*』, its taste is described as mild, sweet and slightly spicy and it is reported to be efficient at eliminating coldness, dysentery, etc (Yoon and Jang 1992).

Chamnamul is reported to harbor isopimpinellin, pimpinelin, 5,8-dioxypsoralen, isobergapten, and anethole, one of the components of fennel seed (Cho 1996). Pimpinellin is a type of furanocoumarin, and has been used for photochemotherapy of mycosis, atropic eczema, psoriasis, etc., including skin-related diseases (Kim 2007). The principal flavors were reported to be isobutanal, β-myrcene, trans-caryophyllene, and trans β-farnesene, and it was examined for use as a spice (Song 1997). With regard to its biological activities, *chamnamul* has been investigated for its anti-thrombosis activity (Kwon et al 2004). As a study for the functional activity of *chamnamul*, antioxidant activity and antimutagenic activity were reported for TA98 and TA100 in an *in vitro* study (Ham 1988).

The flavor components of plants have been suggested as a means of self-defense, and are often reported to evidence biological activities (Lee 2002). Recently, the area of cultivation of wild aromatic edible plants is being enlarged, due primarily to increasing interest in medicinal wild vegetables (Cho 2000). A study of the oil of cultivated and wild chamomile reported a change in the essential oil content in the proportion of pharmacologically active compounds in comparison with the results of the earlier survey, the characteristics of the oils of cultivated and wild chamomile populations remained identical (Szoke et al 2004).

The objectives of the present work were, therefore, to determine the volatile flavor compounds of the essential oil of *chamnamul*, in order to compare them with the biologically active volatile compounds observed after cultivated and wild production.

MATERIALS AND METHODS

Plant materials

Wild *chamnamul* populations were obtained from soily areas of *Jeongseon-gun* near the *Odae* mountain in Korea. The cultivated *chamnamul* was purchased from a farm in *Dunnae-myun Hoengseong-gun*. These were detached and

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Table 1. Analytical conditions of GCD for the identification of volatile flavor compounds

Instrument	Hewlett-Packard G1800B GCD
Detector	GC-MSD
Electron Multiplier Voltage	1600
Column	HP-5 (0.25×300 mm)
Carrier gas	He (0.8 mL/min)
Injector temperature	250°C
Detector temperature	280°C
Split mode	Splitless
Program	40°C (3 min)→3°C/min→ 240°C (5 min)
Injection volume	1 μL
Internal Standard	Nonadecane (10 ppm)
Library	Wiley 275L

subjected to analysis immediately after collection. The reagent used was of 1st grade.

Isolation of volatile compounds

The essential oils of wild and cultivated *chamnamul* were obtained by simultaneous distillation extraction. The fresh *chamnamul* materials (100 g) were coarsely minced and placed in a flask containing 1 L of water, and nonadecane as an internal standard and steam-distilled in a steam distillation extractor for 1 hr. The extraction was concentrated with a Kuderna Danish apparatus and N_2 gas, and the final volume was controlled to 0.25 mL.

GC Analysis

The extracted oils were analyzed by a GC-MSD instrument (Hewlett-Packard, G1800B) linked to a Wiley 275L database under the following conditions (Table 1).

Identification of chemical compounds

The constituents were identified via gas chromatography by comparison of their GC retention time. Further identification was verified by comparison of their mass spectra with those stored in the GC-MSD databases (Wiley 275 libraries). The relative component concentrations were acquired from GC peak areas and calculated on the base of internal standards. The method used was as follows:

$$※ \text{Retention Index (RI)} = [n + \frac{\text{Log X} - \text{Log Y}}{\text{Log Z} - \text{Log Y}}] \times 100$$

n: Carbon number of hydrocarbons
X: Retention time of unknown peak
Y: Retention time of standard hydrocarbons of C(n)
Z: Retention time of standard hydrocarbons of C(n+1)

$$\text{ppm} = \frac{\text{Area of compound} \times \text{amount of I.S.}}{\text{Area of I.S.} \times \text{amount of sample}/10^6}$$

I.S.: internal standard

Table 2. Volatile flavor compounds[1)] of cultivated and wild *Pimpinella brachycarpa*

Peak No.	RT[2)] (min)	RI[3)]	Volatile compounds	Cultivated (ppm)	Wild (ppm)
1	8.73	895	Heptanal		0.25
2	10.05	928	a-Pipene	0.78	20.63
3	12.07	970	β-Pinene	12.42	
4	12.48	978	Sabinene		58.37
5	13.08	988	Myrcene	12.76	6.62
6	13.47	995	1-Phellandrene		1.61
7	13.84	1002	d-3-Carene		9.44
8	14.02	1006	a-Terpinene		0.83
9	14.22	1011	1-Methyl-4-(1-methylethyl)cyclohexene	0.03	14.56
10	14.65	1020	Limonene	5.39	
11	15.52	1033	Trans-β-ocimene		2.78
12	15.57	1040	Bezeneacetaldehyde		3.47
13	16.08	1051	γ-Terpinene	1.37	1.39
14	17.32	1075	a-Terpinolene	0.10	0.43
15	17.79	1083	2-Nonanone		0.18
16	18.17	1090	Linalool	0.12	0.26
17	19.49	1117	Allo-ocimene		1.07
18	20.70	1143	2,6-Nonadienal		0.14
19	21.83	1166	3-Cyclohexen 1-ol		2.25
20	22.44	1178	a-Terpineol		0.47

Table 2. Continued

Peak No.	RT[2)] (min)	RI[3)]	Volatile compounds	Cultivated (ppm)	Wild (ppm)
21	23.69	1203	β-Cyclocitral		0.12
22	25.76	1249	2-Decenal		0.17
23	26.71	1270	Bicyclo[2.2.1]heptan-2-ol		0.37
24	28.48	1307	γ-Elemene		0.20
25	28.72	1312	2,6-Octadienoic acid	0.83	
26	28.98	1319	Bicycloelemene		2.41
27	30.58	1356	a-Copaene	0.17	1.05
28	31.65	1380	β-Elemene	6.26	16.50
29	32.78	1405	β-Caryophyllene	7.87	
30	33.05	1412	β-Cubebene	0.61	3.99
31	33.48	1423	a-Guaiene	0.85	
32	34.04	1437	a-Humulene	2.00	6.81
33	34.92	1458	Trans-β-farnesene		8.36
34	35.88	1481	Germacrene-D		45.73
35	36.01	1484	β-Selinene	38.41	
36	36.20	1488	a-Bergamotene		11.55
37	36.46	1494	β-Bisabolene		0.96
38	36.64	1499	1-Methyl-4-(1,2,2-trimethyl)benzene	6.63	
39	36.71	1500	2.6-Bis(1.1-dimethylethyl)phenol		3.32
40	37.06	1510	d-Cadinene		2.96
41	37.44	1520	a-Cadinene		0.12
42	38.09	1537	Germacrene B		0.10
43	38.61	1550	Nerolidol	2.66	0.34
44	39.02	1561	Endo-1-bourbonanol		3.86
45	39.27	1567	(-)-Caryophyllene oxide	1.85	
46	42.02	1639	T-muurolol		2.99
47	42.04	1640	(+-)-5-Epi-Neointermedeol	3.35	
48	44.63	1709	mintsulfide		0.29
49	45.35	1730	(+)-a-Cyperone	0.73	
50	46.07	1750	Tetradecanoic acid		0.11
51	48.29	1814	Neophytadiene	0.13	0.82
52	49.45	1850	Pentadecanoic acid		0.10
53	50.66	1887	Nonadecane	10.00	10.00
54	51.22	1904	Methyl hexadecanoic acid	0.16	0.14
56	52.41	1941	1,2-Benzenedicarboxylic acid	0.97	1.25
57	52.83	1954	Hexadecanoic acid	0.22	4.39
58	54.66	2010	2-t-Butyl-4-(dimethylbenzyl)phenol	0.11	
59	56.38	2067	9,12-Octadecadienoic acid	0.28	0.10
60	56.59	2074	9,12,15-Octadecatrienoic acid	1.15	0.27
61	57.65	2109	11-Methoxy[a]anthracene		0.05
62	58.12	2126	9,12-Octadecadienoic acid		1.27
63	58.60	2142	Ethyl linoleolate		0.11
64	59.64	2178	Docosane	0.10	
65	62.27	2267	Tricosane	0.10	0.20
66	63.61	2314	1,2-Benzenedicarboxylic acid		0.20
67	65.06	2374	Bis(2-ethylhexyl)hexanedioic acid		1.14
68	65.16	2378	Pentadecane	0.13	
69	67.59	2470	9-Methyl nonadecane		0.15

Table 2. Continued

Peak No.	RT[2] (min)	RI[3]	Volatile compounds	Cultivated (ppm)	Wild (ppm)
70	67.70	2474	2,4-Bis(dimethylbenzyl)phenol	0.18	
71	67.78	2477	Docosane	0.27	
72	68.16	2491	1-Nonadecanal	0.25	
73	68.31	2496	2.4-Bis[dimethylbenzyl]-6-t-butylphenol	0.43	
74	68.41	2500	Methyl docosanoic acid		0.09
75	68.93	2519	1,2-Benzenedicarboxylic acid		2.62
76	70.28	2569	Docosane	0.16	
77	71.57	2622	2-Methyldecalin	-	
78	72.62	2773	2-Chloro-6-hydroxyisonicotinic acid	-	
79	72.68	2776	3-Methyl-2-cyclohexen-1-one	-	

[1]Peak quality was over 90%.
[2]RT: Retention time
[3]RI: Retention index

RESULTS AND DISCUSSION

The components of the oil from the wild and cultivated *chamnamul*, their retention times, and their contents are provided in Table 2, where the components are listed in terms of their elution on the column. The chromatograms are provided in Figs. 1 and 2.

The volatile flavor compounds from the essential oil of cultivated *chamnamul* were identified as 36 different components (Fig. 1) and the major constituents were found to be β-selinene (38.41 ppm), followed by myrcene (12.76 ppm), β-pinene (12.42 ppm), β-caryophyllene (7.87 ppm), 1-methyl-4-(1,2,2-trimethyl)benzene (6.63 ppm), β-elemene (6.26 ppm), limonene (5.39 ppm), T-muurolol (3.35 ppm) etc.

The volatile flavor compoounds of the essential oil of wild *chamnamul* were identified as 56 different components (Fig. 2) and the major constituents were sabinene (58.37 ppm) and germacrene-D (45.73 ppm), followed by a-pipene (20.63 ppm), β-elemene (16.50 ppm), 1-methyl-4-(1-methylethyl) cyclohexene (14.56 ppm), a-Bergamotene (11.55ppm), d-3-carene (9.44ppm), trans-β-farnesene (8.36 ppm), a-humulene (6.81 ppm), myrcene (6.62 ppm). Song et al (1997) reported that the major volatile components of *chamnamul* were β-myrcene, trans-caryophyllene, and trans β-farnesene where the analyzed materials were cultivated. Their results were similar to our results with the cultivated sample.

From these results, β-caryophyllene was identified only in the cultivated *chamnamul,* but trans β-farnesene was identified only in wild *chamnamul*. d-Cadinene and mintsulfide, both of which have a bitter taste, were present in small amounts only in the wild samples. a-Humulene, which has a medicinal effect in wild *chamnamul,* was 3 times higher than in the cultivated samples. Myrcene was identified in

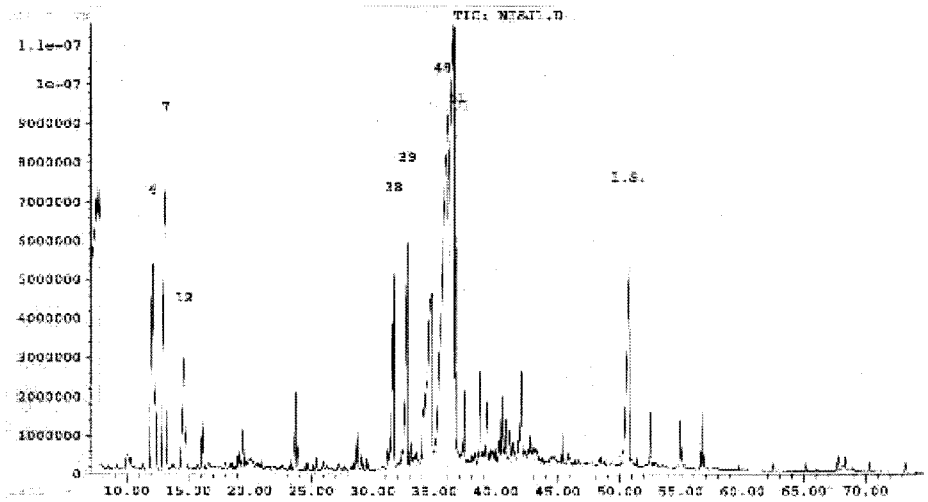

Fig. 1. Gas chromatogram of volatile components from cultivated *Pimpinella brachycarpa.*

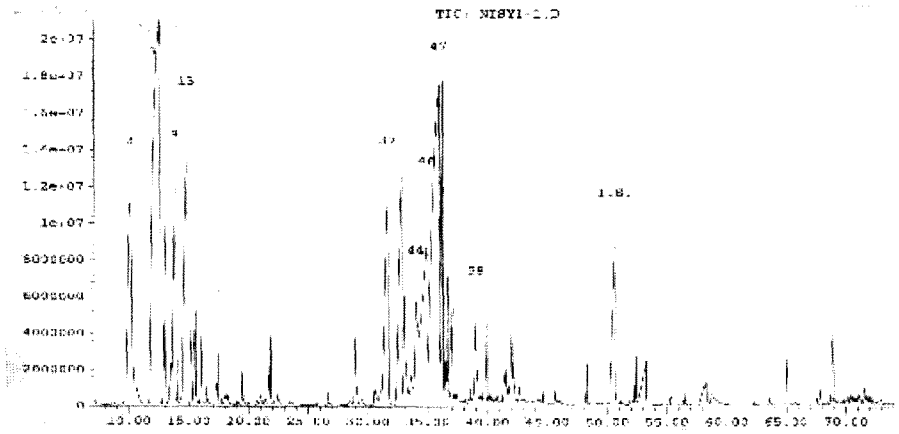

Fig. 2. Gas chromatogram of volatile components from wild *Pimpinella brachycarpa*

both the wild and cultivated samples, but the contents of the cultivated samples were 2 times higher than in the wild samples.

The major component of wild *chamnamul*, sabinene, has been studied as a natural preservative due to its antibacterial effect, and is known to be contained in the needle-shaped leaves of plants such as *Pinus densiflora*--sabinene also has a bitter taste (Kim and Shin 2005).

Limonene, germacrene D, and β-pinene were also the components identified in *Oenanthe stolonifera* DC. in the study conducted by Song and Kwon (1990). *Origanum vulgare* grown in the Euro-siberian area was reported to contain volatile components including caryophyllene, spathulenol, germacrene-D, and a-terpineol, and its methanol extracts were reported to evidence high levels of antioxidative activity and antimicrobial activity (Radusiene et al 2006). β-elemene, belonging to the sesquiterpenes, was also 3 times more abundant in the wild samples than in the cultivated samples, and has been reported to inhibit mouse pancreatic cancer and neoplastic metastasis (Tan et al 2003).

According to our results, it appears that the relative percentage of the identified compounds depends on the area in which the plant is grown. The flavor and medicinal effects may be also influenced by the cultivation method, though the type of vegetable is the same (Hyun et al 2004).

Our study demonstrated similar results, namely that the major aromatic constituents specified in wild *Codonopsis lanceolata*, such as dimethylbenzene, 3-ethyl-5-2- (ethylbutyl)-octadecane, and benzaldehyde were not detected in the domesticated species (Lee et al 1995). Additionally, study of the oils of cultivated and wild chamomile have reported changes in the contents and proportion of pharmacologically active compounds, although the characteristics of the oils of cultivated and wild chamomile populations remained the same (Szoke et al 2004).

However, it appears obvious that further investigations will be necessary to determine the exact contribution of each component to the observed biological activities.

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