JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 781–787 **781**

Volatile Constituents from the Leaves of *Callicarpa japonica* Thunb. and Their Antibacterial Activities

YONG-SUK KIM† AND DONG-HWA SHIN*,§

Faculty of Biotechnology and Research Center for Industrial Development of BioFood Materials, Chonbuk National University, Dukjin-Dong, Jeonju 561-756, Korea

Volatile substances of *Callicarpa japonica* Thunb. were examined for their antibacterial activities against six foodborne microorganisms using the optical densitometer Bioscreen C. Extracts of *C. japonica* were obtained by simultaneous steam distillation and solvent extraction (SDE), and those extracted for 1.5 and 2.0 h at pH 6.0 strongly inhibited the growth of *Bacillus cereus* and *Salmonella typhimurium*; the content of the volatile substances of leaves at these pH levels were 543.1 and 706.7 mg/kg, respectively. All foodborne microorganisms tested were strongly inhibited by the addition of >8% (v/v) of the SDE extracts to broth medium. The major volatile components of the SDE extracts obtained at 1.5 h and pH 6.0 were γ-caryophyllene, 1-octen-3-ol, 2-hexenal, germacrene B, and aromadendrene II, with corresponding peak areas of 44.14, 15.6, 9.86, 5.24, and 4.01%, respectively, and major antibacterial components were 1-octen-3-ol and 2-hexenal. Among the 32 materials identified as volatile flavor components, 2-hexenal, 2,4-hexadienal, 1-octen-3-ol, 2,4-heptadienal, and epiglobulol strongly inhibited microorganism growth. In particular, 2-hexenal (107.52 mg/L) and 1-octen-3-ol (678.64 mg/L) inhibited the growth of most microorganisms tested by >90%.

KEYWORDS: ***Callicarpa japonica*; volatile substances; SDE; antibacterial activity; foodborne microorganisms**

INTRODUCTION

Application of Hazard Analysis of Critical Control Point (HACCP) is a common hygiene control practice in the Korean instant food industry; food-poisoning control depends on this system (*1*). However, for instant food storage refrigeration and the limitation of storage period are applied. Consequently, the addition of preservatives is restricted, and alternative methods are required to prevent food decay and development of foodborne pathogens. Because the materials added to prolong shelf life should not remain in the food, more studies on the use of volatile antibacterial materials for food preservation and the prevention of microorganism development are required (*2*).

Many of the known volatile compounds with antibacterial effect are found in herbs. For example, yeast contamination of food can be prevented by garlic, onion, oregano, thyme (*3*), tea tree (*4*), or summer savory (*5*); an antimold effect can be achieved by adding thyme, garlic, or onion (*6*−*8*); and thyme (*9*), horseradish (*10*), oregano, coriander, and basil have antibacterial effects (*11*). The bactericidal effect of allyl isothiocyanate on *Listeria monocytogenes* has also been investigated (*12*).

Callicarpa japonica Thunb. belongs to the Verbenaceae family and is indigenous to Korea, Japan, China, and Taiwan. The water extracts of its leaves have hemostatic and antibacterial functions, and the extracts of its leaves, stems, and roots are a traditional cure for extravasation, intestinal bleeding, uterine bleeding, lung infection, and tonsillitis (*13*) and are rich in 5,6,7-trimethoxyflavone, which has antiviral (*14*−*16*) and insecticidal (*17*) effects. Kim (*18*) reported that *C. japonica* complex found in East Asia shows diverse morphological variations. Kobaisy et al. (*19*) found that the volatile compounds of *C. japonica* leaves are spathulenol (18.1%), germacrene B (13.0%), and biocyclogermacrene (11.0%) and that these noticeably differ from the volatiles of *Callicarpa americana*.

However, few studies have been conducted on the antibacterial effects of the essential oil of *C. japonica* leaves. Therefore, we undertook the present study to extract the essential oil of *C. japonica* leaves under different conditions, to identify the volatiles present, and to conduct tests of their antibacterial activities, with a view toward increasing the shelf life of instant foods.

MATERIALS AND METHODS

Microorganisms and Cultures. Six different foodborne bacterial species were used. *Bacillus cereus* (ATCC 11778) and *Salmonella typhimurium* (ATCC 14028) strains were grown at 30 °C in nutrient broth or nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.). *Escherichia coli* O157:H7 (ATCC 43894) and *Staphylococcus aureus* (ATCC 25923) were grown at 37 °C and *Listeria monocytogenes* (ATCC 19111) was grown at 30 °C in tryptic soy broth or tryptic soy agar (Difco, Detroit, MI). *Vibrio parahaemolyticus* (ATCC 33844) strain was grown at 37 °C in tryptic soy broth or tryptic soy agar supplemented with 3% (w/v) NaCl. The bacteria were grown for 24 h in sterilized broth medium. A portion of each culture (0.1 mL) was transferred into new broth medium (9.9 mL) and grown for 18 h for the antibacterial experiments.

* Corresponding author (telephone +82-63-270-2570; fax +82-63-270-2572; e-mail dhshin@moak.chonbuk.ac.kr).
† Research Center for Industrial Development of BioFood Materials.
§ Faculty of Biotechnology.

10.1021/jf034936d CCC: $27.50
Published on Web 01/27/2004

Table 1. Antibacterial Activities of the Volatile Essential Oil from *C. japonica* Thunb. versus SDE Extraction Time

	extraction time (pH 6.0)			
microorganism	0.5 h	1.0 h	1.5 h	2.0 h
Bacillus cereus	27.3[a]	55.6	99.6	99.8
Salmonella typhimurium	45.5	70.1	99.4	98.7
Vibrio parahaemolyticus	24.7	38.0	41.3	46.4
Listeria monocytogenes	15.5	29.0	33.1	42.4
Staphylococcus aureus	31.0	30.9	32.3	34.2
Escherichia coli O157:H7	19.0	21.8	29.6	32.6

[a] Growth inhibition rate (%) = 100 − (*B*/*A* × 100), where *A* = total area of growth curve of control by Bioscreen C for a 72 h incubation and *B* = total area of growth curve of treated sample by Bioscreen C for a 72 h incubation. Values represent the mean of three replicates.

Extraction of Volatile Components. The leaves of *C. japonica* were collected from the Jeonju Arboretum (Jeonju, Korea) in September 2001 and washed and stored at −20 °C. Extracts of the volatile compounds in the leaves were obtained by simultaneous steam distillation and solvent extraction (SDE) using the "improved" Likens−Nickerson unit (*20*). After 50 mL of the extracting solvent (redistilled diethyl ether) had been circulated through the apparatus at 36 °C, 100 g of the leaves was ground in a Waring blender (Waring, New Hartford, CT) and added to 1000 mL of distilled water in a round-bottom flask. The mixture was heated at 100 °C under different conditions, as follows. The extraction conditions used for *C. japonica* leaves were varied by using SDE times of 0.5, 1.0, 1.5, and 2.0 h at pH 6.0. The pH was then adjusted to 4.0, 5.0, 6.0 (control) and 7.0 and extracted for 1.5 h. Anhydrous sodium sulfate (∼15 g) was added to remove water, and the ether mixture was cooled to −20 °C for 12 h. The mix was then evaporated to 1 mL using nitrogen flow. Ten microliters of 1-pentanol (*n*-amyl alcohol) was then added to the extract as an internal gas chromatography (GC) standard. The concentrates obtained were tested for antibacterial activity and their volatile components analyzed.

Analysis and Identification of Volatile Constituents. GC (GC-17A V3) and GC-MS (QP5050, Shimadzu Co., Kyoto, Japan) were used for the analysis. Both used a Supelcowax 10 fused silica capillary column (60 m × 0.25 mm; 0.25 μm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The GC oven temperature was maintained at 50 °C for 5 min, then increased to 230 °C at a rate of 2 °C/min, and then held for 10 min. The temperature of the injector was 250 °C and that of the FID detector, 260 °C. The GC split ratio used was 1:60, and 0.5 μL of extract was injected for each run. The mass spectra ranged from *m/e* 28 to 400, and the ionizing voltage was 70 eV. Detected components were identified by comparing the spectra obtained with a mass spectrum library (Wiley NBS 139) and by comparing GC retention indices versus known standards.

Antibacterial Activities of SDE Extracts. *C. japonica* leaf extract (543.1 mg from 1 kg of fresh leaves) was obtained after completely evaporating the diethyl ether under nitrogen atmosphere. Ten percent (v/v) Tween 80 (Showa Chemical Co., Ltd., Tokyo, Japan) in water was then added up to a volume of 0.5 mL. The extract was then sterilized by passing it through a membrane filter (0.2 μm) (*21*, *22*). To determine its antibacterial activity, 5.82 mL of each medium was supplemented with 0.12 mL of the sterilized extract or with 10% Tween 80 as a control and then inoculated with 0.06 mL (10^5−10^6 cfu/mL) of each bacterial strain. The concentration of the dispersion was adjusted to an extract strength of 2, 4, 8, or 10% (v/v) with 10% Tween 80 to produce a total mixture volume of 6.00 mL. Aliquots of these cultures (0.3 mL) were dispensed into Bioscreen C (Labsystem, Helsinki, Finland) (an apparatus for automeasurement of optical density) wells and incubated as described for the respective bacterial strains. Optical density (600 nm) was measured every 12 h for 3 days against the Tween 80 control.

Antibacterial Effect of Extract Component. Thirty-two compounds identified in the volatile extract of *C. japonica* leaves by GC were tested for antibacterial activity. All compounds tested had a purity of >95% except for 2,4-heptadienal (90%). Each volatile compound (the content in *C. japonica*) was dissolved in 0.5 mL of 10% Tween 80 and then added to medium (2% v/v). Antibacterial activity was measured at 600 nm by using Bioscreen C every 24 h for 72 h. Activities were compared against 10% Tween 80 control.

Table 2. Volatile Components of *C. japonica* Thunb. versus SDE Extraction Time at pH 6.0

			peak area[a] (%)				
peak	component	RI[b]	0.5 h	1.0 h	1.5 h	2.0 h	IM[c]
1	acetaldehyde	444	0.94	0.44	0.11	0.09	A, B
2	ethyl acetate	619	0.15	0.08	0.07	0.03	A, B
3	3-methylbutanol	680	0	0	0.06	0.03	A
4	ethyl alcohol	702	1.57	1.91	2.06	0.09	A, B
5	2-ethylfuran	758	0	0.09	0.14	0.06	A, B
6	3-methyl-2-butanone	822	0	0	0.11	0.02	A
7	*n*-valeraldehyde	830	0	0.09	0	0.04	A, B
8	1*R*-(+)-α-pinene	957	0.21	0.16	0.20	0.10	A, B
9	*n*-propanol	1002	0.11	0.09	0.08	0.04	A,B
10	toluene	1038	0	0	0.09	0.03	A, B
11	2,3-pentanedione	1103	0.13	0.06	0.08	0.03	A, B
12	*n*-hexanal	1208	0.72	0.52	0.58	0.03	A, B
13	1-hexyn-3-ol	1285	0	0	0.06	0.02	A
14	(+)-β-pinene	1319	0.61	0.48	0.60	0.29	A, B
15	glycolaldehyde	1357	0	0	0	0.02	A
16	2-hexene	1408	0	0	0	0.03	A
17	*trans*-2-pentenal	1447	0	0	0.05	0.03	A, B
18	2-methyl-4-pentenal	1491	0.16	0.15	0.07	0.08	A
19	1-penten-3-ol	1586	0.36	0.30	0.27	0.15	A, B
20	*trans*-2-heptenal	1873	0.86	0.58	0.51	0.31	A
21	*trans*-2-hexenal	1983	13.30	9.52	9.86	5.86	A, B
22	3-octanone	2223	0.19	0.12	0.29	0.09	A, B
23	1-octen-3-one	2567	2.23	1.56	2.05	0.89	A
24	*cis*-2-penten-1-ol	2695	0.35	0.33	0.35	0.21	A, B
25	*n*-hexanol	2935	0.30	0.20	0.45	0.13	A, B
26	*cis*-3-hexen-1-ol	3186	1.35	1.12	1.99	0.76	A, B
27	3-octanol	3252	0.26	0.20	0.24	0.14	A, B
28	2,4-hexadienal	3350	0	0.14	0.16	0.11	A, B
29	*trans*-2-hexen-1-ol	3357	0.42	0.30	0.62	0.19	A, B
30	1-octen-3-ol	3707	19.55	14.67	15.60	9.70	A, B
31	1,2-cyclohexanediol	3726	0.09	0.13	0.20	0.12	A
32	2,4-heptadienal	3836	0	0.10	0.07	0.12	A, B
33	(−)-α-copaene	4006	0	0.11	0.12	0.14	A, B
34	α-elemene	4303	0	0.05	0	0.08	A
35	linalool	4471	0	0	0.05	0.04	A, B
36	γ-caryophyllene	4835	35.55	45.24	44.14	51.47	A, B
37	α-muurolene	4898	2.29	2.82	2.63	3.43	A
38	α-gurjunene	5017	0.17	0.21	0.19	0.24	A
39	β-chamigrene	5058	0	0	0	0.04	A
40	β-caryophyllene	5183	0	0.16	0.16	0.25	A
41	α-humulone	5368	1.55	1.92	1.80	2.27	A, B
42	α-ylangene	5402	0	0.09	0.06	0.27	A
43	junipene	5562	0	0.11	0.14	0.21	A
44	α-cubebene	5663	0	0.16	0.15	0.24	A
45	germacrene B	5846	5.21	6.80	5.24	8.17	A
46	(+)-δ-cadinene	6022	0.15	0.25	0.22	0.41	A, B
47	β-cedrene	6525	0	0.09	0.12	0.17	A
48	(−)-epiglobulol	7510	0.46	0.38	0.22	0.42	A, B
49	(−)-aromadendrene II	7575	6.42	4.25	4.01	5.01	A, B
50	citronellyl acetate	7945	0.11	0.11	0.11	0.17	A
51	(−)-globulol	8229	0	0	0.07	0.16	A, B
51	(−)-spathulenol	8484	2.32	1.88	1.69	2.34	A
53	aromadendrene I	9112	0.19	0.19	0.10	0.05	A
54	dihydro-α-ionone	9533	0	0.32	0.30	0.47	A
55	farnesene	9744	0	0.16	0.22	0.40	A
56	patchulane	9985	0	0.08	0.10	0.11	A
57	palmitic acid	11105	0	0	0	0.04	A
58	phytol	11295	0.16	0.22	0.30	0.42	A, B
	total peak area (%)		98.44	98.94	99.16	96.86	
	total amounts (mg/kg)		79.3	216.4	543.1	706.7	

[a] Peak area (%) on the gas chromatogram. Values represent the mean of three replicates. [b] Retention index. [c] Identification mode. Components identified by GC-mass are designated A, and the retention indices of the authentic compounds are designated B.

RESULTS AND DISCUSSION

Antibacterial Effect of Leaves Extracted by SDE for Different Times. The antibacterial activities of the *C. japonica* leaf extracts obtained by SDE for 0.5, 1.0, 1.5, and 2.0 h at pH 6.0 are shown in **Table 1**. The growth of *B. cereus* was slightly inhibited in the presence of the 0.5 h extract, whereas the antibacterial activities of the 1.5 and 2.0 h extracts were similar. Also, the growth inhibitory effects on *S. typhimurium* and other

Table 3. Antibacterial Activities of the Volatile Essential Oil from *C. japonica* Thunb. versus the pH of the SDE Dispersion Medium

	extraction pH (1.5 h)			
microorganism	pH 4.0	pH 5.0	pH 6.0	pH 7.0
B. cereus	98.5[a]	95.8	99.6	77.6
S. typhimurium	99.6	97.4	99.4	86.1
V. parahaemolyticus	80.9	75.1	41.3	44.7
L. monocytogenes	33.6	35.1	33.1	32.1
S. aureus	33.6	33.7	32.3	35.8
E. coli O157:H7	37.5	34.9	29.6	36.3

[a] See footnote in **Table 1**. Values represent the mean of three replicates.

strains were lowest for the 0.5 h extract and increased with extraction time. Seo et al. (*23*) found that the initial water extract of hydrolyzed mustard leaves had a weak antibacterial effect, but after 12 h of incubation, the effect increased considerably and reached maximum activity after 24 h, after which it remained constant, which is in line with the results of the present study in which the antibacterial activity increased up to 1.5 h and remained constant thereafter. Therefore, in the latter experiments of the present study, an extraction time of 1.5 h was selected. Moreover, a prolonged heat treatment can break down the effective volatile components (*24*).

Changes in the Compositions of the Volatile Components with Extraction Time. It has been reported that the volatile components of extracts obtained by SDE may differ with the extraction time (*25*) and that this may affect the antibacterial activity (*23*). The volatile components of *C. japonica* leaf extracts are shown in **Table 2**. It was found that as the extraction time increased, the number of volatile components also increased; for example, 33, 47, 52, and 58 components were identified at SDE times of 0.5, 1.0, 1.5, and 2.0 h, respectively. Furthermore, the peak area of the total volatile components increased versus the internal standard as extraction time increased; that is, 79.3, 216.4, 543.1, and 706.7 mg/kg were recorded for extraction times of 0.5, 1.0, 1.5, and 2.0 h, respectively.

The main volatile components (78.85−80.48%) of *C. japonica* leaf extract were identified as γ-caryophyllene, 1-octen-3-ol, 2-hexenal, germacrene B, and aromadendrenepoxide II, accounting for 35.55−51.57, 9.70−19.55, 5.86−13.30, 5.21−8.71, and 4.01−6.42% of the total peak area, respectively. For an SDE time of 0.5 h, the peak area of the low molecular weight volatiles, that is, 2-hexenal (MW 98.15) and 1-octen-3-ol (MW 128.22), were 13.30 and 19.55%, respectively, but these reduced to 5.86 and 9.70% for an SDE time of 2.0 h. On the other hand, the peak areas of the higher molecular weight compounds, γ-caryophyllene (MW 204.36) and germacrene B (MW 204.36), at an SDE time of 0.5 h were 35.55 and 5.21%, respectively, but these increased to 51.47 and 8.17% after an SDE time of 2.0 h. Au-Yeung et al. (*25*) showed in an efficacy test using a Likens−Nickerson extractor that the extraction efficiencies of highly volatile compounds such as allyl isothiocyanate reduced with increasing extraction time, whereas those of nonvolatiles, such as methylpyrazine, increased.

Using the GC-MS, 58 volatile compounds were identified.

Effect of Extraction pH on Antibacterial Activity. The antibacterial activities of the extracts prepared at pH 4.0, 5.0, 6.0 (control), and 7.0 are presented in **Table 3**. Growth inhibitory effect on *B. cereus* and *S. typhimurium* tended to be lower at neutral pH, and at pH <6, the activity did not differ. The growth inhibitory effect on *V. parahaemolyticus* reduced with increasing pH, but activity against other bacterial species was found to be unaffected by extraction pH.

Table 4. Volatile Components of *C. japonica* Thunb. versus the pH of the Dispersion Medium in 1.5 h SDEs

			peak area[a] (%)				
peak	component	RI[b]	pH 4.0	pH 5.0	pH 6.0	pH 7.0	IM[c]
1	acetaldehyde	444	0.18	0.12	0.11	0.14	A, B
2	ethyl acetate	619	0.08	0.08	0.07	0.07	A, B
3	3-methylbutanol	680	0.05	0	0.06	0.14	A
4	ethyl alcohol	702	4.21	3.64	2.06	5.41	A, B
5	2-ethylfuran	758	0.14	0.08	0.14	0.13	A, B
6	3-methyl-2-butanone	822	0	0	0.11	0.05	A
7	*n*-valeraldehyde	830	0.15	0.08	0	0.05	A, B
8	1*R*-(+)-α-pinene	957	0.19	0.15	0.20	0.17	A, B
9	*n*-propanol	1002	0.10	0.07	0.08	0.10	A, B
10	toluene	1038	0.20	0.07	0.09	0.06	A, B
11	2,3-pentanedione	1103	0.11	0.07	0.08	0.08	A, B
12	*n*-hexanal	1208	0.77	0.59	0.58	0.42	A, B
13	1-hexyn-3-ol	1285	0	0	0.06	0.04	A
14	(+)-β-pinene	1319	0.45	0.43	0.60	0.52	A, B
15	glycolaldehyde	1357	0	0	0	0	A
16	2-hexene	1408	0	0	0	0	A
17	*trans*-2-pentenal	1447	0.06	0	0.05	0.06	A, B
18	2-methyl-4-pentenal	1491	0.07	0.09	0.07	0.06	A
19	1-penten-3-ol	1586	0.30	0.23	0.27	0.23	A, B
20	*trans*-2-heptenal	1873	0.68	0.59	0.51	0.24	A
21	*trans*-2-hexenal	1983	9.87	9.15	9.86	6.99	A, B
22	3-octanone	2223	0.32	0.18	0.29	0.30	A, B
23	1-octen-3-one	2567	2.35	1.65	2.05	2.06	A
24	*cis*-2-penten-1-ol	2695	0.37	0.29	0.35	0.31	A, B
25	*n*-hexanol	2935	0.37	0.24	0.45	0.40	A, B
26	*cis*-3-hexen-1-ol	3186	1.57	1.17	1.99	1.50	A, B
27	3-octanol	3252	0.29	0.18	0.24	0.25	A, B
28	2,4-hexadienal	3350	0.20	0.16	0.16	0.09	A, B
29	*trans*-2-hexen-1-ol	3357	0.60	0.37	0.62	0.55	A, B
30	1-octen-3-ol	3707	16.25	12.47	15.60	13.82	A, B
31	1,2-cyclohexanediol	3726	0.20	0.07	0.20	0.17	A
32	2,4-heptadienal	3836	0.07	0.08	0.07	0.09	A, B
33	(−)-α-copaene	4006	0.06	0.11	0.12	0.08	A, B
34	α-elemene	4303	0.07	0	0	0	A
35	linalool	4471	0.35	0.06	0.05	0.05	A, B
36	γ-caryophyllene	4835	38.66	48.57	44.14	41.31	A, B
37	α-muurolene	4898	1.98	2.72	2.63	2.69	A
38	α-gurjunene	5017	0.18	0.19	0.19	0.21	A
39	β-chamigrene	5058	0.05	0	0	0	A
40	β-caryophyllene	5183	0.29	0.17	0.16	0.15	A
41	α-humulone	5368	1.43	1.99	1.80	1.87	A, B
42	α-ylangene	5402	0.31	0.06	0.06	0.08	A
43	junipene	5562	0.43	0.14	0.14	0.14	A
44	α-cubebene	5663	0.06	0.14	0.15	0.13	A
45	germacrene B	5846	2.95	5.51	5.24	5.76	A
46	(+)-δ-cadinene	6022	0.13	0.20	0.22	0.20	A, B
47	β-cedrene	6525	1.08	0.18	0.12	0.15	A
48	(−)-epiglobulol	7510	0.08	0.12	0.22	0.35	A, B
49	(−)-aromadendrene II	7575	4.50	3.30	4.01	6.11	A, B
50	citronellyl acetate	7945	0.16	0.07	0.11	0.19	A
51	(−)-globulol	8229	0.60	0.09	0.07	0.18	A, B
51	(−)-spathulenol	8484	3.06	1.61	1.69	2.84	A
53	aromadendrene I	9112	0.35	0.22	0.10	0	A
54	dihydro-α-ionone	9533	0.66	0.29	0.30	0.52	A
55	farnesene	9744	0.39	0.08	0.22	0.44	A
56	patchulane	9985	0.52	0.13	0.10	0.16	A
57	palmitic acid	11105	0	0.08	0	0	A
58	phytol	11295	0.13	0.29	0.30	0.32	A, B
	total peak area (%)		98.68	98.62	99.16	98.43	
	total amounts (mg/kg)		291.8	311.6	543.1	484.4	

[a−c] See footnotes in **Table 2**. Values represent the mean of three replicates.

Effect of Extraction pH on Extract Volatile Composition. Investigators have shown that for the SDE method volatile components are affected by the salt content of the dispersion media (*26*) and pH (*27*, *28*). In this study, the amount of volatile compounds was also affected by the medium pH (**Table 4**); for example, 53, 50, 52, and 52 compounds were identified at pH values of 4.0, 5.0, 6.0, and 7.0, respectively. The volatile content at pH 6.0 (543.1 mg/kg) was higher than that at pH 4.0 (291.9 mg/kg), 5.0 (311.6 mg/kg), or 7.0 (484.4 mg/kg).

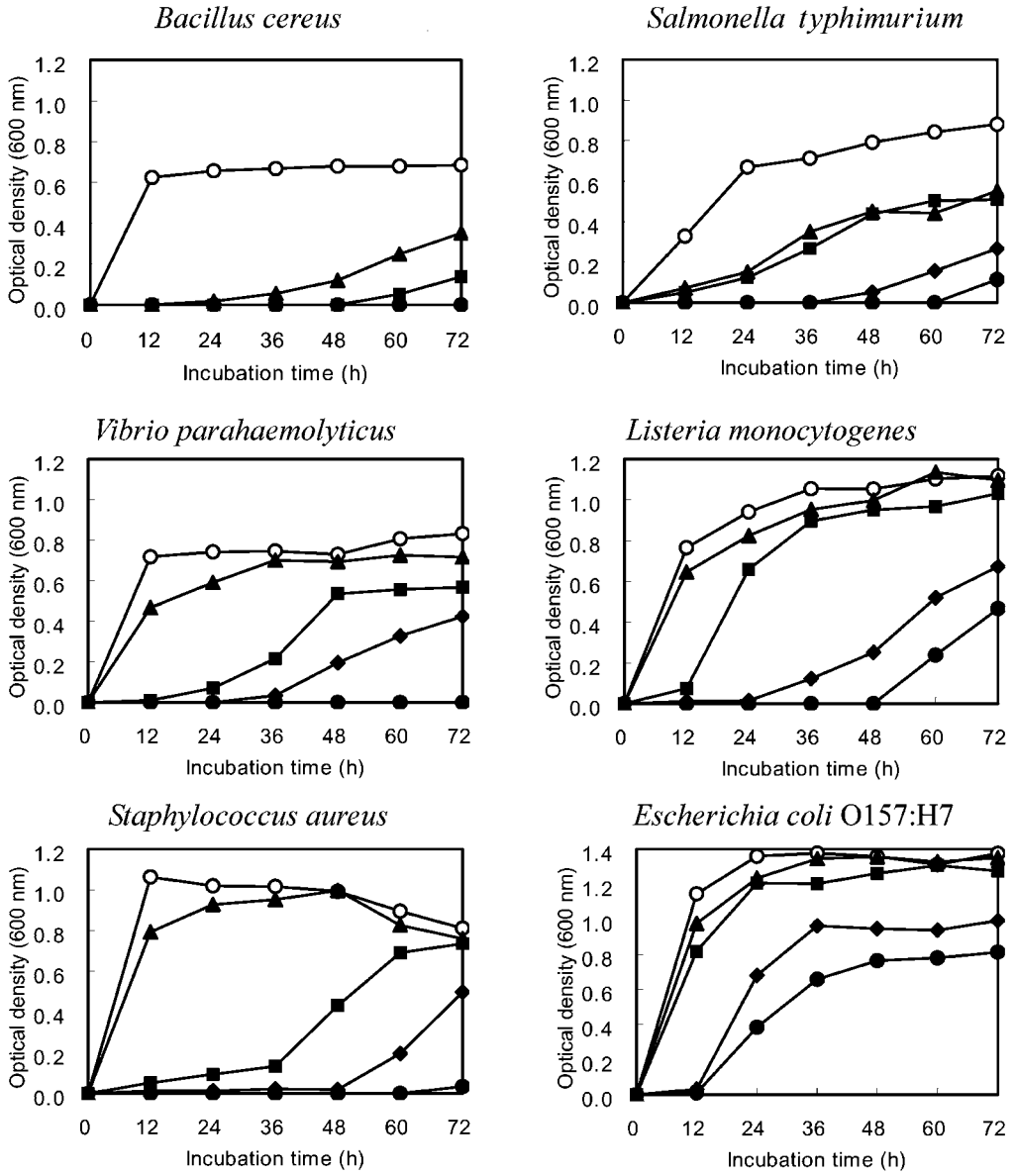

Figure 1. Antibacterial activities of volatile oil obtained by 1.5 h SDE at pH 6.0 from *C. japonica* Thunb. against several foodborne microorganisms: ○, control; ▲, 2%; ■, 4%; ◆, 8%; ●, 10%.

The peak areas of γ-caryophyllene and germacrene B from *C. japonica* leaves were 38.66 and 2.95% of total GC peak area, respectively, at pH 4.0, and these increased to 41.31−48.57 and 5.24−5.76% at pH 5.0−7.0. However, the peak area of 2-hexenal was 9.15−9.87% at pH 4.0−6.0 but decreased to 6.99% at pH 7.0. No significant change in the peak area of 1-octen-3-ol (12.47−16.25%) occurred with pH. The above four compounds and aromadendrenepoxide II were found to be the main volatile compounds in the *C. japonica* leaf extract dispersion at all pH values and represented 67.23−79.00% of the total volatile compound content.

In a previous study, it was found that salt (NaCl) can enhance the steam pressure and increase the number of volatile compounds (*26*). Choi et al. (*29*) found that the volatile compound yield in *Capsella bursa-pastiris* extracts produced by SDE was maximal at pH 7.0 and that at pH 3.0 high levels of pentadecane, octanol, and indole were present. However, in general, minor volatile compounds such as hexanol were unaffected by pH. Nevertheless, this trend between volatile component yields and pH effect (*29*) concurs with the results of the present study.

Effect of SDE Extract Concentration on Antibacterial Activity. Different concentrations of 1.5 h SDE (pH 6.0) extracts (2, 4, 8, and 10% v/v) were tested for antibacterial activity (**Figure 1**).

The growth of *B. cereus* was inhibited for up to 12 h in the presence of 2% extract but increased slowly thereafter. The 4% extract inhibited the growth for up to 48 h, and further increases in concentration inhibited the growth for up to 72 h. The antibacterial effect of the 2 and 4% extracts were weak on the *S. typhimurium* strain compared to the control, and increases in concentration to 8 and 10% were able to inhibit the growth for only up to 36 h. The antibacterial effect of the 2% extract on *V. parahaemolyticus* was poor compared to the control, and the 10% extract was able to inhibit the growth for up to 72 h. A similar effect was found for *S. aureus*. Versus the control, 2 and 4% extracts slightly inhibited *L. monocytogenes*, and at 8 and 10%, the extract showed significant inhibition versus the control. The antibacterial effect of the extract was weak on *E. coli* O157:H7, but a 10% extract showed greater inhibition than the control.

Table 5. Growth Inhibitory Activities of the Volatile Components from *C. japonica* Thunb.

compound (source)	treatment[a] (mg/L)	*B. cereus*	*S. typhimurium*	*V. parahaemolyticus*	*L. monocytogenes*	*S. aureus*	*E. coli* O157:H7
acetaldehyde (F[b])	1.20	15.4[c]	11.1	10.0	2.8	5.3	0
ethyl acetate (S[d])	1.44	0	9.3	4.5	0	6.6	0
ethyl alcohol (S)	104.72	0	3.4	3.9	0	3.2	2.0
2-ethylfuran (A[e])	3.64	0	1.6	2.3	0	4.3	0
n-valeraldehyde (S)	2.36	11.3	4.2	0	0	6.3	0
1*R*-(+)-α-pinene (A)	4.44	0	5.2	10.7	0	20.8	0
n-propanol (S)	1.76	0	2.6	0	0	6.1	0
toluene (S)	1.96	0	0	1.6	0	1.8	0
2,3-pentanedione (A)	1.72	26.9	25.5	39.3	26.2	47.4	9.6
n-hexanal (S)	16.80	19.3	39.1	57.8	14.4	11.3	41.5
(+)-β-pinene (A)	13.04	0	14.9	45.1	2.8	17.6	3.4
trans-2-pentenal (A)	1.84	27.4	39.6	40.7	16.9	12.2	11.0
1-penten-3-ol (A)	5.80	9.3	5.0	1.9	0.4	2.4	0
trans-2-hexenal (A)	247.00	98.8	98.7	100.0	99.7	100.0	99.9
3-octanone (A)	6.32	0	5.1	0	0	5.2	3.1
cis-2-penten-1-ol (A)	10.36	20.4	9.4	10.5	0	8.1	21.9
n-hexanol (F)	9.84	3.6	23.3	27.1	4.2	6.9	16.9
cis-3-hexen-1-ol (S)	43.20	14.3	56.2	73.7	10.3	21.6	41.0
3-octanol (A)	5.32	43.0	20.6	70.7	21.5	52.6	8.9
2,4-hexadienal (A)	3.40	67.3	45.1	99.6	33.6	91.5	14.9
trans-2-hexen-1-ol (A)	13.56	52.5	29.1	38.6	19.2	29.5	31.8
1-octen-3-ol (A)	339.32	54.9	83.5	98.6	32.1	59.2	94.4
2,4-heptadienal (A)	3.40	97.3	60.2	99.1	44.4	80.9	17.4
(–)-α-copaene (F)	4.00	5.9	0.1	0	0	5.7	0
linalool (A)	3.80	8.6	8.2	0	0.6	2.6	2.1
γ-caryophyllene (A)	1470.80	32.1	10.2	26.5	7.2	54.7	12.8
α-humulone (S)	64.76	40.1	6.9	15.7	13.9	6.6	4.6
(+)-δ-cadinene (F)	11.64	20.3	4.3	32.5	15.7	26.3	9.5
(–)-epiglobulol (F)	12.00	63.6	47.4	61.2	94.3	99.4	24.5
(–)-aromadendrene II (F)	143.24	72.3	36.4	45.6	28.0	61.5	24.2
(–)-globulol (F)	4.64	1.9	0.8	0	6.3	8.3	0
phytol (A)	11.92	32.8	1.9	0	0	7.1	0

[a] Calculated on the basis of the contents in *C. japonica* Thunb. [b] F, purchased from Fluka Chemie GmbH (Buchs, Switzerland). [c] See footnote in **Table 1**. [d] S, purchased from Sigma Chemical Co. (St. Louis, MO). [e] A, purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Table 6. Growth Inhibitory Activities of Volatile Components at Various Concentrations

compound	treatment[a] (mg/L)	*B. cereus*	*S. typhimurium*	*V. parahaemolyticus*	*L. monocytogenes*	*S. aureus*	*E. coli* O157:H7
trans-2-hexenal	6.12	90.5[b]	47.1	84.7	31.0	29.2	7.3
	21.52	91.3	63.0	99.6	32.0	28.7	8.2
	43.00	91.8	83.4	100	45.3	44.3	25.5
	61.12	98.7	98.5	99.8	81.2	72.6	47.5
	107.52	100	98.9	99.8	99.4	96.7	89.9
	215.04	100	99.4	99.8	99.8	100	97.3
2,4-hexadienal	0.025	10.3	13.2	6.2	3.9	7.8	0.3
	0.05	9.5	12.9	5.8	3.9	5.3	0.5
	0.12	10.8	12.2	10.4	7.5	6.9	2.1
	0.68	45.3	18.4	20.3	9.2	8.8	1.9
	3.40	71.5	50.3	98.9	32.4	89.4	12.7
	6.80	100	80.6	99.1	41.4	90.9	20.2
1-octen-3-ol	12.48	0	18.2	11.1	5.4	3.6	8.3
	25.52	0	16.7	14.3	6.7	6.6	6.3
	67.88	4.2	17.7	13.5	5.0	5.7	7.9
	135.68	7.6	25.4	21.5	8.2	8.7	8.9
	339.32	54.6	79.1	90.4	27.3	50.2	87.2
	678.64	90.8	98.4	98.8	39.8	80.6	96.5
2,4-heptadienal	0.017	0	5.8	5.6	0.1	0	0.5
	0.068	1.1	8.8	5.8	0.8	0	0.5
	0.34	1.8	8.6	6.1	1.6	2.8	1.3
	0.68	11.0	18.2	11.4	5.9	4.9	4.4
	3.40	92.9	57.8	82.4	34.5	85.6	16.6
	6.80	100	88.6	99.0	51.6	95.5	27.5
(–)-epiglobulol	0.03	0.6	2.2	3.1	5.8	1.7	0.3
	0.24	8.2	3.9	11.4	6.1	2.6	0.7
	1.20	13.9	4.4	11.1	8.2	5.8	0.9
	2.40	18.6	4.9	11.7	10.1	5.9	2.1
	12.0	94.9	39.1	66.4	92.0	99.5	22.6
	24.00	95.7	58.1	65.8	94.3	99.6	26.3

[a,b] See footnotes in **Table 5**.

In this study, significantly different effects of *C. japonica* leaf extracts were not found on the inhibition of Gram-positive *B. cereus*, *L. monocytogenes*, or *S. aureus* strains or on Gram-negative *S. typhimurium*, *V. parahaemolyticus*, and *E. coli* O157:H7 strains. Zaika et al. (*30*) reported that the resistance of Gram-positive strains was higher than that of Gram-negative strains to plant oils. Farag et al. (*31*) and Hussein (*32*) reported that Gram-positive strains were more sensitive to the antibacterial effect of essential oil than Gram-negative strains. However, Dorman et al. (*33*) showed that antibacterial activity depends

on the type of essential oil. Kim et al. (*21*) suggested that the antibacterial activity does not depend on the type of Gram reaction. The findings of this study showed strong antibacterial activity on the Gram-positive (*B. cereus* and *S. aureus*) and Gram-negative (*V. parahaemolyticus*) strains, which supports Kim et al.'s (*21*) report.

Antibacterial Activities of Extract Components. As shown in **Table 5**, 2-hexenal inhibited the growth of all six organisms by 90%, and 2,4-hexadienal inhibited *V. parahaemolyticus* and *S. aureus* by 90%. 1-Octen-3-ol inhibited *V. parahaemolyticus* and *E. coli* O157:H7 by 90% and 2,4-heptadienal, *B. cereus* and *V. parahaemolyticus* by 90%. In addition, epiglobulol inhibited *L. monocytogenes* and *S. aureus*. The antibacterial effects of the major volatile (γ-caryophyllene) of *C. japonica* leaf extracts were in general weak.

Dorman et al. (*33*) found that the antibacterial constituents of black pepper, clove, geranium, and oregano involved thymol, carvacrol, α-terpineol, terpinen-4-ol, eugenol, linalool, nerol, and β-pinene. Weissinger et al. (*34*) found that cinnamic aldehyde and thymol in alfalfa seed and stem inhibited the activity of *Salmonella*, and Inouye et al. (*35*) found that gas-inoculated α-pinene, β-pinene, citronellol, and nerol only weakly inhibited *B. subtilis* and *E. coli*. These results suggest that the low volatilities of these compounds inhibit antibacterial activity.

Effect of Extract Component Concentration on Antibacterial Activity. Different concentrations of components, that is, 2-hexenal, 2,4-hexadienal, 1-octen-3-ol, 2,4-heptadienal, or epiglobulol, were tested for antibacterial activity, as shown by **Table 6**.

2-Hexenal is known to produce a subtle flowery, fruity smell (*36*, *37*) and showed 90% antibacterial activity against *B. cereus*, *S. typhimurium*, *V. parahaemolyticus*, and the other species at concentrations above 6.12, 61.12, 21.52, and 107.52 mg/L, respectively. As for treatment with 2,4-hexadienal, a concentration of 6.80 mg/L inhibited *B. cereus* and *S. aureus* growth, and 3.40 mg/L inhibited *V. parahaemolyticus* growth by 90%. 1-Octen-3-ol is used as an additive to a produce spicy, mushroom-like, fruity flavor at low concentration (*36*, *37*) and was found to inhibit *B. cereus*, *S. typhimurium*, and *E. coli* O157:H7 activities at 678.64 mg/L and to inhibit *V. parahaemolyticus* at >339.32 mg/L by 90%. High levels of *B. cereus*, *V. parahaemolyticus*, and *S. aureus* growth inhibition were recorded in the presence of 3.40 mg/L of 2,4-heptadienal.

The antibacterial activities of the SDE extracts obtained from *C. japonica* against foodborne microorganisms were excellent; thus, it appears to be potentially useful as a modified-atmosphere packing agent to extend the shelf lives of instant foods.

LITERATURE CITED

(1) Chang, D. S.; Shin, D. H.; Chung, D. H.; Kim, C. M.; Lee, I. S. *Food Hygiene*; Jungmoongak: Seoul, Korea, 2002; pp 19−71.

(2) Kim, Y. S.; Shin, D. H. A review—Researches on the volatile antimicrobial compounds from edible plants and their food application. *Korean J. Food Sci. Technol.* **2003**, *35*, 159−165.

(3) Conner, D. E.; Beuchat, L. R. Effects of essential oil from plants on growth of food spoilage yeasts. *J. Food Sci.* **1984**, *49*, 429−434.

(4) Hammer, K. A.; Carson, C. F.; Riley, T. V. In-vitro activity of essential oil, in particular *Melaleuca alterifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. *J. Antimicrob. Chemother.* **1998**, *42*, 591−595.

(5) Gulluce, M.; Sokmen, M.; Daferera, D.; Agar, G.; Ozkan, H.; Kartal, N.; Polissiou, M.; Sokmen, A.; Sahin, F. In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J. Agric. Food Chem.* **2003**, *51*, 3958−3965.

(6) Buchanan, R. L.; Shepherd, A. J. Inhibition of *Aspergillus parasiticus* by thymol. *J. Food Sci.* **1981**, *46*, 976−977.

(7) Yin, M. C.; Cheng, W. S. Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. *J. Food Prot.* **1998**, *61*, 123−125.

(8) Montes-Belmont, R.; Carvajal, M. Control of *Aspergillus flavus* in maize with plant essential oil and their components. *J. Food Prot.* **1998**, *61*, 616−619.

(9) Marino, M.; Bersani, C.; Comi, G. Antimicrobial activity of the essential oil of *Thymus vulgaris* L. measured using a bioimpedometric method. *J. Food Prot.* **1999**, *62*, 1017−1023.

(10) Delaquis, P. J.; Ward, S. M.; Holley, R. A.; Cliff, M. C.; Mazza, G. Microbiological, chemical and sensory properties of precooked roast beef preserved with horseradish essential oil. *J. Food Sci.* **1999**, *64*, 519−524.

(11) Elgayyar, M.; Draughon, F. A.; Golden, D. A.; Mount, J. R. Antimicrobial activity of essential oil from plants against selected pathogenic and saprophytic microorganisms. *J. Food Prot.* **2001**, *64*, 1019−1024.

(12) Ahn, E. S.; Kim, Y. S.; Shin, D. H. Observation of bactericidal effect of allyl isothiocyanate on *Listeria monocytogenes*. *Food Sci. Biotechnol.* **2001**, *10*, 31−35.

(13) Bae, K. H. *The Medicinal Plants of Korea Used for Medicines*; Kyo Hak Publishing: Seoul, Korea, 2000.

(14) Hayashi, K.; Hayashi, T.; Otsuka, H.; Takeda, Y. Antiviral activity of 5,6,7-trimethoxyflavone and its potentiation of the antiherpes activity of acyclovir. *J. Antimicrob. Chemother.* **1997**, *39*, 821−824.

(15) Tsuchiya, Y.; Shimizu, M.; Hiyama, Y.; Itoh, K.; Hashimoto, Y.; Nakayama, M.; Horie, T.; Morita, N. Antiviral activity of natural occurring flavonoides *in vitro*. *Chem. Pharm. Bull.* **1985**, *33*, 3881−3886.

(16) Toshimitsu, O.; Hidacki, O.; Takeda, Y. Antiviral activity of 5,6,7-trimethoxyflavone and its potentiation of the antitherpes activity of acyclovir. *J. Antimicrob. Chemother.* **1997**, *39*, 821−824.

(17) Hosozawa, S.; Kato, N.; Munakata, K. 5,6,7-Trimethoxy flavone from *Callicarpa japonica*. *Phytochemistry* **1972**, *11*, 2362.

(18) Kim, H. Morphological variation of the *Callicarpa japonica* complex in Eastern Asia. *J. Plant Biol.* **1998**, *41*, 283−292.

(19) Kobaisy, M.; Tellez, M. R.; Dayan, F. E.; Duke, S. O. Phytotoxicity and volatile constituents from leaves of *Callicarpa japonica* Thunb. *Phytochemistry* **2002**, *61*, 37−40.

(20) Parliment, T. H. Solvent extraction and distillation techniques. In *Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Dekker: New York, 1997; pp 1−26.

(21) Kim, J. M.; Marshall, M. R.; Wei, C. I. Antibacterial activity of some essence oil component against five food borne pathogens. *J. Agric. Food Chem.* **1995**, *43*, 2839−2845.

(22) Naigre, R.; Kalck, P.; Roques, C.; Roux, I.; Michel, G. Comparison of antimicrobial properties of monoterpenes and their carbonylated products. *Planta Med.* **1996**, *62*, 275−277.

(23) Seo, K. I.; Park, S. K.; Park, J. R.; Kim, H. C.; Choi, J. S.; Shim, K. H. Changes in antimicrobial activity of hydrolyzate from mustard seed (*Brassica juncea*). *J. Korean Soc. Food Nutr.* **1996**, *25*, 129−134.

(24) Kim, K. R.; Zlatkis, A.; Park, J. W.; Lee, U. C. Isolation of essential oil from tobacco by gas co-distillation/solvent extraction. *Chromatographia* **1982**, *15*, 559.

(25) Au-Yeung, C. Y.; MacLeod, A. J. A comparison of the efficiency of the Likens and Nickerson extractor for aqueous, lipid/aqueous, and lipid samples. *J. Agric. Food Chem.* **1981**, *29*, 502−505.

(26) Ebeler, S. E.; Pangborn, R. M.; Jennings, W. G. Influence of dispersion medium on aroma intensity and headspace concentration of menthone and isoamyl acetate. *J. Agric. Food Chem.* **1988**, *36*, 791−796.
(27) Schultz, T. H.; Flath, R. A.; Mon, R.; Eggling, S. B.; Teranishi, R. Isolation of volatile components from a model system. *J. Agric. Food Chem*. **1977**, *25*, 446−449.
(28) Bredie, W. L.; Mottram, D. S.; Guy, R. C. E. Effect of temperature and pH on the generation of flavor volatiles in extrusion cooking of wheat flour. *J. Agric. Food Chem.* **2002**, *50*, 1118−1125.
(29) Choi, H. S.; Lee, M. S. The effect of dispersion medium on intensity of volatile flavor components and recovery of essential oil from *Capsella bursa-pastoris* by steam distillation. *Korean J. Food Sci. Technol.* **1996**, *28*, 827−833.
(30) Zaika, L. L.; Kissinger, J. C.; Wasserman, A. E. Inhibition of lactic acid bacteria by herbs. *J. Food Sci.* **1983**, *48*, 1455−1459.
(31) Farag, R. S.; Daw, Z. Y.; Hewedi, F. M.; El-Baroty, G. S. A. Antimicrobial activity of some Egyptian spice essential oil. *J. Food Prot.* **1989**, *52*, 665−667.
(32) Hussein, A. S. M. Antibacterial and antifungal activities of some Libyan aromatic plants. *Planta Med.* **1990**, *56*, 644−645.
(33) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oil. *J. Appl. Microbiol.* **2000**, *88*, 308−316.
(34) Weissinger, W. R.; McWatters, K. H.; Beuchat, L. R. Evaluation of volatile chemical treatments for lethality to *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.* **2001**, *64*, 442−450.
(35) Inouye, S.; Goi, H.; Miyauchi, K.; Muraki, S.; Ogihara, M.; Iwanami, Y. Inhibitory effect of volatile constituents of plants on the proliferation of bacteria-Antibacterial activity of plant volatiles-. *J. Antibact. Antifung. Agents* **1983**, *11*, 609−616.
(36) Arctander, S. *Perfume and Flavor Chemicals (Aroma Chemicals)*; Det Hoffensbergske Etablissement: Copenhagen, Denmark, 1969.
(37) Bauer, K.; Garbe, D.; Surburg, H. *Common Fragrance and Flavor Materials*, 3rd ed.; Wiley-VCH Verlag GmbH: Weinheim, Germany, 1997.

Received for review August 19, 2003. Revised manuscript received December 9, 2003. Accepted December 21, 2003. This research was supported by the Research Center for Industrial Development of Biofood Materials of Chonbuk National University, Chonju, Korea. The center is designated a Regional Research Center appointed by the Korea Science and Engineering Foundation (KOSEF), Jeollabuk-do Provincial Government, and Chonbuk National University.

JF034936D