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Antibacterial Activity of Volatile Flavor Components from *Houttuynia cordata* Thunb

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Abstract

The volatile flavor components were obtained from the aerial parts of *Houttuynia cordata* by a simultaneous distillation-extraction(SDE) method and bactericidal effects of the volatile flavor components on some strains were examined. Strong antibacterial activities were observed against *Bacillus cereus, Bacillus subtilis, Vibrio cholerae* O-1 and *Vibrio parahaemolyticus*. To further elucidate the effective components in the extract, SDE extract was analyzed by gas chromatography(GC) and gas chromatography/mass spectrometry(GC/MS). A total of 98 volatile compounds were detected. Of these, 90 were confirmed including 6 hydrocarbons(0.34%), 12 alcohols(1.31%), 13 aldehydes(33.81%), 1 acetal(0.01%), 6 esters(1.16%), 2 acids(3.10%), 5 ketones(5.87%), 2 furans(0.06%), 1 phenol(0.18%), 41 terpenes(53.23%) and 3 miscellaneous compounds(0.93%). Major components were determined to be β-myrcene, decanal, cis-ocimene and 2-undecanone.

Key words: volatile flavor components, *Houttuynia cordata*(Yakmomil), antibacterial activity

INTRODUCTION

As a part of 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 *Houttuynia cordata* Thunb.

This plant, commonly known in Korea as 'Yakmomil', is a perennial herb belonging to the *Saururaceae* family that grows mainly in a shady swamp and cultivate broadly in a coastal region of Korea for commercial and/or medicinal purposes. There are 7 species of 5 genera classified from the *Saururaceae* family in North America and Asia. Of these, 2 species(*Saururus chinensis, Houttuynia cordata*) are distributed in Korea(1).

This plant have been used as traditional medicine and folk remedy for meningioma, syphilis, gonorrhea, urethritis, cystitis, cervicitis, pneumonia, water eczema, tracheitis and malignant smallpox in Korea. Various pathogenic bacteria reportedly has a considerable resistance to antibiotics(2,3). Therefore, many researchers have made effort to control or withdraw the use of antibiotics by obtaining novel antibiotics from natural products. A number of studies on antibacterial activity of natural products were reported(4,5).

Recently *Houttuynia cordata* Thunb received much scientific attention as a potential source of the antitumor and antimicrobial agents; more specifically, the volatile and flavonoid compounds of the plant is reportedly to exert a pharmacological activity. The volatile compounds, in particular, are believed to render this plant its distinctive odor as well as physiological activity; however, systematic researches on flavor profiles and practical antimicrobial activity have seldom been carried out.

For this reason, this research was focused on the antibacterial activity of the flavor components of *Houttuynia cordata* Thunb and the identificaion and characterization of volatile flavor components from this plant by GC and GC/MS analysis.

MATERIALS AND METHODS

Materials

Yakmomil(*Houttuynia cordata*) cultivated in Karak (Pusan, Korea), were collected in June, 1996 and transported within 1hr to laboratory and extracted by SDE immediately and stored at 4°C before analysis.

All chemicals involved standard flavor compounds used for the analysis and extraction of the volatile flavor components were purchased from reliable commer-

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cial sources(MERCK, SIGMA, PFALTZ & BAUER, CHEM SERVICE, TCI). Also, all sorts of media and disk used for the screening of the antibacterial activity were purchased from DIFCO(U.S.A.) and TOYO(Japan), respectively.

Simultaneous distillation-extraction(SDE)

The aerial parts of *Houttuynia cordata*(500g) plus 10μg of internal standard(4-decanol) and 1.5L of distilled water were extracted with 100ml of redistilled diethyl ether for 2hr using a modified Likens and Nickerson type SDE apparatus at atmospheric pressure. This procedure was repeated untill utilizing all of the sample (1.85kg). Further details were described by Chung and Kim(6). Extracts were concentrated to 25ml under a gentle stream of nitrogen.

Screening for antibacterial activity

Fifteen species of pathogenic bacteria(ATCC strains and isolates) were used for antibacterial activity test. Tryptic soy broth(TSB ; Difco) and Mueller Hinton agar(Difco) were used for bacterial activation and antibacterial activity test, respectively. For halophiles such as *V. parahaemolyticus* and *V. vulnificus*, NaCl was added to the media to give final concentration of 3%.

Antibacterial activity test was followed by the disk diffusion method of Bauer et al.(7). Sterilized filter paper disks(1.0mm in thickness, 8.0mm in diameter; Toyo Roshi Kaisha, Ltd., Japan) were saturated with volatile flavor components from *Houttuynia cordata* for the sample or saturated with ether for the blank, and then placed on the surface of the medium uniformly spreaded with 0.1ml of each bacterial suspension(1×10^8CFU/ml). The plates were inverted and incubated at 37°C for 18 hours. After incubation, antibacterial activity was determined by the presence of a clear zone on the surface of the agar.

Instrumental analysis(GC, GC/MS)

A Varian model STAR 3600 gas chromatograph equipped with a flame ionization detector(FID) was used for routine analysis. Two μl of SDE extract were injected into a HP-5 crosslinked 5% Phenylmethyl Silicone capillary column(50m length×0.32mm i.d.×1.05μm film thickness, Hewlett-Packard Co.). Nitrogen was used as carrier gas(1.0ml/min), and the split ratio was set to 20 : 1. Oven temperature was programmed from 50°C to 200°C at the rate of 2°C/min with initial and final hold times of 2 and 30min, respectively. In addition, injector and detector temperature were kept at 230°C and 260°C, respectively.

A HP model 5890A series II GC interfaced to a HP model 5989A mass spectrometer was used for MS identification of GC components using the same column and oven conditions listed above. Helium was used as carrier gas(1.0ml/min), and the split ratio was set to 20 : 1. Electron ionization voltage was 70eV. Mass range was 30~500a.m.u., electron multiplier voltage was 2000V.

The volatile flavor components were identified by comparing retention time on GC and mass spectra of unknowns with those of authentic compounds under identical experimental conditions. Tentative identifications were based on standard MS library data(Willey/NBS).

RESULTS AND DISCUSSION

Antibacterial activity

The volatile flavor components from *Houttuynia cordata* were evaluated to determine the antibacterial activity against 15 species of pathogenic bacteria; the extracts showed complete inhibition on the growth of *Vibrio cholerae*, *V. parahaemolyticus*, *Bacillus cereus* and *B. subtilis*(Fig. 1), in particular.

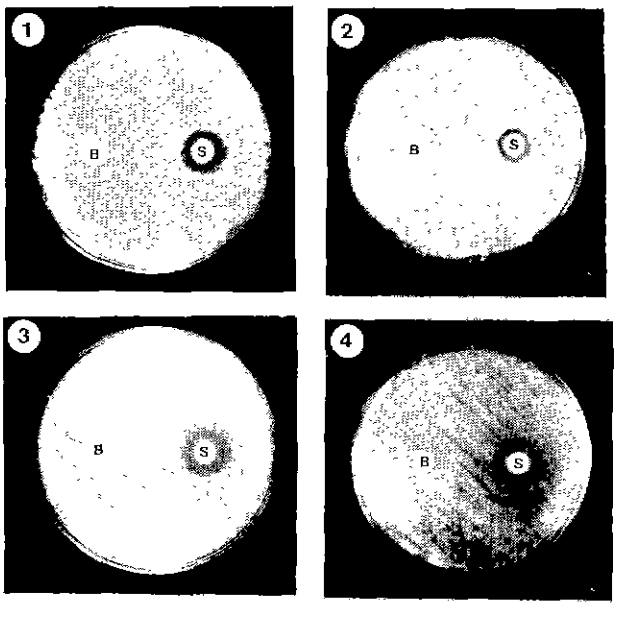

Fig. 1. Antibacterial activities of volatile flavor components from *Houttuynia cordata* against *V. cholerae*(1), *V. parahaemolyticus*(2), *B. cereus*(3) and *B. subtilis*(4).
S, saturated disk with sample ; B, saturated blank disk with ether.

Table 1. Antibacterial activities of volatile flavor components from *Houttuynia cordata* against test strains

Strains	Source	Diameter of inhibition zone(mm)
Bacillus cereus	ATCC 11778	19
Bacillus subtilis	ATCC 6633	16
Corynebacterium xerosis	ATCC 9755	14
Enterobacter cloaceae	ATCC 13047	–[2]
Escherichia coli O157	E32511[1]	–
Listeria monocytogenes	ATCC 15313	14
Salmonella typhi	Isolates	–
Shigella dysenteriae	ATCC 9752	12
Staphylococcus aureus	ATCC 25923	13
Staphylococcus epidermidis	ATCC 12228	14
Vibrio cholerae O-1	Isolates	19
Vibrio parahaemolyticus	ATCC 27519	16
Vibrio vulnificus	Isolates	16
Yersinia enterocolitica	Isolates	–

[1]Provided from College of Veterinary Medicine, Gyeongsang National University

[2]No inhibition zone(8mm, disk diameter)

The inhibitory effects of the extracts against test strains were shown in Table 1. For *Vibrio* sp. such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* and *Bacillus* sp. such as *B. cereus* and *B. subtilis* demonstrated strong antibacterial activity. Other strains such as *Staphylococcus aureus*, *Staph. epidermidis*, *Corynebacterium xerosis* and *Listeria monocytogenes* exhibited considerable antibacterial activity. And also, antibacterial activities were hardly observed in test strains such as *Shigella dysenteriae* and *Yersinia enterocolitica*, whereas *Escherichia coli* O157, *Salmonella typhi* and *Enterobacter cloaceae* were resistant to volatile flavor components from *Houttuynia cordata*.

The bactericidal effect of the extract should be further scrutinized to understand the mechanism; in other words, effective components should first be identified. Thus the following approach was employed : identification of the volatile components by GC/MS followed by selection of possible effective components based upon the literature survey.

Identification of the volatile flavor components

The volatile flavor concentrate obtained from the aerial parts of *Houttuynia cordata* by SDE had a distinctive fishy odor and the approximate yield was 0.009% The gas chromatogram of the whole volatile flavor components is shown in Fig. 2. Ninety compounds which corresponded to 98 peaks in the gas chromatogram were identified by the methods mentioned in 'Materials and Methods'.

These included 5 ketones(5.87%), 1 acetal(0.01%), 6 hydrocarbons(0.34%), 2 furans(0.06%), 6 esters(1.16%),

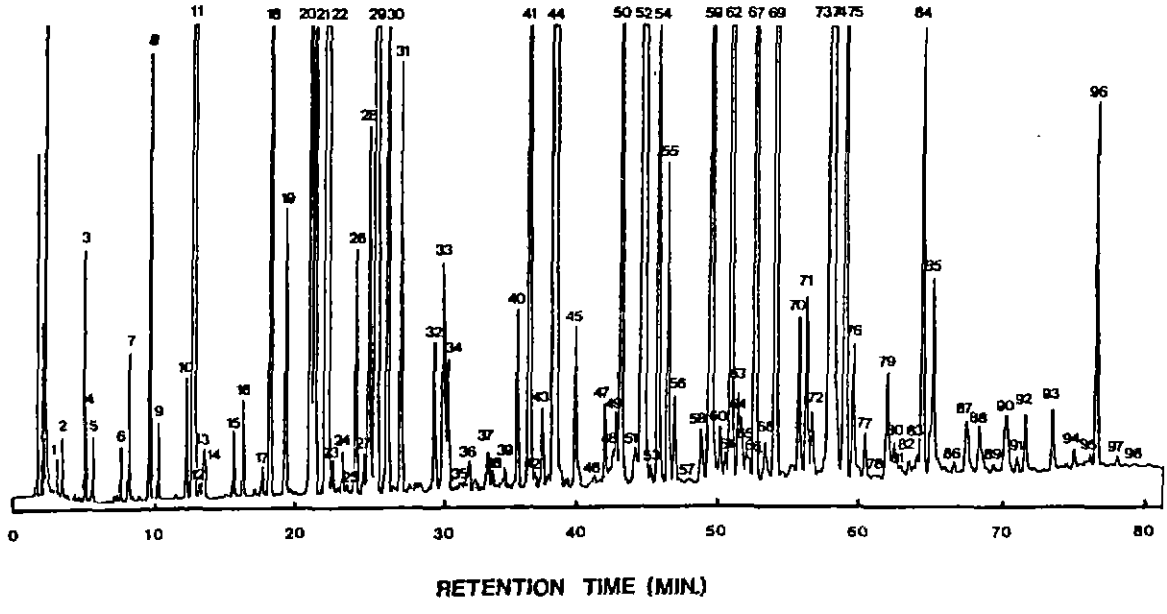

Fig. 2. Gas chromatogram of the volatile flavor concentrate obtained from the aerial parts of *Houttuynia cordata*. Peak numbers correspond to those listed in Table 2.

Table 2. Volatile flavor components in the aerial parts of *Houttuynia cordata*

No	Compound	RT	Method	Area%	No	Compound	RT	Method	Area%
1	3-Butene-2-ol, 2-methyl	3.14	MS	0.009	51	Endobornyl acetate	44.05	GC, MS	0.054
2	Acetic acid, ethyl ester	3.48	MS	0.012	52	2-Undecanone	44.72	GC, MS	3.684
3	1-Penten-3-ol	5.02	MS	0.083	53	Thymol	45.15	GC, MS	t
4	1-Penten-3-one	5.16	MS	0.035	54	Undecanal	45.71	MS	0.867
5	2-Ethyl furan	5.64	MS	0.025	55	Isoterpinolene	46.40	MS	0.363
6	(E)-2-Pentenal	7.58	MS	0.025	56	5-Methyl-3-(1-methylethenyl)-*trans*-cyclohexen	46.87	MS	0.103
7	(Z)-2-Penten-1-ol	8.18	MS	0.068	57	Citronellyl acetate	47.76	MS	t
8	Hexanal	9.63	MS	0.373	58	Neryl acetate	48.80	GC, MS	0.068
9	1,1-Diethoxy ethane	10.24	MS	0.013	59	Geranyl acetate	49.55	GC, MS	0.958
10	(E)-2-Hexenal	12.19	MS	0.090	60	2-Dodecanone	50.12	MS	0.042
11	(Z)-3-Hexenal	12.64	MS	2.985	61	unknown	50.51	MS	0.017
12	(Z)-3-Hexen-1-ol	13.14	MS	0.010	62	Decanoic acid	50.93	GC, MS	3.102
13	(E)-2-Hexen-1-ol	13.39	MS	0.034	63	unknown	51.48	MS	0.094
14	1-Hexanol	13.52	MS	0.035	64	unknown	51.69	MS	0.088
15	Nonane	15.57	MS	0.065	65	2-Dodecanol	52.05	GC, MS	t
16	2-Methyl-1,4-hexadiene	16.29	MS	0.078	66	Eugenol	52.21	GC, MS	t
17	α-Thujene	17.65	MS	0.028	67	Dodecanal	52.68	MS	3.325
18	*α-Pinene*	18.20	GC, MS	0.881	68	*trans*-Caryophyllene	53.41	MS	0.027
19	Camphene	19.31	GC, MS	0.307	69	β-Caryophyllene	54.21	GC, MS	2.778
20	β-Phellandrene	21.06	MS	1.003	70	β-Farnesene	55.92	MS	0.181
21	β-Pinene	21.37	GC, MS	0.865	71	α-Humulene	56.47	MS	0.217
22	β-Myrcene	22.25	GC, MS	27.961	72	β-Selinene	56.85	MS	0.077
23	2-Propyl furan	22.63	MS	0.032	73	*epi*-Bicyclosesquiphellandrene	58.20	MS	0.682
24	α-Phellandrene	23.35	GC, MS	0.024	74	2-Tridecanone	58.23	MS	2.014
25	Δ^3-Carene	23.64	GC, MS	t	75	Germacrene B	59.21	MS	0.959
26	α-Terpinene	24.26	GC, MS	0.255	76	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	59.81	MS	0.176
27	*p*-Cymene	24.84	GC, MS	0.039	77	δ-Cadinene	60.68	MS	0.051
28	*dl*-Limonene	25.20	GC, MS	0.456	78	α-Muurolene	61.05	MS	t
29	*cis*-Ocimene	25.75	MS	12.206	79	4-Octen-3-one	62.30	MS	0.096
30	*trans*-Ocimene	26.53	MS	0.857	80	Nerolidol	62.77	MS	0.018
31	*γ-Terpinene*	27.47	MS	0.487	81	*Dodecanoic acid*	63.17	MS	t
32	α-Terpinolene	29.78	MS	0.210	82	Palustrol	63.83	MS	0.012
33	Linalool +3-(4-methyl-3-pentenyl)furan	30.44	GC, MS	0.337	83	Spathulenol	64.36	MS	0.016
34	Nonanal	30.76	MS	0.156	84	δ-Guaiene+Dodecanoic acid ethyl ester	64.77	MS	0.588
35	2-Phenylethyl alcohol	31.65	GC, MS	t	85	Tetradecanal	65.57	MS	0.308
36	2,6-Dimethyl cyclohexanol	32.29	MS	0.053	86	unknown	67.06	MS	t
37	α-Cyclocitral	33.63	MS	0.060	87	*cis*-Asarone	67.94	MS	0.074
38	Undecane	33.93	MS	0.017	88	α-Copaene	68.87	MS	0.063
39	*cis-p*-2-Menthen-1-ol	34.75	MS	0.012	89	unknown	69.86	MS	t
40	Alloocimene+Borneol	35.72	GC, MS	0.205	90	Farnesol	70.78	GC, MS	0.108
41	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	36.61	MS	1.401	91	Caryophyllene II	71.52	MS	0.014
42	4-Decanol (Internal St)	36.97	MS	0.006	92	unknown	72.14	MS	0.056
43	α-Terpineol	37.55	GC, MS	0.091	93	unknown	74.06	MS	0.062
44	*Decanal*	38.49	MS	25.245	94	1-Hexadecanol	75.57	MS	0.017
45	β-Cyclocitral	39.90	MS	0.208	95	unknown	76.71	MS	0.012
46	(Z)-Citral	41.15	MS	0.009	96	Hexadecanal	77.21	MS	0.394
47	Geraniol	41.92	GC, MS	0.075	97	Neophytadiene	78.71	MS	0.009
48	(E)-2-Decenal	42.50	MS	0.022	98	3,7,11,15-Tetramethyl-2-hexadecene	79.85	MS	t
49	*cis*-L-Carvyl acetate	42.75	MS	0.065					
50	1-Decanol	43.10	MS	0.986					

1 phenol(0.18%), 13 aldehydes(33.81%), 2 acids(3.10%), 12 alcohols(1.31%), 41 terpenes(53.23%) and 3 miscellaneous compounds(0.93%). These volatile flavor components are listed in Table 2 in the order of elution from GC with HP-5 column and the relative contents of each compounds were expressed as the percentage of each peak area to the sum of total peak areas.

Terpenes, the predominant class of the volatile flavor components identified, were composed of 27 monoterpenes, 16 sesquiterpenes and 1 diterpene. The terpenes are ubiquitous in the higher plant. Although the role of these compounds is still being under debate, considerable evidence is accumulating that they play more than one role in nature such as allelopathic agents, antiherbivore agents and antimicrobial agents(8-10).

Both mono- and sesquiterpenes frequently occur in the volatile essential oils of higher plant. The monoterpenes have a considerable range of exploitable properties(including antibacterial, antifungal and anticancer activities); inevitably they are drawing increasing attention in the pharmaceutical industry(11).

The most abundant monoterpenes obtained from *Houttuynia cordata* are β-myrcene(27.961%), *cis*- ocimene (12.206%), terpinen-4-ol(1.401%) and β-phellandrene (1.003%). In addition, α-pinene(0.881%), β-pinene(0.865%), *trans*-ocimene(0.857%), γ-terpinene(0.487%), *dl*-limonene (0.456%), isoterpinolene(0.363%), camphene(0.307%), α-terpinene(0.255%) and α-terpinolene(0.210%) are identified.

The acyclic monoterpene myrcene that has been implicated in analgesic activity and antimicrobial activity was also found in Ginger(12-14), *Artemisia* sp.(9,15, 16), *Poncirus trifoliata*(17) and Fatsia(6). Several monoterpenes such as carene, *p*-cymene, limonene, myrcene, pinene and terpinolene inhibit fungal growth(9). In addition, terpinen-4-ol and α-pinene have the potent repellent properties(9), defence monoterpenes such as linalool, β-pinene, camphene and bornyl acetate are strongly associated with the insect repellent activity(11).

The biosynthesis of several monoterpenes such as myrcene, limonene, α-pinene and β-pinene arises from the monoterpene cyclases reaction. In these reaction, GPP is first ionized and isomerized to enzyme-bound linalyl pyrophosphate(LPP), the tertiary allylic isomer. Ionization of LPP promotes cyclization to the terminal double bond to yield the enzyme-bound α-terpinyl cation, a universal intermediate of these cyclization reactions(10).

The major sesquiterpene obtained from *Houttuynia cordata* are β-caryophyllene(2.778%), germacrene B (0.959%), epi-bicyclosesquiphellandrene(0.682%), α-humulene(0.217%), β-farnesene(0.181%), farnesol(0.108%), β-selinene(0.077%), α-copaene(0.063%) and δ-cadinene (0.051%).

The sesquiterpene caryophyllene has a aphid repellent activity and antifeedant activity(9) and the sesquiterpene alcohol farnesol is phytotoxic. β-Farnesene may be the result of thermal dehydration of farnesol during SDE extraction or GC analysis(16). Several sesquiterpene hydrocarbones such as δ-cadinene, β-caryophyllene, α-copaene and β-selinene have been implicated in allelopathy(8). And β-selinene is main component of the celery seed oil and the traditional paint, Golden Lacquer (18). This compound was also found in *Schizandra chinensis* Bullion(19), *Artemisia apiaceae* Hence (20) and *Eucommiae ulmoides* Oliv(21).

Aldehydes, the second predominant class of the volatile flavor components identified, were composed of 7 alkanals and 4 alkenal such as decanal(25.245%), dodecanal(3.325%), (Z)-3-hexenal(2.985%), undecanal(0.867%), hexadecanal(0.394%), hexanal(0.373%), tetradecanal(0.308%), nonanal(0.156%), (E)-2-hexanal(0.090%), (E)-2-pentenal(0.025%), (E)-2-decenal(0.022%). C_6 compounds such as hexanal, (E)-2-hexenal, (Z)- 3-hexen-1-ol, (E)-2-hexen-1-ol and 1-hexanol were probably formed from the enzyme-induced oxidative breakdown of unsaturated fatty acids including linoleic and linolenic acids(6,22,23).

Decanoyl acetaldehyde has been known to contribute to the characteristic fishy odor in *Houttuynia cordata*. 2-Undecanone(methyl-n-nonyl ketone) among ketones identified has been presumed to be produced from decanoyl acetaldehyde during the extraction process. Likewise, 2,6-bis(1,1-dimethylethyl)-4-methyl phenol identified in volatile flavor concentrate is considered to be a contaminant from the solvent, diethyl ether.

Considering various reports on biologically active volatile components from higher plants, the antibacterial effects of the extract from *Houttuynia cordata* should be the results of coalescent action of several components. Further research is in progress by the authors to elucidate the bactericidal mechanism of volatile components either by the single component or by the mixture of the components to see the synergistic effects.

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