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Separation and recovery of semi-volatile substances of *Cnidii Rhizoma*, *Aucklandiae Radix* and *Amomum Fructus* by reduced pressure collections and GC-MS

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Abstract: When extracting semi-volatile components of herbal medicines using hot water vapor, some substances may react with water vapor or oxygen, and some volatile substances may be lost, when using an organic solvent extraction method has the disadvantage that it may contain a non-volatile material and residual organic solvent. In addition, it is inefficient to separate semi-volatile substances from herbal medicines into each single component and conduct biological activity research for each component to determine the effective ingredient, and some components may be lost in the separation process. In this study, semi-volatile substances evaporated under two pressure-reduced conditions in Chinese herbal medicines such as *Cnidii Rhizoma, Aucklandiae Radix* and *Amomum Fructus* were separated by cooling with liquid nitrogen. Those were analyzed by gas chromatography-mass spectrometry (GC-MS) to identify the components, and this method may be used to study biological activities at the cellular level. The substances separated under reduced pressure, essential oil obtained by simultaneous distillation extraction (SDE) method and substances by using solid phase micro-extraction (SPME) from *Cnidii Rhizoma, Aucklandiae Radix* and *Amomum Fructus* were analyzed by GC-MS. In the case of *Cnidii Rhizoma* and *Aucklandiae Radix*, there were some differences among the essential oil components obtained by SDE and those identified by low temperature capture (CT) and SPME method, these were believed to be produced by some volatiles reacting with water or oxygen at the boiling point temperature of water.

Key words: *Cnidii Rhizoma*, *Aucklandiae Radix*, *Amomum Fructus*, cold trap, GC-MS

1. Introduction

Essential oils of herbal medicines and herbs have long been used as perfumes and food fragrances, the hydrothermal extract of herbal medicines has a limited feature that is difficult to penetrate the blood-brain barrier in the course acting on brain tissue, however interest in this field is growing as studies have shown that semi-volatile oil compounds can pass through the blood vessels of the brain by direct

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administration of some nasal passages.[1-5] The fatigue recovery effect[6] of herbal oils has been attracted attention, and there have been studies of research on obesity treatment effect[7] and cancer treatment[8] of the essence oil of *Cnidii Rhizoma*, antimicrobial activity[9] of food-derived pathogens of essential oils of *Aucklandiae Radix*, and non-alcoholic fatty liver prevention[10] and intestinal mucositis[11] of essential oils *Amomum Fructus*.

Separation of essential oils from these materials was mostly done by steam distillation (SD) using steam.[12] In addition, there are superheated water extraction (SWE),[13] microwave-assisted extraction (MAE),[14] simultaneous distillation extraction (SDE) using water vapor and organic solvent at the same time,[15] organic solvent extraction (SE),[16] and supercritical fluid extraction (SFE).[17]

In order to study the biological activity using medicinal substances after separating them from medicinal herbs, ideally volatile substances using pure and uncontaminated materials should be used, a certain amount (at least 10 mg) should be obtained for cell-level activity studies. However, the extraction method using water vapor may cause some volatile substances in the herbal medicine to react with water or oxygen and deteriorate to other materials. The extraction method using organic solvents may contain residual organic solvents. As a way to solve this problem, in this study, *Cnidii Rhizoma*, *Aucklandiae Radix* and *Amomum Fructus* were volatilized at two different temperatures under reduced pressure without using water or organic solvents, which was recovered by cooling with liquid nitrogen, and the degree of separation was confirmed using GC-MS. In addition, the GC-MS results of two extracts obtained under reduced pressure and essential oils obtained by SDE and adsorption by solid phase micro-extraction (SPME) were compared for the three herbs.

2. Experimental

2.1. Chemicals and reagents

Aucklandiae Radix and *Amomum Fructus* were Chinese herbs and *Cnidii Rhizoma* was Korean herb, and the herbs were used after being refrigerated at 4 °C for refrigeration. The water was deionized water purified by reverse osmosis (Human Corporation, Seoul, Korea), and hexane (HPLC grade) and methylene chloride (MC, HPLC grade) were manufactured by Merck, and anhydrous sodium sulfate (99 %) was Junsei products.

2.2. Cold-trapping Ⅰ (CT Ⅰ)

After measuring the weight of approximately 10.0 g of crushed *Cnidii Rhizoma, Aucklandiae Radix* and *Amomum Fructus* accurately, place them in a flask and maintain a constant temperature and collect volatile substances at a liquid nitrogen temperature for 3 hours in a decompression atmosphere ($(3 \pm 1) \times 10^{-2}$ torr) (*Fig*. 1), part of the collected solution was taken, diluted with MC to a 25 % (v/v) concentration, and 1 μL of it was injected with GC-MS to analyze (CT I-MC). When the collected solution is kept at 4 °C for 24 hours, it is separated by water and oil components. The oil component was separated with a small separatory funnel and the same experiment was conducted three times (*Table* 1).

2.3. Cold-trapping Ⅱ (CT Ⅱ)

After CT I test, put the herb in the collection

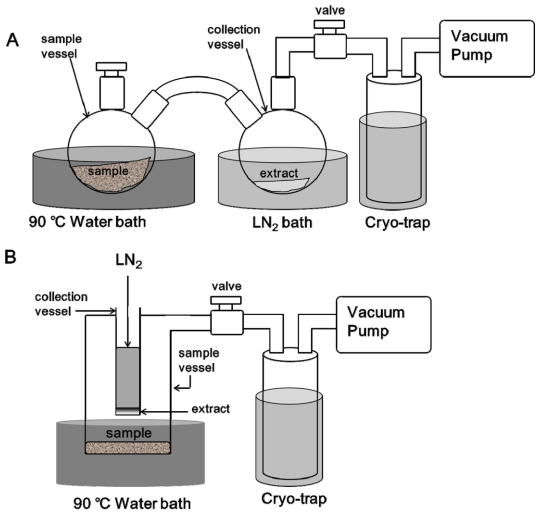

Fig. 1. Schemes for (A) reduced pressure cold trapping I (CT I), (B) reduced pressure cold trapping II (CT II), The cryo-trap protects the vacuum pump from water and volatile organic compounds during extraction.

Table 1. Methods of collecting volatiles in the *Cnidii Rhizoma, Aucklandiae Radix, Amomum Fructus* : CT I, CT II and SDE collection temperature and yield

	Cnidii Rhizoma		*Aucklandiae Radix*		*Amomum Fructus*	
CT I	65 ± 2 °C 10.011 g	0.0201 g 0.20 % (± 0.06 %)	95 ± 2 °C 9.603 g	0.0193 g 0.20 % (± 0.04 %)	40±2 °C 10.064 g	0.0203 g 0.20 % (± 0.04 %)
CT II	95 ± 2 °C 9.196 g	0.0196 g 0.21 % (± 0.0 %)	98 ± 2 °C 8.802 g	0.0153 g 0.17 % (± 0.05 %)	95±2 °C 9.259 g	0.0356 g 0.38 % (± 0.04 %)
SDE	50.002 g	0.464 g 0.93 % (± 0.10 %)	50.001 g	0.386 g 0.77 % (± 0.13 %)	50.002 g	0.534 g 1.07 % (± 0.16 %)

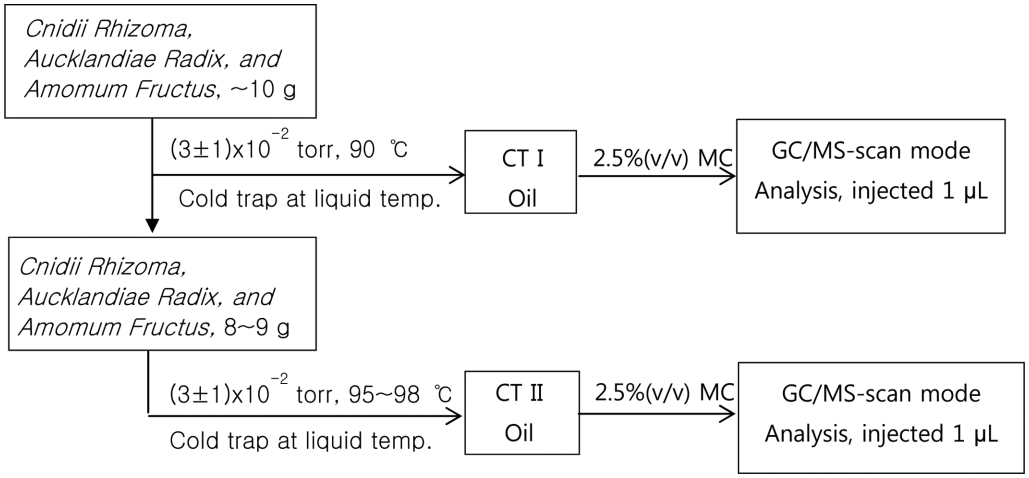

Fig. 2. Overall analytical procedure for CT(cold trapping) I and CT II from *Cnidii Rhizoma*, *Aucklandiae Radix*, and *Amomum Fructus*.

vessel and adjust the temperature with a water bath in a decompression atmosphere ($(3 \pm 1) \times 10^{-2}$ torr), collect the volatile substance at the liquid nitrogen temperature for 3 hours (*Fig.* 1), part of the collected solution was taken, diluted with the MC to a concentration of 2.5 % (v/v), and 1 μL of it was injected with GC-MS to analyze it (CT II-MC). The same experiment was carried out three times (*Table* 1) and the extraction and analysis process was organized in *Fig*. 2.

2.4. Solid phase micro-extraction (SPME) of herbal medicine

After measuring the weight of 1 g of crushed *Cnidii Rhizoma, Aucklandiae Radix* and *Amomum Fructus* accurately, they were transferred to a 20 mL vial, placed in a water bath (95 ± 2) °C, and collected volatile substances using SPME for 5 minutes under the same conditions as *Table* 2, and analyzed with GC-MS. Three identical experiments were conducted on each herb.

Table 2. Solid phase microextraction (SPME) conditions

SPME fiber	85 μm Carboxen/PDMS StableFlex
Sampling temp.	90 °C
Sampling condition	Headspace, static
Sampling time	5 min.
GC desorption time	1 min.
GC desorption temp.	250 °C

2.5. Simultaneous distillation extraction (SDE)

About 50.00 g weight of ground crushed *Cnidii Rhizoma, Aucklandiae Radix* and *Amomum Fructus* herb were accurately measured and put in a 2-L flask, and 800 mL of deionized water was added and

80 mL of hexane was added to the other flask, connected to the SDE device. Heated for three hours in the nitrogen atmosphere and hexane layer was collected. After drying the hexane solution with anhydrous sodium sulfate for 12 hours, the volatile material was removed using a rotary evaporator to obtain the essential oil. The same experiment was carried out three times (*Table* 1) and 1 μL of a 2.5 % (v/v) MC solution was injected directly into the GC-MS for analysis.

2.6. GC-MS

The GC-MS experimental conditions were summarized in *Table* 3 and the component analysis in the TIC was determined by comparing the mass spectrum data of the GC-MS library (W8N08 of Wiley7Nist05) with the reported data.[11] The library matching value were over 90 %. The relative percentage of each component (%) was calculated by dividing the corresponding peak area by the sum of the total area of the peak from the TIC (total ion chromatogram) obtained from the GC-MS.

Table 3. GC-MS conditions

Gas Chromatography	Agilent 7890A
Column Temp	40 °C for 0 min 8 °C/min to 200 °C for 0 min. 10 °C/min to 270 °C for 5 min. 20 °C/min to 300 °C for 10 min.
Column	HP-5MSI (30 m × 0.25 mm × 0.25 μm film thickness) Split ratio - 1 : 10
Mass Spectrometer	Agilent 5975C
Solvent Delay	3.00 min
Ion source temp	230 °C
Injector temp	250 °C
Scan range	35 – 550 m/z

3. Results and Discussion

3.1. CT Ⅰ, CT Ⅱ, SPME and SDE of *Cnidii Rhizoma*

The volatile material was recovered from the liquid nitrogen temperature for 3 hours by heating 10.01g of the *Cnidii Rhizoma* at (65 ± 2) °C under a

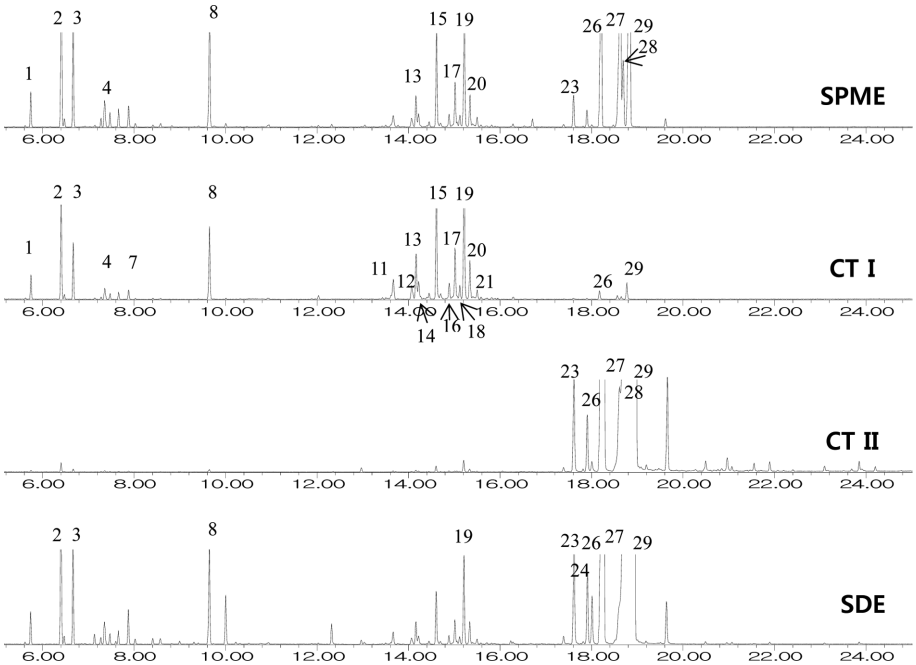

Fig. 3. TIC obtained by GC-MS analysis of samples collecting volatiles from *Cnidii Rhizoma* by SPME, CT I-MC, CT II-MC and SDE-MC methods (Numbers above or near peaks indicate the identified chemicals in *Table* 4. Only numbers of peaks with 1 % or above were shown here).

Table 4. Relative contents from TIC obtained from GC-MS analysis of samples collecting volatiles from *Cnidii Rhizoma* by SPME, CT I-MC, CT II-MC and SDE-MC methods

Retention time(min)		compound	Relative contents (Area %)			
			SPME	CT I-MC	CT II-MC	SDE-MC
1	5.74	α-Pinene	1.35	2.41	-	0.46
2	6.41	Sabinene	10.90	13.81	-	3.31
3	6.67	β-Pinene	4.51	5.35	-	1.46
4	7.36	Limonene	1.36	1.56	-	0.43
5	7.47	β-Ocimene	0.60	0.64	-	0.16
6	7.66	*trans*-Ocimene	0.71	0.83	-	0.20
7	7.88	γ-Terpinene	0.87	1.14	-	0.55
8	9.65	6-Butyl-1,4-cycloheptadiene	5.43	8.31	-	1.72
9	10.01	4-Terpineol	-	-	-	0.83
10	12.32	4-vinyl-2-methoxy-phenol	-	-	-	0.34
11	13.66	β-Elemene	0.77	3.47	-	0.27
12	14.07	α-Copaene	0.58	2.36	-	0.16
13	14.16	β-Caryophyllene	1.48	6.33	-	0.41
14	14.22	α-Cubebene	0.72	2.08	-	0.17
15	14.61	*trans*-β-Farnesene	4.39	12.82	-	0.90
16	14.88	α-Longipinene	0.67	2.24	-	0.17
17	15.02	γ-Curcumene	2.16	6.86	-	0.45
18	15.12	Germacrene D	0.60	1.79	-	0.15
19	15.22	β-Selinene	5.81	17.67	-	1.60
20	15.34	α-Selinene	1.53	4.66	-	0.41
21	15.50	α-Cedrene	0.51	1.23	-	0.10
22	16.28	Germacrene B	0.17	-	-	0.04
23	17.60	Butyl phthalide	1.53	-	1.69	2.25
24	17.90	Butylidenephthalide	0.83	-	0.89	1.38
25	18.01	Allyl phenoxyacetate	-	-	-	0.93
26	18.21	5-Undecene-3-yne	12.12	1.23	17.44	17.13
27	18.63	2-Propenyl phenoxyacetate	13.62	0.62	30.34	19.86
28	18.70	3-Isobutylidenephthalide	3.93	0.49	12.51	-
29	18.84	Ligustilide	22.92	2.12	36.30	44.13
30	20.49	Cyclohexadecane	-	-	0.20	-
31	21.07	Methyl palmitate	-	-	0.24	-
32	21.90	Ethyl palmitate	-	-	0.15	-
33	23.09	Methyl octadeca-8,11-dienoate	-	-	0.09	-
34	23.85	Ethyl linoleate	-	-	0.17	-

reduced pressure ((3 ± 1) × 10^{-2} torr) (CT I). The TIC (*Fig*. 3) obtained by injecting 1 μL of its 25 % (v/v) MC solution into the GC-MS contained 23 compounds including β-selinene (17.67 %), savinene (13.81 %), and *trans*-β-parnesene (12.82 %) (*Table* 4), and the % value in parentheses is relative area ratio (% of that peak area divided by the total peak area), and 20 mg ((0.20 ± 0.06) %) was obtained after removing water. After the CT I experiment, the *Cnidii Rhizoma* was put into the CT II collection vessel and placed in the water bath container (95 ± 2) °C while keeping the pressure at reduced ((3 ± 1) × 10^{-2} torr) condition, and volatiles under these conditions were collected at liquid nitrogen temperature to give 19.6 mg ((0.21 ± 0.05) %). At the TIC obtained by injecting 1 μL of the 2.5 % (v/v) MC solution into the GC-MS, 11

components were identified and the four substances with a large relative area ratio were ligustilide (36.30 %), 2-propenyl phenoxyacetate (30.34 %), 5-undecene-3-yne (17.4 %) and 3-isobutylidenephthalide (12.51 %). These four substances were found in the CT I method extracts 2.12 %, 0.62 %, 1.23 % and 0.49 % (total: 4.46 %) respectively, which means that *Cnidii Rhizoma* is separated under the above decompression capture conditions and contains approximately 5 % of CT I extracts in the CT II collection.

The method of separating the essential oil by heating the *Cnidii Rhizoma* with water is often used, where chemical reactions with high temperature water (the boiling point of water) are likely to result in some substances being converted into other compounds. To verify this, the GC-MS results of oil separated by the SDE method were compared with the GC-MS results obtained by absorbing the volatile components of the *Cnidii Rhizoma* with SPME. After putting about 1.00 g of the *Cnidii Rhizoma* in a 20 mL vial, absorb the volatile substance at (95 ± 2) °C for 5 minutes and analyze it with GC-MS, 26 components were identified, including ligustilide (22.92 %), 2-propenyl phenoxyacetate (13.62 %), 5-undecene-3-yne (12.12 %) and sabinene (10.90 %). Essential oil 0.464 g ((0.93 ± 0.11) %) was obtained from 50.00 g of the *Cnidii Rhizoma* by SDE method and, 1 μL of the 2.5 % (v/v) MC solution of the oil was injected into the GC-MS and 28 components were identified, including ligustilide (44.13 %), 2-propenyl phenoxyacetate (19.86 %), 5-undecene-3-yne (17.13 %). Comparing these 28 components, three new substances were found in the oil from which the *Cnidii Rhizoma* was extracted by the SDE method: 4-terpineol, 4-vinyl-2-methoxy-phenol, and allyl phenoxyacetate, which were not detected by the SPME, CT I and CT II method, and 3-isobutylenephthalide was not detected in oil obtained from SDE method. The reason why substances such as 4-terpineol, 4-vinyl-2-methoxy-phenol, and allyl phenoxyacetate were created is that some of the herbs were created by reacting with high temperature of water during the extraction of the *Cnidii Rhizoma* by the SDE method and 3-isobutylenephthalide is considered to have been dissipated in the SDE process, and further research is required.

In TICs of CT I, CT II, SDE, and SPME, the relative standard deviation of the relative area ratio of peaks ranged from 3.20 to 6.02 %. There was a difference between the GC-MS results of the *Cnidii Rhizoma* and the analysis results of the volatile substances reported by other groups.[7,8,18-25] These differences were found in the ingredients and relative area ratios of volatile substances not only in the case of *Cnidii Rhizoma* but also in the case of *Aucklandiae Radix* and in the case of *Amomum Fructus*, which have been reported to differ according to variables such as variability, cultivation conditions, harvesting times, storage time, extraction and processing methods within the same species.[26-35] Herbal medicines are generally taken by taking hot water extracts, which may cause unusual reactions due to other reactants occurring at high temperatures. It has been reported that hot ginseng extracts ginsenosides.[36]

In TICs obtained by SPME method, the sum of relative area ratio of the peaks of 3.00 to 15.50 minutes was 44.95 %, whereas in the case of oil separated by SDE and for capture separated by CT I method, their sum was 14.25 % and 95.56 %, respectively. This means that in the process of separating the oil by SDE method, more highly volatile substances are removed than by CT I method, and that is why the smell of oil obtained by SDE method is different from that of the powder of *Cnidii Rhizoma* or CT I extract.

3.2. CT Ⅰ, CT Ⅱ, SPME and SDE of *Aucklandiae Radix*

Collect the volatile substances (CT I) at the liquid nitrogen temperature for 3 hours while heating 9.60 g of the *Aucklandiae Radix* to (95 ± 2) °C at the pressure reduction state ((3 ± 1) × 10^{-2} torr), 25 % (v/v) MC solution 1 μL of this solution was analyzed using GC-MS and 28 components were identified, including valerenol (44.9 %), *trans*-caryophyllene (9.61 %), and γ-curcumene (4.03 %) were found ((*Fig*. 4 and *Table* 5) and 19.3 mg ((0.20 ± 0.04) %) was obtained after removing water. After CT I

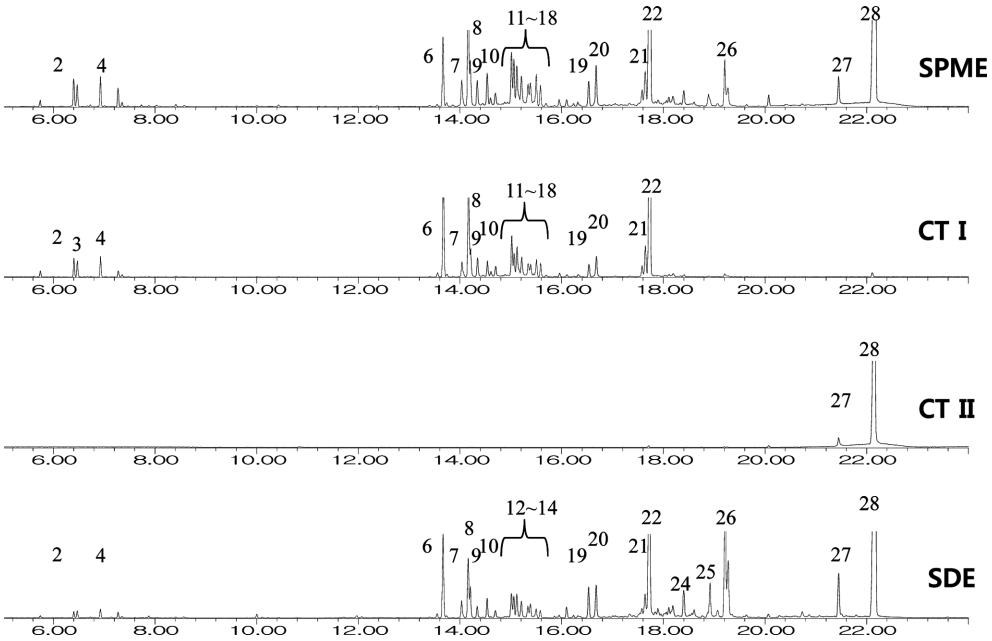

Fig. 4. TIC obtained by GC-MS analysis of samples collecting volatiles from *Aucklandiae Radix* by SPME, CT I-MC, CT II-MC and SDE-MC methods (Numbers above peaks indicate the identified chemicals in *Table* 5. Only numbers of peaks with 1 % or above were shown here).

extract, the herbs was put into the CT II collecting container and heated to (98 ± 2) °C under decompression ((3 ± 1) × 10^{-2} torr) and volatiles under these conditions were collected at liquid nitrogen temperature to give 15.3 mg ((0.17 ± 0.05) %). At the TIC (*Fig*. 4) obtained by injecting 1 μL of the 2.5 % (v/v) MC solution into the GC-MS, dehydrocostuslactone (97.31 %) was identified, and which was not detected in extract obtained by CT I method (*Table* 5). This means that by using CT I and II, the dehydrocostuslactone can be separated by 97.31 % purity.

To check if the volatile components of *Aucklandiae Radix* react to high temperature water and turn into other substances, the results of GC-MS using SPME and the GC-MS analysis of the oil extract obtained by the SDE method were compared. After adding about 1.00g of *Aucklandiae Radix* into a 20 mL vial, the volatile substance was absorbed into the SPME for 5 minutes at (95 ± 2) °C and analyzed with GC-MS, the 28 compounds were identified including dehydrocostuslactone (32.59 %), valerenol (28.40 %) and *trans*-caryophyllene (4.80 %). Essential oil 0.386 g ((0.77 ± 0.13) %) was recovered by SDE method from 50.00 g of *Aucklandiae Radix*. From TIC obtained by injection of 1 μL of its 2.5 % (v/v) MC solution into GC-MS, 27 components were identified including dehydrocostuslactone (36.64 %), valerenol (24.90 %), and β-elemene (4.54 %). Although 1,11-tridecadiene-3,5,7,9-tetrayne was detected in the TIC obtained using the SPME method, no foreign substances were detected in the oil extracted by the SDE method. Relative standard deviations for the relative area ratios of the peaks in the TIC of CT I, CT II, S DE and SPME of *Aucklandiae Radix* ranged from 2.89 to 7.03 %.

Results of analysis of the volatile substance of *Aucklandiae Radix* with GC-MS are reported [9,37-43] and there is a slight difference between the results of this study in the identification of the composition of the volatile substance.

In the TIC obtained with SPME, the sum of the relative area ratios of the peaks from 3.00 to 17.64 minutes was 33.71 %, whereas for SDE-essential oil and the CT I method collections, their sums were

Table 5. Relative contents from TIC obtained from GC-MS analysis of samples collecting volatiles from *Aucklandiae Radix* by SPME, CT I-MC, CT II-MC and SDE-MC methods

Retention time (min)		compound	Relative contents (Area %)			
			SPME	CT I-MC	CT II-MC	SDE-MC
1	5.74	α-Pinene	0.25	0.53	-	0.11
2	6.40	Sabinene	1.11	1.51	-	0.28
3	6.47	β-Pinene	0.84	1.42	-	0.35
4	6.93	α-Phelladrene	1.10	1.59	-	0.40
5	7.35	Limonene	0.19	0.26	-	0.07
6	13.67	β-Elemene	2.92	9.39	-	4.54
7	14.03	Dihydro-α-ionone	1.36	1.90	-	1.07
8	14.16	*trans*-caryophyllene	4.80	9.61	-	3.07
9	14.20	α-Lonone	1.78	2.30	-	1.49
10	14.34	(Z)−α-Bergamotene	1.22	1.97	-	0.60
11	14.53	Geranylacetone	1.45	1.61	-	0.99
12	15.01	γ-Curcumene	2.41	4.03	-	1.23
13	15.06	AR-Curcumene	2.08	2.27	-	1.12
14	15.11	β-Ionone	1.85	3.01	-	1.35
15	15.21	β-Seliene	1.57	2.20	-	0.93
16	15.34	α-Seliene	1.12	1.67	-	0.78
17	15.50	Germacrene A	1.54	1.79	-	0.50
18	15.58	(Z)-γ-Bisabolene	0.90	1.20	-	0.41
19	16.53	γ-Cadinene	1.19	1.28	-	1.66
20	16.67	Caryophllene oxide	2.15	2.25	-	1.98
21	17.64	Dihydro-aplotaxene	1.88	3.33	-	1.69
22	17.74	Valerenol	28.40	44.9	-	24.90
23	18.40	(Z)-α-*trans*-Bergamotol	0.90	-	-	1.67
24	18.88	1,11-Tridecadiene-3,5,7,9-tetrayne	0.68	-	-	-
25	18.92	γ-Costol	0.21	-	-	2.02
26	19.21	β-Costol	2.19	-	-	7.71
27	21.45	3-Phenylcyclohexene	1.32	-	2.69	2.50
28	22.15	Dehydrocostus lactone	32.59		97.31	36.64

24.62 % and 55.12 %, respectively. This is because some low-boiling compounds are lost during the separation and recovery of essential oil components by the SDE method, whereas volatiles with low boiling points are hardly lost by the CT I method.

3.3. CT Ⅰ, CT Ⅱ, SPME and SDE of *Amomum Fructus*

Amomum Fructus (10.06 g) was heated to (40 ± 2) °C under the reduced presure ((3 ± 1) × 10^{-2} torr) and the volatile substances was collected by cooling with liquid niitrogen for three hours (CT I), a GC-MS analysis of 25 % (v/v) MC solution 1 μL of this solution identified 23 components (*Fig*. 5) including α-terpinolene (65.61 %), nerolidol (10.59 %) and *trans*-caryophyllene (6.33 %) (*Table* 6), and 20 mg ((0.20 ± 0.04) %) was obtained after removing water. After CT I extract, the herb was put into the CT II collecting container and heated to (95 ± 2) °C under decompression ((3 ± 1) × 10^{-2} torr) and volatiles under these conditions were collected at liquid nitrogen temperature to give 35.6 mg ((0.38 ± 0.04) %), 1 μL of its 2.5 % (v/v) MC solution was injected into the GC-MS and 12 components were identified between 16.25 minutes and 26.98 minutes including nerolidol (77.12 %), oleic acid (9.46 %) and trendione (5.52 %). Among the compounds isolated by CT II method, the overlapping material with CT I method was

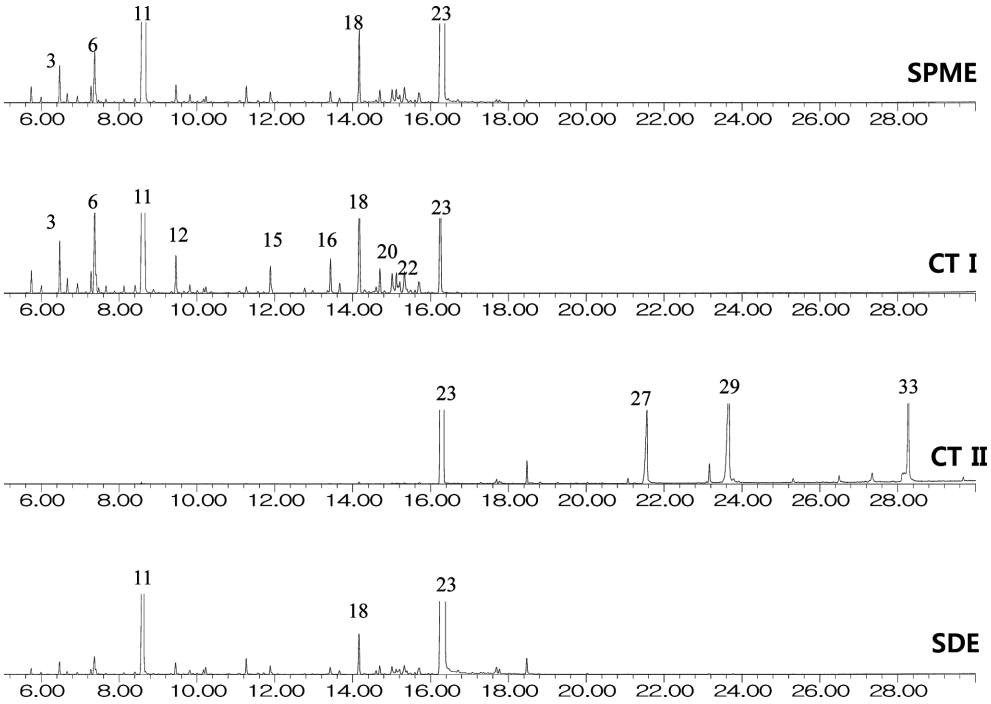

Fig. 5. TIC obtained by GC-MS analysis of samples collecting volatiles from *Amomum Fructus* by SPME, CT I-MC, CT II-MC and SDE-MC methods (Numbers above peaks indicate the identified chemicals in *Table* 6. Only numbers of peaks with 1 % or above were shown here).

nerolidol and α-terpinolene, which contained 10.59 % and 65.61 % in CT I respectively.

After putting about 1.00 g of *Amomum Fructus* into a 20 mL vial, we determined 26 components in TIC analyzed with GC-MS after 5 minutes of adsorption to SPME at (95 ± 2) ℃, three compounds in order of large relative area ratios were nerolidol (54.77 %), and α-terpinolene (34.6 %) and *trans*-caryophyllene (2.10 %). From the *Amomum Fructus* of 50.00 g by SDE method, 0.534 g ((1.07 ± 0.16) %) of oil was obtained, 26 components were identified, including nerolidol (76.98 %), α-terpinolene (16.75 %), and *trans*-caryophyllene (1.14 %) at TIC, obtained by injecting 1 μL of a 2.5 v/v% MC solution into the GC-MS. In the case of *Amomum Fructus*, comparing the results of GC-MS analysis of essential oils obtained with SDE and GC-MS results of collecting volatiles with SPME, the relative area ratios of the components are different, but as in the case of *Cnidii Rhizoma* or *Aucklandiae Radix*, no detected or completely missing substances were found. In addition, 8 substances were identified from CT II method, including oleic acid, which was not extracted by SDE method.

In the TIC obtained with SPME, the sum of the relative area ratios of the peaks from 3.00 minutes to 11.88 minutes was 41.24 %, whereas for SDE-essential oil and the CT I method collections, their sums were 19.35 % and 77.36 %, respectively. This means that relatively well volatile substances in the process of separating and retrieving oils by the SDE method, as in the case of *Cnidii Rhizoma*, and *Aucklandiae Radix*, are removed by the SDE method, and the reason why the smell of oils obtained by the SDE method is different from the smell of *Amomum Fructus* powder and CT I extract. Relative standard deviations for the relative area ratios of the peaks in the TIC of CT I, CT II, S DE and SPME of *Amomum Fructus* ranged from 3.21 to 8.36 %.

There was a difference between the GC-MS

Table 6. Relative contents from TIC obtained from GC-MS analysis of samples collecting volatiles from *Amomum Fructus* by SPME, CT I-MC, CT II-MC and SDE-MC methods

Retention time (min)		compound	Relative contents (Area %)			
			SPME	CT I-MC	CT II-MC	SDE-MC
1	5.74	α-Pinene	0.38	0.73	-	0.13
2	6.01	Camphene	0.16	0.29	-	0.06
3	6.48	β-Pinene	0.95	1.67	-	0.31
4	6.67	β-Myrcene	0.21	0.46	-	0.07
5	6.93	α-Phellandrene	0.17	0.34	-	0.05
6	7.37	β-Phellandrene	1.62	3.71	-	0.56
7	7.41	Eucalyptol	0.16	0.55	-	0.10
8	7.67	β-Ocimene	0.09	0.26	-	0.03
9	8.13	Linalool oxide	0.10	0.27	-	0.05
10	8.41	α-Terpinene	0.15	0.36	-	0.09
11	8.63	α-Terpinolene	34.66	65.61	0.61	16.75
12	9.46	Camphor	0.52	1.38	-	0.32
13	9.82	Borneol	0.25	0.32	-	0.14
14	11.27	*trans*-Genaniol	0.45	0.27	-	0.41
15	11.88	Borneol acetate	0.37	1.14	-	0.28
16	13.43	α-Copaene	0.36	1.26	-	0.24
17	13.67	β-Elemene	0.16	0.42	-	0.13
18	14.17	*trans*-Caryophyllene	2.10	6.33	-	1.14
19	14.70	α-Humulene	0.38	0.91	-	0.24
20	15.01	γ-Selinene	0.55	1.10	-	0.30
21	15.12	Germacrene-D	0.41	0.82	-	0.15
22	15.33	β-Selinene	0.67	1.22	-	0.41
23	16.25	Nerolidol	54.77	10.59	77.12	76.98
24	17.70	5-epi-Neointermedeol	0.14	-	0.21	0.34
25	17.77	α-Gurjunene	0.12	-	0.13	0.18
26	18.47	Nerolidol	0.10	-	0.63	0.53
27	21.56	Palmitic acid	-	-	4.89	-
28	23.16	11-Octadecenoic, methyl ester	-	-	0.77	-
29	23.67	Oleic acid	-	-	9.46	-
30	25.32	Eicosane	-	-	0.21	-
31	26.50	Pinostrobin chalcone	-	-	0.36	-
32	27.35	5-Methoxy-3,7-dihydroxyflavanone	-	-	0.51	-
33	28.28	Trendione	-	-	5.52	-
34	29.68	Neryl acetate	-	-	0.20	-

component analysis of extracts obtained by CT I, CT II, SPME and SDE methods and the GC-MS results of the oil by SDE and hexane extract reported.[44-50] In this study, nerolidol (76.98 %) was the largest relative proportion of essential oils obtained by SDE method, however, previous studies have been reported that camphor (32.3 %) was the largest peak in TIC obtained by injecting essential oils of *Amomum Fructus* by SDE method.[50]

4. Conclusions

For medicinal herbs such as *Cnidii Rhizoma*, *Aucklandiae Radix*, and *Amomum Fructus*, the separation of semi-volatile substances by cooling them at liquid nitrogen temperature under two different capture conditions resulted in the separation of the amounts sufficient to conduct biologically active research at the cell level. The GC-MS analysis

of the extracts separated by CT I and CT II methods of the herbs showed that there were no duplicate substances in the case of *Aucklandiae Radix*, and some substances were slightly duplicated in the case of *Cnidii Rheizoma* and *Amomum Fructus*.

In the case of *Cnidii rhizoma*, three substances were detected only in the essential oil separated by SDE method such as 4-terpineol, 4-vinyl-2-methoxy-phenol and allyl phenoxyacetate, which were not detected in TIC of GC-MS collected and analyzed with SPME, CT I and CT II, and 3-isobutylidenephthalide was not detected only in essential oils extracted with SDE method. In the case of *Aucklandiae Radix*, (*E*)-1,11-tridecadiene-3,5,7,9-tetrayne, which was detected in TIC obtained by SPME method, could not be detected in essential oils extracted by SDE method. These results suggest that some materials reacted with water or oxygen at the boiling point of the water in the process of separating the oil by SDE method, and further research is needed in this field.

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